

University of Crete, Department of Biology



Zebrafish Behavioural Indicators of stress and anxiety



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Behavioural studies in fish

There are numerous fish species used as model organisms to study fish behaviour. Some of them include:

- *Danio rerio* (zebrafish)
- *Notobranchius furzeri* (turquoise killifish)
- *Oryzias latipes* (medaka)
- *Poecilia reticulata* (guppy)
- *Gasterosteus aculeatus* (three-spined stickleback)
- *Pimephales promelas* (fathead minnow)
- *Oreochromis niloticus* (Nile tilapia)
- *Salmo salar* (Atlantic salmon)
- *Cyprinus carpio* (common carp)
- *Dicentrarchus labrax* (European seabass)



Zebrafish as a model organism

Zebrafish entered the scientific spotlight in the early 1970s, thanks to George Streisinger at the University of Oregon.

- Exhibit a wide repertoire of behaviours.
- Its small adult size along with the low-cost housing permits high-throughput screening.
- High fecundity, external fertilization, transparent embryos, and rapid development
- Possess all major neurotransmitter systems, transporters, receptors and hormones.
- Fully sequenced genome with 70-75% of human genes having at least one zebrafish orthologue.
- Readily available video tracking technologies that can be coupled with zebrafish behavioural assays, providing data-rich endpoints (e.g. velocity, distance travelled, three-dimensional spatial and spatiotemporal swim path reconstructions) which are impossible to generate manually.



Zebrafish as a model organism

Because of these advantages, zebrafish are now widely used to study:

- **stress and anxiety**
- **aggression and affiliation**
- **social interaction**
- **learning and cognition**
- **reward processing**



**Zebrafish as a
model organism
for anxiety and
stress research**



Zebrafish as a model organism for anxiety and stress research

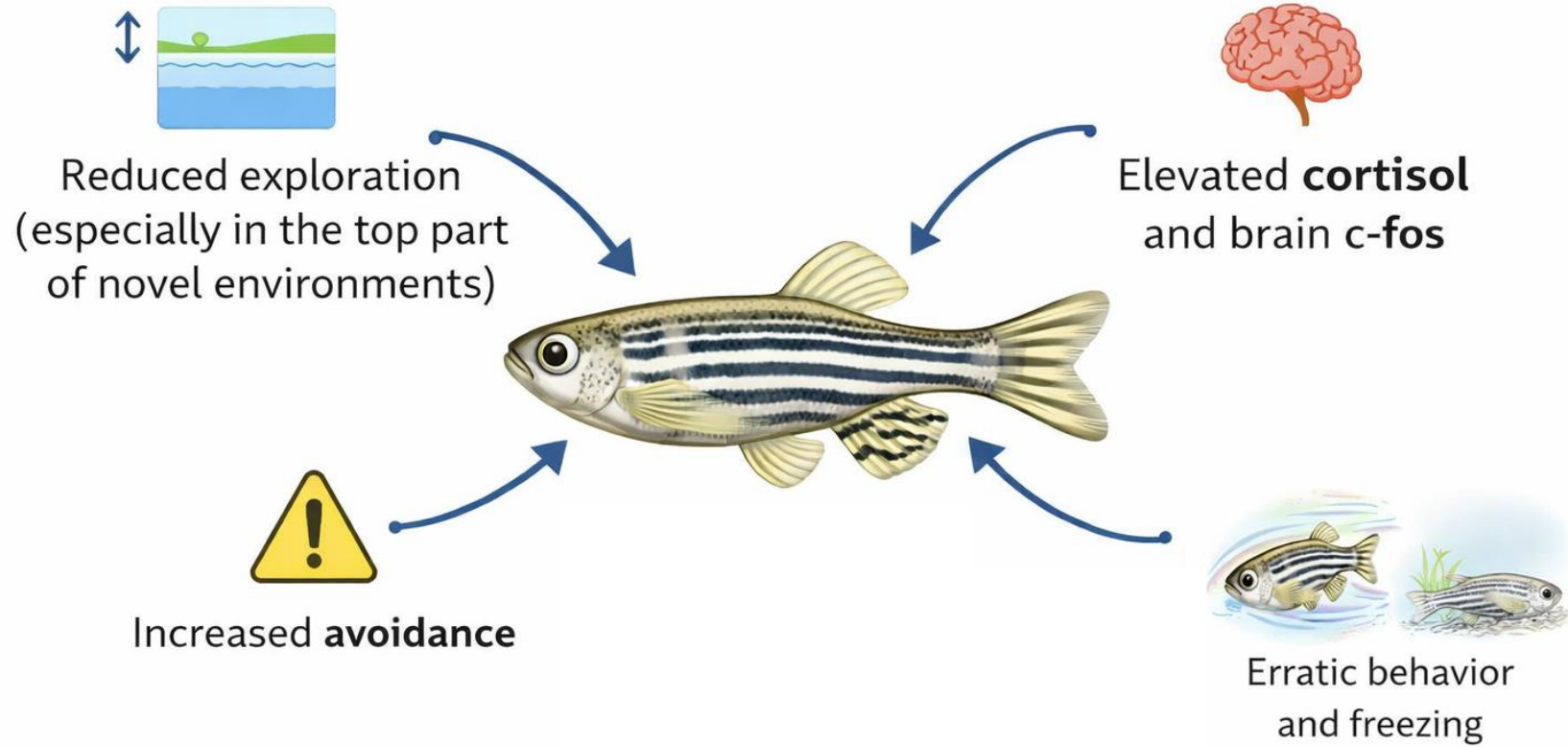
Robust and easily quantifiable **cortisol stress response**.

Clear-cut drug-evoked phenotypes with high predictive validity.

Sensitivity to a wide range of experimental stressors, such as:

- Novelty
- Social isolation
- Confinement
- Predator exposure
- Alarm substance

Anxiety/Fear-related behavior



Behavioural tests for studying stress and anxiety

Tests in this category include:

- Novel tank test (vertical exploration)
- Open field test (horizontal exploration)
- Light/dark preference test
- Exploratory tank test

Novelty

A **novel environment** constitutes a potentially dangerous situation for zebrafish.

As a result, fish exhibit **avoidance behaviour** which serves evolutionary conserved anti-predatory functions.

An animal's **exploratory behaviour** in a novel environment is believed to reflect the **emotional state of animals**.

Novel Tank test

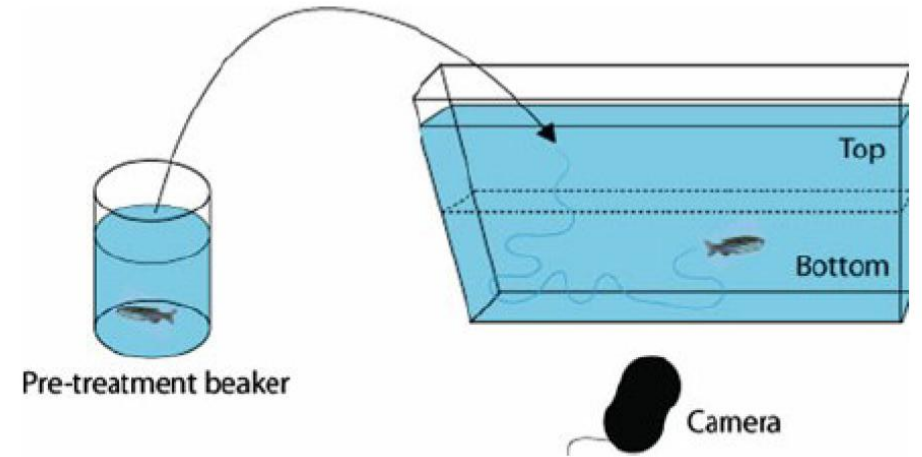
A novelty-based paradigm, which measures vertical exploration.

Zebrafish express a robust anxiety-like response, once introduced to the novel tank apparatus, consisting of:

- Diving to the bottom of the tank (geotaxis)
- Reduced exploration
- Increased freezing
- Erratic movements

These behavioural responses are accompanied by physiological responses:

- Elevated cortisol levels
- Increased breathing
- Increased heartbeat frequency.

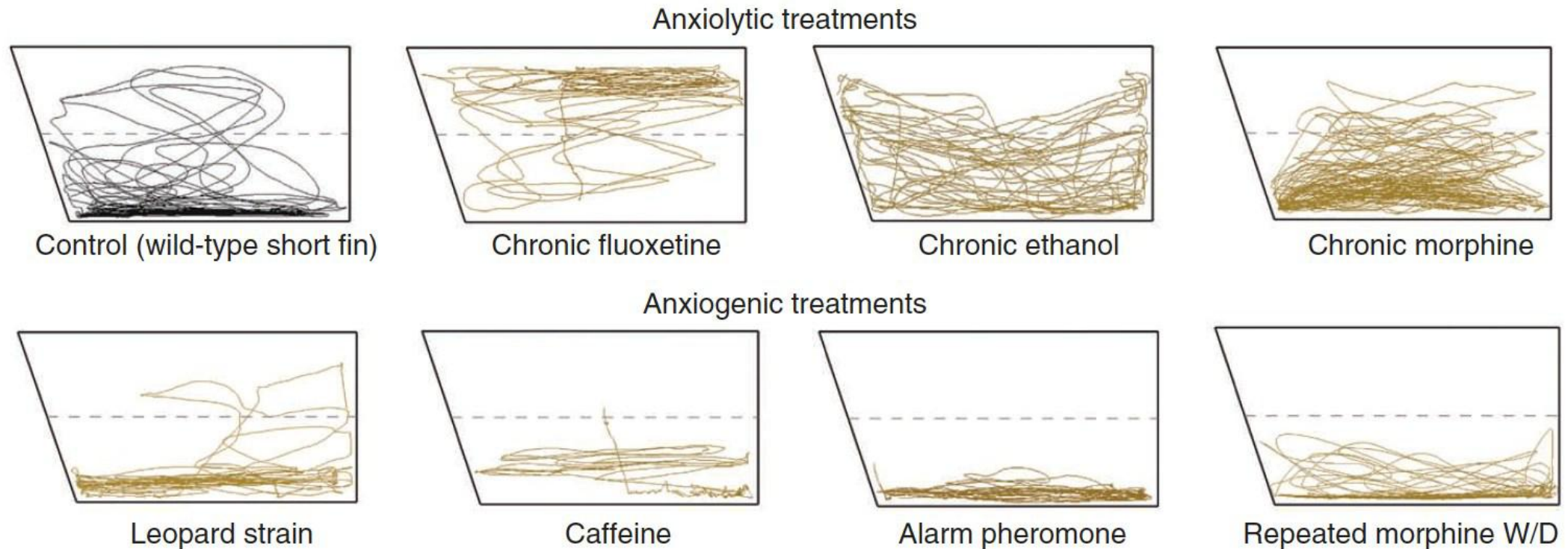


Kalueff *et al.*, 2014

Measured parameters:

- ✓ Time spent in the top part of the tank
- ✓ Transitions to the top
- ✓ Latency to the top
- ✓ Erratic movement
- ✓ Freezing incidents
- ✓ Time spent freezing

Novel Tank test



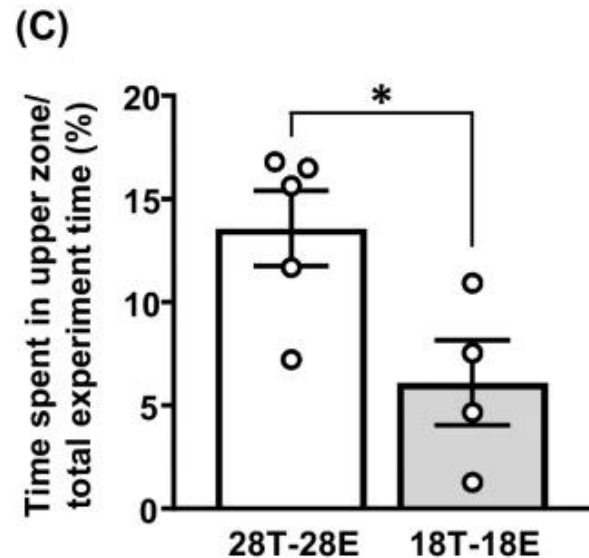
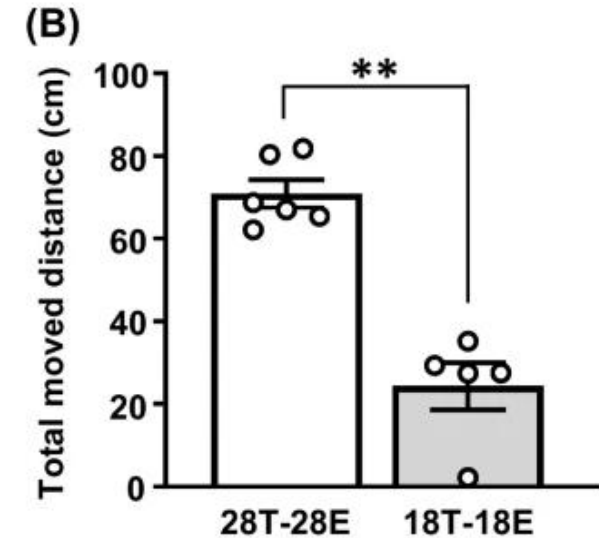
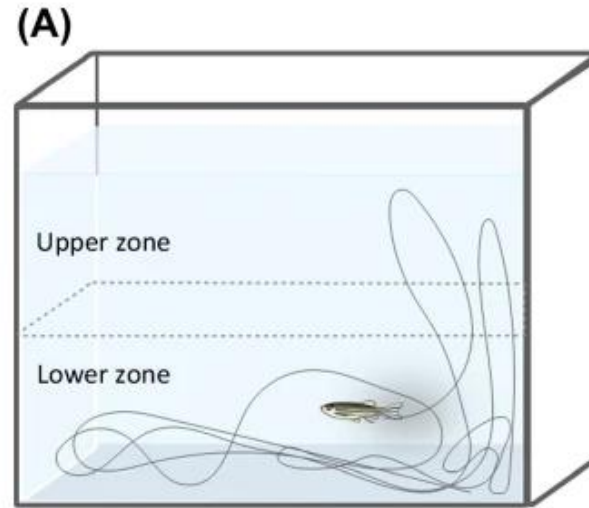
Representative trace of the zebrafish movement in the novel tank test (6-minute trial).

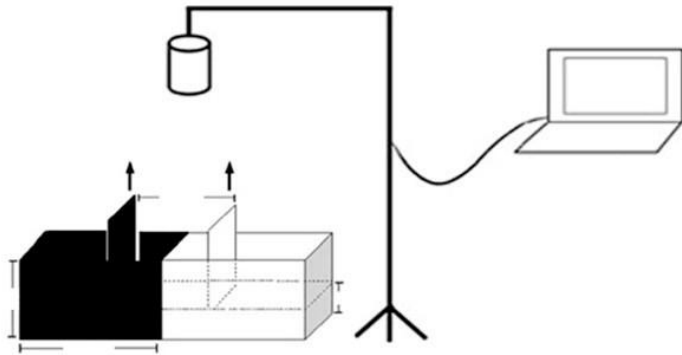
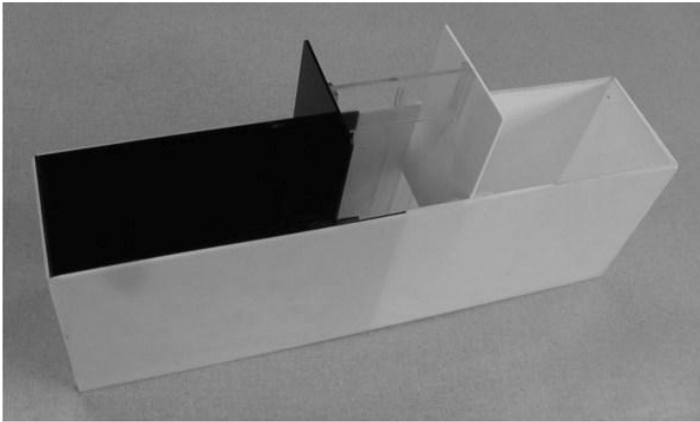
Different experimental manipulations show a decrease or increase in the anxious phenotype, compared to the control tank.

Novel Tank test

The novel tank diving test of adult zebrafish exposed to 28 °C or 18 °C for 24 h.

- (A) Schematic illustration of the novel tank diving test.
- (B) The total moving distance in 18 °C group was significantly shorter than that in 28 °C group.
- (C) The time spent in the upper zone relative to the total experimental time was higher in 28 °C compared to 18 °C.





Measured parameters:

- ✓ Time spent in the white zone
- ✓ Time spent in the black zone

Light/dark preference test

Adult zebrafish, as well as other fish species (e.g. goldfish, guppies, minnows and tilapia), are generally believed to display an **innate aversion to bright and white environments**, and a preference for darker environments.

Fish are left to acclimatize for 3-5 minutes in the centrally isolated compartment.

The separating doors are removed and fish are left to move freely in the tank for 15 minutes.

Exploratory tank test

Quantification of the exploratory behaviour of adult zebrafish.



FIRST DAY

SECOND DAY

8 DAYS OF FREE EXPLORATION

THE DOOR OPENS

A shoal of conspecifics is placed in the first compartment and left there undisturbed for 24 hours.

The next day the experimenter opens the "door" of the second compartment and throws some food pellets to the first and second compartment.



Chronic stress protocols

Protocols that expose zebrafish to unpredictable chronic stress (UCS) conditions.

- Most last for 14 days.
- Fish are exposed to two stressors daily.

The stressors are chosen randomly every day. Some of the stressors applied in protocols of chronic stress include:

- Restraint stress
- Social isolation
- Over-crowding
- Dorsal body exposure
- Tank change
- Cold stress
- Heat stress
- Chasing
- Predator exposure
- Alarm pheromone stress

Chronic stress protocols



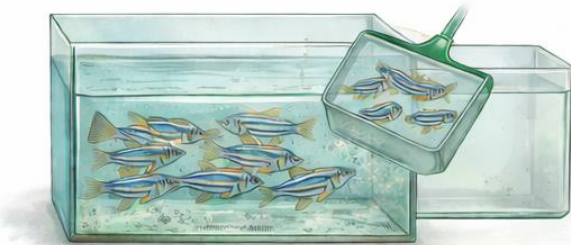
Restraint stress



Social isolation



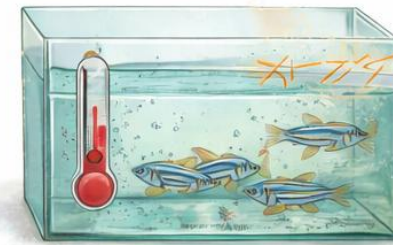
Over-crowding



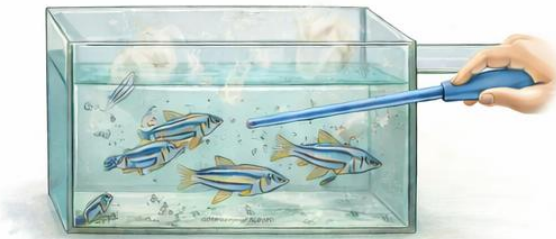
Tank change



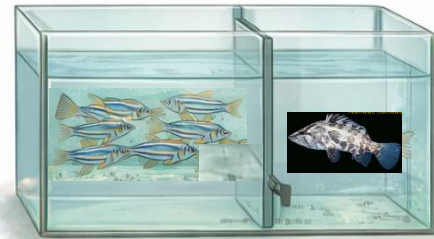
Cold stress



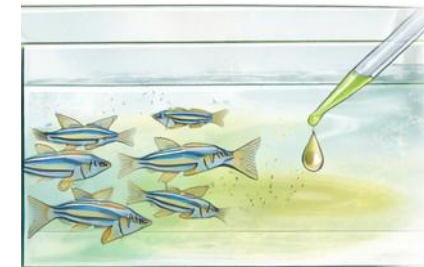
Heat stress



Chasing



Predator exposure



Alarm pheromone stress

Impacts of Chronic Stress in Zebrafish



1. BEHAVIORAL

Locomotion:

- General Hypoactivity
- Increased Freezing

Anxiety Markers:

- Bottom-dwelling

Social / Memory:

- Aggression ↑ (Males)
- Memory Retention ↓
- Variable Shoaling



2. NEUROCHEMICAL

Monoamines:

- Serotonin ↓ (Mood)
- Dopamine (Altered)

Neuroplasticity:

- BDNF ↑
- c-Fos ↓

Metabolism:

- Mitochondrial Dysfunction



3. GENETIC (Brain)

Stress Hormones (↑):

- CRF & GR α / β

Inflammation (↑):

- IL-1 β , TNF- α
- IL-6, COX-2

Neuroprotection (↓):

- hsd11b2 (enzyme loss)

Social behaviour of zebrafish



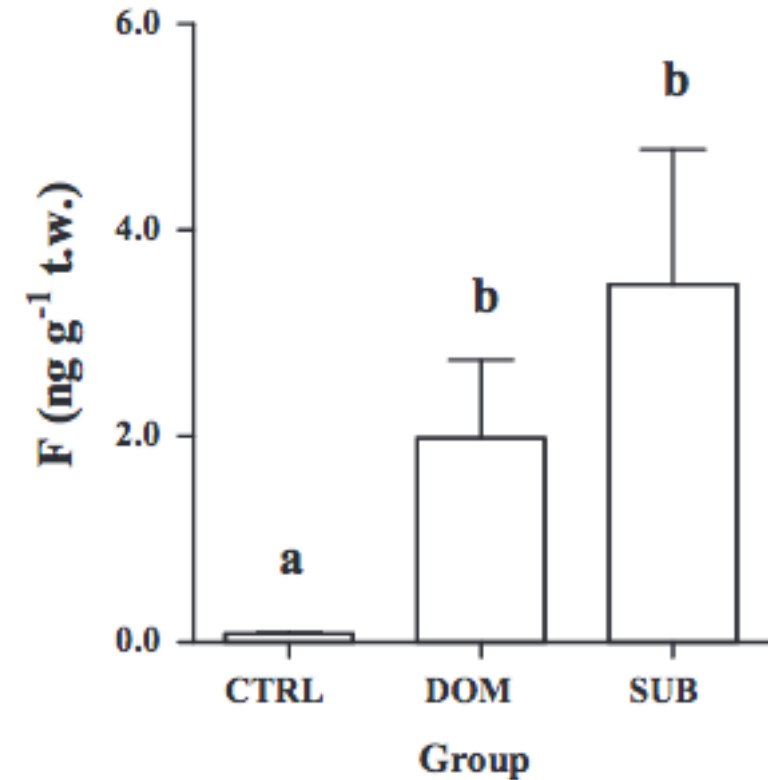
Aggression in zebrafish

Zebrafish are social species that form shoals, but when put in pairs they often exhibit an aggressive behaviour that leads to the establishment of a social hierarchy.

The “winner” of this dyadic interaction is considered the dominant individual, while the “loser” is the subordinate.

The establishment of hierarchy consists a form of social stress for both the dominant and the subordinate, therefore, zebrafish exhibit anxiety-like behaviour.

Both dominants and subordinates show higher cortisol levels compared to controls (Pavlidis *et al.*, 2011).



Aggressive behaviour in zebrafish

Displaying consists of an approach to the conspecific followed by a turn to the left or right with fins erect.

Circling is considered another form of lateral display that may last an extended period and during which the fish rise in the water column.

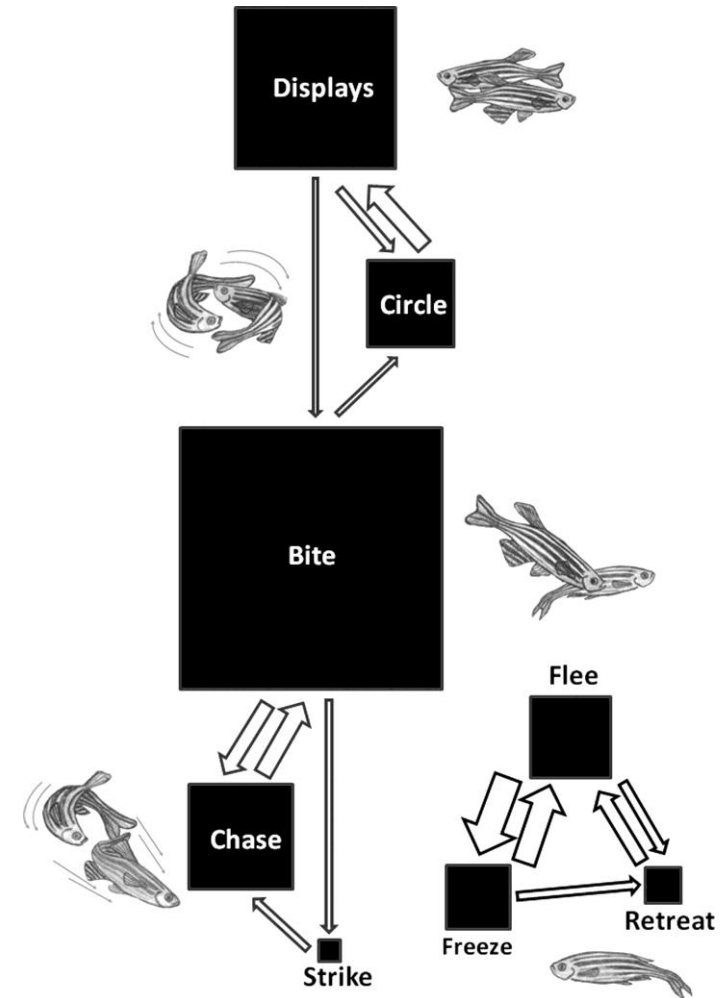
Chasing is a pursuit or a quick approach to an individual.

Bites consist of closing the mouth against another individual.

Freezing is the term that describes the immobility state with retracted fins.

Fleeing is when an individual moves away from a pursuing individual for an extended period.

Dominant behaviour consists mainly of **chasing and biting**, while **subordinate behaviour** is expressed as **fleeing and freezing**.



Experimental tests of aggression

There are two main experimental approaches:

- **Real opponent test**

Two fish are allowed to interact directly.
This produces full behavioural repertoires but carries risk of injury.

- **Mirror-elicited aggression**

Fish interact with their reflection.



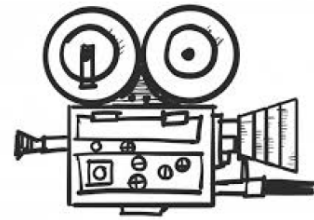
Real opponent test

Real opponent fight: The arena is divided by a removable opaque partition. Once the partition is removed, the two same-sex conspecifics are allowed to interact.



Pairs of same sex adult zebrafish are introduced in 2L tanks

2 h

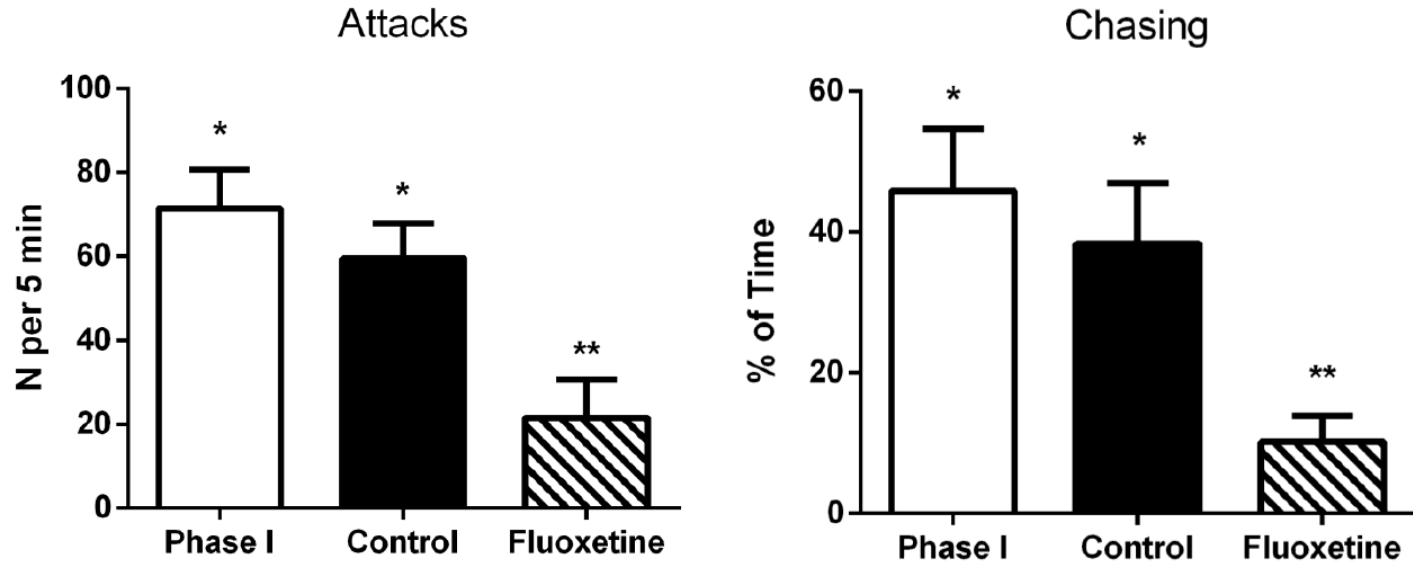


Recording of the pair's behavior for 5 minutes.

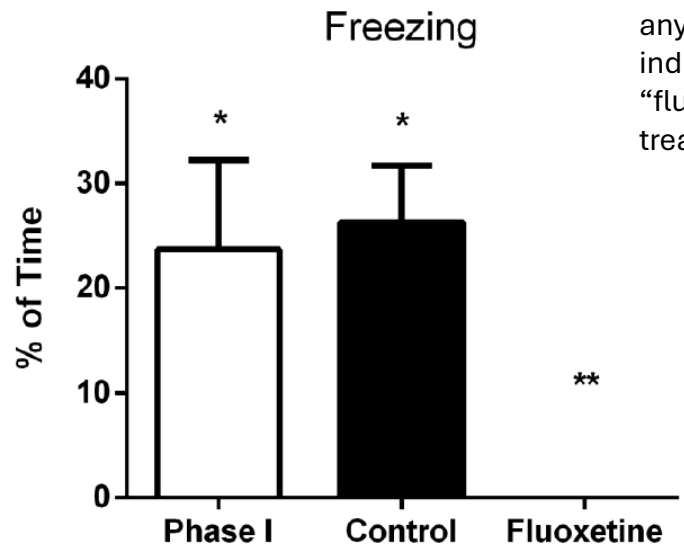
Behavioral analysis and quantification of the **dominant** and **subordinate** behavior

- Number of attacks from the dominant
- Total duration of chasing
- Total duration of subordinate's freezing behavior

A. Dominant Behavior



B. Subordinate Behavior



“Phase I” refers to the part of the experiment prior to any treatment, “control” represents the group of individuals that did not receive any fluoxetine and “fluoxetine” the group that received a fluoxetine treatment.

Fluoxetine, a selective serotonin reuptake inhibitor, is used as an **anxiolytic tool** to alter aggressive behaviour in several vertebrates and as an antidepressant drug in humans.

Pairs of male zebrafish were held overnight to develop dominant—subordinate behaviour, either treated or non-treated for 2 h with fluoxetine (5 mg L⁻¹), and allowed to interact once more for 1 h. Behaviour was recorded both prior and after fluoxetine administration.

Acute exposure to fluoxetine can alter the aggressive behavior of adult male zebrafish.

Decreasing offensive aggression in dominants and by eliminating freezing in the subordinates.

Real opponent test



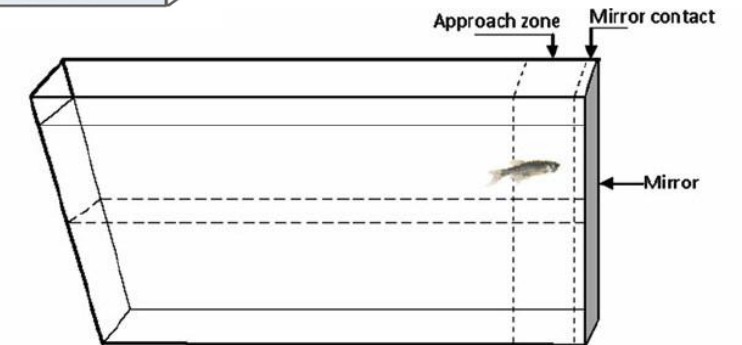
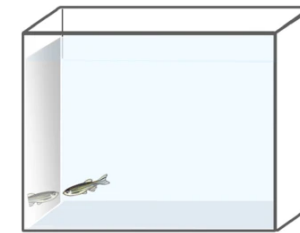
Mirror-elicited fight

Mirror-elicited fight: The arena is divided by a partition which contains one mirror in each side. A second partition is added on each side in order to cover the mirror. To elicit the mirror fight, the outer partitions are removed and the fish are allowed to interact with their own reflection.

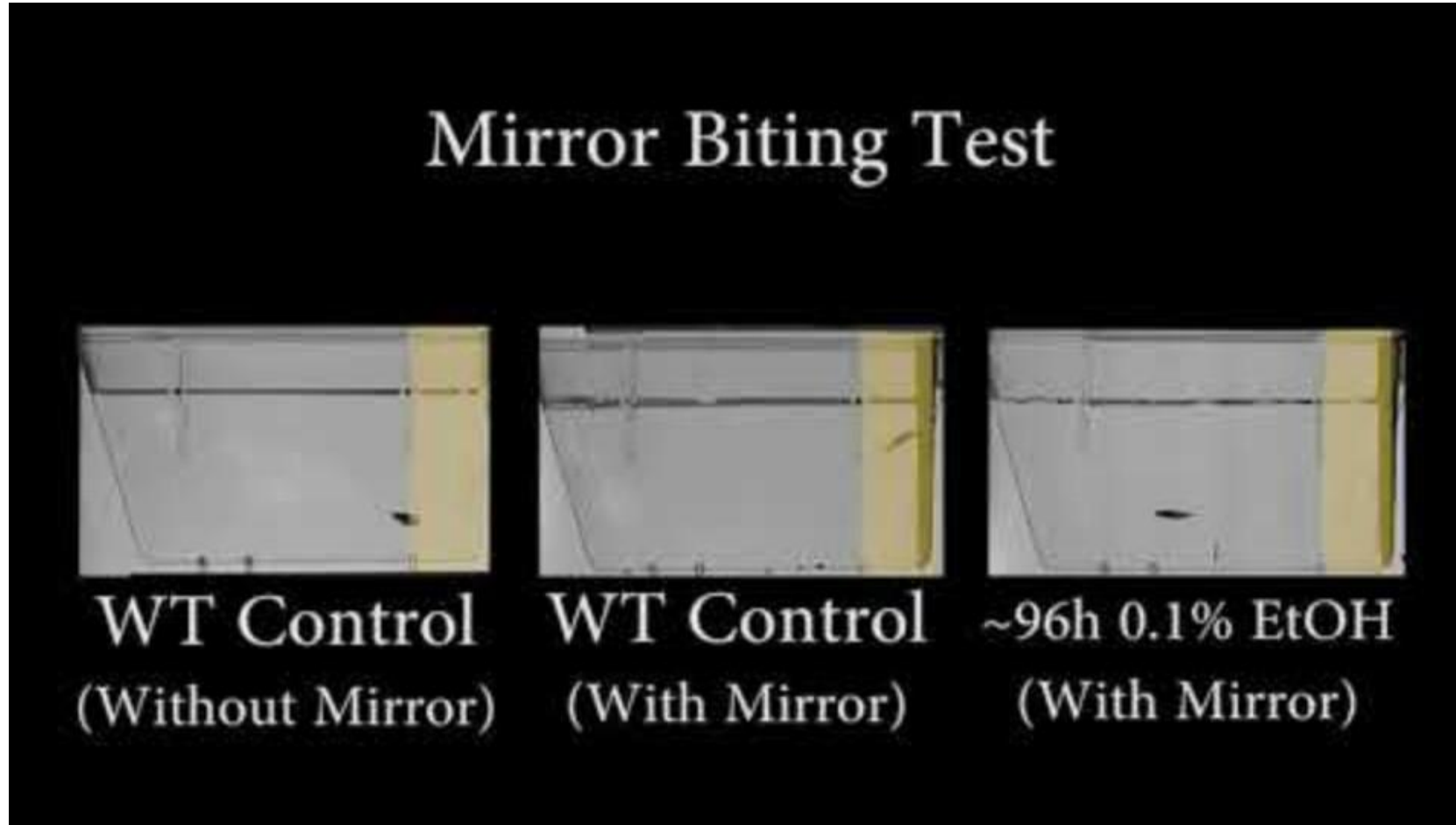
- An established protocol to study social and aggressive behaviour in adult zebrafish.
- Not possible to observe the full repertoire of aggressive behaviour and it has been shown that zebrafish have different endocrinological and genetic responses to a mirror “fight” (Teles *et al.*, 2013), but the risk of injury is avoided.

Measured parameters:

- ✓ Frequency of attacking the mirror
- ✓ Total duration of biting the mirror
- ✓ Number of contacts with the mirror
- ✓ Latency of approaching the mirror



Mirror-elicited fight



0.1 % EtOH → mildly anxiolytic

Social status and anxiety-like behaviour

Zebrafish **anxiety-like behaviour** was assessed in the novel tank test **after the formation of dominant-subordinate hierarchies**.

Ten pairs of animals were subjected to dyadic interactions for 5 days and compared with control animals. After this period, a clear dominance hierarchy was established across all dyads, irrespective of sex.

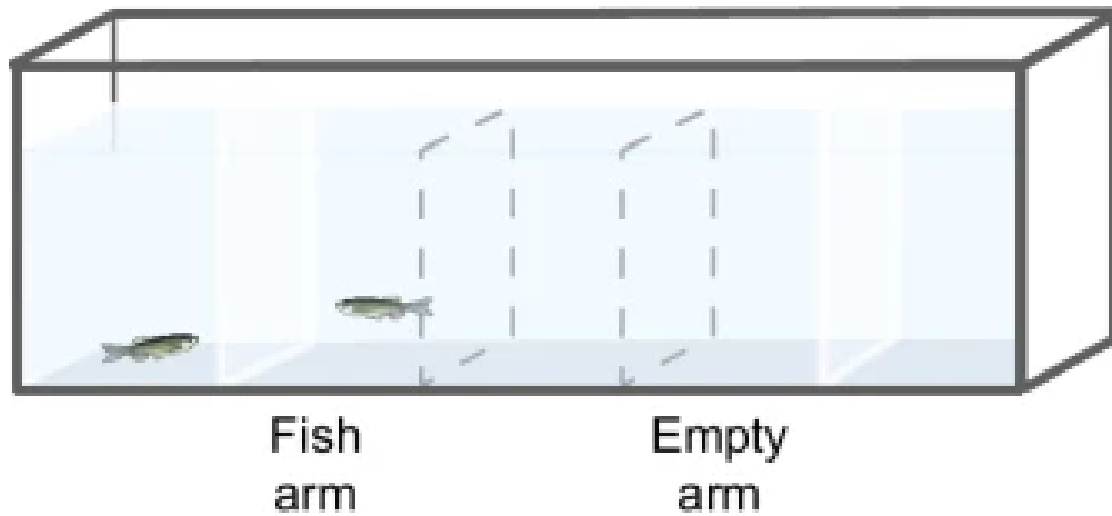
Social status affected parameters of anxiety-like behaviour in the novel tank test, with **subordinate** males and females displaying more **bottom-dwelling**, **absolute turn angle**, and **freezing** than dominant animals and controls.

The results suggest that **subordinate** male and female zebrafish show **higher anxiety-like behaviour**, which together with previous literature suggests that subordination stress is conserved across vertebrates.



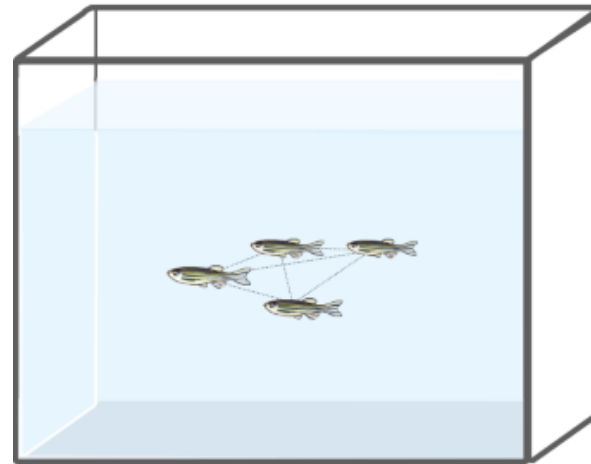
Assessing social behaviour

Social preference test

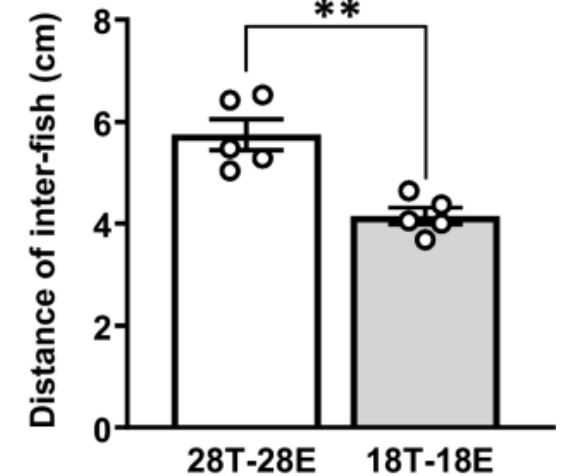


Shoal cohesion test

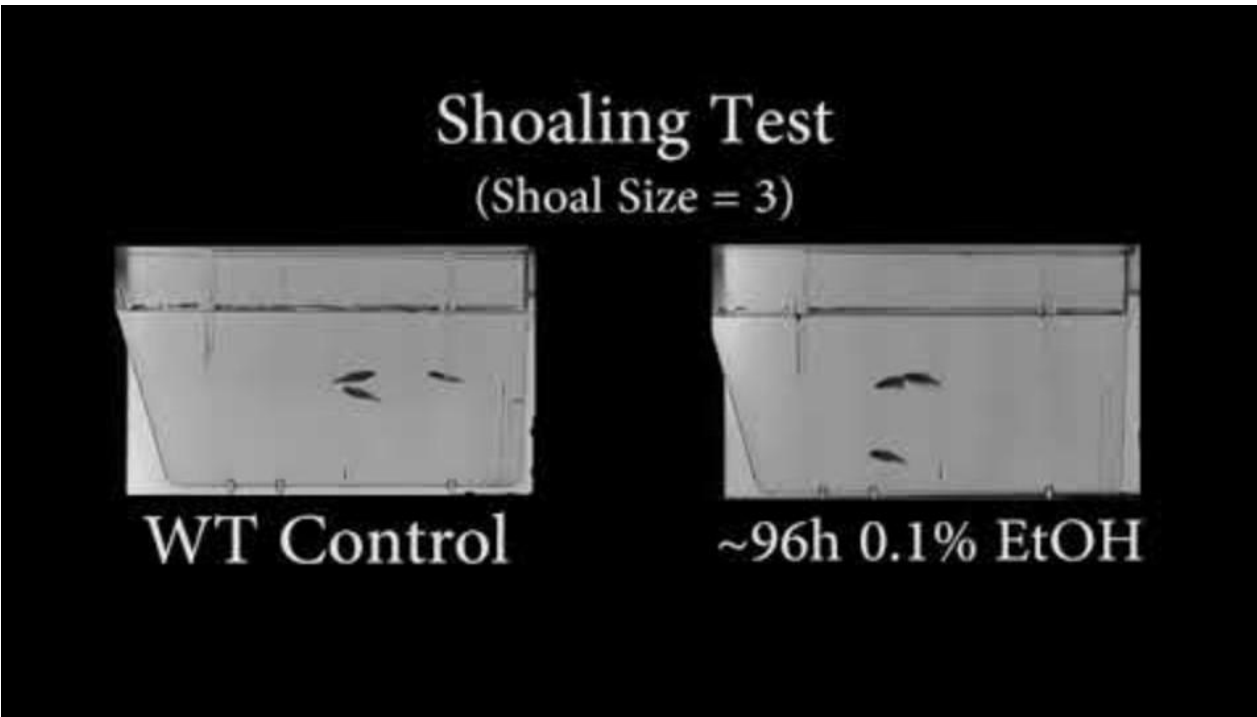
(A)



(B)



Shoal cohesion test



0.1 % EtOH → mildly anxiolytic



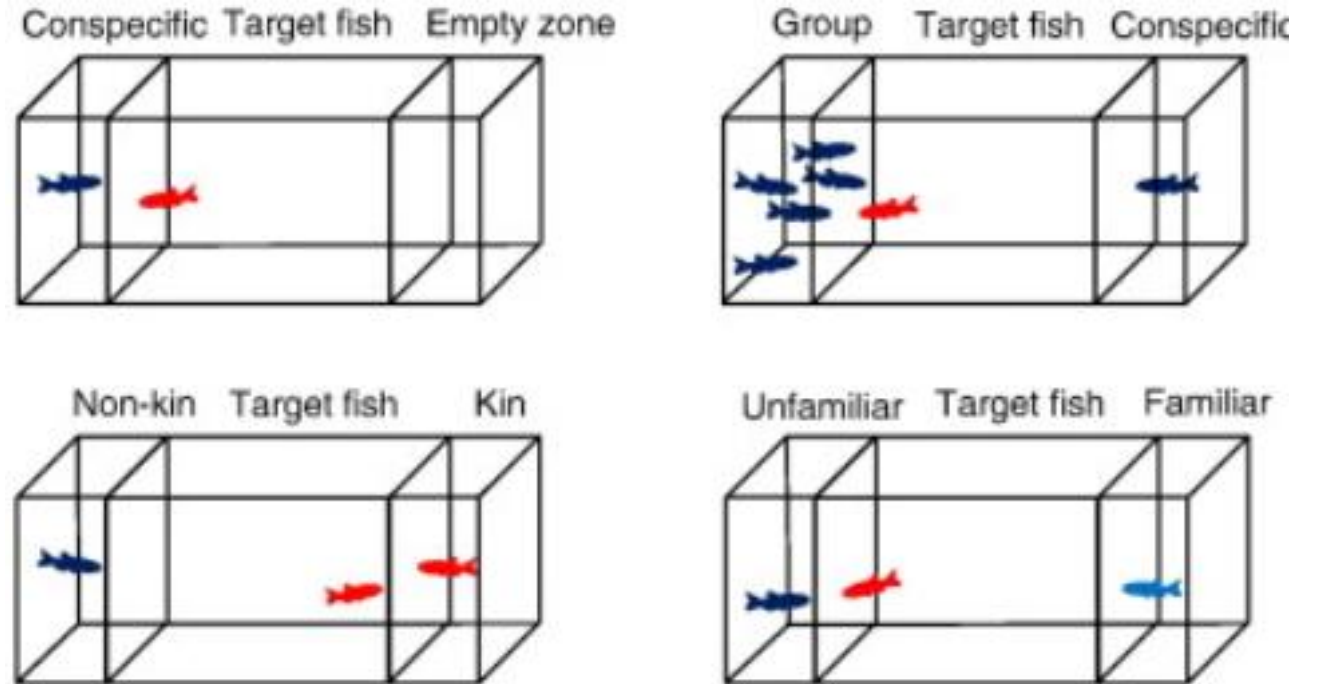
homozygous mutants of *ednraa* gene → social cohesion

Assessing social behaviour

The zebrafish social interaction test in which two unfamiliar zebrafish explore each other (target fish is shown in red).

Note that in the model shown here, the separators are made from glass, providing visual cues sufficient to evoke strong social phenotypes in zebrafish.

In various modifications of this test, holes can be made in the separators (to allow water to pass through), providing additional (olfactory) cues important for evoking social responses in zebrafish.



Assessing social behaviour



Social buffering

Social buffering is a widely observed phenomenon in which the presence of conspecifics ameliorates the effects of aversive experience.

Social buffering of fear/stress is well studied in zebrafish.

The **presence of conspecifics suppresses aggression** elicited by both

- hunger and
- mirror exposure,

implying a novel social buffering mechanism dedicated to aggression modulation.



Social buffering

CNTRL = non-starved group, fish were fed daily with brine shrimp and commercial pellets

HGRY = the starved group, completely deprived of food for six days

M = mirror test

SC = presence of conspecifics

Both **HGRY + SC + M** and **CNTRL + SC + M** groups spent more time in the **Social region of interest** than in the **Mirror Zone** ($P < 0.0001$)

HGRY + SC + M group spent significantly more time in the **Social region of interest** than those in the **CNTRL + SC + M** group ($P < 0.0007$).

The **HGRY + M** group displayed significantly **more mirror bites** than the **CNTRL + M** group ($P < 0.0001$), which is consistent with **elevated aggression-related measures in the absence of social cues**.

In the presence of **both social cues and the mirror stimulus**, both **CNTRL + SC + M** and **HGRY + SC + M** groups **had lower mirror bite counts**.

Notably, the **HGRY + SC + M** group exhibited a **larger decrease in mirror bites** compared to **CNTRL + SC + M** ($P < 0.001$).



SC = presence of conspecifics

M = mirror test



Anti-predator behaviour

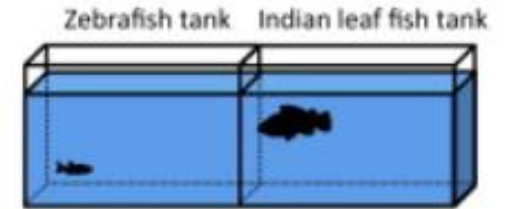
Predator avoidance test

Zebrafish natural predators include among others Indian leaf fish (*Nandus nandus*), freshwater garfish (*Xennentodon* spp.), catfish (*Mystus bleekeri*), as well as avian predators.

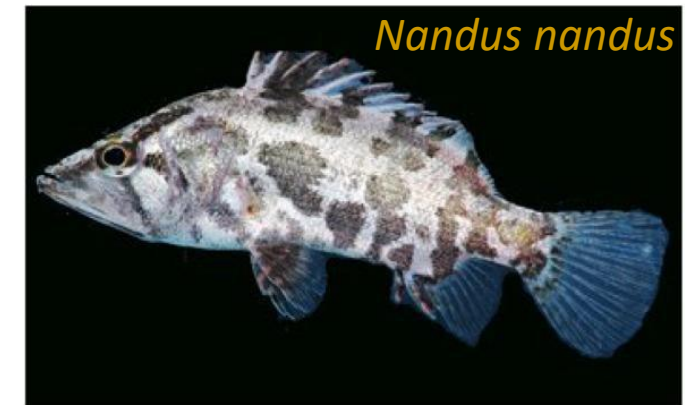
In laboratory conditions, the presence of the Indian leaf fish induces fear-like responses in zebrafish, which consist of (Bass & Gerlai, 2008):

- rapid escape movements
- erratic swimming
- avoidance patterns

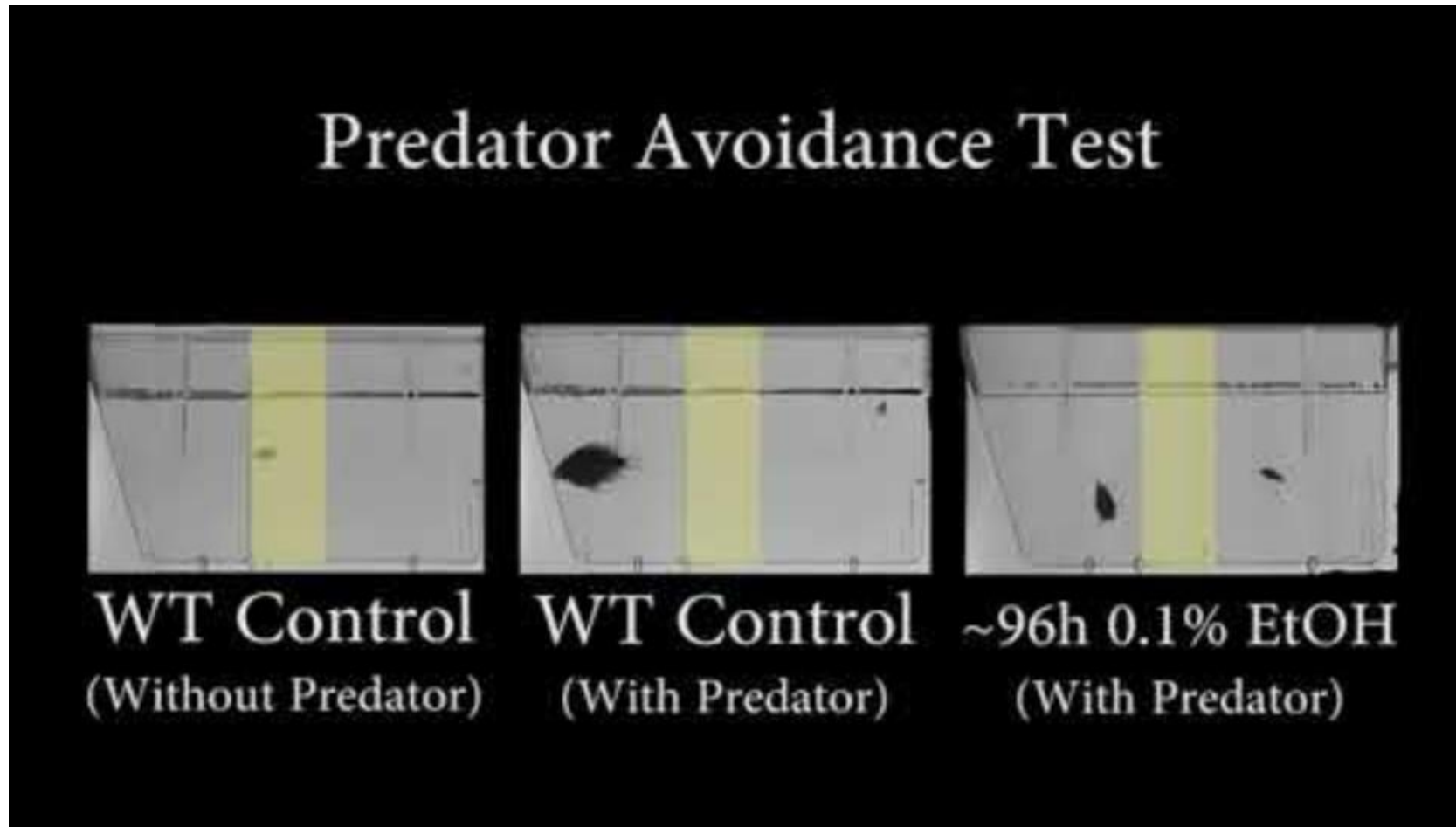
Depending on the species of predator that they are exposed to, zebrafish adjust their defensive and fear-like behaviours (Ahmed *et al.*, 2012).



Kalueff *et al.*, 2014



Predator avoidance test



0.1 % EtOH → mildly anxiolytic

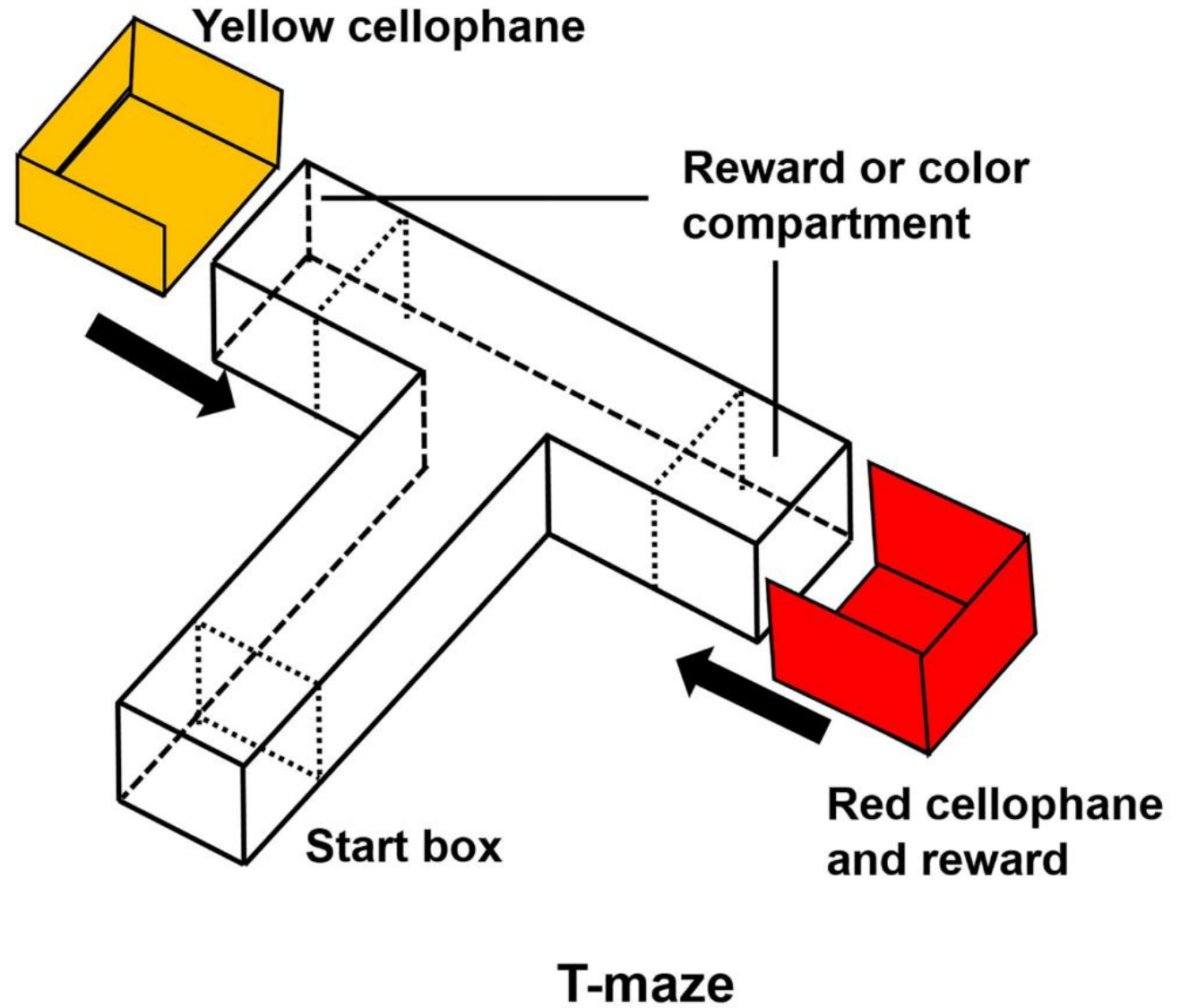
Predator avoidance test

Exposure to both live predator and the robotic model induced a robust avoidance response in zebrafish. Contrary to this, computer-animated images.



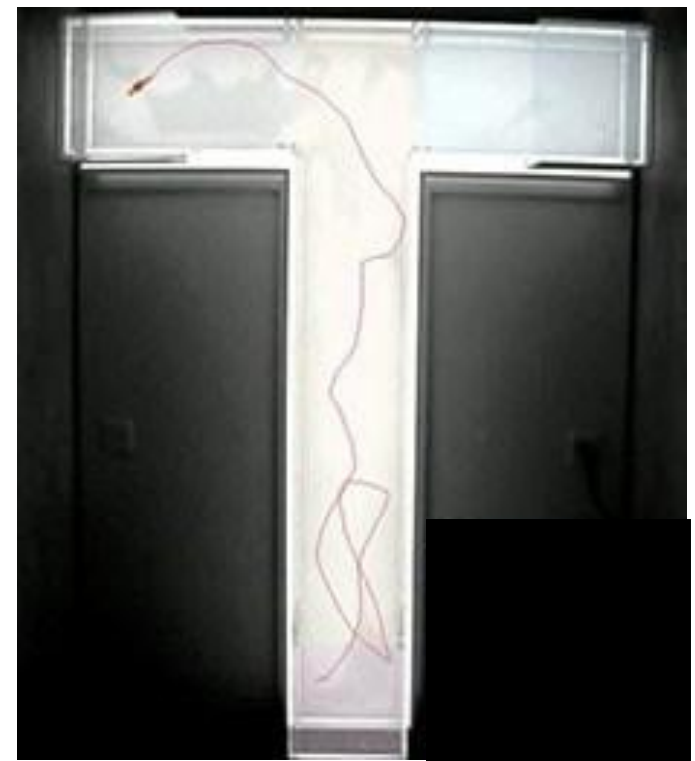
(a) Live red tiger oscar; (b) robotic predator designed after the live oscar; and (c) snapshot of the computer-animated of live Oscar elicit a fear-like behavioural response.

Learning capacity and memory



T-maze

- Learning capacity
- Long-term memory
- Short-term memory
- Memory plasticity

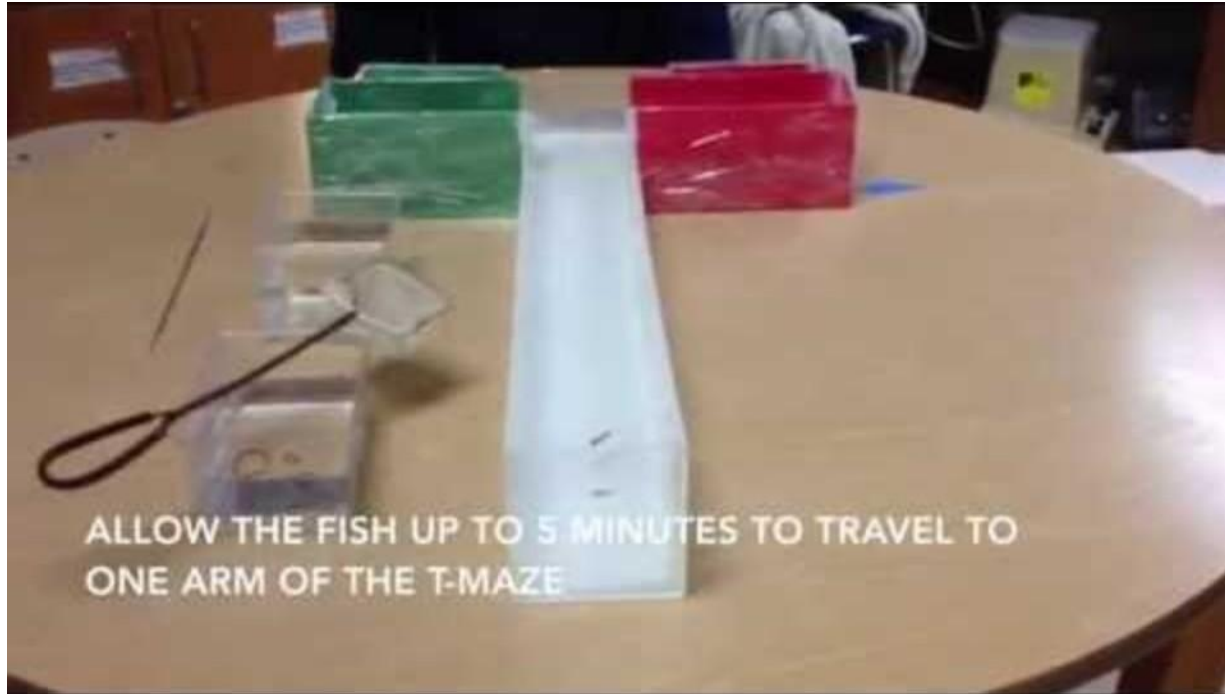


Alternative structure

One arm has an enriched environment which is favourable to zebrafish (plants and marbles).



T-maze



Positive emotions and affective states

Animal welfare includes both pleasant and unpleasant emotions and affective states (Neethirajan, 2022).

The most recent notion suggests that welfare standards for animals under human care should not only ensure a 'life worth living' but may also aim to provide a 'good life', a concept emphasising the importance of positive experiences (Edgar et al. 2013; Wemelsfelder and Mullan, 2014).

Necessity for behavioural tests able to identify positive emotions.

Tactile stimulation	Reduced bottom dwelling, Reduced freezing	Schirmer et al., 2013
Gravel substrate, rocks, and artificial plants	Increased shoaling behaviour, Synchrony and coordination, Reduced aggression, Lack of Distress Signals (diving behaviour or erratic swimming)	Franks et al., 2018
Enriched environment	Judgment bias test (ambiguous locations, ambiguous light cues etc)	Tan et al., 2020
Enriched environment	Judgment bias test (ambiguous locations, ambiguous light cues etc)	Buenhombre et al., 2022
Gravel	Decreased shoaling behaviour (increased relaxation emerging gradually over time in the gravel condition)	Kleinhappel et al., 2023
30 novel objects	Increased shoaling behaviour, Increased group coordination, Curiosity/ exploration (response to object), Reduced aggression	Franks et al., 2023
Virtual demonstration (video) of high and low anxiety	Less time at the bottom of the tank, Reduced erratic behaviour, No freezing episodes, Swimming over the entire area of the tank	Burbano et al., 2023
Music	Touching the water surface (positive exploratory behaviour), Increased shoaling behaviour (following), Synchrony and Coordination (wandering)	Niu et al., 2025

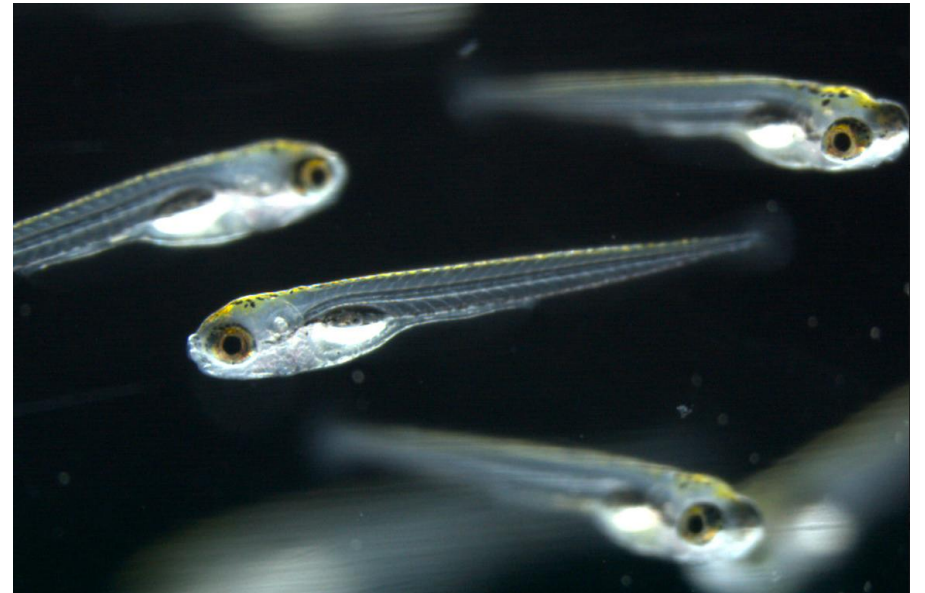
Assessing stress response in zebrafish larvae

Compared to adults, **fewer behavioural protocols** have been established for larvae.

This is mainly because larvae are:

- extremely small,
- develop rapidly,
- and have more limited behavioural repertoires.

However, they offer some major experimental advantages — especially for **developmental biology, genetics, etc.**



Assessing stress response in zebrafish larvae

The most commonly used behavioural tests are:

- Acute stress response to a swirling stressor
- Tapping assay
- Light/Dark preference
- Open field test

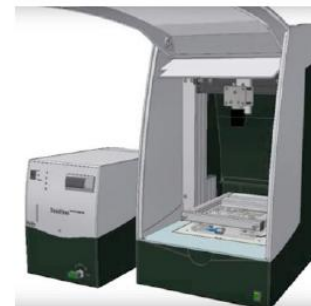
Adult zebrafish used for reproduction



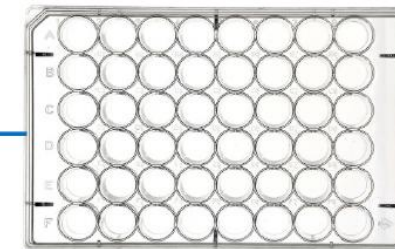
Fertilised eggs



Larvae 5 days post fertilization (dpf)

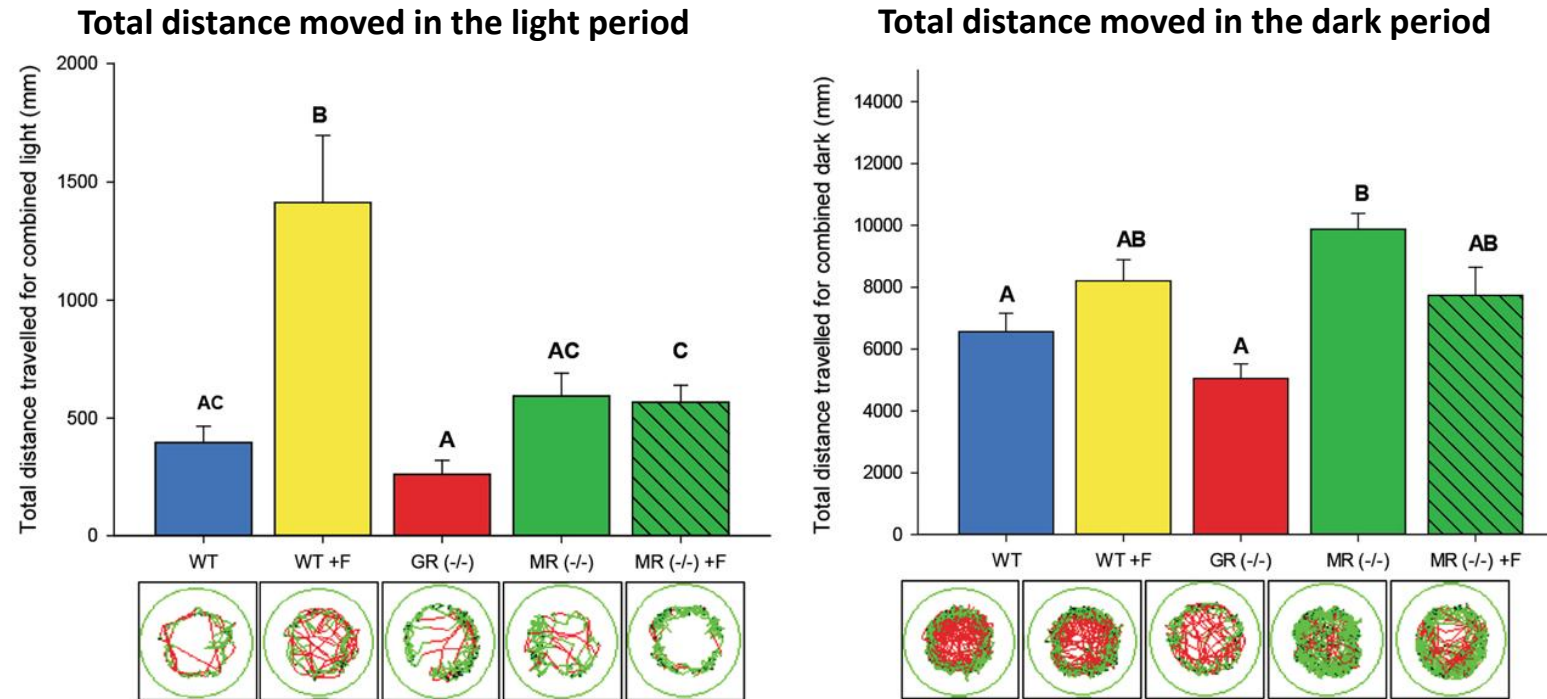


Observation Chamber



Transfer of larvae in cell culture plates

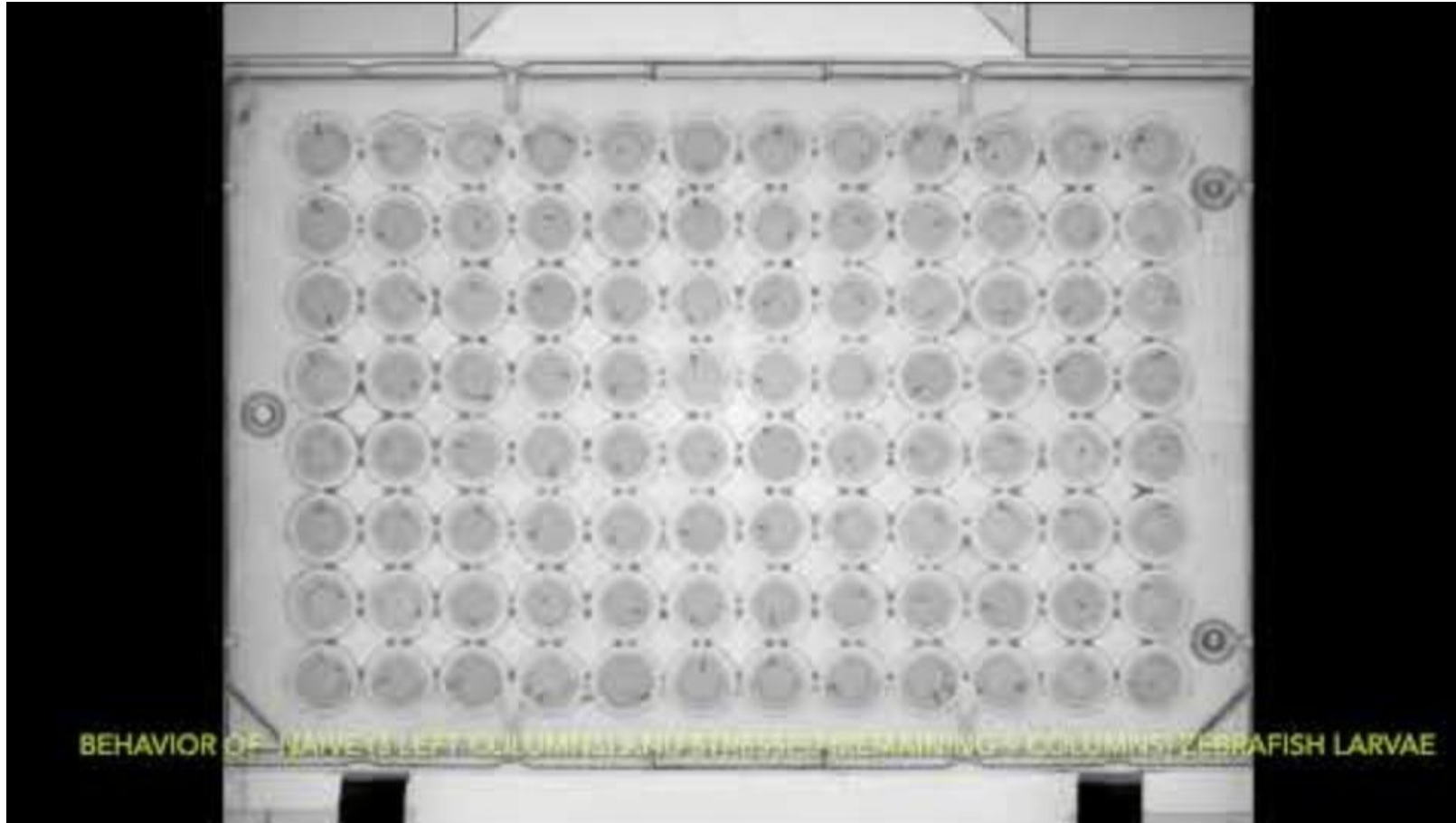
Assessing stress response in zebrafish larvae



Larvae with different genetic manipulations related to genes regulating the HPI axis display differences in light/dark preference and anxiety levels (thigmotaxis).

wildtype [WT], wildtype treated with cortisol [WT + F], GR knockout [GR(-/-)], MR knockout [MR(-/-)] or MR knockout treated with cortisol [MR(-/-) + F]

Assessing stress response in zebrafish larvae



Why study larvae?

Studying larvae is particularly valuable because:

- we can observe how **stress systems develop**
- we can **link genes to behavioural outcomes**
- we can **perform large-scale drug or environmental screening**
- we can **detect early-life effects that influence adult behaviour**

Early developmental stages are often when organisms are most sensitive to environmental conditions.

Although larval behaviour is simpler than adult behaviour, it is **highly informative**.

Larval stress assays provide powerful tools for understanding:

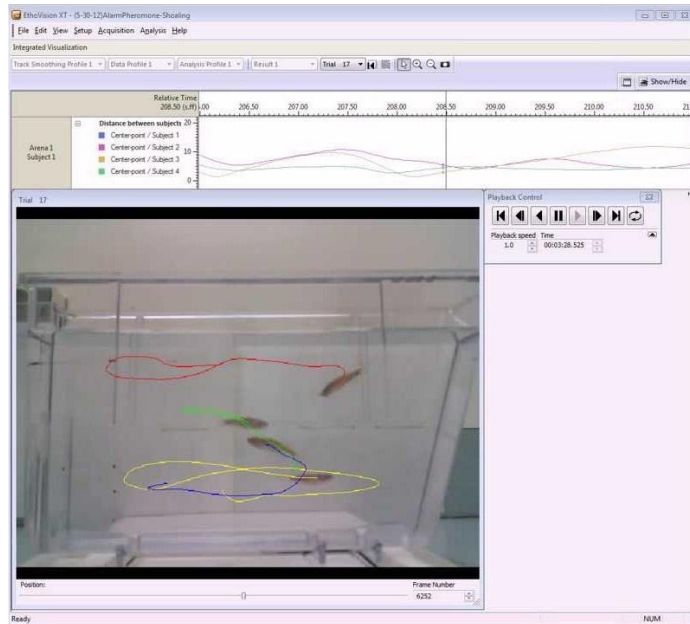
- neurodevelopment
- genetic regulation of stress
- environmental sensitivity
- early indicators of welfare

Because these tests can be performed at very large scale, they are central to modern experimental biology.

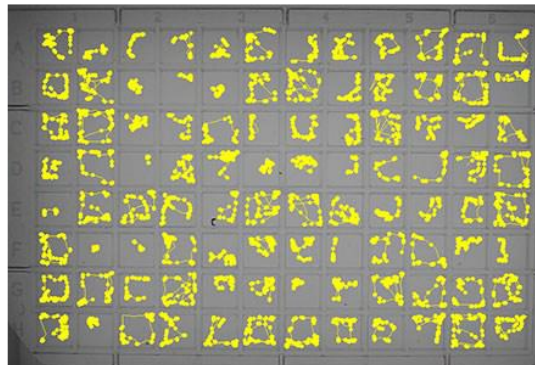


Behavioural analysis software packages

Noldus Information Technology



Ethovision



Daniovision



Viewpoint Behaviour Technology



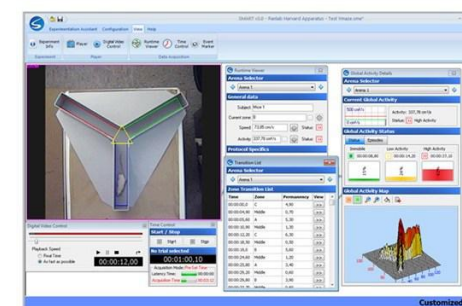
Zebrabox



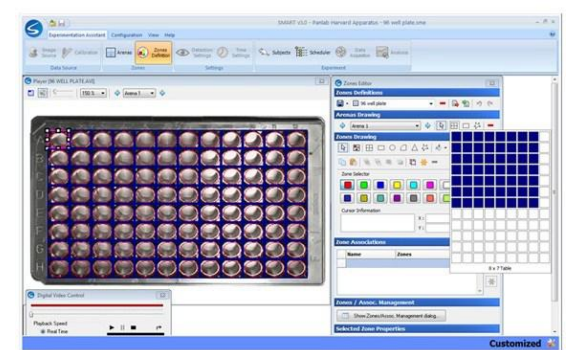
Zebracube

Smart 3.0

Y-maze Spontaneous Alternation



Zebrafish Larvae Activity



So what do all these systems actually do?

They allow researchers to extract quantitative behavioural variables such as:

- total distance travelled
- swimming velocity
- spatial distribution
- freezing duration
- zone preference
- social distance
- exploration patterns

Some systems can even **reconstruct three-dimensional movement trajectories** or analyse behaviour across **hundreds of individuals simultaneously**. This is extremely important for **experimental reproducibility**. Instead of subjective scoring, we obtain objective numerical data that can be statistically analysed and compared across laboratories.



Conclusion

Today we have seen that zebrafish behaviour is not random.

It is structured, measurable and deeply informative.

Through behavioural analysis we can understand:

- stress physiology
- social dynamics
- emotional states
- cognitive function

Zebrafish are not just a laboratory model. They are **a window into how fish experience their environments.**



Thank you!

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References

- Bozi, B., Rodrigues, J., Lima-Maximino, M., de Siqueira-Silva, D.H., Soares, M.C., Maximino, C., 2021. Social Stress Increases Anxiety-Like Behavior Equally in Male and Female Zebrafish. *Front. Behav. Neurosci.* 15. <https://doi.org/10.3389/fnbeh.2021.785656>
- Buenhombre, J., Daza-Cardona, E.A., Sousa, P., Gouveia, A., Cajiao-Pachón, M.N., 2023. Structural environmental enrichment and the way it is offered influence cognitive judgement bias and anxiety-like behaviours in zebrafish. *Anim. Cogn.* 26, 563–577. <https://doi.org/10.1007/s10071-022-01700-x>
- Burbano, D., Senthilkumar, S., Manzini, M.C., 2023. Exploring emotional contagion in zebrafish: A virtual-demonstrator study of positive and negative emotions. *Behav. Processes* 213, 104961. <https://doi.org/10.1016/j.beproc.2023.104961>
- Cachat, J. et al. (2010). Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. *Nature protocols*, 5(11), 1786–1799.
- Demin, K.A., Taranov, A.S., Ilyin, N.P., Lakstygala, A.M., Volgin, A.D., de Abreu, M.S., Strekalova, T., Kalueff, A.V., 2021. Understanding neurobehavioral effects of acute and chronic stress in zebrafish. *Stress* 24, 1–18.
- Facciola, A., Tran, S., Gerlai, R., 2017. Re-examining the factors affecting choice in the light–dark preference test in zebrafish. *Behav. Brain Res.* 327, 21–28. <https://doi.org/10.1016/j.bbr.2017.03.040>
- Faught, E. and Vijayan, M.M. (2018). The mineralocorticoid receptor is essential for stress axis regulation in zebrafish larvae. *Scientific Reports*, 8(1), 1–11.
- Franks, B., Gaffney, L.P., Graham, C., Weary, D.M., 2023. Curiosity in zebrafish (*Danio rerio*)? Behavioral responses to 30 novel objects. *Front. Vet. Sci.* 9. <https://doi.org/10.3389/fvets.2022.1062420>
- Franks, B., Graham, C., Von Keyserlingk, M.A.G., 2018. Is Heightened-Shoaling a Good Candidate for Positive Emotional Behavior in Zebrafish? *Animals* 8, 152. <https://doi.org/10.3390/ani8090152>
- Kalueff, A.V., Stewart, A.M., Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends PharmacolSci* 35(2): 63-75.
- Kleinhappel, T.K., Pike, T.W., Burman, O.H.P., 2023. Changes in group behaviour in response to a preferred environment reflect positive affect. *Sci. Rep.* 13, 10576. <https://doi.org/10.1038/s41598-023-37763-0>
- Kuniyil, A.A., Malik, M.S., Vellattu, R.K., Sivarajan, D., Mannarapurayil, G.K.T., Faisal, F.P., Chathooth, N., Ramachandran, B., 2025. Social maintenance masks induced aggression in zebrafish. *Sci. Rep.* 15, 34396. <https://doi.org/10.1038/s41598-025-17318-1>
- Ladu, F., Bartolini, T., Panitz, S.G., Chiarotti, F., Butail, S., Macri, S., Porfiri, M., 2015. Live predators, robots, and computer-animated images elicit differential avoidance responses in zebrafish. *Zebrafish* 12, 205–214. <https://doi.org/10.1089/zeb.2014.1041>
- Liu, S.-T., Chang, C.-Y., Lee, K.-Y., Tong, S.-K., Huang, H.-L., Chen, H., Horng, J.-L., Chou, M.-Y., 2024. Alternation of social behaviors for zebrafish (*Danio rerio*) in response to acute cold stress. *Fish Physiol. Biochem.* 50, 653–666. <https://doi.org/10.1007/s10695-024-01296-8>
- Neethirajan, S., 2022. Affective State Recognition in Livestock—Artificial Intelligence Approaches. *Animals* 12, 759. <https://doi.org/10.3390/ani12060759>
- Niu, T., Xiang, B., Huang, P., Yang, X., Chai, L., 2025. Effects of music enrichment on the welfare of small ornamental fishes. *Aquac. Rep.* 43, 102950. <https://doi.org/10.1016/j.aqrep.2025.102950>
- Oliveira, R.F., Silva, J.F. and Simões, J.M. (2011). Fighting Zebrafish: Characterization of Aggressive Behavior and Winner–Loser Effects. *Zebrafish*, 8(2), 73–81.
- Pavlidis, M. et al. (2011). Adaptive changes in zebrafish brain in dominant-subordinate behavioral context. *Behavioural brain research*, 225(2), 529–37.
- Schirmer et al., 2013 Shahnawaz Ahmed, Gayathri Venkatesan, Deshapathi Sai Prapul, Abdul Rasheed Md, 2023. Zebrafish as a comprehensive model of neurological diseases. *Int. J. Sci. Res. Arch.* 10, 018–052. <https://doi.org/10.30574/ijrsra.2023.10.1.0696>
- Stewart, A.M., Nguyen, M., et al. (2014). Developing zebrafish models of autism spectrum disorder (ASD). *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 50, 27–36.
- Tan, S.L.T., Handasyde, K.A., Rault, J.-L., Mendl, M., 2020. Insensitivity to reward shifts in zebrafish (*Danio rerio*) and implications for assessing affective states. *Anim. Cogn.* 23, 87–100. <https://doi.org/10.1007/s10071-019-01318-6>
- Teles, M.C. et al. (2013). Social modulation of brain monoamine levels in zebrafish. *Behavioural brain research*, 253C, 17–24.
- Theodoridi, A., Tsalafouta, A., Pavlidis, M. (2017). Acute Exposure to Fluoxetine Alters Aggressive Behavior of Zebrafish and Expression of Genes Involved in Serotonergic System Regulation. *Front Neurosci*, 11, 223.