Assessing social behavior phenotypes in adult zebrafish

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Social deficits

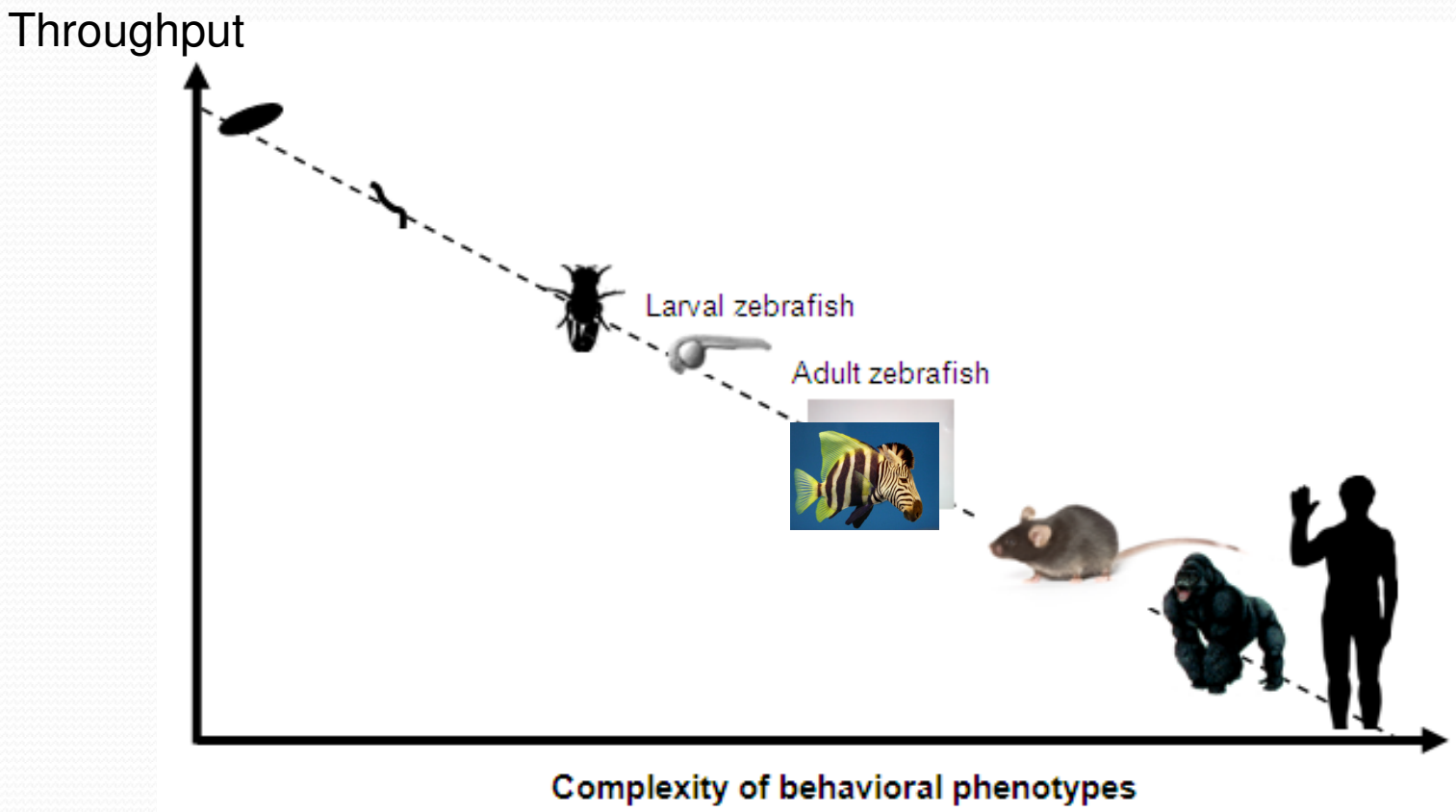
In humans, social deficits are part of multiple brain disorders:

- Autism
- Personality disorders
- Affective disorders (depression, social phobia)
- Schizophrenia

- **It is becoming increasingly important to model social deficits in animals**

- **We need: New behavioral models; New concepts; New markers; New model species**

Behavior vs. throughput



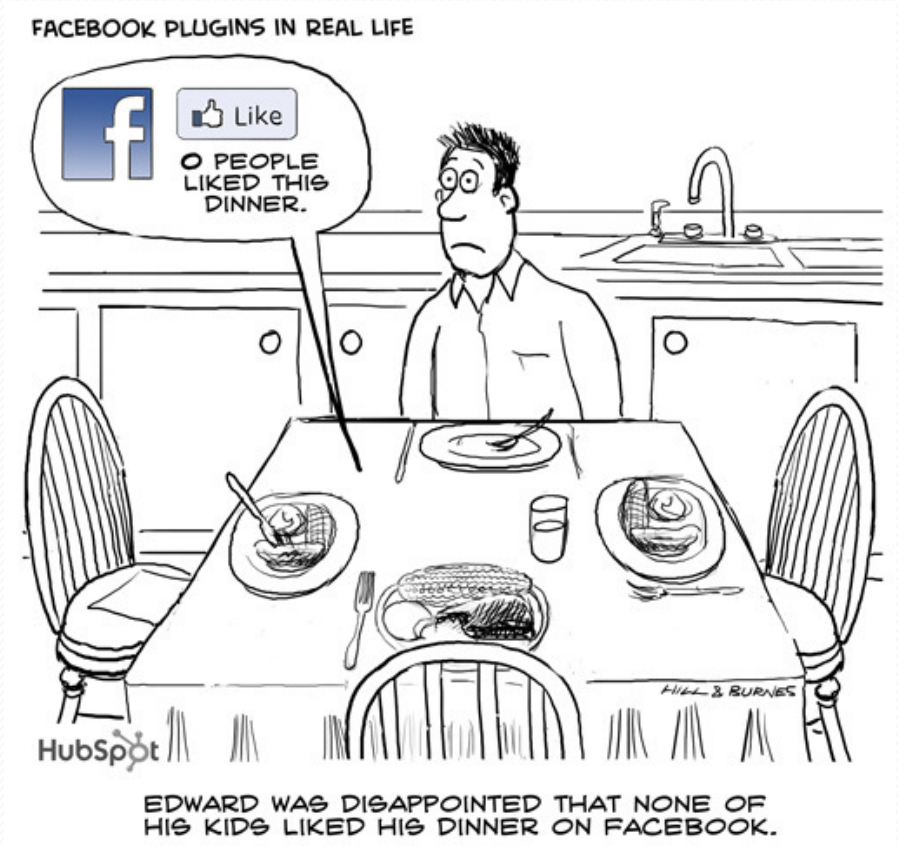
Advantages of zebrafish

- Vertebrate species
- Physiological similarity
- Reproduce quickly and abundantly
- Transparent embryos
- Rapid development
- Fully sequenced genome
- Ease of genetic manipulation
- History of genetic and developmental research
- Low-cost model
- **Highly social animals**

Can zebrafish be a good model?

Time spent together
(< 1 body length)

Behavioral complexity


FACEBOOK PLUGINS IN REAL LIFE
Like
0 PEOPLE LIKED THIS DINNER.
HUBSPOT
EDWARD WAS DISAPPOINTED THAT NONE OF HIS KIDS LIKED HIS DINNER ON FACEBOOK.

- Zebrafish are highly social animals
- They represent an emerging new important model to model normal and pathological social behaviors

- **How to?**

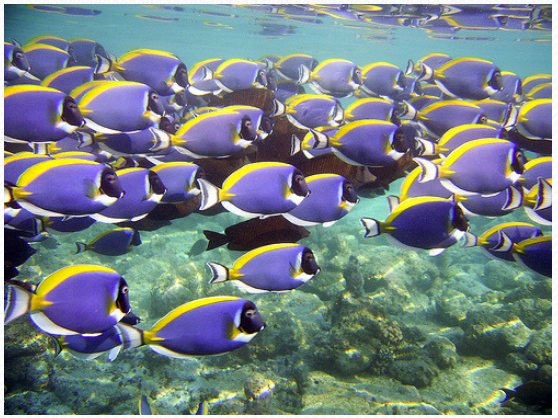
Zebrafish social tests

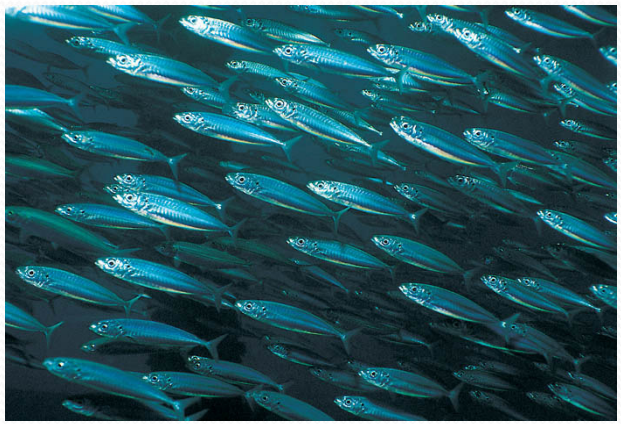
- Zebrafish exhibit robust social behaviors, such as shoaling, boldness, aggression and social preference
- Many 'rodent' social tests can be easily adapted to zebrafish

  - Examples of typical zebrafish tests:
    - Shoaling test
    - Social preference test
    - Mirror biting test

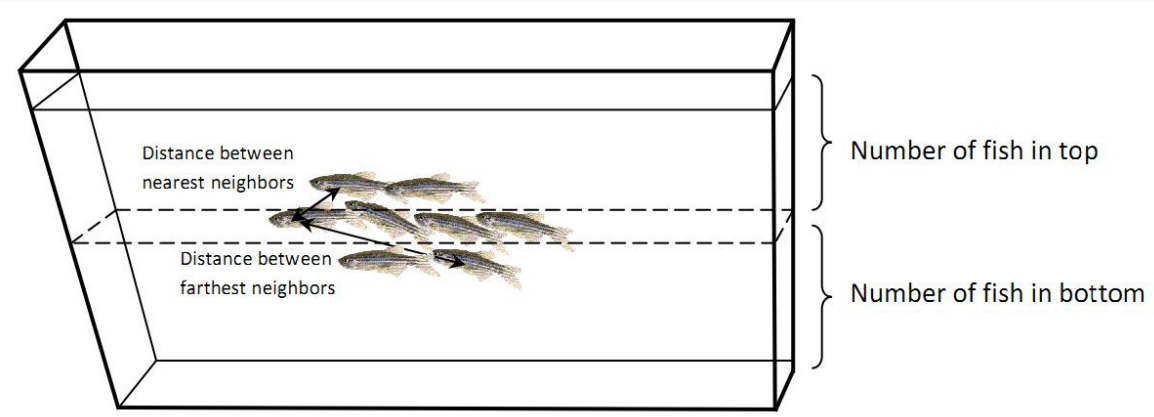
Shoaling behavior

- Very common in fish species
- Represents the complex interaction of animals moving together in coordinated movements
- Fish spend >80% of their time in schools





Shoaling test

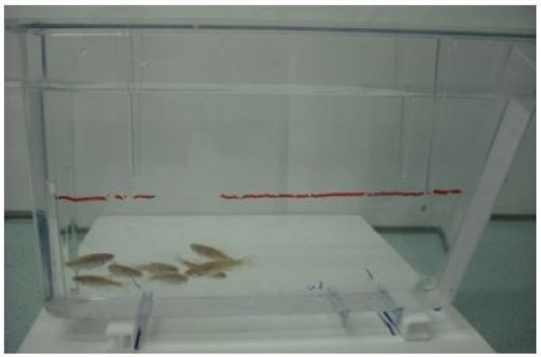


•Expose groups of 8 fish to a drug/treatment in an exposure beaker
•Move fish to the observation tank
•Video-record fish behavior for 3 min by a side-view camera
• Make 8 screenshots within a fixed time interval every 20 s during the last 3 min of the observation period
•Each screenshot should be carefully calibrated and analyzed by trained observers

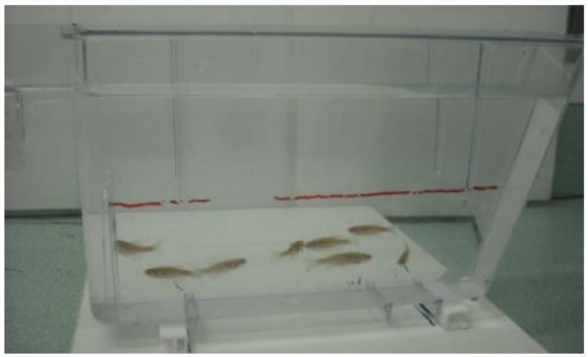
Shoaling test endpoints

Endpoints	Definition
Average inter-fish distance	**Distance between the body center of every member of the shoal**
Average nearest neighbor distance	**Distance for the body center of each fish to the closest neighboring fish**
Average farthest neighbor	Distance for the body center of each fish to the farthest neighboring fish
Top dwelling	Percent of fish in the top (upper half) of the tank
Thigmotaxis	The average distance of the group from the center of the tank
Variance of inter-fish distance	An index reflecting how homogeneously the fish are distributed in the shoal
Shoal area	**The size of the shoal (width and length)**
Excursions from shoal	Number of excursions of individual fish away from the shoal (>4 body lengths)
Duration of excursions	Duration of excursions of individual fish away from the shoal

Shoaling patterns



Tight shoal

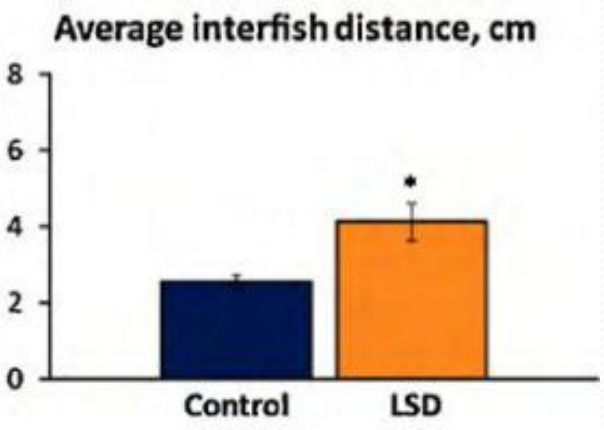


Medium shoal

Loose shoal

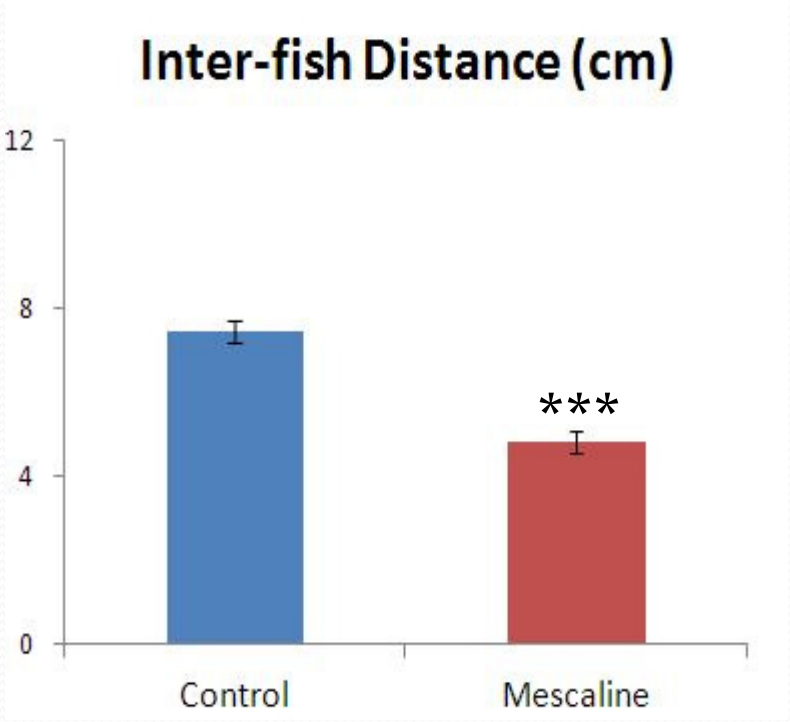
Effects of LSD on shoaling

LSD (250 ug/l) markedly disrupts normal shoaling behavior, significantly increasing the average inter-fish distance

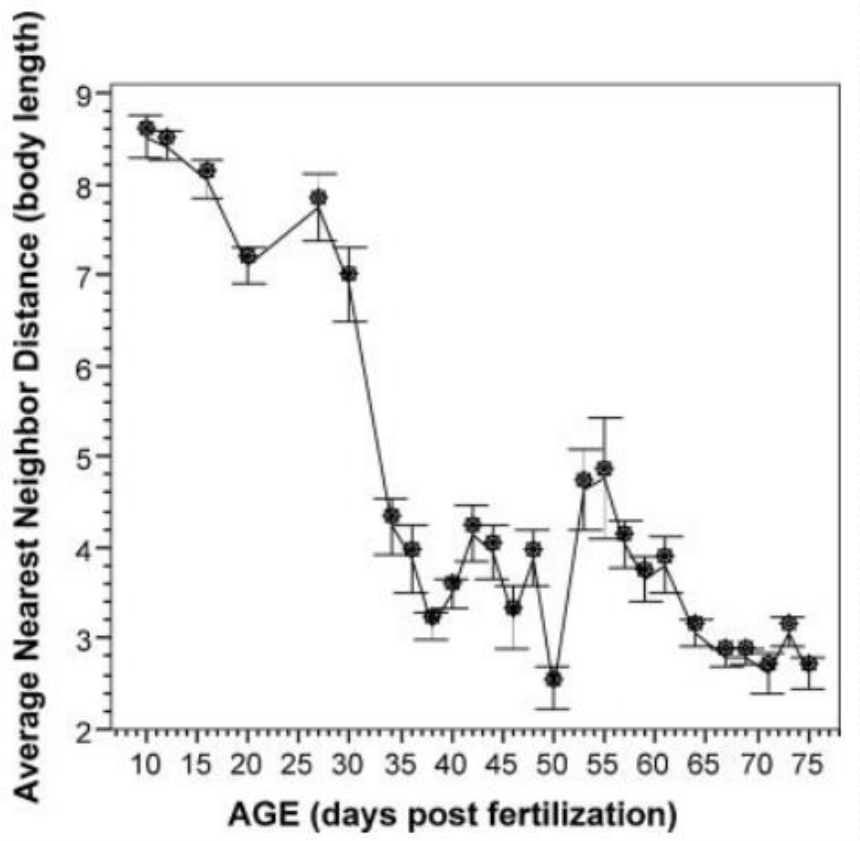


Effects of Mescaline on shoaling

Mescaline (20 mg/L) significanlty zebrafish shoal by decreasing the average inter-fish distance



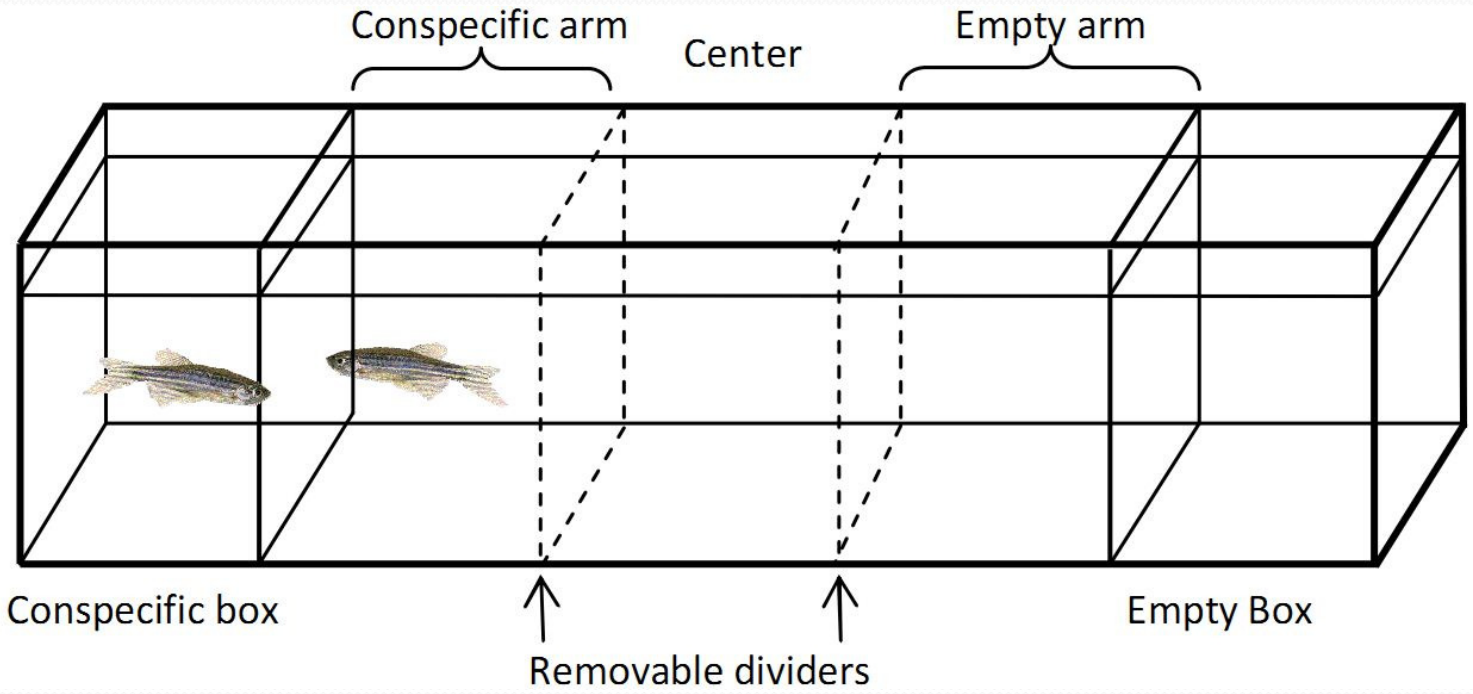
Shoaling changes with age

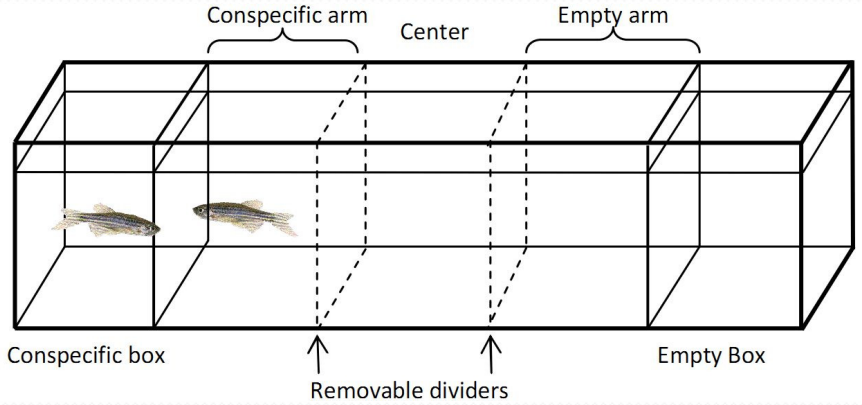


Buske and Gerlai et al., 2011

Social preference

- Zebrafish also display strong preference when placed in a tank with conspecific fish, a trait that can easily be quantified in the two-compartment preference test

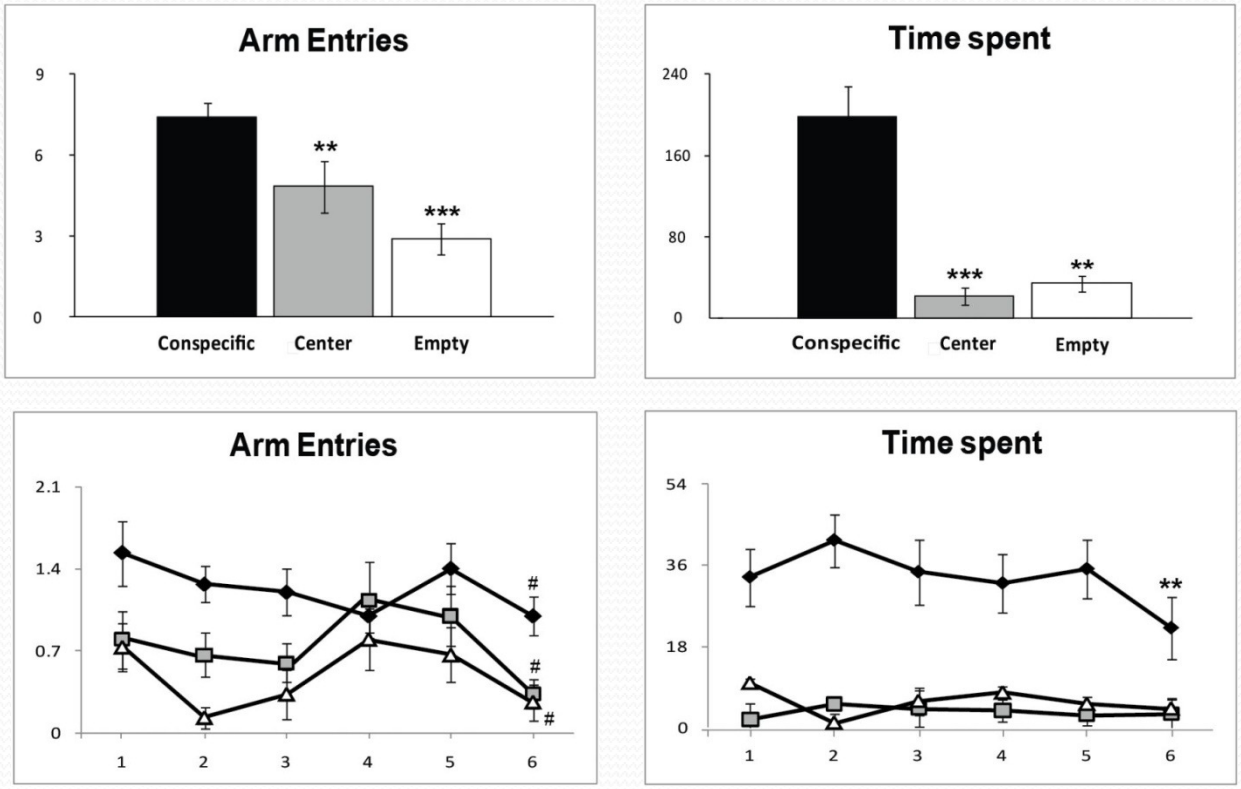



Conspecific arm
Center
Empty arm
Conspecific box
Removable dividers
Empty Box

Social preference endpoints

Endpoints	Definition
Time in target arm	Time spent in the target (conspecific) arm
Time in empty arm	Time spent in the empty arm
Time in center	Time spent in the center of the social preference test apparatus
Target:empty arm time ratio	The ratio between time spent in the target arm to time spent in the empty arm
Target arm entries	The number of entries to the target (conspecific) arm
Center entries	The number of entries to the center of the social preference apparatus
Empty entries	The number of entries to the empty arm
Target:empty arm entries ratio	The ratio between entries to the target arm and entries to the empty arm
Target:total arm entries ratio	The ratio between entries to the target arm and total arm entries

Social preference test

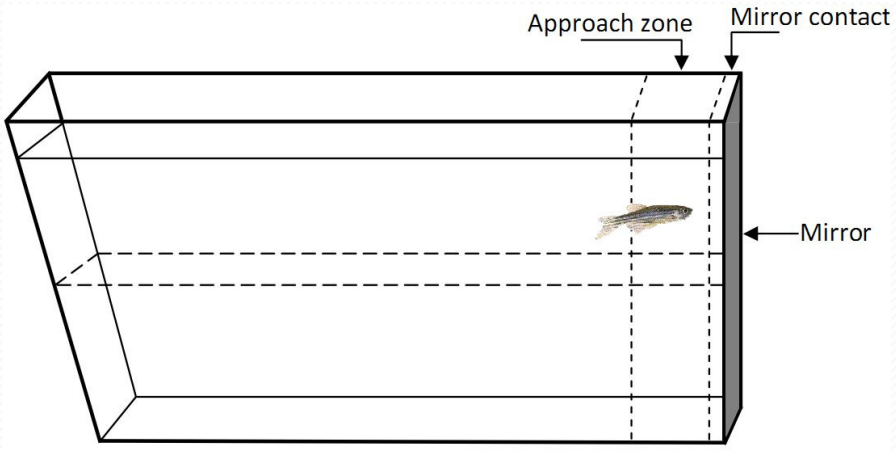


Typical behavior observed in naïve adult zebrafish exposed to the social preference test for 6 min (bar diagrams: ** P<0.01, ***P<0.005 vs. conspecific arm, paired U-test). Fish will generally prefer to spend more time close to a target/conspecific fish In line diagrams, note time-course of behavioral responses, as conspecific arm entries and time change (habituate) over time during the test (** P<0.01, #P = 0.5-0.1 (trend), min 6 vs. min 1, paired U-test).

Mirror biting test

- Mirror image stimulation is a well-established fish paradigm, traditionally used for studying their social/aggressive behavior (Lorenz, 1956)

Mirror biting test



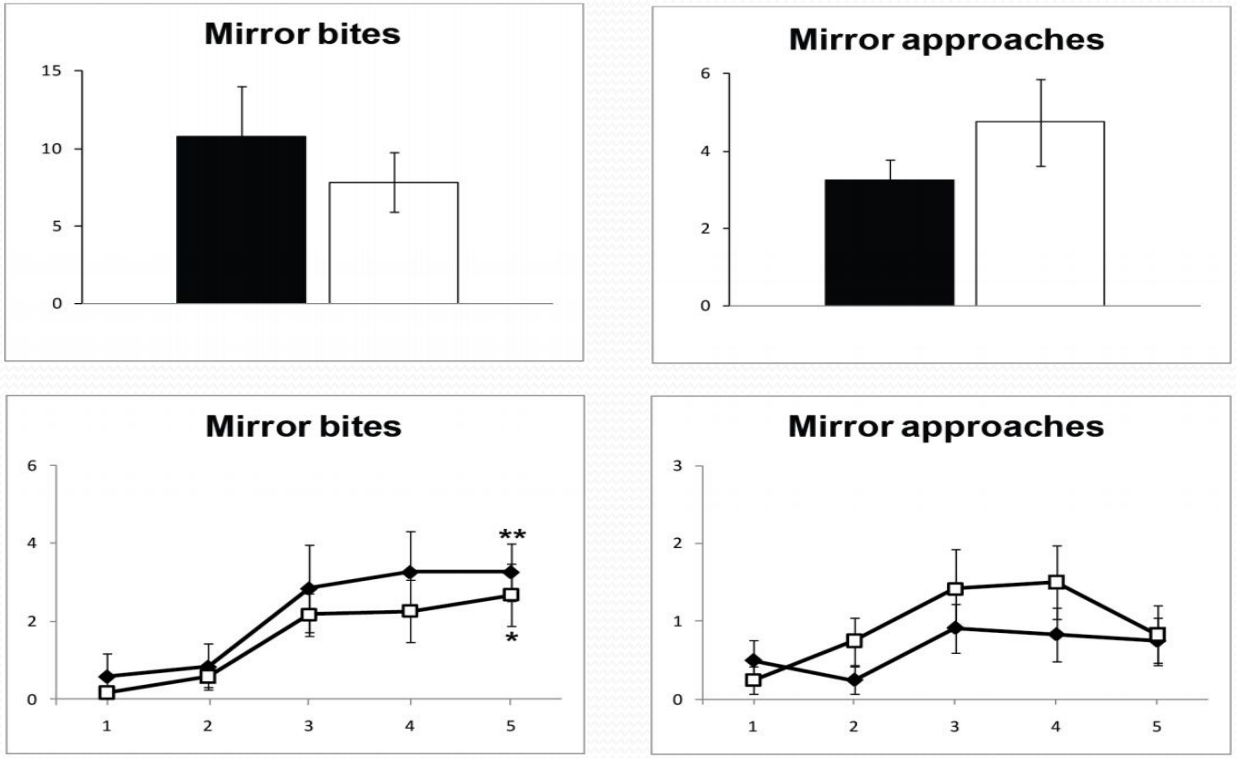
Methodology

- Place the fish in a small tank and leave undisturbed for a long period of time
- Quickly place a mirror into the tank with the fish
- Manually record zebrafish behaviors or use video recording

Mirror biting test endpoints

Endpoints	Definition
Mirror biting frequency	Number of times the fish bite the mirror
Mirror biting duration	Time spent biting mirror
Approaches to the mirror	The number of crossing the line denoting the mirror approach zone, but without mirror contact
Mirror contacts	The number of crossing the line denoting the mirror contact zone (e.g., 0.5 cm from the mirror)
Latency to approach	Time to the first approach to the mirror
Aggressive tail beats	The number of aggressive tail beats against the mirror
Latency to contact	Time to the first contact with the mirror

Zebrafish mirror biting test



Two different modifications of the mirror biting test. In Modification 1 (white bars on bottom diagrams), zebrafish were placed in the novel tank apparatus for a 5-min acclimation prior to introducing mirror and recording fish behavior for 5 min. In Modification 2 (black bars on bottom diagrams), zebrafish were exposed for 5 min to the novel tank test apparatus containing a mirror attached to one of its side walls.

Potential applications of zebrafish tests of social behavior

- Screening of novel drugs for autism and other social behavioral disorders
- Screening of zebrafish mutants relevant to autism
- Testing neurodevelopmental trajectories of social behavior

Summary

- Social phenotypes are a key part of zebrafish natural behavior, and are equally important in the laboratory environment
- Examining shoaling phenotypes, social preference and mirror biting responses provides a better understanding of animal social behaviors
- We need **better, objective tools** to assess social phenotypes in zebrafish
- These tests foster the development of translatable models, contributing to our understanding of human social disorders, such as autism, social phobia and schizophrenia