



Validation of Behaviors in a Zebrafish Model of Autism

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Nature of the Problem

Autism Spectrum Disorder



A neurodevelopmental disorder marked by social and communication deficits as well as repetitive sensory-motor behaviors .

Strong Genetic Component



Altered synaptogenesis

Prevalence

1-in-54 children



Behaviors Associated with ASD

Two Sets of Criteria

1. Deficits in communication and social interaction

Difficulty with:

- Peer interaction
- Relationships
- Nonverbal communication

2. Repetitive Sensory-Motor Behaviors

Stimming

- Hand flapping
- Spinning
- Toe-wiggling

Other: Echolalia, fixative interests, anxiety, hyperactivity, aggression



Screening and Diagnosis

- ADDM taskforce
- Males over 4x as likely as females
- DSM-5
- M-CHAT
- CSBS
- STAT
- ADOS*



Treatment

- Behavior Modification
- Applied Behavior Analysis (ABA)
- BCBA/RBT

- Pharmaceutical Intervention
 - SSRIs and stimulants ⓪ approved by FDA
 - **Risperidone** is the only medication approved specifically to treat irritability in ASD.



Genes Associated with ASD

- Disruption of synaptogenesis
- SYNGAP1, NRXN1, NGLN3, and SHANK3
- PTCH2 and LMX1bb



Zebrafish Model of Autism

“The Father of Zebrafish Research”

History

- Established by Dr. George Streisinger in the 1970s
- First mutant *Golden*
- 117,000 mutants listed on ZFIN database

Benefits of Zebrafish Model

- Ex vivo fertilization and embryonic development
- 200-300 eggs per mating
- Transparent embryos
- Short maturation period
- Social vertebrates
- Homologous gene sequence



1927-1984

Source: IZFS



The background is an abstract, fluid composition of deep blue and magenta-red colors. The patterns resemble ink or paint swirling in water, creating a sense of movement and depth. The colors are most vibrant in the upper left and lower right, fading into darker tones towards the center and bottom.

**SEEKING TO VALIDATE
BEHAVIORS IN A
ZEBRAFISH MODEL
OF AUTISM**

(A Tale of Two Fishies)

An abstract, artistic splash of blue ink on a white background. The ink forms a large, dark blue, cloud-like shape on the left side, which tapers and disperses into smaller, lighter blue droplets and bubbles on the right side. The overall effect is dynamic and fluid.

INTRODUCTION

THESIS

QUESTION

Will zebrafish treated with valproic acid display autistic behaviors analogous to humans with autism spectrum disorder?

HYPOTHESIS

The group treated with VPA will display significantly higher rates of behaviors associated with ASD than the control group.

An abstract, high-contrast image featuring a large, dark blue ink splash or cloud against a white background. The splash has a textured, organic appearance with various shades of blue and black, creating a sense of movement and depth. The text is centered within the splash.

Materials and Methods

Animals

Embryo Collection



Embryos were collected within
10 to 15 minutes of spawning

VPA Exposure

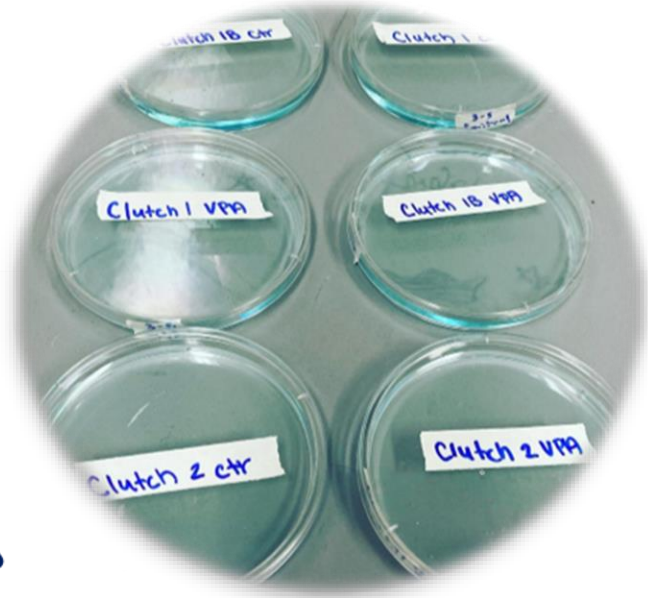


30 ml embryo media treated
with ~~50~~ $50 \mu\text{M}$ of VPA

Housing

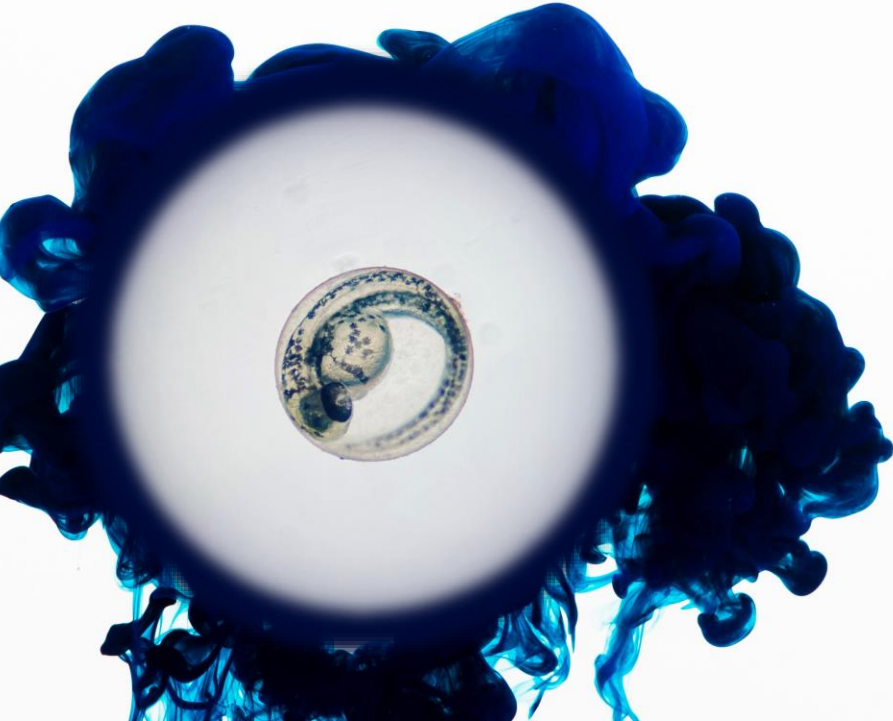


Aquaneering Zebrafish
Aquatic Housing System

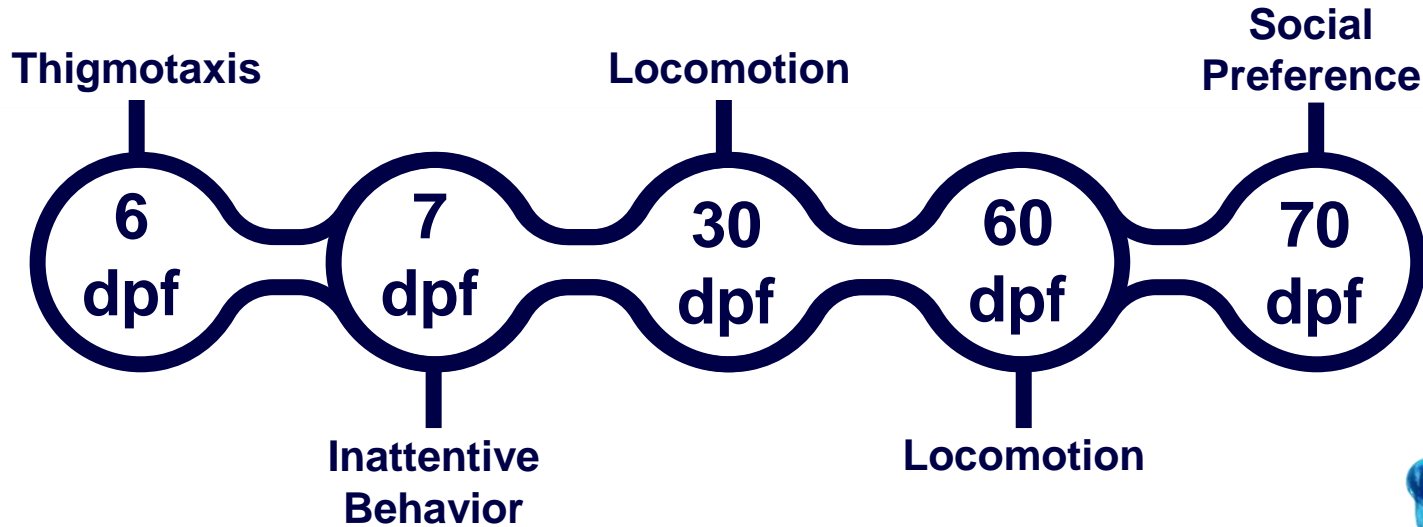


48-hpf VPA-treated

This is my favorite stage of development!



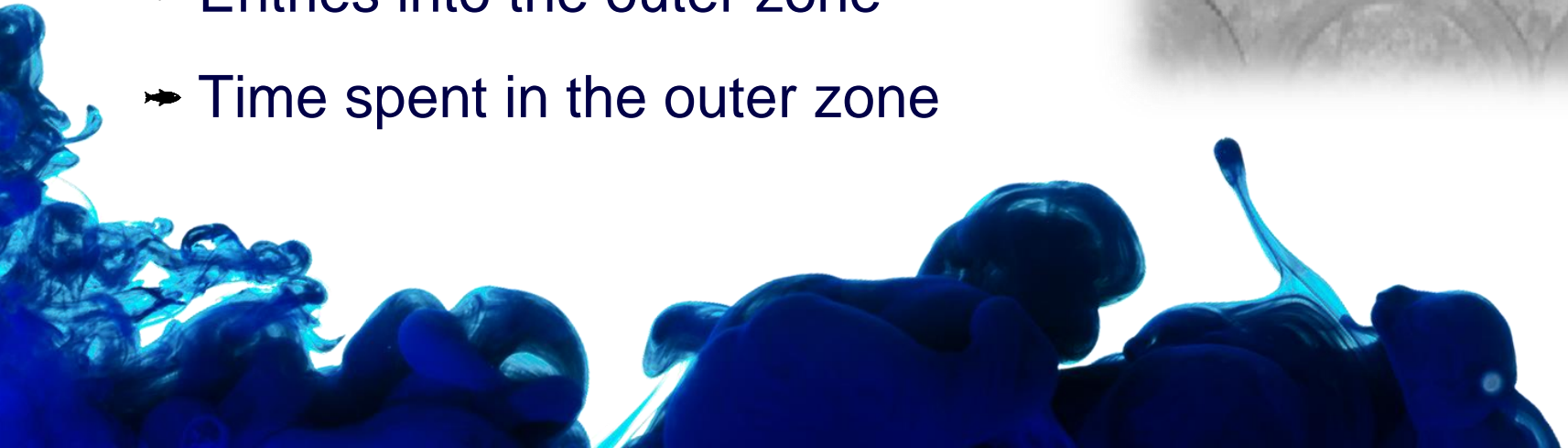
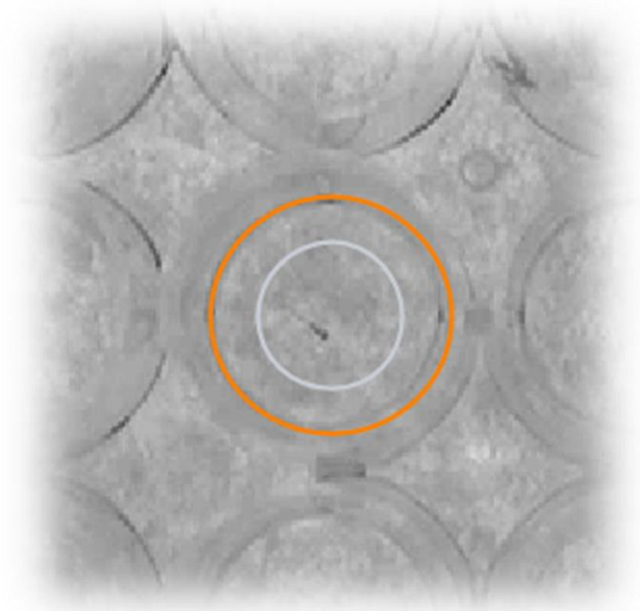
Testing Schedule



*Thigmotaxis and open-field behavior assessments and social preference assessments were based on behavioral assays highlighted *Embryological exposure to valproic acid induces social interaction deficits in zebrafish (Danio rerio): A developmental behavior analysis* by FF Zimmerman et al. (2015). Inattentive behaviors were assessed based on the research of Shubham Dwivedi et al. in the article *Larval zebrafish model for studying the effects of valproic acid on neurodevelopment: An approach towards modeling autism*.

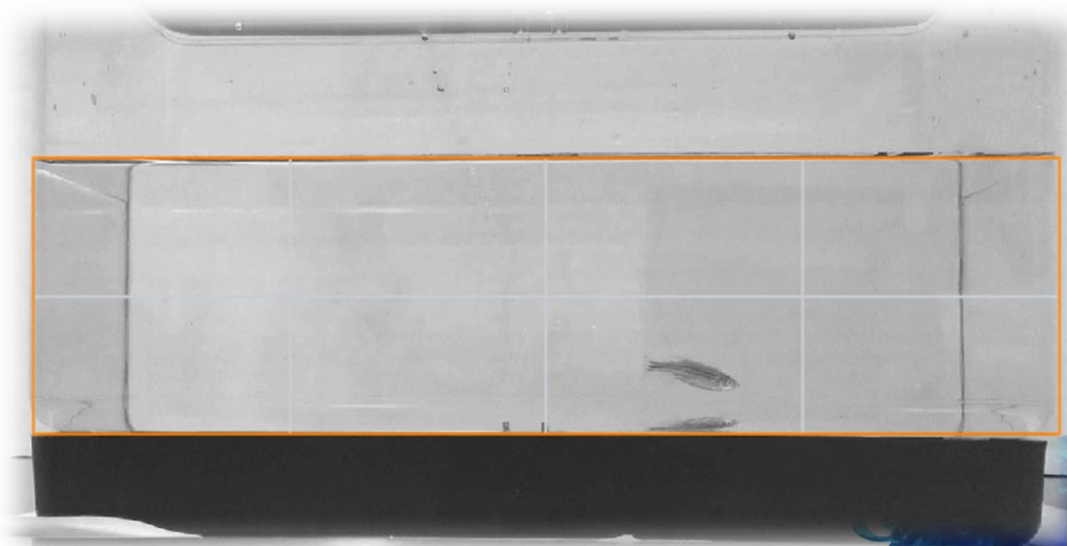
Thigmotaxis

- ➔ 6 dpf
- ➔ “Wall-sticking” behavior
- ➔ 24-well plate/5minute test
- ➔ Entries into the outer zone
- ➔ Time spent in the outer zone



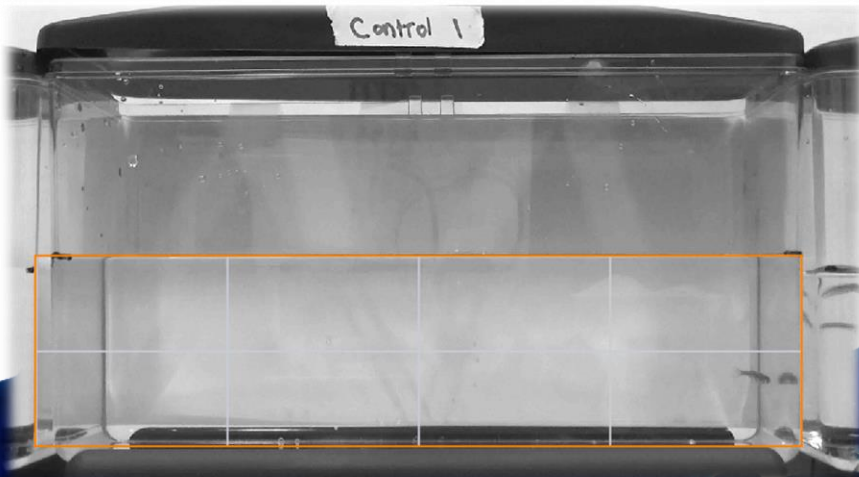
Locomotion

- ➔ 30 dpf and 60 dpf
- ➔ Measure of anxiety
- ➔ More time spent in bottom zone indicates greater level of anxiety



Social Preference

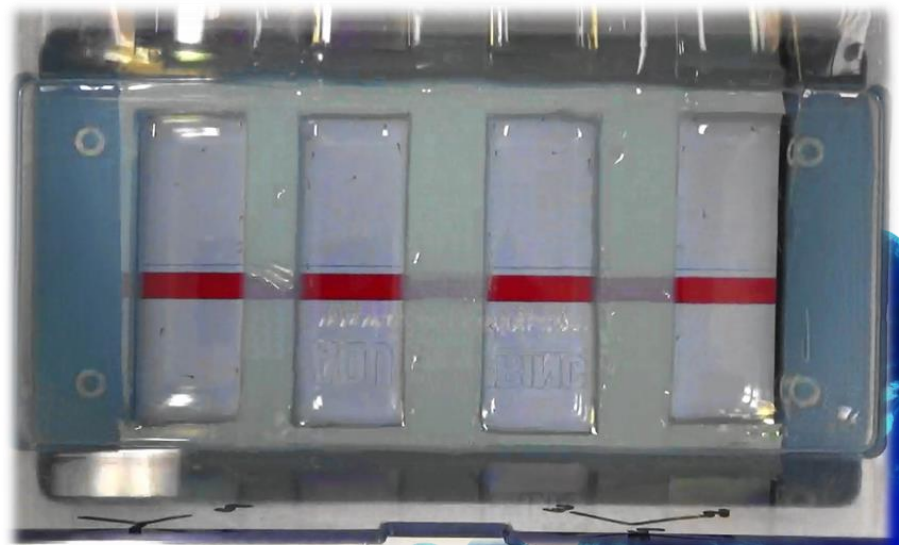
- 70 dpf
- Three tanks
- 4 zones
- Measures shoaling desire
- Time in Zone 1 measure of shoaling desire



Inattentive Behavior

- ➔ 7 dpf
- ➔ Difference in % of larvae in the upper section during aversive stimulus vs acclimation was an indicator of inattentive behavior for the group being tested (Dwivedi, 2019).

$$\begin{aligned} & \% \text{Larvae in upper half over acclimatization} \\ &= \left(\frac{\text{Aversive stimulus} - \text{Acclimatization}}{\text{Acclimatization}} \right) * 100 \end{aligned}$$



7-dpf Inattentive Behavior





RESULTS

Results

Thigmotaxis

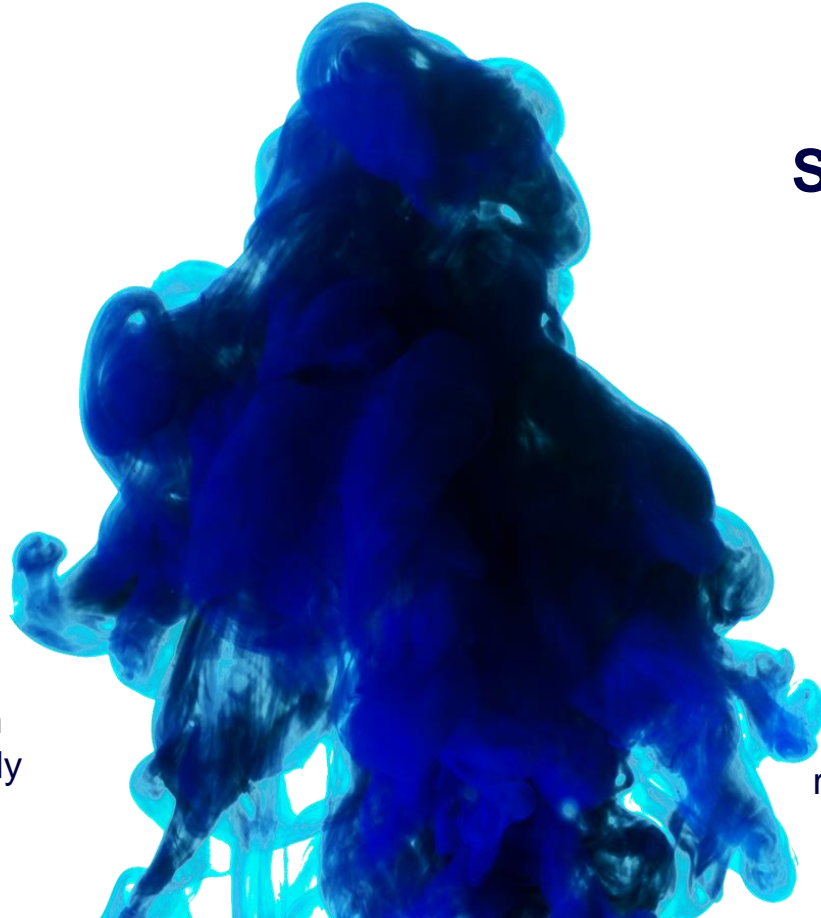
Not statistically significant

All test results not statistically significant

Locomotion

30 dpf SIGNIFICANT

30 dpf time spent in bottom zone was the only statistically significant outcome.



Social Preference

Not Statistically Significant

All test results were not statistically significant

Inattentive Bx

Inconclusive

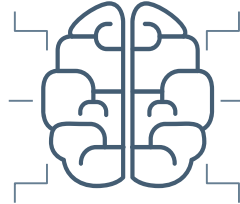
*Note: This test needs to be reperformed and replicated in a different container.

Anxiety Parameters

Thigmotaxis

Not statistically significant

None of the parameters of the 6-dpf testing yielded statistically significant results.



Locomotion

30 dpf statistically SIGNIFICANT

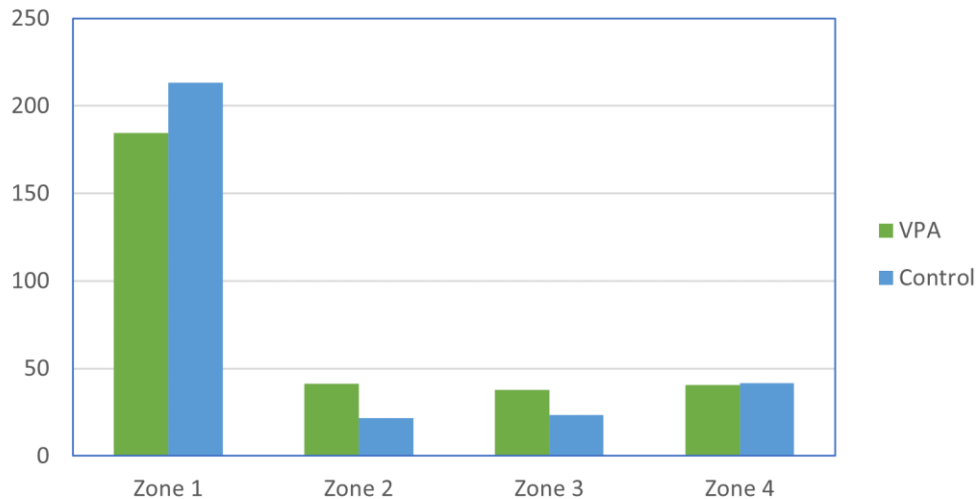
30 dpf time spent in bottom zone was the only statistically significant outcome.

Age	Anxiety Parameter	Control group Means \pm SD	n	VPA group Means \pm SD	n	p-value
6-dpf	Entries in the outer zone	9.4 \pm 5.4	74	11.2 \pm 8.7	81	p > .05
6-dpf	Time spent in outer zone	235.7 \pm 59.7	74	238.2 \pm 49.0	81	p > .05
30-dpf	Time spent in bottom zone	178.7 \pm 47.7 s	18	212.7 \pm 44.0 s	14	p < .05
60-dpf	Time spent in bottom zone	129.7 \pm 65.9 s	12	159.7 \pm 38.2 s	15	p > .05

Social Preference

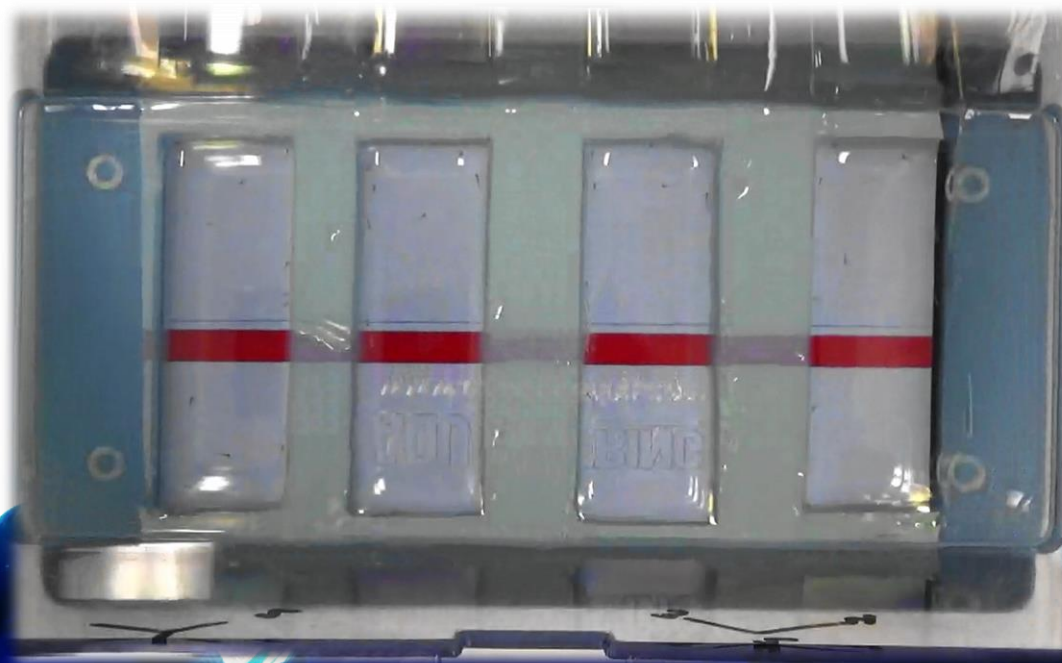
- 70 dpf
- Control fish spent more time on average in the social zone, but the difference was not statistically significant.

70 dpf Social Preference



Inattentive Behavior

- Test results were inconclusive.
- Test requires replication to confirm reliability.
- Logo on the bottom of the tray may have affected validity.



Summary

Statistically Significant

- 30 dpf locomotion
- Time spent in bottom zone

Not Statistically Significant

- 6-dpf thigmotaxis
- 60-dpf locomotion
- 70-dpf social preference

Inconclusive

- 7-dpf inattentive behavior

An abstract, high-contrast image featuring a vibrant blue ink splash or cloud against a stark white background. The ink forms intricate, organic shapes with varying shades of blue, from deep navy to bright cyan. The overall effect is dynamic and artistic.

Recommendations and Discussion

Recommendations

- ➔ VPA administration
- ➔ 2+ semesters for mating and testing
- ➔ Younger fish for mating
- ➔ Zebrafish concentric software
- ➔ Camera apparatus



Discussion

- “It’s fine to celebrate success but it is more important to heed the lessons of failure.” - Bill Gates
- New skills
- Knowledge
- Confidence
- Lasting relationships



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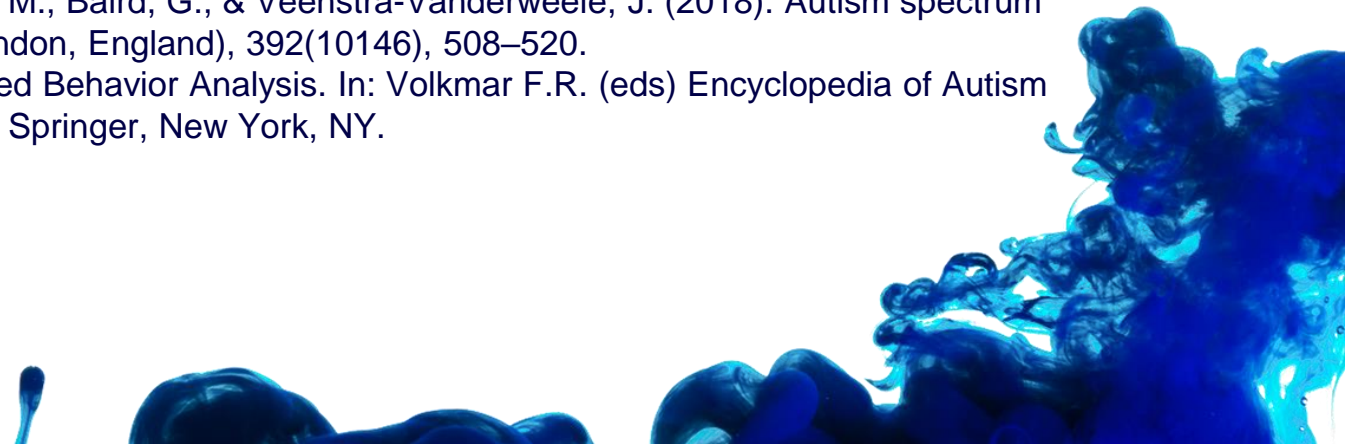
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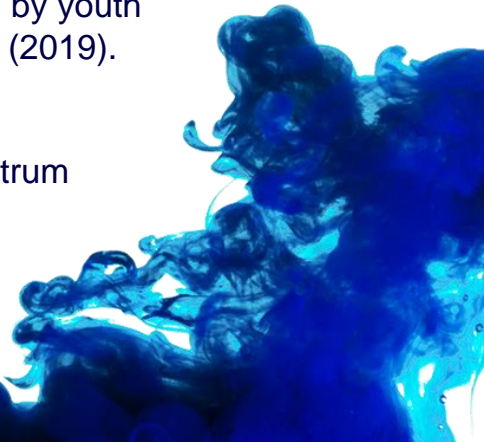
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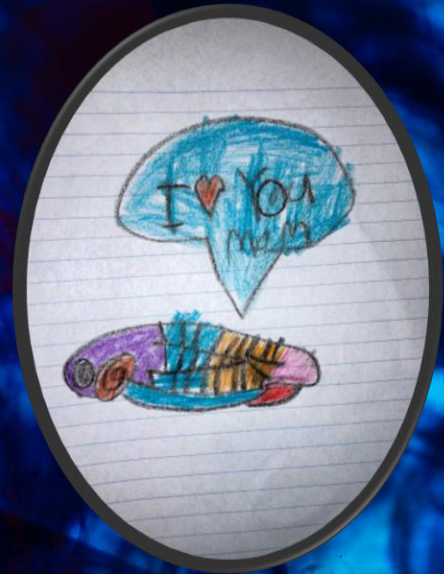
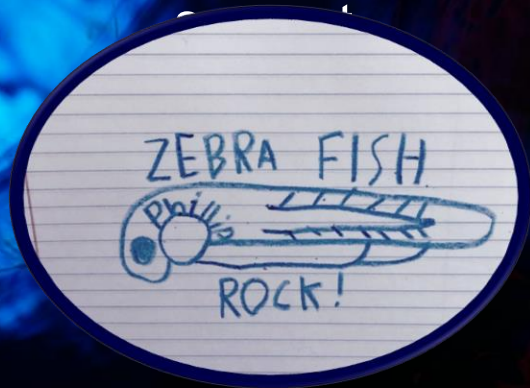
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