Fetal Alcohol Syndrome (FAS) is a condition that can affect the physical health and behavior of developing fetuses as a result of the pregnant mother’s consumption of alcohol. FAS is found in 0.2-1.5 infants out of 1,000 (2). Much like FAS, Autism Spectrum Disorder (ASD) affects behavior, often expressed in impaired social communication and repetitive behaviors. The two conditions can overlap symptoms, and 3% of FAS children also receiving ASD diagnoses (3). Zebrafish are an effective model organism to use for studying FAS and ASD related behaviors due to the ability to non-invasively observe internal processes via microscopy (1). Young zebrafish also develop motor capabilities within 5 days after fertilization. (1). Observing the movement patterns of the young zebrafish exposed to varying concentrations of ethanol solution can reveal the impact of alcohol exposure on development and early behavior. To establish a motor assay protocol that can be used for future alcohol-related and other toxicological experiments in our lab, the experimental methods of past ethanol experiments conducted by Ali et al. (2011) were replicated. 

Hypothesis: Zebrafish embryos exposed to higher concentrations of ethanol will have a smaller average of total distance moved.

### Methods

#### Treatments

- **Embryos raised until 5 days post fertilization.
- Each group exposed to 500μl of E3, 1% Ethanol, or 2.5% Ethanol solutions for 1 hour.
- Embryos rinsed 3 times with E3 to remove ethanol.

#### Data Collection

- Young fish relocated to individual assay well plate
- Fish moved into chamber, 2 minutes to adjust to light before beginning tracking
- Fish movement tracked and recorded via EthoVision detection program, with a cycle of 4 minutes in the light, 4 minutes in the dark, and another 4 minutes in the light.

#### Analysis

- At 24 hours post fertilization, 96 embryos are divided into 3 groups of 32 embryos
- Average total distance moved per fish among treatment groups were recorded
- Any fish with recorded movement greater than or equal to 100,000 mm were considered outliers and removed from analysis
- P-value between treatment groups was calculated via paired t-tests assuming unequal variances

### Results

**Figure 1:** Ethanol treatment has variable effects on swimming behavior in 5dpf zebrafish larvae across 3 experiments

- **A** Results of first experiment; no significant difference in movement
- **B** Results of second experiment; significant difference between E3 and 1% groups and E3 and 2.5% groups; no significant difference between 1% and 2.5% groups
- **C** Results of third experiment; no significant difference in movement

**Table 1:** Average total distance moved per larva treated with vehicle or ethanol.

(A) Results of first experiment; no significant difference in movement

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Distance Moved</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3</td>
<td>1000 ± 200</td>
</tr>
<tr>
<td>0.5%</td>
<td>1050 ± 250</td>
</tr>
<tr>
<td>2.5%</td>
<td>1100 ± 300</td>
</tr>
</tbody>
</table>

(B) Results of second experiment; significant difference between E3 and 1% groups and E3 and 2.5% groups; no significant difference between 1% and 2.5% groups

<table>
<thead>
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</tr>
</thead>
<tbody>
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</tr>
<tr>
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<td>1200 ± 300</td>
</tr>
<tr>
<td>2.5%</td>
<td>1300 ± 400</td>
</tr>
</tbody>
</table>

(C) Results of third experiment; no significant difference in movement

<table>
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</table>

### Future directions

- Analysis of differences in movement between treatment groups in light and dark conditions
- Analysis of differences in velocity and movement time between treatment groups
- Repeat of experiment to confirm significance of difference in movement between treatment groups
- Use motor assay to test effect of environmental factors associated with ASD on neural circuit development

### Acknowledgements

A special thanks to the Carter Lab for their contributions and dedication to this project. Thank you as well to the University of Wisconsin – Eau Claire Biology Department for their support in funding, professional development, and lab space. This project was funded in part by the UWEC Office of Research and Sponsored Programs (ORS) through a Summer Research Experience for Undergraduates grant.

### Citations


### Analysis and Interpretation

Among three separate runs of this experiment across the summer, only one run yielded statistically significant results. The total movement of zebrafish exposed to 1% or 2.5% ethanol solutions was significantly lower than that of zebrafish exposed to E3 (p<0.05), but not significantly different from each other. These results are not true in either of the other experiments performed.

The existence of a significant difference between the control and ethanol groups in at least one of the experiments does imply the existence of a connection between the ethanol exposure and motor movement. However, the other two experiments’ non-significant data prevents the ability to make a definitive claim on the effects of ethanol on motor movement.

Any variability in data collection or experimental procedure in the previous experiments, such as faulty movement tracking and slight differences in the timing of ethanol exposure, must be addressed in future runs of the experiment. It is also possible that this variation is due to differences between clutches.