

Methylphenidate Causes Changes in Zebrafish (*Danio rerio*) Sociability

Aaliyah WilsonFalcone

Saint Joseph's College of Maine Science Department



Introduction

Methylphenidate is a common clinically used medication for the mental disorder ADHD. However, the precise mechanisms of action and potential side effects of the drug is not fully understood in humans. This lack of understanding underscores the importance of studying the effects of methylphenidate on model organisms, like the zebrafish (Brenner et al, 2020).

Objective

Methylphenidate will cause social changes in zebrafish (*Danio rerio*). Although, it is also predicted that the increasing dosage of methylphenidate will cause decreased levels of sociability in zebrafish. Meaning that zebrafish will be less willing to interact with others.

Methodology

Experimental Setup: 4-5L tanks were set up for the experiment, and labeled Tank 1, Tank 2, Tank 3, and Tank 4 (Figure 1).

- Tanks 1, 2 and 3, were equipped with a Top Fin SilenStream PF10 Power Filter and an air pump.
- Each tank was kept between 27-29°C
- A Brinno time lapse camera will be pointed at Tank 4

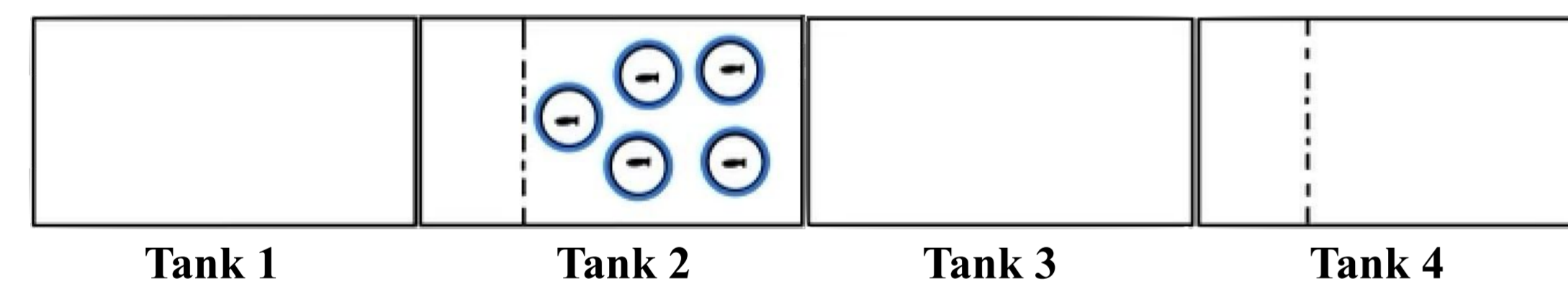


Figure 1: Experimental tank set up. (Tank 1) holding for extra zebrafish, (Tank 2) holding for experimental fish in separate containers, (Tank 3) hold ten shoaling fish, and (Tank 4) the experimental tank, that is divided (see Fig 2). Dotted lines are separators.

Methylphenidate Exposure: Zebrafish (n=5) were individually dosed in a ~100 ml solution with methylphenidate concentration of 0 mg/L, 2 mg/L, or 4 mg/L for 20 min.

Social Testing: After dosed, the zebrafish were rinsed with 10 ml of water and placed into the middle of Tank 4, the experimental tank (Figure 2). The zebrafish were then observed opposite a shoal (n=10) using a Brinno time lapse camera for 20 minutes, first 5 minutes use for acclimation. A shoal is a group of conspecifics.

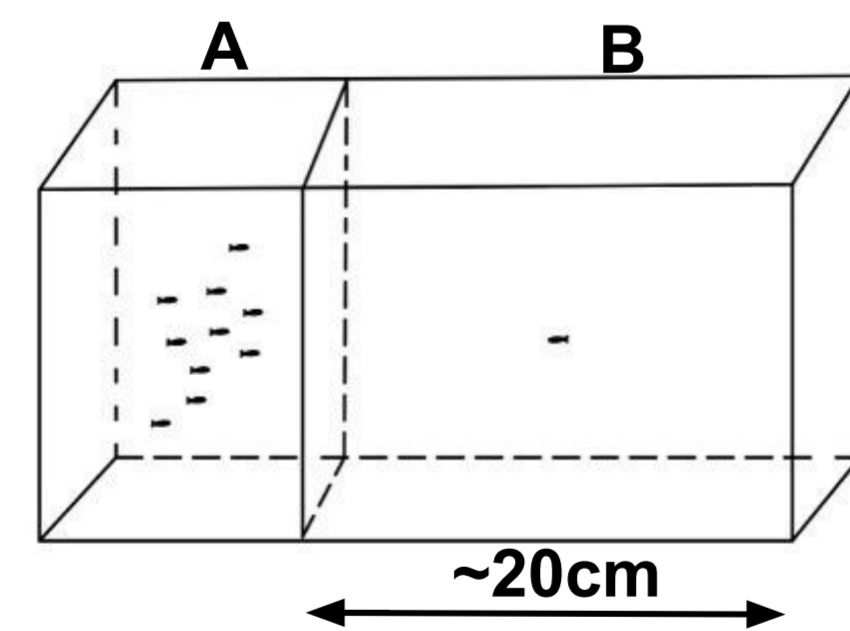


Figure 2: Tank 4, the experimental tank, set up. A was the side for shoaling fish (n=10), and (B) for the experimental fish (n=1)

Analysis

- The zebrafish distance from the shoal was measured over time at different concentration using a Brinno timelapse camera.
- The video from the camera was analyzed by placing 10-2 cm columns (Figure 3) along the side of Tank 4. The columns were used to determine the distance each zebrafish traveled in reference to the shoal.
- An ANOVA test was used to determine whether there was any significant differences between any of the three concentrations (see Fig 3).
- A multiple comparisons test was used to determine any significant difference between each concentration (see Fig 4).

Results

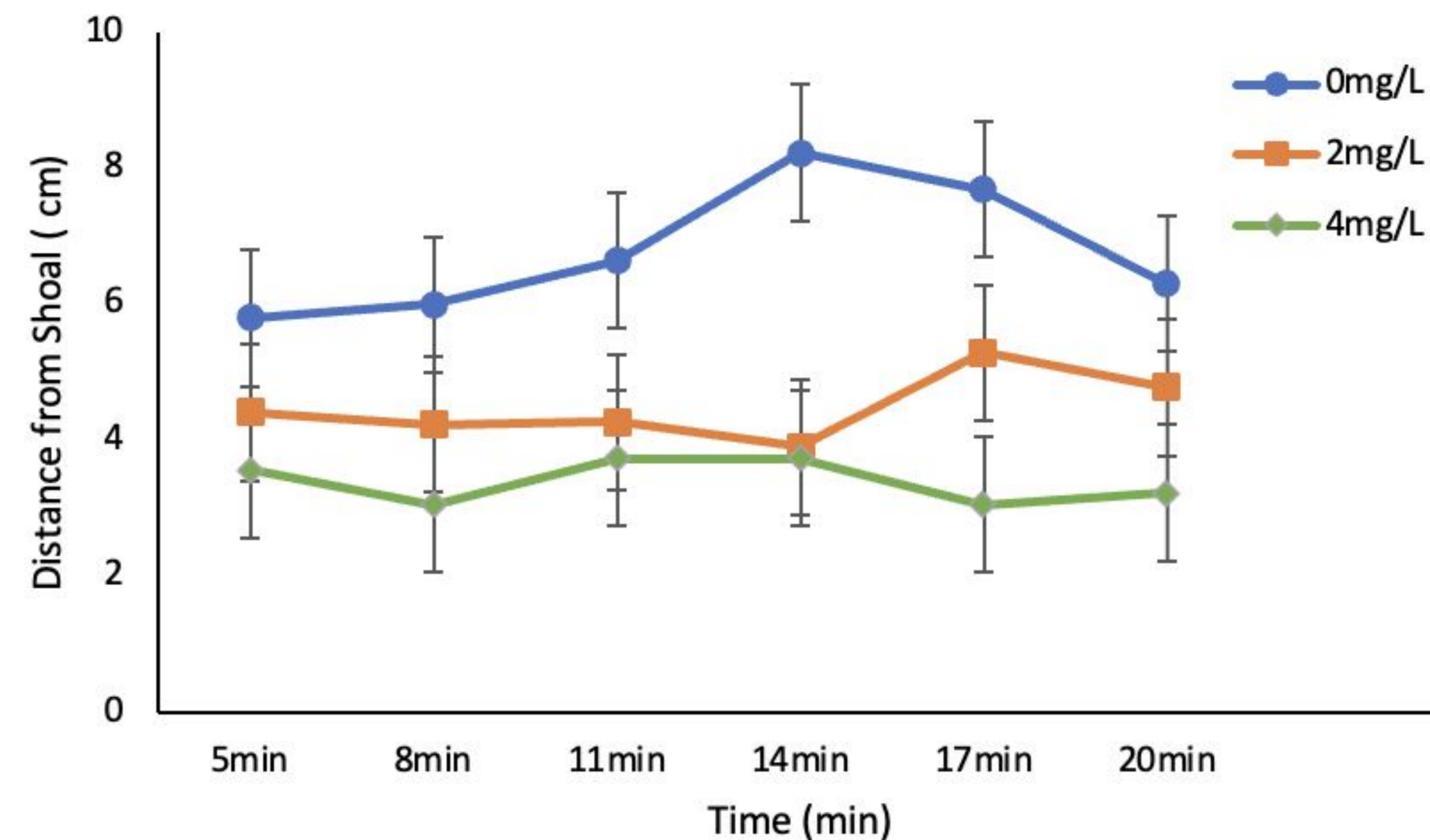


Figure 3: Distance from shoal by concentration of methylphenidate per minute at 0 mg/L, 2 mg/L, and 4 mg/L. Each point and vertical bar indicate the mean and SD (standard deviation). An ANOVA analysis, at the 20 minute mark, resulted in a p-value of 0.0428, which indicates that there is statistical differences among the means (see Fig 4).

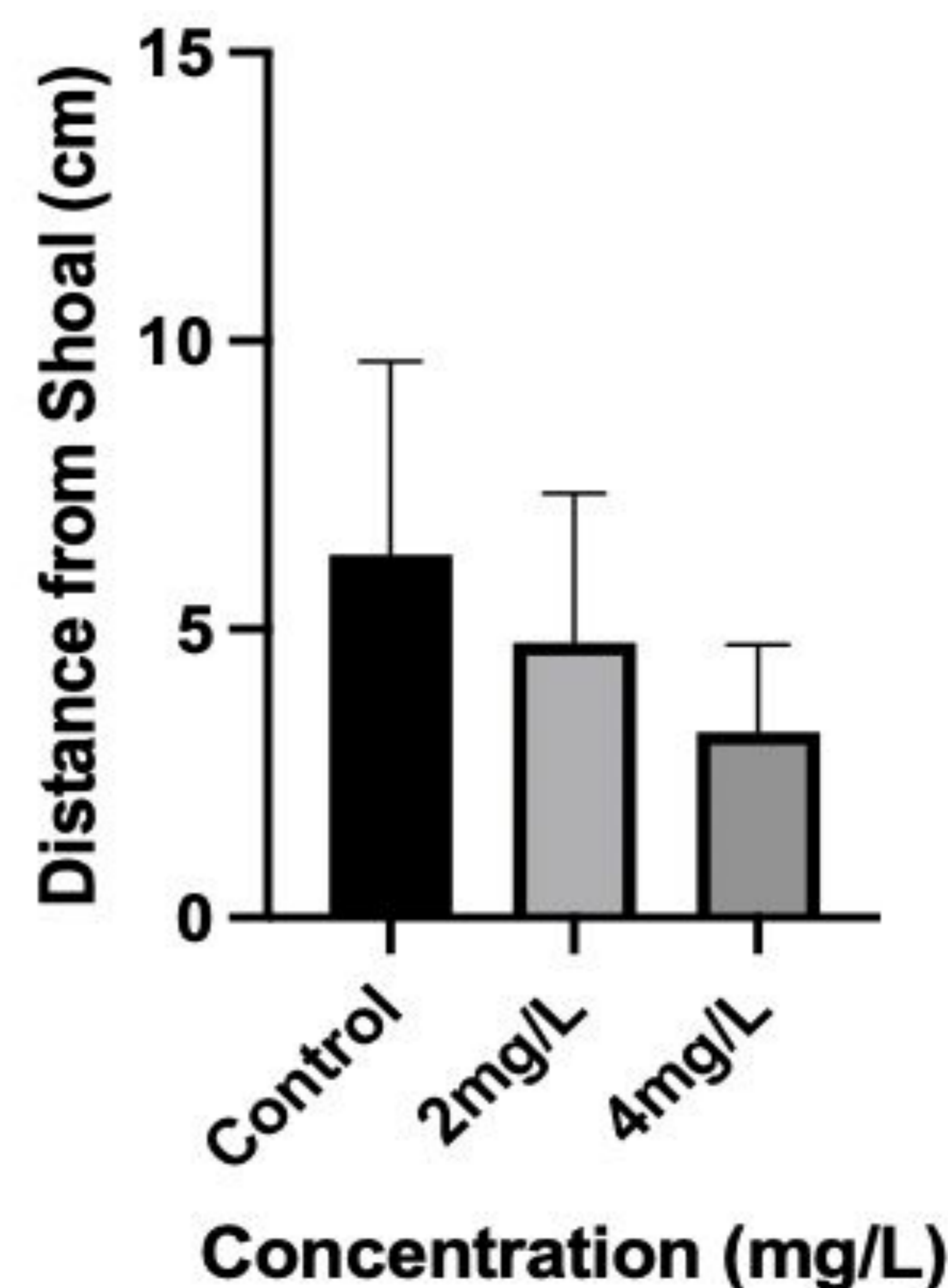


Figure 4: Distance from shoal compared to concentrations at 20 minute during the social shoaling test. The vertical bar indicate the mean and SD (standard deviation). After the ANOVA (see Fig 3) A multiple comparisons analysis resulted in comparing p-values. Control v. 2 mg/L (p=0.502), indicating no significant distances between them. Control v. 4 mg/L (p = 0.026), indicating that there was a significant difference between them. 2 mg/L v. 4 mg/L (p = 0.0172) indicated that there is a significant difference between the concentrations.

Conclusion

- From the control, the doses of 2 mg/L and 4 mg/L had an increased effect on the sociability of the zebrafish (Figure 3). Although, comparing the two doses of 2 mg/L and 4 mg/L, it shows a very minimal change in sociability.
- There was a decrease in distance from the shoal with increased dosage (Figure 4). Although, at 2 mg/L, the margin of error was too great for there to be any statistical differences between the means.
- The null hypothesis was rejected and it was determined that methylphenidate caused changes in the sociability of zebrafish. However, the prediction that the increasing dosage would decrease their social instinct was proven insignificant.
- It was found that increasing the concentration of methylphenidate will have an increasing effect on sociability.

Next Steps

- To determine the fishes maximum effect dose, by testing dosing concentrations higher and lower that what was tested in this experiment
- Another experiment would be to test the activity of the zebrafish after being dosed with various concentrations.

References

- Brenner RG, Oliveri AN, Sinnott-Armstrong W, Levin ED. 2020. Effects of sub-chronic methylphenidate on risk-taking and sociability in zebrafish (*Danio rerio*). *Naunyn-Schmiedeberg's Archives of Pharmacology*. 393(8):1373–1381.
- “ZEBRAFISHFILM.ORG.” *ZEBRAFISHFILM.ORG*, www.zebrafishfilm.org. Accessed 18 Nov. 2023.

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