Embryonic Cannabidiol Exposure Does Not Affect Adult Zebrafish Swimming Performance

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Abstract

Cannabis is used for a variety of reasons such as relieving pain, relieving stress, and reducing nausea during chemotherapy. While cannabis originates from central and south Asia, the drug has become extremely popular in North America. In July of 2001, medicinal use of cannabis was legalized in Canada, and on October 17 2018, recreational use of cannabis was legalized nationally. Many scientific studies have shown the negative effects of cannabis in consumers and of second hand smoke exposure, including lung cancer, respiratory issues, and reduced decision making and cognitive function. Because of the rapid increase in cannabis, high concentrations have filtered into the water treatment facilities and spread into lakes and ponds through pipelines that could potentially cause harm to the fish. While there are studies that have concluded that there are alterations to the fish's neuronal patterns and cardiac systems in zebrafish, there were no reports of how the medical ingredient of cannabis (cannabidiol or CBD) may affect the ability of a fish to swim. Proper swim behaviour is an essential survival characteristic to fish and other marine animals, but when a novel potentially toxic compound is introduced into their environment, impacts to vital biological functions in the organism may occur. This study aimed to investigate the potential effects of cannabidiol on zebrafish by evaluating their critical swimming speed (Ucrit value). Using a swim tunnel, we were able to control the environment and easily identify at what point the fish would be fatigued. Comparisons were made between three different fish tanks: one tank exposed to CBD, and the other two tanks contained a fresh water control and a solvent control. Using both our "p" and "F" stat values, we can conclude that there were no significant differences observed between the three fish tanks. In the future, we hope to analyse the neurology of the fish exposed and complete a fish respirometry measuring the oxygen consumption of CBD exposed fish.

Key words:

embryonic, cannabidiol, exposure, zebrafish, swimming, performance, anova, aquarium, critical swimming speed, CBD, incubator

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Introduction

- Zebrafish (*Danio rerio*) are a widely used model organism for studies involving vertebrate development and gene function because of their rapid rate of reproduction and well-defined embryology. [1]
- Prior research has shown that brief exposure of cannabidiol (CBD-3 mg/L) on embryonic zebrafish results in motor neuron alterations and heart defects in the adult stage. [2]
- The use of swim tunnels allows researchers to study fish physiology and their responses in a controlled environment. [3]
- Critical Swimming Speed (Ucrit) is a measure commonly used to determine the swimming capabilities of fish.[4]

Objective:

1.) Identify if embryonic CBD exposure is associated with impaired adult swim performance as measured by Ucrit.

Exposure to CBD

- Collect the eggs and put them in the incubator.
- Expose the eggs during gastrulation stage in egg water for 5- 5.5 hours.
- Clean the eggs by removing the solution and wash the eggs in egg water x3.
- Put back in the incubator for 5-6 days and let them grow.
- Allow animals to grow to adulthood (at least 3 months of age).

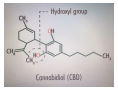

Figure 1. Chemical structure of CBD. (Image from website. [5])

Figure 2. Zebrafish embryos in a petri dish. (Image from website. [6])

Calibrating the swim tunnel

- Shake the individual pieces under water to allow all the air bubbles to escape.
- Turn on the flush pump to release any bubbles trapped in the tube; once clear unplug (repeat step 1 and 2 in the swim tunnel set up).
- Mix and stir 2 drops of dish soap and water in a glass beaker and transfer into 20mL stoppered syringe.
- Using a scoopula, pour 2 mg of green micro beads into the syringe, and lightly shake to disperse all the micro beads.
- Once the beads are in the tunnel, turn on the green laser that connects to the wire instead of the probe.
- On the computer in expert mode, press file, record sequence, name the video, adjust the frames, and press record.
- Once the video has recorded, open Ueye cockpit to upload and analyze the video. The analysis will give the velocity in cm/s and the angle.

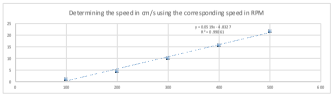

Figure 3. Swim tunnel calibrating chart used to determine what water speed corresponded with the appropriate RPM . The "x" axis is the output in RPM. The "y" axis is the average speed in cm/s.

Euthanization Solution

- Measure, and weigh 0.5g of sodium bicarbonate and 0.25g of MS-222.
- Place the MS-222/bicarbonate into a test tube, seal, and store in the freezer.
- On the day of the experiment, take one test tube from the freezer and mix with approx. 500mL of double distilled water (ddH2O) in a 500mL labeled glass beaker.
- Pour in a small amount of the mixture back into the test tube to ensure all of the powder is used, gently shake, and pour it back into the beaker.
- Use the test tube to stir the mixture and dispose of it in the garbage.

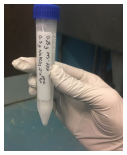

Figure 4. Zebrafish euthanization powder (MS-222) in a plastic test tube.

Swim Tunnel Set Up

- Keep a light flow of water in the aquarium.
- Transport a single zebrafish from one of the three labeled tanks in ¾ of a tank of water in a plastic fish tank.
- Using a fish net, gently guide the single fish from the plastic tank into the tunnel hole.
- Quickly shake the combed end of the swim tunnel to release any air bubbles and push it in, sealing in the zebrafish in the swim tunnel.
- Ensure the tank has air flow and heater to maintain water at approximately 28 °C.
- The flow and speed of the water was 1 BL/s for the acclimation period, and 1.5 BL/s for every step.
- Let fish acclimate for 1.5 hours.
- Increase the speed in 10 minute increments until the fish can no longer swim against the current.

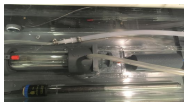

Figure 5. Assembled zebrafish swim tunnel.

Figure 6. The propeller direction must be turned to the left and the acclimating speed should be 160 RPM.

Figure 7. Zebrafish swim tunnel pieces.

Figure 8. Zebrafish swim tunnel propeller box that changes the direction and the speed in RPM.

Results

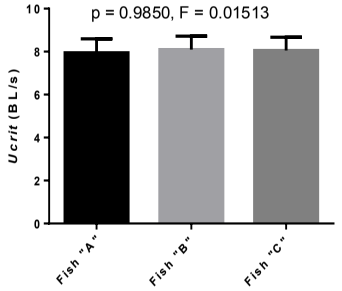

Figure 9. Bar graph used to compare means of treatment groups.

- A 1- Way ANOVA statistical program was used to compare the means of three unrelated and independent treatment groups in regards to a single variable (the CBD treatment group).
- The "p" value indicates the percentage of certainty.
- The "F" value represents the measure of variance.

Conclusion

- There was no significant difference between treatment groups.
- The CBD treatment on the embryos did not affect later stage swim performance.
- Future directions:
  - Analyse the neurology of the fish exposed.
  - Complete fish respirometry measuring Oxygen consumption to determine if metabolism is affected by embryonic CBD exposure.

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