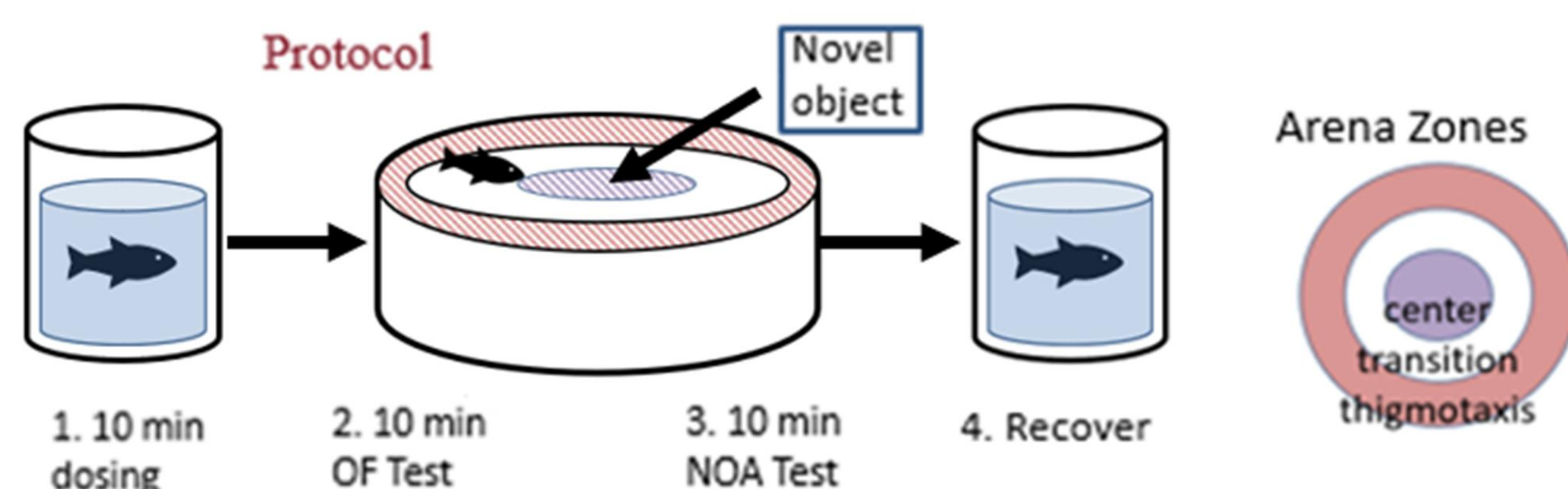


Introduction

In 2016 recreational cannabis use became legal in Canada and this increased ability to investigate cannabinoid products and effects¹. Some compounds present in cannabis substances are terpenes. One of the most common terpenes is pinene, a naturally occurring monoterpene hydrocarbon (C₁₀H₁₆) found in products for both medicinal and personal use². Pinene has different chemical variations such as (-)- α -pinene that research has shown to produce different effects from each other³.

Zebrafish are an excellent model organism; researchers can study their anxious behaviour and boldness using the open field test and novel object approach test⁴. This technique can be used when studying different drugs including other terpenes⁴. This study hypothesized that in both tests (-)- α -pinene will have an impact on zebrafish movement and have them spend more time in the center and transition zones and less time in the thigmotaxis which will show a potential anxiolytic effect (decreasing anxiety while increasing boldness.)

Methods



1. Zebrafish of a wildtype strain bred at MacEwan University were randomly distributed between four experimental groups (~n=15) exposed to either 0% (control), 0.01%, 0.02% or 0.1% concentrations of (-)- α -pinene for 10 minutes.
2. After dosing they were placed in an arena for the open field test for 10 min.
3. Then a colourful LEGO[®] figurine was placed in the center of the arena for the novel object approach test for 10 minutes.
4. Lastly, the zebrafish were placed in a tank to recover and be sexed.

EthoVision software was used to record the distance moved (cm), velocity (cm/s), high mobility (s), immobility (s), meandering (deg/cm) of the fish as well as the time spent (s) in particular zones in the arena divided into the center, transition (middle) or thigmotaxis (outer) zones.

Results

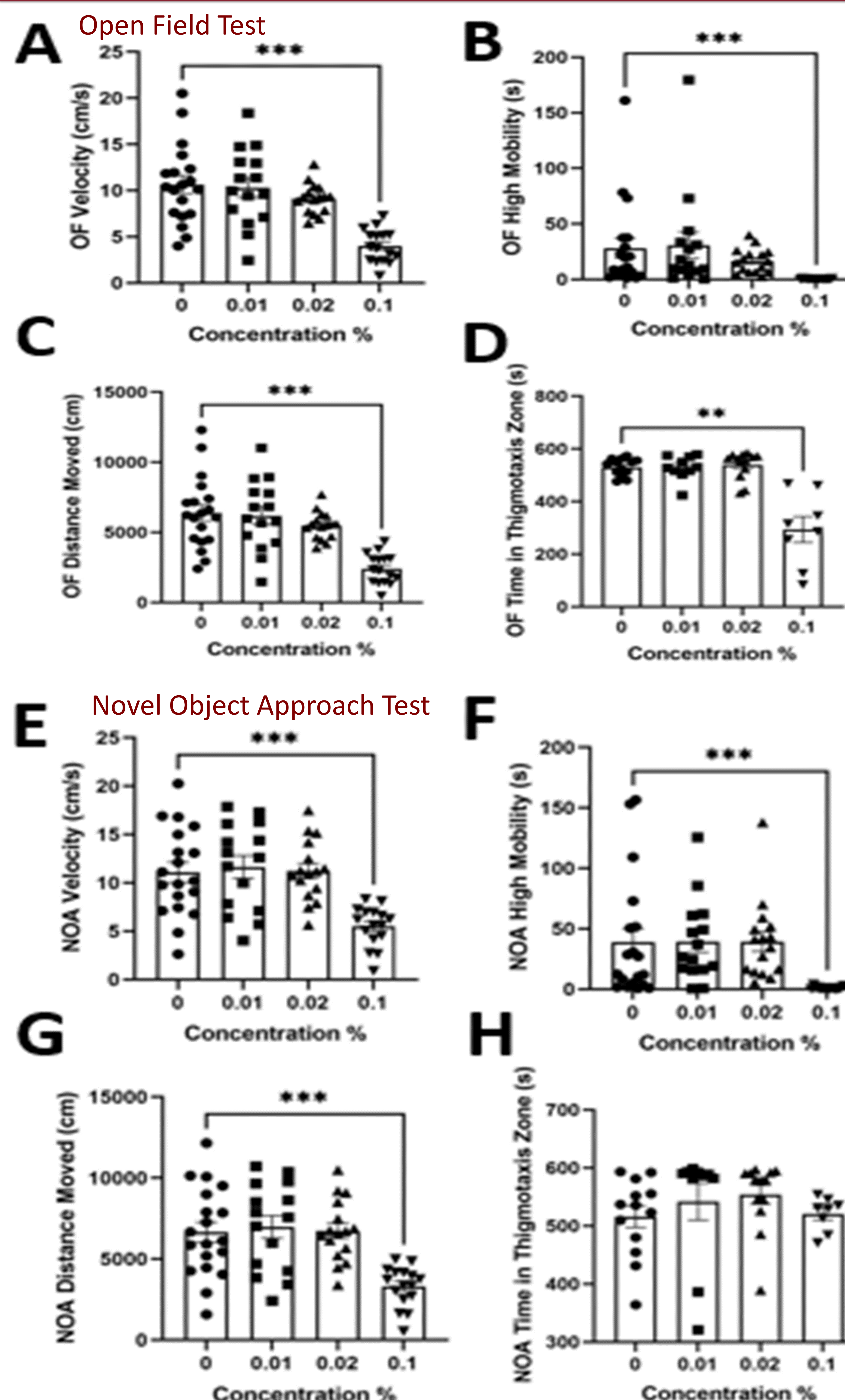
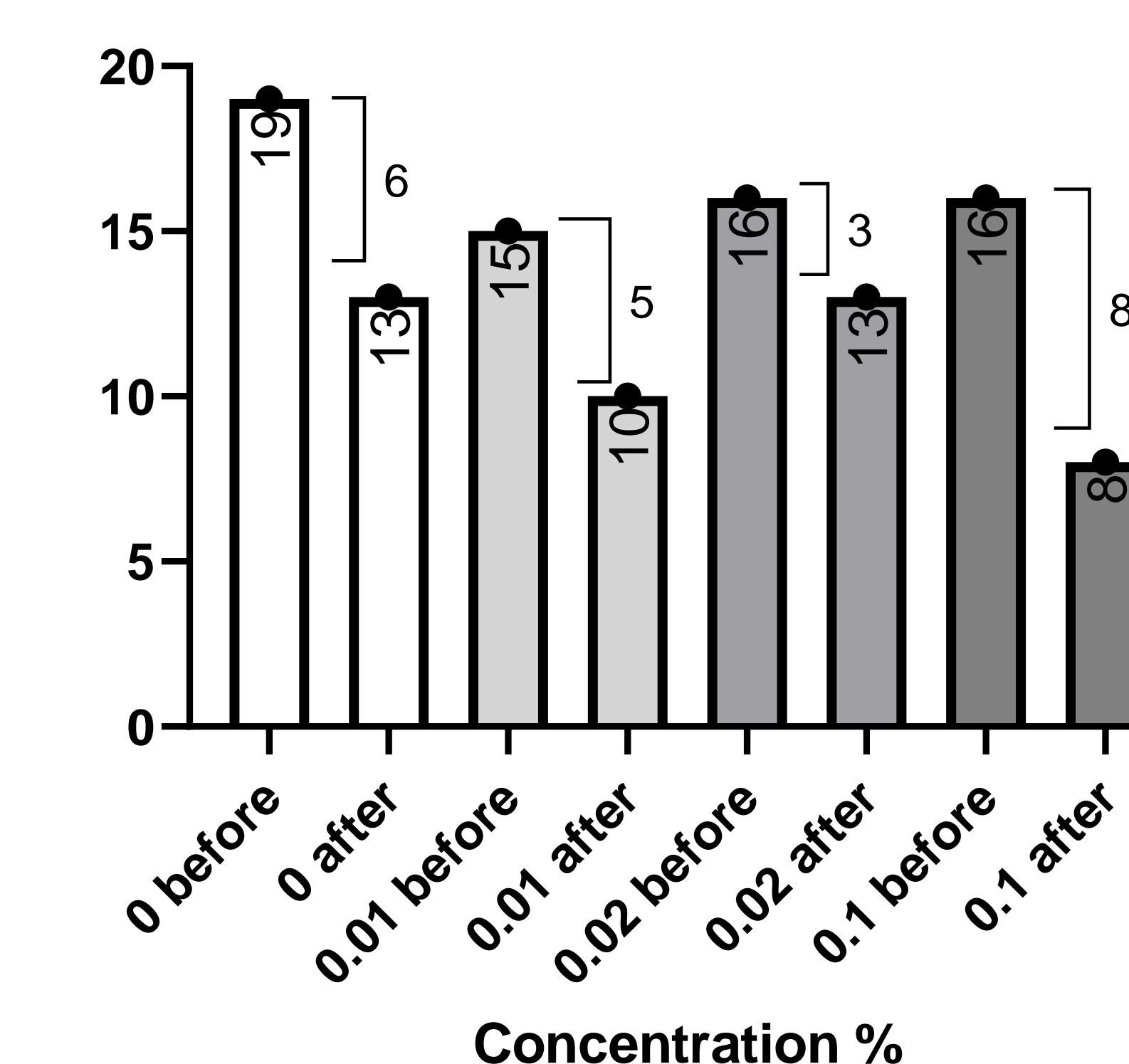


Figure 1. Open Field Test (OF) and Novel Object Approach Test (NOA) Graphs
Open field test results per test group are shown for velocity (A), high mobility (B), Distance moved (C) and time spent in thigmotaxis (D). Novel Object Approach Test results per test group are shown for velocity (E), high mobility (F), Distance moved (G) and time spent in thigmotaxis (H). Fish with >100secs immobility were excluded from Graphs D and H. All data are presented as mean \pm S.E.M. Significant differences between controls and (-)- α -pinene treated groups are indicated ***P<0.01, ****P<0.0001.

Immobility



Conclusions

There is opportunity for further testing and more data analysis to include the immobile fish to look at how it could impact the results (Fig. 2). No significant results were found in meandering for either test, or in the zones for the novel object approach test. Significant differences were seen strongly amongst velocity, high mobility and distance moved among both tests between the control and fish exposed to 0.1% (-)- α -pinene (Fig. 1A- C & E-G). A significant difference was observed in the open field test between the control and 0.1% in the center and transition zone (P < 0.01) and with immobility (P < 0.05). The results suggest that due to the decrease of time spent in the thigmotaxis during the open field test (Fig. D) that 0.1% (-)- α -pinene could have some anxiety reducing potential but may wear off by the novel object approach test or that their boldness is not affected. Overall the results suggest that a higher concentration of (-)- α -pinene has an effect on locomotion decreasing the amount the fish is active. Due to the limits of this study future investigation into other concentrations and terpenes would be valuable.

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