Review of: "Behavioral effects of ethanol in the Red Swamp Crayfish (Procambarus clarkii)"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

Behavioral effects of ethanol on red swamp crayfish (*Procambarus clarkii*). By Gutiérrez et al. (2021). BioRxiv Prepress: https://doi.org/10.1101/2021.06.05.447220

The freshwater crayfish *Procambarus clarkii* has been proven to be a suitable animal model for physiological and toxicological studies of the nervous system. The current study describes the effect of ethanol (EtOH) on the locomotor activity of crayfish in laboratory conditions.

This research found whether EtOH would alter dose-dependent locomotor behavior in juvenile crayfish using immersion time and concentration, measuring distance traveled, speed, and time spent motionlessly.

Following the reading, a few observations were made. The main ones are:

1. About the statistical analysis:

1.1 Do the raw data of the locomotive activity show a normal distribution? If this is the case, the authors should also include these results.

2. As for the results section:

2.1 Authors should clarify whether the effects of EtOH on the locomotor activity of crayfish correspond to 1 hour or only 30 minutes. Because in Experiment 1, they reported that the animals were evaluated in the open field for 30 minutes, and in the first paragraph of Results (Item 3.1), baseline activity and EtOH effects reported locomotor activity corresponding to 1 hour (Fig. 2A).

2.2 Authors should consider including a figure for the temporal course of locomotion. For example, the distance traveled versus time for the group of control animals and animals that received EtOH.

3. On the author's discussion:

3.1 Locomotor activity in basal conditions of crayfish, how is to be compared to other reported studies?

3.2 The significant distance traveled (Fig. 2B), with a lower mean speed in the peripheral region (Fig. 3A). Both, to the central region.

3.3 The concentration of EtOH in the hemolymph of crayfish (Fig. 6) compared to data from other animal models described in other reports.

Finally, some minor observations:

1. In the legend of Fig. 2. What is the meaning of the acronym N.b.?