

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

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The manuscript '**Behavioral effects of ethanol in the Red Swamp Crayfish (*Procambarus clarkii*)**' by Gutierrez et al introduces a new animal model for the study of the effects of alcohol. The authors deserve to be encouraged for the attempt to expand the available tools for alcohol researchers.

General Comments

1. The authors need to be less strict in the housing/care of the crayfish. I had a 350 gallon freshwater tank to house Polkadot Stingray. The odd thing is that I bought one and ended up having three (I unintentionally bought a pregnant female). Before I donated my rays to a zoo, I had two 80 gallon freshwater 'feeder' tanks. Why? Because my rays became depressed if they did not have live food to interact/feed upon. I grew about 30 lbs of red crayfish a month for my rays and jumbo. Reading that you are losing some crayfish during molting, I would just recommend to you to stop treating your crayfish as pristine as you would other lab animals. First, crayfish need dirty water. Crayfish are filter feeders and need bacterium and other micro-organisms from 'natural pond scum' water to be healthy. I would recommended that the authors find the cleanest (no pollution), biological active water in the area to use as the water source for the crayfish. You need to remember, red crayfish love swamps. Also, you need to give some vegetation to the crayfish. They need algae and aquatic plants. If you can find a pond with decaying leaves, pick out the leaves and your crayfish will love eating the grime off the leaves. We always want to make our test subjects the happiest we can (it will generate better data). On special days, give you crayfish Crisco. When I was in Louisiana, the best bait for crayfish was Crisco. To recap, crayfish will eat anything and they need to eat a vary diet to be healthy. Crayfish will fight over discarded submarine sandwiches. Don't be too formal with your subjects, they will live better if they are able to be what they are in the wild.
2. The authors need to consider a much wider usage of red crayfish in research and consider the impact their data would have in other fields. Ecological Pharmacology (the study of drugs of abuse in water ways) could use the behavior alterations described in the current publication (also the parametric assessment of HEC). I would consider included a description of how your data could influence the use of crayfish as sentinel animals for the environment.

Specific Comments for the Manuscript

1. The stats need to be rewritten. If there is a significant interaction term, all single factor analysis is uninterpretable. If

there is a significant interaction term, talking about single between subject factor analysis is pointless. Please read Kepple and Zeddeck, Hayes, or Pedhauser for a clear indication that significant interactions terms preclude the discussion of other factors.

2. Can you indicate somewhere the HEC levels (numeric values) in the crayfish? It would be good if the actual level is available to people. It would provide evidence if there is a linear relationship between the EtOH bath and HEC. It would help future research.
3. Figures 3-5 are messed up. It seems like the authors cropped the figures and some data was cut from B and D.
4. Figures 9 and 10, please have increase the bar size so that the bars touch. It greatly helps with visual assessing the data.
5. Describe the crayfish brain. There is data indicated the brain regions regulating LMA behavior in the crayfish. The authors are encouraged to learn about the brain of the crayfish and understanding the regulatory network of observed behaviors.

References that should be added to the manuscript. There are many crayfish manuscripts that I would recommend to the authors to consider to include in the manuscript.

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From crayfish to humans An evolutionary perspective of addiction<https://doi.org/10.7203/metode.12.18398>

Exposure to a common antidepressant alters crayfish behavior and has potential subsequent ecosystem

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<https://doi.org/10.1002/ecs2.3527>

<https://www.nationalgeographic.com/animals/article/antidepressant-pollution-in-water-may-make-crayfish-bolder>