

Introduction

- Neuropeptide secretoneurin (SN), derived from the precursor protein secretogranin-II (Sg-II), stimulates luteinizing hormone (LH) release from goldfish and mice pituitary^[1,2,3].
- Decreased spawning success and courtship behaviours are observed in Sg-II AB knockout (KO) zebrafish, possibly due to altered LH release.
- Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a pheromone, controlled by LH, which induces spawning ovulatory behaviours in females and reproductive behaviours in males when released into water by females^[4].

Questions

1. Can Sg-II AB knockout (AB-KO) males perceive $PGF_{2\alpha}$?
2. Do pheromones in spawning water of WT-M x WT-F pairs influence contact frequency, contact duration and/or breeding of WT-M x WT-F, WT-M x AB-KO F and AB-KO M x AB-KO F pairs?

Methodology

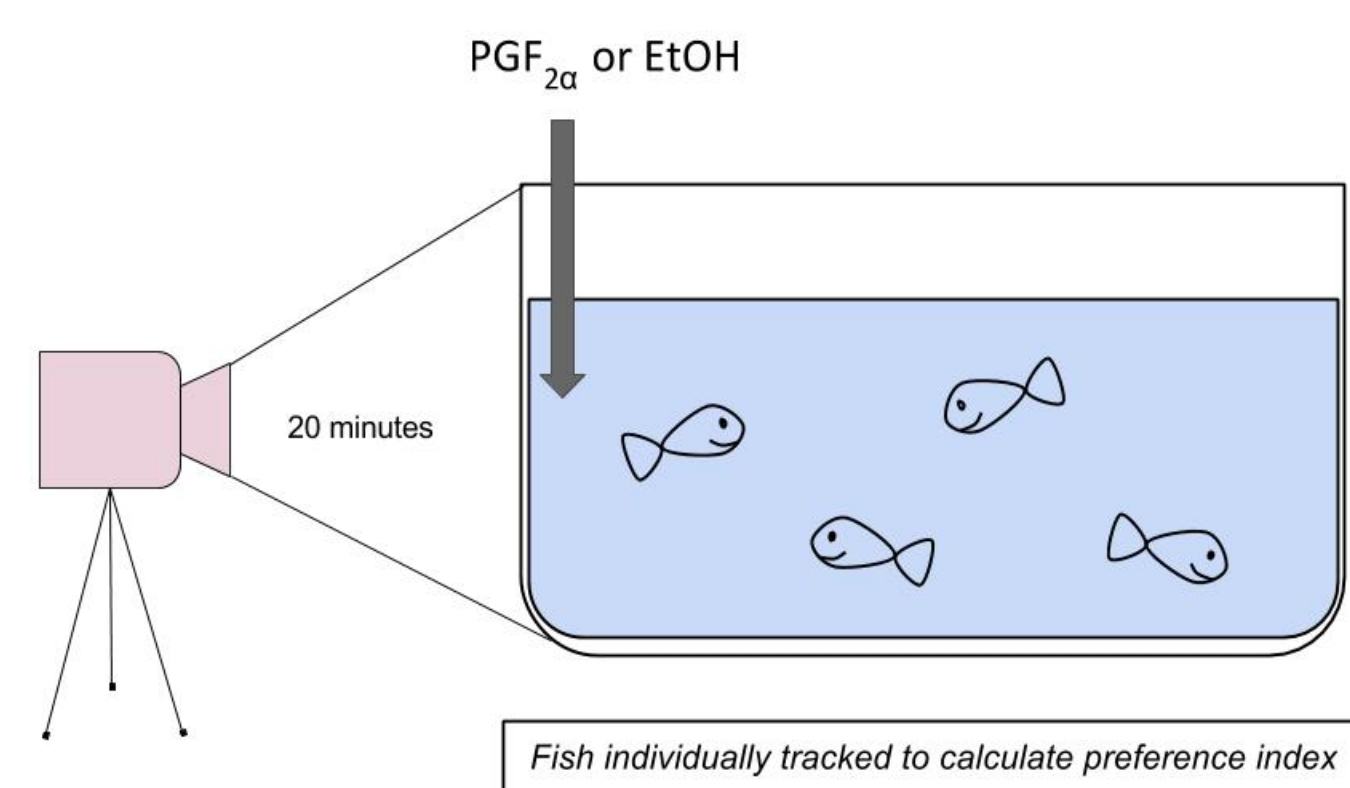


Figure 1. Experiment 1 setup. Four male fish (WT or AB-KO) were placed in a 6L tank. 600 μ L of 10^{-5} M $PGF_{2\alpha}$ was injected into the water through a silicone tube on the left side of the tank. The fish were recorded using viewpoint software for 10 minutes pre and post $PGF_{2\alpha}$ or EtOH (control) exposure. (n=24)

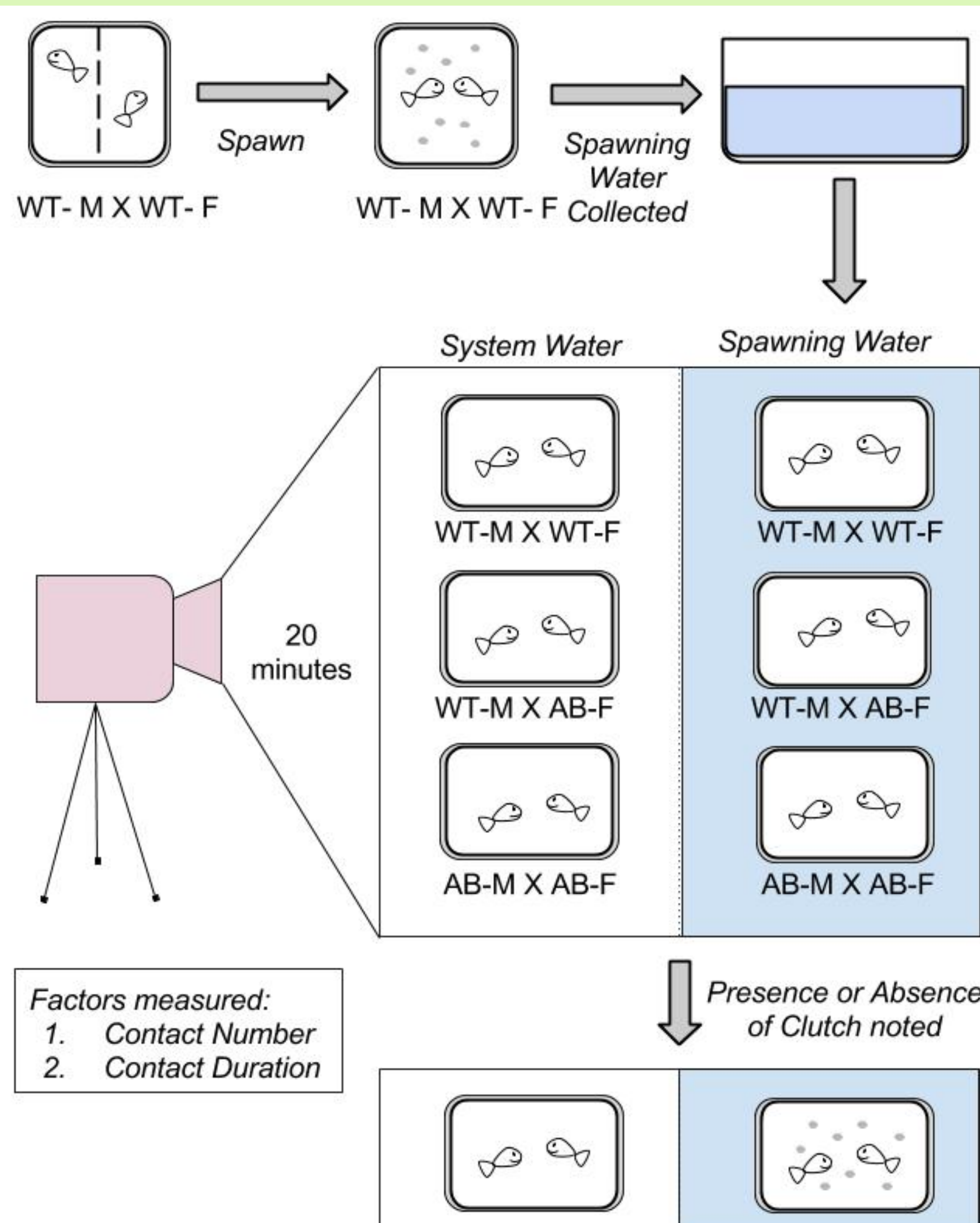


Figure 2. Experiment 2 setup. Male and female fish were paired into their respective crosses in 0.5L breeding tanks overnight, separated by a divider. WT breeding pairs were bred in the morning for collection of spawning water. The pairwise crosses were then subjected to breeding in either control (system) water or spawning water and were recorded for 20 minutes to observe courtship behaviours.

Results

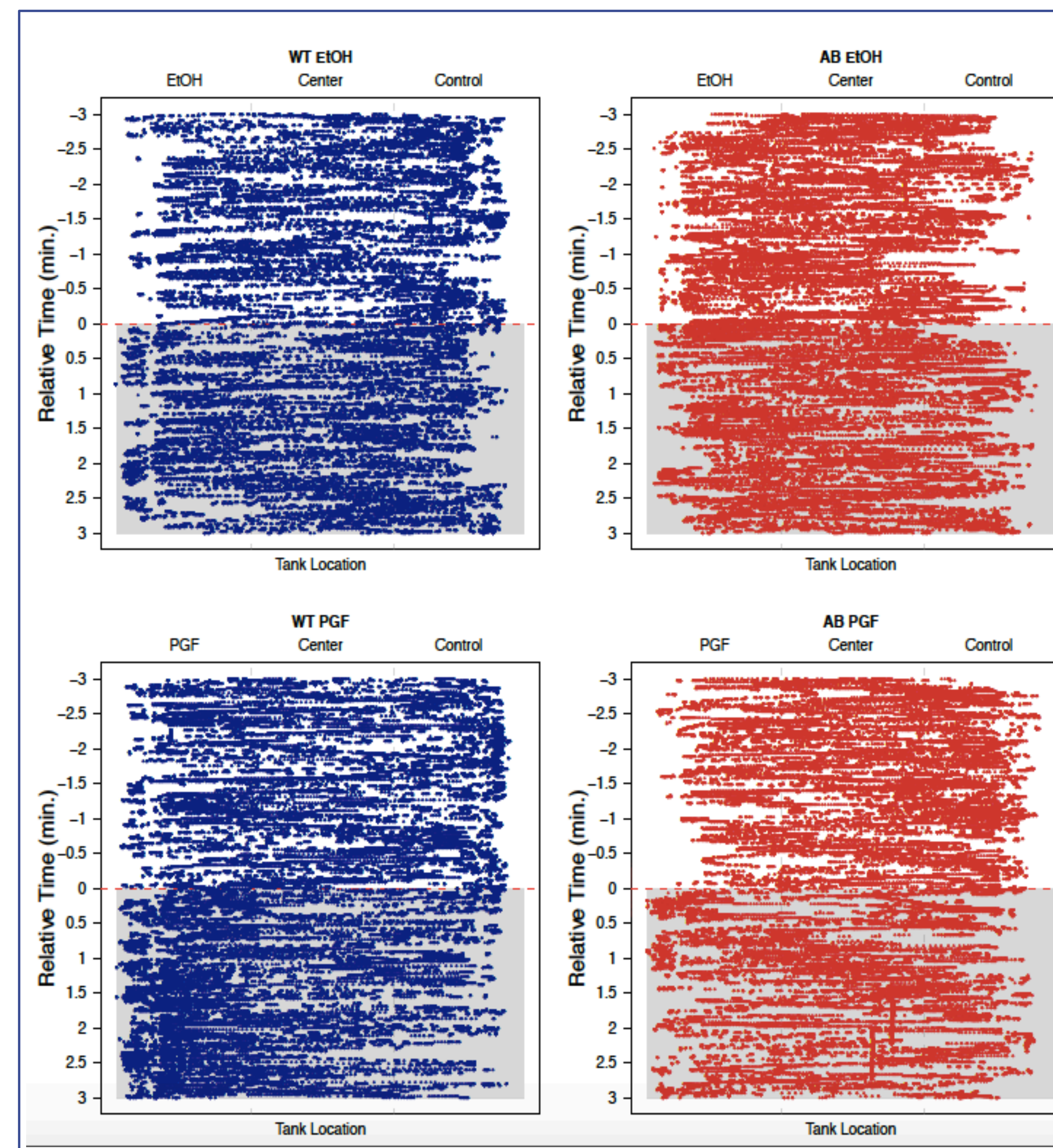


Figure 3. Experiment 1. Location coordinates of the 24 male WT or AB-KO fish in the tank 3 minutes pre- and post- $PGF_{2\alpha}$ or EtOH (control) exposure (n=24). Plots were generated using R.

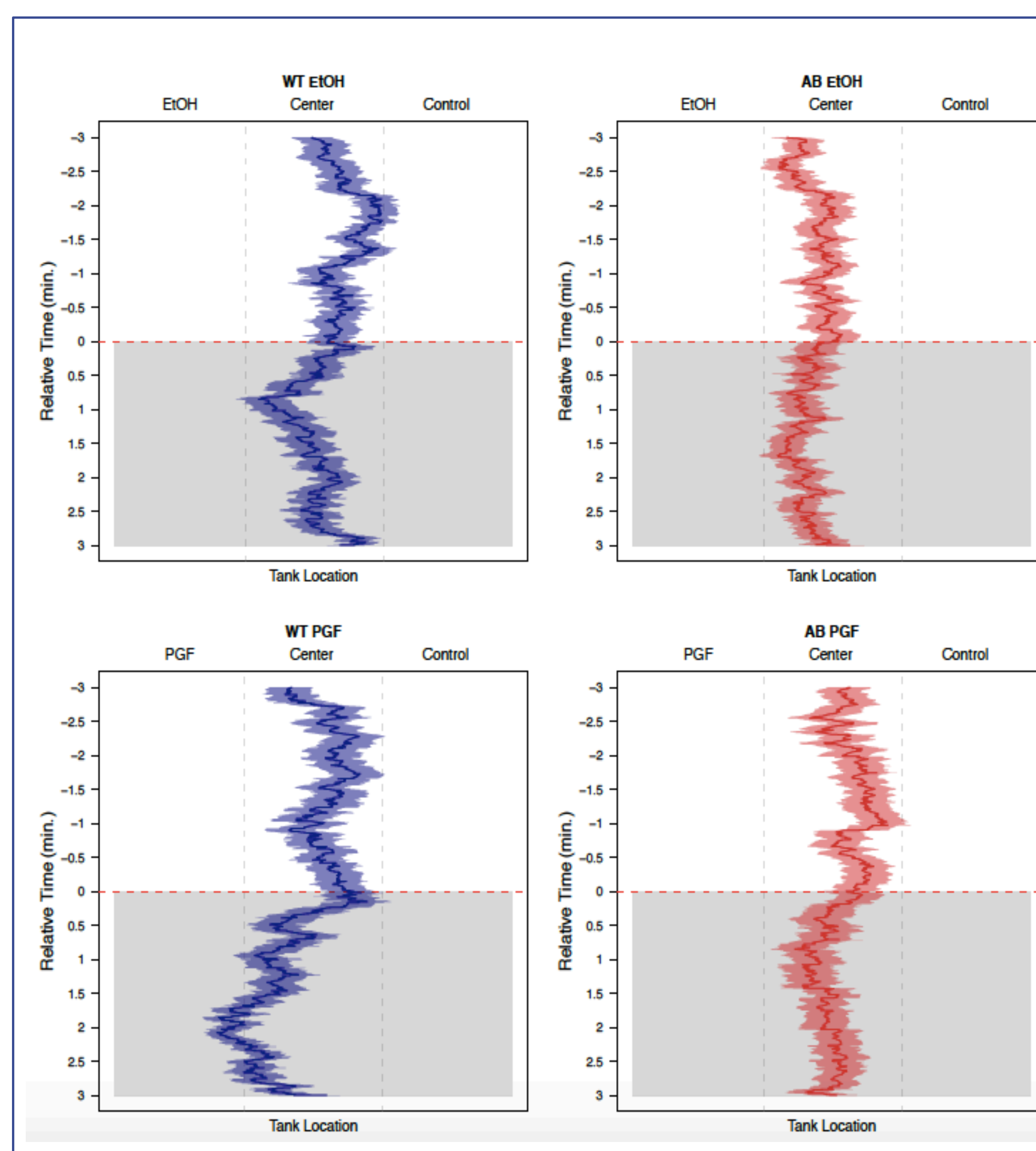


Figure 4. Experiment 1. Mean positions (solid line) and s.e.m. (shading) of the 24 male WT or AB-KO fish in the tank 3 minutes pre- and post- (grey shaded area) - $PGF_{2\alpha}$ or EtOH (control) exposure (n=24). Plots were generated using R.

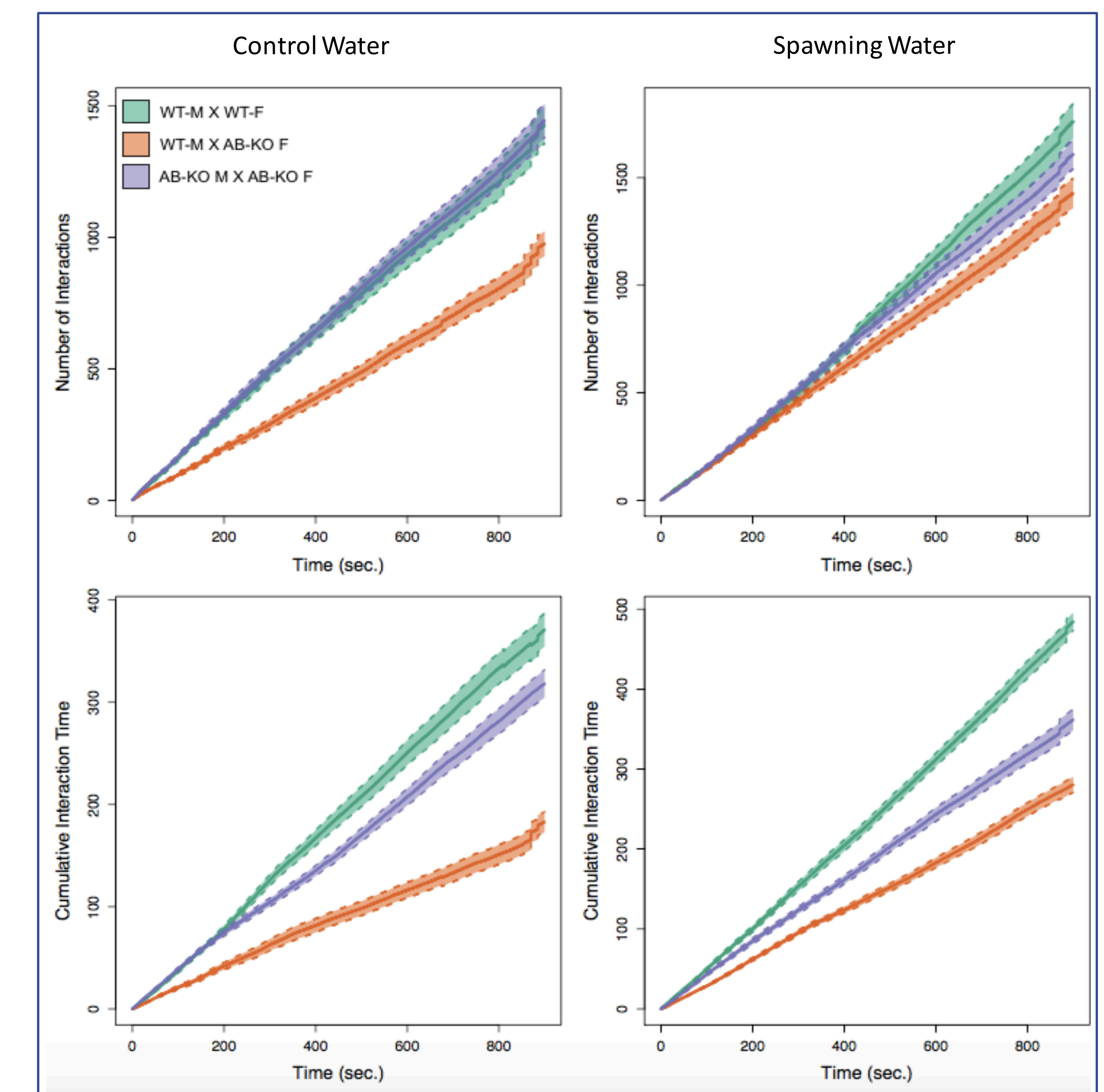


Figure 5. Experiment 2. Number of interactions (A,B) and duration of interactions (C,D) of different pairwise crosses exposed to system water or spawning water vs. time (n=32). Data shown represents the first 15 minutes of video recorded breedings using ViewPoint software and camera. Plots were generated using R.

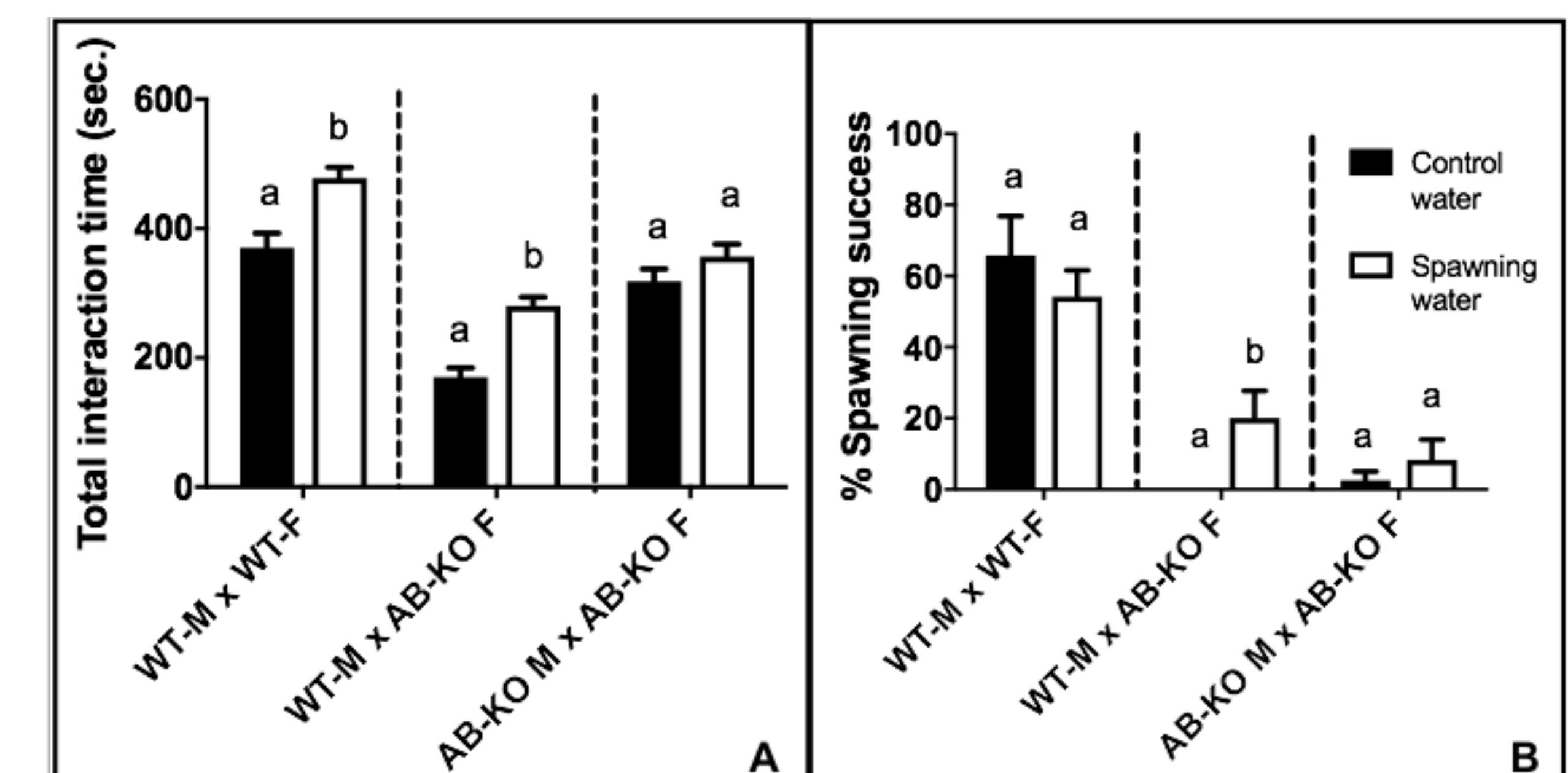


Figure 6. Experiment 2. Spawning success and total interaction time of different pairwise crosses in spawning water vs. control water (n=32). Successful spawning is indicated by the presence of a clutch after 1.5 hours of breeding. Statistical significance is determined using Kruskal-Wallis test (A) and Fischer's exact test (B). Different letters denote statistical significance in breeding between system and spawning water in each respective within line and reciprocal cross.

Conclusion

Experiment 1

- WT male fish preferentially swim towards $PGF_{2\alpha}$ when it is introduced into the tank whereas AB-KO males do not, indicating that perception of $PGF_{2\alpha}$ is lower in AB-KO males.

Experiment 2

- There was a statistically significant increase in spawning success in WT-M x AB-KO F pairs in spawning water as compared to system water, despite their low total interaction time.
- The spawning success of AB-KO M x AB-KO F pairs is significantly lower than WT-M x WT-F pairs in both system water and spawning water, despite having similar total interaction times.
- Further tests need to be done in order to establish a clear relation between interaction time and spawning success.

References

[1] Zhao, E., Basak, A., Wong, A. O. L., Ko, W., Chen, A., Lopez, G. C., Grey, C. L., Canosa, L. F., Somoza, G. M., Chang, J. P. et al. (2009). The Secretogranin II-Derived Peptide Secretoneurin Stimulates Luteinizing Hormone Secretion from Gonadotrophs. *Endocrinology* 150, 2273-2282.

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Acknowledgements

I would like to extend my sincerest thank you to Kimberly Mitchell for her instruction, guidance, patience and hard-work throughout the entirety of this project. A special thanks to Jonathan Dench for his help with the data analysis, without which this project would not have reached completion. Last but not least, thank you Dr. Trudeau for welcoming me into your lab. I am truly thankful to have received this learning opportunity.