The Impacts of Waterborne SSRI and NDRI Exposure on Gulf Toadfish, *Opsanus beta*, Hypoxia Response

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Introduction

- Anxiety and depression are on the rise[1] and antidepressant sales have steadily increased[2]
- Antidepressants enter waterways through poor management of wastewater treatment facilities, agricultural runoff and groundwater contamination[3]
- Common antidepressants include Prozac (active ingredient= fluoxetine: FLX)[4], that inhibits serotonin (5-HT) transporter (SERT), and Wellbutrin (active ingredient= bupropion: BUP) that inhibits the dopamine transporter (DAT), and the norepinephrine transporter (NET)[5] but can also impact 5-HT uptake
- Environmental levels: 0.01 $\mu g \cdot L^{-1}$ FLX and 0.05 $\mu g \cdot L^{-1}$ BUP[6]
- Oxygen (O_2) sensing involves 5-HT[4] ; disruptions in O_2 sensing could impact hypoxia tolerance

Hypotheses

***Series i*:** Chronic exposure of Gulf toadfish to FLX and BUP will decrease reuptake of 5-HT and consequently attenuate the hypoxia response.
***Series ii*:** LOE times will be higher during the second exposure to hypoxia than the first because mechanisms relating to hypoxia will be upregulated

Methods

Series i:

- Fish (n=8) exposed for 5-6 weeks
  - Control: 0 $\mu g \cdot L^{-1}$ FLX or BUP
  - Low: nominal concentrations of 0.1 $\mu g \cdot L^{-1}$ FLX and 0.5 $\mu g \cdot L^{-1}$ BUP
  - High: nominal concentrations of 10 $\mu g \cdot L^{-1}$ FLX and 50 $\mu g \cdot L^{-1}$ BUP
- Loss of equilibrium (LOE) tests conducted

Series ii:

- LOE tests (n = 6 fish) were conducted using a revised, more consistent approach
- Time to LOE was tested again 7-11 days later on the same individuals

Results

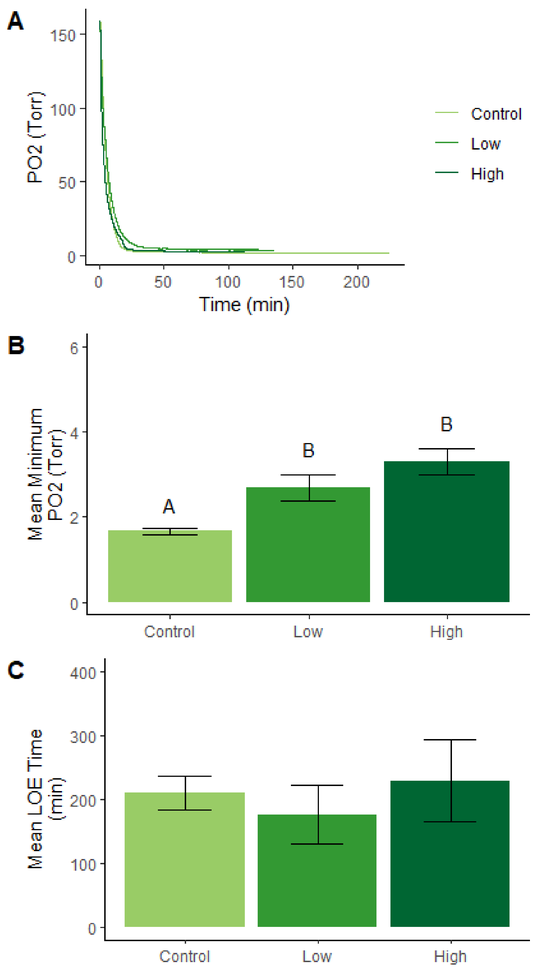

Fig 1: (A) Partial pressure of oxygen (PO_2: Torr) over time for each exposure, (B) the mean minimum PO_2 (Torr) experienced by fish from each exposure (n = 2 LOE trials per exposure); different letters indicate statistically significant differences among means ($p < 0.05$). (C) Mean LOE times (min) for fish from exposure (n=8 fish per exposure). Values are means ± SEM.

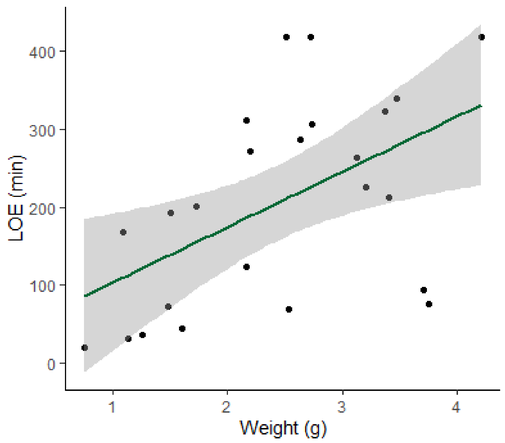

Fig 2: Weight (g) and LOE (min) of all fish in *Series i* with a linear trendline and a shaded region representing a 95% confidence interval.

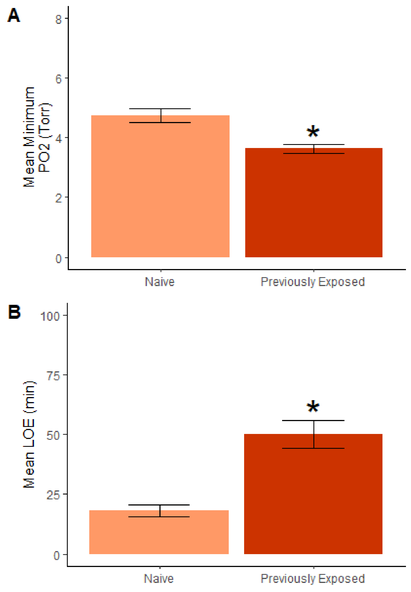

Fig 3: (A) The mean minimum PO_2 (Torr) experienced by fish the first time being exposed to hypoxia (naïve) and the second time being exposed to hypoxia (previously exposed) in Series ii; *$p < 0.05$ compared to naïve fish. (B) Mean LOE time (min) for naïve and previously exposed. Values are means ± SEM; *$p < 0.05$ compared to naïve fish.

Discussion

- ***Series i*:** Our hypothesis was not supported as there was no effect of FLX and BUP treatment on LOE time but there was an impact of weight
- *Series i* results were consistent with other studies
  - In a previous study, waterborne exposure to FLX alone did not impair hypoxia tolerance, but there was increased 5-HT in the plasma that could densensitize 5-HT receptors[4]
- Increased weight resulted in a higher LOE time because larger fish require less O_2 per unit size than smaller fish. Since smaller fish have a higher mass-specific metabolism, they run out of glycogen faster[7]
- Confidence in the O_2 probe was low, which led to variable LOE times and the need for *Series ii*
- ***Series ii*:** Our hypothesis was supported as fish exposed to hypoxia for the first time had lower LOE times than their second exposure
- Constant inflowing nitrogen and 3 O_2 probes were used to increase confidence in O_2 levels, this resulted in lower, more consistent LOE times.
- Slight but significant differences in O_2 levels in *Series ii* oppose effects of treatment (i.e., previously exposed fish were subjected to harsher hypoxia than naïve fish but had higher LOE times)
- Toadfish could be upregulating genes associated with hypoxia tolerance after their first exposure
  - Upregulation of myoglobin and monocarboxylate transporter (transports lactate) have been found in zebrafish[8]
  - Upregulation of anaerobic metabolism and downregulation of cell growth were found in goby fish[9]
- There is likely a hypoxia tolerance threshold for toadfish which is lower than for other fish but can be reached if O_2 levels are low enough
  - Exposure to O_2 levels below this threshold increases tolerance for the next exposure

References

1. Goodwin, R. D., Weinberger, A. H., Kim, J. H., Wu, M., & Galea, S. (2020). Trends in anxiety among adults in the United States, 2008–2018: Rapid increases among young adults. *Journal of Psychiatric Research, 130*, 441-446. doi:https://doi.org/10.1016/j.jpsychires.2020.08.014
2. Aitken, M., Kleinrock, M., Pennente, K., Lyle, J., Nass, D., & Caskey, L. (2016). Medicines use and spending in the US: a review of 2016 and outlook to 2021. *Parsippany: IMS Institute for Healthcare Informatics*.
3. McDonald, M. D. (2017). An AOP analysis of selective serotonin reuptake inhibitors (SSRIs) for fish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 197*, 19-31. doi:https://doi.org/10.1016/j.cbpc.2017.03.007
4. Amador, M. H. B., Schauer, K. L., & McDonald, M. D. (2018). Does fluoxetine exposure affect hypoxia tolerance in the Gulf toadfish, Opsanus beta? *Aquat Toxicol, 199*, 55-64. doi:10.1016/j.aquatox.2018.03.023
5. Stahl, S. M., Pradko, J. F., Haight, B. R., Modell, J. G., Rockett, C. B., & Learned-Coughlin, S. (2004). A Review of the Neuropharmacology of Bupropion, a Dual Norepinephrine and Dopamine Reuptake Inhibitor. *Prim Care Companion J Clin Psychiatry, 6*(4), 159-166. doi:10.4088/pcc.v06n0403
6. Schultz, M. M., & Furlong, E. T. (2008). Trace analysis of antidepressant pharmaceuticals and their select degradates in aquatic matrixes by LC/ESI/MS/MS. *Anal Chem, 80*(5), 1756-1762. doi:10.1021/ac702154e
7. Eric, R. F. (2016). Critical Dissolved Oxygen Tolerances of Whirling Disease-Resistant Rainbow Trout. *North American journal of aquaculture, v. 78*(no. 4), pp. 366-373-2016 v.2078 no.2014. doi:10.1080/15222055.2016.1201556
8. Van der Meer, D. L. M., Van den Thillart, G. E. E. J. M., Witte, F., De Bakker, M. A. G., Besser, J., Richardson, M. K., . . . Bagowski, C. P. (2005). Gene expression profiling of the long-term adaptive response to hypoxia in the gills of adult zebrafish. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 289*(5), R1512-R1519. doi:10.1152/ajpregu.00089.2005
9. Gracey Andrew, Y., Troll Joshua, V., & Somero George, N. (2001). Hypoxia-induced gene expression profiling in the euryoxic fish Gillichthys mirabilis. *Proceedings of the National Academy of Sciences, 98*(4), 1993-1998. doi:10.1073/pnas.98.4.1993

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