Bees Out of Breath: Hypoxia and Insulin Signaling in *Megachile rotundata*

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Introduction

Megachile rotundata are the most commercially managed solitary bee species[1]. These bees undergo diapause as prepupae[2] to survive the winter months. Growth and development are halted, and metabolism is maintained low. Overwintering is a non-feeding stage for these bees, leaving them with limited resources to survive. The insulin signaling pathway is known to be involved in regulating these physiological processes[3] and important for allocating energy resources. Overwintering storage conditions can lead to hypoxic microenvironments for the bees. With limited resources, it is unknown if the insulin signaling pathway changes in response to hypoxic conditions.

Question

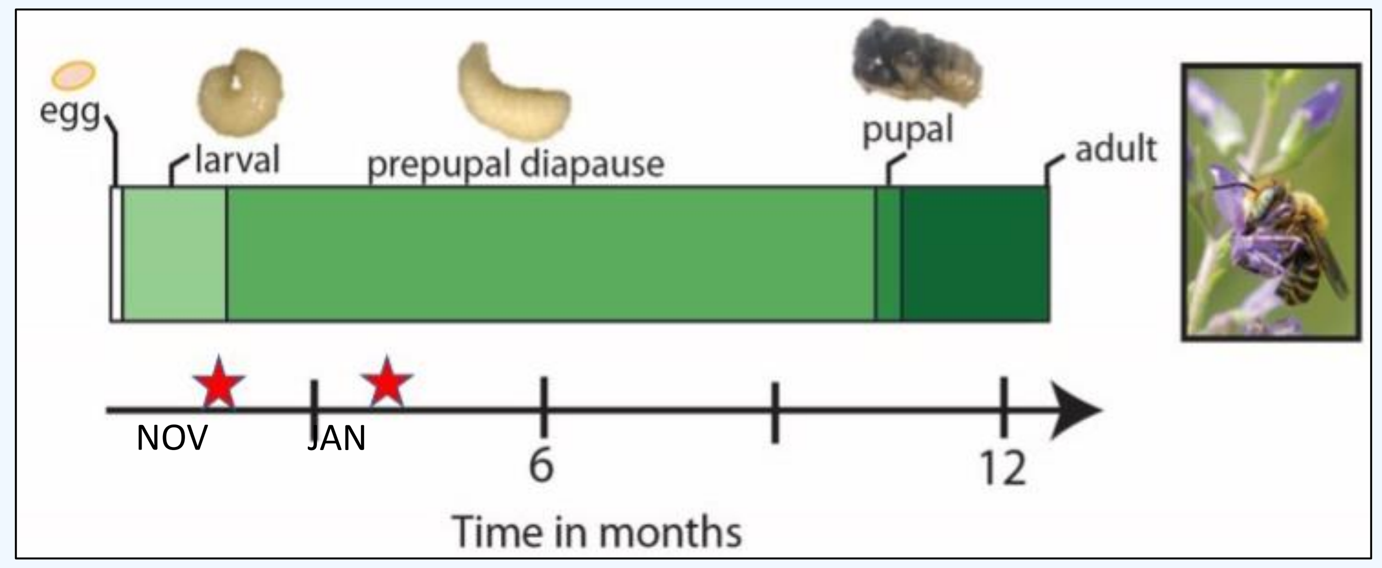
How does gene expression of the insulin pathway change in response to environmental stressors during overwintering?

Hypothesis

Throughout overwintering, gene expression of the insulin pathway varies between hypoxic and normoxic conditions.

Methods

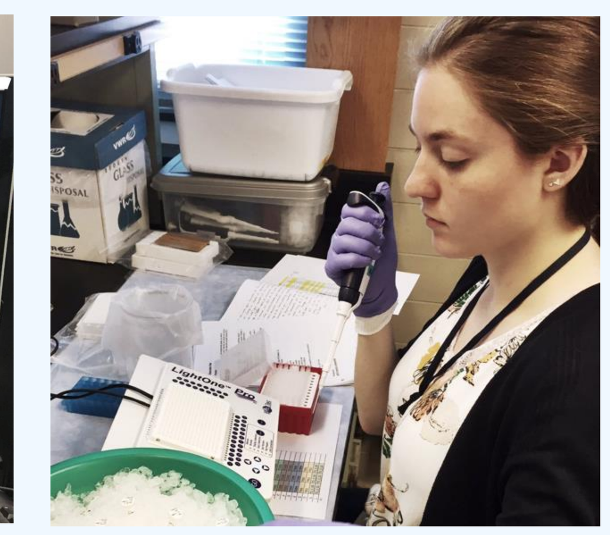
Sample Collecting



Target Genes

	Target Gene 1			Target Gene 2			Target Gene 3			Target Gene 4		
	1	2	3	4	5	6	7	8	9	10	11	12
A	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1	M2_10%_1
B	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2	M2_10%_2
C	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3	M2_10%_3
D	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1	M2_21%_1
E	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2	M2_21%_2
F	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3	M2_21%_3
G	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1	M4_10%_1
H	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2	M4_10%_2
I	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3	M4_10%_3
J	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1	M4_21%_1
K	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2	M4_21%_2
L	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3	M4_21%_3
M	H2O	H2O	H2O	H2O	H2O	H2O	H2O	H2O	H2O	H2O	H2O	H2O
N	CAL. C7S1	CAL. C7S1	CAL. C7S1									
O	CAL. C1S1	CAL. C1S1	CAL. C1S1									
P	CAL. C5S2	CAL. C5S2	CAL. C5S2									

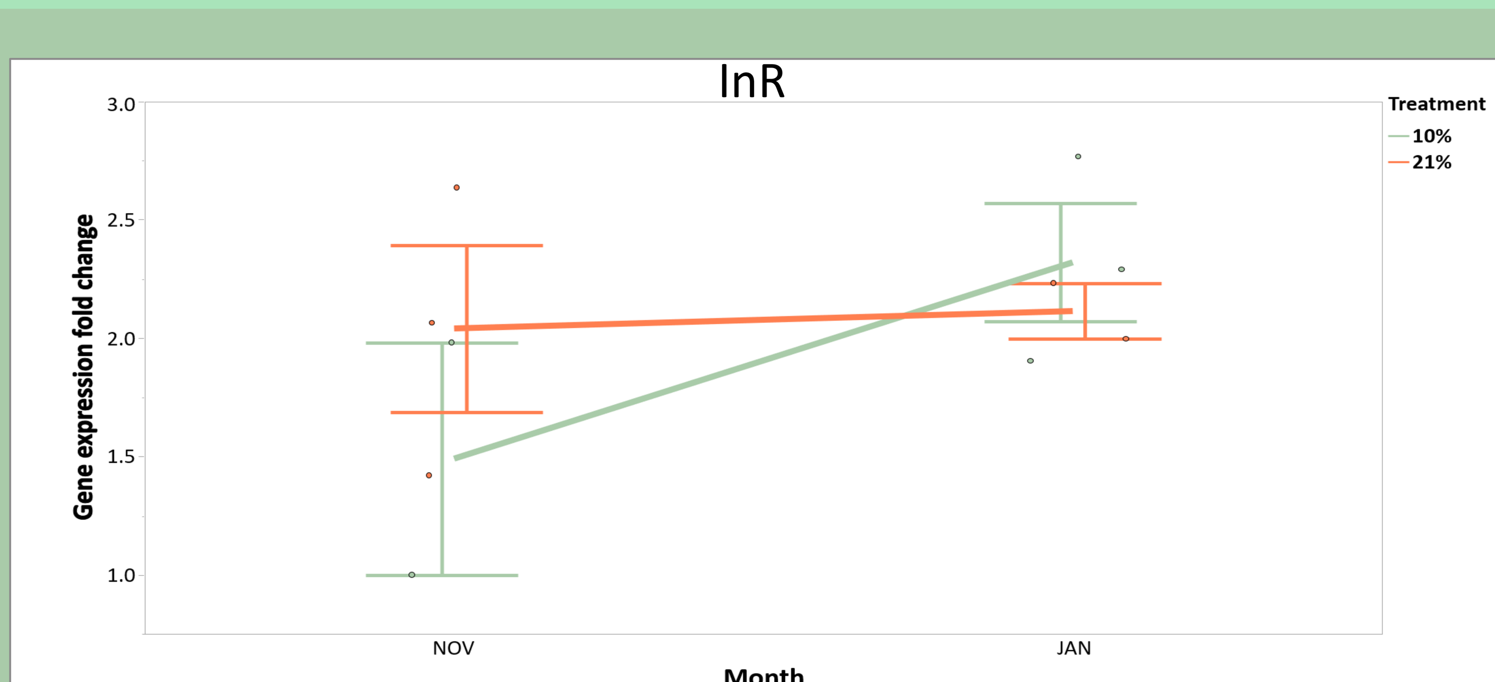
qPCR



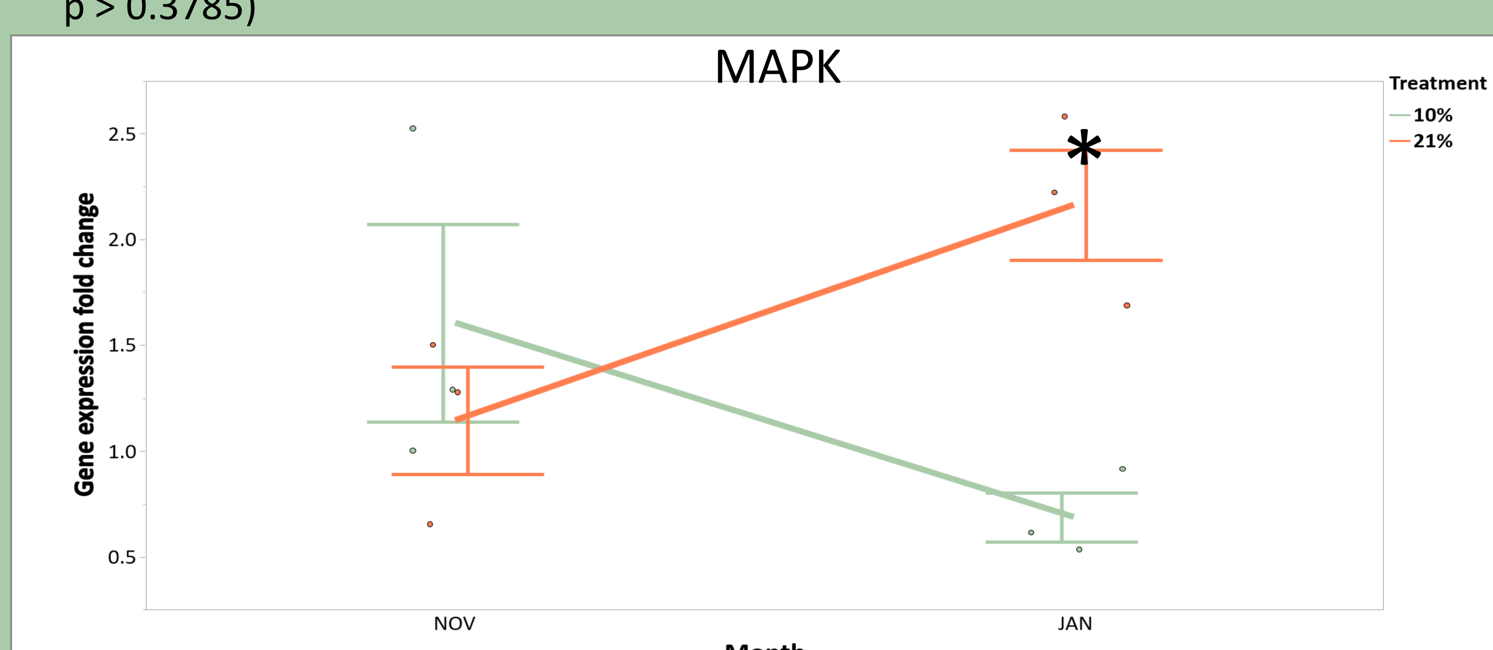
Statistical Analysis

Hypoxia vs. Normoxia
November vs. January

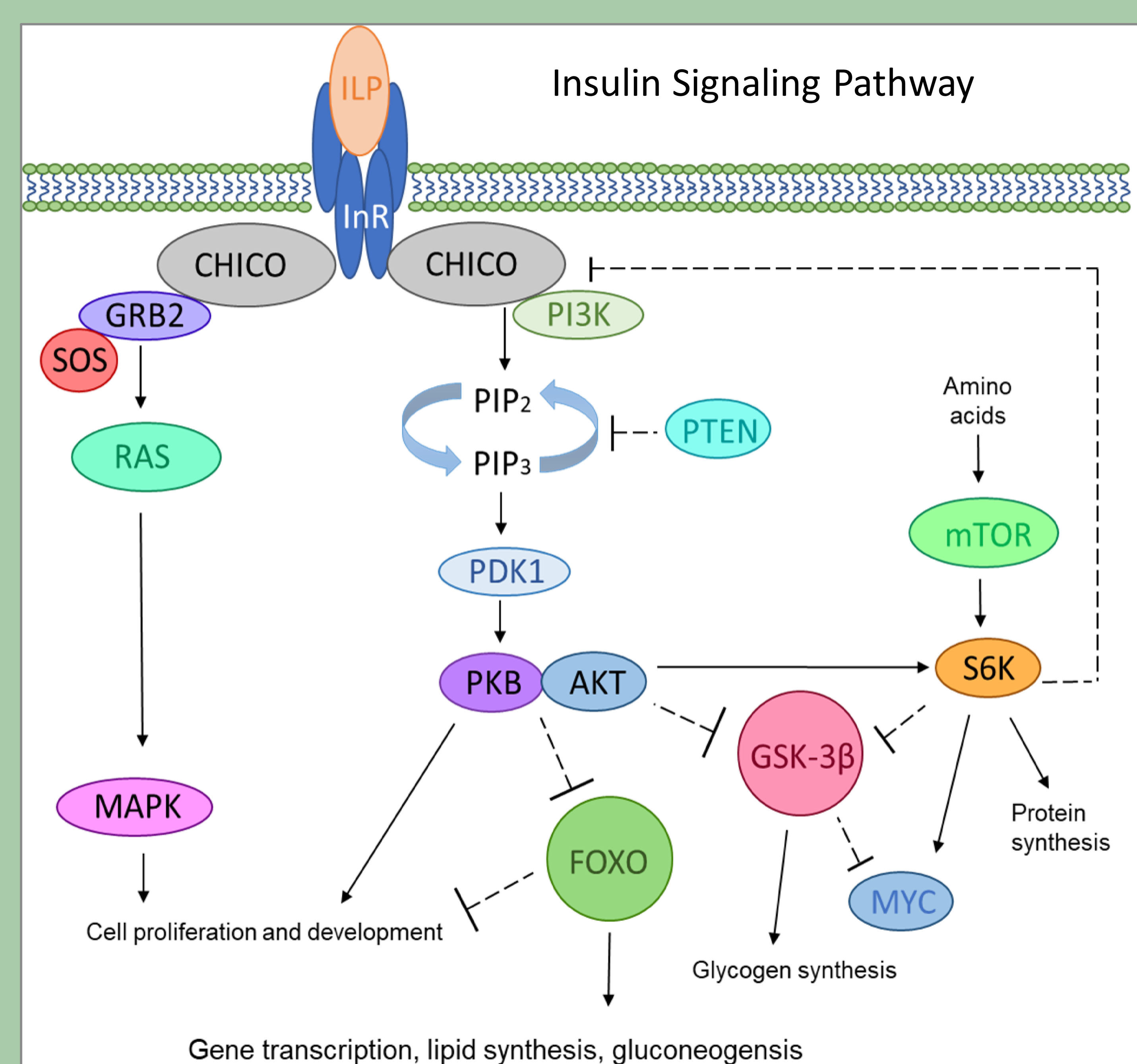
Results

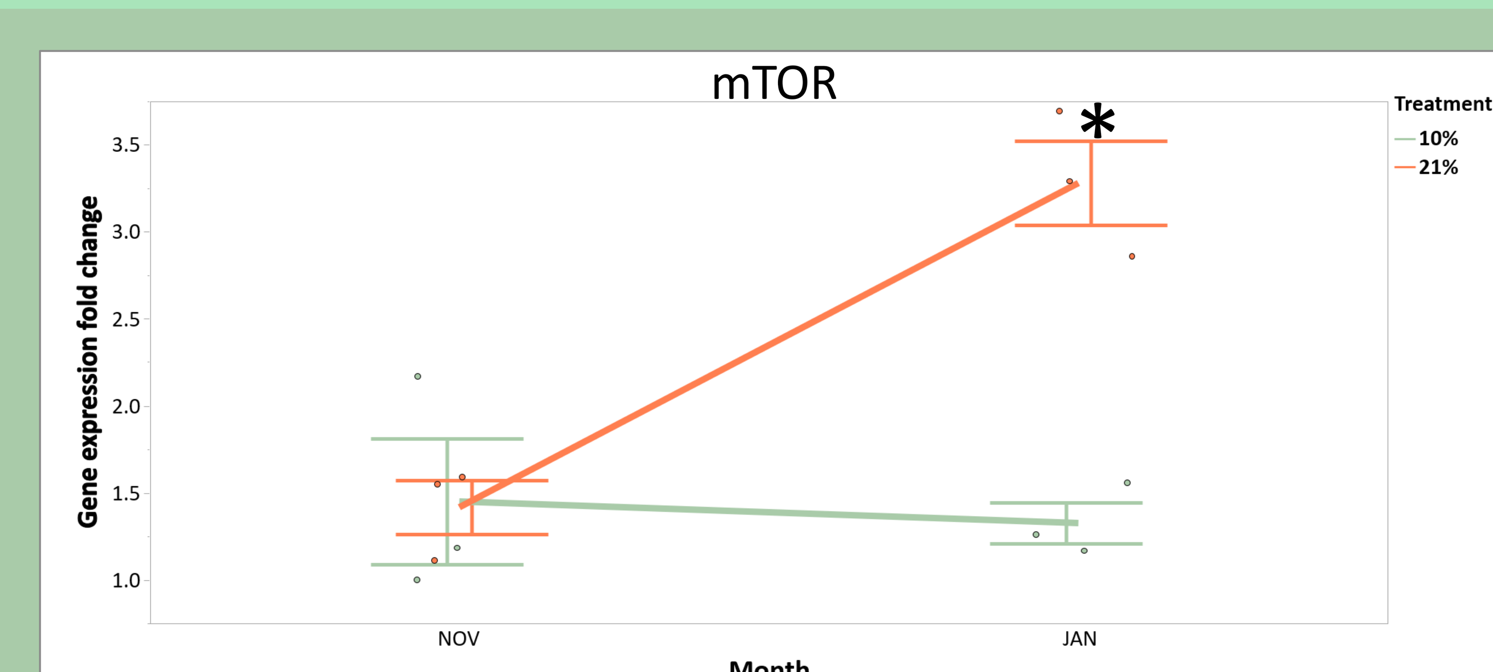


Gene expression for the insulin receptor was not statistically significant between treatments or over time. (Treatment: Z = -0.24019, $p > 0.8102$; Month: Z = 0.8870, $p > 0.3785$)

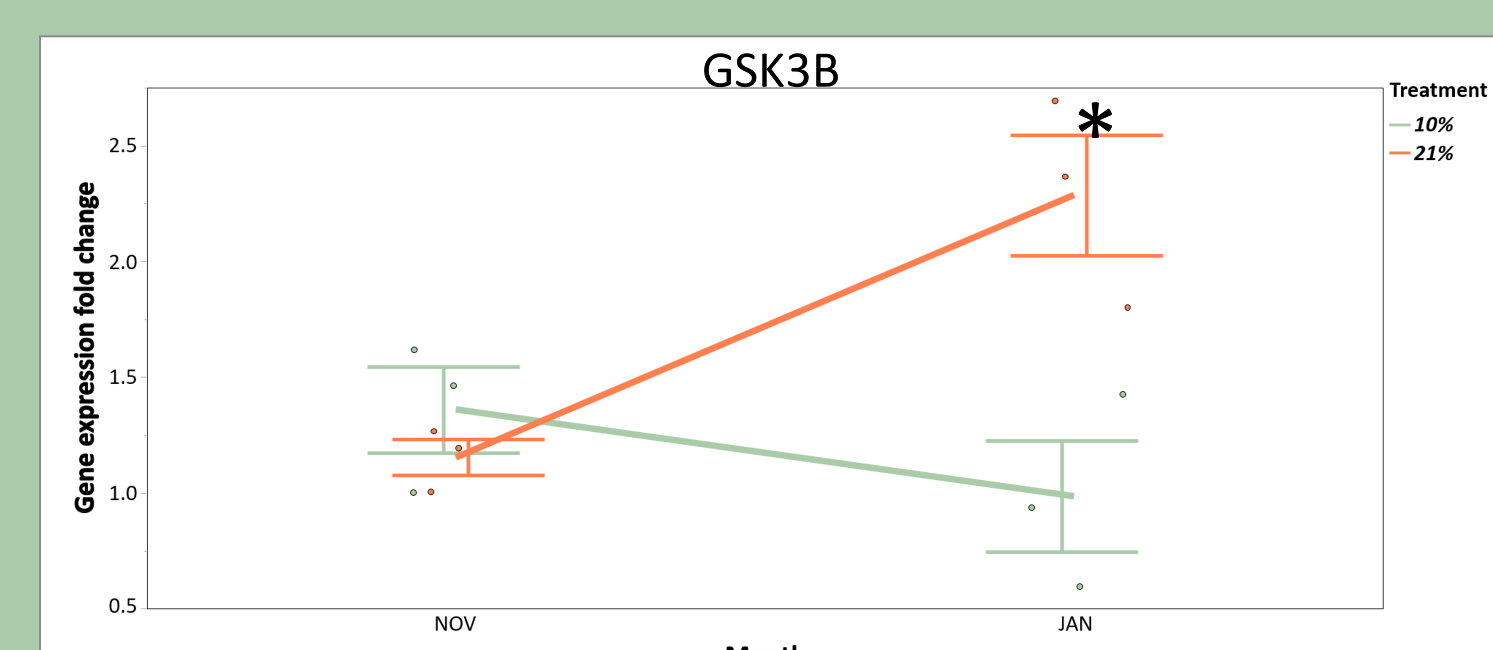


Gene expression for MAP kinase was not statistically significant over time, but was significant between treatments in January ($\chi^2 = 3.8571$, $p < 0.05$).





Gene expression for mammalian target of rapamycin was not statistically significant over time, but was significant between treatments in January ($\chi^2 = 3.8571$, $p < 0.05$).



Gene expression for glycogen synthase kinase 3 beta was not statistically significant over time, but was significant between treatments in January ($\chi^2 = 3.8571$, $p < 0.05$).

Conclusions

- Overall, gene expression was not statistically significantly different over time.
- In January, expression of many genes was higher in normoxic treatment groups than in hypoxic treatment groups.
- Bees enter post-diapause quiescence in January, which could be why a difference can be seen in January between treatments but not November.

Future Directions

- Increase sample size
- Measure gene expression in adult bees
- Include more months in the study
- Test different oxygen concentrations
- Measure proteins

Acknowledgements and Citations

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1. Abdelrahman, H. *et. al.* 2014. Journal of Insect Physiology 64: 81-89.
2. Hahn, D.A. *et. al.* 2011. Annual Review of Entomology. 56:103-121.
3. Sim, C. *et. al.* 2013. Frontiers in Physiology. 189:4.