How Repeated Cold Affects Alfalfa Leaf-Cutting Bees

Naomi Brereton[1], Andre Szejner-Sigal[2], Kendra Greenlee[2]
[1]Canisius University, NY, USA [2]North Dakota State University, ND, USA

Introduction

- *Megachile rotundata*, the alfalfa leafcutting bee, is a heavily managed alfalfa pollinator.
- *M.rotundata* survives the low temperatures of winter by entering diapause, a stage of suspended development, when they have protective mechanisms to tolerate cold.
- Most insects lose their cold hardiness post-diapause.
- Recovering from cold exposure is metabolically costly (Figure.1).
- Finding the metabolic cost of cold recovery for *M.rotundata* is good for predicting how the species may perform in extreme cold climates.

Metabolic Rate and Temperature

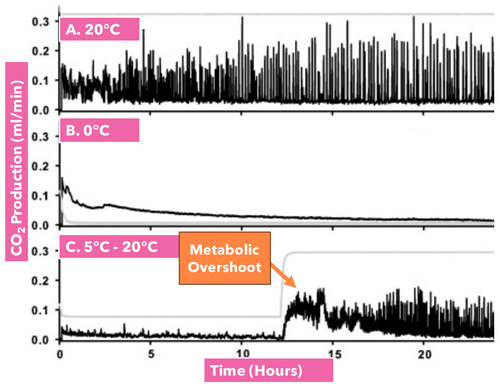


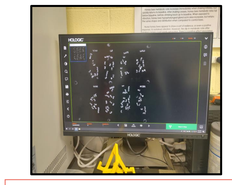
Figure.1. Open flow respirometry recordings of adult beetles, *Alphitobius diaperinus,* exposed to different thermal regimes. During cold recovery, there is a large increase in the volume of CO_2 (C), and it is theorized that this metabolic overshoot (arrow) likely represents the metabolic costs it takes to recover from cold. (Modified from Lalouette et al.2011)

Research question:
What is the metabolic cost of cold recovery especially under repeated cold exposure?

Predictions

- **Prediction 1:** Post-diapausing *M. rotundata* will also have a metabolic overshoot that will show the metabolic cost of cold recovery.
- **Prediction 2:** As the number of times an individual is exposed to cold increases, the metabolic rate will also increase.
- **Prediction 3:** As cold exposure time increases, the longer it takes to emerge as adults.

Methods



Brood cells broken at 29C and x-rayed for viable bees

Use stop flow respirometry on treated brood cells

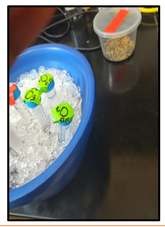
Individuals are weighed in mg after and before respirometry

MR Treatment groups

1. Ice 2 hours (n=15)
2. Ice Overnight (n=15)
3. Control (n=10)

All individuals tracked daily because they are exposed to stress 3 times (once a day) with their metabolic rate recorded using stop-flow respirometry after exposure.

Assign 48 bees to treatment groups



Put treatment group in falcon tubes and put in ice

Emergence Treatment groups

Overnight groups
OR- ice overnight (exposed 3 times, once a day)
O1- ice overnight on day 1
O2- ice overnight on day 2
O3- ice overnight on day 3

Short periods of cold exposure groups
A- ice for 2 hours on day 3
B- ice for 4 hours on day 3
C- ice for 6 hours and 21 minutes on day 3

Results

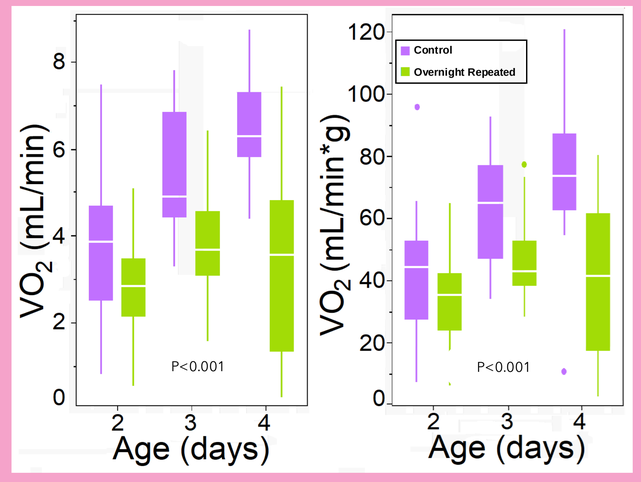


Figure.2 The absolute and mass-specific metabolic rates of the overnight repeated treatment was lower than the control.

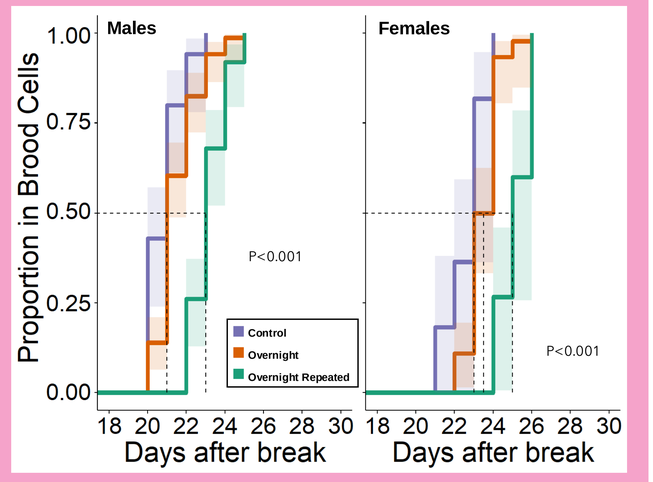


Figure.3 Repeated cold overnight affects emergence by delaying it 2 days compared to the control.

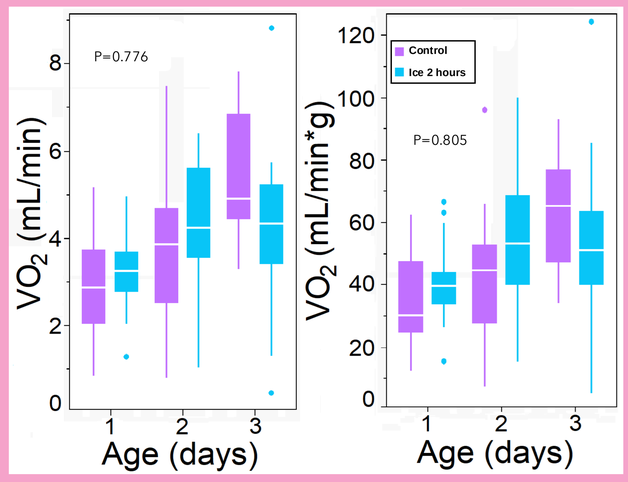


Figure.4 The absolute and mass-specific metabolic rates of 2 hours in ice and control were not significantly different.

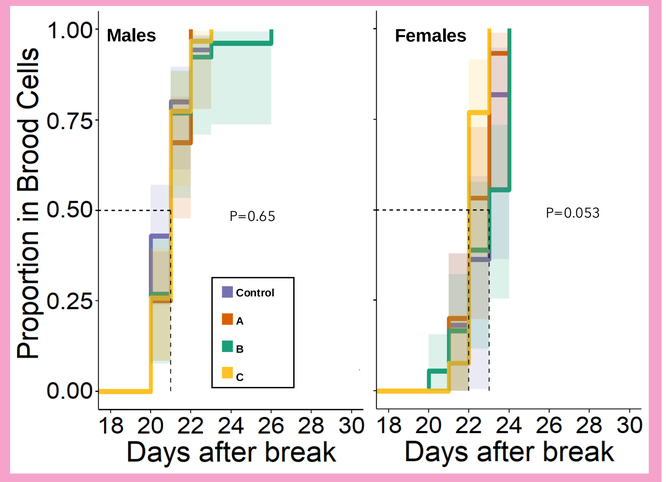


Figure.5 Small periods of cold do not affect emergence.

Conclusion

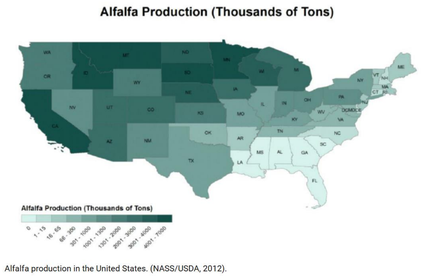
Conclusion 1:
- The metabolic overshoot was not seen after being exposed to treatments. Therefore, the treatments used may not have been stressful enough to induce a significant energetic cost.

Conclusion 2:
- Metabolic rates for controls increased during the first 4 days to support development. Under repeated cold exposure, especially overnight, metabolic rates stayed relatively constant and lower than controls. Contrary to our prediction of an increasing overshoot, lower metabolic rates during key developmental stages may also be a sign of stress.

Conclusion 3:
- Prepupa exposed to 0°C overnight, 3 times (once a day), delayed emergence for 2 days when compared to the controls. This means that the cost of cold recovery slows development.
- Prepupa exposed to short periods of cold had no effect on development , so *M. rotundata* can handle short cold periods at 0°C at least till around 6 hours and 30 min.

Importance

The cold snaps that can occur during spring and the arctic air that moves southward to the U.S. are both possible cold stressors that alfalfa leaf cutting bees must go through, so understanding their cold tolerance is paramount to stakeholders of alfalfa production.



Alfalfa production in the United States. (NASS/USDA, 2012).

Acknowledgements

I would like to thank everyone at the USDA for giving me the resources and support to complete my project.

References

- Earls et al. (2023). Effects of temperature on metabolic rate during metamorphosis in the alfalfa leafcutting bee. *Biology Open*, 12(12).
- Lalouette, et al. (2011). Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 158(2), 229–234.