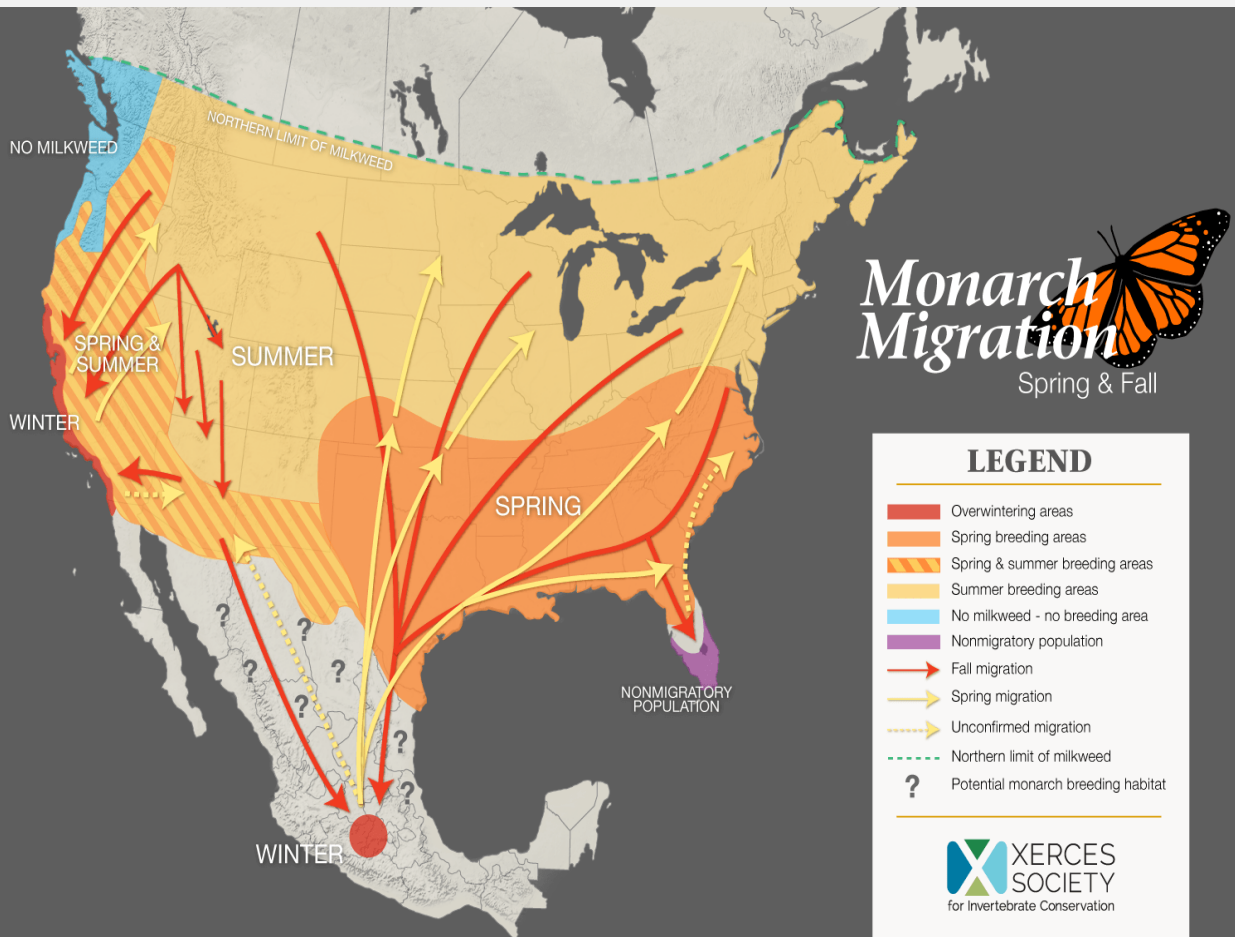


METABOLISM OF THE SPRING FIELD  
CRICKET *GRYLLUS VELETIS* DURING  
FREEZING, THAWING AND  
RECOVERY

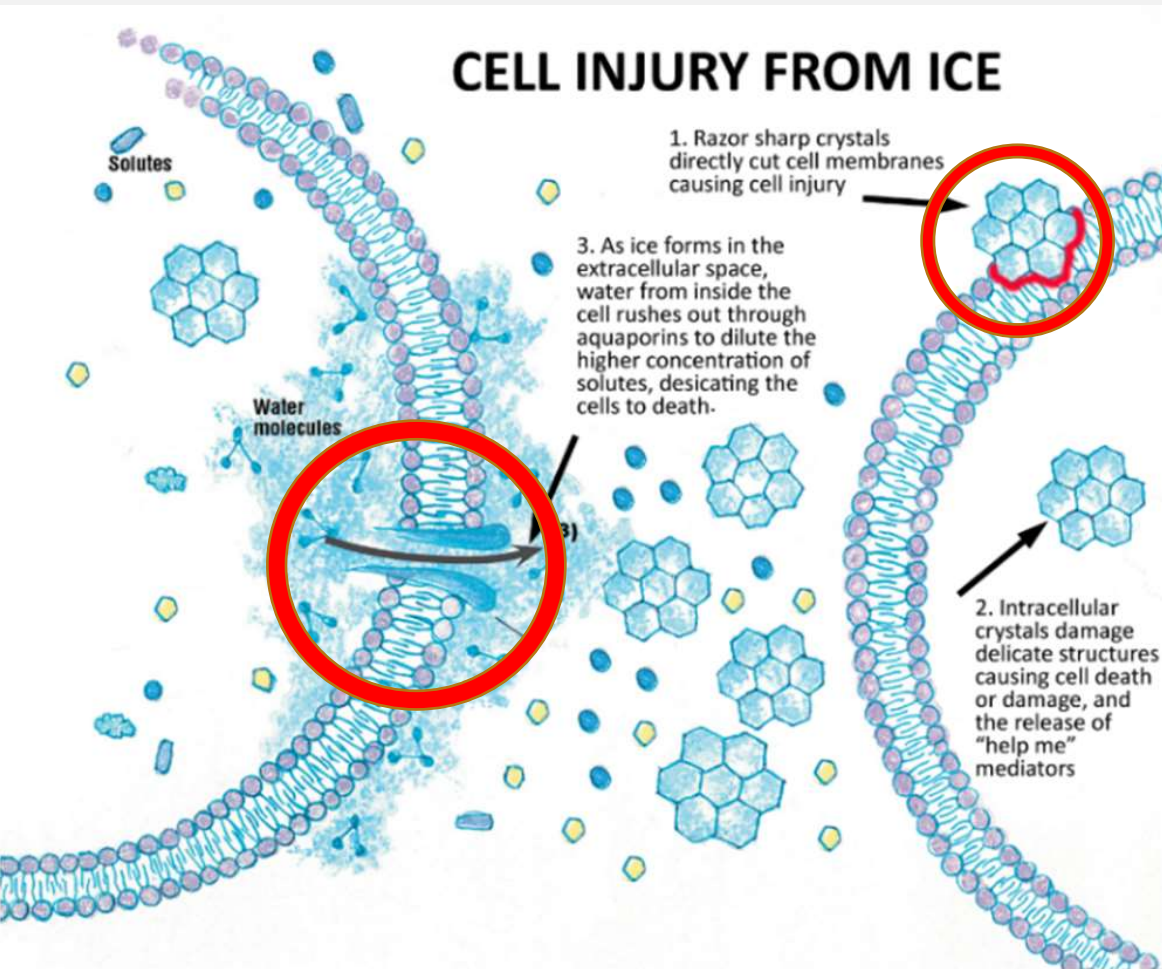
By Julian Moulton

# INSECTS IN WINTER



Freeze Tolerant: An organism that can survive a proportion of their body water freezing

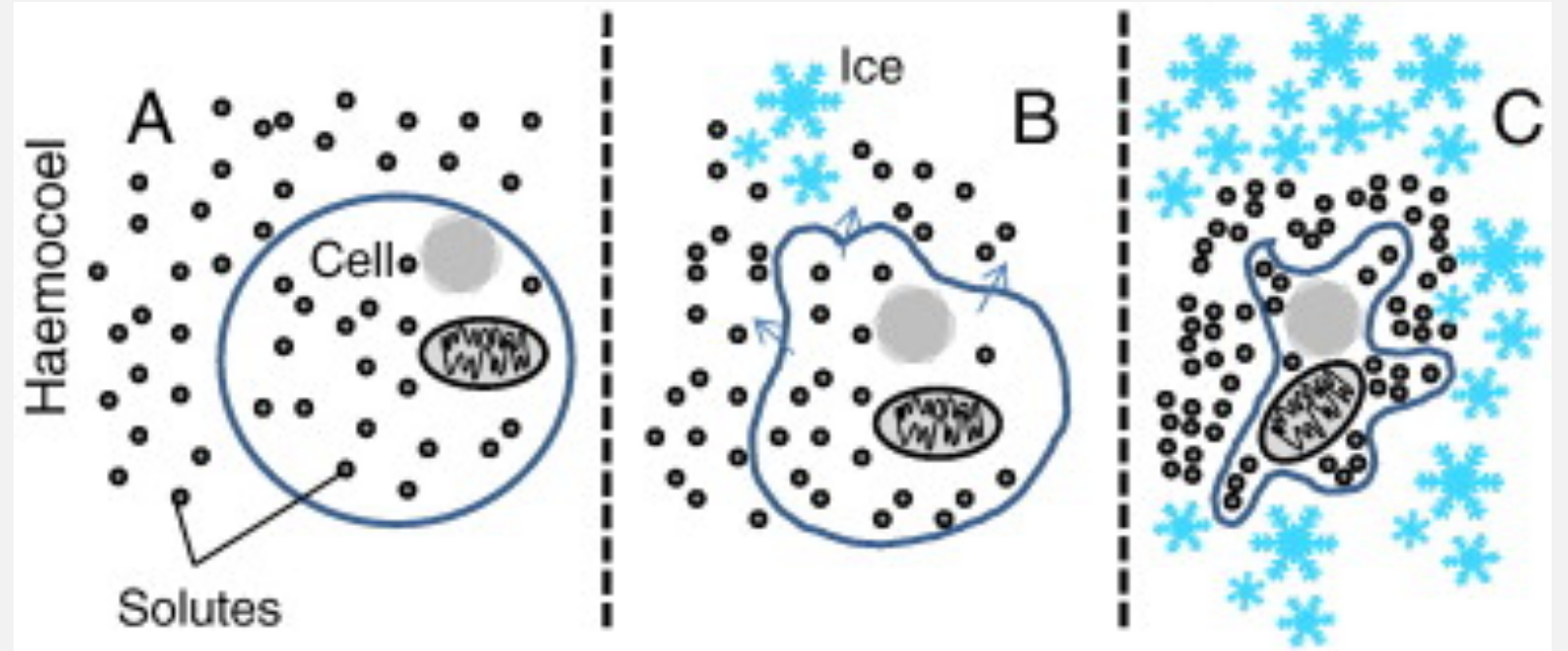
# DANGERS OF LOW TEMPERATURES AND FREEZING



Five main harmful effects of freezing

- Effecting the structure of macromolecules
- Inability to accrue essential nutrients
- Buildup of harmful cations or metabolic byproducts
- Intracellular Ice formation
- Cellular dehydration

# FREEZE TOLERANCE MECHANISMS

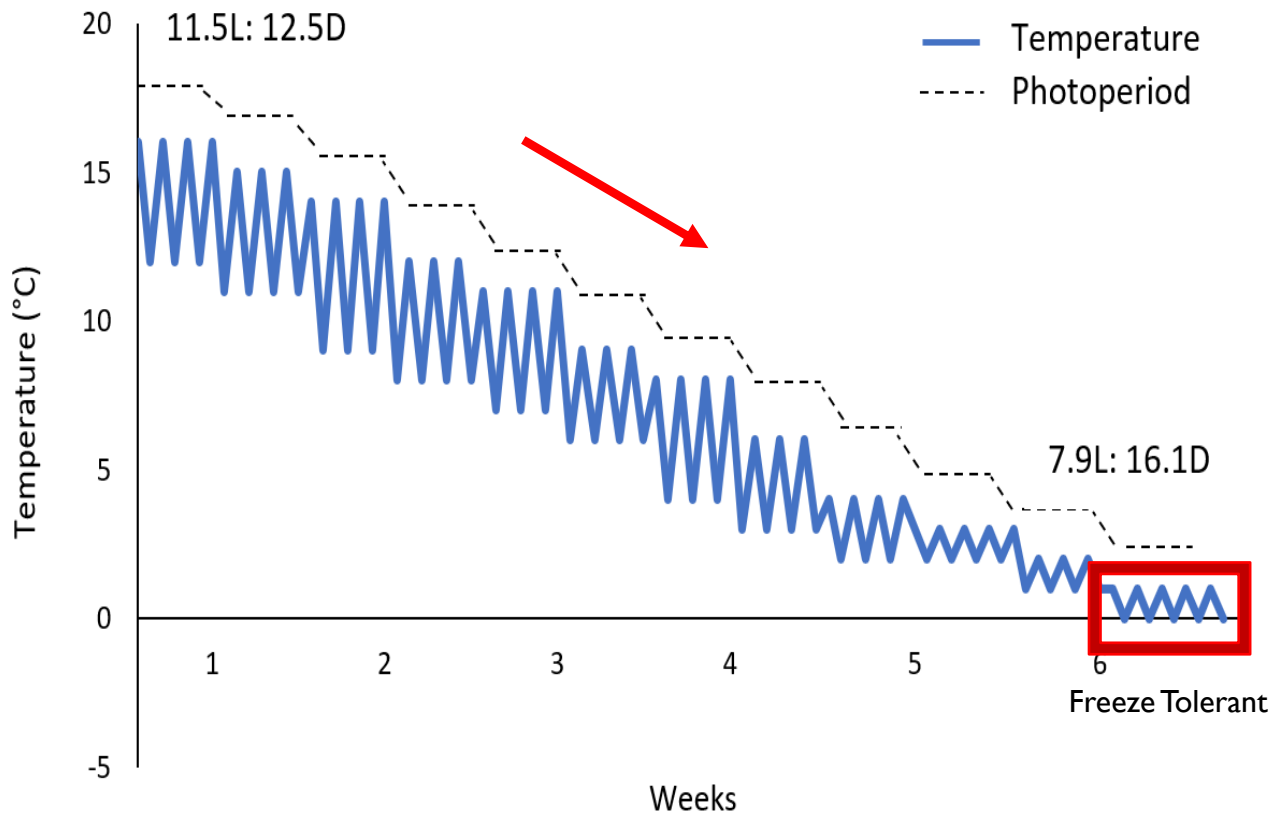


- Control of ice propagation
- Accumulating cryoprotectants
- Metabolic suppression

# SPRING FIELD CRICKET



# SPRING FIELD CRICKET

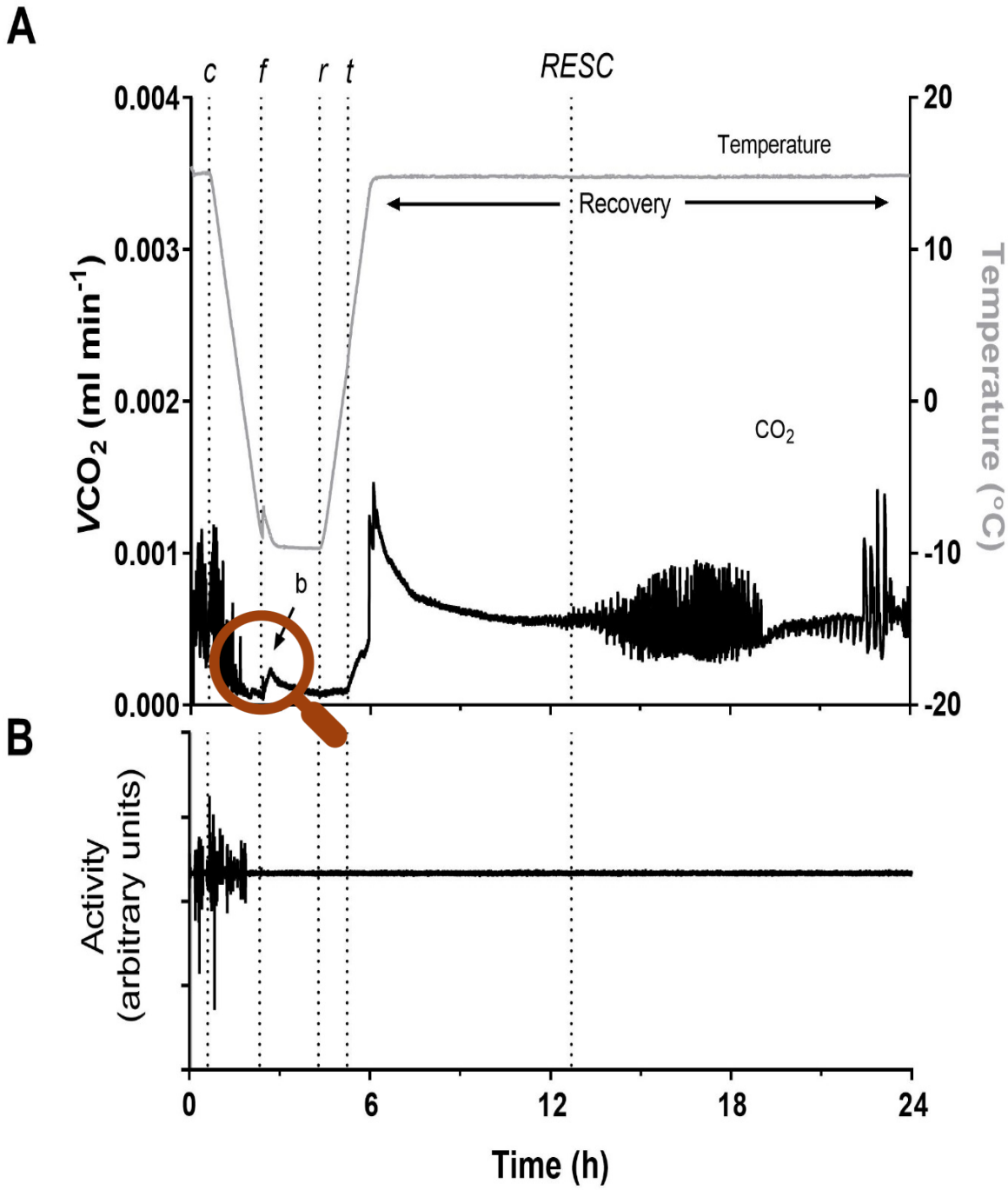


1. What causes the burst of  $\text{CO}_2$  observed at the onset of freezing?

2. What are the short term and long term metabolic costs of freezing?

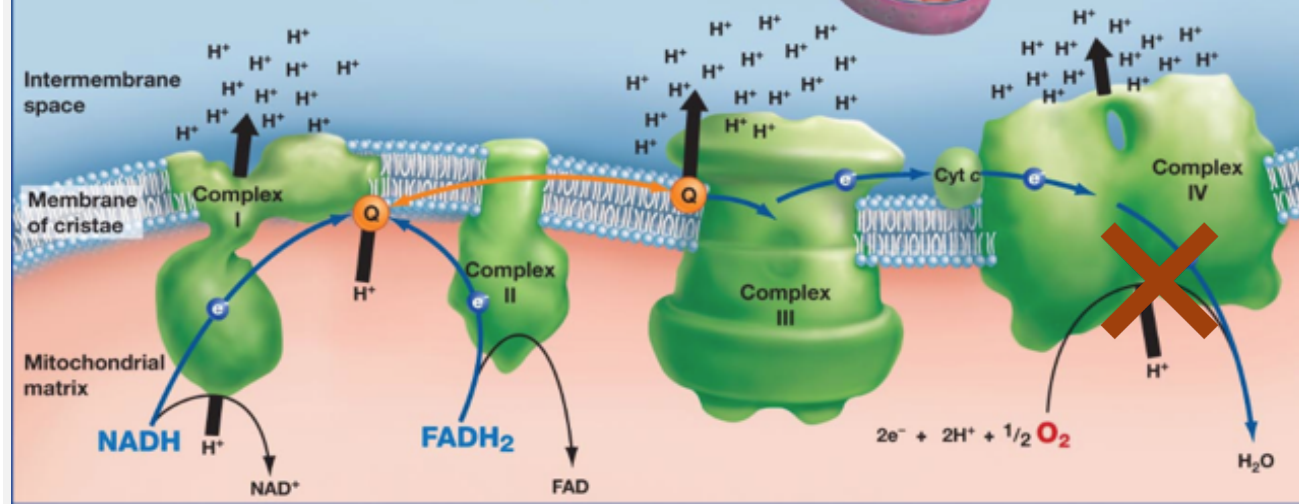
## HYPOTHESES

1. A change in the CO<sub>2</sub> buffering capacity of hemolymph, rather than an increase in metabolism, drives the burst of CO<sub>2</sub> release from *G. veletis* at the onset of freezing.
2. Recovery from freezing has a significant metabolic cost, so crickets that froze will have a higher metabolic rate than crickets that were brought down to -8 °C but did not freeze, as well as crickets that were only acclimated but were kept at 15 °C.

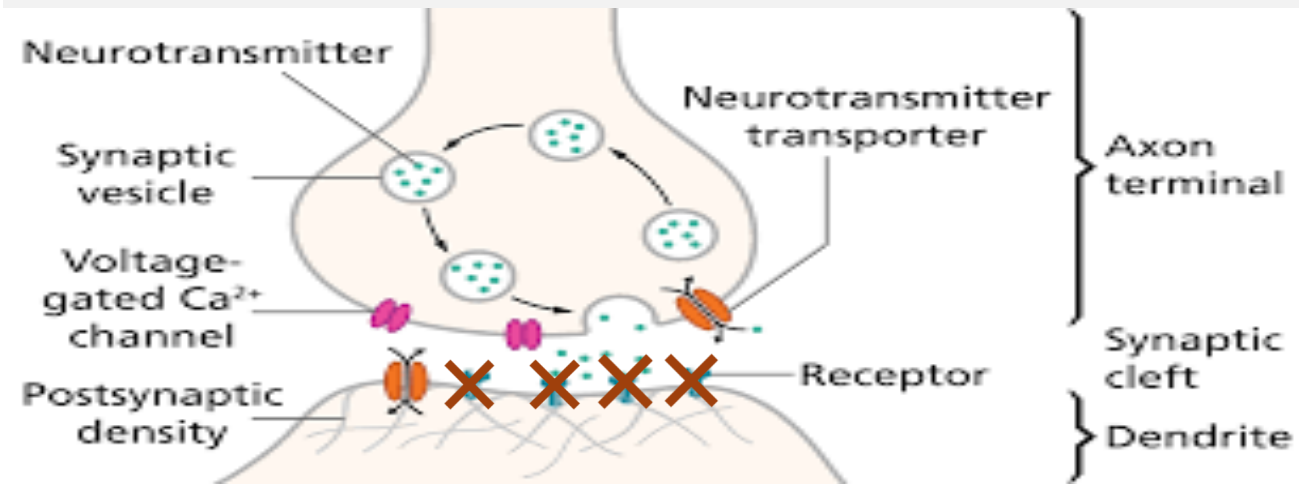


# HYPOTHESIS I

## Effects of cyanide

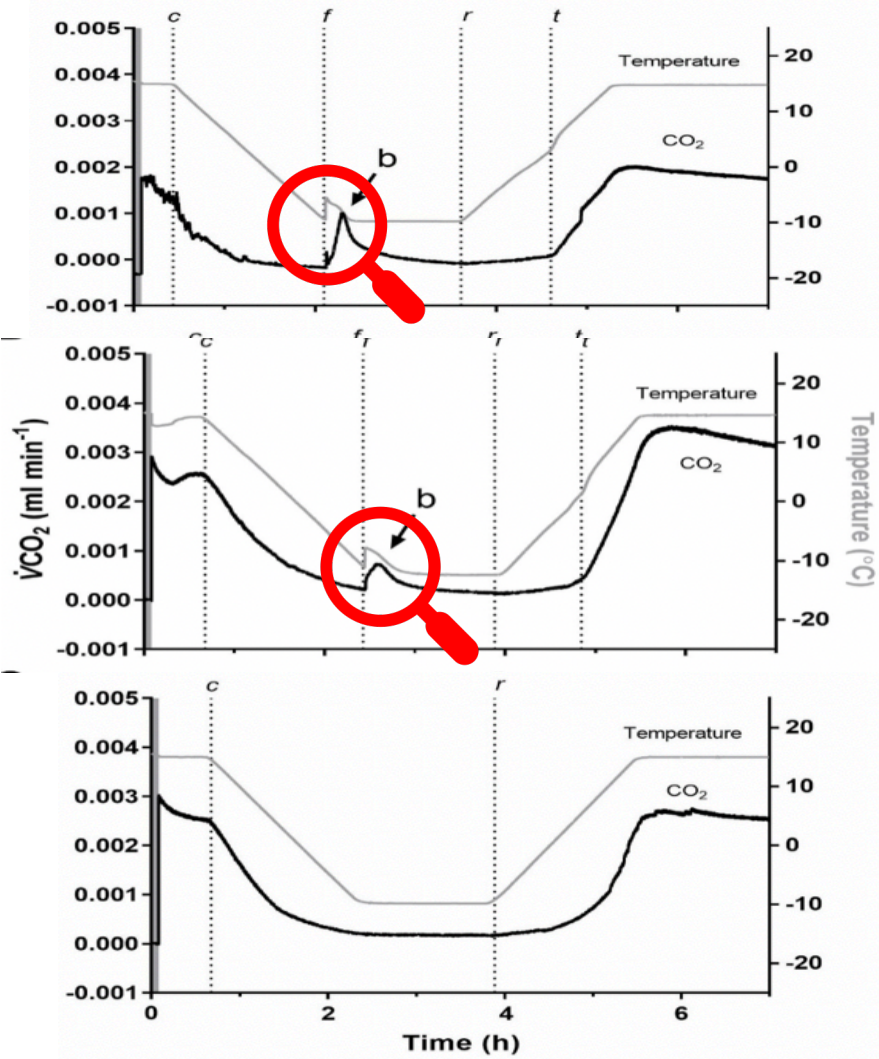


## Effects of Thiacloprid





# HYPOTHESIS I



Thiocloprid Injection

Nervous system response **X**

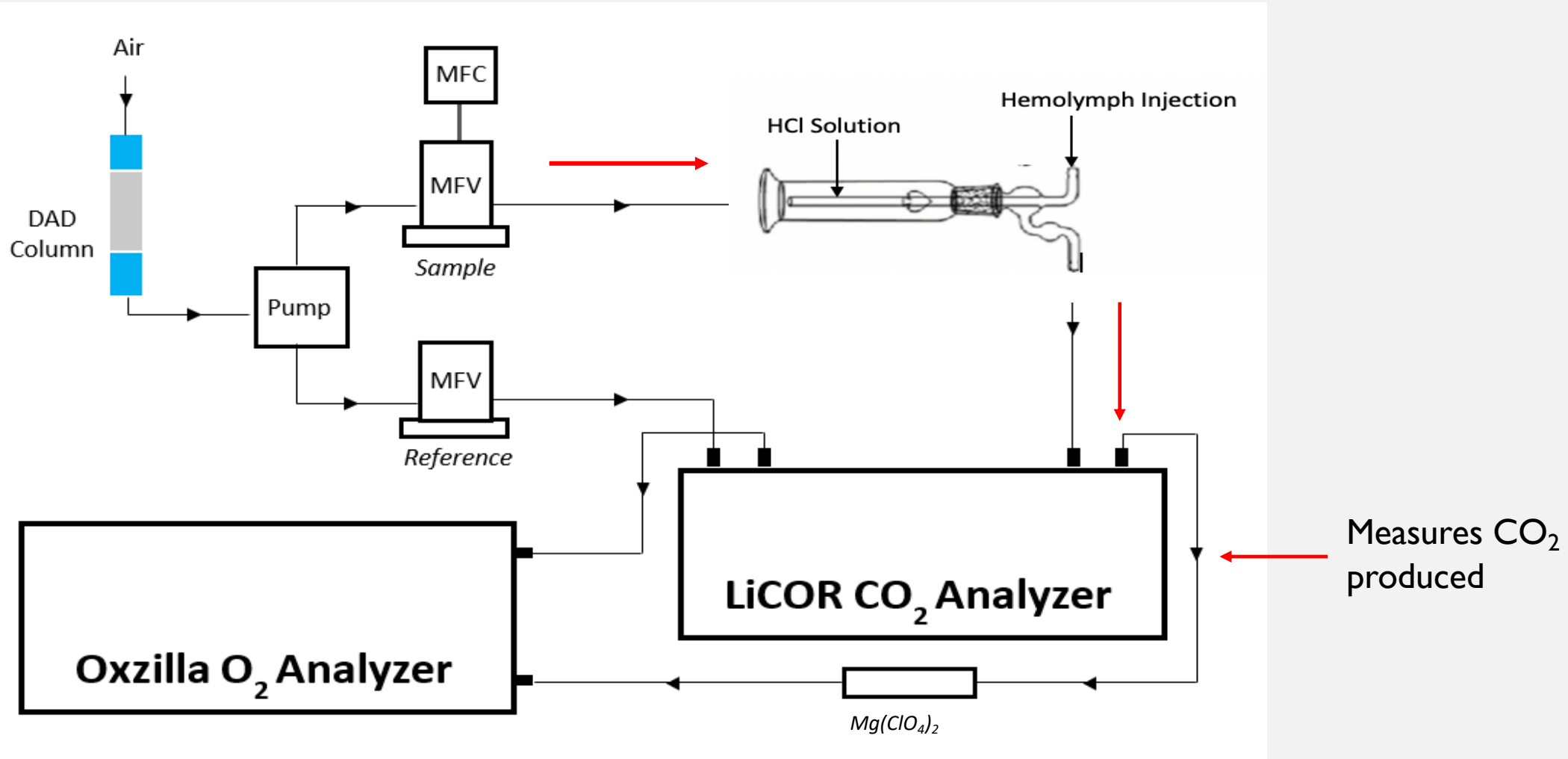
HCN Injection

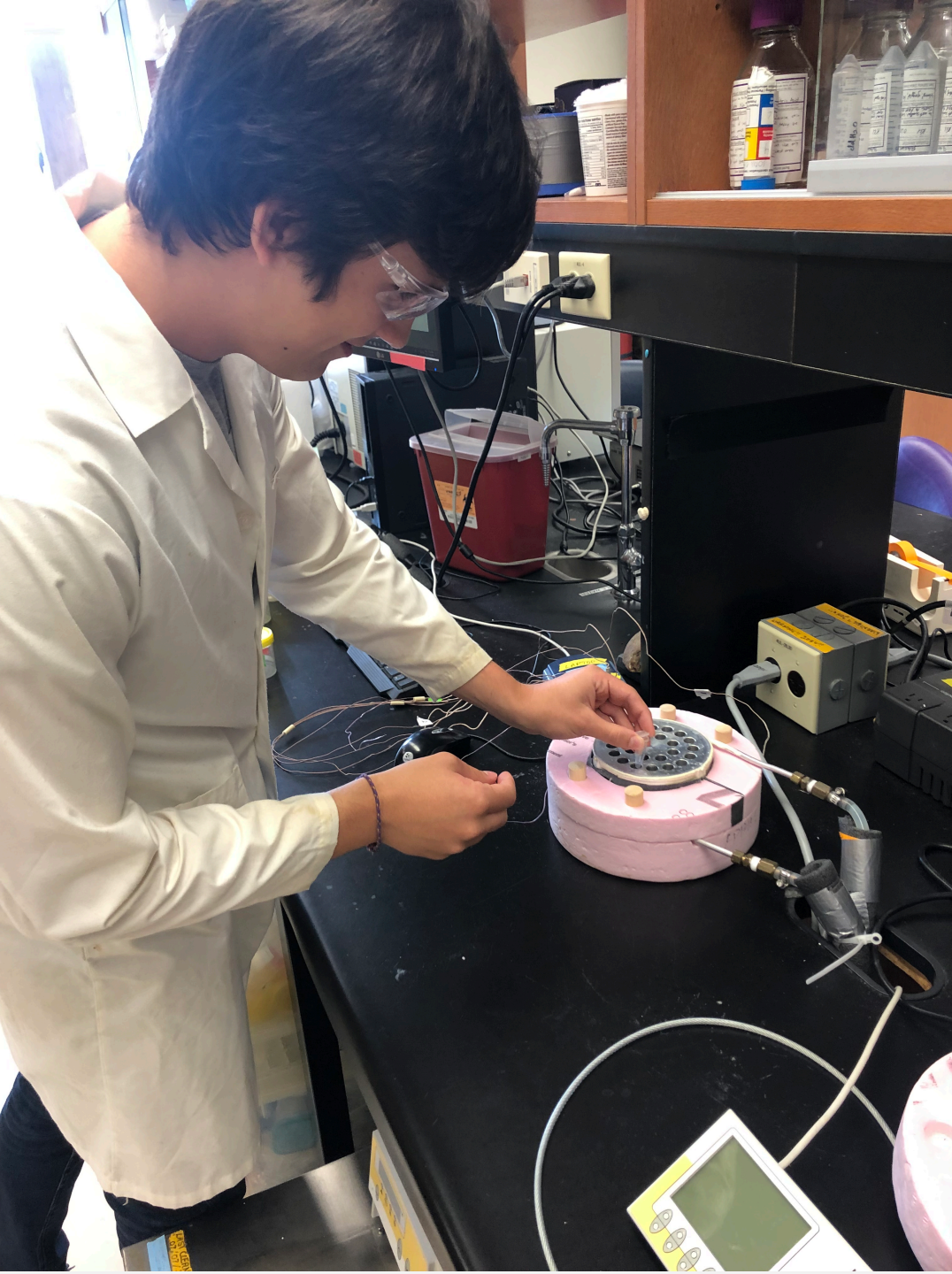
Metabolic response **X**

HCN Injection  
without freezing

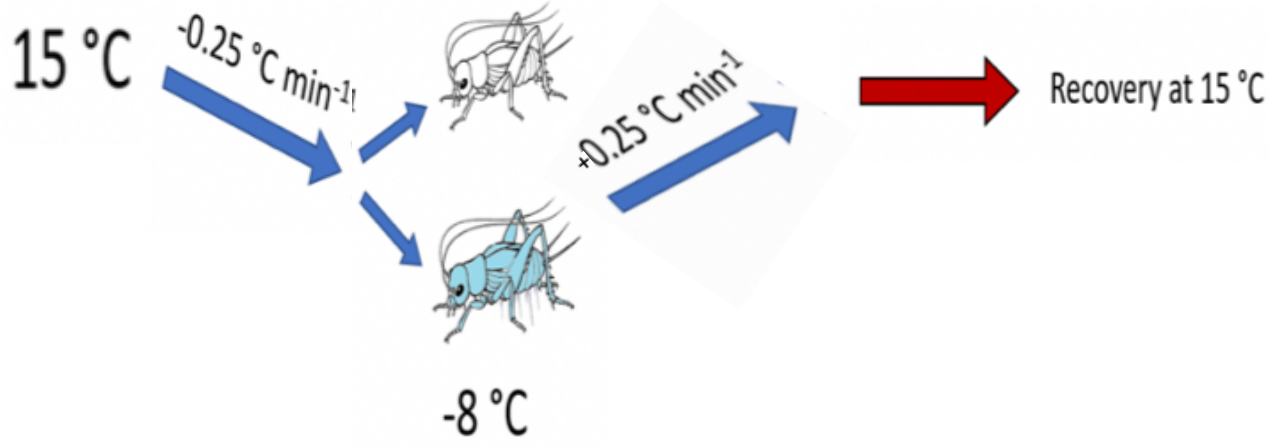
Injection response **X**

# FLOW THROUGH RESPIROMETRY



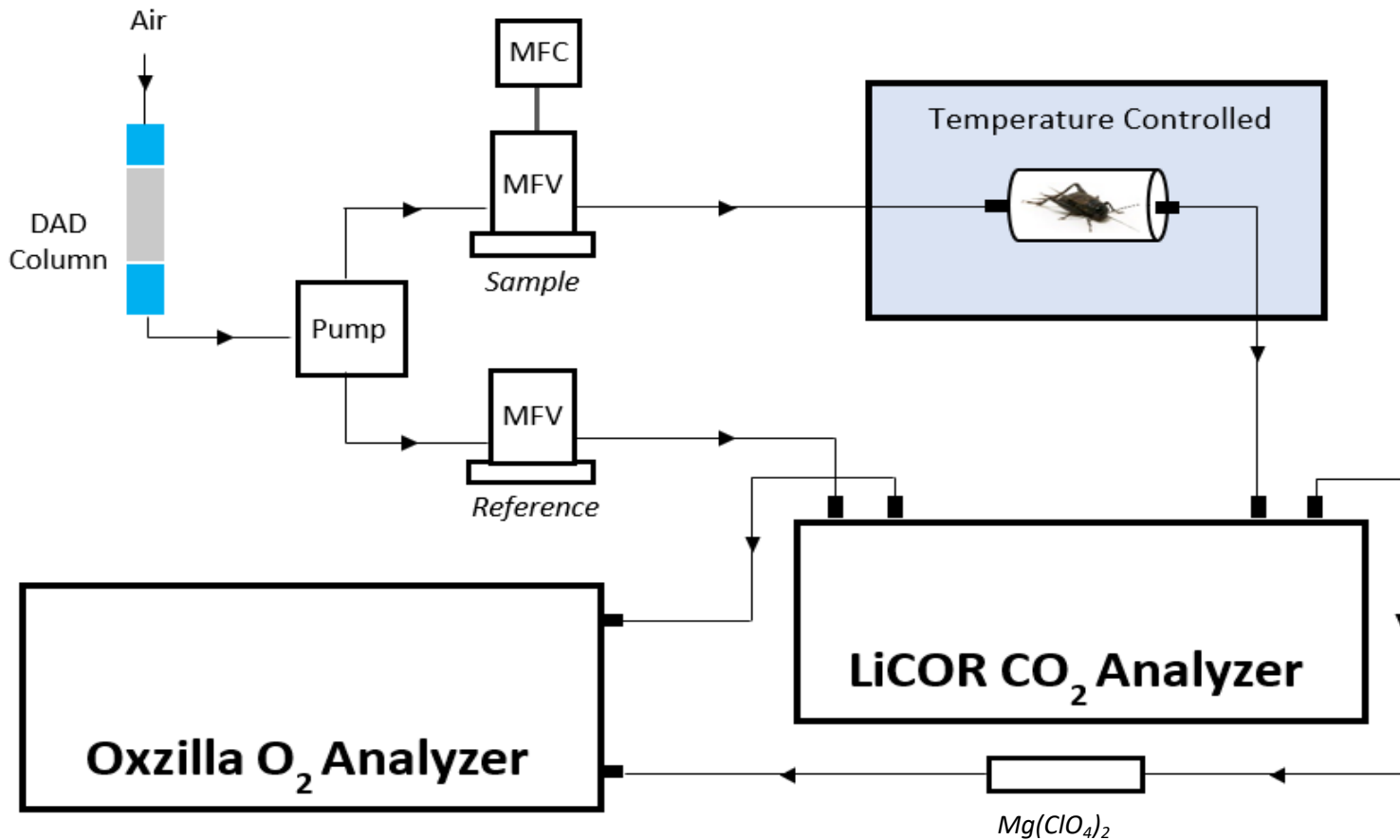


# HYPOTHESIS 2



Bringing the crickets down to  $-8\text{ }^{\circ}\text{C}$  meant about half of the crickets froze and half did not.

# STOP FLOW RESPIROMETRY

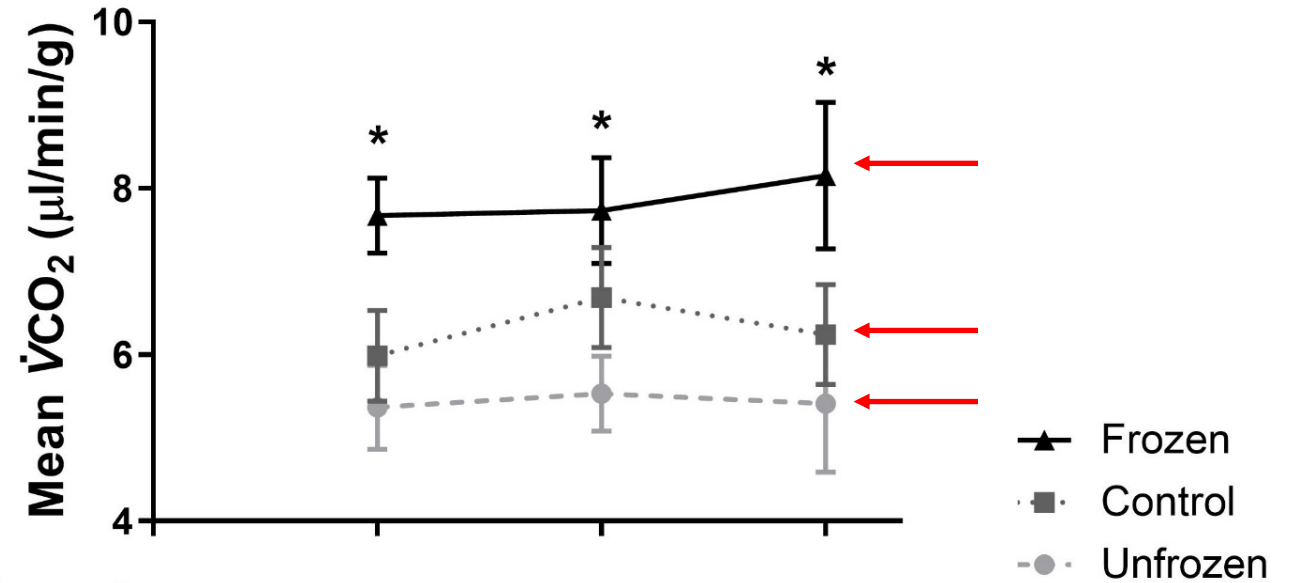


- Placed crickets in respirometer 24, 48, and 72 hours after freezing, and again after molting
- Stop flow respirometry allowed us to test multiple crickets at once and magnifies the differences between metabolic rates

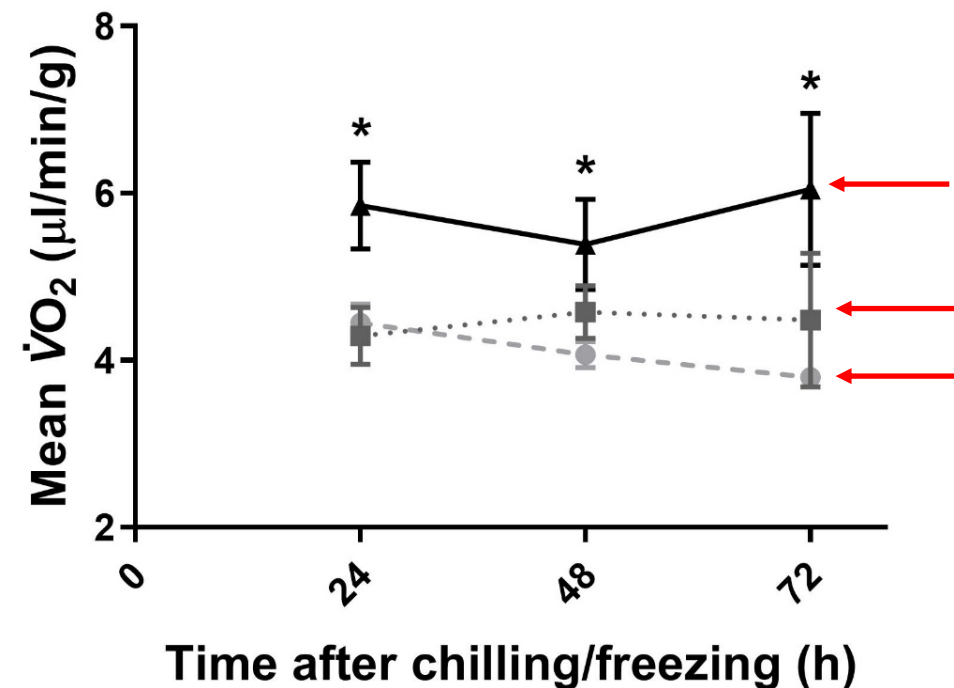
# SHORT TERM RECOVERY

- There was a significantly higher metabolic rate in crickets that froze compared to the control and unfrozen crickets at 24, 48, and 72 hours after being cooled.
- Chilled crickets in general had higher metabolic rates than control crickets, but the difference was not statistically significant

A



B



# LONG TERM RECOVERY

- This difference was not seen when metabolic rates were taken after molting
- Frozen crickets molted later than unfrozen crickets, and many struggled to make it through the molt
- 0 out of 12 frozen crickets survived: survival rate 0%
- 7 out of 14 chilled crickets survived: survival rate 50%
- 5 out of 7 control crickets survived: survival rate 71.4%



# DISCUSSION

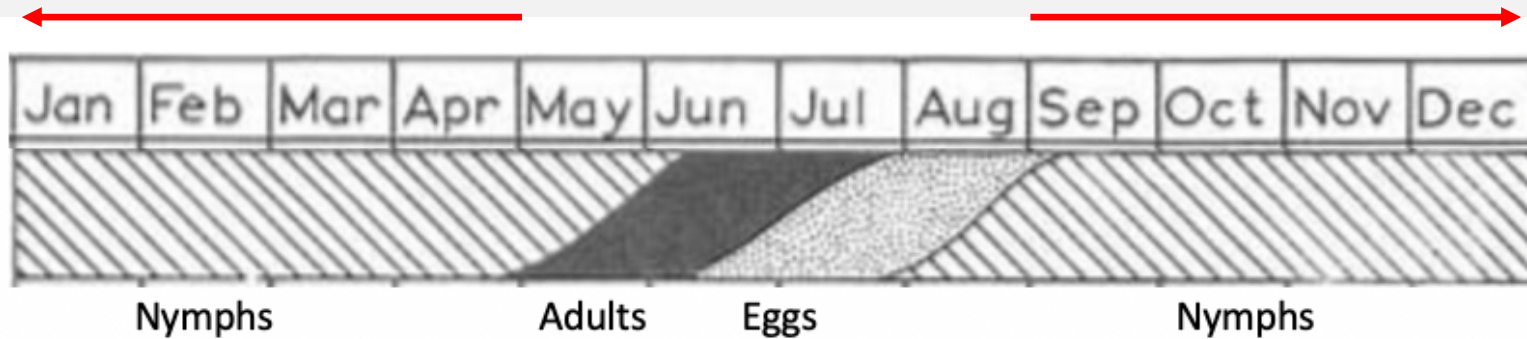
## 3 Main Findings

1. The burst of  $\text{CO}_2$  observed at the onset of freezing is likely the result of dissolved  $\text{CO}_2$  being forced out of hemolymph as it freezes
2. There is a significant metabolic cost associated with freezing and it persists for several days
3. Crickets that froze are less likely to survive until adulthood than crickets that did not freeze



# DISCUSSION

*Life cycle of Gryllus veletis*



- The life cycle of *Gryllus veletis* is dependent on the normal changing of the seasons
- Mechanisms of freeze tolerance need further investigation
- Understanding the cost of freezing could be critical in predicting how freeze tolerant species will be affected by climate change



# ACKNOWLEDGEMENTS



THE SINCLAIR LAB  
**INSECT LOW**  
TEMPERATURE BIOLOGY



**COLORADO**  
**COLLEGE**

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