

# An Investigation of Cold-Induced Barrier Disruption in the Gut Epithelia of *Locusta migratoria*



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## Background

- Chill susceptible insects often succumb to chill coma during cold exposure<sup>1</sup>.

- Extended and/or extreme bouts of cold result in a build up of chilling injuries and death<sup>1</sup>.

- Chilling injuries are often associated with organismal ion imbalance<sup>2</sup>.

- This loss of homeostasis is thought to be at least partly driven by a cold-induced disruption of epithelia barriers in *Drosophila*<sup>3</sup>.

## Key terms

**Chill-susceptible insects** – Suffer negative effects of chilling at temperatures above the freezing point of their bodily fluids<sup>4</sup>

**Chill Coma Recovery Time (CCRT)** – Time to regain coordination following cold stress

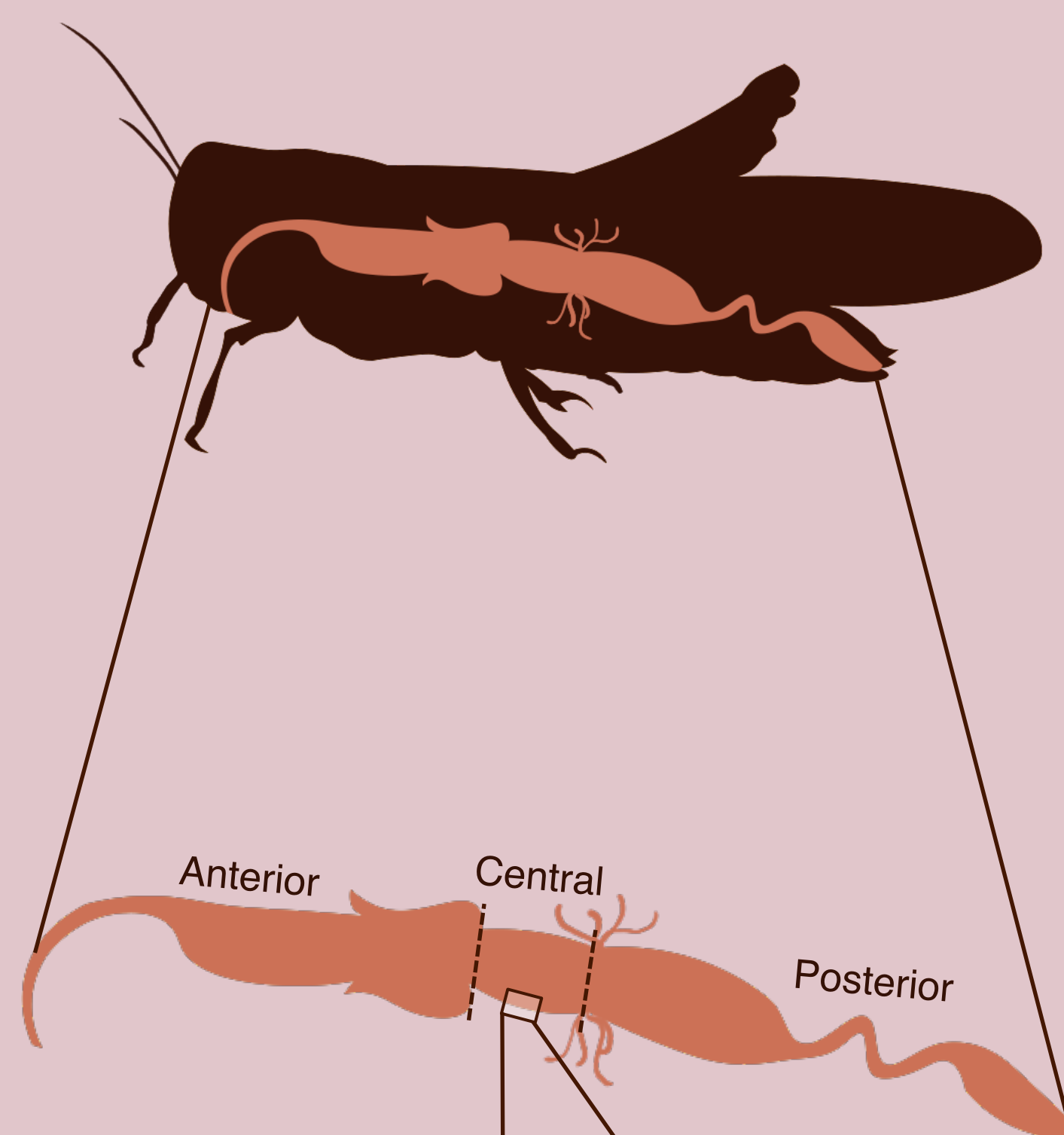
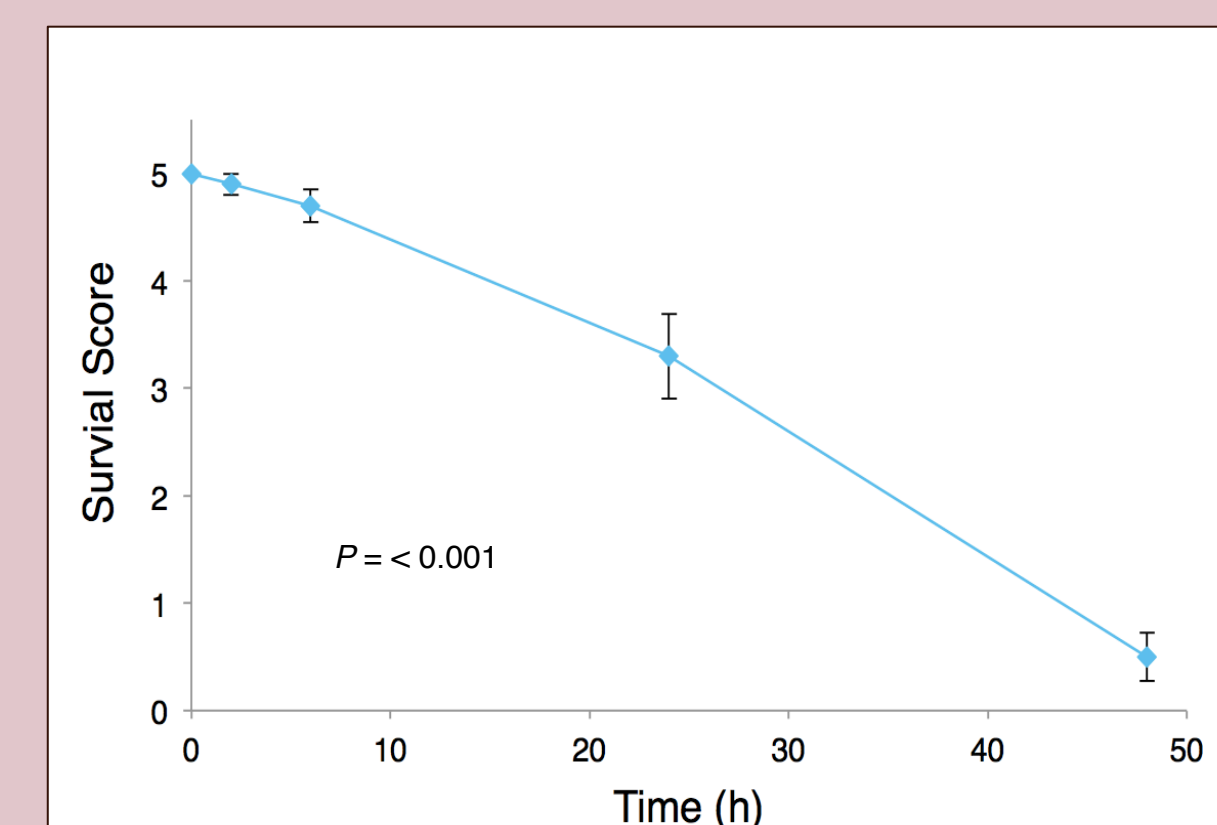
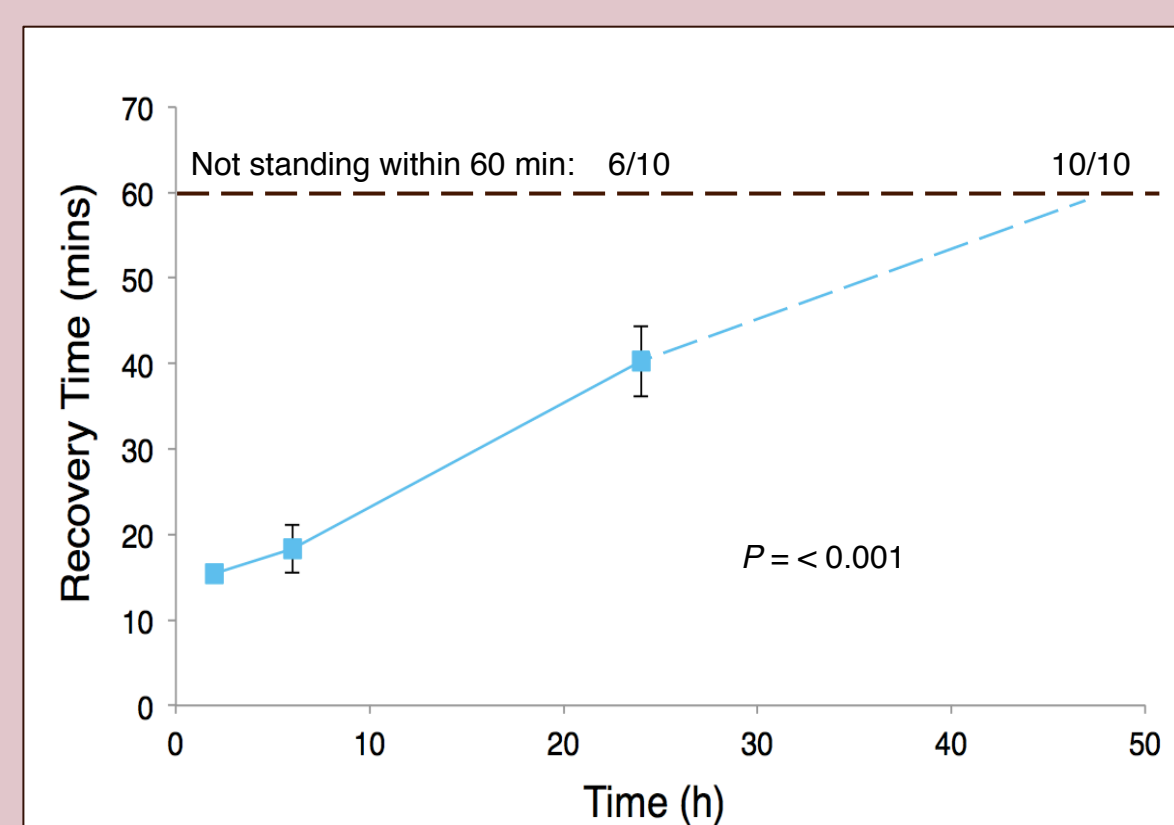
**Survival** – Condition of the organism 24h post-cold exposure

**FITC** – Fluorescently-labeled Dextran; an epithelial barrier marker (paracellular)

**Research Question:** Is locust gut epithelial barrier function maintained during cold stress?

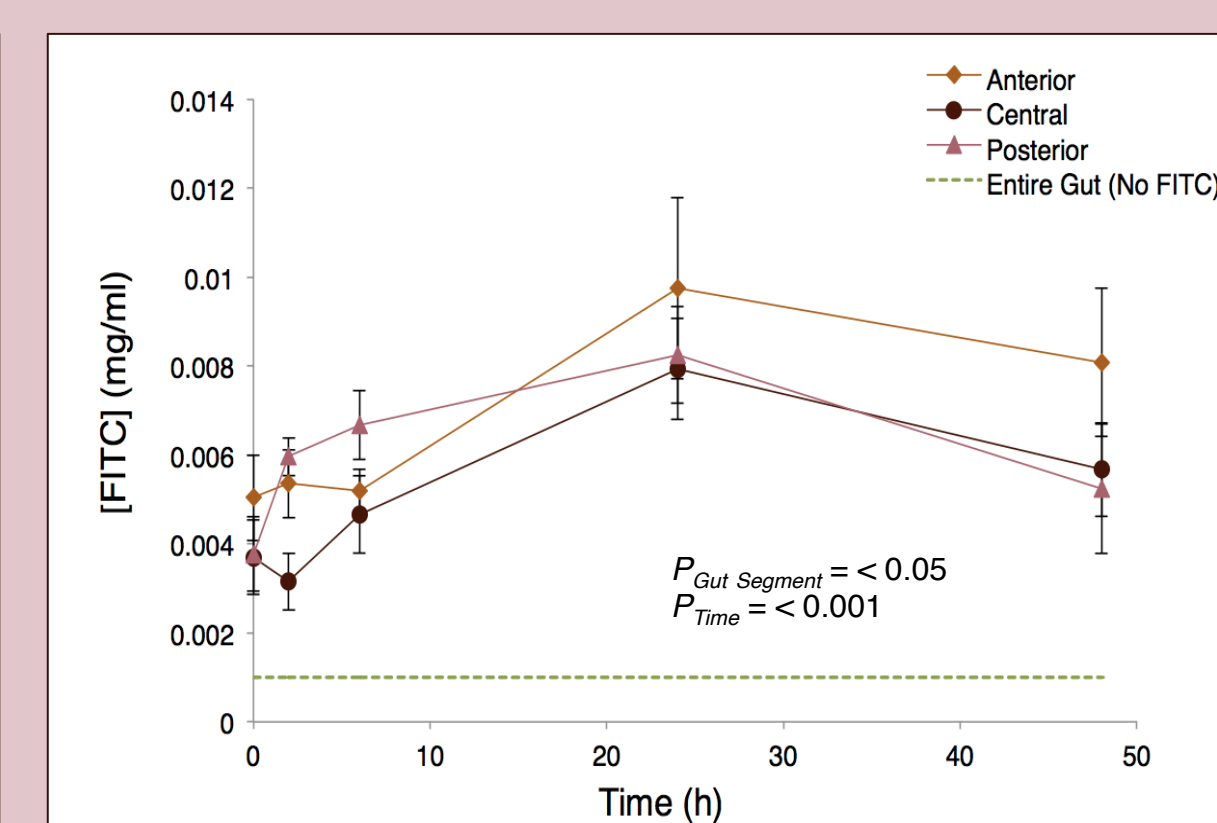
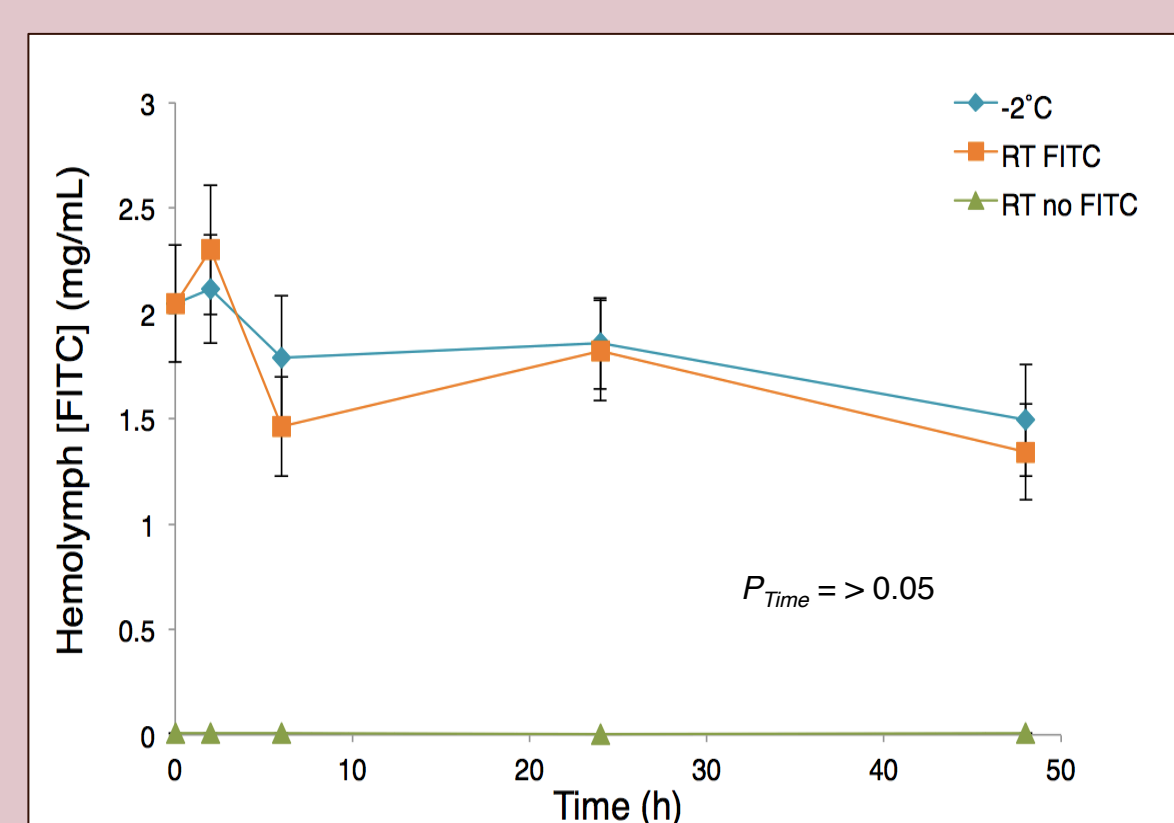


Locust recovery time increased with greater lengths of exposure to -2°C (left). Their probability of survival also decreased over time (right).



**FITC**

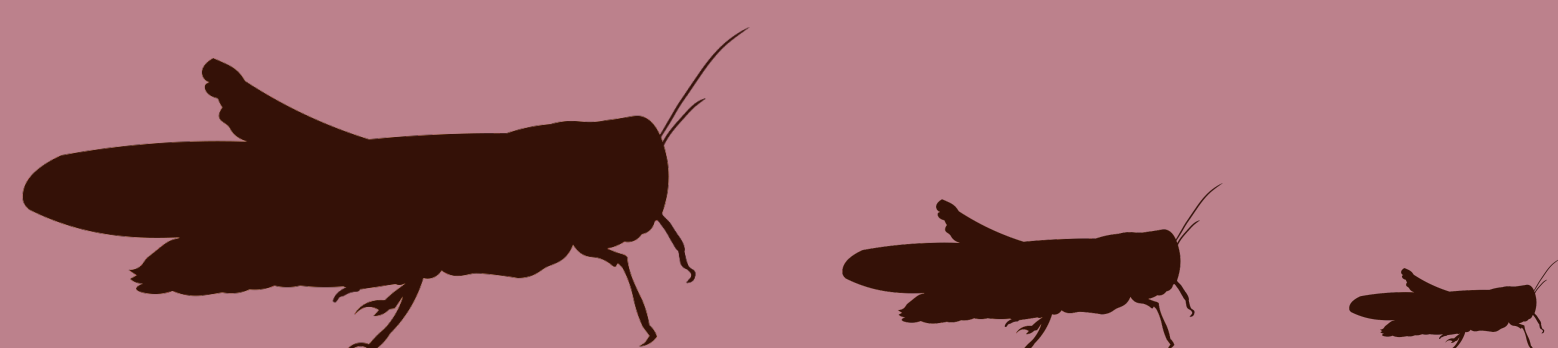
Minimal movement of FITC occurred across the gut epithelia over time in the cold.



## Meet the Locusts

*Locusta migratoria*

- Crop pests found mostly in Africa and Eurasia
- Chill-susceptible insects
- Can experience cold overnight temperatures



## Methods



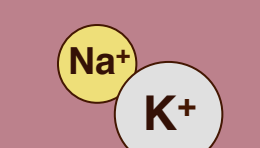
### CCRT & Survival

- Locusts were exposed to -2°C for 2, 6, 24, or 48h
- Observed on removal for CCRT, and after 24h for degree of survival (scored from 0-5; 0: dead, 5: retain pre-cold exposure ability to walk, jump, and/or fly)



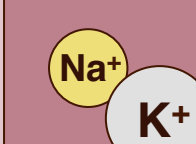
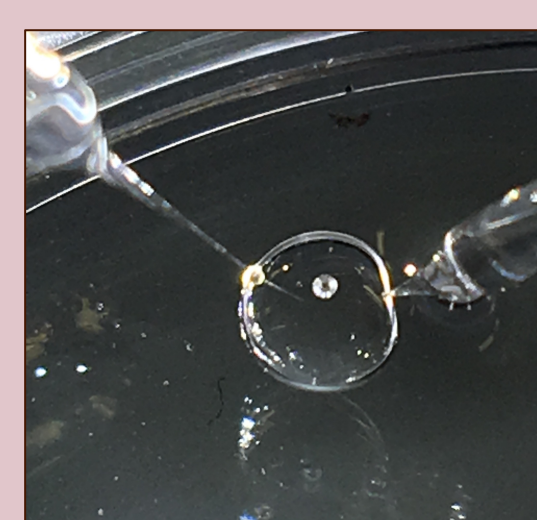
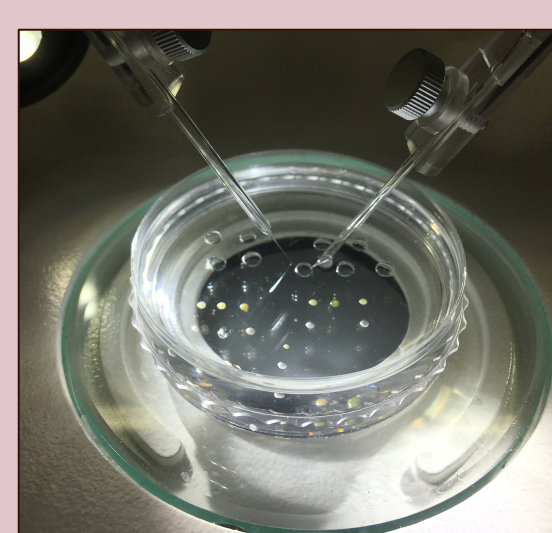
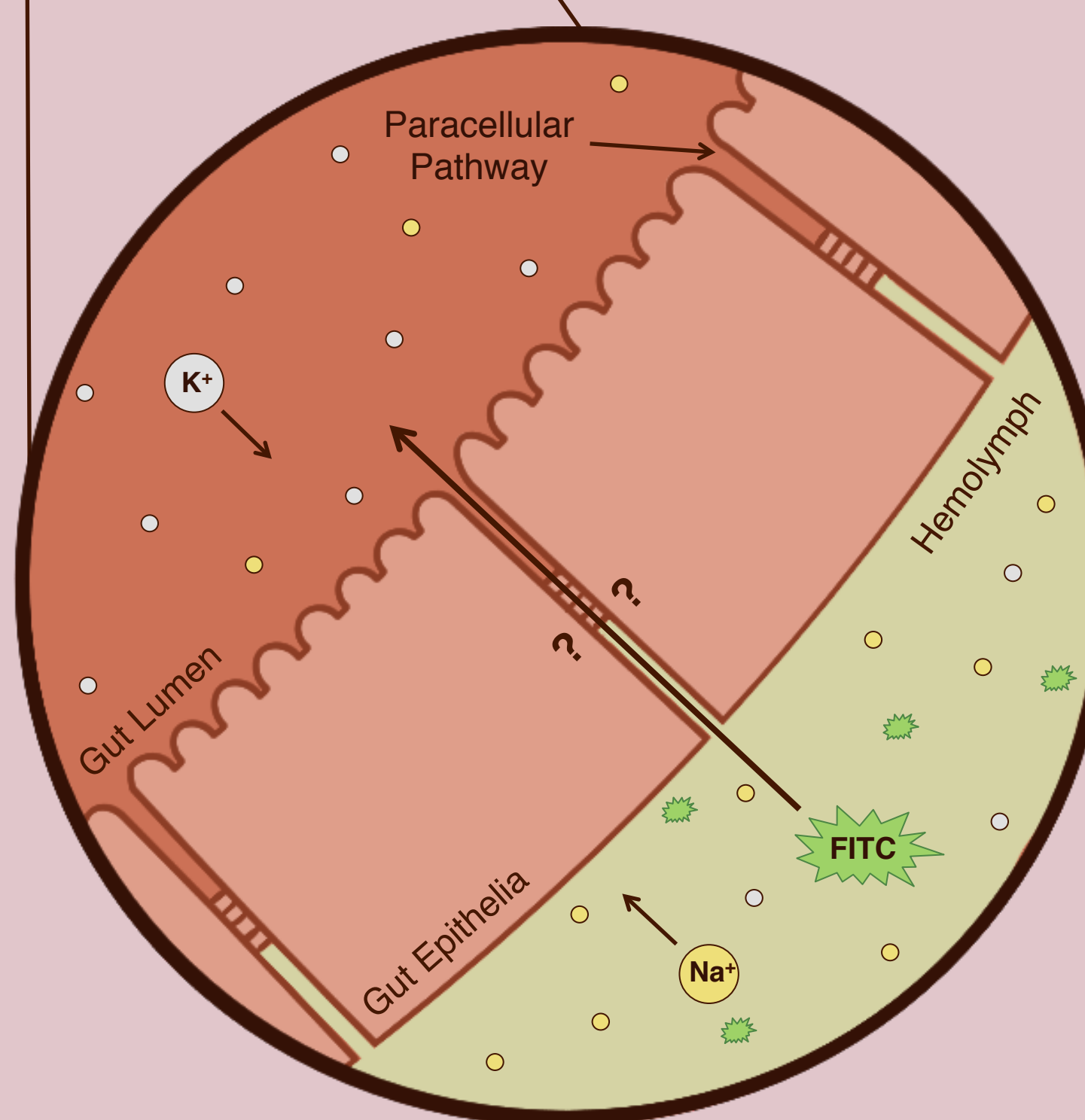
### Visualizing Loss of Barrier Function

- Locusts were injected with FITC and then exposed to -2°C for 2, 6, 24, or 48h
- Samples of anterior, central, and posterior gut segments were independently analyzed for FITC presence using a fluorometer
- Hemolymph samples were analyzed for FITC content using a fluorometer

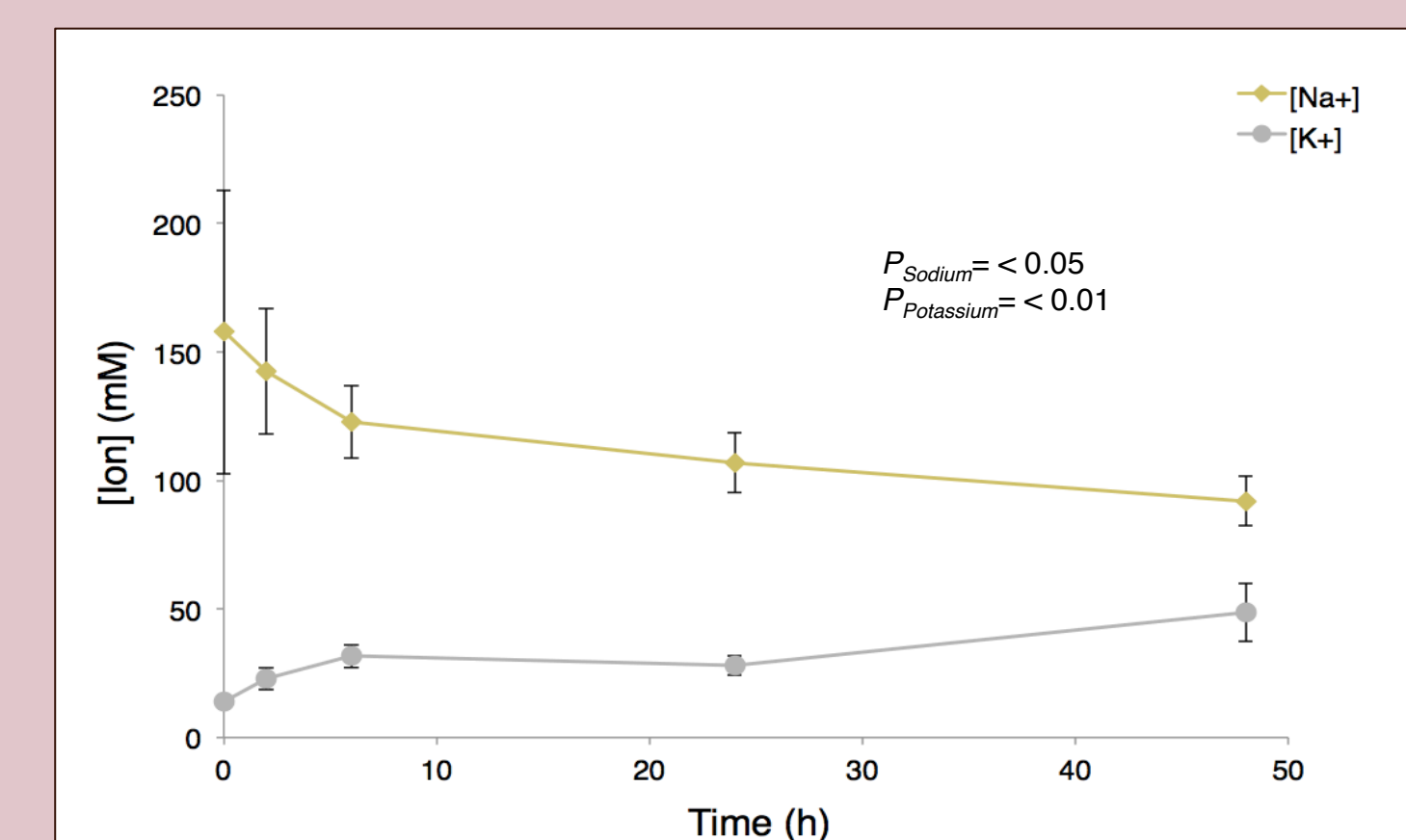


### Cold-Induced Ion Imbalance

- Hemolymph Na<sup>+</sup> and K<sup>+</sup> concentrations were measured using ion-selective microelectrodes (ISME; see photos right)



Increased cold exposure still caused a rise in hemolymph [K<sup>+</sup>] and a fall in [Na<sup>+</sup>]



## Conclusions



Chill susceptibility in the locust colony is confirmed.



Gut barrier function is generally conserved over time of cold exposure, contrary to initial belief.



Cold-induced ion imbalance does still occur within these locusts, indicating barrier disruption<sup>1</sup>. FITC permeability may therefore be limited by its size and charge.

**Next:** Use a temperature controlled Ussing chamber to see if chilling disrupts the electrical resistance of the gut epithelia

## References

1. MacMillan, H.A. & Sinclair, B.J. 2011. Mechanisms underlying insect chill coma. *Journal of Insect Physiology*, 57, 12-20.
2. MacMillan, H.A., Baatrup, E., Overgaard, J. 2015. Concurrent effects of cold and hyperkalaemia cause insect chilling injury. *Proceedings of the Royal Society B*, 282, 1-8.
3. MacMillan, H.A., Yerushalmi, G.Y., Jonusaitis, S., Kelly, S.P., Donini, A. 2017. Thermal acclimation mitigates cold-induced paracellular leak from the *Drosophila* gut. *Scientific Reports*, 7, 1-11.
4. Overgaard, J. & MacMillan, H.A. 2017. The integrative physiology of insect chill tolerance. *The Annual Review of Physiology*, 79, 187-208.

## Acknowledgements

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