Physiological and hormonal response to variation in overwintering conditions in freeze tolerant wood frogs

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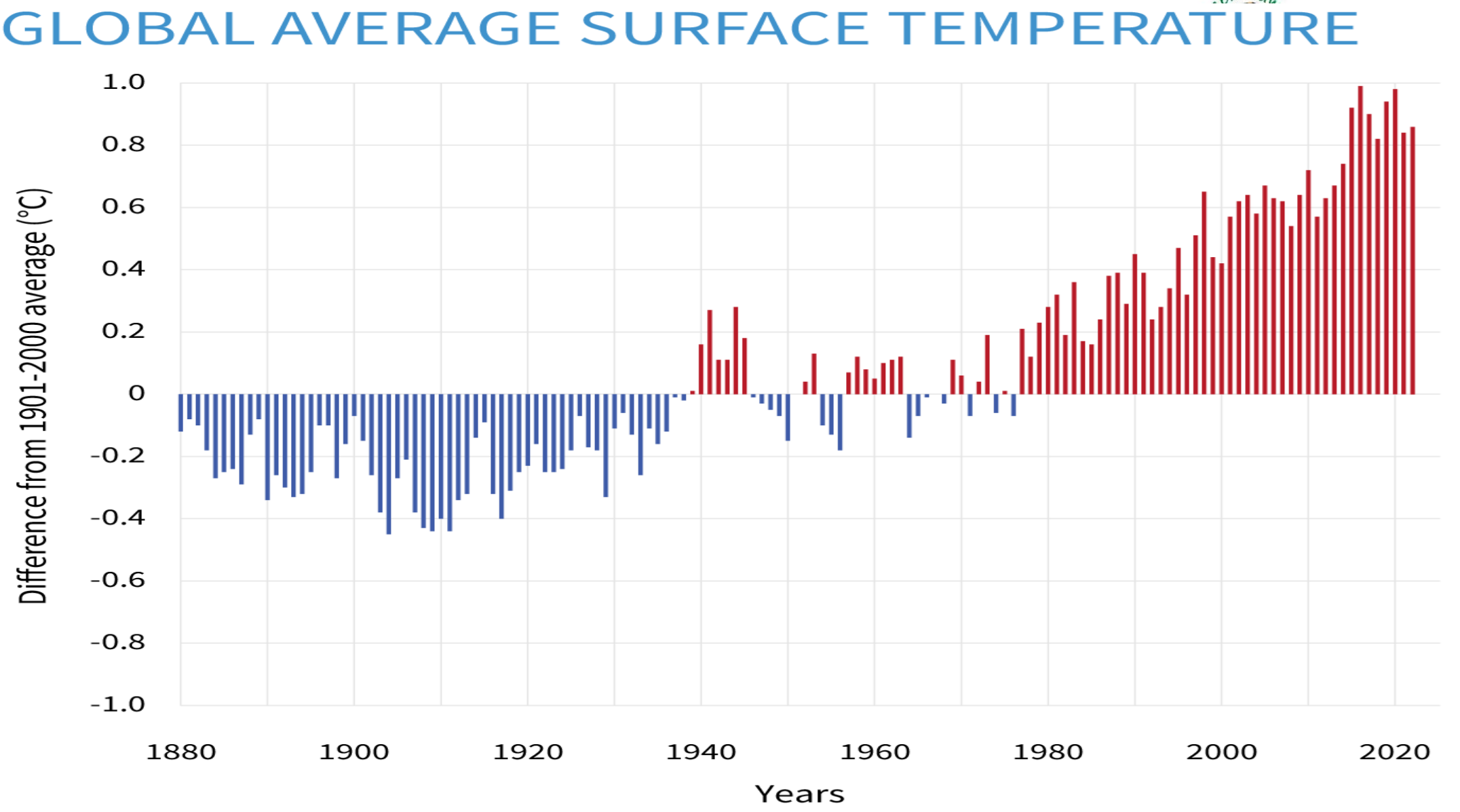
Photo Source: Yara Alshwairikh

Nearly 41% of Amphibian Species are globally threatened

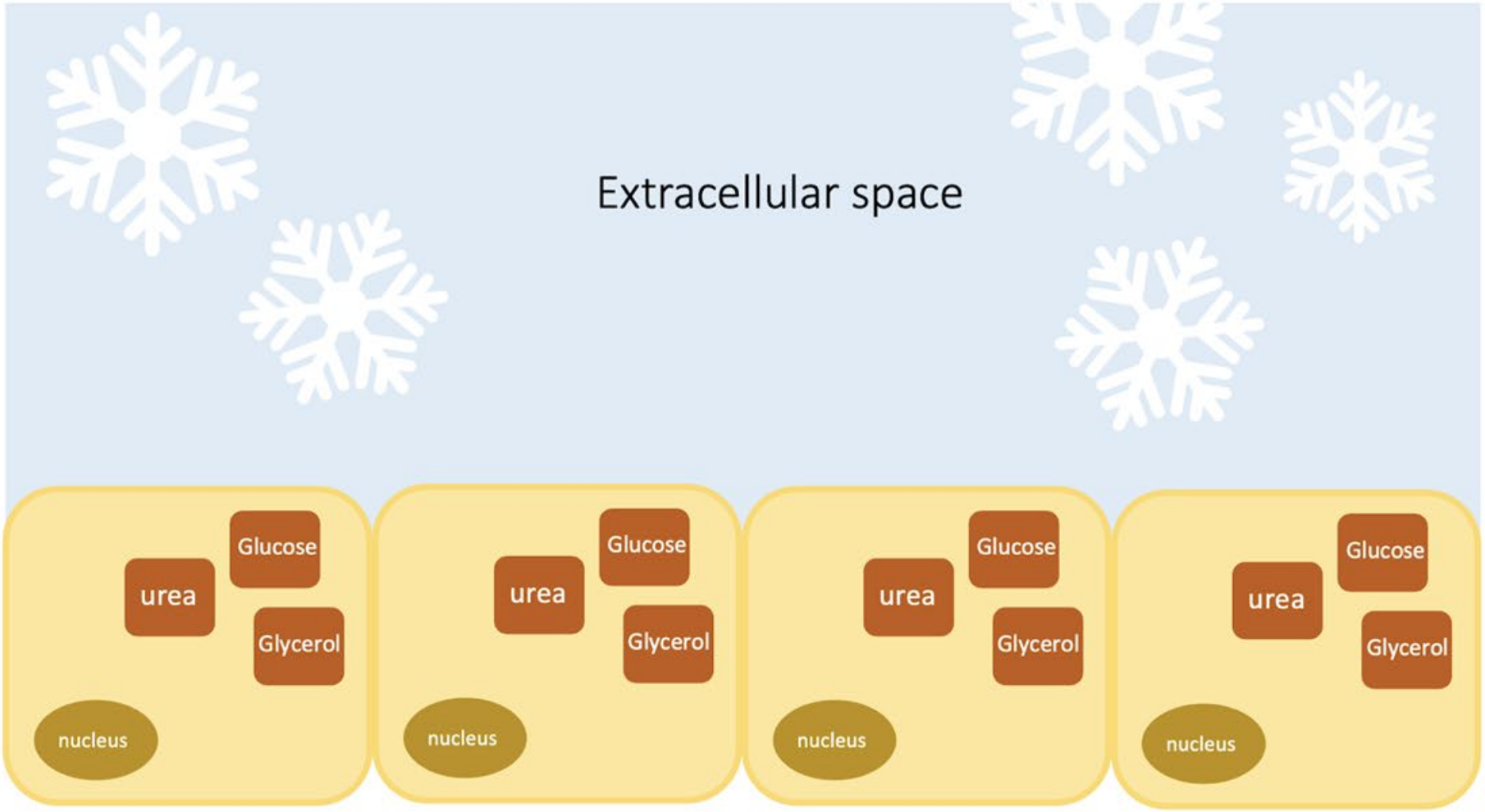


Data Sources: AmphibiaWeb, IUCN. 2017.

How do animals respond to changing climates?

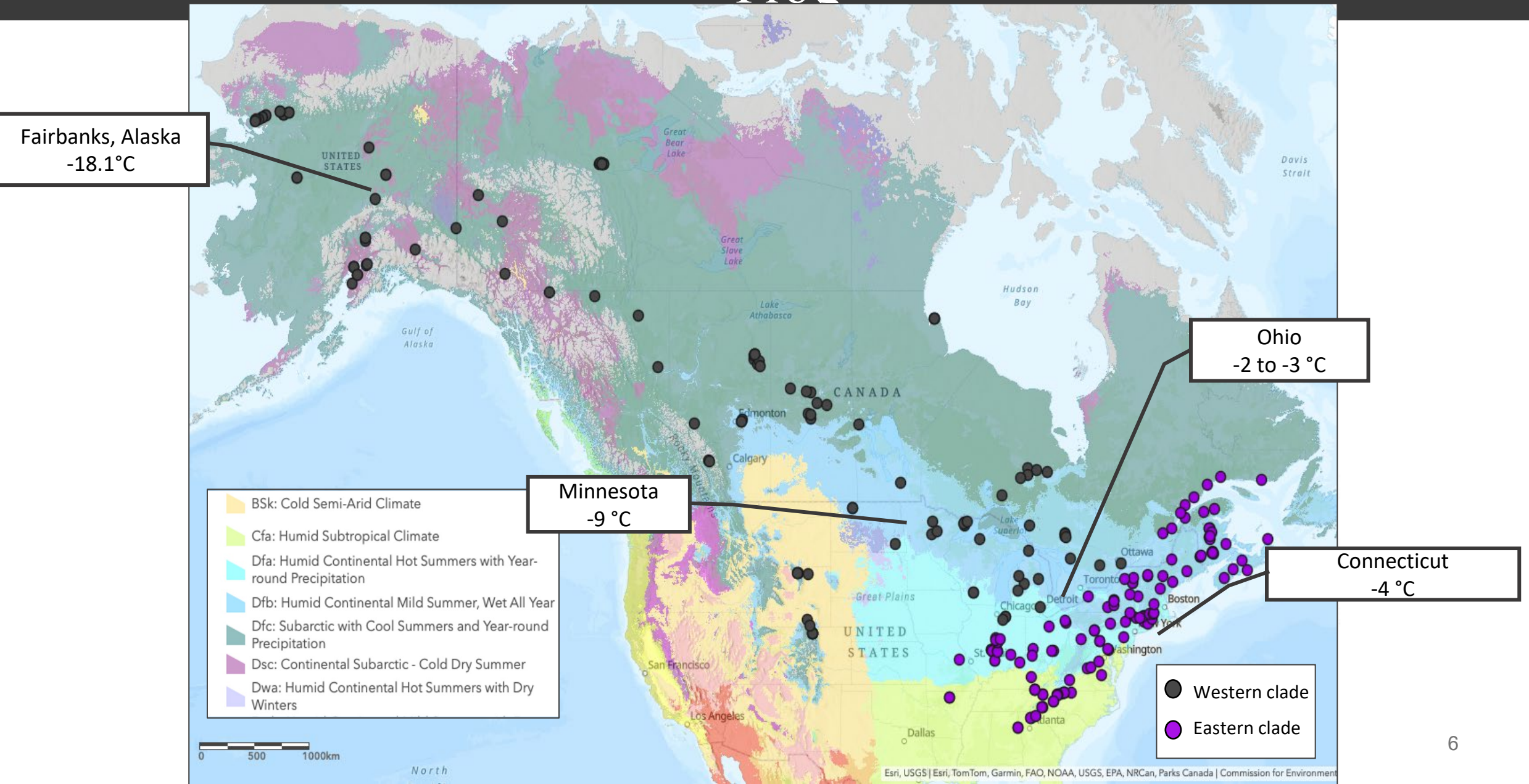
Wood Frog Freezing Adaptation for Overwintering Survival

https://www.youtube.com/watch?v=139NSc53RqQ

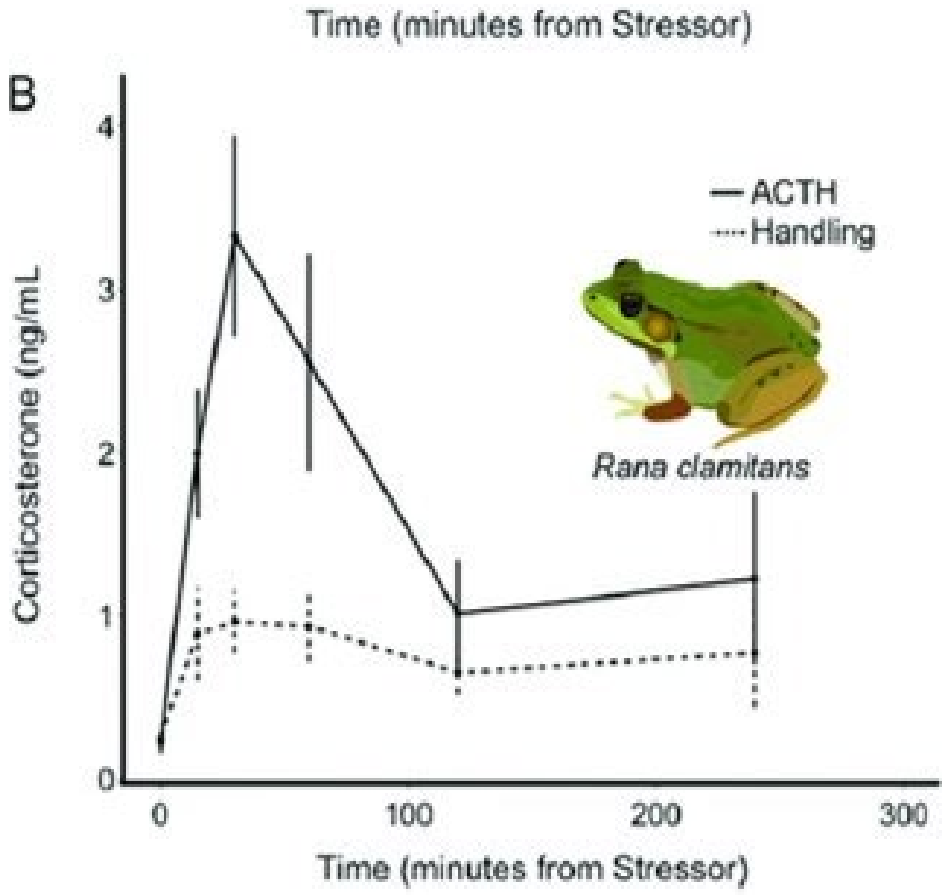


- Glucose is the primary cryoprotectant
- Sourced from glycogen stores in the liver and transported to other organs
- The glucogenic response is activated **immediately** upon freezing

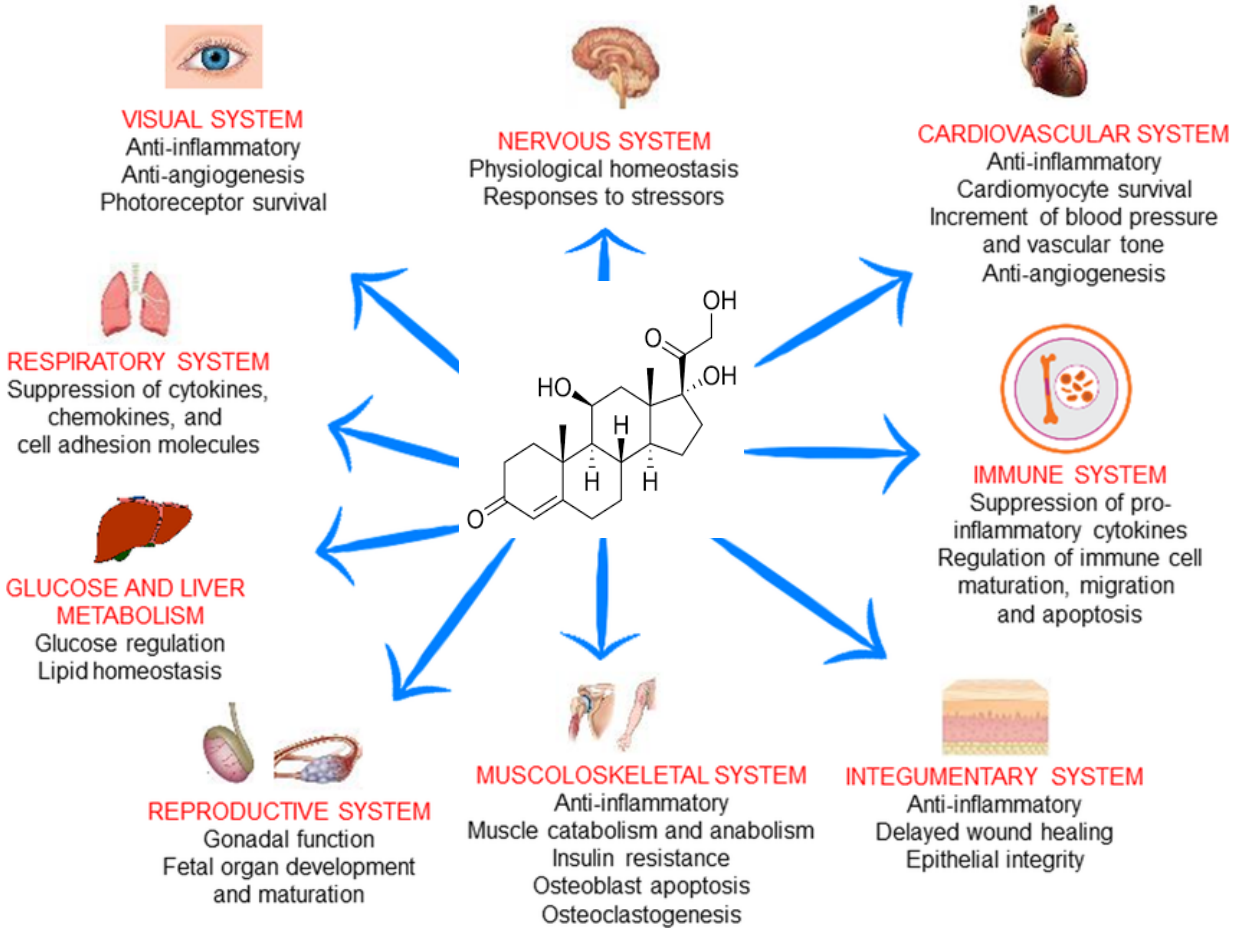
Local adaptation and plasticity: Geographic range of the Wood Frog



Amphibian Hormonal Stress Physiology

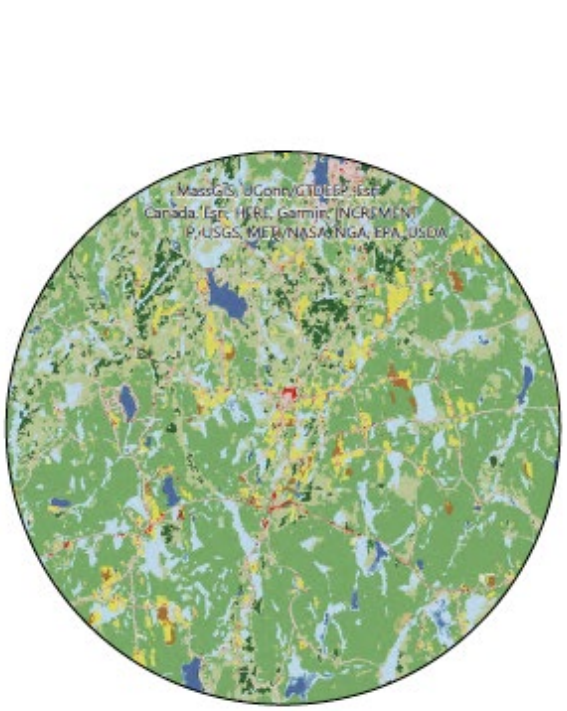


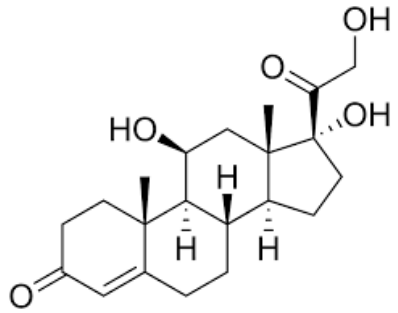
Hammond et. al, 2018



Adapted from: Zefferino et. al, 2020

Environmental impacts on stress physiology



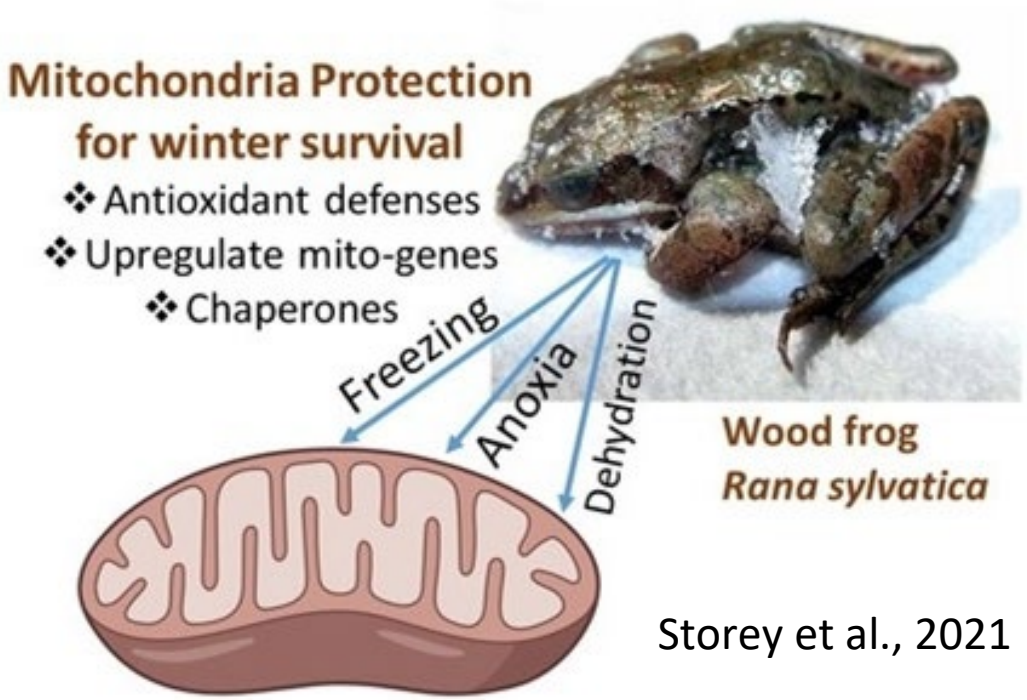


Research Questions & Objectives

<u>Research Question:</u> Does freezing condition and frequency of thawing influence physiological response in wood frogs?

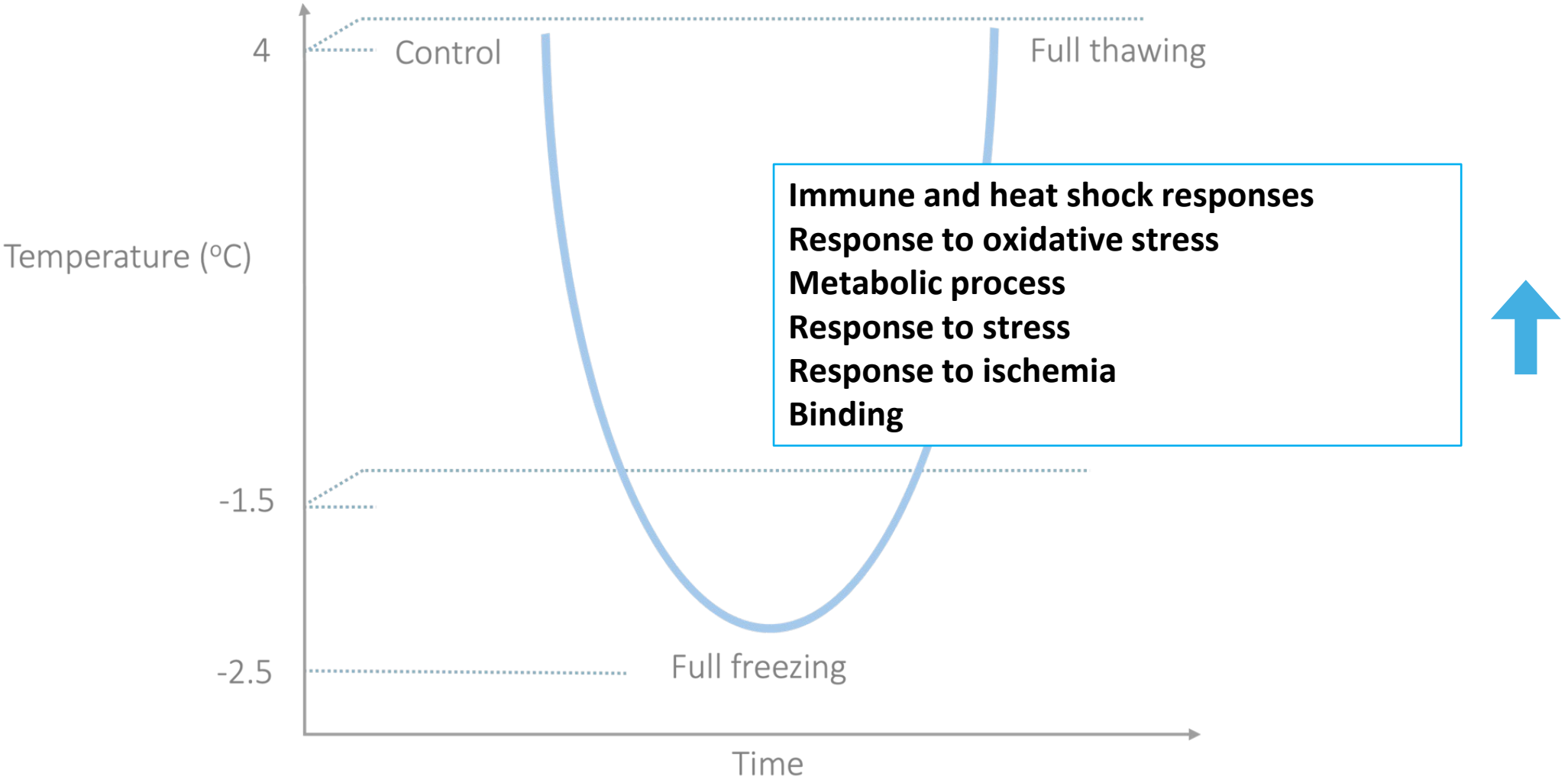
<u>Hypotheses:</u>

- CORT concentrations will be elevated in individuals undergo more frequent freeze/thaw cycles
- CORT increases will correlate with decrease immune blood biomarkers, decreased body condition, and decreased jumping performance



Storey et al., 2021

Upregulated genes in thawing

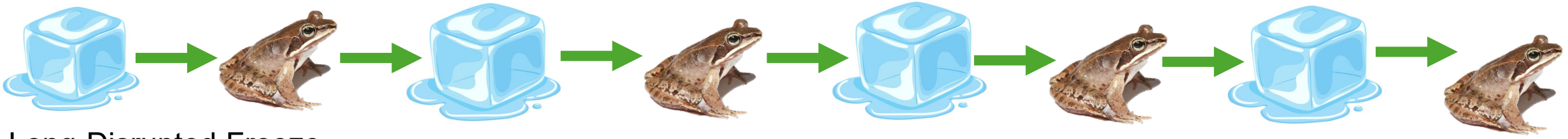


Sampling Frogs & Experimental Setup

Experimental Setup & Conditions

No Freeze

Short-Disrupted Freeze



Long-Disrupted Freeze

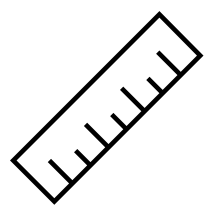
Steady Freeze

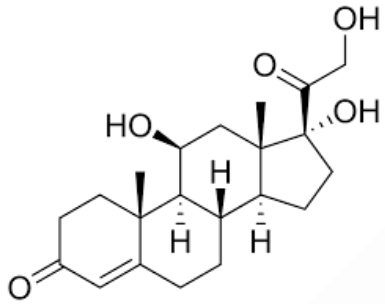
Outcomes of Interest

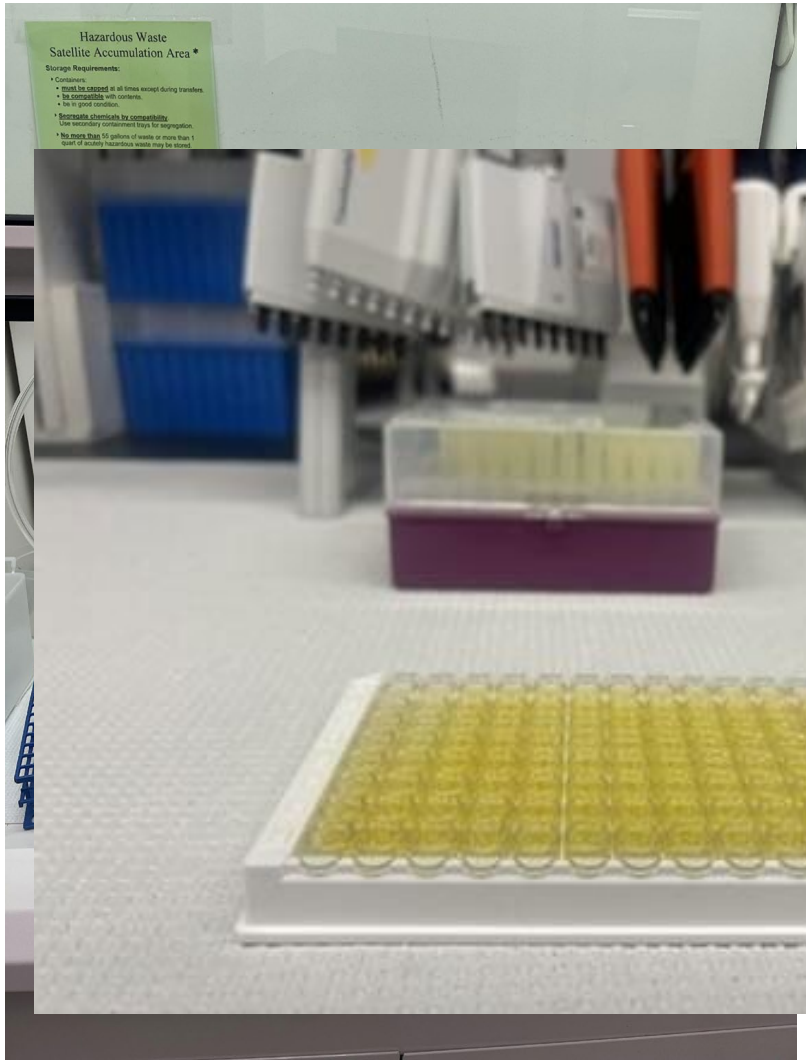
<u>Research Question:</u> Does freezing condition and frequency of thawing influence physiological response in wood frogs?

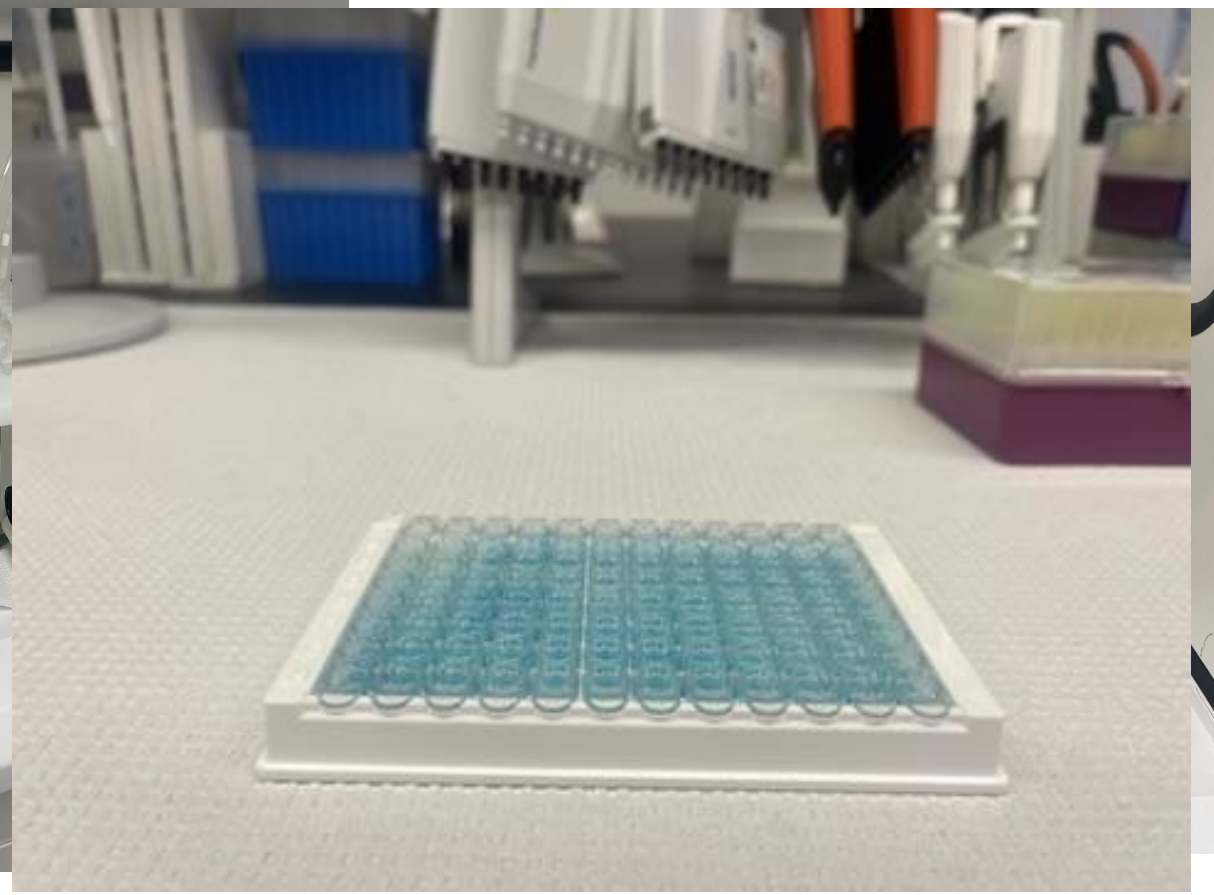
<u>Measured Outcomes:</u>

- CORT assay- pre and post freeze, and during thaw points
- Size and mass- pre and post freeze
- Jumping performance- pre and post freeze
- Blood immune markers- post freeze






Hazardous Waste
Satellite Accumulation Area *
Storage Requirements:
• Containers:
• must be capped at all times except during transfers.
• be compatible with contents.
• be in good condition.
• Segregate chemicals by compatibility
Use secondary containment trays for segregation.
• No more than 55 gallons of waste or more than 1 quart of acutely hazardous waste may be stored



Anticipated Results

Relative to Control Individuals:

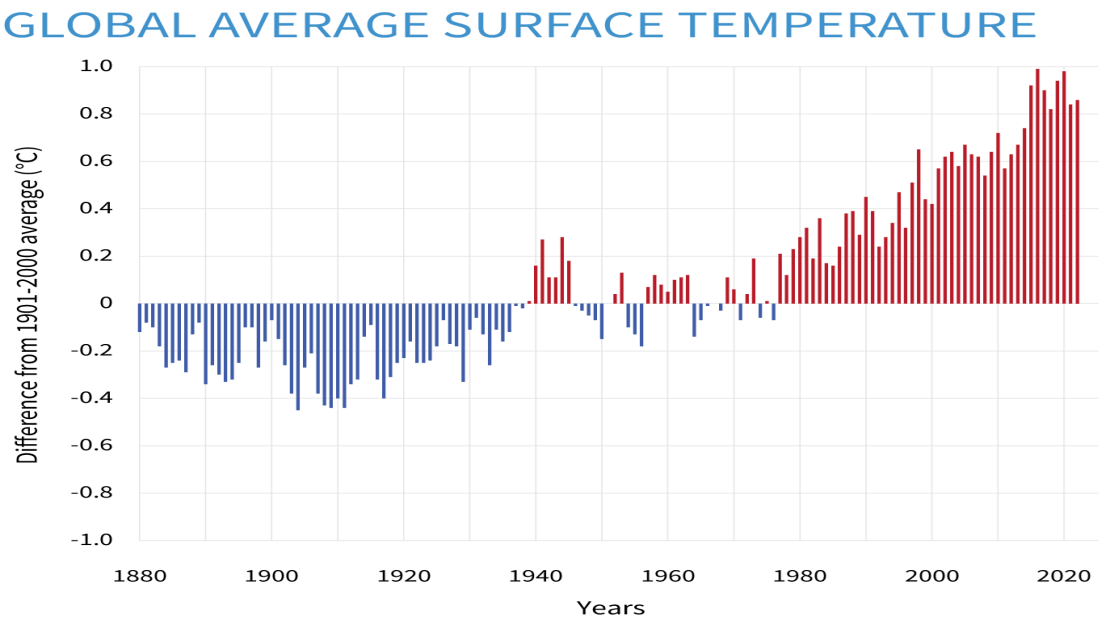
- More frequent freeze/ thaw Increased baseline CORT, Increased stressed CORT response, & Longer recovery
- More frequent freeze/ thaw decrease immune blood biomarkers
- More frequent freeze/ thaw decreased body condition (length & mass)
- More frequent freeze/ thaw decreased jumping performance

Experimental Condition

Relative	No Freeze	Short Disrupted Freeze	Long Disrupted Freeze	Full Freeze
No Freeze	---	↑ CORT & Immune Markers	↑ CORT & Immune Markers	↑ CORT & Immune Markers
Short Disrupted Freeze	↓ CORT & Immune Markers	---	↓ CORT & Immune Markers	↓ CORT & Immune Markers
Long Disrupted Freeze	↓ CORT & Immune Markers	↑ CORT & Immune Markers	---	↓ CORT & Immune Markers
Full Freeze	↓ CORT & Immune Markers	↑ CORT & Immune Markers	↑ CORT & Immune Markers	---

Conclusions &Future Research

- Winters in CT are becoming warmer and more variable leading to more freeze-thaw events and shorter freeze durations
- Variable winter conditions will impact the freeze/thaw cycles of wood frogs both in CT and across their range
- This study expands upon previous biochemical and genetic work and has a direct ties to potential conservation outcomes. This lays a foundation for assessing various physiological responses to adaptation and determining the limits of potential adaptation plasticity



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