Resilience in the Extremes: Tardigrade Cryptobiosis and Neural Recovery

Julia Drushell and Molly Jane Kirk

Faculty Advisor: Joel Rothman

Introduction

The tardigrade, a microscopic animal known for its resilience, can survive extreme environments by entering an inactive state called cryptobiosis.[1] Cryptobiosis is associated with large morphological changes, including changes to the nervous system. Using motor function as a readout of nervous system function, this project aimed to assess the resilience of the tardigrade nervous system following exposure to extreme environments. We have found that tardigrades return to baseline motor function within minutes, suggesting the tardigrade nervous system is extraordinarily stable in the face of large-scale physiological and morphological stress. Ultimately, this work aims to provide insights into neuroresilience with downstream applications to enhance neuroresilience in other more complex organisms.

Tardigrades survive extreme environments

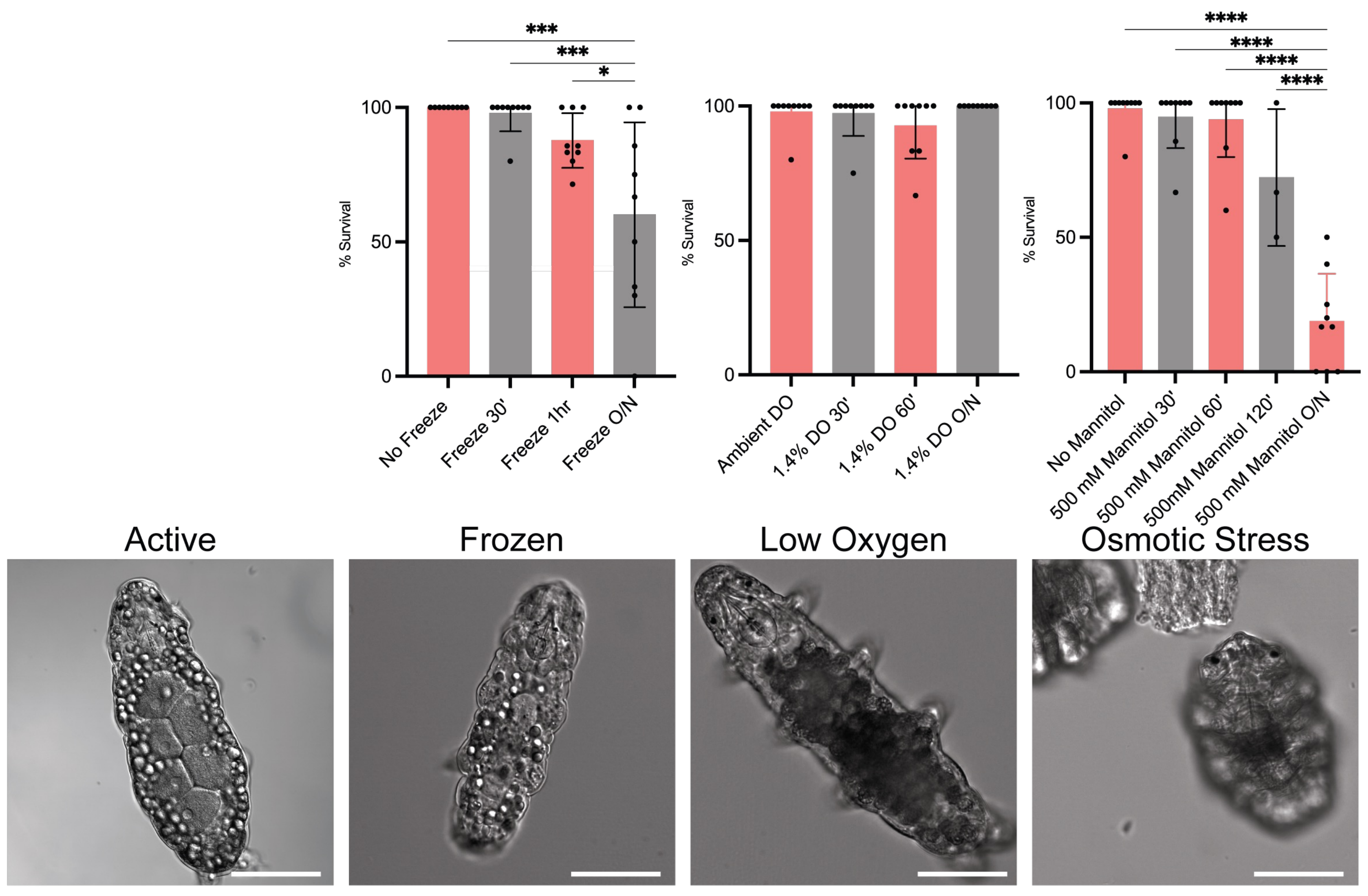


Fig 1. Tardigrades survive freezing, low oxygen, and osmotic stress. Cryptobiosis morphology is dependent on environmental stress.

Cryptobiosis includes gross morphological changes to the nervous system

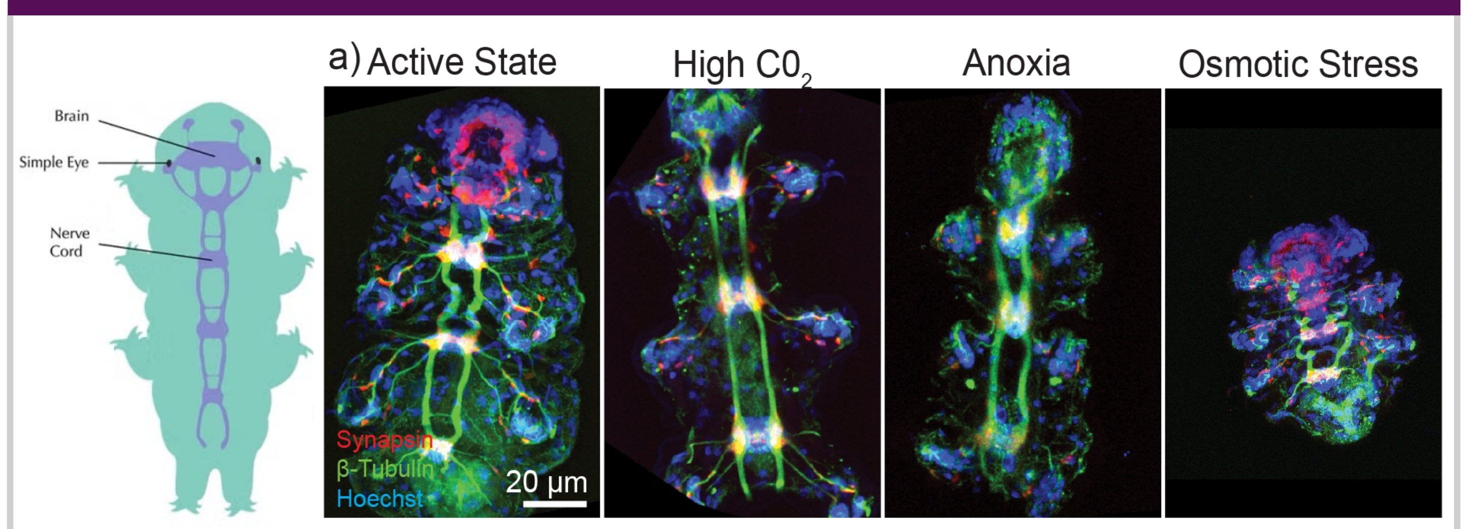
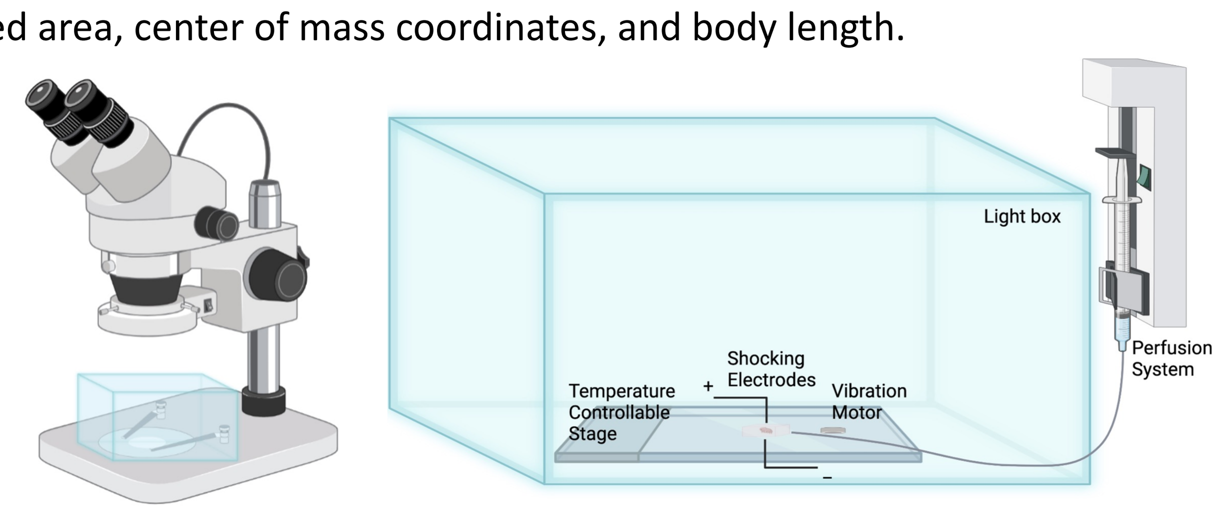


Fig 2. Immunostaining of the tardigrade nervous system across cryptobiotic conditions. Tardigrades were stained with synapsin (synapses, red), β-tubulin (axons, green), and Hoechst (nuclei, blue).

Methods

Tardigrades were cultured in spring water and algae. Prior to experiments, tardigrades were washed 3x with spring water. Survival was determined 24 hours after removal from stress. For behavior experiments, tardigrades were either exposed to 400μL mannitol (0.5M) or CO_2-sparged water (1.4mg/L O_2) with an Arduino-driven perfusion system, frozen in 100μL water at -20 °C, or anaerobically placed in 200μL N_2-sparged buffer incubated with Oxyrase (<0.6mg/L O_2). Videos were recorded with an iPhone on a dissection microscope. Videos were analyzed with Tardigrade Tracker, an image processing system that detected area, center of mass coordinates, and body length.



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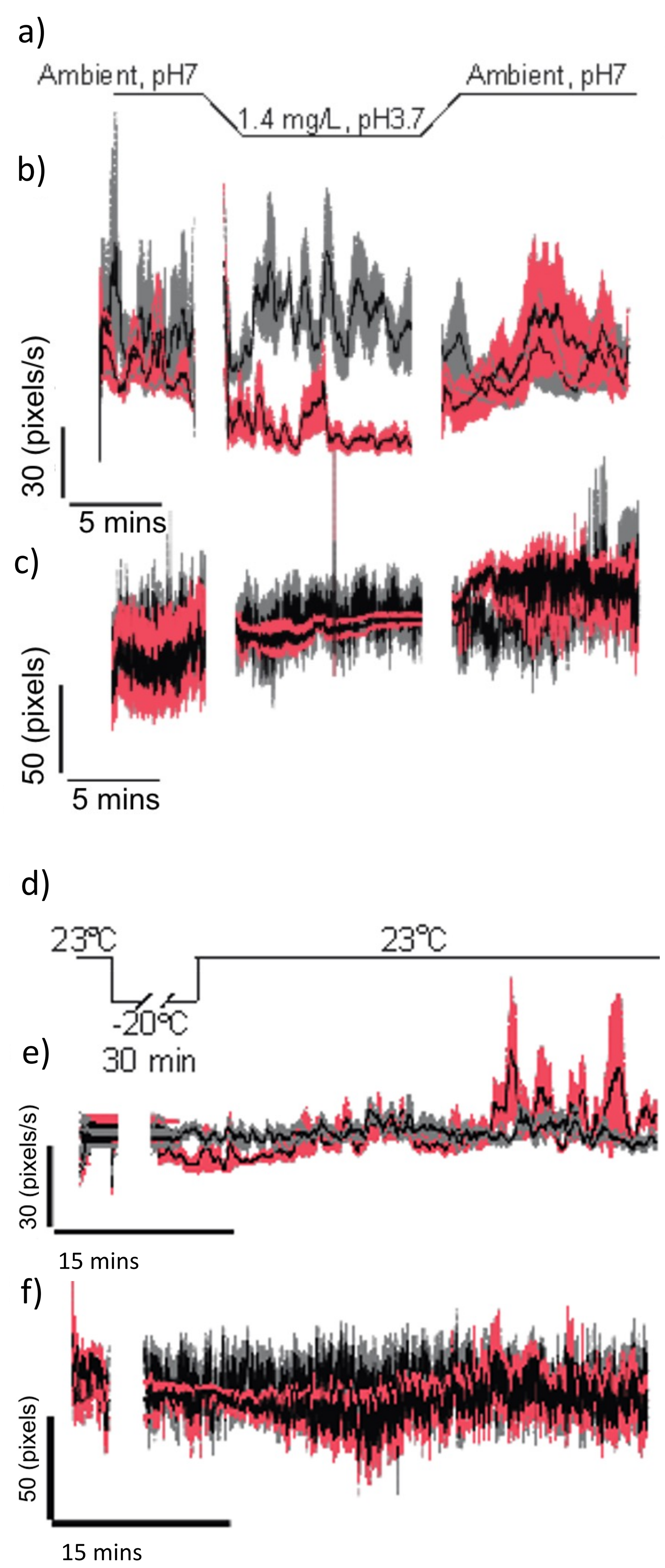
Tardigrades rapidly restore motor function following cryptobiosis



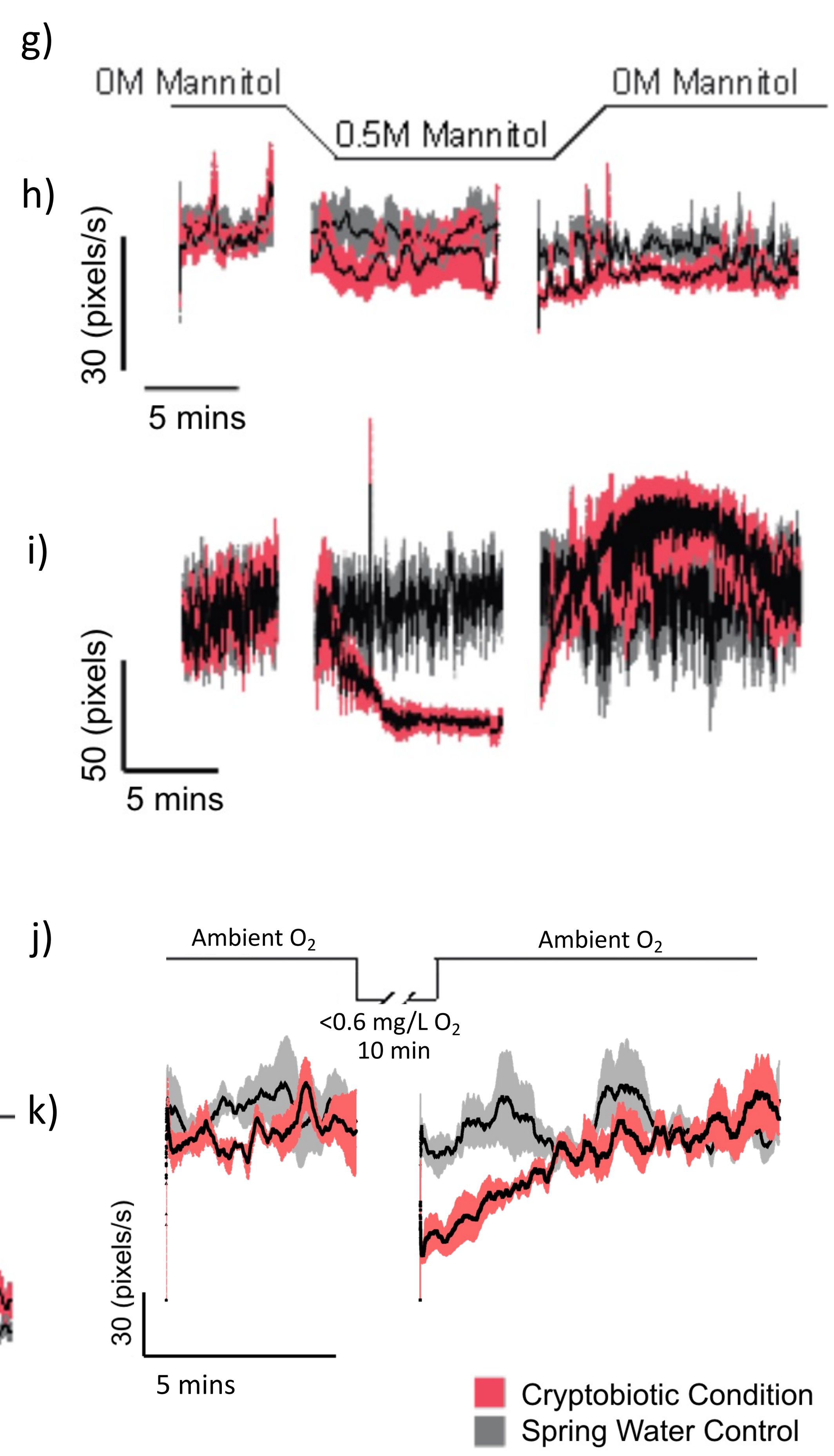


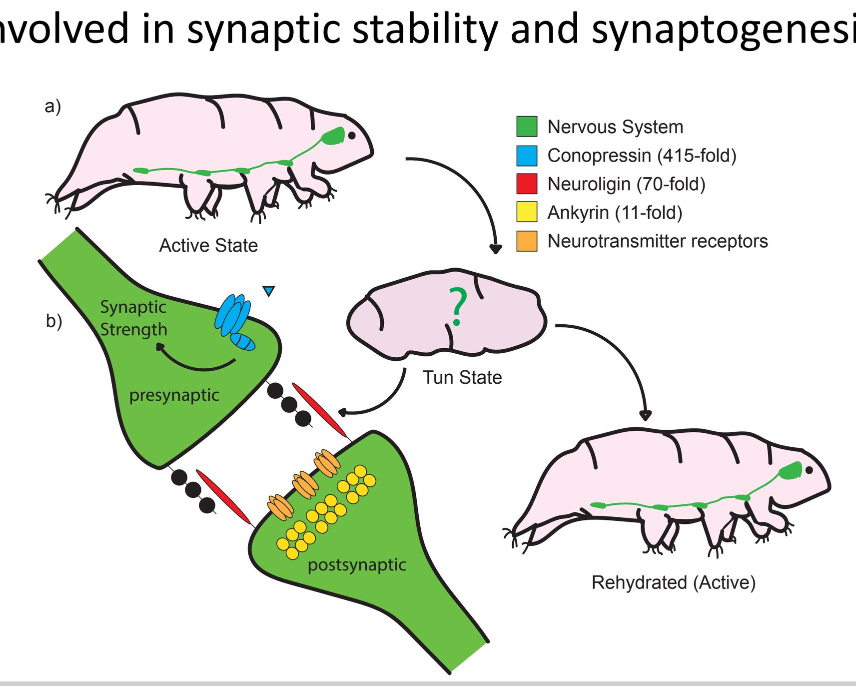
Fig 3. Tardigrades restore average velocity and body length rapidly following cryptobiosis. Impact of CO_2-sparged water on velocity (b) and body length (c). Impact of freezing at -20°C on velocity (e) and body length (f). Impact of 0.5M mannitol on velocity (h) and body length (i). Impact of N_2-sparged buffer on velocity (k). All data represented as mean (black) and SEM (red and grey).

Discussion

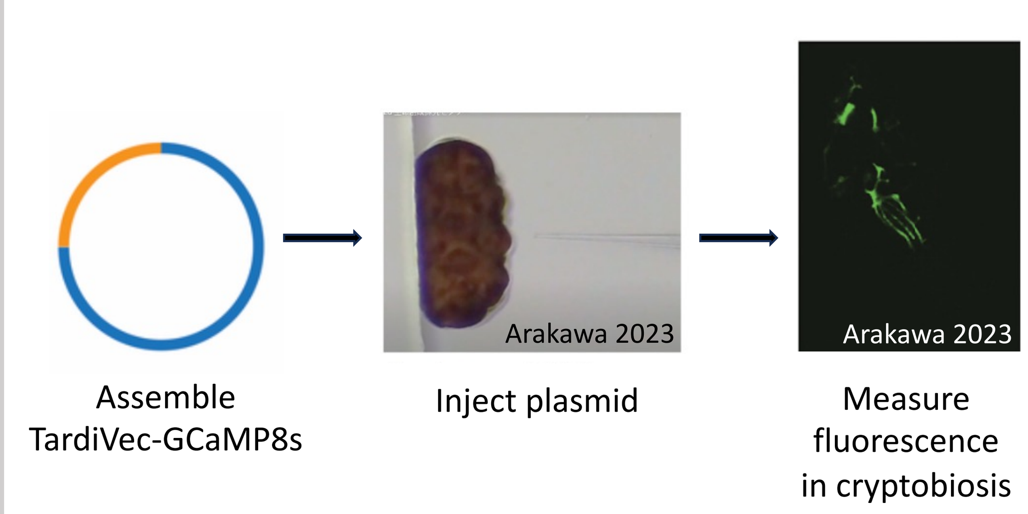
As tardigrades only contain about 200 neurons[2] (compared to humans with 86 billion[3]), they are an ideal and simplistic model for exploring neuroresilience. Despite gross anatomical changes to the nervous system during cryptobiosis, we found tardigrades can restore normal motor function within minutes. As we predict the lengthening and contraction of the nerve tracts during anoxybiosis and osmobiosis may disrupt synaptic connections, the ability of tardigrades to rapidly recover indicates they may possess mechanisms that protect or rebuild synapses. Further investigation of tardigrade synaptic stability may reveal fundamental mechanisms behind neuroresilience and holds promise for translation into more complex organisms.

Next steps

Motor analysis following knockdown of proteins involved in synaptic stability and synaptogenesis



Calcium imaging to investigate neuronal physiology during and in recovery from cryptobiosis



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