Investigating the Osmotic Stress Tolerance of the Tardigrade *Hypsibius exemplaris*
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**Introduction or Overview**

While the use of tardigrades as a model system for the study of extreme environments continues to increase, there is still much to be learned about the mechanisms these resilient animals use to tolerate such harsh conditions. As a result, the development of an accessible model system for the study of tardigrade survival and recovery after exposure to inhospitable conditions is paramount to elucidating the mechanisms of cryptobiosis. Cryptobiosis is a metabolic state in which tardigrades are able to reduce their metabolic activity to a point where it is essentially negligible, which allows them to survive otherwise lethal environmental conditions. Osmotic stress was chosen as the environmental condition of interest for this project due to the wide availability of sodium chloride (NaCl) and the limited amount of literature published on this specific topic.

**Research Hypothesis/Objectives**

I hypothesized that *Hypsibius exemplaris* would demonstrate some degree of osmotic stress tolerance. Consequently, the goals of this study were to determine the lowest concentration of aqueous NaCl that would induce osmobiosis in *H. exemplaris*, the highest concentration *H. exemplaris* could fully recover from, and the highest concentration *H. exemplaris* could tolerate.

**Methodology or Approach**

In a series of experiments focused on immediate or gradual osmotic stress, tardigrades were treated with the following methods:

1) Activity was recorded prior to experimentation
2) Exposure to Osmotic Stress (Immediate or Gradual)
   a) **Immediate Stress:** Tardigrades were exposed to either an aqueous NaCl solution or a control solution (deionized water) for 24 hours
   b) **Gradual Stress:** Tardigrades were acclimated in aqueous NaCl solutions or control solutions (deionized water) for 24 periods that increased in increments of 50 mOsm/kg until the desired experimental concentration was reached, after which tardigrades spent 24 hours at that desired concentration
3) Tardigrade activity was recorded and the tardigrades were washed with deionized water in a three-well glass spot plate
4) Tardigrades were transferred to recovery solutions (deionized water containing *Chlorella* algae) and activity was recorded every 24 hours for two days following this transfer

**Major Outcomes, Results and Conclusion**

My hypothesis was supported because *H. exemplaris* did demonstrate osmotic stress tolerance.

1) Lowest concentration to induce osmobiosis = 100 mOsm/kg
2) Highest concentration that allowed for full recovery = 100 mOsm/kg
3) Highest tolerable concentration = 400 mOsm/kg

*H. exemplaris* was not classified as a tardigrade species with a high tolerance for osmotic stress. Improving this tentative model system by either using another species, such as *Ramazzottius oberhauseri*, or improving upon the experimental procedure could allow for reproducible experiments that could contribute to the future study of adaptations in extreme conditions.