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Limor Raz
Columbus State University

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**REACTION OF TARDIGRADES (*Hypsibius*) TO PHYSICAL
AND CHEMICAL EXTREMES**

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Senior Research Paper

**Reaction of Tardigrades (*Hypsibius*) to Physical
and Chemical Extremes**

Limor Raz

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Columbus State University

Advisor: Dr. Cleveland

Second Reader: Dr. Hendricks


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Survivability and Reaction of *Hypsibius* to Physical and Chemical Parameters

by
Limor Raz

A Thesis Submitted in Partial Fulfillment of
Requirements of the CSU Honors Program

for Honors in the degree of
Biology, B.S.
in
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Thesis Advisor	<u></u>	Date	<u>1/20/05</u>
Committee Member	<u>Heidi Z. Hendricks</u>	Date	<u>7/21/05</u>
Committee Member	<u>Julie Ballenger</u>	Date	<u>7/26/05</u>
CSU Honors Committee Member	<u>Barbara J. Hunt</u>	Date	<u>1/18/05</u>
Coordinator, Honors Program	<u>Barbara J. Hunt</u>	Date	<u>1/18/05</u>

Abstract:

The process of cryptobiosis is characterized by a complete state of body water dehydration and undetectable metabolic activity, which allows tardigrades (*Hypsibius*) to escape environmental stress when conditions become unfavorable. In our research, *Hypsibius* was tested in a laboratory under pH levels of 3 to 10 and a salinity concentration gradient. In the pH experiment, we placed tardigrades in petri dishes with agar solution in an experimental pH and measured the average distance traveled. We expected them to respond favorably to a neutral pH of 7 which is common in most living organisms by showing increased movement. In the salinity experiment we placed the tardigrades in various salt concentrations and measured the number of organisms that were dead, alive and those that transitioned into cryptobiosis. We predicted that tardigrades will enter cryptobiosis in order to maximize their fitness under the most extreme conditions. Results obtained were significant in the salinity experiment, showing that as environmental conditions became more extreme, the rate of transition into cryptobiosis increased accordingly. These findings supported the proposed hypothesis that tardigrades will enter cryptobiosis in order to increase their survival rate in harsh environments, which will otherwise cause death. In contrast, the results in the pH range experiment were not significant. Tardigrades responded favorably and demonstrated increased movement in the pH range of 6 to 8, which leads us to conclude that they may be found in these types of soils.

Introduction:

Only selective organisms such as nematodes, crustaceans and Chironomids are capable of entering cryptobiosis under stressful environmental conditions (Watanabe et al. 2003). Tardigrades, commonly known as “water bears,” also exhibit this unique property of forming a protective shell as they enter cryptobiosis. Belonging to the Phylum Tardigrada, tardigrades are currently classified among the arthropods (Jackson and Raw 1966). They were appropriately named water bears by Thomas Henry Huxley in 1869, due to their inherent pawing behavior which closely resembles the movements of a bear (McInnes and Norman 1996). As their name reveals, tardigrades (*tardus*-slow, *gradu*-step) are slow movers, a property that makes them ideal experimental organisms (Beasley 2001).

There are currently 900 recognized species of tardigrades occupying various ecological niches ranging from terrestrial and marine habitats to freshwater environments (Beasley 2004). Some species live in the water film surrounding leaves of moss and lichen, while others adhere to and feed on aquatic algae at extreme depths (Miller 1999). Once food becomes scarce, tardigrades resort to extreme forms of cannibalism, feeding on nearby tardigrades of their own species (Crowe and Cooper 1971). Likewise, tardigrades are often found in their natural environment living in close association with rotifers and gastrotriches (Mach 2000). Since tardigrade distribution in nature is still unknown, considerable scientific research has been pursued in an attempt to discover their natural habitat.

Mature tardigrades range in size from 0.1mm to 1.2 mm, depending on the species (Middelton 2003). The anatomy of a tardigrade consists of a plump, cylindrical body and four pairs of claw-bearing legs. The claws allow the organism to cling to plant surfaces by secreting a special sugar-like solution. The dorsal side of the body is made from albuminoid, a tough protein which composes the outer cuticle. Periodically, the cuticle is shed to allow for growth as a new one is secreted by the underlying epidermis (Kinchin 1994).

When extreme environmental conditions are introduced, tardigrades survive by forming a protective shell around their body and transitioning into the cryptobiosis state of lowered metabolic activity and desiccation. Once rehydrated, tardigrades will return to an active form of life. The ability to enter the cryptobiotic state is facilitated by trehalose (TRH), a disaccharide. Research conducted by Pereira et al. (2004) revealed the sugar's ability to interact with the cell's phospholipid bilayer by forming hydrogen bonds which stabilize the membrane, thus enabling the cell to resist the disruptive effects of dehydration. Experiments on cryptobiosis conducted by Kinchin in 1994 have demonstrated tardigrade survivability in extreme temperatures ranging from -200°C to 151°C and doses of X-rays 250 times greater than the average mammal. In addition, tardigrades in the cryptobiotic state are also known for their longevity. A discovery of dried moss samples in a botanical museum showed tardigrades reviving upon rehydration after lying dormant for 120 years (Darling 1999).

Based on experimental observations and the latest research, it can be hypothesized that tardigrades could travel to space among the dust on a piece of rock if the earth was destroyed. NASA is currently testing the effects of gravity and space conditions on

tardigrade population densities by measuring their survivability in space (Gilbert et al. 2003). Nevertheless, more research is necessary before tardigrades can be effectively sent to other bodies of the solar system and before the possibility of establishing an artificial ecosystem outside planet Earth can be considered (Mullen 2002). As a result, the goal of the following experiments was to collect data about the extreme conditions which tardigrades can withstand and to validate the factual information given in the literature by testing their reaction under various experimental conditions. Tardigrade response to a pH range and a salinity concentration gradient was tested. Tardigrades alive, dead and those transitioning into cryptobiosis were determined in the salinity experiment according to their characteristic shape and in the pH experiment based on the movement (+/-) in the direction of a specific pH. We hypothesize that tardigrades will enter cryptobiosis in order to increase their survivability in extreme environmental conditions and exhibit increased movement under the pH of 7.

Materials and Methods:

Hydrated tardigrade specimen vials (*Hypsibius*) were obtained from Ward's Biological Company. Each experiment incorporated the use of one vial of tardigrades, with a total of two vials used throughout the experiment. Prior to the experimentation, tardigrade vials were examined to ensure that the organisms were alive. Data gathered in the salinity concentration range experiment was analyzed using the chi-square test and the Pearson correlation coefficient, whereas pH range data was evaluated using ANOVA. Alive tardigrades were distinguished in the salinity experiment by remaining active, floating to the top of the petri dish and displaying their claw-bearing legs. The dead tardigrades, on the other hand, sank to the bottom of the petri dish and did not exhibit any

movement or curled into a ball. Tardigrades that entered cryptobiosis were surrounded by a protective shell and were curled into a spherical ball. These tardigrades were found at the bottom of the petri dish.

pH Range:

The objective of this experiment was to assess tardigrade reaction to specific pH levels by measuring average movement. The null hypothesis is as follows: there will be no significant difference between the different pHs and their influence on tardigrade movement. Thirty-six petri dishes were prepared with a thin layer of nutrient agar solution (a thick layer of agar solution will prevent tardigrade movement during the experiment). Nine treatments of the following pH solutions were tested and labeled accordingly: pH 3, 4, 5, 6, 7, 8, 9, 10 and control, with four trials of each. PH's 1 and 2 were omitted from the experiment due their destructive acidic nature. After sterilizing a spatula and brass cork borer using a Bunsen burner, each petri dish's lid was lifted and the cork was pressed into the agar solution to create four wells. Three equally spaced wells were created along the perimeter of the petri dish and one in the center. The experiment was performed under the most sterile conditions in order to minimize bacterial contamination. The spatula was then used to remove each circular agar piece from each well. Using sterile pipets, two drops of each pH solution were pipetted into each peripheral well of a particular dish. The control dish remained empty at this stage. The tardigrade vial (pH 6) was swirled before the transfer of the specimen, and 3 drops of the solution were pipetted in the center well of each dish. The dishes were then placed on a stationary tray to minimize unnecessary movement and left at room temperature, exposed

to an indirect light source (Beasley 2001). The distance traveled by each tardigrade in each petri dish was measured after seven days by using a ruler. The distance from the center well to the middle of the bacterial colony created by the moving tardigrades was measured in a straight line. Only the closest and farthest radiation lines were recorded and the average movement distance was then calculated for each dish.

Salinity Concentration Range:

This part of the experiment determined tardigrade reaction to different salt concentrations by recording the number of tardigrades dead, alive and those that transitioned into cryptobiosis. The null hypothesis is as follows: there will be no significant difference between the number of tardigrades that remain alive without transitioning into cryptobiosis and the number that transitioned into cryptobiosis at various salt concentrations. Five beakers containing 250 mL of purified water each were prepared and mixed with the following measured sodium chloride concentrations: 0g/mL, 0.1g/mL, 0.3g/mL, 0.5g/mL and 0.7g/mL. The 0g/mL vial was used as a control. A stirring pellet was added to each solution and placed on a stirring plate for one minute in order to create a homogenized mixture. Five mL of each solution were then pipetted to a labeled, grid petri dish along with one mL of tardigrade solution. The petri dish was swirled around to allow for better mixing. A total of five trials were prepared for each treatment following the procedures stated above. The experiment remained in lighted, room temperature conditions for fifteen hours. Tardigrade response to the experimental conditions was then analyzed by recording the number of tardigrades dead, alive and those that entered cryptobiosis.

Results:

A significant difference was found in the salinity concentration range experiment ($X^2 = 142.51$). As the salt concentration increased, the number of tardigrades alive decreased, as the number of tardigrades dead increased in a linear fashion. A positive correlation coefficient of $r = 0.90$ was also calculated between the number of tardigrades dead and those that entered cryptobiosis (Figures 2 and 3). On the other hand, results in the pH range experiment demonstrated the negligible effects of pH on tardigrade movement (1-way ANOVA $F_8=0$, $P=0.05$). Tardigrades remained most active between pH's of 6 and 8 (Figure 1). For additional data tables please refer to Appendix I at the end of the paper.

Discussion:

Tardigrades were able to survive environmental stress by entering cryptobiosis when placed under severe environmental stress. The salinity concentration range experiment supported a strong correlation between the number of tardigrades that died and those that transitioned into cryptobiosis, demonstrating that the organisms used the cryptobiosis mechanism to escape death. Therefore, we can assume that as environmental conditions became increasingly more severe, tardigrade survival rates increased as they transitioned into the cryptobiotic phase. The nature of this relationship supports the literature findings of Kinchin (1994) and confirms the original hypothesis. In addition, our findings parallel those of Bertolani et al. (2004) and correspond to the results obtained in their study on cryptobiosis dormancy in tardigrades. Bertolani et al. concluded that cryptobiotic survivability represented a successfully adaptive strategy in

environments with hostile conditions, thus providing a protective mechanism. Although the exposure to a pH range did not seem to have a significant effect on the tardigrade populations, several trends were observed. It appears that tardigrades prefer neutral pH levels ranging from 6-8 (Figure 1). Since most living organisms prefer the neutral pH of 7 in order to keep their internal mechanisms intact, this finding is not surprising (Miller, 1999). As a result, we can predict that tardigrade distribution in nature will be found in neutral soils with a pH range of 6-8.

Further research is still necessary in order to determine the durability and longevity of the cryptobiotic phase. Before tardigrades can participate in the terra-formation process, a better understanding of the trehalose survival mechanism must be acquired.

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Cryptobiosis in Larvae of *Polypedilum vanderplanki*. *The Journal of*

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Figure 1: The Average Distance Travelled (cm) by Tardigrades under Acidic and Basic pH Solutions (+/- S.E.)

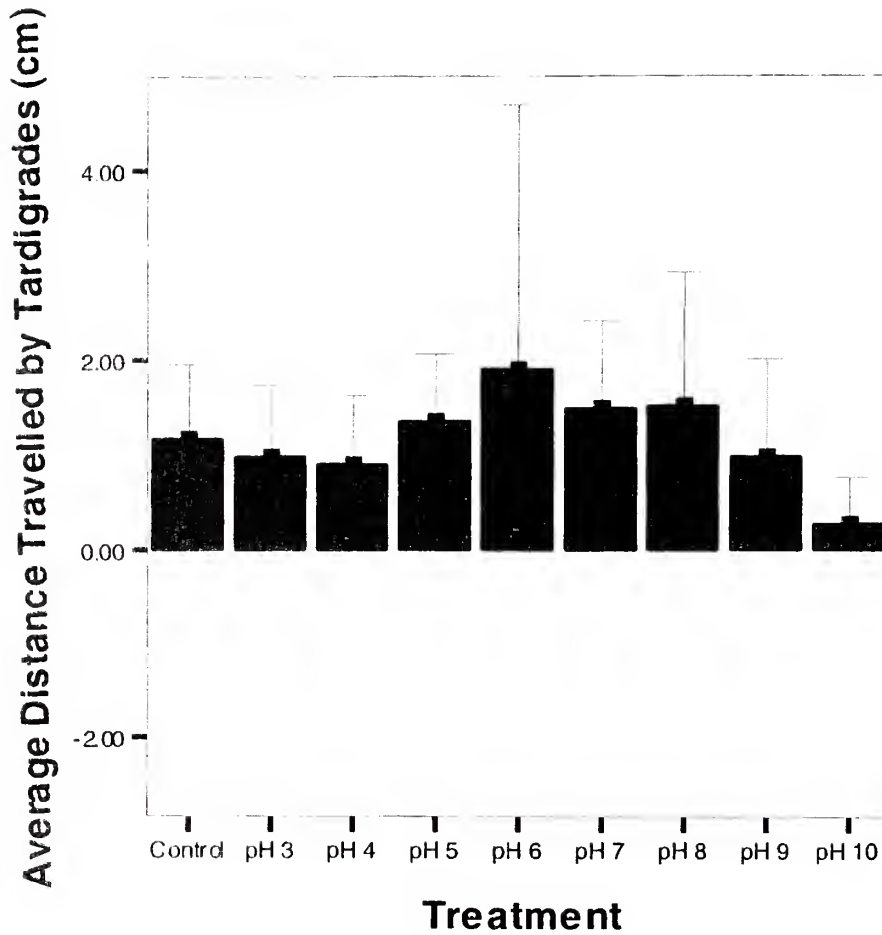


Figure 2: Tardigrade Survivability Rate (+/- S.E) as a Function of Increasing Salinity

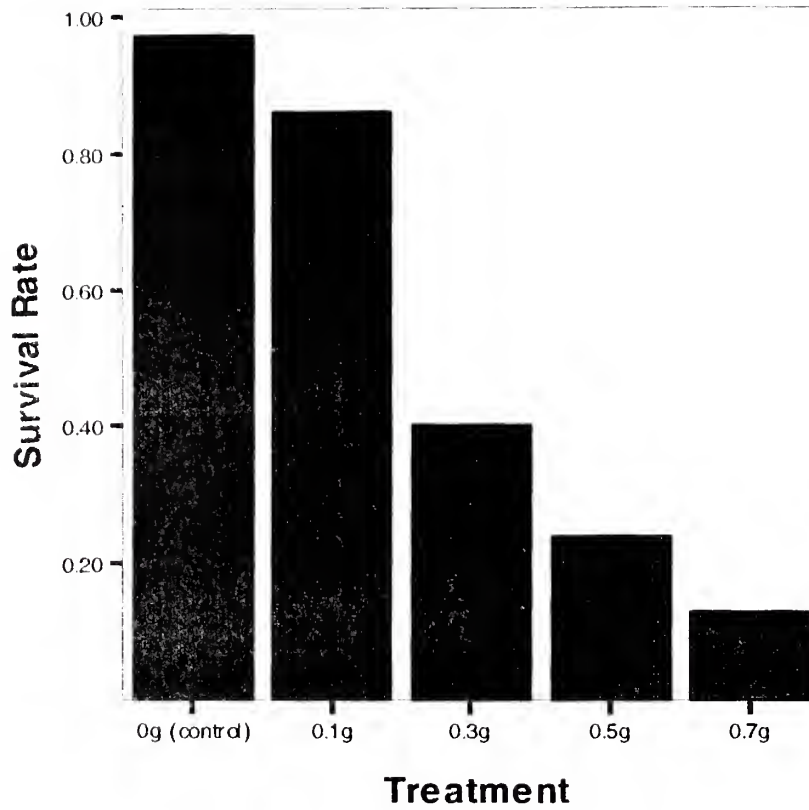
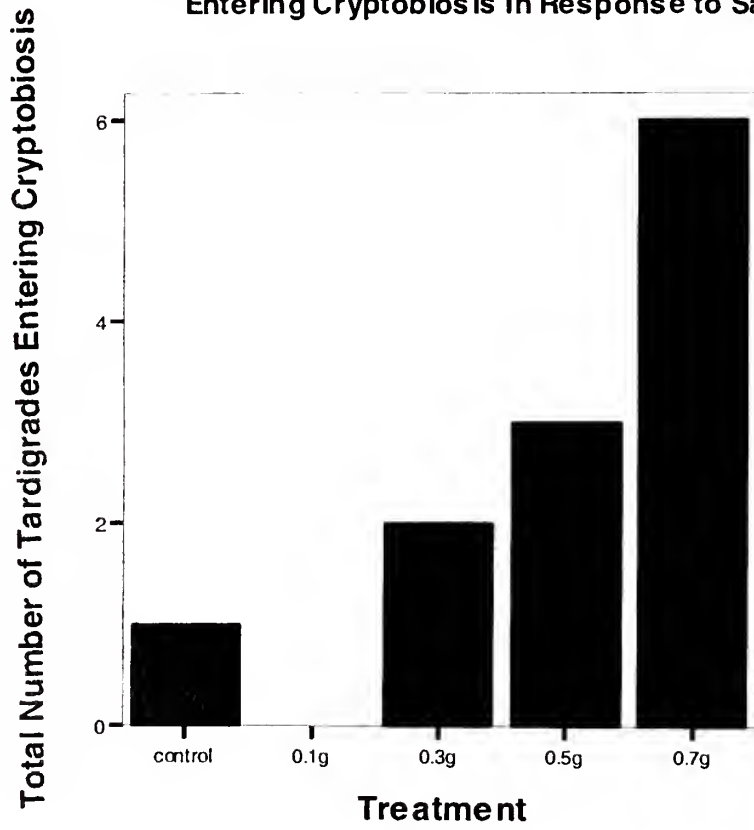


Figure 3: A Summary of the Total Number of Tardigrades (+/- S.E) Entering Cryptobiosis in Response to Salinity Levels



Appendix I:

Table 1: The Average Movement of Tardigrades (cm) in Extreme pH Environments		
Treatment	Average Distance Travelled by Tardigrades (cm)	Average
Control	1.05	1.15
	1.8	
	0.6	
	1.15	
pH 3	1.65	0.975
	0.9	
	0.8	
	0.55	
pH 4	1.5	0.875
	0.9	
	0.7	
	0.4	
pH 5	0.85	1.338
	1.7	
	1.75	
	1.05	
pH 6	0.7	1.138
	1.3	
	4.5	
	1.05	
pH 7	1	1.488
	1	
	1.85	
	2.1	
pH 8	1.8	1.513
	2.15	
	0.2	
	1.9	
pH 9	1.05	0.975
	1.85	
	0.55	
	0.45	
pH 10	0	0.263
	0	

	0.45	
	0.6	

Table 2: Tardigrade Survivability in High Salt Concentration Environments

Treatment	Number of Tardigrades Alive	Number of Tardigrades Dead	Total
Control (0g)	66 (36.18)	2 (31.82)	68
0.1g	50 (30.86)	8 (27.14)	58
0.3g	23 (30.32)	34 (26.68)	57
0.5g	13 (29.26)	42 (25.74)	55
0.7g	8 (33.52)	55 (29.48)	63
TOTAL	160	141	301

Table 3: Total Number of Tardigrades Entering Cryptobiosis in Salty Environments

Treatment	Total Number of Tardigrades Entering Cryptobiosis
Control (20° C)	1
(-70° C)	0
(0° C)	2
(50° C)	3
(70° C)	6
TOTAL	12

