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Limited thermal plasticity in high mountain tropical water bears

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Abstract: Tardigrades, are a phylum of microscopic organisms well known for their resistance to extreme conditions thanks to adaptations such as cryptobiosis, in which they are able to suspend their metabolism until more favorable conditions arise. These organisms are found in a wide range of environments, and high Andean mountains are not the exception, even though weather conditions are highly variable during the day, let alone a month. An experiment was conducted at an altitude of 2600 m in Bogotá, Colombia, to evaluate the resistance of *Macrobiotus* sp. found on trees. Three sets of fifteen tardigrades were exposed to temperatures of 20 °C, 30 °C, and 40 °C, with a heating rate of 0.5 °C/min. The tardigrades were then examined to determine the number of living, dead, and "tun" organisms. At 20 °C, 22% of the individuals were dead; at 30 °C, 35.56% were dead; and at 40 °C, 46.67% were dead. No tardigrades were found in the "tun" state at any temperature. Therefore, the data show an increase in mortality as the temperature rises. However, considering the climatic conditions recorded, studied tardigrades are able to withstand higher temperatures than those they are usually exposed to. The lack of the "tun" state implies that high temperatures, at least at this rate, do not trigger this state, whereas conditions like desiccation might. More research is needed to explore this further.

Key words: Tardigrades, thermotolerance, high temperature, surviving, Andean mountains

Tardigrada is a phylum of free-living microscopic bilaterians within the protostome superclade Ecdysozoa (Jørgensen et al., 2018). These invertebrates, which have four pairs of lobopod legs terminating in claws or sucking disks, are distributed worldwide in freshwater, marine, and terrestrial microhabitats (Nelson, 2002). They are divided into two major evolutionary lineages: eutardigrades and heterotardigrades. Eutardigrades are semiterrestrial and limnic, characterized by the presence of a cloaca, Malpighian tubules, and the absence of sensory organs, which are instead found in heterotardigrades (Møbjerg et al., 2018).

Within the lineage of eutardigrades, the order Parachela is distinguished by the absence of cephalic papillae and the presence of two claws per leg, each with a basal section, as well as primary and secondary branches (Marley et al., 2011). Within Parachela, the family Macrobiotidae is recognized by their Y-shaped diploclaws and an asymmetrical buccal tube with respect to the frontal plane, due to the ventral lamina (Pilato and Binda, 2010). Reviewing the genus *Macrobiotus* under the type species *Macrobiotus hufelandi*, the genus is defined to have lunules connected to the claws by a peduncle, a porous cuticle, and eggs with processes but never aerolated (Kaczmarek and Michalczyk, 2017).

Nowadays, tardigrades are recognized as excellent multicellular model organisms, partly due to their complex structural organization and ease of cultivation in small laboratory facilities (Goldstein, 2022), but primarily for their resistance to extreme conditions. They have developed various physiological strategies to withstand stress conditions such as dehydration (anhydrobiosis), extremely low temperatures (cryobiosis), lack of oxygen (anoxybiosis) and high salt concentration (osmobiosis) (Møbjerg et al., 2011). Among the strategies used to survive harsh conditions are the diapause and cryptobiosis, which Ste-Marie et al. (2022) define as a maximum reduction of metabolism, allowing the organism to enter a basal condition of low energy consumption, referred to as a state of "life in the slow lane." When tardigrades enter cryptobiosis, they are said to be in the "tun" state, during which they contract their legs into the body cavity and reduce their volume (Møbjerg and Neves, 2021). However, limnic species exhibit very low or absent cryptobiotic activity (Guidetti et al., 2011).

In South America, Colombia is a tropical country with diverse geography and no distinct changes in temperature, characterized by one or two rainy and dry (usually from December to February) seasons (Eslava, 1993). Its capital,

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Bogotá presents a high-altitude Andean Mountain Forest stratification, where temperatures can vary by up to 20 °C throughout the day, with low temperatures at dusk and dawn and higher temperatures at noon. Therefore, fluctuations in temperature are expected not only throughout the day but also between months.

Tardigrades in Bogotá are exposed to variable weather conditions as they can be found in moss on trees and soil where they experience hydration-desiccation cycles throughout the day. Consequently, these organisms are expected to have developed broad resistance to such conditions. Therefore, it is anticipated that limnic tardigrades are capable of surviving an increase in water temperature either by remaining alive or entering a "tun state."

Tardigrades used for this study were extracted from different moss samples collected from trees on the Bogotá campus of National University of Colombia (4°38′30.61875″ N and 74°4′57.06839″ W). Samples were taken using tweezers and placed upside down in petri dishes filled with mineral water. After 2–3 hours of hydration, the moss was removed and the petri dish was placed under a stereoscope. Tardigrades were then searched throughout the dish. Once found, the tardigrades were extracted using a micropipette and placed in individual Eppendorf tubes filled with 0.5 mL of mineral water. Each set of 15 individuals was then placed on a heating plate and heated to 30 °C at a rate of 0.5 °C/min. The tubes were then left to cool down and examined 2 and 4 hours later. Each treatment was repeated 3 times at 20 °C, 30 °C, and 40 °C.

Since 135 tardigrades were needed for the study, the moss samples were kept in the laboratory to avoid further harm to moss growth in the environment. Each petri dish remained closed and had cotton swabs with water to maintain humidity above 90%. The temperature of the moss was maintained at an average of 18 °C.

A HOBO® Pro v2 (U23-004) data logger equipped with sensors for both ambient and substrate temperature was deployed to monitor weather conditions. It was left in place for 29 days, from October 18th to November 16th, 2023.

The tardigrades collected exhibit lunules connected to the claws by a peduncle and a buccopharyngeal apparatus without a posterior bend. Therefore, the genus studied was *Macrobiotus* sp. The temperature recorded (Figure 1) shows that environmental temperature averaged 14.7 °C with a minimum of 9.3 °C and a maximum of 25.6 °C, while moss temperature averaged 14.6 °C with a minimum of 9.6 °C and a maximum of 23.1 °C. As water temperature rises, mortality increases: 22.22% were dead at 20 °C; 35.56% were dead at 30 °C, and 46.67% were dead at 40 °C (Figure 2). When in the "tun" state, the body volume decreases and appears oval-shaped. No cases of "tun" were registered at any temperature.

The microhabitat temperature recordings exhibit significant variability throughout the day, reflecting a circadian cycle strongly influenced by the ambient temperatures of Bogotá, with maximum temperatures in the moss reaching around 23 °C. Therefore, treatments at 20 °C and 30 °C represent conditions that are ecophysiologically closer to everyday real conditions,

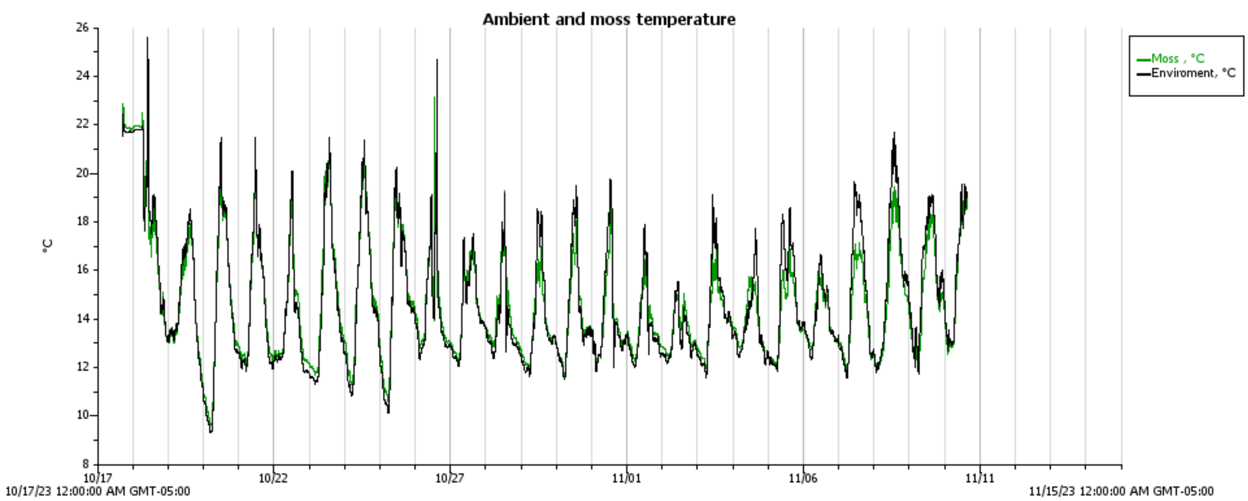

Figure 1. The environmental temperature is depicted in black while the moss temperature is shown in green. Temperatures were recorded every minute from October 18th to November 16th, 2023. Throughout the day, temperatures were not stable. The average environmental temperature was 14.7 °C, ranging from a minimum of 9.3 °C to a maximum of 25.6 °C. The average moss temperature was 14.6 °C, ranging from a minimum of 9.6 °C to a maximum of 23.1 °C.

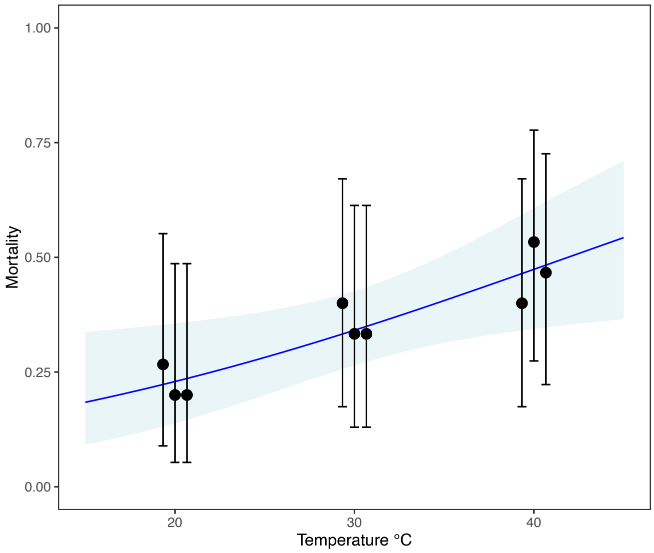

Figure 2. Mortality at each temperature. Three sets of fifteen tardigrades were exposed to temperatures of 20 °C, 30 °C, and 40 °C. As the temperature increases, so does the mortality.

with considerably low mortality rates observed. This supports the notion that the limnic tardigrades studied can withstand higher temperatures (Neves et al., 2020) as demonstrated in previous studies, up to 10 °C higher than their typical exposure in the microhabitat.

Furthermore, mortality increases with higher temperatures, while the "tun" state was not observed in any treatment. It is anticipated that no survivors will be found at temperatures exceeding 50 °C. This observation is consistent with the idea that high Andean tardigrades exhibit limited phenotypic plasticity, defined as "the ability of an organism to change in response to environmental stimuli" (West-Eberhard, 2008). Alternatively, the "tun" stage might emerge as an adaptive mechanism in response to extremely rare and extreme temperatures in the moss habitats where they reside. Finally, the data collected implies that high water temperatures do not induce tardigrades to enter the "tun" state, while conditions like dehydration (and glass-transition temperature) (Hengherr et al., 2009), or possibly a period of acclimatization (Li and Wang, 2005) could trigger this state. Further research is needed to explore these factors in greater detail.

Tardigrades exposed to temperatures like that of the environment show that they are able to withstand sudden changes in water temperature with low mortality rates, even so, high Andean limnic tardigrades show limited phenotypic plasticity to high temperatures. It should be noted that the "tun" state was not observed, implying that high temperatures (until 50 °C) do not trigger this state.

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