Resistance to Extreme Stresses in the Tardigrada: Experiments on Earth and in Space and Astrobiological Perspectives. Lorena Rebecchi[1], Tiziana Altiero[1], Roberto Guidetti[1], Michele Cesari[1], Angela M. Rizzo[2], Roberto Bertolani[1], [1]Department of Animal Biology, University of Modena and Reggio Emilia, Via, Campi 213/D, 41100 Modena, Italy, E-mail: lorena.rebecchi@unimore.it, roberto.bertolani@unimore.it; [2]Department of Molecular Sciences Applied to Biosystems, University of Milan, Via Trentacoste 2, 20133 Milan, Italy.

Extreme habitats are highly selective and require living organisms possessing specific adaptations to stressors. Among them, habitats that unpredictably desiccate and rehydrate can be considered extreme. Tardigrades, together with rotifers and nematodes, are the only metazoans able to enter cryptobiosis surviving desiccation (anhydrobiosis) and freezing (cryobiosis) at any stage of their life cycle, from the egg to the adult [1], [2]. Tardigrades are micrometazoans colonizing often as interstitial organisms marine, freshwater and terrestrial environments. The ability of the so called semiterrestrial tardigrades to enter cryptobiosis in a desiccating habitat allows them to become much resistant to extreme chemical and physical stresses. So far, most studies on the resistance of desiccated tardigrades have focused on their responses to very low or high temperatures, chemical molecules, high pressure [3], [4], [5], [6] (and for review see [2]), ionizing radiations and UV radiations [7], [8], [9]. This ability, and especially radiation and UV tolerance, has lead to propose tardigrades as suitable model in space research [7], [10]. The ability to withstand desiccation in tardigrades (and in other desiccation tolerant organisms) is a complex phenomenon that takes place at every level of the biological organization. Morphological, physiological, biochemical, and molecular constraints are involved in tardigrade anhydrobiosis [2]. For example, the presence of the disaccharide trehalose in tardigrades and its properties allowing life without water are known from long time [11]. As bioprotectants, recently also the Hsp70 proteins, the LEA family type 1 proteins and the antioxidant defences have been evidenced [2], [12], [13],[14]. Nevertheless, studies are still necessary to better understand the mechanisms by which anhydrobiotes and cryobiotes are able to tolerate the total suspension of metabolism due to the complete desiccation or freezing and the evolutionary meaning of life without free water.

Two projects using tardigrades were involved on FOTON-M3 that flew at an altitude between 250-290 km for 12 days in September 2007.

TARSE project [13] analyzed the effects of space environment stresses on the eutardigrade *Paramacrobiotus richtersi* within the spacecraft, allowing for the first time a comparison between hydrated and desiccated animals. Data on survival rate, life cycle, Hsps expression (70 kDa and 90 kDa), genomic DNA integrity (double strand breaks) and antioxidant defences of flown desiccated and hydrated animals, and from specimens in ground control experiments have similar to that observed in ground controls. During the flight mission, starved hydrated tardigrades moulted and females laid eggs. Several eggs hatched and the newborns exhibited normal morphology and behaviour. Microgravity and radiation had no effect on survival or DNA integrity of active tardigrades. Desiccated flown animals had high survival rates (from 79% to 95%) showing a similar survival rate to that observed in ground controls. No visible damages to double strand genomic DNA were observed in all desiccated tardigrades. The relative levels of Hsp70 and Hsp90 of desiccated flight tardigrades deviated significantly from the controls, whereas no differences were detected in Hsps expression between starved hydrated flight tardigrades and their controls. In both hydrated starved tardigrades and in desiccated ones, differences were found in antioxidant enzyme contents and activities between flight and control animals. These antioxidant defences could counteract reactive oxygen species (ROS) and could represent a crucial strategy to avoid damages in desiccation tolerant organisms.

TARDIS project [15] considered desiccated specimens of other two tardigrade species (*Richtersius coronifer* and *Milnesium tardigradum*), directly exposed to the open space environment. Similarly to the data of the TARSE project, desiccated tardigrades of the TARDIS project survived very well when exposed to space vacuum alone, whereas in samples exposed to the most life threatening conditions, a combination of space vacuum and the full spectral range of ultraviolet radiation (UV), only very few specimens of *M. tardigradum* survived. In comparison, in samples exposed to very high UV-A and UV-B doses (7.095 kJ m^{-2}), a high proportion (68%) of *M. tardigradum* specimens revived within 30 minutes after rehydration, but subsequent mortality was high.

Considering the future perspectives offered by space flights, exposures to UV radiation in lab could be of great importance in order to predict the response of multicellular organisms and to elaborate opportune countermeasures to avoid the risk imposed by UV solar radiation. Consequently, we analysed the biological responses of two eutardigrade species (*P. richtersi* and *Ramazzottius oberhaeuseri*) to high UV-B doses, alone or in combination with multiple stressors

(temperature and air relative humidity variations) in two different physiological conditions, namely desiccated and hydrated [16]. Tardigrades were exposed to seven different UV-B doses (from 47.8 up to 406.1 kJ m^{-2}) in a controlled climatic chamber. The results showed that active and anhydrobiotic tardigrades were able to withstand very high doses of ultraviolet radiations. The survival rate of hydrated or desiccated specimens of both species was inversely related to the UV-B doses, with *P. richtersi* that better tolerated the increase of UV-B dose than *R. oberhaeuseri*. Surprising, the tolerance to physical and chemical extreme stresses is not an exclusive property of desiccated tardigrades; in certain environmental conditions (high RH level or low temperature) desiccated tardigrades have a lower or similar withstanding to UV-B irradiation than hydrated ones. This represents a further demonstration of the uniqueness of this animal group in tolerating extreme stresses. In addition, we demonstrated that high temperatures have a strong impact on tardigrade survival during UV-B exposition both in hydrated and desiccated animals. The high resistance to UV radiations in tardigrades can be considered a kind of exaptation or by-product of the evolutionary adaptation to survive desiccation.

Finally, a collaboration for experiments evaluating tardigrade survival in a simulated Mars athmospere is at the beginning.

Results on tardigrades open a window on the future perspective in astrobiology and in their applications. Studies to better understand the mechanisms allowing anhydrobiosis are still necessary, devoted to recognize the presence and the role of further bioprotectants, to characterize genes (and their proteins) allowing dehydration without death, to understand repair mechanisms of damages to biological structures and molecules (especially to DNA) induced by desiccation. The identification of such mechanisms should allow us to use this evolutionary advantage to engineer desiccation tolerance in cells, tissues or animals not naturally anhydrobiotic.

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