How to Kill a Tardigrade – Without Even Trying

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Why Tardigrades?

Tardigrades are small animals that are known for their ability to tolerate extreme desiccation as well as ionizing radiation. The molecular mechanisms underlying such radiation resistance has not been fully characterized, though previous research suggests that tardigrades possess species-specific DNA protection proteins that confer enhanced DNA protection as opposed to enhanced DNA repair. Understanding the molecular basis behind such radiotolerance is critical for space travel beyond the Van Allen Belt, where radiation levels are beyond that which humans can feasibly survive in.

SEM image of a hydrated tardigrade, *Hypsibius dujardini*. Scale bar shows 100 µm increments. Credit: John Varelas

Experimental Design and Purpose

- Quantify the relative amount of DNA damage via detection of 8-oxo-dG per unit of DNA after X-ray irradiation and further elucidate the mechanisms of radiation tolerance in *H. dujardini*
- Do a comparative analysis of DNA damage across three different species
  - Hypsibius dujardini
  - Deinococcus phoenicis
  - Saccharomyces cerevisiae
- Question: Do the nuclei of tardigrades demonstrate the same amount of DNA damage per unit of DNA as *Deinococcus phoenicis* and *Saccharomyces cerevisiae*?

HYPOTHESIS: H. dujardini possess species-specific DNA protection proteins that prevent DNA damage from occurring, thus conferring tolerance to extreme levels of radiation (as opposed to DNA repair mechanisms).

1. BEFORE IRRADIATION
Perform ELISA assay and immunofluorescence microscopy to detect baseline levels of 8-oxo-dG in all three organisms

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2. IRRADIATION
Irradiate all three organisms, do a dose response curve across all three organisms

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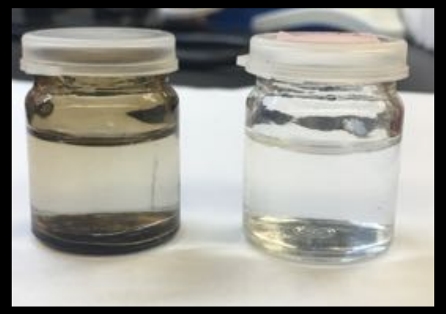
3. POST-IRRADIATION
Perform ELISA assay and immunofluorescence microscopy to detect levels of 8-oxo-dG in all three organisms post-irradiation; live-dead assays using FACS or flow cytometry

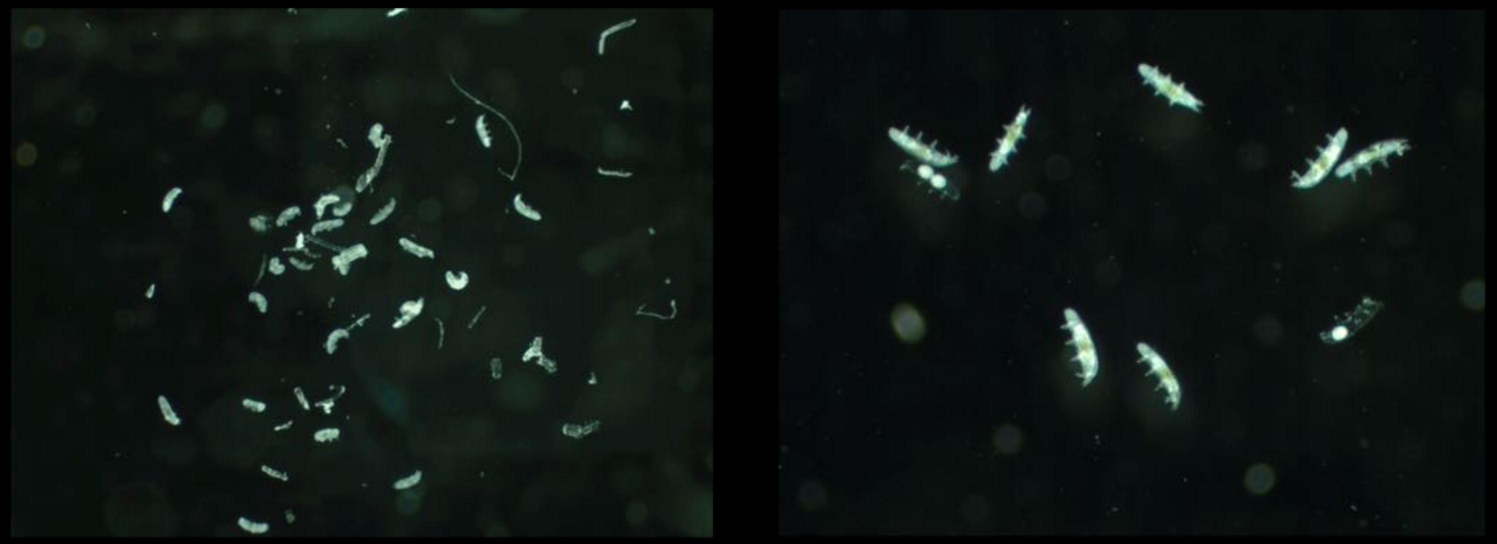
Materials Consideration

Containers in which organisms are irradiated should not shield any radiation or cause organisms to adhere to the surface post-irradiation. For this reason, many containers were tested at 1000 Gy for color change and tardigrade adherence.



Comparative image of an irradiated vessel (left) and a control vessel (right). Concerns of shielding led way to a materials consideration across a variety of different vessel types, as shown in the table.

Container	Active	Tuns	Dead	Color Change	Sticky
Small Tissue Culture Dish	15	5	2	No	No
Small Glass Pyrex Petri Dish	18	3	0	Yes	No
Teflon Cup	All were dead ☹			No	Yes
Plastic Vial (suspect polycarbonate)	22	7	8	No	Yes
Scintillon Glass	26	5 (mainly smaller ones)		Yes	No



Representative images of tardigrades post-irradiation in the small tissue culture dish (left) and the Teflon cup (right), showing a preference for the small tissue culture dish based on tardigrade mortality, dish color change, and adhesiveness.

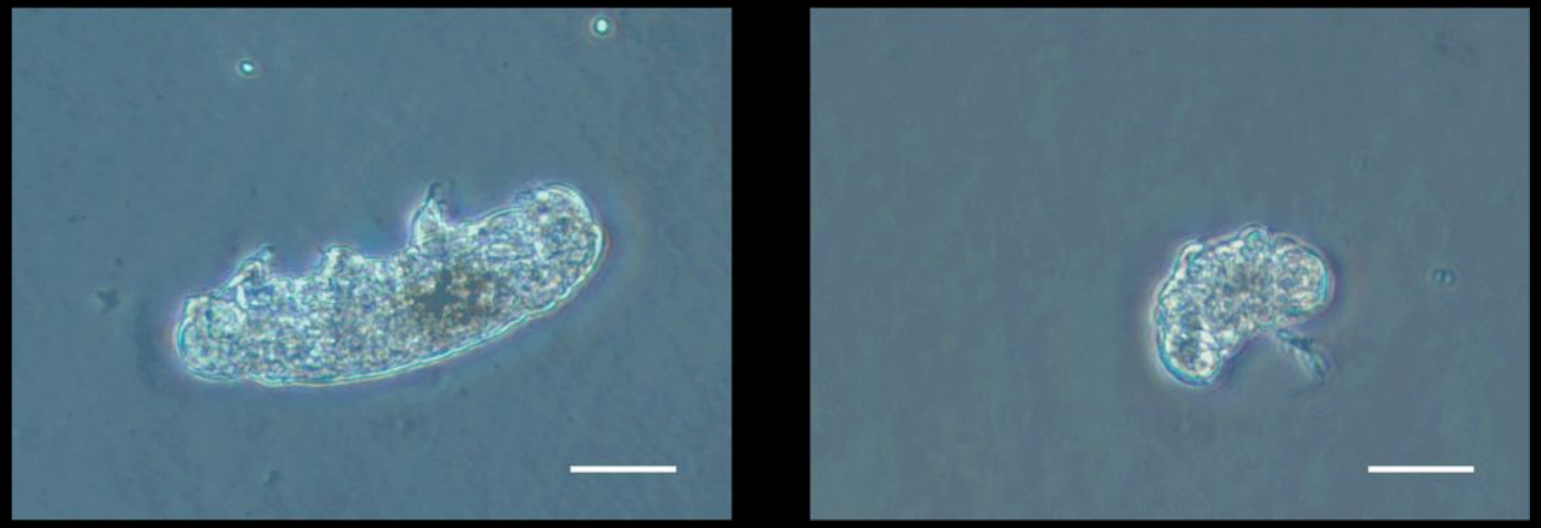
Comparative Analysis Across Species

Hypsibius dujardini
- Genome size: 75 Mb
- LD_{50} = 4180 Gy
- Within the same class as the species of tardigrades that were sent up to space

Deinococcus radiodurans
- Genome size: 3Mb
- Can survive up to 15k Gy of radiation
  - Dosed with 5000 Gy with no decrease in viability
- Experiments will use *D. phoenicis*

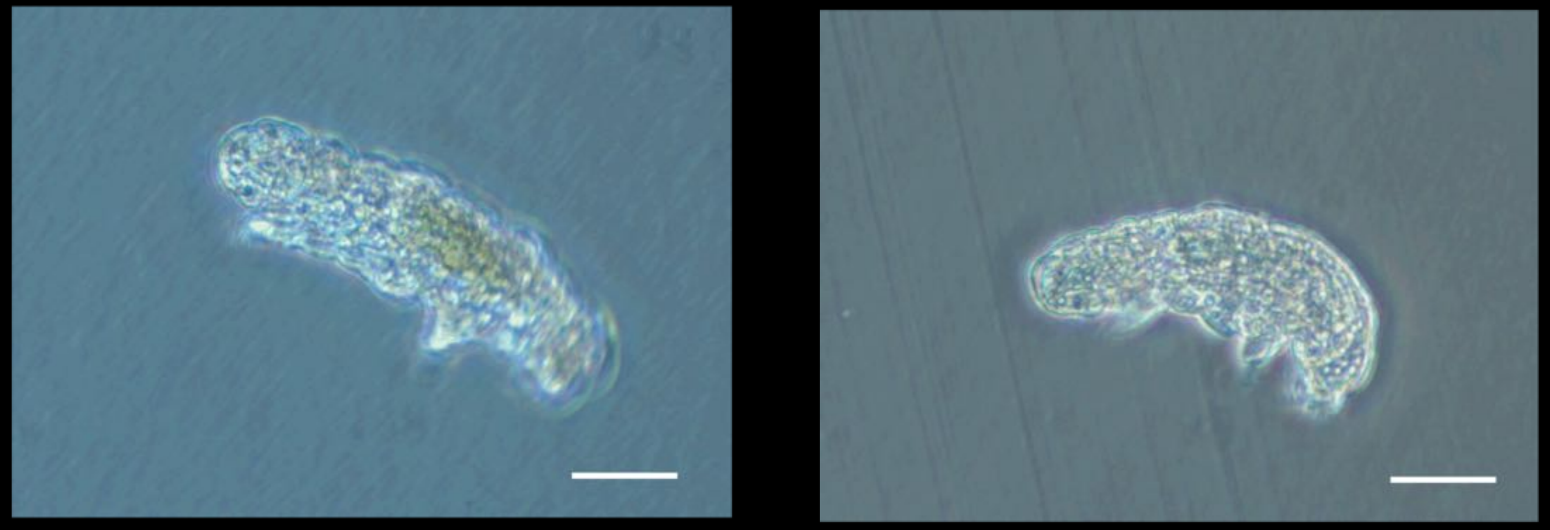
Saccharomyces cerevisiae
- Genome size: 12Mb
- Little radioresistance
  - Will not survive more than 150 Gy
  - LD_{50} = 16.6 Gy

4200 Gy Dose



Representative images of tardigrades post-irradiation at 4200 Gy (LD_{50} for *Hypsibius dujardini*). Irradiated tardigrade (left), control (right). Out of the 100 tardigrades irradiated, only 2 were moving slowly post-irradiation. In the control dish, about 89/100 were moving vigorously. Scale bar shows 100 µm. Max dose rate with parameters: 4.6 Gy/min.

1000 Gy Dose



Representative images of tardigrades post-irradiation at 1000 Gy, the about 250 times what humans can tolerate. Irradiated tardigrade (left), control (right). Out of the 100 tardigrades irradiated, about 90 were moving rapidly post-irradiation. In the control dish, about 73/100 were moving vigorously. Scale bar shows 100 µm. Irradiated glass vial turned brown post-irradiation. Max dose rate with parameters: 4.6 Gy/min.

Rapid Depressurization

About 100 adult Hypsibius dujardini were went to a maximum altitude of about 67,000 feet in a HAB in an OptiCell system with algae and fresh media to see how they fared in a variety of extreme conditions in the metabolically active state. Analysis showed that the tardigrades succumbed to the "bends," causing them to lose various limbs and look deformed. Only about 7 tardigrades were completely intact post-flight; all were found dead. Left shows a normal rounded and dead tardigrade; middle shows a slightly deformed tardigrades that looks squished with its pharynx out of position; right shows a headless tardigrade.

Acknowledgements: The authors acknowledge the support of the NASA Space Biology Program, NASA Ames Space BioSciences Division, and NASA Ames SLSTP Program. We also acknowledge inputs and support from Dr. David J. Smith, Dr. Jonathan Galazka and Dr. Marianne Sowa as well as members of the GeneLab Sample Processing Lab including Valery Boyko, Marie Dinh and Yi-Chun. We also thank Joseph Varelas for his help with the SEM.