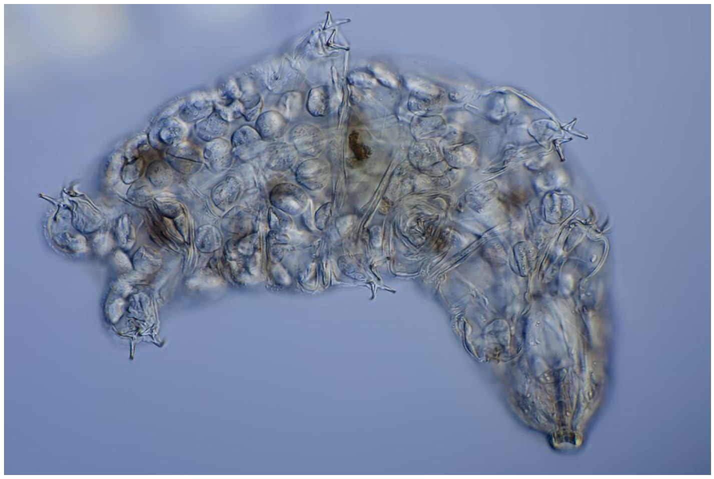
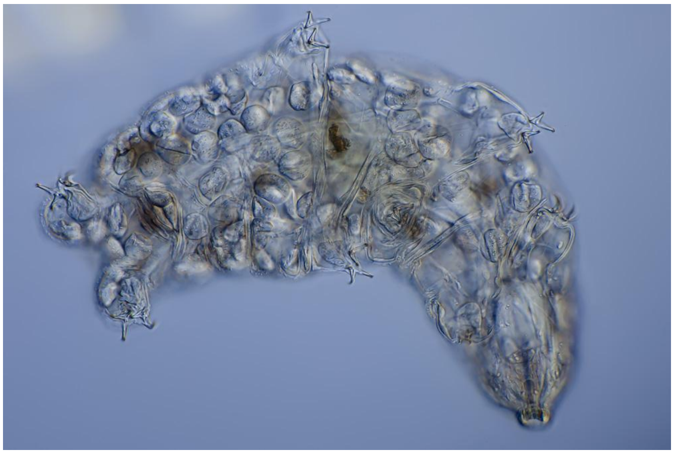
Physiology of Phylum Tardigrada



Kaylyn Flanigan

Physiology is the study of how an organism functions
The branch of biology that deals with the function and processes of a living organism

Introduction



In Invertebrate Zoology this week, our class was given a picture of this organism and we had to guess to which phylum it belonged.

I initially thought it was a nematode based on its body shape; I was wrong. I began looking in our textbook around the nematode chapter. Just before it was a chapter on Tardigrades and Onychophorans. I found an anatomical depiction of a tardigrade that matched the organism on the board.

The mystery was solved, this organism belongs to phylum Tardigrada!

Physiology is the study of how an organism functions
The branch of biology that deals with the function and processes of a living organism
Animal physiology is the scientific study of the life-supporting properties, functions and processes of animals or their parts. The discipline covers key homeostatic processes, such as the regulation of temperature, blood flow and hormones.

Bob Blaylock

Mangus Manske

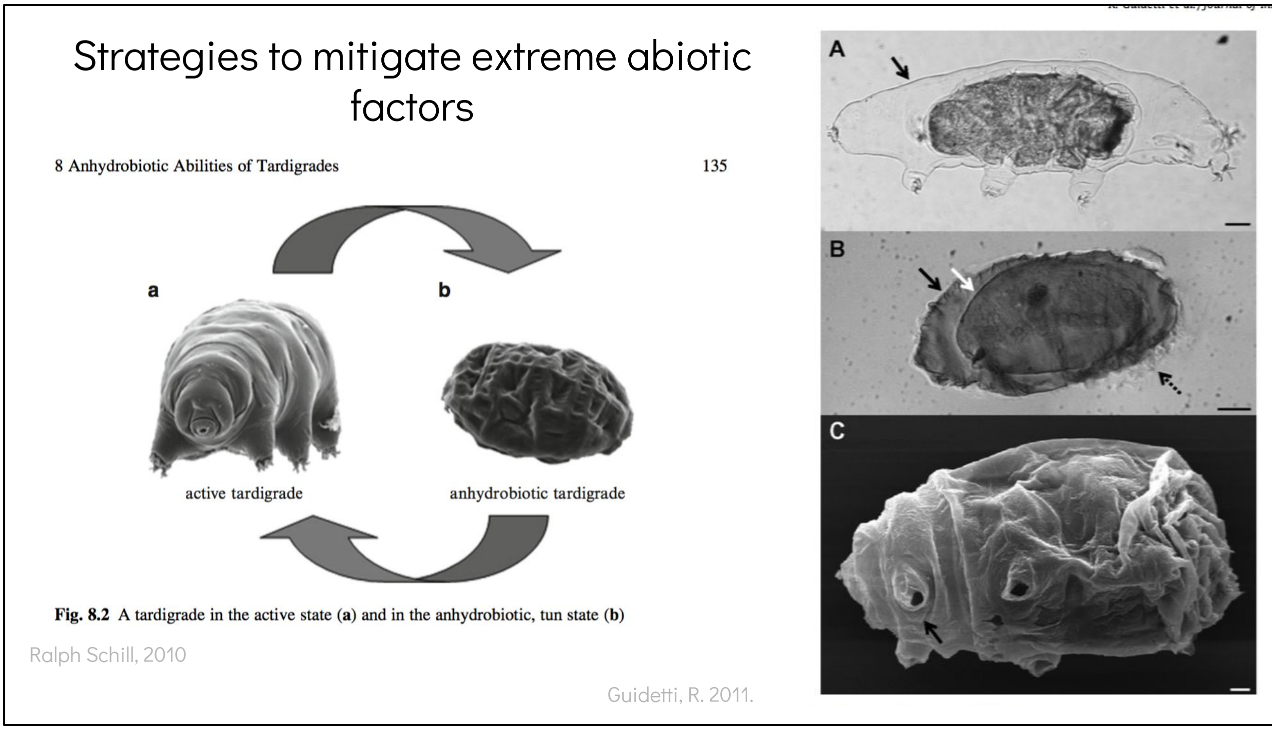
Bobanny

Water-bears, moss-piglets, tardigrades must live within an area that provides a film of water
This includes moss, lichens, terrestrial plants, marine and freshwater[3]

This film of water is necessary to an active life. Eating, breathing, reproducing depends on the presence of the film of water[3]

Tardigrades can live anywhere on the planet encountering extremes. These extremes include super freezing, intense heating, radiation, dehydration, lack of oxygen, and immense pressure[1]

Extreme survivorship is an accolade only reserved for some species of terrestrial tardigrades; marine species do not change as rapidly and thus lost the ability to tolerate extreme abiotic fluctuations[9]



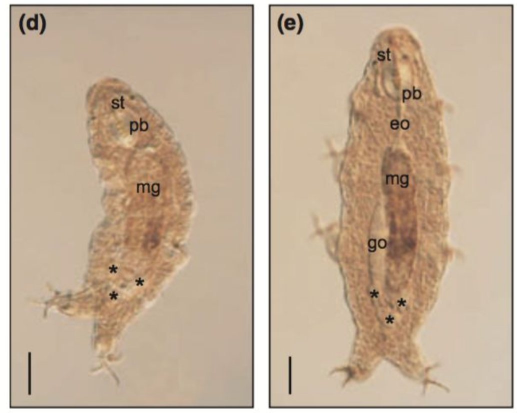
There are three strategies that help the tardigrade mitigate extremes that it is well-known for[10]. Under some extreme conditions the tardigrade can actually mitigate these factors while still in its active state (fig. 8.2 a). These extremes include radiation, freezing, heating, changes in salinity, and drops in oxygen concentration[1]. In the crytobiotic state (tun state) tardigrades can tolerate anhydrobiosis (dehydration) and extreme amounts of pressure (fig. 8.2 b)[1,7]. In the cyclomorphic strategy (Guidetti, 2011 - ABC) some species can tolerate freezing based on seasonal morphs[1].

Clarification:

1. Active State: they are still alive, metabolism is functional
2. Cryptobiotic State: TUN - roll up into a ball; metabolism drops to 0.01% of normal; trehalose (a sugar) is synthesized and replaces water. Tardigrade loses almost all of its water.
3. Cyclomorphic State: Have two phenotypes depending on weather and season to mitigate the stressors of those seasons.
4. Abiotic = non-living (ex. Weather, salinity, water availability)

Tolerating extremes in **active state**

1. **Radiation:** make DNA repairing proteins & damage suppressor proteins[1,4]



Mobjerg et al, 2011

H. crispae osmoregulating - swollen (e)

Tolerating radiation in active state:
- Use of DNA repairing proteins[1]
- Use of damage supressor proteins[4]

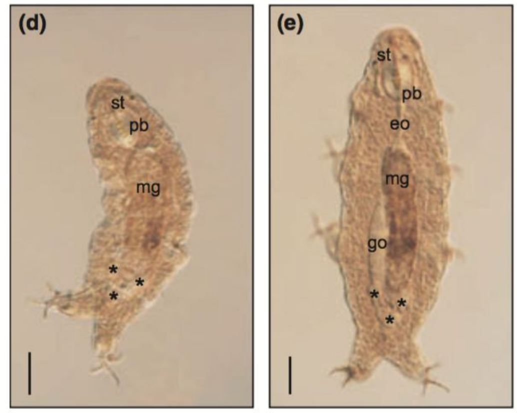
Tolerating freezing in active state:
- Entering **cyclomorphic** state **(seasonal morph)[1]**
- Synthesizing ice-nucleating agents (INAs)[8] - <u>allow the cell to freeze on the *outside* but not the *inside*!</u>

Tolerating heating in active state:
- Use of heat shock proteins (HSPs)[1,5] <u>- stabilize new proteins, ensure the correct folding of proteins</u>
- Vitrification (transition to amorphous solid) of trehalose[6] - <u>the sugar protects vital molecular structures</u>
- Late embryogenesis-abundant proteins ("molecular shields")[5] - <u>LEAs protect proteins from denaturing and sticking together</u>

Tolerating changes in salinity in active state:
- Osmoregulate: maintaining body water relative to solute concentrations outside of cells[1]

Tolerating extremes in **active state**

1. **Radiation:** make DNA repairing proteins & damage suppressor proteins[1,4]
2. **Freezing:** make ice-nucleating agents (INAs)[1,8]



Mobjerg et al, 2011

H. crispae osmoregulating - swollen (e)

Tolerating radiation in active state:

- Use of DNA repairing proteins[1] - function: to repair the DNA
- Use of damage supressor proteins[4] - tolerating stress and destroying some damage pathways

Tolerating freezing in active state:

- Entering **cyclomorphic** state **(seasonal morph)[1]**
- Synthesizing ice-nucleating agents (INAs)[8] - allow the cell to freeze on the *outside* but not the *inside*!

Tolerating heating in active state:

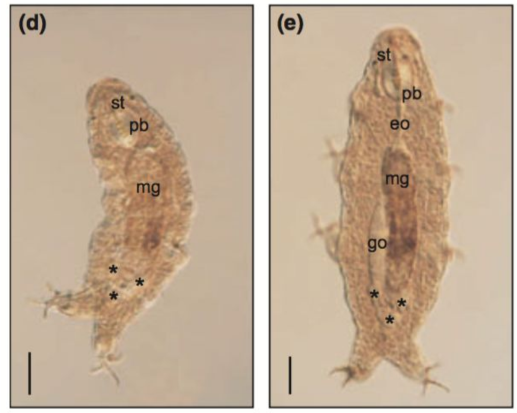
- Use of heat shock proteins (HSPs)[1,5] - stabilize new proteins, ensure the correct folding of proteins
- Vitrification (transition to amorphous solid) of trehalose[6] - the sugar protects vital molecular structures
- Late embryogenesis-abundant proteins (“molecular shields”)[5] - LEAs protect proteins from denaturing and sticking together

Tolerating changes in salinity in active state:

- Osmoregulate: maintaining body water relative to solute concentrations outside of cells[1]

Tolerating extremes in **active state**

1. **Radiation:** make DNA repairing proteins & damage suppressor proteins[1,4]
2. **Freezing:** make ice-nucleating agents (INAs)[1,8]
3. **Heating:** make heat shock proteins, vitrify (to make glass) sugar to maintain important molecules, use molecule shields (LEAs)[1,5,6]



Mobjerg et al, 2011

H. crispae osmoregulating - swollen (e)

Tolerating radiation in active state:
- Use of DNA repairing proteins[1] - function: to repair the DNA
- Use of damage supressor proteins[4] - tolerating stress and destroying some damage pathways

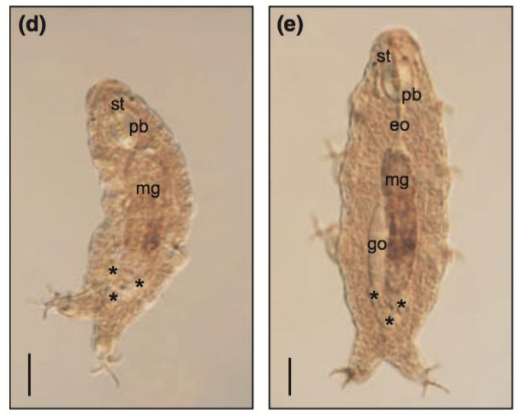
Tolerating freezing in active state:
- Entering **cyclomorphic** state **(seasonal morph)[1]**
- Synthesizing ice-nucleating agents (INAs)[8] - allow the cell to freeze on the *outside* but not the *inside*!

Tolerating heating in active state:
- Use of heat shock proteins (HSPs)[1,5] - stabilize new proteins, ensure the correct folding of proteins
- Vitrification (transition to amorphous solid) of trehalose[6] - the sugar protects vital molecular structures
- Late embryogenesis-abundant proteins ("molecular shields")[5] - LEAs protect proteins from denaturing and sticking together

Tolerating changes in salinity in active state:
- Osmoregulate: maintaining body water relative to solute concentrations outside of cells[1]

Tolerating extremes in **active state**

1. **Radiation:** make DNA repairing proteins & damage suppressor proteins[1,4]
2. **Freezing:** make ice-nucleating agents (INAs)[1,8]
3. **Heating:** make heat shock proteins, vitrify (to make glass) sugar to maintain important molecules, use molecule shields (LEAs)[1,5,6]
4. **Salinity:** osmoregulate - maintain body water relative to solute concentrations outside cell[1]



Mobjerg et al, 2011

H. crispae osmoregulating - swollen (e)

Tolerating radiation in active state:
- Use of DNA repairing proteins[1] - function: to repair the DNA
- Use of damage supressor proteins[4] - tolerating stress and destroying some damage pathways

Tolerating freezing in active state:
- Entering **cyclomorphic** state **(seasonal morph)[1]**
- Synthesizing ice-nucleating agents (INAs)[8] - allow the cell to freeze on the *outside* but not the *inside*!

Tolerating heating in active state:
- Use of heat shock proteins (HSPs)[1,5] - stabilize new proteins, ensure the correct folding of proteins
- Vitrification (transition to amorphous solid) of trehalose[6] - the sugar protects vital molecular structures
- Late embryogenesis-abundant proteins ("molecular shields")[5] - LEAs protect proteins from denaturing and sticking together

Tolerating changes in salinity in active state:
- Osmoregulate: maintaining body water relative to solute concentrations outside of cells[1]

Tolerating extremes in **cryptobiotic (tun) state**

1. Dehydration (anhydrobiosis)[1,2]
2. High pressure[1,2]

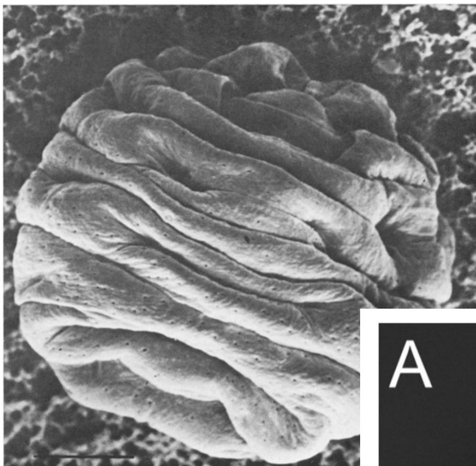
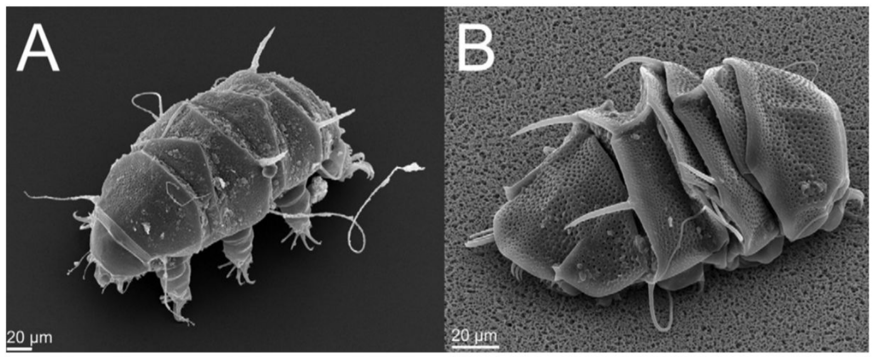


Fig. 1. Scanning electron micrograph of the anhydrobiotic tardigrade, The animal has contracted to form the so called "tun". The bar indicate

Walz, B. 1979



Wełnicz

Fig. 1. Scanning electron micrographs of *Echiniscus granulatus*. Dorsal view of (A) active and (B) tun state.

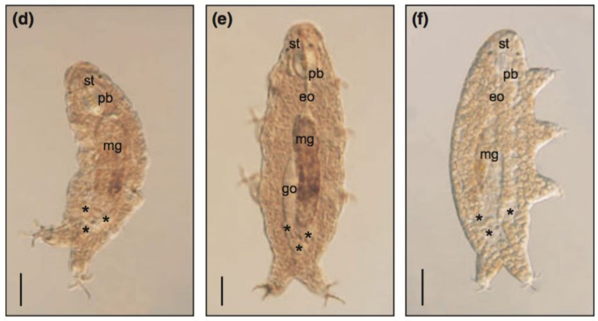
Tolerating anhydrobiosis (dehydration):

- Species will bring their legs in and curl their body laterally to form a ball[1]
- Metabolism will decrease to 0.01% of active metabolism[2]
- 97% of water is expelled from the body[9]
- Trehalose (disaccharide - sugar - carbohydrate) is produced in some species to form a matrix around their membranes, DNA, and proteins to avoid breakdown[1,5]
- Other species produce TDPs (tardigrade-specific intrinsically disordered proteins) to avoid desiccation[11]

Tolerating extreme amounts of pressure:

- Active tardigrades cannot survive >200 MPa[7]
- Tun tardigrades can survive 600 MPa[7]
- Due to tun state being more compact (?)
- Also, DNA repair proteins and matrix forming trehalose could play a role[2,7]

Tolerating extremes in **cyclomorphic** state



Mobjerg et al, 2011

(f) is the freeze-tolerant morph.

Halobiotus crispae exhibits cyclomorphosis - depending on the season, it will have a different phenotype[1]

Tolerating freezing in cyclomorphic state:

- *H. crispae* has a freeze-tolerant morph that occurs seasonally[1]

Similarities

1. **Dehydration → tun state**

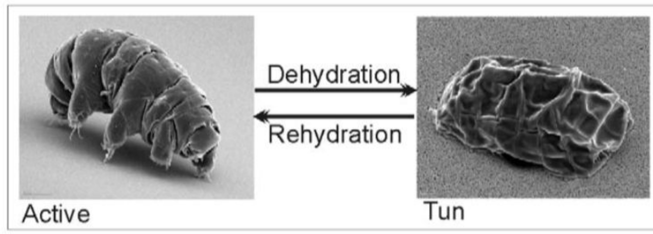


Figure 1. SEM images of *M. tardigradum* in the active and tun state. Tardigrades are in the active form when they are surrounded by at least a film of water. By loosing most of their free and bound water (>95%) anhydrobiosis occurs. Tardigrades begin to contract their bodies and change their body structure into a so-called tun. doi:10.1371/journal.pone.0009502.g001

Schokraie, et al.

Radiation[1,5]

1. *Richtersius coronifer* (after >1,000 Gy, tardigrade becomes sterile)
    a. Desiccated: 1,000 Gy
    b. Hydrated: 5,000 Gy
2. *Milenesium tardigradum*
    a. Desiccated: 5,000 Gy
    b. Hydrated: 8,000 Gy

Similarities

1. Dehydration → tun state
2. **Radiation is mitigated better in active state; pressure is tolerated better in a tun state[1,7]**

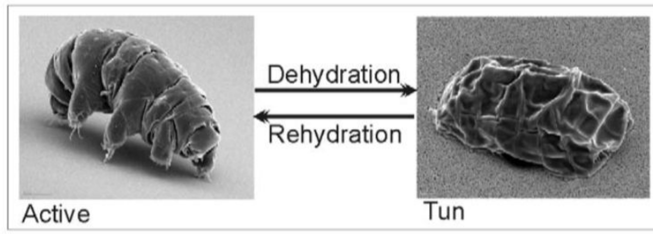


Figure 1. SEM images of *M. tardigradum* in the active and tun state. Tardigrades are in the active form when they are surrounded by at least a film of water. By loosing most of their free and bound water (>95%) anhydrobiosis occurs. Tardigrades begin to contract their bodies and change their body structure into a so-called tun.
doi:10.1371/journal.pone.0009502.g001

Schokraie, et al.

<u>Radiation</u>[1,5]

1. *Richtersius coronifer* (after >1,000 Gy, tardigrade becomes sterile)
   a. Desiccated: 1,000 Gy
   b. Hydrated: 5,000 Gy
2. *Milenesium tardigradum*
   a. Desiccated: 5,000 Gy
   b. Hydrated: 8,000 Gy

Similarities

1. Dehydration → tun state
2. Radiation is mitigated better in active state; pressure is tolerated better in a tun state[1,7]
3. **The length of time spent in the tun state is correlated with the amount of DNA damage[1]**

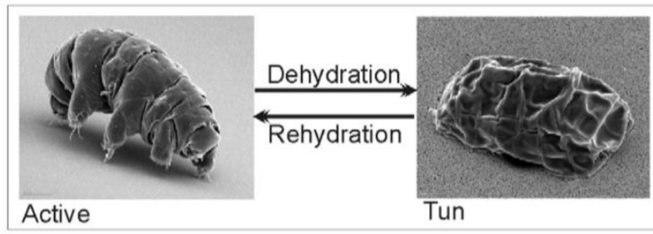


Figure 1. SEM images of *M. tardigradum* in the active and tun state. Tardigrades are in the active form when they are surrounded by at least a film of water. By loosing most of their free and bound water (>95%) anhydrobiosis occurs. Tardigrades begin to contract their bodies and change their body structure into a so-called tun.
doi:10.1371/journal.pone.0009502.g001

Schokraie, et al.

<u>Radiation</u>[1,5]

1. *Richtersius coronifer* (after >1,000 Gy, tardigrade becomes sterile)
   a. Desiccated: 1,000 Gy
   b. Hydrated: 5,000 Gy
2. *Milenesium tardigradum*
   a. Desiccated: 5,000 Gy
   b. Hydrated: 8,000 Gy

Differences (a few...there are many)

1. **Tactics for maintaining homeostasis varies with species**
   a. *Macrobiotus sp.* synthesize trehalose (disaccharide) but it is not measurable in *Milnesium tardigradum*[1,5,6]
   b. Some species produce trehalose while others create tardigrade-species disordered proteins to mitigate desiccation[1,2,11]

There are many species-specific differences across the phylum

Differences (a few...there are many)

1. Tactics for maintaining homeostasis varies with species
   a. *Macrobiotus sp.* synthesize trehalose (disaccharide) but it is not measurable in *Milnesium tardigradum*[1,5,6]
   b. Some species produce trehalose while others create tardigrade-species disordered proteins to mitigate desiccation[1,2,11]
2. **Different species synthesize different bioprotectants**
   a. Some species utilized heat-shock proteins while others rely on DNA repair machinery and LEA proteins that act as "molecular shields" against denaturing proteins[1,5]

There are many species-specific differences across the phylum

Differences (a few...there are many)

1. Tactics for maintaining homeostasis varies with species
   a. *Macrobiotus sp.* synthesize trehalose (disaccharide) but it is not measurable in *Milnesium tardigradum*[1,5,6]
   b. Some species produce trehalose while others create tardigrade-species disordered proteins to mitigate desiccation[1,2,11]
2. Different species synthesize different bioprotectants
   a. Some species utilized heat-shock proteins while others rely on DNA repair machinery and LEA proteins that act as “molecular shields” against denaturing proteins[1,5]
3. **Tolerances for each extreme (dehydration, temperature, pressure, irradiation) have different limits based on species**
   a. *Echiniscus testudo* has a longer cryptobiotic life expansion than *Richtersius coronifer*[1]
   b. *M. tardigradum* can withstand higher irradiation without consequences than *R. coronifer*[1]
   c. *M. tardigradum* had the greatest recovery after extreme warming than any other species[6]

There are many species-specific differences across the phylum

Works Cited

1. Møbjerg, M., et al. "Review: Survival in extreme environments - on the current knowledge of adaptations in tardigrades." *Acta Physiologica* 202: 409-420. 2011.
2. Fox-Skelly, J. "Tardigrades Return from the Dead." *BBC Earth*. 2015. http://www.bbc.com/earth/story/20150313-the-toughest-animals-on-earth
3. Pechenik, J. Biology of the Invertebrates. 7th ed., McGraw Hill Ed., 2015.
4. Hashimoto, T., et al. "Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein." *Nature Communications*, 7: 1-14. 2016.
5. Schill, R.O. "Anhydrobiotic Abilities of Tardigrades." In Dormancy and Resistance in Harsh Environments, ed. E. Lubens et al., 2010.
6. Hengherr, S., et al. "High-Temperature Tolerance in Anhyrdobiotic Tardigrades is Limited by Glass Transition." *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, 82(6): 749-755. 2009.
7. Seki, K. and Masato Toyoshima. "Preserving tardigrades under pressure." *Nature* 395: 853-854. 1998.
8. Westh, P., et al. "Ice-Nucleating Activity in the Freeze-Tolerant Tardigrade *Adorybiotus coronifer*." *Comparative Biology and Physiology*, 99(3): 401-404. 1991.
9. Miller, William. "Tardigrades." *American Scientist*, 99(5): 384-391. 2011.
10. Guidetti, R., et al. "Review: On dormancy strategies in tardigrades." *Journal of Insect Physiology*, 57: 567-576. 2011.
11. Boothby, T., et al. "Tardigrades Use Intrinsically Disordered Proteins to Survive Desiccation." *Molecular Cell*, 65(6): 975-984. 2017.