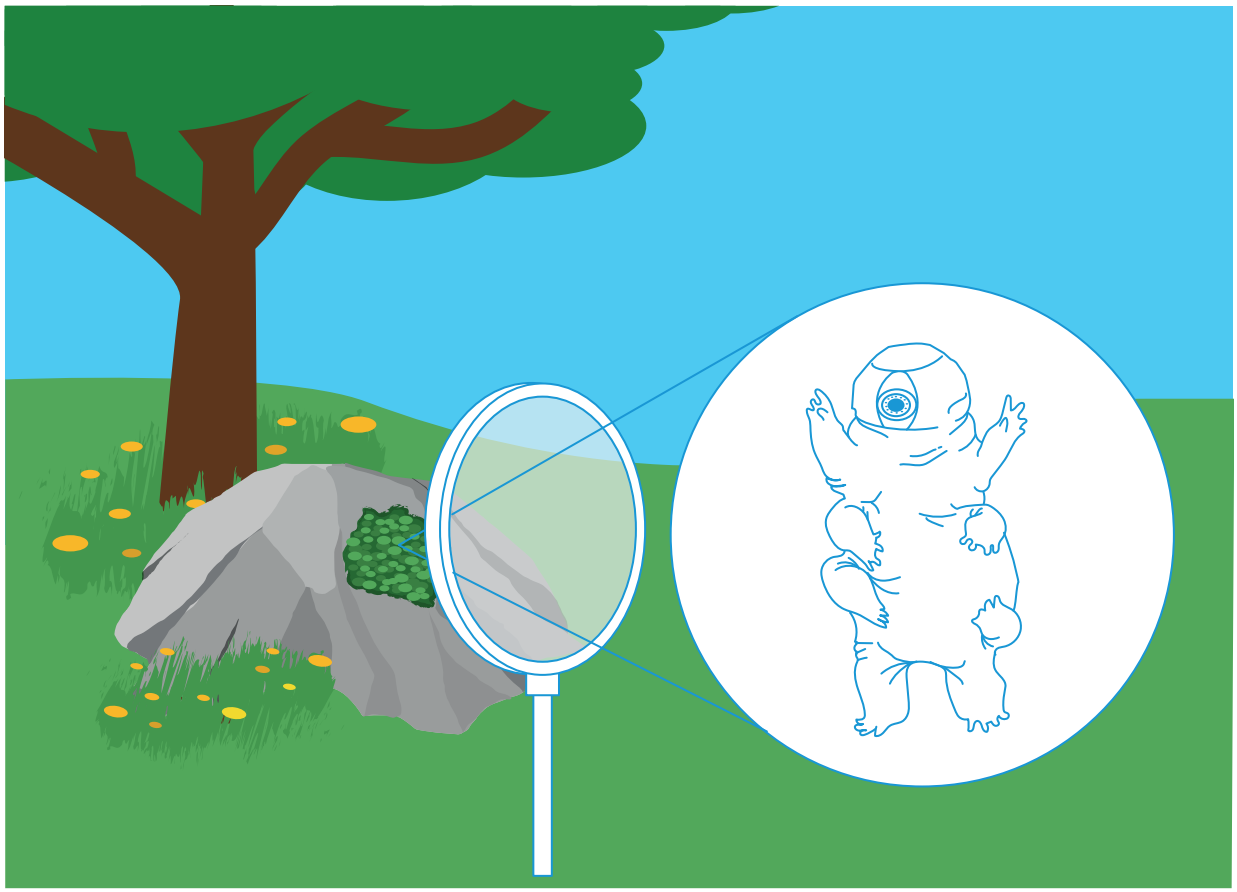
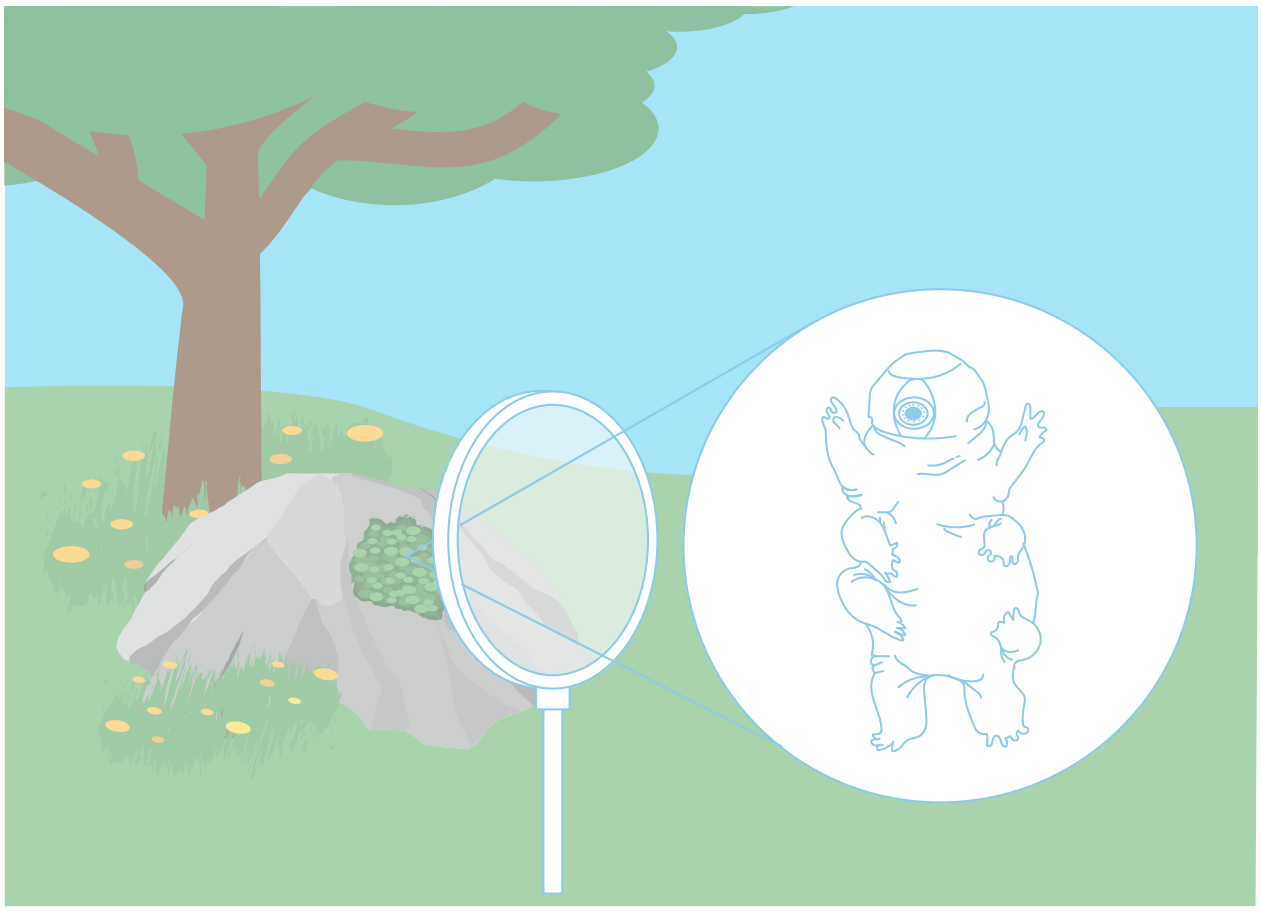
teach with space

→ SPACE BEARS

Lab-experience with Tardigrades





Teacher guide

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→ SPACE BEARS

Lab-experience with Tardigrades

Fast facts

Subject: Biology
Age range: 12-16 years old
Type: student activity, laboratory activities
Complexity: high
Lesson time required: 2 hours and 20 minutes
Cost: medium (10-30 euros)
Location: school laboratory
Includes the use of: living organisms, microscopes, cooker, freezer, chemicals, laboratory equipment

Keywords: Biology, Cryptobiosis, Anhydrobiosis, Cosmic Radiation, Tardigrade, Water Bear.

Brief description

In this set of experimental activities, students will investigate the survival abilities of tardigrades also known as water bears. They will explore how they can collect water bears and which extreme conditions they can simulate in the lab. They will expose collected tardigrades to these extreme conditions and come to a conclusion about which environments they can survive in. The aim of this resource is to test tardigrades' resilience to extreme environmental conditions and link their survival ability to the space environment.

Before beginning this activity, we suggest that you complete the activity *Could life survive in alien environments?* which provides an introduction to life in extreme environments.

Learning objectives

- Learning about tardigrades and the extreme conditions they can survive in.
- Learning about cryptobiosis and how this helps tardigrades survive.
- Investigating the effects of changing a variable in a system.
- Carrying out experiments appropriately, having due regard for the correct manipulation of apparatus, the accuracy of measurements and health and safety considerations.
- Evaluating methods and suggesting possible improvements and further investigations.

→ Summary of activities

Summary of activities					
	Title	**Description**	**Outcome**	**Requirements**	**Time**
1	Collecting the tardigrades	Collecting tardigrades from moss or lichens.	Follow experimental procedure to collect the tardigrades. Plan an experimental investigation.	Completion of the activity *Could life survive in alien environmens?* is advised.	30 minutes +overnight
2	Sending tardigrades to sleep	Transfer the tardigrades from the petri dishes to the small containers and store them in a dry place. The water should desiccate and anhydrobiosis should be induced.	Make observations using microscopes. Know how to identify tardigrades and induce anhydrobiosis.	Completion of activity 1.	30 minutes +overnight
3	Can they endure?	With the tardigrades in anhydrobiosis, students can test their resilience to different extreme conditions.	Conduct an experiment to investigate the effect that different environmental conditions have on tardigrades.	Completion of activity 2.	1 hour
4	Tardigrades in space	Compare the environment on Earth and Mars.	Understand that space is a very hostile environment and life would most likely not survive in these extreme conditions.	none	20 min

→ Activity 1: Collecting the tardigrades

In this activity students will learn how and where they can collect tardigrades. They will carry out these steps and prepare tardigrade samples for the following activities.

Equipment

- Printed student worksheet for each group
- Moss or lichen sample for each group
- Tap water or de-ionized water
- 1 Petri dish for each pair

Exercise 1 – Find the tardigrades

As an introduction provide an overview of tardigrades' properties and discuss which extreme conditions organisms can encounter and survive on Earth and in space or ask the students to investigate independently.

Tardigrades can be collected from moss or lichens samples. The moss collection can be done by the teacher or by the students by following the instructions on the student worksheets. After collection, the moss should dry completely before preparing it to collect the tardigrades.

Exercise 2 – Prepare the moss samples

Divide the class into pairs or groups of 3. The students should choose a moss cushion, which just fits into the petri dish they have and remove most of the loose soil/dirt particles. They should then complete the procedure in their student worksheet.

Exercise 3 – Plan your experiment

The students should plan how they are going to test tardigrades' survival abilities.
The students are asked to list three extreme environmental conditions in which tardigrades can survive.

Examples of answers they may come up with are:
- Extreme temperatures
- No air (different atmospheric conditions)
- High levels of radiation
- No liquid water
- High salinity
- Extreme pH

Discuss the experiments they are about to do. Consider:
- What kinds of experiments can you perform?
- How can these experiments be designed?

The students should complete the title, aim, hypothesis and method sections of the *Investigation Report* in their student worksheet.

→ Activity 2: Sending tardigrades to sleep

In this activity, students will transfer their tardigrades to small containers and induce anhydrobiosis by leaving them to desiccate. Students should understand that tardigrades will enter a new metabolic state, in response to the adverse environmental conditions. It is essential that tardigrades enter this tun state in order to survive these extreme environmental conditions.

Equipment for each pair

- Printed student worksheet for each group
- Microscope and/or loupe
- Small transparent container (coin boxes or similar)
- Pipettes
- Petri dish with soaked moss (from Activity 1)
- Black cardboard or similar to place under the micro aquarium to improve contrast
- Torch

Exercise 1 – Inducing anhydrobiosis

In this exercise students will have to use a small transparent container, like a coin box or similar. The container should have clear walls, similar to glass.

It is advised that the teacher prepares some tardigrades before the lesson in case some groups are unable to extract any from their moss. If the students were unable to find any tardigrades you can discuss why they didn´t find any? Did they collect the wrong type of moss?

In pairs, the students should follow the instructions on the student worksheet to induce anhydrobiosis in preparation for testing the tardigrades' survival abilities. Show some pictures of tardigrades viewed through microscopes so the students know what they are looking for. After squeezing the moss ask the students to observe their samples with a microscope or loupe. They should draw their tardigrade(s) on their student worksheet.

After, the students will have to transfer the tardigrade(s) to their small container(s). The residual water should evaporate slowly, e.g. 6 to 7 hours, with the container almost totally closed. Quicker evaporation will kill the tardigrades.

Before moving to Activity 3 students should revise their experimental plan.

→ Activity 3: Can they endure? Performing the experiments

The students will expose the dried-in samples to different conditions, simulating extreme environments.

Equipment

- Small transparent container(s) with tardigrade(s) sample (from Activity 2)
- Pipettes
- Lab thermometer
- Refrigerator/ Freezer
- Microwave oven
- Hot water or heat source (infra-red lamp or similar)
- Salinity solutions of varying concentrations
- Solutions of varying pH-levels
- Microscopes and/ or loupe

Health and Safety

In the framework of these experiments chemicals and water with high temperatures will be used. Please make sure to enable a safe use of them based on the experiences of the students, the safety and legal guidelines of your school and the equipment available.

For chemicals, please refer to the Safety Data Sheets.

Exercise - Performing the experiments

Each pair should take their samples from Activity 2 and observe the tardigrades using the microscope or loupe. A magnification factor of 10x is sufficient. At this magnification, students should be able to identify some of the main characteristics of the tardigrade tun. Ask them to draw what the tardigrade looks like.

Students should now prepare their experiments. Alongside the different experiments, each group should have a control sample that will simply be revived with tap water at the end.

Figure 1

↑ Tardigrade in the tun state

How to conduct the experiments

Students should record their observations throughout the experiment. Ensure the exposure time remains constant throughout each experiment.

Help the students relate the experimental conditions to real examples e.g. extreme temperatures on the Moon can range from 123 °C in the day to -233 °C at night.

1. Heat

Students should put a drop of hot water on the dried-in sample. The water should remove the tardigrades from the tun state, but due to the high temperature of the water the tardigrades will also be under enormous stress. When the water has cooled down, the students should observe the samples and register the behaviour of the tardigrade. Instead of hot water the students could also use a heat lamp or egg incubator for this test.

Example of temperature conditions to be tested: 40 °C, 60 °C, 80 °C, 90 °C.

2. Cold

Put your sample in the freezer and or/ fridge for several hours or overnight, if possible at different temperatures for example using different fridges, freezers or dry ice. After the samples were exposed to the cold, students should remove them from their tun state.

Example of cold temperatures conditions to be tested:

< -79 °C Dry Ice
-18 °C Freezer
0 °C Water ice
5 °C Fridge

This discussion could be extended to ask students to think about and list what parameters are met for something to be considered alive (made of cells, obtain and use energy, grow and develop, reproduce, respond to their environment, adapt to their environment).

3. Salinity

Prepare solutions with different salinity. Students should add a drop of the solution to their samples and observe their behaviour. The water in the solution should remove the tardigrades from the tun state, but due to the salinity of the water the tardigrades will be under enormous stress. After the completion of the experiment, students should proceed to revive the tardigrades, by adding a drop of tap water.

Some of the moons of Jupiter and Saturn are believed to host subsurface saltwater oceans.

Example of salinity conditions to be tested:

0,9 % Saline – isotonic solution
~3.5% Atlantic Ocean
~34% Dead Sea
~43 % Gaet'ale Pond – saltiest water body on Earth

4. Acidity

Health and Safety

The teacher should supervise this experiment. It includes the handling of solutions with extreme pH.

Prepare solutions with different pH level; students should add a drop to their samples and observe their behaviour. The drop should induce the reviving mechanism, but due to the pH level of the water the tardigrades will be under enormous stress.

After the samples were exposed to the different pH levels, students should revive the tardigrades by adding a drop of tap water.

A wide range of pH conditions can be found throughout our Solar System; from the acidic clouds of Venus and acid lakes of Europa to the alkaline rocks of our neighbouring planet Mars.

pH 3 to 5 – acidic environment
pH 9 to 11 – alkaline environment
pH 7 – Control sample

5. Radiation

To simulate the impact of high radiation in the samples students should place their samples in a microwave oven. Microwaves emit far lower levels of radiation than in space but are suitable as an example for this experiment. Microwaves will also heat the tardigrades, to avoid this a beaker of water can be placed inside the microwave at the same time to absorb the heat. Be careful when removing the water as it will get hot.

They should vary the intensity of the radiation, but the exposure time should be the same in all cases. We suggest starting the experiment by exposing the sample for 30 seconds.
After the samples were exposed to the radiation, students should revive the tardigrades by adding a drop of tap water.

Earth's atmosphere shields us from most of the harmful cosmic radiation. Many Solar System bodies, such as our Moon, do not provide protection from this dangerous radiation. It is something that is monitored closely on the International Space Station (ISS) to ensure the health and safety of astronauts living there.

Example of radiation conditions to be tested: low (~100W), medium (~400W), high (~800W).

After completing their experiments students should observe their samples, take note if the tardigrades are alive and moving or still in their tun state. Some of the tardigrades may have already been revived depending on the experiment carried out. The students should take note of their results and complete an investigation report for their experiment. A report template can be found in their student worksheets.

→ Activity 4: Tardigrades in space

The students will link the experiments they completed to the quest to find life elsewhere in the Universe

Equipment for each pair

- Student worksheet

Results

Compared to Earth, Mars has a very extreme environment. It has a very thin, carbon dioxide rich, atmosphere that does not provide protection from radiation. Atmospheric pressure is very low. Liquid water is unstable on the surface. Despite these harsh conditions there are some terrestrial micro-organisms that could survive on Mars. Tardigrades would most likely be able to survive the environmental Mars conditions for a short time but they would not be able to thrive under those conditions. Tardigrades do not survive well when exposed to high levels of UV radiation so they would need some form of protection to survive for long on Mars.

The ExoMars rover will, for the first time, be able to drill down to a maximum depth of 2 metres beneath the surface of Mars. If life existed on Mars in the past, when it may have been warmer and wetter, here would be the ideal place to find evidence of it as it would be protected from the harsh surface environment.

Space agencies must ensure that they do not bring back to Earth anything harmful from other worlds. At the same time, they must ensure that they do not introduce terrestrial biological contamination to other planets and moons that have potential for past or present life. Space missions take several precautions to avoid cross contamination, space missions are prepared in extremely clean laboratories and they have the legal obligation of comply with planetary protection constraints.

Discussion

Discuss the need to use a control sample and the idea of a fair test. From this you should expand the discussion to include the importance of only changing one variable at a time to isolate the effect of that variable only. Discuss why this is an important and interesting experiment? What can we learn from this? Guide the students to the possibility of life surviving in extreme conditions, especially in space. Be clear with the students that no life has been found anywhere other than Earth and that this experiment only gives some ideas of conditions that tardigrades are able to endure.

If the experiment worked well and the students were able to revive their tardigrades you can discuss the consequences of these findings. What conditions do we normally believe life needs? Do we still think it needs those conditions? You can also discuss other conditions that you think tardigrades could survive in and how you could extend/improve this experiment.

If the students were unable to revive their tardigrades discuss why that may be the case. Discuss the possible limits to tardigrades' tolerance of extreme conditions. They have incredible endurance but cannot survive everything. What does the discovery of tardigrades mean for the search for life elsewhere in the Solar System?

You can also discuss whether there may be other forms of life as resilient as tardigrades. Lettuce seeds and lichen have also survived exposure to space during ESA missions, what else could survive in space?

If the students completed the activity Could life survive in alien environments? prior to this activity, ask the students if their opinion of where life could survive in the Solar System has changed. They can revise the Solar System fact cards and present a more informed decision and relate this to the scientific method.

→ Conclusion

Students should have an understanding of what tardigrades are and what conditions they are able to survive. They should know where to find tardigrades, how to collect them and how to investigate their survival capabilities in a safe and scientifically appropriate way. The students should realise that tardigrades survive these harsh environments but do not function or thrive in them.

Furthermore, students should realize that it is important to have a full understanding of conditions that life can survive in to help understand life and its origins on our own planet as well as aiding the search for life on other worlds.

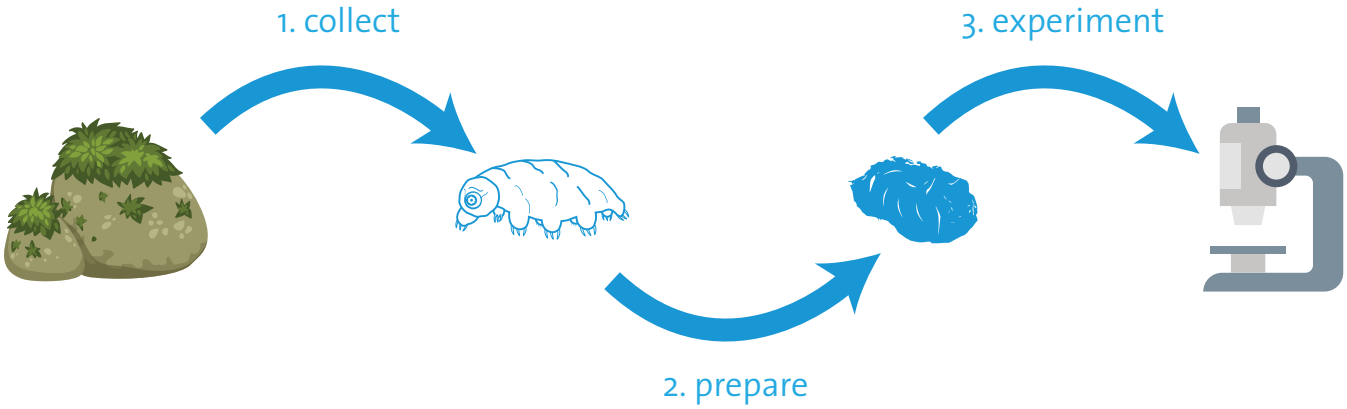
→ SPACE BEARS

Lab-experiences with Tardigrades

→ Introduction

The discovery of organisms that can endure extreme conditions on Earth, similar to those that would be found in space, has made the search for life outside our planet more plausible. Astrobiology seeks to identify the origin of life on Earth and understand if life could exist somewhere else in the Universe.

In this activity, you will test the resistance of tardigrades to extreme conditions, to investigate if terrestrial life could survive the harsh conditions of space.



Tardigrades, or "water bears", are close relatives of arthropods (insects and crustaceans) that can often be found residing in damp mosses and lichens where there is plenty of water. They are extremely small eight-legged animals measuring no more than 1.5 mm in length making them practically impossible to see with the naked eye. Certain species of tardigrade are renowned for their unique survival abilities. They have been found to survive temperatures as high as 150°C and as low as -272 °C, high levels of radiation, extreme pH levels, desiccation, the vacuum of space and high levels of oxygen.

When severely dehydrated (dry conditions) tardigrades enter a state of anhydrobiosis. In this state, known as tun state, there is minimal metabolic activity. Tardigrades can survive in this state for years, or even decades, while exposed to extreme conditions. They can be revived from this state by coming into contact with water again and will continue their lives as normal.

→ Activity 1: Collecting the tardigrades

In this activity you will collect tardigrades from moss or lichens in your local area and prepare your experimental plan.

Exercise 1 – Find the tardigrades

Tardigrades can be found in moss or lichens samples. To collect them, find some sun-dried moss cushions in white rocks, from natural stone walls or from terracotta roof tiles. Many tardigrades prefer calcitic stones as they need some calcite to build up their stiletto teeth. Mosses from forests are less appropriate as many tardigrades prefer mosses that become completely dry every few days. Avoid smelly and permanently damp mosses. Water bears like mosses that are free of bacteria and fungi.

Figure A1

↑ Moss growing on stones is ideal for finding water bears.

1. Tardigrades are often found on wet mosses and lichens. Where, in your local area, might you find tardigrades?

2. Collect a sample of moss (or lichens) that you think may have tardigrades. Store the collected mosses in a way that they can dry completely, e.g. you might expose your samples to direct sunlight or keep them in paper bags in a dry place.

Exercise 2 – Prepare the moss samples

In your group you will have to try and collect tardigrades from your moss (or lichens) sample. Follow the instructions below:

I. Place the moss cushion upside down into the petri dish and fill with tap water or de-ionized water. The moss should start absorbing the water.
II. Continue adding water until the moss is saturated (i.e. it does not absorb any more water) and ensure there is still a few millimetres of water left in the petri dish. Add some water if necessary.
III. Label the petri dish with your names and leave overnight.

Figure A2

↑ Lichens sample in the petri dish.

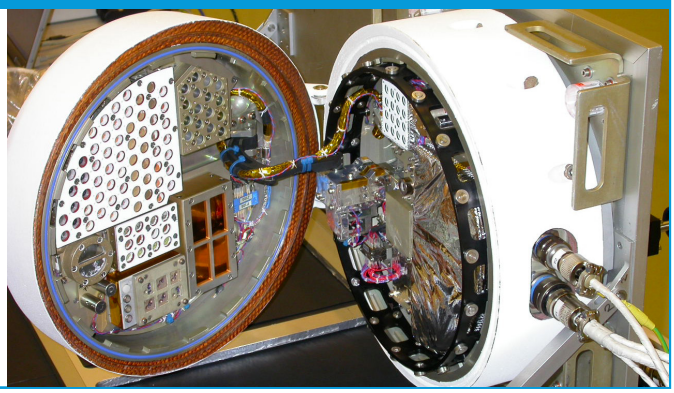
Exercise 3 – Plan your experiment

1. List 3 extreme environmental conditions in which tardigrades can survive.

2. Plan an experiment to test the tardigrade's resilience to one of the environmental conditions you listed in question 1. Complete the title, aim, hypothesis and method sections of the report template.

Did you know?

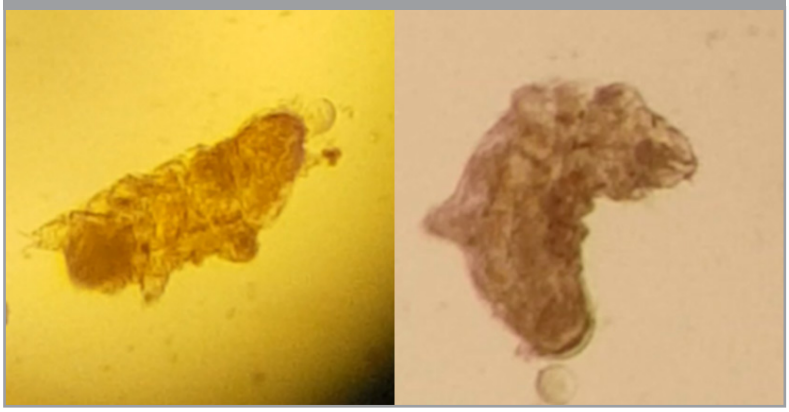
In 2007, as part of ESA's Tardigrades in Space (TARDIS) experiment, 3000 tardigrades were taken into space. They were exposed to the vacuum of space for 12 days where they experienced extreme dehydration alongside high levels of cosmic radiation and survived!



→ Activity 2: Sending tardigrades to sleep

Before proceeding with your experiment you will have to prompt the tun state in the tardigrades. In this activity you will transfer your tardigrades to small containers and induce anhydrobiosis by leaving them to desiccate.

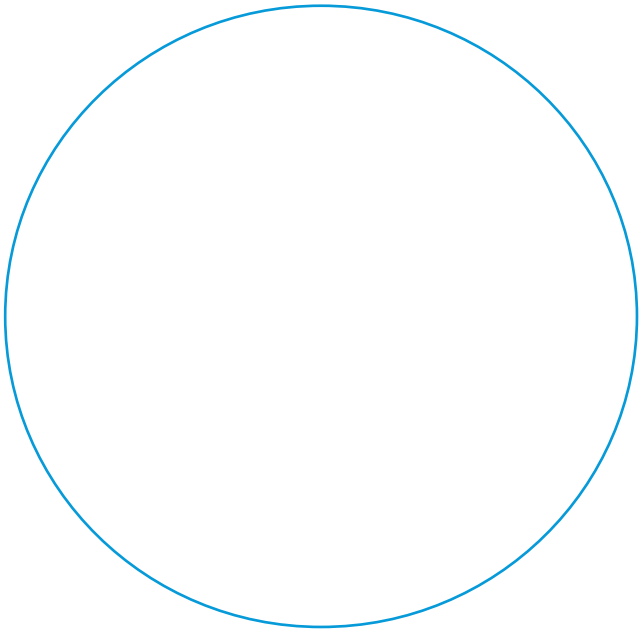
Figure A4



↑ Tardigrades seen with a microscope.

Exercise – Inducing anhydrobiosis

I. Remove the moss cushion from the petri dish. Gently squeeze and shake the moss over your petri dish to remove excess water and shake loose any tardigrades still clinging to the moss.
II. Use your microscope at 20x magnification, or mineralogist's loupe at 10x magnification, to look for tardigrades. Shine a light from the side and put the petri dish on black cardboard to increase the contrast.
III. Use the space below to draw what the tardigrades look like through your microscope.



IV. Use a pipette to extract a tardigrade from the petri dish and transfer it to a small transparent container. Repeat at least 4 more times.
V. Use your microscope to check that the tardigrade has been successfully transferred.
VI. Store your small containers in a warm, dry place overnight to slowly desiccate.
VII. Finalise your plan of how you are going to investigate the survival abilities of your tardigrade(s) and have it approved by your teacher.

→ Activity 3: Can they endure?

In this activity you will expose your tardigrade samples to extreme environmental conditions, as proposed on your experiment plan.

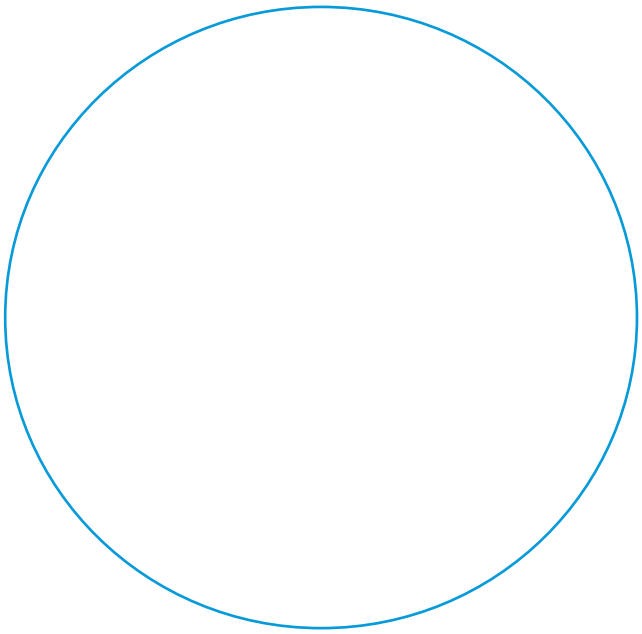
Health and safety

In the framework of these experiments, chemicals and water with high temperature will be used. Follow all safety guidelines in your school.

For chemicals, please refer to the Safety Data Sheets.

Exercise - Performing the experiments

Before you start this exercise you must have decided which environmental condition(s) you wish to simulate and have a plan for how you will create these conditions in your lab.



I. Use your microscope to observe your samples ensuring the tardigrades are in tun state.
II. Use the space on the right to draw what the tardigrade (in its tun state) looks like through your microscope.
III. Prepare the equipment and/or chemical solutions that you will need to simulate your chosen extreme environment(s) (examples: heat, cold, acid, alkaline, radiation, salinity, vacuum).
IV. You should investigate different extremities of a single condition i.e. if you are investigating heat try exposing each tardigrade to a different temperature, for example 40 °C, 60 °C, 80 °C. This will help you find possible limits to the tardigrade's survival abilities.
V. Expose the tardigrades for a set amount of time (make sure this is constant for all tests).
VI. Record any observations during this process.
VII. Use your microscope to see if your tardigrade is alive and moving or still in the tun state. If it is alive and not in distress you can skip to step X. If the tardigrade is still in its tun state or in an extreme environment and in distress, continue with step VIII.
VIII. Open the container and, using a pipette, **gently** place a drop of water on each of your samples.
IX. Close the container taking care to keep the drop of water in the centre.
X. Use your microscope to observe what happens. Try and use a cold lamp where possible as exposure to excess heat at this stage could ruin your results.
XI. Record your results and complete your lab report for this investigation.

At the end of your experiment place the tardigrades back in a humid moss sample and return them to their natural environment.

→ Investigation Report

Title: _____

Aim: _____

Hypothesis:

Method:

Results:

Sample	Environmental condition(s)		Tardigrades alive		Observations*
	Initial	Final	Initial	Final	
Control					

* Environmental conditions to test: temperature, salinity, pH, radiation or pressure

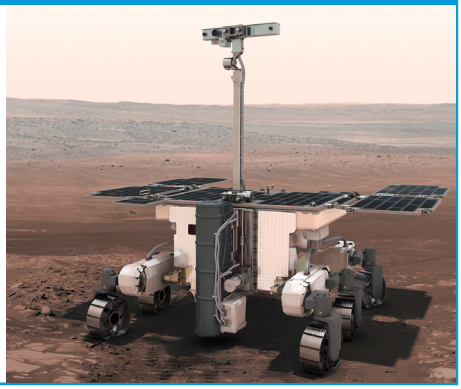
Discussion:

Conclusion:

→ Activity 4: Tardigrades in space

Did you know?

In 2020 ESA, working with the Russian Space Agency (Roscosmos), will launch the ExoMars rover "Rosalind Franklin". The primary goal of the ExoMars programme is to address the question of whether life has ever existed on Mars by landing at a site with high potential for finding well-preserved organic material, particularly from the very early history of the planet. It will carry a drill to collect samples down to a depth of 2 metres and analyse them with next-generation instruments in an on board laboratory.



1. Mars has a tenuous atmosphere composed mostly of CO_2. There is evidence that in the past there was an ocean of water on Mars, which has disappeared with the evolution of the planet. Currently there is no evidence of liquid water flowing on the surface. Temperatures vary between -153 °C to 20 °C.

a. Do you think tardigrades could survive on Mars? Why?

b. The conditions on the surface of Mars are very dry over many tens of thousands of years. Mars is also exposed to much higher levels of radiation than on Earth. Does this present a problem for the possibility of tardigrades surviving there? Why?

c. What type of precautions should be taken to avoid cross contamination of samples?

d. Do you think ExoMars rover will be able to answer the question of whether life has ever existed on Mars?

→ Links

ESA resources

Could life survive in extreme environments?
esa.int/Education/Teachers_Corner/Could_life_survive_in_alien_environments_-_Defining_environments_suitable_for_life_Teach_with_space_B09

ESA classroom resources
esa.int/Education/Classroom_resources

ESA missions

Tardigrades in space (TARDIS) on ESA's orbital Foton-M3 mission :
esa.int/Our_Activities/Human_Spaceflight/Research/Tiny_animals_survive_exposure_to_space

Robotic Exploration of Mars:
exploration.esa.int/mars

Planetary protection: preventing microbes hitchhiking to space
esa.int/Our_Activities/Space_Engineering_Technology/Planetary_protection_preventing_microbes_hitchhiking_to_space

Extra information

Searching for signs of life on Mars
exploration.esa.int/mars/43608-life-on-mars

Ten things you did not know about Mars
esa.int/Our_Activities/Human_and_Robotic_Exploration/Exploration/ExoMars/Highlights/Ten_things_about_Mars

ESA Euronews: Mars on Earth
esa.int/spaceinvideos/Videos/2018/02/ESA_Euronews_Mars_on_Earth

Ted-Ed: Meet the tardigrade
https://www.youtube.com/watch?v=IxndOd3kmSs

Life in extreme environments
https://www.nature.com/articles/35059215