



## Investigating Mosses and Lichens for Tardigrades: an exploration of an extremophile's ecological diversity

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### Abstract

This laboratory procedure uses an inquiry-based approach to give students experience developing a research protocol and hands-on practice with the scientific method. Student-designed, hypothesis-driven, research methods will allow students to develop their skills in project design, data collection, and analysis and allow for flexibility based on course content and academic level. This is ideal for a freshman level introductory biology course but can be scaled up to a higher-level course. The research organisms for this laboratory activity are tardigrades (water bears) which are ubiquitous organisms often found in the water layer that surrounds mosses and lichens. These hardy micro-invertebrates generally measure less than 0.5 mm in length and are extremophiles who survive a variety of environmental conditions making them excellent model organisms to study. The laboratory methods will give students practice in sample collections and introduce or reinforce their skills using a microscope to search for and view the organisms. This simple, inexpensive exercise focuses on developing a hypothesis and research method where students collect moss and lichen samples from a variety of locations. Instructors can also order specimens and set up several laboratory environments for student designed methods when outside collection is not feasible. Inquiry-based learning increases student engagement and has shown to have a positive impact on overall learning. This laboratory engages students in applying the scientific method to develop critical thinking skills and basic laboratory techniques.

**Keywords:** Tardigrades, Extremophiles, Ecology, Inquiry-based learning, Field sampling

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### INTRODUCTION

Across higher education, specifically STEM education, inquiry-based learning (IBL) has gained widespread acceptance and implementation as an effective pedagogical approach. Its value has been further supported by the demand for reform and *Call to Action* in the undergraduate sciences. Specifically, there is a continued push and emphasis on improving student's ability to problem solve and think critically while also helping students gain confidence and a sense of

belongingness, or connectedness, to the field. One increasingly favorable approach to incorporating inquiry-based learning opportunities into science courses is through CUREs, course-based undergraduate research experiences (Auchincloss et al., 2014). However, incorporating such experiences into courses can present a number of challenges for faculty and students including, but not limited to, access to resources, finances, time constraints, class size or differences in course modality (Trautmann and McLinn, 2012).

To address these obstacles and support student-centered, inquiry-based laboratory experiences, online databases or data sets offer a valuable option and have been previously utilized for examining a variety of biological data i.e. soil surveys or animal audio recordings, etc (Kluthe, Mortellaro, and Kluthe, 2022, and Unger, 2022). Online databases are readily available and provide flexible options for engaging students in self-discovery, while developing observational and analytical skills. The online database, iNaturalist, has been recognized as a valuable resource for biodiversity data and research, providing an active approach for students to engage with and learn about biodiversity (Niemiller et al., 2021).

In addition to online discovery, iNaturalist also encourages students to explore the natural world around them through inquiry (Eden, 2023). Outdoor field work has also been shown to significantly improve students' attitude and knowledge towards biology, the natural environment, and a future career in biology (Prokop, Tuncer, and Kvasnicak, 2007). Therefore, the purpose of this lab activity is to use an online database, iNaturalist, for scientific investigations and apply it in outdoor field work. The inquiry-based learning activities will introduce students to the online database to practice self-discovery and observation through accessing, collecting, and analyzing online data. Exploring the species and location information available, students will design and execute their own investigation, applying what they learned from the online sources, to direct their data collection and sampling. Engaging in the unique experiences of outdoor field work, applying what was learned through online inquiry, will provide an increased sense of belonging for students to their program of study and future careers.

## STUDENT OUTLINE

### Investigating Moss and Lichen for Tardigrades: an exploration of an extremophile's ecological diversity Objectives

- To identify a variety of ecosystems that an extremophile could live in
- To utilize online databases to locate biological samples
- To identify geographical features of an ecosystem using online maps
- To develop a hypothesis and protocol based on ecological research
- Demonstrate proficiency in microscope techniques to locate organisms in a sample

### Introduction

In this lab you will explore a fascinating microscopic extremophile called a tardigrade. Tardigrades (also known as water bears) are hardy microinvertebrates within the phylum Tardigrada which generally measure less than 0.5 mm in length. They are excellent model organisms to study because they are extremophiles who can enter into cryptobiosis (this is where metabolic processes stop until more favorable conditions arise) and survive a variety of unfavorable environmental conditions including lack of water, high and low temperatures, and radiation. There are approximately 1000 species of tardigrades. They are ubiquitous organisms found in most ecosystems. The diversity of ecosystems includes mountain peaks to the deep ocean and extreme cold and heat environments. They have been found in rainforests and deserts. Tardigrades have even been to space and survived. Tardigrades are often found in the water that surrounds mosses and lichens so anywhere that you find moss and lichen you have a high probability of finding tardigrades.

This lab will consist of two parts. The first part will introduce you to online databases that will help you locate identified organisms to begin to familiarize you with the range of habitats that tardigrades can be found in. There are several different databases that provide collection information. For our purposes we will use iNaturalist <https://www.inaturalist.org/> because of the ease of use. We will also use Google Earth to determine some geographic information about the ecosystem that the tardigrade was found in. The second part of the lab will be to go out and collect samples in an area where your research has determined that there is a high likelihood of finding tardigrades. Those samples will then be brought back to the lab to see if they contain tardigrades. This part will rely on proficient use of a microscope. If you are successful in finding a tardigrade, you will be able to share your discovery with the world!

### Part 1

Using your computer, open up the website iNaturalist <https://www.inaturalist.org/>. You can create an account or use the website without one. If you find a tardigrade, you may want to have an account so that you can add your finding to the database. Once you have the website up, go to the search icon on the top of the page and type in "tardigrade" then hit enter. Your search results should come back with several different items. Click on the top one, Tardigrades (Phylum Tardigrada). Your results should be like Image 1.

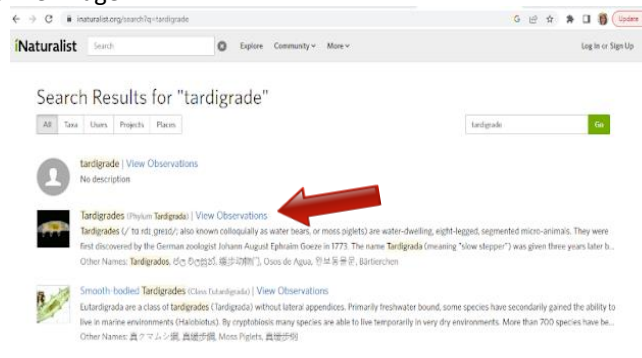


Image 1. Results of the search for tardigrades on iNaturalist.

Next, click on the highlighted Tardigrades next to the image of a tardigrade with a black background. This will bring you to the database that has all the global tardigrade information for the world. The next thing to do is click off the location area. As seen in Image 2.

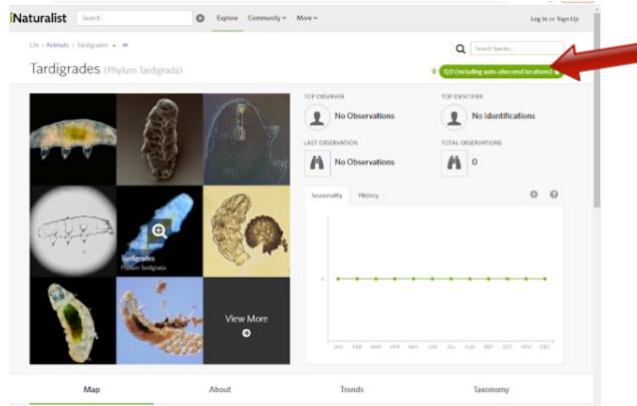


Image 2. The second page of iNaturalist with information on tardigrades. Make sure to click off the green area location to open up the global sample information.

When you click off the location tab you will see that it opens up all the data for tardigrades in the world. Image 3 shows you what the screen would look like. You can scroll just below the graph and pictures and see different tabs; Map, About, Trends, Taxonomy. The map tab will show you all the locations that tardigrades have been found. Take some time and familiarize yourself with this page. Notice that you can see when they are active, history, and life stages.

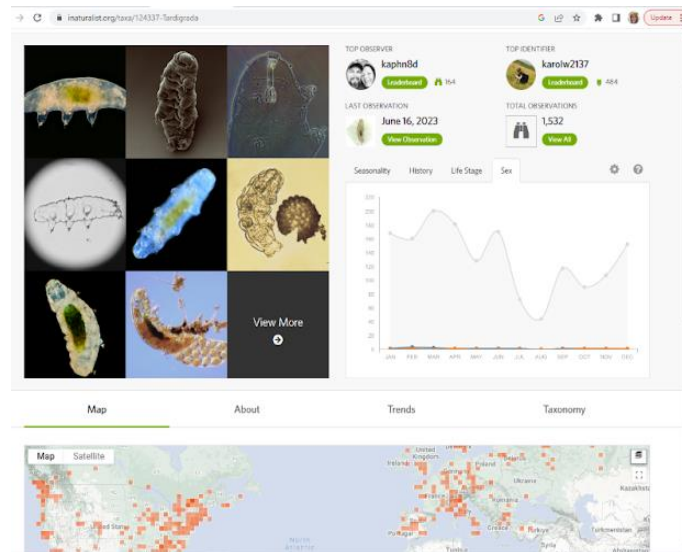


Image 3. Pictures and information about tardigrades from samples taken around the world.

Now you should focus on the map. Zoom into the area of New Jersey. Notice that you can zoom in and out on the map and you can also grab and drag to center the map where you'd like to zoom in. You should notice that there are not many samples that have been recorded in New Jersey. For each sample found, you will need to gather some information. This will be important to help you learn where you might be able to find tardigrades. Click on the red dot that indicates a sample has been recorded. When you do, you'll notice a pop-up box will appear that has more information about the sample. Image 4 shows you what you should see. Notice that the arrow is pointed to View. When you click on the View tab, it will open up

a new page. This is where you will find information needed to fill out Table 1. You will look at the information for all samples in the state. Once you are finished with a sample, you can use the browser back button to take you back to the map view.

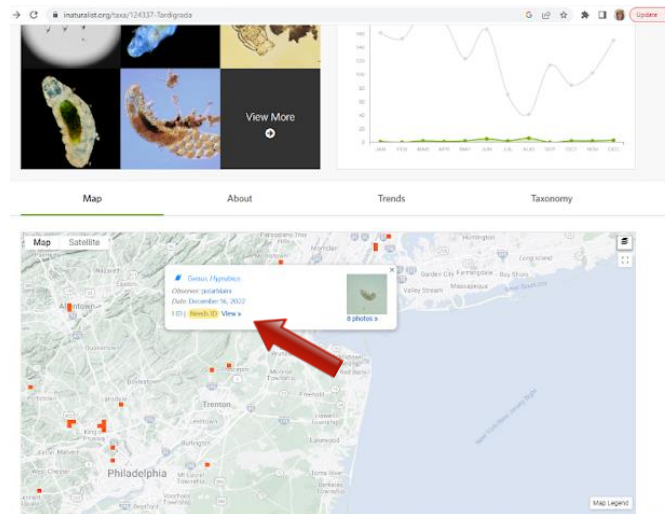


Image 4. Map view from iNaturalist after clicking on a sample location red dot.

Image 5 shows the new page that you will see after clicking on the view tab from the previous pop-up. Notice that you will need to click on the details tab under the map to get specific information on the sample area. This is the information you will need to make an informed plan for the second part of the lab.

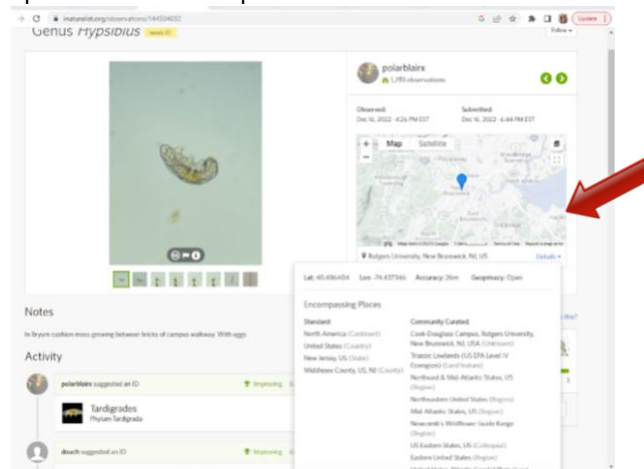


Image 5. This image shows the second page that pops up after hitting view. Notice that the pop-up box appears after hitting details under the map image. This will give specific information on the location of the sample.

Now use the information that is visible to fill in the information in Table 1. You fill in the table for all of the sample locations in the state. Add more lines as needed. Expect that more samples will be added so the images might change slightly. The first line of the table uses information from the New Brunswick sample. The first three columns are information gathered from iNaturalist. You will use Google Earth to get more specific information for the last two columns.

Table 1. Use this table to fill in the information gathered from each of the sample sites on iNaturalist and Google Earth. The first three columns (in italics) are from iNaturalist. The last two columns are from Google Earth.

Sample Name	Date Collected	Lat: Lon:	Elevation	Location description
<i>Hypsibiu (genus)</i>	<i>Dec. 16, 2022 4:26PM</i>	<i>40.486404 -74.437346</i>	27 m	Urban, forest, low elevation, near a river

The next step is to open up Google Earth on your browser. <https://earth.google.com/web/> You can use the web version or the downloaded version if you already have it but the web version will work just fine. We are using Google Earth because we want to take a closer look at the sample location and see what we can learn about the habitat where the tardigrade sample was located. Google Earth will give us the details needed to fill in the last two columns of the table. To do this you will need to take the latitude and longitude data you already collected. You just need the number. Once Google Earth is open you will click on the search bar and insert those numbers. You can copy and paste from the table. Image 6 shows you the Google Earth view with the latitude and longitude entered. Notice the red arrow at the bottom right of the image. This will show you the elevation at the sample site. You will need to have your cursor over the red sample bubble to be most accurate. Record this on your data table. Now that you have a clear image of the area of the sample, look around. What do you notice about the area? Is it urban or rural? Was it near water, a field, a forest, etc.? This will be the information you fill in on the last column of the data table.

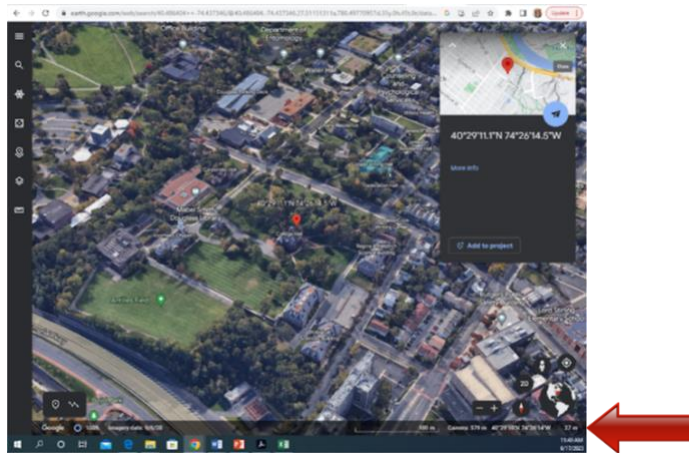


Image 6. Google earth view of the first sample site recorded in the data table. Use this to determine the elevation and more specific habitat details about the sample location.

Once the table is filled in with information from the samples from the state, you are ready to start to make some decisions about where you will try to discover tardigrades. This will require using Google Earth again but focusing on an area near you or outlined by your instructor. Answer the questions below.

1. From reading about tardigrades, where are you most likely to find them? Soil, bark, leaves, moss, etc?
2. Describe the types of habitats the samples in your table came from? What was the elevation? Were there similarities? Differences?
3. Describe the area, including the habitat, that you will be using to collect potential samples of tardigrades? Make a prediction (hypothesis) as to why you think this area will be a good location for finding tardigrades.
4. Give the exact location of your sample site including details as seen in the data table.
5. Write out your protocol for collecting your sample, including the location and site description.

### **Part 2 - Sample Collecting**

In the previous assignment you determined the location of your sample site. Your instructor may give you further guidance and instructions before you go out to collect your sample. Once you are at your destination you will need to collect a small amount of habitat to bring back into the lab. Remember that you are collecting a sample so there will be some disturbance to the area, try to minimize this with your action and the size of sample you collect. The sample collection steps are below.

1. Collect a small patch of moss or lichen (less than 5 square centimeters) and add to your collection container. This could be a plastic bag or small glass or plastic jar with a lid.
2. Add 10 ml of spring water to the sample container and soak overnight (at least 12 hours).

In the lab you will set up your microscope and prepare to look at the sample for any tardigrades found. You may want to look at a prepared slide of tardigrades set up by your instructor before you look at your sample to make sure you are familiar with what you are looking for. Make sure you use good microscope techniques to search the sample. To prepare your slide use the directions below.

1. In the lab you will remove the moss or lichen from the container and squeeze out any excess liquid.
2. Using a plastic pipette add a few drops of the liquid sample to a depression slide and view. Scan the slide so that you are looking at all parts of the sample.



## MATERIALS

This lab takes place both indoors and outdoors. For the first part students will need access to a computer with internet access. The programs being used in this lab may not have full functionality if a student tried to use their phone or tablet and may run into problems so encourage them to use a computer. Students will need a sample container which could be either a plastic bag or small plastic or glass container with a lid. In the lab, the students will need spring water, microscopes, depression slides, and plastic pipettes. It would be a good idea to provide an opportunity for students to view tardigrades under the microscope. Tardigrade samples can be purchased from companies such as Carolina Biological (item # 133960).

## NOTES FOR THE INSTRUCTOR

During this lab students will learn useful skills including using iNaturalist to become citizen scientists and identify areas near them where tardigrades will most likely be found. The example given in this lab was in the authors' home state but can be adapted to locations known to students. Students will learn to use Google Earth and analyze locations in their search for tardigrades.

This lab can be modified in several ways if going out in the field is not a possibility. You may want the students to study tardigrades from ordered samples first, then you can explore the different habitats that they can be found in. You can focus on the morphology and the tardigrade from ordered samples to help understand how these organisms can live in a variety of habitats. You can also modify the iNaturalist section by developing a series of questions about the habitat diversity such as "list the continents that have tardigrades identified", "Locate three different climate zones that have tardigrades". "Find a tardigrade sample in the mountains and another near sea level". This information can be added to a data table to see what kinds of information can be learned about the different habitats of the tardigrade.

If your student finds a tardigrade, they have the opportunity to add that organism to the iNaturalist database. When students enter their images into iNaturalist, several matches may appear regarding the taxonomy of the tardigrade sample entered. If the image does not appear to match exactly, students may enter as "tardigrade" and users of the site can further assist in identification. This lab also reinforces the skills of field biology and microscopy

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