Preference of Amount of Soil Moisture of *Porcellio scaber* Pill Bugs

Animal Behavior

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Introduction

Originating as aquatic beings, terrestrial isopods first colonized land approximately 300 million years ago. Durable and well established, pill bugs are present in Southern European soil, American forests and even Northern Africa. There are two main types of land isopods which include *Armadillidium vulgare* (pillbugs) and *Porcellio scabers* (sowbugs). With their history of being aquatic, terrestrial isopods have not fully adapted to land because they prefer habitats that have moderate temperatures and moist soil. Even though they seek moisture, there is a point where too much moisture is hurtful to them. As a result, their main habitats are in woodlands where it is damp and dark. Under rocks, wood, and logs are where terrestrial isopods are found within the woodlands (Smigel 2008). Terrestrial isopods have bodies that are flat bottomed, segmented, and have 14 legs usually growing up to ½ an inch in length as adults. Their main role in the ecosystem is to decay plant material causing them to be herbivores and scavengers (Missouri Department of Conservation 2021). It is very important to note that Pill bugs are in fact not insects. Pill bugs are actually part of the class Crustacea which relates them to crayfish and lobsters getting oxygen through pseudotrachea, which are white, gill-like organs located on the back of their body. If the pseudotrachea does not stay moist, the organism will die (Wagler 2013).

Isopods are detritivores which are very important for ecosystems. Detritivores feed on dead organic material which also considers them decomposers. With isopods being detritivores, they balance the nutrient input within the surrounding ecosystem through the decomposition of leaves, especially during the fall months. The amount of decomposition and nutrient cycling that occurs is completely dependent on the population densities of the terrestrial isopods. Rainfall determines the abundance of the reproduction of the isopods. When non-optimal rainfall conditions occur such as increased rainfall than normal climate, the population of isopods and isopod reproduction decrease. Changes in climate also affect the time of year isopods reproduce. Breeding of terrestrial isopods is normally in spring to early summer, to then be grown enough in the fall to decompose nutrient rich materials, however, in response to changes in the climate, mating for the isopods shift to earlier in the year and sometimes even later summer to early fall. Long winters cause delays in reproduction which sets back decomposition of other matter. This causes the decrease in decomposition rates from the terrestrial isopods ultimately disrupting the

nutrient cycle in the ecosystem (Zimmer 2005). It is important for the health of ecosystems that the balance of decomposers and producers stays relatively constant.

Terrestrial isopods also have unique responses to changes in their environment. Aggregation behaviors of isopods have been reported to differ depending on environmental stressors such as moisture and temperature. It has been reported that in response to the lack of moisture in the soil, terrestrial isopods aggregate together to prevent the loss of additional moisture. The organisms have also shown behaviors of separation during times of excess moisture in the soil. These aggregation behaviors have shown to buffer the effects of changes in microclimate (Hassall 2010).

These isopods are fascinating because of the insight they can provide on thermoregulation, thus making them excellent test subjects (Refinetti 1984). Thermoregulation allows for organisms to adjust to their changing environment, which pill bugs must do to survive. Since they are typically unable to survive harsh changes in the environment, it is important to note the conditions that they prefer to live in. Previous studies have supported the idea that pill bugs need environments to have moisture in order to thrive, but there is little data supporting how much moisture is best for the pill bugs.

For example, in a study performed in 2011, Schuler and their research team investigated the instances in which Pill bugs acclimated to their environments. They had specifically tested the temperature of the environment, but did not measure moisture levels. Schuler and their team had supported the idea that the Pill bugs had performed the same locomotion whether exposed to large changes in temperatures versus their preferred 10 or 20 degrees Celsius. Since their data had shown such interesting results, it was beneficial that they had utilized statistical testing (Schuler et al., 2011). Again, studies like this still do not define the amount of moisture needed, thus this project is warranted.

To decipher between where the pill bugs actually prefer to live due to their oxygen requirements, it would be advantageous to use statistical testing. Specifically, the Kruskal-Wallis test can allow us to analyze the pill bugs and create quantitative data. This test is a rank-based test to see if there are significant differences between two or more groups of independent variables (Laerd Statistics). By utilizing this statistical test, it will be easy to decide if the data is significant to support the idea that Pill bugs prefer a distinct amount of moisture.

Specific Aims

With the continuous changing of the environment, temperature and rainfall are specific aspects of climate change that can cause detrimental effects on terrestrial isopods such as *Porcellio scabers* (sow bugs). Sow and pill bugs have such a specific habitat in which they can thrive that slight changes in the environment can cause negative effects on the organisms. As a result of climate change, temperature increases. With increased temperature, more moisture is being evaporated from the soils dehydrating the soil of terrestrial isopods. In contrast, climate change also brings increased, heavier, and unpredictable rainfall in some areas of the world increasing the moisture in soils but also in other areas decreased rainfall.

Research has been conducted describing the optimal humidity for the habitat of terrestrial isopods, however, there is little research on the preferred moisture levels of the soil in which the isopods can thrive. Humidity and moisture seemingly parallel each other, however, humidity is the measure of water vapor in the environment while moisture is only the measure of water. With previous studies examining humidity and not moisture, there is a knowledge gap within this field of optimal soil moisture levels.

In this research, we plan to examine the preference of varying moisture amounts in soil for pill bugs. Performing research on the preferred moisture percentage in soils of terrestrial isopods will allow us to receive more information on the effects of climate change affecting these organisms. We hypothesize that if given the choice of 2.5% moisture, 7.5% moisture, 15% moisture, and 22.5% moisture, the isopods will prefer 7.5% moisture. This central hypothesis was formed by researching optimal humidity levels for terrestrial isopods and the distribution of *Porcellio scabers* across the world and the climates of those locations. Our research design and team of investigators are the best possible option to investigate this research because we have previous experience in animal behavior research and are passionate in the concept of the effects of climate change and human impacts on terrestrial organisms. To test the overall hypothesis, the specific aim is proposed:

Aim 1. How much moisture sow bugs prefer in their soil.

Understanding how much moisture sow bugs prefer will be determined by observing the sow bugs under preference testing various moisture amounts in 5 different chambers and how much time they spend in each chamber. We hypothesize that the sow bugs will prefer 3mL of water in their soil which is 7.5% moisture.

Methods

In a 5 chamber arena, 40 mL of soil will be inserted into each chamber with varying amounts of water added. In the center chamber, no water will be added. Using a graduated cylinder to measure water and a pipette to distribute the water equally, the top left chamber will have 1 mL of water (2.5% moisture), the top right will have 3 mL added (7.5% moisture), the bottom left chamber will have 6 mL of water added (15% moisture), and the bottom right will have 9 mL of water added (22.5% moisture). Percent moisture is the ratio of water to soil, i.e. 3 mL water/ 40 mL soil. From a group of approximately 50 *Porcellio scabers* (sow bug), 20 will be selected at random to test and observe. Five pill bugs will be selected at a time and marked with nail polish to aid in identification. The pill bugs will have an acclimation period of 10 seconds and observations will be recorded after the 10 seconds is complete. The amount of time each pill bug spends in each chamber will be recorded over a total of 20 minutes, with five minute increments. The Kruskal-Wallis statistical test will be used to determine the difference between the 4, nonparametric, unpaired groups.

Potential Conclusions

The purpose of this experiment is to test whether *Porcellio scaber* pill bugs prefer soil with varying levels of moisture present in each. Based on previous research that states pill bugs prefer environments with temperate to moist soils, we can potentially conclude that the organisms will choose to burrow in the 7.5% moisture chamber, which supports our original hypothesis. Pill bugs may not prefer the chambers with increased moisture (over 7.5%) due to the fact that the pill bugs could suffocate if their external uropods, which helps them wick up water, gets too saturated (Hornung, 2010).

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