Congregating behavior and response to resource distribution of the Green House Millipede, *Oxidus gracilis*

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Abstract

Background. Congregating behaviors, though common among many animal species, are little studied among millipedes. It is also unclear to what extent abiotic factors influence the distribution and behavior of millipedes. The species *Oxidus gracilis* was surveyed around several streams on the island of Mo'orea along with soil moisture, leaf litter cover, and rock cover. Experiments were designed in order to determine how an innate congregating behavior may affect their distributions.

Methods. Fifteen transects were performed in the field, recording *O. gracilis* abundances and the three environmental factors every 1 m. Forty trials were performed using covered bins filled with soil and five fruits of the Tahitian chestnut tree (*Inocarpus fagifer*). Ten millipedes were placed in the box and left for an hour, after which the number of individuals per fruit was recorded.

Results. It was found that while none of the three environmental factors were strong indicators of the distribution of *O. gracilis*, individuals did in fact demonstrate a tendency to congregate in the experimental trials.

Discussion. The lack of significant relationships with the three environmental parameters suggests a generalist behavior of this millipede species, potentially benefitting its invasive nature. The congregating behavior could potentially be for the purposes of mating or defense. Although not studied in this paper, it is possible that conspecific chemical cues are responsible.

Introduction

Grouping behaviors among different animal species may provide several advantages. Clustering in large groups helps protect populations from predators due to a dilution effect, where a predator can only attack one animal from a group (Mooring & Hart, 1992). This has been described as of the "selfish herd," where risk is distributed from one individual to another when that individual moves closer to others (Hamilton 1971). This same effect has also been found to protect populations from various parasites (Mooring & Hart, 1992). Grouping also helps reproduction by making it easier for individuals to find one another (Dangerfield & Telford, 1993). However, the functions and the mechanisms in which animals congregate are not known for all animals.

Among terrestrial invertebrates, grouping is often observed in the form of swarming, especially in flying insect species (Okubo, 1986). Two well-known examples of this are the swarming of

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honey bees and army ants (Allee, 1926), which happen when a colony's population grows too large and needs to find a new hive (Seeley & Visscher, 2003). Both are examples of social insect groupings with complex societal structures driven by chemical signaling (Conte & Hefetz, 2008). This chemical communication usually consists of pheromones released by individuals and detected by others (Page & Fondrk, 1995).

Most millipede species (Class Diplopoda) have near random distributions when surface active and do not usually exhibit grouping tendencies (Dangerfield & Telford, 1993). However, behavior varies greatly between different species, so it is important to examine possible examples of aggregation on a species by species basis. Fisher (2004) studied millipede distributions on the island of Mo'orea and found that two out of three study species of millipedes were distributed in relation to their resistance to hydraulic stress and submergence in water, which was seen in the form of each species' proximity to water. He found that distributions were related to the different species' hydrodynamic resistance and their ability to utilize resources in freshwater environments (Fisher, 2004). However, Fisher (2004) did not account for any other environmental factors other than stream proximity. Other studies (O'Neill, 1969) have suggested that desiccation is one of the greatest stresses affecting millipede survival, so environmental parameters that relate to millipedes' ability to avoid desiccation need to be examined in further detail. Millipedes are able to avoid desiccation by increased mobility, hiding under leaf litter, and burrowing in moist soil (O'Neill, 1969; Sierwald & Bond, 2007). Therefore, it is necessary to study these other factors in order to construct a more complete charactarization of the ecological influences on millipedes.

On Mo'orea, French Polynesia in the Fall of 2016, it was observed that individuals of the millipede species *Oxidus gracilis*, the greenhouse millipede, would often group together in clusters rather than having an even distribution. This was the species for which Fisher (2004) was unable to find a relationship with stream proximity due to lack of data. It is unknown whether individuals are drawn towards spots of specific environmental parameters, or whether they are actively seeking out other individuals. Or do both factors contribute to their distributions? The distribution and abundance of *O. gracilis* populations were recorded in order to understand the relative importance of soil moisture, leaf litter, and rock cover on its behavior.

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An experimental study was also performed to see whether if in a controlled environment individuals would congregate.

Methods

Study site

Distributions of O. gracilis were examined at three streams between two locations in the Opunohu Valley of Mo'orea: The Three Pines Trail and the Three Coconuts Trail (Fig. 1). Transects began at S 17°32.173' W 149°49.729, S 17°32.045' W149°49.839', S 17°32.709' W149°50.103', each of which marks a separate stream. These locations are characterized by Tahitian chestnut trees (Fabaceae Inocarpus fagifer) and vary in elevation from 119-220 m.

Transect surveys

Eight 10 m transects were performed along a stream at Three Pines, while seven were performed at Three Coconuts along two different streams (Fig. 1). Each transect ran parallel and downstream. This was done to ensure that areas with both high and low soil moisture could be found, since there is a fair amount of soil variability along the streams but usually some spots of high moisture. Transects were started where the trail met the stream and then ran downwards. Where possible, the next transect would be performed 10 meters further downstream from the end of the previous transect. However, in many cases points were selected where a transect could be performed.

Each transect ran within 2 meters of the stream. If this was impossible due to terrain, the transect ran as close to the stream as possible. A 0.25 m^2 quadrat was placed every meter for a total of 10 quadrats per transect. At each quadrat, percent leaf litter and rock cover were determined using a 5x5 grid. Leaf litter was measured first, after which leaves were carefully removed to expose the ground surface. After determining percent rock cover, the total number of individuals of *O. gracilis* were counted, including the ones found underneath small moveable rocks. Only living individuals were counted.

Finally, a soil sample was taken from each quadrat to be taken back to the lab, weighed, and dried. After drying, the soil samples were weighed again. This gave a wet mass and dry mass

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value for each soil sample, which was used to calculate the percent water content by mass for each original sample.

Congregation Experiment

Forty experimental trials were run to determine whether the congregating behavior of *O. gracilis* is unrelated to environmental conditions. Soil was sieved and mixed evenly with water to make a uniform, nearly muddy substrate. This was then laid evenly on the bottom of a plastic bin to create a flat surface. Five fruits of the tree *Inocarpus fagifer*, the Tahitian chestnut, were then placed at evenly spaced positions in the center of the bin on the soil. This was chosen due to the observation that millipedes, especially *O. gracilis*, seem to congregate on these fruits. It was found that without providing any food source or refuge from exposure, individuals would remain in motion and attempt to escape the container. Multiple fruits ensured that the millipedes would settle and that there would be multiple settlement opportunities, creating a potential for individuals to congregate. Ten individuals of *O. gracilis* were placed at ten different spots around the perimeter of the container. The container was then sealed and left for 1 hour. Afterwards, the number of individuals found on each fruit was counted as well as the number of individuals found on the soil. The soil was remixed and the fruits were switched out for different ones after each trial. After each trial of the chestnut fruit experiment the abundances of millipedes at each fruit were recorded from highest to lowest. In this manner, one trial's highest abundance at a fruit was recorded under 'Fruit A', while the lowest was recorded under 'Fruit E'.

Statistical analyses

Linear regressions were performed in R (R Core Team, 2016) for soil moisture vs. *O. gracilis* abundance, leaf litter vs. *O. gracilis* abundance, and rock cover vs. *O. gracilis* abundance to determine to what extent the abundance of *O. gracilis* is related to these three environmental parameters. A Kruskal-Wallis Test was performed in R (R Core Team 2016) on the 10 trials to see if there was a significant difference between the means of Fruits A, B, C, D, and E. A significant difference would indicate that individual *O. gracilis* are typically congregating, while no significance would mean that there is a relatively random distribution of millipedes from fruit to fruit.

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Results

Field Survey

Oxidus gracilis abundances ranged from 0 to 106 individuals per quadrat, with a mean abundance of 10.48 individuals. Soil moisture ranged from 6.67% to 89.6% by mass per quadrat with a mean of 27.1%. Leaf litter ranged from 0% to 97% cover per quadrat with a mean of 40.3%. Rock cover ranged from 0% to 100% per quadrat with a mean of 49.8%. As seen in Figure 2, streamside leaf litter was the strongest predictor of *O. gracilis* abundance, where a slight positive relationship was observed (R^2=0.034, p<0.01, standard error=12.5 on 167 df). Abundance was negatively related to rock cover, though again only slightly (R^2=0.026, p<0.05, standard error=13.2 on 148 df, Fig. 3). Soil moisture, when compared to abundance, had no detectable relationship with *O. gracilis* distributions (R^2=0.0025, p=0.25, standard error=13.6 on 138 df, Fig. 4). However, the low R^2 values of indicates that streamside leaf litter and rock cover are not a strong predictors of *O. gracilis* abundance.

Experimental Study

Millipede counts for group A ranged from 1 to 6 individuals per trial, while the mean number of millipedes found in group A was 3.43. Group B ranged from 0 to 3 individuals per trial with a mean of 1.95. Group C ranged from 0 to 2 individuals per trial with a mean of 0.975. Group D ranged from 0 to 1 individuals per trial with a mean of 0.500. Group E ranged from 0 to 1 individuals per trial with a mean of 0.125. The 'Open' group, which represents the number of individuals not found on a fruit each trial, ranged from 0 to 8 individuals per trial with a mean of 3. Individuals of *O. gracilis* were much more likely to be found at the same location than to be randomly dispersed (Kruskal-Wallis X^2 = 169.22, df=5, p<2.2e-16, Fig. 5). Though a large portion of individuals were found off the fruits, they demonstrated a tendency to congregate when found on the fruits.

Discussion

There was no correlation between *O. gracilis* distributions and soil moisture, leaf litter, and rock cover. However, it is possible that their locations are dictated by proximity to streams, as

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suggested by Fisher (2004). Fisher's study only found trends between other millipede species and stream proximity and could not find enough *O. gracilis* individuals to establish a significant relationship. Therefore, further field surveys should be conducted in order to verify to lack of significance of these three factors as influential factors. Performing more perpendicular transects is particularly important, as it still remains unclear how stream proximity affects *O. gracilis* populations.

An ability to utilize a large spectrum of environmental conditions could potentially aid the species in invading new environments (Seabloom *et al.*, 2003). *Oxidus gracilis*, having originated in Asia, has spread worldwide (Nakamura & Taira, 2005). Being non-native to French Polynesia and most oceanic islands, it would make sense for this species of millipede to be a generalist and have a large range of conditions that it can survive in.

The congregating behavior supported by the experimental data could also play a role in *O. gracilis'* ability to settle in new habitats. As mentioned before, the phenomenon of 'selfish herding' could be at play in the distribution of these millipedes (Hamilton, 1971). By grouping together, it is possible that they are protecting their total population from predation, as predators can only take on a few individuals at a time. On Mo'orea, *O. gracilis* millipedes are potentially susceptible to predation from ant species, which are able to prey *O. gracilis* despite its secretion of poisonous cyanide, so selfish herding may be useful (Brown, 1992; Hamilton, 1971; Taira *et al.*, 2003) It may also give the millipedes a competitive advantage by creating more opportunity for mating due to closer proximity of individuals (Dangerfield & Telford, 1993). However, as this study only established that congregating is occurring, further research needs to be conducted to determine why they are behaving as such. It is possibly due to the release and detection of pheromones by individuals, which could also be for mating purposes (Takeda, 1984). A potential future study could examine the frequency of copulative pairs in areas of high population density as well as analyzing the chemicals and pheromones at play.

As humans continue to alter the natural environment, it is important to understand why certain species have advantages over others and how behavior plays a role. It is predicted that because of increased drought, lower leaf litter quality due to increased atmospheric CO_2, and land cover changes in tropical regions that there will be a decrease in specialist millipede populations (David, 2009). Since *O. gracilis* was found to not be significantly influenced by soil moisture or

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leaf litter, it could be predicted that it will be one of the species that will thrive despite (or even because of) human disturbance. Their survival and advantage over other species could also be amplified by their tendency to congregate (Hamilton, 1971). As such, further research is necessary to verify the overall mechanism of this behavior. This will hopefully help provide a means of understanding the spread of invasive terrestrial diplopods and potentially aid in mitigating their expansion.

Conclusion

The fact that the distributions of *O. gracilis* did not seem to be dictated by soil moisture, leaf litter, or rock cover may indicate that the species is a generalist. The results of this study demonstrate some potential reasons for why *Oxidus gracilis* is so abundant on the island of Mo'orea and why it is so globally widespread. It could also indicate that there are other factors at play that require further investigation. The congregating behavior supported by the experimental results is an interesting case of how much behavior can vary within a group of animals. It thus remains important to avoid generalizations for the behavior of large groups. The results of the study also provide an example of why invasive species are so successful. By utilizing a large range of the resource spectrum, and by clustering, *O. gracilis* millipedes are able to occupy previously unoccupied niches and outcompete the native species of filled niches. It is therefore very important to continue to learn the specifics of invasive species behavior and resource utilization so that we can further find solutions to prevent ecosystem disruption.

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Appendix

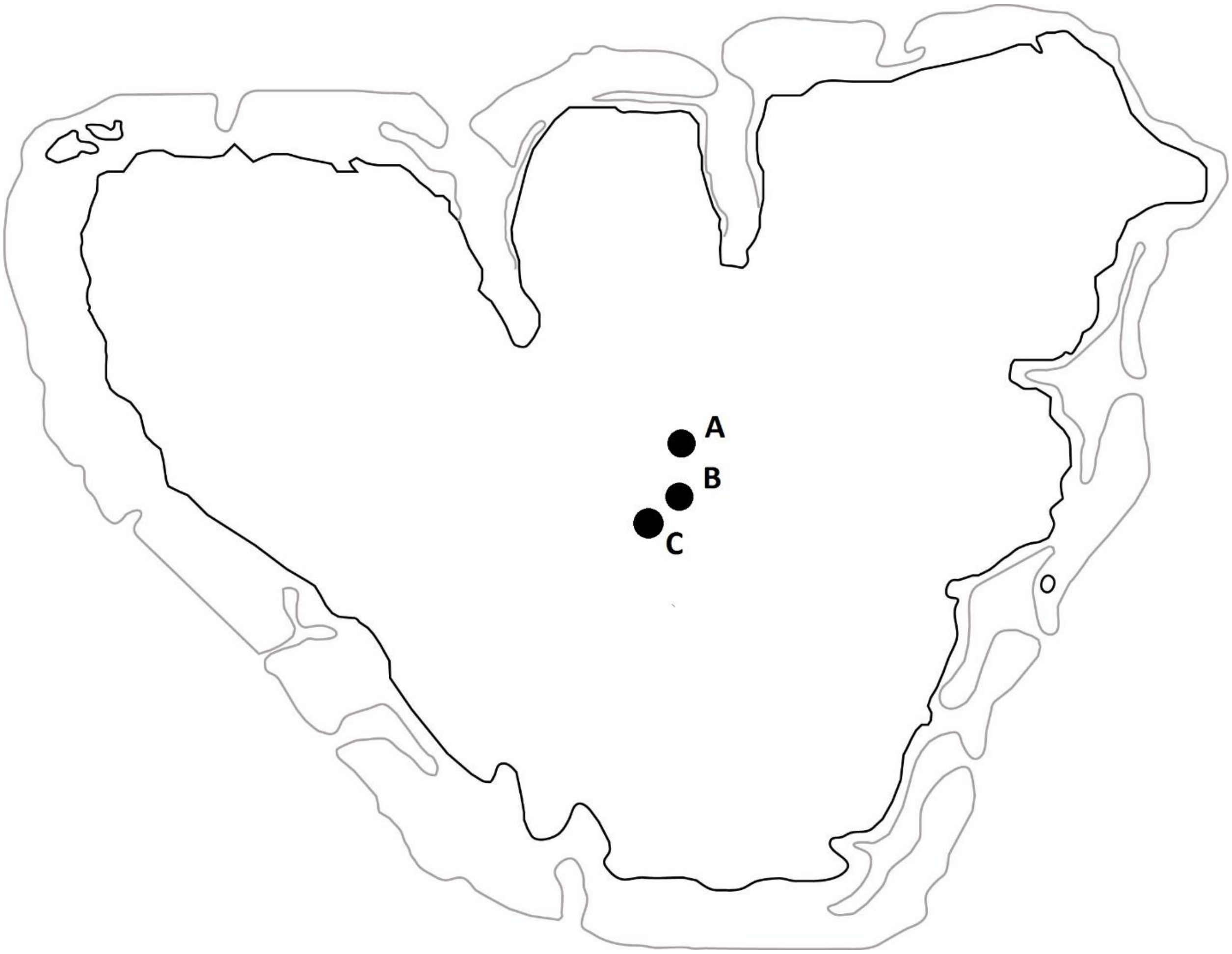

Figure 1. Map of the island of Mo'orea. Points A, B, and C represent the stream sites for the field surveys. (A) Three Pines Trail starting at S 17°32.173' W 149°49.729, elevation 168 m; (B) Three Coconuts Trail starting at S 17°32.045' W149°49.839', elevation 220 m; (C) Three Coconuts Trail starting at S 17°32.709' W149°50.103', elevation 201 m.

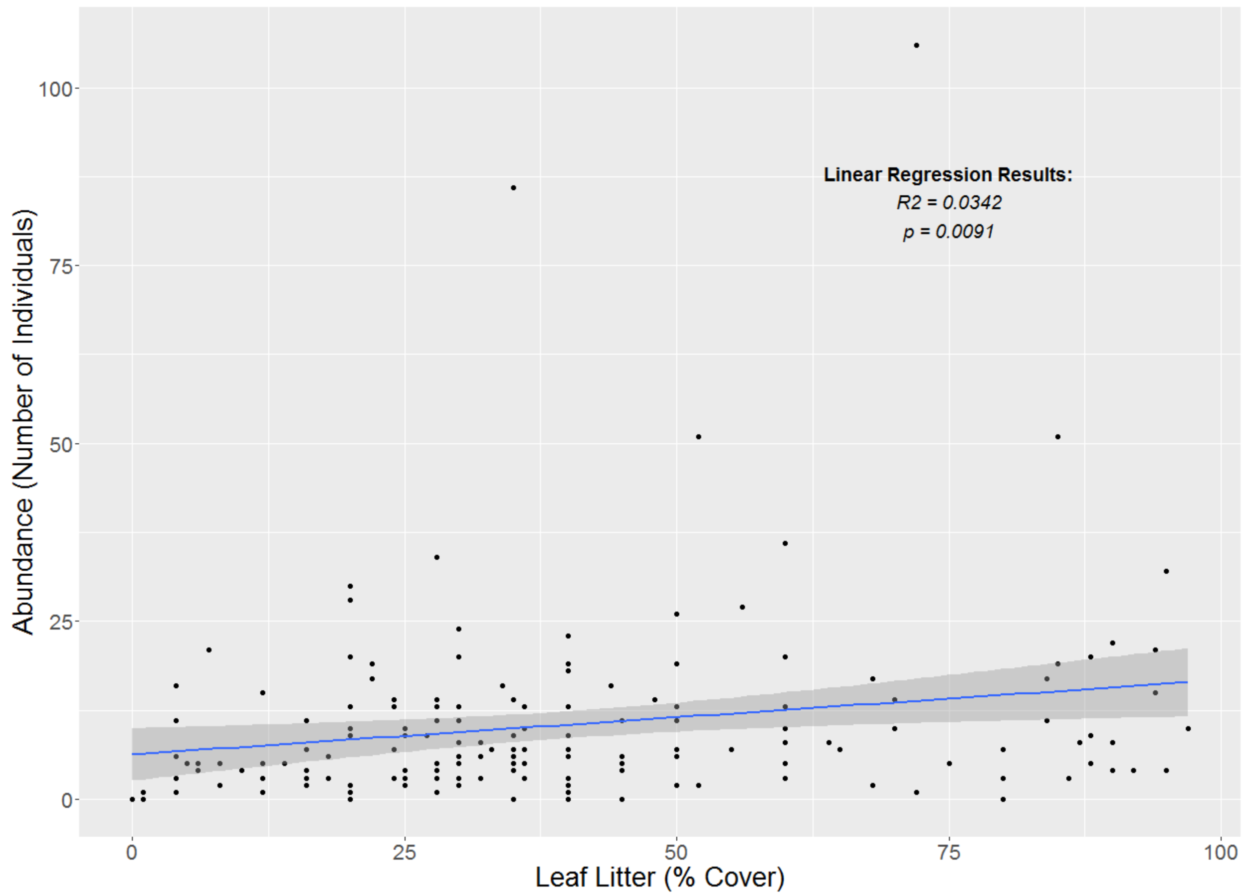

Figure 2. Abundance of O. gracilis in relation to leaf litter. Linear regression indicates relationship but that it accounts for only a small portion of the observed variance.

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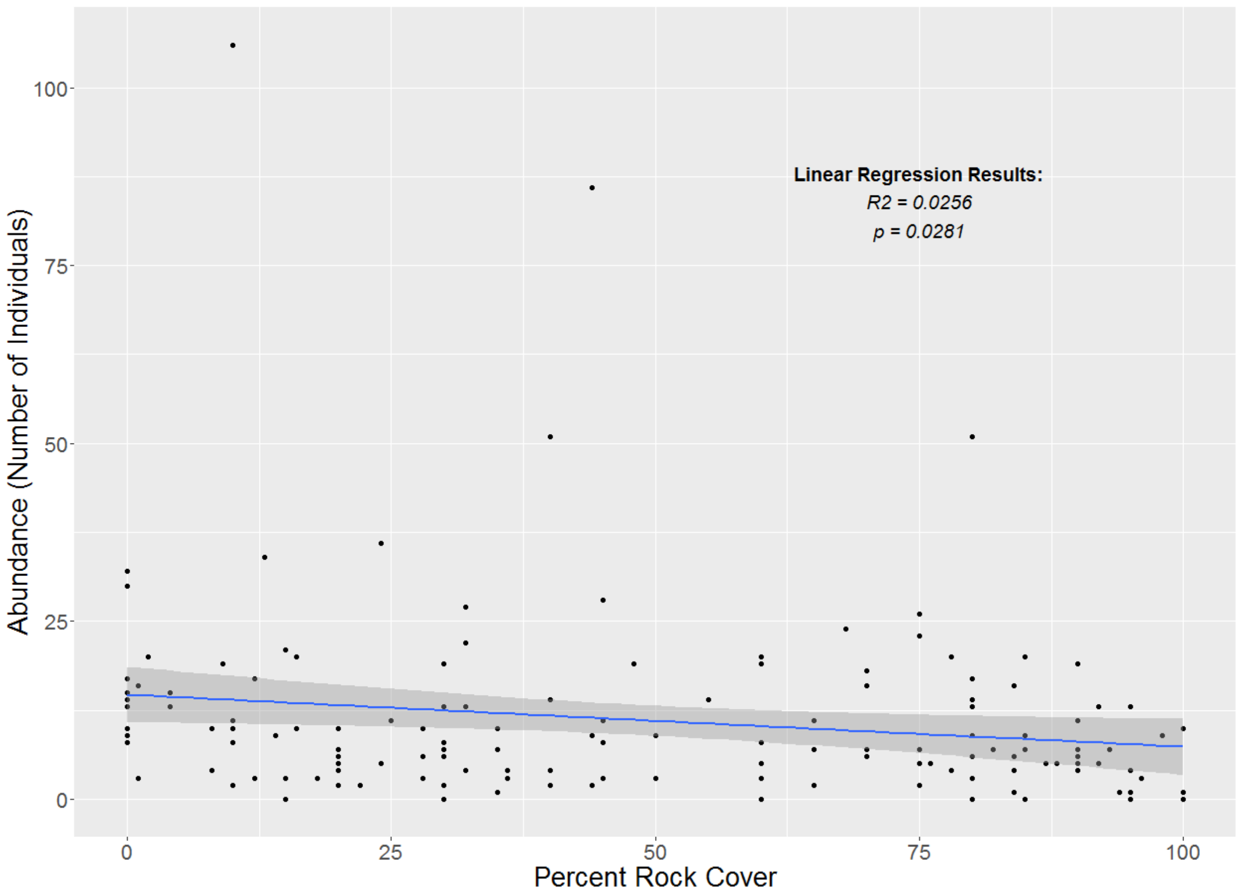

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283 *Figure 3. Abundance of O. gracilis in relation to rock cover. Linear regression indicates*
284 *relationship but a weaker one than seen in Figure 2.*

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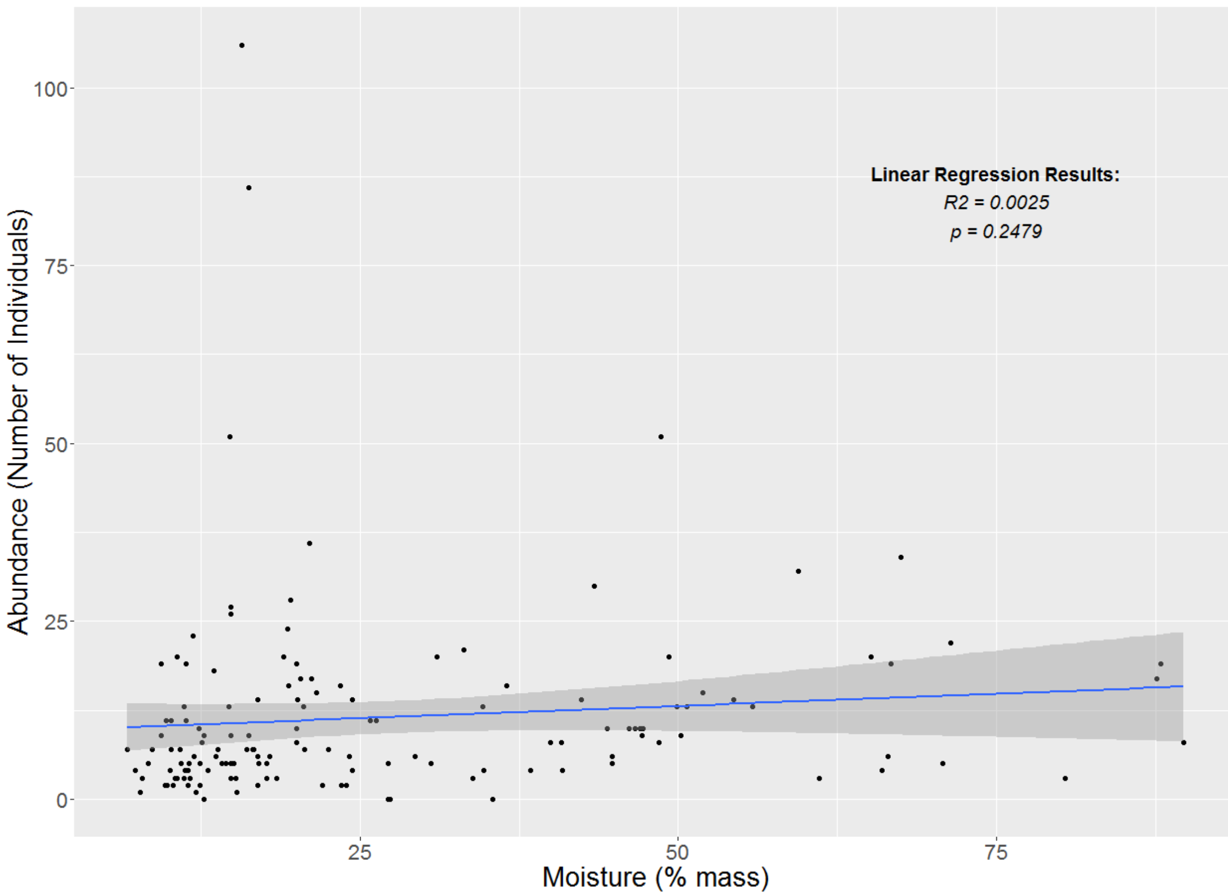

Figure 4. Abundance of O. gracilis in relation to soil moisture. Linear regression indicates that there is no significant relationship and that soil moisture cannot be used as a predictor for millipede abundance.

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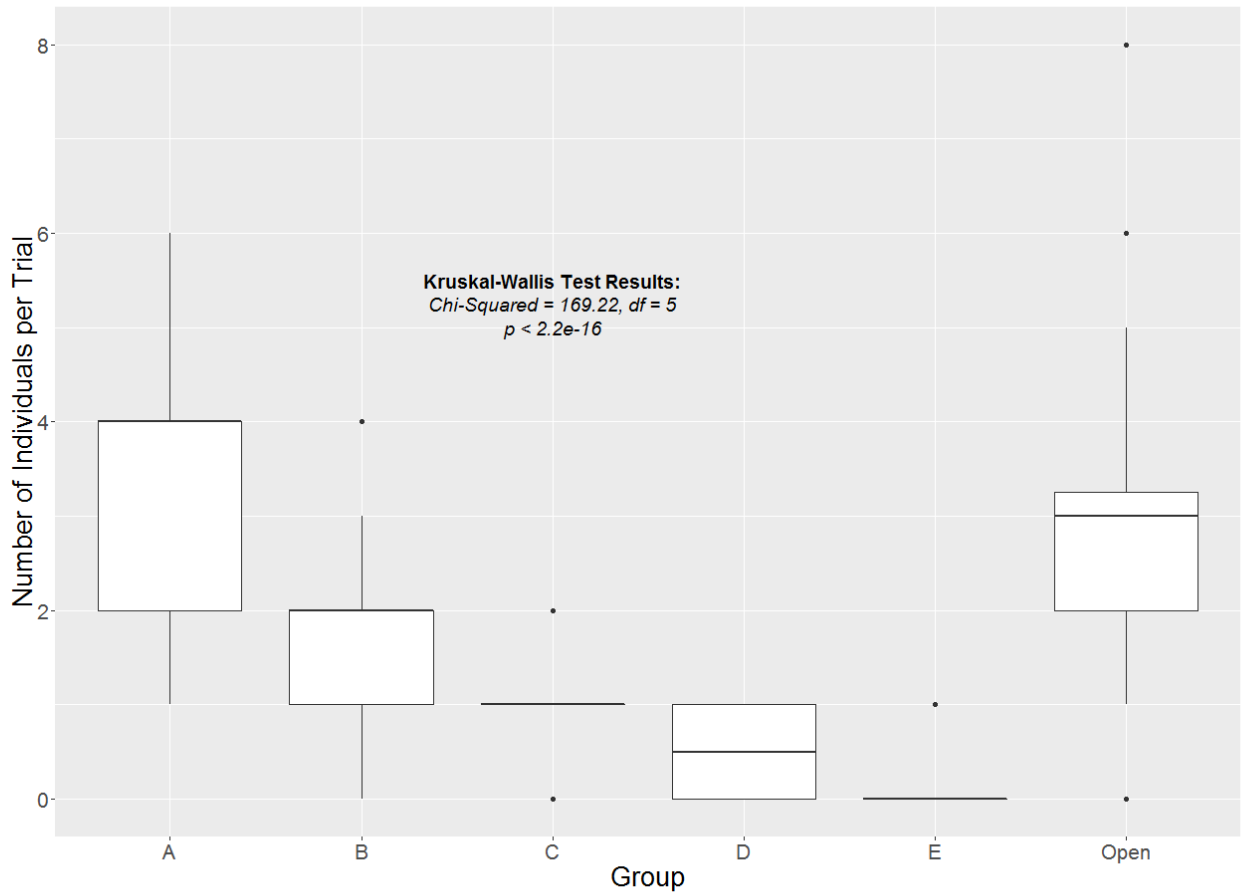

Figure 5. Results from congregation experiment. "Group" indicates that the fruit with the largest abundance of millipedes on it per trial, with "A" having the most individuals and "E" having the least. "Open" indicates the number of individuals per trial not found on any of the fruits. The results indicate that there is a very significant difference between the groups, meaning that a congregating behavior is likely.

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