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Acknowledgements
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The Effects of Environmental Factors on Bromeliad Invertebrate Biodiversity

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ABSTRACT Bromeliaceae is a family of Neotropical plants that retain water between leaves of a rosette arrangement. Each water-retaining tank is referred to as a phytotelma. This particular system is important to consider in the understanding of biodiversity because it creates an ecosystem of its own, providing a habitat for many invertebrates and larvae. In this study, the relationship between environmental factors such as water quality and the biodiversity of invertebrates in epiphytic bromeliads was examined in two different settings. Sample sizes of ten bromeliads were taken from the primary and secondary forests of the Las Cruces Biological Station in Coto Brus County, Costa Rica and compared. Prior to extraction from the trees, temperatures of the water in the inner phytotelmata of the bromeliads were recorded. Parameters including pH and phosphate concentrations in each bromeliad were then measured using approximately 15 mL of bromeliad water, the Testratest Laborett water kit, and the Hanna Instruments phosphate kit. Trends approaching significance were found between the number of species within the bromeliads and maximum tank volume (p=0.06608) as well as the total number of invertebrates within each bromeliad (p=0.06903). The number of species was however correlated to bromeliad distance from the ground (p=0.03215). The number of invertebrate species in bromeliads of the primary and secondary forest was not correlated with the water temperature (p=0.1420), pH (p=0.2826), or phosphate level (p=0.6954), however, these parameters should still be considered in the analysis of invertebrate biodiversity within bromeliads.

INTRODUCTION Under the broad scope of ecology, environmental conditions are examined for their effects on biodiversity and coexistence within communities. The mutualistic relationship between bromeliads and their inhabiting invertebrates is sustained under the control of these environmental factors. Generally, the relationship between bromeliads and invertebrates is important to the study of biodiversity as they rely heavily on each other for reproductive and dietary purposes. Invertebrates help to facilitate the spreading of seeds and fulfill the dietary needs of some carnivorous bromeliads, while bromeliads
provide many invertebrates with a habitat to live and hunt in (Frank 2009).

Many previous studies suggest that environmental factors and water chemistry control mutualism between bromeliads and inhabiting invertebrates. In a study of micro-environmental factors that affect bromeliad fauna, self-induced acidification by the plant was found to affect the types of species that inhabited it (Lopez 2009). In another study by Laessle (1961), sun exposure and leaf litter were shown to have a large impact on water quality within Jamaican bromeliads. Bromeliads in wooded areas exhibited large amounts of dead leaves in the water, which was correlated to high carbon dioxide and low dissolved oxygen readings (Laessle 1961). In our study, the effect of water quality on the biodiversity of invertebrates within the phytotelmata (water tanks) of bromeliads was examined in the primary and secondary forests of the Las Cruces Biological Station in Coto Brus, Costa Rica.

Several parameters may play an important role in determining invertebrate biodiversity within the phytotelmata (water tanks) of bromeliads. Water temperature is vital to tropical plant health, as bromeliads with increased water temperature have also shown a decrease in concentration of oxygen (Guimaraes-Souza 2006). The pH of the water is also important to consider, as pH values of 4.5 and lower have been found to correlate to less bacterial biodiversity as compared to more neutral pH values (Goffredi 2010). Excess phosphate concentrations in water have similar consequences, as they accelerate plant growth and deplete oxygen levels (Fried 2003).

In this study, we wanted to determine whether biodiversity of invertebrates that inhabit bromeliads would differ between the primary and secondary forests of the Las Cruces Biological Station in Costa Rica. While the primary forest is in its original condition with plentiful amounts of plant life, the secondary forest has been regenerated and allows more sunlight to penetrate its forest canopy. We correlated the pH, temperature and phosphate concentration of the water in the phytotelmata with the biodiversity of invertebrates inside the bromeliads. Because exposure of water to sunlight has been previously correlated to lower pH values, the pH of the bromeliad waters in the secondary forest is expected to be lower than that of the bromeliads in the primary forest. Due to the correlation of acidification with lower rates of biodiversity, pH of the bromeliad water is expected to correlate with less biodiversity within the bromeliads, thus less invertebrate biodiversity is expected to be observed in the secondary forest. Excess levels of phosphate are also expected to correlate with depletion of oxygen and less biodiversity in the bromeliads.

METHODS

FIELD SITE

As described in Figure 2, 20 bromeliad samples were collected from the primary and secondary forests of Las Cruces Biological Station in Coto Brus County, Costa Rica during the 15th and 16th of July, 2014. Las Cruces is ~1200 meters above sea level and has an average rainfall of 4 meters per year with daily temperatures from 13 to 26°C (OTS 2013). Data from all 20 individuals were used to correlate maximum inner tank volume with invertebrate biodiversity. Water quality analysis was performed on two samples of 6 bromeliads from each forest because these were the only samples with adequate amounts of water to perform the tests.

PARAMETERS

Temperature, pH, plant length and width, and phosphate concentrations were measured in the water of the bromeliad phytotelmata. To control for the potential effects of rainwater on pH, samples were taken from two bromeliads in the Wilson Garden of the Las Cruces Biological Station over a five-day period to test if the pH changed significantly after rain. No significant effects were found, as the pH of the waters remained at five throughout the test.

SAMPLING PROTOCOLS

All bromeliads (N=20) were selected haphazardly in the primary and secondary forests of Las Cruces Biological Station (Fig. 2). The average dimensions of each were found to be 14 centimeters in width and 20 centimeters in height. Given the assumption that all bromeliad
tanks are cylindrical in shape, the average maximum central phytotelma volume was found to be approximately 135 mL. All samples were epiphytic and grew off of trees found along the trails of the primary and secondary forests. Each bromeliad was on average 160 centimeters above the base of the tree from which it was extracted. Water temperatures from the central phytotelma of the bromeliads were recorded on the 15th and 16th of July 2014. All samples of bromeliads were cut from the trees on the 16th of July. Water samples of six epiphytic bromeliads from the primary and secondary forests (N=12) were collected immediately after returning from the field and measured for pH and phosphate concentrations in the laboratory.

**ANALYSES**

Prior to analysis, all water samples were separated from soil and other contamination using a Clay Adams Compact II CentrifugeTM. The 15 mL water samples from each bromeliad (N=12) were analyzed for pH and phosphate concentrations with a Tetratest Laboret water kit (Blacksburg, VA) and Hanna instruments kit (Woonsocket, RI). Additionally, the width and height of each bromeliad was recorded prior to further analysis (Fig. 3). Bromeliad invertebrates and larvae were individually identified as morphospecies under a microscope and grouped together based on structural similarities. Using Statistica 7 software, the relationships of temperature, tank volume, pH, and phosphate concentrations with biodiversity of inhabiting invertebrates were investigated. Species richness, or the number of different morphospecies in each bromeliad was also correlated species abundance, or the distribution of the number of individuals in each morphospecies. Similarities in the biodiversity of invertebrates were assessed in the two forests using Primer 6 software. Further analyses were performed to decipher which individuals were most alike and which species contributed most to invertebrate biodiversity. CLUSTER analysis was used to group the most similar bromeliads and display their exact similarity percentages. A multidimensional scaling (MDS) plot was also used to visualize similarities among all bromeliad samples, with the most similar samples being within the closest proximity.

**RESULTS**

Seventeen of the bromeliads were identified as *Werauhia gladiolifolio*, 2 were identified as *Aechmea mexicana*, and 1 was identified as *Guzmonla zanhaii*. In total, 42 invertebrates were identified as morphospecies. Of these, 30 were found in the secondary forest, and 20 were found in the primary forest (Fig. 4).

Using CLUSTER analysis, major similarities were found among bromeliads in the primary and secondary forests. There was an 88% similarity among secondary forest bromeliads 7 and 10 and primary forest bromeliads 1 and 9, a similarity of 95% among primary forest bromeliads 2 and 3 and secondary forest bromeliads 1, 4, and 9, and a 95% resemblance among primary forest bromeliad 7 and secondary forest bromeliads 3, 5, 6, and 8 (Fig. 5).

Assuming $\alpha = 0.05$, the number of invertebrate species in bromeliads of the primary and secondary forest was correlated with bromeliad distance from the ground ($R^2=0.3822$, $p=0.03215$), and approaching correlation with maximum tank volume ($R^2=0.2985$, $p=0.06608$) and the total number of invertebrates within each bromeliad ($R^2=0.6302$, $p=0.06903$). The number of invertebrate species in bromeliads was not correlated with the water temperature ($R^2=0.2026$, $p=0.1420$), pH ($R^2=0.1142$, $p=0.2826$), or phosphate concentration ($R^2=0.01598$, $p=0.6955$).

Despite the lack of correlation of invertebrate species with bromeliad water temperature, pH, and phosphate concentration, these parameters contributed to further analysis. In a comparison of bromeliads of the same cluster, all samples were the same species and had very similar water temperatures, pH levels, and invertebrate richness. Secondary forest bromeliads 7 and 10 and primary forest bromeliads 1 and 9 all belonged to the species *W. gladiolifolio*, exhibited average water temperatures of approximately 22.0°C, and contained an average of 4 invertebrate species each. Primary forest bromeliads 2 and 3 and secondary forest bromeliads 1, 4, and 9 exhibited an average pH reading of 6, similar water temperatures of approximately 21.8°C, and average invertebrate
species count of 6. Primary forest bromeliad 7 and secondary forest bromeliads 3, 5, 6, and 8 all belonged to the species *W. gladiolifolio*, had an average pH value of 5.7, and an average invertebrate species count of 8 each.

When all individuals were compared using MDS, primary forest bromeliad 5 differed most from the rest of the samples (Fig. 6). This bromeliad was the only individual of *G. zanhii* and had the lowest amount of invertebrate species of 2. Secondary forest bromeliad 10 also exhibited large differences as it had a high average water temperature of 23.8°C with a low invertebrate species count of 2.

**DISCUSSION**

While we hypothesized that there would be concrete differences between the biodiversity of invertebrates in bromeliads of the primary and secondary forests, the results instead show that there are major similarities among bromeliads in both settings. Most notably, the bromeliad distance from the ground, maximum tank volume, and invertebrate species richness were found to contribute to invertebrate biodiversity in both forests.

No significant relationship was found between invertebrate biodiversity and type of forest, as only 10 more species of invertebrates were found in primary versus secondary forests. This suggests that invertebrates generally do not have a distinctive preference for bromeliads in the primary or secondary forests. Furthermore, the MDS analysis shows that bromeliads in different forests with similar invertebrate species counts are more alike than bromeliads in the same forest with different species counts. A study by Jabiol (2008) also found that the aquatic insects that inhabited *Guzmania. lingulata* were unaffected by bromeliad location.

A strong correlation between invertebrate biodiversity and distance from the ground was found, suggesting that invertebrates are more present in bromeliads that are closer to the treetops. Possible explanations for this include greater access to sunlight and bromeliad ability to accumulate rainwater in the phytotelmata.

The moderate correlation between invertebrate biodiversity and maximum inner tank volume, which was also found by Jabiol (2008), suggests that the inhabiting species have a greater presence in larger bromeliads with greater water retaining capacity. Richardson (1999) also found that invertebrate species richness is highly correlated to plant size, which further confirms the validity of these results, as maximum tank volume generally increases with increasing plant size. Invertebrate richness was also moderately correlated to species abundance, suggesting that competition among invertebrates decreases with an increase in biodiversity of species. These results explain why most of the larger bromeliad samples contained greater amounts of species and invertebrate individuals.

While the water temperature, pH, and phosphate concentrations were not correlated to invertebrate biodiversity, these parameters may still be important for determining the effects of environmental factors on bromeliad invertebrates, as they are indicators of resemblance among all bromeliad samples.

The lack of correlation between the numbers of invertebrate species found in each bromeliad with the pH and phosphate concentrations is likely due to the color of the bromeliad water. The powder-based Tetratest Laborett pH kit and Hanna instruments phosphate kit were designed for clear and colorless water, whereas most of the bromeliad water remained a deep yellow color after centrifuging. Also, the kits were only accurate within 0.5 pH units and 1 mg/L of phosphate, so many of the samples that were classified as having the same pH and phosphate concentrations may actually have had values that were slightly different from one another.

In a previous study by Goffredi (2010) of the effect of pH on bacteria within bromeliads, tank water was found to be greatly affected by the soil surrounding the host tree. Different soil types were found to alter the biochemistry of the tree leaves, which in turn decomposed in the bromeliad, thus affecting the water quality and amounts of bacterial species (Goffredi 2010). In our study, the CLUSTER analysis showed that many bromeliads with similar pH levels also housed very similar invertebrate species, as they were between 88-95% alike. For these reasons, pH and phosphate concentrations may still play
a large role in the invertebrate biodiversity within bromeliads. Future studies of the effects of pH and phosphate concentrations on invertebrate biodiversity would possibly yield significant results using a pH probe and a Hach PHOSPHAX sc phosphate analyzer (Loveland, CO), as these forms of equipment allow for more accurate measurements using much smaller amounts of liquids.

While the CLUSTER analysis showed 95% similarity among 5 bromeliads with very similar water temperatures, no significance was found in the direct correlation of invertebrate biodiversity and average bromeliad water temperatures. This result may have been affected by the times of day that the waters were sampled. Water temperatures were taken between 10:30 a.m. and 2:00 p.m. on the 15th of July and between 8:30 a.m. and 11:00 a.m. on the 16th of July. It is also very likely that there is not a wide enough range of water temperatures in bromeliads of Las Cruces to affect species richness. In a study on the effects of land-use change on larval insect communities within bromeliads, Ngai (2008) found a correlation between temperature and species richness within bromeliads. According to Tilman (1982), increased temperature may also play a large role in competition among species in fresh water habitats. These studies suggest that while little correlation was found in our study, temperature may remain a factor in the invertebrate biodiversity within bromeliads.

**CONCLUSIONS**

Future studies at Las Cruces could focus more on the factors that affect the presence of invertebrates that inhabit epiphytic bromeliads, such as tolerance and competition with other invertebrates. Invertebrates seem to be more present in bromeliads that are closer to the top of the forest canopy with larger tank volumes. For these reasons it may be important to consider the effects of light intensity and inches of rainfall per hour on invertebrate biodiversity. It may also be useful to measure the effects of concentration of tree leaves in bromeliad waters on invertebrate biodiversity, as this may contribute to water acidity and in turn lower pH levels. Laessle (1961) also concluded that leaf litter and sunlight had profound effects on the pH, CO₂, and O₂ concentrations in the tank waters. Algae levels may also be important to consider, as Benzing (1972) found that CO₂ concentrations were higher in bromeliads containing algae, debris, and insects as compared to bromeliads with debris and insects alone. Generally, further studies of communities within bromeliads are important to our advancement in scientific knowledge, as they represent microcosms of larger natural environments.
Figure 1. Photo of an intact epiphytic bromeliad
Figure 2. Map of bromeliad locations in the Las Cruces Biological Station forest (generated by Yerlyn Blanco)
Figure 3. Photo of phytotelmata and dimensions of a bromeliad, where (1) represents phytotelm diameter, (2) represents bromeliad width, and (3) represents bromeliad height from the shortest inner leaf.

Figure 4. Total number of invertebrate morphospecies observed in the primary and secondary forests in Las Cruces Biological Station.
Figure 5. Similarity percentages (Bray-Curtis) of bromeliads from the primary and secondary forests of Las Cruces Biological Station.

Figure 6. MDS analysis of bromeliad individuals in the primary and secondary forests of Las Cruces Biological Station.
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