
-AJ-DAVID-SAVANA-NATE-MORGAN-MARTIN-NATHAN-
-SOPHIE-NEHIR-
-ETHAN-SORAIA-SOFI-JAIRO-MIGUE-
-ADRI-
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Instructors:
Miguel Chaves, PhD
Adriana Arias-Aguilar, PhD

Teaching Assistant:
Jairo Villalobos

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PLANT-FUNGI INTERACTIONS

Percent colonization of mycorrhizae is higher in *Palicourea padifolia* (Rubiaceae) than in, *Quercus insignis* (Fagaceae), and is not correlated to aboveground nor belowground biomass in Monteverde, Costa Rica

Morgan Collins
Department of Biology, Brandeis University, Massachusetts, USA

ABSTRACT

Mycorrhizae are mutualistic plant-based fungi that are essential for the survival of plants and help ensure the preservation of biodiversity. Mycorrhizal associations help plants sequester carbon and are fundamental for plant survival and growth by providing nutrients and protection from drought, pollution, pathogens, and abiotic stressors. Given climate change, increasing greenhouse gas emissions, and deforestation, the health of ecosystems and biodiversity found amongst these forests is threatened. The percent colonization of mycorrhizae in tree saplings and their role in sapling size of two species, 15 samples of *Palicourea padifolia* (Rubiaceae) and 15 samples of *Quercus insignis* (Fagaceae) were investigated at Monteverde, Costa Rica. The percent colonization of mycorrhizae varied significantly between species with *P. padifolia* having an average of 19% and *Q. insignis* with 13% (F=6.487, P=0.017). As sapling height increased, the percent colonization of mycorrhizae of *Q. insignis* also increased (R^2=0.2945, F=5.428, P=0.037) but there was no effect on *P. padifolia* (R^2 = 0.011, F = 0.147, P = 0.708). Additionally, while aboveground biomass (AGB) and belowground biomass (BGB) of all sapling species were strongly correlated, percent colonization of mycorrhizae had no significant effect on either AGB or BGB. This study helps determine tree dependance on and relationship with mycorrhizae and expands upon ecological interactions with the purpose of preserving and protecting the tropical forest.

RESUMEN

Las micorrizas son hongos mutualistas de origen vegetal que son esenciales para la supervivencia de las plantas y ayudan a asegurar la preservación de la biodiversidad. Las asociaciones de micorrizas ayudan a las plantas a secuestrar carbono y son fundamentales para la supervivencia y el crecimiento de las plantas al proporcionar nutrientes y protección contra la sequía, la contaminación, los patógenos y los factores estresantes abióticos. Dado el cambio climático, el aumento de las emisiones de gases de efecto invernadero y la deforestación, la salud de los ecosistemas y la biodiversidad que se encuentra entre estos bosques está amenazada. Se investigó el porcentaje de colonización de micorrizas en árboles jóvenes y su papel en el tamaño de los árboles jóvenes de dos especies, 15 muestras de *P. padifolia* (Rubiaceae) y 15 muestras de *Q. insignis* (Fagaceae) en Monteverde, Costa Rica. El porcentaje de colonización de micorrizas varió significativamente entre especies con *P. padifolia* con un promedio de 19% y *Q. insignis* con 13% (F=6.487, P=0.017). A medida que aumentó la altura de las plántulas, también aumentó el porcentaje de colonización de micorrizas de *Q. insignis* (R^2=0.2945, F=5.428, P=0.037) pero no hubo efecto en *P. padifolia* (R^2=0.011, F=0.147, p=0.708). Además, mientras que la biomasa aérea (AGB) y la biomasa subterránea (BGB) de todas las especies de árboles jóvenes estaban fuertemente correlacionadas, el porcentaje de colonización de micorrizas no tuvo un efecto significativo ni en AGB ni en BGB. Este estudio ayuda a determinar la dependencia y relación de los árboles con las micorrizas y amplía las interacciones ecológicas con el propósito de preservar y proteger el bosque tropical.

INTRODUCTION

Due to increasing rates of climate change, the earth’s ecosystems and biodiversity have become threatened. Climate change decreases cold temperature extremes, increases warm temperature extremes such as intense heat waves, and changes precipitation patterns due to an increasing level of atmospheric greenhouse gases (Milović et al., 2021). This is due to increasing fossil fuel use in industry and results in higher levels of carbon in the atmosphere, trapping in heat and increasing the earth’s overall temperature. However, tropical forests pose as a potential solution as they can store carbon and act as carbon sinks (Milović et al., 2021).

Carbon is stored in tropical forest through photosynthesis of plants. Trees can store carbon in their aboveground biomass (AGB) comprised of leaves, stem, and branches (Milović et al., 2021) as well as in their belowground biomass (BGB) comprised of the root system (Simard and Austi, 2010). Forest soils store one third of the Earth’s carbon, and act as a stable carbon sink because of the carbon stored in the BGB of plants. Most plants can accomplish this carbon sequestration with the assistance of mycorrhizae (Simard and Austi, 2010).

Mycorrhizae are mutualistic plant-based fungi that are essential to biodiversity, ecological stability, and productivity (Sweeny et al., 2022). They are present in about 90% of plants species (Milović et al., 2021). Mycorrhizae are beneficial to vascular plants because they help acquire mineral nutrients and water, protect the plants from pathogens, drought, and pollution, and provide relief from abiotic stressors (Sweeny et al., 2022). In exchange for these benefits, mycorrhizae absorb carbohydrates and carbon from the plant (Sweeny et al., 2022). This mutualism is facilitated by modified absorptive organs made up of plant roots called photobionts, and fungal hyphae called mycobionts. These absorptive organs expand the surface area of plant roots to increase water and nutrient absorption, forming a connection network at community and ecosystem levels (Huey et al., 2020). This relationship of increased nutrient acquisition and protection likely allows for greater growth of plants and therefore increased biomass as well as increasing amounts of carbon sequestration (Thippawan et al., 2023).

There are various species mycorrhizae are known to associate with such as *Quercus insignis* (Southworth, 2013) and types of *Rubiaceae* (Pendleton et al., 1983). *Palicourea padifolia* is a species of *Rubiaceae* prominent in the understory of the tropical rainforest ecosystem (Juárez et al., 2011) and both are crucial species for surviving primary forest ecosystems (Southworth, 2013) (Juárez et al., 2011).

The objectives of this study are to understand how mycorrhizal colonization varies across species as well as how mycorrhizal colonization affects aboveground and below ground biomass. Most existing literature focuses on AGB, often overlooking BGB. Additionally, the influence of mycorrhizae on biomass is not yet well understood. Studying plant mycorrhizal associations is fundamental to better understand the role of tress as carbon sinks, and how tropical forests help to mitigate climate change.

MATERIALS AND METHODS

Study site

Samples were collected on the trails surrounding La Estación Biológica at CIEE Monteverde in two primary locations (Fig. 1) at an elevation of approximately 1500 m. This is classified as a tropical premontane moist forest. Samples were collected approximately 700 m into the "El Principal" trail, and in the forest surrounding the building. The average temperature is 16-18°C and annual rainfall is 3000 mm. AGB and BGB were measured at the laboratory facilities in the CIEE Main Campus in Monteverde, Costa Rica.

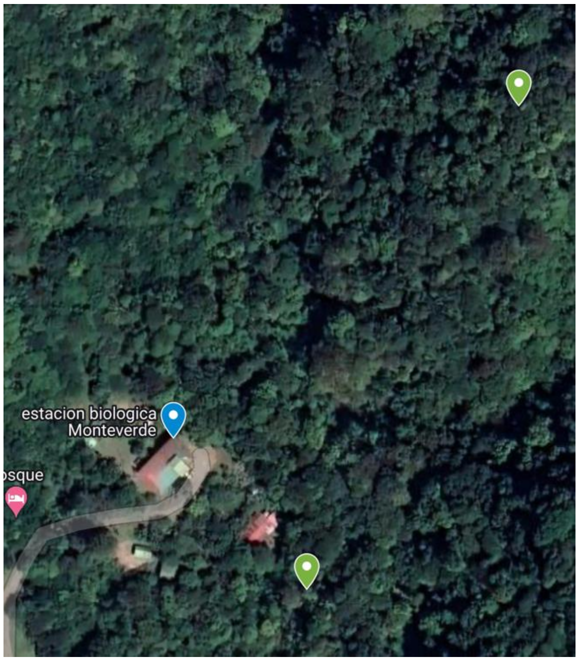

Figure 1. Map of the general sample collection locations (green pins) and primary staining technique location (blue) in Monteverde, Costa Rica.

Sample collection and identification

30 total sapling samples were collected: 15 samples of *Q. insignis* of varying sizes from 4–62 cm and 15 samples of *P. padifolia* of varying sizes from 5–37 cm. First, the species was identified by their morphology, and the height of the sapling was measured with a tape measurer. Then, a hole with approximately a 10 cm diameter and about 20 cm deep was dug surrounding the base of the selected sapling (these holes were larger for larger saplings) (Fig. 2). The dug section was pulled out from the ground, the surrounding dirt was shaken off, and other roots were separated from the sample. The samples were labeled by species of tree, number sample, and transported in a bag to the laboratory for further analyses.

Figure 2. Example of an up-rooted sample used for piloting the collection and staining method before (left) and after (right) clearing the dirt from the roots at Monteverde, Costa Rica.

Determination of percent colonization of mycorrhizae

At the laboratory, I quantified mycorrhizae colonization following the staining technique outlined by Vierheilig et al. (1998). The roots were first rinsed thoroughly to remove the soil until they appeared white. Then, the sapling was set out to dry and a small sample approximately 5 mm long and 1 mm wide was cut using dissection tool. They were then boiled in in 10% KOH in a hot water bath using a heating plate for 6 min and rinsed with water. Then, the root sample was placed in a 10 mL of boiling white household vinegar (5% acetic acid) with 3 drops of 10% ink-vinegar solution for 3 min. The sample was then rinsed by water and placed in vinegar until no dye runoff is present (approximately 2 minutes) (Vierheilig et al., 1998). The root sample was viewed under the light microscope at 10x magnification, and an image was taken through the lens of the root. Then, the grid method was employed to quantify the percent colonization (McGonigle et al., 1990). The image was cropped to the edges of the microscope lens and a grid was overlaid using the app, Grid#. The total number of grid boxes occupied by the visible root were counted. Then, the number of grids containing mycorrhizae (dark blue spots) were counted (Fig. 3) and used to generate a percentage of colonization of mycorrhizae for analysis.

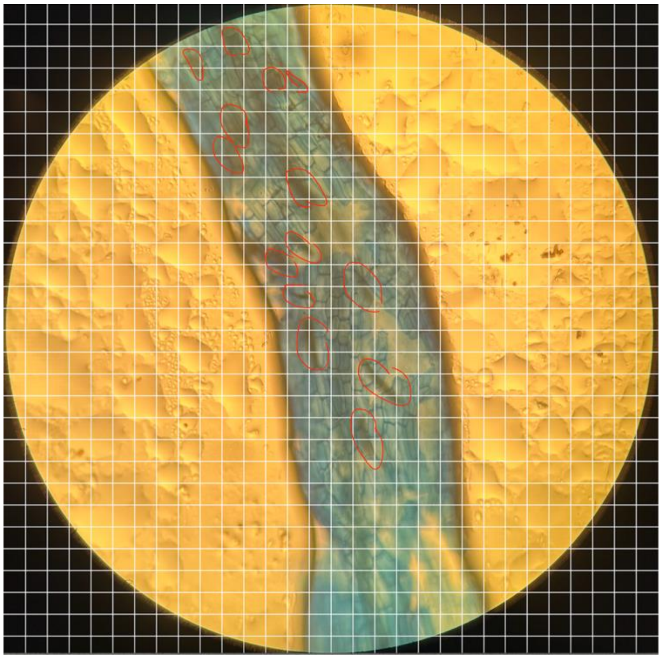

Figure 3. Example of a grid overlaid on a root sample of *Q. insignus* showing the method of calculating the percent mycorrhizae colonization at Monteverde Costa Rica. The individual mycorrhizae (dark blue spots) are circled in red for visualization.

Calculation of AGB and BGB

After the samples dried from washing, they were cut at the root system to separate aboveground (stem and leaves) and belowground (root) parts of the plant. Each sample was placed in paper bags and labeled with the species and number sample. Then, all samples were dried for 48+ hours in an oven at approximately 65°C (Fig. 4). Each sample's AGB and BGB were measured separately using a balance (± 0.01 g) (Fig. 5) and recorded for analysis. A value of 0.01 g was assigned to samples that were too small to be weighed.

Figure 4. Tree sapling samples in brown bags, after being dried out in oven for approximately 48 hours at 65°C at Monteverde, Costa Rica.

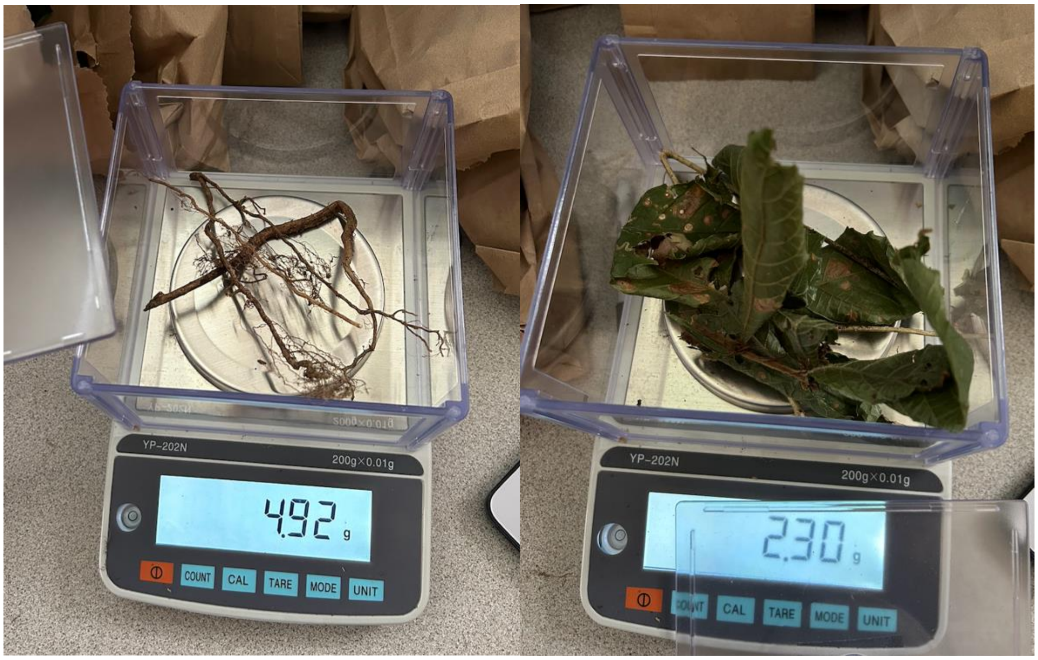

Figure 5. *Q. insignis* (Fagaceae) sample BGB (left) and AGB (right) as they are weighed on a balance after separation at Monteverde, Costa Rica.

Statistical Analysis

All graphs and statistical analysis were performed using R programming software (R Development Core Team 2020). An analysis of variance (ANOVA) was performed to determine if there was significant difference between species percent colonization of mycorrhizae. A linear regression model was also performed to test the effect of plant height on mycorrhizal colonization. An R^2 value was generated to determine how much the variation of percent colonization of mycorrhizae for each species of is explained by plant height.

To understand the relationship between AGB and BGB I conducted a Pearson correlation test.. Lastly, to test the effect of mycorrhizae percent colonization on both AGB and BGB I performed a linear regression model.

RESULTS

Percent colonization by species

Palicourea. padifolia has an average of 19% mycorrhizae colonization, 6% greater than *Quercus insignis* which had an average of 13% colonization (Fig. 6). *P. padifolia* percent colonization of mycorrhizae ranged from 8%-33% while *Q. insignis* ranged from 4%-28%. The percent mycorrhizae colonization between *P. padifolia* and *Q. insignis* saplings was statistically significant (F=6.487, P=0.017).

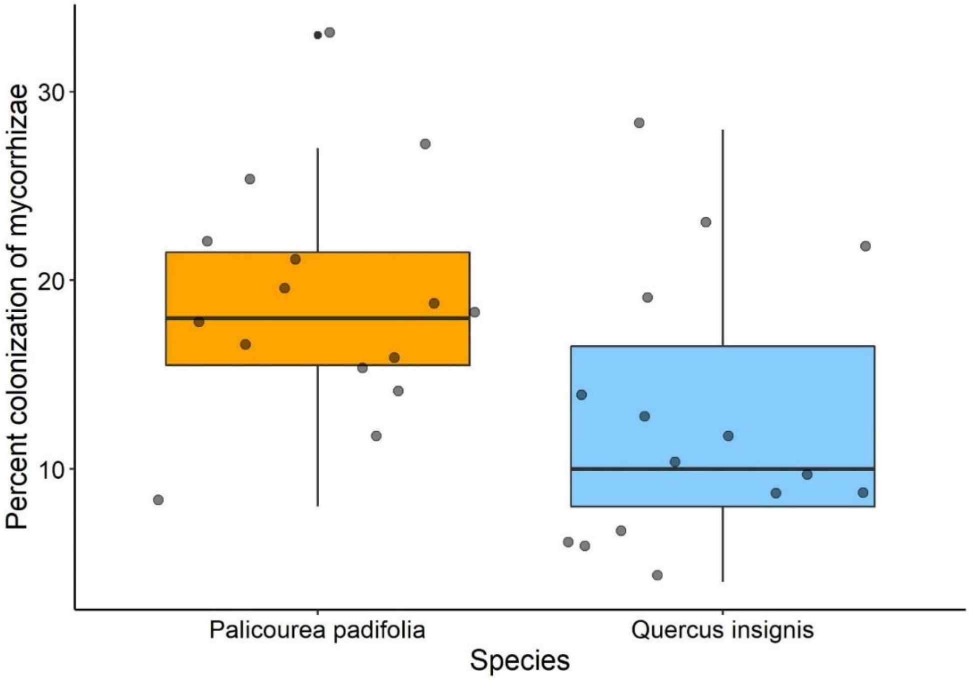

Figure 6. Percent colonization of mycorrhizae in *P. padifolia* (Rubiaceae) and *Q. insignis* (Fagaceae) at Monteverde, Costa Rica.

Percent colonization and sapling height

Plant height was found to have a significant, positive effect on percent colonization of mycorrhizae in *Q. insignis* (R^2=0.2945, F = 5.428, P = 0.037) but had no significant effect on *P. padifolia* (R^2 = 0.011, F = 0.147, P = 0.708) (Fig. 7).

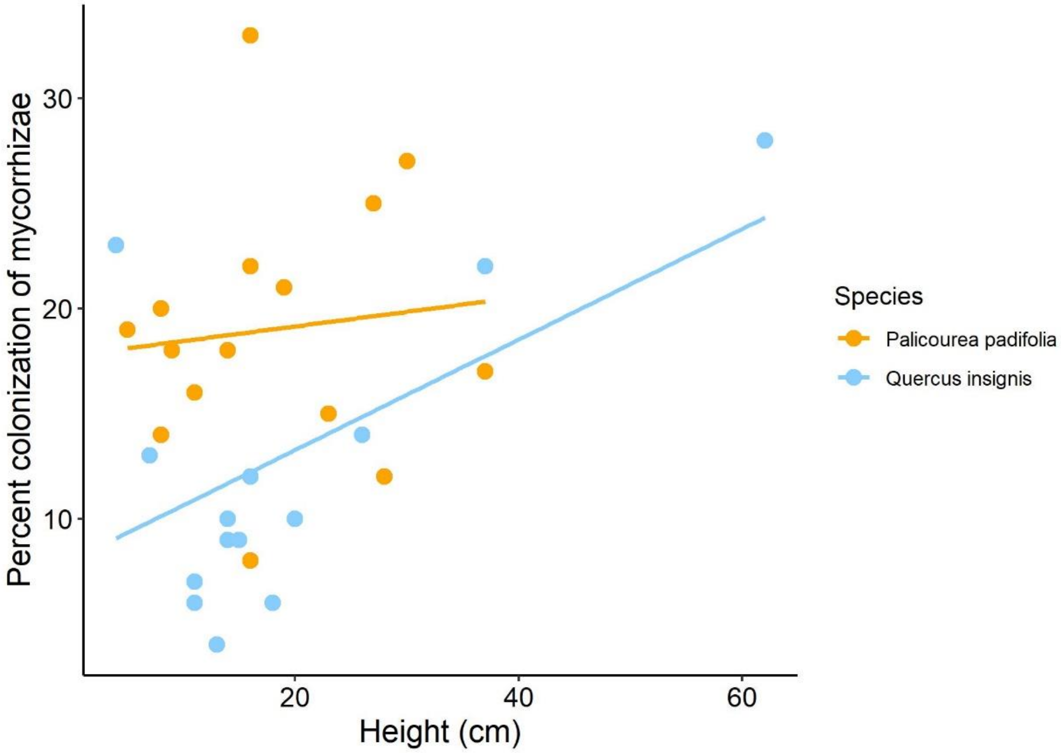

Figure 7. Plant height and percent colonization of mycorrhizae for *P. padifolia* (Rubiaceae) and *Q. insignis* (Fagaceae) in Monteverde, Costa Rica.

AGB and BGB correlation

A strong positive correlation between AGB and BGB (R= 0.808, P < 0.001) for both *P. padifolia* and *Q. insignis* (Fig. 8) was found. This indicates that the variation of AGB can be explained by BGB and vice versa.

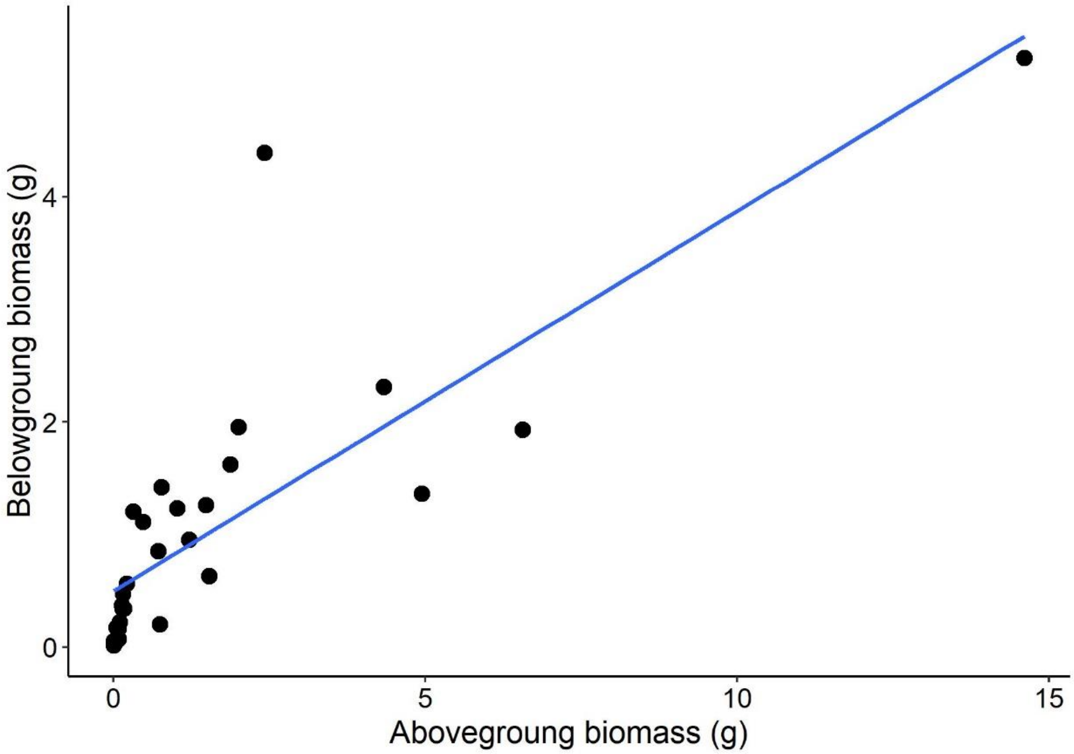

Figure 8. Aboveground biomass (AGB) and belowground biomass (BGB) for both *P. padifolia* (Rubiaceae) and *Q. insignis* (Fagaceae) in Monteverde, Costa Rica.

Percent colonization of mycorrhizae and AGB

The average measured AGB for *P. padifolia* was 0.92g and the average measured AGB for *Q. insignis* was 1.12g. There was no significant effect of percent colonization of mycorrhizae on AGB (Fig.9) when both species were analyzed together (F = 0.010, P = 0.922), indicating variation in AGB cannot be explained by the percent colonization of mycorrhizae. Similar results were found when individual species analyzed separately. *P. padifolia* (R^2= 0.012, F = 0.158, P = 0.698) and *Q. insignis* (R^2= 0.012, F = 0.152, P = 0.703) (Fig. 9). For either species, the results indicate the variation of AGB of is not cannot be explained by percent colonization of mycorrhizae.

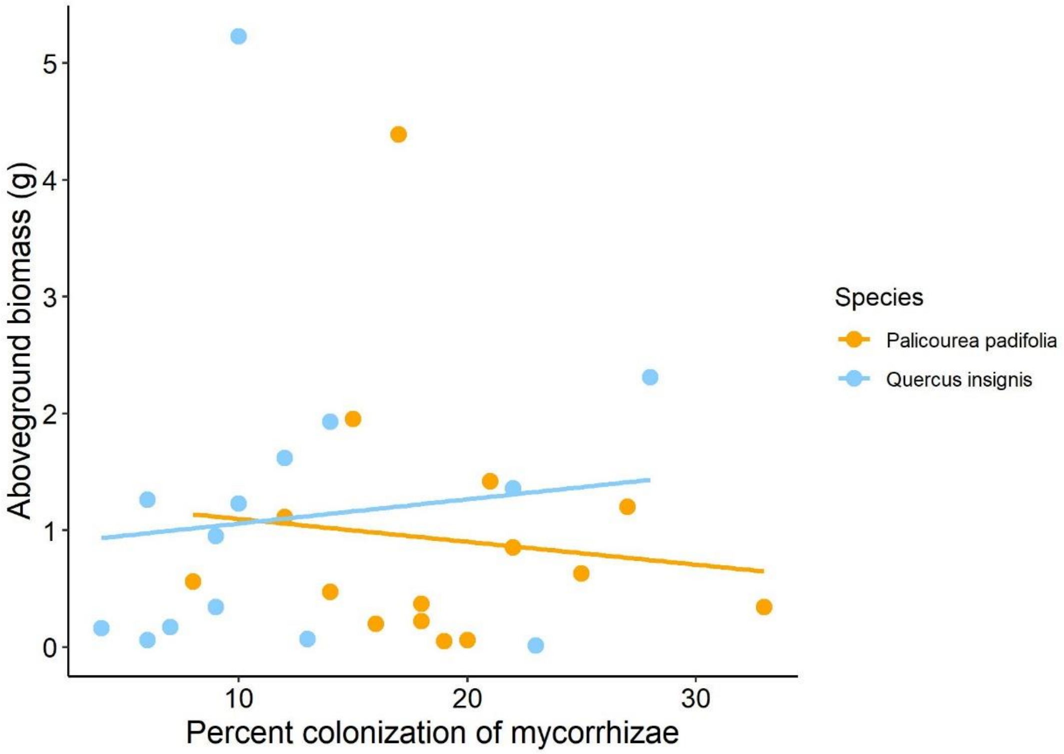

Figure 9. Percent colonization of mycorrhizae on AGB for *P. padifolia* (Rubiaceae) and *Q. insignis* (Fagaceae) in Monteverde, Costa Rica.

Percent colonization of mycorrhizae and BGB

The average measured BGB for *P. padifolia* was 0.66g and the average measured BGB for *Q. insignis* was 2.44g. There was no significant effect of percent colonization of mycorrhizae on BGB (Fig. 10)when both species were analyzed together (F=0.061, P=0.806), indicating variation in BGB cannot be explained by the percent colonization of mycorrhizae. Similar results were found when individual species analyzed separately. *P. padifolia* (R^2=0.004, F=0.050, P=0.826) and *Q. insignis* (R^2=0.022, F=0.287, P=0.601) (Fig. 9). For either species, the results indicate the variation of BGB of is not cannot be explained by percent colonization of mycorrhizae.

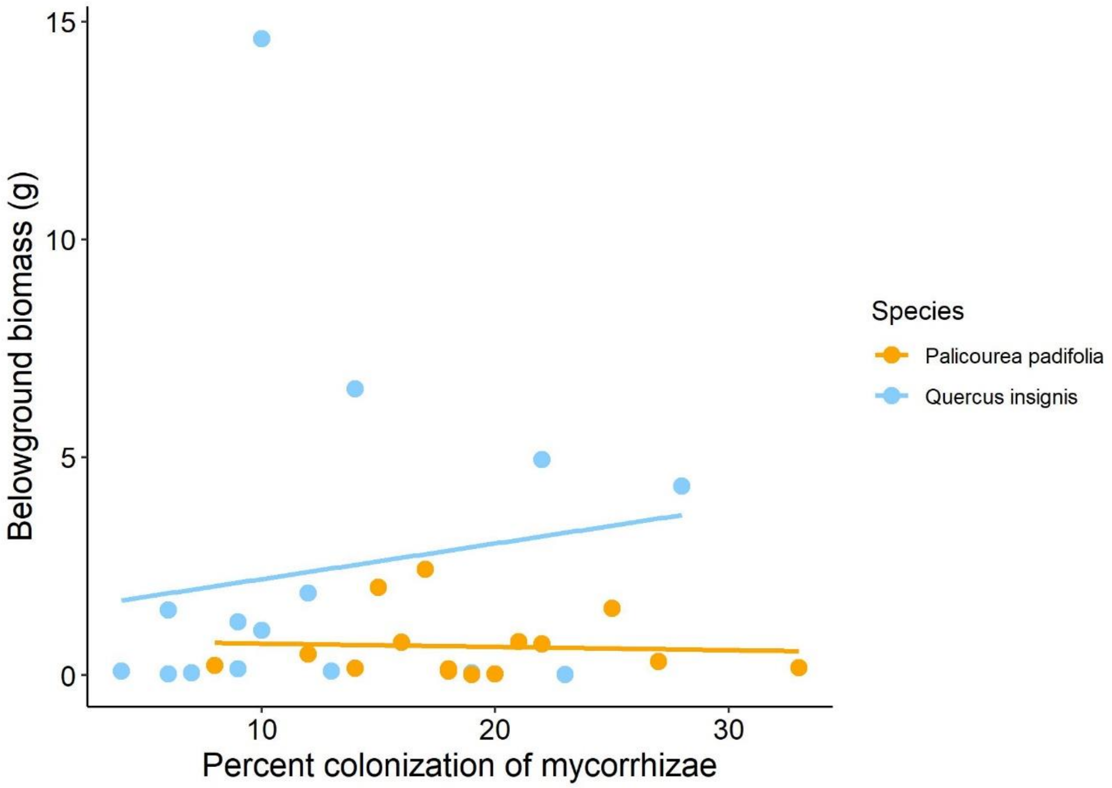

Figure 10. Percent colonization of mycorrhizae on AGB for *Palicourea padifolia* (Rubiaceae) and *Quercus insignis* (Fagaceae) in Monteverde, Costa Rica.

DISCUSSION

This study investigated potential influences of percent colonization of mycorrhizae on tree sapling size for two species. Previous studies demonstrate a range of relationships between plants and mycorrhizae from mutualism to parasitism (Zhang et al., 2012). Species of plants that are mycorrhizal dominant and have typically have high numbers of mycorrhizae, have showed increased height and increased AGB while other species have showed no change and negative influences on height and AGB (Zhang et al., 2012). These differences may be due to arbuscular mycorrhizal fungi (AMF) improving the growth of dominant mycorrhizal species by increasing nutrient absorption of the plant and consequently restraining nonmycorrhizal species and increasing mycorrhizal network (Zhang et al., 2012). Mycorrhizae also increase photosynthetic rates, increasing the likelihood to have increased growth and therefore biomass in species with more mycorrhizal associations.

This study showed a significant difference between percent colonization between sapling species (Fig. 6). *P. padifolia* had increased percent colonization with an average of 19% compared to *Q. insignis* saplings with an average of 13%. This is likely due to differences in mycorrhizal dependency which is known to vary between species and due to environmental factors (Zhang et al., 2012). While the species collected were from the same general areas making various environmental factors very similar such as rainfall and elevation, the proximity of other plants, fungal resources and sunlight availability vary between individual plants. For example, saplings of *Q. insignis* often grew close together in patches while *P. padifolia* were usually further away or sometimes attached to a large parent-plant. Previous studies have also shown mycorrhizae colonization varies across species (Bobo, 2022, unpublished). In one study, percent colonization of different species varied from the most, 51% for *Brosimum alicastrum,* and the least, 16% for *Enterolobium cyclocarpum*, demonstrating the variability in percent colonization of mycorrhizae between species. This is likely due to fugal symbionts preferring a specific tree species (Bobo, 2022, unpublished) and likely played a role in the difference in percent colonization demonstrated between *Q. insignis* and *P. padifolia.*

This study showed a significant effect of sapling height on percent colonization of mycorrhizae for one of the two species tested but no significant effect for the other (Fig. 7). This indicates height of tree saplings can influence the percent colonization of mycorrhizae for specific species and there may be no correlation for others, supporting previous studies (Zhang et al., 2012). Additionally, the age of saplings may play a role as taller plants likely had longer time to accumulate mycorrhizae (Bobo, 2022, unpublished). The species' percent mycorrhizae colonization that was unaffected by height may have been due to differences between species. It is possible an increase in nutrient acquisition due to increased mycorrhizae in *Q. insignis* resulted in higher growth while *P. padifolia* may have been influenced in ways other than height such as thickness or width (Zhang et al., 2012), resulting in no correlation.

Through this study, AGB and BGB were found to be positively correlated regardless of the plant species. This result is consistent with a previous study that found that AGB and BGB are positively correlated in *Leucaena leucocephala* (Fabaceae) (Liu et al., 2018). In other studies, different AGB and BGB ratios have been found under varying coditions, but they are relatively correlated to each other (Liu et al., 2018). Inconsistencies are likely due to varied environmental stressors as well as differences in plant species growth rates.

Due to the high correlation between AGB and BGB, it can be inferred that similar results exist for the effect of percent colonization of mycorrhizae on both AGB and BGB. In many studies, the effect of percent mycorrhiza colonization was studied on AGB alone and inferences can be made regarding BGB. In general, mycorrhizae are typically seen influencing an increase in AGB (Zhang et al., 2012). However, because this is not true for all species, it is possible the species tested were not dependent on mycorrhizae colonization (Zhang et al., 2012). In this study, mycorrhizae showed no significant effect on aboveground biomass when analyzing both species together and individually (Fig. 9). This may indicate nutrient acquisition via the root system and mycorrhizae community is not the only factor influencing sapling AGB for the species tested. Instead, other factors influencing plant growth in tropical climates such as climate (temperature), soil fertility, age, fungal sources/community, proximity to other plants, light access, elevation, and invasive species may play a more crucial role (Asner et al., 2009).

Limitations to this study include the method of measuring BGB and quantifying percent colonization. The root samples collected to measure BGB were clearly not the entirety of the root system as other plant roots were intertwined and many of its own roots were ripped off in the process of removal. A larger hole and less destructive method of estimating BGB may be employed for future research for more accurate estimation. The visibility of each stained root sample varied significantly between saplings. This is likely due to inconsistencies between root type due to the size of the sapling. This influenced the ability to stain the root and therefore visualize and quantify the percent colonization of mycorrhizae as for some samples, visibility was poor and for others, it was high. Proposing a specific section/appearance of the root to stain and sample may alleviate these inconsistencies. Access to other locations also proposes a limitation as the only investigated area was surrounding forests. This means other factors such as surrounding plants, elevation, sun access was varied across samples, potentially effecting results. A single, consistent location with similar environmental factors would limit variability.

Mycorrhizae colonization is not the only factor that might explain plant height, therefore future studies should investigate the roles of other factors such as surrounding plants, other fungal sources, sun access, and soil fertility. Additionally, investigating other factors that may influence AGB and BGB and their underlying mechanisms can also continue the study. One specific species can be selected, and different locations can be used for sampling to measure how environmental factors such as varied light access affects AGB and BGB. Another explanation of this study is rather understanding the effect of percent colonization of mycorrhizae on carbon sequestration. This may help determine the role of mycorrhizae directly regarding climate change and the formation of tropical carbon sinks, and not only biomass.

This study concludes that the percent colonization of mycorrhizae varies between species and is higher for *Q. insignis* than *P. padifolia*. The percent colonization of mycorrhizae is also correlated with sapling height for *Q. insignis*, and not for *P. padifolia*. This supports previous research claims that height and AGB and association with percent colonization of mycorrhizae depend on species and other factors. Similarly, the AGB was not significantly affected by percent colonization of mycorrhizae and showed no correlation for either species, indicating AGB and BGB are likely influenced by other factors requiring further study. However, AGB and BGB were highly correlated with one another, indicating they are influenced by the other and neither are influenced by percent colonization of mycorrhiza for these two species. This research provides insight into the relationship of mycorrhizae in two tree species occurring in tropical primary forests in Costa Rica. Studying the role of mycorrhizae in plant growth and carbon sequestration is fundamental to better understand how tropical forest help us mitigating climate change.

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MACROINVERTEBRATES DIVERSITY

Diversity of the macroinvertebrate community in bromeliad phytotelma along an altitudinal gradient in Monteverde, Costa Rica

Nathan Murthy
University of Utah School of Biological Sciences

ABSTRACT

Altitudinal gradients and their relationships with species richness of flora and fauna are a highly studied area in Biology. Generally, as elevation increases species richness decreases. Within larger ecosystems, smaller and more intimate ecosystems are present. One such example is the phytotelma, an ecosystem formed by the rosette leaf pattern of a bromeliad. This leaf pattern effectively acts as a tank that catches water and debris. Bromeliads are commonly epiphytes, a plant that grows on another plant. Bromeliads provide shelter to several types of macroinvertebrates, and invertebrates that are visible without the use of a microscope. This study aims to describe any differences that altitude causes on bromeliad diameter and phytotelma order richness. Fifteen bromeliads were taken from four different elevation zones spanning 50 m in elevation. For all bromeliads diameter was measured, and then dissected. All macroinvertebrate specimens were identified to their respective order and morphotype using insect. This study found that as elevation increased, bromeliad diameter increased, and order richness decreased.

RESUMEN

Los gradientes altitudinales y sus relaciones con la riqueza de especies de flora y fauna son un área muy estudiada en Biología. En general, a medida que aumenta la altitud disminuye la riqueza de especies. Dentro de los ecosistemas más grandes, existen ecosistemas más pequeños e íntimos. Un ejemplo es el fitotelma, un ecosistema formado por el patrón de hojas en roseta de una bromelia. Este patrón foliar actúa como un depósito que atrapa el agua y los residuos. Las bromelias suelen ser epífitas, es decir, plantas que crecen sobre otras. Las bromelias proporcionan refugio a varios tipos de macroinvertebrados e invertebrados que son visibles sin el uso de un microscopio. Este estudio pretende describir las diferencias que la altitud provoca en el diámetro de las bromeliáceas y en la riqueza de órdenes de fitotelmas. Se tomaron quince bromeliáceas de cuatro zonas de diferente altitud que abarcaban 50 m de altitud. Se midió el diámetro de todas las bromeliáceas y luego se diseccionaron. Todos los especímenes de macroinvertebrados se identificaron según su orden y morfotipo respectivos utilizando insectos. En este estudio se observó que, a medida que aumentaba la altitud, aumentaba el diámetro de las bromeliáceas y disminuía la riqueza de órdenes.

INTRODUCTION

Epiphytes are tree dwelling plants and contribute to an exceptional amount of the diversity worldwide, constituting approximately 10% of the world's vascular plant species (Kress, 1986). The Bromeliaceae family, known as bromeliads, is one of the most diverse and widespread plant families in the Neotropic, with over 3,000 described species (Luther & Sieff, 1998). The leaves of some members of this family are organized in a rosette pattern. This arrangement of leaves can catch water and debris to create a microhabitat, similar to a tank, called the phytotelma (Bruno Corbara et al., 2019).

Within tropical forests, bromeliad tanks can capture up to 50 liters of water per hectare (Romero et al., 2020). The decomposing debris that is caught within the tanks can provide nutrients and substrate for many species (Kitching, 2001). This microhabitat is utilized by several species, including bacteria, protozoa, microcrustaceans, oligochaetes, insect larvae, and Anuran tadpoles (Foissner et al., 2003; Bacigalupo et al., 2006; Martinson et al., 2010; Whittaker et al., 1975).

The changes in elevation that are present in mountainous areas provide an interesting topic of study for bromeliad phytotelm environments (Kageyama, et al., 2003). Previous studies have assessed species diversity of phytotelma along altitudinal gradients. One study looked at elevations of 20 m, 400 m, 910 m, and 915 m and found a mid-elevation spike in species richness at 400 m (Malfatti, 2020). However, the overall trend expressed a decrease in species richness with an increase in elevation (Malfatti, 2020). Another study was able to correlate species turnover with elevation, meaning that as altitude changes there is an increase in substitution of species between communities (Jocque & Field., 2014).

This study aimed to describe the differences, if any, between bromeliad diameter and phytotelma order richness of macroinvertebrates at different elevations in the primary Cloud Forest of Monteverde. Within the cloud forest, as elevation increases, epiphyte growing conditions tend to become more favorable (Melnick, N. L., 2022). Therefore, I hypothesized that as elevation increased, bromeliad diameter would increase. Additionally, as bromeliad growing conditions improved with an increase in elevation, I hypothesized that macroinvertebrate richness would increase as well. Bromeliads harvested were from the *Guzmania* genus. Previous studies have been conducted measuring species richness along a gradient, however these collections were done at sporadic elevations (Malfatti, 2020). This study used consistent intervals of elevation (elevation zones) in order to standardize sample collection along the gradient. Just as it is important to understand changes of ecosystems along altitudinal gradients as a whole, it is also important to understand these differences on a smaller scale like the phytotelma. Understanding this relationship is vital to the protection of the many organisms that use bromeliads and depend upon them.

MATERIALS AND METHODS

Study site

Research took place at the Monteverde Biological Station along the trails Catarata and Principal. The Station is located in Primary Cloud Forest. I took samples from four different elevation ranges. The altitudes of these four sampling sites were distributed accordingly: 1475-1525 m (E1, Fig. 1A), 1575-1625 m (E2, Fig.1B), 1675-1725 m (E3, Fig. 1C), and 1775-1825 m (E4, Fig. 1 D). All bromeliad samples were categorized according to the respective elevation zone.

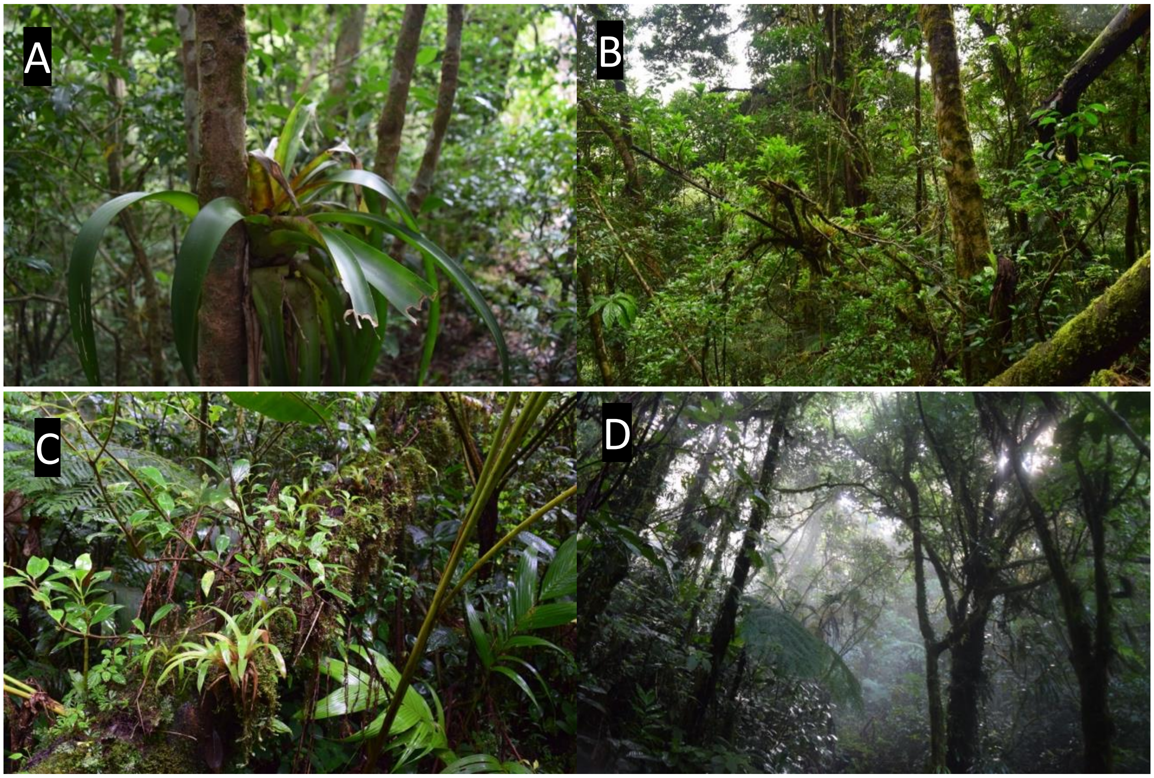

Figure 1.Elevation ranges of the four sampled elevation zones. A) Elevation zone 1:1475-1525 masl); B) Elevation zone 2: 1575-1625 masl; C) Elevation zone 3: 1675-1725 masl; D) Elevation zone 4: 1775-1825 masl.

Specimen Collection

Collections occurred in one elevation zone per day, starting at zone 1. Altitude was tracked using the *My Altitude* app. In each zone, 15 bromeliad individuals that were naturally attached to the branch or trunk of a tree were randomly selected for collection. To ensure randomization, care was taken to collect each bromeliad from different clusters of bromeliads. Additionally, only bromeliads with a diameter greater than 20 cm were collected. Generally, a 20 cm minimum diameter is required for the presence of a water tank in a bromeliad (Jocque & Field, 2014). Diameter was measured from each opposite outermost leaf. Each bromeliad collected was individually bagged to prevent cross contamination of macroinvertebrates.

Data Analysis

Each day after collections, the 15 bromeliads were processed. Processing began with a precise measurement (2 decimal points) of the diameter. Order richness was determined through a dissection of the bromeliad leaves. Orders and morphotypes of macroinvertebrates were identified using a microscope and insect guides. The first occurrence of a morphotype was preserved in ethanol to be used as a reference. A list of orders and morphotypes found per bromeliad and elevation zone was input into an Excel sheet.

Data visualization

To visually describe the general composition of macroinvertebrate orders present in the bromeliad phytotelma across the different elevation zones a treemap was created in R using the package treemapify (ggplot2). The data did not include morphotypes, all morphotypes were combined and attributed to their respective order.

Statistical Analysis

To compare the average values of order richness and bromeliad diameter between elevation zones, a Kruskal-Wallis and the post-hoc Dunn test were performed in R with the ggbetweenstats() function from the {ggstatsplot} package. This test provides insight on whether or not a significant difference is present in a set of data. If present, the next step was to conduct a pairwise comparison using a Dunn test between elevation zones.

RESULTS

Order visualization

Figure 2 offers a clear and intuitive representation of the relative proportions of orders in each elevation zone. This tree map is used only for illustrative purposes and did not involve any statistical analysis.

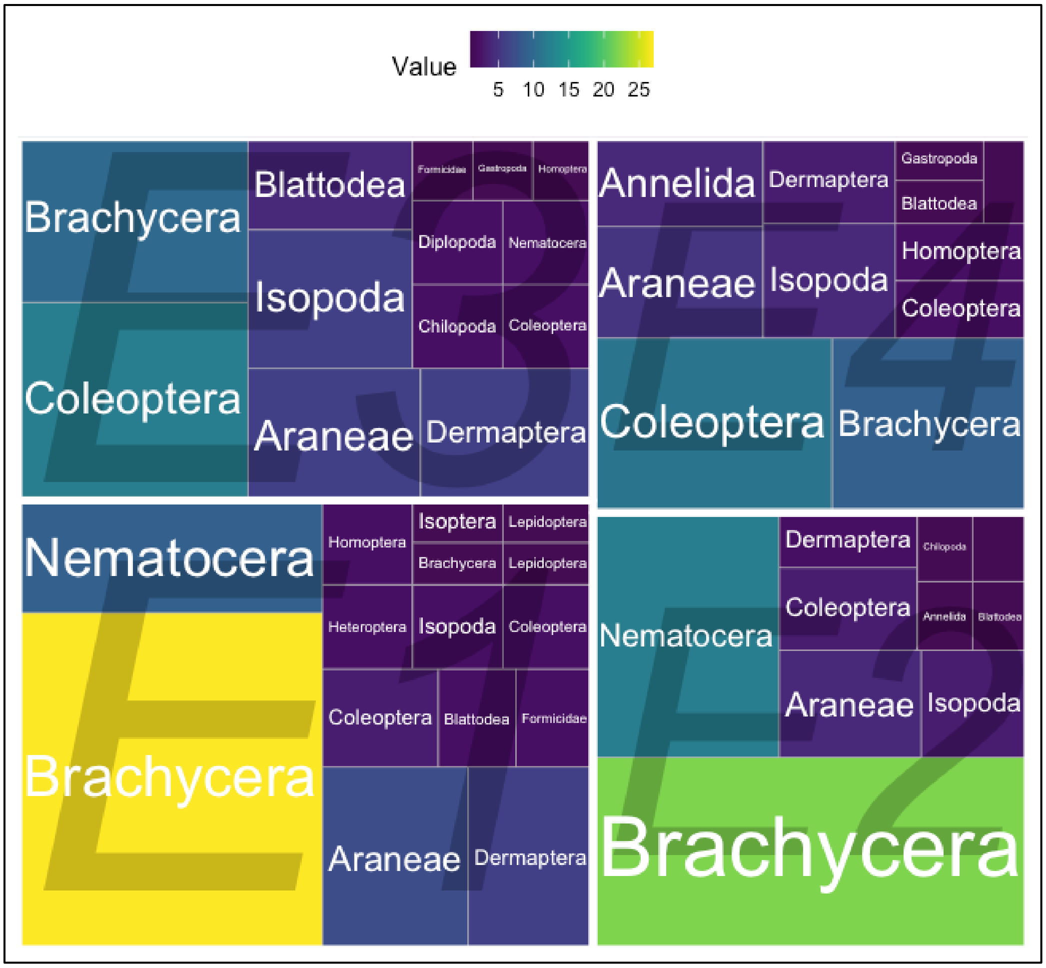

Figure 2. Tree map showing the order of macroinvertebrates present per elevation zone. E1: 1475 m to 1525 m bottom left grid; E2: 1575 m to 1625 m bottom right grid; E3: 1675 m to 1725 m top left grid: and E4: 1775 m to 1825 m top right grid.

Order Richness across elevations

As a general trend, order richness decreased with elevation (Fig. 3). The Kruskal Wallis test showed that there is a significant difference of order richness across elevation zones (X^2Kruskal-Wallis = 7.93, p = 0.05). The Dunn test revealed a significant difference in order richness between zones 1 and 4 (p = 0.05). (Figure 3).

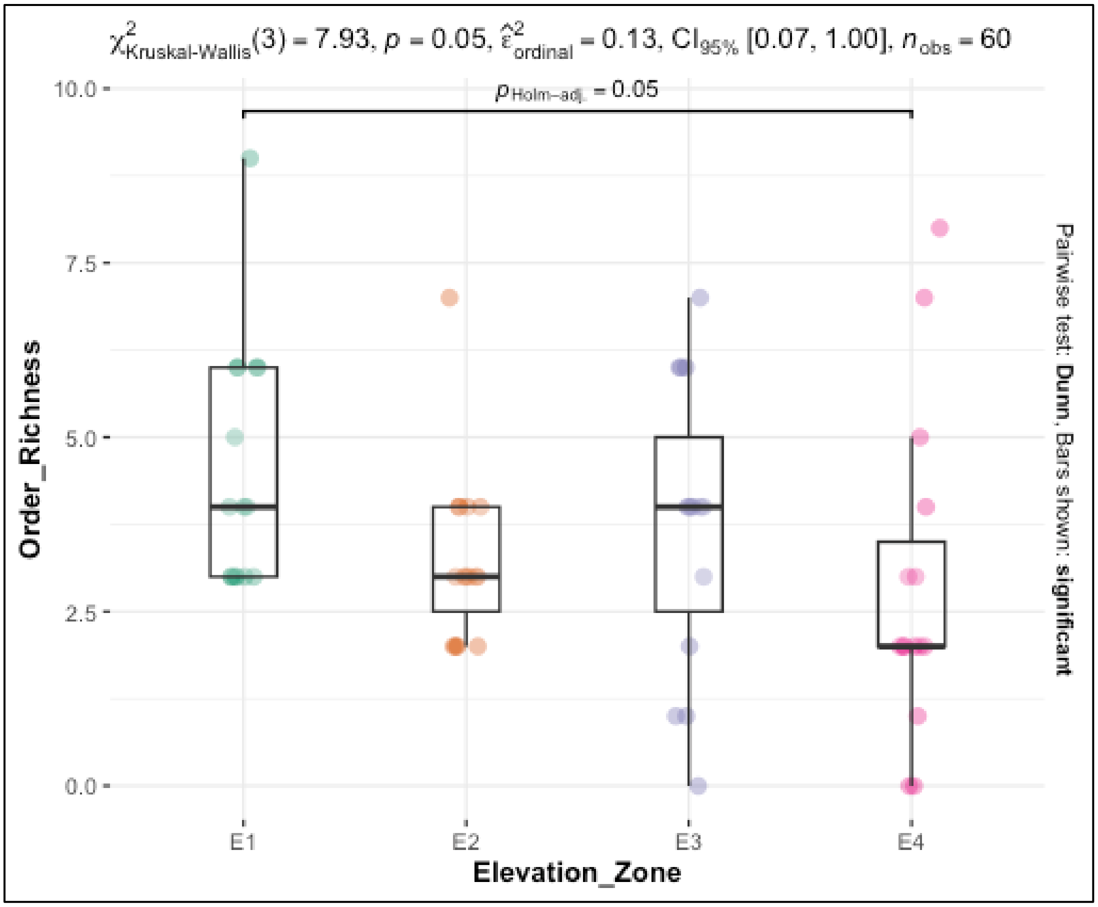

Figure 3. Order richness of phytotelma macroinvertebrates across elevation zones. E1: 1475 m to 1525 m, E2: 1575 m to 1625 m, E3: 1675 m to 1725 m, and E4: 1775 m to 1825 m. Only significant comparisons are shown.

Bromeliad size across elevations

As a general trend, bromeliad diameter increased with elevation (Fig. 4). The Kruskal-Wallis test of diameter across elevation zones indicated that a significant difference was present (X^2Kruskal-Wallis = 20.11, p = 1.61e-04). Subsequently, the Dunn test revealed significant differences between zones 1 and 3 (p = 2.86e-03), zones 1 and 4 (p = 4.65e04), and zones 2 and 4 (p = 0.05) (Fig. 4).

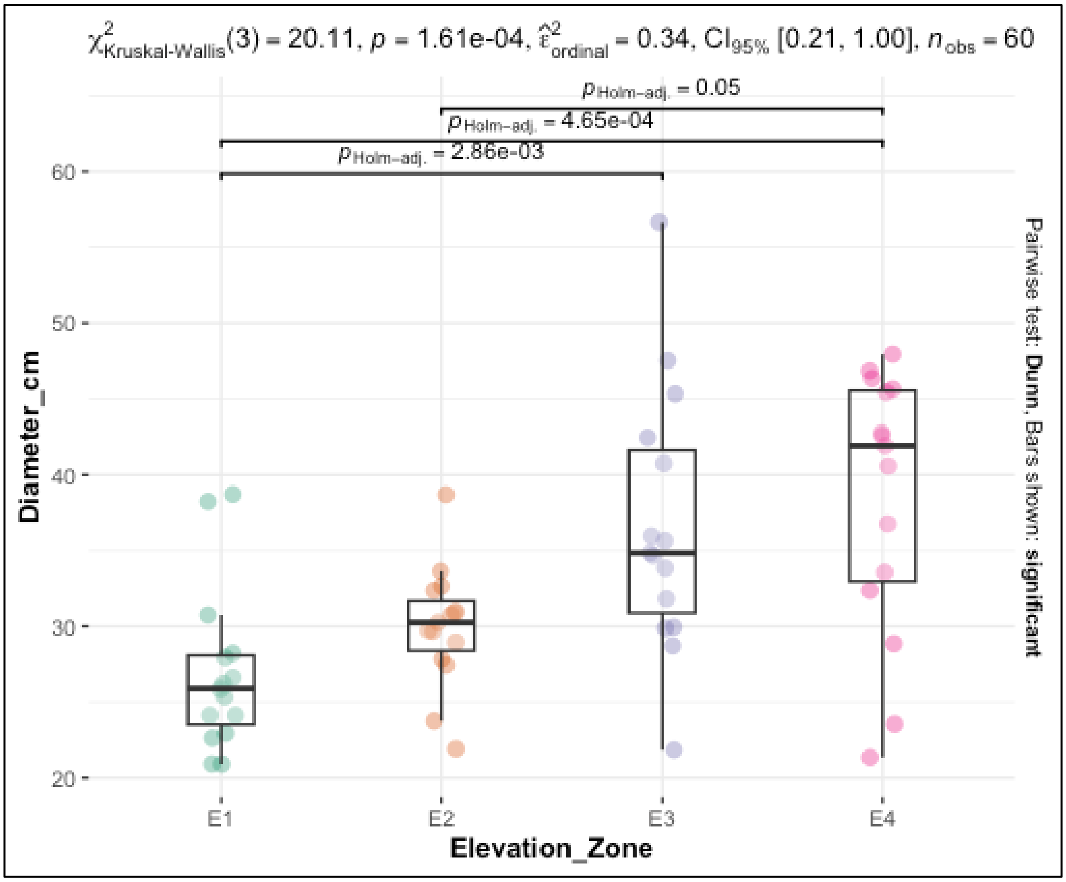

Figure 4. Bromeliad diameter across elevation zones. E1: 1475 m to 1525 m, E2: 1575 m to 1625 m, E3: 1675 m to 1725 m, and E4: 1775 m to 1825 m. Only significant comparisons are shown.

DISCUSSION

Although no statistical analysis was done through the tree map, this graphical display helps us to visualize the species makeup between elevation zones. Elevation zones 1 and 2 contained high amounts of Brachycera and Nematocera (Diptera order) larvae while elevation zones 3 and 4 contained high amounts of Coleoptera larvae. Species composition change could be attributed to change in temperature and also the increase in mist. Brachycera and Nematocera larvae occurrence tended to be replaced by Coleoptera larvae at higher elevations. Studies have been done to assess the effects of fog on the flight of the Culicidae family which are part of the Nematocera order. They found that denser than air environments are capable of affecting a Culicidae’s gyroscopic sensors and wingbeat controllers, causing them to become ineffective (Dickerson et al., 2015). Consequently, the presence of fog creates opportunities for other orders, such as Coleoptera, to thrive.

When looking at order richness, the general trend was that as elevation increased, order richness decreased. My results confirmed previous studies that found a decrease in species richness with an increase in elevation (Malfatti, 2020). My study only measured occurrence, not abundance of macroinvertebrates. Future studies could benefit from measuring abundance of orders and morphotypes. By measuring abundance, it is possible to use statistical analysis to investigate composition similarities (e.g., Morisita Similarity Index) between elevation zones.

When looking at bromeliad diameter across elevation zones, the general trend was that as elevation increased so did bromeliad diameter. Bromeliads and other epiphytes tend to be mist catching specialists. At higher elevations during this study, fog and mist were increasingly visible with an increase in elevation. This creates favorable growing conditions for bromeliads that can be water challenged. This result also interesting because despite an increase in bromeliad diameter with elevation, the order richness decreased.

Studying changes in species composition and richness of the overall ecosystem along elevational gradients is extremely important for the protection of these species. At the same time, it is vital to understand how these factors can affect ecosystems on a smaller scale, like the phytotelma. These results confirm that the general biological trend, as elevation increases, species richness decreases, holds true. Additionally, the order visualization illustrated that species composition changes across elevations. These findings highlight the importance of protecting bromeliad populations at all elevations because bromeliads at one elevation zone do not represent the entire population.

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The Effect of Stream Proximity on Leaf Litter Arthropod Composition in Monteverde, Costa Rica

Savana Vida
Department of Environmental Studies, Brandeis University, Massachusetts, USA

ABSTRACT

Arthropods, characterized by their hard exoskeleton, jointed legs, and segmented bodies, are among the most taxonomically diverse groups on Earth. Arthropods play a fundamental role in the decomposition of plant material and leaf litter. Leaf litter serves as crucial habitat for an array of arthropods, offering shelter, resources, and favorable microclimatic conditions. This study explores the influence of stream proximity on leaf-litter arthropods in a tropical forest ecosystem in Monteverde, Costa Rica. I sampled leaf-litter arthropod abundance and richness using pitfall traps in two habitats: the forest (3 sites) and near a stream (3 sites). The research questions investigated are: **1)** How does stream proximity affect leaf litter arthropod community structure and species richness? **2)** Are there specific arthropod species that demonstrate a strong association with stream proximity? A total of 396 arthropods were collected from both habitats, with forest habitats having more Opiliones, and stream habitats containing more Talitridae. I found that although there are differences in arthropod species composition between forest and stream sites, as shown using a cluster dendrogram, there is no statistical difference between arthropod abundance and richness in stream sites and forest sites. This study advances the knowledge of the effect of bodies of water on arthropod abundance, richness, and composition.

RESUMEN

Los artrópodos, caracterizados por su exoesqueleto duro, patas articuladas y cuerpos segmentados, se encuentran entre los más diversos taxonómicamente en la Tierra. Los artrópodos desempeñan un papel fundamental en la descomposición de material vegetal y hojarasca. La hojarasca proporciona un hábitat crucial para una variedad de artrópodos, ofreciendo refugio, recursos y condiciones microclimáticas favorables. Este estudio explora la influencia de la proximidad al arroyo en los artrópodos de hojarasca en un ecosistema forestal tropical en Monteverde, Costa Rica. El estudio muestrea la abundancia y riqueza de artrópodos de hojarasca utilizando trampas de caída tanto en el bosque como en las cercanías del arroyo. Las preguntas de investigación investigadas son: **1)** ¿Cómo afecta la proximidad al arroyo a la estructura de la comunidad y a la riqueza de especies de artrópodos de hojarasca? **2)** ¿Hay especies de artrópodos específicas que demuestren una fuerte asociación con la proximidad al arroyo? Se recogió un total de 396 artrópodos de ambos hábitats, con los hábitats forestales teniendo más Opiliones, y los hábitats de arroyos conteniendo más Talitridae. Se encontró que no hay diferencias estadísticas entre la abundancia y riqueza de artrópodos en los sitios del arroyo y del bosque. Sin embargo, existen diferencias en el tipo de especies de artrópodos entre los sitios del bosque y del arroyo, como se muestra utilizando un dendrograma de similitud. Este estudio avanza en el conocimiento del efecto de los cuerpos de agua en la abundancia, riqueza y composición de los artrópodos.

INTRODUCTION

The tropics harbor a disproportionate amount of the world's biodiversity including forty thousand species of arthropods (Basset et al., 2012). Arthropods, characterized by their hard exoskeleton, jointed legs, and segmented bodies, are among the most taxonomically diverse animals on Earth (Cole, et al. 2016, Budd & Telford 2009). Arthropods play a fundamental role in the decomposition of plant material and leaf litter, and leaf litter serves as a crucial habitat for many arthropods including spiders, mites, centipedes, and insects. This ecologically vital microhabitat provides them with shelter, food resources, and favorable microclimatic conditions (Lavelle et al. 2012).

Leaf litter, which is dead plant material that has fallen to the ground, plays a critical role in ecosystem dynamics, especially in the tropics. Leaf litter decomposition is essential for nutrient and carbon cycling, consequently, increasing plant productivity, species composition, and carbon storage (Ochoa-Hueso, et al. 2019). Additionally, leaf litter provides vital nutrients and moisture on the forest floor, thus, fostering the growth of seedlings (Johnson, et al. 2002).

While there is limited literature comparing leaf litter composition between streamside and forest habitats, ample research explores the correlation between increased water in tree holes and leaf litter composition. These studies have demonstrated that tree-hole habitats with increased water availability have higher insect colonization rates (Paradise 2004). If water had an effect on insect colonization, it is reasonable to think that larger bodies of water such streams will also influence arthropod assemblages.

This study aims to assess the relationship and influence of stream proximity on leaf litter arthropods in a forest ecosystem. The research questions investigated are: 1) How does stream proximity affect leaf litter arthropod community structure and species richness? 2) Are there specific arthropod species that demonstrate a strong association with stream proximity? Understanding the differences in leaf litter arthropod composition (species richness and abundance) between forest and stream habitats is fundamental to understand the effect of water bodies structuring arthropod assemblages in tropical forests. This research will contribute to the broader literature describing the intricate relationships between freshwater and terrestrial ecosystems.

MATERIALS AND METHODS

Study Site

This study was conducted at the Monteverde Biological Station in Costa Rica in Monteverde, Costa Rica. Samples were collected from three forest plots and three stream plots (Fig. 1). This study occurred in July, during the rainy season in Costa Rica.

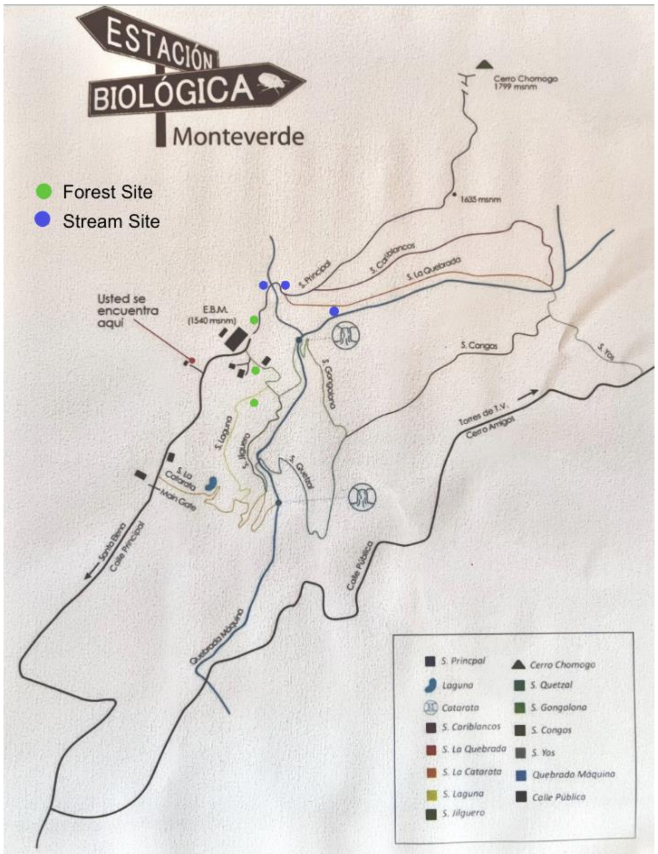

Figure 1. Location of pitfall trap plots on the trail system of the Monteverde Biological Station, Monteverde, Costa Rica.

<u>Collection Method</u>

To sample leaf litter arthropods, pitfall traps were constructed and placed on the at ground level in the forest floor. Each pitfall trap consisted of a cup, containing a solution of hand soap and saltwater, filled roughly halfway up (Fig. 2). In order to prevent debris and rainwater from contaminating the contents in the collection cup, a plastic plate was positioned over the cup held four centimeters above by two skewers. The pitfall traps were collected twice throughout the experiment. The pitfall traps were left in the field for two days before being emptied for further analysis of the leaf litter arthropods. Samples were transferred to a container filled with ethanol at the end of the two-day period.

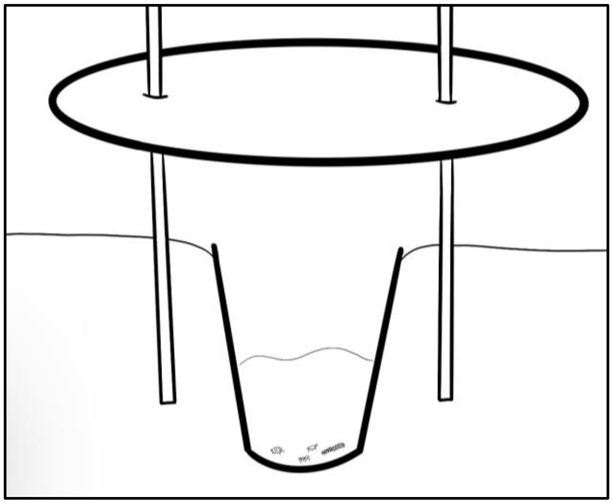

Figure 2: Pitfall trap design. The cup contains a solution of hand soap and saltwater, collecting arthropods.

The collected samples were carefully labeled and transported to the lab for further analysis. Using an insect guide, the arthropods were identified to the taxonomic order, except for Coleoptera and Amphipoda which were identified to the family level. The samples were examined with tweezers to ensure accurate arthropod identification.

Statistical Analyses

To test the effect of habitat type on arthropod richness and abundance, I performed an analysis of variance (ANOVA) using the *aov()* function. Additionally, to assess similarities in arthropod composition between sites, a similarity dendrogram was built using the *vegdist()* and *hclust()* functions of the vegan package (Oksanen, 2019). All analyses were conducted in the statistical programming language R version 4.0.0. (R Development Core Team, 2020).

RESULTS

A total of 396 arthropods were collected and identified from 24 pitfall traps, containing 14 different taxa of arthropods. The forest sites contained the highest abundance of arthropod individuals, having 215 arthropods collected, while the stream sites yielded 181 arthropods. The composition of arthropods varied between the stream and forest sites. At the habitat level, the forest sites contained a higher abundance of Opiliones, with 103 individuals collected, while the stream sites contained only 27 Opiliones (Fig. 3). Stream sites contained a higher abundance of Amphipoda (Talitridae), with 80 individuals collected, while the forest sites only contained 18 Amphipoda (Talitridae). Specifically, stream site 1 contained a majority of the individuals in the Talitridae family, with 68 collected (Fig. 4). At the site level, the stream site-1 contained the highest leaf-litter arthropod abundance, containing 100 individuals. On the other hand, stream site-3 contained the lowest leaf-litter arthropod abundance, harboring only 32 individuals.

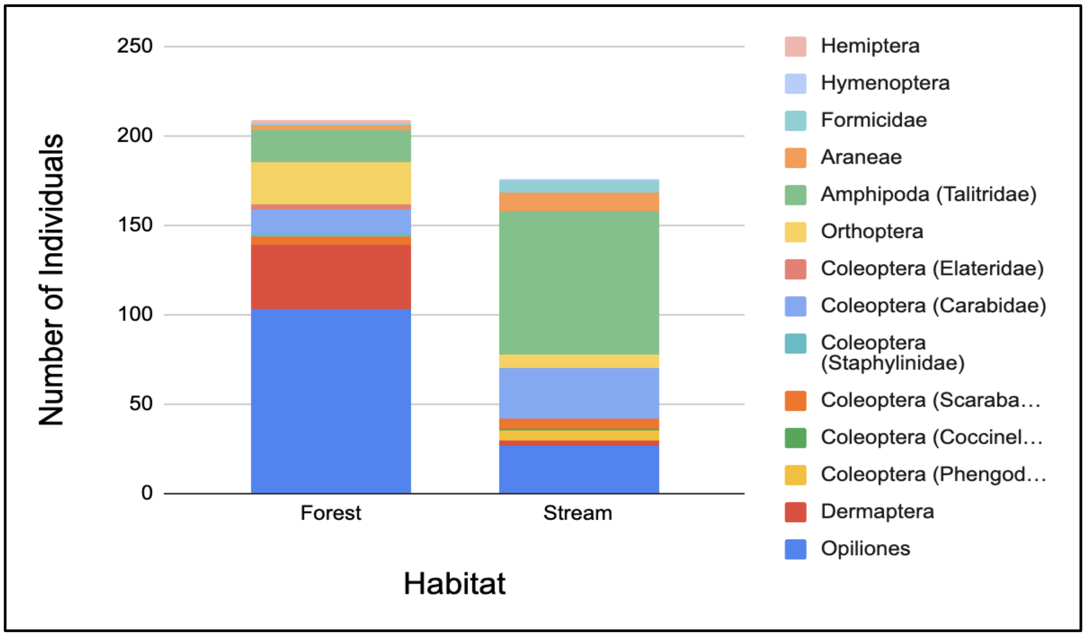

Figure 3: Arthropod abundance in forest and stream habitats at Monteverde, Costa Rica. Each habitat is further separated by the abundance of each taxa collected in each habitat. It is apparent that Opiliones were most common in forest habitats, whilst Amphipoda (Talitridae) were the most abundant in stream habitats.

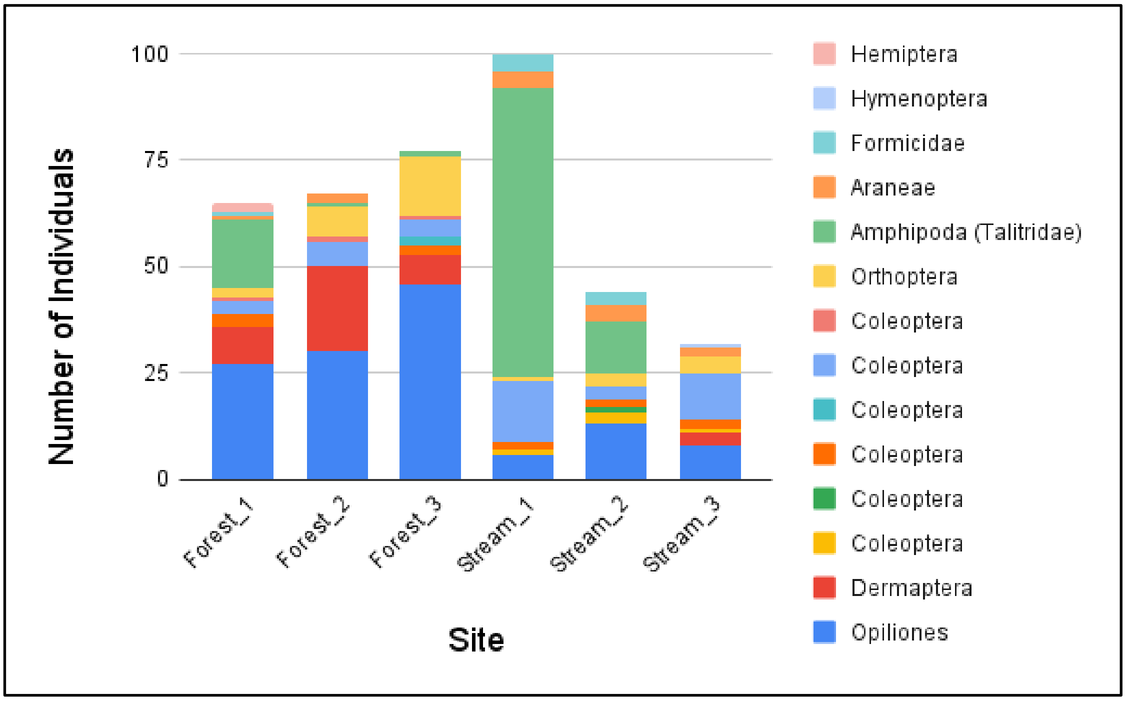

Figure 4: Arthropod abundance in the forest (3 sites) and the stream (3 sites) habitats at Monteverde, Costa Rica. Each site is further divided into sections representing the abundance of each taxa present at the sites.

To determine if stream proximity impacted the abundance and richness of leaf-litter arthropods, I performed an Analysis of Variance (ANOVA) for arthropod abundance and another taxa richness separately. I found that the arthropod abundance differences between forest and stream habitats are not statistically significant (F=0.613, P=0.442) (Fig. 5).

The findings of the ANOVA tests for taxa richness similarly demonstrated that there was no significant difference between forest and stream habitats (F=0.017, P=0.898) (Fig. 6). While the taxa types differed between forest and stream habitats, the number of taxa present was not significantly different.

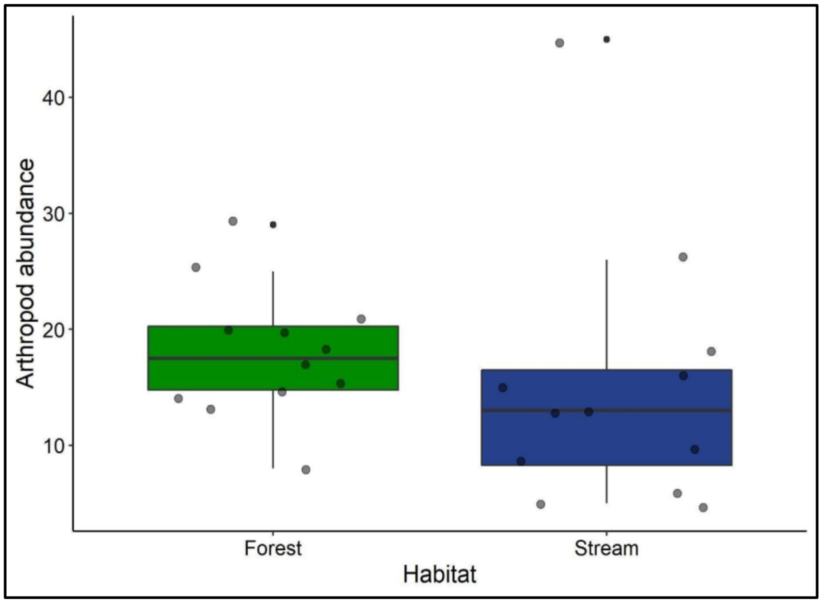

Figure 5. Arthropod abundance in forest and streams habitats in Monteverde, Costa Rica.

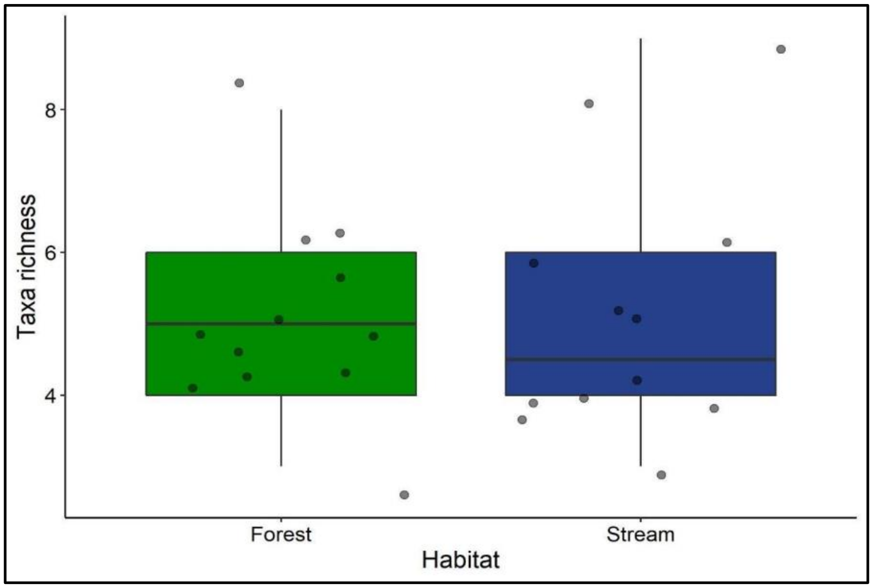

Figure 6. Taxa richness in forest and stream habitats in Monteverde, Costa Rica.

To observe the similarity of the composition of arthropod communities between the six study sites, a cluster dendrogram was created (Fig. 7). The dendrogram was created with the heights of the branches being indicative of the level of similarity between the sites, with a height of zero indicating that the sites have the same composition. Forest site-2 and forest site-3 have the highest similarity with a branch height of 0.3. On the other hand, stream site-1 has the highest dissimilarity compared to the other sites, having a branch height of 0.8. Interestingly, forest site-1 and stream site-2 are second closest in similarity, with a branch height of 0.4.

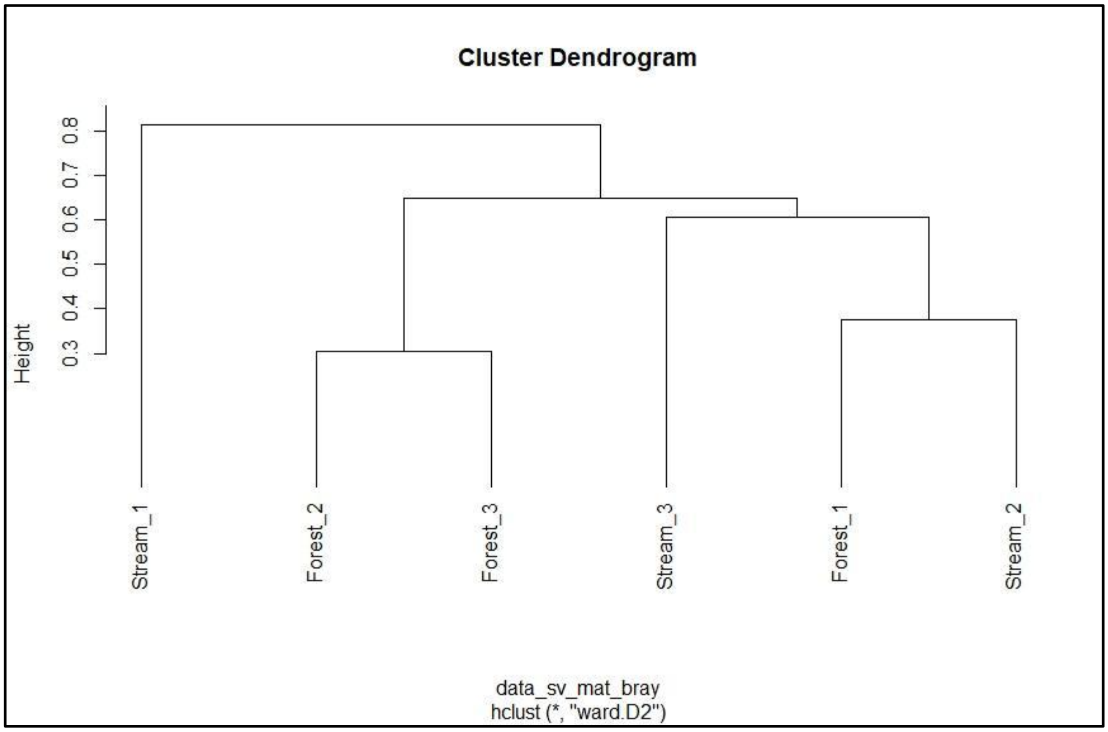

Figure 7. Cluster dendrogram representing the similarity analysis of leaf-litter arthropod communities in Monteverde, Costa Rica. The height indicates how similar the arthropod community of a site is to another.

DISCUSSION

The primary goal of this study was to assess the effect of stream proximity on leaf litter arthropods. This study focused on understanding how stream proximity affects arthropod abundance and richness while also observing whether specific arthropod taxa have a strong association with stream proximity. Arthropods assemblages close to streams were similar in taxa richness and abundance to the forest assemblages. There could have been no difference in arthropod abundance and richness due to the sites being located rather close to one another. In the future, this study might benefit from placing the sites a significant difference from one another.

Although this study did find difference in arthropod abundance of richness, the specific taxa composition between stream and forest sites differed. This suggests that stream proximity might not be the only determinant of arthropod communities in the study area. Other factors, such as microhabitat characteristics, interspecies interactions, and local environmental conditions, may play roles in framing arthropod communities (Lavelle et al. 2012).

The cluster dendrogram provided ample information regarding the similarity in arthropod composition across the six sites. The high similarity between forest site-1 and stream site-2 indicates that these habitats might share other ecological features, apart from proximity to water, that led to them having similar arthropod taxa. The high dissimilarity between stream site-1 and the other sites possibly indicates that stream site-1 contained different ecological factors, such as, temperature, humidity, and microhabitats (Maher et al. 2022). These other ecological factors could possibly have led to such a dissimilarity among arthropod taxa.

The research from this study advances the knowledge of leaf-litter arthropods and the effect streams have on their abundance, richness, and community composition. While significant data was not found on arthropod abundance and richness, this study provided information regarding what species of arthropods are present in each habitat. For example, Talitridae are much more abundant near streams than in the forest. Further research can be added to this study to provide more insight into arthropod composition near bodies of water.

Since limited research remains on the effect of water on leaf-litter arthropods, further research could also include comparing leaf-litter arthropod abundance in areas that receive a significant amount of precipitation and drier areas across larger geographical scales. Additionally, further research could include identifying the leaf-litter arthropods at the species level, rather than solely to the order or family; this could provide a more taxonomically precise assessment of arthropod composition across habitats.

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INSECT ECOLOGY

The Effect of Petiole Size and Fallen Leaf Location of *Cecropias* on the Abundance of Bark Beetles (*Scolytinae, Curculionidae*) in Monteverde, Costa Rica

Nehir Toklu
Department of Organismic and Evolutionary Biology, Harvard University, Massachusetts, USA

ABSTRACT

Understanding the factors that determine the abundance of organisms is a key step to better understand their ecology and natural history. Furthermore, this understanding is important in many aspects of biology: to understand how such compositional differences might shift the ecological role of the species, as well as how this might be affected in our rapidly changing world. Bark Beetles are associated with the petioles of the plant group *Cecropias.* They feed on, hide in, and reproduce within the petioles of fallen Cecropia leaves. The objective of this research is to understand **1)** the effect of leaf size, **2)** the location of leaves (ground vs. vegetation), and **3)** habitat (forest vs. open area) on the abundance of bark beetles in Monteverde, Costa Rica. I found that petiole size is positively correlated with beetle abundance and that leaves in the forest and on top of vegetation had more larvae than leaves in the forest located in the ground. The relationship between beetle abundance and leaf habitat as well as leaf location had no strong differences as the results were not statistically significant.

RESUMEN

Entender los factores que determinan la abundancia de los organismos es un paso clave para comprender mejor su ecología e historia natural. Además, esta comprensión es importante en muchos aspectos de la biología: para entender cómo tales diferencias de composición podrían cambiar el papel ecológico de las especies, así como cómo esto podría verse afectado en nuestro mundo en constante cambio. Los escarabajos de corteza están asociados con los pecíolos del grupo de plantas Cecropias. Se alimentan, se esconden y se reproducen dentro de los pecíolos de las hojas caídas de Cecropia. El objetivo de esta investigación es comprender 1) el efecto del tamaño de la hoja, 2) la ubicación de las hojas (en el suelo versus en la vegetación) y 3) el hábitat (bosque versus área abierta) en la abundancia de los escarabajos de corteza en Monteverde, Costa Rica. Encontré que el tamaño del pecíolo está correlacionado positivamente con la abundancia de escarabajos y que las hojas en el bosque y en la parte superior de la vegetación tenían más larvas que las hojas en el bosque ubicadas en el suelo. La relación entre la abundancia de los escarabajos y el hábitat de la hoja, así como la ubicación de la hoja, no mostró diferencias significativas estadísticamente.

INTRODUCTION

Tropical forest host an astonishingly biodiversity, both in species number and ecological interactions. However, tropical forest are threatened by deforestation, agricultural expansion and climate change. To better protect tropical species it is imperative to first understand the natural history of the species as well their ecological interactions with other organisms. This is true especially when it comes to areas like rainforests, as they foster an overwhelming part of the biological diversity found on Earth (Smith et al., 1977). While this is one of the reasons why tropical rainforests are of most value, it is also one of the reasons they are vulnerable. The intense biodiversity found here is affected by all organisms within it, which are all affected by the human-perpetuated changes the earth faces: global farming, changing ocean chemistry, areas disturbed for agricultural purposes, and so on. Research is an important way science is trying to combat such changes and harm, yet to do so we have to understand the ecological principles that these organisms function in, and the characteristics of the species.

At the tropics, the *Cecropia* trees are important components of the flora, especially in disturbed areas such as roadsides and forest gaps (Janzen, 1983). One example of a valuable species is Cecropias (*Cecropia peltata*). They are an incredibly common species in the tropics, being identified as a pioneer species that has a crucial role in the overturning of the forest composition (Assis and Wittman, 2011). There is a myriad of species associated with Cecropias. Azteca Ants, which live inside the cecropia plant is an example of such interactions. Furthermore, there are other organisms that eat cecropia such as certain bats and birds. The focus of this study is one organism that has a strong relation with Cecropias as well, yet it is organism that is rather understudied, due to its intense diversity. Bark beetles (Scolytinae, Curculionidae) are beetles that live inside the petioles of the old leaves of Cecropias (Jordal, 1998). Cecropias shed their leaves regularly throughout the year, which the beetles use as a habitat in which they feed on the petiole, burrow for protection, and lay their eggs (Wood, 1983). While are extremely abundant, there is a rather small amount of research on the general ecological interactions of the beetles, such as spacial preferences, and mating habits (Jenkins et al., 2017). There are certain studies that focus on topics such as the effects of climate change on beetle species composition and also composition and abundance in relation to petiole abundance (Gandhi, 2008).

The objective of this research is to understand 1) the effect of leaf size, 2) the location of leaves (ground vs. vegetation) and 3) habitat (forest vs. open are) on the abundance of bark beetles in Monteverde, Costa Rica. Because Cecropia petioles vary in size, leaves on vegetation are more isolated than leaves in the ground, and leaves in the forest experience more humidity, I predicted that larger leaves, leaves on the ground, and leaves in the forest will host a higher abundance of bark beetles. Studying bark beetles inhabiting Cecropia leaves is fundamental to better understanding the ecological interactions of Neotropical insects, which in turn can help us to propose management and conservation efforts to better protect tropical biodiversity.

MATERIALS AND METHODS

Collection Sites

This study was conducted at the Estación Biológica Monteverde in Monteverde, Costa Rica. I collected samples from a "disturbed area" around the station buildings where there are two *Cecropia* trees. For the forest specimens, I walked into the forest at the "El Principal" trail. At both forest and open areas habitats, I collected leaves at two positions, on the ground and hanging on surrounding vegetation. In total, I collected 80 *Cecropia* leaves, distributed as 20 forest floor, 20 forests on top of vegetation, 20 disturbed area floor, and 20 disturbed area on top of vegetation (Fig, 1).

Figure 1. Leaves of Cecropia (Urticaceae) on vegetation (left) and on the ground (right) at Monteverde, Costa Rica.

Petiole Size and Beetle Abundance

While previous studies have chosen to store the petioles for around 10 days in order to identify the species of the larvae (Gould, 2021) for the purpose of this project, as identification of each species was not a requirement, the petioles were measured and carved to find the beetles right after collection. The petioles were measured by using a ruler in order to measure the length and width of the petiole. I assessed beetle abundance by carving each petiole and counting the number of adults and larvae. After assessing beetle abundance, beetles were later returned to the forest, close to the *Cecropia* tree where they were collected.

Figure 2. Base of the petiole of a *Cecropia* (Urticaceae) leaf at Monteverde, Costa Rica. The red line depicts how width was measured.

Statistical analysis

To understand the relationship between petiole length and width a Pearson's correlation test was performed. Then, to test the effect of petiole length on bark beetle abundance I ran a Generalized Linear Model with a poisson error distribution. Lastly, to test the effect of habitat and leaf position on bark beetle abundance I ran analyses of variance (ANOVA). All statistical analyses were conducted in the statistical software R (R Development Core Team 2020).

RESULTS

I found that petiole length and width are positively correlated ($R = 0.519$, $t = 5.359$, $P < 0.001$) (Fig. 3). Therefore, the following analyses are conducted only with petiole length. Additionally, I found that petiole length has a significant statistical effect on bark beetle abundance including both adults and larvae ($z = 13.036$, $P < 0.001$) (Fig. 3). There is an exponential relationship between petiole size and total beetle and larvae number, which is found to be statistically significant through a Generalized Linear Model with a Poisson error distribution.

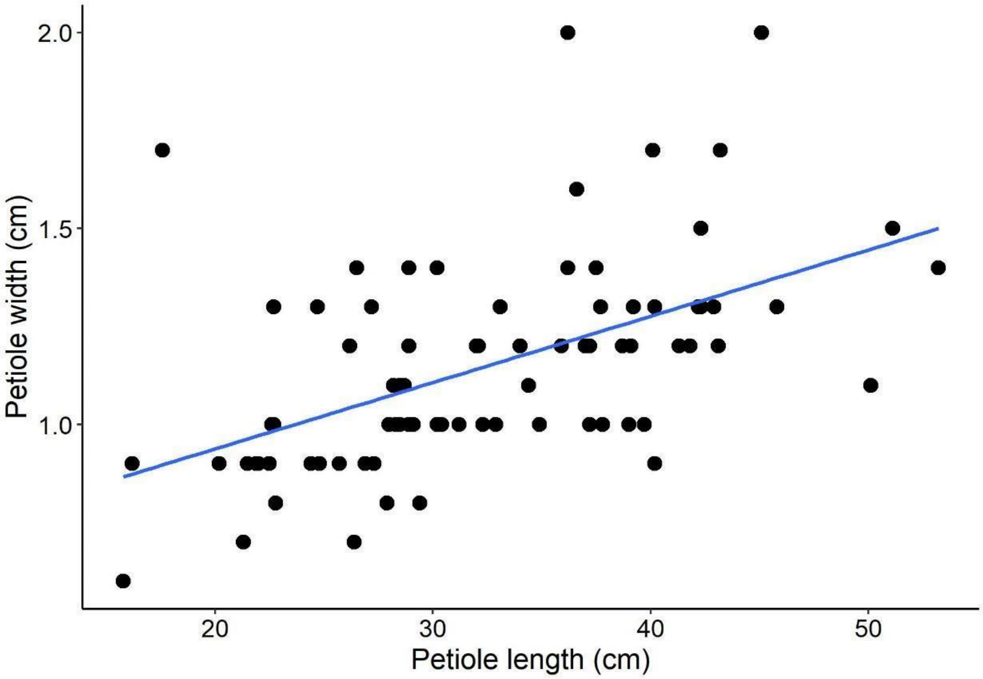

Figure 3. Petiole length and width of *Cecropia* (Urticaceae) leaves at Monteverde, Costa Rica.

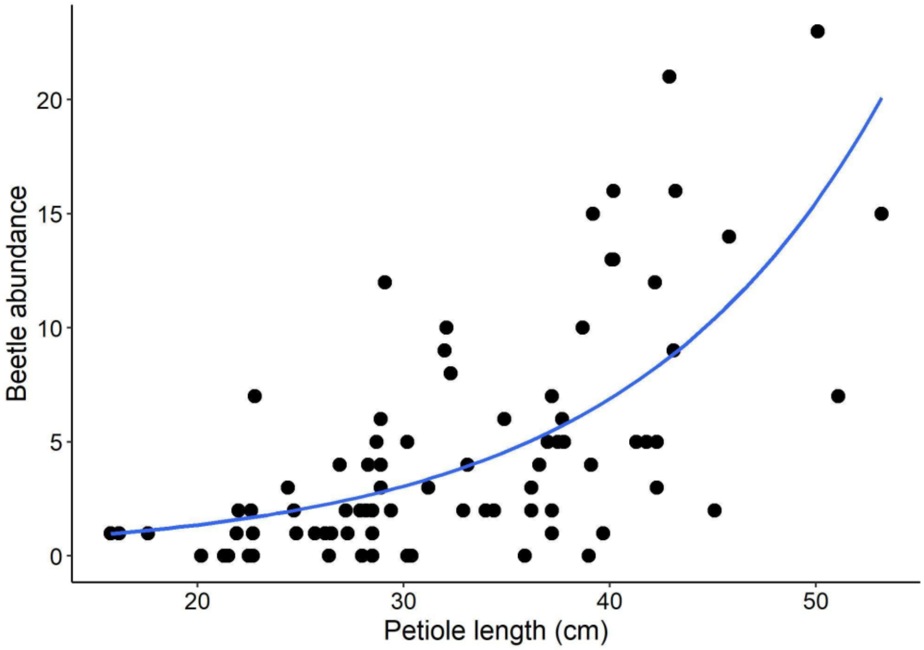

Figure 4. Petiole length and bark beetle abundance of *Cecropia* (Urticaceae) leaves at Monteverde, Costa Rica.

Looking at the different habitats and comparing the beetle abundances, no significant difference was found between a forest habitat and a disturbed area (Analysis of Variance (ANOVA) (F = 0.452, P = 0.504). It appeared that between a disturbed area and an undisturbed forest, there was not any significant difference in abundance. (Figure 2.).

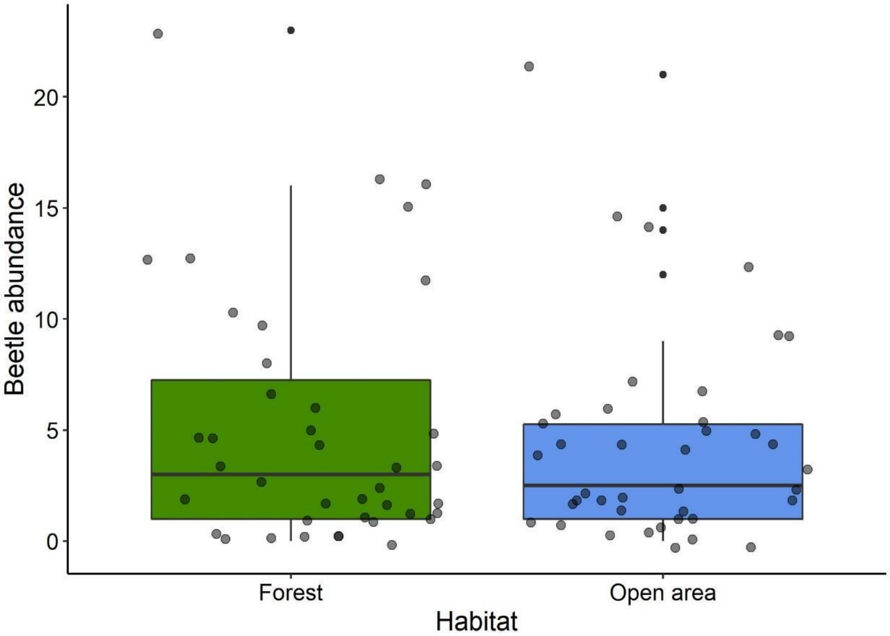

Figure 5. Bark beetle abundance *Cecropia* (Urticaceae) leaves in forest and open area habitats at Monteverde, Costa Rica.

I did not find a difference in bark beetle abundance across leaf positions (F = 0.063, P = 0.802). (F = 0.063, P = 0.802), there was not any significant difference (Figure 6). The same held true in different habitats (Figure 7).

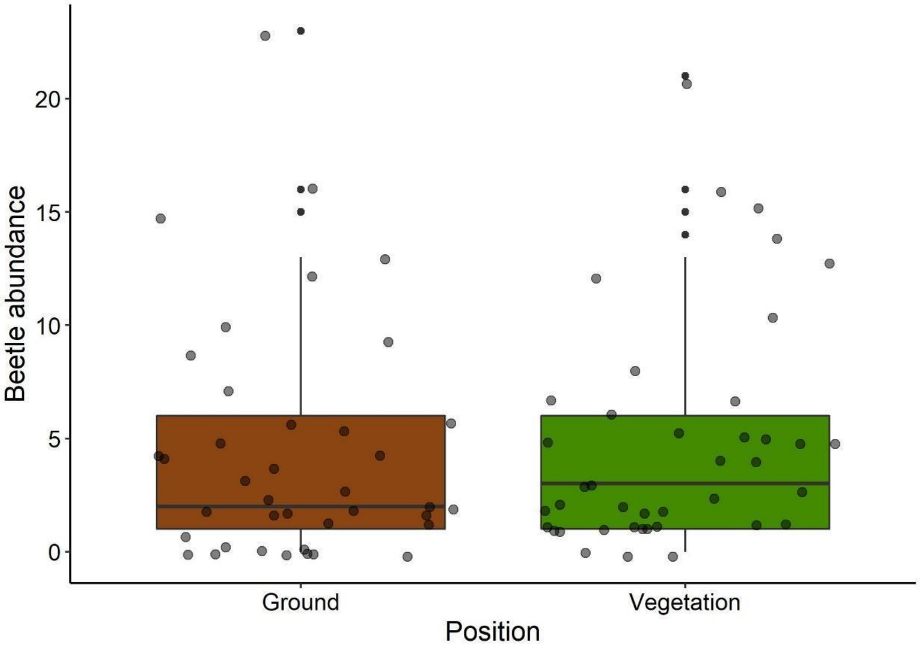

Figure 6. Bark beetle abundance in *Cecropia* leaves found in the ground or hanging in vegetation at Monteverde, Costa Rica.

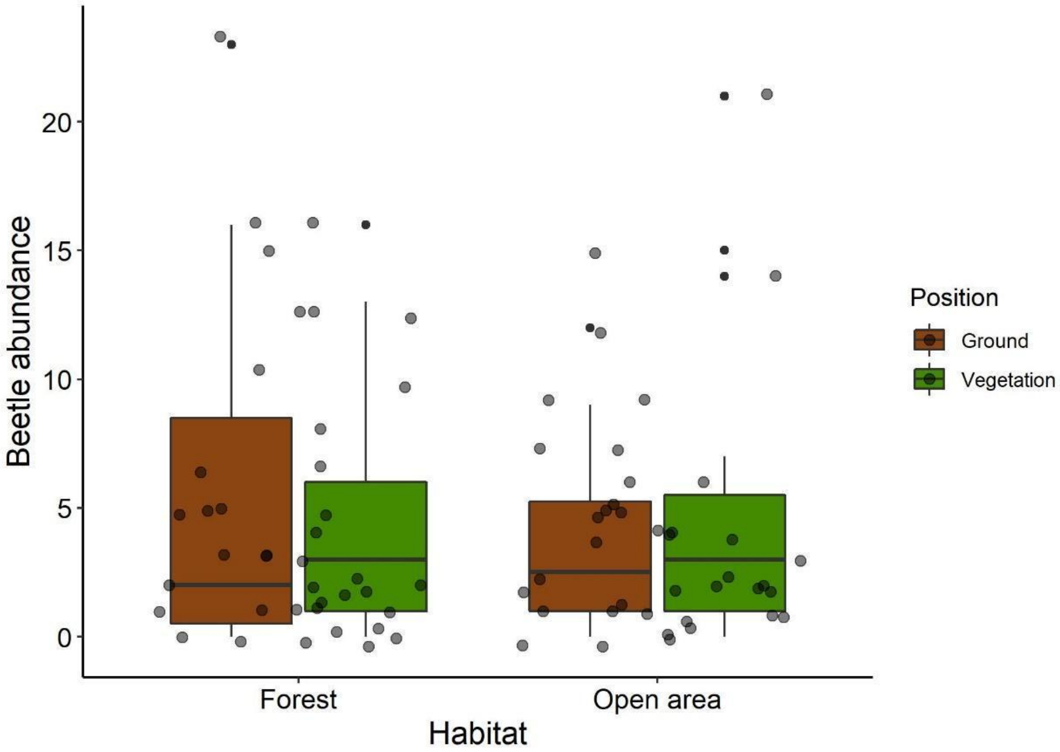

Figure 7. Bark beetle abundance in *Cecropia* leaves found in the ground or hanging in vegetation across habitats (forest and open areas) at Monteverde, Costa Rica.

Looking at the different positions petioles were collected from, there was a closely significant variance between the abundance of larvae in the petioles from the forest ground in comparison to the petioles from the forest vegetation. Conducting an Anova Analysis of Variance (F = 3.195, P = 0.082) there was a closely significant difference in the abundance of the petioles collected from the forest vegetation, showing that larvae are possibly more abundant at a given time in the forest vegetation than anywhere else that was researched.

I observed that bark beetle larvae were more common in leaves hanging in the vegetation in the forest. Therefore, I proceeded to analyze this observation in more detail finding that on average leaves on vegetation had 4.7 larvae whereas leaves on the ground had 3.2 larvae. This difference was marginally significant (F = 3.195, P = 0.082) (Fig. 8).

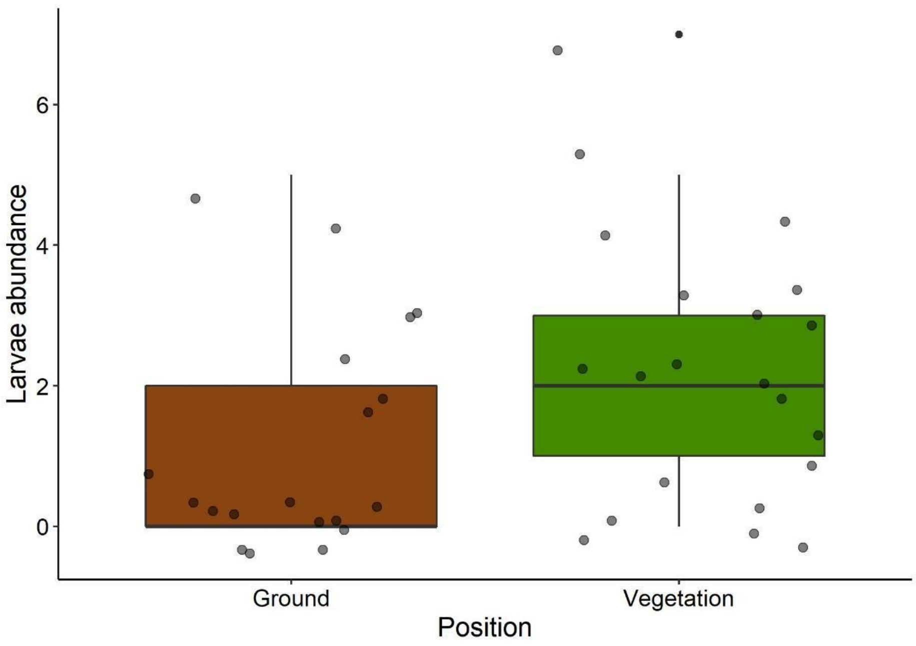

Figure 8. Bark beetle larvae abundance in *Cecropia* leaves found in the ground or hanging in vegetation at the forest habitat at Monteverde, Costa Rica.

DISCUSSION

Size

The clear correlation between size and beetle abundance is rather intuitive, in that as there are more resources available, we could easily expect a higher population. While this is an interesting finding, this correlation gives rise to further questions. How does this mechanism work, is this a behavioral trend? Are there other factors that play a part in the creation of this phenomenon, possibly such as easy mate access due to higher population? Perhaps one of the hypothesis we can form is that size, with increased resources draws more beetles in for the increased resources. This has been observed in other studies, with increased size and species richness in herbivory (Schlinkert et al., 2015). There are other possible reasons that might have additional benefits for the beetles such as easy access to mates that is further drawn by the resource availability.

Habitat

There was no significant difference between the abundance of beetles in different habitats. As previous research has also identified, there hasn’t been a great difference in abundance between different habitats that bark beetles are located at, including close to rivers in comparison to deeper forest habitats (Straus, 2007). Possible reasons for this is the ability of the beetles to travel certain distances as well as choosing to stay around the same tree and traveling to different leaves rather than moving to others (Forsse, 1985).

In the rapidly changing world of today, research points towards almost all organisms being impacted by global warming, other human damage and the changes that they bring, including bark beetles (Bentz et al., 2010). While it is important to identify the organisms and systems that are being primarily harmed by this, it is also important to recognize what actually does not have bad impacts on the organism. In this case, the results show us that there is no significant difference between the abundance of the beetles whether the area they are located at has been disturbed by humans or not. This could be valuable information when it comes to reforestation efforts. Further studies with a larger study group can help in identifying if these results are accurate. Then again, this study does not put climate change, elevation, or other factors that might be affecting the behaviors and resistance of the beetles towards human related damage.

Location and Larvae Abundance

As the results regarding increased beetle abundance in forest vegetation was not totally significant, there needs to be further research to understand if this is actually a trend. If it is, this could give us insights into breeding and egg laying habits of the bark beetles. Perhaps further studies could reveal the evolutionary advantages of preferentially reproducing in vegetation in the forest.

To conclude, in this study I found bark beetle abundance is determined by petiole size but not by leaf position or habitat at Monteverde, Costa Rica. Studying the factors that influence beetle abundance is fundamental to better understand plant-herbivore interactions in the Neotropical forests. This information can serve as a baseline to monitor biodiversity and their responses to climate change.

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Effects of flower usage on locomotion activity of glasswing butterflies (*Ithomia,* Nymphalidae) in Monteverde Cloud Forest, Costa Rica

Sophie Stahl
College of Biology, Honors College Purdue University, Indiana, USA

ABSTRACT

Flight activity in butterflies plays a role in pollination, mating, egg dispersal, predation patterns, and biodiversity. Flight activity is influenced by time, sex, and seasonality. Glasswing butterflies (*Ithomia,* Nymphalidae) are only found in the tropics and contribute to plant genetic diversity within the plant ecosystem as accidental pollinators. There is currently no research on flight activity patterns of glasswing butterflies in response to presence of flowers. By limiting food sources, this research aims to study the effect stressful situations have on the flight activity of glasswing butterflies and the behavior induced as a result. A total of 30 glasswing butterflies were collected in Monteverde, Costa Rica and monitored with portable locomotion activity monitors (pLAM). Half of the butterflies had access to flowers and the other half did not have access to flowers. The study examined movements over time and total movements in the presence and absence of flowers. I did not detect a statistically significant difference in the locomotion activity of glasswing butterflies that used flowers and those that did not (F=0.01, P=0.923). Butterflies with access to flowers had a higher mean of total the ones did not use flowers (1900 vs. 1750 activity events). Butterflies without access to flowers had a higher peak of activity movements during the average timespan (1500 vs. 1100 activity events). Studying the role of flowers in flight activity of glasswing butterflies may help us to understand the ecology of Neotropical butterflies, which in turn can be useful to develop management and conservation efforts for their protection in the tropics.

RESUMEN

La actividad de vuelo en las mariposas juega un papel en la polinización, apareamiento, dispersión de huevos, patrones de depredación y biodiversidad. La actividad de vuelo está influenciada por el tiempo, el sexo y la estacionalidad. Las mariposas glasswing (Ithomia, Nymphalidae) solo se encuentran en los trópicos y contribuyen a la diversidad genética vegetal dentro del ecosistema vegetal como polinizadores accidentales. Actualmente no hay investigaciones sobre los patrones de actividad de vuelo de las mariposas glasswing en respuesta a la presencia de flores. Al limitar las fuentes de alimentos, esta investigación tiene como objetivo estudiar el efecto que las situaciones estresantes tienen en la actividad de vuelo de las mariposas glasswing y el comportamiento inducido como resultado. Un total de 30 mariposas glasswing fueron colectadas en Monteverde, Costa Rica y monitoreadas con monitores portátiles de actividad de locomoción (pLAM). La mitad de las mariposas tenían acceso a las flores y la otra mitad no tenía acceso a las flores. El estudio examinó los movimientos a lo largo del tiempo y los movimientos totales en presencia y ausencia de flores. No detecté una diferencia estadísticamente significativa en la actividad de locomoción de las mariposas glasswing que usaban flores y las que no (F=0,01, P=0,923). Las mariposas con acceso a flores tuvieron una media mayor del total de las que no usaron flores (1900 vs. 1750 eventos de actividad). Las mariposas sin acceso a las flores tuvieron un pico más alto de movimientos de actividad durante el intervalo de tiempo promedio (1500 vs. 1100 eventos de actividad). Estudiar el papel de las flores en la actividad de vuelo de las mariposas glasswing puede ayudarnos a comprender la ecología de las mariposas neotropicales, que a su vez puede ser útil para desarrollar esfuerzos de manejo y conservación para su protección en los trópicos.

INTRODUCTION

Glasswing butterflies (family Nymphalidae) are only found in Tropical America. There are around 350 species, 23 of which belong to the *Ithomia* genus. Characterized by their clear wings, distinct venation, and deep wingbeats, this genus can be found along middle altitudes and is known to seasonally migrate between sea level to over 2,000 meters (Devries & Clark, 1987). The genus *Ithomia* occurs through a diverse range of habitats but is most commonly found in understory forests around flowers of the families Asteraceae and Solanaceae. Both males and females feed on the flower nectar from the previous families throughout the day, but they are more active in the early morning. Additionally, the males obtain pyrrolizidine alkaloids which provide protection against predation and help attract females (Hanson & Nishida, 2016).

Flight activity within the family Nymphalidae is affected by time of day, sexual dimorphism, and seasonality. Butterflies within this family are diurnal and within the genus *Ithomia*, butterflies are most active in the early morning, starting around 8:00 a.m. and peak activity from 9:00 to 9:30 a.m. It is hypothesized that butterflies maintain this schedule in order to avoid more intense solar radiation (Devries & Clark, 1987; Rutowski et al., 1996). Additionally, locomotion tends to vary between males and females. Prior research found that males of the *Asterocampa* genus are more active when the probability of encountering a mate is high. When the chance of encountering a mate is lower, males tend to remain perched on plants for longer periods of time (Rutowski et al., 1996). Females tend to be more active in general than males, especially after the female has eclosed: emerged from her pupal case. It is proposed higher activity in females is due to higher probability of attracting a mate and the need to have a consistent source of amino-acid reserves for proper egg maturation (Rutowski et al., 1996; Torres et al., 2009). Other research has shown that variation in seasons and temperature causes differences in activity among genera in the Nymphalidae family. In the dry season where resources are scarce, butterflies in the genus *Anaea* significantly reduces their population size but maintains the same activity patterns. In contrast, the genus *Myscelia* displays different foraging patterns but do not reduce in population size between the dry and rainy seasons (Torres et al., 2009). Therefore, some genera are more flexible when the seasons change and can switch to other food sources while others are unable to adapt to more stressful situations such as lacking feeding resources.

Butterflies play a crucial role in biodiversity as they are major pollinators, specifically accidental pollinators, and are therefore promoters of genetic plant diversity in their ecosystems. Butterflies have been proven to carry pollen over greater distances and visit a larger variety of plants in different layers of the forest than honeybees (Roy et al., 2021). Current research examines other genera among the Nymphalidae family but lacks information regarding locomotion of the genus *Ithomia* in the presence of food and in stressful situations such as lacking food resources. The objective of this study is to understand how flower usage affects locomotion activity in glasswing butterflies in Monteverde, Costa Rica. As glasswinged butterflies are common in the tropics, further examining any effects the presence of flowers on glasswinged butterflies will allow for further understanding of pollination patterns, migration, predation systems, and mating behaviors. Understanding locomotion activities may help in the conservation of glasswing butterflies and incidentally conservation of their tropical ecosystems.

MATERIALS & METHODS

Study Site

This experiment was conducted at the Biological Station in Monteverde, Costa Rica (10.3026° N, 84.7957° W) from July 17 to 24 of 2023. The Biological Station is a private reserve that contains around 6 kilometers of trails within the tropical forest and aims to protect the cloud forest biodiversity (Fig. 1a). The station sits at around 1500 meters above sea level. The mean annual temperature is 18.25 °C and the total annual rainfall, including mist, is 3176 mm (MINAE, 2023). Therefore, based on the Holdridge Life Zone, Monteverde falls in the Tropical Premontane Wet Forest (Holdridge et al., 1971). Neighboring the Biological Station is the Monteverde Cloud Forest Reserve.

Sampling and Experimental Design

The glasswinged butterflies were collected along the garden trails surrounding the Biological Station main building and found among the flowers of the plant family Asteraceae (Fig. 1b). This family was recognized by their composite flower heads and one-seeded achene fruits (Svoma et al., 2019). The genus *Ithomia* was recognized by having transparent wings, black or orange vein outlining, wingspan of around 28 to 33 mm, and narrow abdomens (Devries & Clark, 1987) (Fig. 1c).

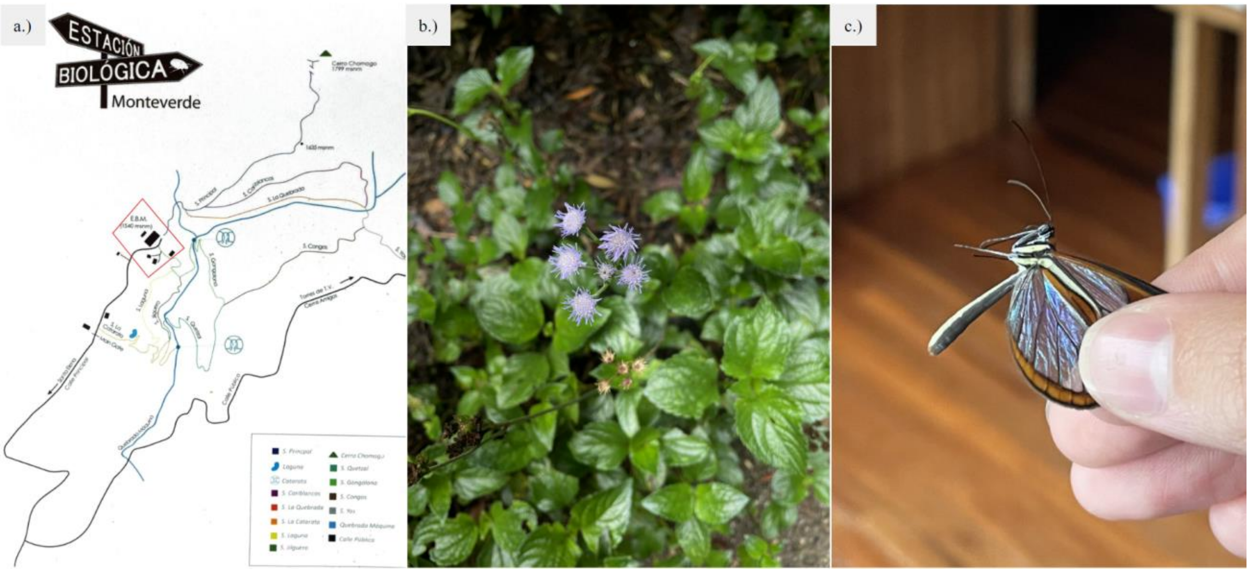

Figure 1. a.) Map of Biological Station in Monteverde, Costa Rica. The glasswings were collected among the gardens within the red box indicated on the map. b.) Flowers of the

Asteraceae family where the glasswings were collected. c.) Glasswing butterfly of the genus *Ithomia* that were collected during the study.

In five days of data collection, I collected a total of six butterflies with a sweeping net each day. There were two mesh cages assembled: both including a sugar water source (1 gram sugar to 4 parts water), the Raspberry Pi system with a camera, and a vase. The vase will either contain water or flowers from the Asteraceae family collected daily (Sondhi et al., 2022). Each day, three butterflies were put in a mesh cage. This study sampled 30 butterflies total for ten trials total (five trials per treatment). Each trial was recorded by the Raspberry Pi system for eight hours between the hours of 8 a.m. and 4 p.m. The Raspberry Pi system was chosen as the portable locomotion activity monitor (pLAM). The Raspberry Pi is designed to track high activity small animals by taking photos and then quantifying the images (Sondhi et al., 2022). The temperature and humidity were recorded each day using a Govee thermometer hydrogmeter (Fig. 2).

Figure 2. a.) The left mesh cage contained the glasswing butterflies without flowers and the right mesh cage contained the glasswing butterflies with flowers. In front of each mesh cage was a Govee thermometer hygrometer. b.) Inside the mesh cage with no flowers contained the Raspberry Pi, sugar water source, and a vase with water. c.) Inside the mesh cage with flowers contained the Raspberry Pi, sugar water source, and a glass container with water and Asteraceae flowers.

Data Analysis

To test the effect of flower usage on glasswinged butterfly species' locomotion activity, we visualized data as movements over time and total movements. Movements over time data were run through preprogrammed scripts (Sondhi et al., 2022). To test the effect of flower usage on butterfly activity I performed an analysis of variance (ANOVA) using the *aov()* function in the statistical software R (R Development Core Team, 2020).

RESULTS

Movements Over Time in Response to Flower Usage

The locomotion, flight patterns, and any other movements from the butterflies were all recorded as activity events by the Raspberry Pi. The total of activity events was compiled every ten minutes for eight hours each trial. Then an average of these compilations was taken between the trials to display the mean amount of movements over the given timeframe (Fig. 3). Both the flower and no flower treatments showed a similar pattern of lower activity events in the beginning and end of the trials. The flowers treatment averaged below 100 activity events in the morning and evening, whereas the treatment without flowers averaged above 100 activity events in the morning but below 50 activity events in the evening. Both groups showed a peak of activity around noon. The flowers treatment averaged above 1000 activity events at their peak, and the group without flowers averaged around 1500 activity events at their peak. Therefore, both treatments displayed an increase in activity reaching noon and a decrease in activity after noon; however, the no flowers treatment was more active than the flowers treatment. No statistical test was performed to see if this difference is statistically significant.

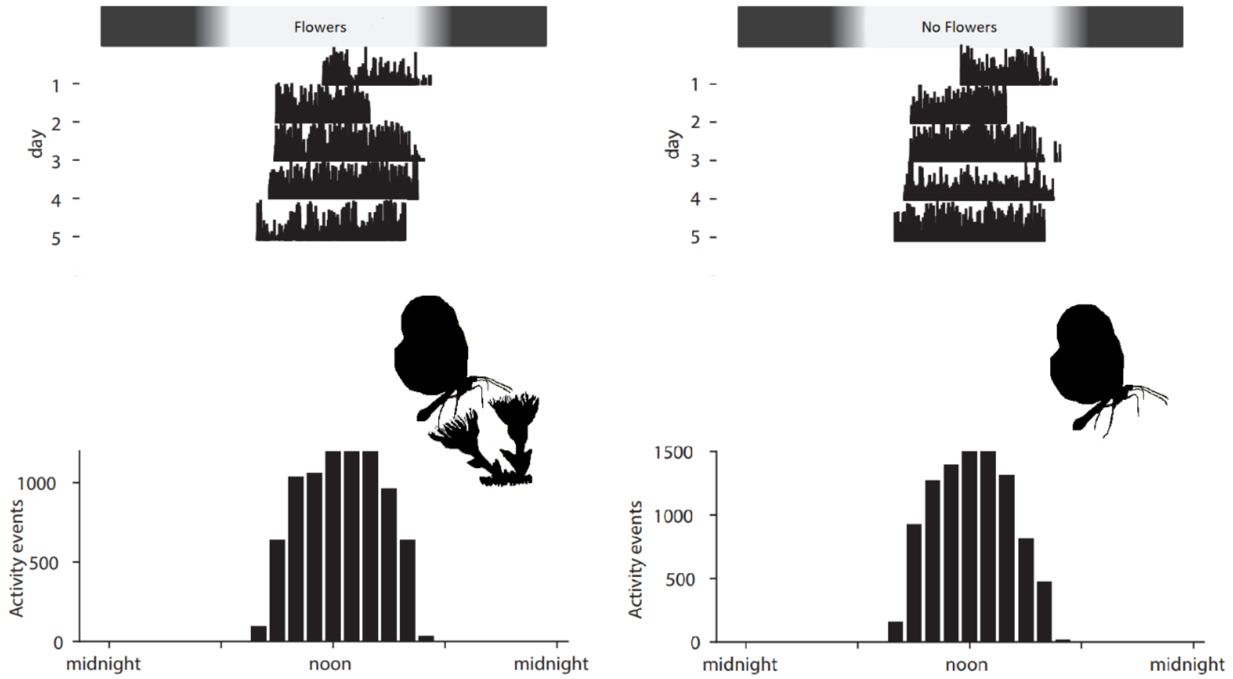

Figure 3. Glasswing butterfly activity events over time in response to flower usage at Monteverde, Costa Rica.

Total Movements in Response to Flower Usage

The group with no flowers had a higher maximum of total activity events than the group with flowers. Additionally, the group with no flowers had a lower minimum of total activity events than the group with flowers. Butterflies in the flower treatment had a median of 1900 activity events, and the butterflies without flowers an average of 1750 activity events (Fig. 4). This difference was not statistically significant (F = 0.01, P = 0.923). This shows that there was no statistically significant correlation in glasswing butterfly locomotion activity and flower presence.

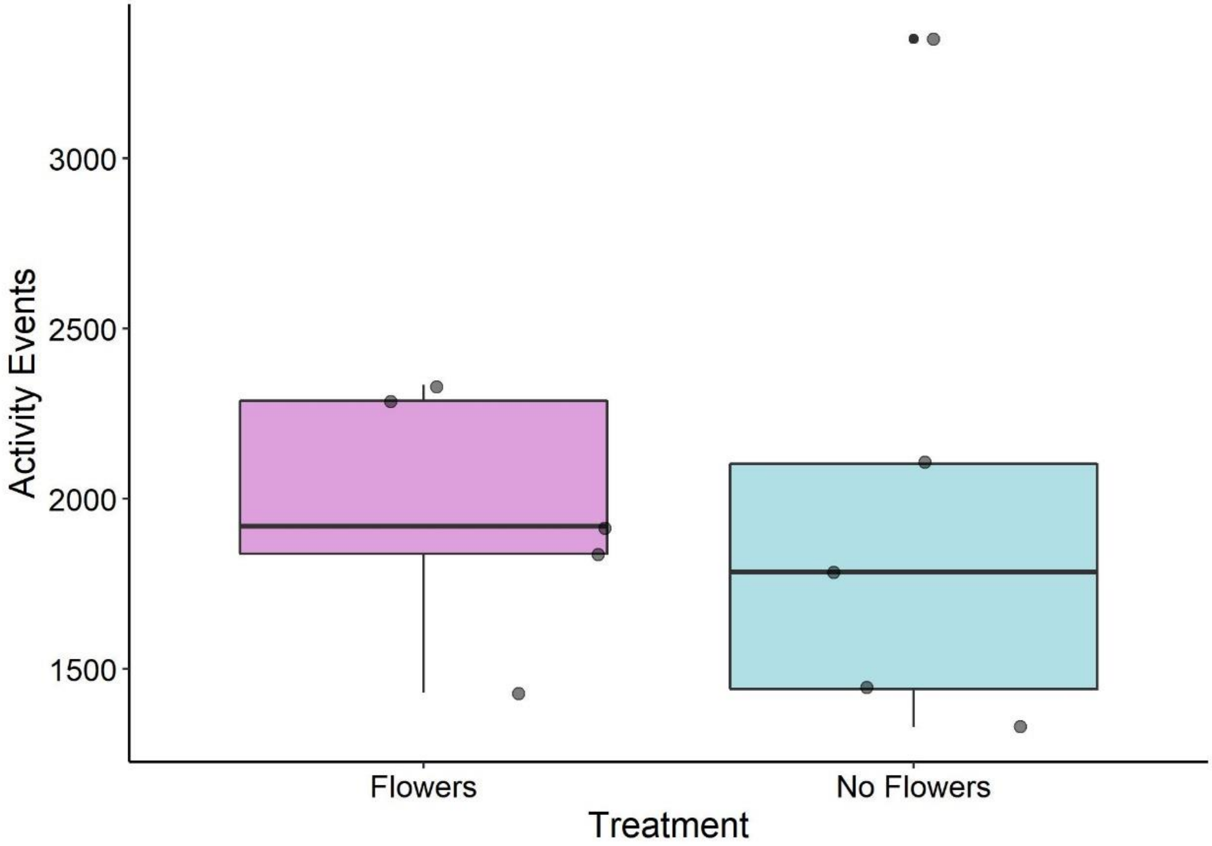

Figure 4. Glasswing butterfly (*Ithomia*, Nymphalidae) total activity events recorded in response to flower usage at Monteverde, Costa Rica.

DISCUSSION

Butterflies were more active at noon and less active during the morning. It was expected that activity events would drop in the evening hours. These results are not consistent with prior research showing that Nymphalidae butterflies are most active in the morning (especially the females) and then activity slowly declines throughout the day (Rutowski et al., 1996). This peak activity during the morning is thought to be due to optimal solar radiation levels, which is especially important for male glasswings who remain perched longer than females (Rutowski et al., 1996). This research shows that absence of flowers may increase the peak number of activity events around midday. The reasoning for why butterflies lacking flowers were slightly more active around midday is unclear. However, the similar activity pattern and ranges of the number of movements seen in both groups could suggest that either both groups were stressed due to change in captivity conditions or flower presence does not contribute to locomotion changes resulting in similar flight patterns throughout the duration of the day.

Regarding the total number of activity events, I initially predicted that butterflies without flowers will be more active than butterflies with flowers due to more attempts to find flowers. However, our results showed the opposite trend, being butterflies with access to flowers slightly more active. As nectar is composed of mostly sucrose, fructose, and glucose, its nutrients provide different benefits to butterflies. Sucrose was found to act as fuel for flying and the other nectar components aid in longevity, lifespan, and reproductive success (Cahenzli & Erhardt, 2012). Therefore, butterflies with access to flowers were slightly more active likely due to the extra energy provided by nectar. However, this activity difference was not statistically significant. Lastly, these results could be affected by a low number of sample size, a larger number of trails would be needed to test this difference with more statistical power. This could be a result of an outlier within one of the trials of the no flowers group.

This study did not control for gender, deaths, temperature, humidity, or natural environment. Picking the butterflies was random and the sex was not considered. However, there are dimorphisms in flight activity between males and females and their age (Rutowski et al., 1996). If there was a group that contained all males, the sample would be expected to have a lower activity event count than a sample that contained all females. There were also three butterflies that died during the experiment among the 15 butterflies in the no flowers group and one death among the 15 butterflies in the flowers group. Therefore, depending on the time the butterflies died and seized moving, this could have a non-quantified effect on the results. Temperature and humidity are known to affect the behavior of some genera among the Nymphalidae family: higher temperature and lower humidity can cause shorter lifespans (Torres et al., 2009). Although temperature and humidity were recorded, they were not kept constant throughout all trials. The temperature and humidity varied daily in the room that contained the mesh cages. Ideally, the temperature and humidity should have been the same for each trial. Finally, captivity could have caused extra stress on the butterflies, also skewing the activity events.

Further research should examine the effects of sex, temperature, and humidity on the locomotion activity of glasswing butterflies. If possible, examining these variables in the natural environment of glasswing butterflies could help avoid the additional causes of stress inflicted by captivity. This study allowed for better understanding of glasswinged butterfly flight patterns under the stressful conditions of food limitations. Research of glasswinged butterflies creates a deeper understanding of the tropics: pollination, genetic diversity, migration patterns, and predation. This deeper knowledge will allow for not only further protection of glasswing butterflies and the plants they pollinate, but also a better understanding of how to conserve the tropics.

ACKNOWLEDGMENTS

I would like to thank the staff of the Monteverde Biological Station and the CIEE Tropical Ecology and Conservation program. I am very grateful for the help of Yash Sondhi; I really enjoyed working with your Raspberry Pies. This work would not have been possible without the unconditional support and patience of Dr. Miguel Chaves and Dr. Adriana Arias-Aguilar. Thank you for helping me learn to enjoy research and the journey that comes with it.

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Effect of Leaf Size on Leaf Miner Herbivory and Positional Preferences of *Palicourea alajuelensis* (Rubiaceae) and *Casearia tacanensis* (Salicaceae) at Monteverde, Costa Rica

Soraia Barros
Department of Biology, Brandeis University, Massachusetts, USA

ABSTRACT

Herbivory, the consumption of plants by insects, occupies a significant role in every ecosystem's food chain as primary consumers. Leaf miners are endophagous herbivores that depend on plants for food and shelter by dwelling within the epidermises of the leaf, creating mines in their path. At the premontane wet forest of Monteverde, Costa Rica, I collected 50 mined leaves from *Palicourea alajuelensis* (Rubiaceae) and 40 from *Casearia tacanensis* (Salicaceae) plants and measured herbivory (total area and percent) for different leaf sizes, as well as preferences for mining between the left or right side of the midvein. The leaf areas ranged from 4-27 cm^2 with an average of 11% mined area for *P. alajuelensis* and 2-40 cm^2 with an average of 16% mined area for *C. tacanensis*. A negative correlation was found between the percent mined area and the total leaf area, with statistical significance at both the community and species levels. Meanwhile, a subtle but not statistically significant preference for the left leaf side was found at the community level and species level (*Palicourea alajuelensis*and *Casearia tacanensis*). No significant result was found for the effect of the total leaf area on the total mined area at the community level and individual level for *Palicourea alajuelensis* and *Casearia tacanensis*. These findings suggest that leaf miners might exploit larger leaves for their resource abundance without substantially altering their feeding habits. This research unveils insights into the behavior of Neotropical leaf miners and the intricate interactions which make up the food chains that sustain entire ecosystems.

RESUMEN

Herbivoría, el consumo de plantas por insectos desempeña un papel significativo en la cadena alimenticia de cada ecosistema como consumidores primarios. Los minadores de hojas son herbívoros endófagos que dependen de las plantas para alimento y hábitat, al habitar dentro de las epidermis de la hoja y crear galerías en su camino. En los bosques húmedos premontanos de Monteverde, Costa Rica, recolecté hojas minadas recolectadas de las plantas *Palicourea alajuelensis* (Rubiaceae) y *Casearia tacanensis* (Salicaceae) para revelar relaciones en el porcentaje y área de herbivoría para diferentes tamaños de hojas, así como preferencias para la minería entre el lado izquierdo o derecho de la nervadura central. Se observó una correlación negativa entre el porcentaje de área minada y el área total de la hoja, con significancia estadística tanto al nivel de comunidad como al de especie individual. Con respecto a la preferencia de lados de la hoja, se encontró una sutil preferencia por el lado izquierdo, sin embargo, estos resultados no fueron estadísticamente significativos al nivel de comunidad ni al nivel de especie (*Palicourea alajuelensis* y *Casearia tacanensis*). No se encontraron resultados significativos para el efecto del área total de la hoja en el área total minada a nivel de comunidad y a nivel de especie para *Palicourea alajuelensis* y *Casearia tacanensis*. Estos hallazgos sugieren que los minadores de hojas podrían aprovechar hojas más grandes debido a su abundancia de recursos sin alterar sustancialmente sus hábitos alimenticios. Esta investigación revela importantes resultados sobre el comportamiento de los

minadores de hojas Neotropicales y las intrincadas interacciones que conforman las cadenas alimentarias que sostienen ecosistemas enteros y los dinámicos bosques en los que habitan.

INTRODUCTION

Many insects rely on plant tissue as a food source and this ecological process is called herbivory (Hanson et al., 2016). Herbivory plays a fundamental role in the food chain of every ecosystem as primary consumers. Herbivory can be characterized in two ways; ectophagy and endophagy. Ectophagy is the consumption of leaves externally and includes insects that consume the plant via sap-sucking and leaf chewing. On the other hand, endophagy is the consumption of the plant internally; examples of endophagous insect behaviors include galling, leaf mining, and stem boring (Sinclair et al., 2010). While ectophagy can cause a widespread consumption of leaf tissue, endophagous insects are smaller in size than ectophagous insects and therefore do not pose such a significant threat to plant biomass (Liu et al., 2015).

Leaf-mining insects, commonly referred to as leaf miners, have evolved this unique type of herbivory where they wedge themselves into the leaves in between two very fine films (epidermis) on both the left and right side of the leaf (Fig 1.). Through this feeding behavior, leaf miners gain benefits like shelter, protection, and nutrients, and are able to evade physical plant defenses by placing themselves inside the leaf (Connor et al., 1997). Leaf miners are most commonly the larvae of beetles (Coleoptera), moths (Lepidoptera), and flies (Diptera), with the majority being caterpillars of minute moths (Hanson et al., 2016). Some species of leaf-mining larvae may carry out their pupation inside the leaf, while others exit the leaf to pupate (Hanson et al., 2016). Though leaf miners reap several benefits from plants, leaf mining herbivory is considered parasitic due to the consumption of photosynthetic materials by the larvae damaging the plants (Schaffer et al., 1997). Leaf miner threats to plant life have caused a search for population control methods against these pests which cause leaf curling and necrosis (Heppner, 1993). However, leaf mining proves to be a very successful method of foliage consumption for larvae.

Leaf miners leave behind conspicuous tracks that are visible to the human eye. Leaf miner tracks can vary in thickness and transparency, additionally, some species of larvae also have varying methods of feces depositing within the leaves (Hanson et al., 2016). The patterns of leaf miners can appear “snake-like” in a back-and-forth movement throughout the leaves. These patterns determine the extent of leaf tissue consumed, affecting the overall herbivory levels, and potentially influencing the fitness and survival of both the leaf miners and the host plants (Morton et al., 2011). Leaf miners can consume the entire interior of the leaf or only a portion (Hering, 2013). The parts of the leaf that are removed to form mines are the parenchyma cells, where most chloroplasts are concentrated within the leaves. If the larvae consume the entire mesophyll, where parenchyma cells are located, the mines will appear completely transparent. Entirely transparent mines are also known as *full-depth* mines. Other mine variations include *upper* or *lower surface* mines, which occur when a larva has consumed only a portion of the parenchyma. Upper and lower surface mines create a leaf that will appear more transparent from one side or the other.

The plant species preferences of leaf miners are relatively well understood in literature, however, there is a gap within this research area regarding their preferences for leaf size and mining location within the leaf. Hespenheide (1991) found that different species of leaf miners exhibited different preferences for leaves at the canopy, lower portions, or none at all. Other variations in miner preference include shaded areas versus sunny areas, though mines located in the shade have greater larval survival and herbivory. Regarding leaf size, Hespenheide (1991) states that smaller leaves are preferred by smaller species, though larger leaves allow for greater larval survival and the coexistence of multiple miners within the same leaf. Bultman & Feath (1986) observed similar results, finding increased larval survivorship in one species of leaf miner with a preference for larger *Quercus emoryi* (Fagaceae) leaves. Leaf miners are exceptional research subjects as they are abundant, have limited mobility, and are able to be studied even after having left the leaf mines. Nevertheless, these advantages of their small size also pose a challenge to understanding the motivations behind their behaviors.

The objectives of this study are to understand 1) the effect of leaf size on leaf-mining herbivory and 2) the preference between the left and right sides of the central leaf vein in *Palicourea alajuelensis* (Rubiaceae) and *Casearia tacanensis* (Salicaceae) in Monteverde, Costa Rica. Given previous studies, the anticipated relationship between leaf size and leaf miner herbivory is that larger leaves will have a greater percentage and area of herbivory and that there is no preference between the left or right sides of the leaf midvein. This research will provide insights about the feeding behavior of leaf miners, specifically about their leaf choices. This research will help to better understand the impact of leaf miners on overall forest structures and strive towards identifying means of controlling their population and harm where necessary.

Figure 1. Unidentified species of leaf miner in *Casearia tacanensis* (Salicaceae) visualized using a microscope in Monteverde, Costa Rica. Also shown are the feces deposits within the mined tracks, present between the epidermises of the leaf.

MATERIALS AND METHODS

Study site

This study was conducted in the Premontane tropical wet forests at the Estación Biológica Monteverde, Costa Rica. The elevation for this site is ~1500 meters. The average annual rainfall in Monteverde per year is ~3,000 millimeters and the mean annual temperature is 16-18 degrees Celsius (Guswa et al., 2007). This took place within a 5-day period from July 17, 2023, to July 24, 2023, containing temperatures averaging at 18 degrees Celsius with frequent rain/mist and windy periods. I collected leave samples of containing leaf miners at the S. Principal, S. La Quebrada, and S. Gongolano trails.

Sampling

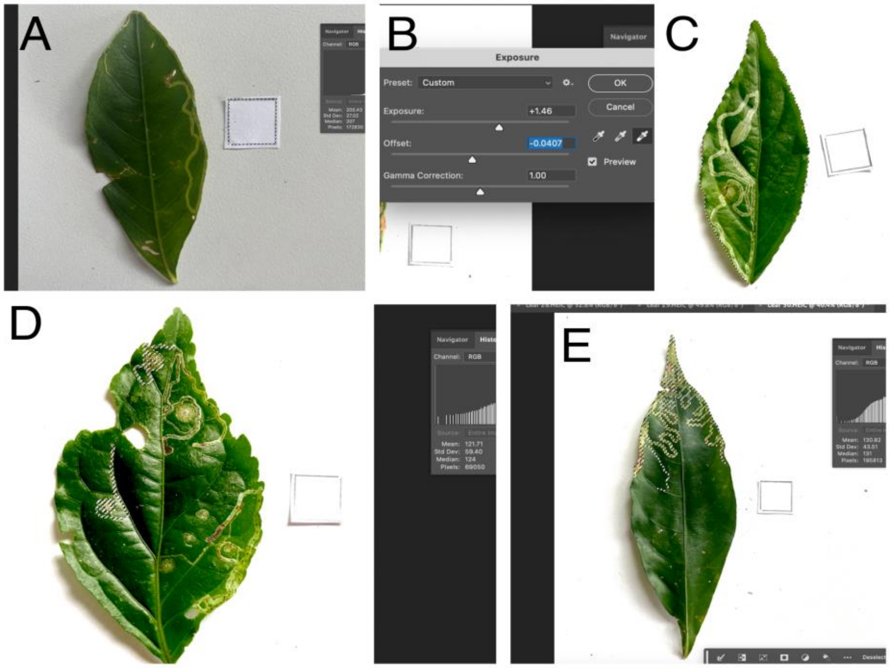
Plants containing visible tracks of leaf miners were collected from the trails within the testing site. During collection, I did not account for different leaf sizes, and rather collected a random sample of leaves from the two plant species. I collected 50 leaves of the species *Palicourea alajuelensis* (Rubiaceae), and 40 leaves of *Casearia tacanensis* (Salicaceae) (Fig 2.). These leaves were removed from their parent plants using plant shears and placed inside of a plastic bag to transport them to the laboratory. In the laboratory, these leaves were then organized by size to ensure enough range of smaller to larger leaves (Fig 3.).

Figure 2. Plants of *Palicourea alajuelensis* (Rubiaceae) (left) and *Casearia tacanensis* (Salicaceae) (right) at the Estación Biológica Monteverde, Costa Rica. Depicted is the difference in leaf mining pattern, where those of the *P. alajuelensis* appear more consistently “snake-like”, whereas the movement of the leaf miners within the *C. tacanensis* leaves contained mined spirals along the center of the leaves.

Figure 3. Display of all leaves of *Palicourea alajuelensis* (Rubiaceae) (bottom) and *Casearia tacanensis* (Salicaceae) (middle left) collected organized by size in Monteverde, Costa Rica.

Quantifying Herbivory

Adobe Photoshop was used to quantify the amount of leaf-mining herbivory per leaf size by converting them into an area (cm²). First, one cm² square of paper was measured with which all leaves were photographed. Photos were taken within 24 hours of collection to ensure leaves would not dry and wilt. The Photoshop Polygonal Lasso tool was used to select the entire traced area of the one cm² paper square. Under the Window tab, the histogram feature was used to count the number of pixels within the selected areas. For an accurate number, the histogram window was refreshed to maintain a cache level of 1in the RGB channel. Then, the object selection tool was used to measure the number of pixels in the entire leaf. Before proceeding to select the mined area, the image contrast was enhanced by adjusting the exposure and offset settings under the Image tab and under adjustments. Increasing the contrast helped the software better differentiate the mined areas from the unmined portions of the leaf. Photoshop's magic wand tool, with frequent adjustments to the tool's tolerance, was used to select the entire area mined on the left of the leaf. Next, the entire leaf was measured, this was done to compare the amount of total mined area located between the left and right sides of the central leaf vein (Fig 4.). To calculate the actual area in cm², values of pixel count from the different leaf components were divided by the number of pixels of the one cm² square paper. All data and calculations were input into Excel for further analysis.



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Std Dev: 27.02
Median: 207
Pixels: 172830

Preset: Custom
Exposure: +1.46
Offset: -0.0407
Gamma Correction: 1.00

Channel: RGB
Mean: 121.71
Std Dev: 59.40
Median: 124
Pixels: 69050

Channel: RGB
Mean: 130.82
Std Dev: 43.61
Median: 131
Pixels: 195813

Figure 4. Steps for quantifying herbivory in order. **A)** Selection of the one cm² drawn paper square showing the value of pixels within the selected area. **B)** Exposure tab is used to edit the contrast of images. **C)** Selection of entire *Casearia tacanensis* leaf. **D)** Selection of leaf-mining on the left side of *Casearia tacanensis* leaf, showing the value of selected pixels. **E)** Selection of leaf-mining on the entire *Palicourea alajuelensis* leaf, showing the value of selected pixels.

Statistical Analysis

To test the effect of leaf size on percent leaf miner herbivory and total mining area I performed generalized linear model (glm) with a gamma error distribution using the statistical programming language R version 4.0.0. (R Development Core Team 2020). To test the effect of leaf side (left and right) on leaf miner herbivory I performed a one-way analysis of variance (ANOVA). Both statistical test were done on the individual plant species level and at the community level (two species together).

RESULTS

Total leaf areas ranged from the smallest leaf with 4 cm^2 to the largest leaf with 27 cm^2 for *Palicourea alajuelensis*. For *Casearia tacanensis*, the total leaf areas ranged from 2 cm^2 for the smallest leaf and 40 cm^2 for the largest leaf. The mined areas averaged at 11% of the total area for *P. alajuelensis* and 16% of the total area for *C. tacanensis.* I found that total leaf area negatively influenced the percentage of the area that was mined by the leaf miners (Fig 5.). Therefore, in both the *P. alajuelensis* and *C. tacanensis* plant species, the larger leaves had less mining activity by a percentage of the total leaf area. This relationship was found to be statistically significant at the community level ($t = 10.310$, $p < 0.001$), and the species level for *P. alajuelensis* ($t = 7.492$, $p < 0.001$), and for *C. tacanensis* ($t = 5.802$, $p < 0.001$). For the effect of total leaf area on the total mined area there was a trend of increased area mined with increasing leaf size, but this relationship was not statistically significant (Fig 6.). Total mined area ranged from 0.5 to 3.5 cm^2 for *P. alajuelensis* and from 0.2 to 3.3 cm^2 for *C. tacanensis*. I did not detect statistically significant differences at the community level ($t = -1.701$, $p = 0.093$), nor at the species level for *P. alajuelensis* ($t = -1.174$, $p = 0.246$), and for *C. tacanensis* ($t = -1.249$, $p = 0.219$).

Alongside findings surrounding leaf size, analysis of leaf miner preference using a one-way ANOVA found that there was no statistically significant difference in the percent mined area between the left or right leaf sides on the community level ($F = 1.199$, $p = 0.275$), nor at the species level for *Palicourea alajuelensis* ($F = 0.785$, $p = 0.378$), and for *Casearia tacanensis* ($F = 0.442$, $p = 0.508$). Although there was a trend in the data showing a slight preference for the left side over the right side in both plant species, this positional preference was found to not be statistically significant (Fig 7.). Therefore, the results do not support that the leaf miners have a preference for a particular side of the leaf midvein.

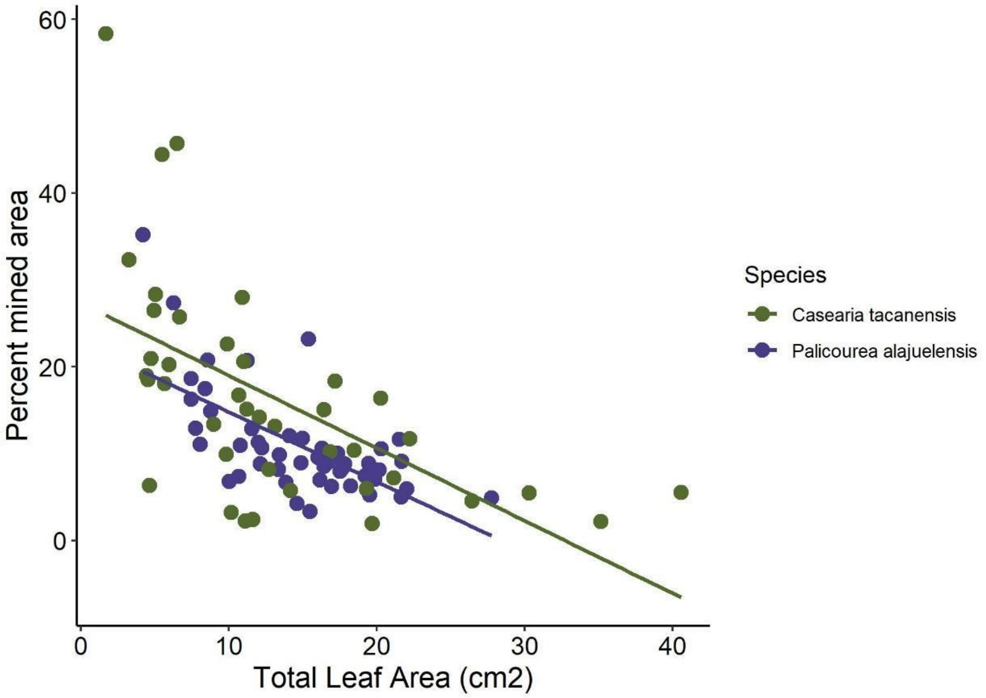

Figure 5. Total leaf area and percent mined for leaves of *Palicourea alajuelensis* (Rubiaceae) and *Casearia tacanensis* (Salicaceae) in Monteverde, Costa Rica.

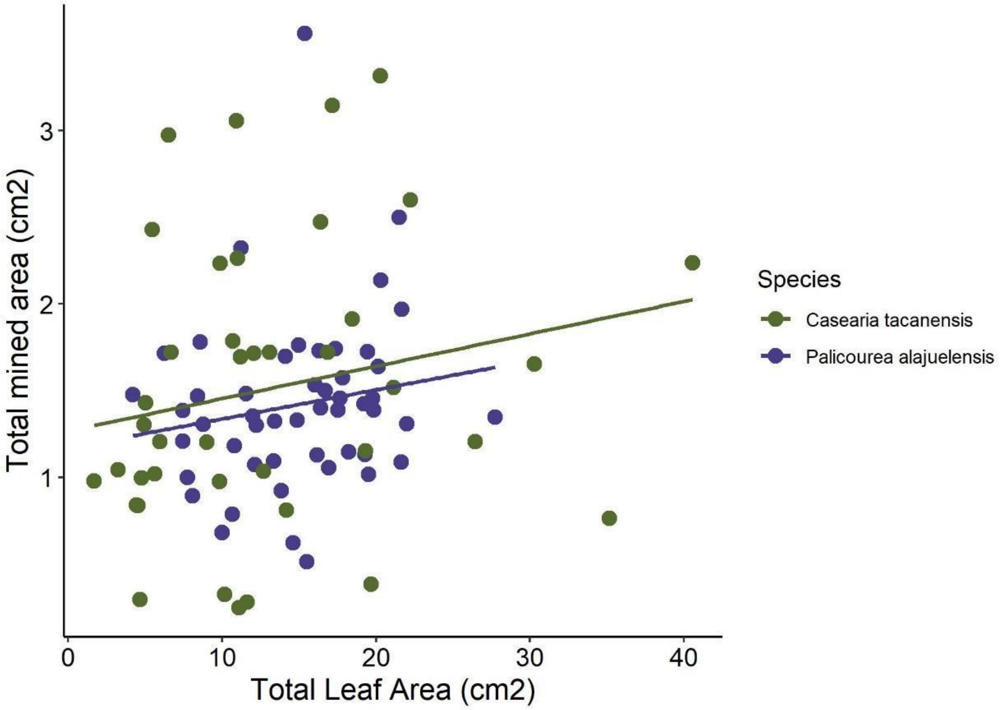

Figure 6. Total leaf area and total mined area for leaves of *Palicourea alajuelensis* (Rubiaceae) and *Casearia tacanensis* (Salicaceae) in Monteverde, Costa Rica.

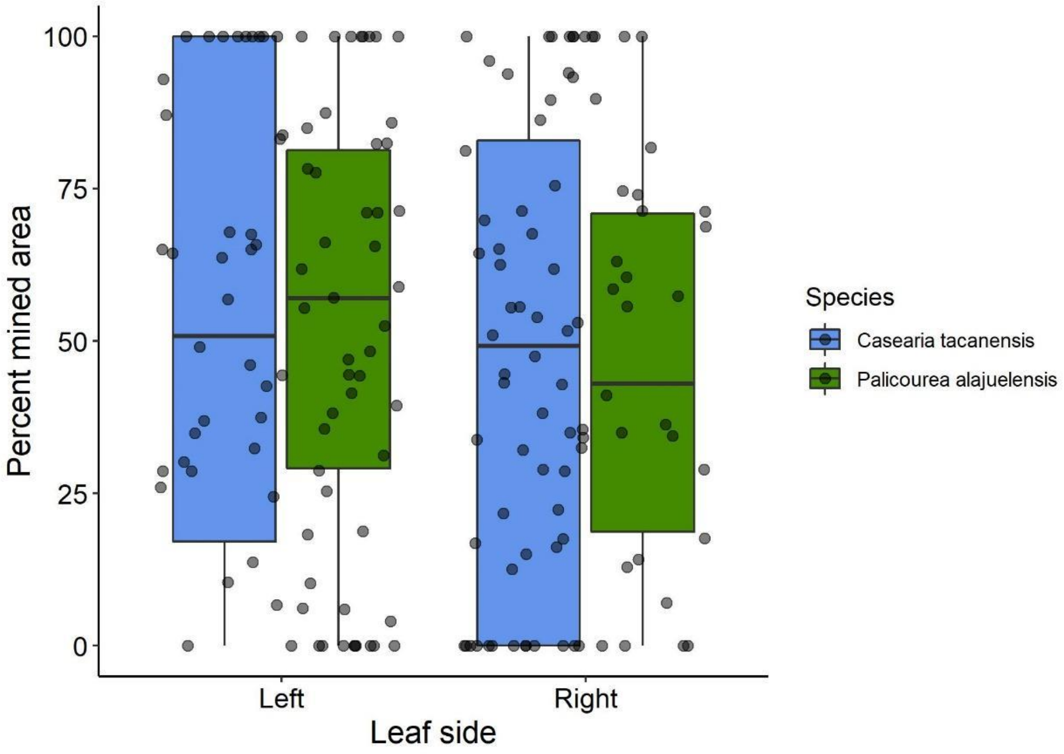

Figure 7. Leaf side and percent mined area for leaves of *Palicourea alajuelensis* and *Casearia tacanensis* in Monteverde, Costa Rica.

DISCUSSION

A key finding in this study is the negative correlation between leaf size and the percentage of the area mined, which contradicts the initial hypothesis that larger leaves would proportionally have more mining. This was true for both the community level and the individual level of the *Palicourea alajuelensis* and *Casearia tacanensis*. The study by Bultman & Feath (1986) explained the preference for larger leaves as being due to the availability of resources with an increased area being positively corresponded with increased survivorship. Although not all species of leaf miners demonstrated such a clear preference for larger leaves (Bultman et al., 1986). It is possible that leaf miners living in larger leaves have a higher survival than if living in smaller leaves, however this was out of the scope of my study. The preference for large leaves from Bultman & Faeth's (1986) study contradicts the significant relationship found in my study which suggested a decrease in the percentage mined area in large leaves. One explanation for this correlation may be due to the short time that leaf miners spend in the leaves, only remaining for about 2-3 weeks until either the beginning or end of their pupation period (Hanson et al., 2016). Leaf miners likely are able to take advantage of a surplus of resources in large leaves but are generally not able to eat more than they need, regardless of leaf size. Therefore, this limit in miner consumption ability explains the trend where the percent of the mined area is smaller in larger leaves, likely due to the overall greater size of the leaf itself. Likely, a preference for larger leaves is a decision made by the parent insect when ovipositing the eggs as a measure to ensure the success of their offspring (Stiling et al., 1987) Preferences for larger leaves are not reflective of the leaf miner itself, but rather a decision made by the female parent.

Regarding the positional preferences on leaf sides, there was a trend found showing a preference for the left side of the leaf in both the *Palicourea alajuelensis* and *Casearia tacanensis* plant species, however this was trend was not statistically significant (Fig. 7). This result is consistent with the hypothesis that leaf miners do not have a positional preference. One reason for not having a positional preference may be the decision of the parent insect to oviposit on one side of the leaf with no prior mining to maximize resource and space availability but also to minimize the likelihood of mines' merging and competition (Stiling et al., 1984; 1987). This parental decision is due to leaf minors being essentially immobile, and thus unable to relocate or escape from predators or competitors. However, the positioning of the larvae is most likely random along an area or side of the leaf where there is limited risk for the offspring. In cases where leaf miners mined on both sides of the leaf, they could only cross over from the top of the leaf to access additional resources on the opposite side of the central vein. An interesting observation from the leaf collections, though, is that many of the leaves from *Casearia tacanensis* contain multiple areas with small spirals. It is predicted that these spirals are where leaf miners are placed by the parent, however, it is interesting that they co-occur on the same leaf at a distance from one another (Fig. 8). This placement may just be a species preference for a leaf miner which requires less unmined space in the leaf.

During the course of this research, limitations were encountered, particularly with the relatively short time frame of the study - 5 days. The time limitation posed a challenge to the depth of knowledge attainable, such as the ability to identify the species of leaf miners or identify their duration and age in the mines. Some biases as a result of these challenges were that mined leaves were collected only from disturbed areas along trails in the specified study, and that leaves were only obtained from visible and accessible plants, meaning either on the forest floor or in the understory. As a result, only full-depth mines were observed, most likely limiting the number of different leaf miner species observed. For future research, many questions remain regarding the symmetry of the mines and the consistency of the patterns made by the leaf miners. Additionally, it would be of interest to differentiate behaviors and patterns of different species of leaf miners and expanding this study to more plant species. Leaf miner herbivory is overall less understood than leaf chewing herbivory in the tropics. Therefore, studying the ecology of leaf miners is fundamental to better understand the whole picture of herbivory in the tropical forests. In conclusion, the captivating world of herbivory, particularly leaf mining, holds vast opportunities for future research. Delving deeper into the intricate interactions of these small yet pivotal organisms can unlock a greater understanding of the food chains that sustain entire ecosystems and the dynamic forests they inhabit.

Figure 8. Leaf of *Casearia tacanensis* (Salicaceaecontaining multiple leaf-mined spirals in separate regions of the leaf at Monteverde, Costa Rica.

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I would like to thank my advisor, Dr. Miguel Chaves, for not only introducing me and teaching me about the world of leaf miners but also for his patience, knowledge, and help throughout the progress of this research project. I am especially grateful to him for all his kindness in completing the statistical analysis required for this project. I would also like to thank Eladio Cruz for lending his expertise in identifying the species of plants relevant to this paper. Without their help, and that of all the staff of the Estación Biológica Monteverde, I would not have been able to carry out this research successfully.

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The effect body and trap size of Wormlions (Vermileonidae) on their predation ability at Monteverde, Costa Rica

AJ Aiken
Department of Biology, Brandeis University, Massachusetts, USA

ABSTRACT

The Vermileonidae larvae, known as Wormlions, have a unique pit-building way of catching prey, in this study I look to find more both about the larvae's pit-building and predation tendencies. The objective of this study was to understand the effect of wormlion size on pit size and their ability to catch prey. I measured Wormlions size (length and weight) and their pit diameter. At the field, I conducted feeding trails to assess their ability to predate ants (5 and 7mm long). It was found that weight and length of Wormlions are strongly correlated, and these two variables have positive effects on both pit diameter and success in predation. This study helps to have a better understanding of the ecology and natural history of the wormlions in the Neotropics.

RESUMEN

Las larvas de Vermileonidae, conocidas como wormlions, tienen una forma única de construir fosas para capturar presas. En este estudio intento averiguar más sobre la construcción de fosas y las tendencias de depredación de las larvas. El objetivo de este estudio era comprender el efecto del tamaño de las larvas en el tamaño de las fosas y su capacidad para capturar presas. Medí el tamaño de los wormlions (longitud y peso) y el diámetro de sus fosas. En el campo, realicé recorridos de alimentación para evaluar su capacidad para depredar hormigas (de 5 y 7 mm de longitud). Añadir datos descriptivos. Se encontró que el peso y la longitud de los Wormlions están fuertemente correlacionados, y estas dos variables tienen efectos positivos tanto en el diámetro de la fosa como en el éxito en la depredación. Este estudio ayuda a conocer mejor la ecología y la historia natural de los wormlions en el Neotrópico.

INTTRODUCTION

Wormlions are the larvae of the fly family Vermileonidae, these larvae are trap-building predators and dig themselves into a burrow, which when a small insect walks over the Wormlions attempt to capture and eat it (Dor 2014). These creatures are found in shaded areas, most of the time nearby ant nests as ants are their primary prey (Bar-Ziv 2018).

The wormlions share their pit trap feeding with a non-related insect group, commonly known as Antlions (Myrmeleontidae). The Antlions are larger than the Wormlions and have a higher success rate of predation (Miller 2018). The research on Wormlions is much less in depth than that of the Antlions, although there are still many things known about these creatures will impact my research. Wormlions prefer smaller particles and a larger depth of substrate in their habitat, and deeper sand leads to better performance in terms of predation (Bar-Ziv 2019). It is not clear in previous research whether there is much intraspecies competition and if this possible competition positively or negatively the entire group of Wormlions (Dor 2014).

The objectives of this research are to understand **1)** the relationship between worm lion length and weight, **2)** the effect of worm lion size on pit size, and **3)** how worm lion size affects predation success.. This will further the insight we have on these interesting creatures and the unique feeding habits that they have. With the increased knowledge coming from research on Wormlions more insight can be accessed on the convergent evolution of the Wormlions with other trap-building predators especially the Antlions. This connection between these families' larvae could tell us much about the natural history of both families as well as to understand the convergent evolution processes in insects.

MATERIALS AND METHODS

Study site

I conducted my study at the Estación Biológica Monteverde at Monteverde, Costa Rica. This study took place from July 17 to July 21, 2023. The elevation of the site is approximately 1500 meters. The Wormlions will be collected near the Estación Biológica under a house, and then taken back to the laboratory for further study.

Experimental Design

I measured the body length and weight, pit diameter and conducted feeding trials on 30 larvae of Wormlions. First a picture was taken of the site with the Wormlion pits and a ruler which was used for calibration, each pit was given a number to be tracked, and the pit diameters were measured using ImageJ. Then ants between the size of 5mm and 7mm were collected in tubes to be used as the prey for the wormlions. For the feeding trial, the ants were dropped into each Wormlion pit where it was recorded if the Wormlions successfully predated and if so, how long it took to capture the ants. Once these trials were done the wormlions were collected from the site and were brought back to the station where they were weighed, and pictures were taken with a ruler to be measured in ImageJ.

Statistical Analysis

To understand the relationship n between Wormlion weight and length I conducted a Pearson Correlation. Then, to test the effect of Womrlions weight on the pit diameter I conducted a linear regression model. Lastly, to test the effect of wormlions weight on their ability to predate successfully I conducted a Generalized Linear Model (glm) with a binomial error distribution. All statistical analyses were performed using the statistical programming language R version 4.0.0. (R Development Core Team 2020).

RESULTS

Correlation Between Wormlion Weight and Length

The Wormlions length ranged from 5.77 mm to 17.56 mm, and the weight from less than 0.001 gram to 0.038 grams. I found that Wormlion body length and weight are positively correlated (R = 0.901, t = 11.56, P < 0.001) (Fig. 1). Since the weight and length of Wormlions were found to be so highly correlated, I will use only weight to test the effect of body size on both pit diameter and success in predation.

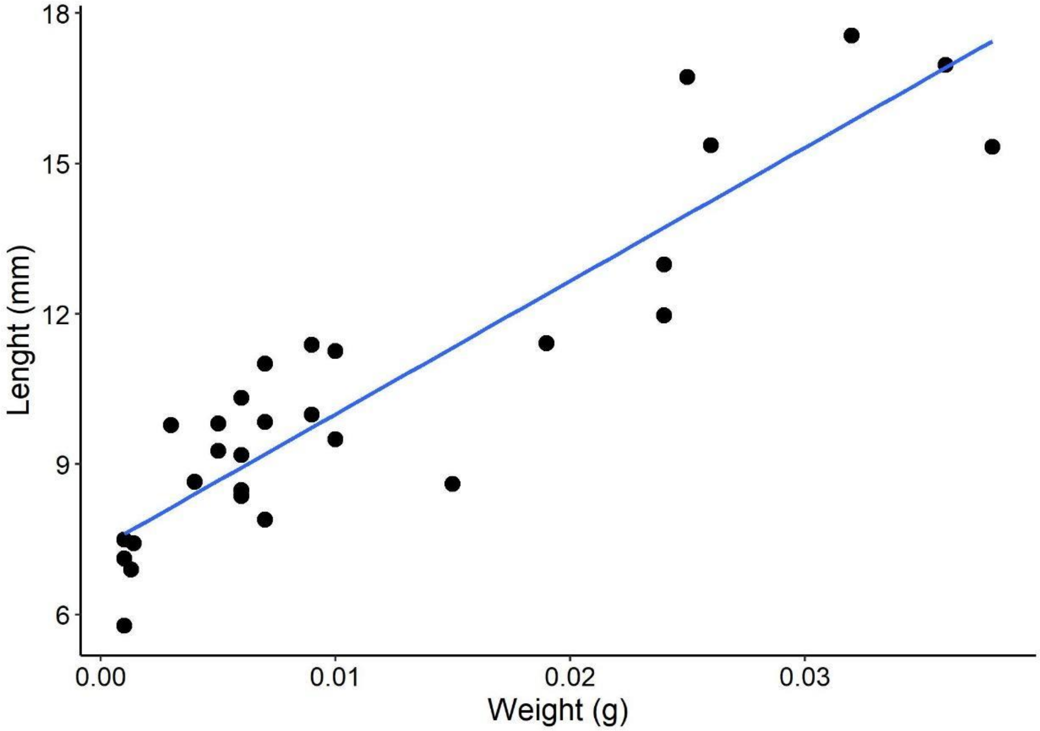

Figure 1. Body weight and length of Wormlions in Monteverde, Costa Rica.

The Effect of Wormlion Weight on Pit Diameter

The pit diameter size ranged from 6.77 mm to 26.56 mm, having a average of 12.48 mm. I found that Wormlion weight has a statistically significant positive effect on pit diameter ($t = 4.065$, $P < 0.001$) (Fig. 2). In other words, larger wormlions built larger pits.

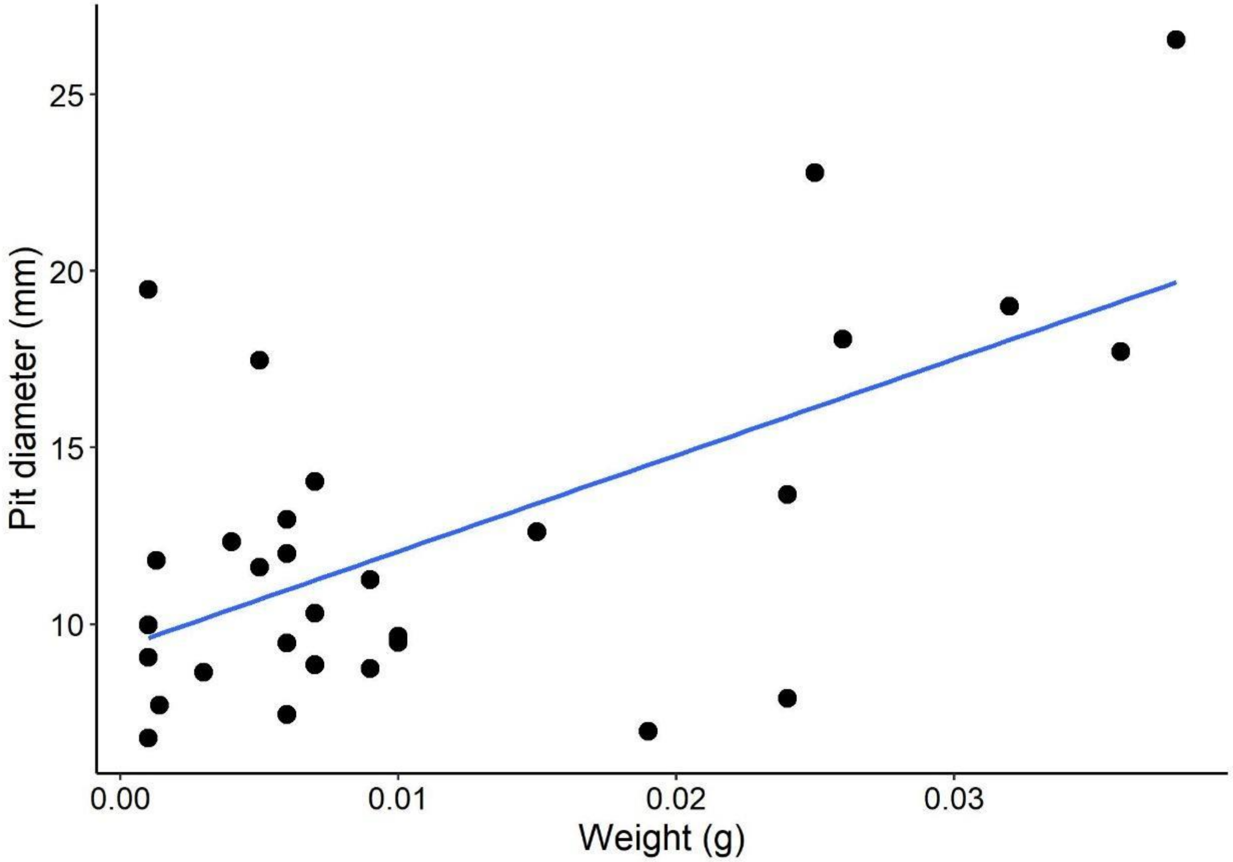

Figure 2. Body Weight and Pit diameter of Wormlions in Monteverde, Costa Rica

Effect of Body Weight on Success of Predation

Out of the thirty feeding trials, 10 wormlions caught their prey successfully. I found that body weight has a statistically significant effect on the success of wormlions to catch a prey ($z = 2.843$, $P = 0.004$) (Fig. 3). This means that larger Wormlions are more likely to successfully predate on this specific size of ant than smaller wormlions.

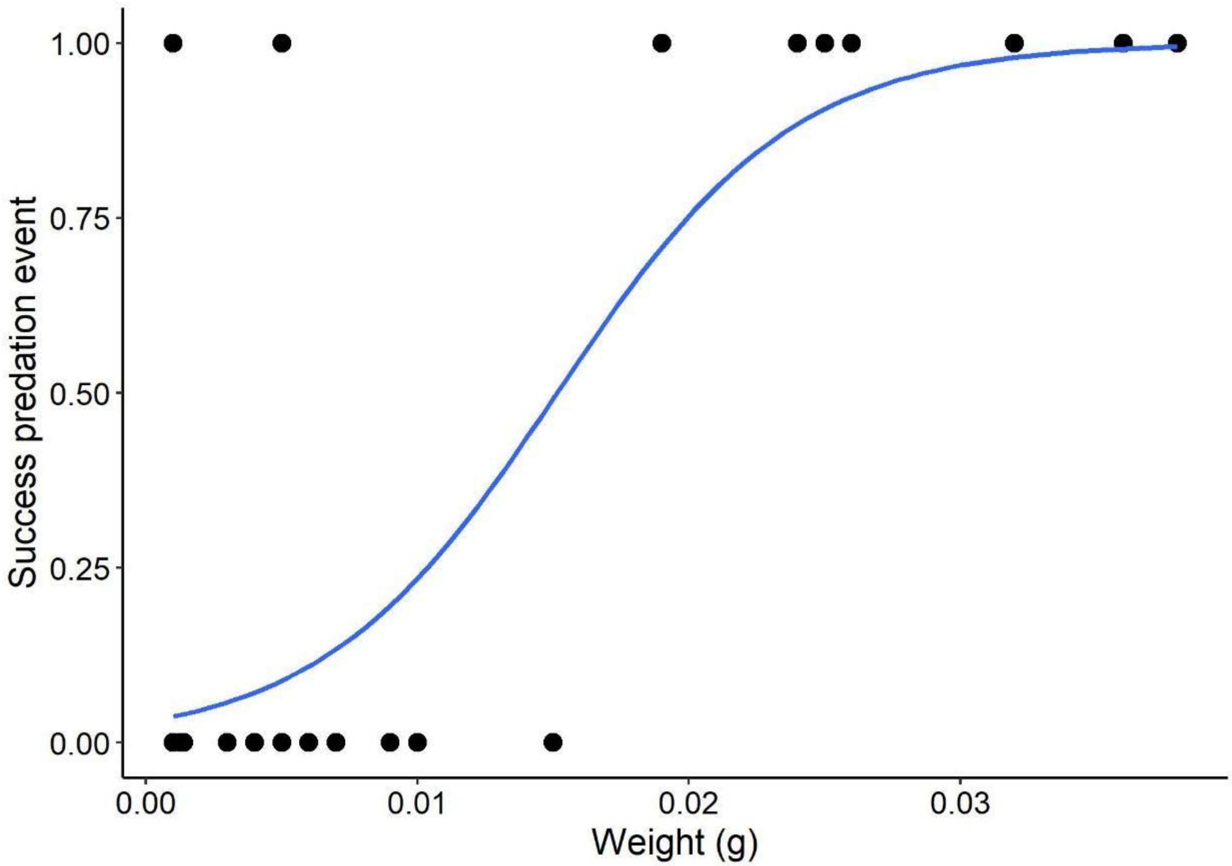

Figure 3. Body weight and success of predation of Wormlion on ants sized 5-7mm in Monteverde, Costa Rica

DISCUSSION

The results of this study show statistically significant positive correlation between Wormlion weight and length, a statistically significant effect of Wormlion weight and length on pit diameter, and a statistically significant effect of Wormlion weight and length on their ability to predate ants sized between 5-7mm.

This study showed that wormlion size has a positive effect on pit size, results are only partially in agreement with previous studies on Wormlions that found pit size and weight were found to only be weakly correlated (Dor 2014. The reasons for this difference could be due to many factors, the larvae in the previous paper were observed in Tel Aviv, Israel, in which the climate or location could have affected the results. Another possibility is that the Wormlions were of different species of Vermileonidae.

In comparison with similar studies done on Antlions the results of this study show that there are base similarities between the two pit-building predators. The Antlions researched were of the species *Morter obscurtus*, it was found that the size of Antlions correlated with the relative size of its prey (Griffiths 1985). This holds true in the data found in this study as only the larger predators were able to predate on the larger-sized ants they were all given. The effect of weight and length on the pit size of Antlions were also studied, again in more details and with more variables in mind as more is known about them. The *Macroleon muinquemaculatus* (Myrmeleotidae) species was studied, it was found that weight and length affected pit size up to a certain point, but there was a limit on this as it was seen that there was a range of maximal optimization of pit size. The tendencies of this species also changed with different variables as in whether the area was disturbed often and if the frequency at which prey visited the area (Griffiths 1986).

There were many limitations in this study, many stem from the short time that was allotted for data collection. First the lack of time led to a relatively small number of larvae that were observed and collected. Also, only a small range of ant size was used in this project, meaning only 10 of the 30 larvae observed and collected captured prey, this meant that the study that was supposed to be done on efficiency of capturing prey could not be done. Another limitation could be that the larvae felt the vibrations that were made by my feet in the collection site and that could have somehow impacted the results. Much of the limitations on this study are due to the lack of background information on this family of larvae, without the background base knowledge it was difficult to know what to study and how to do that effectively.

Further studies of how different masses and pit diameters affect predation of multiple types and sizes of prey would allow for a more comprehensive understanding of the predation behavior of Wormlions. Studies could also be done that use the same methodology as those of Antlions, like comparing trafficked and non-trafficked areas and how that affects the pits the organisms build. This would allow us to further learn about the convergent evolution of these families.

The results of this study are important to understand the effect of body size on pit size and predation success of wormlions. Studying the ecology and natural history of wormlions help us to know better these secretive creatures that are not well studied. Additionally, by comparing this information to the information known about antlions, it provides more information about evolutionary processes such as convergent evolution in insects. These findings along with new findings in the future will allow us to create a broad understanding of these interesting insects and their role in the ecosystems they reside.

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VERTEBRATE ECOLOGY

Vertebrate Composition across Elevational Gradient in Cloud Forests, Monteverde, Costa Rica

Martin Lugwarha Kalere
School for the Environment, University of Massachusetts Boston, MA, USA

ABSTRACT

Vertebrate fauna are the main attraction in Monteverde's tourist filled highlands, however at lower elevations where humans encroach, they are more at risk due to fragmentated habitat areas in need of corridors to ensure healthy cloud forest ecosystem. This study proves that biodiversity is higher than in higher elevations thus more attention needs to be focused on conserving these lower land areas.

RESUMEN

La fauna de vertebrados es la principal atracción en las tierras altas llenas de turistas de Monteverde, sin embargo, en las elevaciones más bajas donde los humanos invaden, están en riesgo debido a las áreas de hábitat fragmentadas que necesitan corredores para garantizar un ecosistema de bosque nuboso saludable. Este estudio demuestra que la biodiversidad es mayor que en elevaciones más altas, por lo que se debe prestar más atención a la conservación de estas áreas de tierras bajas.

INTRODUCTION

Costa Rica is the bridge of the New World, its location and weather conditions make for a unique and ideal recipe for biodiversity on a massive scale, harboring a rich combination of North and South American native but also endemic species. Due to the country's gradual shift from being an agricultural superpower to one that thrives off ecotourism and takes conservation into consideration. The recovery of forest habitat after the late 1990s was possible after heightened regulations from Forestry laws allowed for a decimated Costa Rica full of vast farmed property to regain 60% of forest cover enabling biodiversity to prosper with wilderness abundant (Chomitz, 1999).

The Tilaran mountain range located in northwestern Costa Rica has its upper portion protected (1,500m and up), however this protection is diluted as you go lower in elevation due to the presence of fragmented forest, mainly on the Pacific side which also tends to be drier (Guindon,1996).

Mammals, birds, reptiles, and amphibians of all kinds thrive here, but despite this, many species in the past that were once abundant have been lost due to climatic pressure such as the iconic Golden Toad *(Incilius periglenes)* endemic to Monteverde (Anchukaitis, 2010). This study pertains to the vertebrates that call Monteverde home and how abundant these species are depending on altitude to confirm whether the common notion that there's more life at lower elevations is indeed factual so that it is understood how to better protect these areas more prone to human settlement.

Monteverde is host to an overwhelming array of biodiversity that attracts visitors worldwide. A multitude of life zones coupled with unique weather conditions makes it unique among highland environments.

Elevational Gradient

Elevational gradient studies prove that higher altitudes are colder and possess drier sediments which are directly correlated to reduction in vegetation productivity which as a result means species richness is reduced as well (Kessler, 2009) (McCain, 2007).

Vertebrate Fauna

The cloud forests of Monteverde are world renowned for their species richness, being home to 425 bird species, 101 reptile species, 60 amphibian species, and 120 mammal species. Toucans, sloths, kinkajous, snakes, hummingbirds, tayras, and more elusive inhabitants like the 6 felid species from the mighty Jaguar to the Oncilla are present. These vertebrates of varying shapes and sizes occupy different ecological niches. Monteverde has seven out of twelve life zones in Costa Rica, based on the Holdridge life zones system, so the mix of microclimates and is a paradise for species.

MATERIALS AND METHODS

The Monteverde Biological station is a research facility surrounded by privately-owned primary and secondary forest sitting at 1,538m (about 5,045.93 ft) above sea level. It oversees the Gulf of Nicoya on the Pacific and therefore lies on the drier side of the continental divide.

This is the reason why species on this side differ slightly from ones found in Caribbean conditions. Climate ranges from entirely cloudy to somewhat clearer sunny skies, with rain being prominent every few days or so.

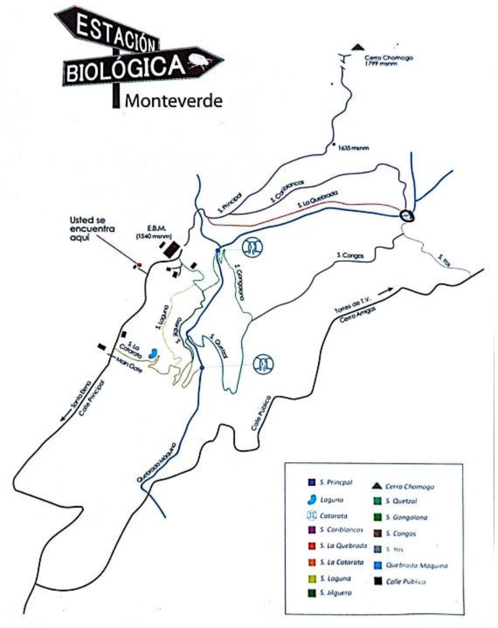

Figure 1. Map source - Estación Biológica Monteverde, Costa Rica.

Camera trap number designated by order of installation:

1) Forest edge – 1,489m

2) Trail – 1,437m

3) Creek intersection – 1452m

4) Canopy gap – 1,496 m

5) Tilaran camera trap – 1,810m

Sampling was done utilizing 5 camera traps in different sites with varying elevations, separated by 40 or so meters in the lowlands (except for No. 5) to capture whatever creature walked or flew within range. The second part of this study required a field component in which observations were done across the sites at different times of day to get as much data as I can in the short timeframe of 5 days. Species were identified visually and captured on camera using a camera but also audible identification with the help of an experienced human guide. Field guidebooks were there for visual confirmation, as many contemporary genera such as birds in the Tyrannidae family look very similar and are challenging to distinguish with the naked eye without experience.

Field observations targeted different clades of organisms depending on the time of day, to ensure data collection when said organism is most active or abundant based on prior research. For example, birding success is most prevalent in the morning (between dawn and 11 am typically), whereas reptiles and amphibians are more likely to appear at dusk or in later hours, most mammals are nocturnal, but others can during the day. There is a lot of variation between the behaviors of these clades and because they can appear anytime, all vertebrates recorded during an intended sampling excursion were noted too. Lastly, elevation was recorded using a GPS device; the highest camera trap set was near the top of Tilaran; No. 5 (1810m), and the lowest was in the creek intersection (circled in Figure 1.) at 1,452m.

RESULTS

Nothing of significance was found in the higher elevation camera trap

Table 1: Field Observations

Location	Species observed	Sampling activity	Elevation	Time of Day	Notes
Site 0	*Momotus lessonii*	Mammals	1475m	1:16 pm	Perched in the canopy
Station	*Sceloporus malachiticus*	Birding	1538m	10:19 am	Basking on wall next to cobwebs
Station	*Penelope purpurascens*	Break*	1538m	6:03 pm	Family with 3 chicks
Site 2	*Cyanolyca cucullata*	Birding	1530m	9:15 am	Perched
Station	*Patagioenas*	Birding	1538m	6:06 am	Nest building
Main road	*flavirostris* *Coragyps atratus*	Herping	1535m	12:29 pm	Soaring over main road

Table 2: Camera Trap Data

Camera Trap #	Species captured	Elevation	Time of Day	Notes
No. 3	*Puma concolor*	1452m	3:31 am	Disappeared into the abyss
No. 3	*Leopardus pardalis*	1452m	3:35 am	Drank on the creek
No. 1	*Canis latrans*	1489m	1:18 pm	Scavenged scraps
No. 3	*Nasua narica*	1452m	12:17 pm	Band foraged across
No. 1	*Dasyprocta punctata*	1489m	6:06 am	Scavenged scraps
No. 4	*Nasua narica*	1496m	9:24 am	Scavenged scraps
No. 1	*Cathartes aura*	1489m	5:29 pm	Scavenged scraps
No. 1	*Nasua narica*	1489m	9:40 am	In an alerted pose
No. 4	*Microsciurus alfari*	1496m	10:12 am	Followed trail past the bait

Figure 2. Camera trap No. 3 (left) Puma (*Puma concolor)*, and No. 1 (right) Coyote (*Canis latrans).*

Figure 3. Camera trap No. 3 (left) Ocelot *(Leopardus pardalis)*, and No. 1 (right) Agouti (*Dasyprocta punctata)*

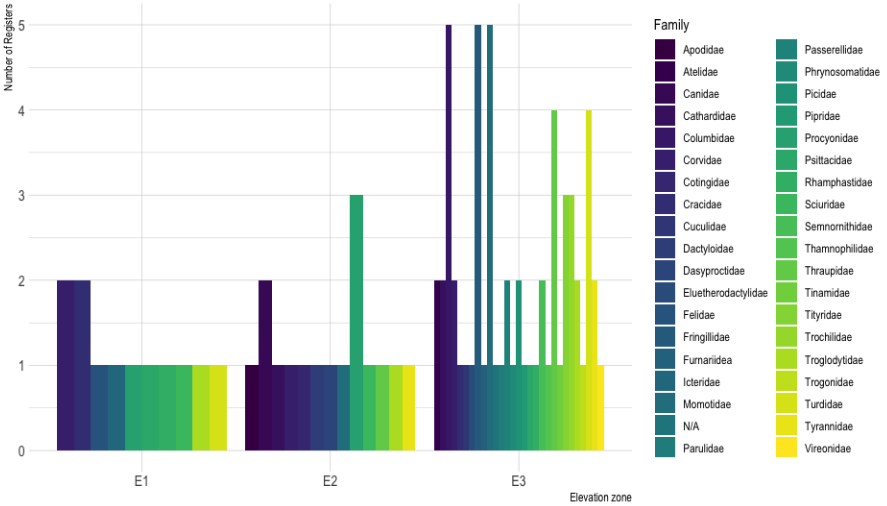

Figure 4. Registers by Family in relation to Elevation

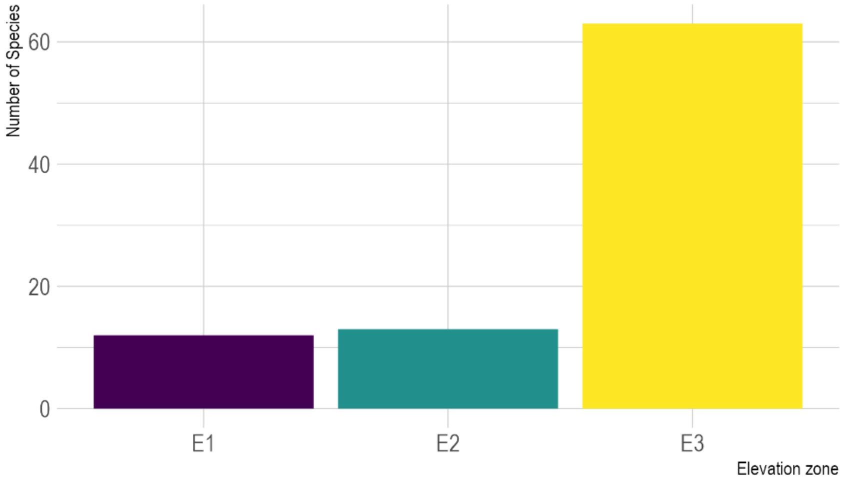

Figure 5. Species in relation to Elevation

MAMMALS

Puma concolor

The scarcity of the Jaguar (*Panthera onca*) has made this cat Monteverde's resident apex predator, anything smaller than it can be turned into a meal. These elusive predators cover a range stretching from southern Alaska, much of western North America (aside from an isolated population in the Florida Everglades) to the southern tip of Chile making it the most widespread large mammal in the New World (Culver, 2000). Areas where Jaguars are absent (typically the North) Pumas are larger which is the opposite going in lower latitudes like in the greater Amazon or Pantanal where the largest Jaguars exist. Exceptions in the South include certain areas in Patagonia, like in the Pampas region where jaguars are absent (Scognamillo, 2003).

Once thought to be uncommon until camera traps showed otherwise (Wainwright, 2007), the individual observed in this study was seen walking on the left side of the stream just a day after the Ocelot's appearance, perhaps following its scent as it disappeared into the abyss.

Leopardus pardalis

This medium sized felid is about twice the size of your typical housecat and is the third largest cat in the country after the Jaguar and the Puma. It's identified by its beautifully dappled coat making them masters of disguise in the underbrush. It is often called "Manigordo" for its enlarged forepaws, compared to its hind ones, and for all intents and purposes it's like a mini-jaguar able to stealthily take down unsuspecting macaws, snakes, iguanas, coatis, monkeys, fish, and crabs, to deer fawns, and young peccaries. (Wainwright, 2007).

Canis latrans

This smaller cousin of the grey wolf (*Canis lupus*) traces its origins back to the southwestern deserts of the United States, where it diverged from its larger cousin about 3.2 million years ago (Flores, 2016). At 7 – 21 kg they're comparable in size to a medium-sized dog. They're opportunistic scavengers and hunters having a wide-ranging diet comprised of carrion, rodents, amphibians, fish, birds, and even grasses, berries and other fruits. They've been known to take down animals as large as adult deer (generally targeting individuals in poor condition). Their cunning and adaptable nature has allowed them to feel at home in any urban environment just as much as in a more natural setting. For this reason, they're also notorious for feeding on people's pets, mainly cats and dogs if given the opportunity, this is especially an issue in large urban environments like in the cities Chicago and Los Angeles (Elliot, 2016). They also face persecution from farmers who regard them as vermin for eating their poultry. Despite the negative connotation that comes with them coyotes are necessary for keeping rodent populations in check. Deforestation has created corridors for this canid to penetrate southward, past the Panama Canal where it is beginning to colonize South America.

An interesting observation to note from this study is the appearance of a second coyote in the first camera trap site, most likely this is the mate of the first individual. Playful and scent marking behavior was observed upon review of the footage, as well as direct evidence of snake predation. The carcass of the dispatched reptile was seen thrown around, ripped apart and rubbed numerous times against their bodies.

Alouata palliata

This large New World monkey is black in color and gets its name from the golden streak of hair found on their flank regions. They live in troops of 10-20 individuals but groups of 40 and larger have been reported. The males of this species can get up to 10 kg and are identified by their larger size compared to females and visibly white scrotums. They are famous for possessing hyoid bones 25% larger than a simian of similar size, this adaptation amplifies their booming calls enabling them to produce some of the loudest recorded vocalizations in the animal kingdom (Wainwright, 2007). As the loudest mammals on earth, they can be heard from nearly 3 miles away and are often heard during the morning or when rainfall ensues. This howling behavior is used as a means of long-distance communication so that there's minimum energy expenditure to risk any kind of physical confrontation (Ceccarelli, 2021). They are also among the most lethargic of New World monkeys, given they feed almost exclusively on leaves (meaning their days are spent eating and sleeping trying to break down this nutrient-poor resource). Therefore, they will augment their dietary habits during the dry season by switching to fruits and seeds when leaves are in short supply (Espinosa-Gómez, 2013). Their diet of leaves and lack of needing huge home ranges makes them less prone to the effects of deforestation compared to other species.

Nasua narica

These highly opportunistic procyonids are diurnal for the most part and are among the most frequently seen mammals in Costa Rica. Their range extends from Arizona to northwestern Colombia near Panama's border. Weighing between 2 and 6 kg both sexes of this species operate separately, Males known as "Coatimundis" live a life of solitude hence the local term "Pizote solos" an alternative term for Lone wolf. Females on the other hand operate in bands of 15-20 individuals sometimes going as high as 40 or 50 which are comprised of adults and their young (including males below the age of 2). Adult males which are larger than females are notorious for committing infanticide, this pressure has made it advantageous for females to compensate in numbers which is usually enough to deter troublesome bachelors (Wainwright, 2007). They eat whatever they can fit in their mouths and will forage on the forest floor, in crevices, and dig up the ground with their claws for fruit, insects, lizards, worms, crabs, snakes, tarantulas, rodents, crocodile and bird eggs, to small mammals (Alves-Costa, 2004). They spend most of their time on the ground but they're also good climbers (but not to the proficiency of a monkey).

BIRDS

The Resplendent Quetzal *(Pharomachrus mocinno)* and the Three-wattled bellbird *(Procnias tricarunculatus)* are favorites among birders. They're both essential seed dispersers of wild avocados seeds and are iconic species that represent cloud forests.

REPTILES

Sceloporus malachiticus

This high elevation specialist is found in elevations of 600-3800m above sea level, choosing to live anywhere from pastures, coffee plantations, to premontane, montane, and subalpine forest (Leenders, 2019). It attributes its success to adaptations designated for mountain life such as the ability to darken its coloration and flatten its ribs to widen surface area in order absorb more solar radiation (Bueter, 2008).

AMPHIBIANS

No amphibians were visually sighted in this study, but they were heard in the lower elevation zones, among them was the Common dink frog *(Diasporus diastemata),* Slim-fingered rain frog *(Craugastor crassidigitus),* and Glass frogs (Centrolenidae).

DISCUSSION

As expected, birds were the most numerous groups of vertebrates recorded by a significant margin, although many of these observations were made at the biological station meaning there is a sampling bias.

Camera traps 2 and 5 didn't yield any vertebrates perhaps due to increased foot traffic in both (more so in the former), choosing spots for these cameras that don't experience as much human activity and leaving them out for longer would have been better for sampling. Box plots couldn't be made due to a lack of more precise sampling (2 camera traps turned up with no vertebrates plus the sampling bias). There was not enough repetition done in the designated sites, hence, repetition over a longer timeframe would have been beneficial for this study.

Despite the shortcomings, this still supports the notion that vertebrates are found in higher concentrations at lower elevations, however more time for sampling is required to solidify these results. Abundant vertebrate concentrations are a good indicator of a highly productive cloud forest ecosystem, and thriving biodiversity is essential for Monteverde's economic growth and health of the ecosystem for generations to come.

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ACOUSTIC ECOLOGY

The effect of human activity and elevation on different soundscapes of the premontane cloud forest of Monteverde, Costa Rica

Ethan Forbes
Department of Ecology & Environment Biology, Purdue University, Indiana, USA

ABSTRACT

As neotropical areas become more urbanized, biodiversity of the environment drops due to many factors, one of which being sound. In this paper, I focus on the growing region of Monteverde, Costa Rica to determine if human activity or elevation is affecting the soundscapes in the region. The areas picked were near The Monteverde Biological Station, with two primary forest sites, and two human sites. The elevation of these areas ranged from 1500m to 1850m. Soundscapes were quantified using the Normalized Difference Soundscape Index (NDSI), Acoustic Complexity Index (ACI), Acoustic Diversity Index (ADI), Acoustic Evenness Index (AEI), and the Bioacoustic Index (BI). Significant differences were found in the soundscape of environments due to elevation and human activity, with the most prominent at the highest elevation. Wind is believed to be the largest contributing factor to the differences found, but human activity still did have a significant impact. From these results, it is clear that soundscapes in primary forests can change drastically. Further research should be done to determine what other factors may influence acoustic diversity and if there are actions that can be taken to increase it.

RESUMEN

Las zonas neotropicales se urbanizan más cada año y la biodiversidad del entorno disminuye a causa de muchos factores, incluyendo el sonido. En este estudio, me centro en la región de Monteverde, Costa Rica para determiner si la actividad humana o la elevación están afectando a los paisajes sonoros. Las zonas elegidas, cerca de la Estación Biológica Monteverde, fueron dos zonas en el bosque primario, y dos zonas antropizadas. La elevación de las zonas varió de 1500m a 1850m. Los paisajes sonoros se cuantificaron mediante el índice de diferencia normalizada del paisaje sonoro (NDSI), el índice de complejidad acústica (ACI), el índice de diversidad acústica (ADI), el índice de uniformidad acústica (AEI) y el indice bioacústico (BI). Se encontraron diferencias significativas en el paisaje sonoro de los entornos, debido a la altitud y a la actividad humana, particularmente en la elevación más alta. Se considera que el viento fue el factor principal de las diferencias, pero hubo diferencias significativas considerando sonidos biológicos.. La actividad humana, la elevación y otros factores provocaron esta diferencia. Es evidente que los paisajes sonoros en los bosques primarios pueden cambiar mucho, incluso en las zonas cercanas. Es necesario investigar más para encontrar otros factores que influyan en la diversidad acústica, y qué acciones pueden incrementarla.

INTRODUCTION

Currently, primary forests across the globe are experiencing habitat and biodiversity loss due to anthropogenic effects. These effects mainly consist of deforestation, fragmentation, introduction of invasive species, and the overexploitation of resources, and are especially apparent in tropical forests (Morris, 2010). Of all the factors influencing this loss, one of the most overlooked is sound and the role it plays. Increases in noise, especially from anthropogenic sources, have been shown to be a threat for many native species in an area (Sordello et al., 2020). These negative effects of sound pollution may also impact different environments within a forest.

The study of soundscapes, and impacts on sounds, is called ecoacoustics. All sounds in an ecoacoustics recording can be broken down into four major categories, geophonies (sounds created from the friction of particles), biophonies (sounds produced by specialized organs of animals), anthropophonies (the sound of human voice), and technophonies (sounds from static or moving machinery) (Farina & Li, 2021). These types of sounds can be measured and analyzed in a variety of ways, of which one is via acoustic indices. These indices include the Normalized Difference Soundscape Index (NDSI) to measure anthropogenic disturbances (Allen-Ankins et al., 2023), the Acoustic Complexity Index (ACI) to determine the amount of noise in an environment (Pieretti et al., 2011), the Acoustic Diversity and Acoustic Evenness Indices (ADI/AEI) for measuring how similar or different all the sounds are (Villanueva-Rivera et al., 2011), and the Bioacoustic Index (BI) for looking specifically at biophonies (Bradfer-Lawrence et al., 2019). All these indices together can give a great perspective about the make-up of any soundscape.

Previous studies have looked at the relationship between noise pollution and biodiversity, and many found that there is a negative correlation. One study from Costa Rica, analyzed the sounds of traffic and how they impacted bird species abundance. It was found that in areas with heavy noise pollution from roadways, bird biodiversity had declined (Arévalo & Newhard, 2011). Another study in Brazil found that marmosets would change their calling patterns in the presence of loud technophonies like mining (Bittencourt et al., 2023). These studies show that biophonies and species abundance can change in the presence of major noise pollution. It could be inferred that this change in species acoustics and abundance could be occurring in other anthropogenic areas, thus changing those soundscapes.

Along with anthropophonies and technophonies causing major changes in species bioacoustics, so too does elevation. A study from southern China found that with an increase in elevation, biodiversity decreases; resulting in the decrease of acoustic diversity (ADI) and the increase of acoustic evenness (AEI)(Chen et al., 2021). The change in these indices represents a decrease in biophonies and biodiversity. However, another study in southwest China found no relationship between elevation and any acoustic index (He et al., 2022). It remains to be seen if how soundscapes and the acoustic indices are affected by elevation in tropical forest.

The mountainous region of Monteverde, Costa Rica, encompasses more than ten thousand hectares of tropical forest and a great biodiversity. With a small town of approximately 6000 people, the major anthropogenic activities in the area are agriculture and tourism. Despite being a rather small anthropized area, the sound caused by these actions may inadvertently be impacting the environment in unknown ways, possibly reducing the overall biodiversity. Furthermore, differences in elevation may also play a role in the soundscape of the area.

In this study, I aim to determine if anthropophonies, technophonies, or elevation significantly impact the soundscape and overall biodiversity of the region. I predict that there will be a decrease in acoustic diversity near human areas, and that with an increase in elevation, the acoustic diversity will also decrease.

MATERIALS AND METHODS

Sampling Sites

Four sampling sites were chosen for sound recording. The sites were picked based on both elevation and whether they were primary forest of human areas (Fig. 1). The four sites were the Human High Elevation (HHE), Human Low Elevation (HLE), Primary Forest High Elevation (PFHE) and Primary Forest Low Elevation (PFLE). The primary forest sites were chosen as areas with less understory coverage so sounds could be properly collected without distortion from objects. The canopy covers were measured for the primary forest sites at both elevations. All sites are classified as premontane moist forests via Holdridge life zone classifications. For the human areas, the audio devices were placed at infrastructures (building and tower) facing towards the forest to record the surrounding environmental sounds. Canopy cover was not recorded for HLE or HHE.

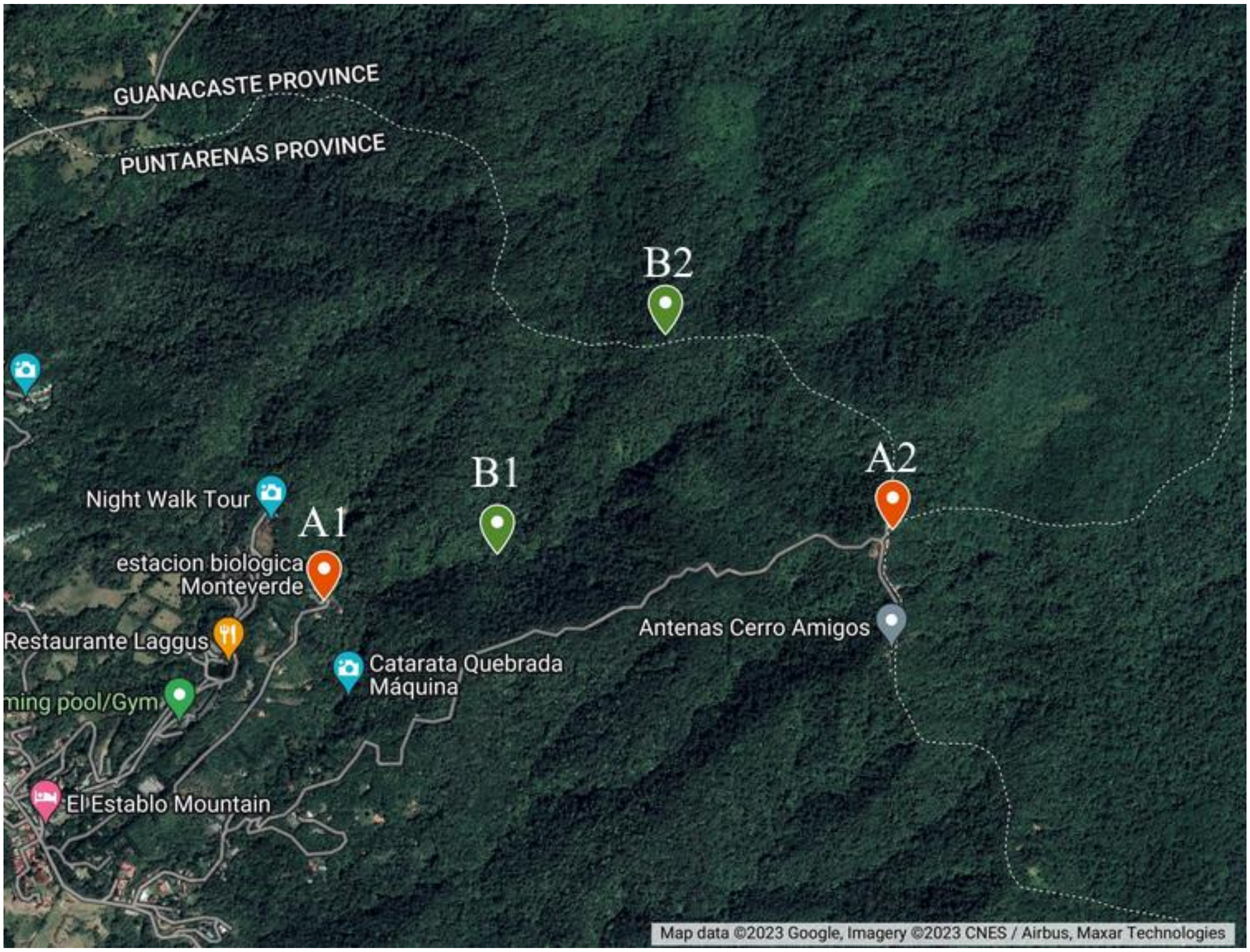

Fig. 1. Map of the four acoustic sampling sites with: (A1) the human low elevation (1491m), (A2) the human high elevation (1843m), (B1) the primary forest low elevation (1491m), and (B2) the primary forest high elevation (1732m).

Audio Data Collection

Sound recordings were collected using two AudioMoth devices. Both devices were programmed with a sampling rate of 48kHz, medium gain, and no filtering. These AudioMoths were set to record 60 seconds every 30 minutes in two periods, once from 11:00 to 16:00 UTC (5:00 to 10:00 CST) and again from 23:00 to 4:00 UTC (17:00 to 22:00 CST). This procedure resulted in 20 audio files each day. Each site was sampled for two days, and all sites were sampled over a period of five days.

Acoustic Indices

The audio files collected were analyzed via the Kaleidoscope Pro Analysis Software by Wildlife Acoustics. Each day of recordings were batch processed, and the data from the Normalized Difference Soundscape Index (NDSI), Acoustic Complexity Index (ACI), Acoustic Diversity Index (ADI), Acoustic Evenness Index (AEI), and Bioacoustic Index (BI) were gathered. NDSI had a frequency range for anthropophonies from 1000 to 2000Hz, and a range of biophonies from 2000 to 24000Hz, ACI and BI frequency ranges were 2000Hz to 24000Hz, and ADI and AEI were both from 0Hz to 24000Hz, with steps of 1000Hz.

Statistical Analysis

Analysis on the relationship between the four sampling sites based on the acoustic indices was conducted in R using the ggstatplot package (Patil, 2021). A Kruskal-Wallis test was run followed by a Dunn-Test for pairwise comparisons, and boxplots were created for each acoustic index in order to determine any significant differences.

RESULTS

Normalized Difference Soundscape Index

Using a Kruskal-Wallis test, it was found that the difference in NDSI between environments was statistically significant (chi-squared = 92.298, df = 3, p-value < 0.001). The human high elevation environment was found to be statistically different from all three other environments (Fig. 2A). The acoustic profile for this index is shown in Figure 2B.

Acoustic Complexity Index

Using a Kruskal-Wallis test, it was found that the difference in ACI between environments was statistically significant (chi-squared = 70.16, df = 3, p-value < 0.001). The human high elevation environment was found to be statistically different from the human low elevation and primary forest low elevation environments. The primary forest low elevation environment was statistically different from all other environments (Fig. 3A). The acoustic profile for this index is shown in Figure 3B.

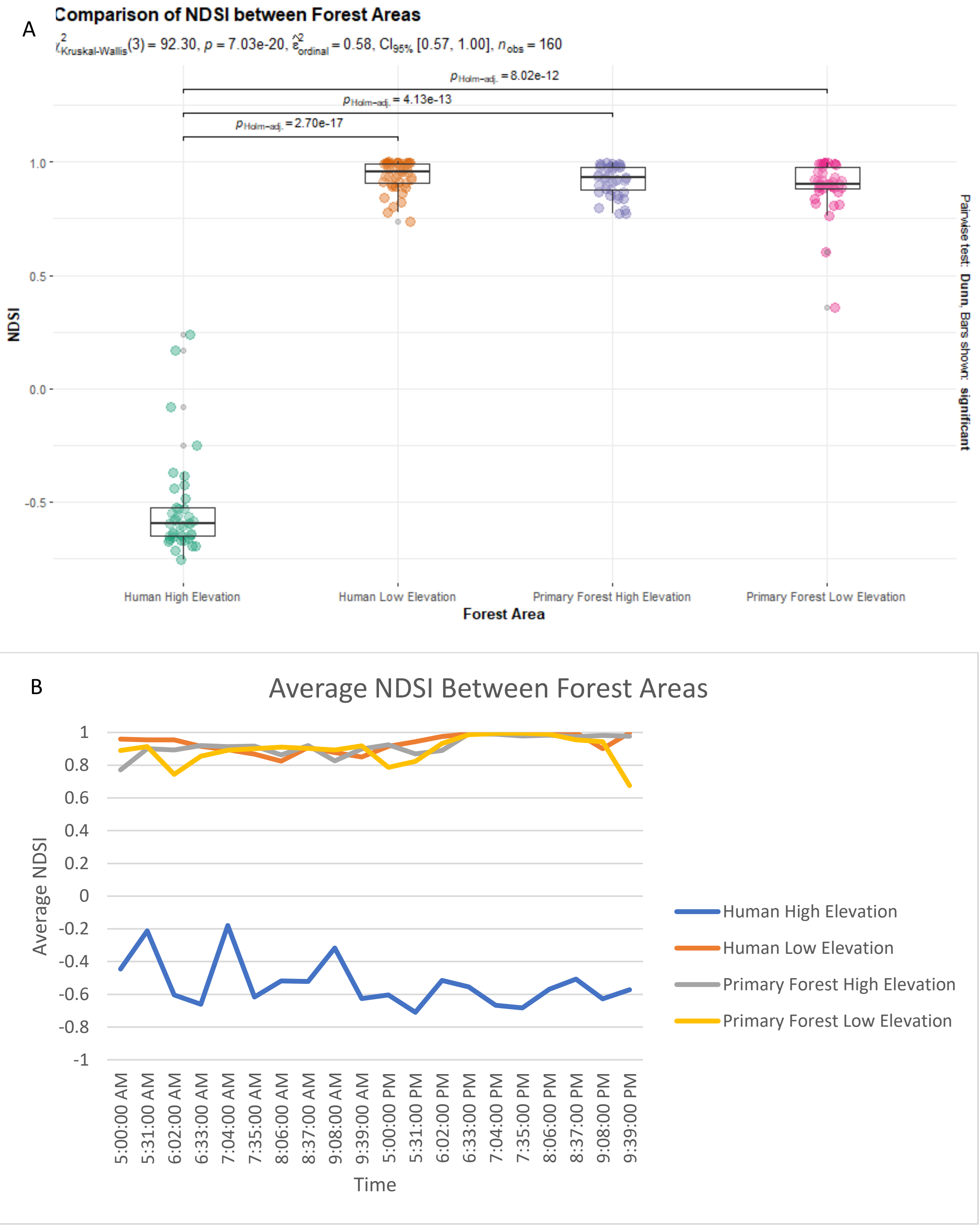

Fig. 2. A) The four environments and their relative statistical difference of NDSI expressed as a box plot. The statistics of the entire model are at the top and the p-value of each comparison is on the graph. B) The average NDSI of each environment during the sampling periods.

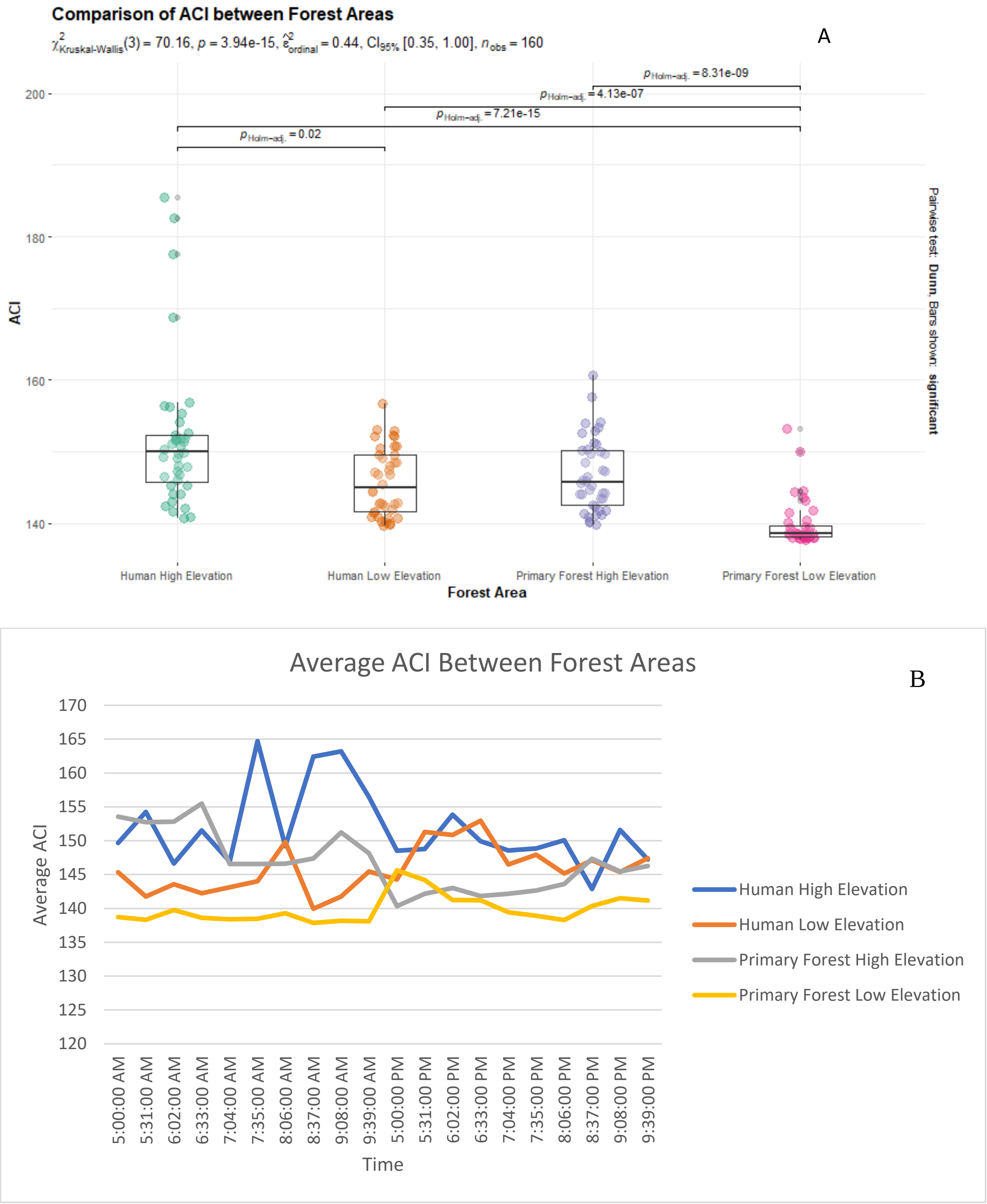

Fig. 3. A) The four environments and their relative statistical difference of ACI expressed as a box plot. The statistics of the entire model are at the top and the p-value of each comparison is on the graph. B) The average ACI of each environment during the sampling periods.

Acoustic Diversity Index

Using a Kruskal-Wallis test, it was found that the difference in ADI between environments was statistically significant (chi-squared = 102.3, df = 3, p-value < 0.001). The human high elevation environment was found to be statistically different from all other environments. The primary forest low elevation environment was statistically different from all environments as well (Fig. 4A). The acoustic profile for this index is shown in Figure 4B.

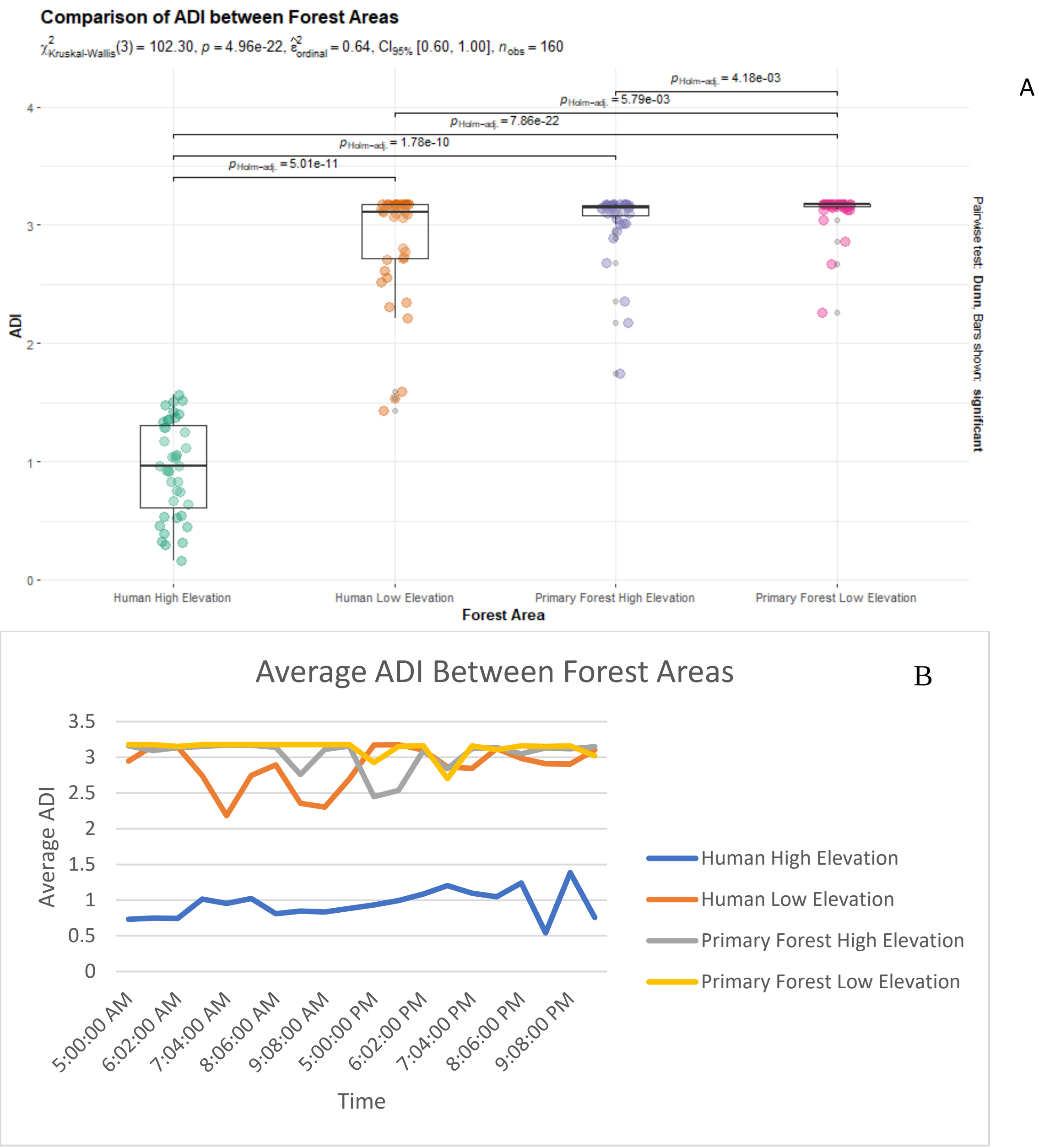

Fig. 4. A; The four environments and their relative statistical difference of ADI expressed as a box plot. The statistics of the entire model are at the top and the p-value of each comparison is on the graph. B; The average ADI of each environment during the sampling periods.

Acoustic Evenness Index

Using a Kruskal-Wallis test, it was found that the difference in AEI between environments was statistically significant (chi-squared = 102.19, df = 3, p-value < 0.001). The human high elevation environment was found to be statistically different from all other environments. The primary forest low elevation environment was statistically different from all other environments as well (Fig. 5A). The acoustic profile for this index is shown in Figure 5B.

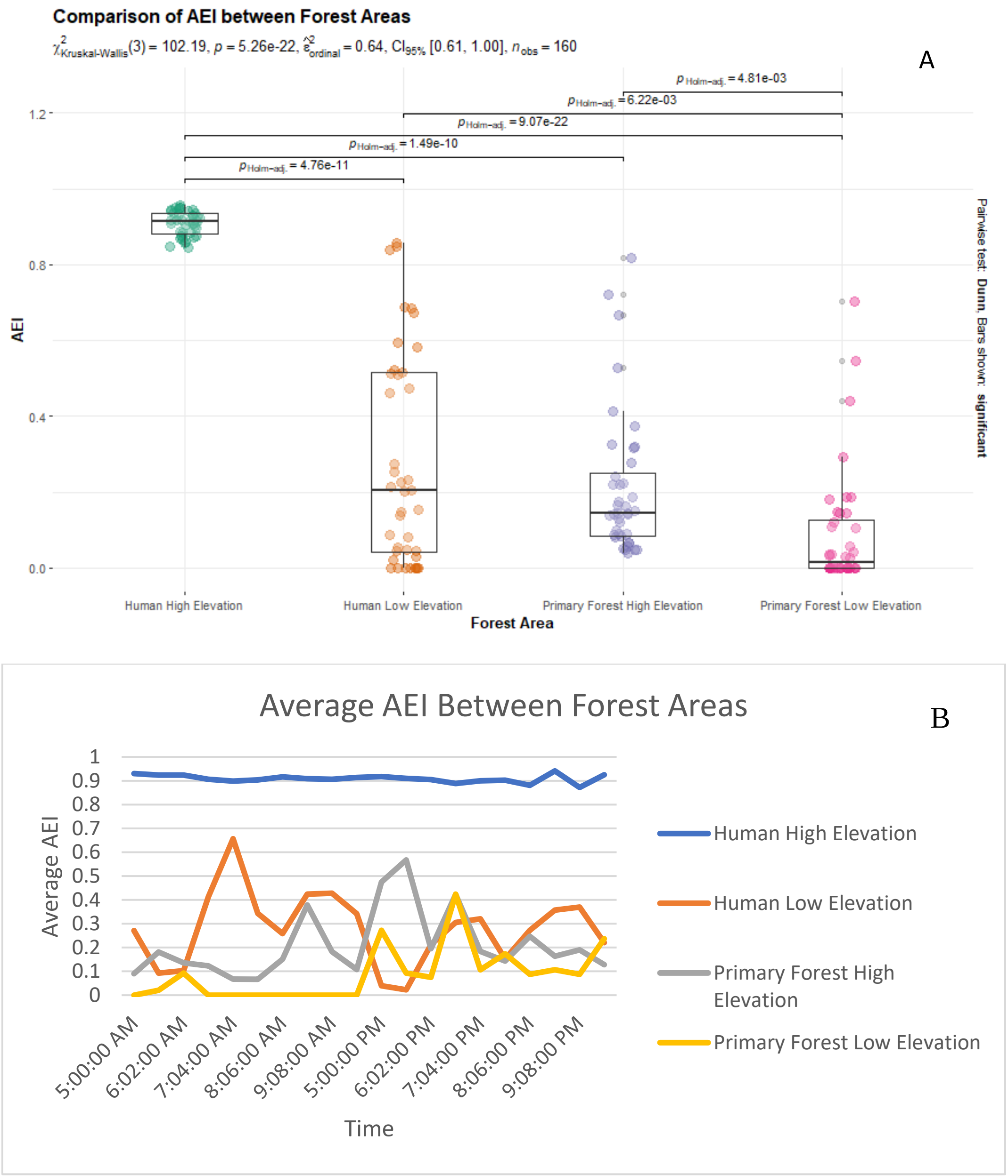

Fig. 5. A) The four environments and their relative statistical difference of AEI expressed as a box plot. The statistics of the entire model are at the top and the p-value of each comparison is on the graph. B) The average AEI of each environment during the sampling periods.

Bioacoustic Index

Using a Kruskal-Wallis test, it was found that the difference in BI between environments was statistically significant (chi-squared = 51.963, df = 3, p-value < 0.001). The human high elevation environment was found to be statistically different from the human low elevation and primary forest high elevation environments. The human low elevation environment was statistically different from all other environments. The primary forest low elevation environment was statistically different from all other environments as well (Fig. 6A). The acoustic profile for this index is shown in Figure 6B.

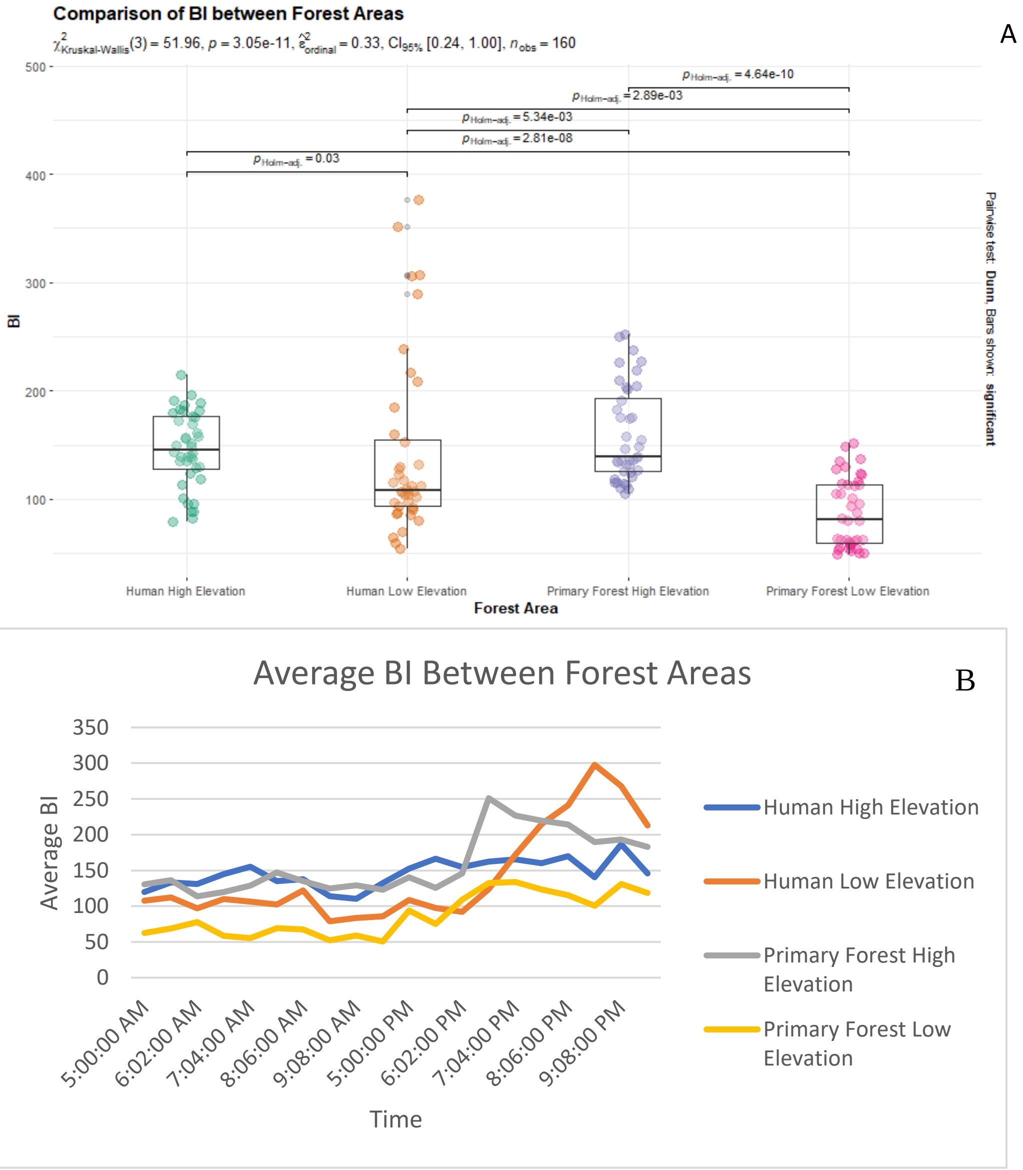

Fig. 6. A) The four environments and their relative statistical difference of BI expressed as a box plot. The statistics of the entire model are at the top and the p-value of each comparison is on the graph. B) The average BI of each environment during the sampling periods.

DISCUSSION

Overall Findings

All models were found to have significant differences between the environments. The environment that was most different in every category was HHE. As can be seen from the boxplots for each acoustic index (Fig. 2,4,5), it was often noticeably higher or lower in score from the others. This wind noise is what may have possibly influenced the significant data found from biophonic, with the wind affecting the vocalizations of the animals, as supported by (Bittencourt et al., 2023). Furthermore, the acoustic data of each environment, while looking similar on the graphs, are often significantly different from each other. This means that there is something in the soundscape leading to these differences, that could be influenced by elevation or human presence.

NDSI

The four environments were found to be significantly different when compared using NDSI data (Fig. 2). The most apparent difference is that between HHE and the others. HHE has a very low score, around -0.6, while the other environments have NDSI score near 1. When analyzing NDSI, a score near 1 indicates an environment dominated by biophonies, while -1 is one dominated by anthropophonies or technophonies. While this may lead one to believe that HHE has a large presence of anthropophonics or technophonic noise, it is likely not the case. HHE having this score is likely due to the massive amount of geophonic noise in the form of wind present in the recordings. This noise dominates the frequencies below 2000Hz and is likely what caused it to score so low for NDSI. In this section, all other environments were found to not be significantly different, meaning anthropogenic noise does not affect these areas as much. This is interesting as it was thought that HLE would have a score similar to HHE since there is human activity there, but this is not the case.

ACI

When comparing the four environments using ACI, there is a statistically significant difference between them (Fig. 3). However, there is no environment that stands out as being extremely different from the rest. This is because ACI looks at frequencies from 2000Hz to 24000Hz, so all anthropogenic noise is gone, and only biophonies remain. These differences indicate that the biophonic activity at these sites is not the same. This could be due to unique animals vocalizations at these sites, or that there was decreased or increased vocalizations.

ADI

For ADI, the model was found to have statistically significant differences between the four environments (Fig. 4). The major outlier is HHE, likely due to the large amount of wind noise. ADI calculates the acoustic diversity of an area, so since there is a massive amount of wind noise all the time, the score is much lower than the others. This model shows that for all other environments, they are quite diverse in their soundscapes. The fact that wind, a geophony, caused a reduction in diversity is supported by another study in Costa Rica. This study looked at roadways and found that bird acoustic diversity was lower near them (Arévalo & Newhard, 2011). These differences indicate that these sites have unique sounds and noises that are not present in the others. This could be influenced by elevation, with unique vocalizations from animals at specific elevations, or of unique noises from anthropogenic sites that would not be heard in a forest.

AEI

AEI is the opposite of ADI. Instead of looking at the diversity, it is analyzing how even a soundscape is. In this case, the model was statistically significantly different, and HHE was the most apparent difference. This indicates that these soundscapes have difference in the similarity of the sounds produced. HLE was also interesting as it had a very large box on the box plot, indicating that the evenness of the area varied throughout the day (Fig. 5. A.). This can be seen on the line graph with large peaks in the morning, likely from anthropogenic noise when waking up (Fig. 5. B.). For HHE, its score was significantly higher than the other environments, and did not have a very large range of AEI values (Fig. 5. B.). This, again, is due to the wind noise. Since it is constant, HHE got very high evenness scores. These findings contradict a study in China that found that AEI did not change due to elevation (He et al., 2022). The change found here may be due to this project occurring in a different region and at a smaller change in elevation.

BI

When looking at BI scores, they have a similar distribution to the ACI scores (Fig. 6 & Fig. 3). This is due to the parameters of the test looking at values from 2000Hz to 24000Hz, eliminating anthropophonies and technophonies. Furthermore, the model found statistically significant differences between the environments. This could indicate differences in intensity, or biophonies measured for these soundscapes. This change could be due to elevation with different species present at different altitudes, or that some species only appear in anthropogenic areas.

In a Broader Context

After looking at all acoustic index models, it is clear that there are significant differences in soundscapes between the four environments. The most different environment was HHE. This area was greatly impacted by the constant noise of wind, which influenced its scores for NDSI, ADI, and AEI. However, when comparing environments using ACI and BI, HHE was not statistically different from some environments. This means that while the wind did change the overall soundscape, it did not influence biophonies very much. From all the acoustic data, different environments in a forest will influence the soundscape of that area. From my analysis, it cannot be determined if this difference is a result of anthropophonies or technophonies because while the human environments differed from the primary forest for some indices, they were also similar at points. A conclusion that can be drawn though is that wind greatly affects how acoustic indices are calculated. Wind led to HHE being significantly different from other environments, but when removed for analyses such as ACI and BI, the environments appeared more similar.

Elevation also played a big role in the make-up of the soundscapes I tested. Wind greatly influenced the data for HHE which can be seen by the large difference in NDSI, ADI, and AEI. Therefore, it can be concluded that elevation did play a role in the differences observed. This is further supported by previous research in China which looked at differences in elevation and how it affected the acoustic indices. It was found that with increases in elevation, ADI would decrease, and AEI would increase. These results were also confirmed in my project as higher elevation environments has lower ADI values and greater AEI values (Figs. 4 & 5) (Chen et al., 2021).

Furthermore, it can be said that even in a small area like Monteverde, soundscapes can drastically differ not only between elevation and human areas, but also within their category. For future studies, sampling of these same areas could result in different acoustic data than collected in this study. More robust studies could also increase the number of samples taken in order to get better data. Furthermore, sampling of each environment all throughout the day could give insight into time periods where acoustic indices are peaking or dropping. Other changes that could be made to this project would be to put the HHE AudioMoth closer to the ground. While it is good practice to put them high in order to not have reflecting sounds, the wind contributed to most of the noise recorded and skewed the results. By having closer to the ground, it would record less geophonies and more biophonies and possibly change the data. Furthermore, looking specifically at different Holdridge life zones in Monteverde and comparing their soundscapes would be interesting. Either way, these data confirmed that there is a difference of soundscapes due to elevation and human activity. Further studies could determine if there are other factors that influence soundscapes, and the direction of the influences. It would be interesting to see how the soundscapes differ in the dry season, when wind is much more common. There are many other factors that could be considered such as how soundscapes change throughout the year, or with weather, or with more robust human activity or elevation changes. These are all ecoacoustics projects that would be incredibly insightful for the conservation and sustainable development of Neotropical areas like Monteverde.

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Acoustic Indices of the Dusk Chorus Acoustic Communities Near Waterfalls in a Tropical Premontane Wet Forest in Monteverde, Costa Rica

David Graber
Department of Biology, University of Southern Indiana, Indiana, USA

ABSTRACT

Acoustic communities are the accumulation of all species in soundscape producing sounds that vary spatially and temporally. The dusk chorus is one of the two heightened periods of sound production during the daylight cycle. Sounds from flowing water, such as those from waterfalls, are significant sources of geophonies that effect acoustic habitats. In a cloud forest of Monteverde, Costa Rica, the impacts of different levels of waterfall geophonies and the proximity to the noise source on acoustic communities were quantified for the first time using the Acoustic Complexity Index (ACI), the Acoustic Diversity Index (ADI), and the Acoustic Evenness Index (AEI). ACI scores of the acoustic communities were found to increase with increasing distance from the waterfall pool. ADI scores were found to vary among treatment groups, but no greater trend was reported. Treatments closer to the source of waterfall geophonies were found to have greater acoustic evenness(lower AEI scores). The amount of biophonies produced by acoustic communities decreased in acoustic habitats closer to waterfalls. More study into the effects of flowing water geophonies on temporal and spatial scales are necessary for the future of waterfall ecoacoustics.

RESUMEN

Las comunidades acústicas son la acumulación de todas las especies en un hábitat acústico que produce sonidos que varían espacialmente y temporalmente. El coro del anochecer es uno de los dos periodos de mayor producción de sonidos durante el ciclo diurno. Los sonidos del agua que fluye, como los de las cataratas, son fuentes importantes de geofonías que afectan a los hábitats acústicos. En un bosque nuboso de Monteverde, Costa Rica, el impacto de las geofonías de las cataratas en las comunidades acústicas con tratamientos de distintos niveles de geofonías se cuantificó por primera vez utilizando el Índice de Complejidad Acústica, el Índice de Diversidad Acústica y el Índice de Uniformidad Acústica. Se observó que las calificaciones del Índice de Complejidad Acústica de las comunidades acústicas aumentaron al aumentar la distancia desde la piscina natural de la catarata. Se observó que las calificaciones del Índice de Diversidad Acústica variaron entre los grupos de tratamiento, pero no se detectó una tendencia pronunciada. Los tratamientos más cercanos a la fuente de las geofonías de las cataratas presentaban una mayor uniformidad acústica (puntuaciones AEI más bajas). La cantidad de biofonías producidas por las comunidades acústicas disminuyó en los hábitats acústicos más cercanos a las cascadas. Son necesarios más estudios sobre los efectos de las geofonías de las aguas corrientes en escalas temporales y espaciales para el futuro de la ecoacústica de las cataratas.

INTRODUCTION

Acoustic communities are the assemblages of species in an environment that are producing sounds and make up a component of the larger soundscape. The soundscape includes not just the biophonies of the acoustic communities, but also the geophonies (sounds from geophysical sources), and technophonies (sounds that are from anthropogenic sources) that can vary spatially within a terrestrial or aquatic environment (Farina & James, 2016). These acoustic communities are known to change over a temporal scale even within the same location with a partitioning of vocalizations by different species at different times throughout the daylight cycle (Farina et al., 2021). Within this temporal partitioning are two periods of increased sound production activity known as the dawn chorus and the dusk chorus (Leopold & Eynon, 1961; Fuller et al., 2016) Anurans and birds are known to be important contributors to acoustic communities with an increase of vocalizations during the dusk chorus (Baker, 2004; Alvarez-Berríos et al., 2016; Lapp et al., 2021).

Geophonies also contribute to the larger soundscape that acoustic communities are located in and include sounds produced by geophysical sources such as wind, rain, earthquakes and waterfalls. Flowing water from streams and waterfalls can provide of source of near constant geophonies lower than 1 kHz but can also have significant sound production above 1 kHz (Brumm & SlabbeKoorn, 2005). In some soundscapes, geophonies from flowing water can be the largest contributor to sounds along mountain stream acoustic habitats (Krause et al., 2011) having a great effect on the acoustic habitats and the acoustic communities present.

Some species of anurans and birds that live near streams and waterfalls have been known to adopt acoustic communication strategies in the presence of flowing water geophonies (Brumm & Slater, 2006; Röhr et al., 2016; Grafe & Tony, 2017). Although flowing water geophonies likely degrade acoustic habitats for acoustic communities by inhibiting acoustic communication, this has not been previously studied. Here, a first attempt was made to describe and quantify the impacts that geophonies from waterfalls have on acoustic communities of a tropical premontane wet forest of Monteverde, Costa Rica. The Acoustic Complexity Index (Pieretti et al., 2011) was used to test the amount of acoustic information from the biophonies within the acoustic communities. The Acoustic Diversity Index (Pijanowski et al., 2011) and Acoustic Evenness Index (Pijanowski et al., 2011) were used to determine the diversity of frequencies present in the soundscape and the evenness of the distribution of these frequencies. A special focus was placed on the temporal period of the dusk chorus for acoustic communities near waterfalls. The effects of the waterfall geophonies sound level were also tested at varying distances. Acoustic communities closer to waterfalls were predicted to have lower acoustic complexities, lower acoustic diversity, and more even distribution of frequencies as a result of the impacts of waterfall geophonies.

MATERIALS AND METHODS

Study site

The study was conducted at the Monteverde Biological Station (10.31892, -84.80842) within the tropical premontane wet primary forests of Monteverde, Costa Rica. The audio monitors were set up at the pools of two waterfalls along the Quebrada Máquina stream system. The waterfall sites (Fig. 1) were selected based on the level of noise from the flowing water and were classified as high background noise treatment site (10.31645, -84.80800), and low background noise treatment site (10.31916, -84.80569).

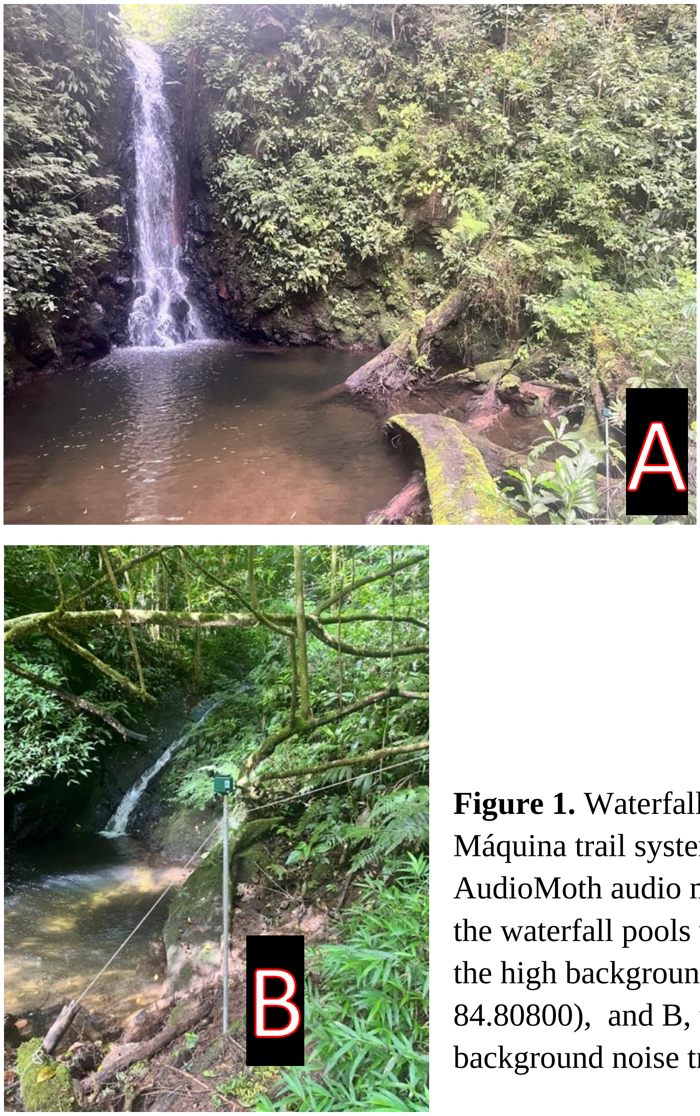


Figure 1. Waterfall pool recording sites along the Quebrada Máquina trail system in Monteverde, Costa Rica. The AudioMoth audio monitors are set at the 0 m distance from the waterfall pools with A, the location of the AudioMoth at the high background noise treatment group site (10.31645, -84.80800), and B, the location of the AudioMoth at the low background noise treatment group site (10.31916, -84.80569).

Recording specifications

Passive recording was conducted using two AudioMoth 1.2.0 audio monitors in IPX7 Waterproof Cases (Open Acoustic Devices). The recordings were done with a sampling rate of 16 kHz as prominent vocalizations of birds and anurans are commonly under 8 kHz (Villanueva-

Rivera et al. 2011). Recording samples were conducted in intervals of 1 minute with 5 seconds between each minute interval. To record the temporal period of the dusk chorus, recording started approximately 30 minutes before the sun set each night from 23:30 until 3:30 UTC. The samples were taken over the course of 6 nights from July 18 to July 23, 2023, during the wet season. The audio monitors were set up from varying distances of the two pools of the waterfalls: 0 m (**Fig. 1**), 10 m and 20 m downstream. This resulted in six different treatments: three acoustic habitats with low background noise and three acoustic habitats with high background noise. The audio monitors were allowed to record two dusk chorus periods before they were moved to the next distance from the waterfall pool. As a result, there were 222 recordings produced per night at each of the two treatment sites.

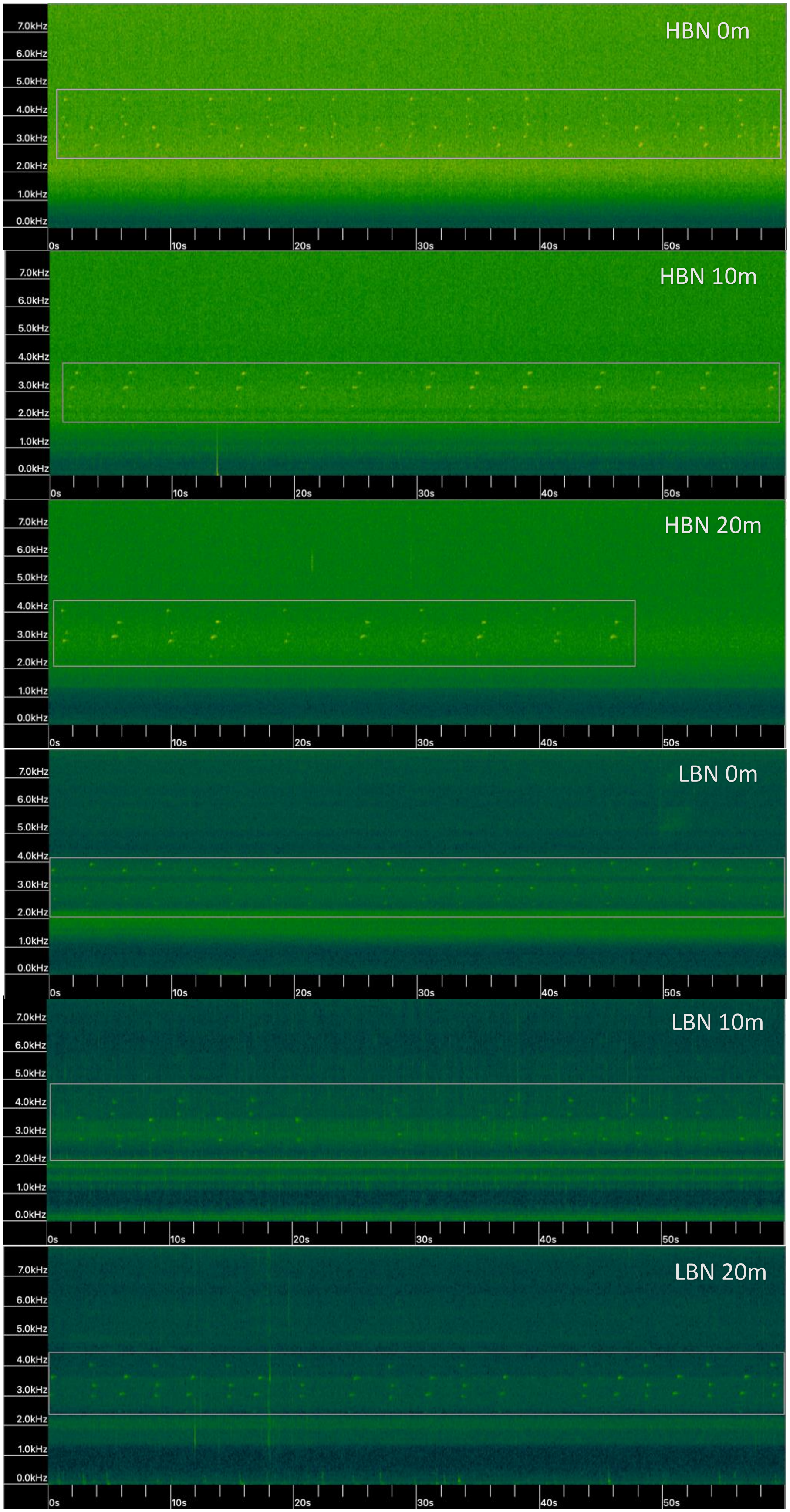
Recording sample analysis

Using Kaleidoscope Pro software version 5.6.1 (Wildlife Acoustics), sound pressure level analysis was conducted on each of the recording samples using the A Weighted band and 94.0 dB adjustment to determine the average, minimum and maximum sound pressure level of each of the 6 treatments during the two dusk choruses. Kaleidoscope Pro software was also used to determine the Acoustic Complexity Index (Pieretti et al., 2011), the Acoustic Diversity Index (Pijanowski et al., 2011), and the Acoustic Evenness Index (Pijanowski et al., 2011), of each of the recording samples. The average scores of all three indices for each of the 222 total 1-minute intervals were calculated across the two dusk chorus sample periods for each of the 6 treatments. To assess the differences between treatments a Kruskal-Wallis H test (Kruskal and Wallis, 1952) was completed using the average values per time interval for each of the three acoustic indices. Then, a Dunn test (Dunn, 1964) was used to make non-parametric pairwise comparisons between each of the three distances within a treatment group (high and low background noise), and across the treatment groups comparing the same distances from the waterfall pool (e.g. 10m high background noise vs 10m low background noise). The P values from the Dunn test were then adjusted using the Bonferroni method (Dunn, 1961).

RESULTS

The sound pressure level average values of the high background noise during the dusk chorus at 0 m, 10 m, and 20 m from the waterfall pool were: 78.20 dB (minimum 76.20 dB, maximum 80.00 dB), 73.85 dB (min. 70.90 dB, max. 78.00 dB) and 69.09 dB (min. 63.80 dB, max. 78.00 dB), respectively. The sound pressure analysis results for the dusk chorus in the low background noise treatment group at distances of 0 m, 10 m, and 20 m were: 57.14 dB (min. 54.2 dB, max. 71.9 dB), 55.94 dB (min. 51.3 dB, max. 75.2 dB) and 54.03 dB (min. 47.5 dB, max. 80.3 dB), respectively. These differences in sound pressure level across treatments were notable when the recording samples were visualized as spectrograms through Kaleidoscope Pro and biophonies remain at the relatively same intensity in comparison to differing levels of waterfall geophonies across the treatments which were found to go across the entire sample range (up to 8 kHz) (**Fig. 2**).

Figure 2. Spectrograms of each treatment exhibiting biophonies of *Catharus fuscater* (Passeriformes, Turdidae). Each spectrogram displays a 60 second recording from each treatment group with the same default spectrogram viewing settings of Kaleidoscope software (higher intensities of sound are indicated in a lighter green than those of low intensity). *Catharus fuscater* vocalizations are outlined on each spectrogram in gray.



The Acoustic Complexity Index (ACI) (**Fig. 3**) was found to vary significantly across the six treatments by the Kruskal-Wallis H test (P < 0.001), as well as all pairwise comparisons conducted with the Dunn test (P < 0.001) (**Table 1**). ACI scores were found to increase at greater distance from the waterfall pool. Moreover, ACI scores were found to vary temporally over the duration of the dusk chorus (**Fig. 4**). For low and high background noise there was a peak around the 50 minute interval at 20 m distance , and around the 70 and 140 minute intervals of the 10 m distance treatments. The 0 m distance treatment had fewer, smaller peaks in ACI scores with one major peak the 210 minute interval of the low background noise level treatment.

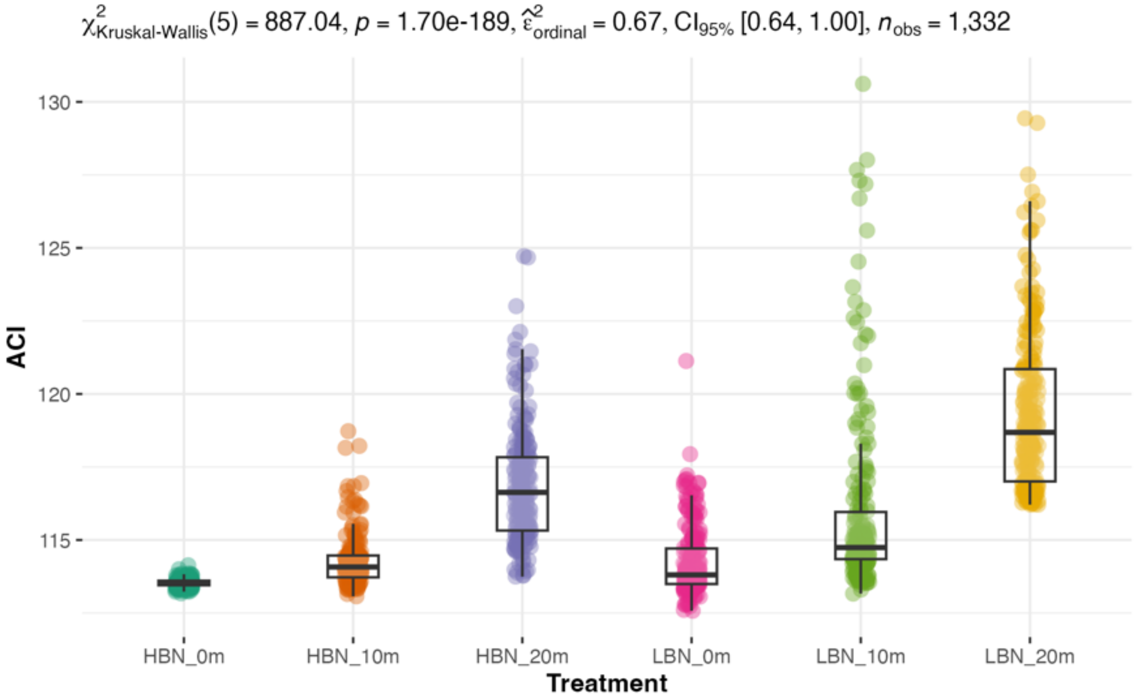


Figure 3. The Acoustic Complexity Index of the dusk chorus acoustic communities in terrestrial acoustic habitats at varying distances from sources of waterfall geophonies in a premontane wet forest in Monteverde, Costa Rica. Each distance from the waterfall pool for both the high background noise (HBN) treatment of 0 m (turquoise), 10 m (orange) and 20 m (purple) and low background noise (LBN) treatment of 0 m (pink), 10 m (green) and 20 m (yellow) are represented separately. The resultant Kruskal-Wallis equation from the ACI results is located above the box plot (P < 0.001).

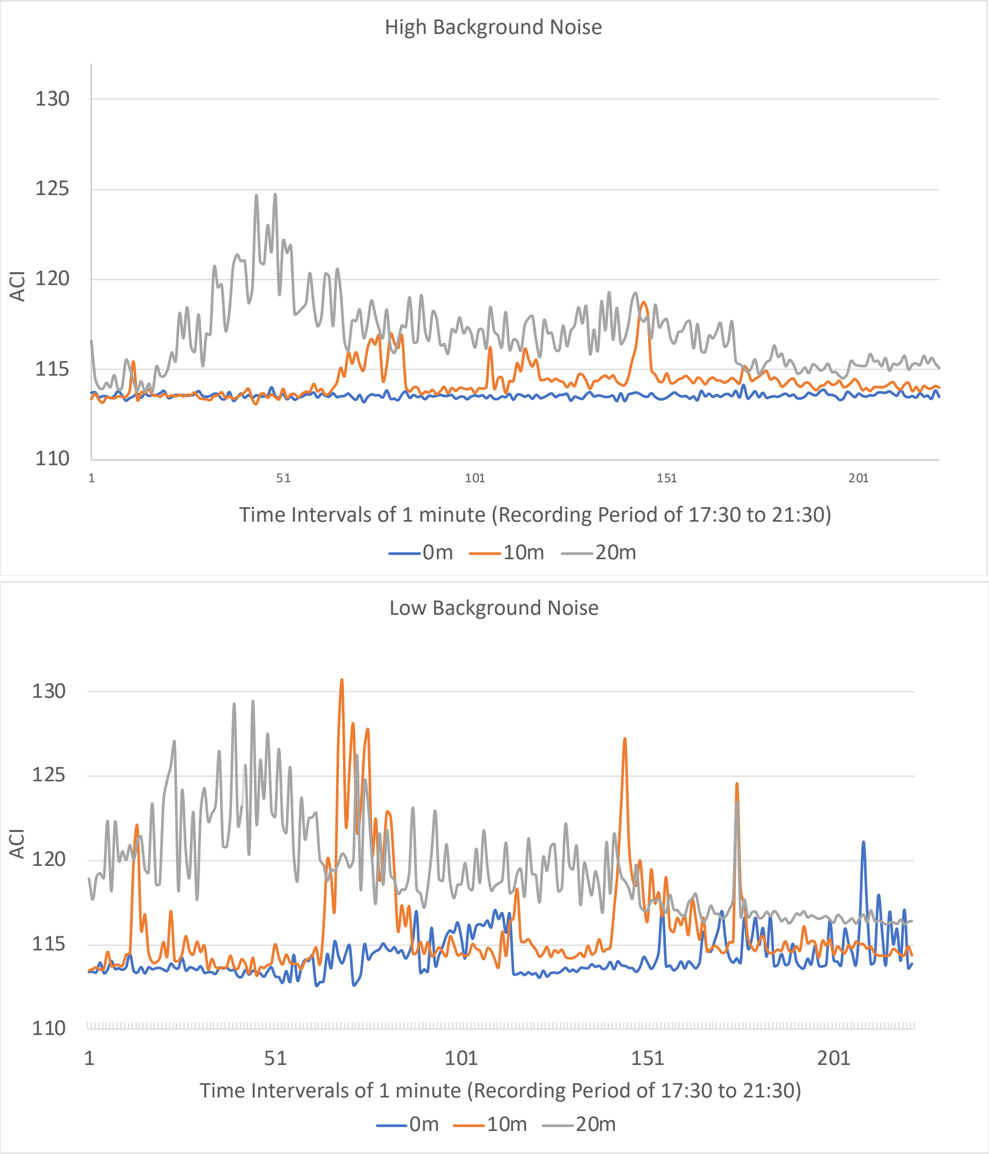


Figure 4. Temporal variations of the Acoustic Complexity Index in 1 minute interval recordings of the dusk chorus in terrestrial habitats at varying distances from sources of waterfall geophonies in a premontane wet forest in Monteverde, Costa Rica. High background noise (top) and low background noise (bottom) levels of waterfall geophony, are represented with the distances from the waterfall pools of 0 m (blue), 10 m (orange) and 20 m (gray) represented separately.

The Acoustic Diversity Index (ADI) was found to vary significantly across the six treatments by the Kruskal-Wallis H test ($P < 0.001$) (**Fig. 5**), as well as most pairwise comparisons ($P < 0.01$) with the exception of the 20 m distance for both background noise groups ($P > 0.05$) **(Table 1**). The distributions of the ADI scores for the 10 m and 20 m low background noise treatments had lower ADI scores than those in the other four treatments.

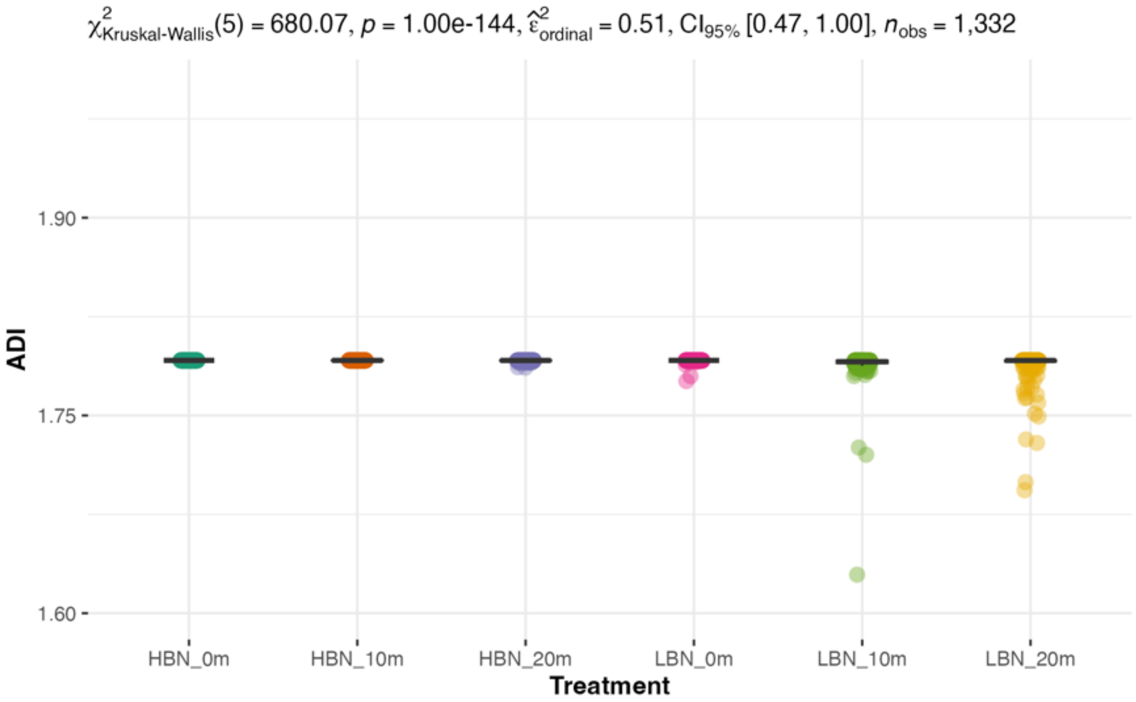


Figure 5. The Acoustic Diversity Index of the dusk chorus acoustic communities in terrestrial acoustic habitats at varying distances from sources of waterfall geophonies in a premontane wet forest in Monteverde, Costa Rica. Treatments are labeled and colored as in Figure 3. The resultant Kruskal-Wallis equation from the ADI results is located above the box plot ($P < 0.001$).

The Gini coefficients reported through the Acoustic Evenness Index (AEI) were found to vary significantly across the six treatments by the Kruskal-Wallis H test ($P < 0.001$) (**Fig. 6**) and, as with ADI scores, most pairwise comparisons were also found to vary significantly (adjusted $P < 0.05$) with the exception of the 20 m distance for two treatment groups ($P > 0.05$) **(Table 1**). The AEI score distributions of the high background noise 20 m, and low background noise 10 m and 20 m treatments were less even than the other treatment groups with the low background noise 10 m treatment exhibiting the most evenness among its recording samples, with a higher AEI score representing less evenness.

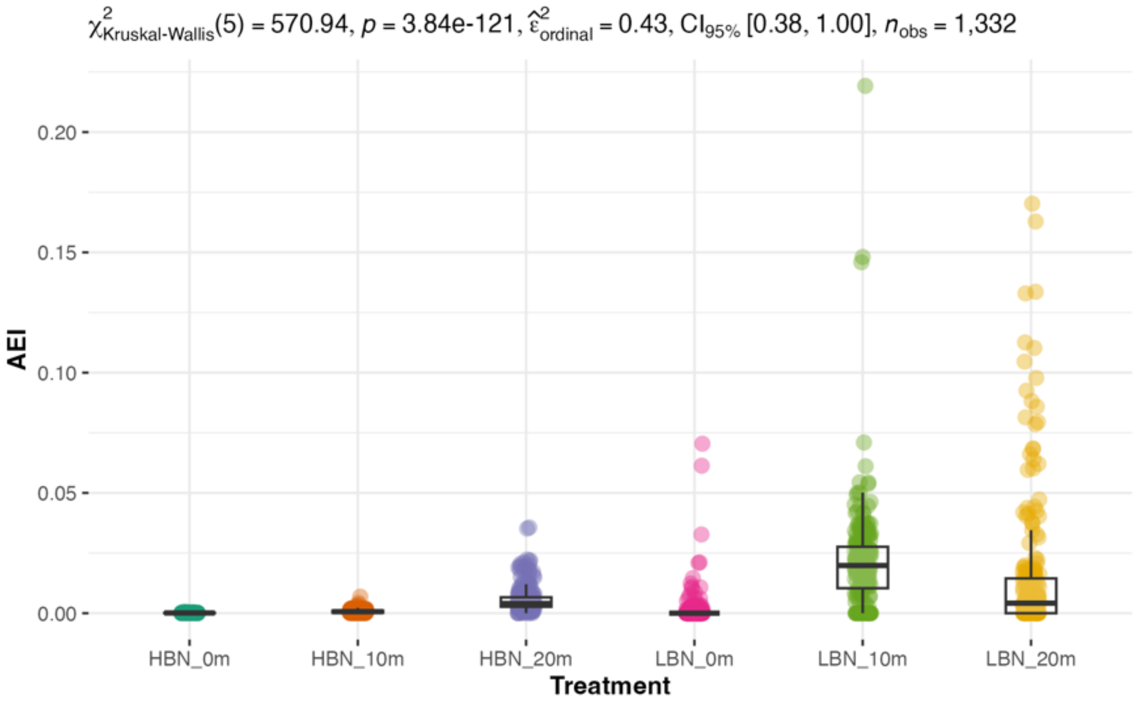


Figure 6. The Acoustic Evenness Index of the dusk chorus acoustic communities in terrestrial acoustic habitats at varying distances from sources of waterfall geophonies in a premontane wet forest in Monteverde, Costa Rica. Treatments are labeled and colored as in Figure 3. The resultant Kruskal-Wallis equation from the AEI results is located above the box plot ($P < 0.001$).

DISCUSSION

The sound pressure level analysis results indicate that the acoustics habitats across the treatments had different noise levels, with the high background noise habitats greater than those of the low background noise. At 0 m and 20 m distance for both groups was the greatest and lowest average noise levels, as expected. Using the average sound pressure level as a proxy for the ambient noise found in each acoustic habitat, comparisons can be made as to how the intensity of waterfall geophonies affected the acoustic communities of the dusk chorus.

The resulting general trend in Acoustic Complexity Index (ACI) (**Fig. 3**) for the dusk chorus acoustic communities was mostly as expected, with an increase in ACI greater the distance from the source of waterfall. This suggests that that acoustic communities produce a smaller amount of biophonies closer to waterfall geophonies, having greater background noise intensity, than those of similar habitats with less geophonies from flowing water. This suggests that waterfall geophonies degrade acoustic habitats. Despite the fact that the sound pressure level was higher at 20 m for high background noise than for low background noise at 0 m, the acoustic habitats near the highest intensity waterfall had higher ACI values. This suggests that geophony intensity may not be the only important factor in acoustic complexity of acoustic communities near waterfalls as the spatial distance from a waterfall an acoustic habitat is located may also impact how waterfall geophonies effect the amount of biophonies produced by these acoustic communities.

The temporal changes in ACI scores (**Fig. 4**)at the same acoustic habitats during only the specific temporal period of the dusk chorus suggests that the amount of biophonies from these acoustic communities near waterfalls may change temporally throughout the entire daylight cycle though more study into the biophonies of the entire daylight cycle of these acoustic communities, as in Farina et al., 2021, is necessary.

Conclusions on the impacts of waterfall geophonies on the Acoustic Diversity Index (ADI) (**Fig. 5**) are complicated by the fact that the index determines the diversity, in frequency bands present, of a recording sample regardless of the source of sound being biophonic, geophonic, or technophonic. Although variation was found among the ADI scores of the 6 treatment groups, no general trend was evident. As the waterfall geophonies had frequencies up to 8 kHz (**Fig. 2**), differences between the acoustic communities affected by differing intensities of waterfall geophonies may not be easily detected with the ADI in the presence of such geophonies, but further research into the application of the ADI in acoustic habitats with high levels of geophonies and technophonies is necessary.

Acoustic communities in habitats with greater intensities of waterfall geophonies generally were more even in there distribution of sound frequencies(**Fig. 6**), indicating more evenness than those in habitats with lower geophony intensities. The AEI scores, like the ADI scores, were also likely affected by waterfall geophonies and their frequencies as the waterfall geophonies were even across the 8 kHz sampling range (**Fig. 2**). Therefore, the ADI and AEI scores may be more helpful in describing the soundscape an acoustic habitat is located in than the acoustic community when high levels of geophonies, or possibly technophonies, are present across the entire sampling breadth of frequencies, though further study is necessary.

The findings of this study are particularly relevant to the increasing levels of sound pollution across acoustic habitats which are known to effect birds and anurans (Kaiser et al., 2011; Roca et al., 2016), as acoustic communication of both groups are also impacted by the presence of waterfall geophonies. Birds and anurans have adopted strategies to mitigate the effects of waterfall geophonies on acoustic communication (Brumm & Slater, 2006; Röhr et al., 2016; Grafe & Tony, 2017), and similar strategies may also be employed in the presence of technophonies that may be introduced into their acoustic habitats. More studies about the effects of waterfall geophonies in different types of terrestrial forest habitats, on the impacts caused by different intensities of flowing water and on the distances from stream sources are necessary. The findings of this study are expected to contribute to the knowledge of acoustic communities surrounding aquatic environments and encourage the study of ecoacoustics within the Neotropics.

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SUPPLEMENTAL MATERIALS

Table 1. The Z-vale and the unadjusted and adjusted P-value results of the Dunn test pairwise comparisons of the Acoustic Complexity Index, Acoustic Diversity Index and Acoustic Evenness Index scores between the three distances from the waterfall pool of 0 m, 10 m and 20 m within the same treatment group, high background noise(HBN) or low background noise(LBN), and between the two treatment groups at the same distance from the waterfall pool. After adjustment with the Bonferroni method, P < 0.05 for all Dunn test comparisons conducted with the exceptions of the comparisons of the ADI and AEI scores of the high background 20 m and low background 20m (P > 0.05) with text in red.

Acoustic Complexity Index			
Comparison	Z	P unadjusted	P adjusted
HBN_0m - HBN_10m	-7.953951	1.81E-15	2.71E-14
HBN_0m - HBN_20m	-20.218794	6.69E-91	1.00E-89
HBN_10m - HBN_20m	-12.264844	1.40E-34	2.10E-33
LBN_0m - LBN_10m	-8.253262	1.54E-16	2.31E-15
LBN_0m - LBN_20m	-18.986018	2.23E-80	3.34E-79
LBN_10m - LBN_20m	-10.732756	7.14E-27	1.07E-25
HBN_0m - LBN_0m	-6.353389	2.11E-10	3.16E-09
HBN_10m - LBN_10m	-6.6527	2.88E-11	4.32E-10
HBN_20m - LBN_20m	-5.120612	3.05E-07	4.57E-06

Acoustic Diversity Index			
Comparison	Z	P unadjusted	P adjusted
HBN_0m - HBN_10m	6.665837	2.63E-11	3.95E-10
HBN_0m - HBN_20m	16.912241	3.66E-64	5.48E-63
HBN_10m - HBN_20m	10.246404	1.23E-24	1.84E-23
LBN_0m - LBN_10m	16.934025	2.53E-64	3.79E-63
LBN_0m - LBN_20m	6.612144	4.00E-08	2.20E-05
LBN_10m - LBN_20m	-4.748459	2.05E-06	3.07E-05
HBN_0m - LBN_0m	3.462366	5.35E-04	8.03E-03
HBN_10m - LBN_10m	13.730554	6.66E-43	9.99E-42
HBN_20m - LBN_20m	-1.264309	2.06E-01	1.00E+00

Acoustic Evenness Index			
Comparison	Z	P unadjusted	P adjusted
HBN_0m - HBN_10m	-5.011302	5.41E-07	8.11E-06
HBN_0m - HBN_20m	-13.202266	8.51E-40	1.28E-38
HBN_10m - HBN_20m	-8.190964	2.59E-16	3.89E-15
LBN_0m - LBN_10m	-18.99453	1.89E-80	2.84E-79
LBN_0m - LBN_20m	-13.774716	3.62E-43	5.43E-42
LBN_10m - LBN_20m	5.219815	1.79E-07	2.69E-06
HBN_0m - LBN_0m	3.210759	1.32E-03	1.99E-02
HBN_10m - LBN_10m	-10.772469	4.64E-27	6.97E-26
HBN_20m - LBN_20m	2.63831	8.33E-03	1.25E-01

Gaps in the highlands: diversity and activity of insectivorous bats in montane cloud forest gaps of Monteverde, Costa Rica

Nathaniel Lindsay
Department of Ecology & Environment Biology, Purdue University, Indiana, USA

ABSTRACT

This study investigates the effects of forest canopy gaps and elevation on bat composition and activity in an undisturbed primary cloud forest. Environmental disturbances, such as canopy gap, play a crucial role in shaping forest ecosystems by introducing structural diversity and heterogeneity. Canopy gaps modify forest conditions by increasing light, moisture, and wind exposure. Using passive acoustic monitoring and sonogram analysis, this study explores how gaps influence insectivorous bat activity and genus-level composition at 1500 &1800masl. There was a statistically significant increase in bat activity from closed canopy to gaps at high elevations. The results show bat genus richness was higher in canopy gaps. There was not a statistically significant difference in bat activity between 1500masl and 1800masl, but genus-specific trends were observed. *Myotis* species demonstrated activity in both canopy types, while *Lasiurus*, *VES45*, and *Eptesicus* genera exhibited higher activity in gaps. This research highlights the ecological significance of canopy gaps in supporting diverse bat communities and offers insight into the influence of forest structure on bat activity.

RESUMEN

Este estudio investiga los efectos de los claros en el dosel del bosque y la elevación en la composición y actividad de murciélagos en un bosque nuboso primario inalterado. Las perturbaciones ambientales, como los claros en el dosel, desempeñan un papel crucial en la configuración de los ecosistemas forestales al introducir diversidad estructural y heterogeneidad. Los claros en el dosel modifican las condiciones del bosque al aumentar la exposición a la luz, humedad y viento. Utilizando monitoreo acústico pasivo y análisis de sonogramas, este estudio explora cómo los claros afectan la actividad de murciélagos insectívoros y la composición a nivel de género a 1500-1800 msnm. Hubo un aumento estadísticamente significativo en la actividad de los murciélagos desde el dosel cerrado hasta los claros en elevaciones altas. Los resultados muestran que la riqueza de géneros de murciélagos fue mayor en los claros en el dosel. No se encontraron diferencias estadísticamente significativas en la actividad de murciélagos entre 1500 msnm y 1800 msnm, pero se observaron tendencias específicas a nivel de género. Las especies de *Myotis* mostraron actividad en ambos tipos de dosel, mientras que los géneros *Lasiurus*, *VES45* y *Eptesicus* exhibieron una mayor actividad en los claros. Esta investigación destaca la importancia ecológica de los claros en el dosel para apoyar comunidades diversas de murciélagos y ofrece una visión sobre la influencia de la estructura del bosque en la distribución de murciélagos.

INTRODUCTION

Environmental disturbances represent important drivers in shaping the forest mosaic by introducing structural diversity and ecosystem heterogeneity (Kricher, 1999; Thom, 2015). This forest structural complexity is a crucial component in determining the community dynamics, biodiversity, richness, and abundance of plants, invertebrates, and vertebrates (Kricher, 1999; Bradbury, 2005; Müller, 2009). Among these disturbances, naturally occurring or anthropogenic forest canopy gaps, hereafter referred to as gaps, are crucial microhabitats that have profound effects on forest communities. Gaps modify forest conditions by increasing light, moisture, and wind exposure (Kricher, 1999). Increased forest heterogeneity and unique gap microhabitat conditions are documented to be an important driver in diversity, richness, and activity of bat species.

Insectivorous bats benefit from forest heterogeneity created by gaps, which offer unique resources such as insect prey, roost sites, and breeding locations (Stein, 2014). Indeed, gaps in temperate and tropical forests show high bat foraging activity compared to intact forest (Crome, 1988; Tena, 2020; Erasmy, 2021). Similarly, temperate forest edge environments foster greater bat foraging activity and variation in species spatial distribution (Menzel, 2002). Particularly for insectivorous bat species, gaps may be significant environments for niche partitioning and diversification (Stein, 2014). Gaps are utilized differentially between insectivorous bats in the open-space foraging, narrow-space foraging, and edge-space foraging guilds, with edge-space foragers increasing activity in gaps (Denzinger et al., 2013; Erasmy, 2021).

In addition to forest heterogeneity, elevation significantly influences bat diversity and activity, likely through its correlation with temperature and precipitation. In tropical regions, bat diversity and activity generally have an inverse relationship with elevation; in temperate regions, bat activity is also inversely related to elevation, however bat diversity is highest at mid-elevations (McCain, 2007). Specifically, in the Neotropics, bat diversity and activity have been observed to peak at mid-elevations (Arias-Aguilar et al., 2020). However, information of bat diversity and activity in the Neotropics is limited beyond elevations of 1560 meters above sea level (masl). Detailed data of the altitudinal trends of highland bat communities (above 1500masl) is needed, especially when considering the potential impacts of climate change and the shifting cloud base on cloud forest species.

Utilizing passive acoustic monitoring, this research seeks to investigate the effects that forest canopy gaps have on bat composition and activity within an undisturbed primary cloud forest. I also aim to study the relationship between high elevations (1500 and 1800masl), composition and bat activity at the genus level. Three primary hypotheses are tested in this study:

(1) Gaps provide optimal conditions for bats, resulting in higher overall bat foraging activity in gaps compared to closed canopy areas.

(2) Because gaps represent forest edge environments, bat genera specialized to edge-space foraging will be more active in gaps compared to other genera.

(3) Elevation influences bat activity at elevations above 1500 masl, therefore there will be lower bat activity at 1800masl compared to 1500 masl.

Through exploration of these hypotheses, this research aims to contribute to a better understanding of how forest canopy gaps and elevation influence bat communities in cloud forest ecosystems. These findings hold implication for conservation efforts, particularly as cloud forest species face increasing pressure due to climate change and shifting cloud base elevations.

MATERIALS AND METHODS

Study Site

This study was conducted at the Monteverde Biological Station in Monteverde, Guanacaste, Costa Rica (10.31866°N, 84.80803°S) from 7/17/2023 to 7/24/2023. The area is classified as Tropical Premontane Moist Forest, according to the Holdridge Life Zone Classification System. The area is dominated by a dense cloud forest with 2500mm to 3500mm of annual rainfall and an annual mean temperature of 16-18° C. Wet season occurs from March to December and dry season occurs from December to March (Nadkarni, 1999). The surrounding forest ranges from 1500 to 1800masl. Sample sites occurred at higher elevation (1650-1800masl) or lower elevations (1500-1650masl).

Experimental Design

To study the effect of canopy condition and elevation on bat diversity and activity, eight sites were sampled: two low elevation gap (LG1 & LG2), two low elevation closed canopy (LC1 & LC2), two high elevation gap (HG1 & HG2), and two high elevation closed canopy (HG1 & HG2) sites. At each site, elevation, percent canopy cover, gap size, and coordinates were recorded (Table 1). Gap and closed canopy sites were chosen using visual inspection and canopy coverage. Areas with canopy coverage above 90% were determined as closed canopy areas. Sites above 1650masl were considered high elevation sites, while sites below 1650masl were low elevation sites. Passive acoustic monitoring with two Audiomoth 1.2.0 was used to collect acoustic data. Data collection occurred over eight consecutive nights. For two consecutive nights, samples were collected at two sites of different canopy conditions and elevations. Audiomoths were placed approximately a meter off the ground. The Audiomoths recorded from 18:00h to 6:00h for 10 seconds every 50 seconds, at a sample rate of 192kHz.

Data Analysis

Acoustic data was processed as sonograms using Kaleidoscope Pro 5.6.1. A combination of Kaleidoscope's Auto Bat ID and manual processing was used to determine bat acoustic activity. Bat echolocation calls were manually identified to the genus level using previous literature as a reference (Arias-Aguilar, et al., 2018), as well as a experienced guide. If genus could not be determined, bats were classified as 'unknown'. Data from Kaleidoscope was organized in Excel and analyzed using R. Bat activity was considered as the number of bats passes per unit, with bat passes representing the number of acoustic files that contained bat echolocation calls. Bat activity was quantified as the average number of bat passes per genus per night (ABP/G/N). ABP/G/N considers the number of bats passes of a single genus in one night at each site. This metric was chosen to summarize bat activity and to generate the highest number of data points for the statistical analysis. The ABP/G/N was compared between elevation (high vs low) and canopy type (gap vs closed) separately, then again between gap and closed canopy with respect to elevation. Additionally, the bat activity of each genus between high and low elevations was compared using ABP/G/N. All comparisons were done using Welsh's t-tests, graphed and visualized as box plots with the ggstatsplot package (Patil, 2021) in R.

Sampling Site	Site Number	Elevation (meters)	Gap Size (m^2)	Canopy Cover (%)	Coordinates
Low Elevation Gap	1	1554	124.5	64.38	10.31972°N, -84.8066°W
	2	1585	115.2	66.46	10.32°N, -84.803561°W
Low Elevation Closed Canopy	1	1582	-	91.42	10.31°N, -84.805°W
	2	1606	-	91.42	10.32055°N, -84.80888°W
High Elevation Gap	1	1757	38.5	71.4	10.32557°N, -84.80157°W
	2	1804	209.05	32.4	10.32555°N, -84.79944°W
High Elevation Closed Canopy	1	1811	-	91.16	10.32388°N, -84.795°W
	2	1786	-	92.72	10.32583°N, -84.79722°W

Table 1: Description of the elevation, gap size, percent canopy cover, and coordinates of each sampling site.

RESULTS

There was a total of 11,053 records made between all sites, 589 of which contained bat activity. Every site, except the high elevation closed canopy site 1, contained bat activity. Between all sites, 4 genera were observed: *Lasiurus, Myotis,* and an unidentified Vespertilionid genus with an Fmin around 45kHz (*VES45*)*,* and *Eptesicus.* High elevation sites contained *Lasiurus, Myotis,* and *VES45*; low elevation sites contained *Lasiurus, Myotis, VES45,* and *Eptesicus (Figure 6).* Gap canopy sites had a higher genus richness compared to closed canopy sites; canopy gap sites contained *Lasiurus, Myotis, Eptesicus*, and *VES45* species, while closed canopy sites contained only *Lasiurus* and *Myotis* species (*Figure 5*). All sampling sites that contained bat activity had bats of unknown genera. Examples of sonograms containing each genus are found in *Figure 6.*

Activity patterns along elevation

The ABP/G/N for high and low elevations were 23.90 and 21.06, respectively, and were not significantly different (t=0.28, p=0.78) (*Figure 1*). Overall, bat activity did not vary significantly by elevation.

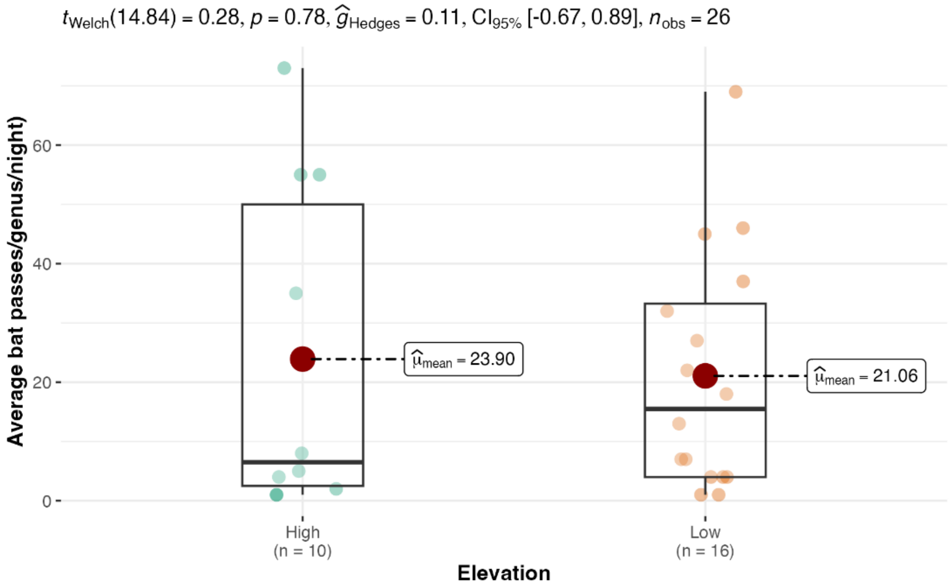

Figure 1. Bat activity, quantified as average number of bat passes/genus/night, compared between high and low elevation (p=0.78).

Activity patterns by canopy coverage

Considering all sites at any elevation, the ABP/G/N values at close canopy sites and gap sites were not significantly different (t=0.19, p=0.85) (*Figure 2*). At low elevations, the ABP/G/N values at closed canopy and canopy gap sites did not differ significantly (t=1.26, p=0.26). However, at high elevations closed canopy and canopy gap sites varied significantly (t=-2.50, p=0.04) in bat activity (*Figure 3*).

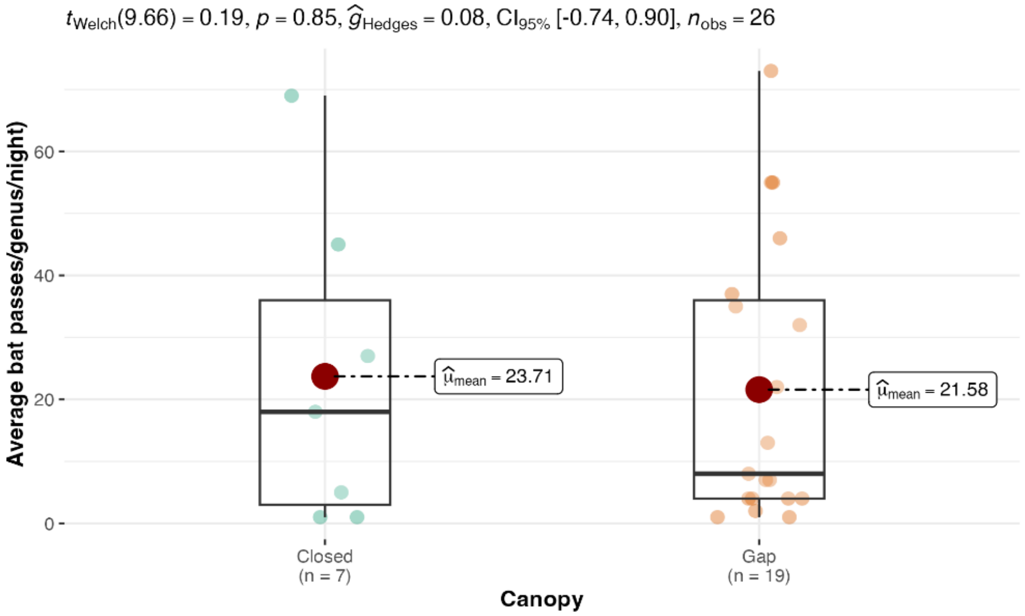

Figure 2. Bat activity, quantified as average number of bat passes/genus/night, compared between closed canopy and gap canopy sites (p= 0.85, p>0.05).

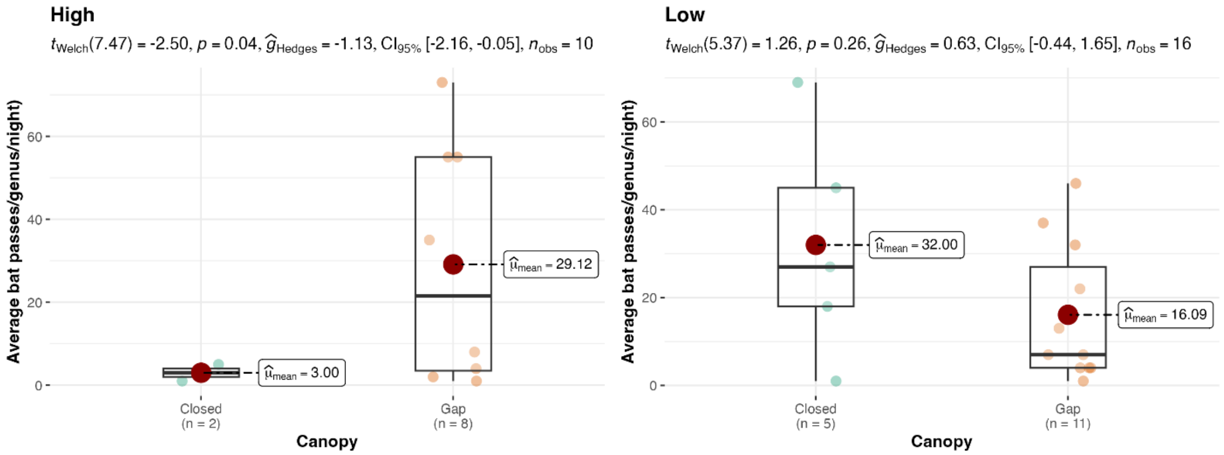

Figure 3. Bat activity, quantified as average number of bat passes/genus/night, compared between closed canopy and gap canopy sites with respect to elevation. High elevation sites: $p = 0.04$, $p<0.05$. Low elevation sites: $p=0.26$, $p>0.05$.

Activity patterns by genus

The composition of bat communities with respect to canopy type is depicted in *Figure 4.* Within closed canopy sites, there was no significant difference in the activity of the genera present ($t= 8.01$, $p=0.12$). However, at gap canopy sites, there is a significant difference in the activity of the genera present ($t= 9.05$, $p=8.76*10^{-3}$). At gap canopy sites, *Myotis* and *Lasiurus* species are more active compared to *Eptesicus* and *VES45* species. *Lasiurus* and *VES45* genera activity differed largely between the closed and gap canopy sites, with both being more active in gap canopy sites. The composition of bat communities with respect to elevation is depicted in *Figure 5.* At high elevations, there was no significant difference in the activity between the genera present ($t=5.48$, $p=0.12$). However, at low elevations there was a significant difference in the activity of the genera present ($t=7.42$, $p=0.04$), with *Myotis* and *VES45* species being more active compared to the other genera. Comparing high and low elevations, *Lasiurus* and *VES45* differed largely in activity between elevations, with *Lasiurus* activity being higher at high elevation and *VES45* activity being higher at low elevations.

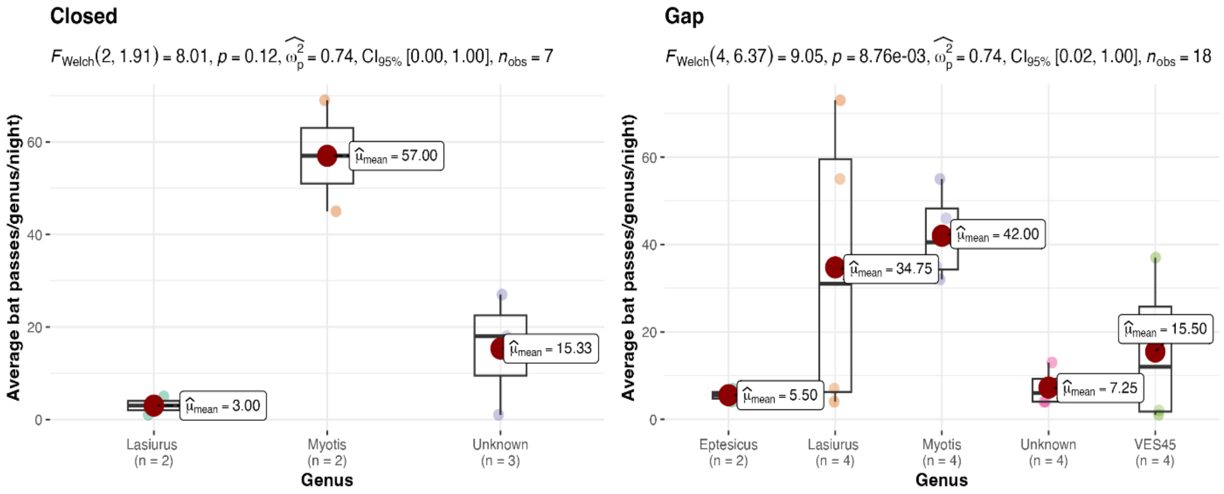

Figure 4. Bat activity, quantified as average number of bat passes/genus/night, compared between genera with respect to elevation. Closed canopy sites: p= 0.12, p>0.05. Gap canopy sites: p=8.76*10^{-3}, p<0.05.

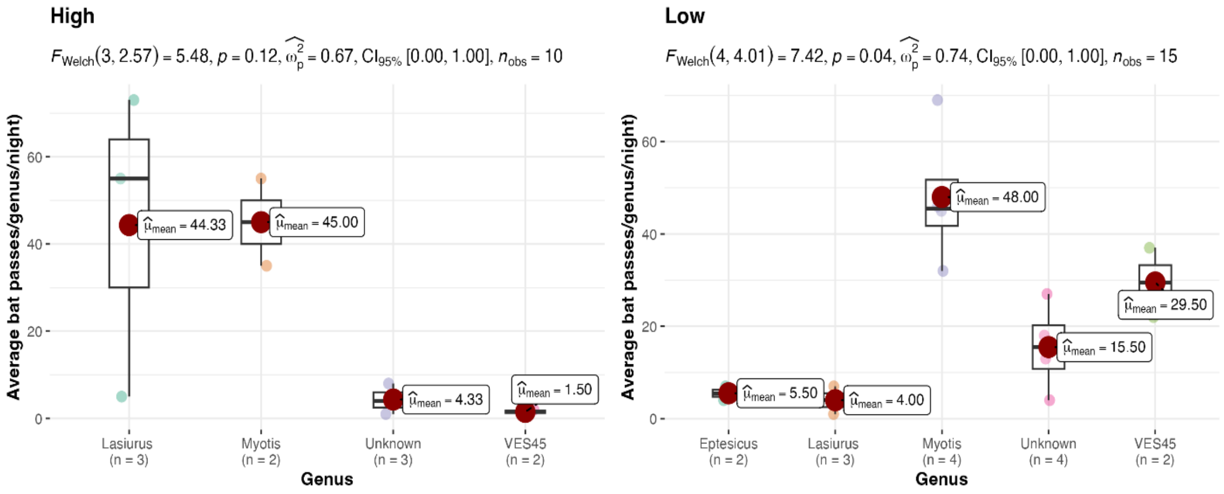

Figure 5. Average number of bat passes/genus/night compared between genera with respect to canopy type. High elevation sites: p= 0.12, p>0.05. Low elevation sites: p=0.04, p<0.05.

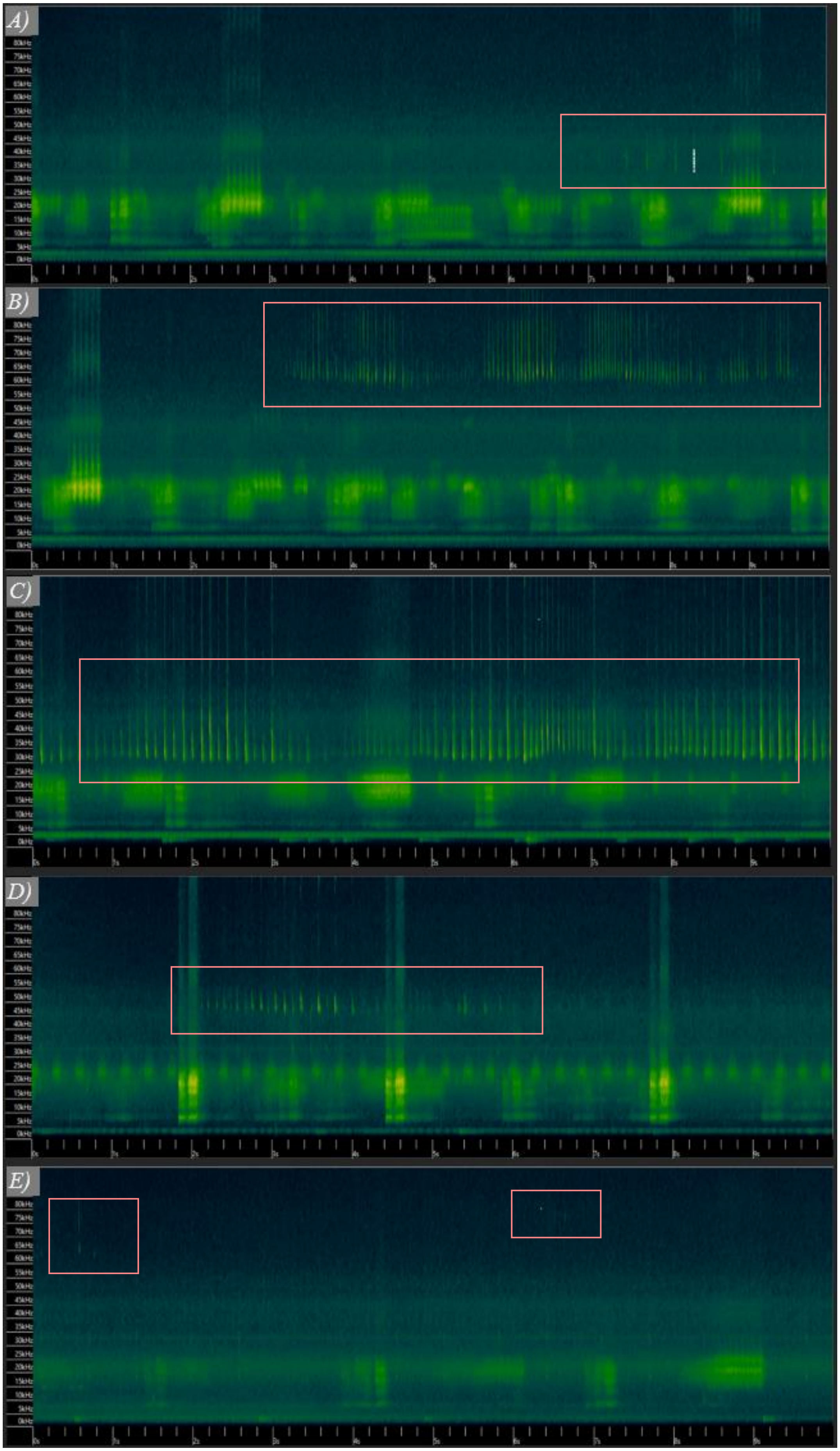
A)
B)
C)
D)
E)
80kHz
75kHz
70kHz
65kHz
60kHz
55kHz
50kHz
45kHz
40kHz
35kHz
30kHz
25kHz
20kHz
15kHz
10kHz
5kHz
0kHz
0s
1s
2s
3s
4s
5s
6s
7s
8s
9s

Figure 6. Examples of sonograms containing activity of all the genera identified. *(A) Eptesicus, (B) Myotis, (C) Lasiurus, (D) VES45, (E)* is one example in which the bat active in the sonogram could not be identified. Bat pulses are outlined in red.

DISCUSSION

General patterns along canopy coverage

The results indicate higher genus richness within canopy gaps compared to closed canopy conditions. Bat activity is found to differ significantly between closed and canopy gap conditions, but only at higher elevations. Moreover, relative bat activity by genera, particularly *Myotis* and *Lasiurus*, is highest under canopy gap conditions. These results support hypothesis (1) that bat activity increases in gaps, however this hypothesis is only supported at high elevations. This result is somewhat consistent with the literature; gaps in temperate and tropical regions generally foster increased bat activity (Crome, 1988; Tena, 2020; Erasmy, 2021). However, this study's results suggest that this trend is only true for high elevation gaps in Monteverde's cloud forest. Like the elevational results, this may be a consequence of the limited scope and limited number of variables tested in this study. Alternatively, this could be due to microclimate differences at higher elevations. As temperature decreases with elevation, so does insect activity (Mellanby, 1939). As gaps create unique microclimates, they may increase temperatures in these relatively cold high elevation sites (Kricher, 1999), representing unique opportunities for increased insect activity. Therefore, the increased availability of feeding opportunities in high elevation gaps compared to low elevation gaps increases bat activity. However, further research is needed to test this hypothesis.

The observed differences in community composition support hypothesis (2) and may relate to bat guilds, the morphologies of each genus, and echolocation call qualities. The coexistence of *Myotis* species in both closed and gap canopy areas can be explained by their classification as both narrow and edge space foragers. The distinctive characteristics of *Myotis* species, such as short round wings and high-frequency, steeply modulated echolocation calls, enable them to efficiently navigate and forage in both the cluttered forest environment and the edge environments created by gaps (Denzinger, 2013). Therefore, their adaptability to varying ecological niches allows them to exploit resources present in both closed forest and gap habitat types. The higher activity of *Lasiurus, VES45,* and *Eptesicus* genera observed in gaps can also be attributed to their classification as edge space foragers. The edge-space forager traits limit their effective use of cluttered forest environments, explaining their prevalence in canopy gaps with edge-space conditions (Denzinger, 2013). These findings are consistent with literature concerning bat activity and temperate forest canopy gaps, wherein gap usage is related to morphology (Crome, 1988) and the presence of gaps does not change the activity of narrow-space foragers but does increase the activity of edge-space foragers (Erasmy, 2021).

General patterns along elevation

Insectivorous bat activity in Monteverde's cloud forest was investigated across different elevations, and the results indicate that bat activity does not exhibit significant differences above 1500masl. This does not support hypothesis (3) that bat activity decreases at elevations above 1500m due to unfavorable conditions. Compared with previous literature on tropical bat activity and elevation, my overall bat activity results are consistent with higher elevations (1500-1800m) having lower bat activity compared to middle elevations. However, my results are inconsistent in the trend that bat activity and elevation are negatively correlated above mid-elevations (McCain, 2007; Weier, 2017). This is possibly because an elevational change of 300m is insufficient to observe significant changes in bat activity. The limited number of sampling nights and data could have also given insufficient statistical power to observe significant results. Additionally, precipitation, temperature, and wind conditions were not considered during data collection, possibly leading to these inconsistent results. Furthermore, while overall bat activity shows no significant difference between high and low elevations, the relative activity of different bat genera varies between elevations. However, these differences in genus-specific elevational trends warrant further investigation though species-level identification to facilitate a more robust comparison with existing literature. Further studies on Monteverde's cloud forest bats should consider weather patterns to determine the exact relationship between bat activity and elevation.

Conclusions

This study's findings provide valuable insight into the relationship between bat composition and activity of Neotropical insectivorous bats in cloud forests. While no elevational trend in overall bat activity was found, there were trends in genus-level activity with elevation. Canopy gaps were found to be hotspots for bat activity, but only at elevations around 1800masl. There were also genus-level differences in activity between gap and closed canopies. These findings highlight the ecological significance of gaps in supporting heterogenous and diverse bat communities.

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