Preliminary Assessment of the Orthoptera Composition of Different Tropical Habitats, within the Madre de Dios Department of Peru.

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Abstract

Orthoptera occupy an important link in food chains and are important biological indicators for ecosystem health. Knowledge about Orthoptera taxonomy and distribution within Peru is limited to brief field guides of common species in certain regions within the country. In this study, an assessment was made of the Orthoptera species richness and diversity of three tropical habitats, characteristic of the Madre de Dios department of Peru. These habitats include *Terra firme*, *Aguajal* and *Regenerating forest*. The habitats were surveyed by running 15 meters transects. During the period of July 30 to August 23 a total of 120 transects were run (40 per habitat). On these transects a total of 275 adult specimens were recorded. These were divided into 66 morphospecies, compromising 24 species of the suborder Caelifera and 42 for Ensifera. *Terra firme* was found to have the highest totals species richness with 33 species, followed by *Aguajal* and *Regenerating forest* with 26 species each. The amount of unique species per habitat was 17 for *Terra firme* (54.8% of total), 22 for *Regenerating forest* (84.6% of total) and 10 for *Aguajal* (38.7% of total). Average species richness was highest in *Regenerating forest* with 3 species per transect, followed by *Aguajal* (1.1 per transect) and *Terra firme* (1.275). Shannon indexes were calculated for each habitat and it was found that *Terra firme* had the highest diversity value (H = 3.21), followed by *Aguajal* (H = 3.08) and *Regenerating forest* (H = 2.61). Further studies could focus their attention on actual species identification, as most specimens in this study were not identified beyond the Family level. A study focusing on environmental variables could better examine their importance when specifically seeking for varying conditions within habitats, which was not done in the current study. A study performed over a longer time period could also capture the effects of seasonality on Orthoptera assemblages.

Introduction

Orthoptera is an order of insects, with includes species that are morphologically adapted for jumping and possess mouthparts made for chewing, as well as the characteristic vocalizations males make to attract potential mates (Borror, Triplehorn & Norman, 1989). The order can be divided in two suborders: Ensifera (katydids and crickets) and Caelifera (grasshoppers). Orthoptera undergo incomplete metamorphosis, where immature individuals resemble adults. Ensifera species are mostly nocturnal where grasshoppers are mostly always diurnal. Most katydids are herbivores, while some species are strictly carnivorous and predatory. Most crickets are omnivorous (Hanson & Nishida, 2016) and grasshoppers are almost exclusively herbivorous. Grasshoppers are abundant insects in both natural and human disturbed habitats, and play an important role in food chains. (New World Encyclopedia, 2012). Katydids are an very important link in vertebrate food chains, being predated upon by many birds, bats, monkeys, lizards and snakes (Hogue, 1993). Grasshoppers are sometimes used as biological indicators for ecosystem health and the efficacy of ecological networks (Latchininsky, Sword, Sergeev, Cigliano, & Lecoq, 2011). Members of both suborders occur almost everywhere in the world but diversity is highest in the tropics (Gwynne, DeSutter, Flook, & Rowell, 1996).

The Madre de Dios department of Peru is located at the southern-east border of Peru. Within this region numerous different tropical habitats exist. One example of a typical habitat is *Aguajal*. *Aguajal* is the name in Peru given to locations with high density of the Aguaje palm (*Mauritia flexuosa*). These palms grow to great heights (35-40 m) and occur on naturally flooded soils. The Aguaje palms are of great economic importance for Peru, as they produce an abundant amount of fruits sold on both the national and international markets (Delgado, Couturier & Mejia, 2007).

Another habitat that plays a key role in the rural economics and forest conservation within Madre de Dios is *Terra firme* forest, through its importance as a source of sustainable income via the harvesting of Brazil Nuts (Hélène, 2017). *Terra firme (*meaning *‘’*solid ground’’) is a forest type characterized by the fact that it is unflooded and well-drained (Balslev, Laumark, Pedersen, & Grández, 2016; Schneider, et al., 2019). Forest canopy of *terra firme* reaches 40 m in height and is very dense, with high species richness. The understory generally receives little light and is more open. Amazonian *terra firme* contains a large amount of above-ground biomass and complex vegetation structure, containing emergent trees, canopy trees, understory trees and shrubs, samplings, seedlings, herb and ferns (Myster, 2016). The soil is made of clay and/or loam with a high amount of organic matter in its upper layer and is alluvial of origin, having been deposited as much as 2.5 million years ago and subsequently uplifted to positions above flood level (Schneider, et al., 2019). Next to these naturally occurring habitats, disturbed environments are also of economic importance. For instance, forest in the neotropics is often cleared and then cropped for several years. After this, the agricultural fields are often converted to artificial pastures for livestock, by planting perennial African grasses. These grasses are aggressive in the way they colonize new areas, and as a result, are widely naturalized in Latin America. In this way, agriculture is temporary stage to the creation of these pastures, that are often maintained by pressures such as grazing and purposeful cutting of secondary growth (Parsons, 1972). The conversion of forests from the Americas to pastures dominated by introduced African grasses causes a dramatic loss of native vegetation (Williams & Baruch, 2000).

Several publications on Peruvian Orthoptera exists, but detailed information on species composition is only available for some localities, such as those from Loreto (Cadena-Castañeda & Westerduijn, 2013) and Huanuco (Westerduijn & Cadena-Castañeda, 2013; Cuespán, Westerduijn & Cadena-Castañeda, 2014). The aim of this study is to make a preliminary assessment of the Orthoptera diversity of different common tropical habitats of the Las Piedras district in the Madre de Dios department of Peru, and to compare the composition of these habitats to draw conclusions about their fitness as living environments for Orthoptera.

Expected results

Ensifera are mostly nocturnal where Caelifera are mostly always diurnal (New World Encyclopedia, 2012) and most Ensifera species only sing at night while Caelifera sing during the day (Borror, Triplehorn & Norman, 1989). (1) Because of this, it is expected that more specimens and species of the suborder Ensifera will be found during nighttime than during the day for all habitats.

Grasshoppers are almost always diurnal and are adapted to live under conditions of abundant light. Higher values for shrub- and sub-canopy layers are assumed to be negatively correlated to the amount of light reaching the lower herb-layer and ground level, where Caelifera species will most likely reside. Ensifera on the other hand, often live on higher vegetation (Borror, Triplehorn & Norman, 1989) and are sing during nighttime and do not desire light to be active. (2) It is thus hypothesized that more specimens and species belonging to the suborder Ensifera will be found in transects with higher cover for shrub-layer, sub-canopy and canopy cover.

(3) In line with the previous hypothesis, it is expected that of the tree study habitats *Terra firme* and *Aguajal* contain the will be found to contain the most species belonging to the suborder Ensifera. The *Regenerating forest* habitat, lacking higher vegetation growth, receives much more light and is expected to harbor more species of the suborder Caelifera instead.

Terra firme forest has a complex vegetation structure, containing emergent trees, canopy trees, understory trees, shrubs, samplings, seedlings, herb and ferns (Myster, 2016), as opposed to the highly monotone landscape of the *Regenerating forest*, which in many cases includes only a herb layer which is entirely dominated by one species of *Brachiaria* grass. Orthoptera is a typical terrestrial order of insects, with very few species adapted to live in wetter environments (Hanson & Nishida). This is expected to result in a lower species richness for the wetter *Aguajal* habitat. However, the *Aguajal* also possesses high vegetation cover for all different heights measured, resulting in a more complex and diverse living environment compared to *Regenerating forest*. (4) Therefore, it is expected that *Terra firme* forest has the highest species richness, followed by *Aguajal* and *Regenerating forest*.

Methods

Habitats surveyed

During this study, three different tropical habitats common in the Madre de Dios region of Peru were studied. These different habitats were surveyed in the direct vicinity of the Alliance For A Sustainable Amazon field site, located North of the city of Puerto Maldonado (12°13'34.9"S 69°06'45.4"W). The study area is located close to the Interoceanic Highway and is situated at the frontier of the forest. This location is situated about 250 meters above sea level. The habitats include *Aguajal*, secondary *Terra firme* forest and *Regenerating forest* patches, all of which are within walking distance from the field site. The forest studied is selectively logged and contains many individuals of the Brazil Nut tree (*Bertholletia excelsa*) that dominate the forest. During this study, the forest property of Alliance For A Sustainable Amazon (ASA), as well as the neighboring property were surveyed. The last one constitutes a Brazil Nut concession that is less selectively logged. The field site is located close to a *Aguajal*, formed around the river stream that flows through it. Around the field site exist many patches of artificial grassland, which are almost entirely composed of one species of grass, colloquially called cattle grass *(Genus: Brachiaria*). This grass is invasive and occurs in high densities in almost every location devoid of actual forest. In the direct vicinity of the field site some of these fields are slowly recovering and secondary growth occurs, as no active management takes place. These areas contain shrubs and short trees with varying degrees of coverage.

Sampling locations

Orthoptera diversity was assessed by sampling the different habitats along short transects within these different environments. Each habitat type was surveyed on different sites. Sites were spread out of the available study area, to create a more representative image of each habitat. Within a period of 4 weeks, 6 sites from all three habitats were sampled. All sites were visited once during the day and at night. In each site a total of 3 transects of 15 meters in length were laid out, except for the first two sites, where 4 transects were laid out per site. This produced a total of 40 transects per habitat (20 during the day and 20 at night). These transects were sampled for both Orthoptera suborders (Caelifera and Ensifera). The locations of the transects were chosen on site to ensure that there was enough space to set up the transects and that the environment is representative for the habitat. Transects belonging to the same site were placed in each others vicinity. This was done by placing transects: (1) In a straight line, where the end of one transect represents the beginning of the next. (2) Side by side, in which case a minimum of 5 meters distance is kept from one transect to another. The starting point of every transect was recorded using a GPS unit (Garmin GPSMAP 64).

Environmental variables

In order to study the causes of potential differences in Orthoptera assemblage between different habitats, some environmental factors within transects were recorded. Vegetation structure was assessed by visually estimating percentage cover of vegetation layers (herb layer 0-50cm, shrub layer 50-150cm, sub-canopy layer >150cm). Canopy cover was assessed by taking photographs of the canopy with a smartphone (Motorola moto c plus). With the use of the program ImageJ (Schneider, Rasband & Eliceiri, 2012), these photographs were converted to binary (black and white), where black pixels represent vegetation and white the sky. The ratio of black to white pixels was then calculated to estimate percent cover. The presence of standing water was noted as YES/NO. These variables were recorded in a 5x5m plot around the midpoint of every transect at 7.5 meters, indicated by marking tape on the middle of the rope used to lay out transects.

Sampling methods in the field

During daytime, transects were walked between 10:00h and 16:00h. Surveying at night occurred between 19:00h and 23:00h. A headlight was used to scan vegetation at night in all habitats, and during the day for *Terra firme* and *Aguajal*. The vegetation surveyed for Orthoptera in the *Regenerating forest* received much more light than the *Terra firme* forest or the *Aguajal*, due to the lack of a high vegetation in most places. For this reason, it was only surveyed during periods of sunshine, when Orthoptera species are most active and easiest to find.

Transects were laid out by attaching one end of a 15 meter rope to nearby vegetation and the other end to the body of the person surveying the habitat. This rope was then straightened by walking to either one of four cardinal directions with the use of a compass (North, East, South or West), where the rope was attached again. The beginning and end points of transects were marked with pink marking tape and the middle with blue marking tape, for easy recognition during night time. Transects were sampled by walking along the rope from one end to the other, in a slow and steady pace, paying attention to the environments and any possible insect movement at every step. Sampling occurred 2 meters to each side. All adult Orthoptera spotted when walking along the transects of *Aguajal* or *Terra firme* were either caught by hand or photographed *in situ*. During trails, it was found that catching Orthoptera by hand in the *Regenerating forest* habitat resulted in a very low catch rate during the day, due to the high level of activity exhibited by Orthoptera as a result of ambient temperatures and sunlight. For this reason a sweepnet was used for this habitat during daytime.

Recording, preparation and identification of specimens

Recording: The recording of specimens took place either after catching individuals (by hand or with a net) and storing them in plastic jars (labeled with site number, transect number, date and time) or by photographing them *in situ* (Nikon D200 with a Sigma 105mm macro lens). Caught specimens were killed by placing them in kill jars with Etylactate and were subsequently stored in entomological envelopes labeled 2019-FLP-OT-XXXX (where the X's represent the specimen number starting from 0001). Photographs are taken of every single specimen with specimen code and a ruler visible in the picture. In addition, pictures were taken of every specimen dorsally, laterally, diagonally from above and from beneath.

Identification: The use of photographic guides of common Orthoptera species from Peru (Cadena-Castañeda & Westerduijn, 2013; Westerduijn & Cadena-Castañeda, 2013; Cuespán, Westerduijn & Cadena-Castañeda, 2014) and other countries of Northern Latin America (Rodríguez, Pinto O., Rodríguez T., & Cadena-Castañeda, 2012) was the first way in which species identification was attempted. If use of these guides failed for identifying specimens at the genus or species level, use was made of additional online resources to aid further identification, these included a Facebook group called ''The Orthopterist Society'' and the service INaturalist, where users can help to identify specimens. Photographs of specimens were submitted to these groups, together with geographical information and date. Suggestions for identifications were compared with other online to verify their validity.

Besides the attempted identification on genus and species level, all specimens were identified to family level. In addition to this all specimens were also divided into morphospecies. This was done by comparing photographs taken of all specimens, and sorting them into different species categories.

Statistical analysis

Generalized linear modeling (GLM) with Poisson regression was used to analyze the data and Shannon indexes were calculated for all habitats as a measure of Orthoptera diversity.

Results

A total of 275 adult specimens were recorded for this study on 120 transects, during the period of July 30 to August 23. Found specimens were divided into 66 morphospecies. These included 24 species of the suborder Caelifera and 42 for Ensifera. These were further divided into 6 different families. Caelifera was divided in

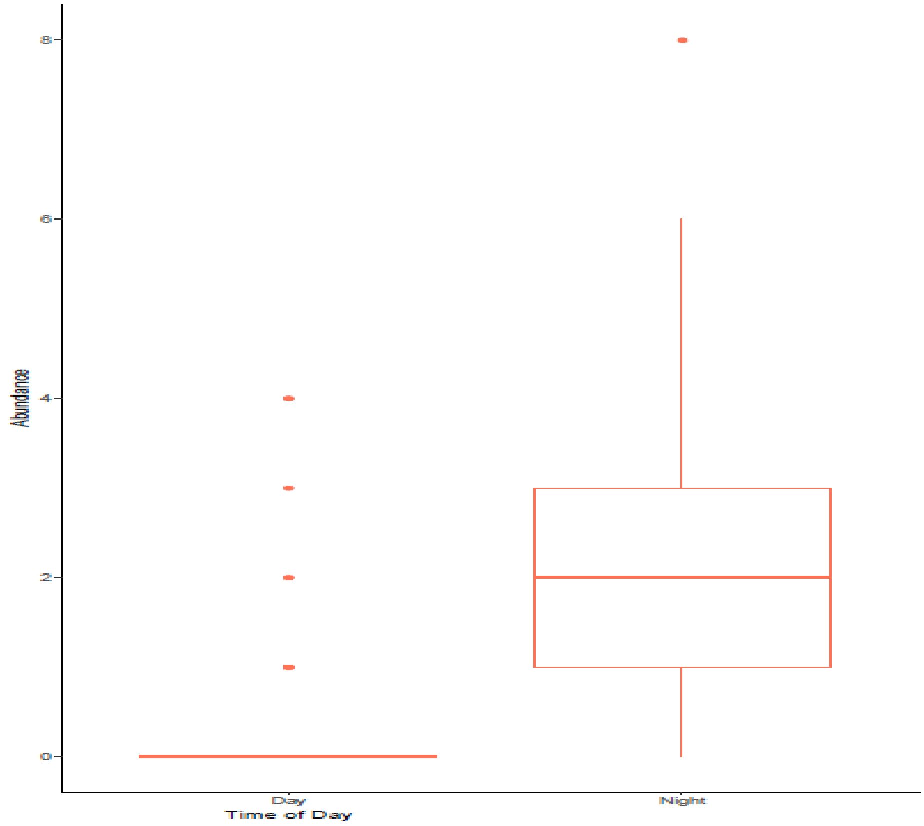


Figure 1: Abundance of Ensifera specimens for day and night transects**.**

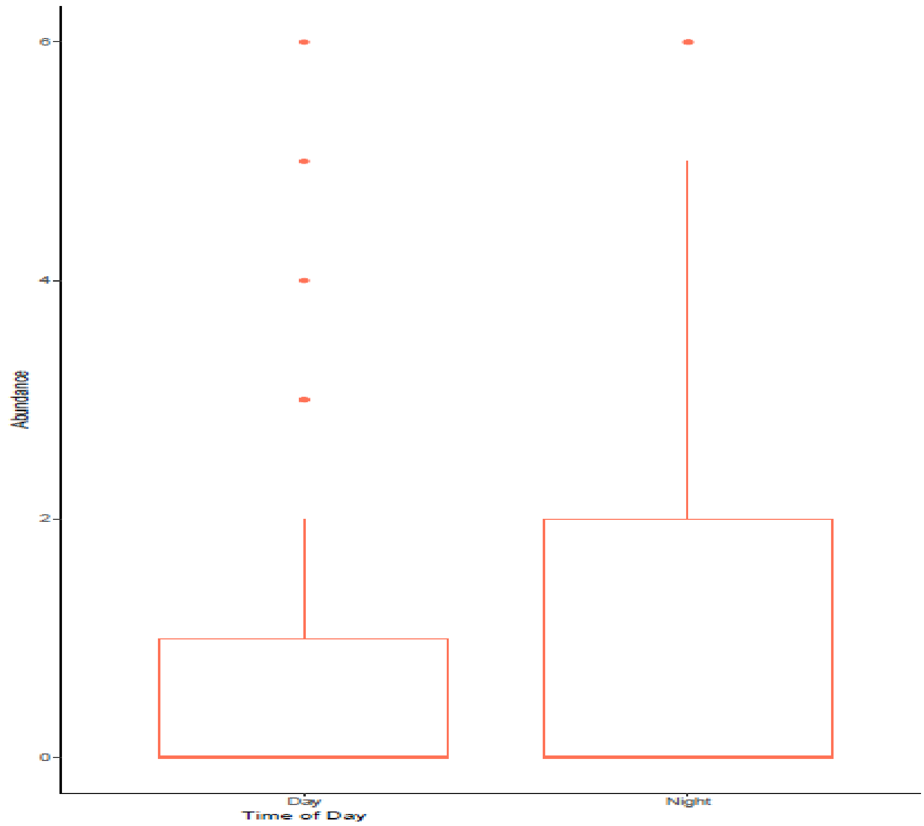


Figure 2: Abundance of Caelifera specimens for day and night transects.

Table 1: Abundance and average abundance of Ensifera and Caelifera for each habitat

	Average Ensifera abundance	Average Caelifera abundance	Total Ensifera abundance	Total Caelifera abundance
Terra firme	0.9	0.5	36	20
Aguajal	0.9	0.35	36	14
Regenerating forest	2.05	2.175	82	87

Table 2: Summary of average species richness, total species richness and number of unique species per habitat. 40 Transects were surveyed for each habitat.

	Average species richness	Average Caelifera species richness	Average Ensifera species richness	Ensifera species richness	Caelifera species richness	Total species richness	Number of unique species
Terra firme	1.275	0.425	0.85	21	10	31	17 (54.8%)
Aguajal	1.1	0.275	0.825	20	6	26	10 (38.7%)
Regenerating forest	3	1.6	1.4	13	13	26	22 (84.6%)

Acrididae (16 species), Tetrigidae (6 species, Eumastacidae (2 species). Ensifera was divided in Tettigoniidae (25 species), Gryllidae (16 species) and Gryllacrididae (1 species).

Differences between day and night

Quantile-quantile plots were used to check the distribution of the data. The data was found to be non-normally distributed. Generalized linear modeling (GLM) with Poisson regression was used for all statistical analyses described from this point forward.

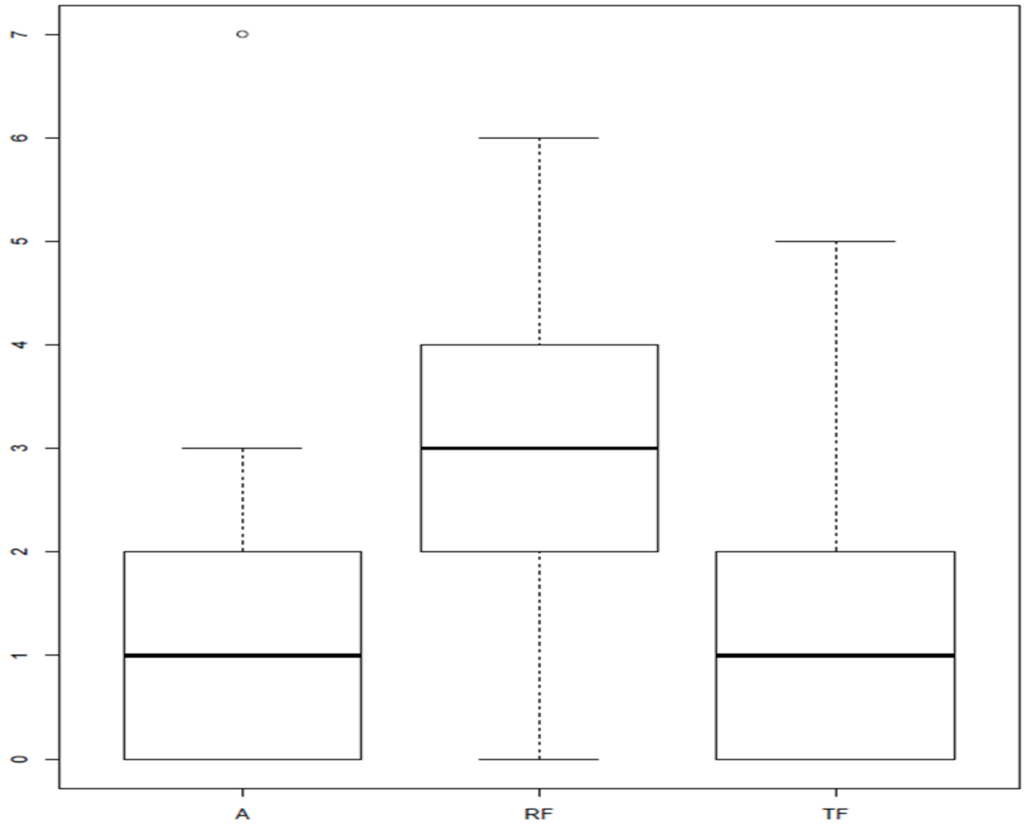
The difference in average abundance between day and night transects for Ensifera and Caelifera was tested for all habitats at once. The difference was found to be highly significant (df=119, y=1.6895, p<001) for Ensifera. This is graphically shown in Figure 1 with the use of a boxplot.

No difference was found between day and night transects for Caelifera (df=119, y=0.2492, p=0.174). See figure 2 for a graph showing the average abundance of Caelifera for day and night.

Abundance and average abundance per habitat

Table 1 shows the number of found Ensifera and Caelifera per habitat, as well as the average abundance. Average abundance was found to be higher for Ensifera (compared to Caelifera) for both *Terra firme* (0.9 and 0.5) and *Aguajal* (0.9 and 0.35). In contrast, the average abundance of Ensifera was found to be almost equal to that of Caelifera (2.05 and 2.175) for *Regenerating forest*. The ratio Ensifera/Caelifera was 1.80 for *Terra firme*, 2.57 for *Aguajal* and 0.94 for *Regenerating forest*.

Figure 3: Average species richness for each habitat.



Species richness and number of unique species

Average species richness of all three habitats was compared. See Table 2 for exact values. The regenerating had a significantly higher average species richness overall. Average Caelifera and Ensifera species richness was also found to be significantly higher in *Regenerating forest*. See Table 3 for a summary of the statistical analyses. Figure 3 shows the differences in average species richness graphically. When looking at total species richness, *Terra firme* has most species found (31), followed *Aguajal* and *Regenerating forest* with 26 species each (see Table 2). The number of unique species for *Terra firme* was 17 (54.8% of total), for Aguajal 10 (38.7% of total) and 22 for *Regenerating forest* (84.6% of total).

Table 3: Summary of statistical analyses regarding average species richness. GLM with Poisson regression. DF=119 in all cases

df= 119	Average species richness	Average Caelifera species richness	Average Ensifera species richness
Terra firme	y = 0.1750 p = 0.596	y = 0.1500 p = 0.4391	y = 0.0250 p = 0.9264
Aguajal	y = 1.1000 p = 0.05	y = 0.2750 p= N.S.	y = 0.8250 p = N.S.
Regenerating forest	y = 1.9000 p = <**0.001**	y = 1.3250 p = <**0.001**	y = 0.5750 p = **0.0352**

Shannon diversity index

To compare the Orthoptera biodiversity of the three study habitats Shannon's H was calculated for each habitat with Shannon's diversity index. A higher H-value indicates a higher evenness and species richness. *Terra firme* was found to have the highest value (H = 3,21). *Aguajal* was second highest (H = 3,08) and *Regenerating forest* was found to be last in terms of diversity (H = 2,61).

Average vegetation cover

Vegetation parameters of every transect were measured. Minimum, maximum and average cover for all four different heights were calculated. These results are displayed in Table 4. The results show that for herb-layer (0-50cm) the *Regenerating forest* habitat had the highest average cover (68.5%). Herb-layer was 35% to 40% at highest, for *Terra firme* and *Aguajal* respectively. In comparison, herb-layer was 40% at minimum for *Regenerating forest* and 95% at most. Shrub-layer (50-150cm) was highest on average in *Aguajal* with 20.3%. Terra firme and Aguajal were 35% at maximum, while Regenerating forest was 80% at the highest. Sub-canopy layer averaged highest in *Terra firme* with 39.3%, ranging from 10-80% for *Terra firme*, 5-45% for *Aguajal* and 0-35% for *Regenerating forest*. Canopy-layer was highest in Terra firme with 81.3%. Canopy cover was 79.6% at average for *Aguajal* and 3.5% for *Regenerating forest*. Canopy cover never went higher than 57.9% for *Regenerating forest* and went to 94.1% and 89.5% for *Terra firme* and *Aguajal*, respectively.

Table 4: Average and full range (min – max) of all measured vegetation layer per habitat

	Herb-layer		Shrub-layer		Sub-canopy layer		Canopy-layer	
	Average	Range	Average	Range	Average	Range	Average	Range
Terra firme	12.5	5-35	17.8	5-35	39,3	10-80	81,3	65.6-94.1
Aguajal	13.5	5-50	20.3	5-35	25.8	5-45	79,6	66.6-89.5
Regenerating forest	68,5	40-95	13.5	0-80	4.3	0-35	3.5	0-57.9

Discussion and Conclusion

The aim of this study was to make an assessment of the Orthoptera diversity of tropical habitats, and to compare their compositions. During the period of July 30 to August 23 a total of 120 transects were laid out, 40 transects each for *Terra firme*, *Aguajal* and *Regenerating forest*. On these transects a total of 275 adult specimens were recorded. These were divided into 66 morphospecies, compromising 24 species of the suborder Caelifera and 42 for Ensifera. These were further divided into 6 different families. Caelifera was divided in Acrididae (16 species), Tetrigidae (6 species) and Eumastacidae (2 species). Ensifera was divided into Tettigoniidae (25 species), Gryllidae (16 species) and Gryllacrididae (1 species).

To compare the compositions of the study habitats, the total species richness, the amount of unique species for each habitat, the average species richness and average abundance per transect and Shannon-indexes were calculated. The total amount of morphospecies collected was 66. *Terra firme* yielded a total of 33 species, *Aguajal* and *Regenerating forest* 26 each. The amount of unique species per habitat was 17 for *Terra firme* (54.8% of total), 22 for *Regenerating forest* (84.6% of total) and 10 for *Aguajal* (38.7% of total). Average species richness was highest in Regenerating forest with 3 species per transect, followed by Aguajal (1.1 per transect) and Terra firme (1.275). Shannon index was calculated for each habitat and it was found that *Terra firme* had the highest value (H = 3.21), followed by *Aguajal* (H = 3.08) and *Regenerating forest* (H = 2.61).

Four hypotheses were created for this study. The first predication stated that Ensifera would be more abundant on night transects and to Caelifera on day transects. It was found that Ensifera abundance was highly significantly ($p<0.001$) for Ensifera when looking at all habitats at once. However, no statistical difference was found for Caelifera. The second hypothesis stated that the ratio Ensifera/Caelifera species and abundance would be higher for increased cover of shrub-layer, sub-canopy layer and canopy layer. This is very difficult to test within habitats, since the range of vegetation cover is small for each habitat. It can only be said that the ratio of Ensifera/Caelifera was found to be higher in *Terra firme* (1.80) and *Aguajal* (2.57) but not in *Regenerating forest* (0.94) and that Ensifera Species richness was found to be higher in *Terra firme* and *Aguajal* than in *Regenerating forest*. This coincides with higher sub-canopy and canopy cover, which largely supports the third hypothesis of higher Ensifera Species richness and ratio for *Terra firme* and *Aguajal*. However, it was also expected that Caelifera species richness would be higher for *Regenerating forest*, which has been found to be equal to that of Ensifera with 13 species both. The fourth hypothesis states that Terra firme yields most species, followed by Aguajal and regenerating forest. This has been found to be the case, although the difference is not that great. A total of 31 species was found for *Terra firme* and a total 26 species for the other two habits. However, it is suspected that increased sampling effort would be more lucrative for finding new species in *Terra firme* and *Aguajal*. This assumption is made on the basis that, while the total number of recorded specimens for *Terra firme* is was the lowest of any habitat (only 56), the number of species was the highest (31). This is 50 for *Aguajal* with 26 different species found. By contrast, for the *Regenerating forest* habitat 169 specimens belonged to 26 species. It is thus suspected that the real Species richness of *Aguajal* and especially of *Terra firme*, are still higher compared to *Regenerating forest* than is found in this study.

Further studies could focus their attention on actual species identification, as most specimens in this study were never identified beyond the Family level. A study focusing on environmental variables could better examine their importance when specifically seeking for varying conditions within habitats, which was not done in the current study. A study performed over a longer time period could also capture the effects of seasonality on Orthoptera assemblages.

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