Habitat Selection of Tree Frogs (Hylidae)

The abundance and distribution of Hylids in three tropical forest areas

Mari-lee Odendaal, 23th December 2016

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HAS University of Applied Sciences, ’s-Hertogenbosch
Research report, 23th December 2016

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Mating Agalychnis callidryas : Mari-lee Odendaal
Dendropsophus ebraccatus: Mari-lee Odendaal
Mating Smilisca baudinii: Oriana Nanoa
Hypsiboas rufitelus: Mari-lee Odendaal
Mating Scinax elaeochroa: Mari-lee Odendaal

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Abstract

Amphibian populations are declining rapidly. Because of their rapid disappearance it is important to understand the factors that influence their survival. Two of the factors amphibians are sensitive to are habitat destruction and water pollution. In this research the focus was on the *Hylidae* (tree frogs) family of the Anurans (frogs). *Hylids* are knows to be very dependent on water, they only become active after heavy rainfall. When it rains, *Hylid* males will start calling to advertise themselves to potential partners. During this study the distribution and abundance of different species was monitored in three different areas. The three areas allowed comparison between high and low elevation habitat, as well as secondary and primary forest habitat. Both acoustic and visual encounters of *Hylids* were used to estimate the *Hylid* abundance and ensure maximum data collection. Visual data, location, distance, time, coordinates, species and gender/age, were collected of every individual. When a *Hylid* was detected acoustically, time and coordinates were noted. The sound of the different species was collected and translated into spectrograms. This was done to get a better understanding on their sounds and on auditory competition between different species. Once every two weeks, water quality was analyzed of one pond in all three the areas to see if this effected the distribution and abundance of species. The pH, phosphorous, nitrate, nitrite, copper, iron, oxygen and ammonia concentrations were determined. Furthermore, distribution maps were made to see where which species were situated. Seven species were detected visually and six were detected acoustically. Visual and acoustic data showed that *Hylid* diversity and abundance was the highest in Tortuguero. The Cerro had a less diverse species composition visually than Tortuguero ($p=0.041<0.05$) and Caño Palma ($p=0.014<0.05$) and Tortuguero had a more diverse auditory species composition than Caño Palma ($p=0.00<0.05$) and the Cerro ($p=0.041<0.05$). Tortuguero also seemed to have the most breed-active *Hylids* of all the study sites. In this area most juveniles and females were found compared to the other two areas, indicating that breeding took place and is still ongoing. The location on which *Hylids* were found was not equally divided between the groups ($p=0.00<0.05$). Instead *Hylids* preferred to sit on leaves over the other locations. Rainfall had an effect on the detection of species as well. Rainfall and species activity were significantly correlated in Caño Palma ($p=0.013<0.05$) and Tortuguero ($p=0.005<0.05$). More *Hylids* were detected after a heavy rainfall. Water quality was also generally better in Tortuguero than in the other areas. A low pH and a high iron concentration was the most alarming water quality results found in all three the areas. Furthermore, distribution maps had shown that Hylids are most abundant near rain puddles. Six species were heard in total, five of which called on the same frequency and thus could be potential competitors for each other.

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1. Introduction

Since the 1980's amphibian populations have declined (Collins & Storfer, 2003). Amphibians are thought to be an indicator species of overall environmental health. Unlike other classes of animals, amphibians get their oxygen partially or entirely through their skin and are dependent on water availability for most of their life cycle (Smith & Sutherland, 2014). One of the threats regarding amphibian decline is habitat loss and alteration (Halliday, 2016). Throughout the world, natural habitats are being destroyed to make way for agriculture and human habitation (Halliday, 2016). The change of land use has resulted in removing habitat or preventing access of amphibians to breeding sites (Collins & Storfer, 2003). Furthermore, the increase in agriculture has resulted in highly eutrophic conditions of water sources (Boyer & Grue, 1995). These conditions are detrimental to amphibians and may be associated with frog embryo mortality or malformations (Boyer & Grue, 1995). Because of the threats regarding habitat alteration and destruction, it has become imperative to fully understand the links between threatened amphibians and their habitats. To estimate the threat of future habitat loss to surviving amphibian populations, the factors that influence population abundance need to be investigated (Osborne et al., 2008).

Hylids are rarely found on the ground. They usually do not descend into the waters of streams, ponds or marshes except during the rainy season in order to breed (Heyer & Savage, 1969). For this reason, they are not an easy group to monitor visually. Males use distinctive species-specific calls to advertise their position to potential mates and rivals (Wells, 1977). These species-specific sounds can be used to estimate their abundance.

During this study, the abundance of *Hylid* species of three different areas was studied. The research was conducted to get a better understanding on environmental factors that influence the species diversity and abundance. Factors such location, water quality, weather data and vocal competition were researched. Furthermore, the amount of males, females and juveniles and distance to a water source were studied to give more clarity on the breeding behavior of *Hylids*. The three areas allowed comparison between high and low elevation habitat, as well as secondary and primary forest habitat. Both acoustic and visual encounters were used to estimate the *Hylid* abundance and ensure maximum data collection.

Additionally, water quality parameters of each study area were compared in order to determine how the factors pH, phosphorous, nitrate, nitrite, copper, iron, oxygen and ammonia affect diversity and abundance of *Hylids*. The pH was measured because many substances dissolved in water are liable to changes caused by the pH levels (JBL, 2016). Moreover, measuring the intermediate stages ammonium, nitrite and nitrate allows certain conclusions to be drawn about the function of the system (JBL, 2016), Ammonia, for instance is a highly toxic substance and concentrations that are too high should be avoided. The nitrate and phosphorous levels indicate how trophic the water is. Furthermore, copper and iron are both trace elements and high concentrations are harmful to aquatic life. It was expected that the combination of primary and secondary forest gives the most diverse range of *Hylid* species and results in bigger populations, and that altitude differences grants a different species composition. Additionally, the area with the most favorable water quality will also house a more diverse range of species and a higher amount of individuals.

The effect of rainfall on the species activity was also studied and was expected to have a significant positive effect on the amount of active *Hylids* (Heyer & Savage, 1969). To finish off the project, spectrograms were made to gain a better understanding of the sounds of different *Hylid* species and to make future projects easier (Hopp, et al., 1998). Furthermore, spectrograms will give an insight into sound competition between *Hylids*, as species calling at the same frequency could be potential competitors to each other.

Furthermore, the breeding behavior of species was compared between the different areas. The composition of males, females and juveniles *Hylids* indicates the activity of a species. For instance, lots of juveniles mean that breeding took place and just a few females indicates that the circumstances are not yet optimal for breeding. Moreover, the distance of an individual to a water source gives insights into the activity of species.

2. Methodology

2.1. Area description

The study took place in three different sites in the Tortuguero area, offering different habitat types. The three areas are shown in figure 2.1. The letter A found on the figure indicates the area Caño Palma, B indicates the Cerro and C shows Tortuguero. The climate of the Tortuguero area is relatively wet and receives 5000 mm rain every year. The average temperature is 26 C° and the relative humidity is 70% (*Lewis., et al*). The first transect was behind Caño Palma research station and has some fragmentation. The second transect, the Cerro, is intermittently used for ecotourism and has some habitat fragmentation as well. It is the only site in the Tortuguero area that offers higher elevation habitat. Both Caño Palma and the Cerro are secondary forests. The third transect was located in the Tortuguero National Park, a protected area that has the least habitat fragmentation of the three sites. It also features a mix of both primary and secondary forest.

Figure 2. 1: Landscape map of the Tortuguero area (OCM Landscape, 2012).

2.1.1. Caño Palma Biological station

The Caño Palma area consist of 40 ha Neotropical secondary rainforest. The forest is characterized by a less developed canopy structure, smaller trees, and less diversity. The canopy is less dense than in the other areas resulting in more light on the forest floor that support ground vegetation. The canopy covers only half of the upper part of the forest and trees are relatively shorter in this area than in the other two. The area is generally wet and is known for flooding. When the Caño Palma canal is very high, neighboring areas will flood creating a swamp-like environment. Appendix 1 figure 1A shows a photo of the Caño Palma area.

2.1.2. The Cerro

The area around the Cerro is fragmented and contains a walking path for tourists. The Cerro is an extinct 119-meter-high volcano (OCM Landscape map, 2012) with a moderate dense canopy cover. The trees found in the Cerro are the tallest and widest compared to the other study sites. Additionally, the forest floor material consists almost exclusively of thick leaf litter. The area in the Cerro is relatively dry compared to the other areas, due to its elevation. Appendix 1 figure 1B shows a photo of the Cerro area.

2.1.3. Tortuguero National Park

Being the only study site containing primary and secondary forest, the National Park hosts a wide diversity of flora and fauna. The National Park has a thick canopy cover and supports trees that are moderately high compared to the other two areas. The walking path consist mainly out of sand and stone. The Park is bordered by a village to the north, ocean to the east, and river to the west. Numbers of temporary rain puddles will appear after a heavy rainfall. This attracts a wide variety of *Anuran* species. Appendix 1 figure 1C shows a photo of the Tortuguero area.

2.2. Species description

According to the IUCN Redlist, thirteen *Hylids* can be found in the Tortuguero area. The species are: *Agalychnis callidryas, Agalychnis saltator, Cruziohyla calcarifer, Dendropsophus ebraccatus, Dendropsophus phlebodes, Hypsiboas rufitelus, Scinax boulengeri, Scinax elaeochroa, Smilisca baudinii, Smilisca phaeota, Smilisca puma, Smilisca sordida* and *Tlalocohyla loquax*. All these species are of least concern. The population status of all the species is stable, except for *C. calcarifer* and *A. callidryas*. The population of these two species is decreasing. General information and photographs of the species found during this project are shown in appendix 2.

2.3. Methods

Visual and acoustic encounter surveys were carried out in a period of twelve weeks, from the 10th of September through the 29th of November 2016. During this period each of the three areas were evaluated and data was gathered once a week on nocturnal surveys (19:00-23:00). The transects were all close to 4 kilometers in length. During a survey, two to five surveyors walked the transect slowly using flashlights to detect *Hylids*. The surveyors would approach the sound location when a group of *Hylids* was heard and male *Hylids* were sometimes challenged by mimicking their sound.

For each survey the starting time, ending time, date, cloud cover, moonlight and rainfall were noted. Appendix 3 shows the weather data and general information of the surveys. Additionally, a map of the location of the different *Hylid* populations and the location of the ponds used for mating were made. The maps show the distribution of *Hylids* in all three of the areas on the rainiest day of the study period. Furthermore, the water quality of the main waterbodies was measured.

2.3.1. Data collection

2.3.1.1. Visual encounter surveys

Visual encounter surveys are commonly used to determine the species and to estimate the abundance of an area. This method is only appropriate for those amphibians that can be seen while walking through a habitat (Ernst & Rödel, 2004). The species, position, height, distance to nearest waterbody, gender/maturity, GPS location and GPS accuracy were noted when an individual was detected in sight of the transect. The location variable was nominal and divided into: Ground, branch, stem, leaf and water. The height of an individual from the ground was estimated. The distance from an individual to the nearest waterbody was estimated and divided into: Less than five meters from water source and 20 or more meters from the water source. Distances between the three ordinal groups (0m, <5m and >20m) were large to make estimations easier. The gender and age was divided into male, female and juvenile.

2.3.1.2. Acoustic encounter surveys

Acoustic encounter surveys were used to count calling male *Hylids*. These counts were used to estimate the relative abundances of all adults and species composition (Zimmermann, 1994). A recorder with the sound of all the male *Hylids* was brought on every survey to make detection easier. All the calling males heard during the transect walks were counted. When an individual or a group of individuals was heard, GPS coordinates, GPS accuracy and species were noted.

2.3.2. Analyzing sound

Each *Hylid* species was recorded during the surveys and their sound was translated into spectrograms. Sounds were recorded with the Zoom H5 recorder. A spectrogram is a visual representation of the spectrum of frequencies in a sound as they vary with time. Spectrograms were made with the software Wavepad (NCH Software, 2012). The time range was 30 seconds for every spectrogram and the decibels were corrected in order to fit in the diagram width. The frequencies of other unwanted sounds were removed from the spectrogram. These spectrograms show which species might compete for sound space and gives a visual presentation of different *Hylid* sounds.

Furthermore, the spectrogram shows the total frequency range of the sound and the range on which the sound is most dominant.

2.3.3. Distribution maps

To compare the three sites, distribution maps of visually and acoustically detected *Hylids* were made. This map included vernal ponds where *Hylids* were active. Three maps were made, one map of each area containing visual and acoustic data of the total amount of Hylids found during the study period. Qgis 2.18.0 (Open Source Geospatial Foundation, 2016) was used to make the distribution maps.

2.3.4. Water quality

The water quality parameters such as pH and temperature were also analyzed to see if there was a difference between water qualities in the three areas. The quality of a random vernal pond in each area was measured every two weeks. The variables size, depth, GPS location and temperature of the waterbodies was noted. Additionally, four bottles with 100 milliliter of the water source was taken to analyze the chemical composition of the sample. The parameters pH, phosphorous, nitrate, nitrite, copper, iron, oxygen and ammonia was measured. The fresh water test kit of JBL was used to test the water elements. The pH, ammonium, nitrite, nitrate, iron, phosphorous, and copper was measured according to the JBL instruction book. The thriving concentration of the parameters can be found in appendix 2 table 4. Table 5 of appendix 2 is used to determine the presence of ammonia, using the pH and ammonium value.

2.4. Statistical analysis

All significance was based on 95% confidence intervals, giving an alpha value of 0.05. The program IBM SPSS statistics 23 (IBM Corporation, 2015) was used to test the confidence intervals.

2.4.1. Species abundance

The Pearson Chi-square test was used to determine if the species varies significantly by study site. The null Hypothesis was “The *Hylid* diversity is independent on the area they live in” and the alternative hypothesis was “The *Hylid* diversity is dependent on the area they live in”. Visual and acoustic data of the individuals of all the *Hylid* species was used to test the dependence. Furthermore, the differences in species abundance between the three areas was determined with the one-way ANOVA. The null Hypothesis was “There is not a difference in species abundance between the three areas” and the alternative hypothesis was “There is a difference in species abundance between the three areas”. Again visual and acoustic data of the individuals was tested for differences.

2.4.2. Species activity

The total amount of calling males was used to determine the activity between different *Hylid* species. This was used to show when which species became active and whether species competed for sound space. The linear regression test was used to show if species had an effect on each other’s calling behavior. The null Hypothesis was “There is not a difference in species activity between survey dates” and the alternative hypothesis was “There is a difference in species activity between survey dates. The linear regression test was also used to see if rainfall had a significant effect on the species activity. The null hypothesis for activeness and rainfall was: “Rainfall had no effect on the amount of calling male *Hylids*” and the alternative hypothesis was “Rainfall had an effect on the amount of calling male *Hylids*”

2.4.3. Preferred location

The locations where *Hylids* were found were tested with the Chi-square test. This test showed whether the groups were equally divided or not. The null Hypothesis was "The amount of *Hylids* found on the different location was equally divided". and the alternative hypothesis was "The amount of *Hylids* found on the different location was not equally divided".

2.4.4. Distance to water

The distance of an individual to water is an ordinal variable. The differences between water distance between the different species and the three areas was measured with the Kruskal-Wallis test. The null hypothesis was: "Distance from individuals from water between species is equally divided" and the alternative hypothesis was: "Distance from individuals from water between species is not equally divided". The null hypothesis for the areas was: "Distance from individuals from water between the areas is equally divided" and the alternative hypothesis was: "Distance from individuals from water between the areas is not equally divided". The Mann-Whitney test was used to test the differences pairwise. This was done to find if there were differences between two specific groups.

2.4.5. Ratio of males, females and juveniles

Differences between the ratio of males, females and juveniles found in the three areas was tested with the independent-sample T-test. The null Hypothesis for the fraction males was "There is not a difference in the fraction males found between the three areas" and the alternative hypothesis was "There is a difference in the fraction males found between the three areas". This was also tested for the ratio females and juveniles. The difference between the total amount of males, females and juveniles was tested with the Chi-square. This test showed whether the groups were equally divided or not. The null Hypothesis was "The amount of male, female and juvenile *Hylids* were equally divided". and the alternative hypothesis was "The amount of male, female and juvenile *Hylids* were not equally divided".

3. Results

3.1. Visual and acoustic Hylid data

3.1.1. Visual species diversity and abundance

The *Hylid* species composition detected visually was not dependent on the study site (p=0.062<0.05). There was however a difference in the species abundance seen between the three areas (p=0.018<0.05). The Cerro had a less diverse species composition visually than Tortuguero (p=0.041<0.05) and Caño Palma (p=0.014<0.05).

Table 3.1 shows the total amount of *Hylids* found per species and area. Eleven samples of *H. rufitelus* were found in Caño Palma, 48% of the total amount of *Hylids* seen, making it the most abundant species for the area. Other individuals detected visually in Caño Palma were five *D. Phlebodes*, three *S. baudinii*, *S. elaeochroa* and one *S. Sordida*. Five species were found in total (Table 3.1). Four species were visually detected in the Cerro, with *S. baudinii* being the most abundant one. Thirteen individuals of the *S. baudinii* were found, 46% of the total amount of *Hylids.* Other individuals found were six *H. rufitelus*, six *S. sordida* and three *A. callidryas* (table 3.1). The area in Tortuguero National Park had the highest *Hylid* diversity. Seven species were detected visually. 70% of the found *Hylids* were *S. elaeochroa*, making it the most abundant species with a population of 150 individuals. The other six species were *H. rufitelus* with 23 individuals, *S. baudinii* with seventeen, *A. callidryas* with thirteen, *D. ebraccatus* with nine, S. sordida with two and *D. phlebodes* with one (table 3.1).

Table 3. 1: The total amount of individuals of different Hylid species seen in the three areas the Cerro, Caño Palma and Tortuguero. The abbreviations of the species are as follows: AC: A. callidryas, DE: D. ebraccatus DP: D. phlebodes, HR: H. rufitelus, SB: S. baudinii, SE: S. elaeochroa and SS: S. sordida

Species	Palma	Palma (%)	Cerro	Cerro (%)	Tortuguero	Tort (%)	Total	Total (%)
AC	0	0,00%	3	10,71%	13	6,05%	16	6,02%
DE	0	0,00%	0	0,00%	9	4,19%	9	3,38%
DP	5	21,74%	0	0,00%	1	0,47%	6	2,26%
HR	11	47,83%	6	21,43%	23	10,70%	40	15,04%
SB	3	13,04%	13	46,43%	17	7,91%	33	12,41%
SE	3	13,04%	0	0,00%	150	69,77%	153	57,52%
SS	1	4,35%	6	21,43%	2	0,93%	9	3,38%
Total	23	100,00%	28	100,00%	215	100,00%	266	100,00%

3.1.2. Acoustic species diversity and abundance

The species composition based on auditory detections, was dependent on the study site (p=0.008<0.05). The species abundance heard was also different between the three areas (p=0.02<0.05). Tortuguero had a more diverse auditory species composition than Caño Palma (p=0.00<0.05) and the Cerro (p=0.041<0.05).

Four of the visually found species in Caño Palma were also detected autitory as shown in table 3.2. 80% of the calling males were also *H. rufitelus*, confirming that this is the most abundant *Hylid* species in the area. H. rufitelus was 68 times heard, *A. callidryas* eleven times, *D. phlebodes* five times and *S. elaeochroa* one time. *A. callidryas* was heard but never seen. (Table 3.2). *H. rufitelus* was the most abundant species based on sound samples found in the Cerro. 53% of the *Hylids* heard were calling *H. rufitelus*. *H. rufitelus* was heard eighteen times, *A. Callidryas* nine times and *S. baudinii* seven times (table 3.2). *S. elaeochroa* was not the most heard species in Tortuguero. *A. callidryas* was 44% of all the *Hylids* heard in Tortuguero and *S. elaeochroa* was 40%. 258 *A.*

callidryas and 235 *S. elaeochroa* were heard. *H. rufitelus* was heard 38 times *D. ebraccatus* 32 times, *S. baudinii* 24 and *D. phlebodes* two times. (Table 3.2).

Table 3. 2: The total amount of individuals of different Hylid species heard in the three areas the Cerro, Caño Palma and Tortuguero. The abbreviations of the species are as follows: AC: A. callidryas, DE: D. ebraccatus DP: D. phlebodes, HR: H. rufitelus, SB: S. baudinii, SE: S. elaeochroa and SS: S. sordida

Species	Palma	Palma (%)	Cerro	Cerro (%)	Tortuguero	Tort (%)	Total	Total (%)
AC	11	12,94%	9	26,47%	258	43,80%	278	39,27%
DE	0	0,00%	0	0,00%	32	5,43%	32	4,52%
DP	5	5,88%	0	0,00%	2	0,34%	7	0,99%
HR	68	80,00%	18	52,94%	38	6,45%	124	17,51%
SB	0	0,00%	7	20,59%	24	4,07%	31	4,38%
SE	1	1,18%	0	0,00%	235	39,90%	236	33,33%
SS	0	0,00%	0	0,00%	0	0,00%	0	0,00%
Total	85	100,00%	34	100,00%	589	100,00%	708	100,00%

3.1.3. Species activity

The graph in figure 3.1 shows the species activity of calling males in Caño Palma during different survey dates, figure 3.2 shows the species activity in the Cerro and figure 3.3 indicates the species activity in Tortuguero National Park. All three the graphs also show rainfall on the secondary vertical axis. Rainfall and species activity were significantly correlated in Caño Palma (p=0.013<0.05) and Tortuguero (p=0.005<0.05).

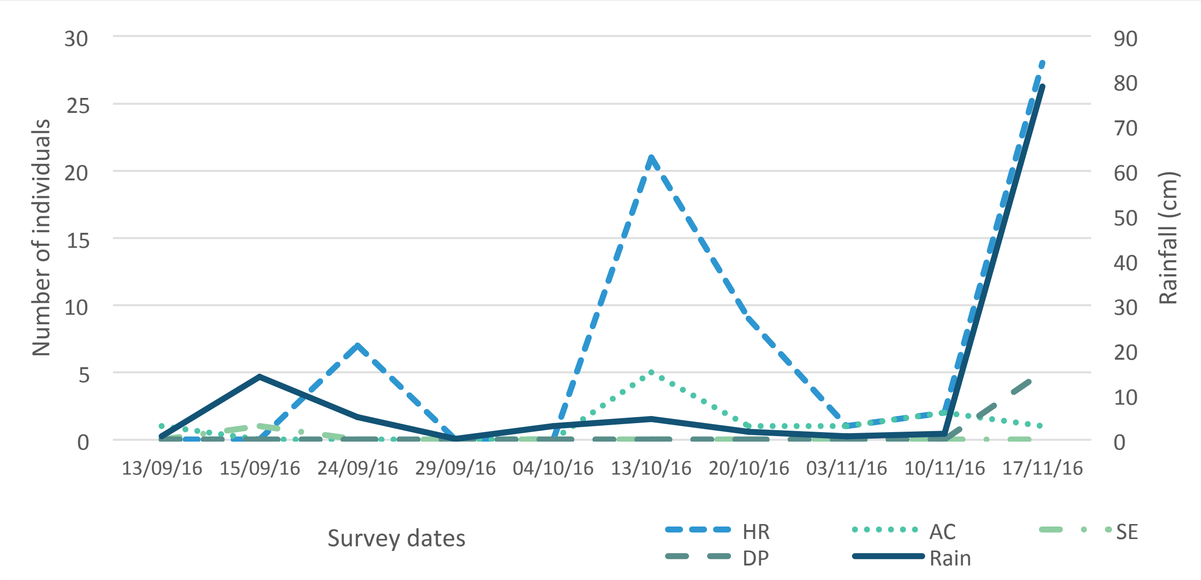

Figure 3. 1: Species activeness and rainfall in Caño Palma on different survey dates. The abbreviations of the species are as follows: AC: A. callidryas, DP: D. phlebodes, HR: H. rufitelus and SE: S. elaeochroa.

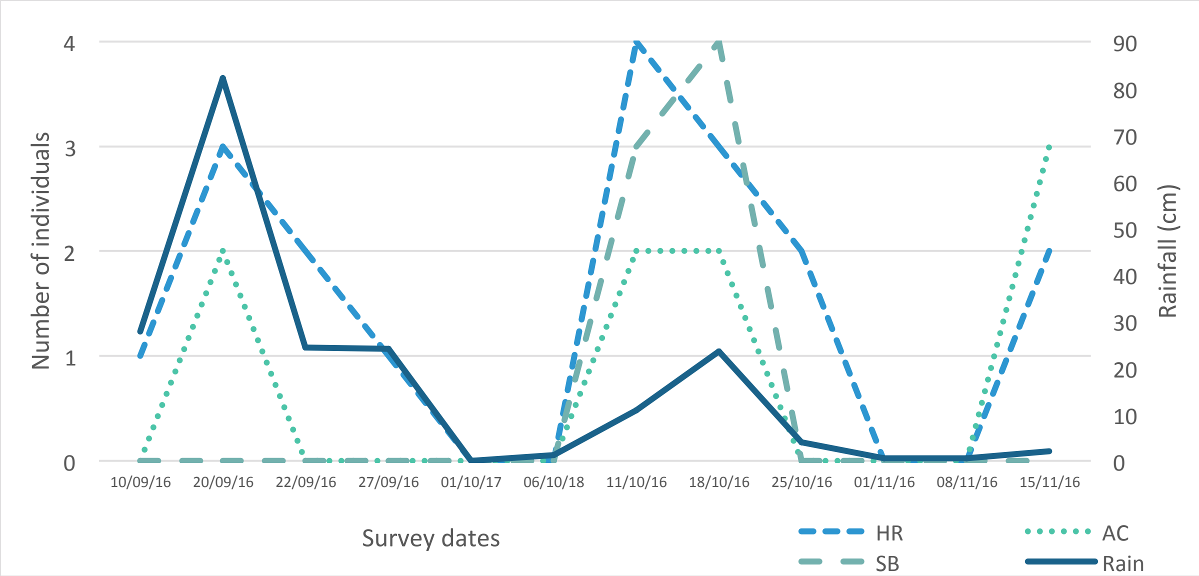

Figure 3. 2: Species activeness and rainfall in the Cerro on different survey dates. The abbreviations of the species are as follows: AC: A. callidryas, HR: H. rufitelus, and SB: S. baudinii.

Competition between calling males from different species was tested for the individuals heard in Tortuguero National Park. In figure 3.3 calling males of *S. elaeochroa* seemed to have a negative effect on the calling males of *A. callidryas*: When males of *S. elaeochroa* increased in activity, the amount of calling males of the *A. Callidryas* decreased. However, this observation was not statistically supported (p=0.672>0.05).

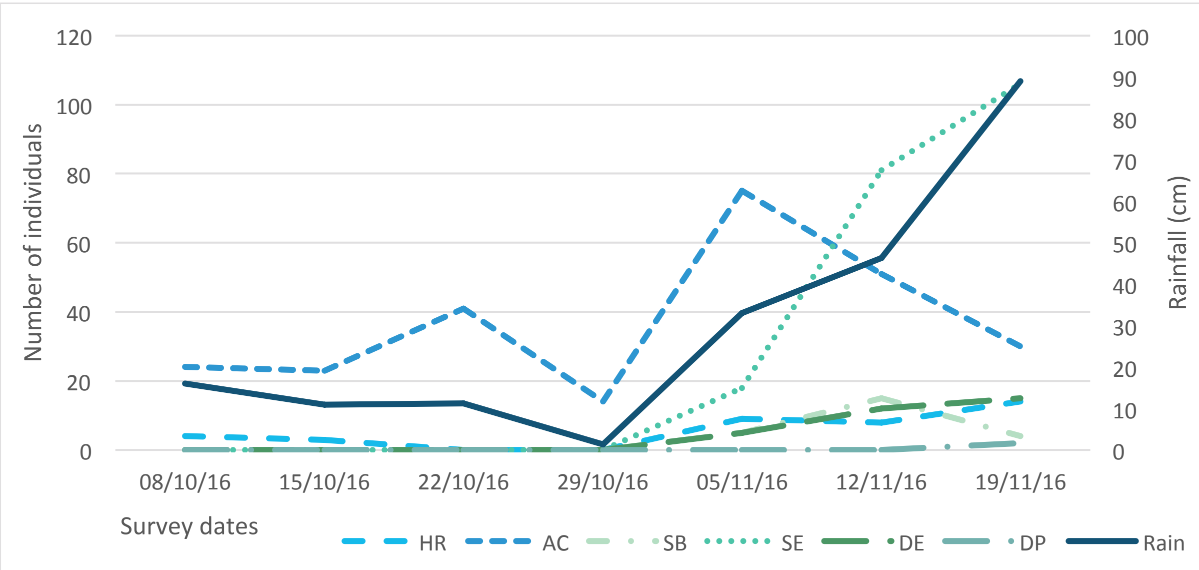

Figure 3. 3: Species activeness and rainfall s in Tortuguero National Prak on different survey dates. The abbreviations of the species are as follows: AC: A. callidryas, DE: D. ebraccatus DP: D. phlebodes, HR: H. rufitelus, SB: S. baudinii, and SE: S. elaeochroa.

3.1.4. Distance to water

The distance of individuals to vernal ponds was significantly different between the three areas (p=0.001<0.05). There was no difference between Caño Palma and the Cerro (0.124<0.05), however, there was between Tortuguero and Caño Palma (p=0.024<0.05). The areas Cerro and Tortuguero had the biggest difference (p=0.000<0.05).

In Tortuguero 73% of the individuals were found directly on the water source. In the Cerro this percentage was 36% and in Caño Palma 35%. 57% of the individuals in Caño Palma were found less than 5 meters from the vernal pond. In Tortuguero 1% of the individuals were found less than 5 meters from the water source and in the Cerro this percentage was 18. 46% Of the individuals in the Cerro were found more than 20 meters from a water source. This percentage was 26% in Tortuguero and 9% in Caño Palma (Figure 3.4). Table 3.3 shows the absolute numbers of individuals found per water distance and area.

Table 3. 3: Absolute numbers of individuals found 0 meters from water, less than five meters from water and more than twenty metres from water in the three areas.

Location	Palma	Cerro	Tortuguero	Total
0 m	8	10	156	168
<5 m	13	5	3	27
>20 m	2	13	56	71
Total	23	28	215	266

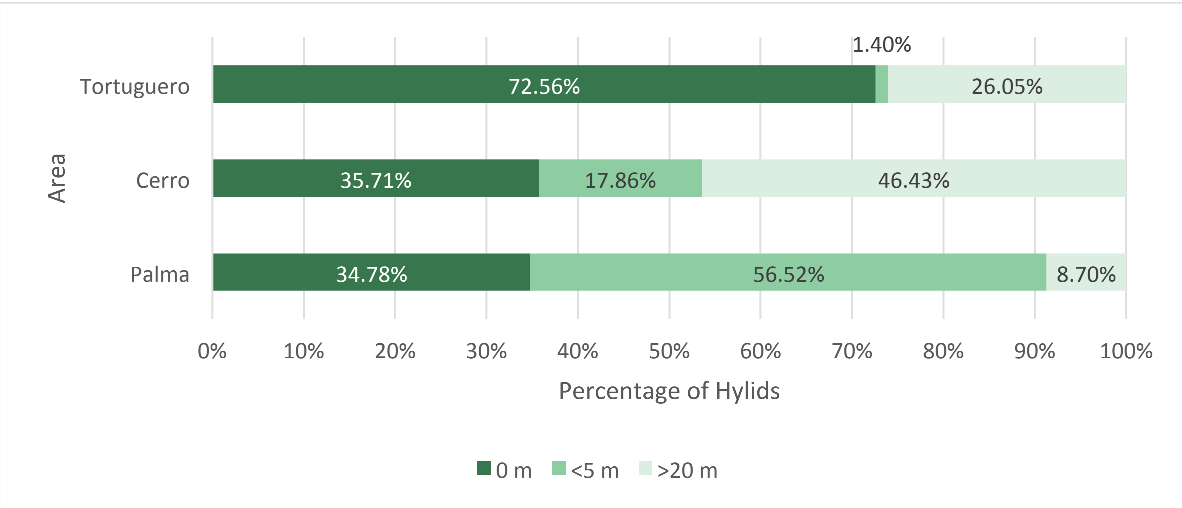

Figure 3. 4: Bar chart of the percentage individuals found on different distances from water (0 meters, less than five meters and more than twenty meters) in the three areas.

The distance of individuals to water was also different between the different species (p=0.00<0.05). *D. ebraccatus* was always directly found on the water source and had a significant difference in distance with the other species (p<0.05), with *D. phlebodes* as an exception (p=0.456>0.05). 89% of the found individuals of *S. sordida* were found more than twenty meters from the water source. This differed from the other species (p<0.05). Table 3.3 shows the absolute numbers of individuals found per water distance and species.

Table 3. 4: Absolute numbers of individuals found 0 meters from water, less than five meters from water and more than twenty meters from water between the different Hylid species. The abbreviations of the species are as follows: AC: A. callidryas, DE: D. ebraccatus DP: D. phlebodes, HR: H. rufitelus, SB: S. baudinii, SE: S. elaeochroa and SS: S. sordida

Species	0 m	<5 m	>20 m	Total
AC	8	2	6	16
DE	9	0	0	9
DP	5	0	1	6
HR	24	11	5	40
SB	15	11	7	33
SE	107	2	44	153
SS	0	1	8	9
Total	168	27	71	266

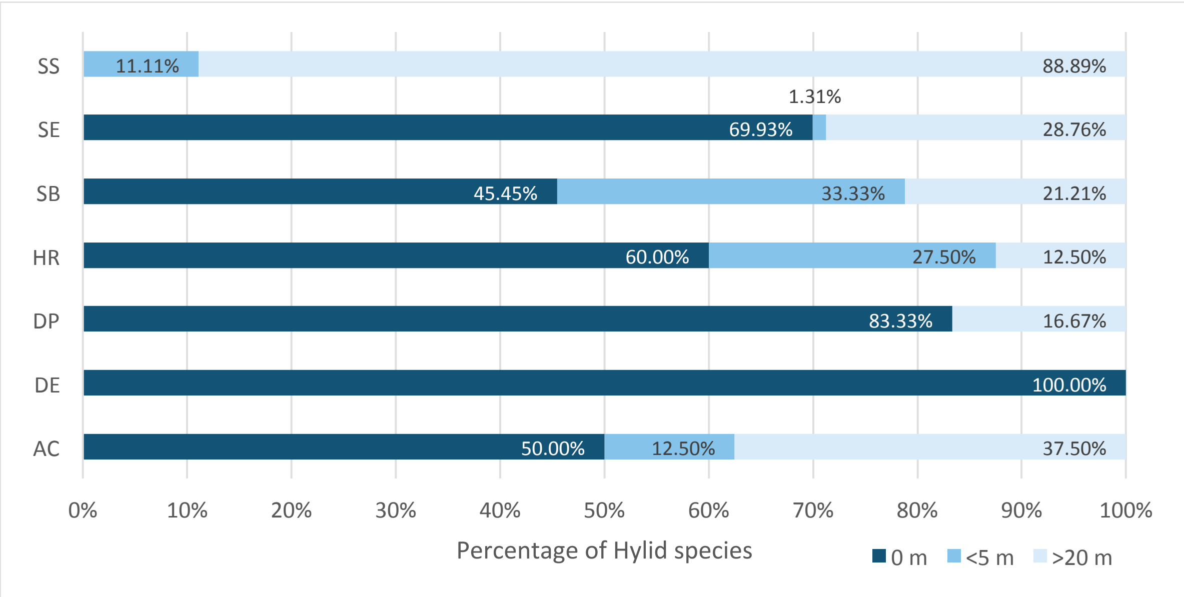

Figure 3. 5: Percentage of individuals found 0 meters from water, less than five meters from water and more than twenty meters from water between the different Hylid species. The abbreviations of the species are as follows: AC: A. callidryas, DE: D. ebraccatus DP: D. phlebodes, HR: H. rufitelus, SB: S. baudinii, SE: S. elaeochroa and SS: S. sordida

3.1.5. Species location

The location on which *Hylids* were found was not equally divided between the groups (p=0.00<0.05). Instead *Hylids* preferred to sit on leaves over the other locations. 199 *Hylids* were found sitting on leaves (Table 3.5), making it 75% of the total amount of *Hylids* found. 11% of the *Hylids* were found sitting on stems, 6% was found in water, 5% on branches and 1% on grass. 2% of the *Hylids* were found sitting on other locations such as walls, trunks, logs and leaf litter (Figure 3.6).

Table 3. 5: Amount of Hylids found on different locations. The location other consist of the following groups: Log, wall, trunk and leaf litter.

Location	Leaf	Stem	Water	Branch	Grass	Other
Hylids	199	30	17	12	4	4

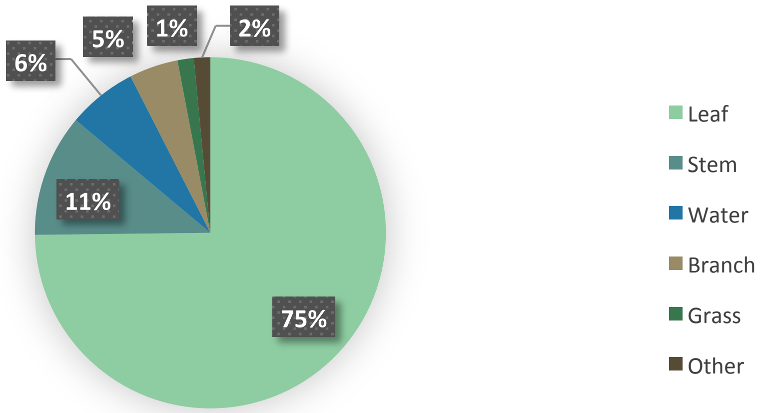

Figure 3. 6: Pie Chart of the percentage of individual Hylids found on different locations. The location other consist of the following groups: Log, wall, trunk and leaf litter.

3.1.6. Males, females and juveniles

Visual detected male *Hylids* were most common in all of the three areas. In Tortuguero 60% of the *Hylids* found were male, in the Cerro this percentage was 65 and in Caño Palma 83% of the *Hylids* were males. The ratio males found in Tortuguero differed significantly with the ratio males found in Caño Palma ($p=0.027<0.05$). The ratio of males found in the Cerro did not differ significantly with the ratio of males found in the other two areas ($p>0.05$). In the Cerro 25% of the *Hylids* found were females, in Caño Palma this was 6% and in Tortuguero 16% females. There were no significant differences found between the three areas ($p>0.05$). In Tortuguero most juveniles (24%) were found compared to the other areas (Figure 3.7). In the Cerro 10% were juveniles and in Caño Palma 11%. There were no significant differences found between the three areas ($p>0.05$). Table 3.6 shows that absolute numbers of males, females and juveniles found in the three study sites.

The total amount of males differed significantly from the total amount of females and juveniles ($p=0.00<0.05$). The amount of females also differed from the total amount of juveniles ($p=0.017<0.05$).

Table 3. 6: The amount of male, female and juvenile Hylids found in the three areas.

Type	Palma	Cerro	Tortuguero	total
Males	15	13	130	158
Females	1	5	34	40
Juveniles	2	2	51	55
Total	18	20	215	253

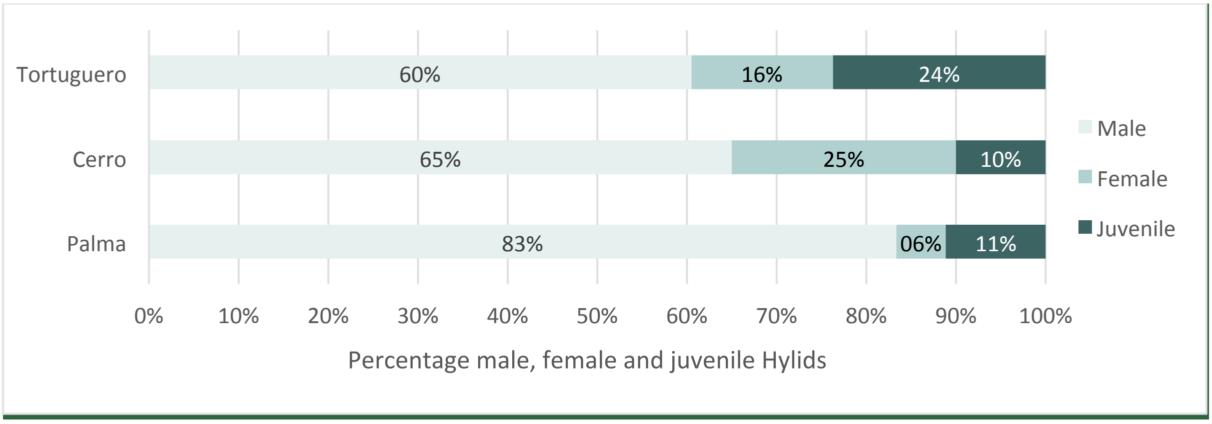

Figure 3. 7 Percentage of male, female and juvenile Hylids found in the three area

3.2. Habitat selection

3.2.1. Water quality

Table 3.7 shows the water quality found in ponds in the three areas. The nitrate, copper and oxygen concentrations found during the water quality analysis were all on a healthy level. The pH was predominately low, except for one pond in Tortuguero. Some ponds had ammonia and nitrite concentrations that were too high. Phosphorous was also sometimes found in a too high concentration. The most alarming result however was the amount of iron found in the ponds. Iron concentrations were almost never on a low level except for two ponds measured in Tortuguero. The overall quality of the ponds found in Tortuguero seemed to be better than the ponds in Caño Palma and the Cerro (Table 3.7). Ammonium concentrations were on a healthy low level because of the low pH (Appendix 3 table 1 and 2).

Table 3. 7: Water quality of ponds in the three areas. Water data taken once every two weeks.

Date	Area	Temp. (C)	pH	NH4 (mg/l)	NO2 (mg/l)	NO3 (mg/l)	PO4 (mg/l)	Fe (mg/l)	Cu (mg/l)	O2 (mg/l)
11/09/16	Cerro	27	6.0-6.5	0.6	0.025-0.05	1.0	0.5	1.5	<0.1	10
01/10/16	Cerro	26	6.5	0.1	0.1	0.5-1.0	<0.1	1.5	<0.1	10
14/10/16	Cerro	26	6.5	0.2	0.01-0.025	<0.5	0.5	1.5	<0.1	10
13/09/16	Palma	26	6.0	0.1	<0.01	0.5-1.0	<0.1	0.8	<0.1	10
29/09/16	Palma	27	5.0	0.05	0.025	0.5-1.0	0.25	1.5	<0.1	10
18/10/16	Palma	26	6.0-6.5	0.1	<0.01	<0.5	<0.1	1.0	<0.1	10
10/11/16	Palma	26	5.0	0.1	<0.01	<0.5	<0.1	1.0	<0.1	10
17/11/16	Palma	26	6.5	0.1	<0.01	<0.5	0.25	0.2	<0.1	10
15/10/16	Tort.	25	7.0	0.2	0.2	1.0	0.5	0.2	0.1	10
05/11/16	Tort.	26	6.5	0.1	0.025	<0.5	<0.1	0.1	<0.1	10
12/11/16	Tort.	26	6	<0.05	<0.01	<0.5	0.5	0.05	<0.1	10

3.2.2. Distribution maps

The maps in figure 3.8, 3.9 and 3.10 shows the visual and auditory distribution of the total amount of *Hylids* found on every survey date. The maps also show which vernal pond is the most popular among *Hylids*. A very blue pond means that choruses of *Hylids* were often found near the pond: The color indicates the amount of surveys on which *Hylids* were found near the pond. Furthermore, the red color in the maps shows the amount of *Hylids* detected visually and auditory: The darker the color the more *Hylids* were found near the same location. All three the maps show that *Hylids* cluster around ponds and are heard mostly around water sources. In some areas of Tortuguero the number of *Hylids* detected near each other acoustically was more than 24, with the highest amount being around 54.

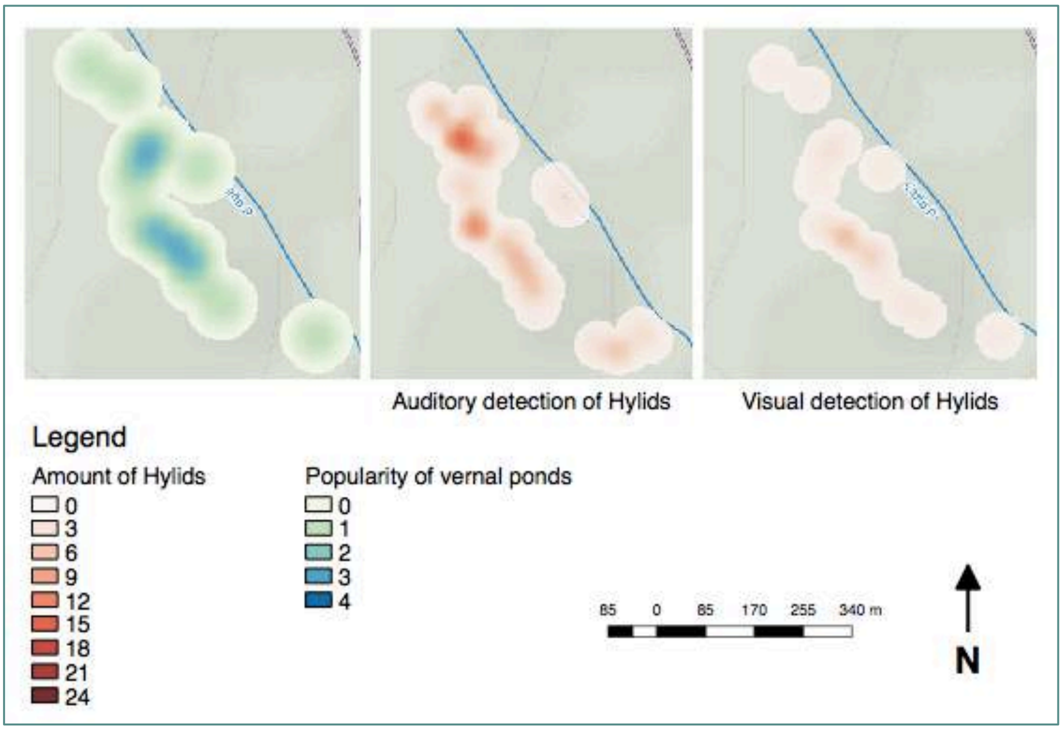

Figure 3. 8: Distribution of auditory and visual Hylids in Cáno Palma.

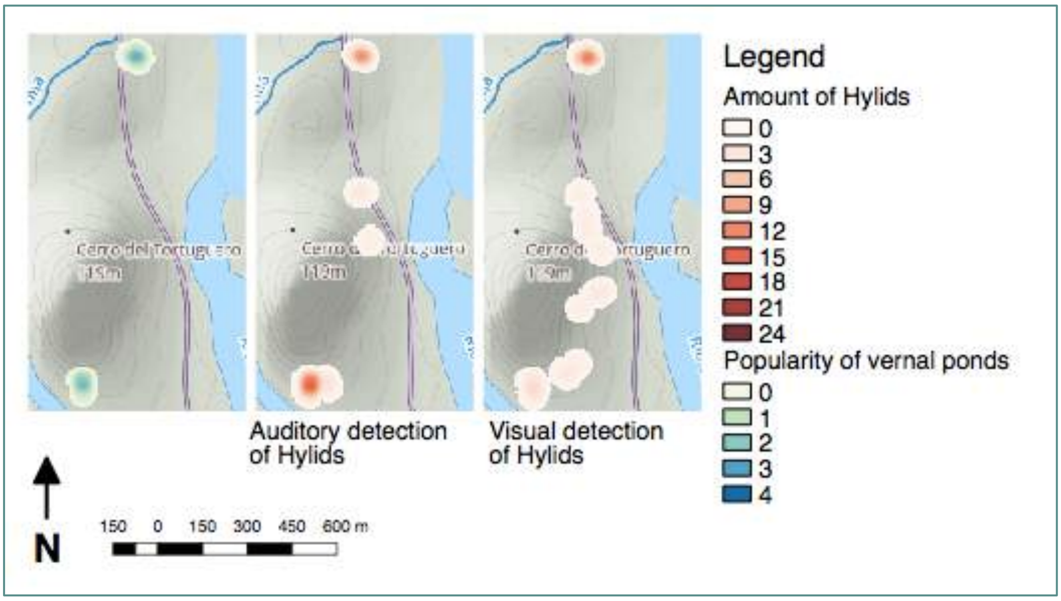

Figure 3. 9: Distribution of auditory and visual Hylids in the Cerro.

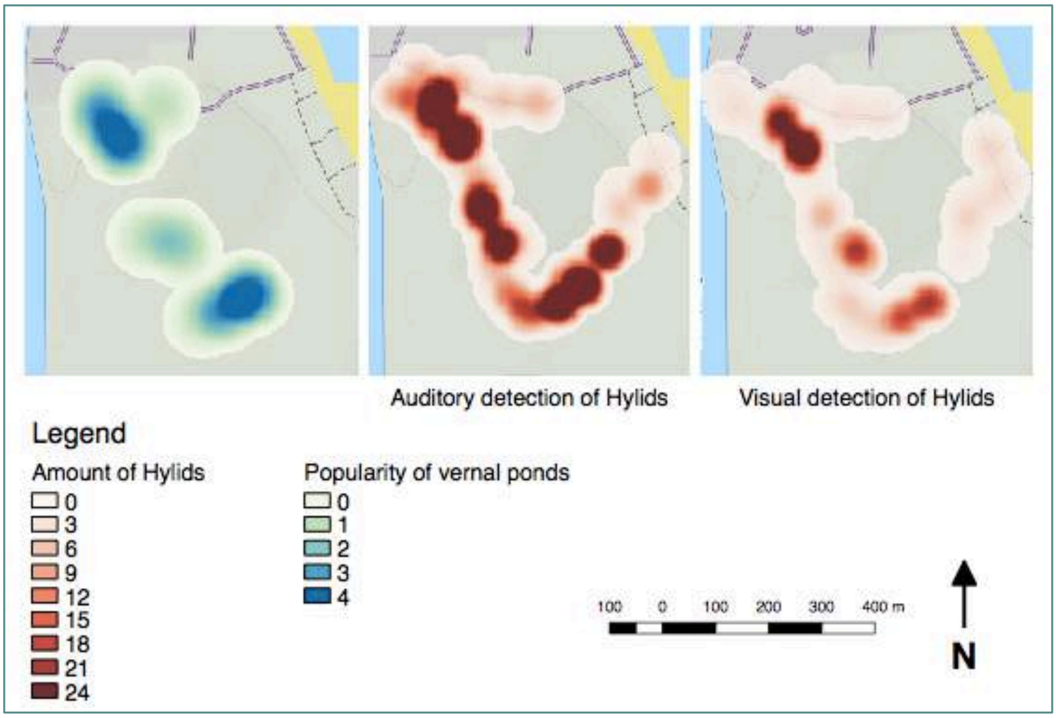

Figure 3. 10: Distribution of auditory and visual Hylids in Tortuguero.

3.3. Hylid sounds

Almost all the *Hylid* species found during this study called at frequencies between 500 and 2500Hz. *D. ebraccatus* called on a very wide frequency range of 0 to 10000Hz, with the dominant sound from 3300 to 6600Hz. *A. callidryas* called on a range of 500 to 2000Hz with a dominant sound on 1000 to 1500Hz. *H. rufitelus* and *S. baudinii* almost called on the same frequencies. *H. rufitelus* called on 700 to 2400Hz and *S. baudinii* on 500 to 2500Hz. The dominant sound of both was on 1000 to 2000/2100 Hz. *S. elaeochroa* called on 1300 to 1800Hz (Figure 3.8). Appendix 3 shows the spectrograms of the different species.

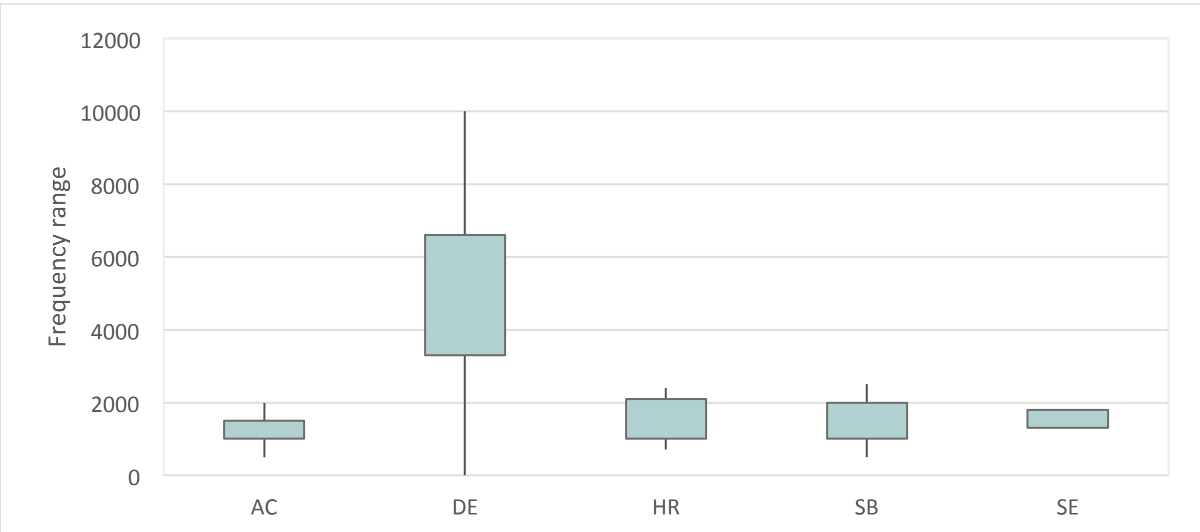

Figure 3. 11: Frequency range of the Hylid species. The abbreviations of the species are as follows: AC: A. callidryas, DE: D. ebraccatus, HR: H. rufitelus, SB: S. baudinii, and SE: S. elaeochroa.

4. Discussion and conclusions

4.1. Habitat selection of species

The Tortuguero National park houses most *Hylid* species and had the highest total amount of *Hylids*. Seven species were found and eight were heard in this area. In Caño Palma five species were found and four species were heard. In the Cerro only four species were found and just three of them were heard. 215 of the 266 *Hylids* were found in Tortuguero and 589 were heard of the 708. This preference was expected since the area is the only one known for its primary and secondary forest. Results also found that general water quality was better in the National Park than in the other areas.

Other factors such as human impact could have had an effect on the *Hylid* abundance in the three areas as well. The Cerro has relatively crowded surroundings. Frequent music coming from the neighboring village could compete with the calling males in the areas, making them difficult to hear for potential mating partners. Tortuguero knows noisy surrounding too. A notably fewer amount of *Hylids* were found on the forest border, next to the village. This same effect was not observed deeper in the forest. According to past research anthropogenic sounds differentially affect amphibian call rate: some anurans will decrease their calling rate while other will increase it (Sun & Narins, 2004). This supports the theory that *Hylids* prefer to live in quieter surroundings.

Moisture of the areas could have had an effect on the species abundance as well. Both Tortuguero and Caño Palma were generally wet areas after a heavy rainfall. The areas flooded when there was a lot of rainfall, connecting all the ponds with each other. Caño Palma was slightly more sensitive to rainfall than Tortuguero. The Cerro However was the highest area, making it the dryest area of the three. Rain that landed in the Cerro would flush to surrounding areas with a lower elevation. Sometimes the Cerro was so dry that there were no ponds to collect water from for water quality analyses.

Another factor that might have an effect on the *Hylid* abundance is the presence of fish in the waterbodies. Tadpoles of *Hylids* may serve as a prey for the fish inhabiting the area, resulting in a smaller frog population. In Caño Palma fish are present in the semi-permanent ponds. These fish were likely to come to inhabit the area after flooding of the area with water from the canal. Caño Palma is the only area where extensive flooding frequently takes place (Semlitsch & Gibbons, 1988).

4.2. Species populations and activity

Seven species were found in total and six species were heard. *S. sordida* was detected visually but not acoustically. One potential reason for this is that they typically breed in the beginning of the dry season, while monitoring took place from the end of the dry season to the beginning of the rainy season. This might also explain why so few species were found.

In general, *H. rufitelus* was the first to be active during the study period, at the end of the dry season. The species would both call on dry days and on heavy rainy days (Figure 3.1, 3.2 and 3.3). More *H. rufitelus* were however heard when rainfall increased. This species seemed to have a moderate abundant population in all of the areas. Visual males were found in all three areas. Females were not often found, indicating that males start calling early in the season. Juveniles were also found in the Cerro and Tortuguero showing that mating did take place.

A. callidryas was the second species to become active during the study period, at the end of the dry season. This species seemed to be quieter when other species became active. This is supported by the fact that *A. callidryas* called at the same frequency as *S. elaeochroa, S baudinii and H. rufitelus* (Figure 3.11). When calling of *S. elaeochroa* increased, the calling of *A. callidryas* decreased (Figure 3.3), seeming like the two compete for sound space. The sound of *A. callidryas* is soft and their call contains a short single note, making it easy for other species to overcome. 44% of the *Hylids* heard in Tortuguero were *A. callidryas* declaring the population to be very stable in the area. The stableness of *A. callidryas* population in the other two areas is questionable. Males and females were found of this species. Juveniles however were rarer to find indicating that the species does not mate early in the rainy season or that they lived high up in the trees.

Moreover, groups of *S. baudinii's* made sounds together and when approached they would stop calling. The species became active after a heavy rain. No juveniles were found of this species. This indicates that mating does not take place early in the season.

S. elaeochroa was by far the most abundant species found in the Tortuguero area. 70% Of the *Hylids* detected visually were *S. elaeochroa* and 40% of the calling males were of this species. *A. callidryas* was 44% of the total amount of *Hylids* found with auditory detection. Counting the calling *S. elaeochroa* males was however difficult. This was due to the fact that individuals did not take a break during their calls and choruses were sometimes too big and noisy to count the exact amount of individuals participating. Juveniles were found early on, showing that this species had mated early in the season.

Furthermore *D. ebraccatus* became active during heavy rains. *D. phlebodes* was the last species to become active during the study period, in the beginning of the rainy season. The population size and mating behavior of these two species is still very unknown in all three of the areas.

4.3. Water quality

The most alarming results found were the iron concentration and the low pH of the water in all the areas. More iron was found in the ponds located in the Cerro and Caño Palma than in the vernal ponds situated in Tortuguero. An explanation for this is that Caño Palma becomes flooded with iron rich water from the canal. The Cerro on the other hand is a drainage area that collects water that run down the old volcano. Water running down the hill contain a lot of sediment which contains iron. There is little know about the toxicity of high iron concentrations on amphibian larvae. It has generally been overlooked as a potential toxin for amphibians. Past research however have shown that a high iron concentration increased the mortality rate of larvae from the Jefferson salamander (Home & Dunson, 1994).

A low pH also has a negative effect on amphibian species. Past studies have shown that a pH lower than 4.5 have a significant effect on the embryonic mortality of certain salamander species. Acidic deposition has been suggested as a cause of a low pH commonly encountered in amphibian habitats (Sadinski & William, 1992).

4.4. Difficulties

Other factors that may have had an effect on the results included weather inconsistency, similar sounds and harsh conditions. As seen in table 3.7 in the result section, water quality for the Cerro was not always measured. This was due to the fact that sometimes no ponds were found in the area because of dry periods. Sound similarities of *D. ebraccatus* and *D. phlebodes* could have had an effect on the results as well. It was sometimes unclear from which species the calling male came. The assumption was based on visual data of the *Hylids*. Moreover, as a result of a hurricane, data gathering had to be a week shorter, resulting in three less surveys.

4.5. Other findings

An interesting finding was the oversize of some individuals. Some *H. rufitelus* individuals found were more than six centimeters in length, while the maximum size for the species is 5.5 centimeters. The biggest *H. rufitelus* found was eight centimeters long. Three *S. baudinii's* found were also bigger than the maximum size for the species. All three of them were ten centimeters while the maximum size is nine. Individuals found of *S. sordida* were also oversized. The maximum size for this species is 5.6 centimeter and the biggest one found was 7.5 centimeters. Appendix 6 includes a picture of an oversized *S. baudinii* with a measurements shown.

4.6. Conclusions

The National Park of Tortuguero, the area containing a mixture of primary and secondary forest had the largest *Hylids* diversity and abundance of all three the areas. The extinct volcano, the Cerro, had the least amount of individuals. These findings were both found with visual and acoustic data. Visual data also showed that the female and juvenile ratio was highest in Tortuguero, indicating that the conditions were more acceptable for breeding than in the other study sites. Furthermore, 75% of all the *Hylids* found visually, were found sitting on a leaf, making it their preferred location.

Moreover, rainfall had a significant effect on the *Hylid* activity, but sound competition between different species was not significantly proven. Spectrograms however show that sound competition could be possible between *A. callidryas, H. rufitelus, S. baudinii* and *S. elaeochroa,* because their calls exist on the same frequency range. Furthermore, the vernal ponds in all three the areas had a low pH of 5 to 6.5 and contained high iron concentrations. Water quality results found from water from Tortuguero were more positive than that of the other areas. Distribution maps show that *Hylids* cluster around ponds and are most often found near water. This was also shown with the visual data on the distance of individuals to water sources. Most of the *Hylids* were found close to the water source. In the Cerro most individuals were found more than twenty meters away from a water source. This was due to the area being very dry and not having any ponds.

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Appendix 1: Research areas

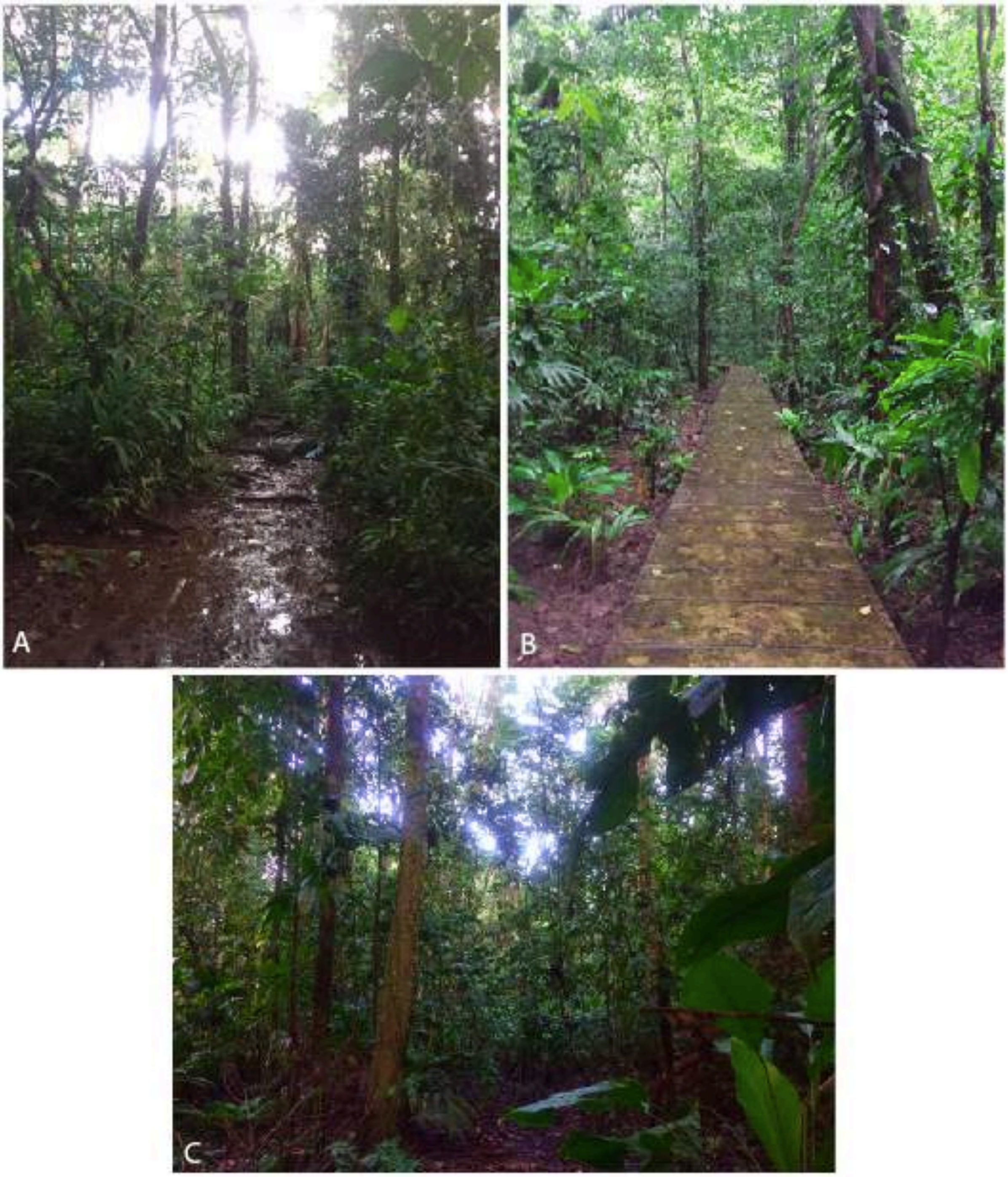

Figure 1: Research areas: A = Cano Palma; B = the Cerro; C = Tortuguero National Park.

Appendix 2 Survey and weather data

Table 1: General survey information and weather data.

Date	Time	Area	Cloud cover(%)	Rainfall(cm)
01/10/16	19:10-21:50	Cerro	0	0
04/10/16	19:10-21:45	Palma	100	3
25/10/16	19:25-21:15	Cerro	25	4
24/09/16	18:50-21:00	Palma	25	5
15/10/16	19:30-21:30	Tortuguero	75	11
15/09/16	19:10-21:30	Palma	100	14
08/10/16	19:40-21:15	Tortuguero	25	16
27/09/16	19:10-21:10	Cerro	75	24
05/11/16	19:20-22:20	Tortuguero	100	33
19/11/16	19:15-22:10	Tortuguero	100	89
29/09/16	19:10-21:00	Palma	0	0.2
08/11/16	19:10-21:45	Cerro	25	0.5
01/11/16	19:10-21:30	Cerro	75	0.6
13/09/16	19:00-21:00	Palma	75	0.8
03/11/16	19:00-21:30	Palma	50	0.8
06/10/16	19:45-21:15	Cerro	100	1.2
29/10/16	19:10-21:35	Tortuguero	0	1.3
10/11/16	19:00-21:55	Palma	75	1.3
20/10/16	19:00-21:20	Palma	50	1.8
11/10/16	19:35-21:15	Cerro	100	10.8
22/10/16	19:25-22:00	Tortuguero	100	11.2
15/11/16	19:05-21:05	Cerro	75	2.0
18/10/16	19:10-21:30	Cerro	100	23.5
22/09/16	19:40-21:45	Cerro	100	24.3
10/09/16	19:15-22:30	Cerro	50	27.7
13/10/16	19:05-21:05	Palma	100	4.6
12/11/16	19:30-22:20	Tortuguero	100	46.3
17/11/16	19:05-21:30	Palma	75	78.8
20/09/16	19:15-21:30	Cerro	100	82.2

Appendix 3: Species description

Table 2: General information on the Hylid species found in the three areas: The Cerro, Caño Palma and Tortuguero (Part 1).

Agalychnis callidryas	**Red-eyed Treefrog**
Size: F: <77mm and M: <59mm	Altitude: up to 1,250m
Distribution: Belize, Colombia, Costa Rica, Guatemala, Honduras, Mexico, Nicaragua and Panama.	
Habitat: inhabits tropical lowland and montane forest, where there is a continuous forest cover. It can live in secondary forest, and even very heavily degraded areas.	
Breeding: occurs during the wet season, beginning with the first rains. Prefers quiet pools with overhanging vegetation as breeding sites.	
Status: least concern and abundant, but decreasing very slowly. They are threatened by habitat loss by the destruction of forests. Also recorded within the pet trade.	

Figure 3: Two mating Agalychnis callidryas in the Cerro. Picture taken by Mari-lee Odendaal

Dendropsophus ebraccatus	**Hourglass Treefrog**
Size: F: <37mm and M: <28mm	Altitude: up to 1,600m
Distribution: Belize, Colombia, Costa Rica, Ecuador, Guatemala; Honduras, Mexico, Nicaragua, Panama.	
Habitat: Of humid tropical forest, including primary and secondary forest and forest edge. Also in heavily disturbed areas where most of the forest has been removed.	
Breeding: The breeding season is largely concordant with the rainy season. The eggs are placed on leaves overhanging temporary pools.	
Status: Least concern and stable: Common to abundant. Very adaptable and has been found in very open landscapes in Costa Rica. The major threat is deforestation.	
Dendropsophus phlebodes	**San Carlos Treefrog**
Size: <28mm	Altitude: 20-620m
Distribution: Colombia, Costa Rica, Nicaragua, Panama	
Habitat: It inhabits lowland humid forest, but may also occur in disturbed habitats. Present wherever there are swampy or wet habitats.	
Breeding: It is an explosive breeder following torrential rains. The eggs are deposited in small masses on the surface of shallow breeding ponds.	
Status: least concern and stable. Within forests it is a relatively rare species. Very adaptable species. Deforestation and pollution are its main threats.	

Figure 2: Dendropsophus ebraccatus sitting on a hand in Tortuguero. Picture taken by Mari-lee Odendaal

Figure 4: Dendropsophus phlebodes sitting on grass in Caño Palma. Picture taken by Mari-lee Odendaal

Table 3: General information on the Hylid species found in the three areas: The Cerro, Caño Palma and Tortuguero (Part 2).

Hypsiboas rufitelus	**Canal zone Treefrog**
Size: M: 39-49mm and F: 46-55mm	Altitude: up to 650m
Distribution: Costa Rica, Nicaragua, Panama.	
Habitat: It inhabits humid lowland forest and tolerates some disturbance. It can be found in open areas, but this needs to be close to forest	
Breeding: reproduces in swamps surrounded by trees.	
Status: least concern and stable. It is locally common in the appropriate habitat. Major threats include habitat loss by the destruction of natural forests.	

Figure 5: Hypsiboas rufitelus sitting on a finger in the Cerro. Picture taken by Mari-lee Odendaal

Scinax elaeochroa	**Narrow headed Treefrog**
Size: F: <40mm and M: <30mm	Altitude: up to 1,200m
Distribution: Colombia, Costa Rica, Nicaragua, Panama	
Habitat: It is primarily a nocturnal species of lowland, and marginally montane, tropical forest. It can also be found in secondary and disturbed forest habitats.	
Breeding: It breeds in temporary ponds during the wet season. Eggs are laid in the pond, or on adjacent vegetation, and the larvae develop in the ponds.	
Status: Least concern and stable. It is a very common species. Deforestation and pollution are its main threats.	
Smilisca baudinii	**Mexican Treefrog**
Size: 47-90mm	Altitude: up to 1,610m
Distribution: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, United States.	
Habitat: Knows a wide variety of habitats. It hides underground, under tree bark, in leaf axils, or in tree holes when inactive.	
Breeding: It breeds in ponds.	
Status: least concern and stable. This is a well-preserved species that can survive even associated with human settlements and altered habitats.	

Figure 5: Two Scinax elaeochroa mating in Tortuguero. Picture taken by Mari-lee Odendaal

Figure 6: Two Scinax baudinii mating in Tortuguero. Picture taken by Mari-lee Odendaal

Smilisca sordida	Drab Treefrog
Size: F: <56mm and M: <36mm	Altitude: up to 1,525m
Distribution: Colombia; Costa Rica; Honduras; Nicaragua; Panama	
Habitat: It inhabits humid lowland and montane forest in the vicinity of rocky streams where it reproduces. It can tolerate substantial disturbance of its habitat.	
Breeding: Breeding occurs during the dry season when streams are low. Males call from rocks in streams. Large numbers of females appear at streams after heavy rains.	
Status: Is of least concern and stable. It is not facing and significant threats.	

Table 4: General information on the Hylid species found in the three areas: the Cerro, Caño Palma and Tortuguero (part 3).

Figure 7: Smilisca sordida sitting on leaf. Picture taken by Mari-lee Odendaal

Appendix 4: Water parameters

Table 1: Water quality parameter for fresh water fish according to JBL.

pH	3.0	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10
NH4 (mg/l)	<0.05	0.1	0.2	0.4	0.6	1.0	1.5	3.0	5.0					
NO2 (mg/l)	<0.01	0.025	0.05	0.1	0.2	0.4	0.6	0.8	1.0					
NO3 (mg/l)	<0.5	1.0	5.0	10	20	40	80	160	240					
Fe (mg/l)	<0.02	0.05	0.1	0.2	0.4	0.6	0.8	1.0	1.5					
PO4 (mg/l)	<0.02	0.05	0.1	0.2	0.4	0.6	0.8	1.2	1.8					
O2 (mg/l)	<0.2	1.0	2.0	4.0	6.0	8.0	10							
Cu (mg/l)	<0.1	0.1	0.2	0.3	0.45	0.6	0.8	1.2	1.6					

	Good
	Acceptable
	Harmful

Table 2: Table to determine the NH3 concentration in water using the pH and NH4 concentration.

		NH4 (mg/l ppm)							
		0.1	0.2	0.4	0.8	1.2	2.0	3.0	5.0
pH	7.0								
	7.5								
	8.0								
	8.2								
	8.4								
	8.6								
	8.8								
	9.0								

	Could be harmful
	Harmful
	Could be lethal
	Absolutely lethal

Appendix 5: Spectrograms

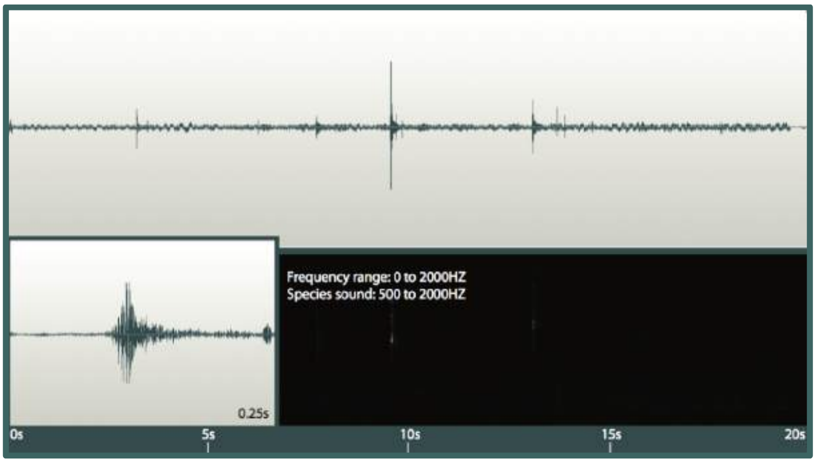

Figure 1: Sound spectrogram of A. callidryas in a 20 second time interval. Frequency range: 0-2000HZ. Species sound: 500-2000HZ.

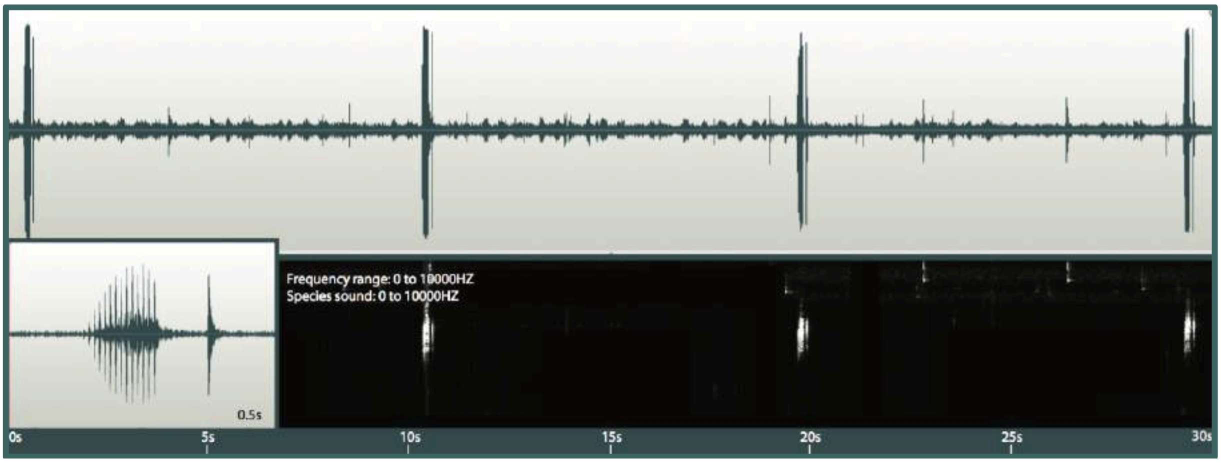

Figure 2: Sound spectrogram of D. ebraccatus in a 30 second time interval. Frequency range: 0-10000HZ. Species sound: 0-10000HZ.

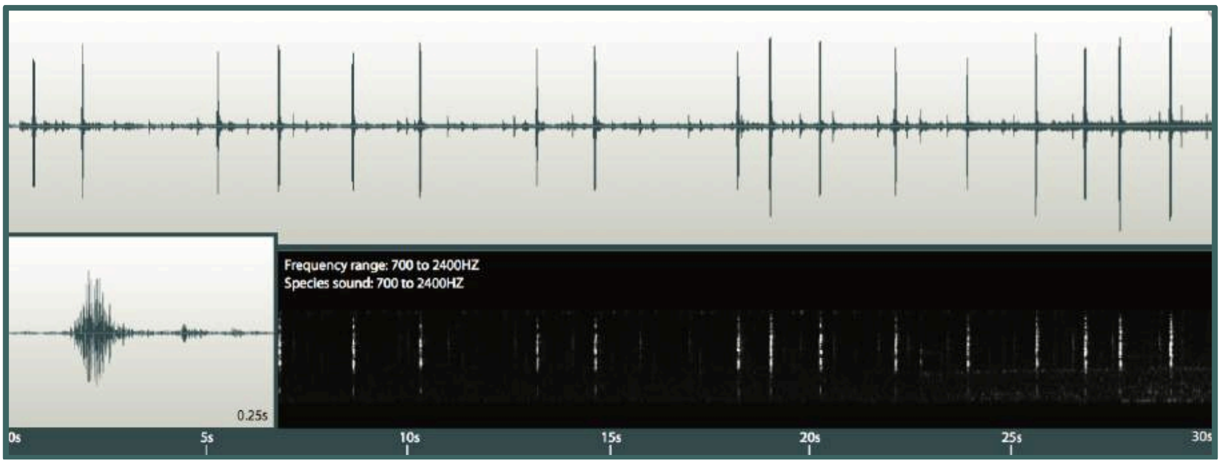

Figure 3: Sound spectrogram of H. rufitelus in a 30 second time interval. Frequency range: 700-24000HZ. Species sound: 700-2400HZ.

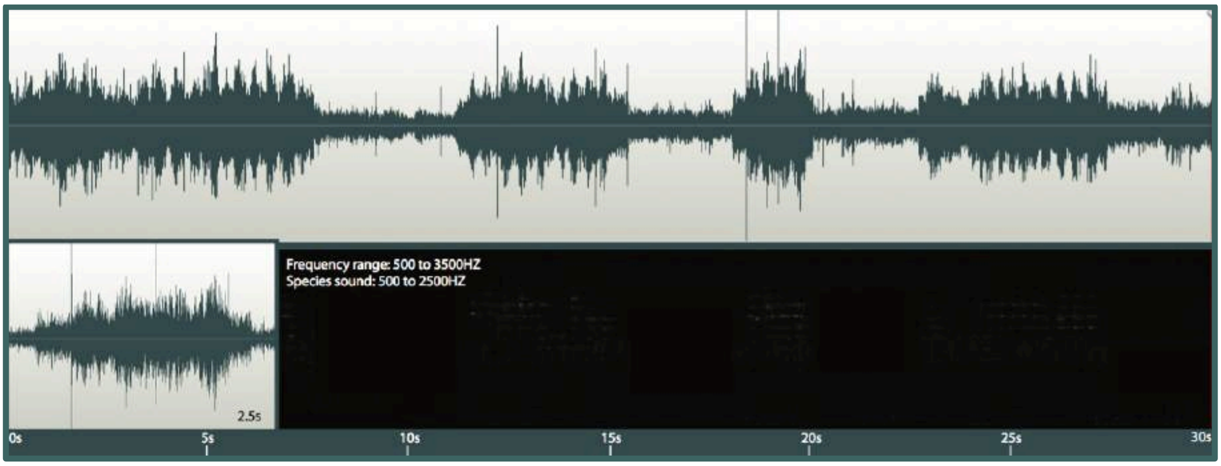

Figure 4: Sound spectrogram of a group of S. baudinii's in a 30 second time interval. Frequency range: 500- 3500HZ. Species sound: 500-2500HZ.

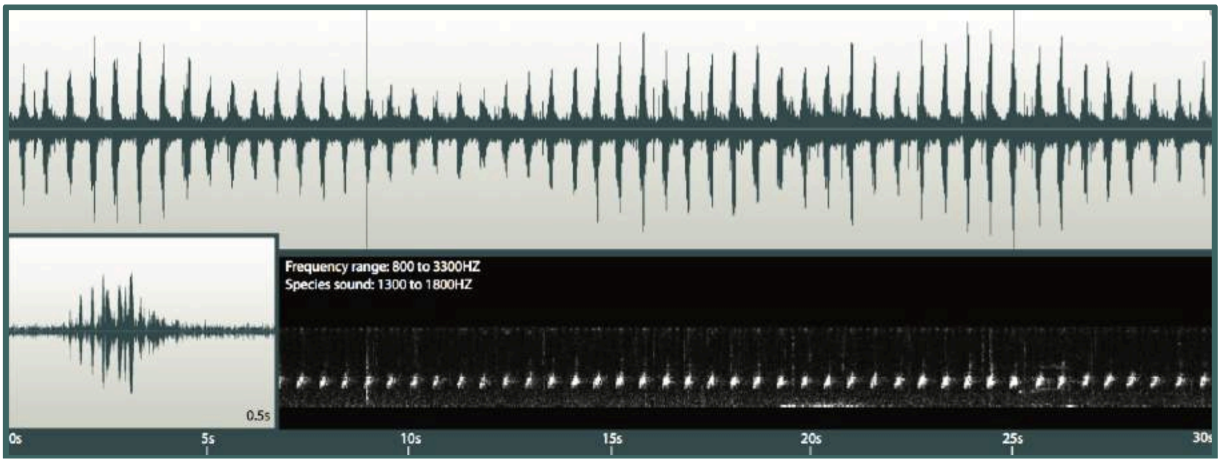

Figure 5: Sound spectrogram of S. elaeochroa in a 30 second time interval. Frequency range: 800- 3300HZ. Species sound: 1300-1800HZ.

Appendix 6: Oversized *S. baudinii*

Figure 1: S. baudinii that is 10 centimeters is size.