Cloudbridge herpetofauna survey in the secondary and primary forest

UNIVERSITY OF APPLIED SCIENCE VAN HALL LARENSTEIN

Cloudbridge herpetofauna survey in the secondary and primary forest

Author:	Jasper van Kessel
Group:	B3NLT
Project:	Period 3, Internship
Study:	Forestry and nature conservation
Supervisors	Tom Gode, Cloudbridge Nature Reserve,
	Brecht Caspers, Van Hall Larenstein University of Applied Scienes
Date of completion:	19-04-2015
School:	University Van Hall Larenstein
Organization:	Cloudbridge Reserve

Foreword

For my studies in Forestry and Nature Management at the Van Hall Larenstein University of Applied Sciences, I carried out research for the Cloudbridge Nature Reserve in Costa Rica. The purpose of this research is to better understand if tropical rainforest maturity correlates with amphibian abundance within the Reserve. The overall objective is to provide Cloudbridge with a better understanding of amphibians in a recovering rainforest.

I would especially like to thank Tom Gode for all the advice he has given me and Linda Moskalyk for her kindness. Furthermore I would like to thank Genevieve Giddy for giving me the opportunity to carry out research in Cloudbridge and sharing her stories, Mat Smokoska for supporting me with collecting the data, and Brecht Caspers from Van Hall Larenstein for supervising me where possible. Lastly I want to thank all the volunteers who helped me with my research, without them I would not been able to book these results.

Summary

In the Cloudbridge Nature Reserve there are several types of forest cover. There is primary forest and relatively immature patches of secondary forest. Comprising the secondary forest is both natural and reforested re-growth. There has not been a study to examine the potential restrictions the amphibians in or immediately surrounding Cloudbridge have to their range.

The main purpose of this research is to better understand if tropical rainforest maturity correlates with amphibian abundance within the Cloudbridge Nature Reserve. The overall objective is to provide Cloudbridge with more information about the amphibians in the Reserve and to gain a better understanding of which species can be found where through more extensive surveying of the property. This will be examined on the basis of sub-questions and the main question: *Does the maturity of the forest cover affect the amphibian abundance and diversity within the Cloudbridge Nature Reserve?*

There are many sampling methods to catch amphibians, and this research uses three different approaches to investigate the main question. The methods undertaken in this study were pitfall traps, plot sampling and night visual encounter surveys.

In this research only frogs were found, all have been found within the night visual encounter surveys. A total of 6 different species of frog were seen visually in the Reserve. In the primary forest 5 species were found within a total of 30 frogs, the natural regrowth yielded 4 species and 20 frogs found were the reforested regrowth only houses 2 species with 3 frogs found. No significant difference has been found between the forest types, although there is a difference, as the result show. That the primary forest houses the most frogs is probably because it is a mature forest and balanced ecosystem compared to the secondary forest. This means that there are more plant species that provide more food and better environment for breeding. Although the natural regrowth closely follows the primary forest in diversity and abundance, this is because the forest has already spent 20 years or longer in recovery.

Most of the frogs fit the description that Savage(2002) gives to their living environment in *The Amphibians and Reptiles of Costa Rica*. One notable difference is that most of the frogs found in the Reserve are normally reported lower in elevation, all except for one. Furthermore the dry weather could have influence the findings of the frogs. Hopefully this research will be undertaken for a longer amount of time to find out if there are more species to be found in the rainy season.

There are some improvements that could be undertaken for continuing research. The buckets could be improved by tunnelling, The VES method can be used in the rainy season, as frogs do not give off calls in the dry season. Furthermore Cloudbridge could improve its incidental database by reporting frogs seen on trail and also by noting forest type.

Index

1. Introduction

Cloudbridge is a private Nature Reserve located in San Gerardo de Rivas, Costa Rica. It was developed as a reforestation effort to provide a wildlife corridor that will link two regions that were separated by pastureland due to homesteading activities. Within the Reserve the one-time pastures have either been actively reforested or naturally regenerated by ecological succession. The diversity of forest types makes it a perfect place to find out whether amphibians repopulate in reforested parts of the tropical forest and if so, in which way (see appendix A. for a map of Cloudbridge Nature Reserve).

Amphibians are special, they have a highly permeable skin, which makes them both vulnerable to losing water and able to absorb water. Their eggs are covered with jelly capsules rather than a hard shell like those of birds.

For these reasons, amphibians require relatively moist environments. Amphibians are common all over the world. Unfortunately, they are now at greater peril than at any time in recent geologic history. Habitats are being lost at alarming rates because of expanding human population, destroying the forest for their benefit or through disease (C. Kenneth Dodd, 2009).

This report will describe the problem statement of the amphibian at Cloudbridge and why this should be researched, followed by a problem description that describes the main question and sub questions as well as the topography, environment and climate. Then the methods undertaken are explained, followed by the analysis, conclusion and discussion. Finally, this report will make several recommendations, as this can be a long-term project.

There are many sampling methods to catch amphibians, and this research uses three different approaches. The methods undertaken in this study were are pitfall traps, plot sampling and night visual encounter surveys. These three methods were selected because they will help provide answer to the research questions.

The main purpose of this research is to better understand if tropical rainforest maturity correlates with the amphibian abundance within the Cloudbridge Nature Reserve. The overall objective is to provide Cloudbridge with more information about the amphibians in the reserve and to gain a better understanding of which species can be found where through more extensive surveying of the property. This will be examined on the basis of sub-questions and the main question: *Does the maturity of the forest cover affect the amphibian abundance and diversity within the Cloudbridge Nature Reserve?*

2. Problem statement

The Cloudbridge Nature Reserve, located in San Gerardo de Rivas Costa Rica, was developed as a reforestation effort to provide a wildlife corridor that will link two regions, that were separated by pastureland due to homesteading events. This allows animals to migrate and plants to disperse more effectively from one place to another. Within the Reserve, the one-time pastures have either been actively reforested or naturally regenerated by ecological succession. The rate of recovery will depend, in part, on the availability of seeds which in turn depends on the proximity of other forest species, and the availability of animals, such as birds, sloths and monkeys, to disperse seeds.

Camera traps placed throughout the Reserve have captured many species of wildlife returning; especially mammals. If and in which way the animals and plants make use of this corridor has yet to be examined.(Cloudbridge Reserve, 2015).

Amphibians are an interesting subject to study because they are good indicators of environmental quality. The skin of amphibians is sensitive and makes them susceptible to environmental contaminants and abiotic factors. Some amphibians only breathe through their skin whereas others have lungs and can breathe through their respiratory system as well as through their skin. Because amphibians are specially adapted for natural environmental conditions, most require a clean and well-established natural environment to flourish.

In the Reserve, there are several types of forest cover. There is primary forest and relative immature patches of secondary forest. The secondary forest is comprised of both natural and reforested secondary regrowth. There has not yet been a study to examine the potential restrictions the amphibians in or immediately surrounding Cloudbridge have to their range.

It has been several years since the last amphibian study was undertaken at Cloudbridge so we hope to see an improvement in species abundance and diversity in the forest types that were previously studied. We are particularly interested to find out, through the various survey techniques, if the secondary forest has become more suitable for amphibians over the last couple of years through the various survey techniques.

The purpose of this research is to better understand if tropical rainforest maturity correlates with the amphibian abundance within the Cloudbridge Nature Reserve. The overall objective is to provide Cloudbridge with more information about the amphibians in the Reserve and to gain a better understanding of which species can be found and where through more extensive surveying of the property.

3. Project description

This research was carried out in the high elevation rainforest in and immediately surrounding Cloudbridge Nature Reserve. It is undertaken in different life stages of the tropical forest, these are the primary forest and secondary regrowth. The secondary forest is comprised of both natural and reforested regrowth. The information that follows from the collected data will later be put into a report.

3.1. Questions

The main question:

Does the maturity of the forest cover affect the amphibian abundance and diversity within the Cloudbridge Nature Reserve?

Sub-questions:

- Which amphibian species are to be found in the different life stages of a cloud forest?
- What is the abundance of the different species in the cloud forest?
- What is the actual ratio of the life stages of the cloud forest?

3.2. Topography, environment and climate

Cloudbridge is a privately owned nature reserve. It is situated in the southern Pacific slope of the Cordillera Talamanca mountain range. The reserve encompasses almost 700 acres of land and has an elevation range between 1500 and 2600 meters although there is not easy access to the upper 700 meters. When Cloudbridge was founded the land mainly consisted of primary tropical forest, secondary forest and farmland (Linda, 2011). Overall, parts of pastureland have either been actively reforested or regenerated by ecological succession.

The Reserve is part of an important bioregion, surrounded by cloud forest and high elevation shrub lands, with a high diversity of species. The Cloudbridge Nature Reserve borders the Chirripo National Park, which has the respectable status of a UNESCO World Heritage Site, due to its high biodiversity and endemism (Cloudbridge Reserve, 2015). The annual rainfall is 4300 mm and occurs mainly from the end of May to December. Temperature reaches between 13.4 and 23.1 degree, depending on the season. (Spek, 2011).

4. Reforestation project

In 2001, Ian and Genevieve (Jenny) Giddy came to Costa Rica to climb a mountain that would deem worthy, mount Chirripo, 3820 meters high. During their climb, they looked over the hills and valleys, and Ian said: 'Look at all the deforestation that happened, why don't we come back here, buy some land, and just let the trees grow'. Coincidently, they found land on the slopes of Mount Chirripo a couple of months later and, in 2002, purchased a 60 hectares of cattle farm bordering Chirripo National Park and the Talamanca Reserve, Their aim was to allow for a wildlife corridor to exist in-between the two areas. Subsequently the reserve was extended with the purchase of more small cattle farms and currently Cloudbridge covers 700 acres.

In May, they started their reforestation project on the Rio trail and the Montana trail, the start of the rainy season (Appendix A). This area was completely deforested. In the first year approximately 10.000 trees were planted, and in the second year they added around 7.000 more trees. All the seedlings came from tree nurseries in the surrounding area. Unexpectedly, there was a loss of 80% of the trees planted on Rio, and 90% of those on Montana. The Montana slope is steep and exposed, so the sun may have influenced the extra 10% of loss.

It was identified that mistakes were made in the beginning of the reforestation process. Firstly, the seedlings came from the surrounding area, however not from the same altitude. Some species that were planted could not, or had great difficulty growing at this higher alleviation. Another was that the workers were only told that they had clear a 1-meter area around the seedlings. However, ferns can and did overgrew the seedlings from more than a meter away but were not cleared because they were outside of the 1-meter range.

After a long learning process with failures and successes, Cloudbridge currently plants 1.000 trees a year and nearly all survive because of the following improvements; Cloudbridge gathers the seeds on site, germinates and plants in plastic bags to be placed in their nursery for 6 months-two years. The soil they are replanted in is enriched with compost, chicken manure of a local farmer and worms. These planting methods improve the health of the seedling. Furthermore, the trees consist of a mixture of 13 native species that naturally grow at this elevation. This includes fast (pioneer) and slow-growing (climax) species. The fast-growing species are competition for the slower species and force them to grow upwards in their normal shape, instead of in a low spreading umbrella shape.

When a tree is planted, cardboard is lain down around the seedling. This cardboard is used for a few reasons. First, it keeps the ground moisturized, especially in the dry season this is of great importance, as it becomes very warm and dry. Secondly, it reduces the growth of weeds and ferns around the seedling. In the rainy season, from July to November, the grass and weeds grow quickly and can overwhelm the baby trees if left unattended. Clearing is required at least once every 4-6 weeks, done with a machete. It takes about two weeks of hard work done by 2 to 4 people to clear the area around all the saplings. Clearing happens around 10 times a year, but with the cardboard in place this is reduced to 6 to 8 times a year, leaving more time for other jobs. To prevent the cutting down of seedlings among the encroaching undergrowth, the trees are planted with stakes.
When worms are added to the soil, they enrich it in a natural way. They break up nutrients into a smaller and more useable size, which is better for the seedlings.

Tree planting only happens beside or on ridges, for several reasons. The ridges are the last area to regenerate naturally, taking well over 35-50 years for pioneers to appear. When they are planted on ridges, nature can do the work to disperse the seeds down the slope. Planting trees on slopes along where most of the trails are, is effective because the grass won't grow

underneath the trees. It currently costs a worker 4 days to mow the edges of a trail, but when the grass is shaded out it will only take one day to do this maintenance. This is not the only benefit of planting trees along the trails, it also creates corridors and a better experience for visitors to Cloudbridge. Overall, the benefit of planting the trees is that the forest regeneration is faster and that the new forest has greater diversity of species.

The Cloudbridge forest map was made especially for this research to show which areas are replanted, and after analysis estimated percentages are given for the types of the forest. Most of the Reserve consists of natural regrowth; approximately 82% of the area. However, this is spilt up into forest that is older than 30 years or younger. The 30+ years forest covers 12% of the natural regrowth, and this will slowly include more of the younger forest over the years. The primary forest cover includes 10% of the total area of the Reserve. The reforested regrowth account for an area of 8%. This is also growing slowly, but rather than taking up more space, it tends to grow more densely.

5. Methods

There are many sampling methods to catch amphibians, and this research uses three different approaches. The three methods undertaken in this study were pitfall traps, plot sampling and night visual encounter surveys. In the following sections, the various procedures will be described. These three methodologies were selected because they will help provide answers to the research questions. The methods were carried out in the three different types of forest cover at Cloudbridge, to see if there is any difference in the abundance of ~~the~~ amphibians. All the sites are included in Appendix B with coordinates, and the pitfall traps are visible on the map (Appendix A)

5.1. Pitfall traps

The difference in the diversity and abundance of leaf-litter amphibians can be measured by employing pitfall traps throughout Cloudbridge's property. Each forest cover (primary, natural regrowth and reforested regrowth) had four pitfall traps placed throughout. To avoid catching the same specimens over and over again, transects were set at least 25 meters apart, whether parallel to one another or heterogeneous.

The traps were checked every day or three days each week (depending on planning), except during weekends when they were closed off. This procedure was followed to give the area some recovery time (Cousineau, 2007). Transects were established uniformly in every life stage of the rainforest.

How to build a pitfall trap

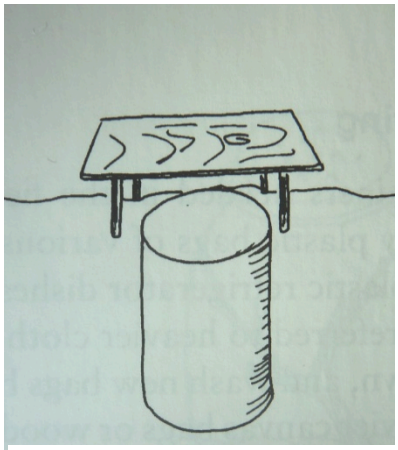


Figure 1: Pitfall traps

A few materials were used: a plastic bucket with a lid and four small stakes. The bucket was entrenched to surface (or you could say ground) level and was covered with a lid, to avoid flooding of the bucket, predation and potential overheating (Figure 1) (Animal Ethics Infolink). The lid was supported above the bucket by the four stakes. Lastly, vegetation from the surrounding area was laid down on top, to make the trap blend in (Cousineau, 2007). This was done with minimum disturbance to the terrain, to prevent human influence in the research.

5.2. Plot sampling (leaf litter and vegetation transects)

The plots were 10 meters by 10 meters. Each corner was flagged and GPS coordinates measured. Plots were established 25m from any potential human disturbance (Michelsohn, 2007). Every Thursday and Friday a plot was inventoried. This type of animal inventory causes a lot of disturbance to the habitat, therefore each plot was only visited once and then had to be left undisturbed (C. Kenneth Dodd, 2009).

Researchers began by working their way through the leaf litter. Each researcher moved at approximately the same pace toward the centre of the plot, capturing any specimens they uncovered. While making their way to the middle of the plot they raked through leaf litter, gently searched under dead woody debris and loose rocks, as well as looked inside root

cavities and bases of vegetation. The time spent searching each site was recorded. We did not have to use a long pole or grabber to turn over debris on the ground as suggested by (Savage, 2002) Venomous species of snakes that have been discovered at Cloudbridge are arboreal, however they come down some times. Nevertheless, so caution was used.

After catching a specimen it would've been placed in a plastic bag, and the time of capture/encounter recorded. After the plot was thoroughly searched, the specimens would've been recorded, measured and released at the site of capture (Savage, 2002). The specimen would not be released until the end of the search to prevent catching the same specimen twice. Specimens would be identified according to Savage(space)(2002) and Michelsohn (2007). For handling, disposable gloves would be used.

5.3. Night Visual Encounter Surveys

Night visual encounter surveys were undertaken throughout the various forest covers. For safety precautions this survey was only carried out along marked trails. The night search was done with headlamps/flashlights by a minimum of two researchers, for safety reasons (Michelsohn, 2007).

The colour of the eye shine may be white, pale yellow, bluish green, pink or red depending on the species. It is possible to be fooled by other conspicuous shines of spiders and moths. After capture, the specimens were identified according to Savage (2002) to the best of our ability.

5.4. Equipment list

The following is a list the materials used for field work:

- Gloves (one pair of heavy garden gloves for fieldwork, especially for the leaf litter and vegetation plots)
- Disposable latex gloves for handling animals.
- Flagging tape
- GPS (Garmin eTrex 20)
- Thermometer
- Head flashlight (night searches)
- Plastic bags with a zip lock
- Duct tape
- Magnifying glass
- Scientific names (Savage 2002)
- Knife

5.5. Process data

The data collected from the fieldwork was digitalised after the work was carried out. For this, the program Microsoft Office Excel was used. With this program, all the dates could be organised and afterward graphs could be made. These graphs and data were then used to write the analysis and, later on, conclusions of the research. In Excel, the t-test was utilized to find out if there was a significant difference between the forest stadia.

6. Analysis

To measure a significant difference between the forest types in amphibian abundance and diversity, the t-test was used. The data used was from night surveys, because no amphibians were found with the other research methods. A summary of all species of amphibian observed in Cloudbridge Nature Reserve in this research, with all the relevant information, is included in appendix A.

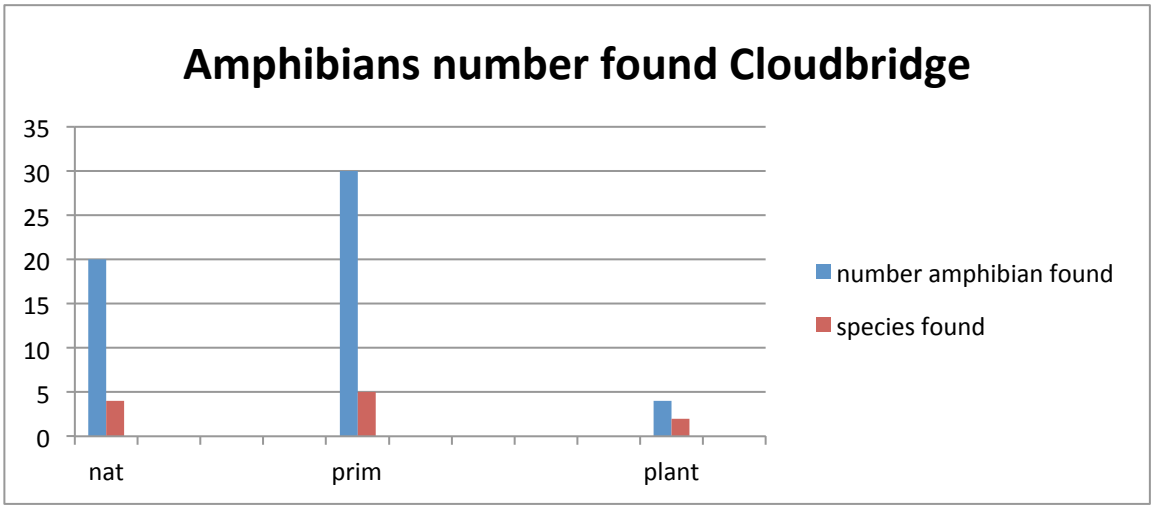


Figure 2: graph of number of amphibian found

A total of 6 different species of frogs were found visually in the Cloudbridge Nature Reserve (Figure 2). No toads or salamanders were identified during this project. As Figure 2 shows, the highest number of species and specimens were found in primary forest - a staggering 30 amphibians from 5 different species. As detailed in table one, most were *Pristimantis (Eleutherodyctyles) ridens* and *Pristimantis (Eleutherodactylus) creuntus*. The natural regrowth followed with 20 amphibians found within 4 species, most of them where *P. creuntus*. The reforested regrowth housed the lowest number of frogs compared to the other two forest types, with 2 species and 3 amphibians found, these were *P. creuntus* and *Craugastor (Eleutherodactylus) fitzingeri*. All of the *P. creuntus* found were juveniles.

The natural regrowth and primary forest showed nearly the same composition of species, with the exception of *Isthmohyla (Hyla) pseudopuma* which was only found in the natural regrowth and *Craugastor (Eleutherodactylus) podiciferus* and *P. ridens* which was only found in the primary forest. In the reforested regrowth only two species were found *P. creuntis* and *C. fitzingeri* , they were also found the primary forest and the natural regrowth.

	Amphibians species found at Cloudbridge	Number of specimens found
Nat	Isthmohyla (Hyla) pseudopuma	1
	Pristimantis (eleutherodactylus) creuntus	12
	Craugastor (Eleutherodactylus) fitzingeri	1
	Espadarana (Centrolene) prosoblepon	5
Prim	Craugastor (Eleutherodactylus) podiciferus	4
	Pristimantis (eleutherodactylus) creuntus	9
	Craugastor (Eleutherodactylus) fitzingeri	6
	Pristimantis (Euleutherodyctyles) ridens	10
	Espadarana (Centrolene) prosoblepon	1
plant	Craugastor (Eleutherodactylus) fitzingeri	2
	Pristimantis (eleutherodactylus) creuntus	1

Table 1: Amphibian species found

There is no significant difference in the biodiversity nor the abundance of species in the different forest types. The significance in species between primary forest and natural regrowth is p=0.64. The significance between primary forest and reforested regrowth is p=0.07. This is coming very close to p</=0.05, which would prove there was a significant difference.
The significance in the abundance of species between primary forest and natural regrowth is p=0.58. The significance between primary and reforested regrowth is p=0.19.

Individuals that were collected outside of dedicated fieldwork were not included in any statistical analyses, as they would give an incorrect representation of the difference between the forest types. As more incidentals were found in the natural regrowth and reforested regrowth than in the primary forest, it would be misleading to include them. A list of extra species found is included in Appendix A as well.

observation
Espadarana (Centrolene) prosoblepon was visually encountered in one site within both the natural regrowth and primary forest, although its calls were heard everywhere. The highest number of calls heard was in the primary forest on the Gavilan trail; however, none were found. The *C. fitzingeri* calls were recognized after one week of rainfall, but when it stopped raining these frogs stopped calling.

7. Discussion

The dry season was extraordinarily dry, with the warmest temperatures ever measured in the Cloudbridge Nature Reserve in the months of February and March temperatures rose to 30 degrees Celsius. At the end of March there were heavy rain showers, which made the amphibians more active and easier to find. Unfortunately the fieldwork was already finished by that time. The dry weather probably had a major influence on the findings, and it is well known that more amphibians will be found during the rainy season.

Two species were found to have suitable habitat throughout the whole reserve, *P. creuntus* and *C. fitzingeri*. Although these species were mostly found in the Primary forested uplands. They are both nocturnal species, as well as the other species found except for *P. podiciferus. P. creuntis* is a common species that is found in low vegetation as well as above in the high leaf litter of the dense primary forests and it is known to be well adaptable (Savage, 2002). Remarkable was that only juveniles of *P. creuntus* were found. This could be because the elderly frogs are in lower number and hiding better.
Savage (2002) explains that *C. fitzingeri* is known to be found in the understory vegetation or bushes along the forest edge. This description fits the findings of this research as the C. fitzingeri was found besides trails or on the edge of the forest were it still has to be reforested. The *C.fitzingeri* is known for giving its calls, but these we only heard after heavy rainfall. Both frogs are known for adapting pretty well to secondary forest areas (Savage, 2002). The frogs are calling after rainfall because they want to set their territory, because it is the start of the rainy season, this is when they are breeding.

E. prosoblepon is common along the margins of fast-moving streams on low vegetation. It was reported in the natural regrowth and primary in this research. Although there is no stream in the primary forest, the highest number of calls was heard there. This is remarkable, because egg deposition usually takes place on upper surface of leaves, moss-covered rocks, or branches up to 3m above water. After 10 days tadpoles hatch from the eggs and drop into the stream (Lui, 2011). This happens in the rainy season from May to November, the males also call during this time (Savage, 2002). Nevertheless, the calls of the *E. prosoblepon* were heard throughout the entire time of survey.
I. pseudopuma was only reported in the natural regrowth. We expected to see this frog more often because it is common in secondary growth as well as in meadows and pastures. Overall it is a common frog in Costa Rica that could be found during the day hiding in tank-bromeliads and in vegetation on the ground (Savage, 2002). Maybe the warm weather caused the only finding in the primary forest.

P. podiciferus and *P. ridens* were only reported in the primary forest. *P. ridens* is a semi arboreal species that was found walking on the ground. Normally it is found in lowlands prominent wet rainforest, a forager in low vegetation that often hides in the leaf litter during the day (Savage, 2002). *P. podiciferus* is the only frog found that is nocturnal as well as diurnal. It tends to be found in primary forest situations as well as in wet lower rainforest. That it was found at high elevation is extraordinary.

Extra species
There were tree species found as incidentals apart from the night survey transects, one frog and two salamanders. The frog is *Craugastor (Eleutherodactylus) andi* was reported in natural regrowth. This frog is on the list of endangered species because of drastic population decline, estimated to be more than 70% over the last three generations, inferred from the apparent disappearance of most of the population, perhaps due to chystridiomycosis (REDLIST, 2014). It is special to find this frog at an elevation of 1634 m because it has only been reported up to 1200m in Savage(2002).

The *Oedipina uniformis* and *Oedipina savagei* were found on the border between natural and reforested regrowth, at an elevation of around 1600 meters. *O. Uniformis* is most commonly found in or under rotten logs, leaf litter, and mats of moss (Savage, 2002). *O. Savagei* is known only on the humid Pacific slopes, in south-western Costa Rica from 1200-1400 m although distribution is poorly known (REDLIST, 2014), as proven by fact that the salamander is found at 1600 m in Cloudbridge.

There are probably more species to be found in the Cloudbridge Reserve, especially in the primary. Most of the frogs are arboreal and could live high up in the trees for most of its live and will never been seen. As the primary forest is in its climax state it is much more developed than the secondary forest. Because of that the primary forest hold the highest diversity of plants of all the forest types. This means that there are more living and breeding areas for different amphibian species, and the diversity and abundance of the food will be higher.

Previous study
The previous studies on amphibians in the Cloudbridge Nature Reserve cannot be used in the analysis here because the data was only found in non-quantitative form. C. Cruentus, C. Fitzingerie and P. podiciferus were reported in other research and in this one as well. C. Cruentus is reported in the primary and secondary forests, while C. Fitzingerie was found in natural regrowth. In this survey both species were found in all three forest types. P. Podiciferus was previously reported in all the forest types, but we only found it in the primary forest.

Furthermore *Craugastor crassidigitus, Diasporis diastema, Diasporis hylaeformis* and *Craugastor sp* have been found in the reserve (see Appendix C.). That these frogs were not re-discovered could mean they have deserted the area, or the season could have an influence on the findings.

Chytridiomycosis Disease
There are many factors leading to amphibian declines. Probably the most important is the habitat destruction, but since Cloudbridge is reforesting land this is of little impact for the amphibians in the Reserve. Another recent factor in amphibian decline is the disease called chytridiomycosis. This pathogen is associated with the global loss of hundreds of species of amphibians and represents a spectacular loss of biodiversity (AMPHIBIAWEB, 2015). It is not known if this disease has reached the Cloudbridge Nature Reserve, and only lab testing can tell. Matt Smokoska, Cloudbridge's resident biologist, believed that he had observed a decline in the Cloudbridge Reserve over the past three years. Most of all in the number of the species found.

Elevation records
There are species present in the Cloudbridge Reserve that are living at higher elevations than previously know, according to Savage (2002). Only one species, *I. pseudopuma*, was found in the elevation limit given by Savage(reported between 1120-2340 m, found at 1700 m). The species found higher than the limits reported in Savage (2002) are *C. podiciferus* reported (reported up to 1520m, found at 2003 m), *C. fitzingeri* (reported up to 1520, found at 1750 m), *P. creuntus* (reported up to 40-1800, found at2003 m), *P. ridens* (reported up to 15-1600 m, found at 1754 m). One species, *E. prosoblepon* (reported up to 20-1900 m, found at 1609 m), was seen visually in the elevation range given by Savage, but calls were heard at 2000 m. The number of amphibian found higher than their supposed range is extraordinary. One of the reasons could be that global climate change is affecting the elevation at which amphibians are able to survive (Wilkinson, 2007). Only a long-term research could explore this hypothesis, because it could differ each year as well.

Discussion of methods
The most carefully and consistently employed survey method was the pitfall trap. However, no species were caught using this technique. This, as mentioned earlier, could be influenced by the dry weather. This method has some main drawbacks, as only leaf-litter individuals will be caught and it samples a very limited area. The advantage is that one section can be measured very thoroughly. This method could be improved for a higher chance of catching amphibians with the use of tunnelling and more buckets, by creating a tunnelling line a couple meters with two buckets on either end.

Night visual encounter surveys were the most effective method, yielding all the species found in this research. This is the best method to identify the most common nocturnal leaf-litter species. During every night survey some specimens were found, although the results varied depending on location. The night searches in the natural regrowth had a more humid environment than the primary forest and planted regrowth. This can influence the results because overall, frogs prefer humid places.

Plot sampling during the day was only undertaken four times due to the dry weather. There was no use in doing more because this method yielded no results. In the rainy season, this could be a more effective method because the frogs may be hiding less, so instead of going in the leaf-litter there would be a better chance of finding them on the leaves.

In the dry season, audio sampling is a ineffective way of collecting data, since frogs only call when it is wet. The one exception during this study was the Emerald Glass frog, which we could hear ~~him~~ during the entire research period. However, audio sampling is effective in the rainy season and should be taken into consideration for further research.

8. Conclusion

The main question:

Does the maturity of the forest cover affect the amphibian abundance and diversity within the Cloudbridge Nature Reserve?

The maturity of the forest cover does affect the amphibian abundance and diversity within the Cloudbridge Nature Reserve, although not with a significant difference. The primary forest houses the most species with the highest abundance. This is probably because the primary forest is in its climax stadia, and because of this it offers the most diversity in plant species. This means that there are more insects, so there is more food for amphibians. It also means that there are more hiding places and breeding places as there more bromeliads and other small watery spots were frogs can breed.

The natural regrowth closely follows the primary forest in diversity and abundance. This forest type is pretty well developed, as most of its sites are the places where 20 years old or more. It has been in recovery for a longer time than the reforested regrowth which houses the least amphibian, probably because it is less developed compared to the other two forest types. There exist invasive grasses and other plants that are not usually found in this habitat that change the dynamic of the ecosystem here. Also the small trees do not provide the cool, moist, shady conditions of a more mature forest. When the forest grows older in the Cloudbridge Nature Reserve there will probably be a higher amphibian abundance and diversity.

Sub-questions:

- What is the actual ratio of the life stages of the cloud forest within the Cloudbridge Nature Reserve?

The actual ratio of the life stage of the tropical rainforest within Cloudbridge are estimated in a map especially for this research. It shows which areas are replanted, and after analysis estimated percentages are given for the types of the forest.
Most of Cloudbridge consists out of natural regrowth; approximately 82% of the area of the Reserve. However, this is spilt up into forest that is older and younger than 30 years. The 30+ years forest covers 12%, and the less than 30 year area covering 88%, of the natural regrowth areas and will gradually include more of the younger forest over the years. The primary forest cover 10% of the Reserve, and the planted regrowth follows with an area of 8%. This is also growing slowly, but it barely takes up more space, as it mostly grows denser.

- Which amphibian species are to be found in the different life stages of a cloud forest?

There were 6 different species found in the different life stages of the Cloudbridge cloud forest. A list of all the species and numbers found during this survey are included in Table 1. The natural regrowth and primary forest show nearly the same composition of species. Of the 5 species found in the primary, C. podiciferes and P. ridens distinguish themselves from the species found in natural regrowth. In the natural regrowth, 5 species are found, and I. pseudopuma was only found in this life stage.
In the reforested regrowth, only two species were found, C. fitzingeri and P. creuntus. They were also found in the primary forest and natural regrowth. There are probably more species to be found throughout the Cloudbridge Nature Reserve, but only longer surveying can prove this.

There is no significant difference between the species diversity of the primary and secondary forest, although there is a difference between the primary forest and reforested regrowth that is nearly significant at p=0.07. There are 4 more species found in the primary than in the planted regrowth, and probably even more are present, but because most of the frogs are arboreal, finding them is very difficult.

- What is the abundance of the different species in the cloud forest?

The abundance of the different species found in the Cloudbridge Nature Reserve are pictured in Figure 2. The primary forest housed the highest abundance, with 30 frogs found from 5 species. The species found most were P. creuntus (found 9 times) and P. ridens (found 10 times). All the other species were found more than once except for E. prosoblepon, although it was heard frequently so more than one were present. There were 20 frogs found in 4 species in the natural regrowth, with P. cruentus found the most (12 times). In the reforested regrowth, the frogs were found only once or twice.

Although there was no significant difference found between the abundance of species in the primary and the secondary forest, there was a small noticeable difference indeed. This probably exists because the primary forest is better developed than the secondary areas, and because of this it houses a higher variety of frogs.

9. Recommendations

Recommendations are of great importance to other researchers that will undertake this survey. In this section there is a plan for how to conduct the research, and suggestions about what can be improved.

Steps to be undertaken by the researcher:

1. Once arrived at the Cloudbridge Nature Reserve it is important to become familiar with the surroundings and environment. It is helpful in knowing where to conduct the night visuals encounter surveys and plot sampling.
2. The buckets (pitfall traps) are normally checked from Monday to Wednesday. On Thursday and Friday daytime plot sampling is undertaken. A night search should be conducted at least once a week, and more often if possible. This is dependent on the volunteers, because it is not allowed to go in the forest alone at night. However, this program can be changed if, for example, the buckets are checked for two weeks, then paused for two weeks for other research ~~or~~ methods. This is possible because the buckets can be closed to animals and do not disturb the environment.
3. All the data that is collected will be put in the 'Cloudbridge Incidentals Database' as well as in the database for this research. This can be found on the hard drive together with this report. This way it is easier to work with the data. **Look at this data before beginning, as you need to know what data to collect**.
4. There are 3 sheets in Microsoft Excel: collected data, t-test, and numbers. All the data that is collected will first be recorded by date in sheet one for further research purposes, so it can be done seasonally as well. After this, enter the data to the other sheets, and do this regularly to prevent a build-up of work at the end.
5. For species identifications the book Savage 2002 is used, found in the library at Cloudbridge.

Steps to be undertaken to improve the research:

- The buckets proved to be fruitless in the dry season. This could be different in the rainy season; however, they could be improved as follows. With the use of tunnelling the change of chatting amphibians will improve. A tunnelling of 3 meters could be used with a bucket on either end, or even one in the middle as well. If this is too much work do it for 3 instead of 6 sites.
- The VES(Vertical Electrical Sounding) method could prove useful in the rainy season. As frogs barely call in the dry season, this method was not utilised. With this method the number of calling males can be measured in a certain area.
- It is possible that plot sampling (day transects) will yield more frogs in the rainy season. This is because these areas will be more humid and the frogs probably don't have to hide to conserve moisture. Do this sampling early in the morning in moist (or you could say) places as this improves the change of finding amphibians.
- The Cloudbridge incidentals database utilizes the trail names to indicate the place an animal was found. Because of this, the data cannot be used for research that examines the difference between forest types. A possible improvement would be to report this by trail and forest type.

10. References

(2014). Retrieved 04 03, 2015, from REDLIST: http://www.iucnredlist.org/details/56414/0

Amphibian ark. (2015). Retrieved 02 04, 2015, from Amphibian ark: http://www.amphibianark.org/the-crisis/amphibians-as-indicators/

AMPHIBIAWEB. (2015, 05). Retrieved 04 05, 2015, from AMPHIBIAWEB: http://amphibiaweb.org/declines/declines.html#why

Animal Ethics Infolink. (n.d.). Retrieved 01 20, 2015, from Animal Ethics Infolink: Cloudbridge reserve

C. Kenneth Dodd, J. (2009). *Apmhibian Ecology and Conservation - A Handbook of Techniques.* Oxford: Oxford University Press.

Cloudbridge Reserve. (2015). Retrieved 01 21, 2015, from http://www.cloudbridge.org/

Cousineau, S. (2007). *Cloudbridge Herpetofauna Study.* Cloudbridge reserve: Cloudbridge reserve.

Linda. (2011, 02 06). *Cloudbridge Reserve Blog*. Retrieved 01 21, 2015, from Cloudbridge Reserve: https://cloudbridgereserve.wordpress.com/about-2/

Lui, K. (2011, 10 05). *Amphibiaweb*. Retrieved 04 03, 2015, from Amphibiaweb: http://amphibiaweb.org/cgi-bin/amphib_query?where-scientific_name=Espadarana+prosoblepon

Michelsohn, M. (2007). *Cloudbridge Herpetological Survey.* Cloudbridge reserve: Cloudbridge.

Savage, J. M. (2002). *The Amphibians and Reptiles of Costa Rica; A Herpetofauna between two Continents, between Two Seas.* Chicago: The University of Chicago.

Spek, M. (2011). *Cloud Rorest recovery.* Cloudbridge: Cloudbridge.

Wilkinson, C. (2007). *Cloudbridge Herpetological Survey.* Cloudbridge: Cloudbridge.

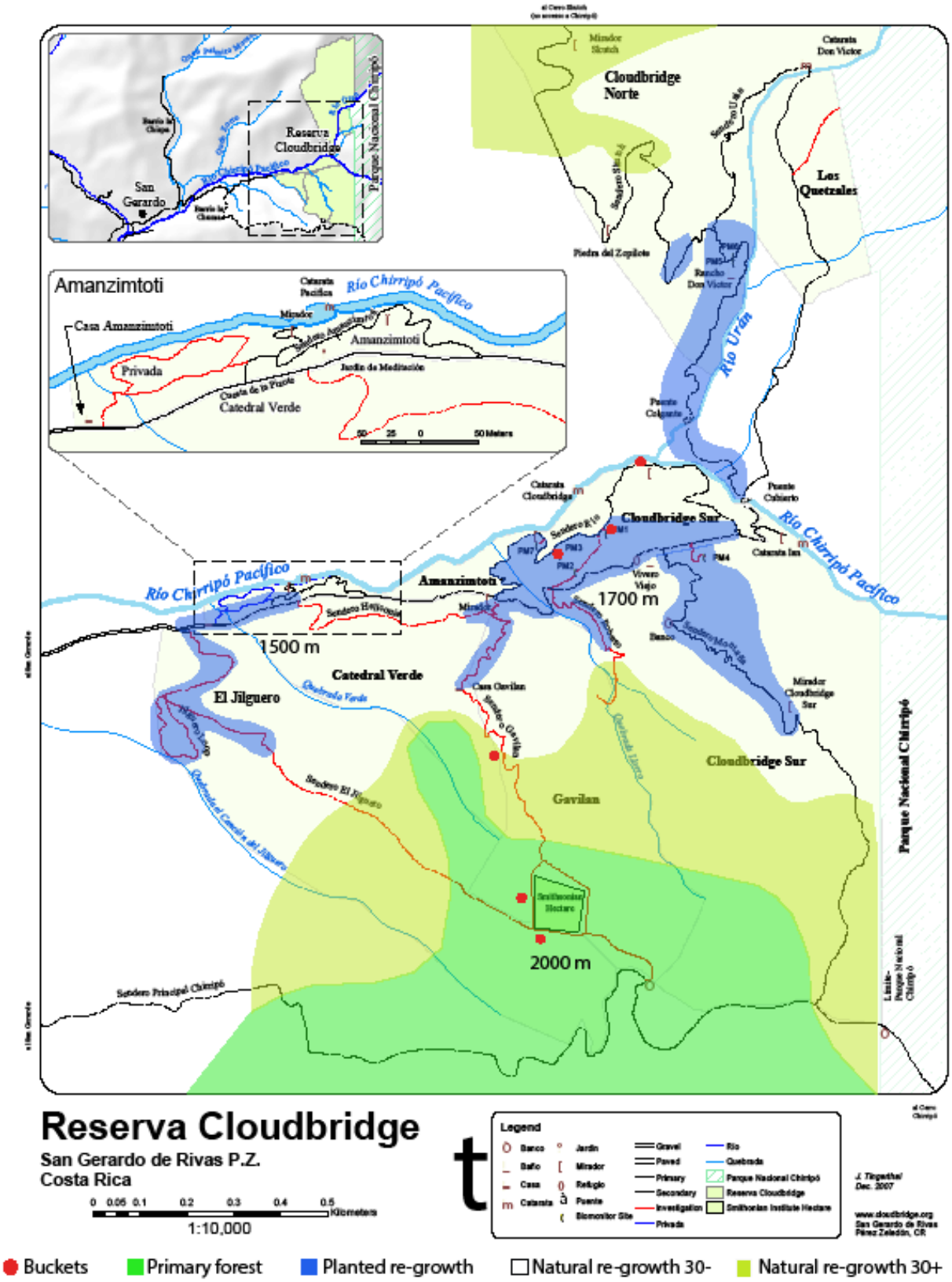
11. Appendix

Appendix A – Cloudbridge forest types

Appendix B – Site and species information

Appendix C – Frogs previously found

11.1. Appendix A. - Cloudbridge forest types



11.2. Appendix B. – Sites and species information

This is a list of all the sites where research has been conducted with the species were found.
N/Z= coordinates
H= altitude
L/M/H=coverage of the different levels of the forest

Pitfall traps:

1. Primary forest, Smithsonian Hectare
   N:09 27 975 Z:083 34 295 H:6520f(1987M)
   L:75% M:75% H:60%
2. Primary forest, Smithsonian Hectare
   N:09 27 999 Z:083 34 200 H:6490f(1978M)
   L:80% M:75% H:60%
3. Natural regrowth, Gavilan trail
   N:09 27 282 Z:083 34 330 H:6170f(1881M)
   L:70% M:50% H:30%
4. Natural regrowth, Sendero Rio
   N:09 27 509 Z:083 34 189 H:5444f(1659M)
   L:50% M:50% H:60%
5. reforested regrowth, East loop
   N:09 27 492 Z:083 34 735 H:5517f(1682M)
   L:70% M:50% H:30%
6. reforested regrowth, West loop
   N:09 27 418 Z:083 34 259 H:5474f(1668M)
   L:80% M:70% H:20%

Plot sampling:

1. Natural regrowth, Rio trail
   Date:19-02-2015 start:7:45 end:8:15
   N:09 27 414 Z:083 34 181 H:5127f(1563M)
   L:80% M:70% H:20%
2. Natural regrowth, Rio trail
   Date:19-02-2015 start:8:45 end:9:15
   N:09 27 417 Z:083 34 264 H:5334f(1625M)
   L:15% M:50% H:85%
3. reforested regrowth, Rio trail
   Date:20-02-2015 start:7:15 end:7:45
   N:09 27 532 Z:083 34 095 H:5618f(1712M)
   L:80% M:40% H:20%
4. reforested regrowth, Rio trail
   Date:19-02-2015 start:8:00 end:8:45
   N:09 27 478 Z:083 34 203 H:5476f(1669M)
   L:40% M:30% H:60%
5. Primary regrowth, Elgeuro
   Date:27-02-2015 start:8:00 end:8:45
   N:09 27 948 Z:083 34 385 H:6350f(1935M)
   L:70% M:60% H:80%

Nigh Visual Encounter Surveys:

1. Primary, Gavilan
   Date: 9-2-2015 start:19:00 end: 20:00
   N:09 27 923 Z:083 34 304 H:6572f(2003M)

- Craugastor (Eleutherodactylus) fitzingeri
- Craugastor (Eleutherodactylus) podiciferus (2*)
- Pristimantis (eleutherodactylus) creutis (2*)

2. Natural regrowth, memorial garden
   Date:18-02-2015 start:19:15 end:20:00
   N:09 27 313 Z:083 34 669 H:5225f(1593M)
   - Espadarana (Centrolene) prosoblepon (3*M/1*F)
   - Pristimantis (eleutherodactylus) creutis (2*)
3. reforested regrowth, Elgeuro trail
   Date:24-02-2015 start:19:35 end:20:05
   N:09 27 186 Z:083 34 697 H:5373f(1638M)
   - Unidentified
4. Primary, Gavilan trail
   Date:05-03-2015 start:19:00 end:20:00
   N:09 27 923 Z:083 34 304 H:6572f(2003M)
   - Craugastor (Eleutherodactylus) podiciferus (2*)
   - Pristimantis (eleutherodactylus) creutis
   - Unidentified
5. Natural regrowth, memorial garden
   Date:18-03-2015 start:18:15 end:19:00
   N:09 27 392 Z:083 34 605 H:5280f(1609M)
   - Pristimantis (eleutherodactylus) creutis (6*)
   - Craugastor (Eleutherodactylus) fitzingeri
   - Espadarana (Centrolene) prosoblepon
6. reforested regrowth, Rio trail
   Date:19-03-2015 start:19:00 end:19:45
   N:09 27 186 Z:083 34 697 H:5373f(1638M)
   - Craugastor (Eleutherodactylus) fitzingeri
7. Primary,Chirripo
   Date:20-03-2015 start:19:00 end:19:45
   Unknown H:1754
   - Pristimantis (eleutherodactylus) creutis (5*)
   - Craugastor (Eleutherodactylus) fitzingeri (5*)
   - pristimantis (Euleutherodyctyles) ridens (10*)
   - Espadarana (Centrolene) prosoblepon
8. reforested regrowth, Rio trail
   Date:25-03-2015 start:19:00 end:19:45
   N:09 27 436 Z:083 34 293 H:5395f(1644M)
   - Pristimantis (eleutherodactylus) creutis
   - Craugastor (Eleutherodactylus) fitzingeri
9. Natural regrowth, Sentianl
   Date:29-03-2015 start:19:00 end:19:45
   Unknown H:1700 m
   - Pristimantis (eleutherodactylus) creutis (4*)
   - Craugastor (Eleutherodactylus) fitzingeri
   - Isthmohyla (Hyla) pseudopuma

Incidentals extra species found:
Frogs:
Craugastor (Eleutherodactylus) andi
Salamander:
Oedipina uniformis
Oedipina savagei

11.3. Appendix C – frogs previously found

	Craugastor podiciferus	*Pristimantis cruentus*	*Craugastor crassidigitus*	*Craugastor fitzingeri*	*Diasporis diastema*	*Diasporis hylaeformis*	*Rhinella marinus*	*Craugastor* sp.	*Sceloporus malachiticus*	*Mabuya unimarginata*	*Norops aquaticus*	*Norops polylepis*	Polychrotid Lizard	*Dendrophidion paucicarinatum*	*Bothriechis nigriviridis*	*Bothrops asper*	*Geophis brachycephalus*	*Dermophis glandulosus*
Amanzimtoti (Riparian)			N		A	A						O			O			
Cloudbridge Norte (All Habitat Types)	N		N	N				T	T									
Cloudbridge Sur Natural Regrowth								T	T, O									
Cloudbridge Sur Plantation			T															
Cloudbridge Sur Secondary Forest	T, O, C	T						T										
El Jilguero Secondary Forest	T, N, O				A	A		T									O	P
Gavilán Primary Forest	T, N	T, N			A			T										
Gavilán Natural Regrowth	P		O	O				O	O									
Heliconia Natural Regrowth			N															
Lower Meadow Plantation									T									
Rio Chirripó Pacifico (Riparian)																		
Sendero Rio (Riparian and Natural Regrowth)													T	O				
San Gerardo de Rivas (outside Reserva Cloudbridge)							N, O			O	O					N	O	