CLOUDBRIDGE
NATURE RESERVE

Assessing the species composition and abundance of anurans in secondary tropical forest

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Summary

1. Within the past century the removal of tropical primary forest worldwide has reached 35-50% of land area. In Costa Rica, a lot of cleared land has now been left for natural re-growth, there is also a scheme put out by the government to pay land owners to conserve land area.

2. Cloudbridge Nature Reserve is a reforestation and conservation effort located in the Southern Pacific slope of the Cordillera de Talamanca mountain range in Costa Rica (elevation 1550m - 2120m). It is classified as premontane wet forest with seasonal climate. Amphibians are highly threatened taxa worldwide, yet are majorly understudied in many areas. As yet, little research has been carried out on the anuran populations that exist at Cloudbridge.

3. Visual encounter and audio surveys were carried out on two trails in Cloudbridge to gain baseline data on species composition and abundance of anurans. Individuals were caught and measured then returned to where they were found. Several environmental variables were measured to see if these had any effect on the counts of individuals.

4. A total of three different species were found which are typical leaf-litter frogs of this habitat type; *Craugastor fitzingeri*, *Pristimantis cruentus* and *Pristimantis ridens*. A further arboreal species was identified through calls, *Diasporus diastema*, although never visually seen. There were significant differences found between counts of species and overall species richness on the two trails. This could be because of environmental factors, although further work needs to be carried out for this to be certain. Rainfall and temperature were found to have a significant effect on the number of calls heard from *D. diastema*.

5. ***Synthesis and applications:*** Cloudbridge contains other unsampled trails which would provide additional information on anuran populations that exist. It is suggested that sampled trails are continually monitored throughout the varying seasons so data on population sizes can be gathered.

Word count: 4564

Introduction

The tropics show patterns of higher species richness for many living organisms comparative to other ecosystems on the Earth (Wiens 2007). However, within the past 40 years, the tropics have had dramatic changes to its original habitat, with an estimated 35-50% of original closed canopy that has been removed for agricultural use (Herrera-Montes & Brokaw 2010). This poses a threat to the tropical community. The lack of current research on some organisms in this habitat means it is hard to understand how human threats may affect the species composition (Young *et al*. 2001). Additionally, the type of response these species will show may be unknown, when little and sometimes nothing is known about them. A lot of conservation work has been focused on conserving primary forest, but due to the large amounts of land that have been cleared, there is an increasing need to understand the important role that secondary forest may play in the sustainability of ecosystems (Barlow *et al*. 2007; Gardner *et al*. 2007).

Environmental deterioration is a problem across the globe. Costa Rica can serve as a good example of reforestation practice, as secondary forest makes up a large proportion of the land. From the mid 1940s to 1980s, the Costa Rican government put out a scheme to encourage less wealthy individuals to own land to farm on. This involved the removal of primary forest for farm use. A large proportion of the Southern Pacific slope of the Cordillera de Talamanca mountain range in Costa Rica was turned into agricultural land (T. Gode 2012, personal communication; REDD 2010). From the 1990s this decision was then turned around to encourage reforestation. Cloudbridge Nature Reserve was established in 2002 with the aim of reforesting the area, so that the vegetation reverted back to its original composition, before the removal of virgin forest. Cloudbridge now consists of over 200 ha of land that has been continually reforested over the last 10 years. As a result, a large majority of the land is secondary forest, but it is possible to see some old growth trees amongst the younger trees.

Cloudbridge provides an opportunity to observe how the animal and plant community will progress, as reforestation occurs over a number of years. Currently there are yearly vegetation assessments around the reserve to gather information and recently, a new project has been set up to monitor vertebrate species. Studies have shown that replanted areas have progressed to a further stage of forest recovery development, compared to naturally regenerated areas, but this was only due to a small number of replanted tree species (Spek 2011). Understanding the roles that species play in the recovery of forests is important in conservation efforts.

Amphibians are a good indicator of forest health due to their sensitivity to environmental changes such as temperature, humidity and water availability (De Souza & Eterovick 2011). Amphibians follow the pattern of increased species richness in the tropical regions, but since the late 1980s, amphibian declines have been noted in many countries around the world and much concern has been raised about the current status of amphibians worldwide. This led to the IUCN Global Amphibian Assessment 2004 (IUCN 2004), which showed that amphibians are more threatened than species of birds and mammals (Stuart *et al*. 2004). Several reasons for the observed declines have been hypothesised including: disease; climate change and land-use change, although no single reason can be attributed as the primary cause (Young *et al*. 2001).

Studies have been carried out to determine the importance that secondary forest can play in the presence of amphibians, as forests comprise a large proportion of their habitat. Gardener *et al*. (2007) found that two-thirds of the species sampled in primary forest also occurred in secondary forests, which supported previous studies. They suggest that secondary forests can become occupied by a subset of native amphibians, which can be valuable to conserving overall species richness in degraded land. Ficetola *et al*. (2008) investigated whether there were differences in abundance of *Eleutherodactylus* frogs in various forests of different levels of disturbance. They found a significant difference in abundance between the various forest types of primary, secondary and pasture and these differences varied among species, despite them being closely related taxa. The high density of *Eleutherodactylus fitzingeri* that was discovered showed that they were able to adapt to dense, low vegetation and therefore able to make a more rapid recovery in secondary forests. The need for pristine habitat becomes a problem for species of anurans that are not direct developing as they have an aquatic lifestage, so require different niches throughout development. Typically, the environments that they rely on are adjacent to one another, however, human interference has caused fragmentation and "habitat splitting" - which is the splitting of riparian or breeding areas with areas of land used for other life history stages. This causes problems for the anurans, as breeding adults need to cross to suitable breeding sites from forested areas. Becker *et al*. (2007) found that habitat splitting is an important factor in the reduction of populations and therefore an important consideration for conservation decisions in areas that have been fragmented.

Many studies have been carried out in certain regions such as North America and Europe, but global generalisations have been made determining why decline has been observed

(Houlahan *et al*. 2000). However, scientists are not certain on the reasons for the observed declines (Pounds *et al*. 2006) and more research is needed in tropical areas that contain high diversity (Houlahan *et al*. 2000). Throughout Latin America, most amphibian declines occurred at higher elevations (500m) and stream-associated species were more sensitive than terrestrial species (Young *et al*. 2001). Gaining knowledge on the current status of amphibians in areas that have not been well researched is important, as this can be added to baseline data. There is also the possibility that endangered and/or new species could be occupying this space. In protected areas for conservation, knowledge of local flora and fauna is also important for informing conservation decisions. More information should be gathered on the state of amphibian populations in areas that are under studied. Cloudbridge offers an opportunity for gathering information on amphibian composition and abundance whilst the forest undergoes transition from newly planted to secondary forests.

Aims

The aim of this project was to gain baseline information on the species composition and abundance of anurans at the reserve. Two field sights were chosen that varied in elevation, vegetation and public use to see if these variables had any effect on the data that was collected.

Materials and methods

Study sites

Cloudbridge Nature Reserve is located in the Southern Pacific slope of the Cordillera de Talamanca mountain range in Costa Rica and the elevation ranges from around 1550m to 2120m. It is classified as premontane wet forest and the climate is seasonal, with a wet season from April – December and a dry season from mid-December to March.

Two trails were chosen at Cloudbridge Nature Reserve South, to carry out fieldwork. The River trail (N 09°28.248’, W 83°34.306’) (Fig. 1) has been reforested in the past 10 years and runs close to the River Chirripó Pacífica, however it is not a riparian zone as the river is a steep drop from the trail. There are patches on the trail where planted trees have failed to establish and grasses dominate the area. The Heliconia trail (N 09°28.120’, W 83°34.552’) (Fig. 1) also runs parallel to Rio Chirripó Pacífica, however its proximity is further away from the river. The vegetation of the trail is the result of natural regeneration from the past 20 years. The River trail is a public trail where as the Heliconia trail is a private trail and isn’t currently being used for any other research, so disturbance is minimal.

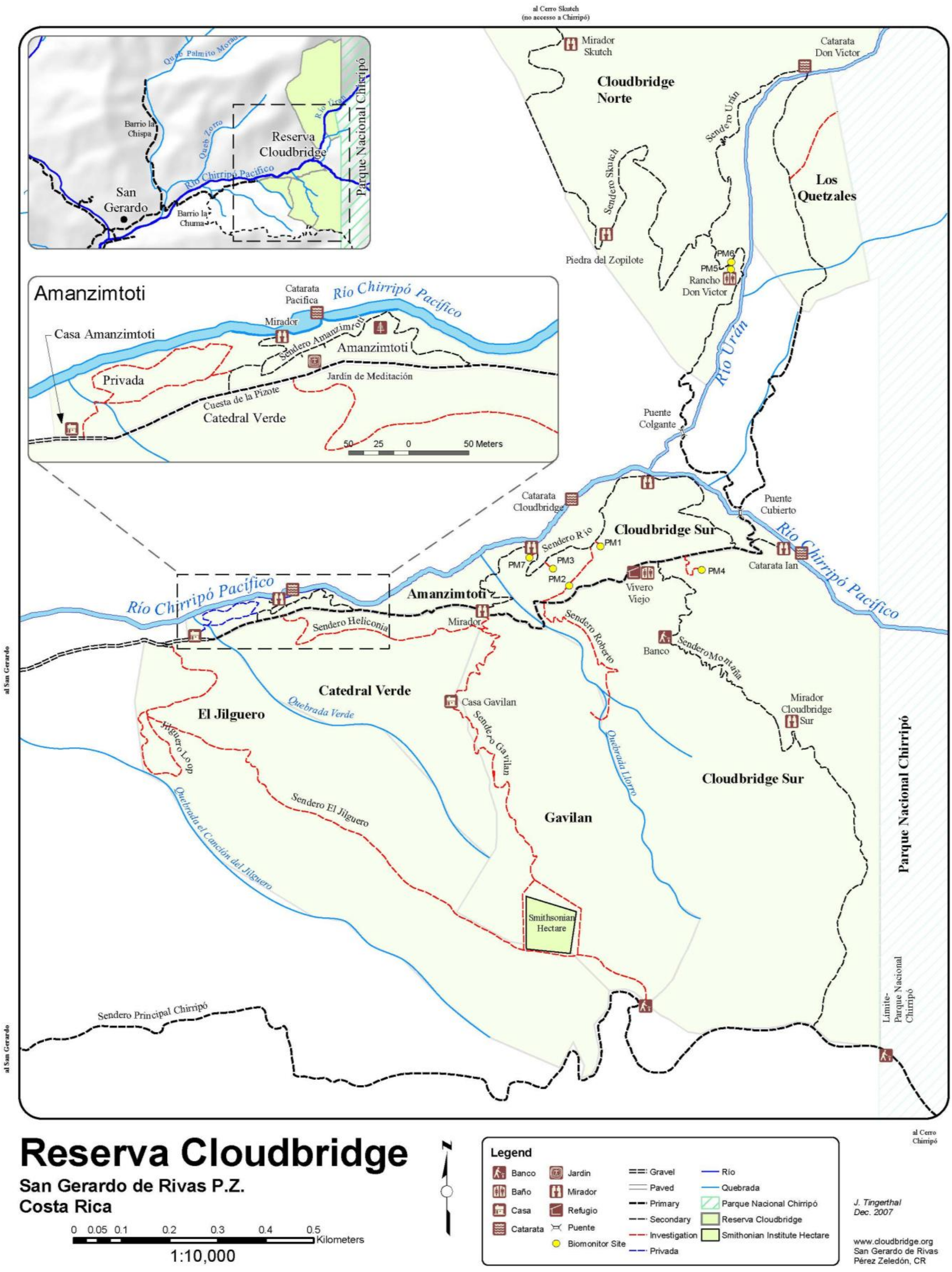

Figure 1 Map of Cloudbridge Nature Reserve. Sendero Rio and Sendero Heliconia trails were used.

Sampling protocol

Each trail was surveyed alternately for 14 nights each, giving a total of 28 survey nights between the 18 June – 31 July 2012. Night surveys were conducted between the hours of 18:00 – 22:30. A 500m section of each trail was covered. Two sampling methods were chosen for this study; visual encounter surveys (VES) and audio surveys. The VES involved a careful search for anurans on the ground and in vegetation (Heyer *et al*. 1994), up to a height that was physically possible to see on either side of the trail. Accessibility on each trail constrained the areas on which transects could be conducted, therefore up to one metre off trail, maximum, was surveyed. The transects were split up into 50m sections and marked with flagging tape as reference points of where anurans were seen. When an anuran was found, it's location on the trail, time it was found, type of vegetation they were on and activity when found, were noted. Individuals were then caught in individual self seal bags for identification. The key in Savage (2002) was used for identification as well as correspondence with external herpetologists. The snout-vent-length (SVL) measurement of individuals was taken whilst they were in the self seal bag. Additionally, any other notable characteristics were recorded e.g. colour markings or any obvious injuries. Photographs were taken of all captured individuals for further identification and reference in the future. Captured individuals were then released back to where they were found.

Audio surveys were carried out simultaneously with the VES in the evening, when calling activity is at its highest (Savage 2002). Six call-point counts were carried out at 100m intervals on the survey trail, where the surveyor would stop for two minutes and count the number of calls heard at that point. Any new calls heard between these points on the survey trail were also recorded. Estimation of distance of calls was difficult within the dense vegetation, therefore approximate distance to the calling individual was not recorded. However, the two species whose calls were recorded were well spaced and therefore individuals were easy to distinguish. All night time surveys were carried out by myself, whilst being accompanied by another researcher for health and safety reasons.

Vegetation surveys were carried out at each 50m point on the trails. The following variables were measured or approximated: canopy cover (using a densiometre); trail width; landform; number of trees with diameter > 10cm within 5m radius of the trail; height of tallest tree within 10m radius (using a clinometre and laser distance measurer); vertical vegetation density (using heights of 0-2m, 2-5m, 5-10m, 10-15m 15m+ and assigning indices of 0 (none), 1 (minimal), 2 (low), 3 (moderate), 4 (high), 5 (very dense)); presence of vegetation strata and approximate ground cover. This was so each trail's vegetation could be described quantitatively and compared. Four measurements of canopy cover, vertical vegetation density and approximate ground cover were taken and then averaged for each point. Daily rainfall and minimum and maximum daily temperature readings were obtained from a meteorological station based at the Casa, *ca* 0.5km from Heliconia trail and *ca* 1km from the River trail.

Data analysis

It would not have been statistically sound to split the 500m section of each trail into individual transects, as the trails were not large enough to have smaller transects far enough apart to be independent of each other. Therefore, each night survey was used as a replicate in the analysis. Generalised linear models (poisson distribution specified), were used to see if rainfall and temperature had an effect on the activity of anurans: for both calls and the number of individuals found through visual encounters. Species from each trail were tested separately so potential differences did not interfere with rainfall and temperature relationships. The abundance of species across the two trails was compared using Kruskal Wallis'. The species richness of each trail using data from the VES and calls was then calculated using Shannon's index and a Kruskal Wallis was used to see if there was significant difference between the two trails. Any individuals that were found after their call was recorded were omitted, so numbers were not duplicated. Vegetation surveys allowed quantitative data to be collected, which was then analysed qualitatively and permits only inferences as to how it may affect the populations of anurans that are found. All statistical analyses were carried out in R (v 2.15.0).

Results

Visual encounter surveys

A total of three different species of frog were found visually on the two trails. No toads were identified during this project. The two species *Craugastor fitzingeri* and *Pristimantis cruentus* were found on both the River and Heliconia trail, but the species *Pristimantis ridens* was found only on the Heliconia trail (Table 1). The abundance of species found through VES on the two trails was significantly different for *C. fitzingeri* (Kruskall Wallis, $\chi^2 = 6.17$, df = 1, p < 0.05) but not for *P. cruentus* ($\chi^2 = 0.02$, df = 1, p = 0.89) or *P. ridens* ($\chi^2 = 1$, df = 1, p = 0.32).

Table 1 Total counts of species found/heard on each trail through visual encounter surveys/acoustic surveys and total species richness of each trail.

	River trail		Heliconia trail	
Species	VES	Audio	VES	Audio
Craugastoridae				
Craugastor fitzingeri	32	99	1	6
Eleutherodactylidae				
Diasporus diastema	-	68	-	77
Strabonmantidae				
Pristimantis cruentus	4	-	3	-
Pristimantis ridens	-	-	1	-
Total number of counts (n)	36	167	5	85
Shannon's diversity index	0.55		0.22	

Only one female of *C. fitzingeri* was found, distinguished by the larger SVL of 41mm (Savage 2002) compared to the male average SVL of 27mm that were caught. Individuals were found on the ground and lower vegetation up to a height of 1.5m. The activity of individuals when found was either perched and calling, or just sitting, on vegetation. The one individual found on the Heliconia trail had a SVL of 24mm and was found calling on a shrub leaf. There were high amounts of colour morphism between individuals. Colours ranged from light brown to dark brown, whilst some had yellow mid-dorsal stripes and some were uniformly brown (Fig. 2).

a) b)

Figure 2 Colour morphism differences in the species *C. fitzingeri* a) male with uniform brown across body, b) male with yellow mid-dorsal stripe.

All *P. cruentus* were found sitting on vegetation between the heights of 1.2m - 8m. All were found sitting on the vegetation, however when one of them was put into the self seal bag it gave off a call, which was not expected for this species. The average SVL on both the River and Heliconia trail was 21mm. Colour morphism was also found in this species (Fig. 3).

a) b)

Figure 3 Colour morphism differences in the species *P. cruentus* a) individual with distinctive yellow cap b) individual with yellow spot on dorsal and yellow heels.

Only one individual of *P. ridens* was encountered throughout the entire study period, on the Heliconia trail. It had an SVL of 20mm and was found sat on a shrub leaf 1.6m off the ground.

Audio surveys

The two species whose calls were recognised and heard on both trails were *C. fitzingeri* and *Diasporus diastema*. There was no significant difference between number of calls at the two sites for either species (Kruskall Wallis, $\chi^2 = 3.48$, df = 1, p = 0.06; $\chi^2 = 0.37$, df = 1, p = 0.54), however the number of calls for *C. Fitzingeri* was much lower on the Heliconia trail than the River trail (Table 1).

Shannon's diversity showed there was a higher diversity on the River trail (0.55) than the Heliconia trail (0.22) and a Kruskal Wallis showed that there was a significant difference between the two trails ($\chi^2 = 7.95$, df = 1, $p < 0.01$).

Environmental variables

Total rainfall throughout the survey period was 603mm. More than half the days had less than 5mm of rain in a day. The average lowest temperature was 13.2°C and the highest was

22.9°C. The daily low temperature had a significant effect on the number of *D. diastema* calls recorded on the River trail (GLM, $z = 2.013$, $df = 10$, $p < 0.05$), but no other significant effects of rainfall and temperature on the number of calls recorded from *C. Fitzingeri* or the number of visual encounters for all other species on the trail. One observation was on a day that had the lowest high temperature of the day in the study period. This was the only day that no frogs were encountered through VES on the River trail. The rainfall had a significant effect on the number of *D. diastema* calls recorded on the Heliconia trail (GLM, $z = 2.632$, $df = 10$, $p < 0.05$), but no other significant effects of rainfall and temperature on the number of visual encounters for all other species on the trail.

The average canopy cover was higher on the Heliconia trail, but there was no significant differences between the two trails (Kruskal Wallis, $\chi^2 = 0.24$, $df = 1$, $p = 0.62$). Vertical density cover was higher on the River trail and was statistically different from the Heliconia trail at two measured levels (ANOVA: 2-5metres, $F_{20,1} = 5.71$, $df = 1$, $p < 0.05$, 5-10metres, $F_{20,1} = 4.37$, $df = 1$, $p < 0.05$). Other vegetation measures showed no significant differences. The Heliconia trail had a higher number of shrubs making up the vegetation strata and trees compared to the River trail. The height of trees was also higher on the Heliconia trail and the trail width was smaller. Average ground cover was higher on the River trail. In the first and last 100m of the River trail that was surveyed, the number of trees was reduced compared to the rest of the trail. These areas had a higher amount of ground vegetation and grasses dominated in the last 100m section. Both trails were mostly midslope of a steep incline in the mountain.

Discussion

Three of the species identified on this survey were new to the Cloudbridge database. *Craugastor fitzingeri* and *P. ridens* are typical ground dwelling species that are found by using the VES method of sampling and *D. diastema* is a common arboreal species. *Pristimantis cruentus* had previously been identified. *Craugastor fitzingeri* is a common frog and is typically found in lower vegetation (Savage 2002). Savage (2002) writes "the advertisement call is a series of short, harsh "clacks" sounding like two stones struck together...", however this was never heard. The call that was heard throughout the study period was more sporadic and could be described as a chirp. There is the possibility that it is used as a form of territorial function but also as a predator defence, as the call is not long and continuous. Höbel (2005) carried out a study looking at the sporadic call in *C. fizingeri* across different populations in Costa Rica. He concluded that the call was highly variable between populations and was representative of an aggressive signal. It is also suggested that the difference between the advertisement call and aggressive signal is an avoidance mechanism, which follows their life-history pattern of cryptic colouration and secretive perch choice.

There was no significant difference in the number of *C. fitzingeri* through VES on the two trails, however, the River trail had 32 counts but the Heliconia trail only had one (Table 1). The observation of increased calls on certain areas of the transect on the River trail, where the lower vegetation density was higher and the canopy was more open, provides the possibility that vegetation composition may determine the number of individuals on the trail. Furthermore, average ground cover and vertical vegetation density was higher on the River trail, which are also characteristics of younger forests, suggesting that younger re-forested areas may also be favoured by *C. fitzingeri* at Cloudbridge. Individuals were found on all types of lower vegetation which suggests that there is no particular plant species that is favoured. Previous studies have shown that populations of *C. fitzingeri* are determined by environmental alterations caused by humans – notably the change in the categorisation of forest (primary/secondary/pasture). Furlani *et al*. (2009) showed that population of *C. fitzingeri* and *D. diastema* were negatively affected by forest alterations, however other studies showed populations of *C. fitzingeri* were found in abundance in secondary forests (Ficetola *et al*. 2008). This suggests that the species of frog is adapted to change in the environment and therefore is more adaptable to changing landscape, resulting in being listed under "least concern" on the IUCN list (www.IUCNredlist.org).

The number of *P. cruentus* was the same on both trails, suggesting that the type of environmental variables at both sites was suitable for them to inhabit. According to Savage (2002) this species does not have a call, but it is possible that the sound that was heard when one of the individuals was in the self seal bag was a distress call. As only one *P. ridens* was found it is hard to make inferences about patterns of their presence. Both *P. cruentus* and *P. ridens* also come under "least concern" on the IUCN list (www.IUCNredlist.org). Colour morphisms of individuals are often prevalent in populations. Captured individuals of *C. fitzingeri* and *P. cruentus* were able to show this variation (Fig. 2 & 3) (Savage 2002). Overall species richness was higher on the River trail and the significant difference between the two sites suggests that the River trail is favoured more by the leaf litter frogs and one arboreal species found in this study.

The lack of visual encounters on the night survey that took place on the coldest recorded day of the survey period, may have been due to amphibians being ectotherms. Studies have shown that calling activity is influenced by abiotic factors such as air temperature, which could have resulted in the lack of encounters (Aichinger 1987). The significant differences found in the number of *D. diastema* calls according to rainfall and temperature show a further possible influence from abiotic factors.

Whilst not directly comparable to other studies, due to location and fieldwork experience, the counts of all species found through VES was low. This could be a result of only having one surveyor on the trail or because the abundance of each species is genuinely low. Seasonal patterns are often observed for anurans and Watling & Donnelly (2002) concludes that the timing of reproduction and patterns of phenology can be attributed to the availability of prey items. The abundance of *P. ridens* in Schlaepfer & Gavin (2001) study was found to increase in the rainy season as well, which they suggest could be a result of prey availability, predation or reproductive success in different habitats. As calling is a form of communication for males attracting females and increase in calling activity has also been found to increase during the rainy season (Höbel 1999), the abundance of individuals may differ according to which season the survey is carried out in. This should be considered when analysing and comparing data in future studies at Cloudbridge.

Recording the calls of the anurans gives a good indication of the presence of species, however it does not provide true population size estimates as records will only represent numbers of males. Calling activity can provide measures of habitat suitability for reproduction (Hilje & Aide 2012), therefore is important to record in areas where little is known on anuran populations to gain an understanding of the health of the populations.

The previous study on anurans carried out at Cloudbridge is not directly comparable due to different trails being surveyed and having only collected three nights worth of data (Cousineua 2007). Not all species were identified down to a species level, however one *P. cruentus* was previously found. This suggests that this species has been in the reserve for at least the previous five years, but population size of this species could have changed in a positive or negative way since the previous study. The species of frogs found in this study are 'direct developing' i.e. they do not have an aquatic life stage. Therefore abundance of water pools will not be central for their reproduction. However, one species found in the previous study was *Hyla pseudopuma*, which has a larval reproductive stage and requires water pools for egg laying (Savage 2002). Reproduction for this species is explosive, with choruses of males gathered around pond sites at the beginning of the rainy season. As none of this species were found during this survey, it is possible that the population that was here before has disappeared, they do not inhabit the areas sampled or they were missed.

The disease chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (Bd), is an infectious disease that can have severe effects on some amphibian populations. It is one of the factors that is thought to be causing worldwide amphibian declines. The symptoms that anurans show when infected can vary widely (Muths & Hero). Although there were no obvious outward signs of infection in the individuals found in this project, laboratory tests would need to be carried out to confirm this. The chytrid fungus has been identified in Costa Rica previously and has been found to persist at higher altitudes throughout the year, compared with lowland areas (Kriger & Hero). It is hypothesised that the spread of Bd can occur through movement of people's boots or equipment, even on birds and invertebrates that are travelling between watersheds (Johnson & Speare 2005). Considering Cloudbridge is open to the public and is at a high elevation, these variables may increase the likelihood that anuran populations are or could be infected in the future, thus having adverse affects on the population sizes.

Conclusions and future work

This study was able to give a representation of the species composition and abundance of individuals on two of the trails at Cloudbridge Nature Reserve, using the methods VES and audio surveys on two species of frogs. There is the strong possibility that there are other arboreal dwelling species that are present on the trails that were not sampled due to non-recognition of their calls. Additionally, if individuals were ground dwelling, there is the possibility of biased searching where individuals are more easily seen sitting on vegetation. An individual was less likely to be seen in dense vegetation, or if their colouration was cryptic, particularly as research was undertaken at night using headtorches for illumination. Despite these possible biases, this study has shown that secondary forests at Cloudbridge are home to forest dwelling species that have been found in similar habitats around Costa Rica (Savage 2002). This also provides the possibility that there are other un-sampled species that could be living here, possibly in lower population sizes or that are endangered.

Cloudbridge has recently collaborated with Global Vision International, which sends four pairs of interns on rotating 10 week placements to the reserve. It is suggested that night time surveys on at least the River trail are carried out simultaneously with other vertebrate surveys, so that continuous data on the population numbers of the anurans can be gathered. The data collected from this project provides a baseline dataset which can be expanded in the future. It would be beneficial to carry out further surveys on other trails and/or study other calls as this will give further information on arboreal dwelling species which are hard to survey using the VES method. This will give a bigger picture of what species are on the reserve. If multiple sites are surveyed with similar vegetation compositions, this will allow statistical inference as to the presence of species.

Long-term studies are also needed to detect changes in anuran populations, as they are able to fluctuate on a yearly bases, therefore any possible long-term declines can only be detected through long periods of study (Lips *et al*. 2010). The reforestation effort that Cloudbridge is experiencing will hopefully increase anuran population numbers and potentially provide new habitats for species that may not already occupy the area.

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