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Monitoring Anuran Populations in Bosque Protector Candelaria: A multi-year comparison of frog populations in an Ecuadorian cloud forest

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A multi-year comparison of frog populations in an Ecuadorian cloud forest

Noblella sp., a potentially new species encountered during this study

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Abstract:

For many years, amphibian populations around the world have been declining due to climate change, habitat loss or change, and diseases such as Ranavirus or the Chytrid fungus. However, there is still a great lack of information regarding the diversity of frogs, especially in the cloud forests of Ecuador where this study was conducted. This study was conducted in April and May of 2017 in the Ecominga Reserve of Cerro Candelaria in El Placer, Ecuador. The objective was to conduct visual-encounter surveys of several sites in the reserve and compare the results to studies that have been conducted in the reserve every spring since 2014 and the fall of 2016. 248 individuals of 20 species were encountered, including two species which were only encountered through auditory data and one that may represent an undescribed species. This is the same number of individuals observed as in the previous study, however this study added two sites of marshland that were areas of high frog density (115 of the 248 individuals were observed in these sites) and suggests that frogs may be more abundant during the dry season, but would need more data collected during the dry season to support this hypothesis. Data were also collected regarding the type of substrate and height at which each frogs were found, in order to determine a preference overall and within species, as well as to compare with similar results obtained in the fall of 2016. Along with the previous study, this study found that frogs were most commonly encountered on leaves, particularly leaves of shrubs and generally at a height of 0-30cm but suggests that more studies be done to better understand the population of anurans in BPCC.

Resumen:

Por muchos años, poblaciones de anfibios en todas partes del mundo han sido disminuyendo debido al cambio climático, perdida o cambia de hábitat, y enfermedades como Ranavirus o el hongo Chytrid. Sin embargo, todavía falta mucha información de la diversidad de ranas, especialmente en los bosques nublados de Ecuador, donde este estudio fue hecho. Este estudio fue hecho en abril y mayo de 2017 en la Reserva Cerro Candelaria de Ecominga en El Placer, Ecuador. El objetivo fue llevar a cabo un censo de visualización en varios sitios en la reserva y comparar los resultados con estudios hecho en la reserva cada primavera desde 2014 y en el otoño de 2016. Este estudio encontró 248 individuos de 20 especies, incluyendo dos especies los cuales solo fueron encontrado con datos auditivos y otra especie que quizás representa una especie nueva. Este número de individuos es lo mismo que en el estudio anterior, sin embargo este estudio añadió dos sitios de pantano con una densidad alta de ranas (115 de los 248 individuos fueron observado en estos sitios) y sugiere que las ranas pueden ser más abundantes durante la estación seca pero más datas son necesarios de la estación seca para apoyar este hipótesis. También datos fueron colectados en relación con el tipo de sustrato y altura en que cada rana fue encontrado para determinar una preferencia de todos y dentro de una especie y para comparar con resultados similares del estudio del otoño de 2016. Junto con el estudio anterior, este estudio encontró que las ranas fueron encontrados más frecuentemente en hojas, particularmente hojas de arbustos y en una altura 0-30cm pero sugiere que más estudios son realizados en BPCC para entender mejor la población de anuras allí.

Topic codes: 601, 609, 614, 624

Keywords: Frog populations, cloud forest, *Pristimantis*, *Noblella*

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Introduction:

Throughout the last several decades, amphibian populations have been observed to be declining across the world, particularly in mountainous areas such as the cloud forests of the Andes (La Marca, 2005). Three of the most common factors causing these declines include habitat loss or alteration, climate change, and disease (Lips, Burrowes, Mendelson, & Parra-Olea, 2005). Due to the fact that amphibians take in oxygen, and therefore contaminants through their skin, they are very sensitive to even small changes in the environment. Additionally, since they live on land and in the water, amphibians are sensitive to changes in both environments. Therefore they can be considered very useful bio-indicators for understanding the total health of an ecosystem. This includes effects produced by climate change as well as other natural and anthropogenic changes in an ecosystem. Juveniles, eggs, and species with larvae that develop aquatically may also be especially at risk (Lips *et al.*, 2005).

Frogs in particular are also threatened by the Chytrid fungus (*Batrachochytrium dendrobatidis*), which was affecting at least 7 species in Ecuador as of 2005 and has likely continued to spread (Bustamante, Ron, & Coloma, 2005). In the lab this fungus has been shown to grow best in cool and humid conditions, making it potentially the most dangerous to montane amphibian populations (Lips *et al.*, 2005). In addition, Stark *et al*. found in 2014 that Ranaviruses, which have been shown to be affecting amphibians throughout the world, had particularly grave effects on the tadpoles and eggs of several species of frogs in the Nicaraguan cloud forest. Additionally, frogs in the Peruvian Andes (including some in the *Pristimantis* genus, which is very prevalent in Ecuador) have shown co-infection between *Batrachochytrium dendrobatidis* and Ranavirus, indicating that as one infection spreads it may facilitate the infection of another (Warne *et al.*, 2016). Changes in climate may also catalyze the spread of these harmful diseases, both of which have high mortality rates, particularly among larvae (Ron & Merino, 2000; Warne *et al.*, 2016).

However, despite these alarming trends, few studies have been conducted to assess amphibian populations in Ecuador, which is the country with the third highest diversity of amphibians (Menéndez-Guerrero & Graham, 2013). According to Bustamante et. al (2005), the

first quantitative study on amphibian decline in Ecuador wasn't published until 2003. Therefore, there is still a lot of work to be done regarding monitoring and studying frog populations in Ecuador, as evidenced by the number of new species described only in the last five years (Batallas & Brito, 2014; Reyes-Puig & Yánez-Muñoz, 2012; Brito, Ojala-Barbour, Batallas & Almendáriz, 2016). These new species mainly belong to the genus *Pristimantis*, a group which is well represented in the area in which this study takes place, with at least 18 of the 24 currently registered species (Reyes-Puig *et al.*, 2013). Assessing these species can help determine their baseline population levels and distribution, two vital pieces of information in order to determine if changes occur in either due to any of the aforementioned factors.

The cloud forests of the Andes have proven to be an environment of especially high diversity and endemism of amphibians, particularly amongst the genus *Pristimantis* (Reyes-Puig & Yánez-Muñoz, 2012). Cloud forests are defined mainly by the persistent presence of clouds or mist and can occur among a wide range of altitudes as well as annual precipitation values (Bubb, May, Miles & Sayer, 2004). This allows for a large variety of microhabitats to exist even within one mountain slope, possibilities which multiply when considering the number of mountain slopes in the Andes range. This study aimed to take a census of the frog population within Bosque Protector Cerro Candelaria (BPCC), a reserve owned by the Ecominga foundation, which acts as a corridor between Sangay and Llanganates National Parks (Figure 1). This reserve is located at 01°28'35.9" S' 78°17'46.6" W and S1 25 46.3 W 78 18 58.4, 01°27'5.9" S, 78°18'29.6"W, with altitudes between 1400 and 3800msnm (Reyes-Puig *et al.*, 2013). Since 2008, 43 species of herpetofauna have been registered within the reserve, 24 of which are frogs and toads (Reyes-Puig *et al.*, 2013). This region of the high Pastaza watershed is an area of particularly high biodiversity due to the convergence of Andean montane forests with tropical lowland forests (such as those in the Amazonian Basin) (Reyes-Puig *et al.*, 2013). BPCC is also home to 40% of the amphibians and reptiles reported in the Pastaza watershed area, as well as 45% of the regionally endemic species (Reyes-Puig *et al.*, 2013). More information about the site can be found under site descriptions in the methodology section. Studies have been conducted in Cerro Candelaria Reserve in spring 2014, spring 2015, spring 2016, and fall 2016. Therefore, one of the objectives of this study was to compare the species richness and abundance of individuals from this study in the spring of 2017, with numbers from previous studies. In addition to the five transect locations used to compare to past studies, this study also took a census of frog populations in two marsh environments to compare numbers of species and individuals between the two and to set them up as areas for future monitoring. This study also explored more specific data regarding each frog encountered, such as the temperature, percent humidity, type of vegetation it was found on, and behaviors it was observed having when encountered.

The objectives of this study can be broken into several parts. First, by using the same transect locations as previous years, this study hoped to compare both species richness and abundance from this year with previous years, within each transect site and overall. Additionally, behavioral data will be compared when possible with previous years, in order to better understand the individual species and to observe any changes. Secondly, this study also looked to compare species richness and abundance found within the various transect locations from this year, in order to better understand the distribution of species and individuals in Bosque Protector Cerro Candelaria and to determine what species and genera are the most and least common. Behavioral data will also be compared within and between species, information that could be useful for future conservation steps. Additionally, species and individuals will be compared

between the two wetland sites and with the other sites to determine if there are differences in species composition. Based on the previous studies, it was expected that the genus *Pristimantis* would be the most common in the reserve, with *P. incomptus* as the most common species. Due to all the previously mentioned factors reducing amphibian populations, it was also expected that fewer individuals and/or species would be encountered in this study than in previous ones.

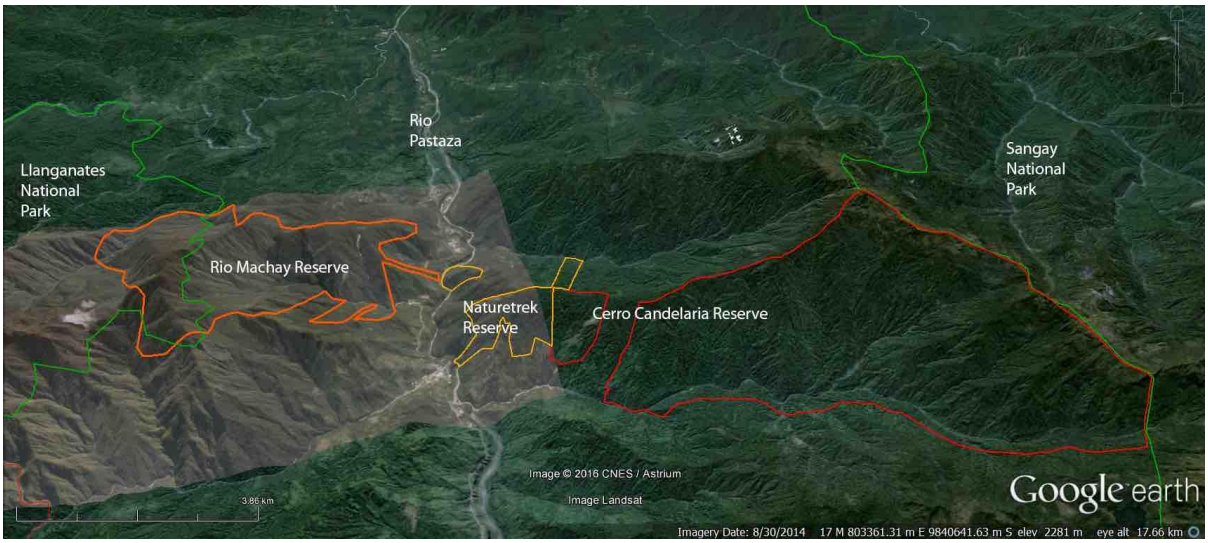


Figure 1. Map of Bosque Protector Cerro Candelaria, showing the boundaries with the neighboring national parks and reserves (Jost, 2015).

Methods:

Materials

- Headlamp with extra batteries
- 30m measuring tape
- 30g scale
- Thermometer with percent humidity measurement
- Digital calipers
- Ziploc bags labeled A-Z
- String
- Marking tape
- Identification book: *Herpetofauna en áreas prioritarias para la conservación: el sistema de reservas Jocotoco y Ecominga*
- Camera
- Alcohol
- Plastic containers
- Field notebook

Methodology

Transect locations were selected based on those that had been measured in previous years. Five trails in Cerro Candelaria were used for this study: Velastegui, Lote G, Machay, San Pedro, and Playas de Chinchin. The park guide from previous years (Luis Recalde) along with

Juan Pablo Reyes-Puig assisted in indicating where the sites were located. A 500 meter transect was measured along each of the five trails during the first day of study at each site. Due to inaccessible terrain on one or both sides of the trail, the transects had to be laid out on the trails themselves. Additionally, each transect was marked at every interval of 20 meters in order to get a better idea of where frogs were found along the transect. Sites were visited 3 times each throughout the study period, generally once a week unless outside factors prevented it. Data were collected generally between the hours of 6:30pm and 10:30pm Monday-Friday, with some exceptions based on trail and weather conditions.

Each night of data collection, the researcher (along with a local guide employed by Ecominga) walked along the transect at a pace of about 40 minutes per 100 meters, looking out 1-2 meters along both sides of the trail. When a frog was encountered, data were taken regarding the time, temperature, and percent humidity. Additionally, observations were made regarding what kind of vegetation the frog was found on, as well as its height from the ground, and any behaviors that were observed, such as perching, singing, or jumping. If the species could be identified in the field that was also noted. The frogs were then captured, placed in a Ziploc bag with air and a leaf, and take back to El Placer. The next day, frogs were measured with calipers to determine their length, and with a 30-gram scale to determine their weight. Photos were also taken and sent to Juan Pablo Reyes-Puig if the species could not otherwise be identified by the researcher or with the help of the book *Herpetofauna en áreas prioritarias para la conservación: el sistema de reservas Jocotoco y Ecominga*. Individuals were then released on the same trail or in a similar habitat with a comparable altitude. Occasionally the researcher captured a frog that appeared to be of a new species or was otherwise of interest to preserve (as advised by Juan Pablo Reyes-Puig). In this case, the frog would be placed in a small jar or plastic container, filled about halfway with 60% alcohol, stored with a label and later given to local expert Juan Pablo Reyes-Puig.

In addition to the five transect locations, data were also taken from two marsh sites. One was located along the first 60 meters of the Playas de Chinchin trail while the other was located a little above the San Pedro trail. Each of these sites was measured in order to determine the approximate area. Observations were also made regarding the vegetation within each of these sites. Data were taken from these sites when visiting the corresponding nearby trails, generally between the hours of 6:30pm and 7:30pm. Due to the large number of frogs within these areas, data were not taken regarding each individual as they were along the transects. Instead, each species was identified and tallied. Temperature and percent humidity were recorded at the beginning and periodically throughout the measuring period. In order to get an idea of the size of the individuals in these sites, measurements were taken for about 10 individuals per species (when possible). This was done in the field or the next day in El Placer using the same techniques as for the individuals found along the transects. Auditory data were also taken when possible, with the help of Juan Pablo Reyes-Puig for species identification, in order to get a more complete census of the number of individuals in BPCC.

Data were analyzed in part using the iNEXT software, developed by Anne Chao *et al.* in order to calculate estimates of the completeness of the sampling of this study, as well as several indices of diversity for each of the 7 sites studied. SpadeR software, also developed by Anne Chao *et al.*, was used to calculate an estimate for the Sorenson similarity index, both between all 7 study sites as well as between each pair of sites.

Site descriptions

Velastegui:

This site was the nearest to El Placer and had an elevation of about 1450 meters above sea level. It was split into two sections, one of 360 meters and the other of 140 meters. In between these sections is an area of naranjilla cultivation. The first 360m section is characterized mainly by a mature secondary forest, with some patches of long grasses along the side of the trail that borders the Pastaza river. The second section of 140m is also primarily secondary forest, but with less dense vegetation due to the presence of large rocky cliffs rising on one side.

Lote G:

This site was the highest altitudinally at about 1800 meters above sea level. It is dominated by primary forest and is absent of any cultivated areas. Vegetation is therefore generally highly stratified, although the terrain drops off steeply along certain parts of the transect, severly decreasing the vegetation. It is not near a river, but several streams and waterfalls intercept the transect area.

Machay:

This site is located about a 6 minute drive from El Placer, across the Pastaza River and near the Machay River. The elevation is about 1500 meters above sea level. It is characterized by a secondary forest, with many pioneer species such as cecropia and balsa still dominant. There are no areas of current cultivation, but effects can still be seen from the agriculture that occured there approximently 25 years ago (Weigel, 2016).

Playas de Chinchin:

This site is located along the main trail from El Placer to the reserve at Cerro Candelaria. The first 60 meters are located in the marsh alongside the Chinchin River (see below for more information). From 60-500 meters, the transect mainly lies alongside secondary forest with some sections of primary forest. However, vegetation directly next to the trail is generally fairy sparse, with a lot of moss and fern-covered cliffs.

Marsh at Chinchin:

This site measures about 55m by 36m, for an overall area of approximately 2000m^2. It is located in the first 60 meters of the transect Playas de Chinchin, alongside the Chinchin River with an elevation of approximately 1600 meters above sea level. Most of the area is flooded with water that drains down from the main trail and from at least one small stream. In the flooded areas, there is a large amount of herbaceous plants, along with a layer of algal growth on the water. As the water starts to recede, shrubs reaching 2-3 meters begin to appear. This area is rich in juveliles, especially from the species *Dendropsophus parviceps*.

San Pedro:

This transect was split into two sections, one of 300 meters and one of 200 meters. The section of 300 meters was located alongside the Pastaza River at an elevation of about 1450 meters above sea level. From 0-140 meters the site is very open and is dominated by large grasses of 2-3 meters. From 140-300 meters there is secondary forest that is recovering from previously being an area of pasture and agriculture. The 200 meter section was located higher at about 1600 meters above sea level along a trail that leads from San Pedro to El Placer. This

section is also primarily secondary forest, dominated by cecropia and balsa, and bisected by several streams and waterfalls that run into the Pastaza River.

Marsh at San Pedro:

This marsh, in contrast to the one along the Chinchin River, is manmade. With an area of about 15m by 30m (approximately 450m^2), it is also much smaller than the the marsh at Chinchin, but resides at about the same elevatino of 1600msnm. It is comprised mainly of two pools of water, one higher than the other, which are connected by a small stream. Around the two pools, the vegetation is dominated by shrubs of 2-3 meters tall. It was observed to be an area of great density of frogs, especially *Chimerella marielene*. There were also several observations of frogs mating, along with eggs in various stages of development.

Results:

Results from this study can be divided into two categories: those collected during this study in the spring of 2017 and those compared between this study and the previous ones from 2014-2016. However, since this study was conducted most similarly to the study in the fall of 2016, some comparisons are made with this study in the section devoted to results from the spring of 2017.

Results from the spring of 2017

During this study period, a total of 248 individuals belonging to 18 species and 4 families were encountered (Table 1). However, two additional species, *Pristimantis pastazensis* and *Gastrotheca testudinea*, were recorded in auditory data, bringing the total number of species observed up to 20 and adding an additional family (Table 1). The one unknown individual was counted towards the number of individuals found but not the number of species. The most individuals were found in the marsh at San Pedro, followed by the transect at Lote G (Table 1). The most common species were *Chimerella marielene* with 79 individuals found, all at the San Pedro marsh, followed by *Dendropsophus parviceps* with 54 individuals found throughout 4 locations, and *Pristimantis rubicundus* with 21 individuals, also in 4 locations (Table 1).

Table 1. Diversity and distribution of frogs found in BPCC in spring 2017.

Species (Family)	Velastegui	Lote G	Playas de Chinchin	Machay	San Pedro	Marsh Chinchin	Marsh San Pedro	Total
Chimerella mariaelenae (Centrolenidae)							79	79
Dendropsophus parviceps (Hylidae)			19		4	22	9	54
Pristimantis rubicundus (Craugastoridae)	16	3	1	1				21
Pristimantis eriphus complex		19						19

(Craugastoridae)								
Pristimantis incomptus (Craugastoridae)	6	1	3	3	3		1	17
Pristimantis bellae (Craugastoridae)		14						14
Pristimantis bicantus (Craugastoridae)		9						9
Hypsiboas almendarizae (Hylidae)			3		2	1	3	9
Pristimantis churuwiai (Craugastoridae)	1		2	5	1			9
Pristimantis conspicillatus complex (Craugastoridae)				5				5
Noblella sp. (Craugastoridae)		3						3
Pristimantis ganonotus (Craugastoridae)		2						2
Pristimantis katoptroides (Craugastoridae)	1							1
Pristimantis sp. 1 (Craugastoridae)	1							1
Pristimantis quaquaversus (Craugastoridae)		1						1
Scinax ruber (Hylidae)	1							1
Nymphargus cochranae (Centrolenidae)			1					1
Rinella margaritifera (Bufonidae)					1			1
Pristimantis pastazensis (Craugastoridae)					Heard			Heard only

Gastrotheca testudinea (Hemiphractidae)							Heard	Heard only
Unknown		1						1
Total	26	53	29	14	11	23	92	248

Additionally, for each frog encountered in a transect site (133 of the 248 total individuals), data were collected regarding the type of vegetation the frogs were found on, the height at which they were found, and the distance along the transect that they were found in. 73.49% of frogs were found on some type of leaf, 34% of which were leaves of shrubs, 18% leaves of herbaceous plants, 13% unspecified types of leaves, 5% epiphytic leaves, and 4% leaves of vines (Figure 2). Leaves were unspecified during the first day of study, after which the methodology was changed to accommodate more specific identification. Additionally, 14% of frogs in transects were encountered on ferns while 6% were found on the ground (Figure 2). 2% or less of frogs were found on club moss, branches, stems, or grass (Figure 2). The previous study from the fall of 2016 also took data regarding the perching point of each frog encountered, although with more general categories. However, similar results can be seen between the two studies. For example, 80.6% of frogs in the fall 2016 study were found on leaves, compared to 73.49% in this study, as well as 12.5% found on ferns in the previous study compared to 14% in this study (Weigel, 2016; Figure 2). One noticeable difference was that only 0.4% of frogs in the fall of 2016 were found on the ground while 6% were found in this study (Weigel, 2016; Figure 2).

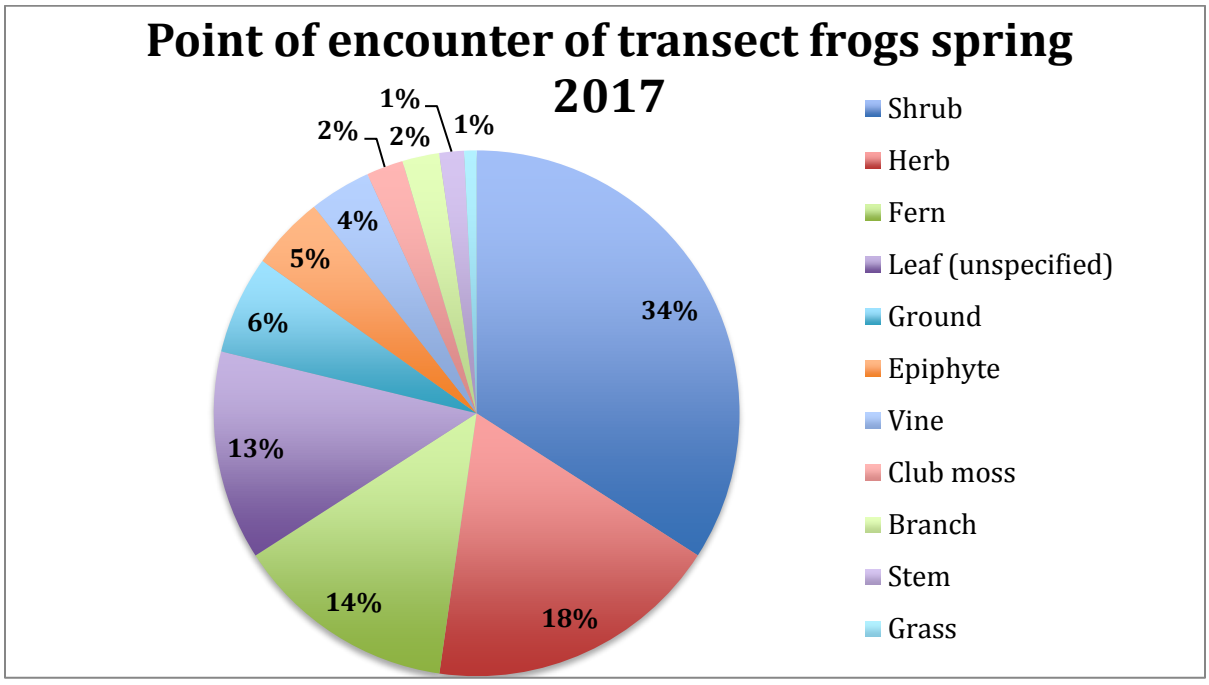


Figure 2. Percentages of the type of vegetation that transect frogs were encountered on.

Some species also seemed to show a preference for a certain type of perching point, while others were more generalized. For example, *P. bellae, P. bicantus, P. churuwiai,* and *P. conspicillatus complex* were all found on at least 5 different substrates in fairly even percentages

(Figure 3). Other species, such as *P. rubicundus* and *P. incomptus* were also found on at least 5 types of vegetation, but had at least 50% which were found on a specific type of vegetation (Figure 3). *Noblella sp. 1* was the only species encountered more than once that was found in only one category; 100% of these frogs were found perching or jumping on the ground (Figure 3).

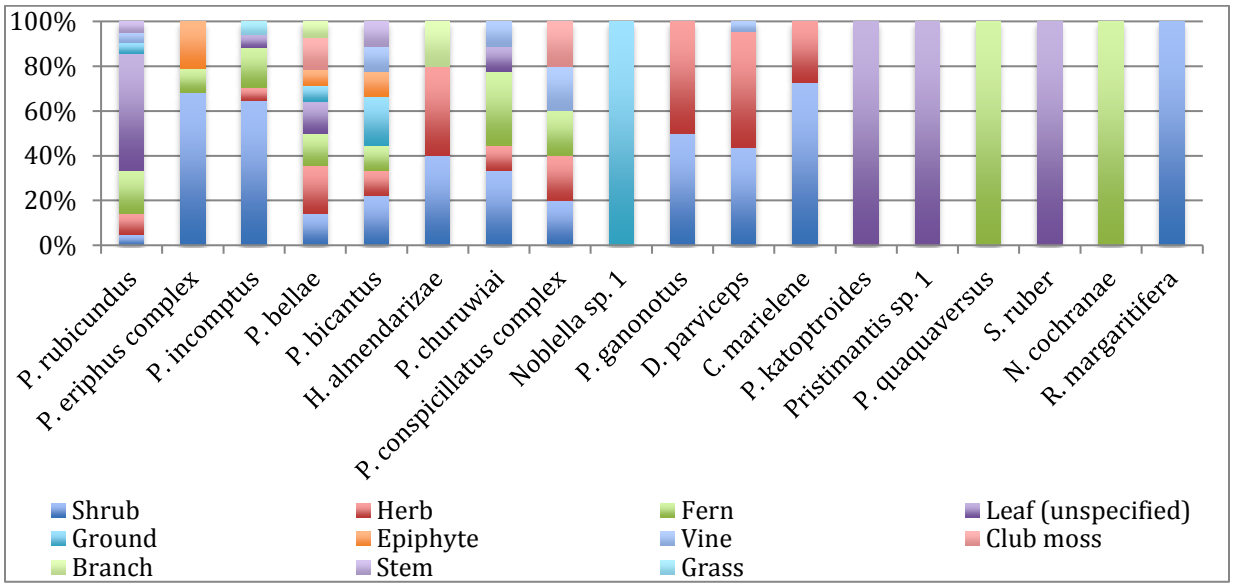


Figure 3. Point of encounter divided by species.

The height at which individuals were found was also recorded. After dividing these heights into 30cm sections from 0-210cm, some patterns among species can be observed. For example, 8 species that were encountered more than once during this study were observed to have at least 50% of individuals found between 0 and 30 centimeters (Figure 4). Some species showed an even higher preference for this height range including *Noblella sp.1* (100%), *P. bicantus* (89%), and *P. bellae* (86%) (Figure 4). *C. marielene* was the only species that showed a majority (50%) preferring a height of 181-210cm (Figure 4). Other species, such as *P. eriphus com, P. incomptus*, and *P. churuwiai* did not show a clear preference for a certain perching height (Figure 4).

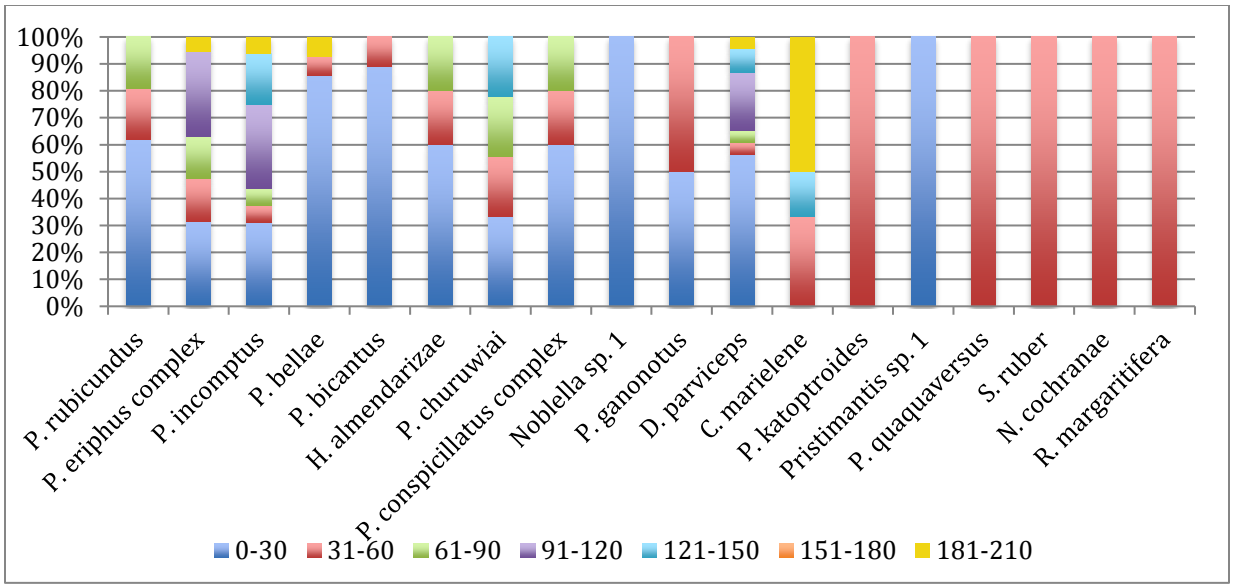


Figure 4. Encounter height divided by species.

Data were also taken regarding the temperature and percent humidity during each collection night. Average temperatures only varied a maximum of 3.05° between sites. Velastegui, the site with the lowest elevation, was found to have the highest average temperature at 19.67°C (Table 2). In contrast, Lote G, with the highest elevation at 1800 meters above sea level was found to have the lowest average temperature at 16.62°C (Table 2). Percent humidity was found to be highest at San Pedro and Machay, with 93.48% and 93.07% respectively (Table 2). The lowest average percent humidity was found at Velastegui with 88.79% (Table 2).

Table 2. Basic information on the climate and elevation of each study site.

Site	Average temperature	Average percent humidity	Elevation
Lote G	16.62	91.58	1800
Playas de Chinchin	16.86	89.48	1600
Chinchin Marsh	16.88	88.84	1600
San Pedro Marsh	18.36	89.6	1600
Machay	18.41	93.07	1500
San Pedro	18.23	93.48	1450-1600
Velastegui	19.67	88.79	1450

Due to these differences in temperature, humidity levels, elevation, and vegetation, different species were found at each site. At Velastegui, 5 different species were found with a total of 25 individuals (Table 3). *P. rubicundus* was by far the most abundant species at this site with 16 individuals (Table 3). Additionally, 6 individuals of *P. incomptus* were found at this site, spread out among the three nights of collection (Table 3). The other three species, all of the genus *Pristimantis*, were only found at this site once, all during the first night of collection (Table 3). The species *Scinax ruber* was technically found outside of the transect area, in the area of naranjilla cultivation that divides the transect, but it was included in the study as it still contributes to the diversity within BPCC (Table 3). At this site, frogs were not found to be

evenly distributed along the 500m transect (Figure 5). Of the 25 twenty-meter transect sections, frogs were found in only 9 of them (Figure 5). Most commonly, frogs were encountered between 40 and 60m (7 individuals) or between 240 and 260m (4 individuals) (Figure 5).

Table 3. Abundance of species and individuals found along the transect Velastegui.

Species	Transect 1	Transect 2	Transect 3	Total
Pristimantis rubicundus	12	2	2	16
Pristimantis incomptus	2	3	1	6
Pristimantis churuwiai	1	0	0	1
Pristimantis katoptroides	1	0	0	1
Pristimantis sp. 1	1	0	0	1
*Scinax ruber**	1	0	0	1
Total	18	5	3	26

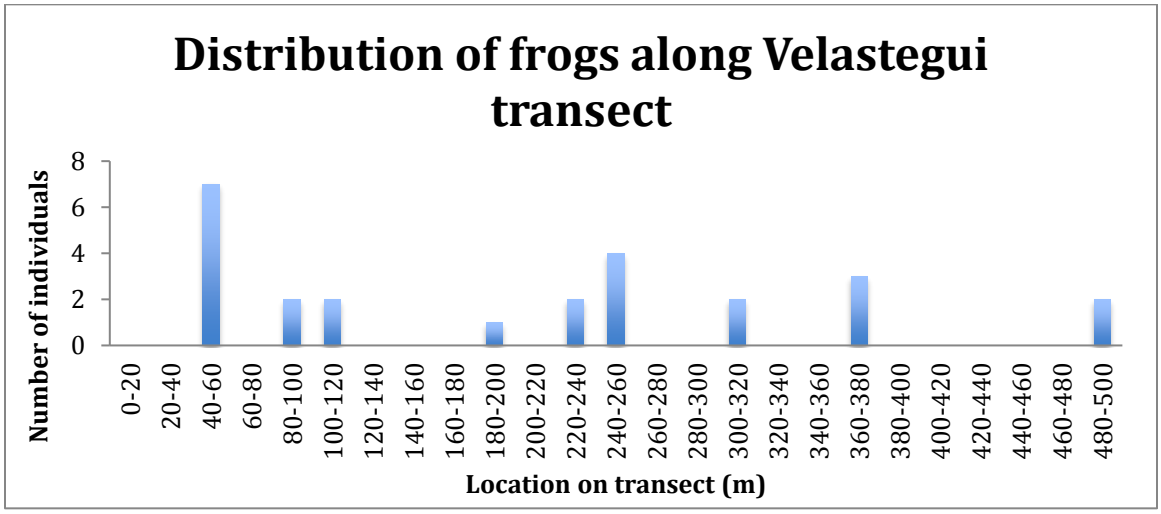


Figure 5. Distribution of individuals along the Velastegui transect.

Lote G was the site with the greatest species diversity, with at least 8 species encountered, as well as the transect site with the greatest number of individuals found (53) (Table 4). *P. eriphus com* was the most common species, found 19 times between the three nights of collection (Table 4). *P. bellae* and *P. bicantus* were also found during each of the three nights, and are the next two most common species, at 14 and 9 respectively (Table 4). *Noblella sp. 1* was encountered three times at Lote G, and potentially represents a new species for this genus (Table 4). The species that was unknown escaped before it could be identified, but was included as partial data regarding its perching height, substrate, and location along the transect had already been recorded. Frogs at Lote G were generally evenly distributed as individuals were found in 19 of the 25 sections of the transect (Figure 6). However, the most individuals were found between 20 and 40 meters and between 80 and 100 meters; each section had 8 individuals when numbers were combined from the three nights of collection (Figure 6).

Table 4. Abundance of species and individuals found along the transect Lote G.

Species	Transect 1	Transect 2	Transect 3	Total
Pristimantis eriphus complex	2	11	6	19
Pristimantis bellae	8	4	2	14
Pristimantis bicantus	4	4	1	9
Pristimantis rubicundus	0	2	1	3
Noblella sp. 1	0	1	2	3
Pristimantis ganonotus	1	0	1	2
Pristimantis incomptus	1	0	0	1
Pristimantis quaquaversus	0	1	0	1
Unknown	1	0	0	1
Total	17	23	13	53

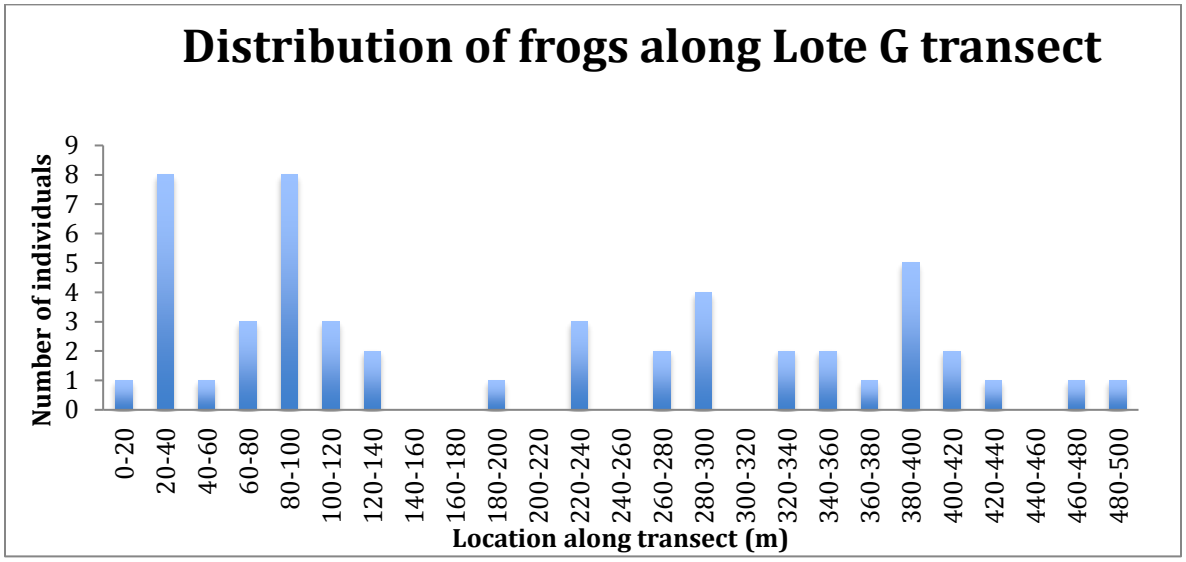


Figure 6. Distribution of individuals along the Lote G transect.

At the transect Playas de Chinchin, a total of 29 individuals belonging to 6 species were found (Table 5). The most common species was *D. parviceps*, found 19 times and on each of the three nights of collection (Table 5). *H. almendarizae* was found three times at this transect location, however each individual encountered was a juvenile, found in the marsh section of the transect (Table 5). *N. cochranae* was found only once, on the first night of collection, but is of interest as this is the first time this species has been recorded at BPCC (Table 5). Frogs were disproportionately found in the first 40 meters of the transect at Playas de Chinchin, with 21 of the 29 individuals found in this section (Figure 7). The remaining 8 individuals were spread throughout 6 sections of the transect (Figure 7).

Table 5. Abundance of species and individuals found along the transect Playas de Chinchin.

Species	Transect 1	Transect 2	Transect 3	Total
Dendropsophus parviceps	7	5	7	19
Hypsiboas almendarizae	2	0	1	3
Pristimantis incomptus	2	1	0	3

Pristimantis churuwiai	1	0	1	2
Nymphargus cochranae	1	0	0	1
Pristimantis rubicundus	0	1	0	1
Total	13	7	9	29

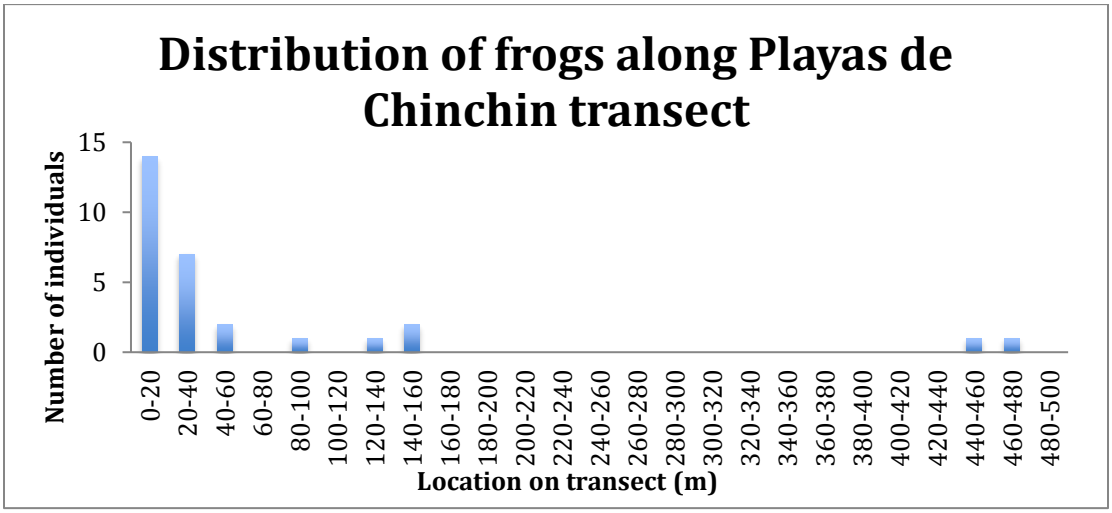


Figure 7. Distribution of individuals along the Playas de Chinchin transect.

In this study, Machay had only 14 individuals of 4 species found throughout the three nights of collection (Table 6). The most common species were *P. churuwiai* and *P. conspicillatus com*, each found 5 times at this site throughout the study period (Table 6). Frogs were distributed among only 8 of the 25 sections of the transect, with the most (3) found between 420 and 440 meters (Figure 8).

Table 6. Abundance of species and individuals found along the transect Machay.

Species	Transect 1	Transect 2	Transect 3	Total
Pristimantis churuwiai	2	2	1	5
Pristimantis conspicillatus complex	2	2	1	5
Pristimantis incomptus	0	1	2	3
Pristimantis rubicundus	1	0	0	1
Total	5	5	4	14

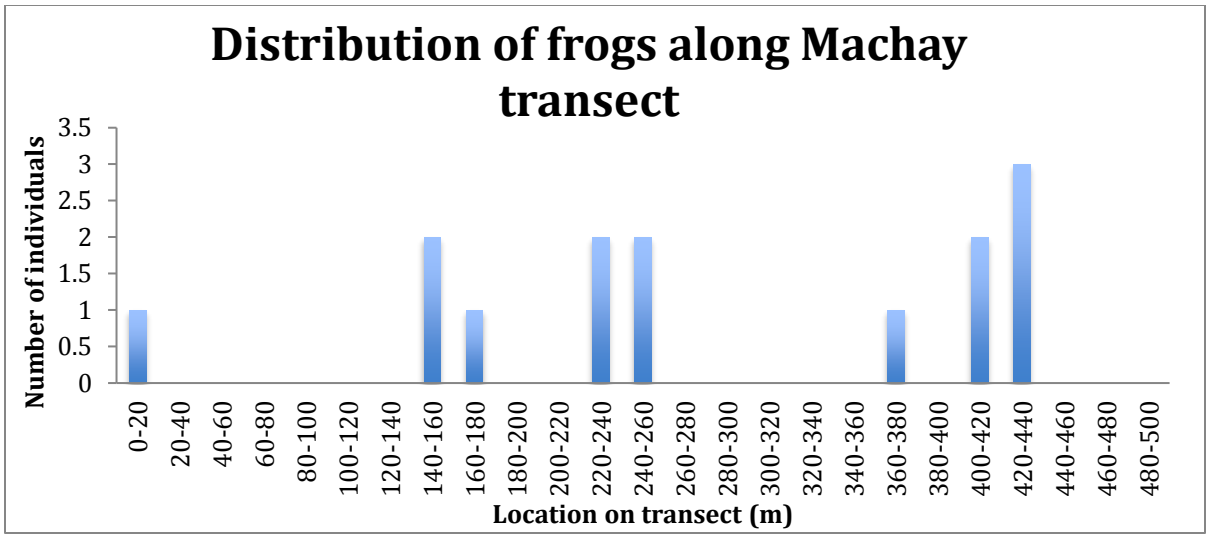


Figure 8. Distribution of individuals along the Machay transect.

The transect at San Pedro had the least number of individuals of the five transect sites, with only 10 individuals of 4 species found throughout the 3 nights of study (Table 7). The most common species at this site was *D. parviceps* found a total of three times on two out of the three nights of study (Table 7). *R. margaritifera* was technically found outside of the transect range, in an area between the marsh and the first 300 meters of the transect, but has been included here to better show the diversity that can be found at BPCC (Table 7). Frogs were distributed throughout 9 of the 25 sections of the transect at San Pedro (Figure 9). The only section where more than one individual was encountered was the last section, between 480 and 500 meters (Figure 9).

Table 7. Abundance of species and individuals found along the transect San Pedro.

Species	Transect 1	Transect 2	Transect 3	Total
Dendropsophus parviceps	3	0	1	4
Pristimantis incomptus	1	2	0	3
Hypsiboas almendarizae	0	1	1	2
Pristimantis churuwiai	1	0	0	1
*Rinella margaritifera**	0	1	0	1
Total	5	4	2	11

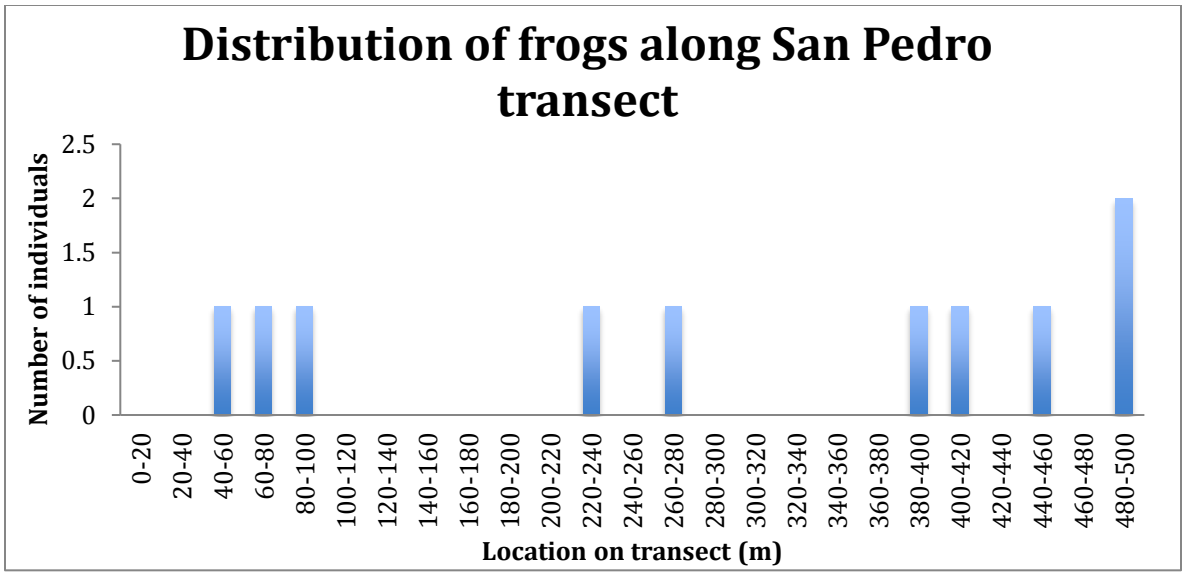


Figure 9. Distribution of individuals along the San Pedro transect.

In addition to the five transect locations, data were taken from two marsh locations. The decision to add this source of data to the study was made after the start of data collection and therefore data is lacking from the first night of data collection at the marsh of Chinchin. However, the first 60 meters of the transect at Playas de Chinchin overlaps with this site, so data could overlap between the two. *D. parviveps* was by far the dominant species found at the marsh, comprising 22 of the 23 individuals (Table 7). The only other species found belonged to *H. almendarizae* (Table 7). However, all of the frogs found in the first 60 meters of the Playas de Chinchin transect technically also belong to the marsh, adding 23 individuals (Figure 7).

Table 7. Abundance of species and individuals found at the marsh of Chinchin.

Species	Night 1	Night 2	Night 3	Total
Dendropsophus parviceps	0	10	12	22
Hypsiboas almendarizae	0	1	0	1
Total	0	11	12	23

The marsh at San Pedro was found to be more diverse than the marsh at Chinchin, with 92 individuals belonging to 4 species (Table 8). However, the dominant species was by far *C. mariaeleane*, contributing 79 individuals to the total (Table 8). *D. parviceps* and *H. almendarizae*, two species that were also found at the marsh at Playas de Chinchin, were found at the San Pedro Marsh (Tables 7 & 8). However, there was one recording of *P. incomptus* in the marsh at San Pedro. (Table 8).

Table 8. Abundance of species and individuals found at the marsh of San Pedro.

Species	Night 1	Night 2	Night 3	Total
Chimerella mariaelenae	21	33	25	79
Dendropsophus parviceps	4	2	3	9

Hypsiboas almendarizae	3	0	0	3
Pristimantis incomptus	1	0	0	1
Total	29	35	28	92

Using iNEXT's asymptomatic analysis, each site that was sampled during this study was analyzed using three different diversity indices. For this program q=0 is a simple measure of species richness, q=1 is the exponential Shannon index, and q=2 is the inverse Simpson index (Chao, A. *et al.*, 2016). The site Lote G was estimated to have the highest diversity using each of the three indices (Figure 10). The marsh at Playas de Chinchin showed the lowest diversity values on the three scales (Figure 10). San Pedro, although it had a lower species richness (q=0) value than Velastegui or Playas de Chinchin, had higher values than both for the exponential Shannon index and the inverse Simpson index (Figure 10).

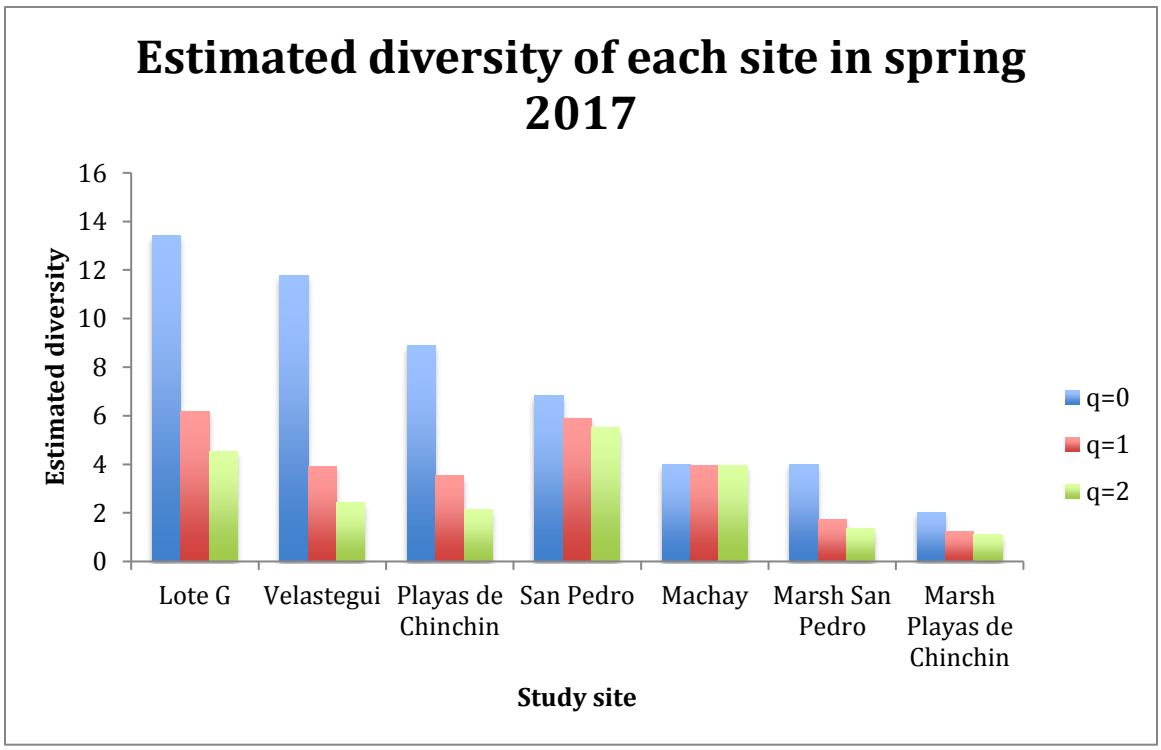


Figure 10. iNEXT estimated diversity of each site sampled during spring 2017 study using 3 diversity indices where q=0 is species richness, q=1 is the exponential Shannon index, and q=2 is the inverse Simpson index.

Sites were also analyzed using SpadeR's multiple community measures in order to estimate the Sorenson similarity index between all of the 7 sites sampled in this study, as well as between each pair of sites. The Sorenson similarity index looks at presence versus absence of species and uses a scale where the closer the value is to 1 the more similar the sites are. The value for all 7 sites was estimated to be 0.1617, indicating a low overall similarity between the sites sampled in this study (Figure 11). The two sites that were found to be the most similar, with a Sorenson similarity value of 0.667, were the two marsh sites (Figure 11). The least similar sites, with a Sorenson similarity value of 0, were between the marsh at Playas de Chinchin and each of three sites: Velastegui, Lote G, and Machay (Figure 11).

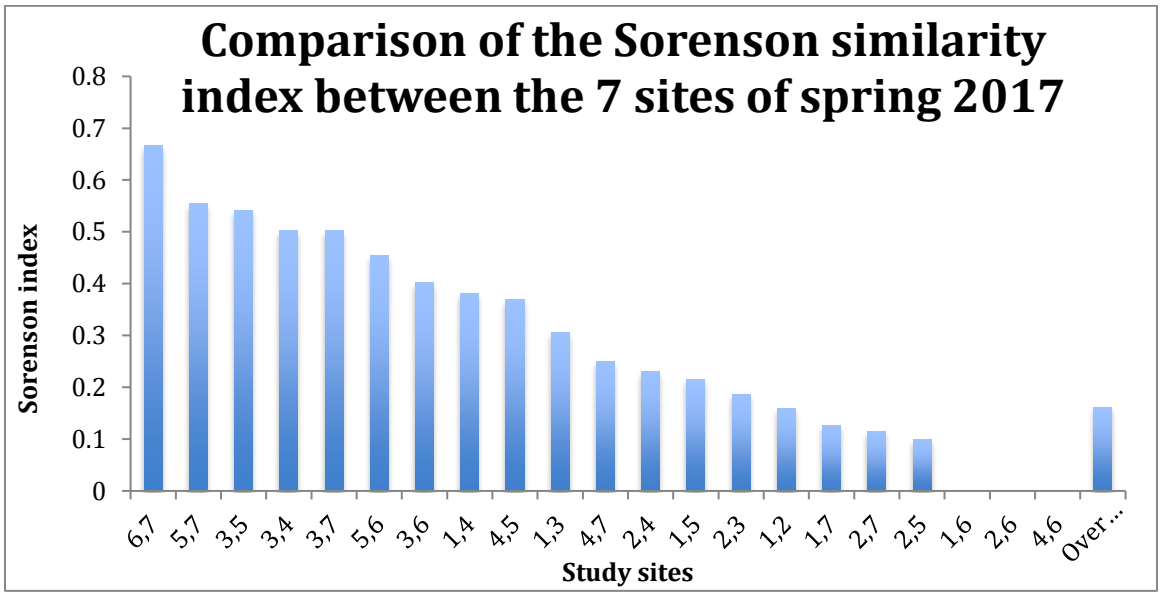


Figure 11. SpadeR estimated Sorenson similarity index for all 7 sites and between each of them where 1=Velastegui, 2=Lote G, 3=Playas de Chinchin, 4=Machay, 5=San Pedro, 6=Marsh at Playas de Chinchin, and 7=Marsh at San Pedro.

When doing a population study, it is useful to know how completely the areas were sampled in order to determine the usefulness of the study and how to improve for future studies. Using the iNEXT program developed by Anne Chao *et al.*, results were obtained estimating the coverage both for each site sampled during this study and for all of BPCC (based on the seven sampled sites). According to these estimates, all sites had a sample coverage of 0.8347 or higher (where 1.0 means that the site was sampled to 100% completeness) (Figure 12). The marsh at San Pedro was found to have the highest estimated sample coverage with a value of 0.9891, while the transect at San Pedro had the lowest estimated sample coverage with a value of 0.8347 (Figure 12). The overall sample coverage from this study was found to be 0.9756 (Figure 12).

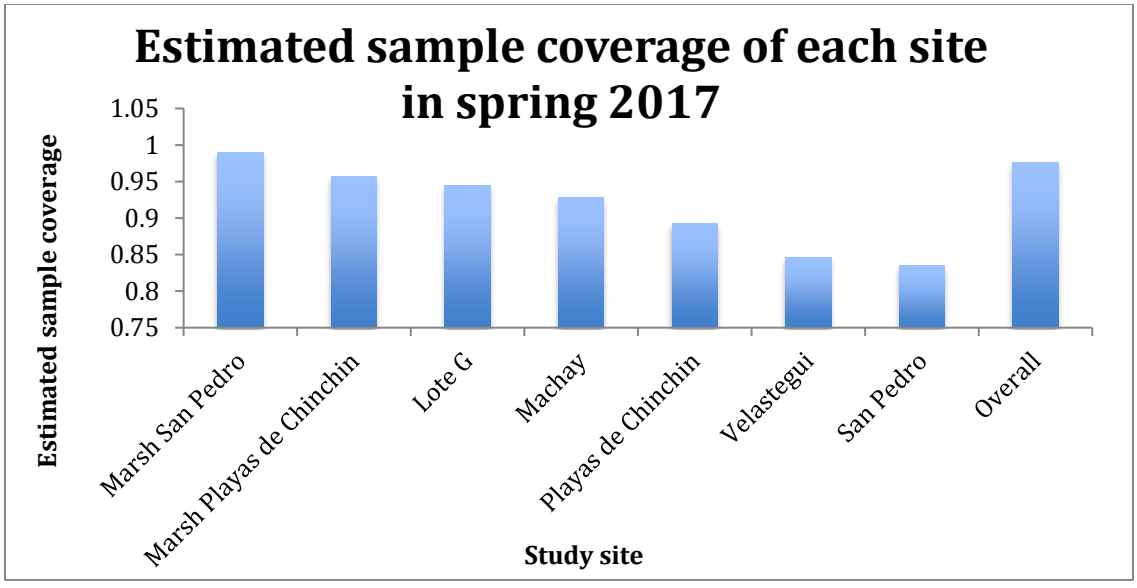


Figure 12. iNEXT estimated sample coverage of each site sampled in spring 2017.

Results compared with previous studies

Another important part of this study was to compare the results with those done in previous years. First, comparing number of species found in each study, more species have been found each year, with the exception of the fall of 2016 (Figure 13). This study found the most species (20) out of any of the previous studies. However, this number includes the two species that were only observed in the auditory data and not visually encountered, as well as two species that were found outside of the transect area (Figure 13).

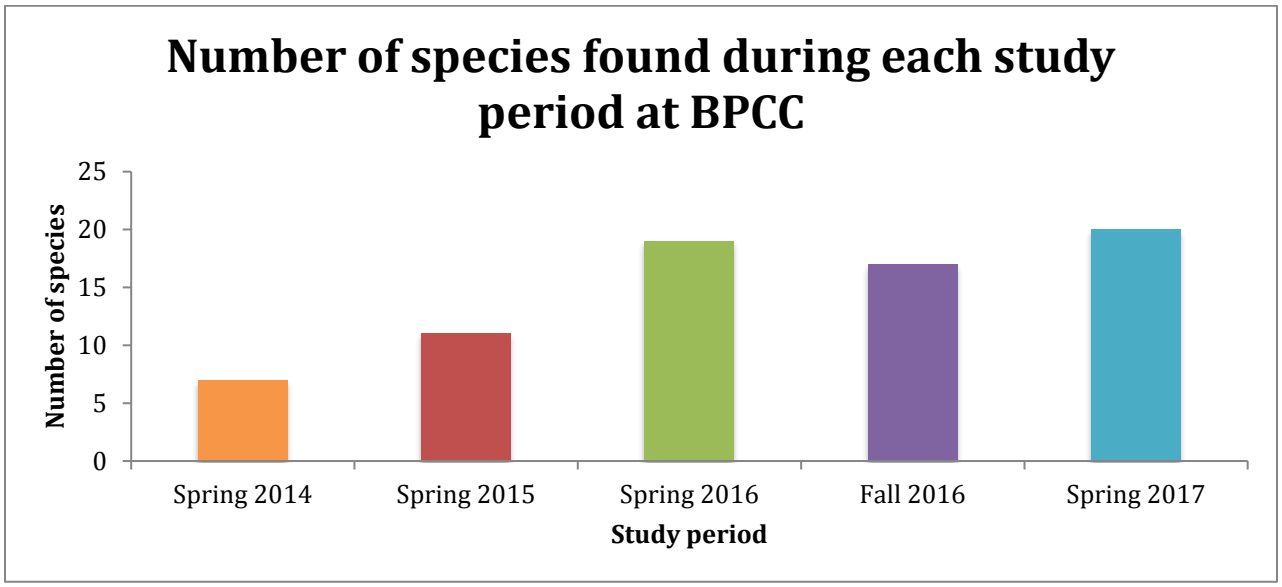


Figure 13. Comparison of number of species found between this study and all previous studies at BPCC.

Number of individuals has also increased over the years, with the exception that the same number of individuals (248) were found in this study as in the previous one (Figure 14). However, these numbers include different sites and different numbers of hours in the field between each of the studies. For example, this study found many individuals in the marsh areas, two sites that were not used in the previous study. Only 133 of the 248 individuals found in this study were found along transects, which is comparable to the 117 individuals found in the spring of 2016 (Figure 14).

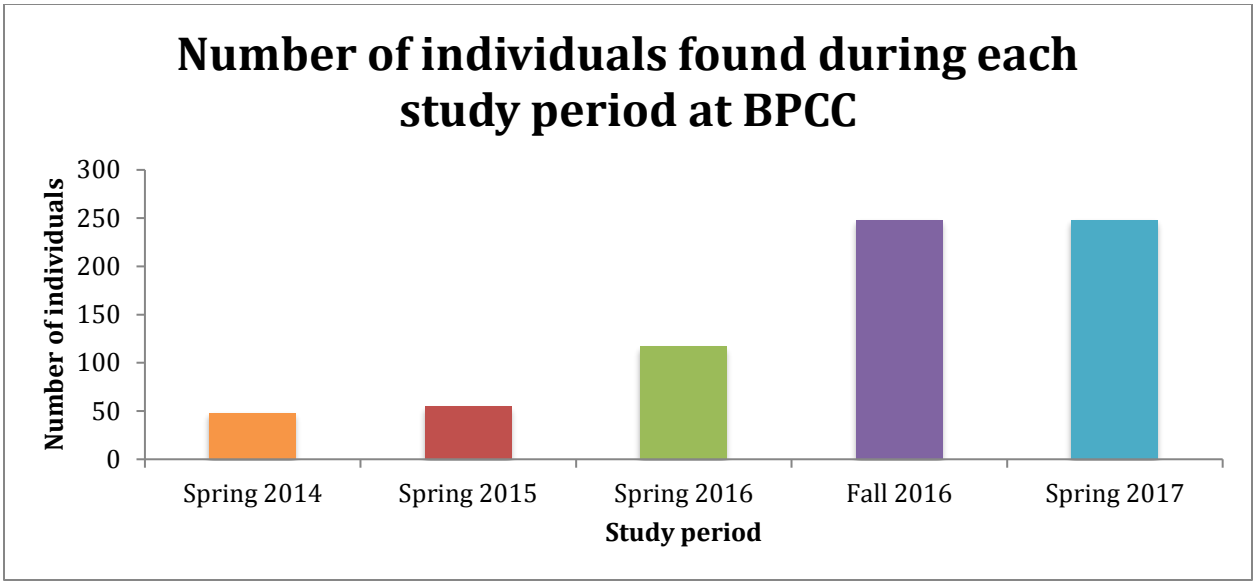


Figure 14. Comparison of the number of individuals found between this study and all previous studies at BPCC.

Additionally, distribution can be monitored according to the location of each transect. Not all the sites covered in this study were included in each of the four previous studies, however each site had been studied at least twice previously. According to these numbers, the study from the fall of 2016 found the highest number of individuals at each of the five sites, with the exception of Lote G where 53 individuals were found in this study compared with 52 in the previous study (Figure 15). San Pedro was also found to be the area of least species richness both in this study and the study from the fall of 2016 (Figure 15). Lote G had the highest number of species for this study as well as the springs of 2016 and 2014, however the fall of 2016 and spring of 2015 studies found the greatest number of individuals at Velastegui (Figure 15).

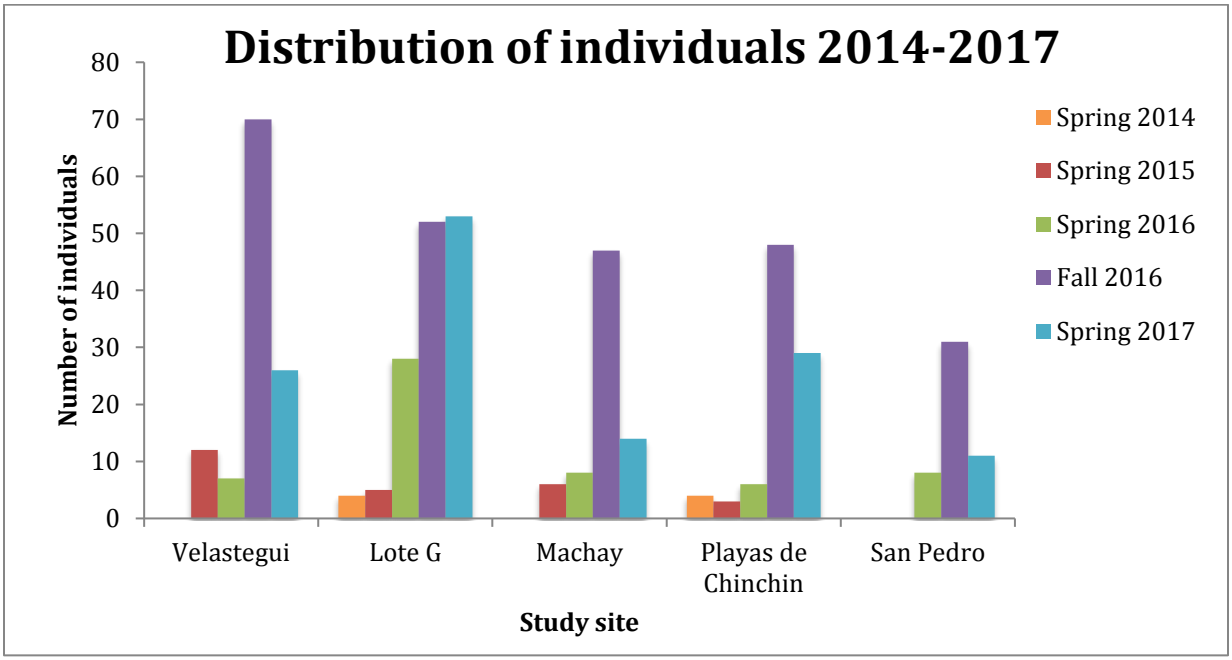


Figure 15. Number of individuals found at each of the 5 transect sites from spring of 2014 to spring of 2017.

Discussion:

This study was overall successful in completing its objectives, although some of the results obtained were different than expected. A total of 248 individuals were observed during this study period, the same number that was observed during the previous study in the fall of 2016 (Figure 13). However, in an effort to improve the study and the types of information gleaned, this study added two locations that were not studied in any of the previous studies. These sites contributed 115 individuals and at least one species that would not otherwise have been found (Tables 7 & 8). Based on the previous studies, it was expected that the genus *Pristimantis* would be the most common in the reserve, with *P. incsomptus* as the most common species. However, due to the presence of these marsh sites and the abundance of individuals within them, the most common species was *C. mariaelenae* (Table 1). Even looking at the individuals found only within the five transect sites, the most common species there was found to be *D. parviceps* (Table 1). Despite this, *Pristimantis* was still overwhelmingly the most common genus from the five transect sites, representing 73.7% of the total individuals found in the transects and 39.9% of the total individuals found in the study (Table 1). These results are comparable to the 65% and 82.7% abundances of *Pristimantis* found in the spring and fall of 2016 respectively (Alverson, 2016; Weigel, 2016). These results are also consistent with a 2008 study that found 20 species of the genus *Pristimantis* in BPCC, 71% of the species of amphibians and reptiles recorded during the study (Yánez-Muñoz & Reyes Puig, 2008). Since *Pristimantis* frogs are direct developing and don't rely on the water to reproduce, they are not frequently found near bodies of water (Yánez-Muñoz & Reyes Puig, 2008). However, one individual of *P. incomptus* was recorded in the marsh at San Pedro. More information regarding the comparison of abundance of species and individuals between this study and the previous ones can be found in Table 9 in the appendix.

One noticeable trend is that number of individuals has significantly increased throughout the years of study (Figure 14). The exception to this is that this study found the same number of individuals as the previous study (Figure 14). However, due to the addition of the two marsh sites, in order to accurately compare number of individuals found with the previous study, only the 133 individuals found in the transects should be considered, a number that is significantly lower than the previous study but is comparable to the 117 individuals found in the spring of 2016 study (Figure 14). One possible explanation for these discrepancies are the number of hours spent in the field in each of these studies. In the spring of 2014, 23 hours were spent in the field, 31.25 in the spring of 2015, 32.5 in the spring of 2016, 63.8 hours in the fall of 2016, and 47.95 in this study (Weigel, 2016). Therefore, it is possible that the study from the fall of 2016 found the most individuals since more hours were spent in the field. However, this could also potentially indicate a difference in frog abundance between the dry season (June to September and December-January) and the wet season (February to May and October-November) (Knapp, MacLeod, & Véloz, 2017). One study found that frogs were more abundant in a Panamanian rainforest during the dry season, when their arthropod prey were also more abundant (Toft, 1980). They also found that species diversity did not change between seasons but did change between study sites, very similar to the results obtained in this study (Toft, 1980). Since there has only been one study conducted in the fall during the dry season in BPCC however, no conclusions can be drawn without further study.

Data were also recorded regarding the perching height and vegetation of each frog encountered on a transect. In both this study and the previous study in the fall of 2016, it was found that leaves were overwhelmingly the most common perching point to encounter frogs (Weigel, 2016; Figure 2). This study also indicated that the leaves of shrubs tended to be the most common type of leaf for frogs to perch on (Figure 2). Frogs were found to most commonly perch at a height between 0 and 30 centimeters (Figure 3). A 2008 study done in BPCC also found that a majority of amphibian and reptilian species (of which the majority are species of frogs) prefer to perch at the lowest strata of the forest, here defined as 0-50cm (Yánez-Muñoz & Reyes Puig, 2008). However, the results obtained in this study could also have been due to the fact that frogs are generally easier to observe on leaves than on the ground. Additionally, due to the high moisture levels in the cloud forest, there is an increased quantity of vegetation, particularly epiphytes, making it more likely to encounter frogs on vegetation than in other types of forests (Fahey, Sherman, & Tanner, 2015). Vegetation also varied a lot even within one transect, which can help to explain the distribution patterns of the frogs along the transects at each site.

However, there are several limitations to this study that restrict the accuracy and completeness of the data. For example, data was only collected during after-dark hours (with the exception of one afternoon spent searching at Lote G with the hopes of encountering more *Noblella sp.*) and therefore nocturnal frogs are those best represented in the study. Additionally, as a visual-encounter type of study, only frogs that were 2 meters or lower were observed during this study, missing any that may prefer to perch higher. Although transects were made in generally the same area as the previous study in the fall of 2016, most of the markers left from the previous study were no longer present. Thus, while data from transects year to year can be compared, the results denoting where along the transect the frogs were found cannot reliably be compared. Additionally, particularly at the site Playas de Chinchin, it is recommended that in the future the transect continue down the principal trail instead of starting in the marsh to avoid overlapping of sites. The transects themselves also present a limitation, as they were all conducted on human-made trails, which could potentially induce edge effects and change the composition of the population (C., Feinsinger, & Crump, 2002). This 2002 study found an abrupt change in the abundance and composition of frogs in the transition from forested area to agriculture, something that could perhaps occur on a smaller scale in the transition from forest to open trail, particularly on those that are more heavily trafficked (C., Feinsinger, & Crump, 2002). Additionally, some of the trails (for example Velastegui) were either bordered or intercepted by areas of agriculture, a much bigger disturbance than a trail which also has the potential to affect the abundance of frogs.

There are also several areas for improvement in the accuracy of data in this study. For example, sometimes when trying to catch specimens to bring back to El Placer to take size measurements and for identification, the frogs escaped before this was possible, reducing data in all categories. Additionally, it is possible that due to poor photo quality, some frogs (especially the juveniles) could have been misidentified. In order to save time in this study, the researchers estimated the heights of perch points of the frogs instead of measured precisely with a measuring tape. In the future, it would be beneficial to actively measure these distances to increase the accuracy. Additionally, although temperature and percent humidity were recorded with the capture of each frog, this means that there are more data points to draw an average from for sites where more frogs were found. Also, although long-term comparative studies are useful to track

changes in populations, all the studies at BPCC were conducted by different researchers and guides, leading to inevitable differences in methodology and ability to find frogs.

Due to the fact that there is still such a lack of information on the frogs in Ecuador (as well as in other places around the world) there is a lot of room for future areas of study. It could be interesting to focus more on the altitudinal differences in frog populations in BPCC, adding transects that are higher than 1800 meters above sea level, the current highest transect evaluated in this and previous studies. Future studies could also add data on the distance of each frog encountered from the path, in order to determine if the path affects the distribution of the frogs. If possible, a transect location (or multiple) away from a path, but with a similar elevation, temperature, and humidity as another transect, should be added in order to compare if the path has a significant effect on frog distribution. Future studies should also continue to study and monitor the marsh sites added in this study, to better determine their composition and to observe any potential changes through the years. Additionally, more marshes could be found, hopefully at varying altitudes, to have a better comparison. Auditory data was also used sparingly in this study, but was useful to identify individuals that were not found immediately along the transects. In the future, an effort should be made to include more auditory data, recording the songs heard at each site. Future studies could also make an effort to better identify all individuals as male, female, or juveniles, something that this study did but didn't get complete enough data to include it.

Although this study had relatively high sample coverage, at 0.8347 or higher for each site, there is still a need to expand studies at this site (Figure 12). These sample coverages are relevant only regarding the methodology used in this study and it is assumed that with other methodologies additional/different species would be found, such as expanding auditory data. Additionally, 35 species have been found throughout the 4 years and 5 studies done in BPCC, significantly more than the 24 indicated in the guide written for the reserve in 2013 (Reyes-Puig *et al.*, 2013). This, combined with the fact that new species are still being discovered (including a potential new species found in this study), indicates that there is still a lot of work to be done to be able to understand the full composition of anurans in BPCC.

References:

Alverson, H. (2016) *Frogs of the forest: Monitoring Anuran populations in Bosque Protector Cerro Candelaria* (Unpublished article (ISP)). SIT, Brattleboro, VT.

Batallas, D. R., & Brito, J. M. (2014). Nueva especie de rana del género Pristimantis del grupo lacrimosus (Amphibia: Craugastoridae) del Parque Nacional Sangay, Ecuador. *Papéis Avulsos de Zoologia (São Paulo)*, *54*(5), 51-62. https://dx.doi.org.library.smcvt.edu/10.1590/0031-1049.2014.54.05

Brito, J. M., Ojala-Barbour, R., Batallas, D. R., & Almendáriz, A. C. (2016). A new species of Pristimantis (Amphibia: Strabomantidae) from the cloud forest of Sangay National Park, Ecuador. *Journal Of Herpetology*, *50*(2), 337-344. doi:10.1670/13-103

Bubb, P., May, I., Miles, L., & Sayer, J. (2004). Cloud forest agenda, UNEP-WCMC, Cambridge, UK. Online at: http://www.ourplanet.com/wcmc/pdfs/cloudforests.pdf

Bustamante, M., Ron, S., & Coloma, L. (2005). Changes in the diversity of seven anuran communities in the Ecuadorian Andes. *Biotropica*, *37*(2), 180-189. doi:10.1111/j.1744-7429.2005.00025.x

C, E. T., Feinsinger, P., & Crump, M. L. (2002). Frogs and a cloud-forest edge in Ecuador. *Conservation Biology, 16(3)*, 735-744. doi: 10.1046/j.1523-1739.2002.00250.x

Camacho-Badani, T., Frenkel, C., Varela-Jaramillo, A. & Ron, S. R. (2013). *Pristimantis conspicillatus*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1387

Chao, A., Ma, K. H., and Hsieh, T. C. (2016) iNEXT (iNterpolation and EXTrapolation) Online: Software for Interpolation and Extrapolation of Species Diversity. Program and User's Guide published at http://chao.stat.nthu.edu.tw/wordpress/software_download/.

Chao, A., Ma, K. H., Hsieh, T. C. and Chiu, C. H. (2015) Online Program SpadeR (Species-richness Prediction And Diversity Estimation in R). Program and User's Guide published at http://chao.stat.nthu.edu.tw/wordpress/software_download/.

Fahey, T., Sherman, R., & Tanner, E. (2015). Tropical montane cloud forest: Environmental drivers of vegetation structure and ecosystem function. *Journal of Tropical Ecology, 32(5)*, 355-367.

In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.

Frenkel, C., Guayasamín, J. M., Yanez-Muñoz, M. H., Varela-Jaramillo, A. & Ron, S. R. (2011). *Pristimantis ganonotus*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1411

Frenkel, C., Páez-Rosales, N., Varela-Jaramillo, A. & Guayasamin, J. M. (2012). *Pristimantis bicantus*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.

Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=7869

Frenkel, C., Páez-Rosales, N., Yánez-Muñoz, M. H., Guayasamín, J. M., Varela-Jaramillo, A. & Ron, S. R. (2013). *Pristimantis eriphus*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1404

Frenkel, C., Páez-Rosales, N., Yánez-Muñoz, M. H., Guayasamin, J. M., Varela-Jaramillo, A. & Ron, S. R. (2014). *Pristimantis quaquaversus*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1469

Guayasamín, J.M., Frenkel, C., Varela-Jaramillo, A. & Cisneros-Heredia, D. F. (2013). *Chimerella mariaelenae*, In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/Vertebrados/Anfibios/FichaEspecie.aspx?Id=1572

Guayasamín, J. M., Varela-Jaramillo, A. y Frenkel, C. (2010). *Nymphargus cochranae*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1201

Jost, Lou. (2016, March 5). Map of EcoMinga corridor between Llanganates and Sangay National Parks. Retrieved from: https://ecomingafoundation.wordpress.com/2016/03/05/landscape-level-conservation-becomes-a-reality-for-ecominga/

Knapp, G. W., MacLeod, M. J., & Vélez, H. P. (2017, April 03). Ecuador. Retrieved from https://www.britannica.com/place/Ecuador/Climate

La Marca, E. (2005). Population status of frogs in the family Dendrobatidae (Amphibia: Anura) in their type localities in the Venezuelan Andes/Estatus de poblaciones de ranas de la familia Dendrobatidae (Amphibia: Anura) en sus localidades tipo en los Andes de Venezuela. *Herpetotropicos: Tropical Amphibians & Reptiles, 2*(2), 73+

Lips, K.R., Burrowes, P.A., Mendelson III, J.R., & Parra-Olea, G. (2005). Amphibian population declines in Latin America: A synthesis. *Biotropica, 37(2),* 222, doi:10.1111/j.1744-7429.2005.00029.x

Ortiz, D. A. Páez-Rosales, N., & Varela-Jaramillo, A. (2013). *Pristimantis bellae*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=8305

Ortiz, D. A. & Ron, S. R. (2014). *Boana almendarizae*. In: Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. y Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=18358

Ortiz, D. A., Ron, S. R., Coloma, L. A. & Páez-Rosales, N. (2017). *Rhinella margaritifera* In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1176

Páez-Rosales, N. (2017). *Pristimantis churuwiai*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=48673

Reyes Puig, J.P., Ramírez, S., Yánez Muñoz, M. H., Mite, M. M., & Recalde, L. (2013). Bosque Protector Cerro Candelaria: Una isla de biodiversidad entre las nubes. In *Herpetofauna*

en áreas prioritarias para la conservación: El sistema de Reservas Jocotoco y Ecominga(Vol. 6, pp. 196-211). Quito, Ecuador: Museo Ecuatoriano de Ciencas Naturales (MECN), Fundación para la Conservación Jocotoco, Fundación Ecominga.

Reyes-Puig, J. P., & Yánez-Muñoz, M. H. (2012). Una nueva especie de *Pristimantis* (Anura: Craugastoridae) del corredor ecológico Llangantes-Sangay, Andes de Ecuador. *Papéis Avulsos de Zoologia (São Paulo), 52*(6), 81-91.

Ron, S. R. & Read, M. (2012). *Dendropsophus parviceps* In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.
http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1292

Ron, S. R. y Read, M. (2013). *Scinax ruber*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.
http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1347

Stark, T., Laurijssens, C., Weterings, M., Spitzen-van der Sluijs, A., Martel, A., & Pasmans, F. (2014). Death in the clouds: Ranavirus associated mortality in assemblage of cloud forest amphibians in Nicaragua. *Acta Herpetologica, Vol 9(1),* 125-127, doi:10.13128/Acta_Herpetol-13516

Toft, C. A. (1980). Seasonal variation in populations of Panamanian litter frogs and their prey: A comparison of wetter and drier sites. *Oecologia (1),* 34.

Varela-Jaramillo, A. & Páez-Rosales, N. (2017). *Pristimantis incomptus*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.
http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1422

Warne, R.W., LaBumbard, B., LaGrange, S., Vredenburg, V. T., & Catenazzi, A. (2016). Co-infection by Chytrid fungus and Ranaviruses in wild and harvested frogs in the tropical Andes. *PLoS ONE, 11(1),* doi: 10.1371/journal.pone.0145864

Weigel, K. (2016) *Comparación del monitoreo del población de Anura en el Bosque Protector Cerro Candelaria: Población de ranas en la Reserva Candelaria de EcoMinga* (Unpublished article (ISP)). SIT, Brattleboro, VT.

Yánez-Muñoz, M. H., Páez-Rosales, N., Frenkel, C., Varela-Jaramillo, A. & Ron, S. R. (2013). *Pristimantis katoptroides* In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.
http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1424

Yánez-Muñoz, M. H., Páez-Rosales, N., Varela-Jaramillo, A. & Ron, S. R. (2011). *Pristimantis rubicundus*. In: Ron, S.R., Guayasamín, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. & Nicolalde, D. A. (2016). AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.
http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1473

Yánez-Muñoz, M. H. and Reyes-Puig, J. P. (2008). Evaluación de la Herpetofauna de las Reservas Biológicas de la Fundación Ecominga". Informe Técnico División de Herpetología Museo Ecuatoriano de Ciencias Naturales. 25, 1–62.

Appendix:

Table 9. Diversity and abundance of species and individuals from all past studies in BPCC.

Species	Spring 2014	Spring 2015	Spring 2016	Fall 2016	Spring 2017	Total
Chimerella mariaelenae			2	2	79	**83**
Dendropsophus parviceps		2	29	23	54	**108**
Dendropsophus sarayacuensis		2		3		**5**
Gastrotheca testudinea					Heard	
Hypsiboas almendarizae				13	9	**22**
Hysiboas calcaratus			8			**8**
Noblella sp. 1			2		3	**5**
Nymphargus cochranae					1	**1**
Osteocephalus verruciger		1				**1**
Pristimantis aff. cremnobates			1			**1**
Pristimantis bellae	5		4	6	14	**29**
Pristimantis bicantus	10		3	1	9	**23**
Pristimantis churuwiai					9	**9**
Pristimantis conspicillatus complex			1		5	**6**
Pristimantis cremnobates		2				**2**
Pristimantis eriphus				14		**14**
Pristimantis eriphus complex	9		7		19	**35**
Pristimantis galdi			3			**3**
Pristimantis ganonotus	1			2	2	**5**
Pristimantis incomptus		18	15	117	17	**167**
Pristimantis katoptroides		1		1	1	**3**
Pristimantis lanthanites		3				**3**
Pristimantis pastazensis				4	Heard	**4**
Pristimantis prolatus			2			**2**
Pristimantis quaquaversus	7	1	2	2	1	**13**
Pristimantis rubicundus	6	17	13	10	21	**67**
Pristimantis sp				5	1	**6**
Pristimantis sp. 2			2			**2**
Pristimantis sp. grupo unistrigatus			1			**1**
Pristimantis sp. nov. chivrivia		7	6	43		**56**
Pristimantis sp.1			10			**10**
Pristimantis ventrimarmoratus		1				**1**
Pristimantis w-nigrum	5		1	1		**7**
Rhinella margaritifera				1	1	**2**

Scinax ruber					1	**1**
Unknown	5*		5*		1	**1**
Total number of species	**7**	**11**	**19**	**17**	**20**	**35**
Total number of individuals	**48**	**55**	**117**	**248**	**248**	**716**

Table 10. Data and climatic information for each night of collection.

Site	Date	Time	Average Temp (°C)	Average Relative Humidity	Weather	Observers
Velastegui	April 17, 2017	6:45-10:30PM	19.26	87.3%	Cloudy	Mindee Goodrum, Luis Recalde, Jordy Salazar, Malika Briggs
	April 25, 2017	6:35-9:05PM	20.74	88.4%	Partly cloudy	Mindee Goodrum, Jordy Salazar, Malika Briggs
	May 3, 2017	6:40-8:36PM	19	90.7%	Partly cloudy	Mindee Goodrum, Jordy Salazar
Lote G	April 18, 2017	6:48-11:30PM	16.79	91.1%	Cloudy with light rain	Mindee Goodrum, Jordy Salazar
	April 24, 2017	7:06-10:54PM	17.1	91.4%	Partly cloudy	Mindee Goodrum, Jordy Salazar
	May 1, 2017	6:35-9:38PM	15.96	92.3%	Cloudy with some mist and rain	Mindee Goodrum, Jordy Salazar
Playas de Chinchin	April 19, 2017	6:21-9:55PM	17.33	89.5%	Cloudy	Mindee Goodrum, Jordy Salazar
	April 26, 2017	6:26-8:52PM	16.67	84.0%	Cloudy	Mindee Goodrum, Jordy Salazar
	May 5, 2017	6:29-8:57PM	16.59	94.9%	Cloudy	Mindee Goodrum, Jordy Salazar
Machay	April 20, 2017	5:57-8:58PM	18.64	93.6%	Raining	Mindee Goodrum, Jordy Salazar
	April 21, 2017	6:15-10:28PM	18.2	92.6%	Cloudy	Mindee Goodrum, Jordy Salazar
	May 3, 2017	6:19-8:50PM	18.4	93.0%	Raining very hard	Mindee Goodrum, Jordy Salazar, Malika Briggs
San Pedro	April 23, 2017	7:54-11:02PM	19.28	91.6%	Rainy/cloudy	Mindee Goodrum, Jordy Salazar, Juan Pablo Reyes Puig
	April 28, 2017	7:47-9:25PM	18.9	92.3%	Rainy/cloudy	Mindee Goodrum, Jordy Salazar
	May 4, 2017	8:05-10:23PM	16.5	96.5%	Partly cloudy	Mindee Goodrum, Jordy Salazar, Juan Pablo Reyes Puig, Malika Briggs
Marsh Playas de Chinchin	April 26, 2017	6:21-7:11PM	16.7	81.0%	Cloudy	Mindee Goodrum, Jordy Salazar
	May 5, 2017	6:26-7:07PM	16.6	94.9%	Partly cloudy	Mindee Goodrum, Jordy Salazar
San Pedro	April 23, 2017	6:54-7:54PM	19.2	88.0%	Rainy/cloudy	Mindee Goodrum, Jordy Salazar, Juan Pablo Reyes Puig
	April 28, 2017	6:36-7:18PM	19	87.0%	Rainy/cloudy	Mindee Goodrum, Jordy Salazar

	May 4, 2017	6:33-7:35PM	16.1	99.0%	Partly cloudy	Mindee Goodrum, Jordy Salazar, Malika Briggs

Species Guide and descriptions:

Chimerella mariaelenae

This species of glass frog can be identified by the dark grey spots on its otherwise yellow-green dorsal and a pale yellow iris surrounded by small black dots (Guayasamín *et al.*, 2013). Adult males are unique in having a small humeral spine (Guayasamín *et al.*, 2013). Like other glass frogs the underside is transparent, allowing the organs to be seen. This species is listed as data deficient by the IUCN.

This species was encountered 79 times in this study, exclusively in the marsh of San Pedro. Due to the fact that this species was only encountered in the marsh, the abundance of individuals in these sites limited full data being taken and so only partial numbers are available for this species. However, of those measured the average length was 21.06mm with a range of 18.58-23.49mm. The average weight was 0.59 grams with a range of 0.4-0.9g. It was most commonly found perching on shrub leaves, although occasionally on herbaceous plant leaves at a height that averaged 143cm. These data correlate with findings of Guayasamín *et al.* (2013) that *C. mariaelenae* is most commonly found on leaves near small rivers in the cloud forest. Males singing, mating pairs, and a few egg deposits were all observed at the San Pedro marsh from this species during the study period.



Dendropsophus parviceps

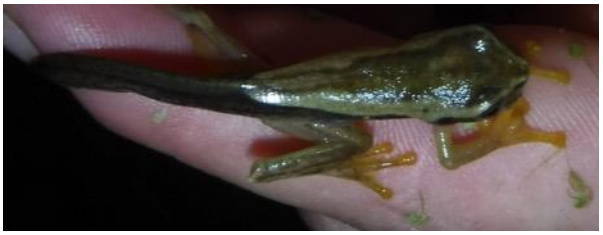
This small frog is typically brown with dark spots that are more visible during the day (Ron & Read, 2012). The underside is a mix of black, grey, and white, with bright orange spots near the calf. Some individuals also have an orange spot in the underarm (Ron & Read, 2012). It is frequently found in the canopy of primary and secondary forests, descending to bodies of water only to mate (Ron & Read, 2012). Females also have large white spots on the neck and side. The IUCN lists this species as of least concern.

This species was found 54 times in this study, primarily in the marsh and the transect at Playas de Chinchin, as well as the marsh and transect at San Pedro. The average length was 18.06mm with a range of 9.93-24.36mm. The average weight was 0.72 grams with a range of 0-1.8 grams. It was most commonly found perching on herbaceous plant leaves and to a lesser extent, leaves of shrubs, at an average height of 60.65cm. This species was occasionally observed mating in the marsh at Playas de Chinchin.

Hypsiboas almendarizae

This frog ranges in color from pale yellow to a reddish-brown, sometimes with long stripes along the back (Ortiz & Ron, 2014). The sides are blue in females and light blue or white in males. The underside is white or yellowish with yellow digits. It is found in primary or secondary forests or open areas on low vegetation 1.5 meters or lower. According to Dubois (2017) this species may now be considered part of the genus *Boana* (Ortiz & Ron, 2014). It is listed as near threatened by the IUCN.

This species was found nine times throughout the study, in both the marsh and transect at San Pedro and Playas de Chinchin. The average length was 44.30mm with a range of 28.07-53.64mm. The average weight was 4.9 grams with a range of 0.6-7.6 grams. It was found on leaves of herbaceous plants as well as shrubs, and was once recorded on a branch, at an average height of 36cm.



Noblella sp.1

Nymphargus cochranae

This glass frog can be distinguished from other similar species by its slightly larger size, a white iris, and the dark spots on its otherwise green back (Guayasamín, Varela-Jaramillo, & Frenkel, 2010). The underside is white except for the lower third which is transparent (Guayasamín *et al.*, 2010). It is listed as of least concern on the IUCN.

This species was encountered only once at the Playas de Chinchin, in the part of the transect that crosses the marsh. The individual measured 23.88mm and weighed 0.5 grams. It was found perching on a fern about 60cm from the ground. This is the first time that this species has been recorded in BPCC.



Pristimantis bellae

This species is typically brown, sometimes with green or red-brown spots (Ortiz, Paéz-Rosales & Varela-Jaramillo, 2013). It has conical tubercles present on the outer parts of the legs as well as on the eyelids (Ortiz *et al.*, 2013). Digits are long with expanded discs at the end (Ortiz *et al.*, 2013). The underside ranges from black with white spots to grey/black with some lighter colored markings (Ortiz *et al.*, 2013). It has not been evaluated by the IUCN.

This species was found 14 times, exclusively at Lote G. The average length was 15.97mm with a range of 9.90-26.58mm. The average weight was 0.66 grams with a range of 0.2-1.5 grams. It was encountered on 7 different types of substrates at an average height of 32.14cm. Ortiz *et al.* (2013) also states that this species can be found perching on a wide variety of low vegetation such as ferns, shrubs, and herbaceous plants.





Pristimantis bicantus

This small frog is typically varying shades of brown, usually with a dark interorbital band and sometimes with an inverted V shape on the back (Frenkel, Páez-Rosales, Varela-Jaramillo & Guayasamin, 2012). Some have alternating dark and light stripes on their limbs. Their underside is often translucent with tinges of red/orange or grey (Frenkel *et al.*, 2012). They are typically found in primary forest, on vegetation of an average height of 32cm (Frenkel *et al.*, 2012). It has not been evaluated by the IUCN.

This species was found a total of 9 times and was restricted to the transect at Lote G. The average length was 13.40mm with a range of 12.07-15.14mm. The average weight was 0.48 grams with a range of 0.2-1.3 grams. It was found on 6 different types of substrates at an average height of 13.33cm.




Pristimantis churuwiai

This frog is typically various shades of brown, with some tones of yellow, orange, or red (Páez-Rosales, 2017). The back legs often have diagonal stripes and the inner muscles have yellow patches, especially on males. The underside is cream colored to brown. This species is typically found in the early hours of the night, on leaves up to 3m high (Páez-Rosales, 2017). It has not been evaluated by the IUCN.

This species was encountered 9 times throughout the study and at every transect site with the exception of Lote G. The average length was 21.19mm with a range of 9.62-30.97mm. The average weight was 0.86 grams with a range of 0-2 grams. It was most commonly found on shrubs or ferns at an average height of 70.56cm.



Pristimantis conspicillatus complex

These frogs are typically brown with green or red tints and an interorbital band (Camacho-Badani, Frenkel, Varela-Jaramillo & Ron, 2013). They also frequently have marks on the back in the shape of an X or an inverted V. Iris is bronze, sometimes with a red stripe. The underside is typically white. Found in primary and secondary forests on low vegetation (Camacho-Badani, 2013). It is considered of least concern by the IUCN.

This complex of species was found 5 times, only at Machay. The average length was 21.27mm with a range of 11.38-32.25mm. The average weight was 0.88 grams with a range of 0.2-2.2 grams. Each individual was found on a different type of substrate, at an average height of 37cm.

Pristimantis eriphus complex

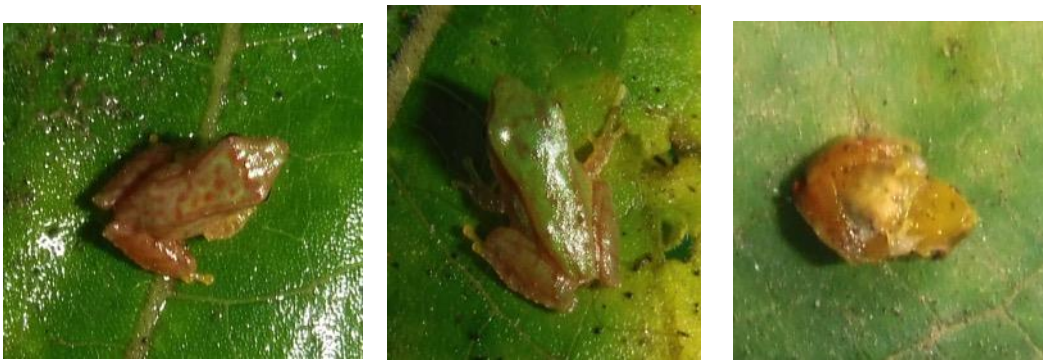
This frog is typically green and spiny with brown or red markings (Frenkel, Páez-Rosales, Yánez-Muñoz, Guayasamín, Varela-Jaramillo & Ron, 2013). The eye is copper or red and the underside of the muscles can be white or yellow, sometimes striped. It is typically found on low herbaceous vegetation (Frenkel *et al.*, 2013). It is considered data deficient by the IUCN.

This species was encountered 19 times throughout the study, only at Lote G. The average length was 17.94mm with a range of 13.14-29.78mm. The average weight was 0.61 grams with a range of 0.2-1.9 grams. It was most commonly found on shrubs at an average height of 73.68cm.

Pristimantis ganonotus

This species can be distinguished by an entirely green back with brown/cream colored eardrums, yellow digits, yellow muscles, and a yellow iris (Frenkel, Guayasamín, Yánez-Muñoz, Varela-Jaramillo & Ron, 2011). Can be found in disturbed areas and in secondary forests, typically on vegetation up to 1.8 meters. Suspected to be diurnal. Listed as data deficient by the IUCN.

This species was only encountered twice during the study, both times at Lote G. Both individuals of this species were juveniles with an average length of 9.2mm and average weight of 0.15 grams. One individual was found perching on a shrub, the other on an herbaceous plant, with an average height of 35cm.



Pristimantis incomptus

This frog is variable in both size and color. Generally brown with green or red tones with creases in the shape of ") (" on the back (Varela-Jaramillo & Páez-Rosales, 2017). The underside is typically grey or varies from brown to cream. The iris is generally bronze. Generally found on

herbaceous plants or small shrubs (Varela-Jaramillo & Páez-Rosales, 2017). Listed as near threated by the IUCN.

This species was found 17 times throughout the study and was the only species to appear at all five transect sites, as well as once at the San Pedro marsh. The average length was 17.00mm with a range of 9.53-25.37mm. The average weight was 0.51 grams with a range of 0-1.3 grams. It was most commonly found on shrubs at an average height of 90.63cm.

Pristimantis katoptroides

The coloration of this species varies from green to brown with darker brown spots (Yánez-Muñoz, Páez-Rosales, Frenkel, Varela-Jaramillo, & Ron, 2013). The underside is white, sometime with brown spots. The groin and underside of the muscles have a bright blue color, sometimes flanked by orange. Found in a variety of habitats from highly disturbed forests to undisturbed primary forests and occasionally near bodies of water. (Yánez-Muñoz *et al.,* 2013). This species is listed as endangered by the IUCN.

This species was found only once during the study, at Velastegui. It was a male measured 21.90mm and weighed 0.8 grams. It was found on an unspecified type of leaf 45cm from the ground.



Pristimantis quaquaversus

This frog varies from cream-colored to a dark brown, occasionally with yellow or orange tones (Frenkel, Páez-Rosales, Yánez-Muñoz, Guayasamín, Varela-Jaramillo, & Ron, 2014). They can have diagonal stripes, an inverted V, or a mid-dorsal band. The underside is white or cream with brown or grey spots. Found in primary or secondary forests on vegetation up to 2.7 meters off the ground.

This species was found only once during the study at Lote G. It measured 19.74mm and weighed 0.4 grams. It was found on a fern 45cm high.


Pristimantis rubicundus

This species varies between green and brown with orange, green, red tones, often with prominent tubercles (Yánez-Muñoz, Páez-Rosales, Varela-Jaramillo, & Ron, 2011). The underside is typically brown with irregular white spots. Digits are elongated with prominent discs on the end. Found in primary or secondary forests on a variety of substrates up to 2 meters high (Yánez-Muñoz *et al.,* 2011). This species is listed as endangered by the IUCN.

This species was encountered 21 times in the study, at every transect site except for San Pedro. The average length was 24.27mm with a range of 7.43-42.48mm. The average weight was 1.8 grams with a range of 0.2-6 grams. It was most commonly found on unclassified types of leaves at an average of 39.05cm.



Pristimantis sp. 1

This species was encountered only once during the study at Velastegui. It measured 14.42mm and weighed 0 grams. It was found on an unspecified type of leaf 10cm off the ground.




Rhinella margaritifera

This species is very variable in color, going between different shades of brown, grey, and red, sometimes with black spots on the back (Ortiz, Páez-Rosales, & Varela-Jaramillo, 2017). The underside coloration is also highly variable, but generally lighter than the back. It is found in a variety of forest habitats and is active during the day and the night. The IUCN lists this species as of least concrn.

This species was encountered only once during the study, at San Pedro between the marsh and transect areas. It measured 29.04mm and weighed 1.6 grams and was found on a shrub.



Scinax ruber

The coloration varies in this species between brown and pale yellow at night to green, bronze, or brown during the day (Ron & Read, 2013). The underside ranges from cream to yellow. The iris is bronze. It is frequently found in disturbed areas on branches, shrubs, or on the ground near water sources (Ron & Read, 2013). It is listed as of least concern by the IUCN.

This species was found only once during the study, in the naranjilla cultivation part between the sections of the Velastegui transect. It measured 32.12mm and weighed 1.8 grams and was found on a leaf of a naranjilla plant.