

HERPETOFAUNAL ABUNDANCE AND SPECIES DIVERSITY IN COASTAL ECUADOR: DIFFERENCES BETWEEN FOREST TYPES, THE ROLE OF ABIOTIC FACTORS AND EDGE EFFECTS

Tropical Conservation, Permaculture & Research Internship

Jama-Coaque Ecological Reserve

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1.0 Abstract

The recent trend of increasing threats to amphibian and reptile populations in the Neotropics, such as deforestation and climate change, are creating a serious need for the implementation of conservation policies. For a better design of these conservation practices, the study of the local herpetofauna is necessary. We approached this problem by carrying out a research project in the Jama-Coaque Reserve in coastal Ecuador, a region characterised by a high diversity of ecosystems but also threatened by high deforestation rates. Firstly, we aimed to (i) compare the abundance and diversity of the herpetofauna in three different ecosystems found in the Reserve, which include tropical moist evergreen forest, premontane cloud forest and a recently reforested area. This was done in order to have a greater understanding of the local species richness and distribution. Secondly, we attempted to (ii) understand the main abiotic factors influencing herpetofaunal abundance and species richness, to assess the potential impact of a climatic or microclimatic change. Finally, we began to (iii) explore the potential role of edges between different types of forests, to shed some light on the effects of local habitat changes on the distribution of reptiles and amphibians. Our data supports a higher biodiversity and herpetofaunal abundance in the reforested area and moist forest compared to the cloud forest. Temperature and distance from water were the only two factors which had a significant (negative) relationship with species richness across ecosystems, while temperature, distance from water and elevation co-varied significantly with herpetofaunal abundance. Finally, distance from the moist forest edge into the cloud forest had a negative relationship with species richness. This is only a preliminary study, the results of which encourage further research to accumulate more data for longer term population trends and species dynamics in other seasons.

Key words: Native forests of coastal Ecuador, herpetofaunal abundance, species diversity, abiotic factors, habitat types, edge effects.

1.1 Resumen

La reciente tendencia al aumento de las amenazas a las poblaciones de anfibios y reptiles en el Neotrópico, como la deforestación y el cambio climático, están haciendo de la implementación de políticas de conservación una necesidad importante. El estudio de la herpetofauna local es necesario para un mejor diseño de las prácticas de conservación. Nos realizamos un proyecto en la reserva ecológica Jama-Coaque en la costa de Ecuador. Esta es una región que se caracteriza por una gran diversidad de ecosistemas, sino también amenazada por las altas tasas de deforestación. En primer lugar, (i) comparamos la abundancia y diversidad de la herpetofauna en tres ecosistemas diferentes que se encuentran en la Reserva, que incluyen bosque húmedo, bosque nublado y una zona recientemente reforestada. Hicimos esto por tener un mayor conocimiento de la riqueza de especies y su distribución. En segundo lugar, intentamos de (ii) entender cuáles son los principales factores abióticos que influyen en la abundancia y riqueza de especies de herpetofauna, para evaluar el posible impacto de un cambio climático. Por último, comenzamos a (iii) explorar el posible efecto del borde entre los diferentes tipos de bosques, para aprender cuáles son los efectos de los cambios de hábitat locales en la distribución de los reptiles

y anfibios. Nuestros datos apoyan la biodiversidad y la herpetofauna mayor abundancia en la zona reforestada y bosque húmedo en comparación con el bosque nublado. La temperatura y la distancia al agua fueron los únicos dos factores que tuvieron una relación significativa (negativa) con la riqueza de especies en los ecosistemas, mientras que la temperatura, distancia al agua y la elevación es relacionada significativamente con la abundancia de herpetofauna. Por último, la distancia desde el borde del bosque húmedo en el bosque nublado tenía una relación negativa con la riqueza de especies. Este es sólo un estudio preliminar, los resultados del cual fomentan más investigación para acumular más datos sobre las tendencias demográficas de larga duración y dinámica de las especies en otras temporadas.

Palabras clave: Bosques nativos del Ecuador, abundancia de la herpetofauna, riqueza de especies, factores abióticos, tipo de hábitat, efectos de borde.

2 Introduction

Biodiversity has an extreme importance in supporting life on Earth, which includes thriving human activities such as medical practices and food production (Urbina-Cardona 2008). Reptiles and amphibians sustain biodiversity by covering key roles in their ecosystems, utilising both terrestrial and aquatic environments (Schneider *et al.* 2001; McCallum 2007). As consumers, predators and prey, they are essential components of their food webs and play a crucial role in a healthy ecosystem. The substantial decline in herpetofaunal abundance and diversity is now a well-known fact between conservation biologists. The Global Amphibian Assessment and the Global Reptile Assessment have expressed serious warning in regards to recent, alarming statistics (Urbina-Cardona 2008). Worldwide, 32.5% and 22% of amphibian and reptile species, respectively, are endangered (Canavero *et al.* 2010), and current trends suggest even more species could become threatened in the near future.

These animals face multiple threats, the main one being habitat loss due to deforestation for agriculture and pasture, human activities that degrade habitat quality and landscape connectivity (Urbina-Cardona 2008). Increasing habitat fragmentation directly correlates with the increasing magnitude of edge-effects, altering environmental conditions such as temperature and humidity, and rendering many species more vulnerable to population declines (Lehtinen *et al.* 2003). Moreover, the rapid invasion of alien species is a further threat to the local biodiversity, as invaders may carry infectious diseases and pathogens such as the Chytrid fungus – an organism causing frog population crashes (Berger *et al.* 1998, Daszak *et al.* 2003). Finally, anthropogenic climate change and pollution are also important causes of biodiversity declines in the neotropics (Lips *et al.* 2005). These factors already had a series of negative impacts on global herpetofaunal populations, such as loss of reproduction sites or even the ability to reproduce, a decline of genetic diversity and changes in behavioural patterns and physiological functions (Urbina-Cardona 2008).

The Neotropic ecozone, located in the tropical areas of the American continents, is inhabited by 30% to 50% of the entire world's reptiles and amphibians (Hamilton and Mouette 2007). Yet, little is known of the ecology and distribution of most species present in these areas, making conservation policies extremely challenging to implement. Due to their extreme biodiversity and the land use practices that are degrading them, the tropical forests of Central and South America are currently

considered a global extinction hotspot. Therefore, it is vital to rapidly develop a better understanding of the ecology, abundance and distribution of the local reptile and amphibian species, in order to quantify the biodiversity loss, inform a wider audience and to implement more effective conservation actions (Soulé and Orians 2001). Because of the great complexity of this region, it is necessary to have a precise knowledge of the taxonomy of the species present in a specific area of interest; this way, appropriate conservation practices can be implemented to protect areas of significant biological importance (Urbina-Cardona 2008). Knowing the spatial distribution of species, and the factors influencing their spatial distribution and abundance, are the keys to identifying and protecting endangered populations.

Located in the Neotropical region, Ecuador is exceptionally diverse in both flora and fauna (Dodson and Gentry 1991). Specifically, biologists consider western Pacific-Ecuador a biodiversity hotspot, exhibiting high levels of endemism. Here, it is possible to find one of the largest remnants of the heavily deforested coastal deciduous and semi-deciduous forests of the Tumbes-Chocó-Magdalena biodiversity hotspot, of which less than 2% of the original forest remains intact (Hamilton *et al.* 2007). High levels of endemism are reached due to the particular climate clinal variations of the region. These variations occur as a result of the corridor's location between the dry lands of Chile and extremely rainy forests of Colombia, and the influence of the patterns of upwelling currents of the Pacific Ocean, El Niño and La Niña, and the Humbolt Current (Conservation International 2006; Dodson and Gentry 1991; Cañadas 1983; Hamilton *et al.* 2007). The cold upwelling of the Humbolt Current causes precipitation, which sheds over the coastal lands in form of fog and increases the humidity levels that vary significantly with elevation (Hamilton and Mouette 2007).

Overall estimates of animal species are difficult to find, but forests of elevation less than 900m are especially characterized by high levels of local endemism. Indeed, various plants and vertebrate species are only found in a single, often very limited area (Lynch and Duellman 1997). The presence of a hill or mountain surrounded by low elevation land results in a separate, island-like ecosystem that often causes these extreme levels of endemism (Hamilton and Mouette 2007). Because of this, it is important to locate where endangered species live, as the loss of even a small area due to deforestation or other land uses may lead to, or have already caused, the loss of several local species (Dodson and Gentry 1991). So far, only very general studies about herpetofaunal species distribution and abundance exist so far. Therefore, more focused and intensive studies in smaller areas are needed.

In the last 50 years coastal Ecuador has been extensively deforested for lumber, banana, and citrus and palm oil plantations (Hamilton *et al.* 2007). Agriculture has been an enormously destructive practice, and vast amounts of land have been cleared for pastures. Intensive agriculture, the most common practice in coastal Ecuador, has been enormously destructive. Even if a forest has not been completely destroyed, selective logging holds a significant enough impact to threaten several species with extinction (Sierra and Stallings 1998). In response to these biodiversity declines, some conservation practices, such as reforestation and old-growth forest restoration, have emerged. These efforts continue to increase, but it remains vital to fully understand how reforestation may affect herpetofaunal diversity, abundance and ecology, to improve its positive effects. By knowing more about the local taxonomical diversity and ecosystem functioning, conservation and restoration practices can be improved (Baratolo *et al.* 2012).

Considering how vital herpetofauna is for the functioning of the ecosystems they live in, the extensive lack of knowledge is shocking and research in the field is greatly needed. Especially considering that amphibians serve as good bio-indicators, a deeper understanding of the local population trends would give extremely useful insight on the health of the local ecosystems (Hamilton and Mouette 2007).

2.1 Research Objectives and Hypotheses

Due to these previously mentioned issues, we decided to begin a long-term monitoring project of the herpetofauna at the Jama-Coaque ecological Reserve (JCR), located near Camarones in the province of Manabí, coastal Ecuador. The JCR was founded in 2007 with the aim of preserving the last remnants of tropical moist evergreen forest and premontane cloud forest of the region. Some areas within the JCR have started to be reforested, and the original size of the protected area has slowly increased. Even though the efforts of preserving these forests have been great, still no extensive herpetofaunal study has been carried out within the Reserve. Therefore, our research objectives were to (i) collect data to improve the current knowledge of the local herpetofaunal community composition, by surveying the cloud forest, moist forest and the reforested areas. This was also done to compare their herpetofaunal biodiversity and abundance. Secondly, we (ii) evaluated the relative influence of temperature, elevation, humidity and distance from water to determine the effects of forest type and abiotic factors on communities of reptiles and amphibians living in the JCR. This second objective intended to shed light over the potential impacts that climatic changes and land modification may have on the local herpetofauna, in order to provide useful information for their conservation. Finally, because of the often extreme climatic and floral difference between moist and cloud forest, we (iii) evaluated the effect of the edge between these two ecosystems. We focused on this to better understand possible influences on the local herpetofaunal species distribution and abundance, and to have an insight on the role of edges in this area, which encompasses different types of ecosystems in a relatively small area.

We hypothesised that (i) the diversity and abundance of species to be higher in the moist and cloud forest compared to the recently reforested area, due to the longer recovery time that a population requires (Heinen 1992). Furthermore, we expected (ii) all the environmental factors that we measured to significantly differ between ecosystems, and that the herpetofauna of the JCR would have significantly co-varied with moisture and elevation, as was already found in Costa Rica (Heinen 1992). We also predicted distance from water to co-vary especially with abundance of individuals, being such an important part of many amphibians' life cycle. However, these were difficult predictions, as factors influencing herpetofaunal diversity and abundance are poorly understood. Lastly, we expected (iii) distance from the moist forest edge to correlate negatively with abundance of species and individuals, as often edges provide a greater variety of vegetation and microhabitat due to intermediate climatic conditions (Yahner 1988).

Ultimately, this project's goal is to improve the general knowledge about the local herpetofaunal abundance and richness, and to provide initial data to begin a long-term monitoring project in support of making more informed future management decisions.

3 Methods

3.1 Study Area

All the research for this study was carried out at the JCR, located at 0°06'56.8" S, 80°07'29.5" W in the Ecuadorian province of Manabí. Situated 19 kilometres south of the equator and 7 kilometres inland from the Pacific Ocean, the JCR contains some of the remaining two percent of original north-western Ecuadorian forests (Ortega-Andrade *et al.* 2010). Due to its location, the JCR rests within the Tumbes-Chocó-Magdalena biodiversity hotspot as well as the Chocó-Manabí biodiversity corridor, which stretches along the Pacific Ocean from Colombia to Ecuador.

Within the 422 ha reserve, study areas were concentrated in three distinct habitat types: tropical moist evergreen forest, premontane cloud forest, and an area that was reforested in 2013 (Figure 1). In the JCR, moist forest elevations range from 230 m to 525 m and are marked by fast growing, large tropical evergreen species, a high canopy, and a 1-3°C higher temperature than found in the cloud forest. Cloud forests, ranging from 525 m to 700 m elevation, are characterised by slower growing, thinner trees, a lower canopy, and an increased presence of mosses and epiphytes. Additionally, cloud forests normally have a higher humidity and lower temperatures compared to moist forests. The reforested area, located at Finca de Madera (outside of the JCR), ranges from 130 m to 300 m in elevation. It includes an increased presence of grasses and weeds, general lack of canopy cover, and temperatures higher than found in the moist forest. During this study, surveyed areas included both primary and secondary moist and cloud forest. Our research took place from the 20th of August until the 18th of September 2014, the period in which coastal Ecuador typically experiences a dry season.

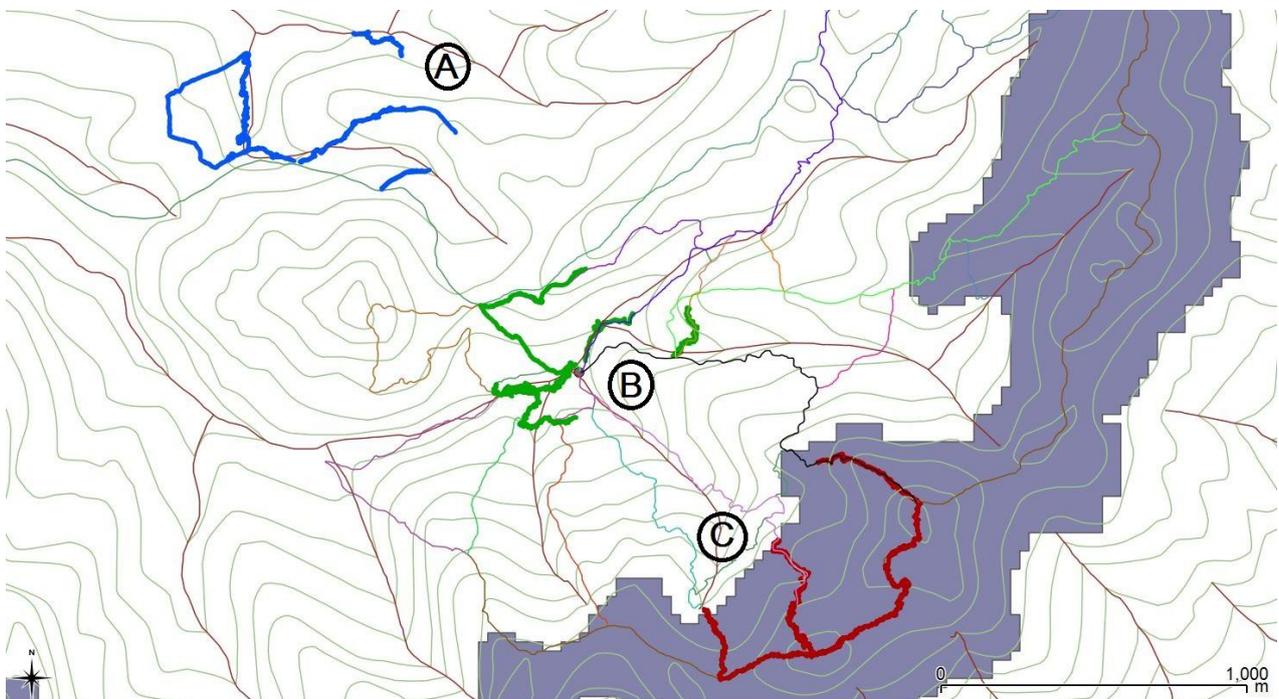


Figure 1. Map of the whole study area built with QGIS software. The letter A indicates the reforested area, B the moist forest and C the cloud forest. The blue, green and red lines indicate the transects that we walked in each of the habitat types. The dark area identifies the cloud forest, the border of which is set at 525m of elevation.

3.2 Survey methods

We used visual encounter surveys along transects as our research method. Transects mainly followed established trails and rivers, in order to minimise human disturbance, with the exception of one newly forged trail within the reforested study site. We performed surveys during both day time (ranging between 10:00 and 15:00) and night time (between 19:30 and 00:00). This provided equal temporal representation of each transect, so that both diurnal and nocturnal species were observed. As much distance as possible was covered during each performed transect. An unequal distance was surveyed during day and night time due to varying weather and trail conditions. We also recorded individuals that were encountered incidentally en route to a transect. We waited at least 24 hours before surveying a transect for a second time.

Transects were surveyed in teams ranging from one to four people; however, the majority of transects were performed with a team of two surveyors. While walking a transect, the first surveyor focused on the trail ahead and areas directly adjacent to the trail while the following member(s) focused on areas perpendicular to the trail and canopy cover (Hamilton, 2008). Upon encountering a specimen, we identified the species and documented the specimen with a photograph taken with a DSLR Nikon D5000, for later confirmation of species identity. Additionally, we recorded the temperature and humidity (AcuRite® digital thermometer) of the sighting location, along with time, microhabitat type and behaviour of the specimen.

3.3 QGIS data processing

A handheld GPS unit (Garmin GPSMAP 64) was used for reporting coordinates and elevation of the encounter site. By entering coordinates into a map of the JCR previously created in QGIS, we calculated the distance from the nearest body of water (usually river or stream) for each individual specimen (e.g. Figure 2). The creation of a map reporting the position of all the specimens found also allowed a preliminary assessment of the species richness and distribution across the three sampled ecosystems.

3.4 Statistical Analysis

3.4.1 Herpetofaunal diversity and abundance

To assess the difference in species diversity and individual abundance between moist forest, cloud forest and reforested area, we calculated the different biodiversity indices illustrated in Table 1. To investigate this we used the whole survey data, including incidental specimens. Because of the uneven sampling effort between the three ecosystems, and the inaccuracy of merely standardising the species richness of different samples by dividing counts by area covered, we calculated the biodiversity indices using the software EstimateS 9 (Gotelli and Colwell 2001; Colwell 2013). EstimateS 9 accounts for the effect of uneven sampling by carrying out randomisation to compute rarefaction: we set it to resample our data 100 times in order to obtain a more accurate mean index (Colwell 2013).

Table 1. Biodiversity indices calculated with the software EstimateS 9, and their estimate (Gotelli and Colwell 2001).

Index	Estimate
S (est.)	Expected number of species represented among m individuals, given the reference sample.
Shannon-Wiener's index	Species richness and evenness.
Simpson's index	Probability of two randomly drawn individuals to belong to the same species.
Alpha diversity	Fisher's alpha diversity index.

3.4.2 Influence of abiotic effects and habitat type

To assess whether temperature, humidity, elevation and distance from water had an effect on the species diversity and abundance of individuals, we used only the survey data coming from transects that were walked both day and night. These transects were then randomly divided into nine 100 m segments for each of the three ecosystems using the software QGIS 2.2.0. We averaged all the abiotic factors present in each of the segments. The abundance and species richness were calculated by summing all the individuals and species encountered in a specific 100 m transect in both daytime and night-time, for the analysis to be accounting for both diurnal and nocturnal species.

We then built two generalised linear models, one to test the relationship between the measured abiotic factors and herpetofauna abundance and the one to test their relationship with species diversity. We performed all the statistical tests, and built all the figures, using the software RStudio v.3.1.0 (R Development Core Team 2013). Microsoft Excel 2013 was also used to building figures (Figure 3 and 6). All the abiotic factors were entered as predictor variables, and they were combined in up to third degree interactions to avoid model over-fitting. Type of habitat (cloud forest, moist forest, reforested area) was also included as predictor variable. We carried out model simplifications through likelihood ratio tests. We subsequently plotted the residuals to check if the assumptions were met (e.g. Lawton and Pratchett 2012). Variance inflation tests (VIF) and residuals plotting to check for normality were also performed upon all the subsequent models.

3.4.3 Analysis of moist forest edge effect

To evaluate the existence of an edge effect between cloud and moist forest, we first divided transects walked in the cloud forest in segments of 40 m, progressively further away from the moist forest. We did this with the aid of the software QGIS (Figure 2). Upon this division, we assessed the species richness and amphibian and reptile abundance for each of the segments of equal distance from the moist forest, summing these characteristics in order to have an overall estimate of the cloud forest biodiversity at that specific distance from the moist forest edge.

We analysed the biodiversity data with two Poisson regressions, estimating first the relationship between distance from the edge and species diversity and then between distance from the edge and herpetofaunal abundance.

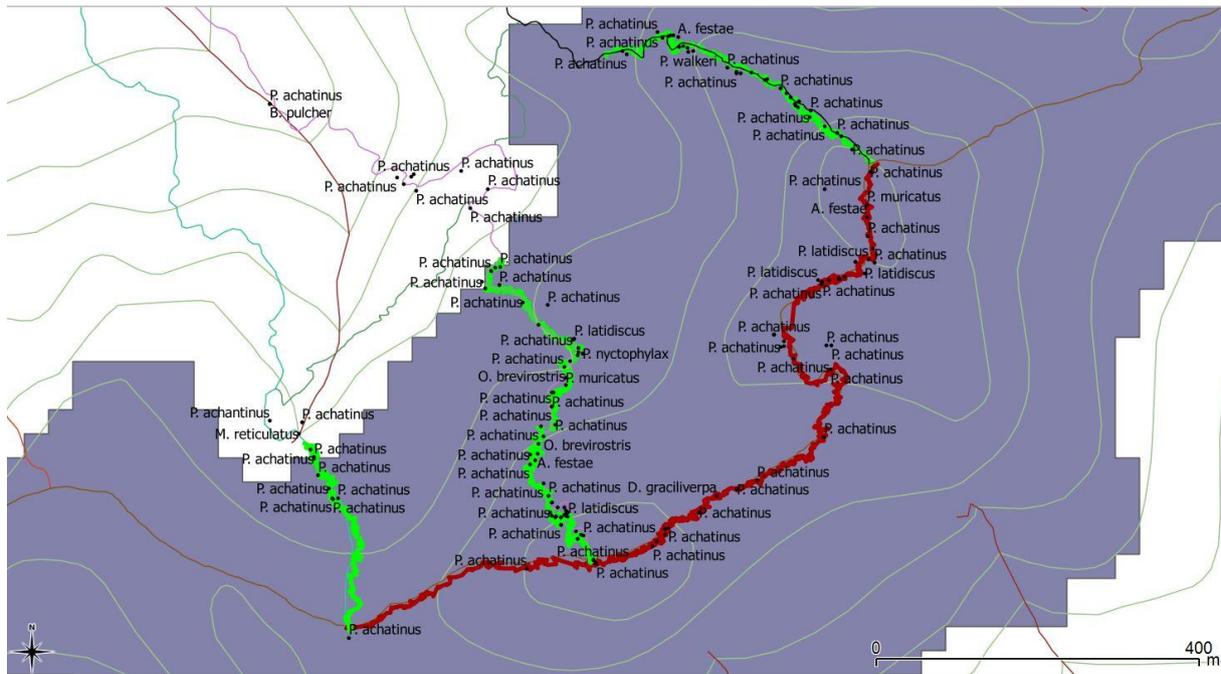


Figure 2. QGIS map of the transects walked in the cloud forest (dark area, the border of which is set at 525 m of elevation). The trails marked in green were divided in segments of 40 m to evaluate the effect of the moist forest edge on the cloud forest herpetofauna. These trails all begin after the transition zone between moist and cloud forest.

4 Results

4.1 Herpetofaunal diversity and abundance

Over a 34-day period we surveyed a total of 11.9 km, including the doubled length of walking the trails for a second time during the night. This distance was divided evenly between the three types of habitats present at the JCR (Table 2).

Table 2. Survey results and sampling characteristics of the three ecosystems in the JCR.

	Cloud forest	Moist forest	Reforested area
Elevational range (m)	519-614	243-534	138-291
Humidity range (%)	86-99	75-94	60-91
Temperature range (°C)	17-22	18-27	21-30
Average distance from water (m) (±SD)	265.68 (± 85.02)	54.73 (± 76.39)	25.77 (± 27.81)
Total transect length (m)	3702	4094	4140
Total transect length covered both day and night (m)	1301	1647	1240

Temperature and humidity were significantly different during day time and night time in all the three areas (e.g. temperature in cloud forest: $F_{1,146} = 15.5$, $p < 0.001$; moist forest: $F_{1,204} = 72.12$, $p < 0.001$; reforested area: $F_{1,128} = 191.7$, $p < 0.001$). Temperature was higher during the day and

lower during the night in all the three forests, and humidity was lower during the day in moist and reforested area but not in the cloud forest, which had an averagedly slightly higher humidity during the day. Temperature was negatively correlated with elevation ($b = - 0.009$; $t_{1,26} = - 5.11$, $p < 0.001$), humidity was positively correlated with elevation ($b = 0.017$; $t_{1,26} = 5.71$, $p < 0.001$), and temperature and humidity were also negatively correlated with each other ($b = - 1.25$; $t_{1,26} = - 5.75$, $p < 0.001$).

Overall, we recorded a total of 27 species, of which 15 were reptiles and 12 were amphibians. Amphibians were the majority of the individuals encountered, and all amphibians found were anurans (Figure 3).

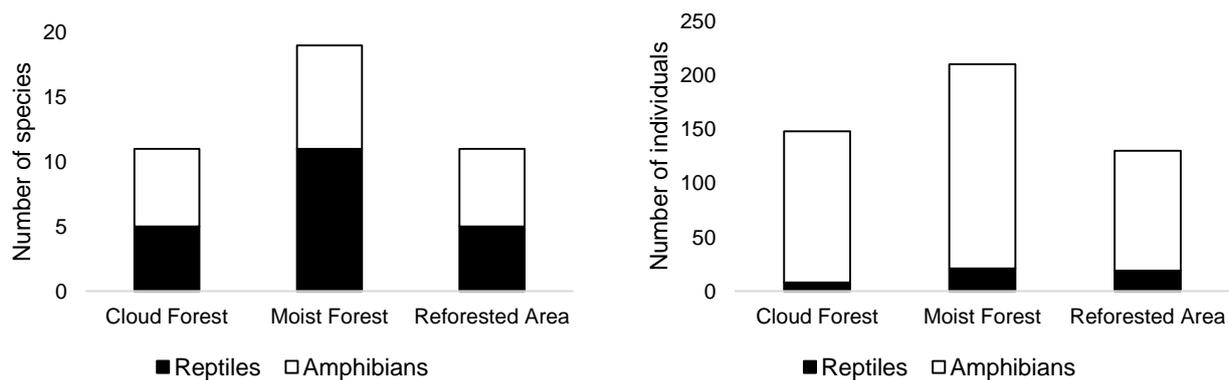


Figure 3. Species richness (left figure) and abundance of individuals (right figure) encountered in the three habitat types, distinguishing between reptiles and amphibians.

Most reptiles and amphibian species were observed in the moist forest, whereas cloud forest and reforested area were equal in terms of their species richness (Figure 3, Table 3). The most abundant species encountered was the frog *Pristimantis achatinus*, with 223 specimens recorded, most of which in the cloud forest. The most abundant reptile species was the lizard *Ameiva septemlineata*, encountered 12 times and only in the reforested area (Table 3). Ten species (seven reptiles and three amphibians) were only encountered once, seven of which were found in the moist forest. The most unexpected finding was *Dendrophidion graciliverpa*, a snake not known yet to inhabit this region.

Table 3. Reptile and amphibian species list (distinguished by the dotted line) encountered during this study.

	Cloud forest	Moist forest	Reforested area	Total
<i>Alopoglossus festae</i>	3	2	0	5
<i>Ameiva septemlineata</i>	0	0	12	12
<i>Anolis binotatus</i>	0	0	2	2
<i>Anolis lyra</i>	0	1	0	1
<i>Anolis peraccae</i>	0	0	1	1
<i>Anolis princeps</i>	0	1	0	1
<i>Bothrops asper</i>	1	5	1	7
<i>Coniophanes dromiciformis</i>	0	1	0	1
<i>Dendrophidion graciliverpa</i>	1	1	0	2
<i>Dipsas andiana</i>	1	5	0	6
<i>Iguana iguana</i>	0	1	0	1
<i>Mastigodryas reticulatus</i>	0	1	0	1
<i>Oxybelis brevirostris</i>	2	0	0	2
<i>Rhinodemmys annulata</i>	0	1	0	1
<i>Stenocercus iridescens</i>	0	2	3	5
<hr style="border-top: 1px dashed black;"/>				
<i>Barycholos pulcher</i>	0	10	27	37
<i>Bufo marinus</i>	0	1	0	1
<i>Epipedobates machalilla</i>	0	36	63	99
<i>Espadarana prosoblepon</i>	1	3	0	4
<i>Hyloxalus awa</i>	0	52	3	55
<i>Hypsiboas rosenbergi</i>	0	2	1	3
<i>Leptodactylus labrosus</i>	0	0	1	1
<i>Pristimantis achatinus</i>	124	83	16	223
<i>Pristimantis latidiscus</i>	9	0	0	9
<i>Pristimantis muricatus</i>	3	0	0	3
<i>Pristimantis nyctophylax</i>	1	0	0	1
<i>Pristimantis walkeri</i>	2	2	0	4
Total	148	210	130	488

Several biodiversity indices were calculated to investigate the differences in herpetofaunal diversity and abundance between cloud forest, moist forest and reforested area (Table 4). The reforested area has most of the highest index values calculated: the Alpha diversity index is the highest between the three ecosystems, as well as the Simpson's and Shannon's indices. The moist forest has very similar diversity values compared to the reforested area (overlapping standard deviations). The cloud forest had overall the lowest values compared to the other two ecosystems.

Table 4. Species richness estimators and biodiversity indices computed for rarefied abundance of individuals (100 randomisations).

	Cloud forest	Moist forest	Reforested area
S est. ($\pm 95\%CI$)	13.67 (10.45-16.89)	22 (17.78-26.22)	27 (22.25-31.75)
Alpha Mean ($\pm SD$)	3.60 (± 0.54)	5.36 (± 0.61)	6.18 (± 0.6)
Shannon Mean ($\pm SD$)	1.41 (± 0.43)	1.74 (± 0.14)	1.85 (± 0)
Simpson's Inverse Mean ($\pm SD$)	2.96 (± 1.12)	3.56 (± 0.95)	3.77 (± 0)

4.2 The influence of abiotic factors in herpetofaunal abundance and species richness

Every time we encountered a specimen, we recorded the temperature, elevation and humidity of the sighting location. Later, the distance of that specimen to the nearest body of water was calculated using QGIS. All these abiotic factors significantly differed between ecosystems (temperature: $F_{2,485} = 223.5$, $p < 0.001$; humidity: $F_{2,485} = 181.6$, $p < 0.001$, distance from water: $F_{2,485} = 607.4$, $p < 0.001$).

We then investigated the relationship between all these abiotic factors with herpetofaunal species diversity through a generalised linear model. Temperature and distance from water were the only factors that significantly related with species richness, and both co-varying negatively with it (Table 5).

Table 5. GLM results for reptile and amphibian species richness of the JCR.

	Estimate	SD	z-value	p-value
Intercept	6.19	1.48	4.18	<0.0001 ***
Temperature ($^{\circ}C$)	-0.211	0.065	-3.22	0.001 **
Distance from water (m)	-0.004	0.001	-3.60	0.0003 ***

The higher the temperature, and the higher the distance from water, the lower the species richness became (e.g. Figure 4). None of the other measured environmental factors had a significant relationship with herpetofaunal species richness.

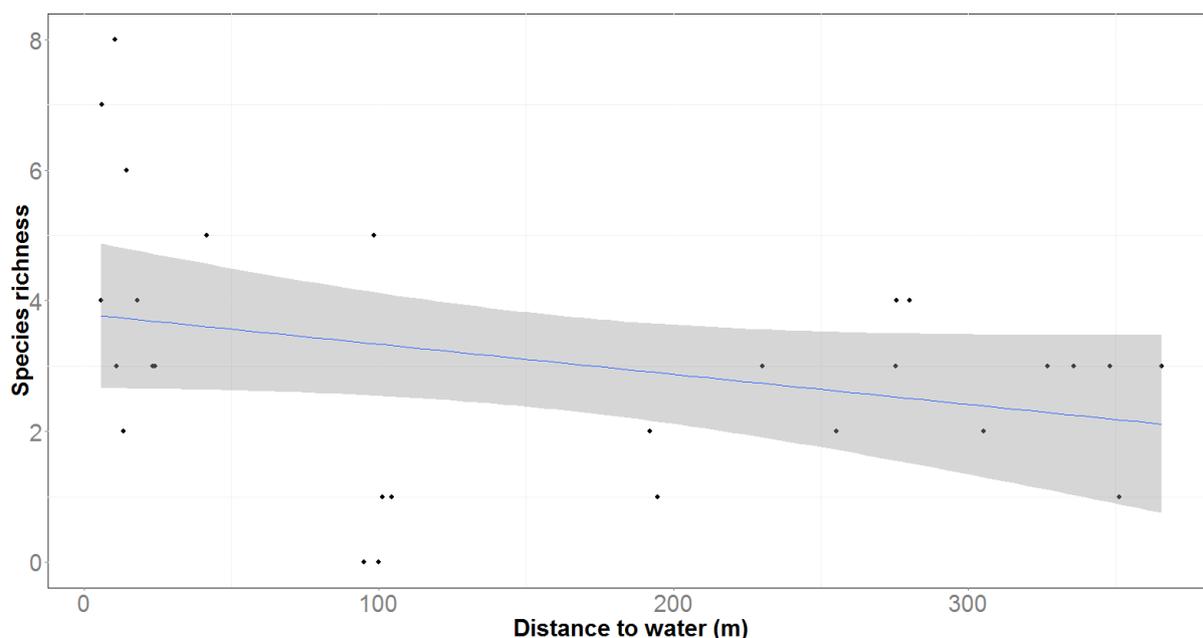


Figure 4. More species of reptiles and amphibians were found closer to a body of water than further away from one of them.

We also investigated the relationship of the measured abiotic factors, and type of ecosystem, with herpetofaunal abundance. This relationship was also analysed through a generalised linear model. Through backward elimination, only temperature, distance from water and elevation were found co-varying significantly with the abundance of reptile and amphibian individuals encountered during the survey (Table 6).

Table 6. GLM results for abundance of reptiles and amphibians of the Jama-Coaque Reserve related to local abiotic factors.

	Estimate	SD	z-value	p-value
Intercept	18.2	3.11	5.85	<0.0001 ***
Temperature (°C)	-0.252	0.052	-4.83	<0.0001 ***
Distance from water (m)	-0.00766	0.00105	-7.25	<0.0001 ***
Elevation (m)	0.00348	0.000822	4.24	<0.0001 ***

Similar to species richness, temperature and distance from water co-varied negatively with abundance of individuals (e.g. Figure 5). Conversely, elevation co-varied positively with abundance of individuals (Table 6).

Additionally, we explored the preferred range of temperature, humidity, elevation and distance from water for each of the species encountered. This allowed us to gain more insight over the influence of these abiotic factors. The graphical output of this supplementary analysis can be found in the Appendix.

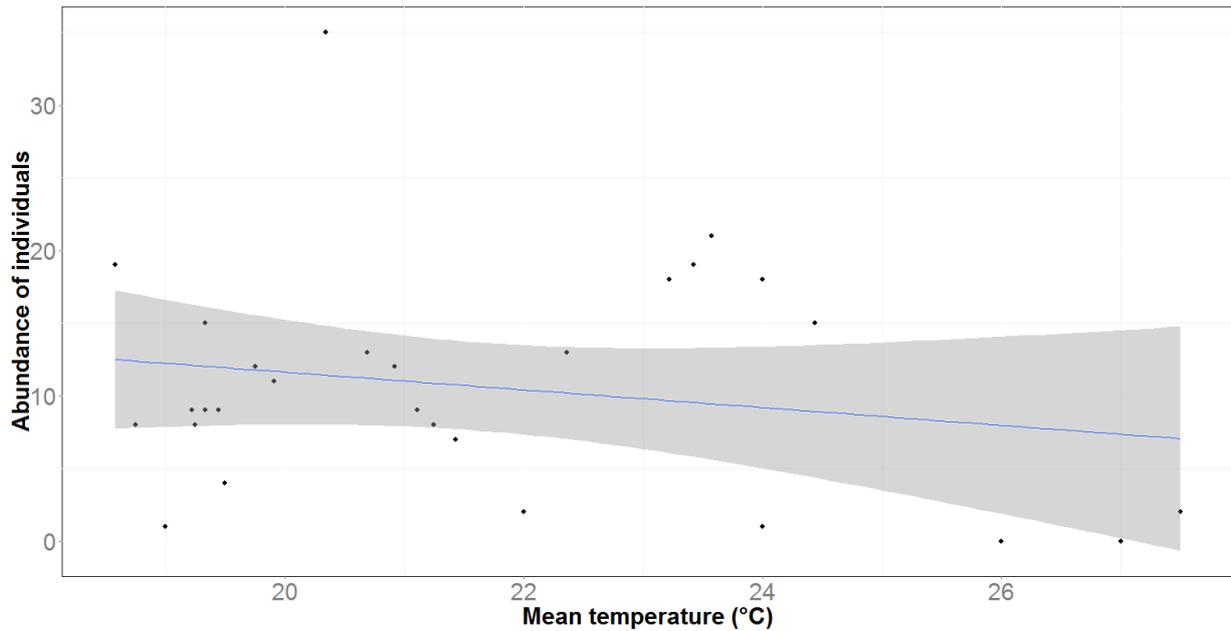


Figure 5. The higher the mean temperature the lower the abundance of reptile and amphibian individuals encountered.

4.3 The moist-cloud forest edge effect

Our third question was whether there is a moist forest edge-effect on the herpetofaunal abundance and species richness. We detected an increasing trend of both abundance and species richness with increasing distance from the moist forest within the cloud forest (Figure 6).

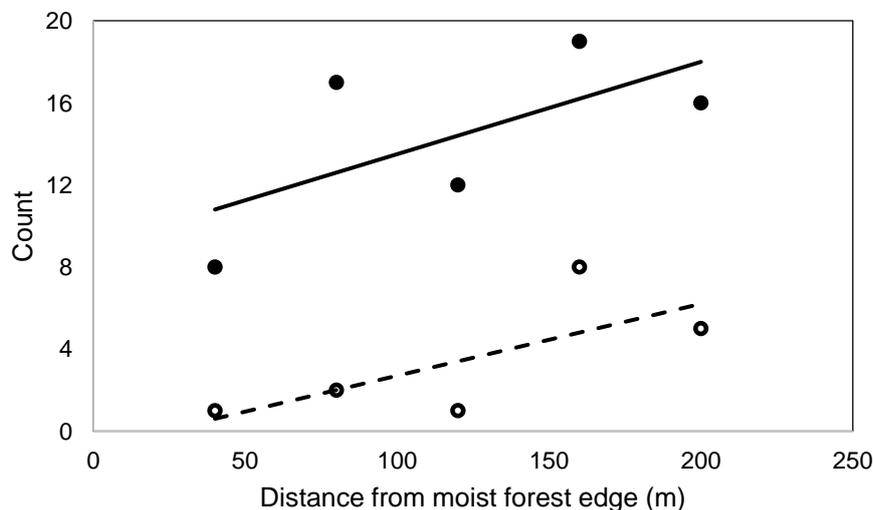


Figure 6. Both species richness (dashed line) and abundance of individuals (solid line) increase with increasing distance from the moist forest edge.

The GLM performed to assess the relationship between the distance to the edge and herpetofauna abundance and species richness confirmed the presence of an edge effect, but only for species richness (Table 7). Abundance of individuals did not significantly co-vary with distance from the edge, whereas species richness had a positive relationship with it.

Table 7. GLM results for the moist forest edge effect on abundance of individuals and species richness.

	Estimate	SD	z-value	p-value
• Abundance				
Intercept	2.27	0.298	7.63	<0.0001 ***
Distance from edge	0.00315	0.00316	1.50	0.135
• Species richness				
Intercept	-0.306	0.779	-0.393	0.695
Distance from edge	0.0112	0.00484	2.30	0.0212 *

5 Discussion

5.1 The biodiversity at the three types of forests at the Jama-Coaque Reserve

Coastal Ecuador has recently undergone a drastic loss of native forested areas mainly due to human land use (Hamilton and Mouette 2007). Therefore, it is vital to increase the research efforts on the biodiversity, ecology and distribution of endangered faunal groups. We assessed the biodiversity and abundance of the herpetofauna present at the JCR during the dry season. Comparing the three types of forests existent at the Reserve, we surprisingly found the reforested area to hold the highest level of biodiversity, followed by the moist evergreen forest. Indeed, all of the calculated biodiversity indices have the highest values for the reforested area, sometimes overlapped by the values calculated for the moist forest (if considering the upper standard deviation range). A higher Simpson's index as well as a higher Shannon-Wiener's index also indicates great species evenness in the reforested area, due to the higher probability of two randomly drawn individuals to belong to the same species. We did not expect this result because generally herpetofaunal populations require a long time to recover after deforestation (Heinen 1992). However, other studies have detected greater species richness in restored habitats compared to reference habitats (Brown *et al.* 2012). Sometimes this was due to the environmental conditions of these restored habitats, such as more open spaces and less predators, and were overall a preferred habitat to colonist species (Brown *et al.* 2012). Most of the amphibian species observed in the reforested area were also observed in the moist forest, apart from some reptile species which may naturally prefer the warmer temperature found in the reforested area.

The high encounter rate of *P. achatinus* (84% of all the individuals found) serves as the primary reason why cloud forest diversity indices are so low. This species seems a very generalist one, having been encountered in a wide range of temperatures, humidity, elevations, and distances from water (see Appendix I). Most importantly, many of the *P. achatinus* found in the cloud forest were juveniles about 1 cm long, consequently very difficult to identify. Therefore, we do not exclude the possibility that many of those juveniles have been mistakenly identified as *P. achatinus*, an error which may have altered our biodiversity estimates. We plan to run additional analysis excluding those individuals and advise future researchers to have care when identifying juvenile amphibians, if to account for them at all.

Additionally, these biodiversity results may be due to experimental design, as well as to factors that we did not measure. One of the most important factors found affecting species diversity is distance from water. Due to environmental limitations, the majority of transects walked in the reforested area was along a river (Table 1). This may have led to biased results, as transects in the cloud forest did not intersect any bodies of water. Also, less dense vegetation, which facilitates carrying out visual encounter surveys, characterises the moist forest and the reforested areas. We often heard frogs in the cloud forest, but the difficulty of moving without causing habitat disturbance proved challenging to find and identify specimens. Since we found a negative relationship between temperature and species richness, and observed a generally higher temperature in the reforested area than in the moist or cloud forest, it seems counterintuitive to observe a higher biodiversity in the generally warmer reforested area. The species present in the reforested area may have a greater tolerance to higher temperatures, or be the least affected by temperature fluctuation. Furthermore, for our analysis we pooled together data collected during night-time and day-time; perhaps the majority of data causing higher diversity indices of the reforested area (and the moist forest) comes from night-time surveys, during a time of lower temperature. A recommendation for a future study would be to compare day-time and night-time data, as this would potentially clarify the results we calculated. Another suggestion would be to attempt methodologies other than visual encounter surveying. We advise the use of pit-fall traps and audio recording, in order to equalise the sampling effort in the three types of forests.

5.2 Herpetofaunal abundance and species diversity and abiotic factors

Our second question investigated whether temperature, humidity, elevation, distance from water and/or habitat type influenced species richness and herpetofaunal abundance. Temperature was the factor most significantly related to species richness, followed by distance from water. Temperature has been often found as a very influential factor in determining herpetofaunal species richness due to its importance in regulating amphibian and reptile physiology and metabolic functions (Duellman and Trueb 1994; Ortiz-Yusty *et al.* 2013; McCain 2010). We found that as temperature increases, species richness and herpetofaunal abundance decrease. This may indicate a great sensitivity of the local reptiles and amphibians to very high temperatures, or show that only a few local species may withstand living actively at higher temperatures. Indeed, past studies have noticed amphibians to be a very sensitive group to extreme climates compared to, for instance, mammals (Owen 1989). This trend may be a seasonal tendency: our survey was carried out during the dry season, the warmest of the year in this area where potentially most herpetofauna becomes active at night. Once more, we recommend future research or analysis to separate and compare day-time and night-time data, as there is often a high variation in daily temperature. Indeed, we observed day-time temperatures are significantly higher than night-time temperatures, and since our analysis did not distinguish the species observed during the night with the ones during the day, it is not surprising that the results may be confusing (e.g. less species in the cloud forest but cloud forest being the generally the coldest). Differentiating the analysis of nocturnal and diurnal species separately could potentially provide a more accurate understanding of what truly influences the biodiversity distribution of the area.

Distance from water was the second factor significantly related to species richness: the further away from water the less the richness of herpetofaunal species. Especially taking amphibians into account, which use water as an important medium for their life-cycle, this trend is quite predictable

(Vallan 2002). Other studies did not find any correlation between reptilian activity and presence of water (e.g. McCain 2010); within the parameters of our study, it is difficult to determine whether the amphibian or the reptile species or individuals are affected differently by this factor. Future analysis should also attempt to compare the effects of these abiotic factors on reptilian and amphibian species separately, in order to have a clearer understanding of their potentially different dynamics.

Finally, elevation had a negative effect on abundance of individuals, but not on herpetofaunal species richness. Because of the negative relationship that temperature and elevation have across ecosystems, perhaps the change in temperature may explain the relationship between herpetofaunal abundance and elevation. Understanding the effect of elevation on the distribution of the species would hold great importance for their conservation, as it would help concentrate efforts in certain areas with specific characteristics (Chettri *et al.* 2010). It is interesting to observe that, contrarily to other studies (e.g. Chettri *et al.* 2010), abundance of individuals was related to elevation but species richness was not. This may be due to most species' ability to withstand the differences found along the elevation gradient, but with more individuals preferring lower elevations due to favourable environmental conditions. Moreover, a significant effect on species richness may not exist because the elevation gradient was not extremely steep (Table 1).

Habitat type and humidity had a significant effect on neither species richness nor abundance of reptiles and amphibians. This is a curious result, as habitat type encompasses several differences in abiotic factors, and humidity is such an important characteristic for an ideal amphibian habitat (Ortiz-Yusty *et al.* 2013). It is possible that, because of the presence of bodies of water, humidity is not a particularly limiting factor (Ortiz-Yusty *et al.* 2013). Moreover, temperature and distance from water could be more strongly influential than habitat type if the resources local herpetofauna needs to sustain life depend more on those specific abiotic characteristics rather than, perhaps, on the type of vegetation.

We divided the survey data into transects with QGIS due to having walked unequal distances in the three types of forests, and to having unequally divided our sampling time between day-time and night-time. This was done to maximise the results of the dry season surveying in the limited time available for data collection. Advice for future work is to carry out the study by dividing the area that researchers wish to cover into equal transects from the start, as we found it challenging to precisely divide the area into transects *post-hoc*.

5.3 The moist forest edge-effect

Our third question inquired whether edges and boundaries between forest types played any significant role on herpetofaunal species richness and abundance. The edge between moist and cloud forest appeared to have a negative effect on both of these variables; however, the distance to the moist forest edge significantly affected only species richness, and in a positive way. This positive correlation revealed that as distance from the moist-cloud forest edge increased, so did the overall species richness. There are many reasons why this correlation was evident in our study, one being the consideration of biases toward human effort and ability. More often than not, cloud forest transects began at the starting boundary (around 550 m of elevation) and were not a continuation of transects performed at the upper boundary of the moist forest. Because of this, surveyors were just beginning to perform a visual encounter survey and may have required some

time to adjust to the practice. As the transect continued, surveyors quickly became used to this survey practice and became more efficient in their work. As a result, surveyors may have missed several specimens or been accustomed to looking for common species.

Other reasons for the above mentioned effect may come from the flora abundance and type in the transition zone, the area between moist and cloud forest. Although most research on edge-effects have centred on habitat fragmentation and deforestation, it may still hold true that a change in forest type, and therefore change in canopy height and density, results in a change of plant growth and floral density (Murcia 1995). Herpetofaunal abundance is shown to correlate with leaf litter depth on transects (Urbina-Cardona 2006). The cloud forest, with an average lower canopy height, may initially be protected from wind and other abiotic factors at the edge with a taller, denser moist forest, therefore reducing the amount of leaf litter on the forest floor. As the distance from this edge increases, abiotic factors can disturb forest flora more and cause increased presence of leaf litter and other debris for favourable microhabitats. Finally, the edge-effect found in our study may simply be a result of the season in which the study took place. Some herpetofauna species have been shown to favour forest edges during the dry season, avoid them completely, or hold no preference (Lehtinen *et al.* 2003). We suggest further research on the edge-effect between forest types taking abiotic and biotic factors more into account. Additionally, the effects of edges between forest types and disturbed areas on herpetofauna in the Neotropics must be further explored, as little consensus and standardised methodology has been achieved (Murcia 1995).

6 Conclusion

Our data supports the higher biodiversity and herpetofauna abundance in the reforested area and moist forest compared to the cloud forest. Temperature and distance from water greatly affected herpetofaunal abundance and species richness, factors often regarded as very influential in studies in different parts of the world (Ortiz-Yusty *et al.* 2013). Higher elevations had less individuals than lower elevations, but there was no significant difference in terms of species richness, possibly because of the low elevation gradient. Humidity had no effect on abundance and species richness, potentially due to the presence of bodies of water around the study sites. Finally, the moist forest edge had a negative effect on reptile and amphibian species richness, which may be due to the characteristics of this transition zone as well as the presence of more predatory species.

This study is the very first comprehensive research on the herpetofauna of the JCR in Manabí, Ecuador. It provided useful data to begin to understand local reptile and amphibian species distribution and ecology in the dry season, as well as provided an updated species list and distribution data for this season. This research was carried out with the intention of future continuation and long term data collection, to gain a thorough understanding of the species dynamics at this reserve. Furthermore, more detailed analysis can be performed in the future with this and forthcoming data. It would be extremely interesting to compare the effects of abiotic factors in different seasons, as well as observe the potentially changing distribution and richness of herpetofaunal species in the different habitats. Lastly, adding average rainfall as another predictor variable would provide interesting results, as it is a very influential factor in other types of ecosystems (Ortiz-Yusty *et al.* 2013).

7 Acknowledgements

We would like to kindly acknowledge the great help of Eva Filicpzyková throughout the whole project, especially during experimental planning and statistical analysis. We also would like to thank Ryan Lynch for helping us with the identification of many of the species we found, and Wouter Hantson for his assistance while using QGIS software. Additionally, we thank Edilberto Marquez for guiding us through some of the trails and opening a new trail at Finca de Madera. Finally, a wholehearted thank to everyone who kept our spirit up throughout the whole project, who joined our surveying team during the long nights, and naturally to Third Millennium Alliance for its current efforts for saving these forests and for making this research project possible.

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9 Appendix I

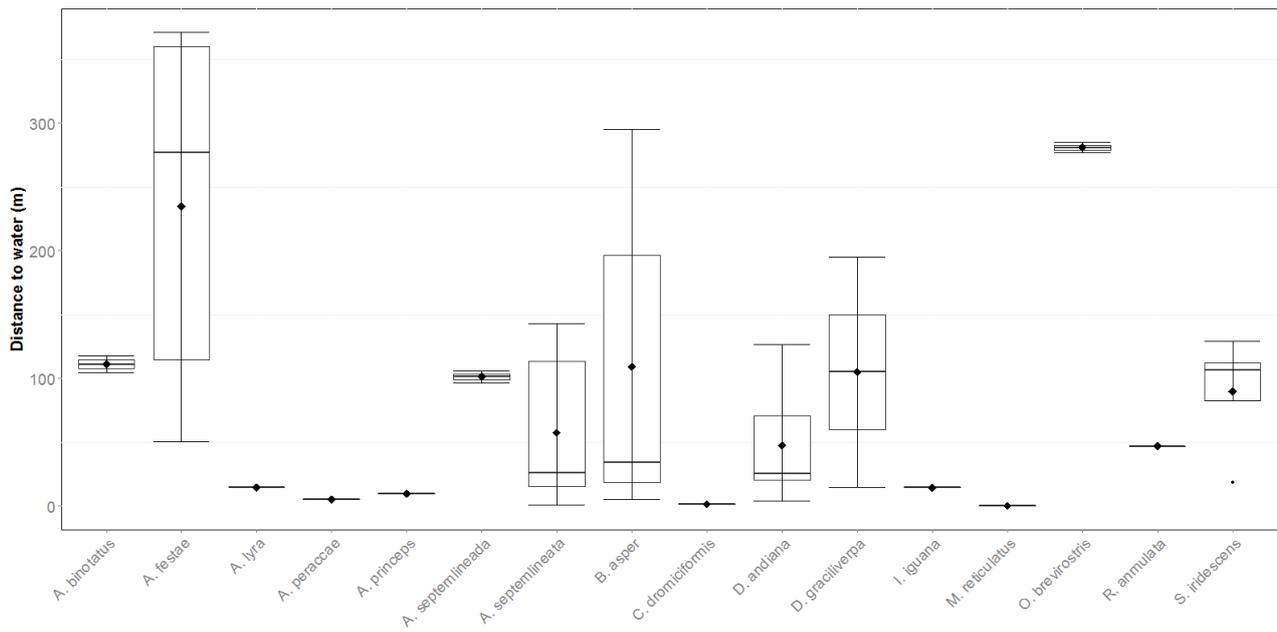


Figure 7. Range of distances to water where individuals of each of the reptile species were found.

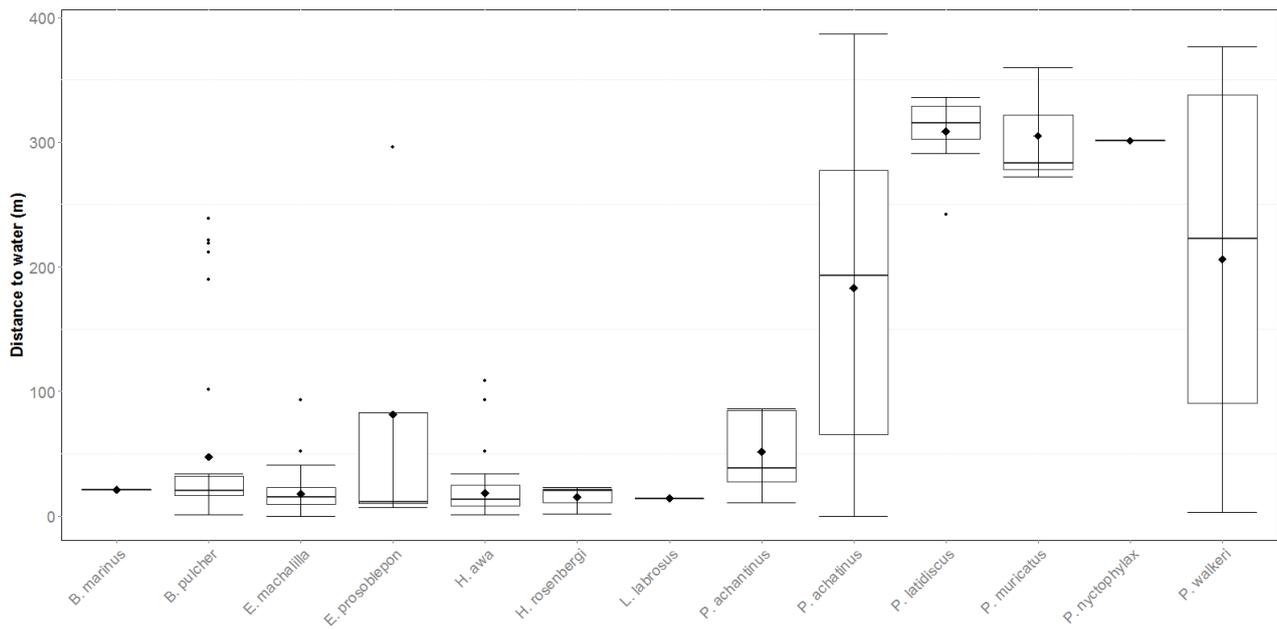


Figure 8. Range of distances to water where individuals of each of the amphibian species were found.

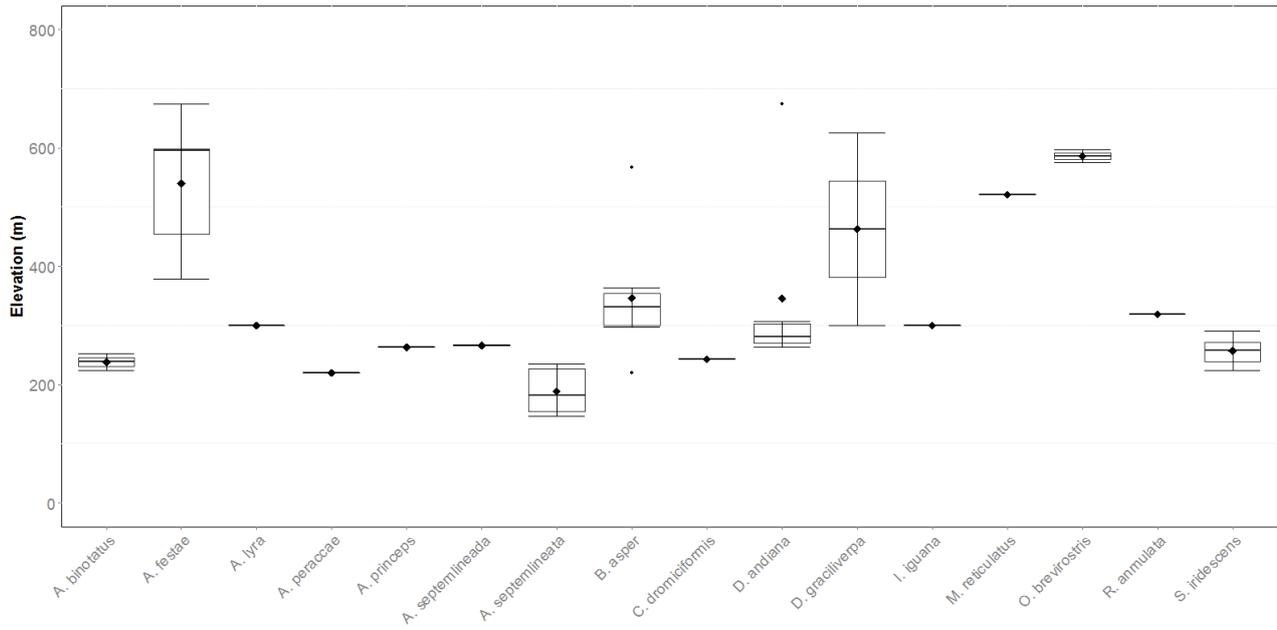


Figure 9. Range of elevations where individuals of each of the reptile species were found.

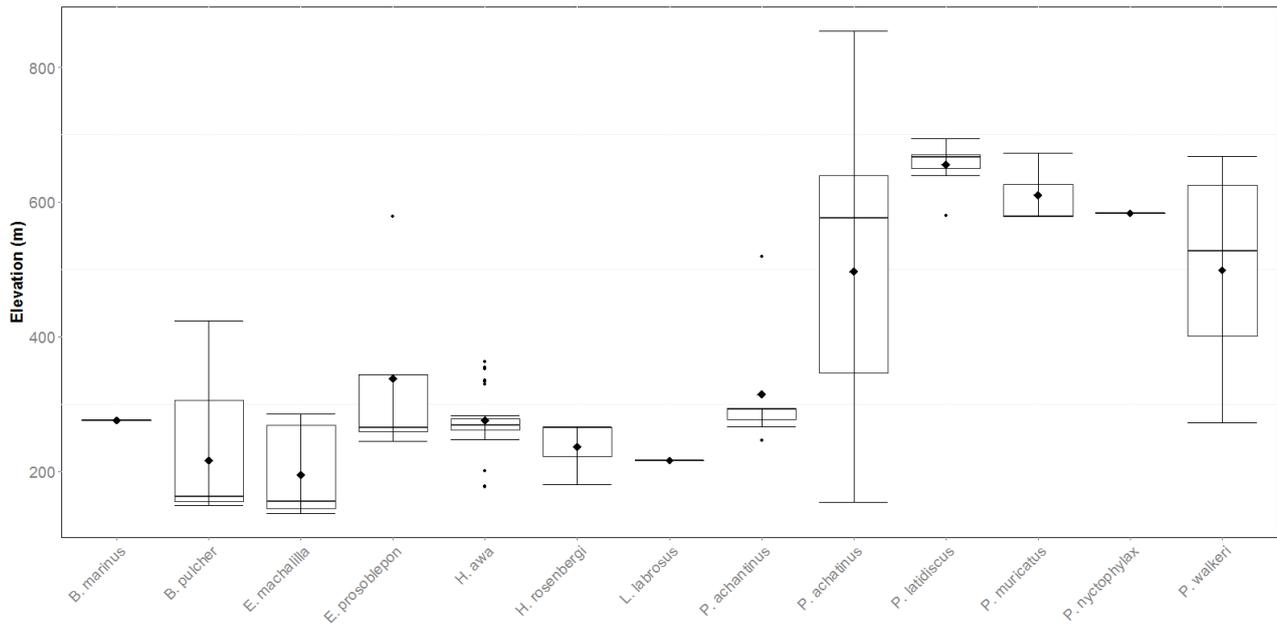


Figure 10. Range of elevation where individuals of each of the amphibian species were found.

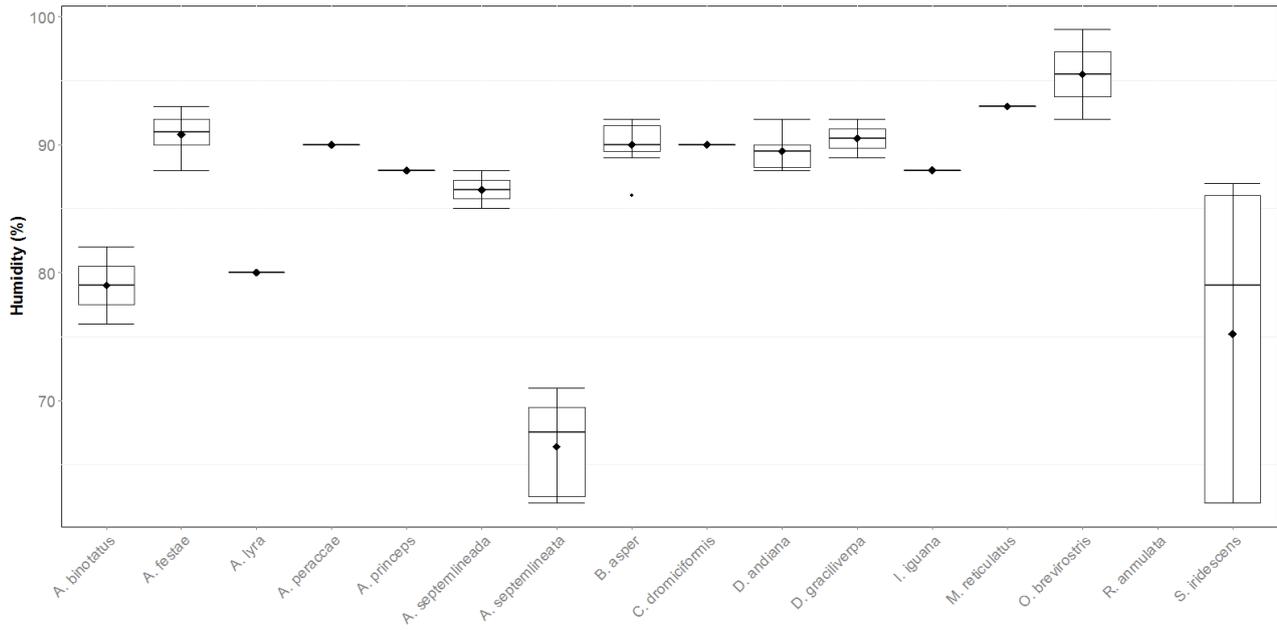


Figure 11. Range of humidity where individuals of each of the reptile species were found.

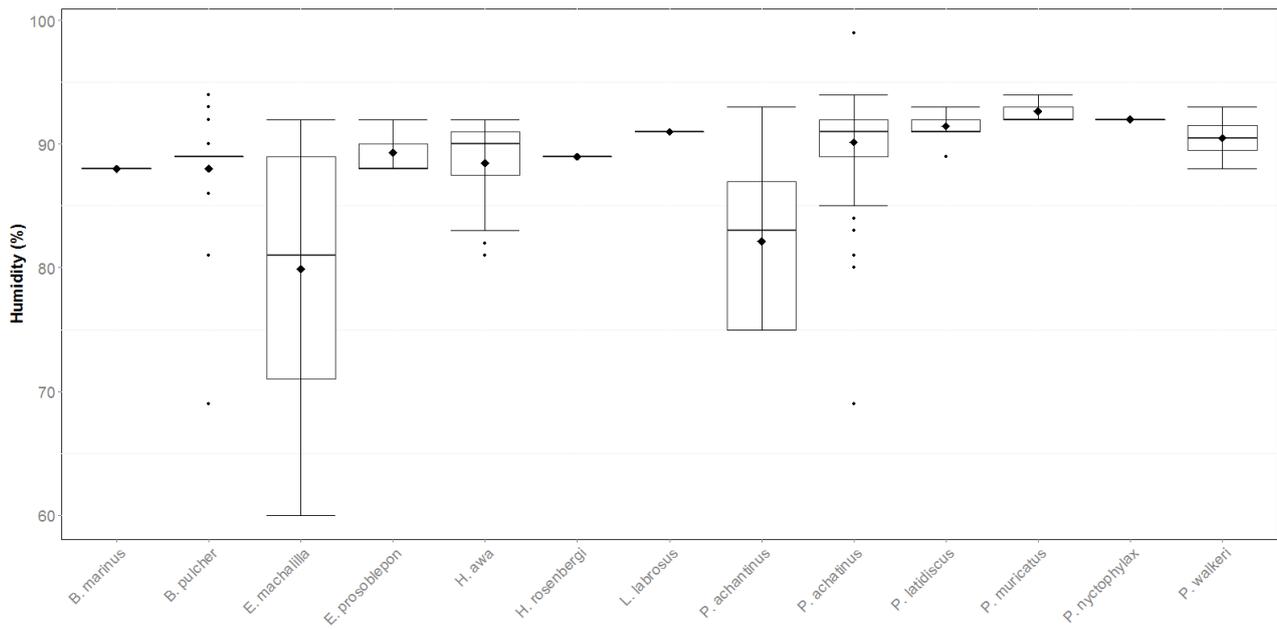


Figure 12. Range of humidity where individuals of each of the amphibian species were found.

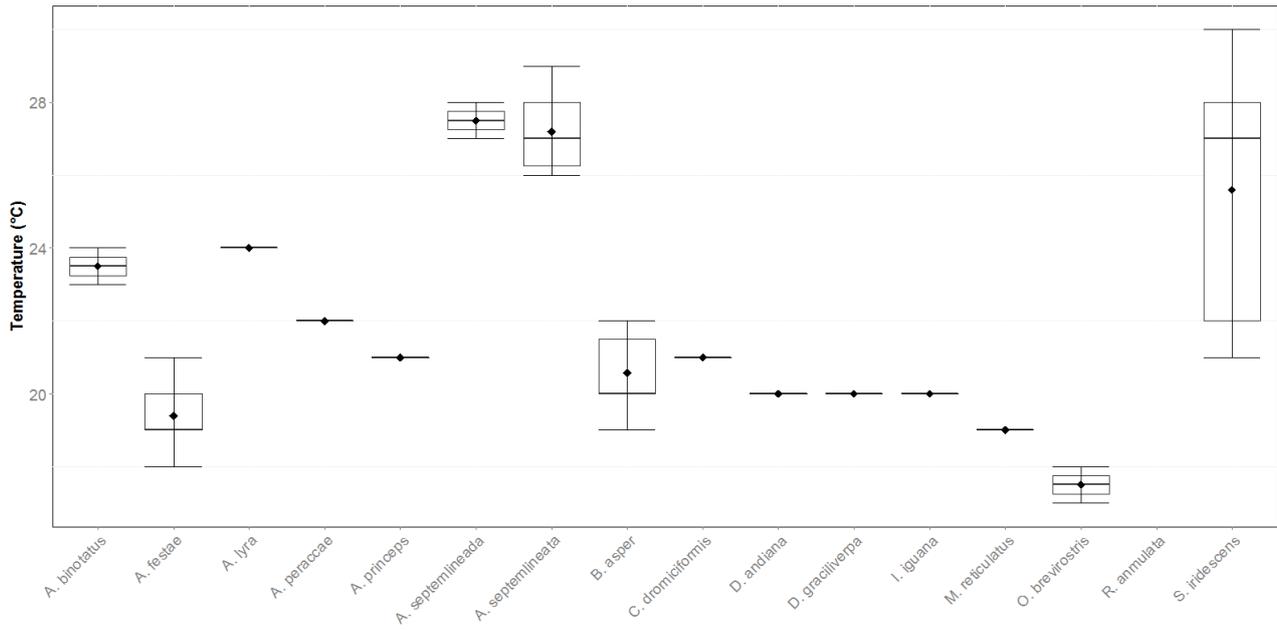


Figure 13. Range of temperatures where individuals of each of the reptile species were found.

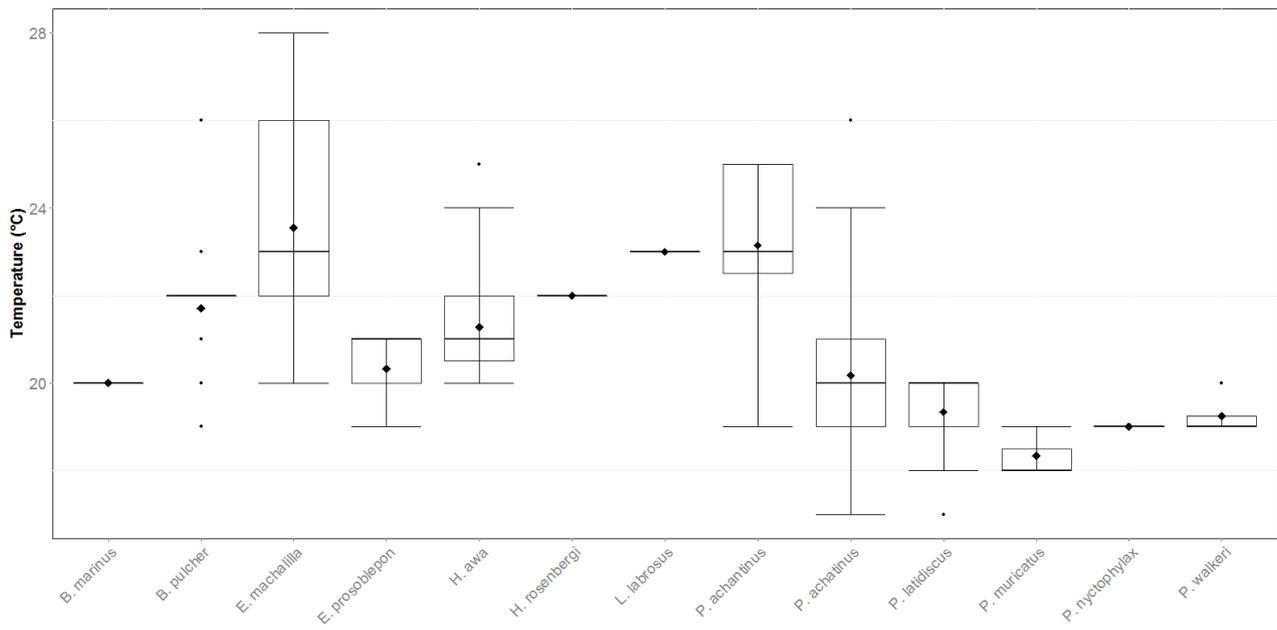


Figure 14. Range of temperature where individuals of each of the amphibian species were found.