Using Multiple Methods to Evaluate Secondary Forests as a Habitat for Amphibians

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Abstract

Amphibians are of important conservation concern due to the extent of their recent declines due to pathogens and habitat loss. Understanding how they can utilize alternative habitats to primary forest, such as secondary forest, is important to understand to best target conservation efforts. This study investigates the ability of secondary forests to be habitat for amphibians, with an emphasis on Hylid amphibians, in the Madre de Díos department of Peru in the Southwest Amazon Basin. Refuge tubes were placed in both primary and secondary forest to sample Hylid diversity, visual encounter surveys using transects were conducted in both environments, and chance encounters were accumulated from each environment. Refuge tube had almost no success, with only one capture in 1,776 trap nights. The abundance of tube surveys and transects surveys were compared and no significant difference was found between the two environments, though a small sample size limited the effectiveness of statistical analysis. Total observations including chance encounters were used to calculate Shannon Weiner Diversity Index values for each environment, with the primary forest having a larger diversity index value. The communities in each environment were distinct, with only one species (*Adenomera andreae*) being found in both. Though providing some data, the results of this study are unfortunately inconclusive and therefore unable to add evidence regarding whether or not secondary forests can function as a habitat for amphibians. Several factors limited the study, including the difficulty of conducting the survey in the dry season and the ineffectiveness of refuge tubes at the study site. Experimentation with refuge tubes could determine if the method is possible in tropical forests, and continued research comparing primary and secondary forest using transects during the wet season is encouraged to better understand amphibian diversity in each.

Introduction

One group of species that has risen to the forefront of conservation concern in recent decades is amphibians, as they are the most rapidly declining among all vertebrates (Collins, 2010). Beyond preserving the diversity of life on Earth, amphibians are important to protect due to their integral role in the world's ecosystems as both predators and prey. The loss of these organisms can therefore prove detrimental to ecosystems as a whole (Whiles et al., 2013). Due to this, amphibian declines have been a focus of recent research. Amphibians are subject to all of the common drivers of biodiversity loss, such as habitat loss, pollution, climate change, and introduced species and pathogens (Collins, 2010). Among these, the factors identified as the largest threats to amphibian populations are the disease Chytridiomycosis, caused by the introduced pathogens *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans*, and habitat loss (Scheele et al., 2019; Gallant et al., 2007).

While the effect of Chytridiomycosis is varies by location, habitat loss is contributing to amphibian declines worldwide (ex. Fulgence et al., 2021; Greenberg et al., 2018; Russildi et al., 2016). One such area facing deforestation threats is the Peruvian Amazon, which contains some of the highest amphibian biodiversity on the planet (Jenkins et al., 2013). This diversity makes the value of amphibian conservation in the region of incredible importance, not just for local ecosystems, but for preserving global biodiversity. Unfortunately, due in large part to deforestation, around a quarter of Peru's amphibians are at least threatened and a further quarter are data deficient, with their conservation status unknown (Catenazzi & von May, 2014). One area subject to deforestation is Madre de Díos, Peru in the Southwest Amazon Basin. Deforestation in this region of the Amazon is increasing (Silva Junior et al., 2021) thanks in part locally to the completion of the Interoceanic Highway (Oliveira et al., 2007). This highway allows for easier access to the area which allows agriculture, which is held as responsible for the majority of deforestation by the Peruvian government (Marquardt et al., 2018; Ravikumar et al., 2016), to more easily clear forests for its operations. This is a trend common in the tropics, where the highest rate of conversion of forest to alternative land uses is observed (Gallant et al., 2007).

Due to the loss of forest caused by land conversion, it is important to understand the contribution of alternative habitats for amphibians to combat their declines, such as secondary forests. Secondary forests are defined as forests that are naturally recovering from alternative land use by humans and are distinct from undisturbed forests (Chokkalingam & de Jong, 2001). These forests have been shown to be able to provide certain ecosystem services like carbon sequestration, hydrological services, and habitat for biodiversity (Tito et al., 2022). Since many old growth forests are disappearing, continuing to investigate the ability of a secondary forest to act as a habitat for amphibians is important in order to understand the capacity they have to contribute to amphibian conservation.

Evidence for Secondary forests acting as a habitat for amphibians is conflicting. Promisingly, secondary forests have demonstrated the ability to harbor similar abundance and diversity to primary forests. Multiple studies have shown secondary forest to house similar amphibian communities in similar abundance to untouched old growth forest (Warren-Thomas et al., 2013; Gillespie et al., 2012) and others have shown secondary forest to maintain, while not equal amounts, the majority of amphibian biodiversity found within old growth forests (Whitworth et al., 2016; Serrano-Rojas et al., 2022). The recovery of amphibian communities to reach this near equivalence has been shown not to take very long (<30 years), indicating that these secondary forests may be able to become adequate habitat relatively quickly (Hernández-Orndóñez et al., 2015). However, one study found secondary forests to contain only half of the species richness of old growth forests (Vallan, 2002) and another also found that, although secondary forests contain similar abundance, they harbor fewer species of amphibians than old growth forest (Gardner et al., 2006). In addition, there is evidence that while some species' recovery may be relatively fast, that recovery for specialist species is actually slow (Acevedo-Charry & Aide, 2019). The conflicting nature of previous research highlights the need for further investigation. Some of this research has been performed in the Madre de Díos department of Peru. One study conducted in the Manu Biosphere Reserve demonstrated that the species richness and diversity in secondary forest were comparable to those in the core protected area, though the species found in each environment did differ (Serrano-Rojas et al., 2022). Another study in the Manu region also found results suggesting that regenerating forest can hold 81% of amphibian diversity found in old growth forest after only 30 years of regeneration (Whitworth et al., 2016). A third found that the difference between the amphibian biodiversity of forests recovering from burns and undisturbed forest found was not significantly different (Warren-Thomas et al., 2013). This is, however, the extent of research on the topic done in the area.

This study plans to further investigate the ability of secondary forests to provide habitat for amphibians, with an emphasis on Hylid amphibians, at Finca las Piedras in Madre de Díos, Peru. Though amphibians in the primary forest of the field station have previously been studied (Gómez Galdos, 2021), it did not explore the secondary forest as a habitat for amphibians. Further, the methods used to survey amphibians have been shown to affect what species are found during surveys and therefore their results (Whitworth et al., 2017). Because of this, continued research at the study site using additional methods is needed to come to understand the primary and secondary forests as habitat. This study will further investigate secondary forests as a habitat for amphibians in the forests of Madre de Díos, Peru in the Southwest Amazon Basin by comparing tree frog (*Hylidae*) abundance and diversity in primary and secondary forests. The secondary forest is predicted to contain less Hylid diversity and abundance compared to old growth forest due to containing less large tree habitat than old growth forest. This will allow for a better understanding of the ability of secondary forests to provide habitat for both this family of amphibians in the region.

Methods

Study Site

This study was performed at Finca las Piedras in Madre de Díos, Peru, which is located in the Southwest Amazon Basin. This region of Peru is home to large amounts of tropical lowland forest and is situated in the Brazil Nut (*Bertholletia excelsa*) corridor, which is a region of forest containing Brazil Nut trees in which the Peruvian government grants concessions to locals to harvest Brazil Nuts. Deforestation in the area is growing and driven by gold mining and agricultural expansion and could continue to grow as roads increase access to remote areas in the department (Gallice et al., 2019). Average annual temperature is 26° C with little variation year-round (Jenkins, 2009). Seasonality in the department is therefore most defined by rainfall, with a wet season from October to April with average rainfall of over 200 mm of rain per month and a dry season from May to September with average rainfall of less than 75 mm of rain per month (Jenkins, 2009). Due to its location just east of the Andes, Madre de Díos experiences cold fronts during the dry season, known as *friajes*, that come from Antarctica via the Andes. This study took place during the dry season and therefore had to contend with both decreased rainfall and *friajes*.

The specific study site, Finca las Piedras, is run by Alliance for a Sustainable Amazon, which works on projects including reforestation, research, and education. The field station contains 10 hectares of secondary, regenerating forest and 50 hectares of primary forest on its property. Though the primary forest has historically been selectively logged, it has not been logged in years and the forest on site represents a mature forest. The secondary forest is seven years of age or younger, as the land only began being reforested in 2016 with the creation of Alliance for a Sustainable Amazon. Land within this secondary forest is varied, with some areas beginning to establish as forests with trees reaching 10m and some young areas with trees planted during the timeframe of the study.

Study Group

The study group, Anurans (frogs), was chosen as they are of dire conservation concern, as they are the most rapidly declining among all vertebrates (Collins, 2010). The study site, in the Southwest Amazon Basin, is one of the world's biodiversity hotspots for amphibians (Jenkins et al., 2013). Therefore, the frog communities in the Madre de Díos department are incredibly diverse and represent eleven families (Villacampa et al., 2017). The family *Hylidae* (tree frogs) make up 40% of the anuran biodiversity of the Madre de Díos department in Peru (Villacampa et al., 2017). Because they make up such a large proportion of the species richness in the area, understanding how they specifically can utilize secondary forests as a habitat is important for understanding how amphibians as a whole can respond. Amphibians have been studied at the site previously (Gómez Galdos, 2021), but not to evaluate their ability to utilize secondary forests.

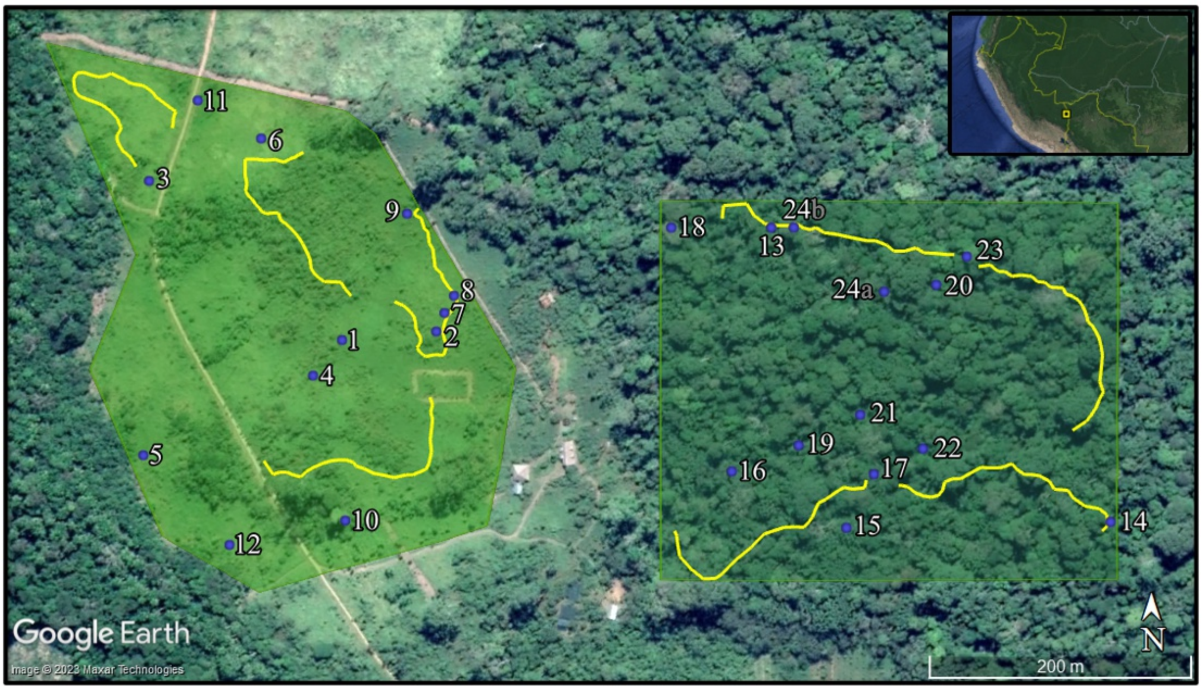
Data collection

Ten hectares of available secondary forest and ten hectares of available primary forest adjacent to the secondary forest were used to compare the Anuran biodiversity in each environment. The plot of secondary forest was an irregular polygon, as it was shaped to match the available ten hectares, while the plot in the primary forest was rectangular. Within each ten-hectare plot, 12 randomly generated points were created by generating random latitude and longitude coordinates using an open access random number generator at *Calculator.net* and pairing the latitude and longitude coordinates based on the order they were generated. This created 24 total sampling locations (See Fig. 1)

Refuge tubes were placed (Boughton & Steiger, 2000) at each randomly generated point. Refuge tubes were made of gray PVC plastic cut to be 60 cm long and 5.4 cm in diameter. Each tube was cleaned before use to keep the inside free of dirt and buildup. Each tube had a hole drilled at 10 cm above the bottom. Each tube was filled with 229 ml of clean water, which is the volume of the tube beneath the hole drilled. Excess water the tubes picked up was released through the hole. Each refuge tube was tied vertically to a suitable tree (> 10 cm in diameter at breast height) within a two-meter radius of the point by stringing rope through two holes drilled at the top of the tube and tying the rope around the tree. The opening on the top of each tube was two meters high on the trunks of the chosen trees. Tubes were secured when necessary by tying an additional knot around the lower part of the tube. This ensured that the tube was vertical and stable to promote the tree frogs' usage of the refuge tubes. If no suitable tree was found within the two-meter radius of the randomly generated point, the radius was expanded to three-meter, four-meter, and so on until a suitable tree was found. Every two to three days the tubes were checked for frogs in the morning between 7 a.m. and 10 a.m. Tubes were checked using a mirror and flashlight to see inside without disturbing the tube. After one week, two additional tubes of the same specifications were placed at each site following the same

Figure 1: Map of Plots, Tube Locations, and Transects.

Figure one shows a map (created using Google Earth Pro®) of the secondary forest plot (light green) and primary forest plot (dark green) with the randomly generated points marked in blue and labeled by number. 24a and 24b represent the initial (a) and adjusted (b) positions of the tubes that were moved by interference from Leafcutter Ants (Subfamily: *Myrmicinae*). Two hundred meter transects are represented by the yellow lines on the map. Satellite imagery is just over four years old.



protocol to increase the number of traps. In total, deployed traps totaled 1,776 trap nights. Tubes at site 24 needed to be moved after 11 days due to interference from leafcutter ants (Subfamily: *Myrmicinae*) that resulted in the ropes tying the poles to the trees being cut up and hauled away. Tubes were moved about 50 meters away to get out of range of the nearby leafcutter nest. Frogs were removed from the tubes using a pole and cloth to guide them out into a clear container that they could be photographed for identification in.

Visual encounter surveys using transects (Rödel & Ernst, 2004) in regenerating forest and primary forest were run during the day and at night to include diurnal and nocturnal species. Four transects of 200 meters were created in each environment (See Fig. 1). Transects in the old growth forest followed the existing trail system. Transects in the regenerating forest were placed at locations where there were walkable trails or that were easily accessible due to some areas being difficult to traverse. The transects chosen represented a balance between the diverse areas of the secondary forest. Performing the surveys involved walking the transects at a deliberate pace (5 meters / min) and identifying and photographing every frog seen for identification. Chance encounters with amphibians within the two plots that were not part of a transect were still photographed and identified to add to the list of known species in the plot, but not included in statistical comparisons similar to Warren-Thomas et al. (2013). Chance encounters from before or after the time frame of the study and chance encounters outside of study activities were not included to avoid biasing the number of encounters towards the primary forest, where more time was spent outside of the study.

Weather data was also collected throughout the timeframe of the study. Highest and lowest daily temperatures were collected within the primary forest and rainfall was measured every day. Weather condition during each tube survey and transect survey was also recorded.

Data analysis

The abundance of tree frogs from each survey day (all 36 tubes) for each environment and the abundance of frogs from each transect (200 m) was determined using the data collected. A Wilcox test was used to compare the abundance values per survey day and transect between the primary and secondary forest. Though planned, due to the low sample size of the data the average Shannon Weiner diversity index values per tube survey and per transect were unable to be calculated and compared between each environment. In place of this, chance encounters were pooled with observations from transects and tube surveys to create large enough samples to calculate Shannon Weiner diversity index values for each environment.

Results

Figure 2A shows the results of transect surveys. No frogs were found during day surveys. The mean number of frogs per survey is higher in the primary forest, but no significant difference is observed between the primary and secondary forest ($p = 0.115$). Figure 2B shows the results of refuge tubes surveys. Only one frog was found within a refuge tube. No significant difference is observed between primary and secondary forest ($p = 0.359$). Figure 2C shows all observations, including chance encounters, from each environment. More frogs were found in the primary forest ($n = 15$) than the secondary forest ($n = 6$), and likewise more species were found in the primary forest ($n = 6$) than the secondary forest ($n = 4$). Six Hylid amphibians were observed in the primary forest while only one was observed in the secondary forest. Shannon Weiner value for the primary forest (1.64) is greater than the corresponding value for the secondary forest (1.24).

Observations

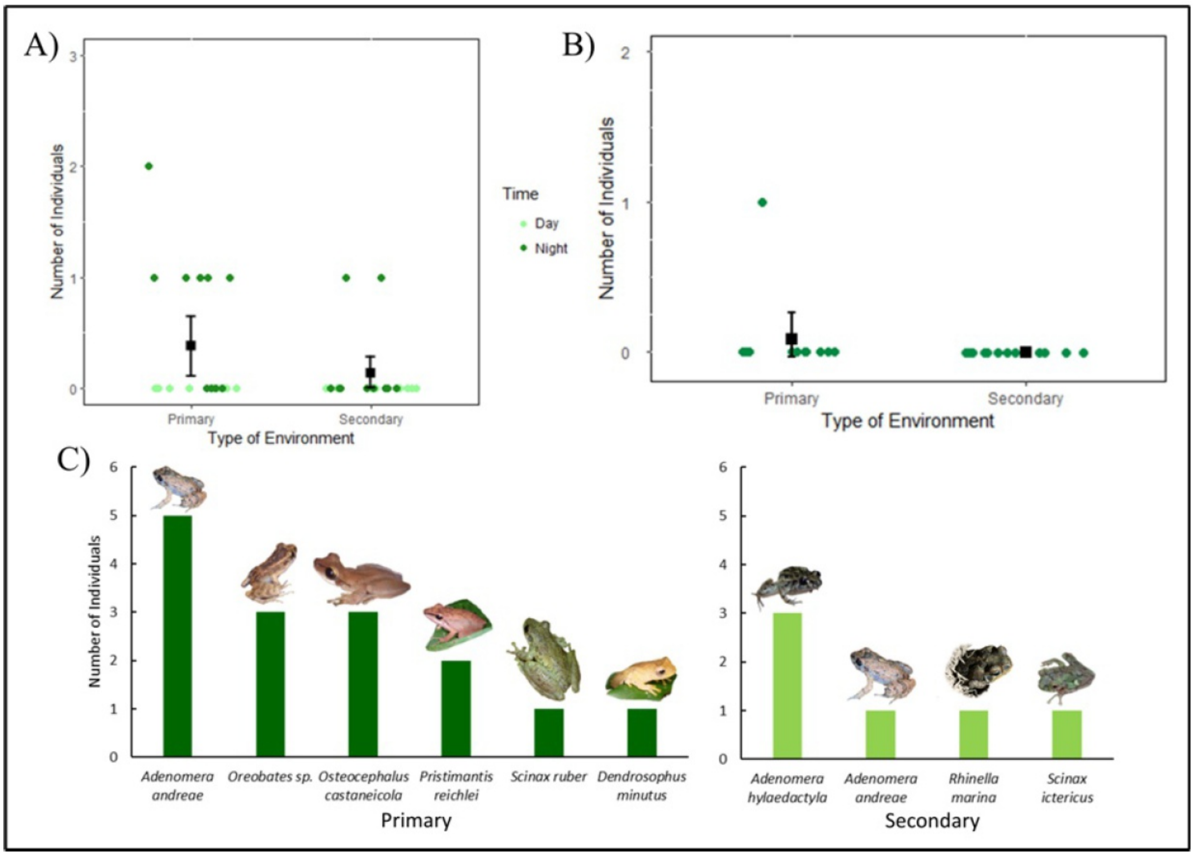
Twenty-one total frogs were observed in total with one coming from refuge tube surveys, nine coming from transect surveys, and eleven coming from chance encounters. The amphibians observed represent nine species and four families (Fig. 4 in Appendix). The species contain both arboreal and terrestrial amphibians, with no aquatic and fossorial species observed. The communities of the two environments contained distinct species, with only one species (*Adenomera andreae*) being found in both environments.

Weather

Average highest temperature within the forest during the study was 26.6ºC, while average lowest temperature was 23.4ºC. No *friajes* were observed during the timeframe of the study, although one occurred both prior to the beginning and immediately after the end of the study period. Only 9.5cm of rainfall occurred during the timeframe of the study. Weather was consistent and almost all surveys were conducted under clear skies, with partly cloudy and overcast conditions observed on few occasions. This means that the conditions, tempertaure, rainfall, and weather, were consistent throughout the study.

Figure 2: Results of Transects, Refuge Tubes, and Chance Encounters.

A) Figure 2A shows the number of individual frogs found during 200 meter transect surveys (n = 20) in the Primary and Secondary Forest. Transect surveys performed during the day (n = 10) are in pale green, while transects performed at night (n = 10) are in forest green, and the mean number of frogs per survey is represented by the black points, with the 95% confidence interval shown by the error bars. B) Figure 2B shows the number of individual frogs found during refuge tube surveys (n = 12) in the Primary and Secondary Forest. The mean number of frogs per survey is represented by the black points, with the 95% confidence interval shown by the error bars. C) Figure 2C shows the total number of frogs found in the primary (forest green/left) and secondary forest (pale green/right), including transect data, tube data, and chance encounters.



Discussion

Transects were run, refuge tubes were placed and checked, and chance encounters were accumulated in both secondary and primary forest to compare amphibian diversity in each environment. No significant difference was observed between the primary and secondary forest from the data collected during refuge tube surveys (Fig. 1B; p = 0.359). Likewise, no significant difference was observed between the primary and secondary forest from the data collected during transect surveys (Fig. 1A). However, the comparison between transects in the primary and secondary forest is nearly significant (p = 0.115) and the data analysis may be limited by small sample size. These data suggest that there is not a great difference between the primary and secondary forests' ability to provide habitat to amphibians. This lines up with what similar studies in the area have found that secondary forests can harbor amphibian diversity comparable to primary forests (Serrano-Rojas et al., 2022; Whitworth et al., 2016; Warren-Thomas et al., 2013). However, larger samples would be required to draw reliable conclusions on the topic.

Pooling encounters from all three methods used (transects, tubes, and chance encounters) allowed for a Shannon Weiner diversity index value to be calculated for each environment (Fig. 1C). The value for the primary forest (1.64) was greater than the value for the secondary forest (1.24), suggesting that the primary forest community represents a more diverse assemblage of amphibians. In addition, more frogs were found in the primary forest (n = 15) than the secondary forest (n = 6), and likewise more species were found in the primary forest (n = 6) than the secondary forest (n = 4). These results suggest that the primary forest, while comparable to the secondary forest, is still a better habitat for amphibians than the secondary forest, which lines up with previous research in the area that showed that secondary forest retained most, but not all of the diversity of a primary forest (Whitworth et al., 2016). Additionally, invertebrates that frequented the refuge tubes add support to this statement, with significantly more invertebrates being found in the primary forest (p = 0.035; Fig. 3 in Appendix).

Observing the pooled encounters also reveals patterns in the species observed in each environment. The communities of the primary and secondary forest contained distinct species, with only one species (*Adenomera andreae*) being found in both environments. This is indicative that the two environments differ in the species that they provide habitat for, suggesting that each environment provides habitat to certain species adapted for that environment. Again, observations from invertebrates observed support this, with the communities found using the tubes differing between the two environments (Fig. 3 in Appendix). This agrees with the findings of Serrano-Rojas et al. (2022) which found secondary forests in the region to contain distinct amphibian communities from those found in primary forests, even though they harbored similar levels of diversity.

One example of this is effect can be seen in the target amphibians of this study, tree frogs. Hylid amphibians were found more in the primary forest (n = 6) than in the secondary forest (n = 1), suggesting that they prefer the environment with more established trees. In addition, the only Hylid frog found in a refuge tube was found in the primary forest. However, the small sample size of this conclusion limits the ability to draw significant conclusions from the data.

The main reason for the limited data in the study that limits the ability to draw conclusions is the dry season. The dry season in Madre de Díos is strong, with a drop in monthly rainfall of over 125 mm from that of the wet season (Jenkins, 2009). Due to the semi aquatic nature of amphibians, this drop in rainfall coincides with decreased amphibian activity, particularly in environments without a water source. This effect is clear when comparing the number of frogs found in this study to a nearby study performed in the area using similar methods. A previous study at the study site conducted at the end of the wet season into the early stages of the dry season found 150 amphibians from 6,000 meters of transects, while this study found only 11 amphibians from 4,000 meters of transects (Gómez Galdos, 2021). However, this study used a different placement of the transects off of trails and was comparing regions of primary forest and therefore did not run any transects in the secondary forest. The placement of transects should not have accounted for the low number of amphibians observed, though, as previous research in the area shows that transects on pre-existing trails are likely to contain more amphibians than off-trail transects (von May & Donnelly, 2009). Another study that augmented their sampling by including both the wet and dry seasons had a greatly increased sample of amphibians (Serrano-Rojas et al., 2022), adding evidence that being limited to the dry season limited this study.

Among the most prominent shortcomings of the study was the use of refuge tubes, which only returned one frog in 1,776 trap nights. The methods the practice was based on reported significantly higher capture rates (Boughton et al., 2000), highlighting the ineffectiveness of the method in this study. A few differences from the specifics of the previous study could be responsible for this. The first is the change in habitat. The previous study was performed in a sub-tropical forest in Florida in the United States while this study was performed in tropical forests. It could be that refuge tubes are ineffective in tropical forests due to an existing abundance of refuge for tree frogs, making the tubes unneeded and unused. Another possibility is the placement of the tubes within 50 meters of water. In the previous study, tubes were placed near bodies of water which are not present in either the primary or secondary forest plots in this study. This could have also limited success as tree frogs, particularly during the dry season, gravitate towards water sources. A third possibility involves the dry season. Many of the tree frogs in the area only descend from the canopy during the wet season to breed (Villacampa et al., 2017), meaning many tree frogs may not have descended from the canopy during the dry season and therefore never come near the tubes. This is supported by the one species that was found in the tube, *Scinax ruber,* which is noted to breed year-round (Villacampa et al., 2017) and therefore descend from the canopy even during the dry season. Further experimentation with this method at the study site would be needed to determine what factor affected the success of refuge tubes in this study.

Though further work investigating different circumstances and how they affect the effectiveness of refuge tubes in forests within Madre de Díos could be useful, it is concluded based on the results of this study that further attempts using them in the methods of this study would continue to be ineffective. Therefore, it is recommended that this method is not re-attempted to survey Hylids at Finca las Piedras unless addressing the hypothesized causes of the poor trap rate. In contrast, further study using transects during the wet season is encouraged to better understand if there is a difference between primary and secondary forest amphibian diversity. Running transects in the wet season would lead to an increased sample size and better illuminate whether or not the two habitats are comparable.

The results of this study are unfortunately inconclusive and therefore unable to add evidence regarding whether or not secondary forests can function as a habitat for amphibians. This is in large part due to the challenges posed performing the study in the dry season away from sources of water. These limitations do pose a concerning outlook for what amphibian communities of the area may look like if predictions of aridification (Alves et al., 2017; Cox et al., 2004; Sampaio et al., 2020; Shiogama et al., 2011) in this region of the Amazon Basin come to pass this century. This highlights the importance of continued forest protection, reforestation, and amphibian research in the area moving forward.

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Appendix

Figure 3: Invertebrates found in Refuge Tubes in Primary and Secondary Forest.
A) Figure 3A shows the number of individual invertebrates found during refuge tube surveys (n = 12) in the Primary and Secondary Forest. The mean number of invertebrates per survey is represented by the point in black, with the 95% confidence interval shown by the error bars. A significant difference is observed between the primary and secondary forest (p = 0.035). B) Figure 3B shows the composition of invertebrates found during refuge tube surveys in the Primary and Secondary Forest. The compositions of each environment is distinct. Groups observed were ants (Family *Formicidae*), termites (Infraorder Isoptera), grasshoppers (Suborder Caelifera), beetles (Order Celeoptera), cockroaches (Order Blattodea), spiders (Class Arachnida), and caterpillars (Order Lepidoptera). Cockroaches were the dominant organism in the primary forest, while caterpillars were the dominant organism in the secondary forest. Termites were only found in tubes in the primary forest.

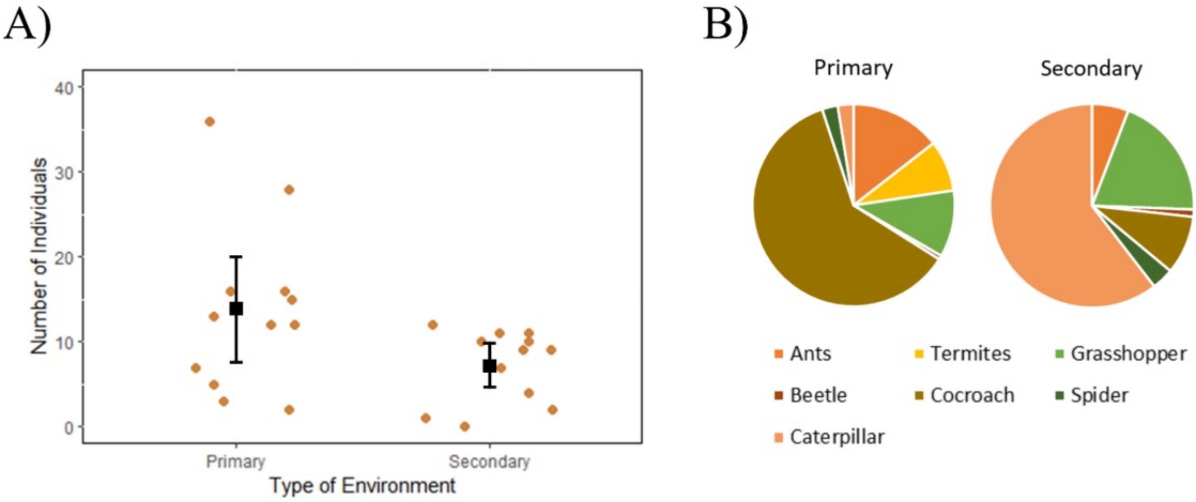

Figure 4: Amphibian Species Observed in the Study.

Figure 4 shows the species that were observed in transects, tubes, and by chance. In order: 1) *Adenomera andreae*, 2) *Adenomera hylaedactyla*, 3) *Dendrosophus minutus* 4) *Oreobates sp.* 5) *Osteocephalus castaneicola* 6) *Pristimantis reichlei* 7) *Rhinella marina* 8) *Scinax ictericus* 9) *Scinax ruber*. The species observed represent four families: *Hylidae, Leptodactylidae, Bufonidae*, and *Craugastoridae*.