

Richness and abundance of amphibians in a forest with different levels of disturbance

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Image by Tess van der Bel

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Abstract

Amphibian populations worldwide are experiencing significant declines, with habitat disturbance as a major contributing factor. This study examines the effects of habitat structure and disturbance history on amphibian richness and abundance in the Peruvian Amazon. Surveys were conducted across three habitats representing a disturbance gradient: a highly disturbed banana plantation, a moderately disturbed secondary forest, and a minimally disturbed selectively logged primary forest. Visual Encounter Surveys (VES) were used to collect data on species richness and abundance, alongside environmental factors such as temperature, humidity, and rainfall. Results revealed that the primary forest supported the highest species richness and abundance, followed by the secondary forest and banana plantation. A strong positive correlation between species richness and abundance was observed, emphasizing the role of habitat complexity in supporting diverse amphibian communities. The banana plantation exhibited reduced amphibian diversity, likely due to its simplified vegetation and limited ecological niches. Regression analyses indicated that environmental variables did not significantly explain variations in amphibian populations, highlighting the importance of habitat characteristics. These findings underscore the critical need to conserve primary forests and rehabilitate disturbed habitats to mitigate biodiversity loss and ensure the survival of sensitive amphibian species in the Amazon.

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1. Introduction

Amphibian populations have experienced a significant global decline in recent decades. In South America, the rate of decline has been even more rapid than the global average (Houlahan et al., 2001). Globally, habitat modification and land-use changes have emerged as significant drivers of biodiversity loss, with amphibians being one of the most severely affected groups (Stuart et al., 2004; Houlahan et al., 2000). In the Amazon rainforest, land-use practices such as agriculture and selective logging have reshaped ecosystems, altering habitat structure, microclimatic conditions, and resource availability (Laurance et al., 2002). These changes have implications for amphibians, who are highly sensitive to even subtle shifts in environmental conditions due to their permeable skin, dependence on aquatic habitats for reproduction, and specialized ecological requirements (Davies et al., 2004; Luz et al., 2021).

One of the major challenges in understanding amphibian declines is the heterogeneity of disturbances across landscapes. In agricultural areas like banana plantations, the replacement of diverse native vegetation with monocultures results in a simplified ecosystem with reduced canopy cover, altered microhabitats, and fewer resources for wildlife (Luz et al., 2021). Secondary forests, often recovering from previous clearing, are characterized by dense understories and increased light penetration, which create distinct environmental conditions compared to primary forests (Zhu, 2002). Meanwhile, selectively logged primary forests, though less impacted than plantations, still experience long-term changes in microhabitats and ecological stability. This gradient of habitat types represents a range of disturbance levels that can significantly influence amphibian populations, but the specific mechanisms driving these effects are not well understood (Griffiths & van Schaik 1993; Bejder et al., 2006).

Amphibians are not only vulnerable to the direct effects of habitat alteration but also to its cascading impacts, such as changes in predator-prey dynamics, reduced water availability, and shifts in vegetation composition. Additionally, the fragmentation of habitats often isolates populations, reducing genetic diversity and making them more susceptible to stochastic events and disease outbreaks (Fisher et al., 2009). These challenges are heightened in regions like the Amazon, where high biodiversity is accompanied by high sensitivity to disturbance.

Amphibians are highly sensitive to environmental changes due to their permeable skin, which allows contaminants and stressors to affect them directly (Davies et al., 2004). Certain amphibian species, particularly those that rely on specific aquatic and terrestrial environments, are especially vulnerable. During larval stages, these species filter large amounts of water through their gills, heightening exposure to toxins and pollutants. Many amphibians also have specialized diets and habitat requirements, making them highly susceptible to environmental disturbances, such as changes in vegetation structure, canopy cover, or hydrological conditions (Mushet et al., 2012). This sensitivity makes them important bioindicators within an ecosystem (Saber et al., 2017).

Despite their ecological importance and status as bioindicators, amphibian populations remain understudied in many tropical regions, particularly in the context of land-use changes. There is a pressing need to explore how different habitat types shape the richness and abundance of amphibian communities. Understanding these relationships is critical for designing conservation strategies that mitigate biodiversity loss in the Amazon and other biodiversity hotspots.

This study aims to address this gap by comparing amphibian communities across a banana plantation, a secondary forest, and a selectively logged primary forest in the Peruvian Amazon. Specifically, it seeks to answer the question: What differences in habitat structure and disturbance history influence the abundance and species richness of amphibians? By examining how varying levels of disturbance influence amphibian populations, this research contributes to understanding the implications of land-use practices on biodiversity.

1.1 Background

The research for my study was conducted at Kawsay Biological Station, situated in the Peruvian Amazon near the city of Puerto Maldonado. Kawsay Biological Station is dedicated to preserving the Amazon rainforest through activities aligned with conservation, such as ecotourism, environmental education, and scientific research. The station hosts various ongoing research projects, with one of the most prominent being their study of the endangered spider monkey. Researchers from around the world visit Kawsay Biological Station to study a wide range of species, contributing to the growing body of knowledge about the Amazon's rich biodiversity. This provides a supportive and dynamic environment for conducting research on amphibians and the factors that may be affecting their populations in the region.

1.2 Research question

What differences in habitat structure and disturbance history influence the abundance and species richness of amphibians?

1.2.1 Main objective

To examine how differences in habitat structure and disturbance history influence the abundance and species richness of amphibians.

1.2.2 Sub-objectives

- To determine whether specific amphibian species are more associated with particular habitat types or levels of disturbance.
- To evaluate the impact of observer bias on the observed trends in amphibian abundance and species richness.

2. Materials and Methods

2.1 Study area

The study area is located on the right bank of the Madre de Dios River in Peru. It is located in the buffer zone of the Tambopata National Reserve. The study area covers 178 hectares and includes four distinct habitat types (figure 1). The primary forest, which was impacted by selective logging until 2017. The secondary forest which is characterized by dense understories with a notable presence of bamboo. A banana plantation, previously used as farmland, and an anthropogenic zone, reflecting areas significantly influenced by human activities.

The region experiences distinct seasonal variation, with a rainy season typically spanning from November to April and a dry season from May to October. It receives an average annual precipitation of 2,459 mm, with a mean relative humidity of 83% and an average annual temperature of 24.9°C (SENAMHI, 2024). Based on the Holdridge life zone classification, the area is primarily characterized as a seasonally flooded subtropical wet forest, situated at an elevation of 150 to 250 meters above sea level (INRENA, 2003).

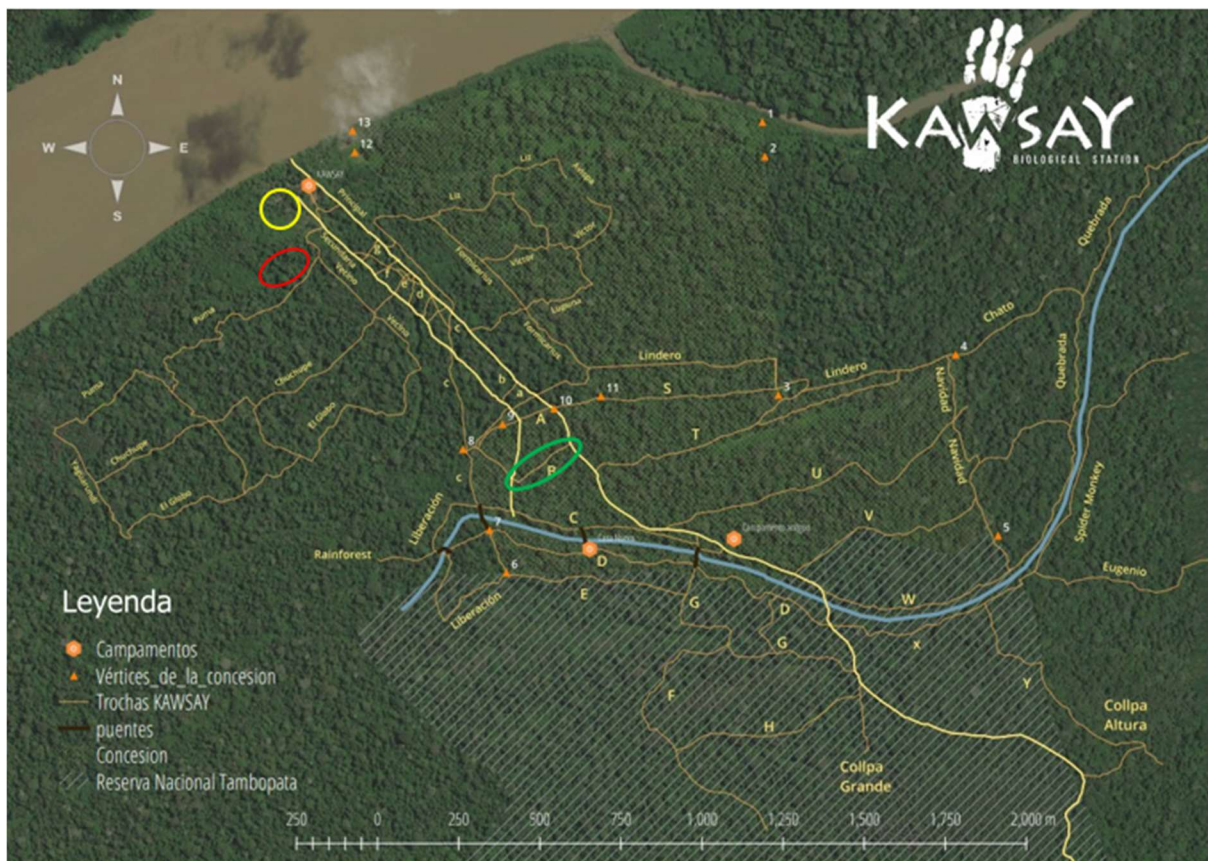


Figure 1. Map of the study area at Kawsay Biological Station, showing the locations of the banana plantation (yellow), secondary forest (red), and selectively logged primary forest (green). Transects (indicated by colored markers) were selected within each habitat type for data collection on amphibian abundance and species richness.

2.2 Project approach

This study examines how habitat disturbance levels affect amphibian populations in the Peruvian Amazon. The research population includes all amphibian species inhabiting three different habitat types: a banana plantation, a secondary forest, and a primary forest. By examining these areas, the study aims to understand how differences in habitat structure and disturbance history influence amphibian richness and abundance.

To gather data, visual encounter surveys were conducted at pre-determined transects within each habitat type. One transect was established in each habitat to account for spatial variability. Observations were carried out exclusively at night, as this is when most amphibian species are active and detectable.

2.3 Methodology

2.3.1 Data collection

To gain a clearer understanding of amphibian populations and the characteristics of the study area, I began with desk research. This involved reviewing relevant literature and past studies on amphibian populations, focusing on key findings related to habitat preferences, amphibian abundance, and distribution. The desk research included an analysis of various research methods used in similar studies, assessing their strengths and limitations, and identifying any gaps in the current knowledge that my research could address. This process also provided insights into amphibian species' responses to different environmental conditions, informing the design of my study.

The primary method for data collection in this study was visual encounter surveys (VES), a technique that allows for systematic observation and counting of amphibian species within selected habitats. Each survey involved walking a 200-meter transect at a slow pace to ensure thorough observation. During the surveys, the search area extended 2 meters on either side of the transect line. Surveys were conducted daily between 21:00 and 23:00, coinciding with peak amphibian activity periods to maximize encounter rates (Barata, 2018). By gathering data on both amphibian abundance and species richness, I conducted statistical analyses to assess how different habitat conditions influence amphibian communities.

These analyses provide valuable insights into the relationship between environmental disturbances and amphibian populations, helping to reveal important patterns. This knowledge is essential in shaping conservation strategies and promoting the protection of amphibian habitats. Ultimately, the findings from this research contribute to a broader understanding of how habitat disturbance influences amphibian populations and support efforts to preserve biodiversity.

2.3.2 Habitat characteristics

These distinct habitats provide a range of disturbance levels, allowing for comparative analysis of their effects on amphibian communities. These habitat types were identified through visual observation and personal communication with local experts and staff at the study site.

Banana plantation

In agricultural areas such as banana plantations, the vegetation structure is typically simplified, with low diversity and limited canopy cover. According to Luz et al. (2021), plantations often replace diverse native vegetation with monocultures, leading to a less complex ecosystem with reduced habitat for wildlife. The structure is dominated by banana plants, with minimal vertical layering.

Secondary forest

Secondary forests are characterized by their dense understories and an increased light penetration due to the partial canopy cover. Zhu (2002) describes secondary forests as having more light availability compared to primary forests, which facilitates the growth of understory vegetation, such as shrubs and young trees. The regeneration of native vegetation is often slower, but these forests still support a relatively high level of biodiversity.

Primary forest

Primary forests generally have a stable environment, with a more complex vertical structure and high canopy cover. Laurance et al. (2002) note that primary forests maintain multiple layers of vegetation, which supports a diverse range of species. Despite historical disturbances like selective logging, these forests still preserve the conditions necessary for a rich biodiversity, including intact microhabitats.

2.3.3 Observer bias

Each night, multiple observers surveyed the same transect. They walked together along the transect but independently observe and record amphibians they encounter. If one observer consistently finds more amphibians than others, this may indicate bias—either because they are more skilled at spotting amphibians, have a better search strategy, or are more familiar with the survey area.

2.3.4 Level of disturbance

To measure levels of disturbance in the three habitat types several indicators and methods based on existing literature and observable ecological features were used.

Banana plantation

The plantation is highly disturbed, with a clear history of human land use for agriculture. The lack of natural vegetation, the presence of monocultures, and the absence of species diversity in comparison to natural forests are key indicators of high disturbance.

Secondary forest

Secondary forests have a moderate level of disturbance as they recover from past land-use activities such as logging or agriculture. Presence of early successional species (e.g., shrubs, saplings) are key signs of disturbance.

Primary forest

Primary forests typically have low disturbance, even though it has been affected by selective logging, which is less frequent in recent years. Old-growth trees, complex vertical structure, and continuous canopy cover with minimal gaps or logging scars indicate low disturbance.

2.4 Data analysis

Microsoft Excel® (2007) was utilized for organizing, storing, and processing field data, while RStudio and SPSS were used for statistical analysis.

2.4.1 Disturbance levels

To compare amphibian abundance across locations with different levels of disturbance, the dataset was structured as follows:

- **Dependent Variable:** Amphibian Abundance (*Scale*) – The number of amphibians recorded per survey.
- **Independent Variable:** Disturbance Level (*Nominal*) – Categorical variable with three levels: primary forest, secondary forest, and banana plantation.

Prior to analysis, data were checked for missing values and outliers. Surveys where no amphibians were recorded were included to ensure an accurate representation of detection probability across locations. Since the amphibian abundance data were count-based and likely non-normally distributed, a Kruskal-Wallis test was chosen.

To assess differences in amphibian abundance across habitat types, pairwise post-hoc tests were conducted since the Kruskal-Wallis test indicated a significant effect of disturbance level. The amphibian abundance data, measured as the number of individuals recorded per survey (scale variable), was compared across the three disturbance levels: primary forest, secondary forest, and banana plantation (nominal categorical variable).

Since the Kruskal-Wallis test only determines whether a difference exists among groups without specifying which groups differ, pairwise comparisons were performed between each combination of habitat types: primary forest vs. secondary forest, primary forest vs. banana plantation, and secondary forest vs. banana plantation.

2.4.2 Regression Analysis

To assess the relationship between environmental factors (temperature, humidity, and rainfall) and amphibian species richness and total abundance, a multiple linear regression analysis was

conducted. Before performing the analysis, data preparation steps were undertaken to ensure the reliability of the results.

Species richness was defined as the number of unique amphibian species recorded per survey, while total abundance was the total number of individuals observed. The independent variables included temperature (measured in degrees Celsius), humidity (expressed as a percentage), and rainfall, which was recorded as a binary variable (0 = no rainfall, 1 = rainfall present). Data were checked for missing values and outliers, and any inconsistencies were addressed before further analysis.

To meet the assumptions of multiple linear regression, normality, homoscedasticity, and linearity of residuals were assessed using diagnostic plots. Multicollinearity was evaluated using the Variance Inflation Factor (VIF), and all predictors were within acceptable limits, indicating low collinearity. Since rainfall was a categorical variable, it was dummy-coded to integrate it into the model appropriately. The final dataset was standardized where necessary to ensure consistency and comparability across variables. Following data preparation, two separate multiple linear regression models were conducted: one for species richness and another for total abundance.

2.4.3 Scatterplot and regression line

To examine the relationship between species richness and total abundance, a Spearman correlation analysis was conducted. This test is appropriate for assessing monotonic relationships without assuming a normal distribution of the data. Species richness was defined as the number of unique amphibian species recorded per survey, while total abundance represented the total number of individual amphibians observed. Both variables were measured on a numeric scale and classified as continuous data.

Before analysis, data preparation steps were performed to ensure accuracy. The dataset was checked for missing values and outliers that could influence the correlation results. Given that Spearman's correlation is a rank-based test, no transformations were necessary. However, the data were inspected for tied ranks. A scatterplot was generated to visually assess the relationship between species richness and total abundance before performing the correlation test.

Once the data were prepared, Spearman's rank correlation coefficient was calculated to determine the strength and direction of the association between species richness and total abundance. This analysis helps evaluate whether habitats with a higher number of species also tend to support larger amphibian populations.

2.4.4 Observer bias

To assess whether observer presence significantly influenced the number of amphibians detected, a generalized linear model (GLM) with a Poisson distribution was conducted. The dependent variable was the number of amphibians found by each observer, while the independent variable was the total number of surveys each observer participated in. The dataset was prepared and analyzed in SPSS using a structured approach. The key variables included: observer (Nominal), people present (String), surveys attended (Scale), amphibians found (Scale). To quantify observer effort, the "people present" column was transformed into a numeric variable ("surveys attended") by counting how often each individual appeared in the dataset. The "amphibians found" variable was computed by summing the number of detections recorded by each observer. This model evaluates whether the number of frogs detected by an individual is significantly associated with their survey participation frequency, accounting for variations in detection effort. The Poisson distribution is appropriate given that amphibian detections represent count data, and the model was tested for overdispersion.

2.4.5 Species accumulation curve

A species accumulation curve was made to assess how species richness increased with additional survey effort. Before analysis, data preparation steps were undertaken to ensure accuracy and consistency. Species richness was treated as a numeric variable on a count scale, representing the number of unique species recorded per survey night. Total abundance was also treated as a numeric variable on a continuous scale, representing the total number of individuals observed. The survey night was recorded as an ordinal variable, as it represented the chronological order in which samples were collected. Data were checked for completeness, and no missing values were identified. To construct the species accumulation curve, species richness was plotted cumulatively against sampling effort, measured as the number of survey nights.

3. Results

Between September 2024 and November 2024, a total of 82 individuals representing 24 species were recorded across the three sites during 51 hours of active searching conducted on different nights. A total of 7 different observers have been engaged in data acquisition. At the banana plantation, a total of 12 individuals from 7 species were observed over 6 nights of active searching, with an average of 2.0 ± 1.24 individuals per night. In the secondary forest, a total of 23 individuals from 8 species were recorded over 6 nights of active searching, with an average of 3.83 ± 1.49 individuals per night. In the primary forest, a total of 49 individuals from 18 species were recorded over 6 nights of active searching, with an average of 8.17 ± 0.83 individuals per night. The most frequently observed species across all sites during the survey period was *Leptodactylus bolivianus*, with 12 recorded observations (Table 1). Further analysis of the relative abundance patterns (Appendix II) revealed that *Scinax pedromedinae* was dominant in the primary forest, contributing 18% to the observations in this habitat. In contrast, *Adenomera cf. andreae* was primarily recorded in the secondary forest, comprising 32% of observations there.

Table 1. Categorization of the observed amphibian species by family and presents their abundance across three locations: primary forest, banana plantation, and secondary forest. The data highlights variations in species distribution, with the family Hylidae being the most represented, while *Leptodactylus bolivianus* was the most frequently observed species overall.

Family	Species	Banana plantation	Secondary forest	Primary forest
Hylidae	<i>Chiasmocleis</i>	0	0	2
	<i>Hyla microdema</i>	0	1	0
	<i>Boana fasciata complex</i>	1	0	6
	<i>Trachycephalus typhonius</i>	0	0	1
	<i>Boana cinerascens</i>	1	0	1
	<i>Dendropsophus leucophyllatus</i>	0	0	1
	<i>Scinax pedromedinae</i>	0	0	9
	<i>Scinax garbei</i>	1	0	2
	<i>Boana punctata</i>	0	0	1
	<i>Scinax funereus</i>	1	0	2
	<i>Scinax icterius</i>	0	0	1
	Phyllomedusidae	<i>Phyllomedusa camba</i>	0	1
<i>Phyllomedusidae tomopterna</i>		0	0	8
Leptodactylidae	<i>Leptodactylus bolivianus</i>	5	5	4
	<i>Leptodactylus sp.</i>	0	3	0
	<i>Adenomera hylaedactyla</i>	1	1	0
	<i>Adenomera cf. andreae</i>	0	7	1
Bufonidae	<i>Hamptophryne boliviana</i>	2	4	2
Dendrobatidae	<i>Colostethus marchsianus</i>	0	1	0
Hempiphractidae	<i>Osteocephalus taurinus</i>	0	0	1
Brachycephalidae	<i>Edalorhina perezii</i>	0	0	3
Hylid	<i>Scarthyla goinorum</i>	0	0	2
	Total	12	23	49

3.1 Richness- Abundance relationship

A Kruskal-Wallis test was performed to compare the abundance of amphibians across locations with different levels of disturbance. The analysis revealed a statistically significant difference in amphibian abundance among the locations, $H(2)=7,39$, $p=0.0025$. This finding indicates that the level of disturbance, as represented by the different location types, significantly impacts amphibian abundance.

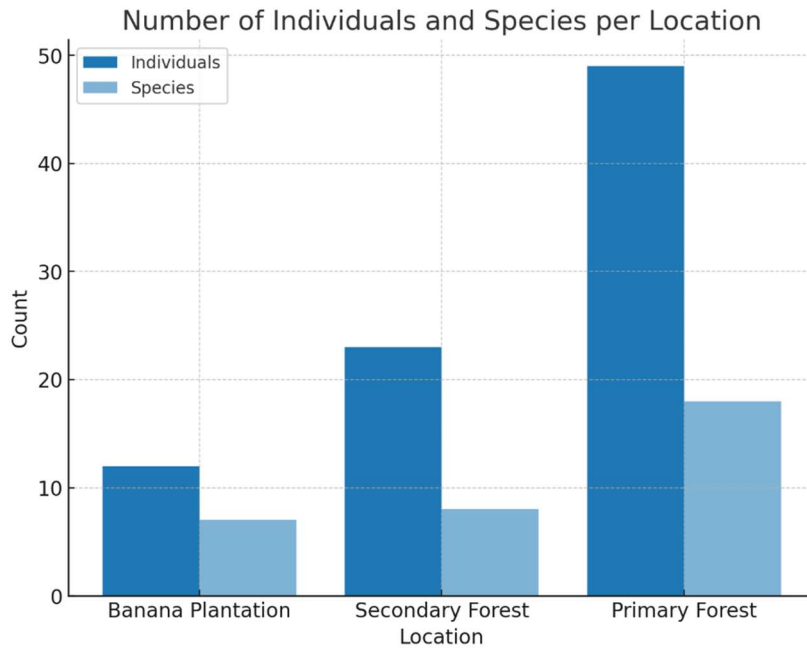


Figure 2. Graph illustrates the number of individuals and species of amphibians observed across three location types: banana plantation, primary forest, and secondary forest. The graph shows higher counts of individuals and species in less disturbed locations. This visualization supports the results of a Kruskal-Wallis test, which found significant differences in amphibian abundance.

Pairwise Comparisons

Post hoc pairwise comparisons using the Mann-Whitney U test provided further insights into these differences:

- Primary forest vs. Banana plantation: A significant difference in amphibian abundance was observed ($U = 3.5$, $p = 0.022$).
- Primary forest vs. Secondary forest: A marginal significant difference in amphibian abundance was observed ($U = 30.5$, $p = 0.051$).
- Banana plantation vs. Secondary forest: No significant difference in amphibian abundance was found ($U = 11.0$, $p = 0.280$).

3.2 Regression Analysis

To assess the relationship between environmental factors (temperature, humidity, and rainfall) and amphibian species richness and total abundance, a multiple linear regression analysis was conducted.

Species Richness

A multiple linear regression model was used to assess the influence of environmental factors (temperature, humidity, and rainfall) on species richness (See appendix II). The model explained only 7.4% of the variation in species richness, as indicated by an R^2 value of 0.074. None of the predictors showed statistically significant relationships with species richness (all p-values > 0.05), suggesting that these environmental variables do not substantially contribute to explaining the variation in species richness in the dataset.

Total Abundance

A similar multiple linear regression model was applied to evaluate the effects of temperature, humidity, and rainfall on total abundance (See appendix III).. The model explained only 5.3% of the variation in abundance, as reflected by an R^2 value of 0.053. Again, none of the predictors were statistically significant (all p-values > 0.05). These results suggest that temperature, humidity, and rainfall do not strongly influence amphibian abundance in the observed location.

The regression analysis results indicate that environmental variables (temperature, humidity, and rainfall) do not significantly explain the variation in species richness and total abundance in the dataset. This suggests that factors such as habitat characteristics (e.g., levels of perturbation) may have a more substantial impact on amphibian richness and abundance.

3.3 Scatterplot and regression line

A scatterplot of species richness (number of unique species) versus total abundance (number of individuals) revealed a strong positive correlation (Figure 3). As total amphibian abundance increased, species richness also tended to increase. The linear trend, represented by the red regression line, showed a clear upward pattern with a confidence interval (shaded in red), indicating the reliability of the observed relationship.

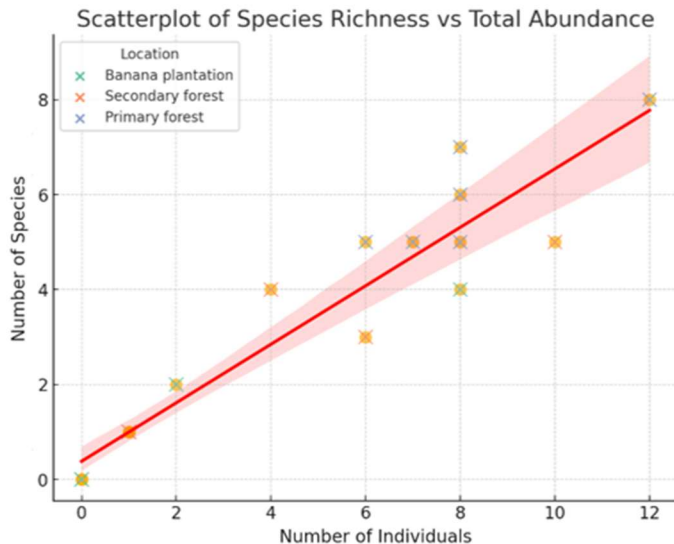


Figure 3. The scatterplot shows the relationship between species richness (number of unique species) and total abundance (number of individuals) across three locations: banana plantation, primary forest, and secondary forest. The line represents the trend, while the shaded region shows the confidence interval.

A Spearman correlation analysis was conducted to assess the relationship between species richness (number of unique species) and total abundance (number of individuals). The results revealed a statistically significant positive correlation (Spearman's $\rho = 0.952$, $p < 0.001$).

3.4 Observer bias

To assess whether there were significant differences in the number of amphibians detected based on observer presence, a generalized linear model (GLM) with a Poisson distribution was conducted. This approach accounts for the count-based nature of the data and models the relationship between survey presence and frog detections.

- **Estimate (β):** 0.038
- **Standard Error (SE):** 0.003
- **z-value:** 12.08
- **p-value:** < 0.001

The analysis revealed a statistically significant relationship between observer presence and the number of frogs detected ($p < 0.05$).

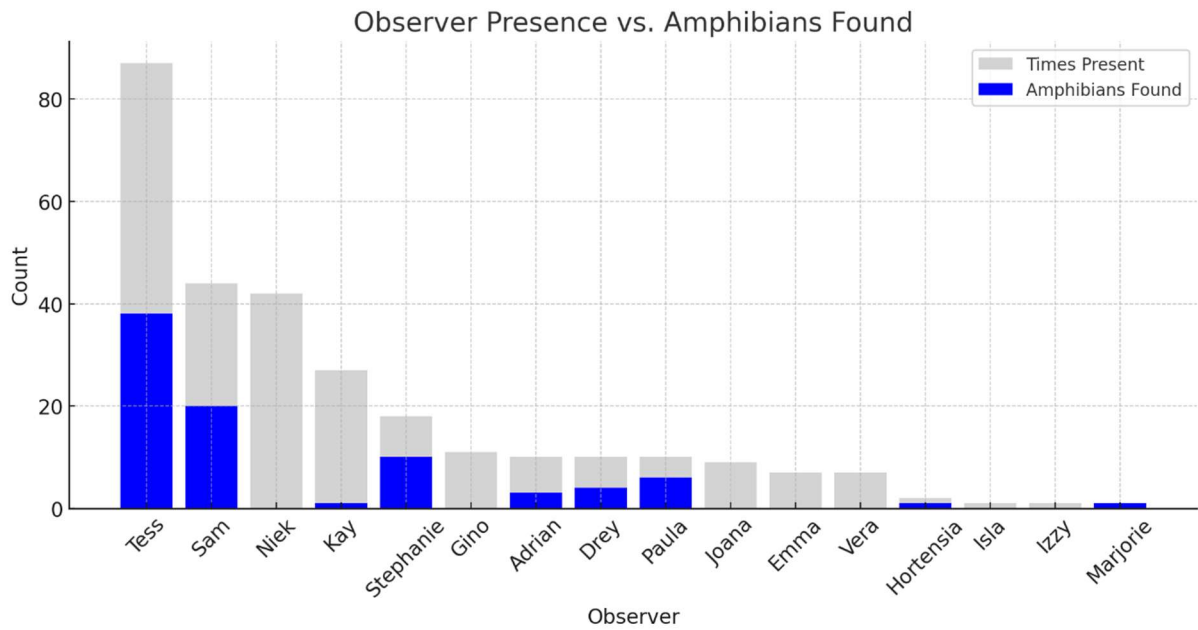


Figure 4. The bar chart displays the number of times each observer was present during surveys (light gray) compared to the number of amphibians they personally detected (blue). This visualization highlights differences in detection rates among observers.

3.4 Species accumulation curve

To examine the rate at which new species were encountered over time, a species accumulation curve was generated. The cumulative number of species increased over the initial sampling nights, with a steep rise in richness before the curve began to plateau. After approximately 14 sampling nights, the accumulation of new species slowed. The final number of species recorded remained stable at 22 species.

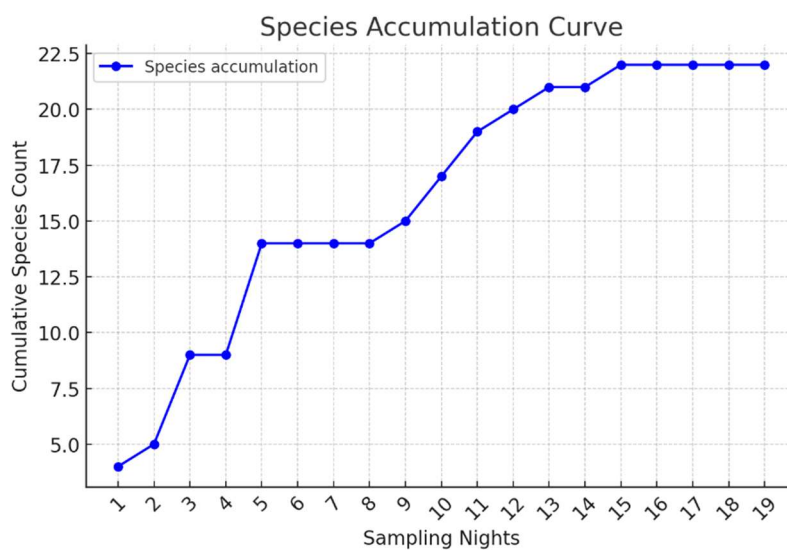


Figure 5. Species accumulation curve showing the cumulative number of species recorded over the course of 18 sampling nights. The curve illustrates how species richness increased with additional survey effort until it reached an asymptote.

4. Methods discussion

The study aimed to investigate how habitat structure and disturbance history influence amphibian abundance and species richness. To achieve this, visual encounter surveys were conducted in three habitats with different levels of disturbance: a banana plantation (highly disturbed), a secondary forest (moderately disturbed), and a primary forest (minimally disturbed). Additionally, it examined observer bias as a potential factor influencing detection rates. The findings provide insights into the dynamics of amphibian communities in different habitats while underscoring methodological challenges that affect interpretation.

Visual Encounter Survey (VES) as a Method

The VES method was chosen for its practicality and effectiveness in tropical environments, particularly for detecting nocturnal species in diverse habitats. It proved reliable for systematically sampling amphibians across habitats with varying structural and ecological characteristics, aligning with Crump and Scott (1994), who emphasized its effectiveness in rainforest environments. Additionally, the adaptability of VES enabled fieldworkers to navigate both open and dense vegetation, ensuring thorough coverage within practical limitations.

However, the findings challenge certain assumptions about habitat openness and detectability. Despite the banana plantation's relatively open structure, amphibian abundance was lower in this habitat compared to the secondary and primary forests. This result diverges from expectations based on visibility advantages and may reflect other ecological factors. One possibility is that the banana plantation lacks critical microhabitats, such as leaf litter, fallen logs, and understory vegetation, which are essential for amphibians (Whitfield et al., 2007a). The absence of these resources likely outweighs the potential detectability benefits of the plantation's open structure.

Additionally, the characteristics of the banana plantation—regular human activity, altered microclimate, and uniform vegetation—may not provide the stability and ecological niches necessary for diverse amphibian communities. Gardner et al. (2007) observed that simplified habitats often support fewer amphibians due to a reduction in available shelter, prey resources, and suitable breeding sites. These factors may explain the relatively low abundance recorded in the banana plantation.

In contrast, the higher abundance observed in the secondary and primary forests aligns with previous studies showing that forested habitats provide critical refuges for amphibians (Lips et al., 2003). Both forests offer a variety of microhabitats and stable conditions conducive to amphibian survival, though the primary forest's greater structural complexity may explain its higher species richness. This supports findings by Whitfield et al. (2007a), who demonstrated that mature forests often support greater amphibian diversity due to their complexity and stable microclimates.

The reliance of VES on visual detection also likely influenced species richness estimates. Cryptic or fossorial species, such as *Adenomera hylaedactyla*, which are less visually conspicuous (Hending et al., 2024), may have been underrepresented in this study. This is particularly true in the primary forest, where dense vegetation provides abundant refuges. As noted by Dorcas and Willson (2009), this bias toward visually prominent species, such as *Phyllomedusa tomopterna*, is a common limitation of VES. This issue could be mitigated in future research by integrating complementary methods such as acoustic monitoring or pitfall traps.

Temporal and Spatial Constraints

The study's temporal limitation, confined to the dry season, likely influenced the results by underrepresenting species that are more active during the wet season. Amphibians in tropical ecosystems often exhibit seasonal activity patterns, with increased abundance and detectability during the wet season when breeding activity peaks (Whitfield et al., 2007b). Similar patterns were observed by Lips et al. (2003), who found significantly higher amphibian abundance and diversity during wetter months in tropical regions. Consequently, the findings of this study should be interpreted as a reflection of dry-season dynamics rather than a comprehensive representation of annual patterns.

The restricted spatial scope, with two transects per habitat, further constrained the ability to capture habitat heterogeneity. Bell and Donnelly (2006) emphasize the importance of spatial replication in tropical forest studies to account for variability in microhabitats, which can significantly influence species distributions. By comparison, this study's limited transects may have overlooked fine-scale habitat differences, particularly in the primary and secondary forests, where structural diversity is high. Expanding spatial coverage in future studies would improve the representativeness of habitat comparisons and yield more comprehensive insights into amphibian distribution patterns.

Observer Bias and Detection Challenges

Observer bias, an inherent limitation of the VES method, likely influenced detection rates in this study. Differences in observer experience, attention, and fatigue can significantly affect amphibian detectability, as documented by Kéry and Schmidt (2008), who observed up to 50% variation in detection rates based on observer expertise. While the use of multiple observers in this study reduced the potential for individual bias, variability in skill levels may still have affected detection consistency. This effect may have been more evident in structurally complex habitats such as the secondary and primary forests.

Studies by Kéry and Schmidt (2008) highlight the importance of addressing this source of bias. Incorporating training sessions or calibration exercises to standardize observer performance could reduce variability and enhance data reliability. Additionally, adopting double-observer methods, where two observers independently assess the same transects, could further minimize bias and improve detection consistency across habitats.

Comparison with Previous Studies

When compared to other research, this study aligns with and diverges from established findings in meaningful ways. The lower amphibian abundance in the banana plantation aligns with Gardner et al. (2007), who documented reduced amphibian diversity and abundance in simplified habitats. This finding reinforces the conclusion that habitat simplification and altered microclimatic conditions negatively impact amphibian communities.

The higher species richness observed in the primary forest compared to the secondary forest mirrors findings by Whitfield et al. (2007a), who demonstrated that mature forests often harbor greater amphibian diversity due to their complexity and stable microclimatic conditions. However, the observed differences in abundance and richness between the secondary and primary forests could have been influenced by the limited number of transects, as larger-scale studies such as Lips et al. (2003) emphasize the importance of spatial replication in capturing habitat variability.

This study's methodological approach also contrasts with multi-method approaches employed by Grant et al. (1992) and Dorcas and Willson (2009), which combined VES with other techniques like acoustic surveys and pitfall traps. While VES alone provided valuable insights, the addition of complementary methods could have enhanced the detection of cryptic and fossorial species, providing a more comprehensive picture of amphibian communities.

5. Results discussion

The findings of this study provide valuable insights into how varying levels of habitat disturbance influence amphibian richness and abundance in the Peruvian Amazon. Amphibian abundance and species richness differed significantly across the three habitats, with the highest values observed in the primary forest, followed by the secondary forest and the banana plantation. These results align with prior studies (e.g., Gardner et al., 2007; Whitfield et al., 2007a) emphasize the importance of habitat complexity and stability in supporting amphibian communities.

Habitat-Specific Trends

The banana plantation exhibited the lowest species richness and abundance. This habitat is characterized by simplified vegetation structure, reduced canopy cover, and frequent human activity, all of which create unfavorable conditions for many amphibian species (Stuart et al., 2004). Similar findings have been documented in agricultural landscapes where monocultures replace diverse native vegetation (Luz et al., 2021). The absence of critical microhabitats such as leaf litter and understory vegetation likely limits the ecological niches available for amphibians, explaining their reduced presence. Species found exclusively in the banana plantation, such as *Adenomera hylaedactyla*, may have physiological or behavioral adaptations allowing them to tolerate such disturbed conditions (Sanchez et al., 2019). These species might exploit the reduced competition or specific microhabitats within the plantation, a phenomenon also noted in Nowakowski et al. (2018). Furthermore, post hoc pairwise comparisons confirmed that amphibian abundance in the banana plantation was significantly lower than in the primary forest but showed no significant difference compared to the secondary forest. This result suggests that while amphibians can persist in the plantation, their populations are constrained by the habitat's limitations.

The secondary forest, with intermediate disturbance levels, supported a higher abundance and species richness than the plantation but lower than the primary forest. Dense understory vegetation and moderate canopy cover create a mix of microhabitats, supporting the establishment of a diverse range of species (Haggerty et al., 2019). This habitat's recovery from past agricultural use may explain the presence of some disturbance-tolerant species, alongside those typically associated with more stable environments. Such findings are consistent with studies highlighting secondary forests as transitional ecosystems that support diverse communities (Zhu, 2002).

The primary forest supported the highest species richness and abundance, highlighting the ecological value of minimally disturbed habitats. The complex vertical structure and stable microclimatic conditions in this habitat provide abundant resources and refugia for amphibians, a pattern supported by Whitfield et al. (2007a). However, selective logging until 2017 might have contributed to microhabitat alterations, potentially affecting the distribution of certain species (Barton & Smith, 2010).

Influence of Environmental Adaptability

Certain species' distributions suggest adaptability to specific environmental factors. For example, *Leptodactylus bolivianus* was found across all habitats, reflecting its generalist nature and ability to exploit a variety of ecological niches (Bernardo et al., 2011). In contrast, *Chiasmocleis* and *Phyllomedusidae tomopterna* were restricted to the primary forest, indicating their reliance on stable and undisturbed habitats. This specialization makes such species particularly vulnerable to habitat alteration, as noted by Davies et al. (2004).

Comparison with Existing Literature

This study's findings align with those of Gardner et al. (2007), who observed reduced amphibian diversity in disturbed habitats, and Whitfield et al. (2007a), who highlighted the biodiversity benefits of mature forests. However, the observed differences in amphibian abundance between secondary and primary forests suggest that microhabitat variability and disturbance history play critical roles, as emphasized by Lips et al. (2003).

Notably, while this study used Visual Encounter Surveys (VES) as the primary method, other studies incorporating acoustic surveys or pitfall traps (e.g., Dorcas & Willson, 2009) have reported higher species richness by detecting cryptic or fossorial species. This methodological limitation should be addressed in future research to provide a more comprehensive understanding of amphibian communities.

Implications for Conservation

The results underscore the importance of conserving primary and secondary forests to maintain amphibian diversity in the Amazon. Protecting primary forests ensures the survival of habitat specialists, while secondary forests play a critical role as refuges and transitional habitats. Efforts to restore degraded areas, such as plantations, by reintroducing native vegetation and reducing human disturbances, could enhance their ecological value for amphibians (Decena et al., 2020).

By comparing amphibian communities across a disturbance gradient, this study contributes to a broader understanding of how land-use changes influence biodiversity, providing a foundation for targeted conservation strategies in tropical ecosystems.

6. Conclusion

This study examined the impact of habitat structure and disturbance history on the richness and abundance of amphibians in the Peruvian Amazon. The findings revealed that minimally disturbed habitats, such as primary forests, supported the highest amphibian species richness and abundance due to their complex vegetation and stable microhabitats. Secondary forests, with intermediate disturbance, provided moderate support for amphibian communities, while highly disturbed banana plantations exhibited the lowest richness and abundance, likely due to their simplified structure and limited resources.

A strong positive relationship between species richness and abundance was identified, emphasizing the importance of diverse habitats for sustaining larger amphibian populations. However, observer bias significantly influenced detection rates, highlighting the need for methodological improvements in future studies. These results stress the critical role of conserving primary forests and rehabilitating disturbed habitats to protect amphibian diversity and maintain ecological balance in the Amazon.

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Appendix I: Examples of Frog Species Observed During Fieldwork



Figure 1. Black-eyed Monkey Frog (*Phyllomedusa camba*), observed in the secondary forest resting on a leaf. This species was photographed during a nocturnal survey conducted on 27-09-2024



Figure 2. Moyobamba Snouted Treefrog (*Scinax funereus*), observed in the banana plantation. This species was photographed during a nocturnal survey conducted on 07-11-2024



Figure 3. Polka-dot Treefrog (*Boana punctata*), observed in the primary forest. This species was photographed during a nocturnal survey conducted on 27-09-2024

Appendix II: Multiple linear regression model species richness

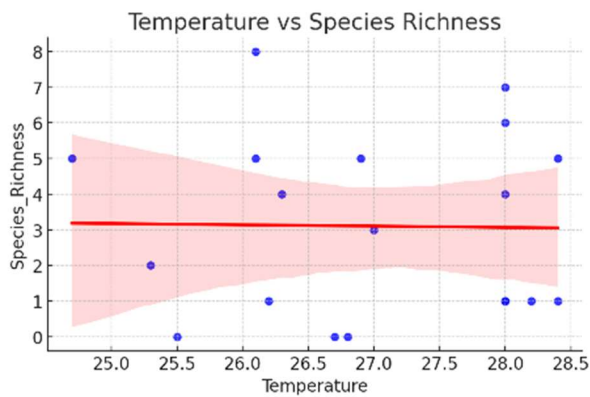


Figure 6. Scatter plot of the relationship between temperature and species richness. The red trend line represents the overall trend, with the shaded red area indicating the confidence interval.

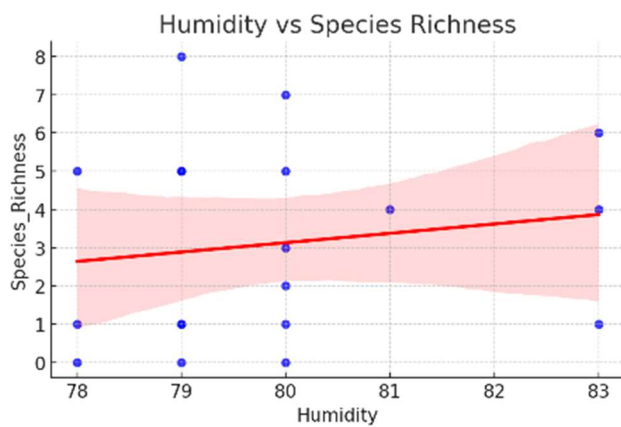


Figure 7. Scatter plot of humidity on the x-axis and species richness on the y-axis. The data points are marked in blue, and a red trend line with a shaded confidence interval is included.

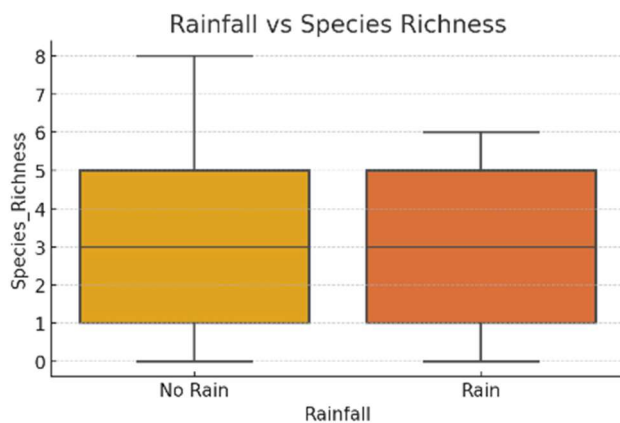


Figure 8. Box plot comparing species richness under two conditions: "No Rain" and "Rain." Each box represents the interquartile range, with a horizontal line indicating the median value. Vertical lines extend from the boxes to show the overall range of data.

Appendix III: Multiple linear regression model total abundance

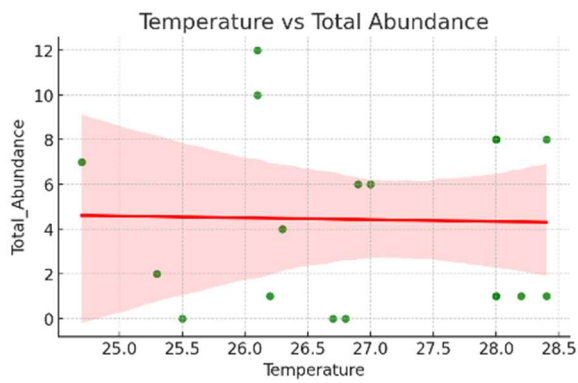


Figure 9. Scatter plot with temperature on the x-axis and total abundance on the y-axis. The data points are green, with a red trend line and a shaded confidence interval.

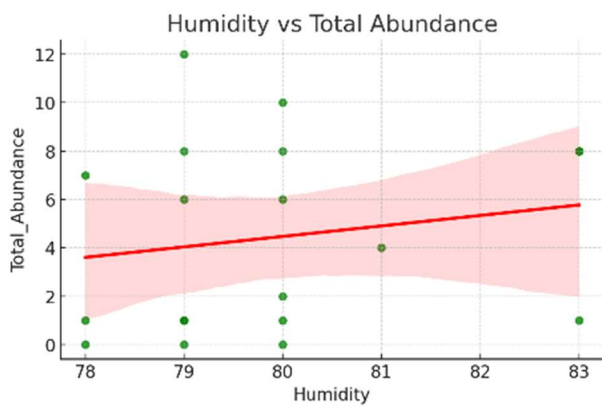


Figure 10. Scatter plot with humidity on the x-axis and total abundance on the y-axis. Green dots represent individual data points, while a red trend line is present along with a shaded confidence interval.

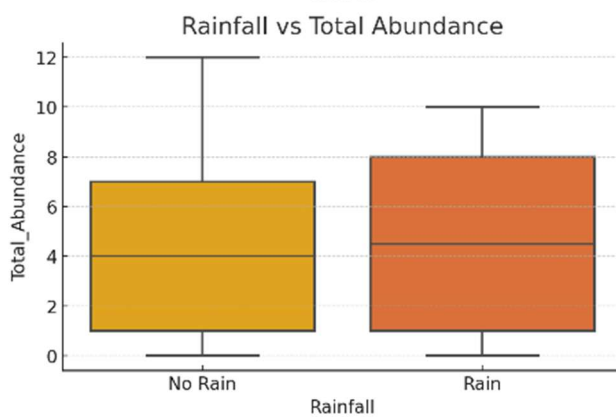


Figure 11. Box plot comparing total abundance between "No Rain" and "Rain" conditions. Each box represents the interquartile range, with a horizontal line marking the median. Vertical lines extend from the boxes to indicate the full range of values.

Appendix IV: Relative Abundance of Amphibians Across Habitats

Table 1. The relative abundance of amphibians recorded at three different habitat types: a banana plantation, a secondary forest, and a primary forest. Relative abundance is expressed as the percentage of individuals observed for each species in each habitat, calculated based on the total number of individuals recorded across all sites.

Family	Species	Banana plantation %	Secondary forest %	Primary forest %	
Hylidae	<i>Chiasmocleis</i>	4.08	0.00	0.00	
	<i>Hyla microdema</i>	0.00	0.00	4.55	
	<i>Boana fasciata complex</i>	12.24	10.00	0.00	
	<i>Trachycephalus typhonius</i>	2.04	0.00	0.00	
	<i>Boana cinerascens</i>	2.04	0.00	0.00	
	<i>Dendropsophus leucophyllatus</i>	2.04	0.00	0.00	
	<i>Scinax pedromedinae</i>	18.37	0.00	0.00	
	<i>Scinax garbei</i>	4.08	10.00	0.00	
	<i>Boana punctata</i>	2.04	0.00	0.00	
	<i>Scinax funereus</i>	4.08	10.00	0.00	
	<i>Scinax icterius</i>	2.04	0.00	0.00	
	Phyllomedusidae	<i>Phyllomedusa camba</i>	4.08	0.00	4.55
		<i>Phyllomedusidae tomopterna</i>	16.33	0.00	0.00
	Leptodactylidae	<i>Leptodactylus bolivianus</i>	8.16	40.00	18.18
<i>Leptodactylus sp.</i>		0.00	0.00	13.64	
<i>Adenomera hylaedactyla</i>		0.00	10.00	4.55	
<i>Adenomera cf. andreae</i>		2.04	0.00	31.82	
Bufonidae	<i>Hamptophryne boliviana</i>	4.08	20.00	18.18	
Dendrobatidae	<i>Colostethus marchsianus</i>	0.00	0.00	4.55	
Hemiphractidae	<i>Osteocephalus taurinus</i>	2.04	0.00	0.00	
Brachycephalidae	<i>Edalorhina perezii</i>	6.12	0.00	0.00	
Hylid	<i>Scarthyla goinorum</i>	4.08	0.00	0.00	
	Total	100%	100%	100%	