

Factors Associated with Distribution of Leishmaniasis

Disease and Vectors in Madre de Dios, Peru

by

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Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
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2015

ABSTRACT

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Abstract

While the Madre de Dios department of Peru has the nation's highest leishmaniasis incidence, limited research on the disease and its sand fly vector exists in the region. Considering the diverse and adaptive nature of sand flies, understanding vector ecology is essential to directing leishmaniasis control efforts. This study addresses this need by investigating the following aims: **1) describe sand fly abundance and biodiversity in communities along the Madre de Dios River; 2) identify environmental and socio-demographic factors associated with sand fly abundance and biodiversity in these communities; and 3) examine ecological risk factors associated with leishmaniasis in the communities.** In order to accomplish these aims, the research team collected sand flies, obtained leishmaniasis case data, conducted community and household surveys, measured soil samples and studied land cover data in communities along the Madre de Dios River. We compared sand fly abundance, sand fly biodiversity and leishmaniasis incidence with soil chemistry, socio-demographic and land cover measures. We ran negative binomial regressions and Poisson regressions to investigate bivariate relationships between dependent and independent variables. We developed a multivariate model that predicts greater potential vector and non-vector abundance in areas with greater forest coverage, lower infrastructure indices and lower soil pH. This study provides an important overview of endemic sand fly fauna in Madre

de Dios and presents an initial description of factors associated with sand fly populations in the region.

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1. Introduction

1.1 *Leishmaniasis*

Leishmaniasis is a neglected tropical disease caused by parasites of the genus *Leishmania* and transmitted by phlebotomine sand flies. The World Health Organization reports that leishmaniasis is endemic in 98 countries and has an incidence of 2 million new cases per year.¹ In Peru there were 6,890 cases reported in 2013, making the national incidence 22.6 cases per 100,000 people.^{2,3} The department of Madre de Dios accounted for 14.8% of those cases, with an incidence of 77.9 cases per 100,000 people.^{2,3} However, poor infrastructure in endemic areas result in underreporting, while a lack of widely available health care makes tracking the site of infection difficult.⁴

As leishmaniasis is a vector-borne disease, the principal risk factors are those that affect an individual's exposure to the vector. These risk factors include socio-demographic factors such as urbanization, poor housing, occupation, time spent outdoors and travel to endemic areas, as well as environmental factors such as rainfall, humidity, temperature, elevation, deforestation and proximity to forests.^{5, 6, 7, 8, 9, 10, 11, 12, 13, 14} Since many of these factors are commonly associated with lower-resource settings, leishmaniasis disproportionately affects people living in poverty.^{7, 15}

Symptoms of leishmaniasis range from skin lesions at the site of infection to deterioration of the nasal cavity to systemic infections and organ failure. The incubation period, clinical manifestation, severity and response to treatment largely depend on the

parasite species and the host immune response.^{16,17} There are about 20 *Leishmania* species that are known to infect humans, and at least six different species have been isolated from humans in Peru (*Leishmania braziliensis*, *L. guyanensis*, *L. lainsoni* and *L. shawi* in Madre de Dios as well as *L. amazonensis* and *L. peruviana* in other parts of Peru).^{11, 18, 19, 20} Different *Leishmania* species are transmitted by different species of vectors and are therefore endemic to different areas. For this reason, risk factors may vary by region.^{8, 9, 11}

1.2 Phlebotomine sand flies

Leishmaniasis is transmitted by the bite of the female phlebotomine sand fly. Sand flies lay their eggs in soil and organic matter.¹¹ While some sand flies have been recording traveling up to 2000 m, the vast majority of sand flies remain within several hundred meters from their breeding site for their entire life.^{11, 21, 22, 23, 24}

While over 800 species of phlebotomine sand fly have been identified, only 98 are known to be capable of transmitting leishmaniasis.^{11, 16} There are 56 suspected vector species for leishmaniasis in the New World, primarily coming from the genus *Lutzomyia*.¹¹ At least 39 different sand fly species have been identified in Madre de Dios, including 22 that are considered vectors for leishmaniasis in other regions.^{11, 20, 25}

Previous studies have shown a wide range of environmental factors that shape the distribution and predominance of sand fly species. In general, rainfall, humidity, temperature, elevation, soil composition, vegetation and seasonality have all been identified as factors that influence sand fly populations in various regions.^{8, 12, 13, 26, 27, 28, 29,}

^{30, 31} Additionally, anthropogenic factors can affect the distribution of sand fly species by favoring species that can better adapt to habitat changes. For example, urbanization, deforestation and land use can alter the distribution of sand fly species.^{9, 11, 27, 32, 33}

Like the parasite they spread, sand flies are very diverse and highly adaptive, with different species occupying a wide range of ecological niches. For example, Singh et al. (2008) reported the different breeding preferences of two sand fly species in India: one preferred soil with neutral pH while the other preferred alkaline soil.²⁸ Meanwhile, Cohnstaedt et al. (2012) noted that some Peruvian sand fly species are found in both sylvatic and domestic habitats, demonstrating the adaptive nature of these species.³⁴ Due to the highly adaptive nature of sand flies and the differing capacity of species to transmit leishmaniasis, identifying endemic sand fly species and measuring their distribution and abundance are important aspects of leishmaniasis surveillance.

Previous studies on sand fly ecology in the Latin American tropics have primarily focused on the effect of environmental change resulting from human activities such as urbanization, deforestation, agriculture and extraction.^{25, 31, 33, 35, 36, 37, 38, 39} The effect of these activities appears to vary by species. Existing research suggests that anthropomorphic habitat change selects for the most adaptive sand fly species. The areas affected by environmental change then become dominated by a few adaptive species, which are often anthropophilic and include vectors of leishmaniasis.^{31, 36, 38}

Other studies have shown that different types of vegetation can influence sand fly populations in Latin American forests.^{31, 32, 40} For example, some sand fly species were associated with different types of vegetation in tropical forests of Latin America.⁴⁰ Meanwhile, other studies have found associations between sand fly populations and forested, agricultural, and urban land cover.^{26, 27, 41}

To our knowledge, only one other study has considered the association of soil chemistry and sand fly populations in South America. Alencar et al. (2011) collected soil samples in the Brazilian Amazon that were slightly acidic (pH 3.9–4.7). However, they did not find a significant correlation between soil pH and sand fly abundance.⁴² Since other studies outside of Latin America have found associations between sand fly populations and various soil types, further research should examine soil chemistry as a potential factor affecting sand fly populations in the Amazon.^{28, 29, 43, 44}

Studies on sand fly ecology in the Amazon often include measurements of biodiversity.^{25, 31, 36, 38, 45, 46, 47, 48, 49, 50, 51} Some studies have found greater sand fly biodiversity in undisturbed forested areas, while reporting that urban areas tend to be dominated by a few species.^{31, 38, 48} Previous research has also shown that vector species are among the most capable of adapting to urbanization and often become the dominant species in urban environments.^{36, 47} Meanwhile, other studies have found that sand fly biodiversity and species richness could result in higher genetic diversity of *Leishmania* parasites.^{48, 52} While no studies have examined the effect of biodiversity on leishmaniasis rates, it is

theoretically possible that greater biodiversity could result in lower rates due to greater competition for blood meals and breeding grounds.

1.3 Study aims

Leishmaniasis infection requires exposure to an infected female sand fly. However, not all sand fly species are capable of transmitting leishmaniasis. For this reason, it is important to understand which sand fly species are present in a region and what factors influence endemic sand fly ecology. In this study, we describe sand fly abundance, sand fly biodiversity and leishmaniasis incidence in Madre de Dios, Peru and compare these measures to socio-demographic indicators, soil chemistry and land cover.

Considering the high burden of leishmaniasis in Madre de Dios, paltry research has been conducted on leishmaniasis and sand flies in this region. In 1995, Perez and Ogusuku studied seasonal distribution of sand flies in a community near Madre de Dios. They collected over 5,000 sand flies from 28 species and reported peaks in sand fly activity around December to January and July to August.⁵¹ In 1998, Lucas et al. examined the geographic distribution of leishmaniasis cases in Peru from 1986 to 1993 and noted a high incidence of both cutaneous and mucocutaneous leishmaniasis in Madre de Dios.⁵³ Rojas-Jaimes characterized the manifestations of leishmaniasis in Madre de Dios in 2012.⁴ The same year, Zorrilla et al. captured 7,381 sand flies from 49 species in communities in the departments of Madre de Dios and Puno. They suggested

that the construction of the Interoceanic Highway had a negative effect on sand fly abundance and biodiversity.²⁵ Also in 2012, Valdivia et al. captured 1,299 sand flies from 33 species in Madre de Dios and tested them for *Leishmania* DNA using PCR. Seven pools of four total species tested positive for *Leishmania* infection. Three pools tested positive for *L. lainsoni*, two tested positive for *L. braziliensis* and two were infected with unidentified *Leishmania* species. This was also the first study to report *Leishmania* infection in *Lutzomyia aurensis*.

The existing literature provides justification for this study by demonstrating the feasibility and importance of leishmaniasis research in similar settings. This study builds on existing knowledge of leishmaniasis and sand fly ecology with the following aims: **1) describe sand fly abundance and biodiversity in communities along the Madre de Dios River; 2) identify environmental and socio-demographic factors associated with sand fly abundance and biodiversity in these communities; and 3) examine ecological risk factors associated with leishmaniasis in the communities.**

Based on previous research, we hypothesize that larger sand fly populations and greater biodiversity will be associated with less community development, higher soil moisture, higher soil pH and forested land coverage. We expect areas with more human activity and less forest coverage to have lower biodiversity, with a greater abundance of vector species relative to non-vector species. For this reason, we also expect to see greater leishmaniasis incidence in these types of areas.

This study adds to the limited research on leishmaniasis and sand fly ecology in Madre de Dios, Peru. The findings of this study will help guide future research and inform public health interventions.

2. Methods

2.1 Setting

The department of Madre de Dios is located in eastern Peru and borders Brazil and Bolivia (see Figure 1). Located in the Amazon jungle, Madre de Dios has a warm and wet climate. No specific and reliable data regarding annual temperature, rainfall or humidity are reported in this region. The population of Made de Dios in 2013 was 130,876.³ The average per capita monthly income in Madre de Dios was 1,161 soles (approximately \$375 USD) in 2013.⁵⁴

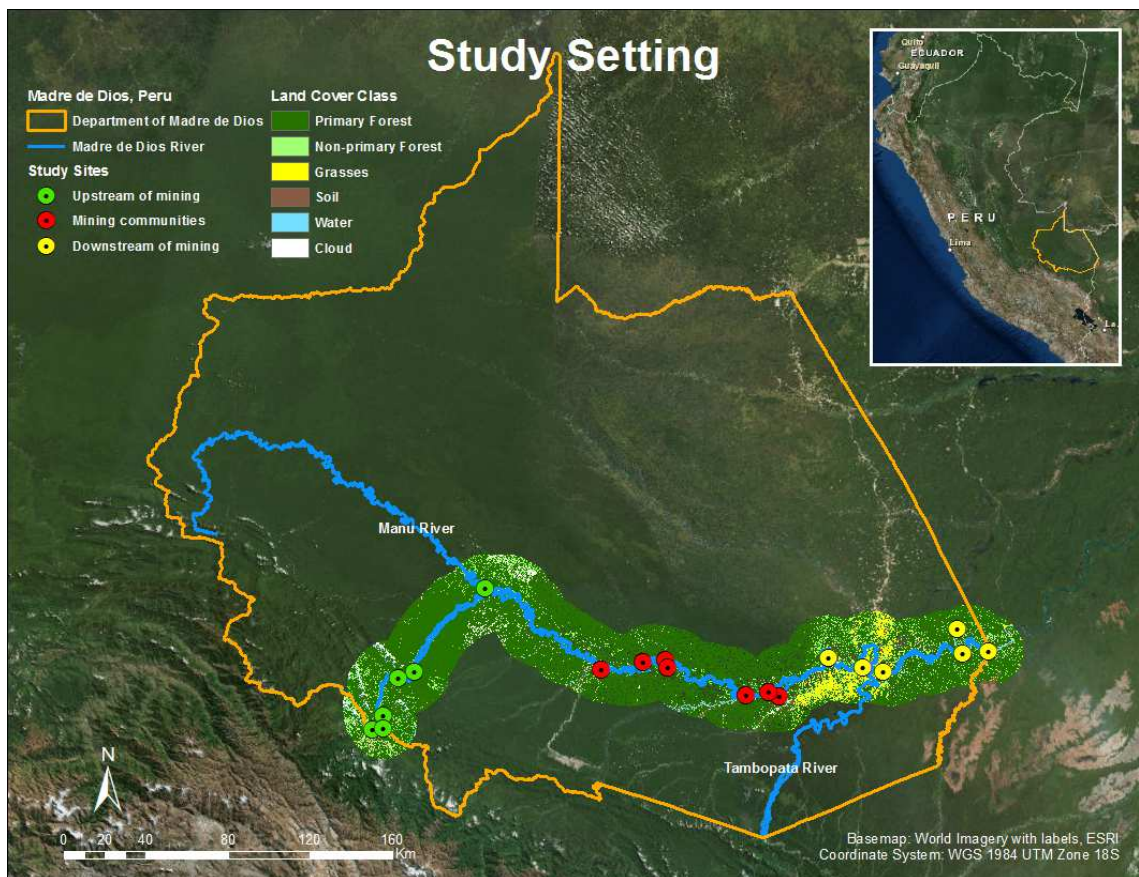


Figure 1: Map of study site

Madre de Dios has the highest incidence of leishmaniasis in the country.^{2,3} There has been significant environmental change in this region due to human activity over the last decade. The construction of the Interoceanic Highway in Brazil and Peru from 2005 until 2012 resulted in rapid population growth and deforestation.⁵⁵ Meanwhile, the proliferation of illegal gold mining along the Madre de Dios River has led to further population growth and environmental degradation.⁵⁶ The high incidence of leishmaniasis and high levels of anthropogenic activity provide an important setting for research on leishmaniasis and sand fly ecology. Sand fly counts and community surveys measuring socio-demographic indicators were collected from June to August 2013. Soil samples, household surveys, leishmaniasis case data and satellite images were collected from June through August 2014.

2.2 Sampling

Nineteen communities along the Madre de Dios River were stratified into three categories: communities upstream of mining, mining communities and communities downstream of mining. Communities were visited in 2013 and revisited in 2014. During the summer of 2014, miners in the mining communities started to go on strikes, preventing the research team from returning to these seven communities in 2014. Ultimately, eighteen communities were visited in 2013 and eleven were visited in 2014. A total of 212 individuals from 45 households participated in household surveys. Table 1 shows which types of data were collected in each community.

Table 1: Data available by community

Community	Leishmaniasis incidence	Sand fly	Community survey	Household survey	Soil chemistry	Land Cover
Upstream of mining						
Villa Carmen	No	Yes	No	No	Yes	Yes
Atalaya	No	Yes	No	No	No	Yes
Salvacion	Yes	Yes	Yes	Yes	Yes	Yes
Shintuya	Yes	No	No	No	Yes	Yes
Itahuania	Yes	Yes	Yes	Yes	Yes	Yes
Boca Manu	Yes	Yes	Yes	Yes	Yes	Yes
Mining communities						
Boca Colorado	Yes	Yes	Yes	No	No	Yes
San Juan Grande	Yes	Yes	Yes	No	No	Yes
CICRA	No	Yes	No	No	No	Yes
Boca Amigo	Yes	Yes	Yes	No	No	Yes
Boca Inambari	No	Yes	Yes	No	No	Yes
Fortuna	Yes	No	Yes	No	No	Yes
Laberinto	Yes	Yes	Yes	No	No	Yes
Downstream of mining						
Tres Islas	Yes	Yes	Yes	Yes	Yes	Yes
Puerto Pastora	Yes	Yes	Yes	Yes	Yes	Yes
Bajo Madre de Dios	No	Yes	Yes	Yes	Yes	Yes
Palma Real	Yes	Yes	Yes	Yes	No	Yes
Puerto Pardo	Yes	Yes	Yes	Yes	No	Yes
Lago Valencia	Yes	Yes	Yes	Yes	No	Yes

2.3 Procedures

A team of Peruvian fieldworkers conducted community and household surveys.

In each community, local leaders were identified to complete the community-level surveys. The leaders drew maps of the community and numbered each household.

Using these maps, three to seven houses were selected for household-level surveys

using a random number generator. In the larger communities, households were

stratified into houses around the town center and houses away from the town center.

The fieldworkers visited selected houses, received informed consent from the household

heads and then administered the surveys. The households did not receive any monetary compensation, but participants were motivated to participate because the research team shared the results of anemia, malaria and mercury exposure tests.

We collected vectors using three Center for Disease Control & Prevention (CDC) light traps and one Shannon trap in each community we visited. CDC light traps attract insects with a light bulb and then trap them with a fan blowing into a collection bag. A Shannon trap consists of a large net under which collectors look for vectors and trap them with a mouth aspirator. CDC light traps were set for one night from 6 p.m. to 6 a.m. and Shannon traps were conducted with 2 to 3 collectors for four hours. Vectors and soil samples were collected at four locations in most communities: CDC light traps were set near the center of the community, near the edge of the community, and in the forest outside the community; and a Shannon trap was set near the center of the community. However, Shannon traps were unable to be placed in four communities due to logistical constraints. GPS coordinates and altitude were recorded for each trap site using a Garmin GPSMAP. After collecting light traps, insects were killed by placing them in a plastic bag with triethylamine for one hour. Sand flies and mosquitoes were separated from other insects in the field and sent to the United States Naval Medical Research Unit No. 6 (NAMRU-6) for morphological identification of species.

To collect soil samples, the ground was cleared of leaves and debris and five centimeters of top soil were sampled using a one-inch diameter soil punch tube. Five

samples were collected within a ten meter radius of each trap site. The samples were homogenized in a plastic bag and five sub-samples were placed in separate 15 ml centrifuge tubes. The remaining soil was used to measure soil texture, following the soil texture field guide from *Wetland Soils: Genesis, Hydrology, Landscapes, and Classification*⁵⁷ (see Appendices 1-2). Soil samples were taken to the NAMRU-6 station in Puerto Maldonado, where they were weighed, air dried on tin foil over 48 hours, and reweighed. Once dried, soil color was measured by matching each sample to color chips in the Munsell soil color chart, which measures hue, value and chroma. After shipping the samples back to Duke University, they were prepared for pH measurement by combining the five sub-samples from each site and sifting them through a 1 mm sieve. 5 ml of 0.01 M calcium chloride dihydrate were added to 2.5 g of soil in order to measure pH with a Brinkmann 665 Dosimat titrator.

The Madre de Dios regional health directorate (DIRESA Madre de Dios) provided data on community population and leishmaniasis cases reported by health posts in fourteen communities from 2011 through 2013.

A land cover raster dataset was prepared from 2014 Landsat 8 images that were downloaded from Earth Explorer. Each pixel was categorized into primary forest, nonprimary forest, grasses, bare soil, water or cloud classes using unsupervised image classification. In order to calculate the proportion of land cover classes near a trap site or community, we created a model in ArcMap 10.2.2 (See Appendix 3). The model created

a separate raster layer for each land cover class and calculated the number of cells within a certain radius for each class. Since land cover can vary greatly in different buffers sizes, buffers of 100 m, 200 m, 500 m, 1000 m and 2000 m were explored for each trap site and buffers of 1000 m, 2000 m and 5000 m were examined for each community.

All study procedures were approved by DIRESA Madre de Dios and NAMRU-6 in Lima, Peru. As a part of a joint agreement between Duke University and NAMRU-6, the procedure was also approved by the Duke Institutional Review Board.

2.4 Measures

2.4.1 Sand fly abundance

Entomological data were categorized according to community, trap, sex and species. Species that have been incriminated as vectors for leishmaniasis in other settings were classified as potential vector species and all other species were categorized as non-vector species. Male sand flies were not included in our analyses because males are not capable of transmitting leishmaniasis and far fewer males were captured than females. The sand fly abundance measures considered in analysis were female potential vector abundance and female non-vector abundance. Female potential vector abundance was defined as the number of female sand flies belonging to potential vector species that were collected in each trap. Female non-vector abundance was the number of female sand flies collected that belonged to non-vector species.

2.4.2 Sand fly biodiversity

Sand fly species and numbers were used to create biodiversity indices within traps and communities. In order to compare our results with the literature, we calculated two standard measures commonly used to quantify biodiversity: the Shannon index and Pielou's index.^{36, 45, 46, 47, 48, 49}

The Shannon biodiversity index, also known as the Shannon-Wiener index, is a measure of biodiversity. It accounts for both species richness, or the number of species in a community, as well as species evenness, or how evenly individuals are distributed between species.⁵⁸ The equation for Shannon's diversity index (H) is:

$$H = - \sum_{i=1}^S p_i \ln(p_i)$$

where p_i is the relative abundance of a species in the sample, which equals the number of individuals in a species divided by the number of individuals in all species. The Shannon index equals zero when only one species is found and generally reaches a maximum around 4.5 in extremely diverse communities. Previous studies in sand fly ecology in the Amazon have reported Shannon indices ranging from 0.1929 to 2.05.^{36, 45}

Pielou's index is a measure of species evenness or equitability.⁵⁸ The equation for Pielou's index (J) is:

$$J = \frac{H}{H_{max}} = \frac{H}{\ln S}$$

in which S is the total number of species captured in the sample. Pielou's index ranges from 0 to 1, with 1 meaning complete evenness among all species. Previous reports of Pielou's index for sand flies in the Amazon have ranged from 0.0900 to 0.7791.³⁶

2.4.3 Leishmaniasis incidence

Fourteen community health posts provided data on leishmaniasis cases and community population in 2011, 2012 and 2013. Community leishmaniasis incidence was calculated by dividing the average number of cases per year by the average community population. Incidence is reported as the number of cases per 100,000 people per year.

2.4.4 Socio-demographic indicators

Separate infrastructure indices were created from the household survey and community survey. Each index was created by adding one point for each infrastructure item or service reported on the survey (e.g., electricity, piped water, presence of a secondary school). In the case of household surveys, the indices were averaged among all households to estimate a community's average household infrastructure. Average household income was calculated in each community by taking the mean of each household's total income as reported in the household survey. We also considered community population size and number of farms as reported on community surveys.

2.4.5 Soil chemistry

The soil measures included in this study were moisture, color, texture and pH. Soil moisture was expressed as the weight of water lost from evaporation divided by the

weight of the dry soil. Soil moisture was calculated for each trap site by taking the mean soil moisture of its subsamples. Soil color was measured in each subsample by recording the hue, value and chroma of the matching soil color chip. Soil hue was calculated for trap sites by taking the mode of sub-samples. Value and chroma were measured by taking the mean of sub-samples. Soil texture from each site was categorized using the soil texture field guide in Appendix 1. These categories were reclassified as fine, medium or coarse based on their locations in the soil texture triangle in Appendix 2. Soil pH for each trap site was measured with a Brinkmann 665 Dosimat titrator.

Community soil moisture and pH were calculated by taking the mean values of the trap sites in each community. Since soil color and texture are categorical, and since there was much variation within community sites, we did not calculate community averages for these variables.

2.4.6 Land cover

Our land cover classes of interest were primary forest, nonprimary forest, grasses and bare soil. Measures for each class were calculated by dividing the number of pixels of a class by the summed number of pixels for all four classes. We were particularly interested in the proportion of primary forest to bare soil. We calculated this measure by dividing the number of primary forest pixels by bare soil pixels. Land cover measures were calculated within several different buffer sizes for traps and communities. The measures included in analysis utilized the 200 m buffer for traps and the 1000 m buffer

for communities. ArcGIS was also used to calculate the distance of each trap from the center of the community and the distance of traps and communities from the river.

2.5 Analysis

After data were collected, entered and prepared for analysis, descriptive statistics were run on individual variables. Associations were then investigated using bivariate analysis between the dependent variables (sand fly abundance, biodiversity indices and leishmaniasis incidence) and independent variables (socio-demographic, soil and land cover measures). Multivariate regressions were run initially with statistically significant variables with a p-value less than 0.20 and then for practically significant variables that were included in our hypotheses. Backwards elimination was used to select a final regression model for each dependent variable.

To analyze sand fly data, we employed a negative binomial regression with random effects. We chose negative binomial regression because our data were over-dispersed, having variance much greater than the mean. The random effect was included because our data were clustered, with multiple traps set in each community. This effect allows clustered observations to be treated independently by introducing a random intercept that varies between clusters (in this case, between communities). We selected the exchangeable correlation structure, which assumes that any two observations within a cluster are correlated, but observations from different clusters have no correlation. The link function we selected was the log function, which provides

output that can be interpreted as incidence density or incidence rate ratio. Since these two terms are interchangeable, this number will be referred to as incidence density in reference to the count of sand flies caught in a collection period. This regression was used for both bivariate and multivariate analysis of sand fly abundance.

Biodiversity data came from the same traps as the sand fly abundance data and therefore required the same consideration for clustering. However, the mean was approximately equivalent to the variance, so negative binomial regression was not required. Instead, we used a Poisson regression with random effects. As with our sand fly abundance regressions, we selected the exchangeable correlation structure and the log link function.

Leishmaniasis data were only available on the community level and therefore had no clustering. For this reason we used a Poisson regression with the log link function for analysis of leishmaniasis incidence. Since the dependent variable was a rate, the beta coefficient was the log of the change in a community's leishmaniasis incidence rate due to a one-unit increase in the independent variable.

For all analyses, a p-value of 0.05 was considered significant. When bivariate regression did not produce any significant results, non-parametric regressions were run by using dichotomous variables instead of continuous variables.

All statistical analyses were conducted using STATA, version 13 (Stata Corporation, College Station, TX).

3. Results

3.1 Sand fly abundance

Table 2 shows results of all dependent variables at the community level. We encountered sand flies in fifteen of the seventeen communities in which we set traps.

Boca Colorado, Tres Islas and Puerto Pastora experienced a cold front at the time traps

Table 2: Leishmaniasis, sand fly abundance and sand fly biodiversity by community

Community	Leishmaniasis Incidence	Potential Vector Abundance	Non-Vector Abundance	H	J
Upstream of mining					
Villa Carmen	-	369	26	1.23	0.43
Atalaya	-	10	8	1.84	0.95
Salvacion	2,943	25	16	2.10	0.88
Shintuya	1,370	-	-	-	-
Itahuania	2,158	23	8	1.99	0.78
Boca Manu	1,174	5	31	1.26	0.65
Mining communities					
Boca Colorado	913	0*	0*	0.00	0.00
San Juan Grande	231	12	3	0.92	0.66
CICRA	-	35	13	2.07	0.81
Boca Amigo	270	7	26	0.87	0.54
Boca Inambari	-	8	88	1.04	0.50
Fortuna	154	-	-	-	-
Laberinto	1,023	3	3	1.67	0.93
Downstream of mining					
Tres Islas	139	0*	1*	0.00	0.00
Puerto Pastora	249	0*	0*	0.00	0.00
Bajo Madre de Dios	-	15	12	1.80	0.82
Palma Real	352	4	11	2.12	0.85
Puerto Pardo	0	19	23	2.26	0.84
Lago Valencia	449	36	16	1.51	0.73

*Catches in Boca Colorado, Tres Islas and Puerto Pastora were reduced due to cold front

Leishmaniasis incidence reported per 100,000 people per year

“-“ indicates no data

Table 3: Total sand fly abundance by community

Community	Villa Carmen	Atalaya	Salvacion	Itahuania	Boca Manu	Boca Colorado	San Juan Grande	CICRA	Boca Amigo
Potential vectors									
<i>L. antunesi</i>	-	-	-	1	-	-	-	1	-
<i>L. aragai</i>	-	-	-	-	-	-	-	-	-
<i>L. auraensis</i>	-	6	-	-	14	-	2	1	65
<i>L. carrerai</i>	38	-	1	1	-	-	-	-	-
<i>L. chagasi</i>	-	-	-	1	-	-	-	-	-
<i>L. choti</i>	-	-	-	-	-	-	-	-	-
<i>L. davisii</i>	1	-	-	15	1	-	12	3	5
<i>L. flaviscutellata</i>	-	-	-	1	-	-	-	-	-
<i>L. hirsuta</i>	8	6	-	5	-	-	-	-	-
<i>L. lainsoni</i>	-	-	-	-	-	-	-	-	1
<i>L. llanosmartinsi</i>	284	1	5	-	-	-	-	16	-
<i>L. paraensis</i>	1	-	6	-	-	-	-	1	-
<i>L. serrana</i>	-	-	-	-	-	-	-	-	-
<i>L. shamoni</i>	-	-	-	-	-	-	-	-	-
<i>L. shawi</i>	40	4	10	-	-	-	-	15	-
<i>L. umbratilis</i>	1	-	-	-	-	-	-	-	-
<i>L. yucumensis</i>	3	-	2	-	4	-	-	1	1
<i>L. yuilli yuilli</i>	4	-	2	-	-	-	-	-	-
Non-vectors									
<i>Brumptomyia</i> spp.	-	-	-	-	1	-	1	-	-
<i>B. avellari</i>	-	-	-	-	-	-	-	-	-
<i>B. galindoi</i>	-	-	-	-	-	-	-	-	-
<i>L. (Evandromyia)</i> spp.	-	-	-	-	-	-	-	-	-
<i>L. (Helcocyrtomyia)</i> spp.	2	5	-	-	-	-	-	-	-
<i>L. (Pressatia)</i> spp.	1	-	-	-	-	-	-	-	-
<i>L. (Psychodopygus)</i> spp.	1	-	-	-	-	-	-	-	-
<i>L. (Trichophoromyia)</i> spp.	2	3	14	3	29	-	2	10	26
Migonei Group	-	-	-	-	-	-	-	-	-
Oswaldoi Group	-	-	-	-	-	-	-	-	-
<i>L. abumaensis</i>	-	-	-	-	-	-	-	-	-
<i>L. barretoii barretoii</i>	1	-	1	-	-	-	-	-	-
<i>L. begoniae</i>	-	-	-	1	-	-	-	-	-
<i>L. beniensis</i>	-	-	-	-	-	-	-	-	-
<i>L. calcarata</i>	-	-	-	-	-	-	-	-	-
<i>L. clitella</i>	1	-	-	-	-	-	-	-	-
<i>L. dreisbachi</i>	-	-	-	1	-	-	-	1	-
<i>L. evangelistai</i>	-	-	-	1	-	-	-	-	-
<i>L. geniculata</i>	1	-	-	1	-	-	-	1	-
<i>L. kirigetiensis</i>	-	3	-	-	-	-	-	-	-
<i>L. lutziana</i>	-	-	-	-	-	-	-	-	-
<i>L. nemorosa</i>	-	-	7	5	-	-	-	7	-
<i>L. nevesi</i>	-	-	-	-	-	-	-	-	-
<i>L. octavioi</i>	-	-	-	-	3	-	-	-	-
<i>L. sherlocki</i>	19	-	2	1	1	-	-	2	-
<i>L. sinuosa</i>	6	-	4	-	-	-	-	11	-
<i>L. sordelli</i>	-	-	-	-	-	-	-	-	-
<i>L. vatterae</i>	-	-	-	-	-	-	-	-	-
<i>L. walkeri</i>	-	-	-	-	-	-	-	-	-
Total	414	28	54	37	53	-	17	70	98

Table 3: Total sand fly abundance by community

Community	Boca Inambari	Laberinto	Tres Islas	Puerto Pastora	Bajo Madre de Dios	Palma Real	Puerto Pardo	Lago Valencia	Total
Potential vectors									
<i>L. antunesi</i>	6	-	-	-	11	2	5	40	66
<i>L. aragaoi</i>	-	1	-	-	-	-	1	3	5
<i>L. auraensis</i>	87	-	-	-	1	1	21	3	201
<i>L. carrerai</i>	-	-	-	-	-	-	-	-	40
<i>L. chagasi</i>	-	-	-	-	-	-	-	-	1
<i>L. choti</i>	-	-	-	-	-	-	1	-	1
<i>L. davisii</i>	3	1	-	-	-	-	7	-	48
<i>L. flaviscutellata</i>	-	-	-	-	-	-	-	-	1
<i>L. hirsuta</i>	-	-	-	-	-	-	-	-	19
<i>L. lainsoni</i>	-	-	-	-	-	-	-	-	1
<i>L. llanosmartinsi</i>	-	-	-	-	-	-	-	1	307
<i>L. paraensis</i>	-	-	-	-	-	-	-	-	8
<i>L. serrana</i>	-	-	-	-	2	-	-	-	2
<i>L. shannoni</i>	-	-	-	-	2	-	-	-	2
<i>L. shawi</i>	-	-	-	-	-	1	7	1	78
<i>L. umbratilis</i>	-	-	-	-	-	-	-	-	1
<i>L. yucumensis</i>	3	2	-	-	-	-	1	-	17
<i>L. yuilli yuilli</i>	-	-	-	-	-	1	-	8	15
Non-vectors									
<i>Brumptomyia</i> spp.	-	-	-	-	-	-	8	-	10
<i>B. avellari</i>	1	-	3	-	9	-	10	-	23
<i>B. galindoi</i>	-	-	-	-	-	-	1	-	1
<i>L. (Eoandromyia) spp.</i>	-	-	-	-	-	1	-	-	1
<i>L. (Helcocyrtomyia) spp.</i>	-	-	-	-	-	-	-	-	7
<i>L. (Pressatia) spp.</i>	1	-	-	-	2	-	-	-	4
<i>L. (Psychodopygus) spp.</i>	-	-	-	-	-	-	-	-	1
<i>L. (Trichophoromyia) spp.</i>	86	3	-	-	4	5	14	16	217
Migonei Group	-	-	-	-	-	1	-	-	1
Oswaldoi Group	-	-	-	-	1	-	-	-	1
<i>L. abunaensis</i>	-	-	-	-	-	1	-	-	1
<i>L. barretoii barretoii</i>	-	-	-	-	-	-	-	-	2
<i>L. begoniae</i>	-	-	-	-	-	-	-	-	1
<i>L. beniensis</i>	-	-	-	-	-	-	1	-	1
<i>L. calcarata</i>	-	1	-	-	-	-	1	-	2
<i>L. clitella</i>	-	-	-	-	-	-	-	-	1
<i>L. dreisbachi</i>	-	-	-	-	-	-	-	-	2
<i>L. evangelistai</i>	-	-	-	-	-	-	-	-	1
<i>L. geniculata</i>	-	-	-	-	-	-	-	-	3
<i>L. kirigetiensis</i>	-	-	-	-	-	-	-	-	3
<i>L. lutziana</i>	-	-	-	-	-	1	-	-	1
<i>L. nemorosa</i>	-	3	-	-	-	8	2	11	43
<i>L. nevesi</i>	1	-	-	-	-	-	-	-	1
<i>L. octavioi</i>	-	-	-	-	-	-	-	-	3
<i>L. sherlocki</i>	-	-	-	-	-	2	-	-	27
<i>L. sinuosa</i>	-	-	-	-	-	-	-	-	21
<i>L. sordelli</i>	-	-	-	-	-	1	-	-	1
<i>L. vatierae</i>	-	-	-	-	1	-	-	-	1
<i>L. walkeri</i>	-	-	-	-	-	-	5	-	5
Total	188	11	3	-	33	25	85	83	1199

were set, substantially reducing the number of sand flies captured in these three communities. In total, we collected 1,199 sand flies (343 male, 856 female) from 47 different species. Table 3 shows the total number of sand flies caught from each species by community. Among female sand flies, 571 belonged to a potential vector species of leishmaniasis, while 285 belonged to non-vector species.

Trap-level results for independent variables and for female sand fly abundance are shown in Tables 4 and 5. Soil pH at trap sites ranged from 3.61 to 6.53, with a mean pH of 4.85. Each trap collected an average of 8.9 female sand flies from potential vector species and 4.5 from non-vector species.

Table 4: Summary statistics of independent variables in traps

Continuous variables	Mean	Min	Median	Max	Variance	Number of observations
Altitude	281.094	170	212.5	621	17284.1	64
Distance from river	842.292	51.909	352.313	6708.57	2425574	64
Distance from community	440.677	11.76	216.39	2536.92	269916	64
Primary forest coverage	35.198	1.235	28.395	96.644	716.151	64
Nonprimary forest coverage	31.647	2.013	31.111	88.591	282.294	64
Grass coverage	15.064	0	0	65.772	464.23	64
Bare soil coverage	18.091	0	10.587	19.433	370.066	64
Primary forest/bare soil	12.168	0.027	2.222	100	568.831	64
Soil pH	4.85	3.61	4.71	6.53	0.709	26
Soil moisture	31.122	15.9	31.994	48.408	78.177	26
Soil hue	2.231	1	3	3	0.985	26
Soil value	5.135	4	5	7.4	0.562	26
Soil chroma	2.985	1.6	3.1	5.2	0.797	26
Indicator variables	Yes	No	Percent Yes		Number of observations	
Fine soil texture	15	11	57.69		26	
Medium soil texture	4	22	15.38		26	
Coarse soil texture	7	19	26.92		26	
Shannon trap indicator	13	51	20.31		26	

Table 5: Potential vector and non-vector abundance of traps

Variable	Mean	Min	Median	Max	Variance	Number of observations
Potential vectors	8.922	0	1	359	2014.33	64
Non-vectors	4.453	0	1	75	109.077	64

Table 6 shows the results of bivariate analysis with negative binomial regression. In our bivariate analyses, we found that the number of female non-vectors captured was significantly associated with a higher proportion of primary forest coverage to bare soil coverage (p-value 0.043). While no independent variables were significantly associated with potential vector abundance, per capita income (0.052), proportion of primary forest to bare soil coverage (0.134) and soil pH (0.149) had p-values below 0.20.

These three variables were included in our initial multivariate regression with potential vector abundance and non-vector abundance. However, backwards elimination did not provide a satisfactory model for either measure (see Table 7). We therefore developed multivariate models that included all of the covariates included in our hypotheses (see Table 8). After backwards elimination, our final model included community infrastructure, soil pH, proportion of primary forest to soil coverage and a Shannon trap indicator. Both potential vector and non-vector models suggested positive associations with proportion of primary forest to soil coverage and negative associations with community infrastructure, soil pH and Shannon traps. However, the p-values in the potential vector model were much lower than the non-vector model.

Table 6: Bivariate analysis of potential vector and non-vector abundance

Independent variable	Potential vector abundance			Non-vector abundance		
	Incidence density	p-value	95% C.I.	Incidence density	p-value	95% C.I.
Upstream of mining	1.047	0.904	0.500 - 2.191	0.884	0.726	0.442 - 1.767
Mining community	1.028	0.944	0.477 - 2.218	1.048	0.891	0.534 - 2.057
Downstream of mining	0.919	0.839	0.409 - 2.068	1.087	0.820	0.530 - 2.228
Shannon trap		Did not converge		0.569	0.167	0.255 - 1.266
Community infrastructure	1.017	0.751	0.917 - 1.127	0.931	0.252	0.825 - 1.052
Community population	1.000	0.561	0.999 - 1.000	1.000	0.245	0.999 - 1.000
Number of households	0.999	0.614	0.997 - 1.002	0.998	0.233	0.996 - 1.001
Number of farms		Did not converge		1.007	0.346	0.992 - 1.023
Per capita income	1.004	0.052	1.000 - 1.007	1.000	0.901	0.996 - 1.003
Household Infrastructure	0.996	0.948	0.870 - 1.139	0.988	0.859	0.868 - 1.125
Altitude	1.001	0.447	0.998 - 1.003	1.000	0.982	0.998 - 1.002
Distance from river	1.000	0.541	1.000 - 1.000	1.000	0.428	1.000 - 1.000
Distance from community	1.000	0.503	0.999 - 1.000	1.000	0.298	1.000 - 1.001
Primary forest coverage	1.004	0.557	0.991 - 1.016	1.007	0.236	0.995 - 1.012
Nonprimary forest coverage	1.006	0.480	0.989 - 1.023	1.002	0.854	0.983 - 1.021
Grass coverage	0.993	0.475	0.976 - 1.012	0.999	0.950	0.984 - 1.015
Bare soil coverage	0.993	0.461	0.974 - 1.012	0.983	0.103	0.963 - 1.003
Primary forest/bare soil	1.014	0.134	0.996 - 1.033	1.018	0.043	1.001 - 1.035
Soil pH	0.592	0.149	0.290 - 1.206	1.012	0.968	0.576 - 1.777
Soil moisture	1.018	0.716	0.923 - 1.124	1.014	0.759	0.929 - 1.107
Soil hue		Did not converge		1.563	0.229	0.755 - 3.236
Soil value	1.150	0.666	0.609 - 2.173	0.803	0.580	0.369 - 1.747
Soil chroma	1.567	0.294	0.678 - 3.621	0.688	0.234	0.371 - 1.275
Fine soil texture	1.113	0.851	0.365 - 3.394	1.229	0.716	0.404 - 3.733
Medium soil texture	0.942	0.940	0.198 - 4.485	0.865	0.846	0.204 - 3.671
Coarse soil texture	0.887	0.867	0.217 - 3.629	0.829	0.795	0.207 - 3.411

Table 7: Multivariate analysis of potential vector and non-vector abundance

Potential vector abundance			
Independent Variable	Model 1: 16 observations	Model 2: 19 observations	Model 3: 53 observations
Per capita income	0.997 (0.561)	-	-
Soil pH	0.426 (0.278)	0.615 (0.183)	-
Forest/soil	1.065 (0.061)	1.062 (0.002)	1.014 (0.134)
Intercept	71.011 (0.417)	1.413 (0.843)	0.339 (0.001)

Non-vector abundance			
Independent Variable	Model 1: 16 observations	Model 2: 19 observations	Model 3: 53 observations
Per capita income	0.996 (0.384)	-	-
Soil pH	0.584 (0.441)	1.041 (0.890)	-
Forest/Soil	1.059 (0.090)	1.038 (0.006)	1.018 (0.043)
Intercept	22.409 (0.498)	0.378 (0.515)	0.399 (0.001)

Table 8: Multivariate analysis of potential vectors and non-vector abundance

Potential vector abundance				
Independent Variable	Model 1: 16 observations	Model 2: 16 observations	Model 3: 16 observations	Final model: 16 observations
Community Infrastructure	0.707 (0.006)	0.741 (0.045)	0.783 (0.048)	0.763 (0.057)
Distance from Community	0.999 (0.081)	-	0.999 (0.222)	-
Soil pH	0.249 (0.001)	0.223 (0.011)	0.255 (0.012)	0.223 (0.015)
Soil moisture	0.927 (0.139)	0.971 (0.618)	-	-
Forest/Soil	1.142 (0.043)	1.096 (0.011)	1.103 (0.002)	1.087 (0.012)
Shannon trap indicator	0.047 (0.009)	0.074 (0.036)	0.090 (0.037)	0.092 (0.043)
Intercept	390786 (0.001)	43074 (0.024)	9209 (0.012)	12792 (0.024)

Non-vector abundance				
Independent Variable	Model 1: 16 observations	Model 2: 16 observations	Model 3: 16 observations	Final model: 16 observations
Community Infrastructure	0.953 (0.641)	0.952 (0.62)	0.959 (0.780)	0.975 (0.790)
Distance from Community	1.000 (0.955)	-	1.000 (0.575)	-
Soil pH	1.178 (0.703)	1.181 (0.693)	0.836 (0.817)	0.942 (0.880)
Soil moisture	0.939 (0.214)	0.939 (0.179)	-	-
Forest/Soil	1.048 (0.188)	1.049 (0.124)	1.031 (0.449)	1.032 (0.308)
Shannon trap indicator	0.320 (0.152)	0.319 (0.151)	0.38 (0.222)	0.381 (0.223)
Intercept	3.871 (0.637)	4.066 (0.606)	1.943 (0.889)	1.242 (0.929)

3.2 Sand fly biodiversity

As shown in Table 2, the Shannon diversity index ranged from 0 to 2.26 and Pielou's evenness index ranged from 0 to 0.95 in the communities. Table 9 shows these data at the trap level, where the Shannon index ranged from 0 to 2.026 and Pielou's index ranged from 0 to 1.

Table 9: Biodiversity indices of traps

Variable	Mean	Min	Median	Max	Variance	Number of observations
Shannon index (H)	1.0447	0	1.126	2.026	0.344	47
Pielou's index (J)	0.856	0	0.895	1	0.022	40

Table 10 shows our bivariate analyses with biodiversity indicators. Since these analyses did not produce significant associations, we also ran non-parametric bivariate analyses in which we replaced continuous variables with dichotomous variables. These also failed to detect significant associations. Similarly, multivariate models incorporating variables from our hypotheses did not produce any significant relationships.

Table 10: Bivariate analysis of biodiversity indices

Independent variable	Shannon Index			Pielou's Index		
	IRR	p-value	95% C.I.	IRR	p-value	95% C.I.
Upstream of mining	1.203	0.528	0.678 - 2.034	0.996	0.991	0.350 - 1.990
Mining community	0.606	0.114	0.325 - 1.129	0.931	0.848	0.452 - 1.922
Downstream of mining	1.389	0.279	0.766 - 2.519	1.079	0.836	0.525 - 2.217
Shannon trap	0.981	0.960	0.467 - 2.062	0.924	0.858	0.392 - 2.179
Community infrastructure	0.990	0.832	0.906 - 1.083	1.007	0.894	0.913 - 1.110
Community population	1.000	0.360	0.999 - 1.000	1.000	0.947	0.999 - 1.001
Number of households	0.999	0.451	0.997 - 1.001	1.000	0.891	0.998 - 1.003
Number of farms	1.003	0.599	0.992 - 1.014	1.001	0.838	0.988 - 1.015
Per capita income	1.001	0.469	0.998 - 1.004	1.000	0.910	0.997 - 1.004
Household infrastructure	0.959	0.534	0.840 - 1.095	1.003	0.971	0.858 - 1.173
Altitude	1.001	0.478	0.999 - 1.003	1.000	0.974	0.998 - 1.002
Distance from river	1.000	0.811	1.000 - 1.000	1.000	0.968	1.000 - 1.000
Distance from community	1.000	0.798	0.999 - 1.000	1.000	0.818	1.000 - 1.001
Primary forest coverage	1.002	0.646	0.992 - 1.012	1.000	0.977	0.988 - 1.012
Nonprimary forest coverage	0.998	0.813	0.982 - 1.014	0.997	0.743	0.978 - 1.016
Grass coverage	1.005	0.429	0.992 - 1.019	1.002	0.825	0.986 - 1.018
Bare soil coverage	0.989	0.223	0.972 - 1.007	1.001	0.890	0.982 - 1.022
Primary forest/bare soil	1.006	0.475	0.990 - 1.022	0.998	0.875	0.978 - 1.019
Soil pH	0.862	0.605	0.490 - 1.514	1.010	0.976	0.531 - 1.922
Soil moisture	1.004	0.897	0.942 - 1.071	0.990	0.797	0.920 - 1.066
Soil hue	0.983	0.947	0.582 - 1.657	0.956	0.894	0.494 - 1.851
Soil value	1.223	0.386	0.776 - 1.927	1.080	0.793	0.609 - 1.913
Soil chroma	1.210	0.489	0.705 - 2.077	1.042	0.895	0.563 - 1.929
Fine soil texture	0.000	-	-	1.072	0.906	0.337 - 3.416
Medium soil texture	0.862	0.843	0.198 - 3.758	1.058	0.944	0.220 - 5.081
Coarse soil texture	1.059	0.922	0.334 - 3.355	0.865	0.842	0.208 - 3.588

3.3 Leishmaniasis incidence

From 2011 to 2013, there were 323 total cases of leishmaniasis reported by the fourteen community health posts providing data. The combined incidence of these communities from 2011 to 2013 was 928.0 cases per 100,000 people. As shown in Table 11, leishmaniasis incidence ranged from zero reported cases in Puerto Pardo to 2,942.7 cases per 100,000 people in Salvacion.

Table 11: Leishmaniasis incidence, cases per 100,000 people per year

Variable	Mean	Min	Median	Max	Variance	Number of observations
Leishmaniasis incidence	816.11	0	400.72	2942.71	744842	14

Unlike our sand fly abundance and biodiversity analyses, our leishmaniasis analyses were conducted on the community level. Bivariate analysis included soil pH and moisture, community infrastructure measures, land cover, sand fly abundance measures and sand fly biodiversity indices. Table 12 shows the output from bivariate analysis of leishmaniasis incidence. No significant relationships were detected in this analysis, in non-parametric analysis or in multivariate analyses.

Table 12: Bivariate analysis of leishmaniasis incidence

Independent variable	Coefficient	p-value	95% C.I.
Upstream of mining	1.620	0.797	-10.702 - 13.943
Mining community	-0.639	0.928	-14.485 - 13.208
Downstream of mining	-1.564	0.872	-20.550 - 17.421
Community infrastructure	0.165	0.846	-1.503 - 1.834
Community population	0.000	0.933	-0.007 - 0.007
Number of households	0.001	0.922	-0.028 - 0.031
Number of farms	0.020	0.814	-0.148 - 0.188
Per capita income	0.004	0.902	-0.060 - 0.068
Household infrastructure	-0.019	0.986	-2.080 - 2.043
Altitude	0.006	0.770	-0.033 - 0.044
Distance from river	0.000	0.973	-0.003 - 0.003
Soil pH	-0.731	0.905	-12.692 - 11.231
Soil moisture	0.084	0.920	-1.562 - 1.731
Potential vectors	0.030	0.904	-0.464 - 0.524
Shannon index	0.524	0.914	-9.036 - 10.084
Pielou's index	3.039	0.923	-58.269 - 64.348
Communities using health post	0.134	0.841	-1.175 - 1.444
Primary forest coverage	3.420	0.900	-50.063 - 56.902
Nonprimary forest coverage	-5.009	0.868	-63.898 - 53.880
Grass coverage	6.406	0.890	-97.464 - 84.653
Bare soil coverage	10.736	0.823	-83.567 - 105.039
Primary forest/bare soil	-0.158	0.908	-2.821 - 2.505

4. Discussion

4.1 Sand fly abundance

Table 13 compares our final multivariate models for potential vector abundance and non-vector abundance. Both abundance measures are positively associated with greater proportion of primary forest to bare soil land coverage. This supported our hypothesis and previous literature.^{31, 38} Both measures are also associated with lower soil pH. This contradicts our hypothesis, but the limited research on the relationship between soil pH and sand flies did not strongly suggest a relationship in either direction.^{26, 42} Potential vector and non-vector abundance were also associated with lower scores in our community infrastructure index. This was consistent with our hypothesis and some previous studies, although other studies found sand fly species associated with development and urbanization.^{31, 32, 33, 43, 59}

Table 13: Comparison of final potential vector and non-vector models

Independent Variable	Potential Vectors	Non-vectors
Community Infrastructure	0.763 (0.057)	0.975 (0.790)
Soil pH	0.223 (0.015)	0.942 (0.880)
Forest/Soil	1.087 (0.012)	1.032 (0.308)
Shannon trap indicator	0.092 (0.043)	0.381 (0.223)
Intercept	12792.36 (0.024)	1.242 (0.929)

One interesting finding from these models is that potential vectors have lower p-values than non-vectors, suggesting greater confidence in associations for potential vectors. This goes slightly against our original hypothesis that non-vectors would be more susceptible to changes in their environment than potential vectors. However, this could be a result of a smaller sample size: we captured 285 female non-vectors, compared to 571 female potential vectors. Furthermore, our bivariate analysis detected a significant relationship between female non-vector abundance and higher proportion of primary forest to bare soil coverage.

While the final model shows significant results for potential vectors, the intercept is curiously high at 12,792.36. This is extraordinarily high for a baseline number of sand flies, and we should be careful not to overestimate the effectiveness of this model.

4.2 Sand fly biodiversity

Besides the three communities that experienced a cold front, all communities produced catches with multiple species of sand flies. The Shannon index in these communities ranged from 0.87 in Boca Amigo to 2.26 in Puerto Pardo. These indices were higher than most other studies reporting Shannon indices of sand fly species, as no other studies in the Amazon reported a Shannon index above 2.05.^{25, 36, 45, 46, 47, 48, 49, 51} Excluding communities affected by cold fronts, the Pielou's evenness index ranged from 0.43 in Villa Carmen to 0.95 in Atalaya, indicating moderate to high evenness among sand fly species in our study setting. Our results suggest slightly more evenness than

previous research in the Amazon, in which Pielou's index has ranged from 0.0900 to 0.7791.³⁶

We did not find any significant associations between our biodiversity measures and our independent variables. This could be due to low statistical power resulting from a small sample size. However, this is one of the first studies to document biodiversity of sand fly populations in Peru.^{25, 51} This novel topic deserves to receive more attention in future research. While there is not yet evidence to support this hypothesis, it is theoretically plausible that higher sand fly biodiversity could lead to more competition for blood meals and breeding space and less opportunity for local dominance of a vector species. This could in turn lead to lower risk of leishmaniasis transmission.

4.3 Leishmaniasis incidence

The average incidence rate from 2011 to 2013 was 928.0 cases per 100,000 people, which is more than ten times that of the Madre de Dios incidence rate and over 40 times that of the national incidence rate.^{2, 3} This is likely a vast overestimation of the community incidence rates, because members of small communities tend to travel to health posts of larger communities such as Salvacion for major health problems. Consequently, the assumed population at risk in some communities would be much smaller than the actual number of people that should be considered at risk of reporting a case to the community health post. However, it is also likely that there are many unreported cases of leishmaniasis in the region as well.⁴

We did not find significant associations between leishmaniasis incidence and any independent variables. This is rather unsurprising, considering our small sample size of 15 communities providing data and the aforementioned circumstances that are likely causing overestimation of incidence rates.

4.4 Implications for policy and practice

Despite finding relatively few significant associations in our analyses, this study still supports some policy recommendations. Since the estimates of leishmaniasis incidence are likely inaccurate, communities could improve surveillance by attempting to more accurately define the catchment of their health posts and by reporting probable locations of leishmaniasis infections. In case communities are unable to provide accurate leishmaniasis data, preventative interventions should be focused on communities that have higher populations of vector species. Communities could potentially predict risk of vector species abundance using predictive models similar to the ones developed in this study. However, further research is necessary to develop more accurate models. Communities could also use these models to look for ways to lower their risk of hosting vector species. For example, Singh et al. (2008) suggested that communities could attempt to alter soil pH by planting weeds that are known to lower soil pH.²⁸

4.5 Implications for further research

While using multiple traps per community helped increase the number of total observations, the clustered nature of the design weakened our study's power. Future

leishmaniasis studies in Madre de Dios should look to improve on this study's sample size and add multiple time points to measure seasonality. Future research should also consider temperature, rainfall and humidity as variables, as these have all been shown to influence sand fly populations in other settings.^{5, 13} Additionally, future studies should test sand flies for infection by *Leishmania* parasites, as this would be the first step toward incriminating species as vectors for leishmaniasis transmission in Madre de Dios.

4.6 Study strengths and limitations

The major strength of this study came from its wide scope and inclusion of diverse communities. This study examined a wide range of variables measuring both environmental and anthropogenic factors that could affect the distribution of sand flies and leishmaniasis. We sampled communities along the Madre de Dios River starting near the region's western border with Cusco across the region to its eastern border with Brazil. While these communities were all small, ranging from under 100 residents to nearly 3,000, we believe that they provided an accurate representation of the Madre de Dios region.

Despite its strengths, this study had numerous limitations. First, the sample size was originally expected to be somewhat small, resulting in a low probability of finding significant associations. Political unrest occurring in 2014 exacerbated the study's low power by preventing us from returning to mining communities for data collection. Additionally, this study is ecological in nature, precluding the study of individual risk

factors for leishmaniasis. Leishmaniasis reporting is also unreliable in the area due to under-reporting and an unknown catchment of health centers in larger communities. There were also many instances of nonresponse to survey questions. Finally, soil measurements were likely biased due to small fluctuations such as variations in natural lighting during soil color measurement.

5. Conclusion

This study is important because it is one of the first studies to describe abundance and biodiversity of endemic sand fly fauna in Madre de Dios, Peru. It reports 1,199 sand flies captured from 47 different species, including 571 females from potential vector species. Shannon diversity indices in communities ranged from 0 to 2.26, a range that is slightly higher than other studies in the Amazon. Pielou's evenness indices ranged from 0 to 0.95, reaching almost complete evenness between species. Bivariate analysis detected a significant association between female non-vector abundance and higher proportion of primary forest to bare soil land coverage. This study introduces a multivariate model that predicts increasing abundance of female potential vectors and non-vectors with less community infrastructure, lower soil pH and higher proportion of forest to soil land cover. Future research should aim to build on this study by improving total sample size and considering the effects of temperature, rainfall, humidity and seasonal variations in sand fly abundance. Future studies should also attempt to test sand flies for *Leishmania* infection in order to better understand the effect of sand fly species on leishmaniasis ecology.

Appendix 1: Soil texture field guide⁵⁷

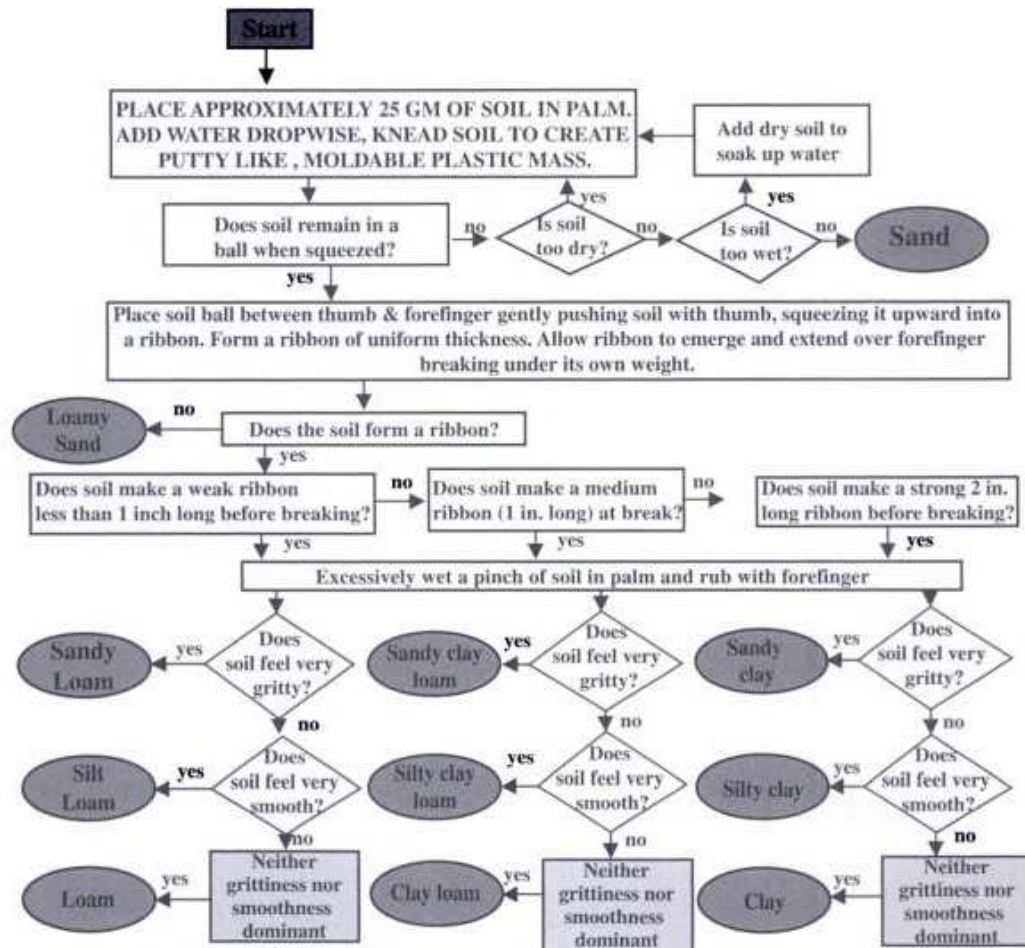
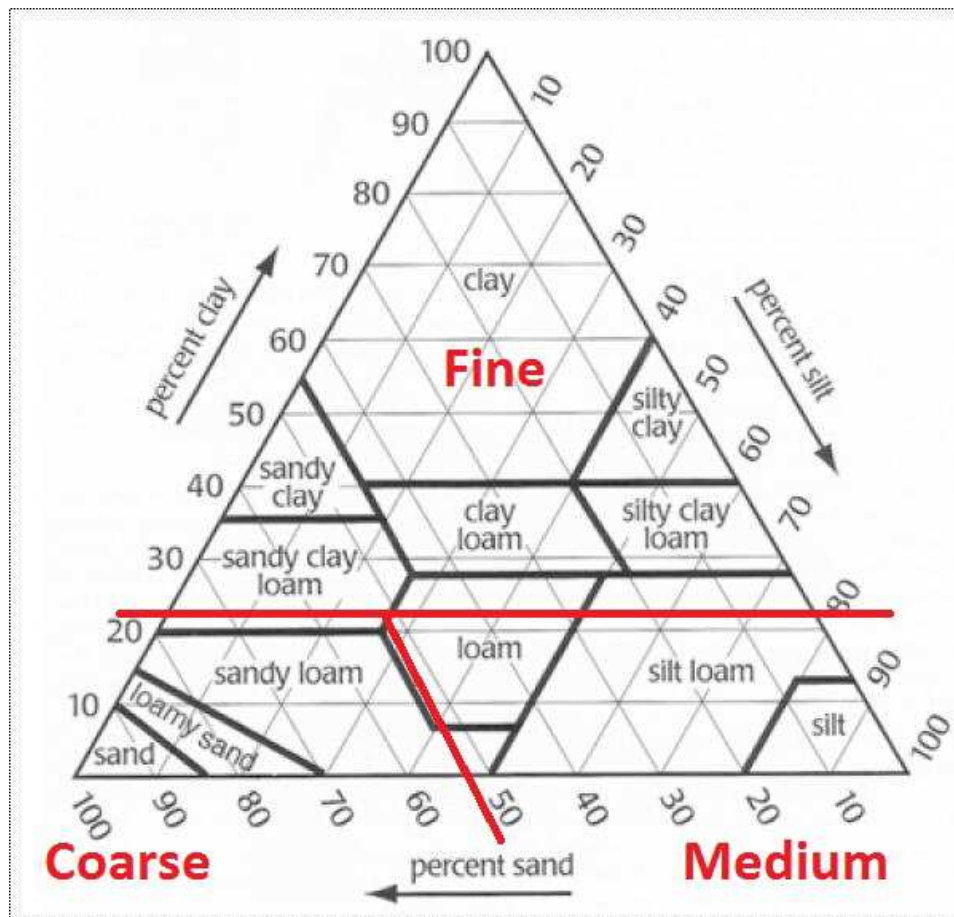
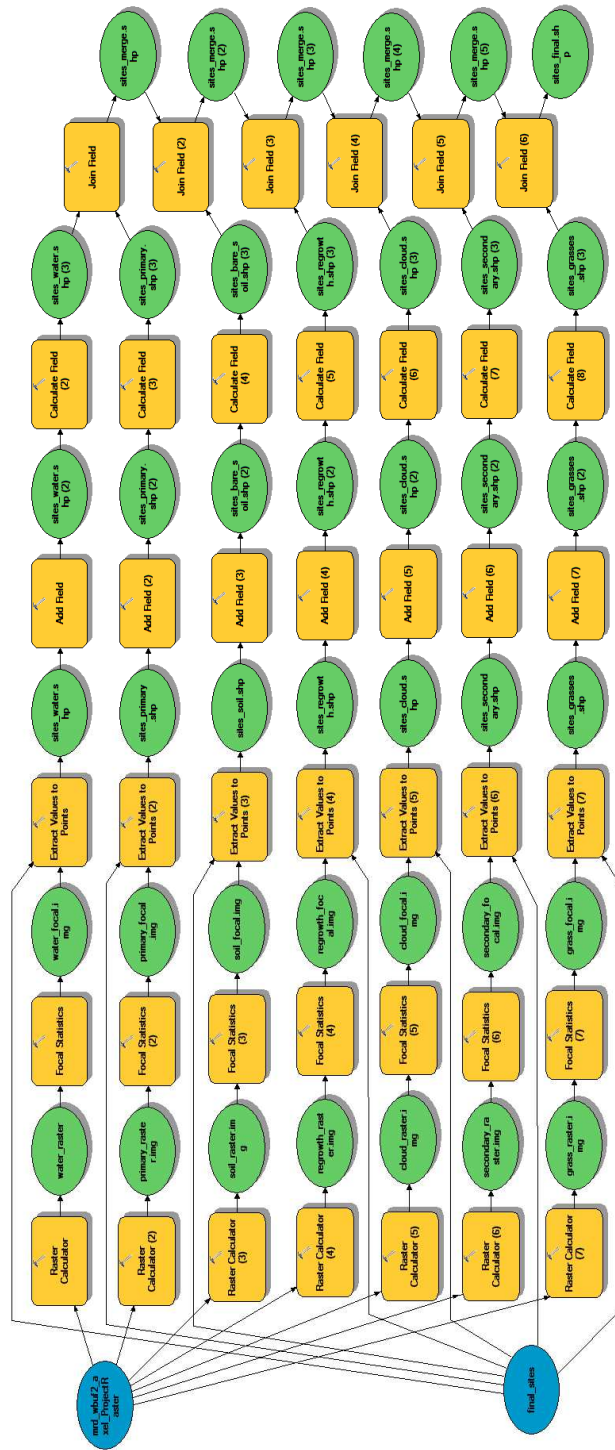


Figure 1.5 Flow chart for estimating soil texture by feel. To estimate soil texture, first wet the soil in the palm of your hand to its state of greatest malleability. It may take a couple of minutes of manipulation to wet the smaller clay aggregates. If the soil gets too wet and puddles, just add more dry soil and rework it to optimum malleability. After the soil is adequately moistened, follow the flow chart by trying to make a ball and then a ribbon of the soil. A soil's ability to hold a ribbon shape reflects its clay content. Grittiness or smoothness of the ribboned soil indicates high content of sand or silt, respectively. Note that no provision has been made for the texture "silt." This omission is not serious because pure silt is uncommon and the difference between silt and silt loam is inconsequential in most routine wetlands work. (Adapted from Thien, S. J. 1979. A flow diagram for teaching texture-by-feel analysis. *J. Agron. Ed.* 8:54-55.)

Appendix 2: Soil texture classification⁵⁷



Appendix 3: ArcGIS land cover classification model



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