

Biases in Amphibian Sampling in the Amazon: Using Infrastructure and Accessibility Data to Identify Sampling Gaps

Abstract: Biogeographic knowledge of Amazonian amphibians presents significant challenges in spatial and temporal coverage, as well as in the taxonomic refinement of their diversity. Despite recent advances, the spatial distribution of sampling and detailed taxonomic knowledge remain limited, potentially causing biases in our understanding of their diversity and distribution. In this study, we conducted a large-scale analysis using an extensive database with 951 species and 213,072 georeferenced occurrence records, distributed across 24,319 sampling points in the Amazon. This analysis aimed to elucidate potential drivers of sampling biases for Amazonian amphibians in the presence of infrastructure factors (cities, hydroelectric dams, and transmission lines) and accessibility (navigable rivers and roads). Among accessibility factors, we found that rivers were the main facilitators in amphibian sampling. On the other hand, roads did not exert a strong influence as expected, due to the late and limited development of land transportation in the region, which has historically been dominated by river transportation. Among the infrastructure factors, both cities and hydroelectric plants had a moderate influence on sampling. The reason for this is that most cities in the Amazon region were established a few decades ago and have limited infrastructure, especially considering the presence of consolidated research centers. Hydroelectric plants have generated extensive databases due to environmental legislation requirements for their installation, but restricted access to information from these reports limited their use in this study. We conclude that Amazonian amphibian sampling exhibits significant geographic bias, attributable to the uneven distribution of research efforts caused by logistical challenges, including accessibility and infrastructure limitations. Overcoming these obstacles requires coordinated efforts between researchers and decision-makers, as well as investment in research infrastructure and data dissemination initiatives, not

only for amphibians, but for all biodiversity in the face of increasing deforestation and climate change.

Keywords: Amazonian amphibians, biodiversity, Linnean deficit, Wallacean deficit, macroecology

Introduction

The synergy between technology and conservation enables scientists and conservationists to adopt effective approaches to biodiversity and ecosystem conservation (Arts et al., 2015; Moreto, 2015; Adams, 2019; Toivonen et al., 2019; Vargas-Ramírez & Paneque-Gálvez, 2019; Sandbrook et al., 2021). Computational simulations allow us to understand global biodiversity patterns, as well as to identify and monitor species dispersal (Michelot et al., 2016; Borowicz et al., 2019), and predict the impacts of human activities and climate change on ecosystems (Hopkiins, 2007; dos Santos et al., 2015; Stropp et al., 2020; Albuquerque et al., 2021; Carvalho et al., 2023). However, the application and robustness of these models depend on the quality of the data used, which can be affected by data collection and availability, tabulation, and taxonomic identification, among other factors (Hortal et al., 2015). Therefore, biodiversity data has biases and gaps that cannot be overlooked (Boakes et al., 2010; Martin et al., 2012; Beck et al., 2014; Hortal et al., 2015; Gueta & Carmel, 2016; Anderson et al., 2020; Hughes et al., 2021).

One of the most important barriers observed in biodiversity data for understanding species distributions is the geographical sampling shortcomings, known as the Wallacean shortfall (Hortal et al., 2015). This occurs when some regions are sampled more extensively than others, resulting in uneven knowledge of biodiversity across geographic space (Boakes et al., 2010; Martin et al., 2012; Amano & Sutherland, 2013; Hortal et al., 2015; Anderson et al., 2016;

Pelayo-Villamil et al., 2018; Hughes et al., 2021; Tessarolo et al., 2021; Zizka et al., 2021; Castro-Souza et al., 2024). Inaccessibility of remote areas, which is common in the Amazonia basin, due to lack of infrastructure such as roads and cities, or even legal restrictions, such as bureaucratic hurdles to access Indigenous lands, may contribute to bias in data collection (Santos et al., 2015; Carvalho et al., 2023). In addition, bias related to accessibility and the tendency to study charismatic group (e.g. mammals and birds; Troudet et al., 2017) or easily identifiable species can lead to an underestimation of the real diversity of a region (Diniz-Filho et al., 2010; Theobald et al., 2015; Amano et al., 2016; Oliveira et al., 2016; Troudet et al., 2017; Hughes et al., 2021). Therefore, this is one of the reasons that the distribution of some groups continues to be neglected, contributing to knowledge biases in mega-diverse regions such as the Amazonia.

The Amazon basin hosts the world's largest tropical forest (Vale and Jenkins 2012; Malhado et al. 2013; Santos et al. 2015) comprising approximately 20% of the planet's terrestrial diversity (Peres et al. 2023) and has contributed to the extensive exchange of evolutionary lineages among different regions and biomes over tens of millions of years (Antonelli et al. 2018; Guayasamin et al., 2024). Consequently, it plays a fundamental role in maintaining biodiversity, ecosystems and regulating global climate (Garda et al. 2010; Aragón 2018; Tigre 2019). However, knowledge about Amazonian biodiversity is still underestimated and influenced by biases and sampling gaps (Hopkins 2007; Santos et al. 2015; Stropp et al. 2020; Albuquerque et al. 2021; Carvalho et al. 2023), leading to inefficiencies in public policies for the conservation of Amazonian biodiversity and ecosystem services. These knowledge gaps arise from the inaccessibility of remote areas, combined with neglect and/or reduced investments in research in the Amazon (Carvalho et al., 2023; Stegmann et al., 2024).

Records of amphibian distribution in the Amazonia are incomplete (Guerra et al., 2020; Fouquet et al., 2021), with significant biases and gaps in the geographic sampling.

Amphibians are one of the three taxa with the highest species richness among vertebrates (AmphibiaWeb 2024; Frost 2024). They have a high endemism index, with approximately 77% in the Atlantic Forest (Vancine et al. 2018) and 82% in the Amazonia (Vacher et al. 2020; Penhacek et al., in press), and face the highest risk of species extinction (40.7%) (Amaral et al. 2019; Luedtke et al. 2023). Furthermore, amphibians are recognized as important indicators of environmental changes (Toledo 2009; Becker et al. 2010; Amaral et al. 2019), due to their high vulnerability to climate change and landscape modification (Amaral et al. 2019; Luedtke et al. 2023). Therefore, amphibians are constantly included in monitoring programs for potentially polluting enterprises, but these data have restricted access (Vaz-Silva et al., 2015). Making this data accessible would contribute to understanding their distribution patterns and evaluating the anthropogenic impacts on biodiversity (Dayrell et al., 2021).

Here, we evaluate the spatial distribution of amphibian sampling in the Amazon, testing the influence of accessibility variables (navigable rivers and roads) and infrastructure (cities, hydroelectric power plants, and transmission lines) as drivers of geographical biases in amphibian knowledge. Given the structural and environmental complexity, as well as the vast territorial extent, we believe that accessibility variables will be the main driver of the geographical knowledge of sampling, followed by the establishment of cities and hydroelectric enterprises such as hydroelectric power plants (HPP) and transmission lines (TL). To facilitate the visualization of sampling patterns, maps were designed to spatially highlight the combined effects of drivers on the distribution of amphibian sampling in the Amazonia, also showing the main sampling gaps. The biases and gaps detected here can serve as alerts to the existence of similar sampling biases in the geographical knowledge of other taxa of terrestrial Amazonian biodiversity, thus contributing to guidance for future biodiversity research and conservation actions.

Materials and Methods

Study Area

The study covered all the Amazonian boundaries proposed by the WWF (WWF 2019). Located in northern South America, the Amazon basin covers an area of approximately 6.5 million km² and includes nine countries: Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guiana, Peru, Suriname, and Venezuela (WWF 2019; Tigre 2019). Although its vast territory contains approximately 50% of all remaining tropical rainforests on the planet, the region also includes limited areas of non-forest vegetation, such as savannas and seasonally flooded grasslands (Schuman et al. 2007, Peres et al. 2010, Castuera-Oliveira et al. 2020). The Amazon basin holds the largest reserves of liquid freshwater, with more than 7,000 tributaries (HidroSHEDS 2024) and about 20% of the world's freshwater flow (Tigre 2019), which also serve as important transport routes and accessibility. Regarding mineral resources, the region has attracted global attention for its vast reserves of aluminum, iron, niobium, and gold, among others. Mineral extraction has an impact on traditional communities, indigenous peoples, and biodiversity (Mello et al. 2013), due to the opening of roads (Laurance et al. 2009) and infrastructure for mining (Siqueira-Gay et al. 2020). Additionally, the vast expanse of arable land and high rainfall have led to a huge expansion of agribusiness, resulting in forest fragmentation, soil, air, and water pollution, and threats to biodiversity and ecosystem services (Fearnside 2015).

Our amphibian occurrences database was created from four primary sources (Fig. 1A): I) "digitally accessible data platforms" (GBIF, SiBBR, SISBIO, Specieslink, and VertNet) collected until February 2022; II) "peer-reviewed articles," consisting of 150 articles published in scientific journals containing information on the occurrence of amphibian in the Amazon; III) "grey literature," which includes technical reports from Environmental Impact Studies/Environmental Impact Reports, as well as records from rescue and monitoring of

amphibians in Hydroelectric Power Plants (HPPs); and IV) "own data," which comprises personal collections of the authors spanning 15 years, from 2007 to 2022, from southern Brazilian Amazon (for more details, see Penhacek et al. in press). Our data contain an extensive database with 163,643 primary records related to 947 species of amphibians.

To this database, we added 52,529 records from 98 species collected between 2011 and 2019 during monitoring and rescue programs at four Hydroelectric Power Plants - (HPPs): Colíder, São Manoel, Sinop and Teles Pires. These data were obtained from the respective licensing agencies: the Mato Grosso State Secretariat for the Environment - SEMA (Colíder and Sinop HPPs) and the Brazilian Institute for the Environment and Renewable Natural Resources - IBAMA (São Manoel and Teles Pires HPPs).

The database underwent a filtering process where records identified above the species level, such as those at the genus (sp), group (gr), related (aff) or confer (cf) levels, were excluded. Then, the remaining species occurrences were taxonomically updated by joining synonyms for the most recent valid species names (according to Frost 2024). Subsequently, each species underwent distribution evaluation using three specialized platforms: AmphibiaWeb (2024), Frost (2024) and IUCN (2024). For more details on data validation (see Penhacek et al. in press). Thus, the final database has 213,072 primary records in 24,319 sampling points in the Amazon, encompassing 951 species (Supplementary Material Worksheet S1).

Accessibility and Infrastructure Variables

To understand the drivers (Fig. 1B) of bias in amphibian samplings, we used five explanatory variables (drivers) related to accessibility (distance to navigable rivers and highways) and infrastructure (cities, hydroelectric power plants, and power transmission lines). We used data from the HydroRIVERS database, specifically focusing on rivers classified within the first five levels of magnitude based on water flow capacity (Lehner and Grill 2013). For highways, we considered federal, state, and/or municipal roads obtained from the Center for

International Earth Science Information Network (CIESIN 2023), Rede Amazônia de
Informação Socioambiental (RAISG 2023), and the Instituto Brasileiro de Geografia e
Estatística (IBGE 2014). We also used information from the following sources: (i) Cities (143
reference urban centers in each location, involving the largest cities in each region), assuming
that they have better infrastructure to support researchers, obtained from Natural Earth Data
(Natural Earth 2023); (ii) Hydroelectric Power Plants (312 Hydroelectric Power Plants, HPPs
and Small Hydropower plants - SHPPs) under construction and/or in operation, obtained from
the Amazon Network of Georeferenced Socio-Environmental Information – RAISG (RAISG
2023); and (iii) power transmission lines (LTs), provided by Arderne et al. (2020). These
shapefiles were incorporated into vector files with a resolution of 0.05° (5 x 5 km in Equator),
containing points representing cities and HPPs/SHPPs, and lines representing the LTs.

Data Analysis

To assess the influence of accessibility and infrastructure variables on amphibian
sampling, we used the Bayesian analysis proposed by Zizka et al. (2020) to compare the
statistical distribution of observed distances (actual occurrences) with a null model (expected
distribution simulated by random sampling). Initially, we evaluated the weight of each variable,
indicating the intensity of bias generated by the presence of each selected variable in the study
area (Figure 1C). We then calculated the correlation between sampling (number of known
amphibian occurrences per grid) and distance (km) for each bias variable within the study area
(Figure 1D).

Finally, we created a spatial projection map that illustrates the combined effects of
accessibility and infrastructure variables on the estimated sampling of amphibian occurrence
records. This map highlights the regions where these variables have a greater effect on sampling
bias, indicating areas that are oversampled (Figure 1E). In this context, we measured the effect

based on the variable with the highest bias, combining it in descending order with the other variables (Supplementary Material Figure S1). These approaches allow us to comprehend the relative influence of different precursor variables of bias on the sampling of amphibians and spatially project their combined effects. This is crucial for better understanding the distribution and representativeness of amphibian occurrence records in the Amazonia, as well as for identifying areas where sampling effort needs to be optimized.

All analysis was carried out in the R Program (R Development Core Team, 2022). Sampbias biases were analyzed using the sampbias package (Zizka et al., 2020). QGIS 3.4 software (Free, 2023) was used to create all cartographic projections, including the bias maps. Figure 1 here

Results

Amphibian sampling distribution in the Amazon

With the robust dataset used here, consisting of over 213,000 occurrence records, our biogeographic projection (Figure 2) revealed an uneven sampling distribution with oversampled (biased) and undersampled (knowledge gap) regions for amphibians in the Amazon, characterizing a strong bias for this taxon in this region.

Figure 2 here

Sampling bias

Sampling is concentrated and strongly biased towards locations close to rivers, followed weaklier by highways, urban centers and hydroelectric plants. In contrast, the proximity of electricity transmission lines (LT) had little influence on sampling (Figure 3).

Figure 3 here

Amphibian sampling data across the Amazon revealed an uneven distribution, with oversampled (biased) and under sampled (knowledge gaps) regions (Fig. 4). Oversampled regions were concentrated especially in the southwest and west regions of the Amazon, near the

Andes Mountain Range (southern Peru to northern Ecuador). In the Brazilian Amazon, this bias was pronounced for almost the entire Madeira River basin in the state of Rondônia, as well as along the upper and lower Tapajos River basin in the states of Mato Grosso and Pará, respectively. Other regions, such as the basins of the Tocantins River in the state of Maranhão, Rio Branco River in the state of Roraima, and along the Amazon River from the confluence of the Negro and Solimoes Rivers in the state of Amazonas to its estuary in the Atlantic Ocean, also presented biases in amphibian sampling (Fig. 4). In Colombia, sampling was biased towards the southeast region near the Vaupés department, which borders Brazil. In Guyana, the bias was observed in the Berbice and Essequibo River basins. Biases were also observed in the northwestern and eastern regions of French Guiana, throughout the northwestern region of Suriname, and in the northern region of Venezuela. In addition to these regions, other smaller areas with sampling biases were observed throughout the Amazonia (Fig. 4).

On the other hand, an extensive area is under sampled in the Amazon basin, highlighting the central-western between the Negro and Solimoes River basins, the middle and upper region of the Xingu River, and the eastern, western, and northern parts of the state of Maranhão, Amapá, and Pará, in Brazil, respectively (Fig. 4). Additionally, we observed a gap in amphibian sampling in the entire central and eastern region of Bolivia, the entire eastern extent of Peru, and the central, northern, and southern regions of Colombia, as well as smaller gaps in the southern regions of Suriname and French Guiana, and the northwest region of Venezuela (Figure 4).

Figure 4 here

Discussion

Our study analyzed the sampling bias of Amazonian amphibians using a robust database that consolidates records for over 11% of global amphibian diversity (Frost 2024). Despite this extensive database, with more than 213 thousand occurrence records, it represents less than 20% of the currently available data for amphibians in the Amazonia (Penhacek et al., in preparation).

This limitation occurs due to absence of geographical coordinates in species records (Wallacean shortfall), taxonomic incompleteness (Linnean shortfall) often found in scientific collection records, digital platforms (e.g., Stropp et al., 2020; Araújo et al., 2022), driven by the high diversity of cryptic amphibian species existing in the Amazon, combined with the scarcity of regional taxonomists.

Even with the robust and extensive database used in this work, we observed that the Amazon is not adequately sampled in all its regions. While we observe sampling concentrations in some regions, mainly on the western edges of the Peruvian and Ecuadorian Amazon and near large rivers such as the Amazon, Madeira, Tapajos and lower Xingu among others (Fig. 1), there are extensive areas that are poorly sampled or neglected throughout the Amazon, but mainly in the southeastern and central western regions of the Amazon (Fig. 4).

The extensive territory of the Amazon, often surrounded by almost inaccessible dense forests, presents challenges for carrying out biological surveys. This increases the sampling deficit “Wallacean deficits” (Hortal et al. 2015), leading to both oversampling in easily accessible areas and undersampling in difficult-to-access areas, creating gaps in our understanding of biodiversity patterns. These gaps found in the Amazon can be attributed to logistical factors, infrastructure limitations, taxonomic challenges, and landscape changes caused by human intervention. Overcoming these obstacles will require coordinated efforts, investments in research and monitoring, and the commitment of countries to United Nations Sustainable Development Goal 15 (‘Life on Land’) of the 2030 Agenda for Sustainable Development, aimed at the conservation and sustainability of the region (UN BR 2030).

Our results revealed significant sampling biases for Amazonian amphibians related to accessibility variables (especially rivers) and urban and industrial infrastructure. As expected, medium and large rivers are the most important factors in amphibian sampling globally in the Amazon. Similar results have been observed for different taxonomic groups studied in the Amazon, including plants (Hopkins 2007, Stropp et al. 2020, Araújo et al. 2022), ants

(Albuquerque et al. 2021) and multi-taxa (Santos et al. 2015, Oliveira et al. 2016, Carvalho et al. 2023). Despite the differences between taxonomic groups, well-sampled areas are consistently those close to rivers and cities. These biases may hinder the understanding of the distribution patterns of Amazonian biodiversity (Daru & Rodriguez, 2023).

Due to the recent surge in road paving and construction projects across the Amazon, along with the consequent need for fauna surveys and impact assessments, during these projects, we initially believed that highways could be a significant driver of sampling bias, as observed for different taxonomic groups such as arthropods, vertebrates, and angiosperms (Oliveira et al. 2016; Andrade-Silva et al., 2022). However, investments in infrastructure in the interior regions of the Amazon are still recent and sporadic (Araújo et al., 2023). Historically, rivers have been and remain the main means of transportation in the Amazon (Hernández-Fontes, et al., 2021), especially in low-lying areas and along wide rivers. In these regions, road construction is considerably hindered by river flooding and soil types, creating significant obstacles to travel during the rainy season. However, during this period, there is an increase in vocalization, foraging, and mating activities of amphibian species. (Bastos and Haddad 2007, Ferrão et al. 2024). This leads herpetologists to prefer sampling during this time, due to the greater local aggregation of species and the increased efficiency in recording amphibian species richness (Ferrão et al. 2024). Therefore, river accessibility is fundamental for reaching remote areas of the Amazon, enabling the cataloging of species from these locations (Carvalho et al., 2023; This study).

The distance from cities (Carvalho et al., 2023) and Hydroelectric Power Plant (Dayrell, et al. 2021) are also considered drivers of biodiversity sampling in the Amazon. However, in this study, the effect of these variables was smaller compared to that of navigable rivers (Fig. 3). Cities with populations greater than 100,000 inhabitants tend to have better infrastructure, including airports, bus terminals and research centers such as universities. Consequently, the number of researchers and research activities near these cities is typically higher, as observed by

Hopkins (2007). Furthermore, sampling in remote and hard-to-reach locations is discouraged due to the high logistics costs and the limited financial resources allocated per km² in the Amazon, especially in the Brazilian region (Fernandes et al., 2017; Barlow et al., 2018; Magnusson et al., 2016, 2018; Hopkins, 2019; Carvalho et al., 2023).

The construction of Hydroelectric Power Plants (HPPs) and Small Hydropower plants (SHPPs) has significantly intensified in the Amazon over the past three decades (Brasil, 2024). Due to their impacts on fauna and flora, and considering the high number n of projects to be implemented in the Amazon (Cavalcante et al., 2021; Dayrell et al., 2021), the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) approved normative instruction (IN 146, dated January 10, 2007). This regulation establishes the necessity for standardized monitoring criteria to assess potential impacts on biodiversity (MMA, 2016). Thus, a large amount of data on biodiversity has been produced in recent years (e.g., Ávila & Kawashita-Ribeiro, 2011; Vaz-Silva et al., 2015; Dayrell et al., 2021). These databases have significant potential in cataloging species and ecological data in previously unexplored or difficult-to-access regions, with great potential to fill gaps in knowledge about biodiversity. However, a large portion of the data obtained during these projects is not adequately available, making it difficult for scientists and decision-makers to obtain and/or use them (Penhacek et al., in press). Therefore, the correct identification and deposition of specimens in scientific collections, standardization of collected data, and the dissemination of results as species list by environmental agencies would make this information more accessible and useful for researchers and environmental managers, enhancing the effectiveness of decision-making and conservation efforts.

Our results also reveal that there is regional variation in the effect of variables that contribute to sampling bias. Although rivers have shown a greater influence on sampling bias globally across the Amazon, there are extensive sampling gaps in areas with high river density, particularly along the middle and upper Xingu basin in the southeastern Amazon, and in the

basins of the Coquetá, Jupara, Jurua, Napo, Solimoes, Purus, Putumayo rivers, among others, in the central-western region of the Amazon (Figure 4). Therefore, in each Amazonian region, different factors may predominantly influence the sampling rate for amphibians.

Finality this study revealed the drivers that explain the sampling bias of Amazonian amphibians, highlighting gaps and challenges in the collection and analysis of biogeographic data. These significant biases, driven by the proximity of navigable rivers, highways, urban centers, and hydroelectric plants, affect the interpretation of species distribution patterns and limit the use of data for predictive modeling, which is essential for biodiversity conservation plans. Moreover, they undermine our understanding of species dispersal, space-time occupation patterns and the effects of human activities and climate change on biogeographic patterns. Thus, the identification of undersampled regions evidenced here, especially in the southwest and central-west Amazon, highlights the need for targeted efforts to fill these gaps to achieve a more complete and accurate representation of amphibian diversity in the region. Such efforts are necessary to protect biodiversity in the Amazon due to the ongoing threats of deforestation and ongoing climate change.

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FIGURAS

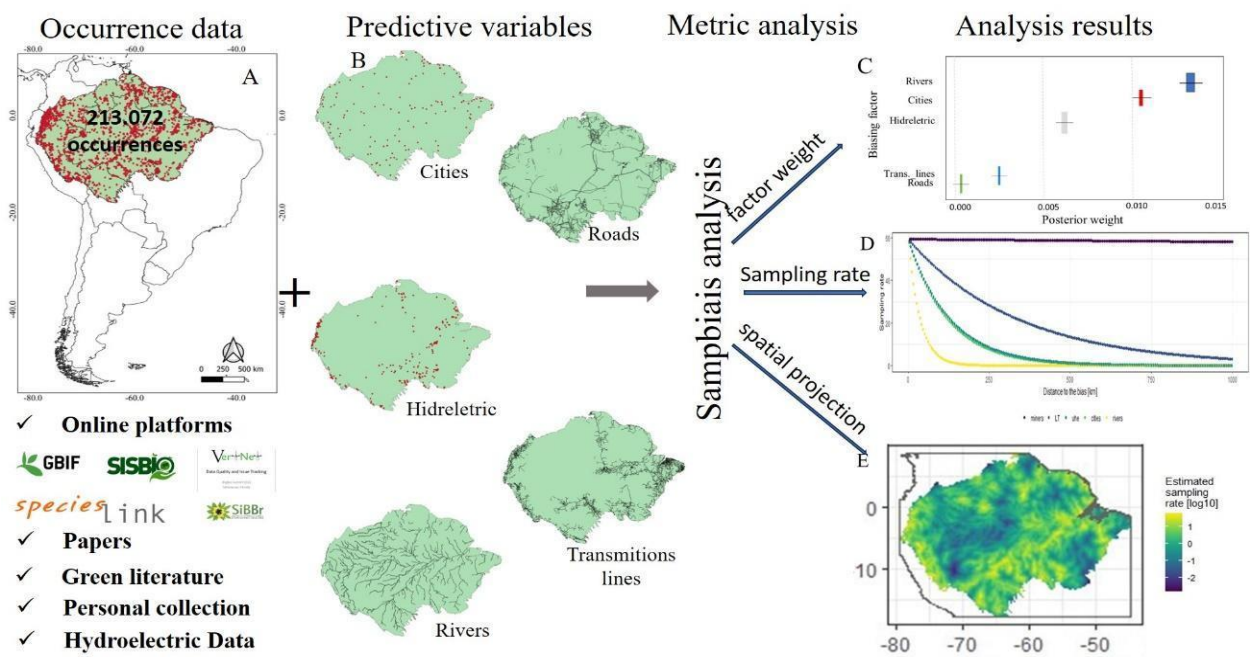


Figure 1. Methodology for evaluating accessibility and infrastructure drivers in Amazonian amphibian sampling biases. Occurrences of amphibian records in the Amazon (A); predictive variables: cities, rivers, hydroelectric plants, roads and transmission lines (B); weights of variables in sampling bias (C); sampling rate as a function of distance for each variable (expected number of occurrences) (D) and; spatial projection of the combined bias effect from accessibility and infrastructure variables (E).

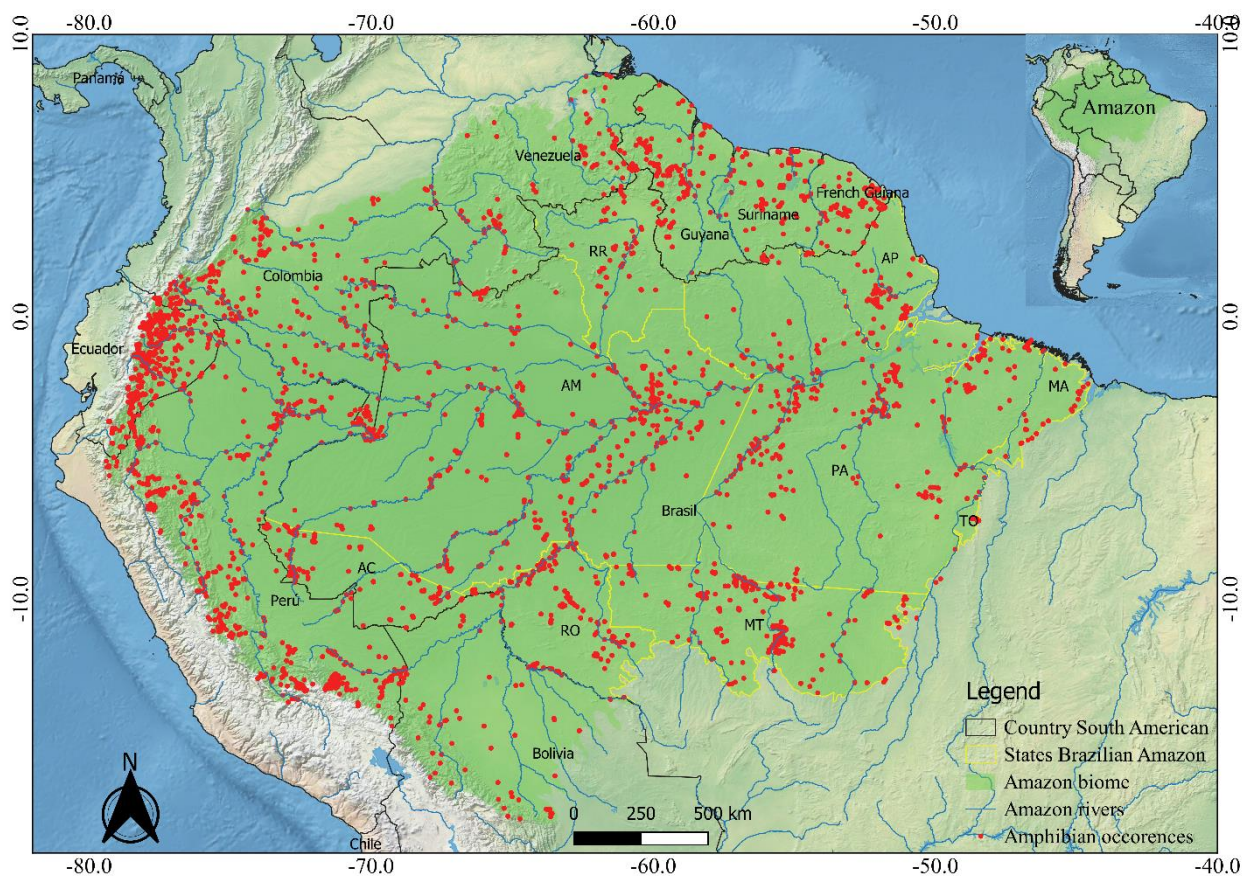


Figure 2. Distribution of the 24,319 amphibian sampling points in the Amazon delimited according to WWF (2019).

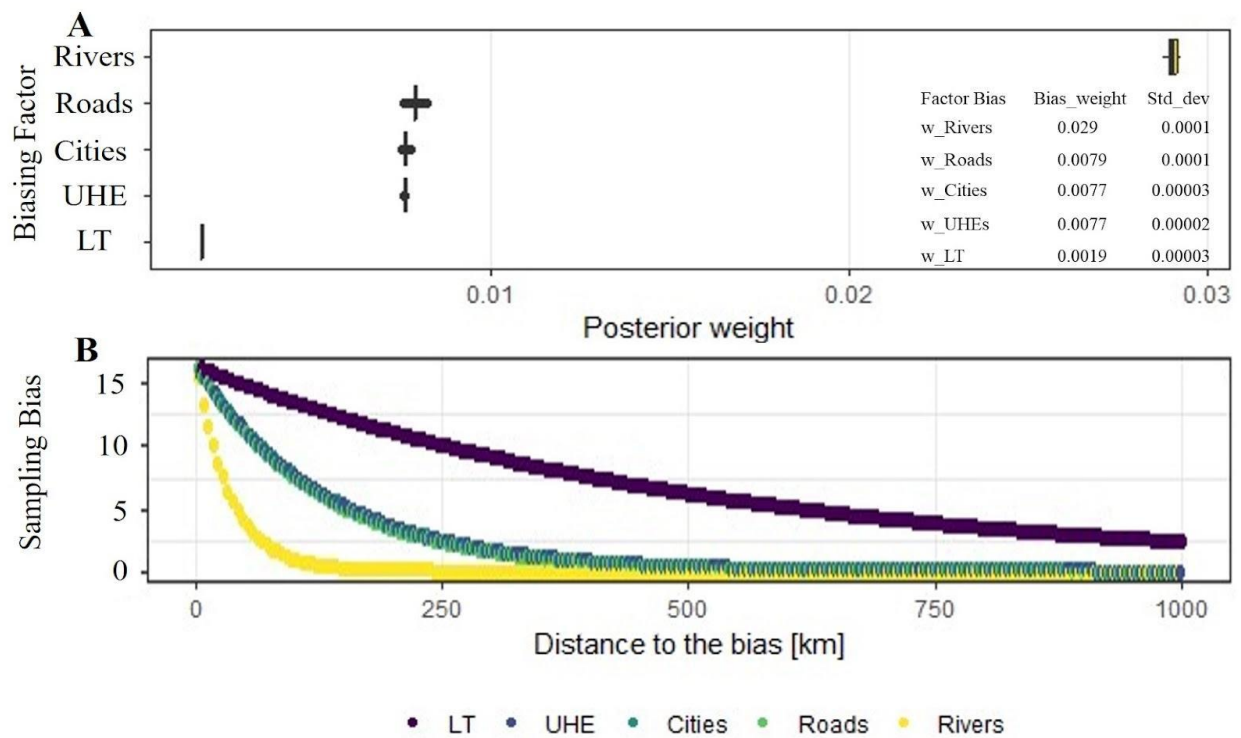


Figure 3. Bias in amphibian occurrences in Amazonia related to accessibility and infrastructure variables. The bias weights (w) represent the influence of each bias variable, and the Factor Bias refers to the specific variable evaluated (A). Sampling rate as a function of the distance to the closest point of each bias variable (the expected number of occurrences) given the inferred sampbias model (B). At the study scale of 0.05 degrees (about 5×5 km), sampbias identified the strongest polarization effect in proximity to rivers, highways, cities, hydroelectric plants, and transmission lines, respectively.

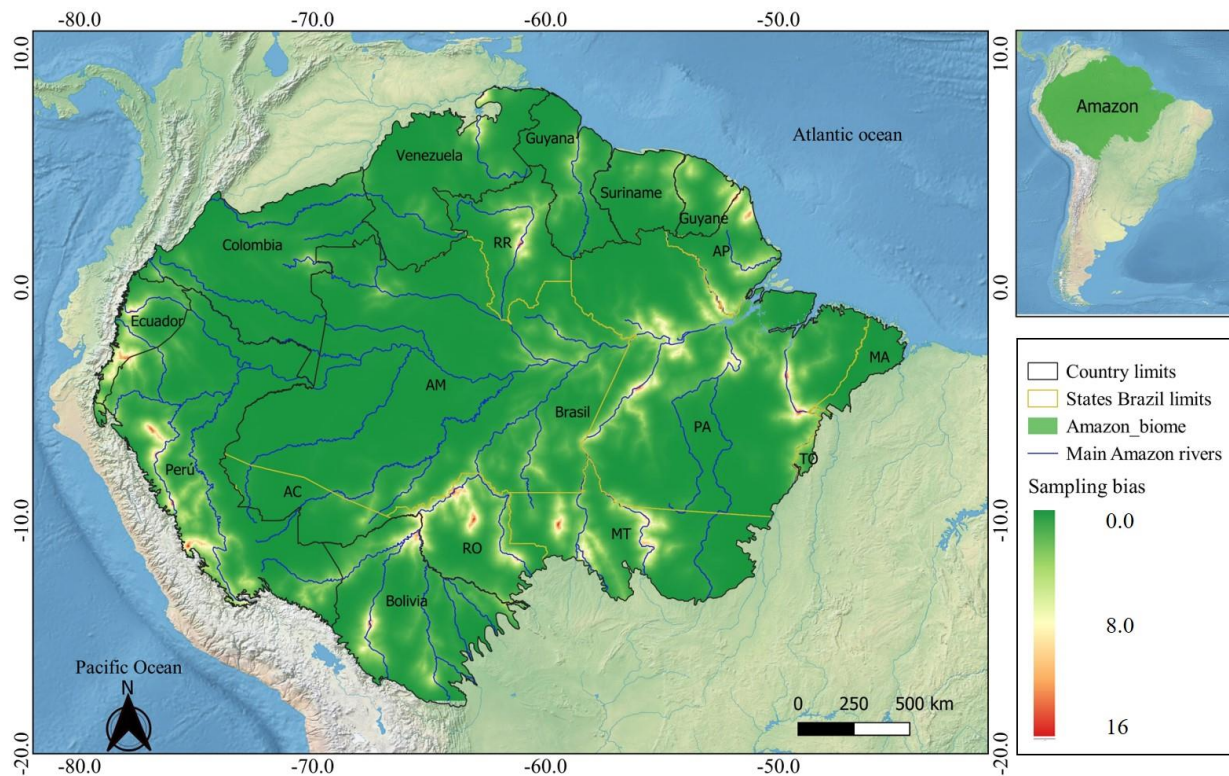


Figure 4. Spatial variation in amphibian sampling intensity across the Amazon basin. Colors show the projection of log10-transformed sampling rates (i.e., number of sample occurrences per cell) when compared to null (stochastic) models. Values close to -3 indicate under sampled areas (gaps), while those near 1 indicate oversampled (biased). The acronyms correspond to Brazilian states, AC - Acre, AM - Amazonas, AP - Amapá, MA - Maranhão, MT - Mato Grosso, PR - Pará, RA - Roraima, RO - Rondônia and TO - Tocantins. Separate effects of the variables can be seen in Supplementary Material figure S1.