# Detailed Methodology

## Study site

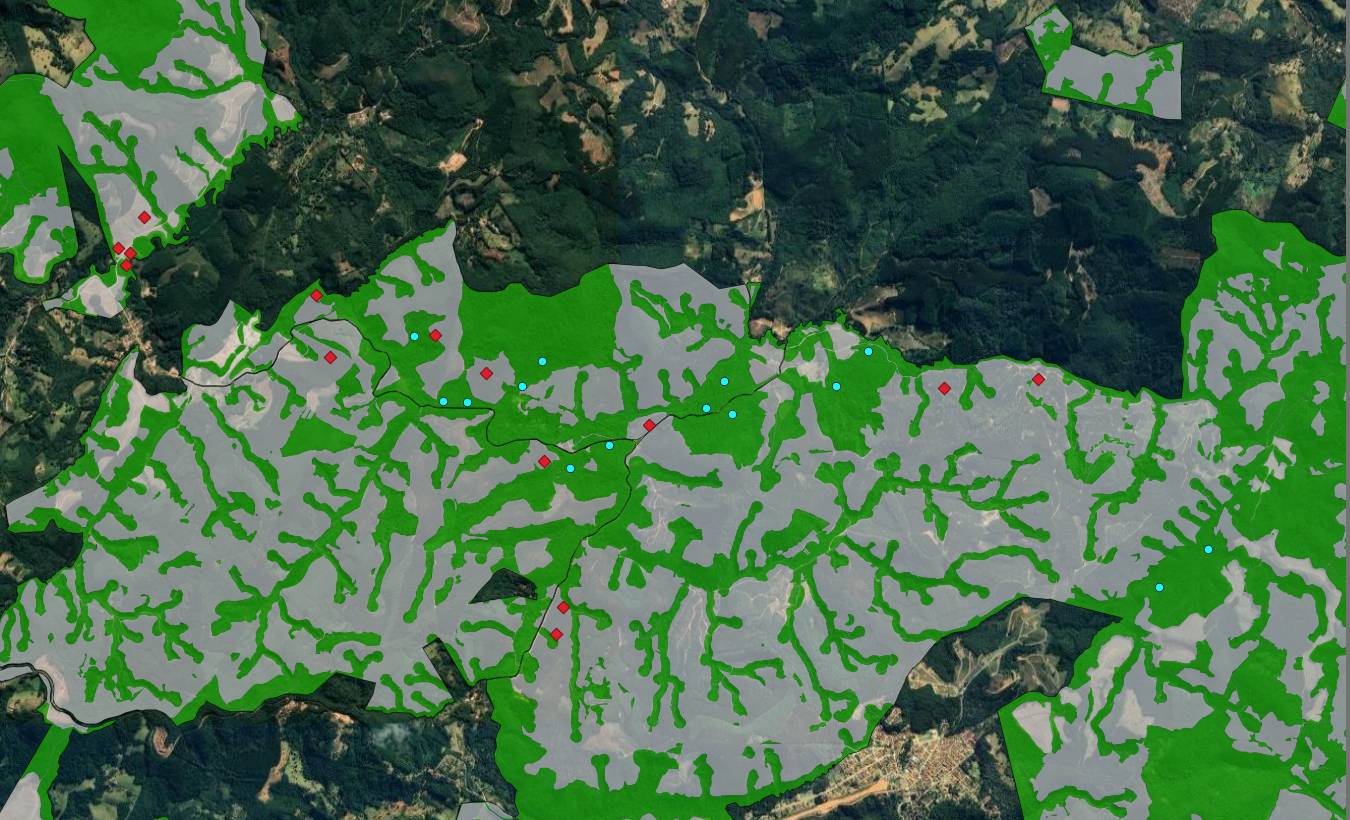
We studied the diet of anurans in an Atlantic Forest in the Camanducaia municipality, Minas Gerais state, Brazil (22°49’37''S, 46°05’26''W, 1257 m above sea level), on the extreme southwest of the Mantiqueira mountain range. The predominant natural vegetation is Mixed Ombrophilous Forest with Araucaria (*Araucaria angustifolia*), but it is located in a mosaic of native forest protected areas and *Eucalyptus* spp. and *Pinus* spp. monocultures (Table A, Figure 1). The region's climate is characterized as Subtropical highland variety (Alvares et al., 2013). Annual rainfall for the area is 1608 mm and mean daily temperatures range from 12.3 ºC to 20.2 ºC (Fick & Hijmans, 2017).

## Sampling methods

Both anurans and invertebrate prey were sampled along 28 600 m transects, evenly distributed between well preserved Atlantic Forest sites and *Eucalyptus* plantation sites. Anurans were found by visual encounter surveys (Crump & Scott Jr, 1994). Sampling occurred from december 2018 to december 2020, once a month, plus one sampling in february 2024, with a random subset of encounters being collected for dietary analyses, with the aim of respecting sampling quotas (SISBIO #59947 and #16593). The snout-vent length (SVL), mouth-width (MW) and weight was measured for all collected individuals using an analog caliper (nearest 0.01 mm) and a spring scale (nearest 0.01g). Anuran specimens were then euthanized using a topical anesthetic (Xylocaine 5%) and injectable solution for large toad individuals (Lidocaine 2g/ml and Epinephrine 2mg/ml), and were then fixed with 10% formaldehyde. We removed stomachs through a small abdominal incision and stored the contents in individualized vials in 70% alcohol.

**Table A:** Sampling locations. Not all sites were sampled for invertebrates, due to logistical problems. At E\_FA and E\_FB, the silviculture was cut during sampling and M\_PA and M\_PB became inaccessible due to road closures over heavy rain.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Site** | **Code** | **Environment** | **Latitude** | **Longitude** |
| Trevo A | E\_TA | Eucalyptus | -22.83041 | -46.05217 |
| Trevo B | E\_TB | Eucalyptus | -22.83324 | -46.06098 |
| Canal A | E\_CAA | Eucalyptus | -22.82509 | -46.07903 |
| Canal B | E\_CAB | Eucalyptus | -22.82033 | -46.08024 |
| Vargem Limpa A | E\_VLA | Eucalyptus | -22.82684 | -46.01931 |
| Vargem Limpa B | E\_VLB | Eucalyptus | -22.82755 | -46.02728 |
| Fazenda A | E\_FA | Eucalyptus | -22.81687 | -46.09632 |
| Fazenda B | E\_FB | Eucalyptus | -22.81424 | -46.09473 |
| Bela Vista A | E\_BVA | Eucalyptus | -22.82639 | -46.0659 |
| Bela Vista B | E\_BVB | Eucalyptus | -22.82343 | -46.07017 |
| Fazenda C | E\_FC | Eucalyptus | -22.8176 | -46.0964 |
| Fazenda D | E\_FD | Eucalyptus | -22.817 | -46.097 |
| 310 A | E\_TRA | Eucalyptus | -22.8446 | -46.0594 |
| 310 B | E\_TRB | Eucalyptus | -22.8467 | -46.06 |
| Capelinha A | M\_CA | Atlantic Forest | -22.83372 | -46.05878 |
| Capelinha B | M\_CB | Atlantic Forest | -22.83194 | -46.05554 |
| Araucária A | M\_AA | Atlantic Forest | -22.82698 | -46.04581 |
| Araucária B | M\_AB | Atlantic Forest | -22.82958 | -46.04512 |
| Ponciano A | M\_PA | Atlantic Forest | -22.84299 | -46.00911 |
| Ponciano B | M\_PB | Atlantic Forest | -22.84003 | -46.00498 |
| Fazenda A | M\_FA | Atlantic Forest | -22.82462 | -46.03363 |
| Fazenda B | M\_FB | Atlantic Forest | -22.82739 | -46.03637 |
| Bela Vista A | M\_BVA | Atlantic Forest | -22.82352 | -46.07201 |
| Bela Vista B | M\_BVB | Atlantic Forest | -22.82545 | -46.06119 |
| Bela Vista C | M\_BVC | Atlantic Forest | -22.8285 | -46.0696 |
| Bela Vista D | M\_BVD | Atlantic Forest | -22.8286 | -46.0675 |
| Araucária C | M\_AC | Atlantic Forest | -22.8291 | -46.0473 |
| Barroso | M\_BR | Atlantic Forest | -22.8274 | -46.0628 |

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## Figure 1: Site sampling and land cover of the study area. Black lines delimit the property area and green overlay represent forest remnants, while grey represent silviculture cover. Red squares are sampled sites in *Eucalyptus* and blue dots are sampled sites in Atlantic Forest remnants.

Frog individuals were preserved in 70% alcohol and will be deposited at the CFBH collection, Universidade Estadual Paulista, Rio Claro, SP, Brazil.

The width and length of each prey item retrieved from an anuran gastrointestinal tract was measured under a stereomicroscope with millimetric paper (nearest 0.5 mm). We then estimated prey volume with the ellipsoid formula (Dunham, 1983): V = 4 \* π \* L \* W23, where V = volume, L = length and W = width. As prey items from stomach content are usually partially digested, we classified prey items into coarse taxonomic categories, usually at sub-Order, with an exception for Formicidae because of their particular morphological and ecological traits and because some anurans are ant specialists (Brunetti et al., 2023). Larvae of all taxa were always categorized separately from the adults. Both non-identifiable (NI) and incomplete items from which measurements could not be estimated were not included in further analyses. We chose this classification approach because it has brought significant insights into trophic networks in the past (Cohen, 1977; Pinto-Coelho et al., 2021), in particular, to anuran-prey trophic interactions (Ceron et al., 2019). Importantly, trophic network structure can be informative regardless of the level of taxonomic resolution of nodes (Guimarães, 2020).

From these data we describe the resource use by anurans as two weighted bipartite matrices (Costa et al., 2007): A, in which aij is the total volume of prey type *j* in the diet of anuran *i* and B, in which bij is the total frequency of prey type *j* in the diet of anuran *i*. To minimize differences in diet composition arising from sampling effort, we only included anuran species with more than five complete prey items per environment in the analyses.

To estimate prey availability, a 150 m transect was established in each sample site, with four trap clusters 50 m apart, including one pitfall trap (400 ml plastic cups) and one glue trap (10 cm x 10 cm) 1.2 m above ground, tied to the closest tree. Traps stayed active for 24 h once in December 2020 and once in February 2024. Sampled invertebrates were preserved in vials in a 70% alcohol solution. All items were measured and identified, following the description for dietary items (see above). Only prey categories identified in at least one anuran species were considered.

## Community composition

To evaluate the effects of the environment on anuran and invertebrate assemblages, we tested for differences between habitat types in abundance (count) and diversity (Shannon’s index, Shannon, 1948). To analyze the effects of the environment on abundance and diversity, a generalized linear model (GLM) was fitted using R version 4.3.3 with the lme4 package (Bates et al., 2015; R Core Team, 2022), adopting poissonand gaussian distributions respectively. We looked for spatial correlation in the data using Moran's I statistic (Li et al., 2007), but results were non-significant (p > 0.05).

We also tested compositional differences between anuran and invertebrates among environments by calculating the Bray–Curtis compositional dissimilarity index (Bray & Curtis, 1957), using the distance function in vegan, between each pair of sample sites (transects). To visualize the dissimilarity patterns, we used NMDS for both anurans and invertebrates. We used perMANOVA in vegan (Oksanen et al., 2022) to test for significant differences in species composition among environments.

## Niche dissimilarity and descriptors of network structure

The heterogeneity of ecological systems can be simplified through the use of network metrics, which reveal the structural patterns of interactions (Guimarães, 2020). To describe the structure of the anuran-prey network we use connectance and modularity: (a) connectance, which describes the proportion of realized interactions in the network, and (b) modularity, which describes sets of predators and prey that interact more with each other than with the rest of the network.

Connectance describes the ratio of the number of interactions recorded relative to the possible number of network interactions, ranging from 0 (non-connected network) to 1 (fully connected network); Once there is a negative correlation between connectance and network size, the number of nodes in a network (Jordano, 1987), we compared both measured connectance in our networks to previously described anuran-prey networks in the literature (Table B). We compiled interactions using previously studied networks in Ceron (2019) via personal correspondence with the author for unpublished data and expanding this database with an additional review of the literature, following the same methods. We searched for studies in the Web of Science and Scopus databases, using the keywords ‘Trophic ecology OR Feed\* OR Diet\*’ and ‘assemblage structure’ and ‘trophic overlap OR trophic plasticity OR niche breadth OR Autoecol\*’ up from 2018 up to 2023. Among these compiled references, we selected only studies presenting data on diet and including at least three syntopic species of anurans and belonging to natural, preserved or recovering habitats. To assess the correspondence between our networks and the literature, we fitted the literature data to a generalized linear model with the form y ∼ log(x) and ran one-sample t-tests for both, *Eucalyptus* and Atlantic Forest networks, to assess the significance of the connectance deviation from the model.

**Table B:** Information of each network reference (data source). country, geographical coordinates (decimal degrees), number of recorded anurans species and prey types, network size (number of anurans + number of prey) and connectance. References 1 to 55 are from Ceron (2019).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Reference** | **Country** | **Latitude** | **Longitude** | **Nº**  **anuran** | **Nº**  **prey types** | **Network**  **size** | **Connectance** |
| 1 | Argentina | -31,671894 | -60,625194 | 6 | 18 | 24 | 0,602 |
| 2 | Argentina | -27,500158 | -58,750208 | 16 | 24 | 40 | 0,414 |
| 3 | Australia | -35,311198 | 148,655701 | 5 | 14 | 19 | 0,520 |
| 4 | Brazil | -22,433447 | -42,983342 | 3 | 25 | 28 | 0,640 |
| 5 | Brazil | -16,230556 | -48,080278 | 3 | 14 | 17 | 0,595 |
| 6 | Brazil | -7,180408 | -35,094242 | 3 | 22 | 25 | 0,697 |
| 7 | Brazil | -10,541669 | -37,058378 | 16 | 20 | 36 | 0,266 |
| 8 | Brazil | -9,65 | -37,666747 | 11 | 20 | 31 | 0,350 |
| 9 | Brazil | -6,585997 | -37,267303 | 16 | 23 | 39 | 0,353 |
| 10 | Brazil | -6,664989 | -40,208611 | 16 | 20 | 36 | 0,303 |
| 11 | Brazil | -6,721503 | -35,189322 | 26 | 21 | 47 | 0,293 |
| 12 | Brazil | -7,416694 | -36,514381 | 16 | 27 | 43 | 0,347 |
| 13 | Brazil | -18,916672 | -48,3 | 4 | 26 | 30 | 0,721 |
| 14 | Brazil | -21,732272 | -43,370233 | 3 | 16 | 19 | 0,542 |
| 15 | Brazil | -14,798778 | -52,642033 | 4 | 15 | 19 | 0,583 |
| 16 | Brazil | -29,383333 | -50,383333 | 7 | 20 | 27 | 0,479 |
| 17 | Brazil | -7,474444 | -38,344167 | 13 | 25 | 38 | 0,338 |
| 18 | Brazil | -20,576886 | -49,316667 | 3 | 20 | 23 | 0,617 |
| 19 | Brazil | -21,664511 | -57,717886 | 12 | 17 | 29 | 0,299 |
| 20 | Brazil | -9 | -41 | 3 | 15 | 18 | 0,733 |
| 21 | Brazil | -15,486694 | -47,689242 | 5 | 17 | 22 | 0,529 |
| 22 | Brazil | -28,134444 | -49,479722 | 3 | 23 | 26 | 0,725 |
| 23 | China | 30,291585 | 122,170978 | 3 | 14 | 17 | 0,810 |
| 24 | Colombia | 10,081033 | -74,001217 | 5 | 19 | 24 | 0,642 |
| 25 | Colombia | 7,05 | -72,950144 | 6 | 14 | 20 | 0,548 |
| 26 | Colombia | 4,833333 | -76,25 | 17 | 20 | 37 | 0,382 |
| 27 | Ecuador | 0,05 | -76,983564 | 64 | 20 | 84 | 0,274 |
| 28 | Ecuador | -0,766886 | -76,1 | 38 | 22 | 60 | 0,291 |
| 29 | Hungary | 46,637302 | 17,142793 | 3 | 21 | 24 | 0,651 |
| 30 | Italy | 42,139225 | 12,102483 | 6 | 21 | 27 | 0,540 |
| 31 | Ivory Coast | 5,528614 | -4,019383 | 4 | 15 | 19 | 0,650 |
| 32 | Malasyia | 1,616667 | 113,583333 | 10 | 10 | 20 | 0,600 |
| 33 | Mexico | 29,462097 | -110,614017 | 3 | 9 | 12 | 0,741 |
| 34 | Nigeria | 5,117247 | 7,78752 | 4 | 22 | 26 | 0,636 |
| 35 | Panama | 9,083333 | -79,833333 | 8 | 4 | 12 | 0,625 |
| 36 | Panama | 9,333333 | -78,916667 | 20 | 4 | 24 | 0,750 |
| 37 | Peru | -9,583333 | -74,8 | 13 | 4 | 17 | 0,846 |
| 38 | Peru | -12,583333 | -69,083333 | 57 | 27 | 84 | 0,270 |
| 39 | Romania | 45,169117 | 27,947175 | 4 | 12 | 16 | 0,896 |
| 40 | Singapore | 1,315169 | 103,816303 | 6 | 22 | 28 | 0,636 |
| 41 | Spain | 40,282414 | -6,661025 | 7 | 30 | 37 | 0,538 |
| 42 | Taiwan | 23,428333 | 120,485 | 5 | 19 | 24 | 0,558 |
| 43 | Uruguay | -34,333608 | -57,000211 | 3 | 12 | 15 | 0,694 |
| 44 | Uruguay | -34,616667 | -55,366669 | 4 | 13 | 17 | 0,712 |
| 45 | Venezuela | 8,616667 | -71,15 | 6 | 26 | 32 | 0,506 |
| 46 | Brazil | -30,235556 | -51.095 | 4 | 22 | 26 | 0,602 |
| 47 | Ecuador | -1,083 | -75,917 | 68 | 35 | 103 | 0,094 |
| 48 | Brazil | -25,507778 | -49,025 | 8 | 26 | 34 | 0,447 |
| 49 | Philipines | 8,973611 | 125,666944 | 4 | 13 | 17 | 0,481 |
| 50 | Philipines | 9,049167 | 125,696 | 5 | 11 | 16 | 0,491 |
| 51 | Brazil | 20,5131 | -54,7277 | 15 | 23 | 38 | 0,319 |
| 52 | Brazil | -1,203889 | -48,302222 | 3 | 15 | 18 | 0,689 |
| 53 | Brazil | -14,798611 | -52,641944 | 3 | 8 | 11 | 0,708 |
| 54 | Mexico | 18,406389 | -96,845833 | 4 | 30 | 34 | 0,565 |
| 55 | Mexico | 22,551944 | -100,08 | 6 | 26 | 32 | 0,500 |
| 56 | Brazil | -10,666667 | -37,416667 | 3 | 12 | 15 | 0,583 |
| 57 | Brazil | -15,589444 | -47,696944 | 6 | 20 | 26 | 0,475 |
| 58 | Brazil | -33 | -52,75 | 3 | 22 | 25 | 0,652 |
| 59 | Vietnam | 12,2011 | 108,2833 | 9 | 28 | 37 | 0,468 |

We measured modularity using the DIRTLPAwb+ algorithm (Beckett, 2016; Liu & Murata, 2010), implemented into the bipartite package (Dormann et al., 2008). To assess the effect of anuran selectivity on network modularity, we used a bootstrapping approach to simulate null model networks. From the total pool of dietary items (prey items consumed), we randomly selected prey items without replacement and assigned them to each species, respecting the observed number of prey items per species and total volume per prey type. We simulated 10,000 networks for each environment using this algorithm and used them to assess the deviation of modularity in observed networks in each environment from modularity when foraging is random.

To evaluate the niche dissimilarity between species, we used the volume-based interaction matrix (Aij) and calculated the Bray–Curtis compositional dissimilarity index between each pair of species. To visualize the dissimilarity patterns, we used a NMDS. We performed a perMANOVA (adonis2 in vegan package) to test for significant differences in dietary composition among the species. We then tested the importance of prey types to the ordering of species visualized on the NMDS through *envfit* from vegan, using proportional contribution from each prey type to the anuran species as a variable (Oksanen et al., 2022). For visual comparison of diet and environmental composition, we also plotted invertebrate communities from each site to the NMDS, but they were not included in the perMANOVA and *envfit* analyses.

Predator specialization

To evaluate dietary specialization amongst anurans in environments, we analyzed the cumulative distribution of each volume-based DAD. We focus on volume because it better captures proportions and dietary patterns (Hyslop, 1980). We calculated the *sp*50 index, which is the minimum number of prey types required to account for 50% of diet. We did it by calculating the half-saturation point of each DAD distribution (Hutchinson et al., 2022). To assess the effect of the environment on predator specialization, we used the non-parametric Kruskal-Wallis test.

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