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Rarefaction, richness estimation and extrapolation methods in the evaluation of unseen plant diversity in aquatic ecosystems

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ABSTRACT

Defining the adequate sampling effort necessary in tropical ecosystems is a sensitive subject. It is virtually impossible to sample every single species in an ecosystem. Thus, sampling must be a compromise between the desired completeness of the dataset and the amount of time and work that is feasible for researchers to do during fieldwork. Here, we used rarefaction procedures and richness estimators to evaluate sampling issues in a coastal lagoon in Ceará state, Brazil. We used 70 plots (1 m^2 each) to perform a phytosociological study in the lagoon and complemented the data with opportunistic floristic observations. We then applied statistical methods to the phytosociological survey to estimate how much of the local plant diversity had been sampled. Extrapolating our sampling accumulation curve to a sampling effort three times larger than the actual sampling done (210 plots), we were able to estimate the cumulative richness expected. After this, we used a different approach and calculated how many plots would be necessary to record a certain proportion of the total plant community. We recorded 49 species using phytosociological plots and 65 species in the floristic compilation. Richness estimators calculated the local alfa diversity to be between 68 and 85 species. We show that sampling 80% of the total local richness would require 100 plots, which is a reasonable sampling effort, but to sample 99% of the species, an extremely large amount of sampling would be needed (580 plots). This is not practical during fieldwork. We show here how rarefaction and extrapolation can be used as a framework to gauge the amount of field effort needed in aquatic ecosystems.

1. Introduction

Tropical ecosystems are known for their high species richness. This creates a problem for those who are sampling biodiversity. No matter how much effort one makes in collecting data, more species are always entering into the sample (Hubbell, 2001), making it virtually impossible to collect all species at a given site (total alfa biodiversity). For this reason, it is necessary for field biologists to recognize the limitations of their sampling methods and to evaluate the reasonable amount of time and work needed before they cease sampling.

Brazil has six major phytogeographical domains ("biomes" *sensu* IBGE, 2004), and of these domains, the Caatinga Phytogeographical Domain (CPD) is the driest, having a semiarid climate and strong rainfall seasonality (Ab'Sáber, 2003; Nimer, 1972). In this area of Brazil, plant communities are subjected to a long dry season, which lasts over six months (Andrade-Lima, 1981; Prado, 2003; Sampaio, 1995). Among the many plant communities that exist within the CPD, the aquatic communities occur in temporary rivers and ponds, which flourish during the rainy season, but are dormant during the dry season.

Most of the Brazilian coast that is located near the Amazon or the Atlantic Rainforests, has humid climate and receives large amounts of annual rainfall, but the northeastern Brazilian coast has very peculiar dry climatic conditions. Some 900 km of the septentrional coast of Brazil (classified as the "Dry Septentrional Coast of Brazil" sensu Ab'Sáber, 2001) borders the Caatinga Phytogeographical Domain and, instead of having a humid climate, it has semiarid or subhumid rainfall regimes (Ab'Sáber, 2006, 2001; IBGE [Instituto Brasileiro de Geografia e Estatística], 2004).

Derived from the idea that dry areas cannot harbor rich aquatic ecosystems, botanists in Brazil have given little attention to the aquatic plant communities in this region of Brazil, a reality that

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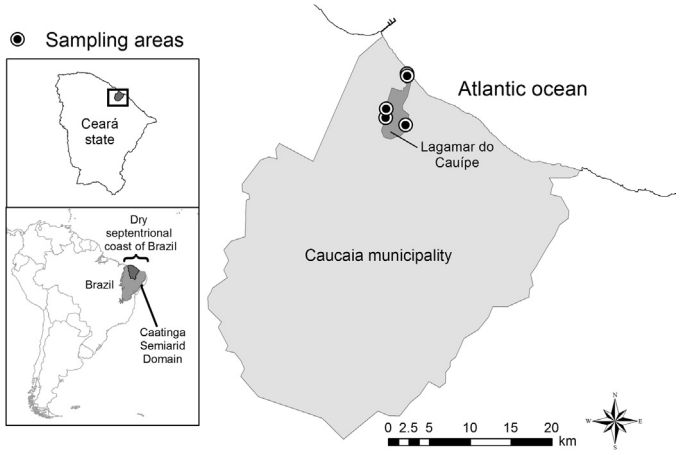

Fig. 1. Geographical location of the study site (3°38'00"S 38°47'30" W) and sampling areas within the Lagamar do Cauípe lagoon. Caucaia municipality, Ceará state, Brazil (Map elaboration: M.F. Moro).

has only recently began to change (Moro et al., 2014a). Recently there has been a number of floristic studies published with data on aquatic plants (see a synthesis in Moro et al., 2014a,b). The earliest of these studies were published only in the last decade (França et al., 2003; Matias et al., 2003), but most of the literature on aquatic plant communities in the semiarid regions of Brazil are focused only on producing species lists. Papers that discuss community dynamics (Tabosa et al., 2012) or phytosociological studies (Matias et al., 2003) are much rarer. Thus, there is a clear need for papers evaluating the ideal sampling effort in order to sample a well represented proportion of the plant communities in these ecosystems.

As floristic and phytosociological studies in the Brazilian semiarid ecosystems are steadily increasing (Moro et al., 2014a), we took the opportunity to develop the framework proposed by Colwell et al. (2012) to evaluate sampling effort issues related to aquatic plant communities using the dry regions of Brazil as a model.

Our aim was to evaluate the adequate sampling effort for these communities by using rarefaction curves, richness estimators and extrapolated species accumulation curves. We used this framework to exemplify how to determine the amount of sampling effort necessary to record a high proportion of the alfa diversity in these ecosystems. Based on our data we show how such a framework can be applied to the understanding of the sampling issues in the semiarid region of Brazil or in any other geographical region.

2. Experimental

2.1. Study site

Our study site was located in the Lagamar do Cauípe coastal lagoon (3°38′00″ S 38°47′30″ W), in Caucaia municipality, Ceará state, Brazil. The lagoon, which is subjected to dry climate, is located on the septentrional Brazilian coast, and borders the semiarid Caatinga Phytogeographical Domain (Ab'Sáber, 2003, 2001; Figueiredo, 1997; Moro et al., 2014b) (Fig. 1). This dry Brazilian coast has an extensive dune system, which is frequently intercepted by river courses. Flooding of the rivers result in flooded areas known as "Lagamares" (coastal lagoons) that occur just beyond the shorelines. We studied one of the largest perennial lagoons on this coast, the "Lagamar do Cauípe" (Fig. 1), as a model site for our approach. Although the Cauípe Lagoon is perennial, its size is reduced during the dry season. The lagoon contains aquatic and amphibian herbaceous plants that are associated with the carnaúba palm (*Copernicia prunifera*), a plant endemic to the dryer areas of Brazil and which occurs in sites subjected to temporary flooding.

2.2. Sampling procedures

We sampled the aquatic plants using 1 m × 1 m plots (1 m^2). We positioned 70 paired plots on the edge of the lagoon, which were distributed along five sampling areas around the lagoon. Thirty-five of the plots were placed near the edge, in the water (submerged plots), and another 35 were placed on the other side of the edge, on land (emerged plots), to sample amphibious aquatic plants (Fig. 2).

For each plot we recorded the number of species and the number of individuals per species. Since plants are modular organisms, and are often capable of vegetative reproduction (a common feature among aquatic macrophytes), we used the concept of a ramet, or an independent and isolated reproductive unit, to define an individual (Harper, 1977). For rooted plants we considered each tussock as one individual, independently of whether or not each tussock was connected to neighbor tussocks. For floating species we considered each isolate floating mat as an individual.

We counted the number of individuals and species in each plot and calculated the following phytosociological parameters of the community: abundance (number of individuals recorded in the plots), absolute density (number of individuals per 100 square meters), relative density, absolute frequency and relative frequency (Brower et al., 1998; Moro and Martins, 2011; Mueller-Dombois and Ellenberg, 1974). The plants were collected following Haynes (1984) and Ceska and Ceska (1986), and deposited in the EAC Herbarium (herbarium of the Federal University of Ceará). We classified each of the species life forms according to Sculthorpe (1967). The list of species from the Lagamar do Cauípe was also complemented by opportunistic records of species observed in the field or deposited in the EAC herbarium, but that were not sampled in the plots.

2.3. Rarefaction and extrapolation procedures and richness estimators

A classic problem in ecology is that it is nearly impossible to sample all species occurring in an area (Hubbell, 2001). In order to estimate the total, asymptotic number of species in our study site we used a group of non-parametric statistical estimators that are based on the number of rare species in the sample to estimate the total number of species at the site (Gotelli and Colwell, 2011). Using EstimateS software (Colwell, 2013), we built smoothed collector curves for the number of species observed in our phytosociological plots, and also estimated the expected total number of species in our study area using the ICE (Incidence-based Coverage Estimator), Chao 2, Jackknife 1 and Jackknife 2 estimators (Gotelli and Colwell, 2011).

To estimate the ideal sampling effort for these ecosystems, we used extrapolation procedures (Colwell et al., 2012) to see how much we would need to keep sampling in order to record the number of species located between the smallest and largest richness suggested by the non-parametric estimators.

Another mathematical approach was proposed by Colwell et al. (2012) (Sample Based Extrapolation – Eq. (20) in their paper) to estimate how many extra sampling units would be required to sample a certain proportion of the total estimated richness. We applied this procedure to calculate how many plots would be needed to sample 80%, 90%, 95% and 99% of the asymptotic richness in our study site.

2.4. Literature survey

To determine how well the aquatic plant communities in the Brazilian dry regions were sampled, we searched the Brazilian botanical literature. We registered all papers dealing with aquatic plants in the semiarid region of Brazil we could find. In our compilation, we included all surveys in the semiarid Caatinga Domain (as