



## Original Research Article

## Assessing sampling sufficiency of network metrics using bootstrap

Grasiela Casas<sup>a,c,\*</sup>, Vinicius A.G. Bastazini<sup>b,c</sup>, Vanderlei J. Debastiani<sup>c</sup>, Valério D. Pillar<sup>c</sup><sup>a</sup> Department of Natural Sciences, Universidade Estadual do Sudoeste da Bahia. Estrada do Bem Querer, km 4, Vitória da Conquista 45.031-900, Brazil<sup>b</sup> Theoretical and Experimental Ecology Station, French National Center for Scientific Research—Paul Sabatier University, 2 route du CNRS, Moulis 09200, France<sup>c</sup> Graduate Program in Ecology, Universidade Federal do Rio Grande do Sul. Bento Gonçalves, 9500, Porto Alegre, Rio Grande do Sul, Brazil

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## ABSTRACT

Sampling the full diversity of interactions in an ecological community is a highly intensive effort. Recent studies have demonstrated that many network metrics are sensitive to both sampling effort and network size. Here, we develop a statistical framework, based on bootstrap resampling, that aims to assess sampling sufficiency for some of the most widely used metrics in Network Ecology, namely connectance, nestedness (NODF- nested overlap and decreasing fill) and modularity (using the QuaBiMo algorithm). Our framework can generate confidence intervals for each network metric with increasing sample size (i.e., the number of sampled interaction events, or number of sampled individuals), which can be used to evaluate sampling sufficiency. The sample is considered sufficient when the confidence limits reach stability or lie within an acceptable level of precision for the aims of the study. We illustrate our framework with data from three quantitative networks of plant and frugivorous birds, varying in size from 16 to 115 species, and 17 to 2,745 interactions. The results show that, for the same dataset, sampling sufficiency may be reached at different sample sizes depending on the metric of interest. The bootstrap confidence limits reached stability for the two largest networks, but were wide and unstable with increasing sample size for all three metrics estimated for the smallest network. The bootstrap method is useful to empirical ecologists to indicate the minimum number of interactions necessary to reach sampling sufficiency for a specific network metric. It is also useful to compare sampling techniques of networks in their capacity to reach sampling sufficiency. Our method is general enough to be applied to different types of metrics and networks.

## 1. Introduction

Understanding how species interact with one another is an important and challenging task for ecologists and evolutionary biologists (Bastazini et al., 2017). Over the recent decades the rise and rapid development of network and complexity science (Albert and Barabási, 2002) has given rise to a new and exciting ecological research program, known as Network Ecology that aims to understand the function, structure, and evolution of ecological systems, using network models and analyses (Borrett et al., 2014). Despite this fast and prolific growth, most of the studies in Network Ecology has mainly focused on the analyses of network structure and robustness (Bascompte and Jordano 2007; Miranda et al., 2013).

Some of the most commonly used metrics to describe the structure of ecological networks are connectance, nestedness, and modularity (Dormann et al., 2009; Miranda et al., 2013), that are used with a bipartite network (i.e., a network which has two discrete groups of species, such as plant and seed dispersers, which interact with members of

the other group, but not within their group), and also with an unipartite network (i.e., a network that has only one group of species that can be connected, such as food webs, and the matrix in this case will have the same number of rows and columns because the species will be repeated). It has been suggested that these structural properties are associated with community stability and ecosystem functioning (Bascompte et al., 2006; Takimoto and Suzuki, 2016). Empirical evidence suggests that most mutualistic networks are nested (Bascompte et al., 2003), and that a highly connected and nested architecture promotes community stability in mutualistic networks. However, in trophic networks this stability is enhanced with compartmented (modular) and weakly connected architectures (Thébault and Fontaine, 2010), as a modular pattern can prevent the spread of perturbations across the network (Stouffer and Bascompte, 2011).

Sampling the full diversity of interactions is a highly intensive effort, and ecologists have now come to realize that most networks published to date may be under-sampled (Chacoff et al., 2012). Nevertheless, sampling may influence the detection of network

\* Corresponding author at: Department of Natural Sciences, Universidade Estadual do Sudoeste da Bahia. Estrada do Bem Querer, km 4, Vitória da Conquista 45.031-900, Brazil.

E-mail addresses: [casas.gr@gmail.com](mailto:casas.gr@gmail.com) (G. Casas), [vpillar@ufrgs.br](mailto:vpillar@ufrgs.br) (V.D. Pillar).

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structures. Most network metrics are sensitive to sampling effort and network size (Dormann et al., 2009). Studying pollination networks, Olesen et al. (2007) found a relationship between network size and nestedness and modularity. In general, if the size of seed dispersal networks increases, connectance decreases (Mello et al., 2011). Bascompte et al. (2003) also found, for plant-frugivore and plant-pollinator networks, that above a size of 50 species, all networks were significantly nested. Consequently, studies with low sampling effort need to be interpreted with caution (Rivera-Hutinel et al., 2012), as low sampling effort can influence the network pattern detected (Vizentin-Bugoni et al., 2016). Regarding pollination network, Chacoff et al. (2012) found that, despite a large sampling effort, their network was under-sampled, as they detected less than 60% of the potential interactions. Seed dispersal mutualistic networks may also be insufficiently sampled, since most of the published datasets describe small networks.

Although a robust and well-designed sampling procedure is essential for the quality of data, the optimality of sample size and/or intensity effort depend on the objectives of the study (Orlaci and Pillar, 1991). A census of the species or interactions in a given area may not be necessary to detect a specific pattern in a network. Similarly, the number of interactions needed to reach sampling sufficiency may be different according to the network metrics and the different types of taxonomic groups within mutualistic and antagonistic networks. The common analyses applied in the literature to evaluate the sampling procedure for mutualistic networks are rarefaction and accumulation curve analyses (Chacoff et al., 2012; Jordano, 2015; Nielsen and Bascompte, 2007; Rivera-Hutinel et al., 2012). Also, Martinez et al. (1999) evaluated the relationship between food-web properties and richness among taxonomic and trophic webs using Monte Carlo simulations and confidence intervals. Here, we developed a statistical framework that aims to assess sampling sufficiency for some of the most used metrics in Network Ecology based on bootstrap resampling.

The bootstrap method (Efron, 1979; Efron and Tibshirani, 1993) is based on the idea that the distribution of observed values in a sample is the best indicator of their distribution in the sampling universe from which the sample was taken. Our framework is similar to the Monte Carlo simulation without replacement proposed by Martinez et al. (1999) in the sense that both approaches involve randomization or reshuffling of the original data. However, we proposed a full bootstrap framework, which is a statistical methodology devised to estimate the sampling distribution of an estimator, in our case network metrics, by randomly sampling with replacement the original sample. Our method is intended to answer the following question: how many interaction events or number of individuals are necessary to be sampled in order to reach stability for a given network metric? We showcase our approach by assessing sampling sufficiency for nestedness, modularity, and connectance for three empirical mutualistic networks that widely ranged in size.

## 2. Methods

### 2.1. Bootstrap resampling technique

We adapted the method of bootstrap resampling from Pillar (1998) to assess sampling sufficiency for network metrics. Our framework is a resampling technique that can generate confidence intervals for each network metric with increasing sample size (i.e., the number of interaction events sampled or the number of observed individuals potentially interacting), which can be used to evaluate sampling sufficiency (Manly, 1992; Pillar, 1998). The sampling units in a sample are taken as “a pseudo sampling universe”, the best available representation of the actual sampling universe from which the sample was taken. Assuming that the sampling units are independent from each other, each new sample obtained by resampling them with replacement is a “bootstrap

sample”.

An interaction matrix is most often built using records of observed interaction events between species of two trophic levels (e.g., plants and frugivorous birds). The interaction events will be independent contingent on the method used for their recording. For instance, a focal plant is selected and then species identities of birds eating its fruits are recorded. These records of  $n$  focal plants are then integrated in an interaction matrix, in which each cell contains the frequencies of interaction events for each plant-bird species pair. In this case, the focal plants may be independent each other, while the interaction events observed on the same plant will not be so. Similarly, when plant species are recorded in the faeces of captured birds (see example in Tables A1 and A2), the  $n$  bird individuals are independent sampling units, while the plant species found in the same bird faeces are not.

However, often the only available information is the network matrix, such as when existing databases are used. In this case, it is not possible to rebuild the actual independent interaction records that were integrated in the network matrix. In order to be resampled, independence between the resampled interaction records will have to be assumed, in which case the total frequency ( $n$ ) of the network matrix is expanded into two columns (e.g., bird species and plant species) and the identities of the  $n$  interaction events are listed in the rows (see Table A3). For example, if a bird species  $A$  has two interaction events with plant species  $X$ , there would be two rows with the same bird and plant names.

The bootstrap resampling algorithm for interaction matrices is the following:

- 1) Randomly select a bootstrap sample of  $n_k \leq n$  sampling units (individuals or interaction events) with replacement from the observed sample (pseudo sampling universe) with  $n$  sampling units;
- 2) Compute the network metric of interest ( $\theta_k$ ) for the bootstrap sample and store the resulting value;
- 3) Repeat steps 1 and 2 a large number of times (say 1,000 times);
- 4) Sort the values of  $\theta_k$  from the smallest to the largest value. Based on this ordering, delimit the confidence limits for a given specified probability  $\alpha$ . For example, with 1,000 bootstrap samples and a probability  $\alpha$  of 0.05 (i.e., a 95% confidence interval), the lower confidence limit at a given sample size will be the value of  $\theta_k$  at the 25th position and the upper limit will be the value of  $\theta_k$  at the 976th position.
- 5) Repeat steps 1, 2, 3, and 4 for a new bootstrap sample size  $n_k + \delta$ , where  $\delta$  is an increase in sample size, repeating the process up to sample size of  $n$  sampling units.

Resampling data according to the bootstrap method will create a frequency distribution for the network metric of interest in samples with increasing size, mimicking the resampling of the sampling universe. The sample is considered sufficient within the range of sample sizes evaluated when the confidence limits reach stability or lie within an acceptable level of precision for the objectives of the study (Pillar, 1998). Stability of the confidence limits indicates that with samples larger than a certain size there is no further gain in precision for the estimation of the analysed network metric. For this, the defined values for  $n_k$  and  $\delta$  should be such that the number of steps up to the total sample size is sufficient to perceive stability of the network metric. The acceptable level of precision is indicated by the magnitude of the confidence interval being lower than the network metric differences that the researcher wishes to detect between samples. The width of the confidence interval indicates precision, which is determined by the chosen level of confidence, and can be used to guide the choice of sample size (Dattalo, 2008).

### 2.2. Network metrics

We assessed sampling sufficiency for three commonly used network

metrics:

- 1) Connectance (C), which is the proportion of realized links in a network relative to the possible number of links (Dunne et al., 2002), with values ranging from 0 to 1. For bipartite networks it is calculated as  $C = L/(I \times J)$ , where L is the number of realized links; I and J are the number of rows and columns in the network matrix, e.g., number of interacting plant and animal species. Connectance only distinguishes whether links are present or absent (unweighted, binary links). Thus, though the information about interaction frequencies is used for the resampling, the frequencies are not considered for the metric.
- 2) Nestedness is characterized by a core of highly connected species (generalists) that interact mainly with each other, and a group of specialist species that interact mainly with the generalist species (Bascompte et al., 2003). We used NODF (nested overlap and decreasing fill, ranging from 0 [non-nestedness] to 100 [perfect nesting]) algorithm proposed by Almeida-Neto et al. (2008), which corrects biases resulting from matrix fill and matrix dimensions. Similar to connectance, information about interaction frequencies is not used for this metric. However, our method is general enough to be applied to different types of metrics, including weighted metrics. We also analysed the data with quantitative connectance and WNODF (Weighted Nestedness metric based on Overlap and Decreasing Fill).
- 3) Modularity is characterized by the degree to which there are groups of nodes (species) that interact more among each other than with other groups (modules) in a network (Guimera and Amaral, 2005). We assessed modules using the QuaBiMo algorithm that computes modules in quantitative bipartite networks, based on a hierarchical representation of species link weights and optimal allocation to modules (Dormann and Strauss, 2013). It ranges between 0 (random network with no modules) to 1 (maximum modularity).

### 2.3. Computer software

The method described here has been implemented in R (R Development Core Team, 2013). Network metrics were calculated using the package Bipartite (Dormann et al., 2008). The bootstrap function and a script with an example are available as Supplementary text A2 and A3, and Table A4, and it is available at <https://github.com/vanderleidebastiani/BootstrapNetwork>.

### 2.4. Examples from mutualistic networks

We illustrate our framework by using data from three quantitative bipartite networks of plant and frugivorous birds (Table 1). Network size in each dataset varied from 16 to 115 species and 17 to 2,745 interaction events. Despite the fact that the original datasets contained quantitative data, we used two unweighted metrics to assess sampling sufficiency (connectance and NODF) that only distinguish whether links are present or absent (binary links), and one weighted (quantitative) metric (QuaBiMo algorithm). We also analysed the data with quantitative connectance and WNODF (Weighted Nestedness metric based on Overlap and Decreasing Fill) (Fig. 3).

For two networks collected by ourselves (named CCS and SS; additional details for methods are in Supplementary Text A1), the birds were captured with mist-nets, banded, and then placed into fabric bags to collect their faeces from the bags. The seeds found in faecal samples were identified to the species level, when possible, to build an interaction matrix between birds and the plant they consumed, with each cell indicating the number of interaction events (the number of times a specific bird species was captured with seeds of a specific plant species found in the faeces) (e.g. Table A4). In this case, the records for each bird individual interactions are available (Tables A1 and A2), which allowed to define the bird individuals as sampling units.

**Table 1**  
Description of the data on plant-frugivore networks used with the bootstrap resampling technique. For CCS and SS, the network data refer to the number  $I_{jk}$  of captures of bird species  $j$  with seeds of plant species  $k$  in the faeces, while for the Schleuning et al. (2011) networks they refer to the number of fruit-eating individuals of a given bird species recorded on a focal plant species.

Network	Habitat type	Location	Method	Bird species	Plant species	Number of interactions	Number of bird individuals
CCS	Atlantic Forest biome (forest-grassland mosaics)	Brazil	Mist net	6	10	17	14
SS	Pampa biome (grassland-forest mosaics)	Brazil	Mist net	15	28	119	100
Schleuning et al. (2011)	Primary and secondary tropical rainforest	Africa, Kenya	Focal plant species	83	32	2745	
Schleuning et al. (2011)	Secondary tropical rainforest	Africa, Kenya	Focal plant species	53	13	568	

For another test case we used the plant-frugivorous birds network described by [Schleuning et al. \(2011\)](#) (Sch; data available from the Interaction Web Database), which was built based on the observation of focal plants, comprising primary and secondary forests and various vegetation strata ([Table 1](#)). We used this network as an example of a large quantitative network. To record bird species feeding on each focal plant species, frugivorous bird visits were recorded at each focal plant individual, but the only available information in the database is the interaction matrix containing the frequencies of each plant-bird species interaction. The interaction frequency was defined as the number of fruit-eating individuals on a plant species independent of fruit handling. We also used, as a separated network, only the data collected in secondary forest areas in [Schleuning et al. \(2011\)](#) ([Table 1](#)). The aim here was to test if the stability for these metrics is reached with a lower sampling effort compared to the entire network of [Schleuning et al. \(2011\)](#), that probably has a high interaction diversity as it comprises primary and secondary forests.

To obtain the bootstrap sample (algorithm step 1), for SS, we started with  $n_k = 10$  bird individuals with replacement, and we repeated the resampling procedure 1,000 times (algorithm step 3). We then increased sample size by  $\delta = 5$  individuals, and the process was repeated with  $n_k + \delta$  up to the maximum number of  $n$  individuals. For the smallest network CCS (with only 14 bird individuals), we started with  $n_k = 7$  and used  $\delta = 1$ . Our aim here was to investigate how many birds were necessary to reach sufficiency for each network metric. In a second analysis, just for comparison, we used as sampling units the interaction events in our own datasets (CCS and SS), with the same  $n_k$  and  $\delta$  used when we resampled bird individuals, and also including the largest network of [Schleuning et al. \(2011\)](#). For the network of [Schleuning et al. \(2011\)](#) (2,745 interaction events) we used  $n_k = 30$  and  $\delta = 50$ , and for the data collected in secondary forest areas in [Schleuning et al. \(2011\)](#) (with 568 interaction events) we used  $n_k = 10$  and  $\delta = 10$ . For all test cases, we used 95% confidence intervals based on 1,000 resampling interactions at each sample size.

### 3. Results

Considering bird individuals as sampling units, the bootstrap confidence limits reached relative stability for the SS network for connectance, nestedness, and modularity within the analysed sample sizes. Therefore, for the SS network the sample was considered sufficient for these metrics ([Fig. 1](#)). The sample of the smallest network (CCS) was not sufficient for any of the analysed metrics, since the confidence limits were wide and unstable with increasing sample size up to 14 bird individuals. For instance, the modularity of the CCS network was expected to lie between 0.44 and 0.77 in 95% of the cases, and the width of the confidence interval was 0.33. This difference is too wide to be indicative of sampling sufficiency compared to the other networks (see detailed results in Supplementary Table A5).

For the SS network, the confidence limits for connectance and modularity generated by the bootstrap reached stability with sample sizes larger than 80 bird individuals. The confidence intervals for nestedness did not reach clear stability but, rather, decreased until the maximum sample size ([Fig. 1](#)). Yet, at the largest bootstrap sample size the width of the confidence interval was 16.22 ([Table A5](#)), which may be an acceptable level of precision depending on the objective of the study.

The results were similar when we considered the interaction events instead of captured bird individuals as sampling units ([Fig. 2](#)). Again, the smallest network (CCS) did not present sufficiency for any of the analysed metrics, since the confidence limit values were wide and unstable with increasing sample size up to 17 interaction events. The SS network was considered sufficient for all analysed metrics, as the bootstrap confidence limits reached stability ([Fig. 2](#); detailed results in Supplementary Table A5). Similar results were expected in these cases, because the matrices using the number of events and the number of

individuals captured in our data were similar (see Supplementary Table A1 for CCS with captured birds as resampled sampling units, and Table A3 for CCS matrix with interaction events as resampled sampling units). We captured most bird individuals with only one plant species in its faeces, and consequently, most recorded interactions (filled cells) comprised one interaction event. Despite the similarity in these results, we show and discuss them because the data available for most networks in the literature and on-line databases unfortunately only allow the extraction of interaction events.

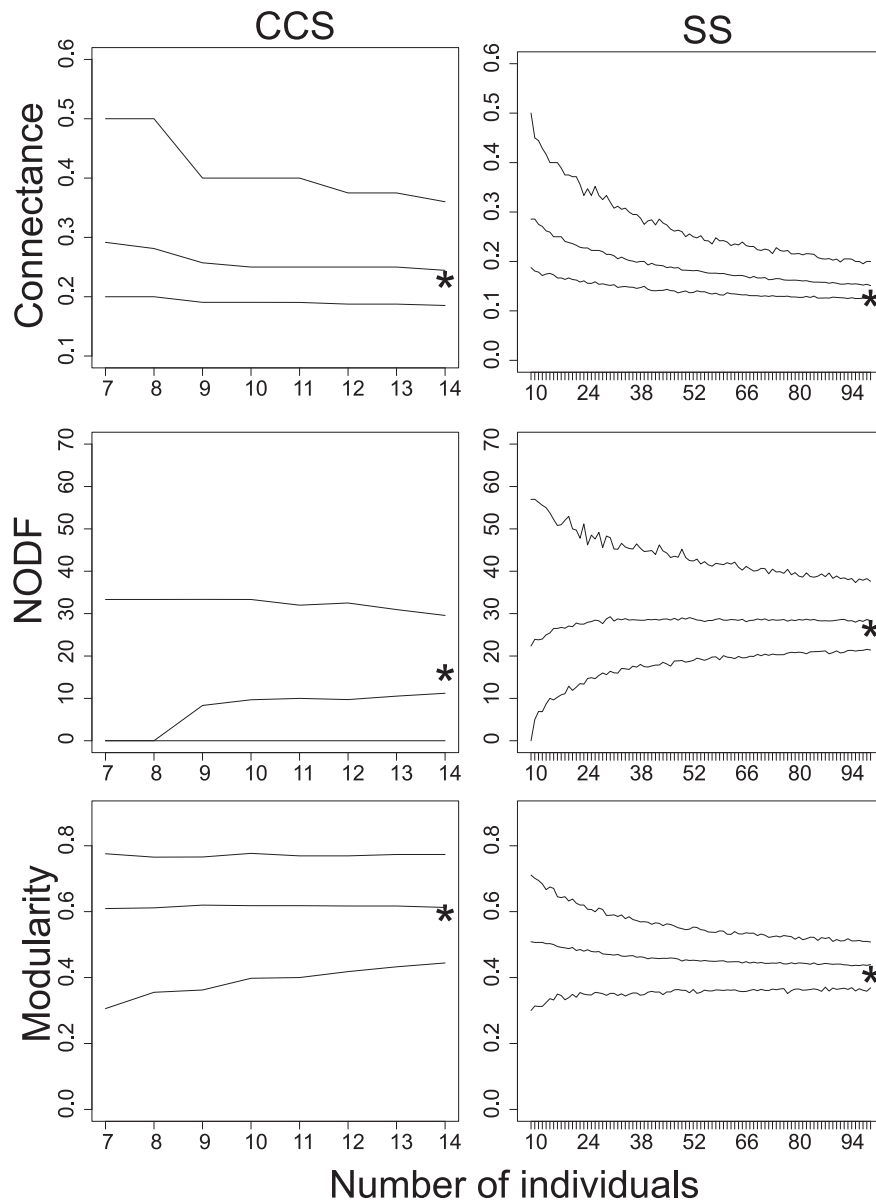
For the largest network ([Schleuning et al. 2001](#)), the stability of confidence limits for connectance, nestedness and modularity was reached, respectively, with sample sizes larger than 280, 580, and 1000 interaction events. When we considered, as a separated network, only the data collected in secondary forest areas in [Schleuning et al. \(2011\)](#) (with 568 interaction events), we found different results: the bootstrap confidence limits reached stability for the metrics connectance, nestedness and modularity, respectively, with sample sizes larger than 100, 150 and 230 interaction events (see Supplementary Fig. A1).

When we reanalysed the data using quantitative connectance and WNODF metrics, considering interaction events as sampling units, the range of confidence limits for almost all networks was smaller compared to the confidence limits using unweighted metrics (see Supplementary Table A5). For example, the bootstrap confidence limits reached stability for CCS network with quantitative connectance ([Fig. 3](#)). This could be an indicative that quantitative metrics are less influenced by sample size than binary metrics. However, for the SS network, the observed connectance value was outside the confidence interval obtained by the bootstrap method ([Fig. A3](#)). We interpret that in this case the network was not sufficiently sampled for the network metric used. Cells that presented one interaction event were less frequent (or did not appear) in the resampling matrices generated by the bootstrap method with replacement, and this difference in the number of filled cells, and consequently in the number of species, probably affected the metric value. Thus, a network matrix presenting many cells with one interaction event, causing the observed metric value to be outside the confidence interval and the median obtained by the bootstrap method, is probably an indicative of sampling insufficiency, in addition to the sensitivity of most network metrics to the number of species and the number of filled cells in the matrix.

### 4. Discussion

The concerns about the effect of sampling effort on network metrics in mutualisms ([Chacoff et al., 2012](#); [Dorado et al., 2011](#); [Nielsen and Bascompte, 2007](#); [Vizentin-Bugoni et al., 2016](#)) and food webs ([Banašek-Richter et al., 2004](#); [Goldwasser and Roughgarden, 1997](#); [Martinez et al., 1999](#)) have grown in the last few years. Here, we develop a statistical framework, based on bootstrap resampling, that aims to assess sampling sufficiency for some of the most widely used metrics in Network Ecology.

The method is general enough to be applied to different types of metrics and networks. However, the type of network metric has to provide a single value at the end of the analysis. For example, modularity involved an optimization method, but we could use it because it gives a modularity  $Q$  value ([Dormann and Strauss, 2013](#)). Further, since the aim is to evaluate sampling sufficiency of network metrics, the network must be a quantitative one because data containing the frequency of interactions is necessary for the resampling procedure (captured bird individuals or interaction events in the test cases). It remains an open question whether our method could be adapted to qualitative networks (binary matrices). The main limitation is the definition of the sampling unit that will be resampled. However, if the only available information is the knowledge that two species do interact, the question about sampling sufficiency for building such network may not apply. Our bootstrap resampling does not add species and interactions to the network; it only resamples the data with replacement, mimicking the

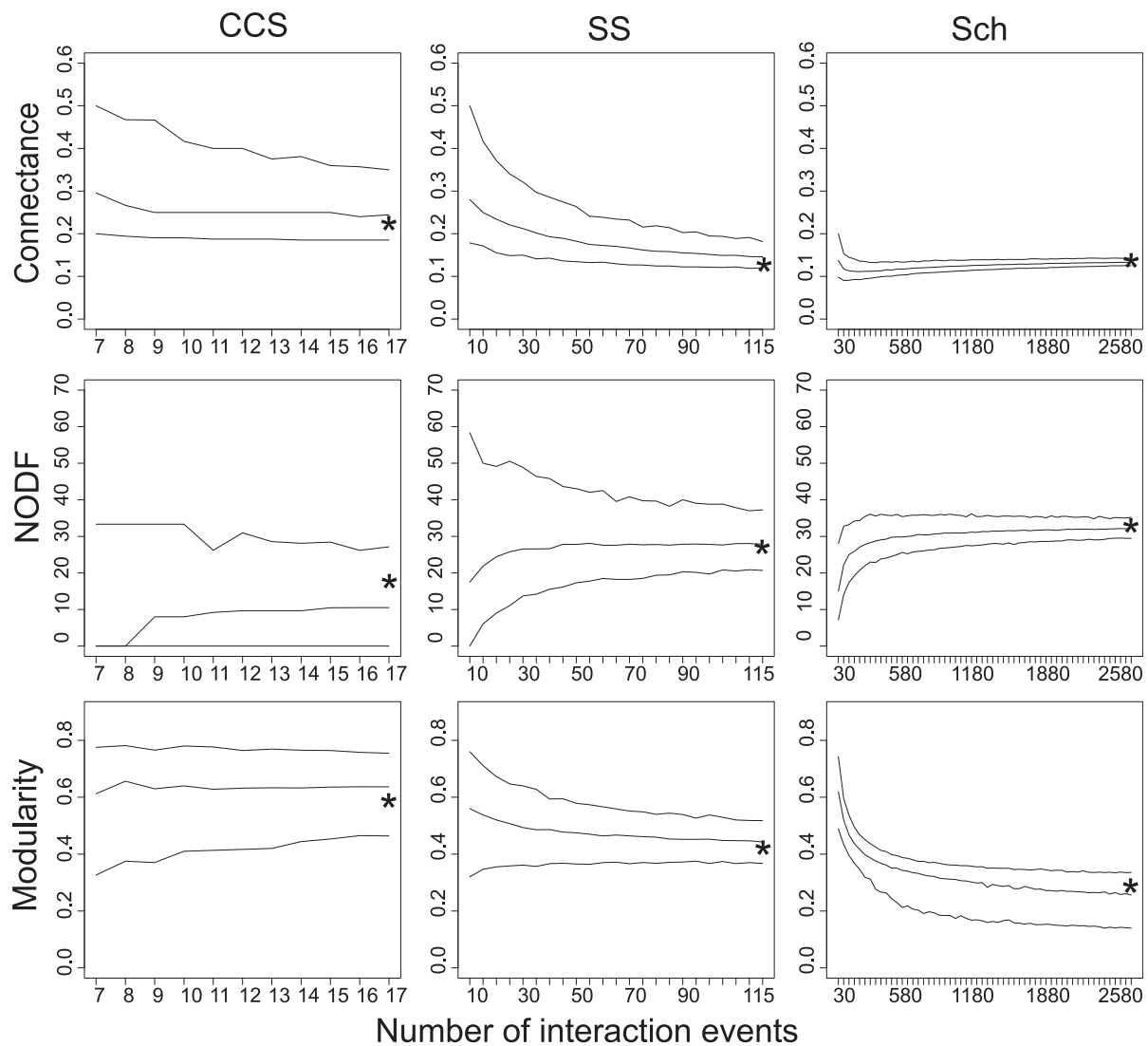


**Fig. 1.** Observed value (star), median and confidence limits of Connectance, Nestedness (NODF) and Modularity metrics using captured bird individuals as sampling units resampled with replacement for two quantitative mutualistic networks (plants and frugivore birds). The 95% confidence intervals as well the median (line in between the confidence limits) were set based on 1,000 resampling interactions at each bootstrap sample size. See Table 1 for detailed information of seed-dispersal networks (bird and plant) CCS and SS.

resampling of the presumed sampling universe represented by the observed network.

With the bootstrap method we are looking for the effect of sampling bias on network metrics. In a different way, previous studies compared different fieldwork sampling techniques and investigated to which extent their conclusions (structural properties of network) were influenced by the way samples were collected. For example, Gibson et al. (2011) analysed the potential bias in network metrics when using time-based observations or transects in plant-pollinator networks, with rarefaction analysis and null models approach. Analogously, the bootstrap method can be used to compare two methodologies in terms of sampling sufficiency. In seed dispersal networks between plants and birds, e.g., the sampling hours or number of observed plant individuals as sample size (through transect or focal-plant methodologies) can be compared with the number of bird individuals captured (with mist net) that need to be sampled in order to reach stability for each network metric.

We assessed sampling sufficiency with the bootstrap method using interaction events as sampling units with three mutualistic networks that differed regarding sampling techniques. In addition, we used the captured bird individuals as sampling units to assess sufficiency for the networks we have collected. The potential advantage of using individual data over interaction events is that often individuals, such as focal plants, are more independent from each other than the interaction events in which these individuals are involved. Independence between sampling units is often an important assumption in data analysis, and the accuracy of the bootstrap method may be affected by lack of independence (Efron and Tibshirani, 1993). Therefore, when it is possible to rebuild the actual independent interaction records that were integrated in the network matrix, with  $n$  focal plants or bird individuals as sampling units, we recommend to use this matrix instead of the matrix with interaction events. Otherwise, if the only available information is the network matrix with the interaction events, independence between the resampled interactions records will have to be assumed. However,



**Fig. 2.** Observed value (star), median and confidence limits of Connectance, Nestedness (NODF) and Modularity metrics obtained by using interaction events as sampling units resampled with replacement for three quantitative mutualistic networks (plants and frugivore birds). The 95% confidence intervals as well the median were set based on 1,000 resampling interactions at each bootstrap sample size. See [Table 1](#) for detailed information of networks.

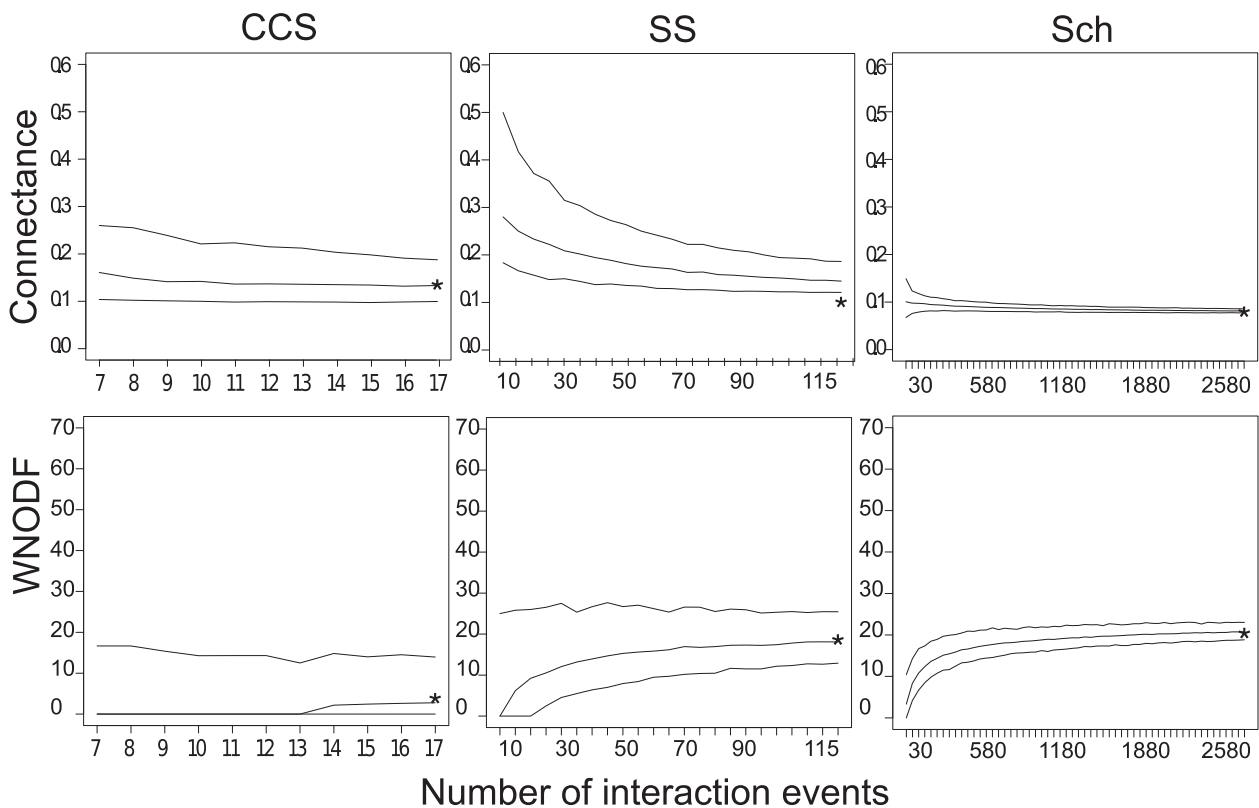
in spite of the advantage of using individuals as sampling units, the data available for most networks in literature and on-line databases unfortunately only allow the extraction of interaction events.

In our study cases, we assessed the stability and the precision of the estimated network metrics with the bootstrap method we propose here. Only for the smallest network (with 14 bird individuals) the bootstrap confidence limits did not reach stability or remained wide up to the maximum sample size, but for the other networks (more than 100 bird individuals or interaction events) the confidence limits reached stability, or could be considered acceptable depending on the objective of the study. Also, we observed across sample sizes a variation of the median of the metric generated by bootstrap resampling, mainly in small samples. We expected that the median would remain relatively stable throughout the process, and only the confidence interval would change considerably with increasing sample size. Since most network metrics are sensitive to sampling effort and network size ([Dormann et al., 2009](#)), probably these metrics are biased, causing this variation in their median across bootstrap sample sizes. Further research, using simulated networks with known properties and dimensions, is needed to evaluate how accurate these network metrics are.

Our results suggest an important point: sampling sufficiency can be reached at different sample sizes for the same dataset depending on the

metric of interest. [Nielsen and Bascompte \(2007\)](#), analysing the sensitivity of connectance and nestedness metrics to variation in sampling effort, also suggested that sampling intensity does not affect all network metrics in the same way, and that nestedness tends to stabilize rapidly with increasing sampling effort. Some confidence limits generated by the bootstrap reached stability with less than 100 individuals or interaction events, meaning that increasing the sample sizes would not affect the conclusions significantly.

However, it has been pointed out that studies of interactions should come from a robust and well-designed sampling procedure, mainly due to the influence of limited sampling effort in network properties ([Chacoff et al., 2012](#); [Dormann et al., 2009](#); [Vázquez et al., 2009](#); [Vizentin-Bugoni et al., 2016](#)). In our results, even though the bootstrap confidence limits for some network metrics reached stability in networks with less than 50 species, the range of confidence limits for the largest network ([Schleuning et al., 2011](#)), with 115 species, was much smaller compared to the other two networks and, consequently, it is considered a more precise sample. Because the study of [Schleuning et al. \(2011\)](#) comprised primary and secondary forests and various vegetation strata, the interactions of this network are heterogeneous (high interaction diversity) and, consequently, the stability for these metrics was reached only with a larger sampling effort compared



**Fig. 3.** Observed value (star), median and confidence limits of quantitative Connectance and Weighted Nestedness (WNODF) metrics obtained by using interaction events as sampling units resampled with replacement for three quantitative mutualistic networks (plants and frugivore birds). The 95% confidence intervals as well the median were set based on 1,000 resampling interactions at each bootstrap sample size. See Table A5 for detailed of observed metric values, median and confidence intervals.

to the other networks.

The bootstrap method we propose here can help network ecologists by indicating the minimum number of interaction events (or other defined sampling units) necessary to reach sampling sufficiency for a specific network metric. It also allows comparing sampling protocols in terms of effort to reach sampling sufficiency. The concerns on the effect of sampling effort on network metrics in mutualistic (Chacoff et al., 2012; Dorado et al., 2011; Nielsen and Bascompte, 2007; Vizentin-Bugoni et al., 2016) and food webs (Banašek-Richter et al., 2004; Goldwasser and Roughgarden, 1997; Martinez et al., 1999) have grown in the last few years. We believe that our method is a significant contribution to assess sampling sufficiency in Network Ecology.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ecocom.2018.09.005](https://doi.org/10.1016/j.ecocom.2018.09.005).

#### References

- Albert, R., Barabási, A.-L., 2002. Statistical mechanics of complex networks. *Rev. Mod. Phys.* 74, 47–97. <https://doi.org/10.1103/RevModPhys.74.47>.
- Almeida-Neto, M., Guimarães, P., Guimarães, P.R., Loyola, R.D., Ulrich, W., 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos* 117, 1227–1239. <https://doi.org/10.1111/j.2008.0030-1299.16644.x>.
- Banašek-Richter, C., Cattin, M.-F., Bersier, L.-F., 2004. Sampling effects and the robustness of quantitative and qualitative food-web descriptors. *J. Theor. Biol.* 226, 23–32. [https://doi.org/10.1016/S0022-5193\(03\)00305-9](https://doi.org/10.1016/S0022-5193(03)00305-9).
- Bascompte, J., Jordano, P., 2007. Plant-animal mutualistic networks: the architecture of biodiversity. *Annu. Rev. Ecol. Evol. Syst.* 38, 567–593. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095818>.
- Bascompte, J., Jordano, P., Melián, C.J., Olesen, J.M., 2003. The nested assembly of plant-animal mutualistic networks. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9383–9387. <https://doi.org/10.1073/pnas.1633576100>.
- Bascompte, J., Jordano, P., Olesen, J.M., 2006. Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* 312, 431–433. <https://doi.org/10.1126/science.1123412>.
- Bastazini, V.A.G., Ferreira, P.M.A., Azambuja, B.O., Casas, G., Debastiani, V.J., Guimarães, P.R., Pillar, V.D., 2017. Untangling the tangled bank: a novel method for partitioning the effects of phylogenies and traits on ecological networks. *Evol. Biol.* 44, 312–324. <https://doi.org/10.1007/s11692-017-9409-8>.
- Borrett, S.R., Moody, J., Edelman, A., 2014. The rise of network ecology: maps of the topic diversity and scientific collaboration. *Ecol. Modell.* 293, 111–127. <https://doi.org/10.1016/j.ecolmodel.2014.02.019>.
- Chacoff, N.P., Vázquez, D.P., Lomáscolo, S.B., Stevani, E.L., Dorado, J., Padrón, B., 2012. Evaluating sampling completeness in a desert plant-pollinator network. *J. Anim. Ecol.* 81, 190–200. <https://doi.org/10.1111/j.1365-2656.2011.01883.x>.
- Dattalo, P., 2008. *Determining Sample Size: Balancing Power, Precision, and Practicality*. Oxford University Press, New York.
- Dorado, J., Vazquez, Diego, P., Stevani, E.L., Chacoff, N.P., 2011. Rareness and specialization in plant-pollinator networks. *Ecology* 92, 19–25. <https://doi.org/10.1890/10-0794.1>.
- Dormann, C.F., Frund, J., Bluthgen, N., Gruber, B., 2009. Indices, graphs and null models: analyzing bipartite ecological networks. *Open Ecol. J.* 2, 7–24. <https://doi.org/10.2174/1874213000902010007>.
- Dormann, C.F., Gruber, B., Fründ, J., 2008. *The Bipartite Package*. Version 0.73. R Proj. Stat. Comput., Vienna, Austria.
- Dormann, C.F., Strauss, R., 2013. Detecting modules in quantitative bipartite networks:

- the QuaBiMo algorithm. arXiv Prepr. arXiv 1304–3218. <https://doi.org/10.1111/2041-210X.12139>.
- Dunne, J.A., Williams, R.J., Martinez, N.D., 2002. Food-web structure and network theory: the role of connectance and size. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12917–12922. <https://doi.org/10.1073/pnas.192407699>.
- Efron, B., 1979. Bootstrap methods: another look at the jackknife. *Ann. Stat.* 7, 1–26.
- Efron, B., Tibshirani, R.J., 1993. *An Introduction to the Bootstrap*. Chapman and Hall, London, UK.
- Gibson, R.H., Knott, B., Eberlein, T., Memmott, J., 2011. Sampling method influences the structure of plant-pollinator networks. *Oikos* 120, 822–831. <https://doi.org/10.1111/j.1600-0706.2010.18927.x>.
- Goldwasser, L., Roughgarden, J., 1997. Sampling effects and the estimation of food-web properties. *Ecology* 78, 41–54. [https://doi.org/10.1890/0012-9658\(1997\)078\[0041:SEATEO\]2.0.CO.2](https://doi.org/10.1890/0012-9658(1997)078[0041:SEATEO]2.0.CO.2).
- Guimera, R., Amaral, L.A.N., 2005. Functional cartography of complex metabolic networks. *Nature* 433, 895–900. <https://doi.org/10.1038/nature03286.1>.
- Jordano, P., 2015. Sampling networks of ecological interactions. *bioRxiv* 025734. <https://doi.org/10.1101/025734>.
- Manly, B.F.J., 1992. Bootstrapping for determining sample sizes in biological studies. *J. Exp. Mar. Bio. Ecol.* 158, 189–196. [https://doi.org/10.1016/0022-0981\(92\)90226-Z](https://doi.org/10.1016/0022-0981(92)90226-Z).
- Martinez, N.D., Hawkins, B.A., Dawah, H.A., Feifarek, B.P., 1999. Effects of sampling effort on characterization of food-web structure. *Ecology* 80, 1044–1055. [https://doi.org/10.1890/0012-9658\(1999\)080\[1044:EOSEOC\]2.0.CO.2](https://doi.org/10.1890/0012-9658(1999)080[1044:EOSEOC]2.0.CO.2).
- Mello, M.A.R., Marquitti, F.M.D., Guimarães, P.R., Kalko, E.K.V., Jordano, P., de Aguiar, M.A.M., 2011. The modularity of seed dispersal: differences in structure and robustness between bat- and bird-fruit networks. *Oecologia* 167, 131–140. <https://doi.org/10.1007/s00442-011-1984-2>.
- Miranda, M., Parrini, F., Dalerum, F., 2013. A categorization of recent network approaches to analyse trophic interactions. *Methods Ecol. Evol.* 4, 897–905. <https://doi.org/10.1111/2041-210X.12092>.
- Nielsen, A., Bascompte, J., 2007. Ecological networks, nestedness and sampling effort. *J. Ecol.* 95, 1134–1141. <https://doi.org/10.1111/j.1365-2745.2007.01271.x>.
- Olesen, J.M., Bascompte, J., Dupont, Y.L., Jordano, P., 2007. The modularity of pollination networks. *Proc. Natl. Acad. Sci. U. S. A.* 104, 19891–19896. <https://doi.org/10.1073/pnas.0706375104>.
- Orlaci, L., Pillar, V.D., 1991. On sample size optimality in ecosystem survey. Eds. In: Feoli, E., Orlaci, L. (Eds.), *Computer Assisted Vegetation Analysis*. Springer, Netherlands, pp. 41–46. [https://doi.org/10.1007/978-94-011-3418-7\\_4](https://doi.org/10.1007/978-94-011-3418-7_4).
- Pillar, V.D., 1998. Sampling sufficiency in ecological surveys. *Abstr. Bot.* 22, 37–48.
- R Development Core Team, 2013. *R: A Language and Environment for Statistical Computing*. R Found. Stat. Comput., Vienna Austria.
- Rivera-Hutinel, A., Bustamante, R.O., Marin, V.H., Medel, R., 2012. Effects of sampling completeness on the structure of plant–pollinator networks. *Ecology* 93, 1593–1603. <https://doi.org/10.1890/11-1803.1>.
- Schleuning, M., Blüthgen, N., Flörchinger, M., Braun, J., Schaefer, H.M., Böhm-Gaese, K., 2011. Specialization and interaction strength in a tropical plant-frugivore network differ among forest strata. *Ecology* 92, 26–36. <https://doi.org/10.1890/09-1842.1>.
- Stouffer, D.B., Bascompte, J., 2011. Compartmentalization increases food-web persistence. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3648–3652. <https://doi.org/10.1073/pnas.1014353108>.
- Takimoto, G., Suzuki, K., 2016. Global stability of obligate mutualism in community modules with facultative mutualists. *Oikos* 125, 535–540. <https://doi.org/10.1111/oik.02741>.
- Thébault, E., Fontaine, C., 2010. Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* 329, 853–856. <https://doi.org/10.1126/science.1188321>.
- Vázquez, D.P., Blüthgen, N., Cagnolo, L., Chacoff, N.P., 2009. Uniting pattern and process in plant-animal mutualistic networks: a review. *Ann. Bot.* 103, 1445–1457. <https://doi.org/10.1093/aob/mcp057>.
- Vizentin-Bugoni, J., Maruyama, P.K., Debastiani, V.J., Duarte, L.D.S., Dalsgaard, B., Sazima, M., 2016. Influences of sampling effort on detected patterns and structuring processes of a Neotropical plant-hummingbird network. *J. Anim. Ecol.* 85, 262–272. <https://doi.org/10.1111/1365-2656.12459>.