

Research

Sampling and asymptotic network properties of spatial multi-trophic networks

Anne McLeod, Shawn J. Leroux, Dominique Gravel, Cindy Chu, Alyssa R. Cirtwill, Marie-Josée Fortin, Núria Galiana, Timothée Poisot and Spencer A. Wood

A. McLeod (<https://orcid.org/0000-0002-4712-9165>) and S. J. Leroux (<https://orcid.org/0000-0001-9580-0294>) ✉ (sleroux@mun.ca), Dept of Biology, Memorial Univ. of Newfoundland, St. John's, NL, Canada. – D. Gravel (<https://orcid.org/0000-0002-4498-7076>), Dépt de biologie, Univ. de Sherbrooke, Sherbrooke, QC, Canada. – C. Chu, Aquatic Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Peterborough, ON, Canada and Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON, Canada. – A. R. Cirtwill (<https://orcid.org/0000-0002-1772-3868>), Dept of Agricultural Sciences, Univ. of Helsinki, Helsinki, Finland. – M.-J. Fortin, Dept of Ecology and Evolutionary Biology, Univ. of Toronto, Toronto, ON, Canada. – N. Galiana (<https://orcid.org/0000-0001-7720-0615>), Theoretical and Experimental Ecology Station, CNRS and Paul Sabatier Univ., Moulis, France. – T. Poisot (<https://orcid.org/0000-0002-0735-5184>), Dépt de Sciences Biologiques, Univ. de Montréal, Montréal, QC, Canada. – S. A. Wood, eScience Inst., Univ. of Washington, Seattle, WA, USA.

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Collecting well-resolved empirical trophic networks requires significant time, money and expertise, yet we are still lacking knowledge on how sampling effort and bias impact the estimation of network structure. Filling this gap is a critical first step towards creating accurate representations of ecological networks and for teasing apart the impact of sampling compared to ecological and evolutionary processes that are known to create spatio-temporal variation in network structure. We use a well-sampled spatial dataset of lake food webs to examine how sample effort influences network structure. Specifically, we predict asymptotic network properties (ANPs) for our dataset by comparing lake-specific network metrics with increasing sampling effort. We then contrast three sampling strategies – random, smallest lake to largest lake or largest lake to smallest lake – to assess which strategy best captures the regional metaweb (i.e. network of all potential interactions) network properties. We demonstrate metric-specific relationships between sample effort and network metrics, often diverging from the ANPs. For example, low sample effort can contribute to much lower and poorer estimates of closeness centralization, as compared to approximations of modularity with similar sample efforts. In fact, many network metrics (e.g. connectance) have a quadratic relationship with sample effort indicating a sampling ‘sweet spot’, which represents optimal sample effort for a close approximation of the ANP. Further, we find that sampling larger lakes followed by smaller lakes is a more optimal sampling strategy for capturing metaweb properties in this lentic ecosystem. Overall, we provide clear ways to better understand the impacts of sampling bias in food-web studies which may be particularly critical given the rapid increase in studies comparing food webs across space and time.

Keywords: fish, food web, lake, metaweb, sampling design



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Introduction

Recent research is uncovering some drivers of spatio-temporal variation in ecological networks (reviewed by Tylianakis and Morris 2017). Specifically, mechanisms pertaining to trait mis-match (Morales-Castilla et al. 2015), spatial scale (Wood et al. 2015), temporal scale (Trøjelsgaard and Olesen 2016) and species composition (Galiana et al. 2018) may explain variation in network structure across systems and the effects of this variation on ecosystem functioning (Gravel et al. 2016). Despite this progress, we still lack an ecological network theory for spatio-temporal variation that may be critical for predicting how ecological networks and the ecosystem services they provide will respond to global changes (Poisot et al. 2015, Valiente-Banuet et al. 2015, Wood et al. 2015, Fortin et al. 2021). Progress towards such a theory hinges on the development of high-quality empirical network datasets that span several spatial locations, sampling periods and systems. Ecologists, however, have long been critical of ecological network datasets for their potential biases due to inconsistent taxonomic resolution, underrepresentation of mobile species and misrepresentation of spatio-temporal variation (Paine 1988, Cohen et al. 1993, Hall and Raffaelli 1993). These debates have demonstrated that accurately capturing empirical network patterns and processes over both space and time is an immensely challenging task (Jordano 2016).

Ecological networks are challenging to measure empirically in part because biased and insufficient sampling generate uncertainties in networks from the species to the interactions themselves (Jordano 2016, Dormann et al. 2017). Sampling bias, for example, is reflected in taxa-focused sampling, such as phyto-centric sampling in plant–animal interaction networks or zoo-centric sampling in plant–frugivore interactions (Schleuning et al. 2012). One outcome of sampling bias is asymmetric interaction matrices (Dormann et al. 2009). Insufficient sampling effort risks missing a) species, b) the co-occurrence of two species or c) the interaction between them (Cirtwill et al. 2019). These three components of sampling error can differentially affect network properties depending on where the omissions occur in the network (Martinez et al. 1999, de Aguiar et al. 2019). Failing to include a taxon in a network has all the known issues inherent to diversity studies (review in Gotelli and Colwell 2001). Yet, it is hard to predict the additional effects of missing species on the overall structure of interaction networks and species-specific interactions, especially because ecological network research rarely reports study-specific sampling sensitivity to species richness estimates (but see Chacoff et al. 2012).

The lack of reporting of sampling effort in network studies is common despite efforts to repurpose species accumulation curves into estimates of the expected number of interactions given the number of observations (Colwell et al. 2004). Similar to species accumulation curves, the interaction accumulation curves reach an asymptote when increasing sampling effort does not result in an increase in the observed number of interactions (Chacoff et al. 2012). Sampling

completeness can then be assessed by calculating how far the observed number of interactions is from this expected asymptotic value. Different sites will have different interaction accumulation curves and this variation in sampling completeness can lead to under- or over-estimating of network properties. Consequently, consideration of sampling completeness is particularly important when analyzing network properties for a collection of sites sampled across space and/or time (Jordano 2016).

A considerable number of studies have measured how sampling impacts properties of bipartite networks. For example, Rivera-Hutinel et al. (2012) demonstrated that many network metrics (e.g. modularity, centralization) were uniquely sensitive to sampling completeness in 10 plant–pollinator networks in a forest-fragmented system in central Chile. For instance, connectance increased with sampling completeness while modularity decreased – demonstrating that we can approximate these network metrics better with increased sampling, but highlighting that increasing sample effort can have opposing results depending on the metric. Of course, in empirical networks there is no way to know the true value of a network metric (Jordano 2016), however, in quantitative niche model simulations, Fründ et al. (2016) recommended collecting at least 20 observations per consumer for accurate estimates of most quantitative bipartite network metrics. Whether such findings for bipartite networks apply to other network typologies such as multi-trophic food webs remain unknown, but similar overlapping sampling biases are present in both network types. For example, sampling of most plant–pollinator networks is centered on plants (Memmott 1999), while antagonistic network sampling is often centered on the predator, identifying prey through observation of interactions, gut content or DNA techniques.

Because of the two-fold uncertainty in presence of both species and interactions, we are left estimating ecological networks that approximate an unknown, and unknowable, reality (Jordano 2016, Cirtwill et al. 2019). Instead, we propose that we can derive a reasonable approximation of these properties using rarefaction. We define ANP as the estimated asymptotic network property (e.g. connectance, linkage density) at a point where increasing sampling effort does not appreciably change the value of the network metric (similar to the asymptotic value of species accumulation curves, Connor and McCoy 1979). Identifying ANPs will help us determine the sample effort required to minimize the uncertainty in the metric of interest, and to ensure appropriate sample effort to distinguish between variation in network properties driven by environmental gradients and variation in network properties driven by sampling artefacts. The advantages to the ANP approach are relevant when we have either many sampling events (temporal sampling) or many sampling locations (spatial sampling), but can also be used to compare a single location with a metaweb (i.e. network of all the potential interactions among species from a regional pool) compiled from direct and indirect evidence of interaction (e.g. literature, feeding trials). The purpose of a metaweb is typically to represent all of the potential interactions among species

from a regional pool whether or not they interact locally. This representation is necessary, for example, for understanding how the introduction of a novel species might modify local webs, to infer interactions that may have been missed during a particular sample, or that are likely to occur after climate change has altered community composition (Gravel et al. 2013, Grünig et al. 2020). Given the current expansion in spatial–temporal network studies (reviewed by Fortin et al. 2021) and application of these spatial–temporal studies for predicting how ecological communities will respond to global changes, the ANP approach will give ecologists a metric to consider how sampling effort may be affecting their conclusions.

Here, we take advantage of a very large dataset of freshwater fish gut contents for 99 lakes in Ontario, Canada to study the impacts of sampling completeness on estimates of trophic network properties. Our first objective is to investigate potential relationships between sample effort and the number of species and/or interactions observed. To do this, we compare the relationship between the numbers of species and/or interactions observed and expected as an increasing number of samples were collected per study lake. Our second objective is to assess the impacts of sampling effort on lake-level ecological network properties and to characterize ANP relationships in this system. Our third objective is to provide an evaluation of three contrasting sampling strategies to best capture the metaweb properties. Given limited time and budgets, ecologists interested in studying ecological networks

across many sites or periods must decide how best to sample the community to capture network properties. To solve this tradeoff, we compare how well prioritizing sampling based on lake size (i.e. from smallest to largest or from largest to smallest or at random) capture the metaweb (Fig. 1). We discuss recommendations to improve the empirical estimates of network properties and the limits to spatio-temporal ecological network comparative studies.

Material and methods

Study system and food web data

Ontario is a large province in Canada with boreal forest in the north, temperate forest in the south and thousands of lakes and rivers. The data were collected as part of the Broad-scale Monitoring Program for Inland Lakes run by the Ontario Ministry of Natural Resources and Forestry (Sandstrom et al. 2013). For this program, fish abundance and fish stomach content information were collected from 741 lakes across Ontario, Canada, between 2008 and 2012. While not all lakes are directly connected, lakes are within a region with similar post-glacial recolonization history (Mandrak 1995) and within the ranges of the diverse fish community we studied. Moreover, due to the sportfishing industry in the region, research suggests there has been a homogenization of species diversity in some regions of the province (Cazelles et al. 2019). A metaweb, therefore, represents a plausible maximal

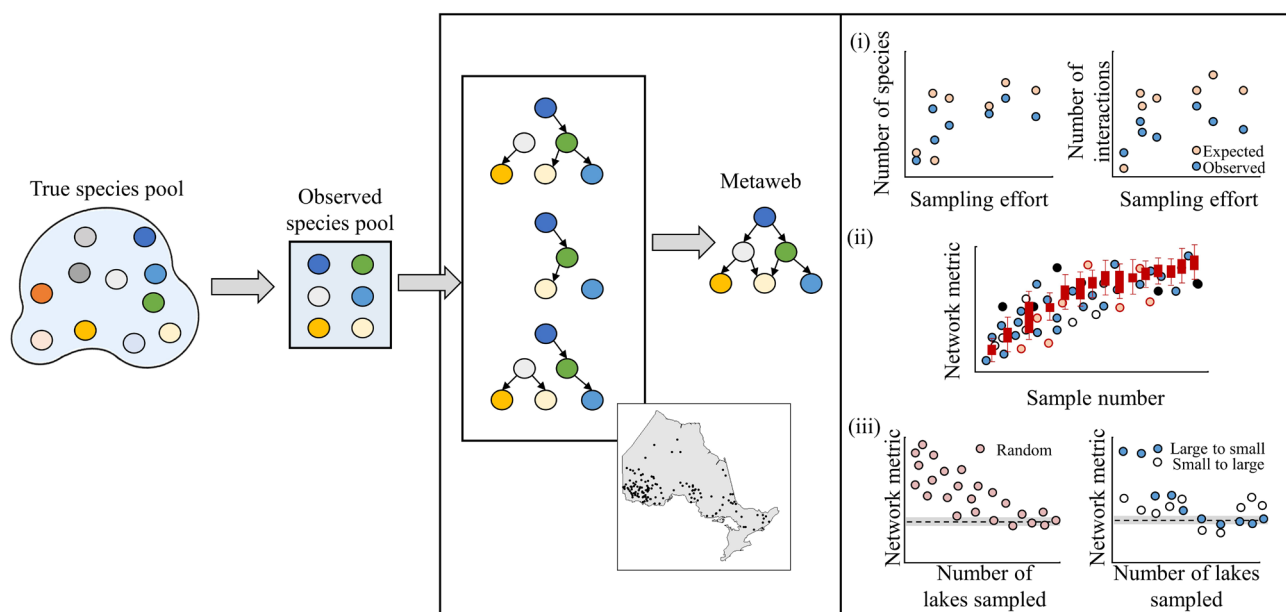


Figure 1. Conceptual diagram of the analyses undertaken to address our three research objectives. First, 1) we investigate potential relationships between sample effort and the number of species or interactions observed by comparing the relationship between expected and observed number of species and interactions as we increase the number of samples collected per study lake ($n = 99$). Second 2), we identify the shape of the relationship between the number of interactions sampled and network properties within a lake, with the aim of finding the best-fit relationship across the majority of lakes. Third 3), we contrast a random lake selection strategy to one which works down from the largest lakes in the sampling area to the smallest lakes, and another which works up from the smallest lakes in the sampling area to the largest lakes to determine which sampling strategy best approximates metaweb properties.

community of the lakes in the province. We used the fish stomach content information to build binary food webs (i.e. presence/absence of interactions) at both the lake and metaweb level. Gut contents do not always represent a single interaction for the same individual can have multiple prey items in its guts, while empty gut contents, or those contents which are not identifiable are ignored. In our analysis, we only considered fish prey to build fish-only food webs. For our analyses, we used a subset of 99 lakes which met the following sample effort criteria: more than five observed interactions, sampling effort sufficient to detect a minimum of 75% of the pairwise interactions expected to occur in each lake (i.e. $S_{obs}/AR_{sp} \times 100$ or $S_{obs}/AR_{int} \times 100 > 75$; see section below for how S_{obs} , AR_{sp} and AR_{int} are calculated), more than two fish–fish interactions observed and more than one unique interaction observed. Lakes that did not meet these criteria have very simple food webs that were not useful for investigating our objectives (275 lakes were rejected because they did not have more than two fish–fish interactions observed or more than one unique interaction observed). Further details on data collection can be found in the Supporting information.

Species and interaction richness

We began by measuring sample-based rarefaction curves for species and interaction richness across our 99 lakes. We reported the number of unique species and interactions observed in the 99 lakes where the lakes all had a different number of species and interactions (i.e. gut contents) sampled. We compared these sample-based rarefaction curves to the expected asymptotic species richness (AR_{sp}) and expected asymptotic interaction richness (AR_{int}) as measured by the abundance-based richness estimator Chao1 (Chao 1987, Chiu et al. 2014; Supporting information). Then, we calculated Chao1 as AR_{sp} to compare the actual species or interactions sampled to the expected number of species or interactions in each lake. This estimate is often used to determine whether sampling efforts have detected a sufficient amount of species or interactions occurring in each lake (Chao 1987, Chiu et al. 2014). AR_{sp} was calculated as:

$$AR_{sp} = S_{obs} + \frac{a_1(a_1 - 1)}{2(a_2 + 1)} \quad (1)$$

where a_1 is the number of species observed once, while a_2 is the number of species observed twice, S_{obs} is the number of observed species and AR_{sp} is the expected asymptotic species richness. AR_{int} was calculated using the exact same method, but using interactions instead of species. We used the vegan package in R to estimate AR_{sp} and AR_{int} (Oksanen et al. 2016). This initial analysis was done to reveal how well our lakes are sampled.

Network properties

There are many measures to characterize ecological networks (recently reviewed by Lau et al. 2017, Cirtwill et al. 2018,

Delmas et al. 2019). In our analysis, we focused on connectance, linkage density, mean prey averaged trophic level (PATL), two centralization metrics (based on degree centrality or closeness centrality) and modularity (Supporting information for definitions). With the exception of the centralization metrics, we chose these metrics because they are commonly reported food web metrics and used in many studies comparing temporal and spatial drivers of food web structure. While centrality measures are reported more often in food web studies than centralization metrics, they are species-level metrics. In our case we use centralization measures for the entire network. These centralization metrics are derived from species-level degree centrality and closeness centrality in that they are the average deviance of individual species degree centrality or closeness centrality scores from the most central species (see Freeman 1978 and Wasserman and Faust 1994 for more details on calculation). In this way they are a measure of how central the most central node is in relation to other nodes and is insensitive to network size and connectance (discussion in Delmas et al. 2019). We randomly selected interactions from each lake without replacement (there are 99 lakes, but the number of interactions in each lake depends on the lake) and recalculated the network metric after each successive interaction draw until all of the interactions for that lake have been used. For example, if a lake had nine interactions, we randomly picked one of those interactions, recalculated the lake-level interaction metric, randomly selected another interaction from that lake, calculated the lake-level interaction metric and continued this process until all nine interactions in the lake had been sampled. This was then replicated 100 times for this lake ($n=900$ network metrics). This process was repeated for all 99 lakes. We assessed the general form of ANP curves by fitting linear, exponential, logarithmic and quadratic models to ANP for each metric for each lake. We fitted trends for four different quantiles (i.e. four groups of lakes based on percent of AR_{int} sampled, P_{int}) and reported trend lines of the best fit model for each quantile. As variance decreases with sample size in these models, the models were fit with variance terms scaled by the inverse standard deviation for that sample size. We assessed evidence in support of linear, exponential, logarithmic and quadratic relationships by reporting the number of times each model was ranked as top model based on AIC.

Comparing sampling strategies to measure network properties

We assessed how three different sampling techniques for the 99 lakes impact estimates of the metaweb properties. Specifically, we contrasted network properties when we sample lakes 1) randomly (100 iterations), 2) from largest to smallest lake and 3) from smallest to largest lake. Similar to above, we computed each network metric as we accumulated lakes under each sampling scheme. For 1) a random lake was drawn and the metric was calculated, another lake was randomly drawn and the composite metric calculated, etc. For 2) and 3) the metric was calculated for the largest (smallest)

lake, then the metric was calculated again for the composite of the largest (smallest) lake and second largest (smallest) lake, etc. We used the metrics for the metaweb as the reference. For each sampling scheme, we reported the number of lakes required to be within and stay within 5, 10 and 30% of the total range of values found in all the networks from the metaweb estimate for each network metric to gain an understanding about how these sampling schemes differ with decreasing precision. We recognize that using the percentage of an absolute value, e.g. for a metric such as modularity which is bounded between 0 and 1, may not be the best method to determine when sampling effort is sufficient to minimize error. Thus, we also calculated the variance using the sum of square difference between the observed value and the metaweb value (Supporting information).

Results

A total of 1131 interactions were observed across the 99 lakes: 116 unique interactions, and 39 different fish species, with an average of $10.77 (\pm 2.98)$ species and $13.02 (\pm 5.77)$ interactions in each lake. There was an average connectance across all lakes of $0.18 (\pm 0.084)$ which was much higher than the metaweb connectance of 0.076. The asymptotic species richness per lake (AR_{sp}) ranged from 3.00 to 38.56, with a mean of $12.30 (\pm 6.29)$ and AR_{int} ranged from 2.00 to 102.81, with a mean of $18.12 (\pm 15.34)$. Further, the deviation between observed and expected is larger for interaction richness than for species richness (Supporting information).

Estimating asymptotic local network properties

We observed large variability in the response of the six focal network metrics to increasing the number of interactions sampled and trends were often different for lakes with few interactions and for lakes with many interactions (Table 1, Fig. 2). Moreover, uncertainty around metric estimates was also metric dependent; some metrics showed large initial uncertainty, but a reduction in uncertainty with increasing sample number (e.g. connectance; Fig. 2), while other metrics retained a high amount of uncertainty even as sample number increased (e.g. degree centralization). No metric appeared to reach a flat trajectory, or asymptote, as we increased the

number of interactions sampled. There was an overall positive relationship between the number of interactions sampled and linkage density. There was a positive relationship between the number of interactions sampled and trophic level and degree centralization but these positive relationships only emerged after sampling ~ 50 interactions. There was no clear trend in the relationship between the number of interactions sampled and connectance and closeness centralization. The relationship between the number of interactions sampled and connectance was negative at low sample sizes whereas the relationship between the number of interactions sampled and closeness centralization was positive at low sample sizes. Finally, there was a negative relationship between the number of interactions sampled and modularity when sample size > 25 and a positive relationship when sample size < 25 (Fig. 2).

An exponential model was the top fit for trophic level, degree centralization, closeness centralization and modularity (Table 1; as there are 99 lakes and eight metrics, we have omitted individual model results). The fit for these top-ranked models was variable; the mean R^2 across all lakes and all network properties was $R^2 = 0.19$ with a standard deviation of 0.05, ranging from $R^2 = 0.13$ for closeness centralization (mean across lakes) to $R^2 = 0.23$ for degree centralization (mean across lakes). A quadratic model, on the other hand, was the best fitting model for the remaining metrics (connectance, linkage density; overall R^2 for metrics where this model was the top fitting model $= 0.56 \pm 0.16$ [mean \pm standard deviation]). For the metrics in which an exponential model was the best fit model, quadratic models were the second best fit, while for metrics in which quadratic models were the best fit, linear models were the second best fit (Table 1, see the Supporting information for an example for one iteration).

Comparing sampling strategies to measure a metaweb

We found positive relationships between number of lakes sampled and linkage density, and closeness centralization (Fig. 3, Supporting information). The estimated linkage density, and closeness centralization metrics were, respectively, within $\pm 10\%$ of metaweb estimates after 84 and 53 samples for random sampling, 39 and 23 samples for largest to

Table 1. Table summarizing the top-ranked model for each network metric. We fit linear (network metric \sim sample number), quadratic (network metric \sim (sample number)²), exponential (network metric $\sim \exp(\text{sample number})$) and logarithmic (network metric $\sim \ln(\text{sample number})$) for each network metric calculated. We completed 100 iterations of random interaction draws for each lake, where one iteration is the consecutive draw of one interaction through to the total number of interactions within that lake, and the network metric is calculated after each interaction draw. The numbers report the number of cases (i.e. lakes) where each model form was ranked as the top model based on AIC (i.e. lowest AIC in set is top ranked model).

Metric	Linear	Quadratic	Exponential	Logarithmic
Connectance	8	91	0	0
Link density	15	84	0	0
Trophic level	0	0	99	0
Degree centralization	0	12	87	0
Closeness centralization	3	3	93	0
Modularity	0	3	96	0

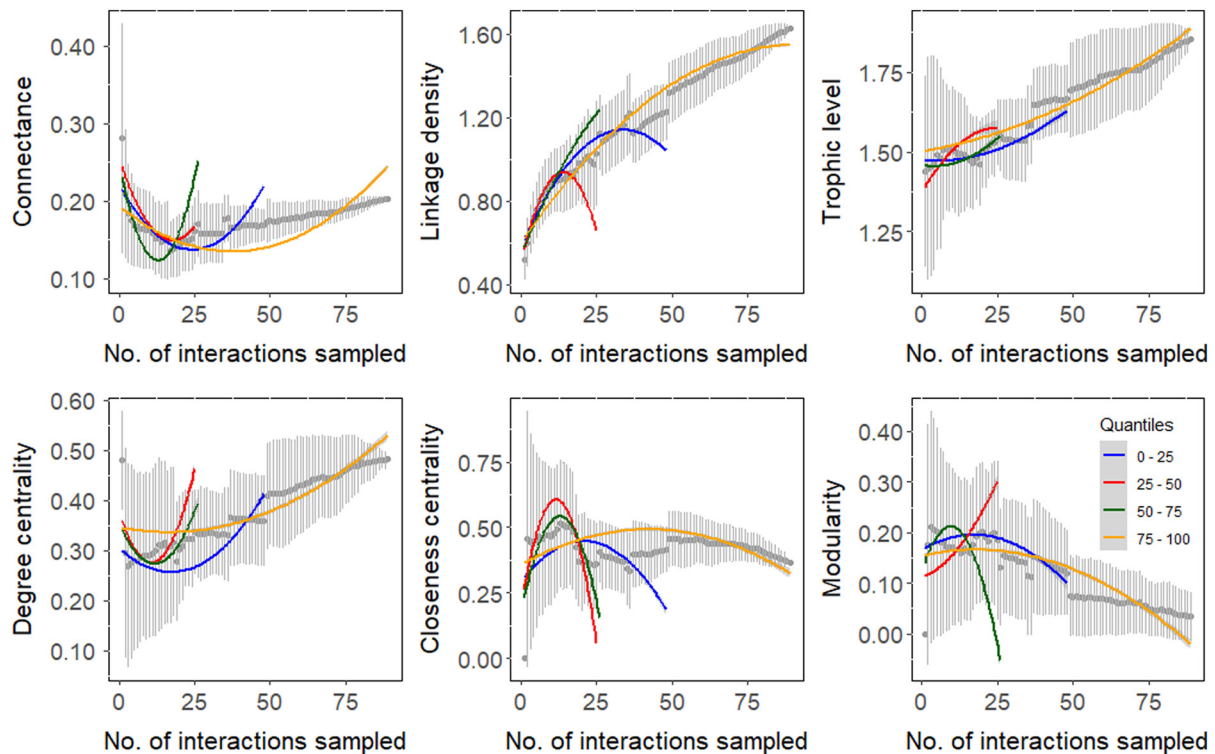


Figure 2. Comparison of how sample effort influences different network metrics. Number of interactions sampled is the number of interactions used to compute the network metric. To generate this figure, we randomly selected interactions from each lake and recalculated the network metric after each successive interaction draw until all of the interactions for that lake have been used. This process is completed 100 times for each lake with the network metric computed after each interaction draw. The grey dots are the grand mean of each metric across lakes (i.e. the average value obtained for the 100 iterations for each lake averaged across all lakes; ± 1 SD) and the coloured lines indicate the trends for the quartiles (i.e. four groups of lakes based on percent of AR_{int} sampled, P_{int}) where 0–25% is blue, 25–50% is red, 50–75% is dark green and 75–100% is orange. Trend lines were fit according to the best fit model for each quartile.

smallest lakes sampling and 73 and 5 for smallest to largest lakes sampling (Supporting information). All three sampling strategies had negative relationships which appeared to asymptote relatively quickly compared to other metrics for connectance, trophic level, degree centralization and modularity (Fig. 3, Supporting information). The estimated connectance, trophic level and degree centralization metrics respectively approached metaweb estimates after 29, 47 and 34 samples for random sampling, 37, 10 and 5 samples for largest to smallest lakes sampling and 12, 29 and 51 for smallest to largest lakes sampling (Supporting information). The best sampling strategy for both closeness centralization and modularity was random, while there was no clear trend for best sampling strategy for degree centralization, and the best sampling strategy for link density and connectance was largest to smallest (Fig. 3). Modularity was the metric with the least variability and required the lowest number samples to fall within $\pm 10\%$ of the range around the metaweb for any sampling scheme. This was due to the low value of modularity in the metaweb. Modularity demonstrated a clear asymptotic relationship with sampling and required relatively low sampling to optimize accuracy of the estimate (Supporting information). Importantly, we found that both the number of lakes that needed to be sampled and the sampling strategy

which approximated the metaweb with the least amount of sample effort were metric-dependent. For example, few lakes were required to estimate modularity with confidence (Supporting information), and while few lakes were required to estimate degree centralization within 30% of the metaweb, almost all of the lakes were required to estimate degree centralization within 5% of the metaweb.

Discussion

Rarefaction is a commonly used technique for ensuring sampling completeness of ecological networks, for separating truly absent interactions from interactions which occur but are simply not observed (Rivera-Hutinel et al. 2012, Vizentin-Bugoni et al. 2014). By applying these rarefaction techniques to well-sampled lentic systems, we show that the distance between expected and observed values is higher for interaction richness than species richness (Supporting information). This suggests that studies which use rarefaction techniques to determine the efficacy of sampling based on species richness – as a stand-in for interaction richness – may be overestimating their sampling efforts. We propose asymptotic network property (ANPs) curves as a way to

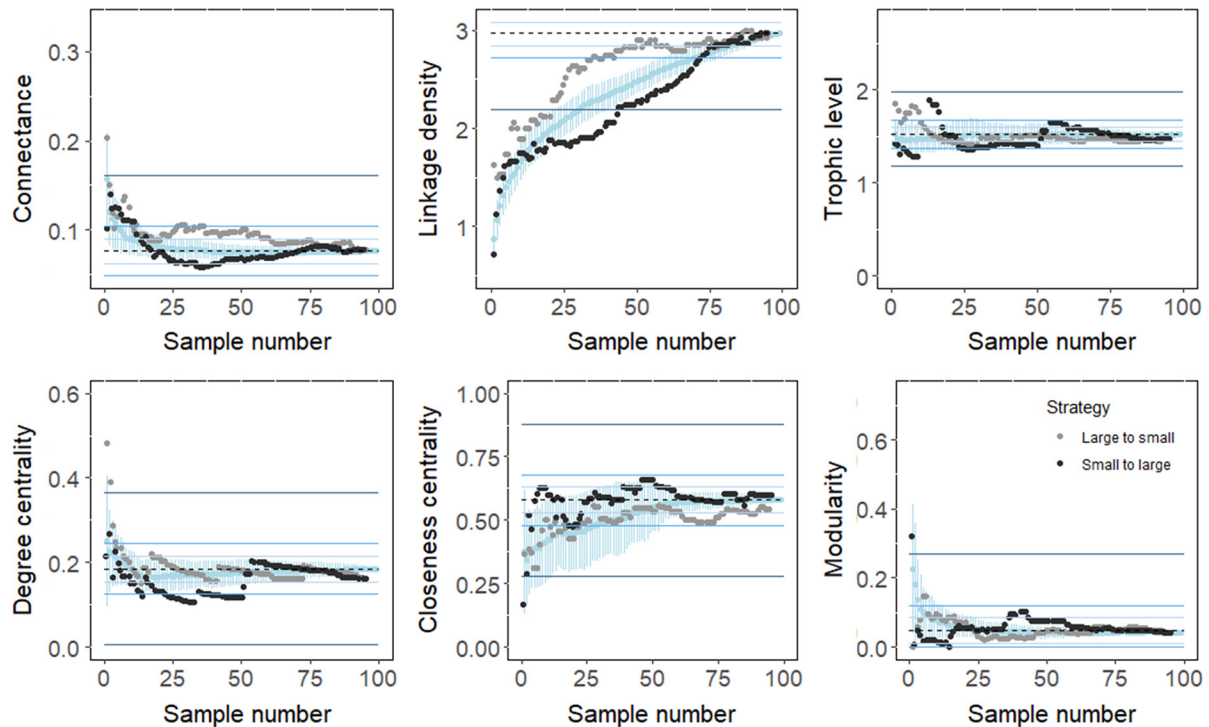


Figure 3. Comparison of three strategies for lake sampling to measure network metrics. Grey dots represent a sampling strategy that starts with the largest lake and adds consecutively smaller lakes, the black dots represent a sampling strategy that starts with the smallest lake and adds consecutively larger lakes, and the light blue circles with error bars are the random sampling strategy. The dashed black line represents the metaweb for the 99 lakes used in this analysis and the light grey, light blue and dark blue bar represents ± 5 , ± 10 , $\pm 30\%$ of the total range around this value, respectively, or the minimum observable value when necessary (i.e. connectance, degree centrality and modularity). In the case of linkage density the error bar for the upper 30% of the range was higher than all of observed linkage densities so the error bar has been omitted. In some cases, the 30% error bars are covering much of the data range because of the large range for that metric in the complete data set (see the Supporting information for complete plots). These range bars assist in visually assessing the number of samples required to estimate the metaweb metric with varying degrees of accuracy.

estimate sample effort required to minimize uncertainty in network metrics. This approach may be particularly useful for the many studies which sample sets of networks over either time or space. ANPs for our lentic system show considerable variability in the relationship between sampling effort and different network metrics. Despite this variability, we demonstrate that a sampling strategy focusing on sampling large lakes first appears to be the best strategy to capture the metaweb, or the set of all potential interactions within this region.

The exercise of measuring network properties is really an attempt to estimate an underlying reality that cannot be quantified (Jordano 2016, Cirtwill et al. 2019). Given that we may never know true network properties, a reasonable approximation may be to estimate asymptotic network properties (ANPs). Underlying this approach is the hypothesis that under an ideal sampling effort, local network properties should converge to a close estimate of the real value. Additionally, this approach is practical because ANPs can be estimated from multiple samples of one location through time, or multiple samples across habitats within a location. Our results provide some of the first evidence to test this hypothesis.

Unsurprisingly, the relationship between sampling intensity and proximity to ANP is metric-dependent. Similar to the study by Rivera-Hutinel et al. (2012) examining the influence of sampling completeness on 10 plant–pollinator networks in Chile, we observe that increasing sampling intensity results in an increased estimate of connectance. The trajectories of the trend lines, however, suggest that these situations of medium sampling intensity may overestimate connectance (Fig. 2). This implies that there is a sampling intensity at which connectance peaks and then declines, which in turn highlights the importance of knowing the true shape of the relationship between sampling effort and network metrics. Specifically, it demonstrates that knowing where each study fits along this sampling gradient is very important and could resolve contradictory results between studies (e.g. debate around the link-species scaling law; Montoya and Solé 2003, Brose et al. 2004, Ings et al. 2009). For example, when examining the results for modularity (Fig. 2), we observe that medium sampling effort (i.e. second and third quantile) results in opposing trends, while both low (blue) and high (yellow) sample effort result in similar trends. Previous studies have suggested that modularity is largely invariant to network size (Dunne et al. 2002, Olesen et al. 2007), however our results suggest this invariance

could simply be a sampling artefact. This observation is further supported by our finding that a quadratic relationship is most frequent across lakes for connectance, and linkage density – suggesting that there is a lake-level ‘sweet spot’, below which too little sampling underestimates a metric (Table 1). Sampling past this sweet spot may, however, also result in underestimates of sampling metrics (note if the parabola is concave-up then sampling both very well and very poorly overestimate the network metric). It is likely that a cubic relationship would emerge if more of our lakes were on the well-sampled end of this spectrum, with an amount of sampling that produces a local minimum (or maximum) before increasing sampling results in a trajectory approaching the ANP.

Individual boreal lake food webs are considered depauperate in species (Gunn et al. 2003). The metaweb of these lakes, however, is considerably larger, suggesting that missing species, whether due to their abundance or to inappropriate sampling techniques, can have a disproportionate influence on estimates of network metrics such as connectance. This is unsurprising since missing a species entails missing all interactions in which it is a part, while missing an interaction is just that – missing one interaction. Incorporating the contributions of an unobserved species to network metrics is rife with complications, which is why many sample effort metrics do not consider it (Macgregor et al. 2017). In particular, this is a strength of determining the ANPs. Similar to using species accumulation curves to estimate true species richness, ANPs could be used to estimate the true network metric, instead of merely sampling completeness. This is of particular importance as studies examining the spatial and temporal variation in networks become more routine (Poisot et al. 2012a, Baker et al. 2015, Trøjelsgaard and Olesen 2016, CaraDonna et al. 2017) to ensure that this spatial and temporal variation captures ecological variation rather than variation driven by sampling artefacts. Of course, this gets us nowhere closer to inferring the specific interactions which are missing. However, combining this approach with recent progress in computational and statistical methods for inferring missing interactions in Desjardins-Proulx et al. (2017) and Cirtwill et al. (2019) may help fill this gap.

Some of the most cited and used trophic network datasets are for freshwater fish (Havens 1992) and marine fish (Brose et al. 2006) communities. There are many methods to determine feeding relationships among fish and the study of fish food web sampling techniques is an active field of study (Bartley et al. 2015, Shipley et al. 2017). For example, one of the most common methods to determine feeding relationships in fish food webs is to use stomach gut content analyses (Hauer and Lamberti 2017). Gut content analyses are based on what organisms eat in a narrow window of time immediately before capture. The width of this window is dependent on the prey item since bony or cartilaginous organisms are harder to digest making them present for longer in the gastrointestinal tract than other softer items. Thus, there should be a partiality in aquatic food webs for predator interactions with these harder-to-digest prey types. But sampling techniques are also often biased to certain types of predators,

causing only species with specific traits to be sampled. For example, gill net surveys have been repeatedly proven to be size- and species-selective (Carol and García-Berthou 2007) with a higher probability of capturing larger, more active species and under-representing sit-and-wait predators (Hansson and Rudstam 1995). This makes it important not only to understand the impact of sampling on network metrics, but also the species-specific traits which sampling is inherently biased against. Determining what traits are more often represented in food web studies could prove critical for developing more accurate trait-based food web models for both local and regional networks (Eklöf et al. 2013, Ho et al. 2019). Combining the results presented here with a traits-based approach for estimating catchability of species in a network could provide a crucial tool for improving network estimates.

Recently, there have been calls to conserve species interactions rather than simply species since the loss of interactions in a community can result in large reductions in ecosystem function (Tylianakis et al. 2010, Valiente-Banuet et al. 2015). However, compiling comprehensive networks across spatial or temporal gradients is both time consuming and costly. Instead, ecologists interested in studying networks must carefully consider how best to maximize sampling effort to fully capture their desired network properties. We demonstrate that, for most network metrics, it may be optimal to focus sampling on larger lakes to best approximate the metaweb in lentic systems. Sampling in the opposite direction (i.e. small to large) will generally require researchers to sample more lakes in order to fall within the desired confidence bounds of the ANP, while the utility of a random sampling strategy is network metric dependent (e.g. intermediate between a size directed sampling approach for connectance, linkage density and trophic level; Fig. 3). Sampling from small to large will result in a quicker accumulation of lakes spanning the region, however, the large lakes likely hold more of the regional species richness. Obviously, the large lake sampling strategy helps to better estimate the range of the metaweb, however, sampling the largest lake many times also will help detect temporal regional rewiring (Poisot et al. 2012b, CaraDonna et al. 2017, Bartley et al. 2019). It is of particular importance to evaluate how targeted sampling strategies will differ from accumulation curves of network properties, which are known to occur in any sampling of a spatially distributed system (Poisot et al. 2012b, Gilarranz et al. 2015).

By assessing the impacts of sampling effort on lake-level network properties and characterizing ANP relationships in this system, we provide a clear example of how sampling strategy can lead to inaccurate representation of network metrics. We also present an approach that can be used to design and evaluate a sampling strategy for the best estimates of network properties. In particular, we demonstrate metric-specific relationships between sample effort and network metrics, often times diverging from the ANP for the system. This highlights the utility of ANPs for assessing sample effort, but also demonstrates the perils of coopting species-diversity metrics as a singular method for assessing sample effort. Deriving a suite of empirical ANPs, as we have done here, from diverse study

systems may be a useful starting point to developing a general model or theory for ANPs. In the end, food web studies that do not report any measure of sampling effort are still much more common than studies that do report on sampling effort. We argue for greater accountability of sampling effects in food web studies and we propose that measuring ANPs may provide one promising tool in this direction. Better accounting of sampling bias in food-web studies is particularly critical given the rapid increase in studies comparing food webs across space and time. Finally, we recognize the balance between limited time and budgets and the increasing need for network studies to better anticipate communities' responses to global change. We demonstrate that focussing on larger lakes, and then moving towards smaller lakes is the most optimal sampling strategy to best capture metaweb properties in lentic ecosystems.

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Author contributions

Anne McLeod and **Shawn J. Leroux** contributed equally to this publication. **Anne McLeod**: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (equal); Software (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Shawn J. Leroux**: Conceptualization (lead); Data curation (equal); Formal analysis (equal); Investigation (lead); Methodology (lead); Project administration (equal); Software (equal); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Dominique Gravel**: Conceptualization (equal); Formal analysis (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Writing – original draft (equal); Writing – review and editing (equal). **Cindy Chu**: Conceptualization (supporting); Data curation (lead); Investigation (supporting); Methodology (supporting); Writing – review and editing (supporting). **Alyssa R. Cirtwill**: Conceptualization (supporting); Investigation (supporting); Methodology (supporting); Writing – review and editing (equal). **Marie-Josée Fortin**: Conceptualization (equal); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing – review and editing (equal). **Núria Galiana Ibáñez**: Conceptualization (supporting); Formal analysis (supporting); Investigation (supporting); Writing – review and editing (equal). **Timothée Poisot**: Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Writing – review and editing (equal). **Spencer A. Wood**: Conceptualization (supporting);

Formal analysis (supporting); Methodology (supporting); Writing – review and editing (equal).

Data availability statement

Data are available from the Figshare Digital Repository: <<https://figshare.com/s/a94ad455871a505d2973>> (McLeod et al. 2021).

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