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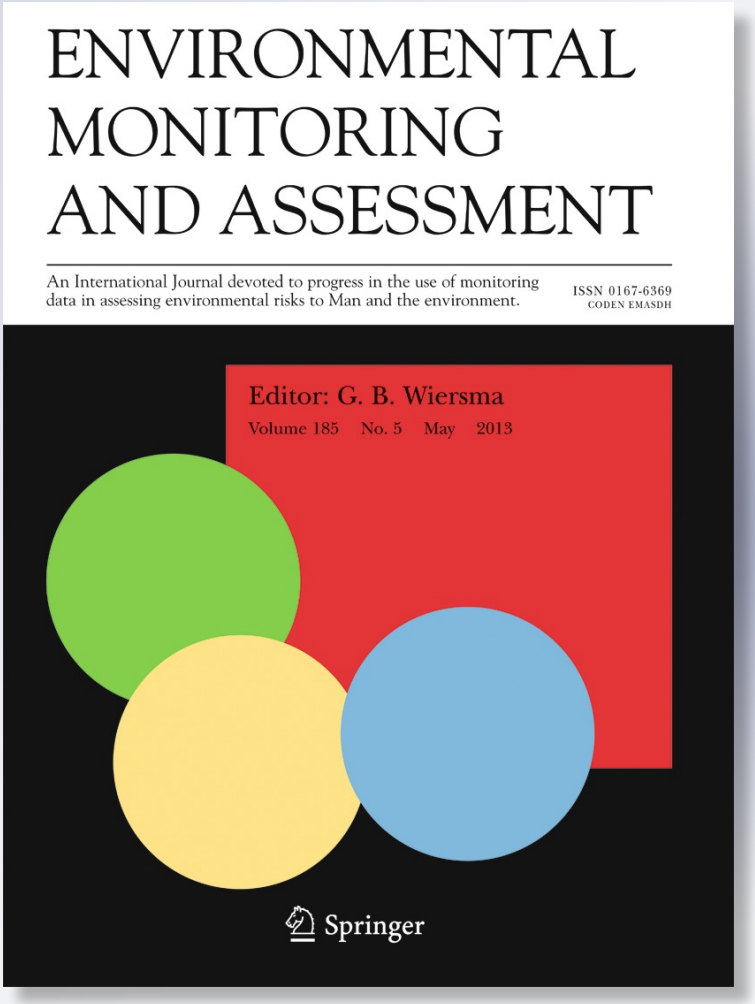

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The problem of using fixed-area subsampling methods to estimate macroinvertebrate richness: a case study with Neotropical stream data

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Abstract Subsampling has been widely applied in the laboratory to process freshwater macroinvertebrate samples. Currently, many governmental agencies and research groups apply the fixed-count approach, targeting a number of individuals per sample, and at the same time keeping track of the number of quadrats (fraction of the sample) processed. However, fixed-area methods are still in use. The objective of this paper was to evaluate the reliability of macroinvertebrate taxonomic richness estimates developed from processing a standard number of subsampling quadrats (i.e., fixed-area approaches). We used a dataset from 18 tropical stream sites experiencing three different levels of human disturbance (most-, intermediate-, and least-disturbed). With 12 quadrats processed (half the sample), the collection curves started to stabilize, and for more than half of the sites studied, it was possible to sample at least 80 % of the total taxonomic richness of the sample. However, we observed that the minimum number of quadrats to achieve 80 % of taxonomic richness was strongly negatively correlated with the number of individuals collected in each site: the fewer the individuals in a sample, the greater the processed proportion of that sample needed to represent it properly. Thus our results indicate that for any given areal subsampling effort (any fixed fraction of the sample), samples with different numbers of individuals will be represented differently in terms of the proportion of the total number of taxa of the whole samples, those with greater numbers being overestimated and those with fewer numbers being underestimated. Therefore, we do not recommend the use of fixed-area subsampling methods alone if the main purpose is to measure and analyze taxonomic richness; instead, we encourage researchers to use fixed-count approaches for this purpose.

Keywords Subsampling methods · Taxonomic richness · Laboratory procedures · Disturbance categories · Stream research

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Introduction

Taxonomic richness is a key measurement for assessing biological assemblage diversity at many spatial scales (Gotelli and Cowell 2001), including macroinvertebrate assemblages (Melo and Froehlich 2001). Taxonomic richness is the basis for many ecological models (MacArthur and Wilson 1967; Lande 1996; Arita and Vazquez-Dominguez 2008) and a common

component of multimetric indices used in ecosystem biomonitoring (Barbour et al. 1999; Klemm et al. 2003; Baptista et al. 2007; Stoddard et al. 2008; Suriano et al. 2011). Thus, taxonomic richness is a cornerstone of both basic and applied studies dealing with macroinvertebrate assemblages, as well as for developing conservation strategies for watersheds (Clarke et al. 2010; Richardson and Whittaker 2010). Accordingly, measuring taxonomic richness, whether expressed in terms of species, morphospecies, genera, or families, is often a key objective when processing samples of freshwater macroinvertebrate assemblages (Clarke et al. 2008).

Despite the importance of taxonomic richness in macroinvertebrate studies, representing taxonomic richness of samples is not an easy task (Vinson and Hawkins 1996). In the laboratory, processing the whole sample is often impracticable because programs and researchers have limited time, money, and personnel resources (Nichols and Norris 2006). In many studies dealing with large spatial extents and high numbers of samples, processing the whole sample is not a viable option (Hughes and Peck 2008). Taking portions of the samples, i.e., subsampling, has been widely applied to resolve this dilemma. There are two major ways to subsample: fixed-count and fixed-area methods (Barbour and Gerritsen 1996). Both can be applied to represent different sampling spatial extents, from microhabitat samples to site composite samples. In fixed-count methods, a fixed number of individuals is randomly picked and identified from the homogenized sample. In the fixed-area methods, also known as fixed fraction and proportional subsampling, the whole sample is homogenized and a fixed proportion of it is then fully processed. This is usually accomplished by spreading the sample in a divided tray and processing a certain number of "quadrats," proportional subdivisions of the tray (Oliveira et al. 2010).

Currently, many governmental agencies and research groups apply the fixed-count approach, targeting a number of individuals per sample, and at the same time recording the number of quadrats processed, to also measure individual densities (e.g., Moulton et al. 2000). The number of individuals processed usually varies between 100 and 500, depending on the study objectives, the amount of available resources, and the agency/research group (Carter and Resh 2001). For example, the United States Environmental Protection Agency applies a combined quadrat, fixed-count (500 individuals), including a large/rare search, in its macroinvertebrate sample processing (Stoddard et al. 2008). However, we doubt whether every research group follows this approach. Fixed-area subsampling has been considered a traditional method for a long time (Barbour and Gerritsen 1996), and recently, many publications presented results on subsampling effort and metric variability for different fractions of the sample processed (e.g., King and Richardson 2002; Petkovska and Urbanic 2010; Oliveira et al. 2010). However, these authors were concerned with many other metrics in addition to taxonomic richness.

The purpose of subsampling methods is to reduce the amount of work in the laboratory and still obtain a dataset not biased by the procedure, capable of reliably representing the samples and precisely answering the research questions (Wrona et al. 1982; Barbour and Gerritsen 1996). Our objective in this paper was to evaluate the reliability of macroinvertebrate taxonomic richness estimates developed from processing a standard number of subsampling quadrats. To do so, we used a dataset from tropical stream sites experiencing three different levels of human disturbance (most-, intermediate-, and least-disturbed).

Materials and methods

Field sampling

We sampled 18 headwater stream sites located in the Araguari River Basin, Minas Gerais, southeastern Brazil, during the dry season of 2009. In this period, discharge is more constant, habitats are most distinct, and macroinvertebrate densities are usually higher (Callisto et al. 2001). Sites ranged from Strahler order 1–3 on 1:50,000 scale maps, with mean wetted channel widths ranging from 1–5 m. The altitudes varied little, ranging from 823 to 954 m. According to the disturbance level of the sites and their catchments, sites were classified a priori as least-disturbed, most-disturbed, and intermediate-disturbed. Least-disturbed sites had clear water, well-developed riparian vegetation, and high in-stream habitat complexity. Their catchments were inside well-preserved areas, some of them inside conservation units, with minor or no human habitation and land use. Most-disturbed sites were inside catchments of small urban areas and/or high agricultural land use. They had poor water

quality and evidence of numerous human alterations in their channels, such as absence of riparian vegetation, presence of trash, pipes, fine sediments, and simplified habitats. Our most-disturbed sites lacked high organic impairment; rather they were characterized as having simplified hydromorphology. Intermediate-disturbed sites, although having little evidence of human alterations of their channels or riparian vegetation, were inside highly disturbed catchments, mostly by agriculture and pasture. Six sites of each disturbance level were sampled. At each site, 11 kick net samples (500 μm mesh, 30 cm mouth width) were taken systematically in a zigzag pattern along the whole site and combined, generating one composite macroinvertebrate sample per site (Peck et al. 2006; Hughes and Peck 2008). A total area of 1 m^2 was sampled in each site, and all samples were preserved in 10 % formalin and stored in tightly sealed plastic buckets.

Laboratory and subsampling procedures

In the laboratory, the samples were first washed through 500 μm sieves to remove much of the mineral substrate (mud, sand, fine gravel, etc.) and larger twigs and leaves. Sample material was then placed in a white enamel tray 36×66×7.5 cm. The tray was half filled with water and the sample was homogenized for 5 min. After that, a metal grid of the same dimensions as the tray was placed upon the sample. This metal grid consisted of 4×6 quadrats (24 in total); each quadrat measured 8.5×10.5 cm and corresponded to nearly 4.2 % of the total sample. The material of each quadrat was carefully removed and stored in plastic jars containing 70 % alcohol. All material that was more than halfway inside a quadrat was considered as part of that quadrat. The macroinvertebrates of each jar were fully sorted and identified to family through use of taxonomic keys (Pérez 1988, Fernández and Domíngues 2001; Costa et al. 2006).

Data analyses

We calculated processing effort (collection) curves for macroinvertebrate taxonomic richness for any given number of quadrats using a randomization technique (1,000 times). We used box plots to show the mean relative richness sampled in each stream along the 24 quadrats processed. We also used the slope of the collection curves to determine when the curves were starting to level off. For each stream site, the slope was calculated by dividing the mean richness predicted in a quadrat n by the mean richness predicted in the quadrat $n+1$. These values expressed the average percentage of richness that is gained by processing a subsequent quadrat. A threshold slope of 3 % was defined as indicating curve leveling. A 3 % slope is less restrictive than a 1 % slope (which occurred with few curves, even when almost all the quadrats were processed) and not as permissive as a 5 % slope (which represents a curve still increasing considerably).

We calculated the minimum number of quadrats (MNQ) for each stream to achieve 80 % of the total richness observed in its whole sample, which we deemed a satisfactory amount in terms of subsampling. The MNQ values were regressed against the number of organisms, equitability, and Shannon–Wiener and Simpson indices of the macroinvertebrate assemblages calculated for the whole samples to verify which sample characteristics influenced the MNQ observed, i.e., which characteristics were responsible for differences in the subsampling effort.

Lastly, we calculated the average number of quadrats necessary to produce 200 and 300 individuals from the stream site samples. These numbers are common goals in many subsampling protocols (e.g., Norris et al. 1995; Carter and Resh 2001; Lorenz et al. 2004). Some protocols require 500 individuals per sample (e.g., Stoddard et al. 2008), but the invertebrate densities of the sites we studied were low and many of our samples did not yield 500 individuals.

Results

We collected 11,994 macroinvertebrate individuals and 66 families from the 18 sites. The Insecta comprised the majority of both individuals (96 %) and families (89 %). The relative abundance of taxa followed the common pattern of stream invertebrate assemblages, with few very abundant taxa and many rare ones. The six most abundant families were, in decreasing order: Chironomidae, Elmidae, Simuliidae, Leptophlebiidae, Leptohyphidae, and Baetidae. Each family was represented by >500 individuals, and together, they included 78 % of all individuals collected in the study. On the other hand, almost half of the taxa collected (31 families) can be considered rare; each family being represented by <0.1 % of all individuals collected.

The majority of the families of the insect orders Diptera (Dixidae, Dolichopodidae, Psychodidae, Phoridae, Muscidae, Syrphidae, Tabanidae, and Stratiomyidae), Heteroptera (Belostomatidae, Gerridae, Veliidae, Mesoveliidae, and Notonectidae), and Coleoptera (Curculionidae, Gyrinidae, Dryopidae, Lutrochidae, Noteridae, Scirtidae, and Ptilodactylidae) were rare.

Biotic metric values, based on entire samples, differed among sites with different disturbance levels (Table 1). Both least-disturbed and intermediate-disturbed sites had higher median values than most-disturbed sites for taxonomic richness, number of organisms, equitability, and Shannon–Wiener and Simpson indices. Median metric values and ranges for least-disturbed and intermediate-disturbed sites were similar, with least-disturbed values being slightly higher in most cases.

The box plots generated from the mean values of subsampling effort for all 18 stream sites show that the curves did not reach an asymptote (Fig. 1a). Some proportion of the richness sampled is gained until the last quadrat (the 24th) is processed, although the gain ratio decreases greatly after the 12th quadrat is processed. We observed an average slope of 3 % or less with 12 quadrats processed (half the sample), for more than half the sites studied (Fig. 1b).

With 12 quadrats being processed, it was also possible to gather at least 80 % of the total richness of the whole samples for at least half of the sites studied. However, we observed from the regressions that the minimum number of quadrats to achieve 80 % of taxonomic richness were strongly negatively correlated with the number of individuals collected in each stream (linear regression, adjusted R^2=0.58, F=24.53, p<0.001, Fig. 2a). The other metrics of the whole site samples (equitability, Shannon–Wiener and Simpson indices) were not correlated significantly with the subsampling effort (p value >0.05 in all cases, Fig. 2b–d).

There was considerable variation in the mean number of quadrats processed needed to achieve a predetermined number of 200 and 300 individuals (Fig. 3). On average, from 3 to 24 quadrats were needed to yield 200 individuals and from 5 to 24 quadrats were needed to yield 300 individuals. Five sites did not yield 300 individuals even when their entire samples were processed. A mean of four more quadrats were needed to produce 300 individuals versus 200 individuals. In sites with high invertebrate densities, an average of two to three quadrats more were needed, and in sites with the lowest invertebrate densities, four to eight more quadrats were needed (Fig. 3). Most-disturbed sites tended to require more quadrats, which were associated with their lower organism densities compared with the intermediate- and least-disturbed sites. On the other hand, the intermediate- and least-disturbed sites included sites with low and high organism densities, and the number of quadrats varied accordingly (Fig. 3).

Discussion

Some authors advocate towards processing entire samples in the laboratory (e.g., Courtemanch 1996; Doberstein et al. 2000). Their argument is that this would be the only way to record all the rare species collected, which represent a major part of macroinvertebrate biodiversity. However, it has been long recognized that subsampling procedures are necessary to complete most studies dealing with large spatial extents and many samples, in this way saving money

Table 1 Median values and ranges (in parentheses) of biotic variables for the entire samples from the 18 study sites (six sites for each disturbance category)

Characteristics of the macroinvertebrate assemblages (whole samples)	Category of human disturbance of the streams		
	Most-disturbed	Intermediate-disturbed	Least-disturbed
Total assemblage family richness	19 (13–32)	29 (21–39)	30 (27–40)
Total assemblage number of individuals	415 (172–986)	780 (184–1,620)	676 (215–1,423)
Total assemblage equitability	0.53 (0.33–0.74)	0.61 (0.52–0.68)	0.62 (0.59–0.71)
Total assemblage Shannon–Wiener index	1.63 (0.89–2.25)	2.10 (1.77–2.25)	2.20 (2.03–2.36)
Total assemblage Simpson index	0.65 (0.38–0.72)	0.79 (0.70–0.82)	0.81 (0.73–0.85)

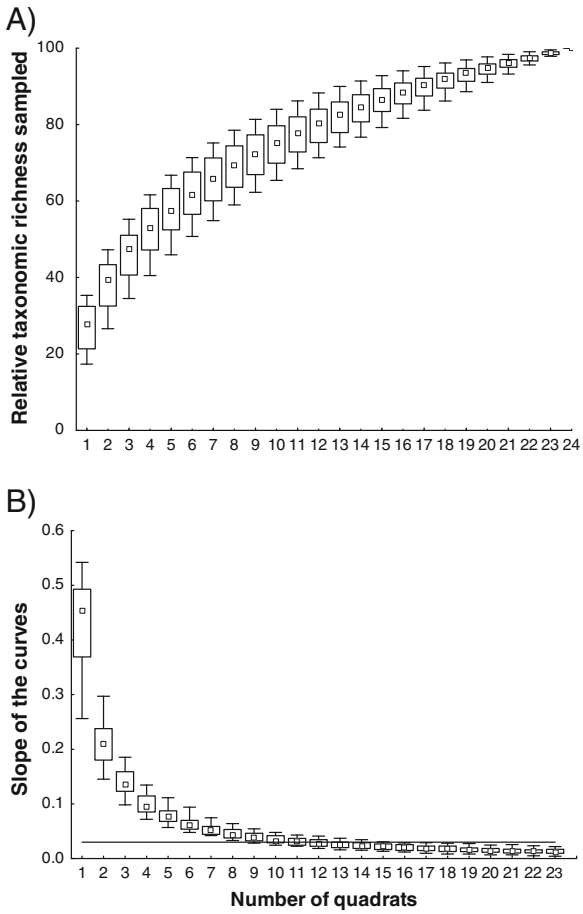

Fig. 1 Subsampling effort (1 to 24 quadrats) versus **A** relative taxonomic richness sampled and **B** curve slope. The *black line* at the bottom of the graph indicates the 3 % slope threshold. In both graphs, the *boxes* represent the median/interquartile of the 18 streams' mean values generated in the randomization procedure (1,000 times per subsampling effort per site). The *error bars* represent the range (maximum and minimum values)

and obtaining viable and timely answers (e.g., Vinson and Hawkins 1996; Hughes and Peck 2008). Currently, an increasing number of research groups are opting for subsampling methods (Carter and Resh 2001) and, besides knowing about the uncertainty associated with the subsampling methods, it is important to ensure that the methods are appropriate and that the data obtained are being properly interpreted.

Hurlbert (1971) distinguished between *numerical species richness* (or simply species richness), calculated as a function between the number of species observed and the number of individuals present in a sample, and *areal species richness* (or species density), calculated as a function of the number of species observed in a given field plot. Some researchers argue that fixed-area subsampling approaches are a solution to standardize areal richness among different samples, enabling comparisons among them (e.g., Courtemanch 1996; Petkovska and Urbanic 2010). However, we observed a strong relationship between the number of individuals in the whole sample and the difficulty in representing its macroinvertebrate areal richness (i.e., to reach some proportion of the total number of taxa in the sample). That is, the fewer the individuals in a sample, the greater the proportion of that sample that must be processed to represent any proportion of its total richness. In our study, for a most-disturbed site with low abundance of individuals, a mean of 16 quadrats were necessary to achieve 80 % of the total richness of the sample. On the other hand, in a least-disturbed site with high abundance of individuals, a mean of nine quadrats sufficed to achieve 80 % of the total richness of the sample; almost half of the number of quadrats needed in the first case. Other characteristics of the samples, like equitability and diversity indices, were not affected by subsampling effort. Initially, we assumed that when few individuals were present in the whole sample, it would be easier to determine sample taxonomic richness by processing few quadrats; however, we observed the opposite. When a sample contained few individuals distributed evenly among the quadrats (the standard subsampling procedure), the taxa were not evenly distributed in the tray. Consequently, we continued to find new taxa even after processing many quadrats. This pattern was strengthened by the existence of many rare families in the samples, as mentioned previously.

Our results indicate that for a given proportional subsampling effort (any fixed number of quadrats), samples with different numbers of individuals will be represented differently, in terms of the percentage of the total number of taxa of the samples. Samples with high numbers of individuals will be better represented than samples with low numbers of individuals. This pattern is likely to create a bias that is difficult to avoid using a fixed-area subsampling approach when one has samples with a wide range of abundances, thereby artificially enhancing statistical differentiation of taxonomic richness between low and high density sites. Therefore, although knowing about areal richness would be useful and ecologically meaningful in some cases (Courtemanch 1996; Gotelli and Cowell 2001), it is only possible to measure and analyze it reliably

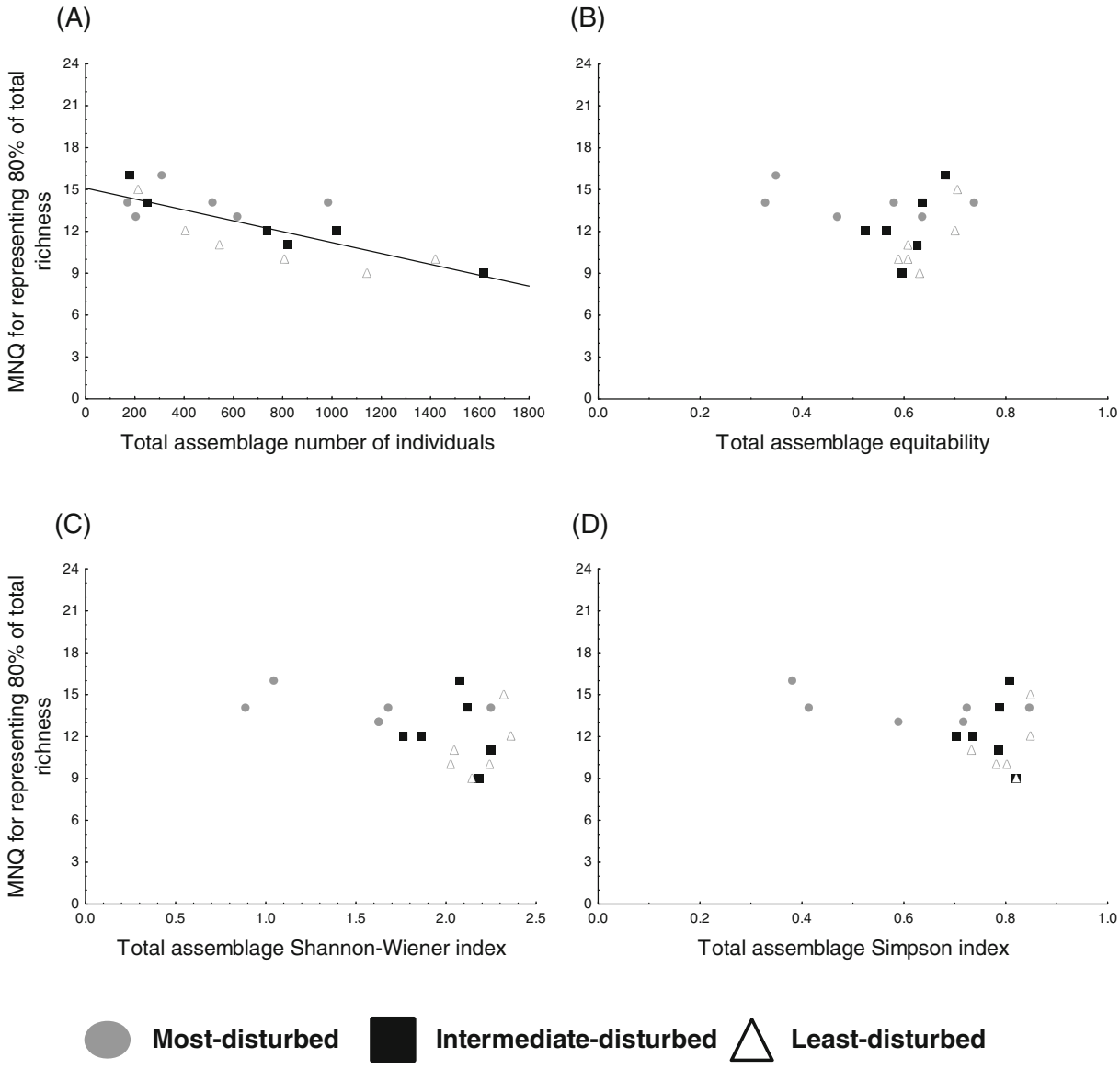

Fig. 2 Minimum number of quadrats (*MNQ*) necessary to represent 80 % of the total macroinvertebrate richness of the samples regressed against **A** total assemblage number of individuals, **B** total assemblage equitability, **C** total assemblage Shannon–Wiener index, and **D** total assemblage Simpson index. The stream disturbance levels are represented by *symbols*

when the entire sample is processed. This finding adds to the list made by Larsen and Herlihy (1998) regarding some practical disadvantages of the use and implementation of fixed-area subsampling approaches.

Sensu Gotelli and Cowell (2001), who revisited established concepts in community ecology (Arrhenius 1921; Preston 1948), stated that taxonomic richness found in a given sample depends on both the area sampled in the field and the number of individuals collected. Gotelli and Cowell (2001) clearly demonstrated that sites usually differ in the "densities" of their richness distribution, and if the number of individuals is not standardized, it is likely to produce erroneous richness comparisons and interpretations. Gotelli and Cowell (2001) compared tree species richness of old-growth and second-growth forests. Given

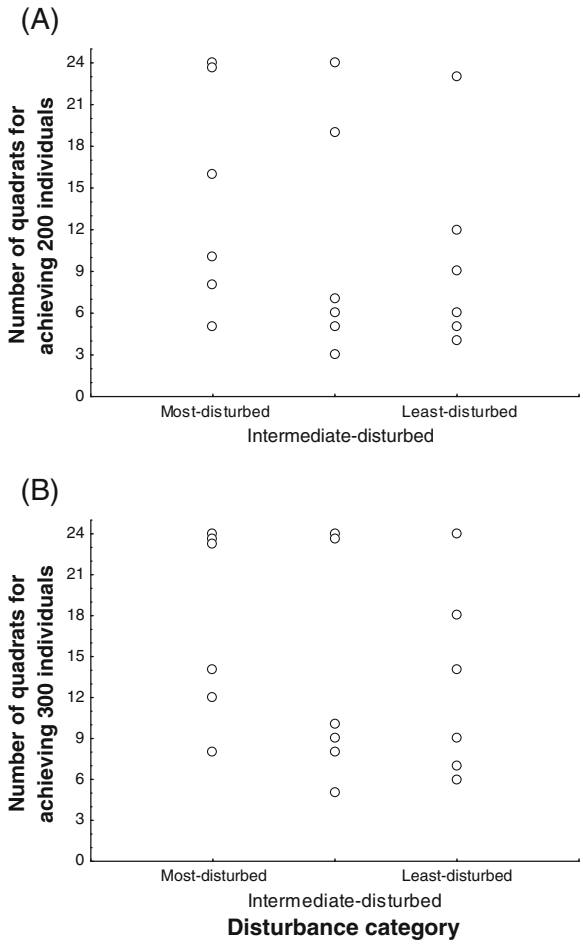

Fig. 3 Average number of quadrats needed to achieve a fixed-count of **A** 200 individuals and **B** 300 individuals from the samples

the same area sampled, they observed greater tree richness in second-growth forests, a pattern that is inverted when the tree density of the plots was also considered and the comparisons calibrated through individual rarefaction. The same analytical artifact was observed in a study with stream macroinvertebrates, regarding the effect of disturbance on the macroinvertebrate richness of artificial substrates (McCabe and Gotelli 2000). Not surprisingly, Barbour and Gerritsen (1996), Vinson and Hawkins (1996), and King and Richardson (2002) found individual rarefaction a more efficient way to compare sites. The standardization of the site area sampled has become a common procedure in field protocols (e.g., Barbour et al. 1999; Hering et al. 2004; Peck et al. 2006; Hughes and Peck 2008; Oliveira et al. 2011). The standardization of the number of individuals has not been used with the same frequency, but increasingly researchers are using rarefaction, statistical estimators, or sampling effort standardization to calibrate statistical comparisons (Cao et al. 2007).

If a given number of individuals is defined (fixed-count method), it is also pointless to set a minimum number of quadrats to process. Because samples usually vary greatly in their individual abundances, the numbers of quadrats necessary to achieve a given number of individuals will also vary greatly. In our study, this varied from as few as three quadrats to the whole sample if the goal was to reach 200 individuals. Setting a minimum number of quadrats can overestimate richness in highly abundant samples, which for instance need fewer quadrats to reach any given count of organisms.

Conclusions

Although measurement of taxonomic density (the number of species found in a certain area) is the goal of some researchers, it is not reliably accomplished though fixed-area subsampling procedures. The consequence of fixing any fraction of the sample is an overestimation of the areal richness in some samples and an underestimation of it in others. Considering this, and also the importance of the use of individual-based rarefaction to compare taxonomic richness of different samples, we do not recommend the use of fixed-area subsampling alone if a key purpose of the research is to measure and analyze taxonomic richness. We encourage researchers, as is being implemented by many groups, to set a given number of individuals per sample as a goal (preferably enough to reach some stability in richness) and at the same time to record the number of quadrats processed, thereby also providing information about individual densities.

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References

Arita, H. T., & Vázquez-Domínguez, E. (2008). The tropics: cradle, museum or casino? A dynamic null model for latitudinal gradients of diversity. *Ecology Letters, 11*, 653–663.

Arrhenius, O. (1921). Species and area. *Journal of Ecology, 9*, 95–99.

Baptista, D. F., Buss, D. F., Egler, M., Giovanelli, A., Silveira, M. P., & Nessimian, J. (2007). A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. *Hydrobiologia, 575*, 83–94.

Barbour, M. T., & Gerritsen, J. (1996). Subsampling of benthic samples: a defense of the fixed-count method. *Journal of the North American Benthological Society, 15*, 386–391.

Barbour, M.T., Gerritsen, J., Snyder, B.D., & Stribling, J.B. (1999). *Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish* (2nd Ed., p. 339). Washington, DC: EPA 841-B-99-002, Office of Water, US Environmental Protection Agency.

Callisto, M., Moreno, P., & Barbosa, F. A. R. (2001). Habitat diversity and benthic functional trophic groups at Serra do Cipó, Southeast Brazil. *Revista Brasileira de Biologia, 61*, 59–266.

Cao, Y., Hawkins, C. P., Larson, D. P., & Van Sickle, J. (2007). Effects of sample standardization on mean species detectabilitites and estimates of relative differences in species richness among assemblages. *The American Naturalist, 170*, 381–395.

Carter, J. L., & Resh, V. H. (2001). After site selection and before data analysis: sampling, sorting, and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies. *Journal of the North American Benthological Society, 20*, 658–682.

Clarke, A., MacNally, R., Bond, N., & Lake, P. S. (2008). Macroinvertebrate diversity in headwater streams: a review. *Freshwater Biology, 53*, 1707–1721.

Clarke, A., MacNally, R., Bond, N. R., & Lake, P. S. (2010). Conserving macroinvertebrate diversity in headwater streams: the importance of knowing the relative contributions of α and β diversity. *Diversity and Distributions, 16*, 725–736.

Costa, C., Ide, S., & Simonka, C. E. (2006). *Insetos imaturos. Metamorfose e identificação*. Ribeirão Preto: Holos.

Courtemanch, D. L. (1996). Commentary on the subsampling procedures used for rapid bioassessments. *Journal of the North American Benthological Society, 15*, 381–385.

Doberstein, C. P., Karr, J. R., & Conquest, L. L. (2000). The effect of fixed-count subsampling on macroinvertebrate biomonitoring in small streams. *Freshwater Biology, 44*, 355–371.

Fernández, H. R., & Domínguez, E. (2001). *Guia para la determinación de los artrópodos bentónicos sudamericanos*. San Miguel de Tucumán: Universidad Nacional de Tucumán.

Gotelli, N. J., & Cowell, R. K. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters, 4*, 379–391.

Hering, D., Moog, O., Sandin, L., & Verdonschot, P. F. M. (2004). Overview and application of the AQEM assessment system. *Hydrobiologia, 516*, 1–20.

Hughes, R. M., & Peck, D. V. (2008). Acquiring data for large aquatic resource surveys: the art of compromise among science, logistics, and reality. *Journal of the North American Benthological Society, 27*, 837–859.

Hurlbert, S. H. (1971). The nonconcept of species diversity: a critique and alternative parameters. *Ecology, 52*, 577–586.

King, R. S., & Richardson, C. J. (2002). Evaluating subsampling approaches and macroinvertebrate taxonomic resolution for wetland bioassessment. *Journal of the North American Benthological Society, 21*, 150–171.

Klemm, D. J., Blocksom, K. A., Fulk, F. A., Herlihy, A. T., Hughes, R. M., Kaufmann, P. R., Peck, D. V., Stoddard, J. L., & Thoeny, W. T. (2003). Development and evaluation of a macroinvertebrate biotic integrity index (MBII) for regionally assessing Mid-Atlantic Highlands streams. *Environmental Management, 31*, 656–669.

Lande, R. (1996). Statistics and partitioning of species diversity, and similarity among multiple communities. *Oikos, 76*, 5–13.

Larsen, D. P., & Herlihy, A. T. (1998). The dilemma of subsampling streams for macroinvertebrate richness. *Journal of the North American Benthological Society, 17*, 359–366.

Lorenz, A., Kirchner, L., & Hering, D. (2004). Electronic subsampling of macrobenthic samples: how many individuals are needed for a valid assessment result? *Hydrobiologia, 516*, 299–312.

MacArthur, R. H., & Wilson, E. O. (1967). *The theory of island biogeography*. Princeton: Princeton University Press.

McCabe, D. J., & Gotelli, N. J. (2000). Effects of disturbance frequency, intensity, and area on assemblages of stream invertebrates. *Oecologia, 124*, 270–279.

Melo, A. S., & Froehlich, C. G. (2001). Evaluation of methods for estimating macroinvertebrate species richness using individual stones in tropical streams. *Freshwater Biology, 46*, 711–721.

Moulton, S.R., Carter, J.L., Grotheer, S.A., Cuffney, T.F., & Short, T.M. (2000). *Methods of analysis by the US Geological Survey National Water Quality Laboratory—processing, taxonomy, and quality control of benthic macroinvertebrate samples*. US Geological Survey Open-File Report 00-212.

Nichols, S. J., & Norris, R. H. (2006). River condition assessment may depend on the sub-sampling method: field live-sort versus laboratory sub-sampling of invertebrates for bioassessmaent. *Hydrobiologia, 572*, 95–213.

Norris, R. H., Hart, B. T., Finlayson, M., & Norris, K. R. (1995). Use of biota to assess water quality. *Australian Journal of Ecology, 20*, 12–27.

Oliveira, R. B. S., Mugnai, R., Castro, C. M., & Baptista, D. F. (2010). Determining subsampling effort for the development of a rapid bioassessment using benthic macroinvertebrates in streams of Southeastern Brazil. *Environmental Monitoring and Assessment, 175*, 75–85.

Oliveira, R. B. S., Mugnai, R., Castro, C. M., Baptista, D. F., & Hughes, R. M. (2011). Towards a rapid bioassessment protocol for wadeable streams in Brazil: development of a multimetric index based on benthic macroinvertebrates. *Ecological Indicators, 11*, 1584–1593.

Peck, D.V., Herlihy, A.T., Hill, B.H., Hughes, R.M., Kaufmann, P.R., Klemm, D.J., Lazorchak, J.M., McCormick, F.H., Peterson, S.A., Ringold, P.L., Magee, T., & Cappaert, M.R. (2006). *Environmental monitoring and assessment program-surface waters: Western Pilot Study field operations manual for wadeable streams*. EPA/620/R-06/003. USEPA. Washington, DC.

Pérez, G. R. (1988). *Guía para el estudio de los macroinvertebrados acuáticos del Departamento de Antioquia*. Fondo Fen. Colombia/Colciencias, Universidad de Antioquia, Colombia

Petkovska, V., & Urbanic, G. (2010). Effect of fixed fraction subsampling on macroinvertebrate bioassessment of rivers. *Environmental Monitoring and Assessment, 169*, 179–201.

Preston, E. W. (1948). The commonness and rarity of species. *Ecology, 29*, 254–283.

Richardson, D. M., & Whittaker, R. J. (2010). Conservation biogeography—foundations, concepts and challenges. *Diversity and Distributions, 16*, 313–320.

Stoddard, J. L., Herlihy, A. T., Peck, D. V., Hughes, R. M., Whittier, T. R., & Tarquinio, E. (2008). A process for creating multi-metric indices for large-scale aquatic surveys. *Journal of the North American Benthological Society, 27*, 878–891.

Suriano, M. T., Fonseca-Gessner, A. A., Roque, F. O., & Froehlich, C. G. (2011). Choice of macroinvertebrate metrics to evaluate stream conditions in Atlantic Forest, Brazil. *Environmental Monitoring and Assessment, 175*, 87–101.

Vinson, M. R., & Hawkins, C. P. (1996). Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. *Journal of the North American Benthological Society, 15*, 392–399.

Wrona, F. J., Culp, J. M., & Davies, R. W. (1982). Macroinvertebrate subsampling: a simplified apparatus and approach. *Canadian Journal of Fisheries and Aquatic Science, 39*, 1051–1054.