Choosing the best non-parametric richness estimator for benthic macroinvertebrates databases

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Eligiendo el mejor estimador no paramétrico para calcular riqueza en bases de datos de macroinvertebrados bentónicos

■ **RESUMEN.** Los estimadores no paramétricos permiten comparar la riqueza estimada de conjuntos de datos de origen diverso. Empero, como su comportamiento depende de la distribución de abundancia del conjunto de datos, la preferencia por alguno representa una decisión difícil. Este trabajo rescata algunos criterios presentes en la literatura para elegir el estimador más adecuado para macroinvertebrados bentónicos de ríos y ofrece algunas herramientas para su aplicación. Cuatro estimadores de incidencia y dos de abundancia se aplicaron a un inventario regional a nivel de familia y género. Para su evaluación se consideró: el tamaño de submuestra para estimar la riqueza observada, la constancia de ese tamaño de submuestra, la ausencia de comportamiento errático y la similitud en la forma de la curva entre los distintos conjuntos de datos. Entre los estimadores de incidencia, el mejor fue Jack1; entre los de abundancia, ACE para muestras de baja riqueza y Chao1, para las de alta riqueza. La forma uniforme de las curvas permitió describir secuencias generales de comportamiento, que pueden utilizarse como referencia para comparar curvas de pequeñas muestras e inferir su comportamiento –y riqueza– probable, si la muestra fuera mayor. Estos resultados pueden ser muy útiles para la gestión ambiental y actualizan el estado del conocimiento regional de macroinvertebrados.

PALABRAS CLAVE. Riqueza estimada. Ríos neotropicales. Gestión ambiental.

■ **ABSTRACT.** Non-parametric estimators allow to compare the estimates of richness among data sets from heterogeneous sources. However, since the estimator performance depends on the species-abundance distribution of the sample, preference for one or another is a difficult issue. The present study recovers and revalues some criteria already present in the literature in order to choose the most suitable estimator for streams macroinvertebrates, and provides some tools to apply them. Two abundance and four incidence estimators were applied to a regional database at family and genus level. They were evaluated under four criteria: sub-sample size required to estimate the observed richness; constancy of the sub-sample size; lack of erratic behavior and similarity in curve shape through different data sets. Among incidence estimators, Jack1 had the best performance. Between abundance estimators, ACE was the best when the observed richness was small and Chao1 when

the observed richness was high. The uniformity of curves shapes allowed to describe the general sequences of curves behavior that could act as references to compare estimations of small databases and to infer the possible behavior of the curve (*i.e* the expected richness) if the sample were larger. These results can be very useful for environmental management, and update the state of knowledge of regional macroinvertebrates.

KEY WORDS. Richness estimation. Neotropical streams. Environmental management.

INTRODUCTION

Species richness is a widely used measure of biodiversity because it is relatively easy to measure and is well understood by researchers, managers and the society (Hellmann & Fowler, 1999). Unfortunately, it depends heavily on sample size, then if we make a simple count of the species present in a sample, surely we will fall into an underestimation, unless we make a census of the community of interest (Hellmann & Fowler, 1999). Because exhaustive sampling is impractical or rarely supported (especially in tropical invertebrate, microbial or plant communities) (Hellmann & Fowler, 1999; Gotelli & Colwell, 2001), the estimation of richness based on available biological inventories has received in the last decade progressively more attention in both theoretical and empirical aspects (Walther & Morand, 1998; Heyer *et al.*, 1999; Petersen *et al.*, 2003; Chao *et al.*, 2005). Much of the importance of these methods lies in that they make comparable the estimates of richness among data sets from different regions, seasons, results of different methodologies or sampling effort (Jiménez-Valverde & Hortal, 2003).

Among the methodologies currently used for this task, four groups can be distinguished: non-parametric estimators, fitting species-abundance distributions, species accumulation curves and species–area curves (Hortal *et al.*, 2006).

Here, I will focus only in the non-parametric estimators. These indices use the abundance or incidence of rare species in the samples to estimate total number of species, using a previously formulated non-parametric model (*e.g.* Chao & Bunge, 2002; Sørensen *et al.*, 2002; Chiarucci *et al.*, 2003; Rosenzweig *et al.* 2003). There are two kinds of non-parametric estimators: abundance and incidence models.

The abundance model considers the number of individuals that represents each species in the sample, and the incidence model considers the number of samples in which each species is present (*i.e.* presence-absence data). These methods use the number of rare species to estimate the possible number of undiscovered species.

Since the abundance and incidence of rare species grow with the increasing of sampling effort, these methods are expected to estimate different species richness as sampling effort increases. Moreover, as different populations have different species-abundance distributions, the estimator performance should depend also on the species-abundance distribution of the data set (Bunge & Fitzpatrick, 1993; Soberón & Llorente, 1993; Colwell & Coddington, 1994; Walter & Morand, 1998). Thus, given a particular data set, the preference for one or another method should be based on the extent that they meet the characteristics of an ideal estimator.

Many authors (*e.g.* Palmer, 1990; Hellmann & Flower, 1999; Walter & Morand, 1998; Hortal *et al.*, 2006) agree that some of these characteristics are: independence of sample size (amount of sampling effort carried out); insensitivity to unevenness in species distributions; insensitivity to sample order (Chazdon *et al.*, 1998) and insensitivity to heterogeneity in the sample units used

among studies to compare richness values obtained from different survey strategies, which is often the case for macroecological studies (Hortal *et al.*, 2006).

Unfortunately, to reach these conditions is a very difficult challenge for an estimator, and also to assess them, especially if quick answers about species richness estimation are needed. However, some simple and practical criteria can offer an overview of the performance of estimators for taxa for which exhaustive statistics assessments have not yet been implemented.

This study seek to recover and revalue some criteria already present in the literature and to provide some tools to apply them in order to assist researchers, managers, and policy makers to choose the most suitable non-parametric estimator of richness of streams macroinvertebrates communities.

Furthermore, such criteria are applied to a large and heterogeneous regional database of benthic macroinvertebrates from a subtropical Andean area. Results are exemplified at two hierarchical levels and several levels of sampling efforts, making such tools applicable to a wide range of situations. Additionally, this work provides valuable information regarding the regional state of knowledge of the taxa analyzed.

MATERIAL AND METHODS

Data set

A large database of benthic macroinvertebrates from the Yungas ecoregion (NW of Argentina, Neotropical region) was used to calculate the richness of Ephemeroptera, Trichoptera, Coleoptera, Diptera and Prostigmata (Hydrachnidia) at family and genera level. This database belongs to the Instituto de Biodiversidad Neotropical (IBN), and includes data of all the specimens collected during a decade by its members, who made significant contributions to the knowledge of macroinvertebrates of northwestern Argentina in both systematic and ecological aspects (Domínguez & Fernández, 2009). Since the specimens were collected with diverse purposes, records

come from a variety of sampling methods. Other orders of macroinvertebrates present in this database were discarded due to low representativeness.

All collection sites available for each order at each taxonomic level were used as units of sampling effort, regardless of the number of visits, season or sampling method used (mist nets, light traps, hand picking, D frame net, Surber net, etc).

For the purposes of this work a "site" was defined as a stretch of a river or stream (rhithron) of approximately 200 m long, with data of geographic coordinates, elevation, ecoregion, and date of collection. This unit of sampling effort was chosen to account for natural levels of sample heterogeneity (patchiness) in the data (Gotelli & Colwell, 2001). However, in order to make comparable estimations among different groups, graphics results were rescaled to the percentage of sites (to family level) or individuals (to genera level) as a measure of sampling effort.

Richness estimators

Six non-parametric estimators were calculated: four incidence estimators (Jacknife 1, Jacknife 2, Chao2 and Incidence-based Coverage Estimator) and two abundance estimators (Abundance-based Coverage Estimator and Chao1).

Incidence or presence-absence estimators, use counts of "uniques" and "duplicates", *i.e.* species that are present only in one or two samples respectively (first-order Jackknife or Jack 1 and Jackknife of second order or Jack 2; Burnham & Overton 1978, 1979). The Chao2 estimator (Chao, 1987; Colwell 1997), takes into account rare species and the total number of species observed in the sample to calculate its richness. The Incidence-based Coverage Estimator (ICE) (Lee & Chao, 1994; Colwell, 1997) calculates both rare and common species, considering as "common" those that occur in more than 10 samples (by default) or a value chosen by the user.

Abundance estimators base their calculations firstly in "singletons" and "doubletons" species, *i.e.* the number of species represented for just one or two individuals (Chao1) (Chao, 1984; Colwell,

1997). Besides singletons and doubletons, the Abundance-based Coverage Estimator (ACE) (Chao *et al.* 1993; Colwell, 1997) considers abundant species, *i.e.* those represented by more than 10 individuals by default.

To proceed, the program requires the upload of the data in the form of an abundance spreadsheet (to calculate abundance and / or incidence estimators) or an incidence spreadsheet (only for incidence estimators). This spreadsheet must specify the number of samples and taxa and the abundance or presence of each taxon in each sample. The program admits six models of spreadsheet that meet these conditions, which is a very convenient option for the user. Data do not require previous statistical transformations.

All estimators were calculated with the program Estimates 7.0.1 (Colwell, 1997) and their mathematical expressions are given in the Appendix.

Evaluation of estimators

Bias and accuracy of the estimated richness are the most popular measures to evaluate estimation methods. To use these measures it is necessary to choose an "a priori" sub-sample size, which is a difficult choice in rich assemblages like macroinvertebrates communities were the estimated richness is strongly dependent on sample size. For this reason, in a study of evaluation of non-parametric estimators applied to benthic macroinvertebrates, Melo & Froehlich (2001) opted for not using such bias and accuracy statistics and instead they used four criteria they argued are more practical and realistic. These criteria were:

1) Sub-sample size required to estimate the observed richness in the total sample. The smaller the sub-sample size, the better performance of the estimator. This characteristic can be interpreted as an indication of the capability of the estimator to reach a reliable value of richness with a small sampling effort.

2) Constancy of the sub-sample size needed to estimate the total observed richness, measured as 1 standard deviation (SD) of the previous criterion (*i.e.* SD of the estimation that equals observed richness in

the minimum percentage of sampling effort).

3) Lack of erratic behavior in curve shape (specifically large variations of estimates for closely similar sub-sample sizes) is considered a greater stability and therefore greater reliability of the estimate. Melo & Froehlich (2001) made a qualitative categorization of this criterion (good stability/bad stability) and here I propose a very simple quantitative tool to measure this characteristic. This tool consists in the sum of the absolute value of the differences between Sobs (i.e. the "observed richness") and the three previous estimates and the three posterior estimations of Sobs. In this way a dimension of the stability of the curve was obtained in a segment of seven items, whose median position is Sobs.

Hereafter I will refer to these first three criteria as "quantitative criteria".

4) Similarity in curve shape through different data sets. This is a very appreciated characteristic since it makes the behavior of the estimator predictable and easy to interpret when it is applied to new data sets. Although it is mentioned by Melo & Froehlich (2001), some additional details presented here would be useful. In this study, uniformity or similarity of the shape of the curves was evaluated qualitatively, considering: tendency of the slope at different levels of sampling effort, presence of peaks of over or under estimation and position of the maximum or minimum estimations respect to level of sampling effort.

The six non parametric-estimators were applied to the dataset and then they were evaluated under the four criteria mentioned in order to provide further evidence to decide on the convenience of applying them to datasets of benthic macroinvertebrates in subtropical areas.

Performance of estimators was assessed according to the following categories: incidence estimators at family level; incidence estimators at genera level; abundance estimators at family level and abundance estimators at genera level.

For each of the quantitative criteria (*i.e.* percentage of samples to achieve Sobs, standard deviation, stability) the incidence estimators were ranked from 1 to 4 according

to the results, being the score 1 assigned to the one with the best performance (*i.e.* estimator that obtained the lowest value in this criterion) and 4 to the one with the worst performance (*i.e.* estimator that obtained the highest value in this criterion). The final scores for each estimator were obtained adding those ranking positions. The same procedure was applied to abundance estimators except that only 1 and 2 scores were possible.

RESULTS

Regarding the three quantitative criteria, performance of estimators varied depending on the taxa considered. In Tables, numbers between parentheses represent the score assigned to each estimator according to the value obtained in each criterion. For example, for incidence estimators at family level under "SD" criterion for the order Coleoptera (Table I), Jack1 obtained the lowest value (1,68) and Chao2 the highest value (6,44), then their scores were (1) and (4) respectively. "Ranking position" is the result of the sum of all scores obtained for each estimator, *e.g.* for ICE in Coleoptera this would be: (3)+(1)+(2)=6, as six is the lowest value, ICE results the estimator with better performance for that order. Estimators having the best ranking positions in most orders are considered the estimators of best overall performance. For each category, these were:
   - Incidence estimators at family level (see Table I): Jack1, is the only one that always took the first or second ranking position.
   - Incidence estimators at genera level (see Table I): Jack1 took the first position in four of the five cases, followed by ICE, with very close scores,
   - Abundance estimators at family level (see Table II): ACE o Chao1 with very close scores,
   - Abundance estimators at genera level (see Table II): Chao1 took the first position in all cases.

Most of the estimators had a different performance depending on the sample under study, except Chao1 -which was always the most stable- and Jack2 which reached the

final value of estimated richness with a lower sampling effort in almost all cases.

Respect to similarity in curves shape, the behavior of estimators shows the following general sequences:

ICE (see Fig. 1a): **General behavior**: This estimator presented its maximum peak followed by a minimum peak in the first 10-20% of samples and then showed a gradual growth up to the final estimate. **General sequence variations**: In Coleoptera, after an overestimation peak there was a progressive increase in the slope of the curve until it achieved the maximum estimated value.

Chao2 (see Fig. 1b): **General behavior**: At family level, the three less sampled orders (Diptera, Prostigmata and Coleoptera) curves showed a very similar pattern, although out of phase in sampling effort. The overall behavior sequence would be: pronounced growth (in the first 10- 20% of the samples), overestimation plateau, and decrease to final value (see Fig. 1). At genus level (see Fig. 1b) -except for Trichoptera- the curves described a similar pattern to that seen in families but much more blurred by the large number of small peaks in the range and because the sequence described occurs at very different levels of sampling for each order. **General sequence variations**: In the better sampled orders (Trichoptera and Ephemeroptera), at family level the curves had a tendency to a continuous growth (*i.e.* without peaks) from the 20% of sampling effort. Trichoptera genera curve, on the other hand, did not show resemblance respect to those described above, as it showed cubic growth from the 66% of samples toward the end of the axis x (R^2=0.99).

Jack1 (see Fig. 1c): **General behavior**: At genus level, there was no clear distinction between well or sub sampled orders, all showing a logarithmic growth. At family level this growth was very pronounced in the three less sampled orders where the curves fit very well to a logarithmic curve (R^2=0.88 Diptera and R^2= 0.99 Coleoptera

Table I. Quantitative evaluation of incidence estimators. Values 1 to 4 between parentheses indicate the score assigned to the estimator in each criterion (% samples, stability, SD). Final ranking position of estimators was obtained summarizing their scores (ss) in each order.

	Estimator	Coleoptera			Diptera			Ephemeroptera			Prostigmata			Trichoptera		
		% samples	Stability	SD	% samples	Stability	SD	% samples	Stability	SD	% samples	Stability	SD	% samples	Stability	SD
Family level	ICE	30,86 (3)	1,61 (1)	3,64 (2)	40,00 (4)	1,60 (1)	1,81 (2)	58,82 (3)	0,21 (2)	0,92 (3)	42,85 (3)	4,86 (4)	2,69 (2)	73,17 (3)	0,32 (2)	0,6 (3)
	Chao 2	25,92 (2)	2,55 (3)	6,44 (4)	24,44 (2)	2,99 (3)	3,21 (4)	100 (4)	0,07 (1)	0,43 (1)	38,09 (2)	3,59 (3)	4,77 (4)	96,34 (4)	0,12 (1)	0,47 (1)
	Jack1	38,27 (4)	1,75 (2)	1,68 (1)	26,66 (3)	1,86 (2)	1,27 (1)	54,90 (2)	0,40 (3)	0,48 (2)	42,86 (3)	3,23 (2)	1,56 (1)	45,12 (2)	0,39 (3)	0,53 (2)
	Jack2	24,69 (1)	2,80 (4)	3,63 (2)	19,30 (1)	4,11 (4)	2,98 (3)	40,20 (1)	0,58 (4)	1,40 (4)	30,95 (1)	2,91 (1)	3,32 (3)	39,02 (1)	0,76 (4)	1,69 (4)
	Ranking position	1. ICE (ss=6) 2. Jack1/Jack2 (ss=7)			1. Jack1 (ss=6) 2. ICE (ss=7)			1. Chao2 (ss=6) 2. Jack1 (ss=7)			1. Jack2 (ss=5) 2. Jack1 (ss=6)			1. Chao2 (ss=6) 2. Jack1 (ss=7)		
Genus level	ICE	35,13 (3)	3,08 (1)	9,31 (4)	31,11 (3)	5,95 (2)	6,82 (2)	48,57 (4)	2,30 (2)	3,71 (2)	43,55 (3)	5,01 (2)	4,18 (1)	50,61 (4)	2,10 (2)	3,01 (1)
	Chao 2	22,97 (1)	12,88 (4)	15,12 (4)	13,33 (1)	15,05 (3)	18,54 (4)	12,38 (2)	7,83 (4)	13,01 (4)	29,03 (1)	11,83 (4)	10,90 (4)	20,99 (2)	2,43 (3)	8,44 (4)
	Jack1	45,94 (4)	5,63 (2)	8,06 (1)	33,33 (4)	8,38 (2)	4,74 (1)	30,08 (1)	1,39 (1)	2,53 (2)	40,32 (2)	4,54 (1)	3,35 (2)	30,86 (3)	1,76 (1)	3,19 (2)
	Jack2	29,73 (2)	9,95 (3)	7,64 (2)	20,00 (2)	17,20 (4)	8,74 (3)	30,10 (1)	3,90 (3)	5,93 (3)	29,03 (1)	7,65 (3)	5,63 (3)	16,05 (1)	8,28 (4)	7,02 (3)
	Ranking position	1. ICE /Jack1/Jack2 (ss=7)			1. ICE (ss=6) 2. Jack1 (ss=7)			1. Jack1 (ss=4) 2. Jack2 (ss=6)			1.Jack1 (ss=5) 2. ICE/Jack2 (ss=6)			1. Jack1 (ss=6) 2. ICE (ss=7)		

Table II. Quantitative evaluation of abundance estimators. Values 1 and 2 between parentheses indicate the score assigned to the estimator in each criterion (% samples, stability, SD). Final ranking position of estimators was obtained summarizing their scores (ss) in each order.

	Estimator	Ephemeroptera			Prostigmata			Trichoptera			Coleoptera			Diptera		
		% samples	Stability	SD	% samples	Stability	SD	% samples	Stability	SD	% samples	Stability	SD	% samples	Stability	SD
Family level	ACE	**100,00** (1)	0,07 (1)	**0,00** (1)	46,43 (1)	1,63 (2)	1,83 (2)	40,90 (1)	0,66 (2)	1,42 (2)	35,82 (1)	4,61 (2)	6,93 (1)	38,09 (1)	**1,90** (1)	3,81 (1)
	Chao1	**100,00** (1)	0,07 (1)	0,45 (2)	85,71 (2)	0,38 (1)	0,72 (1)	**100,00** (2)	0,03 (1)	0,48 (1)	35,82 (1)	3,14 (1)	**7,10** (2)	44,44 (2)	**2,30** (2)	4,37 (2)
	Ranking position	ACE (ss=3)			Chao1 (ss=4)			Chao1 (ss=4)			ACE/Chao1 (ss=4)			ACE (ss=3)		
Genus level	ACE	39,70 (1)	2,87 (2)	5,13 (1)	9,37 (2)	5,02 (2)	3,48 (1)	80,30 (2)	1,75 (2)	1,61 (1)						
	Chao1	39,70 (1)	1,67 (1)	5,74 (2)	43,75 (1)	4,76 (2)	5,83 (2)	66,66 (1)	1,49 (1)	3,86 (2)						
	Ranking position	ACE/Chao1 (ss=4)			Chao1 (ss=4)			Chao1 (ss=4)									

and Prostigmata at family level) that never became asymptotic. **General sequence variations:** At family level in better sampled orders (Trichoptera and Ephemeroptera) and at genus level for Coleoptera, the curves showed a much steeper growth in the first 10% of the sampling where it begins a gradual growth to reach the final value of estimate.

Jack2 (see Fig. 1d): **General behavior:** Curves behaved very similarly to Jack1 in all aspects, except that at the family level presented small peaks during the line of the curves, which anyway did not reverse the general tendency to a logarithmic growth. At genera level, such instability was not that obvious and unlike the family level estimate, three orders achieved an asymptote (Diptera, Ephemeroptera and Prostigmata). There were no important exceptions to this general behavior.

ACE (see Fig. 2a1 and 2b1) and Chao1 (see Fig. 2a2 and 2b2): **General behavior:** at family and genera level they followed a logarithmic growth reaching or tending to asymptote at different levels of sampling
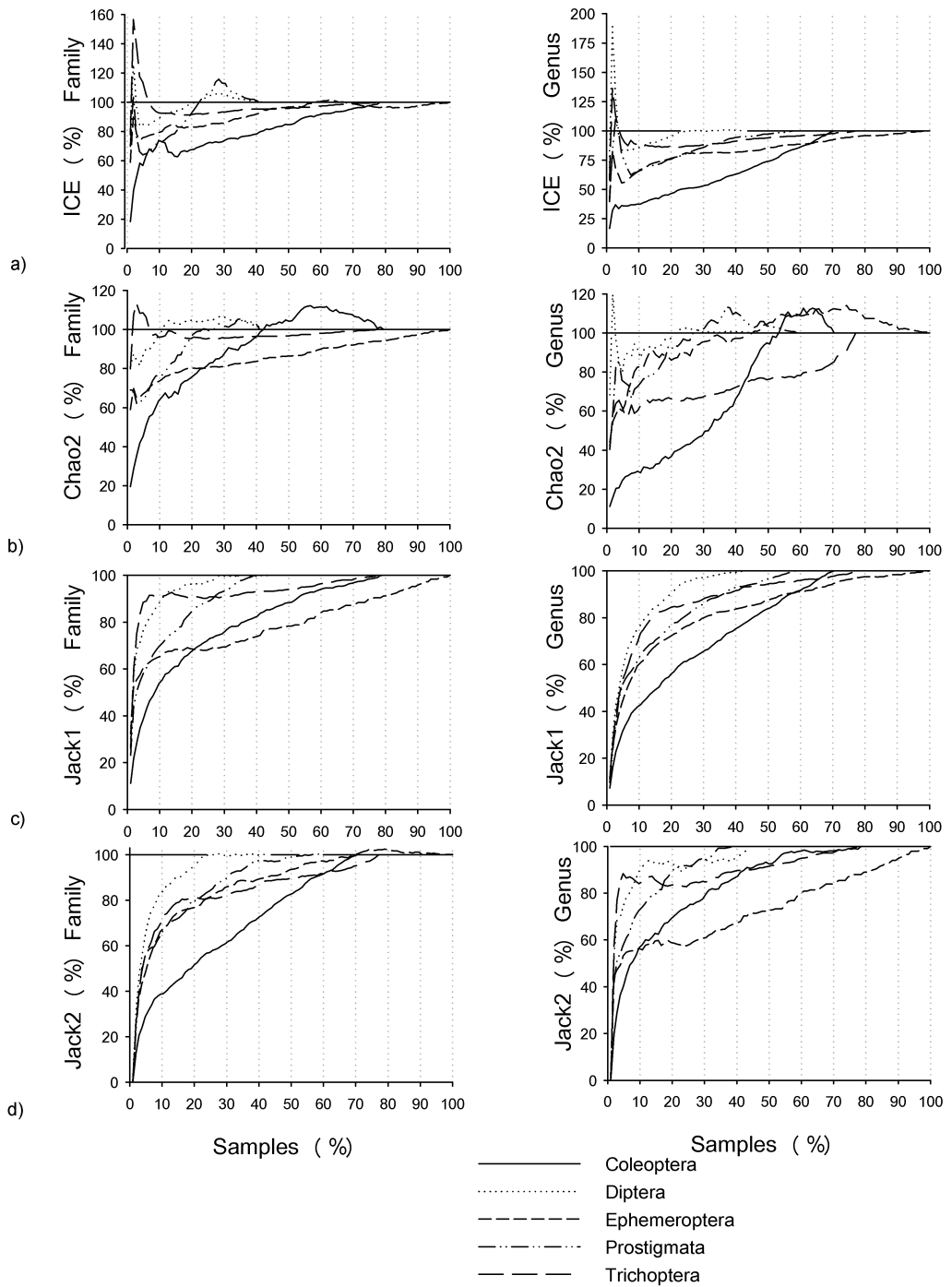
Fig. 1. Incidence estimator's curves for family and genera. a) ICE, b) Chao2, 3) Jack1 and d) Jack2. X axis is re-scaled to percentage of sampling effort: 100%=the maximum value of the larger sample (Ephemeroptera). Y axis is rescaled to the percentage of richness estimated: 100%=final value of richness in each order (fill line).
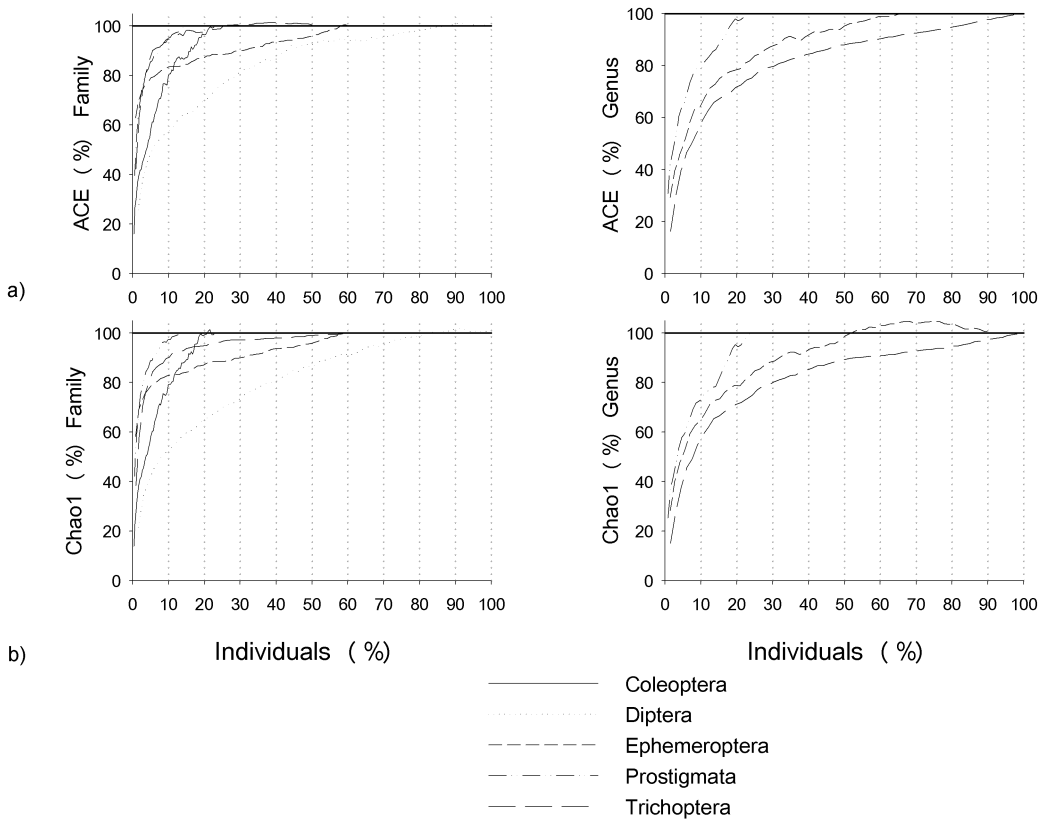
Fig. 2. Abundance estimator's curves for family and genera. a) ACE and b) Chao1. X axis is re-scaled to percentage of sampling effort: 100%=the maximum value of the larger sample (Ephemeroptera). Y axis is rescaled to the percentage of richness estimated: 100%=final value of richness in each order (fill line).

effort in the best sampled orders. In all cases the growth was smooth, with no evidence of peak fluctuation.

Application to the local data set

Incidence data: At the family level (see Fig. 1a) ICE –and Chao2- estimated a final richness value equal to Sobs (Diptera, Ephemeroptera and Trichoptera), suggesting a high level of completeness of these inventories. For Prostigmata, ICE estimates ranged between 15 and 17% above Sobs. For Coleoptera, Jack1 suggested the existence of 30% more families than currently known.

At genus level (see Fig. 1a) ICE was close to Sobs of Diptera and Trichoptera. The estimated richness suggests values between 10% (Trichoptera) and 12% (Ephemeroptera and Diptera) of unknown genera for these inventories, while this value grew to 20% for Prostigmata.

Respect to Coleoptera, Jack1 suggested a very high percentage of unknown families (near 48%).

Abundance data: At family level (see Fig. 2) estimates of ACE and Chao1 indices coincided or were nearly identical to the observed richness (Sobs) for Ephemeroptera, Trichoptera and Prostigmata. For Coleoptera and Diptera at the family level, both estimators suggested an unknown percentage of approximately 35% and 17% respectively.

At genus level (see Fig. 2b), both indices of abundance estimated very similar richness

values for each of the three studied orders, being Prostigmata the group with the highest percentage of unknown genera (15%).

DISCUSSION

The impact of human activities on ecological systems is well illustrated by changes in land use arising from urbanization, whose consequences are a serious threat to biodiversity conservation (Gonzalez-Oreja *et al.* 2010). Ecologists face the challenge of quantifying this impact proposing precise tools and measures to preserve biodiversity. In this context, groups of organisms that act as biodiversity indicators -like many benthic macroinvertebrates- are of great importance as they enable these changes to be monitored (Valladares *et al.*, 2010).

Non-parametric estimators can help to reach inventories in less time and with lower costs (Petersen & Meier, 2003), since they can give a measure of how much sampling effort can be enough to reach a representative inventory. Hence the importance to assess their reliability to adequately adjust them for each group and region. Moreover, when assessing the relevance of a single area in terms of species richness, endemism, or conservation status, the use of richness as a tool for decision making can be misleading without a measure of how complete the lists are (Soberón & Llorente, 1993). Measuring the completeness is then the safest approach for dealing with taxonomic, geographic or ecological biases. This is especially important when inventories are derived from heterogeneous sources such as non-standardized samplings or bibliographic references (Valladares 2010). Anyway, even if the data were imperfect (e.g. museum collection lists), if the appropriate technique is used and a strict interpretation of the results is made, we can evaluate the quality of the lists of species to locate priority areas for conservation (Heyer *et al.*, 1999). The assessment of the completeness of inventories is a powerful tool that non-parametric estimators provide. Moreover, a widely recognized program to calculate them (EstimateS in all versions, Colwell 1994) is freely available on the web, it is very easy to use and does not require the user to make any statistical procedures for calculation.

In this study, among the incidence estimators, the one of better overall performance was Jack1, followed by ICE and Chao2. These results are slightly different from those of Melo & Froehlich (2001) who firstly recommend Jack2 for benthic studies. In the present study Jack2 had a similar performance -but not as good as- ICE at genus level analysis. At the family level Jack2 performance was very poor. In Melo & Froehlich (2001), ICE and Chao2 show a good performance, as in the present study. Gonzalez-Oreja *e.t al.* (2010) studied the performance of non parametric estimators with data of birds of urban areas in the city of Puebla, Mexico. They concluded that Jack1 was the estimator with better general performance, even when they made the evaluation under some criteria they called "hard", *i.e.* criteria that are very rigorous statistically.

Regarding abundance estimators, the analysis did not reveal a better overall performance estimator, but performance depended on whether they were applied to family or genus level. Whereas the number of samples had little or no difference among family and genus level within the same order (see Table II), numerically this could indicate that some estimators have better performance at low richness samples (level of family) and others are more effective when richness reaches higher values observed (genus level). Then, according to the results of this study, a most advisable estimator for low Sobs samples would be ACE, whereas Chao1 would be the best for high Sobs samples.

The curves shapes analysis allow me not to propose the use of estimators of more erratic behavior in the first portion of the sample –like ICE and Chao2- if the sample to be analyzed is small, agreeing in this point with Rico *et al.* (2005).

Nonetheless, from the description of curve shapes it can be said that all are quite stable, being the variations related to the representativeness achieved by each order in relation to sampling effort. This uniformity

in curves form across different data sets and hierarchic levels allowed to describe general sequences that turn out to be very useful from a predictive point of view. That means, for example, that they can act as references to compare estimations of small databases of macroinvertebrates and then infer the possible behavior of the curve –and therefore the expected richness- if the sample were larger.

In a regional context, non-parametric estimates showed good results, *i.e.* high completeness of the inventories of Trichoptera, Ephemeroptera and Prostigmata at both hierarchical levels, while they demonstrated a great lack of knowledge in Coleoptera. These results can be attributed to the fact that only Elmidae is widely studied. Estimates for Diptera produced very good results respect to inventory completeness, however these results should be taken carefully, since it is a very diverse group and intuitively much greater richness than that recorded would be expected in this area. As in Coleoptera, a possible explanation for this could be the low level of determination that can be reached for this group in the region.

CONCLUSION

The results of this study allow to propose the non-parametric estimators Jack1, ACE and Chao1 as excellent tools for both the estimation of richness and as a measure of the completeness of inventories of some of the most conspicuous groups of benthic macroinvertebrates. The application of the estimators to the local data set shows that the best known orders of the region are Trichoptera, Ephemeroptera and Prostigmata, and in a lower degree Coleoptera, while the inventory of the Diptera presented intermediate values of representativeness.

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Appendix. Adapted from EstimateS User´s Guide. Appendix B (http://viceroy.eeb.uconn.edu/EstimateSPages/EstSUsersGuide/EstimateSUsersGuide.htm#AppendixB)

Incidence estimators:

$$Jack1 = Sobs + Q_1\left(\frac{n-1}{n}\right)$$

$$Jack2 = Sobs + \left[\frac{Q_1(2n-3)}{n} - \frac{Q_2(n-2)^2}{n(n-1)}\right]$$

$$Chao2 = Sobs + \frac{Q_1^2}{2Q_2^2} \quad \text{or Chao2} = Sobs + \left(\frac{n-1}{n}\right)\left(\frac{Q_1(Q_1-1)}{2(Q_2+1)}\right)$$

$$ICE = S_{frec} + \frac{S_{infr}}{C_{ICE}} + \frac{Q_1}{C_{ICE}}\gamma_{ICE}^2$$

$$\gamma_{ice} = \max\left[\frac{S_{infr}}{C_{ICE}}\frac{n_{infr}}{(n_{infr}-1)}\frac{\sum_{j=1}^{10} j(j-1(Qj)}{(N_{infr})^2} - 1,0\right]$$

Where:

Q_1 is the number of uniques

Q_2: number of duplicates

n: Total number of samples

S_{freq}: number of frequent species, i.e species present in more than k samples (by default each found in more than 10 samples).

S_{infr}: number of infrequent species, i.e species present in less than k samples (by default each found in 10 or fewer samples).

C_{ice}: Sample incidence coverage estimator

N_{infr}: Total number of incidences (occurrences) of infrequent species

n_{infr}: Number of samples that have at least one infrequent species

Qj: Number of species that occur in exactly j samples (Q_1 is the frequency of uniques, Q_2 the frequency of duplicates)

Q_1: Total frequency of uniques

γ_{ice}: Estimated coefficient of variation of the Q_i for infrequent species

Abundance estimators:

$$\text{Chao}1 = Sobs + \frac{A_1^2}{2A_2^2} \text{ or } \text{Chao}1 = Sobs + \left(\frac{n-1}{n}\right)\left(\frac{A_1(A_1-1)}{2(A_2+1)}\right)$$

$$\text{ACE} = S_{abun} + \frac{S_{rare}}{C_{ACE}} + \frac{A_1}{C_{ACE}}\gamma_{ACE}^2$$

$$\gamma_{ACE} = \max\left[\frac{S_{rare}}{C_{ACE}}\frac{\sum_{i=1}^{10}i(i-1)A_i}{(N_{rare})(N_{rare}-1)} - 1,0\right]$$

Where:

A_1: number of species singletons and

A_2: number of species doubletons

S_{abun}: number of abundant species, i.e. species represented by more than k individuals (by default each with more than 10 individuals).

S_{rare}: number of non abundant species, i.e. species represented by less than k individuals (by default each with 10 or fewer individuals)

C_{ACE}: Sample abundance coverage estimator

N_{rare}: Total number of individuals in rare species

Ai: Number of species that have exactly i individuals when all samples are pooled (F_1 is the frequency of singletons, $F₂$ the frequency of doubletons)

γ_{ACE}: Estimated coefficient of variation of the A_i for infrequent species.