

Evaluation of species richness estimators in studies of diversity involving two larval digenean communities parasitizing snail hosts

Matías J. Merlo · Manuela Parietti · Jorge A. Etchegoin

Received: 26 April 2010 / Accepted: 5 July 2010 / Published online: 22 July 2010
© Springer-Verlag 2010

Abstract The nonparametric estimators of species richness are some of the most widely used extrapolation methods in studies of biodiversity. These studies need relative large samples to achieve total diversity (including rare species), and estimators are routinely used to alleviate this problem. In the existing literature, parasite communities have received little attention. In fact, the present study is the first dealing with parasites in invertebrate hosts. Real data on seasonal diversity of larval digeneans in the snails *Heleobia conexa* and *Heleobia australis* from Mar Chiquita coastal lagoon (Argentina) were used to evaluate seven nonparametric estimators of species richness, observing the behavior of each method and its ability to estimate the richness using different subsample sizes. In addition, estimated species richness was compared with the previously observed species richness in the parasite component communities of both snail hosts, and the minimum sample size, necessary to include all the observed species of larval digeneans, was estimated using the nonparametric estimators. The bootstrap method had the best overall performance in the two communities. Although this estimator required large subsamples to estimate sample richness, it was in general the least biased and most accurate, and it presented the most similar curve shapes with lack of erratic behavior through the seasons. The results obtained from the

comparison between the minimum sample sizes estimated in previous studies of both snail hosts and those suggested by the estimators also support the use of species richness estimators as methods to calculate the minimum sample size in studies of diversity.

Introduction

The effects of climate change, the threat of widespread extinction, and habitat destruction on biodiversity have attracted much attention from researchers. Species richness is the simplest way to describe community and regional diversity, is comparable across communities, and is well understood by researchers, managers, and the public (Hellman and Fowler 1999; Mac Nally and Fleishman 2002; Magurran 2004). Nevertheless, all biologists who sample natural communities are plagued with the problem of how well a sample reflects a community's "true" diversity (Hughes et al. 2001). To deal with this problem, a number of estimation techniques have been developed to extrapolate from the known to the unknown. These techniques can be grouped into three classes: (1) parametric models, (2) nonparametric estimators, and (3) extrapolations of species accumulation curves (SAC; Magurran 2004).

The nonparametric estimators of species richness are some of the most widely used extrapolation methods mainly in studies of free-living animal and plant communities and have been suggested to perform better than SAC and parametric methods (Baltanás 1992; Colwell and Coddington 1994; Walther and Morand 1998; Walther and Martin 2001; Walther and Moore 2005; Hortal et al. 2006). The success of these estimators probably lies in the fact that they are simple to compute and require only data on the presence–absence of all observed species in the samples

M. J. Merlo (✉) · M. Parietti · J. A. Etchegoin
Laboratorio de Parasitología, Departamento de Biología,
Facultad de Ciencias Exactas y Naturales,
Universidad Nacional de Mar del Plata,
Funes 3350,
7600 Mar del Plata, Argentina
e-mail: mjmerlo@mdp.edu.ar

(Colwell and Coddington 1994; Poulin 1998). The observed species richness in the samples is strongly influenced by sampling effort, and the use of extrapolation methods is needed to control for the confounding effects of uneven sampling effort (Walther et al. 1995; Poulin 1998; Foggo et al. 2003; Walther and Moore 2005; Canning-Clode et al. 2008).

In studies of parasite communities, if the sample of host is small or insufficient, only those species with high prevalences will be represented and the values of species richness will not be representative. Therefore, to estimate the richness of a species-poor parasite component community will require a smaller sample of hosts than that needed to estimate the species richness of a richer parasite component community (Poulin 1998). Sampling artifacts can have serious effects on species richness estimates in parasite communities.

The number of parasites recorded in a sample can be highly correlated with the number of hosts examined, and also with host size, population density, and geographic range (Walther et al. 1995). Once controlling for sample size, some of the relationships between host ecological traits and parasite richness lose their significance (Gregory 1990; Walther et al. 1995). Besides controlling the effects of uneven sampling effort, the nonparametric estimators of species richness calculate the number of “rare” species missed in the samples of hosts examined and add this number to the observed species richness (Poulin 1998). In other words, the simulation results of the nonparametric methods “complete” the information about the species composition in a parasite component community derived from samples.

Another of the possible approaches is to estimate the sample size needed to record all the species in a community using traditional methods (e.g., McDonald et al. 1996). Nevertheless, in studies conducted in protected areas with strict regulations regarding the collection of animals and plants and with restriction in the numbers of hosts that can be killed, the minimum sample size required by the traditional methods may be too large to be possible. This is the case of Mar Chiquita coastal lagoon (Buenos Aires province, Argentina), designated a Man and the Biosphere Reserve by UNESCO.

Among the invertebrates inhabiting the lagoon, there are two species of mollusks belonging to the family Cochliopidae, *Heleobia conexa* (Gaillard 1974) and *Heleobia australis* (d’Orbigny 1835). The larval digenean communities in both snail species have been studied exhaustively from 1996 to the present, and these previous studies have revealed that the two species of gastropods combined serve as first intermediate hosts to at least 22 species of digeneans (Etchegoin 1997, 2001; Merlo 2009). The larval digeneans parasitizing the two species of mollusks present different

seasonal patterns of presence/absence, related mainly to the patterns of temporal and spatial distributions of definitive hosts (Etchegoin 1997, 2001; Merlo 2009). To obtain a complete overview of the diversity of larval digeneans in *H. conexa* and *H. australis*, including digenean species present throughout the year and seasonal species, an evaluation of observed richness and sample sizes from previous studies in both snail hosts is needed. For this reason, the purposes of this study were:

1. To evaluate the nonparametric estimators of species richness with real data, observing the behavior of each method and its ability to estimate richness using different subsample sizes;
2. To compare the estimated species richness with the previously observed species richness in the parasite component communities of *H. conexa* and *H. australis*; and
3. To estimate the minimum sample size necessary to detect all the observed species of larval digenean component communities using the nonparametric estimators.

Materials and methods

Study sites and sampling procedures

Data were obtained from a parasitological study dealing with larval digenean communities in two snail species: *H. conexa* (Gaillard 1974) and *H. australis* (d’Orbigny 1835; Cochliopidae). The study was conducted during 1996 in Mar Chiquita coastal lagoon, located in Buenos Aires province (37°46’ S, 57°27’ W). The lagoon is divided into a freshwater zone, characterized by continental water discharge, and an estuarine zone which communicates with the open sea, characterized by mixo-euryhaline waters and great marine water influence (Reta et al. 2001). Two different study sites were selected: “Juan y Juan” (freshwater zone) and Cangrejito channel (estuarine zone). A total of 1,430 specimens of *H. conexa* and 1,353 specimens of *H. australis* were collected seasonally in “Juan y Juan” and in Cangrejito channel, respectively, from the reef-like aggregates of the polychaete *Ficopomatus enigmaticus* (Fauvel 1923; Serpulidae). The numbers of snails collected seasonally in 1996 were: 346, 375, 328, and 381 during the summer, autumn, winter, and spring, respectively, for *H. conexa* and 360, 281, 314, and 398 during the summer, autumn, winter, and spring, respectively, for *H. australis*. In the study sites, random cores (15-cm diameter×15-cm depth) were taken and placed into plastic cups filled with water from the lagoon for transportation. In the laboratory, snails were removed from reefs and measured with a

Vernier caliper (precision, 0.1 mm). Each snail was isolated in a 45-ml plastic cup and exposed to a 100-W incandescent lamp for 48 h to stimulate shedding of cercariae (patent infections). Finally, all gastropods were dissected under a stereomicroscope in order to detect the presence of sporocysts, rediae and immature cercariae (pre-patent infections; Curtis and Hubbard 1990). Shed cercariae, sporocysts, rediae, and immature cercariae were identified according to Martorelli (1986, 1988, 1989, 1990, 1991), Martorelli and Etchegoin (1996), and Etchegoin and Martorelli (1997, 1998).

Data analysis

Data on absence/presence concerning the absence/presence of cercariae in each snail host were grouped seasonally for the two different study sites (and, consequently, for the two species of snail hosts). For each data matrix, 100 randomizations were generated using the software “EstimateS” ver. 8.2 (Colwell 2009). “EstimateS” was used to calculate the mean species accumulation curve for each data set. Seven nonparametric (NP) species richness estimators were evaluated: Chao 1, Chao 2, the first- and second-order Jackknife estimators (Jack 1 and Jack 2), the bootstrap estimator (Boot), the abundance-based coverage estimator (ACE), and the incidence-based coverage estimator (ICE). For a detailed description of these estimators, see Colwell (2009). The program also calculates the number of observed species (S_{obs}) which is equivalent to the species richness accumulation curve.

To evaluate the performance of NP richness estimators, three indicators were used: bias, precision, and accuracy (Hellman and Fowler 1999; Foggo et al. 2003; Walther and Moore 2005; Schoeman et al. 2008). To quantify the performance of the estimators, we compared the curves generated by each estimator in the two study sites.

On the basis of these comparisons, we consider that in the first 30% of the accumulation curves in Juan y Juan and in the first 40% of the accumulation curves in Cangrejito channel, the sampling effort required to achieve good estimates is low. For this reason, we evaluated estimator performance from the 30% and 40% of the curves, respectively.

Bias indicates and quantifies the tendency of an estimator to systematically overestimate or underestimate the true species richness. Basically, bias indicates how close is the mean of the estimates of species richness, derived from the samples, to the true value of species richness (Schoeman et al. 2008).

$$\text{Bias} = \frac{1}{n} \sum_{i=1}^n (S_i - S_{\text{True}})$$

where S_{True} is the true species richness observed and S_i ($i=1, 2, \dots, n$) are the measures of species richness estimated in the $n=100$ randomizations.

Precision can be used to quantify the variance of the estimates resulting from a given procedure. Precision basically indicates how close repeated estimates are to one another based on different species accumulations (Foggo et al. 2003; Schoeman et al. 2008).

$$\text{Precision} = \text{Variance} = \frac{1}{n} \sum_{i=1}^n (S_i - \bar{S})^2$$

where \bar{S} is the population mean of the species richness estimates.

Accuracy indicates how close are the estimates of species richness, made on the basis of many samples from a population, to the true value of species richness. Being a combination of bias and precision, accuracy could be considered the most desirable property of an estimator (Schoeman et al. 2008).

$$\text{Accuracy} = \frac{1}{n} \sum_{i=1}^n (S_i - S_{\text{True}})^2 = \text{Variance}(S) + [\text{Bias}(S)]^2$$

Although bias, precision, and accuracy are widely used to assess the performance of species richness estimators, Walther and Moore (2005) pointed out that comparisons across communities containing different numbers of species are invalid because the magnitude of these measures is related to the number of species in the community under study. These authors provided the following formulations for bias, precision, and accuracy that correct for this scaling effect, rendering measures from various sites more comparable; these adjustments also provide results that are more meaningful in terms of units, being either unitless or expressed as a percentage:

$$\text{Scaled Bias} = \text{SME} = \frac{1}{S_{\text{True}}n} \sum_{i=1}^n (S_i - S_{\text{True}})$$

$$\text{Scaled Precision} = \text{CV} = \frac{100\sqrt{\text{Precision}}}{\bar{S}}$$

$$\text{Scaled Accuracy} = \text{SMSE} = \frac{\text{Accuracy}}{S_{\text{True}}^2}$$

where SME is scaled mean error, CV is the coefficient of variation, and SMSE is scaled mean square error.

To evaluate the performance of richness estimators, Melo and Froehlich (2001) proposed four criteria considered by the authors as more practical and realistic than bias, precision, and accuracy when no subsample size is chosen

a priori to estimate richness. We selected three of the four criteria used by Melo and Froehlich (2001) to evaluate estimation methods: (1) the smallest subsample size required to estimate the observed richness in the total sample, (2) lack of erratic behavior in curve shape, and (3) similarity in curve shape across the sample data sets.

The minimum sample size necessary to include all the observed species was estimated as a linear interpolation of the crossover point where the estimators begin to overestimate the true species richness (Hellman and Fowler 1999).

Results

With a total of 21 observed species, the community of larval digeneans parasitizing *H. conexa* can be considered richer than the community of *H. australis*, which consists of 13 species. Despite the differences in their compositions, both communities showed seasonal fluctuations regarding observed species richness (Table 1).

The species richness estimators utilized in this study presented different results and performances for the two larval digenean communities (Tables 2 and 3). In fact, analyzing the results based on data obtained from the larval digenean community of *H. conexa*, the estimator Boot was the least biased and most accurate in three of the four seasons considered (spring, summer, and autumn). In the winter, the estimator Chao 1 was the least biased and most accurate. With respect to the precision, the estimator Jack 1 was more precise in winter and autumn, while Boot and Chao1 were more precise in spring and summer, respectively (Table 2).

The estimators ACE, ICE, Chao 1, Chao 2, Jack 1, and Jack 2 showed some variability in the number of estimated species in relation to the observed richness: In general, these estimators tended to overestimate the observed richness. In contrast, the estimator Boot produced values of richness slightly lower than the observed richness, but they were consistent for all seasons (Table 2 and Fig. 1).

With respect to the larval digenean community of *H. australis*, the estimators showed more variable results. The estimator Boot was the least biased and most accurate in

Table 1 Seasonal fluctuations in the number of species observed in the larval digenean communities of *H. conexa* and *H. australis* from Mar Chiquita coastal lagoon

Host	Winter	Spring	Summer	Autumn	TNSO
<i>H. conexa</i>	10	14	14	16	21
<i>H. australis</i>	2	9	10	3	13

TNSO total number of species observed

Table 2 Performance evaluation of seven estimators using three indicators: bias, precision, and accuracy

Estimator	Winter			Spring			Summer			Autumn		
	S Bias	S Precision	S Accuracy	S Bias	S Precision	S Accuracy	S Bias	S Precision	S Accuracy	S Bias	S Precision	S Accuracy
ACE	0.1229	4.9012	0.0181	0.4996	17.8088	0.3209	0.4864	13.6478	0.2778	0.4676	13.6281	0.2587
Bootstrap	0.0993	4.8775	0.0027	-0.0130	9.1770	0.0084	-0.0264	11.9542	0.0142	-0.0038	8.6185	0.0074
Chao 1	-0.0080	1.4928	0.0003	0.1713	12.4097	0.0505	0.1225	11.2816	0.0310	0.1325	12.1603	0.0365
Chao 2	-0.0083	1.5035	0.0003	0.1705	12.4052	0.0502	0.1216	11.2825	0.0308	0.1318	12.1527	0.0363
ICE	0.1159	4.6387	0.0161	0.4991	17.8139	0.3205	0.4861	13.6559	0.2775	0.4674	13.6237	0.2584
Jack 1	-0.0083	1.2220	0.0111	0.1521	10.1999	0.0369	0.1449	12.1729	0.0404	0.1485	8.3361	0.0312
Jack 2	0.1044	5.3985	0.0134	0.3654	11.8621	0.1598	0.3418	12.1258	0.1433	0.3134	11.0077	0.1191
Sobs	0.0159	5.4413	0.0138	-0.1413	10.2820	0.0278	-0.1538	11.7587	0.0336	-0.1323	10.2084	0.0253

The four sets of real data utilized in the simulation represent seasonal values of presence/absence of the species included in the larval digenean community parasitizing *H. conexa*

two of the four season considered (spring and summer). ACE and ICE were the least biased and most accurate in autumn and were the most accurate in winter. In winter, Jack 2 was the least biased, but also was the least accurate (Table 3).

As observed in the larval digenean community of *H. conexa*, the estimators ACE, ICE, Chao 1, Chao 2, Jack 1, and Jack 2 tended to overestimate the observed richness when the number of observed species increased. The estimator Boot produced values of richness slightly higher than the observed richness, but they were consistent for all seasons (Table 3 and Fig. 2).

In reference to the three criteria proposed by Melo and Froehlich (2001), in *H. conexa*, the Chao 2 and Jack 2 estimators had good performances in relation to the constancy of minimum subsample size, followed by ACE, ICE, Jack 1, and Boot, but Boot needed the biggest sampling effort. The estimator that had the worst performance was Chao 1, with high minimum sampling effort and high variability (Table 4). Nevertheless, Boot presented the best similar curve shapes with lack of erratic behavior through the seasons, followed by Jack 1, Chao 1, and Chao 2. The estimators ACE, ICE, and Jack 2 did not have curve shapes constant along all samples and showed some erratic behavior (Fig. 1).

In *H. australis*, Jack 1 and Boot had good performances in relation to the constancy of minimum subsample size, and Jack 1 needed lower sampling effort than Boot. The other estimators needed higher sampling effort and had poor constancy (Table 5). Boot presented, as in the larval digenean community of *H. conexa*, the best similar curve shapes with lack of erratic behavior through the seasons, followed by Jack 1, Chao 1, Chao 2, ACE, and ICE. The estimator Jack 2 did not have constant curve shapes along all samples and displayed some erratic behavior (Fig. 2).

Discussion

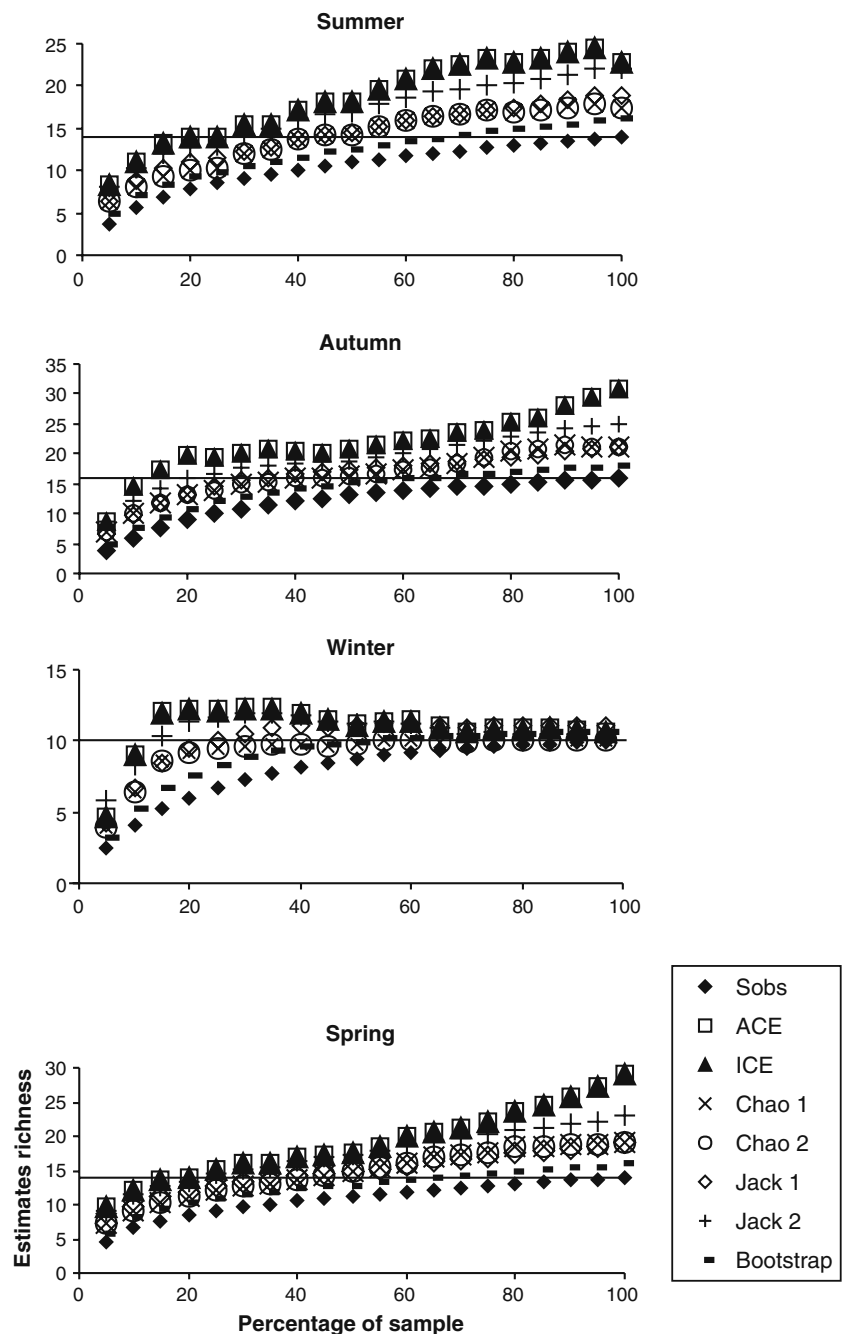
According to our results, the nonparametric bootstrap estimator had the best overall performance in the two larval digenean communities studied. Although bootstrap needed large subsamples to estimate sample richness, this estimator was in general the least biased and most accurate, and it was the best estimator for two of the three previously chosen criteria proposed by Melo and Froehlich (2001). The Chao 1, Chao 2, and Jack 1 methods performed at intermediate level, whichever way we evaluated their performance. The ACE, ICE, and Jack 2 estimators were in general the most biased and least accurate and produced curves with erratic behavior. Particularly, the ACE and ICE estimator had good performance, regarding bias, accuracy, and minimum

Table 3 Performance evaluation of seven estimators using three indicators: bias, precision, and accuracy

Estimator	Winter			Spring			Summer			Autumn		
	S Bias	S Precision	S Accuracy	S Bias	S Precision	S Accuracy	S Bias	S Precision	S Accuracy	S Bias	S Precision	S Accuracy
ACE	-0.0432	3.0480	0.0027	0.4389	5.8490	0.1997	0.3866	2.9162	0.1511	-0.0057	6.0244	0.0036
Bootstrap	0.0527	2.9245	0.0037	0.0215	8.6500	0.0083	0.0347	9.2896	0.0104	0.0110	7.3784	0.0057
Chao 1	-0.0524	4.3750	0.0045	0.0945	6.4698	0.0139	0.1753	6.7304	0.0370	-0.0884	5.7413	0.0106
Chao 2	-0.0524	4.3750	0.0045	0.0939	6.4676	0.0138	0.1744	6.7327	0.0367	-0.0888	5.8236	0.0107
ICE	-0.0432	3.0480	0.0027	0.4386	5.8538	0.1995	0.3727	2.8706	0.1405	-0.0057	6.0244	0.0036
Jack 1	0.1279	4.2805	0.0187	0.2065	7.3981	0.0506	0.2469	7.7873	0.0704	0.1723	6.5251	0.0356
Jack 2	0.0309	21.9480	0.0522	0.3820	5.9374	0.1527	0.4749	7.7883	0.2388	0.3405	8.4738	0.1288
Sobs	-0.0606	5.8643	0.0067	-0.1303	9.9080	0.0244	-0.1465	11.2899	0.0308	-0.1185	8.8248	0.0201

The four sets of real data utilized in the simulation represent seasonal values of the presence/absence of the species included in the larval digenean community parasitizing *H. australis*

Fig. 1 Larval digenean community of *H. conexa*. Curves of accumulation of observed species and estimates for seasonal samples. The total number of species observed in each sample is represented by the horizontal line. S_{obs} number of observed species



sample size required, only when the number of observed species was lower.

To our knowledge, only three studies have applied the nonparametric estimators in studies of parasite diversity and sampling effort (Poulin 1998; Walther and Morand 1998; Zelmer and Esch 1999). All these previous studies were based on real and simulated data obtained from vertebrate hosts (birds, mammals, and amphibians; for a review, see Walther and Moore 2005). In consequence, the present study is the first to estimate species richness of a

parasite community from invertebrate hosts. Our results were consistent with those obtained by Poulin (1998) who stated that the bootstrap estimator is the least likely to provide a greatly inflated estimate and is thus more conservative than either the Jackknife or Chao's estimators. Also, comparable patterns have been obtained by Walther and Morand (1998) using real and simulated parasite data. Nevertheless, further analyses of both real and simulated data sets are required to establish the most reliable estimators for a wide variety of ecological

Fig. 2 Larval digenean community of *H. australis*. Curves of accumulation of observed species and estimates for seasonal samples. The total number of species observed in each sample is represented by the horizontal line. S_{obs} number of observed species

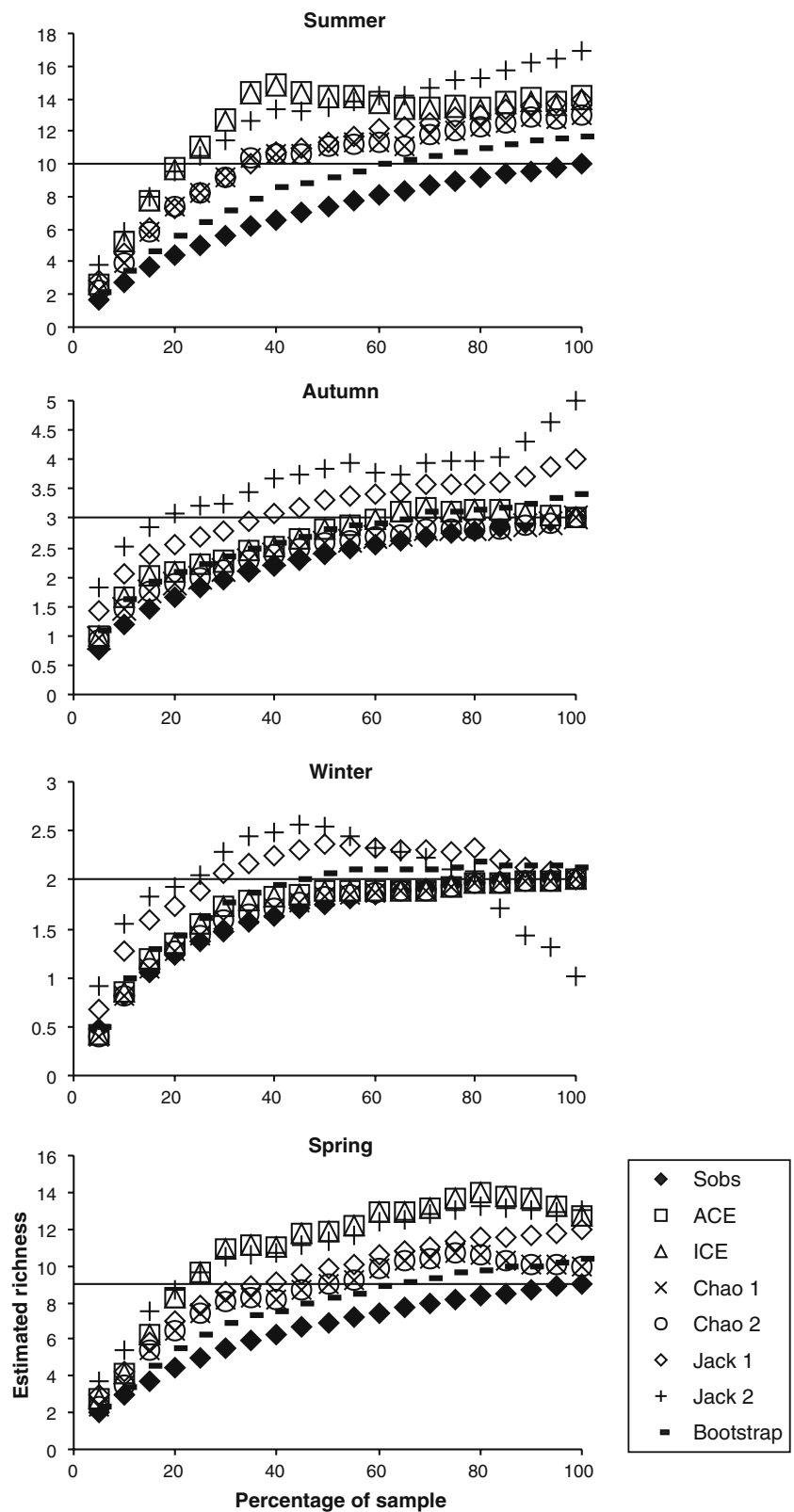


Table 4 Minimum sample size, measured as percentage, required by the seven nonparametric methods to estimate the seasonal observed richness in the larval digenean community of *H. conexa*

	<i>H. conexa</i>	Sample size (%)				\bar{x}	SD
		Winter	Spring	Summer	Autumn		
	Sobs	99.70	100.00	100.00	100.00	99.92	0.15
	ACE	12.20	15.75	25.43	12.80	16.54	6.13
	ICE	12.20	24.93	25.43	12.80	18.84	7.33
	Chao 1	75.00	41.47	43.06	45.60	51.28	15.90
	Chao 2	53.96	41.47	44.22	47.73	46.85	5.39
	Jack 1	23.48	38.06	43.06	34.67	34.82	8.31
	Jack 2	13.72	22.05	27.75	20.53	21.01	5.77
\bar{x} mean of the four values corresponding to each season, SD standard deviation	Bootstrap	51.22	65.88	66.76	63.73	61.90	7.23

communities as this problem is far from resolved (Colwell and Coddington 1994).

As mentioned earlier, in studies conducted in protected areas or in geographical areas with restricted access, the number of specimens that can be sampled represents a real limitation for sampling effort. For this reason, species richness estimators are needed to “complete” the information derived from small samples. With respect to sampling effort, some researchers have proposed different ways of estimating the minimum sample size in ecological studies of larval digenean communities parasitizing snail hosts based on their previous experience with the snail–digenean systems. For example, Lafferty et al. (1994) dissected snails until they found a total of 100 that were infected, and Huspeni et al. (2005) stated that 100 snails proved to be good minimum sample size, sufficient to produce a small variance when estimating prevalence.

To estimate the minimum sampling effort required to reach a satisfactory level of completeness in a survey as judged by the proportion of the species in the area that are detected, species accumulation curves have also proven to be useful (Moreno and Halffter 2000). Walther and Morand (1998) proposed to stop sampling once the variance is <5% of the estimated species richness. And Chazdon et al. (1998) suggested sampling a representative community and then selecting a sample size which incorporates a prede-

termined portion of the total species richness. In the present study, we estimated the minimum sample size necessary to include all observed species as a linear interpolation of the crossover point where the estimators begin to overestimate the true species richness. Similar criteria were used by Poulin (1998), Hellman and Fowler (1999), and Melo and Froehlich (2001).

In previous studies of the larval digenean communities of *Heleobia* spp., we estimated that approximately 300 snails represented a good minimum sample size to estimate species richness (Etchegoin 1997; Merlo 2009). This estimation coincides with the results obtained here with the bootstrap estimator (60%, approximately 300 snails). These results would support the use of species richness estimators as methods to calculate the minimum required sample size in studies of diversity.

In the particular case of parasites, the use of species richness estimators is advisable because their aggregated distributions produce a concentration of parasites in few host individuals and, in consequence, an underestimation of the observed parasite diversity. In studies of diversity of larval digenean communities in snail hosts, the spatial and temporal distributions of the hosts involved in the complex life cycles and the characteristics of the host’s habitat (including biotic and abiotic factors) produce variations in the diversity and in the structure of parasite communities

Table 5 Minimum sample size, measured as percentage, required by the seven nonparametric methods to estimate the seasonal observed richness in the larval digenean community of *H. australis*

	<i>H. australis</i>	Sample size (%)				\bar{x}	SD
		Winter	Spring	Summer	Autumn		
	Sobs	92.99	100.00	100.00	99.65	98.16	3.45
	ACE	96.18	23.37	21.35	60.99	50.47	35.51
	ICE	96.18	23.37	21.62	60.99	50.54	35.43
	Chao 1	96.18	49.25	32.97	98.23	69.16	33.07
	Chao 2	96.18	49.50	32.97	98.23	69.22	33.02
	Jack 1	28.66	36.68	34.59	37.94	34.47	4.11
	Jack 2	71.00	23.37	21.89	19.86	34.03	24.69
\bar{x} mean of the four values corresponding to each season, SD standard deviation	Bootstrap	45.54	62.81	59.73	65.96	58.51	9.01

(Fernández and Esch 1991; Kuris and Lafferty 1994; Etchegoin 1997, 2001; Kube et al. 2002; Fingerut et al. 2003; Huspeni et al. 2005; Poulin 2006; Rogowski and Stockwell 2006; Faltýnková et al. 2008; Thieltges et al. 2008). Due to these variations in time and space, a sample taken without considering such factors may not adequately represent the diversity of the whole community. For this reason, we strongly recommend the use of species richness estimators in the early stages of investigation. Nevertheless, while the bootstrap estimator proved to be the best for the larval digenean communities of *Heleobia* spp., this result cannot be generalized to other studies of diversity of parasites. In each host–parasite system, the estimators should be tested and analyzed in order to select the most suitable for each particular study.

Acknowledgments The authors gratefully thank Dr. Robert Poulin (University of Otago, New Zealand) for reading an earlier version of the manuscript. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET; J.A.E., grant number PIP 114-200801-00001) and Universidad Nacional de Mar del Plata (J.A.E., grant no. EXA 411/08 15/E359). M. J. Merlo and J. A. Etchegoin are members of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

- Baltanás A (1992) On the use of some methods for the estimation of species richness. *Oikos* 65:484–492
- Canning-Clode J, Valdivia N, Molis M, Thomason JC, Wahl M (2008) Estimation of regional richness in marine benthic communities: quantifying the error. *Limnol Oceanogr Methods* 6:580–590
- Chazdon RL, Colwell RK, Denslow JS, Guariguata R (1998) Statistical methods for estimating species richness of woody regeneration in primary and secondary rain forests of NE Costa Rica. In: Dallmeier F, Comiskey J (eds) *Forest biodiversity research, monitoring and modeling: conceptual background and Old World case studies*. Pantheon, Paris, pp 285–309
- Colwell RK (2009) EstimateS: statistical estimation of species richness and shared species from samples, version 8.2. User's guide and application. <http://purl.oclc.org/estimates>
- Colwell RK, Coddington J (1994) Estimating terrestrial biodiversity through extrapolation. *Philos Trans R Soc Lond B* 345:101–118
- Curtis LA, Hubbard KM (1990) Trematode infections in a gastropod host misrepresented by observing shed cercariae. *J Exp Mar Biol Ecol* 143:131–137
- Etchegoin JA (1997) *Sistemas parasitarios presentes en la albufera Mar Chiquita*. Tesis Doctoral, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata
- Etchegoin JA (2001) Dinámica de los sistemas parasitarios. In: Iribarne OO (ed) *Reserva de Biosfera Mar Chiquita: características físicas, biológicas y ecológicas*. Editorial Martín, Mar del Plata, pp 227–250
- Etchegoin JA, Martorelli SR (1997) Description of a new species of *Maritrema* (Digenea: Microphallidae) from Mar Chiquita coastal lagoon (Buenos Aires, Argentina) with notes on its life cycle. *J Parasitol* 83:709–713
- Etchegoin JA, Martorelli SR (1998) Nuevos estadios larvales de digeneos parásitos de *Heleobia conexa* (Mollusca: Hydrobiidae) en Mar Chiquita (Buenos Aires, Argentina). *Neotrópica* 44:41–50
- Faltýnková A, Valtonen ET, Karvonen A (2008) Spatial and temporal structure of the trematode component community in *Valvata macrostoma* (Gastropoda, Prosobranchia). *Parasitology* 135:1691–1699
- Fernández J, Esch GW (1991) Guild structure of larval trematodes in the snail *Helisoma anceps*: patterns and processes at the individual host level. *J Parasitol* 77:528–539
- Fingerut JT, Zimmer CA, Zimmer RK (2003) Patterns and processes of larval emergence in an estuarine parasite system. *Biol Bull* 205:110–120
- Foggo A, Attrill MJ, Frost MT, Rowden AA (2003) Estimating marine species richness: an evaluation of six extrapolative techniques. *Mar Ecol Prog Ser* 248:15–26
- Gregory RD (1990) Parasites and host geographic range as illustrated by waterfowl. *Funct Ecol* 4:645–654
- Hellman JJ, Fowler GW (1999) Bias, precision and accuracy of four measures of species richness. *Ecol Appl* 9:824–834
- Hortal J, Borges PAV, Gaspar CA (2006) Evaluating the performance of species richness estimators: sensitivity to sample grain size. *J Anim Ecol* 75:274–287
- Hughes JB, Hellmann JJ, Ricketts TH, Bohannan BJM (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl Environ Microbiol* 67:4399–4406
- Huspeni TC, Hechinger RF, Lafferty KD (2005) Trematodes parasites as estuarine indicators: opportunities, applications, and comparisons with conventional community approaches. In: Bortone S (ed) *Estuarine indicators*. CRC, Boca Raton, pp 297–314
- Kube J, Kube S, Dierschke V (2002) Spatial and temporal variation in the trematode component community of the mudsnail *Hydrobia ventrosa* in relation to the occurrence of waterfowl as definitive hosts. *J Parasitol* 88:1075–1086
- Kuris AM, Lafferty KD (1994) Community structure: larval trematodes in snail host. *Annu Rev Ecol Syst* 25:189–217
- Lafferty KD, Sammond DT, Kuris AM (1994) Analysis of larval trematode communities. *Ecology* 75:2275–2285
- Mac Nally R, Fleishman E (2002) Using “indicator” species to model species richness: model development and predictions. *Ecol Appl* 12:79–92
- Magurran AE (2004) *Ecological diversity and its measurement*. Princeton University Press, Princeton
- Martorelli SR (1986) Estudio sistemático y biológico de un digeneo perteneciente a la familia Microphallidae Travassos, 1920. I: *Microphallus szidati* sp. nov. parásito intestinal de *Rallus sanguinolentus sanguinolentus* (Aves: Rallidae) e *Himantopus melanurus* (Aves: Recurvirostridae). *Rev Iber Parasitol* 46:373–378
- Martorelli SR (1988) El ciclo biológico de *Levinseniella cruzi* Travassos, 1920 (Digenea, Microphallidae) parásito de los ciegos cólicos de *Rollandia rooland chilensis* (Aves, Podicipedidae) e *Himantopus melanurus* (Aves, Recurvirostridae). *Iheringia* 68:49–62
- Martorelli SR (1989) Estudios parasitológicos en la albufera Mar Chiquita, provincia de Buenos Aires, República Argentina. II. Cercarias (Digenea) parásitas de *Heleobia conexa* (Mollusca: Hydrobiidae), pertenecientes a las familias Schistosomatidae, Haploporidae y Homalometridae. *Neotrópica* 35:81–90
- Martorelli SR (1990) Estudios parasitológicos en la albufera Mar Chiquita, provincia de Buenos Aires, República Argentina. III: Sobre dos cercarias parásitas de *Heleobia conexa* (Mollusca: Hydrobiidae) pertenecientes a la superfamilia Echinostomatoidea. *Neotrópica* 36:55–63
- Martorelli SR (1991) El ciclo biológico de *Microphallus similimus* (Travassos, 1920), comb. n. (Digenea: Microphallidae), parásito de *Heleobia conexa* (Mollusca: Hydrobiidae) y de *Himantopus melanurus* (Aves: Recurvirostridae) en Argentina. *Iheringia* 71:91–98

- Martorelli SR, Etchegoin JA (1996) Cercarias de la superfamilia Opistorchioidea en *Heleobia conexa* (Mollusca: Hydrobiidae) de la albufera de Mar Chiquita. *Neotrópica* 42:61–67
- McDonald TL, Birkes DS, Urquhart NS (1996) Obtaining species: sample size considerations. *Environ Ecol Stat* 3:329–347
- Melo AS, Froehlich CG (2001) Evaluation of methods for estimating macroinvertebrate species richness using individual stones in tropical streams. *Freshw Biol* 46:711–721
- Merlo MJ (2009) Los sistemas molusco-trematode como indicadores ambientales en la laguna Mar Chiquita, provincia de Buenos Aires. Tesis de Licenciatura. Universidad Nacional de Mar del Plata, Facultad de Ciencias Exactas y Naturales
- Moreno CE, Halffter G (2000) Assessing the completeness of bat biodiversity inventories using species accumulation curves. *J Appl Ecol* 37:149–158
- Poulin R (1998) Comparison of three estimators of species richness in parasite component communities. *J Parasitol* 84:485–490
- Poulin R (2006) Global warming and temperature-mediated increases in cercarial emergence in the trematode parasites. *Parasitology* 132:143–151
- Reta R, Martos P, Perillo GME, Piccolo MC, Ferrante A (2001) Características hidrográficas del estuario de la laguna Mar Chiquita. In: Iribarne OO (ed) Reserva de Biósfera Mar Chiquita: características físicas, biológicas y ecológicas. Editorial Martín, Mar del Plata, pp 31–52
- Rogowski DL, Stockwell CA (2006) Parasites and salinity: costly in the threatened species. *Oecologia* 146:615–622
- Schoeman DS, Nel R, Soares AG (2008) Measuring species richness on sandy beach transects: extrapolative estimators and their implications for sampling effort. *Mar Ecol* 29(suppl 1):134–149
- Thieltges DW, Jensen KT, Poulin R (2008) The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology* 135:407–426
- Walther BA, Morand S (1998) Comparative performance of species richness estimation methods. *Parasitology* 116:395–405
- Walther BA, Martin J (2001) Species richness estimation of bird communities how to control for sampling effort? *Ibis* 143:413–419
- Walther BA, Moore JL (2005) The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. *Ecography* 28:815–829
- Walther BA, Cotgreave P, Prince RD, Gregory RD, Clayton DH (1995) Sampling effort and parasite species richness. *Parasitol Today* 11:306–310
- Zelmer DA, Esch GW (1999) Robust estimation of parasite component community richness. *J Parasitol* 85:592–594