Inter-annual variations of true species richness in a subtropical butterfly assemblage: an estimation based on least-biased extrapolations of species accumulation curves

Abstract
More or less strong inter-annual variations of observed species richness are well known in insects in general and in butterfly in particular. Yet, such variations are rarely reliably documented in relevant terms of “true” total species richness (instead of observed). This is because the equity of sampling completeness between annual inventories proves hard to ensure in practice and had long remained difficult to check properly. Accordingly, directly extrapolating the as-recorded inter-annual variations in terms of “true” inter-annual variations of total species richness remains seriously questionable. In other words, which part of recorded inter-annual variations is really attributable to true inter-annual variations of total species richness and which part may (artificially!) results from inter-annual inequity of the level of sampling completeness between successive annual inventories? As neither standardised sampling procedures nor rarefaction procedure at a same sampling size may reliably warrant the equity of sampling completeness, asymptotic estimations of total species richness should imperatively be implemented for each annual inventory. This implies that, among the different estimators of the number of missing species available in the literature, the least-biased one should be adequately selected at first, for each annual inventory. Now that such a procedure of selection is made available, it becomes possible to tackle relevantly the issue. Applying this procedure to the butterfly assemblages at Mount Gariwang-san (south Korea), quantitatively surveyed during seven years by Lee and coworkers (2016), I show that (i) “true” inter-annual variations of species richness may indeed vary in a large range - from simple to double - along successive years; (ii) annual total species richness represents only a limited part - comprised between less than \( \approx 40\% \) and at most \( \approx 80\% \) - of the potential species richness of the site.

Key words: diversity, sampling completeness, nonparametric estimator, Gariwang-san, Korea, Lepidoptera

INTRODUCTION
Most insects assemblages undergo both intra-annual (seasonal) and inter-annual variations of species richness in temperate as well as tropical regions worldwide [1-8]. Due to their attractiveness and relative easiness of determination at the species level, butterfly assemblages have been particularly considered as a convenient model for studying temporal variations of insects' diversity [3-8]. Under tropical climates, the seasonal fluctuations of species diversity may be of variable amplitude while inter-annual variations are sometimes considered as clearly prominent [6]. Yet, the temporal fluctuations of species richness has been much more often addressed at the short time scale, i.e. seasonal variations [1-4, 7] than during longer periods, i.e. substantially longer
time series, involving several successive years (see [8] however). Higher costs investments and shortage of available time to be devoted to such studies are arguably the main reasons for the current scarcity of long-term investigations. For the same reasons, the levels of completeness of the successively scheduled inventories are usually far from being exhaustive, which seriously hamper the significance to be given to as-recorded results, in terms of “true” variations of total species richness. Appropriate extrapolations of species accumulation beyond actually achieved sampling-sizes are thus needed to derive reliable estimates of the true total species richness that occurs at any stage of the time series. Therefore, the current difficulties to obtain relevant extrapolations, providing reliable estimates, urge to implement appropriate procedures involving least-biased extrapolations (thus following the path first initiated by Brose and coworkers [9] and more recently improved by Bégurinot [10-11].

Hereafter, I consider the field data issued from a long-term study of butterfly assemblages at Mount Gariwang-san (South Korea) carried on by Lee and coworkers [12]. As mentioned by the authors, each successive samplings performed along the seven investigated years, remain more or less substantially incomplete. Yet, they offer valuable crude data from which to extrapolate species accumulation and estimate true species richness each year, in order to be able to relevantly address the three following questions:

- how large are the inter-annual variations of the “true” (i.e. total) species richness of the butterfly assemblages at Gariwang-san?
- which proportion of the “overall potential butterfly richness” at the studied site (equated, as a first approximation, to the true species richness cumulated during the seven years) actually occurs at any given year?

Now, as the currently achieved inventories remain substantially incomplete, relevantly answering these two questions requires either (i) to further continue sampling efforts until closely approaching sampling exhaustivity or, (ii) to extrapolate, with minimised bias, the species accumulation process, thereby being in capacity to provide reliable estimation to answer properly these two questions above. Option (i) would, of course, be ideal and, thus, to be privileged insofar as it proves compatible with the available resources in terms of time and costs. Yet, in common practice, option (ii) offers, at least as a first attempt, an economic, convenient and straightforward solution, to be considered at first. Then, before possibly considering the ideal option (i), a third question is to be addressed:

- which additional sampling efforts would be required to closely approach exhaustivity (say, for example, reaching 95% sampling completeness), so as to be in capacity to predict, on a rational basis, the practicability (or not) of option (i): further continue sampling efforts.

MATERIALS AND METHODS

Lee and coworkers [12] conducted a series of samplings of the butterfly fauna at Mount Gariwang-san (South Korea), during 1987 and from years 2010 to 2015. All details relative to the sampling procedure, the environment context, the list of recorded species with their respective abundances are provided in [12] with free access and, accordingly, will not be recalled here. Accounting for species abundances is of prime interest in the perspective of the extrapolation of partial samplings, since abundance data provides
estimates of the numbers $f_1, f_2, f_3, f_4, ..., f_x, ...$ of those species recorded respectively 1-, 2-, 3-, ..., $x$- times in the realised partial sampling. These numbers are required, in turn, to reliably extrapolate the species accumulation curve, as explained below. As substantial numbers of singletons (i.e. species recorded only once) are retained in the inventories performed during each of the seven years (as well as in the seven inventories pooled together), it follows that all these inventories remain substantially incomplete. Extrapolating the species accumulation, beyond the actually achieved sampling sizes, is thus necessary to predict at best the true total species richness of butterfly assemblage for each year.

**Numerical extrapolation of species accumulation beyond the achieved sampling size**

As sampling size increases, the number of recorded species is monotonically growing, at first rapidly and then less and less quickly. The so-called ‘Species Accumulation Curve’ $R(N)$ accounts for the growth kinetics of the number of recorded species $R$ with increasing sampling size $N$ ($N$: typically, the number of observed individuals during sampling). The mathematical expression (and thus the details of the shape) of the Species Accumulation Curve are dependent upon both the total species richness of the sampled assemblage of species and the degree of heterogeneity of the species abundance distribution within the sampled assemblage of species [13]. This would apparently make the extrapolation of the Species Accumulation Curve rather difficult to compute, since both preceding factors are unknown *a priori*. Yet, the numbers $f_1, f_2, f_3, f_4, ..., f_x, ...$ of those species recorded respectively 1-, 2-, 3-, 4-, ..., $x$- times during sampling are directly dependent also upon the total species richness and the degree of heterogeneity of the species abundances. This explains why these numbers $f_1, f_2, f_3, f_4, ...$, may serve as an appropriate basis from which to extrapolate the Species Accumulation Curve, beyond the actual size of the sample under consideration. In particular, the most commonly used estimators of the number of unrecorded species (i.e. non-parametric estimators such as ‘Chao’ and the series of ‘Jackknife’) are all computed from the recorded values of the first numbers $f_x$[14]. In practice, a problem remains however: as already mentioned, each of these different types of estimators provides a substantially distinct estimate and none among these estimators remains consistently the more appropriate. Accordingly the traditional practice has become to consider together all of them without making any choice [15], an admittedly frustrating situation!

Yet, it has been shown recently that although none of the available estimators consistently remains the more accurate[9], each of them may prove, in turn, being the less biased, depending on the value taken by $f_1$ as compared to the other $f_x$[10]. Accordingly, in practice, the most appropriate – i.e. the least biased – estimator of the number of unrecorded species may be selected by comparing the value of $f_1$ to the values of the other $f_x$ for $x > 1$ [10-11]. Selecting this way the least-biased type of estimator thereby provides the best possible estimate of the number $\Delta$ of “missing” species and, in turn, the best estimate of the total species richness $S_t$ of a partially sampled assemblage. In addition, the least biased expression for the extrapolation of the species accumulation curve $R(N)$ is straightforwardly derived.

In practice, the formulations summarised in Appendix 1 provide: (i) the expressions of $\Delta, S_t$ and $R(N)$, according to each of the most commonly used types of nonparametric estimators and (ii) the *key to select*, among these estimators, which one reveals the less
biased and, thereby, which expressions for $\Delta$, $S_t$ and $R(N)$ are the less-biased. Also, in order to reduce the dispersive influence of drawing stochasticity (which inevitably affects the as-recorded values of the $f_x$), it is advisable to regress the as-recorded distribution of the numbers $f_x$ versus $x$.

**RESULTS**

**Extrapolations and asymptotic estimates for each sampled year**

The estimated numbers $\Delta$ of missing (= unrecorded) species, according to each of six types of nonparametric estimators (Jackknife at orders 1 to 5 and Chao), are provided at Table 1 for the seven investigated year. Note that the selected least-biased estimator may differ from year to year: Jackknife type estimators at order 3, 5, 3, 5, 4, 3, 5 are respectively selected for years 1987, 2010, 2011, 2012, 2013, 2014, 2015; Chao and Jackknife 1 and 2 never being selected. And selecting the least-biased estimator in each case is important since, as shown at Table 1, the estimated number of missing species may vary in a wide range for a same sample, typically from simple to double according to estimator type. The least-biased estimations of the ‘true’ (total) species richness, $S_t = R_0 + \Delta$, and the level of sampling completeness (= $R_0/S_t$) are derived immediately: Table 2. Note, incidentally, that the levels of sampling completeness achieved for each of the seven studied years are very weakly related to either sampling-size or total species richness (Figures 1 and 2).

In addition to the asymptotic estimates $\Delta$, $S_t$ and $R_0/S_t$, the full range of extrapolation of the species accumulation curve is computed and made available for each type of estimator (the expression of the least-biased extrapolation being, of course, the one associated to the least-biased estimator). Figures 3 and 4 provide two examples, for years 2010 and 2011 (the least-biased extrapolation in bold). Figures 5 and 6 allow to compare the selected, least-biased extrapolations of the species accumulation curves for six successive years, from 2010 to 2015 inclusively. Note that the six accumulation curves grow at different relative paces and, accordingly, some of them may intersect.

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**Table 1** – The estimated number $\Delta$ of missing (= unrecorded) species, according to six types of nonparametric estimators, Jackknife at orders 1 to 5 and Chao. Estimates are provided for each of the seven annual samplings and, at last, for the seven samples pooled together. The least-biased estimator, selected according to the procedure described, is in bold for each sample.

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Table 2 – The sample size (number of sampled individuals) $N_0$, the number of recorded species $R_0$, the selected, least-biased type of estimator, the corresponding estimated number $\Delta$ of missing (unrecorded) species, the resulting estimated total species richness $S_t$ and the resulting sampling completeness $R_0/S_t$ (%), as computed for each of the seven annual inventories and the pooled inventory. Butterfly fauna at Mount Gariwang-san, field records according to Lee et al. [12] for years 1987, 2010, 2011, 2012, 2013, 2014, 2015.

Incidentally, an important practical consequence of the substantially distinct rates of growth of the numbers of recorded species (Figures 5 & 6) is the irrelevance of the comparison (still too often) made between the numbers of recorded species in several samples, even when the comparison is made at the same size, as already underlined by Lande et al. [16] and more extensively argued by Bégout [17].

Figure 1 – The level of sampling completeness $R_0/S_t$, regressed against the sampling size, for the seven investigated years. While sampling completeness increases of course with sampling size $N_0$ within a same given assemblage, this trend becomes fairly inconsistent when several different assemblages are compared ($r = 0.47$, $n = 7$, $p = 0.29$).
Figure 2 – The level of sampling completeness, \( R_0 / S_t \), regressed against the estimated total species richness \( S_t \), for the seven investigated years. The levels of sampling completeness achieved at each year are substantially independent of the corresponding estimated total species richness (\( r = 0.12, n = 7, p = 0.8 \)).

Figures 3&4 – Extrapolations of the species accumulation curve associated to six types of estimator (Jackknife orders 1 to 5 and Chao; the least-biased extrapolation is in bold) for years 2010 and 2011. The actually achieved sampling is indicated by the grey point. The least-biased estimator (and the associated extrapolation) is Jackknife 5 for year 2010 and Jackknife 3 for year 2011. One particular interest of considering the (least-biased) extrapolation is the possibility to reliably predict the additional sampling effort that would be required to reach any given level of sampling completeness beyond the already achieved completeness (here are indicated the 80%, 90%, 95% completeness levels).
Figures 5&6 – The selected (least-biased) extrapolations of the species accumulation curves for six successive years, from 2010 to 2015 inclusively. For each year, the actually achieved sampling is indicated by the grey point; the selected estimator and the associated extrapolation are Jackknife at order 5, 3, 5, 4, 3, 5 for years 2010, 2011, 2012, 2013, 2014, 2015, respectively. Figure 6 is a zoom of Figure 5, focusing on the beginnings of the extrapolations.

Estimation of the additional sampling efforts required to improve completeness
One major interest of extrapolated species accumulation curves is their capacity to predict the additional sampling effort that would be required to reach any given level of sampling completeness beyond the already achieved completeness. This provides, in turn, a rational basis to decide whether or not it seems worth further continuing the sampling operations, putting in balance the additional effort required and the expected benefit in terms of newly recorded species. And, in this respect, as for the asymptotic estimates above, selecting the least-biased extrapolation is very important to derive
reliable predictions, as shown in Table 3 which exemplifies the large scatter of predictions according to the type of estimator involved. Accordingly, Figure 7 and Table 4 highlight the least-biased estimates of the additional sampling efforts that would have been required to reach different levels of sampling completeness, considering the seven investigated years.

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<td>570</td>
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**Table 3** – The additional sampling effort N required to reach a 95% level of sampling completeness. Predictions differ very strongly according to the type of estimator associated to the extrapolation of the species accumulation curve. The least-biased expectation for the sampling effort required to reach 95% completeness is in bold. Once more, selecting the least-biased estimator features decisive to obtain reliable predictions.

**Figure 7** – The selected, least-biased extrapolations of the species accumulation curves for six successive years, from 2010 to 2015 inclusively. The actually achieved sampling is indicated by the grey point. The estimated additional sampling efforts that would have been required to reach a same 90% level of sampling completeness are indicated for each years. These required additional sampling efforts differ very substantially from one year to the other.

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<td>72%</td>
<td>62%</td>
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Table 4 – Estimated sampling sizes that would have been required to reach increasing levels of sampling completeness (80%, 85%, 90%, 95%) for the inventories conducted at successive years (1987, 2010, 2011, 2012, 2013, 2014, 2015) and for the pooled inventories. Accordingly, approaching sampling exhaustivity would require to consent considerably higher sampling efforts than actually performed. For example, aiming at reaching 95% completeness would have required to multiply the actually achieved sampling sizes by a factor ranging from 8.5 (year 2013) to 16.2 (year 2010). This would have probably exceed by far the resources in time and credit made available to LEE and co-workers [12].

Inter-annual variations of the total species richness of the butterfly assemblage at Mount Gariwang-san

Figures 8, 9, 10, 11 provide a graphical representations of the data given in Table 2, which help visualising the range of inter-annual variations of the species richness of butterfly fauna at Mount Gariwang-san. The estimated total species richness per year may vary substantially (up to a factor 2) and apparently erratically, according to years: Figure 10.

Figure 8 – Histogram providing: (i) below, the number $R_0$ of recorded species, (ii) above, the estimated number $\Delta$ of still unrecorded species, (iii) the sum $R_0 + \Delta$, that is the estimated total species richness $S$, for the seven investigated years and for all seven years pooled together. Butterfly fauna at Mount Gariwang-san, field records according to LEE et al. [12] for years 1987, 2010, 2011, 2012, 2013, 2014, 2015. Data directly derived from Table 2.
Still more interesting is the estimated proportion of the “potential species richness” of the site which actually occurs each year: Figure 11. Here, the “potential species richness” is approximately equated, as a surrogate, to the total species richness estimated for the seven years (1987 & 2010 to 2015) taken together, i.e. 120 species. This proportion of the “potential species richness” actually occurring each year is comprised between a little less than 40% and a little more of 80% of the “potential species richness” of the site. In fact, this figure might be slightly overestimated since the cumulated number of species along seven years might well remain a slight underestimation of the real “potential species richness” of the site. Therefore, a figure comprised between one third and two third of the “potential species richness” might be perhaps more realistic.

Note also that, according to Figure 10, the estimated total species richness does not show any clear temporal trend: the very slight decrease that appears between 1987 and 2015 contributes for less than 2% to the inter-annual variance of species richness ($r^2 = 0.016$).
Figure 10 – Histogram of the estimated total species richness $S_t$ for the seven investigated years and for all seven years pooled together.

Figure 11 – The estimated proportion (%) of the “potential species richness” of the site (estimated to 120 species [at least]) which actually occurs each year. Data directly derived from Figure 8, i.e. on the basis of least-biased extrapolations of total species richness for each year. Along these seven years, the proportion of the “potential species richness” of the site which actually occurs annually varies between 38% and 82% (average: 59%).

**DISCUSSION**

Lee and coworkers [12] have conducted a series of samplings of the butterfly fauna at Mount Gariwang-san (South Korea), during 1987 and from 2010 to 2015. As part of their report, the authors provide, in particular, a detailed account of the inter-annual variations of the recorded species richness. Yet, as mentioned by the authors, these annual samplings are likely more or less incomplete, as confirmed by the significant
proportion of ‘singletons’ in the species lists. Accordingly, any attempt to directly
extrapolate the inter-annual variations of as-recorded species richness in terms of “true”
inter-annual variations of total species richness would be questionable. Specifi-
cally, the involved issue would be: which part of the recorded inter-annual variations is really
attributable to true inter-annual variations of total species richness and which part may
(artificially!) results from inter-annual inequity of sampling completeness between
successive annual inventories?

One alternative solution that may be considered to answer this issue would be, of
course, to further continue sampling operations, each year, so as to closely approach
sampling exhaustivity. While not strictly impossible this ideal procedure would imply
huge sampling efforts, year after year (Figures 3, 4, 7 and Table 4), which, in practice,
may arguably exceed available resources in terms of both time and costs expenditure.

It is therefore necessary to consider the less satisfying, but practically unavoidable
alternative solution: to extrapolate numerically the species accumulation curves up to
their asymptotic levels. Nonparametric estimators may help in this respect, provided
the least-biased type of estimator be selected, separately for each annual survey. This
precaution proves being indispensable, as highlighted by the large scatter between the
estimates issued from the different types of available nonparametric estimators: Figures
5 & 6 and Tables 1 & 3.

Thus, implementing the selection procedure in favour of the least-biased extrapolation
([10-11], see also Appendix 1) provides more reliable expectations of the “true” (total)
species richness, year by year, for the surveyed butterfly assemblages of Mont
Gariwang-san.

As expected, estimated achieved sampling completeness actually vary according to
millesim: from 54% (in 2015) to 72% (in 2012): Table 4. Incidentally, the level of
sampling completeness proves weakly (and non-significantly) related to the sampling
size (Figure 1) and independent of the estimated total species richness (Figure 2). The
selected least-biased estimator also differs from year to year: Jackknife estimators at
order 3, 4, 5 were selected according to studied years and Jackknife order 2 was
selected for the pooled inventories along the seven studied years, while Jackknife 1 and
Chao were never retained (Table 2). As usual, the appropriate selection of the type of
Jackknife estimator proves being important since the estimation of the number of
missed species by inventories most often vary from simple to double (Table 1, Figure 8).

In particular, this seriously question the traditional approaches that consist in either
choosing a priori one given type of estimator, on the basis of alleged assumption as to its
particular appropriateness, or considering all types of estimators together without
choosing among them [15].

The levels of total species richness, derived from estimates of the number of missed
species, substantially vary from year to year: from 46 (in 2014) to 99 (in 2011). That is
up to more than simple to double (Table 2, Figures 8, 10).

The degrees of inter-annual variability of species richness may be quantified by the
“inter-annual species richness ratio”, defined as the ratio (>1) of the estimated total
species richness within any couple of years, successive or not. The histogram of values
taken by this inter-annual species richness ratio at Mount Gariwang-san in the years
2010 to 2015 (Figure 12) provides an estimate of the probabilities for the inter-annual
variability of total species richness being more or less large. Thus, the estimated
probabilities that the ratio exceeds 1.2, 1.4, 1.6, 1.8, 2.0, are 73%, 46%, 26%, 13%, 7%, respectively (Figure 12).

Figure 12 – Distribution of probability for the value of the “inter-annual species richness ratio”, defined as the ratio (>1) of the estimated total species richness within any couple of years from 2010 to 2015 at Mount Gariwang-san.

At last, one additional interesting question is which proportion of the “overall potential butterfly richness” of the site (equated, as a first approximation, to the total species richness considering the seven years pooled together) actually occurs at any given year? Considering the estimations above, the proportion of the “potential species richness” at the sampled site which actually occurs annually varies between 38% and 82% (average: 59%), along these seven years of field survey (Figure 11). This appreciably differs from what would be inappropriately deduced from the crude (non-extrapolated) inventories: based on non-extrapolated data, the proportion of the “potential species richness” would vary from 28% to 58% (average: 42%): Table 2, Figures 8, 9.

Now, from a strictly local point of view, the effective representation of only a limited part (38% to 82%) of the “potential species richness” during each year might raise the paradox of the local perpetuation of those species that are not locally represented during one or several years. The likely reason lies, of course, in the concept of metapopulation, according to which more or less regular flows of exchanges takes place between adjacent localities.

CONCLUSION

While the as-recorded annual species richness of the butterfly fauna at the sampled locality of Mount Gariwang-san was comprised between 29 and 61 species, the actual annual species richness was estimated ranging from 46 to 99 species, using the “least-biased” procedure of extrapolation of species accumulation curves. The total species richness for the seven years pooled together is estimated around 120 species. This figure may arguably be considered as likely approaching the “potential species richness” of the studied locality.
Accordingly, on the basis of these bias-reduced estimates, it becomes possible to address the difficult question of reliably assessing the proportion of the potential species richness of a site which is actually occurring each year on the site. It turns out that, here, only 38% to 82% (average: 59%) of the potential species richness actually occur in any given year.

### Appendices

**Appendix 1 - Bias-reduced extrapolation of the Species Accumulation Curve and the associated bias-reduced estimation of the number of missing species, based on the numbers of species recorded 1 to 5 times.**

Consider the survey of an assemblage of species of size \( N_0 \) (with sampling effort \( N_0 \) typically identified either to the number of recorded individuals or to the number of sampled sites, according to the inventory being in terms of either species abundances or species incidences), including \( R(N_0) \) species among which \( f_1, f_2, f_3, f_4, f_5 \), of them are recorded 1, 2, 3, 4, 5 times respectively. The following procedure, designed to select the less-biased solution, results from a general mathematical relationship that constrains the theoretical expression of any theoretical Species Accumulation Curves \( R(N) \) (see references [10, 11, 18, 19, 20]):

$$ \frac{\partial^x R(N)}{\partial N^x} = (-1)^{(x-1)} \frac{f_{x(N)}}{C_{N,x}} \approx (-1)^{(x-1)} \frac{x!}{N^x} f_0(N) \quad (\approx \text{as } N \gg x) \quad (A.1) $$

Compliance with the mathematical constraint (equation (A.1)) warrants reduced-bias expression for the extrapolation of the Species Accumulation Curves \( R(N) \) (i.e. for \( N > N_0 \)). Below are provided, accordingly, the polynomial solutions \( R_x(N) \) that respectively satisfy the mathematical constraint [1], considering increasing orders \( x \) of derivation \( \frac{\partial^x R(N)}{\partial N^x} \). Each solution \( R_x(N) \) is appropriate for a given range of values of \( f_i \), compared to the other numbers \( f_i \) (according to [10]):

- for \( f_1 \) up to \( f_2 \rightarrow R_1(N) = (R(N_0) + f_1) - f_1.N_0/N 
- for \( f_1 \) up to \( f_2 \rightarrow R_2(N) = (R(N_0) + 2f_1 - f_2) - (3f_1 - 2f_2).N_0/N - (f_2 - f_1).N_0^2/N^2 
- for \( f_1 \) up to \( f_3 \rightarrow R_3(N) = (R(N_0) + 3f_1 - 3f_2 + f_3) - (6f_1 - 8f_2 + 3f_3).N_0/N - (4f_1 + 7f_2 - 3f_3).N_0^2/N^2 - (f_1 - 2f_2 + f_3).N_0^3/N^3 
- for \( f_1 \) up to \( f_2 \rightarrow R_4(N) = (R(N_0) + 4f_1 - 6f_2 + 4f_3 - f_4) - (10f_1 - 20f_2 + 15f_3 - 4f_4).N_0/N - (10f_1 + 25f_2 - 21f_3 + 6f_4).N_0^2/N^2 - (5f_1 - 14f_2 + 13f_3 - 4f_4).N_0^3/N^3 - (f_1 - 3f_2 + f_3 + f_4).N_0^4/N^4 
- for \( f_1 \) larger than \( 4f_2 - 6f_3 + 4f_4 - f_5 \rightarrow R_5(N) = (R(N_0) + 5f_1 - 10f_2 + 10f_3 - 5f_4 + f_5) 

The associated non-parametric estimators of the number \( \Delta_1 \) of missing species in the sample \( [\text{with } \Delta_1 = R(N=\infty) - R(N_0)] \) are derived immediately:
* 0.6 f_2 < f_1 ≤ 2f_2 \rightarrow \Delta_{J1} = f_1 ; \quad R_1(N)

* f_2 < f_1 ≤ 2f_2 - f_3 \rightarrow \Delta_{J2} = 2f_1 - f_2 ; \quad R_2(N)

* 2f_2 - f_3 < f_1 ≤ 3f_2 - 3f_3 + f_4 \rightarrow \Delta_{J3} = 3f_1 - 3f_2 + f_3 ; \quad R_3(N)

* 3f_2 - 3f_3 + f_4 < f_1 ≤ 4f_2 - 6f_3 + 4f_4 - f_5 \rightarrow \Delta_{J4} = 4f_1 - 6f_2 + 4f_3 - f_4 ; \quad R_4(N)

* f_1 > 4f_2 - 6f_3 + 4f_4 - f_5 \rightarrow \Delta_{J5} = 5f_1 - 10f_2 + 10f_3 - 5f_4 + f_5 ; \quad R_5(N)

**N.B.1:** As indicated above (and demonstrated in details in [10]), this series of inequalities define the ranges that are best appropriate, respectively, to the use of each of the five estimators, JK-1 to JK-5. That is the respective ranges within which each estimator will benefit of minimal bias for the predicted number of missing species. Besides, it is easy to verify that another consequence of these preferred ranges is that the selected estimator will always provide the highest estimate, as compared to the other estimators. Interestingly, this mathematical consequence, of general relevance, is in line with the already admitted opinion that all non-parametric estimators provide under-estimates of the true number of missing species [13, 14]. Also, this shows that the approach initially proposed by Brose et al. [9] – which has regrettably suffered from its somewhat difficult implementation in practice – might be advantageously reconsidered, now in light of the very simple selection key above, of far much easier practical use.

**N.B.2:** In order to reduce the influence of drawing stochasticity on the values of the f_x, the as-recorded distribution of the f_x should preferably be smoothened: this may be obtained either by rarefaction processing or by regression of the as-recorded distribution of the f_x versus x.

**N.B.3:** For f_1 falling beneath 0.6 x f_2 (that is when sampling completeness closely approaches exhaustivity), then Chao estimator may be selected: see reference [11].

**Appendix 2 - Regressions on the distributions of recorded values of f_x so as to reduce the consequences of drawing stochasticity**
Figures A2.1 to A2.8—The recorded values of the numbers $f_x$ of species recorded $x$-times (grey discs) and the regressed values of $f_x$ (black discs) so as to reduce the consequence of stochastic dispersion, for the seven studied years and for all years pooled together.

REFERENCES


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