**Title**

A comparison of methods for estimating plant population size

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**Running title**

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**Abstract**

Assessing local population size is one of the most common tasks in biodiversity monitoring.  Population size estimates are not only important for conservation management and population threat assessment; they also enter many other analyses in landscape ecology and conservation. It is therefore concerning that methods for estimating plant population sizes are not standardized. We surveyed the literature and found that the most commonly used methods are counting either all or only flowering individuals on a site, as well as counting individuals in random plots or plots on transects. Often, these methods are combined in the same study, without assurance that they produce comparable results. We therefore conducted a field study, in which we obtained population size estimates from all four methods for six different calcareous grassland species at 18 study sites. Our results demonstrate not only substantial differences between overall count rates generated by the different methods, but methods that surveyed the whole population also systematically yielded lower counts when species were less visible and when the area was larger, suggesting that these methods suffer from biases that could distort species and site comparisons. We conclude that estimates from different methods should not be mixed, and that plot or transect based surveys have likely smaller biases for large areas or poorly visible individuals, and are therefore preferable.

**Key words**

Plant population size, population ecology, conservation, counting, plots, vegetation monitoring, vegetation surveys, field methods

**Introduction**

Estimating population size is of outstanding relevance for many aspects of plant ecology and conservation. For example, population size is directly related to genetic variation, fitness and extinction probability ([Busch and Reisch 2016](#_ENREF_1); [Hooftman et al. 2003](#_ENREF_4); [Leimu et al. 2006](#_ENREF_8); [Matthies et al. 2004](#_ENREF_10)). It is also an essential element of population viability analyses ([Menges 2000](#_ENREF_11)) and population monitoring ([Philippi et al. 2001](#_ENREF_14); [Keith 2000](#_ENREF_7); [Marsh and Trenham 2008](#_ENREF_9)), and a requirement for the implementation of many important conservation policies such as the European Habitats Directive (92/43/EEC) ([Huck et al. 2006](#_ENREF_5)). Consequently, being able to reliably measure population size is a basic requirement for many applications in ecology and conservation.

Given their general importance, it is somewhat surprising that population sizes are currently assessed in very different ways. We performed a literature survey of 143 studies dealing with plant population size, published between 1991 and 2015 (online supplementary S1, Table S1.1). Of the surveyed articles, 32.9% lacked any detailed information on the method applied to determine population size. In most remaining studies, population size was either assessed by counting or estimating the number of individuals at the whole study site (48.2%), or by counting individuals in plots or transects and extrapolating those counts to the area of the study site (7.0%). Moreover, when surveying the whole site, some authors included all individuals, while others only considered flowering plants occurring at the study site (Table1).

These methodological differences are concerning for several reasons. First of all, it is conceivable that different methods will lead to different overall count rates. If that were true, comparisons based on population counts with different methods, for example to prioritize conservation areas or deduce population trends, would be questionable. Even more concerning, however, would be if different methods did not only lead to homogeneously higher or lower count rates, but also to a change of relative proportions when comparing different species or sites. Such a bias could arise, for example, when species or site properties such as visibility or area created different biases for different methods. In this case, analyses based on comparisons between sites and species could be unreliable, even if only one method was used in a study.

To address these questions, we created a test situation where we estimated the population size of six different calcareous grassland species at 18 study sites with four different methods. We included two site-based methods (counting all individuals and counting all flowering individuals at the study site) and two plot-based methods (counting individuals in squares with extrapolation to the site area and counting individuals along transects with extrapolation to the site area) in the study.

We ask the following questions: (i) Are there significant differences in the absolute population size estimates between the four methods? (ii) Is the estimated population size affected by species’ traits such as visibility, i.e. are the methods reliable for comparing between species? (ii) Are differences of population size estimates between methods dependent on site properties such as site area, i.e. are methods consistent for a comparison within sites?

**Materials and methods**

***Study sites and species***

We selected 18 calcareous grasslands in the valleys of the rivers Naab and Laber on the Franconian Alb in Southeastern Germany near Regensburg (online supplementary S1, Table S1.2, Figure S1.1). At each site, we estimated the population size of six different plant species occurring typically in calcareous grasslands in Germany (*Primula veris*, *Polygala comosa*, *Dianthus carthusianorum*, *Sanguisorba minor*, *Salvia pratensis* and *Medicago falcata,* see online supplementary S1, Table S1.3).

Species visibility was described as an ordered categorical variable with three levels: due to their strikingly colored and comparatively large flowers or inflorescences, *P. veris* and  *S. pratensis* are well recognizable, and were therefore rated as high. In contrast, *D. carthusianorum* and *M. falcata* are less conspicuous, and were rated as medium. Least visible are *P. comosa* and *S. minor* since their flowers or inflorescences are small and less bright, and their visibility was therefore rated as low. As an additional covariate, we obtained the area of the studied grasslands using GIS (Arc Info 10.0, Esri), based upon orthorectified aerial photos from 2013.

***Tested methods for population size estimation***

Population size estimates were obtained by field assistants, which were familiar with the flora of calcareous grasslands. All assistants were instructed prior to the data collection to perform counts in the same way. Counts were made at the seasonal flowering peak of the species to ensure their maximum visibility. All plants were counted regardless of their age, including juvenile and adult individuals. Individuals as basic unit for our counts were defined in an ecological sense, which means that spatially separate plants were considered as different individuals. We performed no genetic analyses to check the genetic identity of the individuals, but as all six species considered in this study have short lateral growth, our results should not be affected by confusing genets and ramets due to clonality. Consequently, we also did not test specialized approaches for estimating the size of clonal plant populations.

We used two site-based and two plot-based methods (online supplementary S1, Figure S1.2):

*Counting all individuals at each study site (site al)*

The first site-based method was counting all individuals at each study site by inspecting the whole area through walking loops in a distance of 2-3m. At large sites, the area was divided in subplots which were defined by natural objects (trees, rocks, etc.). All individuals that were seen along the track were counted.

*Counting all flowering individuals at each site (site fl)*

The second site-based method is identical to the first, except that only flowering individuals were counted. Plants with buds or withered flowers were also considered as flowering. For *P. comosa* and *D. carthusianorum,* the number of all individuals was equivalent to the number of flowering individuals, since non-flowering individuals could not be detected in our study.

*Counting individuals in squares with extrapolation to the area of the study site (plot sq)*

The first plot-based method consisted of counting all individuals occurring in 30 1m² squares, that were randomly placed in the study site. The obtained counts were then extrapolated to the entire study site by multiplication with the respective site area.

*Counting individuals along transects with extrapolation to the site area (plot tr)*

The second plot-based method was based on transect consisting of 2x2m plots from north to south. The number of plots per transect depended of the dimension of the study site. For each plot, all individuals were counted. Again, the obtained counts were extrapolated to the entire study site by multiplication with the respective site area.

***Statistical analysis***

We used generalized linear mixed models (GLMM) to analyze the dependence of the population size estimates on method, visibility of the plant species, and log area of the sites (online supplementary S2). We used each predictor as fixed main effect, and included interactions of visibility and area with the estimation method, to test for an influence of method on the effect of visibility and area. Visibility was treated as a numeric variable (i.e. low, mid, high were mapped to values of 1,2,3, respectively). To account for additional effects of site and species, we added random intercepts for both variables. The trivial effect that larger areas will harbor more individuals was corrected for by a log area offset, which scales the estimated population size to the site area. The general model structure, following lme4 notation, was thus

popsize ~ method \* visibility + method \* log(area) + offset(log(area)) + (1|species) + (1|site)

We followed the standard advice to scale and center visibility and log area (but not the area offset) to improve interpretability of the interaction terms. The centering ensures that the effect size estimated for main effects (e.g. methods) applies to a site of mean area, and a species with mean visibility.

Residual checks with the DHARMa R package ([Hartig 2017](#_ENREF_3)) of an initial Poisson GLMM with log-link and an observation-level random effect (to account for overdispersion) revealed substantial zero-inflation. We therefore moved to a zero-inflated negative binomial GLMM, fitted with the R package glmmADMB ([Fournier et al. 2012](#_ENREF_2); [Skaug et al. 2016](#_ENREF_15)). To explore the results of the fitted model, we additionally calculated a Type III ANOVA, and two re-parameterizations of the regression: one regression where methods were summarized into two groups (site and plot), which tests for the significance of overall differences between the site and plot-based methods, and one regression where we specified methods hierarchically into site/group, and then into type A/B within group, to test for the differences within the groups (for site methods flowering / non-flowering, and for plot methods quadrats and transects). We decided against a correction for multiple testing because each of these options tests a distinct hypothesis.

**Results**

The results from the ANOVA demonstrate that all considered factors had a significant effect on population size estimates (Table 2, Figure 1).

The choice of the method had a significant influence on the estimated population size (see regression and ANOVA results in Table 2, highest effect size of all predictors). These differences originate mainly from differences between site-based methods (site al, site fl) and plot-based methods (plot sq, plot tr), which showed differences in population estimates of the order of 2 on the log scale (significant, see plot / size in Table 2), translating roughly to a factor of 10 for the absolute population size. Also the difference between the two submethods plot sq, plot tr was significant, while site al, site fl was not significant.

Concerning the question whether population size estimates depend on species traits, we found that higher/lower visibility was associated to higher/lower population size estimates for the site-based methods as opposed to the plot-based methods (see interaction between visibility and method in Table 2). Differences between submethods were not significant.

We found no significant effect of area for plot-based methods (plot sq, plot tr), which means that population size estimates obtained with these methods were, on average, proportional to area, and population densities thus constant. Conversely, we found a significant negative effect for site-based methods, meaning that the estimated population densities decreased with area. Differences between submethods were again not significant.

**Discussion**

The aim of this study was to test if commonly used methods for estimating plant population sizes in ecological field studies differ in their absolute estimates, in their sensitivity to visibility, and in the impact of the size of the study area. Indeed, we found significant evidence for all three effects in our analysis, with the general tendency that site-based methods find less individuals, lower count rates for less visible species and smaller population sizes for larger sites than the plot-based methods. This demonstrates not only that absolute count rates differ, but also that comparisons between species and sites are likely inconsistent when comparing the results of different methods.

When interpreting our results, it must be kept in mind that we had no absolute reference in our study. Theoretically, it would be possible that the dependence on visibility and habitat area detected by the site-based methods does in fact accurately reflect the true species numbers, while the plot-based methods, for some reason, fail to detect these patterns. The statistical analysis only permits to say that the methods deliver different results, absolutely and dependent on visibility and site area. Common sense, however, paired with the knowledge about how these measurements are performed, suggests to us that it is highly likely that the lower overall count rates of site-based methods, as well as their strong dependence on visibility and area does in fact arise from a measurement bias of these methods.

This interpretation is in line with previous studies suggesting that counting all individuals underestimates population size at large study sites ([Keith 2000](#_ENREF_7)). These results support, therefore, the conclusion that plot-based methods should be preferred, when estimating population size of more frequent species in larger habitats. Although it seems obvious to suspect that counting only flowering individuals would lead to a further reduction of counts ([Hutchings 1991](#_ENREF_6)), this effect was not significant in our data. A possible reason is that there were only few non-flowering individuals present at the time of our study.

In practice, other methods that those considered here are used to estimate population sizes, especially when considering alternative definitions of population size, such as in demographic monitoring, which has been considered more informative than simple population counts ([Hutchings 1991](#_ENREF_6); [Menges and Gordon 1996](#_ENREF_12)). Also, some authors and practitioners have advocated refinements of different methods, for example adaptive cluster sampling ([Philippi 2005](#_ENREF_13)) or an active decision to apply different methods depending on the situation. We cannot say if differences and biases would be reduced by these alterations, but we doubt that demonstrated differences between site-based and plot-based methods would change fundamentally.

Moreover, our study was conducted in calcareous grasslands, which exhibit high species densities. While we think that it is reasonable to assume that results will extrapolate to other ecosystems that provide comparable visual and cognitive challenges for a human observer, we acknowledge that the demonstrated effects may be weaker in less dense habitats with individuals that are well recognizable. In such cases, site-based methods could be as good as or even better than plot-based methods, also because plot-based methods may exhibit higher variability of the estimates at low population density. At which densities and under which conditions such effects occur should be analysed in further studies.

**Conclusions and practical recommendations**

In this study, we tested and compared four essential methods for population monitoring ([Marsh and Trenham 2008](#_ENREF_9)) that are, according to our literature review, applied in many plant ecology and conservation studies. Our study indicates that population size estimates may differ in absolute and relative numbers when different methods are used. We can thus say with confidence that mixing site-based and plot-based estimation methods in the same study, which is often done, is problematic. Erroneous conclusions might arise, for example, when the dependence of population size on area is compared between species that were measured with different methods. Moreover, our results suggest that site-based observations exhibit a bias towards lower population sizes, especially when areas are large and the visibility of a species is poor. We therefore recommend plot-based methods for situations that are comparable to our study.

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**Conflicts of interest**

The authors declare that they have no conflict of interest.

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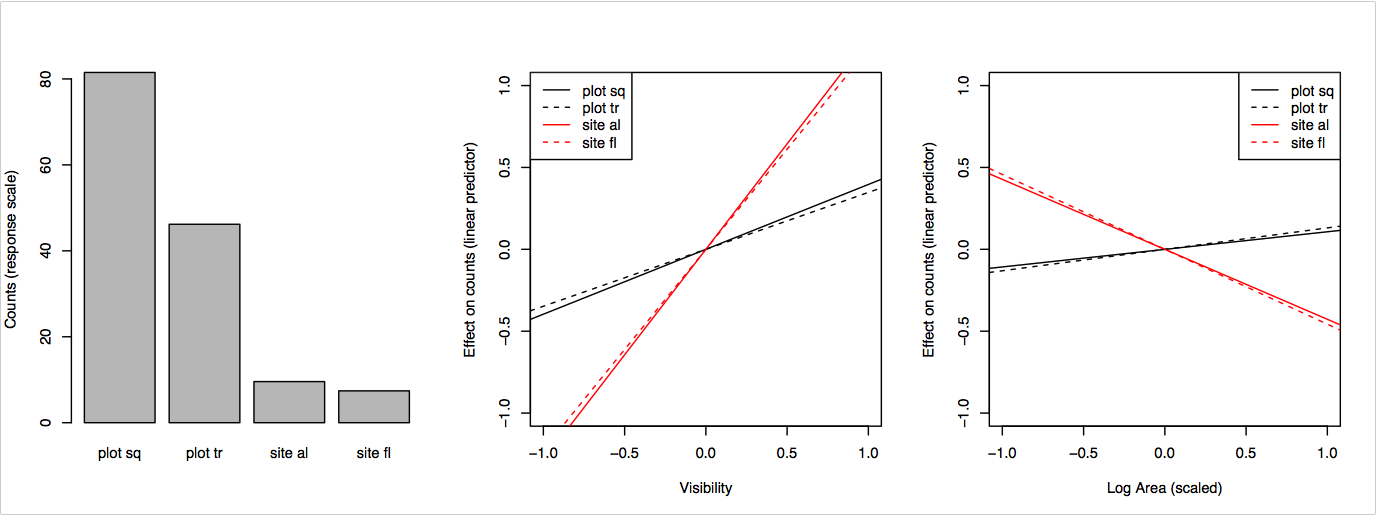
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**Figure captations**

**Figure 1:** Graphical representation of estimated effect sizes (see also Table 1) for method at mean Visibility and Area and an area of 100 (left), as well as the effects of visibility (middle) and log Area (right) for the different methods. Note that the effects of Visibility and Log area effects are displayed at the scale of the linear predictor of the GLMM.

**Figures**

**Figure 1**



**Tables**

**Table 1:** Results from the literature survey on methods used to estimate population size, together with the number (n) and proportion (%) of studies that applied these methods. In total, 143 studies were included in the survey.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Method** |  | **Description** | **(n)** | **%** |
|  |  |  |  |  |
| A |  | No description given | 47 | 32,9 |
| B |  | Counting number of individuals | 20 | 13,9 |
| C |  | Counting number of flowering individuals | 28 | 19,6 |
| D |  | Counting and estimating number of individuals | 7 | 4,9 |
| E |  | Counting number of individuals and extrapolation | 6 | 4,2 |
| F |  | Estimating number of individuals | 8 | 5,6 |
| G |  | Counting number of individuals in plots or transects and extrapolation | 10 | 7,0 |
| H |  | Estimating size of populated area | 10 | 7,0 |
| I |  | Other methods | 7 | 4,9 |
|  |  |  |  |  |
| **Total** |  |  | **143** | **100,0** |
|  |  |  |  |  |

**Table 2:** ANOVA and regression tables for the fitted model.  Additional column plot/site shows regression p-values where methods are grouped into either plot or site. Additional column submethods shows regression p-values for comparison between the submethods within plot and site-based methods. For the latter, other p-values were suppressed (denoted by ///). See supplementary material for extended regression tables and details.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Regression** | | | | **ANOVA** | **plot / site** | **submethod** |
|  |  | **Estimate** | **Std. Error** | **p** | | **p** | **p** | **p** |
| intercept | (plot sq) | -0.2045 | 0.2588 | 0.42945 | n.s. | n.s. | . | /// |
| Method | plot tr | -0.5683 | 0.1454 | 9.3E-05 | \*\*\* | \*\*\* | \*\*\* |
| site al | -2.1423 | 0.1413 | <2E-16 | \*\*\* | \*\*\* | /// |
| site fl | -2.3979 | 0.145 | <2E-16 | \*\*\* | n.s |
| Visibility | (plot sq) | 0.3962 | 0.2508 | 0.11415 | n.s. | n.s. | . | /// |
| Method  x  Visibility | plot tr | -0.0486 | 0.1828 | 0.79036 | n.s. | \*\*\* | n.s. |
| site al | 0.8921 | 0.1756 | 3.8E-07 | \*\*\* | \*\*\* | /// |
| site fl | 0.8271 | 0.1875 | 1E-05 | \*\*\* | n.s. |
| Area | (plot sq) | 0.1075 | 0.1927 | 0.57698 | n.s. | n.s. | n.s. | /// |
| Method  x  Area | plot tr | 0.0233 | 0.1489 | 0.87546 | n.s. | \*\*\* | n.s. |
| site al | -0.5348 | 0.1397 | 0.00013 | \*\*\* | \*\*\* | /// |
| site fl | -0.5646 | 0.1413 | 6.4E-05 | \*\*\* | n.s. |
| Signif. codes:  0 < ‘\*\*\*’ < 0.001 < ‘\*\*’ < 0.01 < ‘\*’ < 0.05 < ‘.’ < 0.1 < ‘n.s ’ <  1 | | | | | | | | |