Plants stand still but hide: imperfect and heterogeneous detection is the rule when counting plants

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Abstract

1. The estimation of population size and its variation across space and time largely relies on counts of individuals, generally carried out within spatial units such as quadrats or sites. Missing individuals during counting (i.e. imperfect detection) results in biased estimates of population size and trends. Imperfect detection has been shown to be the rule in animal studies, and most studies now correct for this bias by estimating detection probability. Yet this correction remains exceptional in plant studies, suggesting that most plant ecologists implicitly assume that all individuals are always detected.
2. To assess if this assumption is valid, we conducted a field experiment to estimate individual detection probability in plant counts conducted in 1x1 m quadrats. We selected 30 herbaceous plant species along a gradient of conspicuousness at 24 sites along a gradient of habitat closure, and asked groups of observers to count individuals in 10 quadrats using three counting methods requiring progressively increasing times to complete (quick count, unlimited count and cell count). In total, 158 participants took part in the experiment, allowing an analysis of the results of 5,024 counts.
3. Over all field sessions, no observer succeeded in detecting all the individuals in the 10 quadrats. The mean detection rate was 0.44 (ranging from 0.11 to 0.82) for the quick count, 0.59 for the unlimited count (range 0.18–0.87) and 0.74 for the cell count (range 0.46–0.94).

4. Detection probability increased with the conspicuousness of the target species and decreased with the density of individuals and habitat closure. The observer's experience in botany had little effect on detection probability, whereas detection was strongly affected by the time observers spent counting. Yet although the more time-consuming methods increased detection probability, none achieved perfect detection, nor did they reduce the effect on detection probability of the variables we measured.

5. *Synthesis.* Our results show that detection is imperfect and highly heterogeneous when counting plants. To avoid biased estimates when assessing the size, temporal or spatial trends of plant populations, plant ecologists should use methods that estimate the detection probability of individuals rather than relying on raw counts.

Keywords: conservation; demography; detectability; field methods; N-mixture; observer effect; plant survey; population monitoring.

Introduction

Population size and the variability of population size over time and space are of central importance in ecology and conservation. These parameters are required to understand ecosystem dynamics (Ripple & Beschta, 2012; Watson & Estes, 2011), assess population viability (Dennis et al., 1991) and trigger conservation policies and actions (e.g. IUCN, 2019). Population size estimates largely rely on counts of individuals, generally made in spatial units such as quadrats or sites (Elphick, 2008; Yoccoz et al., 2001). Therefore, the accuracy of these counts is crucial, as counting errors would result in biased estimates of population sizes and trends.

When carrying out counts, observers can miss some individuals, in effect counting fewer individuals than were present in the sampling units. This phenomenon of imperfect detection, well known in animal studies, leads to underestimating population sizes (Williams et al., 2002). If detection is imperfect but constant over time and space, raw counts can provide unbiased estimates of population trends or spatial variation (Yoccoz et al., 2001). However, if detection varies over time (e.g. across the sampling season or between years) or space (e.g. between habitats), the raw differences between counts performed at different sampling sessions or in different sampling units are a mixture of differences in abundance and detection (Kéry et al., 2009). For example, a decrease in population size over the years may be hidden as observers improve their ability to detect individuals over time (Kendall et al., 1996). Or one might conclude that there are fewer individuals in closed than in open habitats because they are harder to detect in closed habitats (Buckland et al., 2008). Even differences as small as 4–8% in detection dramatically increase the risk of erroneously

concluding that population size varies over time or space (Archaux et al., 2012).

Since the 1970s, many studies on vertebrates have shown that imperfect detection is the rule and that detection probability tends to vary in time and space. Detection probability depends on a number of factors: the observation process (both the observation method and the survey effort), the visibility of the target species, and the characteristics of its habitat (Kéry et al., 2005; Kéry & Schmidt, 2008; Petitot et al., 2014). Consequently, studies including an estimate of detection probability, which corrects for the bias resulting from imperfect detection, have become increasingly common for vertebrates and, to a lesser extent, invertebrates. Yet, they remain exceptional for plants (Kellner & Swihart, 2014), suggesting that most plant ecologists implicitly assume that all individuals are always detected or that differences in detection probability are negligible. It is true that, unlike most animals, plants are immobile, and observers may thus consider it unlikely to miss individuals if careful sampling is conducted. This assumption led John Harper to make the famous statement 'Plants stand still and wait to be counted', comparing the ease of studying plant and animal populations (Harper, 1977).

Although detection has almost never been investigated in plants at the individual level, some studies have investigated it at the species level, assessing species occurrence in a given area using two main approaches: occupancy or time-to-detection designs. In the first approach, the survey duration is fixed, and the detection probability of the species when surveying quadrats or sites is estimated from repeated surveys. In the second approach, the detection probability is estimated based on the survey time required to detect the species. These studies found that the detection of plant species was almost always imperfect and that detection probability varied depending on ecological and observational variables. For

93 example, species detection varied according to the morphology of the target species (Chen
94 et al., 2009, 2013; Garrard et al., 2013), with conspicuous growth forms and phenological
95 stages raising detection probability. A high density of individuals increased the probability of
96 detecting the species (Bornand et al., 2014), while the closure of the surrounding habitat
97 decreased it (Alexander et al., 2012; Garrard et al., 2008). Species detection probability also
98 increased with survey effort (Chen et al., 2009) and often varied between observers
99 (Archaux et al., 2006; Garrard et al., 2008. Although these studies confirm that detection
100 issues exist when assessing the occurrence of plant species, do these issues affect individual
101 detection to a similar extent?

102 Concerning detection at the individual level, the only studies that have been conducted on
103 plants used marked individuals in so-called 'capture–recapture' designs (Lebreton et al.,
104 1992) or the time-to-detection of individuals (Hauser et al., 2022). These studies found that
105 individual detection was imperfect, although individuals were generally searched for in
106 relatively small, intensively surveyed plots (Alexander et al., 1997; Andrieu et al., 2017; Kéry
107 & Gregg, 2003). In most studies using marked individuals, individual detection probability
108 varied with the phenological stage of individuals and sometimes with habitat closure (Moore
109 et al., 2011; Andrieu et al., 2017). Hauser et al. (2022) showed that flowering individuals
110 were detected faster than nonflowering ones, and that the abundance of individuals of
111 species resembling the target species delayed detection. Observers with recent experience
112 in searching for the target species detected it faster, confirming at the individual level what
113 has been demonstrated at the species level. Therefore, it is of particular concern that
114 individual detection probability has never been estimated for counts of unmarked plants,
115 despite this being one of the most commonly used methods for estimating population sizes

and trends in plant ecology (Elzinga et al., 1998; Reisch et al., 2018). In addition, many studies in plant ecology estimate vital rates by counting unmarked individuals per life stage (e.g. Molano-Flores & Bell, 2012; Giljohann et al., 2017). In theory, data from capture-recapture designs could be used to estimate detection probability and correct estimates from counts of unmarked individuals. To our knowledge, this has never been done, probably because it would be very impractical since both observation processes would have to be strictly equivalent regarding detection and because estimating detection probability directly from counts of unmarked plants is usually less labour–intensive (Royle, 2004).

To assess whether imperfect detection of individuals is indeed the rule in plant counts, we conducted a field experiment in which observers counted plants in 1x1 m quadrats, a commonly used quadrat size (Elzinga et al., 1998; Gauthier et al., 2017, 2019). The experiment covered 30 species and 300 quadrats, and involved 158 observers who made 5,024 counts. Our study had three aims: (i) to estimate the detection probability of individuals in plant counts; (ii) to estimate how detection probability varies in time and space; (iii) to determine if increasing the survey effort results in a higher detection probability, and could allow reaching perfect detection. To meet these objectives, we tested whether detection varies according to ecological variables (species conspicuousness, true population density and surrounding habitat closure) and observational variables (counting method and experience of observers), which are likely to vary over time and space, as well as testing varying degrees of survey effort.

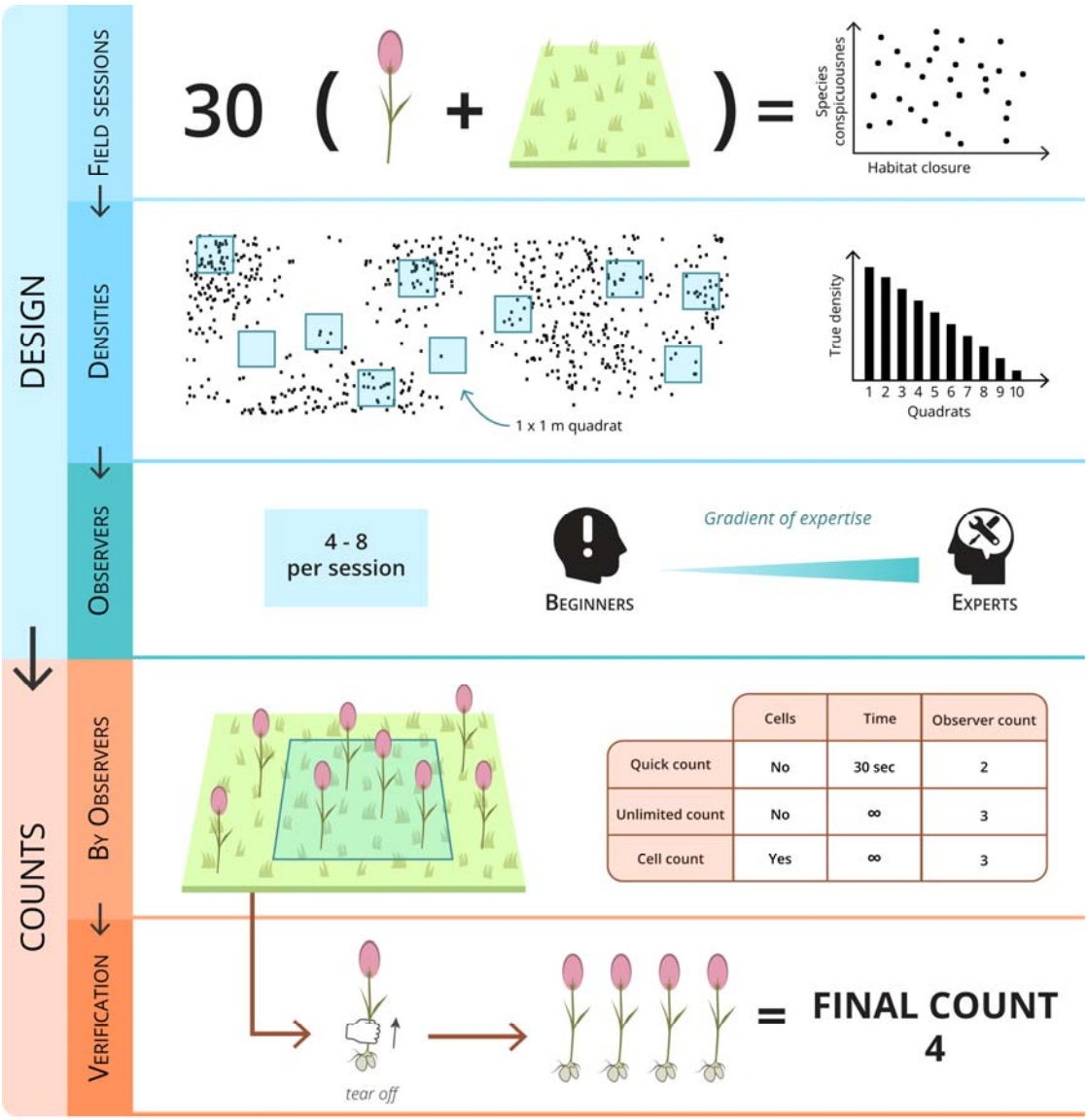
Materials & methods

1 – Selection of sites, species and observers

We selected 30 herbaceous plant species from 24 sites throughout France in Mediterranean, Alpine and temperate climatic regions (Appendix 1). We chose species with various growth forms and phenological stages to cover a gradient from cryptic to conspicuous species. As the experiment focused on counting individuals, we selected only species for which 'individuals' (ramets or genets depending on the species) could be unambiguously differentiated. We avoided species difficult to identify so that all could be identified at first glance without having to check identification criteria on each detected individual. We selected study sites to cover a gradient of habitat closure, from bare ground to dense meadows, to include all possible combinations of species conspicuousness and habitat closure (Fig. 1).

Participants were volunteers selected among our colleagues, researchers, undergraduate students and professionals involved in flora conservation. We selected 4 to 8 observers for each field session, including at least one with several years of field experience in botany, one with no experience in botany, and intermediate profiles. Observers were allowed to participate in up to three sessions, but these sessions had to be several weeks apart. This resulted in 171 participations by 158 unique observers. We conducted the fieldwork from 7 October 2020 to 6 August 2021.

2 – Experiment



	Cells	Time	Observer count
Quick count	No	30 sec	2
Unlimited count	No	∞	3
Cell count	Yes	∞	3

A

B

Figure 1: Diagram of the study's experimental design. We conducted 30 field sessions, each focusing on a different species in a habitat with a different closure level, to cover the widest possible range of combinations of species conspicuousness and habitat closure. For each field session, we placed ten quadrats on the population to form a density gradient and chose 4–8 observers to have a gradient of experience in botany. The observers carried out three counting methods successively (quick, unlimited and cell counts). We then counted the true number of individuals by systematically digging out the individuals detected by the observers and then conducting an additional survey for any individuals that remained unnoticed. Picture A shows the experiment in progress at a Mediterranean site and picture B shows a quadrat with the cells.

Field setup and observer training

The fieldwork consisted of one-day sessions, during which a group of observers counted the number of individuals of a single target species in a habitat at one of the 24 sites. The availability of participants restricted us to organise the field sessions. Whenever we had enough observers available somewhere, we prospected the landscape nearby to find an appropriate site (i.e. easy to access and with a habitat corresponding to what we needed to complete the habitat closure gradient). Then we searched for a suitable species, meeting our general criteria and completing the species conspicuousness gradient. Before the observers' arrival, we placed ten 1x1 m quadrats on the target species population. We chose quadrat locations to ensure that (i) two were free of individuals of the target species (so that observers would not always expect to find individuals in the quadrats), and (ii) they covered a relatively linear gradient of densities up to a maximum of 150 individuals (when possible).

171 It happened regularly that quadrats meant to be empty contained a few individuals and
172 those with the highest densities contained more than 150 individuals. We labelled the
173 quadrats from 1 to 10 and fixed them to the ground. When the observers arrived, we
174 explained how the counts would be conducted and how to identify the target species at all
175 phenological stages and differentiate it from the other species present at the study site. We
176 then trained them to count on additional quadrats to check that everyone had understood
177 the instructions. This training phase lasted until all observers felt comfortable with species
178 identification and the counting methods. The entire training process usually took about an
179 hour.

180 Counting methods

181 Each observer had to count the number of individuals of the target plant in each quadrat
182 using three different methods, requiring a progressively increasing amount of survey time. In
183 the first round, participants had 30 seconds to estimate the number of individuals in each
184 quadrat (the quick count). In the second round, they had to count the number of individuals
185 without any time limit (the unlimited count). We then divided the quadrats into 100 cells of
186 10x10 cm (Fig. 1B), and observers performed the third round, where they had to count the
187 number of individuals in each cell without any time limit (the cell count). This last method
188 forced observers to survey the quadrat homogeneously and has been regularly used in the
189 plant survey literature (e.g. Gauthier et al., 2017, 2019). For the unlimited and cell counts,
190 observers recorded the time they spent counting in each quadrat. To avoid memory effects,
191 after each round we collected the sheets on which the observers recorded their
192 measurements, and observers surveyed the quadrats in a different order, approaching it
193 from a different side at each round. The observers conducted the three counts on the ten

194 quadrats in 1.5 to 5 hours (on average 3.25 hours).

195 Exhaustive count and measurement of covariates

196 After the observers had left, we carried out a final count to determine the true number of
197 individuals present in each quadrat. This was done by searching each 10x10 cm cell for the
198 maximum number of individuals detected by the observers during the third round and
199 collecting them. Once the maximum number of individuals detected by the observers in each
200 cell had been removed, we searched again for any potentially undetected individuals. This
201 final count took between 1 and 7 hours (3 hours on average), depending on habitat closure
202 and the number of individuals of the target species in the quadrats. Despite our verification
203 method, we might have missed a few individuals during the final count, but we will refer to
204 this as the 'true number of individuals' for the sake of simplicity. We calculated the detection
205 probability of individuals by dividing the number of individuals detected by the observers by
206 the true number of individuals present in each quadrat.

207 We measured three variables to investigate their effect on detection probability: an index of
208 species conspicuousness (measured for each field session), an index of habitat closure
209 (measured for each quadrat), and the experience in botany of the observers. For simplicity's
210 sake, we used synthetic indices to characterise species conspicuousness and habitat closure.
211 This allowed us to limit the number of variables and interactions in the model (e.g. species
212 conspicuousness combines the average size of individuals, the presence of vividly coloured
213 flowers, and the interaction of these variables). All the variables and interactions that we
214 investigated in the following analysis are presented in Table 1, as well as the underlying
215 hypotheses. The way we measured the variables is detailed in Appendix 2.

3 – Statistical analysis

In total, 300 quadrats were surveyed during the experiment by on average 5.7 observers, resulting in 5,024 counts. We removed 34 quadrats from the analysis that contained no individuals of the target species, as these do not provide information on detection probability. We withdrew four additional quadrats that unexpectedly contained more than 300 individuals to avoid the leverage effect due to these density outliers (Appendix 3). Keeping these quadrats in the dataset would have led to a reduction in the slope of the density effect, which could result from a nonlinear relationship at high densities, but with only four quadrats, we could not reliably test this hypothesis. In some cases (N = 140, representing 2.8% of the counts), the observers recorded more individuals than were truly present (Appendix 4). We thus carried out the analyses on two datasets, one in which we removed these counts and one in which we set these counts to the true number of individuals. As there was no discernible difference in the results, we present the results of the former analysis in the main text and the latter in Appendix 7. After all filtering steps, 4,319 counts remained in the dataset from which we had removed the observations with excess detections. We also performed the analyses keeping only the experienced botanists to verify that our results were not driven by an excess of novice observers in our sample. Despite some minor differences, our overall results remained unchanged (Appendix 8).

We used a generalised linear mixed model (GLMMs) with a binomial distribution and a logit link function to analyse the correlation between detection probability and the variables we measured and some of their interactions. There could be dependence between detections

238 due to individuals forming clusters. However, observers often detected some but not all
239 individuals within clusters. Furthermore, the aggregation of individuals was not very strong
240 at the quadrat scale. Hence, the dependence between detections is probably relatively
241 small, and we consider the binomial model appropriate. We designed the experiment to
242 disentangle the effects on detection probability of species conspicuousness, habitat closure
243 and survey effort. We had precise hypotheses about the mechanisms explaining these
244 effects (detailed in Table 1) that included interactions between variables, and we collected a
245 large dataset to test all our hypotheses. Therefore, we fitted a single model including all the
246 variables and interactions corresponding to our hypotheses and used their statistical
247 significance for inference. To take into account the repeated measurements at each quadrat,
248 we included random intercepts for the observers, the field sessions and the quadrats (nested
249 within the field session). As the majority of observers participated in only one field session,
250 we did not add a random effect interaction between observers and field sessions.
251 Furthermore, we did not add random effects on the slopes because we had no specific
252 hypothesis that would justify it. We standardised all the variables prior to model fitting and
253 assessed the model's goodness-of-fit using the package DHARMa (Hartig, 2022). We
254 computed the proportion of variance explained by the model and the proportion of variance
255 explained by the model's fixed effects, using the theoretical variance method of Nakagawa
256 et al. (2017). Analyses were performed in R version 3.6.1 (R Core Team, 2019), using
257 packages *lme4* (Bates et al., 2022) to fit the model and *ggeffects* (Lüdecke et al., 2022) to
258 plot the predictions.

259

260 Table 1: Ecological variables (species conspicuousness, habitat closure, true density),

observational variables (counting method, experience in botany, quadrat order, counting time), and interactions between variables tested for their effect on the detection probability of individuals, with our hypothesis about the underlying mechanisms. The measurement method of all variables is detailed in Appendix 2.

Variable	**Description**	**Measurement method**	**Hypothesis**	**Type**	**Observed values**
Species conspicuousness	Conspicuousness of the species at the time of the field session	Expert judgement based on the mean height of individuals and the presence of colourful flowers	Tall and colourful species are easier to detect, which increases their detection probability	Ordinal	1, 2, 3, 4
Habitat closure	Closure of the vegetation in the quadrat	Composite variable created from the vegetation height and the proportion of the area covered by vegetation in the quadrats (Appendix 2)	Dense vegetation masks individuals, which lowers detection probability	Continuous	-1.86–3.54
True density	Number of individuals present in the quadrat	Number of individuals counted in the quadrat by the experimenter at the end of the experiment	High density requires greater concentration and makes it difficult to distinguish between individuals, reducing detection probability	Count	0–295
Counting method	Counting method used	Fixed by the experimental design, each observer successively used the three counting methods on the ten quadrats	Counting methods requiring more survey time yield higher detection probability	Categorical	Quick count, unlimited count, cell count
Experience in botany	General experience in botany of the observer (not specific to the target species)	Self-evaluated by the observers before the experiment using a standardised form	Observers with more experience in botany have higher detection probability	Ordinal	1, 2, 3, 4, 5

Quadrat order	Position of the quadrat in the round	Defined by the experimental design, the order in which the observers investigated the quadrats was fixed in advance and different for each observer and round.	Learning increases detection probability over time, or fatigue decreases it	Continuous	1, 2, 3, …, 10
Counting time	Time spent counting by the observer	Each observer timed the duration of counting	Spending more time increases detection probability	Continuous	Quick count: 0.5 min Unlimited count: 0–11 min Cell count: 0–41.58 min
Species conspicuousness x Habitat closure			Detection probability is less affected by habitat closure for conspicuous species	Interaction term	
Species conspicuousness x Counting method			Detection probability obtained with counting methods requiring more survey effort is less affected by species conspicuousness	Interaction term	
Habitat closure x Counting method			Detection probability obtained with counting methods requiring more survey time is less affected by habitat closure	Interaction term	
Experience in botany x Counting method			The difference in detection probability between experts and beginners is larger for the quick count than for the other two methods	Interaction term	

True density x Counting method			Counting methods requiring more survey time make it easier to distinguish between individuals, which decreases the negative effect of true density	Interaction term	
Quadrat order x Counting method			The effect of fatigue or learning on detection probability is stronger for counting methods requiring more survey time	Interaction term	
Species conspicuousness x Counting time x Counting method		No coefficient is estimated for the quick count as the time was fixed	Spending an additional extra minute further increases detection probability for species that are easy to detect	Interaction term	
Habitat closure x Counting time x Counting method		No coefficient is estimated for the quick count as the time was fixed	Spending an additional extra minute further increases detection probability in sparse vegetation	Interaction term	

265

Results

Over the 30 field sessions, no observer succeeded in detecting all the individuals in all the quadrats. Averaged over all quadrats and observers, the mean detection rate per field session was 0.44 (ranging from 0.11 to 0.82) for the quick count, 0.59 for the unlimited count (from 0.18 to 0.87), and 0.74 for the cell count (from 0.46 to 0.94).

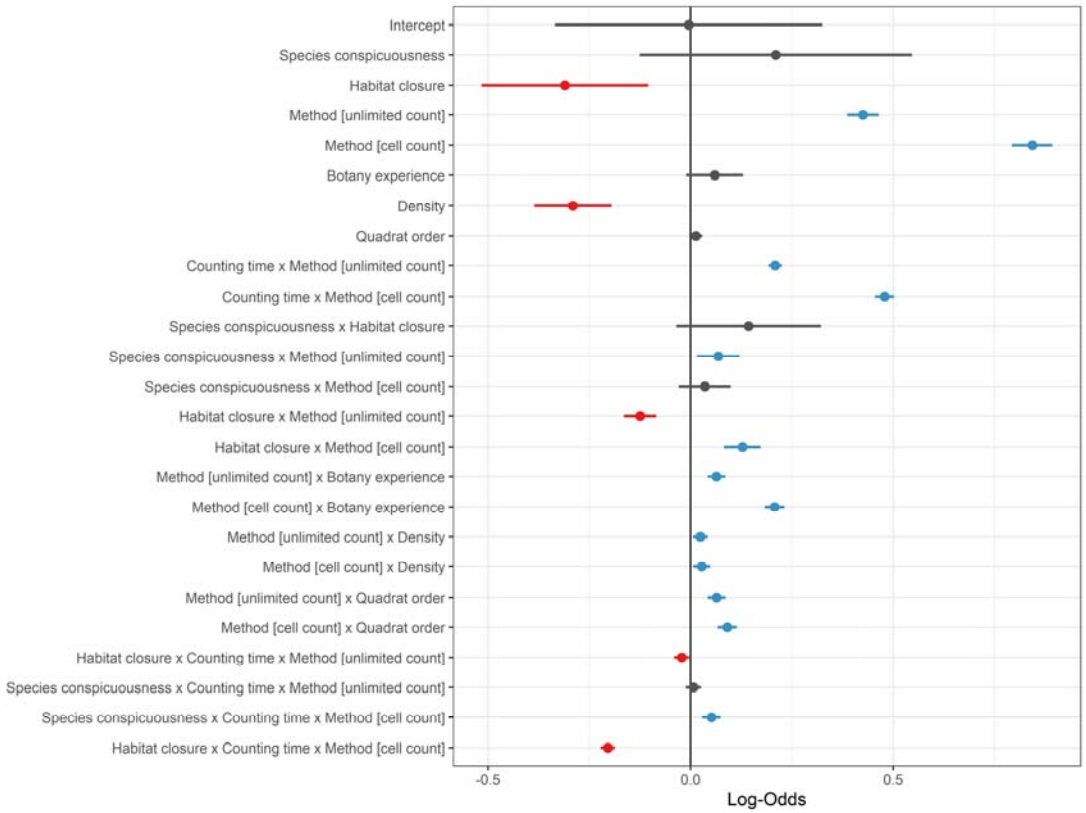


Figure 2: Coefficients of standardised variables in the model. Dots represent estimated coefficients, and lines represent their 95% confidence intervals. Coefficients with a nonsignificant effect are shown in grey. The reference counting method is the quick count, in which observers had 30 seconds to estimate the number of individuals in the quadrat. The coefficients presented for the other two methods indicate the difference (on the logit scale) in mean detection probability obtained with those methods compared to the quick count. The variable 'counting time' appears twice because we manually coded the interaction between the counting time and the counting method, so no slope parameter is estimated for the quick count.

The goodness-of-fit of the fitted model was very good (Appendix 5). All ecological and observational variables were significantly correlated with detection probability in at least some conditions, i.e. for some counting methods or some levels of the other variables (Fig. 2; Appendix 6). Regarding the ecological variables, as expected, the more closed the habitat, the lower the detection probability (Fig. 3). The more conspicuous the species, the higher the detection probability, except in entirely open habitats, where the detection probability was constant and high regardless of the conspicuousness of the species (Fig. 3). Detection probability decreased steeply with the true density, in a similar way for the three counting methods (Fig. 4). Regarding the observational variables, spending more time sharply increased detection probability for the unlimited and cell counts. Habitat closure moderated this trend, i.e. spending an extra minute counting increased detection probability less in closed habitats than in open habitats. Experienced observers achieved slightly higher detection probability than inexperienced observers, and this difference was stronger for the

285 cell count than for the unlimited count. In contrast, there was no significant correlation
286 between experience and detection probability for the quick count. For the unlimited and cell
287 counts, detection probability slightly increased with the position of the quadrat in the round,
288 while there was no correlation for the quick count (Fig. 4).

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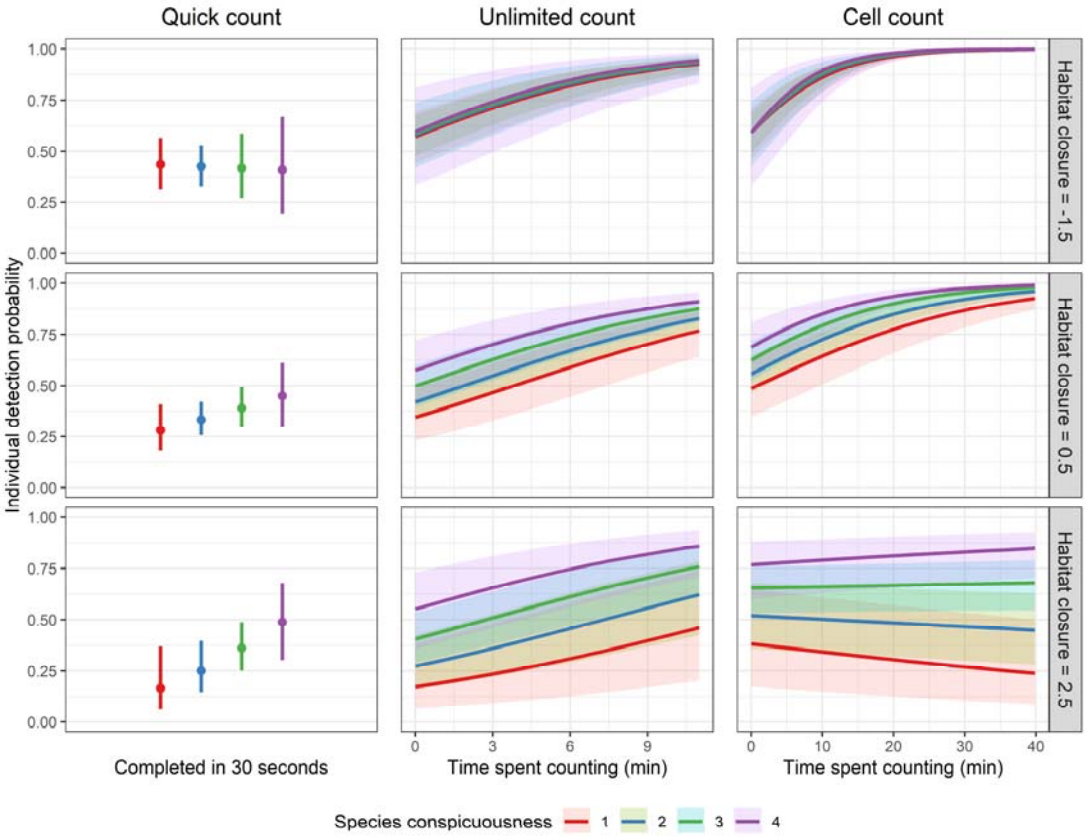

Figure 3: Predicted detection probability depending on species conspicuousness (1 = least conspicuous; 4 = most conspicuous), habitat closure (-1.5 = most open habitats; 2.5 = most closed habitats), the counting method, and the time spent counting. Predictions were made for a quadrat containing 100 individuals of the species of interest, located in the fifth position in the round for an observer who rated his level of experience in botany at 2.5 out of 5. The maximum counting time shown for the unlimited count (11 min) and the cell count (39 min) is the maximum time an observer spent counting on a single quadrat during the experiment. Lines represent the predicted mean detection probability and the shading represents 95% confidence intervals.

290

291 Observers achieved higher detection probabilities with the cell count than with the
292 unlimited count, which yielded higher detection probabilities than with the quick count.
293 However, this came at the cost of a major increase in the time spent counting. The counting
294 time per quadrat was fixed at 30 seconds for the quick count, and the mean counting time
295 averaged over all sessions and observers was 1.8 minutes (ranging from 0.3 to 5.7) for the
296 unlimited count and 8.2 minutes (from 0.9 to 21.6) for the cell count. While the time spent
297 increased, the slope between detection probability and the other variables (e.g. observer
298 experience, vegetation closure) was not reduced. Some variables had roughly the same
299 correlation with detection probability regardless of the counting method (e.g. true density),
300 while others had an even steeper slope (e.g. experience in botany).

301 Within the limits of the counting times we observed during the experiment, the model
302 predicts that reaching a detection probability over 0.95 (i.e. 'almost perfect detection') is
303 only possible by using the cell count for quadrats with a true density below 100 individuals

304 and a counting time per quadrat of at least 20 minutes. In a very open habitat (typically with
305 <7% vegetation cover and median vegetation height <1 cm), this can be achieved no matter
306 the species' conspicuousness. In a less open habitat (e.g. vegetation cover <90%, median
307 vegetation height <6 cm), the target species must be very conspicuous, i.e. a plant above 20
308 cm in height or 10 cm but with colourful flowers.

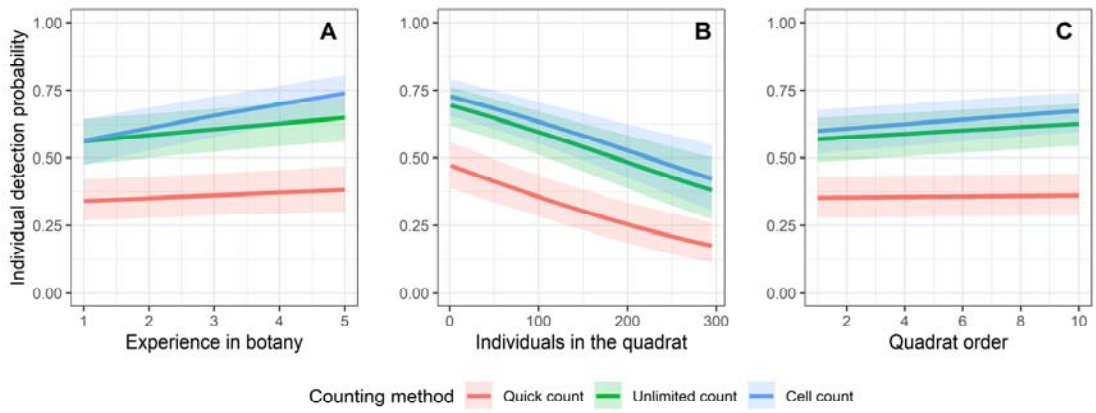

Figure 4: Predicted detection probability of individuals, depending on the experience in botany of the observer (A), the true density (B) and the position of the quadrat in the round (C). The other variables included in the model are held constant at the following levels: species conspicuousness = 2, habitat closure = 0, quick count time = 0.5 min, unlimited count time = 3 min, cell count time = 3 min. Lines represent the mean detection probability of individuals and the shading represents 95% confidence intervals.

309

310 Substantial variation in detection probability between field sessions remained unexplained
311 by the model, even after controlling for species and habitat characteristics, as the variance
312 of this random effect was ${\sigma_{session}}^2 = 0.58$. There was also unexplained variation between
313 quadrats (${\sigma_{quadrat}}^2 = 0.42$). The unexplained between-observer variation was the smallest,

with a variance of ${\sigma_{observer}}^2 = 0.16$. The proportion of variance explained by the model was ${R^2}_{GLMM(c)} = 0.35$, and the proportion of variance explained by the model's fixed effects was ${R^2}_{GLMM(m)} = 0.12$. In other words, detection probability remained heterogeneous even after considering the effect of the seven variables included in the model and accounting for unexplained inter-session, inter-plot and inter-observer variance.

Discussion

This study provides the first estimate of the detection probability of individuals in plant counts, using a detection experiment with a cross-taxa and cross-habitat design. Our results demonstrated that imperfect detection is the rule when counting plants, even in small (1 m²) quadrats with unlimited counting time. Detection probability was also highly heterogeneous and varied with the species, habitat, observer and counting method. Methods involving longer survey time increased the mean detection probability, but did not achieve perfect detection, nor did they reduce the effect of the ecological and observational variables on detection probability. Even after controlling for the effects of all the variables, detection remained highly heterogeneous. Therefore, we argue that plant ecologists should not rely on the use of raw counts and should always take into account detection issues when studying plant populations.

1. Evidence for imperfect detection of plants

In our experiment, while using common plant counting methods (Reisch et al., 2019), not a

335 single observer detected all the individuals present in all the quadrats of a field session. Even
336 with counts unlimited in time, the predicted detection probability under average conditions
337 (i.e. for the average values of all variables included in the model with the unlimited count)
338 did not exceed 0.59 (95% confidence interval: 0.50–0.67). Thus, our results not only confirm
339 that imperfect detection is the rule when counting plant individuals, but also show that
340 detection probability can even be relatively low. Although these values may be surprising,
341 detection issues have been previously documented for plants when searching for a species
342 (e.g. Chen et al., 2013), and a recent study conducted on large quadrats (20x20 m) suggested
343 that imperfect detection may also occur for plant individuals (Hauser et al., 2022).
344 Furthermore, the detection probabilities we found for plants are higher than those
345 commonly found for animals. For example, detection probabilities of 0.04–0.32 have been
346 found in bird point counts (Royle, 2004), 0.07–0.41 for lizards counted along transects (Kéry
347 et al., 2009), 0.06–0.41 for salamanders counted along stream banks (Dodd & Dorazio,
348 2004), and 0.06–0.34 for freshwater mussels searched on riverbeds (Carey et al., 2019).
349 Nevertheless, this does not discount the fact that detection remains far from exhaustive for
350 plants and thus should not be overlooked.

351

352 **2. Effects of the ecological variables**

353 Our results showed that species conspicuousness positively affects detection probability,
354 while habitat closure negatively affects it. This general result was expected, as it has also
355 been observed for plants in occupancy and time-to-detection studies (Alexander et al., 2012;
356 Garrard et al., 2008; Hauser et al., 2022). In open habitats, detection probability was the
357 same regardless of the species' conspicuousness. In contrast, differences between species

358 appeared with increasing habitat closure, reaching up to a 0.42 difference in detection
359 probability between the extreme levels of species conspicuousness in the most closed
360 habitats (estimated difference for the unlimited count with 3 min counting time). It shows
361 that habitat closure drives the detection probability of plant individuals more than species
362 conspicuousness. Furthermore, since both species conspicuousness and habitat closure
363 usually vary over time and space, our results show that heterogeneous detection probability
364 is common and should be considered as the rule rather than the exception. Estimating
365 temporal or spatial trends using raw counts when detection is heterogeneous is likely to
366 yield biased estimates and should therefore be avoided by all means.

367 The negative effect of the density of individuals on detection probability was unexpected in
368 its magnitude (e.g. a predicted detection probability of 0.67 in a quadrat with 20 individuals
369 to 0.37 in a quadrat with 300 individuals, using the unlimited count in average conditions)
370 and occurred regardless of the counting method. Although it would have supported our
371 results, we did not find this phenomenon reported in the literature, even for animal taxa,
372 where density-dependent detection probabilities usually result from changes in individual
373 behaviour in response to density variations (Veech et al., 2016), and not to the observer
374 detecting fewer individuals. In our case, it may be due to the Weber–Fechner law regarding
375 how humans perceive numbers (see Nieder, 2020, for a summary). Indeed, studies on
376 human cognition have shown that it becomes increasingly difficult to estimate the number
377 of items in an area as the number grows, and that there is a general tendency to
378 underestimate it. It is plausible that this phenomenon seldom applies to animal counts, as
379 these do not usually involve counting immobile items in high density, as is the case for
380 plants.

3. Observer effect

The experience in botany of observers had little effect on detection probability compared to the ecological variables and the counting time. In average conditions, the difference in detection probability between novices and the most experienced observers was 0.04 for the quick count, 0.09 for the unlimited count, and 0.17 for the cell count, and confidence intervals largely overlapped except for the cell count (Appendix 6). The advantages of having experience in botany when searching for individuals of a target species may be the ability to more quickly identify an individual once it has been detected, to make fewer identification errors, and possibly to survey the study area more efficiently by knowing where to search for the species (Archaux et al., 2006). These advantages were probably minor in our experiment, since observers were trained to identify the target species before starting counts, all species were easy to identify, and an efficient surveying strategy was of little use in 1x1 m quadrats. In this context, the observers' task can be seen as searching for small objects in a noisy environment, and it seems plausible that their performance was mainly driven by their ability to remain focused during a repetitive task, their motivation, or their fatigue during the experiment. Larger differences between observers might have been observed in larger quadrats, as the surveying strategy might then be important. Overall, it is likely that the variables we studied have a similar effect on the detection probability regardless of quadrat size. In larger quadrats, the observation process has an additional layer of complexity as observers have to walk in the quadrat and might follow different surveying strategies. Furthermore, the differences between observers might have been greater if we had involved participants with recent experience in searching for the target species (Hauser et al., 2022)

404 due to the well-known 'search image' effect, whereby foraging predators improve their
405 probability of detecting the type of prey they are trained to detect (Ishii & Shimada, 2009). If
406 this effect indeed applies to plant counts, population trends estimated from raw counts will
407 be biased if the same observers always make the counts, as their detection probability will
408 increase with time.

409

410 **4. Comparison of the counting methods**

411 As expected, increasing the survey effort, measured by the time spent counting, massively
412 increased detection probability (Archaux et al., 2006; Hauser et al., 2022). In average
413 conditions, the estimated detection probability was 0.36 for the quick count (30 sec counting
414 time), 0.54 for the unlimited count (1.8 min average counting time), and 0.73 for the cell
415 count (8.2 min). In addition, for the unlimited and cell counts, observers spending more time
416 counting achieved a higher detection probability (e.g. for the unlimited count in average
417 conditions, detection probability increased from 0.50 at 1 min to 0.83 at 10 min).

418 The gain in detection associated with counting individuals per cell instead of the whole
419 quadrat could be due to two reasons: the cells force the observer to survey the whole
420 surface area of the quadrat uniformly, and might also reduce uncertainty whether or not an
421 individual has already been counted, which frequently occurs when individuals are close to
422 each other. However, contrary to our expectations, increasing survey effort did not reduce
423 the effect of the ecological variables on detection probability, the most striking being the
424 density of individuals, which had an almost identical effect on detection probability for all
425 three counting methods.

5. Unexplained variance and implications of detection probability heterogeneity

Of the random effects included in the model, the most unexplained variation in detection probability was at the inter-session and inter-quadrat levels. Since we only measured three synthetic variables to characterise the differences between sessions and quadrats (species conspicuousness, habitat closure and true density), we expected that unexplained variance would remain at these levels. For example, we did not measure colour and height heterogeneity, for the habitat or for the target species, although these variables certainly impact detection probability (Garrard et al., 2013; Hauser et al., 2022).

The fact that the unexplained inter-observer variation was the lowest of the random effects is an encouraging result for future studies, as it means that there are few differences in detection probability between observers compared to the differences between species or over a habitat closure gradient. Yet, overall, more than half (65%) of the variance in detection probability remained unexplained by our model. Thus, observers had a heterogeneous detection probability between quadrats even after controlling for several variables, suggesting that factors that vary over time for each observer, such as concentration or motivation, probably explain a large part of the variation in detection probability.

6. Recommendations for counting plants

Our study shows for plants what has been documented many times for animals: that detection probability of individuals is less than one and is usually heterogeneous in space

448 and time, regardless of the counting method and survey effort. Using counts affected by
449 imperfect detection as a proxy for the density of individuals to make comparisons over time
450 and space is only valid under the hypothesis of constant detection. Our findings show that
451 multiple ecological and observational variables cause heterogeneity in detection probability.
452 While observational variables can be standardised, it seems unlikely that ecological variables
453 (i.e. species conspicuousness, habitat closure and true density of individuals) will be constant
454 in any study, even those conducted at limited temporal and spatial scales. Furthermore,
455 trying to achieve almost perfect detection (i.e. >0.95) by increasing survey efforts to fix the
456 issue of detection heterogeneity seems unrealistic given the resource constraints of most
457 ecology and conservation studies. As we studied a wide variety of species and habitats, we
458 trust that our results are transferable to most biomes and species types, provided the
459 scientific context is comparable. The spatial scale must be equivalent in terms of quadrat size
460 (i.e. small enough that it is not necessary to walk inside during the survey) and the studied
461 species must be of a similar life form (e.g. herbaceous species or tree seedlings). We
462 therefore argue that temporal or spatial trends in plant populations should not be estimated
463 using raw counts, as they could then reflect a trend in ecological variables rather than the
464 population's fate.

465 The detection probability depends on the conditions of the observation process and the
466 studied system; thus, it has to be estimated within each study and cannot be extrapolated
467 from our results. Several methods can be used to estimate individual detection probability,
468 and thus estimate unbiased population sizes or trends (e.g. Lebreton et al., 1992; Buckland
469 et al., 2001). In particular, the N-mixture method uses repeated counts of unmarked
470 individuals on sampling units (e.g. quadrats or sites) to estimate detection probability from

471 the differences in the number of individuals detected between counts (Royle, 2004). With
472 this method, estimates of population sizes or trends are more precise if detection probability
473 is high and homogeneous in time and space, if the mean density of individuals is high, and if
474 the sample size, i.e. the number of sampling units and count repetitions, is high (Royle, 2004;
475 Ficetola et al., 2018). Thus, for studies using the N-mixture method, no matter the studied
476 population and the counting method used, we recommend setting a fixed counting time as
477 leaving it to the observer's choice will add heterogeneity to detection probability, resulting
478 in less precise estimates.

479 In terms of allocating survey effort, there are three options: use a more time-consuming
480 counting method to increase detection probability, perform more count repetitions, or
481 survey more sampling units. The only way to be sure of the optimal design is to conduct a
482 pilot study to estimate the mean density of individuals and their detection probability in the
483 population of interest. However, Ficetola et al. (2018) have shown that when the detection
484 probability is above 0.10, the most advantageous strategy is usually to make three count
485 repetitions and to increase the number of sampling units as much as possible. Conversely,
486 when the detection probability is below 0.10, it is better to increase it by using a more time-
487 consuming counting method or to increase the number of count repetitions. Thus, we
488 recommend counting individuals in quadrats without cells with a fixed short counting time
489 (e.g. 0.5–1 min), as this method will yield a detection probability above 0.10 for most species
490 and allow the most sampling units to be surveyed. More time-consuming counting methods
491 should be used only if the study population grows in a very closed habitat and the individuals
492 are inconspicuous, or if travel costs resulting from increasing the number of sampling units is
493 a strong constraint.

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Authors' contributions

J.P., A.B., A.C. and G.P. conceived the study and designed the methodology; J.P. and G.P. organised and conducted the field sessions; J.P. analysed the data and led the writing of the manuscript. All authors critically contributed to the drafts and gave their final approval for publication.

Data availability statement

All data and code used for this work are available from the GitHub repository: https://github.com/JanPerret/Individual_detection_in_plant_counts

Conflict of Interest statement

All authors declare no conflict of interest.

References

Alexander, H. M., Reed, A. W., Kettle, W. D., Slade, N. A., Roels, S. A. B., Collins, C. D., & Salisbury, V. (2012). Detection and Plant Monitoring Programs: Lessons from an Intensive Survey of Asclepias meadii with Five Observers. *Plos One*, *7*(12), e52762. https://doi.org/10.1371/journal.pone.0052762

Alexander, H. M., Slade, N. A., & Kettle, W. D. (1997). Application of mark-recapture models to estimation of the population size of plants. *Ecology*, *78*(4), 1230-1237. https://doi.org/10.1890/0012-9658(1997)078[1230:AOMRMT]2.0.CO;2

Andrieu, E., Besnard, A., Fréville, H., Vaudey, V., Gauthier, P., Thompson, J. D., & Debussche, M. (2017). Population dynamics of Paeonia officinalis in relation to forest closure: From model predictions to practical conservation management. *Biological Conservation*, *215*, 51-60. https://doi.org/10.1016/j.biocon.2017.08.010

Archaux, F., Gosselin, F., Berges, L., & Chevalier, R. (2006). Effects of sampling time, species richness and observer on the exhaustiveness of plant censuses. *Journal of Vegetation Science*, *17*(3), 299-306. https://doi.org/10.1658/1100-9233(2006)017[0299:EOSTSR]2.0.CO;2

Archaux, F., Henry, P.-Y., & Gimenez, O. (2012). When can we ignore the problem of imperfect detection in comparative studies? *Methods in Ecology and Evolution*, *3*(1),

188-194. https://doi.org/10.1111/j.2041-210X.2011.00142.x

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G., Green, P., Fox, J., Bauer, A., & Krivitsky, P. N. (2022). *lme4: Linear Mixed-Effects Models using "Eigen" and S4* (1.1-29).

Bornand, C. N., Kery, M., Bueche, L., & Fischer, M. (2014). Hide-and-seek in vegetation: Time-to-detection is an efficient design for estimating detectability and occurrence. *Methods in Ecology and Evolution*, *5*(5), 433-442. https://doi.org/10.1111/2041-210X.12171

Buckland, S. T., Anderson, D. R., Burnham, K., Laake, J. L., Borchers, D. L., & Thomas, L. (2001). *Introduction to Distance Sampling: Estimating Abundance of Biological Populations*. Oxford University Press.

Buckland, S. T., Marsden, S. J., & Green, R. E. (2008). Estimating bird abundance: Making methods work. *Bird Conservation International*, *18*(S1), S91-S108. https://doi.org/10.1017/S0959270908000294

Carey, C. S., Jones, J. W., Butler, R. S., Kelly, M. J., & Hallerman, E. M. (2019). A Comparison of Systematic Quadrat and Capture-Mark-Recapture Sampling Designs for Assessing Freshwater Mussel Populations. *Diversity*, *11*(8), 127. https://doi.org/10.3390/d11080127

Chen, G., Kery, M., Plattner, M., Ma, K., & Gardner, B. (2013). Imperfect detection is the rule rather than the exception in plant distribution studies. *Journal of Ecology*, *101*(1), 183-191. https://doi.org/10.1111/1365-2745.12021

Chen, G., Kery, M., Zhang, J., & Ma, K. (2009). Factors affecting detection probability in plant distribution studies. *Journal of Ecology*, *97*(6), 1383-1389. https://doi.org/10.1111/j.1365-2745.2009.01560.x

Dennis, B., Munholland, P. L., & Scott, J. M. (1991). Estimation of Growth and Extinction

Parameters for Endangered Species. *Ecological Monographs*, *61*(2), 115-143. https://doi.org/10.2307/1943004

Dodd, C. K., & Dorazio, R. M. (2004). Using counts to simultaneously estimate abundance and detection probabilities in a salamander community. *Herpetologica*, *60*(4), 468-478. https://doi.org/10.1655/03-60

Elphick, C. S. (2008). How you count counts: The importance of methods research in applied ecology. *Journal of Applied Ecology*, *45*(5), 1313-1320. https://doi.org/10.1111/j.1365-2664.2008.01545.x

Elzinga, C. L., & Salzer, D. W. (1998). Measuring & Monitoring Plant Populations. U.S. Department of the Interior, Bureau of Land Management.

Ficetola, G. F., Romano, A., Salvidio, S., & Sindaco, R. (2018). Optimizing monitoring schemes to detect trends in abundance over broad scales. *Animal Conservation*, *21*(3), 221-231. https://doi.org/10.1111/acv.12356

Garrard, G. E., Bekessy, S. A., McCarthy, M. A., & Wintle, B. A. (2008). When have we looked hard enough? A novel method for setting minimum survey effort protocols for flora surveys. *Austral Ecology*, *33*(8), 986-998. https://doi.org/10.1111/j.1442-9993.2008.01869.x

Garrard, G. E., McCarthy, M. A., Williams, N. S. G., Bekessy, S. A., & Wintle, B. A. (2013). A general model of detectability using species traits. *Methods in Ecology and Evolution*, *4*(1), 45-52. https://doi.org/10.1111/j.2041-210x.2012.00257.x

Gauthier, P., Pons, V., Fisogni, A., Murru, V., Berjano, R., Dessena, S., Maccioni, A., Chelo, C., de Manincor, N., Doncieux, A., Papuga, G., & Thompson, J. D. (2019). Assessing vulnerability of listed Mediterranean plants based on population monitoring. *Journal for Nature Conservation*, *52*(125758), 1-11. https://doi.org/10.1016/j.jnc.2019.125758

Gauthier, P., Pons, V., Letourneau, A., Klesczewski, M., Papuga, G., & Thompson, J. D. (2017). Combining population monitoring with habitat vulnerability to assess conservation status in populations of rare and endangered plants. *Journal for Nature Conservation*, *37*, 83-95. https://doi.org/10.1016/j.jnc.2017.03.005

Giljohann, K. M., McCarthy, M. A., Keith, D. A., Kelly, L. T., Tozer, M. G., & Regan, T. J. (2017). Interactions between rainfall, fire and herbivory drive resprouter vital rates in a semi-arid ecosystem. *Journal of Ecology*, *105*(6), 1562–1570. https://doi.org/10.1111/1365-2745.12768

Harper, J. L. (1977). *Population Biology of Plants*. Academic Press.

Hauser, C. E., Giljohann, K. M., McCarthy, M. A., Garrard, G. E., Robinson, A. P., Williams, N. S. G., & Moore, J. L. (2022). A field experiment characterizing variable detection rates during plant surveys. *Conservation Biology*, 36(3), e13888. https://doi.org/10.1111/cobi.13888

Ishii, Y., & Shimada, M. (2009). The effect of learning and search images on predator–prey interactions. *Population Ecology*, *52*(1), 27. https://doi.org/10.1007/s10144-009-0185-x

IUCN Standards and Petitions Committee. (2019). *Guidelines for Using the IUCN Red List Categories and Criteria-Version 14*.

Kellner, K. F., & Swihart, R. K. (2014). Accounting for Imperfect Detection in Ecology: A Quantitative Review. *Plos One*, *9*(10), e111436. https://doi.org/10.1371/journal.pone.0111436

Kendall, W. L., Peterjohn, B. G., & Sauer, J. R. (1996). First-Time Observer Effects in the North American Breeding Bird Survey. *The Auk*, *113*(4), 823-829. https://doi.org/10.2307/4088860

Kery, M., Dorazio, R. M., Soldaat, L., van Strien, A., Zuiderwijk, A., & Royle, J. A. (2009). Trend

estimation in populations with imperfect detection. *Journal of Applied Ecology*, *46*(6), 1163-1172. https://doi.org/10.1111/j.1365-2664.2009.01724.x

Kery, M., & Gregg, K. B. (2003). Effects of life-state on detectability in a demographic study of the terrestrial orchid Cleistes bifaria. *Journal of Ecology*, *91*(2), 265-273. https://doi.org/10.1046/j.1365-2745.2003.00759.x

Kery, M., Royle, J. A., & Schmid, H. (2005). Modeling avian abundance from replicated counts using binomial mixture models. *Ecological Applications*, *15*(4), 1450-1461. https://doi.org/10.1890/04-1120

Kery, M., & Schmidt, B. R. (2008). Imperfect detection and its consequences for monitoring for conservation. *Community Ecology*, *9*(2), 207-216. https://doi.org/10.1556/ComEc.9.2008.2.10

Lebreton, J.-D., Burnham, K. P., Clobert, J., & Anderson, D. R. (1992). Modeling Survival and Testing Biological Hypotheses Using Marked Animals: A Unified Approach with Case Studies. *Ecological Monographs*, *62*(1), 67-118. https://doi.org/10.2307/2937171

Lüdecke, D., Aust, F., Crawley, S., & Ben-Shachar, M. S. (2022). *ggeffects: Create Tidy Data Frames of Marginal Effects for "ggplot" from Model Outputs* (1.1.2).

Molano-Flores, B., & Bell, T. J. (2012). Projected population dynamics for a federally endangered plant under different climate change emission scenarios. *Biological Conservation*, *145*(1), 130–138. https://doi.org/10.1016/j.biocon.2011.10.022

Moore, J. L., Hauser, C. E., Bear, J. L., Williams, N. S. G., & McCarthy, M. A. (2011). Estimating detection-effort curves for plants using search experiments. *Ecological Applications*, *21*(2), 601-607. https://doi.org/10.1890/10-0590.1

Nakagawa, S., Johnson, P. C. D., & Schielzeth, H. (2017). The coefficient of determination R2 and intra-class correlation coefficient from generalized linear mixed-effects models

revisited and expanded. *Journal of The Royal Society Interface*, *14*(134), 20170213. https://doi.org/10.1098/rsif.2017.0213

Nieder, A. (2020). The Adaptive Value of Numerical Competence. *Trends in Ecology & Evolution*, *35*(7), 605–617. https://doi.org/10.1016/j.tree.2020.02.009

Petitot, M., Manceau, N., Geniez, P., & Besnard, A. (2014). Optimizing occupancy surveys by maximizing detection probability: Application to amphibian monitoring in the Mediterranean region. *Ecology and Evolution*, *4*(18), 3538-3549. https://doi.org/10.1002/ece3.1207

R Core Team. (2019). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Reisch, C., Schmid, C., & Hartig, F. (2018). A comparison of methods for estimating plant population size. *Biodiversity and Conservation*, *27*(8), 2021-2028. https://doi.org/10.1007/s10531-018-1522-1

Ripple, W. J., & Beschta, R. L. (2012). Trophic cascades in Yellowstone: The first 15 years after wolf reintroduction. *Biological Conservation*, *145*(1), 205-213. https://doi.org/10.1016/j.biocon.2011.11.005

Royle, J. A. (2004). N-Mixture Models for Estimating Population Size from Spatially Replicated Counts. *Biometrics*, *60*(1), 108–115. https://doi.org/10.1111/j.0006-341X.2004.00142.x

Veech, J. A., Ott, J. R., & Troy, J. R. (2016). Intrinsic heterogeneity in detection probability and its effect on N-mixture models. *Methods in Ecology and Evolution*, 7(9), 1019–1028. https://doi.org/10.1111/2041-210X.12566

Watson, J., & Estes, J. A. (2011). Stability, resilience, and phase shifts in rocky subtidal communities along the west coast of Vancouver Island, Canada. *Ecological Monographs*,

81(2), 215-239. https://doi.org/10.1890/10-0262.1

Williams, B. K., Nichols, J. D., & Conroy, M. J. (2002). *Analysis and Management of Animal Populations: Modeling, Estimation, and Decision Making*. Academic Press.

Yoccoz, N. G., Nichols, J. D., & Boulinier, T. (2001). Monitoring of biological diversity in space and time. *Trends in Ecology & Evolution*, *16*(8), 446-453. https://doi.org/10.1016/S0169-5347(01)02205-4