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Title:

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Date:

2017-09-01

Citation:

Guillera-Arroita, G., Lahoz-Monfort, J. J., van Rooyen, A. R., Weeks, A. R. & Tingley, R. (2017). Dealing with false-positive and false-negative errors about species occurrence at multiple levels. *Methods in Ecology and Evolution*, 8 (9), pp.1081-1091. <https://doi.org/10.1111/2041-210X.12743>.

Persistent Link:

<https://hdl.handle.net/11343/216815>

Dealing with false positive and false negative errors about species occurrence at multiple levels

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1 **Summary**

- 2 1. Accurate knowledge of species occurrence is fundamental to a wide variety of ecological, evolutionary,
3 and conservation applications. Assessing the presence or absence of species at sites is often complicated
4 by imperfect detection, with different mechanisms potentially contributing to false negative and/or false
5 positive errors at different sampling stages. Ambiguities in the data mean that estimation of relevant
6 parameters might be confounded unless additional information is available to resolve those uncertainties.
- 7 2. Here we consider the analysis of species detection data with false positive and false negative errors at
8 multiple levels. We develop and examine a two-stage occupancy-detection model for this purpose. We
9 use profile likelihoods for identifiability analysis and estimation, and study the types of additional data
10 required for reliable estimation. We test the model with simulated data, and then analyse data from
11 environmental DNA (eDNA) surveys of four Australian frog species. In our case study, we consider that
12 false positives may arise due to contamination at the water-sample and qPCR-sample levels, whereas
13 false negatives may arise due to eDNA not being captured in a field sample, or due to the sensitivity of
14 lab tests. We augment our eDNA survey data with data from aural surveys and lab calibration
15 experiments.
- 16 3. We demonstrate that the two-stage model with false positive and false negative errors is not identifiable
17 if only survey data prone to false positives are available. At least two sources of extra information are
18 required for reliable estimation (e.g., records from a survey method with unambiguous detections, and a
19 calibration experiment). Alternatively, identifiability can be achieved by setting plausible bounds on
20 false detection rates as prior information in a Bayesian setting. The results of our case study matched our
21 simulations with respect to data requirements, and revealed false positive rates greater than zero for all
22 species.
- 23 4. We provide statistical modelling tools to account for uncertainties in species occurrence survey data
24 when false negatives and false positives could occur at multiple sampling stages. Such data are often
25 needed to support management and policy decisions. Dealing with these uncertainties is relevant for
26 traditional survey methods, but also for promising new techniques, such as eDNA sampling.

27

28 **Keywords (max 10):** detectability, environmental DNA, identifiability, imperfect detection, monitoring,
29 multiple stages, sensitivity, species occupancy, specificity

30 **Introduction**

31 Understanding spatial patterns and drivers of species occurrence is fundamental to a wide variety of
32 ecological, evolutionary, and conservation applications. An important consideration in such applications is
33 that detection of species is often imperfect in wildlife surveys. Occurrence data are likely to include false
34 absence records, as a species may remain undetected in surveys conducted at sites where it is, in fact,
35 present. One way to deal with this type of error is to collect survey data that are informative about a species'
36 detectability, for instance, by conducting repeat surveys at a set of sites, or by collecting times to detection
37 (Fig. 1 in Guillera-Aroita 2016). Given this type of data, statistical models can be built (MacKenzie *et al.*
38 2002; Tyre *et al.* 2003) to estimate separately, and simultaneously, the probability of species presence
39 (hereafter, occupancy), and the probability of detection of a species at sites where it is present (hereafter,
40 detectability). We refer to these models and their extensions as site occupancy-detection models (SODM).

41
42 In addition to false absences, wildlife surveys may also suffer from false positive errors. For instance, such
43 errors can be important in aural surveys (McClintock *et al.* 2010a; Miller *et al.* 2012b), and in surveys based
44 on the detection of environmental DNA (eDNA; Darling & Mahon 2011; Ficetola *et al.* 2015; Lahoz-
45 Monfort, Guillera-Aroita & Tingley 2016). There are a number of approaches to simultaneously account for
46 false positive and false negative errors in species occupancy estimation (Chambert, Miller & Nichols 2015).
47 These approaches are based on the mixture model in Royle & Link (2006), which describes detections as
48 being generated by either true detections at occupied sites, or false detections at unoccupied sites. This model
49 yields two sets of parameter values as solutions, which are indistinguishable unless prior knowledge about
50 them is available. This identifiability issue can be resolved by augmenting the analysis with additional data
51 that directly informs one or more of the parameters (Miller *et al.* 2011). One such approach involves the use,
52 at least at some of the sites, of a second survey method that produces unambiguous detection data (i.e., is not
53 prone to false positives, though false negatives may still be present). These additional data inform the model
54 by unequivocally *confirming* the presence of the species at those sites with unambiguous detections. Another
55 approach is to conduct independent calibration experiments, to inform the model directly about rates of true
56 and/or false detection. For instance, this idea has been recently applied to account for potential false positive
57 errors in citizen science data (Ruiz-Gutiérrez, Hooten & Campbell Grant 2016).

58

59 In their most basic formulation, SODMs assume that a species is always available for detection during
60 surveys conducted at sites that it uses. In some cases, however, the presence or absence of a species at a site
61 may be somewhat dynamic (“random emigration”), which affects the interpretation of the estimates that a
62 standard SODM yields. In such cases (and assuming here there are no false positives), a standard SODM will
63 estimate different quantities as “occupancy” ($\hat{\psi}$) and “detectability” (\hat{p}), depending on the timing of surveys
64 in relation to the movement of the species. For instance, if separate surveys to a site are timed such that the
65 species had the chance to enter or exit the site between visits, then the model will estimate $(\hat{\psi}, \hat{p}) = (\psi, \theta p)$,
66 where we can think of θ as the proportion of time that the species is present at a site that it uses (or how
67 often it is “available” for detection at the site). In this case, the occupancy parameter represents the
68 probability that the species *uses* a site, and the detectability parameter captures the combined effect of the
69 species being available for detection (i.e. present) at the site, and our ability to detect it at a site when it is
70 present. At the other extreme, in which repeat surveys are conducted close in time, the model estimates $(\hat{\psi},$
71 $\hat{p}) = (\psi\theta, p)$. The estimated occupancy now reflects the combined effect of how likely it is that the species
72 uses the site and how often it is present in it, while the detectability parameter only reflects our ability to
73 detect the species once it is available for detection. For cases between these two extremes, parameter
74 interpretation is less clear.

75
76 Multi-stage SODMs have been developed to estimate separately these three components, ψ , θ and p (Nichols
77 *et al.* 2008). To do so, data that allow teasing apart the different components are needed, which requires an
78 extra dimension in terms of replication. For instance, Nichols *et al.* (2008) analysed occurrence of skinks and
79 salamanders at two scales, by applying multiple independent detection methods within each of several
80 survey occasions at each site. Another approach to tease apart ψ , θ and p is to follow a so-called “robust
81 design” (by analogy to mark-recapture studies), in which replication is applied at two time-scales; a number
82 of “primary survey occasions” separated in time are defined and, within each of those occasions, a number of
83 repeat surveys are conducted relatively close in time (“secondary survey occasions”).

84
85 The concept of availability for detection can be expressed in different ways, depending on the type of survey.
86 For example, in the case of eDNA surveys, availability for detection may be interpreted as whether or not a
87 water sample collected at a site where the species is present contains the species’ eDNA. Even if eDNA is

88 indeed captured in a water sample, it is not guaranteed that it will be detected by PCR (i.e., there is a
89 probability of detection p that is less than one). Where eDNA surveys are conducted following a sampling
90 protocol with multiple PCR runs on multiple water samples, these processes can be separated following the
91 methods of Nichols *et al.* (2008). Here, the water samples can be interpreted as primary survey occasions,
92 and the nested PCR assays as secondary survey occasions, in a robust design. Schmidt *et al.* (2013) followed
93 this approach to study the occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in
94 ponds using eDNA. Similarly, Hunter *et al.* (2015) applied this approach to study Burmese python
95 occurrence. In both cases, availability probability in water samples and per-PCR detection probability were
96 less than one.

97
98 A key limitation of previous multi-stage analyses is that they have assumed that data are free from false
99 positive errors. Although this might be a reasonable assumption in some circumstances, numerous studies
100 have illustrated that some types of surveys can be prone to false positive errors (e.g., McClintock *et al.*
101 2010a; Miller *et al.* 2012b; Ruiz-Gutiérrez, Hooten & Campbell Grant 2016). In this paper, we address this
102 limitation, by developing and testing a model for analysing species detection data with false positive and
103 false negative errors at multiple levels. We first provide a probabilistic and hierarchical description of the
104 model. We then study the ambiguities inherent in the survey data, and the types of additional data that can
105 resolve those ambiguities. We illustrate the applicability of the model through a case study, in which we
106 analyse eDNA detection data from a study of four frog species in southern Australia, in combination with
107 detections from aural surveys and data from calibration experiments. We conclude by discussing limitations
108 and potential future extensions of the methods.

109

110 **Model construction and estimation**

111 In this section, we describe the construction of the model and the approach we take to parameter estimation.
112 To start, we consider only data coming from an ambiguous survey method, i.e. data which may contain false
113 positive errors in addition to false negative errors. Without loss of generality, we consider this to be the main
114 survey method in the study, and hence we refer to its probabilistic description as the “core model”. We then
115 explore how the analysis can be augmented with other data sources to resolve structural identifiability issues.
116 In what follows, we assume a two-stage survey protocol (we later discuss its generalization to more than two

117 stages). In our description, we use eDNA surveys as an example, as the analysis of such data is the
118 motivation for model development.

119

120 CORE MODEL

121 We consider a sampling situation in which sites are sampled on a number of secondary survey occasions,
122 which are nested within a number of primary survey occasions (Fig 1a). In eDNA surveys, this could
123 represent several PCR assays conducted on each of several water samples collected at a site. We can
124 conceptualize the probabilistic description of how such data are generated using a tree diagram, with the
125 parameters characterizing the different stages of the detection process shown in the corresponding branches
126 (Fig. 1b). At the highest level, there is a probability ψ that the species (or its eDNA) occupies/uses the site
127 (and therefore a probability $1 - \psi$ that it does not). Where present, there is a probability θ_{11} that the species
128 is available for detection during the primary survey occasion. In our eDNA example, this could represent the
129 probability that eDNA of the species is captured in a water sample. There may also be a chance (θ_{10}) that
130 eDNA ends up in water samples from sites where the species is absent, due to equipment contamination
131 during the sampling or handling process (in the field and/or in the lab). Where the species is available for
132 detection (e.g. eDNA in the water sample), there is a probability p_{11} of indeed detecting it in a secondary
133 survey occasion (e.g. a PCR run detects the species' eDNA). We consider that (false) detection may also
134 happen where the species is not available for detection, with a probability p_{10} . In the eDNA example, this
135 represents cases where the PCR yields a positive result but the water sample did not contain the species'
136 eDNA. In what follows we use the notation above, denoting the probability of presence/detection at the site,
137 primary sampling occasion level, and secondary sampling occasion levels as ψ , θ and p , respectively. We
138 use subscripts to indicate whether the probability of presence/detection is conditional on presence ('11') or
139 absence ('10') at the immediate upper level. For instance, p_{11} is the probability of detecting the species (first
140 '1') given that it was available for detection (second '1').

141

142 Assuming independence between sampling sites and between sampling occasions (primary and secondary),
143 we can write the probabilistic description of the data as follows:

$$\begin{aligned}
144 \quad L = & \prod_{i=1}^S \left[\psi_i \left\{ \prod_{j=1}^{V_i} \left(\theta_{11} \prod_{l=1}^{L_{ij}} p_{11}^{d_{ijl}} (1 - p_{11})^{\bar{d}_{ijl}} + (1 - \theta_{11}) \prod_{l=1}^{L_{ij}} p_{10}^{d_{ijl}} (1 - p_{10})^{\bar{d}_{ijl}} \right) \right\} \right. \\
145 \quad & \left. + (1 - \psi_i) \left\{ \prod_{j=1}^{V_i} \left(\theta_{10} \prod_{l=1}^{L_{ij}} p_{11}^{d_{ijl}} (1 - p_{11})^{\bar{d}_{ijl}} + (1 - \theta_{10}) \prod_{l=1}^{L_{ij}} p_{10}^{d_{ijl}} (1 - p_{10})^{\bar{d}_{ijl}} \right) \right\} \right], \text{ eqn1}
\end{aligned}$$

146 where S is the number of sites surveyed; V_i is the number of primary sampling occasions (e.g. water
147 samples) at site i ; L_{ij} is the number of secondary sampling occasions at site i on primary sampling occasion
148 j ; and d_{ijl} is the detection/non-detection of the species (a 1 or a 0) at site i on secondary sampling occasion l
149 within primary sampling occasion j . The model parameters $(\psi, \theta_{11}, \theta_{10}, p_{11}, p_{10})$ are as described above and
150 in Fig 1b. We use the bar to denote the complement of an event (i.e. if $d_{ijl} = 1$, then $\bar{d}_{ijl} = 0$). This
151 expression (eqn 1) is the likelihood function for the model. Within the maximum-likelihood framework of
152 inference, we can estimate model parameter values with their uncertainty by identifying which parameter
153 values lead to the largest values of the function. For an example of the likelihood function for a particular
154 site detection history see Appendix S1.

155
156 A perhaps more intuitive hierarchical representation of the model is:

```

157   for  $i$  in 1: $S$  {
158        $z_1[i] \sim \text{Bernoulli}(\psi)$ 
159       for  $j$  in 1: $V[i]$  {
160            $z_2[i, j] \sim \text{Bernoulli}(z_1[i] * \theta_{11} + (1 - z_1[i]) * \theta_{10})$ 
161           for  $l$  in 1: $L[i, j]$  {
162                $d[i, j, l] \sim \text{Bernoulli}(z_2[i, j] * p_{11} + (1 - z_2[i, j]) * p_{10})$ 
163           }
164       }
165   }

```

166 Here, z_1 and z_2 represent latent (imperfectly observed) binary states of the system: z_1 indicates whether the
167 species (or its eDNA) occupies or not the site, and z_2 indicates whether the species is available for detection
168 or not during the primary survey period (e.g. whether eDNA is present in the water sample). The binary
169 variable z_2 can take value 1 through two mutually exclusive paths (Fig 1b), and hence the probability
170 governing its distribution is composed of two additive terms: (1) if the species is present at the site ($z_1 = 1$),

171 z_2 takes value 1 with probability θ_{11} ; (2) alternatively, if the species is absent from the site ($z_1 = 0$), then
172 z_2 takes value 1 with probability θ_{10} . Similarly, the description of how detections (d) are generated involves
173 two mutually exclusive paths depending on the state of z_2 . Note that, in the above, data could be summarized
174 by the number of detections per site and primary survey occasion (i.e. $d_{ij} = \sum_l d_{ijl}$) and the third nested
175 loop (for l in $1:L[i,j]$) removed, replacing the Binomial by a Bernoulli distribution. Hereafter, we summarize
176 the data this way for simplicity.

177

178 IDENTIFIABILITY ISSUES AND PARAMETER ESTIMATION

179 The possibility of false positive errors in a single-stage occupancy-detection model leads to two sets of
180 possible solutions that cannot be distinguished from the data alone (Royle & Link 2006). The likelihood
181 function has the same support at (ψ, p_{11}, p_{10}) and at $(1 - \psi, p_{10}, p_{11})$. Imagine that data are generated
182 following the “correct” model structure (i.e. no model violations) with parameters $\psi = 0.3, p_{11} = 0.7$ and
183 $p_{10} = 0.1$. Regardless of the amount of data we collect, our analysis will not be able to distinguish whether
184 $(\psi, p_{11}, p_{10}) = (0.3, 0.7, 0.1)$ or $(\psi, p_{11}, p_{10}) = (0.7, 0.1, 0.7)$.

185

186 Structural identifiability issues extend in the 2-stage setting. By looking at Fig. 1b, we can see that there are
187 ways in which we can relabel the parameters that yield exactly the same tree diagram. For instance, this is
188 achieved by relabelling ψ as $1 - \psi$, and then interchanging θ_{11} and θ_{10} . Two other alternatives are shown in
189 Table 1. Without additional information, there is possible ambiguity about all five model parameters. The
190 symmetries observed in the tree diagram mean that the likelihood function has several peaks of exactly the
191 same height, and therefore that several solutions are a priori equally plausible.

192

193 Standard maximum-likelihood estimation methods often obtain estimates by identifying a single peak in the
194 likelihood function via numerical optimization, and derive associated standard errors around that point using
195 asymptotic approximations. This approach is not appropriate when fitting models that may suffer from
196 identifiability issues, as results may overlook that a set of alternative competing solutions exist. A safer
197 option is to look at *profile likelihood* functions, and derive confidence intervals based on the concept of
198 likelihood-ratio tests (Morgan 2008, pp. 89-94). The profile log-likelihood function for a given parameter ϕ ,
199 is the value of the log-likelihood function for each value of ϕ , maximized with respect to the other

200 parameters. Confidence intervals can be computed by identifying the parameter values for which the profile
201 log-likelihood function takes values within a given range from the maximum, that is, slicing the log-
202 likelihood at a given depth (e.g. within $3.84/2$ units for a 95% confidence interval of a scalar parameter,
203 where 3.84 is the 5% upper point in a chi-square distribution with one degree of freedom). This approach
204 allows handling situations where confidence intervals may include disjoint sections, as it explores the shape
205 of the likelihood surface away from the maximum. Hence it provides a more appropriate way to analyse data
206 that may suffer from identifiability issues, where the likelihood function can be multimodal. Benefits come
207 at the expense of a greater computational burden, as the method requires a range of function optimizations to
208 construct the profile. However, with current processing capabilities, this is no longer a major limitation.

209

210 In this paper, we use profile log-likelihoods for estimation. We plot the functions and compute associated
211 (potentially disjoint) confidence intervals. We use custom-made code written in R (Appendix S2). We
212 construct the profiles in steps of 0.03 (or 0.01 in the frog analyses; see below). At each step, we run the
213 optimization procedure 25 times (30 for the frog analyses) with different starting values (normally
214 distributed around zero, on the logit scale), to ensure that the true maximum in the function is identified. This
215 is important given that multimodalities in the function can result in numerical optimization procedures
216 getting stuck in local maxima.

217

218 To illustrate the structural non-identifiability of the core model, here we analyse a simulated data set with $S =$
219 250 sampling sites, $V = 3$ primary samples per site, and $L = 3$ nested secondary samples. We set $\psi = 0.7$,
220 $\theta_{11} = 0.7$, $\theta_{10} = 0.1$, $p_{11} = 0.3$ and $p_{10} = 0.05$. Despite the relatively large amount of data, the model
221 cannot identify the true parameter values (Fig. 2). The profile likelihoods for parameters θ_{11} and θ_{10} display
222 four maxima, and symmetry with respect to 0.5. These four peaks are around 0.1, 0.3, 0.7 and 0.9, that is, the
223 four values that are confounded for each of those parameters (θ_{10} , $1 - \theta_{11}$, θ_{11} and $1 - \theta_{10}$; Table 1). The
224 profile likelihoods for parameters p_{11} and p_{10} display two maxima, around 0.05 and 0.3; these peaks reflect
225 the fact that the model cannot distinguish whether each of these parameters take the value of the true p_{11} or
226 that of the true p_{10} (Table 1). The profile likelihood function for ψ is broad and quite flat on top, but in this
227 example there is a hint of two maxima (at ψ and $1 - \psi$).

228

229 RESOLVING IDENTIFIABILITY ISSUES: ADDITIONAL INFORMATION

230 To resolve the structural non-identifiability in the core model, additional information is required. In what
231 follows, we evaluate how different data sources can inform the model to resolve identifiability issues, with a
232 focus on the types of data relevant to our case study. We assess this intuitively, by considering which
233 parameter values are informed by each of the proposed data types, and how this can help distinguish the
234 confounded solutions identified in Table 1. We complement this assessment with the analyses of simulated
235 datasets and inspection of the corresponding profile likelihoods. The conclusions reached are summarised in
236 Table 2.

237

238 One way to help resolve identifiability issues induced by false positives is to collect some data with a second
239 survey method that yields unambiguous detections (i.e. a method with no possibility for false positives;
240 Miller *et al.* 2011). This method could be deployed at a subset of the sites. For instance, the unambiguous
241 detections could be collected via trapping, where individuals are handled and the species can be identified
242 unequivocally, or simply by direct observation. Detections for an unambiguous method confirm the presence
243 of the species at some of the sites, and directly inform about the occupancy probability parameter ψ . This
244 information helps rule out two of the four potential solutions in Table 1, as it indicates whether it is more
245 likely that occupancy takes a given value (ψ , solutions 1 & 3), or its complement ($1 - \psi$, solutions 2 & 4).
246 Yet, with that information alone, we cannot fully identify the detection parameters: we still cannot
247 distinguish the parameters θ from their complement, nor p_{11} from p_{10} .

248

249 Another approach that helps resolve identifiability issues is to run calibration experiments that inform about
250 rates of true and/or false detection. For instance, in the context of our eDNA example, a possible calibration
251 experiment is to run PCR blanks with no template DNA, and then record how frequently PCRs yield a
252 positive result. The data obtained directly informs about the parameter p_{10} , and therefore again help rule out
253 two of the four potential solutions. This time we gain knowledge about which pair of solutions has more
254 support: solutions 1 & 2, or solutions 3 & 4 (Table 1). These calibration data, in combination with data from
255 an unambiguous survey method, resolve the structural identifiability of the core model, i.e. with sufficient
256 data, the true values of all five model parameters can be identified.

257

258 A calibration experiment can also be run that encompasses the handling of water samples across stages. Such
259 an experiment could involve collecting blank water samples in the field (e.g., from a site where the species is
260 known to be absent) or filtering and extracting blank water samples in the lab, then recording how many of
261 the PCR assays yield a positive result. Such an experiment does not provide direct information about a single
262 parameter, but instead provides indirect information about θ_{10} , p_{11} and p_{10} . Where the calibration data
263 consist of a single PCR per water sample, they inform about $\theta_{10}p_{11} + (1 - \theta_{10})p_{10}$. This is the combined
264 probability for the two paths that can ultimately lead to a false detection. It can be shown that this probability
265 takes the same value for solutions 1 & 3, and for solutions 2 & 4. Where the calibration experiment is
266 designed with more than one PCR per water sample, the same ambiguity remains, as the calibration data
267 does not allow distinguishing $(\theta_{10}, p_{11}, p_{10})$ from $(1 - \theta_{10}, p_{10}, p_{11})$. Therefore, combining this type of
268 calibration experiment with an unambiguous survey method is not enough to resolve the structural non-
269 identifiability in the core model.

270

271 We verified our expectations by augmenting our simulated dataset with records from an unambiguous
272 method (two replicates per site), a calibration experiment at the water sample level (50 blank water samples,
273 with one PCR per water sample) and a calibration experiment at the PCR level (50 PCR blanks). We ran
274 analyses with different combinations of data (seven in total). Corresponding plots are provided in Appendix
275 S3; the construction of the extended model used for analysis is presented next.

276

277 EXTENDED MODEL

278 We extend the likelihood function of the core model to incorporate the three additional data sources
279 described above, leading to a joint analysis of all of the data. The data from the unambiguous survey method
280 are treated as detections from an independent detection method, as in other multi-method analyses (Miller *et*
281 *al.* 2011). We assume closure in the occupancy status of sites with respect to the unambiguous method, but
282 the model could be extended easily to account for potential random emigration with data collected following
283 a robust design. The calibration data are considered as a completely independent dataset, and hence their
284 contribution to the likelihood is in the form of separate multiplicative terms. The likelihood of this extended
285 model can be written as follows:

$$\begin{aligned}
286 \quad L &= \prod_{i=1}^S \left[\psi_i \left\{ \prod_{j=1}^{V_i} \left(\theta_{11} p_{11}^{d_{ij}} (1 - p_{11})^{L_{ij} - d_{ij}} + (1 - \theta_{11}) p_{10}^{d_{ij}} (1 - p_{10})^{L_{ij} - d_{ij}} \right) \right\} \{ r_{11}^{\delta_i} (1 - r_{11})^{K_i - \delta_i} \} \right. \\
287 \quad &+ (1 - \psi_i) \left\{ \prod_{j=1}^{V_i} \left(\theta_{10} p_{11}^{d_{ij}} (1 - p_{11})^{L_{ij} - d_{ij}} + (1 - \theta_{10}) p_{10}^{d_{ij}} (1 - p_{10})^{L_{ij} - d_{ij}} \right) \right\} \{ 0^{\delta_i} \} \left. \right] \\
288 \quad &\times \prod_{j=1}^{V^{[c1]}} \left(\theta_{10} p_{11}^{d_j^{[c1]}} (1 - p_{11})^{L_j^{[c1]} - d_j^{[c1]}} + (1 - \theta_{10}) p_{10}^{d_j^{[c1]}} (1 - p_{10})^{L_j^{[c1]} - d_j^{[c1]}} \right) \\
289 \quad &\times \left[p_{10}^{d^{[c2]}} (1 - p_{10})^{L^{[c2]} - d^{[c2]}} \right], \quad \text{eqn 2}
\end{aligned}$$

290
291 where, in addition to the notation already defined, we use K_i and δ_i for the number of replicates and
292 detections with the unambiguous method at site i ; r_{11} for the probability of detecting the species using the
293 unambiguous method; $V^{[c1]}$ for the number of trials in the calibration experiment at the water sample level;
294 $L_j^{[c1]}$ and $d_j^{[c1]}$ for the number of PCRs and detections in blank water sample j ; and $L^{[c2]}$ and $d^{[c2]}$ for the
295 number of PCR blanks and detections in the calibration experiment at the PCR level. For simplicity, here the
296 part of the likelihood corresponding to the core model is expressed in terms of number of detections per site
297 and primary survey occasion (i.e., $d_{ij} = \sum_l d_{ijl}$); similarly, the contribution of the unambiguous method is in
298 terms of number of detections per site (δ_i). Note that the unambiguous method does not need to be
299 conducted necessarily at all of the sites (i.e., K_i can be zero in some sites).

300

301 The hierarchical description of the same model is:

```

302   for i in 1:S {
303       z1[i]~Bernoulli(ψ)
304       for j in 1:V[i] {
305           z2[i,j]~Bernoulli(z1[i]*θ11+(1-z1[i])*θ10)
306           d[i,j]~Binomial(L[i,j], z2[i,j]*p11+(1-z2[i,j])*p10)
307       }
308       δ[i]~Binomial(K[i], z1[i]*r11)           # unambiguous method
309   }
310
311   for j in 1:V[c1]{           # calibration experiment 1

```

```

312         z2[c1][j] ~Bernoulli( $\theta_{10}$ )
313         d[c1][j] ~Binomial (L[c1][j], z2[c1][j] * p11 + (1 - z2[c1][j]) * p10)
314     }
315
316     d[c2] ~Binomial (L[c2], p10)      # calibration experiment 2
317

```

318 This is the model that we use for our case study.

319

320 In addition to the above, there are other ways in which information can be included in the model to resolve
321 identifiability issues. For instance, calibration experiments confirming detection at sites where the species is
322 present are possible. Furthermore, where available, prior knowledge about parameter values can be
323 formalized in the form of a prior distribution; analyses can then be conducted within the Bayesian modelling
324 framework to make use of that prior information.

325

326 Once parameter values are estimated, one can compute the probability of species presence at each of the
327 surveyed sites, conditional on the observed data (equivalent to eqn 1 in Lahoz-Monfort et al. 2016). This
328 quantity is computed as Pr(species presence & observed data)/Pr(observed data). We provide code for this
329 purpose, as calculations are somewhat cumbersome (essentially, they involve the first two lines in the
330 likelihood function in our eqn 2).

331

332 **Case study: using eDNA to survey frogs**

333 We applied the multi-stage model to a case study of concurrent eDNA sampling and nocturnal aural surveys
334 for four frog species (Striped Marsh Frog *Limnodynastes peronii*; Spotted Marsh Frog *L. tasmaniensis*,
335 Southern Brown Tree Frog *Litoria ewingii*; Common Froglet *Crinia signifera*) at seven ephemeral roadside
336 drains in suburban Melbourne, Victoria, Australia (see Tingley *et al.* 2015 for a full description of the study
337 area). Sites were surveyed over a five-month period (August–December 2013). Two visits were generally
338 conducted each month, at each site ($n = 67$ surveys in total, about 10 per site). On each visit, the same two
339 observers conducted an aural survey of the site, by listening for calls during three 3-minute time intervals at
340 the beginning, middle, and end of each site (six of the seven sites were 135 m in length). A species was

341 classified as detected during a survey if it was heard calling at least once. Environmental DNA sampling
342 occurred earlier on the same day that aural surveys were conducted. On each visit, three 500-mL water
343 samples were taken from approximately the same locations where the aural surveys were conducted ($n = 182$
344 water samples in total, about 30 per site). The only exception was one site, which was considerably smaller
345 than the others; only a single water sample was taken and only a single aural survey was conducted at that
346 site, on each visit. We ran four quantitative PCRs (qPCRs) on each water sample (see Appendix S4 for
347 details of eDNA analysis). Field sampling protocols are described in Tingley *et al.* (2015) and Smart *et al.*
348 (2015).

349

350 We treated aural surveys as an unambiguous detection method in our analyses. Some aural surveys can be
351 prone to false positive detections (Miller *et al.* 2012b), but this was extremely unlikely in our case study.
352 Only four species were present in the study area, as confirmed by simultaneous trapping and visual surveys
353 (Smart *et al.* 2015; Tingley *et al.* 2015); each species has a unique call; and the same two observers
354 conducted all surveys. We assumed our system to be closed to changes in occupancy over the sampling
355 period, and treated all water samples and call surveys as survey replicates without temporal considerations.

356

357 To estimate the rate of false positives potentially introduced during lab operations, we ran both water sample
358 and qPCR blanks. In the case of the former, 50 samples of distilled water were filtered and extracted in the
359 same manner as field samples. Four qPCRs were run on each blank sample, for each of the four species ($n =$
360 800 qPCRs). For both *C. signifera* and *L. ewingii*, 1/50 water blanks amplified the species DNA (four and
361 two positive qPCRs, respectively). For *L. peronii* and *L. tasmaniensis*, 4/50 and 2/50 water blanks led to
362 detection (six and three qPCRs, respectively). These records were used as calibration data in the model,
363 indirectly informing about θ_{10} , p_{11} and p_{10} . Our case study assumes that water samples were not
364 contaminated with a species' eDNA in the field.

365

366 To control for potential qPCR contamination, we ran 48 qPCR blanks for each of the four species. None of
367 those qPCRs tested positive for the target species' DNA. This rate (0/48) was used as calibration data for p_{10}
368 in the model.

369

370 The results of our case study perfectly matched those of our simulations (Table 3 and Appendix S5).
371 Parameters were unidentifiable when only the uncertain survey data were used (row 1 in Appendix S5
372 figures). Identifiability issues remained when models only included the unambiguous survey method,
373 calibration data from water sample blanks, or calibration data for p_{10} (rows 2-4). Similarly, there was
374 equivalent support for multiple parameter values when only the unambiguous survey method and calibration
375 data from water sample blanks were included in the model (row 5). Identifiability was not an issue, however,
376 when models included the unambiguous survey method in combination with both types of calibration data
377 (row 8; Table 3). Combining the unambiguous survey method with calibration data for p_{10} , or combining
378 both sources of calibration data, also solved identifiability problems in the core model (rows 6-7).

379

380 Final estimates of false positive detection probabilities for each of the four frog species were obtained by
381 incorporating all available data (i.e., unambiguous survey data, and calibration data at the water sample and
382 qPCR level). This analysis revealed that false positive detection probabilities were greater than zero at both
383 levels, for all four frog species (Table 3).

384

385 **Discussion**

386 We have developed a novel extension of the site occupancy-detection modelling framework (Guillera-Arroita
387 2016) that can be used to estimate species occupancy probabilities that account for potential false positive and
388 false negative errors originating at two different sampling stages. Single-stage occupancy models that account
389 for false positive errors (Miller *et al.* 2011), and multi-stage occupancy models that assume no false positives
390 (Nichols *et al.* 2008), are special cases of the model presented here (Fig. 3).

391

392 We have shown that multiple sources of uncertainty in the data lead to ambiguities in estimation where only
393 simple data sets are available. To avoid parameters being confounded, replication at multiple levels is required,
394 as is the collection of additional data that help tease apart alternative solutions that arise due to the symmetries
395 in the likelihood function. We have shown how, in the two-stage scenario, at least two additional data sources
396 are required to resolve the *structural* non-identifiability in the core model. For instance, this can be achieved
397 by including data from an unambiguous detection method and data from a calibration experiment conducted
398 at the lowest sampling level. In contrast, in the single-stage scenario, one source of additional data is enough

399 for the model to be identifiable. Obviously, the degree to which parameter values can be well estimated in
400 practice depends on the amount of data available. *Practical* non-identifiability may remain where survey data
401 are of limited quantity or quality. In the absence of system-specific data, researchers can also address
402 identifiability issues by setting plausible bounds on false positive rates using prior information (e.g., from the
403 literature) in a Bayesian setting.

404

405 Care must be exercised when attempting estimation from models that may suffer from non-identifiability
406 issues. In this study, we showcase the application of profile likelihood confidence intervals as a safe method
407 for estimation in such situations. Standard maximum-likelihood approaches run the risk of overlooking
408 relevant parts of the parameter space, leading to potentially biased estimation and misreporting of associated
409 uncertainty. There is evidence favouring the use of profile likelihood confidence intervals over those based on
410 point estimates in the context of other ecological modelling applications (e.g., Morgan & Freeman 1989;
411 Cormack 1992). We advocate that this approach be used more frequently, given that nowadays computation
412 time is normally not a limiting factor. Profile likelihood confidence intervals are not only useful when the
413 likelihood function has multiple maxima; another more general benefit is that they can capture asymmetries in
414 the likelihood function that are misrepresented by standard symmetric confidence intervals. Where standard
415 maximum-likelihood estimation approaches are used, analyses should be run multiple times using different
416 initial values for the numerical optimization algorithm that searches for the maximum of the function.
417 Assessing whether different runs lead to different estimated parameter values with similar associated values
418 of the likelihood function provides a sound diagnosis of whether the likelihood function is indeed multimodal,
419 and whether other estimation methods would be more appropriate. We have conducted our analyses within the
420 maximum-likelihood framework of inference, but alternatively one could follow a Bayesian approach.
421 Implementation of the model in programs such as BUGS or JAGS (Plummer 2003; Lunn *et al.* 2009) is in
422 principle straightforward, but applying these general-purpose tools can potentially lead to the same inference
423 problems discussed above (Appendix S6). Where the likelihood is strongly multimodal, the MCMC algorithm
424 may get stuck exploring one of the peaks; results may suggest convergence, even when the algorithm has
425 missed important parts of the posterior distribution. Running several chains with different initial values helps
426 diagnose such situations.

427

428 Our case study of frog eDNA surveys illustrates how the model developed here can be used to account for
429 both false negative and false positive errors in eDNA studies. Several eDNA-based occupancy studies have
430 accounted for false negative errors (Schmidt *et al.* 2013; Hunter *et al.* 2015); in contrast, false positive errors
431 have received far less attention (c.f. Ficetola *et al.* 2015; Lahoz-Monfort, Guillera-Arroita & Tingley 2016),
432 despite early recognition that false positives pose a significant threat to the credibility of the technique (Darling
433 & Mahon 2011). False positive errors in eDNA studies can originate from a variety of sources. Our case study
434 accounted for potential eDNA contamination of water samples due to contamination during filtering and DNA
435 extraction in the lab, as well as for potential false positives due to contamination at the qPCR level. To deal
436 with these errors, we complemented our eDNA survey data with field data collected via an unambiguous
437 detection method and information about lab contamination rates (from calibration experiments). An alternative
438 approach to account for false positives in eDNA studies would be to collect calibration data at sites where
439 species are, and are not, known to occur (Chambert, Miller & Nichols 2015). It is important, however, that the
440 sources of additional data considered indeed inform about the same processes defined by the parameters to be
441 estimated. For instance, a calibration experiment at the water sample-level conducted in the lab cannot inform
442 about the rates of water sample contamination in the field. In practice, some sources of potential eDNA false
443 positives that originate in the field will be particularly difficult to account for. Calibrating the probability of a
444 non-target species transferring the DNA of a target species to an unoccupied site, for example, will likely be
445 difficult (if not impossible) in practice. In contrast, calibration experiments in the lab are relatively easy to
446 carry out, and should become standard practice in eDNA studies. Indeed, it was such experiments that led us
447 to develop a refined protocol for water filtration and DNA extraction that is less prone to false positives (use
448 of disposable syringes and fully-encased filter units).

449

450 The potential range of applications of the multi-stage model extend beyond our specific eDNA case study, and
451 include other types of surveys and study objectives. The multi-stage model could, for instance, be applied to
452 estimate disease prevalence, while accounting for test sensitivity and specificity, as well as for the risk of
453 sample contamination and uncertainty associated with the sampling of individuals. Occupancy-detection
454 modelling has been previously advocated as a useful framework for dealing with uncertainty in disease ecology
455 (McClintock *et al.* 2010b), where it has been applied to study malaria prevalence in wild birds (Lachish *et al.*
456 2012) and chytridiomycosis prevalence in amphibians (Miller *et al.* 2012a), under the assumption of no false

457 positive errors. This assumption, however, might not hold, depending on the type of survey, test, and pathogen.
458 Indeed, research incorporating false positive errors has been identified as an important theme for disease
459 ecology (McClintock *et al.* 2010b). Our model provides a useful step in that direction.

460

461 The ideas presented here could also prove particularly useful for aural surveys of bird species. Temporary
462 random emigration is a common feature of bird surveys, and some researchers have followed a “robust design”
463 to account for it (Nichols *et al.* 2008). Surveys based on aural detections are, however, also prone to false
464 positives, even when conducted by trained surveyors (Miller *et al.* 2012b). The work in this paper thus opens
465 a promising avenue for the analysis of this type of data. For this setting, the structure would need some
466 adjustment. For instance, it would make sense to fix $\theta_{10} = 0$. In our eDNA example, this parameter reflects
467 the probability that a water sample becomes contaminated, that is, the chance that the “species” is present
468 during a primary sampling occasion at a site where the species does not really occur. In contrast, in a bird
469 survey, the species cannot be available for detection at a site where it is absent. Misidentification of other
470 species are possible, and those events could be captured with parameter p_{10} . This simplification of the model
471 structure implies that there are fewer ambiguities in the data. In fact, we believe that in this case there would
472 not be *structural* identifiability issues, even in the absence of additional data; yet, we expect that *practical*
473 identifiability issues are likely to remain, and that robust estimation will indeed still require some additional
474 information. Investigating and applying this modelling approach for bird survey data is an interesting area of
475 future research.

476

477 The work presented here could be extended in other ways. Our code could be used to simulate data and
478 investigate trade-offs in survey design. It is currently unclear, for instance, how best to distribute a fixed survey
479 budget between an ambiguous method, and an unambiguous but costlier or less sensitive survey method. Little
480 guidance is available, and previous recommendations do not reflect the role that unambiguous detections play
481 in resolving identifiability issues (Clement 2016). In addition, with trivial adjustments, our code for estimation
482 can be applied to studies where other arrangements of additional data sources are used to augment ambiguous
483 survey records; for instance, where more than two types of unambiguous detection methods are used, or where
484 data can be classified in different detection states based on their certainty (Miller *et al.* 2011). Future
485 applications of the model could also usefully explore the consequences of disregarding the two-stage model

486 structure presented here, as well as the value of learning about false positive rates in different contexts. In some
487 cases, incorporating effects of covariates on model parameters may provide useful insight into the drivers of
488 false positive and false negative errors, as well as about the drivers of species occurrence.

489

490 Finally, we have focused on studies where sampling is arranged at two levels, but the same principles could
491 be applied to modelling survey data that are collected at three or more levels. Incorporation of additional
492 sampling levels (with their corresponding potential for errors) will increase identifiability issues in the model;
493 therefore, more sources of additional data, informative at different levels, are likely to be required to resolve
494 estimation problems in such cases. Our work showcases how to approach such modelling challenges, in terms
495 of (i) studying and visualising identifiability issues in the model (e.g. by detecting symmetries in the likelihood
496 through tree diagrams and/or plotting of profile likelihoods), and consequently (ii) assessing what forms of
497 additional data can help resolve structural uncertainties. As models become more sophisticated, more care and
498 data are required to make sure that estimation is indeed meaningful. Importantly, these modelling
499 developments should not overshadow the importance of careful survey design and reliable data collection
500 protocols. It is advisable to reduce potential errors during data collection (e.g., by training surveyors and
501 keeping a high standard in laboratory procedures), rather than simply dealing with them later via modelling.

502

503

504 **Acknowledgements**

505 We thank Adam Smart for his contribution to the case study. Museum Victoria kindly provided tissue
506 samples that were used in the case study. G. Guillera-Aroita is the recipient of a Discovery Early Career
507 Research Award from the Australian Research Council (DE160100904). R. Tingley and A.R. Weeks are
508 supported by the Australian Research Council Linkage Scheme (LP140100731). J. Lahoz-Monfort receives
509 support from an Australian Research Council Discovery Project (DP160101003).

510

511 **Author Contributions statement**

512 GGA and JJLM conceived the idea and conducted the statistical developments; GGA wrote the code, ran
513 simulations and led the writing of the manuscript; JJLM and RT contributed critically to the drafts; RT

514 collected and analysed the survey data; ARW and AvR conducted eDNA extraction and analyses; All
515 authors gave final approval for publication.

516

517 **Supporting information**

518 Appendix S1: Example of likelihood construction with annotated explanations

519 Appendix S2: Data, R code for parameter estimation (based on profile likelihoods), and guided examples

520 Appendix S3: Additional plots (simulated data analysis)

521 Appendix S4: eDNA analyses

522 Appendix S5: Additional plots (survey data analysis)

523 Appendix S6: JAGs code and issues with Bayesian analyses

524

525 **Data accessibility**

526 All data used in this manuscript are provided as Supporting information.

527

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594

595

Figures

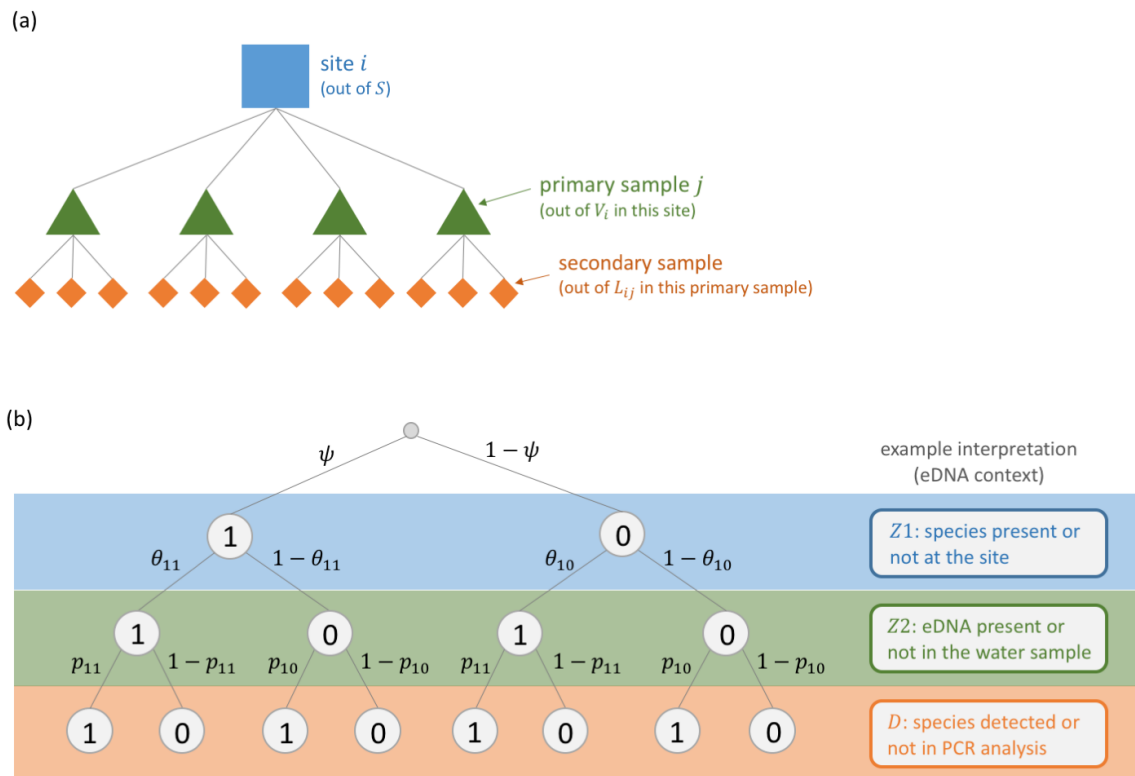


Figure 1 Sampling protocol, latent states and parameters: (a) a sampling situation in which a number of secondary survey occasions (orange diamonds) are embedded within a number of primary survey occasions (green triangles); (b) tree diagram representing data generation, underlying latent states, and associated parameters; the latter are the probabilities of the events indicated by the corresponding branches, e.g. θ_{11} is the probability that eDNA material is present in a water sample that comes from a site where the species was indeed present.

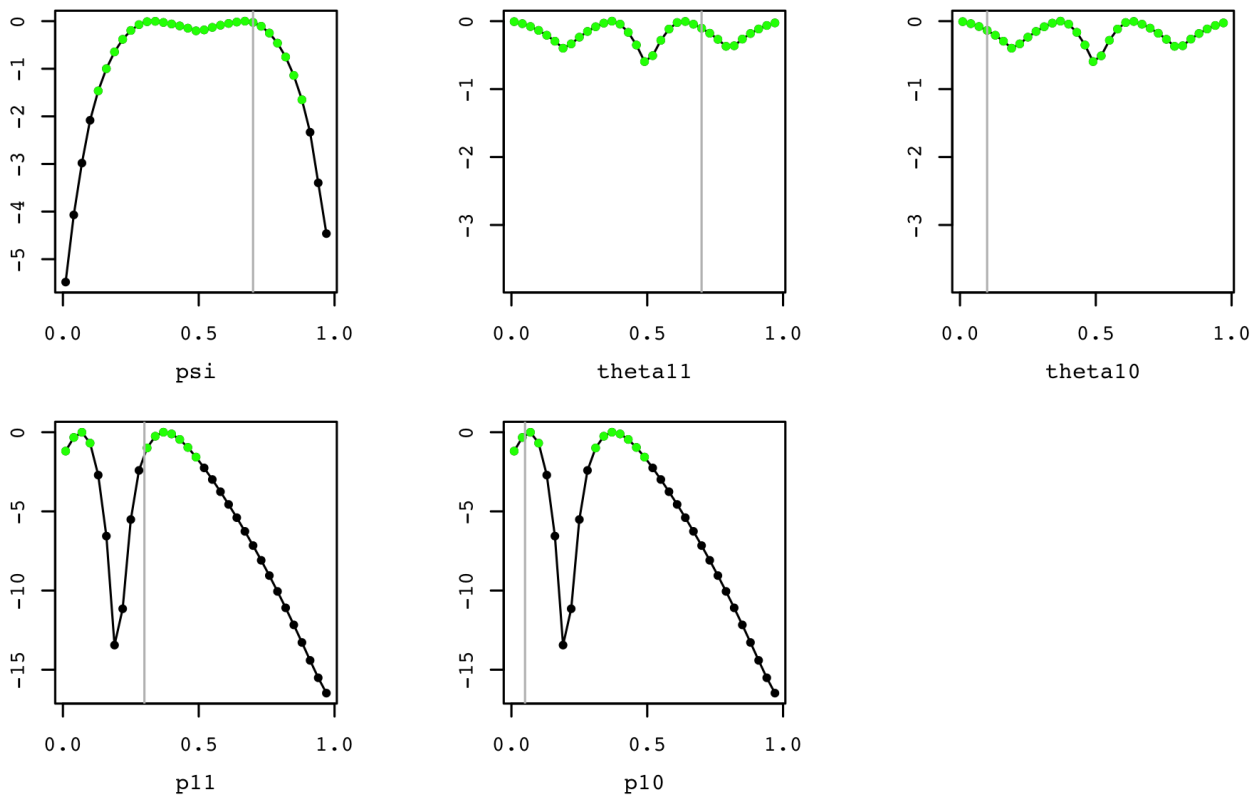


Figure 2 Structural non-identifiability in the core model. The plots show the profile log-likelihood functions for each of the five model parameters (as defined in Fig 1b), for a simulated dataset with 250 sites, 3 water samples per site, and 3 PCR runs per water sample. The true parameters values used in the simulation are indicated by the grey vertical line. The region of parameter values indicated by the green dots correspond to 95% (profile likelihood) confidence intervals.

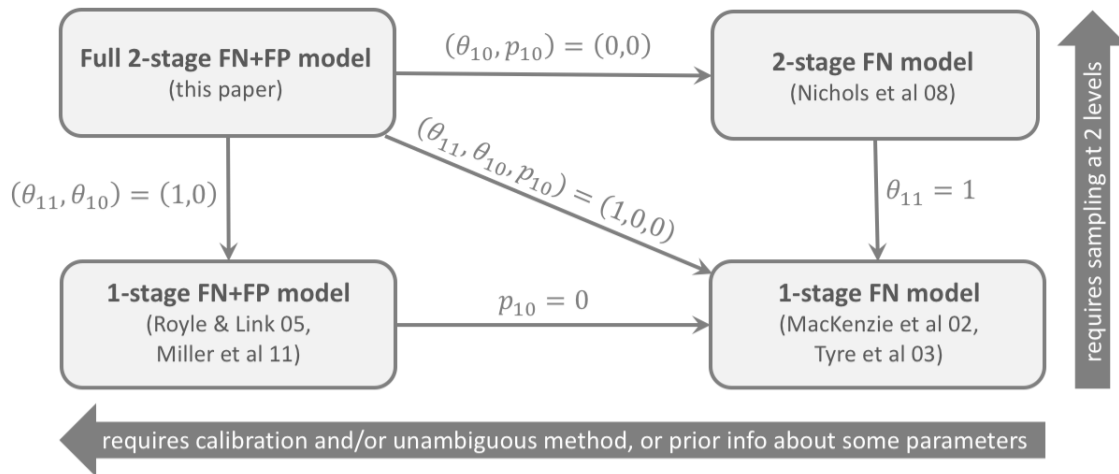


Figure 3 Other models as special cases of the general model described in this paper. Parameter names are as defined in Figure 1.

Tables

Table 1 Solutions that have the same support in the core model.

(1)	ψ	θ_{11}	θ_{10}	p_{11}	p_{10}
(2)	$1 - \psi$	θ_{10}	θ_{11}	p_{11}	p_{10}
(3)	ψ	$1 - \theta_{11}$	$1 - \theta_{10}$	p_{10}	p_{11}
(4)	$1 - \psi$	$1 - \theta_{10}$	$1 - \theta_{11}$	p_{10}	p_{11}

Table 2 Summary of how additional data sources help resolve the structural identifiability problems of the core model.

additional data source	informs about	ambiguity that remains [^]	parameters confounded*	example plot
U	ψ	(1)(3) or (2)(4)	$\theta_{11}, \theta_{10}, p_{11}, p_{10}$	Fig S3.1
C1	$\theta_{10}p_{11} + (1 - \theta_{10})p_{10}$	(1)(3) or (2)(4)	$\theta_{11}, \theta_{10}, p_{11}, p_{10}$	Fig S3.2
C2	p_{10}	(1)(2) or (3)(4)	$\psi, \theta_{11}, \theta_{10}$	Fig S3.3
U+C1	$\psi, \theta_{10}p_{11} + (1 - \theta_{10})p_{10}$	(1)(3) or (2)(4)	$\theta_{11}, \theta_{10}, p_{11}, p_{10}$	Fig S3.4
U+C2	ψ, p_{10}	none	none	Fig S3.5
C1+C2	$\theta_{10}p_{11} + (1 - \theta_{10})p_{10}, p_{10}$	none	none	Fig S3.6

U: unambiguous method, C1: calibration experiment of false positive errors across the two stages (water sample level blanks in the eDNA example), C2: calibration experiment of false positive errors at the lower stage (PCR level blanks in the eDNA example); [^] numbers refer to the solutions in Table 1, e.g. (1)(3) or (2)(4) indicates that, of the four initial solutions supported by the core model, the additional data allows narrowing down the estimation to two of them, either the pair (1)(3) or the pair (2)(4) ; *as per Table 1; Note: the above assumes that for C1, one PCR is run per water sample. Nested designs are also possible (i.e. more than one PCR per water sample), and the implications about identifiability still hold.

Table 3. Model parameter estimates for four frog species at seven sites in Melbourne, Australia.

	<i>Crinia signifera</i>		<i>Limnodynastes tasmaniensis</i>		<i>Litoria ewingii</i>		<i>Limnodynastes peronii</i>	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
ψ	0.86	0.51, 1.00	0.71	0.36, 0.94	0.57	0.23, 0.98	1.00	0.76, 1.00
θ_{11}	0.38	0.31, 0.46	0.60	0.51, 0.68	0.44	0.27, 0.54	0.57	0.50, 0.64
θ_{10}	0.01 [^]	0.01 [^] , 0.06	0.10	0.06, 0.16	0.07	0.01 [^] , 0.12	0.01 [^]	0.01 [^] , 0.04
p_{11}	0.86	0.81, 0.91	0.96	0.94, 0.98	0.88	0.82, 0.93	0.95	0.93, 0.97
p_{10}	0.04	0.03, 0.05	0.05	0.04, 0.06	0.03	0.02, 0.04	0.05	0.03, 0.06
r_{11}	0.44	0.32, 0.56	0.47	0.34, 0.60	0.19	0.06, 0.33	0.54	0.42, 0.65

[^]assessment started at 0.01, so these values should be interpreted as ≤ 0.01 .