NeMO: a flexible R package for nested multi-species occupancy modelling and eDNA study optimisation

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ABSTRACT

Biodiversity monitoring using environmental DNA (eDNA) metabarcoding has expanded rapidly in recent years, offering a non-invasive and widely embraced tool among ecologists and stakeholders. However, eDNA surveys are prone to imperfect detection, and non-detections are often misinterpreted as true absences. Despite its critical implications for biodiversity assessments, detection uncertainty remains rarely quantified in eDNA studies. Occupancy modelling provides a powerful tool to address this limitation, yet it remains underused in eDNA applications, in part due to a lack of accessible and flexible tools. To bridge this gap, we developed NeMO (Nested eDNA Metabarcoding Occupancy), a user-friendly R package for fitting multi-species occupancy models in a Bayesian framework. NeMO explicitly accounts for the nested structure of eDNA metabarcoding studies, typically involving multiple replication steps: collecting several eDNA samples per site and running multiple PCR replicates for each sample. It further incorporates sequencing read counts and different PCR pooling strategies. This framework estimates species occupancy, the probability of eDNA collection when present, the probability of eDNA amplification when collected, and the expected sequence read count when amplified. It also enables users to assess how environmental or methodological covariates affect these probabilities. Importantly, NeMO helps estimate the minimum number of eDNA samples, PCR replicates, and sequencing depth needed to confidently confirm species absence, offering a retrospective tool to evaluate and optimise study design. We demonstrate its utility using a published fish biodiversity dataset from the Rhône River (France). NeMO integrates key modelling features into a single streamlined framework, making it broadly accessible for researchers and practitioners, to rigorously assess

detectability and optimise resource allocation in eDNA metabarcoding surveys. Our results highlight the importance of quantifying detection uncertainty, with significant implications for monitoring elusive species and for guiding robust, cost-effective eDNA sampling strategies.

KEY WORDS

Bayesian modelling, detection, elusive species, environmental DNA, false negatives, metabarcoding

DATA AVAILABILITY STATEMENT

No new data were generated for this study. We used a previously published dataset available on Dryad (DOI: 10.5061/dryad.t4n42rr). The R package developed as part of this research, NeMO, is available on GitHub (https://github.com/bastien-mace/NeMO) and CRAN for installation. The package includes a vignette and an example dataset to guide users in its implementation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

SM and EDT conceived the study. BM analyzed the data and drafted the first manuscript. MR and NR provided essential data for the study. All authors contributed to manuscript revisions and approved the final version.

1. INTRODUCTION

Environmental DNA (eDNA) metabarcoding has emerged as a powerful tool for biodiversity monitoring (Blackman *et al.*, 2024). Unlike traditional survey methods, which often rely on direct observations or specimen collection, eDNA metabarcoding enables the detection of a wide range of species from environmental samples such as soil, water, or air (Taberlet *et al.*, 2012). This approach facilitates biodiversity assessments at the community level, providing a comprehensive view of ecosystem composition (Macé *et al.*, 2024; Stat *et al.*, 2017), including elusive species that conventional surveys may overlook (Beng & Corlett, 2020; Bohmann *et al.*, 2014).

Like any biodiversity assessment approach, eDNA metabarcoding can be prone to detection errors (Cristescu & Hebert, 2018; Darling & Mahon, 2011), with false positives (*i.e.*, species detected despite being absent from the sampled environment) and false negatives (*i.e.*, species not detected despite being present in the sampled environment). False positives can result from contaminations, PCR or sequencing errors (Ficetola *et al.*, 2016), or misidentifications due to incomplete or inaccurate reference databases (Polanco F. *et al.*, 2025). To mitigate this, stringent laboratory protocols (Ficetola *et al.*, 2016), including extraction blanks, PCR and sequencing controls, and bioinformatic filtering pipelines (Mathon *et al.*, 2021), are routinely implemented. Various factors contribute to false negatives, such as low DNA concentration (Barnes *et al.*, 2014), PCR primer biases during amplification (Alberdi *et al.*, 2018), or insufficient sampling effort (Ficetola *et al.*, 2015). Yet, unlike false positives, false negatives are often overlooked in eDNA studies. Absence data are frequently interpreted as true absences, without considering the possibility of undetected presence - an oversight that carries significant ecological implications (Biggs *et al.*, 2015; Pinfield *et al.*, 2019). Indeed, undetected species may lead to underestimated invasion dynamics, inadequate conservation efforts, or distorted community composition assessments.

Occupancy modelling offers a statistical framework to account for imperfect detection by estimating both occupancy and detection probabilities (MacKenzie *et al.*, 2006). Originally developed for traditional field surveys (MacKenzie *et al.*, 2002; Royle & Dorazio, 2008) relying on direct or indirect censuses, occupancy models have since been adapted for eDNA-based studies. These typically follow a hierarchical structure, incorporating multiple nested levels of replication (Takahashi *et al.*,

2023). Specifically, the spatial units under investigation (*i.e.*, sites) are generally sampled through different field replicates (*i.e.*, eDNA samples), with each of these analyzed using multiple PCR replicates. Recognising this structure, Schmidt *et al.* (2013) introduced the first nested occupancy model for single-species eDNA studies, estimating occupancy probability alongside detection probabilities at both the eDNA sample and PCR replicate levels. Building on this foundation, Dorazio & Erickson (2018) refined the approach in their `eDNAoccupancy` R package. Although this package improves accessibility and flexibility, the routine application of occupancy models remains limited in eDNA research, especially in multi-species metabarcoding studies, where non-detections are still frequently interpreted as true absences.

To extend the capabilities of existing models, occupancy frameworks tailored to eDNA metabarcoding studies have recently been introduced (**Table 1**), adapting the multi-species model from Dorazio & Royle (2005). While most of these models were made publicly available as text files, they were not initially designed as user-friendly software, which limited their accessibility to the broader scientific community. A major step forward was made with the development of dedicated R packages for multi-species occupancy modelling in eDNA studies. Fukaya *et al.* (2022) and Diana *et al.* (2024) introduced the `occumb` and `eDNAPlus` packages, respectively, both of which also integrating an important methodological advancement, *i.e.*, the use of sequence read counts instead of solely presence/absence data. By incorporating quantitative sequencing information, these models refine detection probability estimates while keeping some limitations: `occumb` does not account for PCR replication while both are not allowing model comparisons. Building upon these advances, we introduce `NeMO` (Nested eDNA Metabarcoding Occupancy), a versatile R package designed to fully capture the complexity of eDNA metabarcoding datasets. `NeMO` accommodates a broad range of data structures, whether sequence read counts are available or only presence/absence data is provided. It also offers flexibility in handling different PCR replication schemes, whether replicates are individually indexed or pooled before sequencing. By integrating these features, `NeMO` enables users to choose the most suitable model based on the structure and requirements of their dataset. Like the previous occupancy modelling packages, `NeMO` allows the inclusion of covariates at multiple levels, enabling to assess how

environmental and methodological factors influence occupancy and detection probabilities. Additionally, we implement a model comparison tool, a feature present in `eDNAoccupancy` but not yet incorporated into multi-species occupancy frameworks. One key application of occupancy models is optimising resource allocation by estimating here the minimum number of eDNA samples and PCR replicates required to confirm species absence, thereby quantifying the level of imperfect detection in a study. `NeMO` facilitates these calculations using equations from McArdle (1990) and extends this functionality by incorporating the option to estimate the minimum sequencing depth required for robust species detection.

To illustrate `NeMO` capabilities, we apply it to an empirical dataset from Pont *et al.* (2018), demonstrating its usefulness for ecological applications. Our goal is to provide an accessible, flexible, and powerful tool for scientists and stakeholders working with eDNA data.

2. MATERIAL & METHODS

2.1. Description of the nested eDNA metabarcoding occupancy model

eDNA-based surveys are typically conducted at multiple sites, with replication achieved by collecting several eDNA samples per site and running multiple PCRs for each sample. eDNA metabarcoding study designs therefore encompass three nested levels: site, eDNA sample, and PCR replicate (**Fig. 1a**). Additionally, high-throughput sequencing (HTS), which is commonly used to sequence eDNA barcodes (Taberlet *et al.*, 2018), allows to generate sequence read counts for each produced amplicon (*i.e.* DNA fragment from a specific target region that has been amplified and sequenced), and ultimately for each species detected, provided that sequences are successfully assigned. Building on this framework, we adapted models proposed in Dorazio & Erickson (2018) and Fukaya *et al.* (2022) to fit multi-species occupancy and estimate parameters related to i) the probability of species eDNA occurrence at a site, ii) the conditional probability of collecting eDNA from a species in a sample from a site, given that its eDNA is present at the site, iii) the conditional probability of amplifying eDNA from a species in a PCR replicate of a sample, given that its eDNA is collected in the sample, and iv) the expected sequence read count generated for the species. To ensure consistency with existing literature, we adhere as closely as

possible to the mathematical notations introduced by Schmidt *et al.* (2013) and Fukaya *et al.* (2022) for occupancy modelling in the context of the nested eDNA framework.

We consider a community of N entities ($N \in \mathbb{N}^*$), referred to as species for convenience, but which may also refer to taxa, operational taxonomic units (OTUs), amplicons, etc. Biodiversity monitoring of this community was repeated over time across C campaigns ($C \in \mathbb{N}^*$), covering I_c sites ($I_c \in \mathbb{N}^*$), where J_i samples ($J_{ic} \in \mathbb{N}^*$) were collected per site and divided into K_{ijc} PCR replicates ($K_{ijc} \in \mathbb{N}^*$). For each PCR replicate, HTS library preparation generated separate sequence reads. In this framework, the occupancy of species $n \in \{1, \ldots, N\}$ at site $i \in \{1, \ldots, I_c\}$ during campaign $c \in \{1, \ldots, C\}$ is represented by the binary latent variable Z_{nic} which indicate whether eDNA from the species is present ($Z_{nic} = 1$) or absent ($Z_{nic} = 0$). This follows a Bernoulli distribution:

$$Z_{nic} \sim Bernoulli(\psi_{nic}) \tag{1}$$

where $\psi_{nic} \in [0,1]$ is the probability that eDNA from species n being present at site i during campaign c. The detection of species n in sample $j \in \{1, \ldots, J_{ic}\}$ from site i during campaign c is represented by the binary latent variable A_{nijc} which indicate whether eDNA from the species is collected ($A_{nijc} = 1$) or not ($A_{nijc} = 0$). This also follows a Bernoulli distribution, conditional on occupancy:

$$A_{nijc} | Z_{nic} \sim Bernoulli\left(Z_{nic} \cdot \theta_{nijc}\right) \tag{2}$$

where $\theta_{nijc} \in [0,1]$ is the conditional probability of collecting eDNA from species n in sample j from site i during campaign c, given that species' eDNA is present at site i. The detection of species n in PCR replicate $k \in \{1, \ldots, K_{ijc}\}$ from sample j from site i during campaign c is represented by the binary latent variable W_{nijkc} which indicate whether eDNA from the species is amplified ($W_{nijkc} = 1$) or not ($W_{nijkc} = 0$). This also follows a Bernoulli distribution, conditional on eDNA collection:

$$W_{nijkc} | A_{nijc} \sim Bernoulli\left(A_{nijc} \cdot p_{nijkc}\right) \tag{3}$$

where $p_{nijkc} \in [0,1]$ is the conditional probability of amplifying eDNA from species n in PCR replicate k from sample j from site i during campaign c, given that species' eDNA was collected in sample j. The sequence read counts resulting from HTS are observed variable Y_{nijkc}. These counts are conditional on the per-PCR replicate total sequence read count $M_{ijkc} \in \mathbb{N}$ hereafter referred to as 'sequencing depth' for simplicity. The sequencing depth is defined as:

$$M_{ijkc} = \sum_{n=1}^{N} Y_{nijkc} \tag{4}$$

For i, j, k, c where $M_{ijkc} > 0$, the sequence read counts Y_{nijkc} follows a multinomial distribution, conditional on eDNA amplification:

$$Y_{nijkc} | W_{nijkc} \sim Multinomial(\pi_{nijkc}, M_{ijkc}) \tag{5}$$

where $\pi_{nijkc} \in [0,1]$ is the conditional probability of assigning a sequencing read to species n in PCR replicate k from sample j from site i during campaign c, given that species' eDNA was amplified in PCR replicate k. This parameter expresses the relative frequency of the sequence read count for species n under these conditions. It is defined as:

$$\pi_{nijkc} = \frac{W_{nijkc} \cdot S_{nijkc}}{max(1, \sum_{m=1}^{N} W_{mijkc} \cdot S_{mijkc})} \tag{6}$$

where the latent variable $S_{nijkc} \in \mathbb{N}$ is the sequence read count for species n in PCR replicate k from sample j from site i during campaign c, and W_{nijkc} is the amplification detection indicator for the same replicate. The denominator ensures normalisation of probabilities and accounts for cases where the product $W_{mijkc} \cdot S_{mijkc}$ is null (*e.g.*, no amplification or zero read count for all species in the replicate). In such cases, the denominator defaults to 1. To accommodate the discrete, overdispersed, and zero-inflated nature of HTS data, we model the latent sequence read count S_{nijkc} using a zero-inflated negative binomial (ZINB) distribution:

$$ZI_{nijkc} \sim Bernoulli(\varepsilon_{nijkc}) \tag{7.1}$$

$$S_{nijkc} \sim NB\left(\frac{\delta}{\delta + \varphi_{nijkc} \cdot (1 - ZI_{nijkc})}, \delta\right) \tag{7.2}$$

where $\varphi_{nijkc} \in \mathbb{N}$ is the expected sequence read count for species n in PCR replicate k from sample j from site i during campaign c, δ is the dispersion parameter controlling the variance of the distribution, and $\varepsilon_{nijkc} \in [0,1]$ is the zero-inflation parameter. In this formulation, each count is generated from a two-part process. First, a Bernoulli trial with success probability ε_{nijkc} determines whether the observation is a structural zero ($ZI_{nijkc} = 1 \Rightarrow S_{nijkc} = 0$). Then, if the observation is not structurally zero ($ZI_{nijkc} = 0$), the count is drawn from a negative binomial distribution.

The zero-inflation parameter ε_{nijkc} follows a Beta prior:

$$\varepsilon_{nijkc} \sim \mathrm{B}(1,1) \tag{8}$$

and the dispersion parameter δ follows a Gamma prior:

$$\delta \sim \Gamma(\rho, \lambda) \tag{9}$$

where $\rho > 0$ and $\lambda > 0$ are user-defined shape and rate hyperparameters, respectively.

The framework of this occupancy model is summarised in **Fig. 1b.**

The species-level parameters ψ_{nic}, θ_{nijc}, p_{nijkc}, and φ_{nijkc} are modeled as random effects. Each parameter includes an intercept term α and slope terms β associated with covariates that can be incorporated into the model. The linear predictors for these parameters are expressed as follows:

$$logit(\psi_{nic}) = \alpha_{\psi_n} + \sum_{d=1}^{D} \beta_{\psi_{sp_d}} \cdot cov_{\psi_{sp_{nd}}} + \sum_{h=1}^{H} \beta_{\psi_{nh}} \cdot cov_{\psi_{ich}} \tag{10.1}$$

$$logit(\theta_{nijc}) = \alpha_{\theta_n} + \sum_{e=1}^{E} \beta_{\theta_{sp_e}} \cdot cov_{\theta_{sp_{ne}}} + \sum_{l=1}^{L} \beta_{\theta_{nl}} \cdot cov_{\theta_{ijcl}} \tag{10.2}$$

$$logit(p_{nijkc}) = \alpha_{p_n} + \sum_{f=1}^{F} \beta_{p_{sp_f}} \cdot cov_{p_{sp_{nf}}} + \sum_{r=1}^{R} \beta_{p_{nr}} \cdot cov_{p_{ijkcr}} \tag{10.3}$$

$$ln(\varphi_{nijkc}) = \alpha_{\varphi_n} + \sum_{g=1}^{G} \beta_{\varphi_{sp_g}} \cdot cov_{\varphi_{sp_{ng}}} + \sum_{u=1}^{U} \beta_{\varphi_{nu}} \cdot cov_{\varphi_{ijkcu}} \tag{10.4}$$

Here:

- $cov_{\psi_{sp_{nd}}}$, $cov_{\theta_{sp_{ne}}}$, $cov_{p_{sp_{nf}}}$, and $cov_{\varphi_{sp_{ng}}}$ are Boolean or standardised values of covariates associated with species n.
- $cov_{\psi_{ich}}$, $cov_{\theta_{ijcl}}$, $cov_{p_{ijkcr}}$, and $cov_{\varphi_{ijkcu}}$ are Boolean or standardised values of covariates associated with spatial, methodological, or temporal components (*e.g.*, site i, sample j, replicate k, campaign c).
- D, E, F, and G are the numbers of species-level covariates where $(D, E, F, G) \in \mathbb{N}^4$, while H, L, R, and U are the numbers of spatial, methodological, or temporal covariates where $(H, L, R, U) \in \mathbb{N}^4$.

The slope terms of covariates associated with species follow normal priors:

$$\beta_{\psi_{sp.}} \sim \mathcal{N}(0, \tau \mathbf{I}_D), \qquad \beta_{\theta_{sp.}} \sim \mathcal{N}(0, \tau \mathbf{I}_E), \qquad \beta_{p_{sp.}} \sim \mathcal{N}(0, \tau \mathbf{I}_F), \qquad \beta_{\varphi_{sp.}} \sim \mathcal{N}(0, \tau \mathbf{I}_G) \tag{11}$$

where $\tau > 0$ is the hyperparameter controlling the precisions. The intercepts and slope terms associated with spatial/methodological/temporal covariates follow a multivariate normal community-level prior:

$$\begin{bmatrix} \alpha_{\psi.} \\ \beta_{\psi..} \\ \alpha_{\theta.} \\ \beta_{\theta..} \\ \alpha_{p.} \\ \beta_{p..} \\ \alpha_{\varphi.} \\ \beta_{\varphi..} \end{bmatrix} \sim \mathcal{N}(\mu, \Sigma) \tag{12}$$

where μ is the community-mean vector and Σ the positive-definite community covariance matrix, capturing the variance of each parameter and the covariances between them. The community-mean vector μ follows a normal hyperprior:

$$\mu \sim \mathcal{N}(0, \tau) \tag{13}$$

and the community covariance matrix Σ follows as Wishart hyperprior:

$$\Sigma \sim \mathcal{W}(R, \upsilon) \tag{14}$$

where R is a symmetric scale matrix with elements $R_{q_1,q_2} = 1$ if $q_1 = q_2$ and $R_{q_1,q_2} = 0$ if $q_1 \neq q_2$, determining the shape of the Wishart distribution, and $\upsilon \in \mathbb{N}^*$ is the number of random effects.

2.2. `NeMO` R package and associated functions

To analyse empirical data using our proposed model, we employed a Bayesian framework including Markov Chain Monte Carlo (MCMC) sampling, implemented with JAGS (Plummer, 2003). We developed a new R package, `NeMO` (Nested eDNA Metabarcoding Occupancy), to facilitate this process. This package is designed to streamline the analysis of diverse eDNA metabarcoding experimental designs. Our proposed model comprehensively accounts for PCR replicates that are individually indexed for sequencing, a scenario that provides detailed information on presence/absence and sequence read count at the level of individual PCR replicates. However, pooling PCR replicates is a common strategy to reduce costs (Taberlet *et al.*, 2018), but it limits the available information to summarised data. Additionally, while incorporating sequence read counts into occupancy modelling offers a more detailed approach, it also increases model complexity, which may not always be necessary depending on the

study objectives. To accommodate these variations, `NeMO` allows fitting an occupancy model tailored to the chosen modelling protocol with the `Nemodel()` function:

1. 'PCR_rep_seq_read': This model represents the most complex scenario, incorporating eDNA samples, individually indexed PCR replicates, and sequence read counts.
2. 'seq_read': This model simplifies the analysis by omitting PCR replicates indexing (k level) while still considering sequence read counts. It modifies the model described above by replacing W_{nijkc} with A_{nijc} in **Equations 5-6** and excluding **Equation 3**, as well as the calculation of p_{nijkc} and associated random effects.
3. 'PCR_rep': This model focuses on individually indexed PCR replicates without incorporating sequence read counts. Here, W_{nijkc} is replaced with Y_{nijkc} in **Equation 3**, and **Equations 4-9** are excluded, as well as the calculation of φ_{nijkc} and associated random effects.

In addition to fitting occupancy models with the `Nemodel()` function, `NeMO` offers auxiliary tools to facilitate model implementation. It allows the integration of environmental or methodological covariates at any level of the nested model structure. The `covarray()` function standardises these covariates into structured arrays compatible with the model, ensuring flexibility across study designs. The `min_resources()` function calculates the minimum number of samples, PCR replicates, and sequencing depth needed to confirm species absence with a specified confidence level. The `WAIC()` function computes the Watanabe-Akaike Information Criterion (WAIC; Watanabe, 2010) for model comparison. These tools enhance `NeMO` by streamlining data preparation, guiding resource allocation, and aiding model selection. Detailed descriptions of these functions are available in **Appendix S1** and in the package documentation.

2.3. Application to empirical data

We modelled multi-species site-occupancy using a previously published dataset from an eDNA metabarcoding survey of fish conducted during a single campaign in 2016 along the Rhône River and its tributaries (Pont *et al.*, 2018). 80 sites were selected to ensure a representative coverage of the entire river (**Fig. 2a**), and for which a standardised study design was followed, involving 2 eDNA samples per

site and 12 PCR replicates per sample. Details of the sampling and laboratory methods are available in the original publication. The dataset includes sequence read count data for 100 fish species at the selected sites, including false positives – species absent from the Rhône River but detected with eDNA metabarcoding, likely due to human consumption or sewage contamination. Model fitting was performed using `NeMO` v1.0.0 with JAGS v4.3.2 (Plummer, 2003). To illustrate how covariates can be implemented, we included the distance to the sea as a site covariate at the occupancy level (ψ parameter), as fish diversity gradients in the Rhône basin are well documented and particularly pronounced (Tockner *et al.*, 2022). This dataset allowed us to run the `Nemodel()` function with the three different modelling protocols implemented, enabling comparison of model outputs. The raw sequence read count dataset was used for the 'PCR_rep_seq_read' protocol, while PCR replicates were pooled by sample for the 'seq_read' protocol, and sequence read count were ignored and reduced to presence/absence data for the 'PCR_rep' protocol. Three MCMC chains were run in parallel, each with 150,000 iterations, a burn-in period of 50,000 and a thinning of 10. Eventually, we kept 10,000 iterations per chain (30,000 in total). Convergence was checked using traceplots and the Gelman-Rubin statistics $\hat{R}$ (Brooks & Gelman, 1998). The MESO@LR facility at the University of Montpellier was used to run and parallelise the MCMC chains across individual cores on nodes equipped with dual Intel Xeon E5-2680 v4 2.4 GHz processors and 128 GB of RAM.

The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was calculated for each modelling protocol to compare model performance (Fawcett, 2006). This analysis requires prior knowledge of whether a species is present or absent at a given site, which was obtained using an exhaustive list of Rhône fish species supplemented by long-term electrofishing data (2006-2017) described in Pont *et al.* (2018). This dataset was restricted to the 10 years preceding the eDNA sampling (2007-2016). ROC analyses were conducted by river section to account for dams (**Fig. 2a**), using only sections with available electrofishing data. Species were classified as true positives, false negatives, true negatives, or false positives based on fish species list, eDNA and electrofishing detections (see **Appendix S2** for more details). The `pROC` R package v1.18.5 (Robin *et al.*, 2011) was

used to compute and plot ROC curves based on mean species occupancy probabilities from the Z array, providing a comprehensive assessment of model sensitivity and specificity.

Spearman's rank correlation coefficients were calculated between the parameters' link functions to examine whether the species eDNA occupancy probability (ψ) is linked to its probability of collection (θ), amplification (p), and to its expected sequence read count (φ). For species that were recorded in the electrofishing dataset, correlations were also calculated between these parameters and the mean number of catches per species in the Rhône sections prospected over the ten-year survey period. This analysis aimed to investigate whether eDNA occupancy, collection, amplification and expected read count reflects quantitative abundance data. To determine the minimum resources required to confirm that undetected species are truly absent (minimum number of samples J_{min}, minimum number of PCR replicates K_{min}, and minimum sequencing depth M_{min}), we used the `min_resources()` function from the `NeMO` package presented here.

Analyses were conducted using R v4.3.2 (R Core Team, 2023). Unless otherwise stated, values are reported as medians with their 95% highest density intervals (HDIs) across the 30,000 MCMC samples ran with the 'PCR_rep_seq_read' protocol.

3. RESULTS

3.1. Protocol comparison & Model outputs

Model performance was similar across the three modelling protocols, with ROC analysis yielding similar AUC values, ranging from 0.797 for 'PCR_rep_seq_read' to 0.824 for 'seq_read' (**Fig. S3.1**). Estimates remained consistent across all species regardless of the protocol used. However, execution time varied substantially (**Fig. S3.1**). 'PCR_rep' was the fastest, completing in just 2 hours and 30 minutes with the high-performance computing facility, whereas 'PCR_rep_seq_read', the most computationally intensive design, required nearly 19 days to run on the same system. Given the similarity in model outputs among protocols, we focused further analyses on 'PCR_rep_seq_read' results, as this approach provides information at every level of the nested design.

Posterior occupancy for each species at all sites was obtained from the model output by extracting the latent Z array (**Fig. 2b**). Similarly, posterior latent matrices for collection (A),

amplification (W), and read count (S) can be derived, though their visualisation is challenging, as it would require three or more dimensions. Mean occupancy was generally very high when the species was detected with eDNA (close to 1) and very low otherwise (close to 0). However, upstream-downstream occupancy gradients were evident for some species, with certain taxa showing higher occupancy probability downstream, even in sites where they were not detected (*e.g.*, *Barbus meridionalis*, *Cyprinidae – Complex 2*). These occupancy patterns align with the estimates effect of the distance-to-sea covariate (β_ψ, **Fig. 2c**). Based on this estimate, some species can be clearly identified as restricted to upstream sites ($\beta_\psi > 0$: *e.g.*, *Coregonus lavaretus*, *Thymallus thymallus*) or downstream sites ($\beta_\psi < 0$: *e.g.*, *Alosa spp.*, *Chelon ramada*).

3.2. Correlations between parameters

Model outputs provided posterior probabilities for estimates related to eDNA occupancy (ψ), collection (θ), amplification (p), and expected read count (φ). The probability densities varied noticeably among species (**Fig. S3.2**). However, species with high eDNA occupancy probability also tended to exhibit higher detectability (θ, p, and φ). This trend is further supported by positive Spearman's rank correlation coefficients between all parameter pairs (**Fig. S3.3**). Correlations were particularly strong among detection-related parameters – eDNA collection (θ), amplification (p), and sequencing (φ) – ranging from 0.881 (HDI: 0.828 – 0.926) between $logit(\theta)$ and $log(\varphi)$ to 0.961 (HDI: 0.931 – 0.983) between $logit(\theta)$ and $logit(p)$. This indicates that species expected to yield high sequence read count also have eDNA more likely to be successfully amplified in PCR replicates and collected in samples.

Although occupancy and detection parameters were also positively correlated, these relationships were slightly weaker, with coefficients ranging from 0.573 (HDI: 0.482 – 0.655) between $logit(\psi)$ and $log(\varphi)$, to 0.64 (HDI: 0.541 – 0.72) between $logit(\psi)$ and $logit(p)$. As a result, species with high occupancy probability (*i.e.*, occurring in more sites) also exhibited higher detectability across samples, PCR replicates and sequencing reads, and species with low occupancy probability also exhibited lower detectability. The majority of species, like *Rutilus rutilus* (high ψ, θ, p, and φ) and *Misgurnus fossilis* (low ψ, θ, p, and φ) closely followed the general pattern, while *Alosa spp.* and

Scardinius erythrophthalmus diverged (**Fig. S3.3**), illustrating the distinction between rarity (low occupancy) and elusiveness (low detectability). *Alosa spp.* was rare throughout the river (low ψ) but highly detectable (high θ, p, and φ), reflecting its restriction to downstream sites (**Fig. 2b-c**) where its eDNA was consistently detected. In contrast, *Scardinius erythrophthalmus* was common (high ψ) but elusive (low θ, p, and φ). **Figure 3** further illustrates these differences, showing consistently high or low parameter values for *Rutilus rutilus* and *Misgurnus fossilis*, while *Alosa spp.* and *Scardinius erythrophthalmus* display the rare-conspicuous and common-elusive patterns, respectively.

Spearman's correlations were also positive when comparing the mean annual number of catches per species recorded over the ten-year electrofishing survey with parameters ψ, θ, p, and φ (**Table S3.1**). They ranged from 0.4 (HDI: 0.377 – 0.421) for the expected read count (φ) to 0.516 (HDI: 0.456 – 0.571) for occupancy probability (ψ). These results indicate a positive relationship between occupancy probability, detectability, and electrofishing-based abundance estimates.

3.3. Estimation of the minimum technical requirements (eDNA samples/PCR replicates/Sequencing depth) to assess species absence

The minimum number of eDNA samples, PCR replicates, and minimum sequencing depth necessary to ascertain species absence with 95% confidence varied greatly among species (**Fig. 4**). Regarding eDNA sampling and PCR replication, our model shows that the effort in Pont *et al.* (2018) – *i.e.*, 2 eDNA samples and 12 PCR replicates – was sufficient to confirm absence for most species (63%) when not detected (true negatives). In contrast, 18 species would have required additional sampling and PCR technical effort (potential false negatives). Among these, five species required just one additional sample, while they would all have needed more PCR replicates. For some extreme cases, such as *Leucaspius delineatus* and *Lota lota*, the additional PCR replicates required would have been prohibitively high.

The minimum sequencing depth needed to confirm species absence was calculated per sample since individually indexed PCR products were pooled by sample in equal volumes during library preparation, targeting a theoretical sequencing depth of 500,000 reads (Pont *et al.*, 2018). This sequencing depth was sufficient to ascertain species absence, as the median number of samples with

inadequate sequencing depth was zero for all species. However, considering the HDI, one of two samples occasionally lacked sufficient sequencing depth for certain species at some sites (**Fig. S3.4**), but the second sample provided adequate coverage, preventing detection failure within sites.

4. DISCUSSION

We present NeMO, a Bayesian occupancy modelling framework tailored for metabarcoding-based eDNA studies with nested designs. This versatile R package accommodates diverse study designs, handling both presence/absence and sequence read count data, as well as individually indexed or pooled PCR replicates. NeMO accounts for multiple sources of uncertainty across hierarchical levels (collection, amplification, sequencing), and allows users to incorporate covariates influencing occupancy and detection probabilities. Beyond modelling, it enables formal model comparison to assess covariate effects and help optimise study design by evaluating whether sampling effort, PCR replication, and sequencing depth are sufficient to infer species absence. By refining species occupancy estimates and guiding resource allocation, NeMO enhances the reliability of eDNA-based biodiversity assessments, particularly for elusive species.

We applied NeMO to a previously published eDNA metabarcoding dataset investigating fish diversity along the Rhône River (Pont *et al.*, 2018). Regardless of the modelling protocol used, the high AUC value (~ 0.8) in the ROC analysis indicated strong model performance in distinguishing species presence from absence. By accounting for imperfect detection, occupancy probabilities provided more informative insights than raw presence/absence or sequence read count data. Integrating distance to sea as a covariate further refined site-level estimates, successfully capturing well-documented biological gradients (Keith *et al.*, 2020) reflecting species' distributions along the river system. Upstream-associated species exhibited positive estimate values, including *Thymallus thymallus* and *Coregonus lavaretus*, the latter being restricted to alpine lakes. In contrast, downstream-associated species showed negative values, particularly diadromous species such as *Alosa spp.* (anadromous), *Anguilla anguilla* (catadromous), and also estuarine species such as *Chelon ramada*, reflecting their reliance on transitional or marine-influenced habitats.

Positive correlations between occupancy or detection parameter estimates with electrofishing data align with previous studies comparing eDNA with traditional metrics (Muri *et al.*, 2020; Pont *et al.*, 2018, 2023). These findings suggest that occupancy and detection probabilities serve as meaningful proxies for species abundance in this river system. However, these relationships should be interpreted cautiously when surveys differ in temporal and spatial scales. As expected, strong positive correlations were observed among detection parameters (θ, p, and φ), probably due to high eDNA abundance increasing the likelihood of successful collection in a sample, amplification in PCR replicates, and of obtaining a high number of sequencing reads. Correlations between detection parameters and occupancy probability (ψ) were weaker, likely influenced by species-specific biological factors affecting DNA release into the environment, such as activity patterns, reproductive status, or body size (Stewart, 2019). Additionally, methodological factors like primer biases could affect DNA amplification, and sequencing efficiency despite species presence in the site surveyed (Elbrecht & Leese, 2015). Consequently, some species exhibited low detectability and may remain undetected at certain sites they actually occupy. While rare species are often elusive, exceptions exist: some common species remain difficult to detect (*Scardinius erythrophthalmus*), while certain rare species are conspicuous (*Alosa spp.*). This highlights the distinction between rarity and elusiveness, two concepts that should not be confused (Cerqueira *et al.*, 2013). It is worth noting that these concepts should always be contextualised, as a species may be globally rare but regionally common – like upstream or downstream species in the Rhône River – and elusiveness is method-dependent (*i.e.*, a species may be elusive because difficult to detect with one method but readily detectable with another).

`NeMO` enables retrospective evaluation of eDNA surveys by estimating the minimum number of eDNA samples, PCR replicates, and sequencing depth required to confidently confirm species absence. While sequencing depth was consistently sufficient for the species surveyed by Pont *et al.* (2018), about one-third lacked enough samples or PCR replicates to reliably confirm absence. This highlights the inevitable trade-off between resource allocation and species detectability. Although eDNA proved more effective than traditional methods for detecting elusive species – such as *Zingel asper*, *Mugil cephalus* and *Misgurnus fossilis*, which were missed in 10 years of electrofishing – detectability must still be assessed before asserting its superiority. Our results suggest that increased

replication effort could have allowed for the detection of these species at more sites. However, for some low-detectability species like *Leucaspius delineatus*, an 'emerging' species in the Rhône basin, confirming absence would require an impractical number of PCR replicates per sample (>1,000), highlighting the limitations of the selected eDNA study design for certain targets. To improve detection, adjustments to study design could include alternative primers or modified sampling protocols. For example, *Lota lota*, a deep-dwelling nocturnal species (Keith *et al.*, 2020), showed low detectability, likely because sampling was conducted in lentic systems at the surface and during daytime. Therefore, it is essential to cautiously interpret the absence of such species, as it may not reflect true absence. We recommend integrating occupancy models into eDNA studies to validate non-detections and guide replication effort. This approach provides statistical support for filtering low-detection amplicons, as demonstrated by Pont *et al.* (2018), who excluded *Leucaspius delineatus* and *Lota lota* due to single-PCR detections – a decision now supported by `NeMO`. We further show that other species, such as *Salvelinus namaycush* or *Micropterus salmoides*, could have been filtered out as well. Ideally, occupancy models should be used not only for downstream analyses but also during pilot studies to optimise sampling efforts in a statistically-driven way. This approach enables adjustment of eDNA samples, PCR replicates, and sequencing depth to align with study goals and target species. Given the risks of misinterpreting species absences, we recommend comparing community patterns with and without uncertain absences to assess their impact on α- and β-diversity, refining conclusions as necessary. Such practices can improve study efficiency and reliability of biodiversity assessments.

Among the three modelling protocols implemented in `NeMO`, the 'PCR_rep' protocol proved to be the fastest. When sequencing-related covariates are not included, we recommend avoiding models based on sequence read counts – such as the 'seq_read' and 'PCR_rep_seq_read' modelling protocols in `NeMO`, as well as those used in the packages developed by Fukaya *et al.* (2022) or Diana *et al.* (2024) – since they significantly increase computational time without enhancing performance. While our framework accounts for false negatives and detection biases, it does not model false positives, which remain a common issue in eDNA studies. These are often mitigated through stringent bioinformatic pipelines or taxonomic filtering to exclude sequencing artifacts or unexpected detections arising from contamination or diet, which could also lead to false negatives if these filters are too stringent.

Occupancy models that account for false positives do exist (Guillera-Arroita *et al.*, 2017; Lahoz-Monfort *et al.*, 2016), but they generally require additional data to distinguish true from false detections, such as an unambiguous detection method or calibration procedures applied at each stage of the multi-scale sampling hierarchy. These requirements add complexity to already intricate hierarchical models and are often impractical in large-scale metabarcoding studies. Additionally, in lotic systems, species detection may be spatially displaced due to downstream DNA transport, though this effect can be quantified (Perry *et al.*, 2024; Pont *et al.*, 2018). Future developments could thus integrate hydrological transport effects for both freshwater (Gimenez, 2024) and marine systems. More generally, incorporating spatial autocorrelation (Chen & Ficetola, 2019) or species co-occurrence (Rota *et al.*, 2016) could improve inference, but at the cost of greater model complexity and computational demand.

TABLES

Table 1. Scientific papers reporting code or software for multi-species occupancy modelling applied to eDNA metabarcoding data among the 75 papers identified via a literature research query with key-words: [("metabarcoding" OR ("eDNA" AND ("multispecies" OR "multi-species"))) AND "occupancy"] (Web of Science Core Collection on March 13, 2025). Full references are given in Appendix S4.

Publication	Input data	PCR replication level	Covariates implementation	Model comparison	Resource optimisation	Additional features	Package
Doi *et al.* 2019	Presence/Absence	✓	✓	-	-	-	-
Bush *et al.* 2020	Presence/Absence	-	✓	✓	-	-	-
McClenaghan *et al.* 2020	Presence/Absence	✓	✓	✓	-	Species richness (Data augmentation)	-
Gold *et al.* 2021	Presence/Absence	-	-	-	-	-	-
McColl-Gausden *et al.* 2021	Presence/Absence	-	✓	-	-	-	-
Pukk *et al.* 2021	Presence/Absence	-	-	-	-	-	-
Fukaya *et al.* 2022	Sequence read count	-	✓	-	✓	-	occumb
Ji *et al.* 2022	Presence/Absence	-	✓	-	-	Species richness (Data augmentation)	-
Allen *et al.* 2023	Presence/Absence	-	✓	-	✓	-	-
Peixoto *et al.* 2023	Presence/Absence	-	✓	-	✓	-	-
Diana *et al.* 2024	Sequence read count	✓	✓	-	-	Spatial autocorrelation Species correlations	eDNAPlus
Rounds *et al.* 2024	Presence/Absence	-	✓	-	✓	-	-
This study	Presence/Absence OR Sequence read count	✓ individually indexed OR pooled	✓	✓	✓	-	NeMO

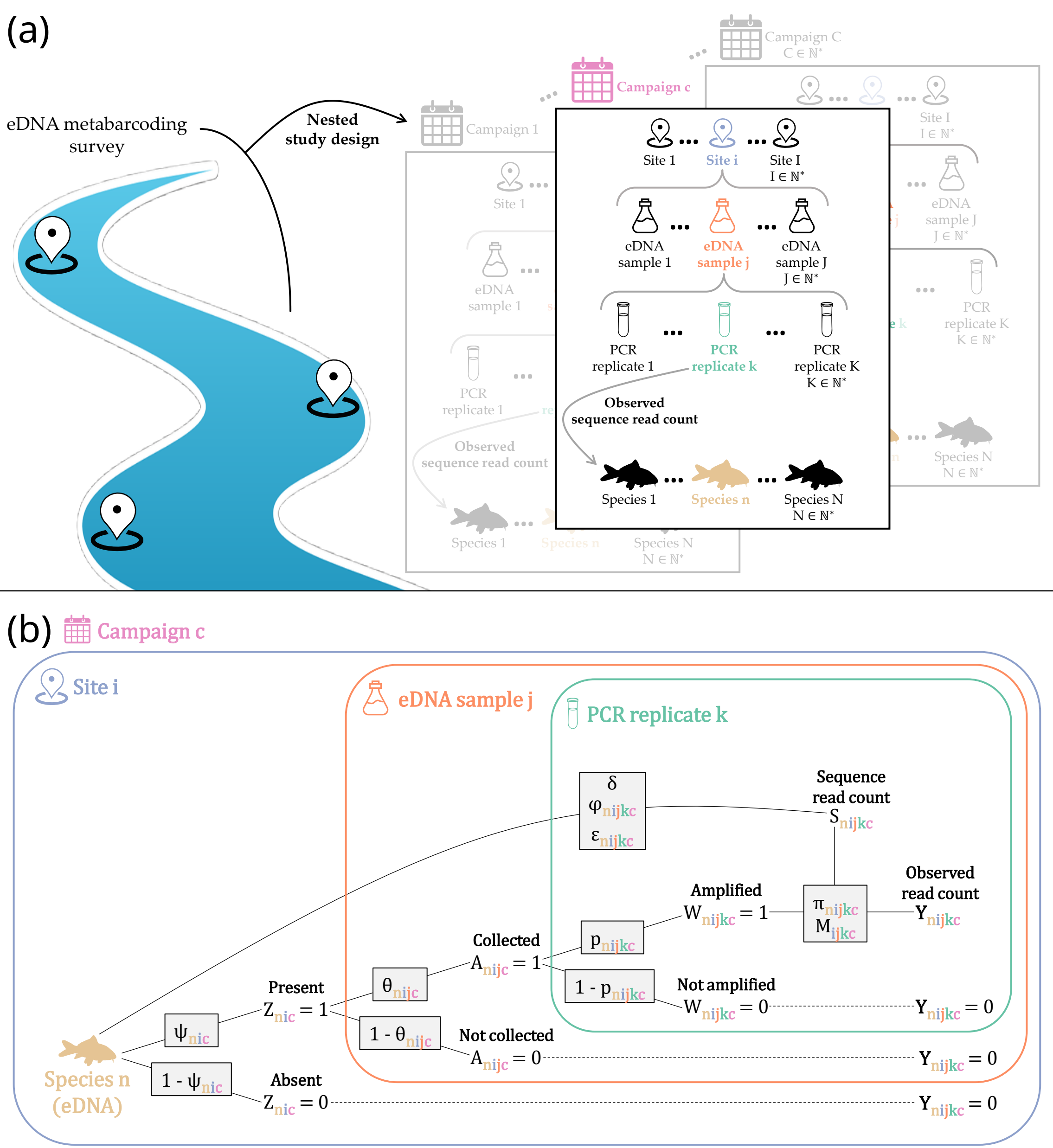
FIGURES

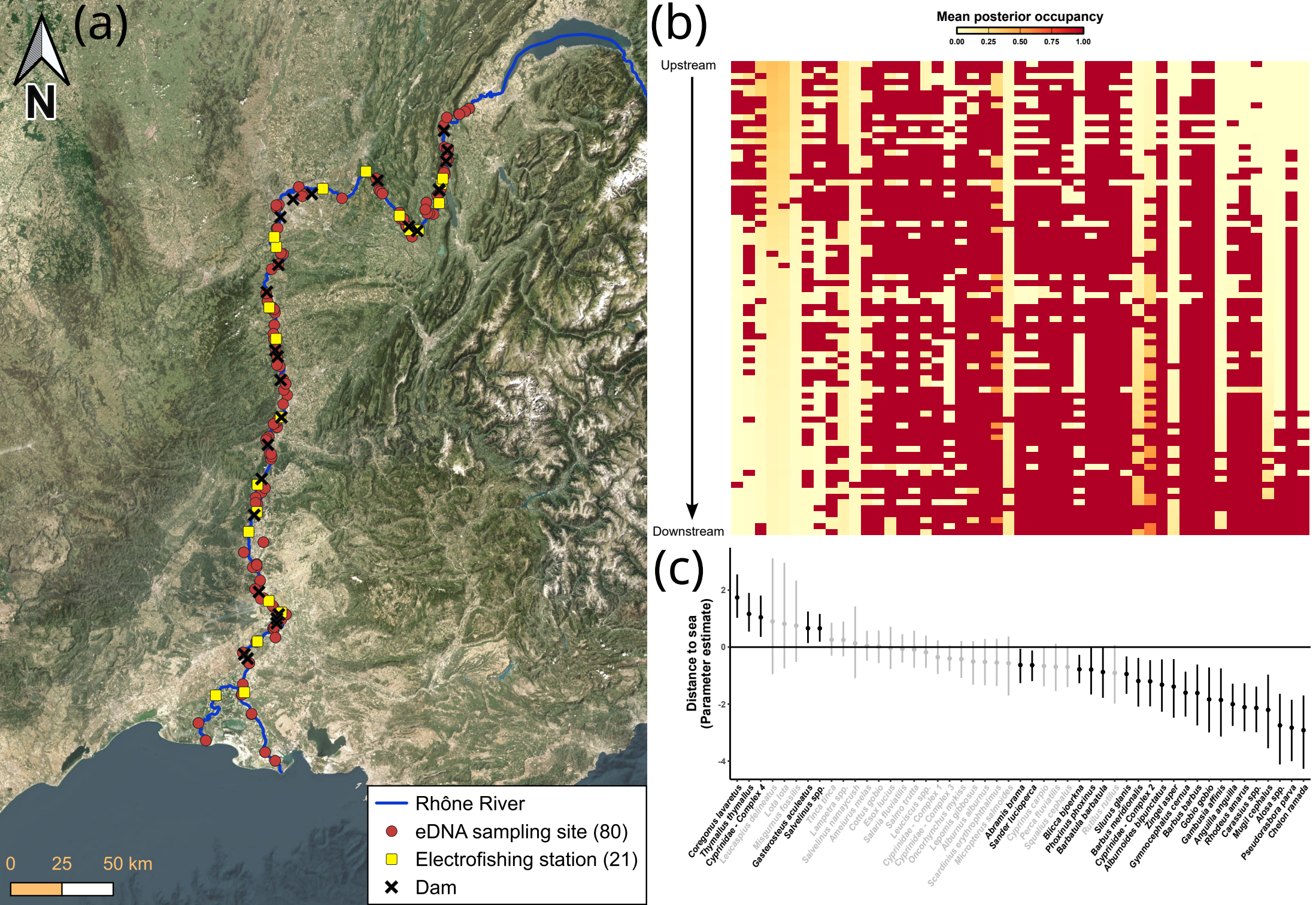
Figure 1. (a) Illustration of a nested study design for an eDNA metabarcoding survey in a river, monitoring N fish species over C campaigns, with replication across sites (I), samples (J), and PCR replicates (K). **(b)** Tree diagram illustrating the nested structure of the occupancy model for eDNA metabarcoding data. During a given campaign c, eDNA from species n may either be present at site i with occupancy probability ψ_{nic} (latent variable $Z_{nic} = 1$), or absent with probability $1 - \psi_{nic}$ ($Z_{nic} = 0$). If present, eDNA may either be collected in an eDNA sample j with detection probability θ_{nijc} (latent variable $A_{nijc} = 1$), or not collected with probability $1 - \theta_{nijc}$ ($A_{nijc} = 0$). If collected, eDNA may either be amplified in a given PCR replicate k with detection probability p_{nijkc} (latent variable $W_{nijkc} = 1$), or not amplified with probability $1 - p_{nijkc}$ ($W_{nijkc} = 0$). If amplified, eDNA is sequenced with frequency π_{nijkc} relative to the total sequence read count M_{ijkc}, generating the observed sequence read count Y_{nijkc}. When eDNA is absent, not collected, or not amplified, the read count is $Y_{nijkc} = 0$. The observed read count depends on the sequence read count (latent variable S_{nijkc}) which follows a ZINB distribution with mean φ_{nijkc}, dispersion parameter δ, and zero-inflation parameter ε_{nijkc}.

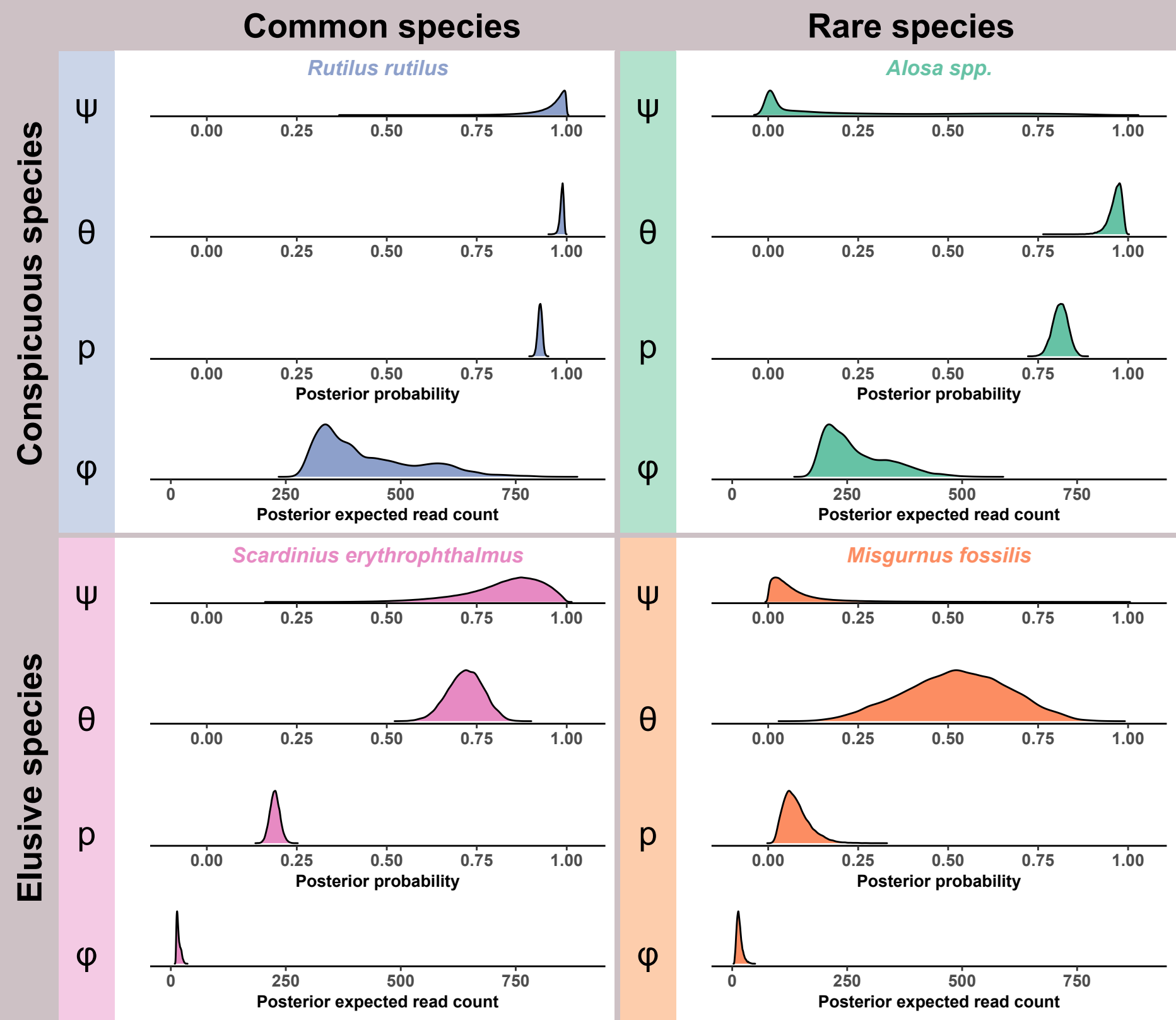
Figure 2. (a) Map of sampling locations along the Rhône River (France). The river thalweg is shown in blue. Red dots denote the 80 sites, including some situated at the confluence of the Rhône and its tributaries, where 2 eDNA samples were collected per site. Yellow squares indicate stations where long-term electrofishing surveys are conducted, and black crosses mark the locations of dams. **(b)** Mean posterior occupancy (Z array) for species inhabiting the Rhône River at the 80 sampling sites. **(c)** Distance-to-sea occupancy parameter estimate (β_ψ) for each species inhabiting the Rhône River. Dots represent the median estimates with their HDI. Species with parameter estimates not overlapping zero are shown in black, while others are in grey. Species identified at the genus level (*spp.*) or as part of complexes are detailed in Table S3.2.

Figure 3. Ridge line plots depicting the densities of posterior probabilities for eDNA occupancy (ψ), collection (θ), amplification (p), and expected read count (φ) for four species inhabiting the Rhône River. The highlighted species are *Rutilus rutilus* (blue), *Alosa spp.* (green), *Scardinius erythrophthalmus* (pink) and *Misgurnus fossilis* (orange). These species are categorised based on (1) their rarity – common species (*Rutilus rutilus* and *Scardinius erythrophthalmus*) exhibit high occupancy probability, while rare species (*Alosa spp.* and *Misgurnus fossilis*) have low occupancy probability – and (2) their detectability – conspicuous species (*Rutilus rutilus* and *Alosa spp.*) display high eDNA collection and amplification probabilities, along with high expected read count, whereas elusive species (*Scardinius erythrophthalmus* and *Misgurnus fossilis*) are characterised by lower eDNA collection and amplification probabilities, as well as lower expected read count.

Figure 4. Tile plot illustrating the minimum number of eDNA samples (J_{min}; in blue), PCR replicates (K_{min}; in green), and sequencing depth (M_{min}; in red) required to ascertain species absence with 95% confidence. Median values are displayed, with their HDI in brackets. For sequencing, values are calculated across all samples and sites, representing summary estimates. The sequencing depth used in the study was always sufficient to ascertain species absence at all sites (see Fig. S3.4). For samples and replicates, grey shading indicates cases where the resources used in the original study (2 eDNA samples and 12 PCR replicates) were sufficient across the entire HDI. Species names are underlined if non-indigenous in the Rhône River, and threatened species are colour-coded according to their IUCN conservation status at the most local scale possible (yellow: Vulnerable; orange: Endangered; red: Critically Endangered). Species identified at the genus level (*spp.*) or as part of complexes are detailed in Table S3.2.


(a)
eDNA metabarcoding survey
Nested study design
Campaign 1
Campaign c
Campaign C
C ∈ ℕ*
Site 1
Site i
Site I
I ∈ ℕ*
eDNA sample 1
eDNA sample j
eDNA sample J
J ∈ ℕ*
PCR replicate 1
PCR replicate k
PCR replicate K
K ∈ ℕ*
Observed sequence read count
Species 1
Species n
Species N
N ∈ ℕ*
(b)
Campaign c
Site i
eDNA sample j
PCR replicate k
Species n (eDNA)
ψnic
1 - ψnic
Present
Znic = 1
Absent
Znic = 0
θnijc
1 - θnijc
Collected
Anijc = 1
Not collected
Anijc = 0
δ
φnijkc
εnijkc
Sequence read count
Snijkc
pnijkc
1 - pnijkc
Amplified
Wnijkc = 1
Not amplified
Wnijkc = 0
πnijkc
Mijkc
Observed read count
Ynijkc
Ynijkc = 0


(a)
N
Rhône River
eDNA sampling site (80)
Electrofishing station (21)
Dam
0
25
50 km
(b)
Mean posterior occupancy
0.00
0.25
0.50
0.75
1.00
Upstream
Downstream
(c)
Distance to sea
(Parameter estimate)
2
0
-2
-4
Coregonus lavaretus
Thymallus thymallus
Cyprinidae - Complex 4
Leucaspius delineatus
Lota lota
Misgurnus fossilis
Gasterosteus aculeatus
Salvelinus spp.
Tinca tinca
Lampetra spp.
Salvelinus namaycush
Ameiurus melas
Cottus gobio
Esox lucius
Salaria fluviatilis
Salmo trutta
Leuciscus spp.
Cyprinidae - Complex 1
Cyprinidae - Complex 3
Oncorhynchus mykiss
Lepomis gibbosus
Alburnus alburnus
Scardinius erythrophthalmus
Microptenus salmoides
Abramis brama
Sander lucioperca
Cyprinus carpio
Perca fluviatilis
Squalius cephalus
Blicca bjoerkna
Phoxinus phoxinus
Barbatula barbatula
Rutilus rutilus
Silurus glanis
Barbus meridionalis
Cyprinidae - Complex 2
Alburnoides bipunctatus
Zingel asper
Gymnocephalus cernua
Barbus barbus
Gobio gobio
Gambusia affinis
Anguilla anguilla
Rhodeus amarus
Carassius spp.
Mugil cephalus
Alosa spp.
Pseudorasbora parva
Chelon ramada


Common species
Rare species
Conspicuous species
Elusive species
Rutilus rutilus
Alosa spp.
Scardinius erythrophthalmus
Misgurnus fossilis
ψ
θ
p
φ
0.00
0.25
0.50
0.75
1.00
Posterior probability
0
250
500
750
Posterior expected read count

Minimum technical requirements

eDNA samples (J_{min}): ≤ 2; 2, 4, 6, 8, 10

PCR replicates (K_{min}): ≤ 12; 40, 80, 120, 160

Sequencing depth (M_{min}): 4000, 8000, 12000, 16000

	eDNA samples (J_{min})	PCR replicates (K_{min})	Sequencing depth (M_{min})
Abramis brama	1 [1; 2]	3 [2; 3]	1 [1; 1]
Alburnoides bipunctatus	1 [1; 1]	2 [2; 2]	6 [1; 187]
Alburnus alburnus	1 [1; 2]	2 [2; 2]	10 [1; 56242]
Alosa spp.	1 [1; 2]	2 [2; 3]	15 [3; 57602]
Barbatula barbatula	1 [1; 1]	2 [1; 2]	17 [3; 71439]
Barbus barbus	1 [1; 1]	1 [1; 1]	12 [3; 74872]
Barbus meridionalis	3 [2; 5]	31 [20; 51]	5856 [533; 122996]
Blicca bjoerkna	2 [1; 2]	4 [3; 4]	35 [5; 94156]
Chelon ramada	2 [1; 2]	4 [3; 4]	97 [8; 102900]
Cottus gobio	1 [1; 2]	2 [2; 3]	70 [8; 113652]
Cyprinidae - Complex 1	1 [1; 1]	2 [2; 2]	16 [4; 100033]
Cyprinidae - Complex 3	1 [1; 2]	3 [2; 3]	45 [7; 117630]
Cyprinidae - Complex 4	3 [2; 5]	22 [16; 31]	1806 [174; 139278]
Gasterosteus aculeatus	2 [2; 3]	7 [6; 8]	1442 [150; 156404]
Gobio gobio	1 [1; 1]	2 [1; 2]	46 [7; 138936]
Gymnocephalus cernua	1 [1; 1]	2 [2; 2]	72 [10; 143004]
Lampetra spp.	3 [2; 5]	14 [11; 18]	3229 [359; 155760]
Leucaspius delineatus	10 [5; 54]	162 [61; 1110]	17231 [1632; 134272]
Leuciscus spp.	2 [2; 2]	6 [5; 6]	578 [65; 170119]
Mugil cephalus	3 [2; 5]	31 [20; 54]	2536 [224; 151794]
Perca fluviatilis	2 [1; 2]	3 [3; 4]	364 [43; 170598]
Phoxinus phoxinus	1 [1; 1]	2 [2; 2]	110 [15; 154876]
Rhodeus amarus	2 [1; 2]	4 [3; 4]	373 [43; 166500]
Rutilus rutilus	1 [1; 1]	2 [2; 2]	32 [6; 140050]
Salaria fluviatilis	1 [1; 2]	3 [3; 3]	308 [39; 183929]
Salmo trutta	1 [1; 2]	3 [3; 3]	212 [27; 179894]
Salvelinus spp.	2 [2; 3]	6 [5; 6]	722 [89; 169894]
Scardinius erythrophthalmus	3 [2; 4]	15 [13; 17]	3256 [417; 187781]
Squalius cephalus	2 [1; 2]	3 [3; 3]	67 [10; 173396]
Tinca tinca	2 [2; 2]	8 [7; 8]	2078 [267; 201754]
Ameiurus melas	2 [2; 3]	9 [8; 10]	214 [7; 82925]
Carassius spp.	2 [2; 2]	5 [5; 6]	986 [81; 124542]
Cyprinidae - Complex 2	3 [2; 4]	30 [23; 41]	3051 [300; 149523]
Cyprinus carpio	2 [1; 2]	3 [3; 3]	258 [27; 153025]
Gambusia affinis	3 [2; 4]	18 [12; 30]	2092 [192; 143813]
Lepomis gibbosus	2 [2; 2]	6 [5; 6]	515 [57; 157288]
Micropterus salmoides	4 [3; 8]	45 [24; 104]	3839 [374; 142300]
Oncorhynchus mykiss	2 [1; 2]	2 [2; 3]	59 [9; 136333]
Pseudorasbora parva	2 [1; 2]	3 [3; 3]	422 [51; 163750]
Salvelinus namaycush	5 [3; 11]	45 [20; 192]	6045 [575; 158543]
Sander lucioperca	2 [1; 2]	3 [3; 3]	288 [37; 183537]
Silurus glanis	1 [1; 2]	3 [2; 3]	210 [28; 184525]
Coregonus lavaretus	2 [2; 3]	6 [5; 7]	226 [18; 105246]
Esox lucius	2 [2; 2]	4 [4; 5]	149 [17; 141617]
Lota lota	7 [4; 23]	96 [39; 406]	10470 [1061; 148271]
Thymallus thymallus	2 [2; 3]	7 [6; 8]	911 [110; 187344]
Misgurnus fossilis	4 [2; 11]	40 [18; 136]	2705 [260; 140182]
Zingel asper	3 [2; 5]	20 [13; 34]	5765 [667; 188959]
Anguilla anguilla	2 [2; 2]	4 [4; 5]	151 [7; 89953]

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