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Research article

Assessing the value of environmental DNA into conservation planning: A case study of freshwater bivalves in France

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ABSTRACT

Environmental DNA (eDNA) is emerging as a valuable tool for generating standardised biodiversity data. Whether used alone or alongside conventional surveys, it is expected that eDNA data can improve conservation planning, but this remains largely untested. Here, we assessed the value of eDNA data for making more robust conservation prioritisation decisions, by comparing errors made when identifying priority areas based on conventional data, eDNA or a combination of both. We collected distribution data for 29 freshwater bivalve species in France from both a conventional source (public databases) and field eDNA surveys. We then used Marxan, a spatial prioritisation tool, to assess how conventional versus eDNA data can influence conservation decisions. Data from both sources were used to model species distributions, considering either all available data (full dataset), eDNA data only (eDNA dataset), conventional data only (conventional dataset), or varying proportions of conventional and eDNA data (mixed datasets: 25/75; 50/50; 75/25). We compared the performance of conservation planning solutions derived from the different datasets against the full dataset, which served as the best approximation to the species' true distributions. For each dataset, we assessed the percentage of species targets met (effectiveness), the efficiency of the conservation solutions, the representation error rates compared to the best attainable information (omission and commission errors), and the overlap between each scenario and the full dataset. The inclusion of eDNA data allowed us to model the distribution of more species, compared to conventional data. However, when using eDNA data alone, fewer species achieved the targets and solutions were less efficient, requiring more areas to be selected. Using either conventional or eDNA data alone was associated with higher commission and omission errors, respectively. Overall, integrating eDNA data with conventional datasets outperformed using conventional data alone, improving the efficiency of conservation solutions.

1. Introduction

Urgent conservation efforts are needed to address species declines in freshwater ecosystems, which have suffered a 30% loss since 1970 (Davidson, 2014). These shocking figures underline the urgent need to improve decision-making processes to prioritise freshwater ecosystem conservation (Nogueira et al., 2021a). Despite growing public support, conservation decision-making is often constrained by limited resources, making high-quality and comprehensive data on biodiversity distributions essential for effective action.

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A vital step towards the conservation of any ecosystem involves the prioritisation of areas that should be protected or where management efforts should be directed (Margules and Pressey, 2000). To undertake such conservation planning exercises, it is necessary to collect data on the spatial distribution of conservation features (ecosystems, species, genes), generally involving conventional field sampling strategies such as electrofishing, traps, acoustics, hand searching, snorkelling, visual census, hand nets, and many others (Beng and Corlett, 2020). Such sampling methods can have several limitations and biases, for example visual surveys often fail to detect species that are small, rare, or otherwise less conspicuous (Edgar et al., 2004). Moreover, conventional surveys require taxonomic expertise (Leese et al., 2018) and often involve killing the target organisms. Conventional methods also tend to be time-consuming and costly (Beng and Corlett, 2020), and so the spatial area surveyed tends to be small, which can result in sparse data (Muenzel et al., 2024). The use of insufficient, outdated or poor-quality data can limit the effectiveness and efficiency of a conservation plan (Hermoso et al. 2015a, 2015b), ultimately failing to address species declines (Nogueira et al., 2021b).

The analysis of environmental DNA (eDNA) fragments collected from the environment (air, soil, water), is becoming a cost-effective and time-efficient tool for the non-invasive detection of organisms (Barnes and Turner, 2016). Its applications are broad and include the early detection of invasive species (Tréguier et al., 2014), the taxonomic identification of commercial species (Collins et al., 2013), and the bioassessment of different ecosystems (Bienert et al., 2012; Mächler et al., 2014). As analytical techniques become faster and cheaper, and given the ongoing biodiversity collapse of freshwater species, eDNA technology could be a key tool to improve inventories and inform conservation decisions (Bird et al., 2024). In this context, the application of eDNA data in Systematic Conservation Planning (SCP) appears particularly promising, as it could improve the mapping of species distributions, especially those that are rare, inconspicuous, or occur in remote areas that are difficult to survey through conventional methods (Muenzel et al., 2024). However, the use of eDNA data in SCP has not yet been fully explored, either on its own or in combination with other sources of spatial distribution data. Combining eDNA samples collected from freshwater with conventional surveys has the potential for higher detection rates and better data quality (Adams et al., 2024; Wang et al., 2021). These two techniques have proven to complement each other and advance the conservation and management of organisms such as freshwater fish (Euclide et al., 2021; Mauvisseau et al., 2020), freshwater mussels (Prié et al., 2023), crayfish (Troth et al., 2020), coral reefs (Muenzel et al., 2024), zooplankton, birds, plants, arthropods, and annelids (Bird et al., 2024), amphibians (Adams et al., 2024), and mammals (Qu and Stewart, 2019). However, conservationists often lack access to both types of data, and the costs and technical skills required to acquire them differ substantially.

In this study, we developed a conservation planning exercise using both conventional and eDNA occurrence data for 29 native freshwater bivalve species across France. Freshwater bivalves provide a relevant case study, as they are facing severe population declines (Lopes-Lima et al. 2017, 2023), and thus improved conservation planning could help to establish new protected areas and prioritise management actions (e.g. habitat restoration or threat mitigation; Carvalho et al., 2017; Game et al., 2013). Our goal was to assess the cost-benefit of using eDNA to inform conservation decisions for freshwater bivalves. We did this by comparing the priority areas selected based on species distribution models that used all available data for all species (conventional and eDNA), as a proxy for the true distribution of the species, with a series of models that considered only conventional data; only eDNA, and a combination of conventional and eDNA data: mix50 (50/50); mix25 (75/25); mix75 (25/75). We expected that the best results would be obtained using a mixture of eDNA and conventional data, thereby overcoming the limitations and potential shortcomings of each individual method (Allen et al., 2021; Bird et al., 2024; Yamamoto et al., 2017). Overall, our study provides guidance on how to incorporate eDNA data into systematic conservation planning to provide more robust conservation recommendations.

2. Material and methods

2.1. Study area

Our study covered the territory of France (540,000 km^2), divided into 4,284 planning units (hereafter referred to as sub-catchments) corresponding to Hydrobasins level 12 (Lehner and Grill, 2013), (Fig. 1). France is located in Western Europe and has a great diversity of landscapes, river types and climatic influences. Altitudes range from sea level to over 4,000 m in the Alps, and the climate is influenced by the Atlantic Ocean and the Mediterranean Sea. In general, temperature increases from the north, characterised by a temperate climate, to the south, with a warmer Mediterranean climate (Meersmans et al., 2012). French rivers are inhabited by 34 native freshwater bivalve species belonging to the families Margaritiferidae, Unionidae and Sphaeriidae (Prié, 2017).

2.2. Data analysis

2.2.1. Conventional data

We compiled data on freshwater bivalve occurrences in France from a public database (Inventaire national du patrimoine naturel - INPN, https://inpn.mnhn.fr/). This database includes data from the literature (both grey and academic), museum collections and scientifically validated observations (Prié et al., 2023). For our analysis, we only considered species that are native to France and whose occurrence records were collected after 2010. For each record, we compiled information on latitude, longitude and species name. We retrieved data for 30 species but excluded one with less than 10 records. Records with identifications at genus or higher taxonomic levels were also excluded. A total of 29 freshwater bivalve species were thus included in the study (Table 1), including threatened species such as *Pseudunio auricularius* (Critically Endangered), *Margaritifera margaritifera*, *Potomida littoralis* (Endangered), and *Sphaerium rivicola* (Vulnerable). For each species, the spatial layer of occurrence records was intercepted with the Hydrobasins level 12 (15 arc-seconds grid resolution) shapefile in QGIS (version 3.14.1), to produce maps of species occurrences at Hydrobasins level 12 resolution. A total of 843 hydrobasins with at least one of the target species were recorded.

2.2.2. Environmental DNA data

The eDNA dataset that we used is an extension of Prié et al. (2023), including 358 additional eDNA sampling sites, for a total of 638 sites. eDNA sampling was performed using a peristaltic pump Vampire Sampler (Bürkle GmbH, nominal flow of 1.1 L.min−1), a VigiDNA® 0.45-µM cross flow filtration capsule (SPYGEN, le Bourget du Lac, France) and disposable sterile tubing for each filtration capsule. The tube inlet was placed a few centimetres below the water surface. Two filtrations were performed in parallel at each site. Water was filtered for approximately 30 min for a water volume of approximately 30 L, or until the filter was clogged. At the end of each filtration, the water inside the capsule was emptied, and the capsule was filled with 80 mL of CL1 Conservation buffer (SPYGEN, le Bourget du Lac, France) and stored at room temperature until extraction.

For DNA extraction, amplification and sequencing we followed the protocol described in Prié et al. (2021). Prior to sequencing, purified PCR products were quantified by capillary electrophoresis and then pooled in equal volumes to achieve an expected sequencing depth of 300,000 reads per each sample and each marker before DNA library preparation. Negative extraction controls and negative PCR controls (ultrapure water, 12 replicates) were systematically performed on each batch of extraction and amplification steps, and they were amplified per

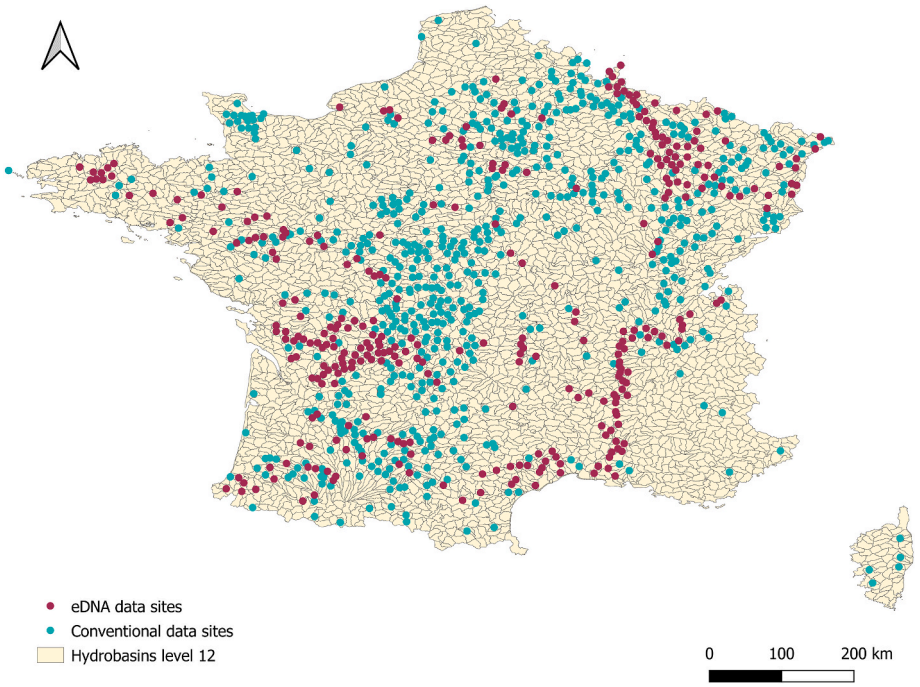

Fig. 1. Map of the study area (France) divided into Hydrobasins level 12 sub-catchments, showing eDNA sampling sites (red) and conventional data records (green).

Table 1
Species sampled with eDNA and conventional data gathered from INPN, conservation status in France, total number of records (eDNA + conventional) for Hydrobasins level 12, the percentage used to set the target for Marxan to achieve across all scenarios tested and respective value of target used.

Species	Conservation status	Records	Percentage	Target
Anodonta anatina	VU	1481	50	741
Anodonta cygnea	VU	1342	50	671
Euglesa casertana	LC	940	30	282
Euglesa henslowana	LC	1060	30	318
Euglesa interstitialis	LC	673	30	202
Euglesa milium	LC	1131	30	339
Euglesa nitida	LC	869	30	261
Euglesa obtusalis	LC	1241	30	372
Euglesa parvula	LC	730	30	219
Euglesa personata	LC	870	30	261
Euglesa pulchella	LC	480	75	360
Euglesa subtruncata	LC	1126	30	338
Euglesa supina	LC	667	30	200
Margaritifera margaritifera	EN	180	100	180
Odhneripisidium moitessierianum	LC	891	30	267
Odhneripisidium tenuilineatum	LC	1127	30	338
Pisidium amnicum	LC	1497	30	449
Potomida littoralis	EN	1308	75	981
Pseudanodonta complanata	EN	768	100	768
Pseudunio auricularius	CR	337	100	337
Sphaerium corneum	LC	698	30	209
Sphaerium lacustre	LC	1133	30	340
Sphaerium nucleus	LC	843	50	422
Sphaerium ovale	LC	535	50	268
Sphaerium rivicola	EN	380	100	380
Unio crassus	LC	1063	50	532
Unio mancus	LC	1345	50	673
Unio pictorum	LC	1300	30	390
Unio tumidus	NT	916	50	458

primer pair and sequenced in parallel with the samples to monitor for potential contaminants. Bioinformatic analysis and taxonomic assignment of sequences were conducted following the methodology outlined in Valentini et al. (2016) using the OBITools package (Boyer et al., 2016). Forward and reverse reads were first merged and demultiplexed, then each sample was segregated into a distinct dataset and dereplicated. Sequences shorter than 20 bp, occurring less than 10 times per sample, or identified as "internal" by the *obiclean* program were excluded. Taxonomic assignments were made using the *ecotag* program in combination with a database retrieved from GenBank V247 and the reference database of Prié et al. (2021). The eDNA sampling plan was opportunistic, taking advantage of various stakeholder projects related to research, water management, and impact studies. A total of 323 hydrobasins with at least one of the target mussel species were recorded by eDNA.

2.2.3. Species distribution models

Species distributions derived from either conventional or eDNA datasets were likely incomplete, missing potential occurrences in Hydrobasins that were neither surveyed nor sufficiently sampled. To fill this gap, we developed species distribution models (SDMs) for each species and then projected the predicted occurrences for all sub-catchments across France. This approach is commonly used to fill gaps in species distributions due to incomplete surveys and usually produces more robust results than using the raw occurrence data (Domisch et al., 2019). SDMs were generated using the algorithms available in the 'biomod2' package implemented in R v3.5.1 (Thuiller et al., 2016). We applied an ensemble forecast (using multiple modelling techniques) with generalised additive models (GAM), generalised linear models (GLM), Maxent and boosted regression trees (GBM) (Araújo and New, 2007). Nine ecological uncorrelated variables (Spearman's correlation <0.7) were extracted for each sub-catchment from HydroBASINS (Lehner and Grill, 2013) and BasinATLAS (Linke et al., 2019) and used as predictors related to the climate (2), physiography (3), hydrology (1), land cover (1), anthropogenic impacts (1) and geology (1) (Table S1 - Supplementary material). We generated 1000 pseudo-absence points for each run and adjusted the weights so that the sum of the weight presences equals the sum of the weight of the pseudo-absences. Data was partitioned into calibration (80%) and validation (20%) sets, and 10 repetitions were performed for model calibration and validation. Models were evaluated using receiver operating characteristic (ROC) curves,

and only models with ROC greater than 0.7 were retained. All suitable models were then used to predict the probability of occurrence in each sub-catchment using the 'BIOMOD_EnsembleModeling' and 'BIOMOD_EnsembleForecasting' functions in the biomod2 package in R (Thuiller et al., 2016). These were then transformed into predicted presence/absence using the optimal threshold function available in the 'Presence-absence' package in R (Freeman and Moisen, 2008).

To test the effect of using different data sources, and variable mixes of conventional versus eDNA data, we developed SDMs for each species using six different datasets. First, we built a model using all available data from both conventional and eDNA sources, a 'full dataset', which was assumed to represent the best approximation to the true species distributions, and thus used as a benchmark to compare the results obtained with reduced datasets. We then built models from datasets including either only conventional data or only eDNA data. Because conventional surveys were conducted over a much longer period and covered a larger area than eDNA surveys, we subsampled the conventional dataset to correct for differences in sampling effort. Specifically, the conventional dataset used in the analysis was produced by randomly selecting 323 out of the 843 Hydrobasins with conventional occurrence records, to simulate the same amount of data available in the eDNA dataset. This process was repeated 10 times, to avoid results becoming overly dependent on a particular configuration of occurrences randomly drawn from the full conventional dataset. Finally, we built combined models with different percentages of eDNA data: mix25 (25:75; eDNA: conventional) with 81 hydrobasins from eDNA and 242 hydrobasins from conventional, mix50 (50:50) with 162 hydrobasins from eDNA and 162 hydrobasins from conventional, and mix75 (75:25) with 242 hydrobasins from eDNA and 81 hydrobasins from conventional sources. In each case, the combined datasets were generated by randomly subsampling 10 times the eDNA and conventional datasets. As mentioned above, the models with ROC greater than 0.7 were retained and used to calculate the probability of occurrence of all species across all sub-catchments, which were then also transformed into predicted presence/absence. As each scenario only included a subset of the total data (except the full dataset), some species with fewer records were not modelled in the SDMs, and so we also compared the number of species modelled across the different datasets.

2.2.4. Identification of priority areas

The predicted species presence-absence data from the SDMs of all six datasets (full, conventional, eDNA and mixed), were used to identify priority conservation areas. This was done using Marxan software, which uses a simulated annealing optimisation algorithm to find an optimal combination of sub-catchments that achieve a set of pre-defined representation targets for all conservation features (genes, species or ecosystems) at minimum cost (Ball et al., 2009; Possingham et al., 2000). Marxan attempts to minimise an objective function that includes the sum of costs of the selected planning units, the sum of penalties for not achieving the representation targets for all conservation features (in our case the species), and penalties for not selecting spatially aggregated sub-catchments. Costs can be related to the area of each planning unit, the presence of invasive species affecting the target species, human disturbance (e.g. Human Footprint Index), investment required to conserve species/ecosystems, or an opportunity cost (e.g. lost socio-economic opportunities in the area) (Ban and Klein, 2009). However, as this study aimed to evaluate the influence of different data sources and in different proportions on the best solutions, we used a constant cost across all sub-catchments to rule out its potential influence (e.g. Hermoso and Kennard, 2012). The representation targets for all species were constant across all scenarios and were defined as a percentage of the number of occurrences (in Hydrobasins level 12) of each species in the full dataset. Percentages were set based on species conservation status in France, with higher values assigned to species of higher conservation concern, to ensure that they were given higher priority than non-threatened species in the final conservation portfolio (Table I). A high and constant SPF (species penalty factor) (SPF = 10) was set for all species to ensure a high penalty for solutions that did not meet the targets for all species. Finally, to ensure that the selected sub-catchments were spatially connected along the river network, we used the boundary file proposed in Hermoso et al. (2011), based on penalties for not connecting sub-catchments longitudinally. As strong connectivity is associated with a higher number of selected sub-catchments (Hermoso et al., 2011) and therefore cost, a connectivity strength modifier (CSM) was calibrated following the recommendations in Serra et al. (2020), resulting in a value of 1 for all datasets. The MARXAN algorithm was run 100 times (1000000 iterations) for each scenario and the best solutions were mapped in QGIS (3.14.1). For the conventional and mixed datasets, as we had 10 replicates each (and therefore 10 Marxan outputs), we calculated and mapped the frequency of selection of each sub-catchment across all replicates.

2.2.5. Comparison of Marxan outputs

We compared the best Marxan solutions for each reduced dataset (conventional, eDNA and mixed) against the benchmark corresponding to the conservation solutions generated using the full dataset, which was assumed to derive from the "true" species distributions. Comparisons were based on commonly used descriptors, including species representativeness (effectiveness), representation error rates (the omission and commission errors in species representation), the efficiency of the Marxan solutions, and the spatial overlap between the solutions.

Species representativeness was estimated as the percentage of species targets met for each conservation solution using the different datasets. We also estimated the percentage of targets that would be met if species not modelled (in the SDMs) for that scenario were included. This indicated the extent to which the sub-catchments selected by Marxan in a given scenario would, by chance, protect species that were not included in the analysis. To do this, we subsetted the list of sub-catchments selected by Marxan using the full dataset, to only those where the species occurred. Then, for each species not included in the initial targets of the reduced datasets conservation plan, we calculated the overlap between the sub-catchments selected according to the full dataset conservation plan, with the ones selected in each reduced dataset. In this way, we estimated the number of occurrences of those species that fell within the Marxan solution even though they were not included in the initial targets.

We also calculated the representation error rates associated with the occurrences of each species in all scenarios planning solutions by comparing the expected (ER) and observed (OR) species representation (error= (ER-OR)/ER), following Hermoso et al. (2015b). The expected representation referred to the number of species occurrences covered by the planning solutions selected by Marxan using each dataset. The observed representation referred to the number of species occurrences in the planning solutions using the full dataset. If the error is positive, it is an omission error, as Marxan incorrectly assumes that the species is absent in the selected sub-catchment, when compared to the full dataset. If the error is negative, it is a commission error, as Marxan incorrectly assumes that the species is present in the selected sub-catchments. It should be noted that in this study, omission and commission errors were calculated relative to Marxan solutions, rather than their more common use in the context of SDMs predictions. We assume that the perfect (idealised) dataset with no errors (commission or omission) would produce the most effective and efficient conservation allocation. As omission errors increase, so do inefficiencies, as we might select more areas than truly needed to cover species targets. In contrast, as commission errors increase, fewer species are actually protected, resulting in lower effectiveness.

The relative efficiency of the Marxan solutions generated using each dataset was calculated by the ratio between the number of sub-catchments required to achieve the conservation goals for each reduced dataset ($NS_{reduced}$) against the number of sub-catchments required for the full dataset (NS_{full}) (efficiency= (NS_{full} - $NS_{reduced}$)/

NS_{full})). We computed a total of 100 Marxan runs for each scenario and calculated the efficiency by averaging results across simulations for the full and reduced datasets (Hermoso et al., 2015b). This measure of efficiency conveys how well a conservation plan can represent the maximum variety of biodiversity at an acceptable cost and compactness (Margules and Pressey, 2000; Serra et al., 2020). If a scenario requires more sub-catchments than the full dataset to achieve its targets, it is less efficient, whereas a scenario that selects fewer sub-catchments than the full dataset is more efficient.

The overlap between the spatial conservation solutions generated using the full and each reduced dataset were calculated using the Jaccard's index. Specifically, the number of shared sub-catchments was divided by the sum of sub-catchments in the full dataset and the number of sub-catchments in the reduced scenario minus the number of shared sub-catchments.

3. Results

3.1. Species distribution models

Using the full dataset, it was possible to produce SDMs for the 29 target bivalve species, while the eDNA and conventional datasets produced SDMs for 27 and 21.9 ± 1.1 (Mean ± SD of the ten random replicates) species, respectively. With the eDNA dataset it was not possible to build models for *Euglesa interstitialis* and *Odhneripisidium moitessierianum*, while using the ten replicates of the conventional dataset it was not possible to build models for *Euglesa interstitialis, Euglesa parvula, Euglesa pulchella, Sphaerium ovale, Sphaerium rivicola* (all ten replicates), *Pseudunio auricularius* (eight replicates), *Pseudanodonta complanata* (seven replicates), *Odhneripisidium moitessierianum* (four replicates), *Sphaerium nucleus* (two replicates), *Anodonta cygnea, Margaritifera margaritifera, and Odhneripisidium tenuilineatum* (one replicate). When considering the mixed datasets, there was an increase in the number of SDMs produced with the increasing percentage of eDNA data included: mix75–26.9 ± 0.7; mix50–26.5 ± 0.5; and mix25–25.5 ± 1.2. (Mean ± SD of the ten random replicates) (Fig. 2). The mix25 dataset failed to model the distribution of *Euglesa interstitialis* (all ten replicates), *Sphaerium ovale* (eight replicates), *Pseudunio auricularius* (seven replicates), *Sphaerium rivicola* (five replicates), *Sphaerium nucleus* (two replicates), and *Pseudanodonta complanata* (one replicate); the mix50 failed to model *Euglesa interstitialis* (all ten replicates), *Sphaerium ovale* (nine replicates), *Sphaerium rivicola* (five replicates), and *Pseudunio auricularius* (one replicate); and the mix75 failed to model *Euglesa interstitialis* (all ten replicates), *Sphaerium ovale* (seven replicates), and *Pseudunio auricularius* (two replicates). The SDMs produced for all datasets achieved a ROC >0.7.

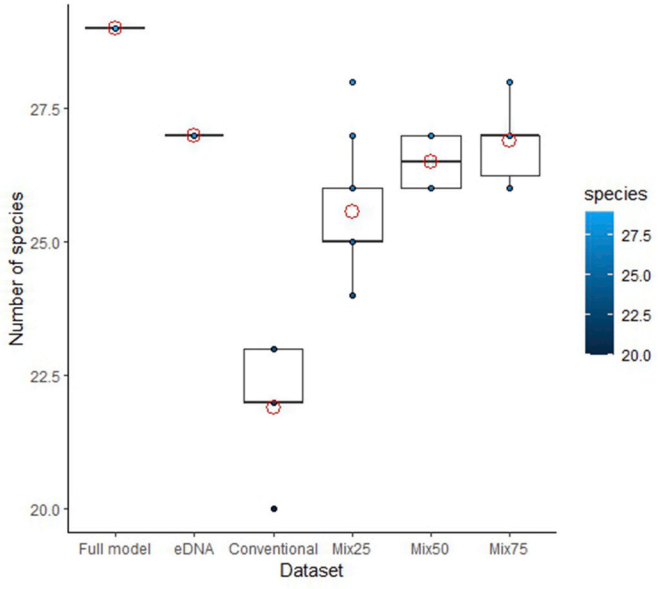

Fig. 2. Boxplots showing the number of species modelled with ensemble SDMs using different datasets. Red circles represent the average values. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

3.2. Comparison of Marxan outputs

The largest number of sub-catchments required to meet the conservation targets were found using the mix25 (2005.6 ± 129.2) and the full (1997) datasets, while slightly lower values were found for the eDNA (1986), conventional (1980.7 ± 149.5), mix50 (1920.4 ± 114.3) and mix75 (1712.8 ± 150.9) datasets.

Regarding species representativeness, the highest target achievement rate was obtained for the full (100%) and the conventional (92.8% ± 6.1) datasets, while the lowest values were obtained for the eDNA dataset (77.8%). The combined datasets produced intermediate representation levels, which were highest for mix50 (90.9% ± 4.3) and mix25 (88.4% ± 4.9), and lowest for mix75 (82.4% ± 5.7) (Fig. 3A). The percentage of representation targets achieved when including the non-modelled species was also higher for the conventional (87.7 ± 2.9) than the eDNA datasets (79.3%), with intermediate values for the combined dataset mix75 (81.7% ± 6.4), while mix25 (88.1 ± 4.8) and mix50 (89.3 ± 4.9) covered a higher percentage of targets (Fig. 3B).

The representation error rates regarding the omission errors were highest for the eDNA dataset and lowest for the conventional and mix75 datasets (Fig. 4A). As the commission error rates were highest for the conventional dataset and lowest for the mix50 dataset (Fig. 4B).

In terms of efficiency, the mix75 scenario was the most efficient, followed by the mix50, while the other scenarios, eDNA, conventional and mix25, showed similarly poorer results (Fig. 5). Given the fact that mix75 and mix50 also selected fewer sub-catchments, it is expected that these are the most efficient, while mix25, the eDNA, and the conventional scenarios require more sub-catchments to achieve conservation targets and are therefore less efficient. In fact, eDNA was the least efficient scenario, with an average of 2038 sub-catchments selected across 100 Marxan runs, compared to the average of 2024 sub-catchments selected with the full model. The number of sub-catchments selected across Marxan solutions was irrespective of the number of species included in the analysis as both eDNA (27 species) and conventional (21.9 ± 1.1 species) scenarios showed lower values of efficiency.

The sub-catchments selected in each scenario are shown in Fig. 6. The spatial overlap between the sub-catchments selected in the full dataset and the mix50 scenarios was highest (0.64 ± 0.07) and lowest between the full dataset and mix25 (0.56 ± 0.04).

4. Discussion

We tested different data acquisition methods to identify priority areas for bivalves' conservation in France. Our results support the idea that eDNA data can be a valuable source to complement conventional surveys and enhance conservation planning exercises. We found that mixed datasets, including both eDNA and conventional data, provided more effective and efficient conservation solutions by improving species representation while maintaining a cost-effective plan. Mixing both datasets, even at different proportions of eDNA and conventional data, mitigated the disadvantages associated with each distributional data source, and outperformed the use of eDNA and conventional data alone.

We modelled the distribution of more species using the full dataset, followed by the eDNA dataset. Using the conventional dataset alone, we produced a smaller number of species distribution models, especially for those with fewer records (rare and threatened species), which are in fact

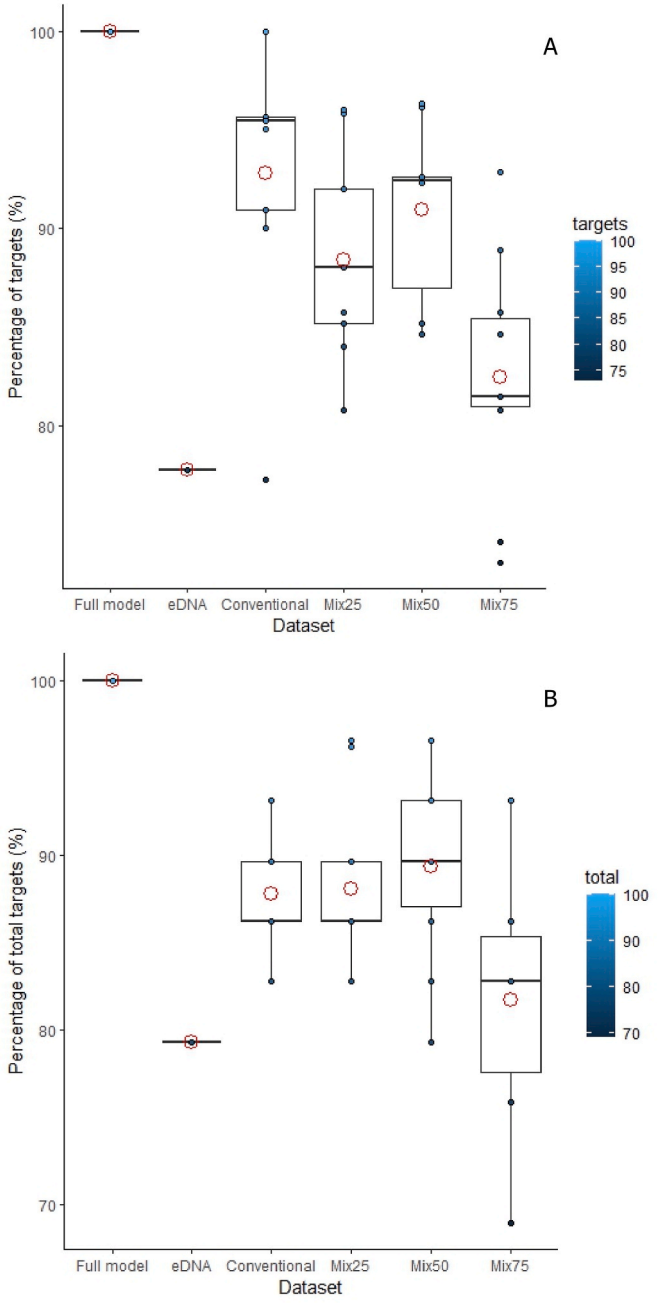

Fig. 3. Boxplots showing the percentage of species targets achieved in Marxan solutions using different datasets (A) and the percentage of species targets achieved in Marxan solutions including the species not modelled in each dataset. Red circles represent the average values. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

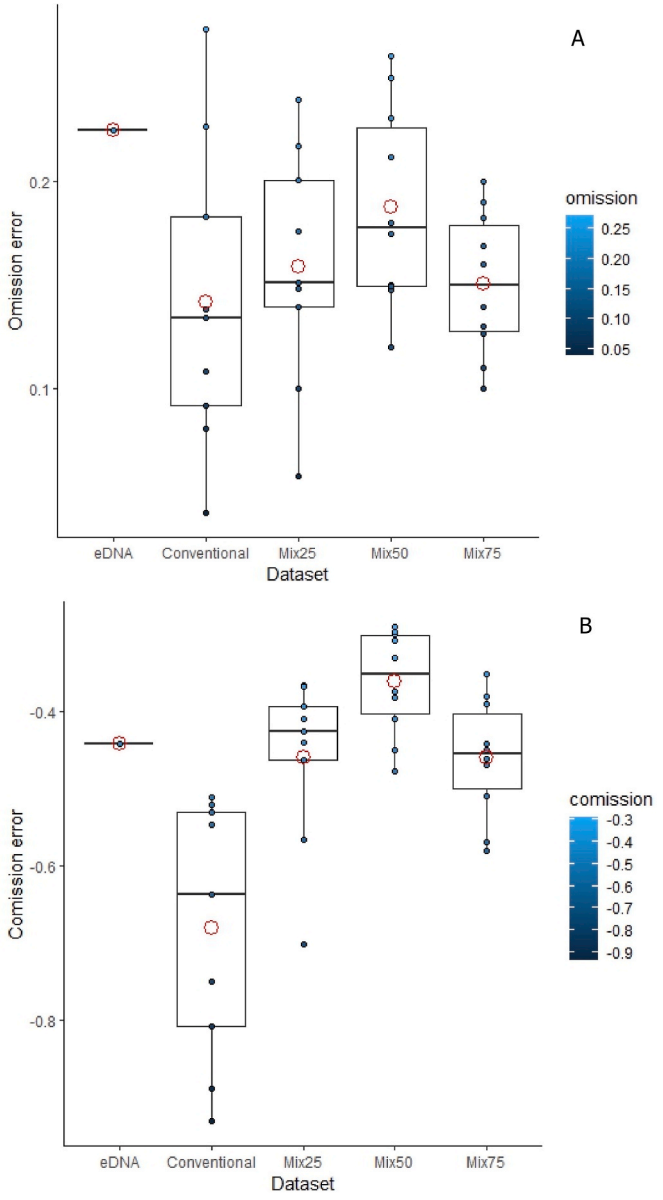

Fig. 4. Boxplots showing the omission (A) and commission (B) errors associated with the species occurrences covered by the planning solutions selected by Marxan using different datasets. Red circles represent the average values. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

the species that deserve more conservation attention. The conventional datasets often failed to model the distribution of species such as *Pseudunio auricularius* (critically endangered) or *Sphaerium rivicola* and *Pseudanodonta complanata* (endangered). Given that rare and threatened species are less common, distribution models that inform conservation plans can be compromised by insufficient records. By adding even a small amount of eDNA data to the conventional dataset, we were able to increase the number of species modelled with the SDMs. This supports the key findings of previous studies, such as Muenzel et al. (2024) who found that eDNA sampling methods were able to model more coral reef species distributions than conventional visual surveys.

As for the Marxan solutions, we found that the sub-catchments selected using the conventional data covered a higher percentage of species targets in the conservation assessments, but when we analysed the percentage of targets that included the species that were not modelled, we see that the combined models (mix25 and mix50) outperformed the conventional scenario. This should be expected, as the conventional dataset was able to model the smallest number of species, and therefore this scenario had a lower species representation than the combined models, when we consider the full species pool.

In addition, priority solutions selected by Marxan based on conventional datasets were associated with higher commission errors. Higher commission errors indicate that the Marxan algorithm selected sub-catchments on the assumption that the species was present and thus

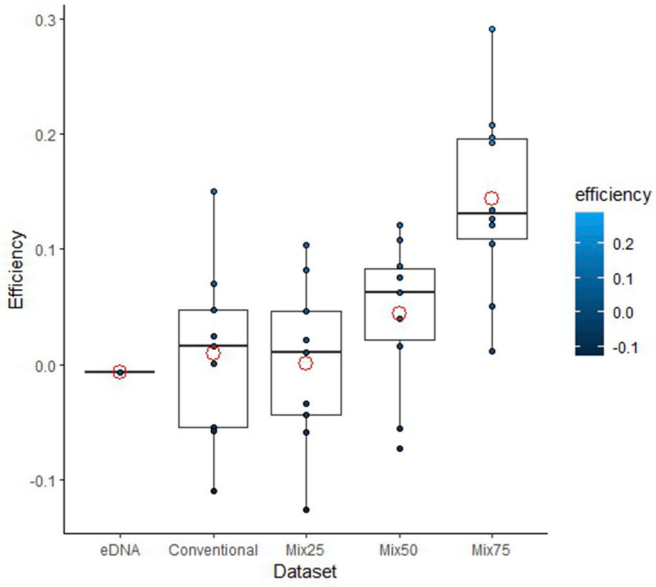

Fig. 5. Boxplots of the efficiency of each planning solution selected by Marxan using different datasets. Red circles represent the average values. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

had already secured the species representation. This may not be the case, as the SDMs constructed using the conventional data to feed Marxan incorrectly indicated the presence of the species in that sub-catchment. We also found that solutions using the eDNA dataset were associated with higher species omission errors. This means that the species distribution models informed Marxan that the species was absent from that sub-catchment, which may not be the case. Therefore, the Marxan algorithm was forced to add unnecessary sub-catchments to ensure the species representation. This is consistent with the results of the efficiency assessment which showed a lower efficiency of the conservation plan using the eDNA dataset. Hermoso et al. (2013) also found that as the omission errors increase, more sub-catchments are selected to meet species representation, reducing the overall efficiency of the conservation plan. Previous studies have also addressed the fact that acquiring more species data through conventional sampling does not automatically lead to better conservation planning outcomes (Hermoso et al., 2015a), and that combining different types of data (e.g. presence and presence-absence) may help reduce omission and commission errors (Hermoso et al., 2015b). Unlike the findings of these previous studies, we did not find higher rates of species representation errors to be associated with the rarer species, but with both common and rare species.

Because conservationists often do not have access to such a large amount and variety of data and given the fact that the acquisition cost and time of eDNA and conventional data are different, they face a trade-off between investment and the quality/quantity of data available for decision-making. Conventional data collection can rely on species records that are free and readily available in public databases but may be associated with higher commission errors. This situation can compromise a true species representativeness, and therefore the effectiveness of the conservation plan. Using only conventional data sources to predict species distributions can also be associated with taxonomic biases, especially for rare or cryptic species, often depends on more complex and expensive protocols, and can be more time-consuming (Thomsen and Willerslev 2015; Yamamoto et al., 2017). Conversely, the use of eDNA alone allows organisms to be identified in a more cost and time effective way, but as shown here, can be associated with higher omission errors. Increasing the rate of omission errors makes our final solution less efficient and potentially compromises our conservation plan. When collecting eDNA data alone, our results can be compromised by some of the limitations faced by eDNA sampling methods, such as the limited and environment-dependent persistence of eDNA (Roussel et al., 2015), sample contamination (Turner et al., 2014), ancient DNA resuspension (Wu et al., 2018), or DNA transport variability, which is particularly important in connected freshwater habitats, as DNA shed by aquatic organisms can be transported and degraded along the river (see Beng and Corlett, 2020 for a detailed description).

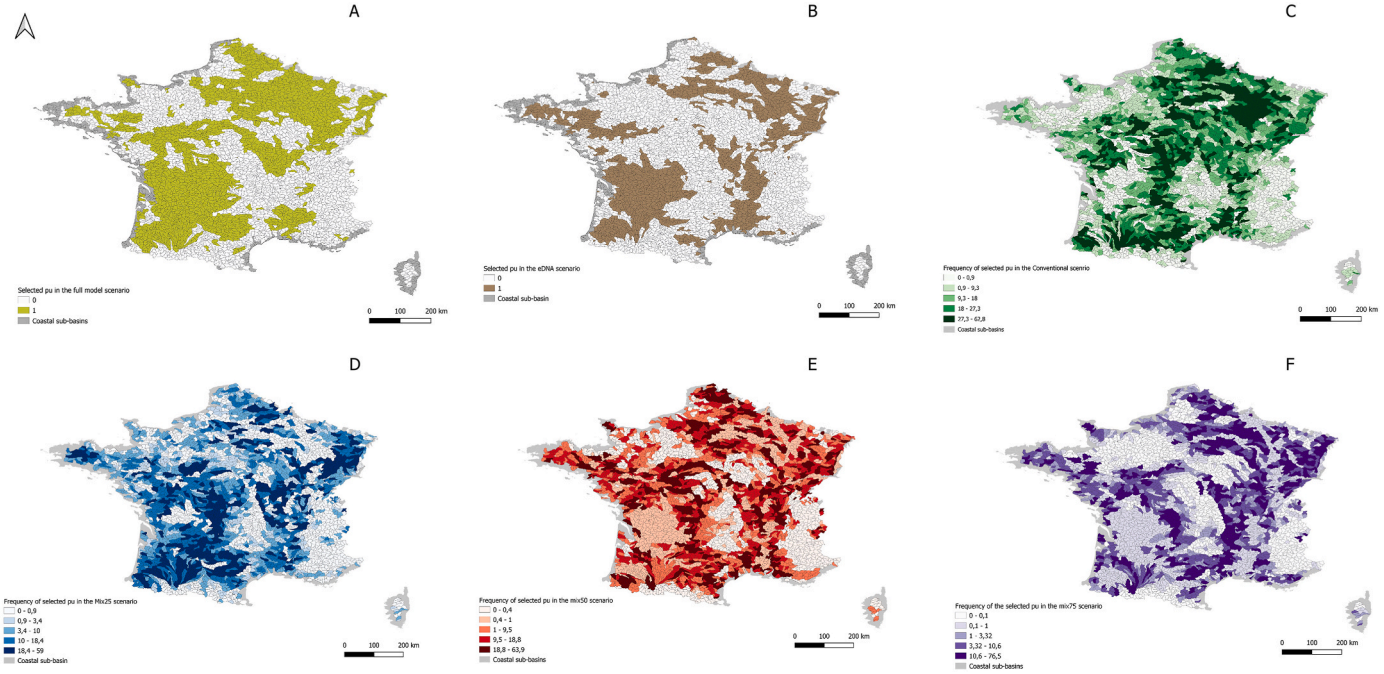

Fig. 6. Maps of the selected sub-catchments (pu; planning units) by Marxan using the full dataset (A), the eDNA dataset (B), the conventional (C), and combined datasets, mix25 (D), mix50 (E), mix75 (F).

Overall, the combined dataset scenarios performed better in terms of efficiency and effectiveness, and according to our Jaccard index results and the maps produced with each of the selected scenario priority areas, the mix50 scenario is the closest to the full dataset, and therefore to the most proximal distribution of the freshwater bivalves in France. Despite being a subset of the full data, reduced datasets have already proved to be successful at designing priority areas adequate at representing species (Hermoso et al., 2013). The use of both types of data (combined models) whenever they are available, provided more robust conservation solutions, as they complemented each other and helped to reduce omission and commission errors and therefore, leading to better outcomes in conservation decisions. Integrating eDNA and conventional species distribution data has the advantage of being applicable to any geographic area, to any ecosystem or species and is particularly useful for improving knowledge in data-poor areas (Adams et al., 2024), and for the coverage of rare species. This approach may therefore improve the representativeness of those species that will benefit most from the success of conservation plans. Nevertheless, we advise thoughtful consideration of potential variations in scale, sample size and spatial distribution of sites from each data source that may affect SDMs predictions and therefore hinder conservation planning outcomes. To the best of our knowledge, this study is the first to combine eDNA and conventional distribution data to select priority areas using Marxan for freshwater species conservation. In a similar study, in marine ecosystems, Muenzel et al. (2024) found that combining eDNA and visual survey data to prioritise areas for coral reef protection in Indonesia was better at informing conservation design, despite the low overlap between areas selected by each method. However, this study did not report information on the efficiency of conservation plans or the representation error rates of eDNA and visual survey data. Adams et al. (2024) also combined eDNA and visual survey data but for prioritising restoration actions for the endangered frog *Rana boylii* in southern Sierra Nevada and found that this mixed approach allowed a reduction in costs while improving the success of reintroductions and management efforts.

Although not intended to provide a true on-the-ground conservation assessment for the region, this work is particularly important to guide future conservation decisions across all ecosystems. As we witness European efforts to include 30% of its territory in protected areas by 2030, eDNA sampling techniques are expected to play a greater role in informing these expansion plans or confirming the presence of those species for which some protected areas are designated. In addition, highlighting a less charismatic (Mammola et al., 2020) taxonomic group such as freshwater bivalves, may help to tilt the 2030 conservation targets towards investment in the protection of such threatened species. Finally, we recommend careful planning of investment in data collection, and before investing in more conventional sampling efforts, conservationists should take advantage of this mixed approach, especially given the declining cost of eDNA methods.

CRediT authorship contribution statement

Joana Garrido Nogueira: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Arnaud Lyet:** Writing – review & editing, Methodology, Formal analysis. **Virgilio Hermoso:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Pedro Beja:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Manuel Lopes-Lima:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Vincent Prié:** Writing – review & editing, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.124852.

Data availability

Data will be made available on request.

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