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Identifying freshwater priority areas for cross-taxa interactions

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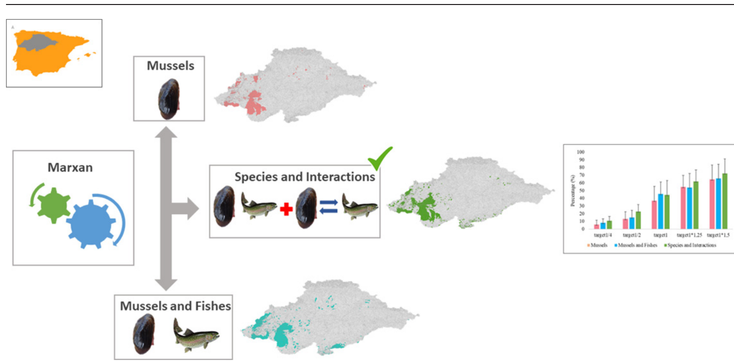
HIGHLIGHTS

- Biotic interactions determine biodiversity patterns and ecosystem functioning.
- Despite its importance, biotic interactions are rarely considered in conservation.
- We demonstrate a novel framework to include biotic interactions in conservation planning.
- We use freshwater mussels and their fish hosts in spatial prioritisation analyses.
- Biotic interactions are less covered when not explicitly included in the analyses.

GRAPHICAL ABSTRACT



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ABSTRACT

Information about biotic interactions (e.g. competition, predation, parasitism, diseases, mutualism, allelopathy) is fundamental to better understand species distribution and abundance, ecosystem functioning, and ultimately guide conservation efforts. However, conservation planning often overlooks these important interactions. Here, we aim to demonstrate a new framework to include biotic interactions into Marxan. For that, we use freshwater mussels and fish interaction (as mussels rely on fishes to complete their life cycle) in the Douro River basin (Iberian Peninsula) as a case study. While doing that, we also test the importance of including biotic interactions into conservation planning exercises, by running spatial prioritisation analysis considering either: 1) only the target species (freshwater mussels); 2) freshwater mussels and their obligatory hosts (freshwater fishes); 3) freshwater mussels, fishes and their interactions. With this framework we found that biotic interactions tend to be underrepresented when the data on both freshwater mussels and fishes is not simultaneously included in the spatial prioritisation. Overall, the priority areas selected across all scenarios are mostly located in the western part of the Douro River basin, where most freshwater

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mussels and fishes still occur. Given the low overlap of priority areas identified here and the current Natura 2000 network, our approach may be useful for establishing (or enlarging) protected areas, especially in light of the EU Biodiversity Strategy for 2030. Also, this work may provide guidance for future habitat restoration and management of main threats to freshwater biodiversity.

1. Introduction

Biotic interactions are fundamental for maintaining natural diversity and ecological processes and are key to understanding species distribution and abundance dynamics, as well as ecosystem functioning (Bairey et al., 2016). Species distribution, population dynamics and ecosystem functioning can be shaped by competition (Brown, 1990), predation (McPeek, 1990), parasitism (Paplauskas et al., 2021), disease (Zipkin et al., 2020), mutualism (Palmer et al., 2010), allelopathy (Rasher and Hay, 2010) and, probably in most cases, a combination of different biotic interactions (Omacini et al., 2001). These biotic interactions are also key from a biodiversity conservation point of view. Species that rely on specialised interactions, as well as on complex life histories, may incur an elevated risk of co-extinction (the extirpation of a taxon caused by the loss of another taxon; Koh et al., 2004; Dunn et al., 2009). The risk is even higher for species that cannot evolve new traits after interactor loss or replace partners in communities exposed to novel environmental conditions due to climate change and/or the introduction of non-native species, among other disturbing factors (Brodie et al., 2014). Given the relevance of biotic interactions for nature conservation, their role should be considered when planning priority areas for biodiversity (Brodie et al., 2014; Linke et al., 2019; Heinen et al., 2020; da Silva et al., 2022).

Resources for biodiversity conservation are often limited and it is essential to identify priorities that guide decision-making on where and how to conserve biodiversity, for establishing new protected areas and/or applying management actions (e.g. restoration of habitats or mitigation of threats; Game et al., 2013; Carvalho et al., 2017). Systematic conservation planning aims to inform decision-making to ensure adequate biodiversity representation in a cost-effective way (Margules and Pressey, 2000; Margules et al., 2002). Conservation planning in freshwater ecosystems has lagged behind terrestrial and marine realms due to the complexity of river connections, the lack of distribution data, and their high spatial and temporal variability (Roux et al., 2008; Collier, 2011; Darwall et al., 2011). Previous studies have included freshwater particularities in conservation planning, i.e., accounting for longitudinal and lateral connectivity along stream networks, the importance of river flow, or using sub-catchments or river stretches as planning units instead of grid cells (Hermoso et al., 2011; Hermoso et al., 2012; Gomes-dos-Santos et al., 2019). However, systematic conservation planning exercises often overlook biotic interactions like predation, competition, parasitism, diseases, or mutualisms that might affect species persistence (but see Decker et al., 2017; Rayfield et al., 2009).

Freshwater mussels (Bivalvia, Unionida) provide a case in point for the importance of considering biotic interactions in conservation planning. This is because they are a highly imperilled taxonomic group worldwide (Lopes-Lima et al., 2017), and they rely on fishes to complete their life cycle (Modesto et al., 2018). Their complex life cycle includes a parasitic stage in which their larvae (glochidia) only develop when attached to a suitable fish species. Therefore, the viability of mussel populations is completely dependent on the presence of suitable fish hosts (Modesto et al., 2018). Some mussel species, such as *Margaritifera margaritifera*, have very narrow host fish ranges, with their glochidia developing in two fish species (*Salmo trutta* and *Salmo salar*), while others like *Anodonta anatina*, being more generalists can use many fish hosts (Taeubert and Geist, 2017; Douda et al., 2013; Dias et al., 2020). Given this dependency, the conservation status of mussel species is highly associated with the distribution and abundance of their host fishes. Therefore, the local decline or extirpation of freshwater fish populations may have a strong negative impact on freshwater mussels (Modesto et al., 2018). While previous studies have prioritised areas for the conservation of mussel species, they mostly used abundance and/or distribution (e.g., Wilson et al., 2011; Leonard et al., 2017), while to the best of our knowledge, none has included the mussel-fish interaction.

Here, we provide a novel framework of incorporating biotic interactions in systematic conservation planning using freshwater mussels and fishes as a model. For that, we use Marxan, a spatial prioritisation tool commonly used for conservation planning, to demonstrate the importance of considering not only the target species (in our case the freshwater mussels), but also their obligatory hosts (freshwater fishes), and their interactions. We focused the study on the Douro River basin, which is the largest one in the Iberian Peninsula and it has relatively diverse native assemblages of both freshwater mussels and fishes (Nogueira et al., 2021a, 2021c), and for which the mussel-fish interactions are very well known (Modesto et al., 2018; Dias et al., 2020). We identified areas in the Douro basin where interactions between freshwater mussels and fishes probably occur by overlapping the mussels and respective hosts distributions, and then evaluated the coverage of these areas under different planning scenarios, using data on either: 1) only freshwater mussel distribution (hereafter 'Mussels' scenario); 2) freshwater mussel and fish distributions (hereafter 'Mussels and Fishes' scenario); and 3) freshwater mussel and fish distributions, as well as their interactions. For the latter case, we treated the areas of potential ecological interactions between freshwater mussels and fishes as additional species (hereafter 'Species and Interactions' scenario). We hypothesise that the 'Species and Interactions' scenario outperforms the others at representing mussel-fish interactions and, therefore, would provide more adequate conservation guidance for mussel species in the Douro River basin.

2. Material and methods

2.1. Study area

The Douro River basin is the largest in the Iberian Peninsula (area 97,603 km^2) flowing westward across north-western Spain and northern Portugal. The Douro River has a total length of 895 km, and its catchment is highly fragmented by multiple dams and weirs, resulting in habitat fragmentation, loss of habitat connectivity, and flow regulation (Sousa et al., 2020). The spread of invasive species (such as *Corbicula fluminea*, *Pacifastacus leniusculus*, *Procambarus clarkii*, *Esox lucius*, *Lepomis gibbosus*, among others), water shortage, and eutrophication are also responsible for the loss of freshwater biodiversity in the Douro River basin (Anastácio et al., 2019; Meira et al., 2019; Sousa et al., 2019). Also, the basin is deeply affected by intensive agriculture, being the main crops associated to cereal, corn, sunflowers, and vineyards. Despite these threats, the Douro River basin holds populations of 4 native freshwater mussel species, some of conservation importance, such as *Margaritifera margaritifera* and *Potomida littoralis* listed in the IUCN Red List of threatened species as Endangered, in addition to *Unio delphinus* (Near Threatened) and *Anodonta anatina* (Least Concern). Also, there are some fish species described for the Douro River basin of conservation importance such as *Cobitis paludica*, *Pseudochondrostoma duriense*, or *Squalius alburnoides*, all endemic species of the Iberian Peninsula currently listed as Vulnerable by the IUCN Red List.

2.2. Mussel and fish field surveys

Field surveys were conducted in June 2017 and June 2018, covering a total of 183 sites throughout the Douro River basin (Fig. 1), to characterise the presence/absence of freshwater mussel and fish species. Each site corresponds to a 100 m river stretch covering all types of habitats (e.g. lentic and

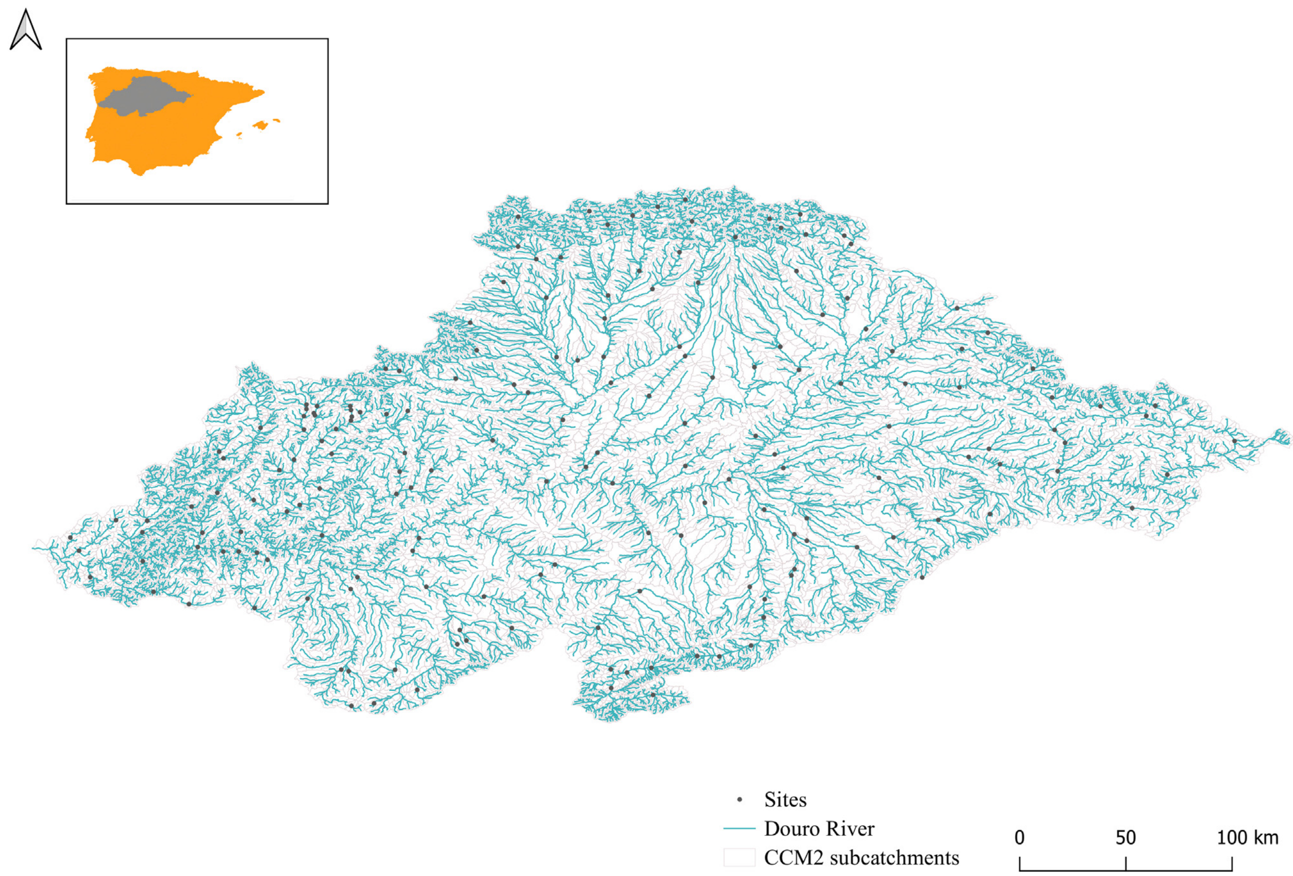

Fig. 1. Map of the 183 sites sampled during June 2017 and June 2018 in the Douro River basin (Iberia).

lotic, banks and centre of the channel) as well as substrate (e.g. pebbles, cobbles, sand, silt, clay, macrophytes).

Freshwater mussels were sampled by snorkelling and by hand searching (following Sousa et al., 2019). These surveys were performed by three divers and mussels were found visually or by hand searching through the bottom when visibility was low, as well as by digging the sediments. Specimens were identified and then returned to their original position. Fish fauna was surveyed by electrofishing using the portable equipment Hans Grassl with a pulsed DC-300-600 V generator (following Nogueira et al., 2021a). Fish were collected and identified to the species level, and then released to the water.

Data regarding the freshwater mussels and their respective fish hosts (hence mussel-fish interactions) is summarised in Table 1. The information about these interactions was gathered from previous studies, some of which performed in the study area. A total of 11 native fish species were collected from the surveys, from which 7 species are suitable hosts and used for this study. There are more fish hosts available for the mussels targeted in this study (e.g. *Anodonta anatina* can develop their gloquidia in other fish species like *Barbus barbus* or *Scardinius erythrophthalmus*; Douda et al., 2013) but we only considered the fish hosts that were surveyed in the study area.

2.3. Data analysis

2.3.1. Modelling species distribution

To identify priority areas for freshwater conservation we considered the sub-catchment as our planning unit, using the Catchment Characterisation Model (CCM2). CCM2 covers the river segments and catchments of Europe based on a digital elevation model with a 100-metre resolution (de Jager et al., 2007). The Douro River basin is divided into 14,542 planning units, each containing the river length and contributing area (with an average 6,7 km^2, ranging from 0,1 km^2 to 174 km^2). Since we did not sample all planning units, and Marxan requires information about species distribution across all planning units, species occurrences (of both mussels and fishes) were modelled and projected to the whole study area. Species presence-absence records were used to build species distribution models (SDMs) with modelling algorithms available in the R package 'biomod2' v3.5.1 (Thuiller et al., 2016) using an ensemble forecasting approach to avoid algorithmic uncertainty (Araújo and New, 2007). Thirteen uncorrelated variables (Spearman's correlation < 0.7) were extracted for each sub-catchment (according to CCM2), and used as predictor variables, intending to describe ecological conditions related to the variability in climate (4),

Table 1
List of freshwater mussel species and respective fish hosts occurring in the study area.

Mussel	Hosts	References
Margaritifera margaritifera	*Salmo trutta*	Lopes-Lima et al., 2017
Unio delphinus	*Achondrostoma oligolepis*	Lopes-Lima et al., 2020
	Luciobarbus bocagei	
	Pseudochondrostoma duriense	
	Salmo trutta	
	Squalius alburnoides	
	Squalius carolitertii	
Anodonta anatina	*Achondrostoma oligolepis*	Douda et al., 2013
	Cobitis paludica	
	Luciobarbus bocagei	
	Pseudochondrostoma duriense	
	Salmo trutta	
	Squalius alburnoides	
	Squalius carolitertii	
Potomida littoralis	*Achondrostoma oligolepis*	Ramos, 2011
	Luciobarbus bocagei	
	Pseudochondrostoma duriense	
	Salmo trutta	
	Squalius alburnoides	
	Squalius carolitertii	

topography (2), hydrology (3), land cover (1), anthropogenic impacts (1) and geology (2). SDMs were fitted using seven different modelling algorithms, namely generalised linear models (GLM), generalised additive models (GAM), boosted regression trees (GBM), classification tree analysis (CTA), flexible discriminant analysis (FDA), multiple adaptive regression splines (MARS) and random forests (RF). For each algorithm, data were partitioned into calibration (80 %) and validation (20 %), 10 repetitions were performed for model calibration and validation. For each species, models were evaluated using the True Skill Statistic (TSS), a measure of overall accuracy of SDMs, ranging from −1 to 1 (Allouche et al., 2006). The predictions that resulted from each algorithm were combined in an ensemble forecast, including only individual models with TSS values above 0.7 (Coetzee et al., 2009). Species probabilities of occurrence were then transformed in presences/absences using the 'optimal threshold' function available in the presence-absence package in R (Freeman and Moisen, 2008). The distribution of each mussel and respective fish host species (Table 1) was used to identify sub-catchments where the ecological interaction of interest could occur. We assumed the mussel-fish host interactions as co-occurrences of a mussel and a suitable fish host species in the same sub-catchment.

2.3.2. Identification of priority areas

Marxan was used to identify priority areas for the conservation of freshwater mussels in the Douro River basin. Marxan uses a simulated annealing optimization algorithm to find an optimal combination of planning units (sub-catchments in our case) that allow achieving a set of predefined representation targets for all conservation features at minimum cost (Possingham et al., 2000; Ball et al., 2009).

$$\text{Objective function} = \sum_{\text{planning units}} \text{Cost} + \sum_{\text{features}} \text{SPF} \times \text{Feature Penalty} + \text{CSM} \sum_{\text{planning units}} \text{Connectivity penalty} \quad (1)$$

To do this, Marxan tries to minimise an objective function (Eq. (1)) that includes the sum of costs of planning units selected, the sum of penalties for not achieving the representation targets for all conservation features, weighted by a Species Penalty Factor (SPF) and penalties for not selecting spatially aggregated planning units, also weighted by a Connectivity Strength modifier (CSM).

Costs can be represented by the area of each planning unit, a measure of human disturbance (e.g. Human Footprint Index) or an opportunity cost (e.g. socio-economic opportunities missed if an area needs to be devoted to a different use than currently used for). Given that the objective of this study was to evaluate the influence of different conservation planning scenarios, we used a constant cost across all sub-catchments to rule out its potential influence from our solutions and make scenarios more comparable (e.g. Hermoso et al., 2012). We used a high SPF (SPF = 10) for all species to ensure that all of them achieve their targets. Finally, to ensure that solutions were connected along the river network we used the boundary file suggested in Hermoso et al. (2011), based on penalties for not connecting sub-catchments longitudinally. Given that strong connectivity comes at a higher number of sub-catchments selected (Hermoso et al., 2011), a connectivity strength modifier (CSM) needs to be calibrated. We calibrated CSM following recommendations in Serra et al. (2020), resulting in a value of 0.02 for all scenarios. We also tested the influence of no connectivity penalty in the results by performing the same analyses but with a CSM value of 0.

2.3.3. Planning scenarios

The framework we present here to include interactions in Marxan, assumes the distribution of areas of potential ecological interactions as additional species in the analyses. This means that we only consider that these 'species' are present when there is a prediction by the models that mussels and hosts co-occur, and that they are known to interact. So, planning units that contain both a mussel and a host fish known to interact, are considered as a presence of that 'species'. To explore the importance of including biotic interactions in conservation planning we tested three different scenarios: i) 'Mussels'; ii) 'Mussels and Fishes'; and iii) 'Species and Interactions'. The 'Mussels' scenario only accounts for mussel distribution; the 'Mussels and Fishes' scenario identifies priority areas based on the distribution of the mussels plus fish host species; and the 'Species and Interactions' scenario included the data on mussels, fishes and their interactions, as described above (20 features; Table 1). By doing so we did not only aim to represent the distribution of each species individually, but also the interactions.

The targets were set using species number of occurrences in the basin and conservation status (target 1). Species with poorer conservation status had higher targets and vice versa. By setting higher targets to species that are more likely to go extinct, we ensure that the models prioritise the areas where these species occur. Regarding the targets of the interactions (that were considered as 'species' in the analyses), we always assumed the conservation status of the most threatened category. For instance, in a mussel-fish pair where one species is Endangered (IUCN Red List) and another is Least Concern, we calculated our target based on the Endangered status. We also tested the influence of the targets in the final solutions by setting different values: half (target1/2), a quarter (target1/4), times 1,25 (target1 ∗ 1.25) and times 1,5 (target1 ∗ 1.5) of the original target 1. These targets were replicated for each of the three scenarios. All analyses were performed for both CSM values of 0.02 and 0. We ran Marxan 100 times and kept the best solution over those runs for comparisons across scenarios.

We measured the interactions representation, i.e., the capacity that each solution has on covering the total number of interactions for each mussel-fish pair. We also measured the spatial overlap between the best solutions by using the Jaccard index. This index measures the similarity between two datasets, ranging from 0 (no similarity) to 1 (total similarity), and is calculated by dividing the number of shared sub-catchments between solutions by the sum of sub-catchments in each solution minus the number of shared sub-catchments in both solutions. Finally, we calculated the spatial overlap between the Natura 2000 network with the results of the Marxan scenarios to determine the percentage of area covered by the currently designed protected areas, using QGIS (3.14.1). We only considered the sub-catchments that have at least 50 % of the area covered by the Natura 2000 network.

3. Results

The total number of possible interactions per sub-catchment, i.e., number of co-occurrences between mussels and their fish host species, range between 0 and 16. Highest values occur in the western part of the catchment (where the number of mussel and fish species is also higher). All scenarios achieve their respective targets, and on average (for CSM value of 0.02), the scenario requiring fewer planning units to achieve them was the 'Mussels', followed by the 'Species and Interactions', and then the 'Mussels and Fishes' scenario (Table 2). For instance, for target 1, the 'Mussels' scenario selects 1328 planning units, the 'Species and

Table 2
Number of planning units selected in the best solutions across the different targets and scenarios.

		'Species and Interactions'	'Mussels and Fishes'	'Mussels'
CSM > 0	target1/4	466	583	226
	target1/2	976	1014	456
	target1	2034	2050	1328
	target1 ∗ 1,25	3043	2962	2282
	target1 ∗ 1,5	3897	3871	2967
CSM = 0	target1/4	410	415	225
	target1/2	835	837	451
	target1	1811	1738	902
	target1 ∗ 1,25	2311	2279	1128
	target1 ∗ 1,5	2823	2813	1354

Interactions' scenario selects 2034, and the 'Mussels and Fishes' selects 2050. As expected, less ambitious targets correspond to fewer planning units selected (Table 2).

The 'Species and Interactions' scenario is the one that represents more interactions across all targets (except for target 1; Fig. 2A). For this target, the average percentage of interactions covered is 45.7 % (±15.6) in the 'Mussels and Fishes' scenario, and 43.4 % (±16.8) in the 'Species and Interactions' scenario, as for the 'Mussels' only 36.9 % (±18.8) of interactions are covered. The interaction *Potomida littoralis* ∗ *Achondrostoma oligolepis* is the one better covered when we apply the target 1, with an average 85 % (±1.9) of its distribution secured by these solutions, while the interaction *Unio delphinus* ∗ *Salmo trutta* only has a 23.2 % (±3.4) coverage. On average, and across all targets, the 'Species and Interactions' scenario covers 42.6 % (±23.1), the 'Mussels and Fishes' scenario covers 37.9 % (±22.4) and the 'Mussels' scenario covers 35.1 % (±22.7) of all interactions, for CSM = 0.02. On average, for CSM = 0, the 'Species and Interactions' scenario covers 37.3 % (±18.5), the 'Mussels and Fishes' scenario covers 36.3 % (± 18.4) and the 'Mussels' scenario covers 28.4 % (±15.4) of all interactions (Fig. 2B).

Despite the lower performance in representing interactions of the 'Mussels and Fishes' and 'Mussels' scenarios, all the scenarios perform well for some: *Anodonta anatina* ∗ *Cobitis paludica*, *Potomida littoralis* ∗ *Pseudochondrostoma duriense*, *Potomida littoralis* ∗ *Squalius carolitertii*, *Potomida littoralis* ∗ *Achondrostoma oligolepis*, *Potomida littoralis* ∗ *Luciobarbus bocagei* and *Potomida littoralis* ∗ *Squalius alburnoides* (Table S1, Supplementary material). On the other hand, for interactions such as *Potomida littoralis* ∗ *Salmo trutta*, both the 'Mussels' and 'Mussels and Fishes' scenarios do not perform so well, being that only the 'Species and Interactions' scenario is able to cover a higher percentage of these interactions.

Regarding the spatial distribution of solutions, all scenarios concentrate most of the selected sub-catchments in the western part of the Douro River basin (Fig. 3). Also, the three scenarios represent patches of connected sub-catchments, with some being selected across the different models. Others, such as the ones selected in the southern part of the Douro River basin, are not present in the 'Mussels' scenario, when we only consider the mussel data.

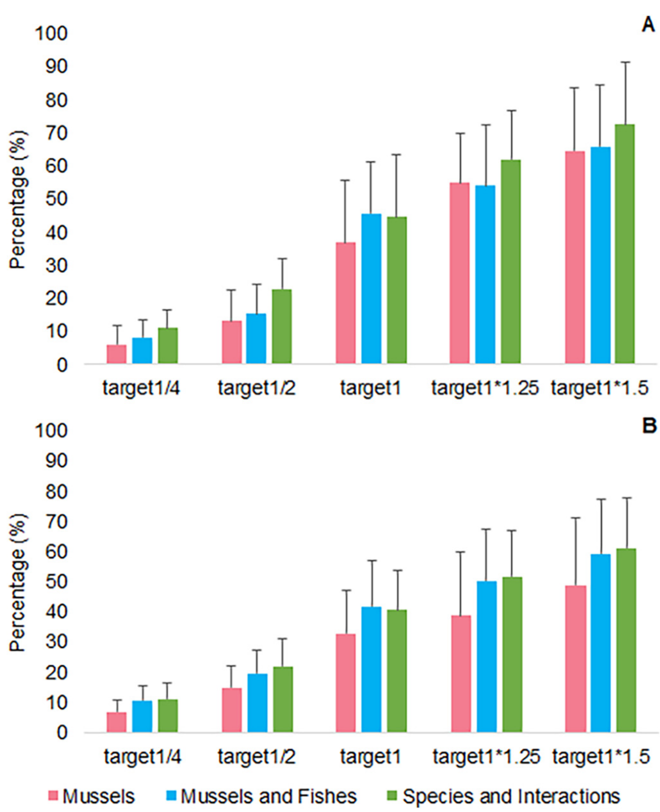

Fig. 2. Average percentage (and standard deviation) of the interactions covered in each of the three scenarios: 'Mussels' (pink), 'Mussels and Fishes' (blue) and 'Species and Interactions' (green), across the different targets for CSM = 0.02 (A) and CSM = 0 (B).

The spatial overlap (Jaccard index) between the three different scenarios is always below 0.5 (except between the 'Mussels and Fishes' and 'Mussels' scenarios for target1 ∗ 1.25; Table 3). Regarding the different targets, the spatial overlap between the scenarios is lower when the targets are also lower, and higher with the target1 ∗ 1.25. Overall, the spatial overlap between the different scenarios is lower when there is no connectivity penalty, and in this case the highest overlaps relate to target1 ∗ 1.5.

The percentage of selected sub-catchments covered by the Natura 2000 network was very low for all scenarios across every target (Table 4). On average, only 5.6 % (±1.3) of the 'Mussels' scenario, 4.2 % (±0.9) of the 'Mussels and Fishes' scenario and 3.8 % (±1.3) of the 'Species and Interactions' scenario overlap the Natura 2000 protected area network.

4. Discussion

We demonstrate a novel way to include ecological interactions in Marxan, using the freshwater mussels and their obligatory fish hosts as a case study. This study also reveals the importance of including biotic interactions in systematic conservation planning exercises. We found that when not explicitly accounted for, biotic interactions for some species tend to be underrepresented, when all the species involved in such interactions are not simultaneously included in the spatial prioritisation. This is one of the few studies that explicitly considers the importance of biotic interactions in systematic conservation planning, as most studies focus on ecosystem functions and services (Heinen et al., 2020; Tylianakis et al., 2010). These are also important topics to acknowledge in the conservation of ecosystems, but for specific species that rely on complex biotic interactions, such as freshwater mussels and fishes, it may be insufficient to prevent extinction. Other studies have included biotic interactions in spatial prioritisation exercises using Marxan (Decker et al., 2017), or Zonation (Rayfield et al., 2009) but mostly focusing on predator-prey interactions. For instance, Decker et al. (2017) included the predator-prey interactions to assess refuge areas for freshwater fishes in lower trophic positions in the Danube River. In that study, the authors included the data about predator-prey interactions by giving a higher cost to the planning units where the predators occur, lowering the probability of Marxan choosing those planning units. In our case, this would not be an option since our objective was to increase the coverage of areas where mussels and fish hosts co-occur and not to Marxan penalise them (and therefore not selecting them). Since our framework incorporates biotic interactions by adding them as additional 'species' in Marxan, we can associate our interactions with a target and a penalty if that interaction is not met. Also, our framework allows the inclusion of multiple interactions, can be used for either increasing or decreasing the overlap between the interactors and can be applied to other types of interactions in terrestrial, freshwater or marine realms. Studies modelling obligatory interactions such as shown here are lacking and to the best of our knowledge no study has incorporated their distribution as a new 'species' in Marxan for spatial prioritisation analysis.

4.1. Relevance of including biotic interactions in systematic conservation planning

A key result of our study is that relying only on the data of the species of conservation focus ('Mussels' scenario) might be insufficient when dealing with the ecological needs of species, such as freshwater mussels, that are dependent on other species to complete the life cycle. By accounting for biotic interactions ('Species and Interactions' scenario), or at least the whole pool of species ('Mussels and Fishes' scenario), areas where these species co-occur are better covered, which is fundamental for the persistence of freshwater mussels. This is especially true when dealing with less ambitious conservation targets, as a combination of low targets and only accounting for

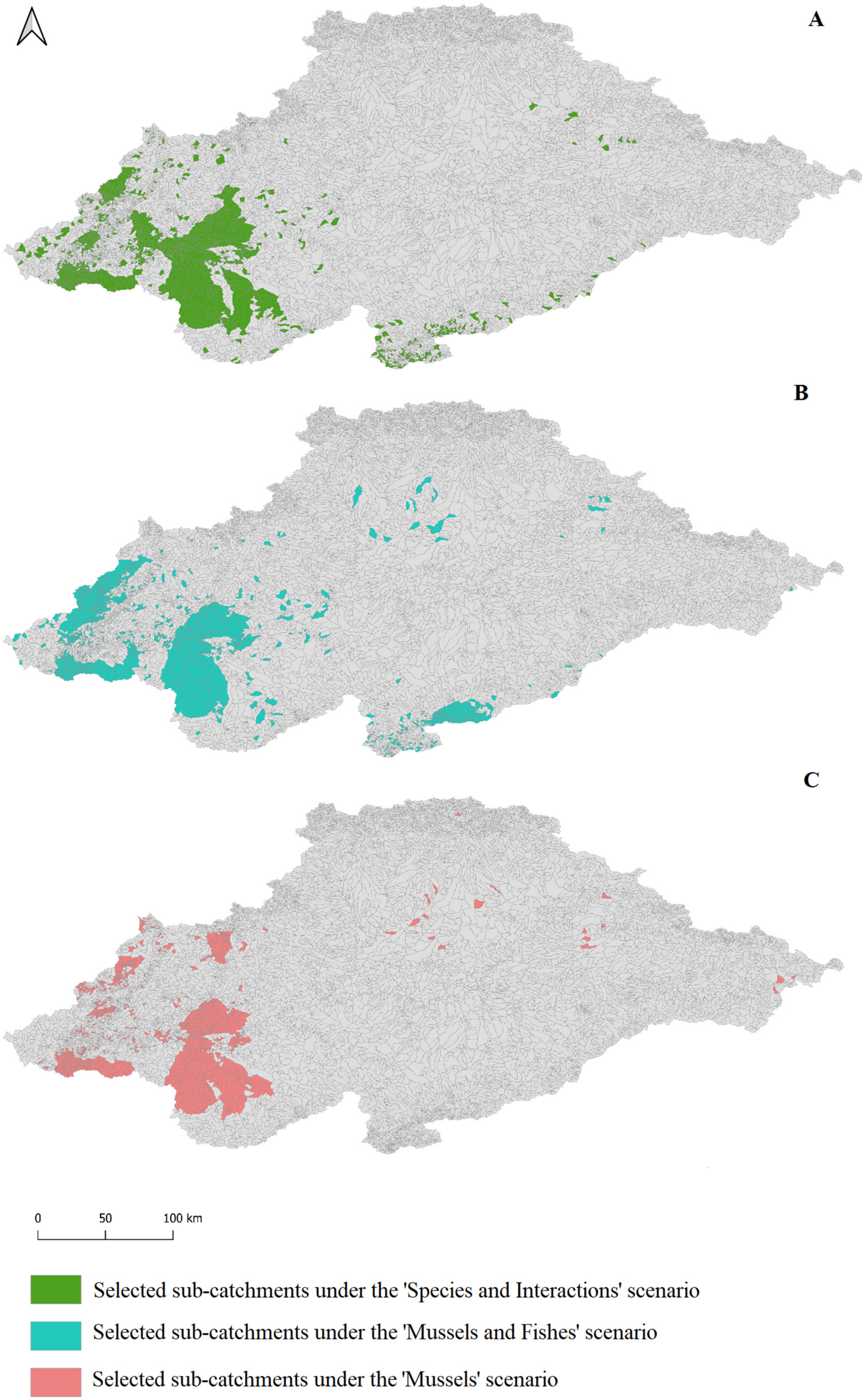

Fig. 3. Map of the selected sub-catchments in the 'Species and Interactions' (A), 'Mussels and Fishes' (B) and 'Mussels' (C) scenarios for target 1 in the Douro River basin.

Table 3
Spatial overlap (Jaccard index) between the best solutions across the different targets and scenarios.

		'Species and Interactions' and 'Mussels and Fishes'	'Species and Interactions' and 'Mussels'	'Mussels and Fishes' and 'Mussels'
CSM > 0	target1/4	0.05	0.07	0.07
	target1/2	0.19	0.15	0.14
	target1	0.43	0.40	0.40
	target1 ∗ 1,25	0.50	0.46	0.54
	target1 ∗ 1,5	0.40	0.40	0.41
CSM = 0	target1/4	0.06	0.05	0.04
	target1/2	0.13	0.11	0.10
	target1	0.27	0.21	0.21
	target1 ∗ 1,25	0.34	0.26	0.26
	target1 ∗ 1,5	0.41	0.31	0.31

freshwater mussels, results in the poorest coverage of mussels-fish interactions. As the targets increase, the number of sub-catchments selected also increases, and therefore the chance of including important areas that contain these interactions is also higher. But, as we should not leave such an important factor for the species' persistence to chance, in addition to selecting proper targets, it should always include at least the data on both the focal species and the interactors. Another aspect to account for is the important role of connectivity because the percentage of interactions covered when we remove the penalty for not achieving connected solutions is lower.

The spatial overlap between the 'Mussels' and the 'Species and Interactions' scenarios, as well as the 'Mussels' and 'Mussels and Fishes' scenarios, are lower than the 'Species and Interactions' and 'Mussels and Fishes' scenarios, showing the spatial mismatch of solutions between using only the mussel's data ('business as usual') and this novel approach of including both freshwater mussels and fishes as well as their interactions. Some of the sub-catchments were selected across all scenarios, as they contain a higher diversity of species as well as rarer mussels and fishes. Also, these areas cover some of the rarest mussel-fish pairs, such as: *Anodonta anatina* ∗ *Cobitis paludica*, *Potomida littoralis* ∗ *Pseudochondrostoma duriense*, *Potomida littoralis* ∗ *Squalius carolitertii*, *Potomida littoralis* ∗ *Achondrostoma oligolepis*, *Potomida littoralis* ∗ *Luciobarbus bocagei* and *Potomida littoralis* ∗ *Squalius alburnoides*. The freshwater mussel *Potomida littoralis* and the loach *Cobitis paludica* are the species of mussel and fish with less presences, respectively, and so, Marxan always selects these sub-catchments to achieve the targets. On the other hand, despite being also rare, some mussel-fish pairs are less covered in the 'Mussels' and 'Mussels and Fishes' scenarios, such as *Potomida littoralis* ∗ *Salmo trutta*, which could be related to the fact that *S. trutta* has a high spatial distribution across the Douro River basin. Given so, Marxan has more flexibility to choose other planning units that contain these species and the probability of missing the areas where *P. littoralis* and *S. trutta* co-occur is higher.

Some of the sub-catchments selected in both 'Species and Interactions' and 'Mussels and Fishes' scenarios are not selected in the 'Mussels' scenario, namely in the southern part of the Douro River basin, which could mean that potentially important areas where mussels and fishes co-occur are disregarded when we do not consider the fishes distribution. Given this, to represent sub-catchments that allow the coexistence of freshwater mussels and their fish hosts, it is important to consider not only the distribution of the mussels but also the fishes, as well as their interactions. Also, a thoughtful consideration of the targets as well as the role of connectivity are fundamental aspects to take into account because if the targets are too low and the CSM values are not considered or are badly calibrated, there is a risk of not properly representing these interactions even if we consider them in the spatial prioritisation analysis.

There are some particularities, such as the strength of interactions, that we did not include in this study, and future studies could address. We treated all mussel-host fish species interactions equally, however, the affinity of each mussel species for different hosts might not be the same (Modesto et al., 2018). These species-specific affinities could be addressed by setting different targets for different mussel-fish interactions. Another important detail to account for when dealing with biotic interactions in systematic conservation planning is the direction of these interactions, as, in our case, mussels depend on fishes to survive but not the other way around. However, recent studies have found that higher densities of mussels (including empty shells) were associated with increasing diversity and density of invertebrates, that may function as a food source to fish species (Ilarri et al., 2018). Finally, it is important to note that this work should not be viewed as a conservation plan for the Douro River basin but as a practical framework to include biotic interactions in systematic conservation planning using this area as a practical case study. A proper conservation plan for the Douro River basin should, ideally, include information about other freshwater focal species, the targets should be negotiated with stakeholders, and the costs, threats and key ecosystem services should be evaluated and included in the overall analysis.

4.2. Management implications

Protected areas often fail to cover freshwater biodiversity (Hermoso et al., 2015). Here, we found that the solutions Marxan presented barely overlap the current Natura 2000 network. This low overlap could be explained by the fact that protected areas are usually designed to manage terrestrial biodiversity and so numerous rare and endemic freshwater species may lack representation. For instance, when assessing the richness and abundance of freshwater fishes in Montesinho Natural Park, a protected area in the Douro River basin, Nogueira et al. (2021a) found that areas with higher diversity and abundance in terms of fish communities were mostly outside the park.

In light of the EU Biodiversity Strategy for 2030 (European Commission, 2020) target to protect at least 30 % of their land and sea, freshwater ecosystems should be considered. There is still much to be done to accomplish this target but works such as this one presents an opportunity to balance the discrepancy between the protected area coverage of terrestrial and marine realms when compared to freshwaters (Juffe-Bignoli et al., 2014). However, establishing (or enlarging) protected areas is often constrained by economic and social factors. Even so, systematic conservation planning outcomes can still be useful to highlight areas where actions such as habitat restoration, environmental education programs, and management of main threats should be developed. The results from the spatial distribution of the best solutions show that the predominant part selected comes from the western part of the Douro River basin. This is probably a reflection of lower human disturbance and higher occurrence of mussels and fishes when compared to the other areas, but still, species face numerous threats. For instance, the spread of invasive species such as the signal crayfish *Pacifastacus leniusculus* (Sousa et al., 2019) is a growing concern in these areas. On the same vein, the impact of extreme climatic events such as droughts (exacerbated by climate change) has caused high mortalities of freshwater mussels in the Douro River basin (Nogueira et al., 2021a, 2021b, 2021c). These threats, combined with the increasing water abstraction used for agricultural purposes, leave freshwater mussels without suitable habitat and more prone to predation (Sousa et al., 2018). Finally, given that freshwater mussels are highly affected by habitat loss and fragmentation, and changes in sediment and water chemistry caused by dams and weirs, it is crucial that both natural river flow and connectivity are improved (Sousa et al., 2020). Habitat fragmentation affects the movement of

Table 4
Spatial coverage of best solutions by the Natura 2000 Network across the different targets. Numbers represent the percentage of the area selected by Marxan covered by the Natura 2000 network.

Scenario	target1/4	target1/2	target1	target1 ∗ 1,25	target1 ∗ 1,5
'Species and Interactions'	2.31	2.36	4.72	4.11	5.41
'Mussels and Fishes'	3.80	2.76	4.29	4.59	5.35
'Mussels'	3.39	6.80	4.42	7.06	6.20

fish hosts across the basin, which means that the mussel's recruitment may be highly impaired. Typically, several of the most suitable fish hosts (e.g. *L. bocagei*, *P. duriense* and *S. trutta*) disperse throughout the Douro River basin, and future studies could investigate the impact of physical barriers in mussel-fish interactions.

5. Conclusion

We demonstrate a novel way to include biotic interactions in systematic conservation planning, using the freshwater mussels and their obligatory fish hosts as a case study. This study also shows the importance of selecting proper targets for species and interactions representation, as well as of considering connectivity between planning units. Furthermore, systematic conservation planning must evolve and account for the intricacies of each conservation plan, as biotic interactions, but also the ecosystem type, and the different levels of conservation features, such as genes, populations, species, or ecosystems. The inclusion of biotic interactions (e.g. mutualisms, parasitism, disease, predation, competition) in systematic conservation planning is very important because the loss of a species is expected to result in the loss of other species that depend on it (co-extinctions) leading to a possible cascading effect across different trophic levels. Such effects will be more severe in obligatory interactions such as the one between freshwater mussels and fishes and so future systematic conservation planning exercises cannot ignore this key aspect.

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CRediT authorship contribution statement

Joana Garrido Nogueira, Manuel Lopes-Lima, Ana Filipa Filipe, Elsa Froufe, Duarte V. Gonçalves, Ronaldo Sousa, Amílcar Teixeira, and Simone Varandas participated in the field work. Joana Garrido Nogueira, Virgilio Hermoso and Janine P. da Silva performed the data analyses. Joana Garrido Nogueira prepared figures and wrote the first draft of the paper. All authors critically contributed to the writing of the paper through comment, additions, and revisions.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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