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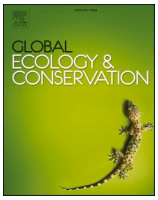

Getting the ‘most out of the hotspot’ for practical conservation of groundwater biodiversity

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ABSTRACT

Conservation planning aimed at halting biodiversity loss has seldom focused on groundwater environments due to the lack of suitable management tools and data. Using harpacticoid crustaceans as a test case, we explore the potential of implementing an approach based on Conservation-Relevant Hotspots for practical conservation of groundwater biodiversity. Conservation-Relevant Hotspots are identified by intersecting species richness, endemicity, and taxonomic distinctness with the aim to minimize the total area to protect. We show that, by targeting five Conservation-Relevant Hotspots that cover only 1.9% of the European land surface, one would protect as much as 44% of the harpacticoid crustacean richness, 93% of its endemicity, and 98% of its taxonomic distinctness. About 28% of the area occupied by these hotspots overlaps with protected areas, which calls for an increase in their protection coverage. Our framework proved a useful tool for conservation planning of environments where spatial or socio-economic constraints occur.

1. Introduction

We are facing a global biodiversity crisis that requires immediate action from all players in society (Lammerant et al., 2019). Recognition of this problem is not new, but biodiversity is still being lost at an unprecedented rate and thus many of

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nature's contributions to humans are being compromised (IPBES, 2019). The most common strategy for halting biodiversity loss is focused on area-based methods (Watson et al., 2014; Maxwell et al., 2020), although not without political and economic concerns (Otero et al., 2020) — e.g., the existence of geopolitical boundaries crossing protected areas and biodiversity hotspots (Liu et al., 2020). Despite these challenges, a successful implementation of transnational protected areas straddling geopolitical boundaries has been achieved in Europe. Since the establishment of the Habitats Directive (92/43/EEC) in 1992, the European Union has acted as an experimental arena for practical conservation, implemented via a capillary network of protected areas. With the new Biodiversity Strategy for 2030, the European Union is now pushing this conservation strategy even further, by proposing to transform 30% of Europe's lands and seas into effectively managed protected areas while bolstering economic development and climate mitigation (European Commission, 2020).

Whereas similar conservation plans are becoming increasingly available for terrestrial, freshwater, and marine ecoregions (Spalding et al., 2007; Abell et al., 2008; Dinerstein et al., 2017), this is rarely the case for their subsurface counterparts, including subterranean ecosystems such as caves and aquifers (Wynne et al., 2021).

Subterranean biodiversity is disregarded partly for regulatory reasons (i.e., its ecological dimension is not regulated) and partly because it is 'out of sight', being hidden and difficult to access. In addition, subterranean communities are dominated by bacteria, protists, and small-sized invertebrates, all understudied compared to other living forms of larger size, especially vertebrates (Troudet et al., 2017; Niemiller et al., 2018). Consequently, and despite the numerous services to humans they provide, subterranean ecosystems and their biodiversity are rarely accounted for in broad-scale conservation agendas (Griebler et al., 2019; Mammola et al., 2019; Boulton, 2020; Sánchez-Fernández et al., 2021; Wynne et al., 2021).

In recent years, we are achieving a better understanding on subterranean biodiversity, but progress is delayed by the difficulty of accessing and sampling subterranean habitats (Ficetola et al., 2019; Mammola et al., 2021b). Owing to this fragmentary knowledge, most attempts of establishing criteria for the protection of subterranean systems have been focused on identifying single-site priorities (e.g., caves, wells, or small aquifers holding great levels of diversity) (Pipan et al., 2020). There are now several examples of prioritization methods to identify such local biodiversity hotspots (different indices are compared in Rabelo et al., 2018). Whereas single-site approaches to subterranean conservation have been dictated by economical and practical constraints (e.g., Devitt et al., 2019; Fattorini et al., 2020), it is now increasingly acknowledged that the protection of single sites does not account for the connectivity among subterranean habitats and the interdependence between surface and subterranean systems (Mammola et al., 2019; Owen et al., 2019). It follows that the delimitation of protected areas of subterranean biodiversity should necessarily account for larger buffering areas (Iannella et al., 2020b). To this end, different authors developed area-based approaches such as grid-based hotspots of groundwater biodiversity (Deharveng et al., 2009; Michel et al., 2009), stygoregions (Stein et al., 2012), or multi-faceted conservation indices (e.g., Fattorini et al., 2020). However, defining criteria for area prioritization is not trivial, insofar as decisions about allocating broad areas for protection often involve conflicts between industry, local communities, and environmental protection agencies (e.g., Wilkinson et al., 2013 for urbanization, Koehnken et al., 2020 for mining, Yang et al., 2020 for cities and farmland). This means that the area that countries are willing to devote to conservation is inevitably very limited and, *ipso facto*, all prioritization approaches should strive to maximize the effectiveness of biodiversity protection while targeting the smallest possible spatial coverage.

Here, we developed an approach for delimiting Conservation-Relevant Hotspots by balancing a trade-off between the effectiveness of protection and the minimization of surface of protected area—that is to say, getting '*the most out of the hotspot*'. We based our approach on three indicators that, collectively, should capture three relevant attributes of the multifaceted biodiversity concept: species richness, endemicity, and taxonomic distinctness (Iannella et al., 2020a). We tested our method using groundwater harpacticoid crustaceans (Crustacea: Copepoda: Harpacticoida), chosen as a model taxon because i) they are widespread in all known groundwater habitat types in Europe; ii) they have typically a limited dispersal capability hence working as indicators of different habitat types (Galassi et al., 2009); and iii) they are one of the few groups of groundwater organisms for which comprehensive distribution data exist (Iannella et al., 2020a, 2020b).

We aimed to i) identify Conservation-Relevant Hotspots based on multiple criteria; ii) evaluate the indirect protection supplied by surface protected areas to groundwater hotspots; and iii) assess the risk of biodiversity loss by combining the intrinsic vulnerability of the groundwater habitats falling within each hotspot and the anthropogenic pressures acting on them.

2. Methods

2.1. Study area and dataset

The study area embraced the European continent, main islands included (longitude min–max = − 31.3 to 65.2°; latitude min–max = 27.6–69.2°). The total surface covers 9489,978 km^2, and embodies a mosaic of 61,275 patches, each representing one out of the three groundwater habitat types as defined by Cornu et al. (2013). The three groundwater habitat types were identified upon the criteria of the groundwater flow type (Cornu et al., 2013), namely: (i) aquifers in consolidated rocks, (ii) aquifers in unconsolidated sediments, and (iii) practically non-aquiferous rocks. The main environmental features of the three groundwater habitat types are related to hydrogeology and retrieved from the International Hydrogeological Map of Europe (IHME; scale: 1:500,000). The groundwater flow is intergranular in unconsolidated sediments, it occurs mainly in small and large fractures in consolidated rocks and is negligible in igneous rocks. Permeability is mainly high in consolidated rocks, high-moderate in unconsolidated sediments and very low in practically non-aquiferous rocks (Iannella et al., 2020a, 2020b).

Each groundwater habitat type includes different subhabitats and microhabitats that can be colonized by different obligate groundwater-dwellers (Boulton, 2020). Karst habitats comprise dripping pools, puddles and trickles which collect the water from the percolation zone of the epikarst (Pipan and Culver, 2013; Kozel and Pipan, 2020) and lakes and perennial subterranean streams in caves which represent the saturated karst (Gibert et al., 1994; Galassi et al., 2017; Di Cicco et al., 2021). Groundwater-fed springs (e.g., Fiasca et al., 2014; Di Lorenzo et al., 2018) may be fed by aquifers in consolidated rocks (karst springs) or aquifers in unconsolidated sediments (alluvial springs). Saturated alluvial aquifers and hyporheic zones of streams and rivers are included in aquifers in unconsolidated sediments (Hancock et al., 2005). Finally, small fissures and fractures in igneous rocks filled with groundwater mainly describe practically non-aquiferous rocks.

We retrieved the occurrences of all subterranean-specialized harpacticoids in each patch (i.e., groundwater habitat type) from the European database (Iannella et al., 2020a, 2020b), namely 3248 occurrences belonging to 408 species. We obtained occurrence data from the European PASCALIS database (Deharveng et al., 2009), the Checklist of the Italian fauna (Ruffo and Stoch, 2005), and bibliographic collections and unpublished data (DMPG). For a few species, we scanned distribution maps from the literature and retrieved the coordinates of occurrence points with ArcMap ver. 10.0 software (ESRI, 2010).

2.2. Conservation-Relevant Hotspots

We carried out an intersection analysis on the ‘hottest hotspots’ of three out of the six indicators formerly used by Iannella et al. (2020a); namely, species richness, endemicity and taxonomic distinctness. We choose these biodiversity facets because they collate multiple dimensions of diversity while being easily applied compared to measures of evolutionary origin and phylogenetic rarity, functional diversity, or niche breadth of groundwater species.

Species richness represents the simplest descriptors of species assemblages and measures the number of species recorded in each site. Endemicity is a species-trait indicator that approximates extinction risk, under the assumption that narrow endemics are more at risk of extinction than wide-range species (Chichorro et al., 2020). We scored endemicity based on the geographic range of each species

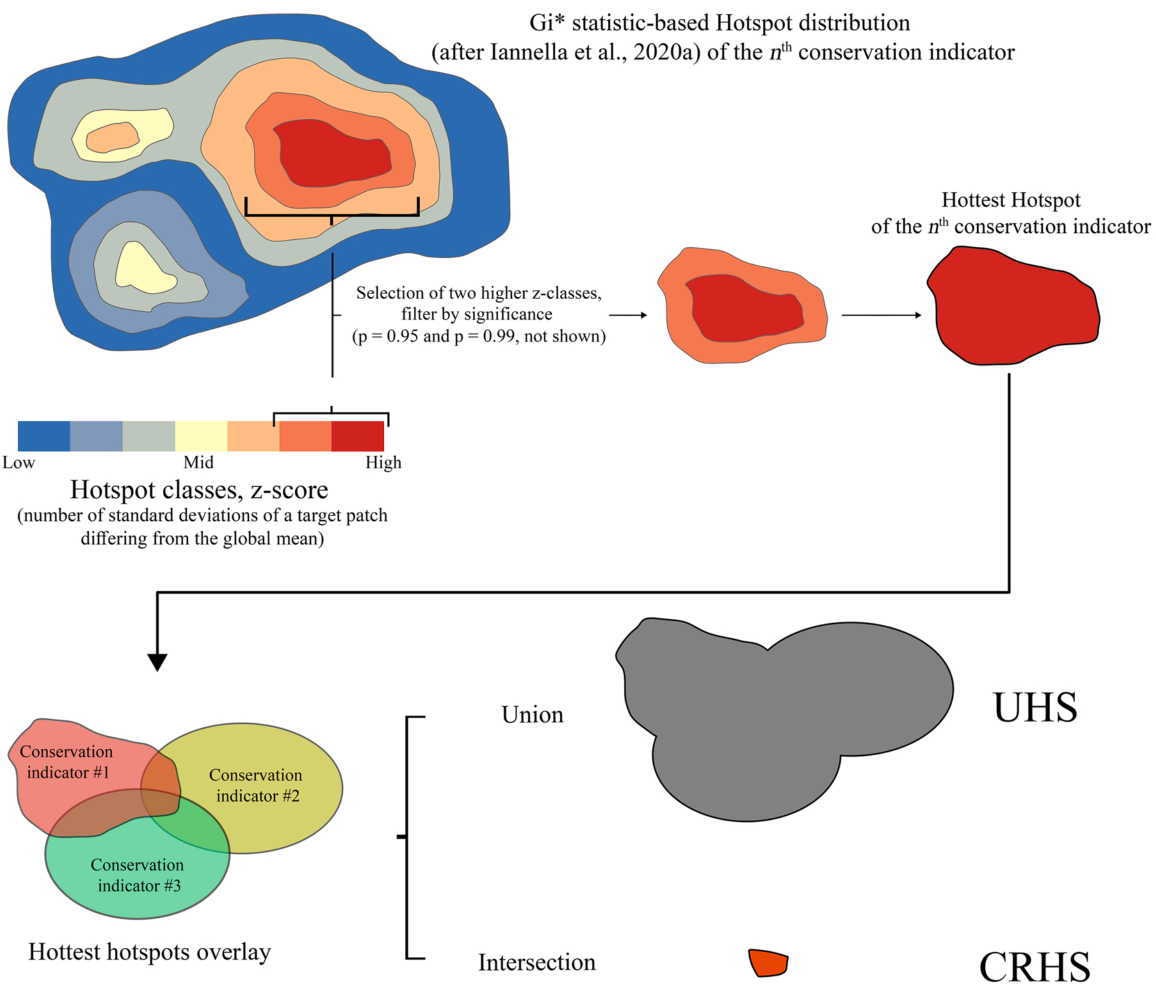

Fig. 1. Graphical scheme showing the processes of intersection and union. The intersection of three areas defined by the three biodiversity indicators contains only the elements that are in the three areas defining the Conservation-Relevant Hotspot (CRHS), and the union of the same three areas contains all the elements contained in either area represented by the geometric Union of Hotspots (UHS).

into five classes as follows: holoendemic (score = 1); euryendemic (score = 2); stenoendemic (score = 3); rhoendemic (score = 4); spot endemic (or microendemic) (score = 5). To minimize the effect of species richness, we computed values of the endemicity indicator as the ratio of the sum of the species' endemicity scores to the number of species occurring in each intersected area. We interpreted taxonomic distinctness (Clarke and Warwick, 1998) as a proxy measure of diversity of evolutionary lineages within each Conservation-Relevant Hotspot. We calculated it as the average taxonomic distance between two randomly selected species through the taxonomic tree of all the species in a dataset with the equation:

$$\Delta^{+}=[\Sigma\Sigma_{i<j}\omega_{ij}]/[s(s\text{-}1)/2]$$

where s = the number of species in the study, ω_{ij} = the taxonomic path length between species i and j. We defined the hotspots of each of these three indicators as the aggregation of patches with values of each indicator clustering together.

We conducted all analyses in ArcMap 10.0 (ESRI, 2010) and with the NCSS software (v. 11).

2.3. Assessment of Conservation-Relevant Hotspots

To identify Conservation-Relevant Hotspots, we combined the three indicators: species richness, endemicity, and taxonomic distinctness, according to a hotspot analysis based on the Gi* statistics by Getis and Ord (1992). In this way, the hotspots of each indicator (aggregation of patches with values of a given indicator clustering together) can be targeted. For each cluster identified as a hotspot, we also obtained a p-value and a z-score. The tool used, implemented in ArcGis 10.0 (Getis and Ord, 1992), applies the Gi* statistics to each patch (i.e., each groundwater habitat type) in the context of its neighbourhood. For each cluster identified as a hotspot, a p-value (indicating the confidence interval to which a hotspot can be identified as such) and a z-score (the number of standard deviations of a patch significantly differing from the global mean) were obtained from the tool. For the aims of the present work, and to be consistent with the previous research dealing with groundwater hotspots (Iannella et al., 2020a, 2020b), the two higher z-score classes were selected also filtered by the two highest confidence intervals ($p = 99\%$ and $p = 95\%$). Both conditions of high p-value and z-score highlighted the 'hottest hotspots' according to the methodology detailed in Iannella et al. (2020a) (Fig. 1).

We subsequently used the ArcMap Intersect tool to compute a geometric intersection of the 'hottest hotspots' of each indicator. We used overlapping features or portions of features to be considered Conservation-Relevant Hotspots—i.e., areas identified by the highest values of species richness, endemicity, and taxonomic distinctness. To allow comparison in terms of area extent between the Conservation-Relevant Hotspots and the 'hottest hotspots' of each indicator, we used the ArcMap Union tool. We computed the geometric Union of the 'hottest hotspots' of the three indicators following the same methodology adopted for measuring species richness, endemicity, and taxonomic distinctness into each Conservation-Relevant Hotspot. We named the united features Union of Hotspots. Note that we incorporated a 6 km distance from each groundwater habitat type into each Conservation-Relevant Hotspot and its respective geometric Union as buffer area because this bend is known to host up to 69% of the groundwater harpacticoid species, as found by Iannella et al. (2020b). We applied this approach to all the analyses performed—for example, when extracting information such as the number of sampling localities or the number of species contained by each hotspot.

2.4. Assessment of the protection coverage

We estimated the protection coverage of each Conservation-Relevant Hotspot and Union of Hotspots as their overlap with protected areas. We obtained spatial data of the protected areas, including national protected Areas (National Parks or Reserves) and Natura 2000 sites, from the European Environment Agency data repository (EEA, 2020a, 2020b).

2.5. Assessment of risk

We assessed the risk of biodiversity loss due to anthropogenic pressures acting on Conservation-Relevant Hotspots by applying the conceptual model provided by the EEA (2020c). This model combines the probability of chemical pollution of a given aquifer, measured as the capacity with which a contaminant introduced at the ground surface can reach underground and diffuse, to its degree of resilience to contamination supplied by its geophysical characteristics. We georeferenced the map of the intrinsic vulnerability of the European groundwater bodies available at the European Union data repository for groundwater quality and vulnerability (EEA, 2020c). In each Conservation-Relevant Hotspot, we scored the intrinsic vulnerability in five classes, from very low (class 1) to very high vulnerability (class 5). We retrieved anthropogenic activities from the land use (100 m resolution) from the Copernicus Land Monitoring Service (2020) website. We translated land use types into "driving forces" (e.g., urban development, industry, agriculture, and other activities) which led to potential pressures below-ground. The magnitude of each pressure was calculated by estimating the potential threats posed to groundwater habitats and their fauna in terms of expected population decline. We assigned potential pressures based on types of surface land use, ranging from very low (class 1) to very high pressure (class 4) (Table S1_EUNIS Habitats). This indirect assessment was necessary because effective population size and sensitivity to pollutants is unknown for most subterranean species. We spatially paired the two maps generated for the intrinsic vulnerability (Fig. S1) and for the anthropogenic pressures (Fig. S2) for each groundwater habitat in each Conservation-Relevant Hotspot. We combined the scores of intrinsic vulnerability and magnitude of the anthropogenic pressures to assess the overall risk. We translated the possible score combinations into a 5 × 4 matrix (Table S2), and for each risk level out the 20 possible combinations we assigned a color-coding: green (low risk), yellow (medium risk), orange (high risk), and red (very high risk).

3. Results

3.1. Conservation-Relevant Hotspots

Using our method (Fig. 1), we identified five Conservation-Relevant Hotspots and their respective Unions of Hotspots in the following areas: i) the French Pyrenees (France); ii) the Alpine arc (Italy) embracing southward the River Po alluvial plain and the External Dinarides (Slovenia); iii) the Central Apennines (Italy); iv) the Balkan Mountains at the boundary between western Bulgaria, north-west Macedonia, and small expansions in Serbia; and iv) the Sardinia Island (Italy) (Fig. 2). These occupied a total area of 180,573 km^2, representing 71% of the overall Unions of Hotspots area (Table 1) and 1.9% of the European surface analyzed in this study.

Of the 408 harpacticoid species, 180 (44%) occurred in the five Conservation-Relevant Hotspots and 209 (51%) and their respective geometric Unions of Hotspots (list of species in Table S3_Species list). In the Conservation-Relevant Hotspots, the mean endemicity score (3.8) and the average taxonomic distinctness (61.8) were only slightly different from the values measured in the UHS (3.9 and 61.3, respectively) (Table 2), and at the European scale (4.1 and 63.3, respectively).

3.2. Protection coverage of the Conservation-Relevant Hotspots

At present, 27.9% of the area occupied by Conservation-Relevant Hotspots overlapped with Protected Areas. Of this, 23.5% was represented by the current Natura 2000 network and 4.4% by nationally designated areas. Individual hotspots, however, showed contrasting overlap values with protected areas: 22% for Sardinia and the Alpine Arc – External Dinarides, 24% for the French Pyrenees, 35% for the Central Apennines, and 60% for the Balkan Mountains (Fig. 3a). Also, the extent of the areas protected by nationally designated areas, Natura 2000 areas, and areas covered by both types of protected areas varied substantially (Fig. 3b). For example, the largest nationally designated areas occurred in the Alpine Arc – External Dinarides whereas the largest areas of Natura 2000 sites occurred in the Central Apennines.

Overall, the five Conservation-Relevant Hotspots encompassed six European countries. The Alpine Arc – External Dinarides hotspot embraced Italy and Slovenia, while the Central Apennines and Sardinia hotspots involved the Italian country only. The French Pyrenees hotspot was located just in France, and the Balkan Mountains hotspot occurred in Bulgaria, and partly in Serbia and Macedonia.

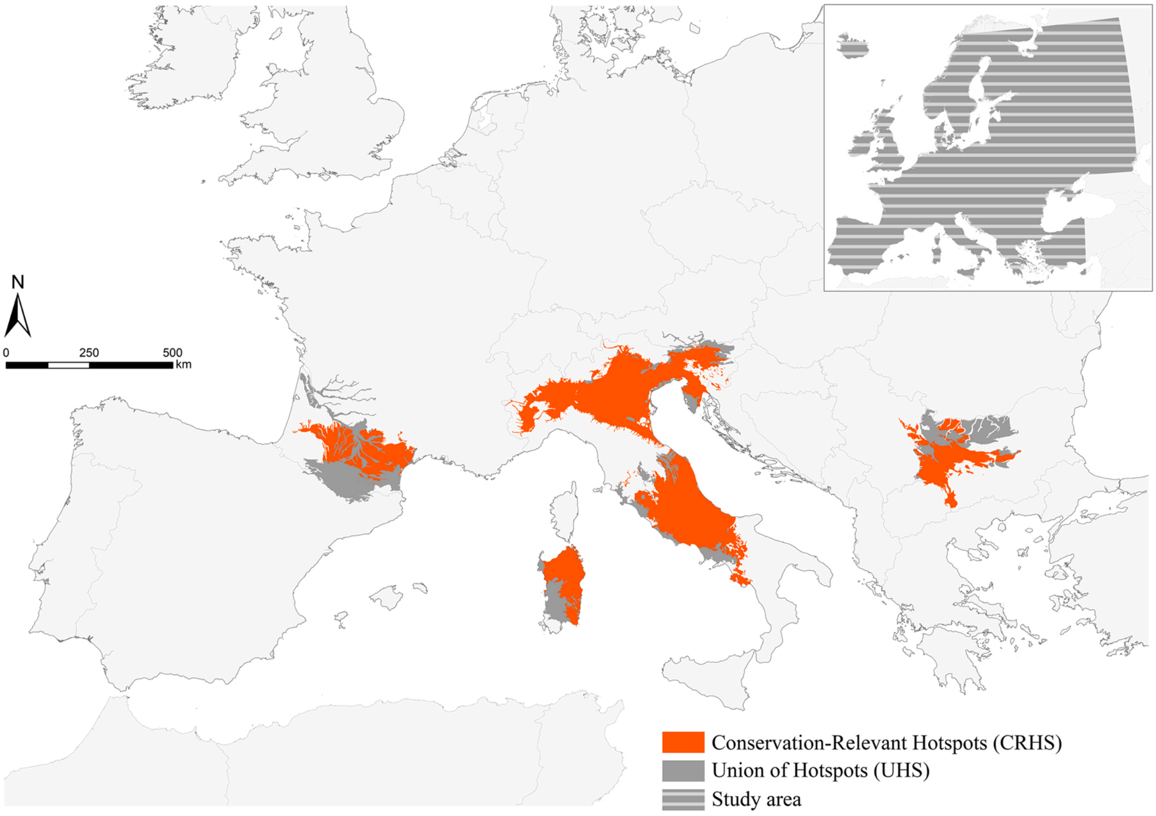

Fig. 2. Conservation-Relevant Hotspots (CRHS) in red and Union of Hotspots (UHS) in dark gray resulting from the intersection and union of the two higher classes of species richness, endemicity and taxonomic distinctness hotspots inferred by Iannella et al. (2020a). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Extent (in km^2) of the Conservation-Relevant Hotspots (CRHS), their respective geometric Union (UHS), and the ratio of their areas (in %).

	CRHS	UHS	CRHS/UHS (%)
Alpine Arc – External Dinarides	70,831	78,288	90%
Central Apennines	48,386	58,528	83%
Balkan Mountains	22,650	40,877	55%
French Pyrenees	24,243	55,182	44%
Sardinia Island	14,463	21,997	66%
Total	180,573	254,872	71%

Table 2
Values of the species richness, endemicity and average taxonomic distinctness indicators calculated for the five Conservation-Relevant Hotspots (CRHS) and the respective geometric Unions (UHS).

	Species richness			Endemicity			Average Taxonomic Distinctness		
	CRHS	UHS	Δ	CRHS	UHS	Δ	CRHS	UHS	Δ
Alpine Arc – External Dinarides	72	72	0	3.4	3.3	0.1	60.6	60.6	0
Central Apennines	43	45	-2	3.8	3.7	0.1	65.4	65.0	0.4
Balkan Mountains	39	42	-3	3.4	3.5	-0.1	63.8	53.0	10.8
French Pyrenees	27	50	-23	3.4	3.8	-0.4	65.4	59.3	6.1
Sardinia Island	22	22	0	4.4	4.2	+ 0.2	62.1	62.1	0
Total	180	209	-29	3.8	3.9	-0.1	61.8	61.3	0.5

Δ represents the difference between the values measured for each indicator in the CRHS and the respective UHS.

Most of the Italian localities of harpacticoid species (64–85%) fell within protected areas, with Natura 2000 sites offering the highest coverage (60%). In Slovenia, protected areas covered about 50% of the hotspot, while protected areas in Bulgaria and France covered about 20% and 3% of the localities of occurrences, respectively. No localities with harpacticoids fell within protected areas in Serbia and Macedonia (Fig. 3c).

3.3. Risk assessment

We assessed varying levels of risk for groundwater biodiversity across the five Conservation-Relevant Hotspots in Europe (Fig. 4a). Most groundwater habitats and species occurred in areas from medium to very high risk (Fig. 4b). The area of CRHS was divided into these two main groups of risk classes, with protected areas mostly covering low/medium risk areas (Fig. 4c). The Alpine Arc – External Dinarides was the hotspot at greatest risk, followed by the French Pyrenees and Central Apennines hotspots.

4. Discussion

4.1. Towards effective subsurface biodiversity conservation

Human pressure on freshwater biodiversity is increasing both above (Albert et al., 2021) and below the ground (Mammola et al., 2019). Regarding surface freshwaters, measures of biodiversity protection have been established (Tickner et al., 2020). This is not the case for groundwaters, where the protection of biodiversity is still at an early stage and, in most cases, confined to single caves or cave systems (e.g., Hutchins, 2018; Devitt et al., 2019). Conversely, the vast majority of groundwater habitats, such as saturated aquifers in consolidated rocks or in unconsolidated sediments and several groundwater-dependent ecosystems, lack formal protection and regulation (Boulton, 2020).

This study intended to provide a practical foundation for extending protection to below-ground diversity. Toward this end, we selected the harpacticoid crustaceans as a test taxon since their distribution and taxonomy is well-known (Iannella et al., 2020a, 2020b) —although some bias may still be claimed regarding the number of records available (e.g., from Belgium, Poland, Bosnia and Herzegovina, Albania) and the number of undescribed species (Table S4_Records_Country). Out of the total harpacticoid species diversity in Europe, 44% was captured in the five Conservation-Relevant Hotspots. An overall enlargement of 29% of these areas, deriving from the geometric union of the hotspots, would involve only a modest increase in species richness (+29 species) and endemicity (+0.1%), and a low decrease in average taxonomic distinctness (− 0.5). Therefore, by protecting a small area accounting for ca. 2% of European surface, one would achieve significant protection for the below groundwater harpacticoid species richness.

Notably, as the Conservation-Relevant Hotspots were generated using the groundwater habitat types assessed by Cornu et al. (2013), this approach should suffice the coverage of hydrogeological units in their entirety, and not only the single localities from which the groundwater harpacticoids were collected. However, these Conservation-Relevant Hotspots currently lack formal protection, insofar as the protected areas network cover just about 28% of their total surface. If such protection might seem acceptable for surface environments (van Rees et al., 2021), it should be noted that the protected areas currently in force were designated for the protection of surface habitats, and thus are not necessarily effective for protecting the vertical dimension of groundwater habitats and their biodiversity (Linke et al., 2019; Sánchez-Fernández et al., 2021).

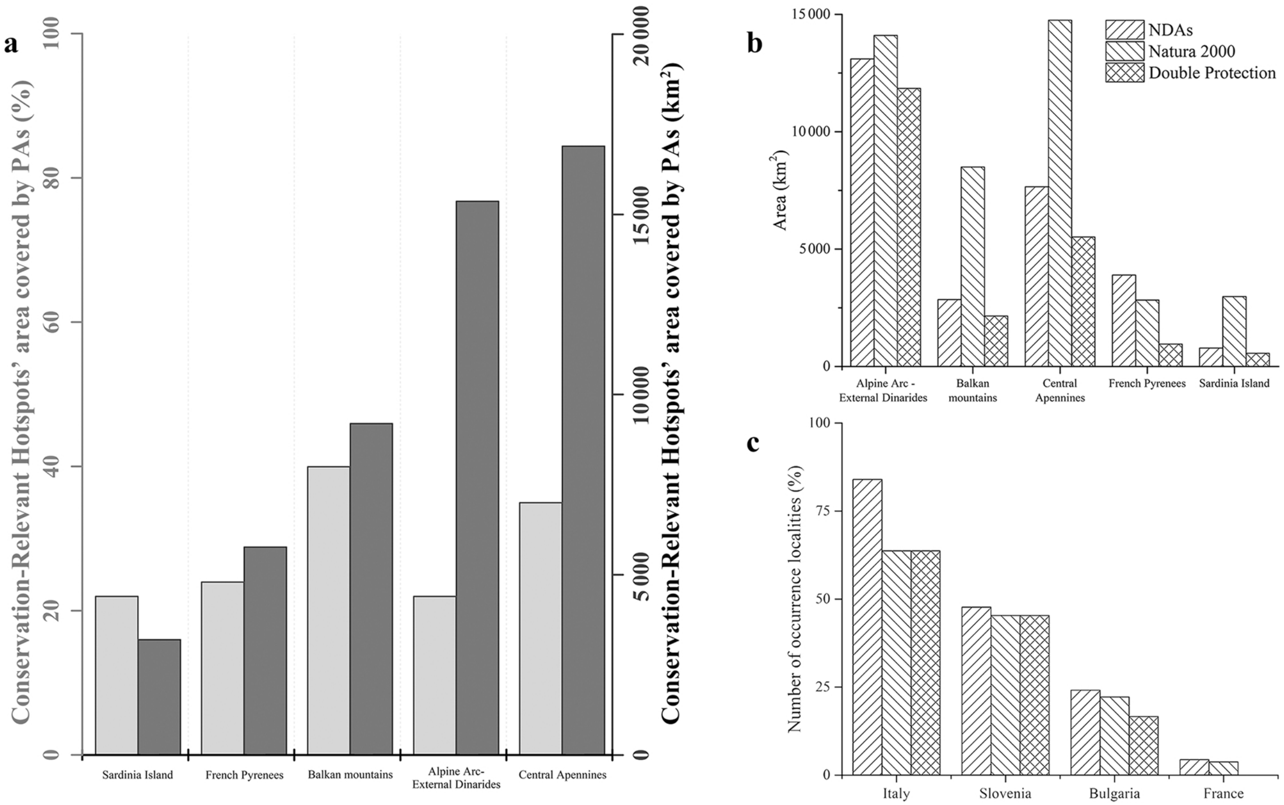

Fig. 3. (a) Protected Areas (PAs) coverage of the five Conservation-Relevant Hotspots (CRHS) in Europe in terms of their total extent (dark gray, km^2) and ratio between Conservation-Relevant Hotspot areas and protected area coverage (light gray, %); (b) Protected areas extent within each of the five European CRHS in terms of coverage by Nationally Designated Areas (NDAs), Natura 2000 sites, and dual protection given by the overlap between NDAs and Natura 2000 network; (c) Number of localities where stygobitic harpacticoid species occur both within the five European CRHS and into the different protected areas divided by country.

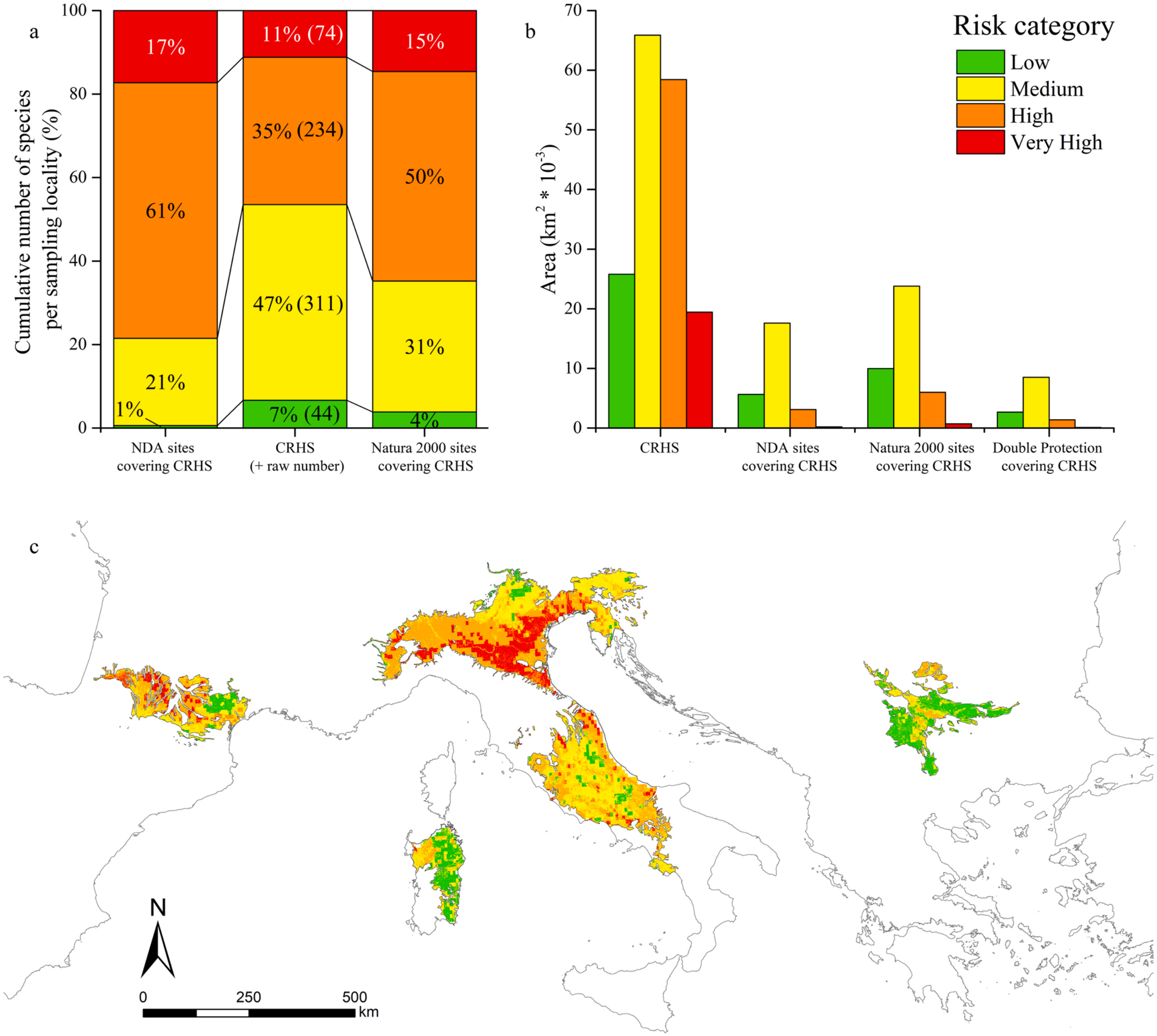

Fig. 4. (a) Cumulative number of specialized subterranean harpacticoid species found in groundwater localities (i.e. the total number of species obtained without removing duplicate records, to foresee the overall exposure to risk) falling into CRHS and in the protected areas covering them, and their associated risk; (b) risk category to which CRHS area and their respective areas covered by protected areas are exposed (in km^2); (c) spatial distribution of risk of groundwater biodiversity loss within the five CRHS. Abbreviations used: CRHS = Conservation-Relevant Hotspots; NDA = Nationally Designated Area.

4.2. Protected area designation for groundwater biodiversity

The designation of protected areas and their management plans should be based on the protection of ecoregions, by simultaneously including species at risk of extinction along with the ecosystem services they provide (van Rees et al., 2021; Sánchez-Fernández et al., 2021; Wynne et al., 2021). In this regard, Abell et al. (2008) highlighted the limits of using surface freshwater ecoregions for their potential extension to the groundwater environment because '*Underground systems [.] may require their own planning framework, as groundwater catchments may not correspond with the surface-water catchments upon which our ecoregions are built*'.

This situation stems from (i) the incompleteness of continental and global species inventories, (ii) the difficulties to assess the multiple dimensions of subterranean ecosystems; (iii) the taxonomic bias affecting many groundwater invertebrate and vertebrate groups; (iv) the geographic bias that vitiates the spatial information associated with many groundwater species, which overall lead to a weakness of the input information for the generation of protected areas, and v) knowledge on the distribution of groundwater functional and phylogenetic diversity. Recognizing the multiple ecological dimensions of groundwater ecosystems in current policies is therefore a clear future direction.

As far as the European Union is concerned, it must be pointed out that the conservation of groundwater habitats is not sufficiently supported by the Habitats Directive. In the EU member states, the Habitats Directive 92/43/EEC does not account for most groundwater habitats, an exception being "Other rocky habitat – 65 Caves not open to the public; Submerged or partly submerged sea caves, and one groundwater-dependent-ecosystem type into: Raised Bogs and Mires" and "Fens-Calcareous fens – 54.12 *Petrifying springs with tufa formation (Cratoneurion)", both listed in Annex I – Natural Habitat Types of Community Interest whose Conservation requires the Designation of Special Areas of Conservation of the Habitats Directive. No groundwater species are listed in Annexes II, III or IV of the same Directive, except for the obligate-groundwater vertebrate known from groundwaters of Europe, *Proteus anguinus* Laurenti, 1768 (Proteidae) and the only known subterranean bivalve *Congeria kusceri* Bole, 1962 (Dreissenidae). Therefore, there is the need to extend the Directive to environments not yet listed in Annex I to achieve unbiased conservation targets (see, e.g., Mammola et al., 2020).

Notably, in subterranean habitats, there are several genera, families, and even orders exclusively known from groundwater. Often, these taxa are recorded from one or few localities and with so small populations that they have been collected only once, and never resampled thereafter. These taxa are likely to contribute unique phylogenetic and functional diversity (e.g., Humphreys, 2000; Galassi et al., 2009; Castelle et al., 2013; Asmyhr et al., 2014), the protection of which can be achieved only by incorporating more extensively subsurface habitats in the protection targets of the European Union.

It must be noted that, in our prioritization exercise, we captured phylogenetic and functional diversities indirectly, by considering endemicity and taxonomic distinctness alongside species richness. Ideally, it is possible to measure these facets of biodiversity directly from phylogenetic data (Tucker et al., 2017) and functional traits (Mammola et al., 2021a), and incorporate diversity indexes into prioritization analyses (Pollock et al., 2020). However, for groundwater organisms in general, and harpacticoid crustaceans in particular, trait data and phylogenetic trees are still scarcely and sparsely available (Galassi et al., 2009; Asmyhr et al., 2014; Karpowicz et al., 2021). A future increase in the availability of similar data will enhance the possibility to routinely account for a multi-pronged view biodiversity in prioritization exercises (Pollock et al., 2020). Provided that the impact of similar prioritization exercises is comprehensively tested (Pressey et al., 2021), it will be possible for practitioners to design protected areas able to simultaneously protect groundwater biodiversity and the important services it provides to humans, especially clean water.

4.3. 'Bending the curve' of groundwater biodiversity loss

An overall seventy percent of the groundwater sites within the five Conservation-Relevant Hotspots assessed in this study fell in aquifers with moderate to very high intrinsic vulnerability and with medium- to high-pressure scores. This condition not only generates impacts to the groundwater habitats, lowering their physical and chemical quality (both ruled by the Directive 60/2000/EC), but also affects their biodiversity. The first step leading to a decisive turnaround involves acquiring knowledge of 'where' the biodiversity hotspots are located, but also of 'how' to protect them. The 'where' and the 'how' should necessarily work in tandem. Land cover changes and the overexploitation of groundwater resources are notoriously the main gross categories of threats to the groundwater fauna (Caschetto et al., 2014; Di Lorenzo et al., 2014, 2015; Devitt et al., 2019; Griebler et al., 2019). It is widely recognized that human footprint is intensifying in places with high biodiversity in terrestrial, freshwater, and groundwater compartments (Dinerstein et al., 2020). Hence, the first step to propose protection and management interventions must necessarily retrace the road already traveled for a better knowledge in various environmental contexts, in order to ensure the possibility of comparative analyses at the crossroad between surface and subterranean systems. However, knowledge for the evaluation of how to 'bend the curve' (Tickner et al., 2020) by reaching the recovery of the groundwater biodiversity is scant. Few studies addressed practical conservation measures in groundwater. Studies so far have focused on European aquifers (Michel et al., 2009) or on selected groundwater species (Devitt et al., 2019). Based on the knowledge derived from these pioneering studies we can only infer that the groundwater fauna is imperiled because of its unique traits—smaller dimension of the populations, longer lifespan, lower reproductive rate, and higher sensitivity to pollutants and climate change (Dole-Olivier et al., 2000; Galassi et al., 2009; Fitzgerald et al., 2021; Chichorro et al., 2020).

5. Conclusions

The approach proposed in this study is expressly addressed to select the smallest areas within the European hotspots of groundwater biodiversity to be protected, balancing conservation, political, and societal needs. This strategy collides with the inability to protect all areas that deserve protection by virtue of the biodiversity that they host. Thus, the area-based approach used is, in oxymoronic words, an "inclusive" extension of the biodiversity hotspot concept, leading to protect the highest value of biodiversity by targeting the smallest areas. This approach guarantees the strict protection of the groundwater habitats and their biodiversity, based on the approximation that if the terrestrial and surface freshwater is covered by protected areas, the risk of groundwater biodiversity loss may be considered lower than in areas that are not protected, for which new tools of protection are urgently needed. Moreover, this approach permits to overcome the limitation of national regulations in terms of delimitation of protected areas, considering the transboundary nature of the hydrogeological units used in this study.

Notoriously, reserve networks should also consider the nature services to humans that biodiversity provides. Whereas it is documented how groundwater ecosystems provide key services in terms of cleaner freshwater and carbon storage, we still lack a precise quantification of these provisions (Boulton et al., 2008; Griebler et al., 2019). Consequently, we currently stand to a point where we cannot optimize the selection of Conservation-Relevant Hotspots toward both the protection of biodiversity and the contributions it provides to human wellbeing (Sánchez-Fernández et al., 2021).

CRediT authorship contribution statement

D.M.P.G. and M.I. had the idea; B.F., T.D.L. and M.D.C. created the dataset and calculated the biodiversity indicators; M.I. and M.B. performed the analyses; D.M.P.G. and S.M. wrote the main draft; all authors discussed the results and contributed to writing.

Declaration of Competing Interest

The authors declare no competing interest.

Data Availability

Data are available in Supplemental Tables S1–S4. For additional information, correspondence could be addressed to dianamariapaola.galassi@univaq.it.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01844.

References

Abell, R., Thieme, M.L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Contreras Balderas, S., Bussing, W., Stiassny, M.L.J., Skelton, P., Allen, G.R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J.V., Heibel, T.J., Wikramanayake, E., Olson, D., Lòpez, H.L., Reis, R.E., Lundberg, J.G., Sabaj Pérez, M.H., Petry, P., 2008. Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. BioScience 58, 403–414. https://doi.org/10.1641/B580507.

Albert, J.S., Destouni, G., Duke-Sylvester, S.M., Magurran, A.E., Oberdoff, T., Reis, R.E., Winemiller, K.O., Ripple, W.J., 2021. Scientists' warning to humanity on the freshwater biodiversity crisis. Ambio 50, 85–94. https://doi.org/10.1007/s13280-020-01318-8.

Asmyhr, M.G., Linke, S., Hose, G., Nipperess, D.A., 2014. Systematic conservation planning for groundwater ecosystems using phylogenetic diversity. PLoS One 9 (12), 115132. https://doi.org/10.1371/journal.pone.0115132.

Boulton, A.J., 2020. Editorial: conservation of groundwaters and their dependent ecosystems: Integrating molecular taxonomy, systematic reserve planning and cultural values. Aquat. Conserv. 30, 1–7. https://doi.org/10.1002/aqc.3268.

Boulton, A.J., Fenwick, G.D., Hancock, P.J., Harvey, M.S., 2008. Biodiversity, functional roles and ecosystem services of groundwater invertebrates. Invertebr. Syst. 22, 103–116. https://doi.org/10.1071/IS07024.

Caschetto, M., Barbieri, M., Galassi, D.M.P., Mastrorillo, L., Rusi, S., Stoch, F., Di Cioccio, A., Petitta, M., 2014. Human alteration of groundwater-surface water interactions (Sagittario River, Central Italy): implication for flow regime, contaminant fate and invertebrate response. Environ. Earth Sci. 71, 1791–1807. https://doi.org/10.1007/s12665-013-2584-8.

Castelle, C.J., Hug, L.A., Wrighton, K.C., Thomas, B.C., Williams, K.H., Wu, D., Tringe, S.G., Singer, S.W., Eisen, J.A., Banfield, J.F., 2013. Extraordinary phylogenetic diversity and metabolic versatility in aquifer sediment. Nat. Commun. 4, 2120. https://doi.org/10.1038/ncomms3120.

Chichorro, F., Urbano, F., Teixeira, D., Väre, H., Pinto, T., Brummitt, N., He, X., Hochkirch, A., Hyvönen, J., Kaila, L., Juslén, A., Cardoso, P., 2020. Species traits predict extinction risk across the Tree of Life. bioRxiv 2020.07.01.183053. ⟨https://doi.org/10.1101/2020.07.01.183053⟩.

Clarke, K.R., Warwick, R.M., 1998. A taxonomic distinctness index and its statistical properties. J. Appl. Ecol. 35, 523–531. https://doi.org/10.1046/j.1365-2664.1998.3540523.x.

Copernicus Land Monitoring Service , 2020. CORINE Land Cover - CLC 2018. ⟨https://land.copernicus.eu/pan-european/corine-land-cover/clc2018⟩ (Accessed 1 August 2021).

Cornu, J.F., Eme, D., Malard, F., 2013. The distribution of groundwater habitats in Europe. Hydrogeol. J. 21, 949–960. https://doi.org/10.1007/s10040-013-0984-1.

Deharveng, L., Stoch, F., Gibert, J., Bedos, A., Galassi, D.M.P., Zagmajster, M., Brancelj, A., Camacho, A., Fiers, F., Martin, P., Giani, N., Magniez, G., Marmonier, P., 2009. Groundwater biodiversity in Europe. Freshw. Biol. 54, 709–726. https://doi.org/10.1111/j.1365-2427.2008.01972.x.

Devitt, T.J., Wright, A.M., Cannatella, D.C., Hillis, D.M., 2019. Species delimitation in endangered groundwater salamanders: implications for aquifer management and biodiversity conservation. Proc. Natl. Acad. Sci. USA 116, 2624–2633. https://doi.org/10.1073/pnas.1815014116.

Di Cicco, M., Di Lorenzo, T., Iannella, M., Vaccarelli, I., Galassi, D.M.P., Fiasca, B., 2021. Linking hydrogeology and ecology in Karst landscapes: the response of epigean and obligate groundwater copepods (Crustacea: Copepoda). Water 13, 2106. https://doi.org/10.3390/w13152106.

Di Lorenzo, T., Cifoni, M., Lombardo, P., Fiasca, B., Galassi, D.M.P., 2015. Ammonium threshold values for groundwater quality in the EU may not protect groundwater fauna: evidence from an alluvial aquifer in Italy. Hydrobiologia 743, 139–150. https://doi.org/10.1007/s10750-014-2018-y.

Di Lorenzo, T., Cipriani, D., Fiasca, B., Rusi, S., Galassi, D.M.P., 2018. Groundwater drift monitoring as a tool to assess the spatial distribution of groundwater species into karst aquifers. Hydrobiologia 813, 137–156. https://doi.org/10.1007/s10750-018-3515-1.

Di Lorenzo, T., Di Marzio, W.D., Sáenz, M.E., Baratti, M., Dedonno, A.A., Iannucci, A., Cannicci, S., Messana, G., Galassi, D.M.P., 2014. Sensitivity of hypogean and epigean freshwater copepods to agricultural pollutants. Environ. Sci. Pollut. Res. 21, 4643–4655. https://doi.org/10.1007/s11356-013-2390-6.

Dinerstein, E., Joshi, A.R., Vynne, C., Lee, A.T.L., Pharand-Deschênes, F., França, M., Fernando, S., Birch, T., Burkart, K., Asner, G.P., Olson, D., 2020. A "Global Safety Net" to reverse biodiversity loss and stabilize Earth's climate. Sci. Adv. 6, eabb2824 https://doi.org/10.1126/sciadv.abb2824.

Dinerstein, E., Olson, D., Joshi, A., Vynne, C., Burgess, N.D., Wikramanayake, E., Hahn, N., Palminteri, S., Hedao, P., Noss, R., Hansen, M., Locke, H., Ellis, E.C., Jones, B., Barber, C.V., Hayes, R., Kormos, C., Martin, V., Crist, E., Sechrest, W., Price, L., Baillie, J.E.M., Weeden, D., Suckling, K., Davis, C., Sizer, N., Moore, R., Thau, D., Birch, T., Potapov, P., Turubanova, S., Tyukavina, A., de Souza, N., Pintea, L., Brito, J.C., Llewellyn, O.A., Miller, A.G., Patzelt, A., Ghazanfar, S.A., Timberlake, J., Klöser, H., Shennan-Farpón, Y., Kindt, R., Lillesø, J.B., van Breugel, P., Graudal, L., Voge, M., Al-Shammari, K.F., Saleem, M., 2017. An ecoregion-based approach to protecting half the terrestrial realm. BioScience 67, 534–545. https://doi.org/10.1093/biosci/bix014.

Dole-Olivier, M.-J., Galassi, D.M.P., Marmonier, P., Creuzé des Châtelliers, M., 2000. The biology and ecology of lotic microcrustaceans. Freshw. Biol. 44, 63–91. https://doi.org/10.1046/j.1365-2427.2000.00590.x.

EEA, 2020a. Data from: Natura 2000 Data - The European Network of Protected Sites. European Environmental Agency (Accessed 1 August 2021). ⟨https://www.eea.europa.eu/data-and-maps/data/natura-11⟩.

EEA, 2020b. Data From: Nationally Designated Areas (CDDA). European Environmental Agency (Accessed 1 August 2021). ⟨https://www.eea.europa.eu/data-and-maps/data/nationally-designated-areas-national-cdda-15⟩.

EEA, 2020c. Data From: European Union Data Repository for Groundwater Quality and Vulnerability. European Environmental Agency (Accessed 1 August 2021). ⟨https://water.jrc.ec.europa.eu/groundwater.html#groundwater-vulnerability⟩.

ESRI, 2010. ArcMap 10.0. ESRI Redlands, CA.

European Commission, 2020. EU Biodiversity Strategy for 2030, Bringing nature back into our lives. ⟨file:///C:/Users/User/Downloads/factsheet-EU-biodiversity-strategy_en.pdf.pdf⟩ (Accessed 1 August 2021).

Fattorini, S., Fiasca, B., Di Lorenzo, T., Di Cicco, M., Galassi, D.M.P., 2020. A new protocol for assessing the conservation priority of groundwater-dependent ecosystems. Aquat. Conserv. 30, 1483–1504. https://doi.org/10.1002/aqc.3411.

Fiasca, B., Stoch, F., Olivier, M.-J., Maazouzi, C., Petitta, M., Di Cioccio, A., Galassi, D.M.P., 2014. The dark side of springs: what drives small-scale spatial patterns of subsurface meiofaunal assemblages. J. Limnol. 73, 55–64. https://doi.org/10.4081/jlimnol.2014.848.

Ficetola, G.F., Canedoli, C., Stoch, F., 2019. The Racovitzan impediment and the hidden biodiversity of unexplored environments. Conserv. Biol. 33, 214–216. https://doi.org/10.1111/cobi.13179.

Fitzgerald, D.B., Smith, D.R., Culver, D.C., Feller, D., Fong, D.W., Hajenga, J., Niemiller, M.L., Nolfi, D.C., Orndorff, W.D., Douglas, B., Maloney, K.O., Young, J.A., 2021. Using expert knowledge to support Endangered Species Act decision-making for data-deficient species. Conserv. Biol. https://doi.org/10.1111/cobi.13694.

Galassi, D.M.P., Fiasca, B., Di Lorenzo, T., Montanari, S., Porfirio, S., Fattorini, S., 2017. Groundwater biodiversity in a chemoautotrophic cave ecosystem: how geochemistry regulates microcrustacean community structure. Aquat. Ecol. 51, 75–90. https://doi.org/10.1007/s10452-016-9599-7.

Galassi, D.M.P., Huys, R., Reid, J.W., 2009. Diversity, ecology and evolution of groundwater copepods. Freshw. Biol. 54, 691–708. https://doi.org/10.1111/j.1365-2427.2009.02185.x.

Getis, A., Ord, J.K., 1992. The analysis of spatial association by use of distance statistics. Geogr. Anal. 24, 189–206.

Gibert, J., Vervier, P., Malard, F., Laurent, R., Reygrobellet, J.-L., 1994. Dynamics of communities and ecology of Karst ecosystems: example of three Karsts in Eastern and Southern France. In: Gibert, J., Danielopol, D.L., Stanford, J.A. (Eds.), Groundwater Ecology. Academic Press, San Diego, pp. 425–450.

Griebler, C., Avramov, M., Hose, G., 2019. Groundwater ecosystems and their services: current status and potential risks. In: Schröter, M., Bonn, A., Klotz, S., Seppelt, R., Baessler, C. (Eds.), Atlas of Ecosystem Services: Drivers, Risks, and Societal Responses. Springer, pp. 197–203.

Hancock, P.J., Boulton, A.J., Humphreys, W.F., 2005. Aquifers and hyporheic zones: towards an ecological understanding of groundwater. Hydrogeol. J. 13, 98–111. https://doi.org/10.1007/s10040-004-0421-6.

Humphreys, W.F., 2000. Relict faunas and their derivation. In: Wilkens, H., Culver, D.C., Humphreys, W.F. (Eds.), Ecosystems of the World: Subterranean Ecosystems. Elsevier, Amsterdam, pp. 417–432.

Hutchins, B.T., 2018. The conservation status of Texas groundwater invertebrates. Biodivers. Conserv. 27, 475–501. https://doi.org/10.1007/s10531-017-1447-0.

Iannella, M., Fiasca, B., Di Lorenzo, T., Biondi, M., Di Cicco, M., Galassi, D.M.P., 2020a. Jumping into the grids: mapping biodiversity hotspots in groundwater habitat types across Europe. Ecography 43, 1825–1841. https://doi.org/10.1111/ecog.05323.

Iannella, M., Fiasca, B., Di Lorenzo, T., Biondi, M., Di Cicco, M., Galassi, D.M.P., 2020b. Spatial distribution of stygobitic crustacean harpacticoids at the boundaries of groundwater habitat types in Europe. Sci. Rep. 10, 19043. https://doi.org/10.1038/s41598-020-76018-0.

IPBES, 2019. Intergovernmental science-policy platform on biodiversity and ecosystem services. In: Díaz, S., Settele, J., Brondízio, E.S., Ngo, H.T., Guèze, M., Agard, J., Arneth, A., Balvanera, P., Brauman, K.A., Butchart, S.H.M., Chan, K.M.A., Garibaldi, L.A., Ichii, K., Liu, J., Subramanian, S.M., Midgley, G.F., Miloslavich, P., Molnár, Z., Obura, D., Pfaff, A., Polasky, S., Purvis, A., Razzaque, J., Reyers, B., Roy Chowdhury, R., Shin, Y.J., Visseren-Hamakers, I.J., Willis, K.J., Zayas, C.N. (Eds.), Summary for Policymakers of the Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. IPBES Secretariat, Bonn, Germany. ⟨https://ipbes.net/sites/default/files/inline/files/ipbes_global_assessment_report_summary_for_policymakers.pdf⟩.

Karpowicz, M., Smolska, S., Swisłocka, M., Moroz, J., 2021. First insight into groundwater copepods of the polish lowland. Water 13, 2086. https://doi.org/10.3390/w13152086.

Koehnken, L., Rintoul, M.S., Goichot, M., Tickner, D., Loftus, A.-C., Acreman, M.C., 2020. Impacts of riverine sand mining on freshwater ecosystems: A review of the scientific evidence and guidance for future research. River Res. Appl. 36, 362–370. https://doi.org/10.1002/rra.3586.

Kozel, P., Pipan, T., 2020. Specialized aquatic subterranean communities are probably most species-rich in the thickest epikarst. Limnologica 81, 125756. https://doi.org/10.1016/j.limno.2020.125756.

Lammerant, J., Grigg, A., Dimitrijevic, J., Leach, K., Brooks, S., Burns, A., Berger, J., Houdet, J., Van Oorschot, M., Goedkoop, M., 2019. Assessment of Biodiversity Measurement Approaches for Businesses and Financial Institutions. EU Business@Biodiversity platform workshop report (Accessed 1 August 2021). ⟨https://ec.europa.eu/environment/biodiversity/business/assets/pdf/European_B@B_platform_report_biodiversity_assessment_2019_FINAL_5Dec2019.pdf⟩.

Linke, S., Turak, E., Asmyhr, M.G., Hose, G., 2019. 3D conservation planning: including aquifer protection in freshwater plans refines priorities without much additional effort. Aquat. Conserv. 29, 1063–1072. https://doi.org/10.1002/aqc.3129.

Liu, J., 2020. Transboundary frontiers: an emerging priority for biodiversity conservation. Trends Ecol. Evol. 35, 679–690. https://doi.org/10.1016/j.tree.2020.03.004.

Mammola, S., Cardoso, P., Culver, Deharveng, L., Ferreira, R.L., Fišer, C., Galassi, D.M.P., Griebler, C., Halse, S., Humphreys, W.H., Isaia, M., Malard, F., Martinez, A., Moldovan, O.T., Niemiller, M.L., Pavlek, M., Reboleira, A.S.P.S., Souza-Silva, M., Teeling, E.C., Wynne, J.J., Zagmajster, M., 2019. Scientists' warning on the conservation of subterranean ecosystems. BioScience 69, 641–650. https://doi.org/10.1093/biosci/biz064.

Mammola, S., Carmona, C.P., Guillerme, T., Cardoso, P., 2021a. Concepts and applications in functional diversity. Funct. Ecol. 35, 1869–1885. https://doi.org/10.1111/1365-2435.13882.

Mammola, S., Lunghi, E., Bilandžija, H., Cardoso, P., Grimm, V., Schmidt, S.I., Hesselberg, T., Martínez, A., 2021b. Collecting eco-evolutionary data in the dark: impediments to subterranean research and how to overcome them. Ecol. Evol. 11, 5911–5926. https://doi.org/10.1002/ece3.7556.

Mammola, S., Riccardi, N., Prié, V., Correia, R., Cardoso, P., Lopes-Lima, M., Sousa, R., 2020. Towards a taxonomically unbiased European Union biodiversity strategy for 2030. Proc. R. Soc. B 287, 20202166. https://doi.org/10.1098/rspb.2020.2166.

Maxwell, S.L., Cazalis, V., Dudley, N., Hoffmann, M., Rodrigues, A.S.L., Stolton, S., Visconti, P., Woodley, S., Kingston, N., Lewis, E., Maron, M., Strassburg, B.B.N., Wenger, A., Jonas, H.D., Venter, O., Watson, J.E.M., 2020. Area-based conservation in the twenty-first century. Nature 586, 217–227. https://doi.org/10.1038/s41586-020-2773-z.

Michel, G., Malard, F., Deharveng, L., Di Lorenzo, T., Sket, B., De Broyer, C., 2009. Reserve selection for conserving groundwater biodiversity. Freshw. Biol. 54, 861–876. https://doi.org/10.1111/j.1365-2427.2009.02192.x.

Niemiller, M.L., Taylor, S.J., Bichuette, M.E., 2018. Conservation of Cave Fauna, with an Emphasis on Europe and the Americas. In: Moldovan, O.T., Kováč, L., Halse, S. (Eds.). Cave ecology Springer, pp. 451–478.

Otero, I., Farrell, K.N., Pueyo, S., Kallis, G., Kehoe, L., Haberl, H., Plutzar, C., Hobson, P., García-Márquez, J., Rodríguez-Labajos, B., Martin, J.L., Erb, K.H., Schindler, S., Nielsen, J., Skorin, T., Settele, J., Essl, F., Gómez-Baggethun, E., Brotons, L., Rabitsch, W., Schneider, F., Pe'er, G., 2020. Biodiversity policy beyond economic growth. Conserv. Lett. 13, 12713 https://doi.org/10.1111/conl.12713.

Owen, D., Cantor, A., Nylen, N.G., Harter, T., Kiparsky, M., 2019. California groundwater management, science-policy interfaces, and the legacies of artificial legal distinctions. Environ. Res. Lett. 14, 045016 https://doi.org/10.1088/1748-9326/ab0751.

Pipan, T., Culver, D.C., 2013. Forty years of epikarst: what biology have we learned. Int. J. Speleol. 42, 215–223. https://doi.org/10.5038/1827-806X.42.3.5.

Pipan, T., Deharveng, L., Culver, D.C., 2020. Hotspots of Subterranean biodiversity. Diversity 12, 209. https://doi.org/10.3390/d12050209.

Pollock, L.J., O'Connor, L.M., Mokany, K., Rosauer, D.F., Talluto, M.V., Thuiller, W., 2020. Protecting biodiversity (in all its complexity): new models and methods. Trends Ecol. Evol. 35, 1119–1128. https://doi.org/10.1016/j.tree.2020.08.015.

Pressey, R.L., Visconti, P., McKinnon, M.C., Gurney, G.G., Barnes, M.D., Glew, L., Maron, M., 2021. The mismeasure of conservation. Trends Ecol. Evol. 36, 808–821. https://doi.org/10.1016/j.tree.2021.06.008.

Rabelo, L.M., Souza-Silva, M., Lopes Ferreira, R., 2018. Priority caves for biodiversity conservation in a key karst area of Brazil: comparing the applicability of cave conservation indices. Biodivers. Conserv. 27, 2097–2129. https://doi.org/10.1007/s10531-018-1554-6.

Ruffo, S., Stoch, F., 2005. Checklist e Distribuzione Della Fauna Italiana. – Memorie del Museo Civico di Storia Naturale di Verona, 2 serie. Sezione Scienze della Vita,, p. 16.

Sánchez-Fernández, D., Galassi, D.M.P., Wynne, J.J., Cardoso, P., Mammola, S., 2021. Don't forget subterranean ecosystems in global climate change agendas. Nat. Clim. Change 11, 458–459. https://doi.org/10.1038/s41558-021-01057-y.

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. BioScience 57, 573–583. https://doi.org/10.1641/B570707.

Stein, H., Griebler, C., Berkhoff, S., Matzke, D., Fuchs, A., Hahn, H.J., 2012. Stygoregions – a promising approach to a bioregional classification of groundwater systems. Sci. Rep. 2, 673. https://doi.org/10.1038/srep00673.

Tickner, D., Opperman, J.J., Abell, R., Acreman, M., Arthington, A.H., Bunn, S.E., Cooke, S.J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A.J., Leonard, P., McClain, M.E., Muruven, D., Olden, J.D., Ormerod, S.J., Robinson, J., Tharme, R.E., Thieme, M., Tockner, K., Wright, M., Young, L., 2020. Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. BioScience 70, 330–342. https://doi.org/10.1093/biosci/biaa002.

Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., Legendre, F., 2017. Taxonomic bias in biodiversity data and societal preferences. Sci. Rep. 7, 9132. https://doi.org/10.1038/s41598-017-09084-6.

Tucker, C.M., Cadotte, M.W., Carvalho, S.B., Davies, T.J., Ferrier, S., Fritz, S.A., Grenyer, R., Helmus, M.R., Jin, L.S., Mooers, A.O., Pavoine, S., Purschke, O., Redding, D.W., Rosauer, D.F., Winter, M., Mazel, F., 2017. A guide to phylogenetic metrics for conservation, community ecology and macroecology. Biol. Rev. 92, 698–715. https://doi.org/10.1111/brv.12252.

van Rees, C., Waylen, K.A., Schmidt-Kloiber, A., Thackeray, S.J., Kalinkat, G., Martens, K., Domisch, S., Lillebø, A.I., Hermoso, V., Grossart, H.-P., Schinegger, R., Decleer, K., Adriaens, T., Denys, L., Jarić, I., Janse, J.H., Monaghan, M.T., De Wever, A., Geijzendorffer, I., Adamescu, M.C., Jähnig, S.C., 2021. Safeguarding freshwater life beyond 2020: recommendations for the new global biodiversity framework from the European experience. Conserv. Lett. 14, e12771 https://doi.org/10.1111/conl.12771.

Watson, J.E.M., Dudley, N., Segan, D.B., Hockings, M., 2014. The performance and potential of protected areas. Nature 515, 67–73. https://doi.org/10.1038/nature13947.

Wilkinson, C., Sendstad, M., Parnell, S., Schewenius, M., 2013. Urban governance of biodiversity and ecosystem services. In: Elmqvist, T., Fragkias, M., Goodness, J., Güneralp, B., Marcotullio, P.J., McDonald, R.I., Parnell, S., Schewenius, M., Sendstad, M., Seto, K.C., Wilkinson, C. (Eds.), Urbanization, Biodiversity and Ecosystem Services: Challenges and Opportunities. Springer, Netherlands, pp. 539–587.

Wynne, J.J., Howarth, F.G., Mammola, S., Ferreira, R.L., Cardoso, P., Di Lorenzo, T., Galassi, D.M.P., Medellin, R.A., Miller, B.W., Sánchez-Fernández, D., Bichuette, M.E., Biswas, J., BlackEagle, C.W., Boonyanusith, C., Amorim, I.R., Borges, P.A.V., Boston, P.J., Cal, R.N., Cheeptham, N., Deharveng, L., Eme, D., Faille, A., Fenolio, D., Fišer, F., Fišer, Z., Gon, I.I.I., Goudarzi, S.M. 'O., Griebler, F., Halse, C., 27, S., Hoch, H., Kale, E., Katz, A.D., Kováč, L., Lilley, T.M., Manchi, S., Manenti, R., Martínez, A., Meierhofer, M.B., Miller, A.Z., Moldovan, O.T., Niemiller, M.L., Peck, S.B., Giovannini Pellegrini, T., Pipan, T., Phillips-Lander, C.M., Poot, C., Racey, P.A., Sendra, A., Shear, W.A., Souza-Silva, M., Taiti, S., Tian, M., Venarsky, M.P., Yancovic-Pakarati, S., Zagmajster, M., Zhao, Y., 2021. A conservation roadmap for the subterranean biome. Conserv. Lett., e12834 https://doi.org/10.1111/conl.12834.

Yang, R., Cao, Y., Hou, S., Peng, Q., Wang, X., Wang, F., Tseng, T.-H., Yu, L., Carver, S., Convery, I., Zhao, Z., Shen, X., Li, S., Zheng, Y., Liu, H., Gong, P., Ma, K., 2020. Cost-effective priorities for the expansion of global terrestrial protected areas: setting post-2020 global and national targets. Sci. Adv. 6. https://doi.org/10.1126/sciadv.abc3436.