GROUNDWATER BIODIVERSITY

Protocols for the ASsessment and Conservation of Aquatic Life In the Subsurface (PASCALIS): overview and main results

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ABSTRACT

The PASCALIS project (EVK2-CT-2001-00121) (2002-2004) was the first comprehensive proposal that specifically addresses the groundwater biodiversity issue at the European scale and represents also an important backbone for research development in groundwater. The main objective was to establish a rigorous and detailed protocol for assessing groundwater biodiversity and to develop operational tools for its conservation. More precisely, a solid unmatched piece of work was provided by the PASCALIS database that reflects our present-day knowledge on stygobiotic biodiversity distribution in six European countries. The cryptic diversity has been explored by using molecular approach on reference taxa and revealed to be a useful tool in the implementation and extension of our knowledge of groundwater biodiversity. Furthermore, a sampling protocol has been assessed, allowing reliable results on species distribution within selected hierarchical units. Results show that species distribution differs from one region to another, and that species similarity between regions is very low due to the high level of endemicity observed. In addition, biodiversity indicators among stygobionts resulted to be a very useful tool to build up a network of reference sites at European scale with reduced costs and sampling effort. Significant improvements have been introduced in the groundwater biodiversity conservation strategy by proposing new methodologies relying on sound scientific results. Finally, the ways to effectively implement a coherent, sustainable and scientific solid conservation strategy have been explored in the framework of EU policies related to biodiversity and sustainable management of groundwater resources.

1. INTRODUCTION

Groundwater plays an important role for the sustainability of many earth ecosystems and a crucial role in human life and socio-economic development (Danielopol *et al.* 2003, Gibert *et al.* in press). Because there are continuously evolving economic, social and cultural pressures on groundwater resources, it is becoming urgent to provide an updated picture of groundwater biodiversity and to propose a global strategy for groundwater management and conservation. In this framework, the main objective of the proposal was to establish a strict and comprehensive protocol to estimate groundwater biodiversity and to elaborate operational tools for its conservation. Knowledge of groundwater biodiversity stands behind that of all other aquatic ecosystems. This puts a major constraint on a successful implementation of its conservation. In this context, the specific scientific objectives of the PASCALIS project were (Gibert 2001):

- to establish the general patterns of groundwater biodiversity at the European scale in creating a large database, firstly to store all information available on subterranean taxa distribution, secondly by incorporating also the new records obtained following the PASCALIS sampling campaigns, to map the groundwater biodiversity at the European scale;

- to improve biodiversity evaluation by exploring genetic diversity of taxa in order to estimate the cryptic biodiversity within selected groups;

- to set up a stratified sampling scheme in order to obtain an optimum field sampling strategy and an unbiased estimate of biodiversity at the regional scale;

- to analyse the patterns of biodiversity and identify potential biodiversity indicators at the regional scale;

- to develop strategies for conservation of groundwater biodiversity in the European socio-economic context by identifying some regions of special biological interest, the spatial scale of relevance for conserving biodiversity within these regions, in order to propose a series of appropriate measures for maintaining their biodiversity.

2. MAIN RESULTS

2.1 Insight on the diversity of stygobiotic species and mapping in southern Europe

Knowledge of the spatial dimension of biodiversity is the basic need to any conservation strategy. Consequently, mapping biodiversity is the fundamental starting point to understand spatial patterns of biodiversity (Gaston and Spicer 2004). Little has been actually achieved on groundwater biodiversity at the European scale (Danielopol *et al.* 2000; Gibert and Culver 2004). Only a few maps for some given taxonomic groups are presently available at national levels in sparse European countries (Juberthie and Decu 1994, 1998, Stoch 2001) but no global representation of national groundwater biodiversity exists. The PASCALIS project attempted to characterize distribution patterns of biodiversity at operational scales for biogeography and conservation.

A flexible biodiversity storage tool has been developed, for the six European countries (Belgium, France, Italy, Portugal, Spain, and Slovenia), as a basis for a mapping strategy to be used for groundwater habitats. Based on the Endemism project (Deharveng 2001), a central database structure has been produced (Appendix 1), comprising 5 tables (Reference, Distribution, Species, Genera, Co-ordinates) of 10 to 25 fields each, and 22 procedures written in the 4D language. The reference table contains 1,756 records. The distribution table has 17,367 records of which 14,780 concern stygobionts. The Species table contains 2,346 species or infraspecific taxa, of which 1,264 are stygobionts (Table 1). The Genera table has 662 genera, most of which are not stygobionts. The Co-ordinate table has 3,971 records. The dataset covers most (likely over 90%) of the published records of stygobiotic taxa in the 6 selected countries.

Different distributional maps were produced: species richness (Fig.1), hotspots of species richness, strict endemism, endemicity scores, range-size rarity scores, taxonomic isolation scores. A map of the residuals of the regression between species richness and site numbers was produced and used to assess the level of knowledge of groundwater stygofauna in southern Europe.

			Family	Genera	Species/subspecies			
Acari			17	32	62			1264
Amphibia			1	1	3			
Annelida		Aphanoneura	1	1	1			
		Oligochaeta	5	20	50			
		Polychaeta	2	2	2			
Cnidaria		Hydrozoa	1	1	1			
Crustacea		Amphipoda	11	28	255		899	
		Branchiopoda	3	4	9			
	Copepoda	Calanoida	1	4	6	290		
	Copepoda	Cyclopoida	1	13	103			
	Copepoda	Gelyelloida	1	1	1			
	Copepoda	Harpacticoida	6	20	180			
		Decapoda	2	3	5			
		Isopoda	6	19	200			
		Mysidacea	2	2	2			
		Ostracoda	6	14	40			
		Syncarida	2	24	92			
		Thermosbaenacea	2	3	6			
Insecta			1	1	2			
Mollusca		Gastropoda	6	43	188			
Porifera			1	1	1			
Protozoa			1	6	7			
Protozoa		Ciliata	1	6	7			
Turbellaria		Temnocephalida	1	4	10			
		Tricladida	2	9	31			

Table 1: Taxonomic distribution of main stygobiotic taxa in Europe (Belgium, Spain, France, Italy, Portugal and Slovenia)

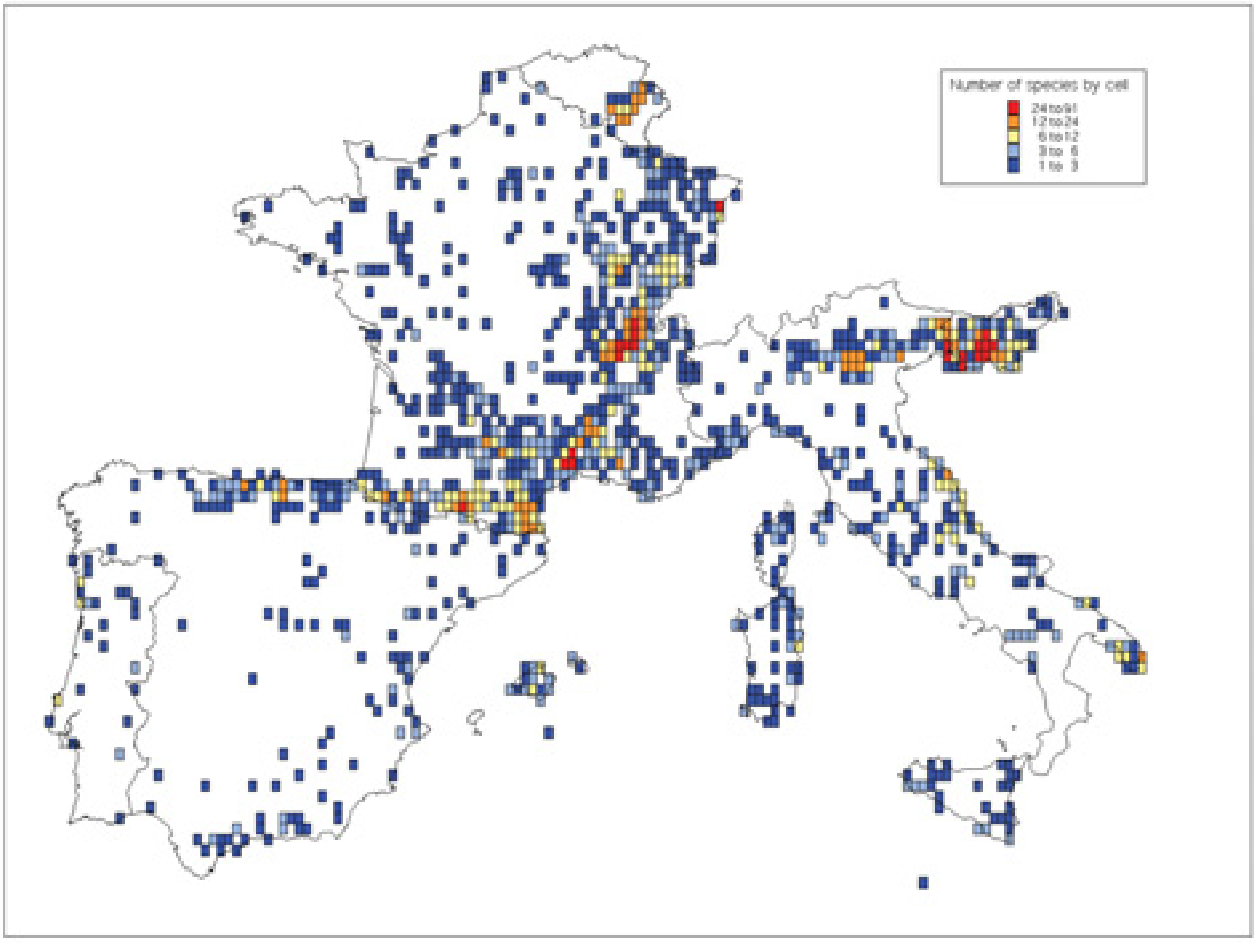

Figure 1 : Species richness in the PASCALIS consortium countries

Present biodiversity distribution patterns are still largely impacted by uneven sampling effort among regions and countries. However, the analysis of these patterns already reveals hotspots and coldspots of highly isolated taxa, using LISA models (Anselin 1995). The highest values are concentrated in a longitudinal area extending from Slovenia to the Mediterranean French coast following the Italian Pre-Alps, while areas of very low taxonomically isolated assemblages are found in the northern part of France. Most of Paleothyrrhenian areas (Sardinia, Corsica and coastal areas of Italy) and part of the Iberian Peninsula show stygobiotic assemblages, poor in terms of number of species but conversely rich in endemics.

The present-day knowledge of the groundwater biodiversity distribution patterns has been deeply enlarged in all the PASCALIS countries, even if further field work is still needed to obtain more information in some poorly sampled regions, such as the Iberian Peninsula, southern Italy and north-western France.

2.2 Hidden diversity in groundwater

In the PASCALIS project, biodiversity assessment essentially relies upon morphologically based identification and traditional taxonomic criteria. However, previous studies showed that groundwater and subterranean habitats as a whole harbour remarkably high numbers of sibling or cryptic species (Stoch 1995). These species cannot be identified by standard morphological analyses, identification keys and only sporadically morphological microcharacters may be used to distinguish closely related species. The awareness about this high cryptic diversity is based on a number of studies on genetic differentiation and population structure (e.g., Avise and Selander 1972, Laing *et al.* 1976, Sbordoni *et al.* 1979, Caccone *et al.* 1986, Sket and Arntzen 1994, Gentile and Sbordoni 1998, Wiens *et al.* 2003, Lefébure *et al.* submitted). Most of these studies used allozymes as genetic markers. These markers are suitable for detecting genetic population structure, gene flow, or boundaries to gene flow, but are less reliable in terms of phylogenetic accuracy, evolutionary rates, and discerning historical events from ongoing processes. Many evolutionary models try to explain the high degree of hidden diversity in subterranean systems (reviewed in Wilkens *et al.* 2001) and the overall diversity of groundwater fauna (Stoch 1995). The commonly recognized models to explain the high level of differentiation in the hypogean environment argue a combination of radiation and multiple invasions. Homoplasy by means of convergence and parallelism seems to be widespread in the subterranean environment. Not less important is the role played by vicariance at different spatial and temporal scales, which may allow the splitting up of an ancestral population and its diversification in different, sometimes morphologically indistinguishable, cryptic species. It is in this general framework, that in the PASCALIS project, four representative taxa have been selected for molecular analyses (Trontelj *et al.* 2004).

- The amphipod ***Niphargus*** is the most widely distributed and diverse in Europe, comprising about 300 species/subspecies. The preliminary phylogenetic hypotheses based on 28S rDNA sequences seem to argue for a paraphyly of the genus. The overall structure of the tree is rather bush-like, with many short internal branches. It is possible that few clades are oversplit. On the other hand, a single species may be composed of distinct, well separated lineages. For example, *Niphargus virei* that shows a weak morphological differentiation among different populations (Ginet 1960), presents an unpredicted molecular tripartite structure based on two independent genes (28S and COI). This suggests that the history of this species might have been eventful, involving phenomena of dispersal and vicariance at various stages of its evolutionary history (Lefébure *et al.* in press).
- The isopod ***Asellus aquaticus*** is an expansive Western Palaearctic species, which has successfully dispersed across Europe (Henry and Magniez 1983). It has a prominent genetic population structure at small geographic distances (<100 km). A. aquaticus can be considered a target “species-group” for clarifying the processes leading to high lineage diversity in restricted areas in the groundwater network (habitat and microhabitat scales). Discrepancies between the morphological-based identification versus the genetic results placed in a phylogeographic scenario were found.
- ***Hirudinea*** belonging to the genera Dina and Trocheta are frequently found in various groundwater habitats, mainly in karstic aquifers. Their systematics is controversial and the specific and generic attributions open to question. Phylogenetic analyses of ITS and COI gene sequences reveal in some cases a strong discrepancy between morphologically-based systematics and phylogenetic relationships among taxa. The cryptic diversity observed in both genera could be the consequence of convergent or parallel evolutionary changes in non-sister groundwater taxa.
- ***Proteus anguinus***, the blind cave salamander is distributed along the Dinaric karst of the western Balkan Peninsula in several

hydrographically isolated areas (Sket 1997). Deep splits between some groups of populations were found in the phylogenetic analysis, indicating the presence of cryptic species.

The molecular approach of selected taxa revealed that cryptic diversity may represent a significant part of groundwater biodiversity at the European scale. The genetic variation can be explained by the multiple cave invasions model or can be the consequence of convergent or parallel evolutionary changes in non-sister groundwater taxa. Convergence may stem from the conservative nature of groundwater in respect to the more changeable epigean freshwater, and may be the result of adaptation to the major environmental describers (Galassi 2001). Convergence toward similar morphological traits may be the consequence of the same response to similar groundwater environments. For this reason, genetically distinct phylogenetic lineages of stygobiotic taxa, that have ceased to exchange genes, may escape clear taxonomic distinction. These results give a good idea about the extent of diversity and endemism that may be overlooked when relying upon traditional taxonomy. From data of the present study, the cryptic biodiversity should be considered as a priority tool for assessing overall groundwater biodiversity.

2.3 Sampling protocols and biodiversity assessment

Due to the inaccessibility of the subterranean environment, planning an efficient groundwater sampling strategy is a difficult task (Gibert *et al.* 1994; Griebler *et al.* 2001). It may not be possible to distribute sampling sites wherever necessary because access is only possible through a limited number of outputs (springs, resurgences) or windows (caves, wells). Apart a few attempts in particular habitats, such as those by Scarsbrook and Halliday (2002) and Boulton *et al.* (2003, 2004) in the porous aquifer, no sampling strategies have been proposed nor tested up to now, to solve such a basic question.

A rigorous sampling protocol has been applied in 6 selected regions (Walloon karst in Belgium, Meridional Jura and Roussillon in France, Cantabria in Spain, Lessinia in Italy and Krim massif in Slovenia) (Malard *et al.* 2002). It is based on a stratified sampling strategy, following a hierarchical scheme, including: region, basin, aquifer type (karst and alluvial), zone in each aquifer (unsaturated zone and saturated zone in karst, hyporheic zone and phreatic zone in unconsolidated sediments) (Fig.2). In each region, 192 sampling sites were selected to assess the groundwater biodiversity.

The main results indicate that the high sampling effort performed during the PASCALIS surveys was not satisfactory in some areas (192 sites per region) due to the high amount of rare species and strict endemics. The stratification considering karstic areas and unconsolidated sediments as different sampling units should be used in every sampling design, being statistically significant in discriminating different species assemblages (Stoch *et al.* 2004).

In order to explore the relationship between the structure of obligate-groundwater assemblages and environmental gradients, multivariate statistical analyses were applied to the environmental and species data sets. Trends of biodiversity were highlighted by the statistical analyses, suggesting the dominant role of geography, paleogeography, habitat structure and water chemistry.

Additive partitioning of groundwater species diversity across nested spatial scales - aquifers, basins, and regions - using species-richness data, was performed; the between-region

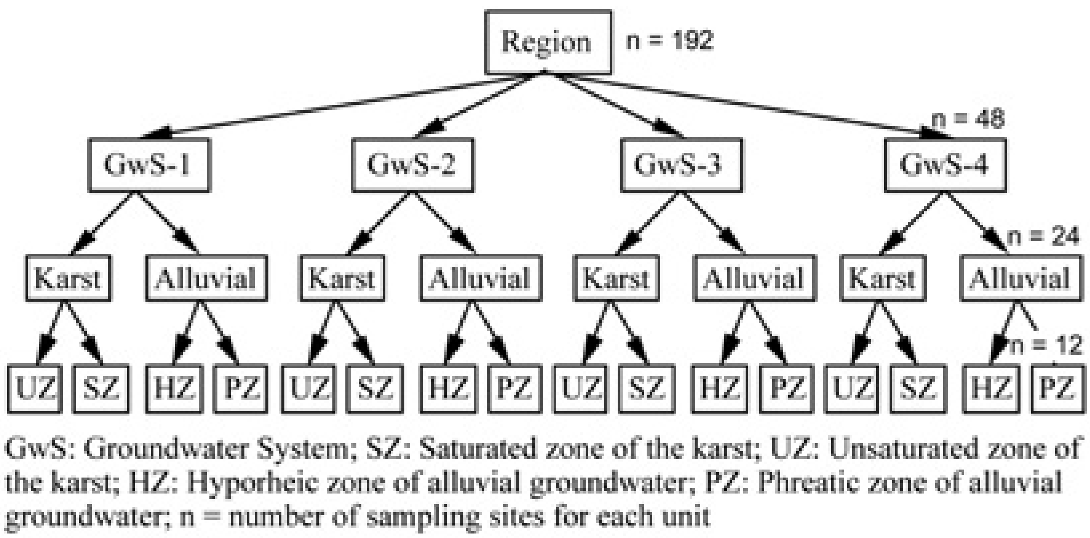

Figure 2 : Hierarchical sampling scheme.

component made by far the highest contribution to the stygobiotic species richness, e.g. community composition varied most importantly over broader spatial scales. The results clearly indicate that the most effective way to preserve stygobiotic diversity in southern Europe is to protect multiple aquifers within different regions and with different environmental features.

2.4 Identification of potential biodiversity indicators and conservation indicators

Species richness is a simple measure of biodiversity and a widely used criterion for biodiversity assessment and conservation. However, data on species richness are reliable only if derived from exhaustive inventories; unfortunately, inventories over large spatial scales are expensive and time-consuming. Predictive models of species richness have been used as an alternative to conduct extensive field studies. These models rely on environmental variables, named “environmental surrogates” of species richness (Araujo *et al*. 2001) or the identification of a representative species group, able to reflect the overall species richness of an entire biota (Pearson, 1994; Pimm *et al*. 2001). These species are named “biodiversity indicators” (Mac Nally and Fleishman 2004).

Sets of environmental parameters, species and higher level taxa were selected as indicators of groundwater biodiversity; multiple regression models and statistically sound information criteria were used to select the indicators and assess their predictive power of species richness. Unfortunately, stygobiotic species vary from one region to another due to the high degree of endemism in groundwater taxa. However, at both European and regional scales, the higher taxa like Gastropoda, Harpacticoida and Amphipoda appear to be significantly correlated with total species richness (Stoch 2004).

Thus, biodiversity indicators are a very useful tool to detect spatial patterns of species richness at large scale, with reduced costs and sampling effort. However, the model should be applied with caution, as the results cannot be extended outside the study area, given the large amount of fine-scale autocorrelation.

A standard method to build conservation indices, based on the information stored in the PASCALIS database and on the grid cells used to map the distribution of species over Europe, has been developed. The degree of endemicity, range-size rarity, habitat selection and taxonomic isolation (included phylogenetic relictuality) were incorporated after normalization in a conservation index. Mean values of endemicity, rarity, and taxonomic isolation (weighted for relictuality) were used to assign a cumulative conservation value to each of the species included in the database. Limitations to this procedure are linked to the high level of endemism of stygobiotic species (more than 83 % of them can be defined as strict endemics) which prevents the selection of a threshold useful for discriminating priority species (Stoch *et al.* 2004).

2.5 Elaboration of an operational conservation strategy

In the European policies, most of the attention regarding groundwater has understandably concerned its use as a safe source of drinking water of primary importance for a large majority of the EU inhabitants. However, it has become increasingly obvious that groundwater should not only be viewed as a drinking water reservoir but also as critical aquatic ecosystems that must be taken into account in the European legislation.

The Habitat Directive (European Commission 2003) has so far mostly neglected groundwater habitats and species (Juberthie 1995, IUCN 2004) and the Water Framework Directive (European Union 2001) did not take groundwater biodiversity into consideration and did not expressly perceive the “good ecological status of ground water” as for surface waters.

As GW ecosystems were never considered when defining priority sites and areas for conservation, there is an evident missing point in excluding stygofauna in the establishment of natural reserves. In order to fill this gap, different methods to select the most efficient network of GW biodiversity reserves, priority species and habitats were tested (De Broyer *et al.* 2004).

2.5.1 The designation of “Groundwater Biological Reserves”

Different methods were tested to design a network of aquifers (as biological groundwater reserves) containing a significant proportion of European groundwater biodiversity (including species of high conservation value) with low-cost procedures. In order to maximize the conservation effectiveness of the network composed of a fixed amount of grid cells, we tested and compared the conservation effects of different cell assemblages, taking into account different parameters, such as species richness, number of endemics, relictuality and conservation value of species contained in the selected cells. The construction of the conservation network, based on complementarity test (between grid cells) offers a more effective approach instead of selection methods based on hotspots of biodiversity and endemism.

A simple method of risk assessment was proposed which combined criteria of aquifer vulnerability and human activities. Vulnerability and human pressure indexes were combined in order to distinguish between 3 distinct types of biological groundwater reserves: healthy, under threat, highly anthropised. Despite several methodological difficulties linked to the paucity of information available for some aquifers, the method proves to be a valuable tool for prioritizing conservation efforts among aquifers of biological interest.

2.5.2 Identification of priority species for groundwater biodiversity conservation

Umbrella species have been selected (Andelman and Fagan 2000) for groundwater biodiversity conservation, according to the basic principle that they are species with ecological requirements encompassing those of the remaining members of a community. By recommending some conservation measures for aquifers hosting a limited number of umbrella species, the conservation effects do benefit to all close-by species. Different ways of selecting umbrella species have been tested aiming at defining a limited number of species covering priority biodiversity hotspots and associated to a high proportion of priority species. A list of 30 taxa that should receive a legal status in order to contribute to a better protection of GW biodiversity has been proposed.

2.5.3 Definition of a list of priority habitats for groundwater conservation

The selection of priority groundwater sites was mostly based on traditional expert selection methods. An innovative approach has been proposed as follows: the reciprocal discrimination of correspondence analysis and hierarchical cluster analysis were used to distinguish between distinct groundwater habitats based on biological data collected (as an example) from 55 aquifers in France. Results show that a spatial hierarchical approach provides a consistent theoretical framework for identifying groundwater habitats comprising dissimilar set of species. Alpha and beta diversity can be used to prioritize conservation efforts among groundwater habitats (Ferreira *et al.* submitted).

The PASCALIS project provides a rare opportunity to propose to European stakeholders a specific Action Plan for the conservation of a unique and neglected part of the European aquatic biodiversity (De Broyer *et al.* 2004). This Action Plan recommends to integrate the groundwater biodiversity protection concern in all relevant European policies, legislation and instruments dealing with conservation and sustainable management of natural resources - in particular water resources (WFD and GW Daughter Directive), nature conservation (Habitat Directive, Natura 2000), - as well as in relevant horizontal legislation and instruments concerning in particular environment, land use, and agriculture.

Relying on the scientific outputs of the PASCALIS project and the expertise of the PASCALIS contributing teams, the following general recommendations are made for the implementation of an Action Plan for the conservation of groundwater (GW) biodiversity in Europe:

REC. 1: TO INTEGRATE THE GW BIODIVERSITY AND ECOSYSTEMS CONSERVATION CONCERN IN ALL RELEVANT EUROPEAN POLICIES

- Considering that GW is not only an exploitable resource but also an ecosystem as all other water bodies;
- Considering the important scientific, heritage and eco-functional value of the European GW biodiversity and ecosystems;
- Noting the present and potential threats they face or may face;
- Considering that GW biodiversity, habitats and ecosystems have been largely overlooked in the current policies implementing the EU biodiversity strategy;

It is recommended:

- to integrate the GW biodiversity and ecosystems concerns in all relevant European policies (legislation and instruments) dealing with biodiversity and sustainable management of natural resources, in particular water resources (Water Framework Directive and GW Directive) and biodiversity conservation (Habitat Directive, Natura 2000), as well as in horizontal environmental legislation and instruments regarding in particular land use, agriculture, environmental impact assessment, and environmental monitoring.

REC. 2: TO COMPLETE THE HABITAT DIRECTIVE POLICY

- Considering the diversity of GW habitats of special conservation interest;
- Noting the high conservation value of most GW species;
- Considering the lack or very limited inclusion of priority GW habitats and species in the Habitat Directive Annexes 1 and 2;

It is recommended:

- To formally include the priority GW species and habitats in the respective Annexes of the Habitat Directive at the next revision opportunity.

Rec. 2.1 : To establish a list of priority GW species for conservation

- Considering that the establishment of a list of priority stygobiotic species at European level as well as at national and regional levels should follow a sound selection protocol taking into account the conservation value of the stygobiotic species (in particular on the basis of criteria of endemism, rarity, and phylogenetic relictuality) and their endangered status;
- Noting that, given the high conservation value of most stygobiotic species and the (potential) endangered status of many of them, a comprehensive list of European GW priority species for conservation does not appear operational for complete inclusion in Annex 2 and that alternative limited selection of representative GW species has to be defined;

It is recommended:

- To rely on a surrogate species approach (such as umbrella species and biodiversity indicators) as an operational conservation strategy to establish a limited list of representative priority species whose habitat conservation allow covering a significant part of the European GW biodiversity.
- To explore new ways of defining species value and of integrating it into site selection process, for a better estimate of site biological value.

Rec. 2.2: To establish a list of priority GW habitats

- Considering that the existing habitat categories relevant for GW in Annex 1 of the Habitat Directive do not acknowledge the diversity of groundwater habitats;
- Considering the complexity of the procedures involved to modify the Habitat Directive and to add some species and habitats to its Annexes;
- Considering that the subdivision of the subterranean hydrosphere in various habitats must be based on sound hydrogeological units taking into account the physico-chemical conditions, and, on the other hand, on the biogeographic spatial and temporal context;

It is recommended:

- To rely on a pragmatic strategy to include priority GW habitats in Annex 1 as it stands by broadening the interpretation of the existing habitat categories relevant for GW and completing the “Interpretation Manual of European Union Habitats” accordingly, before the formal recognition by a revision of the Directive.
- To define the GW (macro)habitats in a regional framework on the basis of the aquifer unit or assemblages of aquifers having identical hydrogeological characteristics and similar palaeoclimatic or palaeogeographic histories, and sharing a suite of characteristic species.

Rec. 2.3 : To develop a European network of priority sites (GW natural reserves) for GWB conservation

- Considering that the appropriate spatial scale to take into account for defining GW natural reserves on ecological and hydrological basis is the aquifer;
- Considering that the most effective way to preserve stygobiotic biodiversity in Europe is to protect multiple aquifers presenting high biodiversity complementarity and different

environmental features within different regions and, there, by maintaining regionally distinctive species-rich assemblages;

- Considering that the European biogeographic regions as referred to by the Habitat Directive do not apply to the GW fauna for which specific biogeographic stygoregions have to be defined;

It is recommended:

- To establish a network of priority aquifers as natural reserves for GWB conservation at European scale but also at national and regional scale, by relying on a sound selection protocol taking into account the intrinsic scientific and heritage value of their biodiversity and by properly assessing (on the basis of the aquifer(s) vulnerability and intensity of human pressure) the risk they are facing in order to define priorities in terms of conservation.

- To attempt to include the maximum number of GW taxa in the priority aquifers at the minimal cost in socio-economical terms by using complementarity approaches for site selection.

- To regularly update, under control of recognized experts, the priority sites network based on a complementarity approach, in order to integrate progress of knowledge on GWB.

- To define biogeographic stygoregions to allow assessing the representativeness of GW habitats and species and to integrate this information into priority species and habitat selection protocols.

- To develop biodiversity modelling in order to compensate the lack of information on biodiversity distribution in many areas that handicaps the habitat selection protocol.

REC. 3: TO INTRODUCE BIODIVERSITY CONCERN AND GOOD ECOLOGICAL STATUS OF GW IN THE WATER FRAMEWORK DIRECTIVE AND THE GW DIRECTIVE POLICY

- Noting that the WFD and the proposed GW Directive consider GW only as a resource and not as an ecosystem, contrary to the surface water systems for which the ecological dimension is duly recognized;

- Recalling that, like surface water systems, GW systems undergo to hydrological, chemical and biological processes which collectively define their ecological status;

- Noting that in the proposed GW Directive the GW protection measures are mainly based on the maintenance of a quantitative equilibrium (balance between GW abstraction and recharge), and on chemical quality (based on threshold concentrations of some GW chemical constituents);

- Considering that the maintenance of a high diversity of GW micro-organisms is crucial to the self-purification processes of GW systems, and that beside micro-organisms, stygobiotic invertebrate and vertebrates can be selectively used as bioindicators of the structural, functional and healthy state of GW ecosystems;

It is recommended

- To elaborate the instruments (including the required expertise and knowledge background) necessary to accurately define the “good ecological status” of GW in the regional context and take it into account in GW management.

- To develop efficient monitoring strategies for GWB and ecosystems (selective biological and environmental indicators, reference sites) in order to ensure a follow-up of the GW populations in selected priority aquifers and of the functional characteristics of the GW ecosystems.

- To consider developing potential monitoring synergies by relying - where relevant - to the reference sites network required by the WFD for monitoring GW resources.

- To improve by appropriate investigations the indicator potential of the GW biodiversity in the characterization of the connectivity with surface waters (and therefore the GW vulnerability) and of the qualitative state of GW.

REC. 4 : TO DEVELOP SCIENTIFIC KNOWLEDGE ON GW BIODIVERSITY AND ECOSYSTEMS

- Considering the large gaps in the scientific knowledge of the nature and distribution of GW biodiversity, its habitats and communities as well as its eco-functional roles;
- Noting that these limitations hamper the development of comprehensive conservation initiatives in favour of GW ecosystems and biodiversity in the framework of European, national or regional policies, as well as the development of a sound ecological approach of groundwater management;

It is recommended:

- To make efficiently available the scientific information on European GW biodiversity and distribution by developing and maintaining a centralized European GWB database.
- To support the establishment of a European platform for GW biodiversity combining the expertise of ecologists, biologists and hydrologists to manage the European GWB database and advise on the implementation of relevant European environmental policies and of a GWB conservation strategy along the lines recommended in this Action Plan.
- Among the priority research topics to focus on, it is suggested:
- To plan large scale sampling surveys to complete the knowledge of the nature and distribution of European groundwater biodiversity at regional and aquifer scale.
- To plan, for reference purposes, in depth all-taxa surveys of selected representative aquifers of different characteristics, which include the assessment of cryptic biodiversity by molecular methods.
- To stimulate the taxonomic study of GW fauna and the development of efficient taxonomic identification tools usable by non-taxonomists.
- To define the European biogeographic stygoregions.
- To develop population dynamics studies of reference GW species in reference sites in order to accurately assess their endangered status.
- To improve the understanding of the structure and function of GW ecosystems in relation to various environmental conditions and anthropogenic influences.
- To assess the role of micro-, meio- and macro-organisms in GW self- purification and biological remediation, as well as their potential as GW quality indicators.
- To strengthen the ecological basis of groundwater management by conducting studies on the sensitivity of GW species to pollution and physical disturbance; the effects of pollutants on biodegradation processes; the ecological recovery of groundwater ecosystem after remediation.
- To improve the assessment of human impact and land-use practices on structural and functional aspects of phreatic, hyporheic, and karstic ecosystems; and the impact of water mining on the functioning of GW ecosystems.

REC. 5 : TO RAISE PUBLIC AWARENESS

- Recalling that the recent Malahide Conference identified the promotion of broader public awareness, understanding and support for the conservation and sustainable use of biodiversity as a critical overarching issue, and recognized that there is an urgent need to mobilise public opinion in support of biodiversity;
- Considering the general lack of knowledge by decision-makers as well as by the general public of the existence, interests and roles of the GW ecosystems;
- Recognising the high heritage, scientific and ecological values of their biodiversity;
- Stressing that an effective conservation policy requires the acceptance, support, and understanding of the socio-economic actors;

It is recommended:

- To undertake systematic campaigns of public awareness targeted towards stakeholders and policy makers in charge of biodiversity conservation, management of natural resources and the environment at European, national, regional and local scale, the water basin agencies and water supply companies, the education- (at all pertinent levels), scientific- and media communities as privileged information relays, the civil society organizations, the general public.
- In these campaigns, to present groundwater fauna and its conservation in the more global framework of groundwater protection, as an essential resource for human welfare.
- To develop educational supports dealing not only with GW biodiversity but with more general aspects of groundwater (geological features of aquifers, water cycle, origin of drinking water, etc.).
- To attempt to designate some GW flagship species which can help in arising people interest on GW ecosystems and biodiversity.

3. CONCLUSIONS

PASCALIS is the first research Programme on groundwater ecology and conservation, funded under the “Global Change, Climate and biodiversity” within the Energy, Environment and Sustainable Development framework Programme of the European Commission. The study of groundwater biodiversity in 6 countries (Belgium, France, Italy, Portugal, Slovenia and Spain) and in 6 selected regions more specifically investigated (Walloon karst, Meridional Jura, Roussillon, Cantabria, Lessinia and Krim massif) allows to draw the following conclusions:

- Synthetic information on groundwater biodiversity becomes available through PASCALIS and constitutes an important backbone for further scientific research. It may also serve as a strong basis for communication about groundwater biodiversity in Europe towards policy makers and other disciplines.
- The subterranean environment is unique if compared to surface environments because it includes a large number of endemic and rare species. Over 83% of the stygobiotic species in the PASCALIS countries can be classified as strict endemics and over 69% are rare.
- At regional scale, for the first time, a rigorous stratified sampling scheme based on a hierarchical approach by examining biodiversity in 6 regions has been applied. General guidelines have been proposed to improve sampling protocols at regional scale, demonstrating the efficiency of a stratification sampling based on aquifer type (karstic vs. porous aquifers). Nevertheless, due to the low similarity between regions and the high level of endemism at sub-regional scale, a stronger sampling effort and a finer stratification, which takes also into account basins, habitat structure, elevation gradient, historical factors, etc, have been strongly recommended.
- Cryptic diversity appears very high in subterranean environments. Cryptic diversity due to cryptic speciation and between non-monophyletic lineages may significantly increase alpha and beta diversity at multiple different spatial scales.
- Considering the biodiversity partitioning, between-region richness made by far the highest contribution (80%) to total richness in Europe. The contribution of alpha and beta diversity to regional species richness probably changes as a function of spatial scale.
- Environmental parameters and higher taxa richness can be used as good indicators and predictors of species richness at regional scale. However, indicators of biodiversity at the species level were different in each region; for this reason, higher taxa were used to predict species richness at the European scale.
- PASCALIS helps to identify regions and aquifers harbouring high biodiversity. The construction of the conservation network, based on complementarity test offers a more effective approach than selection methods based on hotspots of biodiversity and endemism. Reserves should be preferentially defined using aquifers as reference units. Moreover, PASCALIS can help to answer the question to what extent Natura 2000 covers also aquifers of high subterranean biodiversity. Overlay analysis may help to clarify where surface and subsurface biodiversity protection go together and where the most important gaps are traceable.

Follow-up research should focus on further spatial analysis and identification of main pressures for groundwater biodiversity, linking structural and functional aspects, and modelling the most important pressure-response relationships. Better link is needed between hydrological, biogeochemical and biodiversity information of aquifers placed in a land-use scenario, water management and agriculture policies.

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