

Biodiversity indicators in European ground waters: towards a predictive model of stygobiotic species richness

FABIO STOCH*, MALVINA ARTHEAU[†], ANTON BRANCELJ[‡], DIANA M. P. GALASSI* AND FLORIAN MALARD[§]

*Dipartimento di Scienze Ambientali, University of L'Aquila, L'Aquila, Italy

[†]Laboratoire d'Ecologie des Hydrosystèmes, Université Paul Sabatier, Toulouse, France

[‡]National Institute of Biology, Ljubljana, Slovenia

[§]Laboratoire d'Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon, Villeurbanne, France

SUMMARY

1. Estimates of species richness obtained from exhaustive field inventories over large spatial scales are expensive and time-consuming. For this reason, efficiency demands the use of indicators as 'surrogates' of species richness. Biodiversity indicators are defined herein as a limited suite of taxonomic groups the species richness of which is correlated with the species richness of all other taxonomic groups present in the survey area.
2. Species richness in ground water was assessed at different spatial scales using data collected from six regions in Europe. In total, 375 stygobiotic species were recorded across 1157 sites and 96 aquifers. The taxonomic groups collected from more than one site and with more than two species (Oligochaeta, Gastropoda, Cyclopoida, Harpacticoida, Ostracoda, Isopoda, Amphipoda, Bathynellacea and Acari) were used to develop nonparametric models to predict stygobiotic biodiversity at the aquifer scale.
3. Pair-wise correlations between taxonomic groups were low, i.e. variation in species richness of a single taxonomic group did not usually reflect variation of the other groups. In contrast, multiple regressions calculated between species richness of any combination of taxa and extra-group species richness along the six regions resulted in a number of significant relationships.
4. These results suggest that some taxonomic groups (mainly Copepoda and Amphipoda and, to a lesser extent, Oligochaeta and Gastropoda) combined in different ways across the regions, were good biodiversity indicators in European groundwater ecosystems. However, the uneven distribution of taxonomic groups prevented selection of a common set of indicators for all six regions. Faunal differences among regions are presumably related to both historical and ecological factors, including palaeogeography, palaeoecology, geology, aquifer fragmentation and isolation, and, less clearly, anthropogenic disturbance.

Keywords: biodiversity, ground water, indicators, predictive model, stygobionts

Introduction

Species richness is a simple measure of biodiversity and a widely used criterion for conservation planning.

Unfortunately, exhaustive field inventories over large spatial scales are expensive and time-consuming. Considering that it is impractical to monitor comprehensively every taxonomic group living in a habitat or

Correspondence: Fabio Stoch, Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, I-67100 L'Aquila, Italy. E-mail: fstoch@faunaitalia.it

even in a small site, efficiency demands the use of indicators as 'surrogates' of species richness (Pearson, 1994; Prance, 1994; van Jaarsveld *et al.*, 1998; Moritz *et al.*, 2001; Favreau *et al.*, 2006). Moreover, the surrogacy approach allows taxonomic identification of a limited set of species or taxonomic groups and to design monitoring plans based on a restricted data set, bypassing practical impediments in recognising and identifying all members of a community (Mac Nally & Fleishman, 2004). Finally, biodiversity indicators may be of paramount importance in selecting priority areas for conservation, being good candidates as umbrella species, i.e. species whose conservation is expected to confer protection to a large number of naturally co-occurring species (Fleishman, Murphy & Brussard, 2000; Fleishman, Blair & Murphy, 2001; Roberge & Angelstam, 2004).

McGeoch (1998) used the name 'biodiversity indicators' to define a limited suite of taxa, the diversity of which reflects diversities of other taxa. Lindenmayer, Margules & Botkin (2000) extended the definition to (i) species whose presence or absence indicates presence or absence of some other species; (ii) keystone species and (iii) dominant species in a community. More recently biodiversity indicators were simply defined as 'species with occurrence patterns that are correlated with the species richness of a larger group of organisms' (Mac Nally & Fleishman, 2004). Recent work demonstrated that also higher taxa may be good surrogates of species richness (Báldi, 2003), and some taxonomic groups above species level appear to adequately serve the role of biodiversity indicators (Ricketts, Daily & Ehrlich, 2002; Vessby *et al.*, 2002; Vanclaj, 2004).

Unfortunately, with few exceptions (Mac Nally *et al.*, 2002), there is still little empirical evidence to support the expectation that species richness within a particular group is correlated with species richness of co-occurring taxa (Lawton *et al.*, 1998; Ricketts *et al.*, 2002; Vessby *et al.*, 2002). Pair-wise correlations between groups are usually very low as well (Bilton *et al.*, 2006). Although most broad-scale assessments of freshwater biodiversity, which mainly focussed on evaluating environmental quality, have relied on selected indicator taxa (Sauberer *et al.*, 2004; Bilton *et al.*, 2006), it has rarely been explicitly tested how well such putative surrogate taxa reflect the overall community composition (Paavola *et al.*, 2003). Briers & Biggs (2003) examined the mean and range of

cross-taxon correlations between species richness of several insect families of pond macroinvertebrates, and were able to identify Coenagrionidae (Odonata) and Limnephilidae (Trichoptera) as biodiversity indicators, although good correlations were not obtained at taxonomic ranks higher than the family level. In contrast, Heino *et al.* (2003, 2005) criticised the use of single taxonomic groups as indicators of insect biodiversity in headwater streams. Bilton *et al.* (2006), exploring cross-taxon species richness relationships among macroinvertebrates of freshwater ponds, observed that patterns of cross-taxon congruence in species richness were highly variable among taxa and study sites, making the use of a single taxon as a predictor of overall macroinvertebrate species richness problematic. For this reason, Bilton *et al.* (2006), following Su *et al.* (2004), advocated the use of indicators of community similarity between ponds instead of indicators of species richness.

According to Vanclaj (2004), these conclusions may be unnecessarily pessimistic, because such indicators may not be necessarily used to infer species richness within other groups, but rather to extrapolate information on overall species richness. Moreover, Mac Nally & Fleishman (2002, 2004) and Fleishman *et al.* (2005) pointed out that it is unlikely that indicator species from a single taxonomic group will provide information on species richness of the entire biota at spatial scales meaningful for most land-use decisions, suggesting the use of combinations of indicator species. Mac Nally & Fleishman (2004) argued that prediction of species richness should be regarded as a testable hypothesis in the form of a statistical model, i.e. a function of the occurrence of indicator species.

The present study explores the possibility of inferring stygobiotic species richness in ground water using a surrogacy approach, since predictive models of biodiversity indicators in ground water do not currently exist. The large data sets assembled during the PASCALIS project (Gibert *et al.*, 2005), including almost all stygobiotic taxa recorded from European subterranean waters, offers a unique opportunity to investigate whether a limited suite of biodiversity indicators may be identified in groundwater communities. The main goal of this analysis was to develop a statistical model to select potential indicators of stygobiotic species richness based on the assumptions of Mac Nally & Fleishman (2002, 2004). Moreover, the consistency of models across different spatial scales,

using data sets from karstic and porous aquifers from different European regions, are examined.

Methods

Sampling design

The data set was derived from the PASCALIS project as described by Gibert *et al.* (2005). Data were collected following a stratified random sampling in six regions distributed in Europe: the Walloon karst (Belgium), the southern Jura (eastern France), the Roussillon region (southern France), the Picos de Europa, Cantabria (northern Spain), the Lessinian massif (northern Italy) and the Krim massif (Slovenia). In each region, the sampling strategy involved collection of stygobiotic species at 192 sites, evenly distributed among four habitat types: (i) unsaturated karst; (ii) saturated karst; (iii) hyporheic zone and (iv) phreatic zone in unconsolidated sediments, along four hydrogeographic basins. The sampling procedure adopted is reported by Malard *et al.* (2002). The 192 sites were mostly distributed in caves for the unsaturated karst; caves, springs and wells for the saturated karst; hyporheic habitats for the upper porous aquifers; and wells or piezometers for the saturated zone of porous aquifers.

Biological data set

Once collected, groundwater invertebrates were sorted and counted. For each taxonomic group, all specimens were identified at the species level, whenever possible. Only Nematoda and Turbellaria were identified at the genus level and, for this reason, were excluded from further analyses. According to the degree of adaptation and specialisation to life in ground water, each species was assigned to one of the main ecological categories (Gibert *et al.*, 1994): stygobionts (i.e. species strictly confined to the ground-water environment, as they complete their life cycle in ground water, and show morphological and physiological adaptations to subterranean habitats), stygophiles (i.e. species with incipient adaptation to life in ground water, but able to live in both surface and subsurface environments and related ecotones such as springs and the hyporheic zone of streams and rivers), and stygoxenes (i.e. species which enter ground water accidentally through fast or slow infiltration pathways

connecting surface waters to ground water). Only stygobionts were retained for the statistical analyses.

A biological data matrix, based on presence/absence of species, was created for all the stygobiotic species of eleven higher-level taxa: Polychaeta, Oligochaeta, Gastropoda, Cladocera, Calanoida, Cyclopoida, Harpacticoida, Ostracoda, Isopoda, Amphipoda, Bathynellacea, Thermosbaenacea, Acari, Coleoptera collected at each site. For each of the six regions and each taxonomic group, total species richness, mean number of species per site and standard deviation of the number of species per site, and frequency of occurrence were reported (Table 1). Polychaeta, Calanoida, Thermosbaenacea and Coleoptera, each represented in the data set by a single species recorded from one site only, as well as Cladocera, collected with only two rare species, were excluded from data analysis.

Data analysis

Biodiversity indicators are defined as a limited suite of taxonomic groups the species richness of which is correlated with the extra-group species richness, i.e. species richness of all the other taxonomic groups present in the study area. This definition was applied to the taxa listed in Table 1, and the survey unit is given by the sum of all the sampling sites belonging to each habitat type. Within-group species richness was never directly correlated with total species richness, because the two data sets were not independent. Using total richness, S , as the response variable instead of extra-group species richness would artificially enhance the correlation coefficient, especially when dealing with speciose taxa (Briers & Biggs, 2003).

Cross-taxon correlations were calculated using Spearman's rank correlation coefficient. Moreover, the extra-group species richness in each of the six regions was modelled as a function of the within-group species richness. Following Mac Nally & Fleishman's (2004) recommendations, any possible model involving the independent variables (in this case, the within-group species richness of each of the nine higher-level taxa listed in Table 1) and all their possible combinations (i.e. all possible pairs, trios and so forth) were considered, and 2^9 models were tested. Following Culver *et al.* (2003), rank-order multiple-regression was performed, both

Table 1 Distribution of stygobiotic taxonomic groups in the six European regions studied

Taxon	Cantabria				Roussillon				Jura			
	S	Mean	SD	Frequency	S	Mean	SD	Frequency	S	Mean	SD	Frequency
Polychaeta	–	–	–	–	–	–	–	–	–	–	–	–
Oligochaeta	12	0.11	0.38	9.9	17	0.65	1.00	39.6	4	0.11	0.36	9.4
Gastropoda	2	0.05	0.22	5.2	4	0.23	0.47	21.4	6	0.83	0.96	54.2
Cladocera	–	–	–	–	2	0.01	0.10	1.1	1	0.04	0.20	4.2
Calanoida	–	–	–	–	–	–	–	–	–	–	–	–
Cyclopoida	10	0.43	0.66	34.4	12	0.48	0.77	33.7	11	0.84	0.84	60.9
Harpacticoida	10	0.18	0.45	16.1	8	0.11	0.40	8.0	9	0.73	0.89	50.5
Ostracoda	5	0.15	0.48	10.9	9	0.21	0.56	16.6	10	0.84	0.93	55.2
Isopoda	3	0.17	0.37	16.7	8	0.35	0.63	27.3	7	0.33	0.54	29.7
Amphipoda	6	0.11	0.34	10.9	7	0.58	0.72	45.5	10	1.11	0.85	79.2
Bathynellacea	13	0.22	0.59	15.1	4	0.07	0.28	7.0	2	0.13	0.35	12.5
Thermosbaenacea	–	–	–	–	–	–	–	–	–	–	–	–
Acari	6	0.13	0.53	7.8	1	0.01	0.07	0.5	–	–	–	–
Coleoptera	–	–	–	–	–	–	–	–	1	0.01	0.07	0.5
Total	67	1.56	1.83	–	72	2.70	2.72	–	61	4.98	2.93	–

Taxon	Wallonia				Lessinia				Krim			
	S	Mean	SD	Frequency	S	Mean	SD	Frequency	S	Mean	SD	Frequency
Polychaeta	–	–	–	–	1	0.01	0.07	0.5	–	–	–	–
Oligochaeta	3	0.11	0.31	10.9	15	0.26	0.64	16.8	22	0.45	0.68	36.4
Gastropoda	1	0.00	0.07	0.5	2	0.12	0.37	10.7	14	0.61	0.94	36.9
Cladocera	1	0.02	0.16	2.5	–	–	–	–	–	–	–	–
Calanoida	–	–	–	–	–	–	–	–	1	0.01	0.07	0.5
Cyclopoida	7	0.31	0.51	28.2	12	0.85	0.88	59.4	13	1.03	1.00	63.1
Harpacticoida	–	–	–	–	24	0.86	1.00	56.3	18	0.81	1.21	41.7
Ostracoda	5	0.19	0.49	15.8	7	0.12	0.34	11.7	11	0.25	0.64	16.6
Isopoda	3	0.08	0.30	7.9	2	0.04	0.19	3.6	3	0.04	0.20	4.3
Amphipoda	9	0.54	0.69	45.5	12	0.31	0.60	24.9	9	0.51	0.74	38.5
Bathynellacea	–	–	–	–	6	0.07	0.25	6.6	6	0.13	0.41	10.7
Thermosbaenacea	–	–	–	–	1	0.01	0.07	0.5	–	–	–	–
Acari	5	0.05	0.23	5.4	7	0.17	0.41	15.2	8	0.35	0.76	21.9
Coleoptera	–	–	–	–	–	–	–	–	–	–	–	–
Total	34	1.32	1.50	–	89	2.80	2.31	–	105	4.19	3.29	–

S, total species richness; Mean, mean number of species per site; SD, standard deviation of the number of species per site; Frequency, frequency of occurrence (i.e. percentage of sites where a taxonomic group was recorded).

because of the small number of aquifers (16 within each region) analysed, and uncertainties in the distribution of data. Rank regression is well suited to analyse data that have monotonic, but usually nonlinear, relationships. The 'best' models, i.e. those models that optimised fitting errors, were selected for each combination of taxa based on adjusted R^2 values.

Bonferroni corrections (Shaffer, 1995) were applied to correct the alpha level (0.05) when assessing the statistical significance of Spearman's rank correlation coefficients. Unfortunately, the Bonferroni approach is very conservative especially when the number of

comparisons becomes large and when the tests are not independent. Therefore, the less restrictive approximate false discovery rate approach (Benjamini & Hochberg, 1995) was followed with regression models as well; corrections were calculated separately for each combination of taxa within which models were compared.

The entire data set was stored in Microsoft Excel and all the routines to perform correlations and regressions were written by F. Stoch in Microsoft Visual Basic for Applications. Accuracy of results of correlation analysis was tested using SPSS 13.0 for Windows.

Results

In total 375 stygobiotic species were recorded across the 1157 sites and 96 saturated and unsaturated aquifers sampled in the six regions (Table 1). In term of species richness, groundwater assemblages were dominated by the Crustacea Copepoda, with 114 species collected (1 Calanoida, 52 Cyclopoida and 61 Harpacticoida). Oligochaeta were represented by 70 species, but serious limitations to classify the species reliably may have lowered the real number of stygobiotic species. Amphipoda and Ostracoda were represented by 43 and 41 species, respectively, while Isopoda (26 species), Bathynellacea (28), Gastropoda (29) and Acari (19) were relatively species poor. Cladocera were recorded with two species, and Polychaeta, Thermosbaenacea, Coleoptera with one species only, found in a single site.

Total species richness in the study regions ranged from 34 in Wallonia to 105 in the Krim massif. The mean number of species per sample was highly variable, too, ranging from 1.32 in Wallonia to 4.98 in the Jura. The distribution of species richness within the stygobiotic taxa differed among regions as well (Table 1).

Pair-wise cross-taxon Spearman's rank correlations between groups were usually weak (Table 2), i.e. the variation in species richness of a single taxonomic group was usually not representative of the variation of other groups. Moreover, the cross-taxon correlations were highly variable among both taxa and regions. The percentage of significant cross-taxon congruencies per region ranged between 5.7% (Cantabria) and 19.4% (Lessinia), and the correlations between within-group and extra-group species richness calculated for each taxon were weak as well (Table 3).

The results of the rank-order multiple-regressions (best models, selected on the basis of the highest adjusted R^2) are reported in Table 4 and illustrated in Fig. 1. For practical reasons, only combinations of three or less indicators out of a total of nine potential taxa are reported.

This method extracted useful combinations of biodiversity indicators (Table 4), more efficiently for the western regions (Cantabria, Roussillon, Jura) and, to a less extent, for the other three regions. For example, using trios of potential indicators, Copepoda and Amphipoda, together with Gastropoda and Ostracoda, were selected in the western regions. These

groups explained over 70% of the rank-order variation of species richness of the remaining groups found in the same area. In the eastern areas (Lessinia and Krim), Copepoda and Amphipoda were selected as well, together with Oligochaeta and Bathynellacea. Trios of indicators explained over 60% of extra-group species richness rank-order variation. Finally, Cyclopoida and Oligochaeta significantly contributed to explaining species richness of the other groups in the Walloon karst, explaining approximately 53% of extra-group species richness rank-order variation.

Discussion

The results obtained by the present analysis support the contention that some taxonomic groups may be used as biodiversity indicators (Vanclaj, 2004). Reliability of the surrogacy approach is still debatable and questionable in some respects (van Jaarsveld *et al.*, 1998; Favreau *et al.*, 2006). However, although the regressions with most explanatory power include different taxa combinations in different regions, the statistical methodology adopted here strengthens the potential of some taxonomic groups to serve as indicators of overall species richness across European ground waters. This conclusion is supported by the consistency of the biological data set used. In general, Copepoda and Amphipoda appear to be reliable predictors of the residual species richness in almost all the regions analysed. The explanation of such behaviour is probably traceable in the high taxonomic diversification of these crustacean groups in ground water, which probably reflects a wide range of trophic and spatial niche diversification, although niche partitioning is still largely unknown (Galassi, 2001).

Unfortunately, these results also suggest that the selection of biodiversity indicators requires re-calibration, depending on the groundwater region under study. The uneven distribution of various taxonomic groups of stygobionts in European ground waters (Malard *et al.*, 2009; Galassi *et al.*, 2009; Dole-Olivier *et al.*, 2009), absence of some speciose groups from some regions (e.g. stygobiotic Harpacticoida from Wallonia), and geographical variation in the degree of cross-taxon congruence, prevent selection of a single set of indicators able to cover all of the six analysed regions equally well. These observations are in line with the conclusions drawn by Bilton *et al.* (2006), who noticed high variability of indicator groups among the

Table 2 Cross-taxon correlations amongst stygobiotic species richness of the nine taxonomic groups retained for analyses of data from six European regions

Taxa	Cantabria		Roussillon		Jura		Wallonia		Lessinia		Krim	
	R_s	P -value	R_s	P -value	R_s	P -value	R_s	P -value	R_s	P -value	R_s	P -value
Oligochaeta – Acari	0.08	0.779	0.31	0.241	–	–	–0.02	0.945	0.08	0.780	0.03	0.899
Oligochaeta – Amphipoda	0.37	0.162	0.48	0.059	0.55	0.027	–0.27	0.317	0.49	0.054	0.10	0.709
Oligochaeta – Bathynellacea	0.34	0.194	0.21	0.430	0.23	0.387	–	–	–0.17	0.538	0.41	0.116
Oligochaeta – Cyclopoida	0.34	0.191	0.44	0.089	0.15	0.571	–0.16	0.544	–0.39	0.133	0.37	0.162
Oligochaeta – Gastropoda	–	–	0.49	0.055	0.13	0.621	–0.30	0.257	0.52	0.001	–0.12	0.661
Oligochaeta – Harpacticoida	0.44	0.091	0.45	0.080	0.18	0.508	–	–	0.77	<0.001	0.06	0.833
Oligochaeta – Isopoda	0.42	0.107	0.16	0.557	0.15	0.580	–0.43	0.097	0.35	0.189	–0.04	0.888
Oligochaeta – Ostracoda	0.18	0.502	0.64	0.007	0.31	0.238	–0.04	0.888	0.07	0.804	–0.16	0.543
Gastropoda – Acari	0.20	0.450	0.30	0.264	–	–	0.22	0.413	–0.32	0.234	0.26	0.340
Gastropoda – Amphipoda	0.25	0.344	0.15	0.568	0.08	0.768	0.43	0.095	0.83	<0.001	0.71	0.002
Gastropoda – Bathynellacea	0.35	0.182	0.21	0.437	–0.38	0.143	–	–	0.25	0.359	0.28	0.290
Gastropoda – Cyclopoida	–0.01	0.971	0.68	0.004	0.04	0.890	0.44	0.086	–0.53	0.033	–0.06	0.816
Gastropoda – Harpacticoida	–0.10	0.712	0.62	0.010	0.36	0.168	–	–	0.36	0.168	–0.50	0.049
Gastropoda – Isopoda	–0.06	0.832	–0.36	0.175	0.78	<0.001	0.44	0.088	0.69	0.003	0.39	0.137
Gastropoda – Ostracoda	0.49	0.055	0.27	0.314	0.48	0.061	0.32	0.229	–0.08	0.781	0.34	0.193
Cyclopoida – Acari	0.42	0.101	0.09	0.745	–	–	0.21	0.428	0.33	0.212	0.41	0.118
Cyclopoida – Amphipoda	0.13	0.620	0.17	0.526	0.08	0.775	0.61	0.012	–0.31	0.246	–0.04	0.890
Cyclopoida – Bathynellacea	–0.17	0.522	0.29	0.271	–0.23	0.389	–	–	–0.41	0.113	0.40	0.123
Cyclopoida – Harpacticoida	–0.05	0.863	0.73	0.001	0.65	0.007	–	–	–0.29	0.276	0.50	0.051
Cyclopoida – Isopoda	–0.01	0.961	–0.29	0.277	0.43	0.100	0.47	0.067	–0.32	0.230	–0.50	0.047
Cyclopoida – Ostracoda	0.03	0.910	0.51	0.044	0.26	0.321	0.54	0.030	0.16	0.554	–0.36	0.176
Harpacticoida – Acari	–0.35	0.178	0.39	0.133	–	–	–	–	0.27	0.313	0.25	0.342
Harpacticoida – Amphipoda	–0.26	0.323	0.30	0.258	0.12	0.658	–	–	0.38	0.151	–0.41	0.113
Harpacticoida – Bathynellacea	0.73	0.001	0.42	0.104	–0.22	0.422	–	–	0.06	0.827	–0.04	0.892
Harpacticoida – Isopoda	0.18	0.500	–0.15	0.574	0.38	0.152	–	–	0.14	0.597	–0.46	0.072
Harpacticoida – Ostracoda	–0.15	0.583	0.44	0.090	–0.04	0.891	–	–	0.06	0.832	–0.56	0.023
Ostracoda – Amphipoda	0.59	0.017	0.19	0.471	0.31	0.245	0.34	0.197	–0.12	0.652	0.51	0.044
Ostracoda – Bathynellacea	0.03	0.918	0.53	0.034	–0.47	0.069	–	–	–0.28	0.302	–0.03	0.908
Ostracoda – Isopoda	0.27	0.304	–0.09	0.732	0.49	0.054	0.45	0.077	–0.28	0.289	0.60	0.014
Ostracoda – Acari	0.06	0.817	0.23	0.395	–	–	0.05	0.843	0.63	0.008	–0.23	0.384
Isopoda – Acari	0.10	0.708	–0.30	0.256	–	–	–0.09	0.738	–0.49	0.055	–0.22	0.409
Isopoda – Amphipoda	0.47	0.066	0.02	0.945	0.23	0.395	0.35	0.184	0.69	0.003	0.49	0.053
Isopoda – Bathynellacea	–0.07	0.805	–0.37	0.153	–0.41	0.116	–	–	0.39	0.133	–0.15	0.570
Amphipoda – Acari	0.36	0.176	0.18	0.503	–	–	0.12	0.646	–0.29	0.278	0.24	0.374
Amphipoda – Bathynellacea	–0.24	0.364	0.19	0.482	–0.08	0.762	–	–	0.18	0.516	0.25	0.347
Bathynellacea – Acari	–0.25	0.349	0.15	0.572	–	–	–	–	–0.50	0.050	0.40	0.126

R_s , Spearman's rank correlation coefficient; P , probability values.

Significant relationships after Bonferroni correction of alpha for individual tests are shown in bold.

regions they sampled. In the same way, Faith & Walker (1996) and Su *et al.* (2004) observed that the relationships between indicator groups and target groups can be weak or absent in some areas because different factors may drive their distributions. According to Williams (1998), the prevailing factors that might promote particularly tight indicator relationships are: (i) similarity of taxa in terms of ecological requirements; (ii) similar palaeogeographical and palaeoecological events in all regions, which may have led to a common scenario of vicariance and

would result in uniform distribution patterns of taxa across the regions; (iii) similar means for passive dispersal, if any and (iv) similar patterns of biotic interactions, although this last aspect is virtually unknown for groundwater communities (Culver, 1994; Strayer, 1994). Given the above observations, the differences observed in species richness, taxonomic composition and strength of biodiversity indicator relationships across European ground waters are probably related to both historical and ecological factors. Rundle *et al.* (2002) proposed a schematic

Table 3 Correlations between within-group and extra-group species richness in six European regions

Taxon	Cantabria		Roussillon		Jura		Wallonia		Lessinia		Krim	
	R_s	P -value	R_s	P -value	R_s	P -value	R_s	P -value	R_s	P -value	R_s	P -value
Oligochaeta	0.64	0.008	0.71	0.002	0.46	0.075	-0.21	0.428	0.64	0.008	0.18	0.511
Gastropoda	0.42	0.107	0.58	0.018	0.39	0.139	0.42	0.102	0.44	0.085	0.16	0.552
Cyclopoda	0.18	0.516	0.66	0.006	0.41	0.118	0.71	0.002	-0.37	0.160	0.40	0.120
Harpacticoida	0.22	0.403	0.69	0.003	0.43	0.095	-	-	0.53	0.034	-0.30	0.266
Ostracoda	0.28	0.295	0.61	0.013	0.18	0.507	0.53	0.034	0.07	0.800	-0.16	0.565
Isopoda	0.27	0.320	-0.08	0.765	0.48	0.062	0.34	0.193	0.38	0.149	-0.04	0.872
Amphipoda	0.31	0.242	0.46	0.071	0.29	0.279	0.35	0.181	0.49	0.054	0.46	0.074
Bathynellacea	0.15	0.589	0.35	0.185	-0.30	0.265	-	-	-0.09	0.742	0.58	0.019
Acari	-0.08	0.765	0.31	0.245	-	-	0.07	0.796	0.05	0.866	0.43	0.099

R_s , Spearman's rank correlation coefficient; P , probability value.

Significant relationships after Bonferroni correction of alpha for individual tests are shown in bold.

Table 4 Statistical models with two or three potential indicators of extra-group species richness selected using rank-order multiple-regression

Region and taxa	R^2_{adj}	F	$P(F)$	b_1	$P(b_1)$	b_2	$P(b_2)$	b_3	$P(b_3)$
Cantabria									
Amphipoda + Bathynellacea	0.44	6.88	0.009	0.70	0.005	0.48	0.036		
Gastropoda + Harpacticoida + Amphipoda	0.70	12.75	<0.001	0.67	0.003	0.66	0.001	0.41	0.022
Roussillon									
Oligochaeta + Harpacticoida	0.71	19.61	<0.001	0.43	0.016	0.62	0.002		
Gastropoda + Ostracoda + Amphipoda	0.77	17.50	<0.001	0.53	0.002	0.48	0.004	0.35	0.024
Jura									
Gastropoda + Cyclopoda	0.44	6.81	0.010	0.54	0.032	0.54	0.018		
Gastropoda + Cyclopoda + Amphipoda	0.74	15.17	<0.001	0.61	0.0014	0.47	0.004	0.44	0.007
Wallonia									
Oligochaeta + Cyclopoda	0.53	9.60	0.003	-0.17	0.374	0.76	0.001		
Oligochaeta + Cyclopoda + Acari	0.52	6.32	0.008	-0.18	0.379	0.79	0.002	-0.12	0.578
Lessinia									
Oligochaeta + Cyclopoda	0.47	7.67	0.006	0.75	0.004	-0.05	0.818		
Oligochaeta + Cyclopoda + Bathynellacea	0.64	9.93	0.001	0.98	<0.001	0.20	0.335	0.27	0.205
Krim									
Harpacticoida + Amphipoda	0.39	5.72	0.017	0.08	0.743	0.73	0.007		
Harpacticoida + Amphipoda + Bathynellacea	0.63	9.34	0.002	0.00	0.998	0.64	0.004	0.47	0.027

R^2_{adj} , model-adjusted squared correlation coefficient; b_1 , b_2 , b_3 , estimated regression coefficients.

All relationships are statistically significant under the approximate false discovery rate.

representation of the main factors affecting freshwater species pools at different spatial scales. Accordingly, palaeogeographical and palaeoecological events are of primary importance as they shape particular palaeobiogeographical scenarios. These lead to quite different species assemblages, which reflect the different geological and climatic events in distinct geographical areas. For instance, glaciated areas underwent drastic impoverishment of regional species pools during the Quaternary, as in some parts of the Jura massif, where several stygobiotic species show wide ecological tolerance, accompanied by a relatively wide geographical distribution. A more extreme situation occurred in the

Walloon region. Here entire groups of stygobionts are missing, probably reflecting low habitat heterogeneity compared to the other regions examined, together with the strong residual effects of the Quaternary glaciations as the presence of permafrost (Martin *et al.*, 2005). On the contrary, the highest stygobiotic species richness is located in the southernmost regions of Europe, which were much less affected by glaciations. However, it is important to note that the influence of palaeogeography and palaeoecology may date back much further, as for the Lessinian and the Krim massifs, which are characterised by the development of very ancient karst (Boccaletti *et al.*, 1990; Galassi

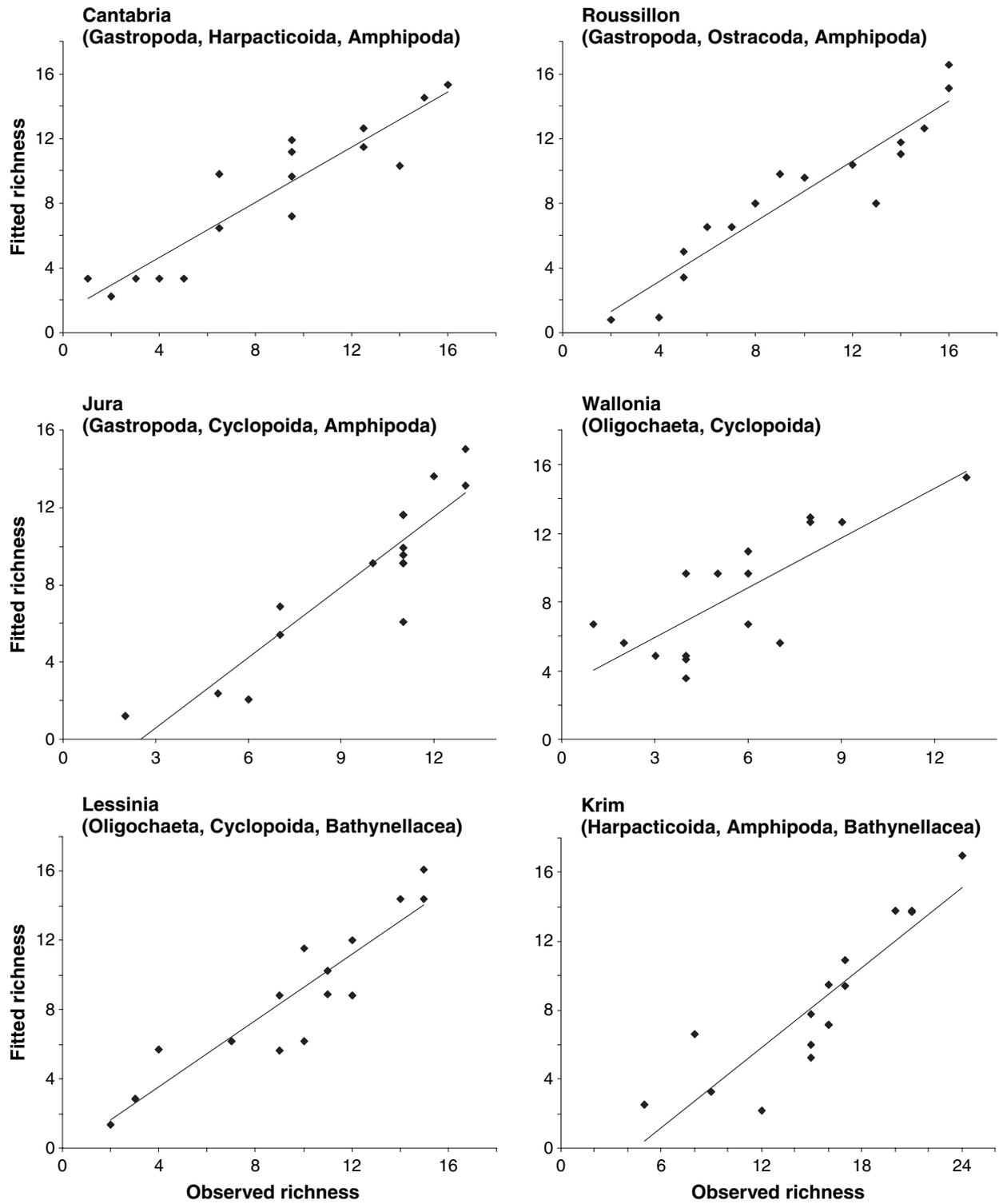


Fig. 1 Fitted versus observed extra-group species richness in six European regions for rank-order multiple-regression models selected on the basis of the highest adjusted R^2 for three indicator groups (two for Wallonia).

et al., 2009) which emerged from the sea in the Tertiary. The old age of these karstic aquifers, together with a less drastic influence of the Quaternary glaciations, led to high diversification of species (especially among Copepoda, Amphipoda and Oligochaeta), which was maintained over time (Galassi *et al.*, 2009).

Influences of other environmental features are superimposed on this basic scenario. These include, for example, habitat heterogeneity and fragmentation, a frequent situation in unsaturated karst, where a high degree of endemism occurs within some taxonomic groups such as Copepoda, Amphipoda, Oligochaeta and Bathynellacea (Pipan & Culver, 2005; Galassi *et al.*, 2009). Equally important is the role of anthropogenic disturbance in affecting composition and structure of the groundwater assemblages (Hancock, 2002; Lafont *et al.*, 2006). An increase in organic matter and nutrient supply alters assemblage composition, leading to population declines or disappearance of some stygobiotic and other sensitive species (Rundle & Ormerod, 1991; Malard *et al.*, 1994; Rundle & Ramsay, 1997; Malard, 2001; Moessleracher, Griebler & Notenboom, 2001; Paran *et al.*, 2005). Finally, although care was taken in the PASCALIS sampling design to distribute sites evenly among aquifers and habitat types, sampling effort and efficiency across the six regions were not exactly the same (Dole-Olivier *et al.*, 2009).

These factors vary in relative importance in different regions. This may allow for greater co-variation of species richness of different taxa in some areas such as the western regions (i.e. Cantabria, Roussillon and Jura), which influences the strength of indicator relationships. Indicator selection models appear to be less efficient in the eastern regions (i.e. Lessinia and Krim), which show higher biodiversity and greater habitat fragmentation (Galassi *et al.*, 2009). Finally, models should be applied with caution to Wallonia, where the effects of Quaternary glaciations and the intensity of land use may have heavily influenced the groundwater assemblages (Martin *et al.*, 2005).

It is still unknown to what extent the different ecological and historical factors shape groundwater assemblages (Stoch, 1995; Ward *et al.*, 1998; Galassi, 2001; Gibert & Deharveng, 2002; Rundle *et al.*, 2002; Galassi *et al.*, 2009) and which factors are key in determining geographical variation of cross-taxon correlations in European ground waters. Towards the goal of drawing general and thus transferable inferences about the nature of ecological assemblages, Mac

Nally & Fleishman (2004) argued for developing and testing hypotheses that explain why a particular set of indicators encompasses fundamental information about a whole community. If this can be achieved, both researchers and natural resource managers striving to improve the monitoring and conservation of stygobiotic biodiversity, may be better informed by reliable studies on selected groups than by impractical attempts to survey the entire groundwater fauna.

Acknowledgments

B. Arconada, R. Araujo, M. Bodon, C. Boutin, A. Camacho, M. Creuzé des Châtelliers, C. Debroyer, W. Decraemer, P. De Laurentiis, A. Di Sabatino, G. & M. Falkner, F. Fiers, M. Ghamizi, N. Giani, R. Ginet, N. Guil, D. Jaume, J. Juget, G. Magniez, F. Margaritora, P. Marmonier, E. Martinez Ansemil, C. Meisch, M. Messouli, A. Navas, S. Prevorčnik, M.A. Ramos, B. Sambugar, B. Sket, J. Van Goethem, F. Velkovrh, K. Wouters are greatly acknowledged for their valuable contributions to data acquisition. Two anonymous reviewers constructively criticised the first draft of the manuscript which helped improve particularly the presentation of statistical results. This study was supported by the PASCALIS project (Protocols for the ASsessment and Conservation of Aquatic Life In the Subsurface) funded by the European Community under its 5th Framework Programme (contract no. EVK2-CT-2001-00121).

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(Manuscript accepted 27 September 2008)