

# Hard rock aquifers and free-living nematodes – an interdisciplinary approach based on two widely neglected components in groundwater research

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## ABSTRACT

The subterranean part of the water cycle takes place in three different types of groundwater bodies: (1) unconsolidated sediments, (2) karstic aquifers and (3) fractured hard rock aquifers. Basically, different aquifer types require different investigation methods. However, the physico-chemical properties of groundwater can aid in the understanding of flow history and aquifer dynamics of each aquifer type. Although groundwater biology is sensitive to hydrochemistry, water temperature and flow velocity, the possible benefits of groundwater animals as ‘hydrogeological agents’ is still rarely considered by hydrogeologists. The main research focus of hydrogeologists and groundwater biologists is shallow groundwater bodies in unconsolidated sediments and karst aquifers. Fractured hard rock aquifers are still poorly considered by both disciplines. This commentary has three purposes: (1) to show the special characteristics of fractured hard rock aquifers in comparison to porous and karst groundwater bodies, and to describe the resulting methodological gap hydrogeologists face when investigating such aquifer types; (2) to give an overview of current groundwater biological research with its focuses and shortcomings, including an introduction of rarely known but common groundwater inhabitants, namely free-living nematodes; and (3) to suggest a combination of methods, supported by a hypothetical example, and the possible benefits this combined knowledge from hydrogeologists and groundwater biologists can bring to both disciplines. Copyright © 2014 John Wiley & Sons, Ltd.

**KEY WORDS** hydrogeology; fractured hard rock aquifers; groundwater biology; free-living nematodes; interdisciplinary methods

*Received 12 November 2013; Revised 12 May 2014; Accepted 22 May 2014*

## INTRODUCTION

From the hydrogeological point of view, the scarcity of methods to investigate hard rock aquifers is a recent and not sufficiently solved problem. This is of increasing relevance as the infrastructural projects crossing the Alps, as well as the need for drinking water supplies in the alpine region, and the political purpose to support alternative and renewable energy sources, such as hydropower or geothermal energy, become of increasing importance in Europe. For a sustainable groundwater management in all these cases, it is necessary to understand the flow systems in hard rock aquifers. With the implementation of biotic analysis and, in particular, with the focus on nematodes, a potential tool could be developed to help overcome difficulties in hard rock hydrogeology.

Addressing the biological viewpoint, the establishment of a uniformly practicable ecological classification system of groundwater habitats is still in great demand not only in

Europe (Boulton, 2009). In Europe, legislation demands the assessment of the chemical and ecological status of surface waters (EU-WFD, 2000), whereas for groundwater, the chemical approach only seems to provide sufficient information regarding general groundwater health (EU-GWD, 2006). This clearly contradicts the fact that only ecologically intact groundwater guarantees essential ecosystem services (ESS), namely to provide intact drinking water quality, which is of utmost importance. However, the maintenance of surface water integrity by groundwater is no less important; this refers particularly to threatened ecosystems such as wetlands (Danielopol *et al.*, 2004). Inconsistency in groundwater fauna patterns, as observed so far and outlined later, might be one major reason an appropriate classification still does not exist.

Various benefits for groundwater biology and also nematology are expected from a potential collaboration with hydrogeologists addressing hard rock aquifers. Groundwater biology can certainly profit from better insights into biota patterns in relation to abiotic complexity as it is relevant in hydrogeology. Furthermore, hitherto (widely) unknown biota patterns from hard rock aquifers can distinctly enhance basic knowledge of

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groundwater biology and also expand general ecological knowledge relating abiotic and biotic information driven from this widely neglected aquifer type. This is also of importance in the context of ESS and functions of the groundwater biota. With respect to the sparse nematological knowledge for groundwater, the information gained from comprehensive investigations covering a range of different lithologies is also of enormous importance in groundwater biology. Better insights into essential geographical patterns as well as the nematode contribution to ESS and functions in groundwater are expected.

In the following, we introduce hard rock aquifers as a special type of hydrogeological setting with special flow conditions and, compared with hydrogeology in unconsolidated sediments, special methodological requirements. The chapter 'biological background' first outlines current research in groundwater biology and finishes with a special view on free-living nematodes. The final chapter illustrates methodological approaches and hypotheses, the latter by a hypothetical example using combined knowledge of hydrogeology, groundwater biology and nematology.

## HARD ROCK AQUIFERS

In hydrogeology, studies on aquifers in unconsolidated sediments and karst systems have a long tradition beginning in the early 19th century. Analytical mathematical approaches to understanding groundwater flow in porous media were established by Darcy (1856). Research in karst hydrogeology began in the early 20th century (e.g. Penck, 1904, Cvijic, 1918 and Piper, 1932). In comparison, hydrogeology of fractured hard rocks is a relatively young discipline becoming of increasing interest in the last decades. Understanding fractured hard rock aquifers is crucial for drinking water suppliers, for tunnelling projects (in both cases especially in mountainous regions) and, as the most recent application, for developing geothermal plants using deep circulation systems within the hard rock basement. In all these cases, hydrogeologists are required to quantify groundwater flow, to delineate catchments and define recharge areas, and to find sustainable ways of groundwater use.

In highly consolidated sediments (e.g. sandstones and siltstones) as well as in magmatic and metamorphic rocks, the circulation of groundwater is confined to structures such as joints, faults or foliation planes and beddings. Matrix porosities due to voids between grains (one of the dominant factors to characterize aquifers in unconsolidated sediments) play a minor role in hard rock hydrogeology. Large areas in Europe such as the mountainous regions of the Central European Alps and the basement under the unconsolidated cover of plains are documented in the International Hydrogeological map of Europe. The so-called consolidated aquifers are classified as high, moderate or low permeable or as practically non-aquiferous rocks on

the basis of pore size and the connectivity of pores (Cornu *et al.*, 2013). This consolidated type of groundwater body can also be described as a hard rock aquifer and what is entitled 'pore size' by Cornu *et al.* (2013) could also be described as 'void size' or 'width of water bearing structures' in fractured hard rock environments. The hydraulic conductivity in these aquifers refers to the presence and frequency of open structures, their width and the connectivity between voids. Structural properties of the bedrock are a function of lithology and former as well as recent tectonic stress (Mortimer *et al.*, 2011). Although these factors can be determined by means of geological field work, it is often difficult to come to reliable predictions about groundwater dynamics, flow history, circulation depth or recharge areas.

One of the problems is that established hydrogeological methods were created to answer questions about groundwater bodies in unconsolidated sediments. Grain size distribution, grain shape and compactness of the layers are the main factors controlling the hydrogeological properties of the aquifer. Thus, investigating the stratification by means of drilling and the dynamics of the water level with a network of observation points are suitable methods to understand groundwater flow in unconsolidated sediments. Because of the homogeneity of hydraulic properties within the layers, it is possible to determine their spatial distribution and to interpolate between groundwater observation points.

Much more so than in unconsolidated sediments, the lithology and, due to this, properties such as solubility and plasticity have an important impact on hydrogeological behaviour of hard rock aquifers. A more or less anisotropic and irregular network of joints and faults, and internal variations of the degree of tectonic exposure make it difficult to interpolate between two stations (e.g. outcrops or wells). In addition, a known and tectonically well-understood structure can act as a favoured flow path or as a hydraulic barrier (Dafny *et al.*, 2013). The hydraulic properties can even differ in one and the same structure and within short distances (Winkler *et al.*, 2010).

As shown in Table I, there is a wide range of possibilities as to how water can move through hard rock aquifers. As a result, a close geological and hydrogeological investigation is required, including detailed mapping, sampling of structural data, sampling of field data from springs and devising a time series of spring discharge, electrical conductivity, water temperature, hydrochemical and isotope data. Whereas geological and structural data as well as discharge curves deliver hints as to the hydraulic permeability of the aquifer, water quality data such as electrical conductivity and hydrochemistry can help to determine the lithologies involved. Isotopic investigations are used to estimate the mean elevation of recharge areas and mean residence times.

Table I. Generalized classification of hard rock aquifers in different (simplified) lithologies.

Lithology	Type of voids	Void size	Flow type	Permeability	Spring type	Mean residence times	Degree of groundwater mineralisation
Limestone	Karst conduits and micro fissures	$\mu\text{m}$ to m	Conduit flow and diffuse flow	High	Few springs, variable discharge	Short for the majority of water	Moderate
Dolostone	Karst conduits and fissures	$\mu\text{m}$ to cm	Diffuse flow	Low to high	Many small springs	Short to long	High
Sandstone	Fissures and pores	$\mu\text{m}$ to cm	Diffuse flow and conduit flow	Moderate	Depends on tectonic exposure	Medium to long	Moderate to high
Siltstone	Fissures and pores	$\mu\text{m}$ to mm	Diffuse flow and conduit flow	Low	Few small springs	Long	Moderate to high
Granite	Fissures	$\mu\text{m}$ to cm	Conduit flow and diffuse flow	Low to high	Depends on tectonic exposure	Short to long	Low
Gneiss	Fissures	$\mu\text{m}$ to cm	Conduit flow and diffuse flow	Low to high	Depends on tectonic exposure	Short to long	Low
Schist	Fissures, beddings, foliation planes	$\mu\text{m}$ to mm	Diffuse flow	Low	Few small springs	Moderate to long	Low to high
Phyllite	Fissures, beddings, foliation planes	$\mu\text{m}$ to mm	Diffuse flow	Low	Few small springs	Long	Low to high

However, a clear and satisfying description of an aquifer, especially in tectonically and lithologically complex environments, such as mountain ranges is still difficult. Results are often ambiguous, and long-term investigations are needed to eliminate short-term influences due to meteorological extremes. Hard rock hydrogeologists are in need of further methods to understand the hydrogeological processes and to confirm their findings. Because different types of voids, variations in void size, different permeabilities and mean residence times create different biotopes, the use of biological indicators may be a promising extension to the range of methods used in hard rock hydrogeology.

## THE BIOLOGICAL BACKGROUND

### *One-sided focuses...*

Groundwater biological research has concentrated on caves, karstic and porous aquifers (e.g. Hahn and Fuchs, 2009; Malard *et al.*, 2009; Larned, 2012), whereas hard rock aquifers have so far rarely been addressed in detail (Stein *et al.*, 2012). This imbalance might be rooted in the history of groundwater biology, which has primarily dealt with certain habitat types from its early beginning on (e.g. caves, springs and wells, and carstic aquifers; Möblacher and Hahn, 2003). Only towards the end of last century porous aquifers have gained an increasing interest (Danielopol, 1989; Stanford and Ward, 1993; Ward *et al.*, 1994; Ellis *et al.*, 1998). Another major imbalance involves the animals investigated either because of habitat inherent conditions or as a result of research interest. Vertebrates (amphibians and fishes), for example, are only able to live in larger pore spaces, as are found in caves and karstic groundwaters (Möblacher and Hahn, 2003); only smaller-sized species and small developmental stages are also observed in porous aquifers' hyporheic fractions (Feral *et al.*, 2005; Kawanishi *et al.*, 2013). In general, arthropods, nematodes and annelids are common groundwater animals (e.g. Humphreys, 2009; Meleg *et al.*, 2012; Gutjahr *et al.*, 2013). Nevertheless, research focuses have been, and still clearly are, on arthropod communities (e.g. Deharveng *et al.*, 2009).

Furthermore, groundwater biology typically investigates patterns and rarely addresses hypotheses and their proofs. This points to a still young discipline according to Larned (2012, and references therein). The lack of attempts to reveal relations between biodiversity (BD), ESS and ecosystem functions (ESF) represents another major shortcoming in groundwater biology, which was already partly discussed by Gibert and Deharveng (2002). Indeed, groundwater biology is still more often concerned with counting species (BD) rather than relating them to functional processes (ESF, but see Boulton *et al.*, 2008).

Thus, no doubt exists that greater efforts are needed in this direction. But admittedly, it might be difficult to evaluate the relation between BD and both ESS and ESF of an often restricted and rare groundwater fauna as mentioned later. But only because species are rare their potential importance for their ecosystems must not be underestimated (Lyons *et al.*, 2005).

*...for a heterogeneous subterranean world*

Nowadays, it is widely agreed that an extreme heterogeneity among groundwater habitats accounts for the enormous heterogeneity among the groundwater biota observed so far (Schmidt and Hahn, 2012; Hahn, 2009, and references therein). When considering only the last decade, several approaches (original research papers, comparing surveys and reviews) have been undertaken, often in order to find common principles and typologies within this heterogeneity (e.g. Hahn, 2009; Humphreys, 2009; Larned, 2012; Stein *et al.*, 2012; Stoch and Galassi, 2010; Boulton, 2009; Boulton *et al.*, 2008; Hancock and Boulton, 2008; Hancock and Boulton, 2009; Humphreys, 2009; Schmidt *et al.*, 2007; Tomlinson and Boulton, 2010; Freshwater biology special issue: 2009/54; Hydrogeological Journal special issue 2009/17/1). These efforts point to the importance of hierarchical scaling among environmental parameters and the aquifer type for faunal patterns. However, the more recent comparison of different aquifer types revealed faunal differences not strictly following their differentiation: the fauna in porous and karstic aquifers were similar (Hahn and Fuchs, 2009), although differences might have been expected because of their different structure and connectivity. More or less pronounced faunal differences between porous and karstic aquifers have been recorded elsewhere (Malard *et al.*, 2009; Stoch and Galassi, 2010).

A priori automatically included in any (spatial) faunal documentation, but not always explicitly mentioned, are temporal aspects that add to complex heterogeneity as ecological and evolutionary determining force (Galassi, 2001). Earth's glaciation history, in particular the last glacier maximum, is considered to be a major driver of groundwater community patterns (Strayer, 1994) and to partly contribute to the geographic distribution patterns of crustaceans (Stoch and Galassi, 2010). They are not only often limited but moreover often endemic (Sket, 1999; Galassi *et al.*, 2009; Gibert *et al.*, 2009; Asmyhr and Cooper, 2012). Because crustaceans represent the major target in groundwater research, their geographic ranges partly hamper the establishment of more general principles in groundwater typology. The high endemism among crustaceans is seen to be caused by allopatric speciation due to habitat fragmentation and also by limited dispersal abilities. Endemism is also reported, for example, for

oligochaets (Achurra and Rodriguez, 2008; Des Chatelliers *et al.*, 2009; Giani *et al.*, 2011) and limited dispersal abilities are basically ascribed to any life in groundwater environments (Botosaneanu, 1986). Not only endemism but also scarcity or even absence of biota (Rouch and Danielopol, 1987) represents another challenge to establishing uniform groundwater typologies using biota patterns.

Compared with surface water, groundwater is characterized by relatively low temperature variability over time and lower resources (Gibert *et al.*, 1994). Because surface water interacts with groundwater via various pathways, they also contribute to the complex heterogeneity in groundwater habitats by affecting abiotic conditions as well as by determining the availability of allochthonous resources. In general, resources in groundwater are attributed to allochthonous production, although exceptions have already been documented: a chemo-autotrophic cave system (Movile cave – Sarbu, 2000) corroborates speculations about a potentially higher importance of autochthonous production in groundwater systems than hitherto assumed. Regardless of which source groundwater communities are sustained from, their organisms are classified into three groups: stygobiontes, which are highly adapted to groundwater habitats and seemingly not able to exist elsewhere, stygophiles, which are able to live in surface and groundwater, and stygoxenes, which are not able to establish permanent populations in groundwater, but might occasionally be found therein (Gibert *et al.*, 1994).

Water temperature, resource availability and oxygen content, and also anthropogenic disturbances, have been investigated with regard to fauna patterns by several studies (e.g. Gibert *et al.*, 1994; Dumas and Lescher-Moutoué, 2001; Dole-Olivier *et al.*, 2009). However, their outcomes do not allow simple inferences about relations between these abiotics characteristics and patterns of the groundwater biota. In a comprehensive field study, measures of three important abiotic parameters (i.e. the standard deviation of water temperature, a five-scaled, semi-quantitative score of the organic content representing the resource availability and oxygen content) were integrated into one index, the so-called groundwater fauna index (GWI) (Hahn, 2006). The relation between this index and patterns of the groundwater biota was analysed, and revealed three size classes of the GWI, which relate reasonably well to the structure of the groundwater fauna (mainly crustaceans as stygophiles and stygobiontes, species richness and abundances). Consequently, the GWI has been considered as a reliable and indicative measure of certain types of groundwater habitats with respect to aboveground–belowground pathways and their exchange processes (low, intermediate and high) influencing the groundwater fauna.

### *Free-living nematodes in general and in groundwaters*

A brief survey on nematodes should mention at least some basics. Nematodes are common in terrestrial and aquatic environments with often highly abundant and diverse communities. Their obviously wide distributional ranges might be explained by their enormous adaptability that allows them to live within a range of conditions – also including environmental stress such as resource depletion, osmotic stress, oxygen depletion, low pH and extreme temperatures (Yeates, 2004; Wharton *et al.*, 2005; Levin *et al.*, 2009; Borgonie *et al.*, 2010; Perissinotto *et al.*, 2010), and also by their potential to use highly variable resources as indicated by the variety in feeding behaviour-related structures such as mouth cavity, head setae and papillae, and amphids (Wieser, 1953).

Although the general importance of nematodes within their habitats is widely accepted and they have also been regularly observed in groundwater (Andrassy, 1978; Boulton *et al.*, 2004; Hahn, 2005; Hahn and Matzke, 2005; Hahn, 2006), they are widely neglected in groundwater research. Most of the sparse data from aquifers represent only total group abundances, lacking detailed observations on finer taxonomic levels. Moreover, these abundance data are considered to inadequately represent actual nematode abundances in groundwater due to the methods used (i.e. too coarse mesh sizes, mostly 74 µm and larger, 40 µm would be at least required to sample nematodes as part of the meiofauna; according to Palmer and Strayer, 1996). Out of 605 aquatic species, Andrassy (1978) lists 76 species, observed exclusively in groundwater, which can thus be considered as stygobiontes. This number is higher than those compiled for certain crustaceans (ostracodes – 68, cyclopoida – 60 and bathynellacea – 41 but see Sket, 1999). Additional nematode species data are available for caves (Barr, 1968; Eder, 1979; Riess *et al.*, 1999; Culver and Sket, 2000). A semi-quantitative survey of several caves in Europe recorded 41 generally common nematode taxa (Austria – 2, Croatia – 4, Italy – 49 and Slovenia – 12 unpublished data courtesy of Fabio Stoch). According to Andrassy (1978), endemism is rare among groundwater nematodes and also generally among aquatic nematodes.

Indications for a potentially hitherto underestimated importance of nematodes in groundwater (but see Mößlacher and Hahn, 2003) might be deduced from a comparison of environments considered extreme, namely the deep sea (Smith and Snelgrove, 2002), glacier rivers (Milner and Petts, 1994) and also groundwater habitats (Rouch and Danielopol, 1997). Low diversity is generally attributed to these habitats and is often indicative for truncated food webs (Gibert and Deharveng, 2002; Giere, 2009). However, the awareness of nematofauna importance increases for the deep sea (Danovaro *et al.*, 2008; Giere,

2009) and glacier rivers (Eisendle, 2008; Eisendle-Floekner *et al.*, 2013). Although these two examples and groundwater are no doubt distinct environments, their resemblance lies within oligotrophy and allochthony often characterizing at least their pristine conditions.

Nematodes are widely applied for assessments of terrestrial and aquatic habitats (Wilson and Kakouli-Duarte, 2009). Aside from common community descriptors including various diversity measures (e.g. Shannon, Evenness and Jaccard), a range of additional measures have been established on the basis of nematodes. The maturity index (MI) incorporates information on their ecological strategies ranging from pronounced colonizers (short generation times, high number of offspring, able to deal with sparse resources and instable habitats and early successional stages) to extreme persisters (long generation times, low number of offspring, adapted to stable and well-developed habitats and later successional stages). The MI was originally developed in terrestrial research (Bongers, 1990) but has also been proven indicative for anthropogenic influences in riverine habitats (Beier and Traunspurger, 2003a, 2003b; Eisendle, 2009; Eisendle-Floekner *et al.*, 2013). Only rarely applied so far in lotic habitats, and also with indicative value, are phylogenetic approaches, such as the taxonomic distinctness (Barbuto and Zullini, 2005; Eisendle, 2009) developed for marine habitat assessment (Clarke and Warwick, 1998, 2001). Finally, the relation of adenophorea and secernentea represents a suitable indicator for pollution of river habitats (Zullini, 1976; Eisendle, 2009). (Although adenophorea and secernentea are no longer systematically valid groups, they are still useful to characterize differences among nematode communities.)

## INTERDISCIPLINARY INTEGRATION OF METHODS

### *Abiotic characterization*

The classification of aquifer types is identical between hydrogeology and groundwater biology, and comprises unconsolidated, karstic and non-karstified hard rock aquifers. Consequently, it should be applied in interdisciplinary studies. Other scales used in biology mostly address solely the geology of an area, whereas finer structures such as geological sub-layers or pore and void sizes are usually not analysed together with the groundwater fauna. A suggestion of hierarchical scales among habitat characterisations is given by Larned (2012), but the finest scales used therein are still too coarse (minimum  $10^{-2}$  m) to actually provide differentiated information on the habitability or inaccessibility of pore spaces with regard to smaller components among the groundwater fauna. However, relevant finer scales belong to the basic characterisations required in hydrogeology (compare

Table I) and no doubt should be used when the fauna is tested as a potential indicator for both hydrogeological and ecological classifications.

In addition, using established hydrogeological methods described in the chapter *Hard rock aquifers* will provide the best possible description of the complete hydrogeological setting (including recharge area, flow velocity, residence times, flow types etc.) in unconsolidated as well as in hard rock aquifers and may enable a more detailed habitat characterization and differentiation.

### Biotic indication

Up until now, the use of the groundwater fauna as a reliable indicator for abiotic habitat conditions has had its shortcomings, as outlined previously. This might be partly due to endemism as well as too coarse habitat characterisations. The use of classification systems in better accordance with lithology can be of higher importance than hitherto assumed, but still needs to be tested. However, the characterization of environmental conditions by means of the GWI together with the fauna is suggested to be a practical and supportive application for hydrogeological requirements with regard to hard rock aquifer analysis.

Nearly lacking endemism among groundwater and surface water inhabiting nematodes might lead to more general and indicative patterns for groundwater habitats than hitherto obtained by the other fauna. In addition, the nematodes' small size (20–50 µm diameter on average) predestines them to reside in (and pass through) extremely fine pores inaccessible to larger animals. Thus, the combined use of nematodes and other groundwater fauna is expected to provide more detailed information on the variety among pore space structures than each group alone would do. Although, for example, large crustaceans point to larger spaces, small nematodes point to co-occurring finer spaces, because as primarily substrate bound animals, they avoid large pore spaces.

Stygobiotic nematode species are considered similarly indicative for water bodies with long residence times and low connectivity as their crustacean equivalents, but with the advantage of lacking crustaceans' geographic restrictions. This favours them to be used in local and regional assessments. In similar geographical independence, the nematofauna is supposed to indicate connectivity with surface waters when the portion of non-stygobiotic adenophorea (typical surface water species as Tobrilidae among them) increases and connectivity with terrestrial habitats when the portion of groups characteristic for terrestrial habitats (secernentea) increases. Endemic species can provide useful indications on aquifer boundaries otherwise difficult to access (Larned, 2012).

In analogy to the GWI, the MI is also suggested as an indicator for short and long-term recharge processes, which entail instable and highly allochthonously influenced (low MI and high GWI), and stable and less allochthonously influenced (high MI and low GWI) habitats, respectively. The numbers of species among nematode feeding types (complexity) are suggested to also give some information on the degree of connectivity (e.g. higher complexity expected with higher degree of connectivity). In addition, nematode community compositions similar (or partly similar) to those described by Zullini *et al.* (2011) are considered indicative for certain lithologies.

The relation of the aforementioned and other (i.e. abundance, diversity, body size, biomass and taxonomic distinctness) nematode community descriptors with abiotic groundwater characteristics including the GWI needs to be tested together with a range of sampling methods, which comprise both pumping and net sampling. Different seasonal hydrological aspects have to be considered by means of taking replicate samples. Net sampling and sample processing procedures should use mesh sizes of at most 40 µm to obtain reliable nematode data. Nematode data from adjacent aquatic and terrestrial surface habitats help to assign flow paths between aboveground and belowground habitats.

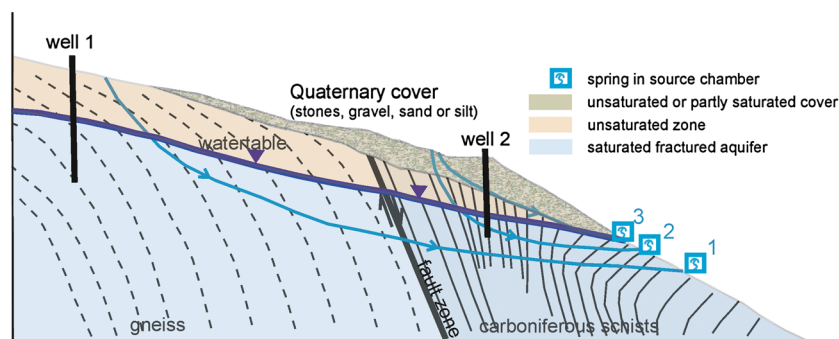


Figure 1. Schematic possible flow paths within an alpine fractured aquifer. Considering the different infiltration areas and percolation depths, three groups of spring waters can be defined (modified after Hilberg *et al.*, 2013).



Table II. Comparison of hydrogeological and biological characters for flow paths 1–3 and wells 1 and 2 in Figure 1.

	Well 1		Well 2	Spring water in source chamber 1	Spring water in source chamber 2	Spring water in source chamber 3
Hydrogeological interpretation	Infiltration topographically high, deep circulation in gneiss and schists	Low infiltration area, mix of water from unconsolidated cover and schists		Infiltration topographically high, deep circulation in gneiss and schists	Infiltration topographically lower, circulation in schists	Infiltration and circulation in unconsolidated cover
Biological characters						
MI	High	Medium		Decreases from chamber 1 to chamber 3		
GW	Low	Medium				
Stygobionts	Dominant	Non-stygobionts and stygobionts		Present	Merely found	Absent
Dominance – faunal elements	Nematodes and others	Nematodes		Nematodes	Nematodes	Nematodes and others
Dominance – body size	Large	Small		Small	Small	Range of various sizes
Complexity nematode feeding types	Low	Medium		Low	Medium	High
Diversity	Low	Medium		Low	Medium	High
Abundances	Low	Medium		Low	Medium	High
Similarity to wells	None	Lowest		To well 1	To well 2	Only minor to well 2
Similarity to surface	None	Few		None	Low	Medium
Secernentea	None	Few		None	Few	Abundant

Grey cells, specific nematode characters; MI, maturity index (Bongers, 1990); GWI, groundwater fauna index (Hahn, 2006).

### A hypothetical example

Hydrogeologists' understanding of the flow history of spring waters and assigning springs to distinct flow paths is of great importance, especially in drinking water protection concerns. The following example (Figure 1), representing a common geological situation in the central European Alps, aims to explain how groundwater biology can be used as an indicator to identify different flow paths and flow history within a hard rock aquifer.

In this geological setting, we find two different hard rock lithologies (gneiss and mica schist) separated by a fault zone. The role of the fault zone is often unclear. It may act as a favourable flow path or as a hydraulic barrier. Gneiss is supposed to provide a comparatively high hydraulic conductivity due to open fractures. In comparison, mica schist shows lower permeability because tectonically induced open fractures are filled with fine grained detritus. The fractured hard rock aquifer is partly covered by unconsolidated slope waste that may act as a porous aquifer.

Three different flow paths might potentially be established in this setting.

- Flow path 1: topographically deeper infiltration area and circulation in mica schist.
- Flow path 2: topographically higher infiltration area, circulation in gneiss as well as in mica schist.
- Flow path 3: circulation in unconsolidated cover, emerging without any contact to the hard rock aquifer.

Because the wells are established within distinct hydrological settings and the flow paths represent different combinations thereof, different faunal structures are expected for the wells and springs in source chambers. They are hypothesized as follows:

- Well 1: high MI, low GWI and typical stygobiotic species (nematodes and other), larger species prevail.
- Well 2: medium MI, medium GWI and non-stygobionts and stygobionts present, small species (nematodes) prevail.
- Flow path 1: the highest MI among the three flow paths, nematodes prevail, stygobionts present; low complexity among nematode feeding types (few species among feeding types), community poor on species and individuals, fauna similar to well 1; no nematofaunal similarity with surface habitats.
- Flow path 2: medium MI, small forms (nematodes) prevail, stygobionts merely present; medium complexity among nematode feeding types; abundances higher than in FP1; species rich community; similarity with well 2; increase of secernentea; low nematofaunal similarity with surface habitats.
- Flow path 3: low MI, nematodes and other fauna, various body sizes; stygobionts no longer present; low

to none similarity with well 2; the highest complexity among nematode feeding types; the highest portion of secernentea; higher nematofaunal similarity with surface habitats.

A survey of the hypothetically exemplified hydrogeological and biological characters is also given in Table II.

## CONCLUSIONS

In this commentary, we dealt with important aspects of groundwater research: the methodological gap in hard rock hydrogeology on the one hand, and two major gaps in groundwater biology, namely difficultly establishing relations between abiotic and faunal characters, and the so far widely neglected free-living nematodes in groundwater biology, on the other hand. In order to bridge some of these gaps in both disciplines and, thus, to enhance the knowledge of each, we introduced an integrated approach combining questions and methods of hydrogeology with those of biology. The fauna patterns should help to reveal conditions relevant in hard rock aquifer research – such as residence times (long, intermediate and short), degree of connectivity between water bodies and between the aquifer and the surface and their lithological pathways. In turn, the integration of characterisations used in hydrogeology and abiotic and biotic data obtained from hard rock investigations are intended to give new insights important for groundwater biology. The outputs of an interdisciplinary approach are thus considered to advance both research disciplines.

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