Technical Report

TR-08-06

Ecology and living conditions of groundwater fauna

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September 2008

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Keywords: Biosphere, Ecosystems, Geo-Chemistry, Biota, Groundwater, Stygofauna, Hydrogeology.

Abstract

This report presents the current state of ecological knowledge and applied research relating to groundwater. A conceptual picture is given of groundwater fauna occurrence in regard to Swedish environmental conditions. Interpretation features for groundwater fauna and applications are outlined. Groundwater is one of the largest and oldest limnic habitats populated by a rich and diverse fauna. Both very old species and species occurring naturally in brackish or salt water can be found in groundwater. Groundwater ecosystems are heterotrophic; the fauna depends on imports from the surface. Most species are meiofauna, 0.3–1 mm. The food chain of groundwater fauna is the same as for relatives in surface water and salt water. Smaller animals graze biofilms and detritus, larger animals act facutatively as predators. A difference is that stygobiotic fauna has become highly adapted to its living space and tolerates very long periods without food. Oxygen is a limiting factor, but groundwater fauna tolerates periods with low oxygen concentrations, even anoxic conditions. For longer periods of time a minimum oxygen requirement of 1 mg/l should be fulfilled.

Geographic features such as Quaternary glaciation and very old Pliocene river systems are important for distribution patterns on a large spatial scale, but aquifer characteristics are important on a landscape scale. Area diversity is often comparable to surface water diversity. However, site diversity is low in groundwater. Site specific hydrological exchange on a geological facies level inside the aquifer, e.g. porous, fractured and karstic aquifers as well as the hyporheic zone, controls distribution patterns of groundwater fauna. For a better understanding of controlling factors indicator values are suggested. Different adequate sampling methods are available. They are representative for the aquifer, but a suitable number of monitoring wells is required.

The existence of groundwater fauna in Sweden is considered as very probable because both *Harpatocoida (Parastenocaris* sp.) and *Nematoda* have been detected in the hyporheic zone in rivers and at shores of the Baltic. In addition, groundwater fauna has been reported from other formerly glaciated areas e.g. Northern Germany, Finland, Iceland, Ireland, North America and Siberia and Alpine regions. Glaciofluvial porous aquifers, especially eskers, and karstic aquifers as well as the hyporheic zone, have proved to offer the greatest chances of successful surveys of groundwater fauna. In Sweden endemic species are not expected to be found, except in karstic aquifers in Gotland and Öland and some parts of the Swedish Mountains. The upper layers of aquifers in crystalline bedrock have only been surveyed at very few sites.

Based on community structures of groundwater fauna, reliable statements on the strength of the surface water impact and the vulnerability of the aquifer are possible. Contacts between different water bodies are displayed by groundwater fauna because groundwater fauna communities mainly reflect the intensity of surface water intrusion at a certain point when compared to hydrochemical data indicating the origin of the water. The information provided by the groundwater assemblages of an aquifer can be used for an ecologically based assessment of groundwater. Ecologically based assessment has provided initial data showing that groundwater fauna is a good marker of mixing between surface water and groundwater at certain depths. Ecologically based assessment has hitherto been used for extraction wells and quality management in drinking water abstraction (standards are still to be established). Groundwater fauna assessments have also proved to be useful in management of wetlands and regulation under nature protection law.

Sammanfattning

Rapporten om grundvattenfauna ger en överblick över det aktuella kunskapsläget för grundvattenekologi och hur forskningsresultaten kan tillämpas. Sammansättningen av grundvattenfaunan tillåter en rad praktiska tillämpningar exempelvis kartläggning av kontakter mellan yt- och grundvatten. Det är känt att djuren kan överleva längre svältperioder i grundvattnet och kortare perioder av syrebrist. Det är också känt att många arter betar av biofilm, men alla funktioner och enskilda arters roll i näringskedjan är långt ifrån kartlagda.

Grundvatten är en dold naturlig livsmiljö och ett av jordens äldsta och största limniska habitat. Grundvattnet är befolkat av en rik och mångfaldig fauna, huvudsakligen meiofauna, 0,3–1 mm. En del arter är relikta dvs mycket gamla och en del arter har sitt ursprung i saltvatten. Uppskattningsvis finns det mellan 50 000 till 100 000 arter i alla jordens grundvattenförekomster. 2 000 arter har hittills upptäckts i Europa. Anpassningen av djuren till sin livsmiljö är stor. Sann grundvattenfauna kallas stygobionta, definitionen är att livscykeln helt utspelar sig i grundvattnet. Stygobionta omfattar många endemiska arter i ofta isolerade grundvattenförekomster. Endemiska arter har framför allt påvisats i karstområden med underjordiska vattensystem i södra Europa och under Australiens öknar. Vid enstaka gruvor i Australiens öken är arter utrotningshotade. För Sverige är det mest troligt att hitta endemiska arter i karstakviferer på Gotland och Öland, i fjällkedjan och i Lappland. Rullstensåsar däremot är ofta så likformade, att det kan vara svårt att avgränsa enskilda grundvattenförekomster från varandra. Grundvattenfaunan som hittats i åsar i Finland har en mycket stor spridning inom Europa. Åsar kan ha varit en spridningsväg för grundvattenfauna efter istiden.

För ekologiska kartläggningar är inte bara stygobionta arter utan hela spektrumet av arter som till viss del trivs i ytnära vatten av intresse. Grundvattenfaunan kan uppträda koncentrerat i enstaka punkter. Detta leder till en låg platsspecifik mångfald. Många arter förekommer ytterst sällsynt. I motsats till detta, är mångfalden inom ett större område ofta jämförbar med artrikedomen i ytvatten. Provtagning av grundvattenfauna kan vara problematiskt eftersom grundvattenförekomster är svårtillgängliga och standardiserad provinsamlingsteknik saknas. Ändamålsenliga redskap för att provta grundvatten och samla in djuren har dock utvecklats. Olika provtagningsmetoder rekommenderas beroende på undersökningens utformning och syfte. Ett specialkonstruerat nät är bra för provtagning under förutsättning att det finns tillräckligt många övervakningsbrunnar/observationsrör, det gäller även för berggrundvatten. Prover från olika nivåer i djupled tas bäst med en cylinderpump med dubbla manschetter. För en översiktlig undersökning av grundvattenfaunan på regional nivå dvs inom ett landskap är specialkonstruerade nät lämpligast. För småskaliga platsspecifika spridningsstudier eller nivåundersökningar i ett relativt ytligt grundvatten rekommenderas däremot små specialkonstruerade fällor som används utan bete. Val av lämplig provtagningsmetod underlättar att ta representativa prover. Undersökningar visar att faunan i ett borrhål är representativ för hela grundvattenförekomsten med förbehåll att mängden grundvattendjur till antal kan vara något förhöjt.

Näringsämnen och syre som förs in från ytvattnet är grundförutsättningar för grundvattnets heterotrofa ekosystem. Beträffande näringskedjan så gäller samma mönster som för meiofauna i ytvatten eller saltvatten, att djuren betar av biofilm och att en del större arter agerar rovdjur. Grundvattenfauna, framför allt ”stygobionts” skiljer sig från annan fauna genom sin anpassning till lagom lite näring och lagom lite syre. Grundvattendjur överlever långa perioder utan näringsämnen och med låga syrehalter, de kan till och med överleva syrefria förhållanden under kortare perioder. Syrehalten blir begränsande när den understiger 1 mg/l. Platsspecifika förhållanden för grundvatten styr förekomsten. Sprickor och porer kan skilja sig inom en och samma aquifer vilket påverkar hydrologisk omsättning och flödesdynamik som båda påverkar utbyte med ytvatten. Småsprickor och porer utgör livsrummet för grundvattenfauna. Samtidigt bidrar grundvattendjuren själva till att hålla porer och småsprickor öppna. Utbyte mellan yt- och grundvattnet är en nyckelfaktor för tillförsel av organiska ämnen och syre, här menas tillförsel av syre i lagom liten omfattning. Detta utbyte formar sammansättningen på artnivå. Andra hydrogeokemiska parametrar har hittills inte visats ha en effekt på förekomsten av grundvattenfauna. Hur föroreningar påverkar grundvattendjur

är föga känt, därför att odling av grundvattendjur hittills inte har lyckats till stor del pga speciella reproduktionsförhållanden. Beträffande dödlighet vid tillförsel av toxiska ämnen är effekterna dvs graden av dödlighet för utsatta grundvattendjur jämförbara med påverkan på ytvattendjur.

Ur ett storskaligt perspektiv inverkar biogeografiska faktorer vilka arter av grundvattenfauna som finns representerade. Det har visat sig att fördelningen av olika arter återger vattendelare som är äldre än Kvartärtiden. På landskapsnivå är geohydrologin en viktig faktor för förekomsten av grundvattendjur. Ogenomsläppliga jord- och bergarter saknar oftast grundvattenfauna i motsats till grundvattenförekomster i lösa sediment, sprickbergarter eller karstområden med hög genomsläpplighet. I akviferer i karstområden, sprickakviferer och okonsoliderade akviferer i sediment samt i glaciofluviala avlagringar har mest grundvattenfauna hittats.. Referenser till sprickakviferer syftar förutom tyska undersökningar huvudsakligen till akviferer i karstområden och okonsoliderade akviferer i sediment syftar på samma sätt till övergångszonen längs vattendrag. Därför är dataunderlaget för slutsatser om grundvattenfauna i sprickakviferer och okonsoliderade akviferer i sediment mer begränsat än antalet referenser utvisar.

Det är sannolikt att grundvattenfauna förekommer i Sverige. Vid undersökningar av ackumulationsbottnar av sjöar och vattendrag och strandnära grundvattenförekomster hittades typiska stygofila (grundvattenälskade) arter, både Harpatocoida *(Parastenocaris* sp.) och Nematoda. Olika *Parastenocaris* arter finns i hela Norden, också på Island och Spetsbergen. Stygobionta arter (grundvattendjur) hittades i andra tidigare nedisade regioner närmare bestämt i Norra Tyskland, Finland, Island, Irland, Nord-Amerika och Sibirien. Förutsättningarna att hitta grundvattendjur i Sverige anses vara bäst i kontaktzonen mellan grund- och ytvatten exempelvis vid ackumulationsbottnar och inom strandnära grundvattenförekomster, likaså i bräckt grundvatten nära havet. Av intresse är också grundvattenförekomster i karstområden och i åsar. Grundvatten i urberg har enbart undersökts i södra Tyskland. Där hittades grundvattenfauna ner till 55 meters djup. Utan undersökningar går det varken att bekräfta eller att utesluta grundvattenfauna i ytnära sprickakviferer i kristallin berggrund i Sverige.

Tidigare undersökningar hade som främsta syfte att hitta nya stygobionta arter. Ett syfte med moderna ekologiska kartläggningar är att se till hela habitatet för att dra slutsatser. I ekologiska kartläggningar inkluderas förutom stygobionta arter också stygofila arter, som koloniserar både yt- och grundvattnet och så kallade stygoxena arter. Stygoxena arter har sin egentliga livscykel i ytvattnet och måste ha transporterats in i grundvattnet. Grundvattenfaunan visar i första hand hur mycket ytvatten som bildar grundvatten på en viss punkt och inte enbart grundvattnets härkomst. Vid hydrologiska förändringar sker ändringar i sammansättningen på artnivå mycket fort. Med allt större inflöde från ytvattnet ökar det totala antalet djur markant. Samtidigt ökar antalet olika arter som hittas, framför allt stygofila och stygoxena grundvattendjur. Stygobionta arter trängs undan, då de inte är anpassade till konkurrens och bra tillgång till syre och näring. Vid undersökning av samtliga arter blir det möjligt att göra tillförlitliga uttalanden om hur stor kontakten mellan yt- och grundvattnet är. Undersökningar av grundvattenfauna vid floden Rhen i Tyskland gav en mycket bra indikation hur och var inströmmande grundvatten blandade sig med flodvatten. Också tidsmässigt visade sig ekologiska kartläggningar av grundvattenfauna vara ett bra verktyg för att kartlägga förändringar när översvämningsområden för risodlingar i Sydkorea undersöktes. Grundvattendjuren reagerade mycket fort på översvämningen respektive torrläggningen. Detta visade sig i ändrad artsammansättning.

Contents

1 Introduction and outline of groundwater fauna research

Groundwater fauna comprises nearly all major taxonomic groups known from limnic surface waters, but it is poorly explored, up to now. The animals live in the pore spaces and fissures of the aquifers. Most of the animals are crustaceans[1], with respect to both abundances and numbers of species. Protozoans, flatworms, tardigrads (waterbears), oligochaetes, nematods, mites, snails and mussels are also quite common in the groundwater; common in sense of a high spatial and temporal heterogeneity inside the groundwater habitat, not in the sense of mass occurrences, see Figure 1-1.

Among insects and even larger vertebrates (fish and amphibians), typical groundwater animals occur. The majority of groundwater animals belong to meiofauna. Meiofauna is defined by a normal size between 0.3–1 mm. However, amongst groundwater fauna some amphipods and isopods reach sizes up to 3.5 cm. Within the group of crustacea groundwater copepods *Parastenocarida* are smallest, with 0.25–0.35 mm. Nematodes may be even smaller and difficult to detect under light microscope. Morphologically, groundwater fauna has adapted to the underground habitat by fitting optimal to their narrow living space in pores. While some taxa predominantly comprise surface-dwelling species, others like the amphipods display the highest taxonomic richness in the groundwater or occur exclusively there, like the *Bathynellacea*. Groundwater is one of the oldest living spaces on earth with relatively consistent environmental conditions over millions of years. Occurrences of relict species are typical for groundwater fauna. A good example is the group of the *Bathynellacea*, which are inferred to live in the groundwater since more than 300 million years. Beside the faunistical diversity, a plethora of microorganisms (bacteria and fungi) is supposed to live in the groundwater. However, exploration of groundwater biodiversity is at it's very beginning and even in central and southern Europe, where investigations are comparatively numerous, new species are described continuously.

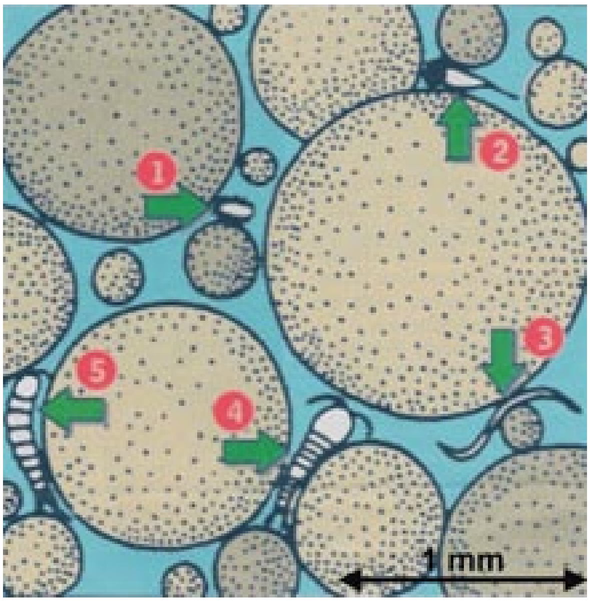

Figure 1-1. *1 = ciliate, 2 = rotatorian, 3 = nematode, 4 = cyclopoid, 5 = harpacticoid. Modified from /Hahn 2002a/.*

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[1] One of the most widespread and diverse group of invertebrates.

According to the heterogeneity in the groundwater (in terms of distribution of organic matter, oxygen and pore size of the matrix), spatial distribution of fauna is often extremely patchy. The patchy distribution of groundwater fauna on a local scale is indicative of the habitat heterogeneity in the subsurface. Additionally, it displays the high degree of adaptation of real groundwater fauna (stygobionts) to the special living conditions in the groundwater. At first view, these distribution patterns together with habitat heterogeneity seem to complicate both sampling and ecological assessment of the groundwater. Actually, the patchy distribution pattern indicates that groundwater fauna reflects the environmental conditions on a local scale. As a consequence groundwater fauna can be used for an ecologically based assessment of groundwater. In the original title the name Stygofauna is replaced by Groundwater Fauna, as an ecological based assessment needs to include all animals found in the habitat, see Section 2.1.1 and 2.1.2. Faunal biocenoses, e.g. living processes of the animals in the groundwater habitat, depend on organic matter (OM) and dissolved oxygen (DO) imported from the surface. The availability of both is the result of the exchange processes between groundwater and surface water. The hydrological exchange has to be considered as the main factor shaping the communities of unpolluted groundwater, as well as site-diversity, see Chapter 5. Strength and direction of surface water/groundwater interactions are influenced by site-specific features like hydrology, depth of the groundwater, hydrogeology, geologic facies, landscape morphology, soils, land use, climate and catchment specifics. Of great interest is the hyporheic zone which exists below and is laterally linked to the channel and banks of many rivers, streams and brooks as well as to lakes and the shores of the Sea. In the hyporheic zone surface water and groundwater exchanges play a crucial role in the biogeochemical transformation of water constituents, mediated by active microbial biofilms /Boulton 2007/. This zone is a typical ecotone zone characterized by a transitional spatial gradient between adjacent ecosystems. The hyporheic zone harbours assemblages of groundwater fauna that graze these biofilms and contribute to secondary production. Groundwater fauna can therefore itself contribute to the porosity of the hyporheic zone.

2 The groundwater habitat

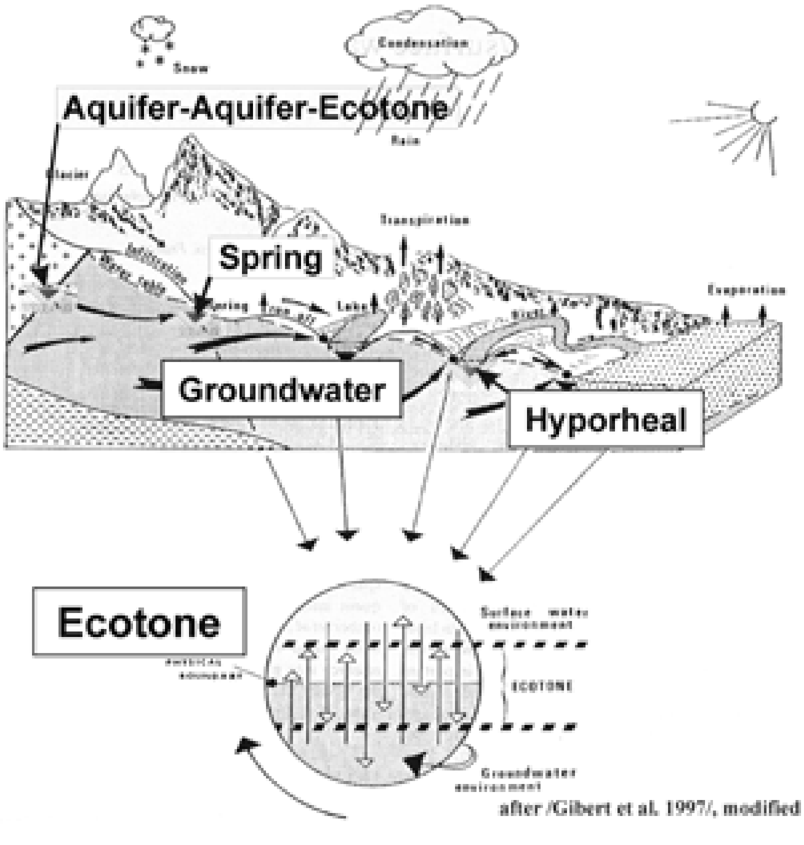
2.1 Ecology in general

A general overview on the groundwater habitat and its ecological particularities is given by /Griebler and Mösslacher 2003, Hahn 2004a, Danielopol et al. 2007/.

Groundwater is a highly diverse biotope. The character of the groundwater depends on site-specific features, see Chapter 1 above, and on the residence time of the water. This diversity is even boosted by the different groundwater – surface water – ecotone zones and groundwater – groundwater ecotone zones, see Figure 2-1. Ecotone zones are the margins of the aquifers, more precisely either the layers at the top of the groundwater body or the layers at the interfaces between two aquifers. A general characteristic of ecotone zones as interfaces between different biotopes is the regulation of the fluxes of energy and matter in landscape i.e. a transitional spatial gradient between adjacent ecosystems. These subsurface ecosystems are characterised by a high taxonomic richness /Marmonier et al 1994, 1995, Gibert et al 1997, Malard et al 2003a, Griebler and Mösslacher 2003/, well documented for the hyporheic zone. The name hyphoreal derives from Greece 'hypo' = under and 'rheos' = river.

Typical groundwater – surface water ecotone zones are found in

1. springs and caves,
2. anthropogenic underground constructions works and mines,
3. the exchange zone under and along rivers, streams, brooks (hyporheic zone) and lakes.



after /Gibert et al. 1997/, modified

Figure 2-1. Groundwater, as a substantial part of the landscape and its water balance, is a very diverse living space for highly adapted species and communities. The pore spaces and fissures of the aquifers are populated by a diverse fauna.

The interfaces between adjacent aquifers are groundwater – groundwater ecotone zones. The most extended groundwater-related ecotone is the groundwater surface, which is the interface between the saturated and the vadose (unsaturated) zone.

On a local scale, the size of pores and fissures influences the flow dynamics and the exchange with surface water, as well as the space available for organisms, thus creating additional structural and environmental heterogeneity /EPA ~1998, Griebler and Mösslacher 2003/. If groundwater fauna is present in the space, the fauna itself contributes to holding pores and fissures open, for example by grazing biofilms partly consisting of bacteria.

There is a fundamental difference between groundwater and surface water ecosystems. Groundwater ecosystems are characterised by permanent darkness. This means that primary production, except some chemoautrophic processes, is negligible. Groundwater systems are mostly heterotrophic habitats. Nutrients have to be imported from the above groundwater (epigean) environment. Heterotrophic organisms require both inorganic and organic compounds (in terms of food and oxygen) as sources for energy production. Their availability is determined by import from the surface. A key factor shaping groundwater communities is the hydrological exchange with surface water /Dôle-Olivier and Marmonier 1992, Brunke and Gonser 1997, Dumas et al. 2001, Griebler and Mösslacher 2003, Hahn and Friedrich 1999, Hakenkamp and Palmer 2000, Malard et al. 2003b/. Groundwater fauna directly depends on the strength of the exchange with surface water. Within the group of groundwater fauna, stygobionts spend their whole life cycle in the habitat. One of their main characteristics is their tolerance to scarcity in regard to food and oxygen supply. Groundwater fauna communities dominated by stygobionts indicate very low contact with surface water, because scarcity in regard to food and oxygen in turn is the result of reduced interactions with surface water.

Although our knowledge of groundwater food webs is extremely poor, it is well accepted that the food base of the communities is particular organic biofilms. These biofilms incorporate dissolved organic matter (DOM). By grazing the biofilms and particular organic matter (POM), groundwater animals enhance microbial activity and prevent the interstices, e.g. tiny crooked channels and pores, of clogging /Boulton 2007, Datry et al. 2005, Hahn 2006/. In that way, groundwater fauna plays an important role for groundwater ecosystems, because they purify the groundwater. Additionally, groundwater fauna may well function as bioindicators as they integrate short-term, mid-term and long-term changes in environmental conditions within an ecosystem /Dumas et al. 2001, Malard et al. 1996, Matzke et al. 2005, Mösslacher and Notenboom 1999, Mösslacher 2000ab/.

Many of the groundwater animals occur exclusively in the groundwater (stygobionts). The term stygobionts is composed of 'bios' the Greece word for 'life' and 'stygo' according to the river Styx in Greece mythology on which the decedents travel to the underworld. Another group of species colonise a wide range of habitats both in the groundwater and in surface waters (stygophilous species) or are exclusively surface-dwelling species occasionally transported into the groundwater by infiltrating surface water (stygoxenous species). The general term used for the animal communities of limnic subsurface waters is groundwater fauna, independent of their composition (in terms of stygobiotic, stygophilous or stygoxenous species).

2.1.1 Stygobionts

Real groundwater fauna consists of organisms, adapted to life in groundwater which means adapting to scarceness. Adapting to scarceness is their evolutionary advantage over their surface dwelling relatives, and resulted in a special life form /Remane 1952/. Stygobiotic animals are blind and whitish or translucent (as an adaptation to darkness). In addition stygobiotic animals are elongated (as an adaptation to the narrow habitat), but most important, their metabolism is reduced.

Figure 2-2. *Stygobiotic and stygophilous copepods : Megacyclops viridis (left animal, body length: 1.3 mm) is a typical ubiquistic stygophilous species living in springs and in the near-surface groundwater, while small, whitish Diacyclops languidoides (right animal) is one of the most common stygobionts. Photo: H. J. Hahn.*

Stygobionts tolerate very long periods without food and low oxygen concentrations. Stygobionts survive even temporary anoxic conditions. The life span is long and the reproduction rate low. Stygobionts are considered to be very poor in interspecific competition, therefore competition is not considered to be an important issue in the groundwater.

These special adaptations enable stygobionts to colonise nearly every groundwater, if the poor size of the matrix is wide enough and a minimum of organic matter and oxygen is available. According to the heterogeneous distribution of these resources, the spatial distribution of fauna is often extremely patchy in the groundwater. The field of research for adaptation issues is quite complex and discussed in detail /Culver 1982, Kane and Culver 1992, Kane and Richardson 1990, Griebler and Mösslacher 2003, Sket 1985/. Adaptation processes when limnic or marine fauna settle into groundwater can be construed either as a refugee into the groundwater habitat or as an active colonisation /Griebler and Mösslacher 2003/.

2.1.2 Stygophilous and stygoxenous species

In contrast to stygobionts, stygophilous and stygoxenous species are more sensitive to the scarcity of food and oxygen. Their occurrence is correlated with the availability of sufficient organic matter and oxygen. Stygophilous and ubiquistic species colonise habitats both in groundwater and in surface waters. Stygoxenous species are exclusively surface-dwelling species occasionally transported into the groundwater. Most of the stygophilous and stygoxenous species are pigmented and have more or less well developed eyes. Their rate of reproduction is much higher compared with stygobionts. In contrast to stygobionts, stygophilous and stygoxenous species compete for nutrition i.e. they have not adapted to the groundwater as a living space in general. If surface water together with nutrients and oxygen infiltrates into the groundwater, food and oxygen will be available. As a consequence, living conditions for stygophilous and stygoxenous species improve. These species invade then into the groundwater and outcompete the stygobionts. Improvement of the environmental conditions in the groundwater in the sense of increased nutrient and oxygen supply means a shift of the communities from stygobiotic to stygophilous and ubiquistic, or even stygoxenous species.

2.2 Taxonomic richness and biodiversity

At the landscape scale, area diversity in groundwater is often comparable to surface water diversity, and taxonomic richness of the groundwater is high. From Europe, 2,000 stygobiotic groundwater species are known so far /Gibert et al. 2005, Hahn 2002a, 2004/. In Europe, taxonomic richness of groundwater is comparable to that in running waters with around 3,000 species known /Illies 1978/. Worldwide 50,000–100,000 species are estimated comprising stygobiotic animals spending all their life cycle subsoil /Culver and Holsinger 1992/. In many regions of Australia, biodiversity of the groundwater is probably higher than surface water diversity (Hahn 2008, pers. com.).

However, in contrast to surface waters, site-diversity i.e. the record of different species in the groundwater from a particular borehole or site is considered to be low. Many species are extremely rare /Fuchs et al. 2006/. This is a result of the high fragmentation and isolation of groundwater habitats and of the patchy distribution of their fauna with many species being extremely rare /Gibert and Deharveng 2002, Hahn and Fuchs 2005/. Average numbers of 2–3 species were recorded per site, which is much lower than in surface water. Site diversity increases with increasing availability of food and oxygen, but as a result of increasing amounts of detritus and oxygen, stygobionts are replaced at first by stygophilous and then by stygoxenous species. To quantify the environmental parameters behind and to predict groundwater communities and their diversity, /Hahn 2006/ proposed a new index, the GW-Fauna-Index. Below it is described as Indicator Values for groundwater fauna, see Section 5.2.

In a regional survey in southwest Germany, 52% of all stygobionts were found in less than 1% of the boreholes /Fuchs et al. 2006/. In other studies 50% of the species occurred in less than 3% and 5% of the sites /Castellarini et al. 2005, Martin et al. 2005/. This rarity is indicative of the high vulnerability of groundwater biodiversity to human impact. Additionally, the stygobiotic biodiversity is inferred to be underestimated by most studies /Fuchs et al. 2006, Martin et al. 2005/.

Consequently, the numbers of stygobiotic taxonomic groups found in a certain area in Central Europe were directly correlated with the numbers of boreholes sampled in this area /Fuchs et al. 2006/. Higher numbers of boreholes sampled within an area or a geological unit provided higher numbers of taxa. One borehole seemed roughly to be equivalent to one taxa often representing one species. It seems that in Central Europe, the number of stygobiotic taxa found in the groundwater in an area is not correlated with the size of the area, but with the number of sites sampled. A first explanation for this perplexing conclusion is the fact that groundwater is both fragmented into different groundwater bodies and heterogeneous. As a result of sampling several thousand groundwater boreholes in Central Europe as well as in Ireland, Korea and Australia, fauna was found to be absent from around 30% of all sample sites (Hahn 2008, pers. com.).

Surveys on groundwater fauna for the regions north of the Quaternary glacial border in the northern hemisphere are restricted to some areas in North America, Ireland, southern Finland and Iceland. The few surveys indicate that groundwater fauna here mainly consists of ubiquists and a few old stygobiotic species /Holsinger 1980, Karaman et al. 1994, Kristjánsson and Svavarsson 2007, Mäkelä et al. 1998, Särkkä and Mäkelä 1998, Strayer 1994/.

3 Cultivation of groundwater fauna in the laboratory

Mesocosm experiments[2] allow for an exact determination of the ecological requirements of communities and species. However, the very most information on groundwater fauna ecology is gathered in the field, but not in the laboratory. This is mainly the result of the difficulties related to the cultivation.

Cultivation of stygobionts is difficult, but not a severe problem. The real problem is breeding. If temperature is constantly low (8–11°C) and food e.g. each kind of organic matter like detritus, fish food, cheese, bread etc, is added extremely sparsely, stygobionts can be cultivated in Petri dishes for years. Since the span of a generation is very long – up to several years – and also larval development is extremely slow, breeding of a sufficiently large population comprising proper cohorts e.g. for ecotoxicological tests, is extremely time consuming. To complicate matters further, numbers of offspring are very low, one or two eggs are quite common for stygobionts e.g. *Bathynellacea* or *Arcticocamptus* species.

It seems that in most cases reproduction rate cannot be increased by increasing temperature since several stygobionts stop reproduction at higher temperatures. An example for adaptation processes is *Paracyclops fimbriatus* a ubiquitous stygophilous. copepod. *P. fimbriatus* was cultivated in a sandy substrate and reduced it's swimming organs as a first step of adaptation of surface water fauna to the groundwater habitat /Stérba and Schmidt 1982/.

Figure 3-1. *Arcticocamptus rhaeticus (length: < 0.5 mm) is a stygobiotic harpacticoid copepod. Typical of stygobites is the low number of eggs. The whole genus Arcticocamptus reproduces with only two egg in a reproduction phase. Photo: A. Fuchs.*

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[2] Mesocosm systems are culture systems with a water volume ranging from 1 to 10,000 m^3. In these often large enclosures a pelagic ecosystem is developed, consisting of a multispecies, natural food chain etc.

4 Sampling methods

4.1 Problems related to sampling groundwater fauna

Sampling of groundwater fauna is difficult by several reasons.

4.1.1 Difficulties to obtain representative samples

Hitherto the highest abundance and greatest taxonomic richness of groundwater communities is found at the margins of the aquifers. When groundwater surface interfaces between adjacent aquifers, e.g. fractured and alluvial aquifers, were investigated, fauna originating both from alluvial and rock was found in the bores /Gibert et al. 1990,1997, Gibert 1991, Griebler and Mösslacher 2003/, see Figure 2-1. Ecotone zones are important for biodiversity /Gibert et al. 1990, 1997, Gibert 1991, Fuchs et al. 2006/.

In general, for sampling animals from ecotone zones, most researchers use existing monitoring boreholes. Monitoring boreholes are constructed for the purposes of hydrogeologists. They are drilled to obtain representative samples from the groundwater of a certain aquifer and therefore installed as far as possible from the margins of this aquifer e.g. the ecotone zones. For ecological purposes the bores should be drilled in particular to obtain representative samples from the ecotone zones. As long as this is not the case the sites selected, the screen width and the packing are often not ideal for fauna investigations. As a result, conclusions about groundwater fauna biodiversity are tentative. For many groundwater ecological studies on a small spatial scale, in particular near the groundwater surface, specific monitoring wells should be installed. For studies on a larger spatial scale, existing groundwater monitoring networks of water administration and authorities or water works are often suitable, with restrictions due to the above mentioned sampling problems.

4.1.2 Patchy distribution of groundwater fauna

Another difficulty when sampling groundwater fauna is the effect of the high spatial and temporal heterogeneity of the groundwater realm, resulting in a patchy distribution of fauna /Gibert 2001, Gibert and Deharveng 2002, Griebler and Mösslacher 2003, Hahn 2004a, Hahn and Matzke 2005, Mösslacher 1998, Mösslacher 2003/. Together with the elevated proportion of are species, it is more or less a question of coincidence, whether a researcher, when sampling groundwater fauna, finds such a spot or not. This could explain the positive relationship between the numbers of boreholes sampled and the numbers of taxa found in a certain area /Fuchs et al. 2006/. At the same time the above statement implies that regional groundwater biodiversity is supposed to be strongly underestimated for most areas

Consequently, either many more boreholes have to be sampled to record representatively the groundwater biodiversity of a certain area, or additional boreholes have to be drilled at sites and depths that are supposed to represent ecotone zones. However, for ecological investigations the use of a sufficient number of boreholes (e.g. between 5 to 10 boreholes on a small scale), provided reliable results in comparative studies /Fuchs et al. 2006, Hahn 2004a, 2005, Mösslacher 2003/.

4.1.3 Lack of standardised sampling techniques

A standard method for sampling subterranean aquatic fauna from springs, the hyporheic zone and deeper groundwater has not yet been developed /Hahn 2002b/. Because of the very different requirements of the great range of existing aquifer types, it might be an idea to suggest different standards for certain environments. Such methods should provide representative samples of groundwater fauna, water and microbiology, while permitting stratified sampling, and nevertheless be economically viable. In particular, this latter issue is a prerequisite for the application of a technique in water management, impact regulation, landscape planning and environmental protection.

For the investigation of groundwater biodiversity, but not for ecological purposes, EU funded the /PASCALIS project ~2001/. The PASCALIS project proposed a variety of methods to be applied for sampling fauna of groundwater and groundwater related habitats e.g. groundwater bodies, caves, springs and hyporheic zones /EU-GWD ~2003, Gibert et al. 2005/. At the present state, for ecological purposes several methods are available. It should be noticed that none of these methods provides really quantitative samples. Additionally, it is difficult to properly estimate the efficiency of these methods, since a complete, quantitative removal of fauna, water and matrix from the aquifer is not possible, or only by way of exception (e.g. freeze coring in the hyporheic zone or shallow groundwater) /Bretschko and Klemens 1986, Danielopol and Niederreiter 1987/.

4.2 Evaluation of sampling techniques

There are three main techniques of sampling groundwater fauna: pumping, net sampling and unbaited traps. From comparative studies; samples taken using all three techniques are considered to be representative of the aquifer. However, this requires an adequate number of bores /Mösslacher 2003, Hahn 2004a, 2005, Fuchs et al. 2006, Bork et al. 2007/. Below an overview of different sampling techniques in use according to /Hahn 2005/.

4.2.1 Pumping

One common technique for sampling groundwater fauna is to pump groundwater and filter animals from it. This method is well established and provides useful data /Boulton et al. 2004/. According to the small mesh size for filtering, 74 µm, it is time consuming and expensive /Hahn 2002b/. The amount of water pumped varies between different studies; from 20 L to 1,000 L /Hakenkamp and Palmer 1992, Malard et al. 1997/. Additionally, pumping is inferred to be selective, under-sampling large or less mobile species due to the filtering effect of the pore spaces in particular in sediments especially when small volumes of water are pumped /Boulton et al. 2004, Dumas and Fontanini 2001, Frazer and Williams 1997, Marmonier 1988, Scarsbrook and Halliday 2002, Williams 1984/.

Discrete sampling by pumping is difficult, even if the sampling level is constrained by the use of packers within a borehole, because both water and animals are not sampled from discrete places /Danielopol and Niederreiter 1987/. Assuming a pore volume of 10–20% for sandy sediments, a 500 L sample of alluvial groundwater, as an example, may originate from a sphere of 160 m diameter or larger /Hölting 1996/. Heterogeneity in the groundwater realm is high. Stratified sampling on a small spatial scale (centimetres) using current techniques does not admit clear statements about the environmental conditions at the places where the animals sampled actually lived /Gibert 2001, Humphreys 1999, Watts and Humphreys 2000/. Furthermore, the finer the sediments, the higher the likelihood of altering sediment structures by pumping.

Repeated sampling at the same site might affect groundwater communities by altering the physical living conditions around /Marmonier 1988, Mauclaire et al. 1998/. Stratified sampling in existing monitoring boreholes is possible by using an air driven double packer piston pump as described by /Danielopol and Niederreiter 1987/.

Figure 4-1. *Double packer piston pump. On the left, the double packer with the two collars, which were pumped up with compressed air to define the stratum where the sample has to be pumped from. Right side: the piston pump unit. Photo: D. Matzke.*

4.2.2 Net sampling

Another method for sampling groundwater fauna is the so-called net sampler or phreatic net (mesh size 74 µm) /Bou 1974, Dumas and Fontanini 2001, Humphreys 1994/. Net sampling is fast and cheap, and, with respect to numbers of taxa and community structure, first evaluations indicate that net samples can be regarded as an equal to pump samples /Dumas and Fontanini 2001, Hahn and Matzke 2005/. On the other hand, stratified sampling is possible only in nested boreholes each having their filter slots at one defined depth. Water samples for chemical parameters can be obtained by a bailer.

Figure 4-2. *Net sampler. Photo: B. Thulin.*

4.2.3 Unbaited traps

Unbaited traps (either empty or filled with sediment, presumably as a living space) have mainly been used in the hyporheic zone /Bretschko and Klemens 1986, Hahn 2002b, 2003, Panek 1991, Scarbrook and Halliday 2002/. The traps consist of an inert plastic chamber with holes in the upper parts and gaskets above the bottom and near the lid of each trap. In general, unbaited traps are suitable for sampling subsurface fauna, e.g. groundwater fauna in the upper part of the groundwater body, often the ecotone zone between surface water and underlying groundwater. Unbaited traps should be preferred for stratified surveys of boreholes drilled at sites and depths that are supposed to represent ecotone zones /Hahn 2005, Bork et al. 2007/. Critical is, if the samples fulfill two prerequisites.

- The fauna inside a trap (or a borehole, which acts as a trap) needs to be representative of the fauna outside.
- The installation of a trap within a borehole should not affect the results of sampling.

4.2.4 Performance appraisal

The hyporheic zone: A statistical comparison of invertebrate densities from samples obtained by different methods is critical. Freeze core techniques were used to quantify the depth distribution of fauna in stony streambeds and running waters /Bretschko and Klemens 1986, Pugsley and Hynes 1983/. Admittedly, sediment-filled traps and pump samples are hardly comparable with one another /Scarbrook and Halliday 2002/. The pumping rate and the volume of pumped samples influence taxonomic richness and invertebrate density per litre /Boulton et al. 2003, Hunt and Stanley 2000/. However, using colonization pots filled with sediment few differences in the taxonomic composition between pumped and trapped samples were found /Scarbrook and Halliday 2002/. Comparing trap samples to quantitative freeze-corer samples, it was found that both methods delivered similar results /Scarbrook and Halliday 2002/. Furthermore, traps seem to discriminate both the hydrochemistry and fauna of different sediment layers within centimetres /Hahn 1996, Hahn 2002c/. In the hyporheic zone of a headwater stream, two types of

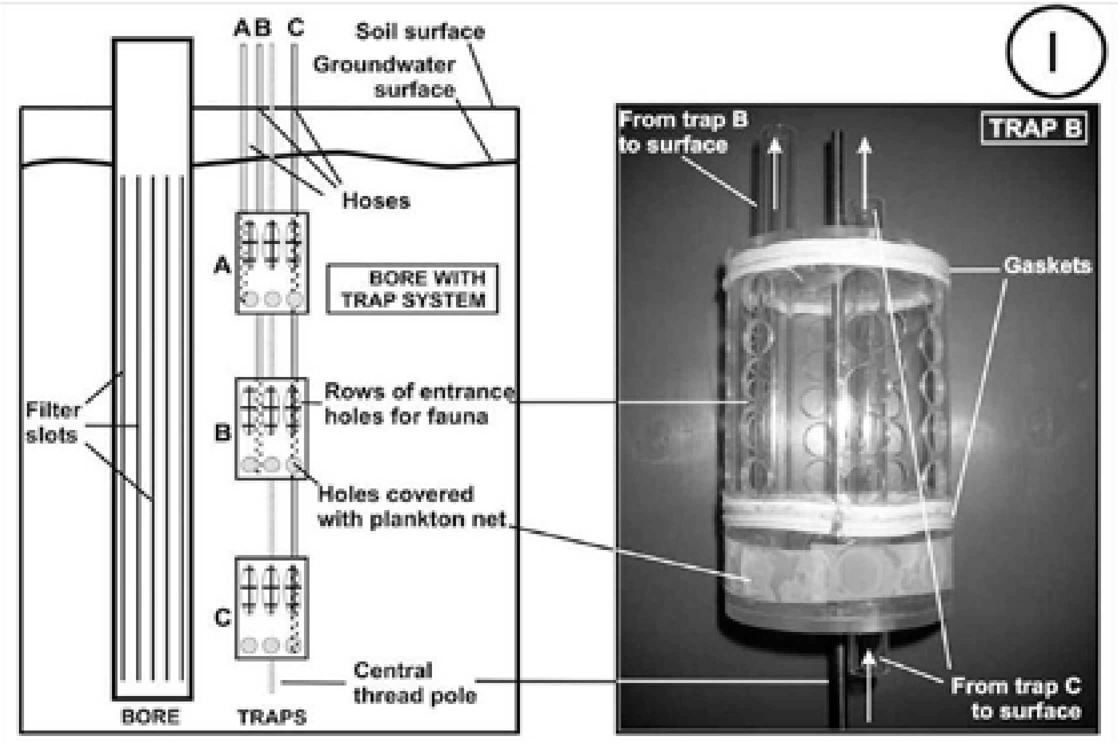

Figure 4-3. *Unbaited traps from /Hahn 2005/.*

unbaited traps were compared, neither of which were sediment-filled /Hahn 2003/. The first type was dug directly into the sediments while the second was fixed within a filter tube previously installed in the sediments – the typical construction of a groundwater monitoring borehole. No differences were detected between the two types of traps, and the effects of the gap between the traps and the bore casing were considered negligible.

The groundwater: recently developed unbaited traps were tested with similar good results as for unbaited traps in the hyporheic zone /Bork et al. 2007, Hahn 2005/. Data evaluation was performed by comparing groundwater fauna obtained using the traps with pumping results. The samples from unbaited traps provided data representative of the aquifer. A critical point is to exclude vertical flow inside an elongated monitoring well, because unbaited traps are used for stratified sampling. Sampling using unbaited traps did not affect the structure of the surrounding sediments, the costs of the traps were moderate and the associated labour was low. Few differences existed between the taxonomic compositions of the stygobiotic fauna at the bottom of boreholes, which act as traps, and the surrounding groundwater /Hahn and Matzke 2005/. However, abundances per litre were higher inside the boreholes compared to the aquifer.

Recommendations

Of the above mentioned three techniques, net sampling is best fitted for large and medium spatial scale studies. Net sampling is also very useful for a screening on a small scale under the prerequisite that the number of monitoring boreholes is sufficient.

If stratified sampling is required for existing monitoring wells, an air driven double packer piston pump is suitable, because of the packer technique preventing vertical flow. For intensive and detailed investigations on a small spatial scale, in particular near the groundwater surface and for repeated sampling, unbaited traps are the best solution, since pumping is time consuming and may affect sediment structure. For evaluation in detail see /Hahn 2005/.

4.3 Groundwater fauna population inside a borehole and in the surrounding aquifer

One of the most important questions in groundwater ecology is to what degree samples from the groundwater are representative of the fauna of the surrounding aquifer. Different studies have used different methods. As mentioned above, surveys require different sampling techniques. The optimal sampling techniques depend to a large extent on the kind of subterranean habitat /Hahn 2002b, Malard et al. 2002/.

Collecting groundwater from boreholes, in particular by pumping and phreatic net, has become the most common method to sample groundwater /Fuchs et al. 2006, Hahn and Matzke 2005/. Monitoring boreholes are in many regions the only sites giving access to the groundwater. The challenge is to get samples from as many boreholes as possible within a limited time, and the fauna and water samples have to be representative of the aquifer. To restrict sampling efforts to the borehole content or even to the bottom of the boreholes helps to reduce sampling-associated time and labour significantly. The essential prerequisite for this is that faunal communities inside boreholes are comparable and representative of the communities outside.

In a comparative study the fauna from the bottom of monitoring wells was investigated and compared with the fauna from the surrounding aquifer which was obtained by pumping /Hahn and Matzke 2005/. They found that community structures (in terms of the proportions of most taxa) were similar both in the aquifer and inside the boreholes. However, fauna was accumulated in the boreholes: total abundances and the numbers of individuals per litre of nearly every taxon were significantly higher inside the boreholes when compared to outside. Also, taxonomic richness of some of the boreholes was higher compared to the aquifer water. This was also the case for groundwater fauna in crystalline bedrock in south-western Germany /Fuchs et al. 2006/. Efforts were made to avoid vertical transports along the well pipe and the bedrock, still such transport is difficult to exclude.

Several studies comparing groundwater in the hyporheic zone and adjacent groundwater aquifers come to similar conclusions. /Boulton et al. 2004/ investigated an exfiltrating site on Ain River in France, where densities of the first one litre of pumped samples were higher than of the subsequent samples, with abundances decreasing from the second to the tenth 1 L sample /Hakenkamp and Palmer 1992, Steenken 1998/. In there they also found much higher densities of groundwater fauna per litre sample volume in the first boreholes sample, respectively, and they claimed that boreholes are preferentially colonized. These results raise the question to what degree the high densities of the first litre were caused by the content of the boreholes. The first litre of a sample "…probably best represents the hyporheic assemblage drifting in the vicinity of the standpipe" /Boulton et al. 2004/.

Results from a study reveal an overrepresentation of the fauna for all three sampling methods /Hahn and Matzke 2005/. The enrichment of organic matter inside the boreholes is considered as the main reason for the accumulation of fauna. Several studies indicate the significance of the amount of detritus as food source, and the existence of a spatial distribution of meiofauna /Brunke and Gonser 1999, Brown et al. 2003, Malard et al. 2003b, Hahn 2006/. The accumulation of fauna inside the boreholes is enhanced, in particular in sparsely populated groundwater biotopes often characterized by a patchy distribution of the fauna /Hahn and Matzke 2005/. This accumulation of fauna also leads to higher taxonomic richness inside the boreholes. They argue that in sparsely populated aquifers, a representative collection of groundwater fauna seems to be possible only by pumping really high amounts of water or by sampling the bottom of the boreholes with pumping or a phreatic net.

At the present state of knowledge, boreholes are assumed to harbour a similar fauna as the aquifer, but in higher abundances and diversity. Further more, sampling boreholes by phreatic nets and traps provide good information on the aquifer fauna and at the same time it reduces time and labour consume compared to pumping techniques.

4.3.1 Endemic species

Groundwater habitats are in general highly fragmented, and degree of endemism is therefore high. However, endemism and diversity are highest in the karstic areas, where evolutionary drift is generally enhanced by the high fragmentation of biotopes. A diverse endemic, exclusively subterranean dwelling fauna is found around the Mediterranean and in the Ponto-Caspic region /PASCALIS ~2001, Gibert et al. 2005, Gibert 2001, Gibert and Deharveng 2002, Griebler and Mösslacher 2003, Malard et al. 1996, Martin et al. 2005, Mösslacher 1998/. In the regions around the Mediterranean, climate changes were not so pronounced as north of the Alps. Consequently, most of the old Tertiary fauna which partially derived from former Thetys, e.g. the Tertiary Ocean from which the Alps were formed, still persists in southern Europe. North of the Alps much of the old fauna was eradicated during the ice ages. In contrast to the Mediterranean region, as a result of the glaciation, degree of endemism is much lower north of the Alps. Furthermore, endemism seems to decrease with increasing distance to the Alps.

Endemism is also an important issue in arid Australia, which is characterised by a very old landscape, without catastrophes like ice ages and volcanism. Here, in the arid climate, former Triassic rivers were reduced to chains of salt lakes, while permanent freshwater occurs only in the underground of the ancient valleys /Boulton 2007, Boulton and Hancock 2006/. Disconnected by the salt lakes, these ancient river systems represent highly fragmented groundwater habitats. As result of this and of the age of the habitats, degree of endemic and relictual species is outstanding. Compared to the surface waters, diversity in the groundwater seems to be much higher in arid Australia (Hahn 2008, pers. com.).

5 Analysis of environmental factors shaping groundwater communities

5.1 Three key factors

The environmental factors that shape distribution patterns and communities of groundwater fauna are best studied on a site scale (while the frame of fauna occurrence is given on a continental and landscape scale), see Chapter 6. /Hahn 2006/ states that the prediction of community structure, abundance and taxonomic richness against environmental parameters is a basic requirement of an ecosystem assessment. The prerequisite for this is the knowledge of the factors shaping the communities. For groundwater ecosystems, their "bad predictability" is a veritable brand mark. Very often, boreholes, which are situated close together and which seem to be very similar in construction, depth and hydrogeology, harbour a different fauna. As an extreme example, a borehole could be well colonized on one sampling occasion, but fauna may be completely absent another time /Hahn 2006/. These phenomena can partly be explained by seasonal changes affecting the groundwater animals because in autumn oxygen content often decreases to only 0,5 mg/l. Due to experience, around 30% of all groundwater samples in Central Europe on different scales are free of groundwater fauna /Fuchs et al. 2006/.

What are the environmental parameters behind? It seems that, under natural conditions, hydrochemistry has no or only minor influence on groundwater fauna – in contrast to the hydrological exchange. These surface water/groundwater interactions control firstly the availability of organic matter and secondly the oxygen content – shaping the communities of the heterotrophic groundwater habitat on a local spatial scale. A third key factor is the size of fractures cracks and fissures respectively pore spaces in sediments.

Figure 5-1. *Nematoda sp. (length: 1.0 mm) from /Fuchs et. al 2006/.*

Both on a landscape and a continental scale, groundwater fauna is considered to depend on the type of aquifer and biogeographic particularities, respectively /Fuchs et al. 2006/. On different spatial levels, fauna distribution depends on additional factors: firstly geography (and related distribution patterns) on the continental level, secondly type and hydraulic conductivity of aquifer on a landscape level and thirdly hydrological exchange with surface water on a very local scale.

The hydrological exchange – its strength and direction – is a result of the site characteristics. Soils, land use, geomorphology, sediment structure, hydraulic conductivity and depth of groundwater table have an effect on the hydrological exchange. All data reflect the surrounding landscape with its typical hydrogeology and climate, directly affecting the landscape's water balance, the groundwater's recharge rate, surface water/groundwater interactions and microbial processes in the soils.

The proportion of stygoxenous species on groundwater communities increase with enhanced food supply /Malard et al. 1994, 1996, 1999, Sket 1999, Culver and Sket 2000/. There are some indications for the importance of organic matter /Bretschko and Leichtfried 1988, Brunke and Gonser 1999, Datry et al. 2005, Gibert and Deharveng 2002, Griebler and Mösslacher 2003, Hahn and Matzke 2005, Strayer et al. 1997/. Also the presence of bacteria might be crucial for some groundwater species /Brown et al. 2003, Brunke and Fischer 1999, Griebler and Mösslacher 2003, Strayer 1994/.

The above statement of the importance of organic matter should be valid both in the groundwater and in the hyporheic zone, but on the whole, the available data on food interactions in the groundwater is scant /Rumm 1999/. In the hyporheic zone, species richness and abundance of groundwater fauna are often positively correlated with both bacterial abundance /Brunke and Fischer 1999/ and organic matter /Strayer et al. 1997/. Similar results were found for the groundwater, where fauna abundance and taxonomic richness correlated with dissolved organic matter (DOM) /Datry et al. 2005/. However, no correlations, neither with bacterial abundance or activity, nor with particular organic matter (POM) was found by /Storey and Williams 2004/. The authors explain their results with low oxygen concentrations.

Comparing oxygen supply and groundwater fauna in several groundwater aquifers, which some showed a strong correlation between groundwater fauna and oxygen, while no correlation was found in others /Malard and Hervant 1999/. Good correlations between fauna and organic matter only were observed in the hyporheic zone, if oxygen concentration were > 1 mg/l /Strayer et al. 1997/. Oxygen is claimed to be important for occurrencies of groundwater fauna, particularly in lower concentrations /Hakenkamp and Palmer 2000/. Correlations of dissolved oxygen (DO) with fauna, if existing, were incongruent, sometimes positive and sometimes negative, depending on the geographical region. DO concentrations < 1.0 mg/l seem to be critical for groundwater fauna, but for concentrations higher than 1.0 mg/l, no correlations between DO and fauna were found. Abundances per sample decreased strongly below 1.0 mg/l; evidently, oxygen is a limiting factor in the groundwater and dissolved oxygen (DO) concentrations below 1.0 or 0.5 mg/l are critical for most groundwater fauna (metazoans). /Hahn 2006/.

On the one hand, the living space required by groundwater fauna is supposed to depend on spatial interconnection and flow patterns within the aquifer. Hydraulic conductivity might be a measure for this. On the other hand fissures and pores even kept open by groundwater fauna itself are decisive for the living space required of species on a very small local scale. Therefore, hydraulic conductivity as a measure of the aquifer does not always correlate with groundwater fauna occurrences in a local scale (Hahn 2008, pers. com.). The task is to establish an estimate on the hydrological exchange of different parts constituting the aquifer with more exact characteristics of the tiny crooked channels and pores where water is transmitted on a facies level. In many geographical regions using the amplitude of water temperature can give a hint. While the temperature of groundwater is nearly constant and corresponds to the annual mean, air temperature and the temperature of surface waters vary due to seasonal effects /Dreher et al. 1997/. There the standard deviation of temperature indicates surface water influence by assuming that the higher the amplitude of temperature is in the groundwater the stronger is the surface water influence. Yet, there are exceptions. In eskers, for example, a relatively deep situated water table, e.g. the top of the groundwater, can have a considerable exchange with surface water without an impact on temperature. This is the case, if the exchange is masked by relatively high water flow in the groundwater of the esker.

The correlations between groundwater fauna on the one hand and hydrological exchange, food and oxygen supply were demonstrated by /Hahn 2006/.

Total abundances increased significantly with enhanced standard deviation of temperature, oxygen content and amount of detritus.

The ocular inspection of the sampling vessel is used as a measure for the amount of detritus. Little detritus correspondences to a situation where the bottom of the sample vessel is slightly covered by detritus; much detritus to the bottom covered by millimetres of detritus; very much detritus to the bottom covered by one centimetre detritus or more.

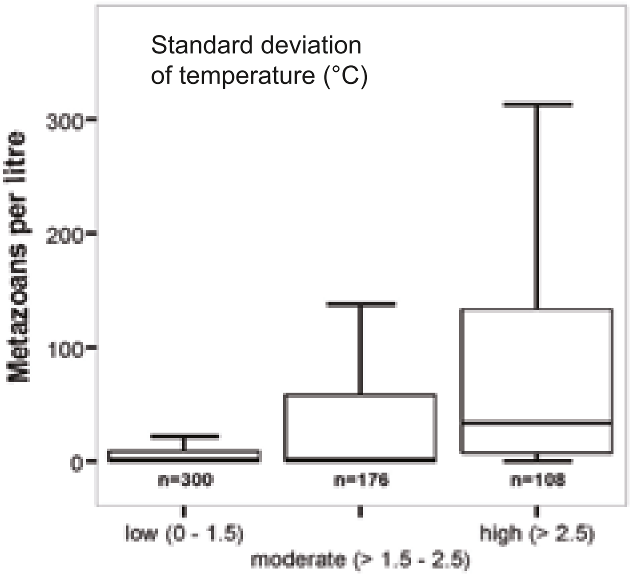

Figure 5-2. Correlations between total abundances of groundwater fauna, e.g. metazoans per litre groundwater versus standard deviation of temperature (SD Temp.) displayed by boxplots: 25 to 75 percentiles, median = black line, n = number of of samples; from /Hahn 2006/.

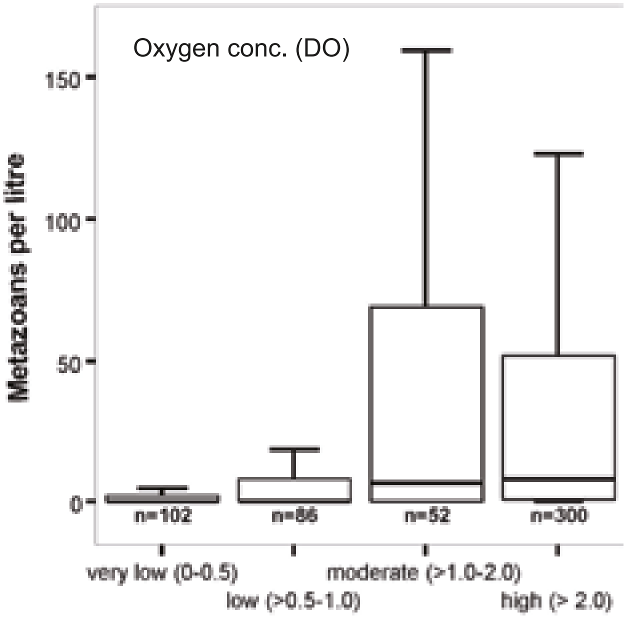

Figure 5-3. Correlations between total abundances of groundwater fauna, e.g. metazoans per litre groundwater versus oxygen concentration (DO) displayed by boxplots: 25 to 75 percentiles, median = black line, n = number of samples; from /Hahn 2006/.

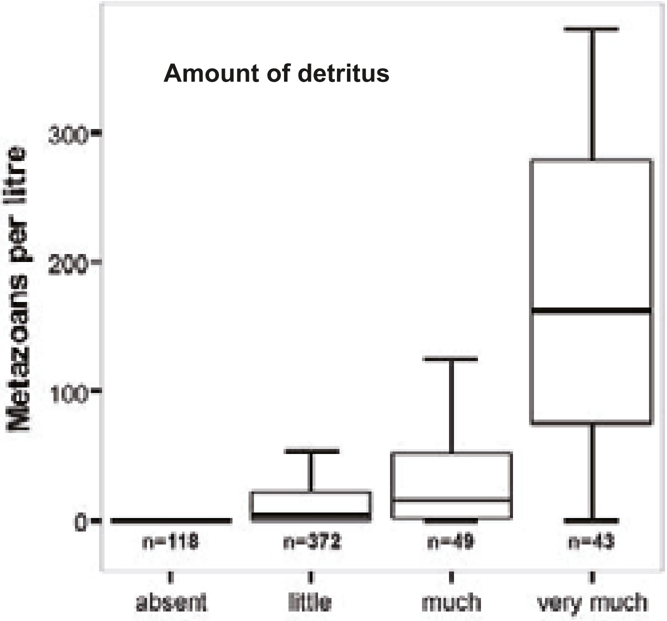

Figure 5-4. Correlations between total abundances of groundwater fauna, e.g. metazoens per litre groundwater versus amonut of detritus displayed by boxplots: 25 to 75 percentiles, median = black line, n = number of of samples; from /Hahn 2006/.

Together with more oxygen and organic matter e.g. DOM and POM, (if food and oxygen supply are sufficiently high), stygophilous and stygoxenous fauna are enabled to durably colonize the groundwater and compete out the stygobionts /Malard et al. 1994, 1996, 1999, Sket 1999/. As a result, community structure, abundance and taxonomic richness of the groundwater fauna reflect the strength of the exchange with surface water, see Section 2.2

5.2 Indicator Values for groundwater fauna

To quantify the ecologically relevant surface water impact on groundwater /Hahn 2006/ proposed the GW-Fauna-Index (below), here described as Indicator Values for groundwater fauna. Numerical numbers are the same.

Indicator Values = $\text{SD Temperature} \cdot \sqrt{\text{DO (mg/1)}} \cdot \sqrt{\text{Amount of detritus}}$

The Indicator Values for groundwater fauna (GW-Fauna-Index) are computed from factors strongly depending on the influence of surface water. Up to now this is standard deviation of temperature, oxygen content and amount of detritus. The standard deviation of temperature indicates contact with surface water. A zero or very low value for standard deviation of temperature would indicate almost no contact with surface water e.g. long retention time for groundwater. In most cases that would not favourite groundwater fauna since food is lacking. Even high inflow from surface water does not support groundwater fauna as the animals are competed out by stygoxenous (non groundwater) species. The oxygen content is important, too. Less than 0.5 ml respectively 1 ml oxygen per litre is regarded as a limiting factor for groundwater fauna. At the same time favour high oxygen contents stygoxenous species. To display the optimal range, the square root value of oxygen content is put into the formula. The third factor in the formula is the amount of detritus. It is measured semi-quantitative.

Due to numerical ranges Indicator Values for groundwater fauna have been divided into three groups. This is an attempt to predict groundwater communities characterised by different intensities of surface water influence:

1. Group I: Indicator Values for groundwater fauna **< 2**
   The groundwater fauna in boreholes or sediments of the hyporheic zone is often characterized by an absence of fauna. The Indicator Values indicate a weak hydrological exchange and a poor food and oxygen supply. If fauna occurs, it consists of stygobionts.
2. Group II: Indicator Values for groundwater fauna **between 2 and 10**
   The groundwater fauna in boreholes or sediments of the hyporheic zone is dominated by stygobionts. These Indicator Values indicate a moderate hydrological exchange and food supply. Oxygen concentrations are moderate to high.
3. Group III: Indicator Values for groundwater fauna **> 10**
   The groundwater fauna in boreholes or sediments of the hyporheic zone is often characterized by taxonomic richness. Typical for the abundant and species-rich fauna are ubiquists, stygophilous and stygoxenous species. Habitats with Indicator Values higher than 10 are strongly influenced by the hydrological exchange with surface water resulting in moderate to high oxygen concentrations and a good food supply.

From all environmental variables the Indicator Values for groundwater fauna (GW-Fauna-Index) best correlated with abundance, taxonomic richness and community structure on a local spatial scale independent of regional characteristics /Hahn 2006/.

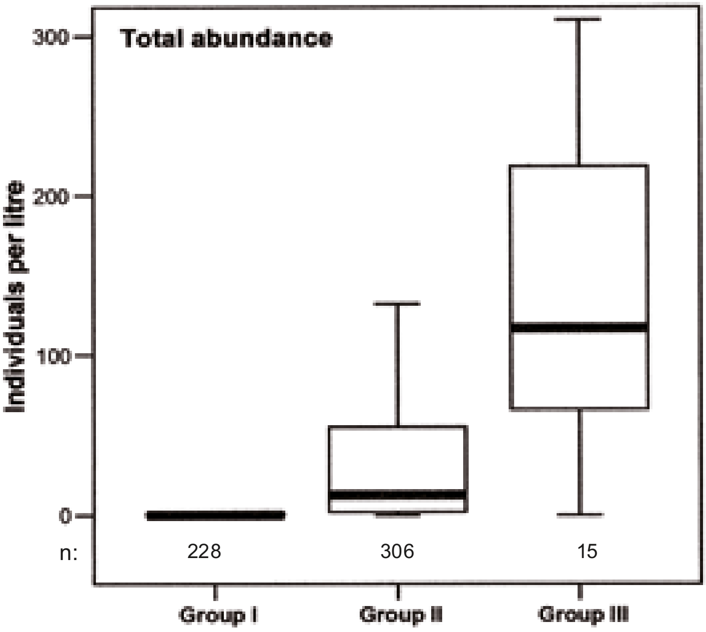

***Figure 5-5.** Total abundance of stygobionts, stygophilous (ubiquists) and stygoxenous summarized in each boxplot. Boxplots: 25 to 75 percentiles median: black line, n = number of samples. Group I represents Indicator Values for groundwater fauna < 2, group II Indicator Values from 2 to –10 and group III Indicator Values > 10; from /Hahn 2006/, modified.*

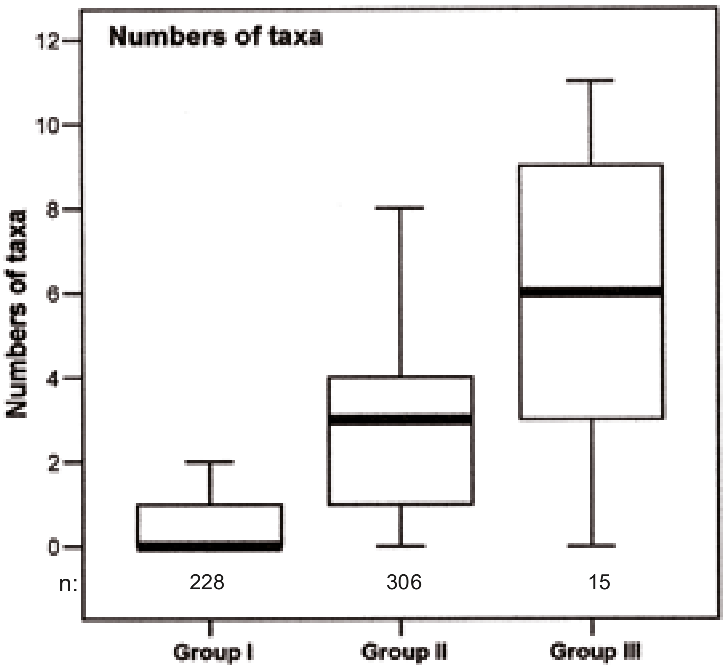

Figure 5-6. *Taxonomic richness of stygobionts, stygophilous (ubiquists) and stygoxenous species summarized in each boxplot. Boxplots: 25 to 75 percentiles, median = black line, n = number of samples. Group I represents Indicator Values for groundwater fauna < 2, group II Indicator Values from 2 to –10 and group III Indicator Values > 10; from /Hahn 2006/, modified.*

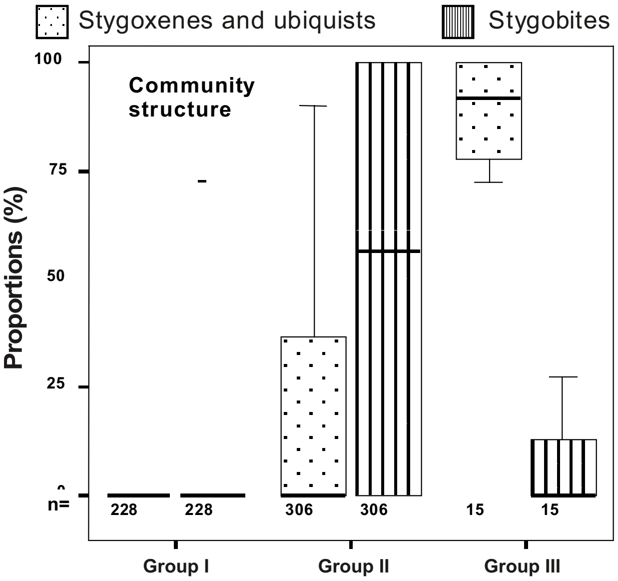

Figure 5-7. *Relative abundances (proportions) of both stygophilous (ubiquists) and stygoxenous species in the left boxes (dotted) and relative abundances of stygobionts in the right boxes (striped). Boxplots: 25 to 75 percentiles median = black line, n = number of samples Group I represents Indicator Values for groundwater fauna < 2, group II Indicator Values from 2 to –10 and group III Indicator Values > 10; from /Hahn 2006/, modified.*

5.3 Further environmental parametres

Since faunal data, under some conditions, scattered widely against the Indicator Values, the objective is to improve Indicator Values by considering not only the quantity of organic matter (OM), but also its quality /Hahn 2006/. Additionally, other factors like bacteria and the size of pores and fractures depending on lithologic and structural characteristics of different geological facies inside the aquifer should be taken into consideration. Hydraulic conductivity did not work for all types of aquifers (Hahn 2008, pers. com.). At the present state of knowledge, Indicator Values ranging from 2 to 10 are the best way to predict reasonable conditions for surveys of groundwater fauna.

In general there are higher abundances in moderately coarse sediments with sufficient fissure and pore space compared to fine sediments /Mösslacher 1998, Strayer et al. 1997/. Larger pore spaces provide more living space for fauna, and they enhance groundwater flow and therefore improve the connectivity between different groundwater habitats and surface water. In aquitards e.g. compact aquifers with no pore spaces available to water flow (interstices in clay are considered to well-filled by just water) do not provide any living space for groundwater fauna. Because of this, aquifers in bedrock may vary in available living space for groundwater fauna. In rocks also fault facies might be of interest /Tveranger et al. 2005/. The minimum oxygen requirement often excludes aquifers at greater depths. Clay covering glacial aquifers may also prevent contacts between surface water and groundwater /Thulin 2006/, see Section 7.2.

The effects of pollutants on groundwater fauna are poorly known and therefore not considered in this report. It seems, that the effects of toxic substances on groundwater fauna are comparable to surface fauna, but depend strongly on the type of pollutant and the species considered /Malard et al. 1994, 1999, Schäfer et al. 2001, Martin et al. 2005, Matzke 2006/. However, a quantitative assessment is difficult, due to the physiological characteristics of stygobiotic fauna. This is related to the problems when cultivating groundwater fauna according to requirements for ecotoxicological tests /Hose 2005, 2007, Humphreys 2007/. The letal ratio is comparable to surface fauna, but also in this case breeding of stygobionts is still one of the challenges for future, see Chapter 3. Besides, in all ecotoxicological studies we know, the stygobionts used were removed from wild living populations.

There are ecological studies about direct and indirect effects of toxic substances on marine ecosystems both microphytobenthos e.g. diatoms and their grazers e.g. meiofauna /Larson et al. 2007, Alsterberg et al. 2007/. These first results elucidate the complexity of reactions within food chains also for groundwater fauna. For laboratory experiments samples were directly taken from shallow marine coastal bays. The meiofauna investigated were harpacticoid copepods and nematodes, with close relatives occurring as groundwater fauna. When CPT (copper pyrithione-an antifouling agent) was added, nematodes were still present in the samples while copepods disappeared and meiofauna in total decreased, while microphytobenthos increased /Larson et al. 2007/.

6 Distribution of groundwater fauna

6.1 Biogeography of Europe

Due to large scale distribution patterns of limnic surface and subsurface fauna, three major biogeographical regions have to be distinguished for groundwater fauna in Europe, which are mainly the result of the Quaternary glaciations periods.

- In those parts of Northern and Central Europe, which were covered by glacial ice shields during Weichsel, Saale and/or Elster glaciations, groundwater diversity within the meio- and macrofauna is low. Endemic species are very rare. However, groundwater fauna surveys from these areas, in particular from Scandinavia, are scant. Most of the groundwater animals recorded to date are supposed to be postglacial recolonisers.
- In the unglaciated parts of Western, Central and Eastern Europe with permafrost soils during ice age, where the climate was very cold and dry during the last ice ages, many of the old tertiary species died out. The majority of the recent groundwater fauna are postglacial recolonisers, but several endemic and relict species survived, and regional biodiversity is generally higher than in the North.
- In contrast to Southern Europe, where the climate stayed moderate during Quaternary glaciations periods, most of the old Tertiary fauna still persists. These regions are characterized by a diverse endemic, exclusively subterranean, dwelling fauna. Here, endemism and diversity are highest in the karstic areas, where evolutionary drift is generally enhanced by the high fragmentation of biotopes.

6.2 Areas influenced by glacial periods

The general pattern of large scale distribution of groundwater described above is widely accepted, but in particular the effects of the glaciation are overestimated. While it in the past was supposed that fauna has become completely extincted below the ice and then partially recolonised these areas /Thienemann 1950/, more recent studies indicate that phreatic meiofauna survived the ice ages /Enckell 1969, Holsinger 1980, Karaman et al. 1994, Kristjánsson and Svavarsson 2007, Mäkelä et al. 1998, Särkkä and Mäkelä 1998, Strayer 1994/. Although information on groundwater fauna north of the glacial border is only available very locally, groundwater fauna has been found to occur much further north than formerly expected, in particular when both stygobionts and stygophilous species are surveyed.

Glacial deposits are currently intensively studied with the aim to understand and reconstruct both the form and flow of former ice by geological evidence and last but not least ice marginal fluctuations during the last glacial for example, /Boulton et al. 2001, Lokrantz and Sohlenius 2006, Siegert et al. 2001, Svendsen et al. 2004/. Deposits from the warm interglacial periods are very scarcely retained. Therefore, we only know that there were at least two periods with ice-free conditions in northern Scandinavia, but both the actual date and duration of the warm periods are unclear /Lokrantz and Sohlenius 2006/. Groundwater fauna may have "survived" cold glacial periods in those areas. Warm interglacial periods between ice advances might have lasted long enough for a recovery and dispersion of the population. As shown above, deposits from warm periods are rare. The discussion, if groundwater fauna might have recolonised formerly ice covered areas either from refugia in karstic region or in bedrock (where groundwater fauna might have survived below the glaciers) or by other spreading mechanism along paths is highly speculative. Especially as we are in the very beginning to survey und understand the distribution of today's groundwater fauna in formerly ice-covered regions.

Scandinavia

The youngest glacial maximum for 20,000 years BP covered almost the whole of Scandinavia /Boulton et al. 2001, Lokrantz and Sohlenius 2006, Siegert et al. 2001, Svendsen et al. 2004/. In most areas, no surveys on groundwater fauna have been done, because of the above mentioned overestimation of glacial effects and the assumption that phreatic meiofauna could not survive glaciations. However, parastenocarid harpacticoids and cyclopoids have been found mainly in sandy beaches bordering lakes and rivers /Enckell 1969, Mäkelä et al. 1998/

The distribution of five *Parastenocaris*, Kessler (harpacticoids, copepoda) species was surveyed in subterranean localities manly from beaches bordering lakes, rivers, the Baltic and the North Sea /Enckell 1969/. To summarize, Enckell investigated the hyporheic zone laterally linked to the channel and banks of many rivers, streams and brooks as well as to shores in the whole Northern Europe including Iceland and Spitsbergen. In Central Europe, *Parastenocaris* species occur prevailingly in the groundwater, but some species were also found in mosses (*P. brevipes*), the interstitial of sandy brooks (*P. germanica*) and in brackish coastal groundwater (*P. phyllura*) /Griebler and Mösslacher 2003/. Many species are regarded to be relicts from Tertiary fauna, which survived cold glacial periods by a retreat into groundwater, as their relatives in the tropic regions live in surface waters. Two of the five *Parastenocaris* species with a sharp contrast in geographical distribution are presented below.

Parastenocaris glacilis Noodt, was restricted to high geographic altitudes often in sediments of small isolated pools and even found in springs. The species was recorded in all Scandinavian countries including Iceland and Spitsbergen /Enckell 1969/.

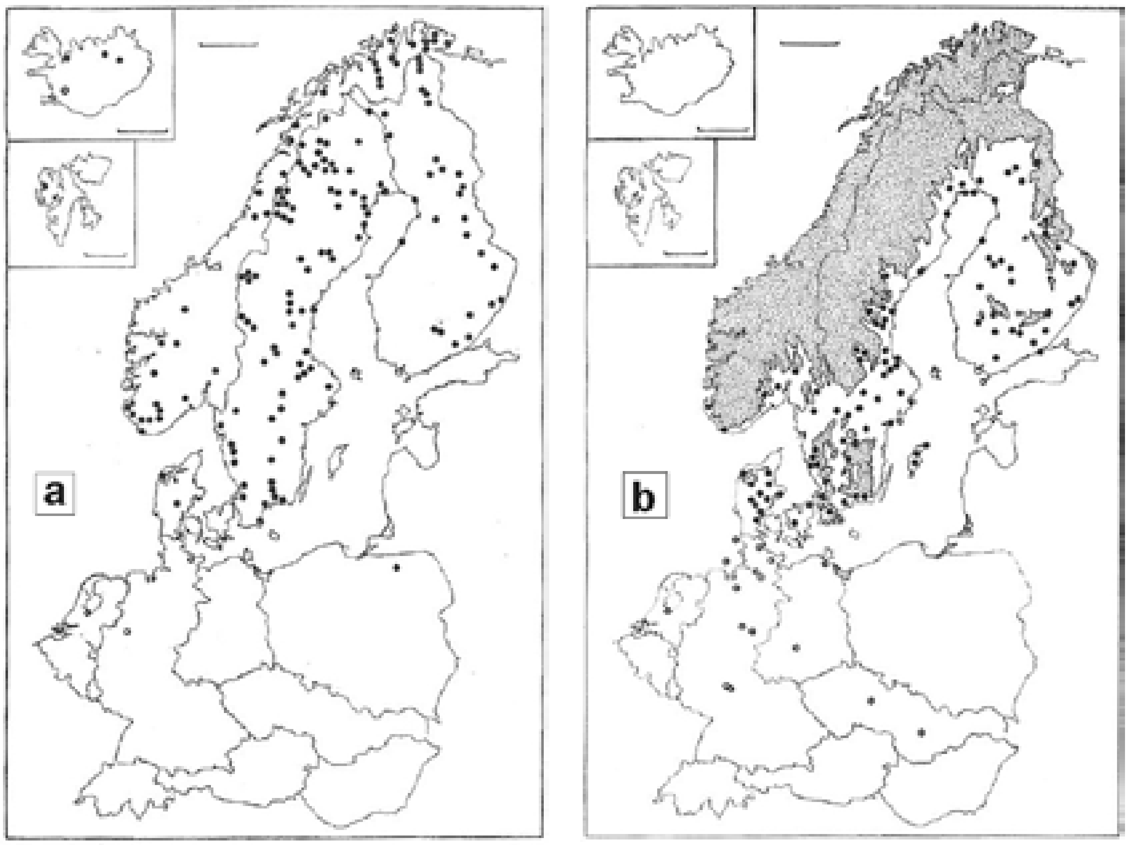

Figure 6-1. *Distribution of a) Parastenocaris glacilis Noodt b) Parastenocaris phyllura Kiefer. Scale = 200 km, from /Enckell 1969/.*

In the Torneträsk area *P. glacilis* was found in sediments in spite of winter temperatures below zero degrees. Proportions of females and males were investigated in sandy beaches during summer season were in balance /Enckell 1969/. A further explanation for spreading patterns of *P. glacilis,* besides up-dwelling from groundwater springs, is ice drifting by storm events. Observations from Sweden's largest lake, Lake Vänern, support this theory. When the ice crusting occurs at high water levels and is succeeded by storm events during early spring in the beginning of the melting period, vegetation and weed along the shores is removed and spread over large distances /Vänerns vattenvårdsförbund ~2004, ~2006/. The same phenomena may have caused the spread of *P. glacilis* when sediments containing the species were linked to bottom ice. This explanation is a hint for possible spreading of other species occurring in sediments and groundwater north of the glacial border.

In contrast to *Parastenocaris glacilis*, *P. phyllura* Kiefer was exclusively recorded in brackish-water groundwater and in the interstitial of beaches beneath the highest shore line /Enckell 1969/.Actual Swedish Species Information (Artdatabanken) tells that there are about 3,500–4,000 species in surface waters e.g. lakes and running waters only in Sweden /Bjerke 2007/. A recolonisation along the hyporheic interstitial of running waters and lakes is not totally out of reach, if stygoxenous and stygophilous species are included along with stygobiotic species. The fact that Baltic fauna was strongly reduced as most species could not adapt to changes in salt content in the postglacial history of the Baltic should not be transferred to other life forms. Diatoms, microalgae, are a consistent part of the food chain. Diatoms adapted fast and with high taxonomic richness to above mentioned changes in salt content in the postglacial history of the Baltic /Thulin et al. 1992, Thulin 1987/.

Survey of nematodes in the hyporheic zone in central/southern Sweden indicate a rich stygophilous fauna /Sohlenius 2007/. Two river basins, both Svartån River and Kilaån River in Södermanland south of Stockholm were surveyed from the springs to the outlets into the Baltic. Nematode fauna sampled was high in abundances and taxonomic richness. Species composition altered downstream along both rivers. These results indicate that nematodes seem to be widespread in the hyporheic zone in central and southern Sweden, see Chapter 2. Additionally, in southern Finland, a typical stygobiont worm, *Troglochaetus beranecki*, has been recorded in eskers /Särkkä and Mäkelä 1998/.

Figure 6-2. *Troglochaetus beranecki (length: 0.7 mm). Photo: A. Fuchs.*

Troglochaetus beranecki Delachaux is an exceptional Archiallnelid, previous an own family, but now classed as a representative for the mainly marine family *Nerillidae* within *Annelida*. *T. beranecki,* average 0, 7 mm long is easy to determine by its ear-like stumps and 7 pairs of parapods /Mösslacher and Hahn 2003/. The modus of movement is creeping or swimming. The stygobiont is omnivorous. In 1920 *T. beranecki* was discovered by Delachaux in Swiss caves and classification was uncertain until /Remane 1952/ found and described relatives from the Mediterranean and the North Sea. *T. beranecki* is a wide spread and common in groundwater both in North America and Europe. *T. beranecki* has been found in groundwater along the riverbeds of Danube, Elbe, Oder, Rhine and Rhone as well as in the gravel of the hyporheic zone of mountain streams to a height of 2,000 m /Steenken 1998/. Hitherto, the north boundary of occurrence was extended to Finnish eskers /Särkkä and Mäkelä 1998/.

Chances to find groundwater fauna in Sweden are best in three habitats according to literature see also Section 7.2.

A. Due to surveys /Klie 1935, Kunz 1938, Noodt 1952, Enckell 1969, Mäkelä et al. 1998, Sohlenius 2007/ the hyporheic zone and adjacent groundwater along shorelines of different freshwater bodies and along the shores of the Baltic can be regarded housing groundwater fauna in particular when stygophilous specis are included. In general this ecotone zone is characterized by a high groundwater fauna biodiversity /Gibert et al. 2005/.

B. Groundwater habitats in karstic aquifers are considered to have facilitated stygobionts to survive in a kind of glacial refugia /Kristjánsson and Svavarsson 2007/. Karstic groundwater aquifers at Öland and Gotland (Lummelundagrottan) in the Western Baltic house are of special interest. The groundwater aquifers on the islands were not affected by postglacial freshwater and marine transgressions because they still contain freshwater. Karstic aquifers and caves are also found in northern Sweden e.g. in Jämtland (Korallgrottan) and in Swedish Lapland (Björkliden).

C. Eskers are important aquifers with groundwater catchments often extending over several surface water catchments in glacial areas. In Finland *T. beranecki* was reported from eskers /Särkkä and Mäkelä 1998/. Both *T. beranecki* as well as other wide spread and common species often regarded as relict forms may have recolonised glaciated areas using eskers as a sort of "interstitial highway".

Characterisation of defined water bodies required by Water Framework Directive /EU-GWD ~2003/ dealt with the problem to define recharge and discharge areas for bedrock aquifers with interconnection in-between them /Thulin 2006/. Interconnection might favourite spreading of groundwater fauna. Yet, for groundwater fauna communities, contacts between bedrock aquifers and surface water is decisive, see Section 5.1. Groundwater fauna requires a minimum of oxygen supply and particular organic matter (POM) which is related to the retention time of the groundwater. Therefore surveys should concentrate on top layers. Survival and occurrence of groundwater fauna in crystalline bedrock is so far not documented for Scandinavia. Findings in Central Europe indicate possible occurrences in the uppermost layers.

Iceland

With *Crangonyx islandicus* the first endemic crustacean and the first stygobiont was described from Iceland /Svavarsson and Kristjánsson 2006/. One year later, a new endemic amphipod family (Crymostygiidae) was reported from Iceland /Kristjánsson and Svavarsson 2007/. Iceland was covered by an ice sheet, but the rebound after deglaciation was much faster than estimated /Ingólfsson et al. 1995/. There was no connection to Greenland or other countries. A survival in subglacial refugia is the best explanation for the above described findings of groundwater species.

Ireland

Until 2006 only two endemic stygobionts amphipods, *Niphargus wexfordensis /*Karaman et al. 1994/ and *Niphargus kochianus irlandicus /*Schellenberg 1932/ were locally known from Ireland. Previously more wide spread and common bathynellid *Antrobathynella stammeri* /Gledhill and Gledhill 1984/ had been found. *A. stammeri* has continental relatives. An Ireland-wide survey of groundwater confirmed the occurrence of *Niphargus kochianus irlandicus* in all parts of the island south of a line Dundalk-Sligo, which comprises around two third of the island's territory. *Niphargus wexfordensis* was found again in the southwest of Ireland, just the part which was not covered by ice during the maximum of Weichselian glaciation. Moreover two wide spread and common stygobiotic species (*Microniphargus leruthi*, amphipoda, and *Fabaeformiscandona breuili*, ostracoda*)* were recorded during the Ireland-wide survey. Around 70% of all groundwater monitoring boreholes investigated were populated by meiofauna, but predominantly by stygophilous, not by stygobiotic species (Hahn 2008, pers. com.).

While it is highly probable that the endemites *Niphargus kochianus irlandicus* and *Niphargus wexfordensis* survived the ice age on the island, the relationship of *Antrobathynella stammeri, Microniphargus leruthi* and *Fabaeformiscandona breuili* to their continental relatives is not evident. Since several hundred thousand years there has not been any connection neither to England, Scotland nor the continent. Because of this, survival of above mentioned species in subglacial refugia or in the not ice covered south-western part has to be assumed.

Northern Germany and Poland

The formerly glaciated parts of Northern Germany and Poland harbour a relatively rich stygobiotic fauna predominantly comprising small species e.g. cyclopoids, parastenocarids, *Troglochaetus beranecki*, mites, but also some amphipods. However, groundwater fauna is impoverished compared to the central mountains further south. On account of salient features of groundwater fauna a number of endemic and relict species are presumed to have survived under the frozen surface or in refugia. Further groundwater species are assumed to have migrated along rivers and streams during recolonisation processes from the south.

In an early survey two different *Parastenocaris* species were recorded in coastal groundwater of the southern Baltic by /Klie 1935, Kunz 1938/. Later, eight stygobiontic species, hitherto only known from southern Germany, were found in the hyporheic interstitial of lakes in Northern Germany as well as sediments along river Elbe /Noodt 1952/. For discussions about a recolonisation of the area the distribution pattern of stygobiotic amphipod family *Niphargidae* is of interest.

Figure 6-3. *Niphargus kochianus irlandicus (length: 5 mm), an endemic amphipod from Ireland. Photo: H J Hahn.*

The distribution pattern was supposed to follow almost exactly the southernmost margin of the Weichselian ice 20,000 years BP /Banarescu 1990/ i.e. no occurrences north of this limit. However,there are several conspicuous exceptions in Northern Germany, Ireland and Belarus (*Crangonyctidae*). In 2006 surveys in Ireland (Hahn 2008, pers. com.) and Iceland /Kristjánsson and Svavarsson 2007/ confirm above mentioned doubts about the ice margin limiting occurrences of groundwater fauna. Ireland and Iceland have not been in connection with the mainland during or after glaciation, so that a recolonisation from the mainland can be excluded. Consequently, groundwater fauna must have survived below the ice. Yet, there is still a lack of knowledge about colonisation and migration issues in regard to groundwater fauna

The Alpine region, effects of glaciations

In the French Jura Mountains, the occurrence of stygobiotic species was found to be correlated with glacial events e.g. Riss-Würm glaciations /Castellarini et al. 2005/. The fauna occurring in areas, which were covered by alp glaciers under Riss and Würm glaciations, is suggested to have been recolonised. Generally there are distribution limits following altitudes /Griebler and Mösslacher 2003/. In all countries of the Alp region, Austria, France, Italy, Slovenia and, Switzerlend etc, stygobionts are well documented. Surveys are concentrated to karstic aquifers and glaciofluvial sediments e.g. the interstitial hyporheic zone along Alpin rivers, se summaries in /Castellarini et al. 2005, Griebler and Möslacher 2003, Malard et al. 1996/. In the prealpine zone of Southern Germany, no differences between formerly glaciated and unglaciated areas could be found, probably the result of the small extension of this zone and a fast postglacial recolonisation /Fuchs et al. 2006/.

Siberia

Siberia is like Scandinavia a white spot with almost no survey of groundwater fauna occurrences. *Stygobromus pusillus* (Crangonyctidae), an amphipod reported from formerly glaciated parts of Siberia was redescribed by /Holsinger 1987/. It’s next relatives are known form North America.

North America

In North America postglacial recolonisation of glaciated areas by fauna was favoured compared to Europe, where the Alps hindered the migration of fauna. Even in North America it was assumed that stygobionts were infrequent north of the glacial border /Strayer et al. 1995/. This hypothesis was confuted by findings stygobiotic amphipod and isopod species in the area; *Stygobromus canadensis*, *Stygobromus secundus* and *Salmasellus steganothrix* respectively are described from the Columbia Icefield in the Canandian Rocky Mountains /Bousfield and. Holsinger 1981, 1980, Bowman 1975, Holsinger et al. 1983/. These species are all regarded as endemic and therefore should have survived below the ice, in particular since the habitats are isolated from each other.

6.3 Hydrography

In limnology, hydrography (in this context affiliation of a site to a certain catchment), is considered to be one of the main parameters influencing the geographical distribution of aquatic fauna. For stygobionts, distribution pattern was for the first-time investigated in south western German (Baden-Württemberg) covering an area of 37,750 km^2 /Hahn and Fuchs 2005, Fuchs et al. 2006/. Baden-Württemberg belongs to two major river basins, the Rhine system and the Danube River. In the Pliocene period, the catchment of the Rhine River was much smaller than today with its origin situated around 150 km north of its present source In the Quaternary, the River Rhine took over large parts of the former upper Danube system together with most of its stygobionts.

The distribution patterns shown in Figure 6-4 clearly indicate that not the recent river systems, but the Pliocene river systems influence geographical distribution of groundwater fauna in Central Europe /Hahn and Fuchs 2005, Fuchs et al. 2006/. Furthermore, most of the species with a restricted distribution are somewhat rare and must be considered as relict forms of the old Pliocene river systems. Both rareness and the relict character are indicative of their potential endangerment.

The central mountains of Germany were not covered by glaciers, except the southern part of the study area. As the climate under Quaternary glaciation periods was very cold and dry with permafrost conditions, that many endemites even in the groundwater habitat may have been extincted.

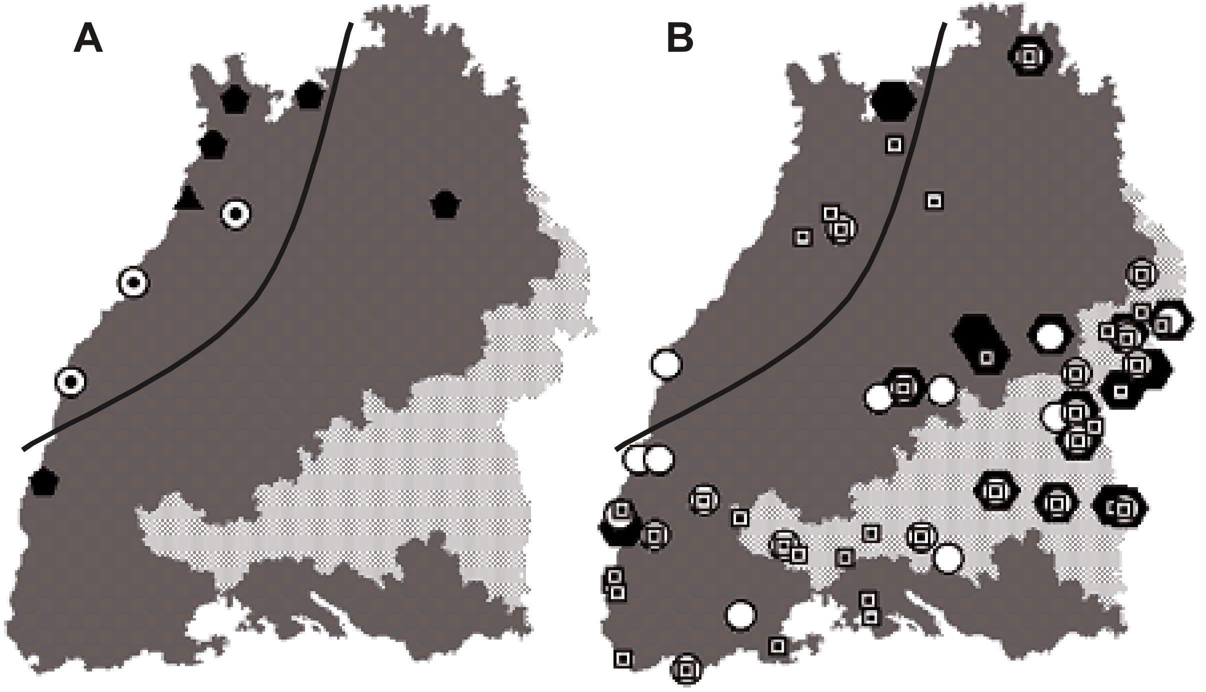

Figure 6-4. *Distribution of selected species in the state of Baden Württemberg, South western Germany. A: ⊙ ⬟ ▲ Species of the Pliocene Rhine system. B ○ ⬢ ⊡ Species of the Pliocene Danube system: Black lines indicate the Pliocene watershed between the Danube and the Rhine. Grey design indicates the present Rhine catchment, dotted design the present Danube catchment. From /Hahn and Fuchs 2005/.*

Figure 6-5. *Mixtaconda laisi (length: 0.6 mm). Photo: H J Hahn.*

6.4 Landscape scale – type of aquifer

On a landscape scale, geology is considered as one of the key factors influencing groundwater communities. The significance of karstic groundwater for a high groundwater fauna biodiversity is often mentioned /Gibert and Deharveng 2002, Griebler and Mösslacher 2003, Hahn 2004b, Steenken 1998/. This approach is supported by /Ronneberger 1975/. Typology for groundwater habitats is focused on the geohydrological type of aquifers /Gibert et al. 1997, Gibert 2001/. Karstic aquifers outside Central Europe are well known for their high biodiversity /Gibert and Deharveng 2002/. Also unconsolidated aquifers are well known for their high biodiversity. In this later case a comparative assessment is extremely difficult, since most data were obtained from hyporheic waters, instead of from groundwater /Hahn and Schmidt 2006/. At the same time, data from fractured, non-karstic, aquifers are very sparse. In south-western Germany, a large scale survey of 304 groundwater boreholes within 13 near-surface geological units were investigated. In monitoring boreholes in granites and gneisses of the Black Forest /Fuchs et al. 2006/ stygobionts were recorded at several levels to a depth of 55 metres.

Faunal data were analysed and grouped by using Bray-Curtis-Index for similarity and Multidimensional Scaling (MDS). Four groups of different hydraulic types of aquifers were identified /Fuchs et al. 2006/. Groups due to significant groundwater fauna content were unconsolidated aquifers, fractured aquifers and karstic aquifers. The separation of these groups in the MDS was highly significant. Aquitards formed a separate group.

1. Fractured aquifers colonized by groundwater fauna refer both to crystalline and sandstone bedrock. The sandstone bedrocks were described as relatively homogenous /Fuchs et al. 2006/.
2. Unconsolidated or porous aquifers in very different geological units, most Tertiary and Quaternary sediments. The groundwater fauna sampled in these aquifers or/and the hyporheic zone can be characterised by high in abundances and taxonomic richness.
3. Karstic aquifers display a groundwater fauna comparable to unconsolidated or porous aquifers.
4. Aquitards in the area consisted of sediments with almost no pore spaces. Aquitards displayed a strongly impoverished groundwater fauna compared to the other aquifers types.

A conclusion from the survey in south-western Germany is that no significant differences in abundance and taxonomic richness were found between unconsolidated (porous), fractured (bedrock) and karstic aquifers /Fuchs et al. 2006/. A second conclusion was that groundwater fauna distribution patterns depend on a very local scale with changes of sizes of pores, fissures and their interjections constituting the living space for groundwater fauna.

7 Applications of groundwater surveys

Groundwater fauna has been used as an ecologically based assessment tool for the monitoring of extraction wells, when surveys are extended from only stygobiotic species to both stygophilous and stygoxenous species, see Section 2.1. Since groundwater fauna react fast to changes in hydrology, they may serve as early warning systems. As several sampling techniques are available, as well as information on their performance, groundwater fauna can be successfully used for ecologically based assessments – in spite of a lack of standards /Daniepol et al. 2004, 2007/.

Fields of applications for an ecologically based assessment of groundwater are:

- Assessment of extraction wells and quality management in drinking water abstraction.
- Management of wetlands.
- Environmental impact assessment of underground construction works or mines.
- Regulation under nature protection law, on a local scale e.g. sites with endemic species, see Section 4.3.1.
- General water management due to the Water Framework Directive and the Daughter Directive on groundwater.

Additionally, groundwater organisms may well function as bioindicators as they integrate short-term, mid-term and long-term changes in environmental conditions within an ecosystem, see Section 2.1.

7.1 Assessment of contacts between surface water and groundwater

A starting point for ecological assessment is to consider groundwater community structure and changes in composition of these communities. Including stygophilous species and stygoxenous species and assuming that the composition of the fauna reflects the influence of surface waters on the aquifer or the hyporheic zone allows for new applications. An increased inflow of surface water has been proved to cause higher abundances, taxonomic richness and proportions of non-stygobiotic organisms /Hahn 2006/. Both phreatic net and unbaited traps were used for sampling. It is important to have a large number of monitoring boreholes available when groundwater fauna is used as a marker for the degree of hydraulic exchange.

7.1.1 Ecologically based assessment using groundwater fauna near the Lower Rhine River, Germany

In a German survey the impact of surface water from the Lower Rhine River and the inflow of an alluvial groundwater were investigated in more detail /Hahn et al. 2007/. At the alluvial Rhine terrace at Flehe Waterworks in Düsseldorf a total of 15 boreholes were sampled for fauna and water.

Six of the bores were spread over the area at depths ranging from 15 m to 21.80 m. Additionally, three rows of bores A, B, C were positioned in a transect near the shore. Each row consisted of three bores 14 m, 17 m and 21 m below the floodplain surface respectively. Placements of boreholes permitted examination of interjection between landside groundwater and surface water of the Rhine River in three dimensions as samples were available from different depths within the hyporheic zone. Prior to analyses, abiotic data were subjected to log transformation and faunal data were subjected to square root transformation. Principal component analysis (PCA) was performed for abiotic factors and fauna factors, respectively. Basic data were derived from Bray-Curtis similarity and then compared using multidimensional scaling (MDS). The MDS technique represents similarities in terms of distances. To test the groups identified, an ANOSIM (analysis of similarity) procedure was applied.

According to physico-chemical data from water analysis two main groups could be distinguished. Data assigned to the first group were characterized by high EC values and increased boron concentrations. The first group represents landside groundwater. The second group represented a mixture of surface water from the Rhine River and alluvial groundwater with high values for dissolved oxygen (DO), nitrate and SAC254 and increased boron concentrations at the same time. High values for dissolved oxygen (DO), nitrate and SAC254 (Spectral absorption coefficient at 254 nm) are distinctive for the influence of Rhine water /Hahn et al. 2007/.

According to the spatial distribution of the fauna, the MDS displays three ecological groups of boreholes. (Rhine water was not included in the analyses, because it was not sampled for fauna.) The three groups were: **(1)** deep, landside groundwater, **(2)** deep alluvial groundwater, which is temporarily in contact with surface water and **(3)** alluvial groundwater interacting with surface water. This grouping is confirmed by an analysis of similarities (ANOSIM) ($r = 0.750$, $p = 0.001$).

For the three ecological groups described, the GW-Fauna-Index and Indicator Values for groundwater fauna were highest near the river and lowest at the most distant sites, reflecting the influence of the Rhine. Even three dimensional differences in surface water influence at different depths could be displayed. The correlation between the GW-Fauna-Index or Indicator Values for groundwater fauna and the distance from the river was strong and highly significant (Spearman correlation $r = -0.819$, $p < 0.001$, $n = 144$). Taxonomic richness and Indicator Values correlated well both for all data and for the aggregated data of the MDS /Hahn et al. 2007/. (Spearman correlation $r = -0.505$, $p < 0.001$, $n = 144$ and $r = -0.682$, $p < 0.005$, $n = 15$, respectively).

Groundwater fauna clearly marked the degree of interference between Rhine water, water of the hyporheic zone and groundwater. Resolution was good enough to state different degrees of mixture between the inflowing alluvial groundwater and Rhine water at certain points e.g. at a higher resolution than hydrochemical data. Change in groundwater fauna composition reflected interjection between landside groundwater and surface water in three dimensions as even different levels of interference in depth could be discriminated.

7.1.2 Ecologically based assessment of flooding events in South Korea

In South Korea ecological assessment by groundwater fauna has also been successful in displaying changes in interfering water masses at certain points /Bork et al. in press/. The flood plain of the Nakdong River is formed by rice terraces. These are subjected to annual flooding events. Even here placement of boreholes permitted examination of interjection between landside groundwater and surface water of the Nakdong River in three dimensions. Of special interest was the flooding of the plain and time series. Samples were available from different depths within the hyporheic zone.

Generally the same methods were applied as for the Rhine samples. However Nakdong faunal data were transformed into ordinal numbers to reduce effects of the two sampling methods applied (traps and kick sampling). In South Korea sampling was conducted using unbaited traps. According to physico-chemical data from water analysis three main groups of groundwater could be distinguished. The PCA discriminates landside groundwater from the adjacent fractured rock aquifers in the south as one group. The group is characterised by high $CaCO_3$ concentrations and a high specific conductance (EC). A second of group Archaean bedrock groundwater is characterised by low $CaCO_3$ concentrations The area is dominated by the Cretaceous Kyõngsang Supergroup with sedimentary and volcanic sequences as well as tertiary intrusions of granites /Reedman and Kim 1992/. According to outline maps /Bork et al. in press, Reedman and Kim 1992/ and these granites are most likely situated at some distance from the site. The third group is constituted by the alluvial groundwater influenced by surface water with high nitrate concentrations according to agriculture. Nakdong River water was distinguished from these waters by high oxygen (DO) concentrations, especially during flooding events. This grouping is nicely confirmed by an ANOSIM ($r = 0.942$, $p = 0.001$) (Hahn 2008, pers. com.). Based on the faunal data, four ecological groups of sites were distinguished: **(1)** enthal Nakdong River water, **(2)** shallow groundwater both from rocky and alluvial aquifers strongly influenced by alluvial surface water, **(3)** deep groundwater from rocky and alluvial aquifers and **(4)** deep groundwater poorly or not influenced by surface water. The separation of the groups is good, although the ANOSIM ($r = 0.669$, $p = 0.001$) indicates some overlapping.

From all abiotic parameters recorded, the Indicator Values for groundwater fauna (GW-Fauna-Index) were most highly correlated with taxonomic richness (Spearman correlation: $r = 0.541$, $p < 0.001$, $n = 260$) and abundance (Spearman correlation: $r = 0.587$, $p < 0.001$, $n = 260$). The values are highest for the benthic and hyporheic sites and lower for the samples from boreholes near or within the deep groundwater. There were significant differences (U-Test: $p < 0.001$, $n = 260$) between the four ecological groups described (Hahn 2008, pers. com.). The patterns found in Korea were quite similar to the situation described for the German site. Groundwater fauna reflected the impact of surface waters by shifting from stygobionts, to stygophilous and stygoxenous species.

In addition to changes in place, groundwater fauna composition also changed over time periods due to flooding events. During flooding the influence of deep groundwater from rocky and alluvial aquifers was temporarily exceeded by alluvial surface water. As a consequence, groundwater fauna composition shifted from real groundwater fauna species (stygobionts) to stygophilous and stygoxenous species. Changes in groundwater fauna composition reflected the degree of interaction between the different groundwater bodies and river water both in space and in time (Hahn 2008, pers. com.).

7.1.3 Evaluation of ecologically based assessment of groundwater

The patchy distribution patterns on a local scale indicate that groundwater fauna reflect the environmental conditions on the particular site. Groundwater fauna communities do not mainly display the origin of the water (like the hydrochemical data), but the intensity of surface water intrusion at certain points. Indicator Values for groundwater fauna still have weaknesses. In the longer term it is hoped that this approach will result in a tool for the assessment of intrinsic groundwater vulnerability and an early warning system for changes in hydrology. The results were independent of the method and the area and displayed ecologically relevant impact of surface water on groundwater assemblages. The ecologically based assessment of groundwater can only be performed on a local scale. Indication of surface water/groundwater interactions is proved to give reliable results when the composition of groundwater fauna is considered /Hahn 2006/

Increased contact between surface water and groundwater causes a change in intensity of surface water intrusion. The new results presented above give evidence that there is a change in groundwater fauna composition as groundwater fauna in turn reflects the impact of surface waters by shifting from real groundwater fauna (stygobionts) to stygophilous and stygoxenous species. The occurrence of stygophilous and stygoxenous species is correlated with a better availability of organic matter and oxygen. These changes in community composition occurred, in accordance with changes in hydrology, within days. At both sites, spatial and temporal distribution of fauna reflected the hydrological conditions, in particular the influence of surface water on groundwater, and fauna reacted fast to changes in hydrology. In contrast, the physico-chemical data predominantly displayed the origin of the water in a greater area (in terms of landside or alluvial) /Hahn et al. 2007, Bork et al. in press/.

7.2 Perspectives and applications for groundwater fauna surveys in Sweden

At the moment there is not much information about groundwater fauna in the regions north of the Quaternary glacial border in the northern hemisphere. However there is evidence for a high probability of the existence of groundwater fauna in Sweden, see Section 6.2 and below. Even surveys in the Alpine regions indicate a high potential especially for groundwater bodies in unconsolidated porous aquifers such as eskers, karstic aquifers and in hyporheic zones along surface waters. This is also true for beaches along the shores of the Baltic /Enckell 1969/. The likelihood of finding endemic species is very low, except in Öland, Gotland and some places in the Swedish mountains and Lapland, see Section 4.3.1. Environmental impact assessments are possible when the whole range of species found in the groundwater is evaluated, not only stygobionts. The focus should be on wide spread and common species, and not on rare and endemic species.

Ecologically based assessment

If stygoxenous, stygophilous or stygobiotic species are found, a site-specific assessment of the quality of contacts between groundwater and surface water can be performed. Such an assessment, as demonstrated in Sections 7.1.1 and 7.1.2, can be performed for small catchments as well. The local scale determines the flow dynamics and the exchange with surface water, as well as the space available for organisms. As mentioned above, groundwater fauna communities do not mainly display the origin of the water (like the hydrochemical data), but the intensity of surface water intrusion at certain points. Environmental impact assessments could benefit from the fact that groundwater fauna composition reflects the degree of contact between surface water and groundwater in the original state. Secondly an impact altering the contact would be reflected by a change in groundwater fauna composition. A return to previous groundwater fauna composition after termination of the environmental impact would prove that the impact did not cause changes within the ecosystem.

The hyporheic zone and adjacent groundwater bodies

Glaciofluvial sediments in the Alpine region and in Canada have proved to be favourable habitats for finding groundwater fauna, as have sediments along rivers and lakes. In Sweden, there are primary surveys of the distribution pattern of five *Parastenocaris* species and of *Nematode* species /Enckell 1969, Sohlenius 2007/. Also taxonomic richness of surface water fauna indicates good conditions for fauna findings especially in ecotone zones in-between the hyporheic zone and groundwater. In the hyporheic zone beneath and along rivers, streams, brooks and lakes and near beaches, surface water can be transmitted more easily. In most areas of Sweden, we find a lot of small unconsolidated aquifers. They are so numerous that initial status characterisation according to Water Framework Directive /WFD 2000/ concentrated on larger groundwater bodies exceeding a minimum quantity /Lång et al. 2005/.

Yet, even in well surveyed areas, data from unconsolidated aquifers often refer to the hyporheic zone and not to adjacent groundwater bodies /Hahn and Schmidt 2006/. The most promising area for finding groundwater fauna is in the ecotone zone at the top of the groundwater beneath the hyporheic zone, if the contact between the water masses is optimal, supplying just enough nutrients and oxygen. Figure 7-1 shows a small theoretical catchment in Sweden to elucidate possible relationships between the Hyporheic zone and an adjacent groundwater body on a small local scale /Thulin 2005/. A starting point is to investigate the hyporheic interstitial along the brook as a habitat for groundwater related fauna. For a) and b) the existence of a typical groundwater – surface water ecotone is not evident. Permeability and a high groundwater level[3]

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[3] In most parts of Sweden the groundwater table is high; exceptions may be a lowering of the groundwater level by groundwater extraction or underground construction and groundwater in eskers with a comparatively low water table.

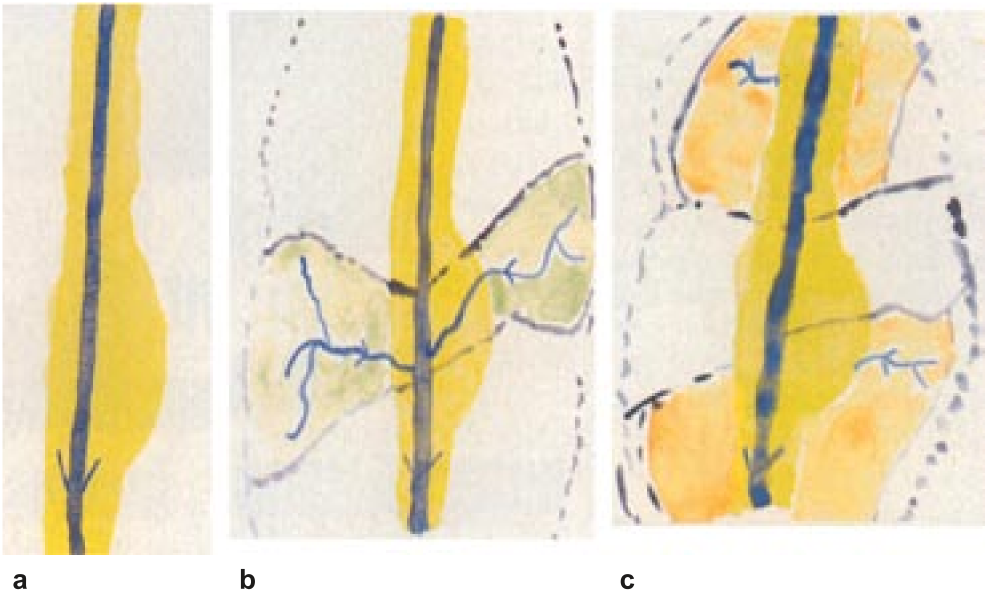

***Figure 7-1.** a) Hyporheic zone along and beneath a brook. The yellow area represents a groundwater body where surface water/groundwater interactions can take place, e.g. an ecotone zone; b) Surface water catchment, represented by greenish areas, where surface water/groundwater interactions can take place c) Groundwater catchment represented by brownish areas, ecotone zone where surface water/groundwater interactions take place; from /Thulin 2005/.*

depend on site specific characteristics of the sediments and rocks. If there is a layer with low hydraulic conductivity like clay between the alluvium and the groundwater body, there may be no transmission of surface water at all. But, if transmission of surface water takes place, biodiversity in the ecotone at the top of the aquifer should be high /Griebler and Mösslacher 2003/. For c) the sudden disappearance of brooks clearly indicates a groundwater catchment of surface waters, i.e. an ecotone zone where surface water directly recharges groundwater and biodiversity of groundwater fauna can be expected to be high. However, this does not imply anything about scenarios a) and b). Ecological assessments are based on the relationship that the stronger the influence of surface waters on the aquifer or the hyporheic interstitial, the higher are abundances, taxonomic richness and proportions of non-stygobiotic fauna.

Karstic aquifers

The probability of finding stygobionts which survived the ice ages in glacial refugia, is claimed to be much higher in karstic aquifers and in eskers compared to other aquifers /Kristjánsson and Svavarsson 2007/. In Sweden we find karstic freshwater aquifers restricted to the islands Öland and Gotland, parts of the mountains (Jämtland) and parts of Lapland in northern Sweden. Surveys of groundwater fauna can be used to prove surface water inflow and vulnerability of the karstic aquifers.

Eskers

Glaciofluvial sediments are highly permeable. Eskers in Sweden are important aquifers especially if they are located in low terrain and above the highest coast limit, and thus have not been subjected to marine transgressions after ice retreat. Groundwater catchments of eskers often extend over several surface water catchments in glacial areas. According to the Finnish reports of *Troglochaetus beranecki* it is most likely that groundwater fauna is to be found in Swedish eskers /Särkkä and Mäkelä 1998/. For eskers too, groundwater fauna can be used for an assessment of intrinsic groundwater vulnerability. Furthermore, it can be used as an early warning system for changes in hydrology, even those caused by changes in water levels during flooding events.

Construction works

Groundwater fauna communities respond fast to even small increases of inflow from surface water, because dissolved oxygen (DO) is enhanced and/or organic matter (OM) becomes more available. Under the influence of surface water, groundwater fauna composition will shift to more stygophilous and/or stygoxenous species and the range of different species to be found will rise. Total numbers of species will also increase. If ecologically based assessment reveals a groundwater community with little contact to surface water and a dominance of stygobionts, it is of interest to investigate the contact conditions after termination of the building. Is there the same degree of (little) contact between groundwater and surface water?

Underground construction works e.g. i.e. road and railway tunnels, culverts for waterpower and fresh- and wastewater, gas-, gasoline and electricity lines, or rock shelters, may change the water flow directions. /Laaksoharju et al. 1999, Kitterød et al. 2000, Mossmark et al. in press/ state a change in vertical water flow with consequences for water contacts between surface water and groundwater and even between different groundwater bodies. Relict groundwater may contribute to high chloride ion concentrations, if the construction generates a flow between adjacent groundwater bodies /Mossmark et al. in press/. If work lasts over longer time periods changed contacts between surface water and groundwater influence the chemical status of the groundwater according to experiments in west Sweden /Mossmark et al. 2007/. Surface water with lower pH and high sulphate concentrations may get into the area and impact the durability and maintenance of the underground constructions /Mossmark et al. in press/. Geological prerequisites for sulphate release are surrounding muscovite gneisses or sediments, or alternatively bedrock with a related composition.

Ecologically based assessment could also indicate a high degree of contact between surface water and ground water before work starts. Such a result would serve as an early warning system. The probability of changes in hydrochemical properties of the groundwater is thought to increase during the construction and operational phases because of high vertical and/or horizontal water flow /Laaksoharju et al. 1999, Kitterød et al. 2000, Mossmark et al. in press/. Water with different pH and/or high sulphate or chloride concentrations may impact the durability and maintenance of the underground construction /Mossmark et al. in press/.

8 Conclusions

Groundwater is an extremely heterogeneous biotope and at the same time characterized by scarcity. Different spatial scales, especially the site specific local scale, have to be considered for an ecologically based assessment of groundwater. On continental and landscape scales the general frame for faunal communities is defined (in terms of regional types of communities). On the local site scale, lithologic and structural characteristics of different geological facies inside the aquifer control the hydrological exchange. Surface water/groundwater interactions control the availability of organic matter and oxygen in the sense of scarcity. Because of these central features of the groundwater fauna assemblages (abundance, taxonomic richness and community structure) are controlled by the site-specific conditions on a local scale. With increasing surface water impact, abundances and taxonomic richness in the groundwater increase, and community structure shifts from stygobiotic to stygophilous or even stygoxenous. Using Indicator Values, with a formula for dissolved oxygen, amount of detritus and standard deviation of temperature, groundwater fauna occurrences and the patchy distribution might be better understood. In German and South Korean sites groundwater fauna correlated well with the Indicator Values for groundwater fauna (GW-Fauna-Index). Although Indicator Values have several weaknesses, this approach could serve as a tool for the assessment of intrinsic groundwater vulnerability providing an early warning system for changes in hydrology. It could then be applied for the monitoring of extraction wells, for the management of mining areas and wetlands and for the ecologically based evaluation of underground construction works like tunnels, culverts and mines. Advances in applications and adjustments of environmental laws are in rapid progress.

Although there are as yet no standards for the ecologically based assessment of groundwater habitats, reliable statements are possible. The strength of the surface water impact, the vulnerability of the aquifer and the hydrogeological conditions are reflected by the groundwater fauna community. Since groundwater fauna react fast (within days and weeks) to changes in hydrology, it is a good marker of mixing between surface water and groundwater. Groundwater communities may serve as early warning systems for those changes. Such applications are possible although there are still some problems related to sampling: while several sampling techniques are available, as well as information on their performance, the main problem is access to the groundwater.

All results are promising for future assessment of contacts between surface water and groundwater by using groundwater fauna in Sweden. Due to the glaciation from the last ice ages, groundwater fauna was often thought to be poor in Scandinavia. All available data indicate that the probability of finding stygobionts is high in formerly glaciated areas, which is true for Sweden too. In general, groundwater fauna is well known from unconsolidated porous aquifers, the hyporheic zone and karstic aquifers. In Finland stygobionts have been surveyed in eskers /Särkkä and Mäkelä 1998/. In addition, findings of *Harpatocoida (Parastenocaris* sp.) and *Nematoda* in the hyporheic zone along rivers, streams, brooks and lakes, and at Baltic shores in brackish groundwater, clearly imply a widespread groundwater fauna for Sweden /Enckell 1969, Mäkelä et al. 1998, Sohlenius 2007/. There is a general lack of surveys in crystalline bedrock. Even in crystalline bedrock perspectives and applications for groundwater fauna surveys in Sweden are good in the upper layers of groundwater bodies, if the minimum oxygen requirement of 1 mg/l is fulfilled. This requirement will be difficult to meet in deep crystalline bedrock aquifers with long residence time for water.

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ISSN 1404-0344
CM Gruppen AB, Bromma, 2008