British
Geological Survey
NATURAL ENVIRONMENT RESEARCH COUNCIL

Groundwater Ecology Literature Review

Groundwater Science Programme

Open Report OR/09/061

BRITISH GEOLOGICAL SURVEY

GROUNDWATER RESOURCES PROGRAMME

OPEN REPORT OR/09/061

Groundwater Ecology Literature Review

L Maurice

Keywords

Groundwater ecology, fauna, biodiversity, ecosystem services

Front cover

Niphargus Aquilex from a River Till (photo by Chris Procter)

Bibliographical reference

MAURICE. L. 2009. Groundwater Ecology Literature Review. *British Geological Survey Open Report*, OR/09/061. 37pp.

Keyworth, Nottingham British Geological Survey 2009

BRITISH GEOLOGICAL SURVEY

The full range of Survey publications is available from the BGS Sales Desks at Nottingham, Edinburgh and London; see contact details below or shop online at www.geologyshop.com

The London Information Office also maintains a reference collection of BGS publications including maps for consultation.

The Survey publishes an annual catalogue of its maps and other publications; this catalogue is available from any of the BGS Sales Desks.

The British Geological Survey carries out the geological survey of Great Britain and Northern Ireland (the latter as an agency service for the government of Northern Ireland), and of the surrounding continental shelf, as well as its basic research projects. It also undertakes programmes of British technical aid in geology in developing countries as arranged by the Department for International Development and other agencies.

The British Geological Survey is a component body of the Natural Environment Research Council.

British Geological Survey offices

BGS Central Enquiries Desk
☎ 0115 936 3143 Fax 0115 936 3276
email enquires@bgs.ac.uk

Kingsley Dunham Centre, Keyworth, Nottingham NG12 5GG
☎ 0115 936 3241 Fax 0115 936 3488
email sales@bgs.ac.uk

Murchison House, West Mains Road, Edinburgh EH9 3LA
☎ 0131 667 1000 Fax 0131 668 2683
email scotsales@bgs.ac.uk

London Information Office, Natural History Museum, Cromwell Road, London SW7 5BD
SW7 2DE
☎ 020 7589 4090 Fax 020 7584 8270
☎ 020 7942 5344/45 email bgslondon@bgs.ac.uk

Columbus House, Greenmeadow Springs, Tongwynlais, Cardiff CF15 7NE
☎ 029 2052 1962 Fax 029 2052 1963

Forde House, Park Five Business Centre, Harrier Way, Sowton EX2 7HU
☎ 01392 445271 Fax 01392 445371

Maclean Building, Crowmarsh Gifford, Wallingford OX10 8BB
☎ 01491 838800 Fax 01491 692345

Geological Survey of Northern Ireland, Colby House, Stranmillis Court, Belfast BT9 5BF
☎ 028 9038 8462 Fax 028 9038 8461
www.bgs.ac.uk/gsni/

Parent Body

Natural Environment Research Council, Polaris House, North Star Avenue, Swindon SN2 1EU
☎ 01793 411500 Fax 01793 411501
www.nerc.ac.uk

Website www.bgs.ac.uk
Shop online at www.geologyshop.com

Foreword

This report is the published product of a review by the British Geological Survey (BGS) on Groundwater Ecology. The report introduces the concept of groundwater ecosystems, discusses why groundwater ecosystems are important, and reviews the literature on sampling techniques and the current understanding of the factors determining the distribution of groundwater fauna.

Acknowledgements

The author would like to thank Anne Robertson of Roehampton University and John Bloomfield at BGS for useful comments on draft versions of this report.

Contents

FIGURES

Summary

Groundwater ecology is the study of ecosystems that occur in the subsurface within groundwater. Groundwater often contains a diverse range of organisms, and those that live in groundwater and generally do not live above the ground surface are called Stygobites. Stygobites species come from several different taxonomic groups of animals. Many animals found in groundwater are Crustaceans (Copepoda, Ostracoda, Amphipoda, Isopoda, Syncarida, Cladocera) but species of Oligocheata and Hirundinea from the phyla Anelida (worms), Mollusca (snails and slugs) and Nematoda (roundworms) also live in groundwater. Groundwater Ecology is important because stygobites provide a unique contribution to global biodiversity. Stygobites have unusual adaptations to their subsurface environment and there is a high degree of endemism therefore they provide insight into fundamental questions of evolution, ecology and biodiversity. Stygobite studies can also be used to investigate past changes in geomorphology and climate, and the distribution of stygobites can inform aquifer characterisation. Groundwater ecosystems also provide important "ecosystem services" due to their role in biogeochemical cycling, and they can enhance contaminant attenuation. Groundwater fauna can also be useful indicators of the environmental health of aquifers. There are however many threats to groundwater ecosystems including habitat removal by opencast quarrying, groundwater abstraction which removes organisms and causes physical and chemical changes to the habitat, and aquifer contamination.

Most records of stygobites in the UK are from caves in the Carboniferous Limestone and boreholes in the Chalk. Ten species of stygobite are known in the UK and Ireland but groundwater organisms have not been well studied, and there may be undiscovered species. The groundwater fauna of many areas of the UK, and many rock types has not yet been investigated. More comprehensive studies have been carried out in other countries and this has lead to insight into the best methods of sampling stygobites, and some understanding of their diversity and distribution in groundwater. Sampling should be designed to take into account variability in geology and hydrogeology as well as regional variations. The best sampling methods in boreholes are net hauling and pumping, and repeated sampling generally increases the number of species found.

Determining the factors controlling the distribution of species in groundwater is difficult because there are many factors which interact in a complex way. Sophisticated computational data analysis techniques are needed to unravel these complexities. Some of the most important regional scale factors thought to determine stygobite distributions are glacial history and geology. The composition of groundwater ecosystems also depends upon the type of aquifer (karstic, porous, fracture, compact), and in karst aquifers whether the ecosystem is in the vadose or phreatic zone. It is believed that local effects, (e.g. aquifer heterogeneity, water chemistry, and the location of the water table), also affects species diversity and abundance in groundwaters.

Future work might include a survey of the groundwater fauna in the UK to assess groundwater biodiversity and investigate the distribution of stygobites in different geologies. Future hydrogeological research areas might include studies of the physical hydrogeological controls on stygobite diversity such as targeted sampling of inflows into boreholes to determine where in the aquifer stygobites live, and the frequency that stygobites enter boreholes. Hydrochemistry research might focus on assessing the "ecosystem services" provided by groundwater ecosystems such as their role in biogeochemical cycling, contaminant attenuation processes, and sustaining surface groundwater dependent ecosystems. Multi-disciplinary studies involving collaboration between hydrogeologists and biologists and ecologists would be particularly beneficial.

1 Introduction

Groundwater Ecology specifically concerns ecosystems that occur within groundwater systems. Obligate groundwater animals (those that live in groundwater and generally do not exist above the ground surface) are known as **Stygobites.** They generally have no eyes, elongated shapes and often have long appendages. They lack pigmentation and are colourless and often translucent (e.g. Figure 1.1). They are slow growing, long lived, and have few young (Gibert, 1994; Humphreys, 2009). Vertebrate stygobites only occur in karst but invertebrate stygobites are widespread in most subsurface environments. They require a physical habitat (which may range in scale from pore spaces between grains in porous aquifers to karstic cave streams), food (bacterial and fungal biofilms which in turn require a source of organic matter in the subsurface), and oxygen or another electron receptor such as nitrate or sulphur (Humphreys, 2009).

Photo by Chris Proctor

Figure 1.1 The amphipod *Niphargus Aquilex*

(Niphargus Aquilex are commonly found in UK groundwaters and are generally 4 to 15 mm long)

Stygobites species come from several different taxonomic groups of animals (Figure 1.2). Most stygobite species are Crustaceans and therefore have a hard external carapace (shell) protecting their body. Stygobite crustaceans are from the classes of Branchiopoda, Ostracoda, Copepoda and Malacostraca (Figure 1.2). Within the Branchiopoda class there are species of Cladocera (water fleas). Within the Malacostraca class there are stygobite species from the Amphipoda, Isopoda and Syncarida orders. Hydracharina (water mites) are also found in groundwater and are also from Phylum Arthropoda but from the Sub-phylum Chelicerata. There are also Oligocheata and Hirundinea stygobite species from the Phylum Anelida (worms), and species of Mollusca (marine and terrestrial snails and slugs) and Nematoda (roundworms).

Other organisms which generally live above the ground surface but can exploit resources in groundwater and seek refuge from unfavourable conditions on the surface in groundwater are called **Stygophiles**, while **Stygoxenes** are organisms that occur accidentally in groundwater environments (Gibert, 1994).

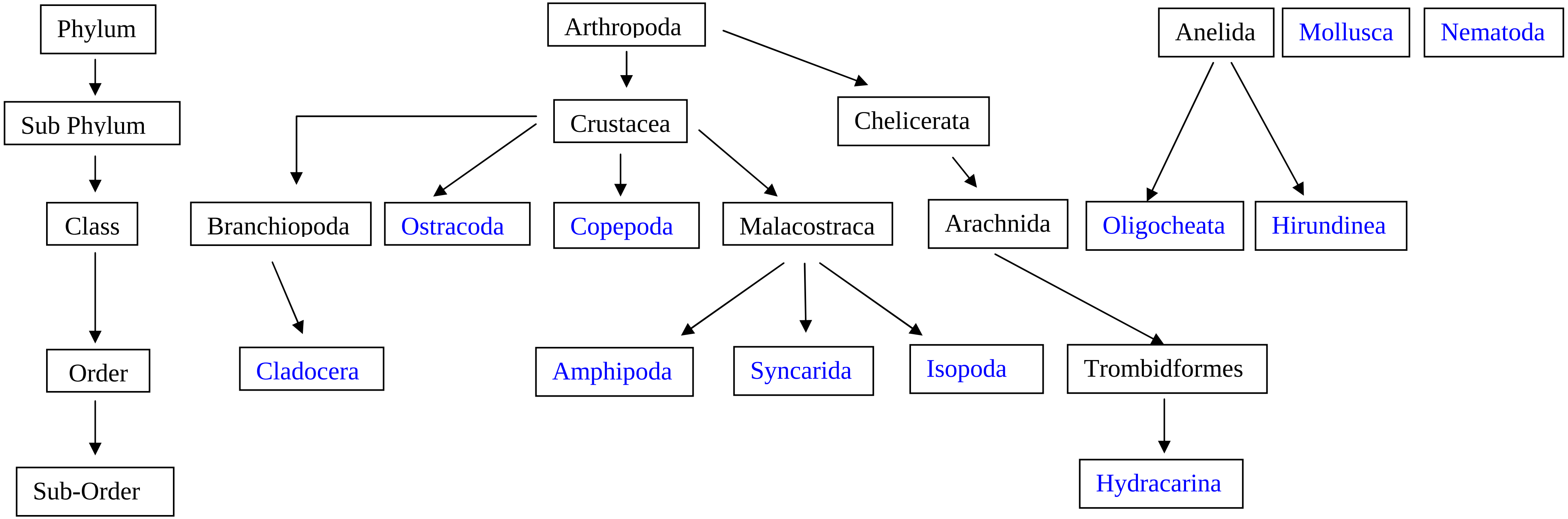

Figure 1.2 The taxonomy of different groups of animals in which stygobite species have evolved

The objective of this report is to provide a context for future studies of groundwater ecology by BGS. The report considers why groundwater ecology is important and reviews previous groundwater ecology studies in the UK and worldwide. This literature is used to discuss sampling techniques and the current understanding of the controls on the distribution of stygobites. Finally, areas of groundwater ecology needing further work and potential research questions are discussed.

Microbial groundwater ecology is not considered in this report, but Griebler and Lueders (2009) provide a comprehensive review of microbial diversity in groundwater including the effects of different types of micro-organisms on different types of pollutants. They also report that there is no evidence for endemic microbiota in groundwater but that microbial communities are different from surface water communities in terms of their physiological capabilities and phylogenetic composition. West and Chilton (1997) also review microbiological activity in groundwaters.

2 Why is Groundwater Ecology important?

2.1 BIODIVERSITY

Recognition of the diversity of organisms in groundwater is relatively recent (Gibert and Culver, 2009). Botosaneanu (1986) reported 6634 species of groundwater stygobites (Gibert and Culver, 2009), and Robertson et al. (2009) cite Gibert and Culver (2004) as suggesting that 7700 stygobites were known in 2000. Danielopol et al. (2000) suggested that in Europe, stygobite species constitute about 40 % of all crustaceans, suggesting that stygobites may play an important role in the overall diversity of crustaceans. During a recent European study (PASCALIS) more than 100 new stygobite species were identified. It is widely accepted that high levels of endemism and low levels of sampling mean that the currently known species are likely to constitute only a small proportion of the true diversity (Danielopol et al., 2000; Gibert and Deharveng, 2002). It is also thought that there are many cryptic species that have not yet been identified (Gibert and Culver, 2009) and in a study of cryptic diversity Trontelj et al. (2009) identified cryptic species and concluded that very few stygobites have large ranges (more than 200 km^2), suggesting that many more cryptic species have not yet been identified. Biodiversity is not just about the number of species, it is also about unusual adaptations of species, and stygobites are therefore important because they have unusual adaptations to living in an environment with no light and limited resources.

Besides the more functional “ecosystem services” provided by groundwater ecosystems and discussed in sections 2.2 to 2.7, it is widely accepted that ecosystems and biodiversity have an inherent value. This includes an “existence value” reflecting the benefit people receive from knowing an environmental resource exists and a “bequest value” reflecting the importance of preserving the natural environment for future generations (Boulton et al., 2008).

Worldwide, assessments of groundwater biodiversity and conservation strategies are becoming increasingly important. In the USA groundwater species are protected under wildlife laws (Humphreys, 2009). In Australia governments recognise the need to protect groundwater ecosystems and require the potential impacts of human activities on groundwater ecosystems to be assessed and monitored (Hancock and Boulton, 2009; Environmental Protection Agency, 2003). Hahn (2009) notes that the European Union groundwater directive (effective from January 2007) recognised the significant contribution of groundwater to ecosystems and states “research should be conducted in order to provide better criteria for ensuring groundwater ecosystem quality and protection. Where necessary, the findings obtained should be taken into account when implementing or revising this directive. Such research, as well as dissemination of knowledge, experience and research findings, needs to be encouraged and funded”.

In the UK, biodiversity in groundwaters is poorly understood yet biodiversity is a major theme in the NERC strategy which notes that climate and other environmental changes make the need for research into biodiversity more pressing. The NERC biodiversity challenges include exploring ecosystems to discover novel biodiversity and increase knowledge of the function, distribution and abundance of biodiversity. It is likely that there are undiscovered species in UK groundwaters and groundwater ecosystems are probably the least well understood UK ecosystem.

Groundwater animals are of particular importance to biologists interested in the fundamental questions of evolution and ecology (Danielopol et al., 2000). Because of the high degree of endemism stygobites are particularly useful animals for understanding the factors determining biodiversity. Groundwater animals are also of interest to biologists because of their high degree of specialisation and adaptation.

2.2 RECONSTRUCTING PAST HISTORY AND CLIMATE

Groundwater ecosystems can exist unchanged for long periods of geological time and species can provide information on the persistence of aquifers through major episodes of climate change such as periods of regional aridity, ice ages and tectonic events at local and regional scales (Humphreys, 2009). For example Humphreys (2009) cites a study that found that in Iceland species in geothermally heated water survived beneath the Pleistocene ice sheet (Bjarni et al., 2007), and a study of the deep artesian Edwards aquifer in Texas which found invertebrate stygobites of marine origin that are relict from a marine inundation during the Cretaceous period (Longley, 1992). Humphreys (2009) also cites a study that found stygobites up to 1 km below the surface in Moroccan karst (Essafi et al., 1998); and Humphreys (2001) found that stygobites have lived for several million years in unconfined aquifers below Australian deserts. Several studies have considered stygobites in the context of large scale biogeographical changes. For example Notenboom (1991) considered marine regressions and stygobite evolution and Boutin (1994) used stygobites to determine the age of the Canary Islands.

2.3 AQUIFER CHARACTERISATION

The structure of groundwater communities can help characterise aquifers for example by indicating the nature of voids within an aquifer, extent of connectivity within aquifers, and the degree and nature of connectivity between a part of an aquifer and surface waters (e.g. Doleolivier and Marmonier, 1992; Hahn, 2006; Arietti and Edwards, 2006).

Pipan and Culver (2006) suggest that copepods in cave drip waters can be used instead of artificially introduced tracers to trace flowpaths in the epikarst. However, unlike artificial tracer testing, this technique cannot demonstrate the location or size of the surface input to the water in a drip, or the travel time along the flowpath. A further complication is that copepod species depend upon hydraulic conductivity, flow rates and chemistry (Galassi et al., 2009a), as well as general connectivity within the aquifer. The study of Pipan and Culver (2006) demonstrates that some species of copepoda are present over wide ranges (although not at all sites) whilst many have very limited lateral extent within cave dripwaters, some species being restricted to one drip. They showed the ranges of different species superimposed on cave maps. However, the paper does not outline how flowpaths can be traced using copepod distributions in dripwaters. If a species is endemic to a small number of drips it is probable that the flowpath feeding those drips is laterally connected. If a drip contains a species that other drips do not contain this may be because the drip receives a source of water that does not feed the other drips, but it is also possible that some other factor has caused the absence of the species (e.g. the species wasn’t present at the time of sampling, or there are other physical or chemical constraints that prevent the species reaching the drip because although the water may come from the same source it has travelled along a different flowpath).

2.4 INDICATORS OF ANTHROPOGENIC IMPACTS ON AQUIFERS

Humphreys (2009) notes that studies have concluded that surveys of groundwater fauna can be used as indicators of environmental health in aquifers, and that stygofauna should be incorporated into groundwater management and protection programmes. Humphreys (2009) cites Hahn (2007) as demonstrating that in the Kolbental Valley in Germany, changes in composition and density of groundwater fauna were better indicators of the effects of groundwater pumping on a surface groundwater dependent ecosystem than other hydrochemical parameters. Galassi et al. (2009a) note that copopods are particularly sensitive to microhabitat characteristics and therefore are good indicators of anthropogenically induced changes in water quality and hydrological regime.

2.5 THE ROLE OF GROUNDWATER ECOSYSTEMS IN HYDROGEOCHEMICAL CYCLING AND CONTAMINANT ATTENUATION

The role of groundwater ecosystems in hydrogeochemical cycling and enhancing groundwater quality, and the functional ecological significance of the biodiversity of groundwater ecosystems is still poorly understood (Boulton et al., 2008). However it is thought that feeding, movement and excretion by groundwater fauna (stygobites and stygophiles) can enhance water purification, bioremediation and water infiltration (Boutlon et al., 2008; Tomlinson and Boulton, 2008). Hallam et al. (2008) found bacteria in the digestive tract of two stygobite crustacean species in Morocco and concluded that bacteria may be a nutritional resource for stygobites. Subsurface fauna graze biofilms, alter pore sizes, and physically transfer material through groundwater (Hancock et al., 2005). It is therefore thought that groundwater fauna may provide important ecosystem services within the hydrogeochemical cycle (e.g. Boulton et al., 2008; Robertson et al., 2009). Groundwater ecosystem processes accelerate the oxidation of organic matter and therefore may be a prime determinant of redox evolution driving many hydrogeochemical processes (Humphreys, 2009). Groundwater ecosystems are thought to provide an important means of attenuating high nitrogen from anthropogenic sources in alluvial aquifers, reducing the nitrogen loading of rivers (Tomlinson and Boulton, 2008).

The literature on the hyporheic zone has not been considered in this report but there are more extensive studies of the role of hyporheic zone faunal assemblages in hydrogeochemical cycling and contaminant attenuation (Anne Robertson, personal communication, 2009).

2.6 AQUIFERS AS REFUGIA FOR SURFACE WATER SPECIES

It is thought that aquifers are important in providing an alternative habitat for organisms in times of environmental change and difficulty. Surface water organisms are believed to find refuge in groundwater during floods and droughts (Robertson et al., 2009). For example Hancock and Boulton (2009) suggest that in the dry Australian climate groundwater can be an important refuge for surface water organisms during dry periods when streams dry up.

3 Threats to groundwater fauna

There are many threats to groundwater fauna and it is likely that anthropogenic activities have caused and will cause the extinction of specialist endemic groundwater species. Threats are discussed by Humphreys (2009) and include:

- Habitat removal by opencast quarrying.
- Groundwater abstraction causing the direct removal of organisms and changes in the physical (e.g. flow speeds and connectivity between aquifer components) and chemical (e.g. saltwater intrusion, changes in dissolved oxygen) nature of aquifers.
- Contamination of aquifers altering the groundwater chemistry.

4 Overview of previous studies of Groundwater Ecology

4.1 UK GROUNDWATER ECOLOGY

The distribution of Ostracods, Copepods, Amphipods and other types of animals in UK groundwaters is described in detail by Proudlove et al. (2003). Many of the results discussed are from caves where most groundwater animals in the UK have been collected. However, Proudlove et al. (2003) also describe some records from wells and boreholes. Groundwater ecology research in the UK has been more recently summarised by Robertson et al (2009) who report that stygobites have been identified at 513 sites in the UK of which 51.6% were from the hyporheic zone, 25.4 % were from aquifers (boreholes, wells, caves and mines), 3 % were from springs and in 19 % of data points the habitat was not recorded.

Only 10 stygobite species have been identified in the UK. Three of these are endemic to Ireland (*Niphargus kochianus irelandicus* and *Niphargus wexfordensis*) or England (*Niphargus glenniei*). However, there have been few investigations of cryptic species in the UK therefore other species may be endemic. Robertson et al. (2009) also report 18 species of water mites which have mostly been found in the hyporheic zone of rivers.

There have been a few records of stygobitic syncarida in the UK. It was previously thought that there are two species of stygobitic Syncarida in the UK: *Antrobathynella stammeri* in Northern England and Scotland, and *Bathynella natans* in Southern England, but it is now thought that all records are actually *Antrobathynella stammeri* (Proudlove et al., 2003; Stubbington et al., 2009). These syncarida are about 1 mm in length and generally live in the hyporheic zones of rivers but they have also been found in caves and springs (Proudlove et al., 2003).

Proasellus cavaticus is the only stygobitic isopoda found in the UK. It looks like an aquatic woodlouse with no eyes or pigment and it has been found in caves in Mendip and South Wales, and in groundwater sites in southern England and Wales (Proudlove et al., 2003). It has been found to be smaller (~ 4mm long) in the vadose one of Mendip caves and larger (~ 8mm long) in the South Wales caves and the phreatic zone of Mendip caves (Proudlove et al., 2003).

Most UK stygobites are Amphipoda which have a shrimp like appearance but lack eyes and pigmentation (see Figure 1.1). *Crangonyx subterraneous* looks very similar to the *Niphargus* species discussed below. It has been found in cave systems in Wales and the Mendips and in boreholes and wells in Wiltshire, Oxfordshire, Hertfordshire, Dorset, Kent, and possibly northeast of Aberystwyth which is north of the Devensian ice limit (Proudlove et al., 2003; see also http://www.freshwaterlife.org/hcrs/species).

There are seven species of *Niphargid* in the UK and Ireland which all look similar and can only be distinguished with certainty under the microscope. *Niphargus aquilex* is the commonest (in 2003 there were 208 records in England and Wales), and *Niphargus aquilex* has been found in wells, boreholes, interstitial gravels and caves (Proudlove et al., 2003). It is generally found in shallow groundwater environments and specimens are also found in rivers, which are thought to have been washed out from shallow groundwater. Most records are from the south of England and Wales, but there are records from County Durham, Worcestershire, Lincolnshire and North Wales to the north of the Devensian ice limit (Proudlove et al., 2003). There are also records from the Isle of Wight and Guernsey. A recent study of cryptic diversity in Europe (Trontelj et al., 2009) demonstrated cryptic molecular diversity with 5 different cryptic species of *Niphargus aquilex* in Greece, Montenegro, 2 different areas in Slovenia, and Belgium, but the UK *Niphargus aquilex* species was not included in the study. *Niphargus fontanus* is found in caves in the Mendips and South Wales, and wells, boreholes and the hyporheic zones in Southern England and South Wales (Proudlove et al., 2003). *Niphargus kochianus kochianus* is most commonly found in the Chalk of Southern England at interstitial sites and boreholes and wells, with only a small number of records from caves, and it is thought to prefer living in deep phreatic environments (Proudlove et al., 2003). *Microniphargus leruthi* is smaller than the other Niphargids and has recently been found for the first time in Ireland (Arnscheidt et al., 2009). It is as yet unclear whether it is a new cryptic species or whether it is the same as the *Microniphargus leruthi* found in Belgium. It has not yet been found in other areas of the UK which may be because it has been mistaken for juveniles of the larger *Niphargid* species (see http://www.freshwaterlife.org/hcrs/species).

The remaining 3 *Niphargid* species are known to be endemic to England or Ireland. *Niphargus Glenniei* is endemic to Devon and Cornwall. It has been found in boreholes and wells in granite, some limestone caves, and some spring sites (Knight, 2009). It has recently been listed as a UK Biodiversity Action Plan species (Knight, 2009). *Niphargus kochianus irlandicus* and *Niphargus wexfordensis* are endemic to Ireland (Proudlove et al., 2003). Recent DNA studies (Hänfling et al., 2009) have demonstrated that *Niphargus kochianus kochianus* and *Niphargus kochianus irlandicus* separated at least 20 million years ago, suggesting that *Niphargus kochianus irlandicus* must have survived the Quaternary glaciations in refugia beneath the ice. In contrast, *Niphargus kochianus kochianus* was found to be quite closely related to the French species, *Niphargus dimorphopus* (Hänfling et al., 2009).

Further detail of these stygobite species can be found in Proudlove et al. (2003) and at http://www.freshwaterlife.org/hcrs. Lee Knight is responsible for the UK Hypogean Crustacea Recording Scheme which is detailed at this website. The website includes a database of all groundwater fauna found in the UK which is freely available. New records of stygobites should be sent to Lee Knight for inclusion in this database.

Arietti and Edwards (2006) carried out a survey of 60 boreholes in the Chalk of the Chilterns. The objective was to investigate whether boreholes were drawing in water from the surface. They found stygobites in all but eleven of the boreholes with specimens identified to the species level in the case of 4 amphipods, but otherwise only the genus level was recorded. Three boreholes without fauna were in the confined chalk, five were in anoxic boreholes in the unconfined aquifer and at 3 sites full sampling and evaluation could not be carried out, leaving only one site in the unconfined aquifer with potentially favourable conditions which did not contain stygobites. Sampling has continued since publication of this report and it appears that stygobites occur fairly ubiquitously in the unconfined Chalk of the Chilterns, with the exception of one valley in the unconfined Chalk with favourable chemical conditions and no stygobite fauna (Marc Arietti, personal communication, 2009).

Arnscheidt et al. (2009) sampled crustacea, basic chemistry and sediment at 106 sites in Ireland to cover a range of geomorphological areas and geologies. Most sites were boreholes or wells and crustacea were found at 57 % of sites. They found 3 ostracods, 2 cyclopods and 1 amphipod (*Microniphargus leruthi*) that were new for Ireland. A more comprehensive survey of groundwater fauna in Ireland is currently being carried out by the same team (http://www.science.ulster.ac.uk/freshwater/STRIVE.html). In another recent study in Ireland (Penk and Knight, 2008) wells, springs, caves and resurgences were sampled. 65 sites were sampled and there were 26 new records from 23 sites. Species presence was investigated rather than abundance and the objective appears to have been to look at general species distributions at the regional level rather than local controls on species distributions.

Groundwater biodiversity is much lower in the UK than in Europe and other areas of the world where groundwater fauna have been sampled and it has been suggested that there may be lower diversity in the UK and other Northern European countries due to local extinctions during glaciations and subsequent slow recolonisation (Proudlove et al., 2003; Dole Olivier et al., 2009a). For example, during the PASCALIS study, far fewer species were found in Belgium than in other countries further south (Martin et al., 2009). However, in the UK, there are records of stygobites north of the last glacial limit, and the extreme lack of data for the UK suggests that the extent of groundwater biodiversity cannot yet be estimated as other species may be present, including other endemics. Figures presented by Proudlove et al. (2003) and Robertson et al. (2009) clearly demonstrate the scarcity of data for England and Wales. Most records are for the Chalk, with some from caves in the Carboniferous Limestone and a small number of records from the Jurassic Limestone and other geologies. There has been no systematic investigation of the groundwater fauna present in the UK. There are no or very few records for many of the major aquifers (the Permo-Triassic sandstones, the Magnesian Limestones, the Lower Greensand and the Jurassic Limestones). Although there are many records of stygobites in caves in the Carboniferous Limestone, there are gaps in this dataset and there are no records from boreholes in the Carboniferous Limestone which are likely to contain fauna that is distinct from cave fauna (Humphreys, 2009). There has also been little work on the groundwater ecology of superficial porous aquifers (although some work has been done on the hyporheic zone), and little work on less permeable strata where it is possible that distinct groundwater fauna are present as found in Germany (Hahn and Fuchs, 2009).

BGS are currently collaborating with Roehampton University on a study of stygobites in Devon and Dorset. The objective is to systematically sample all strata in these counties to improve our understanding of UK groundwater biodiversity.

BGS are also carrying out a pilot study to sample boreholes that were well characterised during LOCAR (Maurice, 2009). The aim is to investigate whether stygobite diversity and abundance in boreholes is related to factors such as permeability, degree of fracturing, vertical flows generated by head differences in boreholes, surface karst, and topographical position; and also to look at whether stygobites live in the water column or just on the bottom, and whether there is a rapid input of stygobites from the aquifer to the borehole. These boreholes will continue to be sampled under different water table conditions to look at seasonal variability in results.

4.2 INTRODUCTION TO WORLDWIDE GROUNDWATER ECOLOGY

There have been much more comprehensive studies of groundwater ecology outside the UK, although the groundwater ecology of some areas of the world remains totally unexplored. An overview of some of the major studies is listed below with more detailed findings of previous studies discussed in Section 5 which covers sampling techniques and Section 6 which provides an overview of the current understanding of the factors determining the distribution of groundwater fauna.

There have been several conferences on groundwater ecology. The first international conference on groundwater ecology was held in Florida in 1992, the second in Atlanta in 1994, and there have been nineteen international symposiums on biospeleology/subterranean biology (see http://www.fi.cnr.it/sibios/symposia.htm). The most recent was in Australia in 2008 and the next is planned in 2010 in Slovenia.

France, Spain, Portugal, Italy, Slovenia and Belgium participated in the PASCALIS project which aimed to investigate the patterns in groundwater biodiversity across Europe, and develop tools for groundwater biodiversity assessment and conservation (Gibert and Culver, 2009). The project compiled existing data and sampled new sites. As of 2006, 5559 samples from boreholes, caves, springs, wells and hyporheic sediments contained stygobites (Deharveng et al., 2009). A series of papers resulting from this work form a special issue of Freshwater Biology (Volume 54, No. 4, April 2009).

There have been many studies of groundwater fauna in Australia (e.g. Humphreys, 2006; Reeves et al., 2007; Hancock and Boulton, 2008). Tomlinson and Boulton (2008) review biodiversity, ecological processes and ecosystem services of subsurface groundwater dependent ecosystems in Australia. A high diversity of groundwater fauna has been found in Australia, especially in arid areas (Humphreys, 2009). Australian researchers have also investigated the efficiency of different methods of sampling groundwater fauna (Eberhard et al., 2009; Hancock and Boulton, 2009).

In the USA there have been many studies of groundwater ecology, and the two international conferences on groundwater ecology were sponsored by the US Environmental Protection Agency. The Edwards aquifer in Texas is known to have a particularly unique and important fauna (Longley, 1992).

In Germany there have been several studies of groundwater fauna which have also investigated sampling methodology (e.g. Hahn and Matzke, 2005; Hahn, 2006; Hahn and Fuchs, 2009).

There are many other reports of stygobites around the world. Two examples include Oman (Botosaneanu, 1997), and Morocco (Essafi et al., 1995; Berrady et al., 2000), but there is information available on stygobites for most countries, and readers with an interest in stygobites in a particular area are advised to undertake country specific searches.

5 Sampling techniques

5.1 SAMPLING DESIGN AND SCALE EFFECTS

One of the difficulties in assessing biodiversity and investigating ecological processes in groundwater is that the factors controlling the distribution of groundwater fauna occur on a wide range of scales (Figure 5.1). At a country or regional scale, historical large scale geological, geomorphological and climatological processes (such as marine transgressions, glaciations, baseflow lowering) may be important in altering the structure of groundwater communities by causing extinction of some species and ingress and adaptation of others. At an intermediate scale the type of aquifer (porous, karstic or fracture) and general chemical and permeability characteristics will determine the groundwater community. At the local scale groundwater communities may be determined by local aquifer heterogeneities, whether sampling is in the vadose or saturated zone, the distance from surface water, the site elevation, and the structure and chemistry of the aquifer in the immediate vicinity of the sampling site. There may also be a sampling bias caused by the type of sampling method used (see Section 5.2). Attempting to answer a question related to any one of the factors affecting groundwater fauna distributions at any of the scales is very difficult because the fauna present will reflect all these processes and the interactions between all these processes and the importance of each individual process varies from site to site.

Hahn (2009) introduces a concept of “georegs” which are geological units within regions. He argues that you cannot simply look at geological units in general because there are regional differences. Hahn (2009) also suggests four hydrogeological classifications at the meso-scale: compact (aquitards), porous, fractured and karstic. At the local scale Hahn (2009) suggests hydrological exchange with surface water is the major criterion for classifying groundwater habitats and suggests three categories: weak, moderate and high hydrological exchange which Hahn (2009) named Oligo-, meso- and eu-alomonic.

The European PASCALIS study also developed a sampling procedure to try to address sampling issues (see Dole-Olivier et al., 2009a for details). Regions of ~ 400 km^2 were selected in each of the 6 participating countries. Within each region 4 basins were sampled, and within each basin a total of 48 samples were taken in two types of aquifer: karst and porous. 12 samples were taken in four hydrogeological habitats. In the karst aquifers 12 samples were taken in the unsaturated zone and 12 samples were taken in the saturated zone. In the porous aquifers 12 samples were taken in the hyporheic zone and 12 samples in the saturated zone. At each sampling site many environmental variables were measured including altitude, geology, hydrological conductivity, pH, SEC, dissolved oxygen, Calcium, Magnesium, Nitrate, Orthophosphate, land cover, and history. Ecological data analysis techniques (in particular niche analysis using the Outlying Mean Index) were used to unravel the controls on the distributions of groundwater fauna (Dole-Olivier et al., 2009b). Deharveng et al. (2009) suggest that although differences in sampling has contributed to the heterogeneity in biodiversity observed in the PASCALIS results, there is evidence for other causes of diversity, and biodiversity hotspots were identified. The local and regional factors determining species distributions in the PASCALIS study sites are discussed further in Section 6.

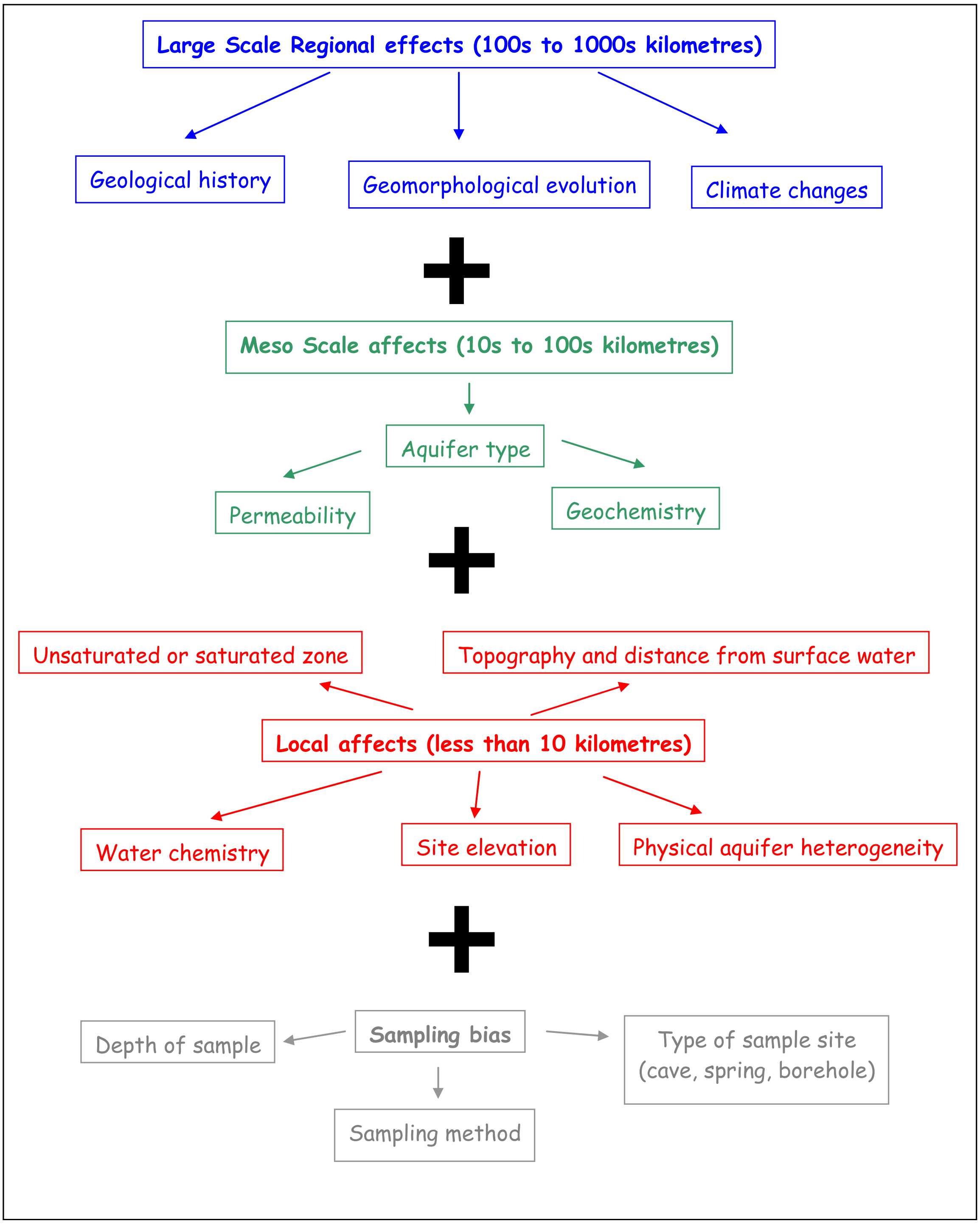

Figure 5.1 Factors affecting stygobite distributions

The objective of many studies is to assess biodiversity and therefore the aim is to sample as many species as possible. Sampling can be designed to increase the likelihood of capturing as much of the diversity as possible. For example, Dole Olivier et al. (2009a) discuss factors that they believe are likely to increase the number of stygobites collected. They suggested that some hydrological conditions are better for sampling, for example springs are best sampled during rising water levels, and the hyporheic zone is best sampled during low flow. They also suggest that selecting the largest caves in karst is best. The correlation between the number of species found and the number of sites sampled suggests a high degree of endemism (e.g. Hahn and Fuchs, 2009; Eberhard et al., 2009; Deharveng et al., 2009).

5.2 PRACTICAL SAMPLING TECHNIQUES

One of the difficulties with data on groundwater fauna is that sampling is undertaken from a variety of different environments and by several different methods, and both the environment and the method may affect the results and yet comparisons are often made between these different types of samples. Samples have been taken from streams, pools and drips in caves, from boreholes, from springs, and from sediments in the hyporheic zone of rivers. Sampling from all sites can be done using a net or a trap while samples in boreholes can also be obtained by pumping. Lee Knight provides a useful guide to methods of sampling groundwater fauna on the Hypogean Crustacea Recording System webpage (http://www.freshwaterlife.org/hcrs/sampling).

Studies in Australia have demonstrated that repeated sampling, and sampling boreholes with a pump detects a much higher abundance and diversity of groundwater fauna than sampling with a net (Eberhard et al., 2009; Hancock and Boulton, 2009). Eberhard et al. (2009) sampled 424 boreholes in alluvial and fractured aquifers in northwest Australia. They found that one net haul collected 33 % of species, whilst 6 net hauls collected 82 % of species. Hancock and Boulton (2009) sampled boreholes in alluvial aquifers in southeast Australia. They report that 10 net hauls collected on average 64 % of the taxa and 44 % of the total abundance, and when combined with 100 litres from pumping the totals rose to 92.5 % and 74.5 % respectively. Hancock and Boulton (2009) concluded that pumping was better than net sampling and that one off sampling does not sufficiently estimate taxa richness or community composition at their study sites, and suggested that survey periods extending beyond 1 year may be needed to assess biodiversity. They also noted that the sampling interval may be important because boreholes act as traps. However, a study by Allford et al. (2008) found that net hauling was a more efficient means of detecting groundwater fauna than pumping as pumping did not capture any more species than net hauling. They compared results from 55 boreholes in a calcrete aquifer in Western Australia using 3 different sampling methods: haul net sampling, pumping and a discrete interval sampler. The study concluded that there was no significant taxonomic bias from the sampling methods and that variability between samples was due to factors other than the sample method used. They found that 10 net hauls captured all the fauna present in a borehole with a decline in capture rates of more common species in subsequent hauls, but rare taxa needed up to seven hauls before they were detected.

Boreholes have been found to have a higher density and diversity of fauna than the surrounding aquifer (e.g. Hahn and Matzke, 2005). This is partly because boreholes act as a trap for fauna washed in through fractures, but perhaps more importantly the bottom of boreholes provides a suitable habitat substrate where sediment and detritus collect and are present in greater quantities than in the surrounding aquifer (Hahn and Matzke, 2005). Hahn and Matzke (2005) compared fauna from inside and outside 20 boreholes in fissured sandstone or alluvial material in southwest Germany. The inside of each borehole was sampled by taking 4 litres of water from the bottom, while the groundwater surrounding the boreholes was sampled by taking 51 litres of water using a piston pump with a double packer system. This study found that abundances of fauna were much higher inside the borehole, although taxonomic compositions were similar except for nematodes and amphipods which were higher inside boreholes. Hahn and Matzke (2005) report that studies of the hyporheic zone by Hunt and Stanley (2000) and Boulton, Dole Olivier and Marmonier (2003) found different communities and densities of fauna in the first litre of sample compared to later samples, also suggesting that fauna inside and outside boreholes differ. However, they also report that Steenken (1998) and Hahn (2003) found no differences in taxonomic composition between boreholes and the surrounding aquifer in the hyporheic zone. However, sampling in porous aquifers is challenging because there can be large differences in species composition over a few metres and the volume sampled will affect the results (Gibert et al. 2009).

If boreholes act as a trap and provide a habitat island with a significantly higher density of fauna than the surrounding aquifer it follows that the abundance and diversity of fauna should decrease with repeated sampling. However, in the study by Hahn and Matzke (2005) repeated sampling one month later revealed similar abundances and diversities to the initial sampling. This suggests that even though boreholes may act as a trap and provide a good habitat substrate, the density of animals passing through the aquifer must be relatively high. This is supported by studies that have shown that abundances and diversity of fauna in groundwater increase with the number of sampling occasions (e.g. Eberhard et al., 2009; Hancock and Boulton, 2009).

In some cases the season of sampling appears to affect the diversity and abundance of groundwater fauna, but this is not always the case. In a study in Western Australia, Eberhard et al. (2009) did not find a significant change in species composition or abundance at individual sites with season, whilst Hancock and Boulton (2009) found that in Eastern Australia more species were present in summer than winter, with densities in autumn and spring more like summer than winter.

Hancock and Boulton (2009) suggest that the location of the sample relative to the groundwater level and the proximity of the sample to the borehole screen are important. They thought that high abundance in two boreholes they sampled in an alluvial aquifer in Australia was because the water level was close to the top of the borehole screen during sampling. They cite a study by Datry, Malard and Gibert (2005) which found a decrease in faunal abundance with depth below the water table in an alluvial aquifer in France. This study found no change in taxa richness with depth, but there were vertical differences in species composition. Hancock and Boulton (2009) also cite another French study of an alluvial aquifer by Mauclaire and Gibert (2001) which found higher taxa richness and total abundance at 1 m than at 4-5 m below the water table.

The bottom of boreholes often has less flow than higher sections, particularly in fractured or karstic aquifers where there may be sections of several metres at the base of the borehole where no fractures are intercepted. Therefore it is possible that water in the bottom of the borehole where groundwater animals live could be different to that of the surrounding aquifer water. This could also be the case if a borehole contains a mixture of waters of different chemistry that have entered the borehole through different fractures. The study by Hahn and Matzke (2005) compared the chemistry of water inside 20 boreholes (1 litre pumped from the base) with water from the aquifer (the 51st litre pumped from a section isolated by packers), and found that there was little difference except in electrical conductance which was higher in the base of the borehole. The electrical conductance inside the boreholes was weakly correlated with the amount of detritus, and Hahn and Matzke (2005) suggested that the higher electrical conductance could be due to microbial decomposition of detritus.

In conclusion, there is a general consensus that repeated sampling is needed to capture more of the diversity and abundance of groundwater fauna. Different sampling methods (net hauling, pumping, trapping and discrete interval sampling) can affect the results obtained and therefore each individual study should have a unique and carefully thought out sample design that enables the required information to be obtained. Repeated net hauls and pumping seem to be the best methods of capturing the greatest diversity. Discrete interval sampling of both chemistry and fauna would be useful in studies aimed at understanding the location of fauna in the aquifer and the chemistry of their habitat.

5.3 DATA ANALYSIS

Ecological studies invariably involve complex systems in which it is difficult to evaluate biodiversity and determine particular causes of species distributions, and to understand interactions between species and their environment. Ecologists have developed and use specialist analytical techniques. Species Diversity and Richness 4 is a programme designed for ecologists to analyse data (Seaby and Henderson, 2006). Details are at:

http://www.pisces-conservation.com/sdrhelp/index.html?specaccum.htm.

This website suggests a series of essential steps for analysing diversity and provides tutorials on different analytical techniques.

Some examples of data analysis techniques used during the PASCALIS study are listed below:

- Linear regression and cluster analysis (Malard et al., 2009)
- A Jack-knife estimator used to estimate species richness using the number of species, the number of sampling sites and the number of endemics (e.g. Derharveng et al., 2009)
- Accumulation curves which plot the cumulative number of species collected against the sampling effort and are used to determine whether most species present in an area have been detected (e.g. Derharveng et al., 2009). Up to a certain point the number of species increases with the sampling effort. When increased effort no longer detects more species the curve reaches an asymptote.
- The relative importance of Regional Species Richness (RSR) to Local Species Richness (LSR) can be used to assess whether regional or local factors are more important in shaping local communities (Malard et al., 2009). If there is a linear relationship between RSR and LSR the implication is that regional processes are more important than local processes.
- Outlying Mean Index analysis (OMI) is used to define environmental gradients along which species lie and can be used to determine the most significant factors determining species distributions (e.g. Dole-Olivier et al., 2009b). This method was developed by Doledec et al. (2000).

6 Current understanding of the factors determining the distribution of groundwater fauna

6.1 REGIONAL SCALE EFFECTS

The discussion below is focused on physical factors that determine the distribution of groundwater fauna. However, biotic factors such as community interactions, mobility, competition, predation and parasitism are also important in determining the diversity and distribution of groundwater fauna.

Large scale long term environmental processes influence the type and abundance of fauna present in groundwater. Culver et al. (2009) discuss the relative importance of vicariance (forced movement of organisms) and dispersal (distribution of taxa by physiological adaptation or locomotion), and concluded that both are important in determining the aquatic fauna present in karst regions. They note that aquatic subterranean species have wider ranges than terrestrial species. They also report that where marine derived species are present in areas near the sea or former sea levels, there is an overall increase in species richness rather than replacement of non-marine species.

Glaciation is thought to affect stygobite communities by causing local extinctions (Hahn and Fuchs, 2009). Species present in areas that have been glaciated are thought to have either survived glaciation in local refugia (e.g. in deep subterranean environments unaffected by permafrost), or to be post glacial re-colonisers that have arrived via long distance dispersal (Hahn and Fuchs, 2009). The effects of glaciation appear to differ from site to site and there is still much uncertainty about how groundwater ecology is affected by glaciation. For example, in the Belgian area of the PASCALIS study, Martin et al. (2009) concluded that the species found indicated that long distance dispersal following de-glaciation was more important than survival of species in local refugia. However, they did find two endemic species in deep subterranean habitats, and they also suggested that there might be more endemic cryptic species, which were not investigated, and therefore survival in local refugia during glaciation may have been more common in this area than the results of this study suggest. The Italian PASCALIS study sites were generally far from glaciated areas but they did not find a decrease in species richness in the areas of unsaturated karst that had been glaciated suggesting that in this area, species found refuge deep in the aquifers during surface freezing (Galassi et al. 2009b). Recent results from a DNA study in Ireland (Hänfling et al., 2009) have shown that the Ireland endemic *Niphargus kochianus irelandicus* diverged more than 20 million years ago, providing fairly convincing evidence that this species has survived multiple glaciations in refugia.

Dole Olivier et al. (2009a) concluded that in karst areas of the PASCALIS study, the main factors influencing biodiversity are mean latitude, Pleistocene events, intensity of karstification (measured by looking at total number of caves and cave lengths), and the degree of fragmentation of karst. This suggests that regional scale affects are important in determining biodiversity in the PASCALIS study areas.

6.2 MESO SCALE HYDROGEOLOGICAL EFFECTS: AQUIFER TYPE

Different types of aquifer (fractured, karstic and porous) provide different physical habitats. Groundwater Ecology studies suggest that aquifer type is important in determining ecological communities although at some sites regional or local factors can be more important than aquifer type. In general it is thought that there are more species in karstic and porous aquifers than in fractured and compact aquifers (Hahn and Fuchs, 2009), and ecological communities are generally different in karstic and porous aquifers (Dole Olivier, 2009b).

In a German study, Hahn and Fuchs (2009) found that the faunal composition of four different types of aquifer (karst, porous, fractured and compact) was different although there were similarities between karst and porous aquifers. Fractured aquifers lacked some species present in karstic and porous aquifers, and contained species absent from karstic and porous aquifers.

During an analysis of the PASCALIS data, Malard et al. (2009) found differences in diversity between karst and porous aquifers suggesting that these communities are controlled by different factors. They thought because porous aquifers tend to be better connected there is more dispersal and less species turnover. They found that in karst aquifers the LSR (Local Species Richness) increased linearly with the RSR (Regional Species Richness) suggesting that regional factors were important in determining local communities, whilst in porous aquifers the LSR levelled off beyond a certain value of RSR.

Dole Olivier et al (2009a) report that in the PASCALIS study overall, in some areas there was no distinction between karst and interstitial species. They suggested that this could be due to an error in geological interpretation because many boreholes in karst areas passed through alluvial deposits overlying the limestones. It is therefore also possible that boreholes were open to both the alluvial deposits and the limestones, or there may have been hydrological connectivity between the two aquifers causing the similarities in karst and interstitial species. They also suggested that if karst voids were sediment filled they might provide a habitat for species that normally prefer interstitial environments. Dole Olivier (2009b) concluded that in these areas, the sampling methods used in the PASCALIS study do not enable a meaningful comparison of karst and porous aquifers.

There seems to be similar species richness in karst and porous aquifers. Dole-Olivier et al. (2009a) present a table summarising the number of species found in each region during the PASCALIS study. 258 species were found in karst aquifers whilst 239 were found in porous aquifers. However, Malard et al. (2009) analysed the PASCALIS data together with data from three other regions in France and found that there were more species in porous than karst aquifers. This suggests that in the French regions studied, porous aquifers had a higher diversity than karst aquifers, as this was not apparent from the data from the PASCALIS study only that is presented by Dole-Olivier et al. (2009a). In a German study, Hahn and Fuchs (2009) also found that abundance and taxonomic richness per sample was lower in karst aquifers than porous aquifers, whilst compact aquifers had the lowest diversity and abundance.

Existing studies suggest that groundwaters from non-carbonate fractured strata with acidic soils leading to lower pH have lower abundance and diversity than carbonate aquifers. In a study by Hahn and Matzke (2005) that sampled a sandstone area in which groundwaters had low electrical conductance, low pH, low nitrate and high dissolved oxygen and an alluvial aquifer with higher pH and electrical conductance, high nitrate and lower dissolved oxygen all samples from the alluvial aquifer contained fauna but many from the sandstone area did not. It is unclear whether the differences in the physical structure (the void size and connectivity) or groundwater chemistry of these aquifers caused the differences in ecology.

The different components of aquifers may also have distinct ecological communities. In karst aquifers there are distinct ecosystems in the conduit part of the aquifer and the karst matrix (Humphreys, 2009). There are also different communities in the epikarst (Pipan and Culver, 2007). Dole Olivier et al (2009a) report similarities between the communities of phreatic and hyporheic habitats in porous aquifers but suggest that this could be because the porous aquifers sampled were in karst areas and were very shallow (and therefore presumably the true phreatic zone was not sampled). Galassi et al. (2009b) found that in the Italian PASCALIS sites there were more species in unsaturated karst and the hyporheic zone of porous aquifers than in saturated karst and porous aquifers. They also suggested that the ancient age of the karst has lead to many endemics due to habitat fragmentation and isolation, whilst in the hyporheic zone of porous aquifers high species richness might be due to spatial variability in environmental conditions.

6.3 LOCAL EFFECTS

Local factors that may affect the groundwater fauna collected include water level and the amount of pressure in the water column, and local physical and chemical aquifer heterogeneity, as well as the affects of using different sampling methods discussed in Section 5. Generally, previous studies have not investigated the effects of local physical aquifer heterogeneity on groundwater ecology. However, many studies have measured chemical parameters and discussed the chemical controls on groundwater ecology, and some have considered the location of the water table. These are reviewed below.

It is thought that alkaline waters with high pH and calcium concentration may contain more fauna than acidic waters (Robertson et al., 2009). Crustaceans, in particular may require calcium for their shells. However it is difficult to determine whether low calcium and low pH limits the development of groundwater ecosystems because in general carbonate aquifers (with high pH and calcium concentrations) have higher permeability and larger and more interconnected voids than fracture aquifers with more acidic groundwaters (sandstones and volcanic aquifers). It is therefore not clear whether it is the chemistry or the physical habitat that limits the development of groundwater ecosystems in fracture aquifers with acidic groundwaters. For example, in Belgium, most species were found in highly permeable geologies with hard waters, although some groups of species were found in areas of low permeability and low calcium concentration (Martin et al., 2009). Penk and Knight (2008) tentatively suggest that Irish species of *Niphargus* prefer SEC of 200 to 720 µS/cm and pH of 6.4 to 8.4, although this is a very wide range of chemistries. They also note that in Southwest England a species of *Niphargus* lives in waters in acidic granite (although no water chemistry is available for the specific sites where the species were recorded). Many porous aquifers and hyporheic sites have low calcium and pH and yet have high diversity and abundance of groundwater fauna suggesting that low calcium and low pH does not limit the development of groundwater ecosystems. For example, Galassi et al. (2009b) found that hyporheic PASCALIS sites in Italy had lower calcium and pH than other types of site, but had a high diversity of fauna.

The role of nutrient concentrations in determining groundwater ecosystems is not well understood. Galassi et al. (2009b) discussed the environmental variables that were important in determining groundwater species found at the Italian PASCLAIS sites and suggested that phosphate was not important, but that there was a weak relationship between nitrate and stygobite species suggesting that groundwater pollution by nutrients might play a minor role in defining groundwater communities. Robertson et al. (2009) discuss the effects of nutrient enrichment of groundwaters. They report that this may lead to an abundance of organisms due to an increase in trophic resources (Wood et al. 2008), but can also lead to a reduction in groundwater biodiversity (Wood et al., 2002). Tomlinson and Boulton (2008) suggest that responses to nutrient pollution may be taxa specific. They report a study by Scarsbrook and Fenwick (2003) in which no syncarids were found in groundwater beneath a sewage bed in New Zealand suggesting that syncarids may be sensitive to high levels of nutrients, although it is not clear that the absence of syncarids was definitely due to the sewage. Tomlinson and

Boulton (2008) also report a study by Dumas and Lescher-Moutoue (2001) in which cyclopoids in a French aquifer were not affected by high nitrate concentrations.

Many groundwater organisms can withstand low oxygen for long periods and some stygobites have even been found beneath hydrogen sulphide layers (Humphreys, 2009). Penk and Knight (2008) also suggest that *Niphargid* species are tolerant of a wide range of dissolved oxygen conditions. However, despite a tolerance of anoxia, dissolved oxygen is widely believed to be a major factor determining the occurrence and distribution of stygobites and stygobites preferentially inhabit groundwaters with high dissolved oxygen concentrations (Strayer, 1994; Tomlinson and Boulton, 2008; Dole-Olivier et al., 2009b; Humphreys, 2009). Hahn (2009) discusses regional and meso scale hydrogeological controls on groundwater habitats, but also suggests that at the local scale hydrological exchange with surface water is essential because it determines the provision of food and oxygen. He suggests that faunal abundance, taxonomic richness and proportions of non-stygobites (stygoxenes and stygophiles) increases with increased hydrological exchange with surface water.

A local supply of organic matter is also important for maintaining groundwater ecosystems. Hahn and Matzke (2005) found that in the aquifer abundances of fauna per litre of water sampled was weakly correlated with the amount of detritus, but inside the boreholes there was no such correlation and concluded that within the aquifer detritus was an abundance limiting factor but that within boreholes enough detritus is available.

Strayer (1994) suggests that groundwater organisms may not be sensitive to changes in water temperature. The apparent survival of *Niphargus kochianus irelandicus* through multiple glacial cycles in Ireland (Hänfling et al., 2009) suggests that this species may be tolerant of cold waters. Gibert et al (2009b) concluded that temperature was not a major factor affecting species diversity in the PASCALIS study areas. However, some studies have found that species have specific temperature requirements. For example, Penk and Knight (2008) suggest that *Niphargids* may have a limited tolerance to temperature although further work is required to determine this conclusively. Results from the PASCALIS study suggested that groundwaters in aquifers at lower altitudes had higher species diversity (Dole-Olivier et al., 2009b), and this could indicate that diversity is affected by temperature because groundwaters at higher altitudes are likely to have lower temperatures.

Different species and taxa are likely to have different environmental habitat requirements. Humphreys (2009) reports a study of Ostracods in Australia by Reeves et al. (2007). This study found that water with low pH, low Eh indicating a reducing environment, or total nitrogen in excess of 10 mg/l rarely contained Ostracods, suggesting that they are sensitive to these environmental variables.

6.4 CONCLUSIONS

There is still a lot of uncertainty about the interaction between regional, meso scale and local effects controlling the distribution of groundwater fauna. Stoch et al. (2009) used the PASCALIS data to try and determine if particular species could be used as indicator species to predict species richness but concluded that the factors determining the variation in groundwater assemblages were still unknown.

Some studies have found that local factors are important whilst at other sites regional factors seem more important. For example, Hancock and Boulton (2008) studied the stygofauna of four alluvial aquifers in Eastern Australia and found that local factors were important in determining species diversity and abundance. Most taxa were collected from boreholes less than 10 m deep with electrical conductivity of less than 1500 µS/cm. Taxon richness decreased with depth below the water table, and boreholes in the alluvium of tributaries of large river systems and near phreatophytic trees contained the most taxa. However, the results of the PASCALIS study suggested that regional factors were more important than local factors in determining groundwater ecology (Gibert et al., 2009b). OMI analysis of the many environmental parameters which were measured during the PASCALIS study suggested that the most important factors determining variability in stygobite assemblages were geology (pore size) and dissolved oxygen, and that altitude and distance of sampling sites from glacial areas were the next most important variables (Dole Olivier et al. 2009b). In the PASCALIS study areas, there seemed to be fairly widespread ecological tolerance to temperature and water chemistry and lack of tolerance to habitat conditions was not thought to be the main cause of endemism in groundwater (Gibert et al., 2009b; Dole Olivier et al. 2009b; Galassi et al., 2009b). Dole Olivier et al. (2009b) concluded that stygobite diversity is dependent on hydraulic conductivity which controls oxygen supply. Overall Gibert et al., (2009b) concluded that hydrogeology, altitude, palaeogeographical factors and human activities interact in a complex way to produce different patterns of species composition and diversity among the PASCALIS regions.

It is clear that despite the extensive work carried out in Europe, the factors controlling the distribution of stygobite species, and how this varies from area to area, are still not well understood.

7 Future work

7.1 LITERATURE RECOMMENDATIONS.

Many groundwater ecology studies have included discussion of remaining research questions and recommendations for future work. Some examples are listed below:

- Danielopol et al. (2000) suggest that important future research areas include investigating the extent of stygobite lineages in karst systems compared to interstitial systems, and whether hotspot areas occur due to a history of numerous climatic and geological changes.
- Gibert et al. (2009b) note that few fissured aquifers have been studied. They also suggest that future work should include improving sampling strategies, development of models of species richness, development of indicators, and studies of functional and genetic diversity.
- Dole Olivier et al (2009a) suggest that there is a need to search for new habitats (fracture aquifers as well as karst and alluvial) and investigate epikarst more thoroughly.
- Dole Olivier (2009b) recommend that more basins, types of aquifer and hydrogeological zones within aquifers are tested to search for currently unknown sources of heterogeneity.
- Hahn (2009) describes a strategy for investigating groundwater habitats but suggests three areas of further work. 1) Determining how to assess the ecology of groundwater related ecotones such as springs, the hyporheic zone, unsaturated karst, and aquatic caves. 2) Investigating whether groundwater ecology depends upon whether the origin of groundwater is river water or non-river water. 3) Reference studies of undisturbed habitats typical of biogeographical, regional and local conditions.
- Humphreys (2009) comments that groundwater sampling is an integration of water from many depths. He suggests that it would be useful to investigate the physicochemical structure of the water column by undisturbed profiling. He also suggests that novel techniques such as filtering aquifer outflows and depth specific sampling of fauna could be useful.
- It is now fairly widely recognised that groundwater ecology is an interdisciplinary subject that should involve both hydrogeologists and ecologists (Hancock et al., 2005; Humphreys, 2009).
- Robertson et al. (2009) identify research priorities in the UK noting the need for a comprehensive and systematic survey of stygobites. They also suggest that genetic studies on UK stygobites are needed to investigate the true biodiversity including cryptic species. They also point out the need for developing interdisciplinary projects and to determine how to monitor the condition of hyporheic and deep groundwater assemblages.

7.2 UK NATIONAL SURVEY

In the UK there is a clear lack of groundwater ecology data, and given the importance of groundwater ecology (outlined in section 2) a systematic nationwide survey of groundwater fauna would be very useful. This survey would provide information on the biodiversity of UK stygobites, provide a basis for understanding ecosystem services, and would act as a baseline enabling future changes in groundwater ecosystems due to climate change or anthropogenic impacts to be recognised. If conservation and assessment of groundwater ecosystems becomes compulsory under European laws then survey data would be useful in determining where and how conservation should be implemented. The survey would highlight what groundwater ecosystem resources there are in the UK and where they are located, enabling biologists, ecologists and hydrogeologists to target future research most effectively. With careful sample design and simultaneous collection/compilation of chemical and hydrogeological information the survey might also provide insight into the controls on the distribution of stygobites.

If a survey is carried out the existing literature suggests that the country should be divided into geological units (major aquifers – Chalk, Lower Greensand, Jurassic Limestones, Permo-Triassic Sandstones, Magnesian Limestones, Carboniferous Limestone, lower permeability fractured strata, superficial porous deposits), and the geological units should be divided regionally along the lines of the “georegs” described by Hahn (2009). It is likely that initial targeting of the least studied areas and aquifers would be the best approach.

7.3 RESEARCH

The science of groundwater ecology offers opportunities to address fundamental science questions. There are many biological (ecological, evolutionary and behavioural) questions which experts in these fields might be interested in. In particular, the high levels of endemism in stygobites make groundwater environments ideal for the study of the fundamental concepts of biodiversity. Studies with combined biological and hydrogeological objectives may be possible.

Some questions of particular hydrogeological interest include:

- Investigating the physical hydrogeological controls on the distribution of stygobites in aquifers: Try to determine where stygobites live by comparing results from different parts of aquifers (vadose zone/saturated zone, springs, boreholes, boreholes of different depths and at different elevations, targeted sampling to determine where organisms enter boreholes, perhaps combined flow logging/tracer testing/packered water sampling). In highly karstic aquifers it would be useful to investigate the distribution of species across different components of the aquifer (epikarst, unsaturated zone, saturated zone, caves, fractures in boreholes, springs). It would also be interesting to investigate a range of aquifers with variable degrees of karstification to determine the role of karstification in determining stygobite distributions (and groundwater ecology in understanding karstification processes).
- Determining the abundance of stygobites in groundwaters and the temporal changes in diversity and abundance: Use repeated sampling of springs, inflows into boreholes, and inflows into caves.
- Investigating the controls on the distribution of stygobites in the Chalk: look at regional and local differences and try to determine where in the aquifer stygobites live.

- Investigating the groundwater ecology of the epikarst and unsaturated zone of the Carboniferous Limestone (combine ecological sampling with looking at flow rates of inputs to caves systems from the surface, the chemistry of these flows and perhaps use tracer tests to define the surface catchment areas of these flows).

References

Allford, A.S. Cooper, J. B. Humphreys, W. F. and Austin, A. D. 2008. Diversity and distribution of groundwater fauna in a calcrete aquifer: does sampling method influence the story? *Invertebrate Systematics* **22**(2): 127-138.

Arietti, M., and Edwards, B., 2006. Groundwater invertebrates in chalk boreholes and their significance in public water supply. Internal report for Three Valleys Water. 40 pp.

Arnscheidt. J., Hahn, H.J., and Fuchs, A., 2009. Aquatic subterranean Crustacea in Ireland: results and new records from a pilot study. *Cave and Karst Science* **35** (1-2): 53-58

Berrady, I., Essafi, K., and Mathieu, J., 2000. Comparative physico-chemical and faunal studies of two thermal springbrooks near Sidi Harazem (Morocco). Annales De Limnologie-*International Journal of Limnology* **36**(4): 261-274.

Bjarni, K., Kistjánsson, B.K., and Svavarsson, J., 2007. Subglacial refugia in Iceland enabled groundwater amphipods to survive glaciations. *Am Nat* **170**:292-296

Botosaneanu L (ed) (1986) Stygofauna mundi. EJ Brill/Dr W Backhuys, Leiden.

Botosaneanu, L. and Stock, J.H., 1997. Stygofauna of Oman .1. A new freshwater stygobiont Cyathura (Isopoda, Anthuridae), from interstitia of coarse wadi sediments in Oman. *Annales De Limnologie-International Journal of Limnology* **33**(2): 79-84.

Boulton, A. J., Fenwick, G.D., Hancock, P.J., and Harvey, M.S., 2008. Biodiversity, functional roles and ecosystem services of groundwater invertebrates. *Invertebrate Systematics* **22** (2): 103-116.

Boulton, A. J., Dole-Olivier M. J. and Marmonier, P., 2003. Optimizing a sampling strategy for assessing hyporheic invertebrate biodiversity using the Bou-Rouch method: Within-site replication and sample volume. *Archiv Fur Hydrobiologie* **156**(4): 431-456.

Boutin, C., 1994. Stygobiology and historical geology – the age of Fuerteventura (Canary Island), as inferred from its present stygofauna. *Bulletin De La Societe Geologique De France* **165**(3): 273-285.

Culver, D.C., Pipan, T. and Schneider, K., 2009. Vicariance, dispersal and scale in the aquatic subterranean fauna of karst regions. *Freshwater Biology* **54**(4): 918-929.

Danielopol, D.L., Artheau, M. and Marmonier, P., 2009. Site prioritisation for the protection of rare subterranean species - the cases of two ostracods from south-western France. *Freshwater Biology* **54**(4): 877-884.

Danielopol, D. L. and Griebler, C., 2008. Changing Paradigms in Groundwater Ecology - from the 'Living Fossils' Tradition to the 'New Groundwater Ecology'. *International Review of Hydrobiology* **93**(4-5): 565-577.

Danielopol, D. L., Pospisil, P. and Rouch, R., 2000. Biodiversity in groundwater: a large-scale view. *Trends in Ecology & Evolution* **15**(6): 223-224.

Datry, T., Malard, F. and Gibert, J., 2005. Response of invertebrate assemblages to increased groundwater recharge rates in a phreatic aquifer. *Journal of the North American Benthological Society* **24**(3): 461-477.

Deharveng, L., Stoch, F., Gibert, J., Bedos, A., Galassi, D., Zagmajster, M., Brancelj, A., Camacho, A., Fiers, F., Martin, P., Giani, N., Magniez G. and Marmonier, P. , 2009. Groundwater biodiversity in Europe. *Freshwater Biology* **54**(4): 709-726.

Doledec, S., Chessel D. and Gimaret-Carpentier, C., 2000. Niche separation in community analysis: A new method. *Ecology* **81**(10): 2914-2927.

Dole-Olivier, M. J., Castellarini, F., Coineau, N. , Galassi, D. M. P., Martin, P., Mori, N., Valdecasas A. and Gibert, J. , 2009a. Towards an optimal sampling strategy to assess groundwater biodiversity: comparison across six European regions. Freshwater Biology **54**(4): 777-796.

Dole-Olivier, M. J., Malard, F., Martin, D., Lefebure T. and Gibert, J., 2009b. Relationships between environmental variables and groundwater biodiversity at the regional scale. Freshwater Biology **54**(4): 797-813.

Doleolivier, M. J. and Marmonier, P., 1992. Ecological requirements of stygofauna in an active channel of the Rhone River. *Stygologia* **7**(2): 65-75.

Dumas, P. and Lescher-Moutoue, F., 2001. Cyclopoid distribution in an agriculturally impacted alluvial aquifer. *Archiv Fur Hydrobiologie* **150**(3): 511-528.

Eberhard, S. M., Halse, S. A., Williams, M. R., Scanlon, M. D., Cocking J. and Barron, H. J., 2009. Exploring the relationship between sampling efficiency and short-range endemism for groundwater fauna in the Pilbara region, Western Australia. *Freshwater Biology* **54**(4): 885-901.

Environmental Protection Agency. 2003. Guidance for the assessment of environmental factors (in accordance with the Environmental Protection Act 1986). Consideration of subterranean fauna in groundwater and caves during Environmental Impact Assessment in Western Australia. No. 54. 12pp.

Essafi, K., Mathieu J. and Pattee, E., 1995. Changes in the structure of subterranean aquatic populations of the karstic system of Oued Zeghzel (Eastern Morocco). *Archiv Fur Hydrobiologie* **135**(2): 195-208.

Essafi, K., Mathieu, J., and Berrady I., 1998. Qualite de l'eau et de la faune au niveau de forages artesians dans les Plaine de Fes et la Plaine des Beni-Sadde: premiers resultats (Water quality and fauna from artesian bores in the Plain of Fes and the Plain of Blessed Sadden). Mem. Biospeol 25: 157-166

Galassi, D. M. P., Huys R. and Reid, J. W., 2009a. Diversity, ecology and evolution of groundwater copepods. *Freshwater Biology* **54**(4): 691-708.

Galassi, D. M. P., Stoch, F., Fiasca, B., Di Lorenzo T. and Gattone, E., 2009b. Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. *Freshwater Biology* **54**(4): 830-847.

Gibert, J., Danielpol, D., and Stanford, J. (Eds), 1994. "Groundwater Ecology". 571 pp.

Gibert, J. and Deharveng, L., 2002. Subterranean ecosystems: A truncated functional biodiversity. *Bioscience* **52**(6): 473-481.

Gibert, J., and Culver, D.C., 2004. Diversity patterns in Europe. In Culver, D.C., and White, W.B. (eds) Encyclopaedia of Caves. Elsevier/Academic Press, Amsterdam, 196-201.

Gibert, J. and Culver, D.C., 2009. Assessing and conserving groundwater biodiversity: an introduction. *Freshwater Biology* **54**(4): 639-648.

Gibert, J., Culver, D.C., Dole-Olivier, M.J., Malard, F., Christman, M.C., and Deharveng, L., 2009. Assessing and conserving groundwater biodiversity: synthesis and perspectives. *Freshwater Biology* **54**(4): 930-941.

Griebler, C. and Lueders, T., 2009. Microbial biodiversity in groundwater ecosystems. *Freshwater Biology* **54**(4): 649-677.

Hahn, H. J., 2009. A proposal for an extended typology of groundwater habitats. *Hydrogeology Journal* **17**(1): 77-81.

Hahn, H. J. and Fuchs, A., 2009. Distribution patterns of groundwater communities across aquifer types in south-western Germany. *Freshwater Biology* **54**(4): 848-860.

Hahn., H.J., 2007. Preliminary ecological evaluation for faunistically justified groundwater monitoring: Interpreting the hydrological exchange. Proc. DWA Seminar “Grundwasserokologie: Praxis and Forschung” Fulda, Germany, Nov 2007.

Hahn, H. J., 2006. The GW-Fauna-Index: A first approach to a quantitative ecological assessment of groundwater habitats. *Limnologica* **36**(2): 119-137.

Hahn, H. J. and Matzke, D., 2005. A comparison of stygofauna communities inside and outside groundwater bores. *Limnologica* **35**(1-2): 31-44.

Hahn, H. J., 2003. Are traps suitable for representative sampling of aquatic meiofauna in both the hyporheic zone and in groundwater? *Limnologica* **33**(2): 138-146.

Hallam, F., Yacoubi-Khebiza, M., Oufdou K. and Boulanouar, M., 2008. Groundwater quality in an arid area of Moroco: Impact of pollution on biodiversity and relationships between crustaceans and bacteria of health interest. *Environmental Technology* **29**(11): 1179-1189.

Hancock, P. J. and Boulton, A. J., 2009. Sampling groundwater fauna: efficiency of rapid assessment methods tested in bores in eastern Australia. *Freshwater Biology* **54**(4): 902-917.

Hancock, P. J. and Boulton, A. J., 2008. Stygofauna biodiversity and endemism in four alluvial aquifers in eastern Australia. *Invertebrate Systematics* **22**(2): 117-126.

Hancock, P. J., Boulton A. J., and Humphreys, W. F., 2005. Aquifers and hyporheic zones: Towards an ecological understanding of groundwater. *Hydrogeology Journal* **13**(1): 98-111.

Hänfling, B., Douterelo-Soler, I., Knight, L., and Proudlove, G., 2009. Molecular studies on the *Niphargus kochianus* group (Crustacea: Amphipoda: Niphargidae) in Great Britain and Ireland. . *Cave and Karst Science* **35** (1-2): 35-40.

Humphreys, W. F., 2009. Hydrogeology and groundwater ecology: Does each inform the other? *Hydrogeology Journal* **17**(1): 5-21.

Humphreys W., 2006, 'Groundwater fauna' paper prepared for the 2006 Australian State of the Environment Committee, Department of the Environment and Heritage, Canberra.

Hunt, G. W. and Stanley, E. H., 2000. An evaluation of alternative procedures using the Bou-Rouch method for sampling hyporheic invertebrates. *Canadian Journal of Fisheries and Aquatic Sciences* **57**(8): 1545-1550.

Knight, L.R.F.D., 2009. The Biodiversity Action Plan (BAP) for *Niphargus glenniei* (Crustacea: Amphipoda: Niphargidae): The first British troglobite to be listed. *Cave and Karst Science* **35** (1-2): 13-18.

Longley, G., 1992. The subterranean aquatic ecosystem of the Balcones Fault Zone Edwards Aquifer in Texas – threats from overpumping. In Groundwater Ecology (J.A Stanford and J.J. Simmons, eds.) pp. 291-300. American Water Resources Association., Bethesda, MD.

Malard, F., Boutin, C., Camacho, A.I., Ferreira, D., Michel, G., Sket, B., and Stoch, F., 2009. Diversity patterns of stygobiotic crustaceans across multiple spatial scales in Europe. *Freshwater Biology* **54**(4): 756-776.

Martin, P., De Broyer, C., Fiers, F., Michel, G., Sablon, R., and wouters, K., 2009. Biodiversity of Belgian groundwater fauna in relation to environmental conditions. Freshwater Biology 54(4): 814-829.

Mauclaire, L. and Gibert, J., 2001. Environmental determinants of bacterial activity and faunal assemblages in alluvial riverbank aquifers. *Archiv Fur Hydrobiologie* **152**(3): 469-487.

Maurice, L. 2009. Investigations of rapid groundwater flow and karst in the Chalk. Unpublished PhD thesis, 453 pp.

Michel, G., Malard, F., Deharveng, L., Di Lorenzo, T., Sket, B. and De Broyer, C., 2009. Reserve selection for conserving groundwater biodiversity. *Freshwater Biology* **54**(4): 861-876.

Notenboom, J., 1991. Marine regressions and the evolution of groundwater dwelling amphipods (crustacea). *Journal of Biogeography* **18**(4): 437-454.

Penk, M., and Knight, L., 2008. Groundwater crustacea of Ireland. A survey of the stygobitic Malacrustacea in caves and springs. Ecoserve Report. 64pp.

Pipan, T. and Culver, D.C., 2007. Copepod distribution as an indicator of epikarst system connectivity. *Hydrogeology Journal* **15**(4): 817-822.

Proudlove, G.S., Wood, P.J., Harding, P.T., Horne, D.J., Gledhill, T., and Knight, L.R.F.D., 2003. A review of the status and distribution of the subterranean aquatic Crustacea of Britain and Ireland. *Cave and Karst Science* **30 (2)**: 51-74

Reeves, J. M., De Deckker P. and Halse, S. A., 2007. Groundwater Ostracods from the arid Pilbara region of northwestern Australia: distribution and water chemistry. *Hydrobiologia* **585**: 99-118.

Robertson, A. L., Smith, J. W. N., Johns, T., and Proudlove, G.S., 2009. The distribution and diversity of stygobites in Great Britain: an analysis to inform groundwater management. *Quarterly Journal of Engineering Geology and Hydrogeology* **42**(3): 359-368.

Scarsbrook, M. R. and Fenwick, G.D., 2003. Preliminary assessment of crustacean distribution patterns in New Zealand groundwater aquifers. *New Zealand Journal of Marine and Freshwater Research* **37**(2): 405-413.

Seaby R. M. and Henderson, P. A. (2006) Species Diversity and Richness Version 4. Pisces Conservation Ltd., Lymington, England.

Stoch, F., Artheau, M., Brancelj, A., Galassi, D.M.P., and Malard, F., 2009. Biodiversity indicators in European ground waters: towards a predictive model of stygobiotic species richness. *Freshwater Biology* **54**(4): 745-755.

Strayer, D.L., 1994. Limits to biological distributions in groundwater. In Groundwater Ecology, Edited by Janine Gibert, Dan Danielopol and Jack Stanford. 287-310.

Stubbington, R., Dunscombe, M.P., and Gledhill, T., 2009. Occurrence of *Antrobathynella stammeri* (Jakobi, 1954) (Crusatcea: Syncarida: Bathynellidae) in the hyporheic zones of two English karst rivers. *Cave and Karst Science* **35** (1-2): 59-62.

Tomlinson, M., and Boulton, A., 2008. Subsurface Groundwater Dependent Ecosystems: a review of their biodiversity, ecological processes and ecosystem services. Australian Government National Water Commission Report. 77 pp.

Trontelj, P., Douady, C. J., Fiser, C., Gibert, J., Goricki, S., Lefebure, T., Sket, B. and Zaksek, V., 2009. A molecular test for cryptic diversity in ground water: how large are the ranges of macro-stygobionts? *Freshwater Biology* **54**(4): 727-744.

West, J. M. and Chilton, P. J., 1997. Aquifers as environments for microbiological activity. *Quarterly Journal of Engineering Geology* **30**: 147-154.

Wood, P. J., Gunn J. and Rundle, S. D., 2008. Response of benthic cave invertebrates to organic pollution events. *Aquatic Conservation-Marine and Freshwater Ecosystems* **18**(6): 909-922.

Wood, P. J., Gunn, J. and Perkins, J. , 2002. The impact of pollution on aquatic invertebrates within a subterranean ecosystem - out of site out of mind. *Archiv für Hydrobiologie* **152**: 223-237