The Groundwater Animals Project

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An investigation into the diversity and distribution of groundwater fauna in England

Project partners:

Roehampton University

British Geological Survey

University of Loughborough

The Freshwater Biological Association

University of Plymouth

Environment Agency

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See also: Groundwater Animals UK: http://new.freshwaterlife.org/web/gwa-uk

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Executive summary

This report describes a collaborative research project investigating groundwater animals in England, led by the Centre for Research in Ecology at Roehampton University and funded by the Esmée Fairbairn Foundation. Groundwater animals are fauna that are found in the aquatic subterranean environment. Those that live exclusively in this environment are termed stygobites, the majority of which are crustaceans, and the focus of this project. Globally, there are probably in excess of 8000 species many of which are endemic. In Britain the species *Niphargus glenniei* is an example, being known only from Cornwall and Devon. Groundwater fauna are vulnerable to local extinctions through anthropogenic impacts such as groundwater pollution and water abstractions.

The project, which commenced in late 2009, consisted of three surveys each designed to explore factors governing the occurrence and distribution of groundwater fauna in England. The main survey focused on boreholes/wells and springs across Devon and Dorset, examining the presence and abundance of stygobites across different geologies and physio-chemical environments. The second survey investigated the stygobites presence on Chalk and Magnesian Limestone geologies either side of the Devensian glacial divide in northern England. The final survey examined differences in hyporheic faunal communities from hydraulically contrasting reaches of the river Piddle, a Chalk stream in Dorset.

The key findings of the surveys were:

1. The presence of stygobites differed significantly between the different geologies surveyed in Devon and Dorset. The probability of finding stygobites was highest for the Chalk, where the greatest species richness and total abundance was also recorded. Geologies with a high degree of fracturing appeared to offer the best habitat opportunities for stygobites but other factors including water chemistry may also play a role.

2. *Niphargus glenniei* was not reported outside Devon but was found in a range of different geologies in the county. Conversely, some other species found in Dorset were not found in Devon. A band of low permeability mudstone between the counties may act as a barrier to the movement of some species. Species found in both counties could be utilising pathways provided by overlying shallow superficial deposits, for their dispersal.

3. Only two stygobitic species were reported from the survey of northern England. None were found in the Magnesian Limestone, with records from the Chalk limited to unconfined strata south of the approximate Devensian glacial limit.

4. On the river Piddle higher species richness and abundance was reported from groundwater gaining (upwelling) reaches, than from groundwater losing (downwelling) reaches. Four stygobitic species were reported from the survey all of which were consistently found in greater abundance in the gaining sections of the river, although this was not a significant difference.

Through the project we hope to have widened the knowledge base on groundwater animals in England and raised their profile through engagement with stakeholder groups. We also hope that the survey findings will assist environmental managers to understand, manage and protect subterranean ecosystems better, as part of a holistic approach to water management and the conservation of this unique group of animals.

1.0 Introduction

1.1 Why is groundwater fauna of interest?

Groundwater is water found in rocks (aquifers) below the earth's surface. It provides an important supply of drinking water for consumption as well as contributing to the base flow of many rivers and wetland habitats. Whilst it is a renewable resource, recharged by rainfall percolating through the soil in to aquifers, it is vulnerable to impacts from pollution and the effects of over pumping. Surprising to many people is that groundwater is also home to a variety of fauna ranging from microbial fauna to meio and macro-invertebrates and even vertebrates (Culver & Pipan, 2009), although the latter are rare and not known from Britain.

The permanent darkness and limited resources present within groundwater provide a unique environment, with many of the fauna found within it occurring nowhere else on earth. The adaption of these animals to such conditions, together with their often sparse populations and slow reproduction rates, make them an important component of global biodiversity. Furthermore, they are poor dispersers, usually having small ranges of often less than 200km^2, so endemics are common (Trontelj *et al*., 2009). This combination of characteristics make them highly vulnerable to local extinctions from anthropogenic effects (Humphreys 2008a), such as pollution and water abstraction pressures. Historically, little attention has been given to this unique group of animals despite evidence that indicates their extremely long persistence through geological time (Kristjansson & Svavarsson 2007). Studies of groundwater fauna are therefore essential both to capture information on current biodiversity, at local, regional and global scales and to help ensure their future protection and conservation.

1.2 Aims & scope of project

This report describes a collaborative research project investigating groundwater fauna in England, led by the Centre for Research in Ecology at Roehampton University and funded by the Esmée Fairbairn Foundation. The project partners comprised; Loughborough University, Plymouth University, the Biological Records Centre's subterranean Crustacea recorder, the British Geological Survey and the Freshwater Biological Association. Further details of the project partnership and supporting organisations are provided in Appendix 1.

The report presents the findings from a baseline survey for groundwater organisms conducted across three different regions of England, utilising a variety of wells, boreholes and springs and the hyporheic zone of rivers to collect samples. The survey regions were chosen through a project scoping exercise identifying them as suitable and practical locations to investigate the distribution and occurrence of groundwater fauna in England. The three regions examined included parts of south-west England, northern England and north-east England. The survey work was designed to address four main project aims, listed below, which focussed upon key issues and questions identified through previous studies and reviews.

1. Determine (a) the habitat requirements, distribution and relative abundance of obligate groundwater organisms in key areas and (b) the impact of the Devensian glaciation on groundwater fauna in northern England.

2. Provide new information to assist in the development of the UK Biological Action Plan (BAP) for the British well shrimp *Niphargus glenniei*, and other groundwater species.

3. Examine and compare the invertebrate assemblages within the hyporheos across river reaches with contrasting contributions from groundwater base flow.

4. Raise the profile of groundwater animals and develop sampling protocols for future monitoring of groundwater organisms, suitable for a national survey.

Placing these aims in context, a review of the subterranean aquatic ecology of England and Wales by the Environment Agency, (Environment Agency 2008), highlighted the need for national surveys and studies of groundwater fauna. The review identified significant knowledge gaps in the known distribution and abundance of subterranean assemblages within Great Britain and the need to understand how environmental variables, such as geology and physio-chemical factors, may help govern the occurrence of groundwater fauna. The report also highlighted the work of Proudlove *et al.* (2003), which recommended surveys of different geologies, utilising the Environment Agency's groundwater monitoring network. Proudlove *et al.* (2003) also identified how the glacial legacy of the British Isles may have influenced the current distribution and diversity of such fauna, recommending surveys across the last glacial divide (of the Devensian glaciation) to help evaluate this theory.

By undertaking this project we hope to begin to fill some of the knowledge gaps on groundwater fauna in Britain and to raise awareness of groundwater biodiversity at a local and national level. The project will also be of benefit in informing future survey efforts and ultimately aid the protection and conservation of the groundwater habitat and the unique species it supports.

1.3 Groundwater and the groundwater ecosystem

Under Article 2 of the Water Framework Directive (WFD) groundwater is defined as "all water which is below the surface of the ground in the saturation zone and in direct contact with the ground or subsoil" (European Commission 2000). In strict legal terms the water inside a lined borehole is arguably not actually groundwater under this definition, but for most purposes such an interpretation is not relevant.

Globally groundwater is the largest supply of freshwater available for human consumption, with 97% of all freshwater (excluding frozen water) being subsurface (Gibert *et al*.,1994). In England and Wales around a third of the supply for drinking water comes from groundwater, although this proportion varies regionally. In the south of England about one half of the supply comes from groundwater, rising to 70% in the south east of the country (Environment Agency 2006). Groundwater is therefore a vital resource which requires and receives protection for its continued use and consumption. This protection has historically concentrated on the chemical constituents and availability of the groundwater, in terms of water quality and water quantity, respectively. Protection is not currently afforded to the biological element of groundwater in European Union member states under the WFD. The Groundwater Directive, a daughter directive of the WFD, does however recognise groundwater as an ecosystem in general terms, conferring a degree of protection by association to surface systems.

The groundwater ecosystem is a unique and complex aquatic environment characterised by extreme but stable conditions that include a lack of light, low temperatures, low oxygen concentrations and low food and nutrient resources (Robertson, *et al*., 2009). It is a physically heterogeneous environment, largely determined by geology, with a range of habitats from small pores and interstices in porous aquifers, to much larger apertures and voids present within fractured aquifers (Maurice 2009). In some geologies, such as karstic limestones where cave development occurs, a further range of habitats exist including; underground streams, isolated pools, deep lakes and flooded passages (Knight and Penk 2010). However, in the absence of light there is no photosynthesis and consequently there is little[1] or no autotrophic primary production within the subterranean environment. Energy sources are therefore almost exclusively derived from the surface as particulate matter or dissolved organic carbon (DOC) that reaches the groundwater via percolation from the surface and laterally through groundwater flow (Humphreys 2008b). Concentrations of DOC, as well as oxygen and water chemistry, can vary significantly within groundwater and sometimes quite sharply, both spatially (with depth) and through time (Humphreys 2006).

1.4 Groundwater fauna & biodiversity

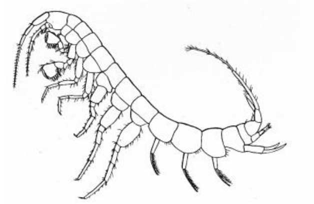
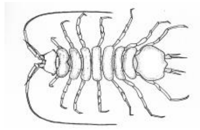
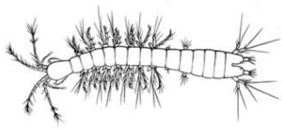
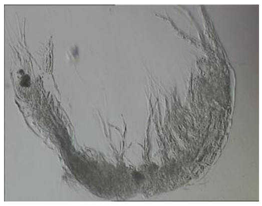
Groundwater animals is a term which can be used to describe organisms that live or are found in groundwater. Gibert *et al.* (1994), describe three groups of such animals, based on their degree of affinity to groundwater; i) those animals that accidently occur in groundwater (stygoxenes), ii) those which inhabit groundwater on a temporary basis (stygophiles) and iii) those which only inhabit groundwater (stygobites). It is this latter group of obligate groundwater animals, sometimes also referred to as stygofauna, which is of principal interest when considering groundwater biodiversity, and is the focus of this project. The vast majority of such fauna are invertebrates of which Crustacea are the dominant sub-phylum. A global estimate of 7700 stygobites is suggested by Deharveng *et al*. (2009), although this figure is likely to be higher now with the discovery of many new species since the publication of this figure. Populations of stygobites appear to be concentrated in “hotspots” of groundwater fauna, and are often associated with karst and other carbonate geologies. Examples include the Dinaric region of the West Balkans (Culver and Sket, 2000) and the Pilbara region of North-west Australia (Eberhard *et al.,* 2005).

There are currently eight stygobitic Crustacea species recorded within mainland Britain, listed and illustrated in Table 1.1 (photographs of each species are provided in Appendix 2). There are also a further two species endemic to Ireland, details of these and the other British species are provided on the Hypogean Crustacea Recording Scheme website (http://www.freshwaterlife.org/hcrs/). Additionally, Proudlove *et al.* (2003) in a review of subterranean aquatic Crustacea list a number of Copepoda and Ostracoda which are reported to have an affinity for groundwater but are also known from other aquatic habitats. Similarly, there are around 18 species of water mite (Hydracarina) known to be associated with the shallow sub-surface waters of riverbeds (the hyporheos) (Gledhill pers. comms. in Robertson *et al.,* 2009). Whilst, such taxa were collected as part of this study they were not the prime focus of the survey work and are not considered in detail in this report. However, specimens of copepods, ostracods, hydracarina as well any worms or nematodes collected during the survey have been retained and it is planned that these will be speciated by relevant experts in due course and reported separately.

In the absence of a comprehensive survey of Britain, there is also the possibility that other species may be discovered in time. Furthermore, since many stygobite species are cryptic, that is they resemble one another morphologically, new species may come to light through genetic studies. Such studies are currently in progress (e.g. Hänfling *et al.*, 2008).

[1] There are some rare examples of chemosynthesis based systems such as the sulphur-based autotrophic ecosystem present in Movile Cave, Romania (Sarbu, 1996)

Table 1.1: Species list of stygobites known from Britain

Species	Order	Generalised view*	Photograph
Niphargus kochianus kochianus (Schellenberg, 1932)	Amphipoda		 *Niphargus aquilex*#
Niphargus fontanus (Bate, 1859)			
Niphargus aquilex (Schiodte, 1855)			
Niphargus glenniei (Spooner, 1952)			
Microniphargus leruthi (Schellenberg, 1934)			
Crangonyx subterraneus (Bate, 1859)			
Proasellus cavaticus (Leydig,1871 sensu Henry, 1970)	Isopoda		 *Proasellus cavaticus*#1
Antrobathynella stammeri (Jakobi, 1954)	Bathynellacea		 *Antrobathynella stammeri*#2

* From Gledhill *et al.* (1993).
Photographs courtesy of Chris Proctor#, Lee Knight #1 & Mark Dunscombe#2

Since these animals only live in groundwater they make a unique contribution to biodiversity. The well shrimp *Niphargus glenniei*, is endemic to England, where it is only known from the counties of Devon and Cornwall. *Niphargus glenniei* was listed in the UK Biological Action Plan (BAP), as a priority species in June 2007. Listing on the plan signifies the UK Government's commitment to the protection of listed species and their habitats. The criteria for selection of *Niphargus glenniei* as a priority species was based on its extremely limited geographical distribution and highly specialised habitat, together with a lack of quantitative data, making threats to the species's populations currently impossible to predict (UKBAP, website). The action plan for *Niphargus glenniei* lists a requirement for species specific monitoring and surveying, providing a clear remit for further study into the distribution and occurrence of the species in the south-west of England (Knight 2009).

Protection of groundwater animals in the UK

Outside the UK BAP for *Niphargus glenniei*, groundwater fauna is poorly protected through any specific legislation, and essentially relies on protection by default via other criteria. For example, some cave systems are Sites of Special Scientific Interest (SSSIs), usually notified on geological grounds but are also an important habitat for groundwater animals. In a few cases the SSSI citation may mention groundwater species but this is not the justification for the notification. As mentioned earlier, the Water Framework Directive does not currently identify groundwater as having a biological quality element, nor does it refer to groundwater ecology. It does however promote a holistic approach to water management recognising the need to deal with water at the catchment scale. For example in paragraph 33 of the preamble to the Directive, it states that measures designed to achieve good water status within a river basin should be co-ordinated, between “surface water and groundwaters belonging to the same ecological, hydrological and hydrogeological system”. It also recognises the role of groundwater in governing the ecological quality of surface waters and terrestrial ecosystems. In this way, groundwater animals might be afforded some protection indirectly through improvements in water management and connectivity between systems.

Elsewhere, the Groundwater Directive (European Commission 2006), a daughter directive of the WFD, makes clear reference to the need for the protection of groundwater dependent ecosystems from deterioration and pollution. It also states within the preamble to the Directive (paragraph 20) that “Research should be conducted in order to provide better criteria for ensuring groundwater ecosystem quality and protection” and that such findings are considered when revising the Directive in future. Whilst stopping short of requiring protection of groundwater fauna it at least acknowledges the biological dimension and promises perhaps more targeted protection in the future. It is hoped that research such as that undertaken by this project will provide some of the necessary evidence, recommended by the Directive, and that future legislation will provide more robust protection for the groundwater habitat and its fauna.

2.0 Survey regions & overview

The aims of the project were addressed through the identification and examination of three separate survey study regions, listed below and illustrated in Figure 2.1.

Region 1: the counties of Dorset and Devon in south-west England

Region 2: parts of county Durham in north-east England

Region 3: parts of north east Lincolnshire and Yorkshire, England

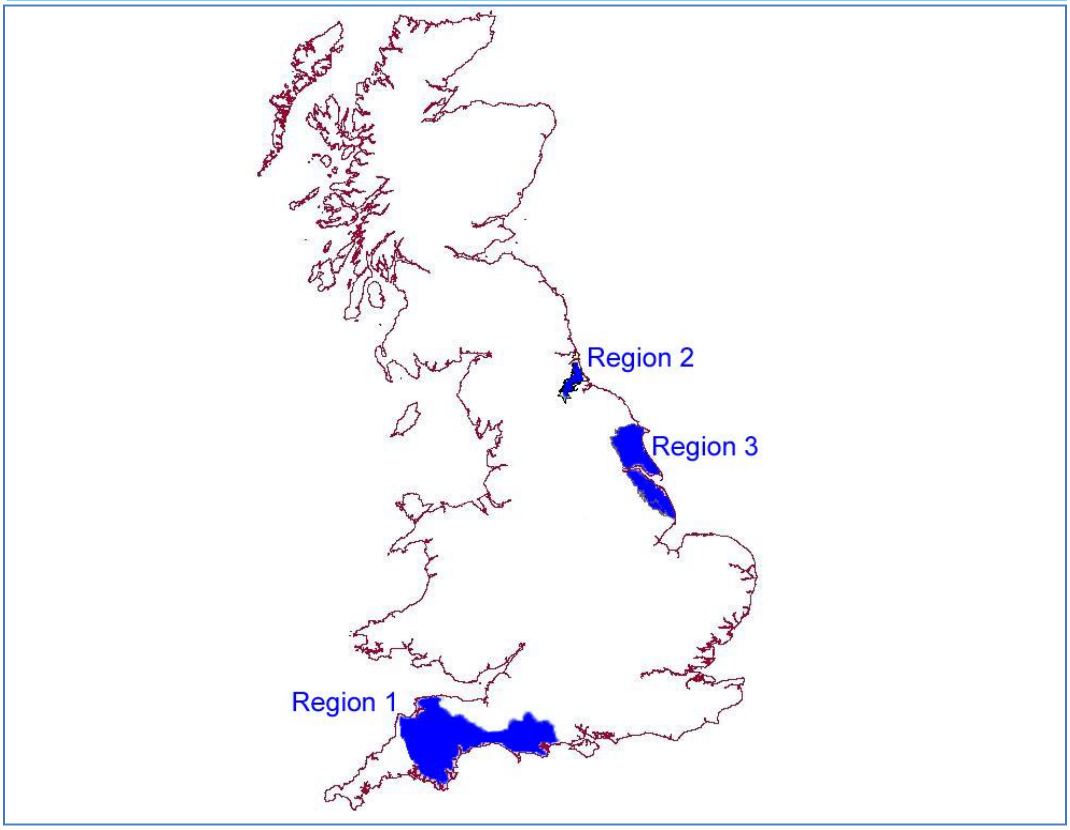


Figure 2.1: Map illustrating the three survey regions comprising the study area.

The majority of the survey work and sampling effort was focused within study Region 1. This was used to investigate the habitat preferences, distribution and abundance of groundwater fauna across a range of different geological units (project aim 1a). It was also used to test and develop sampling protocols (project aim 4) and to further the development of the Biological Action Plan for *Niphargus glenniei* (project aim 2). Additionally, Region 1 was used to investigate hyporheic invertebrate assemblages, examining selected reaches of the river Piddle in Dorset (project aim 3). The Region was the focus for workshops and talks to raise the profile and interest in groundwater fauna.

Regions 2 & 3 were selected to investigate the impact of the last (Devensian) glaciation upon the occurrence and distribution of groundwater fauna in England (project aim 1). The scale of this survey was much smaller than for Region 1 and focused on selected geologies either side of the Devensian glacial divide; i.e. the Magnesian Limestone in county Durham, glaciated during the Pleistocene, versus the Chalk either side of the Devensian glacial limit in North Lincolnshire and Yorkshire.

2.1 Region 1 – Dorset & Devon

2.1.1 Survey area rationale

Devon was selected principally because it is the county from where the majority of records for *Niphargus glenniei* have been recorded (http://www.freshwaterlife.org/hcrs/db). To assist in the development of the UK Biodiversity Action Plan for this species a systematic survey of the county is essential to establish a baseline of the species' distribution, and to gain an understanding of its habitat requirements. Whilst *Niphargus glenniei* is also known from Cornwall, the only county to the west, it is not known how far the species extends eastwards beyond Devon. Dorset, one of the two neighbouring counties to the east of Devon, was chosen to explore the easterly extent of the species. Ideally, Somerset, the other adjacent county, would have been included in the survey area but this was not possible within the time constraints of the project.

There are more than 40 different geological units on the British Geological Survey 1:625000 scale geological map Devon and Dorset. The strata have a range of permeabilities from low to high, and include a variety of both fractured and granular aquifers, as well as carbonate aquifers with varying degrees of kartsification. This variety of physical conditions, together with the different chemical properties associated with these rocks provide a range of environments in which to examine the habitat preferences, abundance and diversity of groundwater fauna within a relatively limited geographical range.

Finally, the river Piddle in Dorset was identified as a suitable river to carry out the investigation of the hyporheic zone (project aim 3). This Chalk stream has been previously well studied (e.g. Arnott, 2009) and recently subject to an in-depth hydrogeological modelling study.

2.1.2 Outline geology

The British Geological Survey 1:625000 scale geological map of the study region is presented in Figure 2.2.

Dorset is dominated by a wide band of Cretaceous Chalk running in a north-east to south-east trend through the middle of the county. To the east of this the Chalk is overlain by more recent sand and clay deposits of the Bracklesham Formation, which stretch all the way to Bournemouth and Poole on the coast at the eastern edge of the county. The Chalk outcrops again in a narrow east-west band in the far south eastern corner of the county before giving way to similarly narrow bands of older material characterised by the Upper and Lower Greensand, Weald clay, Purbeck and Portland limestone. South of the Chalk lie further outcrops of limestone along the coast with the Isle of Portland forming the most southerly point of the county. Moving to the north and west from the main chalk outcrop, a thin band of Upper Greensand and Gault is present, beyond which lie a mixture of outcropping Oolitic limestone and Oxford Clay and Kellaways Formations. In the far west of the county and into Devon the Lias Group dominates the landscape.

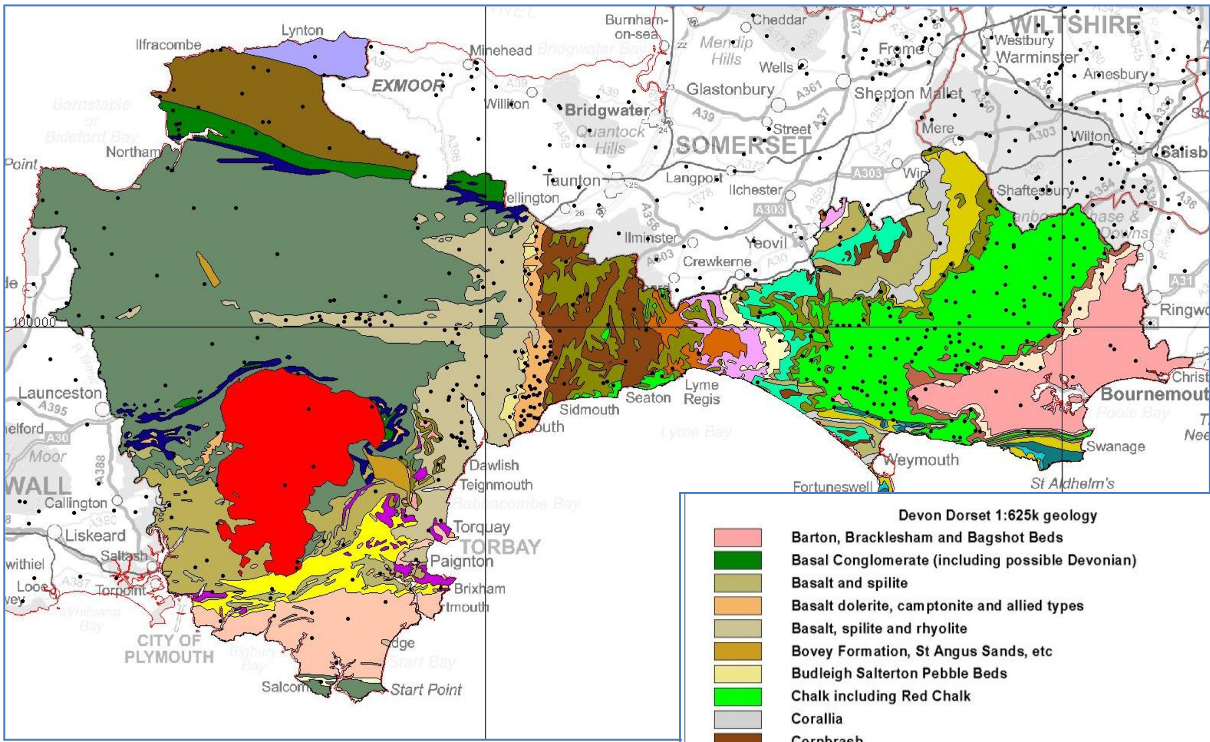


Figure 2.2: Solid geology map of Region 1 (Dorset & Devon) showing location of known borehole and well sampling points (black dots).

Moving further west into Devon the Lias Group gives way to Triassic mudstones interspersed with outcrops of Upper Greensand and Gault clay. Further west, bands of Permian and Triassic sandstones and mudstones running north-south dominate with Permian basal breccias. The latter extends into the centre of the county as a long "finger" running laterally to the west. On the northern and southern coastal fringes of the county, Devonian slates, sandstones and Old Red Sandstone outcrops dominate. At the most southerly tip of the county at Salcombe, there is a small but notable outcrop of metamorphic gneiss and mica schists. The centre of the county is characterised by the Upper Carboniferous Crackington Formation comprising shales and thin subordinate sandstones and siltstones, with thin bands of karstic Carboniferous Limestone present near the edges of the Crackington Formation. Moving south the landscape is dominated by a large igneous intrusion of granite, forming Dartmoor, with smaller extrusions of basalt, sillite and rhyolite occurring within the surrounding area of Devonian slates and sandstones. Also occurring within this area are small, thin bands of kartstified Devonian limestone.

In much of the study area the bedrock geology is present at the surface but in some areas it is overlain by superficial deposits. In many of the river valleys there are river terrace sand and gravel deposits and deposits of alluvium. There are also Clay-with-Flints deposits on some of the higher ground, particularly in east Devon. There are also Peat deposits present on the upland area of Dartmoor in Devon.

2.1.3 Hydrogeology

The diverse geological strata present in Devon and Dorset have a wide range of hydrogeological characteristics. They range from the highly permeable Cretaceous Chalk and Permo-Triassic sandstones which are the two most important water supply aquifers in England to low permeability poorly fractured mudstones and clays with limited groundwater flow.

In carbonate aquifers the rock matrix has very low permeability and groundwater flow is through fractures, and in particular fractures which have been enlarged by dissolution to form fissures (solutionally enlarged fractures that retain the planar geometry of fractures), conduits (more circular shaped voids), and in some cases caves (conduits large enough to enter). The carbonate aquifers present in the study area have varying degrees of karstification with subsurface and surface karst features occurring on an increasingly larger scale from the Jurassic Limestones, to the Cretaceous Chalk to the Devonian and Carboniferous limestones. All these aquifers contain areas of well connected networks of voids that should provide a good habitat for groundwater fauna.

Other permeable strata in the study area are the Permo-Triassic sandstones which are a combined fracture and granular aquifer. Some parts of these deposits comprise poorly consolidated sands and gravels in which groundwater flow is predominantly intergranular - through spaces between the grains. Other parts of the deposits are more consolidated and groundwater flow occurs predominantly through fractures. Permeability of the Permo-Triassic strata in Devon and Dorset is generally lower than in other areas of the UK (Allen *et al*., 1997). Permeability also varies locally depending upon the degree of consolidation and fracturing and the presence of lower permeability mudstone layers that occur within the Permo-Triassic deposits in this area. The water chemistry is also variable with some Permo-Triassic aquifers having carbonate rich waters and others having reducing acidic waters (Allen *et al*., 1997). Other combined fracture and granular aquifers (sands and sandstones) present in the study area include the Cretaceous Upper and Lower Greensand Formations, and parts of the Jurassic Upper and Middle Lias formations.

The Palaeogene aged Barton,Bracklesham and Bagshot Beds comprise sands and silts and clays in which groundwater flow is intergranular. These strata are classified as a minor aquifer, with the highest permeabilities where there are more extensive sand layers present.

Other strata in the study area comprise a range of rocks from sedimentary sandstones, siltstones and mudstones to volcanic rocks of igneous or metamorphic origin. Groundwater flow in these deposits is within fractures that are not enlarged by dissolution. The permeability of the deposits is variable and depends on the number of open fractures present and how connected they are. Permeability is generally higher in sandstones than siltstones and mudstones, and is variable in igneous and metamorphic strata.

To enable broad comparisons to be made between hydrogeology and groundwater fauna, the strata present in the study area were divided into hydrogeological categories as follows:

1) Low permeability strata

1a) Clay/mudstone

Generally very low permeability strata although small yields can been obtained from some boreholes where fractures are present. They include the Jurassic Kimmeridge Clay, Ampthill Clay and Oxford Clay Formations; the Jurassic Kellaways Formation and Lower Lias Group; The Cretacous Weald Clay Formation; The Eocene London Clay Formation.

1b) Metamorphic/igneous strata

Generally very low permeability strata dominated by fracture flow. Includes; Gneiss and mica schists; Hornblende schists; granite, syenite, granophyre and allied types.

2) Granular aquifers (low to moderate permeability)

These are unconsolidated sands, silts and clays in which groundwater flow is intergranular. They include the Palaeogene Barton, Bracklesham and Bagshot Beds and the Palaeogene Bovey Formation and St Angus Sands Formation.

3) Combined granular and fracture aquifers (high permeability).

3a) The Permian and Triassic sandstones.

These comprise the Permian Exeter Group and the Triassic Sherwood Sandstone Group which are separated by the low permeability Aylesbeare Mudstone Group. The Exeter Group and the Sherwood Sandstone Group are made up of fine to coarse grained sandstones and/or conglomerates. The Exeter Group is generally more consolidated and has higher permeability than the Sherwood Sandstone Group (Allen *et al*., 1997).

3b) The Jurassic Upper Lias and Middle Lias Groups.

These deposits comprise sands, sandstones and mudstones and include the Bridport Sand Formation which are high permeability unconsolidated sands.

3c) The Cretaceous Lower Greensand Formation.

Thin sand dominated deposits with moderate permeability but a limited recharge area in the study area.

3d) The Cretaceous Upper Greensand Formation and the Gault Formation.

The high permeability Upper Greensand and low permeability Gault Formation are undifferentiated on the British Geological Survey 1:625000 scale map and therefore remain in the same hydrogeological category despite their different properties. The Upper Greensand comprises sands and sandstones in which groundwater flow and storage can be both intergranular in the less well consolidated deposits and in fractures in the consolidated strata. Groundwater is generally in hydraulic continuity with the overlying Cretaceous Chalk Formation. The underlying Gault Formation comprises low permeability mudstones in which limited fracture flow may occur but it is unlikely that the Gault Formation would provide a good habitat for groundwater fauna.

4) Fractured bedrock: low to moderate permeability

4a) Upper Carboniferous Crackington Formation and Bude Formation.

The Crackington Formation comprises shales with thin subordinate sandstones and siltstones, and the Bude Formation comprises thick bedded massive sandstones with subordinate siltstones and mudstones. The sandstones are well cemented and the primary porosity is low therefore groundwater flow and storage is in fractures. Transmissivity from 53 pumping tests varied from 0.2 to 245 m2/d with an arithmetic mean of 11 m2/d. Groundwater yields from the Bude Formation are slightly higher than those from the Crackington Formation.

4b) Devonian Sandstones.

These include units mapped on the British Geological Survey 1:625000 scale geological map as: the Upper Devonian and Old Red Sandstone and Middle Devonian; Lower and Middle Devonian; Lower Devonian; Middle Devonian; Upper Old Red Sandstone and Upper Devonian. These deposits comprise slates and sandstones. The sandstones are well cemented and have low porosity. Groundwater flow and storage in the slates and sandstones is in fractures, and the permeability of the slates tends to be higher because they are more fractured. Transmissivity from 66 tests in upper, middle and lower Devonian varied from 0.4 to 177 m2/d with an arithmetic mean of about 10 m^2.

4c) Igneous strata.

These include units mapped on the British Geological Survey 1:625000 scale geological map as: Basalt, dolerite, camptonite and allied types; basalt, spillite and rhyolite; Rhyolite, Trachyte, felsite, elvans and allied types; basalt and spillite; Tuff. They comprise small outcrops of low porosity rocks with any groundwater flow via fractures.

4d) Triassic mudstones.

These include the deposits mapped on the British Geological Survey 1:625000 scale map as Keuper Marl, Dolomitic conglomerate and Rheatic. These deposits predominantly comprise low permeability mudstones but thin siltstone and sandstone layers occur and in places these may have moderate permeability, with groundwater flow and storage predominantly in fractures.

4e) Basal conglomerate (Carboniferous, including possibly Devonian).

Mainly low permeability mudstones and shales with higher permeability where fractures are present.

4f) Namurian (Millstone Grit Series).

These comprise sandstones in which any groundwater flow and storage is in fractures.

4g) metamorphic/igneous strata (including granite)

These include units mapped on the British Geological Survey 1:625000 scale geological map as: The largest outcrop is Granite, stenite, granphyre and allied types. Groundwater flow is via fractures and permeability varies depending upon the number and connectivity of fractures present.

5) Carbonate aquifer: low to moderate level karstification

5a) Jurassic Limestones.

These include the deposits mapped on the British Geological Survey 1:625000 scale map as: Portland Beds; Purbeck beds; Corallian; Cornbrash; Great Oolite; Inferior Oolite. These are carbonate rocks with high permeability in fractures enlarged by dissolution.

5b) The Chalk.

The Chalk is the most extensive outcrop in Dorset and as discussed above is a high permeability carbonate aquifer with frequent solutional enlargement of fractures to form fissures and conduits.

6) Carbonate aquifer: high level karstification

These include the Tournasian and Visean (Carboniferous Limestone series); and Devonian limestone. These deposits are limestones with low matrix porosity and high permeability from fracture flow, with significant solutional development forming fissures and caves. Permeability is highly variable with very high permeability in conduits and caves, but permeability can locally appear low where boreholes have not intercepted permeable fractures.

2.2 Region 2 - County Durham

2.2.1 Survey area rationale

This region in county Durham was chosen as it provides the largest single outcrop of Magnesian limestone in the country, and offers potentially suitable habitat for stygobitic fauna. Additionally, the region is an area well within that part of the country which was covered by ice during the Devensian glaciation and therefore suitable for investigation of the impact of glaciation on the British stygofauna community. A map of the Region showing the outcrop of Magnesian limestone and superficial deposits is provided in Figures 2.3 and 2.4.

2.2.2 Geology and hydrogeology of survey area

The Magnesian Limestone is a carbonate aquifer in which the rock matrix has low permeability (Allen *et al*., 1997) and groundwater flow is predominantly in fractures that have been enlarged by dissolution. Small caves are thought to be present in the Magnesian Limestone (Allen *et al*., 1997) suggesting a low-moderate degree of karstification, and indicating that there are likely to be good physical habitats available for groundwater fauna within solutional voids. Permeability is locally very variable. It can be sufficiently high to provide public water supplies but can also be low where boreholes do not intercept fractures. In the study area the Magnesian Limestone is overlain by superficial glacial till deposits which may limit active recharge to the limestone (Fig 2.3 and 2.4)

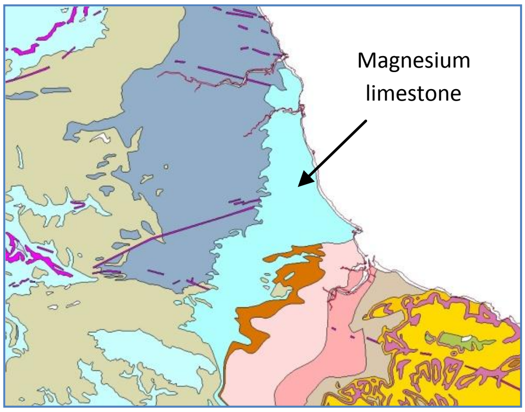


Figure 2.3: Extent of Magnesium Limestone in study Region 2. (based on the British Geological Survey 1:625000 bedrock geology map).

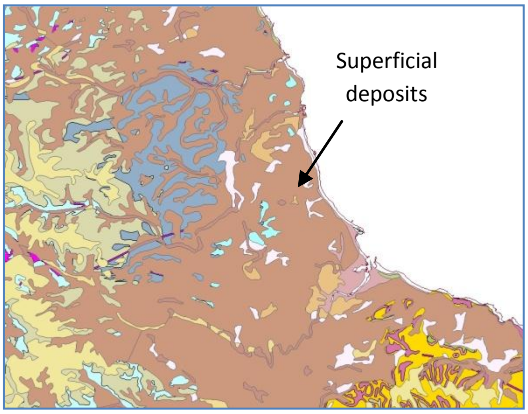


Figure 2.4: Extent of superficial deposits in study Region 2. (based on the British Geological Survey 1:625000 bedrock geology map).

2.3 Region 3 – North east Lincolnshire & Yorkshire

2.3.1 Survey area rationale

This region was selected because it provides a single geological formation (the Chalk) which straddles the Devensian glacial limit. In this way comparison of similar lithologies can be investigated for stygofauna within the context of the Devensian glaciation. The region represents the furthest extent of the Chalk in England and could present some contrasting faunal communities with the Chalk examined further south in study Region 1 and elsewhere. The extent of the Chalk and superficial deposits covering it is illustrated in Figures 5 and 6.

2.3.2 Geology and hydrogeology

The Cretaceous Chalk in northern England has broadly similar geological and hydrogeological characteristics to the Chalk in Southern England. However, the Chalk in Northern England is harder and consequently has a lower matrix porosity than the Chalk in Southern England and it also has a higher permeability which is believed to be due to a higher degree of fracturing (Allen *et al.*, 1997). In Northern England where the Chalk is overlain by extensive superficial glacial till deposits it is confined (Allen *et al.*, 1997) meaning that the water is under pressure. Where the Chalk is confined, dissolved oxygen levels are low because there is limited circulation of water and recharge areas may be several kilometres away. Figure 2.5 shows the extent of the Chalk in Northern England and Figure 2.6 shows where the Chalk is overlain by superficial glacial till deposits (from the British Geolgoical Survey 1:625000 scale geological maps). The blue line marks the approximate glacial extent during the last glacial maximum and is based primarily on the more detailed British Geological Survey 1:10000 scale superficial geology map of the area.

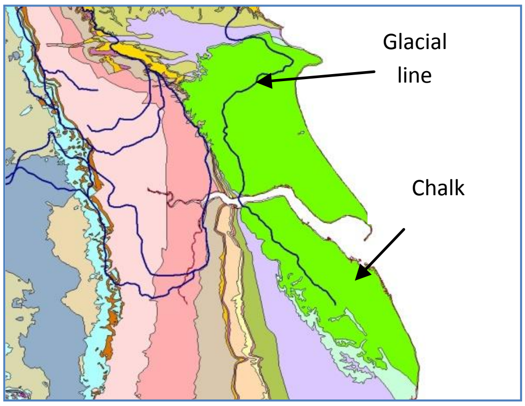


Figure 2.5: Extent of the Chalk in study and glacial divide in Region 3. (based on the British Geological Survey 1:625000 superficial geology map).

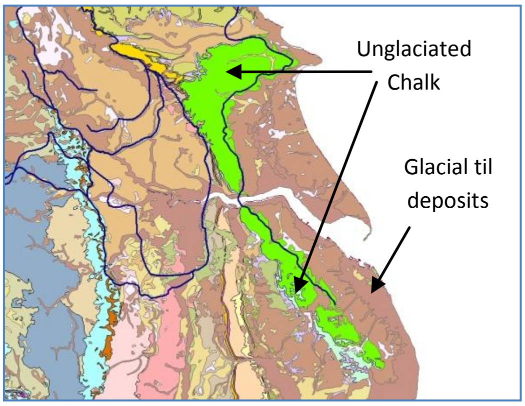


Figure 2.6: Extent of superficial deposits and glacial divide in study Region 3. (based on the British Geological Survey 1:625000 superficial geology map).

2.3.3 Survey areas

To address the aims of the project three surveys were carried out across the three study regions. The main study, which comprised the largest survey of sites, was conducted across Dorset and Devon (survey Region 1). This comprised a survey of nearly 200 boreholes, wells and springs, conducted over eight months. The second survey comprised a more limited study of 38 boreholes in northern England to examine differences in groundwater faunal communities across the Devensian glacial divide. This survey necessitated a study across geographically separated areas and so was conducted across the survey Regions; 2 and 3. The final survey was the most localised survey and was concerned with the hyporheic element of the study (Project aim 3) where the groundwater communities of two hydraulically contrasting sections of the river Piddle were compared. The River Piddle, a Chalk stream in Dorset is located in study Region 1.

The methods and analyses of each of these three surveys are reported under separate titles within the report. A summary of the survey remits, Regions conducted and report section reference is provided in Table 2.1.

Table 2.1 Summary of surveys, locations and report aims

Survey Name	**Survey Area**	**Study Aim**	**Date of survey**	**Report section**
Survey A Dorset & Devon	Region 1	1a, 2 & 4	Sept 2009 - April 2010	3.0 – 5.0
Survey B Northern England	Region 2 & 3	1b, 4	June 2010	6.0 – 8.0
Survey C River Piddle	Region 1	3, 4	Jan-Feb 2011	9.0 – 11.0

3.0 Survey (A) – Dorset & Devon

This survey focussed on identifying and sampling a representative number of boreholes, wells or springs from the different geological units accessible within the study area. The aim was to access and sample a total of 200 sites, split up across the different geologies present and to recover biological and chemical samples from each, as well as recording physical and environmental parameters. The survey work was carried out over eight months from late September 2009 to early May 2010. This period covered the season of aquifer recharge, commencing at a time when groundwater levels are usually approaching their lowest annually, and progressing through the winter recharge period to the annual maximum.

3.1 Survey strategy

As described in Section 2.1 there are in excess of 40 different geological strata mapped at the 1:625000 geolgoical scale within Region 1, and many hundreds mapped at the 1:50000 scale. It was not feasible to sample all geologies individually therefore the sampling strategy aimed to sample a representative number of sites in each of the hydrogeological categories described in Section 2.1.3. These included the six main categories (termed Hydro-Units) and the further 14 subdivisions (termed Hydro-Sub-units). A further two units were created later to cover superficial deposits and mixed aquifer respectively, which were identified post sampling through analysis and review of geological logs. In all, 18 different hydrogeological units were defined.

The surface coverage of the initial 16 Hydro-Units was calculated as a percentage of the whole study area using ArcGIS. This was then used to determine a proportionate number of sampling points derived from the overall target number of 200 points. The different Hydro-sub-units and their respective target number of sampling points are described in Table 3.1 and illustrated in Figure 3.1.

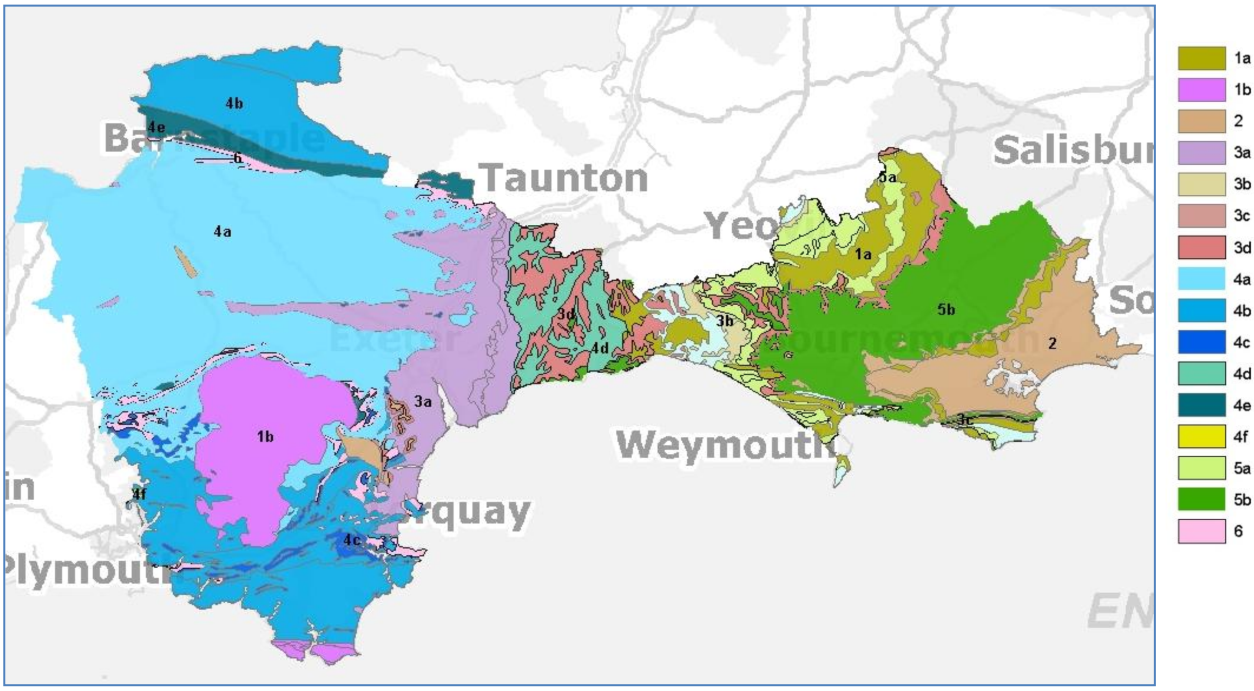


Figure 3.1. The Hydrogeological sub-units identified across study Region 1.

Table 3.1. Generalised description of Hydro-unit with target number sampling points.

Hydro-Unit	Description	Permeability	Level of Karst-ifcation	Percentage surface coverage	No. sample pts	
					Target	Actual
1a	Clay / mudstone	Very low	N/A	5.37%	11	3
1b	Metamorphic / igneous			7.11%	14	13
2	Granular aquifers	Low to mod	N/A	6.35%	13	5
3a	Permian Triassic Sandstones	High	N/A	8.97%	18	19
3b	Upper Lias			1.55%	3	5
3c	Lower Greensand Formation			0.09%	0	0
3d	Upper Greensand Formation			4.65%	9	15
4a	Upper Carboniferous	Low to mod	N/A	28.67%	57	33
4b	Devonian & Old Red Sandstone			14.79%	30	21
4c	Igneous			1.44%	3	6
4d	Triassic mudstones			2.76%	6	1
4e	Basal conglomerate			1.88%	4	4
4f	Namurian (Millstone Grit series)			0.02%	0	0
5a	Portland Bed	High*	Low to Mod	4.03%	8	16
5b	Chalk			10.20%	20	38
6	Carboniferous & Devonian limestone series	High	High	2.13%	4	7

* Chalk has dual porosity (low permeability in matrix with high permeability in fractures)

In some cases the proportion of area covered by the Hydro- unit was so small as to make the sampling of these units, on the basis of 200 site target, unfeasible. In such cases (3c & 4f) these units were ignored in the survey plan. For all the other units the strategy was to identify and sample the target number of sites <u>once</u> during the survey window of September 2009 to May 2010.

3.2 Preliminary site selection

Suitable sampling point locations were identified using the Environment Agency's Boreholes, Wells and Springs (BWS) database and the British Geological Survey's Wellmaster database. Sites were chosen on the basis of accessibility (both in terms of safe access and the ability to sample down the borehole or well with a net) and their location, to achieve a wide as possible distribution of sites within each Hydro- unit. This was not possible in all cases either through lack of boreholes in some units or the borehole's construction. Where shortfalls were encountered springs and tapped boreholes (those only accessible via a tap outlet), were also considered. For most Hydro- units sufficient sampling point locations were identified, the exceptions were Hydro- units 1a, 1b and 4a. Additional sampling points in these units were addressed in the field through map assisted observation and local knowledge.

In all 258 sites were visited, from which samples were obtained from 198. The majority of these were boreholes or wells (164) with the remainder (34) comprising spring sources. The distribution of these was inevitably dictated by their location and accessibility. A map showing all the sites visited is provided in Section 4.0, Figure 4.3.

3.3 Sampling protocol

The following ordered process was adopted at each site visited with variations depending on the sampling point type, dimensions and access arrangements. At each site a record sheet was completed recording the precise sampling details and readings. A copy of the standard sampling record sheet is provided in Appendix 3.

a) Site location data: - the date, time and accurate national grid reference (NGR) were recorded together with the surrounding environmental setting. The latter was recorded on a three point scale to cover the range of environments encountered. Photographs of each site and its surroundings were also taken.

b) Sampling point characteristics: (Boreholes and wells) - the diameter, depth (to groundwater and to the borehole base), height of borehole top above ground and approximate distance to water course, where relevant, were recorded. The purpose of the sampling point (e.g. monitored, disused, pumped) and whether it was an Environment Agency monitored borehole was also noted. At spring sites and where access to the borehole or well was not possible, the borehole dimensions were not recorded.

c) Field chemistry: Using a hand held multi-probe (YSI 600QS)[2], measurements of electrical conductivity, temperature, pH and dissolved oxygen were taken at each sample point. For boreholes or wells, where access was possible, the probe was connected to a 100m reel and lowered to the borehole base. The probe was lifted approximately 0.5m off from the borehole base and left until the readings stabilised. In-situ measurements of groundwater quality were recorded and the probe was then recovered from the borehole. Where access to the borehole base was not possible and in the case of spring sampling points the YSI probe was either placed in a header tank or spring catchpit[3] to record the field chemistry. Failing these options, a sample of groundwater was recovered via a pump or bailer and retained in a bucket into which the YSI was placed (Note: dissolved oxygen measurements recorded this way were deemed unreliable and excluded from the dataset used in the analysis). Deviations from the preferred in-situ method were recorded in the comments box on the site record sheet.

d) Chemical sampling: Groundwater was collected from each sampling point. At boreholes and wells this was usually achieved using a disposable Teflon bailer lowered below the resting water level to the base of the borehole on a cord. Three bailed volumes were taken to fill the sample bottles. Where direct access to the borehole was not possible or the diameter of the borehole too narrow for the bailer, an alternative sampling method was used. This involved either using 19mm Waterra tubing placed down the borehole and pumped by hand or where an in-situ pump and tap was in place a sample was recovered directly from this, taking care to ensure the source preceded any external water treatment process. In some cases these options were not available and the only option was to recover a sample from a header tank to which the borehole was pumped. For springs, groundwater was recovered directly from the spring source or catchpit. On completion of the task all sampling kit was washed with clean water before sampling the next site.

[2] The instrument was calibrated in standard solutions in the laboratory, prior to each sampling event.
[3] Catchpit: a man-made pit, usually of concrete or stone construction, designed to capture spring flow

Groundwater samples were decanted into three separate plastic sample bottles, filled to the top, for the following purposes:

(i) One 1 litre PET[4] bottle for ammoniacal nitrogen and dissolved organic carbon (DOC) analysis.

(ii) Two 10ml Bijou bottles for anion and cation analyses.

Each bottle was labelled with a site reference and dated before being transferred to a cool box. On completion of the day's field work the 1 litre PET bottles were placed in a refrigerated cold store before being transferred to the Environment Agency's laboratory in Leeds[5], usually within 72 hours. The bijou bottles were placed directly in to a freezer and frozen before being transferred in bulk, at the end of all the field work, to the laboratory at Roehampton University[6].

e) Biological sampling: At all sampling sites groundwater samples recovered for chemical analysis were filtered through a dedicated 63µm net to capture any animals present within the water column. Material collected in this way was washed into a receptacle and amalgamated with the main biological sample.

For boreholes and wells the preferred method of sampling used an adapted plankton net lowered to the base of the borehole. This was applied at the majority of sites, with the exception of sites where access to the borehole headworks was not possible or where spring sites were sampled. Four distinct sampling protocols were developed for the various situations, described below:

I. Protocol for the sampling of open boreholes and wells:

A plankton net (63µm mesh), with a screw on filter and a metal weight, the size of which was varied according to the net size, hanging below it, was attached to a 100m cable tape and lowered to the measured base of the borehole. The diameter of the net used varied (3.5-30 cm), using the largest diameter possible, relative to the borehole aperture. Figure 3.2 shows the range of sampling nets used. On reaching the base the net and weight were lifted about a metre back up the borehole and dropped to disturb the bottom sediment. This action was repeated six times before the net was lifted out and removed from the borehole. Material captured in the net and filter was recovered and retained in a plastic sampling bag. The net was then lowered down the borehole again and the procedure repeated a further two times. On completion of the last net haul the net was thoroughly washed down with clean water and a final inspection made of the net and filter to ensure all sample material had been collected.

[4] PET = Polyethylene terephthalate
[5] EA laboratory holds mCerts and UKAS accreditation certificates for these laboratory analyses.
[6] Roehampton University is a non-accredited laboratory.

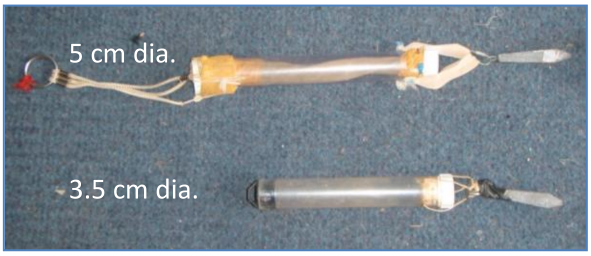


Figure 3.2: Range of plankton sampling nets used for collection of biological samples

***II. Protocol for the sampling of pumped boreholes and wells*:**

Where a net could not be lowered down a borehole or well, an alternative approach, based on the individual site circumstances, was used. For pumped samples a plankton net (63µm mesh) was placed under a tap or other outlet which was then opened fully while the pump was in operation. Although the pump rate could not be controlled the time the pump was running, with groundwater flowing through the net, was standardised at 5 minutes. On completion the net was removed and a final inspect of the net was made to recover all captured material.

III. Protocol for the sampling of header tanks and spring catchpits:

Where no suitable tap outlet was available it was often possible to sample sediment from the base of a header tank using a small hand held pond net (15cm^2 net frame with a 250µm mesh) with attachable handles added, when required, to reach to a maximum depth of about 2.5m. This technique was also used for spring catchpits and some shallow wells. The use of this type of net was justified on the basis of its greater manoeuvrability over the weighted net, where a more effective, controlled sweep of the tank or catchpit could be made. The larger mesh size was selected to avoid clogging of the net, a particular problem in spring catchpits which were frequently blocked with vegetation. The number of net sweeps was tailored to each tank/catchpit depending on the tank dimensions (which varied enormously between sites) and the amount of material present. A standard three net hauls were recovered from each tank/catchpit, as per protocol (1) for borehole sampling. Again, upon completion of sampling a final inspect of the net was made to recover all captured material.

IV. *Protocol for the sampling of spring sources*

Where there was no catchpit, spring samples were recovered either using a Bou Rouch pump or, where ground conditions were unsuitable for the Bou Rouch, a trowel and pond net. The latter involved using the trowel to disturb the sub-surface of the spring source and a pond net to collect the disturbed material, as described by Knight & Penk (2010). Where the Bou Rouch pump (see Figure 3.2) was used this involved hammering a 1.2m long steel pipe (perforated with 5mm holes in the bottom 100mm) into the gravel at the spring source to approximately 50cm below the surface. A hand-operated piston pump was then attached to the top of the pipe and the hand pump operated for a set time of 5 minutes. A pipe net (63µm mesh) was placed over the outflow from the pump via which abstracted material was recovered. A summary of the four sampling protocols, the various net types and other equipment used is presented in Table 3.2.

Figure 3.3. Bou-Rouch pump components (A) and in operation in spring source (B).

Table 3.2. Summary of sampling protocols applied with net types used.

Sample protocol	Sampling method	Net mesh size	Net dimensions	No. sites used at (out of 198)
I	Net down borehole/well	63µm	3.5, 5, 13, 20, 30cm dia.	117
II	Pumped outflow filtered through net	63µm	20cm dia.	21
III	Well base with pond net sweep	250µm	25cm^2	15
	Header tank with pond net sweep	250µm	25cm^2	11
	Spring catchpit with pond net sweep	250µm	25cm^2	29
IV	Trowel and pond net at spring source	250µm	10cm^2	2
	Pipe net from Bou Rouch hand pump.	63µm	Pipe net	3

Post sampling protocol and sampling preservation:

After each net haul or sample sweep, the net was washed down with clean water and the screwed filter end cap (where the plankton net was used) was removed. The net/filter was inspected for visible stygobites using a 10x hand lens. Where specimens were found these were placed in a separate labelled vial preserved in absolute ethanol. The contents of the net/filter were washed into a sterile plastic bag using absolute ethanol. Where used, the filter was reattached to the net and the relevant sampling process repeated. On completion of the last net haul or sweep, a final inspection of the washed net and filter was undertaken ensuring all sample material was collected.

On completion of each sampling event the equipment was thoroughly washed with clean water in preparation for the next sample site. All samples were collected in plastic sampling bags which were sealed and placed in another bag, together with any vials of specimens, sealed again and labelled with the site reference and date. Sample bags were placed directly in a cool box and transferred to a cold store at the end of each day.

3.4 Laboratory analysis

Chemical analysis: The 1 litre PET bottles dispatched to the Environment Agency laboratory were analysed within approximately two weeks from date of dispatch. The analysis was carried out under UKAS accredited analytical techniques. For DOC this was analysed using a Skalar air segmented flow analyser, with a minimum reporting value of 0.2mg/l. Ammoniacal nitrogen was determined by colourimetric analysis using a Konelab discrete analyser with a minimum reporting value of 0.03 mg/l.

The frozen water samples collected in the 10ml bijou bottles were dispatched and stored at Roehampton University Life Sciences laboratory. The thawed samples were analysed for major anions and cations by High Pressure Liquid Chromatography (HPLC) using a Dionex ED40 Electrochemical Detector with a minimum reporting value of 1.00 mg/l.

Biological analysis: Each stored sample was processed through two stacked sieves, descending in order of mesh size from 250μm to 63μm. Clean water was used to wash all the collected material out of the plastic sample bag into the top sieve, where it was gently washed through with water to remove the fine sediment. Small amounts of material from the contents of each sieve were placed in a petri dish with water and inspected under a dissecting microscope. This process was continued until all the sample material had been examined. The majority of the aquatic invertebrates found were identified to the lowest taxonomic level possible and placed in absolute ethanol in separate vials for storage. All vials were labelled with the site identifying details and dated. At the end of the project all samples will be retained at Roehampton University. The taxa specific key used for the identification of Groundwater stygobitic Crustacea was; British Freshwater Crustacea Malacostraca (Gledhill *et al.*, 1993).

3.5 Data management

The data from field record sheets were transferred and stored within a database constructed in Microsoft Excel 2007. The data were divided into separate categories; environmental and field data, chemical data and biological data, and related to a unique sampling reference number. By use of a pivot table and user interface simple data queries were carried out. The, software package PASW Statistics (ver.17) was used for more detailed statistical analysis. A copy of the data will be provided on the project website (http://new.freshwaterlife.org/web/gwa-uk) within two years from the publication of this report.

4.0 Data analysis –Survey (A) Dorset & Devon

4.1. Biological data

This survey focused on recording the presence or absence of stygobitic and other taxa. All the sites surveyed (with one or two exceptions) had not, as far as we are aware, been surveyed for groundwater fauna previously and the data produced therefore represents many new records for stygobitic Malacostraca for Dorset and Devon. As outlined in Section 3.2, the survey also recorded abundance of individual specimens collected and while this is presented here no interpretation of population sizes should be assumed.

Taxa composition: The majority of sites contained a range of different taxon groups with only three sites containing just stygobitic Crustacea and nine sites containing only Stygophilic Crustacea. The number of sites from which each broad taxonomic group were recorded is illustrated in Figure 4.1, as a set of all sites sampled.

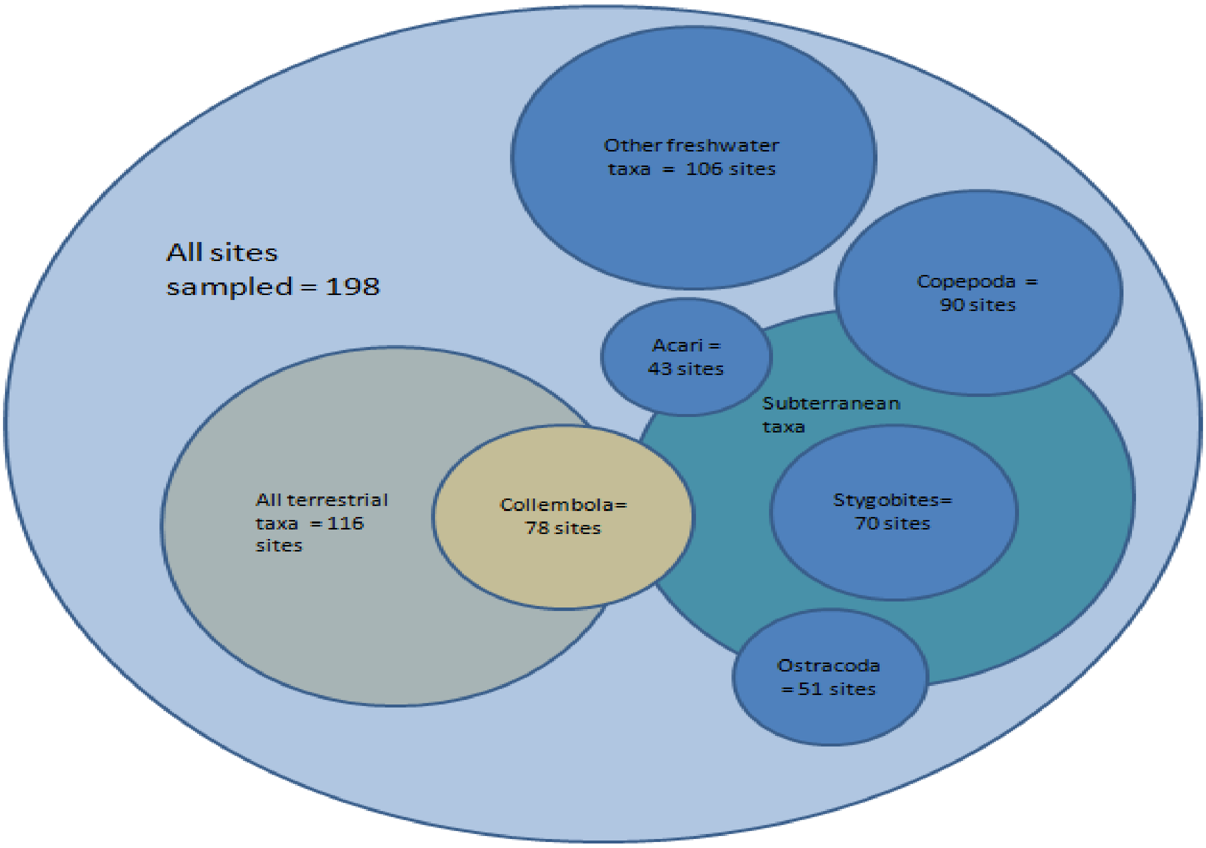


Figure 4.1 Relative proportions of all taxa from all sites sampled

Of the 198 sites visited and sampled three quarters (77%) contained freshwater taxa, many of which also contained terrestrial taxa. Of the remaining portion (23%), 8% contained terrestrial taxa and 15% recorded no taxa. A little over a third of all the sites sampled contained stygobitic Crustacea (35%). Potential stygophilic Crustacea (Copepoda and Ostracoda) were present in just under a half of all samples (49%), whereas Acari were present in about a quarter of the samples (21%). Finally, about a third (39%) of all sites contained Collembola (terrestrial taxa) many of which appeared to lack pigment or eyes, suggesting a troglodyte existence.

Whilst potential stygophilic taxa and Collembola were collected during this survey, and were present in many of the samples recovered, they were not the main focus of this project. This was partly due to time constraints and the expertise required to identify these groups. The taxanomic identification of the specimens collected is currently in progress the results of which will be reported separately at a later stage.

Additionally, there was one record of a freshwater hydra species from an old well in Dorset. The occurrence of freshwater Hydrozoa in subterranean habitats is rare, with the majority of records reported from interstitial habitats near large rivers in central Europe and also from some cave habitats in Australia, North America and Europe (Zagmajster *et al.,* 2011). Records from wells are particularly sparse with one species reported from a well in Switzerland (Chappuis, 1922.)

Stygobitic taxa composition: The survey found all eight stygobitic Crustacea species known from England. The species list and number of sites at which they were found is presented below (Table 4.1) together with their county locality and total abundance. These data are illustrated graphically in Figure 4.2. Here the number of sites where the different species were found is represented as a proportion of all sites where stygobites were present.

Table 4.1. Stygobitic species recorded from the survey of 198 sites in Dorset and Devon.

Species	No. of sites present	Total abundance	Locality
Niphargus kochianus kochianus	24	181	Dorset
Niphargus fontanus	12	28	Dorset
Niphargus aquilex	19	83	Dorset & Devon
Niphargus glenniei	19	79	Devon
Crangonyx subterraneus	12	44	Dorset
Microniphargus leruthi	6	10	Dorset & Devon
Proasellus cavaticus	5	10	Dorset
Antrobathynella stammeri	1	1	Devon

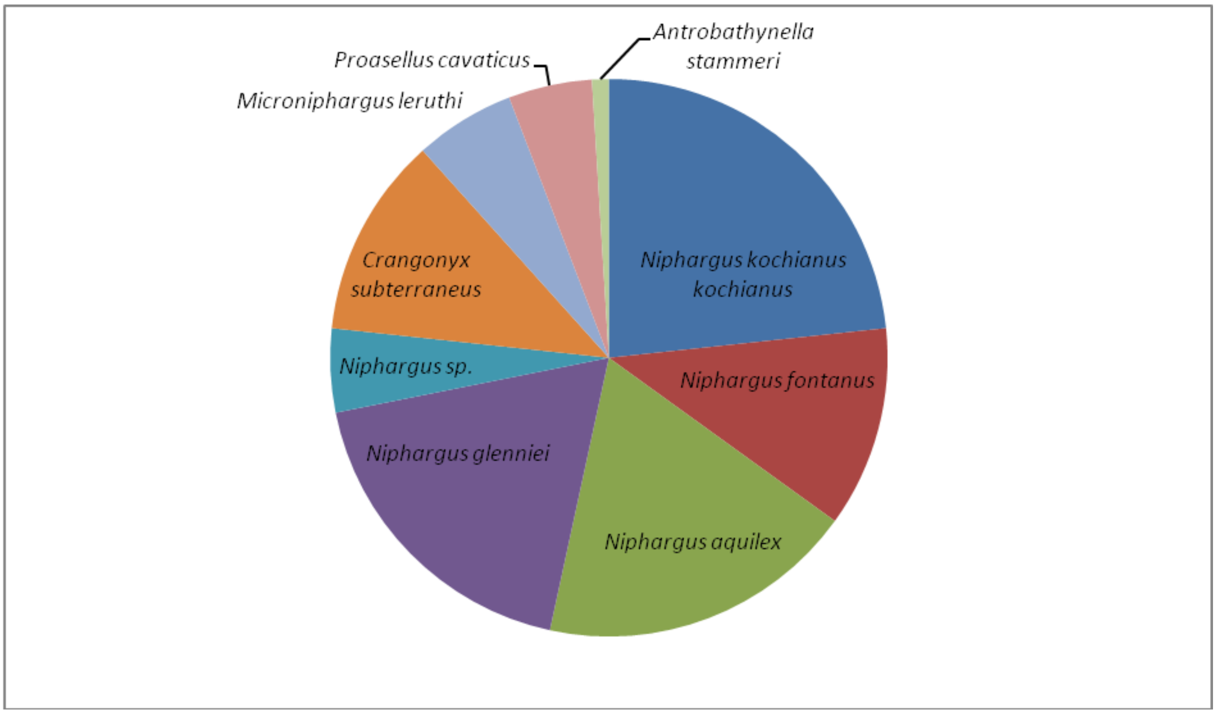


Figure 4.2 Prevalence of stygobitic taxa expressed as a proportion of sites where species recorded

Stygobite presence: Stygobites were recorded in all the sampled hydrogeological units and the superficial deposits (Units 1-7). This was also true for the sub-units (Units1a, 1b etc.), with the single exception of sub-unit 4b (Triassic mudstone). Table 4.2 shows the species present within each hydrogeological unit and the number of sites where stygobites were found in each of these units and sub-units. It also presents a crude measure of the sampling success per unit as a percentage of the number of sites sampled in each unit.

The geographical distribution of all the species recorded, in relation to the hydrogeological units identified across the study region, is illustrated in Figure 4.3 (individual species maps are provided in Appendix 4). Figure 4.3 shows all sampling locations. Only two species, Niphargus *aquilex* and *Microniphargus leruthi* were found in both counties (Table 4.1). *Niphargus glenniei* was exclusively found in Devon, as was *Antrobathynella stammeri* although the latter is also known from elsewhere in Britain. All other species were found in Dorset. Several specimens were not identified due to their poor condition or because they were juveniles. The majority of these specimens were associated with one site in Dorset where a large number of *N. kochianus kochianus* were also recorded.

The distribution of stygobites across the different hydrogeological units and sub-units was not even and was therefore investigated using non-parametric statistics. A Chi-squared cross-tabulation was performed using PASW Statistics (ver.17) to determine whether an association existed between the categorical variables of stygobite presence and the hydrogeological units. This was performed initially with the main hydrogeological-units and then again with the hydrogeological-sub-units. The null and alternative hypotheses were:

H^0 = Hydro-units (or sub-units) are not associated with stygobite occurrence

H^1 = Hydro- units (or sub-units) are associated with stygobite occurrence

For Hydro-units the Pearson Chi-Square value was 13.527 (6 df), p =0.035. For Hydro sub-units the Pearson Chi-Square value was 33.815 (15 df), p=0.040. In both cases at the 0.05 level of significance the null hypotheses is rejected and an association between hydrogeological units or sub-units and stygobite occurrence exists. Some caution should be taken in interpreting these results given the low expected frequencies in a number of the units, that is the number of samples recovered from the different units varied considerably.

The number of sites where stygobites were found appears in Table 4.2. The distribution of each stygobite species is presented in Figure 4.2.

Table 4.2 Stygobite species presence and success rate at sampling sites.

Hydro Unit Ref.	No. of sites sampled in Hydro-Unit **(A^1)**	**Hydro Sub- Unit** Ref	No. of sites sampled in Hydro Sub-Unit t **(A^2)**	**Stygobitic species recorded** in each defined Hydrogeological unit and Sub-unit.	No. sites with stygobites in Hydro Unit **(B^1)**	No. sites with stygobites in Hydro Sub-Unit **(B^2)**	**Sampling efficiency** [No. sites with stygobites / total No. sites sampled] **Hydro unit** (B^1/A^1) x 100%	**Sub Hydro** (B2/A^2) x 100%
1	16	1a	3	*N.kochianus kochianus*	8	1	50%	33%
		1b	13	*N. aquilex, N. glenniei*		7		54%
2	5	-	5	*N. aquilex*	1	-	20%	-
3	39	3a	19	*N. aquilex, N. glenniei, Antrobathynella stammeri*	10	4	15%	21%
		3b	5	*Crangonyx subteraneous*		1		20%
		3d	15	*N.kochianus kochianus, N. fontanus, N. aquilex, N. glenniei*		5		33%
4	65	4a	33	*N. aquilex*	17	3	26%	9%
		4b	21	*N. aquilex, N. glenniei, Microniphargus leruthi*		9		43%
		4c	6	*N. aquilex, N. glenniei*		3		50%
		4d	1	No stygobite records recorded		0		0%
		4e	4	*N. glenniei, Microniphargus leruthi*		2		50%
5	54	5a	16	*Crangonyx subterraneus, Proasellus cavaticus, N. aquilex*	27	3	50%	19%
		5b	38	*N.kochianus kochianus, N. fontanus, Microniphargus leruthi Crangonyx subterraneus, Proasellus cavaticus,*		24		63%
6	7	-	7	*N. glenniei*	1	-	14%	-
7	5	-	-	*N. aquilex, N. fontanus,*	3	-	60%	-
Mixed*	7	-	-	*N. aquilex, N. glenniei*	2	-	28%	-

* Mixed = those sites where examination of logs indicated more than on unit was intercepted within the borehole.

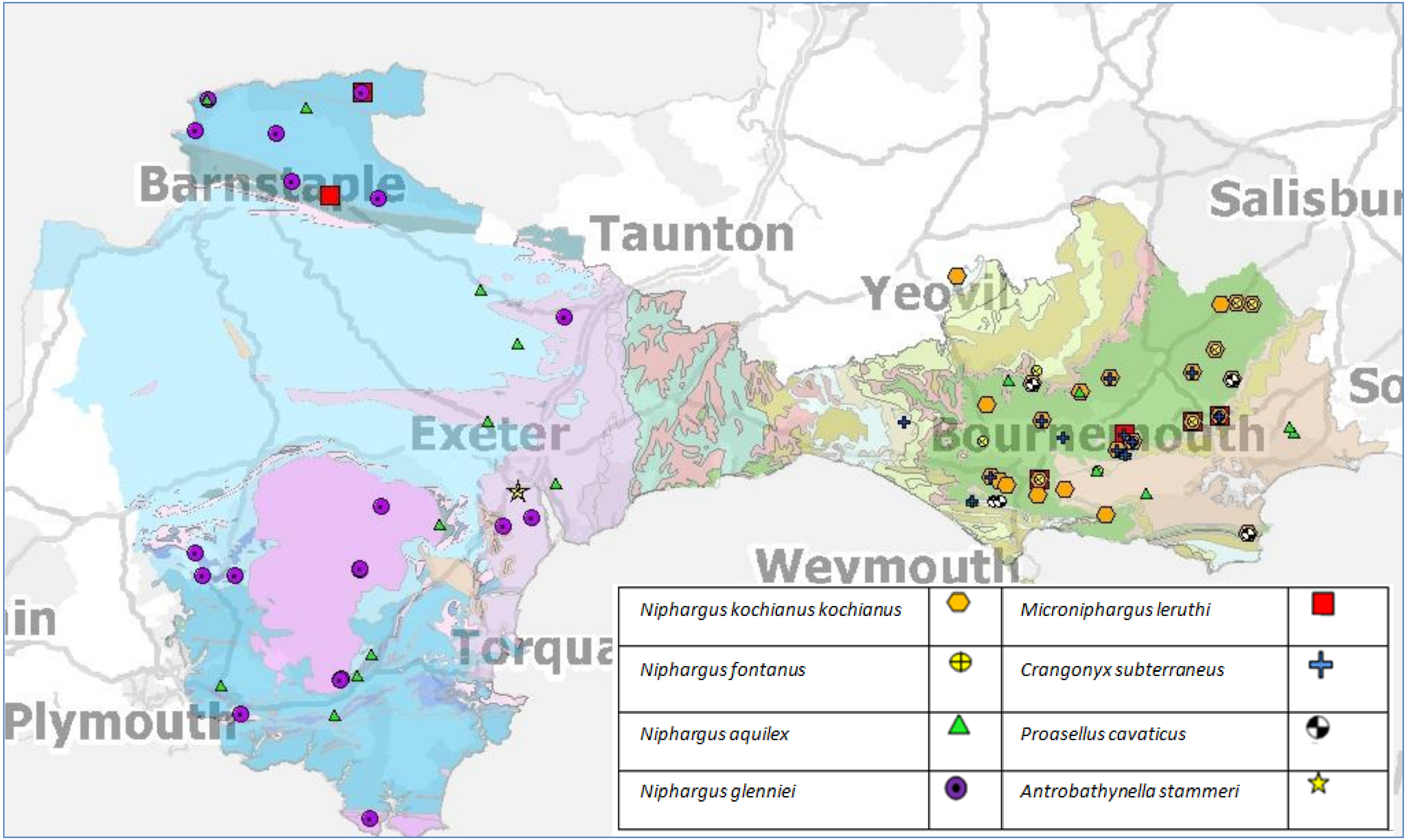


Figure 4.2. Species distribution

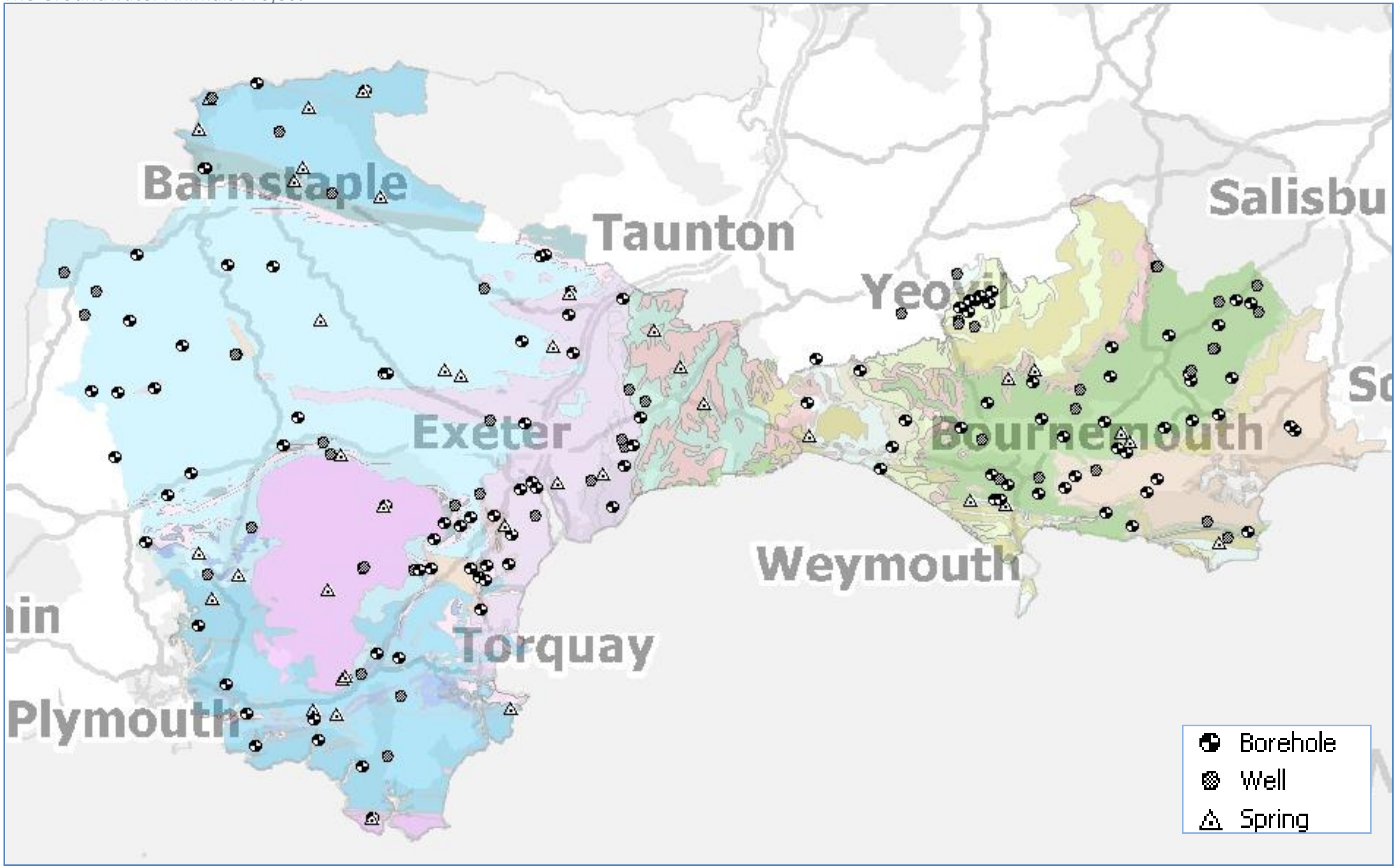


Figure 4.3. All sampling locations

Stygobite abundance: The total abundance of stygobites recorded from the 198 sites sampled was 465. These data were not evenly distributed over the various pre-defined hydrogeological units as might be expected given the different numbers of sites sampled across the various hydrogelogical units. The abundance data was log transformed to produce the following error bar plots illustrating the range of abundances recorded between the different hydrogeological units and sub units (Figure 4.4.).

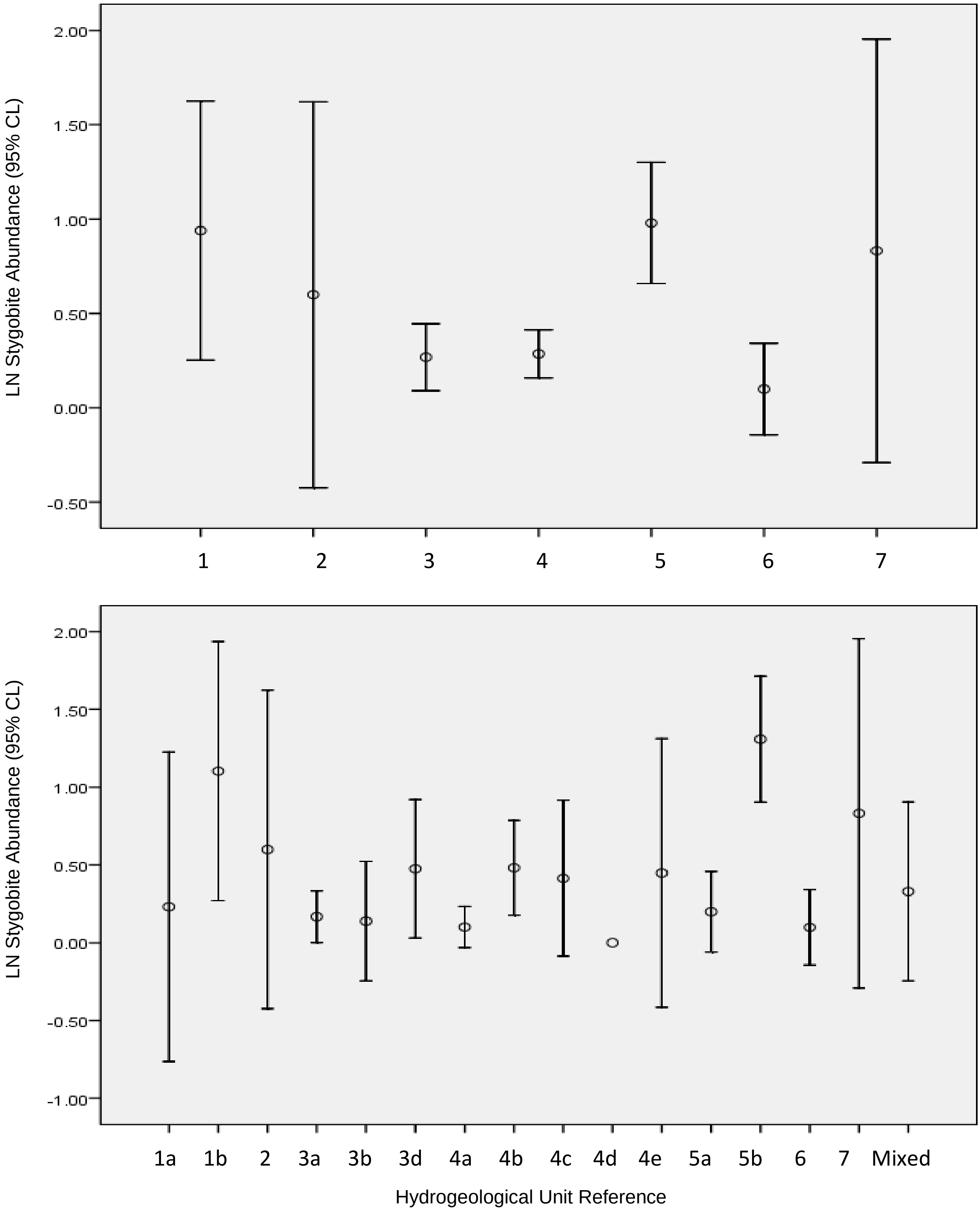


Figure 4.4. Log stygobite abundance (95% confidence limits) across hydrogeological units

Stygobite abundance data are further illustrated spatially in terms of the spread over the various hydrogeological sub-units (Figure 4.5). This is present through a colour gradient, where the darker the unit colour, the higher the total abundance of stygobites on a log scale.

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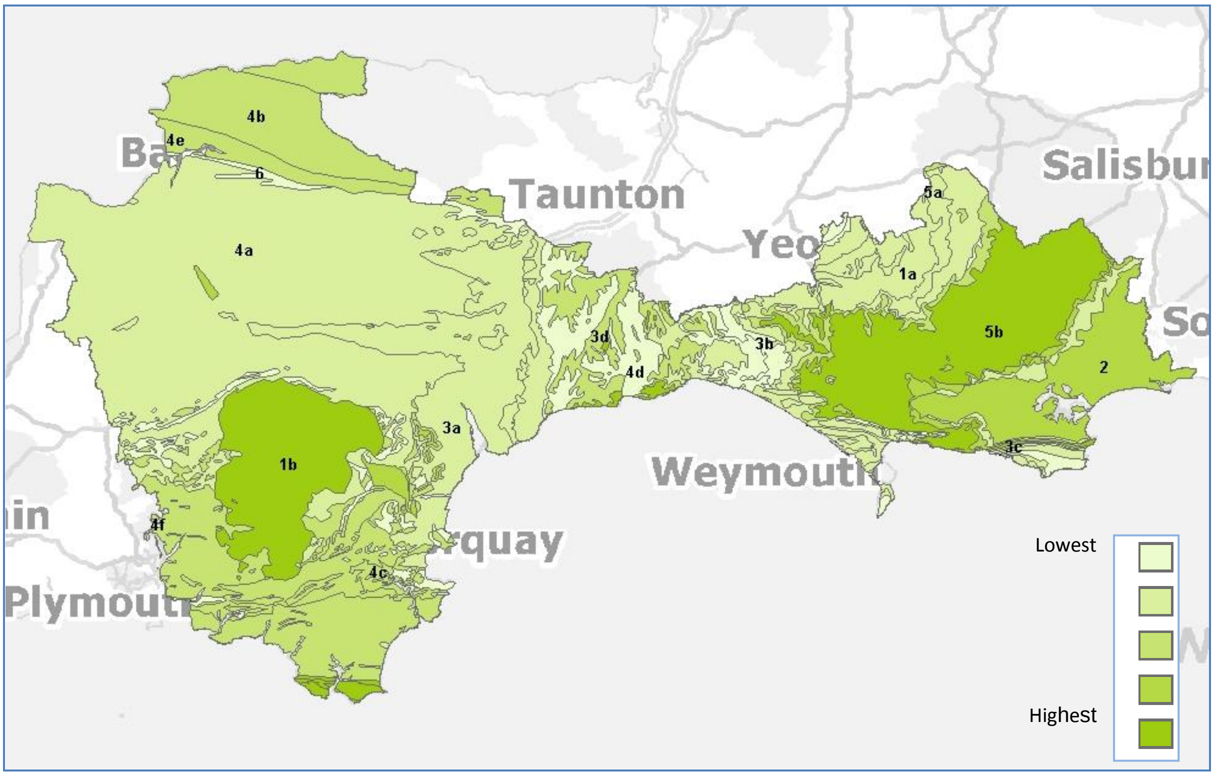


Figure 4.5. Stygobite abundance per hydrogeological sub-unit.

Species abundance: *Niphargus kochianus kochianus* was the most abundant species recorded in the survey (181 individuals) and the most frequently found (in 24 out of 198 sites). The next closest were *Niphargus aquilex* and *Niphargus glenniei*, both with around 80 individuals found at 19 sites. *Antrobathynella stammeri* was the least abundant and least common species, with one individual recorded at one site only. The actual abundance data for each species per hydrogeological and sub-unit is presented in Table 4.3, and illustrated in Figure 4.6.

Table 4.3. Total stygobite species abundance recorded in each hydrogeological unit sampled

Hydro Unit Ref.	Sub- Unit Ref	Species								N Taxa1	N Taxa2	Abundance per sub-unit	Abundance per hydro unit
		N.kochianus kochianus	*N. fontanus*	*N. aquilex*	*N. glenniei*	*Crangonyx subterraneus*	*Microniphargus leruthi*	*Proasellus cavaticus*	*Antrobathynella stammeri*				
1	1a	**1**								**1**	**3**	**1**	**90**
	1b			**37**	**52**					**2**		**89**	
2	-			**7**		**10**				**2**		**17**	**17**
3	3a			**2**	**2**				**1**	**3**	**6**	**5**	**27**
	3b					**1**				**1**		**1**	
	3d	**2**	**10**	**3**	**6**					**4**		**21**	
4	4a			**8**						**1**	**3**	**8**	**38**
	4b			**9**	**12**		**2**			**3**		**23**	
	4c			**1**	**3**					**2**		**4**	
	4d									**0**		**0**	
	4e				**2**		**1**			**2**		**3**	
5	5a			**3**		**2**		**2**		**3**	**6**	**7**	**276**
	5b	**178**	**17**			**41**	**7**	**8**		**5**		**269**	
6	-				**1**					**1**		**1**	**1**
7	-		**1**	**10**						**2**		**11**	**11**
Mixed*	-			**3**	**1**					**2**		**4**	**4**

* Mixed = those sites where examination of logs indicated more than on unit was intercepted within the borehole. Note: (1) Units 3c & 4f were not sampled as the percentage surface area coverage was too small. (2) Greyed out boxes = no species recorded. (3) NTaxa 1= No. of taxa in Hydro-sub units, NTaxa 2= No. of taxa in Hydro-units

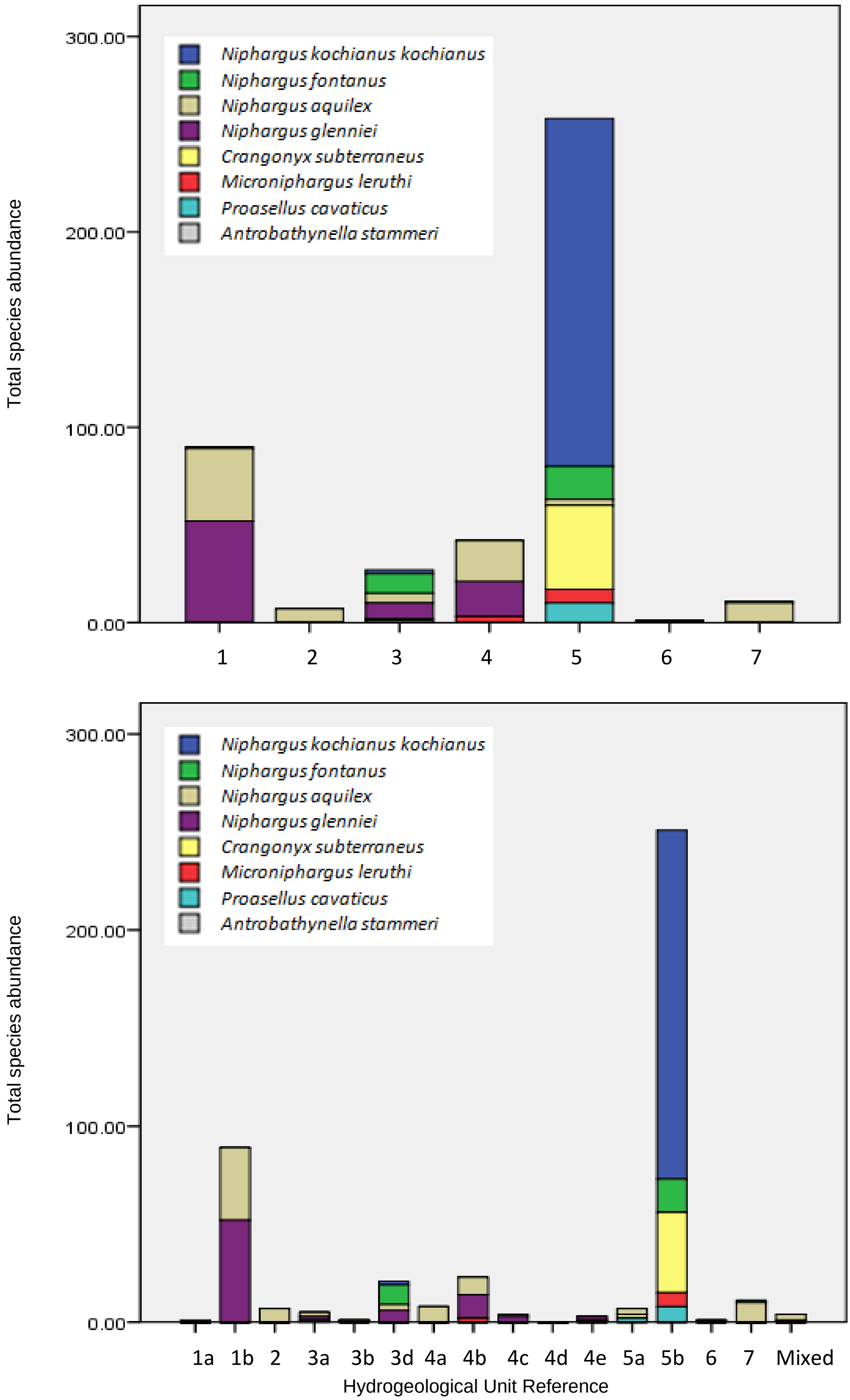


Figure 4.6 Species abundance data grouped for hydro-unit and sub-units

Number of Taxa: With reference to Table F, where the number of taxa per hydrogeological unit is presented, the maximum number of taxa, six, occurred in only two of the hydro-units: the combined granular and fractured aquifers (Unit 3) and the carbonate aquifers with low to moderate level karstification (Unit 5). The mean number of taxa per hydrogeological-unit was three. When examining the number of taxa per sub-unit the highest number of taxa, five, was found in the Chalk (Unit 5b). The number of taxa (NTaxa) found per hydrogeological sub-unit is illustrated spatially in Figure 17 below. This is presented through a colour gradient, where the darker the unit colour, the higher the number of taxa recorded in that unit.

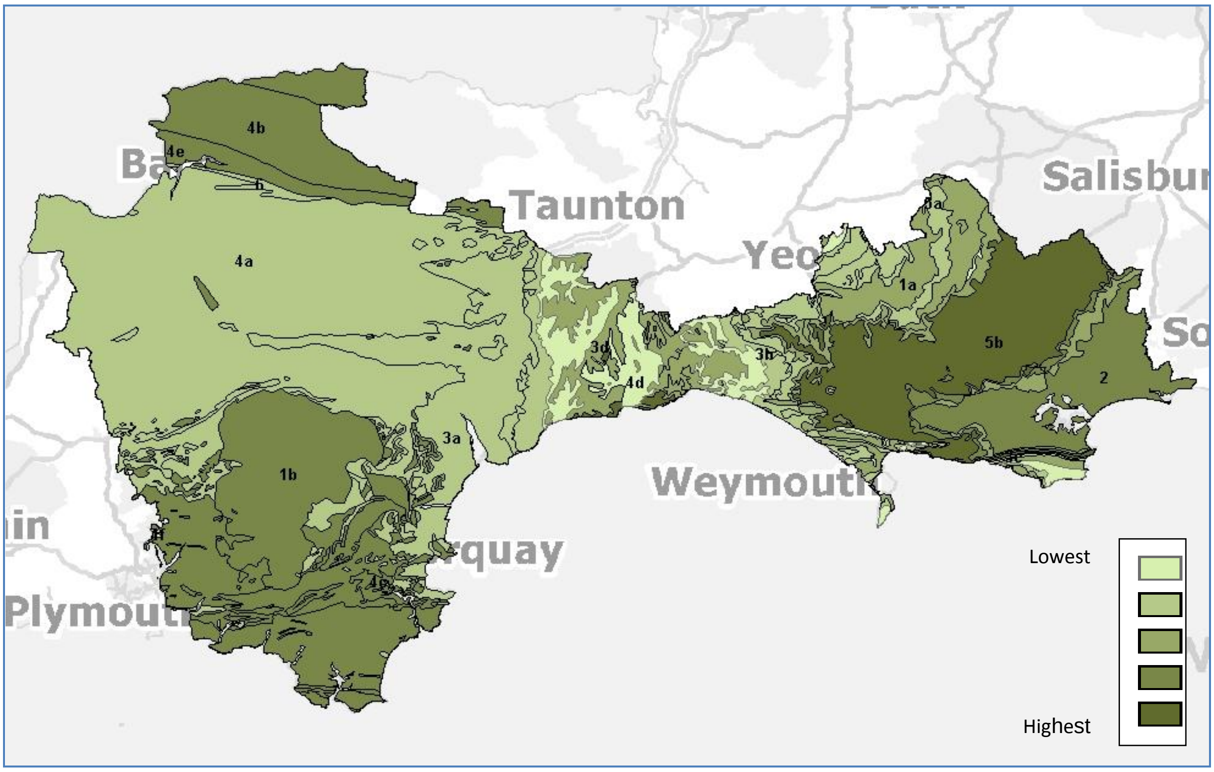


Figure 4.7. Colour gradient for number of stygobitic taxa per hydrogeological sub-unit

The Chalk unit (5b) also recorded the highest number of species for a single site (borehole, well or spring). In all other Units examined no more than two different taxa were found at any one site. The composition of taxa within each of the Chalk boreholes/wells[7] sampled is illustrated in Figure 4.8 by means of individual pie charts showing the relative proportion (abundance) of each taxon at each site, where stygobites were found.

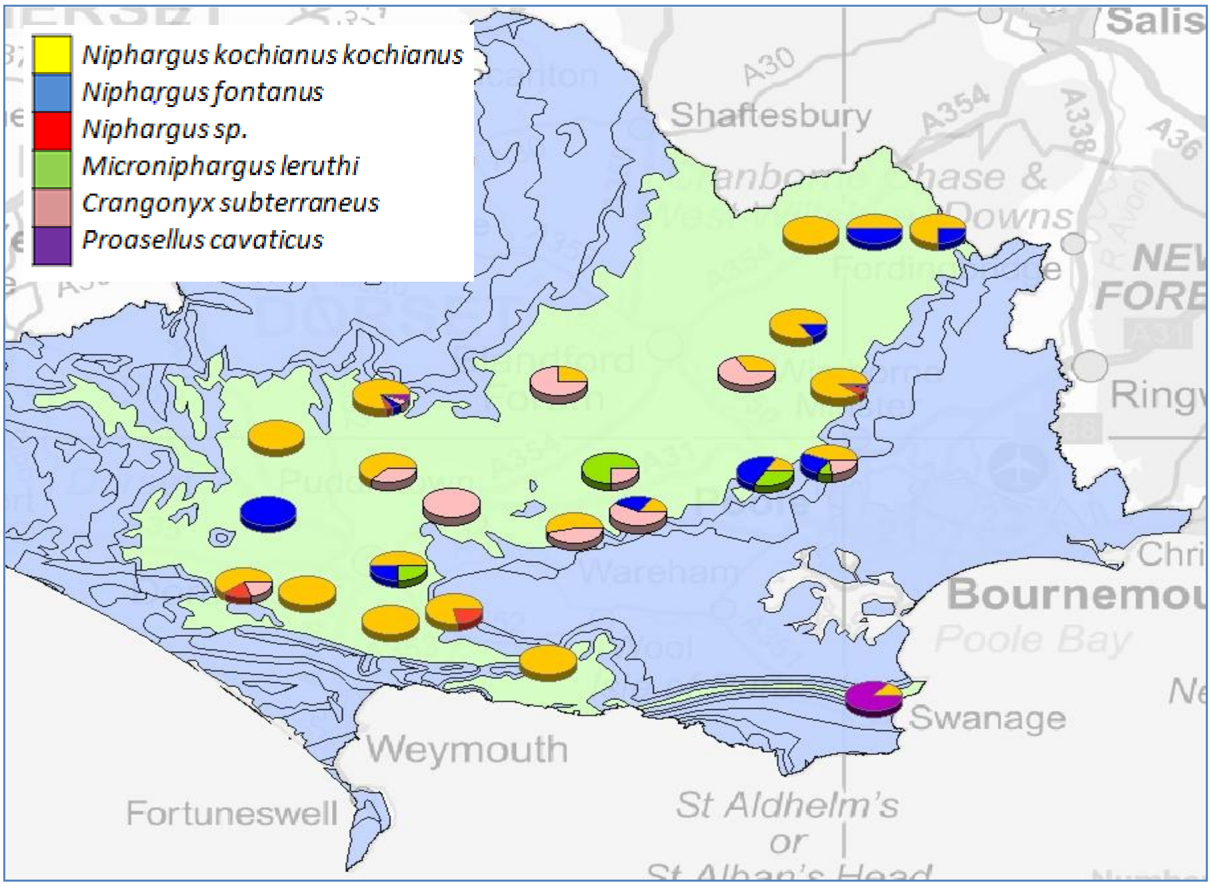


Figure 4.8 Stygobite species composition at sites within the Chalk (unit 5b)

4.2 Chemical data

The chemical data gathered comprised field data (pH, electrical conductivity and dissolved oxygen) and laboratory analysis (major ions and dissolved organic carbon). The reliability of some of the field measurements (principally dissolved oxygen) was identified as a possible source of error, where it could not be measured *in situ* down the well or borehole (these data were identified and removed from the analysis). Where the limit of detection (LOD) was reported a value halfway between zero and the LOD was used (e.g. LOD <0.05 was reported as a value of 0.025).

[7] Note: No spring sites were sampled within the Chalk Unit 5b.

Water chemistry & stygobite presence: The influence of water chemistry on stygobite presence was investigated using Binary Logistic Regression. For this analysis all data (including values interpreted for missing LOD and field chemistry data) were used and processed using PASW Statistics (ver.17). This form of analysis is appropriate for use when predicting a categorical dichotomous variable from a set of predictor variables. It is suited to a mixture of continuous and categorical variables and makes no assumptions about the distributions of the predictor variables. A model was constructed for stygobite presence or absence (1 or 0) to which the categorical predictor variables of the hydrological units (1-7) was added together with the continuous variables of electrical conductivity (EC), dissolved oxygen (DO), temperature, pH, dissolved organic carbon (DOC), ammonical nitrogen (NH4), chloride (Cl), nitrate (NO3), sulphate (SO4), calcium (Ca), magnesium (Mg) and potassium (K).

A test of the full model versus a model with intercept only was found to be statistically significant, $\chi 2(18, N = 198) = 52.538, p < .001$. The model correctly classified 85.2% of those sites where stygobites were absent and 51.4% of those where stygobites were present, giving an overall success rate of 73.27%. Table 4.5 shows the outputs from the analysis: the logistic regression coefficient (B), Wald test ($V\chi 2$), the significance level (p) and odds ratio, for each predictor.

Table 4.5 Logistic binary regression outputs for geo-chemical data

Data Type	Predictor variables	B	Wald χ^2	*p*	Odds ratio
Non-categorical	EC	0.001	2.154	0.142	1.001
	DO	0.048	0.780	0.377	1.049
	Temp	0.015	0.017	0.898	1.015
	pH	-0.251	1.346	0.246	0.778
	DOC	0.011	2.674	0.102	1.011
	NH4	0.238	0.221	0.638	1.269
	Cl	-0.070	5.480	0.019*	0.933
	NO3	0.060	3.287	0.070	1.062
	SO4	-0.051	3.757	0.053	0.950
	Ca	0.028	7.325	0.007#	1.028
	Mg	-0.051	3.762	0.052	0.950
	K	0.034	0.979	0.322	1.035
Categorical	Hydro-Unit 1		10.946	0.090	
	Hydro-Unit 2	-0.576	0.213	0.644	0.562
	Hydro-Unit 3	-2.168	6.584	0.010*	0.114
	Hydro-Unit 4	-0.938	2.224	0.136	0.392
	Hydro-Unit 5	-1.194	1.957	0.162	0.303
	Hydro-Unit 6	-1.988	2.304	0.129	0.137
	Hydro-Unit 7	0.707	0.262	0.609	2.028
	Constant	0.231	0.017	0.897	1.260

***** Significant at 0.05 # significant at 0.01

The relationship between stygobite presence and potential chemical determinands was found to be significant for calcium (*p*<0.01) and chloride (*p*<0.05). Sulphate, nitrate and magnesium were the only other continuous predictors close to significance at the 0.05 level. The Wald Chi-squared statistic tests the unique contribution of each predictor in the context of the other predictors by holding them constant. The values for this statistic show that the contribution of calcium to the overall prediction was the highest. The effect of chloride was found to be less important and in the opposite direction (i.e. lower chloride concentrations).

For the categorical variables of hydrogeological units, the regression compares the contribution of each unit to the first unit identified in the series (i.e. Unit 1). The contribution of the hydrogeological units in predicting the occurrence of stygobites was significant (*p*=0.01) for Unit 3 (the granular and fractured aquifers). The Wald value shows the contribution of Hydro-Units 1 and 3 to the overall prediction were the highest.

Water chemistry & stygobite abundance: The non-parametric Spearman Rank correlation was used to compare the measured chemical determinands against stygobite abundance for all sites. There were significant positive relationships between stygobite abundance and: dissolved oxygen; nitrate; and calcium concentrations. Significant negative relationships were identified between stygobite abundance and chloride and magnesium concentrations (Table 4.6).

Table 4.6 Spearman Rank correlation of selected chemical data and stygobites abundance

Parameter	**Spearman Correlation Coefficient**	**Significant (2-tailed)**	**R^2**	**Significant level (*p*)**
Dissolved oxygen	0.141	0.048	0.02	<0.05
Chloride	-0.195	0.006	0.04	<0.01
Nitrate	0.272	0.000	0.07	<0.01
Calcium	0.223	0.002	0.05	<0.01
Magnesium	-0.203	0.004	0.04	<0.01

4.3 Physical & Environmental data

Stygobite presence: The physical data recorded at each sampling location included a range of measurements (e.g. borehole depth) and categorical classifications (e.g. borehole purpose), as described in Section 3.2.3 and summarised in Table 4.7 below. As with the geochemistry, the relationship between these physical factors and the presence or absence of stygobites was investigated using binary logistic regression. For each categorical variable the regression was directed towards the first category division (i.e. Environment 1), so that each subsequent category in a particular group (i.e. Environment 2 & 3 etc) was compared to the first.

Table 4.7 List of categorical & continuous predictor variables record for each site surveyed.

Category	No	Description
Sampling point type	1	Borehole
	2	Well
	3	Spring
BH purpose	1	Disused
	2	Monitored
	3	Used (pumped)
Mesh size	1	63um
	2	250um

Category	No	Description
Environment	1	Garden/amenity land
	2	Rural land
	3	Roadside & urban

Sampling Protocol *(I-IV)*	1	Net down well
	2	Tap
	3	Pond net sweep
	4	Bou-Rouch/scrape

Continuous variables	Units
BH depth	Metres
BH diameter	Metres
BH volume	Cubic metres
Water column height	Meters
Distance to watercourse	Kilometres

A model was constructed for stygobite presence or absence (1 or 0) to which the five categorical predictor variables and five continuous variables listed above were included. A test of the full model versus a model with intercept only was found to be statistically significant, $\chi^2(15, N = 198) = 26.54$, $p = 0.03$. The model was able correctly to classify 86.3% of those sites where stygobites were absent and 35.5% of those where stygobites were present, giving an overall success rate of 67.1%. The logistic regression coefficient, Wald test and odds ratio for each predictor variable appears in Table 4.8. Employing a 0.05 criterion of statistical significance borehole purpose and sampling point type appeared to have significant effect on stygobite occurrence.

Table 4.8 Predictor variable results from logistic binary regression analysis (Model 1)

Data Type	Predictor variables	B	Wald χ^2	*p*	Odds ratio
Non Categorical	BH depth	-0.028	1.893	0.169	0.972
	BH diameter	0.295	0.196	0.658	1.343
	BH volume	-0.019	1.243	0.265	0.981
	Water column height	0.033	1.942	0.163	1.034
	Distance to watercourse	0.597	1.583	0.208	1.817
Categorical	Sampling point type (1)		5.292	0.071	
	Sampling point type (2)	1.197	1.694	0.193	3.311
	Sampling point type (3)	2.518	5.269	0.022*	12.401
	BH Purpose (1)		12.914	0.002*	
	BH Purpose (2)	1.986	6.843	0.009*	7.285
	BH Purpose (3)	-0.798	1.806	0.179	0.450
	Mesh size (2)	0.717	0.765	0.382	2.049
	Environment (1)		1.366	0.505	
	Environment (2)	-0.483	0.776	0.378	0.617
	Environment (3)	-0.695	1.342	0.247	0.499
	Sampling protocol (1)		0.181	0.981	
	Sampling protocol (2)	0.065	0.002	0.960	1.067
	Sampling protocol (3)	-0.435	0.180	0.672	0.647
	Sampling protocol (4)	-0.336	0.072	0.788	0.715
	Constant	-1.834	5.492	0.019	0.160

***** Significant at < 0.05

For the variables of significance in this model, the odds ratio in terms of stygobite occurrence can be interpreted as:

- The likelihood of stygobites being found in sampling point type 3 sites (springs) is significantly higher than it is for sampling point 2 types (wells).

- The likelihood of stygobites occurring in a borehole, well or springs is significantly higher in those sites which are monitored (BH purpose 2), compared to those which are used/pumped (BH purpose 3).

Because categorical variables (2), (3) and (4) are compared to categorical variable (1) no odds ratio is provided for the latter. However, by examining the relative odds (independent of the logistic regression analysis) of each variable in terms of stygobite presence or absence (see Table 4.9) we can see that the odds for BH purpose (1) are slightly higher than for (2), but lower than (3). Stygobites are therefore most likely to be associated with BH purpose (2 – monitored sites) followed by BH purpose (1 – disused sites) and finally BH purpose (3 – pumped sites). Similarly, the odds ratio for Sampling point (1) are slightly higher than for (2), but lowest for sampling point (3); the likelihood of stygobites occurrence is therefore highest in springs, then wells and least likely in boreholes, from the data analysed.

The occurrence of stygobites, based solely on the calculated odds has been determined for all of the categorical variables, as shown in Table 4.9, although it should be remembered that with the exception of Borehole purpose and Sampling point type, none returned a significant relationships within the model used.

Table 4.9 Calculated odds for stygobite occurrence for all categorical variables identified.

Categorical Variable	**Absent**	**Present**	**sum**	**Odds**			**Likelihood**
Sampling point 1	79	32	111	71	:	29	Least likely
Sampling point 2	32	20	52	62	:	38	Less likely
Sampling point 3	17	18	35	49	:	51	Most likely
BH purpose 1	42	28	70	60	:	40	Less likely
BH purpose 2	32	26	58	55	:	45	Most likely
BH purpose 3	54	16	70	77	:	23	Least likely
Mesh size 1	96	42	138	70	:	30	Less likely
Mesh size 2	32	28	60	53	:	47	More likely
Environment 1	39	21	60	65	:	35	No difference
Environment 2	58	34	92	63	:	37	No difference
Environment 3	31	15	46	67	:	33	No difference
Sampling protocol 1	40	78	118	66	:	34	Likely
Sampling protocol 2	3	18	21	86	:	14	Least likely
Sampling protocol 3	23	30	53	57	:	43	Next likely
Sampling protocol 4	4	3	7	43	:	57	Most likely

Stygobite abundance & physical borehole data: Abundance data for the physical borehole measurements was selected from the full dataset so that only those data with borehole measurements were included. This data was then subject to a non parametric 2-tailed Spearman Rank correlation. No significant relationships were identified between stygobite abundance and the non categorical borehole measurements (e.g. borehole depth etc). Selecting just the sites with actual abundances, scatter plots of abundance versus each borehole measure were created with linear regression lines. No significant correlations were identified but weak positive correlations (R^2 >0.06) were indicated for borehole depth and water column height

Stygobite abundance & environmental data: Each categorical variable identified as an environmental measure was plotted against log stygobite abundance data as error bar plots with 95% confidence levels of the mean. No clear significant relationships were detected.

5.0 Discussion – Survey (A) Dorset & Devon

Stygobites in Devon & Dorset

Of the 198 sites sampled in this survey, stygobites were recorded in about a third of these sites. Their presence and abundance varied considerably between the different geologies sampled, indicating a very patchy distribution of stygobites across the study area.

All eight stygobites species known from England were recorded in the survey, although there was a clear spatial variation in both species richness and composition, with the highest species richness found on Chalk geology. The survey confirmed the known occurrence of *Niphargus glenniei* and *Niphargus aquilex* within Devon but also added records for *Antrobathynella stammeri* and *Microniphargus leruthi* to the species list for the county. In Dorset, additional records for *Niphargus kochianus kochianus, Niphargus fontanus, Crangonyx subterraneus* and *Proasellus cavaticus* were obtained, as well as new records for the county for *Microniphargus leruthi.* The latter species records were amongst the first for the species in Britain, some of the samples having been collected before its discovery in Swildon's cave in Somerset (Knight & Gledhill, 2010 & Knight, 2011). *Niphargus aquilex* was the most widespread species found in the survey, appearing across Dorset and Devon. The reasons for the distribution and occurrence of stygobites reported in this survey are discussed below through the hydrogeological, chemical and environmental factors considered in this study.

5.1 Hydrogeological controls

The occurrence of stygobites differed significantly between the different pre-defined hydrogeological units (aquifers) surveyed. This was true at both the broad hydro-unit scale (where a total of seven units were defined) and at the sub hydro-unit scale (where a total of 18 units were defined). Stygobite occurrence was highest in Chalk (Unit 5b), where the greatest number of individuals was also recorded. The lowest occurrence was in the Triassic mudstone (Unit 4d) but this unit was poorly represented due to a lack of boreholes. A degree of caution should therefore be applied as the number of sites sampled varied between units. Furthermore, the sample sources (i.e. boreholes, wells and springs) and the mechanisms by which they were sampled were not identical. However, the bias in the number of sites sampled in each unit reflected the relative proportions of the surface area covered by each unit within the study region (see Section 3.1). On this basis the data should provide a rough likelihood of finding stygobites in each unit. Possible hydrogeological controls operating at the broad hydro-unit scale and the intermediate sub-unit scale are discussed below.

Stygobite presence and hydrogeology

In line with the findings of Dole-Olivier *et al*. (2006), stygobitic taxa were more commonly associated with the most permeable geological formations; The probability of finding stygobites increasing with increasing permeabilities. These ranged from Unit 1, the very low permeability materials, through to granular strata of moderate permeability, and finally to high permeability fractured aquifers (Units 5&6). Unit 7, a later addition to the pre-defined units, created to account for superficial strata unintentionally sampled, was expected to fit along this continuum in rough approximation to the granular aquifers.

With reference to Table 4.2, the highest probability of finding stygobites in this survey (60%) was actually reported for the superficial deposits (Unit 7), followed by the carbonate aquifers of low-moderate karstification (Unit 5) and the very low permeability strata (Unit 1), both

50%. Conversely the lowest probabilities (~15%) were associated with the highly karstified carbonate aquifers (Unit 6) and the combined granular and fractured aquifers (Unit 3). Of the remaining units; the granular aquifers (Unit 2) and the fracture aquifers (Unit 4), both of low to moderate permeability, had a probability of around 20-30%. At this broad scale the presence of stygobites did not appear to follow the expected pattern, with a distinctly higher than expected probability for finding stygobites in Unit 1, seemingly at odds with the likely restricted habitat opportunities available in a low permeability material. These results are discussed at the hydro-unit and sub unit scale below;

Unit 1 – Very low permeability strata
The unexpectedly high probability of finding stygobites in low permeability material becomes slightly clearer at the sub hydro-unit level, where the unit is divided in to two: Clay and mudstone strata (1a) and metamorphic and igneous strata (1b). Stygobite records for these units were almost exclusively attributed to sub-unit 1b, with only one record for Unit 1a. This latter record is noteworthy since it is from the Dyrham Formation, an interbedded silty mudstone with fine grain sands, where pore spaces are likely to be small and not ideal for stygobites. The presence of stygobites (*Niphargus glenniei*) in Unit 1b is unsurprising given the known association of this species with granite (Knight 2009). Knight (2009) identifies the small body size and laterally flattened shape of *Niphargus glenniei* in aiding its adaption to living in small voids and fissures available within granite. However, the larger *Niphargus aquilex* species was also found at one site on granite, suggesting void space and geochemistry (acidic conditions common to igneous geologies) are not necessarily limiting to the stygobites found in this survey.

Unit 2 - Granular aquifers (low to moderate permeability)
This unit was under represented in the survey with only five boreholes being sampled, due to site availability and the re-categorisation of some sites in to a new category of superficial deposits, following a post survey review. Despite this under representation stygobites (principally *Niphargus aquilex*) were found in 20% of the sites sampled. The sampled aquifers comprised multilayered systems of unconsolidated sands of moderate permeability separated by low permeability clays and silts. Optimal conditions for stygobites in the sands are therefore likely to be reduced by the lower permeability layers limiting the movement and dispersal of stygobites through the entire aquifer.

Unit 3 - Combined granular and fracture aquifers (high permeability).
This unit might be expected to provide suitable habitat for stygobites being dominated by highly permeable sandstones with both intergranular and fracture flow. However, stygobite presence was one of the lowest of any hydro-unit sampled. The Permo-Triassic strata (Unit 3a) in Devon are complicated by faulting and the interleaving of sandstones with lower permeability mudstones, which may exert an influence on stygobite populations. However, it may also be the case that the stygobites commonly associated with boreholes in the Chalk for example are not present in this region due to other geological factors preventing their free movement.

Unit 4 – Fractured bedrock (low to moderate permeability)
Sampling success varied significantly within this unit. The probability of finding stygobites in Units: 4a (Upper Carboniferous formation) and 4d (Triassic mudstone) were the lowest of all units sampled, with no stygobites being found in the Triassic mudstone. However, it should be noted that these units were under represented by the survey due to the limited availability of sites. The absence of wells or boreholes suggests that the groundwater resource is poorly utilised in these units. In contrast, probabilities of stygobite occurrence in Units: 4b (Old Red sandstone/Devonian formation), 4c (extrusive igneous strata) and 4e (Basal conglomerate) were close to 50%. Whilst all units are characterised by low to moderate permeability rocks, with

fracture flow the principal groundwater flow mechanism, the conditions within Units 4a and 4d would seem to be much less suitable for stygobites than the other units.

Units 5&6 - Carbonate aquifers (high permeability)
This unit comprises the Chalk (Unit 5b) and Jurassic Limestone formations (Unit 5a) and the Carbonate aquifers with a high level of karstification (Unit 6). The highest probability of finding stygobites in any unit was reported for Chalk (63%), but much lower probabilities were returned for the Jurassic, Carboniferous and Devonian limestones. This is perhaps surprising given that all these carbonate strata should provide potentially good habitats for groundwater fauna. One possibility is that the limestones are outside the geographical distribution of many of the most frequently found groundwater species – this might be the case in the Carboniferous and Devonian limestones which are located in Devon where several commonly found species appear absent (*Niphargus fontanus, Niphargus kochianus kochianus)*. A possible explanation for the lower numbers of boreholes with stygobites in the limestones in Dorset where these species are present is that the local physical or chemical hydrogeological environment is not favourable to a particular species. A further possibility is that because permeability is more uniformly distributed in the Chalk, there is an increased likelihood of boreholes intercepting permeable fissures in which groundwater fauna live. In contrast other limestone strata may have less frequent solutional fractures and therefore a greater chance that the boreholes sampled did not intercept permeable fissures.

Unit 7 – Superficial deposits
Following a post-survey review of sites it became apparent that some superficial deposits, which were not intended to be considered in this study, had accidently been sampled. In all only five sites were sampled, which significantly under-represents such deposits, within the study region. The occurrence of stygobites, and more specifically *Niphargus aquilex,* in three sites, is not surprising since all sites were close to surface water, where hydraulic connection was likely. *Niphargus aquilex* is commonly associated with shallow groundwater and hyporheic habitats (Robertson, *et al*., 2009) and so its presence was not unexpected. It also highlights the potential for the movement and dispersal of *Niphargus aquliex*, and potentially other species, through the hyporheic corridor, a concept introduced by Stanford and Ward (1993). This might explain the wider distribution of *Niphargus aquliex* across the study region.

Stygobite abundance and hydrogeology
In this study information on stygobite abundances was largely secondary to identifying the presence or absence of stygobites within the study region. Whilst abundance data was collected, caution should be exercised interpreting this information because; firstly, although standardised sampling protocols were used (Section 3.2.3), borehole/well dimensions varied between sites, necessitating the use of different sized nets. Spring sites were also sampled where a different sampling technique was employed and some sites contained pumps, again forcing a different sampling approach. Secondly, each site was only sampled once, and therefore the recorded abundance only provides a snapshot of stygobite numbers at that time. Hancock and Boulton (2008) in a study of alluvial aquifers in southeast Australia found that one off sampling did not estimate sufficiently taxa richness or composition.

With this in mind, only broad conclusions can be drawn from this survey's abundance data. In general the abundance data followed the trend reported for the presence absence data, with greater abundances associated with those units with high reported stygobite presence. The maximum abundance (269 individuals) was consequently found in Chalk (Unit 5b) with the next highest abundance (89 individuals) recorded for the granite (Unit 1b). Of the remaining units, all recorded substantially lower abundances (~20 or less), and in eight of the 16 initial sub-units, abundances of five or less individuals were found. Whilst the numbers of sites sampled in many of these cases were small, the abundances recorded may point to a sparse stygobite population. Hahn and Matzke (2005) found that in a sparsely populated groundwater region in southwest Germany, abundances of fauna were actually higher inside boreholes than outside them. This was thought to be due to the likely more favourable conditions within the boreholes, of low current and probably good detritus food supply. The abundances observed within our survey may therefore actually be an over estimate of the aquifers sampled, although it should also be recalled that this is based on only one sampling event. Eberhard *et al.* (2009) and Hancock and Boulton (2009) both report increasing abundance with the number of sampling occasions.

Geographical distributions of stygobite species and hydrogeology

There was a distinct pattern to the distribution of the eight species recorded in the survey, with a clear division in the species occurring between the two counties. Records for *Niphargus glenniei* and the one record for *Antrobathynella stammeri* were all limited to Devon, whereas *Niphargus kochianus kochianus, Niphargus fontanus, Crangonyx subterraneus* and *Proasellus cavaticus* were only found in Dorset. *Niphargus aquilex* and *Microniphargus leruthi* were the only species found across the study region, in both counties.

Viewed at the broad, hydro-unit, scale no one species was found in all units, with the greatest sampling efficiency recorded in the Chalk, granite and superficial deposits. *Niphargus aquilex* was the most wide spread species appearing in all but the highly karstified carbonate aquifers (Unit 6). *Niphargus glenniei* was the next most widely occurring species, being reported from the granite, as well as fractured, carbonate and combined granular and fractured aquifers.

Species richness was greatest for Units 5 and 3 – the carbonate aquifers with small scale karst and the combined granular and fractured aquifers, respectively. The species composition was similar in both units with *Proasellus cavaticus* and *Microniphargus leruthi* appearing in Unit 5 but not Unit 3. The reverse was the case for *Niphargus glenniei* and *Antrobathynella stammeri* appearing in Unit 3 but not Unit 5. The very high abundance of *Niphargus kochianus kochianus,* in the Chalk (Unit 5b), may help explain the differences in the diversities reported. At the sub-unit level the highest species richness, five, was returned for the Chalk, with the next highest, four, being for the Upper Greensand. In Dorset, the absence of springs at the base of the Lower Chalk suggests downward vertical leakage into the Upper Greensand (Allen *et al.*, 1997). This may provide a pathway for stygobites from the Chalk into the Upper Greensand and could partially explain the species richness reported for the Upper Greensand and the occurrence of both *Niphargus kochianus kochianus* and *Niphargus fontanus* in both strata.

The high presence, abundance and diversity of stygobite species in the Chalk was not unexpected since rich stygofaunal assemblages are commonly associated with calcareous systems (Humphries, 2008). Additionally, other survey data from Chalk (http://www.freshwaterlife.org/hcrs/db) shows similar species diversity from other outcrops within southern England. What distinguishes the Chalk in this survey from other units sampled is the species composition recorded. Many sites within Chalk recorded a number of different species in each borehole, with a maximum number of four species recorded from one borehole. In all other units most sites recorded just a single species. This seems to imply that the Chalk provides optimal habitat opportunities for stygobites, in the study region, as the large fracture networks reported for the Chalk (Allen *et al.,* 1997) would suggest. However, similar habitat opportunities may exist elsewhere, for example in the limestone units in Devon and Dorset. The apparent absence of a rich fauna in these units, may be more a function of what species are able to reach these units, rather than the habitat opportunities they provide.

The pronounced demarcation of species between the counties was not unexpected based on previous surveys (http://www.freshwaterlife.org/hcrs/db) which indicate *Niphargus glenniei* is not known from outside Devon (and Cornwall). Conversely there are no previous records for the other niphargid species (with the exception of *Niphargus aquilex*) or *Crangonyx subterraneus* from Devon. However, this survey's findings show that such geographically separated species actually occur within the same geological strata. *Niphargus kochianus kochianus and Niphargus fontanus* were both found in samples from the Upper Greensand, with *Niphargus glenniei* also being recorded from the same strata but at different locations.

The survey also demonstrated that *Niphargus glenniei*, and *Niphargus aquilex* can be found in a range of different strata including compact fractured aquifers to more granular deposits and highly karstified carbonate aquifers. Consequently they appear to be tolerant of both acidic and alkaline groundwater chemistry. Similarly, *Niphargus kochianus kochianus,* commonly associated with the Chalk (Proudlove *et al*., 2003) may exist in likely sub-optimal conditions, as illustrated by its occurrence within the Dyrham formation, a sandy mudstone of the Lias Group.

Given that *Niphargus glenniei* has been found in a range of aquifers, including the Upper Greensand in Devon and *Niphargus kochianus kochianus* has been found in similar strata but only outside Devon, the question arises as to why neither are distributed more uniformly across the study region. Furthermore, *Niphargus aquilex* and *Microniphargus leruthi* appear to overlap the distributions of the former two species, occurring in much the same strata as them. This suggests that *Niphargus aquilex* and *Microniphargus leruthi* are not governed by the same barrier(s) to dispersal as the other species found seem to be. *Niphargus aquilex* is the commonest niphargid species in Britain, often found in wells and springs and is frequently associated with riverine gravels (Proudlove *et al*., 2003). Its widespread and common occurrence suggests it is able to disperse quite well in comparison to those species more commonly associated with deeper phreatic groundwater. Stanford and Ward's (1993) hyporheic corridor concept, which introduces the idea of a continuous alluvial aquifer system, provides the mechanism by which *Niphargus aquilex* could disperse. In this way *Niphargus aquilex* could have moved into Devon from the east along river channels, from which it has then colonised the underlying solid geology. Whilst less is known of *Microniphargus leruthi*, having only recently been reported from England (Knight & Gledhill, 2010), studies in Ireland suggest some association of this species with interstitial habitats in gravels (Knight & Penk, 2010 and Joerg Arnscheit pers. comms.). Similarly, *Antrobathynella stammeri*, recorded during this survey in Devon, is known from across Britain where it is most often associated with springs and riverine gravels (Proudlove *et al*., 2003). However, *Niphargus glenniei* has been recorded from interstitial gravel on the River Plym on Dartmoor (*http://www.freshwaterlife.org/hcrs/db*). This together with its relatively small size suggests it could potentially utilise superficial deposits for dispersal.

The observed distribution of *Niphargus kochianus kochianus, Niphargus fontanus, Crangonyx subterraneus, Proasellus cavaticus and Niphargus glenniei,* in the study region, suggests that they are unable to use this alluvial transport mechanism effectively for dispersal. A possible explanation for this might be provided by the presence of Triassic mudstone (Unit 4d), which outcrops trending very roughly north-south, from south west Dorset to the north Devon/Somerset coastline (see Figure 5.1). This low permeability strata may provide a geological barrier to the movement of stygobites from the Greensand in the east to the Permo-Triassic sandstones in the west. Interestingly, the Triassic mudstone was the only unit from which no stygobites were recorded in the survey. However, this is based on a very limited sample size and further sampling along the entire length of this unit is needed to provide support to this theory. Additionally, the outcropping mudstone is interspersed by outcrops of Upper Greensand and it would be interesting to examine, through further sampling, the species composition in this area.

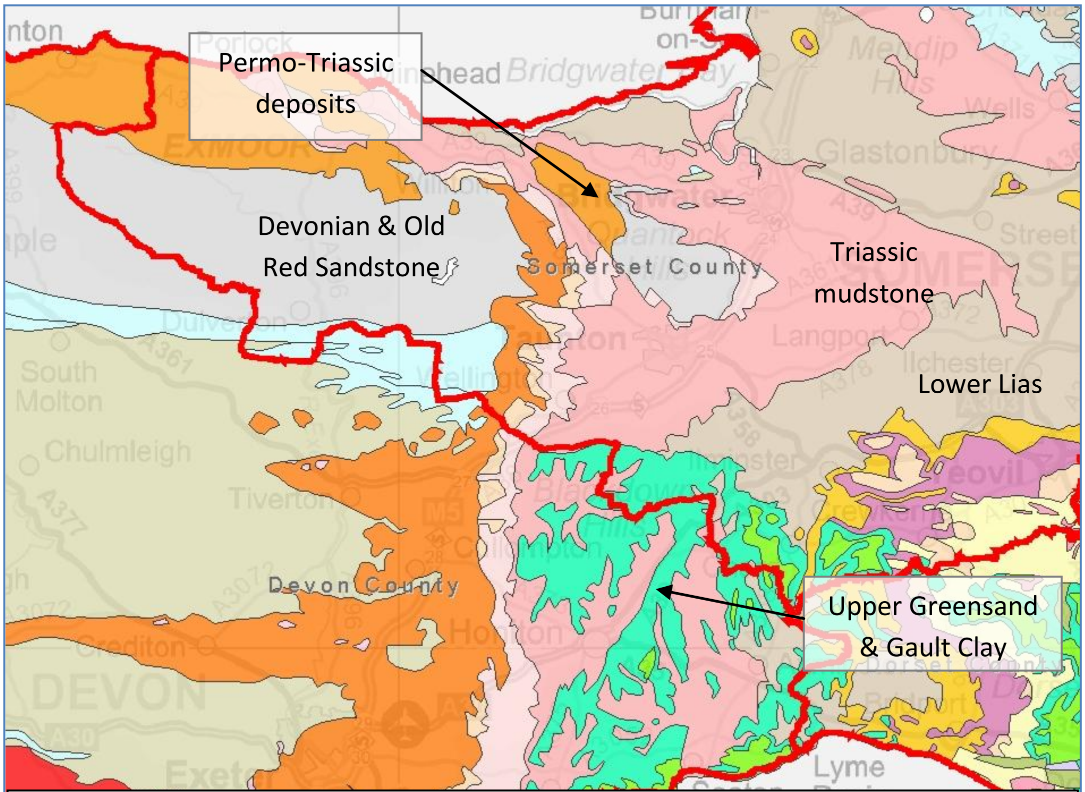


Figure 5.1: Location of low permeability Triassic mudstone and Lower Lias deposits

5.2 Chemical relationships

In this study, calcium and chloride were found to have a statistically significant relationship with the presence or absence of stygobites. For the abundance data significant relationships with calcium, nitrate, dissolved oxygen, chloride and magnesium and were reported. The role of these determinands in relation with stygobite occurrence in this survey is discussed below.

Calcium: The positive correlation of calcium with stygobite occurrence and abundance suggests a relationship between calcium and stygobites. A study by Rukke, (2002) supports this relationship, identifying calcium as an essential component for the development of Crustacea carapaces. Additionally, the highest occurrence of stygobites was from the Chalk, which is typified by groundwater with a high calcium content. However, the range of values for calcium, at all sites (i.e. both those where stygobites were present and absent), in our study, were similar, suggesting the relationship is not a simple one. Indeed, Galassi *et al.* (2009) found a diverse groundwater fauna in low calcium and low pH hyporheic waters, and in our survey *Niphargus kochianus kochianus* and *Niphargus aquliex* were both found in calcium concentrations below 20mg/l, with *Niphargus glenniei* occurring in concentrations below 5mg/l. This suggests high calcium concentrations are not necessarily essential for stygofauna and perhaps the relationship with Chalk, shown in our survey, is more a function of habitat suitability rather than high calcium concentrations.

Nitrate: Stygobites and nitrate concentrations may be weakly related according to studies by Galassi *et al*. (2009). Indeed stygobite presence and abundance was greatest in our survey in the Chalk which had the most frequently elevated nitrate concentrations. Similarly, reports of high stygobite abundance (>300 individuals) in Chalk boreholes with elevated nitrate levels of >14mg/l (as N) (Lee Knight pers. comms.), support this finding. However, as with calcium, this finding may simply underlie the suitability of the Chalk for stygobites rather than any direct relationship between nitrate and stygobite populations. We can conclude however that nitrate does not appear to be limiting to stygobites, within the range of concentrations recorded. Hahn and Matzke (2005) in a study of an alluvial aquifer found stygobites more frequently in high nitrate and low dissolved oxygen waters, compared to sites on a sandstone aquifer with low nitrate but high dissolved oxygen. Although alluvial aquifers were not part of our study, sandstone strata were studied and it is interesting to observe that the Devonian and Old Red Sandstone formations (Unit 4b) were also characterised by low nitrate (median = 3.2mg/l NO_3 as N) and high dissolved oxygen (median 8.52mg/l DO) concentrations. The numbers of stygobites in sites from these strata were also fairly low but, with the exception of the Chalk, this was not uncommon in this survey.

Dissolved Oxygen: Although this dataset was incomplete, due to equipment error and the inability to obtain *in-situ* readings at some locations, a wide range of values were recorded. This was true across the range of hydro-units examined and confirms the spatial heterogeneity of dissolved oxygen (DO) concentrations in groundwater (Malard & Hervant 1999). Using the terminology proposed by Tyson and Pearson (1991), with the ranges as adapted by Malard & Hervant (1999) for oxygen environments, (suboxic, dysoxic and oxic), only one site was suboxic (DO<0.3mg/l). A little under a third of sites were dysoxic (DO>0.3-3.0mg/l), and the remaining two third of sites were oxic (DO>3.0mg/l). No stygobites were found in the suboxic site and the proportion of sites with stygobites in oxic and dysoxic conditions were approximately equal at about a third. No significant relationship between presence absence data and DO concentrations were identified. However, a positive correlation between stygobite abundance and dissolved oxygen was identified. The high abundance of stygobites in Chalk, where 80% of the sites were oxic, may help in part account for this relationship, although the habitat opportunities presented by the Chalk are also an important confounding factor.

Chloride: With the exception of one elevated value (255mg/l), the levels of chloride recorded in the survey were all generally low (min.1.6mg/l, max. 58mg/l, median 9.7). The outlier value was from a sample taken from the Exminster marshes which was unrepresentative of the other sampling environments. This site was flooded at the time of sampling, which may have resulted in some seepage of brackish water from the estuary into the borehole, providing a misleading value. However, excluding this value from the analysis the relationships with stygobite occurrence and abundance remain valid, albeit with slightly reduced levels of significance.

Magnesium: The levels of magnesium recorded in the groundwater samples were all generally low (median 5.2mg/l)) with many readings at the detection limit (0.05mg/l). Whilst stygobites were found throughout the range of magnesium concentrations recorded (54.7mg/l), magnesium concentrations in the Chalk were amongst the lowest detected in the survey. The negative correlation between magnesium and stygobite presence reported may therefore simply reflect the high occurrence of stygobites in the Chalk, for reasons other than magnesium concentrations.

Other chemical factors: Whilst no significant relationships were identified for the remaining chemical parameters measured, the following observations were made:

Dissolved organic carbon (DOC): Stygobites were found in sites with a wide range of DOC concentrations (ranging from <1.0mg/l to ~300mg/l). Where DOC is elevated oxygen will be consumed and further depleted where stygobites are present, utilising these resources (Humphreys, 2008). In this survey the majority of sites had low DOC levels

(<10mg/l) and so patterns between DOC, DO and stygobites could be easily observed. In fact only seven sites had DOC levels over 100mg/l, three were dysoxic (DO>0.3, <3.0mg/l), the remainder Oxic (DO>3.0mg/l), and stygobites were recorded in both the oxic and dysoxic sites.

pH: Stygobites were found in a range of pH conditions in this survey, reflecting the geochemistry of the different aquifers sampled. Robertson *et al.* (2009) report that stygobites are often more common in alkaline waters, than in acidic ones. Our survey appears to agree with this, with higher stygobite presence and abundance in the alkaline Chalk, compared to the granite and other acidic aquifers. Humphreys (2008) notes that the alkaline chemistry of carbonate aquifers, such as the Chalk, appear to be benign to stygobites, whereas the more acidic waters associated with igneous, metamorphic and some sedimentary rocks may be less suitable for stygofauna. This offers a possible explanation of the pattern in stygobite presence and abundances observed in this survey, although the better habitat opportunities presented by the Chalk in comparison to the other strata, perhaps offer a more likely explanation.

5.3 Physical & Environmental relationships

Of the environmental and physical measures recorded only 'borehole purpose' and 'sampling point type' were found to be significantly related to stygobite presence. The abundance data was less informative due to many of the sites having no stygobites present or only very low abundances. The few sites with high abundances (>10 individuals) were limited to the Chalk and granite strata (Units 5b and 1b respectively). The role of these and other factors in governing stygobite occurrence is discussed below:

Sampling point type

This categorical group consisted of the three types of sites which were surveyed: boreholes, wells or springs. The results of the binary logistic regression demonstrated that stygobites were significantly more likely to be found in spring sites compared to the boreholes. It should be remembered however, that the sampling techniques differed between these types of sites and therefore the efficiency of the sampling method may also have a bearing on this finding. The odds ratios suggest stygobites were next most likely to be found from wells and least likely from boreholes. These findings are in general agreement with Arnscheidt *et al.* (2009) who reported greatest sampling success (likelihood of finding stygobites) from dug or excavated wells and springs, compared with constructed boreholes. The abundance data (Figure 4.11) shows a similar trend with greatest abundance associated with the spring sites, with lower comparable abundance recorded for both wells and boreholes.

Borehole purpose

This categorical measure is based on three pre-defined groupings; 'not used', 'monitored' and 'used' - boreholes, wells or springs. 'Not used' were undisturbed/disused facilities, 'monitored' was applied to hydrometric surveillance points occasionally disturbed through water level dipping activities, whereas 'used' was applied to sites frequently disturbed by pumping events. The logistic regression analysis showed stygobites were most likely to be found in 'monitored' sites, followed by 'not used' sites and finally 'used' (pumped) sites. Arnscheidt *et al.* (2009), in a survey in Ireland, found that Crustacea were much more commonly retrieved from more 'natural' sites (wells and springs) than from active abstraction sites. Although not directly comparable to this survey, such findings suggest a linkage with disturbance and pumping regimes. Whilst this might be true for our survey, the results may simply reflect the difficulties we encountered when sampling pumped sites rather than the pumping regime itself.

Borehole design

The apparent preference of stygobites for springs (and wells to some degree) over boreholes may be related to the borehole design and construction, an important factor highlighted for consideration by Hahn & Matzke (2005) when interpreting information on the spatial distribution of stygobites. They also state the importance of knowing the length of the borehole screen when identifying the depth from which stygobites are sampled. Deep boreholes with long screens will sample fauna from the whole length of the borehole and it is therefore difficult to determine the specific depth from which animals are being recovered at these sites. Many of the boreholes in our survey were indeed fairly deep (up to 100m) and detailed construction details were not available. However the logistic regression analysis of borehole depth, diameter, volume and water column height indicated that the likelihood of finding stygobites receded with increasing borehole depth and increasing volume. This negative relationship with borehole depth agrees with observations by Humphreys (2008b) who notes that groundwater animals are mostly restricted to the upper parts of the subterranean ecosystem, whilst acknowledging stygofauna can be found at great depth in some karst systems.

Sampling protocol and mesh size

The sampling protocol consisted of four categories as described in Section 3.2.3; (1) net haul down well/borehole, (2) pumped/tap sample washed through net, (3) pond net sweep of catchpit/header tank, (4) Bou-Rouch & scraped spring samples. Whilst no significant relationship was identified through logistic regression of presence absence data, the odds ratios and calculated odds indicate that stygobites were most likely to be found using method (4), followed by methods (3), (1) and finally method (2). These results are partially skewed by the low number of replicates for protocol 4, and all relate to spring samples. Similarly, protocol (3) relates to those sites collected mainly from springs and perhaps simply reflects the higher sampling success recorded from spring samples. However, protocol (3) also included header tanks from pumped boreholes. The presence of stygobites in about a third of these sites (i.e. pumped to header tank) far exceeded those other pumped sites from which samples were gained directly from a tap. Whilst the sampling success from boreholes was best achieved via a net down the borehole, the sampling of header tanks would appear to be a good alternative where there is no access to the borehole shaft.

Mesh size was not a significant factor in the presence or absence of stygobites. However, the odds data indicate stygobites were more likely to be found using the larger mesh size (250um) and the abundance of individuals was also often higher when using this mesh. Again, a co-correlation with sampling success from springs probably explains this relationship given the larger mesh size was almost exclusively used for sampling spring catchpits and header tanks.

Surrounding environment

This category consisted of three roughly equal sized groups; garden and amenity land, rural land (agricultural and woodland) and urban/roadside land. No significant relationships between stygobite presence or absence were identified through the logistic regression and the odds data indicate no obvious differences between the groups. A similar picture emerges from the abundance data with perhaps the rural group tending towards greater abundance levels. Since the survey was largely conducted over fairly sparsely populated areas, with one or two exceptions, the lack of a relationship with human activities (accepting agriculture as the landscape), is perhaps unsurprising in this survey.

5.4 Conclusions and recommendations

1.0 The presence or absence of stygobites was found to differ significantly between the different hydrogeological units examined. The expected association of stygobites with highly permeable material was demonstrated in part, with high probabilities for stygobite occurrence reported for fractured and granular aquifers and superficial deposits. However, stygobites were also recorded, albeit less frequently, from much lower permeability rocks, such as the granite, where fracture flow is the dominant groundwater transport mechanism. Chalk, which had the highest individual probability of stygobites presence, is of course also dominated by fracture flow due to the low primary porosity of the Chalk matrix. The presence of fracture networks appears to a critical component in dictating stygobites presence in this region.

2.0 Stygobites were reported absent from only one of the hydrogeological units surveyed – the Triassic mudstone. Whilst only one sample was collected from this stratum, it is characterised by very low permeability mudstones and siltstones and would appear to provide the poorest habitat opportunities for stygobites. We suggest that this material which outcrops along the border between Devon and Dorset and extends through Somerset to the North Devon coast, might be acting as a barrier to stygobite movement between Dorset and Devon, and potentially Somerset, although this area was not part of the survey. The presence in Devon *of Niphargus aquliex, Microniphargus leruthi* and *Antrobathynella stammeri*, also known outside Devon to the east, appear to be the exceptions to this theory of restricted movement. We suggest that these species, which can be associated with shallow riverine deposits may be able to move more freely along hydraulically connected hyporheic networks, thus by-passing the underlying lower permeability geology. Other theories on the more widespread occurrence of these species and the apparent isolation of others (e.g. *Niphargus glenniei*) are explored in Proudlove *et al.,* 2003.

3.0 Whilst *Niphargus glenniei* was not found outside Devon, it did occur across a wide range of geologies that were both physically and chemically contrasting. Whilst it was most frequent within the base poor igneous and metamorphic strata it was also present within Devonian Old Red Sandstone, Devonian Limestone, Upper Greensand and Permo-Triassic Sandstone. (It is also known from Devonian limestone caves in Devon, although these were not sampled as part of this investigation). The exception was the Upper Carboniferous Crackington Formation, where it was completely absent. This formation is a mixture of shales, sandstones and siltsones and whilst characterised by low permeability material, fracture flow is the main groundwater flow mechanism, which could provide suitable habitat and Indeed *Niphargus aquilex* was recorded from a few isolated sites. Further study of this unit is needed to examine the apparent absence of *Niphargus glenniei* from this geology.

4.0 Within the survey area the Chalk appears to offer the best habitat for stygobites where the greatest diversity of species and abundance was reported. However, similar diversity (but significantly lower abundance) was also reported collectively from the combined fractured and granular aquifers (Unit 3). In this Unit the Upper Greensand (Unit 3d) and the Permo-Triassic Sandstone (Unit 3a) appear to offer the most suitable conditions. These units are characterised by intergranular flow and fracture flow. The lower abundance of stygobites in these strata may in part be a function of less well developed fracture networks within these units, compared to the Chalk.

5.0 The Upper Greensand which underlies the Chalk, reported species found from the Chalk (in Dorset), as well as species reported from other geologies in Devon. For example *Niphargus glenniei* and *Niphargus kochianus kochianus* were both found in the Upper Greensand (*N. glenniei* on one occasion and *N. kochianus kochianus* on two occasions, neither together). The presence of these species in the same unit might suggest some connectivity between the different units. The loss of Chalk groundwater to the Upper Greensand has been reported in Dorset (Allen *et al.,* 1997) and offers a plausible pathway for stygobite movement. In Devon it is less clear although possible linkage between the Upper Greensand and the Permo-Triassic Sandstone may offer a pathway for *Niphargus glenniei*. However, these are very tentative suggestions and further study is needed to support these theories. To this end we recommend that further stygobite surveys, targeted at sites located within outcrops of Upper Greensand and Permo-Triassic sandstones, are undertaken along the eastern Devon border with Somerset and Dorset. Examining the geology of this border area (see Figure 5.1) it seems possible that *Niphargus glenniei* could be present outside Devon in outcrops of Greensand and Permo-Triassic Sandstone if connective pathways exist.

6.0 There was some evidence for relationships between stygobites and some chemical determinands (e.g. chloride). However, given the fairly limited range in concentrations recorded, it is difficult to interpret these relationships fully. The results are also confounded by the habitat opportunities exhibited by the different geological units. In other instances there was a lack of data, for example no stygobites were found in suboxic environments but there was only one site where such conditions were recorded.

7.0 The use of the sampling facility (i.e. was it subject to disturbance through pumping or monitoring events) may have some bearing on the likelihood of finding stygobites. Sites which were not disturbed through pumping had a higher chance of stygobite occurrence than those sites which were pumped, but this could be due to the difficulties in sampling the pumped sites rather than the fact that the borehole was pumped. Where pumps prevented access to the borehole shaft catchpits and header tanks proved to be a good alternative, provide they preceded any *ex-situ* water treatment mechanism.

6.0 Survey (B) – Northern England

6.1 Survey strategy

The purpose of this survey was to compare the occurrence and diversity of groundwater fauna between geologies either side of the glacial divide in northern England. The survey work examined this through the sampling of selected boreholes in the previously glaciated Magnesian limestone of County Durham (Region 2) and both previously glaciated and un-glaciated chalk in Yorkshire and northern Lincolnshire (Region 3). The survey Regions and surface and solid geology are illustrated in Section 2.0, Figures 2.3 – 2.6.

6.2 Survey protocol

All sites sampled were boreholes which are not actively pumped. At each borehole a net with a 63 µm filter was lowered to the bottom three times using the same protocol as Survey A (Section 3.3). No chemical data were collected, although borehole characteristics and the surrounding typography were noted. The sampled boreholes were often of a small diameter (~5cm) restricting the width of the nets which could be used to recover samples.

Material recovered in each of the 3 net hauls was collected and analysed separately enabling comparison of fauna collected in each individual net. The post sampling and preservation process was identical to Survey A.

6.3 Laboratory analysis

After completion of the survey work all samples were taken back to the laboratories at the British Geological Society (BGS) at Wallingford. Analysis of the samples was carried out by three separate analysers at three locations: the laboratories at CEH Wallingford; the Roehampton University Life Science department and at Lee Knight's laboratory in Devon, using the same protocols described in Section 3.5 for Survey A. The taxa specific key used for identification of Groundwater stygobitic Crustacea was; British Freshwater Crustacea Malacostraca (Gledhill *et al.*, 1993).

7.0 Data analysis – Survey (B) – Northern England

7.1 Magnesian Limestone

Six boreholes were sampled in the Magnesian Limestone. Three net hauls were recovered from each borehole, making a total of 18 samples. A third of these net hauls contained no fauna, the remainder contained mainly freshwater taxa with one or two occasional terrestrial taxa also recorded. None of the samples contained any stygobites. The most abundant taxa found were Oligochaeta (170 individuals) followed by Copepoda (30 individuals), but these taxa were not found universally and only appeared in high numbers on one occasion, each in separate single boreholes.

7.2 Chalk

In the Chalk of north Lincolnshire and Yorkshire (Region 3), a total of 32 boreholes were surveyed, 16 in each county. Again, separate samples were recovered for each of the three net hauls, providing a total of 96 samples to examine. Two stygobitic species were recorded; *Niphargus aquilex* and *Antrobathynella stammeri*.

7.2.1 Taxon composition

In the Lincolnshire Chalk about a third of the 16 sites sampled contained no taxa. In the remaining two thirds of sites a total of 12 different taxa were found. About half of these were aquatic species, the remainder being terrestrial organisms such as Collembola, some of which may have a subterranean existence. The most frequently found aquatic species was the stygobite *Niphargus aquilex*, recorded from six out of the 16 sites surveyed. It was the only stygobitic species recorded from the Lincolnshire Chalk with a total abundance of 40 individuals. The numbers of *Niphargus aquilex* recorded from each net haul, were generally low (median = 2) with one notable exception where 19 individuals were recovered.

In the Yorkshire Chalk a quarter of the 16 sites contained no taxa. At the sites where fauna were found, a total of 27 different taxa were identified. Around a half of these were aquatic species, the other half being of terrestrial origin. The aquatic taxa were however found in far greater abundance than the terrestrial. Of the terrestrial taxa, Collembola were the most common and most abundant. The most frequently found aquatic groups were Oligochaeta (eight sites), followed by Copepoda (four sites). Copepoda were by far the most abundant group (a total abundance of 401 individuals). Two stygobite species were recorded from sites in the Yorkshire Chalk; a single *Niphargus aquilex* was found at one site, with *Antrobathynella stammeri* being recorded from a further two sites with a total abundance of 27. The numbers of *Antrobathynella* individuals recorded from each net haul were fairly consistent, ranging between 1-9, (median = 4).

A summary of the stygofauna recorded from Survey B, is provided in Table 7.1 below. Again, whilst copepods were recorded during this survey they were not identified to species and were not considered further under this survey. They will however, be identified to a higher taxonomic resolution in due course and reported separately at a later stage.

Table 7.1: Summary of species presence and abundance in Survey B, study Regions 2 & 3

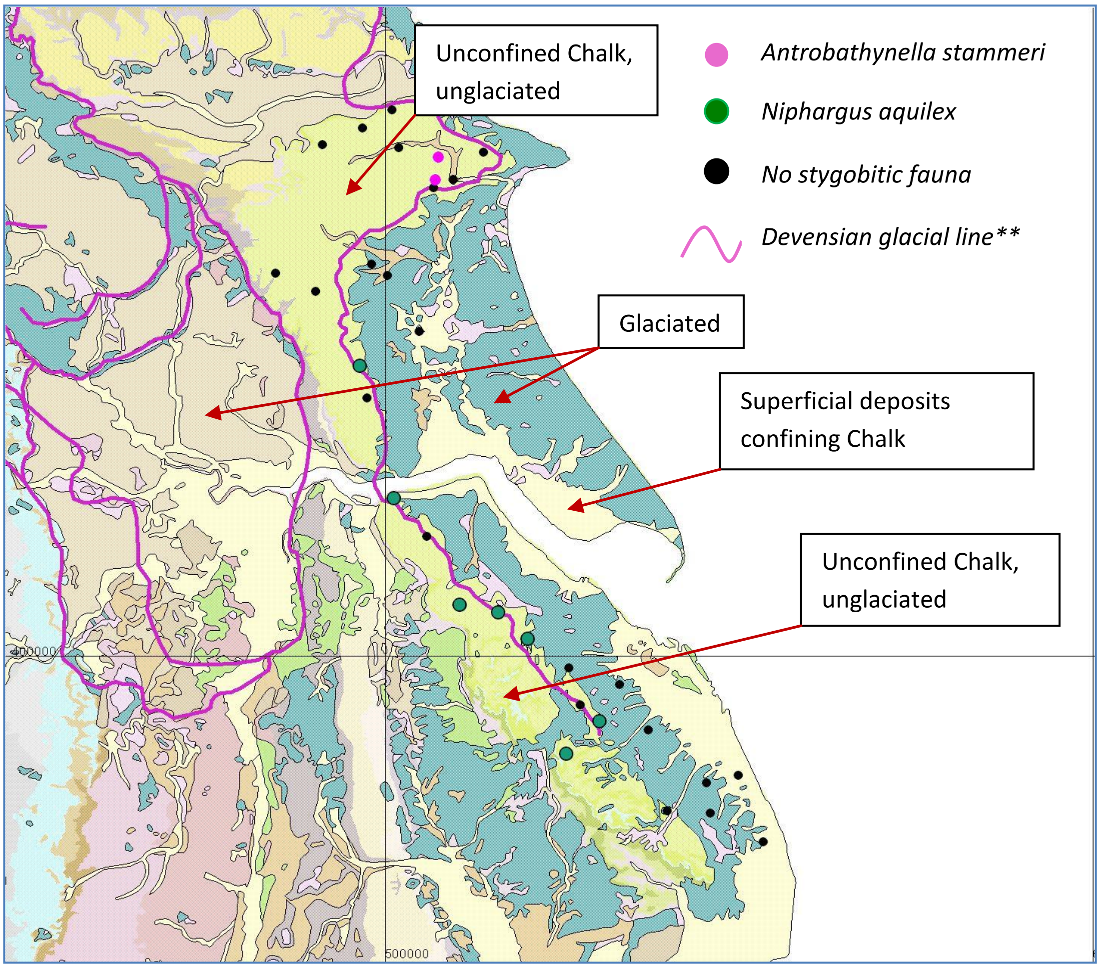
Parameter	Species recorded	Region 2	Region 3	
		Magnesian Limestone	Lincolnshire Chalk	Yorkshire Chalk
No. of sites species recorded from	*Niphargus aquilex*	0	6	1
	Antrobathynella stammeri	0	0	2
Percentage of sites species recorded	*Niphargus aquilex*	0	38%	6%
	Antrobathynella stammeri	0	0%	13%
Total abundance	*Niphargus aquilex*	0	40	1
	Antrobathynella stammeri	0	0	27
Max recorded abundance/ borehole	*Niphargus aquilex*	0	22	1
	Antrobathynella stammeri	0	0	18

7.3 Sampling efficiency

Across all sites sampled where specimens were captured (24 sites) the total number of individuals (terrestrial and freshwater) decreased more often than not (66% of sites) between the first and last net haul. However, the relationship is not statistically significant because the sample size is too small.

7.4 Stygobitic taxa distribution

Figure 7.1 below presents the distribution of the stygobitic fauna recorded in the survey of the Yorkshire and Lincolnshire Chalk. The approximate position of the Devensian glacial limit and the presence of strata confining the Chalk are also depicted. All stygobites records from this survey were located within the unglaciated areas or close to the approximate glacial limit, and in unconfined material.



** The approximate position of the Devensian glacial limit based on British Geological Survey data.

Figure 7.1 Location of stygobites recorded from boreholes surveyed in study Region 3.

8.0 Discussion – Survey (B) – Northern England

8.1 Stygobite distribution

The absence of stygobites from the Magnesian Limestone of County Durham is perhaps not surprising, considering the view expressed by Proudlove *et al.,* (2003), Robertson *et al*., (2009) and others, that the Devensian glaciation might have extirpated populations of subterranean invertebrates. However, there is still some debate and much conflicting evidence over the actual effect of the glaciation on stygobitic populations (see Proudlove *et al*., 2003; Knight & Penk, 2011). In addition only six sites were sampled during the current survey and historically relatively few stygobite records have been reported from this part of the country (http://www.freshwaterlife.org/hcrs/db) and indeed from north of the Devensian limit, supporting the glacial extirpation hypothesis. Of these previous records *Antrobathynella stammeri* is the only species found north of Durham, with records reported from as far north as Cumberland in England and Stirlingshire in Scotland. There is however a single record of *Niphargus aquilex* collected from a well at West Hartlepool in County Durham in 1893, although no specimen exists for verification. The records referred to for *Antrobathynella stammeri* are from superficial deposits, with which the species is commonly associated (Proudlove *et al*., 2003). As mentioned earlier in Section 5.0, *Niphargus aquilex* is also reported as having an association with superficial deposits, so it is possible that both species are present in the region, at least within overlying superficial deposits. Further study of such habitats is needed to explore this possibility.

The presence of *Niphargus aquilex* in the Lincolnshire Chalk and both *Antrobathynella stammeri and Niphargus aquilex* in the Yorkshire Chalk demonstrate the ability of these species to occupy phreatic habitats as well as superficial deposits. Such versatility may help explain their wider known distribution within Britain, compared to the other known species. Indeed, after *Antrobathynella stammeri,* the next most northerly records of any stygobite in Britain are this survey's records for *Niphargus aquilex* from Yorkshire and the unconfirmed, historical record from County Durham. However, neither species was found during the Yorkshire or Lincolnshire surveys in areas where the Chalk was confined by overlying glacial deposits. Furthermore they were not found significantly north of the indicative line of the Devensian glacial limit, although few samples were taken from north of the glacial limit.

8.2 Influences on stygobite occurrence

The absence of stygobites from the confined Chalk strata in the Yorkshire and Lincolnshire surveys is likely to be a function of the dissolved oxygen concentrations (DO) and the availability of organic matter within the system. Hahn (2006) highlights the importance of these factors in governing stygobite occurrence, and how they are in turn dependent on the degree of hydrological connectivity with surface waters.

In unconfined groundwater systems oxygen concentrations can be replenished by the ingress of atmospheric oxygen or by water recharge through the vadose zone. In confined groundwater systems such replenishment cannot occur (Malard & Hervant, 1999). Oxygen concentrations in confined material are therefore naturally and permanently low. Although stygobites have been found in a wide range of DO concentrations, including anoxic environments (Boulton *et al.,* 1992), they do not appear to be able to cope with such conditions indefinitely (Malard & Hervant, 1999). Although dissolved oxygen concentrations were not measured in this survey, it is probable that concentrations within the confined Chalk are a limiting factor to stygobites.

The functioning of the subterranean aquatic ecosystem is dependent on the supply of allochthonous organic matter from surface ecosystems (Foulquier *et al.*, 2010), although chemosynthetic based ecosystems are also known (Sarbu *et al.*, 1996). The availability of dissolved organic matter as a food resource may therefore also be limiting to stygobites in a confined aquifer. This is because in confined aquifers, pathways for dissolved organic matter entering the system will be restricted, compared to unconfined aquifers which have a direct connection to the surface. Additionally, the consumption of any organic matter present in a confined system will further deplete limited dissolved oxygen resources, thereby decreasing biological activity (Humphreys 2006). Whilst dissolved organic carbon (DOC), was not measured in this survey, Goldscheider *et al.* (2006) note that deep and old groundwater, such as that associated with many confined aquifers, can often be virtually free of DOC. Such limited food resources combined with permanently low dissolved oxygen concentrations may explain the recorded absence of stygobites in the confined Chalk in this survey, although more extensive survey work is required to support this assertion.

The absence of stygobites north of the Devensian glacial limit in boreholes from the Chalk of Yorkshire and Lincolnshire is in agreement with a number of other studies, which suggest glaciations have caused local extinctions and that subsequent recolonisation of such areas has been extremely slow (Proudlove *et al*., 2003; Dole Olivier *et al*., 2009). Species reported elsewhere north of the glacial limit (e.g. *Antrobathynella stammeri and Niphargus aquilex,* see Section 8.1 above) must therefore either have survived in refugia (i.e. in deep unfrozen groundwater) or re-colonised later from previously unglaciated areas via long distance dispersal (Hahn and Fuchs, 2009).

8.3 Conclusions and Recommendations

1.0 Based on the results of our limited survey of the Magnesian limestone, stygobite populations appear to be absent from these strata. However, we know from historical records that stygobites have been recorded from both this region and further north. The old unverified record for *Niphargus aquilex,* from County Durham, also suggests that stygobitic species this far north are not restricted to just *Antrobathynella stammeri* and that it is possible other species are present. A more extensive survey of this region is therefore recommended. Given the known association of *Antrobathynella stammeri* and *Niphargus aquilex* with superficial deposits, it would be wise to investigate both shallow and deeper groundwater habitats.

2.0 The absence of stygobites in the Chalk sites north of the approximate location of the Devensian glacial limit supports the theory that stygobite populations may have been extirpated during glacial events. However, because the Chalk in formerly glaciated areas is overlain by low permeability glacial deposits which result in a hydrochemical environment which is likely to be unsuitable for groundwater fauna, it is unlikely that groundwater fauna would have been able to re-colonise these areas following glacial retreat.

3.0 Future studies of groundwater fauna in formerly glaciated areas should focus on aquifers with good connectivity with recharge areas (permeable superficial deposits, or bedrock aquifers which are not concealed by low permeability superficial deposits).

9.0 Survey (C) – River Piddle, Dorset

9.1 Survey strategy

This survey had a distinctly different focus from the previous surveys, examining invertebrate faunal assemblages from shallow depths within the hyporheos of a chalk stream in Dorset. The purpose of this study was to examine how these assemblages differ between hydraulically contrasting sections of the stream; one where groundwater is providing base flow (a gaining section) and one where surface water is being lost to groundwater (a losing section). The river Piddle was chosen for this survey, a well studied stream which has recently been the focus of a detailed groundwater model, constructed by the Environment Agency. The model outputs were made available by the Environment Agency and through discussions with their hydrologist a selection of suitable sites were chosen based on the model predictions and expert judgment.

The experimental design consisted of a total of six sites (A-F), three in gaining reaches (D to F) and three in losing reaches (A to C), all located in the upper part of the river, away from the influence of major abstractions. Each site was located a minimum of 300 m apart, with the losing and gaining sections (D to C) located significantly further away, as illustrated in Figure 9.1. Each site was approximately 25m in length encompassing a shallow riffle pool sequence. All sites were surveyed once on the same day and then again two weeks later. The surveys were conducted between 20th January and 3rd February 2011. On each occasion the surveys commenced with the most downstream reach moving progressively upstream so as not to influence the following reach through disturbance of sediments. With reference to Figure 9.1, the first site sampled on each survey was site F starting with the most downstream sampling point (i.e. sampling point DS1 – in Figure 9.2).

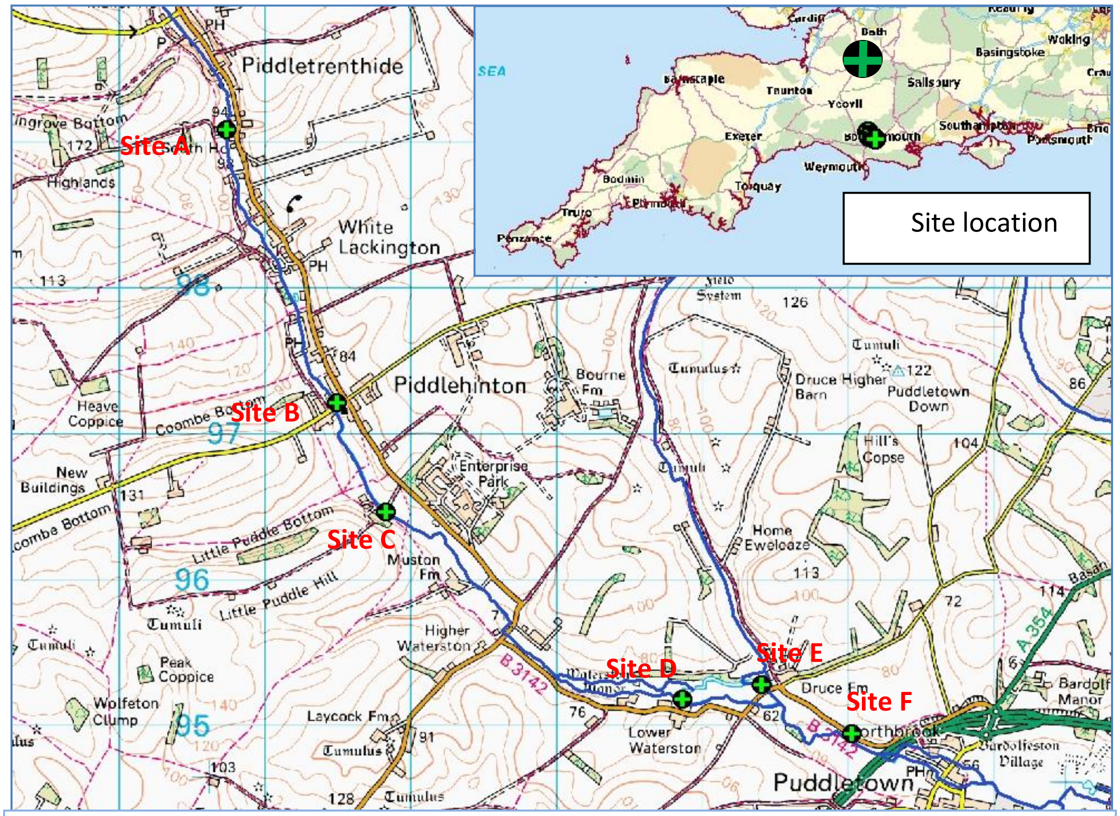


Figure 9.1 Location of sampling sites along the River Piddle, Dorset.

9.2 Survey protocol

a) Site parameters: At each site the length of river to be surveyed was measured out using a reeled tape measure. A GPS unit was used to record grid references for the downstream and upstream points of the reach. Measurements of the average stream depth and width were taken at the upstream, middle and downstream points of the stretch.

b) Site hydrology: At each end of the reach a shallow piezometer was inserted into the river bed. This comprised a narrow transparent plastic tube 1.0 metre in length and perforated at the bottom to a height of 100mm from the base. The pipe was inserted to a depth of 200mm below the base of the river bed using a metal sleeve which fitted over the plastic tube and could then be hammered into the ground, taking the plastic pipe with it. The sleeve was then removed leaving the plastic tube installed at the required depth within the sediment. The piezometers were left in place and at the end of the survey the level of water within the tube was measured relative to the level of the stream surface water level, as either a positive or negative value.

c) Sampling points: The hyporheos was sampled at five locations within each reach on each survey visit in a random linear pattern along the length of the reach within the main flow path. This was achieved using the Bou Rouch pump method (as described for Survey A, Section 3.3 IV). This involves a length of steel pipe being driven into the river bed to a depth, in this case, of 200mm. The steel pipe, measuring 1.2m in length with an internal diameter of 25mm, was perforated at the bottom 100mm with 5mm holes, allowing a discrete window sample to be recovered from the shallow hyporheos.

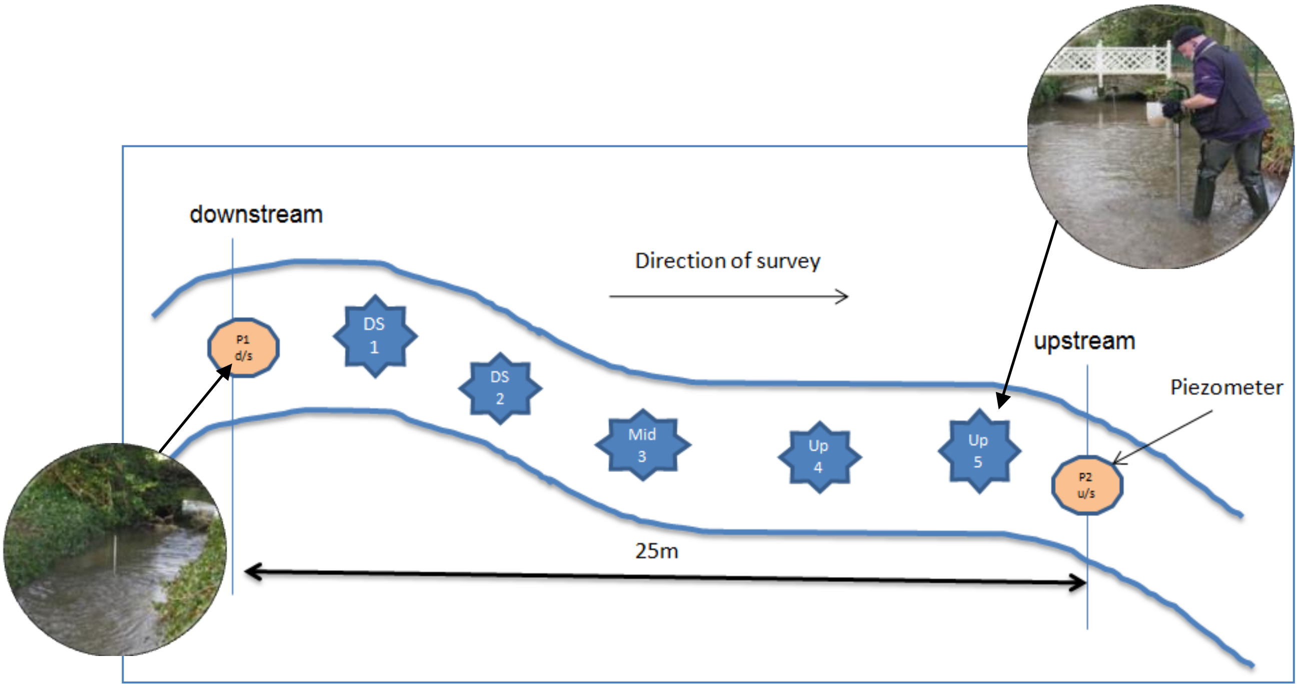


Figure 9.2. Survey plan for hyporheic sampling of river Piddle.

d) Field chemistry: At each site, on both sampling visits, water samples were recovered from three of the five Bou Rouch standpipes prior to pumping, for water chemistry measurements. The standpipes selected were the same at each location, starting with the most downstream point (DS1) followed by the mid point (Mid3) and finally the most upstream point (Up5). Samples of hyporheic water were abstracted from the pipes by lowering a length of plastic tubing to the bottom of the standpipe and slowly drawing the water up through the pipe to the surface using a syringe, thereby minimising the introduction of surface oxygen.

The recovered sample was split into two; one part was used to measure pH, using a handheld (Hanna Instruments HI 9812 pH-EC-TDS) meter, calibrated with standard solutions prior to use at each site. After the pH was measured, this part of the sample was decanted in to two 10ml Bijou bottles, labelled and placed in a cool box. On return from the field the Bijou samples were frozen and then taken to Roehampton University for further chemical analysis. The remaining part of the sample was used to determine the dissolved oxygen concentration [DO], of the hyporheic water. This involved filling a pre-prepared ampule, containing Accuvac Dissolved Oxygen Reagent[8], with the water sample, leaving for 2 minutes for reaction time and then measuring the DO concentration using a Hach pocket colorimeter II.

Temperature measurements of the surface water were recorded using Tiny-Tag data loggers installed at each site for the duration of the two week survey period. The data loggers were installed in the main flow path of the river fixed to a steel stake and covered with bed material. At the end of the survey the loggers were recovered and the recorded temperature profile downloaded to a computer.

e) Biological sampling: Following installation of the Bou Rouch standpipe and the recovery of water chemistry samples (as described in section C above), the Bou-Rouch pump head was attached to the top of the standpipe and first primed with a litre of clean water added to the top of the pump head. The pump was then operated by hand to extract a sample of hyporheic water, along with invertebrate fauna drawn out of the surrounding interstitial habitat by the flow (see Figure 9.2). The sample water was pumped out into a bucket with marked graduations in litres. After 4 litres* had been recovered the sample was taken to the bank-side where it was washed through a 63µm plankton net, The resultant sample was then transferred to a plastic sample bag and preserved in 70% Industrial Methylated Spirits (IMS). The process was carried out at five locations at each site on both sampling visits providing a total of 60 samples in all (i.e. five from each of the six sites, performed twice). The sample bag was sealed and labelled, before being placed in a second labelled bag and stored in a cool box. At the end of each survey day all samples were taken and stored at the FBA River laboratory at East Stoke. On completion of the whole survey the samples were transported to the Life Science laboratories at Roehampton University for analysis to species level where possible (see section 9.4)

*[The figure of 4 litres was derived from previous studies by Kibichii *et al.* (2009) and Boulton *et al.* (2003) which examined optimum sampling volumes and strategies for assessing hyporheic biodiversity using the Bou Rouch method. Ultimately the optimum volume recovered depends on spatial and temporal variables and the number of replicate samples recovered. The studies agree that increasing the sample volume beyond 5 litres results in depletion in invertebrate numbers per litre of sample pumped. Kibichii suggests an optimum volume of between 3-5 litres, depending on the number of replicates, depth of sample and season. He also indicates that a lower number of

[8] EDTA tetrasodium salt, Hydroquinone, alpha-(dinonylphenyl)-omega-hydroxy-poly (oxy-1,2-ethanediyl), L-lysine

samples are needed to capture taxon richness when sampling the ecotone (between the epigean and hyporhoes habitats) than is needed at greater depth within the hyporheos. This is because almost all of the taxa are obtained in the first 3 litres when sampling the ecotone].

9.3 Laboratory analysis

Chemical analysis: The frozen water samples collected in the 10ml bijou bottles and dispatched to Roehampton University were thawed then analysed for major anions and cations. As described in Section 3.4 for Survey (A), high pressure liquid chromatography was used for this analysis, with the same level of reporting values.

Biological analysis: The invertebrate samples were sieved using the same protocols described in section 3.5 for survey A. The samples were sorted using a Bogorov sorting tray under a Nikon DM-10 stereomicroscope. After removal of organisms from the sample, the remaining material was dried at 40^0 C for 24 hours, weighed and ashed in a muffle furnace at 350^0C for 4 hours to ascertain the loosely associated organic matter (LOM). LOM gives a rough approximation of the organic material associated with the sediment within each sample (Pusch *et al*., 1994). The fauna collected were identified to species level where possible in particular crustaceans and macroinvertebrates. Oligochaetae, Diptera, Ostracoda and Nematoda were assigned to groups due to the poor preservation of Oligochaeta and Nematoda and also the predominance of early instar Chironomidae larvae which could not be identified accurately to a higher taxonomic level. The taxa specific keys used for identification of fauna were: Copepoda (Einsle, 1993; Janetzky *et al*., 1996); macroinvertebrates (Edington *et al*., 2005; Elliott *et al*., 1983; Holland, 1972; Hynes, 1993; Wallace *et al*., 2003); Groundwater stygobitic Crustacea (Gledhill *et al.*, 1993).

9.4 Data management

Generalised linear model

Generalised Linear models allow the distribution of the response variable to be fitted correctly whether the distribution follows a Gaussian, Poisson, negative binomial, geometric or gamma distribution (Zuur *et al*., 2009). To accommodate for under- or over-dispersed poisson data quasi-poisson errors were fitted to the model (Wedderburn, 1974). Under or over dispersion occurs when the residual deviance is much greater or much less then the residual degrees of freedom. The use of quasi-poisson errors allows the dispersion parameter to be estimated rather than using the default value of 1 set in a Poisson distribution. Stepwise model selection was performed for each species and higher taxa. The full model with all interactions was reduced in a stepwise manner. The reduced model was compared to the full model using an analysis of deviance test, the optimal model is reached when the model comparison becomes significant indicating no further reduction is possible and the optimum model has been reached. For the analysis, date, losing and gaining sections, were treated as fixed factors. All statistical analyses were performed with R version 2.11.12 (R Development Team, 2010).

Ordination

Detrended correspondence analysis (DCA) was used for the community ordination. DCA is a suitable method for community ordination when environmental data is absent and only species data is available. DCA using the decorana function in the vegan package identifies the first four axes only. The eigenvalues and axis lengths are presented though the eigenvalues in DCA are abstract values that display little information of the dataset. The axis lengths display the most information within the dataset. Axis lengths in DCA are also a measure of beta diversity and axis lengths of 4 or greater indicating a complete turnover of species along the gradient length. All data was input as presence or absence data due to the large number of rare species in the dataset. Ordinations were performed with the Vegan package version 1.8-3 (Oksanen *et al*., 2006). All statistical analyses were performed with R version 2.11.12 (R Development Team, 2010).

10.0 Data Analysis - Survey (C) – River Piddle, Dorset

10.1 Hydrological influence on physico-chemistry

There was a consistent pattern across losing and gaining reaches, with a decrease in both pH and dissolved oxygen in the gaining reaches. This pattern was most apparent for pH, with dissolved oxygen less variable during the second sampling visit (Table 10.1). The number of replicates taken was small therefore results should be judged in this light. Differences in hydraulic head (measured using piezometers at the patch scale) between losing and gaining sections in the shallow hyporheic were detected, though patterns were not consistent across all sites. Where downwelling did occur this was in the losing sections of the Piddle, with the Manor House site (site B) consistent across both replicate studies. The areas displaying strong upwelling properties were located in the gaining sections of the River Piddle. In all gaining sections no negative hydraulic head was detected. The Manor Farm site displayed strong upwelling properties in comparison to the other sites with the pattern consistent across both replicate studies. The majority of sites displayed weak up and downwelling properties in the shallow hyporheic with hydraulic head neutral across many sites. Water flow across the study period was fairly consistent with little diurnal variability. Prior to the first sampling occasion a significant spike in flow occurred in the Piddle catchment as shown in Figure 10.3. Three consecutive pulses of high flow occurred, each increasing in magnitude. Following the high flow period a steady increase in flow occurred up to the first sample date with flow levelling off, then decreasing three days after the first sample date (Figure 10.3). Loosely associated organic matter (LOM) differed significantly between dates and between losing and gaining sections. LOM was significantly higher in the gaining section of the River Piddle (Table 10.2; Figure 10.2) and also decreased significantly between the sampling visits (Table 10.2; Figure 10.2). Dissolved oxygen and pH concentrations were consistent between the two sampling visits with a slight decrease in pH across all sites between visits 1 and 2 and a slight increase in dissolved oxygen between visits 1 and 2. The exception to this was the Watermeadows site (site F), where dissolved oxygen increased substantially between the 20th and the 3rd from 5.7mg/l^{-1} to 11 mg/l^{-1}

Table 10.1 Site location and physico-chemical characteristics of the study sites sampled on both sampling visits. Key: mid = midstream

Site	Losing gaining	National grid reference	pH	DO (mg/l^{-1})	Mean bank width (m)	Mean Depth (cm)			Hydraulic head	
						u/s	mid	d/s	Up stream	Down stream
Visit 1										
South House (A)	Losing	SY7074799055	8.3	11.0	2.65	30	27	17	0.0	0.0
Manor House (B)	Losing	SY7147897241	8.4	9.2	3.6	21	19	15	-5.0	0.0
Little Piddle (C)	Losing	SY7184796469	8.3	11.0	3.2	27	27	14	0.0	0.0
Manor farm (D)	Gaining	SY7400095162	7.3	6.6	5.0	12	12	12	5.0	5.0
Waterson Springs (E)	Gaining	SY7431995176	7.6	11.0	4.93	15	15	11	1.4	0.2
Watermeadows (F)	Gaining	SY7499594935	7.9	5.7	7.83	10	15	16	0.0	0.5
Visit 2										
South House (A)	Losing	SY7074799055	8.1	11.0	2.65	26	30	18	0.5	0.0
Manor House (B)	Losing	SY7147897241	8.0	10.3	3.6	22	15	21	-0.5	0.1
Little Piddle (C)	Losing	SY7184796469	8.2	10.9	3.2	17	27	20	0.0	-0.1
Manor farm (D)	Gaining	SY7400095162	7.3	7.2	5.0	17	14	11	5.0	2.0
Waterson Springs (E)	Gaining	SY7431995176	7.1	10.5	4.93	21	17	32	1.0	1.0
Watermeadows (F)	Gaining	SY7499594935	7.2	11.0	7.83	28	16	22	0.1	0.5

Table 10.2 Generalised linear model results for differences in LOM between sampling dates and between losing and gaining sections of the River Piddle.

	df	± intercept	Standard Error	t-value	p-value
Date	1,57	-0.43	0.09	-4.36	<0.001
Hydrology	1,57	-0.20	0.09	-2.03	0.04

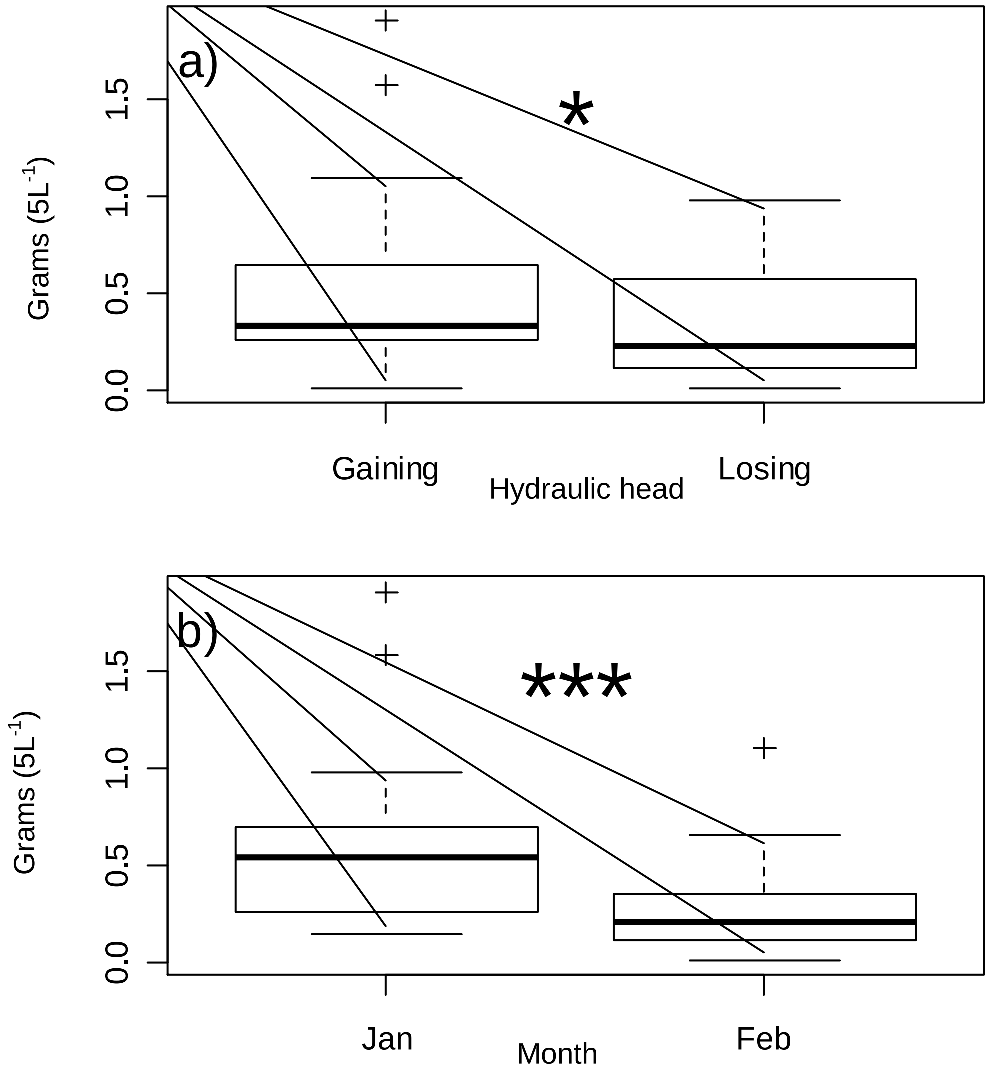


Figure 10.1 Mean amount of LOM recorded in each Bou-Rouch sample collection from a) losing and gaining sections of the River Piddle and b) from both sample dates. Significance results are derived from Generalised linear model results of log10 (n +1) transformed data. Plot results are actual data and not log transformed data.

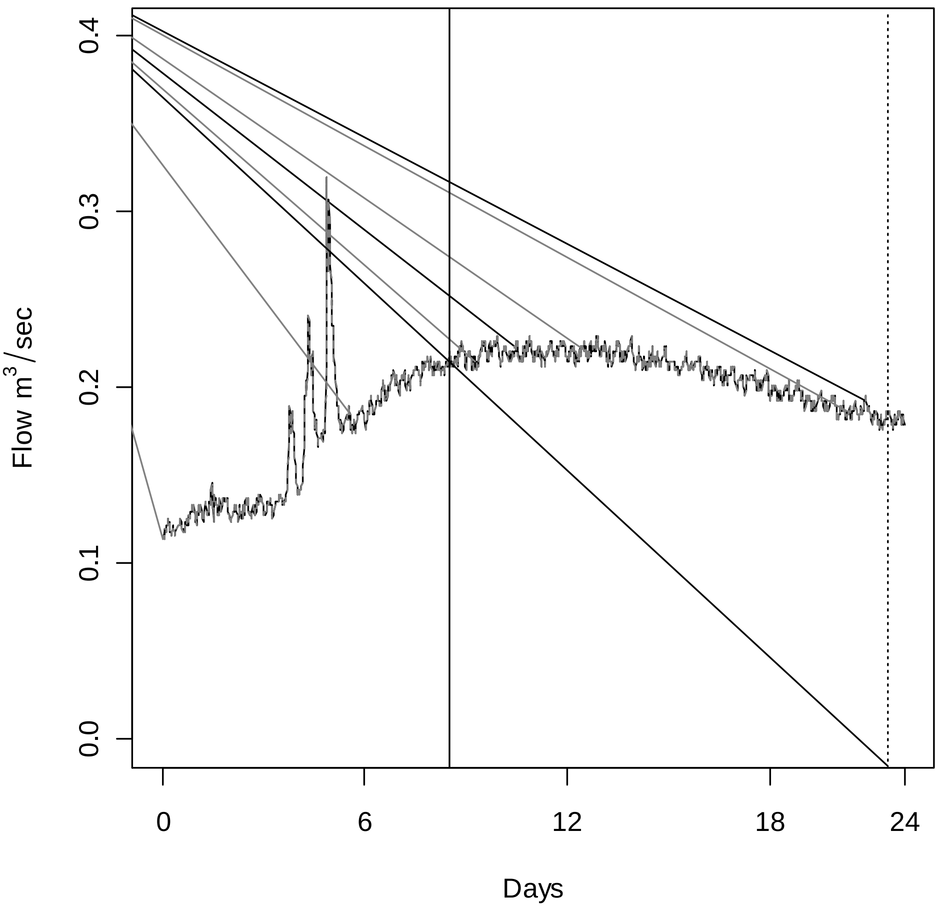


Figure 10.2.Flow data collected from South House (grey line) (NGR SY7074399087) and Little Puddle (black line) (NGR SY7183496467). Flow rate is measured every 15 minutes; first data point (zero) was taken on the 13/01/2011 at 00:00 hours. Vertical black line indicates first data collection and vertical dotted line indicates second data collection.

10.2 Hydrological influence on faunal distribution

Species richness

A total of 41 species and higher taxa were collected during the study on the two sample dates (Table 10.3). Total abundance was dominated by Chironomidae, Oligochaeta and Nematoda making up 25.3%, 28.3% and 15.7% of the total abundance respectively. Stygobite species were collected predominantly from gaining sections of the River Piddle accounting for 1.6% of the total abundance. Stygobite fauna were dominated by *Niphargus aquilex*, collected from both losing and gaining sections, with abundance highest in the gaining section. Other stygobitic species, including *Niphargus fontanus, Antrobathynella stammeri* and *Proasellus cavaticus* were only found in the gaining section. The abundance of *Antrobathynella stammeri* and *Proasellus cavaticus* was low with only two and one specimens found respectively.

Table 10.2 Total abundance of fauna collected from the River Piddle hyporheic study. Numbers given are a total abundance collected from each study reach (5 Bou-Rouch samples) Site codes (A, B, C, D, E, F) are given in Table 10.1.

		Visit 1 (20/01/11)						Visit 2 (3/02/11)					
Taxa		Sample sites											
	Code	**A**	**B**	**C**	**D**	**E**	**F**	**A**	**B**	**C**	**D**	**E**	**F**
Nematoda	Nem	10	22	16	12	62	54	15	34	20	18	133	60
Pisidium sp.	Pis	0	0	0	0	0	0	4	0	2	1	4	14
Hirudinea	Hir	0	0	0	0	0	0	0	2	0	0	4	0
Oligochaeta	Ol	22	8	132	40	130	68	60	30	87	62	133	47
Hydrachnaellae	Hyd	0	0	0	0	0	0	8	0	4	0	0	0
Acari	Ac	0	2	0	0	0	0	2	0	0	2	0	0
Limnocythere sp.	Lino	0	0	0	0	0	0	6	0	0	0	0	0
Ostracoda	Ost	0	0	0	0	0	0	0	0	0	0	0	6
Eucyclops serrulatus	Eucy	0	0	0	0	0	0	0	0	0	0	0	26
Diacyclops languidoides	Dia	0	0	0	0	0	0	0	0	4	0	0	0
Diacyclops sp.	Dia	0	2	0	2	8	4	0	0	0	0	0	0
Atheyella sp.	Ath	8	6	0	2	4	6	0	40	0	0	2	0
Bryocamptus minutus	Br	0	2	2	4	0	0	0	0	0	0	0	0
Bryocamptus sp.	Br	0	2	0	0	0	0	16	0	0	10	13	10
Paracyclops fimbriatus	Pacy	0	0	0	0	0	0	0	6	0	10	18	0
Paracyclops sp.	Pacy	0	4	0	0	0	0	0	0	0	0	0	0
Antrobathynella stammeri	As	0	0	0	2	0	0	0	0	0	0	0	0
Proasellus cavaticus	Pca	0	0	0	0	0	0	0	0	0	0	1	0
Gammarus pulex	Gp	0	6	4	2	0	2	8	2	2	1	0	12
Niphargus aquilex	Niph	0	0	0	2	24	0	2	0	0	0	0	2
Niphargus fontanus	Niph	0	0	0	0	8	0	0	0	0	0	0	0
Isotoma viridis	Iv	0	0	4	0	0	0	0	0	0	0	0	0

Taxa	Code	A	B	C	D	E	F	A	B	C	D	E	F
Baetis Group	Bae	0	6	0	0	2	0	0	0	0	2	0	0
Paraleptophlebia submarginata	Ps	2	0	0	0	0	0	0	0	0	0	0	0
Ephemera danica	Eph	0	2	0	0	0	2	0	0	0	0	0	0
Ephemera sp.	Eph	0	0	2	0	0	0	0	0	0	0	0	0
Caenis sp.	Cae	6	16	0	0	0	0	6	6	0	0	1	1
Leuctra sp.	Leu	0	4	0	0	0	0	0	0	0	0	0	0
Elmis aenea	Eae	0	2	4	0	0	0	0	4	0	0	0	0
Limnius volkmari	Lvo	4	8	0	6	2	0	6	4	14	14	0	0
Oulimnius sp.	Oul	0	8	2	0	0	0	0	2	0	0	0	0
Agapetus sp.	Ag	0	6	2	2	10	0	0	2	4	22	4	1
Tinodes sp	Tin	0	2	0	0	0	0	0	0	0	0	0	18
Hydropsyche sp.	Hyd	0	0	0	0	0	0	0	0	2	0	0	0
Brachycentrus subnubilus	Bs	0	2	0	0	0	0	8	0	3	0	0	0
Limnephilus sp.	Lim	0	0	2	2	2	0	0	0	0	0	0	0
Silo nigricornis	Sni	0	0	0	0	0	0	0	2	0	0	0	0
Tipulidae		0	6	0	0	2	0	2	8	7	0	6	0
Psychodidae	Psy	2	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	Cer	0	10	28	0	4	0	2	4	20	10	5	1
Simuliidae	Sim	0	0	0	0	0	0	0	0	2	0	0	0
Chironomidae	Ch	96	108	32	28	10	24	150	112	42	79	35	13
Species richness	**Spri**	**9**	**23**	**12**	**12**	**13**	**7**	**15**	**15**	**14**	**12**	**13**	**14**

The most abundant microcrustaceans recorded belonged to the genera *Bryocamptus* and *Atheyella*,in the order Harpacticoida, collected from both losing and gaining sections of the River Piddle. The other microcrustacean groups recorded were cyclopoid copepods in the genera *Diacyclops* and *Paracyclops*, also collected from losing and gaining sections and a few Ostracoda recorded from site F. The benthic macroinvertebrate groups collected were dominated by the riffle beetle *Limnius volckmari*, caddis larvae of the genus *Agapetus*, the freshwater shrimp *Gammarus pulex* and *Caenis* mayfly nymphs, all of which were also recorded from both losing and gaining sections of the River Piddle.

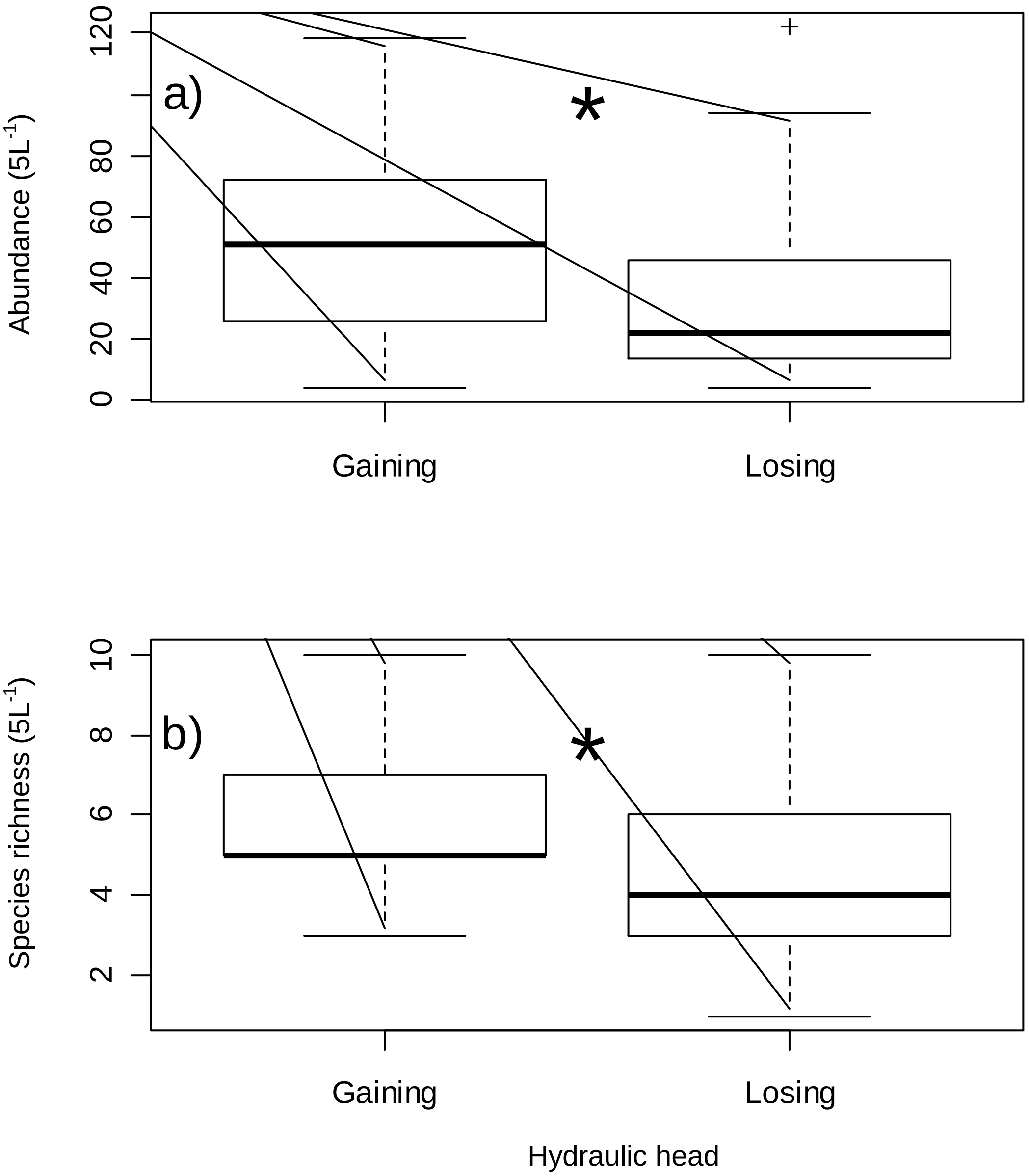


Figure 10.3 Mean total abundance (a) and species richness recorded from each Bou-Rouch sample from losing and gaining sections of the River Piddle. Significance results are derived from generalised linear model results.

Table 10.3 General Linear Model results of comparison between sample dates and losing - gaining sections of the River Piddle. Intercept results for date are for the second sampling visit relative to the first

		df	± intercept	Standard Error	t-value	p-value
Total abundance	Date	1,57	12.57	7.61	-1.65	0.10
	Lose - gain	1,57	-17.65	7.58	-2.32	0.02*
Species richness	Date	1,57	0.81	0.70	1.15	0.25
	Lose - gain	1,57	-1.64	0.65	-2.34	0.02*
Copepoda	Date	1,57	1.00	0.47	2.13	0.04*
	Lose - gain	1,57	-0.44	0.43	-1.02	0.31
Macroinvertebrate species	Date	1,57	0.63	0.63	0.35	1.78
	Lose - gain	1,57	-0.52	0.35	-1.49	0.14
Stygobites	Date	1,57	-2.73	1.04	-2.62	0.009**
	Lose - gain	1,57	-1.26	1.00	-1.27	0.20
Nematoda	Date	1,56	0.57	0.35	1.61	0.11
	Lose - gain	1,57	0.06	0.34	0.17	0.87
Oligochaetae	Date	1,57	0.01	0.33	0.04	0.97
	Lose - gain	1,57	-0.26	0.33	-0.78	0.44
Attheyella crassa	Date	1,57	0.51	0.91	0.56	0.58
	Lose - gain	1,57	-1.33	1.07	-1.24	0.22
Bryocamptus sp.	Date	1,57	1.88	0.74	2.53	0.01*
	Lose - gain	1,57	0.12	0.51	0.25	0.81
Gammerus pulex	Date	1,57	0.85	0.57	1.50	0.14
	Lose - gain	1,57	-1.19	0.61	-1.95	0.06
Caenis sp.	Date	1,57	-0.71	0.67	-1.07	0.29
	Lose - gain	1,57	-1.55	0.82	-1.89	0.06.
L. volckmari (larvae)	Date	1,57	0.57	0.63	0.89	0.38

	Lose - gain	1,57	-0.38	0.62	-0.62	0.54
Cerapotogonidae	Date	1,57	0.15	0.55	0.27	0.79
	Lose - gain	1,57	0.27	0.55	0.49	0.63
Chironomidae	Date	1,57	0.27	0.27	1.01	0.32
	Lose - gain	1,57	-0.96	0.30	-3.26	0.00***

Table 10.4 Detrended Correspondance Analysis axes scores for first four axes of species site associations for the River Piddle data.

	DCA1	DCA2	DCA3	DCA4
Visit 1				
Eigenvalues	0.283	0.221	0.176	0.148
Axis lengths	2.091	2.064	1.700	1.966
Visit 2				
Eigenvalues	0.221	0.183	0.138	0.118
Axis lengths	2.193	1.522	1.634	1.599

Total abundance and species richness significantly increased in the gaining section of the River Piddle (Figure 10.4; Table 10.4). The increase in total abundance was driven by the significantly higher abundance of Chironomidae in the gaining section (Table 10.4). The total abundance of the dominant groups (macroinvertebrates[9] and Copepoda) was higher in the gaining section of the Piddle, though the result was not significant (Table 10.4). The only taxa where abundance was higher in the losing section were Nematoda, Cerapotogonidae and *Bryocamptus* species, though the result was also not significant (Table 10.4). The influence of the flood pulse had a variable effect on the fauna collected, with only stygobite species (with the exception of *Proasellus*) recorded in significantly reduced numbers in the samples collected on the second sampling visit (Table 10.4). Copepoda, in particular *Bryocamptus* species had increased significantly in the second batch of samples (Table 10.4). All other faunal groups were also present in increased abundance in the February samples, though the results were not significant (Table 10.4).

[9] (i.e. benthic macroinvertebrates including stygobites)

Species distribution

The DCA plots showed no clear patterns in species distribution though a weak pattern was evident along the first axis with gaining sites associated with the positive end of the first axis and the losing sites with the negative end of the first axis (Figure 5 & 6). This pattern was replicated across both sampling occasions. The pattern is more evident during the second sampling visit where species were also more strongly associated with the positive gradient of the first axis. The Axis length of the first DCA axis is greater than two indicating a unimodal response curve. The axis length in DCA is also a measure of beta diversity with axis lengths of four SD units or greater indicating that few species are found at both ends of the gradient. The results in this study in visit 1 (axis length = 2.091) and visit 2 (axis length = 2.193) indicate that the majority of species are found all along the first axis with no species turnover apparent.

11.0 Discussion – Survey (C) – River Piddle, Dorset

11.1 Hydrological influence on the hyporheos

The amount of LOM was significantly higher in the gaining section, possibly reflecting a more steady supply of nutrients. Studies have found algal biomass to be greater in the upwelling zones of riffles (Pepin *et al*., 2002) and nitrate and dissolved reactive phosphorus significantly greater in the gaining sections of alluvial rivers (Datry *et al*., 2008; Dent *et al*., 2001). The use of fertilizers within catchments has degraded the water quality of groundwaters in agricultural regions, which also impacts receiving waters such as streams and rivers (Lewandowski *et al*., 2009). The Piddle has seen a year on year increase in nitrate concentrations from 1965-2007 (Howden *et al*., 2009), with nitrate concentrations in the aquifer contributing greatly to the hyporheic and surface water concentrations in the river (Howden *et al*., 2010). This steady flow of nitrates from the aquifer will be relatively stable in the gaining sections of the Piddle, which may also contribute to the increase in LOM in these sections. In freshwaters the mass of dissolved organic matter (DOM) is often greater than that of living organisms (Thomas, 1997). The rate of production facilitating DOM will also increase as base cation and nutrient concentrations increase (Thomas, 1997), with chalk streams in particular characterised by abundant macrophyte growth in the base rich conditions (Holmes *et al*., 1998). While LOM is a coarse measure of organic matter, it will also be higher where production is greater, suggesting that production maybe higher in the gaining section in this study.

Concentrations of pH were consistently lower in the gaining section of the Piddle in comparison with the losing section. Groundwaters typically have lower pH than surface waters due to the production of CO_2 in the soil zone during the breakdown of organic matter (Hiscock, 2007). CO_2 will dissolve in infiltrating groundwater, resulting in the formation of carbonic acid and decreasing the pH in the aquifer relative to the infiltrating surface water. The differences in pH are a strong indication of the presence of upwelling groundwater in the Piddle. Dissolved oxygen while lower in some gaining sections, did not reduce to levels that could be considered deleterious for epigean and groundwater fauna (< $3mg/l^{-1}$) (Malard *et al*., 1999) with conditions oxic in all sample locations.

In this study, patterns in faunal distribution were found between losing and gaining sections of the River Piddle. Total abundance and species richness were significantly higher in the gaining section in comparison to the losing section. This increase in abundance and species richness in the gaining section was consistent for many epigean and hypogean taxa with mean abundances for all main groups, except Nematoda, Cerapotogonidae and *Bryocamptus* species, higher in the gaining section. Gaining sections of streams and rivers are often associated with greater assemblage stability, with inputs of nutrients consistent where groundwater upwells at the surface (Boulton *et al*., 1998; Hayashi *et al*., 2002). Similarly species richness is often greater in the hyporheic zone in gaining sections of river reaches (Datry *et al*., 2007; Malard *et al*., 2003a; Malard *et al*., 2003b). This is primarily due to the increased abundance of groundwater fauna and permanent members of the hyporheos (Malard *et al*., 2003a), which increases the available colonisation pool in the hyporheic zone. In this study while the results at species level were not significant, stygobite species (*Niphargus aquliex*, *Niphargus. fontanus*, *Antrobathynella. stammeri* and *Proasellus cavaticus*) were consistently found in higher abundance in the gaining section of the Piddle. Similarly benthic macroinvertebrate species commonly found in the hyporheic (*Caenis* sp., *Elmis aena*, *Limnius volckmari* and *Oulimnius* sp.) were all found in higher abundances in the gaining section of the River Piddle. Upwelling groundwater will provide a stable environment throughout the year in the hyporheic zone, in comparison to losing sections of streams. Upwelling groundwater will reduce temperature fluctuations, and provide a steady flow of nutrients and DOC, all contributing to the stimulation of hyporheic microbial production (Boulton *et al*., 2006). Flow permanence in the hyporheic zone arising from upwelling groundwater has also been shown to increase taxon richness (Boulton *et al*., 2006; Datry *et al*., 2007; Wood *et al*., 2005).

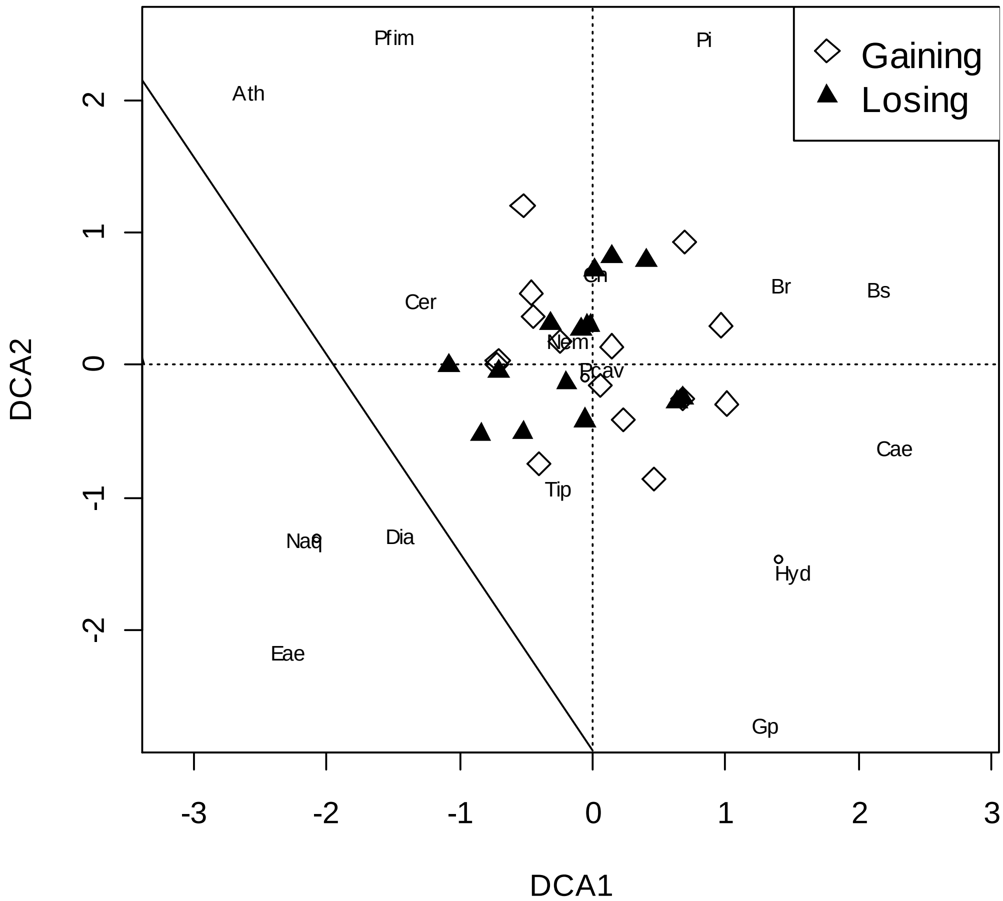


Figure 11.1 Detrended Correspondence Analysis (DCA) ordination of samples derived from losing and gaining sections of the Piddle. Data is presence-absence of species at each sample location. Samples were collected from the River Piddle in Dorset on the 21/01/2011. Species codes are listed in Table 10.3.

The increase in species richness and total abundance in the gaining section of the Piddle in this study supports findings in other studies in different geological settings. The results from this study show how upwelling groundwater can influence species richness and abundance in a system with a limited hyporheic zone. The hyporheic zone of chalk streams commonly exhibits poor connectivity, with surface water penetration found to occur at only shallow depths (10-20cm) in a study on the River Lambourn chalk system (Pretty *et al*., 2006). Groundwater flow accretion at the reach scale on the Lambourn exhibited considerable spatial variation between reaches with gaining and losing reaches evident along the catchment (Grapes *et al*., 2005) Groundwater upwelling dominates hydrological processes in river valleys, with the influence of groundwater upwelling of increasing importance in karstic aquifers where surface flow is reduced (Bonacci *et al*., 2009). The importance of upwelling zones will increase in the future if current climate model predictions for the UK are correct. The current consensus is for high rainfall events across a reduced time period during the winter months and an increase in dry periods during the summer months resulting in a potential decrease in groundwater levels (Jackson *et al*., 2010). High species richness and abundance in gaining sections provides a colonisation source and stable environment for many epigean and hypogean fauna. Quantification of the input of gaining sections in groundwater dominated streams on river biodiversity would provide insights for management and conservation bodies in the preservation of stream biodiversity from future climate impacts.

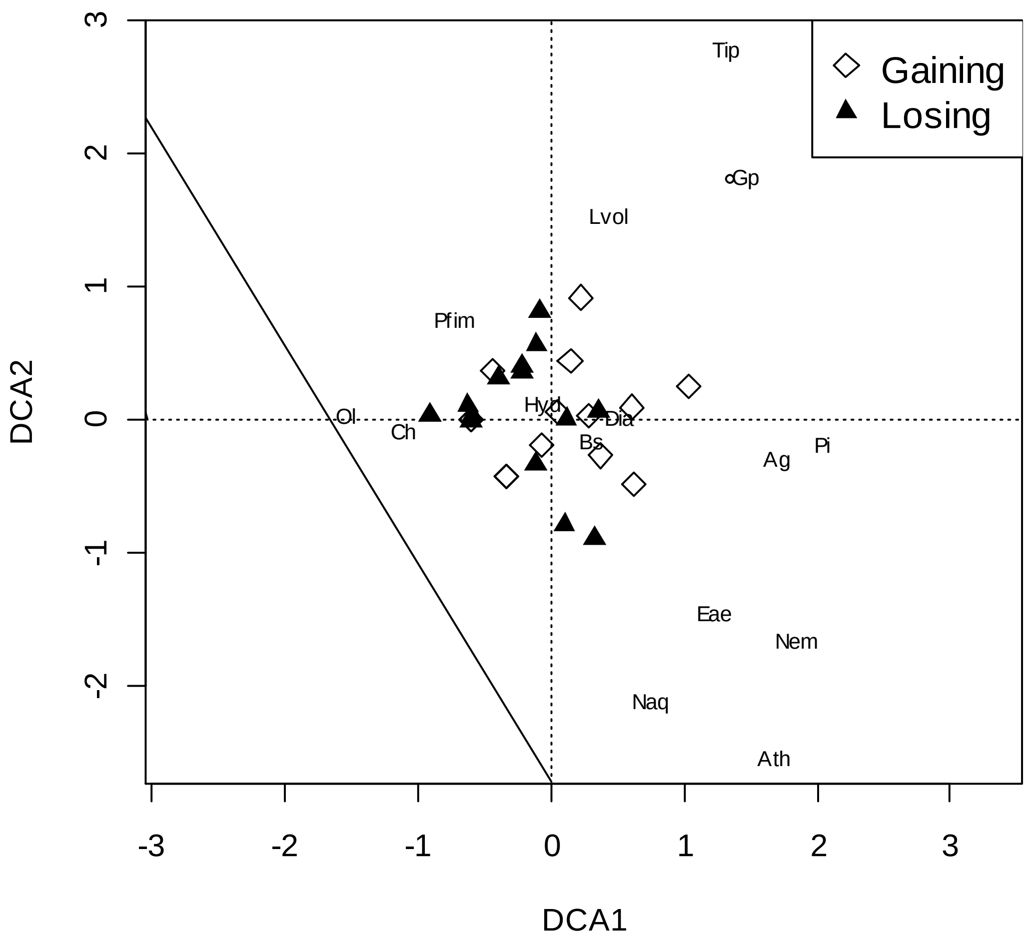


Figure 11.2 Detrended Correspondence Analysis (DCA) ordination of samples derived from losing and gaining sections of the Piddle. Data is presence-absence of species at each sample location. Samples were collected from the River Piddle in Dorset on the 03/02/2011. Species codes are listed in Table 10.3.

The influence of the flood pulse did not affect the majority of species in the hyporheic, with no significant differences between dates for the majority of epigean species. Only Copepods were significantly affected by the flood event and this effect was driven by the genus *Bryocamptus*, with *Atheyella* unaffected. The effect of flood events on meiofaunal sized copepods and chironomids is an increase in drift as flow increases (Palmer *et al*., 1992), with copepods also slow to recover to pre flood abundance levels in the hyporheic zone after high flows (Olsen *et al*., 2005). The surprise was the affect of the flood on stygobite species, with abundance significantly lower two weeks after the flood than directly after it. Studies have shown that stygobite species cope poorly with flood events, with Hancock (2006) finding that stygobite abundance continued to decline after a flood event until they were completely absent 61 days after. Similar studies have shown that both epigean and hypogean fauna are displaced by flood events at depths of up to one metre, with recovery of hypogean fauna within the substrate faster than for epigean fauna (Dole-olivier *et al*., 1992). Studies have shown that physically unstable sites will harbour a community dominated by epigean fauna, with stygobite fauna found away from the disturbance effects of the river in the deeper sediment layers (Marmonier *et al*., 1993). In comparison, the slow recovery of stygobites in this study suggests that flood events in chalk catchments have a deleterious affect on stygobite fauna. The chalk substrate is composed of predominately fine sediments in the benthic and hyporheic sediment layers and is prone to removal during high flow events. The Piddle catchment in particular is characterised by a high proportion of fine grained bed sediment storage in the benthic sediments (Collins *et al*., 2006; Collins *et al*., 2007).

11.2 Conclusions

The influence of small scale hyporheic flow patterns are less evident in chalk streams than are described in hyporheic studies undertaken on coarse highly porous geologies. While small scale flow patterns and associated hyporheic assemblages are not evident in chalk sediments, large scale flow patterns described in the literature are evident in the chalk geology. Implications for increases in species richness and abundance in gaining sections of chalk streams are important for both conservation and management of chalk streams. Recent concerns regarding sedimentation have been highlighted; in particular the impact of sediment on the reproductive cycle of salmonids (Ferreira *et al*., 2009; Malcolm *et al*., 2003). Restoration has been shown to improve sedimentation problems in chalk streams though the fix is only temporary (Pulg *et al*., 2011). The data from this study could inform management of chalk streams, with the dominance of upwelling groundwater possibly contributing to the higher species richness and total abundance in the gaining sections. Virtually all taxa were also recorded at higher abundances in the gaining section, though the result was not significant. Restoration and management of chalk streams could be improved if targeted at gaining sections, which may provide hotspots of biodiversity. This could eventually lead to a more sustainable form of river management if the gaining sections of streams can self regulate, with upwelling water flushing out accumulated sediments.

12.0 Summary

The three surveys conducted as part of this study have expanded our current understanding of the distribution of the groundwater fauna within the study regions and helped fill some of the knowledge gaps relating to the habitat controls influencing their presence.

Eight species of groundwater Crustacea (stygobites) known from England were found in the Dorset/Devon survey. The distribution of these was significantly related to the different geological units investigated. The geologies with a high degree of fracturing appeared to provide the most suitable habitat opportunities (e.g. Chalk) although not all fractured aquifers shared the same species richness or abundance of that associated with the Chalk. Furthermore, there was a distinct division between the species found in Devon and those found in Dorset, despite some geologies offering similar habitat conditions. In our survey only *Niphargus aquilex* and *Microniphargus leruthi* were recorded from both counties, suggesting the other species are restricted in their dispersal. Whilst the reasons for the observed distribution may be multifaceted one possible explanation is that the two species found in both counties are able to utilise superficial riverine deposits for dispersal. (This is probably also true for *Antrobathynella stammeri* which was found in Devon and is known from elsewhere in Britain). The other species recorded are perhaps unable to utilise this pathway effectively and may be prevented from dispersal at depth by the presence of a band of low permeability mudstone which forms a border between Devon and its boundary with Dorset and Somerset. This theory requires further examination for which additional survey work, along the county boundary described, is recommended.

The survey of northern England was limited in terms of the number of sites visited but confirmed the low species richness known from this part of the country. The presence of *Niphargus aquilex* and *Antrobathynella stammeri* in the Yorkshire Chalk demonstrate the ability of these species to occupy phreatic habitats as well as superficial deposits. Such versatility may help explain their wider known distribution within Britain, compared to the other known species. However, in our survey, neither species were recorded from boreholes north of the Devensian glacial limit (although records do exist elsewhere for *Antrobathynella stammeri*). This agrees with previous work which suggests glaciations have caused local extinctions and that subsequent recolonisation of such areas has been extremely slow.

The significant increase in species richness and total abundance of macroinvertebrates from the gaining sections of the river Piddle survey supports findings in other studies in different geological settings. The results from our study show how upwelling groundwater can influence species richness and abundance in a system with a shallow hyporheic zone. We also showed that stygobites (*Niphargus aquilex*, *Niphargus fontanus*, *Antrobathynella stammeri* and *Proasellus cavaticus*) were consistently higher in abundance in the gaining section of the river. These findings have important implications for the conservation and management of chalk streams. Restoration works, for example, could focus on zones of upwelling groundwater which should be self sustaining in flushing out of sediments and acting as biodiversity hotspots.

Groundwater fauna are a unique and often rare group of species which make an important contribution to our national biodiversity. We hope that the findings of our project will help inform and encourage environmental managers, such as the Environment Agency and Natural England, to consider groundwater fauna and subterranean ecosystems, as part of their water management planning and conservation activities.

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Appendices.

1. The project partners and supporting organisations
2. Photographs of the stygobitic Crustacea species found in England
3. Field record sheet template
4. Stygobite distribution of species from Survey (A)

Note: A full data set from the project survey work will be available through the project website within two years of publication of this report. http://new.freshwaterlife.org/web/gwa-uk

Appendix 1. - The project partners

The Centre for Research in Ecology (CRE) at Roehampton University, of which Anne Robertson (AR) is a member, undertakes work to extend our understanding of key environmental systems: soils, fresh waters and canopies and currently comprises 13 members. Anne Robertson has a wide experience of hyporheic and groundwater research. She has recently authored a report ‘A review of the subterranean aquatic ecology of England & Wales’ published by the Environment Agency and has conducted a number of research projects on hyporheic ecology in the UK and abroad, often in conjunction with PhD students.. She has published many research papers on interstitial invertebrates. She is a member of the NERC Hyporheic Zone Network (hydroecology and biogeochemistry core working group) whose mission is to engage with scientists and science end-users working on groundwater - surface water interactions and hyporheic zone processes, and help transfer knowledge from the research to science end-user communities.

Paul Wood (PW) has 16 years of experience undertaking research in the field of groundwater ecology. He has worked extensively within chalk and limestone (Karst) landscapes and in particular on the environmental controls on the distribution of aquatic invertebrate communities. His research has examined the long-term variability of chalk stream macroinvertebrate communities associated with drought and flow permanence in limestone systems. His research has also examined the conservation value and biodiversity of limestone springs and caves. This research has culminated in over 25 peer-reviewed publications to date. He is a core group member of the NERC funded Hyporheic Network (Ecohydrology and Biogeochemistry) group. He is also a co-investigator on a project (lead by Dr J. Arnscheidt at the University of Ulster) examining groundwater biodiversity in Ireland and has recently been awarded funds by the Royal Irish Academy to undertake research on 2 caves in Ireland.

Simon Rundle (SR) is a freshwater ecologist with expertise in sampling and identifying both macro and meiofaunal invertebrates from a range of freshwater habitats, including groundwater systems. His research interests in freshwater ecology centre on understanding which environmental parameters influence the biology and ecology of aquatic species and assemblages and includes experimental laboratory studies and field surveys. The ultimate aim of much of this work is to provide information that can help inform policy makers and conservation bodies. He is currently supervising four PhD students, one of whom is carrying out a survey of sub-surface invertebrates in Scottish rivers (co-supervisors Drs David Gilvear and Nigel Wilby, Stirling University). He is also the co-ordinator of an Esmée Fairbairn funded project evaluating the biodiversity of saline transition zones in lower river reaches.

Lee Knight (LK) is an expert on the collection and identification of groundwater Crustacea in the British Isles and Ireland and is the Biological Recorder for Subterranean Crustacea. Lee has 18 years experience as a freshwater ecologist and has produced, in collaboration with the FBA and with support from the Esmée Fairbairn Trust, a website on hypogean Crustacea (http://www.freshwaterlife.org/hcrs). He has also published a number of papers in this area including on the discovery of *Niphargus glenniei* in West Cornwall. He was responsible for the inclusion of *Niphargus glenniei* in the new UK BAP and has recently undertaken surveys on the groundwater fauna of the Isles of Scilly and of caves, springs and interstitial sites in Ireland (funded by the Irish Heritage Council), complementing the work of Dr J. Arnscheidt at the University of Ulster.

The Freshwater Biological Association (FBA) is a registered charity (no. 214440) founded in 1929, whose aim is to promote the study and application of freshwater biology. It meets this aim through membership, provision of research facilities and information dissemination. The FBA supports professional scientists, managers and students, and includes informed amateurs within its remit, encompassing biological recorders, anglers and those with a general interest in natural history. The FBA is currently running the Recorders and Schemes Project, funded by the Esmée Fairbairn Foundation, which aims to provide support and training for biological recorders in order to enhance our understanding of lesser known freshwater organisms. The FBA is an established publisher, in traditional and electronic formats, and has produced definitive identification keys since the 1940s. Historically known for its identification training courses, it is currently reviving these with considerable success. It has considerable expertise in web-based information dissemination, including design and hosting of websites such as the one detailed in this proposal. The FBA representative for this project is Michael Dobson, its Director.

The British Geological Survey (BGS) provides expert services, consultancy and advice in all areas of geoscience to both the public and private sectors, in the UK and internationally. Its activities include a publicly-funded core programme of long-term strategic geoscientific surveying, monitoring and databasing; a partnership programme of applied geoscience, cofounded by the private and public sectors; and a responsive programme undertaken for clients. Groundwater is a major activity within the British Geological Survey. Our core role in the National Groundwater Survey and as the guardians of the National Groundwater Archive enables us to apply our strategic vision to current groundwater-related issues. BGS' groundwater scientists have extensive expertise in groundwater data and information management and work on a wide variety of groundwater problems including those associated with groundwater resources and environmental management, water quality issues, and groundwater process understanding.

Other organisations engaged with the project & supports

The Westcountry Rivers Trust,
The Devon Invertebrate Forum
The Pengelly Trust.

Environment Agency,
Natural England,
Countryside Council for Wales
Devon Biodiversity Partnership.

Appendix 2. - Stygobitic Crustacea species found in England

Niphargus aquilex

Niphargus glenniei

Niphargus fontanus

Niphargus kochianus kochianus

Proasellus cavaticus

Crangonyx subterraneus

Microniphargus leruthi

Antrobathynella stammeri

Appendix 3 – Field data record sheet

Data sheet for sampling invertebrates from boreholes, wells or springs

Location

Site Name

BH		BWS No.	
Well		NGR	
Spring		Date:	
		Time:	

Argricultural	Woodland	Sub-urban	Urban	Other

Site issues/comments

Physical data

Aquifer code | Strata sampled

Well/BH depth to base		m
Groundwater level		m bgl
Well/BH diameter		m
Approx. distance to river		km

Well/BH/Spring comments

In-situ measurements

Conductivity		us/cm/s
DO		%
DO		mg/l
Temp		oC
pH		
NH4		mg/l

Device used:	submersible	waterra	bailer	BouRouch
Flow through cell used?		Yes/No	Vol. pumped	m3
		Duration well or BH pumped		mins
No. of samples taken				
Sample No. (incl BWS ref)				

Comments on chemical sampling

Biological

			mesh size
Mechanism used	BH Net		63um
	Bailer & net		
	Sub. Pump & net		
	Waterra & net		
	Bou Rouch & net		
	In situ pump		
	Pipe net		

No. of samples taken

Sample No. (incl BWS ref.)

Comments on biological sampling

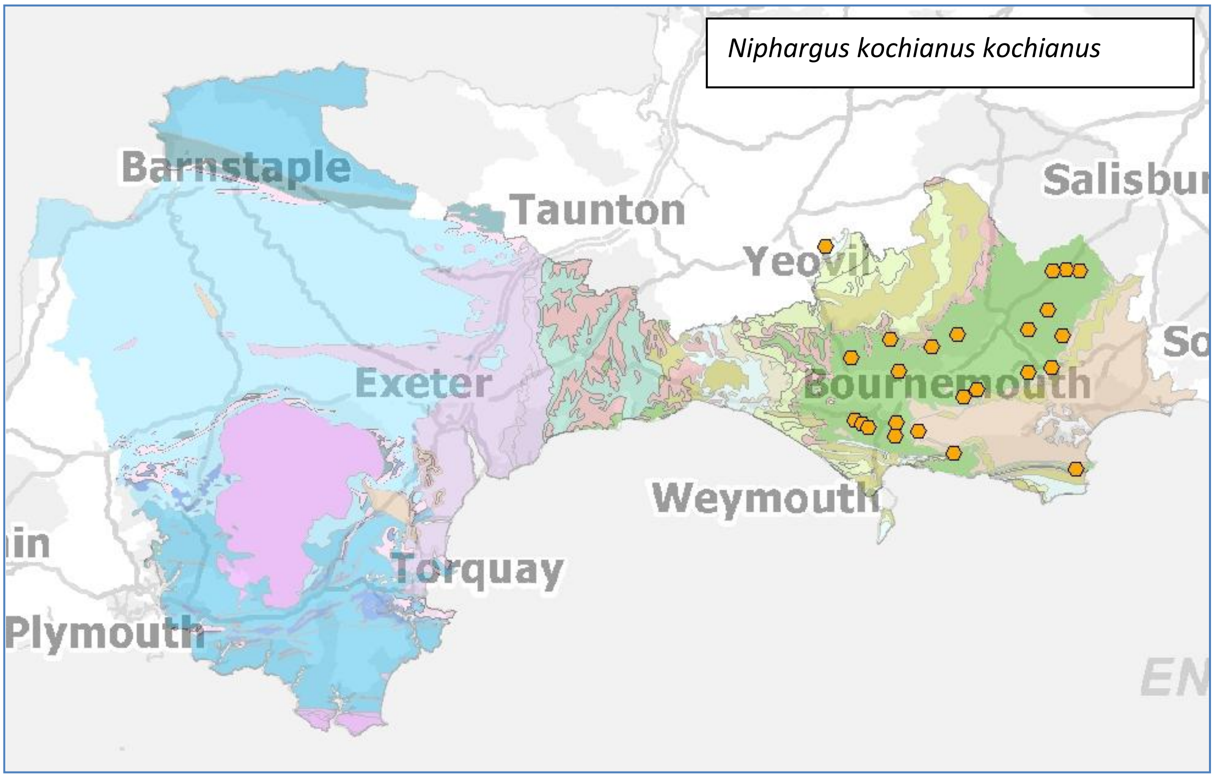
Specimens found

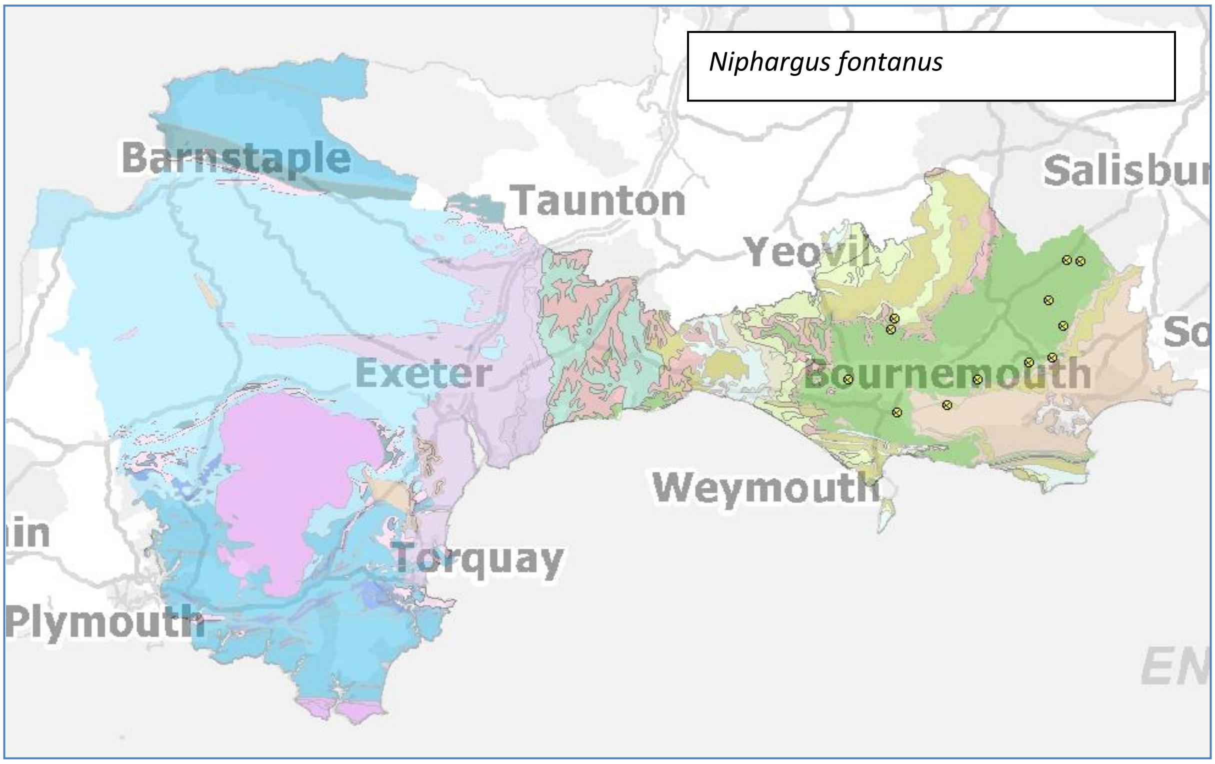
Personel

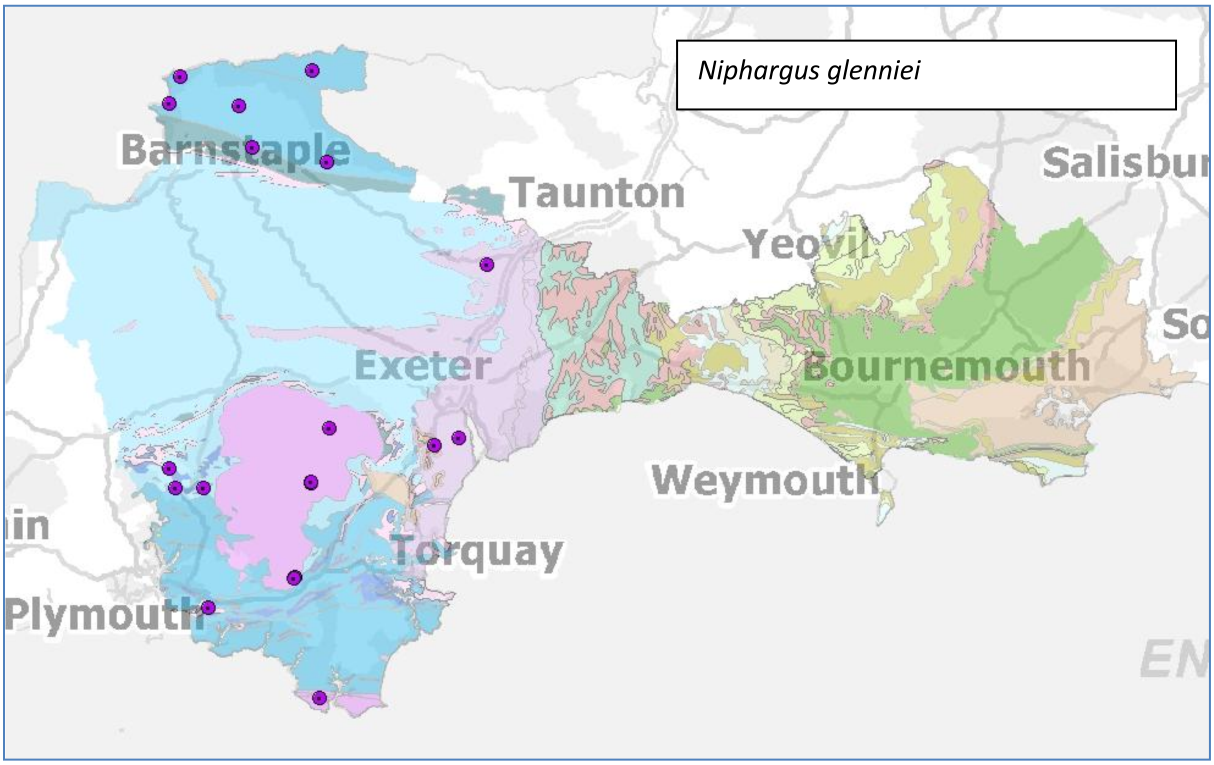
Roehampton University

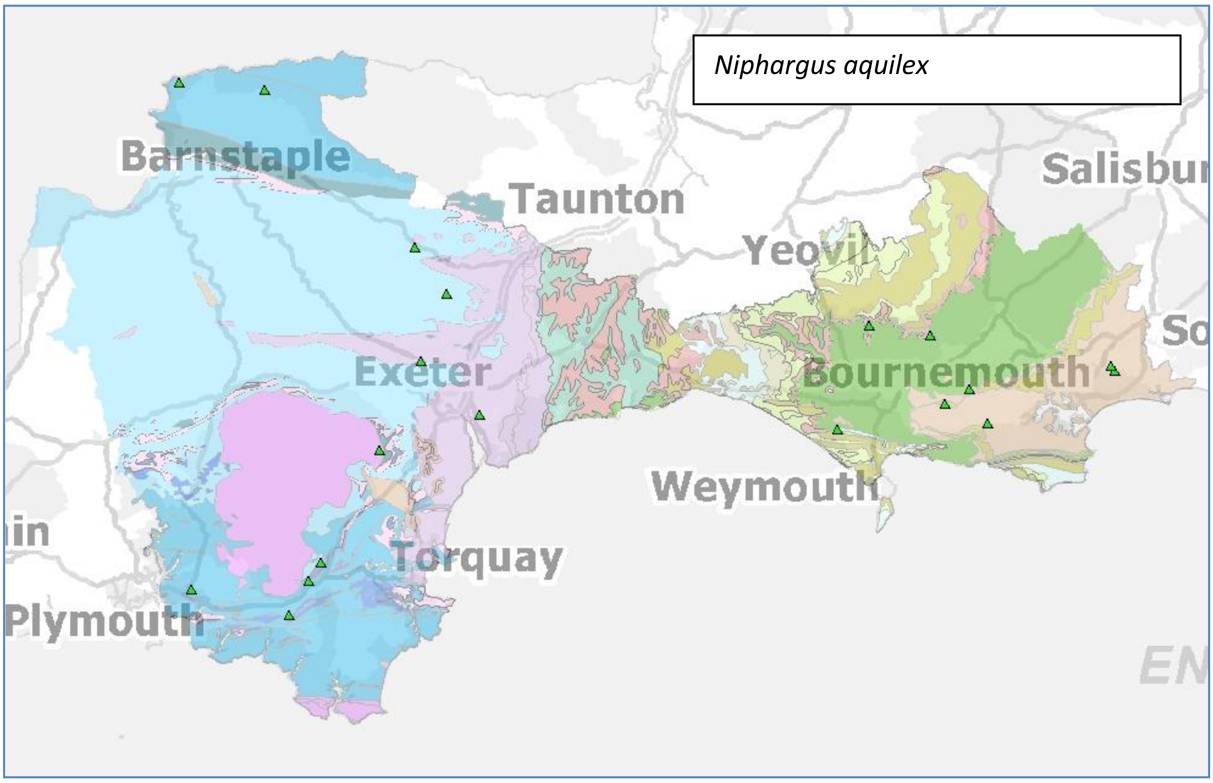
01/04/2010Datasheet

Appendix 4 – Stygobite species distribution maps






Niphargus glenniei
Barnstaple
Taunton
Salisbur
Yeovil
So
Exeter
Bournemouth
Weymouth
in
Torquay
Plymouth
EN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Taunton
Salisbur
Yeovil
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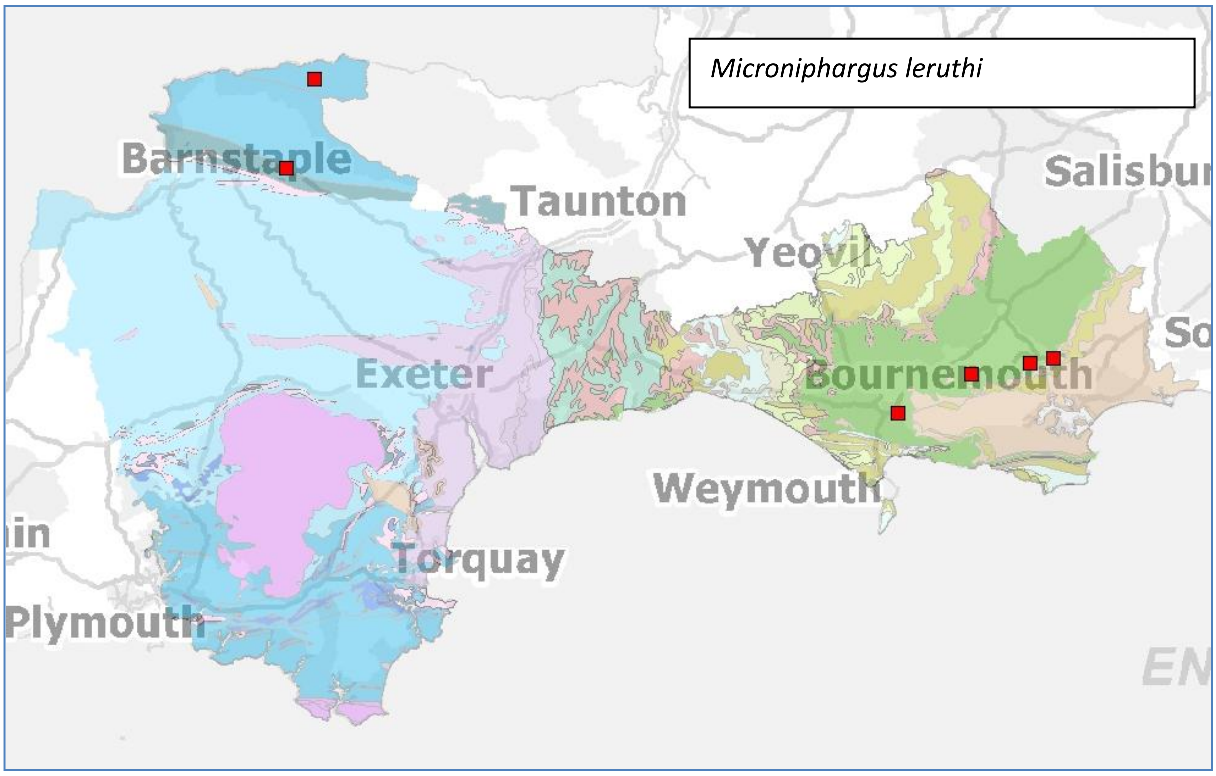
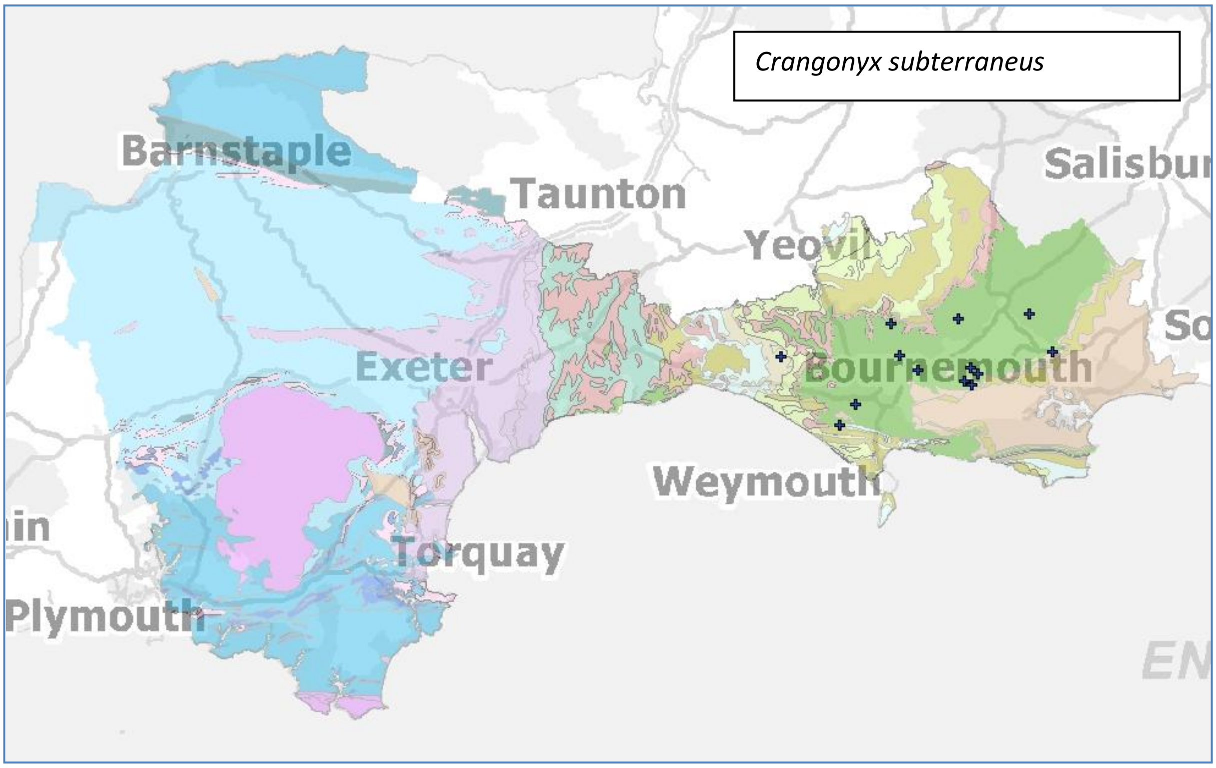
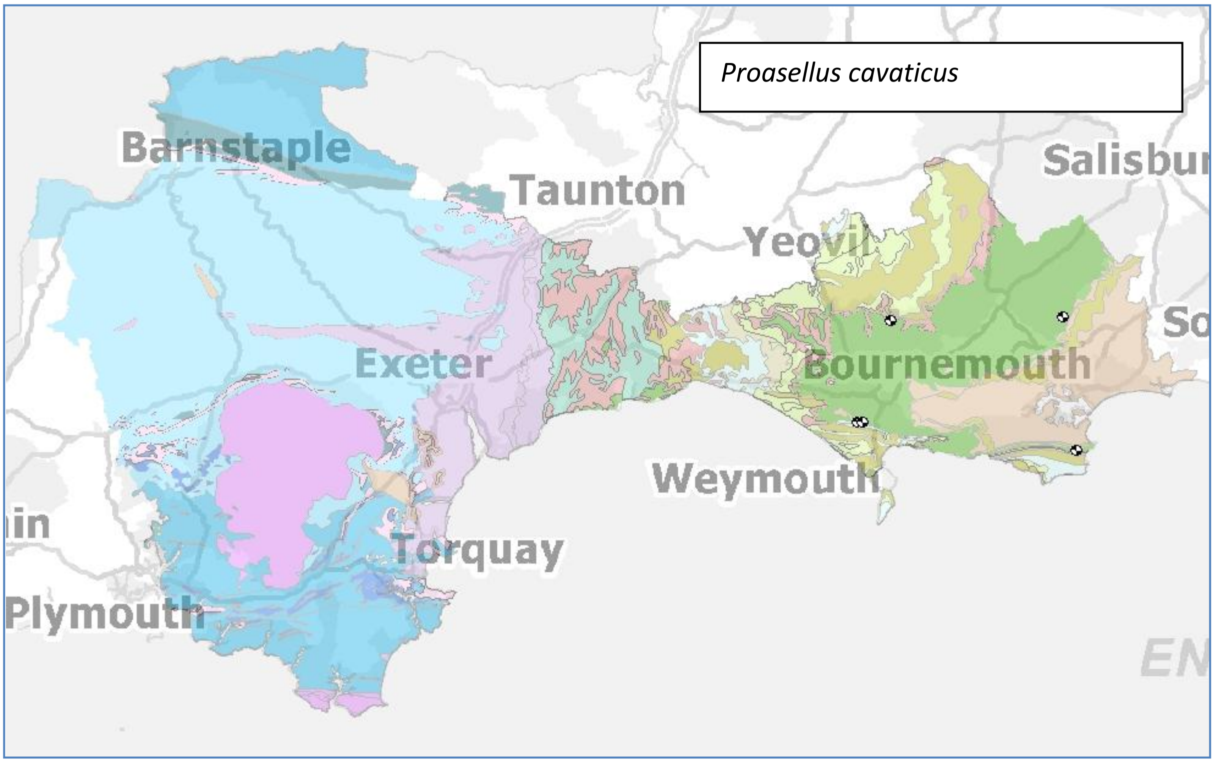
Exeter
Bournemouth
Weymouth
in
Torquay
Plymouth
EN


Microniphargus leruthi
Barnstaple
Taunton
Salisbur
Yeovil
Exeter
Bournemouth
Weymouth
Torquay
Plymouth


Crangonyx subterraneus
Barnstaple
Taunton
Salisbur
Yeovil
Exeter
Bournemouth
Weymouth
Torquay
Plymouth


Proasellus cavaticus
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Taunton
Salisbur
Yeovil
So
Exeter
Bournemouth
Weymouth
Torquay
Plymouth
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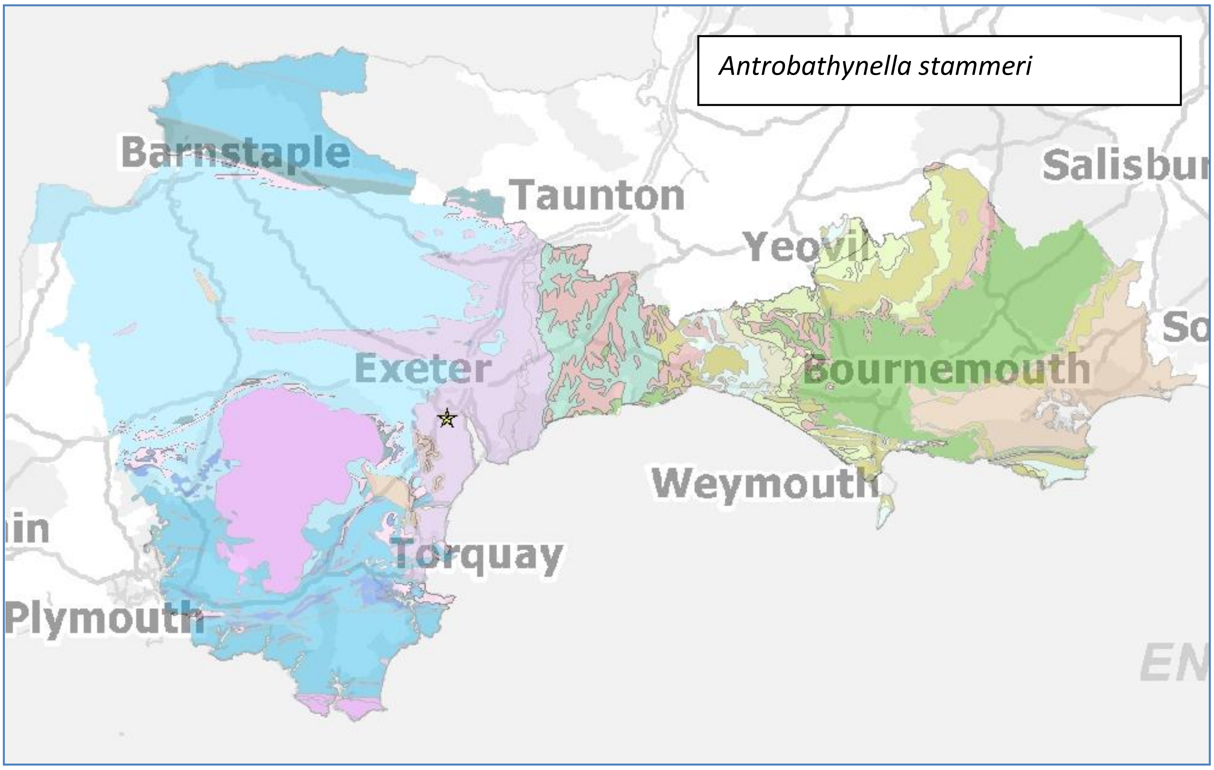
Antrobathynella stammeri
Barnstaple
Taunton
Salisbur
Yeovil
So
Exeter
Bournemouth
Weymouth
Torquay
Plymouth
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