Groundwater Ecology: Invertebrate Community Distribution across the Benthic, Hyporheic and Phreatic Habitats of a Chalk Aquifer in Southeast England

Jessica M. Durkota

Thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

University College London

Declaration of the Author

I, Jessica M. Durkota, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. This work was undertaken with the partial support of the Environment Agency. The views expressed in this publication are mine and mine alone and not necessarily those of the Environment Agency or University College London (UCL).

Abstract

Groundwater is an important resource for drinking water, agriculture, and industry, but it also plays an essential role in supporting the functioning of freshwater ecosystems and providing habitat for a number of rare species. However, despite its importance, groundwater ecology often receives little attention in environmental legislation or research. This study aims to improve our understanding of the organisms living in groundwater-dependent habitats and the influence of environmental conditions on their distribution. Invertebrate communities occurring in the benthic, hyporheic and phreatic habitats were surveyed at twelve sites over four years across the Stour Chalk Block, a lowland catchment in southern England. A diverse range of stygoxenes, stygophiles and stygobionts, including the first record of *Gammarus fossarum* in the British Isles, were identified using morphological and molecular techniques.

The results indicate that under normal conditions, each habitat provided differing environmental conditions which supported a distinctive invertebrate community. While the community recorded in the benthic habitat was characterised by a diverse assemblage of surface water species typical of Chalk streams, the phreatic community comprised a small number of exclusively crustacean stygofauna (such as *Niphargus kochianus* and *Crangonyx subterraneus*) and the hyporheic habitat supported a mixture of surface and groundwater species. Surprisingly, the results indicate that some species, such as *Agapetus fuscipes* (normally considered a surface water taxon), move into the hyporheic habitat in a predictable, seasonal pattern, potentially in response to grazing opportunities. However, the results collected during the high and low flow events which occurred during this study also show the widespread movement of multiple species (such as *Gammarus pulex* and *Niphargus fontanus*) between habitats in response to environmental disturbance. Collectively, these results reflect the movement of fauna longitudinally, laterally and vertically over time throughout the catchment, as though along a continuum rather than between three separate habitats. This suggests that our conceptualisation of lotic functioning should be expanded to better integrate the contribution from groundwater.

The approach taken by this study provides a greater understanding of the full diversity of aquatic invertebrates within this catchment and the way in which their distribution fluctuates across habitats. This study is one of the first to concurrently assess invertebrate distribution across the benthic, hyporheic and phreatic habitats; in addition, the relatively frequent and long-term sampling approach also facilitated a more detailed temporal assessment of these communities. A greater understanding of the distribution and requirements of the fauna inhabiting groundwater-dependent habitats, and their response to environmental change is essential for the conservation of these species and management of lotic ecosystems.

Acknowledgements

Over the course of my study I have received support and encouragement from a number of individuals, only some of whom it is possible to give particular mention to here:

Foremost I would like to thank the UCL community for their advice and support throughout this study: Julian Thompson and Roger Flower for supervising this work (and assistance with freeze coring); Jan Axmacher, Helen Bennion, Anson Mackay and Carl Sayer for their encouragement and advice; Julia Day for the use of her lab and her support in undertaking the molecular analyses; Janet Hope and Tula Maxted for their support in the geography lab and especially to Ian Patmore for his help in fabricating a Bou-Rouch pump from a poor sketch on the back of a napkin; Miles Irving in the Drawing Office for his unfailing assistance in producing diagrams and posters; and to my graduate cohort, James Cheshire, Miriam Fernandez, Tori Gay, William Gray, Caitlin Gutierrez, Charlotte Hall, Regan Koch, Dan Lewis, Luca Marazzi, Luke Mitchell, Matt Owen and Emma Wiik for their support, kindness and good humour throughout this process.

I would also like to thank the wider research community, specifically Anne Robertson, Rachel Stubbington, Tim Johns and Paul Wood for their advice (and enthusiasm) on sampling hyporheic environments; Michael Dobson, Terry Gledhill, Lee Knight, Peter Shaw and colleagues at the Environment Agency for their assistance in the verification of specimen; Jane Newcombe for her help in analysing the microbial samples; Bill Beaumont for his help in facilitating the freeze-core sampling; and Rosie Blackman for her collaboration in drafting the *Gammarus fossarum* manuscript.

I would further like to acknowledge my funders, the Environment Agency, UCL Department of Geography and UCL Graduate School for the opportunities they provided.

Finally, I would like to thank my friends and family for their support (and patience) over the past eight years.

Table of Contents

List of Figures

List of Tables

Chapter 1

A Review of Groundwater Ecology

1.1 Introduction to Groundwater Ecology

Groundwater is recognised as an internationally important resource for public water supply, agriculture and industry, but it also plays an essential role in the functioning of lotic ecosystems (Hancock, 2002; Vorosmarty et al., 2010). Environmental legislation aims to protect groundwater from degradation and over-exploitation; however, it often neglects to consider the ecological role of this resource (Korbel and Hose, 2011). Groundwater contribution to the lotic environment drives nutrient cycling and moderates river temperatures while providing essential habitat for organisms living within the hyporheic zone and the aquifer (Environment Agency, 2009). Despite its importance, groundwater ecology has received relatively little attention in environmental legislation or research, especially in comparison to the ecology of surface waters (Gilvear et al., 2006; Korbel and Hose, 2011). A better understanding of groundwater ecology is therefore essential for the successful management of lotic ecosystems.

This chapter provides a review of current research relating to groundwater ecology. It focuses on groundwater dependent habitats (Section 1.2) and the biological communities they support (Section 1.3), discussing the relative influences of environmental variables on their distribution (Section 1.4). The knowledge gaps identified by this review have been used to inform the aims of this study (Section 1.5), which are outlined in Section 1.6.

1.2 Groundwater Dependent Habitats

Groundwater directly supports a diverse range of ecological communities occupying benthic, hyporheic and phreatic habitats. While there is a wide literature concerning the nature of the benthic habitat, the hyporheic and phreatic habitats are less often considered (Moss, 1998).

1.2.1 Benthic and Hyporheic Habitats

While the benthic habitat encompasses the top layer of the substratum and is influenced by surface water processes, the hyporheic habitat includes the area of interaction between groundwater and surface water and is therefore influenced by both (*sensu* Gibert et al., 1994). The exchange flows that create the hyporheic habitat can occur over any permeable sediment where the pressure head of the surface channel is greater than that of the subsurface.

This change in pressure creates a negative hydraulic gradient which facilitates the downwelling of surface water and subsequent upwelling of groundwater. Hyporheic exchange flows are often catalysed by morphological features which facilitate this exchange such as riffle-pool sequences, meander bends or changes in geological strata. Exchange flows create a spatial and temporal mosaic of physical, chemical and biological gradients which extend vertically below the streambed and laterally into the floodplain (Gilvear et al., 2006). Downwelling surface water drives nutrient cycling and primary productivity as it transports dissolved oxygen and organic matter into deeper layers of the substratum where it is transformed by microbial communities before returning to the surface (Boulton et al., 2010). Hyporheic exchange flows create a highly productive ecotone characterised by thermal regularity, circumneutral pH and gradients of biogeochemical processes. Communities inhabiting the hyporheic environment reflect these continuums and include a range of organisms with varying degrees of surface and groundwater affinity. First described by Orghidan (1959) as the *hyporheic biotope*, after the Greek *hypo* (under) and *rheos* (flow), this community has come to be referred to collectively as the hyporheos (*sensu* Williams and Hynes, 1974). The hyporheos includes a diverse range of bacteria, protozoa, algae, metazoa, fungi and invertebrates which play an important role in nutrient cycling, bioturbation and the redistribution of energy in lotic ecosystems (Boulton et al., 2010; Danielopol et al., 2003; Schmid-Araya et al., 2002).

1.2.2 Phreatic Habitats

The phreatic habitat is found in the aqueous voids and interstices within the aquifer which provide a complex area for communities of microbes and invertebrates, some of which occur nowhere else (Johns and Dunscombe, 2011). Phreatic surveys often focus on boreholes and wells; however, invertebrates and bacteria also inhabit the wider aquifer and have been recorded at depths exceeding 70 meters below the water table (Sorensen et al., 2013). Biological communities are most likely to occur in porous or fractured geologies, such as Limestone or Chalk, as these aquifers provide the space and hydrogeological connectivity required to support these communities (Arietti and Edwards, 2006; Johns and Dunscombe, 2011). Within phreatic habitats, the absence of light precludes photosynthesis and so the basis of the food web is a

combination of carbon transported into the aquifer from the surface and inputs from mineral weathering from within the aquifer (Gregory et al., 2014). However, much of this carbon is oxidized in soil before reaching the aquifer and the microbial communities occurring in phreatic habitats are adapted to live in low-nutrient conditions (Gregory et al., 2014).

1.3 Groundwater Ecology

Organisms that spend all or part of their life cycle in groundwater habitats are classified as stygoxenes, stygophiles or stygobites based upon their affinity to the hypogean environment (Table 1.1). Collectively, these organisms are known as stygofauna, a name derived from the River Styx which divides the Earth from the Underworld in Greek mythology (Hancock et al., 2005).

Table 1.1 Classification of stygofauna (modified after Gilbert et al., 1994)

Classification	Description
Stygoxene	Organisms which have no affinity with groundwater, only occurring there by accident
Stygophile	Organisms which have an affinity to the hyporheic habitat and actively exploit its resources. Comprising: occasional (early instars of organisms which migrate to the surface in later development); amphibiont (dependent on access to both surface and groundwater habitats); and permanent (may complete their lifecycle in either the surface or groundwater habitat) organisms
Stygobiont	Obligate groundwater organisms with all developmental stages occurring in a subterranean habitat and displaying morphological adaptations to this habitat

While epigean fauna include many generalist species that may use the subsurface environment during disturbance events or at the first stages of their life-cycle, stygofauna display morphological, physiological or behavioural adaptations that facilitate their exploitation of groundwater habitats (Gibert et al., 1994). Stygobionts have convergent morphological and physiological adaptations, such as the loss of pigmentation, ocular regression (a reduction (microphthalmia) or absence (anophthalmia) of eyes), vermiform (tubular) body shape, hypertrophy of sensory organs and k-selection reproductive strategies (long lifespans, late maturity and low fecundity) that allow them to inhabit groundwater (Figure 1.1; Gilbert et al., 1994; Robertson et al., 2009).

Figure 1.1 conceptual diagrams of the morphological adaptations of three crustaceans against depth showing a loss of pigmentation, vermiform body shape and ocular regression with depth below the surface, from top: *Gammarus pulex, Crangonyx subterraneus and Niphargus kochianus*. Not to scale.

Stygofauna comprise a diverse range of nematodes, beetles, crustacea and snails that occur across the Americas, Europe, Australia, Asia, New Zealand and Africa (Di Sabatino et al., 2000; Elliott, 2008; Hancock and Boulton, 2009; Hou et al., 2007; Lafont and Malard, 2001; Lafont and Vivier, 2006; Peck, 1998; Shaw et al., 2011). Compared with more than 1,000 species from across Europe, the stygofauna of England and Wales are impoverished in both number and diversity with fewer than 30 recorded species, represented by only the Crustacea and Acari (Figure 1.2; Robertson et al., 2009; Sket, 1999).

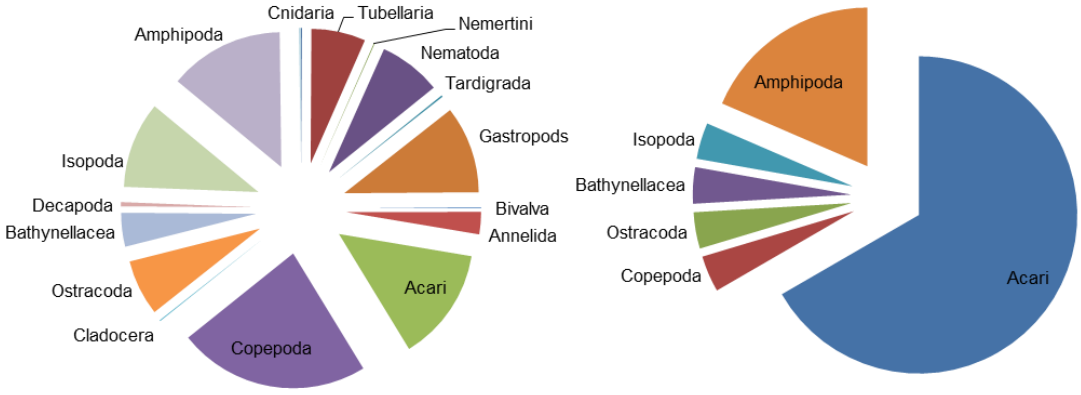


Figure 1.2 Proportional representations of stygofauna in Europe (n=1013 species; left; after Sket, 1999) and in England and Wales (n=27 species; right; data after Robertson et al., 2009)

1.3.1 Microbial Assemblages

Microbial assemblages form an integral part of ecosystem functioning, driving biogeochemical cycling and providing the base of the lotic food web (Findlay et al., 2003; Hall et al., 2012). Many of these communities have physiologically adapted to the groundwater environment by producing extracellular surfaces that allow them to bind with other microorganisms (such as neighbouring bacteria, protozoa, algae and metazoa) and attach to hard surfaces, forming a colony (Findlay et al., 2003; Gounot, 1994). These colonies (biofilms) have an advantage over free living organisms as they provide greater protection from biotic (such as grazing) and abiotic (such as extreme flow events) stressors, as well as a constant source of nutrients derived from surrounding flows and the colony itself (Stanford et al., 1994). Biofilms can be highly organised in structure as their composition often reflects environmental gradients of depth and dissolved oxygen as well as changes in the surface environment, for example, in a study in southern Germany, Zhou et al. (2012) found that the phreatic microbial community was strongly influenced by seasonal changes, increasing in diversity in response to autumn recharge and the associated increase in available organic carbon (Lowell et al., 2009).

1.3.2 Invertebrate Assemblages

Stygofauna are thought to have evolved from surface-dwelling freshwater or marine ancestors that actively or passively colonised the subterranean environment, ultimately becoming isolated from their source populations (Gilbert et al., 1994). The fragmented nature of hydrogeological systems, in addition to glaciation, is likely to have facilitated this process, resulting in restricted distribution and a high degree of endemism in these communities (Dole-Olivier et al., 2009).

The freshwater invertebrate community comprises meiofauna (>55 µm <500 µm) and macroinvertebrates (>500 µm; including “temporary meiofauna” (juvenile macroinvertebrates, *sensu* Robertson et al., 2000). Macroinvertebrates have traditionally received more research attention as they require less specialised sampling and identification skills; however, the meiofauna are thought to comprise a large proportion of the groundwater community as their small size facilitates their colonisation of the interstitial habitat (Stead et al.,

2005). Meiofauna play an important role in the trophic dynamics of lotic ecosystems, providing an essential link between the biofilms on which they graze and the larger invertebrate species that predate them (Hancock et al., 2005). It has been estimated that over five hundred species of water mite and the majority of Copepods known in Europe are stygobites. In Great Britain, the recorded stygo(meio)fauna comprise eighteen species of water mite[1]; one Bathynellacea (*Antrobathynella stammeri*), one Ostracod (*Pseudocandona eremite*) and a selection of Copepods; however, these records are derived from only a few studies in limited geographical areas (Arietti and Edwards, 2006; Robertson et al., 2008; Robertson et al., 2009; Sorensen et al., 2013).

There are over 4,000 species of aquatic macroinvertebrates in Great Britain, of which, eight are considered to be stygofauna (Freshwater Life, 2011). This assemblage is exclusively crustacean, represented by two families of amphipod (Niphargidae and Crangonyctidae) and one isopod (Asellidae). Amphipod species are brooding peracardian crustaceans characterised by a direct development life-cycle (with no independent larval stage) and specialised appendages of differing shapes (Vainola et al., 2008). Although the majority of amphipods occupy the marine environment, some have also colonized an array of freshwater habitats, including groundwater (Vianola et al., 2008). Niphargids are the largest and one of the most widely-distributed groups of freshwater amphipods, comprising over two-hundred epigean and hypogean species; however, the morphology of this group is highly variable and recent genetic analyses on European species has suggested a need for taxonomic revision (Fiser et al., 2008; Lustrik et al., 2011; Vianola et al., 2008). Niphargid species feed opportunistically on detritus, bacteria and fungi (as well as occasionally preying on other invertebrates) and have relatively long life spans, with some species, such as *Niphargus virei* living up to ten years (Freshwater Life, 2011). Currently, five species of Niphargids have been recorded in Great Britain: *Niphargus aquilex* (Schiodte, 1855), *Niphargus fontanus* (Bate, 1859), *Niphargus glenniei* (Spooner, 1952), *Niphargus kochianus kochianus* (Bate,

[1] All of the Acari recorded in Great Britain are of the Hydrachnellae: *Panisellus thienemanni; Thyasella mandibularis; Wandesia racovitzai; Torrenticola Andrei; Monatractides madritensis; Atractides denticulatus; Atractides latipalpis; Atractides acutirostris; Feltria cornuta; Feltria denticulate; Felitria subeterranea; Feltria (Azugofeltria) motasi; Barbaxonella angulate; Lethaxona cavifrons; Kongsbergia clypetata; Stygomomonia laeipes; Neoacarus hibernicus*; and *Hungarohydracarus subterraneus*.

1859) and *Microniphargus leruthi* (Schellenberg, 1934), all of which are stygobites (Freshwater Life, 2011). British studies have suggested that *Niphargus* species have specific habitat affiliations, with *N. fontanus* and *N. k. kochianus* recorded more frequently at greater depths than the more superficial *N. aquilex* (Gledhill, 1977; Gledhill et al., 1993; Sorensen et al., 2013)

The other group of amphipods with hypogean representatives in Great Britain is Crangonyctidae, a similarly widespread but exclusively freshwater family of which the majority are subterranean (Vianola et al., 2008). Two species of *Crangonyx* have been recorded in Great Britain, the epigean *C. pseudogracilis* (Boulsfield, 1958) and hypogean *C. subterraneus* (Bate, 1859). While epigean *Crangonyx* species graze on algae and plant matter, *C. subterraneus* is an opportunistic, omnivorous species which prefers interstitial waters (Freshwater Life, 2011; Gledhill, 1977).

In addition to the Amphipods, one hypogean representative of the Isopoda, *Proasellus cavaticus* (Leydig, 1871), a member of the Asellidae is also regularly recorded in Great Britain (Freshwater Life, 2011). Isopods are also brooding peracardian crustaceans with direct development life-cycles; however, all of their appendages are of similar size and form (Vainola et al., 2008). *P. cavaticus* is also omnivorous and has an exceptionally long life span of up to eleven years (Freshwater Life, 2011). Whilst relatively little is known of the environmental preferences of *P. cavaticus,* it is thought to occur in the deeper saturated zone of karstic aquifers (Martin et al., 2009).

The isolated nature, low fecundity and high endemism of groundwater invertebrate communities suggests that they are particularly vulnerable to disturbance, though the risks to and response of this community to environmental pressures remains largely unknown (Robertson et al., 2009). Groundwater habitats are likely to be at greatest risk from habitat destruction through degradation or depletion of water resources and changes to surrounding biological communities; however, the paucity of long-term monitoring data, tendency of large-scale studies to be undertaken in areas with minimal anthropogenic impact and high numbers of cryptic species have resulted in a knowledge gap surrounding the sensitivities of this community (Dole-Oliver et al., 2009; Dumas et al., 2001; Fiser et al., 2008; Proudlove and

Wood, 2003). Despite these constraints, research interest in stygofauna has increased over the past two decades and conservation measures, such as the designation of *Niphargus glenniei* as a Biodiversity Action Plan and Red Data Book (K5, Insufficiently Known and Endemic) species, have recently been implemented (Robertson et al., 2009). A greater understanding of these species and the factors influencing their distribution is essential for their conservation and management (Stanford and Ward, 1993).

1.3.3 Distribution of Stygofauna

At the landscape scale, the distribution of stygofauna reflects historic patterns of glaciation and geology. In Great Britain, the majority of records for stygofauna occur south of the Devensian glaciation (Figure 1.3) while globally, the distribution of this community reflects the limits of Pleistocene glaciation (Robertson et al., 2009; Stoch et al., 2009).

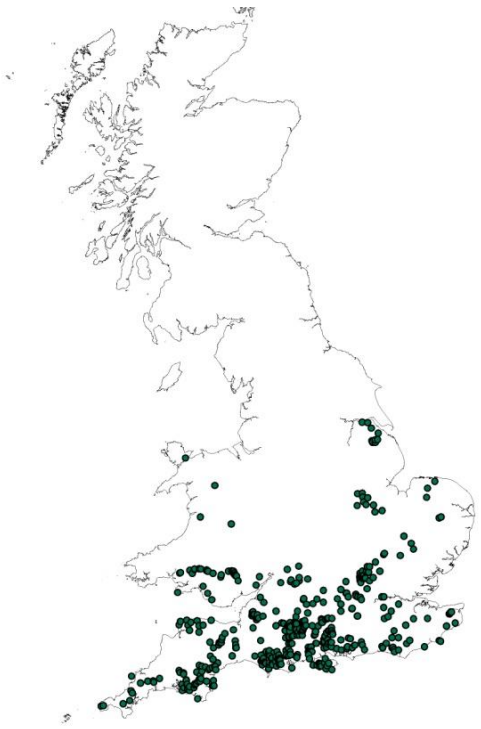


Figure 1.3 Distribution of stygofauna in England and Wales in which each marker represents a record (data interpolated from the Hypogean Crustacea Recording Scheme (2011))

While this distribution suggests a glacial influence, the impact of ice-cover events on groundwater communities is uncertain as recent research has shown that some stygofauna, such as *Crangonyx islandicus* (sp. nov.) and *Niphargus irlandicus* (Schellenberg, 1932) have survived repeated ice-cover events in subglacial refugia in Iceland and Ireland (respectively), suggesting that the

distribution of stygofauna cannot be explained through glaciation alone (Hanfling et al., 2008; Kornobis et al., 2011).

1.3.3.1 Distribution of Stygofauna in Great Britain

In Great Britain, stygofauna are largely restricted to the Chalk and Limestone geologies of England and Wales, although the mechanisms controlling this distribution are unclear. While physical parameters (such as the fissured nature of the strata) and chemical parameters (such as the high levels of calcium carbonate which is important for exoskeleton formation), are both likely to be influential, this distribution may also reflect sampling bias towards the south of the country as a comprehensive survey of Great Britain has not been undertaken (Johns and Dunscombe, 2011; Robertson et al., 2009). Although recent investigations in Scotland (where none were found; Pryce et al., 2010), the Ashdown Forest (southeastern England, Stead et al., 2004), upland Wales (Rundle and Ormerod, 1992), lowland Kent (southeastern England, Wilenchic, 2008), southwestern England (Johns et al., 2015), the Peak District (northern England, Stubbington et al., 2009a) and Ireland (Kibichii et al., 2009), have improved the understanding of stygofauna in the British Isles, comprehensive assessments have lagged behind Europe and North America where alluvial plains and karstic systems better facilitate such research (Pryce et al., 2010).

1.3.3.2 Biogeography in Lotic Ecosystems

A number of conceptual models for understanding and predicting the biogeographical distribution of organisms within lotic ecosystems have been proposed (Figure 1.4). The River Continuum Concept (RCC) was one of the first frameworks to use ecological theory to describe the longitudinal distribution of macroinvertebrates along the continuum of a river from source to mouth (Vannote et al., 1980). This was followed by the Flood Pulse Concept, which included the lateral dimension of the lotic system, highlighting the importance of exchange between the river and its floodplain (Junk et al., 1989). In 1993 Stanford and Ward introduced the Hyporheic Corridor Concept (HCC), which further developed these Concepts to include the hyporheic zone. The HCC describes a subsurface continuum formed by the hyporheic corridor which connects both laterally and longitudinally along the river where areas of vertical hydrological exchange catalyse hyporheic processes like “beads on a string”

(Boulton et al., 1998; Stanford and Ward, 1993). Subsequently, Poole et al. (2006) used the ecological concept of patch dynamics to specifically explain the distribution of hyporheic communities, proposing that areas of hyporheic exchange can be viewed as discrete habitats (patches) in which biogeochemical gradients, resulting from hyporheic exchange flows, create dynamic mosaics of habitat rather than a continuum of zonation. More recently, Hahn (2006) proposed that the distribution of stygofauna should be assessed according to the most influential factors at each scale, suggesting that hydrological exchange flows are appropriate at the micro (site) scale, but that aquifer type should be considered at the macro (reach) scale. While all of these models have attempted to conceptualise and predict invertebrate distribution, they are founded on catchment-scale processes and are difficult to test as the majority of applied research has been undertaken at the site or reach scale; in addition, few consider how these processes change or interact over time.

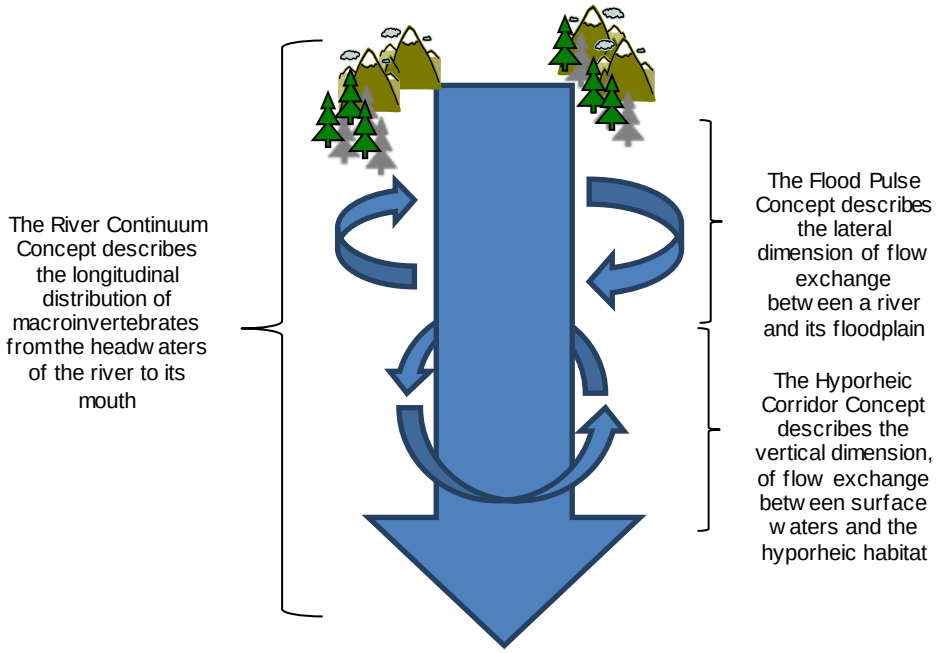


Figure 1.4 A theoretical river depicting biogeographical concepts (River Continuum Concept, Flood Pulse Concept and Hyporheic Corridor Concept) in lotic ecology.

1.4 Environmental Conditions in the Groundwater Environment

The distribution of organisms is limited by environmental parameters (such as geographical range; fundamental niche) and ecological variables (such as competition with other organisms; realised niche); therefore, if an organism could be present in a large area but only occurs locally, its absence may reflect sensitivities to specific conditions (Begon et al., 1995). This premise can be extrapolated to species-specific responses to environmental pressures, allowing

particular organisms or groups of organisms to be used as indicators. Response traits have been applied in the development of biotic indices for the rapid assessment of benthic communities to a number of pressures such as organic pollution (Biological Monitoring Working Party), flow stress (Lotic Invertebrate index for Flow Evaluation) and pesticides (SPEcies At Risk; Beketov et al., 2009; Extence et al., 1999; Moss, 1998). While the response of epigean species to environmental pressures is well established, the response of stygofauna is not, though several studies have suggested biological, physiochemical, chemical and physical influences (Townsend and Hildrew, 1994; Table 1.2).

Table 1.2 Review of potential influences on the distribution of groundwater communities

Factor	Parameter	Influence	Reference
Biological	Competition	Movement of epigean fauna into the hyporheic zone can displace the hyporheos	Danielopol et al., 2003
	Availability of Food	Alterations to food sources influence community composition	Townsend et al., 1983
Physio-chemical and Chemical	Nutrients	Direct impact not well established, indirect influence on food sources	Dumas et al., 2001; Hancock, 2002
	Dissolved Oxygen (DO)	Equivocal, stygofauna likely to be tolerant of low DO but some studies suggest it may be a limiting factor	Dole-Olivier et al., 2009; Hahn, 2006
	Temperature	Reflects surface water influence, potential to influence biota	Hahn, 2006; Korbel and Hose, 2011
	pH	Reflects surface water influence, potential to influence biota	Hancock and Boulton, 2008; Korbel and Hose, 2011
	Conductivity	Reflects surface water influence, potential to influence biota	Hancock and Boulton, 2008
	Geochemistry	Reflects surface and groundwater properties; however, calcium carbonate is essential for crustaceans	Robertson et al., 2009
Physical	Hyporheic Exchange Flows	Proportion of surface and groundwater likely to influence biota in low and high flow conditions	Gilvear et al., 2006
	Depth	Depth of water table as well as boreholes likely to influence biota with biological activity and diversity expected to decrease with depth	Hancock and Boulton, 2009
	Habitat	Dispersal ability of biota limited by hydraulic conductivity	Dole-Olivier et al., 2009
	Altitude	Potential to influence hyporheic exchange flows and biota	Dole-Olivier et al., 2009
	Organic Matter	Reflects availability of food	Hahn and Matzke, 2005; Hahn, 2006
	Aquifer Type	Aquifer properties (confined/unconfined) influence distribution	Arietti and Edwards, 2006; Dole-Olivier et al., 2009; Hahn and Fuchs, 2009
	Geography	Patterns of glaciation may influence distribution	Johns et al., 2015 Dole-Olivier et al., 2009; Varricchione et al., 2005

1.4.1 Physiochemical Variables

While many epigean species have well established tolerances to temperature, pH and dissolved oxygen, little is known of the preferences of their hypogean counterparts. Although the aquifer provides a stable habitat with consistent temperatures, circumneutral pH and relatively low levels of dissolved oxygen, the hyporheic zone is dynamic, with fluctuating physiochemical properties controlled by the relative contributions of surface water and groundwater as well as biological activity (Hendricks, 1993). To adapt to the groundwater environment, stygofauna are likely sensitive to large changes in temperature and pH, but resilient to alterations in dissolved oxygen (Datry et al., 2005).

1.4.1.1 Temperature

Temperature is a master variable of lotic ecosystems, directly influencing epigean community structure and indirectly controlling biogeochemical processes (Environment Agency, 2009). Changes in temperature have been found to significantly influence the structure of both epigean and hypogean communities; however, as groundwater maintains a relatively consistent temperature, it provides stability to phreatic habitats while moderating the thermal regime of receiving surface waters (Environment Agency, 2009a; Hannah et al., 2009). A study by Hannah et al. (2009) that considered water temperatures from surface water, hyporheic and phreatic sites along the River Tern (Shropshire, UK) found that surface water temperatures were heavily influenced by air temperature and were higher during the summer months (1.4 °C) and lower during the winter months (~1.9 °C) than hyporheic water, but that hyporheic water was warmer during the summer months (~2.5 °C) and cooler during the winter months (~4°C) than borehole water. The thermal regularity provided by groundwater is likely to be of increasing importance in moderating river temperatures as some climate change scenarios suggest that increasing surface water temperatures may result in the ‘potamalization’ of epigean communities, referring to a decline in the stenothermic species which are typically found in headwaters or small streams and can only survive within small thermal envelopes, and a corresponding increase in the eurythermic species that are found ubiquitously and are not sensitive to changes in temperature (Dai, 2011; Euro-Limpacs, 2011).

1.4.1.2 pH

Similarly, as groundwater is also expected to be pH stable, it is likely that stygofauna would be sensitive to large fluctuations, particularly if these represent a change in surface water influence. There are few studies on the direct response of stygofauna to changes in pH; however, a study by Townsend et al. (1983) found an indirect response of some species (*Niphargus aquilex*), which replaced epigean fauna (*Gammarus pulex*) at sites in the Ashdown Forest when these locations became more acidic (although this may suggest that stygofauna are more tolerant of low pH, the authors concluded that it was more likely to be an indirect consequence of increases in food source, in this case, iron bacteria).

1.4.1.3 Dissolved Oxygen

It is unlikely that the distribution of stygofauna is heavily influenced by dissolved oxygen as these organisms typically inhabit hypoxic or anoxic environments. Many stygofauna have adapted to these habitats as they are able to expend less energy than their epigean counterparts and utilise anaerobic metabolic functions (Humphreys, 2007). Notenboom et al. (1994) reviewed ecotoxicological studies on groundwater fauna and concluded that ambient oxygen concentrations have a negligible influence on these species within the range of 0.1 to 10.0 mg L^{-1}. However, a study of 18 boreholes in Southwestern Germany found the abundance of stygofauna to be low or completely absent from sites where dissolved oxygen was less than 1.0 mg L^{-1}, suggesting that this may be a minimum threshold (Hahn, 2006).

1.4.2 Chemical Variables

The response of epigean species to nutrient enrichment, geochemistry, industrial pollutants and pesticides is well established and has provided the foundation for environmental regulations encompassing air, land and water; conversely, the sensitivities of stygofauna are not as well established and, in some cases, equivocal (Lafont and Vivier, 2006).

1.4.2.1 Nutrients

The impact of nutrient enrichment, often originating from sewage discharge or agricultural run-off, can result in algal blooms, declines in dissolved oxygen and a shift in ecological communities to favour species that are tolerant of such

conditions in surface waters (Moss, 1998). It is likely that nutrient enrichment interferes with the oligotrophic and hypoxic nature of groundwater (which is often carbon limited), influencing or impairing the oxidation-reduction processes (Notenboom et al., 1994). It has been suggested that stygofauna are more susceptible to nutrient enrichment than their epigean counterparts (Hancock, 2002; Robertson et al., 2009), but it is unclear if this is a direct toxicological response, an indirect effect of subsequent changes in the surrounding environment (Lafont and Vivier, 2006) or increased competition resulting from the migration of epigean taxa (Danielopol et al., 2003; Pepin and Hauer, 2002; Notenboom et al., 1994; Sket, 1999). Within the hyporheic zone, a study of the biological communities inhabiting in the Peak-Speedwell Cavern System (Derbyshire, England) found that two organic pollution events resulted in markedly different ecological responses, in which the first eliminated most taxa while the second resulted in an increased abundance of taxa, which the authors suggested related to an increase in trophic resources (Wood et al., 2008).

The influence of nutrients on fauna within the phreatic habitat is also unclear. A study of 15 boreholes Ariège Aquifer (southern France) found the distribution of groundwater amphipods and isopods to be unrelated to the varying levels of agricultural pollution across the study area where Nitrate concentrations ranged from 20 to 160 mg L^{-1} (Dumas et al., 2001). However, a similar study of 19 boreholes in New South Wales (Australia) found significant differences in stygofaunal richness and abundance relating to differing agricultural practices in which boreholes with low nutrient levels (specifically <2 mg L^{-1}) recorded lower abundance and richness than those with mild nutrient enrichment (2-4 mg L^{-1}; Korbel and Hose, 2011). However, the increase in abundance and richness may be associated with an increase in trophic resources (Dumas et al., 2001).

1.4.2.2 Industrial Pollutants

While all aquatic fauna risk exposure to organic and toxic compounds, such as heavy metals and pesticides, hyporheic communities are at particular risk as they may come into contact with these compounds either from surface run-off or by leaching through soil and into groundwater (Hancock, 2002). While the response to such pollutants is likely to be species specific, stygofauna are considered to be less sensitive to heavy metal pollution (including zinc, copper,

chromium, cadmium and arsenic) than their epigean counterparts as toxicological studies have recorded stygofanua in industrial areas contaminated with heavy metals where corresponding epigean species are absent (Canivet and Gibert, 2002; Humphreys, 2007; Plénet, 1995). It has been suggested that this resistance may be attributed to an ability of stygofauna to bioconcentrate elements such as zinc and copper (Plénet , 1995); however, given the longer lifespan of stygofauna, they are also more likely to bioaccumulate greater quantities of heavy metals (Notenboom et al., 1994).

1.4.2.3 Pesticides and Herbicides

Pesticides and herbicides can be directly toxic to aquatic invertebrates; although epigean crustaceans, such as *Gammarus pulex,* are particularly sensitive to such chemicals, the impact on their hypogean counterparts has not been established (Adam et al., 2009; Cold and Forbes, 2004; Matthiessen et al., 1995). A recent study by Hose (2005) utilised a Species Sensitivity Distribution approach to compare the relative sensitivities of hypogean and epigean taxa to a range of pesticides found in Australian aquifers to derive water quality guidelines for groundwater ecosystems. Their results suggested that hypogean species were more sensitive to some insecticides (Chlorpyrifos) than those recorded in surface waters, but that the inverse was true for some herbicides (Atrazine); however, the study concluded that these responses were likely to be species and toxicant specific and that current groundwater quality guidelines were adequate for the protection of hypogean species (Hose, 2005).

1.4.3 Physical Variables

The distribution of groundwater fauna is partially controlled by physical parameters such as geology, geomorphology and flow, which determine the availability of habitat that can support these species (Boulton, 2007). However, this influence varies over differing spatial scales.

1.4.3.1 Geology and Geomorphological Processes

At the site scale, morphological features that facilitate surface and groundwater exchange, such as riffles, meander bends, springs and boreholes, create gradients of nutrient exchange and dissolved oxygen which result in hotspots of bioproduction and biodiversity (Stanford and Ward, 1993). At the reach scale, the distribution of hypogean fauna is influenced by planform, alluvial sediment

depth and flow patterns (Thulin and Hahn, 2008; Weitowitz, 2012). At the catchment scale, the distribution of groundwater fauna is principally controlled by aquifer type, with the most permeable geological formations supporting the greatest diversity of stygofauna (Dole-Olivier et al., 2009). Within the aquifer, the movement of fauna is limited by pore space and the availability of interconnected habitat, with hydraulic conductivity being the primary determinant of stygofauna presence (Hancock et al., 2005). Surveys in Southwestern England (Arietti and Edwards, 2006), Germany (Hahn and Fuchs, 2009) and France (Dole-Olivier et al., 2009) indicate that aquifer type is the principal factor influencing the distribution of hypogean fauna at the catchment-scale. Highly fractured, porous and karstic geologies, such as Chalk or Limestone, have been found to support greater stygofauna species richness and abundance when compared with other aquifer types (Johns and Dunscombe, 2011; Robertson et al., 2009).

While considerable variation in aquatic communities occurs between sites, reaches and catchments, few studies have considered the influence of scale on community distribution (Dole-Olivier et al., 2009). A recent exception is the study by Johns et al. (2015) which assessed the distribution and composition of stygofauna assemblages at 221 sites across southwestern England and found that aquifer type was more important than groundwater chemistry at this scale.

1.4.3.2 Hydrology and Hydrogeology

Hydrological and hydrogeological variability within lotic ecosystems are primary factors controlling the distribution of fauna and structure of aquatic communities (Datry et al., 2007; Feminella, 1996; Townsend et al., 1983; Wood and Armitage, 2004). While the response of epigean fauna to changes in flow has long been the subject of research, comparatively little is known about how hypogean fauna respond to these same changes, though Thulin and Hahn (2008) suggest that hydrological exchange is the principle factor shaping these communities in unpolluted groundwater (Lewandowski et al., 2009). The relative contribution of surface water and groundwater to hyporheic and phreatic habitats can vary spatially and temporally as a result of natural fluctuations (rainfall, groundwater recharge, permeability, stream bank storage and evapotranspiration) or anthropogenic pressures (discharge and abstraction;

Poole et al., 2006; Williams, 1993). The response of communities occupying these habitats to hydrological or hydrogeological disturbances is dependent on the duration, magnitude and frequency of the event in relation to normal flows (Boulton et al., 2010; James et al., 2008; Stanley and Boulton, 1993).

1.4.3.3 Sediment Characteristics

Hyporheic exchange flows are partially controlled by the hydraulic conductivity of the substratum, which is determined by the size, shape and interconnectivity of interstitial voids (Käser et al., 2009; Pryce et al., 2010). Although hydraulic conductivity varies naturally, high levels of fine sediment (<2 millimeters in diameter) can block interstitial voids (colmation) impeding flow exchange, altering biogeochemical processes and limiting the amount of available habitat for the hyporheos (Boulton, 2007; Environment Agency, 2009; Gilvear et al., 2006; Navel et al., 2010; Varricchione et al., 2005). Under normal conditions, fine sediments are transported downstream either in suspension or as bedload; however, poor agricultural practices, urbanisation, construction and alterations to the hydrological regime can reduce the capacity of a river to transport and scour fine sediments, resulting in colmation (Environment Agency, 2009; Heppell et al., 2009; Wood and Armitage, 1997)

1.5 Aims and Objectives

Despite recent interest in groundwater ecology, the literature reviewed in this chapter suggests that there are still large gaps in our understanding of this environment and the communities it supports. Specifically, much of the literature has focused on a single habitat (or two, by exception) and has been limited in temporal scale. Although there is a wide body of literature which assesses the response of surface water communities to environmental change, the response of organisms in the hyporheic and phreatic habitats is unclear. Moreover, no examples were found of a combined assessment of the response of all three habitats to such change.

This thesis proposes to address these gaps by testing the hypothesis that there are observable spatial and temporal patterns in the distribution of organisms inhabiting groundwater-dependent habitats and that these patterns are influenced by biological, chemical and physical elements. Following an

extended pilot study period to trial different hyporheic and phreatic sampling techniques, comprehensive monitoring of biological and environmental variables was undertaken to inform the two aims of this study through the assessment of their supporting research questions:

Aim 1: Describe the benthic, hyporheic and phreatic habitats and the distribution of the biological communities they support:

(a) Can these communities be described? Given relative paucity of research into groundwater communities and prevalence of cryptic species in this environment, it is expected that some taxa are not understood and that the stygofauna of Britain are under recorded.

(b) Does the spatiotemporal distribution of biological communities reflect the conditions of the three habitats? It is expected that each habitat will provide distinctive environmental conditions, and that the biological communities will reflect each habitat, with minimal movement between horizons.

(c) Do existing conceptual frameworks support the spatiotemporal distribution of biological communities across the three habitats? It is expected that existing theories, such as the Hyporheic Corridor Concept, do not adequately predict the distribution of species as they do not collectively consider all three habitats.

Aim 2: Describe periods of environmental disturbance during the study and the response of the biological communities:

(a) How do the communities respond to disturbance? Disturbance events, such as drought have the potential to alter the environmental conditions of these habitats and structure of their biological communities, it is hypothesised that the response of these communities is habitat-specific.

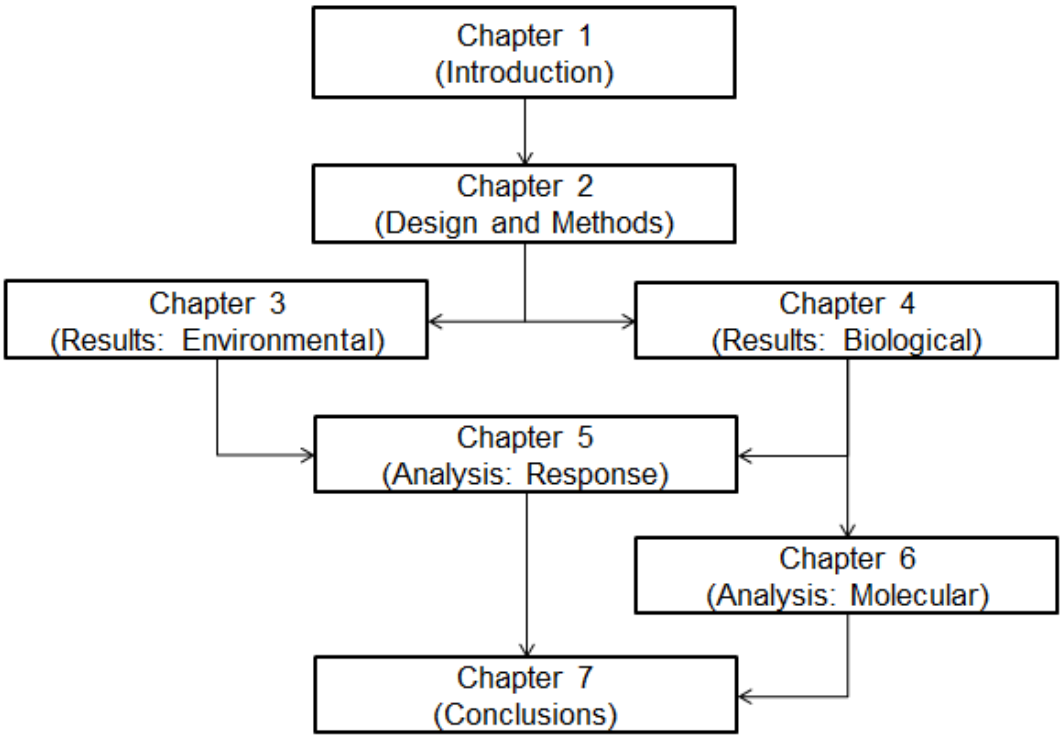
(b) How do the communities recover from disturbance? The impact of a disturbance event depends upon its duration and intensity, it is expected that the recovery of each community is habitat-specific.

1.6 Structure of Thesis

Following a description of methods (Chapter 2), the first aim of the study is informed by the first two data chapters which establish the environmental (Chapter 3) and biological (Chapter 4) descriptions of the benthic, hyporheic and phreatic habitats and their communities. The second aim is informed by the third data chapter (Chapter 5) which assesses the response of these communities to the periods of high and low flow which occurred during the study. The final data chapter (Chapter 6) is beyond the original scope of this work and discusses the morphological and molecular assessment of an

undescribed amphipod recorded from each of the habitats considered by this study. The final chapter (Chapter 7) provides a summary of the study findings and direction for future research. As all chapters contribute to the testing of the hypothesis, cross-referencing occurs throughout the thesis (Figure 1.5).

Figure 1.5 Diagram of thesis structure



Chapter 2

Experimental Design and Methods

2.1 Introduction to Experimental Design and Methodology

The protection and sustainable management of freshwater ecosystems are recognised as international priorities; however, the assessment of these systems is often limited to the biological, chemical and physical status of surface waters, omitting the condition of groundwater and its connection with the surface. Where the status of groundwater is considered, it is conventionally assessed with chemical and hydrogeological parameters, often overlooking biological communities, their diversity and potential to provide information on the condition of this environment (Mermillod-Blondin et al., 2013).

International efforts in Switzerland (Swiss Water Protection Ordinance, 1998) and Australia (New South Wales State Groundwater Dependent Ecosystems Policy) have included ecological criteria in their groundwater policies, but they are notable exceptions (Griebler et al., 2010). Some countries, such as the United States and the United Kingdom have attempted to protect selected groundwater species through the use of wildlife legislation (such as the designation of *Niphargus glenniei* as a British Biodiversity Action Plan (BAP) species), but these species are only a small part of the wider ecosystem. The national freshwater monitoring programme in England does not include any scope for the monitoring of groundwater ecology in hyporheic or phreatic habitats as it has historically focussed on direction provided by the European Union Water Framework Directive (WFD; Council of the European Communities 2000) and subsequent daughter directives (such as the Groundwater Directive (2006/118/EC)) which do not require the assessment of groundwater ecology (though the latter recognises groundwater as an ecosystem and recommends research be undertaken to provide better criteria for ensuring groundwater ecosystem quality). However, a growing body of evidence indicates that benthic, hyporheic and phreatic communities differ in their response to environmental pressures, suggesting that the assessment of a single community does not provide an adequate reflection of the ecosystem as a whole (Boulton et al., 2010; Gilvear et al., 2006).

The paucity of hyporheic and phreatic information has been associated with a lack in methodological standardization, while initiatives such as the Protocols for the Assessment and Conservation of Aquatic Life In the Subsurface

(PASCALIS; Malard et al., 2002), The Hyporheic Handbook (Environment Agency, 2009a) and recent CEN standard on Hyporheic Sampling (CEN, 2011) have attempted to standardise sampling procedures, there is still variance between the number of sites, frequency of collection, sample area and duration of investigations between researchers. While some authors have suggested using exchange flows, rates of biogeochemical activity or diversity of groundwater communities, others have developed indices using biotic and abiotic parameters; however, neither approach has been standardised (Boulton et al., 2010; Hahn, 2006; Korbel and Hose, 2011). A greater understanding of the distribution of species in groundwater dependent habitats and their response to environmental pressures is therefore essential for successfully integrated catchment management.

To address these issues, this study assessed spatial and temporal distribution of organisms throughout benthic, hyporheic and phreatic habitats through the comprehensive multi-year monitoring of biological, chemical and physical variables which are likely to influence these communities (Chapter 1). A natural trajectory experimental design, which collects replicate samples across time to observe ecosystem variables in an unmanipulated environment, was selected for this study as it facilitated the assessment of fluctuations by season and in response to perturbations in a complex groundwater-dependent environment (Gotelli and Ellison, 2004). This chapter details the approach to the study, including the study area (Section 2.2) as well as the approach to sample collection and analysis (Section 2.3).

2.2 Study Area

This study was undertaken in a catchment of the River Stour (Kent) located in Southeast England, specifically focussing on one of its tributaries, the Little Stour. The Little Stour was selected by the Environment Agency, who funded this research, to help inform their management programme for this tributary; however, the study area was expanded to include a larger portion of the catchment, which includes the River Dour, to avoid potentially erroneous generalisations about a single reach. Although the selection of this study area was predetermined by the funding stipulations for this study, the ecological importance of the Little Stour and its response to changes in environmental

conditions has been well documented (Stubbington et al., 2009b; Stubbington and Wood, 2013; Wood and Petts, 1994; Wood and Armitage, 2004).

2.2.1 Stour Chalk Block

The study area is underlain by Chalk geologies typical of Southern England. These fractured, semi-karstic aquifers are characterised by their high rates of transmissivity and hydraulic conductivity as well as consequent exploitation for drinking water (Allen et al., 1997; Environment Agency, 2003). Although typical values for transmissivity in this area are approximately 1500 m^2 d^{-1}, within the study area, boreholes in the Upper Chalk (130 meters) and Middle Chalk (60-80 meters) have the highest rates of transmissivity (and provide better yields) than those located within the Lower Chalk where vertical groundwater flow is impeded by marl layers that force water to issue from springs (Allen et al., 1997). Within the Stour Chalk Block, groundwater flow is anisotropically controlled by geography, flowing North-Northeast/South-Southwest five times faster than West-Northwest/East-Southeast (Allen et al., 1997). These aquifer properties influence groundwater contributions to the surface, recording much higher transmissivity within the valleys than the interfluves and give rise to the Stour and Dour Rivers (Allen et al., 1997).

2.2.2 Stour River Catchment

The Stour is a lowland river typical of Southern England. It comprises two separate headwaters, the Upper Great Stour and East Stour which confluence near Ashford to form the main river (Figure 2.1). The Stour headwaters rise from the Chalk and Lower Greensand, flowing into a Gault Clay valley which results in rapid run-off and a flashy hydrological regime; however, as the river passes through the North Downs it displays features characteristic of a chalk stream with clearer water and greater thermal stability (Environment Agency, 2003). It is in these middle reaches that the river is joined by its major tributary, the Little Stour (Environment Agency, 2003). Downstream of this confluence, the topography of the catchment reduces in gradient and the river becomes wider, deeper and slower flowing, similar in character to a marshland drain. In addition to the Stour River, the aquifer also gives rise to the Dour, which does not join the Stour, flowing instead from the vicinity of the Nailbourne headwaters southward to Dover Harbour (Adams, 2008).

The land use in this catchment, which drains an area of 1081 km^2, is predominantly agricultural but there are also major urban areas including Canterbury, Ashford and Dover (de Vos et al., 2002). Historically, the watercourses of the catchment have been used to power a number of water mills for corn and paper manufacture, resulting in a series of impoundments and areas of canalisation that continue to influence the morphology and hydrology of the catchment. Recent growth in urban areas, such as Ashford, has increased demand in public drinking water abstractions and exacerbated low flows in the catchment; in addition, poor quality sewage treatment works discharges to the middle and lower reaches have resulted in the designation of the Stour as a Sensitive Area (eutrophic; SAe) under the Urban Waste Water Treatment Directive (91/271/EEC; Environment Agency, 2003; Smedley et al., 2003).

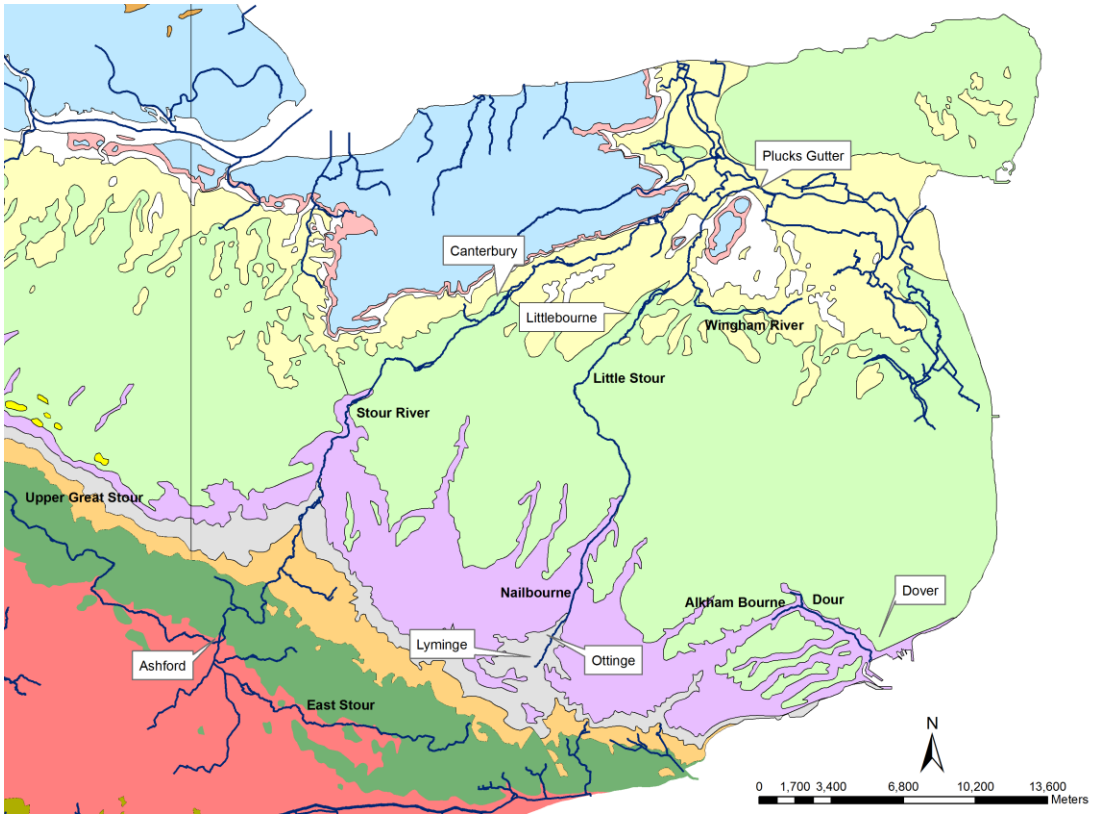


Figure 2.1 The Stour Catchment, its rivers (in blue, labelled in bold typeface), locations of interest and its underlying geology in which: blue denotes London Clay; green, Upper Chalk; purple, Middle Chalk; grey, Lower Chalk; yellow, Thanet Sands; orange, Gault Formation; dark green, Greensands; red, Wealden Clay; pink, Harwich Formation; and white, Lambeth Group (source data from the Environment Agency (National Base Layers, 2014)).

Despite these environmental pressures, the Stour, which flows through the Kent Downs Area of Outstanding Natural Beauty, supports a number of protected species including the Water Vole (*Arvicola amphibius* L.) and White Clawed

Crayfish (*Austropotamobius pallipes,* Lereboullet 1858). The chalk streams within this catchment (the Little Stour and Dour) have been designated under the United Kingdom Biodiversity Action Plan as priority habitats, recognising the importance of these ecosystems. Chalk streams are groundwater-dominated watercourses occurring over Chalk geologies in Northern Europe (England, France, Belgium and Denmark) and New Zealand which provide a specialised habitat typified by stable temperatures, circumneutral pH, low turbidity, high alkalinity and a characteristic, often predictably ephemeral, hydrological regime, which supports a distinctive ecology (Berrie, 1992; Environment Agency, 2004). However, chalk streams such as those in the Stour catchment, are often exploited for anthropogenic use and many suffer from '*Chalk Stream Malaise'*, the deterioration of their characteristics through reduced flows, degraded water quality and colmation (Berrie, 1992; Heywood and Walling, 2003).

2.2.2.1 Little Stour Sub-Catchment

The Little Stour flows for 30 kilometres from its headwaters through the Elham Valley to its confluence with the Stour River at Plucks Gutter, draining a catchment area of 287 km^2 underlain by unconfined (Lower, Middle and Upper) Chalk (Adams et al., 2008; Wood et al., 2000). The Little Stour headwaters, referred to locally as the Nailbourne, rise from a perennial springhead in the Lower Chalk at the well of Saint Ethelburga in the village of Lyminge, before flowing for a few kilometres to the village of Ottinge where the geology changes to Middle Chalk and flow becomes intermittent (Figure 2.2). Historical accounts suggest that the Nailbourne only flows to its confluence with the perennial reach once in seven years and that its springhead only dries during times of extreme drought (Holmes, 2006). This suggests that that the Nailbourne is more of an erratic, intermittent stream than a winterbourne which would be expected to regularly flow throughout the winter and dry during the summer months (Feminella, 1996). This erratic nature may be partially attributed to its Clay substrate as this is likely to impede connectivity with its underlying aquifer.

The perennial section of the Little Stour rises at Well Chapel Spring, a horseshoe-shaped pond located next to monastic ruins near the village of Littlebourne. The Little Stour flows as a characteristic chalk stream for approximately six kilometres until its confluence with the Wingham River where

the channel has been resectioned and displays characteristics of a deep, slow-flowing lowland ditch. The Little Stour joins the River Stour downstream of this confluence at Plucks Gutter where it is pumped into the river as a result of historical subsidence. The flow regime of the Little Stour reflects the contribution from groundwater, with baseflow conditions between August and September and peak discharge occurring between December and February (Stubbington et al., 2009b; Wood et al., 2001; Wood and Armitage, 2004).

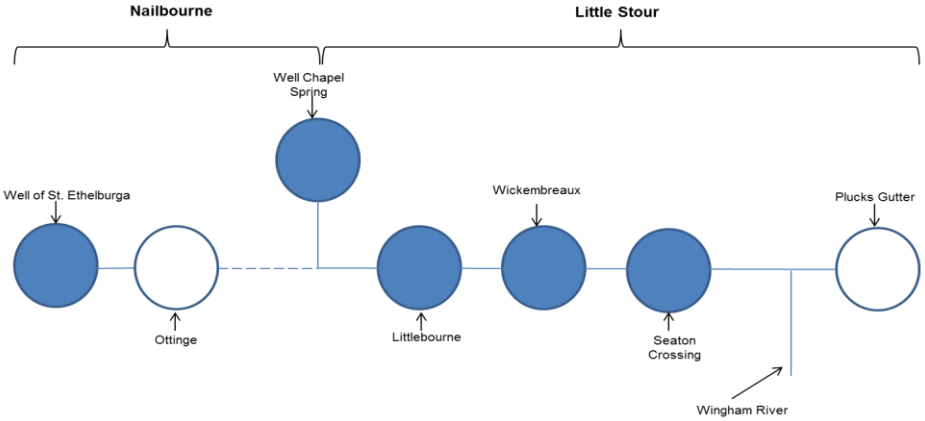


Figure 2.2 Planform of the Nailbourne and Little Stour. Sampling sites are depicted as filled-in circles and sites of interest as hollow circles (though the Well at Chapel Spring was only sampled during the pilot study). Each connecting line represents part of the Nailbourne, Little Stour or Wingham Rivers with interrupted lines showing areas of intermittent flow (not to scale).

The environmental quality of the Little Stour is influenced by historic alterations and current pressures. Historically, the channel has been modified for water cress cultivation and milling, resulting in a legacy of impoundments, artificial pools and canalisation. Presently, the Little Stour suffers from low flows which are likely to be exacerbated by abstraction for public drinking water supply (Environment Agency, 2008). The Little Stour was designated as one of 15 Priority Chalk Rivers impacted by low flows as a result of groundwater abstraction and natural drought by the National Rivers Authority (predecessor of the Environment Agency) in 1993 and the impact of abstraction on the ecology of the river is under investigation by the Environment Agency as part of the Restoring Sustainable Abstraction programme (Environment Agency, 2008; National Rivers Authority, 1993). Annual recharge to the Little Stour is 9.04×10^4 Ml a^{-1}, 27% of which is authorised for abstraction from groundwater; however, this percentage increases to between 30 and 40% during drought periods when the normally perennial sections of the river dry completely (Adams et al., 2008). Although the investigation is ongoing, initial results suggest that even under a scenario of no abstraction, ecologically damaging

drying events would occur following two concurrent years of poor winter recharge (Environment Agency, 2008). Records suggest that the Little Stour flow is 15-20% of what it would be in a pristine state and historic evidence, such as the relic cress beds at Etchinghill and watermill near Lyminge, as well as events such as the Nailbourne Rapids Race (a community boat race) suggest that the flow of the Nailbourne was previously greater (Adams et al., 2008; Barham, 2011; Holmes, 2006). Both historic and current pressures influence the WFD status of the Little Stour Waterbody (GB107040019590) which is designated as a Heavily Modified Waterbody and fails to meet the minimum requirements for fish, dissolved oxygen, hydrology and morphology (Mitigation Measures Assessment; Environment Agency, 2009b).

2.2.2.2 Dour Sub-Catchment

The River Dour is also a groundwater dominated chalk stream, which flows for five kilometres from ephemeral headwaters through the Lydden Valley to Dover, draining a catchment area of 90 km^2 (Adams et al., 2008). The Dour rises at Waters End near Temple Ewell where it flows intermittently to its confluence with its tributary, the Alkham Bourne, and becomes perennial, flowing through Dover and discharging in the English Channel (Figure 2.3; Adams et al., 2008).

The environmental quality of the Dour is also influenced by a series of legacy milling structures, abstraction pressures and urban development (Environment Agency, 2003a). In response to these pressures, the Dour has been identified as one of the top 40 Low Flow Rivers and review of historic records suggests that its current discharge at Crabble Mill is less than 20% of what it would be in a natural, undeveloped state (Adams et al., 2008). During years of below average winter recharge, the Dour headwaters can dry from July to October (Adams et al., 2008). Annual recharge to the Dour is 4.26×10^4 Ml a^{-1}, 83% of which is authorised for abstraction; however, measures such as the Dour augmentation scheme and the Dour Low Flow Alleviation Scheme (which mandates that abstraction for Public Water Supply move from the headwaters to the downstream end of the catchment during periods of low flow) have attempted to alleviate the environmental impact of dry periods (Adams et al., 2008). The WFD status of the upper Dour (GB107040019490) has been influenced by these pressures, as it is designated as a Heavily Modified Water

Body and fails to meet the minimum requirements for fish, hydrology and morphology (Mitigation Measures Assessment; Environment Agency, 2009b).

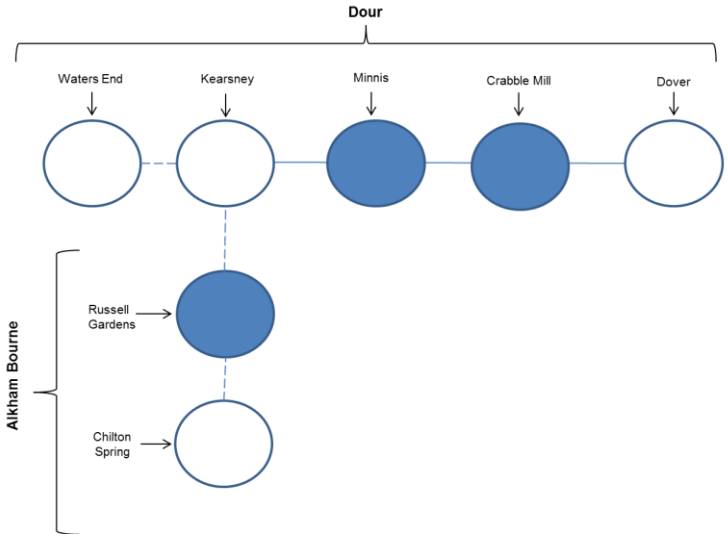


Figure 2.3 The Dour River. Sampling sites are filled-in circles and places of interest as hollow circles (Crabble Mill is the location of the hydrological gauge). Each connecting line represents part of the River with interrupted lines showing areas of intermittent flow (not to scale).

2.2.3 Site Selection

Twenty-three sites located across the Stour catchment were considered for inclusion based upon the availability of stygofaunal records, accessibility, spatial distribution and advice taken from local officers at the Environment Agency and British Geological Survey (Table 2.1; Figure 2.4). Boreholes of >30m depth were excluded due to sampling equipment limitations and the small likelihood of fauna being present beyond this depth (Hahn and Fuchs, 2009). Initial survey results were used to inform continued monitoring based upon environmental characteristics, location, accessibility and the potential for anthropogenic disturbance of sampling equipment. Five paired riverine (benthic and hyporheic) and seven phreatic sites were selected for routine monitoring in this study. The robustness of study could have been improved with the inclusion of additional sites; however, the number of sites was balanced against available resource. The number and location of sites selected follows the approaches suggested by available guidance and literature. For benthic and hyporheic monitoring, the CEN Standard for Sampling Invertebrates in Hyporheic Zone (2011) recommends sampling two riffle-pool sites from two different river reaches, while for phreatic sites, the review by Thulin and Hahn (2008) suggests that five to ten boreholes provide a sufficient ecological assessment.

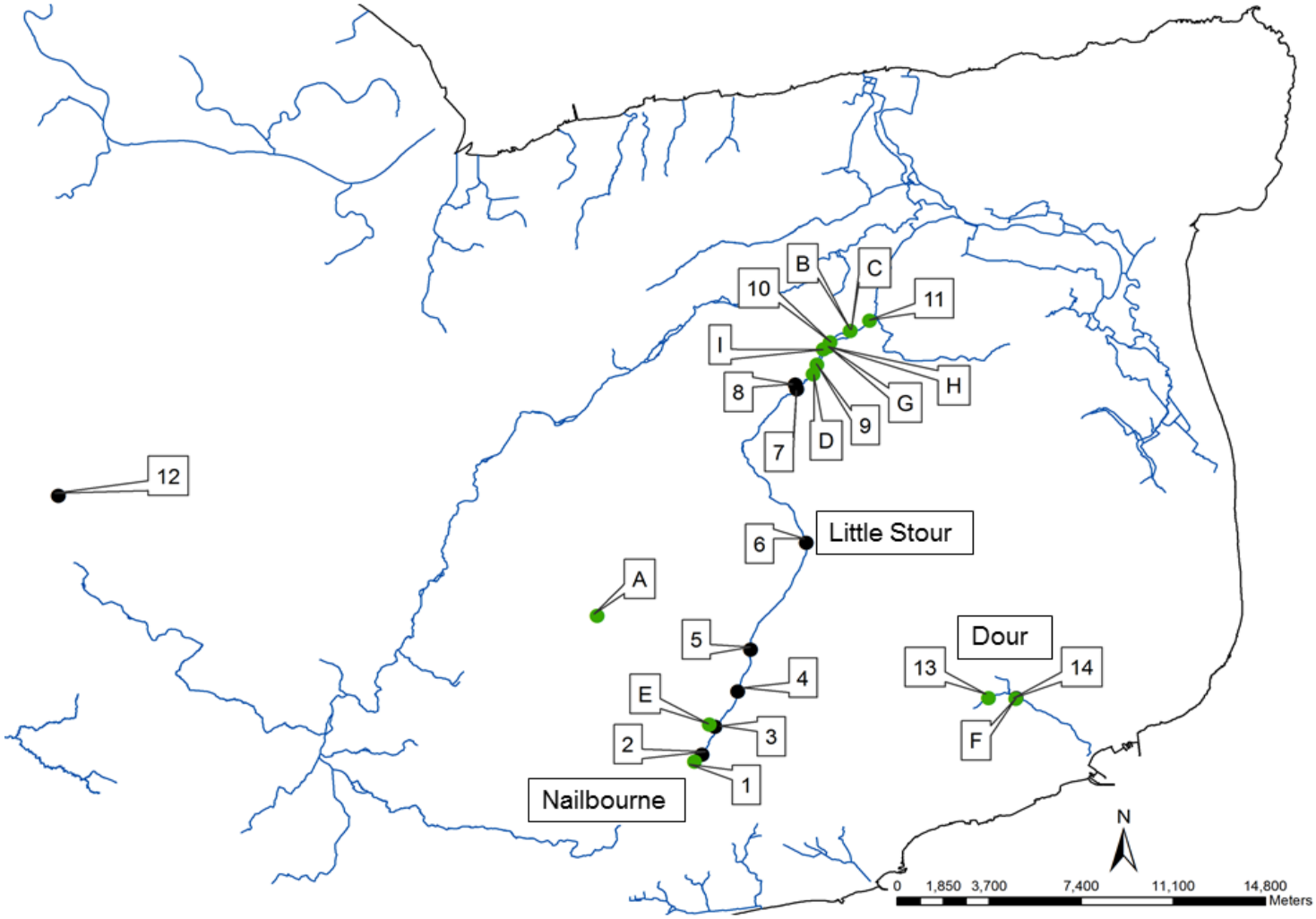


Figure 2.4 The five riverine (numbers) and seven phreatic (letters) sites sampled routinely are displayed in green (n=12). Sites which were only monitored during the pilot period or that were sampled opportunistically at times of high flow are displayed in black (please note sites H and I are obscured).

Table 2.1 Study site names and details of inclusion for routine monitoring throughout the study. Non-routine sites were either sampled as part of the pilot project or opportunistically at times of high flow. Borehole depths are after British Geological Survey and Environment Agency (EA) records, some have corresponding EA environmental monitoring data. Six of the boreholes are 'covered' by a steel plate or similar.

	Site	Name	Selected	Watercourse	NGR	Selection Notes
Riverine (benthic and hyporheic)	1	Ethelburga	Yes	Nailbourne	TR 1617 4090	Springhead, perennial
	2	Lyminge	No	Nailbourne	TR 1646 4120	1 km ds, compacted substrate
	3	Ottinge	No	Nailbourne	TR 1698 4234	3 km ds, access difficult
	4	Elham	No	Nailbourne	TR 1789 4381	4 km ds, access difficult
	5	Grimsacre Farm	No	Nailbourne	TR 1842 4555	6 km ds, clay substrate, intermittent
	6	Barnham	No	Nailbourne	TR 2066 4999	11 km ds, clay substrate, intermittent
	7	Garrington Farms	No	Nailbourne	TR 2027 5635	Clay substrate, intermittent
	8	Well Chapel	No	Little Stour	TR 20218 5655	Springhead, perennial, erratic
	9	Littlebourne	Yes	Little Stour	TR 2108 5737	2 km ds, perennial, historic data
	10	Wickembreaux	Yes	Little Stour	TR 2162 5829	3 km ds, perennial, historic data
	11	Seaton	Yes	Little Stour	TR 2320 5920	5 km ds, perennial, historic data
	12	Lenham	No	Great Stour	TQ 9059 5193	Springhead, clay substrate
	13	Russell Gardens	Yes	Dour	TR 2798 4354	1.5 km ds, historic data, perennial
	14	Minnis	No	Dour	TR 2910 4354	1.5 km ds, heavily modified channels
Phreatic	A	Little Buckett	Yes	Great Stour	TR 1225 4696	Well 30m, uncovered, EA data
	B	Seaton Chalk	Yes	Little Stour	TR 2242 5878	Borehole 3m, covered, EA data
	C	Seaton Gravel	Yes	Little Stour	TR 2242 5878	Borehole 25m, covered, EA data
	D	Silver Dyke G	Yes	Little Stour	TR 2094 5697	Borehole 5m, uncovered, EA data
	E	Cullings Farm	Yes	Nailbourne	TR 1676 4244	Well 25m, covered, EA data
	F	Minnis Borehole	Yes	Dour	TR 2908 4351	Borehole 25m, covered, EA data
	G	Littlebourne G	Yes	Little Stour	TR 2155 5812	Borehole 24m, covered, EA data
	H	Littlebourne C	No	Little Stour	TR 2155 5812	Borehole 19m, covered, no EA data, dry for study duration
	I	Little Stour Farm	No	Little Stour	TR 2134 5801	Well 4m, uncovered, no EA data, connectivity limited

2.2.3.1 Riverine Sites

Five sites on the Nailbourne, Little Stour and Dour were selected for routine paired benthic and hyporheic monitoring. Two of these (10 and 11) were selected for additional, experimental sampling which required the equipment to be left *in situ,* and was facilitated by their relatively secluded locations. All riverine sites displayed features typical of lowland chalk streams such as tufa deposits, characteristic flora (*Hildenbrandia* and *Ranunculus penicillatus* (*pseudofluidans*)) and fish species (*Salmo trutta* and *Cottus gobio*; Figure 2.5; JNCC, 2005).

Figure 2.5 The Nailbourne springhead (left, site 1) and Little Stour (right, site 8)

2.2.3.2 Phreatic Sites

Phreatic samples were collected at extant boreholes and wells across the Stour catchment. Many of these are used by the Environment Agency to monitor groundwater in a 'nest' where boreholes have been drilled into differing strata at near-coincident sites, such as at Seaton Chalk (B) and Gravel (C), which are located less than a meter apart but extend to different depths (Figure 2.6). Where possible, both nested boreholes were sampled, except at Silver Dyke Gravel (D) and Minnis Borehole (F) where the corresponding site was fitted with permanently installed monitoring equipment that inhibited sample collection.

Figure 2.6 Seaton Chalk and Gravel (left, sites B and C) and Silver Dyke Gravel (right, site D)

2.3 Methodological Approach

Sampling methods should provide representative, reproducible results, balancing any limitations against pragmatism, efficiency and effectiveness. Recognising the lack of standardised methods for sampling the hyporheic and phreatic habitats, this section discusses the methods considered, trialled and selected for the collection of these data. Samples were collected bimonthly from January 2009 until September 2012 (with the exception of November 2010 when winter storm conditions precluded collection; phreatic samples were collected from September 2011 to September 2012). Samples were collected at this frequency to encompass a range of hydrological and ecological patterns including peak discharge, baseflow and insect flight times. No guidance is available regarding the optimum frequency of sampling in the hyporheic or phreatic habitats as most previous studies refer to a single sampling occasion or season; as such, this study extends the time series available for these habitats (Griebler et al., 2010; Hahn and Fuchs, 2009; Johns et al., 2015). All of the methods trialled during this study are presented in this section; however, only the results collected using methods selected for routine monitoring were used to inform the statistical analyses in subsequent chapters.

2.3.1 Biological Sampling

Groundwater supports diverse communities in benthic, hyporheic and phreatic habitats. While the methods for sampling benthic communities are well established, there remains a great deal of variability between organisations and individual researchers in sampling hyporheic and phreatic habitats despite initiatives as the PASCALIS Project and the Hyporheic Network (Environment Agency, 2009; Malard et al., 2002). The most frequently utilized methods for sampling in these habitats are summarized in Table 2.2. Discounting approaches which were prohibitively expensive or unsuitable for the characteristics of this catchment, six methods were trialled for sampling the hyporheic habitat and two for the phreatic habitat to select those methods which would be used routinely throughout the study. Methods which provided comparable, consistent and representative samples; were operatable throughout different environmental conditions; allowed for quick and easy sample collection by a minimum number of people; and did not discount ecologically relevant fauna, were selected.

Table 2.2 Review of methods. References for selected methods are discussed in subsequent sections (details for unselected methods: CEN, 2011[+] and Korbel and Hose, 2011[++])

Taxa	Method	Trial	Description	Advantages	Disadvantages	Selected
Epigean Microbes	Cotton Strip Assay	No	A cotton strip is left *in situ*, and tensile strength is tested for microbial activity[++]	Comparable, may be used in rivers and boreholes	Specialised equipment, only surrogate results	-
Phreatic Microbes	Biolog Ecoplate	Yes	Microbial growth on substrates provides a metabolic fingerprint	Simple and comparable	Difficult to use in rivers, expensive	Yes
Benthic Fauna	Suber Net	Yes	Superficial sediment is dislodged to collect drifting invertebrates	Simple, quantitative and low-cost	Provides 'patchy' results	Yes
	Kick-Sweep	Yes	Benthic invertebrates are disturbed and collected in a net	Simple, low-cost, used widely	Semi-quantitative	No
Hyporheic Fauna	Pumping (Bou-Rouch/ Vacuum)	Yes	Interstitial fluid is extracted by pumping from a pipe inserted to depth	Low-cost, semi-quantitative, cosmopolitan	Selective, installation may cause migration, unknown area sampled	Yes
	Stand Pipes	No	Tubes are inserted into the sediment and left *in situ* with subsequent extraction of fauna[+]	Semi-quantitative, low-cost, replicable	Unknown area sampled, may be influenced by anthropogenic disturbance	-
	Coring (Freeze)	Yes	A tube is driven into the substrate before cyrogenic fluid is used to freeze a core	Quantitative, vertical sample with sediment	Difficult, expensive and destructive	No
	Colonisation Pots	Yes	Pots are inserted into the substratum and left *in situ*	Simple and low-cost	Selective, qualitative, difficult to avoid loss on collection	No
	Karaman-Chappuis Pits	No	Fauna collected from pits excavated in exposed sediments[+]	Simple and low-cost	Difficult to replicate, limited to rivers with exposed sediments	-
Phreatic Fauna	Phreatic Net	Yes	A net is lowered into a borehole and raised through water column	Simple and low-cost	Semi-quantitative, limited by well size	Yes
	Phreatic Pump	Yes	A tube is lowered into a borehole, sample is extracted using a pump	Allows for concomitant collection of water sample	Expensive, small aperture wells only, may damage animals	No

2.3.1.1 Riverine Invertebrates

Benthic samples were initially collected using a kick-sweep technique in which fauna residing in this habitat are dislodged by disturbing the substratum and collected in a net; however, this method was replaced by suber-sampling in March 2010 to provide a more quantitative assessment (Murray-Bligh, 1999). A 63 µm mesh size was used in both methods to facilitate the collection of early instars though meiofauna were excluded from statistical assessment.

Hyporheic samples were collected by pumping, artificial substrates and freeze coring (alternative techniques, including Karaman-Chappuis and standpipes, were considered but discounted due to the inhospitable substrate in this catchment and exposure of the sampling locations). Pump-samples were initially collected using a modified Bou-Rouch, comprising a stainless steel pipe attached to a Whale Gusher Urchin Bulkhead Bilge Pump but this was found to provide inadequate suction and was replaced by a standard Bou-Rouch pump in March 2010 (Figure 2.7). In both instances, a steel pipe was driven 30 - 50 centimetres into the substratum using a sledgehammer before priming the pump and extracting six litres of water. This process was repeated four times across the channel at each site. All samples were sieved bankside to a fraction of 63 µm. Several studies have examined the effectiveness of sample volumes and replicates, suggesting a nonlinear relationship between the volume and the number of taxa recovered; however, this relationship remains unstandardized in the literature. The methodology applied throughout this study follows the protocol proposed by PASCALIS (Malard et al., 2002; Boulton et al., 2003; Davy-Bowker et al., 2006; Environment Agency, 2009).

Figure 2.7 Modified (left) and unmodified (right) Bou-Rouch pumps for hyporheic sampling.

Vacuum pumping was undertaken alongside Bou-Rouch pumping during the final year of study at two sites (10 and 11) to assess the relative effectiveness of these methods. Following the techniques of Stubbington et al. (2011), four nests of three polyvinyl chloride (PVC) pipes were driven into the substratum using a steel T-bar to depths of 10, 20 and 30 centimetres below the substratum (Figure 2.8). Reflecting the Bou-Rouch methodology, a six-litre sample was drawn from each pipe using a Whale Gusher Urchin Bulkhead Bilge Pump attached to a rubber hose. Each pipe was capped after sampling to inhibit colonisation by benthic invertebrates. This technique was found to be less efficient than the Bou-Rouch pump and limited by exposure which inhibited the installation (and survival) of equipment at some sites.

Figure 2.8 Vacuum-pumping pipes (left) and artificial substrates contaminated by *Salmo trutta* roe (right), both from site 10.

Artificial substrates were trialled during the pilot study as a method for collecting invertebrates from the shallow hyporheic zone (10-20 centimetres below the substratum; Plénet and Gibert, 1992; Vervier, 1990; Vervier and Gibert, 1991). The substrates comprised two polyvinyl chloride tubes (5 centimetre internal diameter) covered in a variable pattern of pores (10 millimetre diameter) and filled with a mixture of jute rope and river gravels (collected at the site; Figure 2.8). Substrates were buried for a period of 28 days (±2) to allow for colonization following the disturbance caused by installation (Coleman and Hynes, 2004; Scarsbrook and Halliday, 2002). This method was successful in the collection of hyporheic invertebrates; however, it was discontinued during the pilot study as the substrates were repeatedly found to have been used as spawning habitat by Brown Trout (*Salmo trutta*), inhibiting their colonisation by invertebrate fauna and the collection of meaningful data.

Freeze-coring was trialled during the pilot study as an alternative method of sampling the hyporheic habitat. At each site, a pointed copper pipe (3 centimetre internal diameter) was driven vertically into the stream bed to a minimum depth of 30 centimetres and left *in situ* for approximately 72-hours to allow for recolonisation (Olsen and Townsend, 2005; Varricchione et al., 2005). During this resting period, the pipes were capped using a balloon to inhibit the ingress of rainwater. Prior to sampling, a metal baffle was placed around the pipe to inhibit the flow of warming surface water around the core. Approximately two litres of liquid nitrogen were funnelled into each pipe over a period of approximately ten minutes, causing the surrounding sediment to freeze and adhere to the pipe, forming a sediment core (Figure 2.9; Scarsbrook and Halliday, 2002). The cores were removed using a tripod and hand winch before thawing the resulting core over a tray split into 10-centimeter fractions. To provide statistically robust samples, cores were taken from three locations at sites eight and ten; however, as this method failed to capture a representative assemblage of fauna at all horizons when compared with other methods, it was not selected for continuation. The sediment collected using this method was used to describe the substratum (Section 2.3.2.5).

Figure 2.9 Photographs of freeze core sampling (left, site 8) and thawing core (right)

2.3.1.3 Phreatic Invertebrates

Inertial pumping and phreatic netting were trialled as methods for sampling invertebrates in this environment. Inertial pump samples were collected using a

Power Pump II (Waterra Pumps Limited) following the methods outlined by Hancock and Boulton (2009). This method was trialled during the pilot study but discontinued as it was found to be ineffective in the sampling of wells with wide apertures as the pumping mechanism was not able to draw water. Conversely, the netting method, in which a weighted net (Institut Für Grundwasser Ökologie, 63-µm mesh) was lowered to the bottom of the borehole, raised and lowered ten times, was found to successfully capture fauna equally at all sites (Figure 2.10; Malard et al., 2002).

Figure 2.10 The phreatic net (left), small (middle, site G) and large (right; site E) aperture bores

Previous studies have found the inertial pump and phreatic net to have comparable sampling efficiencies, and as such, there is high confidence in the selection of the netting method (Allford et al., 2008; Dole-Olivier et al., 2009; Dumas and Fontanini, 2001; Hancock and Boulton, 2009).

2.3.1.4 Invertebrate Sample Processing

Following collection, invertebrate samples were processed, identified and enumerated. Samples were preserved using industrial methylated spirits or ethanol (with the exception of the Acari which were preserved in Koenikes Fluid) as appropriate (Bartsch et al., 2007). Samples were wet-sieved to a fraction of 63 µm and sorted under a Leica MZ75 microscope. Identification was undertaken using a Leica MZ75 or DMLB microscope and standard Freshwater Biological Association keys and specialist texts. Wherever possible, invertebrates were identified to species level; however, some early instar larvae, damaged specimen and taxonomically demanding groups (Diptera) were left at higher levels of taxonomic resolution. Taxa were verified by specialists at the Environment Agency (South East Region Analysis and Reporting Teams), Roehampton University (Peter Shaw), the Freshwater Biological Association (Terry Gledhill), Freshwater Life (Lee Knight) and APEM (Michael Dobson). All

records of stygofauna were supplied to the Hypogean Crustacea Recording Scheme following verification. One species, *Gammarus* sp. could not be identified morphologically and was assessed molecularly (Chapter 6).

2.3.1.6 Phreatic Microbiology

In addition to invertebrate samples, a representation of the microbiological community was also collected from phreatic water samples to provide a reflection of biofilm growth, an invertebrate food source, at each site. The microbiological community was assessed using Biolog EcoPlates, a method which measures microbial community functionality through the utilization of different carbon sources. Each EcoPlate consists of 96 wells, comprising three sets of 31 different carbon substrates (polymers, carbohydrates, carboxylic acids, amino acids, amines and phenolic compounds) and 1 control well with no substrate (Table 2.3).

Limited resources allowed for the analysis of microbiological samples from only two site visits, in March and September 2012. Each EcoPlate well was inoculated with 150 µl of phreatic sample and incubated in the dark for six days at 20^{o}C to allow the bacterial flora to respire and reduce the tetrazolium dye (which is not metabolized by fungi) within each well to a purple formazan. The development of colour over time was measured spectrophotometrically as absorbance at 595 nm using a BioLog OmniLog microplate reader at the University of Surrey (Janniche et al., 2012; Lee et al., 2010). Contamination was assessed using a sterile control plate as a blank which was clear for all wells during both spring and autumn.

Table 2.3 Table of one-third of one EcoPlate (the 32-wells comprising the analysis for one sample)

Water (no substrate)	β-Methyl-D-Glucoside	D-Galactonic Acid γ-Lactone	L-Arginine
Pyruvic Acid Methyl Ester	D-Xylose	D-Galacturonic Acid	L-Asparagine
Tween 40	i-Erythritol	2-Hydroxy Benzoic Acid	L-Phenylalanine
Tween 80	D-Mannitol	4-Hydroxy Benzoic Acid	L-Serine
α-Cyclodextrin	N-Acetyl-D-Glucosamine	γ-Hydroxybutyric Acid	L-Threonine
Glycogen	D-Glucosaminic Acid	Itaconic Acid	Glycly-L-Glutamic Acid
D-Cellobiose	Glucose-1-Phosphate	α-Ketobutyric Acid	Phenylethylamine
α-D-Lactose	D,L-α-Glycerol Phosphate	D-Malic Acid	Putrescine

2.3.2 Sampling of Environmental Parameters

The literature reviewed in Chapter 1 informed the selection of chemical and physical parameters likely to influence the benthic, hyporehic and phreatic communities or indirectly provide information on the exchange of flows between surface and groundwater. The selected variables exceed those recommended by the PASCALIS project as they include parameters specific to the Chalk catchment, such as Strontium, which may be used as a signature of groundwater influence (Malard et al. 2002).

2.3.2.1 Physiochemical Parameters

Physicochemical parameters, including temperature, pH, conductivity, dissolved oxygen, total alkalinity and turbidity were measured in the field. All values were recorded as the mean of three measurements for each parameter. Temperature, pH, conductivity and dissolved oxygen were measured *in situ* at riverine sites and in the bailer at phreatic sites using interchangeable probes attached to a Hach HQ Series Portable Meter. Dissolved oxygen was not measured in riverine hyporheic samples as the results would have been unduly influenced by the (pump) sampling method. Total alkalinity was determined at each site using an acidimetric titration Hach test-kit in which a few drops of phenolphthalein were added to 50 millilitres of water (turning it to a blue-green colour) before a titrant, H_2SO_4, was gradually added until the end point (a change of colour to violet) was reached. Total alkalinity ($CaCO_3$) was calculated by multiplying the number of drops required by the sample volume, accounting for the molarity of the acid. Turbidity was measured using a Thermo Orion Turbidity Meter, which passed light of known wavelength through the sample of water to assess the quantity of suspended particulates. However, turbidity was not measured for hyporheic or phreatic samples as the results would have been unduly influenced by the sampling procedure.

2.3.2.2 Chemical Parameters

Water samples were collected from the benthic, hyporheic and phreatic horizons to assess nutrient concentrations and geochemical composition throughout the final year of the study. All samples were processed using Whatman GF/C Glass Microfibre Filters before being frozen until analyses could be completed in the UCL laboratories. Assessment of contamination was

undertaken for samples collected in the field as well as the laboratory using blanks. Nutrient analyses for Phosphate (PO_4^{3-}) and Nitrate (NO_3^-) were undertaken in the using a Hach Lange DR2800 spectrophotometer. Phosphate was measured according to the ascorbic acid method while Nitrate was measured according to the cadmium reduction method. Geochemical analysis was undertaken using acid-washed vials, centrifuged and fixed with 0.1M Nitric acid prior to being analysed for Calcium, Magnesium, Sodium, Potassium and Strontium using a Varian 720-ES Inductively Coupled Plasma Optical Emission Spectrometer. Iron was initially included as part of the geochemical analysis; however, initial results were found to be biased by sampling method (specifically collection using the steel Bou-Rouch pump).

2.3.2.3 Hydrology and Hydrogeology

To assess the influence of hydrology on riverine invertebrate assemblages, wetted perimeter, depth, flow velocity and discharge were determined for each site at the time of sample collection. Discharge (Q) was calculated by multiplying the mean velocity of the water (V; measured at a number of locations as determined by width across the channel using a Valeport impeller flow meter (BFM 002 S-N 1855) positioned at 0.6 of the water depth from the surface of the water) by the area of the channel (A; calculated by multiplying the wetted width by mean depth) after Fetter (2001). The calculated discharges were contextualised using long-term continuous hydrological data provided by the Environment Agency (from the Littlebourne Ultrasonic Gauge on the Little Stour at NGR TR 21120 57510 and from the Crabble Mill v-notch weir on the Dour at NGR TR 30043 43019) and metrological data from the Met Office (Manston weather station 51° 35' N, 1° 34'E; 49m above mean sea level). Hydrogeological conditions over the study period were assessed using water level data (mAOD) collected by the Environment Agency as part of their routine groundwater level monitoring programme at each of the phreatic sites. These data were used to contextualise the conditions of the study period and calculate deviation from the long-term average for further analysis (LTA).

2.3.2.4 Vertical Hydraulic Gradient

Vertical hydraulic gradient (VHG) was calculated for two of the riverine sites (10 and 11) using minipiezometers to provide an indication of hyporheic exchange

flows during the final year of the study. Minipiezometers (polyvinyl chloride pipes measuring 70cm in length) which were installed to a depth of approximately 30 centimetres using a steel T-Bar and were left *in situ*, the depth of the water in each minipiezometer was measured on each sampling occasion using a tape measure to determine hydraulic head and calculate VHG following the methods described by Dahm and Valett (1996; in which a negative VHG reflects an area of downwelling surface water and a positive VHG reflects upwelling groundwater; Figure 2.11).

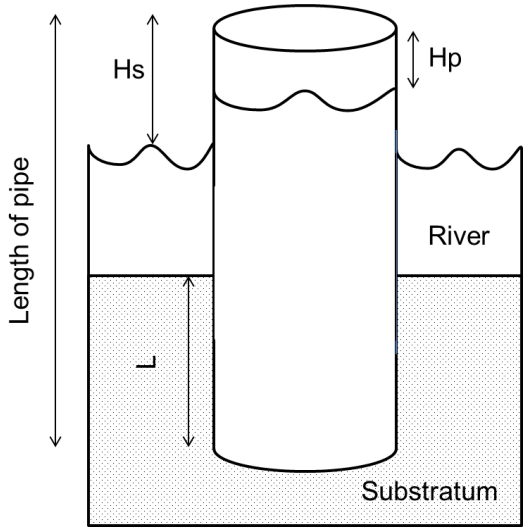


Figure 2.11 Variables used to calculate VHG at an upwelling site in which Hs is the distance from the top of the piezometer to the river surface; Hp is the distance from top of the piezometer to the surface of the water within the piezometer; L is the depth of the pipe in the substratum and VHG = (Hs-Hp)/L (Dahm and Valett, 1996).

2.3.2.5 Substrate Characteristics

The composition of the substratum is an important facet of habitat quality for aquatic organisms in riverine habitats (Boulton, 2007). Sediment samples were collected as a by-product of freeze coring and divided into four horizons: 0-10 centimetres; 10-20 centimetres; 20-30 centimetres; and >30 centimetres on site by thawing the sample over a splitting box prior to the removal of all macroinvertebrates (with the exception of empty Trichoptera cases and mollusc shells). The samples were returned to the UCL laboratory where they were sieved in a nested Wentworth column using apertures of 4.0 millimetres; 2.0 millimetres; 63 micrometres; and 38 micrometres to determine grain size after Bunte and Abt (2001; Figure 2.12). The results were used to describe the percentage of fine sediment by depth, calculate hydraulic conductivity (after Blaschke et al., 2003) and determine sortedness (after Fetter, 2001). In

addition, the percentage of organic content was determined for each horizon using a loss-on-ignition calculation in which a representative sub-sample was extracted, dried, weighed and then heated at 550°C before re-weighing, with the difference between weights being used as a representation of organic content

Figure 2.12 Sediment sample collected from a frozen core at site 8 following separation. From top left: cobbles (>4mm); gravel-pebbles (>2mm); sand (>63 µm); silt (>38 µm); clay (<38 µm).

2.3.3 Summary of Monitoring Data

Due to uncertainties in the sampling methods and site suitability, a long pilot study was required to establish fixed monitoring which could provide comparable results suitable for statistical analyses. The pilot study for riverine sampling extended from January 2009 to January 2010 during which time a number of methods and sites were trialled. Paired benthic and hyporheic sampling, including the collection of physiochemical information, was undertaken using fixed sampling methods from March 2010 until September 2012. The pilot study for phreatic sampling was undertaken in September 2011 to trial different sites and methods. Monitoring was undertaken routinely at seven sites in this habitat from November 2011 to September 2012 (only twice for microbiological samples). Chemical sampling was limited to the final year of this study for all habitats due to resource constraints. The results of this monitoring programme are presented in Chapters 3, 4 and 5 with further analysis and discussion in subsequent chapters.

2.3.4 Statistical Approach

The data collected during this study have been analysed to test the suitability of the study hypothesis using the approach outlined by Zuur et al. (2010) in which: (i) the data were explored using basic visualisation techniques such as box

plots to identify outliers; (ii) variability in the results was assessed (using an analysis of variance); (iii) normal distribution was tested and used to determine the selection of further multivariate techniques; (iv) large numbers of null values were approached using specialised techniques such as (zero-inflated) Generalised Linear Models; (v) collinearity (correlation) between covariates was identified; (vi) relationships between response variables and individual covariates were explored; (vii) interactions were considered; and (viii) observations of the response variables were considered for their independence. This approach was selected as it facilitates the analysis of vastly different data sets using standard metrics (such as Alpha (α-) diversity) across the three habitats (Magurran and McGill, 2011). All analyses were undertaken using R Statistical Software (version 2.15.0) and the Vegan Package (version 2.0-10) unless otherwise noted.

Chapter 3

Environmental conditions across the benthic, hyporheic and phreatic habitats

3.1 Introduction

This chapter describes the physiochemical (Section 3.2), chemical (Section 3.3) and physical (Section 3.4) environmental conditions recorded in the benthic, hyporheic and phreatic habitats during this study, considering the results both independently and collectively (Section 3.5). The results have been used to support the aims of this study: (1) to describe the distribution of fauna between the three habitats and assess the influence of environmental variables on these communities; and (2) describe the response of the biological communities to environmental change (Chapters 4-5).

3.2 Physiochemical Environmental Conditions

Physicochemical parameters including temperature, pH, conductivity, dissolved oxygen, alkalinity and turbidity were measured in paired benthic and hyporheic samples at the five riverine sites on the Little Stour (1, 9, 10 and 11) and Dour (site 13) as well as from the seven phreatic sites in the wider catchment (A-G). Benthic and hyporheic samples were collected bimonthly from March 2010 to September 2012 (from September 2010 for sites 11 and 13; no samples were collected in November 2010 from any site; Site 1 was dry in November 2011) and phreatic samples were collected from November 2011 to September 2012). Samples were collected after the methods discussed in Section 2.3.2.1 and the results are reported as the mean replicate value unless otherwise noted. The results are used to assess the spatiotemporal variability in physiochemical conditions within each habitat and between habitats.

3.2.1 Temperature

Water temperature was recorded across the three habitats on each sampling occasion. Benthic temperatures ranged from 6.5 to 17.1 °C and reflect an association with air temperatures, with the lowest values recorded during the winter months and highest values during late summer and early autumn (Site 11, January 2011 and September 2012 respectively; Figures 3.1 and 3.4). Although shading varied markedly between sites, spatial differences were not significant; however, temporal variability was significant and reflects a seasonal pattern ($F=11.38$, $p=0.001$; Tables 3.1 and 3.2). These results are consistent with long-term Environment Agency monitoring which suggests an average

surface water temperature on the Little Stour of 12.2 ºC (n=144 at Littlebourne, 2000-2011) and 10.9 ºC on the Dour (n=86 at Minnis, 2000-2007).

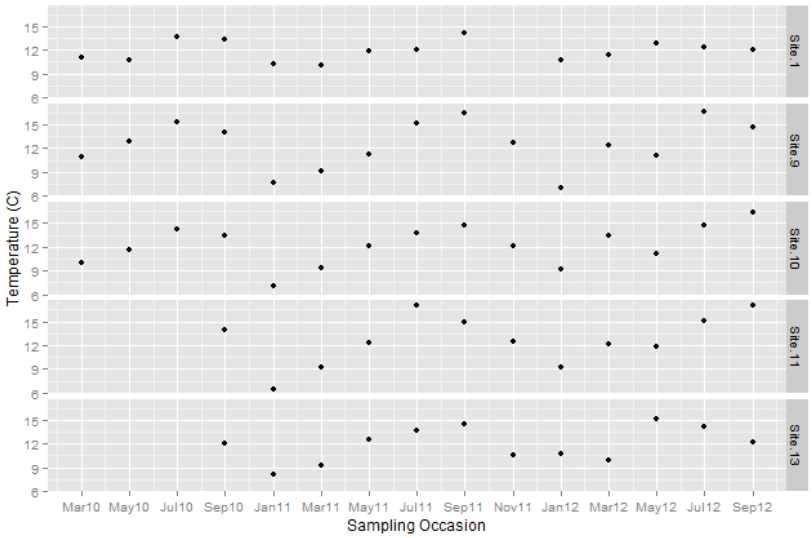


Figure 3.1 Benthic temperatures at the five riverine sites from March 2010 to September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

A similar, but more stable, seasonal pattern was observed in hyporheic temperatures which ranged from 8.0 to 16.0 °C (Site 9, January 2011 and September 2011 respectively; Figure 3.2). As with the benthic habitat, the results indicate significant temporal but not spatial variability, and reflect a seasonal pattern (F=10.06, p=0.001; Tables 3.1 and 3.2). Water temperature was significantly different between the benthic and hyporheic habitats (F=10.74, p=0.001).

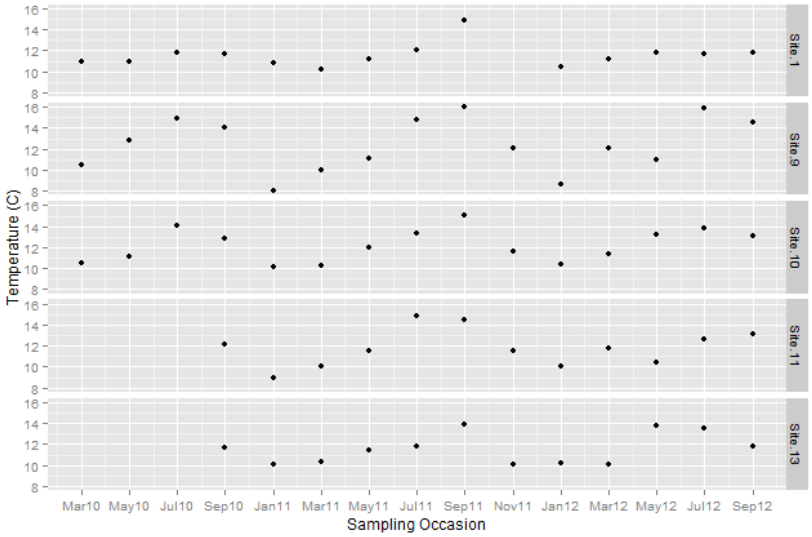


Figure 3.2 Hyporheic temperatures at the five riverine sites from March 2010 to September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

Although limited to a single year of data, phreatic temperatures also reflected a seasonal pattern with temperatures ranging from 11.0 to 18.2 ºC (site E, January 2012 and site G, July 2012, respectively; Figure 3.3). These results do not indicate significant spatial variability, but there are significant differences between sampling occasions (F=8.14, p=0.001; Tables 3.3 and 3.4). The temperatures recorded in the phreatic habitat were higher and more variable than anticipated (10-11ºC), and may reflect a high degree of hydrological exchange in this catchment (Bloomfield et al., 2013; Thulin and Hahn, 2008).

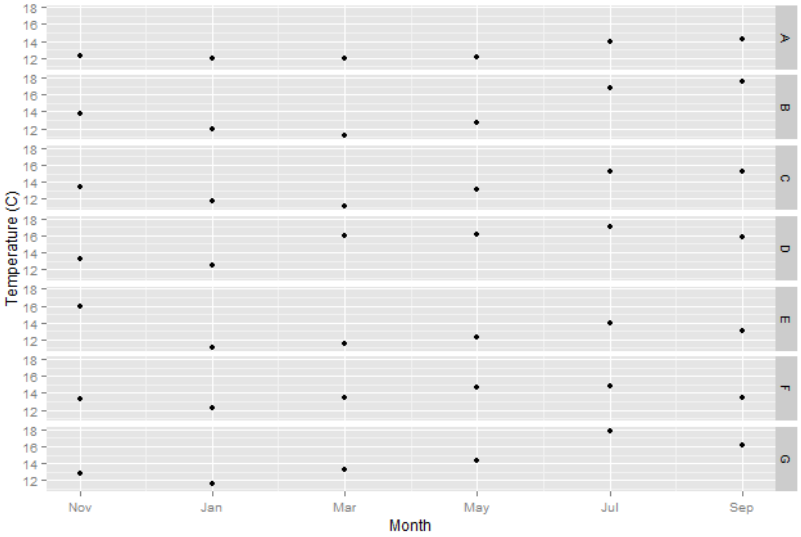


Figure 3.3 Temperatures at the seven phreatic sites from November 2011 to September 2012.

Benthic temperatures were more variable and closely associated with air temperature, with cooler values during the winter (0.1 to 1.7 °C) and warmer values during summer (0.1 to 1.6 °C). Although phreatic temperatures were only recorded during the final year, they suggest the greatest degree of thermal stability (±4.2 °C) between these habitats but also consistently the highest temperatures (Figure 3.4). Given the thermal stability of groundwater, it would be expected that the habitats with the greatest amount of groundwater influence record the least variability between temperatures and the results of this study support this assumption (Section 1.4). However, it should follow that habitats with greater groundwater influence would be warmer during the winter and cooler during the summer, as found in a similar three habitat comparative study by Hannah et al. (2009), but the results of this study only support this assumption for the hyporheic and benthic habitats, the phreatic temperatures do not. The reason for this difference in phreatic temperatures is unclear; however, it may have been confounded by the drought experienced during the final year

of the study which may have resulted in higher than expected temperatures as a result of decreased hydrogeological connectivity and increased residence time in the boreholes during this period (Section 3.4).

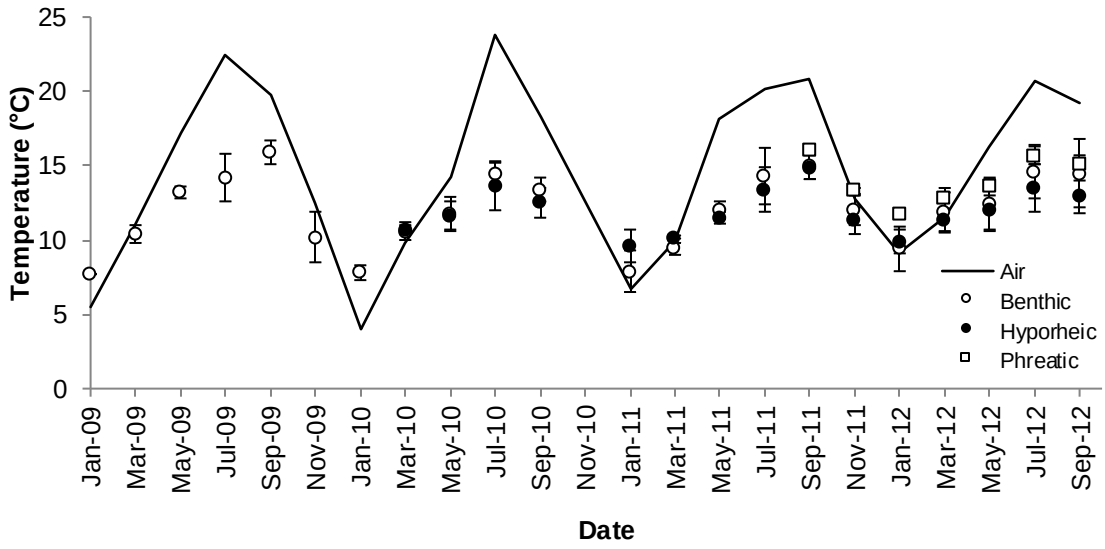


Figure 3.4 Mean monthly maximum daily air temperatures at the Manston Weather Station (Met Office, continuous line, annual average of 10.4 °C; n=45) and mean water temperature (±1 SE) measured in the benthic (n=69), hyporheic (n=69) and phreatic habitats (n=41).

These results indicate that each habitat provides distinctive thermal conditions which vary seasonally but not spatially, suggesting a degree of thermal stability throughout the study area. The benthic habitat provides the broadest thermal envelope and highest variability while the phreatic habitat provides the most restricted thermal envelope and least variability.

3.2.2 pH

Sample pH was recorded on every sampling occasion in each of the three habitats. Benthic pH values reflect a longitudinal spatial distribution, with the lowest levels recorded in the headwaters (6.2 at site 1 in January 2012), and increasing with distance downstream (maximum 8.1 at site 10 in March and September 2012; Figure 3.5). This spatial variability is significant ($F=15.1$, $p=0.001$); however it did not vary by sampling occasion (Table 3.1 and 3.2). Hyporheic pH values also varied spatially ($F=11.05$; $p=0.001$) along a similar gradient (ranging from 7.0 at site 9 in March 2010 to 7.8 at site 10 in July 2012) but not temporally (Figure 3.6; Tables 3.1 and 3.2). Although similar, the results indicate significant differences between the pH of the benthic and hyporheic samples ($F=13.48$; $p=0.001$), reflecting differing environmental conditions by habitat. However, despite the significant spatial differences in both the benthic

and hyporheic samples, pH was not found to be significantly different between the sites on the Little Stour and Dour.

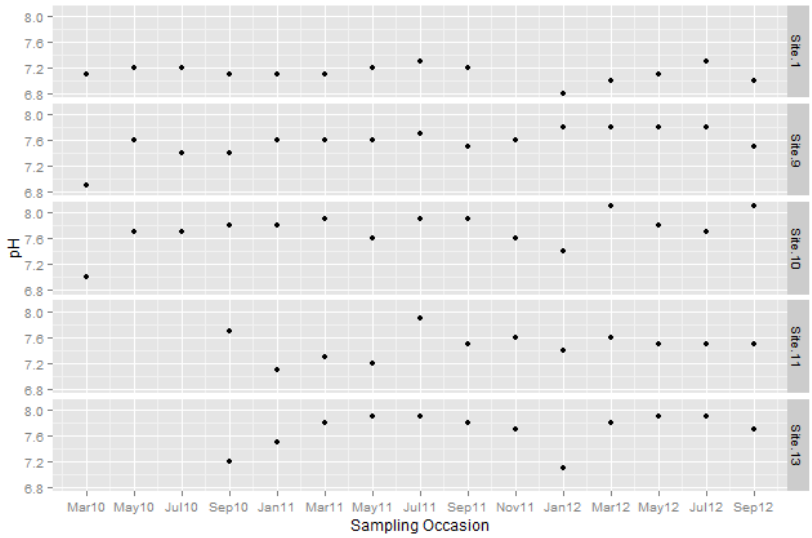


Figure 3.5 Benthic pH at the five riverine sites between March 2010 and September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

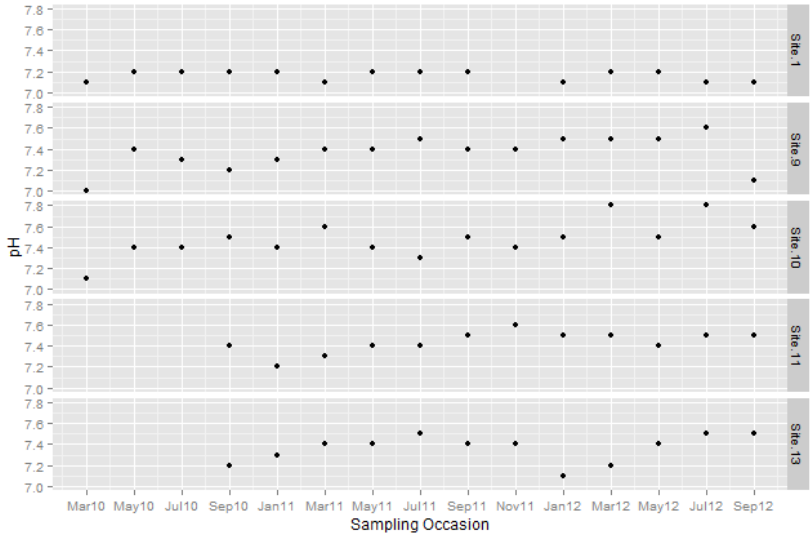


Figure 3.6 Hyporheic pH at the five riverine sites between March 2010 and September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

Within the phreatic habitat, pH varied spatially but not temporally ($F=7.88$; $p=0.001$), with the lowest values recorded at sites at the western edge of the catchment (6.7 at site A, January 2012), increasing towards the east (8.6 at site D, July 2012; Figure 3.7; Tables 3.3 and 3.4). Despite these differences, the pH values recorded during this study were circumneutral, as would be expected in a chalk stream environment (Berrie, 1992).

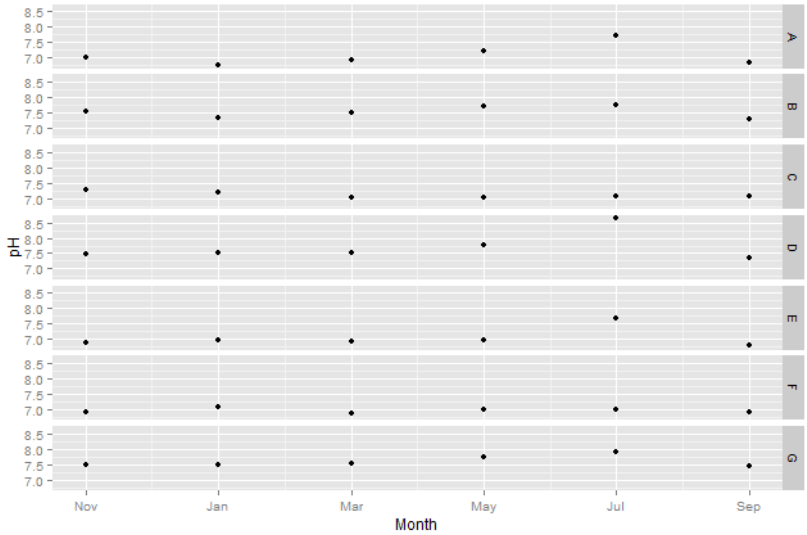


Figure 3.7. pH at the seven phreatic sites from November 2011 to September 2012

Values of pH were temporally stable and circumneutral across the study area, but differed spatially along a longitudinal gradient across the catchment and by habitat with the greatest stability recorded in the hyporheic habitat.

3.2.3 Conductivity

Conductivity was recorded on every sampling occasion across the three habitats. The values recorded in the benthic habitat reflected a longitudinal spatial distribution, with the highest values recorded in the headwaters and decreasing with distance downstream (747 µS cm^{-1}, site 1 January 2012; minimum 504 µS cm^{-1}, site 9 September 2010; Figure 3.8). These results vary spatially (F=13.4; p=0.001) but were temporally stable (Table 3.1 and 3.2).

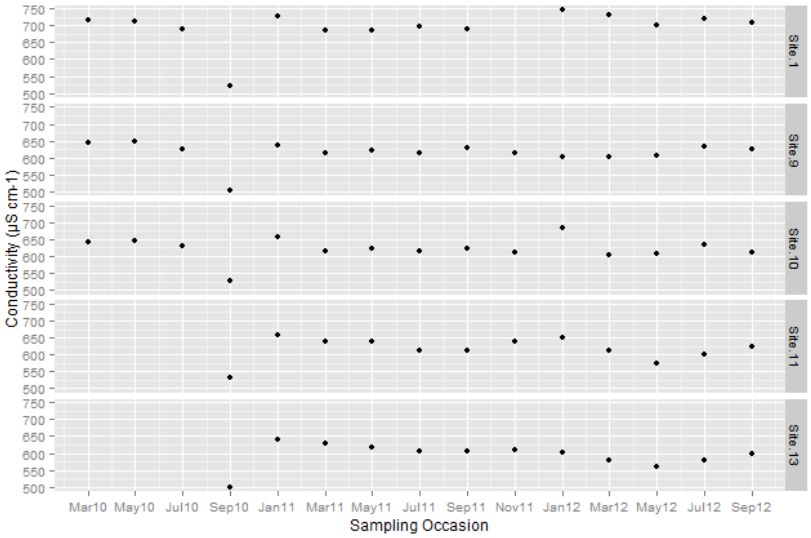


Figure 3.8 Benthic conductivity at the five riverine sites from March 2010 to September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

A similar spatial pattern was observed in the hyporheic results with the highest values recorded in the headwaters, decreasing with distance downstream (700 µS cm^{-1}, site 1 July 2011; though the lowest values were recorded at site 13, 508 µS cm^{-1}, September 2010; Figure 3.9). Hyporheic conductivity varied spatially (F=31.7, p=0.001) but not temporally, and was significantly higher at riverine sites on the Little Stour (sites 1, 9, 10 and 11) than the Dour (site 13) in both benthic and hyporheic habitats (F=3.23; p=0.001; Tables 3.1 and 3.2).

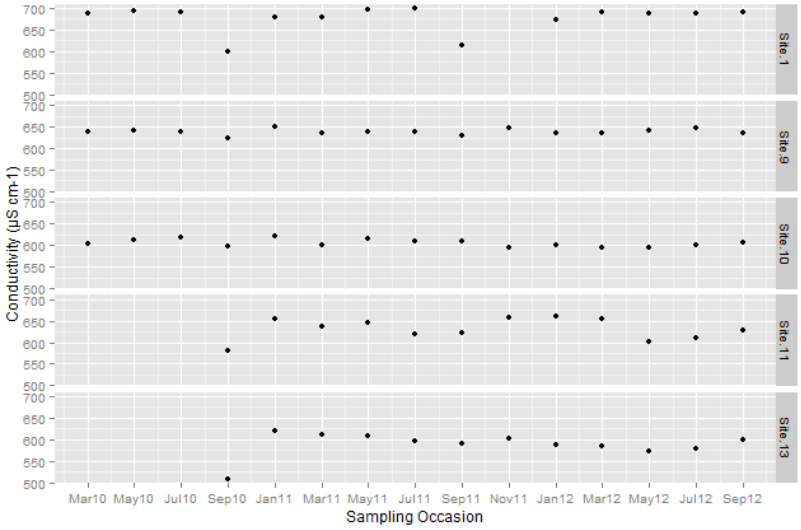


Figure 3.9 Hyporheic Conductivity at the five riverine sites from March 2010 to September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

The results from the phreatic habitat were spatially variable (F=16.77, p=0.001), distribution did not follow the gradient reflected in the riverine sites as values were highest at site C (at the downstream end of the catchment; maximum 694 µS cm^{-1}, November 2011) and lowest at site G (located just upstream of site C; minimum 458 µS cm^{-1}, July 2012; Figure 3.10; Tables 3.3 and 3.4).

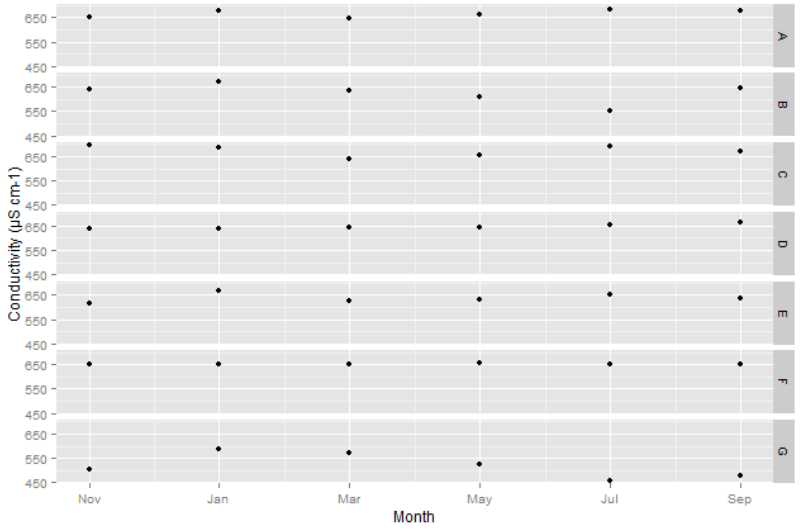


Figure 3.10 Phreatic conductivity at the seven sites from November 2011 to September 2012

Viewed collectively, the conductivity recorded during this study was relatively high for a riverine environment but not dissimilar to other studies in Chalk catchments, such as on the River Lambourn (England) where hyporheic values were found to exceed 540 µS cm^{-1} against a chalk groundwater baseline of nearly 600 µS cm^{-1} (Allen et al., 2010). Minimum, maximum and average readings from the benthic, hyporheic and phreatic habitats were very similar (630, 629 and 631 µS cm^{-1}, respectively), reflecting little difference in conductivity between habitats.

3.2.4 Dissolved Oxygen

Dissolved oxygen was recorded in the benthic and phreatic habitats on each sampling occasion (hyporheic measurements were not taken due to the undue influence of the sampling method). Benthic dissolved oxygen concentrations reflect a significant longitudinal spatial distribution ($F=7.78$; $p=0.001$) with the lowest concentrations recorded in the headwaters, increasing with distance downstream (site 1, 6.5 mg L^{-1}, September 2010; 14.3 mg L^{-1} site 9 in March 2012; Figure 3.11; Table 3.1). This variability is expected as lower dissolved oxygen is associated with the intermittency of the headwaters but, even allowing for this, the results suggest that all of these sites are well oxygenated and are consistent with long term Environment Agency monitoring near site 9 ($\bar{x}$=10.66 mg L^{-1}; n=109; 2000-2011; Feminella, 1996). Benthic dissolved oxygen concentrations also reflected a significant seasonal pattern ($F=2.50$; $p=0.008$) with the lowest concentrations recorded at the end of the hydrological year when discharge was lowest and highest concentrations at the beginning of the hydrological year when discharge was highest (Table 3.2).

Dissolved oxygen concentrations were markedly lower in phreatic habitats ranging from 1.54 mg L^{-1} (site C, September 2012) to 9.86 mg L^{-1} (site D, July 2012; Figure 3.12). While these concentrations also varied significantly by site ($F=14.41$; $p=0.001$), there is no apparent pattern to their distribution within the catchment, suggesting site-specific influences (Table 3.3). Unlike the benthic habitat, there are not significant differences between sampling occasions, suggesting greater stability (Table 3.4). While this may be due in part to the limitations of single year of phreatic sampling, it is notable that this coincided with a period of drought, when dissolved oxygen would be expected to be

depleted, especially if hydrogeological connectivity were reduced (Section 3.4). These results suggest that the phreatic sites considered in this study are comparatively well oxygenated as similar studies that have focussed on this habitat recorded many concentrations of less than 1.0 mg L^{-1}, a concentration suggested as a minimum threshold for groundwater invertebrate communities (Hahn, 2006). Collectively this suggests that these sites are well oxygenated but that dissolved oxygen differs between the benthic and phreatic habitats.

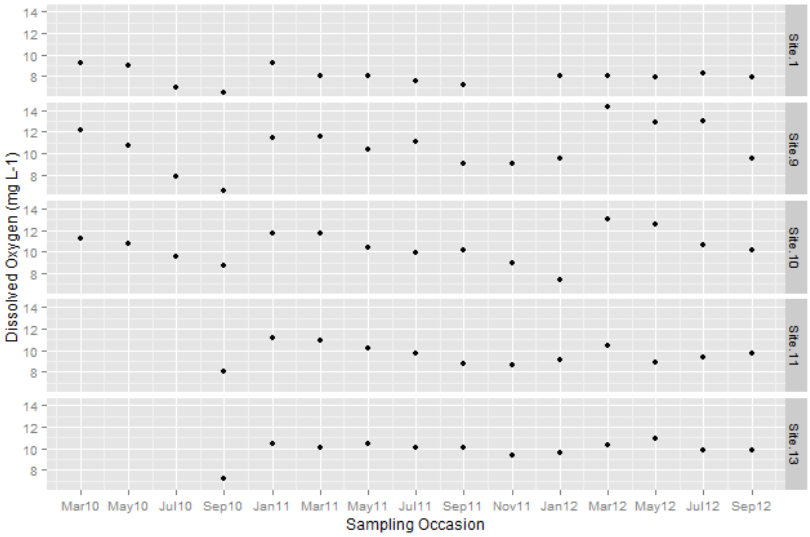


Figure 3.11 Dissolved oxygen at the five benthic sites between March 2010 and September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

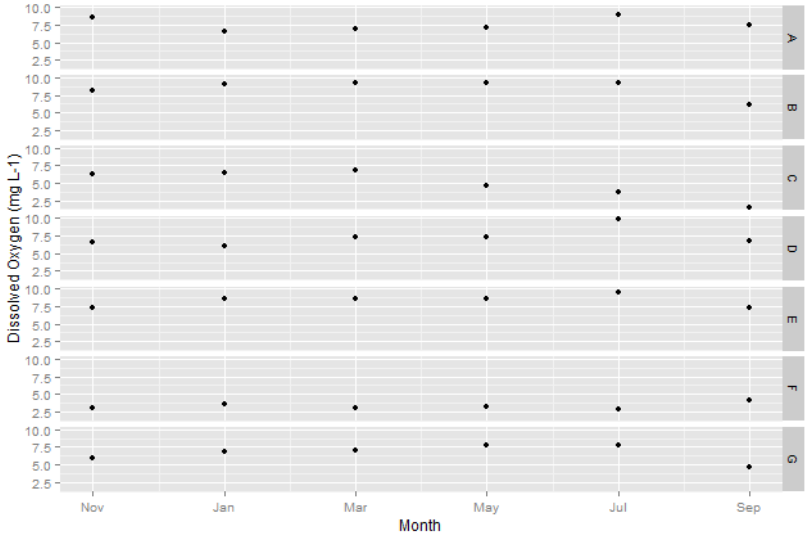


Figure 3.12 Dissolved oxygen at seven phreatic sites from November 2011 to September 2012

3.2.5 Alkalinity

Alkalinity was recorded across the three habitats on each sampling occasion. Benthic concentrations ranged from 174 to 382 mgL^{-1} and, with an average of 254 mgL^{-1} (n=69), are consistent with similar long-term Environment Agency

monitoring near site 9 ($\bar{x}$=240 mg L^{-1}; n=108; 2000-2011; site 10 in September 2012 and site 1 in January 2011, respectively; Figure 3.13).

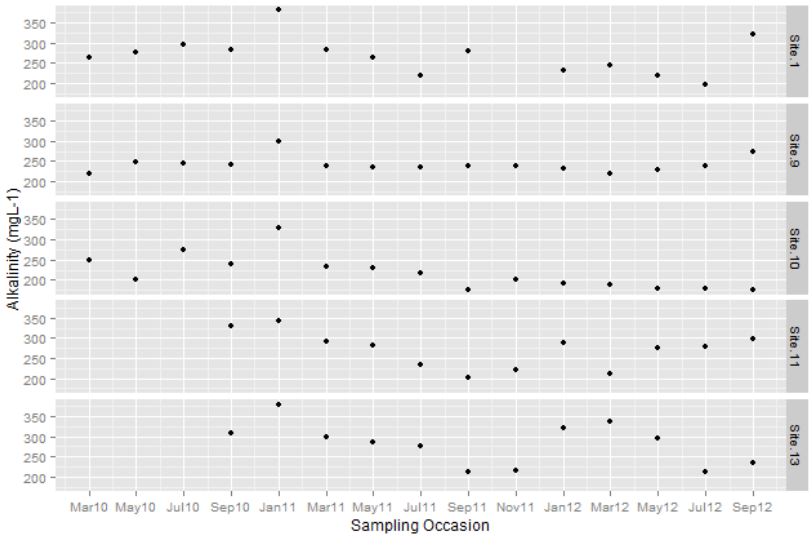


Figure 3.13 Benthic alkalinity at the five riverine sites from March 2010 to September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

Hyporheic concentrations were similar and ranged from 164 to 338 mg L^{-1} (average of 258 mg L^{-1}; n=69; site 10, September 2011 and site 11 in January 2011, respectively; Figure 3.14). In both habitats, alkalinity varied spatially (F=5.06, p=0.001; F=12.74, p=0.001, respectively) and reflected a longitudinal pattern, with higher concentrations in the headwaters, decreasing with distance downstream (Table 3.1). Benthic and hyporheic concentrations also varied temporally (F=3.07, p=0.001, F=2.02, p=0.03, respectively), suggesting a seasonal pattern in which concentrations are highest during the autumn and winter, decreasing during the spring and summer (Table 3.2).

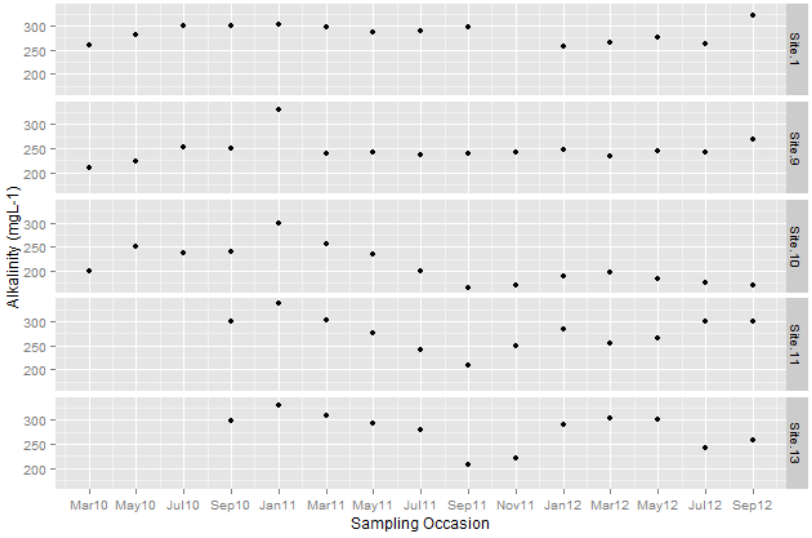


Figure 3.14 Hyporheic alkalinity at the five riverine sites from March 2010 to September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

Alkalinity concentrations were slightly lower in phreatic habitats than in the riverine environment, ranging from 89 to 332 mgL^{-1} (site C in May 2012 and site B in September 2012, respectively), with an average of 204 mgL^{-1} (n=41; Figure 3.15). Phreatic alkalinity concentrations varied spatially, with those recorded at site C being markedly lower than the others (F=5.21; p=0.001; Table 3.3); however, temporal variability was insignificant (Table 3.4). These results are unexpected as the high alkalinity of surface waters in chalk streams is thought to be influenced by their carbonate geology and it should follow that the alkalinity of groundwater would be higher, this is made more surprising by the seasonal pattern in the distribution of alkalinity patterns in the benthic and hyporheic habitats (EPA, 1997). The reason for this is unclear, particularly as it does not follow the same pattern as pH, but may be confounded by the drought conditions during the final year of the study which recorded elevated temperatures or an undue influence from surface conditions, such as rainwater infiltration, at specific sites (Section 3.4; Darling et al., 2012).

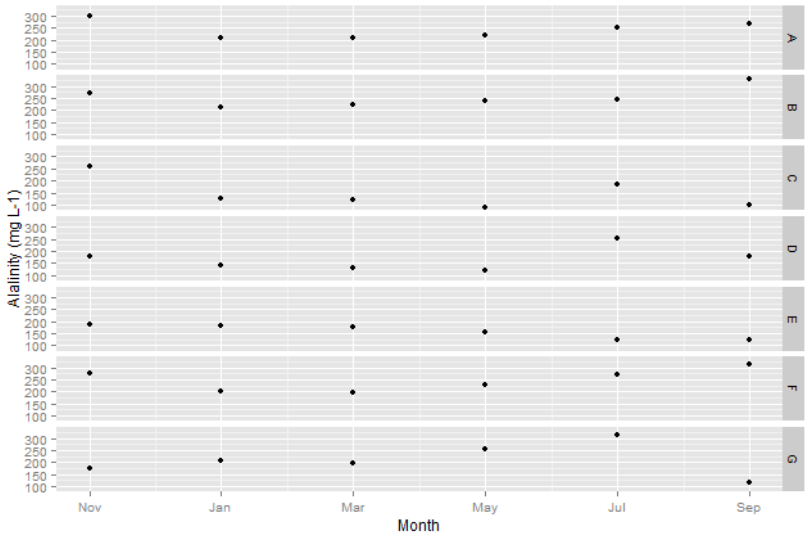


Figure 3.15 Alkalinity at the seven phreatic sites from November 2011 to September 2012

3.2.6 Turbidity

Turbidity was only measured in benthic habitats as the hyporheic and phreatic sampling methods precluded the collection of representative samples. The results ranged from 0.02 to 5.4 NTU (site 1 in September 2011 and site 13 in September 2012, respectively) with an average of 1.57 NTU (n=69). Although the spatial and temporal variance between these records is not significant, values were generally higher in the headwaters, particularly during periods of lower discharge (Tables 3.3 and 3.4). The average results are consistent with

what would be expected for a chalk stream (<3.00 NTU) and suggest good water clarity (Feminella, 1996).

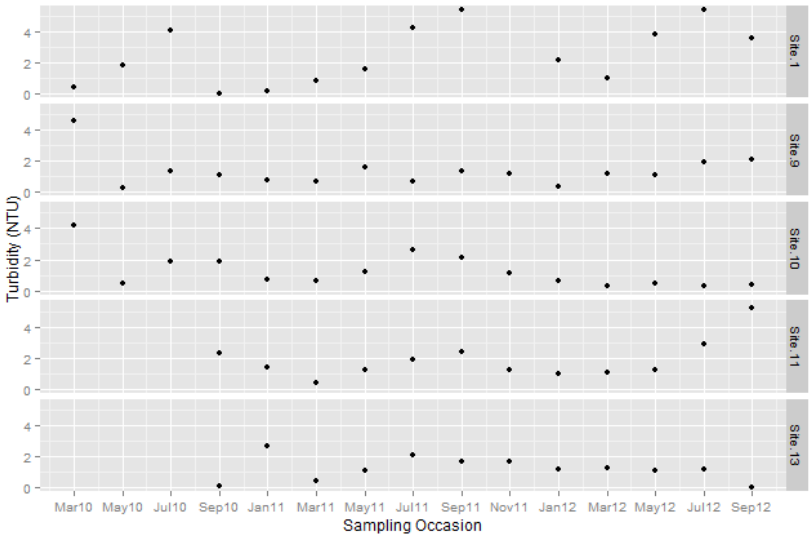


Figure 3.16 Benthic turbidity at the five riverine sites from March 2010 to September 2012. Sampling began at sites 11 and 13 in September 2010. Site 1 was dry in November 2011.

3.2.7 Summary of physiochemical conditions

The physiochemical results are characteristic of a chalk stream environment and are consistent with long-term monitoring in this catchment. Spatially, the results reflect a high degree of variability for alkalinity, conductivity, pH and dissolved oxygen which, in the benthic and hyporheic habitats follows a clear longitudinal gradient from the headwaters to the downstream sites, suggesting that the location of a site within the catchment strongly influences its environmental conditions. Temporally, strong seasonal variability was recorded in the benthic and hyporheic habitats for temperature, dissolved oxygen and alkalinity but not the phreatic habitat, suggesting greater (expected) stability in the aquifer. Collectively, the results indicate physiochemical diversity between sites, sampling occasions and depths, with each habitat providing specific conditions which differ their range and stability of temperature, pH, alkalinity and dissolved oxygen.

Table 3.1 Mean benthic (Ben) and hyporheic (Hyp; Bou-Rouche) physio-chemical results by site (±SE; March 2010-September 2012; n=69 and n=69) in which change has been analysed using a one-way ANOVA with * and ** indicating significance of $p<0.05$ and $p<0.001$ (respectively) and ns $p>0.05$.

Parameter	Habitat	1	9	10	11	13	Spatial Change
Temperature (°C)	Ben	11.9 (±3.7)	12.5 (±0.8)	12.2 (±0.7)	12.7 (±0.9)	11.9 (±0.6)	ns
	Hyp	11.5 (±0.3)	12.4 (±0.7)	12.2 (±0.4)	11.8 (±0.5)	11.6 (±0.4)	ns
pH	Ben	7.1 (±0.0)	7.6 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	7.7 (±0.1)	**
	Hyp	7.2 (±0.0)	7.4 (±0.0)	7.5 (±0.1)	7.4 (±0.0)	7.4 (±0.0)	**
Conductivity (µS cm^{-1})	Ben	696 (±14)	615 (±10)	622 (±9)	617 (±10)	593 (±11)	**
	Hyp	676 (±8)	639 (±2)	606 (±2)	631 (±7)	589 (±8)	**
DO (mg L^{-1})	Ben	8.0 (±0.2)	10.6 (±0.5)	10.4 (±0.4)	9.6 (±0.3)	9.8 (±0.3)	**
Alkalinity (mg L^{-1})	Ben	268 (±12)	243 (±5)	217 (±11)	271 (13)	281 (±16)	**
	Hyp	286 (±5)	247 (±7)	212 (±10)	276 (±10)	277 (±11)	**
Turbidity	Ben	2.5 (±0.5)	1.4 (±0.3)	1.3 (±0.3)	1.9 (±0.4)	1.2 (±0.2)	ns

Table 3.2 Mean benthic (Ben) and hyporheic (Hyp; Bou-Rouche) physio-chemical results by sampling occasion (±SE; March 2010-September 2012; n=69 and n=69) in which change has been analysed using a one-way ANOVA with * and ** indicating $p<0.05$ and $p<0.001$ (respectively) and ns $p>0.05$.

Para-meter	Hab-itat	Mar 10	May 10	Jul 10	Sep 10	Jan 11	Mar 11	May 11	Jul 11	Sep 11	Nov 11	Jan 12	Mar 12	May 12	Jul 12	Sep 12	Temporal Change
Temp (°C)	Ben	10.6 (±0.3)	11.8 (±0.6)	14.4 (±0.5)	13.4 (±0.4)	7.9 (±0.6)	9.4 (±0.2)	14.3 (±0.8)	14.3 (±0.6)	15.0 (±0.4)	12.0 (0.5)	9.4 (±0.7)	11.9 (±0.6)	12.4 (±0.8)	14.6 (±0.7)	14.5 (±1.0)	**
	Hyp	10.7 (0.2)	11.6 (0.6)	13.6 (0.9)	12.5 (±0.4)	9.6 (±0.5)	10.2 (±0.1)	11.5 (±0.2)	13.4 (±0.7)	14.9 (±0.4)	11.3 (±0.4)	9.9 (±0.3)	11.3 (±0.3)	12.0 (±0.6)	13.5 (±0.7)	12.9 (±0.5)	**
pH	Ben	7.0 (±0.1)	7.5 (±0.1)	7.5 (±0.1)	7.4 (±0.1)	7.4 (±0.1)	7.5 (±0.2)	7.5 (±0.1)	7.7 (±0.1)	7.6 (±0.1)	7.6 (±0.0)	7.3 (±0.2)	7.7 (±0.2)	7.6 (±0.1)	7.6 (±0.1)	7.6 (±0.2)	ns
	Hyp	7.1 (±0.0)	7.3 (±0.1)	7.3 (±0.1)	7.3 (±0.1)	7.3 (±0.0)	7.4 (±0.1)	7.7 (±0.0)	7.4 (±0.1)	7.4 (±0.1)	7.5 (±0.1)	7.3 (±0.1)	7.4 (±0.1)	7.4 (±0.1)	7.5 (±0.1)	7.4 (±0.1)	ns
Cond (µS cm^{-1})	Ben	668 (±25)	668 (±22)	649 (±21)	518 (±7)	665 (±17)	637 (±13)	637 (±13)	630 (±17)	631 (±15)	619 (±7)	657 (±27)	626 (±27)	610 (±24)	633 (±24)	632 (±20)	**
	Hyp	645 (±24)	650 (±24)	649 (±22)	582 (±20)	644 (±11)	634 (±14)	641 (±16)	633 (±18)	613 (±6)	626 (±16)	626 (±17)	625 (±19)	617 (±20)	625 (±19)	633 (±16)	ns
DO (mg L^{-1})	Ben	10.9 (±0.9)	10.1 (±0.6)	8.1 (±0.8)	7.3 (±0.4)	10.5 (±0.7)	10.8 (±0.4)	9.9 (±0.5)	9.7 (±0.6)	9.0 (±0.5)	9.0 (±0.2)	8.7 (±0.4)	11.2 (±1.1)	10.6 (±1.0)	10.2 (±0.8)	9.4 (±0.4)	*
Alkalinity (mg L^{-1})	Ben	244 (±13)	242 (±22)	272 (±15)	280 (±18)	347 (±15)	268 (±14)	259 (±12)	237 (±11)	222 (±2.2)	219 (±8)	250 (±23)	240 (±26)	240 (±21)	221 (±17)	260 (±26)	**
	Hyp	224 (±18)	251 (±17)	264 (±19)	278 (±14)	320 (±8)	281 (±14)	266 (±12)	250 (±16)	223 (±22)	220 (±18)	254 (±18)	251 (±17)	254 (±20)	244 (±20)	264 (±26)	*
Turbidity (NTU)	Ben	3.1 (±1.4)	0.9 (±0.5)	2.5 (±0.8)	1.1 (±0.5)	1.2 (±0.4)	0.6 (±0.1)	1.3 (±0.1)	2.3 (±1.3)	1.6 (±0.7)	1.3 (±0.1)	1.1 (±0.3)	1.0 (±0.2)	1.5 (±0.9)	2.3 (±0.9)	2.3 (±1.0)	ns

Table 3.3 Mean phreatic physio-chemical results by site (±SE; 2011-2012; n=41) in which change has been analysed using a one-way ANOVA with * and ** indicating overall significance of $p<0.05$ and $p<0.001$ (respectively) and ns reflecting $p>0.05$.

Parameter	A	B	C	D	E	F	G	Spatial Change
Temperature (^oC)	13.2 (±4.2)	14.0 (±6.4)	13.3 (±4.2)	15.1 (±4.6)	13.0 (±4.9)	13.7 (±2.6)	14.3 (±6.3)	ns
pH	7.06 (±0.32)	7.52 (±0.19)	7.12 (±0.11)	7.71 (±0.48)	7.04 (±0.32)	6.97 (±0.08)	7.61 (±0.17)	**
Conductivity ($\mu S\ cm^{-1}$)	655 (±87)	627 (±123)	673 (±55)	647 (±28)	640 (±50)	651 (±5)	521 (±110)	**
DO ($mg\ L^{-1}$)	7.5 (±2.1)	8.6 (±3.2)	4.9 (±4.8)	7.3 (±3.7)	8.3 (±2.3)	3.3 (±1.1)	6.7 (±3.1)	**
Alkalinity ($mg\ L^{-1}$)	239 (±34)	253 (±44)	146 (±65)	166 (±49)	158 (±28)	248 (±47)	210 (±68)	**

Table 3.4 Mean phreatic physio-chemical results by sampling occasion (±SE; 2011-2012; n=41) in which change has been analysed using a one-way ANOVA with * and ** indicating overall significance of $p<0.05$ and $p<0.001$ (respectively) and ns reflecting $p>0.05$.

Parameter	November	January	March	May	July	September	Temporal Change
Temperature (^oC)	13.3 (±0.2)	11.8 (±0.2)	12.6 (±0.7)	13.6 (±0.6)	15.7 (±0.6)	15.1 (±0.6)	**
pH	7.3 (±0.1)	7.2 (±0.1)	7.2 (±0.1)	7.3 (±0.1)	7.7 (±0.2)	7.1 (±0.1)	ns
Conductivity ($\mu S\ cm^{-1}$)	636 (±23)	655 (±12)	630 (±10)	625 (±18)	620 (±85)	633 (±26)	ns
DO ($mg\ L^{-1}$)	6.5 (±0.8)	6.8 (±0.7)	7.0 (±0.8)	6.8 (±0.8)	7.4 (±1.1)	5.4 (±0.8)	ns
Alkalinity ($mg\ L^{-1}$)	243 (±22)	182 (±13)	179 (±15)	186 (±25)	235 (±23)	204 (±38)	ns

3.3 Chemical Environmental Conditions

Chemical parameters, including nutrients and geochemistry, were measured in the benthic and hyporheic habitats at the five riverine sites on the Little Stour (sites 1, 9, 10 and 11) and Dour (site 13) as well as at the seven phreatic sites in the wider aquifer (sites A-G) during the final year of the study (November 2011 to September 2012).[2] Analysis for pesticides and herbicides was not included in this study; however, monitoring undertaken on behalf of the Environment Agency suggests that the study area is of good quality with little pesticide or herbicide pollution (Entec, 2008). The results are used to describe the spatiotemporal variability in the chemical conditions of each habitat.

3.3.1 Nutrients

Nutrients, Nitrate (N) and Phosphate (P) were measured across the three habitats. The results indicate that nutrient levels within the study area are consistently lower than expected for a high alkalinity lowland river, suggesting that the sites considered are relatively unimpacted by nutrient pressures but that they are within the range expected to influence the distribution of stygofauna (Defra, 2014; Section 1.4).

In the benthic habitat, Nitrate concentrations ranged from 1.8 to 5.7 mg L^{-1} (site 13 in July 2012 and site 1 in January 2012, respectively) with an average concentration of 3.34 mg L^{-1} (n=29) and varied spatially, with the highest concentrations in the headwaters at site 1 and lowest concentration on the Dour (site 13; F=7.82; p=0.004), which may reflect subtle differences in land use between these catchments, especially as site 1 is located in a managed parkland (Figure 3.17; Table 3.5). Nitrate concentrations did not vary significantly by sampling occasion suggesting that the source of nitrate is unlikely to be from seasonal agricultural inputs (Table 3.6). Phosphate concentrations in the benthic habitat were low at all sites on all sampling occasions, averaging 0.16 mg L^{-1} (n=29), with the exception of an outlier at site 1 of 1.43 mg L^{-1} in July 2012, which is likely to reflect an isolated incident, potentially related to the management of the park surrounding this site (Figure 3.18). Phosphate concentrations did not significantly vary spatially or temporally (Tables 3.5 and 3.6).

[2] Blanks were assessed with no results above the level of detection for all parameters.

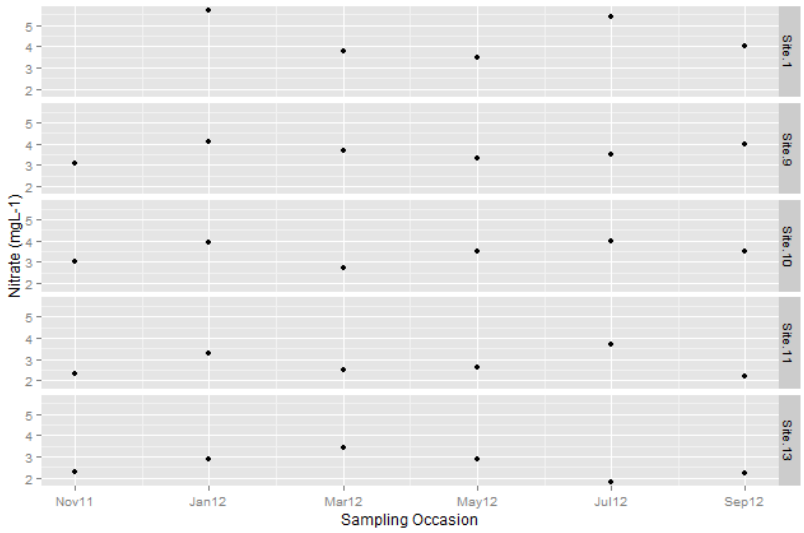


Figure 3.17 Benthic Nitrate at the five riverine sites between November 2011 and September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

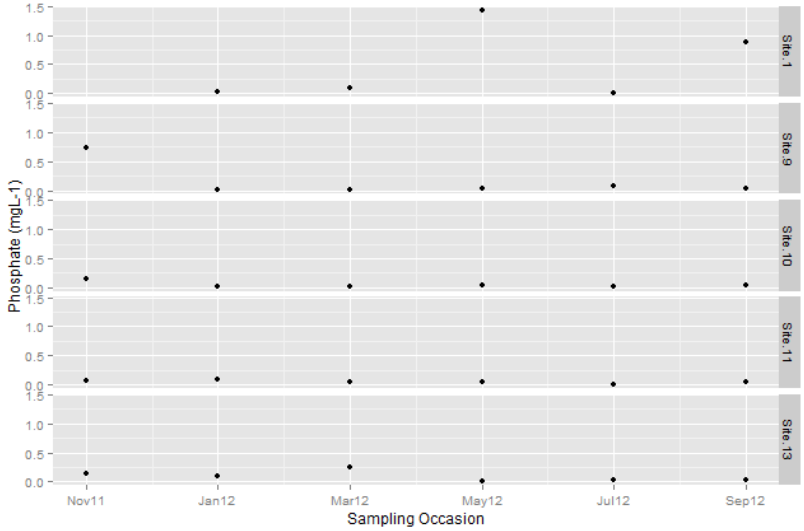


Figure 3.18 Benthic Phosphate at the five riverine sites between November 2011 and September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

Nitrate in the hyporheic habitat was slightly lower than in the benthic habitat with concentrations ranging from 0.6 to 4.7 mg L^{-1} ($\bar{x}$=3.02 mg L^{-1}; n=29) and Phosphate from 0.02 to 0.90 mg L^{-1} ($\bar{x}$=0.13 mg L^{-1}; n=29; Figures 3.19 and 3.20). Surprisingly, no significant spatial or temporal variance was found for Nitrate or Phosphate in the hyporheic habitat (Tables 3.5 and 3.6), nor between the benthic and hyporheic habitats.

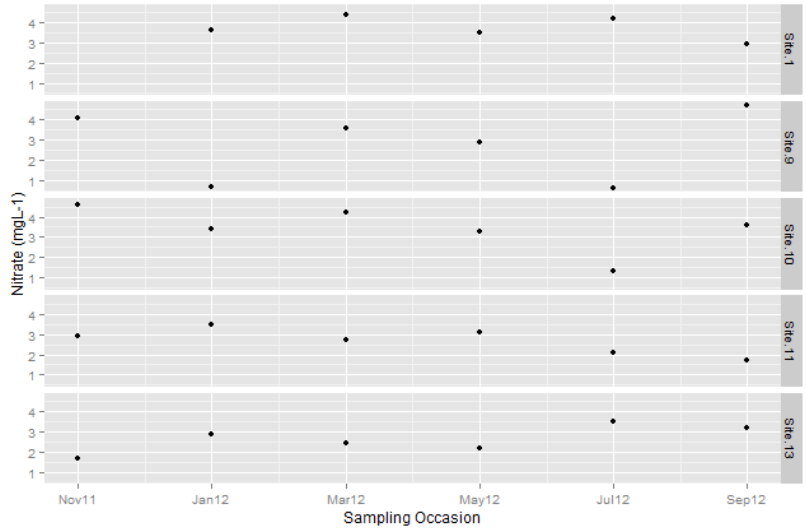


Figure 3.19 Hyporheic Nitrate at the five riverine sites between November 2011 and September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

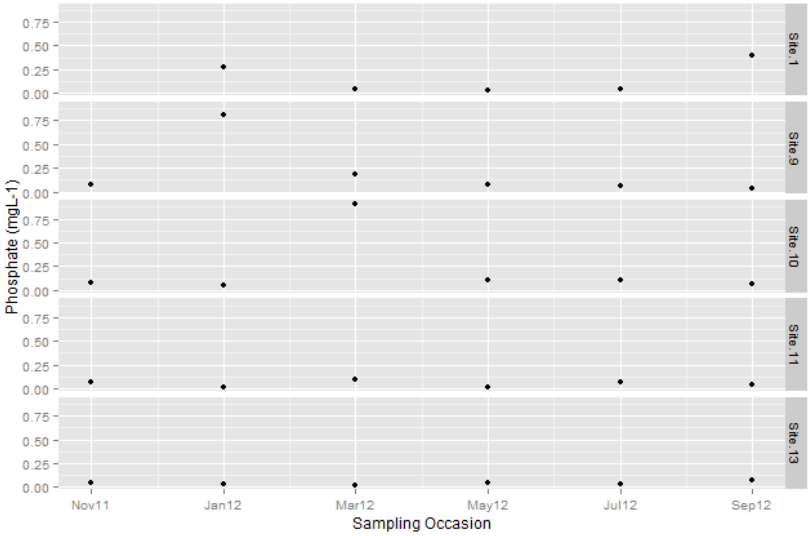


Figure 3.20 Hyporheic Phosphate at the five riverine sites between November 2011 and September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

Nutrient concentrations in the phreatic habitat were, surprisingly, slightly higher than in the surface waters. Nitrate ranged from 0.50 to 8.8 mg L^{-1} (site D, January 2012 and site B, November 2011, respectively) with an average of 3.05 mg L^{-1} (n=41; Figure 3.21). Phosphate ranged from 0.01 to 1.59 mg L^{-1} (with the maximum recorded at site 1 in May 2012). The average concentration for all sites was $\bar{x}$=0.29 mg L^{-1} (n=41; Figure 3.22). Both Nitrate and Phosphate varied spatially in the phreatic habitat but not temporally, suggesting site-based influences on the nutrients within this habitat (Tables 3.7 and 3.8).

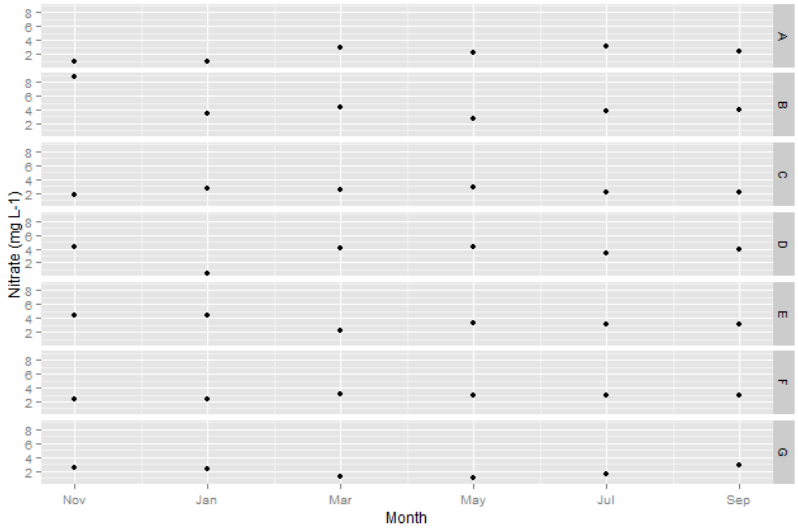


Figure 3.21 Nitrate in the seven phreatic sites from November 2011 to September 2012.

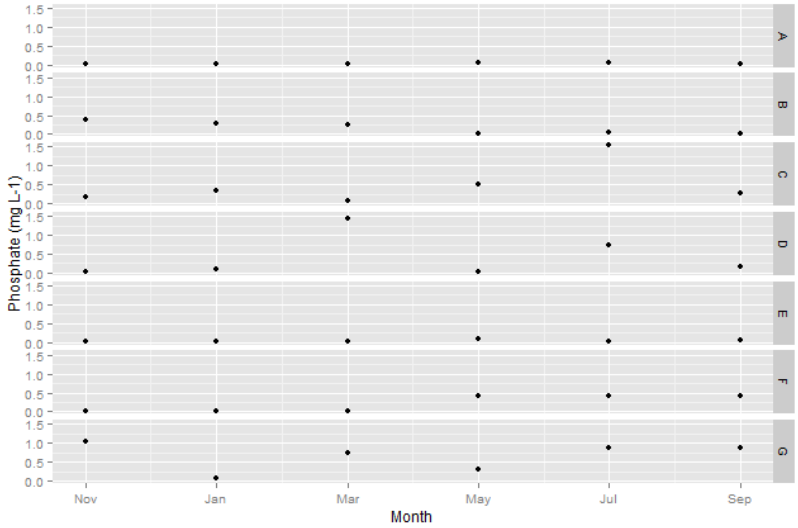


Figure 3.22 Phosphate in the seven phreatic sites from November 2011 to September 2012

3.3.2 Geochemistry

Geochemistry was described by measuring Calcium (Ca), Potassium (K), Magnesium (Mg), Sodium (Na) and Strontium (Sr) concentrations throughout the final year of the study across the three habitats.

3.3.2.1 Calcium

Calcium varied spatially but not temporally in the benthic, hyporheic and phreatic habitats ($F=4.47$, $p=0.008$; $F=3.74$, $p=0.02$; $F=6.47$, $p=0.001$, respectively; Tables 3.5-3.8).[3] Ranges (66-141 mg L^{-1}) and means ($\bar{x}$=108, 109 and 106 mg L^{-1}, respectively) were similar between all three habitats and were consistent with long term Environment Agency monitoring on the Stour Chalk Block ($\bar{x}$=112 mg L^{-1}, 1995-2008; Entec 2008).

[3] The level of detection for Calcium in this study is 0.0235 mg L^{-1}

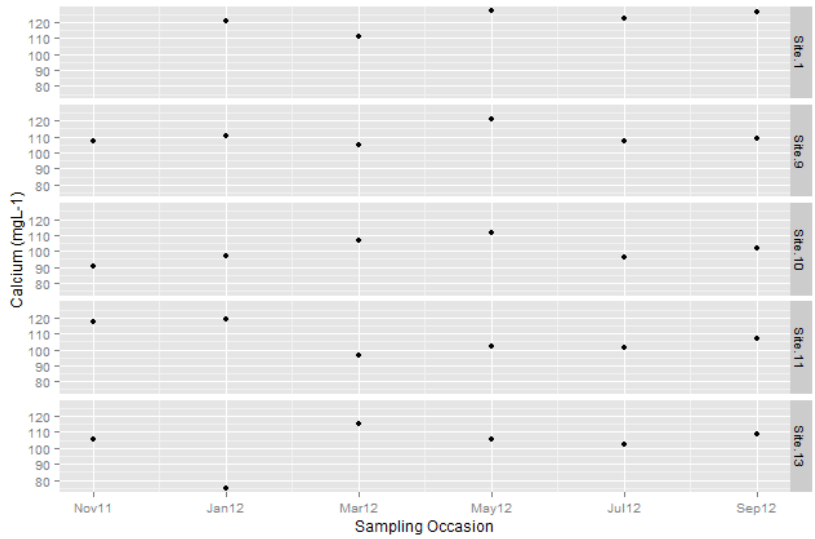


Figure 3.23 Benthic Calcium at the five riverine sites between November 2011 and September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

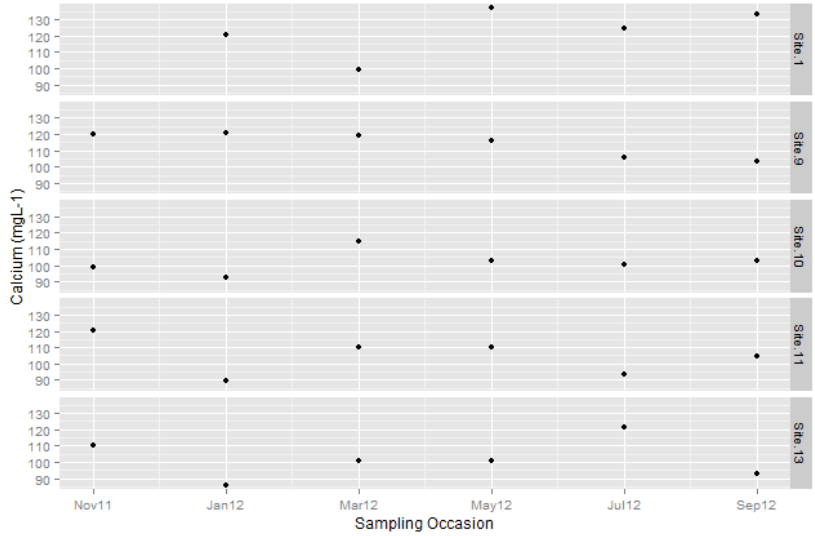


Figure 3.24 Hyporheic Calcium at the five riverine sites between November 2011 and September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

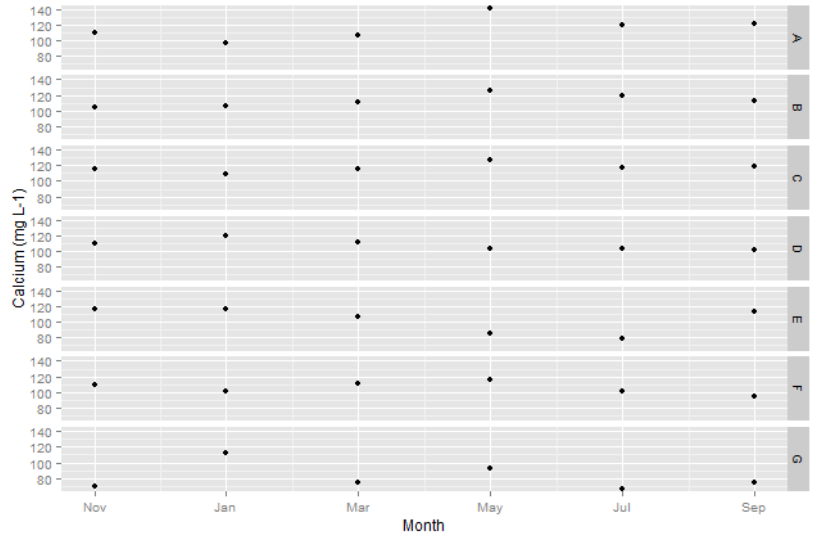


Figure 3.25 Calcium from the seven phreatic sites from November 2011 to September 2012.

Calcium derives from the dissolution and diagenesis of the Chalk geology and the concentrations recorded in this study likely reflect groundwater contribution, with the highest concentrations recorded in the western part of the study area near the headwaters (Sites 1 and A) and lowest in the easterly, downstream part of the catchment (Sites 11, 13 and G; Figures 3.23-3.25; Robins, 1998).

3.3.2.2 Potassium

Potassium concentrations did not vary spatially or temporally in either the benthic or hyporheic habitats; however, they varied both spatially ($F=2.34$, $p=0.05$) and temporally ($F=5.23$, $p=0.001$) in phreatic habitats (Tables 3.5-3.8). Potassium concentrations were lowest in the benthic waters, ranging from 0.09 to 0.33 mg L^{-1} ($\bar{x}=0.14$), and slightly higher in hyporheic waters where the results ranged from 0.10 to 0.72 with a mean of 0.20 mg L^{-1} (Figures 3.26 and 3.27). The highest results were recorded in phreatic habitats where they ranged from 0.14 to 1.59 mg L^{-1} ($\bar{x}=0.43$ mg L^{-1}) and were highly variable between sites and sampling occasions (Figure 3.28).

Potassium concentrations notably increased at phreatic sites during July 2012 (with the exception of sites D and E). The reason for this increase is unclear but may be a reflection of the aquifer responding to the post drought recharge event and the recovery of groundwater levels; however, these results are confounded by the lack of temporal variability in the results in the benthic and hyporheic habitats, particularly as Potassium is used as a signature of surface water influence, specifically in agricultural areas where it is a common constituent of fertilisers (Section 3.4; Bartley and Johnston, 2006; Hahn and Fuchs, 2009). While these results are striking, the Potassium concentrations recorded during this study are within the expected range for inland freshwaters and are unlikely to be toxic to aquatic communities (Talling, 2010).

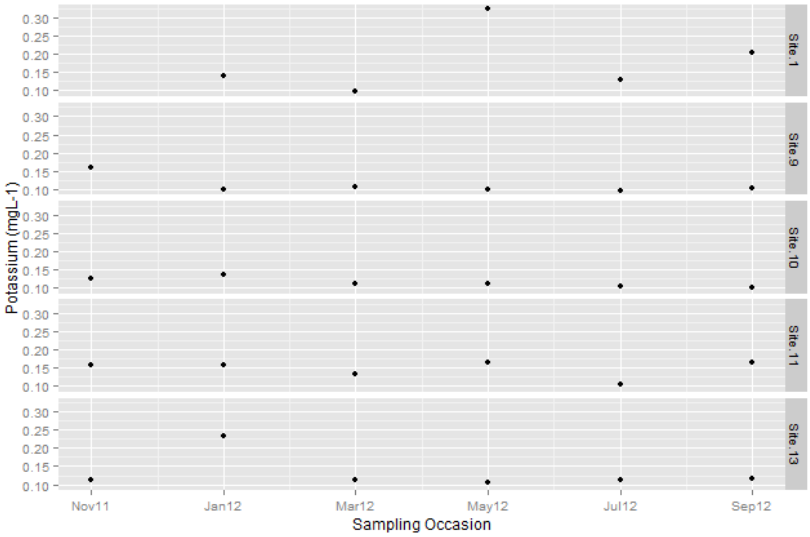


Figure 3.26 Benthic Potassium from the five riverine sites from November 2011 to September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

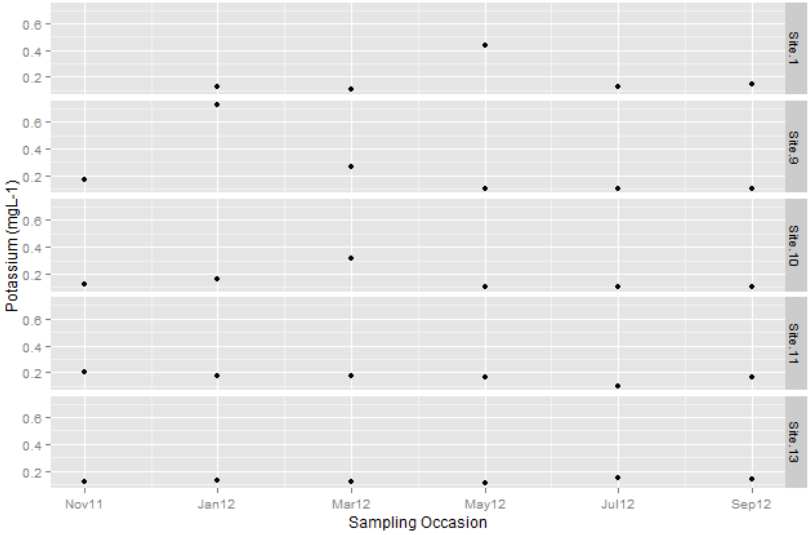


Figure 3.27 Hyporheic Potassium from the five riverine sites from November 2011 to September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

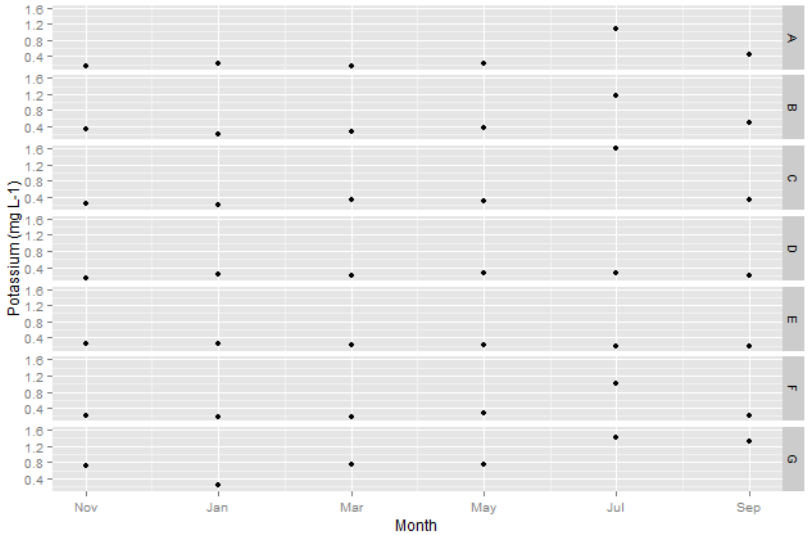


Figure 3.28 Phreatic Potassium at the seven sites from November 2011 to September 2012.

3.3.2.3 Magnesium

Magnesium concentrations varied spatially but not temporally in the benthic, hyporheic and phreatic habitats (F=13.95, p=0.001; F=5.77, p=0.002; F=67.61, p=0.001, respectively; Figures 3.29-3.31; Tables 3.5-3.8).[4] Ranges (2.0-4.8 mg L^{-1}) and means ($\bar{x}$=2.5, 2.6 and 2.9 mg L^{-1}, respectively) were highest in the phreatic habitat but slightly lower than long term Environment Agency monitoring from the Stour Chalk Block ($\bar{x}$=3.34 mg L^{-1}, 1995-2008; Entec 2008).

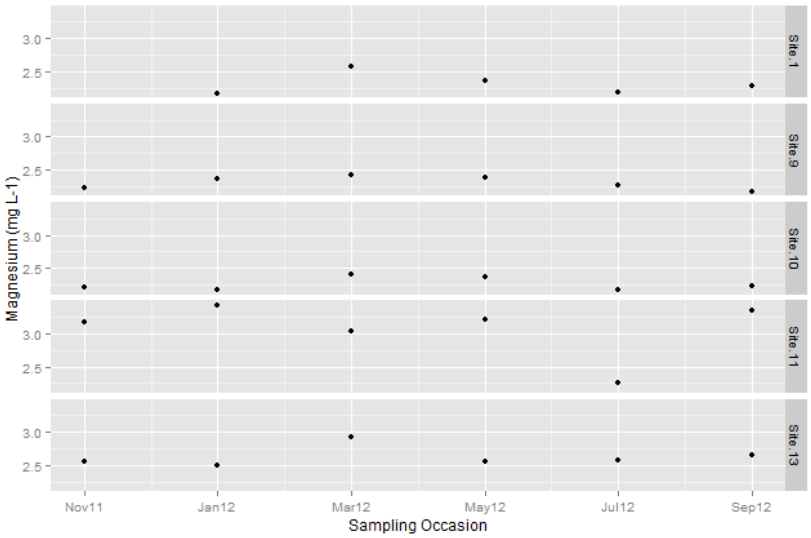


Figure 3.29 Benthic Magnesium from the five riverine sites from November 2011 to September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

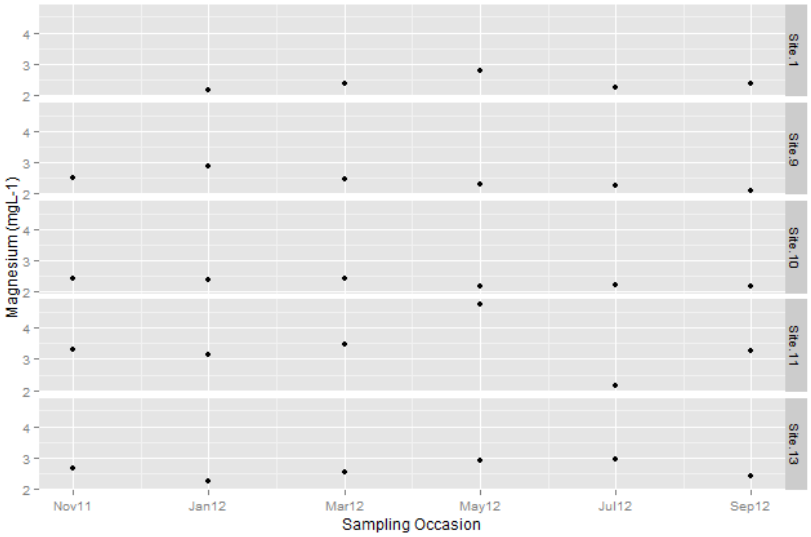


Figure 3.30 Hyporheic Magnesium from the five riverine sites from November 2011 to September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

[4] The level of detection for Magnesium for this study is 0.0005 mg L^{-1}

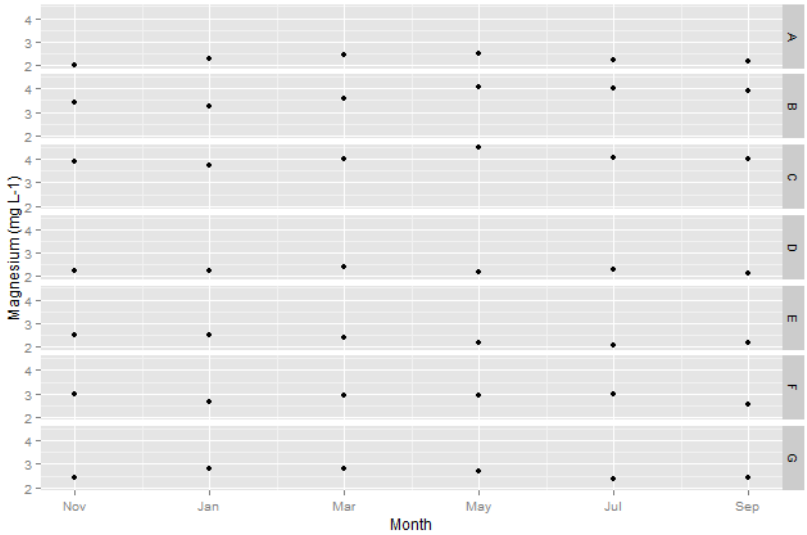


Figure 3.31 Magnesium at the seven phreatic sites from November 2011 to September 2012.

This variability by depth is expected as Magnesium, like Calcium and Strontium, derives from the dissolution of the Chalk geology and therefore, should be highest in the phreatic habitat. However, it would be expected to follow that the highest concentrations would be recorded at sites located in the headwaters, closest to the areas of geological outcrop but instead, the highest concentrations were recorded at the furthest downstream sites on the Little Stour near Seaton (Sites 11, B and C) in all three habitats. The reason for this is unclear, but may be attributed to the leakage of Magnesium from the overlying strata which could accumulate as the water flows down gradient (Robins, 1998).

3.3.2.4 Sodium

Sodium ranges (0.41-1.36 mg L^{-1}) and means ($\bar{x}$=0.68, 0.70 and 0.76 mg L^{-1} across the benthic, hyporheic and phreatic, respectively) were similar between habitats. Sodium concentrations varied spatially but not temporally in benthic ($F=3.50$, $p=0.02$) and phreatic ($F=12.57$, $p=0.001$) habitats, but neither spatially nor temporally variable in hyporheic habitats (Tables 3.5-3.8).[5] In both benthic and hyporehic habitats, sodium concentrations were lowest at site 13 on the Dour and highest at the downstream sites (10 and 11) of the Little Stour (Figures 3.32 and 3.33). Conversely, concentrations in the phreatic habitat were highest on the Dour (site F) and lowest at the sites located nearest to the Nailbourne headwaters (site A; Figure 3.34).

[5] The level of detection for Na in this study is 0.0002 mg L^{-1}

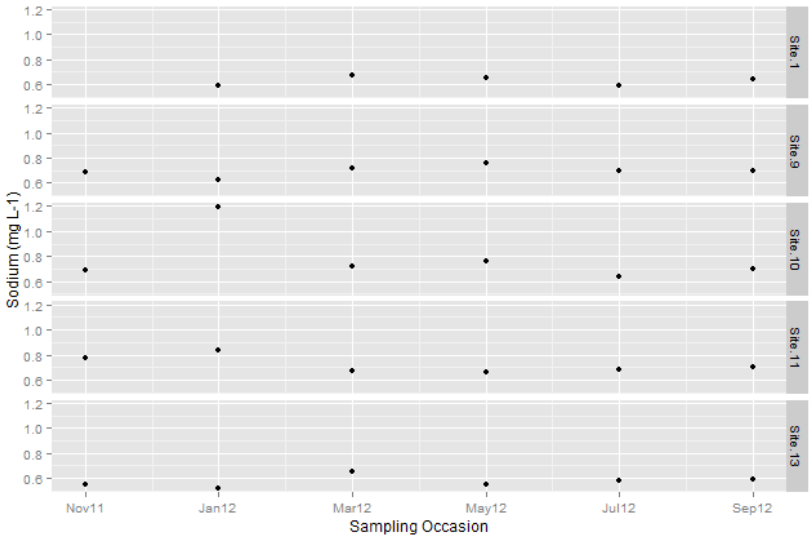


Figure 3.32 Benthic Sodium from the five riverine sites from November 2011 to September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

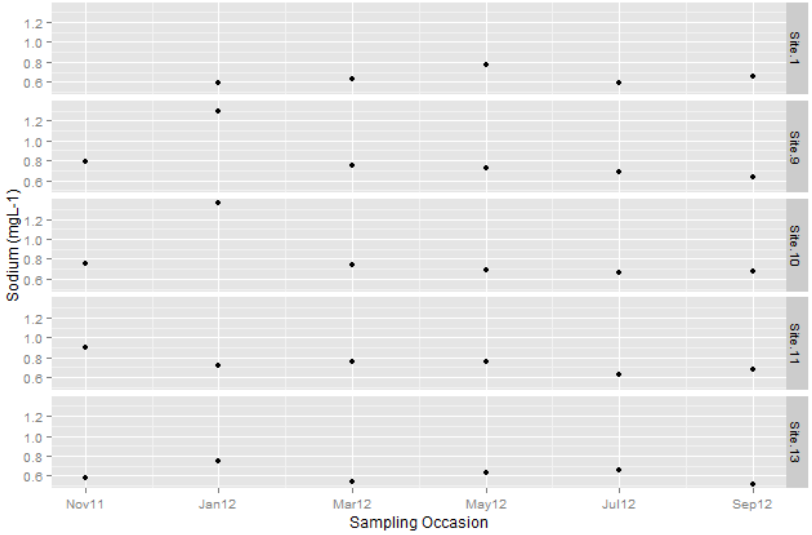


Figure 3.33 Hyporheic Sodium from the five riverine sites from November 2011 to September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

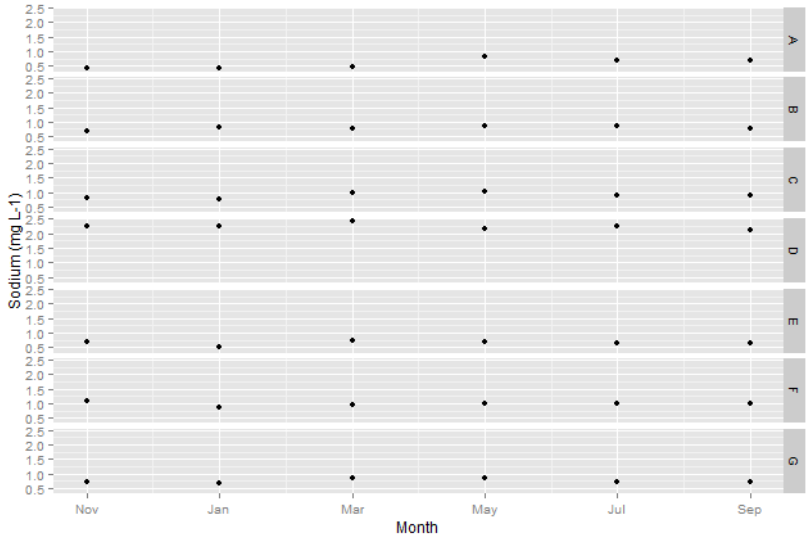


Figure 3.34 Sodium from the seven phreatic sites from November 2011 to September 2012.

These results are surprising and could reflect complex interactions in which the groundwater is modified by ion exchange as it flows down the hydraulic gradient so that Ca and Mg are replaced by Na from the minerals in the aquifers matrix, particularly where it is confined by overlying clays (Robins, 1998).

3.3.2.5 Strontium

Strontium ranges (0.15-0.39 mg L^{-1}) and means ($\bar{x}$=0.25, 0.20 and 0.25 mg L^{-1} in benthic, hyporheic and phreatic respectively) were similar between habitats and consistent with long-term Environment Agency monitoring on the Stour Chalk Block ($\bar{x}$=0.27 mg L^{-1}, 1995-2008; Entec 2008). Strontium varied spatially but not temporally in all three habitats (F=21.69, p=0.001; F=14.34, p=0.001; and F=12.74, p=0.001, respectively; Tables 3.5-3.8).

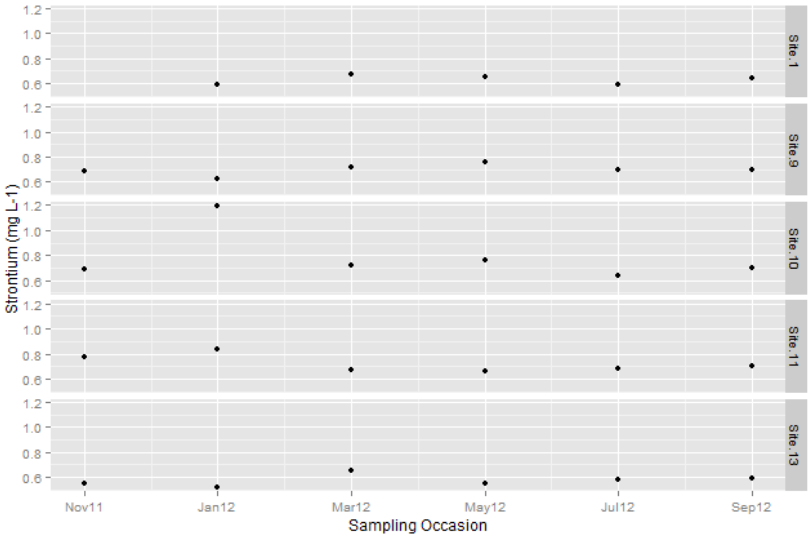


Figure 3.35 Benthic Strontium from the five riverine sites from November 2011 to September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

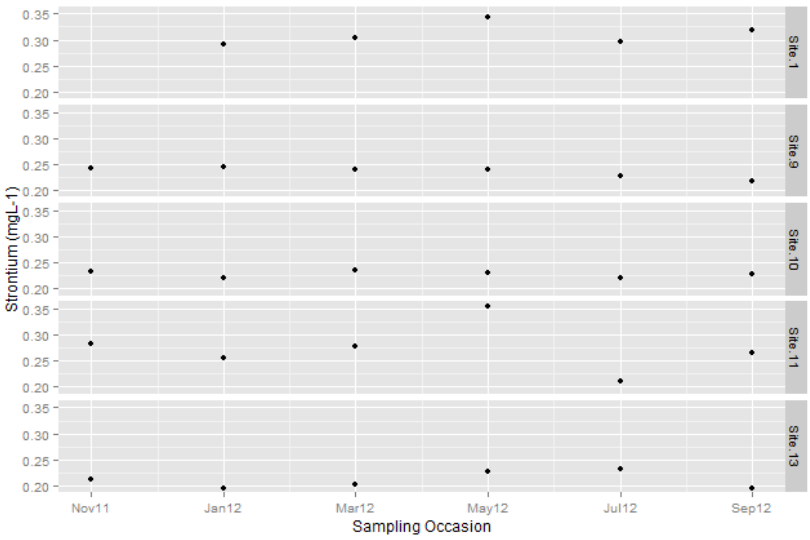


Figure 3.36 Hyporheic Strontium from the five riverine sites from November 2011 to September 2012 (no samples were collected at site 1 in November 2011 as it was dry).

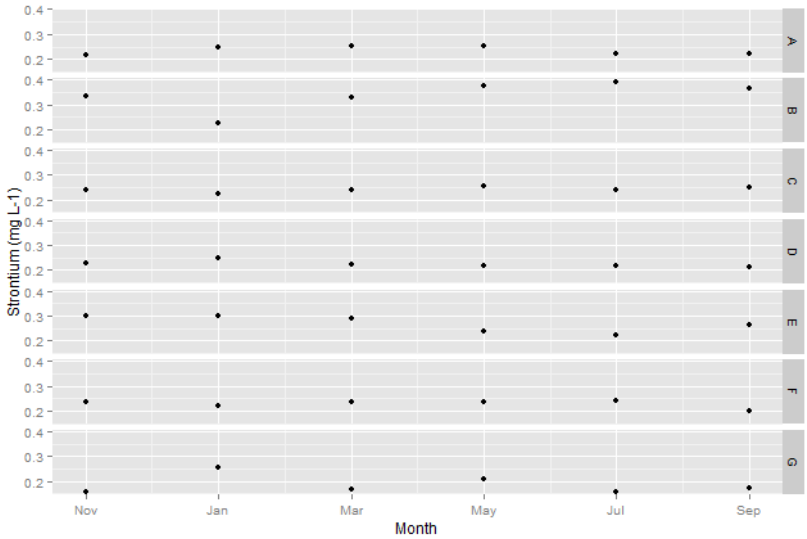


Figure 3.37 Strontium from the seven phreatic sites from November 2011 to September 2012.

Spatial patterns in Strontium concentrations are similar to Calcium as both derive from the dissolution and diagenesis of the Chalk geology, with the lowest concentrations recorded on the Dour and in gravel boreholes (sites 13 and G) and highest concentrations in the headwaters and Chalk boreholes (sites 1 and B; Figures 3.35-3.37; Robins, 1998; Smedley et al., 2003).

3.3.3 Summary of chemical environmental conditions

The nutrient and geochemical results are characteristic of a relatively unpolluted chalk stream environment and were consistent with long-term monitoring in this catchment. Spatially, the results reflect a high degree of variability for parameters which are closely associated with the chalk geology (Ca and Sr) in which there is a longitudinal gradient from the headwaters to the downstream sites, suggesting that the location of a site within the catchment heavily influences its environmental conditions. However, not all of these parameters varied as would be expected, suggesting that there are a number of complex interactions within the aquifer that may not be fully explained by the variables recorded. Temporally, the results indicate minimal variability for all parameters in the three habitats, suggesting stable chemical conditions across the year and between depths. This is noted with the exception of a spike in Potassium which significantly increased in the phreatic habitat in July 2012, which may be the result of a recharge event following the drought break in spring 2012 (Section 3.4). Collectively, the results suggest minimal differences between depths, suggesting that there is more variation between sites than by depth or sampling occasion.

Table 3.5 Mean benthic and hyporheic (Bou-Rouch) chemical results for sites 1, 9, 10, 11 and 13 (±1 SE; 2011-2012; n=29) in which change has been analysed using a one-way ANOVA with * and ** indicating overall significance of $p<0.05$ and $p<0.001$ (respectively) and ns reflecting $p>0.05$. All results are reported in mg L^{-1}.

Parameter	Habitat	1	9	10	11	13	Spatial Change
N	Benthic	4.5 (±0.4)	3.6 (±0.2)	3.4 (±0.2)	2.8 (±0.2)	2.6 (±0.2)	*
	Hyporheic	2.8 (±0.7)	2.4 (±0.4)	3.1 (±0.3)	3.4 (±0.4)	3.4 (±0.5)	ns
P	Benthic	0.5 (±0.3)	0.2 (±0.1)	0.1 (±0.0)	0.1 (±0.0)	0.1 (±0.0)	ns
	Hyporheic	0.2 (±0.1)	0.1 (±0.0)	0.1 (±0.4)	0.2 (±0.2)	0.1 (±0.0)	ns
Ca	Benthic	121.8 (±2.3)	110.2 (±2.4)	100.6 (±3.2)	107.3 (±3.8)	102.0 (±5.7)	*
	Hyporheic	107.8 (±6.4)	101.5 (±4.1)	113.5 (±4.4)	119.5 (±6.0)	103.3 (±4.0)	*
K	Benthic	0.2 (±0.0)	0.1 (±0.0)	0.11 (±0.0)	0.15 (±0.01)	0.13 (±0.02)	ns
	Hyporheic	0.2 (±0.1)	0.1 (±0.0)	0.2 (±0.0)	0.2 (±0.1)	0.1 (±0.0)	ns
Mg	Benthic	2.3 (±0.1)	2.3 (±0.0)	2.3 (±0.0)	3.1 (±0.2)	2.6 (±0.1)	**
	Hyporheic	2.5 (±0.1)	3.1 (±0.4)	2.5 (±0.1)	2.7 (±0.2)	2.4 (±0.2)	*
Na	Benthic	0.6 (±0.0)	0.7 (±0.0)	0.78 (±0.1)	0.72 (±0.03)	0.57 (±0.02)	*
	Hyporheic	0.8 (±0.1)	0.8 (±0.1)	0.6 (±0.0)	0.7 (±0.1)	0.7 (±0.0)	ns
Sr	Benthic	0.3 (±0.0)	0.2 (±0.0)	0.2 (±0.0)	0.3 (±0.0)	0.2 (±0.0)	**
	Hyporheic	0.3 (±0.0)	0.3 (±0.0)	0.2 (±0.0)	0.3 (±0.0)	0.2 (±0.0)	**

Table 3.6 Mean benthic and hyporheic (Bou-Rouch) chemical results by sampling occasion (±1 SE; 2011-2012; n=29) in which change has been analysed using a one-way ANOVA with * and ** indicating overall significance of $p<0.05$ and $p<0.001$ (respectively) and ns reflecting $p>0.05$. All results are in mg L^{-1}.

Parameter	Habitat	Nov-11	Jan-12	Mar-12	May-12	Jul-12	Sep-12	Temporal Change
N	Benthic	2.7 (±0.2)	4.0 (±0.5)	3.2 (±0.3)	3.2 (±0.2)	3.7 (±0.6)	3.2 (±0.4)	ns
	Hyporheic	3.3 (±0.7)	2.8 (±0.5)	3.5 (±0.4)	3.0 (±0.2)	2.3 (±0.7)	3.2 (±0.5)	ns
P	Benthic	0.3 (±0.2)	0.1 (±0.0)	0.1 (±0.0)	0.3 (±0.3)	0.1 (±0.0)	0.2 (±0.2)	ns
	Hyporheic	0.1 (±0.0)	0.2 (±0.2)	0.2 (±0.2)	0.1 (±0.0)	0.1 (±0.0)	0.1 (±0.1)	ns
Ca	Benthic	105 (±6)	105 (±9)	107 (±3)	114 (±5)	106 (±4)	111 (±4)	ns
	Hyporheic	112 (±5)	102 (±8)	109 (±4)	114 (±7)	109 (±6)	107 (±7)	ns
K	Benthic	0.1 (±0.0)	0.2 (±0.0)	0.1 (±0.0)	0.2 (±0.0)	0.1 (±0.0)	0.1 (±0.0)	ns
	Hyporheic	0.2 (±0.0)	0.3 (±0.2)	0.2 (±0.0)	0.2 (±0.1)	0.1 (±0.0)	0.1 (±0.0)	ns
Mg	Benthic	2.6 (±0.2)	2.5 (±0.2)	2.7 (±0.1)	2.6 (±0.2)	2.3 (±0.1)	2.5 (±0.2)	ns
	Hyporheic	2.7 (±0.2)	2.6 (±0.2)	2.7 (±0.2)	3.0 (±0.5)	2.4 (±0.2)	2.5 (±0.2)	ns
Na	Benthic	0.7 (±0.1)	0.8 (±0.1)	0.7 (±0.0)	0.7 (±0.0)	0.6 (±0.0)	0.7 (±0.0)	ns
	Hyporheic	0.8 (±0.1)	0.9 (±0.2)	0.7 (±0.0)	0.7 (±0.0)	0.6 (±0.0)	0.6 (±0.0)	ns
Sr	Benthic	0.2 (±0.0)	0.3 (±0.0)	0.3 (±0.0)	0.3 (±0.0)	0.2 (±0.0)	0.3 (±0.0)	ns
	Hyporheic	0.2 (±0.0)	0.2 (±0.0)	0.3 (±0.0)	0.3 (±0.0)	0.2 (±0.0)	0.2 (±0.0)	ns

Table 3.7 Mean phreatic chemical results for sites A-G (±1 SE; 2011-2012; n=41) in which change has been analysed using a one-way ANOVA with * and ** indicating overall significance of p<0.05 and p<0.001 (respectively) and ns reflecting p>0.05. All results are reported in mg L^{-1}.

Parameter	A	B	C	D	E	F	G	Spatial Change
N	2.18 (±0.84)	4.55 (±2.15)	2.33 (±0.39)	3.38 (±1.46)	3.26 (±0.78)	4.13 (±2.60)	1.92 (±0.75)	*
P	0.04 (±0.02)	0.17 (±0.16)	0.48 (±0.54)	0.42 (±0.61)	0.04 (±0.04)	0.17 (±0.19)	0.66 (±0.38)	*
Ca	116.03 (±15.30)	113.27 (±8.22)	117.38 (±5.67)	108.97 (±7.23)	100.02 (±17.01)	105.63 (±7.62)	81.29 (±17.44)	**
K	0.38 (±0.36)	0.48 (±0.35)	0.50 (±0.54)	0.21 (±0.05)	0.21 (±0.02)	0.35 (±0.33)	0.86 (±0.44)	*
Mg	2.27 (±0.19)	3.71 (±0.33)	4.03 (±0.26)	2.25 (±0.10)	2.26 (±0.18)	2.84 (±0.19)	2.58 (±0.21)	**
Na	0.59 (±0.18)	0.79 (±0.07)	0.90 (±0.10)	0.65 (±0.07)	0.62 (±0.06	0.96 (±0.07)	0.76 (±0.08)	**
Sr	0.23 (±0.02)	0.34 (±0.06)	0.24 (±0.01)	0.23 (±0.01)	0.26 (±0.04)	0.23 (±0.02)	0.19 (±0.04)	**

Table 3.8 Mean phreatic chemical results by sampling occasion (±1 SE; 2011-2012; n=41) in which change has been analysed using a one-way ANOVA with * and ** indicating overall significance of p<0.05 and p<0.001 (respectively) and ns reflecting p>0.05. All results are reported in mg L^{-1}.

Parameter	November	January	March	May	July	September	Temporal Change
N	4.3 (±1.4)	2.4 (±0.5)	2.9 (±0.4)	2.8 (±0.4)	2.9 (±0.3)	3.8 (±0.8)	ns
P	0.4 (±0.2)	0.1 (±0.1)	0.4 (±0.2)	0.4 (±0.1)	0.4 (±0.2)	0.2 (±0.1)	ns
Ca	103 (±7)	109 (±3)	105 (±5)	113 (±8)	101 (±8)	106 (±6)	ns
K	0.3 (±0.9)	0.2 (±0.0)	0.3 (±0.1)	0.3 (±0.1)	1.0 (0.2)	0.5 (±0.2)	**
Mg	2.8 (±0.3)	2.8 (±0.2)	2.9 (±0.2)	3.0 (±0.3)	2.9 (±0.3)	2.8 (±0.3)	ns
Na	0.7 (±0.1)	0.7 (±0.1)	0.8 (±0.1)	0.8 (±0.1)	0.8 (±0.1)	0.8 (±0.1)	ns
Sr	0.2 (±0.0)	0.2 (±0.0)	0.3 (±0.0)	0.2 (±0.0)	0.2 (±0.0)	0.2 (±0.0)	ns

3.4 Physical Environmental Conditions

Meteorology, hydrology, hyporheic exchange flows, sediment characteristics and hydrogeology were assessed to describe the physical environmental conditions of the study area and contextualise these conditions over time. The results are used to describe the spatiotemporal variability in the physical conditions of the benthic, hyporheic and phreatic habitats and to identify potential perturbations.

3.4.1 Meteorology and Hydrology

Long-term average rainfall data from the Met Office (Manston Weather Station; 48.98 mm; 1934-2012; n=858) have been used to contextualise hydrological conditions during the study period. These results reflect two periods of abnormal conditions, including a winter period of above average rainfall in 2009-10 and an extended period of below average rainfall in 2011 (Figure 3.38).

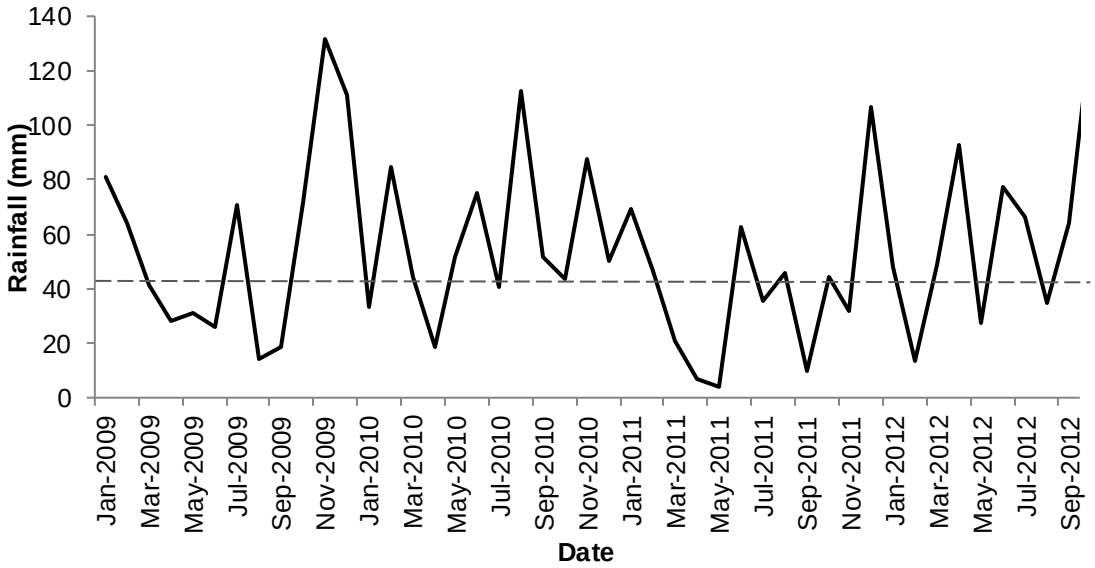


Figure 3.38 Monthly rainfall observations at the Manston Weather Station (Met Office) during the study period (solid line) plotted against the long-term average rainfall (48.98 mm) for this station (dashed line; 1934-2012; n=858).

Continuous Environment Agency monitoring data were used to assess the response of the Little Stour and Dour rivers to these periods of abnormal rainfall. Under normal conditions, the flow regime in this catchment is typical of a groundwater-dominated chalk stream, with predictable baseflow conditions between August and September and peak discharge occurring between December and February (Stubbington et al., 2009b; Wood et al., 2001). This assessment suggests four distinct periods of flow conditions which deviated

from normal (expected) discharge pattern and suggest a clear response to these periods of abnormal rainfall, including: the spate flows of spring 2010; short-term drying in autumn 2010; drought period of 2011-12; and drought break from spring 2012 until the end of the study (Figure 3.39).

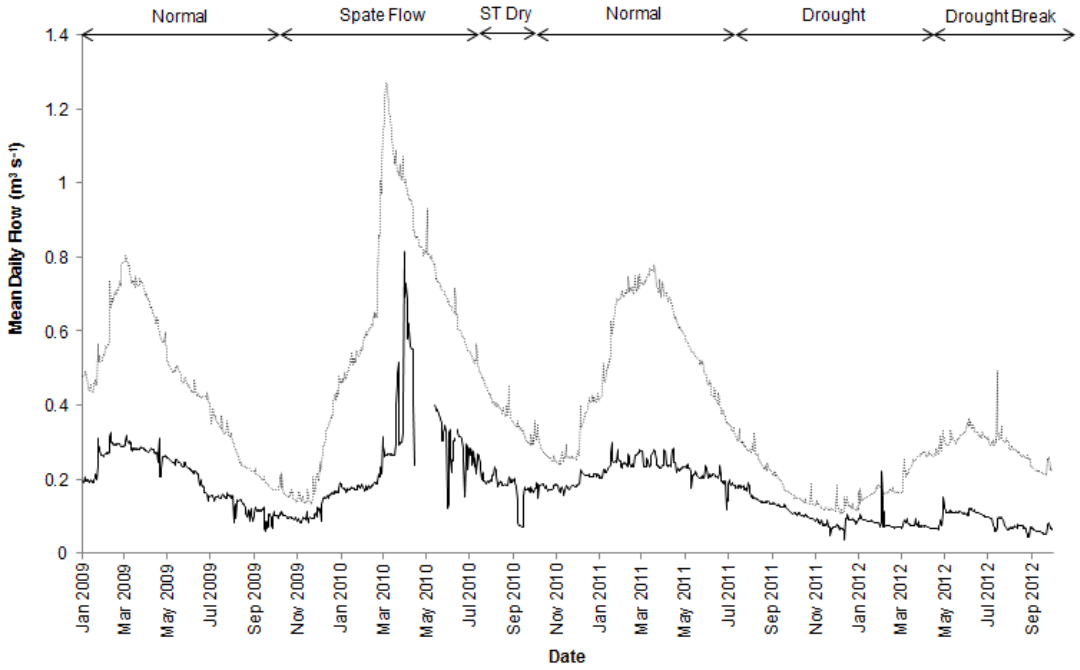


Figure 3.39 Hydrograph of mean daily flow (m^3 s^{-1}) recorded by the Environment Agency at the gauging station at Littlebourne on the Little Stour (solid line, near site 9) and at Crabble Mill on the Dour (dotted line, near site 13) from January 2009 to September 2012. Headings refer to notable periods of flow experienced within the context of the study (in which ST Dry refers to Short-Term drying period). The gap in April 2010 on the Little Stour reflects an absence of data.

The period of above average rainfall was followed by a spate flow which peaked on both rivers during the spring of 2010, recording the highest mean daily discharges of the study period in March 2010 (1.27 m^3 s^{-1} and 0.81 m^3 s^{-1}, on the Dour and Little Stour, respectively). During this high flow period the Nailbourne, notably, also flowed along its entire length to its confluence with the Little Stour. These spate flows overwhelmed the wastewater treatment system on the Little Stour and resulted in the overpumping of untreated sewage directly to the watercourse (between sites 6 and 7) in March and April 2010 (Chapter 5).

Similarly, the period of below average rainfall resulted in drought conditions both locally and nationally that peaked before breaking in April 2012 (Marsh et al., 2013). The lowest discharges recorded during the study period occurred during this low flow period in November 2011, just before peak discharge would be expected during normal conditions (mean daily 0.11 m^3 s^{-1} and 0.04 m^3 s^{-1}

on the Dour and Little Stour, respectively). While perennial flow was maintained on the Little Stour and Dour sites throughout the study, site 1 in the Nailbourne headwaters dried completely in November 2011. While these results suggest that this drought period may not have been as locally severe as previous droughts (1949, 1991-92 and 1996-97) which caused parts of the Little Stour to dry completely, nationally the 2011-12 drought was considered to have been more intense than previous droughts in 1975-76, 1995-97 and 2003-06, particularly in groundwater-dependent catchments owing to limited recharge during the two preceding winters (reflected in the short-term drying that occurred in autumn 2010; Marsh et al., 2013; discussed in Chapter 5).

Continuous Environment Agency monitoring data were also used to create flow duration curves for the Little Stour and the Dour before and during the study period to contextualise hydrological conditions and calculate flow statistics for further assessment (Table 3.9; Figure 3.40). The results reflect the expected flow regime for a chalk stream environment, with large differences between the Q_5 (flow exceeded 5% of the time, a high flow threshold) and Q_{95} statistics (flow exceeded 95% of the time, a low flow threshold; Sear et al., 1999). However, the Q_1 and Q_5 statistics were much higher during the study than the antecedent period, reflecting the above average rainfall and subsequent spate flows. Conversely, there is little difference between the antecedent and study period low flow statistics as the Q_{95} and Q_{99} discharges are similar on both watercourses, suggesting regular periods of low flow.

Table 3.9 Discharge statistics ($m^3\ s^{-1}$) from the Little Stour and Dour rivers representing flows during the antecedent (2004-2008) and study (2009-2012) time periods. The difference between the discharge statistics during these time periods is not significant on either the Little Stour or Dour (student t-test). Data after routine Environment Agency monitoring, as previous.

Discharge Statistic	Little Stour		Dour	
	Antecedent	Study Period	Antecedent	Study Period
Q1	0.34	0.52	0.74	1.14
Q5	0.28	0.30	0.68	0.81
Q10	0.22	0.28	0.62	0.73
Q50	0.15	0.17	0.37	0.34
Q90	0.09	0.07	0.17	0.16
Q95	0.08	0.07	0.12	0.14
Q99	0.05	0.05	0.10	0.12

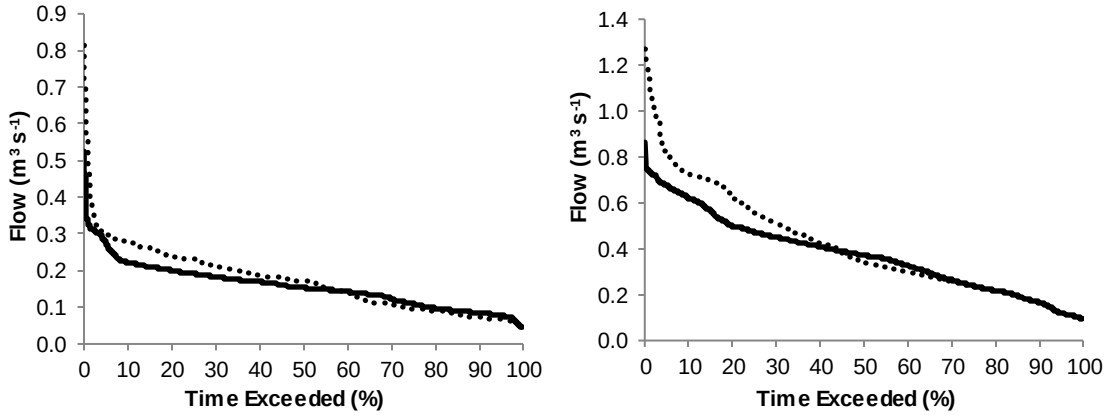


Figure 3.40 Flow duration curves of Environment Agency data from the Little Stour (left) and Dour (right) in which antecedent flows during the five years prior to this study are the sloid line (January 2004 – December 2008; n=1726 and n=1827, respectively) and the flows during the study are the dotted line (January 2009 – September 2012; n=1335 and n=1369, respectively).

Continuous Environment Agency monitoring data over the study period were also used to calculate the Baseflow Index (BFI), to quantify the contribution of groundwater to the Little Stour and Dour. The BFI considers the ratio of baseflow to the total channel discharge to produce a value of 0.10 to 0.99 in which lower values reflect flashy, surface-water dominated catchments and higher values reflect stable, groundwater-dominated catchments; Gustard et al., 1992; Wahl and Wahl, 2007). The BFI values for both watercourses were high (BFI=0.88, n=1335 and 0.98, n=1369 respectively; data as Figure 3.40), an expected result as Chalk streams typically record BFI values ~0.90 as a product of the high groundwater component of their discharge (Sear et al., 1999). These results suggest that the similarity between the antecedent and study period low flow statistics reflects the stabilising effect of the high contribution of groundwater to this catchment. At the site scale, the hydrological metrics recorded independently at riverine sites as part of this study suggest an expected longitudinal gradient of hydroperiodicity with those located in the headwaters recording much smaller discharges than sites located further down the catchment (Table 3.10).

Table 3.10 Hydrological statistics at riverine sites from spot sampling data collected during sampling visits (March 2010-September 2012) in which discharge was calculated by multiplying average flow measurements by the wetted area (Q=VA) after Fetter (2001).

Site	1	9	10	11	13
Median Discharge (m^3 sec^{-1})	0.07	0.17	0.26	0.44	0.23
Maximum Discharge (m^3 sec^{-1})	0.62	1.01	0.90	1.21	0.73
Minimum Discharge (m^3 sec^{-1})	0.00	0.04	0.10	0.10	0.09
Minimum wetted width (m)	0.0	5.8	4.1	3.6	5.5

3.4.2 Hyporheic Exchange Flow

Vertical hydraulic gradients were recorded at sites 10 and 11 during the final year of the study to assess the hyporheic exchange flow at these sites. The results indicate strongly downwelling water at both sites over the winter period, followed by upwelling water in July, suggesting that these sites are more dependent upon groundwater in the summer months (Figure 3.41). This is consistent with the levels recorded in the phreatic habitats, which recovered from drought conditions in July 2012, and reflect the groundwater contribution to the surface (Section 3.4.4).

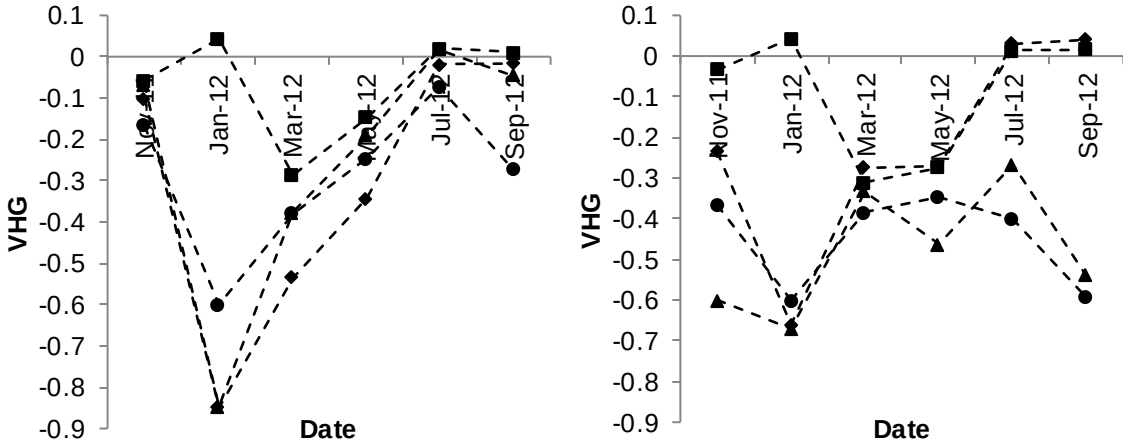


Figure 3.41 Vertical Hydraulic Gradient measurements at four replicates within site 10 (left) and site 11 (right) from November 2011 to September 2012 (after Dahm and Valett, 1996).

3.4.3 Sediment Characteristics

Grain size distribution and percentage organic matter were measured from the sediments collected by the freeze coring to characterise the substratum (site 10; July 2010). Comprehensive substrate data are only available for this site; as such, these results, and their representativeness, are limited (Section 2.3.1.1). Coarse sediments (>2mm) were dominant at all depths (Table 3.11; Fetter, 2001). The percentage of fines (<2mm), organic matter and sortedness were greatest in the superficial horizons and decreased with depth while hydraulic conductivity increased with depth (Figure 3.42).

Table 3.11 Average grain size of three freeze core replicates collected at site 10 in July 2010.

Size Class	Depth (cm)			
	0-10	10-20	20-30	>30
>4 mm	70%	66%	78%	81%
>2 mm	7%	15%	11%	10%
>63 um	21%	19%	11%	8%
>38 um	1%	1%	0%	0%
<38 um	0%	0%	0%	0%

These results suggest a high potential for exchange flows as well as inhibition from fine sediment accumulation. The variability between replicates suggests dynamic physical conditions within this site which may be attributed to the macrophyte growth at the time of sampling or the preceding period of spate flows which may have scoured some of the fine sediments from the substratum.

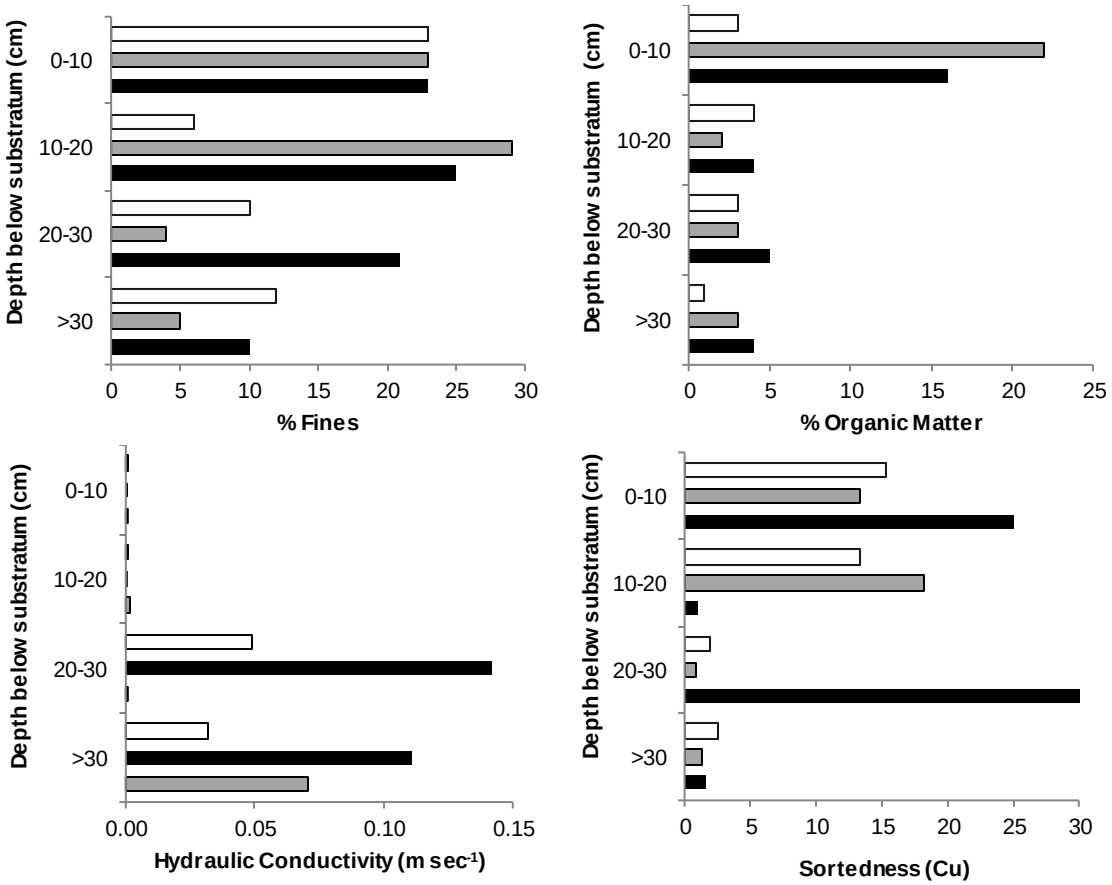


Figure 3.42 Sediment characteristics three replicate freeze cores from site 10 displaying: % fines (<2mm), % organic matter, hydraulic conductivity (in which $K=0.0116*D_{10}^2 * (0.7+0.03(T))$ after Blaschke et al., 2003) and sortedness (in which $Cu = D_{10} / D_{60}$ after Fetter, 2001).

3.4.4 Hydrogeology

During periods of normal rainfall, hydrological conditions in this aquifer are predictable and follow an expected seasonal pattern, with peak levels occurring during the early part of the calendar year before recessing to base levels at the end of the hydrological year (Sear et al., 1999). Figure 3.43 shows a similar pattern between phreatic sites but differing amplitudes of water level fluctuations. This pattern is most striking following the period of below average rainfall in 2011 which resulted in low recharge and a departure from the expected seasonal pattern with many sites recording minimum levels early in 2012 and maximum levels during the following July, contrary to what would normally be expected.

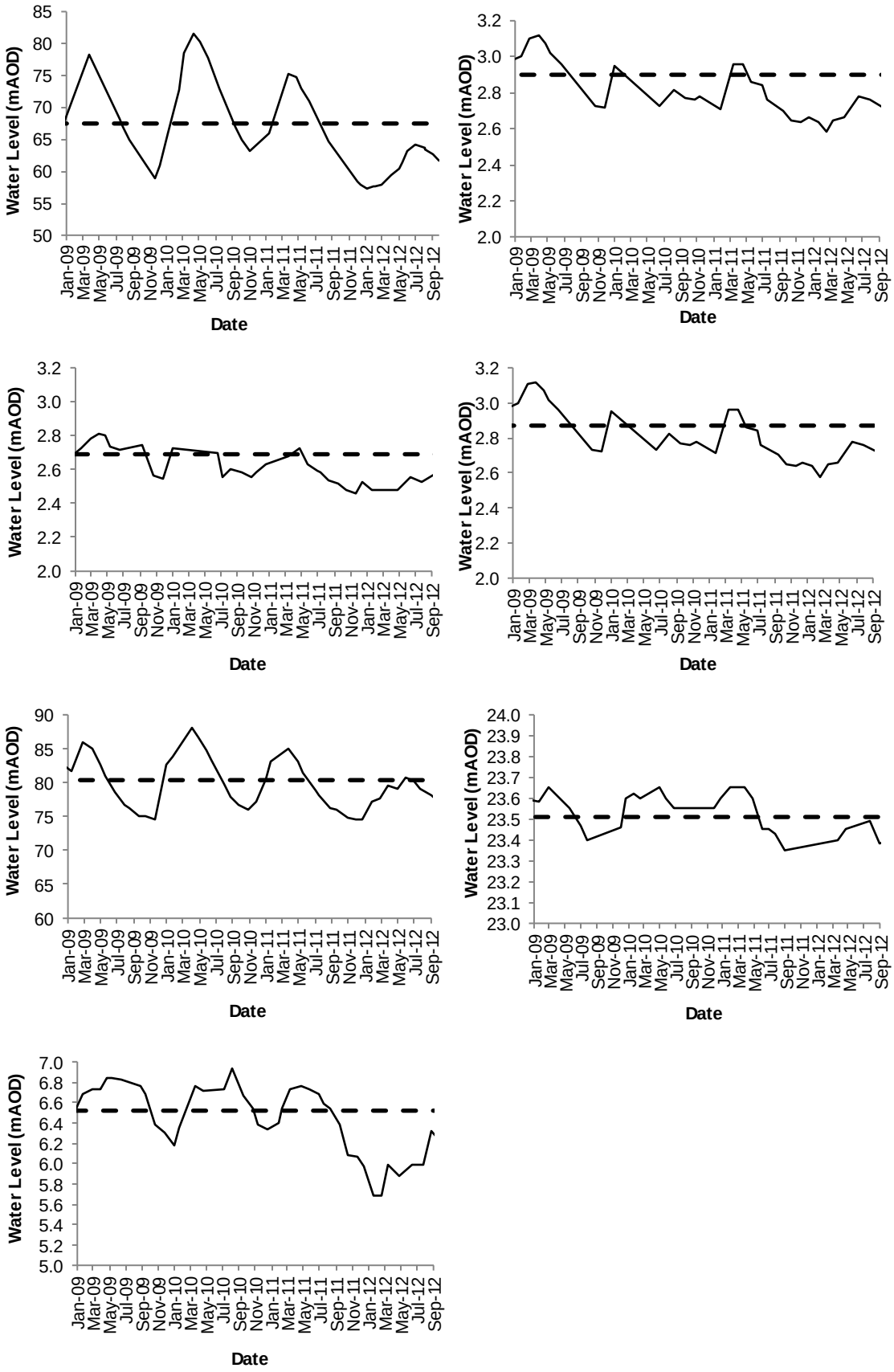


Figure 3.43 Water level observations (Environment Agency) over the study period (solid line) plotted against the long-term average levels (2000-2012) for each site (dashed line). From top left, site A (LTA=67.00; n=109); site B (LTA=2.85; n=118); site C (LTA=2.69; n=111); site D (LTA=2.87; n=118); site E (80.37; n=143); site F (23.51, n=125); and site G (6.52; n=102)

3.4.5 Summary of physical environmental conditions

The results suggest that study area flow regime is typical of a lowland groundwater-dominated Chalk catchment, with a strong seasonal pattern in which discharge normally peaks during winter and low flows occur during summer, with degrees of permanence along the watercourses. However, contextualising the rainfall, river flows and water level observations that occurred over the study period using long-term Met Office and Environment Agency data suggests that two abnormal meteorological periods influenced the environmental conditions within this catchment during the study. The period of above average rainfall in 2009-10 resulted in spate flows throughout the study area and much higher Q_1 and Q_5 discharge statistics during the study than the antecedent period. Similarly, the period of below average rainfall in 2011 resulted in drought conditions throughout the study area which were extraordinary as they created an extended period of low flow over the winter months when discharge would normally be highest. Changes in the environmental conditions of the benthic, hyporheic and phreatic habitats resulting from these two abnormal periods varied by depth and site location. Considering the drought period, discharge began to recover from May 2012 while the upwelling of groundwater in the hyporheic habitat (as measured by VHG) and the water level observations within the phreatic habitat lagged slightly, beginning their recovery in July 2012, suggesting that this catchment responds quickly to recharge events.

3.5 Discussion and Summary of Environmental Conditions

This chapter assesses the environmental conditions measured in the three habitats. The results have been considered independently and collectively within a framework of two research questions to inform the aims of this study.

3.5.1 Does each habitat provide distinct environmental conditions?

The conditions recorded in the benthic, hyporheic and phreatic habitats were assessed using Principal Components Analyses to identify spatial and temporal variance in the multivariate dataset. These analyses were undertaken for each habitat individually before considering all habitats collectively. In both cases, abiotic environmental variables were first tested for normality and square-root transformed prior to analysis (Zuur et al., 2010).

3.5.1.1 Benthic

The environmental conditions of the benthic habitat are spatially variable, reflecting a longitudinal gradient across the catchment from the headwaters to downstream, and vary seasonally. Considering the physiochemical results from the study period, the first axis of the PCA ordination reflects this spatial distribution as variables associated with the headwater sites (site 1; conductivity, turbidity and alkalinity) were related to positive values and negative values were related with variables characteristic of sites downstream (dissolved oxygen, discharge and pH), this axis explained 34% of the variability (Figure 3.44a).[6] The second axis explained a further 24% of the variability and was related to sampling occasion in which the lowest values were associated with variables reflecting the summer months and baseflow (temperature; July and September) and the highest values with variables reflecting the winter months and peak discharge (alkalinity; January and March; Figure 3.44b)

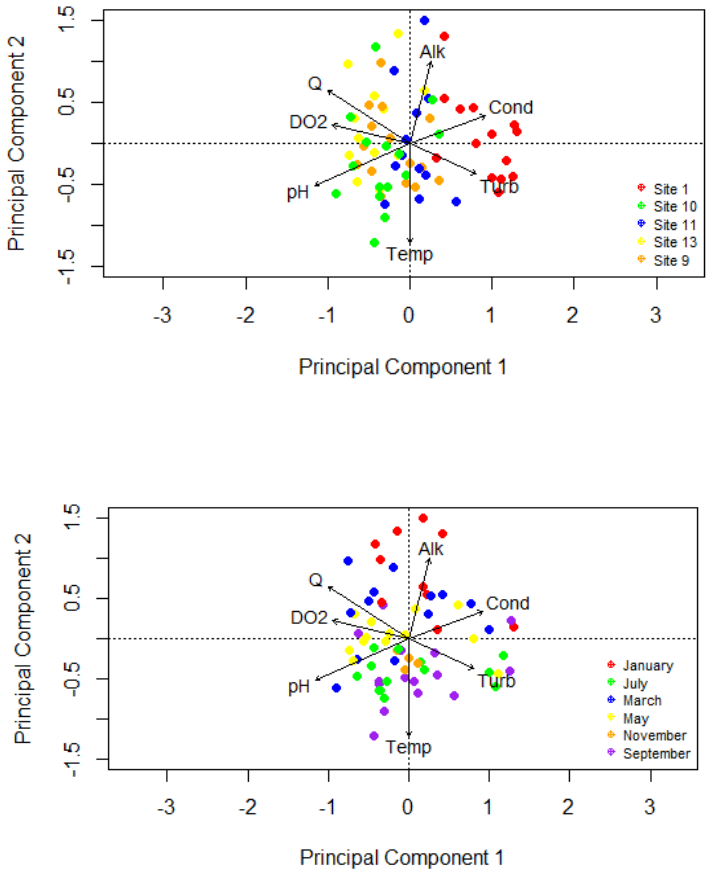


Figure 3.44 PCA ordination of transformed physiochemical results from the benthic habitat across five sites (March 2010-September 2012) shown by site (a; top) and season (b; bottom).

[6] Eigenvalues for axes 1-4: 2.366; 1.698; 1.065; 0.849 and proportion of variance explained for axes 1-4: 0.338; 0.243; 0.121; 0.055

The addition of chemical variables in the final year of the study further supports this spatial pattern. The first axis of the resulting PCA, which explained 36% of the variability, depicts physiochemical and chemical variables along the longitudinal gradient with positive values associated with headwater variables and high groundwater contribution (conductivity, turbidity, Sr, Ca) and negative values associated with sites located further downstream and greater surface water influence (discharge, dissolved oxygen, pH; Figure 3.45a).[7] The second axis, which explained a further 16% of the variability was associated with Mg and Na but does not suggest a temporal influence as previous (Figure 3.45b).

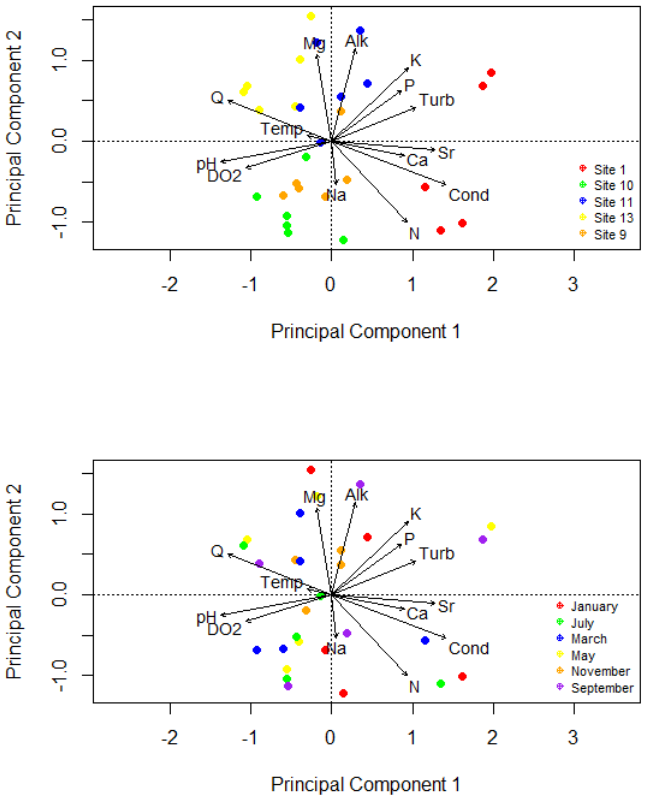


Figure 3.45 PCA ordination of transformed benthic physiochemical and chemical results across five sites (November 2011-September 2012) by site (a; top) and season (b; bottom).

3.5.1.2 Hyporheic

The preceding sections indicate that environmental conditions in the hyporheic habitat are spatially variable and suggest a longitudinal gradient across the catchment from the headwaters to downstream, and that they vary seasonality. Considering the physiochemical results from the study period, the first axis of

[7] Eigenvalues for axes 1-4: 4.989; 2.287; 1.506; 1.270 and proportion of variance explained for axes 1-4: 0.356; 0.163; 0.108; 0.091

the PCA ordination, which explained 34% of the variability, reflects this spatial distribution as variables associated with the headwater sites (specifically conductivity and alkalinity at site 1) were associated with negative values and positive values were associated with variables characteristic of downstream sites (discharge and pH; Figure 3.46a).[8] The second axis explained a further 24% of the variability and reflects seasonality as the results from winter months were associated with positive values for discharge and alkalinity and those collected during the summer were associated with negative values for temperature (Figure 3.46b).[9]

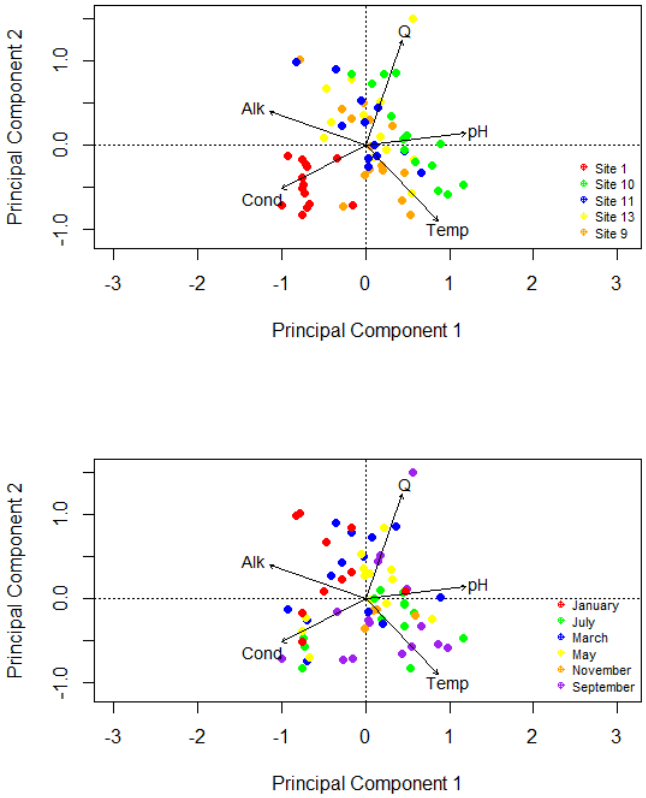


Figure 3.46 PCA ordination of transformed physiochemical results from the five hyporheic sites (November 2011 to September 2012) by site (a; top) and season (b; bottom).

The addition of chemical variables in the final year of the study further supports this spatial pattern. The first axis of the resulting PCA, which explained 26% of the variability, depicts physiochemical and chemical variables along the longitudinal gradient with positive values associated with sites located at the downstream sites where there is greater surface water influence (discharge,

[8] Eigenvalues for axes 1-4: 2.366; 1.698; 1.065; 0.849 and proportion of variance explained for axes 1-4: 0.338; 0.243; 0.121; 0.055

[9] Eigenvalues for axes 1-4: 3.094; 2.624; 1.556; 1.095 and proportion of variance explained for axes 1-4: 0.258; 0.219; 0.130; and 0.091

pH) and negative values associated with sites located in the headwaters which have a greater groundwater influence (geochemistry, conductivity; Figure 3.47a).[10] The second axis explained a further 22% of the variability but was not strongly associated with any of the variables and does not suggest a temporal influence as previous, reflecting stability in this habitat (Figure 3.47b).

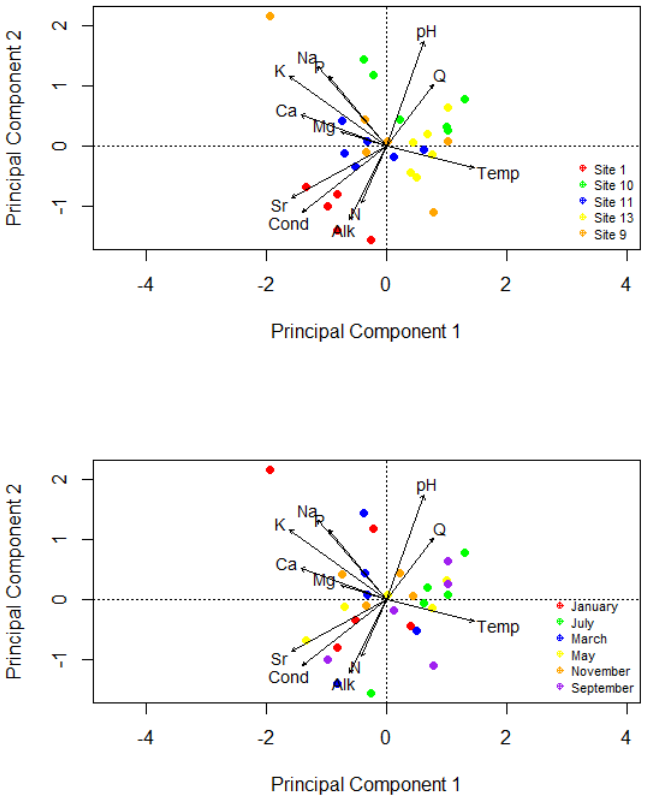


Figure 3.47 PCA ordination of transformed physiochemical and chemical results from five hyporheic sites (November 2011-September 2012) by site (a; top) and season (b; bottom).

3.5.1.3 Phreatic

The preceding sections suggest that the environmental conditions of phreatic habitat vary spatially but are (broadly) temporally stable. Considering the physiochemical and chemical results from the final year of the study, the PCA ordination reflects this distribution with distinctive clustering of sites, especially those located in the headwaters to the western side of the catchment (sites A, D and E) which recorded much higher dissolved oxygen concentrations than the other sites (Figure 3.48a).[11] Unlike the results from the benthic and hyporheic

[10] Eigenvalues for axes 1-4: 3.095; 2.624; 1.556; 1.095 and proportion of variance explained for axes 1-4: 0.258; 0.219; 0.130; 0.091

[11] Eigenvalues for axes 1-4: 3.389; 2.721; 1.862; 1.203 and proportion of variance explained for axes 1-4: 0.261; 0.210; 0.143; 0.093

habitats, no temporal influence was apparent in the phreatic habitat, suggesting stability in this habitat (Figure 3.48b).

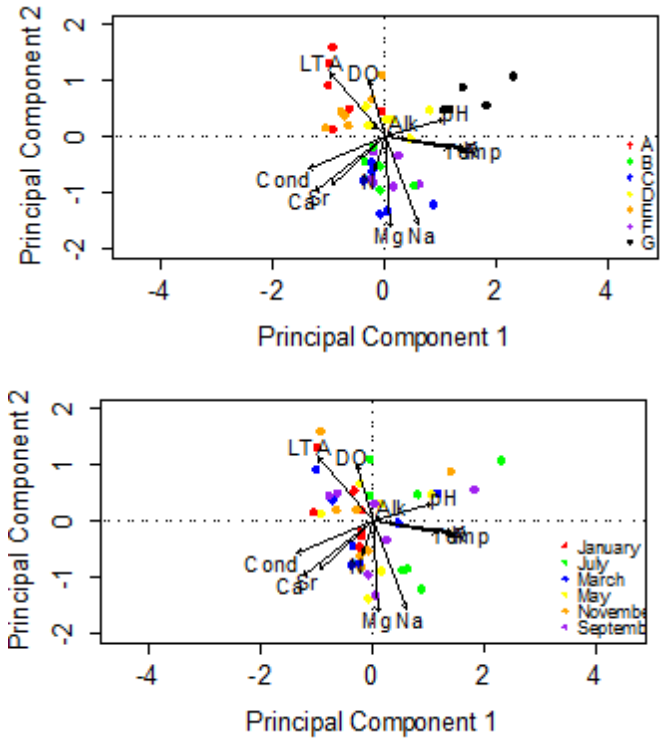


Figure 3.48 PCA ordination of transformed physiochemical and chemical results from the seven phreatic sites (November 2011 - September 2012) by site (a; top) and season (b; bottom).

3.5.1.4 Summary of Environmental Conditions across the Three Habitats

The preceding sections describe the environmental conditions of the benthic, hyporheic and phreatic habitats and suggest that they each provide distinctive environmental conditions and stability with regard to physiochemical parameters; however, both nutrients and geochemistry are similar by depth.

Considering the physiochemical and chemical results from all three habitats over the final year of the study, the first axis of the PCA, which explains 34% of the variability, reflects the relative contribution of groundwater, with variables associated with headwater sites located to the western edge of the catchment (conductivity, geochemistry) recording positive values and those associated with surface water dominated sites to the eastern side of the catchment (pH) recording negative values (Figure 3.49).[12] The resulting ordination shows some clustering of the phreatic sites but a great deal of overlap between the results from the three habitats, suggest that, while environmental conditions do differ between habitats (especially for the physiochemical variables), they provide a

[12] Eigenvalues for axes 1-4: 2.560; 1.948; 1.258; 1.174 and proportion of variance explained for axes 1-4: 0.233; 0.177; 0.114; 0.107.

continuum between habitats. These results may be attributed to the high degree of groundwater-dominance in this catchment or that there is mixing within the aquifer which masks variations, as has been found in other Chalk catchments in southern England (Darling et al., 2012). This relative homogeneity provides stability to the study area, reflected in the minimal temporal variability across all three habitats despite periods of abnormal conditions.

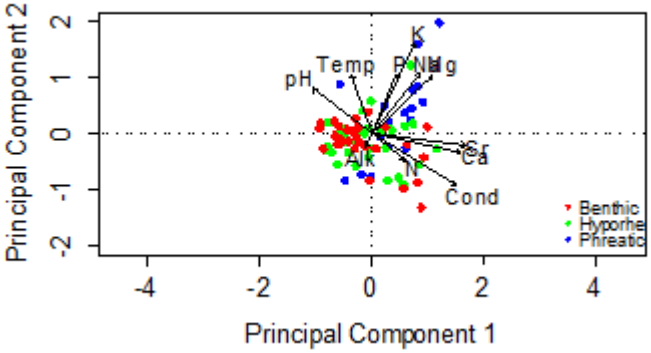


Figure 3.49 PCA ordination of transformed environmental results from the benthic, hyporheic and phreatic habitats (November 2011 to September 2012)

3.5.2 Did periods of environmental disturbance occur during the study?

Assessment of external monitoring data indicates that two abnormal periods of rainfall resulted in disturbance events during the study period which influenced the environmental conditions in the study area. These disturbances include spate conditions during spring 2010 and drought conditions in 2011-2012. Contextualising these results within the long-term records for this study area suggests that the spate flows were particularly notable in their intensity, while the drought was similar, if less intense than previous low-flow events.

3.5.5 Summary

This chapter has described the physiochemical, chemical and physical environmental conditions of the benthic, hyporheic and phreatic habitats and assessed their spatial and temporal variability. The results are characteristic of a chalk stream environment and are consistent with long-term monitoring in this catchment. As expected, the results indicate that each habitat provides distinctive physiochemical conditions; however, chemical conditions are similar throughout all habitats and may reflect the dominance of groundwater contribution to this catchment. These conditions vary spatially, particularly with reference to the location of the site within the catchment as well as temporally,

particularly with reference to seasonality and groundwater contribution. The results were also considered within the context of long-term records to identify periods of abnormal conditions, and indicated that two distinct disturbance events occurred over the course of this study. Further analysis of the biological communities occupying the benthic, hyporheic and phreatic habitats and their relationship to environmental conditions is considered in Chapter 4. Further analysis of the responses of these communities to the disturbance events is undertaken in Chapter 5.

Chapter 4

Distribution of biological communities in benthic, hyporheic and phreatic habitats

4.1 Introduction

This chapter describes the biological communities recorded in benthic (Section 4.2), hyporheic (Section 4.3) and phreatic (Section 4.4) habitats throughout the study. The results are used to describe the individual species and communities occupying these habitats, how they vary, and to identify environmental conditions which influence their distribution. The results were considered for each habitat independently and collectively within a framework of research questions (Section 4.5).

4.2 Communities Recorded in Benthic Habitats

Samples from the benthic habitat were collected at five riverine sites on the Little Stour (Sites 1, 9, 10 and 11), and Dour (Site 13), all of which were paired with hyporheic samples at the same sites (Section 2.2). Benthic samples were collected bimonthly from March 2010 to September 2012 (from September 2010 for Sites 11 and 13; Section 2.3). Samples were analysed to describe the assemblage (Section 4.2.1), determine spatiotemporal variability in its distribution (Section 4.2.2) and identify potential relationships with the environmental conditions of the benthic habitat (Section 4.2.3) before consideration within the research questions framework (Section 4.2.4).

4.2.1 Benthic Invertebrate Community Description

Over 6,000 individuals representing 67 macroinvertebrate taxa were recorded from 68 benthic samples over the study period. *Gammarus pulex* dominated the benthic invertebrate community, accounting for 25% of total invertebrate abundance. The four further macroinvertebrate taxa comprising the largest proportions of the community were: *Agapetus fuscipes* (16%); Chironomidae (8%); *Asellus aquaticus* (5%); and *Baetis rhodani* (4%). While no stygobiontic organisms were recorded at these sites during the main sampling programme, one *Niphargus aquilex* (Site 8, March 2009) and two eyeless *Gammarus* sp. (Site 1, May 2009) were collected during the pilot study.

The composition of the benthic invertebrate community included a number of species typical of chalk streams (such as *A. fuscipes* and *Drusus annulatus*) as well as a variety of species associated with different hydrological conditions, from intermittent (larvae of *Helodes* sp.) to perennial flow (*B. rhodani, Elmis aenea* and *Piscicola geometra*; Smith et al., 2003; Townsend et al., 1983; Wood

et al., 2005a). The taxonomic composition of the benthic community is similar to that recorded previously during routine monitoring undertaken by the Environment Agency (Little Stour and Dour catchments, 1966-2012) or from the literature (Holmes, 2006; Stubbington et al., 2005; Stubbington and Wood, 2013; Stubbington et al., 2015; Wood et al., 1998). However, a number of new taxa for this catchment were recorded at Site 1 which is located in the headwaters and has not been routinely surveyed previously. The new records include the Dytiscid beetles *Agabus didymus* and *Hygrotus (Coelambus) confluens* and the Hydrophilid beetle *Anacaena limbata (ovata)* which are associated with temporary waters and are commonly recorded in southern England (Eyre et al., 1990; Jefferies, 2003). One novel species, *Gammarus* sp., was also recorded in the benthic community and is discussed in Chapter 6.

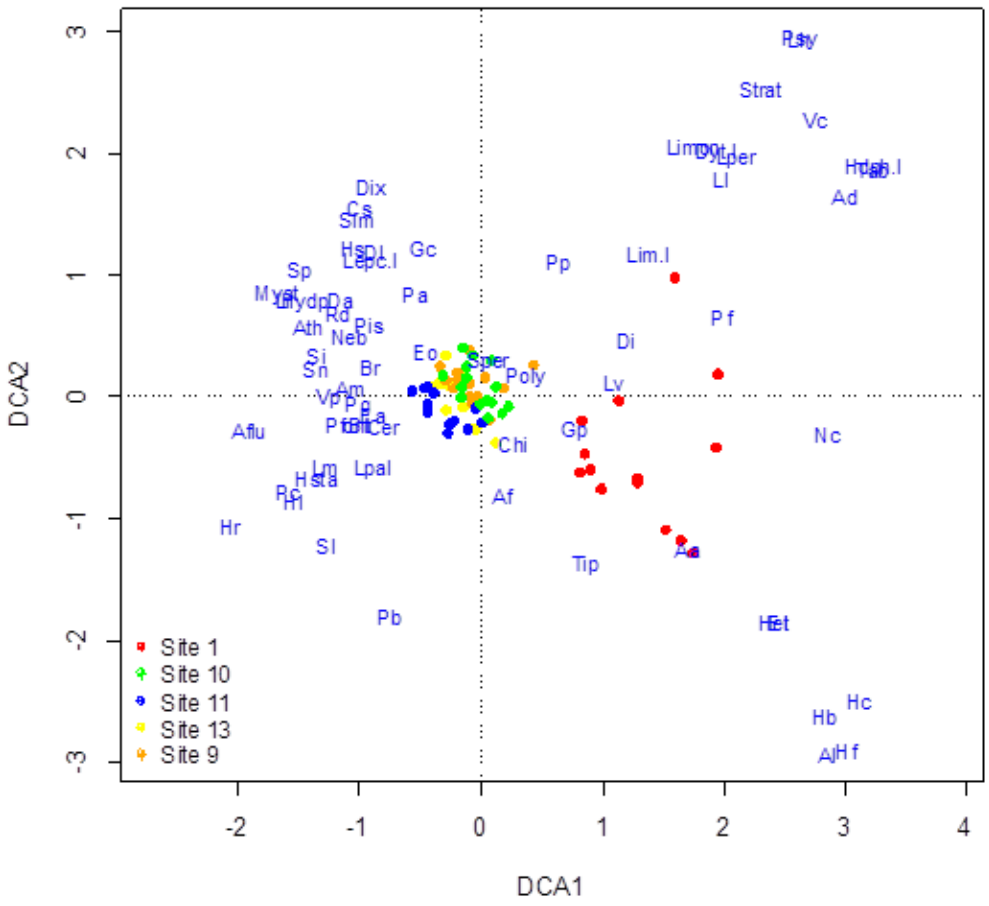


Figure 4.1 Detrended correspondence analysis of macroinvertebrates collected from benthic habitats from March 2010 to September 2012 (species abbreviations are listed in Appendix I).

Detrended correspondence analysis (DCA) was used to explore variability in the benthic invertebrate community. The first axis of the resulting ordination (Figure 4.1), which explained 23% of this variation, suggests a longitudinal gradient in

which higher scores are related to species recorded at Site 1 (such as *Nemoura cinerea* (Nc) and Hydrophilidae larvae (Hdph.l)), some of which are associated with temporary waters (such as *A. didymus* (Ad) and *H. confluens* (Hc); or can persist during periods of low flow (such as *Helophorus brevipalpis* (Hb) and *A. limbata (ovata)* (Al); Stubbington et al., 2009a; Williams 2006).[13] Lower scores were related with species only recorded at the furthest downstream site (Site 11; *Halesus radiatus* (Hr) and *Ancylus fluviatilis* (Aflu)). The results from Site 13 (on the Dour) plot near the centre of the ordination, suggesting this community is similar in composition to the middle reach of the Little Stour.

4.2.2 Benthic Community Variability

Data exploration using community metrics suggests spatial and temporal variance in abundance and diversity (richness). Scores were highest during the spring and early autumn and lowest during late autumn and winter. This suggests that the largest number and diversity of invertebrates use the benthic habitat during periods of relatively stable discharge (Figures 4.2 and 4.3). Spatially, Site 1 differs from this pattern as abundance and diversity peaked during high summer and were lowest immediately after this location dried completely in November 2011.

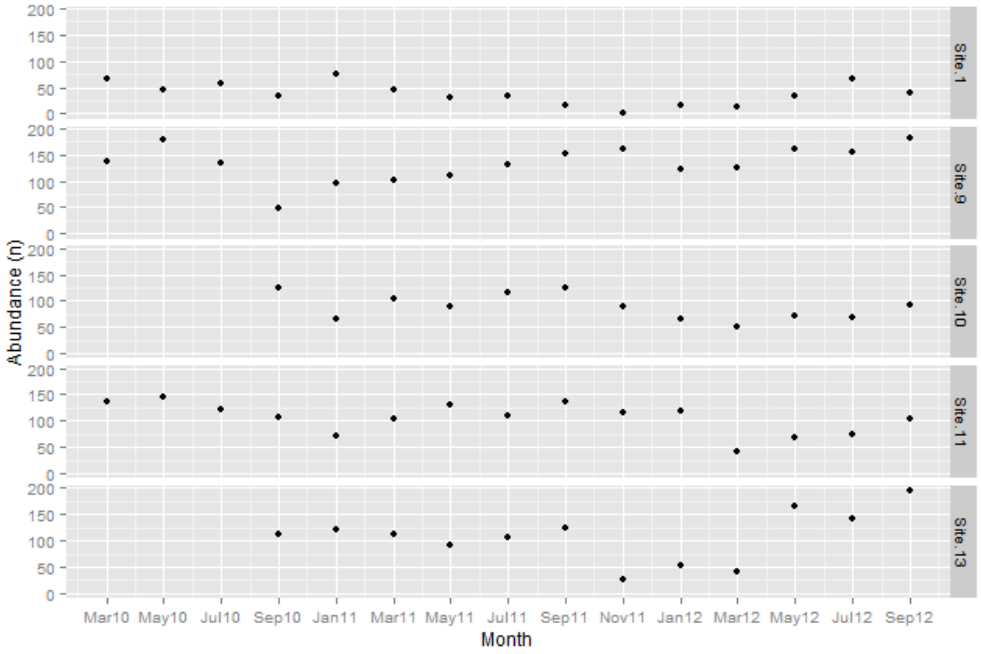


Figure 4.2 Macroinvertebrate abundance (n) recorded in benthic samples from March 2010 (Sites 1, 9 and 10) or September 2010 (Sites 11 and 13) to September 2012. No samples were collected at any Site in November 2010. Site 1 was dry in November 2011.

[13] Eigenvalues for the first four axes: 0.231; 0.130; 0.100; 0.082 and associated axis lengths: 2.522; 2.263; 2.717; 1.356

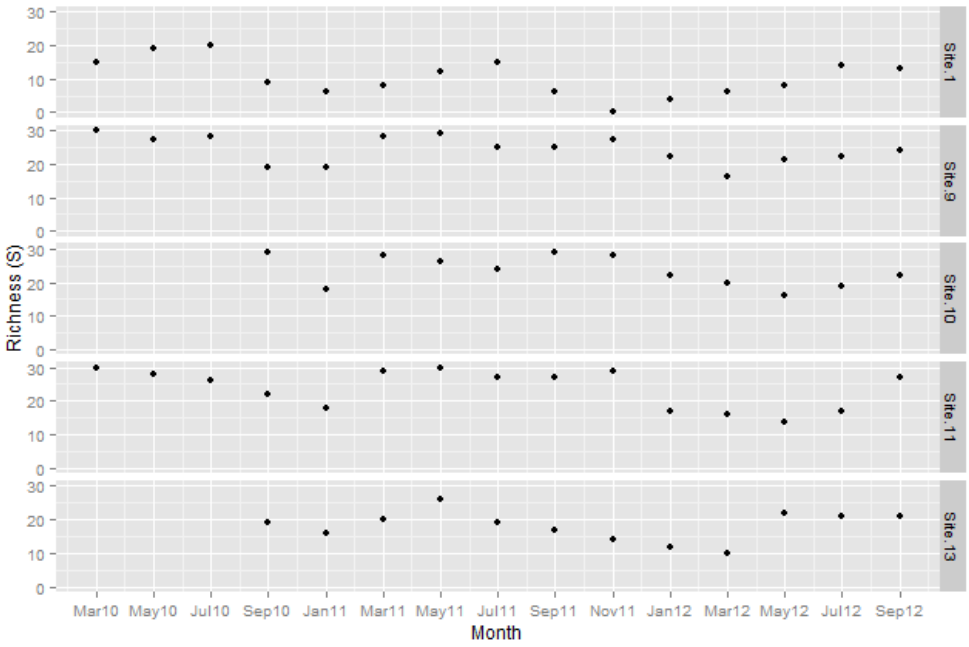


Figure 4.3 Macroinvertebrate richness (S) recorded in benthic samples from March 2010 (Sites 1, 9 and 10) or September 2010 (Sites 11 and 13) to September 2010. No samples were collected at any Site in November 2010. Site 1 was dry in November 2011.

4.2.2.1 Spatial variability of the Benthic Macroinvertebrate Community

Macroinvertebrates were recorded at all sites on all sampling occasions over the study period, except at Site 1 when it dried completely in November 2011. Four community metrics were calculated to assess variability in the spatial distribution of this community (Table 4.1). Significant spatial variance was observed for abundance (F=33.54; p=0.001) and all diversity metrics (S: F=33.57; p=0.001; H: F=22.82; p=0.001; and D: F=16.61; p=0.001), indicating site-level influences on the composition of this macroinvertebrate community.

Table 4.1 Benthic macroinvertebrate community and diversity (Shannon-Wiener (H) and Simpsons (D)) metrics (Sites 1, 9 and 10, March 2010-September 2012; Sites 11 and 13, September 2010 – September 2012) presented as the study average ± 1 SE. Spatial change was assessed using a One-Way Analysis of Variance (ANOVA), in which ** indicates p<0.001, * p<0.05 and ns p>0.05.

Metric	Site					Spatial Change
	1	9	10	11	13	
Abundance (n)	38.27 (±5.64)	133 (±9.06)	88.75 (±7.12)	105 (±7.61)	107.25 (±14.32)	**
Richness (S)	10.34 (±1.47)	24.13 (±1.08)	23.42 (±1.32)	23.8 (±1.50)	18.08 (±1.31)	**
Diversity (H')	1.67 (±0.20)	2.51 (±0.06)	2.51 (±0.91)	2.42 (±0.09)	2.25 (±0.05)	**
Diversity (D)	0.68 (±0.07)	0.88 (±0.01)	0.86 (±0.02)	0.85 (±0.02)	0.86 (±0.01)	**

While abundance and diversity are similar between sites 9, 10, 11 and 13 (with no significant difference found between the Little Stour and Dour), site 1 is a notable exception as all metrics were consistently lower. This is likely a reflection of both the limitations of the physical capacity of this relatively small and isolated location (which lacks hydrological connection with the main river during most years) and the indirect difficulties for some species to persist at this location without specific adaptations to withstand periods of low (or no) flow. These results are consistent with previous research that suggests habitats in intermittent headwater and spring systems (such as site 1) support lower diversities than those located in perennial locations (Wood et al., 2005a).

Between all sites, changes in abundance were driven principally by fluctuations in the number of *Gammarus pulex* individuals, a species recorded at all sites in all collected samples with abundances ranging from 1 to 164 individuals. While the diversity metrics are similar between Sites 9, 10, 11 and 13, there are notable differences in the composition of these assemblages which reflect site-specific influences on the distribution of individual species. Specifically, the damselfly *Calopteryx splendens* was only recorded at Site 10 and is likely to be associated with the more abundant riparian vegetation at this location (Hofmann and Mason, 2005) and the caddisfly *Halesus radiatus* was only recorded at Site 11, likely a reflection of the relatively deeper water and available pool habitat preferred by this species at this downstream location (De Brouwer et al., 2016).

4.2.2.2 Temporal Variability of the Benthic Macroinvertebrate Community

Four community metrics were also calculated to assess variability in the temporal distribution of the benthic community. Although the previous results suggest seasonal patterns at the individual sites (Figure 4.2 and 4.3), the results indicate that neither abundance nor diversity varied significantly over the study period (Table 4.2). These results are surprising given the significant variability in physiochemical conditions, particularly temperature and dissolved oxygen, at these sites over the study period, and specifically during the periods of spate and drought flows (Section 3.4).

Table 4.2 Benthic macroinvertebrate community and diversity (Shannon-Wiener (H) and Simpsons (D)) metrics for samples collected from September 2010 to September 2012 (March-July 2010 were excluded as the number of sites sampled was not comparable). All results presented as the study average ± 1 SE. Temporal change was assessed using a One-Way ANOVA (**=p<0.001, * =p<0.05 and ns=p>0.05. Temporal variability was insignificant for all metrics.

Sampling Occasion	Metric							
	Abundance (n)		Richness (S)		Diversity (H')		Diversity (D)	
Sep-10	85.8	(±18.4)	19.6	(±3.22)	2.34	(±0.20)	0.86	(±0.03)
Jan-11	85.6	(±10.5)	15.4	(±2.4)	1.95	(±0.27)	0.75	(±0.09)
Mar-11	93.8	(±11.8)	22.6	(±4.00)	2.18	(±0.31)	0.77	(±0.08)
May-11	90.4	(±16.9)	24.6	(±3.25)	2.58	(±0.08)	0.89	(±0.01)
Jul-11	98.6	(±16.9)	22	(±2.19)	2.42	(±0.06)	0.86	(±0.02)
Sep-11	111.0	(±24.1)	20.8	(±4.22)	2.29	(±0.29)	0.83	(±0.06)
Nov-11	78.4	(±30.0)	19.6	(±5.61)	2.16	(±0.55)	0.73	(±0.18)
Jan-12	75.0	(±20.4)	15.4	(±3.4)	1.89	(±0.32)	0.73	(±0.09)
Mar-12	54.6	(±19.1)	13.6	(±2.48)	2.18	(±0.17)	0.87	(±0.02)
May-12	100.2	(±26.6)	16.2	(±2.54)	1.91	(±0.18)	0.74	(±0.05)
Jul-12	101.6	(±19.4)	18.6	(±1.44)	2.29	(±0.09)	0.84	(±0.02)
Sep-12	122.6	(±29.1)	21.4	(±2.34)	2.39	(±0.06)	0.87	(±0.01)
Temporal Change	ns		ns		ns		ns	

Macroinvertebrate abundance was lowest in March 2012 and highest in September 2012, coinciding with the peak and post-drought periods (respectively), though these differences were not significant (Figure 4.4; Section 3.4). The range of abundances recorded during each sampling occasion, as well as the number of outliers suggests that while median abundance is relatively stable over the study period, there are large fluctuations in the abundance of particular species at individual sites. This is most notable in September 2012, when some of the lowest as well as the highest levels of abundance were recorded and was attributed to fluctuations in *G. pulex*.

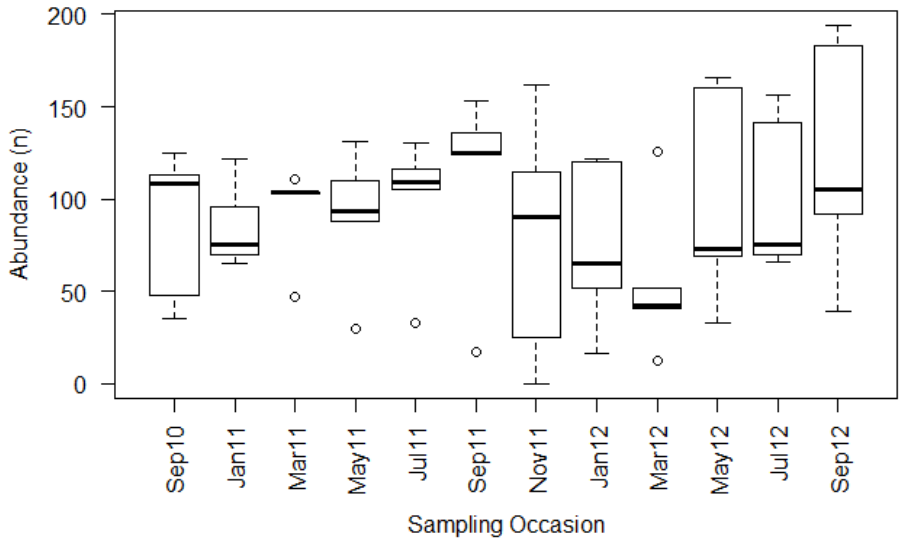


Figure 4.4. Benthic macroinvertebrate abundance (n) at Sites 1, 9, 10, 11 and 13 from September 2010 to September 2012 (March-July 2010 excluded as incomparable). The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

Similarly, macroinvertebrate diversity follows an expected seasonal pattern during the first portion of the study period, with the highest values recorded during the spring and autumn, before declining during the drought and then recovering, with median values recorded in September 2012 similar to those in September 2010 (Figure 4.5). Although not significant, these results suggest a response to changing environmental conditions beyond expected seasonality.

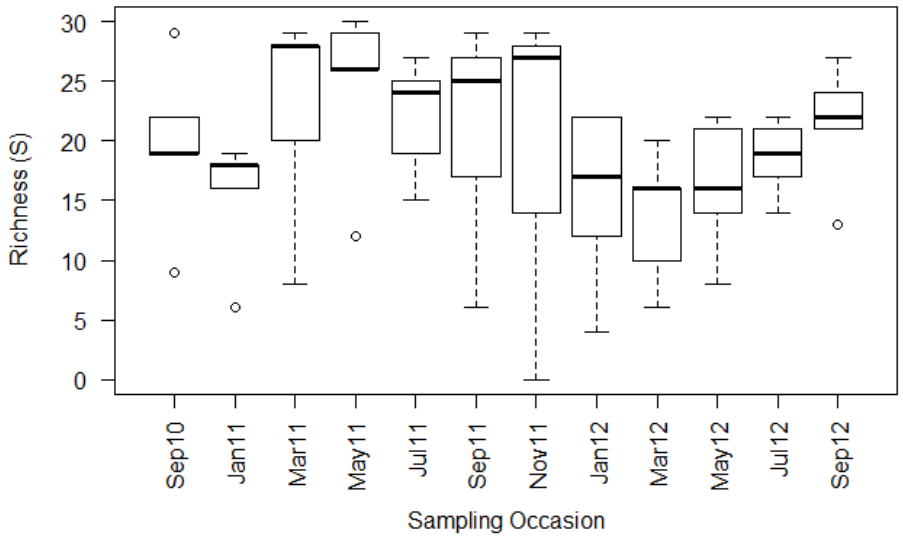


Figure 4.5 Benthic macroinvertebrate richness at Sites 1, 9, 10, 11 and 13 from September 2010 to September 2012 (March-July 2010 excluded as the number of sites is incomparable). The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

4.2.3 Benthic Assemblages in relation to Environmental Parameters

Multivariate analyses and ordination were used to investigate the relationships between the benthic macroinvertebrate assemblages and the environmental conditions of this habitat. The results are discussed with reference to the community as a whole and to individual species.

4.2.3.1 Benthic Community in Relation to Environmental Parameters

The Detrended Correspondence Analysis (DCA) suggests that it is not clear if the results from the benthic habitat are linear or unimodal as the longest axis value was of intermediate length (Section 4.2.1); as such, direct gradient analyses were tested using both linear and unimodal methods to determine the best fit (Leps and Smilauer, 2003; ter Braak and Smilauer, 2002). In both cases, a constrained approach (Redundancy Analysis (RDA) and Canonical Correspondence Analysis (CCA)) was selected over an associated unconstrained method (such as Correspondence Analysis) as this facilitated extraction of variation explained by the measured environmental parameters (Leps and Smilauer, 2003). In both cases, abiotic variables were tested for normality using the Shairo-Wilk test and were square-root transformed prior to analysis. In addition, due to the high number of null values in the biological results, both models were run with (log(x+1)) transformed count data to enhance fit (Zuur et al., 2010). Both models were run twice, first to account for all of the biological and physiochemical results recorded over the study period (2010-2012) and again to account for the biological, physiochemical and chemical results which were only available during the final year of the study. The results of these parallel analyses indicate that while both the RDA and CCA models were significant and provided similar results, the RDA produced a better fit and is therefore discussed below.

Considering biological and physiochemical data collected throughout the study period, the RDA explains 30% (r^2=0.295) of the species variance, with the first two axes explaining 22% of this variance.[14] The model fit was tested using an analysis of variance which indicated that it was significant (F=2.590; p=0.001). The importance of individual variables was assessed using Monte Carlo

[14] Eigenvalues for axes 1-4: 0.539; 0.095; 0.072; 0.049 and proportion explained for axes 1-4: 0.188; 0.033; 0.025; 0.017; total inertia= 2.861 (constrained=0.845; unconstrained=2.016); total proportion = 1.00 (constrained = 0.295; unconstrained=0.705)

permutation testing, which indicated that discharge (F=8.992; p=0.001); temperature (F=5.875; p=0.001); dissolved oxygen (F=3.634; p=0.001); conductivity (F=1.982; p=0.035); and alkalinity (F=2.334; p=0.023) were significant (Legendre and Legendre, 2012). The model was re-run omitting the environmental variables which were not significant (turbidity: F=1.222; p=0.221 and pH: F=1.094; p=0.299) but this did not enhance the fit. Due to the marked differences in community composition at Site 1 (Section 4.2.1), the model was also re-run omitting the results from this Site; however, this did not improve the fit of the model.

The resulting ordination reflects the importance of the measured physiochemical variables as well as the spatial influence of site locations within the catchment on the distribution of species in the benthic habitat (Figure 4.6). The arrangement of the vectors and the relatively long lengths of discharge (Q), dissolved oxygen (DO2) and conductivity (Cond) reflect their importance in explaining benthic community variability. The inverse relationship between pH and conductivity as well as between dissolved oxygen and alkalinity reflects a longitudinal spatial influence. Both pH and dissolved oxygen were lower in the headwaters and increased with distance downstream (Sections 3.2.2 and 3.2.4), while conductivity and alkalinity were highest in the headwaters and decreased downstream (Section 3.2.3 and 3.2.5). This spatial distribution is also reflected in the clustering of sites, most notably with the headwater community recorded at Site 1. The clustering of taxa near the centre of the ordination reflects the high degree of overlap between samples. However, the ordination also suggests some species-specific relationships, such as between *Serratella ignita* (Si) and discharge (in which *S. ignita* is positively associated with higher discharges), and between Chironomidae (Chi) and pH (in which higher abundances were recorded at the downstream sites where pH was higher). The results of the RDA suggest that while the measured variables do not fully explain the distribution of macroinvertebrates in this habitat, they reflect the high degree of spatial variability in this community.

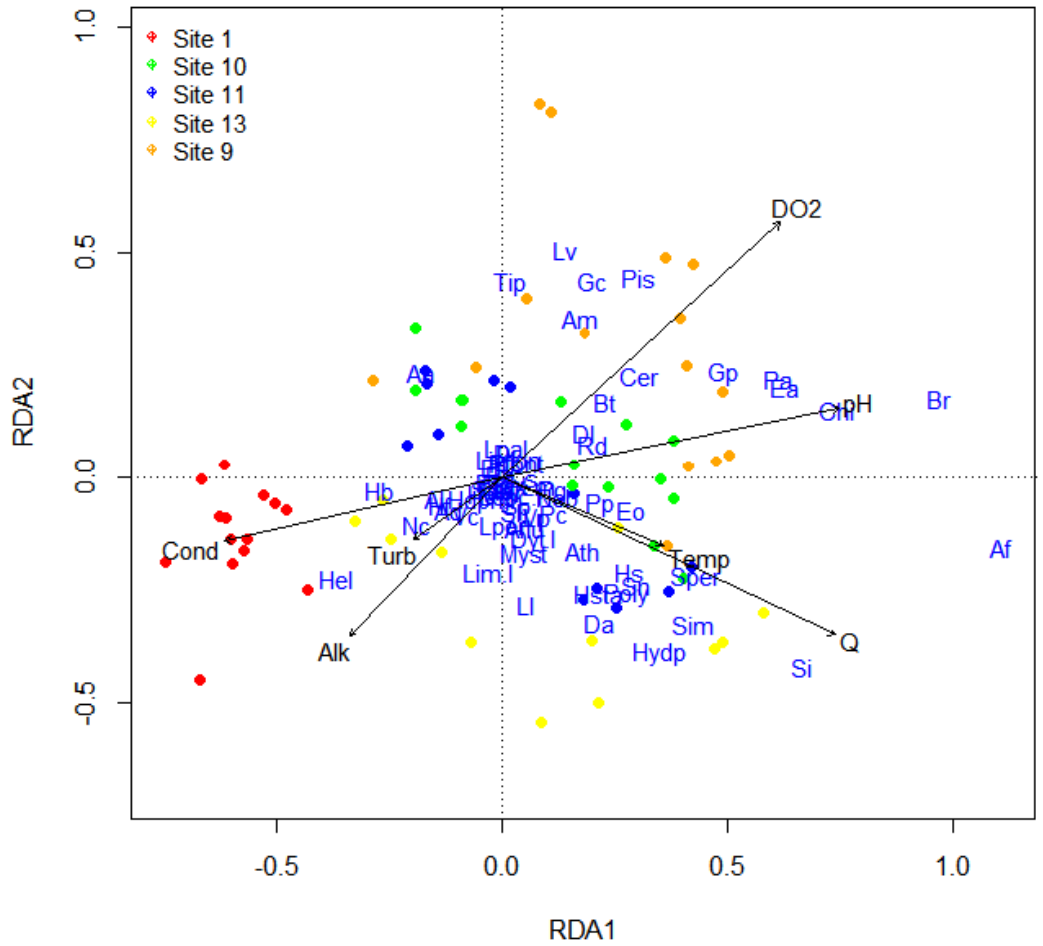


Figure 4.6 RDA triplot of macroinvertebrate species (blue, transformed count data after Appendix I) ordination with respect to physiochemical environmental variables (vectors, square-root transformed; September 2010-2012).

A second RDA was fitted to assess biological, physiochemical and chemical data collected from the benthic habitat during the final year of the study using the same approach (Figure 4.7). Considering all variables, the model explained 63% (r^2=0.625) of species variance (with the first two axes explaining over half of this variance).[15] As previous, the fit of the model was tested using an analysis of variance that indicated it was significant (F=1.669; p=0.006) and the importance of individual variables was assessed using Monte Carlo permutation testing which indicated that discharge (F=4.622; p=0.001), temperature (F=3.717; p=0.004) and dissolved oxygen (F=2.219; p=0.043) were significant (Legendre and Legendre, 2012). The model was re-run omitting the environmental variables which were not significant and the samples from Site 1, but neither enhanced the fit. The RDA suggests that while the measured

[15] Eigenvalues for axes 1-4: 0.607; 0.310; 0.166; 0.139 and proportion of variance explained for axes 1-4: 0.236; 0.121; 0.065; 0.033; total inertia= 2.57 (constrained=1.606; unconstrained=0.963); total proportion = 1.00 (constrained = 0.625; unconstrained=0.375)

variables do not fully explain the distribution of the invertebrate community, they do account for more of the variance than the first RDA; however, as none of the chemical variables were found to be significant, this is likely a result of the increased influence of physiochemical variables (especially those related to the drought period such as discharge, temperature and dissolved oxygen) during the final year of the study rather than the inclusion of chemistry. It is also important to note that the two time periods differ in the assemblage considered as some species, such as *Calopteryx splendens* (Cs), *Agabus didymus* (Ad), and *Hygrotus (Coelambus) confluens* (Hc), were absent from the final year of the study and others, such as *Potamopyrgus antipodarum* (Pa) and *Elmis aenea* (Ea), recorded restricted spatial distributions following autumn 2011.

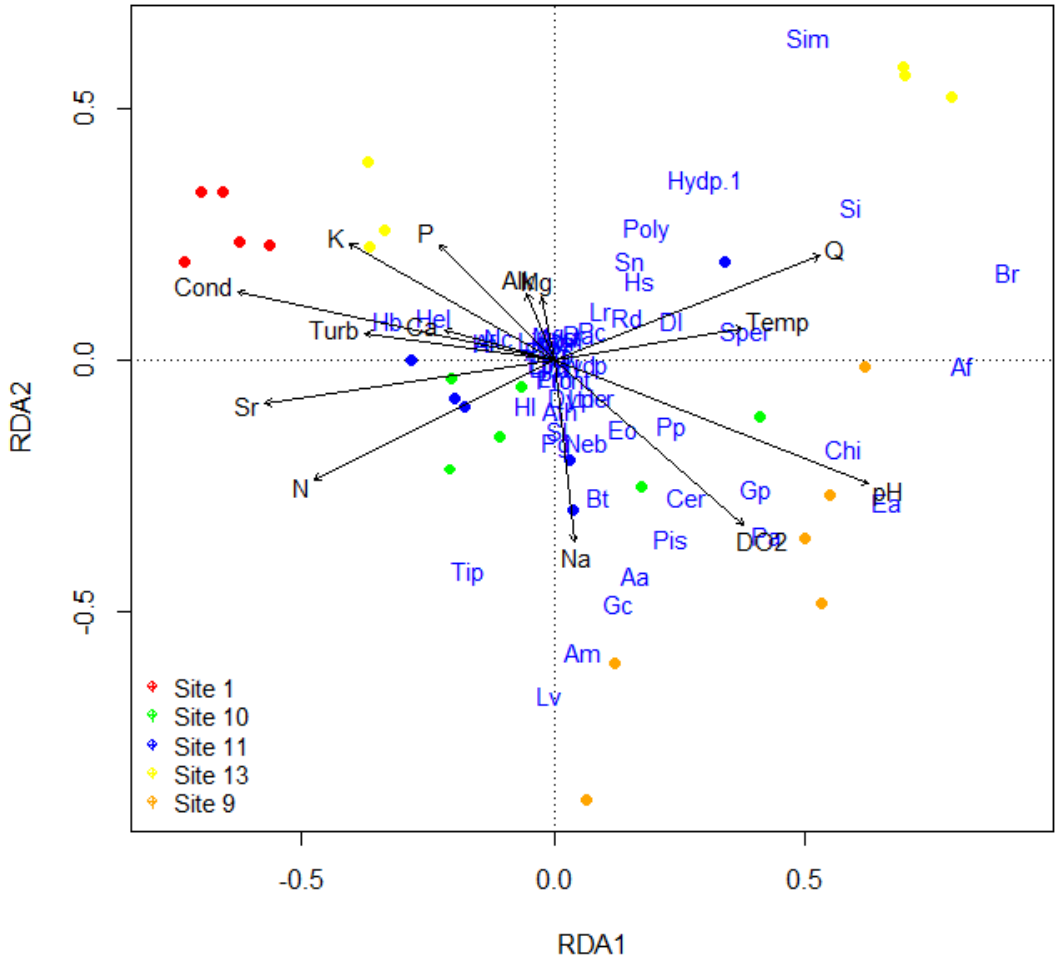


Figure 4.7 RDA triplot of species (blue, transformed count data) ordination with respect to physiochemical and chemical environmental variables (vectors, square-root transformed) from the final year of the study (November 2011-September 2012).

The resulting ordination, which considers all physiochemical, chemical and biological results from the final year of the study, reflects the importance of the measured variables as well as the spatial influence of site locations within the catchment on the distribution of species in this habitat. The ordination is similar

to the previous RDA in the distinctive clustering of sites (particularly Site 1), species-specific relationships (such as between *S. ignita* and high discharge) and in the influence of longitudinal distribution between the headwaters and downstream sites (reflected by the inverse relationship between pH and conductivity as well as dissolved oxygen and alkalinity), suggesting that these patterns were constant throughout the study. However, the second model suggests additional relationships, such as a closer association between high discharge and flow-sensitive species (such as *Baetis rhodani* (Br)) and an inverse relationship between *Agapetus fuscipes* (Af) and turbidity which supports previous research on the Little Stour which found that *A. fuscipes* is sensitive to fine sediment deposition (Brittain and Saltveit, 1989; Wood and Armitage, 1999). These relationships are likely to have been exacerbated by the low flows experienced during the final year of the study. Although not significant, some of the associations between the measured variables are also of interest, such as the inverse relationship between discharge (Q) and strontium (Sr) which likely results from differences in surface water and groundwater respectively, with the latter expected to contain higher concentrations of Sr from the Chalk aquifer which would be diluted at locations with higher contributions of surface water or during periods of higher flows (Smedley et al., 2003).

4.2.3.2 Benthic Species in relation to Environmental Parameters

Generalized linear models (GLMs) were used to assess the relationship between environmental variables and the distribution of the most dominant individual species recorded in the benthic habitat. As this assessment was undertaken using untransformed biological count data, the models were initially fitted using a Poisson reference distribution; however, while this was appropriate for *Agapetus fuscipes*, due to over-dispersion caused by the high variance in the results for *Gammarus pulex, Asellus aquaticus* and *Baetis rhodani*, both a quasi-Poisson and a negative binomial error structure were also tested for these species, the latter of which was found to provide the best fit (Table 4.3; Leps and Smilauer, 2003). GLM performance was tested using all variables but was found to be enhanced by omitting geochemical and nutrient variables. The results indicate that each of these species respond to differing environmental conditions which are discussed below.

Table 4.3 GLMs of key taxa and square-root transformed environmental variables. Fit was tested using an ANOVA and Chi-Square test (α=0.05). Over-dispersion was tested by dividing the residual deviance by residual degrees of freedom. Significant results (p<0.05) are in bold.

Variable	*Gammarus pulex*	*Agapetus fuscipes*	*Asellus aquaticus*	*Baetis rhodani*
Temperature	0.290	0.078	0.963	**0.002**
Conductivity	0.330	0.539	0.543	**0.015**
Alkalinity	0.875	0.517	**0.004**	**0.001**
pH	0.960	0.955	0.309	0.494
Discharge	0.208	**0.050**	**0.001**	**0.001**
Turbidity	**0.003**	0.101	0.758	0.878
DO2	**0.023**	0.136	0.257	0.108
Goodness of Fit	0.082	0.205	0.064	0.262

Gammarus pulex was recorded at all sites on all occasions in abundances of 1 to 68 individuals (except Site 1 in November 2011). Abundance was highest in early 2012 (Sites 9 and 10) and lowest in September 2011 at Site 1, coinciding with periods of high and low flow respectively (Figure 4.8; Section 3.4.1). Periods of low abundance in the benthic habitat corresponded with an increase in abundance in the hyporheic habitat (discussed further in Sections 4.3 and 4.5). The periodicity of these episodes suggests that such changes are not associated with life history or seasonality but with external environmental factors, specifically surface water discharge.

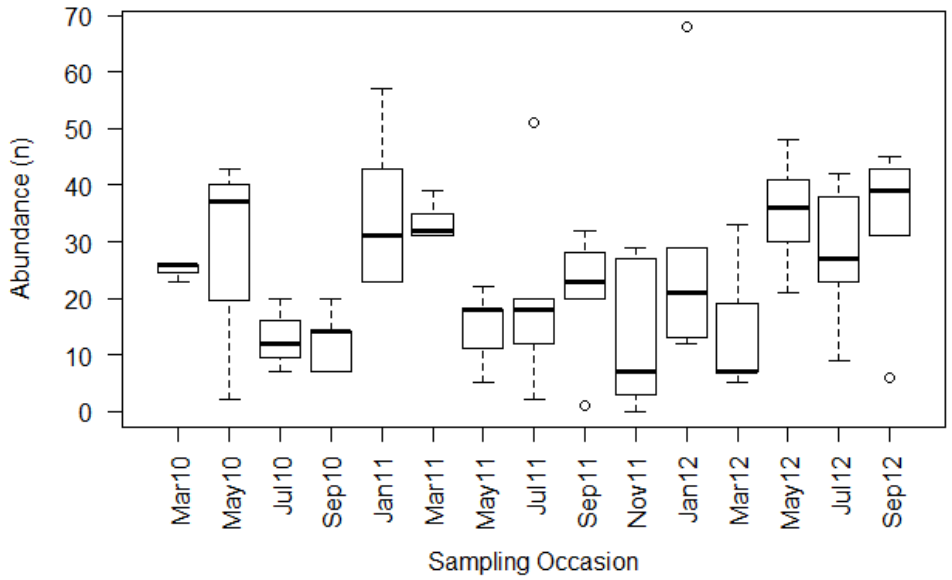


Figure 4.8 *Gammarus pulex* abundance in benthic samples from all riverine sites. Abundances from March, May and July 2010 reflect samples from Sites 9, 10 and 11 only. The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

Agapetus fuscipes was recorded at all riverine sites in abundances of up to 56 individuals (Figure 4.9). Abundance varied seasonally, and was highest during the summer months and lowest during the winter as expected for a univoltine species with summer emergence (Becker, 2005). The GLM indicates a significant relationship with flow but it is unclear if this reflects seasonal trends or a response to the drought period. Spatial differences in abundance are much lower prior to the drought but more variable just before the drought peak, a trend that continued following the drought break, suggesting that the distribution of this species was negatively influenced by the drought but recovered rapidly at some downstream sites following the resumption of normal flows (Section 3.4.1). This response is likely to be a function of the life cycle plasticity of *A. fuscipes* and its active seeking of refugia during dynamic discharge periods (Giller and Malmqvist, 1998; Nijboer, 2004). Previous research has suggested that *A. fuscipes* is a stenothermic species which is particularly vulnerable to high temperatures and climate change (Domisch et al., 2011). While it is initially surprising that no relationship was found between temperature and *A. fuscipes* distribution in this study, the work by Domisch et al. (2011) used air temperature as a surrogate for water temperature, potentially neglecting the potential of the hyporheic habitat to provide a refuge and/or the role of groundwater contributions to the benthic habitat to mitigate water temperature changes and, therefore, the distribution of this species.

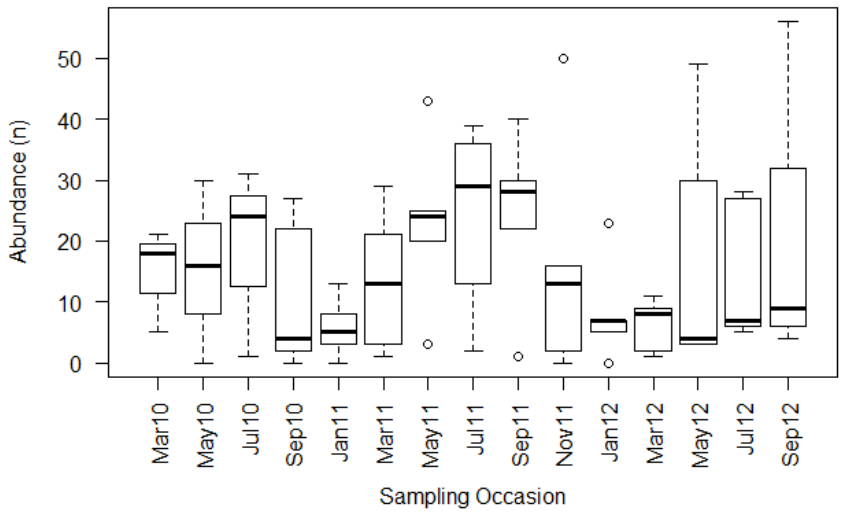


Figure 4.9 *Agapetus fuscipes* abundance in benthic samples from all riverine Sites. Abundances from March, May and July 2010 reflect samples from Sites 9, 10 and 11 only. The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

Asellus aquaticus was regularly recorded at all riverine sites in varying abundances (Figure 4.10). The GLM indicates that the distribution of *A. aquaticus* is associated with both discharge and alkalinity; however, it is not clear if this is a direct association or a response to the drought period. *Asellus aquaticus* was also regularly recorded in hyporheic samples, suggesting fluidity in habitat preferences at these sites, with vertical movement occurring both to feed opportunistically and, potentially, in response to unfavourable environmental conditions.

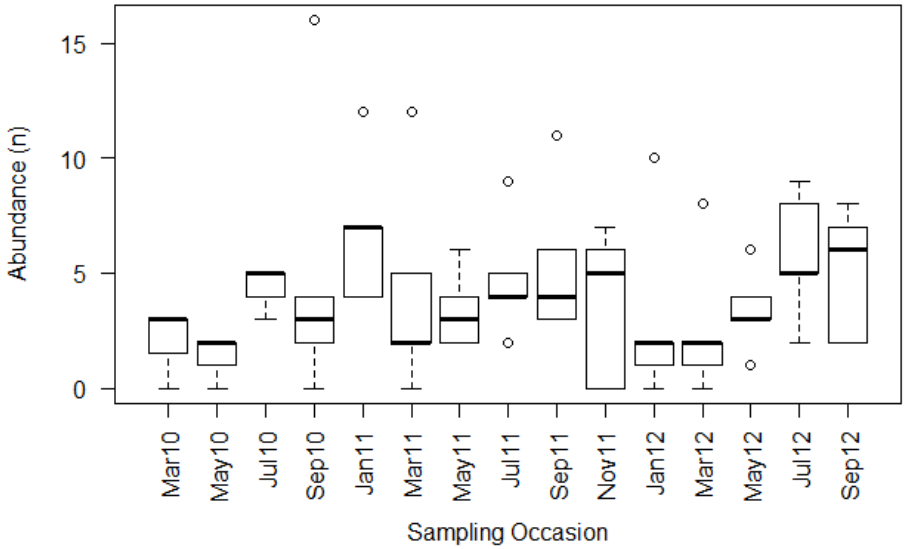


Figure 4.10 *Asellus aquaticus* abundance from benthic samples from all riverine Sites. Abundances from March, May and July 2010 reflect samples from Sites 9, 10 and 11 only. The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

Baetis rhodani was recorded on at least one occasion at each riverine site. Abundance peaked at 22 individuals (Site 13, September 2012) and followed an expected seasonal pattern with the lowest numbers recorded during the winter months (Figure 4.11; Elliott, 2013). The GLM indicates *B. rhodani* distribution is associated with discharge, temperature, conductivity and alkalinity but, as with the previous species, it is unclear if these relationships are related to expected seasonality or a response to periods of low flow. Consistently higher abundances occurred between sites during the spring and summer prior to the drought with subsequent variable distribution, suggesting that this species was negatively influenced by the period of low flow; however, it recovered rapidly in some downstream sites following the drought break. This response is likely to be a function of the life cycle plasticity of *B. rhodani* and quick drift response

which facilitates its resilience during periods of unfavourable conditions (Brittain and Saltveit, 1989). This result is similar to previous studies which found *B. rhodani* to drift during periods of low flow but recover quickly upon flow resumption (Wood et al., 2005a; Wood et al., 2005b). As no *B. rhodani* were recorded in the hyporheic habitat during this study, other longitudinal refugia, such as pools or springwells (such as Site 8) may have been utilised. Phenological studies on similar mayfly species (*Baetis bicaudatus* and *Ephemera danica*) found them to be very sensitive to changes in temperature, responding by drifting or seeking refuge in areas of cooler groundwater (Everall et al., 2015; Harper and Peckarsky, 2006). It is possible that the groundwater contribution to the sites considered in this study may facilitate the recovery of *B. rhodani* in this catchment following periods of low flow and provide a buffer to changes in water temperature.

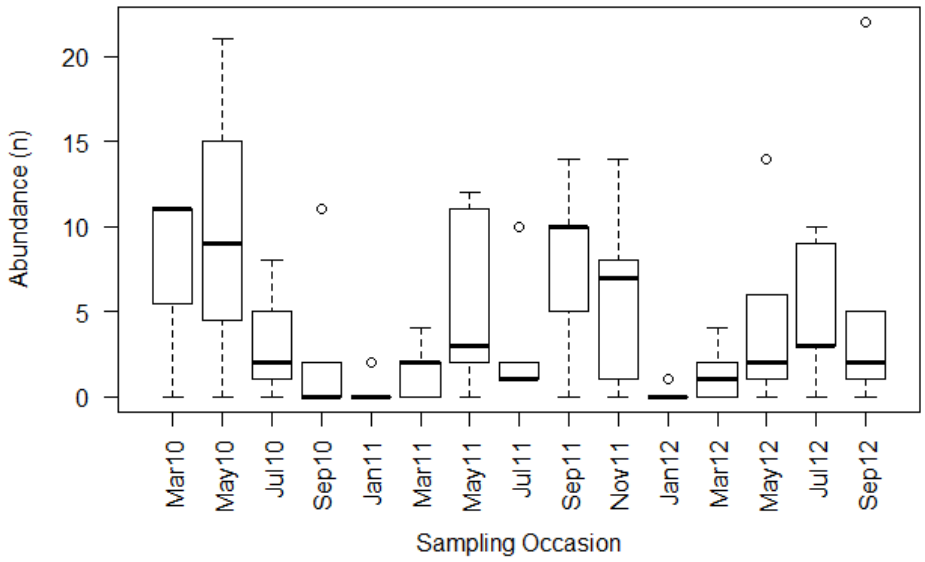


Figure 4.11 *Baetis rhodani* abundance from benthic samples from all riverine Sites. Abundances from March, May and July 2010 reflect samples from Sites 9, 10 and 11 only. The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

4.2.4 Discussion of Benthic Communities

The results presented above have been assessed within the context of current literature (Chapter 1) and the aims of this study to inform discussion of the research questions.

4.2.4.1 Can the benthic macroinvertebrate community be described?

The benthic invertebrate community comprised an assemblage of insects, beetles, crustaceans, molluscs, annelids, turbellarians and arachnids typical of a chalk stream which, with the exception of some specialist headwater species, is consistent with previous studies and long-term monitoring in this catchment (as discussed in Section 4.2.1). No stygobiontic species were recorded from the benthic habitat during the study period (although some were recorded during the pilot study). The distribution of these taxa follows a longitudinal spatial gradient from the headwaters to the downstream sites that may reflect specific environmental conditions or preferences of individual taxa.

4.2.4.2 Does the benthic community vary spatiotemporally?

The composition of the benthic community was spatially variable, reflecting the distinctive conditions between sites, but temporally stable. Both abundance and diversity varied by site, suggesting that the distribution of macroinvertebrate taxa in the benthic habitat is determined by environmental characteristics at the site (or within-site) scale. This is particularly notable in the intermittent headwaters (Site 1), which recorded consistently lower abundances and diversity than the other, perennial sites. However, both abundance and diversity were temporally stable, a result that is surprising given the seasonal life cycles of many of the recorded species and the environmental conditions recorded over the study period. The lack of temporal response to these environmental conditions, with specific reference to spate and drought flows, suggest that these were ramped (rather than stepped) disturbances, which may have provided the benthic community with an opportunity to respond to this change and recover quickly either through the utilisation of trait-based adjustments in life cycle (*Agapetus fuscipes*) or by exploiting refugia through vertical (*Gammarus pulex*) or longitudinal (*Baetis rhodani*) migration. Alternatively, it may also be a reflection of the lack of sensitivity in these metrics to assess such a response.

4.2.4.3 Are species distributions related to environmental parameters?

The results indicate that the composition of the macroinvertebrates occurring in the benthic habitat is strongly influenced by environmental parameters and that this influence increased during the drought period. Specifically, the distribution

of these species is significantly influenced by changes in discharge and related physiochemical variables (such as dissolved oxygen). In addition, the distribution of species is also influenced by variables indirectly associated with changes in discharge, including dissolved oxygen and temperature.

4.2.4.4 Summary of the Benthic Community

As expected, the macroinvertebrate assemblage recorded in the benthic habitat comprised a diverse range of surface-dwelling taxa typical of lowland chalk stream environments. The distribution of this community was directly influenced by discharge and associated environmental variables, particularly dissolved oxygen and temperature, most notably during the final year of the study which coincided with a drought period. The community was dominated by species with traits that facilitated their persistence (either through life cycle adaptations or migration) during periods of flow pressure; however, the influence of these environmental variables was site and species-specific.

4.3 Communities Recorded in Hyporheic Habitats

Samples from the hyporheic habitat were collected at five riverine sites on the Little Stour (Sites 1, 9, 10 and 11), and Dour (Site 13), all of which were paired with benthic samples collected at the same sites (Section 2.2). Hyporheic samples were collected bimonthly between March 2010 and September 2012 using a Bou-Rouch pump (from September 2010 for Sites 11 and 13; Section 2.3). The results were analysed to describe the community (Section 4.3.1), determine spatiotemporal variability in this distribution (Section 4.3.2) and identify potential relationships with the environmental conditions of this habitat (Section 4.3.3), before consideration within the research questions framework (Section 4.3.4). Due to the difficulty of sampling this habitat, other methods were trialled but were found to be unsuitable (Section 2.3.1), while the results of these alternative methods have not been analysed further, they are of contextual interest and are presented in Section 4.3.1.1.

4.3.1 Hyporheic Invertebrate Community Description

Over 1000 individuals representing 21 invertebrate taxa of both surface and groundwater affiliations were recorded from 68 hyporheic samples. The community recorded in the hyporheic habitat comprised a mixture of normally

benthic species which only occur in this habitat by accident (stygoxenes), occasional hyporheos which have an affinity with this habitat (stygophiles) and groundwater specialists (stygobionts). *Gammarus pulex* dominated the hyporheic macroinvertebrate community, accounting for 72% of total abundance (Table 4.4). The three further taxa comprising the largest proportions of the community were: *Agapetus fuscipes* (9%); Chironomidae (6%); and Elmid beetles (*Elmis aenea*, *Limnius volckmari* and *Oulimnius*; 3%). Three stygobiontic crustacea (*Crangonyx subterraneus*, *Niphargus aquilex* and *N. fontanus*) were recorded in the hyporheic habitat and comprised 1.6% of total macroinvertebrate abundance. Four meiofauna taxa, which have not been considered in further analysis, accounted for 4% of this community.

Table 4.4. Description of taxa recorded in hyporheic habitats (March 2010 to September 2012 (n=68)). Mean density = (n individuals/n litres per Site)/ number of samples); modified after Datry et al., (2005). Meiofauna have not been included in further statistical analyses.

	Taxon	Community Dominance (%)	Mean Density (n/L)	Abundance Range (n)	Positive Samples (%)
Stygo-biont	*Crangonyx subterraneus* (Cs)	0.4	<0.01	1-4	3
	Niphargus aquilex (Na)	0.7	<0.01	1-3	9
	Niphargus fontanus (Nf)	0.5	<0.01	3-3	3
Stygox-ene	*Erpobdella octoculata* (Eo)	0.3	<0.01	1-2	3
	Dicranota (Di)	0.1	<0.01	1-1	1
	Serratella ignita (Si)	0.1	<0.01	1-1	1
Stygophile	*Agapetus fuscipes* (Af)	8.5	0.06	1-18	43
	Asellus aquaticus (Aa)	1.8	0.01	1-3	21
	Chironomidae (Chi)	6.1	0.04	1-5	56
	Limnius volckmari (Lv)	0.7	<0.01	1-3	9
	Oulimnius (Oul)	0.3	<0.01	1-1	4
	Elmis aenea (Ea)	1.5	0.01	1-2	19
	Gammarus pulex (Gp)	71.6	0.5	1-48	100
	Helodes sp. (Hel)	1.4	0.01	1-4	13
	Nemoura cinerea. (Nc)	0.4	<0.01	1-2	6
	Sericostoma personatum (Sp)	0.7	<0.01	1-2	10
Meiofauna	Copepoda (-)	0.6	<0.01	1-2	7
	Oligochaeta (-)	1.8	0.01	1-3	21
	Ostracoda (-)	2.2	0.02	1-5	25
	Hydracarina (-)	0.3	<0.01	1-2	3
	Hydra oligactis (-)	0.1	<0.001	1-1	1

Detrended correspondence analysis (DCA) was used to explore variability in the hyporheic invertebrate community and its distribution (Figure 4.12). The first axis of the ordination, which explained 34% of the variation, reflects the groundwater affiliation of these species with the stygoxenes (such as *Dicranota* (Di); and *Serratella ignita* (Si)) recording the highest scores and the stygobionts (such as *Crangonyx subterraneus* (Cs) and *Niphargus aquilex* (Na)) the lowest

scores.[16] The second axis, which explained 30% of this variation, reflects the spatial differences between sites, with the lowest scores attributed to the headwaters (Site 1) and higher scores to those further downstream. The clustering of samples at Site 1 is similar to the results of the benthic analysis but the reduction in overlap between Site 13 and the other sites suggests that the hyporheic habitat on the Dour differs from the Little Stour.

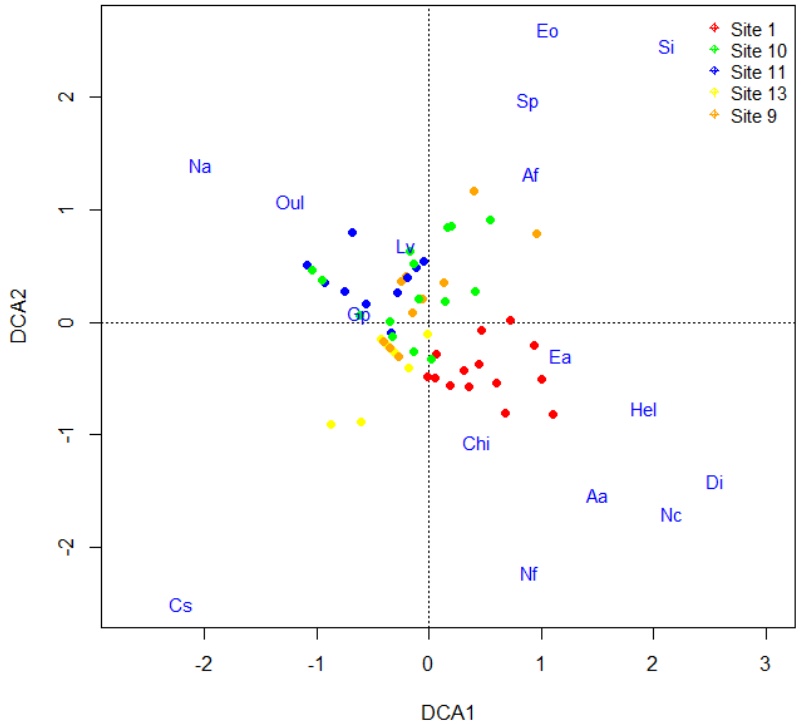


Figure 4.12 Detrended correspondence analysis of macroinvertebratres collected from hyporheic habitats from March 2010 to September 2012 (species abbreviations after Table 4.4).

4.3.1.1 Results of additional hyporheic sampling methods

Although the results from other hyporheic sampling methods are not directly comparable and have not been included in subsequent analyses (Section 2.3), it is notable that both the vacuum pump and the artificial substrate methods recorded very different invertebrate assemblages to those presented above. Specifically, both of these methods recorded a greater diversity of benthic species (including *Baetis rhodani* and *Potamopyrgus antipodarum*) than samples collected with the Bou-Rouch pump. These results reflect the selective nature of these methods which include bias by size, pumping strength and artificial enhancement of connectivity between the surface and subsurface.

[16] Eigenvalues for axes 1-4 in the DCA: 0.34; 0.30; 0.19; 0.14 and corresponding axis lengths: 2.19; 2.08; 1.57; 1.43.

Considering all hyporheic samples collected over the entire study, a total of 33 stygobiontic individuals from four species (including *Gammarus* sp.) were recorded (Table 4.5). However, it is notable that *Proasellus cavaticus* was not recorded at any site during this study despite previous records from this catchment (Stubbington 2009b). Spatially, at least one stygobiont was recorded in the hyporheic habitat at each of the five riverine sites, indicating that these locations represent areas of connectivity with groundwater. Spatially, the results suggest site-level influences on the distribution of these species as all but one (Site 11) supported only a single species. Temporally, the most frequently recorded stygobiont was *Niphargus aquilex*.

Table 4.5 Records of all stygobiontic species recorded from the hyporheic habitat using all trialled methods (including Artificial Substrates, the modified (m) Bou-Rouch pump, Bou-Rouch pump and vacuum pumping at 10, 20 and 30 centimetres below the substratum) over the course of the entire study (January 2009-September 2012).

Date	Method	Site	Taxa			
			Crangonyx subterraneus	*Niphargus aquilex*	*Niphargus fontanus*	*Gammarus* sp.
Jan-09	Artificial Substrate	9				1
Sep-09	Artificial Substrate	11				1
Sep-09	(m)Bou-Rouch	11				5
Nov-09	Artificial Substrate	1			1	
Jan-10	Bou-Rouch	11				2
Jan-11	Bou-Rouch	1			3	
Jan-11	Bou-Rouch	13	4			
Jan-11	Bou-Rouch	10		1		
Jan-11	Bou-Rouch	11		1		
Mar-11	Bou-Rouch	1			3	
Sep-11	Vacuum (20)	11				2
Sep-11	Bou-Rouch	11		1		
Nov-11	Vacuum (20)	10		1		
Nov-11	Bou-Rouch	10		3		
Nov-11	Bou-Rouch	11		1		
Jul-12	Bou-Rouch	13	1			
Jul-12	Bou-Rouch	10		1		
Jul-12	Vacuum (20)	11		1		

4.3.2 Hyporheic Community Variability

Macroinvertebrate abundance and richness varied by site and sampling occasion with the greatest abundance and diversity of individuals occupying the hyporheic habitat during the summer and early autumn and lowest numbers during the later autumn and winter (Figures 4.13 and 4.14). These results correspond with periods of baseflow at the end of the hydrological year, suggesting that the hyporheic habitat is used by the greatest number and

diversity of invertebrates during periods of low surface water discharge. The greatest variation in both metrics occurred at Sites 10 and 11, which are located at the downstream end of the Little Stour while Site 13, located on the River Dour, recorded the least variability in both abundance and diversity.

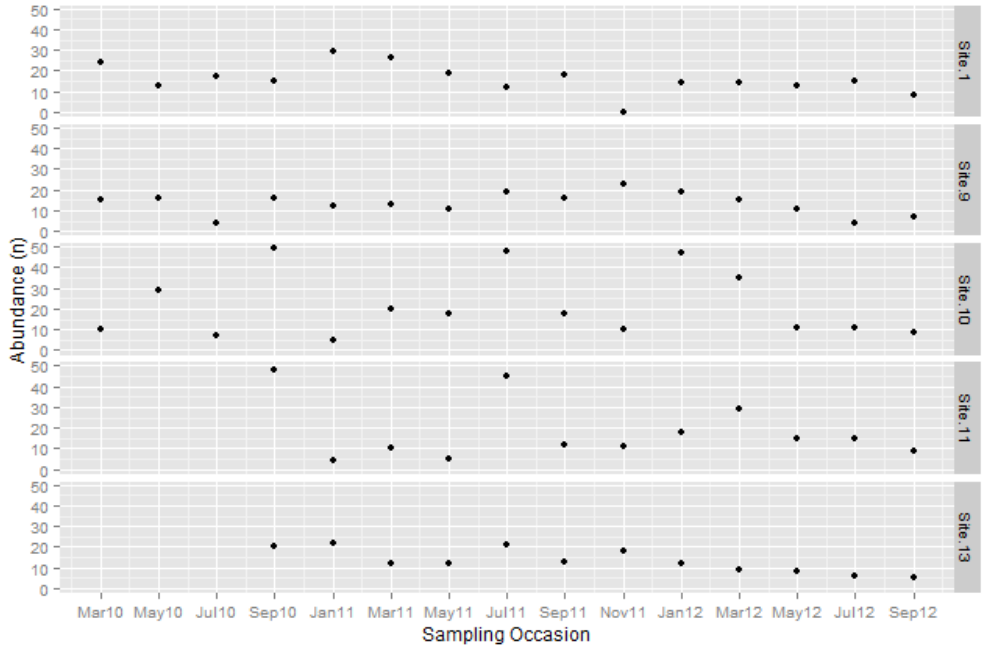


Figure 4.13 Macroinvertebrate abundance (n) recorded in hyporheic samples from March 2010 (Sites 1, 9 and 10) or September 2010 (Sites 11 and 13) to September 2012. No samples were collected at any Site in November 2010. Site 1 was dry in November 2011.

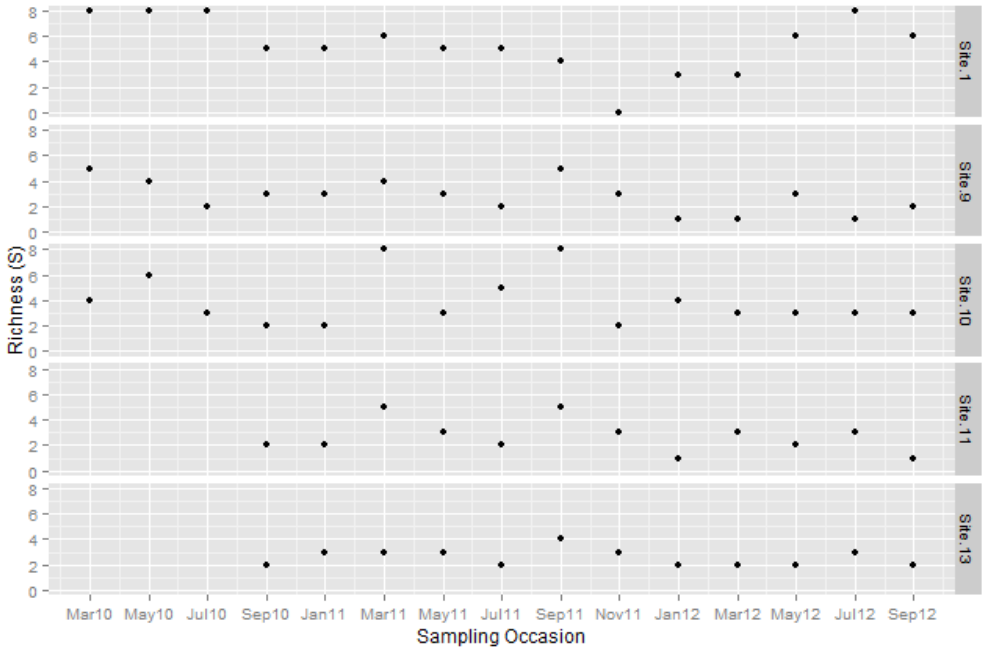


Figure 4.14 Macroinvertebrate richness (S) recorded in hyporheic samples from March 2010 (Sites 1, 9 and 10) or September 2010 (Sites 11 and 13) to September 2010. No samples were collected at any Site in November 2010. Site 1 was dry in November 2011.

4.3.2.1 Spatial variability of the Hyporheic Macroinvertebrate Community

Four community metrics were calculated to assess variability in the spatial distribution of the hyporheic macroinvertebrate community. The results indicate significant differences in diversity between sites, suggesting high spatial variability in the composition of the invertebrate assemblages (Table 4.6).

Table 4.6 Hyporheic macroinvertebrate community and diversity (Shannon-Wiener (H) or Simpsons (D)) metrics for samples collected from March (Sites 1, 9 and 10) or September (Sites 11 and 13) 2010 to September 2012. All results are presented as the study average ± 1 SE. Spatial change was assessed using a One-Way Analysis of Variance (ANOVA) with ** indicating p<0.001, * p<0.05 and ns p>0.05.

Metric	Site					Spatial Change
	1	9	10	11	13	
Abundance (n)	15.8 (±1.84)	13.4 (±1.40)	21.8 (±4.08)	18.42 (±4.23)	13.17 (±1.69)	ns
Richness (S)	5.34 (±0.58)	2.80 (±0.34)	3.93 (±0.51)	2.67 (±0.38)	2.58 (±0.19)	***
Diversity (H')	1.29 (±0.15)	0.61 (±0.07)	0.83 (±0.12)	0.58 (±0.13)	0.63 (±0.06)	***
Diversity (D)	0.64 (±0.14)	0.36 (±0.06)	0.46 (±0.06)	0.36 (±0.13)	0.40 (±0.06)	*

Macroinvertebrates were recorded at all sites on all sampling occasions (except Site 1 in November 2011) over the study period. Variability in abundance, driven by fluctuations in the *Gammarus pulex* population, was not found to be site specific ($F=1.63$; $p=0.18$). Conversely, the diversity of the macroinvertebrate community was found to vary spatially by all diversity metrics, with Site 1 consistently recording a more diverse community than the other sites (S: $F=7.25$, $p=0.007$; H': $F=5.89$, $p=0.0004$; D: $F=3.11$, $p=0.02$). These results indicate significant differences between the communities recorded in the Little Stour (Sites 1, 9, 10 and 11) and Dour (Site 13) catchments for all metrics (n: $df=68.17$, $p=0.0001$; S; $df=73.32$, $p=0.0001$; H': $df=124.26$; $p=0.0001$; D: $df=68.08$, $p=0.0001$), supporting the results of the DCA.

Spatial variance in diversity reflects differences in the community composition at these sites. The hyporheic community recorded at Site 1 included a number of species unique to this location (though not this habitat) including *Asellus aquaticus* and *Niphargus fontanus* which increased the diversity of this site. This result is unsurprising as the character of Site 1, an intermittent headwater, differs from the others and would be expected to support a different assemblage; however, these species are not considered to be spring specialists

(Wood et al., 2005a). While *A. aquaticus* is typical of intermittent habitats, *N. fontanus* is a stygobiont associated with the phreatic habitat (and recorded as such during this study; Iversen et al., 1978; Wood et al., 2005a). The composition of the hyporheic community at Site 13 included some species which were unique to this site, such as the stygobiont *C. subterraneous* which was exclusive to the hyporheic habitat at this location.

4.3.2.2 Temporal Variability of the Macroinvertebrate Community

The same four metrics were also calculated to assess temporal variability in the distribution of the macroinvertebrate community. The results indicate significant differences in temporal variability of invertebrate abundance ($F=6.66$, $p=0.01$) but suggest that the diversity of this community is largely stable (S: $F=1.12$, $p=0.30$; H: $F=0.27$, $p=0.61$; D: $F=0.18$, $p=0.68$; Table 4.7).

Macroinvertebrate abundance was highest in September 2010 and July 2011, which also marked the greatest variance in abundance between sites. These results were driven by an increase in *Gammarus pulex* individuals into the hyporheic habitat and coincide with the onset of seasonal flow recession (Section 3.4.1). These results indicate temporal variance in abundance but not diversity, suggesting that taxa normally present at each site increase their use of the hyporheic habitat at specific times. The lack of spatial variability in abundance suggests that this response is similar between sites.

Table 4.7 Hyporheic macroinvertebrate community and diversity (Shannon-Wiener (H) and Simpsons (D)) metrics from September 2010 to September 2012 All results are presented as the study average ± 1 SE. Temporal change (TC) was assessed using a One-Way ANOVA with ** indicating $p<0.001$, * $p<0.05$ and ns $p>0.05$.

Sampling Occasion	Metric			
	Abundance (n)	Richness (S)	Diversity (H')	Diversity (D)
Sep-10	29.6 (±7.76)	2.8 (±0.58)	0.39 (±0.17)	0.20 (±0.09)
Jan-11	14.4 (±4.86)	3.00 (±0.55)	0.63 (±0.06)	0.35 (±0.07)
Mar-11	16.2 (±2.97)	5.2 (±0.86)	1.31 (±0.16)	0.69 (±0.05)
May-11	13.0 (±2.55)	3.4 (±0.40)	0.85 (±0.03)	0.53 (±0.05)
Jul-11	29.0 (±7.31)	3.2 (±0.74)	0.66 (±0.20)	0.36 (±0.10)
Sep-11	15.4 (±1.25)	5.2 (±0.74)	1.17 (±0.14)	0.61 (±0.06)
Nov-11	12.4 (±3.91)	2.2 (±0.58)	0.54 (±0.18)	0.34 (±0.12)
Jan-12	22.0 (±6.38)	2.2 (±0.58)	0.28 (±0.13)	0.15 (±0.07)
Mar-12	20.4 (±4.93)	2.4 (±0.40)	0.34 (±0.09)	0.18 (±0.03)
May-12	11.6 (±1.17)	3.2 (±0.74)	0.69 (±0.22)	0.39 (±0.11)
Jul-12	10.2 (±2.27)	3.6 (±1.17)	0.91 (±0.33)	0.52 (±0.16)
Sep-12	7.6 (±0.75)	2.8 (±0.86)	0.70 (±0.29)	0.44 (±0.15)
Temporal Change	*	ns	ns	ns

4.3.3 Hyporheic Assemblages in relation to Environmental Parameters

Multivariate analyses were used to investigate relationships between the hyporheic macroinvertebrate community and the environmental conditions of this habitat. The results are discussed with reference to the assessment of the community as a whole and to individual species.

4.3.3.1 Hyporheic Community in relation to Environmental Parameters

Ordination techniques were used to assess the relationship between environmental variables and the distribution of the macroinvertebrate community following the same procedure as described in Section 4.2.3.1. As with the benthic community, the DCA indicated that the distribution of these species was neither linear nor unimodal as the longest axis value was of intermediate length and the results were assessed using both CCA and RDA techniques for both the whole study and the final year. As with the results from the benthic habitat, the RDA provided the best fit for both hyporheic datasets and is presented below.

Considering biological and physiochemical data collected throughout the study, the RDA explains 23% (r^2=0.23) of the species variance, and most of this variance is explained by the first two axes (19%).[17] The fit of the model was tested using an analysis of variance that indicated that it was significant (F=3.35; p=0.001). The importance of the variables was assessed using Monte Carlo permutation testing which indicated that discharge (Q; F=7.456; p=0.001), conductivity (Cond; F=6.569; p=0.001) and pH (F=2.162; p=0.038) were significant (Legendre and Legendre, 2012). The model was re-run omitting the insignificant variables (temperature: F=0.421; p=0.885 and Alkalinity F=2.187; p=0.056) but this did not enhance the fit.

The resulting ordination reflects the importance of discharge and conductivity; however, the arrangement of the vectors, and principally that none of the variables plot parallel to the axes, implies poor explanatory ability of this model (Figure 4.15). Despite this, the ordination suggests a strong spatial influence as shown by the inverse relationship between pH and conductivity as well as

[17] Eigenvalues for axes 1-4 in the first RDA: 0.055; 0.030; 0.005; 0.002 and proportion explained for axes 1-4: 0.124; 0.065; 0.016; 0.005; total inertia= 0.395 (constrained=0.918; unconstrained=0.303); total proportion = 1.00 (constrained = 0.233; unconstrained=0.767)

alkalinity and discharge with the former being lowest in the headwaters and increasing with distance downstream (Section 3.2.5) and the latter being highest in the headwaters and decreasing with distance downstream (Section 3.2.3). Similarly, the inverse relationship between discharge and alkalinity is spatial as well as temporal, with lower discharge in the headwaters associated with higher contributions from the aquifer and therefore higher alkalinity (Sections 3.2.5 and 3.4.1). The species associated with these variables reflect an expected spatial distribution, specifically with the headwater community recorded at site 1. Notably, neither of the two most dominant species, *G. pulex* and *A. fuscipes,* were strongly associated with any of the vectors in this ordination, suggesting a ubiquitous distribution throughout this habitat.

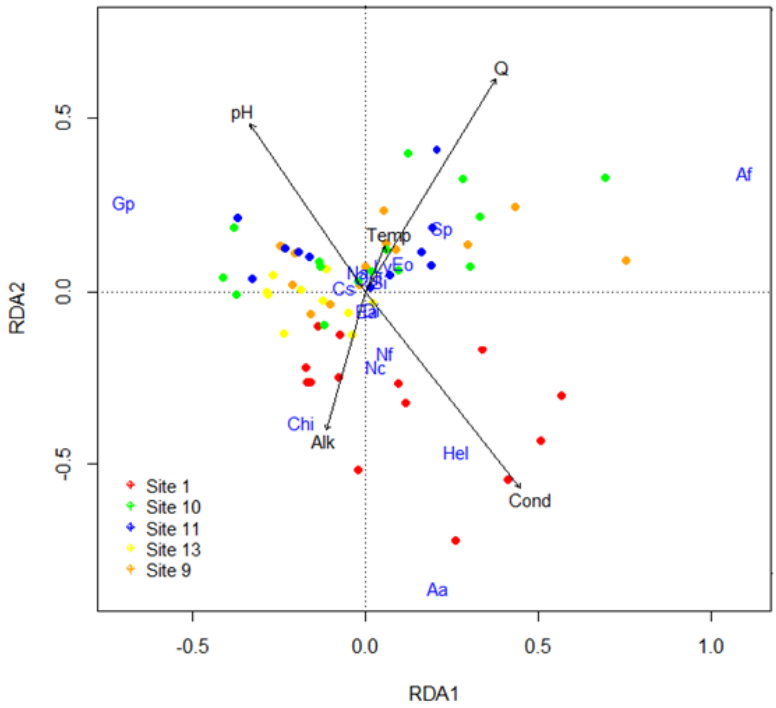


Figure 4.15 RDA ordination of species (blue, transformed count data after Table 4.4) and physiochemical variables (vectors, square-root transformed; September 2010-2012).

Considering biological, physiochemical and chemical data collected from the hyporheic habitat during the final year of the study, the RDA indicates that the model can explain 43% (r^2=0.43) of the species variance, and that most of this variance is explained by the first two axes (30%).[18] However, the fit of the model was tested using an analysis of variance which indicated that it was not significant (F=1.02; p=0.483), though the results are of interest and are explored

[18] Eigenvalues for axes 1-4: 0.061; 0.022; 0.010; 0.007 and proportion explained for axes 1-4: 0.240; 0.088; 0.040; 0.029; total inertia = 0.254 (constrained=0.110; unconstrained=0.144); total proportion = 1.00 (constrained = 0.434; unconstrained=0.566)

below. Monte Carlo permutation testing was used to select the most significant parameters in the data set to run a reduced model but this did not significantly enhance the fit (Legendre and Legendre, 2012). The permutation testing suggests that conductivity (F=2.436; p=0.057), temperature (F=2.041; p=0.082) and pH (F=1.746; p=0.150) had the greatest influence on species distribution and that none of the chemical parameters recorded significantly affected the distribution of species in the hyporheic habitat over this period. These results contrast with those above as discharge was not found to be significant (F=0.409; p=0.819) despite the temporal period considered coinciding with drought conditions. In addition, this ordination also differs from the previous models as it does not suggest patterns of groundwater affiliation in species distribution or spatial clustering of sites (Figure 4.16). These differences may be attributed to the change in species composition during the final year of the study or in the homogenisation of conditions between sites. The difficulty in fitting both of these models reflects the complexity and variability of this community. The marked difference between the first and second models highlights the importance of multi-year sample collection in hyporheic studies.

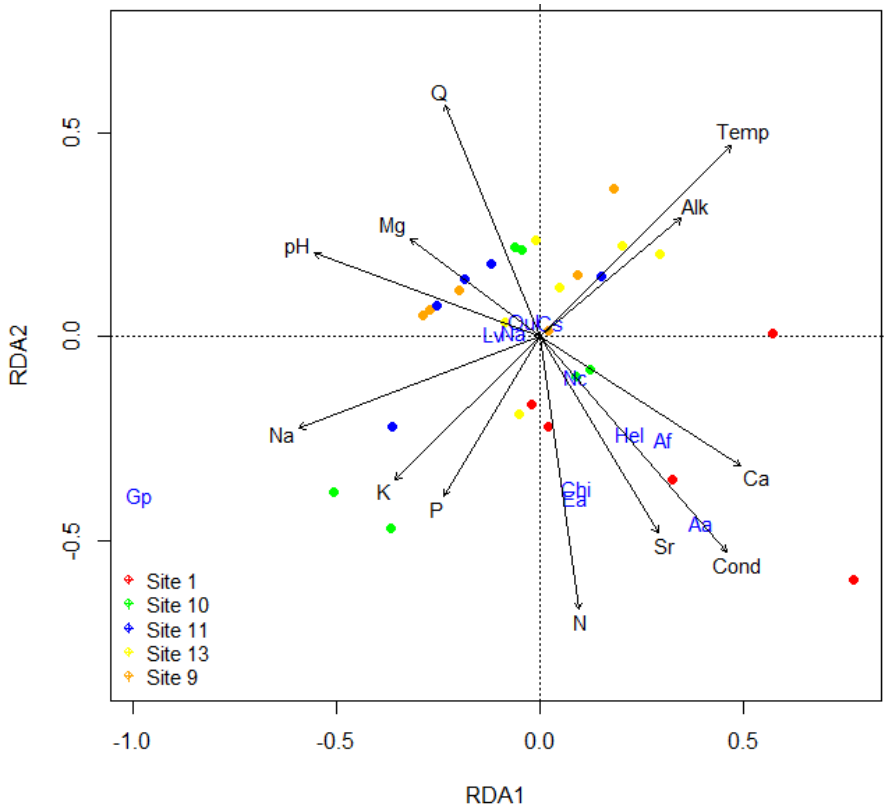


Figure 4.16 RDA ordination of macroinvertebrates (blue, transformed count data after Table 4.4) with physiochemical and chemical environmental variables (vectors, square-root transformed; November 2011 to September 2012).

4.3.3.2 Hyporheic Species in relation to Environmental Parameters

Generalized linear models (GLMs) were used to assess the relationship between environmental variables and individual species distribution. As this assessment was undertaken using untransformed biological (count) data, the GLM was initially fitted using a Poisson reference distribution; however, due to overdispersion caused by the high variance in the results, both a quasi-Poisson and a negative binomial error structure were also tested, the latter of which was found to provide the best fit and is presented below (Table 4.8; Leps and Smilauer, 2003). GLM performance was enhanced by excluding geochemical and nutrient variables. GLMs were intended to be run for the most abundant species and the stygobionts; however, this could not be undertaken on *C. subterraneus* or *N. fontanus* as both were only recorded on two occasions. The results suggest differing ecological preferences for each species and species-specific influences on macroinvertebrate distribution across this habitat.

Table 4.8 GLMs for the presence/absence of key taxa tested against square-root transformed environmental variables assuming a negative binomial error structure. The fit of the model was tested using an ANOVA and Chi-Square test (α=0.05). Over-dispersion was tested by dividing the residual deviance by the residual degrees of freedom. Significant results are emboldened.

Variable	*Gammarus pulex*	*Agapetus fuscipes*	*Niphargus aquilex*
Temperature	0.745	0.250	**0.047**
Conductivity	**0.021**	**0.005**	0.726
Alkalinity	0.997	**0.007**	0.962
pH	0.122	0.468	0.056
Discharge	0.101	**0.001**	0.912
Goodness of Fit	0.24	0.78	0.99

Gammarus pulex was recorded at all sites on all occasions in abundances of 1 to 48 individuals (Figure 4.17). The abundance of *G. pulex* was highest in September 2010, July 2011 and January 2012, coinciding with periods of low flow (Section 3.4.1).

Episodes of high abundance in the hyporheic habitat corresponded with a decrease in abundance in the benthic habitat at all riverine sites (Sections 4.2). The periodicity of these episodes suggests that such changes in abundance are not associated with life history or seasonality but with external environmental factors, specifically surface water discharge (although the results of the GLM do not suggest that this is significant).

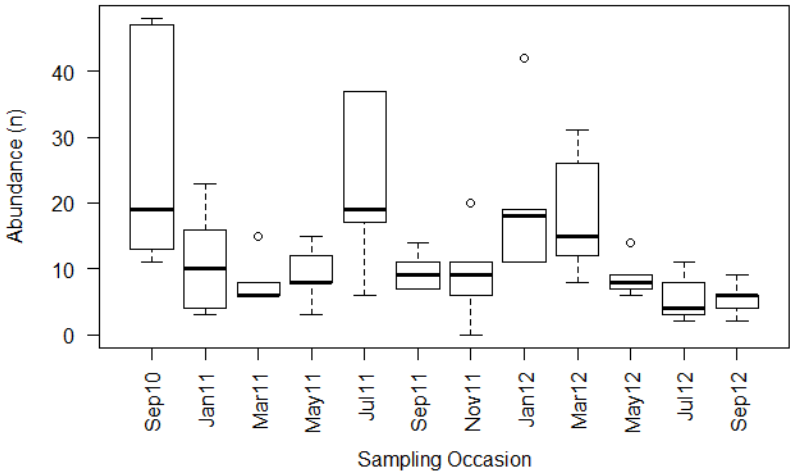


Figure 4.17 *Gammarus pulex* abundance from hyporheic samples at all five riverine Sites (1, 9, 10, 11 and 13) from September 2010 to September 2012. The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

Agapetus fuscipes was recorded in the hyporheic samples at all five riverine sites; although its affinity with this habitat is less clear than for other species, it was recorded consistently both spatially and temporally and, at times, in high densities, suggesting that it behaves as a stygophile in this catchment (a finding which aligns with previous interstitial records on the Little Stour; Stubbington et al., 2015). The abundance of *A. fuscipes* in the hyporheic habitat suggests a seasonal pattern with numbers increasing most notably from the winter through the spring period (Figure 4.18). This pattern may reflect a vertical movement into the substratum for food as this species feeds primarily on biofilms and most intensively over this period (Becker, 2005). However, this pattern is only represented during the first two years of the study as the abundance of *A. fuscipes* collapsed during the final year. The results of the GLM indicate an association with discharge, conductivity and alkalinity which may reflect the marked influence of the drought period on this species during the final study year. These results suggest that the normally benthic *A. fuscipes* utilises the hyporheic habitat seasonally, likely in response to biofilm availability, but that changes in environmental conditions can inhibit this use either directly through the survival of this species during perturbations or indirectly through a change in food availability.

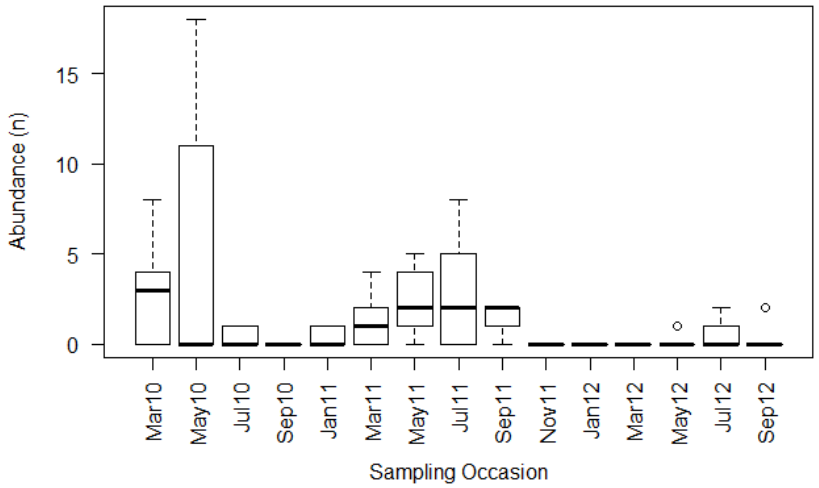


Figure 4.18 *Agapetus fuscipes* abundance in hyporheic samples from all riverine Sites. Abundances from March, May and July 2010 reflect abundances from Sites 9, 10 and 11 only. The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

The spatial distribution of stygobionts in the hyporheic habitat was site-specific as *Crangonyx subterraneous* was only recorded on the Dour (Site 13), *Niphargus aquilex* only at the downstream end of the Little Stour (Sites 10 and 11) and *N. fontanus* only in the headwaters of the Little Stour (Site 1). It was anticipated that the greatest abundance and diversity of stygobionts would be recorded during periods when groundwater contribution to surface water is greatest and these organisms could enter the shallow interstitial environment through upwelling groundwater (Plénet et al., 1995; Stubbington et al., 2015). However, the temporal distribution of stygobionts did not fit this pattern, as their highest abundances (both considered individually and pooled) were recorded during the winter of 2011 which coincided with the lowest river flows (Section 3.4.1) and below average groundwater levels (Section 3.4.2) of the drought period (Figure 4.19). This pattern does not align with the wider hyporheic community or with that of *Gammarus pulex*, suggesting that factors influencing this distribution are unclear. Although a GLM was fitted independently for *Niphargus aquilex*, the most frequently recorded stygobiont, this suggested only a very weak relationship with temperature and no relationship with discharge. These results suggest that the variance in the stygobiontic portion of the hyporheic community cannot be explained using the environmental variables considered in this study.

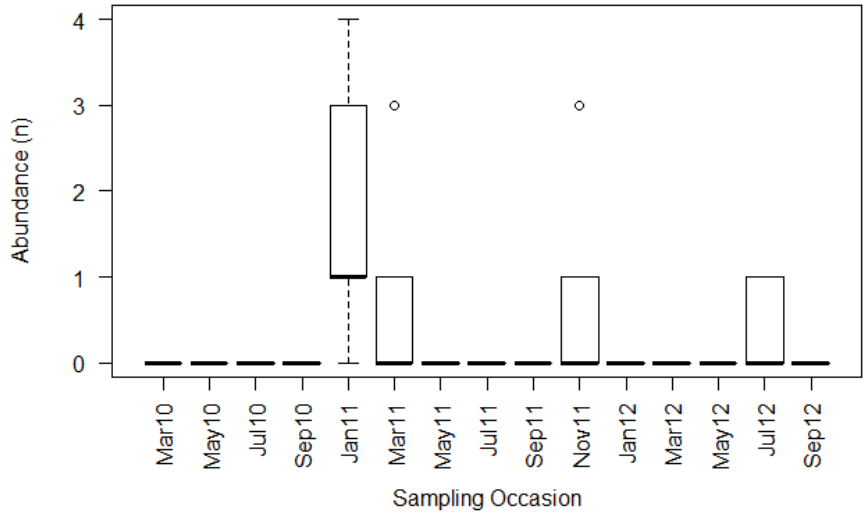


Figure 4.19 Pooled abundance of stygobionts in hyporheic samples (abundances from March, May and July 2010 reflect abundances from Sites 9, 10 and 11) from March 2010 to September 2012. The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month. While no stygobionts were found in most samples, three species were found across Sites 1, 10, 11 and 13 in January 2011

4.3.4 Discussion and Summary of Hyporheic Communities

The results presented above have been assessed within the context of current literature (Chapter 1) and the aims of this study to inform the discussion of the research questions.

4.3.4.1 Can the hyporheic macroinvertebrate community be described?

The macroinvertebrate community recorded in the hyporheic habitat comprised a mixture of stygoxenes, stygophiles and stygobionts. Three of the recorded species are stygoxenes (the mayfly *Serratella ignita,* dipteran *Dicranota* and leech *Erpobdella octoculata*), normally benthic taxa which were only recorded on isolated occasions in very small numbers (although it should be noted that *S. ignita* has been regularly recorded in the hyporheic habitats of the River Lathkill, Derbyshire; Stubbington et al., 2010). A further three species are stygobionts (*C. subterraneus*, *N. aquilex* and *N. fontanus*), obligate groundwater organisms and occasional occupants of the hyporheic habitat (Section 1.3). While the majority of taxa recorded in the hyporheic habitat were stygophiles, normally benthic species which have an affiliation with groundwater, either as permanent, occasional or temporary hyporheos (*sensu* Williams and Hynes, 1974). Some of these species, such as the Cnidarian *Hydra oligactis*, are regularly recorded in hyporheic habitats and may spend their entire life cycle in a groundwater

environment (Lee and Johns, 2012). Other taxa, such as *Sericostoma personatum,* Chironomidae and *Asellus aquaticus,* regularly exploit the hyporheic habitat by burrowing into the substrate to feed (Mermillod-Blondin et al., 2002; Wagner, 1991). Some species, such as *Gammarus pulex* and the Elmid beetles, have been found to actively move between benthic and hyporheic habitats, particularly in response to unfavourable flow conditions (Elliott, 2006; Marchant, 1995; Wood et al., 2010). The hyporheic affiliations of the stonefly *Nemoura cinerea,* larvae of the Scirtidae beetle *Helodes* and caddisfly *Agapetus fuscipes* are not clear in the literature (Hynes, 1976; Pacioglu and Moldovan, 2016; Rasmussen, 1979; Stubbington et al., 2010). However, the consistent frequency and abundance in which they were recorded during this study suggests that they are regularly resident in the hyporheic habitat within this catchment.

4.3.4.2 Does the hyporheic community vary spatiotemporally?

The hyporheic community sampled in this study did vary spatially, particularly with regard to differences in composition between headwater and downstream sites. Although diversity varied by site, abundance did not, suggesting species-specific exploitation of the hyporheic habitat on a site-specific basis. These findings are similar to previous studies (Section 1.4) which suggest that the composition of the hyporheic community is determined by environmental characteristics at the site (or within-site) scale. The community also varied temporally. Although diversity was stable, abundance fluctuated, suggesting species-specific variability in the utilisation of this habitat over time. While the abundance of some species, such as *Agapetus fuscipes*, suggests a seasonal pattern, for other species, such as *Gammarus pulex,* it coincides with specific environmental changes. The temporal aspect of these results is of particular note to this field given the paucity of long-term hyporheic studies with frequent sampling regimes (Section 2.3).

4.3.4.3 Are hyporheic assemblages related to environmental conditions?

The results suggest that the distribution of the hyporheic community can be related to environmental conditions, specifically with reference to physiochemical variables including discharge, pH and conductivity. Changes in the abundance and diversity of individuals recorded in this habitat suggest that

it is used by the greatest number and diversity of invertebrates during periods of low discharge, coinciding with the onset of seasonal flow recession. These results are driven principally by marked increases in the number of *Gammarus pulex* individuals recorded in the hyporheic habitat during periods of low flow (and corresponding decreases in the number of these individuals in the benthic habitat), suggesting a direct influence of discharge on this community.

Conversely, while it is unlikely that the community responds directly to changes in either pH or conductivity as a result of ecological tolerance, this relationship could be an indirect response to broader environmental factors (Section 1.4) as both can be used as an indication of surface water influence in the hyporheic habitat (Hancock and Boulton, 2008; Korbel and Hose, 2011). Both pH and conductivity varied spatially but not temporally during this study (Section 3.2.2 and 3.2.3) indicating that the changes to surface water influence were site-specific. The influence of this spatial variability on the hyporheic community may be a reflection of hyporheic exchange flows and therefore habitat quality. Alternatively it might provide an indication of the influence of these variables (specifically pH) on hyporheic biofilms and therefore invertebrates, such as *A. fuscipes*, which rely on them as a food source (Townsend et al., 1983).

These results are confounded by the lack of a relationship between the hyporheic community and geochemical variables; however, this may be expected as all of the sites were located over broadly the same geological matrix. The results of this study are similar to other studies which also found species distribution to not be associated with geochemistry but should be assessed with caution as the geochemical sampling was limited to the final year of the study and overlapped with drought conditions (Gibbins et al., 2016).

4.3.4.4 Summary of the Hyporheic Community

The hyporheic community comprised a mixture of stygoxenes, stygophiles and stygobionts but was dominated by stygophiles, specifically *Gammarus pulex*. The distribution of species within the hyporheic habitat was dependent upon site-specific conditions but no association was found between stygobionts and the measured environmental conditions. The results indicate that the presence and abundance of stygophiles in the hyporheic habitat can be related to

seasonality (and life history) or changes in environmental conditions. These results suggest that the hyporheic environment provides a unique habitat to a variable community of temporary and permanent residents.

4.4 Communities Recorded in Phreatic Habitats

Samples from the phreatic habitat were collected from seven boreholes and wells located throughout the Stour Chalk Block (Sites A-G) to assess the spatiotemporal variability of their microbial and invertebrate communities (Section 2.2). Microbial samples were collected twice, during the spring and autumn of 2012, while invertebrate samples were collected bimonthly from November 2011 to September 2012 (Section 2.3). The results were analysed to describe these communities and their variability (Sections 4.4.1 and 4.4.2) as well as identify potential relationships with the environmental conditions of this habitat (Section 4.4.3). The findings are discussed with reference to the aims and objectives of this study (Section 4.4.4).

4.4.1 Microbial Community Description and Spatiotemporal Variability

The functional diversity of the microbial communities was measured using Biolog Ecoplates. The spatiotemporal variability of the microbial community was assessed using Average Well Colour Development (AWCD), richness (S), diversity (Shannon-Wiener (H)) and substrate utilization for all samples. The results indicate significant spatial but not temporal differences in these metrics, potentially reflecting the stability of this habitat (Table 4.9).

Table 4.9 Microbial community metrics by site (A-G) in which change has been assessed using a One-Way Analysis of Variance with * indicating $p<0.05$ and ns $p>0.05$. Measurements from the control (blank) wells within the plates were markedly lower than the measurements for non-control sites, suggesting that these results represent genuine microbial activity in relation to differing carbon sources (Section 2.3.1.6; Table 2.3).

Metric	Season	Site							Spatial Change	Temporal Change
		A	B	C	D	E	F	G		
AWCD	Spring	0.91	1.35	0.69	1.36	0.34	0.33	1.36	*	ns
	Autumn	1.02	1.27	0.93	1.25	0.56	1.32	1.27		
Richness (S)	Spring	25	28	29	31	24	14	29	ns	ns
	Autumn	28	31	26	31	21	29	30		
Shannon-Wiener (H')	Spring	3.2	3.32	3.34	3.36	2.96	3.1	3.4	ns	ns
	Autumn	3.23	3.4	3.21	3.37	3.24	3.33	3.35		

The AWCD is an expression of microbial activity that integrates cell density and the diversity of substrate utilisation in which higher values suggest greater microbial activity and functional diversity (Equation 4.1; De Lipthay et al., 2004;

Janniche et al., 2012). Considering all sites, the AWCDs (0.33-1.65) were slightly higher than expected for groundwater samples (0.60) but this may result from the temperature of incubation (20 °C) being higher than average groundwater temperatures occurring within this habitat ($\bar{x}$ = 14.1 °C; n=49; Choi and Dobbs, 1999). While the results indicate that sampling occasion (season) was not significant, there were significant differences in AWCD by site (F=3.96; p=0.04) indicating spatial differences in the distribution and functioning of sampled microbial communities. This is particularly notable for Site E which recorded a comparatively low AWCD on both occasions.

$$AWCD = [\sum(C\text{-}R)] / n$$

Equation 4.1 Average Well Colour Development in which a corrected absorbance value (where R is the absorbance of the control well and C is the absorbance of each well (optical density measurement), both at day 6) is divided by the number of substrates (n=31; Lee et al., 2010; Weber et al., 2007).

Community diversity was assessed using richness (S), a secondary calculation which reflects the number of carbon sources metabolized within a given time. Richness was calculated after Janniche et al. (2012) in which a source was considered to be metabolized when the corrected absorbance value met or exceeded 0.25. This measure of diversity did not vary spatially or temporally.

Microbial community diversity was also calculated using the Shannon-Wiener (H') diversity index in which Pi is the relative use of one specific carbon source calculated as the ratio between the absorbance of each substrate and the sum of absorbance in all substrates (all absorbance values read at day 6 and corrected using the blank, negative values were set to zero) with higher values reflecting a greater diversity of utilized carbon sources, and therefore functional diversity (De Pithay et al., 2004). The results were as expected for aerobic groundwater and ranged from 2.96-3.41 suggesting that diversity was not influenced by site or sampling location, with all sites supporting a consistently diverse flora (Janniche et al., 2012).

While the diversity calculations suggest that many of the carbon sources were utilised and that all of the sites supported a diverse flora, additional analyses were undertaken to identify differences in the utilization of carbon sources by different functional groups to better understand the resilience of the microbial community. Preferential carbon source utilization was identified in substrates

where Pi ≥0.032 before sources were divided into broad functional groups of polymers, phenolic compounds, carbohydrateoxylic acids, carbohydrates, amino acids and amines (Choi and Dobbs, 1999; Janniche et al., 2012). The results indicate high substrate utilisation among all samples, suggesting that the microbial community is diverse and resilient as it can utilize differing carbon sources (Figure 4.20).

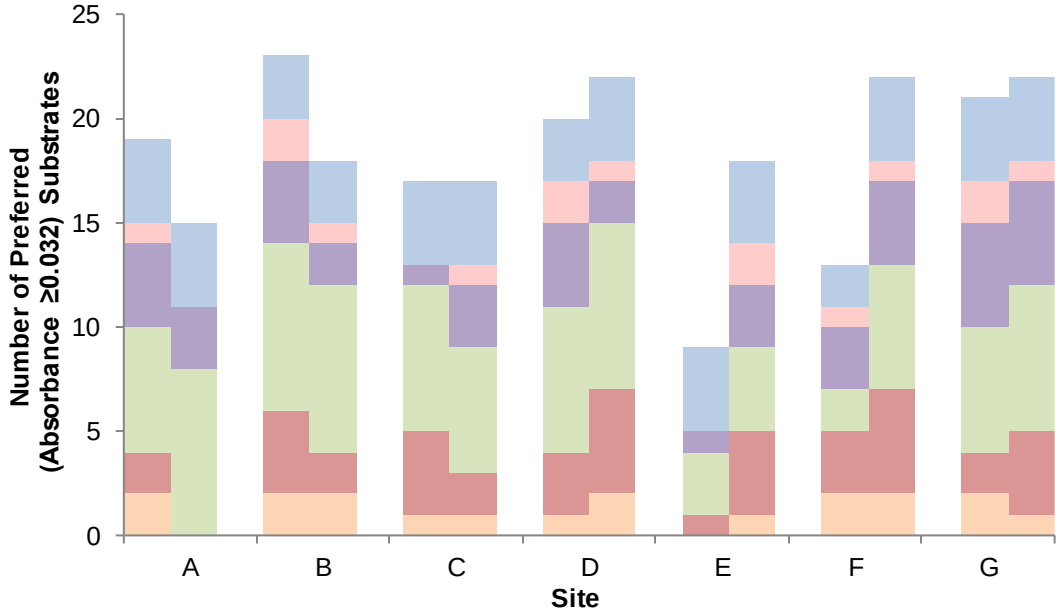


Figure 4.20 Preferred substrates of phreatic microbial communities in the spring (left) and autumn (right) by Site represented as the summed number of carbon sources used preferentially (in which Pi >0.032) at day 6 by functional group in which polymers are represented in blue; phenolic compounds in pink; carbohydrateoxylic acids in purple; carbohydrates in green; amino acids in red and amines in orange (after Choi and Dobbs, 1999 and Janniche et al., 2012).

These results suggest that the microbial communities at these sites are spatially variable. This spatial variation is driven by the notably smaller AWCD results from Site E in both the spring and autumn samples. The reason for this difference is not clear but could be attributed to the low recharge and subsequently higher temperatures recorded at this site during these sampling occasions (Sections 3.2.1 and 3.4.2). While care should be taken when inferring the lack of temporal variance given the limited sampling occasions, these results do represent vastly different conditions of peak drought and autumn recharge when influence beyond normal seasonality would be expected. The lack of variance between these two occasions suggests a surprisingly stable microbial community, the resilience of which may be attributed to relatively high community diversity. Based on previous studies, it is expected that these

microbial communities would be positively associated with supporting groundwater fauna (Brunke and Fisher, 1999; Hahn, 2006).

4.4.2 Invertebrate Community Description and Spatiotemporal Variability

Forty-three macroinvertebrate individuals representing four stygobiontic crustacean species (*Niphargus kochianus, N. fontanus, Crangonyx subterraneus* and *Gammarus* sp.*)* were recorded from phreatic samples collected during this study (Table 4.12). A further fifty-six individuals of *Acanthocyclops sensitivus* (a common stygobiontic Cyclopidae copepod) were recorded at all seven phreatic sites but excluded from further analysis as this species is a member of the meiofauna (Proudlove et al., 2003; Section 2.4). Two Collembola species, *Folsomia candida* (a cosmopolitan, unpigmented, blind springtail) and *Heteromurus nitidus* (a cosmopolitan springtail which is not blind or unpigmented despite its troglobite affiliation) were also recorded at Sites A, D, E and F but have not been considered further as they are terrestrial and are likely to have inhabited the walls or casings of the sites rather than the aquatic environment (Fountain and Hopkin, 2005; Hopkin, 2007; Wilson, 1975).

Table 4.10 Abundance and location of macroinvertebrate species recorded from the seven phreatic Sites (A-G) during this study (November 2011 to September 2012).

Species	Total Abundance	Sites Supporting
Niphargus kochianus (Nk)	21	A, B, C
Niphargus fontanus (Nf)	7	B, C, D, G
Gammarus sp. *(Gs)*	1	A
Crangonyx subterraneus (Cs)	15	B, C, F

Niphargus kochianus was the most abundant macroinvertebrate (comprising nearly half of all individuals recorded from this habitat) while *N. fontanus* was the most widespread (recorded from four of the seven sites). Within this study, only *N. kochianus* was exclusive to the phreatic habitat as *N. fontanus, Gammarus* sp. and *C. subterraneus* were also recorded in benthic and/or hyporheic habitats. Detrended correspondence analysis (DCA) was used to explore variability in the phreatic macroinvertebrate community (Figure 4.21). The resulting ordination does not suggest a gradient of distribution, instead it reflects the convergence between records as many of the results completely overlap spatially (as both Sites B and G recorded samples with only a single *N.*

fontanus individual) or temporally (as only a single *N. kochianus* individual was recorded at Site A on three separate occasions).[19]

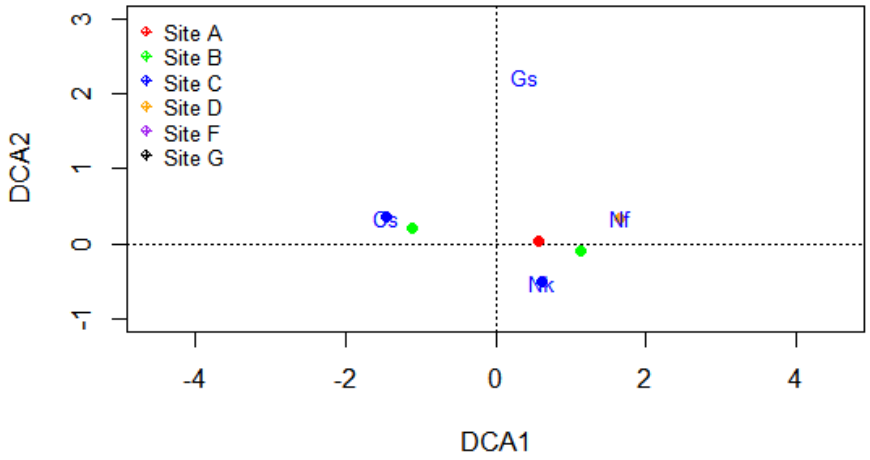


Figure 4.21 Detrended correspondence analysis of phreatic macroinvertebratres collected from November 2011 to September 2012 (abbreviations after Table 4.12). No macroinvertevrates were recorded at Site E on any sampling occasion. Please note the complete overlap in species records between many sites and sampling locations which are obscured in the plot.

With few exceptions, phreatic macroinvertebrate abundance and richness varied little by site or sampling occasion (Figures 4.22 and 4.23). While many null values were recorded over the study period, the highest abundance was recorded in March 2012 (Site B; n=12). Richness did not exceed a value of 2 at any site on any occasion.

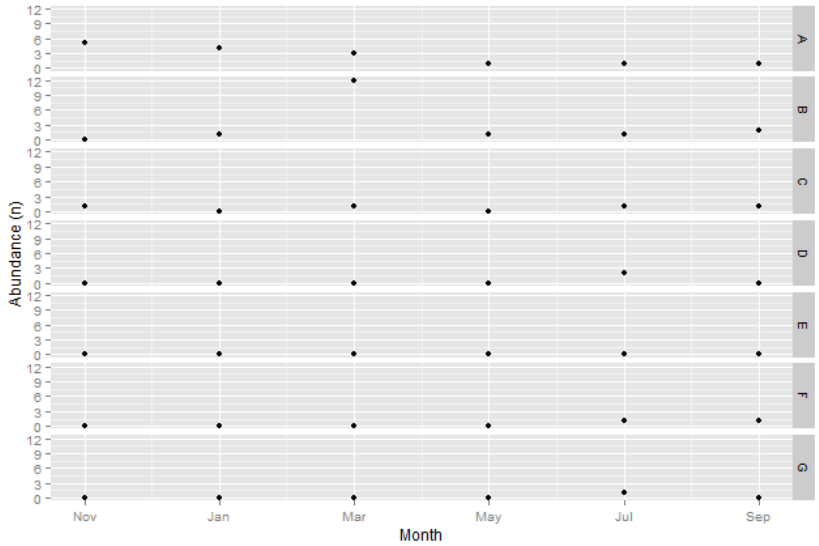


Figure 4.22 Macroinvertebrate abundance ((n) individuals/sample) recorded at the seven phreatic sites (A-G) over the study period (November 2011 – September 2012).

[19] Eigenvalues for axes 1-4 in the DCA: 0.861; 0.394; 0.729; 0.729 and corresponding axis lengths: 3.100; 0.869; 1.628; 1.628.

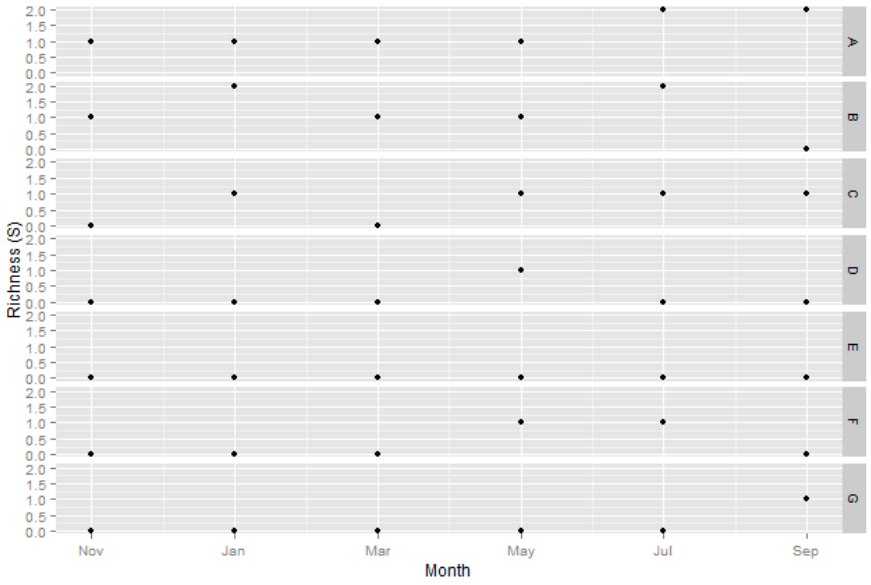


Figure 4.23 Macroinvertebrates richness (S) recorded at the seven phreatic sites (A-G) over the study period (November 2011-September 2012).

4.4.2.1 Spatial variability of the Phreatic Macroinvertebrate Community

Five community metrics were calculated to assess variability in the spatial distribution of macroinvertebrates occupying the phreatic habitat. The results suggest significant differences in the number of positive samples, abundance and richness of this community between sites (Table 4.11).

Macroinvertebrates were recorded at six out of the seven phreatic Sites (86%) over the study period; however, there was significant variability in the consistency between sites. For example, positive samples were recorded on every occasion at Site A but only once at Sites D and G ($F=6.182$; $p=0.001$). No macroinvertebrates were recorded at Site E. While these findings are consistent with similar studies in England and Germany where stygofauna were found to be absent from 30% of sampled boreholes, the reason for this absence at Site E is not clear (Hahn, 2006; Hahn and Fuchs, 2009; Johns et al., 2015; Thulin and Hahn, 2008; Weitowitz, 2012). Site E is not materially different from the other sites in location, design or environmental parameters (Sections 3.2, 3.3 and 3.4); however, the microbiological community at this site is markedly less diverse than the others (Section 4.4.1) and could reflect poor availability of food sources for the invertebrate species present at the other phreatic sites which graze on biofilms (Thulin and Hahn, 2008).

Table 4.11 Phreatic macroinvertebrate community and diversity (Shannon-Wiener (H) and Simpsons (D)) metrics. All results are presented as the study average ± 1 SE (n=6), except he number of positive samples (sum). Spatial change assessed using a One-Way Analysis of Variance (ANOVA) with ** indicating p<0.001, * of p<0.05 and ns p>0.05. Some diversity metrics could not be calculated due small sample sizes.

Metric	Site							Spatial Change
	A	B	C	D	E	F	G	
Positive Samples (n)	6	5	4	1	0	2	1	**
Abundance	2.57 (±0.61)	2.83 (±1.85)	0.67 (±0.21)	0.34 (±0.34)	0	0.34 (±0.21)	0.17 (±0.17)	**
Richness (S)	1.29 (±0.18)	1.17 (±0.31)	0.67 (±0.21)	0.17 (±0.17)	0	0.34 (±0.21)	0.17 (±0.17)	**
Diversity (H')	0.16 (±0.11)	0.19 (±0.13)	na	na	0	na	na	na
Diversity (D)	0.27 (±0.12)	0.65 (±0.20)	na	na	0	na	na	na

Macroinvertebrate abundance also varied spatially (F=3.29; p=0.001) with notably higher averages at Sites A and B (Figure 4.24). While most positive samples reflect a range of one to four individuals (per sample), it is notable that ten *C. subterraneus* and two *N. kochianus* were recorded at Site B in March 2012 (Figure 4.24). The cause of this increase in *C. subterraneus* is unclear. While this could be related to the marked change in water levels following the breaking of the drought and a sudden increase in the groundwater level at this site (exceeding the long term average; Section 3.4.2), it is surprising that similar changes in abundance were not recorded at any other site, especially at Site C which is paired with Site B (Section 2.2). Similarly, macroinvertebrate richness also varied by site (F=5.11; p=0.001) and was consistently highest at Sites A, B and C (Figure 4.25). These results are consistent with similar studies in England and Germany which found one to three groundwater species per site (Thulin and Hahn, 2008; Griebler et al., 2010; Weitowitz, 2012).

These results indicate that the macroinvertebrate community is spatially variable with the greatest number of positive samples, abundance and diversity recorded at Sites A, B and C. The reason for the large number of individuals at these sites is not apparent given the geographical distance between Site A (located upstream of the headwaters in the western-most part of the catchment) and Sites B and C (paired Sites located at the downstream end of the catchment), their varying depths and differing environmental conditions (Section

2.2); however, the number of positive samples and richness of these samples is consistent with similar phreatic studies.

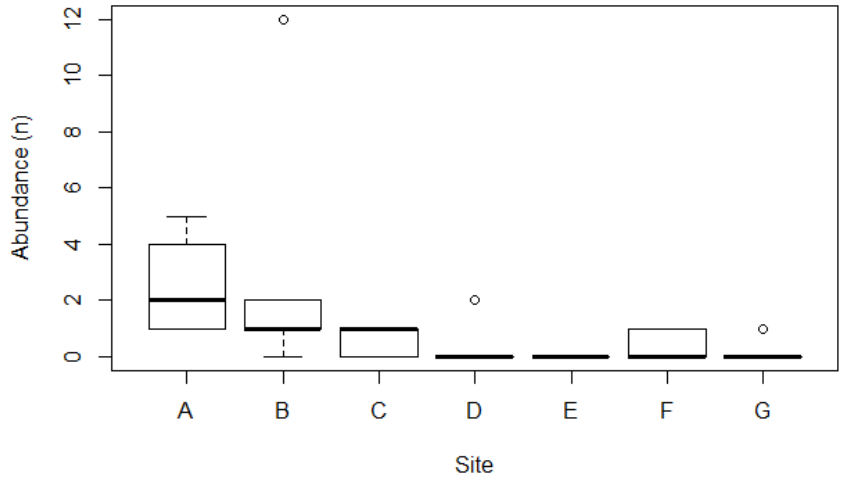


Figure 4.24 Macroinvertebrate abundance ((n) individuals per sample) recorded at the seven phreatic sites (A-G) over the study period (November 2011 to September 2012). The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

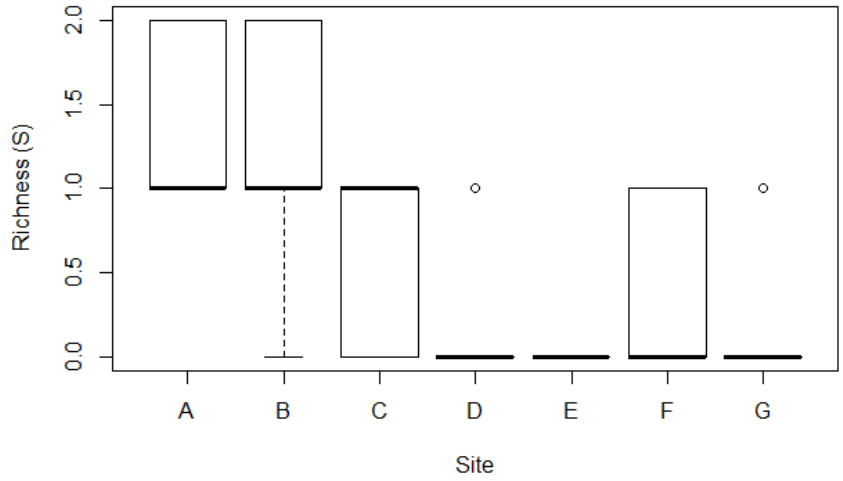


Figure 4.25 Macroinvertebrate richness (S) at the seven phreatic sites from November 2011 to September 2012. The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile and minimum and maximum abundance recorded each month.

4.4.2.2 Temporal Variability of the Macroinvertebrate Community

The same five metrics were also calculated to assess temporal variability in the distribution of the macroinvertebrate community; however, these results indicate no significant temporal differences in the abundance or richness of this community between sampling occasions (Table 4.12).

Table 4.12 Phreatic macroinvertebrate community and diversity (Shannon-Wiener (H) and Simpsons (D)) metrics as the study average ± 1 SE (n=6), except the number of positive samples. Temporal change has been assessed using a One-Way Analysis of Variance (ANOVA) with ** indicating overall significance of p<0.001, * of p<0.05 and ns p>0.05. Analyses were not undertaken on diversity metrics due to the small sample size.

Metric	Sampling Occasion						Temporal Change
	Nov	Jan	Mar	May	Jul	Sep	
Positive Samples (n)	2	2	3	2	6	4	ns
Abundance	0.75 (±0.62)	0.63 (±0.50)	2.00 (±1.48)	0.25 (±0.16)	0.88 (± 0.23)	0.63 (± 0.26)	ns
Richness (S)	0.38 (±0.26)	0.25 (±0.16)	0.50 (±0.27)	0.25 (±0.16)	0.75 (±0.16)	0.63 (±0.26)	ns
Diversity (H')	0.06 (±0.06)	-	0.06 (±0.06)	-	-	-	na
Diversity (D)	0.20 (±0.10)	-	0.15 (±0.08)	-	-	-	na

There is a marked increase in the number of positive samples in the final months of the study which may be related to the drought break and recovery of groundwater levels in July and September (Section 3.4.4). Macroinvertebrate abundance was lowest in May and highest in March but did not vary significantly by sampling occasion (F=0.77; p=0.60), despite the noted increase at Site B during the latter (Figure 4.26).

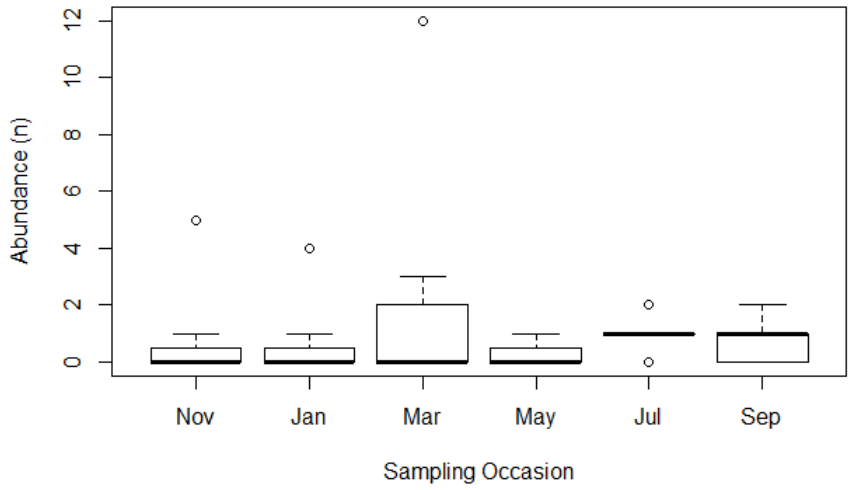


Figure 4.26 Total macroinvertebrate abundance from the six sampling occasions (November 2011 to September 2012) at all phreatic sites (A-G). The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

Macroinvertebrate richness did not exceed two species during any one sampling occasion; although overall richness was highest in March and July, this variance was not significant (Figure 4.27; F=1.66, P=0.16). These results suggest that the distribution of the groundwater macroinvertebrate community is

not temporally variable. While this is consistent with the expectations associated with the stable habitat provided by the aquifer (Section 1.3), it is surprising given the severity of the drought which occurred during the study period and suggests that this habitat may provide a refuge during periods of low flow.

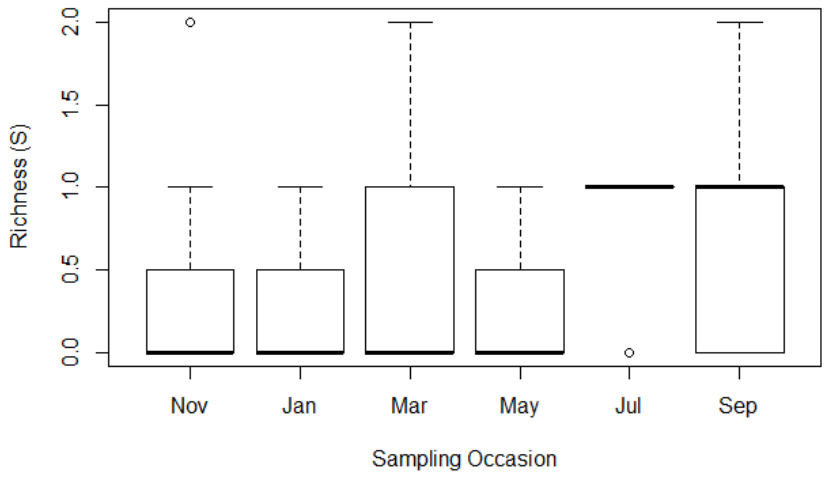


Figure 4.27. Macroinvertebrate richness (S) from the six sampling occasions (November 2011 to September 2012) at all phreatic sites (A-G). The plot illustrates the median (thick black line), first (bottom of each box) and third (top of each box) quartile as well as the minimum and maximum abundance recorded in each month.

4.4.3 Phreatic Assemblages in relation to Environmental Parameters

Multivariate analyses were used to investigate relationships between the phreatic macroinvertebrate community and the environmental conditions of this habitat. The results are discussed with reference to the community as a whole (Section 4.4.3.1) and to individual species (Section 4.4.3.2).

4.4.3.1 Phreatic Community in Relation to Environmental Parameters

Ordination techniques were used to assess the relationship between environmental variables and the distribution of the phreatic macroinvertebrate community following the same procedure as described in Section 4.2.3.1. As with the other habitats, the DCA indicated that the distribution of these species was neither linear nor unimodal as the longest axis value was of intermediate length and so the results were assessed using both CCA and RDA techniques, but the RDA provided the best fit.

The RDA indicates that the global model can explain 51% (r^2=0.51) of species variance and that most of this variance is explained by the first two axes

(43%).[20] The fit of the model was tested using an analysis of variance which indicated that it is significant (F=2.28; p=0.003). The resulting ordination suggests that temperature and change in water level against the long term average (LTA; Section 2.3) are the most important variables influencing the distribution of the macroinvertebrate community (Figure 4.28). The importance of these variables in explaining the distribution of this community was assessed using Monte Carlo permutation testing which indicated that both temperature (Temp; F=2.85; p=0.03) and LTA were weakly significant (F=3.00; p=0.04; Legendre and Legendre, 2012). The distribution of individual species suggests differing responses to the measured variables but that *N. fontanus* is the most cosmopolitan as it is located near the centre of the ordination. The ordination also displays the spatial variation in these results, showing the distinct clustering at Site A and the outlying record at Site B.

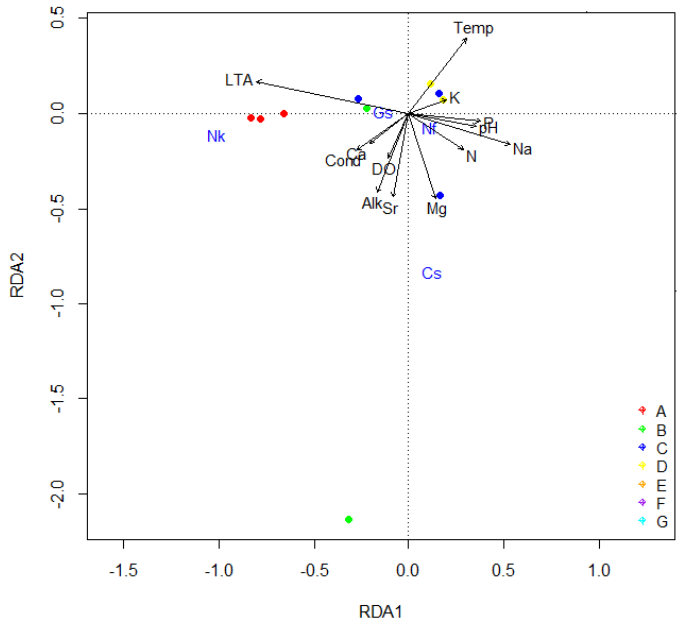


Figure 4.28. RDA triplot of species (blue, transformed count data, notation after Table 4.12) ordination with respect to physiochemical and chemical environmental variables (vectors, square-root transformed; November 2011- September 2012). Some sites (F and G) are obscured due to the overlap between results.

4.4.3.2 Phreatic Species in Relation to Environmental Parameters

Generalized linear models (GLMs) were used to assess the relationship between environmental variables and the distribution of individual species (Table 4.13). The GLMs tested the influence of (square-root) transformed environmental variables on the presence or absence of individual species using

[20] Eigenvalues for axes 1-4 in the RDA: 0.77; 0.64; 0.01; 0.00 and proportion explained for axes 1-4: 0.30; 0.13; 0.08; 0.00; Total Inertia= 0.93 (constrained=0.048; unconstrained=0.045); Total Proportion = 1.00 (constrained = 0.51; unconstrained=0.49)

a binomial error structure. This approach was selected instead of using count data to help mitigate the effects of outliers and control for the semi-quantified methodological approach to sampling (Section 2.3; Leps and Smilauer, 2003). Difficulty was experienced in fitting the GLMs; as such, the model for each species was run against each parameter individually, rather than with all of the parameters at the same time. While this difficulty is attributed to the small number of positive records in this habitat, it also suggests that the results should be regarded with caution and no further analyses have been pursued. The results suggest differing ecological preferences for each species, with species-specific influences on macroinvertebrate distribution across this habitat.

Table 4.13 GLMs of phreatic macroinvertebrate species presence/absence tested against square-root transformed environmental variables assuming a binomial error structure. Model fit was tested using a Chi-Square test (α=0.05). Significant results (p<0.05) are emboldened. A GLM was not fitted for *Gammarus* sp. as only one individual was recorded in this habitat on one occasion. Goodness of fit was determined by re-running the models using only the significant variables (excepting *C. subterraneus*).

Variable	*Niphargus kochianus*	*Niphargus fontanus*	*Crangonyx subterraneus*
Temperature	0.26	**0.05**	0.27
DO2	0.69	0.48	0.92
Conductivity	0.10	0.18	0.35
Alkalinity	0.31	**0.04**	0.06
pH	0.14	0.06	0.98
N	0.34	0.82	0.68
P	**0.05**	0.09	0.72
Ca	0.07	0.67	0.60
K	0.67	**0.01**	0.89
Mg	0.81	0.14	0.06
Na	0.14	0.61	0.08
Sr	0.32	0.28	0.28
LTA	0.06	0.18	0.89
Goodness of Fit	0.73	0.99	-

Niphargus kochianus was recorded at Site A on every sampling occasion as well as at Site B in March and Site C in September, suggesting that the distribution of this species is more spatially than temporally variable. The results of the GLM suggest a weak relationship with phosphate; however, this is likely to be a reflection of the geographical location of Site A (which is upstream of the catchment headwaters and recorded lower levels of phosphate than the other Sites, Section 3.2.3), than an ecological tolerance.

Niphargus fontanus was recorded only from phreatic samples collected during the months of July and September and only at sites located within a close

proximity of the river (B, C, D and G), suggesting that its distribution is both spatially and temporally variable. The results of the GLM suggest a relationship with temperature, alkalinity and potassium. Significant peaks in temperature and potassium were recorded in the phreatic habitat during the months when *N. fontanus* was recorded from these sites (Section 3.3). While the increase in water temperature during these months follows an expected seasonal pattern, the reason for the notable increases in alkalinity and potassium are likely associated with the recharge events which occurred at the end of the drought period (Section 3.3). Assessment of *N. fontanus* records from both riverine and phreatic habitats during this study, suggests that this species may use hyporheic corridors to exploit phreatic refugia during periods of adverse conditions and particularly in response to higher temperatures.

Crangonyx subterraneus was recorded at sites B, C or F during all sampling occasions except September 2012, suggesting that the distribution of this species is both spatially and temporally variable. The results of the GLM do not suggest significant relationships with any of the measured variables, a surprising result given the increase in the abundance at Site B in March 2012.

4.4.4 Discussion and Summary of Phreatic Communities

These results, including the proportion of sites supporting macroinvertebrates and the abundance and richness of this community, are as expected for a phreatic community, suggesting that further assessment be undertaken.

4.4.4.1 Can the phreatic macroinvertebrate community be described?

The community recorded in the phreatic habitat comprised four species of stygobiontic crustacean, three of which (*Niphargus kochianus; Niphargus fontanus* and *Crangonyx subterraneus*) are typical of a carbonate aquifer in this geographical location (Johns et al., 2015). The presence of *Gammarus* sp. is an unexpected and novel record (which will be discussed in more detail in Chapter 6). Considering the wider stygobiontic assemblage of Great Britain, it is notable that no individuals of *Microniphargus leruthi* or *Proasellus cavaticus* were recorded despite previous records from Western Kent. However, the absence of *Niphargus glenniei* is unsurprising as this species is only known from the western areas of Great Britain (Johns and Dunscombe, 2011; Weitowitz, 2012). It is also notable that no *Niphargus aquilex*, Acari or oligochaetes were recorded

in any of the phreatic samples, despite their presence in nearby benthic and hyporheic samples throughout this study (and commonly in phreatic habitats elsewhere; though it has been suggested that *N. aquilex* prefers shallower habitats; Section 1.3; Hahn, 2006; Johns et al., 2015).

Within this community, *Niphargus kochianus* was the most abundant species while *N. fontanus* was the most widespread. Only *N. kochianus* was exclusive to the phreatic habitat. While this exclusivity is consistent with similar studies which have suggested that *N. kochianus* prefers phreatic habitats (Martin et al., 2009), it suggests that the other species may utilise hyporheic corridors to exploit more multiple habitats.

4.4.4.2 Does the phreatic community vary spatiotemporally?

The results suggest that this community varies spatially but not temporally. Although positive results were recorded at all but one Site (E), there was significant variance in the number of positive samples, abundance and diversity between sites. The highest number of positive samples, abundance and diversity was recorded at Sites A, B and C, although the reason for this is not apparent in their geographical locations, physical structure, varying depths or the variables assessed by this study. By these same metrics, the reason for the absence of macroinvertebrate fauna at Site E is also not apparent; however, the microbiological results at this location were much poorer than the others which suggests that this may be partially attributed to a lack food sources for the invertebrates recorded across the aquifer (Brunke and Fisher, 1999; Hahn, 2006; Thulin and Hahn, 2008).

The lack of temporal variance in this community is consistent with the relatively stable habitat provided by the aquifer. However, it is surprising given the severity of the drought which occurred during the study period and the direct impact this had on groundwater levels throughout the catchment (Section 3.4.2). This finding is of particular interest as it is based on six separate sampling occasions and differs from previous literature in which samples were limited to one or two occasions alone (Dole-Olivier et al., 2009; Hahn and Fuchs, 2009; Johns et al., 2015; Martin et al., 2009).

The results from individual taxa indicate that some spatiotemporal variability may be species-specific. While the presence of *N. kochianus* varied spatially but not temporally, the presence (*N. fontanus*) and abundance (*C. subterraneus*) of the other species varied both spatially and temporally. As the only species recorded exclusively in the phreatic habitat during this study, it is likely that the distribution of *N. kochianus* is controlled by site-specific variables in the groundwater environment. Conversely, as the other species were also recorded in the superficial habitats and their distribution varied both spatially and temporally, it is likely that their distribution is controlled by influences from both the groundwater and surface water and that, in some cases, these species may utilise the phreatic habitat as a refuge.

4.4.4.3 Are phreatic assemblages related to environmental parameters?

The results indicate that the phreatic community is influenced by environmental parameters, specifically temperature and changes in water level, suggesting that hydrological exchange is a driving factor in the distribution of this community. While this may result from the particular period considered and the drought which occurred, these findings are also consistent with previous phreatic studies which were not undertaken during drought conditions and found that hydrological exchange was the principle factor shaping communities of unpolluted groundwater (Thulin and Hahn, 2008; Section 1.5.2). These findings differ from other phreatic studies as neither dissolved oxygen nor nutrients were found to be influential, although this is likely a product of the study area as all sites recorded relatively high (>1.0 mg L^{-1}) levels of dissolved oxygen and low levels of nutrients (Section 3.3; Dole-Olivier et al., 2009; Hahn, 2006; Hahn and Fuchs, 2009; Stein et al., 2010).

4.4.4.4 Summary of the Phreatic Community

The macroinvertebrate community recorded in the phreatic habitat comprised four species of stygobiontic crustacean. With the exception of the single *Gammarus* sp., the composition and diversity of this community as well as the number of sites with positive samples is typical of this type of aquifer. The community is spatially but not temporally variable. As expected, the spatiotemporal distribution of invertebrates in the phreatic habitat reflects the distinctive conditions of this environment and the distribution of species is

influenced by environmental conditions, specifically by temperature and changes in water level. However, contrary to expectations, these influences are species-specific and may reflect the migration of some taxa, specifically *N. fontanus*, into the phreatic habitat during adverse conditions.

4.5 Invertebrate Community Distribution across Three Habitats

Following the independent analysis of the communities occupying the benthic, hyporheic and phreatic habitats, the results were assessed collectively to describe the distributions of the constituent taxa (Section 4.5.1), variability in their distribution (Section 4.5.2) and association with environmental variables (Section 4.5.3). The discussion of this assessment is considered within the context of recent literature (Chapter 1) and the research questions addressed in this study. As geochemical and phreatic samples were only collected during the final year of the study, the following analyses are all based on records from November 2011 to September 2012 unless otherwise stated.

4.5.1 Community Descriptions

The results indicate that each habitat supported a distinct invertebrate assemblage. The benthic assemblage comprised exclusively epigean taxa, many of which were typical of a chalk stream environment, which were influenced in their distribution along a longitudinal spatial gradient from the headwaters to the downstream Sites (Section 4.2.4.1). The phreatic assemblage comprised exclusively stygobiontic taxa typical of a carbonate aquifer with a large degree of overlap between sites (Section 4.4.4.1). The hyporheic assemblage comprised a mixture of stygoxenes, stygophiles and stygobionts which were influenced in their distribution first along a gradient of groundwater affiliation and secondly along a longitudinal spatial gradient from the headwaters to the downstream sites. However, while the majority of these taxa were also recorded in either in the benthic or phreatic assemblages, some, such as *Niphargus aquilex,* were exclusive to the hyporheic habitat (Section 4.3.4.1). Although there is a great deal of overlap in the composition of assemblages between the benthic and hyporheic habitats as well as between the phreatic and hyporheic habitats, only one species, *Gammarus* sp. was recorded in all three (and is discussed further in Chapter 6). These results suggest that each of the habitats supported a distinct invertebrate community

whose distribution was influenced either by its affiliation with groundwater, location within the catchment or both.

Detrended correspondence analysis (DCA) was used to explore the variability in the distribution of invertebrate assemblages recorded in each habitat (Figure 4.29).[21] The first axis of the resulting ordination, which explained 76% of the variation, reflects the groundwater affiliation of the recorded species. The highest scores associated with stygobionts (such as *N. fontanus* (Nf) and *N. kochianus*) and the lowest scores with the epigean taxa.

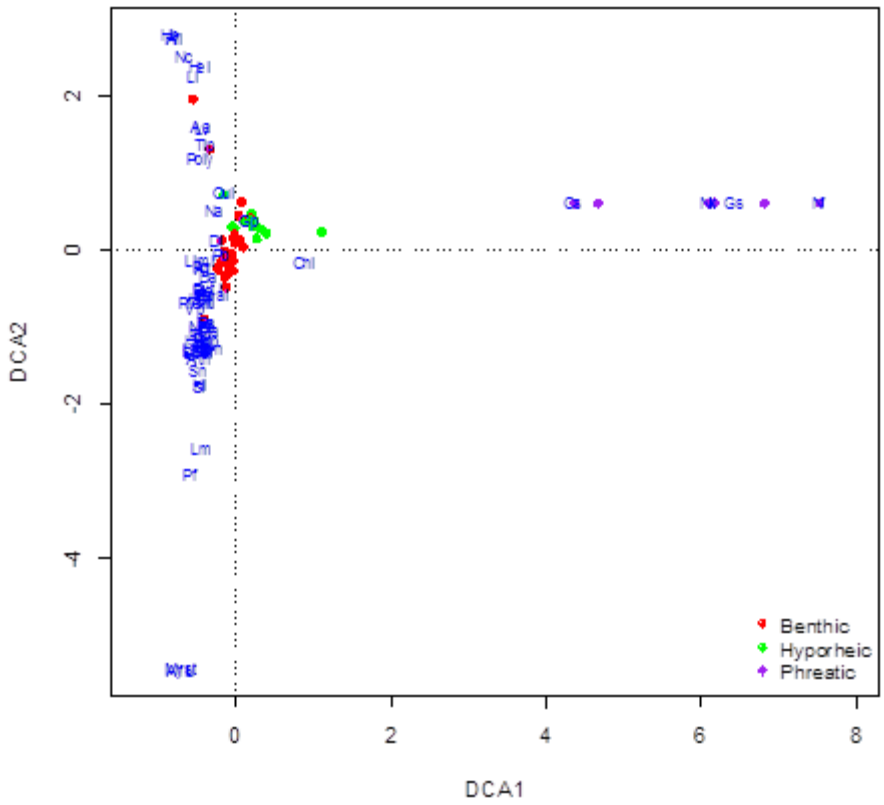


Figure 4.29 Detrended correspondence analysis of macroinvertebratres collected from all three habitats from November 2011 to September 2012 (species after Appendix I).

It would have been expected for *N. aquilex* (Na), a stygobiont, to be associated with a high score on this axis; however, its low score reflects its behaviour within this catchment during this study where it was recorded exclusively in the hyporheic habitat (with the exception of a single benthic record during the pilot study). The distribution of taxa along the second axis suggests a spatial influence with the highest scores associated with taxa recorded at the

[21] Eigenvalues for axes 1-4 in the DCA: 0.761; 0.248; 0.154; 0.290 and corresponding axis lengths: 8.089; 2.841; 0.892; 2.001

headwater sites (such as *Helodes* larvae (Hel)) and lower scores associated with taxa only recorded at Sites further downstream (such as the caseless caddisfly *Polycentropus flavomaculatus* (Pf)). Indirectly, the second axis also reflects changes to the temporal distribution of these species as *P. flavomaculatus* was distributed throughout the Little Stour catchment during the study but confined to the two furthest downstream sites during the final year. This change is likely a reflection of the low flows experienced during the final year as, while *P. flavomaculatus* can physiologically withstand periods of low dissolved oxygen, it requires deeper water in which to hunt prey since it deploys its spun silken threads to form snares which trap small animals (Philipson, 2010). These collective results provide support for the independent habitat assessments in reflecting the influence of both groundwater affinity and location within the catchment to species distribution.

4.5.2 Spatiotemporal Variability across the Three Habitats

When assessed independently, the benthic, hyporheic and phreatic habitats each supported distinctive invertebrate assemblages. When considered collectively, the results suggest that they function as a continuum between the surface and groundwater environments. While some species were only recorded in a single habitat, the most abundant and dispersed species were regularly recorded in multiple habitats, suggesting that these species have behavioural or physiological traits which facilitate this dominance. This is most notable in the overlap between benthic and hyporheic assemblages, which were both dominated by *G. pulex.* Despite its benthic affiliations, this species has previously been recorded at depths over two meters below the substratum during periods of disturbance and has been found to migrate into spring sites during periods of low flow (Boulton, 2003; Dole-Olivier and Marmonier, 1992; Stubbington and Wood, 2013). Considering the entire study period, assessment of *G. pulex* abundance as a ratio between paired benthic and hyporheic samples shows a similar pattern at all five riverine sites, mirroring the catchment hydrograph over this period as greater abundances were found in subsurface samples during periods of low flow (Section 3.4.1; Figure 4.30).

These results suggest that *G. pulex* actively migrates into the subsurface during periods of low flow when surface water availability declines. The dominance of

G. pulex within these communities suggests that such behaviour is advantageous to the persistence of this species during periods of adverse environmental conditions, such as low flow. Similarly, the results presented in Section 4.4.3.2 suggest the potential for species like *Niphargus fontanus* to move between the hyporheic and phreatic habitats during periods of adverse conditions, and specifically in response to high temperatures. These results suggest that, when viewed collectively, these habitats should be viewed as an ecological continuum which is utilised by species during periods of unfavourable conditions. This goes a step beyond the notion of the hyporheic habitat as a refuge and suggests instead a hyporheic corridor, supporting the suggestion by Williams et al., (2010) that the hyporheic habitat is a transitional interface between the surface and groundwater.

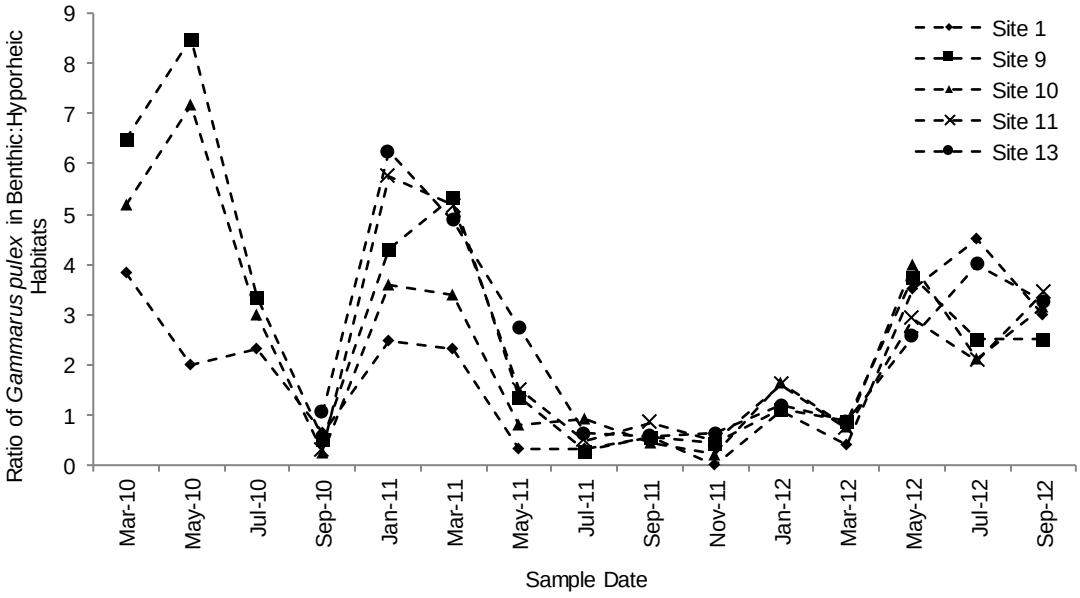


Figure 4.30 Ratio of *Gammarus pulex* in benthic and hyporheic habitats at the five riverine Sites from March 2010 to September 2012.

4.5.3 Distribution in Relation to Environmental Variables

The composition of the benthic, hyporhiec and phreatic communities was associated with the environmental variables considered by this study. Ordination analysis was undertaken to assess patterns in species distribution and the relationship between this and the environmental variables. Following the same multivariate approach presented earlier in this chapter, the results of the DCA were used to inform model selection (CCA given the relatively long DCA axis length) and the environmental and biological data were transformed (square-root and log(x+1), respectively) to enhance fit (Leps and Smilauer,

2003; ter Braak and Smilauer, 2002; Zuur et al., 2010). The results indicate that the model was significant (p=0.001) and explained 24% of species variability (r^2=0.236).[22] The importance of environmental variables in explaining the distribution of macroinvertebrates throughout these habitats was assessed using Monte Carlo permutation testing which indicated that conductivity (F=2.86; p=0.001); potassium (F=5.29; p=0.001); Mg (F=1.74; p=0.008); pH (F=2.21; p=0.003); nitrate (F=1.42; p=0.03); and temperature (F=1.31; p=0.039) were all of significance (Legendre and Legendre, 2012). The model was re-run omitting insignificant variables (alkalinity, phosphate, calcium, sodium and strontium) but this did not enhance the fit. The resulting ordination suggests a gradient between benthic and hyporheic samples and some overlap between these and the phreatic samples (Figure 4.31).

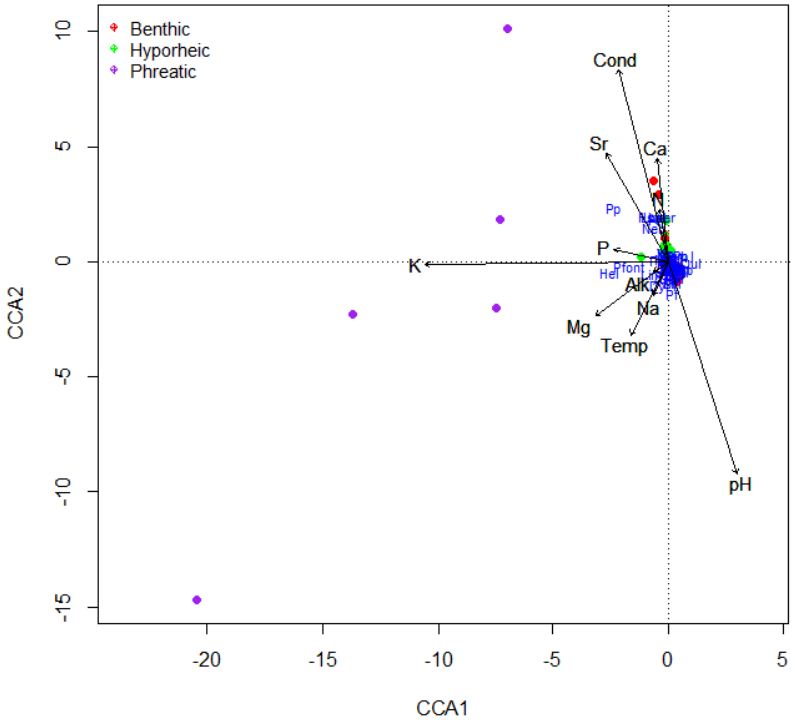


Figure 4.31 Canonical Correspondence Analysis ordination of transformed biological and environmental results from the benthic, hyporheic and phreatic habitats (November 2011 to September 2012; species abbreviations after Appendix I)

The inverse relationship between pH and conductivity supports the results from the independent assessment of the benthic and hyporheic habitats, reflecting the longitudinal gradient between the headwaters, which recorded a lower pH and higher conductivity, and the downstream Sites, which recorded a higher pH

[22] Eigenvalues for axes 1-4 in the CCA: 0.34; 0.23; 0.15; 0.11 and proportion explained for axes 1-4: 0.07; 0.05; 0.03; 0.02; Total Inertia= 4.66 (constrained=1.102; unconstrained=0.236); Total Proportion = 1.00 (constrained = 3.56; unconstrained=0.76)

and lower conductivity. Interestingly, many of the phreatic samples were associated with the potassium vector. While potassium was stable in the benthic and hyporheic habitats, it increased significantly (from 0.2 or 0.3 to 1.0 mg L^{-1}) in the phreatic habitat in July 2012 (Section 3.3.2.2), coinciding with a peak in the number of positive phreatic samples (Section 4.4.2.2). Potassium is used in groundwater studies as a signature of surface water influence, but previous studies have found that spikes in groundwater can reflect recharge events, especially in highly connected systems (Bartley and Johnston, 2006; Hahn and Fuchs, 2009; Stuart and Smedley, 2009). The dramatic increase in phreatic potassium is likely to reflect recharge following the drought break. The concurrent increase in positive phreatic samples may result from the passive movement of animals through the aquifer as part of this recharge event and recovery of groundwater levels following the drought.

4.5.4 Summary

This chapter has described the biological communities occupying the benthic, hyporheic and phreatic habitats and assessed the relative influence of environmental variables on these communities, fulfilling the first aim of this study. While each habitat supported a distinct invertebrate assemblage, when viewed collectively, the three habitats functioned as a continuum between the surface and groundwater environments, with most species being recorded in multiple habitats. While this migration between habitats is expected for some species (such as *Gammarus pulex*), it is surprising for others, such as the normally benthic *Agapetus fuscipes* which behaves as a stygophile in this catchment. Although this distribution may be attributed to the traits of individual species, it may also be a behavioural response to the history of flow stress in this catchment and the resulting selection in assemblage. The distribution of communities and species throughout these habitats was found to relate to environmental variables, specifically those which were directly or indirectly associated with flow. The concurrent sampling of these three habitats is a novel approach in this field, particularly as these samples were collected at a bimonthly frequency for more than a year. The results indicate that this approach provided a greater understanding of the full diversity of invertebrates within the catchment and of the way in which their distribution fluctuates both seasonally and in response to environmental change.

Chapter 5

Community Response to High and Low Flow Disturbances

5.1 Introduction

Variability in hydroclimatology is a primary influence on the distribution of invertebrate communities in lotic ecosystems (Dole-Olivier and Marmonier, 1992). The findings of this study indicate that invertebrate community distribution throughout the benthic, hyporheic and phreatic habitats of the study area is related to changes in environmental conditions, specifically those which are directly or indirectly related to hydrology. Two periods of abnormal rainfall occurred during the study period resulting in a period of high flow in spring 2010 and a drought in 2011-2012. These disturbances are of particular interest in exploring the relationships between invertebrate distributions across the three habitats. This chapter assesses changes in environmental conditions and the response of the invertebrate communities to these changes with reference the period of high flow (Section 5.2) and drought (Section 5.3) in fulfilment of the second aim of this study.

5.2 Invertebrate Community Response to High Flows

Periods of high flow increase flow velocity, amplify hydrological connectivity, restructure habitat and alter abiotic conditions (Lake, 2003). Invertebrate communities are influenced by these disturbances directly, as epigean fauna may be carried downstream by faster flowing surface waters and hypogean fauna may be swept into hyporheic or benthic habitats by increased groundwater discharge, or indirectly through changes in environmental conditions (Vervier and Gibert, 1991). While some invertebrates may be dislodged and lost during periods of high flow, others may persist using trait-based adaptations through resistance (when species are able to remain in the impacted area) or resilience (when species recover through recolonisation following the use of refugia, upstream crawling or active drift; Hildrew and Giller, 1994; Verdonschot et al., 2014). The Flood Refuge Hypothesis, an extension of the Hyporheic Refuge Hypothesis, suggests that some normally benthic taxa actively migrate into hyporheic sediments during periods of high flow (Wililams and Hynes, 1974; Boulton et al., 2004).

The response of invertebrates in benthic and hyporheic habitats to high flows is equivocal. Some authors have suggested that hyporheic fauna are displaced either by the event itself or by epigean taxa seeking refuge (Culver and Pipan,

2014; Olsen et al., 2010; Williams and Hynes, 1974). A study on the River Rhone, France by Dole-Olivier and Marmonier (1992) assessed the vertical distribution of fauna in benthic and hyporheic habitats over a series of spates and found a downward movement of both epigean and hypogean taxa during these periods, with *Gammarus pulex*, a normally epigean species, migrating as far as 2 meters into the sediment. However, similar studies have found no alteration in vertical distribution during such events (Giberson and Hall, 1988; Olsen and Townsend, 2005). Communities occupying phreatic habitats have also been found to respond to periods of high water levels. An experimental study across a glaciofluvial aquifer in France found the abundance and richness of the invertebrate community was significantly higher in boreholes treated with artificially increased recharge rates than in reference sites; however, it was suggested that this was an indirect result of increased dissolved organic carbon rather than a response to increased recharge (Datry et al., 2005).

High flows have the potential to alter the structure of benthic, hyporheic and phreatic communities and their recovery to pre-disturbance conditions is likely dependent upon the amplitude and duration of the event, disturbance regime and season (Dole-Olivier and Marmonier, 1992). It is expected that the high flows that occurred during this study altered environmental conditions and reduced the abundance and diversity of the biological communities.

5.2.1 High Flow Period

A period of above average rainfall occurred between October 2009 and March 2010, resulting in high flows between February and July 2010 which peaked on the first of April 2010 (1.19 m^3 s^{-1}; Figures 3.38 and 5.1). The discharges recorded during this period were much higher than the long-term average (0.18 m^3 s^{-1}, recorded by the Environment Agency near Site 9, February 2003-September 2012, n=337,708) and matched the previous discharge maximum recorded in February 2003 (1.19 m^3 s^{-1}). Flow velocities recorded during this period ranged from 0.32 to 0.55 m s^{-1} and also exceeded the long term average (0.15 m s^{-1}, as previous). Groundwater levels were also higher than expected over this period. Water table levels were, on average, 9% above their site-specific long term averages as recorded by the Environment Agency (between

0.009 (site C) and 14.55 (site A) mAOD above the LTA for sites with more than one record over this period; Section 3.4.4).

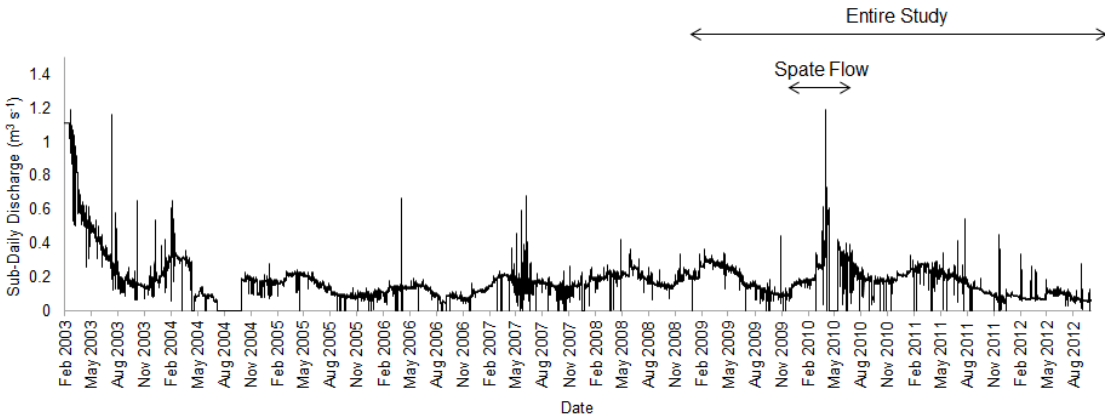


Figure 5.1 Hydrograph of sub-daily (15 minute) discharge (m^3 s^{-1}) recorded by the Environment Agency on the Little Stour (near Site 9) from the inception of this gauge (February 2003) until the end of the study period (September 2012).

Notably, during the high flow period, the Nailbourne flowed along its entire length to its confluence with the Little Stour, an occurrence historical accounts suggest only occurs once in seven years. High flows also overwhelmed the wastewater treatment system, resulting in the pumping of sewage to the river at Patrixbourne (30 March – 26 April) and Bishopsbourne (1 April – 25 April) both located between sites 6 and 7 on the Nailbourne (Section 2.2; Figure 2.5; Southern Water, *pers*. *comm*., 6 July 2010). These occurrences suggest that the above average rainfall resulted in an extraordinary high flow period.

5.2.2 Sample Collection during the High Flow Period

Physiochemical and biological samples were collected from benthic and hyporheic habitats on the 27th of March (prior to the discharge of sewage) and the 1st of May (following cessation of pumping; 2010) at sites 1 (upstream of the pumping), 9 and 10 (downstream of the pumping) as part of the routine monitoring for this study. Additional benthic samples were collected at sites 4, 6 and 7 (Figure 2.5) alongside the routine monitoring in March to assess the response of the communities Nailbourne channel (which would normally be dry) to the resumption of flow; unfortunately, hyporheic samples could not be collected at these additional sites due to the thick layer of clay and terrestrial grasses which comprised the substratum. To assess the potential impact of sewage discharge on the biological communities, additional benthic and hyporheic samples were also collected on the 2nd of April (2010) at sites 1, 9 and 10. The high flow period predates the collection of phreatic samples.

5.2.3 Results and Analysis of the High Flow Period

A sub-set of the results from this study which were collected during the high flow period (from March, May and July 2010) as well as the additional biological samples from April of the same year were assessed to identify changes in environmental conditions and in the composition of the benthic and hyporheic communities. Unfortunately, due to the divergence in sampling methods (Section 4.3), it is not possible to directly compare these samples with those collected prior to March 2010; however, it is possible to compare the results with those from spring 2011, a period of normal flow (Figure 3.39).

5.2.3.1 Environmental Conditions

Surface water discharge was the same as the long-term average for the Little Stour in January 2010 (0.18 m^3 s^{-1}); however, it was dramatically higher from March to July, recording values between the antecedent Q_1 (0.34 m^3 s^{-1}) and Q_{10} (0.22 m^3 s^{-1}; Section 3.4.1; Table 5.1). Variance in physiochemical parameters from the benthic and hyporheic habitats followed expected seasonal patterns and was similar to values of a normal flow year, with the exception of temperature, which was on average more than a degree higher during March 2010 than March 2011 (Section 3.2). This difference is not associated with air temperature as this was slightly lower in March 2010 than March 2011 (9.8 °C and 10.0 °C, respectively), but is instead a likely reflection of increased groundwater quantities, which contribute warmer water to the surface habitats.

Table 5.1 Mean environmental variables at sites 1, 9 and 10 in the benthic (Ben) and hyporheic (Hyp) habitats during the high flow period (March-July 2010) and following year (March 2011).

Variable	Mar-10		May-10		Jul-10		Mar-11	
Habitat	Ben	Hyp	Ben	Hyp	Ben	Hyp	Ben	Hyp
Discharge (m^3 s^{-1})	0.32	-	0.33	-	0.23	-	0.25	-
Temperature (°C)	10.6	11.3	11.8	12.7	14.4	11.9	9.5	10.2
DO (mg L^{-1})	10.9	-	10.1	-	8.1	-	10.5	-
pH	7.0	7.1	7.5	7.3	7.4	7.3	7.5	7.4
Conductivity (µS cm^{-1})	668.0	644.7	668.3	649.7	649.0	648.7	638.7	639.7
Alkalinity (mg L^{-1})	244.3	223.7	241.7	251.3	271.7	263.7	251.3	265.0

5.2.3.2 Benthic Assemblage

Fifty-six taxa were recorded in the benthic habitat at sites 1, 9 and 10 between March and July 2010 (n=4). The most commonly recorded species was *Gammarus pulex* (comprising 19% of this community), followed by *Agapetus fuscipes* (15%), Chironomidae (10%), Oligochaeta (8%) and *Baetis rhodani* (7%). The three sites which did not normally support flow (4, 6 and 7) were

surveyed in March 2010 and recorded six taxa: *Asellus aquaticus*, Chironomidae, *Gammarus pulex*, Limnephilidae larvae (taxonomy at indistinguishable instar), *Limnius volckmari* and Oligochaeta, all of which were routinely recorded upstream at Site 1 and downstream at Site 9. No stygofauna were recorded at any of these sites during this period.

Abundance and richness followed a similar pattern at sites 1, 9 and 10, decreasing markedly from March 2010, before increasing in May (sites 9 and 10) or July (Site 1; Table 5.2). This decline is more pronounced at the perennial, downstream sites (9 and 10) than in the intermittent headwaters (Site 1).

Table 5.2 Biological metrics from sites 1, 9 and 10 collected during the high flow period, including additional sample collection in April 2010.

Variable	Site	Mar-10	Apr-10	May-10	Jul-10
Abundance	1	67	59	47	56
	9	136	33	178	133
	10	136	36	144	121
Richness	1	15	13	19	19
	9	30	20	27	28
	10	29	17	27	25

Invertebrate abundance varied between March and July, with the highest values recorded in May and lowest in April (both at Site 9); however, site and sampling occasion were not found to be significant in explaining this variability when tested using a two-way analysis of variance. The marked decline in abundance observed in April 2010 corresponded with peak discharge and reflects a brief, but large reduction in the number of *Gammarus pulex* in the benthic habitat. However, if additional samples had not been collected in April, this decline would have not been identified as values had recovered by May (Figure 5.2)

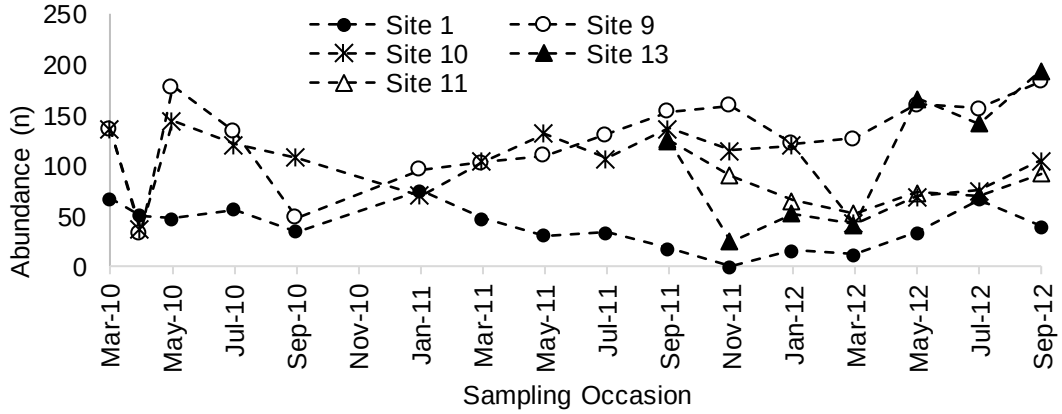


Figure 5.2 Benthic invertebrate abundances recorded at sites 1, 9, 10, 11 and 13 over the study period. Site 1 was dry in November 2011, recording no benthic fauna.

Invertebrate richness also varied between March and July 2010, with the highest values recorded in March (Site 9) and lowest values in April (Site 1) and varied by site (p=0.01, F=11.89) and sampling occasion (p=0.03, F=7.35). The decline in richness observed in April 2010 corresponded with peak discharge and reflects the loss of a number of species from each site (Figure 5.3). This decline was most notable at Site 10 where a number of species, including *Limnephilus lunatus, Hydropsyche siltalai, Asellus aquaticus*, and *Glossiphonia complanata* were recorded in March as well as May and July but not in April. A similar pattern was observed at sites 1 and 9. The species which persisted at these sites during April included pollution intolerant species such as the cased-caddisflies *Silo nigricornis* and *Sericostoma personatum* (larger caddisflies which carry relatively heavy mineral-based cases that could facilitate their resistance during high flow), suggesting that the decline in richness is not associated with the sewage discharge.

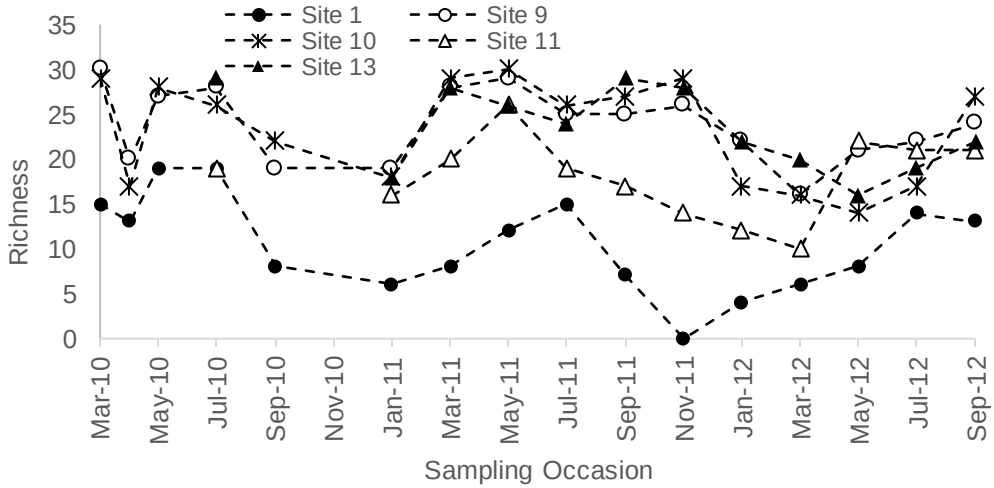


Figure 5.3 Benthic invertebrate richness recorded at sites 1, 9, 10, 11 and 13 over the study period. Site 1 was dry in November 2011, recording no benthic fauna.

5.2.3.3 Hyporheic Assemblage

Thirteen taxa were recorded in the hyporheic habitat at sites 1, 9 and 10 between March and July 2010 (n=4). The most commonly recorded species was *Gammarus pulex* (comprising 40% of this community), followed by *Agapetus fuscipes* (37%), Oligochaeta (4%), Scirtidae larvae (Helodes; 4%) and Chironomidae (4%). No stygofauna were recorded from the hyporheic habitat during this period.

Invertebrate abundance varied between March and July; however, contrary to the benthic habitat, a notable increase was recorded at sites 9 and 10 in April

2010 (although neither site nor sampling occasion were found to be significant when tested using a two-way analysis of variance; Figure 5.4).

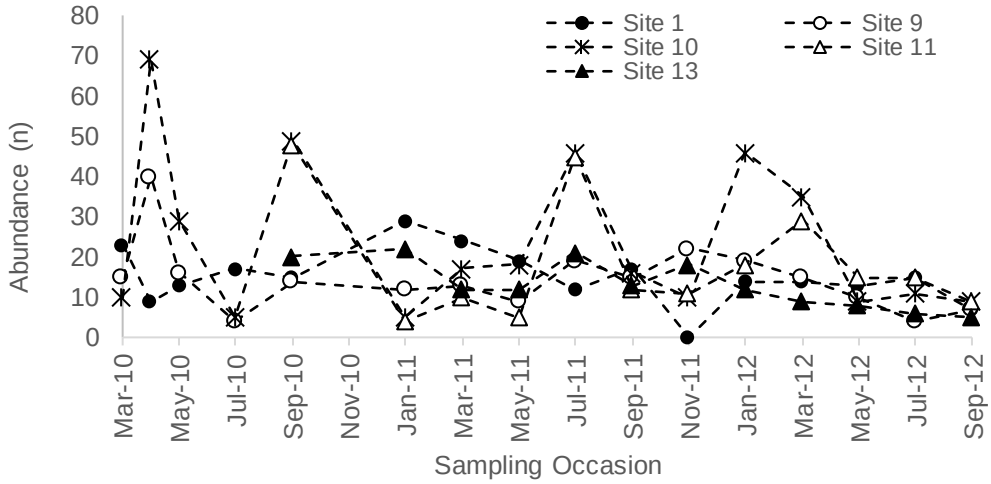


Figure 5.4 Hyporheic invertebrate abundance recorded at sites 1, 9, 10, 11 and 13 over the study period. Site 1 was dry in November 2011, recording no hyporheic fauna.

The marked increase in abundance observed in April 2010 corresponds with peak discharge and reflects a brief, but large increase in the number of *G. pulex* and *Agapetus fuscipes* in the hyporheic habitat (Figure 5.5).

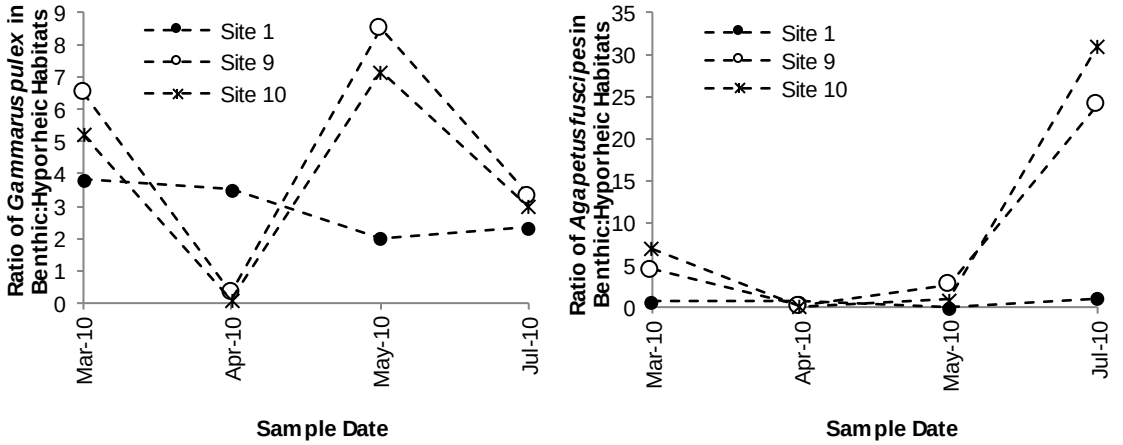


Figure 5.5 Ratio of *Gammarus pulex* (left) and *Agapetus fuscipes* (right) in the benthic and hyporheic habitats between March and July 2010.

Conversely, invertebrate richness declined over this same period (Figure 5.6), but did not vary significantly by site or sampling occasion (when tested using a two-way analysis of variance). The decline in richness observed in April 2010 corresponds with peak discharge and the increase in the abundance of *Gammarus pulex* and *Agapetus fuscipes* in the hyporheic habitat, which may have displaced other species.

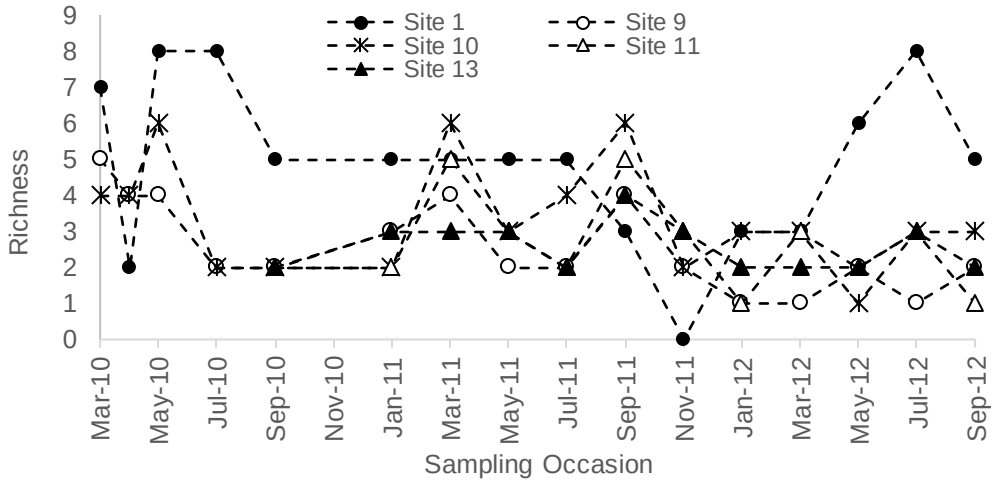


Figure 5.6 Hyporhiec invertebrate richness recorded at sites 1, 9, 10, 11 and 13 over the study period. Site 1 was dry in November 2011, recording no hyporheic fauna.

5.2.4 Summary and Discussion of the Response to the High Flow Period

The results have been assessed to determine if the high flow period altered environmental conditions (Section 5.2.4.1) or abundance or diversity of the communities in the benthic and hyporheic habitats (Section 5.2.4.2).

5.2.4.1 Did environmental conditions change as a result of the high flows?

The period of above average rainfall resulted in an intense spate flow. During this time, flow velocities, river discharge and groundwater levels were higher than the long-term average. The results suggest that hydrological connectivity was amplified longitudinally (as the Nailbourne flowed along its entire length to its confluence with the Little Stour) and vertically (as increased water temperature in the hyporheic and benthic habitats suggest greater connectivity between the aquifer and surface water) during this period; however, no change was detected in other abiotic variables.

5.2.4.2 How did the invertebrate communities respond to the high flows?

The invertebrate communities recorded in the benthic and hyporheic habitats responded to the high flows but there was no significant change in the calculated metrics, suggesting minimal impact. In the benthic habitat, both abundance and diversity declined during the high flow period, specifically coinciding with peak discharge, but recovered to pre-disturbance values within a month. These findings are similar to those of Williams and Hynes (1974) who also recorded an active movement of benthic invertebrates into the hyporheic habitat during spate conditions and a reversal of this migration within days of the end of the spate. In the hyporheic habitat, diversity decreased but

abundance briefly increased, reflecting the movement of normally benthic species, specifically *G. pulex* and *A. fuscipes*, into this habitat and providing support for the Flood Refuge Hypothesis. It is surprising that no stygofauna were recorded during this period as the environmental results, specifically temperature, results suggest a greater upwelling of groundwater over the study area which would have been expected to dislodge these taxa.

The impact of this disturbance was mitigated by the quick recovery of these communities, which is attributed to species-specific persistence. The continued presence of some cased-caddisflies, specifically *S. nigricornis* and *S. personatum*, in the benthic habitat suggests that they were able to resist being displaced by the high flows. These results differ from those of *L. lunatus*, which was absent at sites where it was normally recorded (specifically during April). However, this may be expected as previous studies have found that *L. lunatus* is more vulnerable to high flow velocities as its case is half-organic, half-mineral (unlike *S. nigricornis* and *S. personatum*) and it drifts downstream when flow velocities exceed 0.10 m^3 s^{-1} (Verdonschot et al., 2014). Similarly, the movement of *G. pulex* and *A. fuscipes* from the benthic to the hyporheic habitat during the high flow period suggests that the exploitation of vertical refuges facilitates the resilience of these species. These results support previous studies which found both of these species to actively migrate to deeper habitats during periods of high flow (Dole-Olivier and Marmonier, 1992; Nijboer, 2004)

5.2.4.3 Summary

The period of high flow altered environmental conditions and the invertebrate communities of the benthic and hyporheic habitats. The response of the invertebrate communities was species-specific and differed between habitats; however, the impact of the disturbance was mitigated by the trait-based resilience and resistance of these species which facilitated the recovery of these communities, with the results indicating no significant or long-term effect.

5.3 Invertebrate Community Response to Low Flows

Droughts are natural, unpredictable disturbance events that reduce the water available to the environment over an extended period of time, resulting in a contraction of lotic habitat, disruption in hydrological connectivity (longitudinally

along the river corridor, laterally to the riparian area and vertically into the hyporheic habitat) and change in abiotic conditions (Lake, 2003; Ledger et al., 2016). Droughts can have marked effects on aquatic communities, directly through the reduction in habitat (and connectivity between habitats) and indirectly through increases in fine sediment accumulation, alteration in food resources, elevated water temperatures and decreased dissolved oxygen (Lake 2003). The response of aquatic communities to these changes varies. While some species can be lost if they become trapped or stranded in conditions exceeding their tolerance, others may persist using species-specific physiological, behavioural or life strategy adaptations (Lake, 2003). Some of these traits allow taxa to resist drought conditions by remaining in an impacted area while others are resilient and will recolonize the area when normal conditions resume having temporarily exploited refugia or reproduce using desiccation-resistant eggs (Chessman, 2015; Lake, 2003; Stubbington et al., 2009a). Drought impact on aquatic communities depends upon its duration and intensity, and is often associated with reductions in abundance and richness (Lake 2003; Stubbington et al., 2015).

The Hyporheic Refuge Hypothesis has been used to describe the active migration of normally benthic species into hyporheic sediments during periods of low flow (Williams and Hynes, 1974). However, while some studies have supported this hypothesis (Marchant, 1995; Wood et al., 2005a), others have found no changes in the vertical distribution of invertebrates (James et al., 2008; Olsen and Townsend, 2005), or have found the number of normally benthic species in the hyporheic habitat to decrease (del Rosario and Resh, 2000). While many of these studies focus on the potential downward migration of benthic species during droughts, a study by Wood et al., (2010) found that the abundance of both epigean and hypogean taxa (such as *Gammarus pulex* and *Niphargus aquilex*, respectively) increased in the hyporheic habitat in response to elevated surface and hyporheic water temperatures during a supra-seasonal drought on the Little Stour (2006). This suggests that refuge seeking taxa may migrate downward as well as upward. While research on the response of the phreatic community to drought is limited, an experimental study in Australia assessed the distribution of stygofauna in relation to groundwater

drawdown and found the response to be taxon-specific but that all taxa eventually became stranded (Stumpp and Hose, 2013).

Droughts have the potential to alter the structure and composition of benthic, hyporheic and phreatic communities. It is expected that the low flow period that occurred during this study altered the environmental conditions of the study area and reduced the abundance and diversity of the biological communities.

5.3.1 Low Flow Period

An extended period of below average rainfall occurred in spring 2011 and resulted in drought conditions that peaked before breaking in April 2012 (Section 3.4.1). The lowest discharges recorded during the study occurred in November 2011, just before peak discharge would be expected during a normal flow year (mean daily flow=0.11 m^3 s^{-1} and 0.04 m^3 s^{-1} on the Dour and Little Stour, respectively as recorded by the Environment Agency; Section 3.4.1). Groundwater levels were also lower than expected over this period. At the sites considered in this study, water table levels were, on average, 5% below their site-specific long term averages as recorded by the Environment Agency (between -0.13 (site F) and -9.72 (site A) mAOD below the LTA; Section 3.4.4). This suggests a departure from the anticipated seasonal pattern, with many sites recording their minimum levels early in 2012 and maximum levels during the following July, contrary to what would be expected (Section 3.4.4). While perennial flow was maintained on the Little Stour and Dour sites throughout the study, Site 1 in the Nailbourne headwaters dried completely in November 2011 which has historically only dried during extreme droughts (Holmes, 2006; Section 2.2). However, as previous droughts (1949, 1991-92 and 1996-97) caused parts of the Little Stour to dry completely, this drought may not have been as severe (Section 3.4.1). Although in isolation this appears to be a seasonal drought, the period of low rainfall in autumn 2010 resulted in a short-term drying event in November 2010 which was a precursor to the low flow period and, these collectively suggest a supra-seasonal drought across the study area (*sensu* Lake, 2003; Figure 3.39).

5.3.2 Results and Analysis of the Low Flow Samples

Routine results from the benthic, hyporheic and phreatic habitats were assessed to understand the response of these communities to the drought. Unlike the high flow period, no additional samples were collected during the drought (Section 5.2.2). No benthic or hyporheic samples could be collected from Site 1 in November 2011 due to the lack of water. The results were analysed within three distinct time periods: normal flow conditions (November 2010 - July 2011); drought (September 2011 - March 2012) and drought-break (May - September 2012; Figure 3.39).

5.3.2.1 Environmental Conditions

The environmental conditions in each of the three habitats altered as surface water flow and groundwater levels declined over the drought period. While alkalinity, pH and conductivity were not influenced by the low flow period, there were fluctuations in temperature (Sections 3.2.2, 3.2.3 and 3.2.5). Water temperature in the benthic and hyporheic habitats was higher in January and March 2012 than the same months in the preceding year, and at many sites, in both habitats, the values recorded during March were higher than those recorded in May, which is contrary to the expected seasonal pattern (Table 3.2; Section 3.2.1). While phreatic temperature was only recorded during the final year of the study and cannot be compared with a non-drought year, the values were higher and more variable (ranging from 11.0 (site E) to 18.2 ^{o}C (site G)) than what would be expected for groundwater (annual average of 10-11 ^{o}C) and may reflect longer residence times resulting from a reduction in hydrological connectivity in this habitat (Bloomfield et al., 2013; Section 3.2.1). Surprisingly, there was no decline in dissolved oxygen in either the benthic or phreatic habitats during this period, with the highest values of the study recorded in March 2012 (Section 3.2.4). No significant temporal variability in geochemical parameters was detected across the three habitats, suggesting stable chemical conditions despite the drought period and its break, with the exception of a spike in potassium in the phreatic habitat in July 2012, which has been attributed to a recharge event (Section 3.3.2).

5.3.2.2 Benthic Assemblage

Fifty-two taxa were recorded in the benthic habitat at sites 1, 9, 10, 11 and 13 between September 2011 and March 2012 (n=4; no taxa were recorded at Site 1 in November 2011 as it was dry). The most commonly recorded species was *Gammarus pulex* (comprising 27% of this community), followed by *Agapetus fuscipes* (16%), Chironomidae (9%), Oligochaeta (7%) *Asellus aquaticus* (5%) and *Baetis rhodani* (4%). No stygofauna were recorded in any benthic samples during this period.

Macroinvertebrate abundance varied between sites during this period with numbers declining first at the headwater sites (1 and 13) in November 2011 and then at the sites located further downstream (9, 10 and 11) in March 2012 (Figure 5.2). Abundance at all sites increased after the drought broke in March 2012, suggesting that the benthic community responded quickly to the change in conditions. Considering the period from the onset of the drought (September 2011) to the end of the study period (September 2012), the results indicate significant differences in abundance by site ($p=0.001$, $F=13.47$) and, weakly, by sampling occasion ($p=0.04$, $F=2.64$) when tested using a two-way analysis of variance (data after Section 4.2.2). Spatially, these results are similar to those recorded over the whole study period which found significant differences by site (Section 4.2.2.1) but not between sampling occasions (Section 4.2.2.2). These changes in abundance correspond with fluctuations in the number of *Gammarus pulex* recorded in the benthic habitat which show large declines in November 2011 and March 2012, before a large increase across all sites in May 2012, suggesting a quick recovery of the numbers of this species following the breaking of the drought (Figure 4.8). This is similar to the response of *Agapetus fuscipes,* which also decreased markedly in November 2011 but recovered in number at a few downstream sites following the drought break (Figure 4.9). The results for *A. fuscipes* are notable as the abundances in spring 2010 and 2011 are markedly (though not significantly) higher than those in spring 2012, suggesting that this species was adversely effected by the period of low flow, though it is not possible to discern the long-term impact without data from 2013 (Section 4.2.3.2).

Macroinvertebrate richness was also lower over the drought period than in comparable months in previous years (Figure 5.3). Broadly, richness declined from November 2011 to May 2012. The results from previous years would suggest that richness would be expected to be at its highest during the spring and autumn months; however, richness during the drought period, and most notably March and May 2012, was amongst the lowest over the entire study. Considering the period from the onset of the drought (September 2011) to the end of the study period (September 2012), the results indicate significant differences in richness by site ($p=0.001$, $F=14.45$) but not sampling occasion ($p=0.07$, $F=2.26$) when tested using a two-way analysis of variance. This decline reflects the loss of a number of regularly recorded species, most notably rheophilic taxa such as *Baetis rhodani* during spring 2012 (Section 4.2.3.2).

5.3.2.3 Hyporheic Assemblage

Nine taxa were recorded in the hyporheic habitat at Sites 1, 9, 10, 11 and 13 between September 2011 and March 2012 (n=4; no taxa were recorded at Site 1 in November 2011 as it was dry). The most commonly recorded species was *G. pulex* (comprising 84% of this community), followed by Chironomidae (7%), *Agapetus fuscipes* (3%), Oligochaeta (2%) and *Niphargus aquilex* (2%).

Invertebrate abundance in the hyporheic habitat varied but peaked at downstream sites (10 and 11) in January and March 2012 over the drought period (Figure 5.4). These results suggest an inverse pattern to the abundances recorded in the benthic habitat, which decreased at the same sites over this same period (Figure 5.2). Over the course of the study period, abundance in the hyporheic habitat was broadly static but increased at some sites during the summer months in 2010 and 2011. The results from the drought period are notable as they suggest similar peaks but during the winter and early spring, a pattern only observed previously during the high flow disturbance period. However, this variability was not found to be significant by site or sampling occasion when tested using a two-way analysis of variance (Section 5.2.3.3). The marked increase in abundance corresponds with the peak drought period and reflects a brief, but large, increase in the number of *Gammarus pulex* in the hyporheic habitat (Figure 5.5; Figure 4.30).

Invertebrate richness was broadly static over this same period (Figure 5.7), and did not vary significantly by site or sampling occasion when tested using a two-way analysis of variance. Interestingly, richness at Site 1 increased dramatically following its complete drying in November 2011, and recorded 8 taxa, the highest hyporheic diversity at any riverine site throughout this study, matched only at the same site following the period of high flows. The reason for this is unclear but may be attributed to an influx of invertebrates recolonizing the site following disturbance. *Niphargus aquilex* was the only stygofauna recorded in the hyporheic habitat during the low flow period, with the highest abundances occurring in September and November 2011 at sites 10 and 11, coinciding with the start of the drought period.

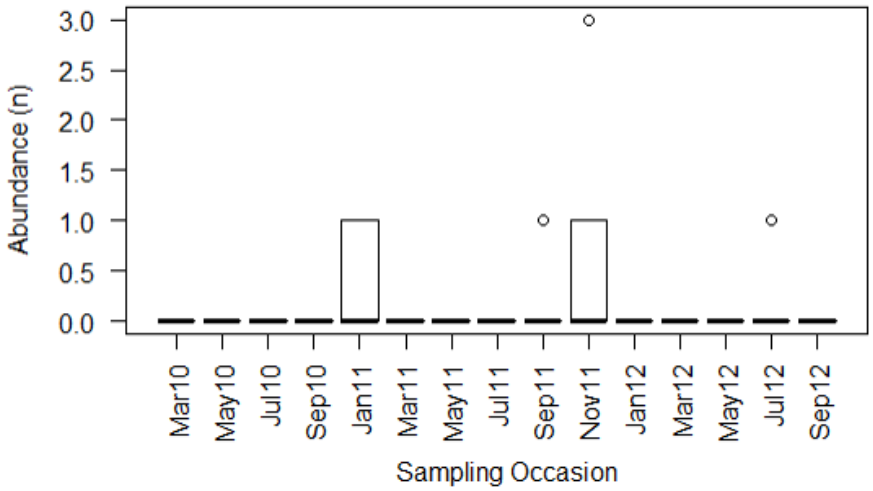


Figure 5.7. *Niphargus aquilex* abundance in hyporheic samples (Sites 1, 9, 10, 11 and 13) across the study period.

5.3.2.4 Phreatic Assemblage

Four species were recorded in the phreatic habitat at sites A-G between September 2011 and March 2012 (n=4; September samples only collected at sites A and E), including *Niphargus kochianus, Niphargus fontanus, Gammarus* sp. and *Crangonyx subterraneus.* The most commonly recorded species over this period, both in abundance and distribution, was *Niphargus kochianus*. Macroinvertebrate abundance was highest in March 2012, resulting from the large increase in the number of *Crangonyx subterraneus* at Site B (Figure 5.8). The reason for this increase is unclear from the measured variables but it may relate to the migration of this species from the river environment (where it was recorded in the hyporheic habitat) to the phreatic habitat at a time which coincides with the drought peak and the refuge seeking behaviour of other species. Despite these variations, richness remained static over this period and

neither abundance nor richness were found to vary significantly by site or sampling occasion when tested using a two-way analysis of variance.

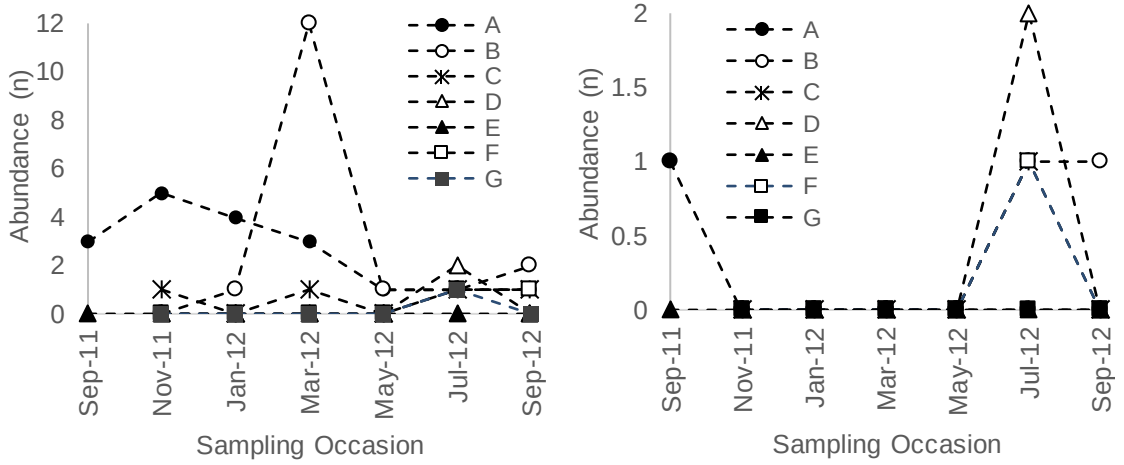


Figure 5.8 Abundance of all phreatic macroinvertebrates (left) and of just *N. fontanus* (right) across the 7 phreatic sites from September 2011 (Sites A and E only) to September 2012. Note that the *N. fontanus* graphic obscures the record of one individual at Site C in July 2012.

The greatest number of positive samples was recorded in July and September 2012 (n=6 and 4, respectively), the first positive macroinvertebrate samples during this study at a number of sites (Table 4.14). This increase coincides with the recovery of groundwater levels (July 2012) following the breaking of the drought (April 2012) and lags behind the recovery of surface water flows (May 2012; Section 3.4.5). Recharge events are expected to increase hydrological connectivity, and therefore facilitate the movement of fauna throughout the catchment. Many of these positive samples exclusively recorded individuals of *N. fontanus*, a species recorded in both the hyporheic and phreatic habitats in this catchment during this study, whose abundance and distribution increased markedly in July and September 2012 (Figure 5.9). While the distribution of *N. fontanus* was found to be associated with potassium, alkalinity and temperature it is not clear if the sudden increase in its presence results from an active migration into the phreatic habitat, potentially in response to elevated riverine water temperature, or a passive displacement resulting from the recharge events. Assessment of *N. fontanus* records from riverine and phreatic habitats suggests that this species may use hyporheic corridors to exploit phreatic refugia during periods of adverse conditions.

5.3.2.5 Desiccation

Only Site 1, the perennial springhead of the Nailbourne dried completely during this study. Drying occurred in November 2011, and inhibited the collection of

both benthic and hyporheic samples. Discharge from the springhead resumed in time for sample collection in January 2012. Within the benthic habitat three organisms were recorded following the resumption of flow, *Asellus aquaticus* (n=1); Chironomidae (n=1); *Gammarus pulex* (n=12); and Oligochaeta (n=2; Table 5. 3). This assemblage mirrors the paired hyporheic sample which recorded *Asellus aquaticus* (n=1), Chironomidae (n=2), and *Gammarus pulex* (n=11). In March, two further species, *Agapetus fuscipes* and the beetle *Elmis aenea* were also recorded at the site, both of which have also been found to migrate into the hyporheic habitat during periods of adverse conditions (Williams and Hynes, 1974; Section 5.2.3.3). Conversely, the dytiscid beetle *Agabus didymus* and net-spinning caseless caddisfly *Polycentropus flavomaculatus* were absent during the drying event and did not return during the study period. The absence of the large-bodied, net-spinning predator, *P. flavomaculatus* at this site reflects a decline throughout the study area during the low flow period, likely a result of a reduced ability to snare prey in low flow conditions and in keeping with previous studies which have indicated that this species is adversely impacted by drying events (Section 4.5.1; Lancaster and Ledger, 2015). However, the absence of *A. didymus* is surprising as many *Agabus* species obtain oxygen from the air and have a cuticle which inhibits water loss; in addition, previous studies have suggested their potential resistance to drying events through the persistence of desiccation-resistant eggs (Holdgate, 1956; Stubbington et al., 2016). These results suggest a species-specific response to desiccation.

The invertebrate response to desiccation at Site 1 is of particular interest as recolonisation sources are limited. Longitudinal flow in this channel had not resumed by January, therefore it is unlikely that these individuals originated from downstream sources. Given the similarity between the assemblages recorded in the benthic and hyporheic habitats, (both of these taxa had been recorded from both habitats during this study), it is likely that these individuals migrated into the substratum during the drying event and emerged when surface flow resumed. All of the taxa recorded in the January samples have also been found to emerge from experimental trials involving the rehydration of dry sediments, suggesting physiological traits which enable their persistence to short-term drying events (Stubbington et al., 2016). When viewed together, this

suggests that these species were able to persist at this site during desiccation through a combination of refuge seeking behaviour and physiological traits.

Table 5.3 Taxa recorded from benthic (B) and hyporheic (H) habitats at site 1 between September 2011 and September 2012. No samples were collected in November 2011.

Taxa	Sampling Occasion													
	Sep-11		Nov-11		Jan-12		Mar-12		May-12		Jul-12		Sep-12	
	B	H	B	H	B	H	B	H	B	H	B	H	B	H
Agabus didymus	1													
Agapetus fuscipes	1						1		3	1	5	2	6	2
Anacaena limbata ovata											3		1	
Asellus aquaticus	11	2			1	1	2	1	1	1	5	2	8	1
Ceratopogonidae									1					
Chironomidae		1			1	2	2		1	3	1	2	2	1
Gammarus pulex	1	14			12	11	5	12	21	6	9	2	6	2
Elmis aenea								1				2		
Helodes							1		2	1	2	2	4	
Helophorus brevipalpis											29		3	
Hydrobius fuscipes											3		1	
Limnephilus lunatus											1			
Limnius volckmari									1		3		1	
Nemoura cinerea	2										1	1	3	
Oligochaeta	1				2		1		3	1	2	2	1	1
Polycelis											1		2	
Polycentropus flavomaculatus	1													
Tipula											1		1	

5.3.3 Summary and Discussion of the Response to the Low Flow Period

The results have been assessed to ascertain if the low flow event altered environmental conditions and reduced the abundance or diversity of benthic, hyporheic or phreatic communities.

5.3.3.1 Did Environmental Conditions Change as a Result of the Event?

The period of below average rainfall created drought conditions in which river discharge and groundwater levels were below the respective long-term averages. Longitudinal connectivity was reduced across the study area as Site 1 dried entirely for a short period. This event was related to changes in abiotic conditions, specifically with increased water temperature in all three habitats. The thermal stability provided by groundwater may mitigate periods of elevated temperatures for aquatic invertebrates, particularly during droughts. Previous studies have found elevated temperatures to be a key driver of invertebrate response during periods of low flow (Section 1.4; Stubbington and Wood, 2013). These results indicate that the period of low flow altered environmental

conditions, resulting in a disturbance; however, the impact of this disturbance was likely mitigated by the timing of the drought, which occurred during the winter months and avoided peak summer temperatures.

5.3.3.2 Did the Invertebrate Community Respond to the Disturbance Event?

The invertebrate communities recorded in the benthic, hyporheic and phreatic habitats responded to the low flow period. The benthic community declined in abundance and diversity, with large reductions in the number of dominant species and the loss of a number of regularly recorded rheophilic species from these sites. However, the results also indicate a rapid recovery of some benthic taxa, specifically *G. pulex*, following the breaking of the drought, suggesting minimal long-term impact on these species. Some species, such as *P. flavomaculatus*, were absent during the low flow period and did not return before the end of the study. These results suggest that the response to and recovery from low flows was species specific, with taxa displaying physiological and behavioural adaptations persisting through the event.

Conversely, macroinvertebrate abundance in the hyporheic habitat increased over this period, principally reflecting an influx of *G. pulex* individuals. These results are similar to those of Boulton and Stanley (1995) who found an increase in hyporheic abundance during periods of low flow as well as a number of previous studies that have focused on the migration of *G. pulex* into deeper habitats during periods of low flow (Smith et al., 2003; Wood and Armitage, 2004; Stubbington et al., 2010). However, unlike previous studies, these results also suggest similar downward migration of *A. fuscipes* in response to low flows, a behaviour which is likely to have facilitated its recovery at Site 1 following the short-term drying event, but may be unique to this catchment (Section 4.3.3.2). While richness was broadly static, some notable species, such as *Niphargus aquilex*, were only recorded at the beginning of the drought period, potentially in response to habitat contraction. These results are similar to those discussed by Wood et al., (2010) who found the abundance of both surface and groundwater taxa to increase in the hyporheic habitat during periods of drought and suggest that taxa may migrate downward as well as upward during periods of adverse conditions.

Phreatic macroinvertebrate abundance and richness were also largely static over this period with the exception of a large increase in the number of *Crangonyx subterraneus* in March 2012, coinciding with the lowest surface water flows recorded during the drought. Similarly, the abundance and spatial distribution of *N. fontanus* increased in July and September 2012, coinciding with the recovery of groundwater levels. Both of these species were regularly recorded in the riverine and phreatic samples during this study and these marked changes suggest that they may be utilising hyporheic corridors actively in search of refugia during periods of adverse environmental conditions or passively in response to habitat contraction and a reduction in connectivity.

Considered together these results suggest that low flow conditions altered the structure of the invertebrate communities across this catchment but that this response varied by habitat and species. The greatest impact was recorded in the benthic habitat where the drought period was associated with a reduction in abundance and diversity of the invertebrate community. These metrics remained largely static across the hyporheic and phreatic communities with the exception of large influxes of specific taxa which coincided with specific hydrological milestones, including the onset of the drought, its peak and subsequent recharge events, and therefore times when the aquatic habitat contracted or expanded. These results mirror the environmental conditions recorded during this period in which greater stability was related to greater depth. Although the results suggest recovery for some taxa immediately following the resumption of flow, previous studies suggest that full recovery in this catchment may take up to two years (Wood and Petts, 1999; Stubbington et al., 2009b). The impact on these communities is likely to have been minimised by the occurrence of the drought during the winter rather than summer months.

5.3.3.3 Summary

The low flow period reduced hydrological connectivity, surface water flow and groundwater levels, altering some abiotic conditions (specifically water temperature). The invertebrate communities of the benthic, hyporheic and phreatic habitats responded to this event. The benthic community reduced in abundance and diversity in response to the low flows as expected, but abundance in the hyporheic habitat increased episodically reflecting the vertical

movement of taxa into this habitat from both the surface and (likely) deeper waters, providing support for the Hyporheic Refuge Hypothesis. Although the abundance and diversity of the phreatic community remained stable over the low flow period, there were episodic changes in the distribution and abundance of individual species which coincided with the drought peak and recovery of groundwater levels, and suggest the potential for lateral movement of some species into this habitat from the riverine environment. These results indicate that invertebrate composition of the benthic, hyporheic and phreatic habitats altered in response to low flows but that this response was habitat and species-specific. The movement of some taxa vertically or laterally in response to the event suggests that all three habitats should be considered in the assessment of the impact of drought.

5.4 Summary

This chapter has described the environmental conditions and biological communities of the benthic, hyporheic and phreatic habitats in relation to the high flow and low flow disturbances that occurred during the study period, fulfilling the second aim of this study. As expected, the biological communities occupying these habitats responded to changes in hydrological connectivity which were amplified by the high flows and reduced by low flows. Despite dramatically different conditions during these two disturbances, the response of the invertebrate communities was similar and characterised by refuge seeking behaviour, specifically into the hyporheic habitat. Contrary to expectations, the recovery of the invertebrate communities to both disturbances was species-specific rather than habitat-specific, and the impact of these disturbances was mitigated by the resilience and resistance of specific taxa which facilitated their recovery. The invertebrate response to these disturbances also varied by depth which, when viewed collectively, suggests that these three habitats functioned as a continuum both vertically and laterally, particularly during periods of disturbance.

Disturbance is an importance mechanism in ecosystem dynamics and succession and it is likely to increase in a future of changing climatic conditions (Arnell and Gosling, 2013). It is important for environmental managers to understand how species respond to disturbance and to facilitate natural

mitigation and recovery where possible. While existing theoretical frameworks such as the Hyporheic Refuge Hypothesis and the Flood Refuge Hypothesis are useful in explaining the response of some species to disturbance events, further development could be undertaken to better incorporate the phreatic habitat and its community. As noted in Chapter 4, the concurrent sampling of three habitats is a novel approach in this field, and the results indicate that this provided for a greater understanding of the full diversity of invertebrates within the catchment and of the way in which their distribution fluctuates in response to disturbance events. This is of particular importance to stygofauna which are more vulnerable to disturbance than their epigean counterparts due to their restricted distribution, poor competitive ability, low migration potential and slow reproductive rates (Hoekstra et al., 1994; Hose, 2005; Robertson et al., 2009).

Chapter 6

Morphological and Molecular Taxonomy: Identifying Cryptic Species

6.1 Introduction

Fourteen individuals of a previously undescribed amphipod were collected during this study (hereafter referred to as *Gammarus* sp.). Although these individuals morphologically resembled *Gammarus pulex* (Linnaeus, 1758) they lacked both pigmentation and eyes, characteristics that have not previously been described among British freshwater amphipod populations (Gledhill et al., 1993). As identification could not be confirmed using morphology, molecular analyses were used to determine the identity of these organisms and their relation to other Gammaridae. The importance of accurate identification of organisms cannot be understated as understanding the distribution and sensitivities of different species forms the foundation of biological conservation and environmental management. This Chapter outlines the current understanding of Gammaridae in Britain (Section 6.2), the identification of *Gammarus* sp. using morphological and molecular techniques (Section 6.3); and discussion (Section 6.4) and summary (Section 6.5) of the findings.

A number of stygofauna have recently been described as new to science. While many of these discoveries result from increased sampling effort, others have arisen from improved molecular techniques (Hou et al., 2013; Ozbek et al., 2013). The use of both morphological and molecular analyses is important in the study of stygofauna as these taxa display particularly high rates of morophological convergence (Gibert et al., 1994; Witt et al., 2006).

As the groundwater environment is characterised by its limitations in light, space and oxygen, stygofauna often display adaptations such as ocular regression, hypertrophy of sensory organs and loss of pigmentation which reflect their habitat (Robertson et al., 2009). These adaptations often result in convergent evolution and high numbers of cryptic species within stygofaunal communities, creating uncertainty in identification using morphological characters alone (Lefere et al., 2006). Recent molecular studies have increased certainty in the identification of stygofauna for both genera and species. A morphological and phylogenetic review of *Niphargus* (Crustacea: Amphipoda), the largest genus of freshwater amphipods (primarily stygofaunal), found high levels of convergence for entire suites of physical characters, implying a high degree of morphological homoplasy and doubt in the validity of all

morphologically-based sub-groupings (Fiser et al., 2008). These studies indicate the importance of using both morphological and molecular techniques to identify the *Gammarus* sp. and understand its relation to other Gammaridae. The morphology of the *Gammarus* sp. suggests three possible identities for this taxon: (1) an eyeless Gammaridae not known to Britain; (2) a morphological variant of *G. pulex*; or (3) a species new to science.

6.2 The Gammaridae

The Garmmaridae comprises over 200 species distributed across North America, Europe and Asia (Hou et al., 2007; Karaman and Pinkster, 1977). Phylogenetic studies suggest that this family originated during the Palaeocene in the Tethyan region, diversifying from saline to freshwater habitats in the Middle Eocene (Hou et al., 2011). Seventeen Gammaridae species have been recorded in Britain, three of which, *G. dubeni*, *G. lacustris* and *G. pulex,* inhabit freshwater, and none of which display reduced or absent eyes (Hadfield, 2002). Although this family includes several examples of blind species recorded outside of Britain, none of the available descriptions account for the *Gammarus* sp. suggesting that the identification of this taxon using morphological characters alone is insufficient (Table 6.1).

The Gammaridae includes a number of established polymorphic species which display localised phenotypic plasticity in response to environmental conditions (Hadfield, 2002; Karaman and Pinkster, 1977; Ozbek et al., 2013). For example, *G. minus* (Say, 1818), a eutroglophilic species found in caves, springs and streams in North America displays significant morphological divergence, but insignificant genetic variation, between populations recorded in subterranean and benthic habitats (Carlini et al., 2009; Fong 1989). Studies within wild *G. minus* populations suggest that intraspecific morphological variation relates to ecological factors such as predator pressure and habitat availability. Benthic populations of *G. minus* have been found to have larger eyes in areas inhabited by fish than those where fish are absent, while subterranean populations have significantly reduced eye sizes (Glazier and Deptola, 2011). Laboratory studies on *Gammarus pulex* have found similar displays of phenotypic plasticity in which eye size was significantly changed in accordance with regulated light levels over a single generation (Hadfield, 2002). These studies suggest that it is

possible that the *Gammarus* sp. is a morphological variant of *Gammarus pulex* which reflects an adaptation to hyporheic and hypogean habitats.

Table 6.1 Morphological and molecular records of blind freshwater Gammaridae.

Species	Habitat	Location	Identification	Reference
G. xianfengensis	Cave	China	morphology	Hou and Li, 2002
G. lichuanensis	Cave	China	morphology and molecular	Hou and Li, 2002
G. kesanensis	-	Turkey	morphology	Ozbek et al., 2010
G. vignai	-	Europe	morphology	Pinkster and Karaman, 1978
G. pulex polonensis	-	Poland	morphology	Karaman and Pinkster, 1977
G. aoculus	-	China	morphology	Hou and Li, 2003
G. translucidus	Cave	China	morphology and molecular	Hou et al., 2004
G. comosus	Cave	China	morphology and molecular	Hou et al., 2005
G. abstrusus	Cave	China	morphology and molecular	Hou et al., 2006
G. albimanus	Cave	Macedonia	morphology and molecular	Hou et al., 2011
G. sketi	Spring	Macedonia	morphology and molecular	Wysocka et al., 2013
G. ustaoglui	-	Turkey	morphology	Özbek and Guloglu, 2005
G. minus	Cave	US	morphology and molecular	Carlini et al., 2009
G. sp.	River	Ireland	morphology	University of Ulster, 2013
G. fossarum	Cave	Europe	morphology	Escorze and Milla, 1992
G. baysali	Cave	Turkey	morphology	Ozbek et al., 2013

6.3 Identification Methods and Results

Following sample collection, the specimens were described morphologically and analysed using molecular techniques. All procedures were undertaken according to the standard methodologies of the UCL Geography Department and the UCL Research Department of Genetics, Evolution and Environment.

6.3.1 Sample collection

Fourteen specimens of the *Gammarus* sp. were recorded from the benthic, hyporheic and phreatic habitats over the course of this study using the methods described in Section 2.3 (Table 6.2).

Table 6.2 Records of *Gammarus* sp. over the study period

Site	Method	Habitat	Date	Abundance
Site 9	Artificial Substrates	Hyporheic	Jan-09	1
Site 1	Kick Sample	Benthic	May-09	2
Site 10	Artificial Substrates	Hyporheic	Sep-09	1
Site 10	Bou-Rouch	Hyporheic	Sep-09	5
Site 10	Bou-Rouch	Hyporheic	Jan-10	2
Site 10	Vacuum Pump (20 cm)	Hyporheic	Sep-11	2
Site A	Phreatic Net	Phreatic	Sep-11	1

Additional *G. pulex* specimens were collected to serve as controls for the molecular assessment. These were collected from benthic habitats using a kick sweep technique in each of the sub-catchments of the study area at sites 1 (Nailbourne); 10 (Little Stour): and 13 (Dour); in addition, one further *G. pulex* specimen was collected from a catchment outside of the study area (the River Adur in Hassocks, West Sussex, NGR TQ 30675 16043; Section 2.3). All control specimens were preserved in ethanol and frozen until processed.

6.3.2 Morphological Description

Specimens of *Gammarus* sp. were described morphologically using standard keys (Eggers and Martens, 2001; Gledhill et al., 1993; Karaman and Pinkster, 1977). Validation was undertaken by specialists at the Freshwater Biological Association (Terry Gledhill), the Hypogean Crustacea Recording Scheme (Lee Knight) and/or APEM (Mike Dobson).

While consistently pale, *Gammarus* sp. varied in opaqueness as well as size, ranging from 1757 µm to 12806 µm as measured from head to urosome using an eyepiece graticule. Detailed photographs of distinguishing morphological features including the shape of the gnathropod hands, urosome and antennae were taken using a Leica DMLB microscope (Figure 6.1).

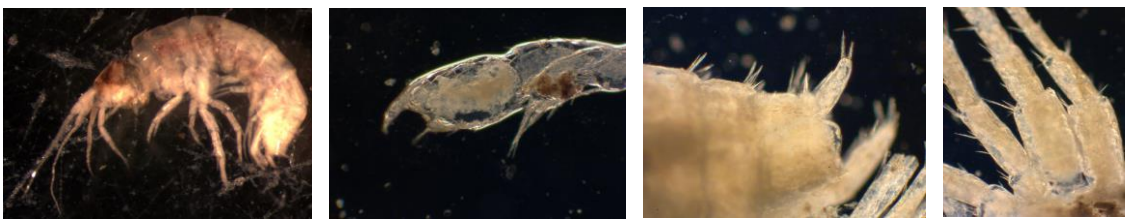


Figure 6.1 Photographs of *Gammarus* sp. collected at site 10 in September 2011, from left: entire (dissecting microscope); gnathropod; urosome and antennae base (4x/0.10 objective).

6.3.3 Molecular Identification

Laboratory protocols were informed by the review of previous molecular and phylogenetic studies on amphipods (Table 6.3). The methodology utilised by Hou and et al. (2007) was selected as the species considered were most closely aligned to *Gammarus* sp. and Cytochrome Oxydase Subunit I (COI) was used because this region has the broadest taxonomic coverage for macroinvertebrates in sequence databases and is most widely used for this group (Blackman et al. 2017).

Table 6.3 Methodologies described in the literature for the sequencing of COI in amphipods

Organism	Sample	Size (bp)	Primers 5'-3'	PCR Reaction	PCR	Reference
G. fossarum, G. pulex, G. wautieri, G. lacustris, G. roeseli and *G. marinus*	Entire	376	COIa-H and COI-Gf	50 µL (10 mM Tris-HCl; 50 mM KCl; 1.5 mM $MgCl_2$; 50 mM each dNTP; 0.5 U Taq; 1 µL each primer)	35 cycles (30s at 93 °C, 30s at 50 °C), ext. for 60s at 72 °C	Meyran et al., 1997
G. pulex, G. gallicus; G. fossarum	Leg	710	LCO1490 and HCO2198	10 µL (1 µL DNA, 200 pM each primer; 1 x NH_4 reaction buffer; 2.5 mM $MgCl_2$; 0.6U Taq; 80 mM dNTPs)	94 °C for 3m then 40 cycles (15s at 94 °C, 20s at 53 °C and 60s at 72 °C) and 180s ext. at 72 °C	Hadfield, 2002
Niphargus virei	Entire	450	COI-virei-F6 and COI-virei-R7	50 µL (0.2 dNTPs; 2mM $MgCL_2$; 0.4 µM primers; 2.5 U Taq; PCR Buffer; 200 ng DNA)	35 cycles (30s at 94°C, 30s at weakest primer Tm, 30s at 72 °C), ext. for 60s at 72 °C	Lefebure et al., 2006
Gammarids	Head	500	LCO1490 and HCO2198	25 µL (0.15 µL Taq; 2.5 µL PCR buffer (2.0 mM $MgCl_2$); 0.8 µL dNTP; 1 µL each primer; 1 µL DNA)	60s at 94 °C, 5 cycles (30s at 94 °C, 90s at 45 °C and 60s at 72 °C), then 35 cycles (30s at 94 °C, 90s at 51 °C, 60s at 72 °C), ext. for 5m at 72 °C	Hou et al., 2007
G. clarus, G. hypolithicus, G. parvioculus, G. nekkensis	Head	656	LCO1490 and HCO2198	As Hou et al., 2007	As Hou et al., 2007	Hou and Li. 2010

6.3.3.1 DNA Extraction

Of the fourteen *Gammarus* sp. specimens, only three were suitable for molecular identification as the others had not been preserved in ethanol (Table 6.4). Genomic DNA was extracted from these specimens using the DNeasy Blood and Tissue Kit (QIAGEN) in accordance with the published protocol (Purification of Total DNA from Animal Tissue (Spin Column); Weiss et al., 2014). For each specimen, approximately 25 mg of tissue was added to 180 µl of ATL buffer and 20 µl of Proteinase K, centrifuged and then incubated for two hours at 56°C, before mixing and incubating at the same temperature for a further hour. Following incubation, 200 µl AL buffer and 200µl ethanol were added and the resulting mixture pipetted into a mini spin column before adding buffers AW1, AW2 and AE to produce a primary extraction and a second elution. The success of the extraction was tested using a Nanodrop Spectrophotometer (ND 8000 v. 2.0) which assessed the quality of the protein in the samples using the manufacturers published protocol (ThermoScientific T009 Technical Bulletin 260/280 and 260/230 Ratios). The results suggest that all specimens, with the exception of three *Gammarus* sp. individuals (J4, J5 and J7), were viable for further analyses.

Table 6.4 DNA extraction results for *Gammarus* sp. and control specimens

Specimen	Type	Site	Habitat	Date	Sample	ng µL^{-1}	260/280	260/230
H1	Control	Adur	Benthic	May-13	Legs	45.31	1.89	1.3
H2	Control	Adur	Benthic	May-13	Body	281.3	2.09	1.94
H3	Control	Adur	Benthic	May-13	All	251.3	2.04	1.37
H4	Control	Adur	Benthic	May-13	Head	165.2	2.03	1.53
J1	Control	10	Benthic	May-13	Legs	62.07	2.06	0.87
J2	Control	13	Benthic	May-13	Legs	37.42	2.16	0.62
J3	Control	1	Benthic	May-13	Legs	124.2	2.08	1.16
J4	*G*. sp.	A	Phreatic	Nov-11	All	4.8	2.35	0.12
J5	*G*. sp.	10	Hyporheic	Sep-11	Legs	21.01	1.59	0.35
J6	*G*. sp.	10	Hyporheic	Jan-10	All	70.85	1.55	0.61
J7	*G*. sp.	10	Hyporheic	Sep-11	Body J5	5.05	2.33	0.12

6.3.3.2 PCR Amplification

Polymerase chain reactions (PCR) were used to amplify the CO1 region of the mitochondrial gene using 25-µL reactions (12.75 µL double distilled water; 2.5 µL Bioline 10xNH_4 Reaction Buffer; 1.25 µL Bioline $MgCl_2$ (50 mM); 0.25 µL DNTP; 0.25 µL Bioline TAQ (DNA Pol 5 ulul); 1.0 µL LCO1490; 1.0 µL HCO2198; 6 µL DNA; Alberts et al., 2002; Folmer et al., 1994; Hou et al., 2007).

PCR was performed after the methods suggested by Hou et al. (2007; 60s at 94ºC; then 5 cycles of 30 seconds at 94 ºC, 90 seconds at 45 ºC and 60 seconds at 72 ºC; then 35 cycles of 30 seconds at 94 ºC, 90 seconds at 51ºC and 60 seconds at 72 ºC; with a final 5 minute extension at 72 ºC) for all samples except the *Gammarus* sp. for which the annealing temperature was increased to 50 ºC to help minimize double banding.

The success of the PCR was assessed with gel electrophoresis, a method which uses an electric field to force the DNA through a matrix in which proteins of different sizes are separated into distinct bands (Alberts et al., 2002). The movement of the PCR products (4 µL of PCR product and 1 µL Bioline 5x DNA Loading Buffer) through the gel (0.5 g Agarose: 50 µL Tris/Acetate/EDTA Buffer plus 2 µL Ethedium Bromide) was assessed under an ultraviolet light to allow for the bands of DNA to be examined against standard markers (Bioline Hyper Ladder I). The results suggest that the four control specimens collected from the study area (J1-3) and one collected from outside the study area (H2) as well as one *Gammarus* sp. (J6) were suitable for sequencing (despite the apparent double banding) while the remaining *Gammarus* sp. (J4, J5 and J7) were not (Figure 5.2). The reason the remaining *Gammarus* sp. were not viable for sequencing likely relates to the poor yield of DNA during extraction which may be attributed to poor quality preservation during morphological assessments.

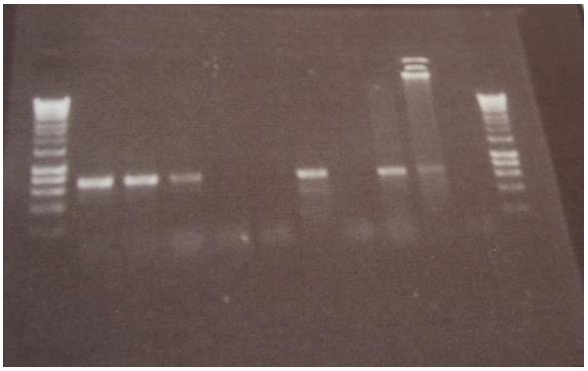


Figure 6.2 Gel electrophoresis results from all samples following PCR. From left: Control Ladder; J1; J2; J3; J4; J5; J6; J7; H2; Positive Control; Negative Control; Control Ladder.

6.3.3.3 Sequencing

Prior to sequencing, the PCR templates were purified using MicroClean (5ml 5M NaCL; 0.1ml 1M tris-HCL pH8.0; 0.02 ml 0.5M EDTA; 20g PEG 8000; 0.086 ml 2M MgCl2; and distilled water), centrifuged and resuspended. Sequencing was undertaken using BigDye (v3.1) in accordance with standard UCL

protocols. Sequences, which were obtained for five specimens, were edited, aligned and blasted using Mesquite 2.71 and Sequencher 3.1.

6.3.3.4 Results of Molecular Assessment

The sequencing results identify four of the specimens, including the *Gammarus* sp. (J6) as *G. pulex* and one specimen (J3; the control specimen from site 1) as *Gammarus fossarum* (Koch, 1836; Table 6.5).

Table 6.5 Results of the sequencing analysis for each specimen

Specimen	Primer	Base Pairs	Size (Base Pairs)	Quality (%)	Species
H2	LCO	656	671	94.5	*Gammarus pulex*
	HCO	652		94.9	
J1	LCO	650	676	96.0	*Gammarus pulex*
	HCO	664		95.8	
J2	LCO	660	674	98.0	*Gammarus pulex*
	HCO	661		95.3	
J3	LCO	653	675	98.2	*Gammarus fossarum*
	HCO	661		96.1	
J6	LCO	600	683	77.8	*Gammarus pulex*
	HCO	648		80.7	

Despite the overlap in the sequence result, there is little confidence in the identification for specimen J6 due to the small amount of extracted DNA and double banding following the PCR; as such, it is not clear if this specimen represents an eyeless form of the *G. pulex* species complex or if the result reflects cross-contamination (although no contamination in any control sample was detected at any point during these analyses). However, as no other specimen of this description yielded an adequate quantity of extracted DNA, it is not possible for the identity to be confirmed within the remit of this study.

Conversely, the identification of specimen J3 as *G. fossarum* is certain as there is high confidence in the molecular results and low risk of contamination (as there are no records of *G. fossarum* in the United Kingdom and molecular analyses have not been undertaken on any other amphipod specimen in this laboratory). Following the completion of this analysis, the remainder of this specimen was reassessed independently using molecular techniques and confirmed as *Gammarus fossarum* (Blackman et al., 2017; Appendix II).

6.4 Discussion

Morphological and molecular analyses were undertaken to determine the identification of *Gammarus* sp. recorded during this study and its relation to other Gammaridae.

6.4.1 Species Identification

The results indicate that the identity of the *Gammarus* sp. cannot be confirmed within the remit of this study. The morphology of this taxon is similar to *G. pulex* but it lacks both pigmentation and eyes, suggesting that a genetic distinction between it and morphologically typical *G. pulex* would be expected. However, this is not supported by the molecular results which suggest that this specimen represents an eyeless, phenotypic variation of *G. pulex*.

6.4.2 Relation to other Gammaridae

The results of this study suggest that this is a novel record of *G. fossarum* in Britain, raising a number of important questions regarding its current distribution, which is thought to be limited to central Europe and Asia Minor (Feckler et al., 2012; Karaman and Pinkster 1977; Figure 6.3).

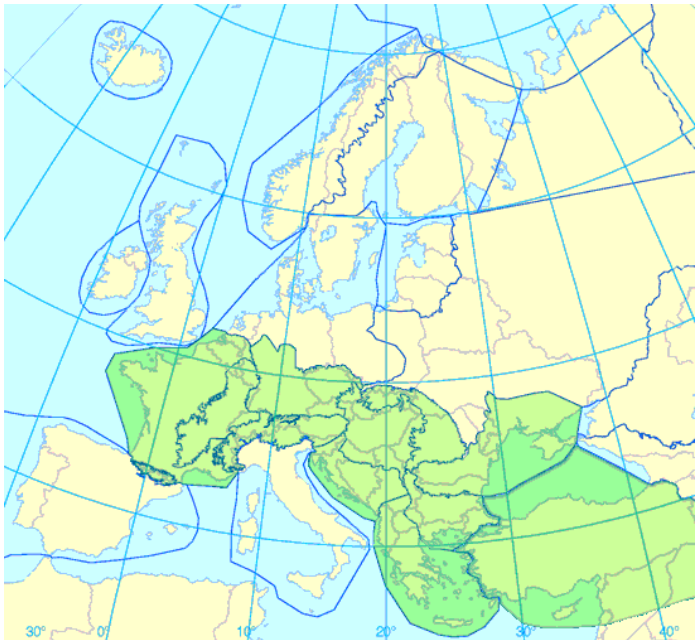


Figure 6.3 *Gammarus fossarum* distribution by Eco-Region (ER2, 4-12 including the Pyrenees, Alps, Dinaric Western Balkans, Hellenic Western Balkans, Eastern Balkans, Western Highlands, Central Highlands, Carpathians, Hungarian Lowland, Pontic Province and Western Plains) after Schmidt-Kloiber and Hering, 2012.

While the results confirm both the morphological and molecular identification of *G. fossarum*, they also reflect the similarities between this species and *G. pulex*. Due to the minor morphological differences between these two species, (in the antennae setal arrangement, mouthpart shape and relative length of the rami) *G. fossarum* had previously been considered a sub-species of *G. pulex*; although these species are now known to be distinct, *G. fossarum* is the closest genetic relative of *G. pulex* (Hadfield, 2002; Karaman and Pinkster, 1977; Mayer et al., 2012). While these two species are sympatric, *G. fossarum* is

outcompeted by *G. pulex* and tends to prefer headwater habitats with higher current velocities and cooler temperatures, away from the lower river reaches where *G. pulex* is more prevalent (Karaman and Pinkster, 1977; Pockl et al., 2002; Siegismund and Muller, 1991). The habitat preferences and morphology of *G. fossarum* also enable it to exploit spring and hyporheic environments more readily than *G. pulex* as the shape of its mouth parts enable the scraping of biofilms (unlike *G. pulex* which are more suited to shredding and the predation of free-swimming organisms), an important food source in groundwater environments (Mayer et al., 2012). Within this study, *G. fossarum* was only identified by chance at a single site (1), located in the headwaters of an intermittent stream. It is possible that *G. fossarum* populations in Britain have not previously been recorded as competition with *G. pulex* may have driven them into deeper hyporheic habitats which are not normally included in freshwater sampling programmes. Alternatively, it is possible that *G. fossarum* has recently been introduced to Britain (Blackman et al., 2017). These results also suggest the possibility that some of the *Gammarus* sp. specimens which were not viable for sequencing could be eyeless variants of *G. fossarum*, which is known to be polymorphic (Feckler et al., 2012; Meyran et al., 1997). Previous studies have suggested the existence of a European subspecies (*G. fossarum subterraneus*) which displays reduced eyes and pigmentations (no further molecular descriptions are available; Escorze and Milla, 1992).

6.5 Summary

This study attempted to apply molecular techniques to identify a cryptic stygofauna. Although the identification of this specimen was inconclusive, the results suggest that further work, beyond the remit of this study, should be undertaken to determine its identity. However, the results of this study do represent the first record of *G. fossarum* in Britain and further morphological and molecular work, again beyond the remit of this study, should be undertaken to ascertain the current distribution of this species (Appendix II) and its relation to other populations, specifically those on mainland Europe, which Weiss et al. (2014) have suggested comprise several clades and require taxonomic revision. Both of these results support the first aim of this study and reflect need for further work to understanding the British stygofauna.

Chapter 7

Summary, Conclusions and Implications for Future Management

7.1 Introduction

This chapter outlines the findings of this study (Section 7.2) and, based upon analysis of its strengths and limitations, makes suggestions for further work to expand upon this research topic (Section 7.3). The conclusions (Section 7.4) provide a summary of the key outcomes and achievements of this thesis.

7.2 Summary of Key Findings

This thesis aims to describe the ecological community occupying groundwater dependent habitats (benthic, hyporheic and phreatic) and to assess the response of these communities to environmental change by testing the hypothesis that there are observable spatial and temporal patterns in the distribution of organisms inhabiting groundwater-dependent habitats and that these patterns are influenced by biological, chemical and physical elements.

7.2.1 Aim 1: Describe the benthic, hyporheic and phreatic habitats and the distribution of the biological communities they support

The physiochemical, chemical and physical results recorded by this study are consistent with long-term monitoring in this catchment and indicate that the study area is characteristic of a chalk stream environment (Chapter 3). The benthic, hyporheic and phreatic habitats varied in their physiochemical parameters with reference to spatial distribution within the catchment and temporally in relation to seasonality and groundwater contribution, but nutrient concentrations and geochemistry were similar by depth. The overlap in environmental conditions between habitats suggests that, under normal hydrological conditions, they should be viewed as a continuum between the surface water and groundwater environments.

7.2.1.1 Can these communities be described?

Given the relative paucity of research into groundwater communities it was expected that the number of British stygofauna was under recorded; however, the record of *Gammarus fossarum* is surprising and remarkable as this species was found in the benthic habitat of a water course which has been studied and monitored previously.

7.2.1.2 Does the spatiotemporal distribution of biological communities reflect the conditions of the three habitats?

As expected, each habitat provided distinctive environmental conditions and the biological communities reflected these conditions. The benthic assemblage comprised epigean taxa, many of which were typical of a chalk stream environment, and were distributed along a longitudinal gradient from the headwaters to downstream reaches. The phreatic assemblage comprised exclusively stygofauna typical of a carbonate aquifer, the distribution of which was species-specific and associated with differences in water level and temperature. The hyporheic assemblage comprised a mixture of stygoxenes, stygophiles and stygobionts which were distributed primarily along a gradient of groundwater affiliation and then along a longitudinal gradient from the headwaters to downstream reaches. While the majority of these taxa were recorded either in the benthic or phreatic assemblages, some, such as *Niphargus aquilex,* were exclusive to the hyporheic habitat. Although there was overlap in the composition of assemblages between the benthic and hyporheic habitats as well as between the phreatic and hyporheic habitats, only one taxon, *Gammarus* sp. was recorded in all three. Interestingly, the temporal resolution of this study suggests that some species, such as *Agapetus fuscipes,* utilise the hyporheic habitat seasonally (likely to facilitate grazing), suggesting an active, predictable exploitation of multiple habitats.

7.2.1.3 Do existing conceptual frameworks support the spatiotemporal distribution of biological communities across the three habitats?

While existing theoretical frameworks such as the Hyporheic Corridor Concept are useful in predicting and explaining the longitudinal distribution of species along the river corridor, the results from this study suggest that further development is needed to better incorporate the phreatic habitat and its community in our conceptualisation of lotic function. Specifically, while some species were only recorded in a single habitat, the most abundant and dispersed species were regularly recorded in multiple habitats, both routinely and in response to disturbance events, suggesting that these species have behavioural or physiological traits which facilitate their use of these habitats and

dominance of these communities. The collective assessment of the longitudinal, lateral and vertical movement of fauna suggests that these three habitats are being used as a continuum rather than in isolation and that conceptual frameworks should be updated to integrate surface and groundwater.

7.2.2 Aim 2: Describe periods of environmental change that occurred during this study and the response of the biological communities

Two disturbance events were identified over the course of the study including a period of above average rainfall which resulted in a high flow event in spring 2010 and an extended period of below average rainfall which resulted in a low flow period from 2011-12 (Section 3.4). Contextualising these results within the long-term records for this study area suggests that the period of high flow was particularly notable in its intensity, while the drought was similar, if less intense, than previous periods of low-flow, likely due to its occurrence during winter, rather than summer months

7.2.2.1 How do the biological communities response to disturbance?

The period of high flow amplified hydrological connectivity, increasing flow velocities, discharge and groundwater levels, notably facilitating the flow of the Nailbourne along its entire length to its confluence with the Little Stour and altering abiotic conditions (specifically water temperature; Section 5.2). The invertebrate communities recorded in the benthic and hyporheic habitats responded to the high flows but there was no significant change in the calculated metrics, suggesting minimal impact. In the benthic habitat, both abundance and diversity declined during the disturbance, specifically coinciding with peak discharge, but recovered within a month. In the hyporheic habitat, diversity decreased but abundance briefly increased, reflecting the movement of normally benthic species, specifically *G. pulex* and *A. fuscipes*, into this habitat.

The period of low flow reduced hydrological connectivity, reducing river discharge and groundwater levels below their respective long-term averages, causing some study sites to dry completely and altering abiotic conditions (specifically water temperature; Section 5.3). The benthic community reduced in abundance and diversity in response to the low flows, with some species recovering quickly following the resumption of normal discharges. Abundance in

the hyporheic habitat increased episodically while diversity remained constant, reflecting the vertical movement of normally benthic taxa (specifically *G. pulex* and *A. fuscipes*) into this habitat from both the surface and (likely) deeper waters specifically during periods of high and low flow. The phreatic community remained stable during this period with the exception of episodic changes in the distribution and abundance of individual species (specifically *Crangonyx subterraneus* and *Niphargus fontanus*) at times which coincided with large changes in surface discharge and suggests a lateral movement of these species from the riverine environment to the aquifer.

7.2.2.2 How do the communities recover from disturbance?

Contrary to expectations, the response and recovery of the invertebrate communities to both disturbances was species rather than habitat-specific, with the recovery of some species associated with behavioural and physiological persistence strategies. Specifically, species, such as *Gammarus pulex*, which were found to exploit multiple habitats, appeared to recover more quickly and across a broader spatial scale than others. While the literature includes a number of examples of species migration into different habitats in response to periods of high or low flow, this study is novel in its consideration of all three habitats during disturbances at both ends of the hydrological spectrum (please see references in Chapter 5). The movement of some taxa vertically and/or laterally in response to changes in flow suggests that all three habitats should be considered in assessing the impact of these disturbances and recovery of the catchment. While existing theoretical frameworks such as the Hyporheic Refuge Hypothesis and the Flood Refuge Hypothesis are useful in explaining the response of some species to disturbance events, further development could be undertaken to better incorporate the phreatic habitat and its community.

7.2.3 Summary

The findings indicate that the study has achieved its aims. The results suggest support of the hypothesis as they identify spatial and temporal patterns in the distribution of organisms inhabiting groundwater-dependent habitats and that these patterns are influenced by biological, chemical and physical elements. The novel approach taken by this study to concurrently assess the benthic,

hyporheic and phreatic habitats suggests that while all three are distinct, they also function as a continuum between surface and groundwater.

7.3 Recommendations for Future Work

This study detected spatial and temporal patterns in the distribution of invertebrates inhabiting the benthic, hyporheic and phreatic habitats and identified environmental variables that influenced this distribution. However, both the strengths and limitations of this approach should be considered in the development of this research area and in the application of these findings to the management of the groundwater environment. The field of groundwater ecology is still in a nascent stage and, while this study helps to address some of the gaps, there are a number of opportunities to expand this research in future.

7.3.1 Further development of research in groundwater ecology

The primary strength of this study is its concurrent spatial assessment of the benthic, hyporheic and phreatic habitats which facilitated the comparative analysis of the taxa occupying these habitats and their response to environmental conditions. It is recommended that such a design be applied to future research as it facilitates a more holistic assessment of data throughout the catchment and allows for the consideration of changes in both the surface and groundwater environment. Although it was necessary for the Little Stour to be the focus of the study area due to the premise of funding for this research, the inclusion of sites in the Nailbourne headwaters and on the Dour was intended to provide a spectrum of comparable environmental conditions. While spatial differences were detected between these sites, comparison of these results to another catchment, particularly one without a history of flow stress, would be of great interest.

Temporally, a further strength of this study was its duration, which established a baseline during a normal flow year for the benthic and hyporheic habitats and facilitated the assessment of community response to disturbance events. The collection of phreatic samples was limited to the final year of the study which overlapped with low flow conditions and may have confounded some of the results as the drought started before a baseline had been established. It is recommended that future research establish a concurrent baseline for all three

habitats. While routine bimonthly sample collection was more frequent than what has been suggested in much of the literature (especially for phreatic studies), the augmented monthly sampling which occurred during the high flow period detected important changes in the composition of the invertebrate communities, suggesting limitations in bimonthly sample collection.

This study was designed using a natural trajectory, rather than manipulative, approach due to the difficulties in replicating complex groundwater interactions in a controlled manner. However, this approach also limited the study to its observational findings which were challenging to interpret for both the hyporheic and phreatic communities due in part to some of the current research gaps in this field. Controlled microcosm experiments paired with observational studies could be used to establish a better understanding of the physiological and behavioural response of stygofauna to thresholds of environmental change.

A comprehensive review of sampling methods was undertaken prior to this study, with many experimental techniques trialled during its pilot phase (Section 2.3). However, a number of these methods failed to collect statistically meaningful data (or simply failed). Biologically meaningful data were collected in the benthic habitat using a suber sampler (as this better facilitated assessments of abundance but negated comparison with long-term Environment Agency monitoring data as these are collected using a timed kick-sweep technique); in the hyporheic habitat using a Bou-Rouch pump (although sampling was very difficult at times given the compacted substrate); and the phreatic habitat using phreatic net. It is unfortunate that microbiological sampling in the phreatic habitat was limited to two occasions as it provided interesting results which suggested a potential relationship with the presence of macroinvertebrates, suggesting that further research would benefit from concurrent microbiological sampling to explore this potential relationship.

The multidisciplinary approach of this study facilitated the assessment of the results. This was particularly important in aiding understanding of the connectivity between biological communities and the potential association of surface flow events and changes in groundwater communities. While the biological and physiochemical variables were found to be the most relevant in describing these communities and their response to environmental change,

geochemistry was also helpful in understanding the influence of groundwater throughout the catchment and it would be recommended that it be included in further studies.

The discovery of *Gammarus fossarum* was an unexpected development in this study (which highlighted the importance of fixing invertebrate specimens in a preserving liquid that does not inhibit molecular identification). Future research would be greatly enhanced though the processing of stygofauna using both morphological and molecular techniques. The greatest potential for further research is in the development of molecular techniques as these can be used to clarify the taxonomy of invertebrate fauna, specifically to better understand the eyeless forms of *Gammarus pulex* and the extent and provenance of *Gammarus fossarum* in the United Kingdom.

Finally, temporal limitations excluded the assessment of meiofauna (specifically the Acari) and (with regret as they comprised a large part of the benthic community) the speciation of the Chironomidae. Our understanding of benthic, hyporheic and phreatic communities could be greatly enhanced through a better understanding of all of the taxa they support.

7.3.2 Management of the groundwater environment

Groundwater is recognised as an internationally important resource for public water supply, agriculture and industry, but it also plays an essential role in the functioning of lotic ecosystems (Hancock, 2005; Mermillod-Blondin et al., 2013; Vorosmarty et al., 2010). Environmental legislation aims to protect groundwater from degradation and over-exploitation; however, it often neglects to consider the ecological role of this resource (Korbel and Hose, 2011). The response of groundwater dependent communities to changes in groundwater quality or quantity is not well understood; however, this study suggests clear responses in these communities to environmental change, specifically to disturbances precipitated by abnormal periods of rainfall. Integrated catchment management could be improved by the consideration of the hyporheic and phreatic communities in addition to conventional benthic monitoring. Previous authors have proposed applying the model adopted by the WFD to assess the condition and inform the management of groundwater ecosystems (Griebler et al., 2010). This approach would establish groundwater ecosystem typologies; select

biological elements; derive a reference status; and develop assessment models based upon understood threshold values and response mechanisms (Griebler et al., 2010). If such a model were adopted, the results of this study could be used to aid in the development of a typology for a chalk aquifer and inform the approach to monitoring and assessment.

7.4 Conclusions

This study addressed a number of important gaps in the understanding of fauna occupying groundwater dependent habitats. The concurrent assessment of the benthic, hyporheic and phreatic communities represents a novel approach which demonstrates the importance of connectivity between these habitats and suggests that they should be viewed as a continuum. The disturbance events that occurred during the study period altered this connectivity, amplifying it during the period of high flow and reducing it during the period of low flow, but in both instances, the response of the invertebrate communities was a similar seeking of refuge (vertically and laterally) between habitats. These results suggest that the assessment of a single habitat limits our understanding of the catchment, the interactions between its communities and their response to changing environmental conditions, potentially confounding the conventional approach to environmental management. This study is also one of the first long-term assessments of the phreatic habitat and the findings (particularly the fluctuations in the number of positive samples, abundance and diversity of this community) reflect the importance of monitoring which exceeds a single occasion or season. Finally, the discovery of *Gammarus fossarum*, the first such record in the British Isles demonstrates the importance of combining both conventional morphological and innovative molecular techniques to further ecological understanding.

References

Adam O, Badot PM, Degiorgi F and Crini G. 2009. Mixture toxicity assessment of wood preservative pesticides in the freshwater amphipod *Gammarus pulex* (L.). *Ecotoxicology and Environmental Safety* 72(2): 441-449.

Adams B, Baxter K, Buckley D, Cunningham J, Hopson P, Dochartaigh BO, Packman, Robinson V, Sage, R, Smedley, P, Shaw P, Waring P, Warren G and Watson S. 2008. *The Chalk aquifer of the North Downs.* Research Report RR/08/02. British Geological Survey: Nottingham.

Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P. 2002. *Molecular Biology of the Cell.* Garland Science: New York.

Allen DJ, Brewerton LJ, Coleby LM, Gibbs BR, Lewis MA, MacDonald AM, Wagstaff SJ and Williams AT. 1997. *The Physical Properties of Major Aquifers in England and Wales.* British Geological Survey Technical Report WD/97/34, Environment Agency R&D publication: 8.

Allen DJ, Darling WG, Gooddy DC, Lapworth DJ, Newell AJ, Williams AT, Allen D and Abesser C. 2010. Interaction between groundwater, the hyporheic zone and a Chalk stream: a case study from the River Lambourn, UK. *Hydrology Journal* 18(5): 1125-1141.

Allford A, Cooper SJB, Humphreys WF and Austin AD. 2008. Diversity and distribution of groundwater fauna in a calcrete aquifer: does sampling method influence the story? *Invertebrate Systematics* 22:127-138.

Arietti M and Edwards B. 2006. *Groundwater invertebrates in chalk boreholes and their significance in public water supply*. Three Valleys Water: Hatfield

Barham. 2011. *Nailbourne Aquatic Society.* Accessed: 17 June 2011. Available: http://www.barham-kent.org.uk/Aquatic%20Society.htm.

Bartley P and Johnston P. 2006. *Eutrophication from Agricultural Sources*. Report 2000-LS-2-M2. Environmental Protection Agency: Dublin.

Bartsch I, Davids C, Diechsel R, Di Sabatino A, Gabrys G, Gerecke R, Gledhill T, Jager P, Makol J, Smit H, van der Hammen H, Weigmann G, Wohltmann A, Wurst E, Gerecke R. 2007. *Chelicerata: Araneae, Acari I. Susswasserfauna von Mitteleuropa 7/2-1:1-388.* Spektrum Akademischer Verlag: Heidelberg.

Becker G. 2005. Life cycle of *Agapetus fuscipes* (Trichoptera, Glossosomatidae) in a first-order upland stream in central Germany. *Limnologica – Ecology and Management of Inland Waters* 35(1-2): 52-60.

Begon M, Harper JL and Townsend CR. 1995. *Ecology: Individuals, Populations and Communities.* Second Edition. Blackwell Science: Oxford.

Beketov MA, Foit K, Schafer RB, Schriever CA, Sacchi A, Capri E, Biggs J, Wells C and Liess M. 2009. SPEAR indicates pesticide effects in streams. Comparative use of species- and family-level biomonitoring data. *Environmental Pollution* 157: 1841-1848.

Berrie AD. 1992. The chalk-stream environment. *Hydrobiologia* 248: 3-9.

Blackman RC, Constable D, Hahn C, Sheard AM, Durkota J, Hänfling B and Handley LL. 2017. Detection of a new non-native freshwater species by NDA metabarcoding of

environmental samples – first record of *Gammarus fossarum* in the UK. *Aquatic Invasions.* 12.

Blaschke AP, Steiner KH, Schmalfuss R, Gutknecht D and Sengschmitt D. 2003. Cloggin processes in hyporheic interstices of an impounded river, the Danube at Vienna, Austria. *International Review of Hydrobiology* 88 (3-4): 397-413.

Bogan MT, Boersma KS and Lytle DA. 2013. Flow intermittency alters longitudinal patterns of invertebrate diversity and assemblage composition in an arid-land stream network. *Freshwater Biology* 58: 1016-1028.

Boulton AJ and Foster JG. 1998. Effects of buried leaf litter and vertical hydrologic exchange on a hyporheic water chemistry and fauna in a gravel-bed river in northern New South Wales, Australia. *Freshwater Biology* 40(2): 229-243.

Boulton AJ, Findlay S, Marmonier P, Stanley EH and Valett HM. 1998. The functional significance of the hyporheic zone in streams and rivers. *Annual Review of Ecology and Systematics* 29: 59-81.

Boulton AJ. 2003. Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages. *Freshwater Biology* 48: 1173-1185.

Boulton AJ. 2007. Hyporheic rehabilitation in rivers: restoring vertical connectivity. *Freshwater Biology* 52: 632-650.

Boulton AJ, Fenwick, GD, Hancock PJ and Harvey MS. 2008. Biodiversity, functional roles and ecosystem services of groundwater invertebrates. *Invertebrate Systematics* 22(2): 103-116.

Boulton AJ, Datry T, Kasahara T, Mutz M and Stanford JA. 2010. Ecology and management of the hyporheic zone: stream-groundwater interactions of running waters and their floodplains. *Journal of the North American Benthological Society* 29(1): 26-40.

Brittain JE and Saltveit SJ. 1989. A review of the effect of river regulation on mayflies (Ephemeroptera). *Regulated Rivers: Research and Management.* 3:191-204.

Brunke M and Fischer H. 1999. Hyporheic bacteria – relationships to environmental gradients in a prealpine stream. *Archiv fur Hydrobiologie* 146(2): 189-217.

Bunte K and Abt SR. 2001. *Sampling Surface and Subsurface Particle-Size Distributions in Wadable Gravel- and Cobble-bed Streams for Analyses in Sediment transport, Hydraulics and Streambed Monitoring.* General Technical Report RMRS-GTR-74.United States Department of Agriculture, Forestry Service, Rocky Mountain Research Station, Fort Collins, Colorado.

Canivet V and Gibert J. 2002. Sensitivity of epigean and hypogean freshwater macroinvertebrates to complex mixtures. Part I: laboratory experiments. *Chemosphere* 46(7): 999-1009.

Carlini DB, Manning J, Sullivan PG and Fong DW. 2009. Molecular genetic variation and population structure in morphologically differentiated cave and surface populations of the freshwater amphipod *Gammarus minus. Molecular Ecology* 18: 1932-1945.

CEN (European Committee for Standardization). 2011. *Standard for Sampling Invertebrates in the Hyporheic Zone of Rivers* (draft). Proceedings from the Loughborough CEN meeting 13-14 June 2011.

Chessman BC. 2015. Relationships between lotic macroinvertebrate traits and responses to extreme drought. *Freshwater Biology* 60:60-63.

Choi KH and Dobbs FC. 1999. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *Journal of Microbiological Methods* 36(3): 203-213.

Cold A and Forbes VE. 2004. Consequences of a short pulse of pesticide exposure for survival and reproduction of *Gammarus pulex. Aquatic Toxicology* 67(3): 287-299.

Coleman MJ and Hynes HBH. 2004. The vertical distribution of the invertebrate fauna in the bed of a stream. *Hydrobiologia* 400: 55-62.

Culver DC and Pipan T. 2014. *Shallow Subterranean Habitats: Ecology, Evolution and Conservation*. Oxford University Press: Oxford.

De Brouwer JHF, Besse-Lotoskaya AA, ter Braak CJF, Kraak MHS and Verdonschot PFM. 2016. Flow velocity tolerance of lowland stream caddisfly larvae (Trichoptera). *Aquatic Sciences:*1-7

De Lipthay J, Johnsen K, Albrechtsen H, Rosenberg P and Aamand J. 2004. Bacterial density and community structure of a sub-surface aquifer exposed to realistic low herbicide concentrations. *FEMS Microbiology Ecology* 49:

de Vos E, Edwards SJ, McDonald I, Wray DS and Carey PJ. 2002. A baseline survey of the distribution and origin of platinum group elements in contemporary fluvial sediments of the Kentish Stour, England. *Applied Geochemistry* 17: 1115-1121.

Dahm CN and Valett HM. 1996. Hyporheic Zones. In: *Methods of Stream Ecology.* F Hauer and GA Lamberti (eds). Academic Press: New York.

Dai AG. 2011. Drought under global warming: a review. *Wiley Interdisciplinary Reviews – Climate Change* 2(1): 45-65.

Danielopol DL, Griebler C, Gunatilaka A and Notenboom J. 2003. Present state and future prospects for groundwater ecosystems. *Environmental Conservation* 30(2): 104-130.

Darling WG, Gooddy DC, Morris BL and Peach DW. 2012. The hydrochemistry of a Chalk aquifer during recovery from drought. *Quarterly Journal of Engineering Geology and Hydrogeology* 45: 473-486.

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

Datry T, Larned ST and Scarsbrook MR. 2007. Responses of hyporheic invertebrate assemblages to large-scale variation in flow permanence and surface-subsurface exchange. *Freshwater Biology* 52: 1452-1462.

Davy-Bowker J, Sweeting W, Wright N, Clarke RT and Arnott S. 2006. The distribution of benthic and hyporheic macroinvertebrtes from the heads and tails of riffles. *Hydrobiologia* 563:109-123.

Del Rosario RB and Resh VH. 2000. Invertebrates in intermittent and perennial streams: is the hyporheic zone a refuge from drying? *Journal of the North American Benthological Society* 19: 680-696.

Department for Environment, Food and Rural Affairs (Defra). 2014. Water Framework Directive implementation in England and Wales: new and updated standards to protect the water environment. Defra Report PB14163.

Di Sabatino A, Gerecke R and Martin P. 2000. The biology and ecology of lotic water mites (Hydrachnidia). *Freshwater Biology* 44: 37-62.

Dole-Olivier M-J and Marmonier P. 1992. Effects of spates on the vertical distribution of the interstitial community. *Hydrobiologia* 230: 49-61.

Dole-Olivier MJ. 1998. Surface water / groundwater exchanges in three dimensions on a backwater of the Rhone River. *Freshwater Biology* 40: 93-109.

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

Domisch S, Jaehnig SC and Haase P. 2011. Climate-change winners and losers: stream macroinvertebrates of a submontane region in Central Europe. *Freshwater Biology* 56: 2009-2020.

Dumas P and Fontanini G. 2001. Sampling fauna in aquifers: a comparison of net-sampling and pumping *Archiv fur Hydrobiologie* 150(4):661-676.

Dumas P, Bou C and Gibert J. 2001. Groundwater macrocrustaceans as natural indicators of the Ariège Alluvial Aquifer. *International Review of Hydrobiology* 86(6): 619-633.

Eggers TO and Martens A. 2001. Bestimmungsschl ussel der Sußwasser-Amphipoda (Crustacea) Deutschlands. *Lauterbornia*, 42: 1–68.

Elliott, JM. 2006. Critical periods in the life cycle and the effects of a severe spate vary markedly between four species of elmid beetles in a small stream. *Freshwater Biology*51(8): 1527-1542.

Elliott, JM. 2008. The ecology of riffle beetles (Coleoptera: Elmidae) *Freshwater Reviews* 1: 189-203.

Elliott, JM. 2013. Contrasting dynamics from egg to adult in the life cycle of summer and overwintering generations of *Baetis rhodani* in a small stream. *Freshwater Biology* 58(5): 866-879.

Entec (for the Environment Agency). 2008. *Environment Agency – Southern Region Groundwater Body Groundwater Quality Reports: East Kent Stour Chalk Groundwater Body Final Report*. Environment Agency: Winchester.

Environment Agency. 2003. *The Stour Catchment Abstraction Management Strategy*. Environment Agency: Worthing.

Environment Agency. 2004. *The State of Englands Chalk Rivers.* Report Number HO-07/04-2K-BIAB-EAD-EP207A. Environment Agency: Bristol.

Environment Agency. 2008. *Restoring Sustainable Abstraction: options identification, appraisal and agreement stage plan for the Little Stour River Alleviation of Low Flow Scheme*. Environment Agency: Addington (Kent).

Environment Agency. 2009a. *The Hyporheic Handbook: a handbook on the groundwater-surface water interface and hyporheic zone for environment managers*. Integrated catchment science programme science report SC050070. Environment Agency: Bristol.

Environment Agency. 2009b. *South East River Basin Management Plan, South East River Basin District Annex B.* Environment Agency: Bristol.

Environmental Protection Agency (EPA). 1997. *Volunteer Stream Monitoring: A Methods Manual.* United States Environmental Protection Agency Report Number EPA 841-B-97-003.

Escorze CM and Milla AS. 1992. *The Natural History of Biospeleology*. CSIC: Madrid.

Euro-Limpacs. 2011. *Climate Change and Freshwater.* EU Contract GOCE-CT-2003-505540. Accessed: 9 May 2011. Available: www.climate-and-freshwater-info

Everall N, Johnson MF, Wilby RL, Bennett CJ. 2015. Detecting phenology change in the mayfly *Ephemera danica:* responses to spatial and temporal water temperature variations. *Ecological Entomology* 40: 95-105.

Extence CA, Balbi DM and Chadd RP. 1999. River flow indexing using British benthic macroinvertebrates: a framework for setting hydroecological objectives. *Regulated Rivers: Research and Management* 15: 543-574.

Eyre MD, Foster GN and Foster AP. 1990. Factors affecting the distribution of water beetle species assemblages in drains of eastern England. *Journal of Applied Entomology* 109: 217-225.

Feckler A, Thielsch A, Schwenk K, Schulz R and Bundschuh M. 2012. Differences in the sensitivity among cryptic lineages of the *Gammarus fossarum* complex. *Science of the Total Environment* 158-164.

Feminella JW. 1996. Comparison of benthic macroinvertebrate assemblages in small streams along a gradient of flow permanence. *Journal of the North American Benthological Society* 15(4): 651-669.

Fetter CW. 2001. *Applied Hydrogeology*. Fourth Edition. Prentice Hall.

Findlay SEG, Sinsabaugh RL, Sobczak WV and Hoostal M. 2003. Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnology and Oceanography* 48(4): 1608-1617.

Fiser C, Sket B and Trontelj P. 2008. A phylogenetic perspective on 160 years of troubled taxonomy of *Niphargus* (Crustacea: Amphipoda). *Zoologica Scripta* 35(6): 665-680.

Folmer O, Black M, Hoeh H, Lutz R and Vrijenhoek. 1994. *Molecular Marine Biology and Biotechnology* 3(5): 294-299.

Fong DW. 1989. Morphological evolution of the amphipod *Gammarus minus* in Caves: quantitative genetic analysis. *American Midland Naturalist* 121(2): 361-378

Fountain MT and Hopkin S. 2005. *Folsomia Candida* (Collembola): A “standard” soil arthropod. *Annual Review of Entomology* 50:201-222.

Freshwater Life. 2011. *Hypogean Crustacea Recording Scheme.* Accessed: 9 May 2011. Available: http://www.freshwaterlife.org/hcrs.html

Gibbins CN, Grant J, Malcolm IA and Soulsby C. 2016. Influence of groundwater chemistry on hyporheic invertebrate assemblages is revealed by fine-scale sampling. *Fundamental and Applied Limnology* 187(3): 207-221.

Giberson DJ and Hall RJ. 1988. Seasonal variation in faunal distribution within the sediment of a Canadian Shield stream, with emphasis on responses to spring floods. *Canadian Journal of Fisheries and Aquatic Science* 45: 1994-2002.

Gibert J, Stanford JA, Dole-Olivier M-J and Ward JV. 1994. *Basic attributes of groundwater ecosystems and prospects for research in Groundwater Ecology* [eds] Gibert J, Danielopol DL and Stanford JV. Academic Press Limited: London.

Giller PS and Malmqvist B. 1998. *The Biology of Streams and Rivers.* OUP: Oxford.

Gilvear D, Rundle S and Griffin I. 2006. *River biodiversity: a preliminary study of the hyporheos of Scottish gravel bed rivers*. Scottish Natural Heritage Commissioned Report Number 216 (ROAME Number F05AC604)

Glazier DS and Deptola TJ. 2011. The amphipod *Gammarus minus* has larger eyes in freshwater springs with fish predators. *Invertebrate Biology* 130(1): 60-67.

Gledhill T. 1977. Numerical fluctuations of four species of subterranean amphipods during a five-year period. *Crustaceana* (Supplement 4): 144-152.

Gledhill T, Sutcliffe DW and Williams WD. 1993. *British Freshwater Crustacea Malacostraca: A Key with Ecological Notes.* Freshwater Biological Association: Cumbria.

Gotelli NG and Ellison AM. 2004. *A Primer of Ecological Statistics.* Sinauer Associates: Sunderland.

Gounot AM. 1994. Microbial ecology of groundwaters. In *Groundwater Ecology* [eds] Gibert J, Danielopol DL and Stanford JV. Academic Press Limited: London.

Gregory SP, Maurice LD, West JM and Gooddy DC. 2014. Microbial communities in UK aquifers: current understanding and future research needs. *Quarterly Journal of Engineering Geology and Hydrogeology* 47:145-157.

Griebler C, Stein H, Kellermann, C, Berkhoff S, Brielmann H, Schmidt S, Selesi D, Steube C, Fuchs A and Hahn HJ. 2010. Ecological Assessment of Groundwater Ecosystems – vision or illusion? *Ecological Engineering* 36: 1174-1190.

Gustard, A., Bullock, A., Dixon, J. M. 1992. *Low flow estimation in the United Kingdom*. Wallingford, Institute of Hydrology, 88pp. (IH Report No.108)

Hadfield SJ. 2002. Genetic structure and colonisation history of European and UK populations of *Gammarus pulex.* Phd Thesis, University of Hull. Unpublished.

Hahn HJ and Matzke D. 2005. A comparison of stygofauna communities inside and outside groundwater bores. *Limnologica* 35: 31-44.

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

Hahn HJ and Fuchs A. 2009. Distribution patterns of groundwater communities across aquifer types in south-western Germany. *Freshwater Biology* 54:848-860.

Hall EK, Besemer K, Kohl L, Preiler C, Riedel K, Schneider T, Wanek W and Battin TJ. 2012. Effects of resource chemistry on the composition and function of stream hyporheic biofilms. *Frontiers in Microbiology* 3:1-14.

Hancock PJ. 2002. Human impacts on the stream-groundwater exchange zone. *Environmental Management* 29(6): 763-781.

Hancock PJ, Boulton AJ and Humphreys WF. 2005. Aquifers and hyporheic zones: towards an ecological understanding of groundwater. *Hydrogeology Journal* 1(3): 98-111.

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

Hancock PJ and Boulton AJ. 2009. Sampling groundwater fauna: efficiency of rapid assessment methods tested in bores in eastern Australia. *Freshwater Biology* 54: 902-917.

Hanfling B, Douterelosoler I, Knight L and Proudlove G. 2008. Molecular studies on the *Niphargus kochianus* group (Crustacea: Amphipoda: Niphargidae) in Great Britain and Ireland. *Cave and Karst Science* 35(1): 35-40.

Hannah DM, Malcolm IA and Bradley C. 2009. Seasonal hyporheic temperature dynamics over riffle bedforms. *Hydrological Processes* 23: 2178-2194.

Harper MP and Peckarsky BL. 2006. Emergence cues of a mayfly in a high-altitude stream ecosystem: potential response to climate change. *Ecological Applications* 16: 612-621.

Hendricks SP. 1993. Microbial ecology of the hyporheic zone: a perspective integrating hydrology and biology. *North American Benthological Society* 12(1): 70-78.

Heppell CM, Wharton G, Cotton AC, Bass JAP and Roberts SE. 2009. Sediment storage in the shallow hyporheic of lowland vegetated river reaches. *Hydrological Processes* 23: 2239-2251.

Heywood MJT and Walling DE. 2003. Suspended sediment fluxes in chalk streams in the Hampshire Avon catchment, U.K. *Hydrobiologia* 494: 111-117.

Hoekstra JA, Vaal MA, Notenbloom J and Slooff W. 1994. Variation in the sensitivity of aquatic species to toxicants. *Environmental Contamination and Toxicology* 53: 98-105.

Hofmann TA and Mason CF. 2005. Habitat characteristics and the distribution of Odonata in a lowland river catchment in eastern England. Hydrobiolgia 539(1): 137-147

Holdgate MW. 1956. Transpiration through the cuticles of some aquatic insects. *Journal of Experimental Biology* 33: 107-118.

Holmes NTH. 2006. *Environmental Quality Appraisal of the Little Stour Catchment.* Alconbury Environmental Consultants: Huntingdon. (unpublished).

Hopkin SP. 2007. *A Key to the Collembola (Springtails) of Britain and Ireland.* Field Studies Council: Shrewsbury.

Hose GC. 2005. Assessing the need for groundwater quality guidelines for pesticides using the species sensitivity distribution approach. *Human and Ecological Risk Assessment* 11: 951-966.

Hou Z and Li S. 2002. Two new species of troglobitic amphipod crustaceans (Gammaridae) from Hubei Province, China. *The Raffles Bulletin of Zoology* 50(1): 27-36.

Hou Z, Jinzhong F and Li S. 2007. A molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochrondrial and nuclear gene sequences. *Molecular Phylogenetics and Evolution* 45:596-611.

Hou Z and Li S. 2010. Intraspecific or interspecific variation: delimitation of species boundaries within the genus *Gammarus* (Crustacea, Amphipoda, Gammaridae) with description of four new species. *Zoological Journal of the Linnean Society* 160: 215-253.

Hou Z, Sket B, Fiser C and Li S. 2011. Eocene habitat shift from saline to freshwater promoted Tethyan amphipod diversification. *Proceedings of the National Academy of Sciences.* Early Edition: 1-6.

Hou Z, Li J and Li S. 2013. Ten new *Gammarus* species (Crustacea: Amphipoda: Gammaridae) from Yunnan-Guizhou Plateau, China. *Zootaxa* 3687(1): 001-095

Humphreys W. 2007. Comment on assessing the need for groundwater quality guidelines for pesticides using the species sensitivity distribution approach by Hose (2005). *Human and Ecological Risk Assessment: An International Journal* 13(1): 236-240.

Hynes HBN. 1976. Biology of Plecoptera. *Annual Review of Entomology.* 21:135-153

Iversen M, Wiberg-Larsen P, Birkholm Hansen S and Hansen FS. 1978. The effect of partial and total drought on the macroinvertebrate communities of three small Danish streams. *Hydrobiologia* 60(3): 235-242.

James ABW, Dewson ZS and Death RG. 2008. Do stream macroinvertebrates use instream refugia in response to severe short-term flow reduction in New Zealand streams? *Freshwater Biology* 53: 1316-1334.

Janniche GS, Spliid H and Albrechtsen H-J. 2012. Microbial Community-Level Physiological Profiles (CLPP) and herbicide mineralization potential in groundwater affected by agricultural land use. *Journal of Contaminant Hydrology* 140-141: 45-55.

Johns T and Dunscombe M. 2011. *The Groundwater Animals Project: an investigation into the diversity and distribution of groundwater fauna in England.* Esmée Fairbairn Foundation Report by Roehampton University, London.

Johns T, Jones JI, Knight L, Maurice L, Wood P and Robertson A. 2015. Regional-scale drivers of groundwater faunal distributions. *Freshwater Science* 34(1): 316-328

Joint Nature Conservation Committee (JNCC). 2005. *Common Standards Monitoring Guidance for Rivers*. Accessed: 28 October 2012. Available: jncc.defra.gov.uk/pdf/csm_rivers_mar05.pdf.

Junk WJ, Bayley PB and Sparks RE. 1989. The flood pulse concept in river-floodplain systems. In *Proceedings of the International Large Rive Symposium (LARS).* Canadian Special Publication of Fisheries and Aquatic Sciences.

Karaman GS and Pinkster S. 1977. Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea-Amphipoda) Part I. *Gammarus pulex*-group and related species. *Bijdragen Tot de Dierkunde* 47: 1-97.

Käser DH, Binley A, Heathwaite AL and Krause S. 2009. Spatio-temporal variations of hyporheic flow in a riffle-step-pool sequence. *Hydrological Processes* 23: 2138-2149.

Kibichii S, Baars J-R., and Kelly-Quinn M. (2009) 'Optimising sample volume and replicates using the Bou-Rouch method for the rapid assessment of hyporheic fauna'. *Marine and Freshwater Research*, 60 (1): 83-96.

Knight, L. 2011. *Metadata for the dataset Hypogean macro-Crustacea records.* National Biodiversity Network. Accessed: 30 October 2011. Available: data.nbn.org.uk/datasetInfo/taxonDataset.jsp?dsKey=GA000514

Korbel KL and Hose GC. 2011. A tiered framework for assessing groundwater ecosystem health. *Hydrobiologia* 661: 329-349.

Kornobis E, Palsson S, Sidorov D, Holsinger JR and Kristjansson BK. 2011. Molecular taxonomy and phylogenetic affinities of two groundwater amphipods, *Crangonyx islandicus* and *Crymostygius thingvallensis,* endemic to Iceland. *Molecular Phylogenetics and Evolution* 58: 527-539.

Lafont M and Malard F. 2001. Oligochaete communities in the hyporheic zone of a glacial river, the Roseg River, Switzerland. *Hydrobiologia* 463: 75-81.

LaFont M and Vivier A. 2006. Oligochaete assemblages in the hyporheic zone and coarse surface sediments: their importance for understanding of ecological functioning of watercourses. *Hydrobiologia* 564(1): 171-181

Lake PS. 2003. Ecological effects of perturbation by drought in flowing waters. *Freshwater Biology* 48: 1161-1172.

Lancaster J and Ledger ME. 2015. Population-level responses of stream macroinvertebrates to drying can be density-independent or density-dependent. *Freshwater Biology* 60(12): 2559-2570.

Lefebure T, Douady CJ, Gouy M, Trontelj J, Broilays S and Gibert J. 2006. Phylogeography of a subterranean amphipod reveals cryptic diversity and dynamic evolution in extreme environments. *Molecular Ecology* 15: 1797-1806

Lee EH, Kim JY, Kim SY, Koo SD, Lee KS, Ko DC, Yum BW and Cho KS. 2010. Comparison of microbial communities in petroleum-contaminated groundwqater using genetic and metabolic profiles at Kyonggi-Do, South-Korea. *Environmental Earth Sciences* 60:371-382.

Legendre P and Legendre L. 2012. *Numerical Ecology (3rd Edition).* Elsevier: Oxford.

Leps J and Smilauer P. 2003. *Multivariate Analysis of Ecological Data using CANOCO*. Cambridge University Press: Cambridge.

Lewandowski J, Lischied G and Nutzmann G. 2009. Drivers of water level fluctuations and hydrological exchange between groundwater and surface water at the lowland River Spree (Germany): field study and statistical analyses. *Hydrological Processes* 23: 2117-2128.

Lowell JL, Gordon N, Engstrom D, Stanford JA, Holben WE and Gannon JE. 2009. Habitat heterogeneity and associated microbial community structure in small-scale floodplain hyporheic flow path. *Microbial Ecology* 58: 611-620.

Lustrik R, Turjak M, Kralj-Fiser S and Fiser C. 2011. Coexistence of surface and cave amphipods in an ecotone environment (spring area). *Contributions to Zoology* 80: 133-141.

Magurran, A.E. & McGill, B.J. (2011) Challenges and opportunities in the measurement and assessment of biological diversity. Biological Diversity: Frontiers in Measurement and Assessment (ed. by A.E. Magurran and B.J. Mcgill), pp. 1-7. Oxford University Press: Oxford.

Malard F, Dole-Olivier MJ, Mathieu J and Stoch F. 2002.*Sampling Manual for the Assessment of Regional Groundwater biodiversity. European Project: Protocols for the Assessment and Conservation of Aquatic Life in the Subsurface (PASCALIS).*Pascalis: University of Lyon.

Marchant R. 1995. Seasonal variation in the vertical distribution of hyporheic invertebrates in an Australian upland river. *Archiv fur Hydrobiologie* 134(4): 441-457.

Marsh T, Parry S, Kendon M and Hannaford J. 2013. *The 2010-2013 drought and subsequent extensive flooding*. Centre for Ecology and Hydrology: Wallingford.

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

Matthiessen P, Sheahan D, Harrison R, Kirby M, Rycroft R, Turnbull A, Volkner C and Williams R. 1995. Use of a *Gammarus pulex* Bioassay to measure the effects of transient Carbofuran runoff from farmland. *Ecotoxicology and Environmental Safety* 30(2): 111-119.

Mayer G, Maas A, Waloszek D. 2012. Mouthpart morphology of three sympatric native and non-native Gammaridean species: *Gammarus pulex, G. fossarum* and *Echinogammarus berilloni* (Crustacea: Amphipoda). *International Journal of Zoology* 2012: 1-23.

Mermillod-Blondin F, Foulquier A, Maazouzi C, Navel S, Negrutiu Y, Vienney A, Simon L and Marmonier P. 2013. Ecological assessment of groundwater trophic status by using artificial substrates to monitor biofilm growth and activity. *Ecological Indicators* 25: 230-238.

Mermillod-Blondin F, Gerino M, Creuze des Chatelliers M and Degrange V. 2002. Functional diversity among three detritivorous hyporheic invertebrates: an experimental study in microcosms. *Journal of the North American Benthological Society* 21(1): 132-149.

Meyran JC, Monnerot M and Taberlet P. 1997. Taxonomic status and phylogenetic relationships of some species of the genus Gammarus (Crustacea, Amphipoda) deduced from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 8(1): 1-10.

Moss B. 1998. *Ecology of Freshwaters: Man and Medium, Past to Future*. Blackwell Science: Oxford.

Murray-Bligh J. 1999. *Procedure for collecting and analyzing river macro invertebrate samples.* Report Number BT001, issue 2.0*.* Environment Agency: Bristol.

National Rivers Authority. 1993. *Low flows and water resources: facts and figures on the top forty low flow rivers in England and Wales*. National Rivers Authority: Bristol.

Navel S, Mermillod-Blondin F, Montuelle B, Chauvet E, Simon L, Piscart C and Marmonier P. 2010. Interactions between fauna and sediment control the breakdown of plant matter in river sediments. *Freshwater Biology* 55: 753-766.

Nijboer R. 2004. The ecological requirements of *Agapetus fuscipes* Curtis (Glossosomatidae), a characteristic species in unimpacted streams. *Limnologica* 34: 213-223.

Notenboom J, Plénet S and Turnquin MJ. 1994. Groundwater contamination and its impact on groundwater animals and ecosystems. In: Gibert J, Danielopol DS and Stanford JA (eds) *Groundwater Ecology*. Academic Press: San Diego.

Olsen DA and Townsend CR. 2005. Flood effects on invertebrates, sediments and particulate organic matter in the hyporheic zone of a gravel-bed stream. *Freshwater Biology* 50: 839-853.

Orghidan T. 1959. Ein neuer Lebensraum des unterirdischen Wassers: Der hyporheische Biotop". *Archiv fur Hydrobilogie* 55: 392-414.

Ozbek M and Camur-Elipek B. 2010. *Gammarus kesanensis* sp. nov., a new blind amphipod species from Turkey (Amphipoda, Gammaridae), with a key to the eyeless Gammarus species of Europe. *Zootaxa* 2399: 51-60.

Ozbek M, Yurga L and Kulkoyluoglu O. 2013. *Gammarus baysali* sp. nov., a new freshwater amphipod species from Turkey (Amphipoda: Gammaridae). *Turkish Journal of Zoology* 37:163-171.

Pacioglu O and Moldovan OT. 2016. Response of invertebrates from the hyporheic zone of chalk rivers to eutrophication and land use. *Environmental Science and Pollution Research* 23:4729-4740

Peck SB. 1998. A summary of diversity and distribution of the obligate cave-inhabiting faunas of the United States and Canada. *Journal of Cave and Karst Studies* 60(1): 18-26.

Pepin DM and Hauer FR. 2002. Benthic responses to groundwater-surface water exchange in two alluvial rivers in northwestern Montana. *Journal of the North American Benthological Society* 21(3) 370-383.

Plénet S. 1995. Freshwater amphipods as biomonitors of metal pollution in surface and interstitial aquatic systems. *Freshwater Biology* 33: 127-137.

Plénet, S., J. Gibert, and P. Marmonier. 1995. Biotic and abiotic interactions between surface and interstitial systems in rivers. Ecography 18:296–309.

Plénet S and Gibert J. 1992. A floodplain spring: an ecotone between surface water and groundwater. *Regulated Rivers: Research and* Management 7: 93-102.

Philipson GN. 2010. The effect of water flow and oxygen concentration on six species of caddis fly (Trichoptera) larvae. *Proceedings of the Zoological Society of London* 124(3): 547-564.

Pinkster S and Karaman GS. 1978. A new blind *Gammarus* species from Asia Minor, *Gammarus vignai* n.sp. (Crustacea, Amphipoda). *Quaderni di Speleologia, Circolo Speleologico Romano* 3:27-36.

Pockl M, Webb B and Sutcliffe D. 2002. Life history and reproductive capacity of *Gammarus fossarum* and *G. roeseli* (Crustacea: Amphipoda) under naturally fluctuating water temperatures: a simulation study. *Freshwater Biology* 48(1): 53-66.

Poole GC, Stanford JA, Running SW and Frissell CA. 2006. Multiscale geomorphic drivers of groundwater flow paths: subsurface hydrologic dynamics and hyporheic habitat diversity. *Journal of the North American Benthological Society* 25(2): 288-303.

Proudlove G and Wood PJ. 2003. The blind leading the blind: cryptic subterranean species and DNA taxonomy. *TRENDS in Ecology and Evolution* 18(6): 272.

Proudlove G, Wood PJ, Harding PT, Horne DJ, Gledhill T and Knight LRFD. 2003. A review of the status of the subterranean aquatic Crustacea of Britain and Ireland. *Cave and Karst Science* 30(2): 53-74.

Pryce D, Willby N and Givear D. 2010. An investigation into the hyporehic zone of gravel bed rivers in Scotland and its associated fauna. *Scottish Natural Heritage Commissioned Report* Number 397.

Rasmussen K. 1979. The Invertebrate fauna of a helocrenous spring area in eastern Jutland, Denmark. *Flora og Fauna* 85(3-4): 71-81.

Robertson AL, Rundle SD and Schmid-Araya. 2000. Putting the meio-into stream ecology: current findings and future directions for lotic meiofaunal research. *Freshwater Biology* 44: 177-183.

Robertson AL, Smith JWN, Johns T and Proudlove GS. 2008. *A Review of the Subterranean Aquatic Ecology of England and Wales.* Science Report SC030155/SR20. Environment Agency: Bristol.

Robertson AL, Smith JWN, Johns T and Proudlove GS. 2009. The distribution and diversity of stygobites in Great Britain: an analysis to inform groundwater management. *Quarterly Journal of Engineering Geology and Hydrogeology* 42: 359-368.

Robins NS. 1998. *Groundwater Pollution, Aquifer Recharge and Vulnerability.* Geological Society of London: London.

Rundle, SD and Ormerod, SJ. 1992. The influence of chemistry and habitat features on some microcrustacea of some upland welsh streams. (1992) *Freshwater Biology* 26(3): 439-452.

Scarsbrook MR Halliday J. 2002. Detecting patterns in hyporheic community structure: does sampling method alter the story? *New Zealand Journal of Marine and Freshwater Research* 36: 443-453.

Schmid-Araya JM, Hildrew AG, Robertson A, Schmid PE and Winterbottom J. 2002. The importance of meiofauna in food webs: evidence from an acid stream. *Ecology* 83(5): 1271-1285.

Schmidt-Kloiber A and Hering D (eds). 2012. The Taxa and Autecology Database for Freshwater Organisms, version 5.0. Available: www.freshwaterecology.info. Accessed: 4 August 2013.

Sear DA, Armitage PD and Dawson FH. 1999. Groundwater dominated rivers. *Hydrological Processes* 13: 255-276.

Siegismund HR and Muller J. 1991. Genetic structure of *Gammarus fossarum* populations. *Heredity* 66: 419-436.

Sket B. 1999. The nature of biodiversity in hypogean waters and how it is endangered. *Biodiversity and Conservation* 8: 1319-1338.

Shaw P, Dunscombe M and Robertson A. 2011. Collembola in the hyporheos of a karstic river: an overlooked habitat for Collembola containing a new genus for the UK. *Soil Organisms* 83(3): 507-514.

Smedley PL, Griffiths K, Tyler-Whittle R, Hargreaves R, Lawrence AR and Besien T. 2003. *Baseline Report Series (5) The Chalk of the North Downs, Kent and East Surrey.* Groundwater Systems and Water Quality Commissioned Report CR/03/033N. National Groundwater and Contaminated Land Centre Technical Report NC/99/74/5. British Geological Survey: Nottingham.

Smith H, Wood PJ and Gunn J. 2003. The influence of habitat structure and flow permanence on invertebrate communities in karst spring systems. *Hydrobiologia* 510: 53-66.

Sorensen JPR, Maurice L, Edwards FK, Lapworth DJ, Read DS, Allen D, Butcher AS, Newbold LK, Townsend BR and Williams PJ. 2013. *Using boreholes as windows into Groundwater Ecosystems. PLOS ONE* 8(7): e70264

Southern Water. *Pers. Comm.* Email. *Nailbourne River request for information*, Southern Water Reference CCMS 156529. 6 July 2010.

Stanford JA and Ward JV. 1993. An ecosystem perspective of alluvial rivers: connectivity and the hyporheic corridor. *Journal of the North American Benthological Society* 12(1): 48-60.

Stanford JA, Ward JV and Ellis BK. 1994. Ecology of the alluvial aquifers of Flathead River, Montana. In *Groundwater Ecology* [eds] Gibert J, Danielopol DL and Stanford JV. Academic Press Limited: London.

Stanley EH and Boulton AJ. 1993. Hydrology and the distribution of hyporheos: perspectives from a Mesic River and a desert stream. *Journal of the North American Benthological Society* 12(1): 79-83.

Stead TK, Schmid-Araya JM and Hildrew AG. 2004. The contribution of subsurface invertebrates to benthic density and biomass in a gravel stream. *Archiv fur Hydrobiologie* 160(2): 171-191.

Stead TK, Schmid-Araya JM, Schmid PE and Hildrew AG. 2005. The distribution of body size in a stream community: one system, many patterns. *Journal of Animal Ecology* 74: 475-487.

Stein H, Kellermann C, Schmidt SI, Brielmann H, Steube C, Berkhoff SE, Fuchs A, Hahn HJ, Thulin B and Griebler C. 2010. The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater quality. *Journal of Environmental Monitoring*

Stoch F, Artheau M, Brancelj A, Galassi DMP and Malard F. 2009. Biodiversity indicators in European groundwaters: towards a predictive model of stygobiotic species richness. *Freshwater Biology* 54: 745-755.

Stuart ME and Smedley PL. 2009. Baseline groundwater chemistry: the Chalk aquifer of Hampshire. *British Geological Survey Open Report, OR/09/052.*

Stubbington R, Greenwood AM, Wood PJ, Armitage PD, Gunn J and Robertson AL. 2009a. The response of perennial and temporary headwater stream invertebrate communities to hydrological extremes. *Hydrobiologia* 630: 299-312.

Stubbington R, Wood PJ and Boulton AJ. 2009b. Low flow controls on benthic and hyporheic macroinvertebrate assemblages during supra-seasonal drought. *Hydrological Processes* 23: 2252-2263.

Stubbington R. 2011. *The Hyporheic Zone as a Refugium for Benthic Invertebrates in Groundwater-Dominated Streams.* PhD Thesis, Loughborough University.

Stubbington R, Wood PJ, Reid I and Gunn J. 2011. Benthic and hyporheic invertebrate community responses to seasonal flow recession in a groundwater-dominated stream. *Ecohydrology* 4(4): 500-511

Stubbington R. and Wood PJ. 2013. Benthic and interstitial habitats of a lentic spring as invertebrate refuges during a supra-seasonal drought. *Fundamental and Applied Limnology.* 182(1): 61-73.

Stubbington R, Boulton AJ, Little S and Wood PJ. 2015. Changes in invertebrate assemblage composition in benthic and hyporheic zones during a severe supraseasonal drought. *Freshwater Science* 34(1): 344-354.

Stubbington R, Gunn J, Little S, Worrall TP and Wood PJ. 2016. Macroinvertebrate seedbank composition in relation to antecedent duration of drying and multiple wet-dry cycles in a temporary stream. *Freshwater Biology* 61: 1293-1307.

Stumpp C and Hose CG. 2013. The impact of water table drawdown and drying on subterranean aquatic fauna in in-vitro experiments. *PLoS ONE* 8(11): e78502.

Talling JF. 2010. Potassium – a non-limiting nutrient in fresh waters? *Freshwater Reviews* 3: 97-104.

ter Braak CJF and Smilauer P. 2002. *Canoco Reference Manual.* Microcomputer Power: New York.

Thulin B and Hahn HJ. 2008. *Ecology and living conditions of groundwater fauna* Technical Report produced for the Swedish Nuclear Fuel and Waste Management Company (TR-08-06).

Townsend CR, Hildrew AG and Francis J. 1983. Community structure in some southern English streams: the influence of physicochemical factors. *Freshwater Biology* 13: 521-544.

Townsend CR and Hildrew AG. 1994. Species traits in relation to a habitat template for river systems. *Freshwater Biology* 31: 265-275.

University of Ulster. *A Blind Gammarus from County Fermanagh*. Accessed: 16 June 2013. Available: www.science.ulster.ac.uk/gei/A-blind-Gammarus-from-County.html.

Vannote RL, Minshall GW, Cummins KW, Sedell JR and Cushing CE. 1980. The River Continuum Concept. *Canadian Journal of fisheries and Aquatic Sciences* 37: 130-137.

Vainola R, Witt JDS, Grabowski M, Bradbury JH, Jazdzewski K and Sket B. 2008. Global diversity of amphipods (Amphipoda: Crustacea) in freshwater. *Hydrobiologia* 595: 241-255.

Varricchione JT, Thomas SA and Minshall GW. 2005. Vertical and seasonal distribution of hyporheic invertebrates in streams with different glacial histories. *Aquatic Sciences* 67: 434-453.

Verdonschot PFM, Besse-Lotoskaya AA, Dekkers TBM and Verdonschot RCM. 2014. Directional movement in response to altered flow in six lowland stream Trichoptera. *Hydrobiologia* 740: 219-230.

Vervier P. 1990.Study of aquatic community dynamics in a karstic system by the use of artificial substrates. *Archiv fur Hydrobiologie* 119(1): 15-33.

Vervier P and Gibert J. 1991. Dynamics of surface water/groundwater ecotones in a karstic aquifer. *Freshwater Biology* 26(2): 241-250.

Vorosmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Gidden S, Bunn SE, Sulllivan CA, Reidy-Liermann C and Davies PM. 2010. Global threats to human water security and river biodiversity. *Nature* 467: 555-561.

Wagner, R. 1991. The influence of the diel activity pattern of the larvae of Sericostoma personatum (Kirby and Spence) (Tricoptera) on organic matter in stream-bed sediments – a laboratory study. *Hydrobiologia* 224:65-70.

Wagner R and Schmidt HH. 2004. Yearly discharge patterns determine species abundance and community diversity: Analysis of a 25-year record from the Breitenbach. *Archiv fur Hydrobiologie* 161(4): 511-540

Wahl TL and Wahl KL. 2007. *A computer program for determining an index to base flow* [online]. Lakewood, CO: US Department of the Interior. Available from: http://www.usbr.gov/pmts/hydraulics_lab/twahl/bfi/ [Accessed 1 December 2012].

Wallace ID, Wallace B and Philipson GN. 2003. *A key to the case-bearing caddis larvae of Britain and Ireland*. Freshwater Biological Association, Scientific Publication Number 61:Ambleside.

Water Framework Directive – UK Technical Advisory Group (WFD-UKTAG). 2014. *UKTAG River Assessment Method Benthic Invertebrate Fauna - Invertebrates (General Degradation): Whalley, Hawkes, Paisley and Trigg (WHPT) metric in River Invertebrate Classification Tool (RICT).* SEPA, Stirling.

Weber KP, Grove JA, Gehder M, Anderson WA and Legge RL. 2007. Data transformations in the analysis of community-level substrate utilization data from microplates. *Journal of Microbiological Methods* 69: 461-469.

Weiss M, Niklas Macher J, Seefeldt MA and Leese F. 2014. Molecular evidence for further overlooked species within the *Gammarus fossarum complex* (Crustacea: Amphipoda). *Hydrobiologia* 721: 165-184.

Weitowitz D. 2012. *Distribution and Abundance of Stygobites in the North Downs Chalk of Kent, UK*. MSc Dissertation, University of Oxford. (unpublished).

Wilenchic C. 2008. *An evaluation of the efficiency of different sampling methods to collect macroinvertebrates from the hyporheic zone of a river*. MSc Dissertation, Loughborough University. (unpublished).

Williams, DD and Hynes HBN. 1974. The occurrence of benthos deep in the substratum of a stream. *Freshwater Biology* 4: 233–256.

Williams DD. 1993. Nutrient and flow vector dynamics at the hyporheic/groundwater interface and their effects on the interstitial fauna. *Hydrobiologia* 251: 185-198.

Williams DD. 2006. *The Biology of Temporary Waters.* Oxford University Press: Oxford.

Williams DD, Febria CM, Wong JCY. 2010. Ecotonal and other properties of the Hyporheic Zone. *Fundamental and Applied Limnology* 176(4): 349-364.

Wilson J. 1975. The effect of low humidity on the distribution of *Heteromurus nitidus* (Collembola) in Radford Cave, Devon. *Transactions of the British Cave Research Association* 2(3): 123-126.

Wood PJ and Petts GE. 1994. Low flows and recovery of macroinvertebrates in a small regulated chalk stream. *Regulated Rivers: Research and Management* 9: 303-316

Wood PJ and Armitage PD. 1997. Biological effects of fine sediment in the lotic environment. *Environmental Management* 21(2): 203-217.

Wood PJ, Barker S and Stokes J. 1998. *Macroinvertebrate Survey of the Little Stour.* Limestone Research Group, University of Huddersfield for the Environment Agency, Report: 200005752.

Wood PJ and Armitage PD. 1999. Sediment deposition in a small lowland stream – Management Implications. *Regulated Rivers – Research and Management* 15(3): 199-210

Wood PJ and Petts GE. 1999. The influence of drought on chalk stream macroinvertebrates. *Hydrological Processes.* 13: 387-399

Wood PJ, Agnew MD and Petts GE. 2000. Flow variations and macroinvertebrate community responses in a small groundwater-dominated stream in south-east England. *Hydrological Processes* 14: 3133-3147.

Wood PJ, Hannah DM, Agnew MD and Petts GE. 2001. Scales of hydroecological variability within a groundwater-dominated stream. *Regulated Rivers: Research and Management* 17:347-367.

Wood PJ and Armitage PD. 2004. The response of the macroinvertebrate community to low-flow variability and supra-seasonal drought within a groundwater dominated stream. *Archiv für Hydrobiologie* 125: 1-20.

Wood PJ, Gunn J, Smith H and Abas-Kutty A. 2005a. Flow permanence and macroinvertebrate community diversity within groundwater dominated headwater streams and springs. *Hydrobiologia* 545: 55-64.

Wood PJ, Toone J, Greenwood MT and Armitage PD. 2005b. The response of four lotic macroinvertebrate taxa to burial by sediments. Arch. Hydrobiol. 163(2) 145-162

Wood PJ, Gunn J and Rundle SD. 2008. Response of benthic cave invertebrates to organic pollution events. *Aquatic Conservation: Marine and Freshwater Ecosystems* 18: 909-922.

Wood PJ, Boulton AJ, Little S and Stubbington R. 2010. Is the hyporheic zone a refugium for aquatic macroinvertebrates during severe low flow conditions? *Fundamental and Applied Limnology* 176(4): 377-390.

Wysocka A, Grabowski M, Sworobowicz L, Burzynski A, Kilikowska A, Kilikowska A, Kostoski G and Sell J. 2013. A tale of time and depth: intralacustrine radiation in endemic *Gammarus* species flock from the ancient Lake Ohrid. *Zoological Journal of the Linnaean Society* 167(3): 345-359.

Zhou Y, Kellermann C and Griebler C. 2012. Spatio-temporal patterns of microbial communities in a hydrogically dynamic pristine aquifer. *FEMS Microbiology Ecology* 81: 230-242.

Zuur, AF, Ieno EN and Elphick CS. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* 1:3-14

Appendix I – Taxonomic List (and Abbreviations)

Class/Order	Family	Taxon	Abbreviation
Copepoda	Cyclopidae	Acanthocyclops sensitivus	-
Coleoptera	Dytiscidae	Agabus didymus	Ad
Trichoptera	Glossosomatidae	Agapetus fuscipes	Af
Coleoptera	Hydrophilidae	Anacaena limbata ovata	Al
Gastropoda	Planorbidae	Ancylus fluviatilis	Aflu
Isopoda	Asellidae	Asellus aquaticus	Aa
Isopoda	Asellidae	Asellus meridianus	Am
Trichoptera	Leptoceridae	Athripsodes spp.	Ath
Ephemeroptera	Baetidae	Baetis rhodani	Br
Gastropoda	Bithyniidae	Bithynia tentaculata	Bt
Odonata	Calopterygidae	Calopteryx splendens	Csp
Diptera	Ceratopogonidae	Ceratopogonidae	Cer
Diptera	Chironomidae	Chironomidae	Chi
Copepoda	-	Copepoda	-
Malacostraca	Crangonyctidae	Crangonyx pseudogracilis	Cp
Malacostraca	Crangonyctidae	Crangonyx subterraneus	Cs
Seriata	Dendrocoelidae	Dendrocoelum lacteum	Dl
Diptera	Pediciidae	Dicranota	Di
Diptera	Dixidae	Dixidae	Dix
Trichoptera	Limnephilidae	Drusus annulatus	Da
Coleoptera	Dytiscidae	Dytiscidae (larvae)	Dyt.l
Coleoptera	Elmidae	Elmis aenea	Ea
Diptera	Syrphidae	Eristalis tenax	Et
Clitellata	Erpobdellidae	Erpobdella octoculata	Eo
Diptera	Fanniidae	Fanniidae	Fan
Megaloptera	Gammaridae	Gammarus fossarum	Gf
Megaloptera	Gammaridae	Gammarus pulex	Gp
Malacostraca	Gammaridae	Gammarus sp.	Gs
Clitellata	Glossiphoniidae	Glossiphonia complanata	Gc
Trichoptera	Limnephilidae	Halesus radiatus	Hr
Coleoptera	Haliplidae	Haliplus lineatocollis	Hl
Clitellata	Glossiphoniidae	Helobdella stagnalis	Hsta
Coleoptera	Scirtidae	Helodes sp.	Hel
Coleoptera	Hydrophilidae	Helophorus brevipalpis	Hb
Hydrozoa	Hydridae	Hydra oligactis	-
Arachnida	Hydrachnidiae	Hydracarina	-
Coleoptera	Hydrophilidae	Hydrobius fuscipes	Hf
Coleoptera	Hydrophilidae	Hydrophilidae (larvae)	Hdph.l
Trichoptera	Hydropsychidae	Hydropsyche siltalai	Hs
Trichoptera	Hydroptiloidea	Hydroptila	Hydp
Coleoptera	Dytiscidae	Hygrotus (Coelambus) confluens	Hc
Trichoptera	Leptoceridae	Leptoceridae (larvae)	Lepc.l
Ephemeroptera	Leptophlebiidae	Leptophlebia sp.	Lept
Trichoptera	Limnephilidae	Limnephilidae larvae	Lim.l
Trichoptera	Limnephilidae	Limnephilus lunatus	Ll
Trichoptera	Limnephilidae	Limnephilus marmoratus	Lm
Coleoptera	Elmidae	Limnius volckmari	Lv
Diptera	Tipulidae	Limoniidae	Limon
Gastropoda	Lymnaeidae	Lymnaea palustris	Lpal
Gastropoda	Lymnaeidae	Lymnaea peregra	Lper
Trichoptera	Psychomyiidae	Lype reducta	Lr

Class/Order	Family	Taxon	Abbreviation
Trichoptera	Leptoceridae	Mystacides spp.	Myst
Coleoptera	Dytiscidae	Nebrioporus elegans	Neb
Malacostraca	Niphargidae	Niphargus aquilex	Na
Malacostraca	Niphargidae	Niphargus fontanus	Nf
Malacostraca	Niphargidae	Niphargus kochianus	Nk
Plecoptera	Nemouridae	Nemoura cinerea	Nc
Oligochaeta	Oligochaeta	Oligochaeta	-
Ostracoda	-	Ostracoda	-
Coleoptera	Elmidae	Oulimnius	Oul
Gastropoda	Physidae	Physa fontinalis	Pfont
Clitellata	Piscicolidae	Piscicola geometra	Pg
Bivalvia	Sphaeriidae	Pisidium	Pis
Gastropoda	Planorbidae	Planorbis planorbis	Pp
Trichoptera	Polycentropodidae	Plectrocnemia brevis	Pb
Tricladida	Planariidae	Polycelis sp.	Poly
Trichoptera	Polycentropodidae	Polycentropus flavomaculatus	Pf
Trichoptera	Limnephilidae	Potamophylax cingulatus	Pc
Gastropoda	Hydrobiidae	Potamopyrgus antipodarum	Pa
Diptera	Psychodidae	Psychodidae	Psy
Diptera	Ptychopteridae	Ptychopteridae	Pty
Trichoptera	Rhyacophilidae	Rhyacophila dorsalis	Rd
Trichoptera	Sericostomatidae	Sericostoma personatum	Sp
Ephemeroptera	Ephemerellidae	Serratella ignita	Si
Megaloptera	Sialidae	Sialis lutaria	Sl
Trichoptera	Goeridae	Silo nigricornis	Sn
Trichoptera	Goeridae	Silo pallipes	Spa
Diptera	Simuliidae	Simuliidae	Sim
Diptera	Stratiomyidae	Stratiomyidae	Strat
Diptera	Tabanidae	Tabanidae	Tab
Diptera	Tipulidae	Tipula	Tip
Gastropoda	Valvatoidea	Valvata piscinalis	Vp
Hemiptera	Veliidae	Velia caprai	Vc

Appendix II – *Gammarus fossarum* Manuscript

Aquatic Invasions (2017) Volume 12 Article in press

Research Article CORRECTED PROOF

Detection of a new non-native freshwater species by DNA metabarcoding of environmental samples—first record of *Gammarus fossarum* in the UK

Rosetta C. Blackman[1,*], Drew Constable[2], Christoph Hahn[1,3], Andrew M. Sheard[4], Jessica Durkota[5], Bernd Hänfling[1] and Lori Lawson Handley[1]

[1]*Evolutionary and Environmental Genomics Group, School of Environmental Sciences, University of Hull, Cottingham Road, Hull, HU6 7RX, UK*

[2]*Environment Agency, Bromholme Lane, Brampton, Huntingdon, Cambs, PE28 4NE, UK*

[3]*Institute of Zoology, University of Graz, Universitaetsplatz 2, A-8010 Graz, Austria*

[4]*Independent author, Beverley, UK*

[5]*Department of Geography, University College London, Gower Street, London, WC1E 6BT, UK*

Author e-mails: rosiecblackman@gmail.com (RCB), drew.constable@environment-agency.gov.uk (DC), C.Hahn@hull.ac.uk (CH), andysheard@me.com (AMS), j.durkota@ucl.ac.uk (JD), B.Haenfling@hull.ac.uk (BH), L.Lawson-Handley@hull.ac.uk (LLH)

**Corresponding author*

Received: 23 September 2017 / Accepted: 20 March 2017 / Published online: 24 April 2017

Handling editor: Michal Grabowski

Abstract

We report the discovery of a non-native gammarid, *Gammarus fossarum* (Koch, 1836) (Crustacea, Amphipoda), in UK rivers. *Gammarus fossarum* is a common freshwater gammarid in many parts of mainland Europe, but was previously considered absent from the UK. *Gammarus fossarum* was detected in a number of UK rivers following DNA metabarcoding of a mini-barcode region of the *COI* gene in macroinvertebrate kick samples, and environmental DNA (eDNA) from water and sediment samples. Subsequent morphological analysis and standard DNA barcoding showed that the species could be reliably identified and separated from *Gammarus pulex* (Linnaeus, 1758), the most dominant and widespread native freshwater gammarid in the UK. Our data demonstrate extensive geographical coverage of *G. fossarum* in the UK, spanning distant river catchments. At present there is no data to confirm the likely origin of *G. fossarum*'s introduction. Subsequent re-examination of historic archive material shows the species to have been present in the UK since at least 1964. This study is among the first to demonstrate the potential of eDNA metabarcoding for detection of new non-native species.

Key words: environmental DNA, metabarcoding, passive detection, early warning, cryptic species, Gammaridae, non-native

Introduction

Amphipods are successful invaders in freshwater ecosystems, with many invasive non-native species (INNS) having been observed to adversely impact indigenous species within Europe over the last century (Bij de Vaate et al. 2002; Grabowski et al. 2007). The introduction of non-native amphipods may not only lead to displacement of native congeners (e.g. Dick and Platvoet 2000; MacNeil and Platvoet 2005; Kinzler et al. 2009), but may also impact on ecosystem structure and functioning (MacNeil et al. 2011; Piscart et al. 2011; Constable and Birkby 2016) and introduce novel pathogens to newly colonised areas (Bacela-Spychalska et al. 2012).

Once non-native species are widely established, efforts to reduce their impacts are often problematic, hence management strategies are strongly focused on preventing introductions or spread (e.g. the "check, clean, dry" campaign in the UK). Early detection is key to such strategies, either to improve the success of eradication programs or to prevent further establishment and dispersal (Roy et al. 2014; Dejean et al.

2012). For freshwater macroinvertebrates, INNS detection methods typically rely on sampling programmes and morphological identification. However, the standard UK monitoring method for macroinvertebrates, a three minute kick sample, will typically recover 62% of families and 50% of species at a site (Furse et al. 1981). This can present considerable challenges when dealing with rare or elusive species. Morphological identification can also prove difficult when identifying taxonomically similar or cryptic species, or juvenile life stages, and is highly dependent on the taxonomical expertise of the investigator. Emerging molecular detection methods may provide significant benefit for detecting non-native species in aquatic environments (Darling and Mahon 2011; Lawson Handley 2015).

One new and rapidly developing method is the use of environmental DNA (eDNA) (Taberlet et al. 2012a, b; Rees et al. 2014; Lawson Handley 2015), which refers to cellular or extracellular DNA that can be extracted directly from environmental samples without prior separation of taxa (Taberlet et al. 2012a). Environmental DNA has been successfully used in numerous studies to detect specific taxa using a targeted approach based on standard or quantitative PCR (Dejean et al. 2012; Dougherty et al. 2016). In an alternative approach, called "metabarcoding", entire species assemblages are analysed by PCR with broadly conserved primers, followed by Next Generation Sequencing (NGS: see Lawson Handley 2015; Hänfling et al. 2016; Port et al. 2016; Valentini et al. 2016 for further detail). Environmental DNA metabarcoding has been successfully used in a small number of studies, for example, to describe entire communities of vertebrates (e.g. Lawson Handley 2015; Hänfling et al. 2016; Port et al. 2016; Valentini et al. 2016) and invertebrates (Deiner et al. 2016) from marine, lake and river samples. Metabarcoding has excellent potential as an early warning tool for detection of non-native species from samples collected from invasion pathways or natural/semi-natural habitats (Mahon and Jerde 2016; Lawson Handley 2015). For example, the technique was recently used as an early detection method for screening ship ballast, and detected non-indigenous zooplankton in Canadian ports (Brown et al. 2016). Environmental DNA metabarcoding has also identified non-native fish species present in samples from the live bait trade (white perch, *Morone americana* (Gmelin, 1789) Mahon et al. 2014) and in river samples (northern snakehead, *Channa argus* (Cantor, 1842) Simmons et al. 2015). However the number of applications of metabarcoding for detection of non-native species has so far been limited.

In this paper we describe the detection of *Gammarus fossarum* (Koch, 1836), a newly recognised freshwater amphipod to the UK, using macroinvertebrate community and eDNA metabarcoding. The species was found in several UK rivers following a preliminary non-targeted sampling programme for macroinvertebrate communities based on metabarcoding of a 313 bp mini-barcode region of the cytochrome c oxidase subunit I (*COI*) gene, and was subsequently confirmed using a combination of morphological analysis and standard full-length *COI* DNA barcoding (via Sanger sequencing). This study demonstrates the power of eDNA metabarcoding for detection of non-native species in natural habitats.

Methods

Metabarcoding surveys

Sampling

Field surveys were carried out in March 2015 within 8 UK river catchments (Figure 1, Maps A–H, excluding E). At each site (n = 65) environmental variables including water depth, width, substrate type and surrounding habitat were recorded. Three sample types were collected at each site: a three minute macroinvertebrate kick sample (Murray-Bligh 1999) for identification by microscopy analysis and high molecular weight DNA extraction from pools of individuals; and water and sediment samples were collected for eDNA extraction. Two litres of water was sampled from the surface by collecting 4 × 500 ml from points across the river width using a sterile bottle. Sediment samples were collected from points across the river width using a trowel, and the material was placed in a 42 fluid oz. sterile Whirl-pak® bag (Cole-Palmer, Hanwell, London). All sampling equipment was sterilized in 10% commercial bleach solution for 10 minutes then rinsed with 10% MicroSol detergent (Anachem, UK) and purified water between samples. Sample bottles filled with ddH_2O were taken into the field and later filtered as sample blanks.

Macroinvertebrate community sample processing

All macroinvertebrates from each kick sample were sorted and identified to the lowest taxonomic level possible, before being stored in sterile 50 ml falcon tubes filled with 100% ethanol. For DNA extraction, samples were dried to remove the ethanol and the entire macroinvertebrate community was lysed in a Qiagen Tissue Lyser® with Digisol (50mM Tris, 20M EDTA, 120 mM NaCl and 1% SDS) (3 × 30 sec). Samples were then incubated overnight at 55 °C with SDS and Proteinase K. DNA from a 200 µl subsample

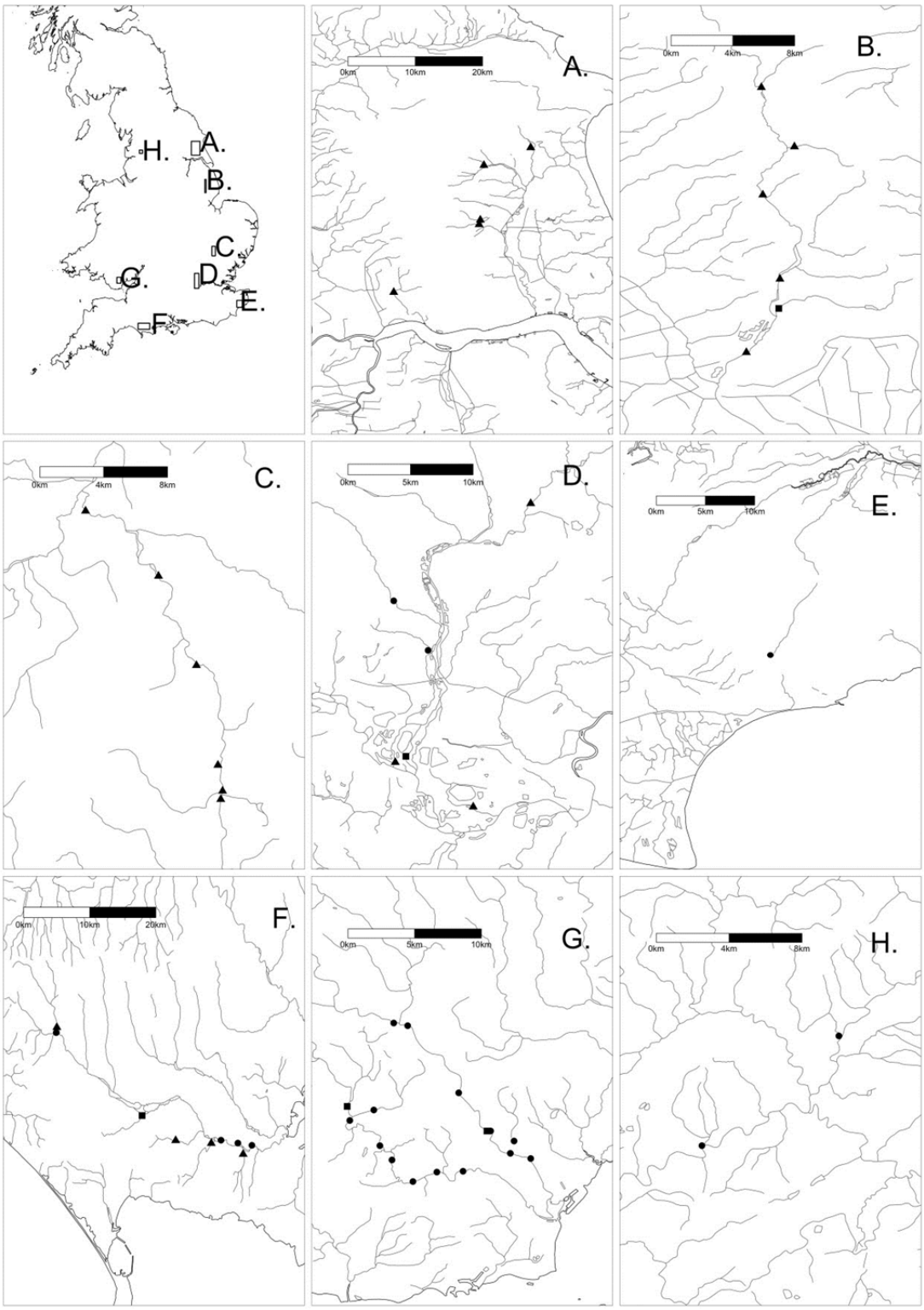


Figure 1. Distribution of Gammaridae species detected during this study. ● – *Gammarus fossarum*, ▲ – *Gammarus pulex* and ■ – both species present. A – River Hull, B – River Bain, C – River Cam, D – River Colne, E – Nailbourne, F – River Frome, G – Rivers Taff and Ely and H – River Ribble. See supplementary information Table S1 for further site information (Pebesma et al. 2005; Wickham. 2009; Bivand et al. 2013; Bivand et al. 2016; Gallic. 2016) Contains OS data © Crown copyright and database right (2016).

of the lysed tissue was extracted using the DNeasy Blood & Tissue Kit® (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Environmental DNA sample processing

Water samples were filtered within 24 hours through sterile 47 mm diameter 0.45 µm cellulose nitrate membrane filters and pads (Whatman, GE Healthcare, UK), using Nalgene filtration units attached to a vacuum pump. Sediment samples were stored at −20 °C within 12 hours of sampling. The sample was defrosted, mixed and 200 ml of sediment placed in a sterile measuring cylinder with 500 ml of molecular grade water, then inverted 10 times and left to stand for 30 s, the supernatant was then poured off into a sterile container. This procedure was repeated twice. Two hundred and fifty millilitres of the supernatant was then prefiltered through sterile 20 µm filter paper (Whatman, GE Healthcare, UK), and the filtrate subsequently filtered through 0.45 µm cellulose nitrate filters, as for the water samples. Filter papers were stored in sterile petri dishes at −20 °C until extraction. Filtration blanks (2 L purified water) were run before the samples for each filtration run to test for contamination at the filtration stage (n = 5). Filtration equipment was sterilized in 10% commercial bleach solution for 10 minutes then rinsed with 10% MicroSol detergent and purified water after each filtration.

Environmental DNA from both water and sediment samples was extracted using PowerWater® DNA Isolation Kit (MoBio Laboratories, Inc. Carlsbad, USA) following the manufacturer's instructions.

PCR, library prep and sequencing

We chose to use *COI* for metabarcoding because this region has the broadest taxonomic coverage for macroinvertebrates in public sequence databases and is the most widely used DNA barcode for taxonomic discrimination in this group. A 313 bp fragment ("mini-barcode") was targeted using the primers described in Leray et al. (2013). For library preparation we used a nested tagging protocol, modified from the Illumina 16S two-step metabarcoding protocol (Illumina 2011) as outlined in Kitson et al. (2015).

In the first step, PCRs were performed with modified versions of the primers jgHCO2198 TAIA CYTCIGGRTGICCRAARAAYCA and mICOIintF GGWACWGGWTGAACWGTWTAYCCYCC (Leray et al. 2013). In addition to the standard primer sequence, primers included one of eight unique forward or 12 unique reverse 8-nucleotide Molecular Identification Tags (MID), plus a bridge site, which acts as a binding site for PCR 2 (see Kitson et al. 2015 for full details). PCRs were carried out in 25 µl volumes with MyFi High-Fidelity Taq (Bioline, UK) containing: 10 µM of each primer, and 2 µl of undiluted DNA template. PCRs were performed on an Applied Biosystems Veriti Thermal Cycler with the following profile: initial denaturation at 95 °C for 1 min, followed by 45 cycles of denaturation at 98 °C for 15 s, annealing at 51 °C for 15 s and extension at 72 °C for 30 s, with a final extension time of 10 min at 72 °C. This included PCR and filtering blanks (n = 3 and n = 5, respectively) and single species positives: *Triops cancriformis* (Bosc, 1801) (n = 2) and *Harmonia axyridis* (Pallas, 1773) (n = 2). PCR products were confirmed by gel electrophoresis on a 2% agarose gel stained with ethidium bromide. PCRs were carried out three times and then pooled. Pooled PCR products were then purified using the E.Z.N.A Cycle Pure Kit® (VWR International, Leicestershire).

In the second PCR step, Illumina adapters and additional forward and reverse MID tags were added in a second PCR with 10 µM of each tagging primer and 2 µl of purified PCR product. PCR settings were: initial denaturation at 95 °C for 3 min, followed by 12 cycles of denaturation at 98 °C for 20 s, annealing at 72 °C for 1 min and extension at 72 °C for 5 mins, with a final extension time of 10 mins at 4 °C (Kitson et al. 2015).

Samples were then classified into five categories based on the strength of band produced on ethidium bromide-stained agarose gels. Negative controls (including filtration blanks) produced no bands on the agarose gel so were categorised with samples with the lowest band strengths when being added to the library. All positive control (i.e. extracted tissue) samples were categorised as high band strength. Volumes of the samples were then pooled according 5 band strength categories: 10 µl for the lowest band strength, then decreasing volumes of 8 µl, 6 µl, 4 µl, and 2 µl for increasing band strength. The library was then pooled and cleaned using AMPure XP beads following the recommended manufacturer's protocol (Agencourt AMPure XP, Beckman Coulter Inc. US). The library was run at a 12 pM concentration on an Illumina MiSeq, at the in-house facility at the University of Hull, using the 2 × 300 bp V3 chemistry.

Specimen confirmation – microscopy and standard DNA barcode sequencing:

Verification of the results from DNA metabarcoding was carried out using a combination of morphological identification and standard DNA barcoding (by Sanger sequencing).

Gammarus fossarum is a well-studied diverse species complex, which has three well established cryptic species

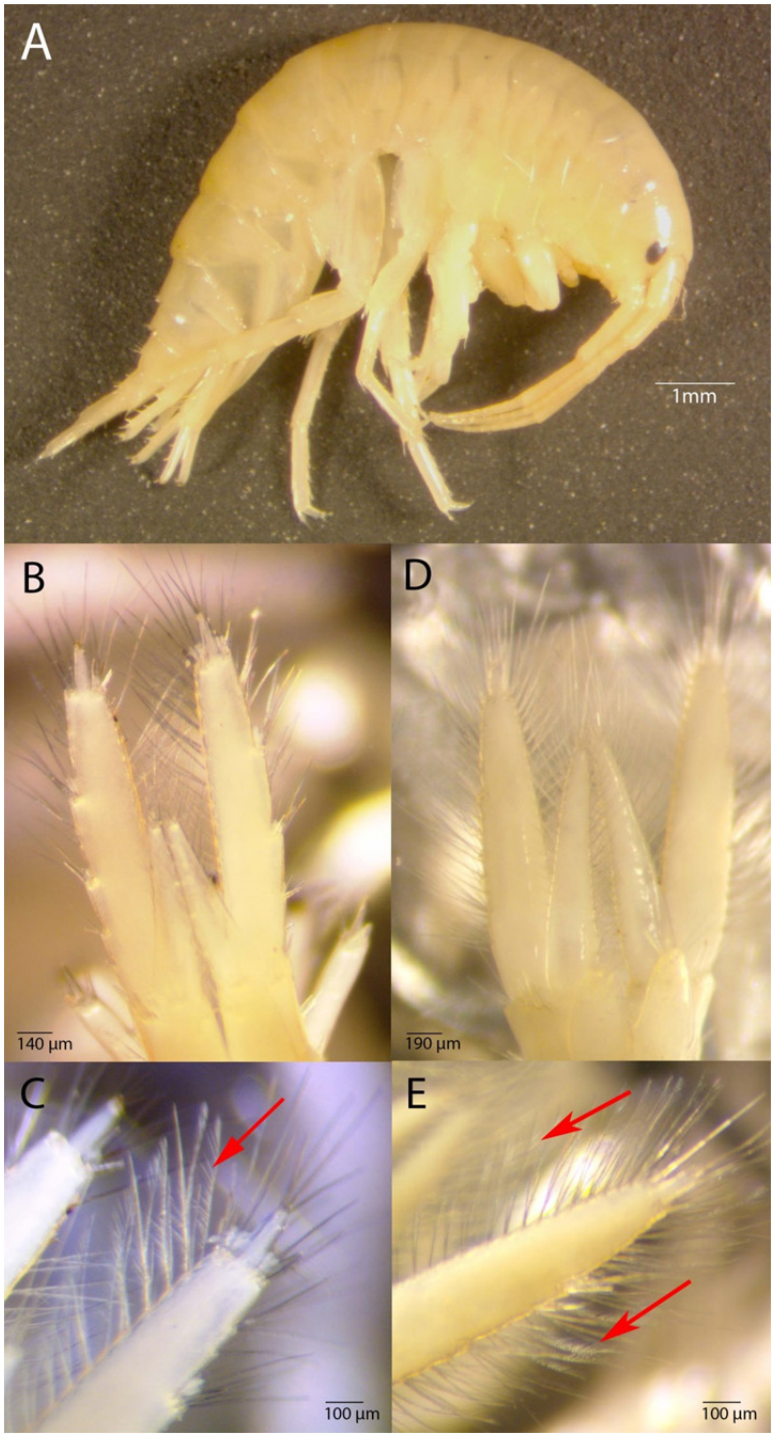


Figure 2. Picture of *Gammarus fossarum* found in the River Taff, UK, 7/6/2016, A) male adult specimen, B) male uropod III and C) male plumose hairs on inside of exopod of uropod III (↗); and picture of male *Gammarus pulex* features for comparison D) uropod III and E) plumose hairs on inner and outer edge of exopod of uropod III (↗) (Photographs by D. Constable).

(types A, B and C) with a further 36–53 different cryptic lineages being identified through phylogenetic studies (Weiss et al. 2014; Copilaş-Ciocianu and Petrusek 2015). Species within this complex are known to differ in their ecology both in terms of their environmental requirements and geographic distributions (Copilaş-Ciocianu and Petrusek 2015; Eisenring et al. 2016). The *G. fossarum* complex belongs to the *G. pulex*-group, which means it has small oval or kidney shaped eyes (less than twice as long as wide)

and the pereopods 5–7 are armed with spines and few setae (Pinkster 1972). Within the UK, these features alone would help to separate it from *G. duebeni*, *G. tigrinus* and *G. zaddachi*. It can be distinguished from all five known UK freshwater *Gammarus* residents by examining uropod III. In *G. fossarum* the ratio length of the endopod versus the exopod is about 0.5, whilst in the other five it is >0.5, typically 0.75 (see Figure 2B and 2D respectively). Another feature of *G. fossarum* is that only the inside margin of the exopod has plumose setae, whilst the other five have plumose setae on both inner and outer margins (see Figure 2C and 2E respectively). The latter feature should however be used with caution, as plumose setae on the outer margin of the exopod can show up in very old males of *G. fossarum* (Meijering 1972).

A *post hoc* morphological examination of UK *Gammarus* specimens was carried out to confirm the presence of *G. fossarum*. Since the entire macroinvertebrate samples from the original sampling program had been lysed for metabarcoding, new specimens were collected by hand net from two catchments where *G. fossarum* was detected by metabarcoding in close proximity to previously sampled sites; River Taff, Wales (n = 38) on 7/6/2016 and River Frome, England (n = 39) on 27/6/2016. Additional, archived specimens obtained from the Nailbourne (Little Stour catchment), England (n = 2) on 20/4/2013, were also analysed; (see Table 1 and Figure 1, Maps: E, F and G). Collected individuals were then subject to morphological examination and identified using Karaman and Pinkster (1977), Eggers and Martens (2001) and Piscart and Bollache (2012).

Microscopic identification was carried out on all specimens collected for morphological confirmation. Both *G. fossarum* (n = 37) and *G. pulex* (n = 1) were identified from individuals collected from the River Taff and only *G. fossarum* (n = 39) was found in a sample from the River Frome. Standard DNA barcoding was performed on some of the individuals identified morphologically as *G. fossarum* (n = 3) and *G. pulex* (n = 1) from the River Taff, and *G. fossarum* from the Nailbourne (Little Stour catchment) (n = 2). DNA was extracted using the DNeasy Blood & Tissue Kit® (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The full length *COI* DNA barcoding fragment was amplified (Folmer et al. 1994) using the following protocol: PCRs were performed in 25 µl volumes with MyTaq (Bioline, UK), 10 µM of each primer and 2 µl of DNA template. The PCR profile consisted of: initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 50 °C for 15 s and extension at 72 °C for 10 s, with a final extension time of 10 min at 72 °C. PCR products were checked on agarose gels and commercially sequenced using HCO2198 (Macrogen Europe, Amsterdam, Netherlands).

Bioinformatics

Processing of Illumina read data and taxonomic assignment were performed using a custom bioinformatics pipeline (metaBEAT, v.0.97.7-global; see Github reference 1) as described previously (Hänfling et al. 2016), with minor modifications. For each sample, raw Illumina sequences were filtered to retain only read pairs containing the expected forward/reverse in-line barcode combination (perfect matches only) using the program *process_shortreads* from the Stacks v1.20 program suite (Catchen et al. 2013) and subsequently quality trimmed using the program Trimmomatic v0.32 (Bolger et al. 2014). Specifically, read quality was assessed across 5 bp sliding windows starting from the 3'-end, and reads were clipped until the per window average read quality reached a minimum of phred 30. Any reads shorter than 100 bp after the quality clipping were discarded. To remove PCR primers and spacer sequences the first 30 bp of the reads was clipped off. Remaining sequence pairs were merged into single high quality reads using the program FLASH v1.2.11 (Magoč and Salzberg 2011). For any read pairs not merged successfully, only the forward read was retained for downstream analyses. Sequences were clustered at 97% identity using vsearch v1.1 (see Github reference 2). Any clusters represented by less than three sequences were excluded from further analyses, as these likely represent sequencing error. Each of the remaining distinct sequence clusters was collapsed to a single representative sequence (aka centroid). Only centroid sequences of the expected length as determined by the primers (313 bp ± 5%) were retained for downstream analyses. To obtain a final set of non-redundant (nr) queries for taxonomic assignment, centroid sequences across all samples were clustered globally at 97% identity using vsearch v1.1. The global set of nr queries was subjected to a BLAST (Zhang et al. 2000) search (blastn) against a custom reference database consisting of gammarid sequences from Weiss et al. (2014) and two *CO1* sequences from *T. cancriformis* (GenBank accession numbers EF189678.1 and JX110644.1) and *H. axyridis* (accession numbers KU188381.1 and KU188380.1), respectively. Taxonomic assignment was performed using a lowest common ancestor (LCA) approach. In brief, after the BLAST search the algorithm identifies the most significant matches

to the reference database (top 10% bit-scores) for each of the query sequences. If only a single taxon is present in this list of matches then the query is assigned directly to this taxon. If more than one taxon is present, the query is assigned to the lowest taxonomic level that is shared by all taxa in the list. Queries yielding best BLAST matches below a bit-score of 80 or with less than 85% identity were binned as "unassigned". To assure full reproducibility of our analyses we have deposited the entire workflow in an additional dedicated Github repository (see Github reference 3). To reduce the possibility of false positives based on our single species positive samples and in order to obtain a conservative estimate of the distribution of *G. fossarum* in the UK, we only report *G. fossarum* as present at a given site if it was supported by at least 1% of the total quality trimmed reads per sample.

Phylogeny

Phylogenetic analysis was performed to further confirm the identity of the putative *Gammarus* sp. sequences obtained as part of the current study. We downloaded a previously published *CO1* dataset (Weiss et al. 2014; Copilaş-Ciocianu and Petrusek 2015) from Genbank, comprising 89 sequences of *G. fossarum*, six *G. pulex* (Linnaeus, 1758) sequences and a single sequence each from four further outgroup species (*G. balcanicus* (Schaferna, 1922), *G. glabratus* (Hou and Li, 2003), *G. roeselii* (Gervais, 1835) and *G. tigrinus* (Sexton, 1939) (Radulovici et al. 2009; Hou et al. 2011; Feckler et al. 2012; Weiss et al. 2014). This set of previously published sequences was extended by the sequences obtained via standard full-length DNA barcoding and mini-barcode metabarcoding. Prior to phylogenetic analysis we extracted the most abundant sequence, i.e. haplotype, from each sample from the initially obtained 97% sequence clusters assigned to *G. fossarum* and *G. pulex*, respectively. Nucleotide sequences of *G. fossarum* and *G. pulex* used in the phylogenetic analysis were deposited in Genbank (GenBank accession KY464959–KY464977). Phylogenetic analysis was performed in the Reprophylo environment (Szitenberg et al. 2015). In brief, sequences were aligned using the program MAFFT v7.123b (Katoh and Standley 2013) and the alignment was trimmed using the program trimAl v1.2rev59 (Capella-Gutiérrez et al. 2009). Maximum-likelihood tree inference was performed using RAxML v8.0.12 (Stamatakis 2014). The full, detailed analysis is provided as Jupyter notebook in the dedicated Github repository (Github reference 3), which also contains the alignment underlying the phylogenetic tree and further supplementary information.

Comparison of data from eDNA/DNA and microscopy analysis

A correlation was performed to compare the Gammaridae abundance data generated from the kick sample microscopy analysis and the DNA/eDNA metabarcoding. Specifically, the relationship between DNA/eDNA data (read count) and data from microscopy analysis (biomass calculated from average Gammaridae specimen weight) was investigated by calculating Pearson's Correlation Coefficient in R v3.1.3 (R Core team 2013). Note that *G. fossarum* and *G. pulex* sequencing data have been combined here as the species were not distinguished during the initial morphological determination.

Results

Metabarcoding survey

The total sequence read count passing quality control, before removal of chimeric sequences, was 4,290,271. We quantified the level of possible contamination using sequence information from single species positive samples, which enabled us to choose a suitable threshold level (1% of total sample reads) for filtering and removal of low level contamination. This conservative threshold is comparable to recent, similar studies (e.g. Hänfling et al. 2016; Port et al. 2016). After applying this threshold, over the 195 samples the total read count was 933,457.

Gammarus fossarum was detected in 28 sites in total: 25 via metabarcoding, 1 site by morphological identification, 1 site by standard DNA barcoding and 1 site by morphological identification and DNA barcoding (See Table 1 and Supplementary material Table S1). Of the 25 metabarcoding samples, *G. fossarum* was found in: 25 DNA macroinvertebrate samples, 8 water eDNA samples and 9 sediment eDNA samples. *G. pulex* was detected in 27 of the sites in the metabarcoding DNA macroinvertebrate samples only and a single site using Sanger sequencing.

A full breakdown of gammarid sequences per sample and proportion of gammarid biomass per sample are included in Supplementary material Table S1. A further 36 freshwater macroinvertebrate families were detected by metabarcoding: data from these non-gammarid species form part of a wider macroinvertebrate data set which is being analysed separately and will be published elsewhere.

The average read count of the samples with gammarid species present was 3512. At those sites the proportion of *G. fossarum* reads per sample ranged from 1.68 – 100% in the macroinvertebrate DNA,

Table 1. Specimen identification and identification method for morphologically identified and DNA barcoded specimens. (*Specimens collected from the River Frome were subject to morphological identification only. **Specimens collected from Nailbourne were DNA sequenced only due to damaged specimens).

Unique ID	Catchment	Site Name	Coordinates		*G. fossarum*		*G. pulex*	
			Lat	Long	Micro-scopy	DNA sequen-cing	Micro-scopy	DNA sequen-cing
DC003	Taff	Forest Farm Country Park	51.516	−3.242	✓	✓		
DC004	Taff	Forest Farm Country Park	51.516	−3.242			✓	✓
DC005	Taff	Forest Farm Country Park	51.516	−3.242	✓	✓		
DC006	Taff	Forest Farm Country Park	51.516	−3.242	✓	✓		
DC007-045	Frome	East Stoke	50.681	−2.185	✓*			
JD001	Nailbourne	Adj Saint Ethelburga well	51.126	1.087		✓**		
JD002	Nailbourne	Adj Saint Ethelburga well	51.126	1.087		✓**		

1.67 – 55.35% in the water eDNA and 1.59 – 18.05% in sediment eDNA samples (Table S1). Similarly, *G. pulex* reads ranged from 1.65 – 97.41% in the DNA macroinvertebrate samples. There was a significant positive correlation between the percentage of *Gammarus* biomass in the sample, and the percentage of *Gammarus* sequence reads (Pearson's $r = 0.747$, df = 46, $P = 1.098 \times 10^{-9}$, Supplementary material Figure S1). Importantly, *Gammarus* sequences were detected when gammarids constituted as little as 2.6% of the total biomass (Table S1).

Verification of Gammarus fossarum by microscopy

Gammarus fossarum was not identified morphologically in any samples surveyed in March 2015 prior to metabarcoding. Of the 38 gammarid specimens recovered from the River Taff on 7/6/2016, 37 *G. fossarum* morphological identifications were made. Adult males ranged between 8–12 mm (n = 21) and adult females 7–10 mm (n = 15). Four females were ovigerous. The other gammarid specimen encountered was a male *G. pulex* (13 mm). Of the 39 gammarid specimens collected from the River Frome on 27/6/2016, all were identified as *G. fossarum* morphologically. Adult males of this population ranged from 8–11.5 mm (n = 24) and adult females 7–9 mm (n = 15). Again, four ovigerous females were recorded. The relative abundance of size distribution in the two sampled populations can be seen in the Supplementary information (Figure S2). The two individuals collected from the Nailbourne on 20/4/2013 were not verified using microscopy as the specimens were too heavily damaged for morphological identification.

The size ranges encountered for *G. fossarum* fall within the expected range for the species, with Goedmakers (1972), Pinkster (1972), Karaman and Pinkster (1977) and Piscart and Bollache (2012) reporting that the largest males typically reach 14–15 mm.

Verification of Gammarus fossarum by DNA barcoding

Morphological identifications were confirmed by DNA sequencing for specimens collected from the River Taff (n = 4): 3 specimens of *G. fossarum* and a single *G. pulex*. The individuals collected from the Nailbourne (n = 2) were also both identified as *G. fossarum* using subsequent DNA barcoding (see Table 1).

Phylogeny

The phylogeny (Figure 3) is congruent with the findings of the morphological identification. The *G.* cf. *fossarum* and *G.* cf. *pulex* sequences cluster with their respective lineages (identified in Weiss et al. 2014; Copilaş-Ciocianu and Petrusek 2015). *Gammarus fossarum* sequences obtained by both metabarcoding and standard DNA barcoding show little divergence and cluster together in the phylogeny, indicating closely related sequences. The *G. fossarum* sequences obtained in the current study group with high statistical support within Clade 11, as defined using the distance based Automatic Barcode Gap Discovery (ABGD) approach in Weiss et al. (2014). Sequences further group in a subclade with samples from southwestern Germany, Southern Black Forest and Eastern Sauerland in Germany, i.e. clade 14, as delineated using the tree-based GMYC in Weiss et al. (2014). Aligning the UK *G. fossarum* specimens within Clade 11 confirms previous studies which show this clade to be the most widely distributed across Europe within the species complex (Copilaş-Ciocianu and Petrusek 2015; Weiss and Leese 2016).

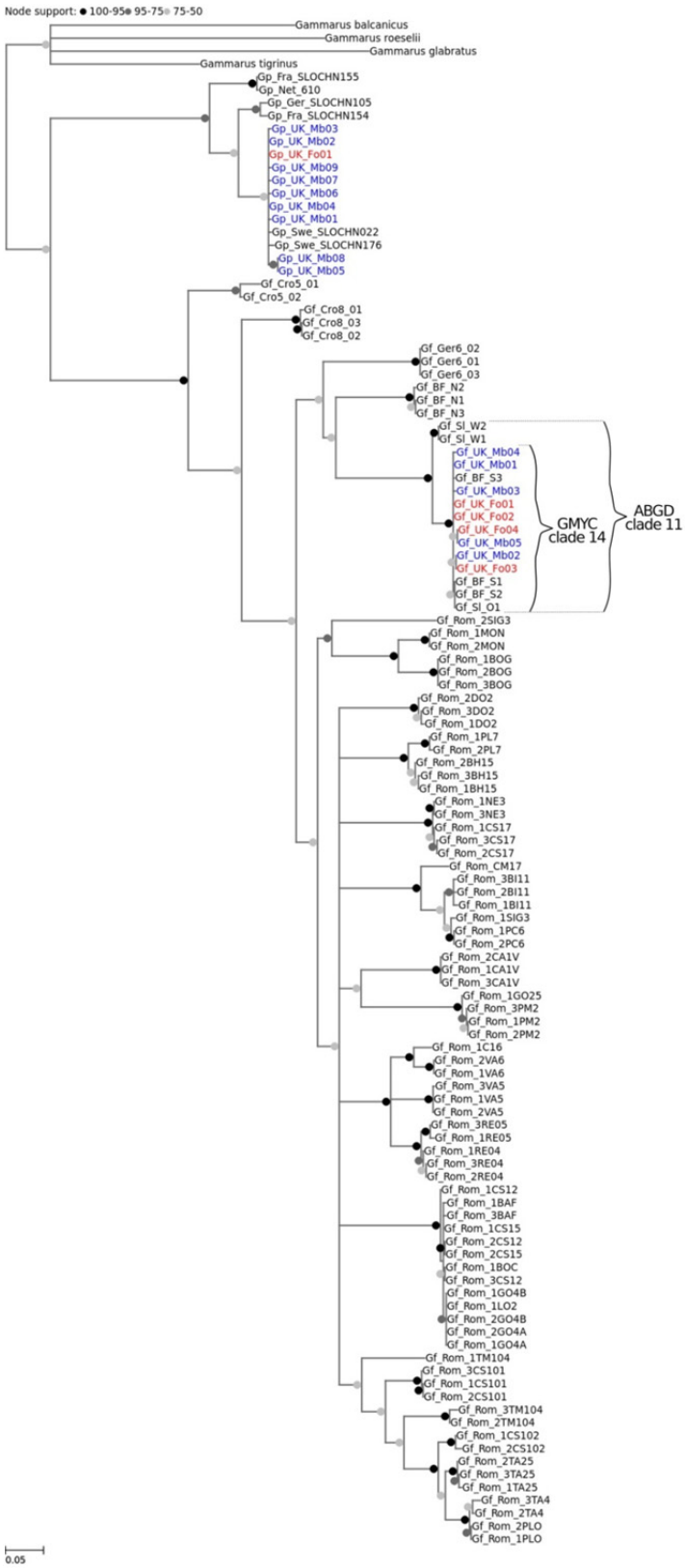


Figure 3. Maximum likelihood phylogenetic tree for the *COI* gene – based on sequences obtained from previously published and newly obtained Gammaridae sequences. The mini-barcode (metabarcoding) and standard *COI* barcode sequences from this study are represented in blue and red, respectively. (See supplementary material Table S2, for accession numbers and origin of individual sequences). GMYC – General Mixed Yule Coalescent, ABGD – Automatic Barcode Gap Discovery (Puillandre et al. 2011) indicate the approaches used by Weiss et al (2014) to detect the different clades in their study.

Discussion

Non-targeted detection by direct and environmental DNA metabarcoding has the potential to revolutionise early warning systems for non-native species, but this utility of the new technology has so far been demonstrated only a limited number of times (Mahon et al. 2014; Brown et al. 2016). In this study, *G. fossarum,* a newly recognised non-native species for the UK, was detected during the course of a wider metabarcoding survey of macroinvertebrate communities. The identification of *G. fossarum* was subsequently confirmed by microscopy and standard DNA barcoding. The sequences generated from this study indicate that the UK populations of *G. fossarum* sampled here fall within the previously identified Clade 11, *sensu* Weiss et al. (2014), of this highly diverse species complex (Figure 3). Importantly this is the most widely distributed clade within the *G. fossarum* complex (Weiss et al. 2014; Copilaş-Ciocianu and Petrusek 2015; Weiss and Leese 2016).

Gammarus fossarum was found in seven distant river catchments within the UK, indicating a widespread distribution (Figure 1). Initial detection of *G. fossarum* was made using non-targeted metabarcoding of macroinvertebrate DNA, water eDNA and sediment eDNA samples. Of the sites where *G. fossarum* was detected using this method (n = 25), *G. fossarum* was detected in all 25 DNA macroinvertebrate samples (100%), in 8 of water (32%) and 9 sediment (36%) samples. The lower detection of *G. fossarum* in eDNA samples compared to macroinvertebrate samples is not surprising due to the dilution of eDNA and effects of flow on DNA availability in lotic systems.

At 23 of the 28 sites (including *post hoc* samples) where *G. fossarum* was present it was the only Gammaridae species detected. This suggests it is not only widespread in the UK but could also be the dominant gammarid in some locations, possibly even having displaced the native *G. pulex* locally. With the new species discovery, recent re-examination of historical archived gammarid samples was undertaken from available Environment Agency and Natural History Museum (NHM), London, collections. Material from the Environment Agency had overlooked records of *G. fossarum* dating back to 2005 from the River Len, Maidstone, Kent (51.2619ºN; 0.56451ºE) whilst re-examination of material from the NHM revealed the earliest record to date, 1964 from the River Darent, Kent. This shows that *G. fossarum* has remained undetected and overlooked by conventional means for a substantial length of time.

Gammarus fossarum is indigenous and widespread in mainland Europe, and typically inhabits springs and upper reaches of mountainous streams, with *G. pulex* being more dominant in lower river sections (Nijssen 1963; Goedmakers 1972; Karaman and Pinkster 1977; Chen et al. 2012). This distribution pattern is linked to *G. fossarum*'s comparative preference for shallower streams and higher current velocities, and its reduced tolerance of low dissolved oxygen conditions (Meijering 1971; Peeters and Gardeniers 1998). It may also be found in middle sections of rivers and is able to coexist with *G. pulex* (Janetzky 1994; Piscart and Bollache 2012; Copilaş-Ciocianu et al. 2014). In such areas of coexistence, *G. fossarum* will often occupy faster flowing areas where vegetation is sparse or absent, and *G. pulex* will be found near marginal shore zones, with reduced currents and rich vegetation growth (Karaman and Pinkster 1977). The distributions of *G. fossarum* in this study covered a range of habitats, mainly lowland rivers (altitude <90 m) with the exception of the Nailbourne spring, adjacent to Saint Ethelburga Well and Maiden Newton on the Upper Frome, with altitudes of 106 m and 109 m, respectively (see Supplementary information). The river depths at *G. fossarum* locations were shallow, seldom reaching more than 20 cm. It is important that further exploration of UK upland systems is undertaken as the sites surveyed for this study were mostly lowland, and at this stage are an indication of habitat suitability rather than preference for *G. fossarum* in the UK. Of our five study sites where *G. fossarum* and *G. pulex* coexisted, all had a mean depth >20 cm and featured both fast and slow currents as well as vegetative marginal areas, however there appears to be no other pattern in the distribution of sites where both species were found to co-exist. Four of the five sites were from the metabarcoding samples, the percentage read count for both species varied substantially, hence no species dominance can be inferred from this data (see Supplementary material Table S1).

Gammarus fossarum is the third non-native freshwater gammarid to be found in the UK within the last six years, following the discoveries of *Dikerogammarus villosus* in 2010 (MacNeil et al. 2010) and *Dikerogammarus haemobaphes* in 2012 (Aldridge 2013). The record is rather unforeseen and the species has not been included on the UK's non-native species watch list with more focus being placed on Ponto-Caspian species that have invaded western Europe (Gallardo and Aldridge 2015). A detailed risk assessment of the threat that *G. fossarum* poses to native Gammaridae within the UK does not currently exist; further research into how *G.*

pulex and *G. fossarum* co-exist within UK habitats should be carried out to decide if this action is warranted. However, the importance of this discovery as a new non-native species to the UK should not be overlooked as it has important implications for future ecological assessments.

In conclusion, we detected a newly recognised non-native species to UK fauna using non-targeted DNA metabarcoding, and confirmed its presence using microscopy and standard DNA barcoding. It is well known that the effectiveness of INNS control or management relies heavily upon early detection (Lodge et al. 2006; Vander Zanden et al. 2010). In future, for other species, non-targeted monitoring of high risk invasion pathways using eDNA may ensure that early eradication or containment are possible management options (Davis 2009; Hulme 2009; Jerde et al. 2011; Thomsen et al. 2012; Lawson Handley 2015). It is important that future research should now focus on establishing the true distribution, ecology and potential implications of *G. fossarum* within the UK, as well as exploring how the non-targeted eDNA metabarcoding approach can be used to detect non-native species.

Acknowledgements

This work was funded by the UK Environment Agency. We are particularly grateful to Dr Kerry Walsh and Dr Alice Hiley for initiating the metabarcoding study and for support throughout. We gratefully acknowledge Amanda Arnold, Frances Attwood, Charlotte Davey, Carole Fitzpatrick, Tim Jones, Mel Lacan, Ellen Moss and Paul Nichols for providing invaluable help during field work, Julia Day, Robert Donnelly, Helen Kimbell, James Kitson and Graham Sellers for contributing to laboratory work and Miranda Lowe and Ben Price from the Natural History Museum, London and Susan Skipp from the Environment Agency for allowing access to archive material. We would like to thank F. Leese and anonymous reviewers for their constructive criticism on the initial submission which greatly helped strengthen the manuscript. The views expressed in this paper are our own, and not necessarily of the institutions we represent.

References

Aldridge DC (2013) GB non-native organism rapid risk assessment for *Dikerogammarus haemobaphes* (Eichwald, 1841). http://www.nonnativespecies.org/downloadDocument.cfm?id=870 (accessed 12 August 2016)

Bacela-Spychalska K, Wattier RA, Genton C, Rigaud T (2012) Microsporidian disease of the invasive amphipod *Dikerogammarus villosus* and the potential for its transfer to local invertebrate fauna. *Biological Invasions* 14: 1831–1842, https://doi.org/10.1007/s10530-012-0193-1

Bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1159–1174, https://doi.org/10.1139/f02-098

Bivand RS, Pebesma EJ, Gomez-Rubio V (2013) Applied spatial data analysis with R, Second edition. Springer, NY. http://www.asdar-book.org

Bivand RS, Keitt T, Rowlingson B (2016) rgdal: Bindings for the Geospatial Data Abstraction Library. R package version 1.1-10. https://CRAN.R-project.org/package=rgdal

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A Flexible Trimmer for Illumina Sequence Data. *Bioinformatics* 30: 2114–2120, https://doi.org/10.1093/bioinformatics/btu170

Brown EA, Chain FJJ, Zhan A, Maclsaac HJ, Cristescu ME (2016) Early detection of aquatic invaders using metabarcoding reveals a high number of non-indigenous species in Canadian ports. *Diversity and Distributions* 22: 1045–1059, https://doi.org/10.1111/ddi.12465

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: A Tool for Automated Alignment Trimming in Large-Scale Phylogenetic Analyses. *Bioinformatics* 25: 1972–1973, https://doi.org/10.1093/bioinformatics/btp348

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: An Analysis Tool Set for Population Genomics. *Molecular Ecology* 22: 3124–3140, https://doi.org/10.1111/mec.12354

Chen W, Bierbach D, Plath M, Streit B, Klaus S (2012) Distribution of amphipod communities in the Middle to Upper Rhine and five of its tributaries. *BioInvasions Records* 1: 263–271, https://doi.org/10.3391/bir.2012.1.4.04

Constable D, Birkby NJ (2016) The impact of the invasive amphipod *Dikerogammarus haemobaphes* on leaf litter processing in UK rivers. *Aquatic Ecology* 50: 273–281, https://doi.org/10.1007/s10452-016-9574-3

Copilaş-Ciocianu D, Grabowski M, Pârvulescu L, Petrusek A (2014) Zoogeography of epigean freshwater Amphipoda (Crustacea) in Romania: fragmented distributions and wide altitudinal variability. *Zootaxa* 3893: 243–260, https://doi.org/10.11646/zootaxa.3893.2.5

Copilaş-Ciocianu D, Petrusek A (2015) The Southwestern Carpathians as an Ancient Centre of Diversity of Freshwater Gammarid Amphipods: Insights from the *Gammarus fossarum* Species Complex. *Molecular Ecology* 24: 3980–3992, https://doi.org/10.1111/mec.13286

Davis MA (2009) Invasion Biology. Oxford University Press, Oxford, UK, 288 pp

Darling JA, Mahon AR (2011) From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environmental Research* 111: 978–988, https://doi.org/10.1016/j.envres.2011.02.001

Deiner K, Fronhofer EA, Mächler E, Walse JC, Altermatt F (2016) Environmental DNA reveals that rivers are conveyer belts of biodiversity information. *Nature Communications* 7: 12544, https://doi.org/10.1038/ncomms12544

Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E, Miaud C (2012) Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* 49: 953–959, https://doi.org/10.1111/j.1365-2664.2012.02171.x

Dick JTA, Platvoet D (2000) Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proceedings of the Royal Society B: Biological Sciences* 267: 977–983, https://doi.org/10.1098/rspb.2000.1099

Dougherty MM, Larson ER, Renshaw MA, Gantz CA, Egan SP, Erickson DM, Lodge DM (2016) Environmental DNA (eDNA) detects the invasive rusty crayfish *Orconectes rusticus* at low abundances. *Journal of Applied Ecology* 53: 722–732, https://doi.org/10.1111/1365-2664.12621

Eggers TO, Martens A (2001) A key to the freshwater Amphipoda (Crustacea) of Germany. *Lauterbornia* 55: 1–68

Eisenring M, Altermatt F, Westram AM, Jokela J (2016) Habitat Requirements and Ecological Niche of Two Cryptic Amphipod Species at Landscape and Local Scales. *Ecosphere* 7: e01319, https://doi.org/10.1002/ecs2.1319

Feckler A, Thielsch A, Schwenk K, Schulz R, Bundschuh M (2012) Differences in the Sensitivity among Cryptic Lineages of the *Gammarus fossarum* Complex. *The Science of the Total Environment* 439: 158–164, https://doi.org/10.1016/j.scitotenv.2012.09.003

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA Primers for Amplification of Mitochondrial Cytochrome c Oxidase Subunit I from Diverse Metazoan Invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299

Furse MT, Wright PD, Armitage I, Moss D (1981) An appraisal of pond net samples for biological monitoring of lotic macro-invertebrates. *Water Research* 15: 679–689, https://doi.org/10.1016/0043-1354(81)90160-3

Gallardo B, Aldridge DC (2015) Is Great Britain heading for a Ponto-Caspian invasional meltdown? *Journal of Applied Ecology* 52: 41–49, https://doi.org/10.1111/1365-2664.12348

Gallic E (2016) LegendMap. https://github.com/3wen/legendMap

Goedmakers A (1972) *Gammarus fossarum* Koch, 1835: Redescription based on neotype material and notes on its local variation (Crustacea, Amphipoda). *Bijdragen Tot de Dierkunde* 42: 124–138

Grabowski M, Jazdzewski K, Konopacka A (2007) Alien crustacea in Polish waters – Amphipoda. *Aquatic Invasions* 2: 25–38, https://doi.org/10.3391/ai.2007.2.1.3

Hänfling B, Handley LL, Read DS, Hahn C, Li J, Nichols P, Blackman RC, Oliver A, Winfield IJ (2016) Environmental DNA Metabarcoding of Lake Fish Communities Reflects Long-Term Data from Established Survey Methods. *Molecular Ecology* 25: 3101–3119, https://doi.org/10.1111/mec.13660

Hou Z, Sket B, Fiser C, Li S (2011) Eocene Habitat Shift from Saline to Freshwater Promoted Tethyan Amphipod Diversification. *Proceedings of the National Academy of Sciences of the United States of America* 108: 14533–14538, https://doi.org/10.1073/pnas.1104636108

Hulme PE (2009) Trade, Transport and Trouble: Managing Invasive Species Pathways in an Era of Globalization. *The Journal of Applied Ecology* 46: 10–18, https://doi.org/10.1111/j.1365-2664.2008.01600.x

Illumina (2011) Preapring 16S Ribosomal RNA Gene Amplicons for Illumina MiSeq system. Illumina technical note

Janetzky W (1994) Distribution of the genus *Gammarus* (Amphipoda: Gammaridae) in the River Hunte and its tributaries (Lower Saxony, northern Germany). *Hydrobiologia* 294: 23–34, https://doi.org/10.1007/BF00017622

Jerde CL, Mahon AR, Chadderton WL, Lodge DM (2011) "Sight-Unseen" Detection of Rare Aquatic Species Using Environmental DNA. *Conservation Letters* 4: 150–157, https://doi.org/10.1111/j.1755-263X.2010.00158.x

Karaman GS, Pinkster S (1977) Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea, Amphipoda). Part I. *Gammarus pulex*-group and related species. *Bijdragen Tot de Dierkunde* 47: 1–97

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* 30: 772–780, https://doi.org/10.1093/molbev/mst010

Kinzler W, Kley A, Mayer G, Waloszek D, Maier G (2009) Mutual predation between and cannibalism within several freshwater gammarids: *Dikerogammarus villosus* versus one native and three invasives. *Aquatic Ecology* 43: 457–464, https://doi.org/10.1007/s10452-008-9206-7

Kitson JJN, Hahn C, Sands RJ, Straw NA, Evans DM, Lunt DH (2015) Nested metabarcode tagging: a robust tool for studying species interactions in ecology and evolution. *bioRxiv* 035071, https://doi.org/10.1101/035071

Lawson Handley L (2015) How will the "molecular revolution" contribute to biological recording? *Biological Journal of the Linnean Society* 115: 750–766, https://doi.org/10.1111/bij.12516

Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ (2013) A New Versatile Primer Set Targeting a Short Fragment of the Mitochondrial COI Region for Metabarcoding Metazoan Diversity: Application for Characterizing Coral Reef Fish Gut Contents. *Frontiers in Zoology* 10: 34, https://doi.org/10.1186/1742-9994-10-34

Lodge DM, Williams S, MacIsaac HJ, Hayes KR, Leung B, Reichard S, Mack RN, Moyle PB, Smith M, Andow DA, Carlton JT, McMichael A (2006) Biological Invasions: Recommendations for U.S. Policy and Management. *Ecological Applications* 16: 2035–2054, https://doi.org/10.1890/1051-0761(2006)016[2035:BIRFUP]2.0.CO;2

MacNeil C, Platvoet D (2005) The predatory impact of the freshwater invader *Dikerogammarus villosus* on native *Gammarus pulex* (Crustacea: Amphipoda); influences of differential microdistribution and food resources. *Journal of Zoology* 267: 31–38, https://doi.org/10.1017/S0952836905007351

MacNeil C, Platvoet D, Dick JTA, Fielding N, Constable A, Hall N, Aldridge D, Renals T, Diamond M (2010) The Ponto-Caspian "killer shrimp", *Dikerogammarus villosus* (Sowinsky, 1894), invades the British Isles. *Aquatic Invasions* 5: 441–445, https://doi.org/10.3391/ai.2010.5.4.15

MacNeil C, Dick JTA, Platvoet D, Briffa M (2011) Direct and indirect effects of species displacements: an invading freshwater amphipod can disrupt leaf-litter processing and shredder efficiency. *Journal of the North American Benthological Society* 30: 38–48, https://doi.org/10.1899/10-056.1

Magoč T, Salzberg SL (2011) FLASH: Fast Length Adjustment of Short Reads to Improve Genome Assemblies. *Bioinformatics* 27: 2957–2963, https://doi.org/10.1093/bioinformatics/btr507

Mahon AR, Nathan LR, Jerde CL (2014) Meta-Genomic Surveillance of Invasive Species in the Bait Trade. *Conservation Genetics Resources* 6: 563–567, https://doi.org/10.1007/s12686-014-0213-9

Mahon AR, Jerde CL (2016) Using Environmental DNA for Invasive Species Surveillance and Monitoring. *Marine Genomics* 1452: 131–142

Meijering MPD (1971) Die *Gammarus*-Fauna der Schlitzerländer Fließgewässer. *Archiv für Hydrobiologie* 68: 575–608

Meijering MPD (1972) Physiologische Beiträge zur Frage der systematischen Stellung von *Gammarus pulex* (L.) und *Gammarus fossarum* Koch (Amphipoda). *Crustaceana* Suppl., 3: 313–325

Murray-Bligh JAD (1999) Procedure for Collecting and Analysing Macroinvertebrate Samples, Quality Management Systems for Environmental Monitoring: Biological Techniques BT001 Version 2.0. Environment Agency, Bristol, UK, pp 40–45

Nijssen H (1963) Some notes on the distribution and the ecology of the amphipod *Gammarus fossarum* Koch, 1835, in the Netherlands (Crustacea, Malacostraca). *Beaufortia* 10: 40–43

Peeters ETHM, Gardeniers JJP (1998) Logistic regression as a tool for defining habitat requirements of two common gammarids. *Freshwater Biology* 39: 605–615, https://doi.org/10.1046/j.1365-2427.1998.00304.x

Pebesma EJ, Bivand RS (2005) Classes and methods for spatial data in R. R News 5(2). http://cran.r-project.org/doc/Rnews/

Pinkster S (1972) On members of the *Gammarus pulex*-group (Crustacea-Amphipoda) from western Europe. *Bijdragen Tot de Dierkunde* 42: 164–191

Piscart C, Bollache L (2012) Crustacés amphipodes de surface (Gammare d'eau douce). Association Française de Limnologie, Thonon les Bains, 113 pp

Piscart C, Mermillod-Blondin F, Maazouzi C, Merigoux S, Marmonier P (2011) Potential impact of invasive amphipods on leaf litter recycling in aquatic ecosystems. *Biological Invasions* 13: 2861–2868, https://doi.org/10.1007/s10530-011-9969-y

Port JA, O'Donnell JL, Romero-Maraccini OC, Leary PR, Litvin SY, Nickols KJ, Yamahara KM, Kelly RP (2016) Assessing Vertebrate Biodiversity in a Kelp Forest Ecosystem Using Environmental DNA. *Molecular Ecology* 25: 527–541, https://doi.org/10.1111/mec.13481

Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, automatic barcode gap discovery for primary species delimitation. *Molecular Ecology* 21: 1864–1877, https://doi.org/10.1111/j.1365-294X.2011.05239.x

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Radulovici AE, Sainte-Marie B, Dufresne F (2009) DNA Barcoding of Marine Crustaceans from the Estuary and Gulf of St Lawrence: A Regional-Scale Approach. *Molecular Ecology Resources* 9: 181–187, https://doi.org/10.1111/j.1755-0998.2009.02643.x

Rees HC, Maddison BC, Middleditch DJ, Patmore JR, Gough KC (2014) The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology* 51: 1450–1459, https://doi.org/10.1111/1365-2664.12306

Roy HE, Peyton J, Aldridge DC, Bantock T, Blackburn TM, Britton R, Clark P, Cook E, Dehnen-Schmutz K, Dines T, Dobson M, Edwards F, Harrower C, Harvey MC, Minchin D, Noble DG, Parrott D, Pocock MJO, Preston CD, Roy S, Salisbury A, Schönrogge K, Sewell J, Shaw RH, Stebbing P, Stewart AJA, Walker KJ (2014) Horizon Scanning for Invasive Alien Species with the Potential to Threaten Biodiversity in Great Britain. *Global Change Biology* 20: 3859–3871, https://doi.org/10.1111/gcb.12603

Simmons M, Tucker AJ, Chadderton WJ, Mahon AR (2015) Active and passive environmental DNA surveillance of aquatic invasive species *Canadian Journal of Fisheries and Aquatic Sciences* 73: 76–83, https://doi.org/10.1139/cjfas-2015-0262

Stamatakis A (2014) RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* 30: 1312–1313, https://doi.org/10.1093/bioinformatics/btu033

Szitenberg AMJ, Blaxter ML, Lunt DH (2015) ReproPhylo: An Environment for Reproducible Phylogenomics. *PLoS Computational Biology* 11: e1004447, https://doi.org/10.1371/journal.pcbi.1004447

Taberlet P, Coissac E, Pompanon F, Brochmann C, Willerslev E (2012a) Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* 21: 2045–2050, https://doi.org/10.1111/j.1365-294X.2012.05470.x

Taberlet P, Coissac E, Hajiabaei M, Rieseberg LH (2012b) Environmental DNA. *Molecular Ecology* 21: 1789–1793, https://doi.org/10.1111/j.1365-294X.2012.05542.x

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E (2012) Monitoring Endangered Freshwater Biodiversity Using Environmental DNA. *Molecular Ecology* 21: 2565–2573, https://doi.org/10.1111/j.1365-294X.2011.05418.x

Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF, Bellemain E, Besnard A, Coissac E, Boyer F, Gaboriaud C, Jean P, Poulet N, Roset N, Copp GH, Geniez P, Pont D, Argillier C, Baudoin JM, Peroux T, Crivelli AJ, Olivier A, Acqueberge M, Le Brun M, Møller PR, Willerslev E, Dejean T (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology* 25: 929–942, https://doi.org/10.1111/mec.13428

Vander Zanden MJ, Hansen GJ, Higgins SN, Kornis MS (2010) A pound of prevention, plus a pound of cure: early detection and eradication of invasive species in the Laurentian Great Lakes. *Journal of Great Lakes Research* 36: 199–205, https://doi.org/10.1016/j.jglr.2009.11.002

Weiss M, Leese F (2016) Widely Distributed and Regionally Isolated! Drivers of Genetic Structure in *Gammarus fossarum* in a Human-Impacted Landscape. *BMC Evolutionary Biology* 16 (July): 153, https://doi.org/10.1186/s12862-016-0723-z

Weiss M, Macher JN, Seefeldt MA, Leese F (2014) Molecular Evidence for Further Overlooked Species within the *Gammarus fossarum*. *Hydrobiologia* 721: 165–184, https://doi.org/10.1007/s10750-013-1658-7

Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A Greedy Algorithm for Aligning DNA Sequences. *Journal of Computational Biology: A Journal of Computational Molecular Cell Biology* 7: 203–214, https://doi.org/10.1089/10665270050081478

Github References:

Github reference 1: https://github.com/HullUni-bioinformatics/metaBEAT; v.0.97.7-global

Github reference 2: https://github.com/torognes/vsearch

Github reference 3: https://github.com/HullUni-bioinformatics/Blackman_et_al_Gfossarum_UK. DOI: https://doi.org/10.5281/zenodo.495075

Supplementary material

The following supplementary material is available for this article:

Table S1. Specimen identification, identification method and site information for metabarcoding samples.

Table S2. Information on specimens from own and published studies that were used in the phylogenetic tree.

This material is available as part of online article from:

http://www.aquaticinvasions.net/2017/Supplements/AI_2017_Blackman_etal_SupplementaryTables.xls

Figure S1. Correlation of the % *Gammarus* biomass in the sample, and the percentage *Gammarus* sequence reads.

Figure S2. Frequency distribution of body length of male and female *Gammarus fossarum* individuals collected from the River Taff and the River Frome.

This material is available as part of online article from:

http://www.aquaticinvasions.net/2017/Supplements/AI_2017_Blackman_etal_SupplementaryFigures.pdf