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An Ghníomhaireacht um Chaomhnú Comhshaoil

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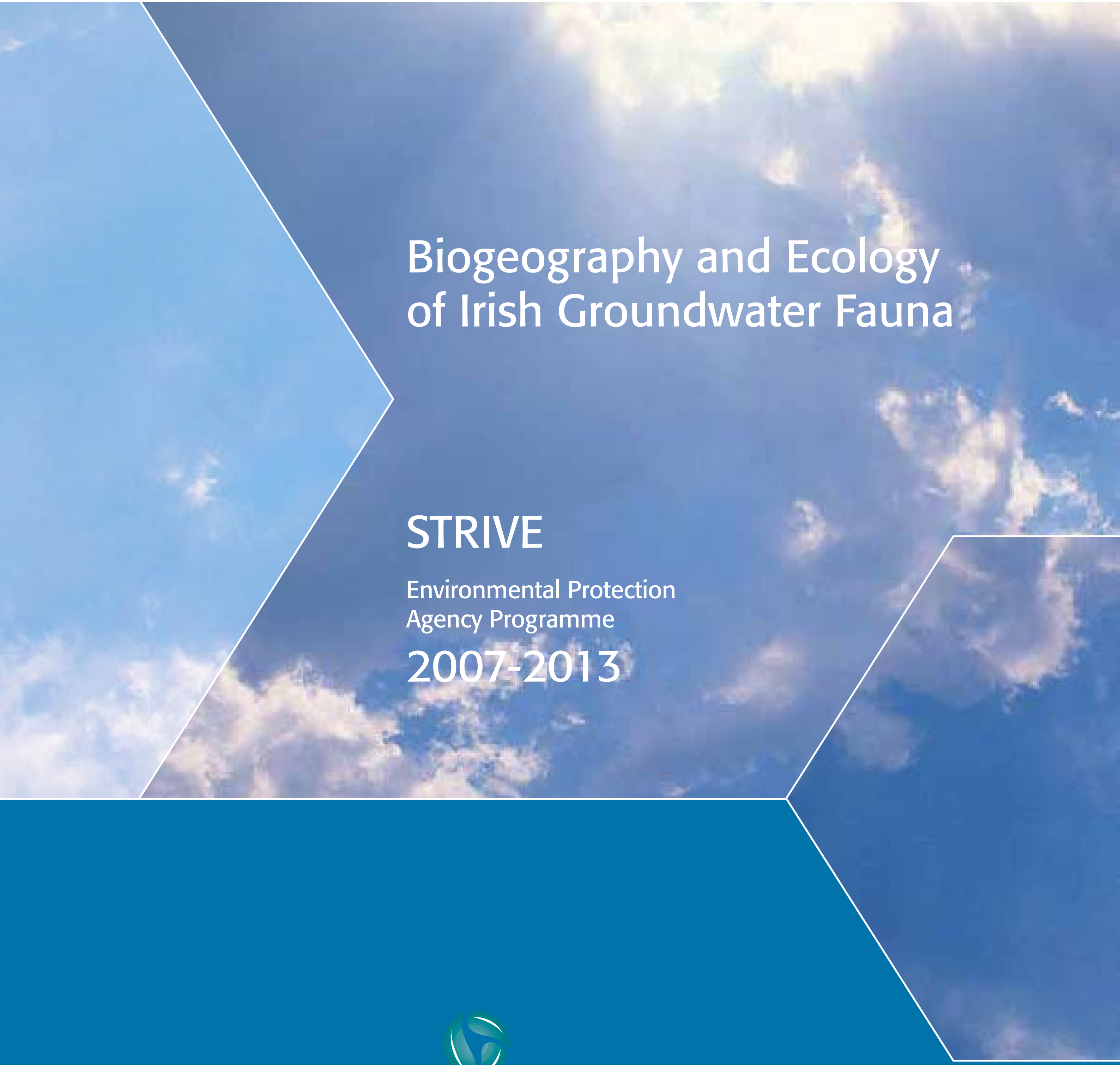

Biogeography and Ecology of Irish Groundwater Fauna

STRIVE

Environmental Protection
Agency Programme

2007-2013

SSTI
Strategy for Science, Technology and Innovation

Comhshaol, Pobal agus Rialtas Áitiúil
Environment, Community and Local Government

Environmental Protection Agency

The Environmental Protection Agency (EPA) is a statutory body responsible for protecting the environment in Ireland. We regulate and police activities that might otherwise cause pollution. We ensure there is solid information on environmental trends so that necessary actions are taken. Our priorities are protecting the Irish environment and ensuring that development is sustainable.

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EPA STRIVE Programme 2007–2013

Biogeography and Ecology of Irish Groundwater Fauna

Assessment of the Distribution, Structure and Functioning of Subterranean Fauna within Irish Groundwater Systems

(2007-W-MS-1-S1)

STRIVE Report

Prepared for the Environmental Protection Agency

by

University of Ulster
National University of Ireland, Galway
University of Koblenz–Landau
Loughborough University

Authors:

Jörg Arnscheidt, James Dooley, Karin Eriksson, Catherine Hack, Hans Jürgen Hahn, Tara Higgins, Thomas Kieran McCarthy, Caitríona McInerney and Paul Wood

ENVIRONMENTAL PROTECTION AGENCY
An Ghníomhaireacht um Chaomhnú Comhshaoil
PO Box 3000, Johnstown Castle, Co. Wexford, Ireland

Telephone: +353 53 916 0600 Fax: +353 53 916 0699
Email: info@epa.ie Website: www.epa.ie

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The EPA STRIVE Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Details of Project Partners

Jörg Arnscheidt
School of Environmental Sciences
University of Ulster
Coleraine, BT52 1SA
UK
Tel.: +44 28 70124401
Email: j.arnscheidt@ulster.ac.uk

Karin Eriksson
School of Environmental Sciences
University of Ulster
Coleraine, BT52 1SA
UK
Tel.: +44 0 28 70124401
Email: Eriksson-K@email.ulster.ac.uk

Hans Jürgen Hahn
University Koblenz-Landau
Institute of Environmental Science
University of Koblenz-Landau, Campus Landau
Fortstrasse 7, Building G
D-76829 Landau
Germany
Tel.: +49 6341 28031211
Email: hjhahn@uni-landau.de

Thomas Kieran McCarthy
Department of Zoology
Martin Ryan Institute
National University of Ireland, Galway
Galway
Ireland
Tel.: +353 91 492333
Email: tk.mccarthy@nuigalway.ie

Paul Wood
Loughborough University
Department of Geography
Loughborough, LE11 3TU
UK
Tel.: +44 (0)1509 223012
Email: p.j.wood@lboro.ac.uk

James Dooley
School of Biomedical Sciences
University of Ulster
Coleraine, BT52 1SA
UK
Tel.: +44 28 70124944
Email: jsg.dooley@ulster.ac.uk

Catherine Hack
School of Biomedical Sciences
University of Ulster
Coleraine, BT52 1SA
UK
Tel.: +44 28 70124944
Email: cj.hack@ulster.ac.uk

Tara Higgins
Office of Climate Change, Licensing & Resource Use
Environmental Protection Agency
PO Box 3000
Johnstown Castle Estate
Wexford
Ireland
Tel.: +353 53 9160600
Email: T.Higgins@epa.ie

Caitríona McInerney
School of Environmental Sciences
University of Ulster
Coleraine, BT52 1SA
UK
Tel.: +44 0 28 70124401
Email: c.mcinerney@ulster.ac.uk

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Executive Summary

Groundwater is a very important resource for securing Ireland's water supply. In some counties it even represents the main water source for human consumption. Groundwater environments not only store water, but also function as ecosystems. The purpose of this Environmental Protection Agency (EPA)-funded research project was therefore to provide baseline data on the biogeography of Irish crustacean groundwater fauna and its ecology. With the exception of an earlier pilot investigation, this research represents the first systematic and detailed attempt at an island-wide survey and thus is a significant step towards filling the knowledge gap identified by the European Groundwater Directive (2006/118/EC) with regard to groundwater ecology.

Currently in Ireland, seven crustacean species are classified as obligate groundwater fauna. One of them was discovered during this survey and is a species new to science (*Moraria* sp.). Other survey highlights were the first record of the harpacticoid *Parastenocaris vicesima* and the first record of a blind *Gammarus* specimen from stream-bed sediments, which may represent a genetic strain of *Gammarus lacustris* with special adaptation to life in the darkness of the hyporheic zone. Evidence for the existence of several cryptic species among groundwater amphipods and copepods may enhance subterranean aquatic biodiversity in Ireland even further.

The biogeographic distribution of Irish groundwater fauna reflects the island's hydrogeological setting. Poorly productive aquifers have very limited hydrologic connectivity with adjacent higher productivity aquifers and are, thus, effective dispersal barriers for groundwater fauna. Therefore, they appear to set boundaries for the biogeographic range of obligate groundwater animals, for example by preventing Niphargidae from dispersing into most groundwater environments of Northern Ireland. Comparison of biogeographic ranges also suggests that species associations with bedrock geology can vary between individual species of the same taxonomic family. While *Niphargus wexfordensis*, for example, has also been recorded from areas with volcanic bedrock, the other Irish Niphargidae were absent from this geological setting.

Crustacean abundance in groundwater displayed strong correlations with hydrology related parameters, such as water table dynamics and electrical conductivity, which can be used as a proxy for hydrologic exchange with surface water. Increased faunal abundance during dry periods with falling groundwater levels appeared to be the consequence of a concentration effect. Increased abundance in zones with higher exposure to exchange with surface water was presumably caused by improved food availability from surface environments. The most promising application of groundwater fauna monitoring would therefore be in bioindication of surface water intrusion (i.e. groundwater vulnerability). Obligate groundwater crustaceans tolerated a low oxygen environment, but did not occur in environments with saturation levels of 5% or lower. The most frequently encountered animal *Niphargus kochianus irlandicus* also displayed a low tolerance of elevated salt concentration and is therefore unlikely to inhabit coastal margins that are exposed to saline intrusions.

Genetic investigations revealed that this endemic and therefore uniquely Irish crustacean was separated from other European species several million years ago and must have survived all Quaternary glaciations in Ireland. Based on this genetic assessment, it needs to be elevated from its current taxonomic subspecies ranking to species level. It should be renamed as *Niphargus irlandicus*. The species has at least three genetic lineages, whose biogeographic ranges are partially separated by poorly productive aquifers. The geothermal refuge hypothesis proposes that the animals survived under the ice cover in zones where geothermal activity led to the rise of warm water through deep bedrock faults. Supporting evidence for this hypothesis are the accessibility of warm and tepid springs within each lineage's geographic range and the species' lack of adaptation to water temperatures below 4°C. It should be tested whether the hypothesis

can also be applied to other endemic groundwater animals in Ireland and whether it may even be applicable to groundwater fauna in formerly glaciated non-volcanic areas beyond Ireland.

After this island-wide project, more intensive regional surveys are needed for further advances in the knowledge of the biodiversity of Irish subterranean aquatic fauna. Aquifers adjacent to large rivers and eskers would appear to be particularly promising targets for future surveys. A sampling effort of six sampling sites per aquifer is deemed sufficient for the good representation of faunal diversity.

Investigations into the impact of hydrology and hydrogeology parameters on groundwater fauna require long-term monitoring of groundwater fauna. Therefore, the recommendation is to identify sets of long-term monitoring sites in at least two different aquifer types (Fracture flow and Intergranular flow), which should be sampled at least every 2 months for several years. Finally, an improved understanding of groundwater ecosystems also requires further autecological studies of groundwater organisms.

For practical applications, water engineers may be interested to note that annual monitoring of abundance of crustacean groundwater fauna and surface water fauna within distribution networks for water transport from groundwater sources could provide early warning signals with regard to microbial growth, surface water intrusion and related contamination risks.

1 Introduction

Even though taxonomic description of the larger Irish groundwater organisms commenced in the early 20th century, information on their biogeography, biodiversity and ecology remains limited. Therefore, this research project aimed to take stock of the crustacean groundwater fauna present in Ireland. After a brief introduction to the groundwater environment and groundwater ecology, this report will provide information on the biogeography of groundwater crustacean fauna in Ireland and explore the fauna's temporal dynamics in two different aquifers. It will then investigate aspects of the ecology of the uniquely Irish species, *Niphargus kochianus irlandicus,* before concluding with its genetic analysis, which provides a deep insight into the consequences of glaciation and the species' survival during these periods.

1.1 Groundwater: Ecosystems of Vital Importance to Humanity

Groundwater is a vitally important water resource. On a worldwide scale, non-saline groundwater represents 24.7% of current freshwater resources (Kotwicki, 2009). This proportion may rise dramatically if climate change diminishes the volume of freshwater stored in the world's polar ice caps and glaciers, especially since many large rivers depend on the latter.

In the Republic of Ireland, groundwater abstraction accounts for up to 26% of the total water supply (EPA, 2008). Figures for Northern Ireland are lower and represent about 15% of the total water supply (Robins, 1997). Regionally, there is considerable variation, for example groundwater sources in Co. Roscommon supply 75% of its total water consumption (EPA, 2007).

With continued expansion of industrial, agricultural and domestic demands on water resources, groundwater usage seems set to rise in the future. This imposes the need for protection of aquifers and groundwater-dependent ecosystems, and monitoring and management of resources to ensure quality and quantity according to the requirements of the Water Framework Directive (WFD). In recent years, the Environmental Protection Agency (EPA), the Geological Survey of Ireland (GSI) and the Geological Survey of Northern Ireland (GSNI) have invested significant resources in mapping and classifying the status of Ireland's groundwater bodies and in modernising groundwater monitoring networks (Daly et al., 2007; Daly, 2009). Almost 20% of the water research programme for the implementation of the WFD in Ireland, funded and co-ordinated by the EPA, has focused on groundwater and on groundwater-dependent ecosystems (Wemaere et al., 2009).

For many regions within Europe, historic data on groundwater quantity and chemical water quality are available; however, the European Groundwater Directive (2006/118/EC) identified a knowledge gap regarding groundwater ecology and encourages European Union (EU) Member States to undertake research in this sub-discipline of ecology.

The ecosystem nature of groundwater is only starting to receive wider recognition. Biomass estimates (Whitman et al., 1998; Kheshgi et al., 2000) may serve as a useful indicator of the ecosystem's importance in our planet's biosphere. Groundwater micro-organisms are tiny and usually do not occur at great densities. Yet, all of their organic carbon together represents a quantity equal in size to 3.7–36% of the total organic carbon contained in the world's terrestrial plants. The enormous microbial biomass suggests that, albeit often ignored, groundwater is indeed a very important type of ecosystem on a global scale.

1.2 The Groundwater Environment in Ireland

The main aquifers found in Ireland comprise Quaternary deposits and Palaeozoic bedrock formations, including Carboniferous limestone and sandstone, Devonian red sandstone and Ordovician volcanics (EPA, 2005). Palaeozoic limestone and sandstone underlie more than 50% of the country (Fitzsimons and Misstear, 2006). Even though Ireland has a complex geology, its groundwater contains mainly calcium, magnesium and hydrogen-carbonate (Daly et al., 2000), because limestone bedrock and limestone-derived subsoils are very common. Absolute ion concentrations are quite low, suggesting that monitored Irish groundwater bodies contain relatively shallow and young groundwater. With very few exceptions, oxygen is available and pH values are not extreme (EPA, 2008).

Outside a few specific areas in Ireland where there is mixing with warm water rising fast along faults from great depths, the temperature of shallow groundwater remains close to the mean annual soil temperature, between 11°C and 12.7°C (Garcia-Suarez and Butler, 2006), and exhibits very little seasonal change.

The predominant groundwater flow horizons in Ireland are situated within the top 30–40 m below the ground surface (Daly, 1995); extensive karstification in some limestone aquifers has produced conduit systems with very rapid groundwater flow rates (Coxon and Drew, 1999). Most Irish bedrock aquifers are dominated by fracture flow; there is only one important sandstone aquifer representing porous groundwater flow; sand and gravel aquifers with intergranular flow underlie only 2% of the country (EPA, 2005).

1.3 Characteristics of Groundwater Fauna

Several of the major taxonomic groups commonly encountered in surface water habitats also have a strong subterranean presence (e.g. Marmonier et al., 1993; Sket, 1999). For the whole world, Botosaneanu (1986) listed about 7,000 known aquatic subterranean species. With future exploration, their numbers are estimated to rise to between 50,000 and 100,000 (Culver and Holsinger, 1992). Crustaceans have been the most successful invaders of groundwater habitats. They currently represent more than 40% of animal species known in groundwater worldwide.

Groundwater biodiversity exhibits several peculiarities, including high proportions of either phylogenetic or distributional relics (‘living fossils’) and a high proportion of endemic species (Gibert and Deharveng, 2002). Constraints, including scarce food resources and often low oxygen levels, have forced groundwater animals to evolve remarkable adaptations in their physiology and life cycles. These features mean that the aquatic subterranean fauna ranks among the most precious biological heritage on our planet, but they may also increase the risk of extinction due to anthropogenic disturbance.

Groundwater fauna may occur in all types of aquifers with voids of suitable size and an imported or ‘in situ’ supply of energy. The habitats represent three key types of aquifers: karstic, fissured and porous. Fauna inhabiting subterranean aquatic environments primarily consists of small invertebrates (except in caves and in karst areas, where voids are large enough for vertebrates to occur), and displays various degrees of adaptation to subterranean life.

Gibert et al. (1994) classified fauna based on the phenology and presence or absence of organisms within groundwater habitats. Fauna with no affinity to groundwater are called stygoxenes (= alien to groundwater); those species that can spend part or their entire life cycle in subterranean aquatic environments are called stygophiles (= friendly with groundwater), while obligatorily subterranean species are called stygobites (= living in groundwater).

Stygobites have successfully adapted to the dark, nutrient-poor and often spatially constrained subterranean habitats. They are typically small, eyeless and without colour. However, in some instances remnant eye structures are still visible, yet they have no pigment, suggesting that there has been a selection for degenerative mutations at regulatory loci, while structural genes remained intact (Culver et al., 1995). These animals seem to have originated from epigean ancestors of marine, freshwater and semi-terrestrial habitats at different times and in a variety of ways (Reddy, 2002).

There is a very high proportion of endemic species among groundwater fauna and a strong tendency towards cryptic diversity. Trontelj et al. (2009) analysed stygobite species with large geographic ranges and concluded that they would have to be split into many tentative cryptic lineages. Most of these lineages had ranges <200 km in length, only one extended to 700 km. Small geographic ranges of macro-stygobites would seem to be the rule and not the exception.

Traditionally, the high number of endemic species among stygobites has been explained with vicariance, i.e. separation of populations by external events, and the low dispersal ability of groundwater fauna. Current biogeographic distribution patterns of subterranean animals often seem to result from a complex mixture of processes involving the dispersal capabilities of species, external vicariant events separating formerly coherent populations, and rates of evolution and extinction (Porter, 2007). The biogeography of subterranean species assemblages may also reflect hydrological drainage patterns of the past. For example, Fuchs et al. (2006) found that groundwater fauna species assemblages differed markedly between the preglacial drainage basins of the Palaeo-Rhine and the Palaeo-Danube, even though the drainage patterns of current surface watersheds are different to those of the past. While there is evidence for a low dispersal ability of relatively large subterranean crustaceans such as amphipods, this does not seem to hold true for smaller fauna such as ostracods (e.g. Danielopol et al., 1994).

With regard to biodiversity, there seems to be consensus among ecologists that cave sites or aquifers with more than 20 subterranean species represent subterranean hotspots of biodiversity (Culver and Sket, 2000; Deharveng et al., 2009). In Europe, such hotspots have so far emerged in Slovenia and north-eastern Italy, the French Pyrenees, the Cévennes in southern France, in the Rhine River valley in north-eastern France (Deharveng et al., 2009) and in the floodplains of the River Danube (Danielopol and Griebler, 2008). In Ireland, targeted surveys have only just begun. Therefore, we are in an exciting age of discovery with regard to groundwater fauna and a significant amount of research is required, before we can begin to understand this island's groundwater ecosystems.

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2 All-Ireland Survey for Crustacean Groundwater Fauna

2.1 Aims and Research Questions

The work programme aimed to generate baseline data on the spatial distribution (biogeography) of groundwater crustacean fauna in Ireland. In addition, the sampling activity for the survey aimed to provide other work programmes with specimens for experimental purposes and genetic analysis.

Research questions:

1. What is the biogeographic distribution of groundwater fauna in Ireland?
2. Is there evidence for the association of individual groundwater taxa with particular geographic regions, aquifer types, hydrological or chemical conditions?
3. What is the biodiversity of Irish groundwater fauna in comparison with other regions in Europe?

2.2 Introduction

First records of subterranean animals in Ireland date back to 1863, but no details of the geographic position were provided. The first recorded sampling site was Templeogue, Dublin, in 1899, where *Niphargus kochianus irlandicus* was discovered in a well. Soon afterwards more specimens were recorded from the bottom of a lake with groundwater influx (Kane (1904) cited in Proudlove et al., 2003). From the late 1930s onwards, knowledge of Irish subterranean fauna has greatly increased due to the sampling efforts of the Cave Research Group of Great Britain (CRG), led by Audrey Glennie and Mary Hazelton (Hazelton, 1974), and due to pioneering work by Mary Crichton for the Irish Biological Records Centre. Biogeographic patterns of Irish subterranean fauna have, however, remained largely obscure, because of accessibility issues and an imbalance in sampling effort, with the majority of historic sampling sites located in caves of karstic areas. In Ireland, these features are spatially clustered. Any exploration of subterranean fauna in the pursuit of classic biospeleology within caves therefore has a strong bias towards regions with these clusters. In order to achieve a more comprehensive and better balance in geographic coverage, recent survey activities have focused predominantly on other types of sampling sites and groundwater habitats (Arnscheidt et al., 2008; this project) or have at least included some non-cave sites (Knight and Penk, 2010). However, there is obviously a higher availability of suitable sampling sites in areas with regionally or locally important aquifers. Hence, a sampling bias towards such regions is almost unavoidable in any island-wide survey.

2.3 Study Area

A total of 235 sites were sampled for the all-Ireland survey of subterranean aquatic crustaceans in 2008 and 2009. Sampling sites comprised boreholes, wells, springs, caves and a river bank. Most of the sites were selected following the advice of EPA and GSNI staff. Almost all sites were directly accessible; however, samples from three wells could only be taken by filtering the discharge from traditional handpumps, which had once served the public water supply in small towns and villages, but are now often only preserved as decorative features. Figure 2.1 shows the geographical distribution of sampling sites. The most even spread of sampling sites was achieved among boreholes, while wells and springs were much more clustered, for example most springs sampled were in counties Mayo, Galway and Clare. Traditional dug wells usually had a top cover, which provided an effective entrance barrier to surface water species. Other wells represented the most heterogeneous group of sampling sites. On maps many of the wells were marked as ‘holy wells’. These were either unfurnished or had well shafts constructed from a wide variety of materials and in many different styles. Most of the wells did not show signs of an active water discharge. Their water surface was often near ground level and exposed to the atmosphere. This feature, which they share with most springs, provides opportunities for colonisation by surface water fauna, particularly species that can fly and deposit their eggs on the water surface. With regard to the two cave sites

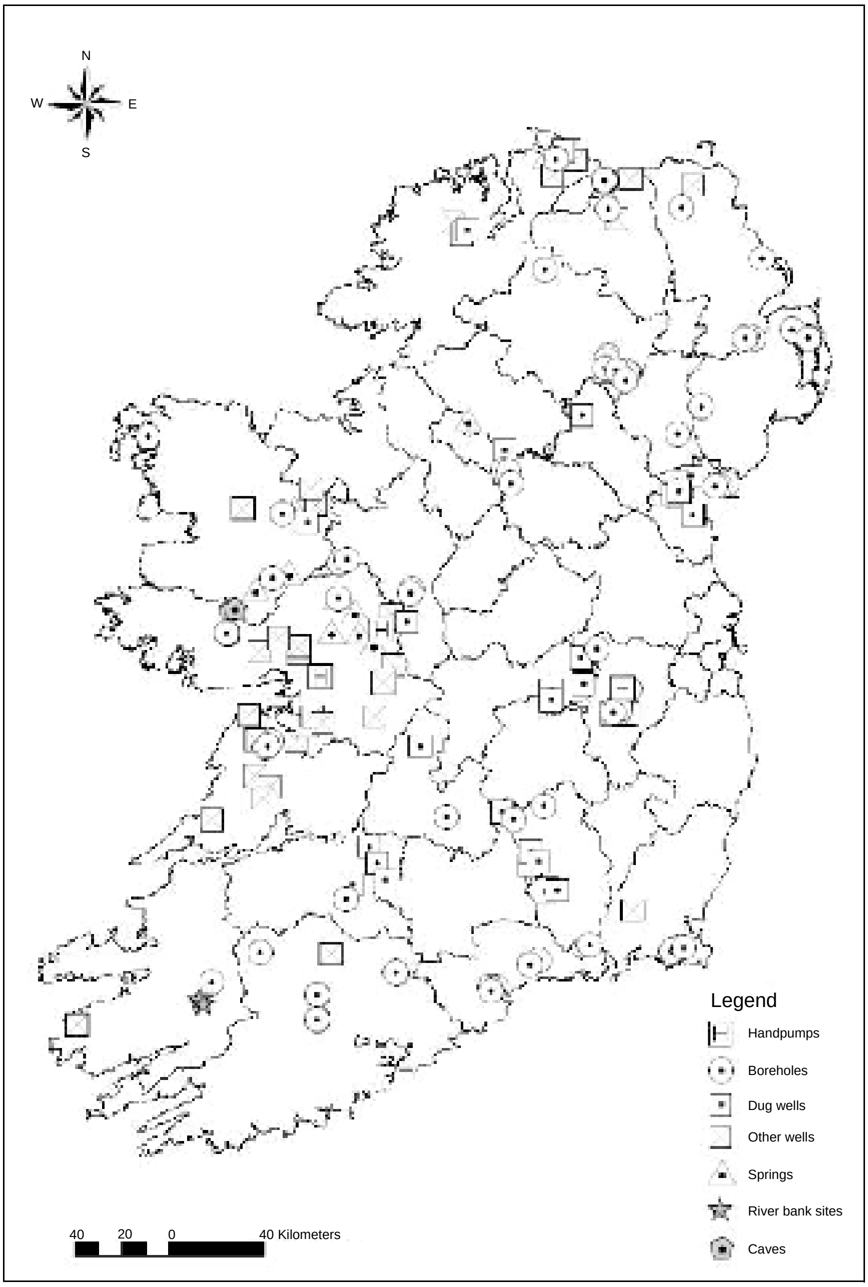

Figure 2.1. Survey map exhibiting the types of sampling sites for the 2008–2010 survey.

sampled, it should be noted that samples were obtained from an area inside the caves, which was close to the cave entrance. As a consequence, these sites could also be accessed by surface water species.

While the sampling programme aimed to cover all major bedrock geology groups in Ireland, most sites represent limestone due to the island's extensive cover of limestone rock units. In August 2010, an additional 18 sites centred on streams and rivers were sampled for hyporheos.

2.4 Methods

2.4.1 Non-biotic parameters

Geo-referenced data were recorded with a Garmin eTrex® GPS. Water level depths were determined with a Type 10 van Walt dip meter (100 m). Where possible, this device was also used to determine the total depth by loss of tension on the dipper cable. In depths up to 30 m, oxygen concentration and saturation were determined with a Hach HQD40 and a Hach IntelliCAL™ LDO® probe. Water samples were retrieved in a polytetrafluoroethylene (PTFE) bailer with a slow-release valve. Electrical conductivity and pH were measured in water retrieved with the bailer using a Hach sensION 156 with a conductivity cell and a Hach Platinum pH electrode. In samples retrieved from depths greater than 30 m, oxygen measurements were taken in the bailer water. Water temperature measurements were recorded for all field measurements. The water temperature value was taken from the oxygen meter in sampling depths up to 30 m and from the conductivity meter for greater sampling depths, as these were associated with the smallest errors due to temperature change caused by the sampling process. Filtered water samples (0.45 µm cellulose acetate) were preserved in 2% ANAL-R nitric acid. For hyporheic samples, oxygen was measured with a Hach HQ10 meter with LDO® probe. Other parameters were measured in a polyethylene (PE) beaker using the instruments described above.

2.4.2 Biological sampling (boreholes, wells and springs)

As the aim of the island-wide survey was to achieve a representative and well-balanced geographic coverage, sampling activity primarily targeted dug wells and boreholes. Sampling at such sites takes advantage of the tendency of groundwater organisms to become trapped in the still water at the base of wells and boreholes. Once they are inside these structures they tend to sink to the bottom sediments, where they seem to remain alive for considerable time. Using net samplers to catch those animals is classified as a passive sampling method rather than sampling which involves pumping groundwater and retaining the animals by filtration over gauze/netting (Malard et al., 2002).

Due to its ease of use, the method of choice employed in this survey was passive sampling with a modified Cvetkov net sampler (Cvetkov, 1968; Fuchs, 2007). The equipment is lightweight, comfortably fits into a backpack and is thus even ideal for sampling sites that can only be reached on foot. The sampler (Fig. 2.2) can be operated from a fishing rod for wells up to 200 m deep. The sampler consists of a small 63-µm net, with an aperture diameter of 47 mm for the smallest nets. An aperture diameter of 140 mm was used in wells with suitably large shaft dimensions.

The net is funnel shaped and is attached to a centrifuge tube. The tube carries lead weights at its lower end, which serve to suspend sediment and the organisms within it (Fig. 2.2). Moving the net up and down through the water first helps to suspend organisms from the bottom into the water column (bottom weight). These are then filtered from the water

Figure 2.2. Net samplers for boreholes (top) and wells (bottom).

column (net) and concentrated in the centrifuge tube, which acts as a sample reservoir. After sampling the tube is exchanged and closed with a screw cap for sample storage. While this survey method has been very effective, it should be noted that for most sites examined drilling records or other documentation of furnishing of boreholes and wells could not be obtained. As a consequence, it is not always clear if the absence of groundwater organisms from a particular site is the result of aquifer properties or the construction details of the sampling site.

Springs were sampled by kick sampling with a 63-µm net and, where applicable, by picking animals from stones that had been placed in a sampling tray.

Samples were sorted prior to conservation under Swift Dual Mag or Olympus SD30 microscopes and further identified under Olympus SZX16 and Olympus BH2 microscopes. Samples were preserved in 70% ethanol.

2.4.3 Biological sampling (hyporheos)

A perforated stainless steel pipe with a tip (Fig. 2.3) was driven into the stream bed, so that the uppermost perforation holes were at a sediment depth of at least 5 cm. The deepest holes were at a maximum depth of 30 cm, depending on how far the equipment could penetrate. A bilge pump evacuated a mixture of sediment and water from the pipe. Sampling was continued until water became clear. The sediment water mixture obtained from the pump was poured into a bucket and elutriated and filtered through a net with a maximum mesh size of 63 µm. Sediment retained by the net was stored in 70% ethanol. In the laboratory, samples were sorted in Bogorov counting chambers and identified under microscopes as described above.

2.5 Results

2.5.1 Chemistry

Low values for electrical conductivity at almost 12% of all sampling sites suggest that a large proportion of water resulted from very recent recharge or exposure of the sampling site to direct rainwater input. There was no indication of saline intrusions. With few exceptions, pH values did not vary far from a circumneutral range; the minimum pH was 5.9. More than 30% of the sampling sites had oxygen saturation values of less than 5%. Figure 2.4 displays histograms for all three parameters and compares the data set for all sites with those at which the most frequently encountered

Figure 2.3. Hyporheic sampling pipe in a Burren stream.

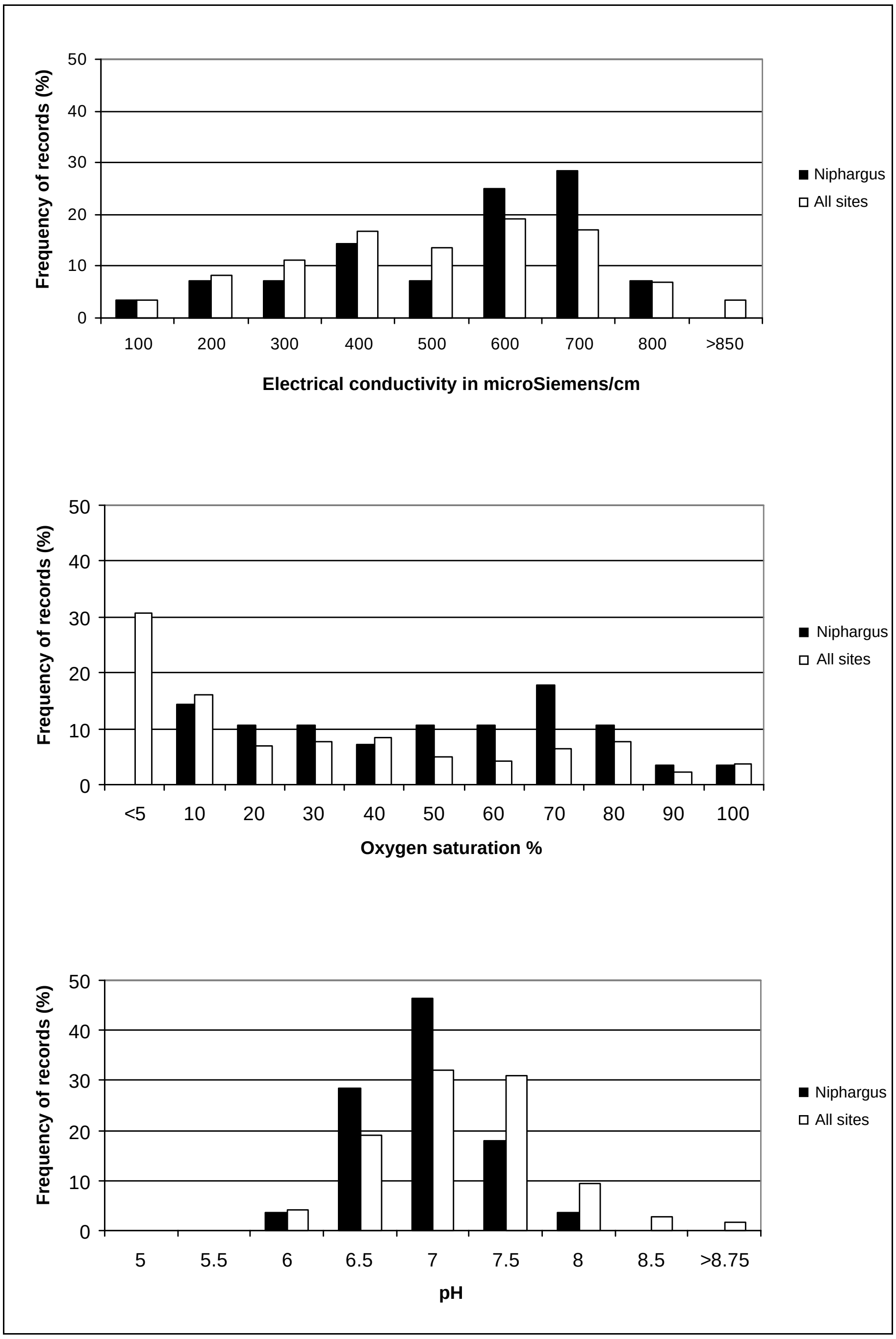

Figure 2.4. Comparative histograms of water chemistry parameters for all sampling sites and those with records for *Niphargus k. irlandicus*.

stygobite species *Niphargus k. irlandicus* was recorded during the sampling campaign. *Niphargus k. irlandicus* was absent from sites with an oxygen saturation <5%. Nevertheless, the presence of *Niphargus k. irlandicus* at sampling sites did not indicate a distinct preference for high oxygen values. There was a slight tendency towards more records from sites with lower pH values, although there was no apparent preference with regard to electrical conductivity.

2.5.2 Biology: boreholes, wells and springs

There were marked differences between different types of sampling sites (Table 2.1). Boreholes represented the majority of sampling sites, but only 26% of them yielded crustacean fauna. Crustacean

Table 2.1. Summary of sampling success during the 2008–2010 survey according to the type of sampling site.

	Total	BH	H	DW	W	S	C	R
Crustaceans	**96**	34	1	19	31	8	1	2
Fauna excl. crustaceans	**6**	5	0	0	1	0	0	0
No fauna	**135**	94	2	24	10	4	1	0
Total	**235**	**133**	**3**	**43**	**42**	**12**	**2**	**2**

BH, borehole; H, handpump; DW, dug well; W, well, excl. dug wells; S, spring; C, cave; R, river-bank sediment.

records were obtained from 44% of sampled dug wells, as well as from 76% of other wells and 67% of sampled springs. Examination of the map of sites identified a few patterns with regard to sampling success (Fig. 2.5). Sampling in areas of karstified bedrock with conduit flow yielded the highest chance of success, while the success rate decreased in areas associated with poorly productive aquifers. However, irrespective of aquifer type, crustacean records were obtained from most areas with extensive sampling activity.

Tables 2.2–2.6 list all the crustacean taxa recorded during the 2008–2010 survey.

The *Gammarus* species are surface water species (Table 2.2), with *G. lacustris* considered a glacial relict and *G. duebeni celticus* and *G. pulex* representing part of the freshwater fauna in springs. All three Irish niphargid amphipod species have been recorded during this survey. *Niphargus wexfordensis* is now also known from Kildare. The sites in which *Microniphargus leruthi* has been recorded suggest a wide geographic distribution in Ireland. Yet with 30 sampling sites, *Niphargus k. irlandicus* represents the vast majority of records. It was most frequently encountered in the West of Ireland during this survey, even though high abundances have been recorded for a sand and gravel aquifer in Kildare subject to the temporal sampling scheme (Chapter 3). Its known biogeographic range now extends into Co. Fermanagh (west of the Upper Lough Erne).

Among the Copepoda recorded during the survey, the three *Diacyclops* species (Table 2.3) are frequently associated with groundwater, although these species are also documented to occur in surface water habitats (Schminke and Gad, 2007). Cross-breeding studies have shown evidence for cryptic species in *Diacyclops bicuspidatus* (e.g. Monchenko, 2000). The Irish specimens show morphological variations, which also suggest cryptic speciation (Fabio Stoch, 2010, Università di L'Aquila, Italy, personal communication). Subterranean *Diacyclops* may thus be genetically different from animals found in surface water environments.

Acanthocyclops venustus is usually found in acidic boggy waters or groundwater, while the other *Acanthocyclops* species occur in many types of small waterbodies. *Acanthocyclops robustus* can even have pelagic populations in large lakes (Schminke and Gad, 2007). *Eucyclops serrulatus* lives in all types of aquatic biotopes. It is frequently encountered in groundwater and the hyporheic zone (Schminke and Gad, 2007).

Paracyclops fimbriatus has been recorded in many different types of surface and subterranean aquatic environments. *Paracyclops poppei*, albeit much rarer, seems to display a similar pattern (Schminke and Gad, 2007).

Taxonomic identification of *Microcyclops* is notoriously difficult. Only a few species appear to be associated with groundwater. Two *Microcyclops* species were recorded by Fryer (1993) in Yorkshire. Both (*Microcyclops bicolor* and *Microcyclops varicans rubellus*) lived in ponds; the Irish specimens therefore may also not be stygobites.

For the remaining cyclopoid Copepoda species identified during the current survey, there appears to be no particularly strong association with subterranean environments.

Juveniles of *Parastenocaris* had already been recorded during the pilot survey in 2006, but could not

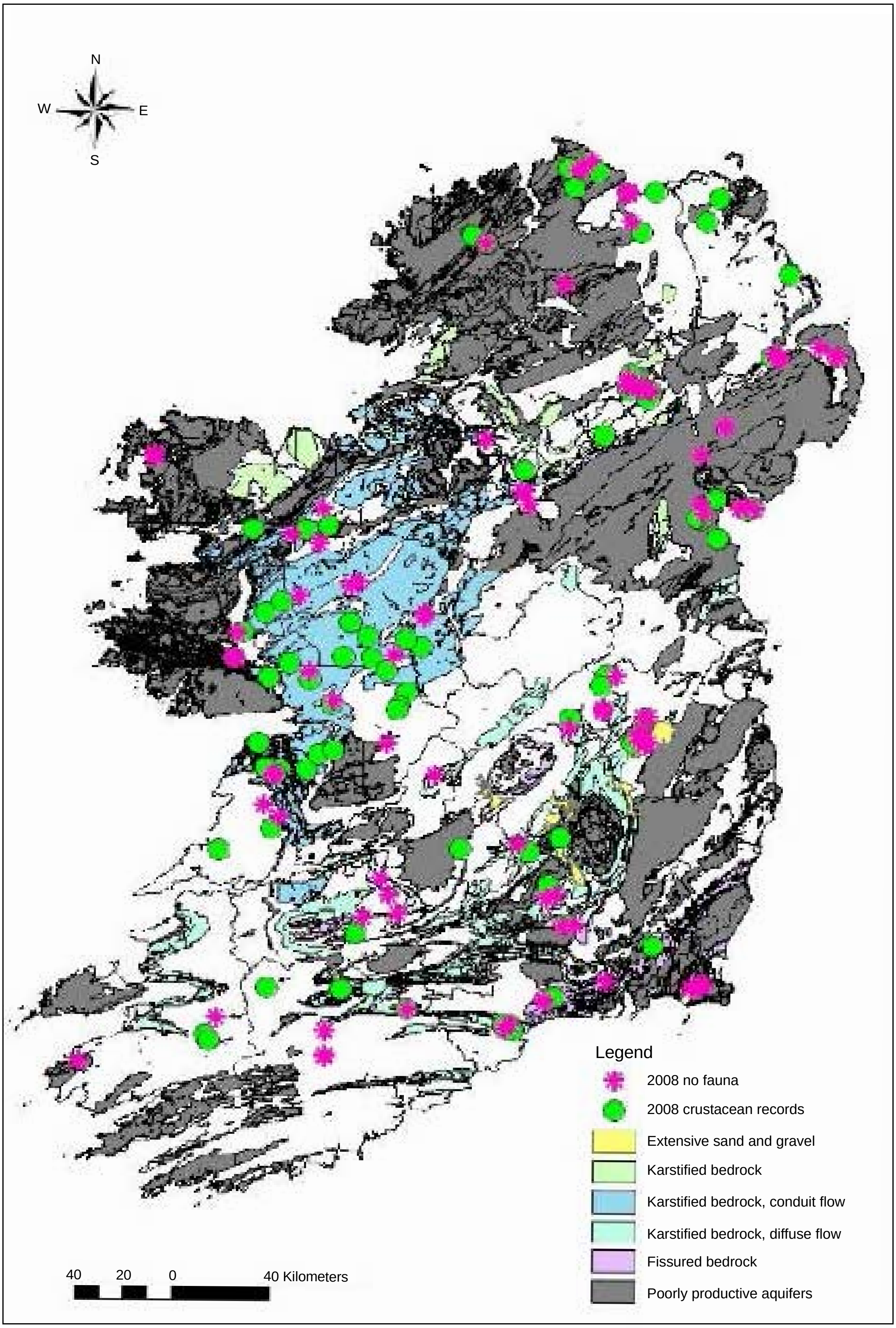

Figure 2.5. Survey map exhibiting sampling sites with records of crustaceans and those with no faunal records. Hydrogeology data were provided by the Geological Survey of Ireland and the Geological Survey of Northern Ireland.

Table 2.2. Taxa of Amphipoda recorded during the 2008–2010 survey.

Family	Species
Gammaridae	*Gammarus duebeni celticus* Stock & Pinster, 1970
Gammaridae	*Gammarus lacustris** Sars, 1863
Gammaridae	*Gammarus pulex* Linnaeus, 1758
Niphargidae	*Microniphargus leruthi* Schellenberg, 1934
Niphargidae	*Niphargus kochianus irlandicus* Schellenberg, 1932
Niphargidae	*Niphargus wexfordensis* Karaman, Gledhill & Holmes, 1994

*Hyporheic survey only.

Table 2.3. Taxa of cyclopoid Copepoda (Family: Cyclopidae) recorded during the 2008–2010 survey.

Species
Acanthocyclops robustus (Sars, 1863)
Acanthocyclops venustus (Norman & Scott, 1906)
Acanthocyclops vernalis (Fischer, 1853)
Cyclops furcifer Claus, 1857
Cyclops strenuus (laevis) Fischer, 1853
Cyclops sp.
Diacyclops bicuspidatus s.str. (Claus, 1857)
Diacyclops bisetosus (Rehberg, 1880)
Diacyclops languidus (Sars, 1863)
Eucyclops serrulatus (Fischer, 1853)
Megacyclops latipes group
Megacyclops viridis (Jurine, 1820)
Microcyclops sp.
Paracyclops affinis (Sars, 1863)
Paracyclops fimbriatus (Fischer, 1853)
Paracyclops sp.

Table 2.4. Taxa of harpacticoid Copepoda recorded during the 2008–2010 survey.

Family	Species
Parastenocarididae	*Parastenocaris vicesima* Klie, 1935
Phyllognathopodidae	*Bryocamptus pygmaeus* (Sars, 1863)
Phyllognathopodidae	*Moraria* nov. sp.

Table 2.5. Taxa of Isopoda (Family: Asellidae) recorded during the 2008–2010 survey.

Species
Asellus aquaticus Linnaeus, 1758
Proasellus meridianus (Racovitza, 1919)

Table 2.6. Taxa of Ostracoda recorded during the 2008–2010 survey.

Family	Species
Candonidae	*Candona candida* (Müller, 1776)
Candonidae	*Cyclocypris globosa* Sars, 1863
Candonidae	*Cyclocypris serena* (Koch, 1867)
Candonidae	*Fabaeformiscandona breuili* (Paris, 1920)
Cyprididae	*Herpetocypris chevreuxi* (Sars, 1896)
Cyprididae	*Potamocypris* sp.
Cyprididae	*Potamocypris villosa* (Jurine, 1820)
Darwinulidae	*Darwinula stevensoni* (Brady & Robertson, 1870)

be identified to species level. The current survey, however, yielded adult specimens from the River Flesk, Co. Kerry. These have been identified as *Parastenocaris vicesima.* To our knowledge this represents this species' first Irish record. Recently, also, *Parastenocaris phyllura* has been recorded by Kibichii et al. (2010) for Ireland. Both of these species have been found in Britain as well (Proudlove et al., 2003). They are known to occur in beach and river sands and have some tolerance of salinity (Schminke and Gad, 2007). Animals of the genus *Moraria* seem to depend on subterranean environments (Schminke and Gad, 2007). The *Moraria* species from Antrim is new to science and will be described in co-operation with Italian copepod specialist Fabio Stoch.

Asellus aquaticus and *Proasellus meridianus* (Table 2.5) have both been frequently recorded from Ireland as surface water animals. Blind specimens whose morphological diagnostic features resemble those of *Asellus aquaticus* have been recorded in Crag Cave, Co. Kerry (Wood and Arnscheidt, unpublished data).

The ostracod *Fabaeformiscandona breuili* (Table 2.6) is classified as a groundwater animal (stygobite) by Meisch (2000). It was initially recorded for Ireland during the 2006 survey (Arnscheidt et al., 2008). All other ostracod species in this list have previously been recorded for Ireland. Among them, only *Cyclocypris globosa* is considered to be a rare species, which appears to prefer slightly acidic conditions (Douglas and McCall, 1992; Meisch, 2000).

An overview of the frequency of records for individual taxa is provided in Table 2.7. *Niphargus k. irlandicus* was by far the most frequently encountered crustacean species, with 30 records. Very few other taxa were recorded five times or more. A species' record profile with regard to types of sampling sites may indicate its tendency to inhabit subterranean habitats. The stygobite *Niphargus k. irlandicus* had a strong presence in boreholes. Most records of the stygophile species *Diacyclops bicuspidatus* came from boreholes and dug wells, while the stygoxene species *Gammarus pulex* occurred only in a spring and a non-dug well.

2.5.3 Biology: hyporheic survey

Sampling activity focused on the retrieval of macroscopic crustaceans and therefore the campaign centred on selected areas. *Niphargus k. irlandicus* occurred in the hyporheos of a Burren stream at the borderline between shale and limestone, where the

Table 2.7. Frequency of records from the 2008–2010 survey according to type of sampling site (excl. hyporheic survey programme).

	Total	BH	H	DW	W	S	C	R
Asellus aquaticus	**11**	4			5	2		
Proasellus meridianus	**2**	1				1		
Gammarus duebeni celticus	**5**				2	1	1	1
Gammarus pulex	**2**				1	1		
Microniphargus leruthi	**6**	6						
Niphargus k. irlandicus	**30**	13	1	2	11	3		
***Niphargus* sp.**	**1**			1				
Niphargus wexfordensis	**2**	1			1			
Acanthocyclops robustus	**1**	1						
***Acanthocyclops* sp.**	**8**	4		1	2	1		
Acanthocyclops venustus	**3**			1	2			
Acanthocyclops vernalis	**4**	2			2			
Cyclops furcifer	**1**				1			
Cyclops strenuus (laevis)	**1**				1			
***Cyclops* sp.**	**10**	3		3	4			
Diacyclops bicuspidatus	**11**	5		3	3			
Diacyclops bisetosus	**2**	1			1			
Diacyclops languidus	**3**	1	1		1			
Eucyclops serrulatus	**5**	3		1	1			
***Megacyclops* (*latipes* group)**	**1**	1						
Megacyclops viridis	**1**	1						
***Microcyclops* sp.**	**3**			2	1			
Paracyclops affinis	**1**	1						
Paracyclops fimbriatus	**4**	1			2	1		
***Paracyclops* sp.**	**1**	1						
Bryocamptus pygmaeus	**2**					1		1
***Bryocamptus* sp.**	**4**			1	3			
***Moraria* n. sp.**	**1**				1			
Parastenocaris vicesima	**1**							1
Candona candida	**1**				1			
***Candona* sp.**	**9**	2		2	4	1		
Cyclocypris globosa	**2**			1	1			
Cyclocypris serena	**1**				1			
Darwinula stevensoni	**1**			1				
Fabaeformiscandona breuili	**1**				1			
Herpetocypris chevreuxi	**1**					1		
Potamocypris* sp. *indet	**1**				1			
Potamocypris villosa	**1**				1			

BH, borehole; H, handpump; DW, dug well; W, well, excl. dug wells; S, spring, C, cave; R, river-bank sediment.

Figure 2.6. Hyporheic sampling sites and locations for important hyporheic records.

stream was resurging due to rising groundwater (St Brendan's well at Lisdoonvarna, Fig. 2.6).

A blind *Gammarus* specimen was encountered in the hyporheos of a stream in Fermanagh, east of Upper Lough Erne (Fig. 2.6), which links two small lakes. Specimens whose eye pigments have not been activated will usually darken after the exposure to light even following preservation. The Fermanagh specimen's eye facets, however, have remained colourless (Fig. 2.7), which suggests that it has truly lost its pigment. Even though there is only one specimen so far, this is probably the first evidence of the existence of an Irish *Gammarus* strain, which lives in hyporheic/subterranean environments and therefore can exist without eyesight. The individual seems only half-grown and shares characteristics with *Gammarus lacustris*. The posture of the blind specimen, with its concave curve along its back, a feature it shares with niphargids, is rare for *Gammarus* (Fig. 2.8) whose back usually has a convex curve even when preserved. No *Anthrobathynella* specimen has been found during this campaign despite considerable sampling efforts.

2.6 Discussion

2.6.1 Sampling sites with low oxygen saturation

Even though elements of Irish groundwater fauna can tolerate quite low oxygen saturation, the evidence suggests that more than 30% of all sites were 'unavailable' for fauna due to extremely low oxygen levels in the water. In some cases, this will actually reflect the prevailing conditions within the aquifer, for example in the coastal aquifer at Magilligan on the North Antrim coast. At other sites, low oxygen may, however, have been an artefact, for example caused by little water exchange in boreholes that are not pumped regularly and by microbial decomposition of organic matter accumulated in the borehole sump. At

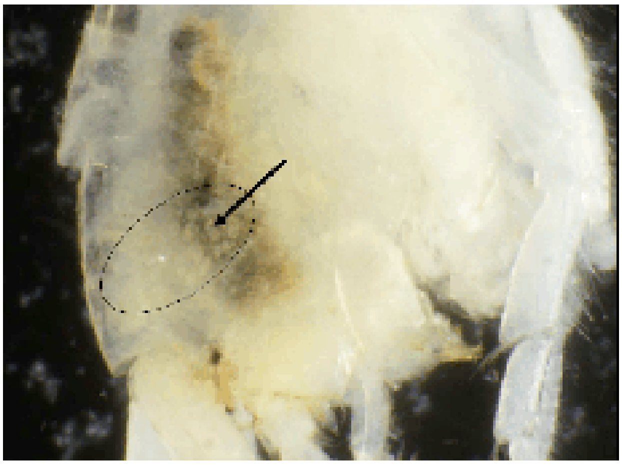

Figure 2.7. Blind *Gammarus* from Fermanagh. In this picture the animal is standing on its head, i.e. antennae and mouth parts are orientated towards the bottom of the picture. The eye is encircled. The arrow points at an eye facet without pigment.

Figure 2.8. Blind *Gammarus* from Fermanagh. The dorsal (back) side has a slightly concave curve. The black patch represents a part of the gut.

the latter sites, any trapped fauna would die and decompose.

Some of the sampled borehole fields had only been established very recently as part of the new EPA groundwater monitoring infrastructure. In principle, these are very attractive survey sites because they are exceptionally well documented. Yet, the short interval between construction and sampling visit may have had a negative impact on sampling success. Furthermore, some of the new fields are located in areas with poorly productive aquifers. While these were previously under-represented in Ireland's groundwater monitoring, they may also have less groundwater fauna per se. Nevertheless, these monitoring fields should be sampled again, now that they have been in operation for more than 1 hydrological year.

2.6.2 Biogeographic boundaries for groundwater fauna in Ireland

Based on morphological identification criteria, three groundwater amphipod species can be differentiated in Ireland. A detailed genetic study would be extremely useful in order to clarify the phylogenetic positions of *Niphargus wexfordensis* and *Microniphargus leruthi*, as well as to investigate the potential existence of cryptic species. The occurrence of *Microniphargus* remains a conundrum, as it suggests an unusually wide geographic range for this species. Outside Ireland its known biogeographic distribution comprises areas in Belgium (Schellenberg, 1934), Luxembourg (Hoffmann, 1963), Germany (Spangenberg, 1973) and England (Knight and Gledhill, 2010). If it was not genetically very distinct from continental or English specimens, it would raise the question of how and when it got to Ireland (Chapter 5).

This survey has confirmed the existence of a general biogeographic divide for Irish groundwater amphipods. To the north, their geographic range is confined by a string of poorly productive aquifers extending north of a line from Dundalk to Sligo. Those aquifers, which mostly lie under substantial layers of glacial till, form a natural barrier for a northward expansion from any glacial refugia in more southern regions. Future investigations of limestone aquifers near Upper Lough Erne may still lead to very slight northward advances of the biogeographic boundaries for niphargids, but any major extensions of their currently known range would come as a surprise due to the geographic coverage achieved by recent sampling campaigns. There is a similar band of poorly productive aquifers separating the midlands from the south, which is, however, not continuous and thus leaves some space for colonisation pathways (Chapter 5). Productive aquifers in the south-east appear quite isolated because they are framed by poorly productive aquifers, but may be accessible for colonisation from southern and western directions.

2.6.3 Association of fauna with bedrock geology, hydrogeology and vulnerability

With regard to associations of Irish groundwater amphipods with geological settings, some differences are emerging. It has to be considered, though, that some records for *Niphargus k. irlandicus* predating 1994 may have contained the two other species *Niphargus wexfordensis* and *Microniphargus leruthi*. With the exception of a site in bordering sandstone areas, records of *Niphargus k. irlandicus* are associated with limestone geology (Fig. 2.9). Water chemistry data suggest that this is not likely to be caused by a high sensitivity towards acidity (Fig. 2.4). Outside of limestone areas *Niphargus wexfordensis* has also been recorded in settings of volcanic or metamorphic bedrock geology (Fig. 2.10). Due to the small number of records, it is currently not clear whether this species has indeed less specific requirements regarding the bedrock units it inhabits or whether its actual habitats in non-limestone areas are, for example, alluvial or glacial sediments. A similar profile and the same uncertainty apply to *Microniphargus leruthi* (Fig. 2.11), which is often associated with sand and gravel sites. The majority of records for Irish niphargids are associated with regionally important aquifers (Fig. 2.12), suggesting that these animals are well adapted to the following flow regimes:

- Karstic with diffuse flow;
- Karstic with conduit flow;
- Fracture flow in fissured bedrock; and
- Intergranular flow in sand and gravel.

From a biogeography perspective, there appears to be a sufficient number of records in aquifers of local importance, interspersing aquifers of regional importance to suggest that the former did not present any insurmountable barriers for geographic expansion by dispersal after population contractions caused by Quaternary glacial activity. Outside the actual refuge areas, any site with a record of groundwater amphipods must have been accessible via postglacial colonisation pathways within a continuous groundwater habitat at some point in time. Some of these connections may have been cut off after colonisation, for example through sea level rise, so that populations may have become isolated over time. This is a likely scenario for some coastal aquifers, for example on the Cooley Peninsula.

Many more colonisation pathways via surface waters are available to stygophile species. As a result, it is not surprising that the geographic range of species such as *Diacyclops bicuspidatus* extends to the north coast and into regions with poorly productive aquifers (Fig. 2.13). While the same colonisation pathways would also be available to isopods such as *Asellus aquaticus*, they seem to have more specific requirements regarding the structure of the aquifer, i.e. they were only found in karstic settings (Fig. 2.14). This

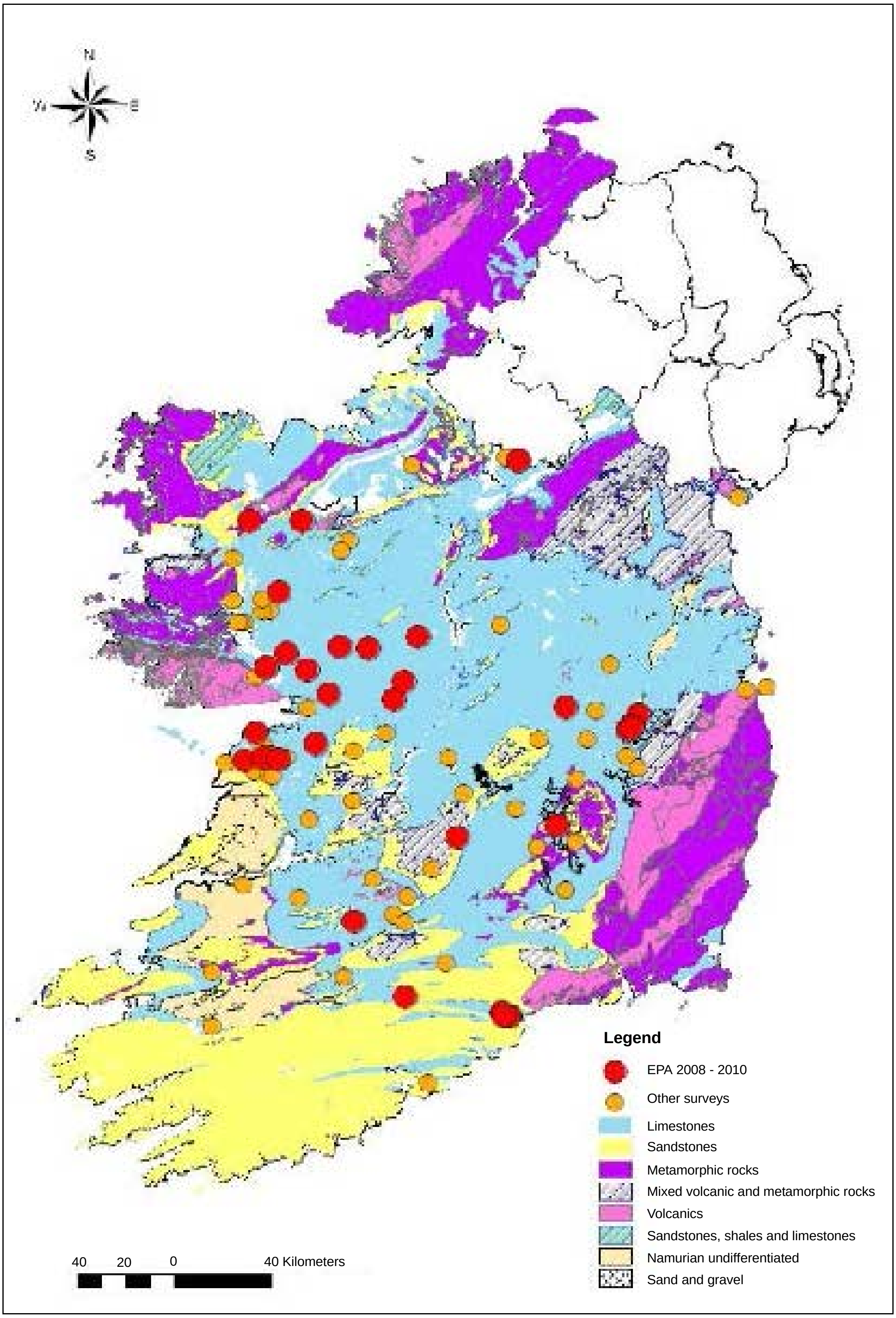

Figure 2.9. Map of known *Niphargus k. irlandicus* records, rock units and regionally important sand and gravel deposits. Data from other surveys are from the British Cave Research Association (BCRA) biological recorder database, Knight and Penk (2010) and Arnscheidt et al. (2008). Geological data were provided by the Geological Survey of Ireland.

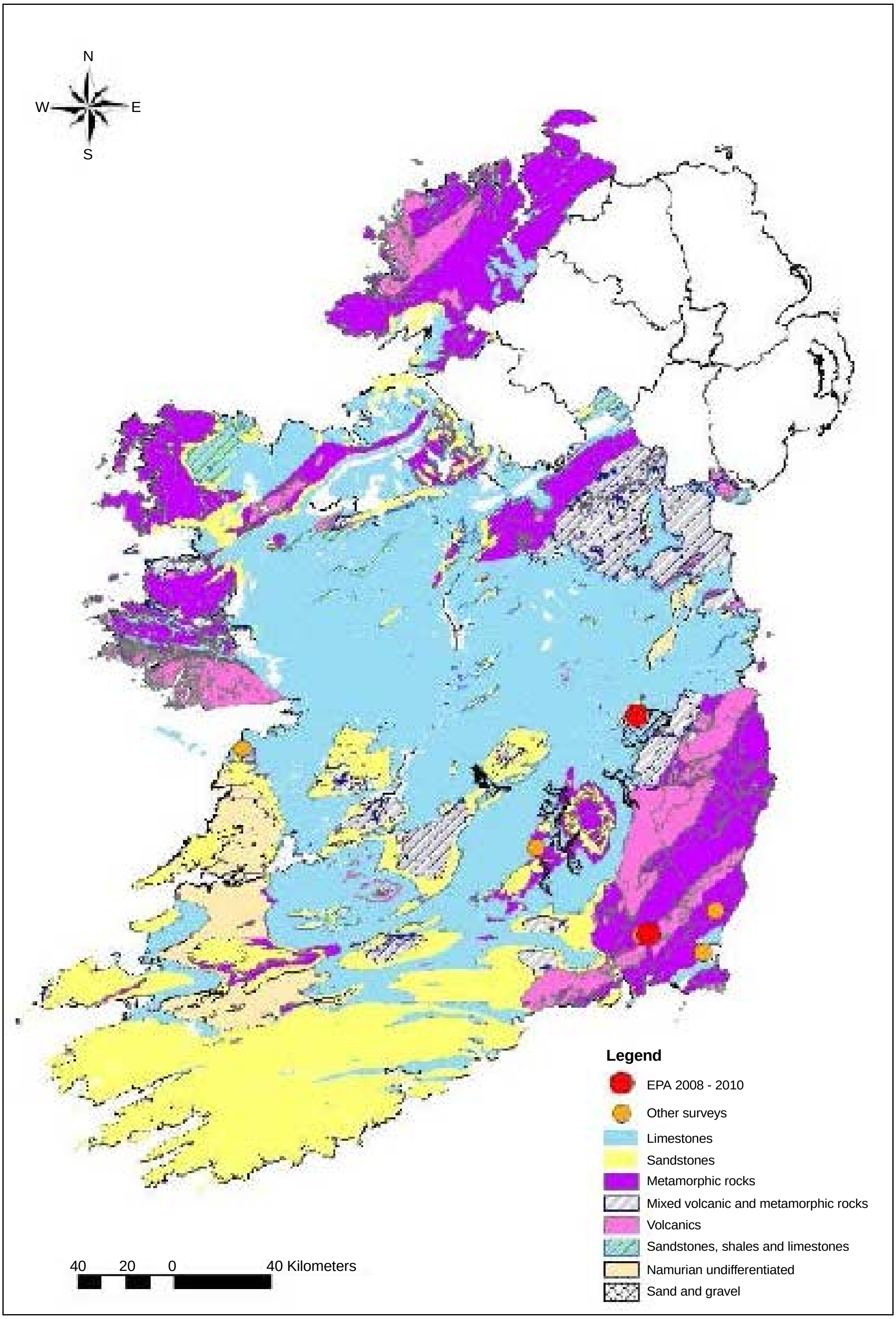

Figure 2.10. Map of known *Niphargus wexfordensis* records, rock units and regionally important sand and gravel deposits. Data from other surveys are from the British Cave Research Association (BCRA) biological recorder database, Knight and Penk (2010) and Arnscheidt et al. (2008). Geology data were provided by the Geological Survey of Ireland.

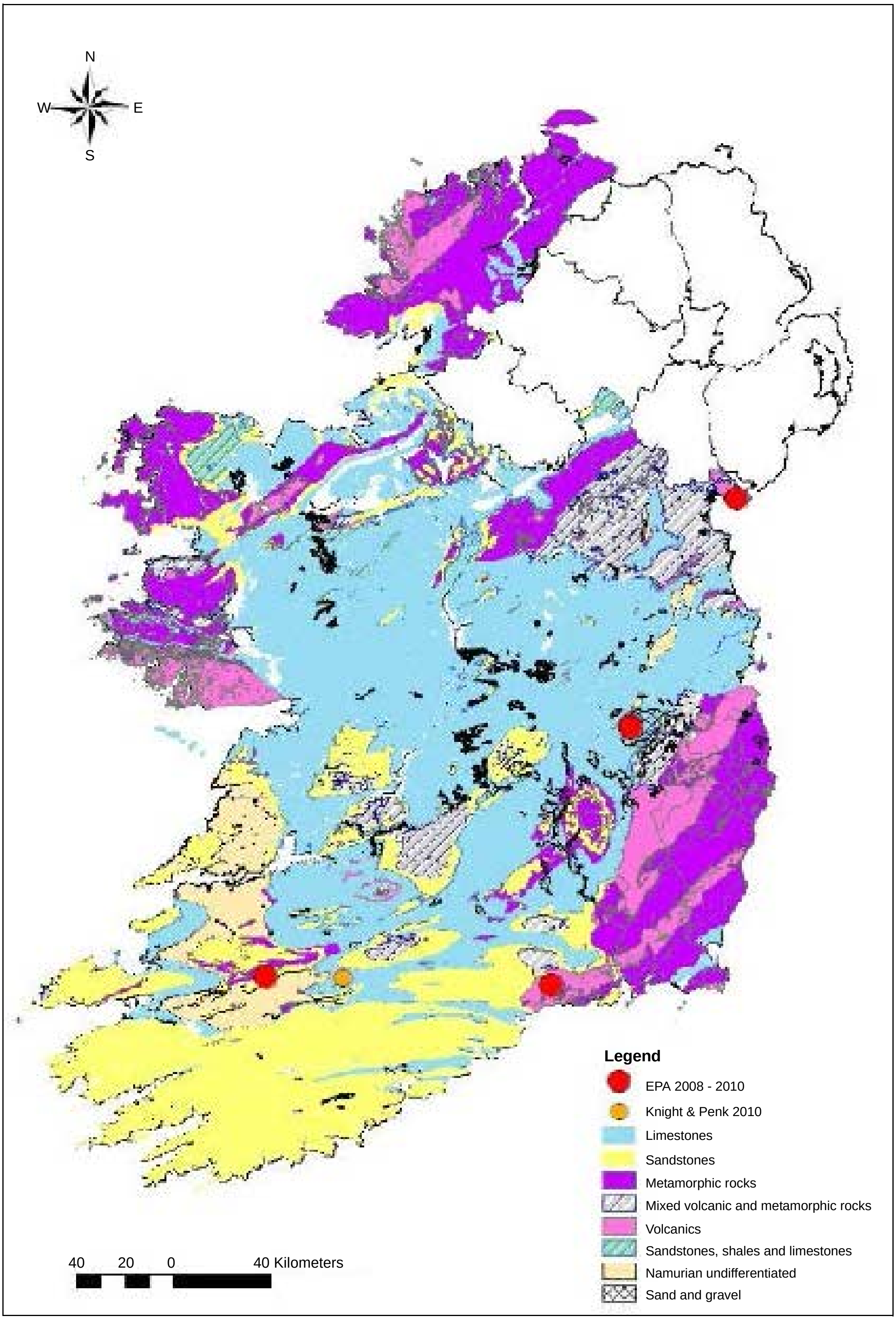

Figure 2.11. Map of known *Microniphargus leruthi* records, rock units and regionally important sand and gravel deposits. Data from other surveys are from Knight and Penk (2010) and Arnscheidt et al. (2008). Geology data were provided by the Geological Survey of Ireland.

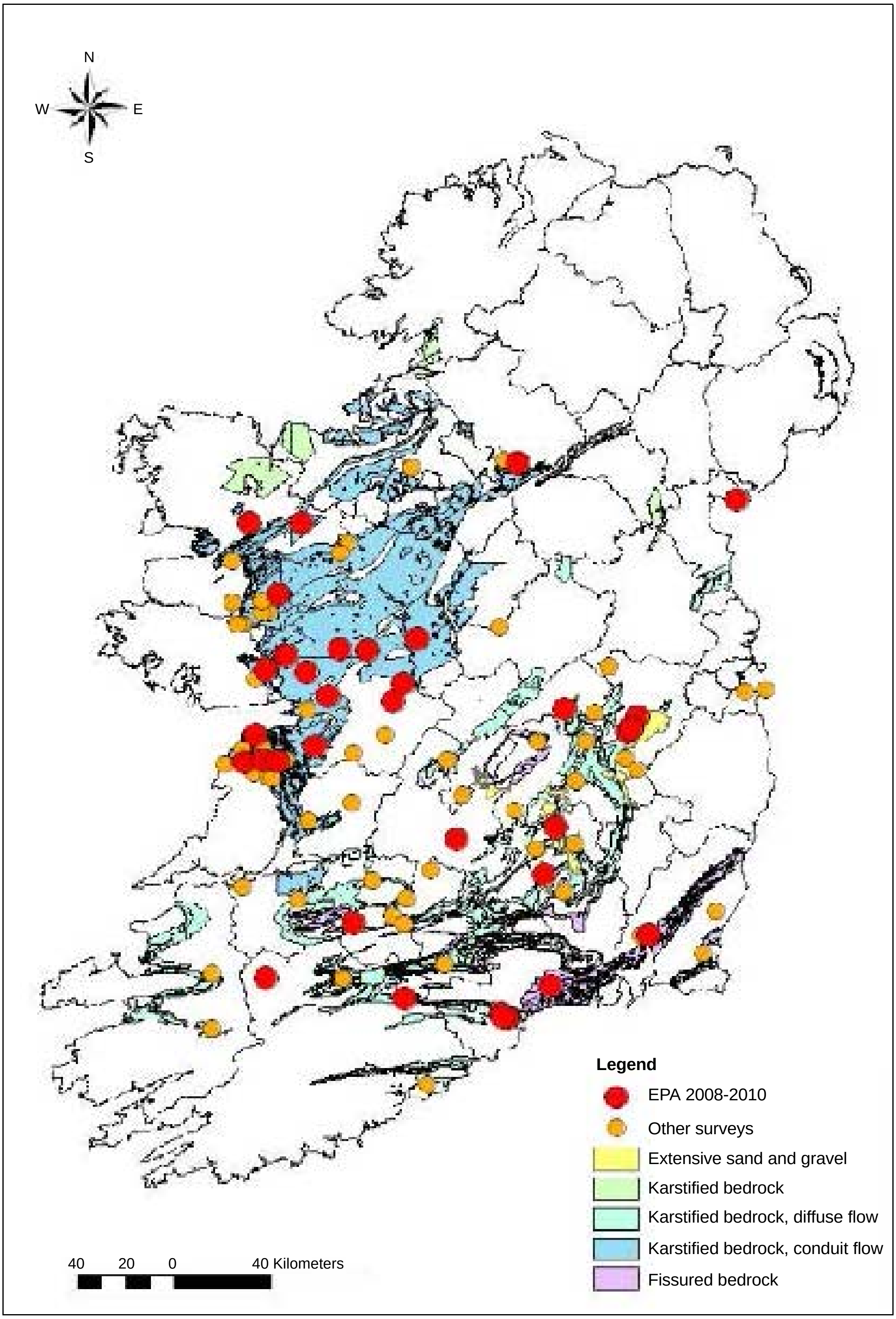

Figure 2.12. Map of known Niphargidae records and regionally important aquifers. Data from other surveys are from the British Cave Research Association (BCRA) biological recorder database, Knight and Penk (2010) and Arnscheidt et al. (2008). Hydrogeology data were provided by the Geological Survey of Ireland and the Geological Survey of Northern Ireland.

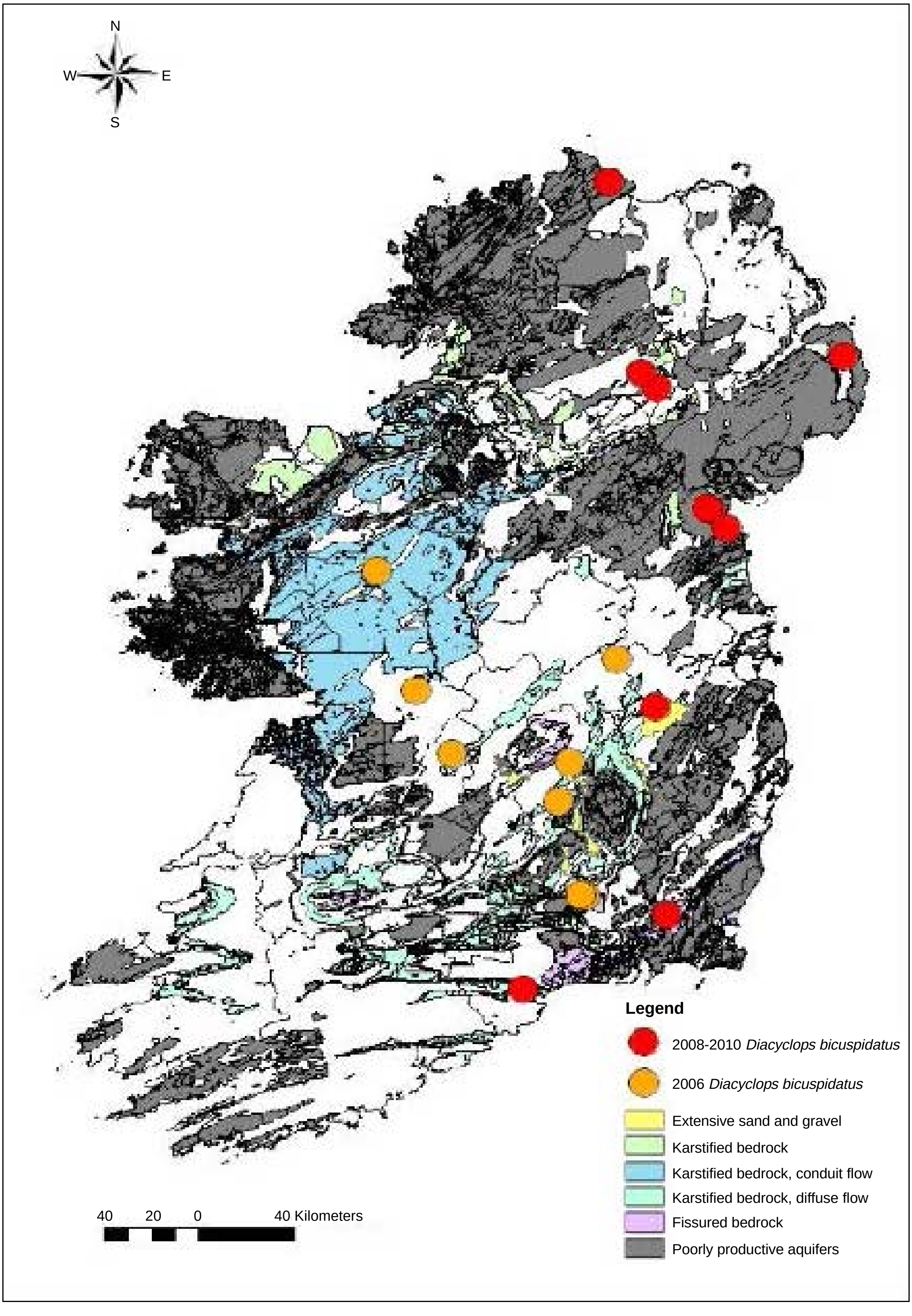

Figure 2.13. Map of known *Diacyclops bicuspidatus* records and regionally important aquifers. 2006 data are from Arnscheidt et al. (2008). Hydrogeology data were provided by the Geological Survey of Ireland and the Geological Survey of Northern Ireland.

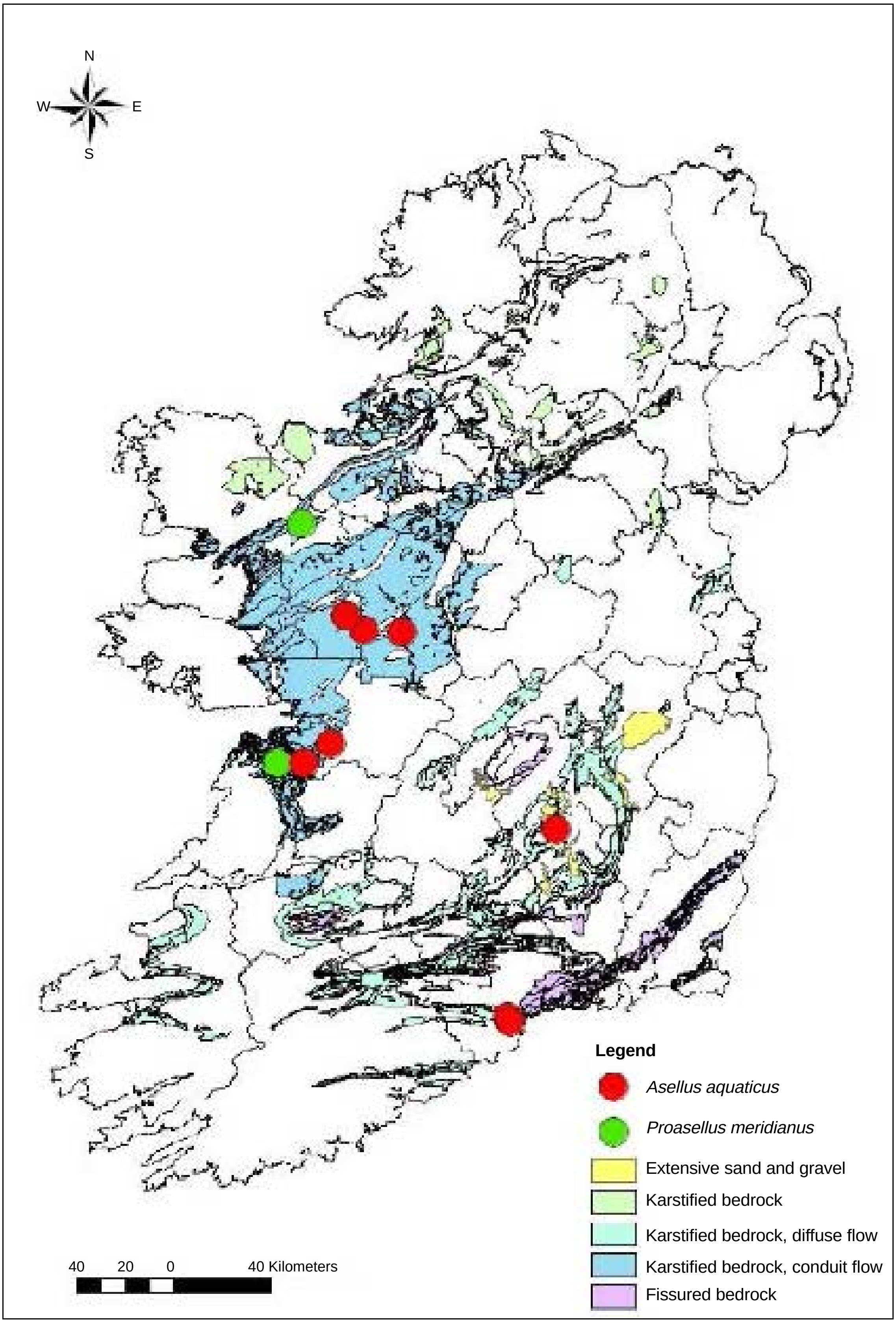

Figure 2.14. Map of records for Asellidae from the 2008–2010 survey and regionally important aquifers. Hydrogeology data were provided by the Geological Survey of Ireland and the Geological Survey of Northern Ireland.

is likely to reflect a preference for sites with very high vulnerability towards contamination from the surface, because in such a setting, with relatively high transfers of organic carbon from surface environments, availability of food resources would be higher than in well protected aquifers. *Proasellus meridianus* seems to have a less stygophilic tendency than *Asellus aquaticus*, as it was only recorded in springs; its biogeographic range within Ireland appears to be limited to the west of the island.

2.6.4 Biodiversity of Ireland's groundwater fauna

For Ireland, there are records of three species of stygobite amphipods (*Niphargus k. irlandicus, Niphargus wexfordensis* and *Microniphargus leruthi*), one stygobite harpacticoid copepod (the newly discovered *Moraria* species), two stygobite ostracod species (*Fabaeformiscandona breuili* and *Fabaeformiscandona wegelini*) and the elusive syncarid *Anthrobathynella stammeri*, bringing the total up to seven species. Even when the numerous stygophile species are included, groundwater faunal diversity is much lower in Ireland than the biodiversity hotspots around the Mediterranean Sea. This is most likely the consequence of this island's glacial history. Yet there have been only a few targeted investigations to date, all of which have led to new discoveries, such as the blind *Gammarus* and the *Moraria* species, the latter of which is new to science. More species are likely to be recorded and discovered in future surveys, especially if they focus on individual regions.

2.7 Conclusion

- The biogeographic distribution of Irish groundwater fauna reflects the island's hydrogeological setting. For stygobite species with weak dispersal abilities, areas with poorly productive aquifers appear to be dispersal barriers confining their geographic range.

- Species associations with bedrock geology vary between different species of the same taxonomic order. Examples are *Niphargus k. irlandicus* and *Niphargus wexfordensis*. While both species have been recorded from limestone areas, only *Niphargus wexfordensis* has also been recorded from areas with volcanic bedrock. Stygobite animals tolerate a low oxygen environment, but do not occur at saturation levels of 5% or lower. Surface water fauna with stygophile tendencies can occur in settings with very high groundwater vulnerability and would therefore have a potential for verifying vulnerability classification by bioindication.

- Although Ireland does not appear to be a biodiversity hotspot for groundwater fauna, the endemic nature of some of its groundwater species makes them unique. There is still no indication that we may already know the full extent of biodiversity in Ireland's aquatic subterranean crustacean fauna. New surveys are very likely to result in new/first records for Ireland or species new to science.

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3 Temporal Monitoring Programme

3.1 Aims and Research Questions

The work programme aimed to generate baseline data with regard to identifying seasonality in abundance and diversity of groundwater fauna retrieved from wells and boreholes in two different aquifer types.

Research questions:

1. Which sampling season is likely to yield the best results with regard to abundance and diversity of groundwater fauna?
2. How many sampling sites are necessary to characterise a groundwater body with regard to abundance and diversity of groundwater fauna?
3. Are correlations between non-biotic parameters and fauna strong enough to be useful for hypothesis building/testing?
4. Do observed patterns vary across different types of aquifers?

3.2 Introduction

Spatial and temporal abundance and diversity patterns for invertebrates have only recently received more attention in the study of groundwater ecology due to the problems associated with accessing fauna. Most results are available from hyporheic zones along surface water–groundwater gradients, while studies of true groundwater sites have been relatively rare to date.

Malard (2001) has provided an example of the potential of temporal monitoring of groundwater invertebrates for revealing patterns of contamination in karst aquifers. Hahn (2005) was able to demonstrate the effect of groundwater abstraction on the fauna inhabiting a sand and gravel aquifer, and investigations in Australian alluvial aquifers have proved that realistic estimates of groundwater invertebrate biodiversity can only be achieved with several sampling occasions at each individual sampling site (Hancock and Boulton, 2009).

Evidence suggests that the number of groundwater species in an aquifer may be quite accurately reflected by samples obtained from boreholes (Hahn and Matzke, 2005) or traps in piezometers (Hahn, 2005). However, proportional taxonomic composition could display significant differences between the environment inside and outside a borehole. Hahn and Matzke (2005) sampled more amphipods and fewer copepods from boreholes than from the surrounding sand and gravel aquifer. They reported that boreholes were generally very efficient traps for groundwater invertebrates and particulate organic matter as their potential food source. Therefore, total abundance of groundwater invertebrates tends to be higher at borehole sites than in the surrounding groundwater environment. Pumping studies of boreholes have repeatedly demonstrated that the abundance of invertebrates per unit volume of water declined with increasing sampling volume, for example Steenken (1998). In terms of sample yield/effort, it is therefore efficient to concentrate the sampling effort on the water within boreholes.

If evidence from hyporheic environments could be transferred to groundwater environments, investigations by Panek (1991) and Wagner and Feio (2001) would suggest that food scarcity may lead to increased searching activity and thus a higher probability of entering a trap. Therefore, abundance in boreholes may not only be a function of the invertebrates' standing crop, but also a reflection of their activity patterns.

3.3 Study Area

The temporal survey was conducted in two different aquifer types (sand and gravel, limestone). Table 3.1 provides some characteristics of the sampling sites.

3.3.1 Curragh sand and gravel aquifer to the east of the town of Kildare in Co. Kildare

This aquifer in a lowland setting is classified as regionally important 'Rg' (GSI, undated a). Sand and gravel deposits are of glacio-fluvial or glacio-lacustrine

Table 3.1. Details of sampling sites from the temporal monitoring 2009–2010.

Site	Name	Easting	Northing	Type	Borehole diameter (m)	Total depth (m)	Screen length (m)	Screen depth (m)
C1	Tully BH1	273313	210923	BH	0.15	9.12	0.34	
C2	Kildare Chilling Factory	274788	212224	BH	0.05	14.38	0	
C3	MB30	275042	213190	BH	0.15	10.75	0.31	12–24
C4	MB29	275043	213065	BH	0.15	24.40	0.27	9.3–13.3
C5	MB09	277368	217300	BH	0.18	11.75	0	
C6	MB37	277433	217126	BH	0.18	13.47	0	14.2–18.2
D1	Dungarvan Area J	221339	95043	BH	0.18		0.38	
D2	Dungarvan Area D	224000	93225	BH	0.18	47.78	0.22	
D3	Dungarvan Area G	221595	93225	BH	0.18		0.19	
D4	Waterford Crystal Factory	224501	93076	BH	0.18	45.05	0.10	
D5	Dungarvan PWS (Ballinamuck)	223631	94709	BH	0.25	23.20	–	
D6	Dungarvan PWS (Springmount)	224638	92840	Well	2.5 x 2.5	4.97	–	

BH, borehole; PWS, public water supply.

origin with a usual thickness between 20 and 40 m and these deposits rest on limestone bedrock. They are primarily covered by sandy till with moderate to low hydraulic conductivity. The Curragh sand and gravel aquifer has a high recharge coefficient (81–85%) and was classified as highly vulnerable (Misstear and Brown, 2010). Sampling sites were selected along a south-west/north-east transect (Fig. 3.1). There was no known water abstraction from any of them. C1 was close to the area where the aquifer discharges towards the south-east at springs in the Japanese Gardens, Co. Kildare (Hayes et al., 2001), groundwater at C2 was presumed to flow in the same direction. Water from C3 and C4 was presumed to flow towards the Pollardstown Fen/Milltown feeder and so does water from C5 and C6, albeit from the other side of the Fen (Misstear and Brown, 2010). Site C3 was close to Site C4 and so was Site C5 to C6. The reason for their selection was that they sampled different depths.

3.3.2 *Dungarvan limestone aquifer at the town of Dungarvan in Co. Waterford*

This regionally important aquifer was classified as Rkd (GSI, undated b), i.e. as karstic with diffuse flow. Bedrock geology consists mainly of pure unbedded Dinantian limestones. Figure 3.1 shows the location of sampling sites. The Ballinamuck site (D5) was a source for drinking water production, with a reported mean abstraction rate of 5,928 m^3/day. There were four boreholes, with one pumping at a time (EPA, 2011a). The Springmount site (D6) was a karst spring, which had been modified to augment the source at the Ballinamuck site. Augmentation from Springmount was not necessary between 2006 and 2011 (EPA, 2011b). The sampled site was an artesian dug well, which had been established in the 1980s, but has not been pumped since the late 1990s. Sampling Sites D1–D3 were boreholes from exploratory drilling activity with no known groundwater abstraction. Borehole D4 fell out of use in 2007, when the Waterford Crystal factory closed down. The recharge coefficient for the Dungarvan aquifer was calculated as 90% from data in GSI (1998). The cover of the aquifer varied from moderately permeable sandy tills of 0–10 m thickness to less than 1 m rock cover. As a result, groundwater vulnerability classification in this area varied from moderate to extremely vulnerable (GSI, 1998).

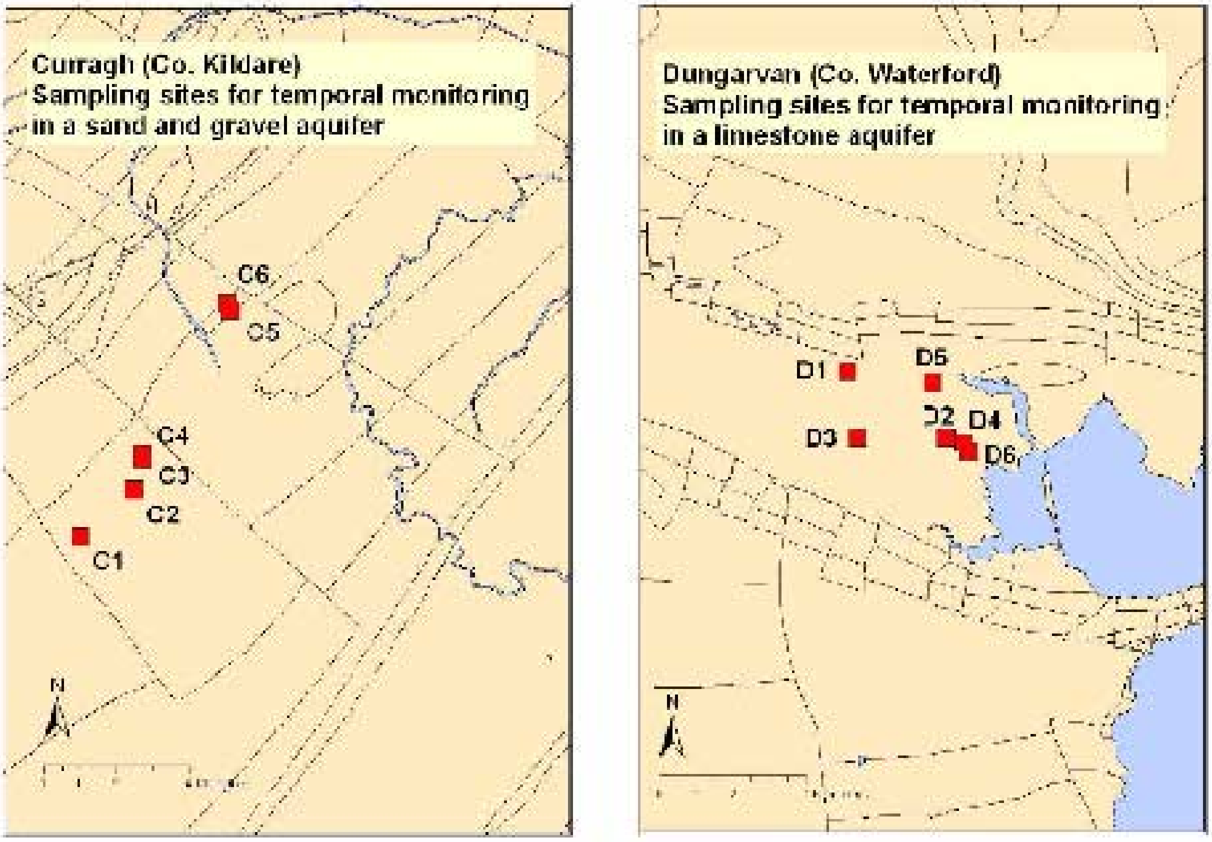

Figure 3.1. Maps of the sampling sites for temporal monitoring at the Curragh sand and gravel aquifer (left) and the Dungarvan limestone aquifer (right).

3.4 Methods

Six sites were selected from each aquifer. Sampling took place six times at each site between June 2009 and June 2010. Methods for sampling and laboratory analysis were the same as those for sampling of boreholes and wells outlined in Chapter 2.

Field measurements of physical and chemical parameters were compared with results in the EPA groundwater monitoring database containing results from the years 1993–2008 (Curragh: EPA measurements from sites at Pollardstown Fen and Curragh Camp; Dungarvan: EPA data for Ballinamuck and Springmount PWS[1]). EPA data for Dungarvan often represented post-treatment (chlorination) values.

Data analysis was undertaken within SPSS 19. It investigated non-parametric correlations between hydrological or chemical parameters and abundance of crustacean fauna. Only sites with any records for Niphargidae and Cyclopoida from the sampling period were included in this analysis in order to avoid a bias due to any potential unknown construction details preventing fauna access. In most instances, a Spearman rank correlation analysis was performed. For seasonal correlations the very first sample was excluded from the analysis, because the duration of the borehole or well sampling period for this sample was undefined and thus could have introduced bias.

3.5 Results

3.5.1 Chemistry

Oxygen saturation was lower at the karstic limestone aquifer sites than at the sand and gravel aquifer sites.

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[1] PWS, Public water supply

In comparison with minimum and maximum values from the EPA database (Fig. 3.2a–d), one Curragh site (C6) had lower values for oxygen saturation from August 2009 to January 2010. At Dungarvan, oxygen saturation even fell below 10% at some sites. Two sites (D1, D2) had lower oxygen saturation during the whole monitoring period; one site (D3) had lower values between August and November 2009. Electrical conductivity was generally higher at the Curragh sites than at the Dungarvan sites. At Curragh boreholes with relatively shallow depths, electrical conductivity values were occasionally lower than the minimum value of 384 µS/cm recorded in the database for EPA monitoring sites of this aquifer. At Site C3, this happened three times. In January, electrical conductivity values at all Dungarvan sites were lower than the minimum database value of 381 µS/cm for EPA monitoring sites of this karstic aquifer. During the sampling period, such low conductivity results occurred repeatedly at Sites D1–D3.

3.5.2 *Abundance of crustacean fauna*

Figures 3.3 and 3.4 illustrate that abundance patterns varied considerably between individual sites, for example peaks occurred in different seasons even for sites in close proximity to each other. Combined totals for each aquifer, however, reveal more regular patterns. For example at Dungarvan, niphargid numbers peaked in summer and declined in winter (Fig. 3.3). There were peaks in summer and autumn at

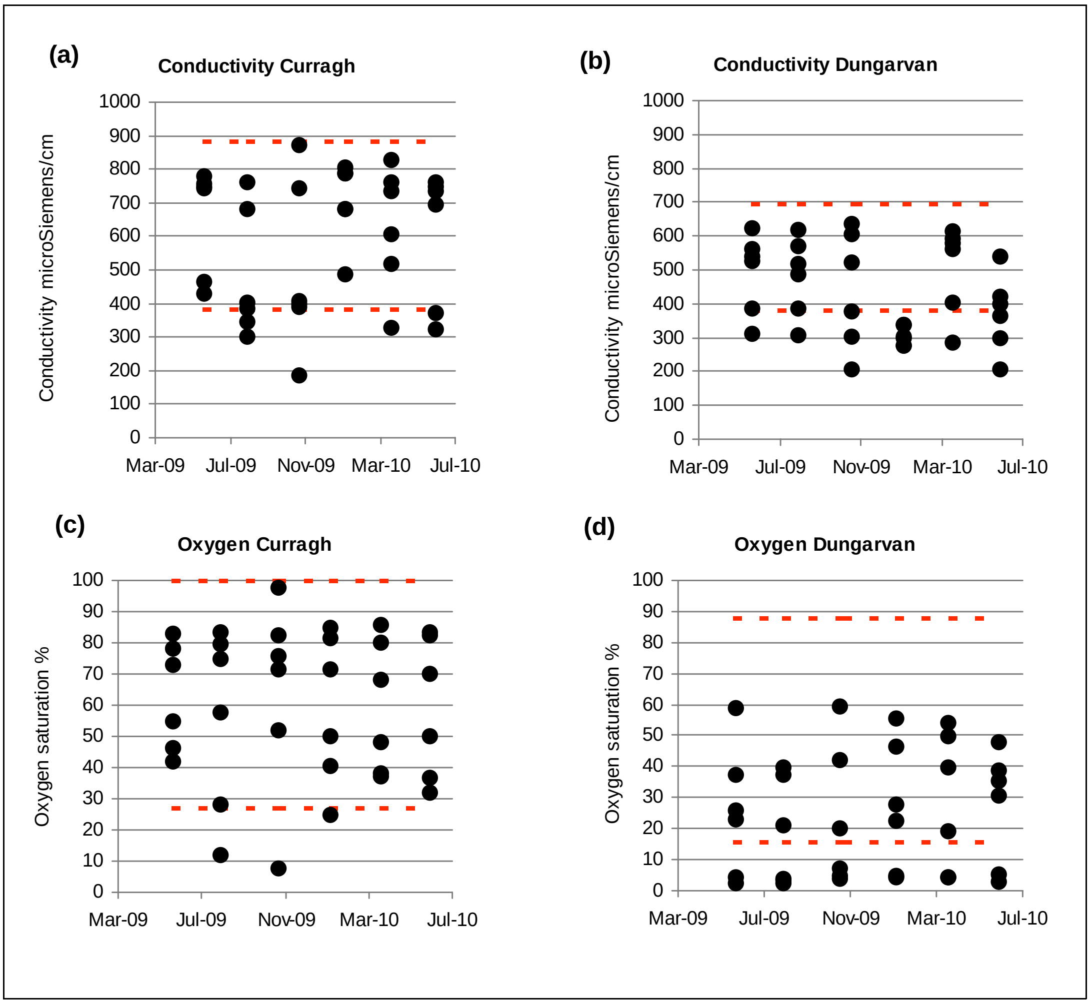

Figure 3.2. Scatter plots showing results of electrical conductivity measurements from the (a) sites in the Curragh, (b) sites at Dungarvan and results for oxygen saturation from the (c) sites in the Curragh and (d) sites at Dungarvan. Broken lines indicate maximum and minimum values from results for Environmental Protection Agency monitoring sites in the respective aquifers.

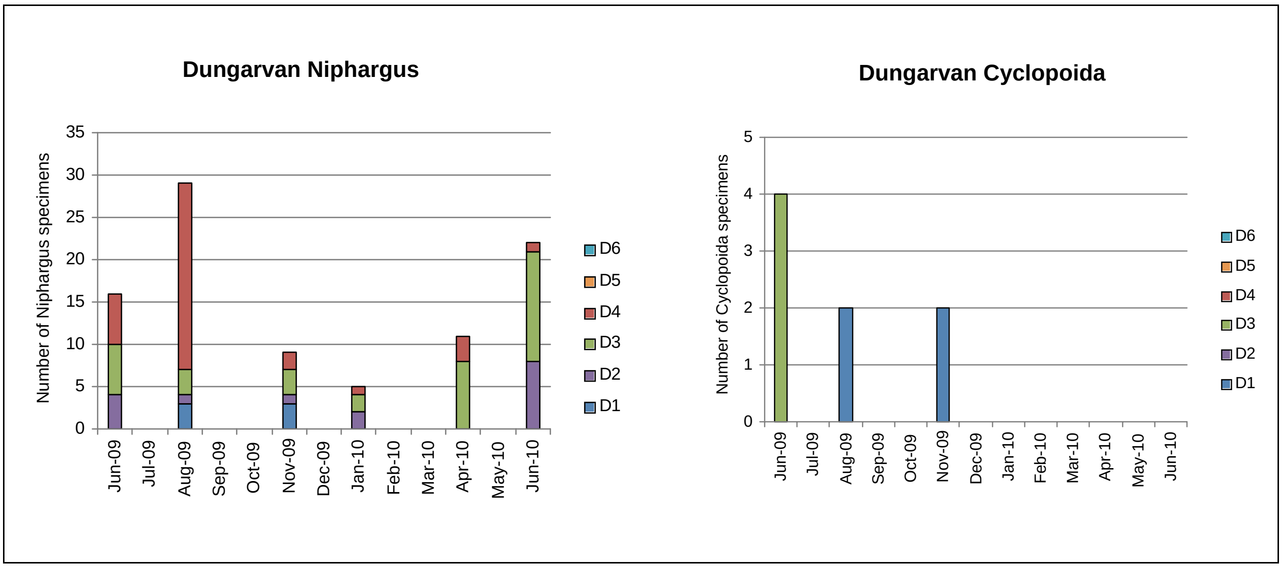

Figure 3.3. Bar charts with results of abundance for major taxonomic crustacean groups at the Dungarvan sampling sites. No fauna was recorded from Sites D5 and D6.

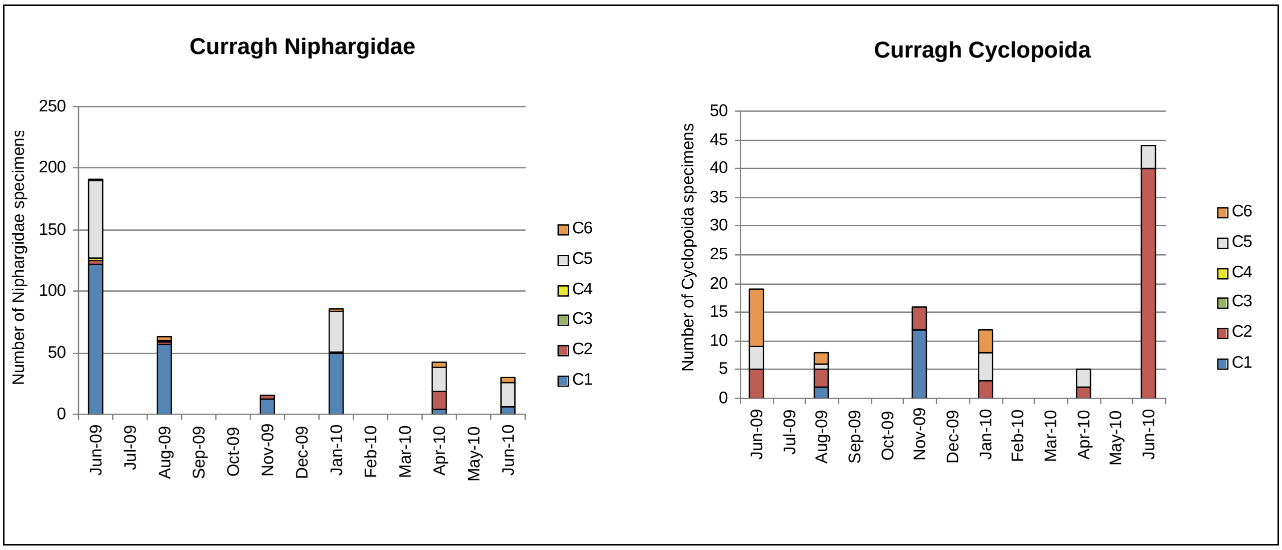

Figure 3.4. Bar charts with results of abundance for major taxonomic crustacean groups at the Curragh sampling sites.

the Curragh sites and there appears to be some synchronicity between Niphargidae and Cyclopoida abundance in this sand and gravel aquifer (Fig. 3.4).

At Dungarvan Sites D5 and D6, no animals were recorded, with the exception of *Asellus aquaticus*. Among crustaceans the only taxonomic groups with sufficiently high numbers for correlation analysis were Niphargidae and Cyclopoida; the number of Cyclopoida was only sufficient at the Curragh sites, but not at Dungarvan. For the seasonal analysis (Table 3.2), only the correlation between the depth of the water level below ground level and the number of Niphargidae specimens at the Dungarvan sites was significant ($p < 0.05$).

At the Curragh sites, a strong negative correlation between the depth range and the site-specific total of Niphargidae specimens was recorded and also a strong positive correlation between the range of

Table 3.2. Spearman rank correlation analysis results for individual aquifers: arithmetic means of non-biotic parameters at individual sampling dates are analysed for correlation with pooled abundance totals from the same date.

Aquifer	Parameter	Taxon	N	ρ	P value
Dungarvan	Water table depth	Niphargidae	5	0.900	0.037*
Dungarvan	2 months rainfall prior to sampling	Niphargidae	5	–0.400	0.505
Dungarvan	2 months rainfall – evaporation prior to sampling	Niphargidae	5	–0.700	0.188
Dungarvan	Conductivity	Niphargidae	5	0.500	0.391
Curragh	Water table depth	Niphargidae	5	–0.327	0.591
Curragh	2 months rainfall prior to sampling	Niphargidae	5	0.700	0.188
Curragh	2 months rainfall – evaporation prior to sampling	Niphargidae	5	0.300	0.624
Curragh	Conductivity	Niphargidae	5	0.400	0.505
Curragh	Water table depth	Cyclopoida	5	0.200	0.747
Curragh	2 months rainfall prior to sampling	Cyclopoida	5	–0.300	0.624
Curragh	2 months rainfall – evaporation prior to sampling	Cyclopoida	5	–0.200	0.747
Curragh	Conductivity	Cyclopoida	5	–0.100	0.873

*Significant for p < 0.05.

electrical conductivity values and the site-specific total of Cyclopoida was observed; both correlations were significant for p < 0.05 (Table 3.3).

At Dungarvan, a strong positive correlation between the range of oxygen saturation values and the site-specific total abundance of Niphargidae specimens missed the significance threshold by an extremely narrow margin of 0.001 (Table 3.3). A higher abundance of Niphargidae was recorded at Dungarvan sites, with lower frequencies of low oxygen saturation (Fig. 3.5).

3.5.3 *Diversity of crustacean fauna*

A total of six samples from each site yielded a maximum diversity of three crustacean species per site (Table 3.4). The Curragh aquifer sites assemble all three currently known Irish niphargid amphipods and at least two cyclopoid copepod species. For the Dungarvan sites, so far, only one *Niphargus* species, two cyclopoid copepod species and the isopod *Asellus aquaticus* have been recorded. The latter species is commonly known from surface water environments. It has a tendency to move into subterranean environments, where it is then encountered near springs and caves. The cyclopoid species are known to occur in surface and subterranean waters, while the niphargids all represent true groundwater species.

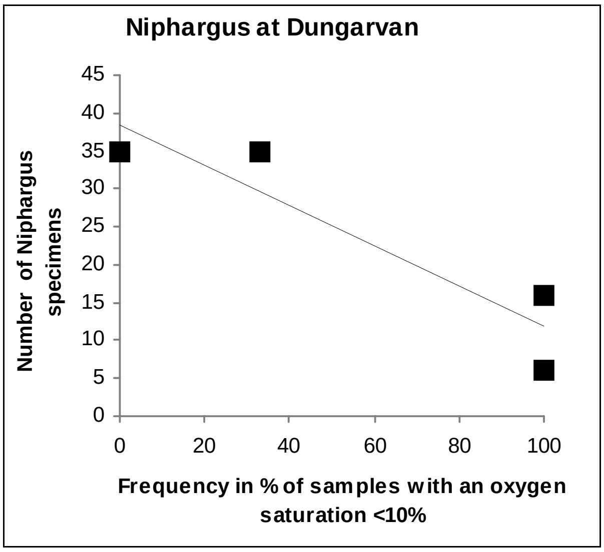

Figure 3.5. Scatterplot showing the frequency of water samples with oxygen saturation values below 10% for individual Dungarvan sites and the number of *Niphargus* specimens from those sites.

Table 3.3. Spearman rank correlation analysis results: arithmetic means of non-biotic parameters at individual sampling sites are analysed for correlation with abundance totals from the same site.

Aquifer	Parameter	Taxon	N	ρ	P value
Curragh	Depth range	Cyclopoida	6	–0.448	0.373
Curragh	Conductivity mean	Cyclopoida	6	0.609	0.200
Curragh	Conductivity minimum	Cyclopoida	6	0.160	0.762
Curragh	Conductivity range	Cyclopoida	6	0.870	0.024*
Curragh	Oxygen saturation mean	Cyclopoida	6	–0.203	0.700
Curragh	Oxygen saturation minimum	Cyclopoida	6	0.058	0.913
Curragh	Oxygen saturation range	Cyclopoida	6	–0.058	0.914
Curragh	Temperature range	Cyclopoida	6	0.279	0.592
Curragh	Depth range	Niphargidae	6	–0.883	0.020*
Curragh	Conductivity mean	Niphargidae	6	0.371	0.468
Curragh	Conductivity minimum	Niphargidae	6	–0.314	0.544
Curragh	Conductivity range	Niphargidae	6	0.771	0.072
Curragh	Oxygen saturation mean	Niphargidae	6	–0.429	0.397
Curragh	Oxygen saturation minimum	Niphargidae	6	–0.314	0.544
Curragh	Oxygen saturation range	Niphargidae	6	0.406	0.425
Curragh	Temperature range	Niphargidae	6	0.696	0.125
Dungarvan	Depth range	Niphargidae	4	–0.211	0.789
Dungarvan	Conductivity mean	Niphargidae	4	0.738	0.262
Dungarvan	Conductivity minimum	Niphargidae	4	0.211	0.789
Dungarvan	Conductivity range	Niphargidae	4	0.738	0.262
Dungarvan	Oxygen saturation mean	Niphargidae	4	0.738	0.262
Dungarvan	Oxygen saturation minimum	Niphargidae	4	0.500	0.500
Dungarvan	Oxygen saturation range	Niphargidae	4	0.949	0.051(*)
Dungarvan	Temperature range	Niphargidae	4	–0.183	0.718

*Significant for $p < 0.05$; (*) narrow miss.

3.6 Discussion

Groundwater chemistry analysis is normally performed after pumping at least several borehole volumes, so as to make sure that the water that is analysed has been retrieved from direct contact with the aquifer. There was no active pumping undertaken in this study, so borehole conditions may have come to bear on the chemistry results. Curragh electrical conductivity results are relatively consistent with database records and there is no evidence for ‘short circuit’ flow of water from the surface through cracks or gravel packs around boreholes in this aquifer. For Dungarvan sites, such a possibility cannot be excluded with the same certainty. Yet, it has to be taken into consideration that the database contains values from sites with groundwater abstraction. In comparison to sites with little water movement, water abstraction would be expected to narrow the range of analysis results for many parameters due to the mixing along forced preferential flow paths. It is therefore plausible that low conductivity values at Dungarvan sites reflect local heterogeneity due to groundwater recharge. Pumping

Table 3.4. Crustacean species recorded from individual sampling sites.

Site	Species
C1	*Eucyclops serrulatus*
C1	*Niphargus kochianus irlandicus*
C2	*Microniphargus leruthi*
C2	*Eucyclops serrulatus*
C3	*Microniphargus leruthi*
C4	*Niphargus kochianus irlandicus*
C5	*Niphargus kochianus irlandicus*
C5	*Acanthocyclops vernalis*
C6	*Acanthocyclops vernalis*
C6	*Niphargus wexfordensis*
D1	*Diacyclops bicuspidatus* (s. str.)
D1	*Niphargus kochianus irlandicus*
D2	*Niphargus kochianus irlandicus*
D3	*Niphargus kochianus irlandicus*
D4	*Diacyclops languidus*
D4	*Niphargus kochianus irlandicus*
D4	*Asellus aquaticus*
D5	*Asellus aquaticus*
D6	*Asellus aquaticus*

for water abstraction and subsequent treatment processes can lead to gas exchange and aeration of groundwater. Hence, results from in situ measurements would be expected to fall below post-treatment measurements at the Dungarvan water sources. Low oxygen saturation values can, however, also reflect decomposition of organic material trapped and accumulated in the borehole sump.

When compared with the all-Ireland survey, results from temporal monitoring suggest that repeated sampling will not lead to a much higher diversity of crustacean species being recorded at individual sites. However, sampling more sites per aquifer seems to increase the diversity of species found in the respective hydrogeological unit. While it remains speculative to propose a minimum number of sampling sites for biodiversity studies in Ireland, results suggest that sampling more sites is not likely to result in a steep biodiversity increase for any of the two aquifer types.

Absence of fauna from some sampling sites at the Dungarvan limestone aquifer may be a consequence of abstraction of large water volumes currently or in the recent past. If abstraction was to lead to a decrease in faunal abundance, the number of recorded specimens at the Dungarvan site of the Waterford Crystal factory suggests that total eradication is not necessarily the consequence. Animal records from the Curragh site at the former chilling factory would also seem to support this conclusion.

Results from both aquifer types indicate that the Irish summer is a good sampling season, if the survey is focused on boreholes, since the highest seasonal abundances were recorded at that time of year. Four to six sites with fauna can apparently be sufficient to reveal consistent seasonal abundance patterns for subterranean crustaceans in an individual aquifer. However, the question remains whether such patterns resemble (a) actual changes in abundance, (b) the animals' activity patterns, or (c) the frequency of passive transport by water, thus indicating seasonal hydrological changes. Considering the slow reproductive cycle and a comparatively low fecundity of groundwater crustaceans, hypothesis (a) would currently seem to be the least likely. Under field conditions, it is difficult to discriminate between (b) and (c) (active migration and passive transport). While there does not seem to be any published experimental evidence for changes in seasonal activity patterns, the response of groundwater fauna to hydrological processes has been suggested by several authors (e.g. Schmidt et al., 2007).

Interestingly, all evaluated parameters that were significantly correlated with crustacean abundance can be taken as proxies for hydrological processes. A site's wide range of electrical conductivity values indicates an environment where low conductivity water from recent groundwater recharge mixes with 'older' high conductivity groundwater. Curragh sites with these characteristics had significantly higher abundances of cyclopoids. Exposure to 'new' recharge water would also imply an import of bio-available organic carbon from the soil cover, thus slightly increasing availability of potential food resources, which would be attractive for both groups of animals. In an indirect way, high crustacean abundance could thus also indicate groundwater vulnerability to pollution. In the karstic limestone aquifer, exposure to 'new' recharge water is likely to be prevalent along flow paths with occasionally high velocities, which these animals might find difficult to withstand. This would explain the absence of a significant correlation between abundance and the range of electrical conductivity for this aquifer type.

Groundwater level is a parameter that can be directly related to hydrological processes. In the Dungarvan aquifer, the water level fell with decreasing recharge during the summer, while niphargid abundance rose. Dispersal of niphargids during times of rising water tables and thus a dilution effect with regard to their abundance (more space becomes available to the same number of animals) would provide an explanatory hypothesis. There is qualitative evidence for this, as the animals have frequently been observed in non-permanent cave pools in periods with higher water tables fed by karstic aquifers (personal observation by Tara Higgins (2008) and Karin Eriksson (2009)). When the water table retreats, the animals have to move down with it, i.e. they become more concentrated in the remaining space. In this concentration phase, they would also be more likely to become trapped in a borehole. At a sand and gravel site, Hahn (2005) observed a rising abundance of copepod species at a site that had a falling groundwater table due to abstraction. He suggested a vertical abundance gradient (abundance decreases with increasing groundwater depth) as an explanation.

Freedom of movement for larger groundwater animals such as niphargids can be expected to depend on aquifer properties such as porosity and permeability. When compared with karstic aquifer flow path networks, a filtering effect of a sand and gravel aquifer matrix would make it much harder for groundwater animals to keep up with the downward movement of the water table. In sand and gravel aquifers, there is thus potential for an increased risk of larger animals 'being left behind' and becoming desiccated, thus preventing them from accumulating large abundances at sites with high water table dynamics.

Water chemistry results from EPA groundwater quality monitoring suggest the existence of patches with low oxygen saturation in the Dungarvan aquifer. Low oxygen saturation can indicate relatively high microbial respiration activity, a low rate of water exchange or both. Microbial activity indicates the presence of bioavailable organic carbon sources, i.e. potentially increased availability of food. Moreover, sulfide-metabolising bacteria near boundaries of oxic and anoxic conditions have been suggested as a potentially important food source for subterranean invertebrates (Dov Por, 2007). Therefore, aquifer zones with low oxygen saturation cannot be immediately discounted as wholly unattractive for crustaceans, especially since some of them are able to tolerate periods of low oxygen saturation (Malard and Hervant, 1999). Nevertheless, the strong positive correlation between niphargid abundance and the range in oxygen concentrations at Dungarvan sites would indicate that *Niphargus k. irlandicus* at least prefers to live in the proximity of zones with high oxygen saturation. If the duration of periods with low oxygen saturation is a critical parameter for the animals' survival, temporal monitoring results will be far more conclusive than results based on one visit per site with regard to species-specific tolerance of low oxygen saturation, even if the latter comprise many survey sites.

3.7 Conclusions

- Temporal monitoring results suggest that sampling in the summer months (June–August) will yield the highest abundance and diversity of groundwater crustaceans.

- Sets of at least six sampling sites per aquifer are likely to yield optimal results for one-off surveys of groundwater fauna in Irish boreholes.

- Hydrology-related parameters appear to be strongly correlated with faunal abundance.

- There were notable differences in faunal response to individual parameters between aquifer types, for example groundwater-level fluctuations only had a strong negative correlation with abundance of Niphargidae in the sand and gravel aquifer, but not in the karstic aquifer.

Results presented here suggest that much is to be gained from seasonal monitoring in terms of understanding the functioning of groundwater fauna and its response to hydrological conditions. Based on the evidence of hydrology's importance for faunal abundance and the difference in faunal response across aquifer types, testable hypotheses for laboratory or targeted field studies can be built. Long-term monitoring at a few selected aquifers would be particularly valuable in detecting annual and inter-annual trends. Recent cave studies have underlined the importance of long-term data sets for understanding the dynamics of subterranean fauna (Wood et al., 2008).

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4 Autecology of *Niphargus k. irlandicus*

4.1 Aims and Research Questions

The work programme aimed to provide the first investigations into the ecological niche occupied by the endemic Irish groundwater shrimp *Niphargus k. irlandicus*.

Research questions:

1. What is the temperature tolerance of *Niphargus k. irlandicus*?
2. What is the tolerance of *Niphargus k. irlandicus* with regards to salinity?
3. Which food resources can be exploited by *Niphargus k. irlandicus*?

4.2 Introduction

4.2.1 Temperature tolerance

Invertebrates are ectotherms, i.e. their body temperature is regulated by their surrounding environment. Hence, body temperature of groundwater invertebrates is largely controlled by groundwater.

Annual groundwater temperature amplitudes are usually much smaller than those of surface water temperature. From a depth of between 15 and 40 m below ground, the temperature amplitude of groundwater narrows to <0.01°C and this is considered as the start of the isothermic zone (Griebler, 2003). Even though its amplitude may be wider than at isothermic conditions, the temperature of shallow groundwater generally remains close to the mean annual soil temperature. For Ireland, Garcia-Suarez and Butler (2006) reported annual mean soil temperatures at 100 cm depth, which ranged between 11°C (Armagh) and 12.7°C (Valentia Observatory). In exception to this general expectation, shallow groundwater may be warmer in areas of geothermic activity, which are, however, quite rare in Ireland. The warmest recorded water temperature for an Irish spring is 25°C (Reynolds et al., 1998).

Biological parameters of an organism's behaviour and physiology tend to vary with body temperature. The temperature range that an animal tolerates is defined by the critical temperature minimum and maximum. Somewhere between these two values lies the thermal optimum, where the animal displays the optimal performance with regard to its behavioural and physiological functions (Angilletta et al., 2002) (Fig. 4.1). The experimental approach in this project

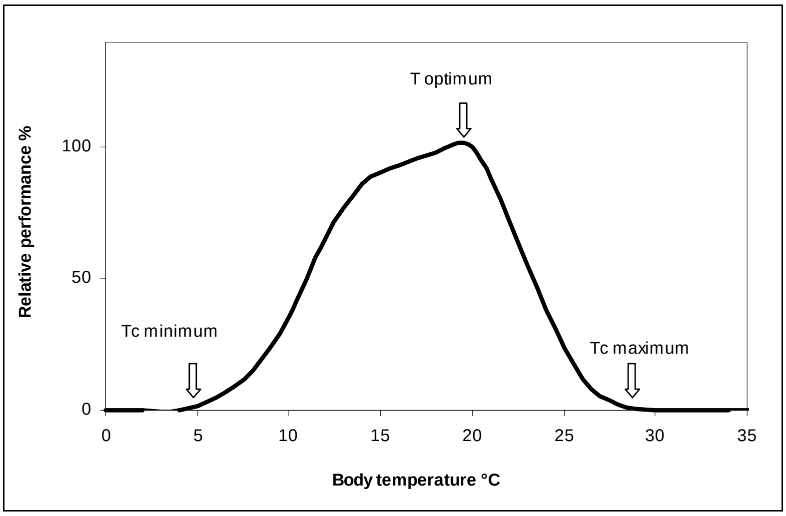

Figure 4.1. Theoretical form of relationship between body temperature and performance parameters such as locomotion (Tc, critical temperature) after Angilletta et al. (2002).

was based on the assumption that, in the absence of other stimuli, free-moving ectotherm animals, such as *Niphargus k. irlandicus*, would position themselves on a thermal gradient, so that they remain close to their thermal optimum.

4.2.2 *Salinity tolerance*

Due to their high hydraulic conductivity, subterranean habitats in karst limestone rocks located along the coasts are usually also connected to the sea. The term 'anchialine habitats' is used to designate bodies of haline waters with subterranean connections to the sea. Such habitats exhibit noticeable marine and terrestrial influences (Stock et al., 1986). Groundwater abstraction can greatly promote the intrusion of saline water in coastal limestone aquifers. Saline intrusion currently represents a threat to the low-lying karst limestone aquifers of west Clare and south-west Galway, where it can result in a substantial salinity gradient ranging from freshwater (<0.5 psu) to oligohaline–limnic (0.5–5 psu). With a high probability of sea level rises resulting from climate change, many more regionally important coastal aquifers of Ireland are likely to become inundated with saline waters in the future, affecting both their drinking water resource potential and ecology (Sweeney et al., 2008). With greater distance from coastal areas, vulnerable aquifers may still become subject to increased salt concentrations, for example due to leaching of fertilisers, irrigation practices and road salting.

There is a great lack of data on the salt tolerance of groundwater crustaceans. The only comparative study appears to be by Mösslacher (2000), who investigated the sensitivity of groundwater fauna towards elevated potassium chloride concentrations, which may be a consequence of artificial fertiliser application in agriculture. Mösslacher tested and compared the sensitivity of surface water and groundwater species of Isopoda, Copepoda and Ostracoda and, within each group, stygobite species were among the most sensitive.

While most known *Niphargus* species appear to be largely confined to freshwater environments, there are some records that suggest a potential for an extended tolerance towards intermediate salinity within this species complex. For example, Gottstein et al. (2007) recorded two *Niphargus* species in habitats with salinities between 5 and 10.4 psu in Dalmatian coast anchialine caves and Vonk et al. (1999) found a *Niphargus* species in south-west Turkey at 18,800 µS/cm.

With an increasing risk of rising salinity in some Irish aquifers due to anthropogenic activities and climate change, and in view of the particular vulnerability of endemic groundwater populations, it was considered worthwhile investigating the salinity tolerance of *Niphargus k. irlandicus*. As an added benefit, these investigations would also reveal whether brackish water environments may have the potential to function as refuge zones for the species' survival during Quaternary glaciations.

4.2.3 *Feeding behaviour*

Information on the feeding behaviour of *Niphargus* species is extremely sparse. Many references in the literature appear to be based on assumptions rather than direct observation, food trial experiments or an analysis of functional morphology. Omnivory seems to be most frequently assumed for *Niphargus* species. Rumm (2007) summarised the feeding behaviour of *Niphargus fontanus* in laboratory trials. This species fed on algal trichomes, detritus, oligochaetes, excrements and *Alnus* and *Acer* leaf varieties, not all of which are likely to be available in groundwater environments. Predation and cannibalism have been observed for *Niphargus timavi* in a laboratory study by Luštrik et al. (2011). In probably the first forensic science case study on groundwater animals, Vanin and Zancaner (2011) recently reported *Niphargus* feeding on the dead body of a drowned man. The latter study gives testament to a tendency of *Niphargus* species to feed on carrion.

4.3 Study Area

Test organisms were collected from the Bunatober Spring in Co. Galway and boreholes at the Japanese Gardens and at Milltown, Co. Kildare.

4.4 Methods

4.4.1 *Temperature tolerance*

Experiments were conducted in a darkroom, whose sole light source was a darkroom light. Animals were tested in a purpose-built temperature gradient chamber, whose construction followed the same design principles as the one used by Schregelmann, which is described in Brielmann et al. (2011). An acrylic glass chamber with interior dimensions of 400 × 40 × 50 (length, width, height in mm) was equipped with a Peltier element at one end and two heating plates at the other. The floor was clad with black water-resistant sandpaper. The chamber was then positioned at a slope with an angle of 13.5°, so that the Peltier element was at the lower end and the heating plates were at the upper end. At a height of 23 mm, seven thermistors were inserted 55 mm apart from each other. Feedback loops from the thermistors closest to either the Peltier element or the heating plates controlled cooling and heating with the aid of a Campbell CR 10X data logger, in order to achieve a stable temperature gradient. Convection currents in the chamber were studied in separate experiments with green food dye, which was introduced into the chamber near the Peltier element at the bottom or the heating element at the top. The distribution of the dye due to convection was photo documented, and flow patterns and velocities were later analysed from these images. Temperature profiles (Fig. 4.2) were documented with a mobile temperature probe on a long thin flexible cable. In the evening prior to the start of exposure experiments, the chamber was filled with tap water, which had been aerated throughout the day. The test organisms comprised *Niphargus k. irlandicus* specimens from the Japanese Gardens and Milltown, Co. Kildare, which had been fed and kept at 10°C in the laboratory for several months. For each experiment, four to six *Niphargus* specimens were introduced into the chamber through a port at the higher end. They were discharged so that they dropped into the 10–12°C zone, i.e. the temperature that represents the water temperature in most boreholes where *Niphargus* is found. For the first set of experiments, animal positions along the longitudinal axis were recorded at hourly intervals (6 records per animal) with the help of a small LED torch after an initial introduction period of 2 h (Set 1). For the second set of experiments, recording of positions started 12 h after introduction to the chamber (Set 2).

Occasionally test organisms fell into coma at the cold end or were incapacitated at the hot end of the chamber. Animals that swam into the hot zone usually died, while those that had fallen into a coma at the lower end usually recovered after removal from the chamber at the end of each experiment. If any animal appeared to show no movement at all, due to coma or incapacitation, further sightings for the respective specimen were disregarded. At the end of each experiment, oxygen saturation was recorded with a Hach HQ10 LDO® probe after careful mixing of water in the chamber to break the thermal gradient.

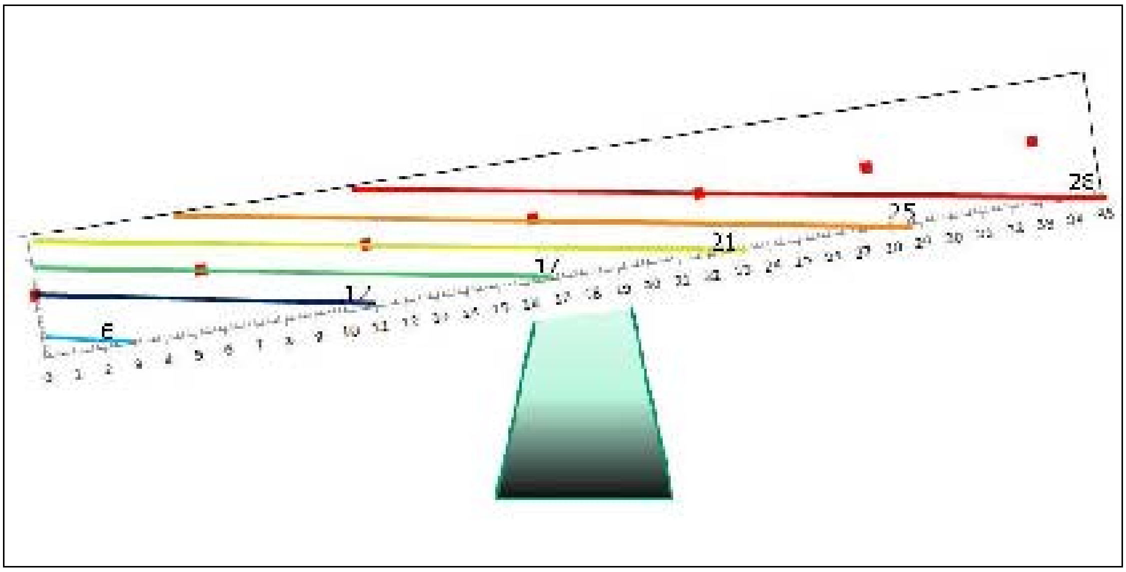

Figure 4.2. Schematic depiction of a typical temperature gradient in the experimental chamber.

4.4.2 Salinity tolerance

4.4.2.1 Video observation

Because some niphargids have been reported to show a pronounced stress response to both low and high intensities of light, for example with the increase of oxygen consumption by *Niphargus stygius* being significantly greater at higher light intensity (Simčič and Brancelj, 2007), infrared video recording was used for animal observations. Animals were videoed in darkness using a digital camcorder in night vision mode connected to a PC for live video capture. An infrared LED light source was provided (Fig. 4.3). The tested *Niphargus* specimens did not show any response to infrared light, which facilitated its use in the current video recording experiments.

Live video was captured on the PC using Ulead Video Studio software. Motion tracking software (MaxTraq, Innovation Systems, Columbiaville, MI) was used to measure the direction and velocity of the animals' movements (Fig. 4.4). The custom-arranged video set-up represented the best compromise between providing the animals with sufficient space to move around, while allowing for the production of sharp images.

At the end of the 14-day acute transfer salinity bioassay with the *Niphargus k. irlandicus* specimens

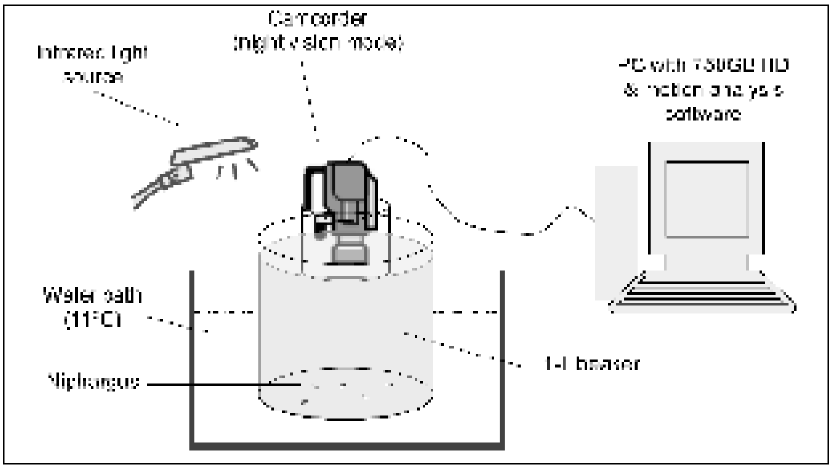

Figure 4.3. Laboratory set-up for video observation of *Niphargus*.

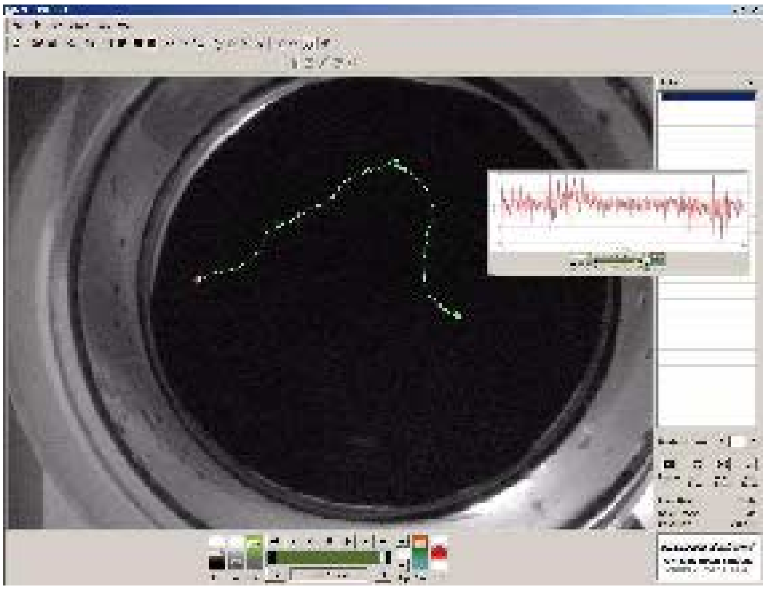

Figure 4.4. Tracking of *Niphargus* movement using MaxTraq motion analysis software.

from County Galway, locomotory activity (swimming, crawling) in the remaining live animals was measured. Animals were videoed first in darkness for a period of 5 min and subsequently in exposure to ambient room light for 3 min. Recordings were analysed using MaxTraq motion analysis software (Innovation Systems, Columbiaville, MI) in order to quantify whether salinity had non-lethal detrimental effects on the animals in terms of their swimming/crawling movements.

4.4.2.2 Bioassays

After collection from the sampling site, adult specimens of *Niphargus* from Bunatober Spring, Co. Galway, were kept in aerated spring water in darkness for 48 h. For the salinity bioassays, synthetic seawater (35 psu) was diluted with filtered water, collected from Bunatober Spring (0.35 psu), to the following final concentrations: 0.35 (control), 1, 2, 3, and 4 psu. For the experiments, 12–14 adult animals were transferred from the lab culture using a wide-mouthed pipette to duplicate 1-l bottles containing the salt solutions (resulting in 10 bottles containing 130 animals in total). Bottles were incubated in darkness at 11°C for a period of 2 weeks. Constant gentle aeration was provided via an air stone positioned close to the top of the bottles. Bottles were checked for mortalities at 1.5, 3, 6 and 12-h intervals for the first 24 h and at 12-h intervals thereafter, and dead animals removed using a wide-mouthed pipette and their body length measured under a dissecting microscope.

After several unsuccessful attempts to gather a sufficient number of specimens from Galway, a ‘replication’ bioassay was performed with specimens from sites at the Japanese Gardens and at Milltown, Co. Kildare, which had been kept in aerated tap water for several weeks prior to testing. Animals were fed weekly with aquarium fish food and crabmeat before experiments. As one of the aims was to determine LC_{50} and LC_{100} values (concentrations leading to 50% and 100% mortality, respectively), the range of tested concentrations was extended for this bioassay. Due to the restricted quantity of test organisms, concentrations of 1 and 2 psu were not replicated. A total of 160 animals were used in the second bioassay, with solutions of 0.35 psu (control), 3, 4, 5, 6, 7, 8, 9 psu. Salinity of all individual salinity treatments within the experiments was checked using a Hach sensION 156 with a conductivity cell.

4.4.3 Feeding behaviour

Feeding trials were performed in batches; five to ten animals were kept in 500 ml water with aeration at 10°C. Trials were conducted in the dark. Observations were made on an Olympus SZX 16 with the photo/video camera DP72. Tested animals came from the Japanese Gardens and Milltown sites in Co. Kildare. Bait was dyed with Rose Bengal.

4.5 Results

4.5.1 Temperature tolerance

There were slow convection currents, which separated the waterbody into two halves (Fig. 4.5). From a lateral

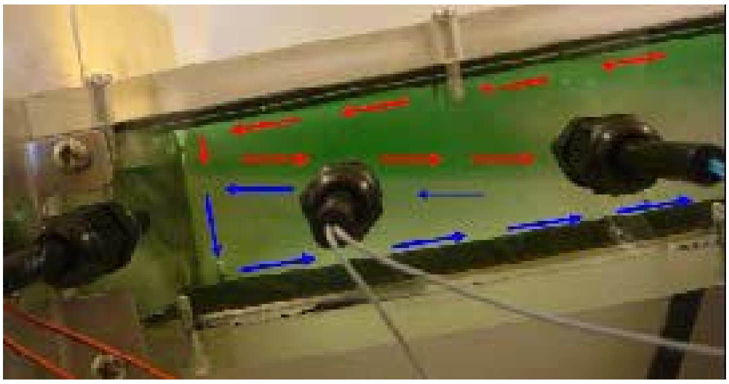

Figure 4.5. Hydrodynamics within the temperature gradient chamber. The image shows convection currents near the lower end with the Peltier element. Convection currents (arrows) were traced with green food dye.

perspective, the dividing line between the two halves was close to a diagonal line from the top corner at the cold end to the bottom corner at the warm end. With regard to the *Niphargus* specimens, the most relevant hydrodynamic feature probably was a slow and low turbulence current near the floor. This current moved water from the cold end to the warm end with a velocity <0.2 mm/s (0.75 m/h).

Valid observations were made between 4.6°C and 33.2°C. Below 4°C, specimens seemed to fall into coma for the remaining observation time. Above 35°C, specimens became incapacitated and died. After mixing and thus breaking the temperature gradient at the end of experiments, oxygen saturation values ranged from 60% to 79%; the minimum concentration was 5.5 mg/l.

Both sets of recorded temperatures for positions that animals were observed at had similarly shaped non-normal distributions (Figs 4.6–4.7). Data set 2 had a lower median value (12.7) than Data set 1 (16.1) and thus came closer to the average shallow groundwater temperature in Ireland. However, the Mann–Whitney U-test indicated that the difference was not significant, U = 1,935, p = 0.143. Median temperature for the combined data set was 15.3°C (Fig. 4.8). The combined data set represents 429 observations.

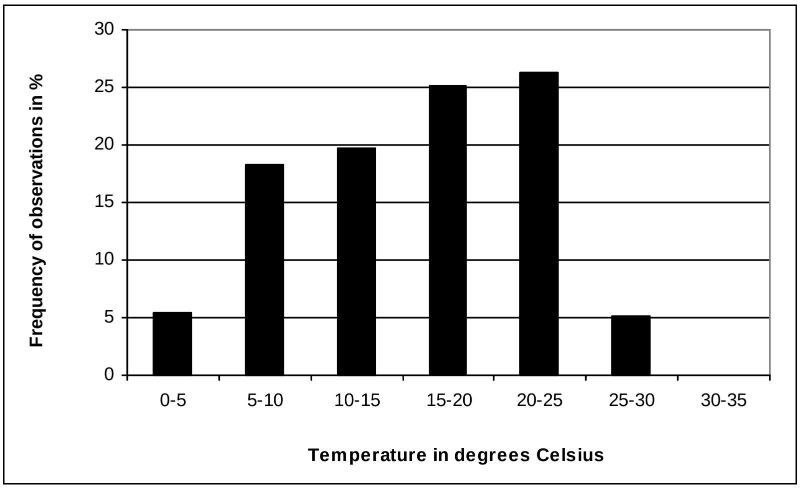

Figure 4.6. Observation Data set 1 for temperature gradient chamber experiments with observations between 2 and 8 h after introduction of animals to the chambers.

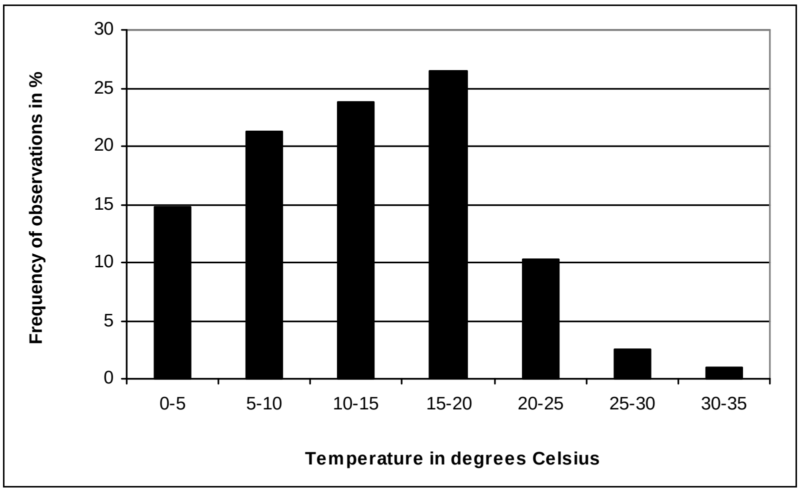

Figure 4.7. Observation Data set 2 for temperature gradient chamber experiments with observations between 12 and 18 h after introduction of animals to the chambers.

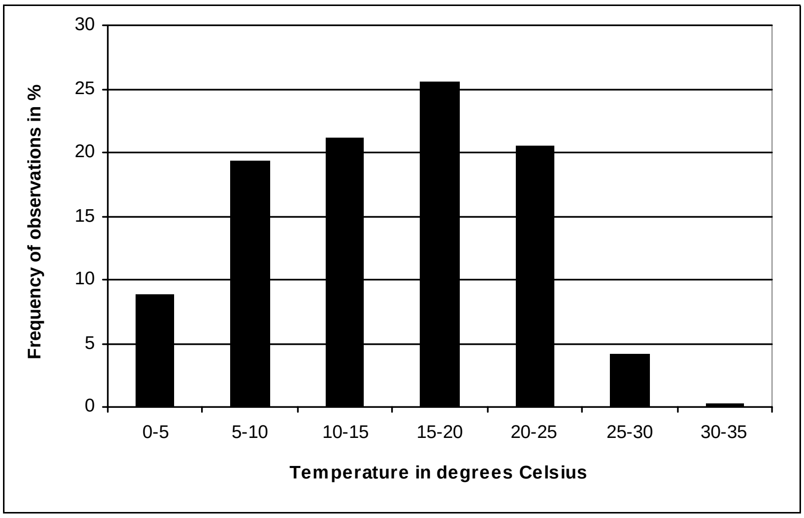

Figure 4.8. Combined data set (Data set 1 and Data set 2) for temperature gradient chamber experiments.

4.5.2 Salinity tolerance

Table 4.1 lists summary parameters that characterise the test organisms from both counties with regard to body length measurements. Both assays used males and non-ovigerous females. The range of body lengths suggests that not all of them may have been fully mature, but data on growth curves are still lacking for this species. An F-test (ANOVA) showed that, with regard to variance of length measurements, there were highly significant differences between both samples (F = 3.27; df 84, 112; $p < 0.001$).

At the end of the bioassays for Galway test organisms, individuals in the 4 psu jars showed reduced movement, as measured with MaxTraq motion analysis software, compared with the locomotory activity of animals in the controls (Fig. 4.9), in both darkness (p = 0.008) and ambient light (p = 0.039) (Wilcoxon signed rank test).

Table 4.1. List of parameters, which summarise body length measurements for samples of test organisms from salinity bioassays with *Niphargus k. irlandicus.*

	Galway	Kildare
Mean length (mm)	3.88	3.82
Median length (mm)	4.00	3.82
Standard deviation (mm)	0.52	0.94
Minimum length (mm)	3.00	1.35
Maximum length (mm)	5.13	6.12
Sample size (n)	**113**	**85**

For the Galway bioassay, sudden transfer of animals to saline solutions of 1–4 psu did not result in any immediate deaths in the first 24 h. After 14 days, >90% of animals at 1–3 psu, and all animals in the controls, remained alive (Fig. 4.10). At 4 psu, mortalities increased steadily between Days 1 and 5, then ceased except for a further small increase in mortalities on Day 13 to reach 37.5%.

Results for the Kildare bioassay exhibited a steady increase in mortality for concentrations of 3–5 psu after 14 days (45–60%). There was then a steady 'mortality drop' (encircled area in Fig. 4.10) from 30% to 20% for concentrations of 6–8 psu. At a concentration of 9 psu, however, mortality peaked at 70%. Again, there were no immediate fatalities within the first 48 h. Mortality within individual treatments commonly showed a slow steady increase towards the end of the experiment (with plateaux for 7 and 9 psu during the final 3 days of exposure).

4.5.3 Feeding behaviour

Niphargus k. irlandicus was observed to ingest fine detritus material. In a feeding trial with dyed crabmeat, seven out of eight animals had fed on the meat within 12 h. Evidence for this feeding activity was the subsequently red colour of their gut content (Fig. 4.11). With regard to feeding on dead *Niphargus* specimens in another trial, test animals seemed to hesitate. For

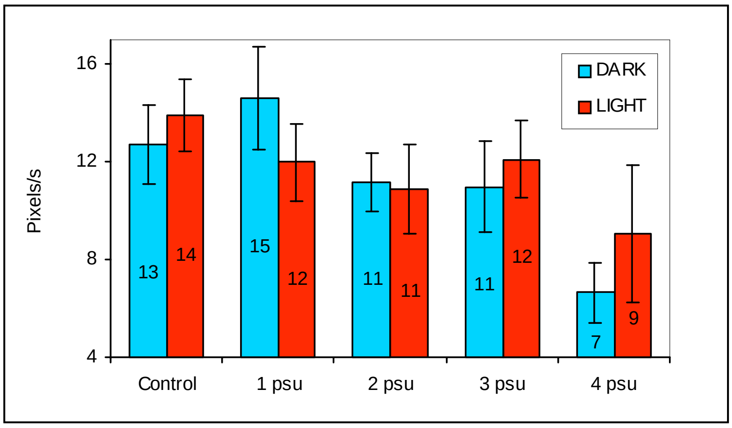

Figure 4.9. Locomotory activity profiles of animals in darkness and ambient light following the salinity bioassays, which were measured using MaxTraq motion tracking software.

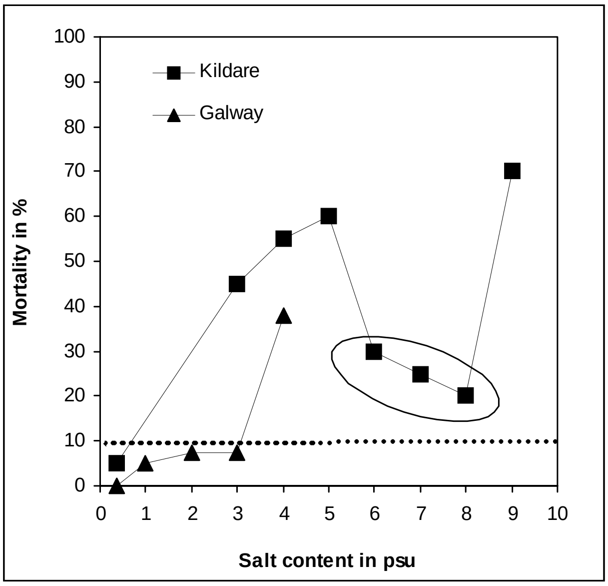

Figure 4.10. Plot of results from salinity bioassays for *Niphargus k. irlandicus* samples from Kildare and Galway. The dotted line marks 10% as the acceptable threshold for non-significant mortality effects. Encircled is the 'mortality drop' referred to in the text.

Figure 4.11. Specimen of *Niphargus k. irlandicus*, which has fed on crabmeat that had been dyed with Rose Bengal. Remains of the red crabmeat in the intestine are visible due to the transparent body tissue of *Niphargus*.

the whole 7-day trial period, there was no evidence of cannibalistic feeding on carrion. Video observation showed coprophagic behaviour with regard to its own faeces. Animals were also observed to feed on aquarium fish food flakes, which suggests that they would be able to process similar flakes, for example from microbial mats, if available. No *Niphargus k. irlandicus* specimen was ever observed attempting to catch or eat a live copepod.

4.6 Discussion

4.6.1 Temperature tolerance

Oxygen saturation levels remained too high throughout the experiments to restrict the animals' activity. The near chamber floor current seems to have been too slow to introduce any bias towards warmer temperatures. No drifting animals were observed throughout the experiments. The variety of positions that specimens assumed did not suggest that they were trying to reduce drag.

Specimens of *Niphargus k. irlandicus* did not appear to show a very active response to warmer or colder temperatures outside the range that they had been kept at prior to the experiments. Even moving into the 'hot zone' at the top end did not evoke an immediate flight reaction. The species apparently does not tolerate temperatures above 34°C. Even brief exposure seems to be fatal. As a reaction to temperatures below 4°C, animals stopped all movements, yet full recovery was possible; freezing was fatal. Slowing down movements at temperatures near 4°C may actually have introduced a small bias towards lower temperatures in these experiments, because animals would have been very slow to move out of these zones. Even though the difference between medians for Data sets 1 and 2 was not significant, there was a notably smaller percentage of observations at temperatures >20°C in Data set 2. A 12-h time interval between the introduction and recording of observations would seem to be the preferable experimental set-up, because the test animals' exploratory movements through the test chamber were quite slow. While results from these experiments provide an indication of temperature preference for the tested species, they cannot be used to provide a definite description of the temperature range that facilitates long-term survival of the species.

With respect to the latter, long-term experiments would be necessary that would include measures of the animals' fitness and reproductive success (Issartel, 2005b, 2006). In order to assess the potential long-term effects of temperature change on groundwater fauna, feedback mechanisms from other temperature responses of the groundwater ecosystem would have to be taken into account. For example, it needs to be considered how altered microbial growth rates in aquifers would affect oxygen concentrations and food resources that are available to invertebrates (Brielmann et al., 2011).

For a presumably stenothermic environment, such as groundwater, it would seem reasonable to assume that its fauna would not need to be equipped with very sensitive organs for temperature sensing. In an environment with a narrow temperature range, there would be little need for an ability to detect temperature gradients fast enough to trigger an immediate flight reaction should the animals encounter temperatures detrimental to their well-being. This could explain why *Niphargus k. irlandicus* individuals apparently did not show any alarm when they happened to move into hot or cold zones.

When comparing two different *Niphargus* species with regard to differences in their behavioural activity at low temperatures, Issartel et al. (2005a) found that activity patterns reflected whether or not the biogeographic range of the individual species extended into formerly glaciated areas. While *Niphargus rhenorhodanensis*, which is frequently found in formerly glaciated areas, did not show signs of reduced locomotory activity at low temperatures, *Niphargus virei*, which is only present in previously unglaciated areas, became almost totally inactive near 3°C. The failure of the Irish species *Niphargus k. irlandicus* to show any locomotory activity at temperatures below 4°C is therefore very interesting and may provide important clues as to where this species may have survived during glaciation. Its temperature preference would not seem indicative of a species that would be particularly well suited to survive in near surface water of a glacial environment, because a long-term survival in water near freezing point seems highly questionable. In spite of an early record from a lake, this Irish species would not even be particularly well adapted to live on the bottom of deep stratifying lakes in the temperate zone, where hypolimnetic temperatures reach 4°C. From the few studies available, the preferred temperature range of *Niphargus k. irlandicus* does not seem to be unusual among *Niphargus* species. Similarly to the already mentioned cold water response of *Niphargus virei*, a cold coma at water temperatures below 5°C has recently also been reported for another *Niphargus* species, *Niphargus opinatus* (Brielmann et al., 2011).

Issartel et al. (2006) have demonstrated that *Niphargus rhenorhodanensis* can even function in water below freezing point. Super-cooled water can, for example, enter the ground in a glacier's meltwater zone. Due to meltwater's decrease in flow velocity after infiltration it is likely to form basal ice below ground (Tweed et al., 2005). Coming into contact with ice or frozen ground carries the risk of crystallisation of an invertebrate's body fluids and subsequent death. Preadaptation to cold temperatures, however, results in physiological changes (Issartel et al., 2005b), which increase the resilience of *Niphargus rhenorhodanensis* against contact freezing. Whether a preadaptation period would result in a similar cold resilience of *Niphargus k. irlandicus* remains to be tested. Yet with a similar temperature tolerance profile to this Irish species *Niphargus virei* did not show any sign of the respective physiological change during a preadaptation period (Issartel et al., 2005b).

4.6.2 Salinity tolerance

Reduced mobility is a common response in invertebrates to a variety of stressors, which reflects reduced energy expenditure, thereby extending the survival of the animals. For example, slower locomotory activity has also been shown as a stress response to experimental hypoxia in the hypogean amphipods *Niphargus rhenorhodanensis* (Hervant et al., 1995) and *Niphargus virei* (Hervant et al., 1996), in the subterranean isopod *Stenasellus virei* (Hervant et al., 1997) and in cave populations of the amphipod *Gammarus minus* (Hervant et al., 1999).

Freshwater animals must expend additional energy for osmoregulation in a saline or brackish environment. Experimentally increased salinity was shown to result in reduced mobility in the freshwater amphipod *Gammarus roeseli* (Piscart et al., 2007). For *Niphargus k. irlandicus*, decreased locomotory activity of specimens in the Galway sample shows the same effect in water of a salinity >3 psu (Fig. 4.9). This decrease is evidence for a stress response.

In acute transfer toxicity bioassays with crustaceans it is not uncommon to accept mortalities of ≤10% as non-significant effects, for example ISO 6341 (1996). Data in Fig. 4.10 suggest that a salinity of 3 psu, which corresponds to electrical conductivity values $\kappa_{25°C}$ of <6,000 µS/cm, still represents the 'no observed adverse effect level' (NOAEL) for the Galway sample, while *Niphargus* from the Kildare sample seems to have a lower tolerance and is likely to have an LC_{50} value between 3 and 4 psu. This may indicate that Irish *Niphargus* in karstic aquifers close to the coastline may have evolved a slightly higher salinity tolerance than other populations further inland. Apart from the need for further replication for a rigorous test of this hypothesis, potential consequences of a reliance on samples from natural populations with comparatively wide size ranges will have to be taken into account. The latter shortcoming, which was unavoidable in this project, means that specimens in several developmental stages with varying sensitivities towards the stressor may have been included in the test, with obvious implications for the test outcome. As there was a significant difference in the variance of body length data between the Galway and Kildare samples, it is not unlikely that they also differed with regard to the proportional representation of individual developmental stages.

The mortality drop as a response to intermediate salinity for the Kildare sample is too consistent over a range of 6–8 psu to represent a random artefact. While only future replication will provide the certainty required to dismiss the possibility of it being a non-random artefact, it should be noted that U-shaped dose response curves are frequently encountered in biology (Calabrese and Baldwin, 2001). With regard to salinity responses, U shapes have been reported for freshwater insect emergence (inverse U shape) and for the metabolism response in early life stages of estuarine shrimps (McNamara et al., 1983). Rather than suggesting a wide tolerance for *Niphargus k. irlandicus* with regard to intermediate salinities, which would be in line with the latter reference, mortality data for the Kildare sample may merely indicate step changes in the metabolic response to rising salinity. Up to 5 psu, the animals may function with relatively little physiological change while showing signs of stress. Salinities between 6 and 8 psu may trigger a shock reaction, which results in an almost complete shutdown of physiological activity, thus requiring less osmoregulation and energy expenditure. This would help to avoid increased fatalities. Higher salinities may then surpass another threshold, which may then leave no other response option but excessive energy expenditure, which then leads to the successive rise in fatalities.

In natural conditions, mortality is likely to be determined by the combined effects of many abiotic and biotic variables rather than the isolated effect of any single stressor such as salinity. Multifactorial analysis would be an experimental strategy to overcome this problem. An acute toxicity bioassay does not take account of a stressor's sublethal effects, which may threaten a population's long-term survival, for example by decreasing its reproductive success. Direct measurements of physiological changes on a molecular level can investigate such effects. Both experimental approaches, however, require significantly more laboratory resources than were available for the current investigations.

Kefford et al. (2004) reported that for freshwater macroinvertebrates there was a close correspondence between the LC_{50} for an acute transfer bioassay and the maximum field salinity from the species' sampling records. This suggests that results from the acute transfer bioassays performed for *Niphargus k. irlandicus* are likely to be a reasonable estimate for the species' salinity tolerance under field conditions.

4.6.3 Feeding behaviour

Although *Niphargus k. irlandicus* could theoretically occupy the top of subterranean food webs in aquatic environments, there is no evidence yet for predatory or cannibalistic behaviour. As expected, the species is polyphagous (excrements, fine detritus and carrion), which is typical for fauna living in environments with a limited supply of food resources.

4.7 Conclusions

- *Niphargus k. irlandicus* does not tolerate temperatures below 4°C and above 34°C.

- Without prior adaptation, animals do not tolerate salt contents higher than 2–3 psu. However, there may be slight regional differences with regard to salinity tolerance.

- The species is polyphagous. It responds quickly to the availability of carrion and does not display a strong tendency towards cannibalism.

It is striking that *Niphargus k. irlandicus* does not appear to be well adapted to exposure to cold surface water or seawater intrusions. This has important implications with regard to the availability of potential refuge areas and the species' survival during Quaternary glaciations.

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5 Phylogenetics and Phylogeography of *Niphargus k. irlandicus*

5.1 Aims and Research Questions

This work programme aimed to investigate the phylogeography of the endemic groundwater crustacean *Niphargus k. irlandicus* and to clarify its phylogenetic position compared with morphologically convergent taxonomic units in the *Niphargus* genus from England and mainland Europe.

Research questions:

1. How genetically divergent is *Niphargus k. irlandicus* compared with populations of *Niphargus kochianus* in England and mainland Europe?
2. Should *Niphargus k. irlandicus* be reclassified as an independent species?
3. Do spatial patterns of genetic structure and diversity exist in *Niphargus k. irlandicus*?
4. What does phylogeography suggest with regard to the glacial survival of *Niphargus k. irlandicus* in Ireland?

5.2 Introduction

Traditionally, a widespread eradication of groundwater fauna during glaciations has been assumed to have occurred in northern Palearctic and Nearctic regions (Thienemann, 1950; Strayer, 1994). Evidence for this is a sharp biodiversity gradient for groundwater fauna that decreases from unglaciated southern to formerly glaciated northern areas in both regions. There have, however, been records for stygophile and stygobite species from areas north of the palaeomargin delineating the maximum extent of Quaternary ice coverage (Thulin and Hahn, 2008; Maurice, 2009). The presence of stygophile species in these areas may be due to postglacial recolonisation from unglaciated areas by comparatively fast dispersal in surface waters, from which they may have accessed their present subterranean habitats. Cold-adapted or euryhaline species may even have survived at the ice margins. Due to the low dispersal capability of most stygobite species, their occurrence in formerly glaciated areas suggests that they probably survived there throughout glacial periods. In the case of the endemic *Niphargus k. irlandicus*, it has been repeatedly noted that the present-day geographic range in Ireland extends far into formerly glaciated areas (Costello, 1993; Proudlove et al., 2003). Similarly, *Niphargus wexfordensis* and *Microniphargus leruthi*, the two other species of Niphargidae that have been recorded in Ireland, also exhibit biogeographic patterns that extend into formerly glaciated areas (Karaman et al., 1994; Arnscheidt et al., 2008; Knight and Penk, 2010; Chapter 2 of this report). The taxonomic classification of the Niphargidae based solely on morphology has been described as troubled, due to frequent morphological convergence and cryptic speciation (Fišer et al., 2008). Resolving taxonomic confusion will only come from a complete revision of the Niphargidae group based both on taxonomy and phylogenetic analysis, which also has the potential to discover previously unidentified cryptic species. To this end, Hänfling et al. (2008) were the first to investigate the phylogenetic divergence between the endemic Irish *Niphargus k. irlandicus* and the British *Niphargus k. kochianus*. Their study was based on the 28s rRNA and mitochondrial cytochrome *c* oxidase subunit I (COI) genes and analysed a very limited number of specimens available at the time. The current study extends their research to include a larger and more comprehensive sampling design, thereby facilitating rigorous testing of the hypotheses. Samples of *Niphargus k. irlandicus* encompassed almost the entire geographic range in Ireland, while a large number of the (according to traditional morphology-based taxonomy) closely related subspecies *Niphargus k. kochianus* from England and *Niphargus k. kochianus* and *Niphargus k. dimorphopus* from Belgium were also included in the study. Moreover, in addition to genetic analysis of the 28s rRNA and COI genes the 16s rRNA gene was also included, thereby increasing the robustness of the results generated.

5.3 Study Area

Niphargus k. irlandicus was collected from boreholes, hand pumps, natural springs and wells between 2008 and 2010 during the all-Ireland survey (Chapter 2). Samples (n = 118) from 17 sites in 10 counties (Fermanagh, Mayo, Galway, Clare, Tipperary, Cork, Waterford, Offaly, Kildare, Louth) were included in the genetic analysis. Irish sampling sites were located within Carboniferous limestone and sand and gravel aquifers. In addition, *Niphargus k. kochianus* (n = 65) was collected from 10 borehole sites in four English regions (Berkshire, Dorset, Hertfordshire, Gloucestershire). The boreholes have been well characterised for their hydrogeology and are located within chalk and Jurassic limestone geology, corresponding to two important aquifer types in England. *Niphargus k. kochianus* samples were collected by the University of Roehampton and the British Geological Survey and were made available for genetic analysis as part of an ongoing collaborative agreement. DNA sequence data for samples of *Niphargus k. kochianus* and *Niphargus k. dimorphopus* from the Meuse Valley in Belgium were also included in this study. The data represent samples from sites in sand and gravel aquifers. The DNA sequences became available as part of an ongoing collaborative agreement with the Royal Belgian Institute of Natural Sciences. Lastly, samples of *Niphargus fontanus* from Dorset, UK, and *Niphargus aquilex* from Somerset, UK, were provided by Lee Knight, Hypogean Crustacea Recording Scheme. Further details of samples and sampling locations are provided in Table 5.1.

5.4 Methods

5.4.1 Sample preservation and genomic DNA isolation

Samples collected with a modified Cvetkov net sampler (Chapter 2) were preserved in 70% ethanol. Samples were sorted under the microscope and the *Niphargus k. irlandicus* specimens were separated out and stored at –20°C until further processing for genomic DNA isolation. For comparative purposes, samples of the taxonomically closely related subspecies *Niphargus k. kochianus* from England and *Niphargus k. kochianus* and *Niphargus k. dimorphopus* from Belgium were also included in the study. Genomic DNA was isolated from the whole organism using the Nucleon® PhytoPure® kit (Genprobe), with slight modifications. Preliminary studies indicated that contaminant DNA (from gut contents, etc.) did not present a significant problem with a minimal risk of its amplification during polymerase chain reactions (PCRs) (2%). The resulting DNA was resuspended in 20 µl of sterile water and stored at –20°C. DNA concentrations were quantified using a NanoDrop ND-1000 spectrophotometer and samples were diluted when necessary to a concentration of ~50 ng/µl.

5.4.2 DNA amplification and sequencing

Genetic variation of *Niphargus k. irlandicus* was assessed at two mitochondrial genes, COI and 16s rRNA, and a nuclear gene, 28s rRNA. Polymerase chain reactions were undertaken in 30-µl reaction volumes containing ~12.5 ng DNA, 200 µM dNTPs, 2.5 mM $MgCl_2$, 1 × PCR reaction buffer (Invitrogen), 10 pM of each primer and 1.5 U *Taq* DNA polymerase (Invitrogen). Thermal cycling conditions consisted of an initial denaturation (5 min at 94°C), followed by 40 cycles of 1 min at 94°C, 1 min at the optimal annealing temperature and 2 min 30 s at 72°C, and a final extension step of 10 min at 72°C (Table 5.2). The PCR primers implemented in the multi-gene analysis are listed in Table 5.2. Additional PCR primers were designed for COI and 28s rRNA genes from *Niphargus k. irlandicus* DNA sequences using PrimerSelect^{TM} (Lasergene 4.0, DNAStar Inc.). Following amplification, an aliquot of the PCR product (5 µl) was visualised on a 1.5% agarose gel containing ethidium bromide and sized against a 100-bp DNA size ladder (Invitrogen). For successful PCRs, the remaining PCR product volume (25 µl) was purified using a Wizard® SV Gel and PCR Clean-Up System (Promega). For samples that failed to amplify, an alternative PCR primer set was implemented in a repeat PCR experiment. Purified PCR products were bi-directionally sequenced (Macrogen Inc., Republic of Korea) using non-standard conditions. Both DNA template amount (5 µl) and cycling parameters (annealing time, cycle number) were increased to try to maximise sequencing reaction success. For the 16s

Table 5.1. Site locations of the *Niphargus irlandicus* and *Niphargus kochianus* samples from Ireland, England and Belgium included in this study.

Country	Species	Population	Latitude	longitude	Site abbreviation
Ireland	*Niphargus irlandicus*	Ireland: Athenry, Galway	53°16'44" N	8°45'27" W	Ath_6001
Ireland	*Niphargus irlandicus*	Ireland: Ballymacmague, Waterford	53°00'21" N	7°40'56" W	Bal_5001
Ireland	*Niphargus irlandicus*	Ireland: Barnaderg, Mayo	53°27'15" N	8°41'05" W	Bar_12001
Ireland	*Niphargus irlandicus*	Ireland: Borrisoleigh (Tin 83), Tipperary	52°45'14" N	7°57'21" W	Tip_1
Ireland	*Niphargus irlandicus*	Ireland: Coole, Clare	53°31'32" N	8°50'36" W	Coo_7001
Ireland	*Niphargus irlandicus*	Ireland: Derrylin, Fermanagh	54°08'48" N	7°34'06" W	Der_4001
Ireland	*Niphargus irlandicus*	Ireland: Fermoy, Teagasc, Cork	52°09'38" N	8°16'27" W	Fer_1
Ireland	*Niphargus irlandicus*	Ireland: Japanese Gardens, Kildare	53°08'38" N	6°54'04" W	Jap_3001
Ireland	*Niphargus irlandicus*	Ireland: Kilcoona, Galway	53°25'49" N	9°01'11" W	Kil_8007
Ireland	*Niphargus irlandicus*	Ireland: Milltown, Kildare	53°12'02" N	6°50'32" W	Mil_1001
Ireland	*Niphargus irlandicus*	Ireland: Moycullen, Galway	53°22'33" N	9°08'44" W	Moy_10001
Ireland	*Niphargus irlandicus*	Ireland: NUIG Research Station, Clare	53°02'15" N	9°03'48" W	NUG_2; UCG_9002
Ireland	*Niphargus irlandicus*	Ireland: TC3, Clare	53°02'23" N	9°09'10" W	TC3_233
Ireland	*Niphargus irlandicus*	Ireland: Tullokyne, Galway	53°22'33" N	9°08'48" W	Tul_11001
Ireland	*Niphargus wexfordensis*	Ireland: Milltown, Kildare	53°12'04" N	6°50'38" W	Nwexford1
Ireland	*Microniphargus leruthi*	Ireland: Geashill, Offaly	53°14'05" N	7°17'28" W	Mleruthi Geashill
Ireland	*Microniphargus leruthi*	Ireland: The Bush, Louth	53°59'54" N	6°11'43" W	Mleruthi Bush
England	*Niphargus kochianus*	England: Corfe Mullen, Dorset	50°47' 07"N	2°02'02" W	Nkoch
England	*Niphargus kochianus*	England: Barcombe farm, Dorset	50°49'40" N	2°25'42" W	ENG_Bar
England	*Niphargus kochianus*	England: Brightwalton Holt, Berkshire	51°29'49" N	1°22'57" W	ENG_Bri
England	*Niphargus kochianus*	England: Calversley Farm, Berkshire	51°28'28" N	1°11'28" W	ENG_Cal
England	*Niphargus kochianus*	England: Cowdown, Berkshire	51°33'25" N	1°19'22" W	ENG_Cow
England	*Niphargus kochianus*	England: Crane Bridge, Cotswolds	51°40'41" N	1°55'14" W	ENG_Cra
England	*Niphargus kochianus*	England: Digswell, Herfordshire	51°49'21" N	0°12'13" W	ENG_Dig
England	*Niphargus kochianus*	England: Martinstown well, Dorset	50°41'53" N	2°29'41" W	ENG_Mar
England	*Niphargus kochianus*	England: Throop, Dorset	50°44'24" N	2°14'57" W	ENG_Thr
England	*Niphargus kochianus*	England: Warmwell, Cressbeds, Dorset	50°41'11" N	2°21'36" W	ENG_War
England	*Niphargus kochianus*	England: West Lodge, Dorset	50°46'45" N	2°24'30" W	ENG_Wes
Belgium	*Niphargus kochianus/dimorphopus*	Belgium: Meuse Valley			

Table 5.2. List of PCR primer pairs and sequencing primers used in this study.

Gene	Primer name	Direction	Tm (°C)	Primer (5'–3')	DNA sequence source; citation
COI	Nkocirl_CO1_F	Forward	55	GGGTCTGTTGATTTAGGTATTTTT	*Niphargus irlandicus*; this study
	Nkocirl_CO1_R	Reverse		TGCGCGAGTATCAACATCT	*Niphargus irlandicus*; this study
COI	Nkocirl_CO1_F	Forward	50	GGGTCTGTTGATTTAGGTATTTTT	*Niphargus irlandicus*; this study
	COI-virei-Rtot	Reverse		TACATCTATTCCTACAGTAAAT	*Niphargus virei*; Lefébure et al. (2006b)
COI	Nkocirl_CO1_F	Forward	53	GGGTCTGTTGATTTAGGTATTTTT	*Niphargus irlandicus*; this study
	COI-virei-Rard	Reverse		CCAATAGCAAGTATGGCGTA	*Niphargus virei*; Lefébure et al. (2006b)
COI	LCO1490	Forward	50	GGTCAACAAATCATAAAGATATTGG	Arthropoda; Folmer et al. (1994)
	HCO2198	Reverse		TAAACTTCAGGGTGACCAAAAAATCA	Arthropoda; Folmer et al. (1994)
16s	F-77	Forward	58	TAAGATTTAGTGTACAAATTGCCCGTC	*Niphargus rhenorhodanensis*; Lefébure et al. (2006b)
	R-1000	Reverse		GGCTCAAGCCGATTTGAACTCAAATCATGT	*Niphargus rhenorhodanensis*; Lefébure et al. (2006b)
28s	Nkocirl_28S_F	Forward	58	AGCGGGGCATCGGTACTTCCTTC	*Niphargus irlandicus*; this study
	Nkocirl_28S_R	Reverse		CACCCGCCGCTTACTTGCTTTCA	*Niphargus irlandicus*; this study
28s	Aaqu_28s_F	Forward	58	CAAGTACCGTGAGGGAAAGTT	*Asellus aquaticus*; Verovnik et al. (2005)
	Aaqu_28s_R	Reverse		AGGGAAACTTCGGAGGGAACC	*Asellus aquaticus*; Verovnik et al. (2005)
28s	28S_Seq_F*	Forward		AAACACGGGCCAAGGAGTAT	*Niphargus* sp.; Flot et al. (2010)
28s	28S_Seq_R*	Reverse		TATACTCCTTGGCCCGTGTT	*Niphargus* sp.; Flot et al. (2010)

Tm, annealing temperature; *sequencing primers.

and 28s rRNA genes, external PCR primers used in the PCR reaction were utilised for sequencing. For the COI gene, internal sequencing primers from within conserved regions of this hyper-variable gene were utilised for sequencing.

5.4.3 DNA sequence quality control for nuclear mitochondrial pseudogenes (numts)

Mitochondrial genes (e.g. COI, 16s rRNA) have the potential to translocate into the nuclear genome, thereby creating non-functional copies known as pseudogenes or numts (Song et al., 2008; Buhay, 2009), whose open reading frames contain coding errors such as stop codons, point mutations and or insertions/deletions. Due to the conservation of primer binding sites, both the target and nuclear sequences of mitochondrial origin (numts) are co-amplified simultaneously during PCR. This is problematic when analysing DNA sequence data sets, because data sets will also contain numt DNA sequences. Currently, no technical protocols are available to eliminate the problem of numts. Redesigning PCR primers to increase specificity has proven ineffective against numts (Moulton et al., 2010). Removal of chromatograms containing multiple peaks reduces the number of numts from a data set; however, it does not eliminate them altogether. It is therefore crucial to quality control (QC) mitochondrial genetic data to ensure the removal of numts prior to phylogenetic analysis (e.g. Song et al., 2008). This rationale also applies to published data contained in public databases (e.g. GenBank). These databases are non-curated, archival sources of primary data that rely on the submitter to indicate the presence of a numt. Studies have highlighted a prevalence of numts in public data sources that lack annotation (e.g. Davis et al., 2010). Thus, when utilising published data for comparative phylogenetic analysis, it is also necessary to QC data for numts.

5.4.4 DNA sequence analysis and bioinformatic identification of numts

Chromatograms for the forward and reverse DNA sequences were assembled and corrected in CodonCode Aligner (LI-COR Inc.). Poor-quality sequence data and chromatograms containing double or triple nucleotide peaks were excluded. Sequences were compared in a nucleotide Basic Local Alignment Search Tool (BLASTn) analysis against the GenBank nucleotide collection to confirm gene and DNA origin. Relevant DNA sequence data for the 16s rRNA, 28s rRNA and COI genes for *Niphargus* were collated from GenBank. Multiple sequence alignments of *Niphargus* DNA sequences for each gene were carried out using ClustalW in MEGA 5 (Tamura et al., 2011). A bioinformatic strategy was then implemented for the detection and removal of numts. Aligned mitochondrial DNA sequences were translated to amino acid sequences, utilising the invertebrate mitochondrial genetic code in MEGA 5. The *Niphargus* sequences were visually inspected for stop codons, frame-shift mutations and large insertions/deletions (~20 bp). Mitochondrial DNA sequences with evidence of coding errors typical of numts were subsequently removed from the data set. Final DNA sequence alignments for the 28s rRNA (n = 149; length 843 bp), COI (n = 147; length 881 bp) and the 16s rRNA (n = 165; length 958 bp) genes were analysed.

5.4.5 Phylogenetic, molecular evolutionary and genetic diversity analyses

The software DnaSP v5 (Librado and Rozas, 2009) was used to estimate descriptive statistics of genetic diversity: number of haplotypes (H), haplotype diversity (h) and nucleotide diversity (π). For locations where multiple samples were of the same haplotype, a consensus sequence for each haplotype present was used in further analyses. Evolutionary relationships amongst *Niphargus* taxa were investigated using the neighbour-joining (N-J) method in MEGA 5. N-J phylogenetic trees were constructed using the maximum composite likelihood method and the option ‘pairwise deletion’ (see MEGA, 2012). Branch support for the nodes was estimated by performing 500 bootstrap replicates. Pairwise comparisons of genetic distances (da), calculated based on the number of net nucleotide substitutions per site between populations, were estimated in DnaSP v5. Based on a molecular clock approach and node depths, time of genetic divergence was calculated using the equation $T = da / 2 \times \mu$ where μ is the rate in number of substitutions per site per year (Nei, 1987). This approach remains contentious, because the rate of genetic change can vary between genes. In this study, different rates of change dependent on the gene under investigation were applied, in order to improve calibration accuracy. Minimum and maximum *T* values were estimated based on an interval of divergence rate assumed to be 1.40–2.60% of substitutions/site per million years for the COI (Knowlton and Weigt, 1998), 0.53–2.20% for the 16s rRNA (Stillman and Reeb, 2001) and the global clock rate of 1.25% for the 28s rRNA gene (Ketmaier et al., 2003). This study evaluated whether lineages could be defined as cryptic species by applying the threshold of a 0.16 substitution/site in the COI gene to delimit new or uncertain species (Lefébure et al., 2006a).

5.5 Results

5.5.1 *Phylogenetic analysis of* Niphargus *taxa*

Results of the overall phylogenetic analysis are presented in the N-J trees in Figs 5.1–5.3. Branch nodes for distantly related *Niphargus* species have been compressed for illustrative purposes (dark triangles) and, in the figures, *Niphargus k. irlandicus* has already been renamed *Niphargus irlandicus*. Results are congruent between all three genes. Analysis for the 28s rRNA gene comprised a total of

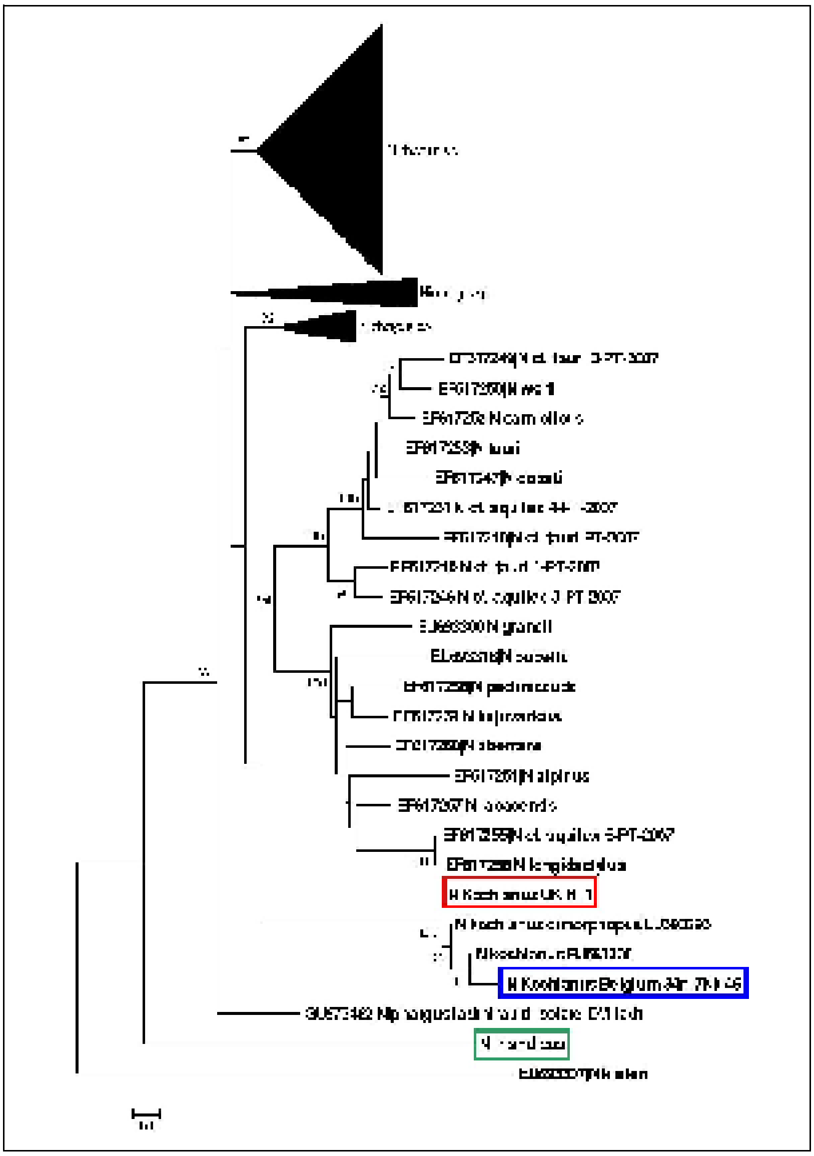

Figure 5.1. Neighbour-joining phylogenetic tree of 28s rRNA sequences, including *Niphargus irlandicus* samples from Ireland (green), *Niphargus kochianus* from England (red) and Belgium (blue) and published data; displayed are bootstrap support values (>70%).

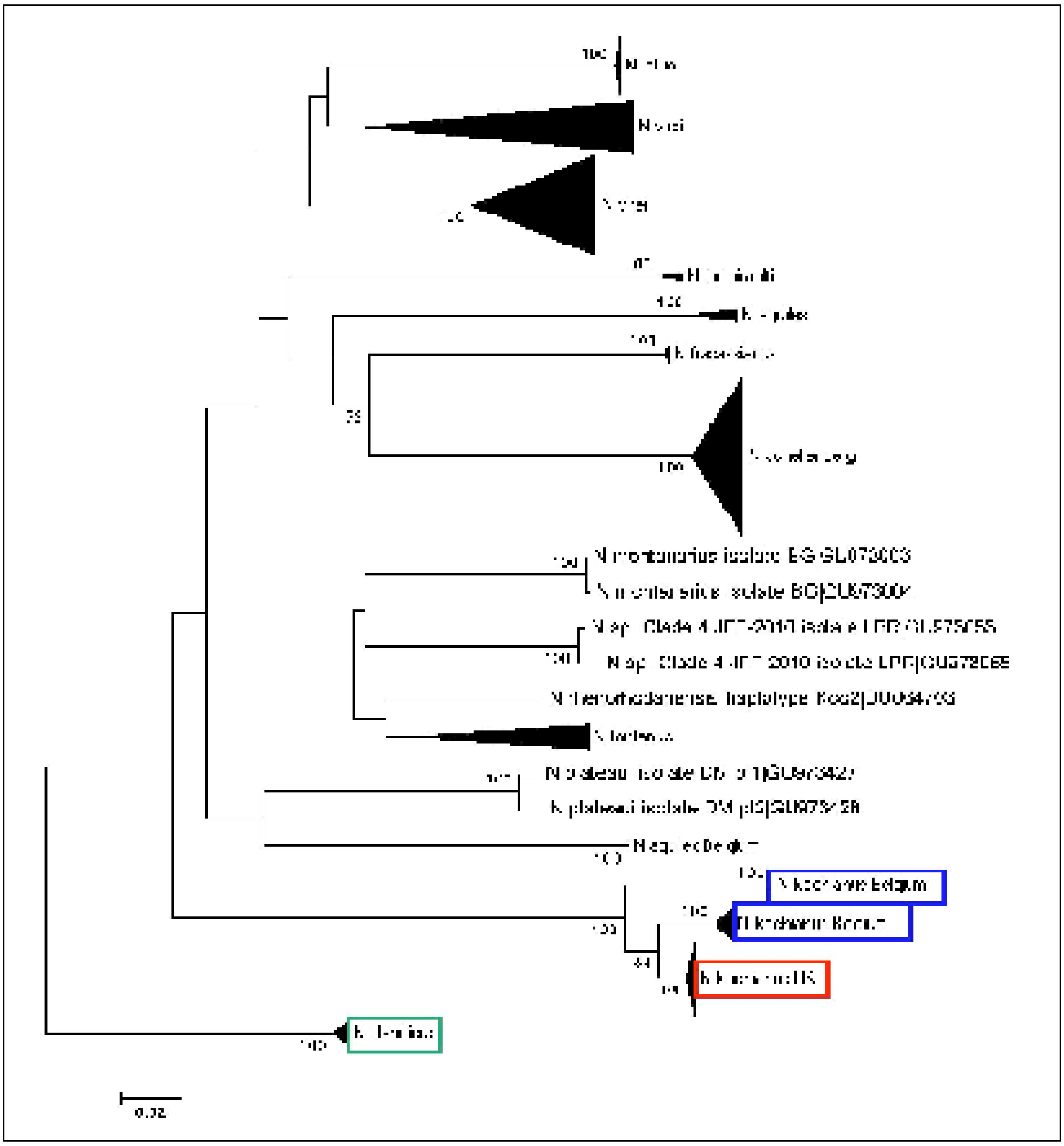

Figure 5.2. Neighbour-joining phylogenetic tree of cytochrome *c* oxidase subunit I (COI) sequences, including *Niphargus irlandicus* samples from Ireland (green), *Niphargus kochianus* from England (red) and Belgium (blue) and published data; displayed are bootstrap support values (>70%).

149 DNA sequences with an alignment length of ~843 bp. Within the 28s rRNA phylogeny, *Niphargus k. irlandicus* and *Niphargus kieferi* occupy a basal position (Fig. 5.1). Both species are ancestral and therefore very ancient compared with the other species. The Belgian and English *Niphargus kochianus* subspecies cluster together to form a group with the highest probability. *Niphargus k. irlandicus* is highly divergent from the *Niphargus kochianus* subspecies and clearly does not belong to this taxonomic group. The net 28s rRNA sequence divergence between *Niphargus k. irlandicus* and *Niphargus k. kochianus* was 0.2188, which translates to a divergence time of 8.75 million years based on a molecular clock (Table 5.3). Analysis for the COI gene comprised a total of 147 DNA sequences, with an alignment length of ~881 bp. Within the COI phylogeny, *Niphargus k. irlandicus* again occupies a basal position (Fig. 5.2). Once more, the Belgian and English *Niphargus kochianus* subspecies cluster together to form a group with high probability. Two Belgian subspecies *Niphargus k. dimorphopus* and

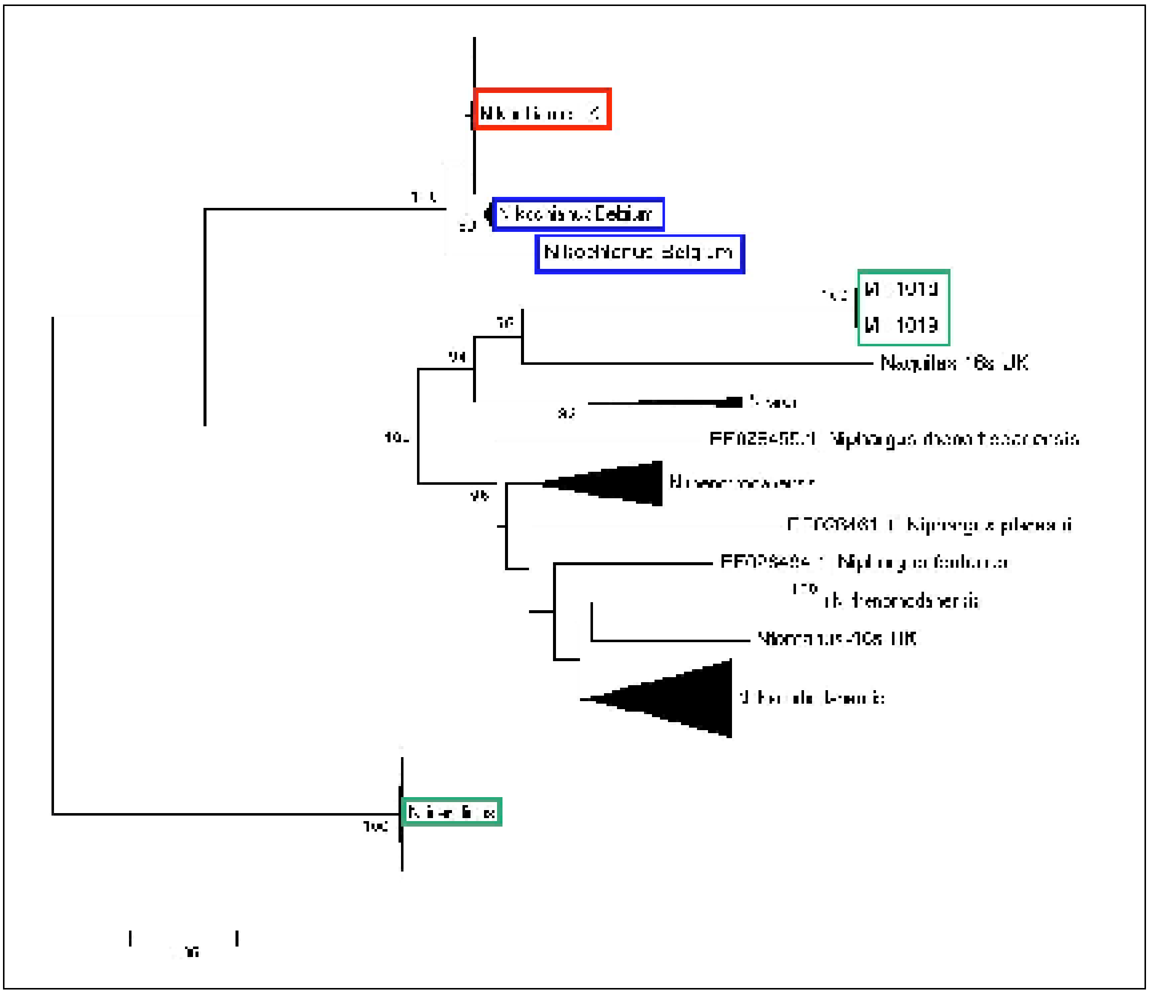

Figure 5.3. Neighbour-joining phylogenetic tree of 16s rRNA sequences, including *Niphargus irlandicus* samples from Ireland (green), *Niphargus kochianus* from England (red) and Belgium (blue) and published data; displayed are bootstrap support values (>70%).

Niphargus k. kochianus are clearly resolved with this phylogenetic analysis. *Niphargus k. irlandicus* is highly divergent and does not belong to the *Niphargus kochianus* subspecies taxonomic group. The net COI sequence divergence between *Niphargus k. irlandicus* and *Niphargus k. kochianus* was 0.1549 which translates to a divergence time interval of between 2.97 and 5.53 million years based on a molecular clock (Table 5.3). *Niphargus k. irlandicus* is sufficiently separated from *Niphargus k. kochianus* to qualify as an independent species according to the threshold of a 0.16 substitution/site in the COI gene to delimit new species (Lefébure et al., 2006a). Thus, *Niphargus k. irlandicus* should be renamed and taxonomically reclassified as *Niphargus irlandicus*. In this report, this new name shall therefore be used from here on.

Analysis for the 16s rRNA gene comprised a total of 165 DNA sequences with an alignment length of ~958 bp. Within the 16s rRNA phylogeny, *Niphargus irlandicus* again occupies a basal position (Fig. 5.3). Once more, the Belgian and English *Niphargus kochianus* subspecies cluster together to form a group with the highest probability. Amongst the Belgian samples, the two subspecies *Niphargus k. dimorphopus* and *Niphargus k. kochianus* are clearly resolved with this phylogenetic analysis. *Niphargus irlandicus* is highly divergent and does not belong to the *Niphargus kochianus* subspecies taxonomic group. The net 16s rRNA sequence divergence between *Niphargus irlandicus* and *Niphargus k. kochianus* was 0.2452, which translates to a divergence time interval of between 5.84 and 23.13 million years based on a

Table 5.3. Estimate of the net sequence divergence (*da*) and time of genetic divergence (*T*) between *Niphargus irlandicus* and *Niphargus k. kochianus* and amongst *Niphargus irlandicus* genetic lineages based on a molecular clock approach. An interval for *T* (years) was estimated utilising a maximum and minimum rate applied for the individual genes (see Section 5.4.5).

Pairwise comparison	Gene	*da*	*T* min	*T* max
1) *Niphargus irlandicus* vs *Niphargus k. kochianus*	16s	0.2452	5,838,571	23,133,962
	COI	0.1549	2,977,885	5,530,357
	28s	0.2188	8,751,200	
2) *Niphargus irlandicus* genetic lineages (16s rRNA)				
Lineages 1 vs 2	16s	0.0030	70,476	279,245
Lineages 1 vs 3	16s	0.0048	113,571	450,000
Lineages 2 vs 3	16s	0.0041	96,667	383,019
3) *Niphargus irlandicus* genetic lineages (COI)				
Lineages 1 vs 2	COI	0.00839	161,346	299,643
Lineages 1 vs 3	COI	0.00721	138,654	257,500
Lineages 2 vs 3	COI	0.00830	159,615	296,429

molecular clock (Table 5.3). Time of divergence estimates between *Niphargus irlandicus* and *Niphargus k. kochianus* for each gene all predate the last glacial maxima of the Quaternary period. Interestingly, two 16s rRNA DNA sequences from Milltown, Kildare, (Mil-1018, Mil-1019) form an independent group with high probability. This finding indicates that a second species, other than *Niphargus irlandicus*, was identified amongst the Irish 16s rRNA DNA sequences. The second species' nearest relative is *Niphargus aquilex* from England. Lastly, the 16s rRNA DNA sequence results for *Microniphargus leruthi* seem to indicate that the specimens analysed did not belong to an independent species. Unfortunately, DNA sequence data for the other two genes are currently unavailable and it would be necessary to confirm findings for each of these for the two species.

5.5.2 *Comparison of genetic diversity amongst* Niphargus irlandicus *populations*

A summary of the molecular diversity statistics for COI and 16s rRNA is presented in Tables 5.4 and 5.5, respectively. A total of seven haplotypes were observed for the COI gene, with an overall haplotype diversity of 0.594 and nucleotide diversity of 0.00255. Nine of the 11 populations sampled each had a single COI haplotype, with the exception of two populations – Barnaderg (Mayo) and Ballymacmague (Waterford) had greater genetic diversity, with each site having two different COI haplotypes. Overall for the COI gene, Ballymacmague (Waterford) displayed the highest haplotype diversity (h = 0.500) and nucleotide diversity (π = 0.00059). For the 16s rRNA gene, a total of 18 haplotypes were observed, with an overall haplotype diversity of 0.862 and nucleotide diversity of 0.00276. Eight of the 14 populations sampled had a single 16s rRNA haplotype each. Six populations (Barnaderg (Mayo), NUIG Research Station (Clare) Japanese Gardens (Kildare), Milltown (Kildare), Ballymacmague (Waterford) and TC3 (Clare)) had between two and five haplotypes each, therefore displaying greater genetic diversity. Overall, for the 16s rRNA gene, the population TC3 (Clare) displayed the highest haplotype diversity (h = 1) and nucleotide diversity (π = 0.00537). DNA sequence data for the 28s rRNA comprised a single haplotype. As the 28s rRNA gene displayed no nucleotide variation (i.e. only one haplotype), an inter-population comparison of genetic diversity was not possible.

Table 5.4. Comparison of population genetic diversity of *Niphargus irlandicus* between sites for the COI gene: number of individuals (N), number of haplotypes (H), haplotype diversity (*h*), nucleotide diversity (π) and genetic lineage grouping.

Population	N	H	*h*	π	Lineage
Barnaderg, Mayo	5	2	0.400	0.00047	1
Kilcoona, Galway	6	1	0	0	1
Tullokyne, Galway	5	1	0	0	1
NUIG Research Station, Clare	3	1	0	0	1
Japanese Gardens, Kildare	20	1	0	0	1
Milltown, Kildare	3	1	0	0	1
Derrylin, Fermanagh	1	1	0	0	1
Borrisoleigh (Tin 83), Tipperary	1	1	0	0	2
Fermoy, Teagasc, Cork	1	1	0	0	3
Ballymacmague, Waterford	4	2	0.500	0.00059	3
TC3, Clare	1	1	0	0	3

Table 5.5. Comparison of population genetic diversity of *Niphargus irlandicus* between sites for the 16s rRNA gene: number of individuals (N), number of haplotypes (H), haplotype diversity (*h*) nucleotide diversity (π) and genetic lineage grouping.

Population	N	H	*h*	π	Lineage
Barnaderg, Mayo	7	4	0.810	0.00123	1
Kilcoona, Galway	8	1	0	0	1
Moycullen, Galway	2	1	0	0	1
Tullokyne, Galway	5	1	0	0	1
NUIG Research Station, Clare	6	4	0.800	0.00107	1
Coole, Clare	1	1	0	0	1
Japanese Gardens, Kildare	34	5	0.786	0.00107	1
Milltown, Kildare	3	3	1	0.00143	1
The Bush, Louth	1	1	0	0	1
Derrylin, Fermanagh	5	1	0	0	1
Borrisoleigh (Tin 83), Tipperary	1	1	0	0	2
Ballymacmague, Waterford	7	2	0.286	0.00031	3
Fermoy, Teagasc, Cork	1	1	0	0	3
TC3, Clare	2	2	1	0.00537	1, 3

5.5.3 Within-species genetic divergence of Niphargus irlandicus

Results of a within-species genetic divergence analysis of *Niphargus irlandicus* are presented in the N-J trees in Figs 5.4 and 5.5. Results for the COI and 16s rRNA genes are congruent and each identified three genetically distinct lineages amongst the *Niphargus irlandicus* samples with high probability. Genetic lineages and their groupings by populations are defined in Tables 5.4 and 5.5. In addition, the geographical distribution of the genetic lineages is mapped in Fig. 5.6. Lineage 1 is the largest group and

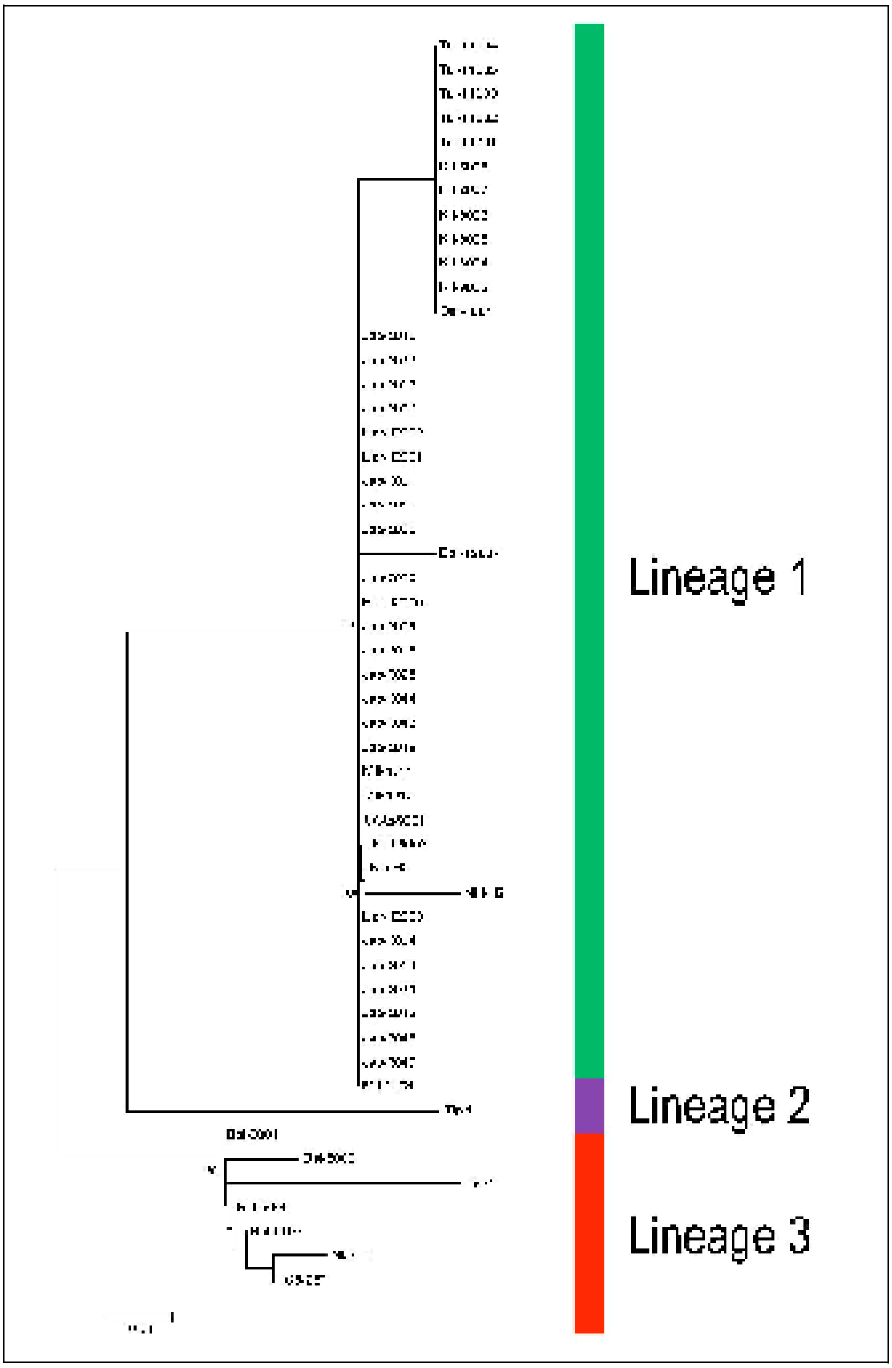

Figure 5.4. Neighbour-joining phylogenetic tree of all cytochrome *c* oxidase subunit I (COI) sequences from *Niphargus irlandicus* samples from Ireland, including haplotypes from Hänfling et al. (2008); displayed are bootstrap support values (>70%). Haplotypes identified from Hänfling et al. (2008) comprising Nki-H1 located in Cork, Nki-H2 located in Doolin (Clare) and Nki-H3 located in Pigeon Hole (Galway); displayed are bootstrap support values (>70%).

comprised 11 populations geographically located in the more northerly populations sampled in Counties Mayo, Galway, Clare, Kildare, Louth and Fermanagh. Lineage 2 comprised a single population located at Borrisoleigh (Tin 83) in Tipperary in the south-west. Lineage 3 comprised three populations geographically located in the furthest south and south-west in Counties Waterford, Clare and Cork. Interestingly, the sampling location TC3 in Clare contained individuals for both genetic Lineages 1 and 3. Thus, the

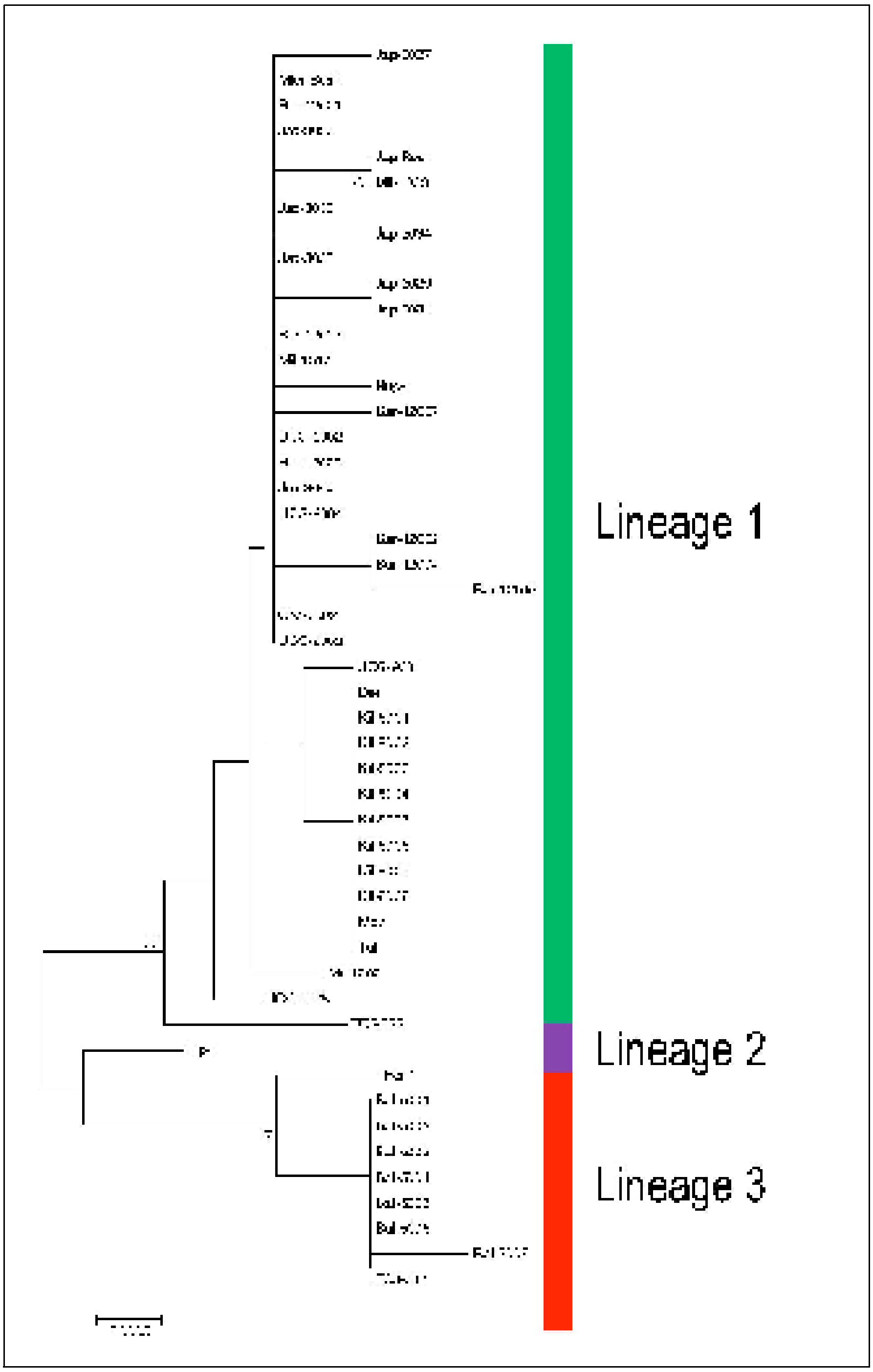

Figure 5.5. Neighbour-joining phylogenetic tree of all 16s rRNA sequences from *Niphargus irlandicus* samples from Ireland; displayed are bootstrap support values (>70%).

geographic ranges of individual genetic lineages clearly occupy three separate geographical areas in Ireland, with some overlap in Clare (see Fig. 5.6). Net sequence divergence between the three *Niphargus irlandicus* genetic lineages was relatively similar for all comparisons for both mitochondrial genes. For the COI gene, sequence divergence averaged 0.008, which translates to a divergence time interval of between 153,205 and 284,523 years before present (BP) (Table 5.3). For the 16s rRNA, sequence divergence averaged 0.0039, which translates to a divergence time interval of between 93,571 and 370,754 years BP (see Table 5.3). Time of divergence estimates between the three genetic lineages all predate the last

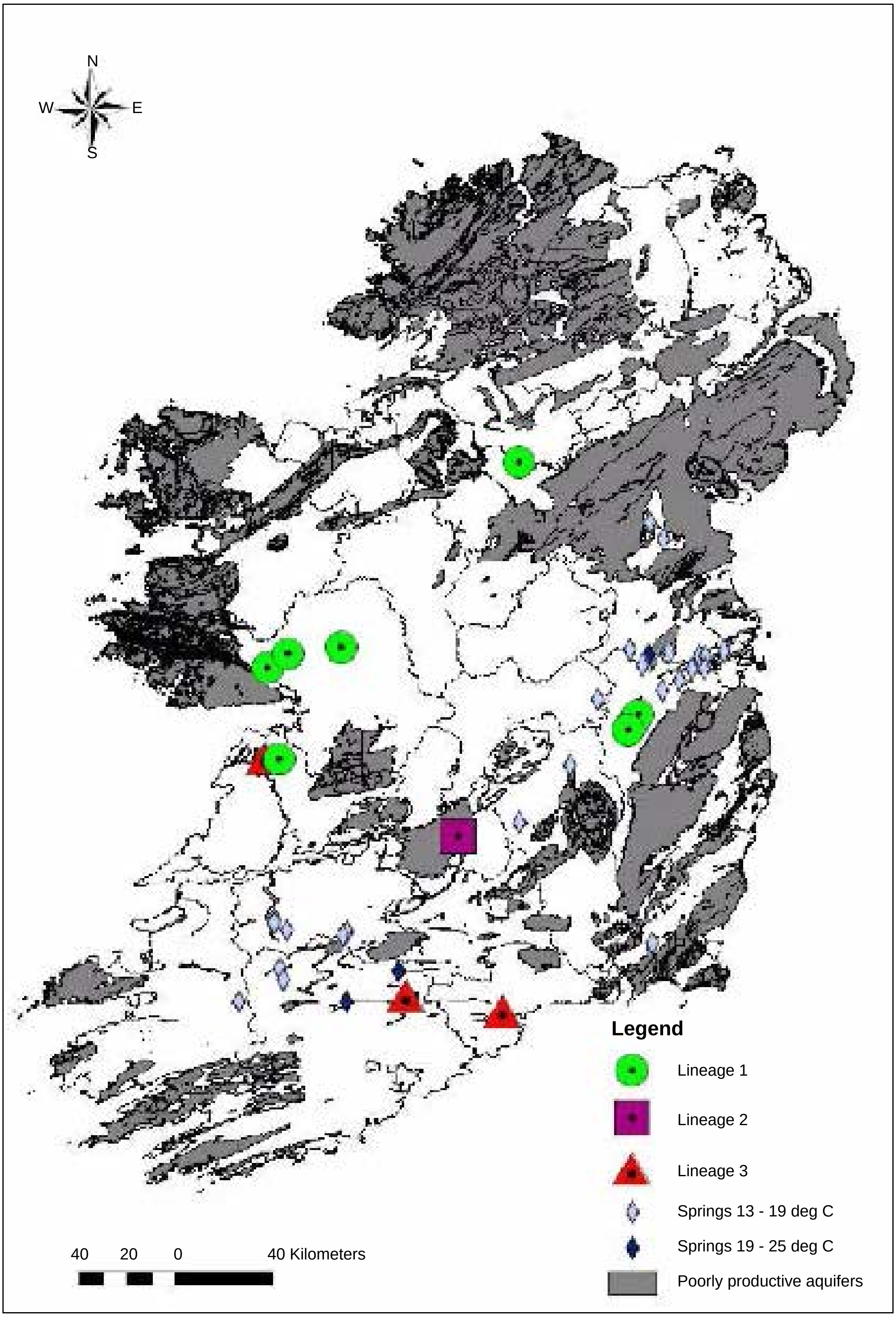

Figure 5.6. Geographic distribution of *Niphargus irlandicus* genetic lineages, poorly productive aquifers (data were provided by the Geological Survey of Ireland and the Geological Survey of Northern Ireland) and the distribution of warm and tepid springs in Ireland (georeferences and temperature data for springs from Goodman et al., 2004).

glacial maxima of the Quaternary period and coincide with the penultimate glaciation, known in Ireland as the Munsterian glaciation.

5.6 Discussion

5.6.1 *Phylogenetic analysis and species diversity of* Niphargidae in Ireland

Niphargus is a highly speciose genus with morphologically convergent taxa. Species identification in this invertebrate group is difficult, especially for juveniles. For the most thorough morphological approach to species identification carried out in this study, specimens were dissected and mounted on microscopic slides. These specimens could not be used for genetic analysis, however, as chemicals used for mounting specimens are destructive to DNA. Thus, morphological and genetic analysis of the same specimen was not possible. For taxonomically challenging groups such as Niphargidae, discrepancies will inevitably occasionally arise between genetic and morphological classification, especially when only a very small number of specimens is available. In this study, DNA sequence analysis provided a useful tool for both species identification and taxonomic assessment. Firstly, individuals morphologically identified as *Microniphargus leruthi* did not appear to be genetically distinct from *Niphargus irlandicus*. This was either a case of misidentification or it represents preliminary evidence suggesting that Irish animals morphologically classified as *Microniphargus leruthi* may not be a distinct species, but rather an ecotype of *Niphargus irlandicus*. *Microniphargus leruthi* specimens have been recorded from Ireland, England, Belgium, Luxembourg and Germany (Chapter 2). Compared with other niphargids, *Microniphargus leruthi* therefore has an extremely large geographic range of ~1,400 km (Trontelj et al., 2009). Considering this large range, it might not be unexpected if considerable genetic heterogeneity is later determined between geographically separated populations. If this potential finding is confirmed, it would be another reflection of the need to update diagnostic keys for the Niphargidae. Moreover, two specimens identified as *Niphargus irlandicus* from Milltown in Kildare were found to be genetically distinct from *Niphargus irlandicus*. Phylogenetic analysis revealed that the unidentified cryptic species was most closely related to *Niphargus aquilex* in England. Thus, this may be an entirely new species in *Niphargus* taxonomy. Alternatively, the specimens may be representative of unidentified specimens of *Niphargus wexfordensis*. Further investigation of the Milltown site is required, with additional data for taxonomy as well as genetic analysis, before any firm conclusions can be drawn.

5.6.2 *Phylogenetic analysis and time of divergence of* Niphargus irlandicus

Phylogenetic analysis of *Niphargus* taxa provided a comprehensive review of the taxonomic status of *Niphargus irlandicus*. Inclusion of samples from *Niphargus kochianus* in England and Belgium facilitated the determination of the levels of genetic divergence amongst subspecies in this group. Results were congruent with both mitochondrial (COI, 16s rRNA) and nuclear (28s rRNA) genes. The multi-gene analysis revealed that *Niphargus irlandicus* is highly divergent from all other investigated *Niphargus* taxa and is ancient by comparison. This was evident by its basal position in each of the phylogenetic trees. Consequently, *Niphargus irlandicus* was highly divergent from the *Niphargus kochianus* subspecies in both England and Belgium. Net sequence divergence between *Niphargus irlandicus* and *Niphargus k. kochianus* from England was relatively high in the 16s rRNA (*da* = 0.25), COI (*da* = 0.16) and 28s rRNA (*da* = 0.22) genes. Observed levels in the COI gene were equivalent to the threshold of a 0.16 substitution/site to delimit new species under the phylogenetic species definition of Lefébure et al. (2006a). Thus, based on this criterion, *Niphargus k. irlandicus* qualifies as a separate species. The results from this study corroborated those of Hänfling et al. (2008) who determined a similar net genetic divergence between *Niphargus irlandicus* and *Niphargus k. kochianus* (28s rRNA, *da* = 0.28) and also suggested that *Niphargus k. irlandicus* should be reclassified taxonomically as *Niphargus irlandicus*.

Estimation of the time of divergence between *Niphargus irlandicus* and *Niphargus k. kochianus* ranged between 2.9 and 23.1 million years BP. This early separation far predates the last ice age, which began approximately 40,000–30,000 years BP and reached its last glacial maximum (LGM) 27,000–

23,000 years BP. Regarding the phylogeography of *Niphargus irlandicus*, the hypothesis of a postglacial recolonisation of this species from outside of Ireland is clearly refuted. Evidence from all three genes suggests that *Niphargus irlandicus* has been present in Ireland for many millions of years and therefore has survived the Quaternary glacial cycles in refugial populations. Vicariant events accounting for the separation of *Niphargus irlandicus* from related *Niphargus* groups are likely to include the neogenic uplift of Britain and Ireland and the formation of the Irish Sea.

5.6.3 Phylogeographic patterns and genetic diversification in **Niphargus irlandicus**

Niphargus irlandicus exhibits many genetic characteristics typical of refugial populations, lending further evidence to support the argument that it survived in Ireland. Firstly, refugial populations that evolve in isolation for millennia often become highly genetically divergent through the processes of lineage sorting and random genetic drift. Correspondingly, *Niphargus irlandicus* is highly genetically divergent from all other *Niphargus* taxa. Secondly, refugial populations can undergo population expansion and diversification, leading to an accumulation of high genetic diversity. Similarly, results suggest that *Niphargus irlandicus* has undergone significant genetic diversification during its evolution as an endemic species for millions of years in Ireland. *Niphargus irlandicus* exhibits far greater genetic diversity compared with other niphargids. Moreover, *Niphargus irlandicus* could be separated into three genetically divergent lineages. Evidence for this separation is supported and independently corroborated with results for two mitochondrial genes (COI, 16s rRNA). In addition, net sequence divergence between each of the genetic lineages was relatively similar for each of the genes, averaging 0.4% and 0.8%, respectively. Time of genetic divergence between the *Niphargus irlandicus* genetic lineages dated to between 70,000 and 450,000 years BP for the 16s rRNA and between 140,000 and 300,000 years BP for the COI gene. Thus, the three genetic lineages diverged at least 45,000 years before the LGM of the Midlandian glaciation. Rather, time of divergence of the *Niphargus irlandicus* genetic lineages appears to coincide with the penultimate glaciation – the Munsterian glaciation. This is a novel finding. Fascinatingly, the genetic lineages appear to be spatially distributed into separate areas of Ireland (see Fig. 5.6). Lineage 1 was the largest group and encompassed the northern half of Counties Mayo, Galway, Clare, Kildare, Louth and Fermanagh. Lineage 2 was smaller and encompassed Tipperary in the south-west. Finally, Lineage 3 was geographically located in the far south and south-west and encompassed Counties Waterford, Clare and Cork. Coexistence of Lineages 1 and 3 at a site in Co. Clare suggests this area may be a contact zone of the two lineages where admixture might be occurring. In conclusion, populations of the groundwater amphipod *Niphargus irlandicus* are highly genetically structured. This finding was not observed amongst *Niphargus kochianus* from England, which appears to be a more recently colonised species (results not shown). The high level of genetic structuring observed in *Niphargus irlandicus* is most likely attributable to genetic changes that have accumulated over millions of years during its existence in Ireland. As the three identified genetic lineages appear to have evolved in geographically separate areas, it is highly likely that each lineage has developed local adaptations that are unique to the physiochemical and geological conditions of that area. Such local adaptations might, for example, involve the modification of an organism’s physiology, behaviour or morphology.

The genetic divergence of *Niphargus irlandicus* into separate genetic lineages was caused by discontinuous gene flow between the three geographical areas: (1) midlands, (2) Tipperary, and (3) far south and south-west. Discontinuous gene flow occurs when there is a lack of demographic exchange. Hence, barriers to dispersal must have existed (and may still exist) between *Niphargus irlandicus* populations in the different areas, but the factors that caused them are not known. Restricted dispersal of groundwater fauna between different surface water drainage systems does not seem to be responsible. This was evident as the geographic distribution of genetic lineages does not correspond to delineations of both past and present-day surface water drainage systems. As an impact of the LGM and subsequent deglaciation, major rivers were diverted to their present-day routes. Prior to the LGM, the River Shannon flowed in a more southerly direction but was later diverted north of Limerick (Lamplugh et al., 1907). Similarly, the River Liffey previously flowed in a more southerly direction, but was later diverted through Dublin (Farrington, 1929). Regardless, neither the past nor present-day delineations of the drainage systems of the Shannon and the south-eastern river basins seem to correspond to the geographic areas where the three *Niphargus irlandicus* genetic lineages are found. Alternatively, bedrock geology, hydrogeology, phylogeographic history and geothermal activity may all have a part in explaining patterns of gene flow in *Niphargus irlandicus*.

5.6.4 *Restricted gene flow in* Niphargus irlandicus *due to bedrock geology and hydrogeology*

Successful and unimpeded dispersal of groundwater fauna most likely depends upon the presence of a continuous suitable habitat between one area and the other. The incidence of low permeability strata could therefore act as a barrier to dispersal and hence limit gene flow. This has been recently demonstrated for the subterranean invertebrates *Collembola* within an unconsolidated aquifer (Sullivan et al., 2009). Ireland's bedrock geology is mostly comprised of either calcareous or siliceous units (e.g. Fig. 2.9). Both types of parent material lend different hydrogeological properties to the rock units derived from them. Calcareous bedrock often has fractures, which may be further eroded by the acidity of rainwater to form karstic features. Large fissures and conduits in karst systems facilitate extensive and efficient water flow that can achieve rapid travel times (Daly et al., 2002). Conversely, unless extensive fissuring occurred, many siliceous bedrock systems are far less permeable to water and hence are often poorly productive as aquifers. Dispersal of groundwater fauna is therefore likely to be more efficient within calcareous bedrock, compared with many siliceous bedrock units due to the low permeability of the latter. Results of the present study indicate that Lineage 1 occurs throughout central Ireland, mainly within limestone aquifers as well as a sand gravel aquifer underlain by limestone bedrock. Correspondingly, Lineage 3 also seems to be associated with limestone aquifers; Lineage 2 was identified in the marginal area of a limestone aquifer. It is noteworthy that the geographic areas located between Lineages 1 and 3, and equally Lineages 2 and 3, are partially separated by poorly productive aquifers in mostly siliceous bedrock (Fig. 5.6). Thus, genetic divergence of Lineages 1 and 3 and Lineages 2 and 3 may, in part, be explained by restricted gene flow due to the occurrence of geological dispersal barriers of low permeability. The hydrogeology of poorly productive aquifers may have impeded the dispersal of groundwater fauna, particularly along a north–south axis. According to present-day bedrock distributions, Lineages 1 and 2 are not entirely separated by poorly productive aquifers. However, we cannot exclude the possibility that Lineages 1 and 2 were separated by siliceous bedrock in the past, which could have subsequently been eroded by weathering, denudation or the movement of large glaciers over the last 300,000 years (Greenwood and Clark, 2009). Alternatively, there is also the possibility that Lineage 2 genetically diverged because of small-scale isolation effects due to its existence on the margins of a limestone aquifer. Interestingly, results seem to suggest that the continuous distribution of calcareous bedrock between areas with regionally important aquifers has perhaps maintained dispersal along an east–west axis within the geographic ranges of the larger Lineages 1 and 3. Predominant fault and fracture systems in Ireland also occur in an east–west direction, as well as geomorphological features from previous glaciations that are mostly comprised of sand and gravel (e.g. eskers, moraines). These geological features could have provided a continuous suitable habitat across most of central and some parts of southern Ireland, thereby facilitating dispersal routes for groundwater fauna along an east–west axis.

5.6.5 *Persistence of* Niphargus irlandicus *in Ireland throughout its glacial history and the influence of geothermal activity on the provision of refugia*

Niphargus irlandicus has persisted within the island of Ireland for millions of years, including the entire Quaternary period. In all, the Quaternary period has comprised three major ice advances, which occurred at approximately 0.45 Ma[2], 0.24 Ma and most recently 40,000 years BP. The maximum extent of the ice sheets and whether ice-free areas have existed in

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[2] Ma, millions of years ago or megaannum.

Ireland are a subject for some debate. Certainly, the evolution of Irish ice sheets in the course of the last glaciation has been a highly dynamic process centred around several ice domes and with a long series of advances and retreats (Clark et al., 2010). There is strong evidence for large fluctuations of ice sheet coverage and high mobility of the Irish ice sheet margins in rapid response to a rather unstable climate (Clark et al., 2012). Geologists have long held the view that parts of Ireland's south-west may have remained without ice cover during the last glacial period. This hypothesis was based on the existence of the southern Irish end moraines, as these were believed to outline the southern limits of the LGM. In addition, this theory was also based upon the existence of deposits attributed to the penultimate glaciation located further south. However, Hegarty (2004) has since shown that the end moraine idea is unfounded and that the features in question represent pro-glacial outwash instead. Additionally, detailed stratigraphic investigations recently carried out along the south coast by Ó'Cofaigh et al. (2010) have demonstrated that indeed the overlying drift material was not deposited during the penultimate glaciation, but by the last ice sheet instead. In conclusion, it now appears that much, if not all, of southern Ireland was covered by ice during the LGM. It is reasonable to suppose that the island of Ireland has been entirely covered by ice during the previous glaciations as well. This idea is difficult to reconcile, however, given the occurrence of Lusitanian fauna within Ireland and Portugal, but nowhere in-between. Attempts to explain the disjunct distributions of these faunal elements have given rise to various biogeographical hypotheses based upon the supposed existence of ice-free refugial zones for fauna in south-west Ireland during the last ice age. Phylogeographic evidence for the Natterjack toad *Bufo calamita*, however, now rather supports a postglacial migration into Ireland, thereby refuting the south-west Ireland glacial refuge hypothesis for this species (Rowe et al., 2006). In a comparable study on aquatic invertebrates, Krebes et al. (2010) investigated the phylogeography of the amphipod *Gammarus duebeni*. The Irish clade of this surface water crustacean was shown to be of pre-Quaternary origin. Hence, this species is very likely to have persisted continuously in or around Ireland throughout glacial periods. The observed high salinity tolerance of its genetic lineages would have facilitated glacial survival along the coastal margins of ice sheets. From coastal margins, it could then have dispersed into glacial lakes and the freshwater habitats that were formed as the legacy of retreating ice.

The results of this study indicate that survival of *Niphargus irlandicus* along coastal margins or in glacial lakes, however, is highly improbable. When tested, *Niphargus irlandicus* displayed intolerance towards both raised salinity and lowered water temperatures (see Chapter 4). Thus, the chances of survival for *Niphargus irlandicus* by moving from one ice-free area to another within a dynamic glacial landscape would also seem to be unlikely when considering the limited dispersal capability of this type of obligate groundwater fauna. The authors therefore argue for the persistence of groundwater fauna in Ireland throughout the ice ages in sub-glacial refugia. Present-day genetic lineages of *Niphargus irlandicus* are probably representative survivors of populations belonging to separate refugia and their geographic ranges may have repeatedly contracted and expanded due to Quaternary glacial cycles.

There may be some evidence to suggest that geothermal activity in the subsurface has been responsible for creating and maintaining sub-glacial refugia for groundwater fauna within Ireland throughout the Quaternary period. Areas of the earth's crust can have particular properties that cause relatively fast regional or localised heat transfers from deep in the earth's crust. Such thermal anomalies near the ground surface can result in the presence of warm springs. Formation of warm and tepid springs is likely to occur through the rapid deep circulation of recharge water from the surface along geologic fractures/faults from depths of several hundred metres. This mechanism has been proposed as the 'Mallow convection model' (Goodman et al., 2004). Warm and tepid springs are invariably an important component of groundwater. In Ireland, comprehensive explorations have recorded warm springs and water in monitoring boreholes in the south-west near Mallow, to the south-east of the Iapetus suture (which runs from the Shannon estuary to Louth) and also near Kildare and Kilbrook in the midlands (McKerrow and Soper, 1989;

Goodman et al., 2004). Groundwater temperatures in these warm springs can be elevated between 3°C and 12°C above average groundwater temperatures of the surrounding areas, with some seasonal variations (Goodman et al., 2004). For example, spring water temperatures depend on the depth from which the water rises, and also on the extent of its mixing with colder groundwater from shallower depths (Aldwell and Burdon, 1986; Brück et al., 1983). Furthermore, the temperature of spring waters is affected by surface temperature, causing water temperature in the Mallow spa spring to drop from 22°C to 17°C during winter time (Aldwell et al., 1985). Thus, discharge from such springs will have been colder during glacial periods. Geothermal heating of groundwater in Ireland has probably persisted throughout the glacial periods, irrespective of whether there was ice cover. This is reasonable to assume because geothermal springs can occur and discharge in areas of continuous permafrost such as a contemporary landscape with glacial conditions like those of the Tibetan plateau (see Ge et al., 2008). According to calculations by these authors, Tibetan geothermal springs must be fed by sub-glacial recharge. A similar mechanism is likely to have existed in Ireland during the ice ages. Considering geologic timescales, individual warm springs in Ireland may of course have been rather transient in nature with respect to their discharge point and regime. Nevertheless, subterranean zones connected to the geologic fracture/fault systems that circulate relatively warm groundwater are likely to have persisted throughout the ice ages and most likely provided sub-glacial and subterranean refugia for surviving species such as *Niphargus irlandicus*.

In terms of habitat persistence, the geothermal refuge hypothesis would therefore fit well with the estimates for the time of genetic divergence in *Niphargus irlandicus*, which predates the last ice age. It is striking that warm and tepid springs occur in the geographic range of each individual lineage (Fig. 5.6). All three lineages would therefore have had access to these potential refugia, allowing them to survive throughout all of the Quaternary glacial periods in Ireland. The results of this study are particularly interesting, considering that there are only a few other geographic areas with examples of endemic styobiotic amphipod or isopod crustaceans that may have survived in sub-glacial refugia. This includes two amphipod species (*Stygobromus*) and one isopod species (*Salmasellus*) from Castleguard Cave in Alberta, Canada, ~500 km north of the glacial limit (Bousfield and Holsinger, 1981; Holsinger, 1981; Holsinger et al., 1983), *Stygobromus* species from Siberia (Holsinger, 1987) and *Crangonyx islandicus* and *Crymostygius thingvallensis* from Iceland (Kristjánsson and Svavarsson, 2007). The Canadian cave is located in Banff National Park, which is also known for its geothermal activity and its hot springs. Glacial survival of the Icelandic groundwater fauna has already been attributed to the geothermal activity in this hotspot of volcanic activity (Kornorbis et al., 2010).

The geothermal refuge hypothesis represents a modification of the sub-glacial refuge hypothesis. It proposes specific regions that could have provided persistent refugial habitats under ice cover. The geothermal refuge hypothesis has the potential to be tested more extensively in Ireland. For example, a more detailed genetic analysis of *Niphargus irlandicus* could be undertaken, in order to determine more fine-scale patterns of dispersal routes. These may reveal that population expansions occurred from the postulated refugia identified in this study. The results from such a study should also be of interest to hydrogeologists, as they could reveal patterns of long-term connectivity between Irish aquifers. In order to increase confidence in the geothermal refuge hypothesis, phylogeographic analysis of at least one other endemic obligate groundwater species (e.g. *Niphargus wexfordensis*) should be undertaken, so as to determine whether its phylogeography exhibits a pattern similar to that of *Niphargus irlandicus*.

5.7 Conclusions

- *Niphargus kochianus irlandicus* has genetically diverged from *Niphargus kochianus* at least 2.9 million years ago.
- It should therefore be elevated from its rank of a subspecies to that of a species and should be renamed *Niphargus irlandicus*.
- This Irish species has at least three geographically separated genetic lineages. It is likely that each lineage has developed unique

local or regional adaptations to the physiochemical and geological conditions it occurs in. This finding has important implications, as ecosystem management of groundwater should aim at preserving the genetic diversity of its fauna. Ideally, effects on each genetic lineage would therefore receive separate consideration. Great care needs to be taken where decisions on groundwater abstraction and resource development may have impacts on small and genetically distinct populations. This may be relevant to Lineage 2. The authors anticipate that *Niphargus irlandicus* will prove useful in the future as a model system to investigate natural selection in the genomes of groundwater fauna located within different geological settings.

- *Niphargus irlandicus* has survived for millions of years and throughout all Quaternary glaciations within Ireland. The identification of three distinct genetic lineages suggests that they have survived in at least three different refugia. These were probably sub-glacial and subterranean and are likely to be located in aquifers that receive warm water due to geothermal activity. The 'geothermal refuge hypothesis' proposed in this study warrants further investigation with genetic tools in order to verify the location of refugial areas and the directions of colonisation pathways starting from those refugia.

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6 Conclusions and Recommendations

As a synthesis of historic sampling records and an effort in the collection of new baseline data, this project hoped to represent a new beginning in the research into Ireland's groundwater fauna. This final chapter briefly summarises the study's limitations and the main conclusions from its results. Unsurprisingly for a new beginning, limitations and conclusions alike lead to new research questions, which are addressed in the recommendations below.

6.1 Groundwater Ecology: Hydrogeology and the Biological Sciences

Data of the geographic range for individual species of Irish groundwater fauna or their genetic lineages are starting to reflect hydrogeological features in the Irish landscape. The clearest example of this is the most frequently encountered Irish groundwater animal – *Niphargus irlandicus*. To a large extent the presumed northern limit of its biogeographic range can be rationally explained as a confinement by bedrock geology with no groundwater or very low groundwater productivity. The geographic areas occupied by this species' individual genetic lineages are separated by geological features with similar hydrogeological properties. They appear to have functioned as barriers for the animal's dispersal and genetic exchange, thus preserving genetic differences between those lineages. In turn, the phylogeography of *Niphargus irlandicus* is very likely to reflect patterns of long-term hydrological connectivity involving all aquifers within the geographic range of individual genetic lineages that may be difficult to expose with conventional chemical tracers.

Recommendation:

- Future groundwater ecology research projects should involve hydrogeologists and biologists.

6.2 Ireland's Unique Endemic Fauna and its Groundwater Ecosystems

It is now certain that Ireland possesses at least one species of truly indigenous groundwater crustaceans – *Niphargus irlandicus* – which has a long history of survival in Ireland and is genetically very distinct from congeneric species in other parts of Europe. The estimates for its genetic divergence time from those European species coincide with the time line for the geological records of those processes that separated the island of Ireland from Britain and mainland Europe. Therefore, it is not unlikely that other Irish species of obligate groundwater crustaceans (stygobites) may be genetically equally unique among the European groundwater fauna.

Recommendations:

- Phylogenetics of the remaining species of Irish groundwater amphipods should be investigated.
- *Niphargus irlandicus* could become the flagship species for raising awareness that the groundwater environment actually represents ecosystems and not merely facilities for natural water storage. Increased recognition of groundwater's ecosystem status by water professionals and Ireland's general public alike could be very helpful in promoting efforts to protect it from pollution and in encouraging sustainable use of its resources.

6.3 Ice Age Survival of Groundwater Fauna: the Geothermal Refuge Hypothesis

Genetic results for one Irish groundwater crustacean species (*Niphargus irlandicus*) have confirmed that it has survived glaciations within Ireland in several geographically distinct refugia. The geothermal refuge hypothesis, as proposed in this report, links sub-glacial survival to zones of geothermal activity, where heat transfer from great depths occurs due to the fast rise of water along vertical fault lines. Even throughout glaciations, water in the vicinity of these geological features will very likely have remained warm enough for the survival of groundwater organisms. A lack of adaptation to water temperature below 4°C in *Niphargus irlandicus* and accessibility of warm and tepid springs within each lineage's geographic range provide supporting evidence for the hypothesis.

Recommendations:

- Phylogeographic investigations should be conducted for the other Irish groundwater amphipod species in order to test whether they display similar patterns.
- Further phylogeographic investigations on *Niphargus irlandicus* should aim to elucidate patterns of genetic drift within geographic areas occupied by the individual lineages.
- Universal applicability of the geothermal refuge hypothesis should be tested on similar groundwater organisms in non-volcanic and formerly glaciated areas outside Ireland.

6.4 Biodiversity

This survey has yielded a new species of the genus *Moraria*, a new first record for Ireland (*Parastenocaris vicesima*) and other interesting records, whose genetics should be investigated to clarify their phylogenetic position (e.g. the blind hyporheic *Gammarus lacustris* specimen). Some historic sampling successes (*Anthrobathynella*) could not be repeated in spite of targeted search efforts. As a consequence of the island's glacial history, Ireland's subterranean aquatic fauna appears to be much less diverse than biodiversity hotspot regions around the Mediterranean Sea. Diversity may, however, be higher than this project's results suggest. The survey's deliberate focus on groundwater sites without much direct surface water influence limited its scope to explore potential habitats of as yet undiscovered species. An intensive survey of aquifers in the vicinity of large rivers and a taxonomic extension of investigations towards worms (Annelida) would very likely result in further new first records for Ireland.

Recommendation:

- The further exploration of the diversity in Ireland's aquatic subterranean fauna needs a dedicated survey targeting aquifers in the vicinity of major rivers and eskers. Taxonomically it should extend to worms and crustaceans.

6.5 Presumed Absence of Groundwater Fauna

This project's survey activities have advanced the knowledge of Irish groundwater fauna by extending the focus of attention to regions that lack large numbers of caves. For example, the presumed northern limit for the biogeographic range of Irish *Niphargidae* now appears much more certain because, in spite of a targeted search effort, there are still no *Niphargus* records from north of Upper Lough Erne.

Considerable uncertainties remain, however, with regard to the presumed absence of fauna from individual aquifers. Many older boreholes lacked drilling records and information on furnishing, including features such as screens, which could have prevented access by groundwater fauna. Due to unfortunate time constraints, sites of the modern EPA monitoring network often had to be sampled shortly after installation. This may have inflated the percentage of sites without crustacean fauna.

While considerable efforts were made to achieve coverage of all regions to a similar extent, it has to be recognised that some regions remain, for example in the south-east, where relatively few sampling sites could be accessed during this survey. In addition, project results have also highlighted regions of particular scientific interest that should receive special attention in future surveys (e.g. suspected transition zones between individual genetic lineages of *Niphargus irlandicus*).

Recommendations:

- Sites of the modern EPA monitoring network should be resampled for groundwater fauna in the near future in order to confirm results, especially with regard to absence of fauna.
- There should be more intensive regional surveys in areas of particular scientific interest or with comparatively sparse survey coverage.

6.6 Autecology

For Ireland's groundwater amphipod species *Niphargus irlandicus*, this project has identified a temperature tolerance of 4–34°C, a tolerance threshold of 2–3 psu towards increased salinity, a tolerance threshold of 5% oxygen saturation, and has made observations on feeding behaviour. Acute toxicity tests proved to be difficult because of the long exposure times needed due to the slow metabolism for groundwater organisms. Salinity experiments highlighted the importance of the animals moulting process (either moulting may be induced by stress or there is a sensitivity increase during moults). All experiments were constrained by the supply of test organisms. Autecology experiments are essential for an improved understanding of groundwater ecosystems and future research needs to explore aspects of behaviour, fecundity, nutrition and environmental tolerance.

Recommendations:

- Autecology needs to be extended towards more species of groundwater organisms.
- Laboratory breeding needs to be established for a sufficient supply of test organisms.
- Tests on pollution tolerance need to monitor physiological or molecular stress indicators.
- It should be investigated whether individual lineages of one species differ significantly in their environmental tolerance.

6.7 Effects of Water Abstraction

Results from the more frequently monitored sites indicate that large-scale groundwater extraction appears to result in decreasing abundance of crustaceans regardless of the hydraulic features of the aquifer. At present it is neither known whether a modification of pumping rates or depths of abstraction may mitigate these effects, nor how long it takes for groundwater fauna abundance to recover after pumping. Water abstraction from greater depths may not only avoid the extraction of fauna, but may have the added benefit of an improved microbial water quality (because aquifer zones avoided by fauna may have less food resources, i.e. bioavailable organic carbon) and may provide better safeguards against short-term pollution. However, depending on local circumstances, the trade-off against increased drilling costs and potentially disadvantageous changes in chemical water parameters may be prohibitive.

Recommendation:

- Sites that are not in permanent use or are about to be decommissioned could be used for experiments to test mitigation effects and recovery times. The knowledge gained from such experiments could become invaluable for the management of sites with extremely rare faunal elements in order to preserve the current biodiversity (e.g. *Niphargus irlandicus* Lineage 2).

6.8 Effects of Hydrology and Climate Change

Temporal monitoring in two different aquifer types has demonstrated that the abundance of groundwater organisms appears to correspond to hydrology-related parameters. Observations were, however, limited to repeat sampling visits within one 12-month period. Only long-term monitoring can reveal how groundwater recharge, water table fluctuations, hydrologic exchange with surface water and other important hydrology parameters impact on groundwater fauna. Such data sets would be extremely valuable, especially with the prospect of climate change leading to more extreme weather conditions, for example droughts causing large groundwater table fluctuations.

Recommendation:

- Sets of sites for long-term groundwater fauna monitoring should be identified that represent the variety of aquifer types (minimum: fracture flow and intergranular flow). For each aquifer, six sites with significant faunal presence should be sampled at least six times per year.

6.9 Potential Applications in Water Quality Monitoring

Groundwater crustaceans were found to be widely present in shallow groundwater. While they have not been reported as the direct cause of hygienic problems, they can pass through conventional filter barriers quite easily and water users may find their appearance in drinking water objectionable. Many of these species are specially adapted to live in oligotrophic environments. They are therefore also potentially capable of inhabiting drinking water distribution networks, with a preference for low velocity zones in water tanks, roofed reservoirs and sand filters. High abundances would only be observed where food sources are available and could therefore indicate areas with aggregations of organic material, which would also provide resources for microbial growth and water contamination.

An abundance of stygophile surface water fauna (e.g. *Asellus aquaticus*) would indicate hydrologic exchange with surface water and hence vulnerability to short-term pollution incidents, for example after rainfall.

Recommendation:

- Distribution networks should be monitored for the existence of groundwater and surface water fauna as early warning indicators for potential contamination. Unless there is a history of transgressions for microbial water hygiene parameters, annual monitoring would suffice.

Acronyms

BLASTn	Nucleotide Basic Local Alignment Search Tool
BP	Before present
BCRA	British Cave Research Association
COI	Mitochondrial cytochrome *c* oxidase subunit I
CRG	Cave Research Group of Great Britain
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EPA	Environmental Protection Agency
EU	European Union
GSI	Geological Survey of Ireland
GSNI	Geological Survey of Northern Ireland
LC_{100}	Concentration leading to 100% mortality
LC_{50}	Concentration leading to 50% mortality
LED	Light-emitting diode
LGM	Last glacial maximum
Ma	Millions of years ago or megaannum
$MgCl_2$	Magnesium chloride
N-J	Neighbour-joining
NOAEL	No observed adverse effect level
numt	Nuclear mitochondrial pseudogene
PCR	Polymerase chain reaction
PE	Polyethylene
psu	Practical salinity unit
PTFE	Polytetrafluoroethylene
PWS	Public water supply
QC	Quality control
rRNA	Ribosomal ribonucleic acid
WFD	Water Framework Directive

An Ghníomhaireacht um Chaomhnú Comhshaoil

Is í an Gníomhaireacht um Chaomhnú Comhshaoil (EPA) comhlachta reachtúil a chosnaíonn an comhshaol do mhuintir na tíre go léir. Rialaímid agus déanaimid maoirsiú ar ghníomhaíochtaí a d'fhéadfadh truailliú a chruthú murach sin. Cinntímid go bhfuil eolas cruinn ann ar threochtaí comhshaoil ionas go nglactar aon chéim is gá. Is iad na príomhnithe a bhfuilimid gníomhach leo ná comhshaol na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhuanaithe.

Is comhlacht poiblí neamhspleách í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) a bunaíodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnú Comhshaoil 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaoil, Pobal agus Rialtais Áitiúil.

ÁR bhFREAGRACHTAÍ

CEADÚNÚ

Bíonn ceadúnais á n-eisiúint againn i gcomhair na nithe seo a leanas chun a chinntiú nach mbíonn astuithe uathu ag cur sláinte an phobail ná an comhshaol i mbaol:

- áiseanna dramhaíola (m.sh., líonadh talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh., déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- diantalmhaíocht;
- úsáid faoi shrian agus scaoileadh smachtaithe Orgánach Géinathraithe (GMO);
- mór-áiseanna stórais peitreail;
- scardadh dramhuisce.

FEIDHMIÚ COMHSHAOIL NÁISIÚNTA

- Stiúradh os cionn 2,000 iniúchadh agus cigireacht de áiseanna a fuair ceadúnas ón nGníomhaireacht gach bliain.
- Maoirsiú freagrachtaí cosanta comhshaoil údarás áitiúla thar sé earnáil - aer, fuaim, dramhaíl, dramhuisce agus caighdeán uisce.
- Obair le húdaráis áitiúla agus leis na Gardaí chun stop a chur le gníomhaíocht mhídhleathach dramhaíola trí comhordú a dhéanamh ar líonra forfheidhmithe náisiúnta, díriú isteach ar chiontóirí, stiúradh fiosrúcháin agus maoirsiú leigheas na bhfadhbanna.
- An dlí a chur orthu siúd a bhriseann dlí comhshaoil agus a dhéanann dochar don chomhshaol mar thoradh ar a ngníomhaíochtaí.

MONATÓIREACHT, ANAILÍS AGUS TUAIRISCIÚ AR AN GCOMHSHAOL

- Monatóireacht ar chaighdeán aeir agus caighdeáin aibhneacha, locha, uiscí taoide agus uiscí talaimh; leibhéil agus sruth aibhneacha a thomhas.
- Tuairisciú neamhspleách chun cabhrú le rialtais náisiúnta agus áitiúla cinntí a dhéanamh.

RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN

- Cainníochtú astuithe gáis ceaptha teasa na hÉireann i gcomhthéacs ár dtiomantas Kyoto.
- Cur i bhfeidhm na Treorach um Thrádáil Astuithe, a bhfuil baint aige le hos cionn 100 cuideachta atá ina mór-ghineadóirí dé-ocsaíd charbóin in Éirinn.

TAIGHDE AGUS FORBAIRT COMHSHAOIL

- Taighde ar shaincheisteanna comhshaoil a chomhordú (cosúil le caighdéan aeir agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíochtaí comhshaoil).

MEASÚNÚ STRAITÉISEACH COMHSHAOIL

- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar chomhshaol na hÉireann (cosúil le pleananna bainistíochta dramhaíola agus forbartha).

PLEANÁIL, OIDEACHAS AGUS TREOIR CHOMHSHAOIL

- Treoir a thabhairt don phobal agus do thionscal ar cheisteanna comhshaoil éagsúla (m.sh., iarratais ar cheadúnais, seachaint dramhaíola agus rialacháin chomhshaoil).
- Eolas níos fearr ar an gcomhshaol a scaipeadh (trí cláracha teilifíse comhshaoil agus pacáistí acmhainne do bhunscoileanna agus do mheánscoileanna).

BAINISTÍOCHT DRAMHAÍOLA FHORGHNÍOMHACH

- Cur chun cinn seachaint agus laghdú dramhaíola trí chomhordú An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Freagrachta Táirgeoirí.
- Cur i bhfeidhm Rialachán ar nós na treoracha maidir le Trealamh Leictreach agus Leictreonach Caite agus le Srianadh Substaintí Guaiseacha agus substaintí a dhéanann ídiú ar an gcrios ózóin.
- Plean Náisiúnta Bainistíochta um Dramhaíl Ghuaiseach a fhorbairt chun dramhaíl ghuaiseach a sheachaint agus a bhainistiú.

STRUCHTÚR NA GNÍOMHAIREACHTA

Bunaíodh an Ghníomhaireacht i 1993 chun comhshaol na hÉireann a chosaint. Tá an eagraíocht á bhainistiú ag Bord lánaimseartha, ar a bhfuil Príomhstiúrthóir agus ceithre Stiúrthóir.

Tá obair na Gníomhaireachta ar siúl trí ceithre Oifig:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig um Fhorfheidhmiúchán Comhshaoil
- An Oifig um Measúnacht Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáide

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag ball air agus tagann siad le chéile cúpla uair in aghaidh na bliana le plé a dhéanamh ar cheisteanna ar ábhar imní iad agus le comhairle a thabhairt don Bhord.

Science, Technology, Research and Innovation for the Environment (STRIVE) 2007-2013

The Science, Technology, Research and Innovation for the Environment (STRIVE) programme covers the period 2007 to 2013.

The programme comprises three key measures: Sustainable Development, Cleaner Production and Environmental Technologies, and A Healthy Environment; together with two supporting measures: EPA Environmental Research Centre (ERC) and Capacity & Capability Building. The seven principal thematic areas for the programme are Climate Change; Waste, Resource Management and Chemicals; Water Quality and the Aquatic Environment; Air Quality, Atmospheric Deposition and Noise; Impacts on Biodiversity; Soils and Land-use; and Socio-economic Considerations. In addition, other emerging issues will be addressed as the need arises.

The funding for the programme (approximately €100 million) comes from the Environmental Research Sub-Programme of the National Development Plan (NDP), the Inter-Departmental Committee for the Strategy for Science, Technology and Innovation (IDC-SSTI); and EPA core funding and co-funding by economic sectors.

The EPA has a statutory role to co-ordinate environmental research in Ireland and is organising and administering the STRIVE programme on behalf of the Department of the Environment, Heritage and Local Government.

epa
Environmental Protection Agency
An Ghníomhaireacht um Chaomhnú Comhshaoil

ENVIRONMENTAL PROTECTION AGENCY
PO Box 3000, Johnstown Castle Estate, Co. Wexford, Ireland
t 053 916 0600 f 053 916 0699
LoCall 1890 33 55 99
e info@epa.ie w http://www.epa.ie

SSTI
Strategy for Science, Technology and Innovation

Comhshaol, Pobal agus Rialtas Áitiúil
Environment, Community and Local Government