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GROUNDWATER CRUSTACEA OF IRELAND

A survey of the stygobitic Malacostraca in caves and springs

Report

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ABSTRACT

Survey work on the stygobitic Crustacea (Malacostraca) species of Ireland was carried out in early spring 2008. The survey, which concentrated on springs and caves added 26 new records from 23 new locations and expanded the known distribution of three of the four known Irish species. Most of the records were of *Niphargus kochianus irlandicus*, the commonest and most widespread of the Irish stygobitic Crustacea. The Carrowmore cave system in County Sligo, is the northernmost record for this species in Ireland and for the genus *Niphargus* in Europe. *Niphargus wexfordensis* was discovered at four new locations: springs in County Wexford and County Kilkenny and two caves (Polldubh, County Clare and Central Cave, County Cork). The sole record of the tiny *Microniphargus leruthi* was from riverine interstitial gravels on the Dripsey River, County Cork. This is only the second record of this species from Ireland, following its discovery in County Cork in 2006. Sixty five sites were sampled in total and taxa lists include epigean (surface-dwelling) taxa found within the groundwater habitats.

INTRODUCTION

THE GROUNDWATER ECOSYSTEM

It is estimated that approximately 22% of freshwater is stored underground, which, excluding glaciers and icecaps, constitutes 97% of the freshwater that is potentially available for human use, the remainder being lakes, rivers, swamps and soil moisture (Quevauviller, 2008). Groundwater is present, to some extent, in all rock formations but is most abundant in strata that have sufficient permeability and porosity to allow the movement and storage of water, i.e. to form aquifers. Aquifers are usually bordered above by an unsaturated zone, containing both air and water and below by impermeable strata such as shale. Aquifers can be broadly classified into three main types: karstic, porous and fractured.

Karst consists of rock types that are readily dissolved by water and have specific features, such as caves and sinkholes associated with them. The main type of karst rock is limestone (calcium carbonate). Much of Ireland is underlain by limestones, many of which are karstified. Other karst found in Ireland includes dolomite (magnesium and calcium carbonate) and a small area of chalk in County Antrim. It is the solutional action of water over thousands of years that creates the complex system of conduits, varying from micrometers to meters in scale that permeate the rock and form the aquifer. Within the karst the groundwater environment can be divided into two distinct ecotones, the water-saturated zone and above this the vadose, or unsaturated zone.

Porous aquifers are present in unconsolidated sediments, such as sand and gravel and are usually associated with alluvial deposits underlying watercourses (valley aquifers). Alluvial aquifers consist of two ecotones, the deeper, water-saturated phreatic zone and the hyporheic zone, the zone of interaction between the groundwater and the surface water in the watercourse above. Other porous aquifers consist of hillslope and perched upland aquifers.

As the name suggests, fractured or fissured aquifers are present in rock formations that have undergone substantial fracturing due to processes such as tectonic and volcanic activity etc. and include rocks such as granite, shale and basaltic lava.

Based on hydro-geological characteristics and the value of the groundwater resource, Ireland's land surface has been divided into nine aquifer categories, of which the most important are 'karstified bedrock', 'fissured bedrock' and 'extensive sand and gravel' (DELG, EPA & GSI, 1999).

Environmental management objectives for groundwater resources have traditionally focused on chemical and bacterial water quality (potability) and have failed to recognise their potential as critical aquatic ecosystems. The European Water Framework Directive indirectly deals with groundwater ecosystems by stating that "the status of a body of groundwater may have an impact on the ecological quality of surface waters and terrestrial ecosystems associated with that groundwater body." Within the introduction to the new European Groundwater Directive mention is made of the requirement for further research into groundwater ecosystems and their consideration in groundwater management decisions. Similarly, despite the Rio Convention of 1992 highlighting the consideration of biodiversity as a major duty of governments towards future generations, the biodiversity of subterranean habitats has been commonly neglected or even ignored.

The European Protocols for the ASsessment and Conservation of Aquatic Life In the Subsurface (PASCALIS) has gone some way to address this omission and has identified two major constraints for the successful implementation of a protection policy for groundwater biodiversity: the current incomplete state of knowledge on groundwater biodiversity and the lack of a rational conservation strategy. The ultimate goal of the project is to propose a specific Action Plan for the conservation of groundwater biodiversity at the European level (PASCALIS, 2008).

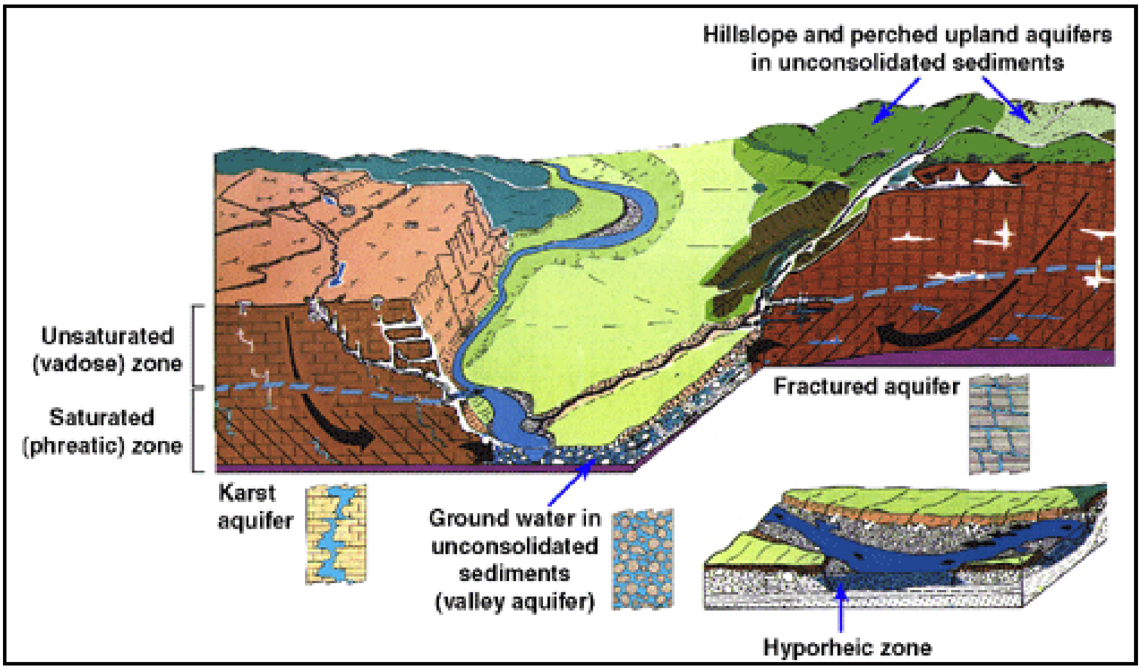

Figure 1. Aquifer types (reproduced from Dole-Olivier *et al.*, 2008).

The primary characteristic of the groundwater environment is the lack of light and primary production (photosynthesis) and a general reduction in the diversity of habitats, due primarily to the absence of vegetation. However, physical heterogeneity still exists in karst and fractured aquifers due to the diversity of void shape and size. Within the karst vadose zone there can be a variety of different habitats present within caves, ranging from underground streams and rivers to small isolated pools and deep lakes. The interstitial habitat (porous aquifers) is even more unfavourable, with only one habitat type, the pore space being available. However, even the pore space can have considerable heterogeneity, such as the size and arrangement of grains, the void size and the different physical and chemical qualities of aquifers (Gibert *et al*., 1994). Other groundwater characteristics include little or moderate annual and daily temperature fluctuations, low trophic inputs and low oxygen conditions in the interstitial and deep phreatic.

Due to the lack of primary production, most underground ecosystems rely on the input of exogenous energy coming underground in the form of organic matter originating from the surface, mostly detritus washed in by streams and percolating water from above. Fungi and bacteria are the primary consumers and frequently form bio-films on calcite formations in caves and on the granular surface in the interstitial habitat. They are then in turn grazed upon by larger micro-organisms and invertebrates and form the lowest tier in simple subterranean food webs. Many subterranean invertebrates are generally polyphagous in their diets due to the scarcity of food. Gibert (1986) found that *Niphargus rhenorhodanensis* fed on small aquatic invertebrates and various terrestrial organisms washed underground but that clay and its associated bacteria and fungi are its basic source of nutrition. Jefferson (1989) and Chapman (1993) discuss the possibility of endogenous food webs in British caves, arising from chemo-autotrophic bacteria and bacteria utilising fossilised hydrocarbons in limestone. Jefferson (1969 & 1976) also discusses the possibility of some organisms absorbing organic compounds direct from solution.

Hypogean (subterranean) organisms display biological, morphological, physiological or behavioural adaptations that appear to be linked to the limitations of their environment. These include lack of pigmentation, ocular regression and hypertrophy of sensory organs, with appendages tending to be long and numerous, with highly developed chemical and mechanical receptors also usually present (Gibert *et al.*, 1994). Because of the small pore space, many interstitial animals tend to have long, thin body shapes and a small size in comparison to epigean relatives. Due to the energy-poor environment many hypogean organisms have life histories involving delayed maturity, an increase in longevity, fewer, larger eggs in comparison to epigean species and reduced motor and reproductive output (Gibert *et al.*, 1994).

The inhabitants of the groundwater can be classified into three groups (based on Gibert *et al.*, 1994). Stygoxenes are organisms that have no affinities with the groundwater environment but occur accidentally in caves and alluvial sediments, washed in from above. Stygophiles have greater affinity with groundwaters and actively exploit the resources therein or use the environment as a refuge from predators and floods. They include the early instars of some aquatic epi-benthic invertebrates and taxa such as some species of Copepoda which spend their entire life in the groundwater but can also be found in epigean habitats. Stygobites are obligate hypogean species, specialized to a subterranean existence and frequently showing morphological adaptations described above. The stygobites of Britain and Ireland are represented by two stygobitic Oligochaeta, 16 species of water mites (Hydracarina) found in the interstitial and several species of Malacostraca Crustacea.

Research work in recent decades has shown that far from being biological "deserts", groundwater habitats are surprisingly rich in diversity, containing almost all the major taxonomic groups encountered in surface water habitats and harbouring an impressive number of phylogenetically isolated animals. It is likely that, on a global scale, the groundwater biota is virtually unknown.

Many groundwater taxa are of great age and some ancient lineages were probably established in groundwater prior to the separation of North America from Eurasia during the Mesozoic, or even the breaking up of Pangea during the Jurassic (Gibert *et al.*, 1994, Ward *et al*., 2000 and Dole-Olivier *et al.*, 2008). Many groundwater species were comparatively protected from the major climatic changes and other catastrophes that led to massive extinctions of the epigean fauna. Although groundwater systems are inter-connected, many taxa appear to show low dispersal abilities and habitat fragmentation may have been sufficient to promote reproductive isolation (Barr & Holsinger, 1991). These three factors have led to high degrees of endemism within the groundwater fauna. Recent developments in DNA studies have shown that subterranean habitats can harbour very high numbers of morphologically similar but genetically different 'cryptic' species. The notes on *Niphargus kochianus irlandicus* below highlight recent genetic research in this field. These high levels of endemism within groundwater fauna mean that many species can face extinction due to anthropogenic disturbances (Dole-Olivier *et al.*, 2008). Within Ireland such disturbances include threat to groundwater from diffuse agricultural pollution and decreasing groundwater levels arising from over-abstraction from an increasing population as well as future shifts in rainfall patterns due to global climate change.

BACKGROUND TO THE PROJECT

As mentioned above, the Crustacea form an important component of the stygobitic fauna of the British Isles and Ireland. *Gammarus duebeni celticus* and several species of cyclopoid Copepoda: *Eucyclops serrulatus*, *Megacyclops viridis* and *Acanthocyclops venustus* have been recorded as stygophiles in Ireland (Proudlove *et al.*, 2003) but the most widely distributed and diverse group are the stygobitic Malacostraca, of which four species have been recorded from Ireland. The small syncarid *Antrobathynella stammeri* is only known from one site on the River Flesk. Outside of Ireland it is known from England, Scotland, Germany, Austria, Italy, Romania and the Czech Republic (Proudlove *et al.*, 2003) and has recently been discovered in Belgium (Fiers, 2007). The two niphargids *Niphargus kochianus irlandicus* and *Niphargus wexfordensis* are taxa endemic to Ireland and are not known to occur elsewhere. The tiny *Microniphargus leruthi* has only been recorded once in County Cork in 2006 (Arnscheidt *et al.*, in prep.)

The first stygobite record for Ireland was in 1863, when Kinahan collected several specimens of *Niphargus kochianus irlandicus* from an old well, sunk in limestone in Dublin. His notes mention that "a few individuals had also been observed many years ago" (Bate & Westwood, 1863). In the following years the species was recorded from a well at Templeogue, on the outskirts of Dublin, from the bottom of Lough Mask and from West Meath, near Mullingar in 1910. The next record was not until 1956, with further records in the 1960s and 1970s, many from caves in the Burren, County Clare (Hazelton, 1974). There were no further records until 1997 when it was recorded by K. Thorne as abundant in St. Patrick's Well, a covered 'holy well' near the River Liffey, Morristown, Co. Kildare. In 2003 it was recorded by EcoServe in the same well, during a survey of thermal springs in Ireland (EcoServe, 2003). Two further records from Co. Clare included a single specimen collected by A. O'Connor from the margins of Treed Turlough in 2002 and in 2005 Knight collected specimens from the Doolin River Cave on the Burren (HCRS, 2008).

In 1980 a new species of aquatic stygobite Crustacea was found in a well at Kerloge, County Wexford, which was later described as *Niphargus wexfordensis* (Karaman *et al.*, 1994). This species was recorded at the same location in 1986 and 1987. Later re-examination of the material collected by Knight from the Doolin River system subsequently found that as well as *Niphargus kochinaus irlandicus*, at least one of the specimens was identified as *Niphargus wexfordensis*, previously unknown outside of County Wexford.

Antrobathynella stammeri was collected by T. & J. Gledhill from a gravel bank beside the River Flesk, near Killarney in 1982 (Gledhill & Gledhill, 1984) and has not been recorded since (HCRS, 2008).

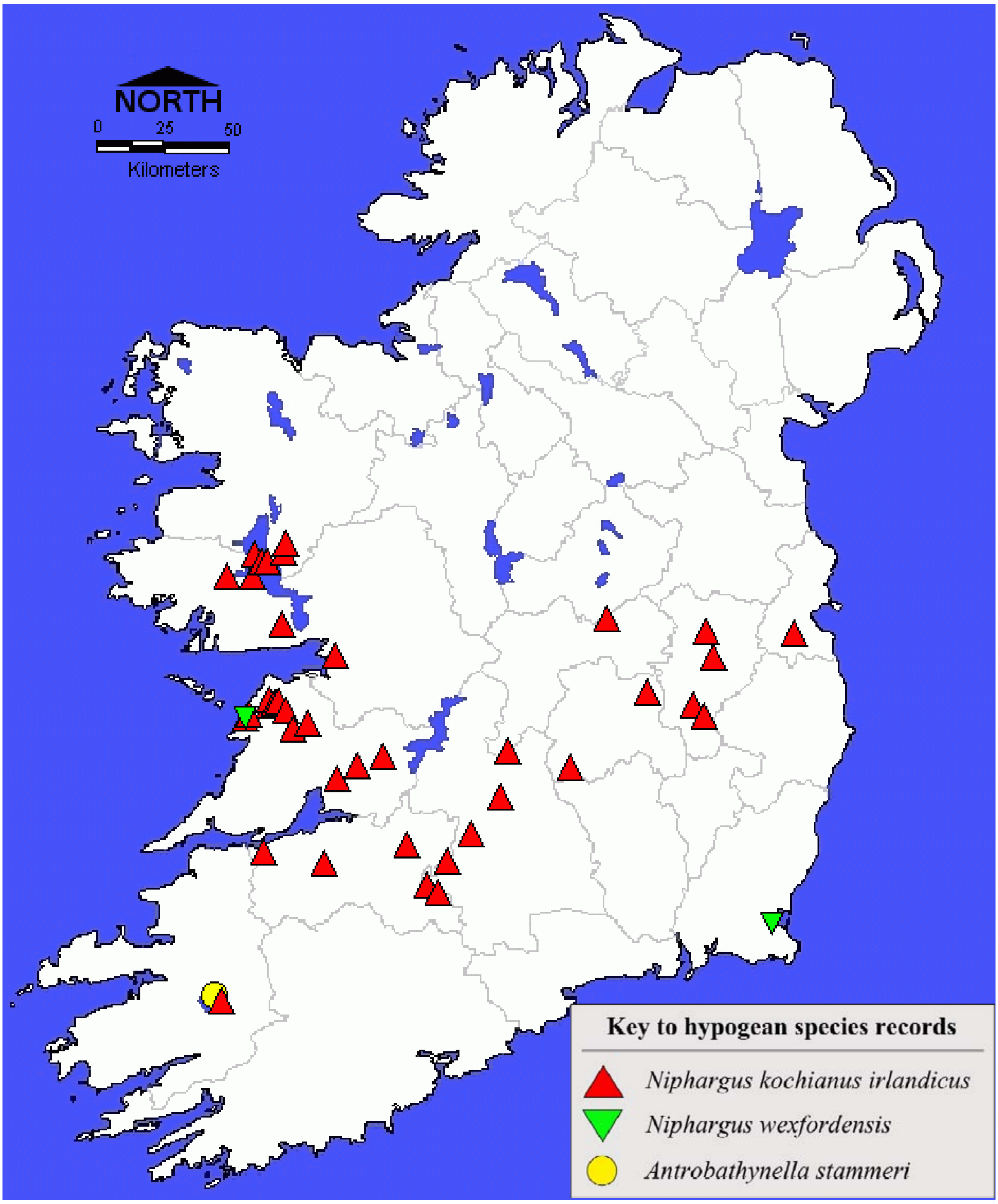

Figure 2. Map of the distribution of previously published records of stygobitic Crustacea in Ireland.

The above paragraphs highlight the lack of systematic recording of the stygobitic Crustacea in Ireland and the urgent need for research into both the distribution of the group and the possible occurrence of new species within the country. The lack of systematic recording is primarily due to the difficulties of sampling the groundwater habitat, which is mostly inaccessible to man. Access to groundwater is only really achievable via wells, boreholes, entry into caves and where it comes to the surface as springs or the resurgences of underground rivers. The fauna of the shallow hyporheic zone can also be sampled along watercourses using various methods. Records for the Irish stygobitic Crustacea previous to the current survey are included in Appendix 2. These are held by the Biological Records Centre at Wallingford, England and the Hypogean Crustacea Recording Scheme (www.freshwaterlife.org/hcrs.html). The records show that from 1863 to 2005 *Niphargus kochianus irlandicus* has been recorded 62 times from 39 locations. *N. wexfordensis* has been recorded four times from two locations and *Antrobathynella stamerri* has been recorded once from the River Flesk. The first record of *Microniphargus leruthi* has not yet been published (Arnscheidt *et al.*, in prep.) and has not been included in Appendix 2.

The diversity of hypogean Crustacea in Britain and Ireland is impoverished in comparison to that of mainland Europe. It has generally been accepted that this is the result of the last glaciation (known as the Devensian in Britain and the Midlandian in Ireland). At its maximum extent, between 25,000 and 15,000 years ago it covered most of Ireland and Wales, northern England and Scotland, with permafrost conditions present in the south. It is assumed that the ice would have sterilised the environment beneath it and would have removed sources of food from the surface environment. This theory would suggest that most of the British and Irish stygobitic Crustacea populations were established by animals migrating from un-glaciated areas in Europe (dispersal hypothesis). The main pattern of distribution of the British species would seem to support this hypothesis, with most records occurring in the southern part of England. It is thought that the cave systems of South Wales were re-colonised by migration from areas such as the Mendip Hills in North Somerset.

However, there are several notable exceptions to this, including *Antrobathynella stammeri* in the Midland Valley of Scotland and several records of *Niphargus aquilex* north of the glacial limit. Similarly, in Ireland most of the *Niphargus kochianus irlandicus* records are north of the Midlandian glaciation. Current thinking is that the hypogean fauna actually survived in un-frozen groundwater within the tundra (tundral refugia) and beneath the ice-sheet (sub-glacial refugia). The recent discovery of two endemic species of hypogean Crustacea in Iceland, *Crymostygius thingvallensis* and *Crangonyx icelandicus* (Krisjánsson & Svavarsson, 2007) proves that stygobitic Crustacea can survive beneath ice. It is now thought the populations of South Wales survived beneath the ice in the ancient cave systems there, rather than having become established by re-colonisation at the end of the Devensian glaciation. Proudlove *et al.* (2003) discuss the geological and geomorphological explanations for the distribution of the British and Irish stygobitic Crustacea.

The sub-glacial refugia hypothesis is certainly more plausible with regards to the Irish fauna, as the question of how freshwater subterranean taxa may have colonized the country is even more complex than that of Britain. Yalden (1999) describes a 100 m deep seawater channel separating Ireland from Britain and there appears to be little evidence of the necessary land bridge to facilitate colonization. One possible theory was the existence of a mobile land bridge that moved northward with ice recession. This would have been low-lying and possibly tidal (Proudlove *et al.*, 2003). It is likely that the Irish hypogean fauna have been isolated from the rest of Europe for some considerable time, which would explain both the paucity of the fauna and the high proportion of endemism. This hypothesis is supported by recent research on the DNA of *Niphargus kochianus irlandicus* discussed in the species' account below.

The initial application for funding from the Heritage Council was for a systematic survey of selected groundwater sites throughout Ireland, with a focus on the province of Munster, where most of the previous records originated. However, after the initial application, information on important survey work being carried out by Dr. Joerg Arnscheidt of the University of Ulster came to light. Arnscheidt has been awarded funding by both the Royal Irish Academy and the Environmental Protection Agency to carry out systematic surveys for groundwater fauna in 2008 and 2009. He carried out a pilot survey in 2006 which has produced some very interesting results (Arnscheidt *et al.*, in prep.). This survey represented the first systematic survey of groundwater sites in Ireland and included the recording of *Niphargus wexfordensis* at a second site in County Wexford, the discovery of the tiny stygobitic amphipod *Microniphargus leruthi* in County Cork and the recording of *Niphargus kochianus irlandicus* at several new locations, as far north as the Dundalk Peninsula, the most northerly record for *Niphargus* in Europe. The 2006 survey concentrated on sampling wells and boreholes with no work carried out on springs, interstitial sites and caves. After consultation, the 2008 RIA survey will sample two caves, Marble Arch, County Fermanagh and Crag Cave, County Kerry. A further four caves are planned to be included in the EPA STRIVE survey, which will take place over three years from 2009 to 2011. Thus it was decided that the current survey, for the Heritage Council would concentrate on primarily sampling caves and springs, with the inclusion of some hyporheic (interstitial) sites. This would both complement the work being carried out by Ulster University and avoid duplication.

Much of Ireland is underlain by limestone, although not all of this is karstified. Nonetheless, almost every county in Ireland has at least one cave or more, although their number and size varies greatly. The main areas of cave development in Ireland are: the Blackwater Valley limestone, mostly in County Cork but with extensions into the counties of Tipperary and Waterford; the caves around Castleisland in County Kerry; the Burren in County Clare, the most famous of Ireland's areas for sport cavers; Aille River Cave in County Mayo; the Sligo-Leitrim area; and the Fermanagh-Cavan area. Information on these areas is available through guide books, covering Fermanagh (Jones, 1974 and the more recent Jones *et al.*, 1997), Clare (Mullan, 2003) and Cork (Oldham, 2003) and the now out-dated Coleman (1965), covering all of Ireland. Further information is available from the cave database of Ireland, on the University of Bristol Spelaeological Society website (www.ubss.org.uk), from the Speleological Union of Ireland (www.cavingireland.org) and on the website of the Geological Survey of Ireland (www.gsi.ie).

STYGOBITIC CRUSTACEA SPECIES ACCOUNTS

Antrobathynella stammerri (Jakobi, 1954)

The superorder Syncarida is a freshwater group that evolved from marine stock and has the total absence of any vestige of a carapace or cephalic shield. It consists of three orders. Members of the Anaspidacea are restricted to Tasmania and Australia, where they are found in streams and lakes on the surface, as well as springs and caves. The small order Stygocaridacea contains four genera and seven species, all found in interstitial waters in South America and New Zealand. The order Bathynellacea was the first to move into groundwaters and originated by neoteny from epigean ancestors (Hobbs, 2000). It is the largest of the three orders with some 200 species, mostly recorded from temperate regions, although some are known from tropical areas. With the exception of two species in Lake Baikal, all members of the Bathynellacea are stygobites and confined to freshwater, except for two species of *Hexabathynella* which will also tolerate brackish water. With the exception of several Australian species, they are mostly tiny animals, about 1mm long, with a thin, elongated body shape adapted to interstitial and phreatic habitats.

Little is known of bathynellid biology. They are believed to feed on detritus, bacteria and fungi and their locomotion consists of a combination of swimming and crawling. Post embryological development is abbreviated, with only two phases, a larval (parazoeal) phase and a juvenile (bathynellid) phase, supporting the idea that they evolved by neoteny. The developmental period for German specimens of *Antrobathynella stammeri* averages 9 months (Gledhill *et al.*, 1993). The closely related genus *Bathynella* demonstrates the extreme in K-reproductive strategy by producing a single, large yolky egg (Hobbs, 2000).

Antrobathynella stammerri is a very small, eyeless crustacean, approximately 1 mm long and 0.1 mm in diameter. It is more or less colourless with a long, thin body, highly developed to suit the interstitial habitat from which it is generally recorded. The body is elongate with 14 trunk segments (8 thoracic and 6 abdominal) and the head is longer than broad. Each of the thoracic limbs, with the exception of the last, is two-branched. All but the first and last abdominal segments are without appendages. The 1st antenna is un-branched, whilst the 2^{nd} has a small branch (Figure 3).

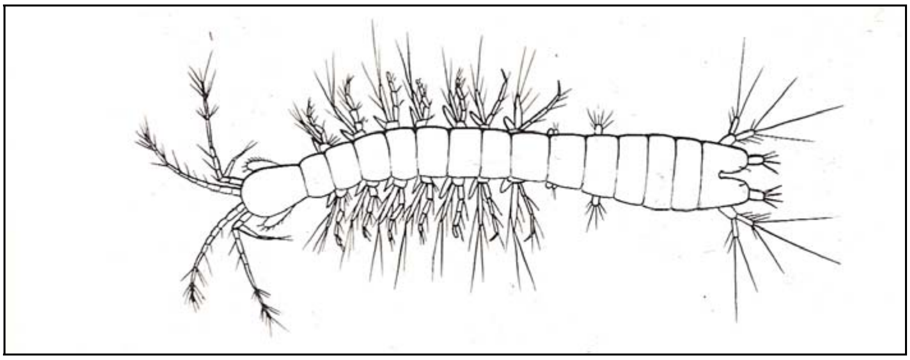

Figure 3. *Antrobathynella stammeri* (drawing from Gledhill *et al*. 1993, after Thienemann).

A single specimen of *A. stammeri* was collected in November 1982 from a gravel bank on the side of the River Flesk, near Flesk Castle, Killarney in County Kerry (Gledhill & Gledhill, 1984). The specimen was collected using a Bou-Rouch pump after a single specimen of *Niphargus kochianus irlandicus* was also collected from the same location using the Karaman-Chappuis method. This remains the only record of *Antrobathynella* from Ireland.

The genus *Niphargus*

The genus *Niphargus* is known from the Palaearctic region west of the Caspian Sea. It is thought that diversification and speciation within the genus began in the basins of the Paratethys Sea during the Tertiary Period, from which European fresh waters were subsequently colonised via brackish subterranean waters. This is supported by the fact that the largest number of taxa and those most differentiated morphologically and ecologically are found in the Danubian – Carpathian region and the northern parts of the Balkan Peninsula. There is a reduction in the number of taxa with distance from this area. Of the 252 taxa included in *Niphargus*, only 5% inhabit surface waters, including the bottoms of lakes (Gledhill *et al.*, 1993).

Niphargus species are generally considered to be saprophagous, living on plant and animal detritus, much of which is washed into the subterranean habitat from the surface. However, some are predaceous on other invertebrates, including juvenile niphargids. *Niphargus fontanus* has been observed preying on the stygobitic isopod *Proasellus cavaticus* in Welsh caves and niphargids can be captured in traps baited with meat or cheese. Due to the scarcity of food in the underground habitat it is likely that they are opportunistic feeders, eating whatever they encounter.

Karaman & Ruffo (1986) recognised three morphological types of niphargids. Species from cave habitats are generally large, with long antennae and a stout *Gammarus* – like body. Those from phreatic and interstitial habitats are often less than 5 mm long, with either a slender and elongated body, often with small non–contiguous coxae, or with a stout body and large contiguous, or partly–overlapping coxal plates. They suggest that body size in phreatic and interstitial habitats is related to differing types of locomotion through different physical media. British specimens of *Niphargus fontanus* certainly follow this pattern, with cave specimens tending to be large and robust and specimens from interstitial habitats especially, being much smaller and thinner.

Life cycles in niphargids may be relatively long. *Niphargus virei* has 13 moults over 3 years to reach sexual maturity and may live for up to 10 years. The numbers of eggs carried by breeding females varies between species but niphargids generally have few eggs. Gledhill & Ladle (1969) studied the life cycle of *Niphargus aquilex* in exposed gravel beds of the Oberwater, a soft-water stream in the New Forest, southern England. *N. aquilex* was observed to have two generations each year, with mature males and females exceeding 20% of the population. In *N. aquilex* the number of eggs per female ranges from 1 to 7 (mean 2.9) and is linearly correlated with body length. Small species of *Niphargus* and surface-dwelling gammarids (4-6 mm in length) carry similar numbers of eggs (5-7) but the ratio of egg number to body size increases more rapidly in the gammarids. Large mature *Niphargus* have only half the number of eggs carried by large gammarids of similar size (Ginet, 1960).

Niphargus kochianus irlandicus **(Schellenberg, 1932)**

The first Irish niphargid record, from Dublin in 1863, was of *Niphargus kochianus irlandicus*. Schellenberg (1932) differentiated the Irish specimens as a separate sub-species from the British *Niphargus kochianus kochianus*, a decision upheld by Stock & Gledhill (1977).

The *Niphargus kochianus* group is differentiated from other British and Irish niphargids by the sub-acute palmar angle of the propodus of gnathopods 1 and 2 and the acute posterodistal angle of epimeron 3. The telson lobes lack lateral spines, although 3 - 4 distal spines are present. *N. kochianus kochianus* and *N. kochianus irlandicus* primarily differ in the shape of the propodus; an extensive fringe of D-setae on the mandible palp article 3 of *N. kochianus irlandicus*; a single dorsal spine on either side of urosome segment 2 in *N. kochianus kochianus* (3 – 4 spines in *N. kochianus irlandicus*); and the more acute posterodistal angle on epimeron 3 in *N. kochianus kochianus*. A series of photographs in Appendix 3 illustrate these features.

Niphargus kochianus irlandicus is endemic to Ireland and is one of the two *Niphargus* species currently known to be present. Although *N. kochianus kochianus* and *N. kochianus irlandicus* are morphologically very similar the two have been separated from each other by the Irish Sea for at least 10,000 years and share no genetic continuity. Recent DNA analysis carried out by Proudlove & Hanfling (2008) has shown that they are not in fact related and that they last had a common ancestor over 10 million years ago. This leads to the conclusion that the Irish populations have been isolated in Ireland for this period and must have survived many Pleistocene glacial cycles in sub-glacial refugia. From the results of the analysis it would appear that *N. kochianus irlandicus* is more closely related to the British and European species *Niphargus fontanus* and it is possible that this represents the common ancestor from which members of the *kochianus* group diverged. On the basis of this research, *Niphargus kochianus irlandicus* should be elevated to species rank. It would appear that morphology is not always the best method for taxa separation, highlighting the importance of future DNA studies in identifying ‘cryptic’ taxa amongst the stygobitic Crustacea.

N. kochianus irlandicus has been recorded from numerous localities across central Ireland, extending into County Kerry in the south west and as far north as County Galway in the west and the Dundalk Peninsula in the east. Unlike most other British niphargids, most of these records are within areas fully glaciated during the Midlandian. Costello (1993) suggests that it might be a pre-glacial relict species, having survived beneath the ice in sub-glacial refugia, an assumption supported by the DNA evidence above. Habitats that *N. kochianus irlandicus* has been recorded from include wells, riverine gravels and caves, the latter mostly in County Clare. It has also been recorded in the bottom sediments of Lough Mask. Both Schellenberg (1932) and Stock & Gledhill (1977) noted slight morphological differences between specimens from Lough Mask and those from caves and wells, although these were not thought sufficient enough to form the basis of a new taxon. However, in light of the new DNA evidence separating *N. kochianus irlandicus* from *N. kochianus kochianus*, some new research into this subject is required. As part of the EPA STRIVE project planned by the University of Ulster, a research group in Galway will be collecting specimens from Lough Mask.

Figure 4. *Niphargus kochianus irlandicus* from springs in County Clare (sites CL 2, CL8 & CL 9).

Niphargus wexfordensis (Karaman, Gledhill & Holmes, 1994)

Niphargus wexfordensis is endemic to Ireland and is one of the two *Niphargus* species currently known to be present. It was first recorded from a well in the garden of a house at Kerloge, County Wexford in 1980. This was thought to be the only known location for this endemic Irish species until 2006, when it was recorded from a second site in County Wexford by Arnscheidt. (Arnscheidt *et al.*, in prep.).

As mentioned above, recent re-examination of some of the material (prompted by the observations of C. Fišer, University of Ljubljana and the results of the current survey) collected by Knight from the Doolin River Cave in 2005 has since revealed at least one of the specimens, previously regarded as *Niphargus kochianus irlandicus*.to actually be *Niphargus wexfordensis*. This specimen had been overlooked as the species was not known to occur outside of County Wexford and was previously unknown from the cave habitat.

Niphargus wexfordensis was described by Karaman *et al*. (1994), who noted its similarities with the British species *Niphargus glenniei*. *N. glenniei* also has a very limited distribution, being confined to the far south west of England and has only recently been discovered in West Cornwall, the first record outside of the county of Devon (Knight, 2001). The similarities between the species are primarily a reduced number of D-setae on article 3 of the mandible palp, a small size and the outer ramus distinctly shorter than the inner on uropod 2 (although the rami are sub-equal on uropod 1). It differs from *N. glenniei* in the telson being cleft to almost half its length (three-quarters in *N. glenniei* and *N. kochianus irlandicus*), with each lobe bearing 3 distal spines and a pair of plumose setae (no spines and 3 distal setae in *N. glenniei*) and differences in the setation of the mandible palp articles 2 and 3. The main features that separate the species from *N. kochianus irlandicus* include the telson cleft and mandible palp, mentioned above and the more rounded shape of the gnathopods, with gnathopod 2 propodus normally larger than that of gnathopod 1. This latter feature means that superficially *N. wexfordensis* resembles small specimens of *N. fontanus*, another factor that could indicate this species as being the possible common ancestor of the Irish fauna. A series of photographs in Appendix 4 illustrates the features described above.

Figure 5. *Niphargus wexfordensis* from site KK2b, holy well, tributary of Nuenn River headwaters, County Kilkenny.

Microniphargus leruthi (Schellenberg, 1934)

Prior to its discovery at a site in County Cork in 2006 (Arnscheidt *et al.*, in prep.), *Microniphargus* was unknown from Britain and Ireland. It was previously thought to have a very limited area of distribution, between the Ardennes and the northern Rhine region and has been recorded from Engihoul Cave and wells near Liège, Belgium and wells in Siegburg and near Bonn in western Germany (Karaman & Ruffo, 1986). The discovery of *Microniphargus* in southern Ireland has great significance in our understanding of the distribution of the stygobitic Crustacea. The species' small size means that it has probably been overlooked in the past and it is very likely to be present in the British Isles, especially along the east coast. This report represents a big jump in the known distribution of the species from the Ardennes of Belgium. County Cork lies to the south of the Midlandian limit.

Figure 6. *Microniphargus leruthi* from interstitial gravels on the Dripsey River at Dripsey Bridge, County Cork (site CO4).

Microniphargus leruthi has specific characters, such as its small size (2mm) and the shape of the mandiblular palp, telson and gnathopods that set it apart from other genera in the family Niphargidae (Karaman & Ruffo, 1986). The third segment of the mandidular palp does not have a fringe of small bristles. The gnathopds are slightly longer than broad and appear rather rectangular. The telson is as long as wide, has a V-notch (as opposed to a cleft / split in other species) to about halfway and each lobe ends in a single spine. Other differences include the pleopods with two branches and the laccinia of the maxilla with a maximum number of seven spines (Arnscheidt, pers. comm.). These features are illustrated in a series of sketches and some of them are presented in photographs in Appendix 5.

METHODOLOGY

SURVEY STRATEGY

Field work was carried out between 31st March and 13th April 2008. The current survey aimed to primarily investigate caves and springs. The sampling strategy for the project was thus based around the main areas of cave development in Ireland (Figure 7). A few other minor caves were also selected in areas of interest outside the main areas of karst.

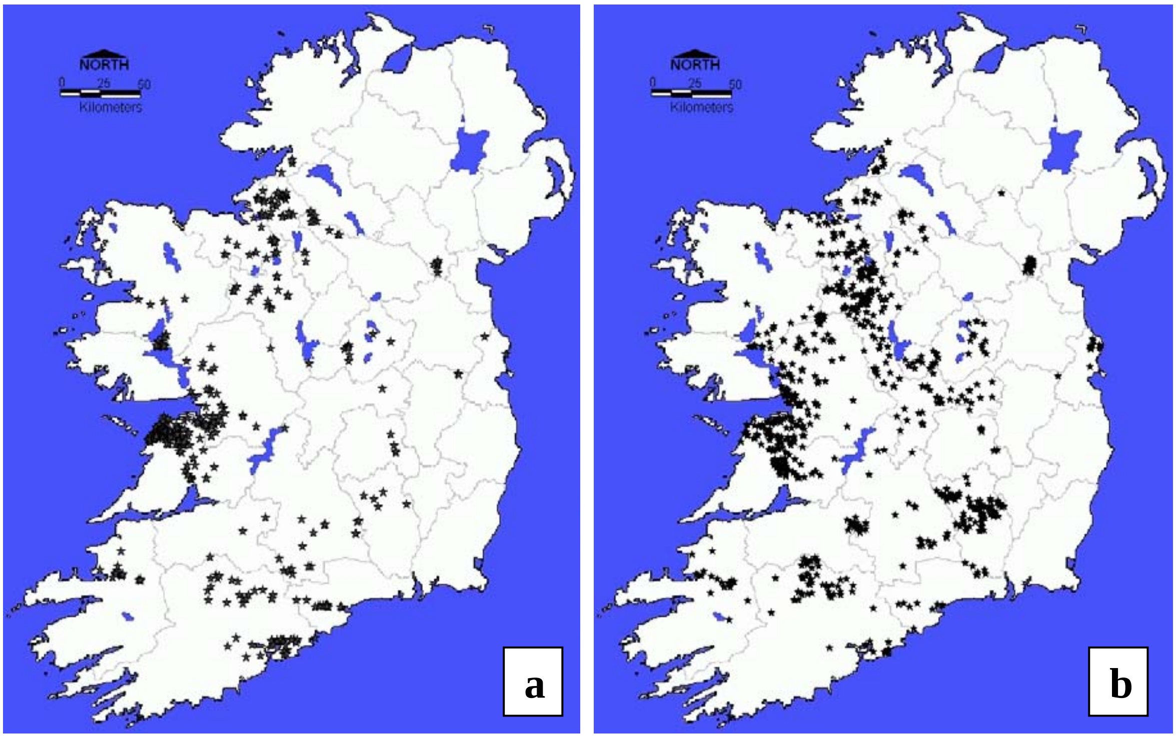

Figure 7. Distribution of caves (a) and springs (b) in the Republic of Ireland (based on data from GSI).

Springs were also targeted for sampling within and near to the cave areas. Springs were a more unknown factor in comparison to caves, as less information is available on them and it was often unclear whether sampling would be possible prior to a field visit. Information was gathered on a fairly large number of springs in each area and the number visited/sampled then depended on accessibility, time available and suitability for sampling. A lot of the information on springs was downloaded from the GSI web. However, many of these springs tended to be rather small and unsuitable for sampling and/or were difficult to locate. It soon became apparent that the best way to locate springs was to target "holy wells".

Irish holy wells are predominately springs, as opposed to wells *per se* and can take many forms, from small inconspicuous rock hollows on desolate hills, to rather ornamental shrines sheltering the spring source. Many of the holy wells in Ireland arise from clean limestone strata and are perfect for sampling using a variety of methodologies described below. Another advantage is that they are clearly marked on the Ordnance Survey Ireland 1:50,000 Discovery series of maps and are relatively easy to find.

Six of the holy wells (KK3, CL4, CL8, MO5, CA1 and WX7) were static pools of water, fed by groundwater seepage rather than an active spring flow. These locations were categorised as wells rather than springs. Three major resurgences (MO4, CA2 and FE2) were also sampled.

A few hyporheic (interstitial) sites were also sampled on an opportunistic basis. These sites were mostly side and point bars of gravel/sand and pebble/cobble ERS (Exposed Riverine Sediments) beside watercourses.

Altogether, approximately 100 sites were visited during the survey representing 13 counties. Of these, 65 sites were sampled: 21 caves, 27 springs (including flowing holy wells), 6 wells (static holy wells), 3 major resurgences and 8 interstitial (Table 1.1 in Appendix 1). Photographic records of representative sites are presented in Appendix 6.

SAMPLING SITES

County Kilkenny

Dunmore Cave National Monument (KK4) is situated on the Castlecomer plateau in a small, isolated outcrop of limestone. Most of the cave is developed as a show cave and is illuminated by artificial lighting. Two sections of the cave could be considered to be true wild (undeveloped) cave: the section beyond the low bedding plane in Haddon Hall and Crystal Chamber, the latter reached by a narrow passage beneath the main part of the show cave.

Four springs (McKeons Bar – KK1, upper reaches of River Nuenn - KK2a & b, Newtown North – KK5) and one well (source of Nuenn River – KK3) were also sampled.

County Tipperary

The Mitchelstown Caves are situated in a small strip of Carboniferous limestone on the southern side of the Galtee Mountains, a northern extension of the Blackwater Valley limestone (Oldham, 2003). Mitchelstown cave, also known as the New Cave has been developed as a show cave. Survey work was not carried out in this cave as permission was refused by the owner. Nearby is a second cave, which has remained undeveloped due to the dangerous 30 foot entrance pitch. This is the Old Cave (TP2), also known as Desmond's Cave, which was surveyed during this study.

One holy well (near Crannagh – TP1) was also sampled.

County Cork

Mogeeley Cave (CO1) is located in a small, overgrown quarry near Carrigshinagh Farm. Lake Cave and Central Cave are both located in the eastern section of the disused Carrigacrump Quarry near Cloyne. This quarry used to contain eight caves, four of which have been covered with blocks and other tipped debris. Deep pools and flooded rifts that connect with the phreatic water table are present in most of the caves. Central Cave (CO3a) has two entrances leading to a flooded rift at the end of the cave. Lake Cave (CO3b) is situated in the eastern wall of the quarry, much of which has been quarried away since the cave's discovery. It now only consists of the large lake chamber beneath a low, wide arch; consequently most of the cave is within the threshold zone (affected by daylight).

One spring (Rostellan – CO2) and one interstitial site (Dripsey River – CO4) were also sampled.

County Kerry

The Crag Cave system, near Castleisland consists of a total of 3.81km of stream and side passage. The middle part of the system has been developed as Crag Show Cave. The main stream in the system is fed by leaks from the bed of the Ahroe Stream/Glanshearoon River, to the north-west and by several sinks in the limestone at various locations. Crag Quarry Cave and Rift/Blocky Cave are upstream of the show cave, whilst downstream are Crag Cave JK's, Crag Lower Cave, Crag Cave 2, Crag Cave 3 and Crag Cave 4, after which the stream rises as the Anglore Stream (Jones, 1995). Although permission was given by the owner, Crag Show Cave was not visited as survey work in this cave was to be carried out by the Ulster University group later in 2008. Sampling was carried out in Crag Cave 2 (KE3c), Crag Lower Cave (KE3b) and JK's Cave (KE3a).

The small (147 m) Kilmurry Cave (KE4) is a relatively recent discovery to the east of Castleisland. The cave lies in upper Viséan massive mudbank limestone in the Vale of Tralee (Jones, 1995). The low entrance is in the bottom of a heavily overgrown disused quarry filled with tipped rubbish.

Three interstitial sites (Owengarriff River – KE1a & KE1b, Flesk River– KE2) were also sampled.

County Clare

Three caves were surveyed in the county: Faunarooska (CL10), Polldubh (CL11) and Pollballiny (CL12). The caves are located fairly close to each other on the north eastern side of Slieve Elva in the Burren. Almost all the caves in this area are active swallow holes, fed by streams draining off the Namurian rocks. With the exception of the Polldubh system, which rises to form the headwaters of the Coolagh River, none of the streams have proven resurgences (Mullan, 2003).

Six springs (Killinaboy – CL1, Fahee North – CL2, Eanty More – CL3, Gleansleade – CL5, Derrynavaahagh – CL7, Cloughmore – CL9), two wells (Berneens – CL4 and Gragan East – CL8) and one interstitial site (Aille River – CL6) were also sampled.

County Galway

The sampling in County Galway was concentrated on the Cong Peninsula, close to the border with County Mayo. Water from Lough Mask, at a higher elevation, runs beneath the peninsula into the lower Lough Corrib via a series of underground conduits in the limestone. The water sinks at a series of swallow holes on the shores of Lough Mask in Dringeen and Castle Bays, to rise at Hatchery, Ellechrissaun and Curreighnabannow springs, around the village of Cong. Hatchery and Curreighnabannow springs were inaccessible due to deep water and steep overgrown banks, in the case of the latter. The more accessible Ellechrissaun springs (GA1) rise at several points in a natural limestone amphitheatre.

Pigeon Hole Cave (GA2) is a deep opening, providing access to a short section of the underground passages carrying the water from Lough Mask. Sampling was also carried out in Ballymaglancy Cave (GA3).

County Mayo

A planned survey of the Aille River Cave system was abandoned due to high river flows and predicted bad weather. A short section of stream passage at the base of a cliff (MO2) near the main entrance was sampled instead.

The River Aille emerges from the system as a deep pool near Bellaburke. Upstream of this pool a channel, several hundred metres long and terminating in a rocky hollow carries extra flow in very wet conditions. This was dry at the time of the survey. At the downstream end of this channel, were two small pools (MO4) with gravel substrate overlying compacted clay.

Two springs (Caheredmond – MO1, Baloor West – MO3) and one well (Ross West – MO5) were also sampled.

County Roscommon

Pollawaddy Cave (RO1) is a small cave in the Oakport Limestone formation near Lisacul village, on the southern flank of a ridge above flat, marshy fields. At 45.5m long, it is the longer of two caves known in the county (GSI website).

Three springs (Gortaganny – RO2, Moor – RO3, Carrick-on-Shannon – RO4) were also sampled. The Gortaganny spring discharges into Lough Errit, close to a pumping station.

County Sligo

The Carrowmore cave system contains 250 m of passage entered via two deep pot holes Pollnagollum and Seighmairebaun (SO1), the latter was used to access the system.

Two springs (Carrownyclowan – SO2, Ummeryroe – SO3) were also sampled.

County Leitrim

One spring (Carrowrevagh – LE3) and two interstitial sites (stream flowing into Lough Gill – LE1, Bonet River – LE2) were sampled.

County Cavan

The source of the River Shannon (CA2), a deep water-filled pot was investigated but could not be sampled with the available equipment. Sampling was carried out in the channel just downstream of the pool.

One well (Tober – CA1) was also sampled.

County Fermanagh

The four caves sampled in the county (Upper Cradle Hole – FE4, Pollnagollum (of the boats) – FE3, Pollbwee – FE5 and Bruce's Pot – FE6) are situated fairly close to each other near the Marble Arch system in the Cuilcagh Mountain area of Fermanagh. These are all formed in pure carbonate mudbank-type limestone (Jones, 1974).

Upper Cradle Hole (FE4) lies in a large, L-shaped shake hole, at the southern end of which the Owenbrean River emerges for a short length, flowing east to west to its downstream sump. Pollbwee (FE5) and Bruce's Pot (FE6) are part of the Monaster Cliffs system above the Owenbrean River, which sinks at the base of the cliffs.

One resurgence, Tullyhona Rising (FE2), which rises from a cave system of the same name and one spring (Cavaneagh – FE1) were also sampled.

County Wexford

Sampling many of the holy wells in Co. Wexford proved quite difficult, as the substrate predominately consists of a thin layer of gravel overlying fine silt and sand or clay. Such material tends to clog the holes of the Bou-Rouch pipe (see below and Appendix 7), making pumping impossible.

Five springs (Kiltilly – WX1, St. Machan's Well – WX3, Kilnew – WX4, Knockanevin – WX5 and St. Killians Well – WX6), one well (St. Garvan’s Well, near Kilgarvan — WX7) and one interstitial site (River Slaney at Enniscorthy – WX2) were sampled.

HABITATS SURVEYED

A variety of equipment and sampling methodologies were used during the survey to sample the groundwater habitats. For more detailed descriptions see Appendix 7. Each of the habitats is dealt with separately in the following sections.

Caves

Subterranean rivers and streams

Subterranean streams and rivers were sampled using a combination of kick and sweep sampling (net fitted with a 250 µm mesh collecting bag), both underground and at the point of resurgence from a system. A drift net (250 µm mesh) was occasionally anchored in place (using rocks) at the resurgence as the surveyor entered the cave along the stream. Benthic taxa dislodged by the movements of the surveyor were then washed into the net, which was examined for fauna after exiting the cave. Additional manual searching (described below) was also carried out at the margins and in areas of slack water.

Small pools, gour pools, dripping/trickling water on calcite slopes etc.

These habitats were sampled using manual searching, simply looking for fauna and collecting specimens using forceps or a pipette. Entomological and feather-light forceps were used to avoid damaging specimens. Animals in thin/trickling films of water were collected using small paint brushes, minimising damage. Care was taken to avoid disturbing the sediment at the bottom of pools as this could quickly cloud the water and make manual searching impossible. This also meant that manual searching was only effective in small pools in which the whole of the bottom could be viewed from the edges. In slightly larger pools a hand-net, fitted with an additional handle section if required, was used to sweep through the water and substratum at the bottom. Net sweeping was also sometimes used to supplement the manual search in small pools if no taxa were found, as *Niphargus* will often bury itself in the substrate.

Large pools/underground lakes, sumps and flooded passages

Manual searching and kick / sweep sampling with a net, fitted with an additional handle as required were used to sample the shallow margins of such waterbodies. The deeper sections were sampled using a trawl net (zooplankton net) attached to a length of rope and fitted with lead weights, to enable the net to both sink to the bottom and be thrown from the edge of the waterbody effectively. Care had to be exercised when deploying the net to make sure that it did not become snagged on submerged rocks.

Figure 8. Sweep netting in the margins of The Lake, Crystal Chamber, Dunmore Cave, County Kilkenny (KK4).

Springs (wells and resurgences)

Many of the springs encountered consisted of groundwater upwellings through gravel and sand. These were sampled using the Bou-Rouch method at the point of issue. If circumstances did not permit this, then the Bou-Rouch pump was deployed as near to the point of issue as possible downstream (**Error! Reference source not found.**). The Bou-Rouch method involves abstracting sub-surface water using a piston pump, fitted with a collecting net and attached to a perforated steel pipe driven into sediments (for further details see Appendix 7).

In some circumstances Bou-Rouch sampling was not possible at a spring due to its physical characteristics. Some of the springs on the Burren (e.g. sites CL2 and CL3) consisted of water flowing out from between limestone strata and at the issue point there was only a thin layer of gravel and sand overlying bedrock. Other springs consisted of fine sand and / or silt which rapidly clogged up the holes of the Bou-Rouch pipe and made pumping impossible. At such sites a degree of improvisation was implemented and sampling consisted of kick and sweep sampling with a hand net, the placement of a drift net or a combination of the above. At site CL 5, a small spring consisting of a muddy seepage the Karaman-Chappuis method was used. One of the most effective methods employed was placing a drift net just downstream of the issue point and then disturbing the substrate with a hand-held garden weeding rake as far into the spring issue point as could be physically reached and as deep into the substrate as the rake would go. The resulting plume was then swept with a hand net and the contents of the drift net examined. Often the issue point was so small that the drift net could not be put in place, in which case a hand net was held downstream during the above procedure instead.

Holy wells consisting of stagnant pools of water were sampled by net sweeps or the use of a zooplankton trawl net (KK3). The three major resurgences were treated as large springs and were investigated with a combination of sweep and kick sampling.

Figure 9. Bou-Rouch sampling of a spring at site KK1.

Hyporheic (riverine interstitial) sites

These were primarily side and point bars of exposed riverine sediment (ERS) on rivers. They were sampled using a combination of both the Bou-Rouch and the Karaman-Chappuis methods, which proved to be more efficient than using either method in isolation. The Karaman-Chappuis method involves digging a pit in sediments until the water table is reached and filtering out the water as it percolates into the hole. For further details see Appendix 7.

After the initial Karaman-Chappuis pit was dug and the water filtered several times, the Bou-Rouch pipe was then hammered into the substrate at the bottom of the pit. The water was then pumped through a pipe net, to filter out any fauna and into a jug so that it could be thrown into the nearby river and the interstitial flow into the hole was maintained.

Figure 10. Combined Karaman-Chappuis & Bou-Rouch sampling on ERS (CO4).

SAMPLE PROCESSING

After each sampling event, the contents of the net were placed in a black-lined tray and examined in the field. Specimens of stygobitic Crustacea were picked out with entomological forceps and placed in a vial, whilst epigean fauna were mostly identified in the field and returned to the habitat.

Specimens picked out in the field were placed in vials of 70% IMS (Industrial Methylated Spirits) solution for transportation back to the laboratory and examination beneath a stereoscopic microscope. If further magnification was required a compound microscope was also used. For long-term storage approximately 5% by volume of glycerol was added, to prevent complete dehydration should the tubes become damaged. Representative specimens of each taxon, in good condition, were selected from certain samples for sending to the National Museum of Ireland as reference material. The bulk of the material was kept by Knight and is available for examination upon request.

As most samples from subterranean and interstitial habitats contain little organic matter, examination of many of the samples was possible in the field. The samples were generally free of the plant matter, detritus and empty shells and cases etc. that make samples from epigean freshwater so difficult and time-consuming to process. However, some of the samples contained large amounts of sand and gravel and were preserved in 70% IMS and retained for more detailed analysis in the laboratory.

These samples were processed using a combination of decantation, sieving and the modified floatation technique, developed by Anderson (1959). A fraction of the sample was placed in a large tray and a sugar solution (approximately 1 kg granulated sugar per gallon of solution) was introduced, causing organic matter to float to the surface, due to differences in the specific gravities of the organic matter and the mineral substrate. The supernatant was then poured through a fine mesh (250 μm) sieve and smaller fractions of the sieved mater were sorted in a large Petri dish beneath a stereoscopic microscope. Specimens were then picked out for identification. Standard Freshwater Biological Association (FBA) taxonomic keys were used for the identification of freshwater epigean fauna. Hypogean Malacostraca were identified using the following publications: *Niphargus kochianus irlandicus*: Gledhill *et. al*. (1993); *Niphargus wexfordensis*: Karaman *et. al*. (1994); *Microniphargus leruthi*: Sminke (2007). Identification of *Microniphargus* was confirmed by T. Gledhill of the FBA.

PHYSICO-CHEMICAL SAMPLING

A basic suite of physico-chemical parameters was determined and recorded *in situ* using hand-held instruments. However, this was not always possible as measuring equipment could not be taken into some of the more demanding cave passages due to a risk of damage. Parameters measured included:

- dissolved oxygen (as % saturation and concentration in mg L^{-1}),
- temperature (ºC),
- pH,
- conductivity (μS cm^{-1}).

RESULTS

GROUNDWATER HABITATS AND FAUNA

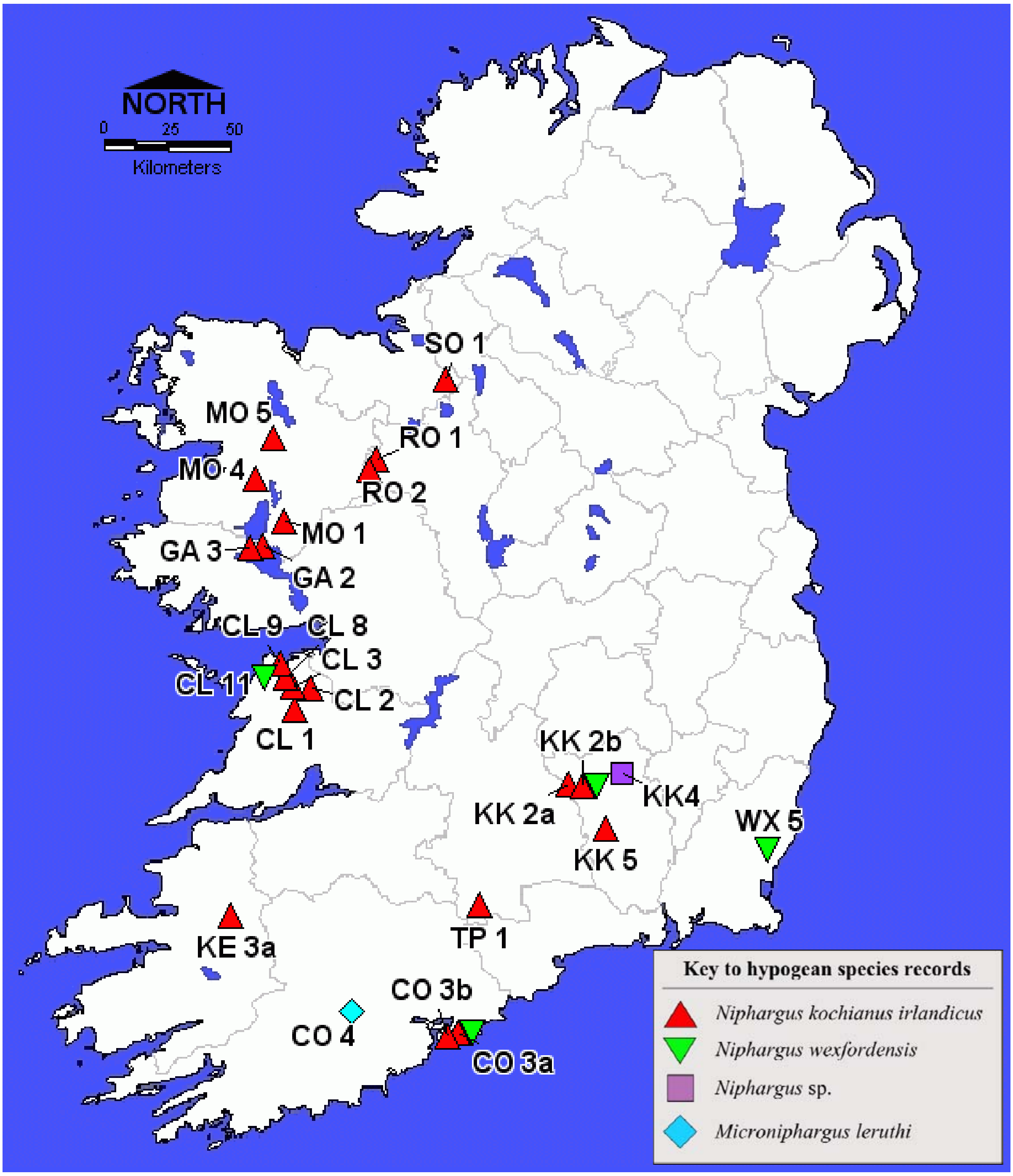

Figure 11. Records of hypogean Crustacea generated during the study.

The survey produced 26 records of hypogean Crustacea. *Niphargus kochianus irlandicus* was recorded from 20 locations including seven records from caves, one record from a river resurgence; two from stagnant holy wells and ten from springs. *Niphargus wexfordensis* was collected from four locations, including two from springs and two from caves. *Microniphargus leruthi* was recorded at one location from the interstitial waters within exposed riverine gravels. In addition, one unidentified juvenile *Niphargus* specimen was recorded from a cave. These records are illustrated in Figure 11 above and listed in Table 1.3 (Appendix 1). Table 1.4 (Appendix 1) lists all of the taxa recorded during the survey, including epigean fauna.

This survey had a qualitative character and species presence was investigated rather than their abundance. No quantitative methods were deployed; therefore the results produced may not always be a good indicator of the population size and should not be interpreted as such, except where stated. The survey results are further elucidated in the county sections below.

County Kilkenny

Gour pools with troglophilic Collembola on the surfaces were observed toward the end of Haddon Hall and in the Crystal Chamber within the Dunmore Cave system (KK4). During the field sorting of a sample from the large, deep pool of water located in The Crystal Chamber, a single juvenile *Niphargus* sp. was the only fauna recorded. However, this specimen was lost during transferral to the sample pot and could not therefore be identified further. Within the illuminated section of the cave troglophilic springtails (Collembola) and pot worms (Enchytraeidae) were present in some of the pools.

Two juvenile *Niphargus kochianus irlandicus* and a single specimen of *Niphargus wexfordensis* were collected from spring KK2b (upper River Nuenn). A single specimen of *N. kochianus irlandicus* was recorded from spring KK2a (upper River Nuenn), found during the lab analysis of the sample. Zooplankton net trawls within the chamber of the well KK3 (source of River Nuenn) collected five Copepoda. A single *N. kochianus irlandicus* specimen was recorded during the analysis of the sample from the heavily overgrown (predominately watercress, *Rorippa nasturtium-aquaticum*) holy well at Newtown North (KK5). Only epigean fauna was recorded from spring KK1 (McKeons Bar).

County Tipperary

Six specimens of *Niphargus kochianus irlandicus* as well as some epigean fauna were recorded from the holy well near Crannagh (TP1) – four in the field and two found later in the lab during analysis of the sample. One of these specimens was sent to the National Museum of Ireland.

The Old Cave (TP2) at Mitchelstown contained good habitat for stygobitic Crustacea (e.g. gour pools and pools on the mud floor) throughout. However, only troglophilic Collembola were observed on the water surfaces. The Great East Chamber sloped at an angle of 35° down to deep pools which were probably contiguous with the phreatic water table. Sampling in one of these pools found no fauna.

County Cork

Much of the cave passage in Mogeeley Cave (CO1) carried a surface stream that sank in the floor of the quarry and consequently the floor of the cave was very muddy and strewn with debris carried in by the stream. A few gour pools were evident below calcite formations, with occasional troglophilic Collembola, but most of the fauna consisted of epigean benthic species washed in by the stream and stranded in pools on the floor.

Just inside the north entrance to Central Cave (CO3a), within the threshold zone there was a high, flooded rift. Sweeps with a net in this deep pool collected a juvenile *N. kochianus* and a single specimen of *N. wexfordensis.* No further taxa were collected from the flooded rift at the end of the cave.

The lake in Lake Cave (CO3b) was heavily contaminated by droppings from the many birds that use the cave for shelter. Sweeps and kicks around the margins of the lake collected eleven specimens of *Niphargus kochianus irlandicus*, three of which were sent to the National Museum of Ireland.

Only epigean fauna were recorded from Rostellan Spring (CO2). Sampling of ERS on the Dripsey River (CO4) produced early instar larvae of various benthic invertebrates and also four specimens of *Microniphargus leruthi*, two of which were later sent to the National Museum of Ireland.

County Kerry

Crag Cave JK's (KE3a) was largely made up of passage carrying the main Crag streamway, although towards the north there was a substantial length of higher, dry passage. Epigean benthic fauna were seen in the stream, as well as a few specimens stranded in pools within the dry part of the cave. Stygophilic *G. duebeni* and *A. aquaticus* were present in this section, as well as two specimens of *N. kochianus irlandicus* in a small pool. A strong smell of diesel/oil was noticeable at the northern end of the cave, near to the show cave and many of the pools had oil on the water surface and within the sediment.

Crag Lower Cave (KE3b) mostly consisted of a passage carrying the main streamway, in which epigean benthic fauna were observed. A few gour pools on ledges above the stream contained stygophilic *Gammarus duebeni* and *Asellus aquaticus*, which had probably been washed into the system and then left stranded as water levels in the stream subsided. These specimens were lacking pigmentation, with the *Gammarus* also having white eye facets. Some of the *Asellus* specimens examined showed ocular regression with a lack of eye spots.

Crag Cave 2 (KE3c) was a very short cave containing the main streamway. A drift net anchored in the resurgence, whilst work was carried out further upstream in the system collected only epibenthic invertebrates.

Kilmurry Cave (KE4) was very muddy and held many pools left over from small, ephemeral streams that run through the cave in wet conditions. These pools held epigean fauna, primarily *G. duebeni*, *Crangonyx pseudogracilis* and *Asellus aquaticus*, many of them without pigmentation. Sampling in the short length of active streamway, in the rift at the end of the cave collected many epigean benthic invertebrates, suggesting that this was near to the surface sink.

The hyporheic sites on the KE1a, KE1b (Owengarriff River) and KE2 (River Flesk) recorded mostly epigean aquatic invertebrates, with some terrestrial fauna and Copepoda.

County Clare

Four specimens of *Niphargus wexfordensis* were observed in small pools by an inlet, close to the northern entrance (entrance B1g in Mullan, 2003) of the Polldubh Cave system (CL11). Three specimens were collected, of which two were sent to the National Museum of Ireland.

Faunarooska Cave (CL10) mostly consisted of active streamway, in which *G. duebeni* (some with sygophilic features) were observed. Some small, isolated pools were present in the higher, dry passages towards the end of the cave but no fauna was noted, although stygophilic *G. duebeni* were seen in pools in an ephemeral inlet.

Caddis larvae (*Plectrocnemia* spp.), *G. duebeni* (many stygophilic) and white flatworms (*Phagocata vitta*) were seen in the stream in Pollballiny Cave (CL12). A few gour pools were present on ledges above the stream, with troglophilic Collembola on the water surfaces.

Niphargus kochianus irlandicus specimens were collected from five of the eight holy wells sampled: Killinaboy (CL1), Fahee North (CL2), Eanty More (CL3), Gragan East (CL8) and Cloughmore (CL9), with the holy well at Gragan East being particularly productive. Twenty one specimens were recorded there, three of which were sent to the National Museum of Ireland. The hyporheic site (CL6) on the Aille River produced only Collembola, Oligochaeta and 1st instar of the mayfly *Baetis* spp.

County Galway

Most of the Pigeon Hole Cave (GA2) lay within the threshold zone, with darker passage downstream. Drift net and kick sampling recorded epi-benthic invertebrates, whilst sweeps in a deep pool, beneath boulders in the dark zone collected large numbers of *G. duebeni*. Bou-Rouch sampling in the bed of the underground river collected a cyclopoid copepod and four specimens of *N. kochianus irlandicus*, one of which was sent to the National Museum of Ireland.

Most of Ballymaglancy Cave (GA3) appeared to be active autogenic streamway, with occasional pools on calcite ledges above the water. Stygophilic *G. duebeni* were observed in some of these pools and a single *N. kochianus irlandicus* was observed in a silt-lined pool in an inlet near the entrance. A further two specimens were collected in net sweeps in the resurgence. Sampling of the Ellechrissaun Springs (GA1) collected only epigean benthic invertebrates and no stygobitic fauna.

County Mayo

Sampling of site MO2 (near Aille River Cave system) using a drift net and sweep sampling recorded only epigean, riverine species.

Disturbing the gravel and sweeps with a net in the pools at site MO4 (River Aille resurgence) collected three Copepoda, epigean benthic fauna and four *N. kochianus irlandicus*, two of which were sent to the National Museum of Ireland.

The holy well at Ross West (MO5) consisted of a small chamber containing stagnant water and large amounts of leaf litter and algae. Eleven specimens of *N. kochianus irlandicus* were collected, five of which were sent to the National Museum of Ireland.

Twenty specimens of *N. kochianus irlandicus* were collected from the holy well at Caheredmond (MO1) and sampling of a silty spring at Baloor West (MO3) collected only epigean fauna.

County Roscommon

A stream sank at the entrance of Pollawaddy Cave (RO1) but soon disappeared further in. Another stream, in which stygophilic *G. duebeni* were observed was present in a rift at the end of the cave. It was not determined if this was the same watercourse. Just past the second, tight squeeze, at least twenty specimens of *N. kochianus irlandicus* were observed in a small pool. Fifteen specimens were collected, of which four were sent to the National Museum of Ireland and three to the National Museum Northern Ireland. Further into the cave, in a dead-end passage there were two pools. The first of these pools contained in excess of 100

Niphargus spp. The second pool, beyond this was not investigated as this would have disturbed the population in the first pool, which occupied the width of the passage. The second pool was likely to have contained similar numbers.

Bou-Rouch sampling in the gravel shallows at the issue point of the spring at Gortaganny (RO2) collected no fauna. Netting in a small water-filled pit next to the pumping station collected two specimens of *N. kochianus irlandicus*. No stygobite fauna were found in the springs at Moor (RO3) and near Carrick-on-Shannon (RO4).

County Sligo

The Seighmairebaun entrance to the Carrowmore cave system (SO1) was a deep vertical pot carrying a small stream from the surface. *Plectrocnemia* spp., *G. duebeni* and *Phagocata vitta* were seen in small pools collected on ledges in the pot. At the bottom there was horizontal stream passage and just before this was reached, a small crawl to the left ended in a static pool, which appeared to be unconnected to the stream. A single specimen of *N. kochianus irlandicus* was collected from this pool.

No stygobite fauna were recorded from the springs at Carrownyclowan (SO2) and Ummeryroe (SO3).

County Leitrim

Only epigean benthic species were recorded at hyporheic sites on a stream flowing into Lough Gill (LE1), the Bonet River (LE2) and from the spring at Carrowrevagh (LE3).

County Cavan

Only epigean fauna were recorded from the holy well at Tober (CA1). Sampling in the channel just downstream of the source of the River Shannon (CA2) collected riverine benthic species only.

County Fermanagh

Pollnagollum (FE3) was mostly active streamway, with dry chambers above the stream, towards the end of the cave (the Polldowncog section). A small inlet in this section contained *G. duebeni*. In Rift Chamber there were some gour pools around a large calcite boss. These pools were lined with silt and contained detritus. Despite being good habitat for stygobitic Crustacea no fauna was observed in them.

Upper Cradle Hole (FE4) was predominately active streamway, with some pools on rocky ledges just above the water, which contained troglophilic Collembola and *G. duebeni*. More troglophilic Collembola were observed on the surfaces of crystal-lined gour pools on a calcited ledge high above the stream.

Pollbwee (FE5) consisted of mostly flooded passage, leading off from a lake at the bottom of the entrance pitch. This lake was fed by small inlets from the surface and contained a lot of detritus washed in from above. Abundant *G. duebeni*, some showing stygophilic characteristics and several species of Limnephilidae cased caddis larvae were present around the shallow margins of the lake, sheltering in the detritus and feeding upon it.

Bruce’s Pot (FE6) contained silt-lined pools on the floor and gour pools beneath calcite flowstone, although no fauna was seen in them. Large *G. duebeni* were collected from the deep flooded rift at the end of the cave.

Although the spring at Cavaneagh (FE1) appeared to be a suitable habitat for sygobitic fauna, only epigean species were recorded. Sampling of the Tullyhona Rising (FE2), in the patches of gravel amongst boulders, both on the surface and into the dark zone of the cave system itself produced only epigean benthic invertebrates.

County Wexford

A single *N. wexfordensis* was collected from the spring at Knockanevin (WX5). This was the only location where the Bou-Rouch pump could be used successfully.

Asellus meridianus and Chrironomidae larvae were recorded from St. Garvan’s Well (WX7), a small stone chamber that is apparently spring-fed as there is an overflow channel to a nearby stream. At the time of the survey there was no discernible flow.

Collembola were recorded from the hyporheic site on the River Slaney (WX2) and only epigean species were recorded at Kiltilly (WX1), St. Machan's Well (WX3), Kilnew (WX4) and St. Killians Well (WX6).

PHYSICO-CHEMICAL PARAMETERS

All physico-chemical parameters recorded *in-situ* are presented in Table 1.2 (Appendix 1). Their relevance with regard to hypogean fauna records is highlighted below.

The conductivity range recorded during the survey was 70 to 1,080 µS cm^{-1}. Readings from the sites where *Niphargus* spp. were found ranged between 200 and 720 µS cm^{-1} and records were evenly distributed within this range. Water from site CO4, where *Microniphargus leruthi* was recorded had a conductivity level of 230 µS cm^{-1}, within the lower range recorded during the survey (Figure 12a).

The pH range recorded during the survey was 4.9-8.4. Readings from the sites where *Niphargus* spp. were found ranged between 6.4 and 8.4, thus records coincided with the pH values away from the lower extreme of the range observed and were mostly from waters with a pH between 7.0 and 8.5. *Microniphargus* was recorded at a pH of 6.8 (Figure 12b).

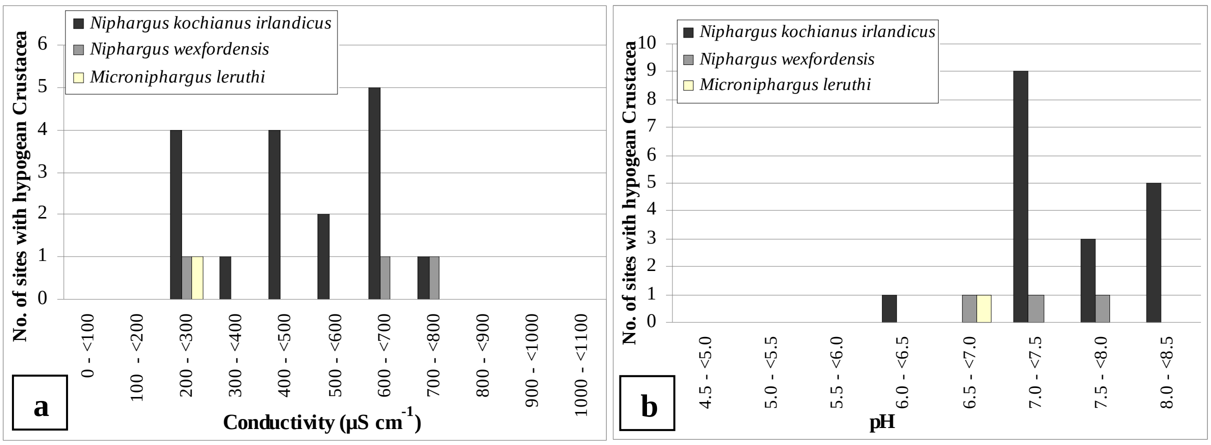

Figure 12. Hypogean Crustacea records in relation to the conductivity (a) and pH (b) range observed in the study.

The range of dissolved oxygen (DO) saturation of water recorded during the survey was 14-132% and records of *Niphargus* spp. occurred within the whole range. They peaked toward the upper limit and most records of *Niphargus* spp. were made from waters with a dissolved oxygen saturation of 80-100% (Figure 13a). Similar was true for dissolved oxygen concentration, which ranged between 1.5-15.3 mg l^{-1}, with most records of *Niphargus* spp. from waters with a value of 10-12 mg l^{-1}. *Microniphargus* was recorded at a DO level of 45% (or 4.2 mg l^{-1}).

The temperature range of water recorded during the survey was 6.5-17.1°C. Readings from the sites where *Niphargus* spp. were found ranged between 6.5 and 11.8°C, thus records coincided with the lower part of the temperature range observed. Although quantitative methods were not employed during the survey, it was observed that *Niphargus kochianus irlandicus* occurred at higher abundance within the temperature range of 9 to 11°C. *Microniphargus* was recorded at a temperature of 13.3°C, higher than those of any *Niphargus* spp. records in this study (Figure 13b).

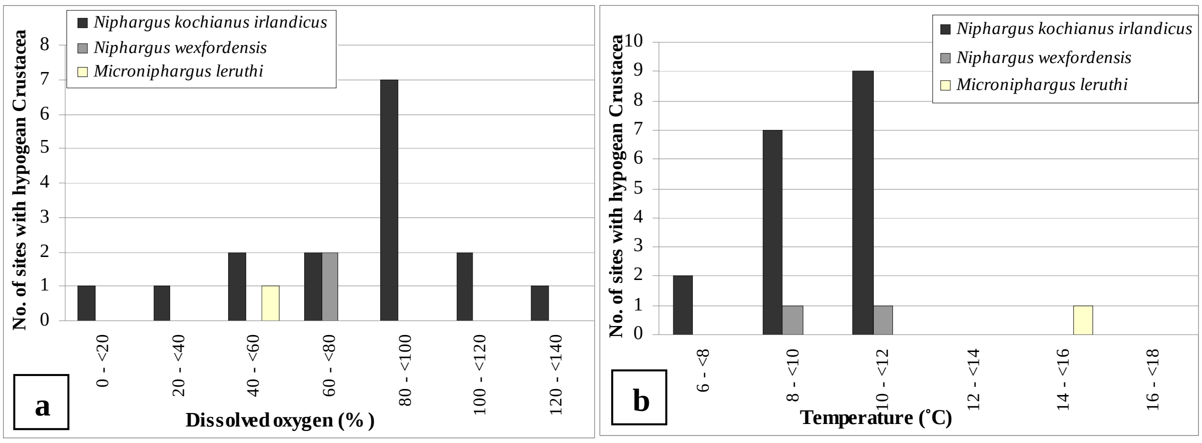

Figure 13. Hypogean Crustacea records in relation to the dissolved oxygen (a) and temperature (b) range observed in the study.

DISCUSSION

STYGOBITE AQUATIC FAUNA OF IRELAND

With 26 new records for stygobitic Crustacea from 23 new locations, this study has significantly contributed to the known distribution of Irish groundwater fauna (Appendix 2).

Niphargus kochianus irlandicus was previously reported from 39 locations. During the current survey 20 new records for this species were generated. Apart from the record from the Pigeon Hole Cave in Co. Galway, the remaining 19 locations were previously unknown. These include six caves, two stagnant holy wells, a river resurgence and ten springs. The survey has extended the known latitudinal distribution limits of *N. kochianus irlandicus* both to the south and north and the species is now known to occur from Carrigacrump Caves, Co. Cork to Carrowmore cave system, Co. Sligo. It appears to be the most common Irish niphargid species, with a range covering most of the Leinster, Munster and Connaught provinces.

The large numbers of *N. kochianus irlandicus* observed in Pollawaddy Cave, Co. Roscommon represent the largest cavenicolous population of *Niphargus* recorded to date in Britain and Ireland (HCRS, 2008 & Knight pers. obs.). Cavernicolous populations are often ephemeral, being washed out of crevices in strata following heavy rain and often travelling back into these crevices if conditions are unsuitable or as pools dry out. Nonetheless, despite its small size Pollawaddy Cave harbours an important population of stygobitic Crustacea.

Niphargus wexfordensis was previously reported only from Co. Wexford and it was assumed to be limited in its distribution to the south-east corner of Ireland. During the current study *N. wexfordensis* was found elsewhere, in counties Kilkenny, Cork and as far north-west as Co. Clare, a significant advance in the knowledge of the species' distribution. *N. wexfordensis* was recorded from four new locations, including two springs and two caves.

The cave records, from Polldubh on the Burren and from Central Cave (Carrigacrump Caves) in Co. Cork, were thought to be the first records for this species from the cave habitat. The Central Cave record and that from the spring on the Nuenn River, Co. Kilkenny were also thought to be the first observed occurrences of *N. wexfordensis* co-existing with *N. kochianus irlandicus*. As previously mentioned, these results (as well as the observations of C. Fišer) led to the re-examination of the material collected by Knight from the Doolin River Cave, Co. Clare in 2005 and the subsequent discovery of a *N. wexfordensis* specimen amongst what was previously thought to be a single species sample of *Niphargus kochainus irlandicus*. Most of the older records of *N. kochianus irlandicus* were collected from caves on the Burren in the 1960s and 70s and represent the majority of the records prior to 2006. The above results call into question the identity of the specimens represented in these earlier records and the fact that at least some of these might be of *N. wexfordensis*, a species unknown to science at the time.

Microniphargus leruthi collected from interstitial riverine gravels on the Dripsey River in Co. Cork was only the second record for this species from Ireland, and it confirmed its earlier finding from the same county (Arnscheidt *et al.*, in prep.). This represents a major leap in its previous known distribution in the Ardennes region, Belgium and the northern Rhine basin in Germany. The specimens collected match the described morphology of *Microniphargus leruthi* but further molecular work is required to assess whether the Irish specimens are indeed the same species, especially due to the large gap in the distributional pattern. Due to its small size it is likely that *Microniphargus* is under-recorded and might have been overlooked in Britain, western Belgium and Holland. However, future studies might reveal that the Irish specimens are in fact a new, cryptic species previously unknown to science. A project addressing the phylogeny of the Niphargidae is in progress at the University of Ljubljana and is likely to produce some interesting results (Fišer pers. comm.).

Site KE 2, gravel / sand ERS on the River Flesk, County Kerry, was near the location at which *Antrobathynella stammeri* was recorded in 1982 but only epigean fauna were found. Thus the attempt to confirm the previous finding of *Antrobathynella* was not successful and its discovery by Gledhill & Gledhill (1984) remains the only known record of the species in Ireland.

Stygophilic *Gammarus duebeni* and *Asellus aquaticus* were recorded in many of the caves during the survey and exhibited adaptations to the subterranean habitat, such as the lack of pigmentation and white eye facets in some of the *Gammarus*. A few *Asellus* specimens from Crag Lower cave, Co. Kerry showed ocular regression with a lack of eye spots. Such adaptational traits have been recorded in stygophilic populations of Crustacea in Britain and elsewhere. For example, eyeless *Gammarus pulex* have been recorded in Lathkill Head Cave in the English Peak District (Proudlove *et al.*, 2003).

Distribution of the genus *Niphargus*

It is now apparent that the genus *Niphargus* occurs over a much wider area in Ireland than previously thought. The record from the Carrowmore cave system in County Sligo represents a new northern limit for the known distribution of the genus *Niphargus* within Ireland. It is also the northernmost record of the genus *Niphargus* in Europe at 54°07’44.87’’N and 08°15’02.15’’W (or Irish Grid 183666 320031). Despite a considerable sampling effort it has not been found further north. Several of the caves and springs sampled in counties Fermanagh, Leitrim and Cavan contained suitable habitat for stygobitic Crustacea and further south similar sites produced records. This is similar to the previous findings of Arnscheidt, who has not found it north of the Dundalk Penninsula (Arnscheidt *et.al*. in prep.)

The hypothesis that the distribution of *Niphargus* in Europe is limited by the last glaciation obviously does not appear to apply in Ireland. With most of the records being north of the last glacial limit, currently known distribution of the genus poses an interesting puzzle. The distribution of *Niphargus* in Ireland suggests that it either re-colonized Ireland more rapidly, survived in sub-glacial refugia or both.

Approximately 40% of the island of Ireland and 50% of the Republic is underlain by porous limestone (Karst Working Group, 2000). Much of it is contiguous and therefore potentially providing connectivity for hypogean Crustacea between different areas of habitat as well as abundant refugia.

If the dispersal hypothesis is assumed to be correct and that the distribution of Niphargidae in Europe represents re-colonisation from the unfrozen south, then a possible theory is that this same connectivity in Ireland has led to a more rapid re-colonisation of areas that were previously glaciated (from a small area of tundral refugia in the extreme south) than occurred in Britain and mainland Europe. Over there porous strata might be more separated and northwards migration of stygobitic Crustacaea might have had to take place in the shallower gravels of the hyporheic zone, where the species would be subject to greater variations in physico-chemical conditions compared to those in the deeper phreatic zone. It is thought that in Britain *Niphargus aquilex* (the most superficial of the British species and a frequent inhabitant of the hyporheic) might have re-colonised parts of the north in this way, as it has been recorded from several locations north of the Devensian limit. The possibility of the caves of South Wales having been re-colonised from the Mendip Hills of Somerset has already been mentioned in the introduction. Thus the Irish *Niphargus* could have managed a more rapid movement northwards than their European and British relatives.

On the other hand there are several pieces of evidence that support the refugial hypothesis. Physiological studies on some French species have shown that *Niphargus* is very resistant to starvation, hypoxia and sub-zero temperatures (Malard & Hervant, 1999; Hervant & Renault, 2002; Issartel *et al*., 2005; Issartel *et al*., 2006). Most groundwater habitats have relatively constant environmental conditions and, in the temperate zone are always above zero, thus these adaptations might remain from earlier times when many species were forced to live in sub-glacial conditions. It is known that unfrozen water, containing oxygen and nutrients (from bacteria etc) can exist beneath ice (Bhatia *et al*., 2006; Tranter *et al*., 2005). Other indications include the discovery of endemic stygobitic species in Iceland, although these could have survived in thermal pockets in a volcanically active land mass and the initial results of recent DNA studies on *Niphargus kochianus irlandicus* (Proudlove & Hanfling, 2008) mentioned in the introduction section (stygobitic Crustacea species accounts). These imply that the Irish taxa have been isolated for several million years and must have survived several Pleistocene glaciations. The existence of British *Niphargus aquilex* and *Antrobathynella* north of the glacial limit might have resulted from survival in refugia rather than re-colonisation. If the refugial hypothesis is shown to be correct, then perhaps refugia were more readily available further north to the Irish species than to their British and European relatives.

Both hypotheses have their flaws when attempting to explain the distribution of the Irish taxa. If the refugial hypothesis is correct then surely refugia would have been available in the karst of Fermanagh, Cavan and Leitrim? If the dispersal hypothesis is correct then what has prevented movement further north?

The first answer could be temporal in that the re-colonisation is a continuous, slow process that has currently reached as far north as County Sligo and is still progressing northwards. Alternatively the process has been interrupted by some form of barrier.

The degree of karstification of limestones and hence the development of fissures can be reduced where there are inter-bedded shale layers which restrict water movement and where strong deformation causes the re-sealing of fractures with crystalline calcite (Karst Working Group, 2000). Several karst areas in Fermanagh are known to lack cave development due to extensive faulting that has led to dolomitization and the Castle Archdale-Belhavel Fault system (part of a structure extending from County Mayo to Scotland) runs through the north of the area (Jones *et. al.,* 1997). It could be suggested that the belt of mountains and uplands that stretches across the northern part of Ireland might form a barrier to the re-colonisation process but this does not explain the absence of hypogean Crustacea from karstified areas at the southern edge of these uplands. An alternative suggestion could be that drainage in the area forms a hydrological barrier to the movement of the groundwater fauna. Following heavy rainfall on the uplands, the subsequent large amounts of water draining to the south could flush hypogean Crustacea out of the subterranean conduits and hence restrict the development of significant populations.

PHYSICO-CHEMICAL ENVIRONMENT

It is hard to extrapolate too much detail on the physico-chemical preferences of the stygobitic crustacean fauna of Ireland from a rather limited dataset over a wide geographical area.

However, based on the results of this study, Irish *Niphargus* spp. appear to prefer groundwaters with a conductivity level of approximately 200-720 µS cm^{-1} and pH of 6.4-8.4.

Ion-deficient waters challenge the ability of aquatic amphipods to maintain sufficient ionic concentration in their tissue and body fluids (Glazier & Sparks, 1997). In addition, low buffering capacity of ion-poor waters makes them prone to acidification with consequent deleterious physiological effects to fauna (Sutcliffe & Hildrew, 1989; Glazier & Sparks, 1997). A comparison of different gammarid species showed elevated metabolic rates under ion-deficient conditions (Sutcliffe, 1984) and in surface waters distinct changes in faunal composition occur below pH 5.7-5.4, with Crustacea becoming scarce or absent (Sutcliffe & Hildrew, 1989). Such conditions could be limiting the survival success of Irish *Niphargus* spp. However, in South West England *Niphargus glenniei* is known to occur on acidic granite geology both in the far west of Cornwall and at a reasonably high elevation on Dartmoor (Knight, 2001). These areas are known to be low in both pH and conductivity, although no water chemistry data is available for the specific sites from which the species was recorded.

No photosynthesis occurs in hypogean waters and there is limited free gas exchange with the atmosphere, thus groundwaters are often characterised by low oxygen levels. *Niphargus kochianus irlandicus* was recorded from water with a dissolved oxygen saturation as low as 14% (or 1.5 mg l^{-1}). Wide tolerance of *Niphargus* spp. to the dissolved oxygen conditions found during the present study might be a reflection of adaptation to the demanding conditions of the groundwater environment.

Underground waterbodies are characterised by a fairly constant temperature. The current study recorded *Niphargus spp*. at a temperature maximum of 11.8°C and *Microniphargus* at 13.3°C. With limited data recorded conclusions cannot be drawn from the results. However, they suggest that 12°C is the upper limit for Irish *Niphargus* and that *Microniphargus* might display a slightly higher thermal tolerance, although it is equally likely that the results represent the higher temperatures found within the interstitial zone (as opposed to groundwater) from which *Microniphargus* was recorded. *Niphargus kochinaus irlandicus* occurred at higher numbers within the 9 to 11°C range and this might indicate optimal conditions for this species. However, such hypotheses require further verification.

FURTHER IMPLICATIONS

The combined results of the current study (carried out predominantly on cave and spring habitats) and those of Arnscheidt of the University of Ulster (focused on wells and boreholes) are expected to compliment each other and build upon the previous records to produce a comprehensive database of the biodiversity and distribution of the stygobitic Crustacea of Ireland. This will serve to highlight the importance of the groundwater environment as an important aquatic ecosystem worthy of protection.

Further work is required to re-assess the known distribution of *N. wexfordensis*, with a focus on its occurrence in the caves of the Burren and elsewhere. Work is already underway to track down and re-examine material collected from caves in the 1960s and 70s. Intensive survey of the Burren caves in particular is required to assess if *N. wexfordensis* is widespread in the cave habitat and regularly co-exists with *N. kochianus irlandicus*, as the initial results from the current study and the subsequent re-examination of material collected in 2005 from the Doolin River system imply. In Slovenia it is quite common for two or more species to co-exist in the same locality (Fišer pers. comm.) and to a lesser extent the same has been recorded in some British caves (HCRS, 2008).

Collection of specimens for subsequent DNA studies will also provide more information on the phylogentic relationship of the two Irish *Niphargus* and might indicate the existence of other cryptic taxa that have previously been overlooked. It is possible that molecular clock data, timing the divergence of the endemic Irish taxa from others may help to elucidate the timing of a land bridge between Ireland and Britain (Proudlove *et. al.*, 2003). Understanding the phylogeny of the Irish *Niphargus* in relation to British species might help to provide insight into their evolution and bio-geography, which is currently little understood. The results of detailed phylo-geographical studies, such as that being undertaken as part of the PASCALIS project (Proudlove *et. al.*, 2003) and by the University of Ljubljana (Fišer pers. comm.) will hopefully answer the questions behind the distribution of the Niphargidae.

Both *Antrobathynella stammerri* and *Micronipahrgus leruthi* are small species that are likely to be under-recorded in Ireland and future surveys, focusing on the interstitial habitat are likely to produce further records.

Further ecological studies are required to investigate the affinity of certain species for particular physio-chemical conditions; tolerance to stress factors, including groundwater contamination and nutrient enrichment; and food web interactions. Only with this knowledge will it be possible to protect the stygobitic fauna of Ireland's aquifers and caves and appropriately address the sustainable use of groundwater resources with implications for issues such as natural purification processes and the quality of drinking water supply.

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APPENDIX 1: Survey data

Table 1.1 Sampling sites (ERS – exposed riverine gravels); asterisk (*) next to the site code indicates sites with stygobite Crustacea records.

Date sampled	County	Site code	Site type	Site name	Irish Grid coordinates			Land use	Site description	Sampling methodology
31.03.08	Kilkenny	KK 1	spring	Holy Well, Near McKeons Bar	S	35658	63413	roadside	Small well block with stream below	Bou-Rouch
31.03.08	Kilkenny	*KK 2a	spring	Holy Well, Nuenn River headwater trib	S	35151	62550	pasture	Small spring issuing from the bank	Bou-Rouch
31.03.08	Kilkenny	*KK 2b	spring	Holy Well, Nuenn River headwater trib	S	35141	62521	pasture	Spring forming a short tributary	Bou-Rouch
31.03.08	Kilkenny	KK 3	well	Nuenn River source	S	34298	61974	roadside	Well collecting chamber at source of Nuenn River	trawl net
31.03.08	Kilkenny	*KK 4	cave	Dunmore Cave	S	50940	64950	beneath pasture	Most of cave is a showcave, Haddon Hall and Crystal Chamber undeveloped. Lake in Crystal Chamber	visual, sweep & trawl net in lake in Crystal Camber
31.03.08	Kilkenny	*KK 5	spring	Holy Well, Newtown North	S	46759	44830	pasture	Spring overgrown with macrophytes *(Rorippa* sp.)	Bou-Rouch
01.04.08	Tipperary	*TP 1	spring	Holy Well, Near Crannagh Br	R	97601	15102	mixed woodland, surrounded by pasture	Spring in woodland	Bou-Rouch
01.04.08	Tipperary	TP 2	cave	Mitchelstown Old Cave (Desmond's Cave)	R	9239	1676	scrub, surrounded by pasture	Plenty of good pools throughout cave. Deep pools in Great East Chamber	manual, sweep & trawl net in deep pools
01.04.08	Cork	CO 1	cave	Mogeeley Cave	W	96659	74552	deciduous woodland (old quarry), surrounded by pasture	Small cave most of which is flooded by stream in high water	visual
02.04.08	Cork	CO 2	spring	Rostellan spring	W	88629	66405	marshy scrub, surrounded by arable	Small spring in marshy hollow	Bou-Rouch
02.04.08	Cork	*CO 3a	cave	Carrigacrump Caves,Central Cave	W	90201	65272	scrub, disused quarry	Contains large flooded rifts	manual, sweep
02.04.08	Cork	*CO 3b	cave	Carrigacrump Caves, Lake	W	90283	65276	scrub, disused quarry	One large chamber containing lake	sweep & kick in

Date sampled	County	Site code	Site type	Site name	Irish Grid coordinates			Land use	Site description	Sampling methodology
				Cave						lake margin
02.04.08	Cork	*CO 4	interstitial	Dripsey River, Downstream of Dripsey bridge	W	48776	73842	pasture	Cobble / gravel ERS	Bou-Rouch, Karaman-Chappuis
03.04.08	Kerry	KE 1a	interstitial	Owengarriff River, 200m Upstream from Muckross Lake	V	9626	8484	deciduous woodland	Grave / pebble ERS beside main channel and in dry oxbow	Bou-Rouch, Karaman-Chappuis
03.04.08	Kerry	KE 1b	interstitial	Owengarriff River, 100m Upstream from Muckross Lake	V	9615	8485	deciduous woodland	Cobble/gravel ERS beside river	Bou-Rouch, Karaman-Chappuis
03.04.08	Kerry	KE 2	interstitial	River Flesk, Upstream Bridge at Ballyspillane	V	98761	90384	urban	Gravel/sand ERS	Bou-Rouch, Karaman-Chappuis
03.04.08	Kerry	*KE 3a	cave	Crag Cave, JK Cave	R	01382	11394	pasture	Cave contains main Crag System streamway	manual, sweep
03.04.08	Kerry	KE 3b	cave	Crag Cave, Lower Cave	R	01382	11394	pasture	Cave contains main Crag System streamway	manual, sweep
03.04.08	Kerry	KE 3c	cave	Crag Cave, Crag Cave 2	R	01388	11283	pasture	Short cave, contains main Crag System streamway	drift net
03.04.08	Kerry	KE 4	cave	Kilmurry Cave	R	054	094	pasture	Many pools on the floor left from ephemeral streams running through cave	manual, sweep
04.04.08	Clare	*CL 1	spring	Holy Well, Near Killinaboy	R	25752	91466	pasture	Issues from amongst boulders	Bou-Rouch
04.04.08	Clare	*CL 2	spring	Holy Well, Fahee North	R	31777	99612	moorland pasture	Issues from limestone bedrock substrate compacted fines overlain with gravel	sweep, drift net
04.04.08	Clare	*CL 3	spring	Holy Well, Eanty More	M	25336	00488	moorland pasture	Water percolating from limestone bedrock	sweep, drift net
04.04.08	Clare	CL 4	well	Holy Well, Berneens	M	22994	02955	limestone pavement	Small (spring-fed?) rock hollow containing stagnant water	sweep
04.04.08	Clare	CL 5	spring	Holy Well, Glensleade	M	22749	00572	marshy pasture	Small muddy trickle from boulders into stagnant marsh area	Karaman-Chappuis
05.04.08	Clare	CL 6	interstitial	Aille River, Downstream Lisdoonvarna bridge	R	13488	97934	urban	Pebble/gravel ERS	Bou-Rouch, Karaman-

Date sampled	County	Site code	Site type	Site name	Irish Grid coordinates	Land use	Site description	Sampling methodology
								Chappuis
05.04.08	Clare	CL 7	spring	Holy Well, Derrynavaahagh	M 17734 06113	pasture	Spring seeping from between limestone strata	sweep, drift net
05.04.08	Clare	*CL 8	well	Holy Well, Gragan East	M 21978 04199	moorland pasture	Small (spring-fed?) well chamber with stagnant water filled with leaf litter and small pockets of muddy sand	sweep
05.04.08	Clare	*CL 9	well / spring	Holy Well, Cloughmore	M 20092 09816	moorland pasture	Enclosed well chamber (ca. 1.2m diameter), with quite brisk flow from a rock-channeled spring mouth	sweep
05.04.08	Clare	CL 10	cave	Faunarooska	M 1421 0453	moorland	mostly active streamway	manual
05.04.08	Clare	*CL 11	cave	Polldubh	M 1376 0376	coniferous plantation	mostly active streamway	manual
05.04.08	Clare	CL 12	cave	Pollballiny	M 1394 0438	coniferous plantation	mostly active streamway	manual
06.04.08	Galway	GA 1	spring	Ellechrissaun Springs, Cong	M 14143 55279	deciduous woodland	Major resurgence (from Lough Mask), springs emerge at several points through boulders and limestone cliffs	sweep, kick, drift net
06.04.08	Galway	*GA 2	cave	Pigeon Hole Cave	M 13275 55423	mixed woodland	Cave contains major river resurgence from Lough Mask	sweep, drift net, Bou-Rouch
06.04.08	Galway	*GA 3	cave	Ballymaglancy Cave	M 11548 55153	pasture	Most of cave active streamway, with occasional pools on calcite ledges	manual, sweep
06.04.08	Mayo	*MO 1	spring	Holy Well, Caheredmond	M 21410 65027	pasture	Issuing into a field ditch	Bou-Rouch
07.04.08	Mayo	MO 2	cave	Aillle River Cave	M 06790 81008	pasture	Cave is mostly major active streamway, sampled small stream near entrance	sweep, drift net
07.04.08	Mayo	MO 3	spring	Baloor West	M 08387 82011	pasture	Silty spring	sweep
07.04.08	Mayo	*MO 4	resurgence	River Aille Resurgence, Bellaburke	M 09807 81687	pasture	Main resurgence is deep pot. Sampled pools at end of dry channel upstream of main resurgence	sweep, Bou-Rouch
07.04.08	Mayo	*MO 5	well	Holy Well, Ross West	M 1675 9775	moorland	Small chamber containing stagnant water, lots of filamentous algae and leaf litter	sweep
07.04.08	Roscommon	*RO 1	cave	Pollawaddy Cave	M 57317 89238	pasture	Small cave in ridge above marshy flatlands	manual, sweep
08.04.08	Roscommon	*RO 2	spring	Holy Well at Gortaganny, shore of Lough Errit	M 54340 85016	lake / roadside	Spring on shores of Lough	Sweep, Bou-Rouch

Date sampled	County	Site code	Site type	Site name	Irish Grid coordinates			Land use	Site description	Sampling methodology
08.04.08	Roscommon	RO 3	spring	Holy Well, Moor	M	68801	81470	marshy pasture	Muddy spring in flat marshland	sweep
08.04.08	Roscommon	RO 4	spring	Holy Well, Near Carrick-on-Shannon	G	89844	12205	lake / roadside	Small chamber with channel downstream	sweep
08.04.08	Sligo	*SO 1	cave	Carrowmore System	G	83666	20031	coniferous plantation (clear-fell)	Most of cave vertical pot carrying surface stream with streamway at bottom of pot	manual, sweep
08.04.08	Sligo	SO 2	spring	Carrownyclowan	G	82059	21579	moorland	peat/mud covered with some pebbles and cobbles	sweep
08.04.08	Sligo	SO 3	spring	Ummeryroe	G	81565	19619	pasture	Spring issuing into a pool with quite brisk flow	sweep
09.04.08	Leitrim	LE 1	interstitial	Small Stream Flowing into Lough Gill	G	79595	34553	deciduous woodland	Stream substrate consisted of thin layer of gravel on top of clay	sweep
09.04.08	Leitrim	LE 2	interstitial	Bonet River, Downstream New Bridge	G	86952	71231	moorland pasture	Two gravel/pebble banks downstream of bridge	Bou-Rouch, Karaman-Chappuis
09.04.08	Leitrim	LE 3	spring	Carrowrevagh	G	88399	48753	moorland	Stream issuing from lower end of boulder channel, dry upstream	sweep, Bou-Rouch
09.04.08	Cavan	CA 1	well	Holy Well at Tober	G	99697	29440	roadside	Stagnant pool, filled with leaves	sweep
09.04.08	Cavan	CA 2	resurgence	Source of the Shannon	H	05334	31755	pasture	Deep water-filled pot. Sampled just downstream, gravels filled with silt and fine sand	Bou-Rouch, sweep, kick
09.04.08	Fermanagh	FE 1	spring	Holy well at Cavanveagh	H	12033	35956	deciduous woodland	Small spring - gravels filled with fine sand and silt	sweep, Bou-Rouch
10.04.08	Fermanagh	FE 2	resurgence	Tullyhona Rising	H	15335	33785	mixed woodland	Stream rising from cave system with gravel amongst boulders	sweep, Bou-Rouch
10.04.08	Fermanagh	FE 3	cave	Pollnagollum (of the boats)	H	12782	33876	moorland	Cave mostly active streamway with good gour pools, lined with silt and some detritus	manual, trawl net
10.04.08	Fermanagh	FE 4	cave	Upper Cradle Hole	H	12055	34127	moorland	Cave mostly active streamway, some ledges above stream with pools; mostly rock-lined, some with patches of gravel	manual
10.04.08	Fermanagh	FE 5	cave	Pollbwee	H	11989	33591	moorland	Lake at bottom of pitch fed by inlets from surface, edges contained a lot of surface	manual, trawl net

Date sampled	County	Site code	Site type	Site name	Irish Grid coordinates			Land use	Site description	Sampling methodology
									detritus	
10.04.08	Fermanagh	FE 6	cave	Bruce's Pot	H	12013	33497	moorland	Lots of silt-lined pools on floor and some below calcite flows with some gour pools	manual, trawl net
12.04.08	Wexford	WX 1	spring	Holy well at Kiltilly	S	97733	58713	pasture	Small stone hole with water with quite strong flow	sweep
13.04.08	Wexford	WX 2	interstitial	River Slaney, Downstream Bridge at Enniscorthy	S	97469	39882	urban	Gravel/pebble ERS	Bou-Rouch, Karaman-Chappuis
13.04.08	Wexford	WX 3	spring	Holy Well, St. Machan's Well	T	16589	39646	deciduous woodland	Spring water entering stone chamber and overflowing as small gravelly spring	sweep
13.04.08	Wexford	WX 4	spring	Holy Well,Near Kilnew	T	14543	37816	roadside	Well collecting chamber which overflowed into small channel	sweep
13.04.08	Wexford	*WX 5	spring	Holy Well, Near Knockanevin	T	09428	36724	pasture	No sign of well per se but small stony stream near road	Bou-Rouch
13.04.08	Wexford	WX 6	spring	Holy Well, St. Killians Well, near Ballysilla Br	T	03911	31505	deciduous woodland	Small stone chamber (no discernible flow here) which overflows to stone trough at roadside, chamber full of leaves	sweep
13.04.08	Wexford	WX 7	well	Holy Well, St Garvans Well, Nr. Kilgarvan	S	89213	22596	arable	Stone well chamber, no discernible flow, overflows to nearby stream via silty channel (dry at time of visit)	sweep

Table 1.2. Physico-chemical water parameters taken *in-situ* (nd = not determined); asterisk (*) next to the site code indicates sites with stygobite Crustacea records.

Site code	Site type	Conductivity (µS/cm)	pH	Dissoilved oxygen (%)	Dissoilved oxygen (mg/L)	Temperature (˚C)
KK 1	Spring	660	7.0	90	10.2	9.9
*KK 2a	Spring	670	7.1	102	12	9
*KK 2b	Spring	710	7.1	71	8	9.4
KK 3	well	670	7.2	57	6.3	10.5
*KK 4	Cave	540	8.2	95	10.4	10.1
*KK 5	Spring	720	7.1	48	4.5	11.2
*TP 1	Spring	500	7.3	14	1.5	10.1
TP 2	Cave	510	7.5	101	9.2	17.1
CO 1	Cave	nd	nd	nd	nd	nd
CO 2	Spring	1080	7.2	70	7.6	10.9
*CO 3a	Cave	640	7.4	73	7.1	11.8
*CO 3b	Cave	670	7.2	81	8.5	10.8
*CO 4	Interstitial	230	6.8	45	4.2	13.3
KE 1a	Interstitial	nd	nd	nd	nd	nd
KE 1b	Interstitial	70	7.4	97	9.7	10.1
KE 2	Interstitial	310	7.1	65	6.7	13.1
*KE 3a	Cave	nd	nd	nd	nd	nd
KE 3b	Cave	nd	nd	nd	nd	nd
KE 3c	Cave	430	7.3	94	9.8	11.9
KE 4	Cave	nd	nd	nd	nd	nd
*CL 1	Spring	420	7.4	85	9.4	10.3
*CL 2	Spring	450	7.7	96	10.7	10.1
*CL 3	Spring	380	8.2	97	10.7	10.3
CL 4	well	290	7.8	55	6.3	11.9
CL 5	Spring	nd	nd	nd	nd	nd
CL 6	Interstitial	780	7.3	30	3.4	8.4
CL 7	Spring	390	8.1	102	11.6	9.4
*CL 8	Well	280	8.3	77	8.7	9.4
*CL 9	Well / Spring	430	7.9	99	11	10
CL 10	Cave	nd	nd	nd	nd	nd
*CL 11	Cave	nd	nd	nd	nd	nd
CL 12	Cave	nd	nd	nd	nd	nd
GA 1	Spring	260	8.3	99	11.3	9
*GA 2	Cave	260	8.3	97	11.3	8.5
*GA 3	Cave	250	7.8	100	11.8	7.6
*MO 1	Spring	680	7.1	52	5.9	9.2
MO 2	Cave	520	7.4	79	9.2	7.6
MO 3	Spring	570	6.7	77	9	8.5
*MO 4	Resurgence	460	8.4	132	15.3	6.5
*MO 5	Well	200	6.4	34	3.7	9.1
*RO 1	Cave	nd	nd	nd	nd	nd
*RO 2	Spring	630	7.1	87	9.5	8.2
RO 3	Spring	520	7.9	101	10.9	9.9
RO 4	Spring	460	7.3	79	8.1	10.6
*SO 1	Cave	nd	nd	nd	nd	nd
SO 2	Spring	90	4.9	101	10.7	8.4
SO 3	Spring	nd	nd	nd	nd	nd
LE 1	Interstitial	500	7.4	91	9.9	7.4
LE 2	Interstitial	100	7.6	81	9.2	8.7

Site code	Site type	Conductivity (μS/cm)	pH	Dissoilved oxygen (%)	Dissoilved oxygen (mg/L)	Temperature (˚C)
LE 3	Spring	170	7.6	98	11.1	8.2
CA 1	Well	180	6.6	35	3.9	7.6
CA 2	Resurgence	160	7.6	99	11.1	8.4
FE 1	Spring	550	7.1	70	7.9	9
FE 2	Resurgence	240	8.1	97	10.7	7.8
FE 3	Cave	nd	nd	nd	nd	nd
FE 4	Cave	nd	nd	nd	nd	nd
FE 5	Cave	nd	nd	nd	nd	nd
FE 6	Cave	nd	nd	nd	nd	nd
WX 1	Spring	190	6.0	nd	nd	nd
WX 2	Interstitial	260	7.2	nd	nd	nd
WX 3	Spring	650	8.0	nd	nd	nd
WX 4	Spring	620	7.4	nd	nd	nd
*WX 5	Spring	280	6.9	nd	nd	nd
WX 6	Spring	630	7.3	nd	nd	nd
WX 7	Well	230	6.1	nd	nd	nd

Table 1.3. List of stygobite Crustacea species recorded during the survey.

Site code	Site type	*Niphargus* sp.	*Niphargus kochianus irlandicus*	*Niphargus wexfordensis*	*Microniphargus leruthi*
KK 2a	Spring	-	*x*	-	-
KK 2b	Spring	-	*x*	*x*	-
KK 4	Cave	x	-	-	-
KK 5	Spring	-	*x*	-	-
TP 1	Spring	-	*x*	-	-
CO 3a	Cave	-	*x*	*x*	-
CO 3b	Cave	-	*x*	-	-
CO 4	Interstitial	-	-	-	*x*
KE 3a	Cave	-	*x*	-	-
CL 1	Spring	-	*x*	-	-
CL 2	Spring	-	*x*	-	-
CL 3	Spring	-	*x*	-	-
CL 8	Well	-	*x*	-	-
CL 9	Spring-well	-	*x*	-	-
CL 11	Cave	-	-	*x*	-
GA 2	Cave	-	*x*	-	-
GA 3	Cave	-	*x*	-	-
MO 1	Spring	-	*x*	-	-
MO 4	Resurgence	-	*x*	-	-
MO 5	Well	-	*x*	-	-
RO 1	Cave	-	*x*	-	-
RO 2	Spring	-	*x*	-	-
SO 1	Cave	-	*x*	-	-
WX 5	Spring	-	-	*x*	-
	No. of records	***1***	***20***	***4***	***1***

Table 1.4 List of all invertebrate taxa recorded during the survey.; asterisk (*) next to the site code indicates sites with stygobite Crustacea records.

Site code	Site type	Invertebrate fauna
KK 1	Spring	Chironomidae, *Pisidium casertanum*, Oligochaeta, Ostracoda, *Polycelis felina*
*KK 2a	Spring	Oligochaeta, *Asellus sp*. (tiny juv.), Ostracoda, **1 juv. *Niphargus kochinanus irlandicus***
*KK 2b	Spring	Oligochaeta, Collembola, *Polycelis felina,* Chironomidae, *Asellus aquaticus*, **1 *Niphargus wexfordensis*, 2 *Niphargus kochianus irlandicus (juveniles)***
KK 3	Well	5 Cyclopoidea
*KK 4	Cave	Collembola, Enchytraeidae, **1 juv. *Niphargus sp.***
*KK 5	Spring	Collembola, *Asellus aquaticus,* Chironomidae, *Polycelis felina*, *Succinea / Oxyloma sp.* ***1 Niphargus kochinaus irlandicus***
*TP 1	Spring	Oligochaeta, Chironomidae, *Dixa maculata / nubilipennis, Velia sp*. (nymph), Oribatei, *Polycelis felina*, **6 *Niphargus kochinaus irlandicus***
TP 2	Cave	Collembola
CO 1	Cave	Collembola, *Elmis aenea, Hydroporus*, Hydracarina, *Pisidium,* Oligochaeta
CO 2	Spring	Oligochaeta, *Polycelis felina, Gamarus duebeni*
*CO 3a	Cave	**1 *Niphargus kochianus irlandicus* (juvenile), 1 *Niphargus wexfordensis***
*CO 3b	Cave	**11 *Niphargus kochianus irlandicus***
*CO 4	Interstitial gravels	*Esolus parallelepipedus, Elmis aenae, Polycelis felina*, Oligochaeta, Nematoda, *Polycentropus flavomaculatus, Leuctra sp*., Collembola, Chironomidae, *Caenis rivulorum*, Ostracoda, *Palpomyia / Bezzia* type, *Hemerodromia* type, Chloroperlidae sp., Ostracoda, Cyclopoidea, **4 *Microniphargus leruthi***
KE 1a	Interstitial gravels	Oligochaeta, Chironomidae, Chloroperlidae, *Cyphon sp., Palpomyia / Bezzia* type, *Sphaeromias* type, *Phagocata vitta, Leuctra sp., Baetis sp., Dicranota sp*. 4 Cyclopoidea, 1 Harpaticoidea
KE 1b	Interstitial gravels	*Leuctra sp*., Chironomidae, *Elodes sp., Sphaeromias* type., Chloroperlidae, Oligochaeta, Collembola, *Limnius volckmari*, Cyclopoidea
KE 2	Interstitial gravels	*Baetis sp.*, Oligochaeta, *Stenus sp.*, Collembola, Cyclopoidea
*KE 3a	Cave	G*ammarus duebeni, Asellus aquaticus* (both inc. troglophilic specimens), Chironomidae, Oligochaeta, Ceratopogonidae, **1 *Niphargus kochianus irlandicus* (+ 1 headless juv.)**
KE 3b	Cave	*Gammarus duebeni*, *Asellus aquaticus* (both inc. troglophilic specimens)
KE 3c	Cave	*Gammarus duebeni, Baetis sp. Asellus aquaticus*, Chironomidae, Oligochaeta.
KE 4	Cave	*Gammarus duebeni, Crangonyx pseudogracilis, Asellus sp*., Copepoda, Ostracoda, Chironomidae, Oligochaeta, *Leuctra sp.*, Heptageniidae, Ceratopogonidae, *Baetis sp.*
*CL 1	Spring	*Baetis sp.*, Oligocheata, *Asellus meridianus,* Chironomidae, **2 juv. *N. kochianus irlandicus.***
*CL 2	Spring	*Gammarus duebeni*, Heptageniidae, Polycentropodidae, Plecoptera, *Elmis aenea*, **9 *N. kochianus irlandicus.***
*CL 3	Spring	*Gammarus duebeni,* **1 *N. kochianus irlandicus.***
CL 4	Well	Chironomidae, *Heptagenia sp*. (1 specimen)
CL 5	Spring	Chironomidae, Oligochaeta
CL 6	Interstitial gravels	Collembola, Oligochaeta, *Baetis sp.*
CL 7	Spring	*Dugesia polychroa gp., Elodes sp*., Chironomidae, *Gammarus duebeni*, Collembola, *Pericoma* type, Oligochaeta, *Tinodes sp., Leuctra hippopus,* Ostracoda, *Molophilus sp.*

Site code	Site type	Invertebrate fauna
*CL 8	Well	**21 *Niphargus kochianus irlandicus***
*CL 9	Spring - well	**8 *Niphargus kochianus irlandicus***
CL 10	Cave	*Gammarus duebeni*
*CL 11	Cave	**4 *Niphargus wexfordensis***
CL 12	Cave	Collembola, *Plectrocnemia sp*.? *Gammarus duebeni, Phagocata vitta*
GA 1	Spring	Philopotamidae, Perlodidae, Perlidae, Simuliidae, Baetidae, *Asellus sp*., *Gammarus duebeni*
*GA 2	Cave	*Gammarus duebeni, Baetis sp*., Simuliidae, Collembola, Cyclopoidea, **4 *N. kochianus irlandicus***
*GA 3	cave	*Gammarus duebeni*, **3 *Niphargus kochianus irlandicus***
*MO 1	spring	Chironomidae, *Asellus aquaticus*, Oligochaeta, Collembola, **20 *N. kochianus irlandicus***
MO 2	cave	Chironomidae, Perlodidae, Limoniidae, *Asellus meridianus, Gammarus duebeni, Pisidium sp.*, Oligochaeta,
MO 3	spring	*Plectrocnemia sp.? Pisidium sp., Baetis sp.*, Chironomidae, Limonidae, Oligochaeta, *Asellus sp.*
*MO 4	resurgence	Chironomidae, *Asellus aquaticus*, Oligochaeta, *Siphlonurus sp*., Cyclopoidea, **4 *N. kochianus irlandicus***
*MO 5	well	Oligochaeta, *Agabus bipustulatus*, **11 *Niphargus kochianus irlandicus***
*RO 1	cave	*Gammarus duebeni,* ***>100 Niphargus kochianus irlandicus***
*RO 2	spring	*Asellus sp., Siphlonurus sp.*, Nemouridae, **2 *N. kochianus irlandicus***
RO 3	spring	*Gammarus duebeni*, Polycentropodidae, *Agabus bipustulatus, Pisidium sp.*, Oligocaheta, Chironomidae, Nemouridae, *Asellus aquaticus, Micropterna sp.*?
RO 4	spring	*Gammarus duebeni, Asellus aquaticus,* Oligocghaeta, *Pisidium sp.*
*SO 1	cave	*Plectrocnemia sp. ? Gammarus duebeni, Phagocata vitta,* **1 *Niphargus kochianus irlandicus***
SO 2	spring	*Agabus sp*., Perlodidae, Chironomidae, *Phagocata vitta*, Polycentropodidae
SO 3	spring	Chironomidae, Oligochaeta, *Elmis aenea, Nemurella picteti, Phagocata vitta, Pisidium casertanum, Plectrocnemia sp*., Limnephilidae.
LE 1	interstitial	*Gammarus duebeni*, Philopotamidae, Polycentropodidae, Chironomidae
LE 2	interstitial	Collembola, Oligochaeta, *Caenis rivulorum, Esolus parallelepipedus*, Nematoda
LE 3	spring	*Gammarus duebeni*, Polycentropodidae, Baetidae
CA 1	well	*Pisidium sp*., Copepoda, Cladocera, Polycentropodidae, *Chrysopilus sp.*, Culicidae, *Haemopis sanguisuga*
CA 2	resurgence	*Baetis sp., Gammarus duebeni,* Oligochaeta, Chironomidae.
FE 1	Spring	*Gammarus duebeni, Plectrocnemia sp., Pisidium sp.*, Chironomidae.
FE 2	resurgence	Baetidae, Perlodidae, Oligochaeta, Heptageniidae, *Gammarus duebeni*.
FE 3	Cave	*Gammarus duebeni*
FE 4	Cave	*Gammarus duebeni*, Collembola
FE 5	Cave	*Gammarus duebeni*, Limnephilidae
FE 6	Cave	*Gammarus duebeni*
WX 1	Spring	Chironomidae, Oligochaeta, Collembola, *Phagocata vitta*
WX 2	Interstitial gravels	Collembola
WX 3	Spring	Chironomidae

Site code	Site type	Invertebrate fauna
WX 4	Spring	*Plectrocnemia sp.*
*WX 5	Spring	Dytiscidae sp., *Gammarus duebeni,* **1 *Niphargus wexfordensis***
WX 6	Spring	*Gammarus duebeni, Leuctra sp*., Polycentropodidae, *Sericostoma personatum*, Ostracoda, Chironomidae, *Polycelis felina*.
WX 7	well	Chironomidae, *Asellus meridianus*

APPENDIX 2: Irish stygobite Crustacea records

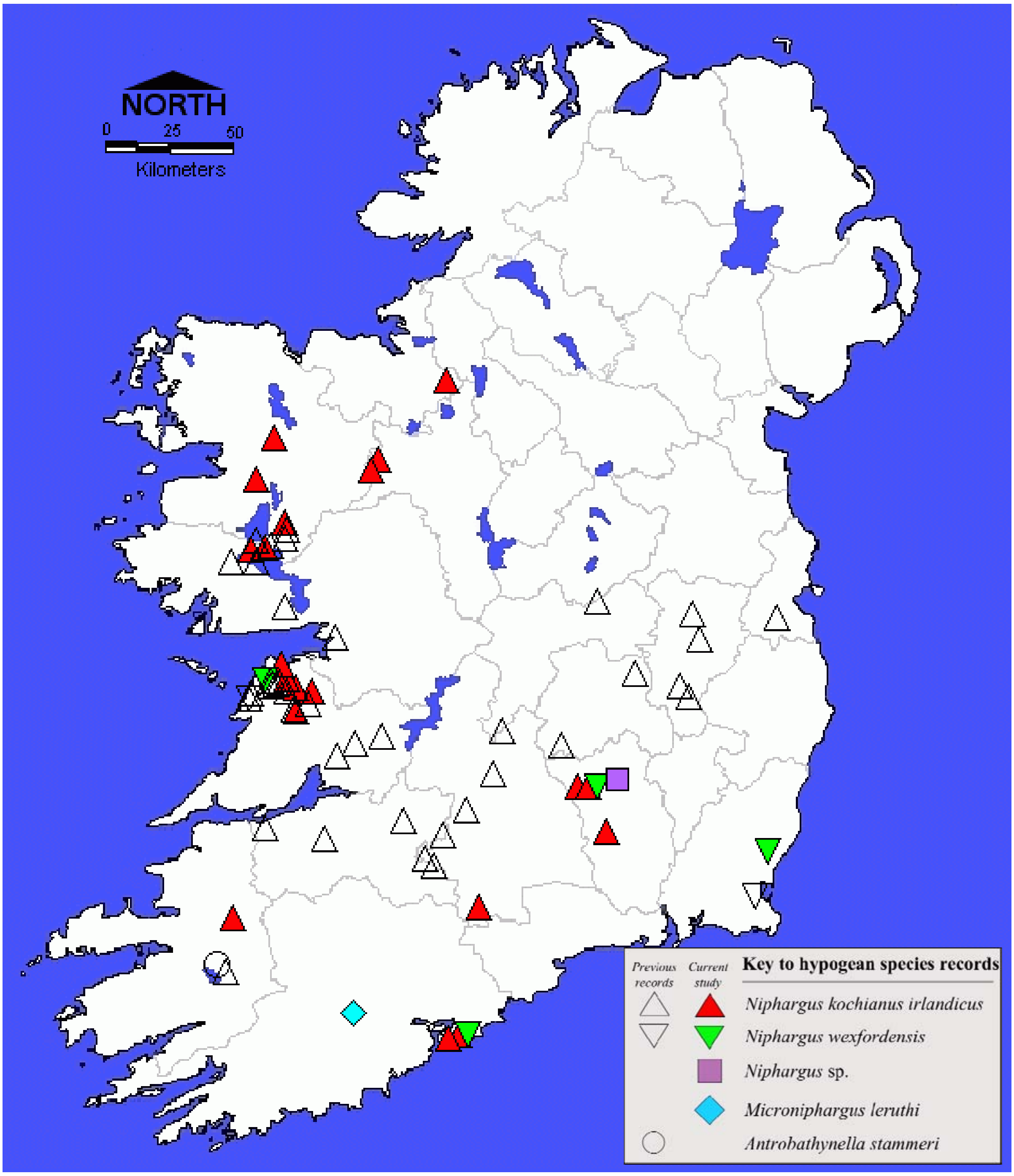

Figure 2.1. Map of the distribution of previously published records of stygobitic Crustacea in Ireland and the results of the current study.

Table 2.1. Irish hypogean aquatic Crustacea records by counties, including the results of this study (*). For more details go to www.freshwaterlife.org/hcrs.

Year	Grid Reference	County	Location
Anthrobathynella stammeri			
1982	V 987 902	Kerry	River Flesk, Killarney, Nr. Flesk Castle
Microniphargus leruthi			
2008*	W 4878 7384	Cork	Dripsey River, Downstream Dripsey Bridge
***Niphargus** sp.*			
2008*	S 50940 64950	Kilkenny	Dunmore Cave
Niphargue kochianus irlandicus			
1966	M 1596 0164	Clare	Pollcragreagh
1956, 1968, 1970, 1971	M 1610 0375	Clare	Poulnagollum Cave
1969	M 1820 0189	Clare	Cullaun Two, Western Poulacapple
1956	M 1927 0295	Clare	Gragon West Cave, Eastern Poulacapple
2005	R 0745 9686	Clare	Doolin River Cave, Fisherstreet Pot
1956	R 0846 9830	Clare	Doolin Cave, Aran View Passage, Aran View Swallet
1966	R 2220 9933	Clare	Cave of the Wild Horses, Kilcorney
1966	R 2514 9240	Clare	Ballycasheen Cave (Fergus River Cave)
1963	R 42 74	Clare	Quin
1963	R 49 79	Clare	Tulla
1963	R 59 82	Clare	Bodyke
2002	R 306 947	Clare	Treed Turlough
2008*	R 25752 91466	Clare	Near Killinaboy
2008*	R 31777 99612	Clare	Fahee North
2008*	M 25336 00488	Clare	Eanty More
2008*	M 21978 04199	Clare	Gragan East
2008*	M 20092 09816	Clare	Cloughmore
2008*	W 90201 65272	Cork	Carrigacrump Caves,Central Cave
2008*	W 90283 65276	Cork	Carrigacrump Caves, Lake Cave
1863, 1899, 1904	O 13 28	Dublin	Dublin, Templeogue
1956, 1974, 2008*	M 13275 55423	Galway	Pigeon Hole Cave
2008*	M 11548 55153	Galway	Ballymaglancy Cave
1979	M 41 20	Galway	Clarinbridge
1904, 1907	M 0 5	Galway	Lough Mask
1907	M 0 5	Galway	Kanes Cave
1956, 1974	M 1328 5542	Galway	Pigeon Hole Cave
1974	M 21 32	Galway	200 m SE of Moycullen
2008*	R 01382 11394	Kerry	Crag Cave, JK Cave
1982	V 987 902	Kerry	River Flesk, Near Flesk Castle
1964	N 75 01	Kildare	Fontstown
1997, 2003	N 8261 1922	Kildare	St Patrick's Well, Morristown
1964	S 79 97	Kildare	Ballitore
1964	N 80 29	Kildare	Clongowes

Year	Grid Reference	County	Location
2008*	S 35151 62550	Kilkenny	Nuenn River headwater trib
2008*	S 35141 62521	Kilkenny	Nuenn River headwater trib
2008*	S 46759 44830	Kilkenny	Newtown North
1964	N 58 06	Laois	Ballybrittas
1964	S 29 78	Laois	Rathdowney
1962	R 68 49	Limerick	Cahirconlish
1962	R 14 46	Limerick	Glin
1962	R 37 42	Limerick	Rathkeale
1962	R 83 43	Limerick	Oola
1974	M 1 5	Mayo	Krussaun Spa Well
1974	M 10 58	Mayo	Lough Mask, Nr. Saint Island
1974	M 1529 5568	Mayo	Kelly's Cave
1974	M 21 59?	Mayo	2 km W-SW of Ballylassa
1974	M 22 62	Mayo	2.5km NW of Kilmaine
2008*	M 21410 65027	Mayo	Caheredmond
2008*	M 09807 81687	Mayo	River Aille Resurgence, Bellaburke
2008*	M 1675 9772	Mayo	Ross West
1910	N 43 34	Meath	Near Mullingar
1964	S 06 84	Offaly	Dunkerrin
2008*	M 57317 89238	Roscommon	Pollawaddy Cave
2008*	M 54340 85016	Roscommon	Gortaganny, shore of Lough Errit
2008*	G 83666 20031	Sligo	Carrowmore Cave System
1962	R 92 53	Tipperary	Hollyford
1962	S 03 67	Tipperary	Borrisoleigh
1962	R 76 34	Tipperary	Emly
1962	R 80 31	Tipperary	Kilross
2008*	R 97601 15102	Tipperary	Near Crannagh Br
Niphargus wexfordensis			
2005	R 0745 9686	Clare	Doolin River Cave, Fisherstreet Pot
2008*	M 1376 0376	Clare	Polldubh Cave
2008*	W 90201 65272	Cork	Carrigacrump Caves,Central Cave
2008*	S 35141 62521	Kilkenny	Nuenn River headwater trib
1980, 1986, 1987	T 05 19	Wexford	Kerloge
2008*	T 09428 36724	Wexford	Near Knockanevin

APPENDIX 3: *Niphargus kochianus irlandicus* micrographs

Plate 3.1. ***Niphargus kochianus irlandicus*** **– mandible palp article 3 setae.**

Plate 3.2. ***Niphargus kochianus irlandicus*** **– telson.**

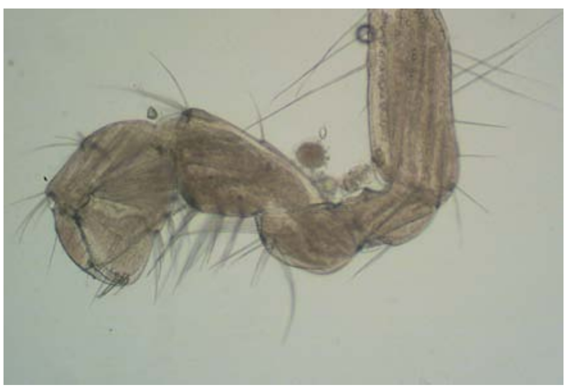

Plate 3.3. ***Niphargus kochianus irlandicus*** **– gnathopod 1.**

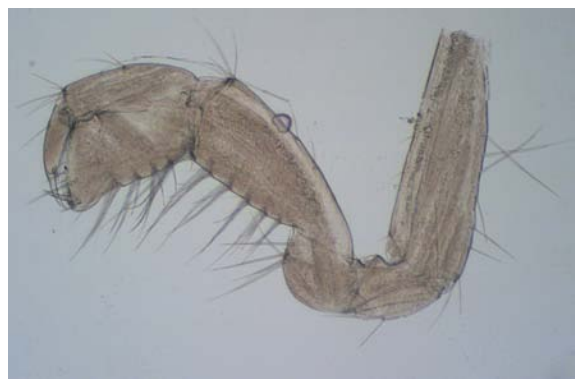

Plate 3.4. ***Niphargus kochianus irlandicus*** **– gnathopod 2.**

Plate 3.5. ***Niphargus kochianus irlandicus*** **– epimeral plates 2 and 3.**

Plate 3.6. ***Niphargus kochianus irlandicus*** **– urosome.**

Plate 3.7. ***Niphargus kochianus irlandicus*** **– head in lateral view.**

Plate 3.8. ***Niphargus kochianus irlandicus*** **– female with eggs.**

Plate 3.9. ***Niphargus kochianus irlandicus*** **– egg.**

APPENDIX 4: *Niphargus wexfordensis* micrographs

Plate 4.1. ***Niphargus wexfordensis*.**

Plate 4.2. ***Niphargus wexfordensis*** **– head in lateral view.**

Plate 4.3. ***Niphargus wexfordensis*** **– telson.**

Plate 4.4. ***Niphargus wexfordensis*** **– mandible palp.**

Plate 4.5. ***Niphargus wexfordensis*** **– gnathopod 2.**

Plate 4.6. ***Niphargus wexfordensis*** **– gnathopod 1.**

APPENDIX 5: *Microniphargus leruthi* micrographs

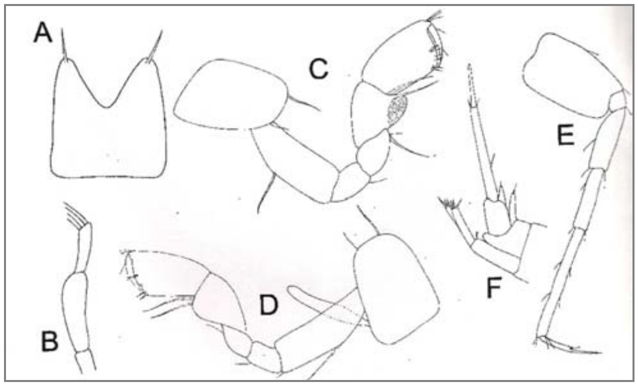

Plate 5.1. *Microniphargus leruthi* – Sketches of diagnostic morphological features (from Schminke, 2007, modified after Schellenberg, 1942): A- telson (dorsal), B- mandile palp, C- gnathopod 1, D- gnathopod 2, E- pereopod 7, F- urosome (distal view).

Plate 5.2. *Microniphargus leruthi* – urosome.

Plate 5.3. *Microniphargus leruthi* – mandible palps.

Plate 5.4. *Microniphargus leruthi* – gnathopod 1.

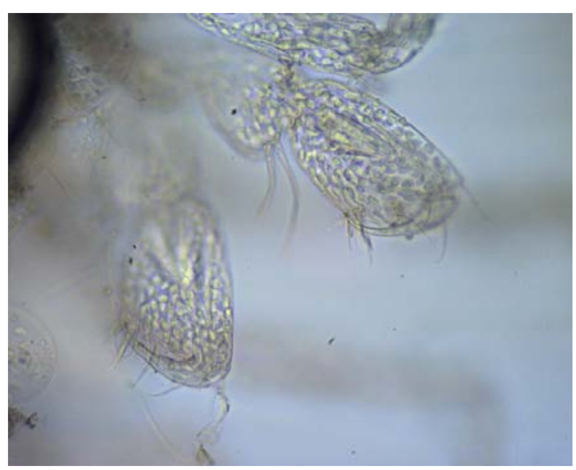

Plate 5.5. *Microniphargus leruthi* – gnathopods 2.

APPENDIX 6: Photographs of selected survey sites

Wells

Plate 6.1. Gragan East, Co. Clare (CL8).

Plate 6.2. Tober, Co. Cavan (CA1).

Plate 6.3. Berneens, Co. Clare (CL4).

Plate 6.4. Ross West, Co. Mayo (MO5).

Plate 6.5. Cloughmore, Co. Clare (CL9).

Plate 6.6. Nuenn River source, Co. Kilkenny (KK3).

Interstitial sites

Plate 6.7. Aille River, Lisdoonvarna bridge, Co. Clare (CL6).

Plate 6.8. Owengarriff River, Co. Kerry (KE1B).

Plate 6.9. Stream flowing into Lough Gill, Co. Cavan (LE1).

Plate 6.10. Bonet River, New Bridge, Co. Cavan (LE2).

Plate 6.11. River Flesk, Ballyspillane bridge , Co. Kerry (LE2).

Plate 6.12. Dripsey River, Dripsey bridge, Co. Cork (CO4).

Springs

Plate 6.13. Tributary of Nuenn River headwater, Co. Kilkenny (KK2A).

Plate 6.14. Killinaboy, Co. Clare (CL1).

Plate 6.15. Fahee North, Co. Clare (CL2).

Plate 6.16. Eanty More, Co. Clare (CL3).

Plate 6.17. Carrowrevagh, Co. Leitrim (LE3).

Plate 6.18. Derrynavaahagh, Co. Clare (CL7).

Caves

Plate 6.19. Mitchelstown Old Cave, Co. Tipperary (TP2).

Plate 6.20. Dunmore Cave, Haddon Hall, Co. Kilkenny (KK4A).

Plate 6.21. Carrigacrump Caves,Central Cave, Co. Cork (CO3a).

Plate 6.22. Pigeon Hole Cave, Co. Galway (GA2).

Plate 6.23. Bruce's Pot Cave, Co. Fermanagh (FE6).

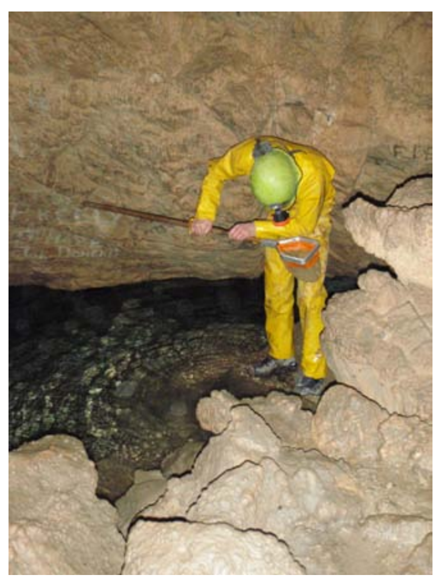

Plate 6.24. Dunmore Cave, Crystal Chamber, Co. Kilkenny (KK4B).

Resurgences

Plate 6.25. Tullyhona Rising, Co. Fermanagh (FE2).

Plate 6.26. River Aille Resurgence, Co. Mayo (MO4).

Plate 6.27. Source of Shannon, Co. Cavan (CA2).

APPENDIX 7: Hypogean sampling equipment and methodologies

Net sampling

A wide suite of sampling nets can be used to capture stygobite fauna and selection of an appropriate one largely depends on the circumstances, such as site accessibility or desired mesh size (Plate 7.1). When sampling underground habitats, choosing the size of the net mesh aperture requires a balance between capturing smaller sized specimens and maintaining a good flow of water through the net, preventing it from becoming clogged up by silt and debris. The standard UK Environment Agency kick sampling/pond net with a mesh of 1 mm is likely to retain larger specimens, but small/juvenile niphargids and bathynellids might be lost. A mesh of 250 µm is generally more suitable for stygobitic Crustacea sampling. Choosing the right net and technique is often subject to trial and error.

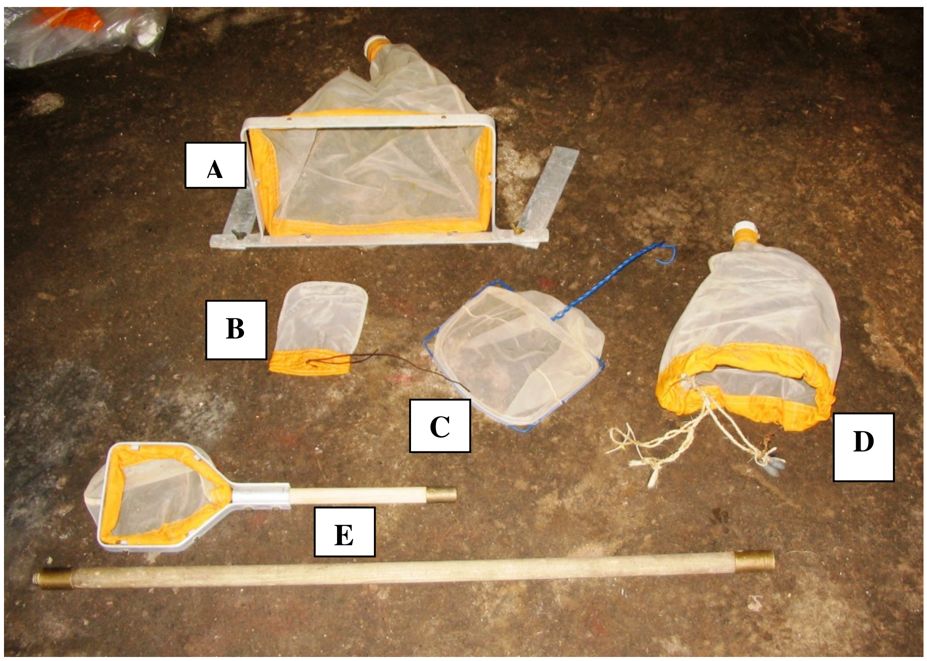

Plate 7.1. Nets for use in sampling hypogean aquatic habitats. A: drift net, B: pipe net (with draw-string), C: aquarist's hand net, D: zooplankton trawl net (with lead weights), E: hand net (with optional extended handle section, more than one can be fitted if required).

The Bou-Rouch method (Bou & Rouch, 1967)

This is the main method used to sample the shallow interstitial by groundwater ecologists and proved to be very effective in sampling groundwater up-wellings in gravely Irish springs. The equipment consists of a zinced iron pipe, 3 cm in diameter and 1.2 m in length. At one end is a spiked tip, with rows of holes above this. At the other end is a lip or thread. The pipe was driven into sediments with a hammer (a cap is used to protect the thread or lip) and once at the desired depth a piston pump was fitted on the end (Plate 7.2). The interstitial water was then pumped through a pipe net, fitted around the pump's nozzle, which retained fauna and gravel carried in the water.

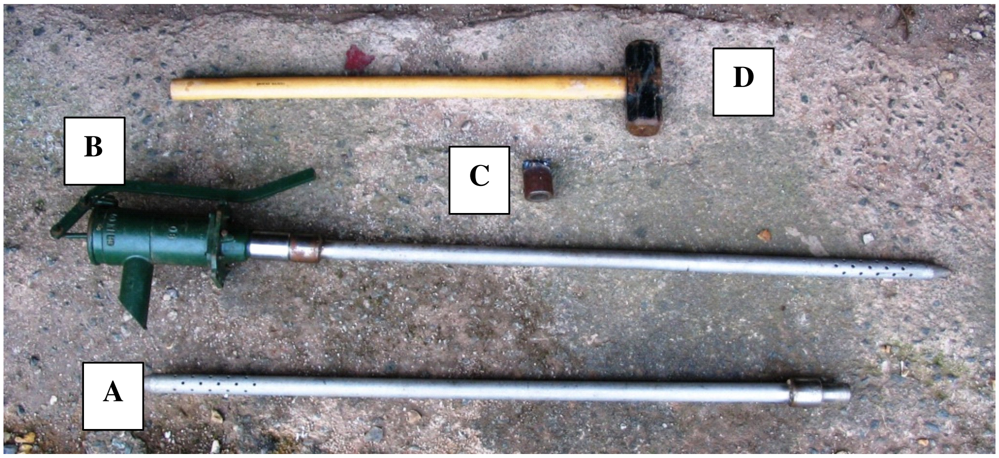

Plate 7.2. Bou-Rouch sampling equipment. A: perforated zinced iron pipe, B: piston pump attached to pipe, C: protective striking cap, D: hammer.

The Karaman-Chapuis method

Karaman (1935) and Chappuis (1942) both developed a method for sampling the fauna in the water beneath gravel banks at the margins of rivers and streams. It simply involves digging a hole into the gravel until the water level is reached. Interstitial water then flows into the hole until it reaches equilibrium with the subsurface water table. Interstitial flow into the hole is maintained by removing the water with a jug and filtering it through a net, to collect any invertebrates dislodged by the current from the surrounding gravel.