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A pilot study of the biota of percolating cave waters in Britain: a proxy for the epikarst aquifer

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Abstract: Dripping water habitats within caves (drips or adjacent drip-fed pools) were investigated as a surrogate for sampling the epikarst aquifer. This was conducted in three geographically dispersed caves in Britain – Ogof Draenen in South Wales, Swildon's Hole in the Mendips and Baker's Pit in Devon. Trays were placed at four locations in each cave to collect and filter water dripping from above over a period of six years (2016–2022), as well as periodic sampling of nearby drip-fed pools. Glacial scouring of the land surface is likely to have resulted in relatively poor development of the epikarst above Ogof Draenen, in comparison to the unglaciated regions in which Swildon's Hole and Baker's Pit are located, enabling some comparisons to be made. Swildon's Hole and Ogof Draenen had previously been the subject of systematic surveys of their aquatic fauna in vadose streams and pools, whereas ephemeral streams in Baker's Pit were sampled for the first time during the course of this study.

A variety of Ostracoda, Copepoda, Isopoda and Amphipoda species, some of which can be classified as either stygophilic (occur in groundwater habitats but also known from surface aquatic habitats) or stygobitic (obligate groundwater inhabitants), were found in the water drips and drip-fed pools. There was significant overlap in species composition of the biota collected in trays filtering dripping water, nearby drip-fed pools and vadose streams in the three caves. However, several species found exclusively in the trays and pools do not appear to occur regularly in vadose aquatic habitats within the three caves, suggesting they are potential epikarst inhabitants. These include the cyclopoid copepods *Graeteriella unisetigera* (E. Grater, 1908), *Diacyclops languidoides* (Lilljeborg, 1901) and *D. bisetosus* (Rehberg, 1880); the harpacticoid copepods *Bryocamptus echinatus* (Mrázek, 1893), *B. zschokkei* (Schmeil, 1893), *B. pygmaeus* (G.O. Sars, 1863), *B. typhlops* (Mrázek, 1893), *Altheyella crassa* (G.O. Sars, 1863) and *Parastenocaris* sp. and the ostracods *Fabaeformiscandona breuili* (Paris, 1920) and *F. wegelini* (Petkovski, 1962).

Two stygobitic Ostracoda species *Fabaeformiscandona breuili* and *F. wegelini*, connected with the epikarst environment, were for the first time confirmed from Britain in this study. A potentially exclusive new inhabitant of the epikarst is a not yet determined representative of the genus *Parastenocaris* (Copepoda) from Baker's Pit.

This study demonstrates that wider investigations would have great potential for making new discoveries and developing a better understanding of the British epikarst biota.

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Introduction

The physical properties of the epikarst

The concept of water stored and flowing horizontally at a shallow depth in the upper layers of karst was known and defined by Birot (1966) as the "subcutaneous flow," water flowing laterally through the upper, weathered layer of limestone (Gunn, 1983).

The term epikarst first came into use in the early 1990s and was defined by delegates at an epikarst workshop as:

> "*Epikarst is located within the vadose zone and is defined as the heterogeneous interface between unconsolidated material, including soil, regolith, sediment, and vegetative debris, and solutionally altered carbonate rock that is particularly saturated with water and capable of delaying or storing and locally rerouting vertical infiltration to the deeper, regional, phreatic zone of the underlying karst aquifer.*" (Bakalowicz, 2004).

Epikarst (which is also known as the subcutaneous zone) is the zone of highly weathered karstified bedrock lying immediately beneath the land surface, usually just a few metres (3–10m on average) thick (Culver and Pipan, 2014). Most commonly it lies beneath a soil layer, but it can also be exposed at the surface (Bakalowicz, 2004; Williams, 2008). It represents the boundary region between the soil and the bedrock in karst, and is commonly honeycombed with small fractures, dissolution pockets and dissolutionally enlarged fissures, caused by chemical dissolution, physical stress release (a result of seasonal climatic variations or seismic activity), and tree root penetration. The resulting enlargement of rock joints and cracks creates a more permeable and porous zone lying above the more compact underlying bedrock (Bakalowicz, 2019). Epikarst permeability decreases with depth, and soil can restrict or plug some of the fissures at its base. Also, the epikarst overlies the water infiltration zone, which is intersected by locally enlarged vertical fractures and conduits.

The base of the epikarst thus acts like an aquitard, resulting in a perched aquifer, the epikarst aquifer, which can hold a considerable volume of water close to the surface (Bakalowicz, 2019). This drips slowly through the ceilings of subterranean voids below or percolates downwards into conduits within the underlying limestone strata.

One of the principal characteristics of the epikarst is that it displays considerable heterogeneity, with remarkable variations in the residence time of the water passing through its network of semi-isolated dissolution pockets, and in its water chemistry (Brancelj, 2005; Bakalowicz, 2019). Early studies of epikarst hydrology showed that part of the rainwater percolating into the epikarst infiltrates directly and quickly through wide fractures and vertical conduits, either from dispersed infiltration at the karst surface or from point infiltration through sinkholes. The remainder is stored within the karst aquifer, where some is utilized by penetrating plant roots, some percolates slowly through the fine crack and rock porosity (slow infiltration), and some is later flushed away into the vertical conduits of the underlying infiltration zone (the doline-shaft system) during heavy rain and resultant epikarst overflow (delayed infiltration). Point and direct fast infiltration water flows do not pass through the epikarst, with dolines and shafts instead allowing direct vadose flows to reach the conduit system below rapidly, in essence acting as holes through the epikarst and overflow drainage when it becomes saturated (Bakalowicz, 2019).

The storage capacity of the epikarst is determined by its connectivity and thickness, the average porosity and the rainfall pattern in a region. The time delay between rain or snow melt and an increase in drip discharge in cave passages below can be very short (less than an hour to a few hours), as recorded by long-term measurements of precipitation and drip discharge in the cave Velika Pasica in Slovenia (Brancelj, 2015). However, other studies have shown far longer residence times for water in the epikarst, varying between three to 160 days in the epikarst above White Scar Cave in Yorkshire (Bottrell and Atkinson, 1992) and even as much as 0.58 to 10000 years (average 2.97 years) in the epikarst above Postojnska Jama, Slovenia (Mandic *et al*., 2013).

Flow from the epikarst supplies the small tributaries of cave streams, often maintaining their flow throughout the year, and small temporary or permanent springs emerging on hillsides (epikarst or helocrene springs). Slow leakage paths from the epikarst also maintain seepage to many stalagmites throughout the year (Williams, 2008).

Epikarst is best developed in temperate regions that experience moderate rainfall. In many tropical and arid regions 'case hardening' occurs, when secondary deposition of carbonate in pores immediately beneath the surface causes conditions of very low primary and secondary porosity in the upper layers of the carbonate rock. Also, epikarst does not develop well in coralline limestone and chalk sequences, because their primary porosities are so high overall that rapid downwards movement of meteoric water leaves little opportunity for dissolution to occur (Culver and Pipan, 2014). In some temperate areas, especially in formerly glaciated regions, the epikarst is reduced in thickness or might even be absent, because it has been scoured away by glacial action (Williams, 2008).

Investigations of the epikarst fauna

Pipan and Culver (2013) provide a useful global overview of research into the epikarst fauna over the previous 40 years. Sampling of water drips originating directly from the epikarst, using special equipment, has provided new insights into the structure and function of communities there (Brancelj, 2004, 2015; Culver *et al*., 2019), confirming that a specialised fauna occurs in the habitat. However, a distinction should be made between "*species collected from the epikarst*" and "*epikarst specialists*". The first group includes all species collected from drips, including those washed into the epikarst from permanent and temporary aquatic habitats on the surface, as well as those living in the epikarst aquifer. The latter includes a few stygobitic species that are specialized to the epikarst habitat. Both groups are essentially present as "sink populations" in water drips entering the vadose or phreatic zones (Brancelj, 2002).

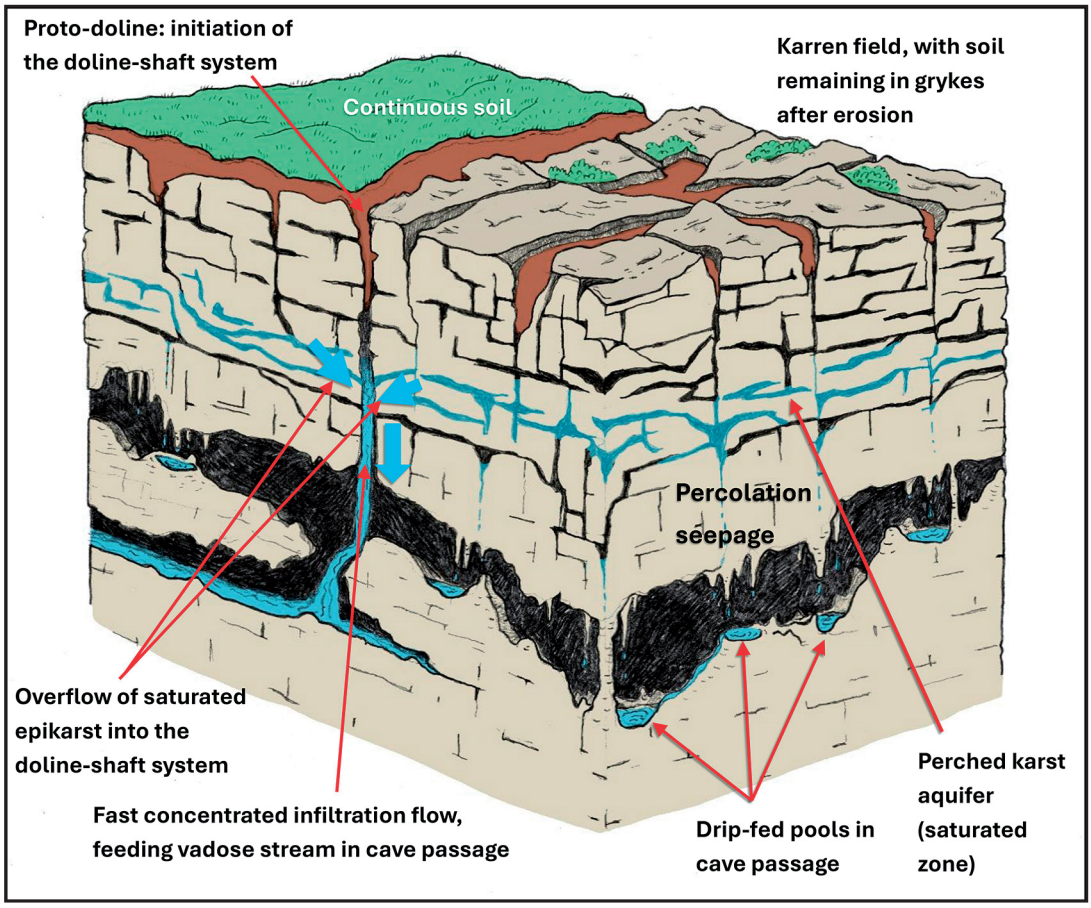

***Figure 1**:*
Representational diagram of the epikarst showing structure, flow paths and their relation to cave passages beneath.
[Figure drawn by Dominika Wróblewska, based on Figure 1 in Bakalowicz (2019), adapted from Mangin (1975).]

The first records of epikarst species date back to 1930, when three species, now recognized as typical epikarst inhabitants, *Diacacylops hypogeus* (Kiefer, 1930), *Speocyclops infernus* (Kiefer, 1930) and *Morariopsis scotenophila* (Kiefer, 1930), were discovered in the Škocjanske Jame system (Höhle von St. Canzian in German) in Slovenia (Kiefer, 1930). It was documented that the samples were collected from a drip pool (actually a man-made basin) near the entrance of Tominčeva Jama (Tominz Grotte in German, part of the Škocjanske Jame system) where the thickness of rock above the cave ceiling is about 60m.

The first long-term sampling of drip water from the epikarst was performed by Rouch in caves within the karst Massif du Badget in the Pyrenees, where he recorded the epikarstic fauna along with some ecological parameters. Although he filtered dripping water in several shallow caves, at the time the habitat was not described as "*epikarst*" (Rouch, 1968; 1986). Following this, many papers were published that documented the results of single-visit-sampling events of different habitats in caves, including drip-fed pools filled exclusively by water from the epikarst, related either to pure taxonomy or combined with measurements of environmental parameters. After 2000, research was undertaken in several countries on different continents, including Europe (France, Italy, Portugal, Romania, Slovenia, Spain), the USA, Brazil, and Asia (Thailand and Vietnam) (Pipan and Brancelj, 2001, 2004a, 2004b; Brancelj, 2002, 2015; Pipan, 2005; Pipan and Culver, 2005, 2007; Meleg *et al*., 2011; Booyanusith *et al*., 2018; Bruno *et al*., 2018; Culver *et al.*, 2019). Detailed comparisons of the fauna collected directly from drips with that of drip-fed pools with different substrates indicated that the fauna of the latter varies over time, and thus was a biased sample of drip water; thus, direct sampling of drips is more representative of the epikarst fauna in general. Over time the fauna remaining in drip-fed pools consists of only the top predators or generalist species, as first noted in Velika Pasica Cave, Slovenia (Brancelj, 2002, 2015; Culver *et al*., 2019).

Intensive and systematic research of the epikarst fauna commenced with a project undertaken in Velika Pasica in 2000, when three different habitats, one of which was filled exclusively by dripping water, were sampled eight times over the course of a year (Brancelj, 2002). At the same time, Pipan and Brancelj (2001) started a study in six large caves, across different regions of Slovenia, in which drip-fed pools and percolating water were sampled either weekly or monthly, using a combination of funnels and filtering bottles beneath drips (as described in materials and methods below) and pumping and filtering of the water in drip-fed pools. The results revealed high numbers of copepod species collected in the vadose zone (20–60m below surface) that had been washed-out from the overlying epikarst. Over the study period there were considerable differences in drip rates and copepod abundance between individual sampling points, as evidenced by statistically significant ($p<0.05$) positive Spearman correlations between discharge (drip-rate/precipitation) versus abundance at many, but not all, drips (Pipan and Brancelj, 2001, 2004a; Pipan, 2005). These differences might be due to an important percolation threshold leading to the formation of a temporary aquifer within the epikarst, as well as the heterogeneity of the habitat, with non-productive drips essentially by-passing the aquifer altogether (Pipan, 2005).

The only known long-term (lasting May 2006 – August 2014) study in which a number of environmental parameters (discharge, air and water temperature, pH, electrical conductivity, major ions, fauna composition and abundance) were also measured was that involving four dripping points, fed by the epikarst aquifer in Velika Pasica, where abiotic parameters were measured on a 6-hour scale and biological samples were collected every 14 – 30 days. The results showed considerable variation in the species composition of individual sampling points, indicating that species were not evenly distributed within the epikarst, as well as positive and negative correlations between species and various chemical parameters. Discharge and hydrological dynamic were the main parameters determining the faunal composition at three of the points, with some species preferring low discharge and a slow hydrological dynamic and others preferring the reverse (Brancelj, 2015).

According to the research described in the preceding paragraphs, the aquatic epikarst fauna is composed predominately of stygophilic or stygobitic taxa in the Copepoda (Cyclopoida and Harpacticoida; 75–90% of specimens collected), Ostracoda and Syncarida. Less common components include members of the Turbellaria, Nematoda, Rotifera, Gastropoda, Isopoda, Amphipoda and Coleoptera (Brancelj, 2015; Culver *et al*., 2019). Amongst the Copepoda, the most common epikarst representatives within the Cyclopoida include the genera *Speocyclops* Kiefer, 1937 (Europe), *Bryocyclops* Kiefer, 1927 (Asia, North America) and within the Harpacticoida *Elaphoidella* Chappuis, 1928 (Europe, Asia, North America), *Bryocamptus* Chappuis, 1929 (Europe, North America), and *Parastenocaris* Kessler, 1913 (so far only collected from some drips and drip-fed pools in Europe and Japan, but members of the genus are distributed in groundwater habitats worldwide). Although present in epikarst communities there appear to be no species amongst the Ostracoda limited to the habitat. Syncarida recorded from the epikarst include several closely related and geographically limited, but distinct genera: *Iberobathynella* Schminke, 1973 (Iberian Peninsula); *Siambathynella* Camacho, Watiroyram, Brancelj, 2011 (Thailand); *Sketinella* Camacho, 2005 (Vietnam), and *Paraeobathynella* Camacho, 2005 (Vietnam); although overall it is a globally distributed group. Large-bodied taxa recorded from the epikarst include the Isopoda genera *Caecidotea* Packard, 1871) (USA) and *Proasellus* Dudich, 1925 (Europe) and the Amphipoda genera *Stygobromus* Cope, 1872 (USA) and *Niphargus* Schiødte, 1847 (Europe).

All the previously mentioned genera include species also known from other habitats, such as springs and the hyporheos (the interstitial habitat underlying river valleys), not just from the epikarst. Conversely, there are a few species known exclusively from the epikarst within one or a few geographically restricted areas, although (considering the insufficient sampling of the habitat) more are likely to occur. Amongst the Copepoda these species include: *Siamcyclops cavernicolus* Boonyanusith, Sanoamuang and Brancelj, 2018 (Cyclopoida, Thailand); and several species in the genus *Paramorariopsis* Brancelj, 1992 (Harpacticoida, Slovenia). The harpacticoid genus *Morariopsis* Borutzky, 1931 also includes four species known exclusively from drips and drip-fed pools in caves (one species in Japan, one in Croatia, and two species in several caves in Slovenia (Brancelj, 2002; Karanovic and Abe, 2010)); the remaining two species in the genus occur in the depths of Lake Baikal, Siberia (Borutzkii, 1964). The Amphipoda epikarst specialists include *Niphargobates orophobata* Sket, 1981 (Slovenia) and *Niphargobatoides* (formerly in the genus *Niphargobates) lefkodemonaki* (Sket, 1990) (Crete). The same restricted distribution is also exhibited by the syncarid *Batubathynella malaya* Sars, 1929 (Malaysia) and two Coleoptera species known only from drip-fed pools, *Trogloguignotus concii* Sanfilippo, 1958 (Dytiscidae, Venezuela) and *Troglelmis leleupi* Jeannel, 1950 (Elmidae, DR Congo) (Botosaneanu, 1986; Culver *et al*., 2019).

Previous putative records of epikarst fauna in Britain

Although there have been some hydrogeological studies of the epikarst in Britain and Ireland (e.g. Friederich and Smart, 1981, 1982; Bottrell and Atkinson, 1992), its biota is virtually unknown. Chapman (1993) does not mention the epikarst because, at that time, the term was only just coming into use. He does (page 47) describe the 'Superficial Underground Compartment' (SUC) and mentions that parts of this habitat can become temporarily flooded, which he also described as 'amphibious mesocaverns.'

> "*As soon as mesocaverns develop an airspace, they become available for colonization by terrestrial cavernicoles. However, cracks and anastomoses are extremely flood-prone, often filling up with water each time there is heavy rainfall at the surface. Vertical cracks probably flush more violently, but remain water-filled for shorter periods than horizontal cracks, and this may result in some differences in their faunas*."

This sounds very similar to an early description of the epikarst as it is now understood.

There are numerous records of stygobitic Crustacea and copepods in drip-fed cave pools on *Hazelton* (the database of biological records held by the BCRA: https://bcra.org.uk/biology/) and the database of the Hypogean Crustacea Recording Scheme (HCRS: https://hcrs.brc.ac.uk/hcrs-database), though these records encompass species also found widely in other habitats.

A population of stygobitic Crustacea (primarily *Proasellus cavaticus* (Leydig 1871), with sporadic records of *Niphargus fontanus* Spence Bate 1859 and stygophilic *Gammarus pulex* (Linnaeus 1758) occurs in the pools fed by percolation water in Barnes' Loop in Swildon's Hole, far above the level of the main stream (Chapman, 1993; Knight, 2011); although numbers of the latter two species have declined significantly in recent years. However, no study has been carried out to indicate whether the pools are fed by drip water or fracture flow, or a combination of the two. The occurrence of *G. pulex*, albeit specimens with a long residence time, would suggest some direct surface connections, which might be active only at times of high rainfall.

In the lake at the lowest point in Pen Park Hole (Bristol) there is a large population of the stygobitic amphipod *Niphargus kochianus* Spence Bate 1859, which has been known for some years and is the only confirmed recent occurrence of this species in a cave. *Niphargus kochianus* has long been considered a phreatobite in Britain (Proudlove *et al.,* 2003) and mostly it is known only from the deep groundwater habitat (i.e. phreatic zone). In a recent survey of Pen Park Hole, Knight (2014, 2017) found a single specimen of *N. kochianus* in a pool in the First Chamber, and several specimens in a pool in Upper East Passage. The former pool is close to the bottom of the entrance shaft and the latter in a passage beneath. Both locations are far above the uppermost reaches of the lake (which fluctuates considerably with groundwater levels), suggesting a possible epikarst origin for the specimens.

***Figure 2**: Map of southern Britain showing the locations of the three study sites.*

The first specimens of *Antrobathynella stammeri* (Jakobi 1954) in Britain (recorded at the time as *Bathynella natans* (Vejdoysky 1882)) were collected in 1927 and 1932 by A.G. Lowndes from a tub that was fed by water dripping from the roof of a tunnel in Pickwick Quarry, one of the Bath Stone mines near Corsham in Wiltshire (Lowndes, 1932a). Although this species has been recorded from a variety of other habitats, including cave streams, springs and the hyporheos, these first records could indicate that it also occurs in the epikarst, a habitat for which its elongated body and tiny size would make it particularly suited.

To date there are no known investigations in British caves and mines in which drips have been sampled directly using the funnel method developed by Brancelj (2004), or any other investigations of the epikarst fauna.

Study sites

Although there are no published comparative studies of its occurrence across Britain, there is likely to be considerable variability in epikarst development both within and between British karst regions, reflecting lithology, geological structure and the local history of Quaternary glaciation (J. Gunn, pers. comm). For comparative purposes, three caves in geographically separated areas (South Wales, the Mendip Hills of Somerset, and Devon) were selected for the trial study (Fig.2). Ogof Draenen in Wales and Swildon's Hole in the Mendip Hills contain permanent vadose streams, whereas Baker's Pit in Devon contains three ephemeral streams. Active stream caves are virtually absent in Devon, where most of the cave systems have formed by dissolution, apparently owing little of their development to corrasion by sinking streams (Gunn, 1994).

Within Ogof Draenen there are several distinct subterranean streams with various smaller tributaries, fed either by percolating water or by seasonal seepages from the overlying moorland via shallow dolines. The aquatic fauna of the various streams was sampled by Knight *et al*. (2018)in a study that also included limited sampling of pools throughout the cave, the seepages feeding dolines proven to be connected to the cave system, and the known resurgences. A surface stream sinks into the Swildon's Hole entrance, becoming the main stream that flows throughout the cave to the terminal sump, while being augmented by four main autogenic tributaries along its subterranean course. Both the allogenic main stream and its autogenic tributaries were sampled for their aquatic fauna by Knight (2011). Because no systematic survey of aquatic habitats has been carried out in Baker's Pit, the ephemeral streams in the cave were sampled in winter as part of the current study, to supplement the records of aquatic taxa on the *Hazelton* and HCRS databases and those reported in previous literature.

The three study sites differ in geology and in their glacial history. Other than parts of the extreme south in Pembrokeshire and the Gower, almost all of Wales, including the caves of the North Crop, was glaciated in the Pleistocene, including during the last (Devensian) glaciation (Catt *et al*., 2006). The Mendip Hills were not glaciated during the Devensian, but were certainly covered with ice during earlier glacials, including the Anglian (MS12, 478ka), which reached as far south as London and the Bristol Channel, along the north Devon coastline, and just north of the Cornish coastline (Catt *et al.*, 2006), with its glacial maximum passing through the northern isles of the Scillies archipelago (Cullingford, 1998). The far Southwest (Devon and Cornwall) was not glaciated, although periglacial, tundra-like conditions with a deep permafrost would have been evident (Gunn, 1994).

Ogof Draenen in southern Wales is Britain's second longest cave, with more than 70km of mapped passage spanning a vertical range of 148m, located near the southern edge of the Brecon Beacons, within the Dinantian Carboniferous limestone sequence of the North Crop (Farrant and Simms, 2011). The system contains an extensive network of vadose streams, fed

almost entirely by percolating water (autogenic), and higher-level relict passages. Considering the area's glacial history, epikarst is likely to be poorly developed above Draenen and absent where cave passages extend beneath Carboniferous (Namurian) sandstones of the Marros Group (formerly referred to as the "Millstone Grit Series") (J. Gunn, pers. comm.). This was confirmed at three of the sites within the cave during the current study. The fourth (Site 1, see below) was close to the margin of the Marros Group sandstones, which are overlain in turn by Westphalian Coal Measures (Maurice and Guilford, 2011); thus, some epikarst development might be present above this location.

Swildon's Hole is the longest cave within the Dinantian limestones of the Mendip Hills in Somerset, with a length of 9.2km, encompassing a vertical range of 167m (Gray *et al*., 2013). An allogenic stream that sinks into the entrance, is joined by several smaller autogenic tributaries along its length.

Despite earlier Pleistocene glaciation during the Anglian, it is expected that a well-developed epikarst is present above Swildon's Hole (J. Gunn, pers. comm.). Friederich and Smart (1982) studied autogenic percolation inflows in the nearby GB Cave and observed that some percolation inputs showed a rapid response to rainfall, indicating high transmission capacity in the fissures feeding them, with those sites showing the largest and most rapid response exhibiting rapid recession, indicating they drew on limited storage. Conversely some inflows showed relatively little response to rainfall, with gently sloping recessions, indicating a relatively large amount of storage. Although the epikarst concept was not recognized at the time, this sounds very much like the flow characteristics of point and direct fast infiltration coupled with slow infiltration, characteristic of overlying epikarst.

Baker's Pit, combined with the connecting Reed's Cavern is the longest system in the largest outcrop of Mid Devonian karst at Buckfastleigh, Devon, with the entire system consisting of some 3.6km of passages, over a vertical range of 21m (Oldham, 1986), occupying much of the hill behind Higher Kiln Quarry. As this area of Britain was not glaciated, a well-developed epikarst is expected to be present above the cave (J. Gunn, pers. comm.). Within Baker's Pit there is an ephemeral channel that flows through the First Chamber into Drain Chamber and then Stream Chamber, at the bottom of which it enters a sump, before resurging within Dart River Cave on the right-hand bank of the River Dart in the valley below. Two other ephemeral streams, flowing down the Great Rift and from the small waterfall beneath the PCG Extensions respectively, join the former flow in Stream Chamber and the combined waters then enter the terminal sump at the bottom of the chamber.

For a comprehensive list of aquatic fauna previously recorded from the three caves see Supplementary Table S1 on-line, which includes data from: Knight *et al*. (2018) Draenen; Knight (2011) Swildon's; and for Baker's Browne *et al*. (1970) and Griffiths and Fishpool (1991); as well as historical records on *Hazelton* and the HCRS databases.

Materials and methods

As a surrogate for sampling the epikarst aquifer, dripping water habitats within caves (drips and adjacent drip-fed pools) were investigated in three geographically dispersed caves in southern Britain.

Brancelj (2004) and Pipan and Brancelj (2001, 2004a) perfected a series of sampling techniques to examine the drip fauna (see figures 3 and 4). A funnel is positioned beneath a drip to catch it and channel the water into a plastic collecting container; in some investigations a whole set of fine mesh netting was positioned to cover a group of several dripping stalactites and then channel the water to a single funnel (Brancelj, 2015). The collecting container has holes cut in two of the sides, about 1 to 1.5cm above the bottom of the container, which are covered with a very fine mesh (60 to 100µm).

The funnel and container are left *in situ* and as water drips from the cave ceiling, sometimes carrying aquatic fauna from the epikarst within it, it accumulates in the container which is designed so that a 1cm-deep layer of water collects at the bottom, enough for small copepods to live in, whilst the rest of the water is filtered and escapes, thus quite large volumes of dripping water can be filtered passively, and their fauna sampled by this method.

Figure 3*: Trays used to sample dripping water.*
Top row (left to right)*: tray design, tray fitted with funnel, pouring sample into vial of preservative.*
Bottom row (left to right)*: Site 4, Ogof Draenen, tray without funnel wedged in fissure; Site 3, Baker's Pit, Site 1, Baker's Pit.*

***Figure 4**: Filtering bottle (Brancelj, 2004) and bailing jug. The bottle has mesh-lined holes on two sides and can be carried inside the jug to protect the mesh screens during transportation between sampling sites.*

For the trial to study fauna originating from drips in British caves, collecting trays were placed at four locations in each of the three geographically spread caves, two of which have previously been surveyed for aquatic fauna in streams and pools within the systems. Each collecting tray consisted of a plastic box 17.5cm by 14.5cm with two windows (approximately 8cm by 3cm) cut in opposite sides (2.5cm above the bottom of the box) and lined with 63µm mesh. A single funnel was positioned in the tray to catch and channel most of the water dripping from above (Fig.3). The trays were emptied periodically, approximately every three to four months, although the time periods were not fixed. The contents of the trays were poured into a filtering bottle (design as specified in Brancelj (2004), Fig.4) to reduce the volume and concentrate the sample, then poured into a vial and topped up with ethanol for transportation back to the laboratory.

The cave systems and locations of the collecting trays are listed in Table 1, along with estimations of their depth beneath the land surface. The equipment was deployed in late January and early February 2016 and removed in late March and early April 2022. With the exception of Site 4 in Draenen, all of the trays were placed beneath water dripping from stalactites above. At the location of Site 4 there were few such areas and instead this last tray was placed in a fissure that channelled trickling water. When deployed, the tray was wedged into the fissure to intercept the trickling flow and no funnel was required. The discharge rates of the drips were not measured, although seasonal oscillations were noted, thus the samples were likely to be an amalgamation of both rapid and slow infiltration flows.

***Figure 5**: Drip-fed pool sampling.*
***(Above)**: pool at Site 3, Ogof Draenen;*
***(Below)**: sampling large pool at Site 4, Baker's Pit. Pools being pumped with a bilge pump and the water passed through a filtering bottle to concentrate the sample.*

In addition to the drip-collecting trays, drip-fed pools in the vicinity of each of the trays were also sampled on four occasions, during the deployment and removal of each tray and in March 2019 and March 2021. Some drip-fed pools were occasionally dry during sampling events (for details see captions for Supplementary Tables S2, S3 and S4). The water in the larger pools was bailed using either a small hand-operated bilge pump or a plastic bailer, whilst constantly agitating the bottom sediment, and the water filtered through the same collecting bottle described above (see Brancelj, 2004). Samples were then preserved in pure ethanol *in situ* for transportation to the laboratory.

Cave	Ordnance Survey grid reference / lat-long (decimal degrees)	Site Locations	Approximate distance below surface
Ogof Draenen	SO 2463 1178	1: Under dripping stal at first set of formations in Gilwern Passage	90m
study period:	51.799566 −3.0943909	2: Stream Passage above Lamb and Fox Chamber	20m
13/2/2016 to		3: On sandy bank on corner by entrance to Straw Grotto	10m
02/04/2022		4: In fissure above stream upstream of Straw Grotto	10m
Swildon's Hole	ST 5312 5131	1: Water Chamber, on ledge near exit of Dry Way	40m
study period:	51.259013 −2.6732101	2: Ledge at beginning of Barnes' Loop	90m
06/02/2016 to		3: Old Grotto near junction of Long and Short Dry Way	30m
25/03/2022		4: Small chamber at end of New Grotto	5m
Baker's Pit	SX 7420 6649	1: Bear Cave, at top of stal slope to lower pool	20m
study period:	50.484680 −3.7745879	2: Ledge in Swildon's Rift	20m
23/1/2016 to		3: End of Crystal Corridor	30m
18/04/2022		4: Half way along Crystal Corridor, near large pools on floor	30m

***Table 1**: Locations of the four sampling sites in each of the three cave systems. The location names are those used on the 1997 Chelsea Spelaeological Society's survey of Ogof Draenen; in Gray* et al. *(2013) for Swildon's; and on the 1966 revised survey of the Baker's Pit–Reed's cave system.*

Cave		Ogof Draenen			Swildon's Hole			Baker's Pit		
		Trays	Pools	Streams	Trays	Pools	Streams	Trays	Pools	Streams
TAXA	**TYPE**									
TRICLADIDA										
PLANARIIDAE										
Phagocata vitta (Duges, 1830)	sty.ph.	1								
NEMATODA	indet.	16		X	5	1	X			
OLIGOCHAETA	indet.	54	14	X	32	30	X	4	5	4
GASTROPODA										
HYDROBIIDAE										
Potamopyrgus antipodarum (J.E. Gray, 1843)	epig.					1				
CRUSTACEA										
AMPHIPODA										
Niphargus glenniei (Spooner, 1952)	stygo.							9	7	2
Niphargus aquilex Schiödte, 1855	stygo.									2
Microniphargus leruthi Schellenberg, 1934	stygo.	1		X		1	X			
Gammarus pulex (Linnaeus, 1758)	epig.			X		1	X			
ISOPODA										
Proasellus cavaticus (Leydig, 1871)	stygo.	3	2	X		3	X			
COPEPODA										
CYCLOPOIDA										
Graeteriella unisetigera (E. Graeter, 1908)	sty.ph.				1			8	2	
Graeteriella sp. (*c.f. boui*??)	sty.ph.	1								
Diacyclops languidoides (Lilljeborg, 1901)	sty.ph.	1						1		
Diacyclops bisetosus (Rehberg, 1880)	sty.ph.								8	
Diacyclops sp.	epig.							1		
Paracyclops fimbriatus gp. (Fischer, 1853)	sty.ph.	18	4	X		15	X			2
Tropocyclops prasinus (Fischer, 1860)	epig.			X				1		
Megacyclops viridis (Jurine, 1820)	sty.ph.					23				
Eucyclops serrulatus (Fischer, 1851)	sty.ph.			X				5		
Cyclopoida sp. (indet.)	indet.				1			1		5
HARPACTICOIDA										
Bryocamptus echinatus (Mrázek, 1893)	sty.ph.	18	5							
Bryocamptus zschokkei (Schmeil, 1893)	sty.ph.	2				2		4		
Bryocamptus pygmaeus (G.O. Sars, 1863)	sty.ph.	8				3				
Bryocamptus typhlops (Mrázek, 1893)	sty.ph.				5					
Bryocamptus sp.	indet.							3		
Attheyella crassa (G.O. Sars, 1863)	sty.ph.					9				
Parastenocaris sp.	indet.							1		
Harpacticoida sp. (indet.)	indet.				1					
OSTRACODA										
Fabaeformiscandona breuili (Paris, 1920)	stygo.	2								
Fabaeformiscandona wegelini (Petkovski, 1962)	stygo.					5				
Potamocypris fulva (Brady, 1868)	sty.ph.					41				
Ostracoda sp. (indet.)	indet.	4				50				
ACARI										
HALICARIDAE										
Soldanellonyx chappuisi Walter, 1917	sty.ph.	1			3		X			
EPHEMEROPTERA										
CAENIDAE										
Caenis sp. (1st instar)	epig.					1				
TRICHOPTERA										
HYDROPSYCHIDAE										
Diplectrona felix McLachan, 1878	epig.	1								
POLYCENTROPODIDAE										
Plectrocnemia geniculata McLachlan, 1871	epig.	7		X			X			
Plectrocnemia sp.	epig.	5		X			X			
DIPTERA										
EMPIDIDAE										
Chelifera sp.	epig.	1					X			
CHIRONOMIDAE										
Chironomidae sp.	epig.	12		X			X			
COLEOPTERA										
SCIRTIDAE										
Elodes sp. (larvae)	epig.				2					
ELMIDAE										
Limnius volckmari (Panzer, 1793)	epig.	7								

***Table 2**:*
*Cumulative data for the drip trays, drip-fed pools, and vadose streams in each cave throughout the study period (January 2016 to April 2022). Abbreviations in the "**Type**" column refer to ecological classification: epig. epigean; sty.ph. stygophile; stygo. stygobite; indet. insufficiently known. The '**Streams**' column includes data from Knight* et al. *(2018) for Draenen, and Knight (2011) for Swildon's, with an "***X***" denoting the presence of the taxon in the stream habitat for comparative purposes. The stream data for Baker's are those collected during the current study.*
*More detailed data for Draenen, Swildon's and Baker's is available in **Supplementary Table S1**, which includes the full taxa lists for the two respective studies, and historical Hazleton data for Baker's, including those species not listed in Table 2. Nematoda, Oligochaeta and Chironomidae were not determined further. "Paracyclops fimbriatus gp." represents both* Paracyclops fimbriatus *and cf.* P. chiltoni. *More-detailed data, of taxa and numbers collected from the trays and drip-fed pools on particular dates are provided online as **Supplementary Table S2**.*

In order to provide a degree of comparison with the stream fauna, the ephemeral streams in Baker's Pit were surveyed on 24th January 2018 after several weeks of wet weather when the streams exhibited substantial flow. The same method as that employed in the vadose stream surveys conducted in Swildon's (Knight, 2011) and Draenen (Knight *et al*., 2018) was used, involving kick sampling, for a timed period of three minutes, with an FBA-pattern pond net fitted with a 250µm collecting bag. This was undertaken at four locations in the lower passages of the cave:

- A: Main stream at terminal sump;
- B: Tributary stream at the waterfall beneath the PCG Extensions;
- C: Tributary stream in the Great Rift;
- D: Pooled water in Drain Chamber.

In the laboratory, samples were washed gently through a stacked set of small sieves of mesh sizes 250, 150 and 63µm, and separated aliquots were placed in a large petri dish for sorting and the picking out of specimens with forceps or pipette under an XTL-101 stereo-zoom microscope (GX Microscopes). Except for the Nematoda (round worms), Oligochaeta (segmented worms) and Chironomidae larvae (Diptera), which were not determined further, specimens were identified to the lowest taxonomic level, dependent on maturity and available taxonomic keys. AB identified the Copepoda, NM the Ostracoda and LK the rest of the aquatic taxa. Specimens were retained in the private collections of the authors.

The taxa were afterwards grouped into four ecological types, based on the best knowledge of their affinities for the subterranean environment. They were designated as:

- epigean (epig.), ubiquitous, *i.e.* dominant in epigean (surface) habitats, but can also persist for some time in the subterranean environment, although unable to form persistent breeding populations; all aquatic insect larvae (Ephemeroptera, Trichoptera, Diptera, Coleoptera) were included within this category;
- stygophiles (sty.ph.), *i.e.* taxa found in surface habitats but which are also capable of forming breeding subterranean populations; many are intimately connected with springs or in other near-subsurface habitats;
- stygobites (stygo.), *i.e.* obligatory subterranean dwellers;
- ecology insufficiently known (indet.), for taxa not determined beyond order or genus, which might have constituted several species.

Results

See Table 2 and Table 3.

A total of 33 distinct taxa (e.g. where Harpacticoida sp., *Bryocamptus* sp. and *Bryocamptus echinatus* (Mrázek, 1893) present in the sample, then only one would be counted as distinct), 18 in Ogof Draenen, 18 in Swildon's Hole and nine in Baker's Pit, were recorded in the trays collecting drip water and drip-fed pools. The number of specimens ranged from one to 54 over the duration of the study, although most taxa were represented by just one to five specimens, testament to the scarcity of the fauna in the dripping water. Of the 33 taxa, 10 were epigean species, 16 stygophiles, six stygobites and three (Nematoda, Oligochaeta and *Parastenocaris* sp.) were given an "insufficiently known" category as they were not determined further and are likely to have included species that could fit into any of the preceding three categories.

Number of taxa	Ogof Draenen	Swildon's Hole	Baker's Pit
Only in trays	18	6	8
Only in pools	4	14	4
In trays and pools	18	18	9
Not recorded in streams	11	11	7

Table 3*: Comparison of taxa between habitats in each cave.*

Only Oligochaeta were present in all three caves, across all the habitats. The harpacticoid copepod *Bryocamptus zschokkei* (Schmeil, 1893) was recorded in the trays in Draenen and Baker's, and pools in Swildon's. The cyclopoid copepod *Paracyclops fimbriatus* group (Fischer, 1853) was also present in all three caves but only in the stream habitat. Aquatic Insecta larvae were only recorded in Draenen and Swildon's Hole, mostly in the streams but also including small numbers in the trays (sites 4 and 2 in Draenen, and Site 1 in Swildon's) and in the drip-fed pools at Site 3, Swildon's.

A total of 18 taxa, including three stygobites, were collected in the trays in Ogof Draenen, whereas four taxa (also present in the trays) were collected from drip-fed pools. Eleven of these taxa were not recorded in the vadose streams by Knight *et al.* (2018), including *Phagocata vitta* (Duges, 1830), *Graeteriella* sp., *Diacyclops languidoides* (Lilljeborg, 1901), *Bryocamptus echinatus* (Mrázek, 1893),, *B. zschokkei*, *B. pygmaeus* (G.O. Sars, 1863), *Fabaeformiscandona breuili* (Paris, 1920), *Soldanellonyx chappuisi* Walter, 1917), *Diplectrona felix* McLachlan, 1871, *Chelifera* sp. and *Limnius volckmari* (Panzer, 1793).

In Swildon's Hole six taxa were collected in the trays and 14 (including three stygobites) recorded in the pools. Twelve of the taxa present in the pools were not recorded in the trays and 11, present in both the trays and pools, were not recorded in the vadose streams by Knight (2011), including *Potamopyrgus antipodarum* (J.E. Gray, 1843), *Graeteriella unisetigera* (E. Graeter, 1908), *Megacyclops viridis* (Jurine, 1820), *Bryocamptus zschokkei, B. pygmaeus, B. typhlops* (Mrázek, 1893), *Attheyella crassa* (G.O. Sars, 1863*), Fabaeformiscandona wegelini* (Petkovski, 1962)*, Potamocypris fulva* (Brady, 1868), *Caenis* sp. and *Elodes* larvae.

In Baker's Pit eight taxa were collected in the trays and four from drip-fed pools, including the stygobite *Niphargus glenniei* (Spooner, 1952), and four taxa from the ephemeral streams. *Diacyclops bisetosus* (Rehberg, 1880) was recorded only in the pools, whereas *Paracyclops fimbriatus* gp. and a second stygobitic amphipod *Niphargus aquilex* Schiödte, 1855 were only present in the streams. Seven of the taxa, all Copepoda, recorded in the trays and pools were absent from the streams, including *G. unisetigera, D. languidoides, D. bisetosus, Tropocyclops prasinus* (Fischer, 1860), *Eucyclops serrulatus* (Fischer, 1851), *B. zschokkei* and *Parastenocaris* sp.

More detailed data, of specific taxa collected on particular dates and at each site within the three caves are provided online as *Supplementary Tables S2 to S4*.

Discussion

The subterranean fauna of the British Isles is known to be considerably poorer in species diversity compared to that of more southern latitudes in Europe, primarily due to the legacy of the Pleistocene glaciations (Proudlove *et al*., 2003; Robertson *et al*., 2008). To date investigations of the aquatic fauna of British caves has centred mostly on vadose streams, with occasional sampling of drip-fed pools, and no clear indication of any connection with overlying epikarst. The present study, involving the direct sampling of dripping water over a prolonged timeframe with fine-mesh nets in three caves has improved knowledge of the distribution of various species in subterranean aquatic habitats.

Overall numbers of specimens and species diversity were sparse throughout the study, with many sampling visits often recording no fauna in some of the trays. The sampling of small volumes of slowly percolating water was always going to yield low numbers of specimens, compared to similar studies conducted elsewhere in non-glaciated regions, hence the long-time frame for the study. However, it has still added two newly confirmed stygobitic ostracod species, (*Fabaeformiscandona breuili* and *F. wegelini*), with possibly a third new species of harpacticoid copepod in the genus *Parastenocaris*, although it is highly unfortunate that the single specimen was too badly damaged to identify further. Despite collection from the trays every three to four months, predation and mortality of specimens collected in the trays cannot be ruled out. In retrospect, having more frequent collection times, such as the 14-to-30-day intervals used by Barncelj (2015) in Valica Pasica, might have increased the number of specimens, but this was not feasible logistically, especially given the distance between the caves. Often in the summer month the trays were either dry or many times altogether devoid of fauna, indicating a genuine scarcity of biota in the dripping water.

Considering the epikarst is rarely thicker than 10m, this would suggest that sites 4 in Swildon's and 1 and 2 in Baker's were sampling the epikarst and little else, with the remaining sites collecting water and fauna from both the epikarst aquifer and fissures in the underlying vadose infiltration zone. With the possible exception of the relatively deep (90m below the surface) Site 1 in Draenen, epikarst was potentially absent or truncated above the other three shallower sites due to local glaciation. Although there were localized variations in the composition of the biota between all three caves, within the caves there appeared to be few discernible differences in the fauna between individual sites at varying depths (see Table S2).

Aquatic habitats (mostly vadose streams) in two of the caves (Ogof Draenen and Swildon's Hole) were the subject of intensive sampling in the past, the data from which has been included in Table S1 to enable a useful comparison between the fauna collected during the current study and any differences between it and that of the more permanent vadose aquatic habitats. However, note that the comparisons have some limitation imposed by differences in the sampling method of the earlier studies and the mesh size of the nets employed, discussed beloThe most abundant specimens collected in the trays sampling dripping water were the Nematoda and Oligochaeta, two taxa with long thin bodies common in wet soil, from which they are likely to have been washed down into the percolating waters.

Most of the taxa recorded (16 distinct taxa out of 33 recorded in total) from the trays and pools were small (1–3mm) Crustacea, encompassing the groups Cyclopoida and Harpacticoida (Copepoda) and Ostracoda. Most of these species occur widely in subterranean waters and, except for the stygoxene *Tropocyclops prasinus* (Fischer 1860), can be regarded as stygophilic (Proudlove *et al.*, 2003; Knight and Mori, 2022; Knight *et al*. in prep). Of particular interest are the stygobitic ostracods *Fabaeformiscandona breuili*, recorded just once from the tray at Site 4 in Draenen and *F. wegelini,* recorded once in pools at Site 3, Swildon's, the first occurrence of each species in Britain, as detailed in Knight and Mori (2022). As in many other similar studies sampling percolating waters elsewhere in Europe and across the globe, the Copepoda were the most abundant and diverse group recorded in the trays (Brancelj and Culver, 2005; Culver *et al*., 2019). Ostracoda were more commonly recorded in pools in Swildon's, suggesting they might not have originated from the dripping water. Amongst this group of Crustacea, the copepods *Paracyclops fimbriatus* gp. and *Bryocamptus echinatus* were the most abundant in Draenen; *P. fimbriatus*, *Megacyclops viridis* and the ostracod *Potamocypris fulva* in Swildon's and the copepod *Graeteriella unisetigera* in Baker's Pit. As expected, the total number of subterranean copepod species (12 stygophilic taxa) was low compared to that recorded in southern Europe, where 8–15 stygobitic species were recorded across six caves by Pipan (2005) and 14 stygobitic species by Brancelj (2015) during a long-term study in Velika Pasica Cave.

The only potential stygobitic copepod recorded in the current study was a single specimen, collected in the tray at Site 3, Baker's, in the harpacticoid genus *Parastenocaris*, which was too damaged to determine to species and could represent a species new to science. Both *Parastenocaris vicesima* (Klie 1935) and *Proserpinicaris phyllura* (Kiefer, 1938) (formerly placed in the genus *Parastenocaris*) have been recorded in Britain from fine sand at the sources of springs and are likely to be psammobitic (Proudlove *et al.*, 2003). Kibichii *et al*. (2010) recorded *P. phyllura* from Ireland and *P. vicesima* was recorded from amongst riverine sand on the banks of the River Flesk in Ireland by Arnscheidt *et al*. (2012). Unidentified *Parastenocaris* juveniles were also collected from two boreholes in an earlier survey (Arnscheidt *et al.*, 2008). *Parastenocaris* has not previously been recorded from British caves (Botosaneanu, 1986) and more specimens are required from Baker's Pit for further taxonomic study.

The harpacticoid copepod *Bryocamptus typhlops* is rare in Britain and although regarded by some European authors as a stygobite (e.g. Kiefer, 1959; Pipan and Brancelj, 2004a,b), it also occurs in surface habitats in Britain (Gurney, 1932) and other countries such as Austria (Gaviria, 1998) and Belgium (Fiers and Ghenne, 2000). It was collected for the first time underground in Britain in the tray at Site 1, Swildon's Hole on two occasions during the current study.

The cyclopoid copepod *Graeteriella unisetigera* is known almost exclusively from springs and caves in southern Europe, where it is regarded as stygobitic. It was frequently collected from helocrene springs and the interstitial habitat during the PASCALIS project assessing the biota of sub-surface habitats across Europe. In Britain, before the current study, it was known only from a few surface habitats, such as wet moss; Gurney (1933) lists it from just two sites, amongst wet peat on the slope of Boars Hill, Oxford and from moss on a wet rock at Tall-y-Llyn in North Wales. These records suggest that the species is only likely to be stygobitic in the south (Proudlove *et al.*, 2003). Within Britain, it is still a rare species, known from just a few sites, with the first subterranean records in the current study, where a single specimen was collected in the tray at Site 1, Swildon's and small numbers were also recorded in the trays at sites 1 and 2 in Baker's. A further specimen of *Graeteriella* was also recorded from the tray at Site 1, Draenen but was unfortunately too damaged to determine beyond genus. In Belgium the species was previously known from caves and a well in the provinces of Namur and Liège, until Fiers and Ghenne (2000) found it to be widespread in the humid leaf litter of beech forests, with its distribution in Europe showing a close correlation with that of beech. Such a habitat could make this a prime source population for being carried into the perched epikarst aquifer by percolating water.

Other taxa of particular interest included the stygobitic amphipods *Niphargus glenniei, N. aquilex*, and *Microniphargus leruthi* Schellenberg 1934, and the stygobitic isopod *Proasellus cavaticus*. Single specimens of *M. leruthi* were recorded in the tray at Site 1, Draenen and the pools at Site 2, Swildon's. With the exception of *Microniphargus*, which is 1 to 2mm in length, the remaining three species are relatively large-bodied organisms that are not likely to be true inhabitants of the small void spaces making up the epikarst aquifer, instead inhabiting the larger water-filled fissures of the vadose zone. *Niphargus glenniei* was recorded in small numbers in the trays and pools in Baker's and whilst mature individuals can reach 3mm in size, all of the specimens recorded in the trays at sites 1 and 3, were smaller juveniles. During the study *Niphargus aquilex* (along with the copepod *Paracyclops fimbriatus* gp.) was recorded only in the ephemeral streams in Baker's, but occurs widely in pools

throughout the cave (Browne *et al.*, 1970, *Hazelton* and HCRS data). The larger size of *N. aquilex*, (up to 10mm) might exclude it from the epikarst habitat. *Proasellus cavaticus* was recorded in small numbers in the trays at sites 2 and 4 in Draenen, both above streamways and likely to have larger fissures feeding the water flow, as well as in pools in both Draenen and Swildon's. Other notable elements of the fauna included the stygophilic flatworm *Phagocata vitta* in Draenen and the halicarid mite *Soldanellonyx chappuisi* Walter 1917, present in both Draenen and Swildon's.

In Ogof Draenen several of the taxa recorded in the trays and drip-fed pools had not previously been recorded in the cave streams by Knight *et al.* (2018). *Phagocatta vitta, Diplectrona felix, Limnius volckmari*, and *Plectrocnemia* sp. caddis larvae are predominately surface taxa sometimes washed into caves, all of which were recorded in the tray at Site 4, which, as mentioned earlier, was likely to have been sampling fast infiltration and was also close to the surface. Similarly, chironomids, and to a lesser degree *Chelifera*, are small elongate aquatic Diptera larvae that are also frequently washed into caves. *Diplectrona* larvae were recorded at one of the sinks above Draenen by Knight *et al.* (2018). However, it should be noted that the stygobitic ostracod *Fabaeformiscandona breuili*, as well as the stygophilic copepod *Diacyclops languidoides* and mite *Soldanellonyx chappuisi* were also recorded in this same tray, possibly during delayed infiltration. It is noteworthy that the stygophilic ostracod *Cavernocypris subterranea* (Wolf 1920), which was recorded at several stream sites by Knight *et al*. (2018), was not recorded in percolating waters, thus its colonization of caves might rely on other vectors than water infiltration. In addition to caves, *C. subterranea* is often found in springs and the interstitial habitats of lakes and rivers, preferring cold (6–12ºC) and slow flowing water (Meisch, 2000; Mori and Meisch, 2012).

Despite the probable lack of well-developed epikarst above most of Draenen, there would appear to be several copepod taxa (*Graeteriella, Diacyclops languidoides, Bryocamptus echinatus*, *B. zschokkei*, *B. pygmaeus*) and the ostracod *F. brueili* that were recorded only in percolating water. However, it should be noted that Knight *et al*. (2018) conducted their survey using a net fitted with a 250µm mesh collecting bag, which might have been too coarse for sampling such small species as the harpacticoid copepods, as opposed to the 63µm mesh fitted to the trays and filtering bottle in the current study.

In Swildon's Hole, several species not recorded by Knight (2011) in the streams were present either in the trays or drip-fed pools. The hydrobiid snail *Potamopyrgus antipodarum,* mayfly nymph *Caenis* sp. and marsh beetle larvae (*Elodes* sp.) are surface-dwelling taxa. Both *Elodes* larvae and a single specimen of *P. antipodarum* were collected from the tray at Site 1, possibly at a time of fast infiltration. The same can also be said for the single *Gammarus pulex* specimen recorded in the tray at Site 2, Barnes' Loop, a stygophilic population of which has existed intermittently at this location for some time, as discussed in the Introduction above. The single 1st instar *Caenis* nymph was collected from the pools at Site 3. These pools are on a small ledge close to a nearby stream, thus at times of very high flow the adjacent stream might well overspill into these pools and introduce lotic taxa. The ostracod *Potamocypris fulva* was recorded just once, in fairly high numbers, in this same group of pools at the same time as the *Caenis* nymph and is believed to have colonized the habitat via the same vector, rather than via dripping water.

Although Knight (2011) also carried out his sampling in the Swildon's streams using a 250µm mesh net, it could be the case that the copepods *Graeteriella unisetigera, Megacyclops viridis, Bryocamptus zschokkei, B. pygmaeus, B. typhlops* and *Attheyella crasssa* are limited to percolating water habitats in this cave. However, as discussed below, repeat sampling of the streams in all three caves using a net fitted with a 63µm mesh will be required to determine this with certainty.

In Baker's Pit, several Copepoda, including *Diacyclops languidoides, Eucyclops serrulatus* (Fischer, 1851), *Tropocyclops prasinus*, *B. pygmaeus* and *Parastenocaris* were recorded only in the trays, whereas *G. unisetigera*, although present in pools at Site 4, was also most recorded frequently in the trays.

The results from sampling dripping water therefore indicate that there are several ostracod and copepod taxa that were recorded in the trays and pools only, suggesting that they could be inhabitants of the epikarst/fissure network above the cave systems. At this stage, based only upon relatively limited sampling sites and data, it is problematical to ascertain whether these taxa can be regarded as typical of the epikarst habitat in Britain. This is especially so because a larger mesh-size net was used for sampling the watercourses in each cave system, reflecting the practicalities of sampling streams in which fast flows and waterborne debris can potentially clog finer-mesh nets. However, a 250µm mesh size has been proven to be effective at collecting species such as *P. fimbriatus, T. prasinus* and *E. serrulatus* from cave streams, along with the even smaller *Soldanellonyx* mites and harpacticoid copepods, possibly because they become enmeshed in debris. Fast-flowing habitats are likely to be disadvantageous to many smaller organisms unless they can find refuge in the substrate, which could be another factor, aside from predation by larger species, explaining their absence from the sampled cave streams. Thus, this could suggest that the static conditions offered by the perched epikarst aquifer, and pools fed by percolating water are the only viable habits for such small species underground. Although one must also consider that the communities of pools represent "sink populations" of individuals washed-in by accident (Brancelj and Culver, 2005; Culver *et al*., 2019).

The picture is complicated by the fact that there are few specialist stygobitic species in Britain, with all of the taxa discussed also known to occur in a variety of other sub-surface habitats, including springs and the gravel beds of watercourses. The exception to this might be the two species in the genus *Fabaeformiscandona*, which have not been confirmed in Britain until this study, although there are records of both species from boreholes in Ireland (Arnscheidt *et al.*, 2008, 2012) and possible, unconfirmed, records of *F. breuili* from springs in northwest Scotland (Boomer *et al.*, 2006), as well as possible synonymy with the ostracod species *Candona wedgewoodii* (Lowndes, 1932), described by Lowndes (1932b) from a pool fed by an overflowing drip-fed tub in Corsham stone mines, as discussed in Knight and Mori (2022). *Fabaeformiscandona breuili* is also known as fossil valves within Holocene deposits of several river catchments (Knight and Mori, 2022).

One would expect a degree of taxonomic variation between the three caves due to differences in geographical location. However, despite the lack of well-developed epikarst above most of the Draenen sites (due to its glacial legacy), there was still a suite of Ostracoda and Copepoda species collected from percolating waters in this cave. This could indicate that some of these species can maintain populations in overlying wet soil or sediments as well as the epikarst aquifer. Alternatively, they could also be present as source populations in ephemeral aquatic habitats on the surface, although this requires further investigation.

Whilst these initial results are of great interest, especially including as they do the first British records of two stygobitic Ostracoda (*Fabaeformiscandona*) and probably one Copepoda (*Parastenocaris*), much more work is required to elucidate if there is a fauna unique to the epikarst habitat here. Future studies should be much more intensive in nature, incorporating more frequent collection of fauna from the trays to reduce predation, and enclosing larger areas of dripping stalactites with fine mesh nets that channel the flow to a central collecting container,

enabling the sampling of considerably greater volumes of water, similar to studies such as those carried out elsewhere by Pipan (2005), Pipan and Culver (2007) and Brancelj (2015). These studies should also target shallow cave passages, preferably within 10m of the surface, so that the epikarst aquifer is sampled in isolation, rather than sampling water originating from both the aquifer and fissures in the vadose zone (infiltration zone) immediately beneath.

Conclusions

Initial investigations of percolating water habitats, as a surrogate for sampling the perched epikarst aquifer, in three British caves have provided some new results. There appears to be a suite of Ostracoda and Copepoda species, many of which can be classified as stygophilic or stygobitic, that whilst present in percolating water habitats, either collected in trays directly sampling dripping water or drip-fed pools, do not appear to be regular inhabitants of streams within the same caves, suggesting that they could possibly be regarded as inhabitants of the epikarst in Britain. These species included the cyclopoid copepods *Graeteriella unisetigera, Diacyclops languidoides* and *D. bisetosus;* the harpacticoid copepods *Bryocamptus echinatus, B. zschokkei, B. pygmaeus, B. typhlops, Altheyella crassa* and *Parastenocaris* sp.; and the ostracods *Fabaeformiscandona breuili* and *F. wegelini*; the latter two species confirmed from Britain for the first time in this study (Knight and Mori, 2022).

Aside from the two ostracods and *Parastenocaris*, all the above species are also known from other aquatic habitats in Britain, both surface and subterranean. These habitats include wells/boreholes, streams and pools in the vadose zone, and the interstitial (gravels at spring sources, the hyporheic zone beneath river channels, aquifers in unconsolidated sediments) (Proudlove *et al.*, 2003; Knight and Mori, 2022; Knight *et al*., in prep); although the occurrence of some of these species in other habitats could potentially represent "sink populations" originating from the fissure network. Despite the lack of epikarst habitat above much of Ogof Draenen, some of the species recorded there were also recorded in the other two caves, suggesting that the species might alternatively use deep wet soil layers as a surrogate for a true epikarst aquifer.

Since there are few stygobitic species in Britain, many have rather generalist ecology and distributions, which coupled with how little is still known about British subterranean biology means that much that has been written on the topic remains rather speculative at this stage. Thus, it is difficult to ascertain if the listed species can be regarded as representative of a British epikarst fauna, without further investigation, including repeat sampling of the vadose streams in the three caves using a net fitted with a 63µm mesh to enable more valid comparisons between the habitats.

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Supplementary information

Supplementary information relating to this Paper is available via the BCRA Website as an On-line Appendix (pp S1–S13) within the on-line instance of:

***Cave and Karst Science*, Volume 51, Number 2**.

The On-line Appendix comprises additional data tables:
[Table S1, Table S2, Table S3 and Table S4]
The tables are referenced within the main text above.

***Note** that the supplementary material is optimized primarily for on-screen viewing.*
Biota of percolating cave waters in Britain: a proxy for the epikarst aquifer