The impact of pollution on aquatic invertebrates within a subterranean ecosystem - out of sight out of mind

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Abstract

Pollution within a subterranean ecosystem caused gross staining of stream passages within a cave, although no visible impact was observed outside of the system. The source of the pollutant was identified in the surface catchment using water tracing experiments, and the direction and destination of water and pollutants within subterranean passages determined. Impacted sites and control sites were identified within the cave and compared to pre-disturbance data for the impacted cave and an adjacent system. The abundance and diversity of invertebrate taxa at impacted sites, downstream of the pollutant source, were significantly lower than in the pre-disturbance community and unaffected control sites.

Keywords:- subterranean ecosystem, cave, pollution, water tracing, invertebrates.

Introduction

The impacts of pollutants on aquatic invertebrates in groundwater dominated environments have been poorly studied. Acute and chronic toxicity tests have not been used in groundwater ecotoxicology, primarily due to the difficulties in culturing hypogean animals in an experimental design (NOTENBOOM et al., 1994). However, the interaction between the faunal communities within riverine aquifers and the adjacent surface waters have been demonstrated (e.g. BRETSCHKO, 1992; PLENET et al., 1996; MARMONIER et al., 2000). Caves, and the water which passes through them, provide opportunities to assess the impact of pollution on groundwater dominated ecosystems, although few studies have been undertaken to date (ELLIOTT, 2000).

Cave environments and the communities that occupy them are widely considered to be stable when compared to epigean systems (CULVER, 1985; WARD & PALMER 1994). The absence of autotrophic organisms means that heterotrophic organisms have to rely on food resources that are usually scarce in groundwater dominated habitats (GIBERT et al., 1994; POULSON & LAVOIE, 2000). Subterranean environments are typified by low abundances and diversities of organisms when compared to surface ecosystems (HOSLINGER, 1988). Due to isolation and evolutionary adaptation to cave environments some organisms are endemic to individual caves (CULVER et al., 2000; SARBU, 2000) and some have evolved unique feeding strategies and resistance to starvation in response to the limited food resources (CULVER 1985; HERVANT et al 1999; HÜPPOP, 2000). In addition, some diverse chemoautotrophically driven aquatic ecosystems have also been recorded, challenging historic views regarding trophic structures in the natural environment (SARBU et al., 1996).

Disturbance and pollution as a result of human activity in the surface catchments of subterranean systems can seriously degrade and threaten these fragile and often unique ecosystems (SIMON & BUIKEMA, 1997; TERCAFS, 1992). The vulnerability of cave ecosystems to disturbances, especially as a result of pollution from agricultural and industrial activities, has been widely noted (CULVER et al., 2000; GUNN et al., 2000) although little quantitative data exists (SIMON & BUIKEMA, 1997). This paper examines the aquatic invertebrate community before and after a severe pollution episode. Data are presented for impacted and unaffected (control) sites within Peak Cavern, Castleton, Derbyshire, and the adjacent Speedwell Cavern.

Study Site

The Peak-Speedwell Cavern system is the longest (c. 20 km) in the English Peak District and has the greatest vertical range of any cave in England (c. 290 m). The system has a history of development extending back at least one million years, longer than the majority of other British karst sites (WALTHAM et al., 1997), and its national importance is recognised by the designation as a Site of Special Scientific Interest - the Castleton Caves SSSI (Fig. 1).

Peak Cavern is fed largely by autogenic recharge (water that has only had contact with limestone bedrock and the overlying soil) from rainwater which infiltrates through the loessic soils of the limestone catchment, approximately 8.4 km^2 in area (HARDWICK, 1995). The waters percolate through, and are integrated in the unsaturated (vadose) zone before entering the cave via three main feeders, Far Sump (A), Ink Sump which feeds directly into Lake Passage (B) and Main Stream Inlet (C) (Fig. 2).

In contrast to Peak Cavern, Speedwell Cavern is largely fed by allogenic recharge (water that originates on non-limestone) from twelve streams that have their source on a Millstone Grit catchment of just over 5 km^2 (HARDWICK, 1995). The streams sink on, or shortly after, crossing onto the Carboniferous Limestone and flow through both open and flooded cave passages before entering Speedwell Cavern at two points, Main Rising and Whirlpool Rising (BOTTRELL & GUNN, 1991; GUNN, 1991) (Fig. 1).

The major landuse within both the autogenic (limestone) and allogenic (non-limestone) catchments is agriculture with livestock grazing on both improved and unimproved pasture. However, a large limestone quarry, which ceased to operate in 2000, accounts for 4.5% of the land-use in the autogenic catchment with a further 6% being land associated with former underground and surface lead mines (pits and waste tips), and surface extraction of vein minerals (HARDWICK, 1995).

The Pollution Episode

Between March and July 1999, cavers reported an orange-red staining in the water emerging from Ink Sump into Lake Passage (B) in Peak Cavern (Fig. 2). However, it is likely that the problem predates this since high water conditions during the winter and early spring prevented entry into this part of the cave prior to March. The water flows through the Peak Cavern system, and the main streamway was stained orange-red for approximately 1000 m downstream of Ink Sump. The area around the emerging water in Lake Passage was covered in a gelatinous biofilm that sloughed off when disturbed. Cave divers confirmed that the material was present in two sumps downstream, Buxton Water Sump (D) and the Peak Cavern Rising sump (upstream of F on Fig. 2). The latter is the source of the surface stream emerging from the cave, Peakshole Water. However, no visible evidence of the pollution was detectable in the watercourse outside of the cave. Detailed examination of the autogenic catchment suggested that the most likely source of the pollution was ‘agricultural material’ being stored on unimproved pasture prior to spreading on the land. This material was a mixture of paper pulp and filter plant sludge from the

production of paper mixed with organic rich peat from a water treatment works. Runoff from the material was similar in colour to that in Peak Cavern and was observed to enter and sink in an area of closed depressions close to Hollandtwine Mine (Fig. 2).

Methods

Following the identification of the potential source of the pollution an attempt to determine the direction, destination and rate of movement of runoff entering the depression was undertaken using water tracing compounds. The dye tracers used were fluorescein (CI 45350 Acid Yellow 73) and rhodamine WT (CI Acid Red 388). Since unequivocal results were required, relatively large quantities of dye, 1200 g fluorescein (2.0 L of solution at 60% weight/volume) and 500 g rhodamine WT (2.5 L of solution at 20% weight/volume) were injected into the depression and flushed with approximately 10900 L of water.

Monitoring of the water passing through the subterranean system was undertaken at underground sites in Peak Cavern (A, B, C, D and E), at the three risings from the system (F, G and H), Pindale Sough (I) (a sough is an old lead mine drainage level) close to the point where it discharges into the Peakshole Water, Kronstadt Sough (M), and four sites in the village of Bradwell (J, K, L and N), two of which are thought to discharge water from soughs (see Fig. 2 for details). Each site was monitored using activated charcoal detectors and water sampling. The raw water samples were analysed directly on a Hitachi F4500 luminescence spectrofluorimeter using a synchronous scanning method with a 20 nm separation between excitation and emission. The activated charcoal detectors were eluted and the resulting solution analysed using the same spectrofluorimeter.

The invertebrate community was sampled monthly over a 36-month period (January 1997 – December 1999; 26-months pre-disturbance and 10 months post-disturbance) from 21 sites (Fig. 1). Benthic invertebrates were sampled using a 0.05 m^2 cylinder sampler over a 30-second period disturbing the substratum to a depth of 5cm. In addition, larger substratum particles were inspected for attached fauna. Sampling could not be undertaken at all sites each month due to the flooding of some subterranean passages, particularly during the winter months (total n = 340). The sample sites encompassed 3 disturbed sites, downstream of the suspected contamination input (n = 52 pre- and 27 post-disturbance); 3 control sites, known to be hydrologically unconnected to the suspected contaminated water source (n = 52 pre- and 27 post disturbance); 6 control sites in Speedwell Cavern (n = 101 pre- and 54 post disturbance); and an additional 9 sites which were sampled at irregular intervals (n = 27). All specimens were preserved in the field and returned to the laboratory for sorting and identification to species level wherever possible.

To examine the impact of the pollution episode on the invertebrate community the abundance (individuals/m^2), the number of taxa per sample and the Shannon-Wiener diversity index were offered as independent variables within a one-way analysis of variance (ANOVA). Invertebrate samples from within Peak Cavern, prior to and following the disturbance, and for impacted and control sites were analysed to provide replication in space and time. In addition, samples from the unaffected adjacent cave system, Speedwell Cavern, were analysed to examine the differences between the two caves.

Water temperature (°C), conductivity (μS/cm), dissolved oxygen (mg/L) and pH were measured in the field using a portable YSI 600R water quality probe following the detection of the pollution within the Peak Cavern system. Replicate water samples collected from within the cave and from the springs were analysed for nitrate and phosphate concentrations. In addition, water samples were collected for microbial analysis in pre-sterilised polypropylene bottles. Samples were spread onto plates of yeast extract agar (YEA) and used to determine heterotrophic microbial concentrations. Plates were incubated at 22°C +/- 1°C for 72 hrs and at 37°C for 24 hrs (HMSO, 1994). The lower incubation temperature encourages the growth of soil and water micro-flora while the higher temperature provides an indication of bacteria that may be derived from human or other animal sources. Morphological, cultural and biochemical identification tests were carried out following standard methods (COLLINS et al., 1995).

Preliminary analysis of the physico-chemical characteristics of water for the entire sampling period indicated significant differences in nitrate (mean values: Peak Cavern 2.35 mg/L; Speedwell Cavern 3.0 mg/L; $P < 0.01$ ANOVA) and phosphate (mean values: Peak Cavern 0.63 mg/L; Speedwell Cavern 0.38 mg/L: $P < 0.01$ ANOVA) concentrations between the caves. However, no other significant differences between the autogenic water from Peak Cavern (mean values: water temperature 8.1°C; conductivity 320 μS/cm; dissolved oxygen 11.85 mg/L; pH 7.4) and the allogenic water from Speedwell Cavern (mean values: water temperature 8.1°C; conductivity 337 μS/cm; dissolved oxygen 10.82 mg/L; pH 7.3) were recorded. In addition, there were no significant differences between samples from pre- and post disturbance or control and impacted sites within Peak Cavern. Similarly, there were significant differences between the microbial characteristics of Peak Cavern and Speedwell Cavern, but not between control and impacted sites within Peak

Cavern. Heterotrophic plate counts of Colony Forming Units (CFUs/mL) were significantly lower in Peak Cavern at 22°C (mean annual values 2495-4268 CFUs/mL; P <0.001 ANOVA) and 37°C (92-475 CFUs mL^{-1}; P < 0.001 ANOVA) than in Speedwell Cavern (mean annual values 6855-26498 CFUs/mL at 22°C and 412-9961 CFUs/mL at 37°C). This clearly demonstrated that the pollution episode did not result in any medium to long-term detectable impacts on either water quality or the microbial community, and as a result this data was not used in any further analysis.

Results

The majority of the injected tracer, and hence any pollutant entering the depressions in the area of dye injection, followed two main routes. First, north-east, through Ink Sump into Lake Passage in the Peak Cavern system and secondly, east, through an unexplored subterranean conduit to three soughs in the Bradwell area (J, L and M in Fig. 2). In addition, relatively small volumes of the tracer solution targeted the upstream end of Far Sump (A) in Peak Cavern, Russet Well (H) - almost certainly via the streamway in Speedwell Cavern, and Pindale Sough (I). The travel time to Lake Passage (B) was 7-10 days giving a minimum velocity of 155-221 m/day. The travel time to A, and by inference areas upstream of Far Sump, was similar. The travel time to the soughs in Bradwell (J, L, and M) was also 7-10 days, but the distance was significantly greater giving a minimum velocity of 387-589 m/day. The minimum tracer velocity in the largely open, but partially flooded, passages between Lake Passage (B) and the Peak Cavern Rising (F) was 643 m/day. The maximum concentrations of fluorescein and rhodamine WT in water samples from the Peak Cavern Rising were 5143 and 2476 ng L^{-1} giving dilutions of 16,400 and 13,900 respectively. As the water from Lake Passage accounts for less than half of the discharge from Peak Cavern, it can be assumed that under the conditions that prevailed at

the time of the experiment any pollutant entering the depressions would be diluted by 7000-8000 times by the time it arrived at Lake Passage.

Altogether, 31 taxa were recorded in the subterranean passages of the Peak-Speedwell Cavern system (25 taxa in Speedwell and 21 taxa in Peak) (Table 1). The abundance of fauna in Peak Cavern prior to the pollution was typically lower (mean abundance = 287.8/m^2) than that of Speedwell Cavern (mean abundance = 432.2/m^2) (Fig. 3). Following the pollution, abundance was markedly reduced at impacted sites (mean abundance = 52.9/m^2), although it was comparable to pre-disturbance values at control sites (mean abundance = 275.1/m^2). One-way analysis of variance (ANOVA) clearly demonstrated differences between the abundance of invertebrates within Peak Cavern prior to and following the pollution episode (Table 2). Detailed examination of the invertebrate community indicated that the abundance, number of taxa and the Shannon-Wiener diversity index were significantly different prior to and following the disturbance at sites impacted by the pollution within Peak Cavern. In marked contrast, there were no significant differences recorded at unaffected control sites within Peak Cavern. In addition, no differences were recorded before and after the pollution episode within Speedwell Cavern, although there were significant differences between Peak Cavern and Speedwell Cavern throughout the study period (Table 2).

The aquatic invertebrate community at sites in Peak Cavern was dominated by Copepoda and Oligochaeta prior to the pollution (Table 1 and Fig. 4a). Following the pollution, the invertebrate community at impacted sites was almost entirely composed of Oligochaeta (Fig. 4b). In contrast, sites in Speedwell Cavern contained a more varied community throughout the sampling period including: Oligochaeta, Chironomidae

larvae, Copepoda and *Gammarus pulex* (Fig. 4c). Several taxa were abundant at individual sites within the respective caves:- *Pisidium nitidum* (Site 11 on Fig. 1), *Pisidium personatum* (both Bivalvia: Pisidiidae) (Site 2), *Gammarus pulex* (Amphipoda: Gammaridae) (Site 11; and Site 14 prior to the pollution) and *Hydroporus ferrugineus* (Coleoptera: Dytiscidae) (Site 3). In addition, *Thaumalea verralli* (Diptera: Thaumaleidae) were common and abundant in small trickles in the threshold zone of Peak Cavern (Site 10). The remaining taxa occurred infrequently and never contributed more than 5% of any sample.

The pollution episode had a marked impact on the number of taxa at sites hydrologically connected to the pollutant pathway compared to control sites (Table 2 and Fig. 4b). In March 1999, when the pollution was first detected, only dead specimens of the terrestrial Oligochaeta, *Lumbricus terrestris*, were recorded, stranded and decaying on exposed sediments at the margin of the streamway, at sites downstream of the pollution source. While *L. terrestris* were recorded in samples downstream of Ink Sump prior to the pollution, the increased numbers were the result of polluted water carrying them into the cave. Some 9 months after the first detection of the pollution, only three additional taxa were recorded at impacted sites (2 tubificid worms: *Limnodrilus hoffmeisteri, Tubifex tubifex* and the amphipod *G. pulex*), although abundance and community composition at control sites were comparable to the pre-disturbance community (Table 1 and Table 2). In contrast, the invertebrate community of the sites examined in Speedwell Cavern appeared to be unaffected by the pollution episode.

Discussion

Pollution of groundwater dominated ecosystems is a multifactorial problem that has been widely recognised as a potential threat to the sustainable utilisation of this important resource (NOTENBOOM et al., 1994; WATSON et al., 1997). Degradation of groundwater differs substantially from pollution of surface waters with respect to its occurrence and duration. The problems associated with the identification, measurement and prediction of pollution have stimulated considerable interest in the field of groundwater quality (MAYER et al., 1993; MALARD et al., 1996; FRITCH et al., 2000), although comparatively little data exist specifically for caves. One of the primary concerns associated with the contamination of groundwaters is that pollutants can pass into the soil or aquifer directly and/or from diffuse sources within the catchment unseen. It may take some time before it is possible to detect any impact, by which time severe degradation of the resource and ecosystem may have occurred, and it may take even longer to trace the source (GUNN, 1991; FELS, 1997).

Pollution incidents within cave environments frequently go undetected due to the difficulty of identifying the source of the pollutant. Within the Castleton karst, historic monitoring of the Peakshole Water identified degradation of the aquatic invertebrate community as a result of cypermethrin pollution from agricultural sources, primarily sheep dips, and highlights a long history of disturbance. An important aspect of the present investigation is that while clear impacts on the Peak Cavern streamway and invertebrate community have been demonstrated, the pollution would probably have gone unnoticed had it not been possible to gain access to the cave, and was only made possible due to an ongoing biological survey (WOOD & GUNN, 2000). Routine water sampling did not indicate the presence of any pollutant within the subterranean

ecosystem and without continuous monitoring it is unlikely that it would have been possible to do so, especially under high flow conditions.

Crustacea are frequently the numerically dominant aquatic faunal group in subterranean habitats (HOBBS, 2000) and formed a relatively large proportion of the invertebrate populations in both caves in the current investigation. Crustacea were absent from impacted sites for 9 months following the first detection of the pollutant, at which point *G. pulex* was recorded again. SIMON & BUIKEMA (1997) found that Amphipods and Isopods were absent from grossly polluted subterranean waters in Banners Corner Cave (Virginia: USA), demonstrating the vulnerability of these organisms to pollution and their value as indicator organisms in subterranean environments. Organic pollution potentially increases the volume of food resources available within subterranean ecosystems, although most troglobitic organisms are excluded since they are unable to compete with epigean fauna carried into caves (SKET, 1999) or groundwater aquifers (BOULAL et al., 1997) with pollutants. At those sites impacted by pollution in this study large numbers of *L. terrestris* were found dead at the margins of the streamway suggesting a toxic effect on some members of the invertebrate community. However, during the recover period two aquatic Oligochaeta (*Limnodrilus hoffmeisteri* and *Tubifex tubifex*: Tubificidae) were the first to re-colonise, and are widely known to be tolerant of organic pollution in surface waters (MASON, 1996). There are no known true stygobitic fauna within Peak or Speedwell Cavern, although the larvae of *Hydroporus ferrugineus* Stephens (Coleoptera: Dytiscidae) may be an obligatory subterranean life-stage that has only been recorded from these two caves (ALARIE et al., 2001). Sampling invertebrate communities in subterranean environments, and water emerging from springs and old mines (soughs), should be undertaken with care. Unnecessary and repeated disturbances

can severely degrade the subterranean environment and can lead to the extinction of individual cave populations (KNIGHT & WOOD, 2000).

Speedwell Cavern did not appear to experience any significant changes associated with the pollution episode, even though the water tracing experiment indicated that small volumes of the pollutant probably reached the cave. This reflects the rapid transfer of water from sinking streams carrying large numbers of epigean invertebrates and a wide range of organic material from the surface catchment into the cave (GUNN et al., 2000). The invertebrate community within Speedwell Cavern has adapted to this dynamic environment and is able to exploit short-term increases in organic material. In contrast, the percolation water within Peak Cavern typically carries a low organic content, and this fact accounts for the lower diversity and abundance of invertebrate taxa, and the lower microbial concentrations (CFUs/ml) recorded. The differences between the two caves resulting from allogenic and autogenic water clearly demonstrates the need to examine individual cave systems even over small spatial scales.

Conclusions

Caves and other subterranean environments are vulnerable to anthropogenic impacts within their surface catchments. The invertebrate community within Peak Cavern was seriously degraded by contaminated runoff from 'agricultural material' being stored in the surface catchment. Clear differences were recorded between samples before and after the detection of the pollution at impacted sites. There were no significant differences recorded at unaffected (control) sites suggesting the existence of refugia from which recolonisation and recovery could take place. The link between sinking streams and springs is relatively easy to demonstrate, although the pathways followed by

percolation water have been more difficult to determine historically. Water tracing experiments facilitated the identification of the pollution source within the Peak Cavern catchment. However, the detection of pollution in the majority of subterranean environments is rare since there is often no visible sign of the 'problem'. The pollution in the current investigation was clearly a point-source, with contaminated runoff entering well defined depressions. However, medium to long-term impacts may occur as a result of the spreading of material on the catchment surface and the diffuse entry of pollutants into groundwater dominated ecosystems (GOODDY et al., 2001). Recovery of the invertebrate community did not occur during the study period although ongoing research will specifically monitor it.

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List of Figures

Table 1. Invertebrate fauna recorded in Speedwell Cavern and Peak Cavern: prior to the pollution (pre-disturbance) and at impacted and control sites following the pollution episode.

	SPEEDWELL CAVERN	PEAK CAVERN (Pre-disturbance)	PEAK CAVERN (Impacted)	PEAK CAVERN (Control)
PLANARIIDAE				
Crenobia alpina	X			X
Phagocata vitta	X			
GASTROPODA				
Lymnaea peregra		X		X
BIVALVIA				
Pisidium nitidum		X		X
Pisidium personatum	X			
OLIGOCHAETA				
Aporrectodea rosea	X			
Enchytraeidae	X	X		X
Limnodrilus hoffmeisteri	X	X	X	X
Lumbriculus variegatus	X	X		
Lumbricus terrestris	X	X	X [a]	X
Spirosperma ferox	X	X		X
Stylodrilus sp.	X	X		
Tubifex tubifex	X	X	X	X
CRUSTACEA				
CLADOCERA				
Alona quadrangularis	X			
COPEPODA				
HARPACTICOIDA	X	X		X
CYCLOPOIDA				
Acanthocyclops bicuspidatus lubbocki		X		X
Acanthocyclops gigas	X			
Acanthocyclops venustus	X	X		X
Acanthocyclops vernalis	X	X		X
Acanthocyclops viridis	X			X
Eucyclops agilis		X		X
GAMMARIDAE				
Gammarus pulex	X	X	X [b]	X
EPHEMEROPTERA				
Baetidae	X			
COLEOPTERA				
Hydroporus ferrugineus	X	X		X
DIPTERA				
Chironomidae				
Chironominae				
Polypedilum sp.		X		
Orthocladiinae				
Brillia modesta	X			
Parametriocnemus stylatus	X			
Rheocricotopus fuscipes	X	X		X
Tanypodinae				
Thienemannimyia group	X			
Simuliidae	X			
Thaumaleidae				
Thaumalea verralli		X		X

a – all specimens of *Lumbricus terrestris* recorded during the 5 months following the detection of the pollution were dead; b - *Gammarus pulex* were recorded again at impacted sites 9 months after the detection of the pollution.

Table 2. One-way analysis of variance of: invertebrate abundance (individuals/m^2), number of taxa per sample and Shannon-Wiener diversity index, between (a) Peak Cavern and Speedwell Cavern, and (b) pre- and post pollution data.

	df	Abundance F	Taxa F	Shannon-Wiener F
a) Peak Vs. Speedwell Cavern:				
All samples - Peak and Speedwell Cavern	1	31.87***	21.37***	19.51***
Pre – pollution	1	26.53***	15.62***	12.87***
Post – pollution	1	36.22***	27.91***	22.16***
b) Pre-pollution Vs. post pollution:				
Peak Cavern – all sites	1	6.54*	4.88*	4.63**
Peak Cavern – control sites	1	0.65	0.27	0.11
Peak Cavern – impacted sites	1	70.22***	56.28***	47.81***
Speedwell Cavern – all sites	1	3.83	1.38	1.64

(*** = P <0.001; ** = P <0.01; * = P <0.05)

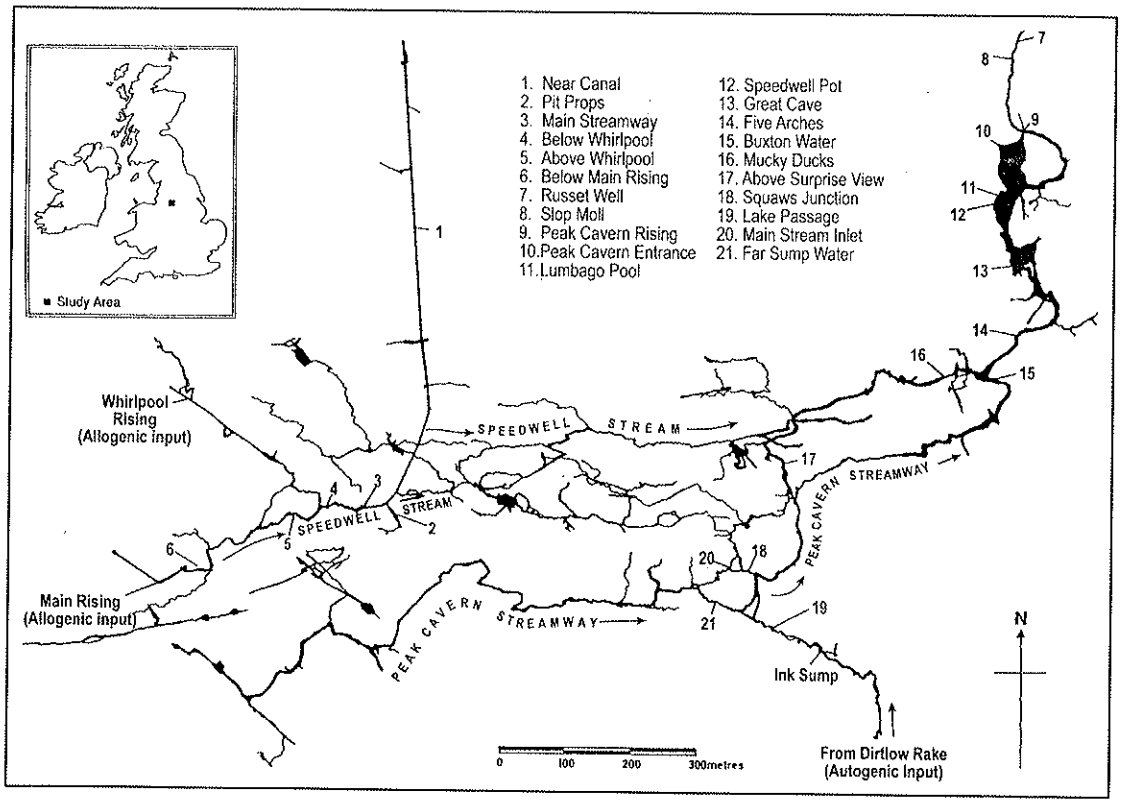

Fig. 1. The Peak – Speedwell cave system indicating the location of invertebrate sample sites (1–21) and additional points of interest. Arrows indicate streamflow direction.

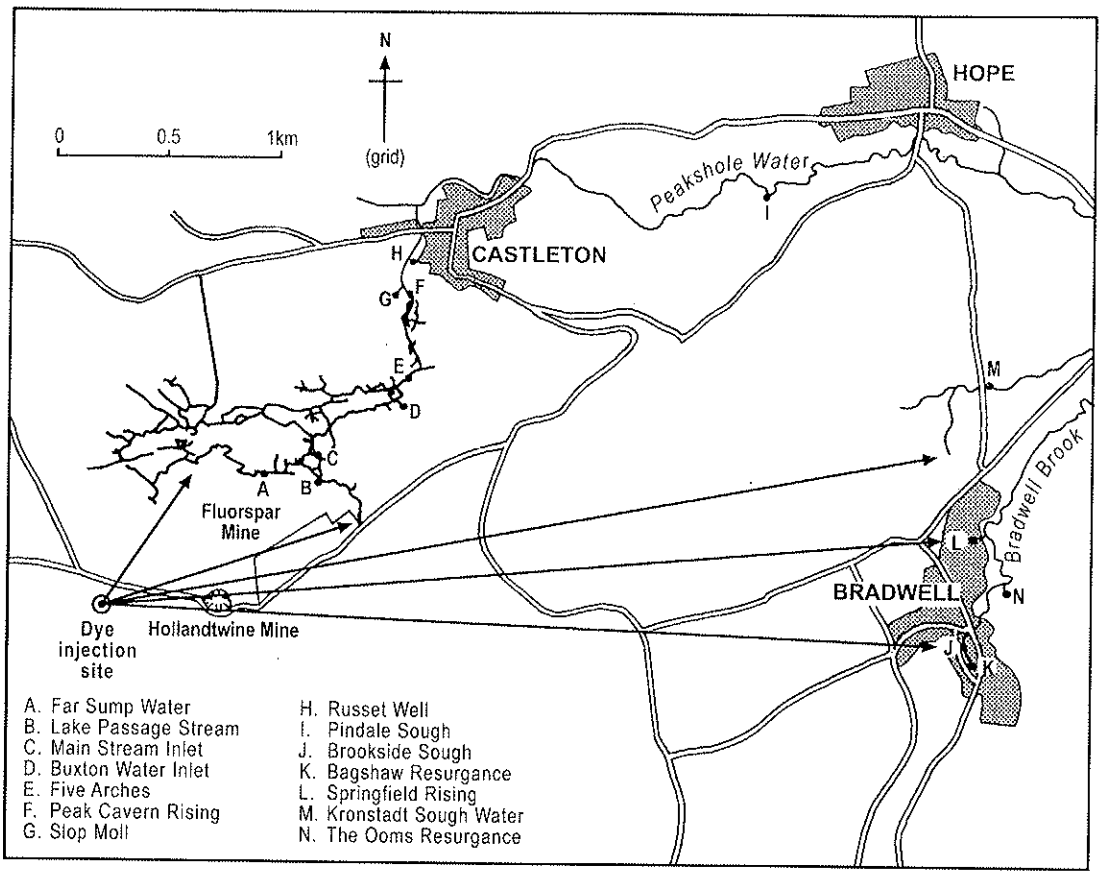

Fig. 2. Water tracing sample sites within the Peak – Speedwell Cavern system, indicating the dye injection point and location of charcoal detectors (A – N) within the caves and surrounding spring risings.

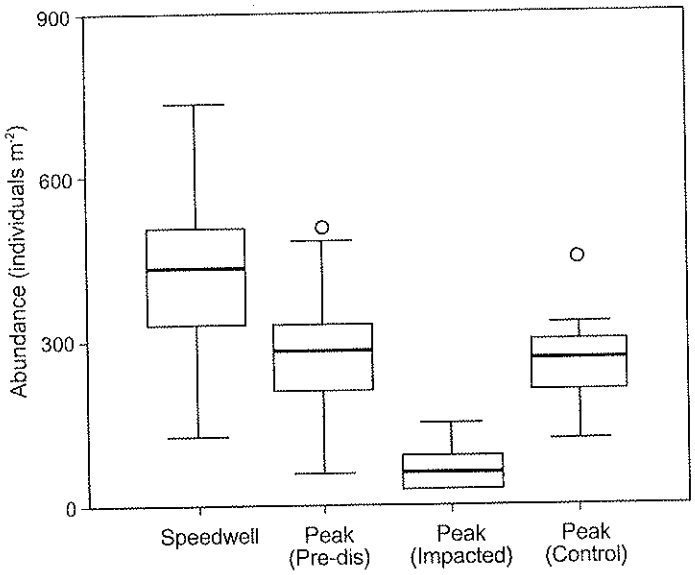

Fig. 3. Box-plot of invertebrate abundance (individuals/m^2) in Speedwell Cavern (all samples) and Peak Cavern: pre-disturbance, impacted and control sites. The solid line at the centre of the box indicates the median value, the box encapsulates the interquartile range and the outer lines span the range. O = outliers.

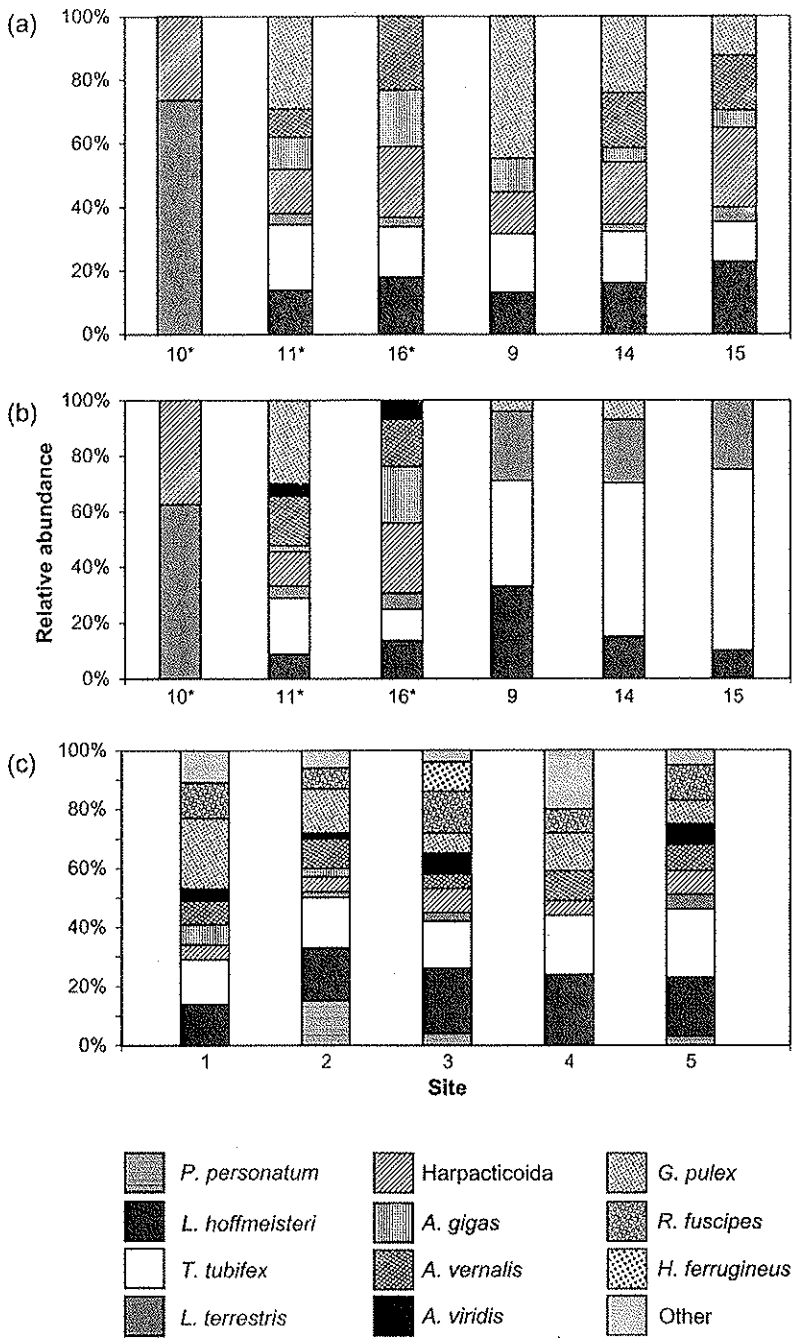

Fig. 4. Pattern of relative abundance of common invertebrate taxa at permanent sample sites in **(a)** Peak Cavern prior to the pollution episode (Jan 1997–Feb 1999), **(b)** Peak Cavern following the pollution episode (March 1999–Dec 1999); all specimens of *Lumbricus terrestris* recorded in the 5 months following the detection of the pollution were dead, and **(c)** sample sites in Speedwell Cavern (Jan 1997–Dec 1999). * Indicates control sites. Taxon abbreviations are as follows *Pisidium personatum, Limnodrilus hoffmeisteri, Tubifex tubifex, Lumbricus terrestris, Acanthocyclops gigas, Acanthocyclops vernalis, Acanthocyclops viridis, Gammarus pulex, Rheocricotopus fuscipes* and *Hydroporus ferrugineus*.