First In, Last Out: Should Aquifer Ecosystems be at the Vanguard of Remediation Assessment?

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ABSTRACT: Diverse assemblages of invertebrates are obligate inhabitants of groundwater which, with microbial and protozoan communities, occasionally vertebrates, comprise groundwater ecosystems. They are found to one kilometre depth in karstic, alluvial and fractured rock aquifers and may depend on allocthonous or chemoautotrophic energy sources, and may be found under oxic and anoxic conditions. Groundwater ecosystems may be comparable in diversity and dynamics to some surface ecosystems. The maintenance or recovery of groundwater ecosystems should be the goal of groundwater management and site remediation, as in surface ecosystems. Stygofauna may serve as sentinels for groundwater contamination and as indexes of restoration. They may influence restoration work directly through their physical activity or indirectly through their ecological interactions with microbial communities. By focussing on the ecosystem rather than the contaminant there are possible circumstances under which restoration may be contraindicated. It may be useful to include the status of groundwater as a contaminant.

KEYWORDS: groundwater ecosystems, invertebrates, stygofauna, sentinels, restoration.

INTRODUCTION

The potential role of invertebrates, largely crustaceans, is ignored in groundwater remediation studies. These, together with microbes and protozoa, comprise the aquifer ecosystems that are the first to be impacted by groundwater contamination, and may be the last to be relieved of its consequences. Hence, aquifer ecosystems, or their members, may serve both as an indicator of the onset of contamination and of their eventual remediation. Just as in surface ecosystems, should the restoration of aquifer ecosystems be the ultimate measure of groundwater remediation?

I present a brief background to groundwater ecosystems and discuss the possible utility of considering the restoration of groundwater ecosystems as a goal in remediation work, rather than the usual restricted scope of removing the contaminant(s), albeit sometimes utilising microbial means. While making reference to the key world literature, where possible I draw on indicative Australian studies and, for brevity, often cite only the volume in edited works.

SUBTERRANEAN ECOSYSTEMS

Life forms may occur several kilometres below the Earth's surface and this subterranean world is the largest terrestrial biome (Gold 1992). A specialised invertebrate fauna, occasionally vertebrates, occurs in these subterranean ecosystems, the health of which is paramount as the greater part of the world's freshwater occurs as groundwater. My references to groundwater ecosystems incorporates this wider view of subterranean ecosystem, rather than the microbial, occasionally protozoan, assemblages usually referred to in the context of remediation work. However, in some aquifer ecosystems only microbial elements will occur.

Animals are mostly restricted to the upper parts of subterranean ecosystems, that region commonly the target of remediation work. Nonetheless, a diverse stygofauna (Longley 1992) may be found up to at least one kilometre depth (Essafi *et al.* 1998); stygofauna is known in Australia from between 1 and 150 m depth (Humphreys 2000).

Subterranean waters are conveniently separated into groundwater and hyporheic waters that occur below river channels (Jones and Mulholland 2000) forming a broad ecotone between surface water and groundwater. Stygobites are the obligate inhabitants of groundwater, collectively comprising the stygofauna (Gibert *et al.* in Gibert *et al.* 1994). Stygobites have a convergent morphology—distinct from the vermiform tendencies of interstitial fauna—exhibiting a reduction or loss of eyes, pigments and hardened body parts (they are commonly translucent), and they have enhanced non-optic sense organs (Culver *et al.* 1995). Stygofauna tend to be slow-growing, long-lived and have few young, attributes that make them difficult to study but efficient bioaccumulators (Plenet *et. al.* 1992) and slow to recover from reductions in their populations. Groundwater ecosystems are not semi-deserts, occupied by rare, effete lineages, but are dynamic systems comparable in complexity to surface ecosystems (Rouch 1977, Gibert *et al.* 1994).

Groundwater ecosystems commonly comprise locally endemic species—species restricted to a small geographic area—and may include species belonging to ancient relictual lineages. In northwestern Australia stygal assemblages occur that largely comprise lineages known elsewhere only from groundwaters on either side of the North Atlantic—they are surviving Mesozoic communities (Humphreys 2000). Other stygofaunas comprise lineages distributed amongst fragments of the supercontinents (Poore and Humphreys 1998, Wilson and Keable 1999). Despite widespread relict geographical and phyletic lineages, there is also active colonisation of stygal habitats and some lineages may repeatedly enter or emerge from the stygal systems through evolutionary time (Culver *et al.* 1995).

Types and Locations of Groundwater Habitats

Groundwater ecosystems are of wide extent, occurring in karst, basalt, alluvial and in fractured rock matrices (Malard *et al.* 1996). Karst systems are typically of restricted extent, especially in Australia, and the contained stygal inhabitants may be confined to a particular cave or karst region (Wilkens *et al.* 2000). In contrast, alluvial aquifers may form a system of interconnected pathways that through time form a global pathway of dispersal (Stanford and Ward 1993), comparable to that proposed to follow the mid-oceanic ridges (Boxshall 1989).

Australia has the usual array of karstic and alluvial aquifers containing stygofauna (Humphreys 1999a, Hamilton-Smith and Eberhard in Wilkens *et al.* 2000). But, in addition, there are anchialine systems—near coastal groundwaters influenced by marine tides but without surface connection with the sea (Humphreys 1999b)—as well as novel faunas in both fresh and saline groundwater calcrete aquifers (Humphreys 1999a, in press).

Globally, aquifer ecosystems were best studied in karst regions of carbonate rocks which characteristically exhibit open conduit flow that causes the particular remediation problems associated with non-Darcian systems. Recent research has focussed on alluvial aquifer ecosystems, owing to their potential impacts on surface water and their perceived role in protecting groundwater from contaminants (Gibert *et al.* 1994, Jones and Mulholland 2000).

Anchialine systems exhibit sharp clines in temperature and salinity below which occur layers of hydrogen sulphide, sub- to anoxic conditions, chemoautotrophic energy production and a specialised fauna (Humphreys 1999b, Pohlman *et al.* in Wilkens *et al.* 2000) that is vulnerable if the stratification is disrupted. Remediation work would need to maintain the vertical stratification of the ecosystem and this requirement may generalise to any system exhibiting marked stratification, such as found in calcrete aquifers in the Australian arid zone (Watts and Humphreys in press, Humphreys in press).

Groundwater Ecology in Australia

In the late 19th century Charles Chilton, working mostly in New Zealand and Australia, was a global pioneer of groundwater biology (Hurley 1990). However, the groundwater fauna (stygofauna) of Australia, together with that of Africa, is now especially poorly known (Marmonier *et al.* 1997). Most work has been conducted on cave faunas (Hamilton-Smith and Eberhard in Wilkens *et al.* 2000, Humphreys 2000) and work other than in caves is recent (Humphreys 1999a, in press). A measure of the poverty of information can be drawn from Australia having changed within the last decade from being considered depauperate in stygofauna to being stygofauna rich. For example, Australia has the world's two most species rich stygal amphipod communities, Barrow Island (an anticline of Miocene limestone), and at Ethel Gorge, a groundwater calcrete deposit of Tertiary age on the Fortescue River. Furthermore, a small part of the Western Shield contains more species of stygal diving beetles (Dytiscidae) that the remainder of the world (e.g. Watts and Humphreys in press).

Apart from faunistic studies, there is meagre knowledge of aquifer ecosystems in Australia. Some research has been conducted on an anchialine system (Humphreys 1999b) and on hyporheic systems (Boulton in press), peripheral to groundwater ecosystems. However, even in the absence of detailed knowledge of Australian groundwater ecosystems, research elsewhere (e.g. Gibert *et al.* 1994), and general principles dictate that they provide 'ecosystem services' which, rather than endangered species, is the reason for maintaining biodiversity. Interference with groundwater ecosystem 'services' in the absence of knowledge of the associated processes and dynamics may involve the risk of destabilizing such systems.

Energy sources

Until recently subsurface microbial communities were thought to be supported by organic matter transported from the surface along flow paths or from organic matter deposited in sediments at their formation (Krumholz 2000). Over the last ten years microbial communities have been found in a wide variety of situations, often deep into the earths surface, and which are energetically dependent on non-traditional sources of energy (Table 1). Similarly, stygofauna has also been considered dependent on the downward percolation of energy fixed by the photosynthetic pathways of green plants which is processed by decomposers or trapped by the biofilm to form the foundation of the subterranean ecosystem. Recent work has shown that a variety of stygal ecosystems may also be dependent on energy derived *in situ* along chemoautotrophic pathways, being fixed, for example, by methanogens and sulphur bacteria. In the artesian Edwards Aquifer, Texas (Longley 1992), and in Movile Cave in Romania (Sarbu in Wilkens *et al.* 2000), sulphides, respectively of petroleum and magmatic origin, support chemoautotrophic ecosystems (Poulson and Lavoie in Wilkens *et al.* 2000). These ecosystems are analogous with those associated with both hydrothermal and cold deep sea vents (Gebruk *et al*. 1997). Chemoautotrophy has also been demonstrated in Frasassi Cave (Sarbu *et al.* in Wilkens *et al.* 2000), and strongly implicated in anchialine systems (Humphreys 1999b, Pohlman *et al.* in Wilkens *et al*. 2000).

Table 1. The location and energy sources of some subsurface microbial communities (Krumholz 2000).

Location	Energy source
1, Heterotrophic anaerobes	Permeable sandstone adjacent to organic-rich deposits (lignite

	rich Eocene sediments; organic rich Cretaceous shales and Cretaceous clays containing organic materials and fermentative bacteria.).
2, Igneous rock no organic source apparent.	Basalt and granite-rich subsurface (10^4-10^6 cells ml^{-1}; lithotrophic bacteria growing on chemically generated H_2.
3, Deep oceanic sediments	Anaerobic metabolism and sulphate reduction.

Groundwater ecosystems are generally energy poor and the addition of energy to the system can reduce the competitive advantage that stygofauna has under these oligotrophic conditions and permit the invasion of surface species (Malard *et al.* 1996). Contaminants may boost energy levels in groundwater and enhance microbiological (Haack and Bekins 2000) and probably macroinvertebrate productivity but excessive levels of organics, for example of sewage, may reduce populations (Sinton 1984). Although many contaminated sites are anaerobic as a result of bacterial utilization of oxygen (Haack and Bekins 2000), this need not preclude the presence of macroinvertebrates as many stygal species are adapted to extremely low, even anoxic conditions (Hervant *et al.* 1998, Humphreys 1999b).

Function role of Invertebrates

Recent studies suggest that the variability in subsurface geochemical and hydraulic conditions influence the sub-surface microbial community structure (Haack and Bekins 2000) in a manner comparable to that found in stygal communities (see Gibert *et al.* 1994, discussion in Humphreys 1999b, Watts and Humphreys in press).

By analogy with surface ecosystems, invertebrates have numerous potential function roles in groundwater systems, but as yet experimental evidence is largely lacking. These include the maintenance of voids — suggested also for protozoa (Haack and Bekins 2000) — the alteration of redox gradients, enhancing the release of organic carbon and the cycling of nutrients (which often limit bioremediation processes: Haack and Bekins 2000), promotion of biofilm activity (*inter alia* through grazing, nutrient cycling and improved hydraulic flow paths), the provision of favourable sites for microbial activity (see also Gebruk *et al.* 1997 below), the acceleration of cycling, movement and mass transfer of energy and materials through the sediments, alteration of population size and community structure through predation ("top-down" effects) and as prey ("bottom-up" effects). These issues are discussed and referenced more fully by Boulton (2000: Table II). While it is recognised that consortia of microorganisms can work, by mechanisms yet to be identified, to breakdown contaminant plumes (Haack and Bekins 2000), it has yet to be recognized that stygofauna may be expected similarly to interact with biofilms, and other expressions of microbial diversity, so as to promote or to restrict their activity and the community diversity in groundwaters.

Microbial Communities

Diverse microbial communities occur in groundwaters (Haack and Bekins 2000, Humphreys 1999b), and the complexity of these ecosystems is greatly enhanced by the presence of protozoans, which can reach densities of up to 10^4 g^{-1} in contaminant plumes. Predation by protozoans may control bacteria abundance (density protozoa: bacteria is 1: 10^3) and structure microbial communities (Haack and Bekins 2000). Even in deep granite aquifers sulphur bacteria may occur at densities of 10^4-10^6 cells ml^{-1} groundwater (Krumholz 2000.) but it is not known whether their replication rate is sufficient to support stygofauna.

Microbial communities in groundwater are influenced by the strong vertical and horizontal geochemical gradients in the groundwater (Haack and Bekins 2000), analogous to effect found in stygofaunal communities (Rouch and Danielopol 1997, Humphreys 1999b, Watts and Humphreys in press). These marked gradients require that the sampling intervals be determined at a scale appropriate to the gradients and sometimes this necessitates very fine scale spacing of sampling units (Humphreys 1999a). The presence of sharp gradients offers the potential for invertebrates (as well as microorganisms: *ibid*.) to sample across the interface into contaminant plumes otherwise inimical to life, even of utilizing microbial intermediaries, as occurs with some species utilizing the hot vent ecosystems of the deep ocean; interfaces so marked that many shrimps may be scarred from the scolding water (Gebruk *et al.* 1997).

Stygal and hyporheic communities may also change on a fine scale due, for example, to sometimes subtle changes in hydraulic conductivity, water flow characteristics or oxygenation (Rouch and Danielopol 1997). Such work on invertebrates reinforces the work on subsurface microorganisms that substantiates the need to understand the structure of both communities and populations in contaminant plumes in order to interpret degradation processes (Haack and Bekins 2000). As subsurface bacteria and stygofauna coexist they are subject to many of the same conditions and constraints, but, because the studies are rarely juxtaposed either physically or intellectually, there is little interplay between the relevant research workers.

Ecosystem Restoration and monitoring

In surface ecosystems, the restoration or maintenance of ecosystem functions, often measured as biodiversity, is a common goal — sometime a legal requirement — in environmental management. Many groups of animals are equally, even more, diverse in groundwater as in surface water (Rouch and Danielopol 1997, Sket 1999, Danielopol *et al*. 2000) so there is a strong argument to apply the same ultimate management goal to both surface and groundwaters. To achieve this goal understanding of the composition, distribution and processes of aquifer ecosystems needs to improve substantially.

In addition to serving as the standard to which remedial work should strive, intact groundwater ecosystems have other attributes of potential interest to those involved in groundwater remediation. Firstly, they may have a functional role in the remediation process, particularly through their interaction with microbial communities (see below). Secondly, they can serve as sentinels of change in groundwater ecosystems. For example, heavy metals can present a risk for human health and the aquatic environment. Interstitial and hypogean macroinvertebrates bioaccumulate heavy metals from sediments in a manner very responsive to water fluxes and can be used as sensitive sentinels to trace heavy metal pollution in aquifers (Plenet *et. al.* 1992). Groundwater fauna fit well with the sentinel system suggested by the Committee on Animals as Monitors of Environmental Hazards of the U.S. National Research Council (1991); they have a measurable response to the pollutants, they occur in the areas of the aquifer affected, the fauna is easily enumerated and sometimes the population size is adequate for sampling purposes. While the data were collected from the hyporheic zone the results can be extended to the aquifers (Plenet *et. al.* 1992).

Stygofauna lack 'resting' stages so their presence is evidence of permanent groundwater. It can be inferred from this permanence and the distribution of obligate stygal lineages between continents, that some groundwater communities have existed throughout geological eras (Humphreys 2000, Poore and Humphreys 1998). Hence, the loss of these communities, or elements of them, as a result of anthropogenic impacts would imply that the magnitude and/or rate of perturbation to these systems is greater than that experienced through these geological eras — sentinels indeed!

By focusing on the ecosystem, rather than the contaminant, there is scope for a range of circumstances in which the remediation of aquifer ecosystems may be unnecessary, even damaging. For example, remediation may destroy the halocline in salinity stratified waters and this is likely to disrupt anchialine ecosystems (Humphreys *et al.* 1999). Further, ecosystems dependent, for example, on sulphides of magmatic or petroleum origin for chemoautotrophic energy, or which are dependent on petrochemicals (see above), may not require remediation from some contaminants.

The status of groundwater—the level, direction and magnitude of flow—may have profound effects on the location and composition of subterranean populations (e.g. Rouch and Danielopol 1997, Watts and Humphreys in press). While these attributes could act synergistically with recognised contaminants, the attributes themselves can be contaminants when they fall outside normal variation as a result of anthropogenic agents. In which case, the status of groundwater may fall legitimately under the umbrella of remediation studies. The following trivial example suggests that these hydrological factors may result in profound change to stygal ecosystems and thus may be expected to influence the effects of contaminants (see also Gibert *et al.* 1994, Rouch and Danielopol 1997).

In the Australian arid zone, large spatial and temporal changes in water quality occur that result from the hydrogeochemical processes in the groundwater flow. Episodic rainfall, characteristic of the arid zone, may rapidly recharge calcrete aquifers causing the groundwater tables fluctuates widely, accompanied by large surface changes in salinity (Watts and Humphreys in press), and probably in oxygen, changes that may be expected to have important effects on biogeochemistry (Baker *et. al.* 2000), as well as stygofauna.

CONCLUSIONS

The widespread occurrence of groundwater invertebrate faunas suggests that the target for remediation work should be to restore the diversity and functioning of the intact groundwater ecosystem. Indeed, such faunas may provide a sensitive indicator of the presence of contamination and the success or otherwise of remediation. Under special circumstances there is the potential that remediation work itself could damage stygal ecosystems. Finally, it may be rewarding to investigate whether the endogenous communities of bacteria (Krumholz 2000) and macroinvertebrates (Sinton 1984) in groundwater can usefully be harnessed jointly to facilitate bioremediation.

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