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Protistan communities in aquifers: a review

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Abstract

Eukaryotic microorganisms (protists) are a very important component of microbial communities inhabiting groundwater aquifers. This is not unexpected when one considers that many protists feed heterotrophically, by means of either phagotrophy (bacterivory) or osmotrophy. Protistan numbers are usually low ($<10^2$ per g dw of aquifer material) in pristine, uncontaminated aquifers but may increase by several orders of magnitude in aquifers subject to organic pollution. Small flagellates (typically 2–3(5) μm in size in situ) are by far the dominant protists in aquifers, although amoebae and occasionally ciliates may also be present in much lower numbers. Although a wealth of new taxonomic information is waiting to be brought to light, interest in the identity of aquifer protists is not exclusively academic. If verified, the following hypotheses may prove to be important towards our understanding of the functioning of microbial communities in aquifers: (1) Differences in swimming behavior between species of flagellates lead to feeding heterogeneity and niche differentiation, implying that bacterivorous flagellates graze on different subsets of the bacterial community, and therefore play different roles in controlling bacterial densities. (2) Bacterivorous flagellates grazing on bacteria capable of degrading organic compounds have an indirect effect on the overall rates of biodegradation.

Keywords: Amoeba; Aquifer; Ecology; Flagellate; Groundwater; Protist; Protozoa; Subsurface

Contents

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1. Introduction

Protists are eukaryotic, single-celled microorganisms comprising groups commonly referred to as algae, protozoans and lower fungi. This paper will deal mostly with 'protozoan' protists, i.e. heterotrophic forms which enclose their food in membranous vacuoles. These protists occur in almost every habitat which provides them with an adequate food source and sufficient moisture. Many also have the ability to survive under desiccation and other unfavorable conditions by undergoing a reversible process of encystment.

In recent years the ecology of protists has been receiving an increasing amount of attention in a variety of habitats. Many protists are now known to be effective predators, feeding on bacteria or other microorganisms (including other protists), and therefore playing a key role in the so-called microbial loop [1]. Nonetheless, the study of protistan ecology is still much less advanced than, for instance, the ecology of terrestrial metazoans or plants. The current state of flux of protistan systematics and taxonomy could offer a partial explanation for this situation. Several contrasting classification schemes of protists have been proposed in recent years [2–5]. The number of protistan species described to date – estimated as 40 000 in the case of extant protozoan protists [6] – may represent but a small proportion of the actual total [6]. This view is supported by the fact that new taxa are constantly being discovered as new habitats are investigated [7–12]. In some groups, particularly the naked flagellates and naked amoebae, fundamental difficulties with observation and identification are brought about by the small cell size and the paucity of readily observable characters of taxonomic value.

On the whole the terrestrial subsurface is extremely poorly studied from the point of view of its characteristic protistan communities. In this respect caves [13–15] and soils [16–21] fare slightly better than aquifers, for which little is known about the distribution, identity, and ecological role of protists. This paper aims to give an overview of the current state of knowledge of protistan communities in aquifers as a basis for future developments in this area of research.

2. Historical overview

There appear to be only 20 publications or so dealing entirely or extensively with protists in aquifers. In the earliest observations known to us [22], in addition to a number of metazoans, 70 species of protists were reported in well waters in Prague, Czech Republic, 8 amoebae, 4 heliozoans, 3 sporozoans, 12 flagellates (including 1 autotroph), and 33 ciliates. A similar study was conducted a few years later in Lille, France [23], where several species of metazoans and 33 species of protists were found: 12 amoebae, 2 heliozoans, 4 flagellates (including 2 autotrophs), and 15 ciliates. Other similar investigations were carried out in Basel, Switzerland [24], where many metazoans and 11 species of protists were found: 5 amoebae, 1 (autotrophic) flagellate, and 5 ciliates; and also in other regions of continental Europe, where numerous metazoans and 56 spe-

cies of protists were found: 28 amoebae, 4 heliozoans, and 24 ciliates [25].

The early investigations are of great historical interest owing especially to the detailed taxonomic identifications. However, they were based on the examination of water samples pumped from pre-drilled wells, and the possibility of unwanted microbiological contamination during sampling cannot be ruled out. This casts a reasonable doubt on the extent to which the species found were representative of the true protistan communities in the aquifers. A step forward in this direction was taken during investigations on coastal groundwaters on Hiddensee island, Germany [26–28]. These studies relied not only on pumped water samples from pre-drilled wells but also freshly drilled core material. In addition to a number of metazoans, a total of 61 species of protists were found. All of these were ciliates, identified at least down to the genus level. A total of 10 ciliates were found exclusively in the pre-drilled wells, suggesting that the remaining 51 species were representative of the true aquifer communities.

Following a lack of studies lasting nearly 30 years, the basis for modern research on aquifer protists was provided by a study carried out in Segeberger Forest, Schleswig-Holstein, Germany [29]. Here, in addition to numerous morphotypes of bacteria and 8 fungi, 10 unnamed morphotypes of protists (including a flagellate, an amoeba and a ciliate illustrated using light micrographs) were found in samples obtained aseptically from 1 year old wells.

At about the same time, microbiological investigations on aquifers were becoming increasingly numerous [30–33]. Some studies on sediments from sandy aquifers suggested that protists could be absent from these environments [30–32]. However, a few years later evidence was provided for the presence of a range of protists in both the unsaturated and the saturated zones of a pristine (uncontaminated) site in Oklahoma, USA [34]. Except for one recent investigation documenting the presence of ciliates in pumped groundwater samples [35], subsequent studies have also adopted the approach of examining freshly drilled core material [36–42]. These studies have invariably reported the presence of protists (mostly flagellates and amoebae). The focus was mainly on the distribution and abundance of protists, and little taxonomic information was provided.

The most recent investigations, which have also adopted the general approach of examining freshly drilled cores of aquifer sediments, have been centered on an unconfined sand-and-gravel aquifer on Cape Cod, MA, USA, contaminated as a result of a 60 year discharge of treated sewage effluent [43]. This is the US Geological Survey Toxic Substances Hydrology Programme Research Site, informally referred to as the 'Cape Cod aquifer'. Information on the location and physicochemical characteristics of the aquifer has been summarized elsewhere [43]. Initially the focus was on quantitative aspects and transport behavior [44–47]. Recently, taxonomic information has also been provided on protists from the Cape Cod aquifer [43].

3. Methodology

3.1. Sampling

Sampling must be carried out in a way which minimizes the risk of microbiological contamination at all stages of sample collection and manipulation. When using water samples pumped from pre-drilled wells, the possibility should be borne in mind that non-indigenous microbes may have been introduced in the subsurface during drilling and well development [37,48]. However, samples may be suitable for microbiological analysis if adequate precautions are taken [49].

Groundwater samples may represent water collected over a large volume of the subsurface [49]. Should this be undesirable to the investigator, multi-level samplers (MLS) make it possible to collect groundwater samples from discrete zones [49]. The suitability of MLS for studying microbial populations that inhabit the sandy aquifer sediments that characterize the contaminant plume at the Cape Cod site has been demonstrated [50]. The MLS are installed by allowing the natural formation material to collapse around the samplers, thereby precluding the need for introduction of foreign material (e.g. bentonite) commonly used to seal the space between formation material and well casings. Because of the sharp chemical gradients within the contaminant plume, MLS have the advantage of sampling discretely at depth, unlike larger diameter observation

wells that are typically screened over considerable intervals. Also, the small diameter (6.5 mm) of the sampling tubes minimizes the physical and chemical disturbance to the groundwater habitat and allows pumping from the surface. This precludes the need for down-well pumps that can introduce atmospheric gases and surface-associated contaminants.

Drilled cores of aquifer material are necessary to sample aquifer microorganisms attached to sediment surfaces [49]. Ensuring that cores are microbiologically representative can be demanding because of inherent difficulties with the drilling process. Drilling through consolidated geological substrata requires the use of fluids to cool the drill bit, and these fluids may represent a source of contamination [49]. Dry drilling techniques are preferable since they do not require fluids; however, their applicability is generally limited to unconsolidated substrata such as sandy sediments [49].

During investigations on the Cape Cod aquifer [43,44,46] a dry drilling technique was used which minimizes the risk of contamination. A wireline piston core barrel [51] was used in conjunction with a hollow stem auger drill. To prevent contamination from the non-sterile core sleeve, sediment was removed from the central portion of the core; previous research [52] indicated that there was no significant difference between epifluorescence direct counts of protists in sediments collected in non-sterile and sterile core sleeves ($P = 0.05$). The core barrel was removed from the ground and the ends were immediately capped with plastic caps wiped with 95% ethanol. In order to minimize the risk of contamination, both ends (about 0.15 m) of each 1.5 m core were cut away and discarded immediately after collection. The barrel was wiped with 95% ethanol in the area to be cut, and a pipe cutter wiped with 95% ethanol was used to make all cuts. Plastic caps wiped with 95% ethanol were immediately placed on the ends of the core after cutting was complete, and secured with several wraps of electrical tape.

3.2. *Enumeration of protists*

Methods for enumerating protists fall into two broad categories: indirect (culture) and direct methods. The dilution culture method [53–55] in conjunction with the Most Probable Number (MPN) model [56] has been used in several investigations on aquifer protists [34,36,38–40,42]. It entails serial dilutions of samples in a culture medium which will encourage growth of the protists to be quantified. The concentration of protists in the original samples can be estimated statistically if the dilution ratios, the number of replicate cultures and the number of cultures with protistan growth are known. Concentrations of trophic versus encysted cells can also be estimated by comparing counts before and after acidification [53,54,57], although this procedure has attracted several criticisms [18,58] (see also Section 6). Another disadvantage of the dilution culture method is that the culture media and bacterial prey used may favor those species of protists well adapted to growing under those conditions, while they may be unsuitable or detrimental for other species [59]. For this reason, using bacteria not indigenous to the samples to be enumerated is undesirable. In the case of small protists a further disadvantage is that the cells to be counted may be difficult to detect visually during the examination of culture wells, which is routinely performed at low magnification.

Compared to culture methods, direct enumeration methods have the advantage of being less selective and a priori more representative because they estimate abundance in the original samples chemically fixed as soon as possible after collection. There are now well-established direct methods based on epifluorescence microscopy (see below), making it possible to enumerate a variety of microorganisms in fixed samples after staining them with suitable fluorochromes [60,61].

Epifluorescence direct counts of flagellates from the Cape Cod aquifer [44] required preliminary extractions of flagellates from core samples to maximize the recovery of surface-associated forms. Such extractions are desirable whenever sandy sediments are investigated for the presence of protists. Quantitative extractions were carried out using mechanical agitation of core material suspended in buffer in polyethene bags [52]. The supernatant was filtered on polycarbonate filters, stained with DAPI or primulin according to established procedures [60,61], and flagellates were enumerated. In the senior author's laboratory an extraction procedure based on Percoll density gradient centrifugation [62] was also tested on uniprotistan sediment-water cultures of an

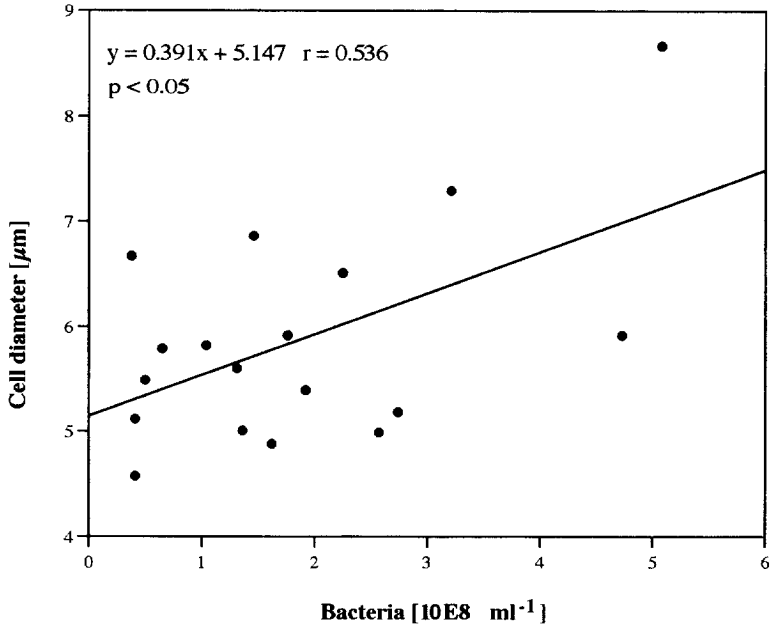
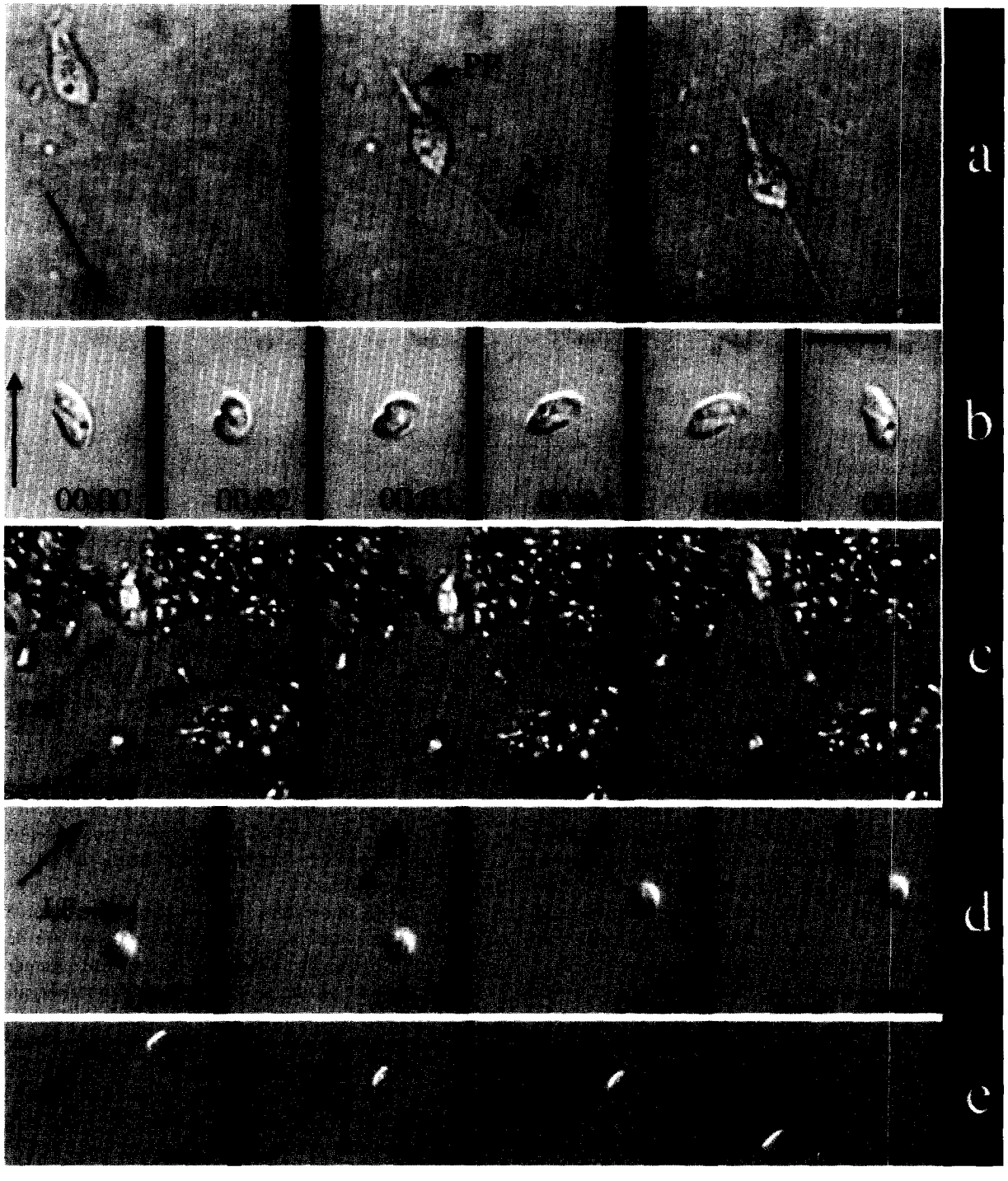


Fig. 1. Relationship between bacterial availability in culture and cell diameter (mean of 50 measurements) of an undescribed bacterivorous flagellate isolated from the Cape Cod aquifer.

hitherto undescribed flagellate from the Cape Cod aquifer. Unpublished results suggest that the maximum efficiency in separating and recovering flagellate cells from sediment particles is about 70–75%. Other applications of Percoll density gradient centrifugation to aquifer protists include buoyant density determinations to predict transport behavior [63].

In spite of their disadvantages, indirect counting methods are usually the only option for enumerating naked amoebae. These protists are often very small, translucent, and firmly attached to sediment particles, making them very difficult to enumerate by direct counting methods. Therefore, amoebae from Cape Cod were quantified using a modified version of the enrichment culture method [64], replicated using 3 different culture media. For each medium, a 10-fold dilution series was made of the aquifer sediment. Care was taken to resuspend the sediment grains before each dilution step. Aliquots of diluted sediment were inoculated into the wells of tissue culture plates containing culture medium. For each medium and each dilution, three replicate 24-well plates were used. For enumerating encysted amoebae, replicate plates were prepared using aquifer sediment which had been treated overnight with 2% HCl and neutralized with 2% NaOH [57]. All plates were incubated at 18°C in the dark and scanned after 1–2 weeks to check for growth of fast-growing amoebae. After 3–4 weeks incubation, amoebae were enumerated using an inverted phase contrast microscope at ×400 magnification. For each amoeba present in a well, it was assumed that at least one amoeba cell of the same species had been present in the initial aliquot of inoculum.

Although fluorescent rRNA binding probes in conjunction with epifluorescence microscopy are becoming increasingly widespread as tools for enumerating and identifying a wide range of microorganisms [65,66] they have not yet been applied to aquifer protists on a routine basis. During preliminary unpublished work carried out in the senior author's laboratory, a universal eukaryotic probe consisting of a 5′-fluorescein-labelled, 16 base long oligonucleotide (5′-GGGCATCACAGACCTG-3′) was used successfully on uniprotistan cultures of a novel, hitherto undescribed heterotrophic flagellate isolated from aquifer sediments from the Cape Cod site, and also on mixed (enrichment) cultures of



aquifer flagellates from Cape Cod. The probe only stained flagellate protists. Bacterial cells present in the cultures were unstained, and there was no non-specific binding of the probe to polycarbonate filters

Fig. 2. Swimming behavior at 20°C of flagellates from the Cape Cod aquifer, illustrated using digital film technology at 1/25th of a second. The time (bottom right-hand corner) is in seconds:frames. Arrows other than those pointing to morphological details indicate the direction of movement. Scale bars = 20 μm (a), 10 μm (b, c, e), or 5 μm (d). a: *Cercomonas* sp., creeping movement. PP = pseudopodium, AF = anterior flagellum. b: *Cryptaulaxoides vulgaris*, swimming freely. c: *Cryptaulaxoides vulgaris*, attached to sediment by means of the long (posterior) flagellum (LF) and then swimming away. d: *Spumella guttula* swimming freely along a circular track; LF = long flagellum. e: *Spumella elongata* swimming freely along straight tracks.
←

used for recovering flagellates. Counts were comparable to those obtained using conventional fluorochromes.

3.3. Cultivation

Cultures are very useful for microscopical observations but very few aquifer protists have been brought into culture to date. The section which follows is based on cultivation of flagellates from the Cape Cod aquifer [43].

Standard aseptic techniques were used at all stages of cultivation including handling of core samples, isolation and subculturing in a laminar flow cabinet. Enrichment cultures were prepared as soon as possible after the freshly drilled cores of aquifer material arrived at the laboratory. For this purpose, subsamples of core material of 0.1 g each were transferred to petri dishes containing 15 ml of soil extract medium with added minerals (50 ml soil extract, 200 mg KNO_3, 20 mg $K_2HPO_4 \cdot 3H_2O$, 20 mg $MgSO_4 \cdot 7H_2O$, distilled water to 1000 ml) or 1% w/v Cerophyl-Prescott's infusion medium [67]. Standard sterile techniques were used, and care was taken to ensure that the dishes were not inoculated with material from the outermost 2 cm of the cores. Uniprotistan, non bacteria-free cultures were obtained by using serial dilution-to-extinction or single-cell micropipetting techniques. Uniprotistan cultures were maintained on the same medium (soil extract or Cerophyl-Prescott) used for the enrichment cultures from which they had been isolated originally. All cultures were incubated in the dark at 18–21°C. In order to avoid selecting bacterivorous flagellates based on prey preference, no bacteria were added to the natural bacterial flora present in the enrichment cultures.

3.4. Observation

Aquifer protists are best observed using both light and electron microscopical techniques. Bright-field, differential interference contrast and phase-contrast light microscopy are all useful for observing live, unfixed cells. To characterize swimming behavior flash photomicrography is helpful but video recordings [68] and digital film technology [69] are preferable. The latter makes it possible to analyze movement on the computer at a time interval of 1/25th of a second using high-resolution, real-time digitizers which convert moving analogue video clips to computer files (Fig. 2). A prerequisite to the observation of swimming behavior is the availability of a temperature-controlled microscope stage because motion in protists may be affected by temperature.

Whole mounts for transmission electron microscopy can be prepared using standard procedures involving recovery of protists on coated grids, fixation with OsO_4 vapour and shadowing [43]. For scanning electron microscopy, the standard procedure of double fixation with glutaraldehyde and OsO_4 followed by chemical dehydration, critical point-drying and sputter-coating – as used for instance for marine and freshwater flagellates [70–73] – is adequate.

4. Taxonomic composition of protistan communities

While the early investigations [22–25,74] were essentially taxonomic accounts, the modern ones usually gave little taxonomic information, with one exception [43]. This is regrettable because identifying aquifer protists may yield much useful information not only taxonomically but also ecologically. Different species may differ in their ecological characteristics, and therefore play different roles in the functioning of aquifer ecosystems. If there are species which can be used as biological indicators or are potentially pathogenic, it becomes essential to identify them reliably. Reliable identifications are also essential to gain insight into relationships between diversity (in the sense of species richness) and organ-

ic contamination. Such relationships are very poorly understood not only in the case of protists, but also microorganisms in general [48]. In the presence of pollution microbial diversity often decreases [48]. By contrast, in the Cape Cod aquifer a higher diversity of flagellates and amoebae was observed in organically contaminated sites compared to pristine, uncontaminated sites [43]. It was hypothesized that the greater abundance and variety of food sources in the contaminant plume (bacteria, colloidal and dissolved organic matter) was capable of supporting a larger number of protistan species [43]. In order to test this hypothesis it will be necessary to investigate feeding modes in the various species of protists found, the flagellates in particular since all of the amoebae observed are bacterivorous.

4.1. Flagellates

Flagellates have been found in the majority if not all of the modern investigations, and therefore appear to be the most widespread protists in aquifers. This contrasts with the fact that there is just a single modern study dealing specifically with their taxonomic identity [43]. Flagellate species identified to date in the modern investigations are the following:

- bodonids: *Bodo edax, Bodo minimus, Bodo* sp. and a putative bodonid ([43], and Novarino, unpublished);
- cercomonads: *Cercomonas* spp. [34,43];
- chrysomonads: *Spumella elongata, Spumella guttula* [43];
- cryptomonads: *Goniomonas truncata* [43];
- pelobiontids: *Mastigamoeba/Mastigella* sp. (Novarino, unpublished);
- incertae sedis: *Cryptaulaxoides vulgaris* (as *Cryptaulax vulgaris*) and *Cyathomonas emarginata* [43].

Flagellate identification is based mainly on morphological characters. This poses difficulties owing to the small cell size, the small number of readily observable characters of taxonomic value and the variable quality of original taxonomic descriptions, most of which date back to the pre-electron microscope era. Further difficulties are caused by the so-called ambiregnal issues, whereby the nomenclature of many flagellates is regulated both by the Zoological and Botanical Codes of Nomenclature [75–77]; and the fact that many flagellate species may still be awaiting discovery [6].

Morphological characters used to identify flagellates from the Cape Cod aquifer [43] included the following: cell size, cell shape, ability to form pseudopodia, flagellar ornamentation and relative flagellar length, presence and position of contractile vacuoles, presence or absence of ejectile organelles, and presence or absence of scales. Different combinations of characters were used for different species [78]. Only a few species were identified because most appear to be new [43].

Flagellates enumerated from Cape Cod sediment cores were about 2–3(5) μm in size [44], while flagellates identified in laboratory cultures reached about 10 μm [43]. In bacterivorous flagellates such size differences may be related to the scarce availability of bacteria in the aquifer, the typical free-living bacteria:protists ratio in the aquifer being low, i.e. 10^0–10^2 [46]. This idea is consistent with the fact that in laboratory cultures the size of bacterivorous flagellate cells may increase with increased food availability (Fig. 1). This prompts for some caution in using size as a taxonomic character. In the course of unpublished work carried out in the senior author's laboratory, it was observed that cell shape may also be affected by food availability.

In accordance with existing information [68,79], behavioral features such as swimming modes were also found to be useful for taxonomic purposes [43]. Thus the morphologically similar chrysomonads *Spumella elongata* and *Spumella guttula* have different swimming tracks: *S. elongata* swims along short straight lines with frequent changes of direction, while *S. guttula* swims along circular lines (Fig. 2). Another behavioral difference between these species is that *S. guttula* is capable of temporary attachment to solid surfaces by means of a posterior protoplasmic filament, whereas *S. elongata* appears incapable of doing so [43].

Flagellates from the Cape Cod aquifer can be subdivided into three broad categories based on swimming behavior and movement:

1. Creeping flagellates (e.g. euglenids, *Cercomonas*, *Mastigamoeba/Mastigella*), mostly as-

sociated with solid surfaces, they are often able to produce pseudopodia and exhibit amoeboid movements;
2. Flagellates swimming actively in the pore-water and never or rarely attached to solid surfaces (e.g. *Spumella elongata*, some bodonids); and
3. Flagellates which can not only swim actively in the pore-water but are also capable of temporary attachment to solid surfaces (e.g. *Spumella guttula, Cryptaulaxoides/Cryptaulax vulgaris*).

Examples of swimming behavior are illustrated in Fig. 2, obtained using digital film technology [69].

4.2. Amoebae

Amoebae have been reported in a high proportion of modern studies [29,34,38–42], but little taxonomic information has been provided. One study reported a new family of the order Aconchulinida, although a formal diagnosis was not given [34]. In the Cape Cod aquifer, naked amoebae belonging to genera *Acanthamoeba* Volkonsky, *Hartmannella* Alexéieff emend. Page, *Mayorella* Schaeffer, *Rosculus* Hawes, *Vahlkampfia* Chatton and Lalung-Bonnaire, and *Vannella* Bovee or *Platyamoeba* Page were found in addition to a few unidentified forms ([43], and Butler, unpublished). Characters used for identification were those adopted in a widely used taxonomic key [67].

4.3. Ciliates

Ciliates were found only in a small minority of modern studies [29,35,42]. Taxonomic information was provided in one study where 38 species were found, with the dominant six species belonging to the genera *Acineria* Dujardin, *Aspidisca* Ehrenberg, *Cinetochilum* Perty, *Colpidium* Stein, *Glaucoma* Ehrenberg, and *Holosticha* Wrzesniowski [35].

Ciliates were not found in the Cape Cod aquifer [43]. This is believed to reflect their inability to be transported [43] as a result of their typical cell size being much greater than optimal size for particle transport through the aquifer sediments. Flow-through column experiments with microspheres [80] and small-scale in situ transport experiments with 2–6 μm flagellates from the aquifer [47] showed that the optimal size for particle transport through the Cape Cod aquifer sediments is ~2 μm. Very high rates of immobilization for ciliate-sized (≥10 μm) protists can be predicted for the Cape Cod aquifer sediments based on colloidal-filtration theory [81]. Furthermore, straining would preclude advective movement of protists larger than 20 μm [82].

4.4. Autotrophs and other protists

Very little is known about the significance of autotrophic protists in aquifers. Autotrophic protists and algae (unidentified forms of autotrophic flagellates, green algae and diatoms) were found in deep subsurface sediments [38]. Since the investigated sites were likely to be influenced by surface recharge and streams cutting into the upper formations, the origin of autotrophs was possibly attributed to surface populations moving into the permeable subsurface formations [38].

A dozen or so species of autotrophic protists and algae (euglenids, green algae and diatoms, most of which were identified at least down to the genus level) were found in water samples from the Edwards Aquifer, Texas, USA [83]. Here again, their presence was attributed to surface recharge importation [83].

Heliozoans [22,23,25] and sporozoans [22] were found during early investigations. However, their presence has not been reported since (with the exception of the well-known occurrence of *Cryptosporidium* oocysts), and their significance is virtually unknown.

5. Quantitative occurrence

Despite their common occurrence in aquifers, protists are present in very low numbers at pristine sites, with concentrations ranging from $< 10^0$ to 10^2 cells per g dw of aquifer material [41]. By contrast, elevated numbers of protists (up to 10^5 per g dw of aquifer material) have been recorded in at least three organically contaminated sites [40,42,44]. However, individual results may not be directly comparable because different enumeration techniques were used, i.e. the dilution culture method [40,42] and epifluorescence direct counts [44].

The most common protists in aquifers are flagellates and amoebae. Numerically, flagellates are generally dominant. In particular, in the Cape Cod aquifer counts of amoebae in both uncontaminated and organically contaminated sites were low relative to the number of flagellates and were in the order of 10^{-1} to 10^{0} amoebae per g dw of aquifer material (Butler, unpublished). These results are consistent with those reported previously for the same two sampling sites [44]. In the Cape Cod aquifer, the much lower numbers of amoebae compared to flagellates could be partly explained by hypothesizing that surface-associated flagellates (e.g. *Cercomonas*, *Mastigamoeba*/*Mastigella*) are out-competing the amoebae for surface-associated bacterial prey.

In an aquifer contaminated by aviation fuel elevated numbers of amoebae were reported in areas of contamination and where biotreatment was occurring [42]. The abundance of amoebae was roughly equal to that of the flagellates [42], reflecting perhaps the preference of these organisms for the culture conditions used during enumeration. In contrast to the results obtained on the Cape Cod aquifer (Butler, unpublished), the numbers of amoebae were roughly equal to numbers of flagellates in both contaminated and uncontaminated parts of the aquifer [42].

6. Encystment

Many protists are able to survive desiccation and other unfavorable conditions by undergoing a reversible process of encystment [84]. Cysts are known to occur in aquifer flagellates and amoebae ([78], and Butler, unpublished), although their quantitative importance in situ relative to trophic (active) cells is still unclear. Previous investigations reported that 3–40% of the flagellate population in the Cape Cod aquifer was encysted, and at the majority of sites $>70\%$ of organisms were in an active, trophic state [46]. Further observations (Butler, unpublished) have also suggested that amoebae populations from the Cape Cod aquifer are in an active rather than an encysted state. It is possible, however, that acid treatment may inactivate cysts resulting in unreliable estimates of the relative numbers of encysted and trophic cells [18,58].

7. Hypotheses on niche differentiation

The ecological principle of competitive exclusion [85] implies that for two or more species to coexist in the same environment their respective ecological niches must be differentiated. In the protists this issue is relatively poorly understood. In the case of autotrophic planktonic protists it has led to the well-known concept of the 'paradox of plankton' – numerous species coexisting in an apparently unstructured environment, all of which compete for the same sort of materials [86]. The three dimensions of niche space – food, habitat, and time – in protozoan protists have been discussed and illustrated in the literature [87].

It is hypothesized here that the mechanisms by which niche differentiation is achieved in aquifer flagellates include feeding heterogeneity. Phagotrophic flagellates could exploit different subsets of the bacterial community as a source of food, and therefore play different roles in controlling bacterial densities. The mechanisms by which this is achieved may include differences in flagellate swimming behavior. Creeping flagellates are best suited to ingesting bacteria loosely associated with sediment particles. Flagellates capable of swimming actively in the pore-water are more likely to intercept unattached bacteria directly. Certain actively swimming flagellates may attach temporarily to solid surfaces, and this could give rise to an increase in the efficiency with which they feed on unattached bacteria [88].

The hypothesis is in agreement with experimental work on flagellates from environments other than aquifers, showing that surface-associated species mostly graze attached/aggregated bacteria but not unattached bacteria, whereas the opposite is true for freely swimming species [89,90]. It is also consistent with the following observations:

1. Interspecific differences in swimming behavior appear to be constant under equivalent conditions [43].
2. Several species of flagellates found in aquifers are known, or believed to be, bacterivorous [43].
3. Bacteria in aquifers may live in association with sediment particles or in the pore-water [91–93].

Niche differentiation based on feeding heterogeneity could also be achieved by adopting osmotrophic rather than phagotrophic nutrition. Bacterivory has not been observed in all of the flagellate species from the Cape Cod aquifer [43]. Furthermore, in the Cape Cod aquifer the typical free-living bacteria:protists ratio is low, i.e. 10^0–10^2 [46]. In agreement with the possibility that numbers of heterotrophic flagellates may not always correlate strongly with bacterial numbers [94], these observations have led to the hypothesis that at least some flagellates in the Cape Cod aquifer may be relying on alternative or additional food sources e.g. colloidal and dissolved organic matter [43]. This needs to be evaluated against the generally accepted view that osmotrophic nutrition in free-living protozoan protists is unlikely to have a significant nutritive role [87].

8. Geographical distribution

Modern studies on aquifer protists have been carried out mostly in North America [34,36,38–47,78], so it is difficult to comment on geographical distribution. However, in the senior author's laboratory work is in progress on the isolation and characterization of flagellates from aquifer material collected by two of us (R.A.M. and B.T.) in various locations in Israel. Preliminary unpublished observations have shown that several flagellates found in the Cape Cod aquifer are also present in material from Israel. This suggests a possible cosmopolitan distribution, in agreement with analogous findings on a wide range of freshwater and marine flagellates [95].

9. Conclusions

Our knowledge of protistan communities in aquifers is at an early stage. Nonetheless, some important conclusions can be derived already:

1. Protistan communities in aquifers are usually dominated by heterotrophic flagellates.
2. Aquifer flagellates are typically small in size: 2–3(5) μm in situ, up to about 10 μm in laboratory cultures. The size of bacterivorous flagellates may be influenced by food availability.
3. Differences in swimming behavior can be detected between different species of aquifer flagellates.
4. Population densities are usually low ($< 10^2$ cells per g dw of aquifer material) in pristine, uncontaminated sites but may increase by several orders of magnitude in the presence of organic pollutants.
5. Diversity (in the sense of species richness) appears to be higher in the presence of organic pollutants.

Given that flagellates are the most important aquifer protists, we feel that the following hypotheses could represent a basis for future developments in this area of research:

1. Differences in swimming behavior between species of flagellates lead to feeding heterogeneity and niche differentiation. This implies that bacterivorous flagellates graze on different subsets of the bacterial community, and therefore play different roles in controlling bacterial densities.
2. Bacterivorous flagellates grazing on bacteria capable of degrading organic compounds could have an indirect effect on the overall rates of biodegradation.

There are several other topics in need of urgent attention, particularly the relative importance of encysted versus trophic cells, and the relationships between diversity and organic pollution. Progress in all of these areas will depend ultimately on the development of suitable quantitative methods and improved taxonomy, making it possible for aquifer protists to be enumerated and identified simultaneously. As methodology progresses, aquifer protists will undoubtedly offer many exciting opportunities for pure and applied research well into the next Millennium.

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References

[1] Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. and Thingstad, F. (1983) The ecological role of water-column microbes in the sea. Mar. Ecol. Progr. Ser. 10, 257–263.

[2] Margulis, L., Corliss, J.O., Melkonian, M. and Chapman, D.J. (1990) Handbook of Protoctista. Jones and Bartlett, Boston, MA.

[3] Cavalier-Smith, T. (1993) Kingdom Protozoa and its 18 phyla. Microbiol. Rev. 57, 953–994.

[4] Corliss, J.O. (1994) An interim utilitarian (user-friendly) hierarchical classification and characterization of the protists. Acta Protozool. 33, 1–51.

[5] Patterson, D.J. (1993) Protozoa: evolution and systematics. In: Progress in Protozoology – Proceedings of the IX International Congress of Protozoology, Berlin, 25–31 July 1993 (Hausmann, K. and Hülsmann, N., Eds.), pp. 1–14. Gustav Fischer Verlag, Stuttgart.

[6] Vickerman, K. (1992) The diversity and ecological significance of Protozoa. Biodiv. Con. 1, 334–341.

[7] Foissner, W. (1993) *Corticocolpoda kaneshiroae* n.g., n. sp., a new colpodid ciliate (Protozoa, Ciliophora) from the bark of Ohia trees in Hawaii. J. Eukar. Microbiol. 40, 764–775.

[8] Foissner, W. (1995) Tropical protozoan diversity – 80 ciliate species (Protozoa, Ciliophora) in a soil sample from a tropical dry forest of Costa Rica, with descriptions of 4 new genera and 7 new species. Arch. Protistenk. 145, 37–79.

[9] Foissner, W. (1996) Faunistics, taxonomy and ecology of moss and soil ciliates (Protozoa, Ciliophora) from Antarctica, with description of new species, including *Pleuroplitoides smithi* Gen. N., Sp. N. Acta Protozool. 35, 95–123.

[10] Fenchel, T., Bernard, C., Esteban, G., Finlay, B.J., Hansen, P.J. and Iversen, N. (1995) Microbial diversity and activity in a Danish fjord with anoxic deep-water. Ophelia 43, 45–100.

[11] Petz, W. (1994) Morphology and morphogenesis of *Strombidium kryalis* nov. spec. (Ciliophora, Strombidiida) from Antarctic sea-ice. Arch. Protistenk. 144, 185–195.

[12] Petz, W. (1995) Morphology and morphogenesis of *Thigmokeronopsis antarctica* Nov. Spec. and *T. crystallis* Nov. Spec. (Ciliophora, Hypotrichida) from Antarctic sea-ice. Eur. J. Protistol. 31, 137–147.

[13] Gittleson, S.M. and Hoover, R.C. (1969) Cavernicolous Protozoa: review of the literature and new studies in Mammoth Cave, Kentucky. Ann. Spéléol. 24, 737–767.

[14] Delhez, F. and Chardez, D. (1970) Protozoaires des grottes de Belgique. Ann. Spéléol. 25, 107–137.

[15] Gittleson, S.M. and Hoover, R.C. (1970) Protozoa of undergroundwater in caves. Ann. Spéléol. 25, 91–106.

[16] Sandon, H. (1927) The Composition and Distribution of the Protozoan Fauna of the Soil. Oliver and Boyd, Edinburgh.

[17] Stout, J.D. (1967) Protozoa. In: Soil Biology (Burges, A., Ed.), pp. 149–195. Academic Press, New York.

[18] Foissner, W. (1987) Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. In: Progress in Protistology (Corliss, J.O. and Patterson, D.J., Eds.), Vol. 2, pp. 69–212. Biopress, Bristol.

[19] Ekelund, F. and Ronn, R. (1994) Notes on Protozoa in agricultural soil with emphasis on heterotrophic flagellates and naked amebas and their ecology. FEMS Microbiol. Rev. 15, 321–353.

[20] Foissner, W. and Wanner, M. (1995) Protozoa of soil. In: Protistological Actualities (Proceedings of the Second European Congress of Protistology Clermont-Ferrand, 1995) (Brugerolle, G. and Mignot, J.-P., Eds.), pp. 128–135. Imprimeries Couty, Clermont-Ferrand.

[21] Darbyshire, J.F. (Ed.) (1994) Soil Protozoa. CAB International, Wallingford.

[22] Vejdovsky, F. (1882) Tierische Organismen in den Brunnewässern von Prag. Selbstverlag, in Commission von Franz Rivnac, Prague.

[23] Moniez, R. (1889) La faune des eaux souterraines du Département du Nord et en particulier de la ville de Lille. Rev. Biol. Nord Fr. 1, 81–94.

[24] Chappuis, P.A. (1924) Die Fauna der unterirdischen Gewässer der Umgebung von Basel. Arch. Hydrobiol. 14, 1–88.

[25] Spandl, H. (1926) Die Tierwelt der unterirdischen Gewasser. Speläol. Monogr. 11, 1–235.

[26] Münch, H.-D. and Petzold, H.-G. (1956) Zur Fauna des Küstengrundwassers der Insel Hiddensee. I. Chemisch-physikalische Verhältnisse und ihr Einfluß auf die Grundwasserfauna. Wissensch. Zeit. Ernst Moritz Arndt-Univ. Greifswald, Math.-naturwissensch. Reihe 5, 413–429.

[27] Petzold, H.-G. (1956) Zur Fauna des Küstengrundwassers der Insel Hiddensee. II. Holotriche Ciliaten, Nematoden und Gastrotrichen aus dem Küstengrundwasser. Wissensch. Zeit. Ernst Moritz Arndt-Univ. Greifswald, Math.-naturwissensch. Reihe 5, 429–433.

[28] Münch, H.-D. (1956) Zur Fauna des Küstengrundwassers der Insel Hiddensee. III. Spirotriche und peritriche Ciliaten, Rotatorien und Archianneliden aus dem Küstengrundwasser.

Wissensch. Zeit. Ernst Moritz Arndt-Univ. Greifswald, Math.-naturwissensch. Reihe 5, 433–436.
[29] Hirsch, P. and Rades-Rohkohl, E. (1983) Microbial diversity in a groundwater aquifer in Northern Germany. Dev. Ind. Microbiol. 24, 183–200.
[30] Wilson, J.T., McNabb, D.L., Balkwill, D.L. and Ghiorse, W.C. (1983) Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. Ground Water 21, 134–142.
[31] Ghiorse, W.C. and Balkwill, D.L. (1983) Enumeration and morphological characterization of bacteria indigenous to subsurface environments. Dev. Ind. Microbiol. 24, 213–224.
[32] Harvey, R.W., Smith, R.L. and George, L.H. (1984) Effect of organic contamination upon microbial distributions and heterotrophic uptake in a Cape Cod, Mass., aquifer. Appl. Environ. Microbiol. 48, 1197–1202.
[33] Beeman, R.E. and Suflita, J.M. (1987) Microbial ecology of a shallow unconfined ground water aquifer polluted by municipal landfill leachate. Microb. Ecol. 14, 39–54.
[34] Sinclair, J.L. and Ghiorse, W.C. (1987) Distribution of Protozoa in subsurface sediments of a pristine groundwater study site in Oklahoma. Appl. Environ. Microbiol. 53, 1157–1163.
[35] Foissner, W. (1996) Ciliates in rapid gravity filters of waterworks exploiting deep groundwaters. Microsc. Res. Tech. 33, 12–22.
[36] Beloin, R.M., Sinclair J.L. and Ghiorse, W.C. (1988) Distribution and activity of microorganisms in subsurface sediments of a pristine study site in Oklahoma. Microb. Ecol. 16, 85–97.
[37] Ghiorse, W.C. and Wilson, J.T. (1988) Microbial ecology of the terrestrial subsurface. Adv. Appl. Microbiol. 33, 107–172.
[38] Sinclair, J.L. and Ghiorse, W.C. (1989) Distribution of aerobic Bacteria, Protozoa, Algae, and Fungi in deep subsurface sediments. Geomicrobiol. J. 7, 15–31.
[39] Sinclair, J.L., Randtke, S.J., Denne, J.E., Hathaway, L.R. and Ghiorse, W.C. (1990) Survey of microbial populations in buried-valley aquifer sediments from northeastern Kansas. Ground Water 28, 369–377.
[40] Madsen, E.L., Sinclair, J.L. and Ghiorse, W.C. (1991) In situ biodegradation: microbiological patterns in a contaminated aquifer. Science 252, 830–833.
[41] Sinclair, J.L. (1991) Eukaryotic microorganisms in subsurface environments. In: Proceedings of the First International Symposium on Microbiology of the Deep Subsurface, 15–19 Jan. 1990, Orlando, Florida (Fliermans, C.B. and Hazen, T.C., Eds.), pp. 3-39–3-51. WSRC Information Services, Aiken, SC.
[42] Sinclair, J.L., Kampbell, D.H., Cook, M.L. and Wilson, J.T. (1993) Protozoa in subsurface sediments from sites contaminated with aviation gasoline or jet fuel. Appl. Environ. Microbiol. 59, 467–472.
[43] Novarino, G., Warren, A., Kinner, N.E. and Harvey, R.W. (1994) Protists from a sewage-contaminated aquifer on Cape Cod, Massachusetts. Geomicrobiol. J. 12, 23–36.
[44] Kinner, N.E., Bunn, A.L., Harvey, R.W., Warren, A. and Meeker, L.D. (1991) Preliminary evaluation of the relations among protozoa, bacteria, and chemical properties in sewage contaminated ground water on Cape Cod, Massachusetts. In: USGS Toxic Substances Hydrology Program Proc. Techn. Meeting, WRI report 91-4034 (Mallard, G.E. and Aronson, D.A., Eds.), pp. 141–143.
[45] Harvey, R.W., Kinner, N.E., Bunn, A. and MacDonald, D. (1992) Transport of protozoa through an organically contaminated sandy aquifer. First International Conference on Ground Water Ecology, U.S. Environmental Protection Agency, American Water Resources Association, pp. 111–118.
[46] Kinner, N.E., Bunn, A., Warren, A. and Harvey, R.W. (1993) The distribution of protozoa in an organically contaminated aquifer. In: Integrated Soil and Sediment Research: A Basis for Proper Protection (Eijsackers, H.J.P. and Hamers, T., Eds.), pp. 677–678. Kluwer Academic, Dordrecht.
[47] Harvey, R.W., Kinner, N.E., Bunn, A., MacDonald, D. and Metge, D. (1995) Transport behavior of groundwater protozoa and protozoan-sized microspheres in sandy aquifer sediments. Appl. Environ. Microbiol. 61, 209–217.
[48] Chapelle, F.H. (1993) Ground-water Microbiology and Geochemistry. John Wiley and Sons, New York.
[49] Fredrickson, J.K. and Phelps, T.J. (1997) Subsurface drilling and sampling. In: Manual of Environmental Microbiology (Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D. and Walter, M.V., Eds.), pp. 526–540. ASM Press, Washington.
[50] Smith, R.L., Harvey, R.W. and LeBlanc, D.R. (1991) Importance of closely spaced vertical sampling in delineating chemical and microbiological gradients in groundwater studies. J. Contam. Hydrol. 7, 285–300.
[51] Zapico, M.M., Vales, S. and Cherry, J.A. (1987) A wireline piston core barrel for sampling cohesionless sand and gravel below the water table. Ground Water Monit. Rev. 7, 74–87.
[52] Bunn, A.L. (1992) Techniques for Enumerating Protozoa in Saturated Subsurface Sediments. Ph.D. Dissertation, University of New Hampshire, Durham, NH.
[53] Singh, B.N. (1946) A method of estimating the numbers of soil protozoa, especially amoebae, based on their differential feeding on bacteria. Ann. Appl. Biol. 33, 112–120.
[54] Singh, B.N. (1955) Culturing soil protozoa and estimating their numbers. In: Soil Zoology (Kevan, D.K., Ed.), pp. 104–111. Butterworths, London.
[55] Darbyshire, J.F., Wheatley, R.E., Greaves, M.P. and Inkson, R.H.E. (1974) A rapid micromethod for estimating bacterial and protozoan populations in soil. Rev. Ecol. Biol. Sol 11, 465–475.
[56] Hurley, M.A. and Roscoe, M.E. (1983) Automated statistical analysis of microbial enumeration by dilution series. J. Appl. Bacteriol. 55, 159–164.
[57] Anderson, R.V., Elliot, E.T., McClellan, J.F., Coleman, D.C., Cole, C.V. and Hunt, H.W. (1978) Trophic interactions in soils as they affect energy and nutrient dynamics. III. Biotic interactions of bacteria, amoebae, and nematodes. Microb. Ecol. 4, 361–371.
[58] Berthold, A. and Palzenberger, M. (1995) Comparison between direct counts of active soil ciliates (Protozoa) and most probable number estimates obtained by Singh's dilution culture method. Biol. Fertil. Soils 19, 348–356.
[59] Berninger, U.-G., Caron, D.A., Sanders, R.W. and Finlay,

B.L. (1991) Heterotrophic flagellates of planktonic communities, their characteristics and methods of study. In: The Biology of Free-living Heterotrophic Flagellates (Larsen, J. and Patterson, D.J., Eds.), pp. 39–56. Clarendon, Oxford.

[60] Porter, K.G. and Feig, Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25, 943–948.

[61] Caron, D.A. (1983) Technique for enumeration of heterotrophic and phototrophic nanoplankton, using epifluorescence microscopy, and comparison with other procedures. Appl. Environ. Microbiol. 46, 491–498.

[62] Starink, M., Bär-Gilissen, M.J., Bak, R.P.M. and Cappenberg, T.E. (1994) Quantitative centrifugation to extract benthic protozoa from fresh-water sediments. Appl. Environ. Microbiol. 60, 167–173.

[63] Harvey, R.W., Metge, D.W., Kinner, N. and Mayberry, N. (1997) Physiological considerations in applying laboratory-determined buoyant densities to predictions of bacterial and protozoan transport in groundwater: Results of in-situ and laboratory tests. Environ. Sci. Technol. 31, 289–295.

[64] Rogerson, A. and Laybourn-Parry, J. (1992) The abundance of marine naked amoebae in the water column of the Clyde Estuary. Est. Coastal Shelf Sci. 34, 187–196.

[65] DeLong, E.F. (1993) Single-cell identification using fluorescently labeled, ribosomal RNA-specific probes. In: Handbook of Methods in Aquatic Microbial Ecology (Kemp, P.F., Sherr, B.F., Sherr, E.B. and Cole, J.J., Eds.), pp. 285–294. Lewis, Boca Raton, FL.

[66] Lim, E.L., Caron, D.A. and DeLong, E.F. (1996) Development and field application of a quantitative method for examining natural assemblages of protists with oligonucleotide probes. Appl. Environ. Microbiol. 62, 1416–1423.

[67] Page, F.C. (1988) A New Key to Freshwater and Soil Gymnamoebae. Freshwater Biological Association, Ambleside.

[68] Vørs, N. (1992) Heterotrophic amoebae, flagellates and heliozoa from the Tvärminne area, Gulf of Finland. Ophelia 36, 1–109.

[69] Novarino, G. and Roberts, D.M. (1994) Digital film as an aid for studying live protists. Binary 6, 197–203.

[70] Novarino, G. (1991a) Observations on *Rhinomonas reticulata* and *Rhinomonas reticulata* var. *eleniana* var. nov. (Cryptophyceae), with comments on the genera *Pyrenomonas* and *Rhodomonas*. Nord. J. Bot. 11, 243–252.

[71] Novarino, G. (1991b) Observations on some new and interesting Cryptophyceae. Nord. J. Bot. 11, 599–611.

[72] Novarino, G., Lucas, I.A.N. and Morrall, S. (1994) Observations on the genus *Plagioselmis* (Cryptophyceae). Crypt.-Alg. 15, 87–107.

[73] Novarino, G. and Lucas, I.A.N. (1993a) A comparison of some morphological characters in *Chroomonas ligulata* sp. nov. and *C. placoidea* sp. nov. (Cryptophyceae). Nord. J. Bot. 13, 583–591.

[74] Javorovsky, A. (1893) Fauna studzienna minsta Krakowe i Lwowa napisat. Spravozd. Komm. Fizy. Krakau.

[75] Novarino, G. and Lucas, I.A.N. (1993b) Some proposals for a new classification system of the Cryptophyceae. Bot. J. Linn. Soc. 111, 3–21.

[76] Novarino, G. and Lucas, I.A.N. (1995) A zoological classification system of cryptomonads. Acta Protozool. 34, 173–180.

[77] Novarino, G. (1996) Notes on flagellate nomenclature. I. *Cryptaulaxoides* nom. nov., a zoological substitute for *Cryptaulax* Skuja, 1948 (Protista *incertae sedis*) non *Cryptaulax* Tate, 1869 (Mollusca, Gastropoda) non *Cryptaulax* Cameron, 1906 (Insecta, Hymenoptera), with remarks on botanical nomenclature. Acta Protozool. 35, 235–238.

[78] Novarino, G., Warren, A., Kinner, N.E. and Harvey, R.W. (1994) Protists from a sewage-contaminated aquifer on Cape Cod, Massachusetts, U.S.A. In: Second International Conference on Ground Water Ecology, U.S. Environmental Protection Agency American Water Resources Association, Atlanta, Georgia, U.S.A., 27–30 March 1994 (Stanford, J.A. and Valett, H.M., Eds.), pp. 143–151. AWRA, Herndon, VA.

[79] Woodcock, H.M. (1916) Observations on coprozoic flagellates: together with a suggestion as to the significance of the kinetonucleus in the Binucleata. Phil. Trans. R. Soc. Lond. B 207, 375–412.

[80] Harvey, R.W., Kinner, N.E., MacDonald, D., Metge, D.W. and Bunn, A. (1993) Role of physical heterogeneity in the interpretation of small-scale laboratory and field observations of bacteria, microbial-sized microsphere, and bromide transport through aquifer sediments. Water Resource Res. 29, 2713–2721.

[81] Rajagopalan, R. and Tien, C. (1976) Trajectory analysis of deep-bed filtration with the sphere-in-cell porous media model. J. Am. Inst. Chem. Eng. 22, 523–533.

[82] Harvey, R.W. (1991) Parameters involved in modelling movement of bacteria in groundwater. In: Modelling the Environmental Fate of Microorganisms (Hurst, C.J., Ed.), pp. 89–114. American Society for Microbiology, Washington, DC.

[83] Kuehn, K.A., O'Neil, R.M. and Koehn, R.D. (1992) Viable photosynthetic microalgal isolates from aphotic environments of the Edwards Aquifer (Central Texas). Stygologia 7, 129–142.

[84] Sleigh, M.A. (1989) Protozoa and other Protists, 2nd edn. Edward Arnold, London.

[85] Hardin, G. (1960) The competitive exclusion principle. Science 131, 1292–1298.

[86] Hutchinson, G.E. (1961) The paradox of the plankton. Am. Naturalist 95, 137–145.

[87] Fenchel, T. (1987) Ecology of Protozoa. The Biology of Free-living Phagotrophic Protists. Springer, Berlin.

[88] Fenchel, T. (1986) The ecology of heterotrophic microflagellates. In: Advances in Microbial Ecology (Marshall, K.C., Ed.), Vol. 9, pp. 57–97. Plenum, New York.

[89] Caron, D.A. (1987) Grazing of attached bacteria by heterotrophic microflagellates. Microb. Ecol. 13, 203–218.

[90] Sibbald, M.J. and Albright, L.J. (1988) Aggregated and free bacteria as food sources for heterotrophic microflagellates. Appl. Environ. Microbiol. 54, 613–616.

[91] Hirsch, P. and Rades-Rohkohl, E. (1988) Some special problems in the determination of viable counts of groundwater microorganisms. Microb. Ecol. 16, 99–113.

[92] Hazen, T.C., Jiménez, L., López de Victoria, G. and Fliermans, C.B. (1991) Comparison of bacteria from deep subsur-

face sediment and adjacent groundwater. Microb. Ecol. 22, 293–304.

[93] Albrechtsen, H.J. (1994) Distribution of bacteria, estimated by a viable count method, and heterotrophic activity in different size fractions of aquifer sediment. Geomicrobiol. J. 12, 253–264.

[94] Gasol, J.M. and Vaqué, D. (1993) Note: Lack of coupling between heterotrophic nanoflagellates and bacteria: A general phenomenon across aquatic systems? Limnol. Oceanogr. 38, 657–665.

[95] Vørs, N. (1993) The biogeography of marine-water column heterotrophic flagellates: Do special rules apply? In: IX International Congress of Protozoology, July 25–31 1993, Berlin, Germany, Book of Abstracts, Abstract 518. German Society of Protozoology and German Society of Parasitology.