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Special issue:
Protists as Bioindicators of Past and Present Environmental Conditions

Seven Reasons Why Protists Make Useful Bioindicators

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Abstract. To introduce the special issue this paper reviews the use of protists as bioindicators. Seven key advantages of protist bioindicators are highlighted, namely: environmental sensitivity, functional importance, distribution, size and numbers, response times, ease of analysis and preservation potential. Protist bioindicators have been used in a wide range of contexts from monitoring ecosystem restoration to fire history, and particularly environmental pollution. Most major protist groups have been used as bioindicators with diatoms, foraminifera and testate amoebae particularly widely studied. To increase uptake of protist bioindication methods in routine environmental monitoring technique development should consider the needs of stakeholders from an early stage. Papers in this special issue reflect the diversity of both protist life and possible bioindicator applications.

Key words: Indicator, biomonitoring, environmental sentinel, Protozoa, algae.

A BRIEF HISTORICAL DIGRESSION

Nineteenth century coal mines were dangerous places to work: a coal miner in the USA in 1900 had a 1 in 10 chance of dying in a mine accident during their working life (US Department of Labour 2013). Particular dangers came after underground fires and explosions when many miners died a mysterious death from a colourless, odourless gas the miners called 'white damp' and which we now know to be carbon monoxide (CO). Safety lamps alerted miners to other hazards like methane and carbon dioxide but were useless for the detection of CO. Chemical tests were possible but took time, were not always reliable and required a laboratory (Burrell 1914). Scientists tasked with this problem hit upon a novel solution, perhaps the first widely applied example of a bioindicator. It was found that small mammals and birds with rapid metabolisms succumbed to the effects of CO much more quickly than humans, showing signs of distress or inertia after just a few minutes (Burrell 1914). Miners carrying a caged canary into dangerous areas were able to detect the presence of CO and leave before suffering serious symptoms. While this story is well-known it is worth repeating here as it illustrates many of the best features of bioindication. Coal-mine canaries were extremely successful bioindicators because the response was rapid, unambiguous and of direct relevance to the health of the miners and canaries were relatively cheap and easy to deploy underground (O'Brien *et al*. 1993, Stephen and Ribble 2001). It was not until 1986 that canaries were phased out in British mines as the technology for portable gas

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analysers improved and became cheaper. This paper and this special issue address whether protists could be similarly useful bioindicators. The paper addresses the aims of bioindication, the existing use of protist bioindicators, the reasons why protist bioindicators have potential to be useful and the action which is needed for protist bioindicators to become widely applied.

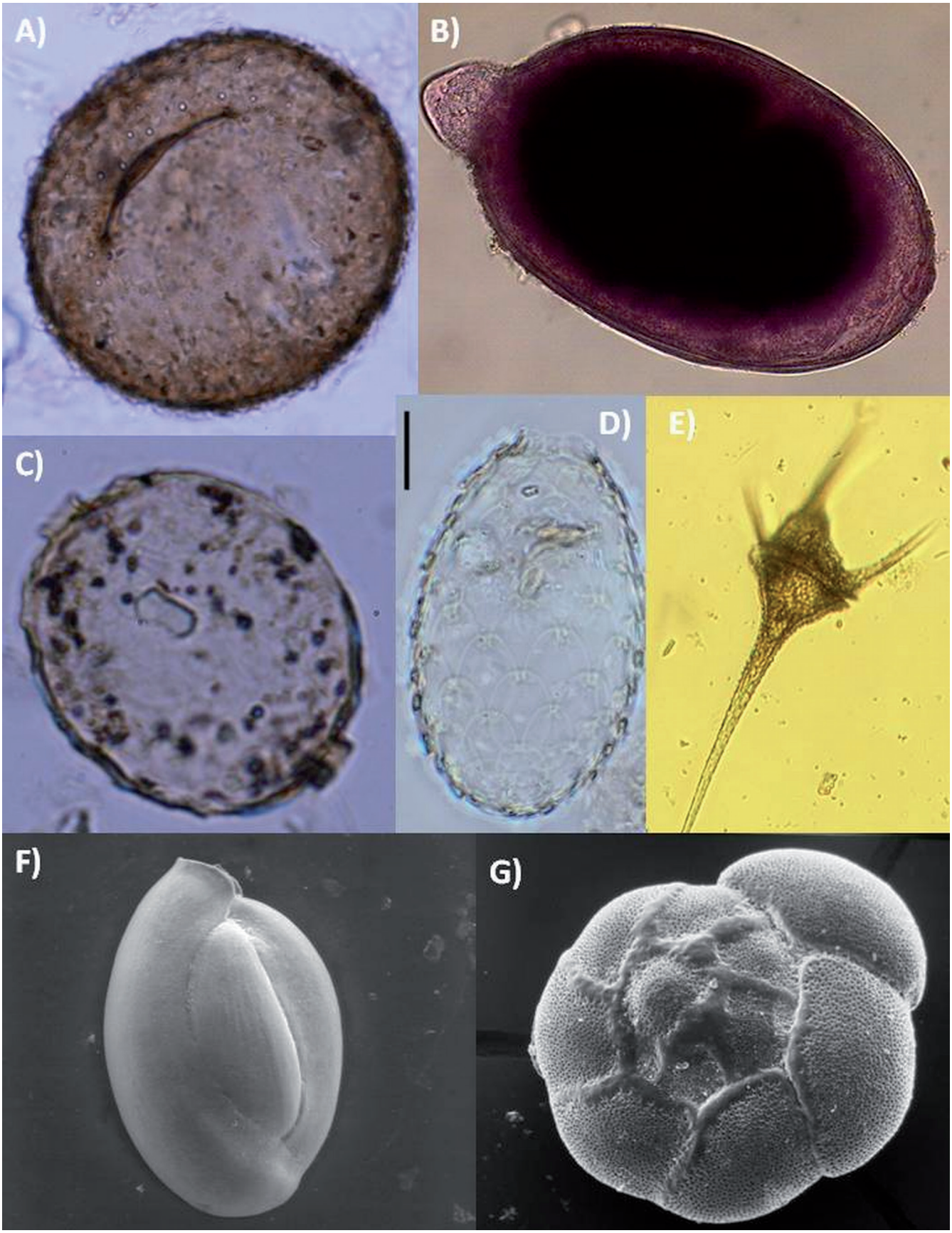
WHAT DO WE MEAN BY BIOINDICATION?

Before examining whether protist bioindicators might be able to emulate the success of the mine canary it is worth taking a step back and considering what exactly we mean by bioindication. Bioindication is a term used frequently but rarely defined; indeed it is often used in different ways in different contexts to mean quite different things! (O'Brien *et al*. 1993). In general terms the aim of bioindication is simply 'the use of biota as indicators of environmental state' but within this broad definition aims, methodology and practise vary greatly. McGeoch (1998) argues that three broad categories of bioindicators can be recognised based on their aims and objectives: *environmental*, *ecological* and *biodiversity indicators*. Although these categories may be considered somewhat artificial, and certainly overlap, they provide a useful framework in which to consider the diversity and differences in bioindication approaches. *Environmental indicators* aim to quantitatively or semi-quantitatively indicate the value of an environmental variable often by studying the response of organism phenotype, population size and community structure. An example of such a study is the paper in this volume by Barnett *et al*. which aims to use protist communities to quantitatively infer sea-level; variability along gradients is studied and used to develop a predictive model for use elsewhere. This category of bioindicators also encompasses approaches such as bioaccumulators: organisms which accumulate a chemical (usually a pollutant) to indicate environmental exposure (such as the use of shell-fish to indicate TBT pollution: Morcillo *et al*. 1999) and environmental sentinels, organisms which are introduced with the deliberate aim of indicating the presence or level of a pollutant (such as the use of bioluminescence by genetically modified bacteria to indicate the presence of mutagenic pollutants: Podgórska and Węgrzyn 2006).

By contrast to environmental indicators *ecological indicators* focus on the impacts of an environmental factor rather than the level. While this distinction is subtle – and some indicators may fill both roles – it is an important one as the criteria for efficacy is different. In the case of environmental indicators the primary criterion for an effective indicator is simply an ability to establish the presence or level of an environmental factor, in the case of ecological indicators the aim is for the indicator to demonstrate impacts on a broader range of organisms or ecosystem parameters. The paper by Valentine *et al*. (this volume) illustrates a potential ecological indicator; the use of testate amoebae to monitor peatland restoration. While testate amoebae may directly indicate environmental factors such as water table and pH (and therefore also provide an environmental indicator) they are of particular value because the position of testate amoebae at the top of the microbial foodweb means that changes in testate amoebae can be argued to represent changes in a broader suite of organisms.

The final category of bioindicators is *biodiversity indicators* in which the aim is to focus on the diversity of one group of organisms as a surrogate for the diversity of a broader range of organisms (McGeoch 1998). This is an important topic in conservation biology where a major question is how to direct conservation efforts to maximise preservation of biodiversity; the diversity of all groups cannot be feasibly studied in all areas so there is a desire to identify indicator organisms as proxies for ecosystem biodiversity (Schulze *et al*. 2004, Kati *et al*. 2004). Whether this is indeed possible is still a source of debate (Prendergast *et al*. 1993) and such studies have paid little attention to microbial diversity. Critical here is the open question of whether microorganisms have biogeographies; if all species can be found everywhere there is a suitable environment

Fig. 1. Some of the diversity of protists represented in this special issue. **A** – the testate amoeba *Bullinularia indica* (photo: Julie Valentine), test is approximately 170 µm diameter; **B** – the foraminifer *Allogromiid* spp. (photo: Anna Sabbatini); **C** – the testate amoeba *Amphitrema wrightianum* (photo: Julie Valentine), test is approximately 60 µm diameter; **D** – the testate amoeba *Tracheleuglypha dentata* (photo: Julie Valentine), scale bar: 10 µm;. **E** – the dinoflagellate *Ceratium hirundinella* (photo: Jane Fisher), cell length 280 µm; **F** – the foraminifer *Pseudotriloculina rotunda* (photo: Maria Pia Nardelli); **G** – the foraminifer *Ammonia tepida* (photo Fabrizio Frontalini).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(c.f. Fenchel *et al*. 2004) then they are unlikely to be suited to biodiversity indication but if this is not the case then there may be a substantial and under-studied conservation issue (Cotterill *et al*. 2008, Heger *et al*. 2011).

QUESTIONS AND APPROACHES

The investigation of protists as bioindicators has a history almost as long as the study of the organisms themselves (e.g. Kolkwitz and Marsson 1908) but interest in the topic has increased greatly in recent decades. Studies discussing protist bioindicators – or at least mentioning bioindication in the context of protists – are numerous but unevenly distributed between protist groups, ecosystems and applications. A brief review of the literature (Table 1) suggests that the largest proportion of studies are in the context of water quality, mostly using ciliates, foraminifera and diatoms. Studies of soil and atmospheric pollution and climate, particularly testate amoebae as climate proxies, are well represented while other potential applications such as monitoring the success of ecosystem restoration or fire history are much rarer.

The vast majority of bioindicator studies using protists have used community structure as the response variable; however there is no intrinsic reason for this bias. Bioindicator studies in other groups of organisms have exploited a much broader range of variables encompassing behavioural, physiological, biochemical and genetic change. There are some examples of such studies in protists, for instance Díaz *et al*. (2006) report the use of a fluorescence method to monitor the bioaccumulation of heavy metal deposits within ciliate cells, a promising potential environmental indicator, but such studies are rare. There is undeniably great potential for genetic data to contribute to protist-based bioindication but this is currently under-utilised (Lara and Acosta-Mercado 2012).

Table 1. Contexts for protist bioindication; an overview of 100 papers.

Category	Number of articles*
Water quality (including wastewater treatment)	37
General/Reviews/Methodologies	30
Atmospheric and soil pollution	11
Climate-related	9
Other variables/Multiple variables	6
Restoration ecology	3
Agriculture	2
Fire	2

*List based on 100 top returns from Google Scholar search for 'protist bioindicators' ignoring some irrelevant entries. Classification inevitably encompasses some subjective decisions and may well be biased towards those groups where the term 'protist' is more widely used – for instance towards ciliates but against diatoms.

SEVEN REASONS WHY PROTISTS MAKE USEFUL BIOINDICATORS

Protists are a diverse polyphyletic group present in a wide range of environments; potential bioindicator applications are similarly varied. Making generalisations about protist bioindication is difficult however I believe that seven key features make protists particularly valuable in this role.

Environmental sensitivity

Essential to any environmental indicator is that it is sensitive to forms of environmental change which are of scientific, conservation or policy interest, with environmental pollution perhaps the most frequent target. There is much evidence for the sensitivity of protists to many types of environmental change. For instance, if we consider the protist group with which I am most familiar: the testate amoebae, previous studies suggest sensitivity to climate (Jassey *et al*. 2011), sulphur (Payne *et al*. 2010), ozone (Payne *et al*. 2012), nitrogen (Gilbert *et al*. 1998), CO_2 (Mitchell *et al*. 2003), heavy metals (Nguyen-Viet *et al*. 2008), particulate pollutants (Meyer *et al*. 2010), agricultural land use (Heger *et al*. 2011) and fire (Turner and Swindles 2012) amongst many others. Similarly lengthy lists could be compiled for most other protist groups. This sensitivity to environmental change provides the essential prerequisite to the use of protist bioindicators.

Functional importance and trophic position

While the environmental sensitivity of protists makes them potentially-useful *environmental* indicators it is their functional importance and trophic positions which makes them potentially interesting *ecological* indicators. Photosynthesis by autotrophic protists is very important in many ecosystems, for instance diatoms may

account for 40–45% of oceanic net primary production (Mann 1999). Major changes in marine diatom communities are highly likely to have an impact on both species at a higher trophic level and on ecosystem-scale processes. Heterotrophic protists also play critical roles in food-webs as the most important microbial predators in aquatic systems (Sherr and Sherr 2002), typically occupying intermediate trophic positions preying on prokaryotes and ultimately being preyed upon by invertebrates. This link to both lower and higher trophic levels means that changes in the protist communities are likely to be sensitive to both bottom-up control through food-supply and top-down control through predation and therefore indicators of wider ecosystem change. In the case of both autotrophic and heterotrophic protists their functional importance makes their analysis potentially representative of changes in a broader suite of organisms and processes.

Distribution

While there is an increasing weight of evidence to show that some protist species have restricted distributions it is also clear that the protist groups themselves have very broad distributions. If we again consider the testate amoebae, studies of soils around the world have identified diverse testate amoeba communities in environments ranging from Vietnamese rain forests (Balik 1995) to sub-Antarctic islands (Smith 1972) and to the Arizona desert (Bamforth 2004). Perhaps even more remarkably many taxa are consistently found in such diverse environments (in the case of these three cited studies four species: *Difflugia lucida*, *Euglypha rotunda*, *Phryganella acropodia* and *Trinema enchelys*). This widespread distribution of both protist groups, and many individual species within those groups means that an indicator based on the abundance or phenotype of a species in one region can potentially be applied in another; a key advantage in an era when environmental policy is increasingly set at a supra-national level.

Size and numbers

Protists range in size from approximately 1 μm diameter in the case of the diminutive marine alga *Ostreococcus tauri* (Chrétiennot-Dinet *et al*. 1995) to over 10 cm in the case of the largest xenophyophores (Lecroq *et al*. 2009) but most taxa are in the range of 5–200 μm. Largely as a consequence of this small size protist numbers can be vast. For instance, even in the wet, acidic and nutrient-poor environment of peatlands there may still be 120,000 microalgae, 46,000 testate amoebae, 28,000 heterotrophic flagellates and 700 ciliates in each gram of soil (Gilbert and Mitchell 2006). For bioindication this means that even a very small sample of water, soil or sediment is likely to contain more than enough abundance and diversity for meaningful community quantification reducing sampling disturbance, improving potential spatial resolution and easing the logistical difficulties of sample movement and storage.

Response times

Generation times of protists are typically short. For instance some figures reported in the literature show generation times of as little as four hours for the testate amoeba *Phryganella acropodia* (Beyens and Meisterfeld 2001), seven hours for freshwater heterotrophic flagellates (Laybourn-Parry and Walton 1998) and marine ciliates (Dolan 1991) and 18 hours for Antarctic marine diatoms (Spies 1987). While the environments considered and methodologies behind these figures vary greatly they make the point that under optimal conditions protist reproduction can be very rapid indeed. This speed of response combined with sensitivity to many forms of environmental change mean that protist communities are capable of rapid reorganisation and may allow high-frequency biomonitoring.

Ease of analysis

A key advantage of protists as bioindicators is their ability to be studied by relatively simple means. All of the studies reported in this special issue identify protists using relatively simple preparation methods and light microscopy. I do not mean to under-state the difficulties involved with microscopic identification of protists: morphological taxonomies of many groups leave much to be desired (Mitchell and Meisterfeld 2005, Heger *et al*. 2009), researchers with taxonomic knowledge of less-studied groups are rare and becoming more so (Cotterill and Foissner 2010) and it is clear that morphospecies do not map easily to genotypes with the probability of considerable cryptic diversity in many groups (Mann 1999, Hayward *et al*. 2004, Beszteri *et al*. 2007). However, the fact remains that it is possible for an experienced observer with cheap and simple methods to identify consistent morphospecies in many protist groups. This is not the case for many other microorganisms. Molecular methods certainly offer advantages but are not always essential, giving protists all the advantages of using microorganisms as bioindicators without the necessity for specialised facilities and expensive analyses.

Preservation potential

Many protist taxa including dinoflagellates, chrysophytes, foraminifera, diatoms and testate amoebae produce hard body parts which are resistant to decomposition. In favourable sedimentary environments it is possible to reconstruct the community structure after hundreds, thousands or even millions of years and thereby qualitatively or quantitatively reconstruct environmental change. Protist proxies have become among the most important tools for understanding long-term environmental change, particularly in the Quaternary (Lowe and Walker 1997). Indeed, the increasing use of protists by palaeoecologists has been an important motivator for more ecological research on some groups of protists over recent years. Although protists may respond rapidly to short-term change the assemblages studied by palaeoecologists typically integrate changes over longer-periods; a powerful but rarely-used approach is the simultaneous analysis of living and fossil communities to allow contemporary change to be put in a longer-term context.

MOVING BEYOND ACADEMIA

Most protist bioindicators discussed in the literature (Table 1) have only been applied in the context of scientific research. Scientific studies are vital to identifying and developing methodologies but if these methods are to really contribute to solving the world's environmental problems they need to move out of academia and into the 'real world'; they need to be developed to the point where they cease to be research projects and become routine tools. I believe that the development of methods should focus more on the needs of those who will habitually apply them. Few methods have made the transition to routine application. Various schemes have been proposed for the systematic recording and interpretation of protist data and quantitative indices proposed for, for instance: aquatic protozoa and water quality (Jiang and Shen 2003); protozoa and sewage treatment sludge (Madoni 1994); and foraminifera and coral reef health (Hallock *et al*. 2003) but take-up of such methods is variable. Perhaps the approach nearest to widespread application is the use of diatoms to monitor water quality where systems have been implemented as part of routine water quality monitoring (e.g. Prygiel and Coste 1993, Kelly 1998, Kelly *et al*. 1998, Wu 1999).

No doubt part of the reason for the limited impact of protist bioindicators relates to the generally low profile of protists among ecologists, and even more so amongst the general public. Despite the increasing scientific realisation that microbes dominate global biodiversity and environmental processes (*c.f.* Nee 2004) protists have been neglected to the point where even the term 'microbes' is often used as a synonym for 'prokaryotes' (Caron *et al*. 2009). This is clearly to ignore an important component of the ecosystem; studies of bacteria in isolation are the microscopic equivalent of studying gazelle populations whilst ignoring lions! However, beyond this general bug-bear of all protistologists I believe it is also the case that protist bioindication has not made more of an impact because insufficient attention has been paid to what features of a bioindicator are essential, which desirable and which unnecessary. There is an inevitable taxonomic bias in the bioindicator literature: entomologists favour insects as the best indicators, botanists plants and ornithologists birds (McGeoch and Chown 1998); as a testate amoeba researcher I naturally favour testate amoebae as the perfect bioindicator! However, as applied scientists we need to recognise that our personally-favoured indicator groups may not be the most appropriate in all circumstances. I believe that protists as a group have many advantages as bioindicators but we should recognise that there will be cases when other organisms are superior: for instance the size of protists is an advantage for the small size of samples required and spatial resolution offered but there will certainly be instances when it is more important to have indicators which can be studied easily in the field without microscopy. Such trade-offs call for strict attention to the properties of the ideal indicator for any given problem. Definition of such standards requires the active involvement of end-user communities and the impartial evaluation of potential indicators against pre-agreed criteria. The criteria will depend on the particular issue being investigated but will require consideration of many of the key questions in Table 2.

THE CONTRIBUTION OF THIS VOLUME

The range of papers in this volume reflects the diversity of protists and their potential bioindicator applications. One of the most widely used protist groups

Table 2. Some key questions to be considered in criteria-setting for bioindicator selection.

1	What should the indicator show?
2	How frequently will the indicator be analysed?
3	What period of time should the response represent?
4	What spatial resolution is required in results?
5	How large a sample can be removed?
6	What identification skills are available?
7	What is an acceptable cost?
8	What equipment is available?
9	Does the same indicator need to be applicable across a large area?

in bioindication is the foraminifera and four of these papers investigate various bioindication contexts. Frontalini *et al*. (this volume) discuss the spatial ecology of foraminifera in Lake Varano, a coastal lagoon in southern Italy and show the presence of distinct communities, particularly associated with differences in salinity. As well as indicating physical conditions foraminifera may also be valuable for indicating anthropogenic disturbance, particularly by pollution. For instance Nardelli *et al*. (this volume) discuss the impact of zinc on the foraminifer *Pseudotriloculina rotunda,* showing impacts on biomass and growth but, contrary to expectations, not on test deformities. Despite the vast numbers and wide dispersal of protists, research effort is unevenly distributed with many groups very lightly studied. One such group may be the monothalamous foraminifera which are frequently not included in foraminifer studies as they do not preserve in sediments and due to their difficult taxonomy and difficulty of analysis. Sabbatini *et al*. (this volume) present a review of this group with a particular focus on the Adriatic and make a case for the importance of more widespread study.

A key advantage of several groups of protists is their fossilisation potential. Garcia *et al*. (this volume) provide an example of the widespread use of protists as bioindicators on longer time-scales to reconstruct Holocene environmental change in the Bay of Biscay from sediment cores. Garcia *et al*. also study the living foraminifera of their study site; such modern analogues have become increasingly important in palaeoecology. Another example is provided by Barnett *et al*. (this volume) who report a study of salt-marsh testate amoebae from Norway with the aim of improving interpretation of palaeoecological records. Testate amoebae are also the topic of the paper by Valentine *et al*. (this volume) who present a study of the use of testate amoebae in monitoring peatland restoration. Many peatlands in Western Europe are degraded by human activity and considerable conservation effort is focused on restoring these degraded sites. Testate amoeba communities may indicate the success of these efforts in raising water tables and producing microbial communities which are similar to those prior to drainage. The final paper of the special issue by Fisher *et al*. (this volume) considers a protist group (dinoflagellates) in parallel with cyanobacteria in freshwater lakes. The authors show that the dinoflagellates may have greater value as indicators of nutrient conditions than previously appreciated.

CONCLUSIONS

Protists make up a substantial proportion of all life on earth with huge numbers and vast genetic and phenotypic diversity in almost all habitats. It would be extremely surprising if protists *did not* have some value as bioindicators. However, the particular advantages of protist bioindicators have perhaps not been well-communicated to non-protistologists and the methods have had relatively limited impact on routine biomonitoring. In this paper I have tried to synthesise the advantages which protist bioindicators offer and suggest some ways by which we can encourage their more widespread use. However, of course, protists are fascinating organisms for many reasons, not least their undoubted functional importance in many ecosystems, and their value as bioindicators is just one argument for why we should study them.

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