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REVIEW

As trees walking: the pros and cons of partial sight in the analysis of stream biofilms

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Background – The microscopic world of the freshwater biofilm is a complex association of organisms from prokaryotes to metazoans. Understanding the relationships between these organisms, and between them and their environment, is complicated by the processes by which biofilms are studied. Whilst it is possible to observe and minutely describe the individual organisms which comprise biofilms, inter-relationships within the 'community' are often destroyed during sample collection and investigation under the microscope. Ecologists often focus on particular groups of organisms (e.g. diatoms) and interrogate data using multivariate statistics. This offers valuable insights that enable us to understand how associations of particular taxonomic groups respond to key environmental gradients yet offers an essentially abstract view of the microscopic world.

Approach – In this essay we contrast the great detail achieved when we see and describe individual cells with the gross approximations necessary when the response of communities is considered. A focus on the diatom assemblage (one part of the intricate biofilm community) and the use of multivariate statistics to interpret responses along ecological gradients offers opportunities to understand environmental change in space and time but at the expense, perhaps, of local detail which may account for some of the unexplained variation in models. We cannot envisage a change in approach in the near future but, instead, encourage a greater awareness of the complexity of stream biofilms to better inform interpretation.

Key words – Ecology, biofilms, imaging, periphyton, phytobenthos, algae, diatoms.

INTRODUCTION: 'SEEING' WITH A MICROSCOPE?

The aim of the natural scientist is to gain a better understanding of the living world. In the case of the microscopic world, this understanding cannot be achieved through direct observation but requires the use of sophisticated optical equipment in order to see individual cells and detail fine structures. Whilst these techniques should enhance our understanding of the organisms or habitats we are studying, there is also a risk that these manipulations can draw us away from an appreciation of the living organism, its behaviour and interactions within the microhabitat.

Hacking (1981) asked: do we see with a microscope? He argued that when we look through a microscope we see objects that are not "… physical things in a literal sense, but merely by courtesy of language and pictorial imagination". A key part of his argument is that 'seeing' in the microscopic world requires interventions. The lenses in our microscopes are one type of intervention, of course and without these we would not be able to resolve small features with clarity. But this is just the start: we often use stains to adjust the optical properties of certain parts of cells, making it both easier to see these and, in many cases, to infer their composition (iodine binding to starch is a good example). The study of

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diatoms depends upon high resolution mountants, which make the task of viewing structural details much easier. This follows a stage in which the cellular contents are dissolved away by the action of strong oxidising agents. So routine is this stage that we fear that some diatomists are in danger of forgetting that the empty glass shell is not the reality that they seek.

This dependence upon technology has another consequence: insights at the microscopic level reflect the technology available at the time at which the observations were made. In 1921, Fred Taylor reported that the literature on diatoms is 'extensive' (Taylor 1921). He cited the 10 plates published by Rabenhorst in his *Süsswasser Diatomaceen* (1853), quoting "it is wonderful how much was seen and accurately recorded with instruments that would now be despised and rejected". Over 150 years later, we ask how the plethora of sophisticated instruments available to us now have enhanced our ability to 'see' the microscopic world.

It is appropriate to raise these issues in a Festschrift dedicated to Eileen Cox. Her core work, as a diatom taxonomist, provides an illustration of how an understanding of what we 'see' with a light microscope can be enhanced by deeper knowledge of diatom structure gained through use of Scanning Electron Microscopy (SEM) (Cox 1979) and culturing (Trobajo et al 2006, Rose & Cox 2014). She has, to a greater extent than most of her peers, recognised the limitations of restricting study to the silica frustule of diatoms. In particular, she recognised the importance of 'soft' structures such as plastids (Cox 1981) and mucilage tubes (Cox 1975) and, more recently, molecular genetic evidence (Trobajo et al. 2009). Her book on identifying live diatoms (Cox 1996) was a bold and underappreciated attempt to incorporate this way of thinking into mainstream ecological analyses.

In this essay, we extend Hacking's consideration of 'seeing' with a microscope from the perception of individual objects (microscopic algal cells, in our case) to developing an appreciation of the interrelationships amongst cells in freshwater biofilms, and between these cells and their environment. These challenges start with the collection of the sample itself. We then go on to consider how a synergism between conventional 'data', broader 'knowledge' and, crucially, imagination can enrich our understanding of natural systems.

THE DILEMMA OF VIEWING MICROBIAL COMMUNITIES

The issues Hacking (1981) described when observing isolated objects are further compounded when trying to understand the interrelationships of individual cells within communities such as biofilms. The routine sampling procedure for diatoms, for example, involves brushing the surfaces of stones with a toothbrush or a similar device (Kelly et al. 1998) in order to remove the biofilm. Subsequent processing to examine diatoms removes organic matter and soft-bodied organisms but, in the process, epiphytes are removed from their hosts and, in many cases, colonies and filaments are fragmented into isolated cells. The statistical methods that are used to infer environmental conditions assume a random distribution of organisms (or, in the case of diatoms, their cell walls) whereas their natural condition is anything but random (fig. 1). Methods for viewing other groups of algae, whilst less destructive, still lead to considerable disruption in the journey from a biofilm growing on a submerged surface in a lake or stream to a wet mount on a microscope slide. The shallow depth of field possible at high magnifications means that any structure that does survive this journey is viewed in two dimensions rather than three. Add to this a range of optical microscopical techniques – phase contrast, fluorescence, differential interference contrast, and even the ability to view objects at far greater light intensities than they ever encounter naturally – we are left facing a paradox: we are viewing, identifying, measuring and counting objects that are distorted versions of the entities that they represent; yet, at the same time, we convince ourselves that we are improving the quality of our insights.

This paradox can be partially resolved in two ways. First, following a series of conventions for viewing organisms makes it easier to match them to illustrations and descriptions in the taxonomic literature and this greater taxonomic insight outweighs the problems faced when trying to understand ecological patterns (Gillett et al. 2009). Second, this approach meets the demand for a rigorous quantitative approach to freshwater ecology by providing suitable feedstock for multivariate statistical programs (see below) which has, over the years, provided sufficient valuable insights into the recent and past state of the environment (Bennion et al. 1996, Kelly et al. 2008, Hausmann et al. 2016) to justify itself. However, the paradox cannot be wholly resolved: ecologists studying the macroscopic world have a reference point: they can look at their data and visualise the communities it describes, even if they did not perform the original survey. They know how the species relate to one another – they know which are trees, which are epiphytes, ground layer herbs, or whatever. The ecologist of the microscopic world, by contrast, can see the individual components of communities but rarely has comparable knowledge about species associations and interactions.

OTHER WAYS OF 'SEEING'

Multivariate statistical approaches, themselves, deserve consideration in an essay on 'seeing' the microbial world. Since pioneering work in the 1960s (Whittaker 1967), ecologists have used multivariate statistics as a powerful tool for, in particular, understanding how communities change along ecological gradients. Ordination methods, for example, use the similarity in composition of assemblages to arrange them along one or more environmental gradients. The resulting graphs are a representation of the real world in abstract terms: they are not representational in the way that Constable or Corot's paintings present scenes that indicate the relationship between pictorial elements that can be related to our own experiences. We see parallels in ordination plots with those art movements of the late 19th and 20th centuries which removed ('abstracted') pictorial detail to reveal (the artists argued) the hidden truths of life (Gompertz 2012). These graphs present scientists with information about the systems they study that transcends what is possible by simply describing the organisms present and their interrelationships.

Developments in gradient analysis (such as constraining axes so that they only display variation that is described by certain variables, e.g. ter Braak & Prentice 1988) led, in turn, to models that allow the response of an assemblage to key environmental pressures to be investigated. Such models underpin modern palaeoecology (Birks et al. 1990) and ecological status assessment (Kelly et al. 2008). In the case of diatoms, these have influenced policy (Derwent & Wilson 2012) and, at a more local level, provide essential information for regulators concerned with the implementation of water-related legislation across Europe (Poikane et al. 2016). Diatoms became the focus of this work for a number of practical reasons (Kelly et al. 2015) but, in both cases, the objective is not to 'see' the microscopic world but, rather, to gain insights into broader environmental patterns. Diatoms have become no more than a tool through which scientists and bureaucrats 'see' something else altogether. The state-of-the-art', in other words, is a utilitarian and highly reductionist view of the microscopic world.

Whilst it has been considered that diatom-based assessments are generally more strongly related to key environmental gradients than other organism groups (Hering et al. 2006), it is rare for the relationship between biology and stressor to explain more than half the total variation. In such situations, it is possible to partially explain the unexplained variation in statistical terms (Soininen & Eloranta 2004, Jamoneau et al. 2017) and to acknowledge this when making decisions (Kelly et al. 2009). However, this, again, is a utilitarian 'fix' that does not attempt to understand the reasons behind the observed variation. Is it possible that part of the problem is that we do not 'see' the real *in vivo* state of biofilm communities?

UNDERSTANDING BIOFILM STRUCTURE

The combination of analytical methods that focus on cleaned diatoms and a powerful suite of statistical tools to interpret these data results, we believe, in a form of 'tunnel vision' in which all peripheral information about biofilms is lost.

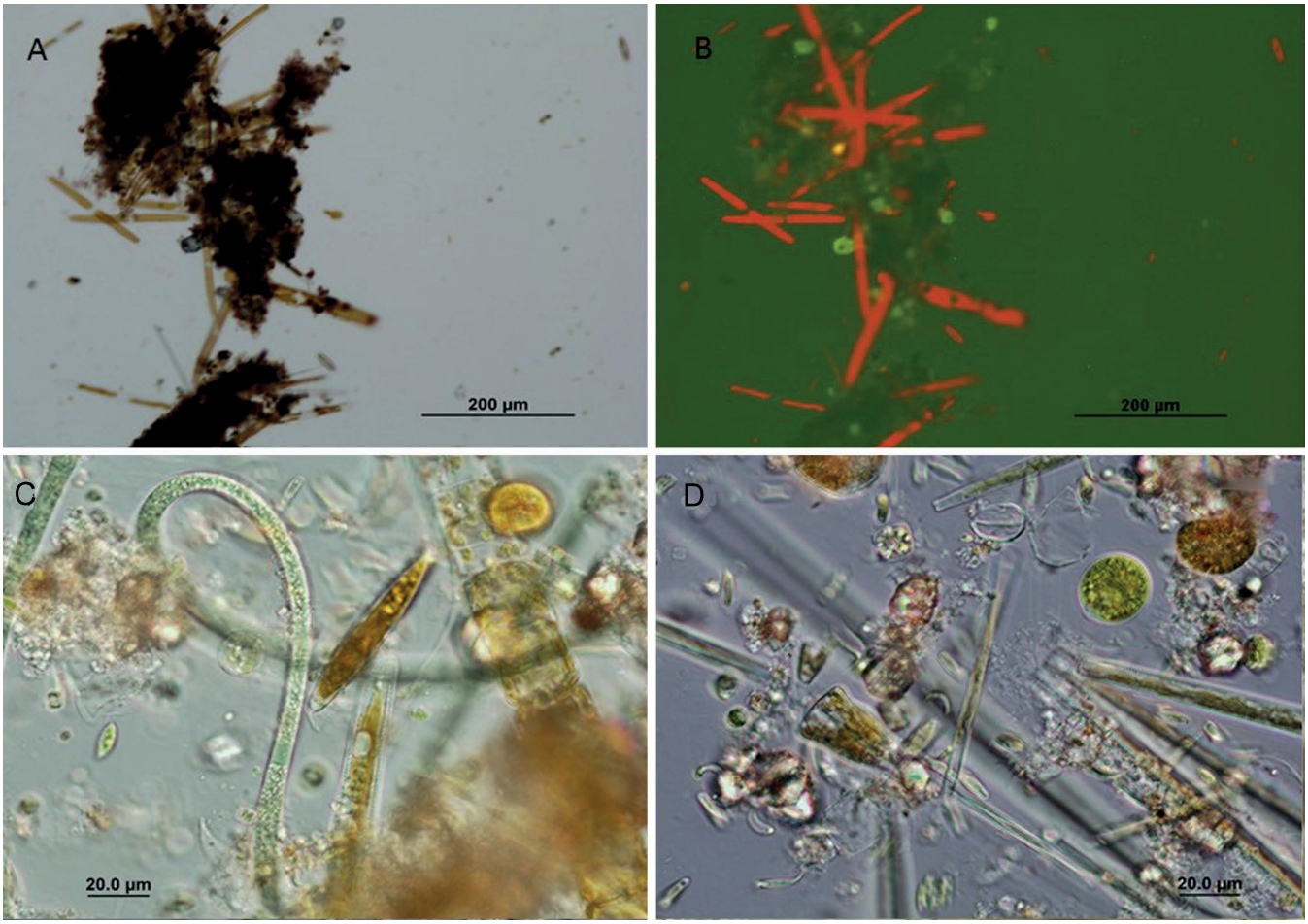

Figure 1 – Illustrations of the complex, jumbled and non-random world of freshwater biofilms as encountered with the light microscope: A, representatives of several autotrophic groups live in, on and around organic and inorganic debris, along with fungi, bacteria, heterotrophic protists and higher organisms: epilithic biofilm from Winford Brook (latitude: 51.36697, longitude: -2.621881). Organic material obscures many live cells; B, using chlorophyll autofluorescence imaging, location of pigmented cells is revealed; C, closer inspection of dispersed biofilm material reveals single-celled and filamentous diatoms intertwined with other phototrophs such as filaments of cyanobacteria; D, colonies of *Scenedesmus* and live and empty cells of dinoflagellates are located amongst the diatoms and mineral particles. Light microscopy was undertaken using a Leica DM LB2 microscope (Wetzlar, Germany) with an Olympus DP70 camera (Hamburg, Germany).

Witness the widespread use of the term ‘diatom community’ (122 occurrences in titles of papers since 1970, according to Web of Science, August 2018) as if no other alga, fungi or heterotrophic protists and metazoans had any relevance to life in the subaquatic world.

It is not hard to see why this predilection for cleaned diatom samples has evolved. Not only has the diatom cell wall proved to be a very sensitive means of interrogating phylogenetic information, but the roots of modern quantitative methods lie in palaeoecology, for whom diatom cell walls were (until recently) one of the few parts of the algal community that could be analysed from lake sediments. We do not mean to suggest identification and enumeration of diatoms is a trivial task but ask readers to compare the view of a cleaned diatom assemblage from a river or lake biofilm with the same sample in its fresh state. In the fresh state, there will be inorganic and organic particles, many of which may occlude the diatoms that you are trying to identify (fig. 1). Some of the diatoms may be motile, serenely gliding under a particle or out of the frame of view before you have a chance to name or count them (meanwhile, others have glided into your field of view …). Then there are other algae, ranging from tiny spherical unicells to substantial filaments; some genera of the latter often bear diatoms (and other algae) as epiphytes (fig. 2) whilst others do not. Naming many filamentous green algae beyond genus requires reproductive structures which are often not present. Bacteria, including cyanobacteria, and fungi are also likely to be present. Then there are the heterotrophic protists (fig. 3) and other microfauna, which may play an important role as grazers, exerting a top-down control on the algae. Grazers further compound the issue of viewing ‘dead’ taxa as they will expel the partially digested remains (i.e. providing us with a ‘partially cleaned’ diatom cell!) back into the biofilm. You may find Chironomidae larvae browsing amongst the algal filaments and organic particles, as well as representatives of other invertebrate orders; some of which may even bear zoophytic algae. The minimalist world of cleaned diatoms may well seem like a welcome relief after trying to make sense of the diverse, complex and forever shifting world of ‘fresh’ samples.

The term ‘partial sight’ in the title was chosen to reflect the ambiguity between this world and the limited view (al-

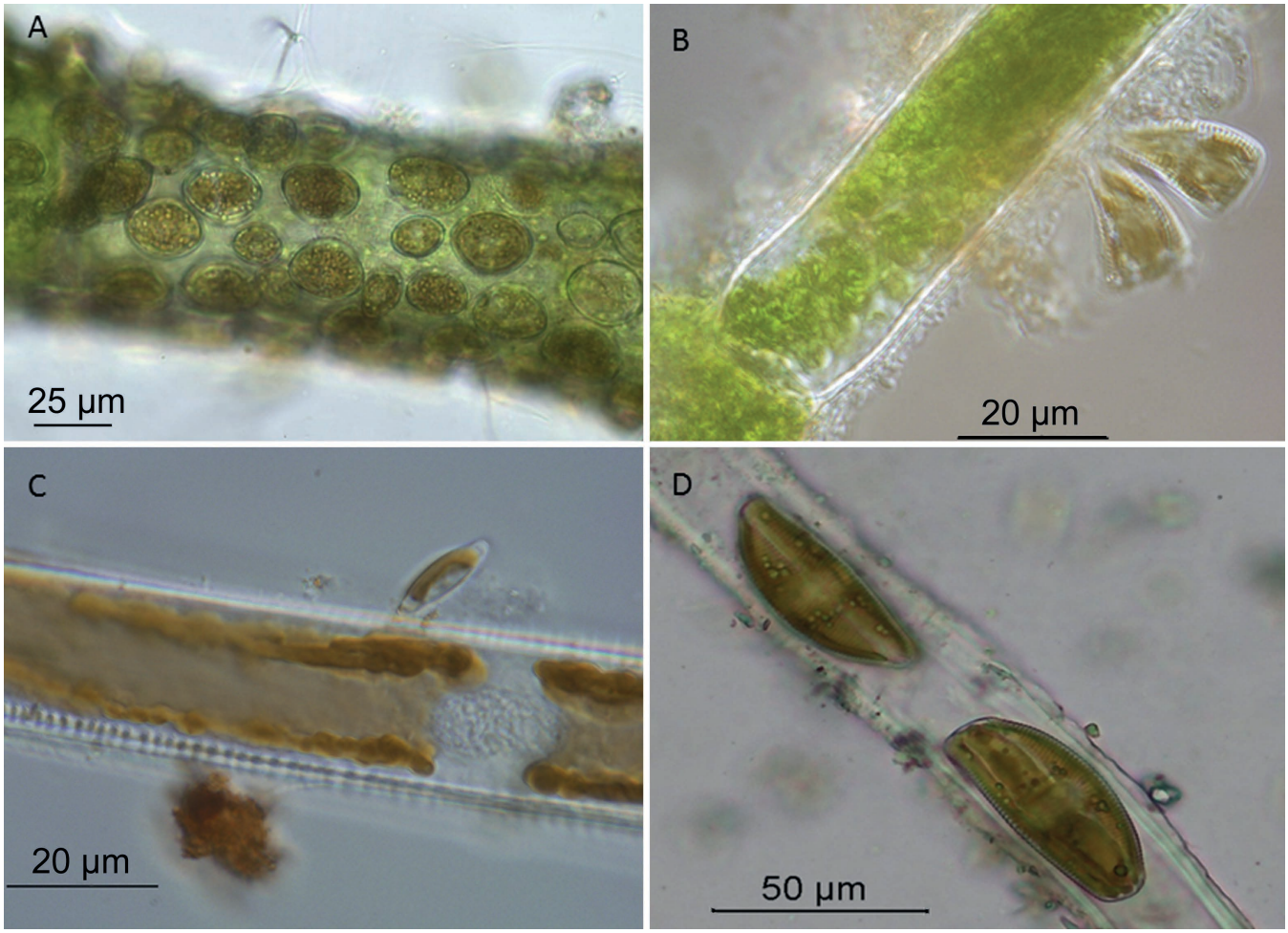

Figure 2 – Digestion of biofilms, necessary for detailed identification, leads to loss of information on host-epiphyte associations; A, closer inspection of live material reveals the presence of diatoms as epiphytes on other freshwater algae e.g. *Cocconeis pediculus*; B, *Rhoicosphenia abbreviata* attached to filaments of green algae; C, diatoms also act as hosts for other diatoms e.g. *Achnanthidium minutissimum*; D, removal of other organic material leads to other information loss e.g. presence of tube-dwelling species of diatoms such as *Encyonema* sp. Samples A & D taken from streams in Baden-Württemberg, Germany; samples B & C taken from Winford Brook, North Somerset, UK.

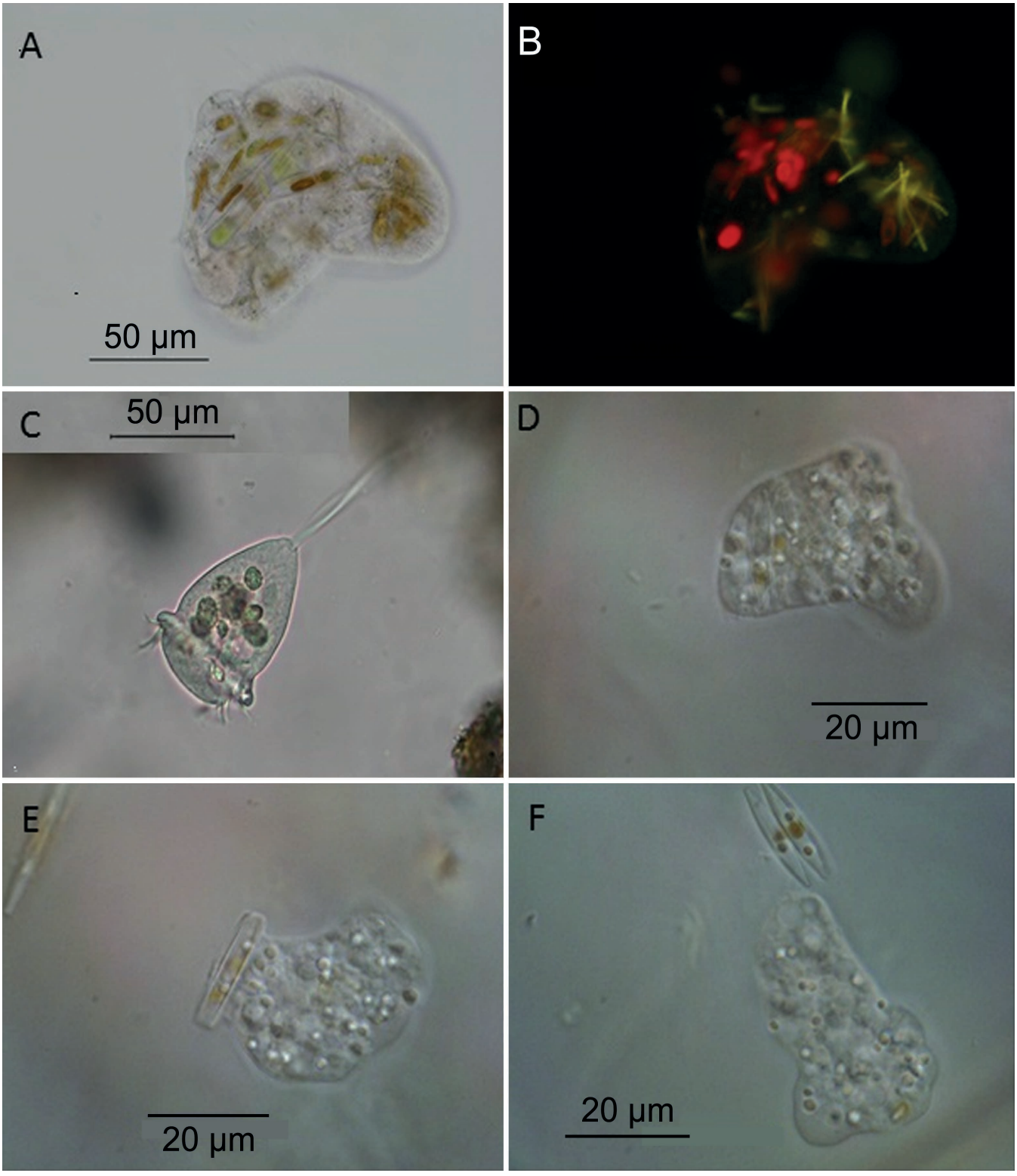

Figure 3 – We know very little about the selectivity of heterotrophs, yet many species are found within biofilms: A, a ciliate has consumed a variety of live pennate and centric diatoms and cyanobacterial filaments; B, algae autofluorescing red and cyanobacterial filaments yellow within the ciliate; C, other protists e.g. *Vorticella* select relatively smaller soft-bodied green algae; D, this amoeboid protist had previously consumed two relatively large diatoms; E, some reorganising of the cell contents is required to shuffle these engulfed cells to the periphery; F, exocytosis takes place to release the partially digested cells, and the amoeba rapidly moves away. This sequence of events lasted a few minutes. A–B & D–F taken from biofilm material from Winford Brook, North Somerset, UK; C taken from the Danube at Zimmern, Baden-Württemberg, Germany.

beit with many applied benefits) afforded by cleaned diatoms. We also take the opportunity to pose the question of whether there is information amidst this complexity that could be applied by ecologists to refine their insights. At the same time, we recognise that the term 'partial sight' could also be applied to the relatively limited role that specialists in one group of organisms play in the overall decision-making process. Legislation such as the Water Framework Directive (WFD) of the E.U. has ushered in a new era of ecological awareness in aquatic management in Europe but it also requires science to be integrated into national bureaucracies that have to balance claims for increased sensitivity against the need for consistency across whole countries, all on very tight budgets.

Functional groups

The simplest way to incorporate information on interactions within biofilms is to assign algal species to 'functional groups' or 'guilds' such as 'motile', 'stalked', 'adnate' etc. (Katoh 1992, Molloy 1992). This, in turn, depends upon observations of live material and allocation of taxa to one of a number of categories. In practice, such assignments are often performed at genus level, although exceptions occur. For example, the diatom *Nitzschia acicularis* (Kütz.) W.Sm. belongs to a 'motile' genus within biofilms, but is a frequent component of the phytoplankton of some lowland rivers in the UK and elsewhere, whilst *Didymosphenia geminata* (Lyngb.) M.Schmidt is usually seen living on the end of long self-generated stalks, but there are periods in its life cycle when cells are free-living and very definitely motile. Many species of *Encyonema* Kütz. live in mucilage tubes but some species are more often seen free-living. Several workers, including ourselves (Rosenkranz et al. unpublished data), have looked for patterns in the distribution of functional groups along ecological gradients such as nutrients or in response to toxins, in particular using diatoms (Steinman et al. 1992, Gottschalk & Kahlert 2012, Rimet & Bouchez 2012, Law et al. 2014, Tapolczai et al. 2016, Riato et al. 2017) though results are not always particularly conclusive.

General trends that have been observed include an increase in the proportion of motile diatoms along enrichment gradients (Passy 2007, Law et al. 2014). However, this has never really been converted into a take-home message that might inform the decisions that a catchment manager might use, and so rarely forms part of routine assessment methods. Significantly, the functional groups approach is not used in any WFD assessment method for benthic algae, apart from, in a few cases, differentiating between planktonic and benthic taxa, though even this can be fraught as not all species can be neatly categorised, either because of knowledge gaps or a

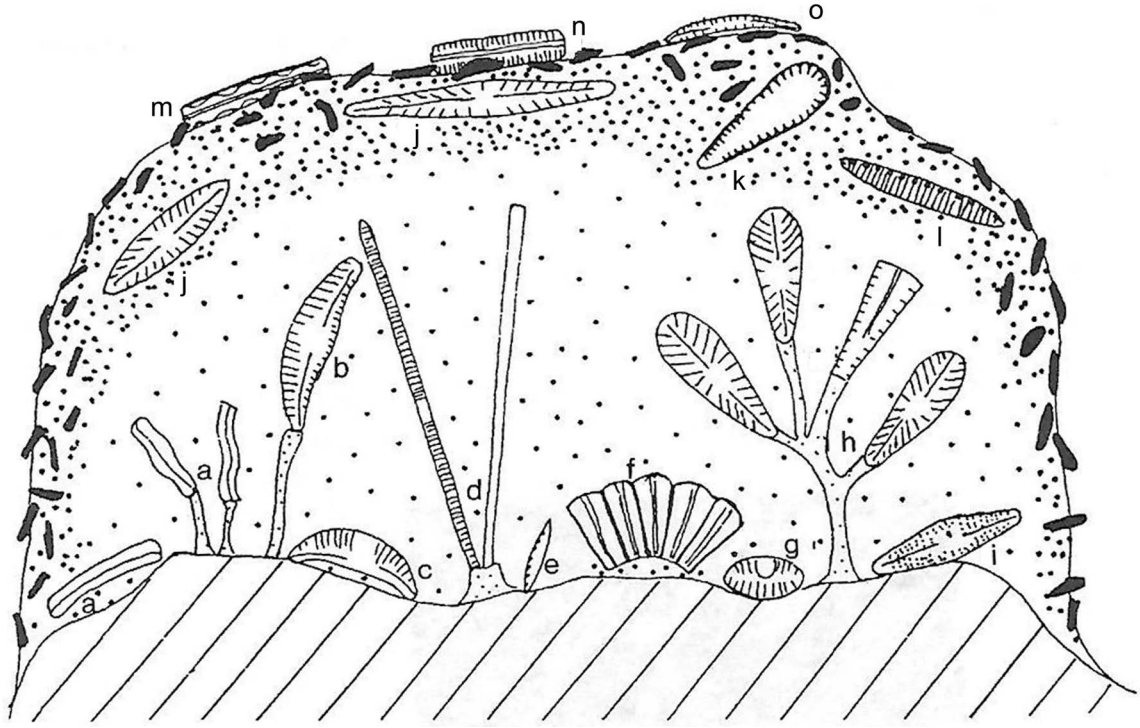

Figure 4 – Visualisation of the epilithic diatom flora on a stone surface together with the overlying silt and its associated flora (adapted from Round 1993): a, *Achnanthidium minutissimum*; b, *Cymbella* sp.; c, *Amphora pediculus*; d, *Ulnaria ulna*; e, *Nitzschia* sp.; f, *Fragilaria* sp.; g, *Planothidium lanceolatum*; h, *Gomphonema*; i, *Luticola goeppertiana*; j, *Navicula* sp.; k, *Surirella* sp.; l, *Nitzschia* sp.; m, *Cymatopleura* sp.; n, *Navicula* sp.; o, *Amphora* sp. Note that many generic names have changed since this diagram was produced. Where possible, these have been updated but names such as "*Fragilaria*" could now encompass a number of different genera.

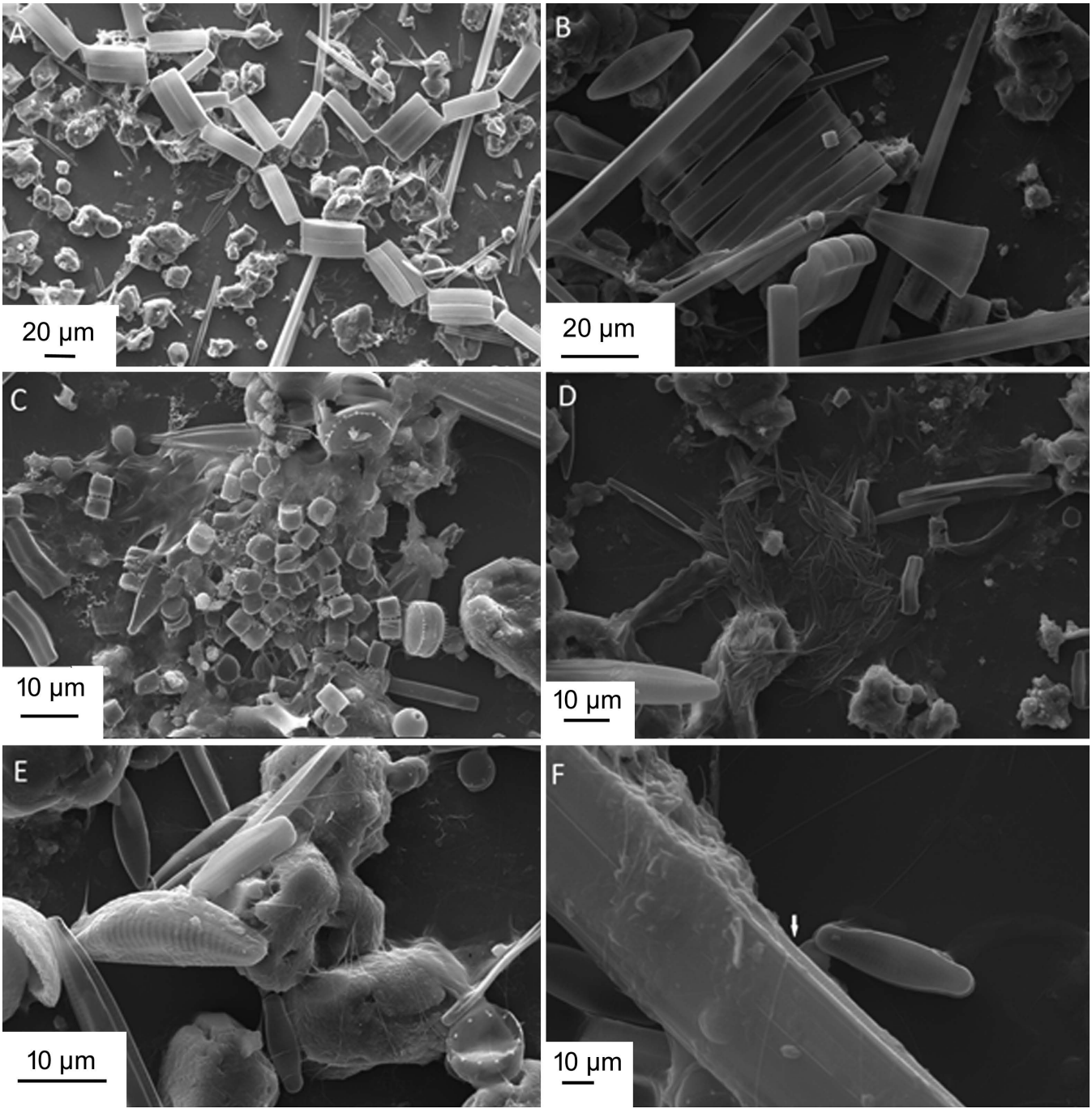

Figure 5 – Use of Scanning Electron Microscopy on an early stage biofilm colonised on a glass slide, located in an outdoor river mesocosm, inoculated with fresh river water from Winford Brook uncovers 'hidden' complexity in a 4-day old biofilm: A, relatively long chains of *Diatoma* form a framework for this biofilm with chains of *Fragilaria* providing further structural complexity; B, pennate diatoms frequently dominate the biofilms; C, on closer inspection, patches of the biofilm include many centric diatoms which may be a significant component of these biofilms; D, relatively small, weakly silicified cells are often 'missed' in routine sampling, though aggregates here may be numerically dominant; E, binding of algae and mineral particles together with extracellular polymeric substances (EPS) leads to stability within this early-stage biofilm; F, closer inspection of this large motile *Nitzschia* shows the presence of *Achnanthidium;* the latter genus being typically considered as adnate and associated with colonising of bare material, firmly attached to another diatom by means of EPS (white arrow), conferring 'motility' to this species within the biofilm. Images were taken with a Zeiss Evo 15 ESEM, shot in high vacuum mode with an SE1 detector.

genuine ability to exist in both suspended and attached situations; another key example being some species of *Aulacoseira* Thwaites (Krammer & Lange-Bertalot 1991). Thus, whilst a logical extension of the basic 'name and count' approach to analysing diatoms, might offer some potential insights, the use of functional groups is still problematic, not least in the underpinning of knowledge about relevant categories and how taxa should be allocated to these. The approach is still, mostly, 'blind' to other groups of algae which can form a significant part of the system, as well as to all heterotrophic organisms present in the biofilm. By way of illustration, some diatoms can be found living on green algae within biofilms, whilst other species can be found living on motile diatoms (fig. 2). Such observations highlight the difficulties of assigning species to a limited number of guilds, as these species may actually be experiencing very different environment if growing as epiphytes on other algae or directly on surfaces.

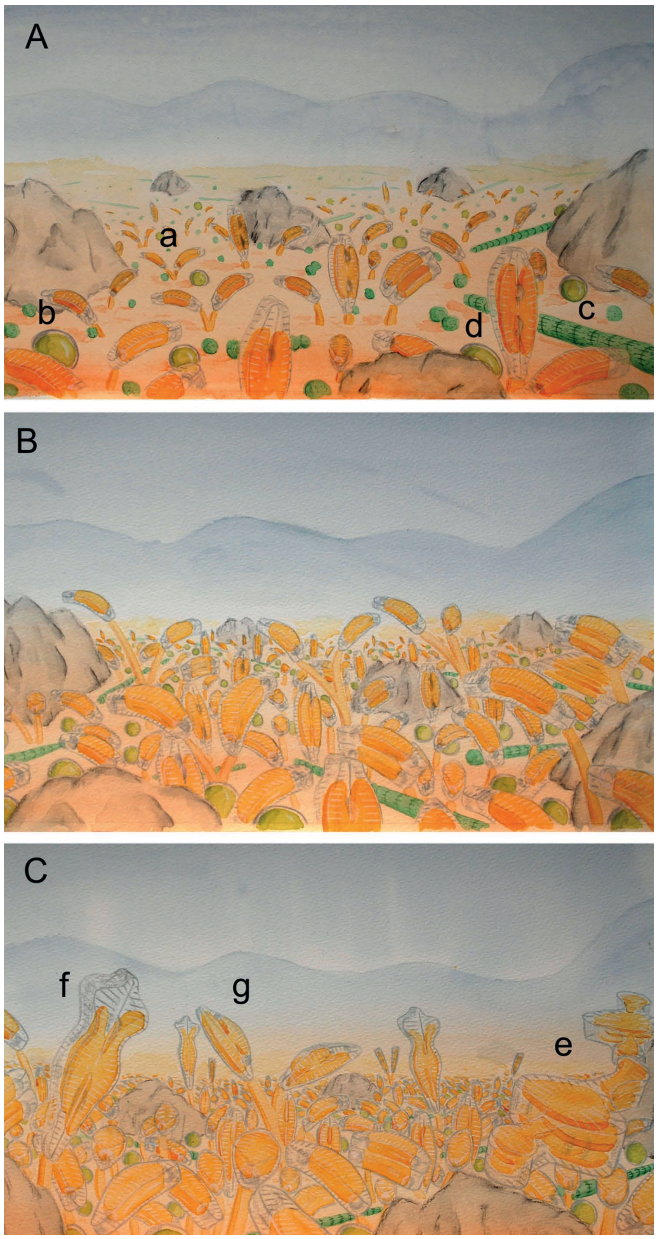

Figure 6 – The colonisation of bare rock surfaces by algae in Wastwater, visualised from experimental data in King (1999): A, a sparse community of colonising algae after two weeks; B, after three weeks much of the space has been filled in but there has been little change in composition. C, after six weeks, however, competition for resources leads to a situation which favours stalked and filamentous diatoms. Key to taxa: a, *Achnanthidium minutissimum*; b, spherical non-motile green algae; c, narrow *Phormidium*-like cyanobacterial filament; d, *Gomphonema parvulum* complex; e, *Tabellaria flocculosa*; f, *Gomphonema acuminatum*; g, *Cymbella* sp. This figure is not covered by the Creative Commons License (CC BY 4.0) of this paper and copyright is held by Martyn Kelly.

Recognising three-dimensional structure

Our understanding of the three-dimensional structure of biofilms has developed over time. Mann (2015) described preparations made by Lothar Geitler, in 1925, of rootlets of *Phragmites* from a German lake to demonstrate the arrangement of epiphytic diatoms. Patrick & Roberts (1979) and Round (1993) went on to visualise these as if a 'forest' within a mucilage matrix, though without the presence of other algae (fig. 4). These representations served to demonstrate that the 'niche' of diatom species is more than just a unimodal response to chemical pressures. Biggs et al. (1998), Yallop & Kelly (2006) and others developed this to emphasise a more dynamic understanding of biofilm structure which, in turn, opened up further possibilities. Recognising that stalked diatoms, for example, have a competitive advantage over many other diatoms in situations where light is low was the first step in looking beyond straightforward responses to gradients, allowing consideration both of three-dimensional structure and of the role of time. The light climate that is available to pioneer organisms, for example, will be very different to that for those at a more advanced stage of succession, whilst hydrological factors and top-down control from grazers (fig. 3) will also play a role (Biggs & Lowe 1994). The three-dimensional organisation of organisms remains, however, the most elusive aspect of biofilm structure, largely because natural communities are invariably destroyed or, at best, greatly distorted during the sample collection and analysis phases, whilst those grown on artificial substrata are often different in composition to nearby communities on natural surfaces. This aspect of biofilms has been little exploited in applied ecological studies, perhaps because it is less amenable to the reductive approach that characterises most studies of biofilms. There is, however, potential here, at the very least, to place the outcomes of quantitative analyses into context.

SEM has been used to good effect to understand three-dimensional structure (Lamb & Lowe 1987, Blenkinsopp & Lock 1994, Rimet et al. 2009). However, the need to fix, dehydrate and vacuum-coat with gold prior to examination can introduce distortions (more so for soft-bodied algae) and SEMs present opaque, monochrome worlds (fig. 5), which means that 'seeing' the microscopic world in this way is not always straightforward either. In practice, SEM images are most powerful as one of a suite of approaches that allows the reader/viewer to construct a mental image of the submerged microscopic world. Rimet et al. (2009), for example, used quantitative analyses based on cleaned diatoms, identified

and enumerated using light microscopy, to establish a narrative of how biofilms respond to changes in their ambient environment, with SEMs offering qualitative insights that enriched the understanding offered by these analyses. Other visualisation techniques that have been used with success to understand biofilms include confocal laser-scanning microscopy (Battin et al. 2003) and epifluorescence microscopy (Barranguet et al. 2004, Bar-Zeev et al. 2012). Neu et al. (2010) review these and other advanced imaging techniques.

A further possibility is to gather all available information on both a sample and the organisms it contains and to visualise the microscopic world of biofilms in an artistic way (Kelly 2012). The illustrations of biofilms from artificial surfaces submersed in Wastwater (Cumbria, UK) presented here (fig. 6) offer similar 'interpretations' of data – in this case quantitative analyses of diatoms and soft-bodied algae (King 1999) – and show how a 'forest'-like growth, with an upper storey of stalked diatoms (*Gomphonema acuminatum* Ehrenb. and species of *Cymbella* C.Agardh) to emerge from the *Achnanthidium minutissimum* (Kütz.) Czarn.-dominated 'ground layer'. This approach sidesteps the distortions introduced by SEM and other imaging techniques whilst, admittedly, adding another: imagination.

CONCLUSIONS

This essay is about 'seeing' with a microscope, building, in particular, on Hacking's (1981) conclusion that images observed with a microscope make sense only because of interactions that go beyond patterns of light hitting the retina. 'Seeing', in other words, is a higher-level process that involves interactions with the brain and with the specimen itself. Ecologists, however, have to go beyond consideration of individual specimens, building these individual observations up to give a picture of the biological community that was present at the time of sampling. The default, we have argued, is a reductionist, albeit useful, view of the microscopic world.

Why do we need to challenge this approach? Ecologists who study the macroscopic world have direct experience of how organisms relate to one another. They are able to look at a list of species from a site and build a mental image of the community which can then form the basis of their interpretation; albeit typically obtaining only the above-ground view. Ecologists of the microscopic world are moving, aided by multivariate statistics, to a more abstract view of the world. Their outputs, nonetheless, still benefit from interpretation – from understanding the significance of the rise or fall of a particular species over time or between two sites. It is rarely possible (or necessarily desirable) to build information other than the response to a few key ecological gradients into models, so those who interpret outputs need to call upon knowledge and experience if they are to offer an informed opinion. Above all, we need to challenge the perception that qualitative information is less relevant than quantitative, that descriptive, observational accounts constitute 'soft' science in contrast to 'hard' quantitative studies. The truth is often quite different, as Fryer (1987) eloquently argued.

How do we achieve this nuanced understanding from the limited data upon which ecologists of the microscopic world depend? This is done, we believe, by mentally hybridising the data with external information, partly from the literature (although this type of information is very diffuse) and partly from experience. Even simplistic diagrams such as that of Round (1993) can provide initial 'hypotheses' of biofilm structure against which taxa lists can be compared in mental 'thought experiments' that either organise the taxa in a list into a meaningful three-dimensional arrangement or alter the hypothesis or both. These can be further challenged and refined by the use of more sophisticated visual imaging approaches (e.g. SEM, confocal and epifluouresence microscopy), as well as by more detailed analyses of biofilm structure and function. Over time, these mental 'schemata' develop and adapt to different circumstances. This may not sound like hard 'science' but is, in fact, rooted in the psychology of perception (Gombrich 1977) which, itself, is influenced by Popper's (1962) theories of 'conjectures and refutations'.

Developing this richer understanding of biofilm structure, however, needs time and, more particularly, opportunities for those who collect the basic data to assimilate the primary and secondary literature and to make their own observations from live material (our assumption here is that most European data on the condition of biofilms is derived primarily from analyses of cleaned diatoms). Our concern is that diatom analysis has become a streamlined process, managed by organisations with limited resources and often out-sourced through competitive tendering. This may produce reliable feedstock for the models on which ecological assessment is based, but is there sufficient time to acquire the peripheral experience and knowledge that underpins interpretation? We know this is only part of the story. As we enter a new era where molecular barcoding might replace analysis by light microscopy in some contexts (Vasselon et al. 2017, Hering et al. 2018), this situation is likely to become more acute. Whilst the basis for identification, and the equipment required, are very different (Mann et al. 2010), barcoding is really little more than a variant of the traditional 'name and count' approach, carrying the same challenges for ecologists as light microscopy. In the automated 'high throughput' laboratories, the first time that a biologist encounters the sample may be as a list of Latin binomials on a spreadsheet. In the transition period, the first generation of biologists responsible for interpreting results may be those who learned their craft with light microscopes but, as time passes, organisations will see little benefit in training staff in traditional identification skills or in utilising their local site knowledge. Some have promoted the option of abandoning traditional taxonomy altogether, preferring 'molecular operational taxonomic units' instead (Visco et al. 2015), at which point any attempt to understand the subtleties in biofilm organisation will be lost completely.

In some ways, perhaps, this is an inevitable end-point of a series of decisions – mostly logical when viewed separately but which, cumulatively, have led the study of benthic microscopic algae in rivers along a road to perdition. The focus on the silica cell wall of diatoms has, undoubtedly, led to an impressive understanding of taxonomy and phylogenetics and to a number of ecological insights too. But this has come at a cost to a broader understanding of the structure and functioning of benthic biofilms. Yet, at the same time, the living biofilm presents numerous challenges to anyone

attempting to collect quantitative data on composition. The paradox can be at least partially resolved by accepting that how we 'see' benthic biofilm communities requires integration of direct microscopical observations with a wider range of indirect knowledge than is the case when dealing with individual cells. It is possible to construct an impression of what a microbial community may look like if we had the perspective of a chironomid larvae pushing its way through the tangle of filaments and stalks, or were able to swim above it, as if it were a kelp forest, and this qualitative – and partially imagined view – is a valuable adjunct to the hard data that is the cornerstone of modern quantitative science.

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