

Microalgal (Microphytobenthic) Biofilms in Shallow Coastal Waters: How Important are Species?

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Microphytobenthic biofilms, dominated by diatoms, are important components in many intertidal and shallow water marine environments. Much of our understanding of their functioning comes from studies that have not treated species composition as a significant factor. Conversely, many studies concerned with species distribution have not encompassed our understanding of the dynamic nature of biofilms. This paper presents data to show there are significant differences in the behaviour and physiology of different taxa of diatoms within the same biofilm, and that species respond differently to the external nutrient environment. These data indicate that consideration of the importance of the species-niche (both autecological and synecological) can be beneficial to both taxonomy and ecology.

In many marine and estuarine environments, large areas of shallow intertidal and subtidal habitats are dominated by microalgal assemblages. These are known by a number of terms, epipsammon, epipelon, periphyton, benthic microalgae and microphytobenthos (Round et al. 1990). Although these communities are mixed assemblages containing representatives of many algal groups, those communities found on muddy, and to some extent, sandy sediments are dominated by diatoms (Admiraal 1984; Underwood and Kromkamp 1999). Overall species richness of marine benthic diatoms is quite high, particularly over geographical scales (Witkowski et al. 2000), yet within a particular environment, it is more usual to find only a few (20+) taxa that are numerically dominant within assemblages (Admiraal and Peletier 1980; Colijn and Dijkema 1981; Underwood 1994; Thornton et al. 2002).

The ecology of these systems has been extensively studied, in particular estuarine intertidal habitats, saltmarshes and in shallow coastal seas, primarily the Baltic. From these studies we know that in ecological terms, microphytobenthic biofilms are extremely important in ecosystem functioning. The primary production of microphytobenthos can be very high, and this can contribute a significant proportion of the autochthonous carbon production in estuarine and shallow lagoon environments (Underwood and Kromkamp 1999; Cahoon 1999). Microphytobenthos also mediate a whole range of biogeochemical processes, such as nutrient exchange and nitrogen cycling and attenuation of nitrogen loads (Dong et al. 2000; Thornton et al. 2002). This is because biofilms occur at the interface between the water column and the sediment, and within the relatively narrow zone, high rates of biological activity can result in oxygen supersaturation, release of organic carbon exudates, anaerobic conditions and rapidly changing chemical gradients (Underwood and Kromkamp 1999). Microphytobenthos can sequester phosphorous, silicates and nitrogen from the water column and both enhance, and inhibit, denitrification and coupled nitrification-denitrification (Sundbäck et al. 1991; Rysgaard et al. 1994; Dong et al. 2000). As such, extensive areas of

biofilms can significantly attenuate the flux of nutrients through coastal areas (Thornton et al. 2002). Another property of biofilms is their ability to increase the stability of sediments, usually through the production of mucilage (extracellular polymeric substances, EPS) (Smith and Underwood 1998, 2000; de Brouwer and Stal 2001), which acts to increase the critical erosion stress required to initiate erosion of flocs from the sediment bed (Widdows et al. 2000; Tolhurst et al. 1999; Underwood and Paterson 2003). Thus biofilm-inhabited sediments are more difficult to erode, and biofilm-inhabited sediments more rapidly trap and hold, fine sediments (Underwood and Paterson 1993, 2003).

As this brief overview makes clear, diatom-rich biofilms play a very important role in the ecology of shallow-water systems. However, a study of the literature clearly shows that functional studies on biofilms predominantly ignore species composition, and treat the assemblage as a black box — “a biofilm.” Conversely papers dealing with species distribution tend to rely on a very descriptive approach to defining the environment, usually in terms of nutrient concentrations, salinity and temperature. These latter (state) variables tend to be taken as a valid description of the potential niche of a species, without regard to the changeable nature of these variables over short term (hours - fluxes of nutrients rather than concentrations, diel fluctuations in temperature) and medium term (days — changes in tidal exposure period, meteorological conditions) time scales.

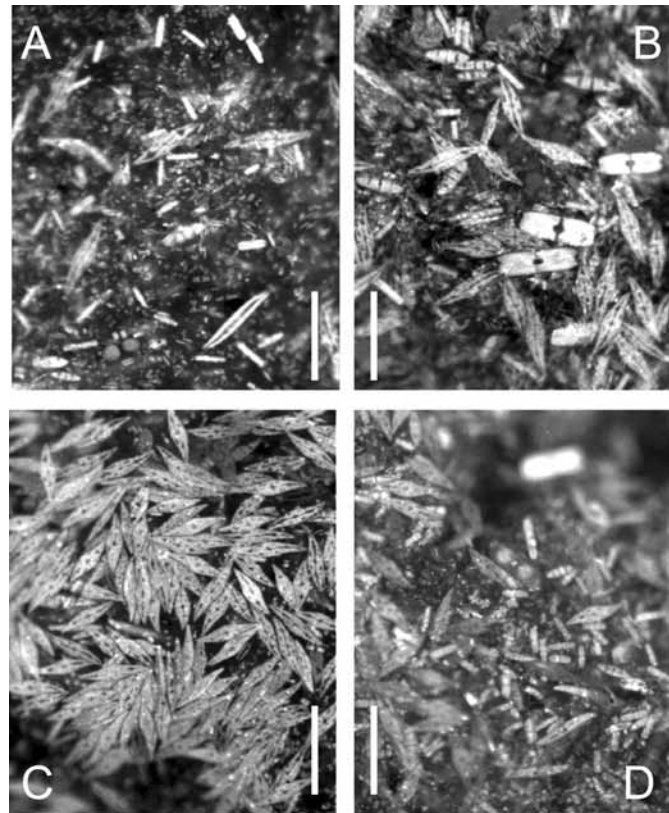
Can knowledge about the species of diatoms in a biofilm improve on our understanding of biofilm processes? Why should functional ecologists be interested in species composition, and therefore by association, require a good and robust taxonomy? This paper aims to show that, at least in two areas, photosynthesis and response to nutrients, species-information can improve our understanding of biofilm function.

PHOTOSYNTHESIS AND DIFFERENCES BETWEEN BIOFILMS

In general, it has been shown that light intensity influences the rate of photosynthesis and overall primary production of biofilms (Underwood and Kromkamp 1999). This reasoning underlies many models that predict benthic primary production on the basis of irradiance data and biomass (Serôdio and Catarino 2000). However, this view is currently being reassessed. This is partly because some studies are based on using slurry systems for measuring photosynthesis. Using a slurry (a mixture of biofilm and sediment) removes the 3D structure of the biofilm and prevents cells from following their natural migration patterns. Placing cells in suspension means that they behave like phytoplankton and respond to light accordingly (Underwood and Kromkamp 1999). Recent developments in imaging technology have now made it possible to measure the photosynthetic performance of different cells within intact biofilms (Oxborough et al. 2000). In the natural environment, microphytobenthos show complex behaviours in response to light. In a study of microphytobenthos in the Tagus estuary, Perkins et al. (2001) showed that during tidal exposure when light levels are low, cells accumulate at the surface, but migrate away from the surface at high irradiances. This was described as a type of behavioural photoacclimation. In the Tagus estuary (Miles and Sundbäck 2000; Perkins et al. 2001), and Colne estuary (Perkins et al., unpublished data), this can result in highest rates of photosynthesis early in the morning, despite light levels increasing towards midday, as cells position themselves in an optimum light climate within the biofilm. These movements can be very rapid, and the surface community can change from a biofilm dominated by diatoms to one dominated by euglenoid algae in 10 minutes as light intensity increases (Perkins et al. 2002). Similar patterns of migration and migration responses of different microphytobenthos have been shown using low temperature electron microscopy coupled with measurements of spectral reflectance off mudflats (Paterson et al. 1998). Such micro-migrations are superimposed over

the tidal migration of cells to and from the sediments surface. Fig. 1A–D shows the change in species composition at the same point on the surface of a biofilm over a diel exposure period. Substantial changes in species composition are evident, so that measurements over a period of time will be measuring the photosynthesis of different populations of diatoms. Imaging has revealed that individual taxa can have significantly different photosynthetic efficiencies (the rate at which captured light energy is converted to electrons and used in photosynthesis). Oxborough et al. 2000 showed that the photosynthetic efficiency of three diatom species (*Pleurosigma angulatum* (Quekett) W. Sm., *Gyrosigma littorale* (W. Sm.) Griffith et Henfrey and *Plagiotropis vitrea* (W. Sm.) Kuntze differed from each other at lower light intensities, and all three species were significantly less efficient than *Euglena* sp. at higher irradiances ($> 500 \mu\text{mol m}^{-2} \text{s}^{-1}$). Differences between species also appear during tidal exposure cycles. Fig. 2A shows the composition of a surface biofilm during a diel exposure period. Three diatom taxa dominated this particular biofilm (*P. angulatum*, *P. vitrea* and *Nitzschia dubia* W. Sm.), with small *Nitzschia* spp becoming abundant at the end of the tidal period. At the beginning of the day, the three diatom taxa had the same photosynthetic efficiency, but by midday, all three taxa showed reduced efficiencies, but also that there were significant differences in photosynthetic efficiency between species (Fig. 2B). These differences will combine to produce overall biofilm functioning, and may even be selective for particular species under different conditions. These hypotheses need to be tested, but it is evident from these data that all cells within a biofilm do not behave the same. Thus ecological function is a property that requires knowledge of species composition (including good taxonomic resolution).

This requirement to incorporate species and assemblage information with measures of community functioning is clearly shown in a study of tropical microphytobenthos. Measurements of photosynthetic efficiency, characterisation of the light climate within the sediments, algal biomass and maximum rates of relative electron transport (a proxy measure for photosynthesis) were found to be significantly different between microphytobenthos from different habitats (Underwood 2002). The conventional explanation (in the absence of any species composition information)



FIGURES 1A–D. Temporal changes in the surface composition of a microphytobenthic biofilm from the Colne estuary, UK, over a tidal exposure period taken using a microscope-based, fluorescence imaging system (Oxborough et al. 2000). All images are of the same area at (A) 07.30 h, (B) 11.00 h, (C) 15.30 h, (D) 18.00 h. Scale bar = 200 μm .

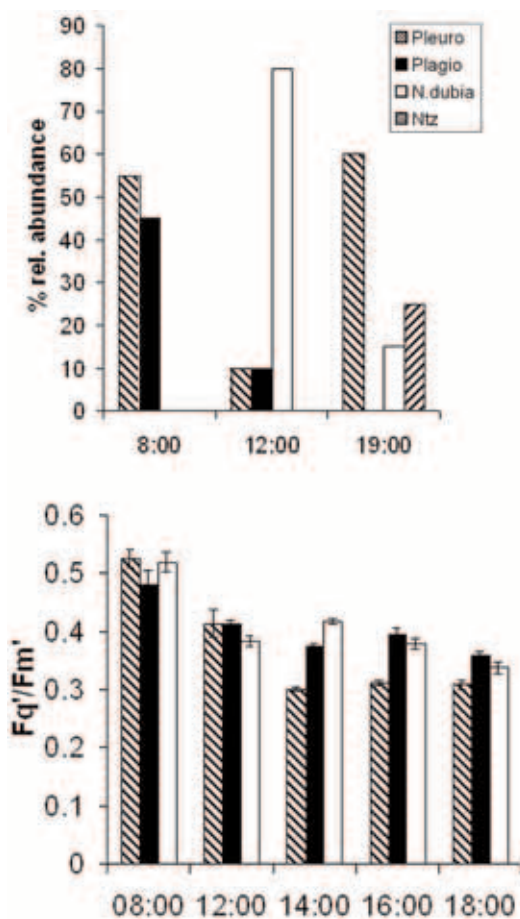


FIGURE 2. (A). Relative abundance (%) of 4 taxa of benthic diatom present at the surface of a biofilm at 3 periods over a diel cycle. (B) Measures of individual cell photosynthetic efficiency (Fq' / Fm') showing significant differences between taxa during the later part of the diel period. Values means \pm SE, $n = 10$ or greater.

Navicula species. The shallow subtidal biofilms in the coral sands and seagrasses had a different flora, dominated by *Diploneis*, *Amphora*, *Psammodictyon*, *Tryblionella* and *Mastogloia* and showed little evidence of vertical migration in response to light stress or tidal rhythms. Thus the differences in biofilm function can also be mapped onto differences in species composition as well as the more conventional explanations of nutrient limitation or photoacclimation.

SPECIES AS NUTRIENT INDICATORS

There are increasing data to support the hypothesis that microphytobenthos can be used as indicators of environmental conditions, particularly nutrient concentrations and salinity. This is leading to the development of trophic indices for marine and estuarine systems similar to those currently available for freshwaters. The estuarine environment is a particular challenge in this respect,

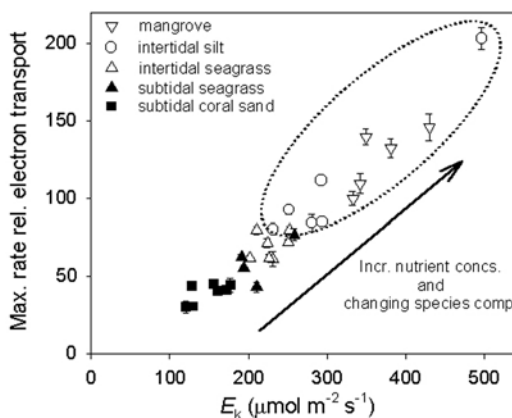


FIGURE 3. Relationships between the maximum relative electron transport rate and the light saturation parameter E_k for microphytobenthic biofilms from 5 different tropical marine habitats, showing the significant correlation with nutrients, but also changing species composition and the occurrence of vertical migration within particular biofilms (dotted ellipse). (Redrawn from Underwood 2002).

would be to explain the significant correlations between maximum rates of relative electron transport (a proxy measure for photosynthesis) and E_k (the half light saturation coefficient) with nutrient concentrations in terms of nutrient limitation causing biofilms to be less efficient. However, this significant “nutrient photosynthesis” relationship also corresponded directly with differences in species composition between biofilms (Fig. 3). Biofilms showing the highest values of photosynthetic parameters also exhibited strong vertical migration in response to light and comprised many large, motile taxa, such as *Hantzschia virgata* (Roper) Grun., *Berkeleya scopulorum* (Bréb.) Cox and

partly because of the significant human impacts on estuarine habitats, due to proximity of large centres of human habitation and industry (Hessen 1999; Nedwell et al. 1999), but also because of the wide range of conditions estuarine taxa have to tolerate. Thus estuarine microphytobenthos appear to be tolerant of a fluctuating range of conditions (Admiraal 1984). In the Baltic Sea, various diatom taxa have been shown to be sensitive to the well-defined and constant salinity gradient present in the Baltic Sea (Snoeijs 1993; Snoeijs and Vilbaste 1994; Snoeijs and Potopova 1995; Snoeijs and Kasperovièiene 1996; Snoeijs and Balashova 1998). This stable gradient may allow quite narrow species niches to be defined, but it would be incorrect to attribute the same salinity niche to one of these taxa if it was found in a meso- or macrotidal estuarine environment. However, even in the more variable environment of tidal estuaries, species shifts with nutrients can be demonstrated. A number of field studies, long-term data sets, experimental-nutrient enrichments, experimental studies in mesocosms and on isolated cultures have all indicated that some taxa (e.g., *Fallacia pygmaea*, *Navicula salinarum*) have greater tolerance to high nutrient concentrations and organic loads and may potentially be indicators (Sullivan 1976, 1981; Admiraal 1984; Peletier 1996; Underwood et al. 1998; Sullivan 1999). It is clear that broad scale patterns of species variability reflect changed environmental conditions (sediment type, water flow, salinity). However, more detailed studies are needed to demonstrate that the diatom community at a particular site will change if nutrient loads increase or decrease (Peletier 1996). In particular, experimental testing of hypotheses derived from correlative field measurements of species occurrence and environmental variables, to demonstrate causes and mechanisms for changing species composition is needed. For example, Figure 4 shows the maximum intrinsic growth rate (μd^{-1}) of cultures of *Navicula phyllepta* Kützing isolated from the Colne estuary, U.K. and grown in a range of nitrogen con-

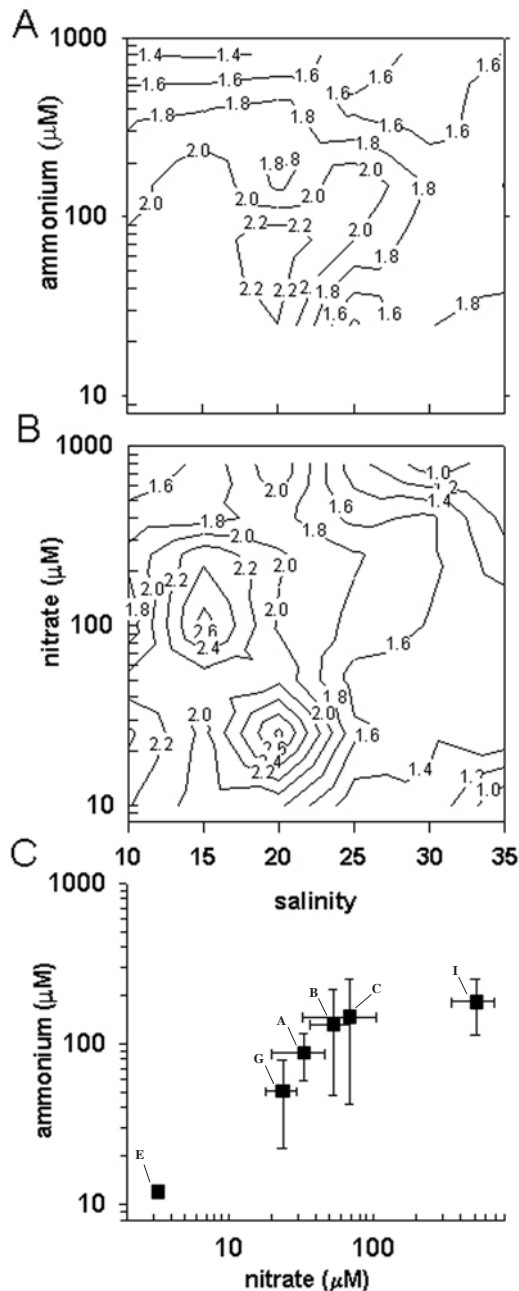


FIGURE 4. Maximum intrinsic growth rates (μd^{-1}) of *Navicula phyllepta* grown in culture in a range of ammonium and salinity (4A) or nitrate and salinity (4B) conditions. (4C) Occurrence of various diatom assemblages related to ammonium and nitrate concentrations over a 12 month period in the Colne estuary, UK. Assemblages A, B and C were dominated by *N. phyllepta*. (Redrawn from Underwood and Provot 2000 and Thornton et al. 2002.)

centrations (ammonium and nitrate) at different salinities. *Navicula phyllepta* showed maximum growth within an inorganic nitrogen range of between 50–300 μM and at salinities between 15–25 ppt (Fig 4A, 4B). These culture data agree closely with field data, where assemblages dominated by *N. phyllepta* were present in the estuary when nitrate and ammonium concentrations were within this range (Fig 4C). Underwood and Provot (2000) showed that three taxa of *Navicula* had different, but overlapping nitrogen and salinity optima, and these may be partly an explanation for field patterns. Such experimental approaches do need to consider the physiological responses of cells to changing environments, particularly the ability of cells to adapt to changing conditions.

CONCLUSIONS

There are good reasons to encourage a “marriage” of taxonomy with ecology. For the reasons outlined above, the ecological importance of biofilms has meant that substantial funding has been provided for scientific investigations of biofilm functioning in the marine environment. Such studies have been quick to embrace new technologies to increase the resolution of measurement down to the microscale and now it is possible to measure and visualise the environment at a scale comparable with that of individual diatom cells. This provides a great opportunity to link ecosystem functioning with knowledge of individual species. Up till now, much of this ecological research has tended to ignore species composition as a factor in biofilm functioning. New techniques, such as *in situ* quantitative PCR and using fluorescently-labelled markers to identify species, provide a mechanism for taxonomic expertise to interface with functional studies. This does require diatom taxonomy to broaden its view on what characteristics of diatoms are important taxonomically and a willingness to “widen” the species concept away from the rigid morphological definition of a species. The “marriage” will be harmonious when separations of species on taxonomic grounds are found to match, in some way, the functioning of those taxa in the environment. There is plenty of scope within the marine microphytobenthos to investigate the concept of the species-niche in its broadest sense (both autecological and synecological) and to use this information in both taxonomy and ecology.

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