Graham J. C. Underwood and Mark Barnett

What determines species composition in microphytobenthic biofilms?

Abstract

Despite significant advances over the past few years in our understanding of biofilm physiology and functioning, we still have a very patchy and unclear picture of what influences the species composition of biofilms. Much of our understanding of biofilm 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. Nutrients are a strong contender for an environmental variable that influences species composition. Yet, much of the available data comes from mechanistic floristic surveys that do not really inform as to the driving mechanisms determining species composition. So does the local nutrient environment really select for particular taxa? Can we separate out the effects of nutrients, sediment particle size, species physiology and other, perhaps, unmeasured, covariables that help to define the synecological niche? In this paper we give a brief review of some of the main areas that influence species composition, and look at approaches where these different issues have been addressed.

Introduction

Microphytobenthos play an important role in the ecology of many coastal habitats. Abundant biofilms are present on intertidal sand and mudflats (Underwood and Kromkamp 1999), within saltmarsh creeks and amongst saltmarsh plants (Sullivan 1999), and, where water clarity is sufficient to permit light penetration, on subtidal sediments (Sundbäck and Jönsonn 1988; Underwood 2002). The primary production of microphytobenthos ranges between 50-875 g C m^{-2} a^{-1} and the activity of the films on the sediment surface influences nutrient transformations within the sediments (nitrification, denitrification) and nutrient exchange across the sediment-water inter-face (Underwood and Kromkamp 1999; Thornton *et al.* 1999; Dong *et al.* 2000; Rysgaard-Petersen 2003).

Microphytobenthos consist of a number of phylogenetic groups, with diatoms, cyanobacteria and euglenophytes being the most common. Diatoms are ubiquitous and are usually dominant in terms of biomass, but there are situations when other photosynthetic microorganisms are more abundant (Cahoon 1999). This paper will

generally discuss patterns in diatom abundance, where there is a reasonable literature. Our knowledge of the dynamics of the other algal groups in microphytobenthos is patchy in comparison, and more research is needed on the role of other taxonomic groups (Sullivan and Currin 2000). The diatom composition of microphytobenthic mats changes both seasonally and spatially within estuaries. Spatial changes are usually related to the salinity (and associated nutrient) gradients within estuaries. From such studies some general patterns concerning species distribution are revealed (Sullivan 1999; Underwood and Kromkamp 1999). Yet, intercomparison between studies is often difficult due to differences in the major taxa found in different estuaries, or differences in nomenclature and identification by different authors. The high level of synonymy in benthic diatom taxonomy and the problems of standardisation of taxonomy between different sets of literature also confound these problems.

There is a requirement to understand the niches of estuarine diatoms. It has been known since the 1970's that the species within a biofilm do not function identically. Round (1979) found differences in the pattern of migration of different species within biofilms in Mass. Harbour. Different taxa of microphytobenthos change their position within the biofilm in response to a number of variables, of which probably the best studied is irradiance. *Euglena* and diatoms rapidly change position at the surface of sediments as light intensities increase and decrease (Paterson *et al*. 1998; Perkins *et al*. 2002; Kingston 1999). Single cell PAM fluorescence imaging has shown that *Euglena* has significantly higher operating efficiencies than diatoms within the same biofilm at higher irradiances (Oxborough *et al*. 2000), and such a strategy for *Euglena* fits with its observed pattern of occurrence, being commoner during the summer months (Underwood 1994). Different taxa migrate to the surface of a biofilm at different times throughout a diel tidal exposure and different species exhibit different photosynthetic efficiencies at the same irradiance (Perkins *et al*. 2002; Underwood *et al*. 2005). This indicates that species may function quite differently. By accepting the premise that the species composition influences biofilm function (Underwood *et al*. 2005), it becomes important to be able to predict what species we might find in a particular environment. Can this be done with any certainty?

Accurate description of the niche of organisms has been a problem in biology for a long period. Charles Darwin, in his published *Journal of the Voyages of the Beagle* (first pub 1839), wrote,

> 'we do not steadily bear in mind, how profoundly ignorant we are of the conditions of existence of every animal, nor do we always remember, that some check is constantly preventing the too rapid increase of every organism being left in a state of nature. …we feel so little surprise at one, of two species closely allied in habits, being rare and the other abundant in the same district, or again, that one *(species)* should be abundant in one district and another, filling the same place in the economy of nature, should be abundant in another district, differing very little in its conditions. If asked how this is, one immediately replies that it is determined by some slight difference in climate, food or the number of enemies: yet how rarely, if ever, we can point to the precise cause and manner of action of the check. We are, therefore, driven to the conclusion, that causes generally quite unappreciable by us, determine whether a given species shall be abundant or scanty in numbers.' *(Darwin 1860)*.

Despite the major advances in our understanding of biofilm functioning in the last 20 years (since Admiraal, 1984), these cautionary words of Darwin are highly valid when applied to our current understanding of the distribution and abundance of microphytobenthic taxa.

Large scale differences in MPB species composition between estuaries

At what spatial scale do differences in biofilm species composition in estuaries occur? Diatom assemblages from the Severn, Colne and Blackwater estuaries, U.K. were compared. These studies took place over a number of years; Severn estuary 1990-1991, Blackwater 1992-1995, Colne estuary 1996-1998, and further details are given in Underwood (1994; 1997; 2000) and Thornton *et al*. (2002). The studies all had a similarity of approach and taxonomic standardisation permitting intercomparison (Table 1). Multivariate analysis was applied to relative abundance (RA) data and also to biomass-weighted RA data (RA of each taxon in a sample multiplied by the sediment Chl *a* concentration for that sample.

PCA of relative abundance data

Principal components 1-4 derived from relative abundance data for the diatom assemblages for the three estuaries explained 54.9% of the variation in the data set (Table 2). The data for each of the estuaries sampled generally clustered together, with the Colne and Blackwater (Northey Island) estuaries separated from the Severn estuary by PC1, with PC2 separating Colne from Blackwater samples (Figure 1). The major gradient (PC1) was due to high relative abundances of *Navicula pargemina*, *N. flanatica*, *Nitzschia epithemioides*, *C. signata* and *Rhaphoneis* found in the Severn estuary samples, compared to high RA's of *N. phyllepta*, *N. salinarum*, *Plagiotropis vitrea* and other species found in the Colne-Blackwater complex (Figure 1). Lower shore samples from the Blackwater estuary showed a greater overlap with Colne estuary mudflat samples than those from the upper saltmarsh. Colne mudflat samples tended to have higher RA of *Plagiotropis, N. gregaria*, *Staurophora amphioxys* and *N. rostellata*, while the saltmarsh sites in the Blackwater estuary were characterised by higher RAs of *Nitzschia frustulum, N. sigma, C. closterium* and *Amphora marina* (Figure 1).

Principal component 1, derived from species abundance data, was significantly negatively correlated (Table 2) with sediment ash free dry weight, chl *a*, and total and colloidal carbohydrate concentrations (all environmental variables that represent a gradient of increasing organic content, nutrient availability and microalgal biomass in the sediments), and negatively correlated with salinity. The same environmental variables (with the addition of temperature) were also negatively correlated with PC2. Thus *N. phyllepta*, *N. frustulum*, *Cylindrotheca closterium* and *N. salinarum* show greater relative abundances in more eutrophic sites and at higher temperatures, while *C. signata*, *N. epithemiodes* and *N. pargemina* also favour warmer conditions, but have higher relative abundances in less eutropic conditions. The following diatom taxa, *Raphoneis minutissima*, *N. flanatica*, *Coscinodiscus* may thus be representative of lower temperatures, higher salinity and lower microphytobenthic biomass and nutrient conditions.

Table 1. Median (minimum-maximum) relative abundance (RA) values of benthic diatom species (taxa occurring at least once at over 1% RA) and median (min-max) values for environmental variables for 3 different U.K. estuaries.

Taxon	code in Figs1-4	Severn	Blackwater	Colne
Navicula phyllepta Kütz.	NPHY	2 (0-64)	35 (5-75)	17 (0-97)
N. pargemina Underwood & Yallop	NPAR	23 (0-97)	5 (5-75)	ND
N. flanatica Grun.	NFLA	0 (0-58)	5 (5-35)	5 (0-64)
N. salinarum Grun.	NSAL	0 (0-5)	5 (5-75)	0 (0-51)
N. cincta Ehrenb.	NCIN	0 (0-2)	15 (5-75)	0 (0-12)
N. rostellata Kütz	NROST	0.5 (0-20)	5 (5-75)	2 (0-48)
N. digitoradiata (Greg.) A. Schmidt	NDIGIT	0 (0-1)	5 (5-35)	0 (0-13)
N. gregaria Donkin	NGREG	2 (0-59)	5 (5-75)	3 (0-57)
N. diserta Hustedt	NDIS	ND	5 (3-35)	0 (0-10)
Fallacia pygmaea (Kütz.) Stickle & D.G. Mann	FPYG	0 (0-1)	ND	0, (0-10)
Amphora salina W. Smith	ASAL	0 (0-2)	5 (5-35)	0 (0-4)
A. marina W. Smith	AMAR	ND	5 (5-35)	ND
Nitzschia sigma (Kütz) W. Smith	NSIG	0 (0-3)	5 (5-75)	ND
Nitzschia frustulum (Kütz) Grun.	NFRU	1 (0-65)	15 (5-75)	1, (0-9)
Nitzschia epithemiodes Grun.	NEPI	4 (0-82)	ND	ND
Amphiprora paludosa W. Smith	AMP	0 (0-23)	ND	ND
Cylindrotheca closterium (Ehrenb.) Reimann & Lewin	CCLOS	0 (0-76)	15 (5-75)	ND
Cylindrotheca signata Reimann & Lewin	CSIG	1 (0-94)	ND	ND
Staurophora amphioxys (Greg.) D.G. Mann	STAURO	0 (0-50)	ND	0 (0-31)
Pleuosigma angulatum (Quekett) W. Smith.	PANG	0 (0-21)	5 (5-75)	0 (0-7)
Gyrosigma limosum Sterrenburg et Underwood	GLIM	0 (0-19)	ND	0 (0-82)
Plagiotropis vitreae (W.Smith) Cleve.	PLAG	ND	ND	5, (0-100)
Raphoneis minutissima Hustedt	RAPH	4 (0-37)	ND	0, (0-35)
Cymatosira belgica (Grun.) Van Heurck.	CBELG	ND	ND	0, (0-25)
Coscinodiscus sp.	COSC	2 (0-26)	ND	ND
water content %		60 (31-79)	68, (34-84)	ND
ADFW %		10 (3-20)	15 (4-43)	ND
salinity ‰		29 (15-39)	33 (16-210)	33 (8-34)
temp $^\circ$C		16 (1-28)	19 (4-30)	13 (4-24)
Chl a (μg g^{-1})		11 (1-206)	80 (9-295)	40 (3-219)
total carbohydrate (mg g^{-1})		19.6 (2-46)	30.1 (45-131)	2.7 (0.5-14)
colloidal carbohydrate (μg g^{-1})		250 (0-5334)	3055 (584-6345)	35 (4-2568)

Table 2. The percent variation explained by principal components (PC) 1-4 of the relative abundance (RA) and biomass-weighted relative abundance (RA^B) of microphytobenthic diatom assemblages from the Severn, Colne and Blackwater estuaries and the correlation between each PC and environmental variables. Values in **bold** are significant at p < 0.05 or less.

	% var.	AFDW	salinity	Temp	Chl *a*	Total carbo.	colloidal carbo.
RA							
PC1	27.6	**-0.46**	**-0.32**	0.07	**-0.57**	**-0.34**	**-0.70**
PC2	10.8	**-0.36**	**-0.32**	**-0.34**	**-0.42**	**-0.30**	**-0.59**
PC3	9.0	0.01	-0.08	**0.40**	**0.27**	**0.12**	**0.14**
PC4	7.5	0.10	0.10	**0.12**	0.06	0.01	0.05
RA^B							
PC1	22.8	**0.603**	**0.318**	0.085	**0.731**	**0.538**	**0.797**
PC2	10.8	-0.043	**-0.113**	**0.263**	**0.333**	**0.158**	0.092
PC3	9.8	**0.357**	**0.344**	**0.118**	**0.175**	**0.198**	**0.367**
PC4	5.8	**0.133**	**0.180**	0.024	**0.211**	**0.126**	0.107

The clustering of samples by estuary evident in PC1 and PC2 was not present with principal components 3 and 4. PC3 and 4 explained an additional 16.5% of the variation in the data set, and samples from the 3 estuaries showed substantial overlap. Temperature, sediment Chl *a* content and total and colloidal carbohydrate content all correlated positively with PC3, and temperature with PC4. Thus the main gradient across Figure 2 (from lower left to upper right) is a seasonal gradient of increasing temperature and biomass. The species vectors can be placed in 3 groups, low biomass-temperature winter taxa (*Raphoneis* , *N. flanatica*, *Coscinodiscus*, *N. frustulum*, a mid temperature-biomass range of taxa (*N. cincta*, *N. sigma*, *N. pargemina*, *P. angulatum*, *Stauroneis* and *Plagiotropis*) and summer taxa (*N. phyllepta*, *N. gregaria*, *C. signata* and *Nz. epithemioides*). This major gradient present in species composition is seen across all the three estuaries investigated, as can be seen from the scatter of samples from each estuary across the graph.

PCA of biomass-modified RA data

Modification of the relative abundance data by a biomass factor resulted in a less pronounced separation of samples with PC1 and PC2 (Figure 3) into clusters for each estuary (as was seen in Fig 1), and an increase in scatter for all estuarine data. PC1 and 2 explained 33.6% of the data set, with the Blackwater and Colne samples intermingled, and some overlap between the Severn, Colne and Blackwater data. The incorporation of biomass into the analysis factor removed the low biomass taxa (*Raphoneis*, *Coscinodiscus*, *Cymatosira* etc.) from the list of significant species relating to PC 1 and 2, with increasing significance of a number of commonly occurring
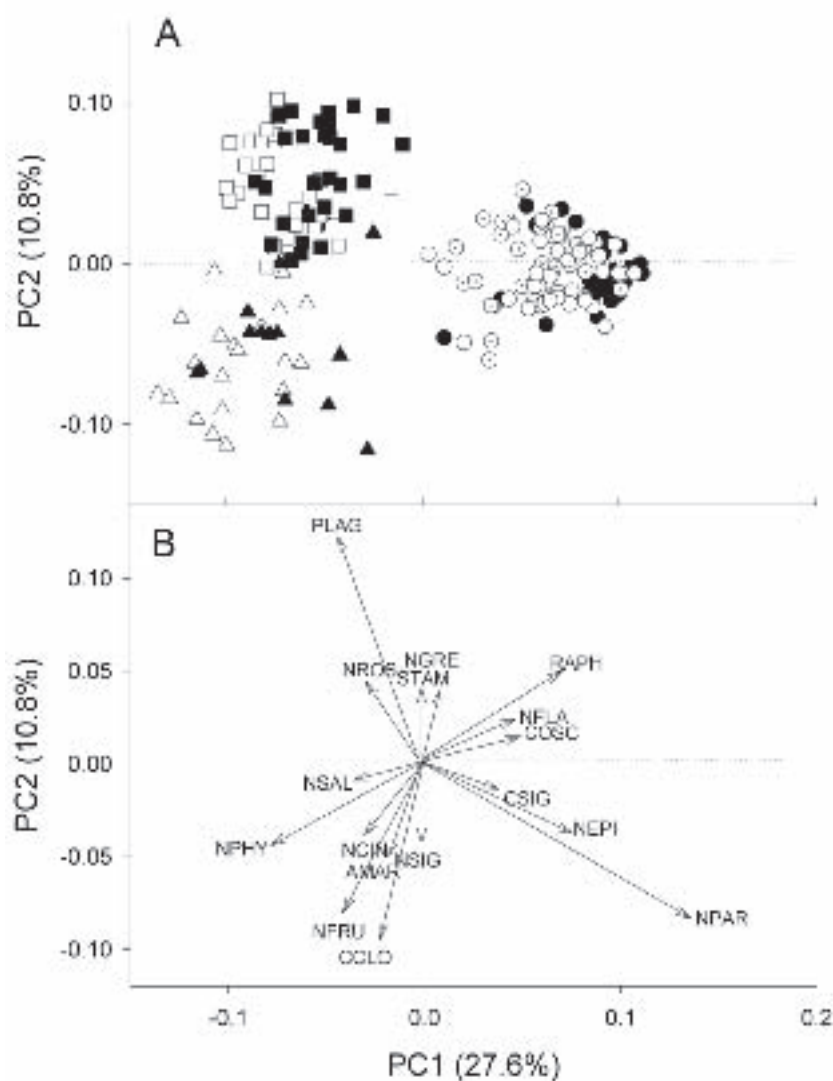
Figure 1. (A). Principal components analysis scatter plot of components 1 (PC1) and 2 (PC2) derived from relative abundance values of microphytobenthic diatom species composition in samples from sites in 3 U.K. estuaries. (■) Colne estuary sites 1 and 2, (□) Colne estuary sites 3 and 4 (Thornton *et al.* 2002), (▲) Blackwater Estuary upper marsh sites (△) Blackwater estuary lower marsh and mudflat sites (Underwood 1997), (○) Severn estuary Aust, (⊙) Severn estuary, Portishead, (●) Severn estuary, Sand Bay (Underwood 1994). (B). Vectors showing the direction of increasing relative abundance of particular diatom species. Only species with vectors significantly correlated ($p < 0.05$) with either PC1 or PC2 are shown. For species codes see Table 1.
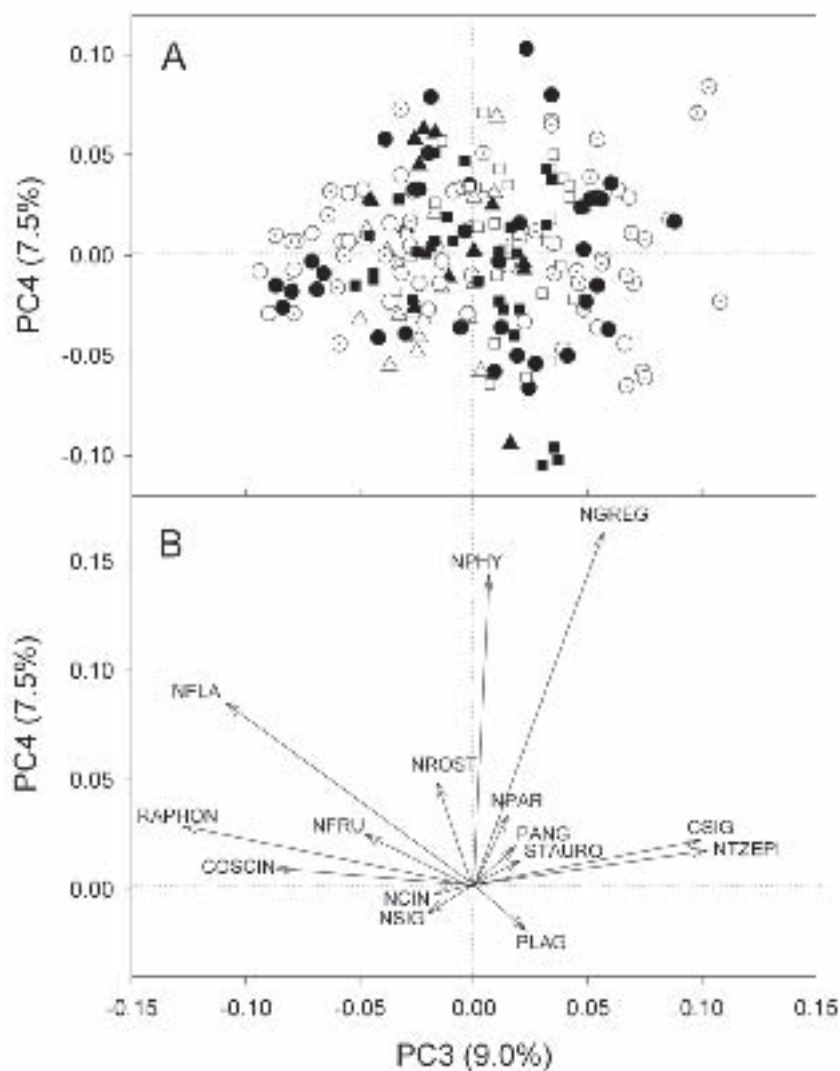
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Figure 2. (A). Principal components analysis scatter plot of components 3 (PC3) and 4 (PC4) derived from relative abundance values of microphytobenthic diatom species composition in samples from sites in 3 U.K. estuaries; (■, □) Colne, (▲, △) Blackwater, and (○, ☉, ●) Severn estuaries. For detailed key see Figure 1. (B). Vectors showing the direction of increasing relative abundance of particular diatom species. Only species with vectors significantly correlated (p < 0.05) with either PC3 or PC4 are shown. For species codes see Table 1.
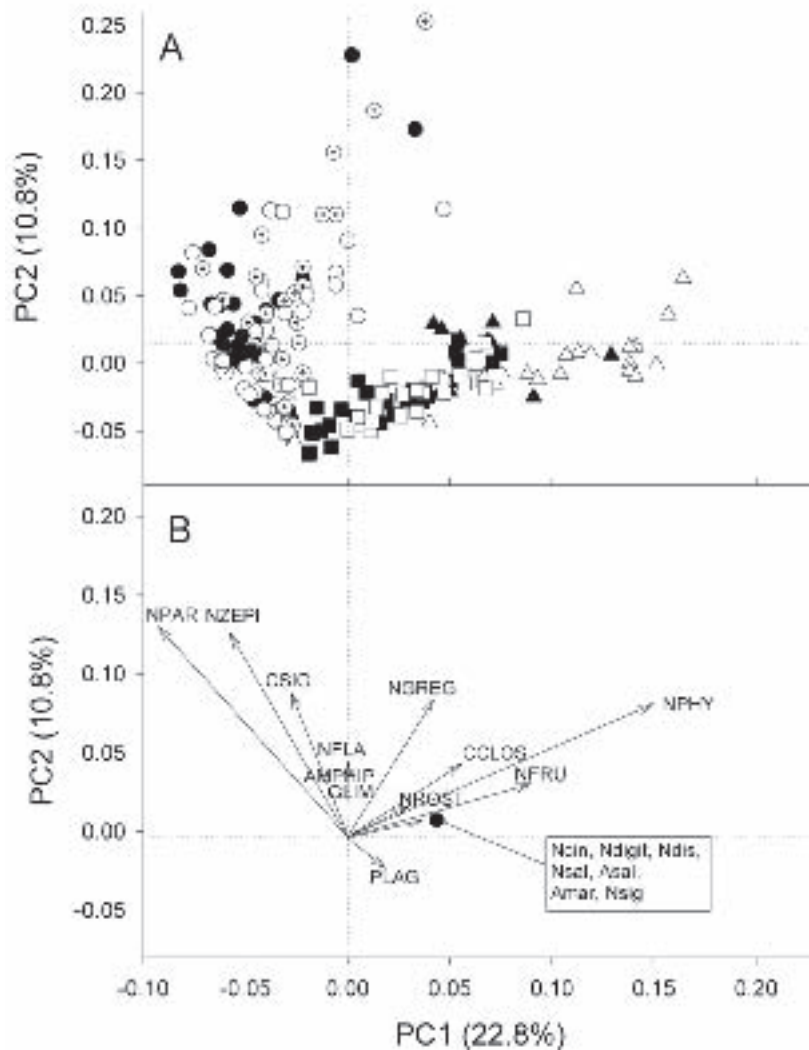
Figure 3. (A). Principal components analysis scatter plot of components 1 (PC1) and 2 (PC2) derived from biomass-weighted relative abundance values of microphytobenthic diatom species composition in samples from sites in 3 U.K. estuaries; (■, □) Colne, (▲, △) Blackwater, and (○, ⊙, ●) Severn estuaries. For detailed key see Figure 1. (B). Vectors showing the direction of increasing relative abundance of particular diatom species. Only species with vectors significantly correlated ($p < 0.05$) with either PC1 or PC2 are shown. For species codes see Table 1.

(though often in low relative abundance) taxa that were present when the microphytobenthic biomass was higher (e.g. *Navicula cincta*, *N.rostellata*, *N. digitoradiata*, *N. diserta*, *Amphora salina*, *A. marina*, *Nitzschia sigma*). These taxa are common members of the assemblages, but usually do not appear within the set of important taxa due to low relative abundance. PC1 was significantly correlated with the same set of biomass/organic richness as in Figure 1 (Table 2), while PC2 was negatively correlated with temperature and positive with Chl *a* and total carbohydrate concentrations. Thus the effect of biomass-normalisation on PC1 and PC2 was to reduce the separation into estuary-specific clusters, widen the scatter of the data set, remove taxa associated with very low biomass and increase the significance of commonly found taxa yet that usually have a fairly low relative abundance.

PC3 and 4 explained a further 15.6% of the biomass-adjusted data, and separated out the upper saltmarsh samples from the other mudflat samples, and separated the Colne from the Blackwater samples (Figure 4). PC3 was positively correlated with biomass and organic content variables, as well as salinity. Taxa responding positively to this gradient (*Amphora marina*, *Cylindrotheca closterium*, *Nitzschia sigma* and *N. frustulum*) were common in the saltmarsh assemblages sampled at Northey Island. Separation of samples along PC4 was also correlated with a gradient of increasing salinity and organic content, with lower estuary samples tending to have a higher PC4 score. These two gradients of salinity place *N. phyllepta* and *N. gregaria* as taxa abundant in lower salinity samples, and *Pleurosigma angulatum* and *N. rostellata* and the cluster of 'saltmarsh' taxa more characterstic of higher salinity samples (Figure 4).

By incorporating a biomass-factor into these analyses, some of the taxa that were acting to separate out estuaries become less important (for example, winter taxa and tychoplankonic diatoms in the Severn estuary), and the UK East coast estuarine sites show more overlap. But there are still large differences in the flora of these sites, with some species characteristic of high biomass conditions in one estuary not common in another. For example, in the Severn, *N. pargemina, Nz. epithemioides, C. signata* while on the east coast, *N. phyllepta, N. gregaria, C. closterium* and *Nz. frustulum* are dominant taxa. This is despite the ranges of environmental data measured for these three estuaries showing significant overlap (Table 1) so it is not immediately obvious what factor may be causing differences in species composition. Monthly nutrient data for the Severn and Blackwater sites were not measured during those studies, but area-normalised nutrient loads for all 3 estuaries have been calculated (Table 3; Nedwell *et al*. 2002). These data would suggest greater similarities between the Severn and Blackwater estuaries, not a pattern observed in the floristic data. This would suggest that nutrient loads don't appear a good predictor at this very broad geographical scale.

This example illustrates the care that must be taken in drawing conclusions about nutrient status and estuarine diatom flora. The Severn estuary epipelic diatom flora (see also Oppenheim 1991) does appear to differ from that found in UK southern North Sea estuaries. The flora of the latter estuaries is quite similar to the flora of Dutch and Flemish estuaries (Colijn and Dijkema 1981; Peletier 1996; Sabbe 1993). Whether this is evidence for regional floristic differences is a hypothesis that needs
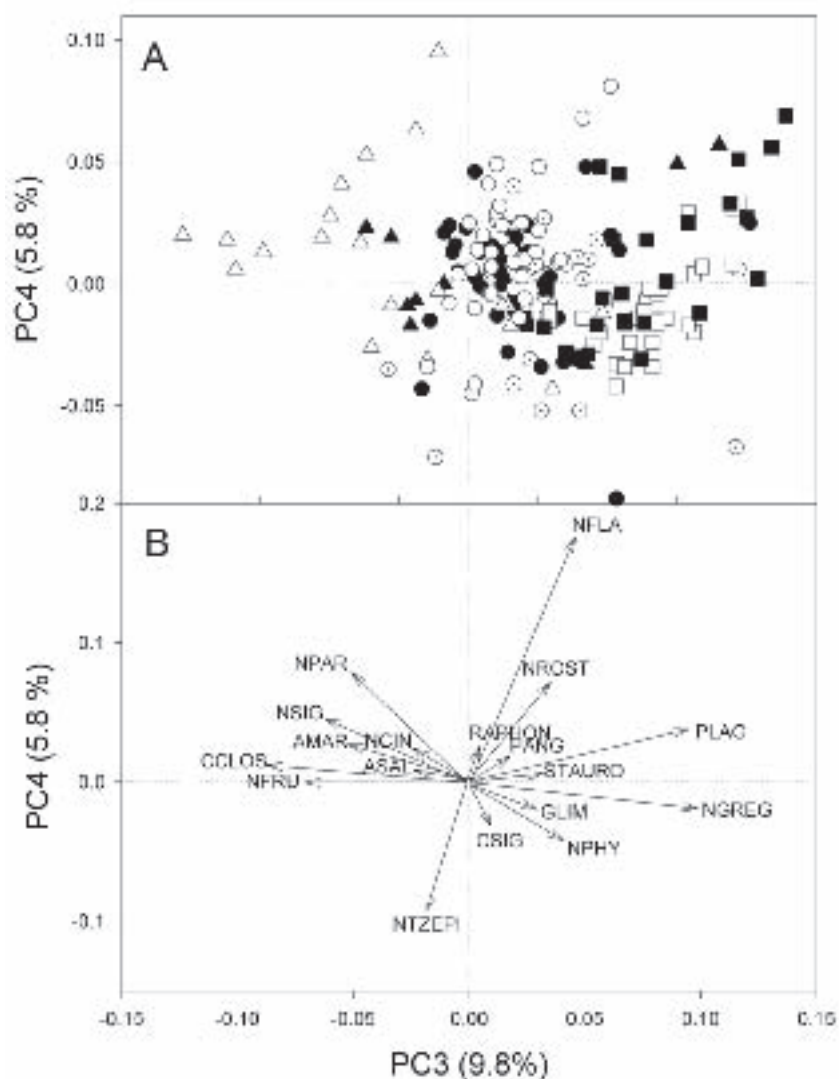
Figure 4. (A). Principal components analysis scatter plot of components 3 (PC3) and 4 (PC4) derived from biomass-weighted relative abundance values of microphytobenthic diatom species composition in samples from sites in 3 U.K. estuaries; (■, □) Colne, (▲, △) Blackwater, and (○, ⊙, ●) Severn estuaries. For detailed key see Figure 1. (B). Vectors showing the direction of increasing relative abundance of particular diatom species. Only species with vectors significantly correlated (p < 0.05) with either PC3 or PC4 are shown. For species codes see Table 1.

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Table 3. Estuary area-normalised loads of nitrate and ammonium (Mmol N km^{-2} y^{-1}) for the Blackwater "Colne and Severn" Estuaries in 1995-1996 (from Nedwell *et al.* 2002).

Load (Mmol N km^{-2} y^{-1})	N-NO$_3^-$	N-NH$_4^+$
Blackwater	4	0.06
Colne	2	1
Severn	5	0.6

investigating. Interestingly, the Severn estuary also has a benthic fauna different from other UK estuaries, attributed to the physical dynamics of the estuary (Warwick *et al.* 1991). Is disturbance a selective force for the dominant diatom taxa in the Severn estuary?

Many benthic marine diatom species appear to have a cosmopolitan distribution (Witkowski *et al.* 2000), though some genera do show higher diversity in some biogeographic areas (e.g. *Mastogloia* in the tropics). However, great care needs to be taken in drawing conclusions concerning global or even regional distribution patterns. Many regions are undersampled, and taxonomic difficulties are present in poorly described floras. For example, *Navicula flanatica* is a common European mudflat taxon that tends to be more dominant in assemblages during the cooler months of the year (Colijn and Dijkema 1981; Admiraal *et al.* 1984; Underwood 1994; Thornton *et al.* 2002). Yet, apparently the same species (identical in the morphological features used to identify diatoms) is common within mangrove and muddy intertidal sediments in Fiji, where temperature do not drop below 25 °C, and often exceeds 40 °C (Underwood, unpublished observations). Combined taxonomic, physiological and genetic studies are needed to clarify our definitions of species and to determine intraspecific variability in niche widths.

Within estuary patterns of species distribution

Salinity and nutrients

As well as broad, regional patterns of species difference, changes in the relative numbers of different taxa are very evident along much shorter gradients (Figure 5, Table 4). Within estuaries, the estuarine gradient is, characterised usually by a decrease in nutrient concentrations and increasing salinity (Underwood and Kromkamp 1999; Thornton *et al.* 2002). Salinity has been implicated as a major driver of species distribution (Van Dam *et al.* 1994; Watt 1998), and there do appear to be distinct brackish water and marine species. In the stable salinity gradient of the Baltic Sea, species occur in narrow salinity bands (Snoeijs 1993; Snoeijs and Vilbaste 1994; Snoeijs and Potapova 1995; Snoeijs and Kasperovičiene 1996; Snoeijs and Balashova 1998; Stachura-Suchoples 2001), but other estuarine taxa have a wide tolerance to salinity (Admiraal 1984; Cox 1995). In meso- and macrotidal estuaries, there is wide fluctuation in the
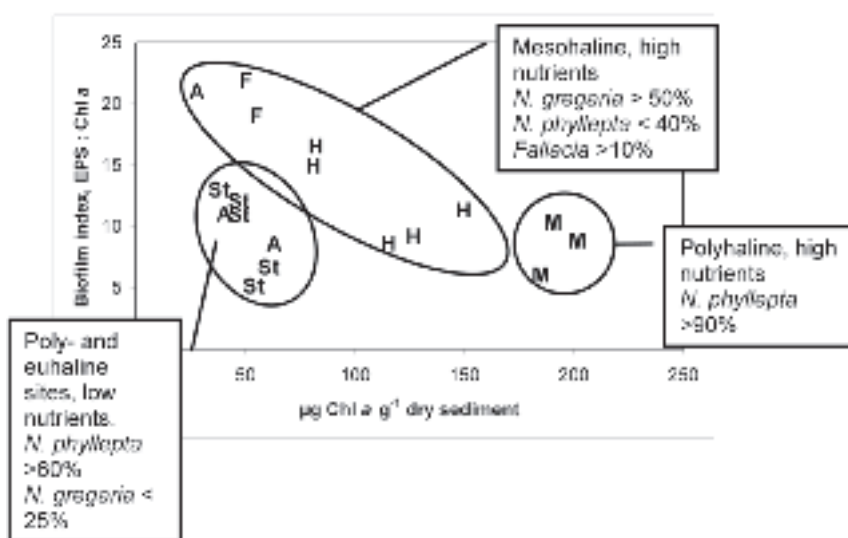
Figure 5. Scatter plot of sediment Chl *a* content and biofilm index (ratio of EPS: Chl *a* content) from 5 sites (F, St, A, H, M) in the Colne-Blackwater estuary complex, indicating major species composition of the different biofilms. For site details see Table 4.

Table 4. Chemical conditions at 5 sites in the Colne (F, S, A, H) and Blackwater (M) estuaries, Essex.

(µM)	Fingringhoe (F)	St Osyth (St)	Alresford (A)	Hythe (H)	Maldon (M)
Overlying water					
Salinity (‰)	12	32	28	7	22
T.Ox.N.	274 ± 45.1	11.0 ± 1.18	26.0 ± 2.12	649 ± 48.3	297 ± 46.5
Phosphate (µM)	17.7 ± 2.54	0.17 ± 0.07	1.37 ± 0.16	34.0 ± 2.12	31.9 ± 6.72
Ammonia (µM)	14.2 ± 1.60	22.7 ± 3.61	9.76 ± 1.20	79.6 ± 16.3	52.8 ± 3.77
Silicate (µM)	125 ± 22.4	15.3 ± 0.88	30.5 ± 3.20	247 ± 8.21	131 ± 37.2
Porewater					
Salinity (‰)	7.9 ± 0.33	35.5 ± 0.16	33.5 ± 0.81	4.0 ± 0.0	15.8 ± 0.20
T.Ox.N.	7.31 ± 1.95	1.01 ± 0.90	6.26 ± 0.77	13.8 ± 8.59	6.24 ± 6.24
Phosphate (µM)	65.6 ± 4.36	12.0 ± 0.86	30.5 ± 5.36	112 ± 14.6	68.0 ± 22.0
Ammonia (µM)	29.3 ± 6.29	15.1 ± 2.40	152 ± 32.8	630 ± 57.5	232 ± 36.7
Silicate (µM)	338 ± 15.7	18.0 ± 3.39	56.1 ± 13.5	643 ± 105	418 ± 138

salinity regime over the spring-neap tide cycle and also annually. Such variation is likely to select for species tolerant to changing salinities. Patterns in salinity also co-vary with nutrient concentrations and loads (Underwood *et al.* 1998; Ribeiro *et al.* 2003), so it is difficult to separate out the effect of each variable, though multivariate analyses can be used (Oppenheim 1991; Thornton *et al.* 2002).

Experimental approaches can be used to manipulate environmental variables and determine the response of different populations of species. Underwood *et al.* (1998) manipulated ammonium concentrations in tidal mesocosms and found that certain taxa showed significant treatment-related changes in population density. Ammonium concentrations may play an important role in determining species composition due to the toxic effects of ammonia (Admiraal 1977) under conditions of high pH. Similarly sulphide probably has a selective effect on assemblage composition (Sullivan 1999). Concentrations of these ions are high in organically-enriched sediments, and species such as *Fallacia (Navicula) pygmeae* and *Navicula salinarum* appear particularly tolerant of sulphide and ammonia (Peletier 1996; Sullivan 1999; Table 4; Figure 5). A series of nutrient enrichment experiments carried out on saltmarshes on the eastern seaboard of the USA also found shifts in species composition due to nitrogen enrichment (Sullivan 1999), while a major reduction in nutrient loads in the Ems-Dollard estuary over a 10-year period have resulted in significant changes in the diatom flora (Peletier 1996).

Culturing experiments have also shown significant differences in growth rates of estuarine species across a range of nitrate, ammonium and salinity conditions with evidence of separation of the autecological niches of *Navicula phyllepta*, *N. salinarum*, *N. perminuta* and *Cylindrotheca closterium* across this matrix of conditions (Underwood and Provot 2000). These experimental data agree with known distributions of some taxa. For example, experimental data suggests that *N. phyllepta* is a mesotrophic species, favouring nitrate and ammonium concentrations in the range of 100-400 µM, which matches the occurrence of assemblages dominated by this taxon in the Colne estuary (Thornton *et al.* 2002). The relative abundance of *Navicula phyllepta* also shows an inverse relationship with that of *N. gregaria* across a wide nutrient range (Figure 6). Both taxa are common in a range of estuaries and are commonly found together.

Grazing

A very under-researched area is the impact that grazing might have on assemblage species composition. Grazing has been shown to have very significant impacts of the species composition of epiphytic and epilithic assemblages, both in freshwater habitats and on rocky shores, with smaller, more adpressed taxa having an advantage under grazed conditions. However, though there are numberous studies that have demonstrated that grazing can significantly reduce biomass of estuarine microphytobenthos (e.g. Sullivan and Currin 2000), detailed grazing studies concentrating of species composition are generally lacking. Grazing experiments with *Nereis diversicolor* and *Corophium volutator* have shown a shift in species composition, compared to ungrazed controls (Smith *et al.* 1996), and recently it has been shown that certain
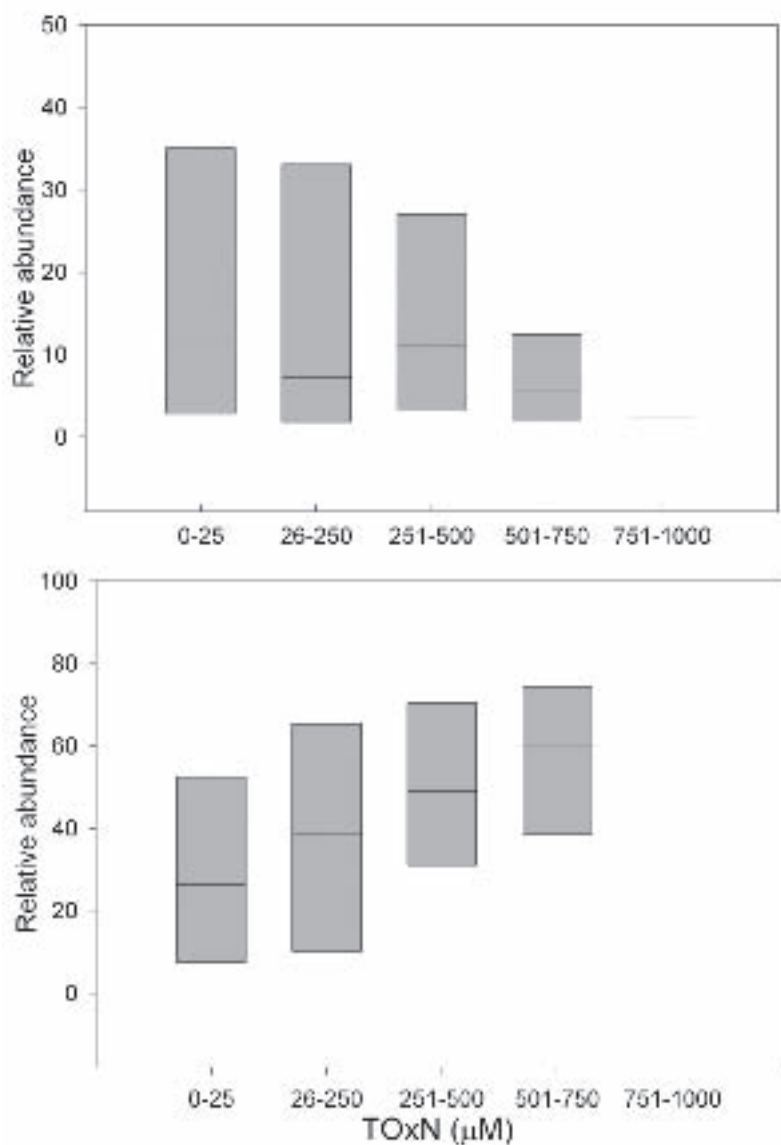
Figure 6. Box plots (median value, 25 and 75% quartiles) of the relative abundance of (A) *Navicula gregaria* and (B) *Navicula phyllepta* across a range of total oxidised nitrogen (T.Ox.N) concentrations in the Colne, Great Ouse, Conwy and Orwell estuaries, U.K.

ciliate species select actively different taxa of epipelic diatoms (Hamels *et al.* 2004). Whether these grazing interactions are sufficiently intense to result in substantial species shifts *in situ* remains to be determined (i.e., the relative importance of top-down versus bottom-up effects in determining microphytobenthic species composition).

Sediment type

Benthic diatoms are often separated into epipsammic and epipelic taxa, depending on their mode of attachment and motility in sediments. Epipsammic species are usually very small, virtually non-motile, and live closely attached to depressions and crevices in sand grains by pads or short stalks. Epipelic diatoms are non-attached and are highly motile (Round *et al.* 1990). In the natural environment, the distinction between these two growth forms is blurred, as sands and muds are found together and an assemblage can consist of both epipelic and epipsammic taxa (Hamels *et al.* 1998). Some large, motile naviculoids, e.g. *Navicula rostellata*, *N. peregrina* and *N. arenaria*, are found in sandy muds, and sand can support dense populations of motile *Hantzschia* species (Underwood 2002; Kingston 1999) as well as the smaller, more typical epipsammic taxa. Where sands are scoured and moved by currents, the density of epipelon is reduced (Sabbe 1993; Hamels *et al.* 1988; Vilbaste *et al.* 2000,) and epipsammic species dominate the assemblage. In estuaries, the location of sandflats and mudflats will depend partly on hydrodynamics (Dyer 1998; Flemming 2000) and in partially-mixed estuaries (the common NW European system), sandier sediments will be more abundant towards the mouth of the estuary (Viles and Spencer 1995). Thus spatial changes in diatom flora along estuarine gradients may also reflect increasing sediment grain size, as well as increasing salinity and a dilution of riverine nutrient inputs.

A cautionary example

The temptation to assume that variables measured in a study are the causative factors in determining environmental patterns is very strong, as Darwin (1860) (*op.cit.*) pointed out. Yet, it is not always clear what the real causative factors are. As an example, data on microphytobenthic species composition, nutrient conditions, photophysiological responses of microphytobenthos and behavioural characteristics of different biofilms from various shallow water habitats in Fiji are summarised in Figure 7. Signficant differences in species community composition were found between different intertidal and subtidal sites, with sites of similar physical characteristics clustering (by diatom species) together, even though geographically separate. Microphytobenthos showed a significant gradient of light acclimation and potential maximum rates of photosynthesis across these sites, which also correlated with a significant gradient in water column nutrient concentrations (see Underwood 2002 for details). Clear patterns of tidally-linked endogenous rhythms of vertical migration were present in intertidal biofilms, but not recorded in subtidal biofilms, and there were also significant differences in carbohydrate and relative EPS content across these gradients. It is not possible to determine which, if any, of these variables is
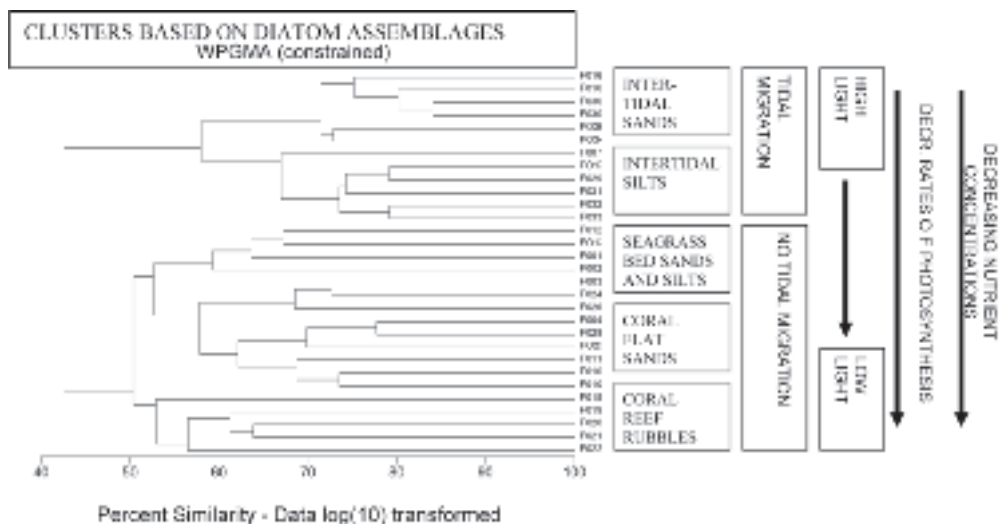
Figure 7. Dendrogram of sample clusters based on percentage similarity in diatom species composition for 30 benthic assemblages from intertidal and shallow subtidal sites in Fiji. The different site and biofilm characteristics (vertical migration, photophysiology etc.) for these clusters are indicated (Underwood 2002).

causative of the patterns in species distribution observed. What is interesting is that differences in ecologically-important properties of biofilms, for example potential primary production and EPS concentrations) are also related to differences in species composition (Cahoon 1999). It might be that the species profile changes biofilm properties? This suggests that greater attention needs to be paid to the species composition of biofilms. While more descriptive studies on spatial and seasonal patterns in species composition will be useful, manipulative studies are also needed to determine species-specific responses to environmental variables. Combination of these data will allow more accurate understanding of what determines species composition in estuarine microalgal biofilms.

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