An Investigation of the Periphytic Diatom Assemblages in Grazing Marsh Ditches: Application to Assessment of Ecological and Conservation Status of Grazing Marsh Ditch Systems

Technical Report Part 2: Diatom assemblages in grazing marsh ditch systems in England and Wales: results from surveys undertaken in 2008-2009 and overall synthesis from 2007-2009.

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Species of the genus Rhopalodia © Marian Yallop

Executive Summary

1. A survey of the periphytic diatoms of selected ditches from grazing marshes in Suffolk, Kent, Sussex, Norfolk, Essex, Wales (Anglesey and Gwent) and the Somerset and Avon Moors and Levels was undertaken during 2007-2009. This project is linked to a detailed investigation into the current status of aquatic invertebrate and plant communities of grazing marsh ditch systems in England and Wales.

2. Periphytic diatom assemblages were sampled from selected macrophyte stems growing in ditches. One hundred and thirty five samples were analysed. A total of 214 diatom species were recorded. This data set provides the first detailed record of the diatom flora of the grazing marsh ecosystems in the UK. The data will be passed to the National Biodiversity Network.

3. The revised Trophic Diatom Index tool (DARLEQ) devised for freshwater rivers was applied to obtain estimates of the ecological status of the ditches. Assessment was based on the relative abundance of the diatom assemblages. Preliminary results indicated that ditches spanned a range of ecological status from poor to high. However, some samples contained diatoms that are relatively uncommon in freshwaters and currently there is insufficient information on their nutrient tolerance or other environmental preferences hence their use in the assessment of ecological status is tentative.

4. Due to the natural variability in diatom assemblages it is recommended that temporal replicates be obtained from more of the grazing marshes before reliable estimates of the ‘true’ ecological status can be made.

5. The majority of diatom species recorded were typical of those commonly found in rivers and their nutrient preferences are well known though some were more typical of lakes and brackish waters and in some cases, their tolerance to nutrients is uncertain.

6. Differences were found between metrics derived using the diatom composition of ditches from different geographical areas. Whilst the main driver appears to be salinity, other variables may account for the differences in species composition.

7. Evidence presented here illustrates the utility of including diatoms as a signature for overall ecosystem health in the grazing marshes. These data provide a benchmark allowing assessment of changes in the ecological status of grazing marsh ditches to be made in the future.

1. Introduction: Background and aims

This study of periphytic diatom assemblages (a proxy for phytobenthos) in grazing marsh ditches had four aims:

1) to extend the present knowledge of diatom communities in ditches
2) to provide data for subsequent exploration of the possible relationships between diatom, macrophyte and invertebrate assemblages in ditch systems
3) to act as a surrogate for the programme of water chemistry analysis
4) to constitute a pilot study for extending the Water Framework Directive assessment methodology to diatoms of ditch systems

This study formed part of a programme of surveys carried out by Buglife – The Invertebrate Conservation Trust, to assess the condition of the aquatic biota of grazing marsh ditches in England and Wales.

The Water Framework Directive's (WFD), (European Union, 2000) Programme of Measures (PoM), will be applied to Natura 2000 sites, some of which (e.g. Nene Washes, Ouse Washes, Norfolk Broads) contain ditch systems. A WFD compliant assessment methodology is required for the phytobenthos of ditches. In the UK, we have recently developed a conceptual model and predictive tool (DARLEQ) to assess changes in the assemblages of phytobenthos along a pressure gradient of eutrophication (nutrients and organic pollution), (Yallop & Kelly, 2006; Kelly *et al*., 2008). The DARLEQ tool for rivers (formerly called the DARES tool) and lakes (formerly referred to as the DALES tool) enables assessments to be made of their ecological status). The tool assesses changes in the composition of attached diatoms communities (predominantly epilithon), which play a key role in the primary productivity of inland aquatic ecosystems. Diatoms are used as proxies for phytobenthos in the tool. The application of this tool to ditch ecosystems was assessed during this study. The river based tool was applied based on the assumption that rivers represent the most appropriate analogy to the ditch ecosystems. However, it is possible that some ditches share more characteristics with lakes. The lakes tool will be applied as a part of further ongoing investigations.

In this report the findings of the 2008-2009 surveys on diatom flora of ditches are presented and an overall synthesis of the results from the 2007-2009 is compiled.

2. Methods

2.1 Study sites in 2008-9

Samples of phytobenthos were collected from 94 sites from ditches in Sussex, Norfolk, Essex, Suffolk, Kent, Anglesey and the Somerset and Avon Moors and Levels during 2008-2009. To investigate inter-annual variation in diatom assemblages, samples were taken from the Somerset and Avon Moors and Levels from sites sampled during the first part of the survey in 2007 (see Yallop, 2008, Report Part 1). Field sampling methods followed standard protocols (for detailed methodology see: http://craticula.ncl.ac.uk/dares/methods.htm).

2.2. Sample processing

Subsamples of the periphytic biofilms were cleaned to digest the organic matter following a standard protocol (for detailed methodology see: http://craticula.ncl.ac.uk/dares/methods.htm). A slide was made up for each sample and, where possible, over 300 diatom valves identified in each sample by reference to standard works (Krammer & Lange-Bertalot, 1986-2004). The percentage relative abundance of each species was determined. Each diatom species in the DARLEQ database has been assigned a sensitivity s value according to its tolerance to nutrients (Kelly *et al*., 2008). There are five groups with s values ranging from 1 (very nutrient sensitive) through to 5 (very nutrient tolerant). However, some species were recorded that are not in the database and appropriate s values had to be based on our current understanding of their environmental preferences. It is important to note that over the duration of this project, the Trophic Diatom Index tool was being further developed and refined in compliance with requirements for the Water Framework Directive. The analyses for the samples collected in 2007 were based on a 'trial' version of the new predictive tool, the TDI3. Further refinements to the TDI were carried out subsequently, including taxonomic revisions and adjustments to expected reference conditions for unimpaired sites. All samples have now been analysed using the latest version of the tool the TDI4.

2.2.1 Diatom metrics

Metrics were derived from the data set, including:

- Trophic Diatom Index, (TDI), which is a measure of nutrient/organic pollution status varying from 1 – 100 where 100 indicates hypereutrophic
- Ecological Quality Ratio (EQR) ranging from 0 – 1 and measuring deviation from reference site where no deviation = 1
- % motile valves (an indication of silt and biofilm maturity)
- % organic pollution tolerant valves
- Species richness
- Evenness and diversity (Shannon-Wiener as $\log_e$)
- Salinity (H) ranging from 1 = fresh, to 4 = brackish
- Oxygen tolerance (O) where 1 = can only tolerate high oxygen and 5 = tolerates low oxygen

- pH (R), 1 = acidophile to 5 = alkalibiontic
- N (nitrogen uptake metabolism), where 1 = N autotroph to 4 = obligate N heterotroph, dependent on sources of organic N. (H,O,R and N values assigned after van Dam & Mertens, (1993) and weighted by the relative abundance of each species in a sample.

2.3 Data Analyses

Detrended Correspondence Analysis (DCA) was used to examine spatial patterns in the data set. Relative abundance values were square-root transformed prior to analysis and rare taxa were down-weighted. The DCA was performed on a reduced dataset where all species that were present in two or fewer samples and with a relative abundance of less than 1% were removed. Correlations were undertaken to compare the samples scores of the first two axes of the DCA with a number of environmental variables and other derived biotic metrics obtained from the plant flora. Analysis of variance (or the nonparametric form, Kruskal-Wallis) was carried out to explore differences between selected metrics across the marshes.

The DARLEQ diatom tool was used to calculate the TDI. Ecological Quality Ratios (EQR's) were produced by comparing the observed TDI with that expected to be obtained if the site was at reference conditions i.e. in the absence of any eutrophication pressure (Kelly *et al*., 2008). Alkalinity values were required to compute the EQR values. A regression equation was used to obtain estimates of the ditch alkalinity values derived from matched alkalinity/conductivity from UK river sites (regression equation used y = 0.339x-7.7395, r^2 = 0.82, where x = conductivity µS cm^{-1}; Kelly, unpublished data). Many of the ditches sampled during 2008-2009 were brackish. The relationship between alkalinity and conductivity will be confounded by the presence of salts other than $CaCO_3$.

3. Results of the 2008-2009 survey

3.1 Diatom species: diversity, richness and environmental preferences

A total of 214 diatom taxa were identified from the ditches sampled during 2008 and 2009 in the south of the England and Anglesey adding a further 38 taxa to this list recorded for 2007. A spreadsheet of the diatom species has been compiled and will be made available in due course). Ditch samples were categorised to look for major trends. For interpretation of their geographical differences, ditches were assigned to groups as follows: Somerset and Avon Moors and Levels; Anglesey (Wales; Kent; Norfolk; Suffolk; Essex and Sussex. Only two sites were sampled in Wales in this second part of the survey and were not included in the 'site-specific' analysis due to the small sample size.

A number of the additional taxa are typically associated with brackish waters and indicate the saline intrusion associated with a number of the ditches e.g. *Rhopalodia* spp., *Amphora coffeaeformis*, *Bacillaria paxillifera* and *Tabularia* spp., (Figure 3.1).

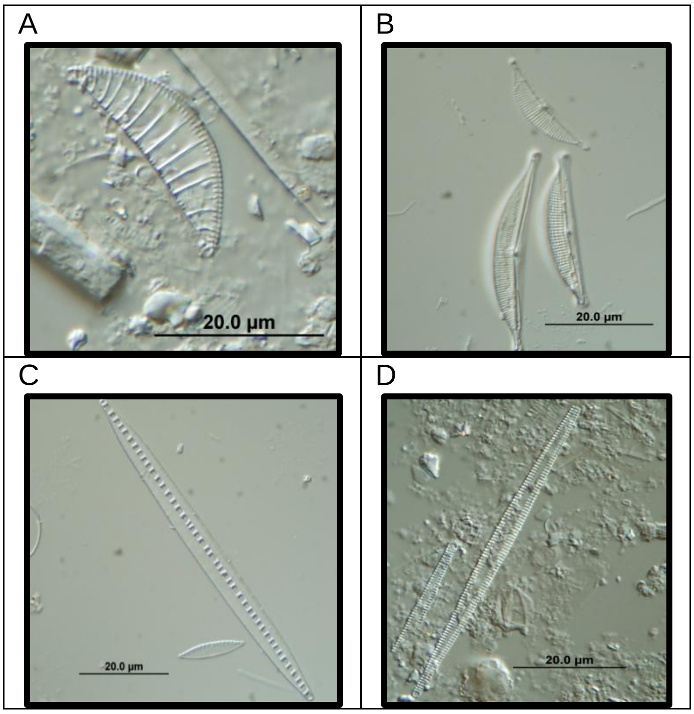

Figure 3.1 Photomicrographs of selected diatoms, more typical of brackish conditions, sampled from macrophytes in ditches: A = *Rhopalodia acuminata*, B = *Amphora coffeaeformis*, C = *Bacillaria paxillifer* and D = *Tabularia fasciculata.*

*S*pecies richness ranged from 5 species (PVY52) in a sample from a ditch in the Pevensey Levels, East Sussex to 48 species (FLG6) in Fleggburgh Marsh, Norfolk (Table 1). The diatom sample taken from *Glyceria maxima* in ditch PVY52 was very unusual being dominated almost entirely (relative abundance (RA), > 95%) by the motile diatom *Nitzschia paleacea.* This is a very common species, typical of eutrophic waters of and tolerant of high electrolyte content and tolerant of strongly polluted water (Cox,1996). In the early stages of colonisation, biofilms tend to be dominated by adnate or stalked forms of diatoms and the motile species will colonise at a later stage weaving through the biofilm (Yallop & Kelly, 2006). For this reason, the low species richness and dominance by a motile species is atypical and may be an indication of less favourable environmental conditions. One possibility could be the presence of sediments which may get trapped and accumulate on stems of the macrophytes. A second example of dominance by a motile species in the biofilm was recorded for ditch MTH8 in Anglesey, Wales where the diatom biofilm was dominated by *Nitzschia capitellata* (RA > 90%), (Figure 3.2); a species well known for its tolerance to extreme pollution (Cox, 1996; Della Bella et al., 2007).

By contrast, the diatom sample from *Sparganium erectum* in FLG6, was genera rich including a number of species from the genera *Achnanthidium*, *Amphora*, *Bacillaria*, *Cocconeis*, *Ctenophora, Cymatopleura,* *Denticula, Epithemia, Tryblionella, Synedra, Surirella, Stauroneis, Rhoicosphenia, Planothidium Nitzschia, Navicula, Karayevia, Gomphonema, Fragilaria* and *Eunotia*.

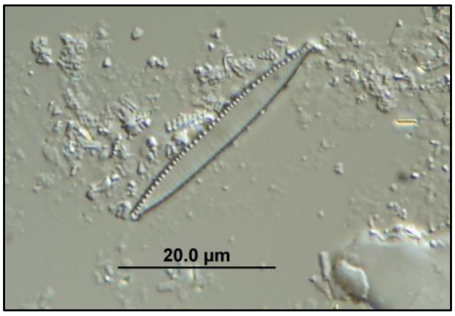

Figure 3.2 Valve view of the benthic diatom *Nitzschia capitellata.*

A number of other sites were associated with a particularly low species richness including DWS37 (6 species) and WDM20 (8 species), both in Kent. The former site was notable for the dominance of the centric diatom species *Cyclotella c.f meneghiniana.* Many of the centric diatoms are planktonic and are not included in measures of ecological status using benthic diatoms (Figure 3). However, for this particular species has been described as species that is littoral and 'rarely planktonic' (Cox, 1996). Possibly further consideration should be given to the inclusion of this species in the benthic community. However, it rarely occurred in other samples from the ditches. It's presence is likely associated with an autumnal bloom in the water column. The species produces long mucilaginous extrusions and coupled with the presence of spines on the frustules, may cause the cells to become entangled in the *Sparganium* (Liz Haworth, pers.comm).

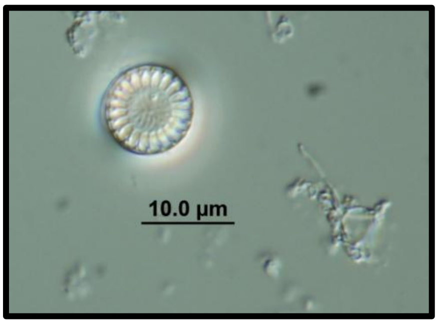

Figure 3.3 Valve view of the centric diatom *Cyclotella c.f meneghiniana.*

Ditch WDM20 was dominated by two adnate diatoms including *Amphora pediculus* and *Epithemia adnata* (Figure 3.4). *Amphora pediculus* is a widespread species in rivers and may be epiphytic on other diatoms, though was not common in the ditch samples analysed here. *Epithemia adnata*, conversely is uncommon in the river samples used for the calculation of the TDI (Kelly et al., 2008) and is common in ditches. The latter species is an epiphyte tolerating high electrolyte and brackish conditions. It was a relatively common member of the epiphytic assemblages of the ditches sampled during this survey and was frequently the most dominant genus. Diatoms in the genera *Rhopalodia* and *Epithemia* are known to house nitrogen-fixing cyanobacterial endosymbionts (DeYeo et al., 2004) and the dominance of these diatoms in certain ditches may provide a useful indication of the nutrient status or possibly reflect a change in the typical ratio between the major ions, nitrate and phosphate, critical for plant and algal growth.

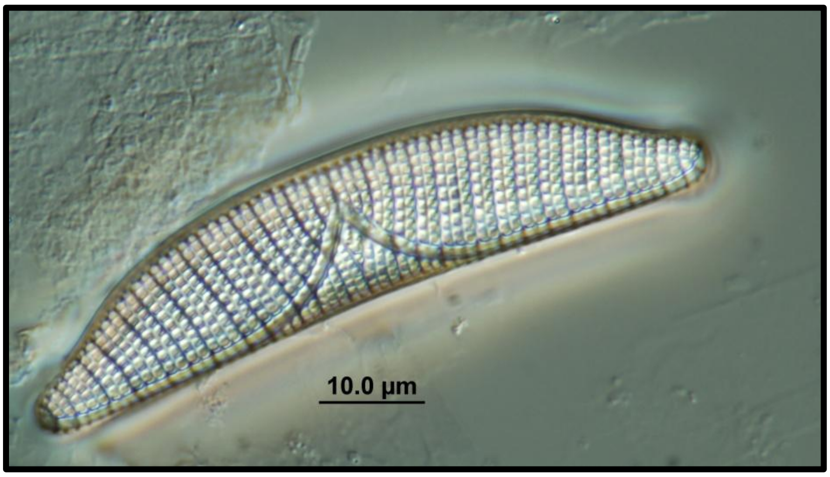

Figure 3.4. Frustule of *Epithemia adnata*

3.2 Ecological status of ditches sampled between 2008 and 2009

The revised trophic diatom index (TDI) indicated ditches ranging from Poor to High Ecological status. However, it is important to emphasise that some of the 's' values used for particular diatom species are not reliable as we have insufficient information on their nutrient preferences. This is a particular problem where the samples are dominated by diatoms that are not commonly recorded in the freshwater flora. For that reason, some of the information on ecological status has to be regarded as tentative until we can obtain more information on their nutrient tolerance. There was a significant difference in the TDI between sampling location of the ditches (ANOVA, $F = 3.99_{(5,77)}$, $p = 0.003$). *Post hoc* Tukey tests indicated that the mean TDI of ditches in Somerset (47.4 ± 10.8) and Sussex (48.6 ± 9.7) were significantly lower than means for ditches in Essex (63.3 ± 7.0) indicating a better ecological status of the former ditches. Once again these results must be regarded as provisional for reasons already stated. The mean percentage of motile valves ranged from 14.8 ± 7.8 in Suffolk to 38.6 ± 28.5 in Essex. No differences were found in the percentage of motile valves between sites (Kruskal Wallis $H = 6.36$, $p = 0.231$). The lowest mean percentage of organic tolerant valves was recorded for ditches in Suffolk (4.6 ± 3.4) and the highest in ditches in Essex (17.9 ± 15.8). No differences were observed between the mean percentage of organic tolerant valves between sites ($H = 7.77$, $p = 0.169$). There was a significant positive correlation between conductivity and TDI across sites (Spearman Rank Correlation, $p = 0.014$), (Figure 3.5). One anomalous outlier in the data set is the sample from site CHY42. The conductivity measured at this site was 7880 µS cm^{-1} and the diatom sample was collected from *Bulboschoenus maritimus*. Observations of the raw material obtained from this site indicated a large amount of flocculant polymeric material with large number of *Rhopalodia gibba* cells intermingled in the floc. This diatom species is widespread in standing and slow flowing waters (Cox, 1996) though is not commonly recorded in diatom samples from streams (Kelly et al, 2008) and was not recorded in the ditches samples in Somerset and Avon Moors and levels and Gwent Levels during 2007 (Yallop, 2008). For this reason we may not have a realistic measure of the trophic indicator status of this species. At present, it has been assigned an s value of 1 for rivers and 2 for lakes in the DARLEQ database. The lake s value is likely a better indicator for this species given that it is typically found in slow flowing waters or lakes. This example serves to highlight the problems in obtaining reliable estimates of the TDI, and therefore measures of ecological status when samples are dominated by less well known taxa in terms of their nutrient signatures. Also, further work is required to obtaining information to compile other metrics e.g. the Van Dam metrics (see methods) for less known taxa and results must be regarded with caution.

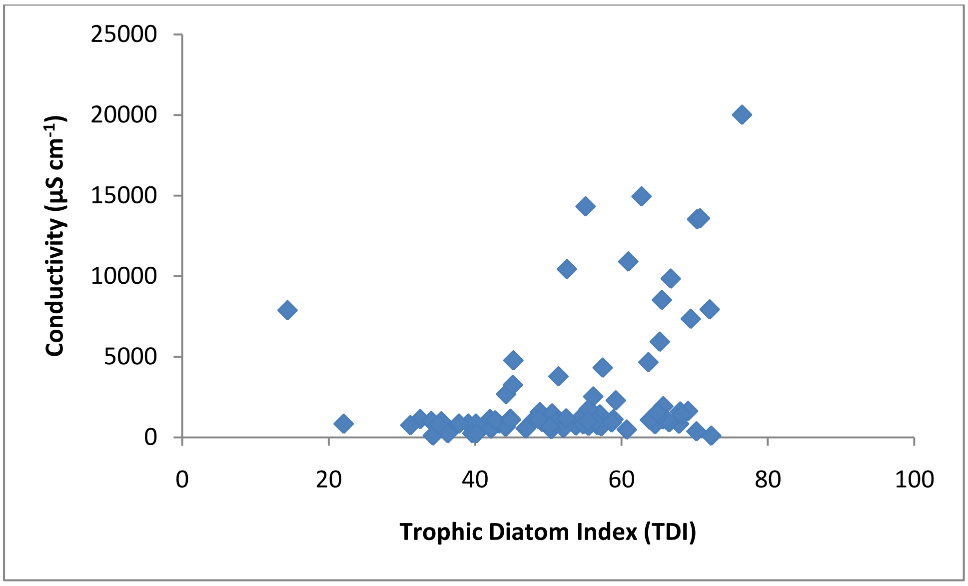

Figure 3.5. Relationship between conductivity of ditchwater and the TDI, based on the relative abundance of periphytic diatoms taken from ditches sampled during 2008 and 2009. The data point to the far left of the plot is taken from site CHY42.

3.3 Indicators of eutrophication linked to plant origin during 2008-2009

Diatom-derived biofilms from each site were regrouped according to macrophyte origin disregarding geographical location. Six categories were defined including biofilms on *Bulboschoenus spp. Sparganium spp.*, *Phragmites spp., Typha spp., Glyceria spp.*, and a sixth category for ‘other genera’ plants; average

TDI indices were derived using the diatom assemblages (Table 3.1). Significant differences were recorded for the TDI depending on plant origin (ANOVA, F = 2.76,$(_{5,79})$, p = .024); *post hoc* analysis indicated that the TDI derived from epiphytic diatoms growing on *Phragmites spp* was higher than the TDI obtained from diatoms taken from *Typha spp*. Conductivity measurements were frequently much higher on samples taken from *Typha* plants compared to those growing on *Phragmites* though the uncertainty attached to the 's' values from the more brackish diatom species, means these results must be regarded with some caution. No differences were found in the percentage of motile diatoms associated with different plant types (ANOVA, p = 0.22) or in the percentage of organic tolerant valves (p = 0.72).

Table 3.1 Relationship between Trophic Diatom Index (TDI) and macrophyte origin of diatoms collected from ditch sites during the 2008-2009 field campaign.

Plant Category	TDI (mean ± SD)	Conductivity (µS cm^{-1}) (mean ± SD)
Bulboschoenus maritimus	55.7 ± 15.4	8753 ± 6118
Sparganium erectum	52.5 ± 11.4	999 ± 486
Phragmites australis	59.6 ± 8.2	3220 ± 3457
Typha latifolia	43.4 ± 11.8	729 ± 306
Glyceria maxima	45.7 ± 6.7	616 ± 96
'Other'	48.8 ± 17.1	2752 ± 3036

7.4 Ordination of diatom assemblages and spatial patterns in species composition across sites sampled between 2008-2009.

A detrended correspondence analysis was undertaken on all sites sampled during 2008-2009. A preliminary analysis indicated two major outliers in the ordination. Outliers were removed to examine spatial patterns between the other ditch samples before running a second DCA. The gradient length of the first axis was > 3.5 indicating good separation and the first three axes explained 16% of the cumulative percentage variance in the species data. The sites were identified according to ditch location (Figure 3.6).

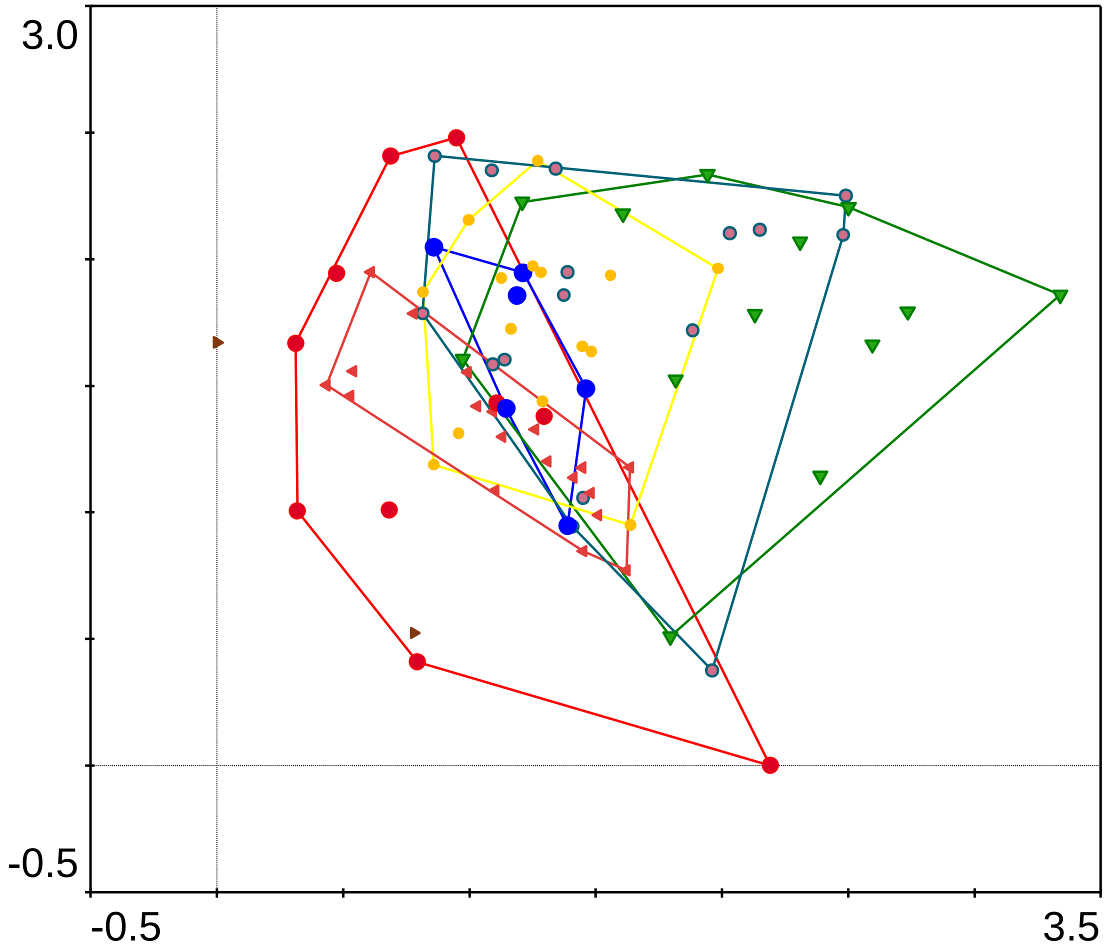

Figure 3.7 Axis 1 and 2 of a DCA of ditch samples categorised according to geographical location based on relative abundance of diatom species assemblages. Red circles = Sussex, Blue circles = Suffolk; downward pointing green triangles = Essex; Yellow circles = Norfolk; pink/orange left-handed triangles = Somerset and Avon Moors and Levels; Brown right pointing triangles = Anglesey; Pink circles = Kent.

Ditch samples from Essex were located primarily to the right of the ordination on Axis 1 whilst sites from Sussex, with one exception, were located to the left and there was little overlap between the two envelopes indicating very different diatom assemblages and inferring different environmental conditions. The southern most datapoint for Sussex was taken from Amberley Wildbrooks in West Sussex and was again was unusual being dominated (72% RA) by *Nitzschia acicularis*, a diatom that is common and is typically recorded as planktic but can be epipelic (mud growing) and tolerant of heavy pollution. It was rarely recorded in other samples. Ditch samples from Essex and Sussex were more diverse, whilst diatom samples from ditches in Suffolk and Somerset and Avon were clustered more centrally hence samples from the latter two geographical areas did not show much variation between samples. The two Welsh diatom samples from ditches in Anglesey had very disparate diatom communities and were spatially separated along Axis 2. However, the number of sites in each geographical location is variable and more samples from less well represented sites are required before firmer conclusions could be drawn relating to heterogeneity within geographical location.

The DCA samples scores from Axis 1 and 2 were correlated with selected variables to account for the ditch diatom sample distribution. A positive correlation was recorded for the percentage of motile valves and DCA Axis 1 sample scores ($p < 0.001$) indicative of more motile species, (which are typically, though not always an indication of nutrient tolerance) to the right of the ordination. There were also strong positive correlations between the Van Dam metric for salinity (H) and conductivity ($p < 0.001$) with DCA Axis 1. A greater relative abundance of acidophilic species was found in a few of the samples, notably PBB3 from West Sussex with 17% *Eunotia mondon* and MTH2 from Anglesey, dominated by *E.pectinalis* providing strong evidence of an acidic influence in the these sites (Figure 3.8). Again, along Axis 2, there was a strong positive correlation with the % of motile diatoms and with the van Dam metric for salinity ($p < 0.001$). However, many of the variables correlated with each other and teasing out the major drivers will require further investigation.

Figure 3.8. Examples of two acidophilic diatom species with *E.pectinalis* (left) and *E.monodon* (right).

It has been noted that a number of sites had a relatively high abundance of diatoms for which we have less reliable indicators of their tolerance to nutrients or other pressures within the UK. One of the most frequently occurring genera was *Epithemia*. This genus was also common in the sites from the Somerset and Gwent Levels sampled in the first survey of 2007(see Part 1, Yallop. 2008). The presence of nitrogen fixing endosymbionts in this species complex suggests that sites in which this species is common may have lower concentrations of inorganic sources of nitrogen. In the previous report, (Yallop, 2008), it was considered that sites with a relatively high abundance of *Epithemia*, (and consequently relatively high TDI values) may not be a reliable estimate of ecological status due to the lack of information about the tolerance of this species to eutrophication. It was suggested that the ‘s’ value allocated for this species in the DARLEQ database for the predictive tool was too high (see Report 1 for further details). For the analyses in this second report, the value for s was revised to match that assigned to lake sites in the DARLEQ lake tool. The rationale for this was that *Epithemia* is much more common in lake sites than in river sites. At least three species were found including *E.sorex*, *E.turgida* and *E.adnata* in the 2008-2009 survey. *E.sorex* is known to be tolerant to brackish conditions though was also recorded in freshwaters.

The sample scores for DCA Axis 1 obtained from the diatom samples collected between 2008-2009 correlated positively with conductivity (0.633, $p < 0.001$). Conductivity measurements taken in the during the field sampling surveys ranged from 100 to 20,000 $\mu S\ cm^{-1}$. Highest values were measured in sites from Essex. A number of diatom species with a preference for saline waters were recorded in these sites. For example, the marine diatom *Amphora coffeaeformis* reached a high relative abundance in a number of the Essex ditches including HDL1 (44% RA), and VFB13 (48%). Only sparse records exist for the distribution of this species in the UK database, and we do not have sufficient data with which to derive reliable indicator values for nutrient tolerance. In order to include this species in the analyses, it was assigned a generic code of AM9999 and given an 's' value of 4, which is a typical score for species in this genus, though this needs further verification. This species was positioned to the far right in the ordination on Axis 1 and at just about the central position along Axis 2. Likely, this distinctive species would be a good indicator of salinity. A second species *Tabularia fasciculata* also reached a high relative abundance in a limited number of samples e.g. GRN17 in Kent, where a relative abundance of 17% was recorded. This species was located to the top right of the ordination along with sites from Essex. This diatom was typically found with another relatively long pennate species *Ctenophora pulchella.* Both of these species may be useful indicators of environmental conditions.

7.5 Inter-annual variation of ditches on the Somerset and Avon Levels

To provide an indication of the natural variability at sites, repeat samples were taken from sites on the Somerset and Avon Moors and Levels to examine the inter-annual variability of diatoms, over the short-term in areas not considered to be subject to any marked change in management other than normal cleaning processes. The TDI values obtained for the sites during the first survey are *not* directly comparable with the values obtained in this second survey. Ongoing method refinements were underway as part of Phase 2 of the development of the TDI predictive tool and the revised tool was used to analyse the data from 2009-2009. The variability between samples collected from these sites between 2007-2009 is revisited in the final part of the report (section 4). Further, problems associated with small sample sizes were experienced with a number of the repeat site samples taken during the 2008-2009 and these had to be removed from the analyses.

Diatom Surveys 2008-2009: key points.

- The diatom species composition of the ditches across the south of England and Anglesey (Wales) revealed a high species richness.
- The species recorded were mostly typical of the epilithic diatom assemblages found in rivers across the UK but a number of additional species were recorded which are more typical of saline conditions or more typically associated with lakes as epiphytes of aquatic plants.
- Application of the revised DARLEQ tool indicated sites ranging from poor to high ecological status, though results are regarded as tentative because some samples were dominated by the rarer taxa where their nutrient tolerance is not certain.
- More reliable estimates are needed for environmental tolerances of diatom species that are relatively uncommon in the ditch diatom flora, particular brackish species.
- Ordination techniques indicated that whilst ditches from some geographical areas clustered together in the ordination, others were more disparate in their species composition indicating considerably more spatial heterogeneity in diatom composition and therefore reflecting greater variation in ecological status of those ditches.

Section 4 Classification of ditches according to their periphytic diatoms flora: a synthesis from sites sampled during 2007-2009.

4.1 Methods

A database of the relative abundance of 134 diatom samples taken from ditch systems of grazing marshes in Somerset and Avon Moors and Levels, Gwent and Anglesey (Wales) Kent, Sussex, Suffolk, Essex and Norfolk was compiled. A list of the marshes surveyed during is recorded in Table 4.1. Some samples were removed from the analyses due to insufficient sample size. Details of the methods used and results from the individual surveys are presented in Part 1 and Part 2. In this final section of the report a synthesis is carried out using all of the data obtained from the survey in 2007 in Somerset and Avon and Gwent, and the 2008-2009 survey in Anglesey, Sussex, Suffolk, Somerset and Avon, Norfolk, Essex and Kent. Refinements were made to the predictive TDI diatom tool between the two survey periods. Here, the revised TDI tool is applied to the entire data set from all the sites sampled from England and Wales between 2007 and 2009.

Table 4.1 Location and number of the sub-set of ditches sampled for diatoms during the 2007-2009 surveys.

Marshes	Year Sampled			County	Number of ditches sampled		
West Sedgemoor	2007	2008	2009	Somerset	6	4	4
Kings Sedgemoor	2007	2008	2009	Somerset	4	1	2
Moorlinch	2007	2008	2009	Somerset	4	3	5
Chilton and Catcott Moors	2007			Somerset	4		
Tealham and Tadham Moors	2007			Somerset	4		
Pawlett Hams	2007			Somerset	4		
Kenn, Nailsea and Tickenham	2007			Avon	4		
Southlake Moor	2007	2009		Somerset	3	1	
Non-SSSI sites	2007			Somerset	2		
Caldicot Level	2007			Gwent	8		
Wentlooge Level	2007			Gwent	4		
Broomhill Levels	2008			Kent/East Sussex*	1		
East Guldeford Level	2008			Kent/East Sussex	2		
Walland Marsh	2008			Kent/East Sussex	1		
Fairfield Level	2008			Kent/East Sussex	3		
The Dowels	2008			Kent/East Sussex	2		
All Hallows	2008			Kent	1		
Grain Marshes	2008			Kent	1		
Seasalter/Graveney	2008			Kent	3		
Cooling Marshes	2008			Kent	1		
Shorne Marshes	2008			Kent	2		
Chetney Marshes	2008			Kent	1		
Halstow Marshes	2008			Kent	2		
Amberley Wildbrooks	2008			Sussex	2		
Pulborough Brooks	2008			Sussex	1		
Pevensey Levels	2008			Sussex	9		
Malltraeth Marsh	2008			Anglesey	2		
Fambridge Marsh	2009			Essex	3		
Rainham Marsh	2009			Essex	3		
Vange and Fobbing Marsh	2009			Essex	3		
Brightlingsea Marsh	2009			Essex	2		
Hadleigh Marsh	2009			Essex	2		
Sizewell Marsh	2009			Suffolk	4		
Shotley Marsh, Orwell Estuary	2009			Suffolk	2		
Buckenham Marsh	2009			Norfolk	2		
Upton Marsh	2009			Norfolk	3		
Cantely Marsh	2009			Norfolk	2		
South Walsham Marsh	2009			Norfolk	1		
Oby Marsh	2009			Norfolk	3		
Limpenhoe Marsh	2009			Norfolk	2		
Upton Broad	2009				3		
Fleggburgh Marsh	2009			Norfolk	2		

*samples pooled with Kent for analysis

4.1.1 Data harmonisation

During the development of the new predictive TDI tool, a number of data harmonisation exercises were undertaken and a revised list of taxa was determined. As part of the DARLEQ tool development, ring-tests were undertaken by a team of expert taxonomists. As a result, some 'problematic taxa', which proved difficult to distinguish using light microscopy, should be pooled prior to further analysis. The ditch flora has similarly been screened and taxa pooled, where appropriate, prior to further analyses.

4.1.2 Data Analyses

Selected environmental variables were taken from the spreadsheets compiled for the larger botanical and invertebrate survey to compare with distribution patterns and metrics obtained from the diatom flora. TWINSPAN (Two-way Indicator Species Analysis, (Hill, 1979) was adopted to produce a classification of the diatom samples using CAP, Pisces). Data were square-root transformed prior to analysis and rare species which were found in less than 3 of the samples were removed. Five pseudospecies cut levels were used on the relative abundance data (0, 5, 10, 20 and 40 relative abundance). Preliminary DCA screening of the entire data-set indicated outliers which and these outliers were removed to allow further discrimination between samples. End groups identified during the TWINSPAN analysis were described according to a suite of environmental characteristics and further ordination techniques were applied.

4.2 Results

4.2.1 Classification of the ditches based on diatom flora sampled during 2007-2009.

Using TWINSPAN analysis seven end-groups were identified (Table 4.2). The geographical location of samples within each of the grazing marshes is indicated.

Table 4.2. TWINSPAN end groups based on relative abundance of periphytic diatoms on emergent macrophytes from grazing marsh ditches.

	A	Bi	Bii	Ci	Cii	D	E
Sussex	2	1	1	**4**	3		
Essex	**9**		3				
Suffolk			2	3			1
Norfolk	2	**1**	**11**	2			
Kent	**8**		5	2			
Somerset and Avon		**10**		**6**		**15**	**27**
Gwent			1			6	4
Anglesey					2		
Total	21	12	23	17	5	21	32

The first split resulted in two groups of 78 and 53 respectively. The main indicator species for the first split was *Lemnicola hungarica*, and small *Navicula* species which were relatively more abundant in samples from the group of 53 diatom ditch samples. The group of 53 were predominantly located in Somerset and Avon Moors and Levels and Gwent, and were dominated, though not exclusively, by samples collected during 2007 assigned to groups D and E. The group of 78 was split into Group A (21 samples) and a further group of 57 samples with saline tolerant species including *Tabularia fasciculata*, *Amphora coffeaeformis* and *Navicula tripunctata* favouring Group A (mostly from Kent and Essex). The group of 57 samples was further subdivided into a group of 35 (TWB), due to frequent representation of *Achnanthidium minutissimum*, *Navicula veneta*, *Amphora veneta* and *Amphora iniarensis* and a group of, 22 (TWC) with no particularly strong indicators. Further splits for each group further resolved spatial patterns. Importantly, repeat samples, collected during 2008 and 2009 from specific Somerset and Avon Moors and levels were mostly associated with Twinspan Group Bi, notable for low representation of *Nitzschia amphibia*, *Achnanthidium minutissimum* and *Rhoicosphenia abbreviata*.

The end-groups were characterised by a suite of selected environmental variables (Table 4.3).

Table 4.3 Selected biotic and abiotic characteristics of TWINSPAN end-groups: TDI is the Trophic Diatom Index indicating eutrophication where higher values indicate relatively more eutrophication; the % motile valves may indicate sediment deposition on the plants or may be an indication of maturity of biofilm; the % organic tolerant valves may indicate organic contaminants in the system; the % of saline valves indicates those species with known tolerance or preference for saline conditions. Numbers in bold are the highest values within each category. Values are mean ± SD.

Group and Site Variables	**TWA**	**TWBi**	**TWBii**	**TWCi**	**TWCii**	**TWD**	**TWE**
TDI	59.8±12.9	60.8±6.6	59.1±10.3	54.8±9.1	38.8±20.3	**64.3±5.4**	62.4±9.5
% motile valves	**41.47±24.6**	28.5±21.8	17.6±14.1	3.6±5.3	30.0±35.0	34.3±19.4	30.0±16.8
% organic tolerant valves	15.8±9.8	16.8±19.9	8.93±11.4	0.5±1.2	22.9±38.2	20.7±14.4	**24.5±17.2**
% saline valves	**19.4±20.5**	2.7±3.2	4.8±7.0	0.3±0.9	0.5±0.6	4.3±5.3	1.1±1.3
Water depth (cm)	59.3±36.8	55.0±16.5	65.0±26.9	**70.3±25.3**	67.0±14.1	62.4±24.5	58.0±17.3
Silt depth (cm)	52.8±32.0	**80.8±28.2**	62.2±23.9	65.9±33.1	38.0±25.0	57.9±43.0	74.1±35.0
Conductivity (µS cm^{-1})	**6932±5955**	857±258	2109±2146	1116±718	190±78.0	695±284	763±196
pH	**7.7±0.6**	7.3±0.4	7.3±0.5	7.2±0.3	6.5±0.5	7.4±0.4	7.0±0.3
Aquatic Plant Species	10.4±5.5	**20.0±2.3**	14.7±5.3	18.7±4.0	19.0±5.5	18.2±3.7	16.1±3.9
No. Diatom Taxa	24.7±8.9	22.9±5.6	24.5±6.1	13.2±5.3	22.8±7.2	**31.3±5.4**	22.9±6.6
% cover emergents	30.93±33.7	35.70±46.0	42.18±38.2	29.5±35.41	30.5±25.0	14.4±19.9	**47.9±32.4**
% samples with > 30% cover of *Lemna*/*Azolla*	10	2	17	18	0	52	**66**

The split between TWINSPAN groups A and B from groups D and E separates sites with a lower % cover of floating plants (*Lemna* and *Azolla*). Many of the samples in Groups D and E scored either a 4 or 5 for the % cover of floating plants assessed on the DAFOR scale where 4 = > 30% cover and % = > 70% cover (Nick Stewart, pers.comm). The diatom *Lemnicola hungarica* was an indicator species for the first division of the TWINSPAN analysis and this species is frequently found growing on *Lemna* spp. Presence of a relatively large percentage of *Lemnicola* valves in a ditch sample could indicate contamination from *Lemna* (though this was not evident in field derived samples) or it could indicate that the valves from *Lemna* may act as an inoculum for colonisation of other plants growing in the ditches. Duckweed species are frequently associated with eutrophication. Crucially, it is the presence of *Lemnicola* in the diatom samples that caused the split of many of the 2007 diatom samples taken from the Somerset and Avon Moors and Levels and assigned to groups D and E, from the 2008/9 samples (when they were largely assigned to TWBi end-group). The reason for the predominance of floating duckweed in 2007 and the low cover in subsequent sampling years is not clear. The mean Trophic Diatom Index was higher in Twinspan groups D and E compared to sites where floating duckweed and *Azolla* were relatively uncommon although the mean range of TDI values across the end-groups was small, the exception being the TDI for TWCii, though the sample size for this group was very small. The diatom *Lemnicola* was originally assigned an 's' value of 5 in the DARLEQ database indicating a tolerance to high nutrients. Samples with a relatively high abundance of this species will give an estimate of relatively low ecological status. The overall percentage of ditch cover (% cover emergents/floating mats) was highest in ditches in TWE marking them as sites with the least open water and greater silt depth, and were similar to ditches assigned to TWINSPAN end groups A,C and G2 based on the more extensive ditch survey on macrophytes sampled over the same time period (Drake *et al*., 2010). Such ditches are considered to be in predominantly a late stage in the hydrosere (Nick Stewart, pers. comm.) which could indicate a late stage in the cleaning cycle or a low maintenance regime (Drake *et al*., 2010). By contrast, ditches in TWD had the lowest % cover of emergents and interestingly had the highest average number of diatom species. It is hypothesised that ditches in a late stage in the hydrosere may become more specialised in terms of their diatom flora and environmental conditions become more extreme or less favourable e.g. less light, less flow, steeper oxygen gradients etc. In the more extensive macrophyte surveys it was considered that ditches dominated by floating duckweeds were 'predominantly located in the western marshes of Gwent and Somerset (Drake *et al*., 2010) hence these could potentially be less favourable and be less speciose. Further research will be required to test this hypothesis.

The strong brackish influence is also apparent and particularly so for TWINSPAN group A where the higher conductivities and highest % saline valves were recorded. Sites with higher salinity were mostly located in North Kent and Essex marshes in TWA and TWBii. Two sites in Norfolk had a high conductivity also contributing to the higher mean for group TWBii. TWA and TWBii also had a lower average number of aquatic plant species. Most samples from Norfolk were assigned to TWBii indicating a more distinctive diatom flora.

4.2.2 Ordination of ditches.

Detrended correspondence analysis (DCA) of the samples, based on the relative abundance of diatoms was carried out on the dataset from 2007-2009. The first two axes have eigenvalues of 0.266 and 0.178 respectively and the first three axes explain 15% of the cumulative percentage variance of the species data. Figure 4.1 identifies the sampling sites according to conductivity (as a surrogate for salinity) along gradient along DCA Axis 1.

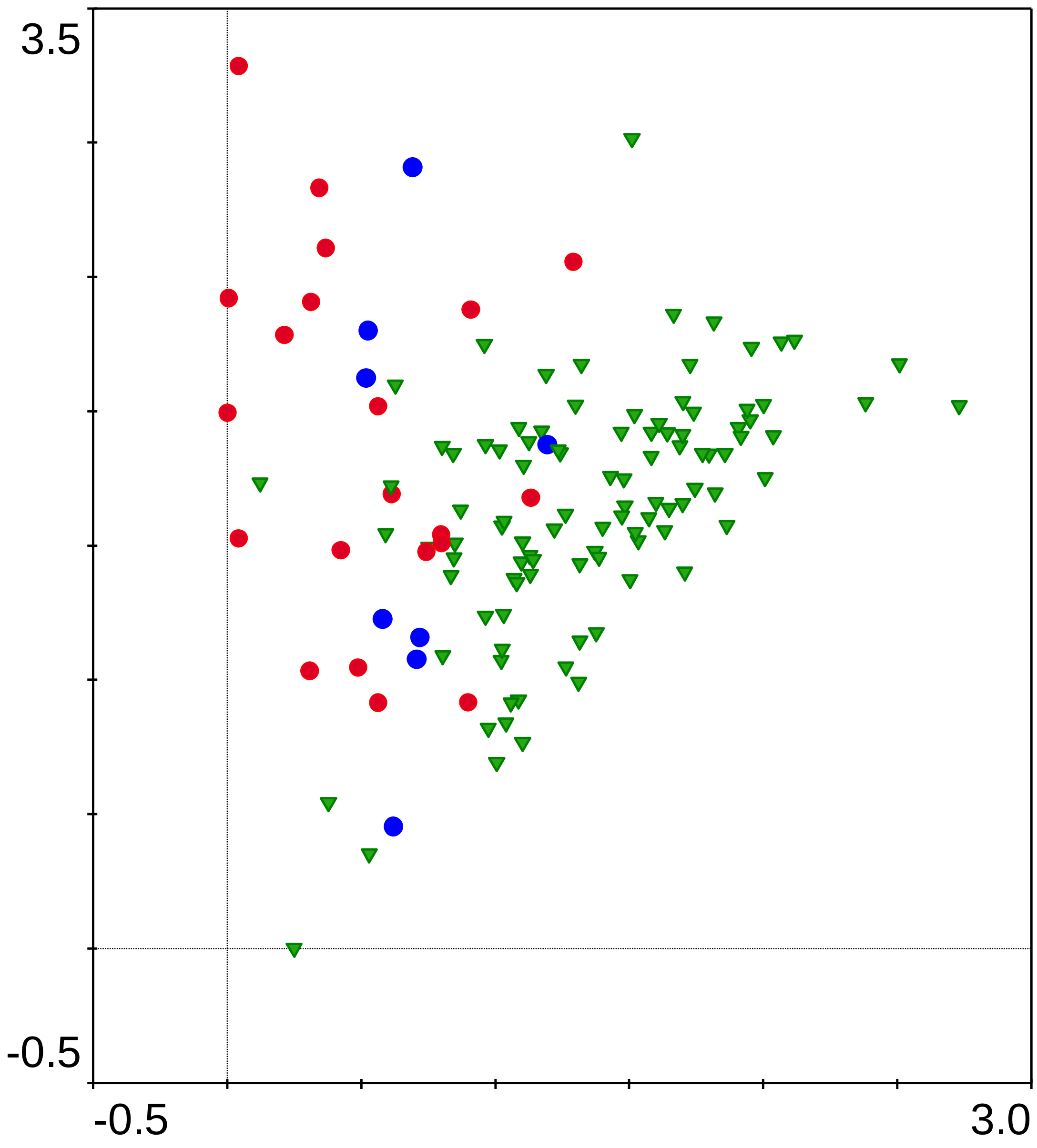

Figure 4.1 Axis 1 and 2 of a Detrended Correspondence Analysis (DCA) of periphytic diatom samples in ditches from grazing marshes sampled between 2007 and 2009 indicating the gradient of conductivity (as a surrogate measure of salinity). Red filled circles = sites with conductivity equal to or higher than 2000 µS cm^{-1} ; blue filled circles are sites with intermediate conductivity between 1250 and 2000 µS cm^{-1}; green triangles are sites with conductivity below 1250 µS cm^{-1}).

Whilst a negative conductivity gradient is seen along Axis 1, there is a large spread of sites from each category of salinity along Axis 2 indicating that other environmental variables are influencing diatom composition. The DCA was structured according to TWINSPAN categories to further highlight patterns according to group characteristics (Figure 4.2).

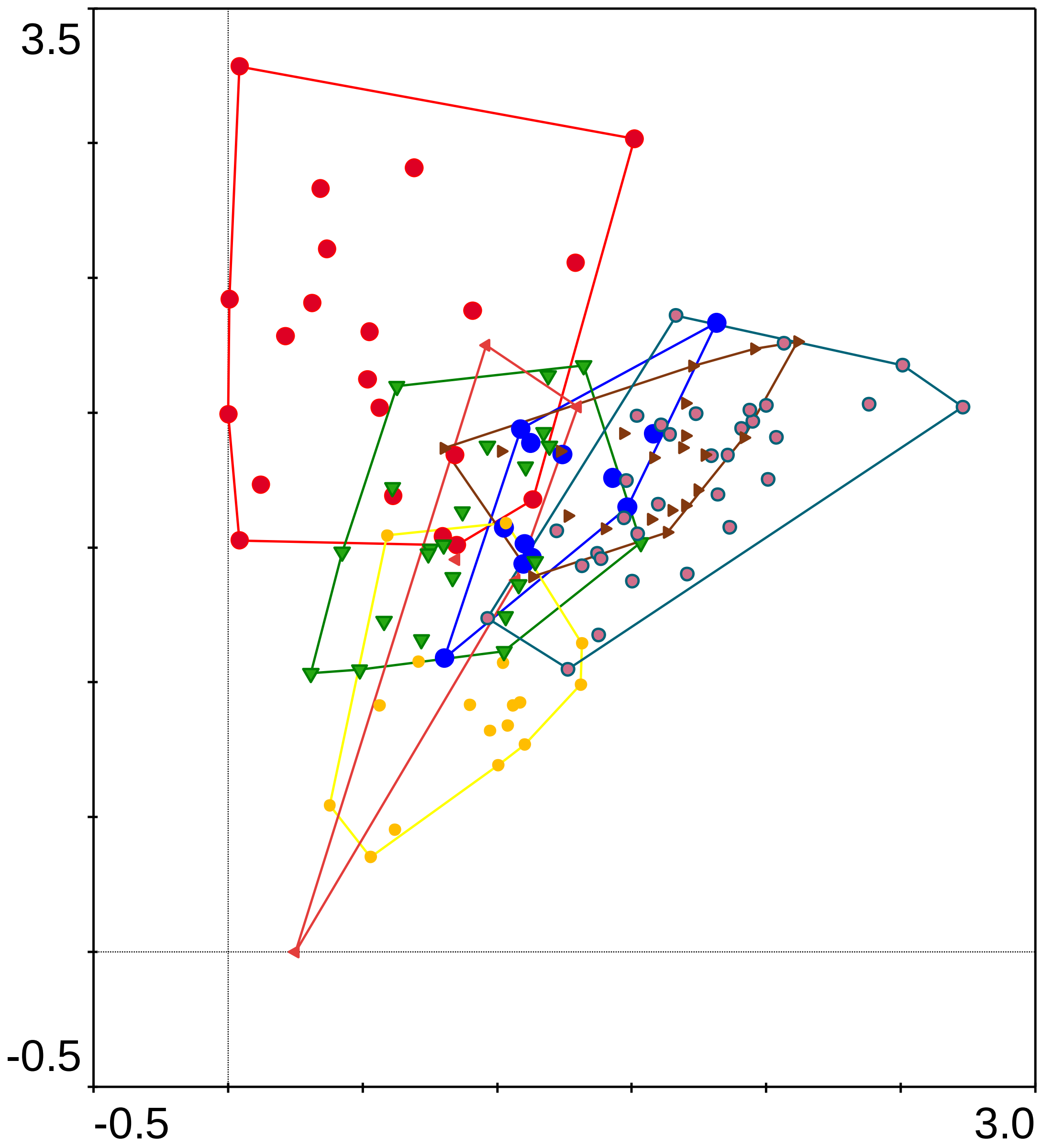

Figure 4.2. Axis 1 and 2 of the Detrended Correspondence Analysis (DCA) of periphytic diatom samples from selected location in ditches from grazing marshes sampled between 2007 and 2009 indicating boundaries (envelopes) for TWINSPAN end groups where Red filled circles = TWA; blue filled circles = TWBi; green downward pointing triangles = TWBii; Yellow filled circles = TWCi; left handed pink triangles = TWCii; right handed brown triangles = TWD and pink filled circles = TWE.

Sites to the top left of the ordination assigned predominantly to TWA mostly came from Essex and Kent. Whilst the majority of samples in TWA were either of high or intermediate conductivity, there were two notable exceptions, the two sites from Sussex with low conductivity. Many of the diatom samples in TWBii are located at the bottom of Axis 2. This group was characterised a relatively low mean number of aquatic plant species and the second highest mean for the % cover of emergent species. The clear separation of TWA from the rest of the samples demonstrates the clear overriding influence of salinity on the composition of the diatom flora.

A similar categorisation is seen in the ordination plot identifying diatom sampling according to their sampling origin (Figure 4.3) indicating a degree of 'geographical distinction'. For example, there is little overlap in the envelopes delineating diatom assemblages sampled in Somerset and Avon compared to that from Essex indicating a very different diatom assemblage between these areas. A distinctiveness in the macrophyte flora of brackish ditches, particularly in the east of England was also highlighted in the plant survey report (Drake *et al*., 2010).

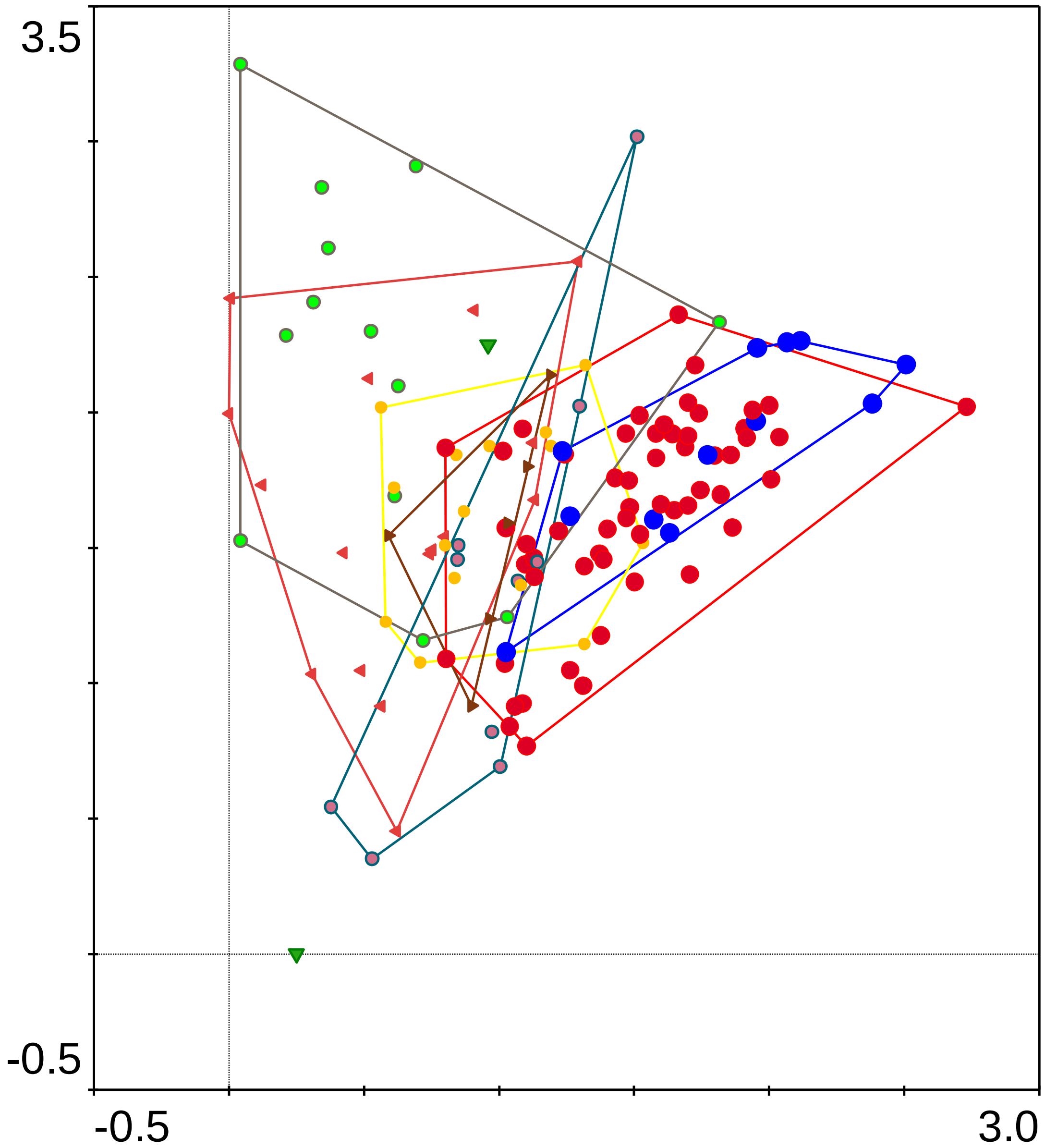

Figure 4.3 Axis 1 and 2 of the Detrended Correspondence Analysis (DCA) of periphytic diatom samples from selected location in ditches from grazing marshes sampled between 2007 and 2009 highlighting by means of envelopes, the relationships between diatom samples according to location of sampling. Red filled circles = Somerset and Avon; blue filled circles = Gwent; green downward pointing triangles = Anglesey; Yellow filled circles =Norfolk; left handed pink triangles = Kent; right handed brown triangles = Suffolk; pink-filled circles = Sussex and green filled circles = Essex; green downward pointing triangle = Anglesey.

6.3 Aspects of Host specificity

The diatom samples were taken from many different plant species. In order to assess if there was evidence of host specificity in this larger data set, samples were assigned to their respective plant groups to look for patterns (Figure 4.4). Clusters of diatoms sampled from different plant types can be found within small areas of the ordination and therefore it would appear that a similar composition of diatoms can be found regardless of the substrate on which they are grown. These findings further contribute to the body of evidence supporting the 'neutral substrate hypothesis' (Yallop et al., 2008; Cejudo-Figuiras et al., 2010). However, some plants have a more restricted distribution e.g. the salt tolerant plant *Bulboschoenus maritimus*, situated to the left of the ordination where a more specialist flora of brackish diatom species might be expected. However, it is noted that there is lower representation of some plant types. Diatom samples from *Sparganium* and *Phragmites* are spread throughout the ordination illustrating the utility of these plant types as sampling substrates to infer site ecological status by means of their associated diatom assemblages.

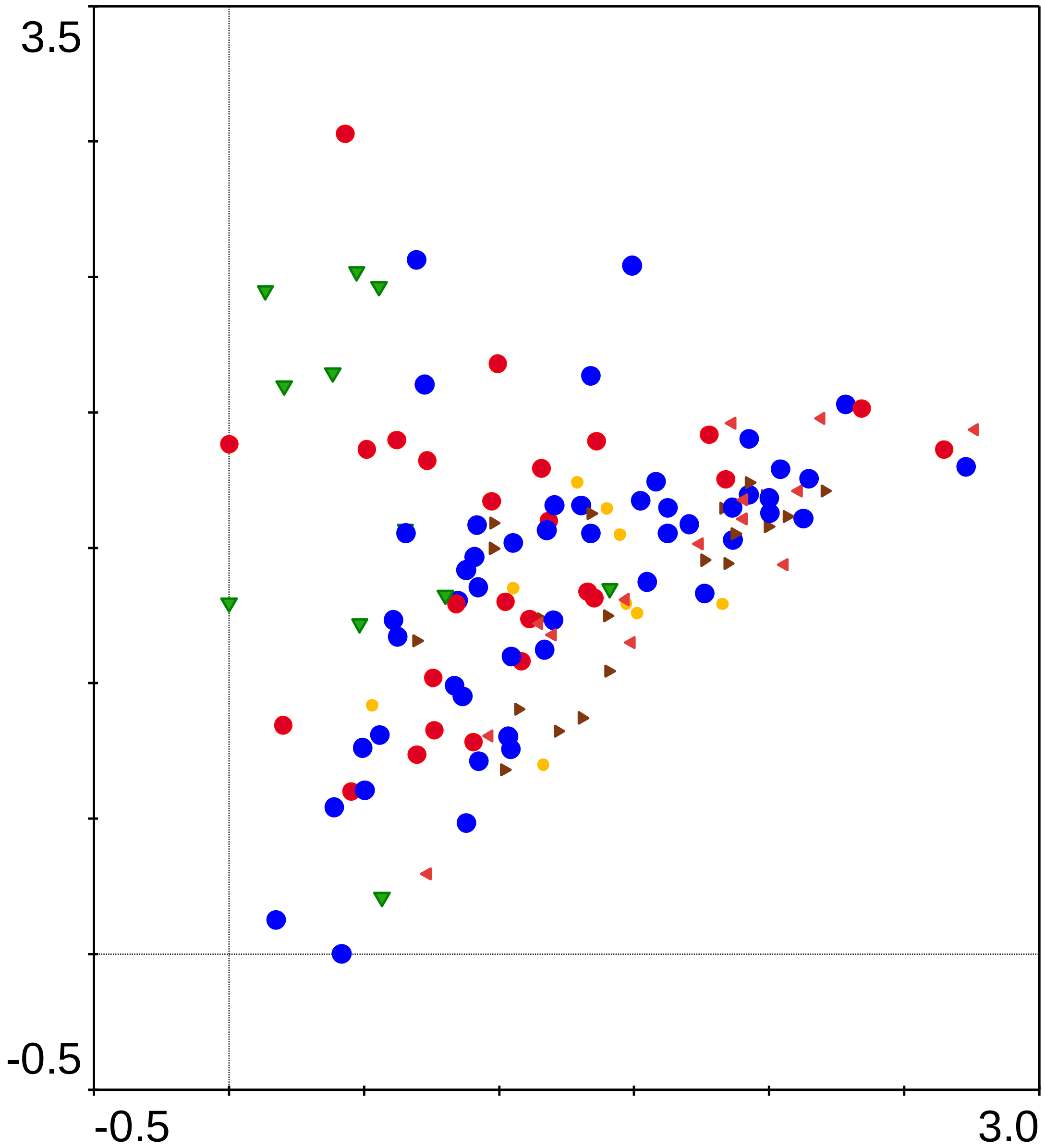

Figure 4.4 Axis 1 and 2 of the Detrended Correspondence Analysis (DCA) of periphytic diatom samples from selected location in ditches from grazing marshes sampled between 2007 and 2009. Symbols indicate allocation to host plant species where red filled circles =*Phragmites* spp.; blue filled circles = *Sparganium* spp. TWBi; green downward pointing triangles = *Bulboschoenus* spp.; Yellow filled circles = *Typha* spp.; left handed pink triangles = *Glyceria* spp.; right handed brown triangles = other plant groups.

Whilst the host plants themselves may be distributed according to environmental preferences there is no clear evidence of any spatial patterns in the distribution of diatoms that can be linked to the host plants and further validating the use of a variety of emergent macrophytes as substrates for sampling diatoms.

4.4 Evidence for Eutrophication from the diatom signal

Diatoms have frequently been used in the assessment of environmental pressures in freshwater rivers and lakes. They have been used, for example to assess changes in eutrophication above and downstream of sewage treatments works in compliance with the Urban Waste Treatment Directive (UWWTD). More recently, diatoms (as a proxy for phytobenthos) have been used as one of the 'biological elements' to assess the ecological status of lakes and rivers in the UK, and across Europe in compliance with the Water Framework Directive (WFD). An Ecological Quality Ratio (EQR) is quantified using the diatom tool and is used to assign each site to one of five categories of ecological status ranging from Bad to High, where high indicates little change from pristine conditions. For the purposes of this report, the TDI was obtained from an assessment of the ditch diatom flora. To obtain an indication of nutrient pressures the sites were assigned to one of four categories on the ordination plot (Figure 4.5) to look for evidence of eutrophication. These categories are considered as relative rather than absolute due to the uncertainty of 's' values associated with flora that are not commonly recorded in freshwater rivers.

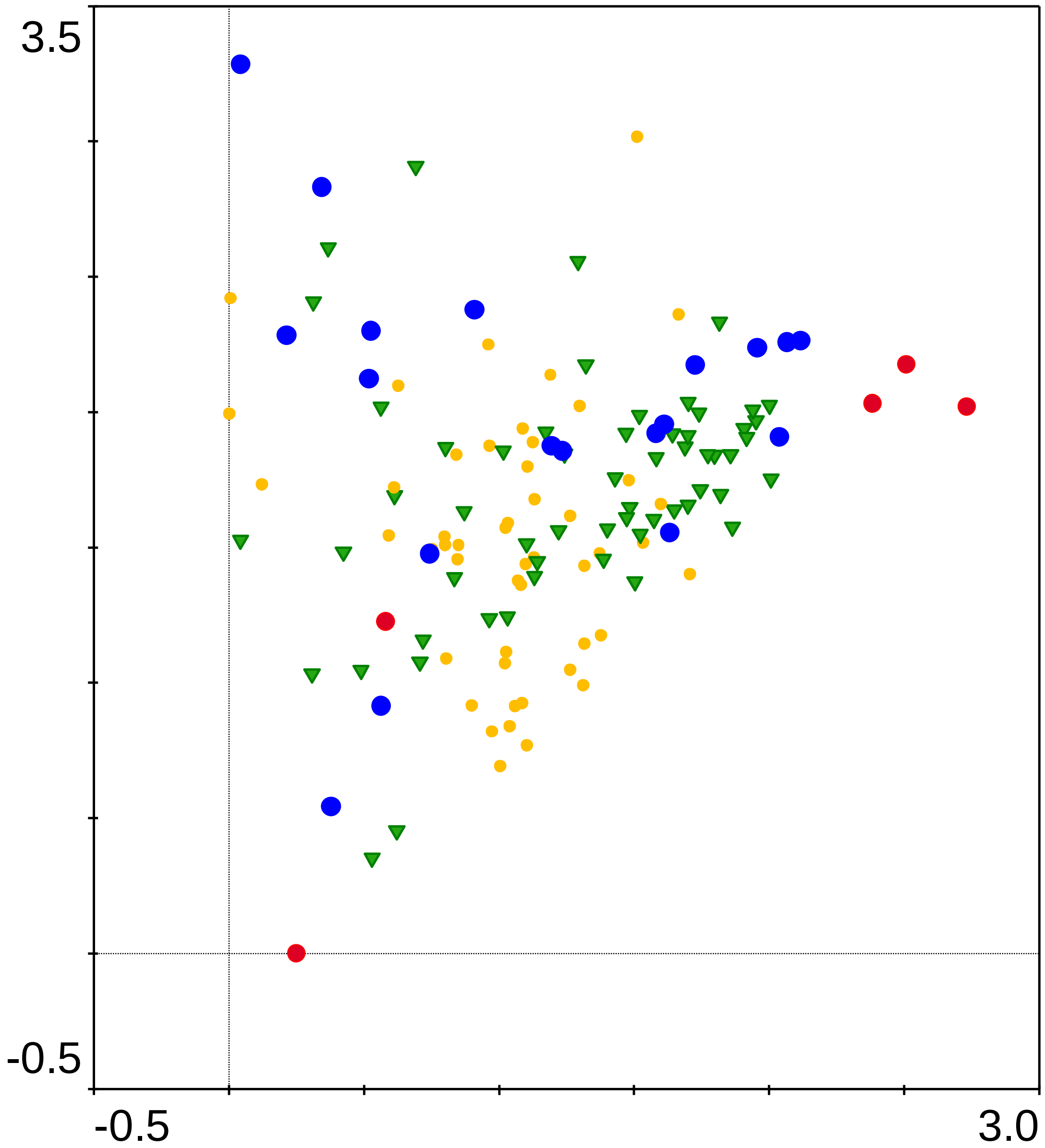

Figure 4.5 Axis 1 and 2 of the Detrended Correspondence Analysis (DCA) of periphytic diatom samples from selected location in ditches from grazing marshes sampled between 2007 and 2009 showing inferred nutrient status. Symbols indicate allocation to categories of inferred nutrient loading, based on the TDI, where red filled circles = very high nutrients; blue filled circles = high nutrient loading; green downward pointing triangles = intermediate nutrient concentrations and yellow filled circles = relatively lower nutrients.

Only five samples, of which three were from Wales, one was from Somerset and Avon and one from Norfolk (TTM21, WLL22, CCL51, OBY14, MTH8) were considered to reflect much higher nutrient concentrations relative to other sites The three diatom samples with very high inferred nutrient concentrations to the right of the ordination were all sites where the cover of *Lemna* and *Azolla* was relatively high and these were dominated by *Lemnicola hungarica*. The other two sites were dominated by either *Rhoicosphenia abbreviata*, an epiphyte on blanket weed, *Cladophora* or by *Epithemia*, for which 's' values need to be resolved. The over-riding effect of salinity may mask other associations. Further analyses e.g. variance partitioning will likely lead to further resolution of the factors influencing the diatom distribution whilst also acting as a valuable signature of the status of the waterbodies. However, many of the sites would not appear to be heavily impacted by eutrophication. Due to the natural variation in diatom assemblages, and based on evidence obtained from replicate sampling in rivers and lakes, it is advised that a number of samples are collected from one site over a period of time (Kelly *et al*., 2009). It is therefore recommended that more temporal replicates are obtained before reliable estimates of ecological status are obtained.

Synthesis of diatom surveys 2007-2009: key points

- The epiphytic diatom assemblages on emergent plants from ditches across Southern England and parts of Wales are speciose with over 216 taxa recorded.
- Many samples were species-rich averaging circa. 25 species in freshwater sites.
- There was a strong brackish influence and sites of higher conductivity > 2000 µm cm^{-1}) were less speciose, with circa 20 species on average.
- Brackish species e.g. *Amphora coffeaeformis* were frequently recorded in some of the samples and these are rarely recorded in river diatom flora.
- Some epiphytic diatoms, typically found in lakes are not well represented in river flora e.g. *Rhopalodia* spp. and *Epithemia* spp. were dominant in some of the samples.
- A few samples were depauperate in diatom flora or were dominated by motile diatom species for reasons unknown.
- Diatom samples with a very skewed, species poor flora can have an undue influence and confound interpretation of general patterns.
- The % of cover of *Lemna/Azolla* was an important criterion separating groups of diatom samples possibly linked to the presence of the diatom *Lemnicola hungarica* .
- Typically diatom samples from the west of England and Gwent supported a different flora to those from whilst salinity was the major driver separating sites, other environmental influences caused a clear east-west separation in the diatom communities.
- A number of diatom species were identified that provided useful indications of water quality.
- Application of the revised TDI enabled separation of sampling sites in terms of the relative position within the spectrum of eutrophication though more temporal replicates are needed before reliable estimates of ecological status can be obtained.

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