Distribution of aquatic vascular plants in lowland rivers: separating the effects of local environmental conditions, longitudinal connectivity and river basin isolation

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SUMMARY

1. Changes in species distributions along rivers have rarely been observed independently of changes in environmental conditions and meaningful comparison between different catchments is made difficult by the limited geographical distribution of species. This study presents a new approach to quantify the effect of the spatial structure of lowland river networks on aquatic plant distribution and to explore the potential underlying processes using species life-history characteristics.
2. Twenty-five species of aquatic vascular plants recorded in 62 sites across five calcareous river basins were used to investigate (i) the temporal turnover of plant species, (ii) the habitat utilisation of species, (iii) the trade-offs between different plant life-history characteristics and (iv) the relationship between species life-history characteristics and habitat utilisation.
3. The annual plant turnover within a 3-year period was, although significant, extremely low. It suggests that results from spatial surveys conducted over 3 years should not be undermined by temporal changes.
4. Spatial connectivity along and between rivers was more important than in-channel physical characteristics in shaping species assemblages. Neither chemical factors (ammonium, phosphate) nor extrinsic biotic competitors (filamentous green algae) significantly influenced plant distribution.
5. The most common combinations of life-history characteristics were neither related to environmental conditions nor to spatial isolation. Instead, they could reflect natural selection processes associated with larger scales than those considered in this study.
6. Plant distribution was most strongly related to the dispersal and regeneration abilities of the plants, supporting the hypotheses relating to longitudinal connectivity. The hypothesis that different growth forms would be associated with different in-channel physical features was not verified. As expected, there were no substantial differences in plant life-history characteristics between river basins.

Keywords: dispersal, habitat, spatial connectivity, species traits, three-table ordination

Introduction

There are a number of factors that influence the distribution of species and communities in nature. By far the best studied of these is the influence of local environmental conditions on species occurrence (the niche concept of Hutchinson, 1957). However, the degree of connection or isolation of an area has an additional, important effect on observed patterns of species distribution and community structure, particularly in a spatially complex or fragmented ecosystem (MacArthur & Wilson, 1963). Rivers are particularly

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good examples of spatially complex ecosystems, with obvious isolation between catchments and strongly directional connectivity within a catchment resulting from the flow of water (Wallace, 1853, p. 467; Shelford, 1911; Butcher, 1927).

New statistical procedures have shown the importance of spatial structure for species distribution in a river network, independently of local environmental conditions, although so far no studies have centred on aquatic plants (see e.g. Angermeier & Winston, 1998; Johnson & Belk, 2001; Joy & Death, 2001; Olden, Jackson & Peres-Neto, 2001; Magalhães, Batalha & Collares-Pereira, 2002). Some studies have tried to disentangle longitudinal gradients in aquatic plant composition from intrinsic downstream changes in habitat characteristics; however the results were rather qualitative or confounded by other factors (e.g. Entz, 1961; Wiegleb, 1980, 1984).

Actual patterns of species distribution are also influenced by evolutionary history and colonisation events. For example, two river basins emanating from the same mountains but running in opposite directions can be found to harbour a different set of species in similar habitat conditions (Burton & Odum, 1945). Studies relating organisms to the longitudinal gradient of rivers may, therefore, have limited predictive power outside their geographical area. This has led ecologists to search for methods which are not based on species identity alone (Shelford, 1911). The river continuum concept (Vannote *et al.*, 1980) incorporated developments from a number of disciplines, including systems theory, geomorphology and species life-history concepts. It offers a good overall framework through which predictions about the location along the river continuum of different types of organisms, such as different types of primary producers, can be made. For example, aquatic vascular plants tend to grow well within a relatively narrow stretch of the theoretical river continuum, where the river bed is relatively stable (Riis & Biggs, 2003) and where light availability for photosynthesis is good (Edwards & Owens, 1962; Kelly, Thyssen & Moeslund, 1983), conditions commonly found in small calcareous lowland rivers. This functional approach does not, however, make explicit predictions about the population dynamics of the different types of organisms found in rivers.

Townsend & Hildrew (1994) made specific predictions about the position of general life-history char-

acteristics in theoretical river habitat 'templets', based on habitat heterogeneity and temporal variability. The river habitat templet concept was then extensively and independently tested within the Rhône river and its floodplain using a range of taxa, including aquatic plants (Statzner, Resh & Dolédec, 1994). It has been used as a framework in many studies of which several were based on primary producers in a riverine context (Wiegleb, Brux & Herr, 1991; Bornette *et al.*, 1994; Muotka & Virtanen, 1995; Biggs, Stevenson & Lowe, 1998). The strong phylogenetic convergence in aquatic plants (Cook, 1990) has encouraged the grouping of species by their life-history characteristics and its relationship to habitat utilisation (see Willby, Abernethy & Demars, 2000 for a recent account). Further studies have now been able to formally link local environmental conditions, species distribution and species life-history characteristics (Dolédec *et al.*, 1996; Legendre, Galzin & Harmelin-Vivien, 1997; Lavorel *et al.*, 1998; Lavorel, Rochette & Lebreton, 1999). Therefore, it is now possible to investigate the role played by the spatial structure of the river network, independent of local environmental conditions, on species composition, and to explore underlying processes using species life-history characteristics.

The aims of the present investigation were (i) to study the temporal turnover of plant species, (ii) to separate the effects of local environmental conditions, longitudinal connectivity and river basin isolation on species distribution, (iii) to investigate trade-offs between species life-history characteristics and (iv) to test the relationship between species life-history characteristics and factors driving the species distribution. It was hypothesised that the spatial effect, independent of environmental conditions, is a significant influence of isolation under the same environmental conditions. It was expected that plant dispersal and regeneration abilities would explain the effect of the spatial isolation. Different morphological plant characteristics were expected to occur in deep/silty *versus* shallow/gravelly river stretches.

Material and methods

Study area

The study area consists of lowland calcareous river basins (maximum elevation 95 metres OD) situated in the county of Norfolk, eastern England (Fig. 1) and
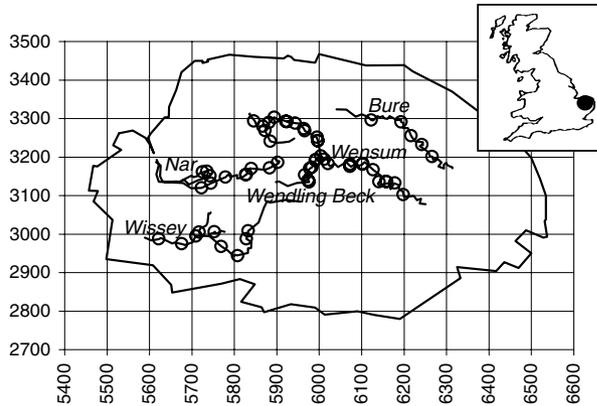
Fig. 1 Location of sampling sites (open circles) and rivers drawn down to their freshwater limits on the 10×10 km^2 National Grid Reference of Great Britain.

characterised by Upper Chalk solid geology overlain by quaternary deposits (chalk boulder clay; glacial sands and gravel). The catchments are dominated by pasture and arable with scattered woodlands (Boar *et al.*, 1994). The impact of water abstraction on river discharge is negligible in the rivers Nar, Bure and Wensum (Hiscock *et al.*, 2001). The rivers have long been engineered to drain the surrounding land more efficiently; channels have been re-directed, widened and weirs inserted to create impoundments. These mill weirs are not now in general use, but still create ponded conditions upstream and cause silt accumulation. The engineering works and weirs have removed the covariation between channel cross-section area (m^2) and discharge (m^3 s^{-1}), generally observed in natural river channels (Leopold, 1962). This has been a key factor in this study as it has allowed the separation of the impact of longitudinal connectance from local physical environmental conditions.

Site location and characteristics

Sixty-two river stretches, each about 500 m^2, in five rivers were selected (Fig. 1). The sites were approximately evenly distributed along the water courses. Pairs of sites were also sampled above and below the main sewage treatment works and weirs in the River Wensum catchment area. The River Wensum and the Wendling Beck were surveyed during summer 1999 and the other rivers (Nar, Wissey, Bure) during summer 2000. A subset of 12 sites in 2000 and 16

sites in 2001 were resampled in the Wensum and the Wendling Beck to investigate the temporal variability of the species composition. Each site was chosen to be physically homogeneous (in terms of depth-velocity) and was largely unshaded.

Plant survey

Plants were surveyed by snorkelling in a zigzag fashion going in an upstream direction for about 40 min (although no strict time limit was imposed) to achieve an exhaustive survey within each site. An index of cover-abundance (*sensu* Braun-Blanquet, 1932) was allocated for each taxon. Herbarium sheets of all taxa recorded for this study have been deposited in the University of Leicester Herbarium (LTR). Nomenclature follows Stace (1997). As non-flowering specimens of *Ranunculus trichophyllus* and *R. penicill-atus* ssp. *pseudofluitans* could not be separated, they were aggregated, along with the flowering specimens of the two species. An isozyme technique was developed to identify the non-flowering individuals of *Callitriche* species (Demars & Gornall, 2003). *Callitriche obtusangula* and *C. platycarpa* were found very frequently and were aggregated because it was not possible to give a separate cover-abundance score for each species where, as often observed, both grew at the same site. *Lemna minor* and *L. minuta* were pooled for the analysis as *L. minuta* was probably overlooked in the first summer.

The Braun-Blanquet index of cover-abundance was coded numerically from 1 to 6. Taxa recorded in less than three sites (rare and transient species) were eliminated as were all the strictly emergent riparian species (e.g. *Glyceria maxima*; *Phragmites australis*; *Typha latifolia*) to exclude statistical and sampling bias. Riparian emergent plants might reflect the environmental conditions of the bank more than the in-stream conditions. This resulted in the species table 'sites × species'. For the subset of sites sampled repeatedly in the Wensum and Wendling Beck, species were retained if they occurred at least twice (either across sites or years). This resulted in the species turnover table 'replicated sites × species'.

Habitat variables

Local environmental variables. The percentage cover of the substrate types (gravel, sand, silt, clay) and shade

intensity were assessed visually and coded as follows: 1, <20; 2, 20–40; 3, 40–60; 4, 60–80; 5, >80%. Mean width (0.8–20.9 m) and mean depth (0.1–2.0 m) were also included in the analyses as general descriptors.

Phosphate (11–3600 µg L^{-1} PO_4-P) and ammonium (16–434 µg L^{-1} NH_4-N) concentrations used in analyses were, for most sites, an average of values determined monthly by the Environment Agency during 1990–99. Two 'snapshot' water samples (winter and summer) were collected from sites not covered by Environment Agency monitoring.

The cover-abundance of filamentous green algae was recorded as a biological environmental variable, as they are known to compete with aquatic vascular plants. It was quantified using the Braun-Blanquet index and coded in the same way as for the vascular plants.

Spatial structure. Two alternative solutions were used to code the spatial structure of a river network for statistical analysis: a catchment approach and a spatial connectivity approach. For the catchment approach, catchments were binary coded as a dummy variable and the distance from river's source to site (hereafter referred to as 'distance') was measured from 1 : 50000 scale maps. For the spatial connectivity approach, the river network was binary-coded to represent the spatial connectivity of the sites (as suggested in Legendre & Legendre, 1998, p. 47 and 768). Fig. 2
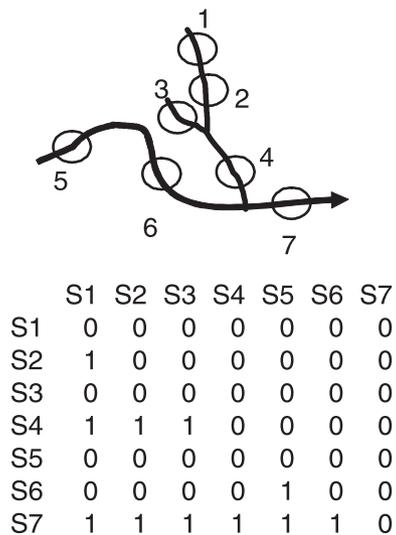
illustrates how the interconnection of the sites was coded. Both the local environmental variables and spatial structure were included in the habitat table 'sites × habitat characteristics' for subsequent analyses.

Species life-history characteristics

Species traits (coding life-history characteristics) were taken from the species attribute table of Willby *et al.* (2000). This table has been constructed to take into account the phenological plasticity of aquatic plant species. Traits are generally subdivided into a range of states (or modalities) that are referred to as attributes. For example, 'small <1 cm^2' is an attribute of the trait 'leaf area'.

Data analysis

Data exploration. All the data in the present study were centred and standardised. All ordinations were run on correlation matrices. A principal component analysis (PCA) was run using the habitat table to investigate the strength of the habitat variables and their inter-relationships. Linear Pearson's product-moment correlation coefficients were calculated between pairs of variables.

Another PCA was run using the species table to investigate how the species relate to each other. The appropriateness of the linear ordination method was confirmed by first running a correspondence analysis in which ordination axes length of <3 SD were obtained (Jongman, ter Braak & van Tongeren, 1995, p. 154; ter Braak & Šmilauer, 1998, p. 37).

Linking species with habitat variables. Redundancy analyses (RDAs), were performed (i) to investigate which variables from the habitat table would significantly explain the species composition (Monte Carlo permutation test); (ii) to include in the final RDA model a set of habitat variables that significantly reduced the total residual sum of squares (through a stepwise selection procedure); and (iii) to quantify the variance partitioning of the selected habitat variables by running a set of partial RDAs. A Bonferroni correction (see Cabin & Mitchell, 2000) was applied (Type I error, $\alpha \leq 5\%$ chosen in the present study) because of the high number of repeated tests (high probability of having some tests

	S1	S2	S3	S4	S5	S6	S7
S1	0	0	0	0	0	0	0
S2	1	0	0	0	0	0	0
S3	0	0	0	0	0	0	0
S4	1	1	1	0	0	0	0
S5	0	0	0	0	0	0	0
S6	0	0	0	0	1	0	0
S7	1	1	1	1	1	1	0

Fig. 2 Sites (S1-S7) interconnected by a river network can be binary-coded.

resulting as significant by chance alone). The significance of the axes of the final RDA model (named RDA1) was also tested with the Monte Carlo permutation test (1000 unrestricted random permutations).

Spatial connectivity approach. The spatial variables of the catchment approach had the advantage of allowing the separation of 'distance' (longitudinal connectance) from the five modalities of 'catchment' (basin isolation). An second approach was taken, of substituting the spatial variables of the habitat table (namely 'catchment', 'distance' and its collinear vector 'width') by the site scores from the principal PCA axes extracted from the spatial connectivity matrix. The advantage of this was to better quantify the overall effect of the spatial structure on species composition. This is because there was no longer a dummy variable (catchment) and all spatial variables (i.e. PCA axes) were linearly independent.

Linking attributes with habitat variables. The 'double CCA' approach (Lavorel *et al.*, 1998, 1999) was used to test whether attributes were related to habitat variables, although here a linear model was more appropriate, i.e. 'double RDA'. A habitat × species correlation table was produced by the RDA1 model (ter Braak & Šmilauer, 1998, p. 180), and then transposed into the species × habitat table. Species scores from the principal axes of a PCA of the attribute table were used to reduce the number of potential predictive variables (58), this choice being based on the pattern of decay in eigenvalues and the amount of variance explained by the combined axes. These axes could be seen as natural combination of attributes (NCAs; Lavorel *et al.*, 1999). This resulted in the NCA matrix 'species × NCAs'. NCAs allowed the investigation of the major trade-offs in aquatic plant attributes. Then RDAs were performed (i) to investigate which NCAs (all linearly independent from each other) would significantly explain the projection of habitat variables in the species space; (ii) to run the final RDA model (named RDA2); and (iii) to test the number of significant axes. In order to produce an easy-to-interpret triplot, the NCAs were then translated back to the original attributes themselves by taking the matrix product between the attribute scores of the attribute matrix PCA and the NCA scores of the RDA2 model (Lavorel *et al.*, 1999). The 'NCAs × habitat variables' correlation table produced by the RDA2 model was also translated back to an 'attributes × habitat variables' table using the same principle.

Species turnover. The potential effect of time alone on the species composition was also investigated with RDA. The Monte Carlo random permutation test was based on a random cyclic shift restricted to the replicated time series, which consisted of each resurveyed site. Partial RDAs enabled changes in composition between different combinations of years: 1999–2000, 2000–2001 and 1999–2001 to be examined. The analyses were also repeated on presence-absence species matrices. The potential effect of time alone on the NCAs was also investigated.

Further considerations. All the multivariate analyses were performed with CANOCO 4.0 (ter Braak & Šmilauer, 1998) and all the graphs were drawn in CANODRAW 3.0 (Šmilauer, 1992), except the triplot ordination diagram, which was partly drawn in EXCEL. Correlation tables produced by the RDAs were found to summarise better the information and were thought to be clearer than ordination diagrams with up to four significant axes. The percentage of explained variance for the different factors was expected to be relatively low overall, because of the inherent spatial autocorrelation in river networks, the subtlety of the gradients investigated, the relatively small number of species, the natural environmental stochasticity, and nonlinearity of biotic responses to environmental variation.

Results

Data exploration

Relative independence of the geomorphological, chemical and biotic gradients. The first three axes of the habitat PCA ordination diagram (Fig. 3) showed four relatively independent gradients: geomorphological (gravel, silt, depth), chemical (phosphate and ammonium), biotic (algae) and spatial ('distance', 'catchment'). Pairwise correlations confirmed that gravel was negatively related to silt ($r = -0.85$). River width was positively correlated with 'distance' ($r = 0.86$). Depth however was not strongly related to silt ($r = 0.52$) or 'distance' ($r = 0.54$). Phosphate and ammo-
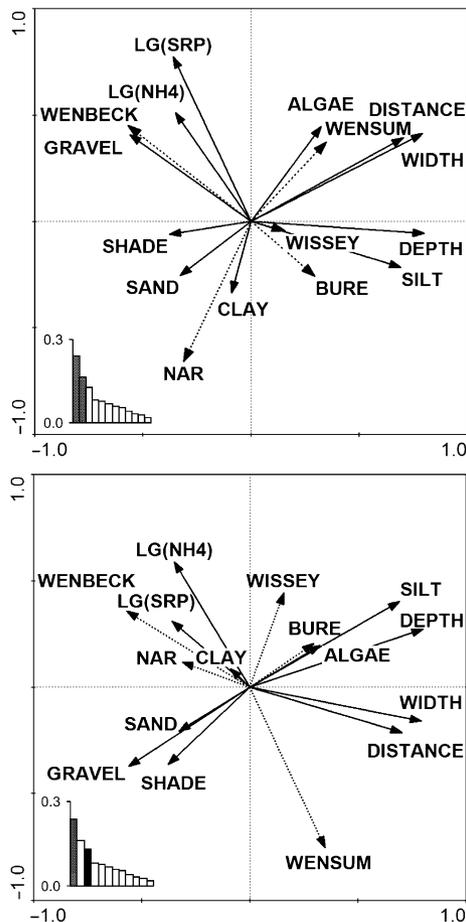
Fig. 3 Habitat variables projection into the PCA ordination diagram. Insets depict the pattern of change in eigenvalues and highlight which axes are displayed (shaded chart bar). Axis 1 is horizontal in both diagrams.

nium were not strongly related to each other ($r =$ 0.55).

Species interrelationships. The species PCA ordination diagrams (Fig. 4) showed how the species relate to each other along the first three axes. Along the first axis there is a clear shift from *Veronica*, *Callitriche platycarpa/obtusangula*, *Zannichellia* and *Ranunculus penicillatus/trichophyllus* to *Ranunculus circinatus*, *Nuphar*, *Elodea nuttallii* and *Sagittaria sagittifolia*. Along axis two, species shifted from *Myriophyllum spicatum*, *Schoenoplectus lacustris* through to e.g. *Glyceria fluitans/notata/declinata*, *Sparganium erectum*, *Lemna trisulca* and *Elodea canadensis*. A few species were strongly and positively associated with axis three such as *M. spicatum*, *Potamogeton crispus*, *Oenanthe fluviatilis*, *Zannichellia*, *Callitriche* and *Potamogeton pectinatus*.
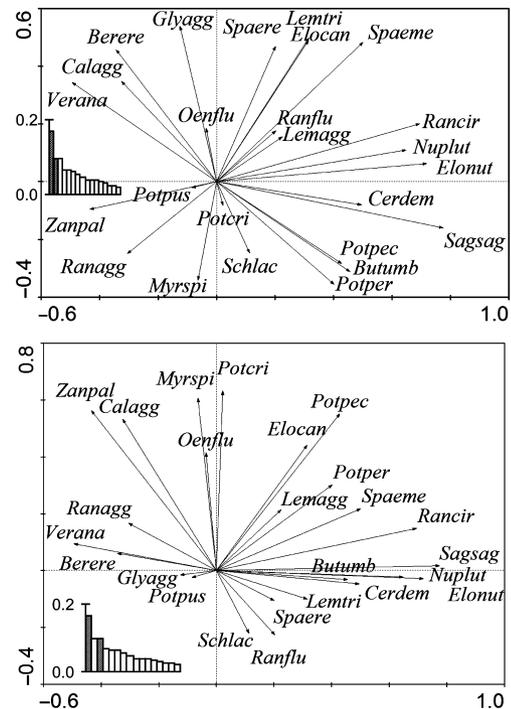
Fig. 4 Species projection into the PCA ordination diagram. Insets depict the pattern of change in eigenvalues and highlight which axes are displayed (shaded chart bar). Axis 1 is horizontal in both diagrams. See Table 2 for full names of taxa.

Species turnover

The RDA model based on the 'species turnover table' for 12 sites showed that a significant change of species composition had occurred from 1999 to 2001 ($P < 0.013$). However, this species turnover was slow because the changes in species composition between 1999 and 2000 ($P > 0.37$) and between 2000 and 2001 ($P > 0.28$) were not significant. The RDA model based on the 'species turnover table' with 16 sites explained 3% of the overall variance and confirmed the significant shift between year 1999 and 2001 ($P < 0.001$). Analyses ran on the presence–absence species matrices gave similar results. There was however no trend in attribute change over the years 1999–2000–2001 ($P > 0.58$).

Linking species with habitat variables

Catchment approach. After Bonferroni correction, species composition was significantly related to 'distance', width, depth, silt, gravel and catchment, but not to algae, nutrient levels, shade, sand and clay (Table 1).

Table 1 Percentage of variance explained by each habitat variable using redundancy analysis: singly and after stepwise selection. Significance tested by running 1000 unrestricted Monte Carlo random permutations.

	Singly	P	After stepwise selection	P	Selection order
			Percentage of explained variance		
Distance	11.1	<0.001	11.1	<0.001	1
Width	10.7	<0.001	1.2	0.522	8
Depth	9.3	<0.001	5.3	<0.001	2
Wensum	5.3	<0.001	4.0	<0.001	3
Silt	5.0	<0.001	2.8	0.002	6
Gravel	4.1	0.001	1.0	0.654	9
Nar	3.9	0.004			
Wissey	3.9	0.004	2.7	0.007	4
Wend. Beck	3.3	0.011	2.7	0.006	5
Bure	3.0	0.033	1.6	0.198	7
Algae	2.8	0.039			n.s.
Shade	2.8	0.045			n.s.
LG (NH$_4$-N)	2.4	0.112			n.s.
Sand	2.2	0.132			n.s.
LG (PO$_4$-P)	2.2	0.148			n.s.
Clay	0.7	0.944			n.s.

n.s., not significant

Width and gravel do not appear significant after forward selection ($P = 0.52$, $P = 0.65$, respectively). Four variables ('distance', 'catchment', depth and silt) explained 30.2% of the variance in species composition (Table 1, stepwise selection), which was equivalent to 75% of the variance explained by all supplied variables. The first four axes of the RDA were statistically significant (Fig. 5). Distance was positively related to the first axis but negatively related to the second axis. Silt and depth were positively related with the first and second axes. Silt was also positively related to the third axis. The dummy variable 'catchment' was mostly related to axes two and four. Gravel and width were also represented on the RDA diagram although they were not included in the final analysis (Fig. 5). Representation of the explanatory variables with arrows in the RDA diagram grossly simplifies the complex patterns from which they emerge. Contour lines illustrate this complexity and show that changes in distance, depth and silt were remarkably orthogonal (Fig. 6). The effect of 'catchment' was independent of the effect of the other variables ($P > 0.1$; Fig. 7), but the effect of depth and silt significantly overlapped with the effect of 'distance' ($P < 0.003$; Fig. 7). No significant additional effect ($P > 0.23$) of nutrients, algae and
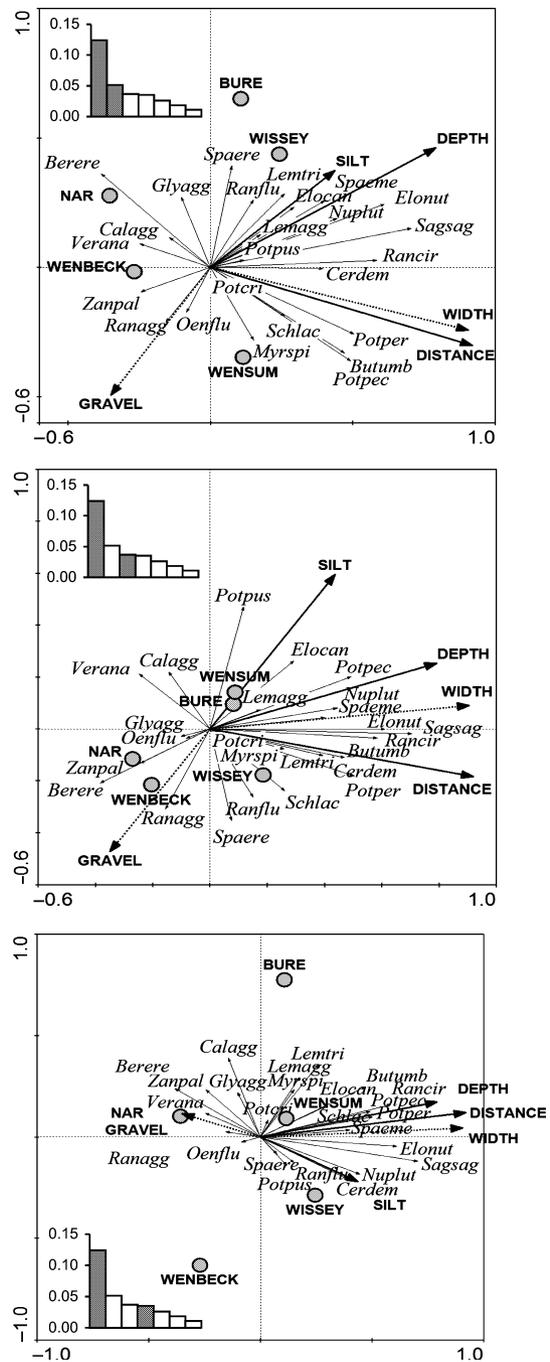

Fig. 5 Projections of species and habitat variables into the RDA ordination diagram. Insets depict the pattern of change in eigenvalues and highlight which axes are displayed (shaded chart bar). Axis 1 is horizontal in both diagrams. Note the similarity with Fig. 4. See Table 2 for full names of taxa.

shade taken together was detected. The species × habitat correlation table produced by the RDA1 model enables the relationship between each species and every habitat variable included in the final model to be
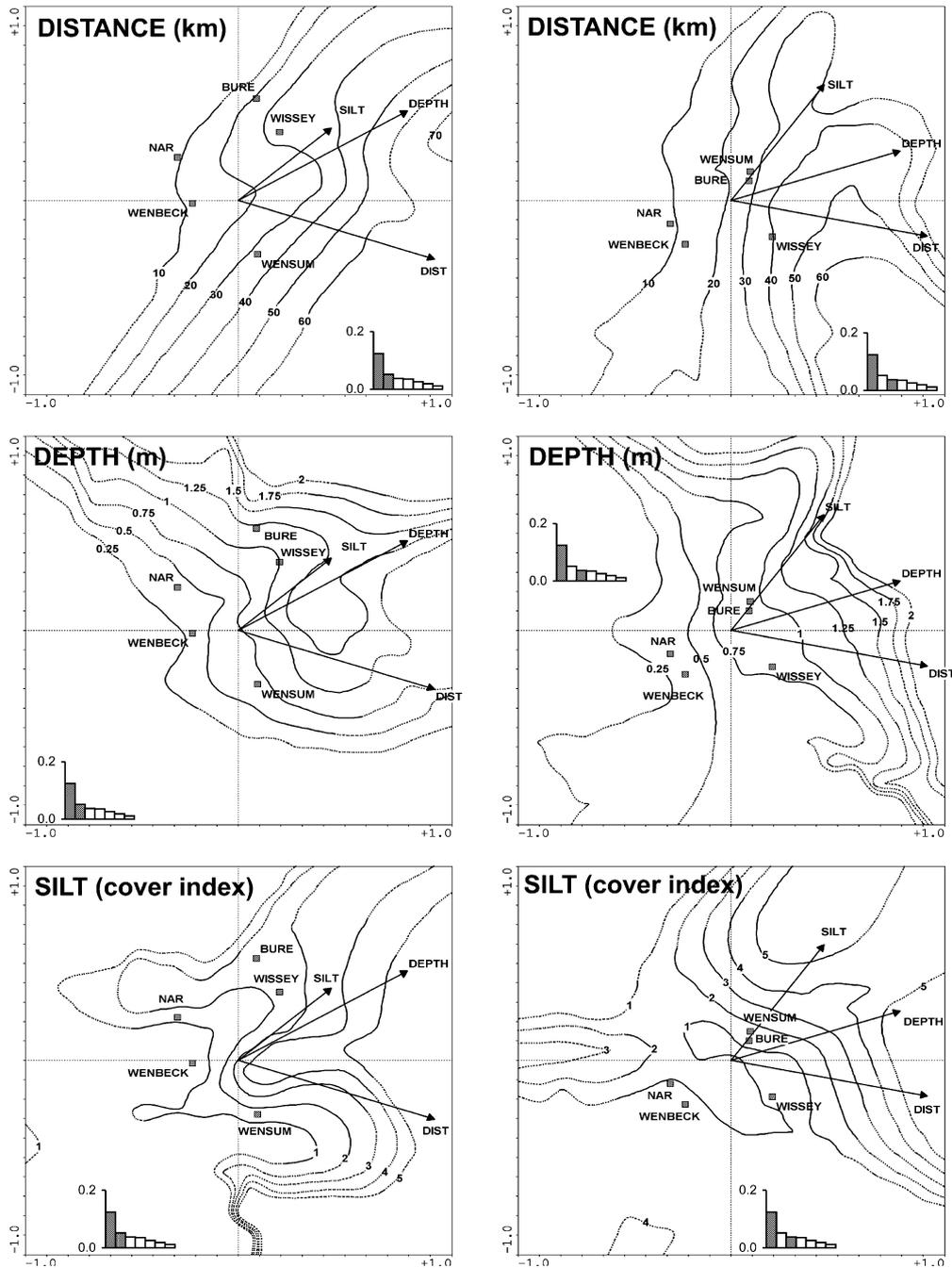
Fig. 6 Projection of the habitat variables along the three axes of the redundancy analysis using the catchment based approach. The smoothed contour lines (see Šmilauer, 1992, p. 37–43) illustrate the independence of the environmental gradient 'distance', depth and silt. Insets depict the pattern of change in eigenvalues and highlight which axes are displayed (shaded chart bar). Axis 1 is horizontal in all diagrams.

examined in detail (Table 2). The cut-off for displaying positive and negative associations was $r > \pm0.2$. These associations should therefore only be considered as weak trends, especially when the species percentage of

occurrence was low (see Table 2). It can be seen from Table 2 and Fig. 5 that some species were associated more with one catchment than another (e.g. *Ranunculus fluitans*, *S. erectum*) while others seemed to be
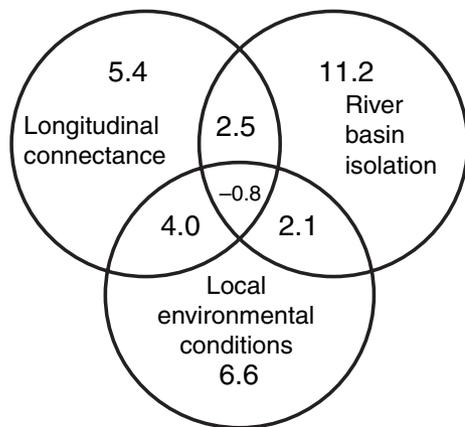
Fig. 7 Variance partitioning (expressed as percentage of variance explained) of the effects of local environmental conditions, longitudinal connectance and river basin isolation on aquatic plant distribution, using a catchment based approach.

one (or few) habitat variable(s), such as *S. lacustris* with 'distance', *R. fluitans* with catchment, and *O. fluviatilis* with gravel.

Spatial connectivity approach. The spatial connectivity matrix was reduced to the site scores of the initial six PCA axes explaining 77% of the variance (Fig. 8). The RDA1 model based on the spatial connectivity matrix gave very similar results compared with the catchment approach. The final model explained 30.1% of the variance (71% of the variance explained by all supplied variables). The spatial components (see method) and the environmental variables (depth, silt) explained 18.2 and 6.3% of the variance respectively, and a further 5.6% of the variance ($P < 0.001$) was explained by both (Fig. 9).

Selection of variables. The constrained ordination diagrams of the species (RDA1 models) were similar to the unconstrained species PCA ordination diagram (see Figs 4 & 5). As the axes were strongly constrained by a limited set of exploratory variables, it meant that the selection of exploratory variables was good.

indifferent (e.g. *S. lacustris, Zanichellia palustris, Nuphar lutea*). Some species were associated with deep/silty sites (*E. canadensis, R. circinatus*) while others were not (e.g. *R. penicillatus/trichophyllus, Zannichellia palustris*). Of particular interest were the species associated with

Table 2 Correlation table showing positive and negative trends ($r > 0.2$) between species and habitat variables, derived from redundancy analysis

| Species	Depth	Silt cover	Distance	Bure	Nar	Wensum	Wissey	Wendling Beck	% Occurrence
Berula erecta	o	o	−	+	+	−	o	o	32
Butomus umbellatus	+	o	+	o	−	+	o	o	19
Callitriche platycarpa/obtusangula	o	o	o	+	o	o	o	−	84
Ceratophyllum demersum	o	+	+	o	o	o	+	o	5
Elodea canadensis	+	+	o	+	o	o	o	o	42
Elodea nuttallii	+	+	+	o	−	o	+	o	21
Glyceria fluitans/notata/declinata	o	o	o	+	+	−	o	o	6
Lemna minuta/minor	+	o	o	o	o	o	o	−	66
Lemna trisulca	+	o	o	+	o	o	o	o	11
Myriophyllum spicatum	o	o	o	o	−	+	o	o	37
Nuphar lutea	+	+	+	o	o	o	o	o	23
Oenanthe fluviatilis	o	−	o	o	o	o	o	o	11
Potamogeton crispus	o	o	o	o	o	o	o	o	26
Potamogeton pectinatus	+	o	+	o	−	+	o	−	48
Potamogeton perfoliatus	+	o	+	o	o	+	o	−	34
Potamogeton pusillus	+	+	o	o	o	o	o	o	3
Ranunculus penicillatus/trichophyllus	−	−	o	o	o	o	−	+	40
Ranunculus circinatus	+	+	+	+	−	o	o	o	19
Ranunculus fluitans	o	o	o	o	o	−	+	o	10
Sagittaria sagittifolia	+	+	+	o	−	o	+	o	23
Schoenoplectus lacustris	o	o	+	o	o	o	o	o	6
Sparganium emersum	+	+	+	+	o	o	o	o	55
Sparganium erectum	o	o	o	o	+	−	+	o	69
Veronica anagallis-aquatica	o	o	−	o	o	o	o	o	52
Zannichellia palustris	−	−	o	o	o	o	o	o	32

o, no trend.
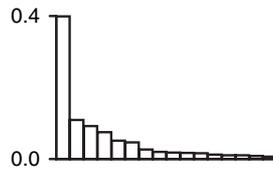
Fig. 8 Pattern of change in eigenvalues of the first 16 PCA axes extracted from the spatial connectivity matrix.
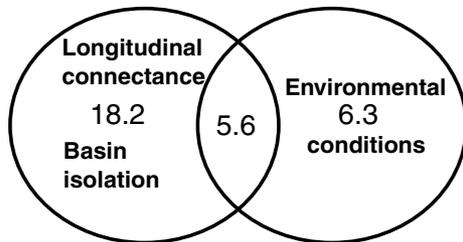

Fig. 9 Variance partitioning (expressed as percentage of variance explained) of the effects of local environmental conditions and spatial isolation on aquatic plant distribution, using a spatial connectivity matrix approach.

Trade-offs between aquatic plant life-history characteristics

The main patterns of variation along the NCAs were summarised by six NCAs explaining 66% of the attribute variance of the 25 species present (see
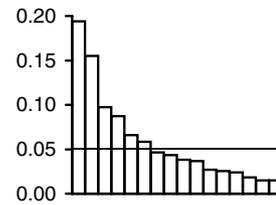

Fig. 10 Pattern of change in eigenvalues of the first 16 PCA axes extracted from the species attribute table.

Fig. 10; Table 3). The main pattern of variation along NCA1 was from multiple apical growth point, small leaves, reproduction by fragmentation, water pollinated, medium body size to attributes including high root : shoot biomass, plastic growth form, waxy leaf texture, single basal growth point, vegetative reproduction by stolons. On NCA2, the trend was from anchored, submerged soft non-waxy leaves growth form, large body size, and high number of reproductive organs, through to free floating surface growth form, small size, very high number of reproductive organs, vegetative reproduction by budding, single basal growth point, low flexibility and small fruit size. These trends reflected the strongest gradient in the variability of species' attributes investigated here.

Table 3 Natural combination of attributes (NCAs) describing major trade-offs in the life-history characteristics of 25 aquatic vascular plant species growing in lowland calcareous rivers. Trade-offs are interpreted as positive or negative loadings on the first six axes of a principal component analysis of the species × attribute table. Note that the species given as examples do not necessarily possess all the listed attributes.

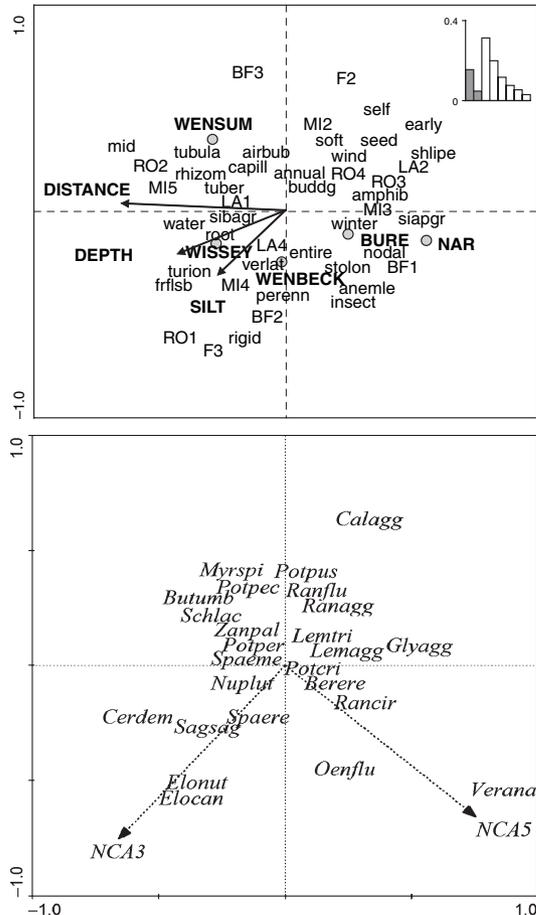| NCAs	Negatively related attributes	Positively related attributes
NCA1	Multiple apical growth point, small leaves, reproduction by fragmentation, water pollinated, medium body size (e.g. *Ranunculus circinatus*, *Elodea canadensis*, *Ceratophyllum demersum*)	High root : shoot biomass, plastic growth form (anchored, with floating, emergent or heterophyllous large leaves), waxy leaf texture, single basal growth point, vegetative reproduction by stolons (e.g. *Schoenoplectus*, *Sagittaria*, *Potamogeton pectinatus*)
NCA2	Anchored, submerged growth form with soft non-waxy leaves, large body size, high number of reproductive organs (e.g. *Ranunculus fluitans*, *Potamogeton perfoliatus*, *Myriophyllum spicatum*)	Free floating surface growth form, small size, very high number of reproductive organs, vegetative reproduction by budding, single basal growth (e.g. *Lemna minor/minuta*)
NCA3	Medium size fruit, self pollination, early production of reproductive organs	Low number of reproductive organ, large fruit size, rigid leaves
NCA4	Capillary leaves, perennial	Entire leaf, annual, pollination by air bubbles,
NCA5	Mid period of production of reproductive organs, medium number of reproductive organs, high body flexibility	None
NCA6	None	Medium body size, biennial/short lived perennial, and anchored heterophylly growth form

Fig. 11 Triplot ordination diagram based on a 'double redundancy analysis' showing the relationship between species, attribute and habitat variables. Inset depicts the pattern of change in eigenvalues and highlight which axes are displayed (shaded chart bar). See Table 2 for full names of taxa and Table 4 for attribute codes.

Linking aquatic plant attributes with habitat variables

The first two canonical axes of the final RDA2 model (Fig. 11) were constrained using NCA3 and NCA5 as explanatory variables, which explained 20.2% of the variance of the habitat variables ($P = 0.007$). The other four NCAs were not significant ($P > 0.3$). The remaining axes were unconstrained and explained a large part of the remaining variance (Fig. 11). The relationships between the attributes, species and habitat variables can be illustrated with a triplot ordination diagram (Fig. 11) and the correlation table 'attribute × habitat variables' (Table 4). Table 4 can be read in the same way as Table 2, with the same cut-off level for association ($r > \pm 0.2$). For example,

within the trait 'growth form', the attribute 'free floating submerged' was positively associated ($r > 0.2$) with the habitat characteristics 'distance' and 'depth'. This was because of species such as *Ceratophyllum demersum*, *E. canadensis* and *E. nuttallii* being associated with 'distance' and/or depth (see Table 2; Fig. 11). The strength of the associations between attributes and environmental and spatial variables can be further examined on the triplot (Fig. 11). 'Single apical growth point', 'medium leaf area', 'biennal/short lived perennial' were all negatively related to depth and distance, and positively related to the catchment of the River Nar (Table 4; Fig. 11). 'Self pollination' and 'early period of production of reproductive organ' were negatively related to distance, depth and silt. Other attributes were positively associated with the latter, such as 'fruit size >3 mm' and 'low number of reproductive organs year^{-1} individual^{-1}'. There seemed to be some trade-off within traits (e.g. turion versus seed) and between traits (e.g. 'fruit size' versus 'number of reproductive organs year^{-1} individuals^{-1}'; 'amphibious' versus 'water as gamete vector') (see Table 2; Fig. 11).

Discussion

This inductive approach enables the definition of the realised niche (*sensu* Hutchinson, 1970) of species, characterised by their response along three gradients: biotic (e.g. competitors, grazers), chemical (e.g. nutrient availability) and physical (e.g. depth, frequency of peak flows). One basic assumption of such a study is that population dynamics are governed mainly by autecological processes, i.e. the intrinsic ecology of each species rather than by biotic interactions (Townsend & Hildrew, 1994). The major strength of this study was that the survey design allowed the analysis of the effect of one type of environmental factor independently of the others. This contrasts with previous investigations in which the environmental gradients were inseparable (e.g. Wiegleb, 1984).

Species turnover

Analyses carried out on presence–absence data confirmed that the change in species composition was genuine species turnover, not merely a change in

Table 4 Correlation table showing positive and negative trends ($r > 0.2$) between attributes and habitat variables. Morphology index is calculated as the sum of scores for potential height + potential lateral extension where each component is allocated scores of 1, <1 cm; 2, 1–10 cm; 3, 10–40 cm; 4, 40–100 cm; 5, >100 cm.

Traits	Attributes	Codes	Distance	Depth	Silt	Bure	Nar	Wensum	Wissey	Wendling Beck
Growth form	Free floating surface	frflsr	o	o	o	o	o	o	o	o
	Free floating submerged	frflsb	+	+	o	o	o	o	o	o
	Anchored, floating leaves	anflle	o	o	o	o	o	o	o	o
	Anchored, submerged leaves	ansule	o	o	o	o	o	o	o	o
	Anchored, emergent leaves	anemle	o	o	o	o	o	o	o	o
	Anchored, heterophylly	anhete	o	o	o	o	o	o	o	o
Vertical shoot architecture	Single apical growth point	siapgr	–	–	o	o	+	o	o	o
	Single basal growth point	sibagr	o	o	o	o	o	o	o	o
	Multiple apical growth point	muapgr	o	o	o	o	o	o	o	o
Leaf type	Tubular	tubula	+	o	o	o	–	o	o	o
	Capillary	capill	o	o	o	o	o	o	o	o
	Entire	entire	o	o	o	o	o	o	o	o
Leaf area	Small (<1 cm^2)	LA 1	o	o	o	o	o	o	o	o
	Medium (1–20 cm^2)	LA 2	–	–	o	o	+	o	o	o
	Large (20–100 cm^2)	LA 3	o	o	o	o	o	o	o	o
	Extra large (>100 cm^2)	LA 4	o	o	o	o	o	o	o	o
Morphology index (score)	2	MI 1	o	o	o	o	o	o	o	o
	3–5	MI 2	o	o	o	o	o	o	o	o
	6–7	MI 3	–	o	o	o	+	o	o	o
	8–9	MI 4	o	o	o	o	o	+	o	o
	10	MI 5	+	o	o	o	–	+	o	o
Rooting at nodes		nodal	o	o	o	o	o	o	o	o
High below-above-ground biomass		root	o	o	o	o	+	o	o	o
Mode of reproduction	Rhizome	rhizom	+	o	o	o	–	o	o	o
	Fragmentation	fragmn	o	o	o	o	o	o	o	o
	Budding	buddg	o	o	o	o	o	o	o	o
	Turions	turion	+	+	o	o	o	o	o	o
	Stolons	stolon	o	o	o	o	o	o	o	o
	Tubers	tuber	o	o	o	o	o	o	o	o
	Seeds	seed	–	–	o	o	–	o	o	o
Number of reproductive organs/year/individual	Low (<10)	RO 1	+	+	+	o	o	o	+	o
	Medium (10–100)	RO 2	+	o	o	o	–	+	o	o
	High (100–1000)	RO 3	–	–	o	o	o	+	o	o
	Very high (>1000)	RO 4	o	o	o	o	o	o	o	o
Perennation	Annual	annual	o	o	o	o	o	o	o	o
	Biennial/short lived perennial	shlipe	–	–	o	o	+	o	o	o
	Perennial	perenn	o	o	o	o	o	o	o	o
Evergreen leaf		winter	o	o	o	o	o	o	o	o
Amphibious		amphib	–	o	o	o	o	o	o	o
Gamete vector	Wind	wind	o	o	o	o	o	o	o	o
	Water	water	+	o	o	o	o	o	o	o

Table 4 (*Continued*)

Traits	Attributes	Codes	Distance	Depth	Silt	Bure	Nar	Wensum	Wissey	Wendling Beck
	Air bubble	airbub	o	o	o	o	o	o	o	o
	Insect	insect	o	o	o	o	−	o	o	o
	Self	self	−	−	−	o	o	o	o	o
Body flexibility	Low (<45°)	BF 1	−	o	o	o	+	−	o	o
	Intermediate (>45–300°)	BF 2	o	o	o	o	o	o	o	o
	High (>300°)	BF 3	o	o	o	o	o	+	o	o
Leaf texture	Soft	soft	o	+	+	o	o	o	o	o
	Rigid	rigid	o	o	o	o	o	o	o	o
	Waxy	waxy	o	o	o	o	o	o	o	o
	Non-waxy	nowaxy	o	o	o	o	o	o	o	o
Period of production of reproductive organ	Early (March–May)	early	−	−	−	o	+	o	o	o
	Mid (June–July)	mid	+	+	o	o	−	+	o	o
	Late (August–September)	late	o	o	o	o	−	o	o	o
	Very late (post September)	verlat	+	o	o	o	o	o	o	o
Fruit size	<1 mm	F 1	o	o	o	o	o	o	o	o
	1–3 mm	F 2	o	−	−	o	o	o	o	o
	>3 mm	F 3	+	+	+	o	o	o	o	o

o, no trend.

species cover-abundance. The temporal variance of species composition however was small and it is therefore unlikely that it has confounded the results of the spatial survey spread over 2 years.

Phosphorus stripping at two sewage treatment works (76% reduction at East Dereham, Wendling Beck and 64% reduction Fakenham, River Wensum) started in autumn-winter 1999. However, it had, only a limited effect on the in-stream phosphorus loads because of the contribution of diffuse sources (Demars, 2002). The observed significant change in species composition is more likely to have been because of stochastic population extinction and colonisation (such as described by Butcher, 1933; Wiegleb, Herr & Todeskino, 1989), a conclusion that is reinforced by the fact that no shifts in species life-history characteristics were detected.

Inference 1. The annual turnover of aquatic plants is slow and reflects stochastic processes. This suggests that spatial surveys conducted over 3 years should not be undermined by temporal changes.

Linking species with habitat variables

Chemical variables. An important factor to consider is carbon availability (Gessner, 1959, p. 196; Entz, 1961; Sculthorpe, 1967, p. 115; Wright & Mills, 1967; Smith, 1993). The excess partial pressure of carbon dioxide [$EpCO_2$, expressed here as x times atmospheric pressure (atm)] typically decreases with distance downstream in spring fed chalk streams (e.g. Entz, 1961; Massabuau & Fritz, 1984). In this study, however, $EpCO_2$ was constant (at about 5–7 atm) over 93% of the stream distance studied and therefore did not appear to be related to 'distance', although in the remaining 7% represented by the headwaters $EpCO_2$ was slightly higher (about 15 atm; B.O.L. Demars, unpubl. data). Aquatic plants have alternative strategies to uptake inorganic carbon. Two are particularly relevant here: first, half of the species found during the survey can produce floating and/or emergent leaves during the growing season, gaining access to atmospheric CO_2; second, some species can uptake bicarbonate (Steemann Nielsen, 1946; Sand-Jensen, 1983; Kohler & Schoen, 1984; Maberly & Madsen, 2002). As alkalinity was similar across sites (4.5–5.4 meq L^{-1}) and uncorrelated to $EpCO_2$ (B.O.L. Demars, unpubl. data), bicarbonate content of the water did not differ

significantly between sites. Therefore, dissolved inorganic carbon was extremely unlikely to impact the plant composition across the studied area. Nitrate concentrations were high at all sites and year-round (annual average 6.4–12.9 mg L^{-1} NO_3-N) and would therefore not be related to the plant distribution, as found in previous studies (e.g. Kohler, Vollrath & Beisl, 1971; Kohler, Wonneberger & Zeltner, 1973; Melzer, 1980). Dissolved phosphate and ammonium in the water did not exert a significant effect on species composition (Table 1). Clarke (2000) and Clarke & Wharton (2001) also failed to link species to sediment nutrient (N, P) characteristics in British lowland rivers. These studies along with the results presented here seem to contrast with other recently published works, which suggest that aquatic plant assemblages do respond to phosphate/ammonium enrichment in lotic ecosystems (e.g. Carbiener *et al.*, 1990; Robach *et al.*, 1996; Dawson & Szoszkiewicz, 1999; Thiébaut & Muller, 1999). These studies, however, failed to separate the effect of one factor such as changes in carbon availability (cf. Massabuau & Fritz, 1984; Carbiener *et al.*, 1990), conductivity (Dawson & Szoszkiewicz, 1999), or pH (Thiébaut & Muller, 1999) from phosphate/ammonium enrichment. Others have been more cautious about the potential causality of the impact of phosphate/ammonium enrichment on plants but have suggested that the plants are bioindicators (e.g. Amoros, Bornette & Henry, 2000; Kohler & Schneider, 2003). Ecological stoichiometry has revealed that neither nitrogen nor phosphorus limit the primary productivity of aquatic plants in calcareous lowland areas (see review by Haslam, 1987, p. 168–169; Demars & Harper, 2002a, p. 85–86). This is in accordance with the older view that flowing water systems are 'eutrophic' because of the continuous renewal of the medium (Ruttner, 1926; Odum & Hoskin, 1957). Moreover, ammonium toxicity has not been experimentally verified even in cases where excessively high concentrations were used (see original findings of Glänzer, 1974). Given that correlation does not mean that there is a causal relationship, a reassessment of the literature is desirable. Indeed this issue is currently of practical importance as conservation decisions could be based on incorrect interpretation of ecological data (e.g. Mainstone, Parr & Day, 2000). Moreover, the production of seemingly solid indices may mislead the policy-makers and conservation agencies who do not have the expertise to interpret the simplified indices in a way which reflects the complexity of nature.

Biotic variables. Only limited growth of filamentous algae occurred during the three, drought-free, years of this study. Their growth can, however, be substantial under low flow conditions (Demars & Harper, 1998), meaning that long-term annual surveys at selected sites would be helpful to understand the dynamics between vascular plants and filamentous green algal growth (Wade *et al.*, 2002). Although there are many other possible biotic interactions such as interspecific competition (e.g. Gopal & Goel, 1993) and those involving epiphyton and grazers (e.g. Lamberti *et al.*, 1995; Jones *et al.*, 2000; Sand-Jensen & Jacobsen, 2002; Elger & Willby, 2003), these were thought to play a more limited role than filamentous algae in the present study.

Physical variables. No difference in water temperature (mean and amplitude) was detected between sites during the 1990s (Environment Agency, Norwich, U.K., unpubl. data). Substrate types, depth and velocity gradients are all related in rivers of this kind (Kemp, Harper & Crosa, 1999, 2002). Therefore it was only necessary to record two of them to characterise the physical habitat. The substrate types recorded were known to reflect the physico-chemical properties of the sediment (Wiegleb, 1984; Demars & Harper, 2002b). Physical environment factors had a marked effect on the species composition (see Tables 1 and 2) and essentially confirmed earlier findings (e.g. Butcher, 1933; Haslam, 1978; RSPB, NRA & RSCN, 1994).

Inference 2. River bed characteristics influenced plant distribution, not the water chemistry or biotic competition from filamentous algae.

Spatial structure

The effect of the spatial structure of the river network on aquatic plant species composition was detected independently of the impacts of environmental factors (Figs 7 & 9). It was checked that mean current velocity was not related to 'distance' (B.O.L. Demars, unpubl. data). The two approaches (based either on 'catchment' or spatial connectivity matrix) confirmed that the effect of the spatial structure on plant distribution was overwhelming. The implications of these findings are that the plant composition at a site may be due less

to external environmental factors and more to intrinsic properties of species such as their colonisation abilities (see below).

The detection of a significant overlap in explained variance of the effects of 'distance' and the physical habitat (depth and silt) showed that the effects of these variables could only be partially separated. Therefore, despite all the efforts made in the survey design, there was still some spatially-structured environmental variation (Figs 7 & 9; Legendre, 1993). However, this was small and thus did not compromise the central aim of the study, namely to separate the effects of local environmental conditions, longitudinal connectivity and river basin isolation.

Where it was possible to identify *R. trichophyllus* and *R. penicillatus* ssp. *pseudofluitans* to species level, the two species were never recorded within the same stream. It is therefore likely that their aggregation for the analysis has resulted in statistical bias. This was also observed to a lesser extent with the aggregated species of *Lemna* and *Callitriche* in the upper catchments. Further efforts should focus on the identification of non-flowering or non-fruiting populations (e.g. Demars & Gornall, 2003).

Inference 3. Spatial structure is the single most important factor explaining plant distribution in lowland rivers.

Trade-offs between aquatic plant life-history characteristics

The first two NCAs (Table 3) that resulted from the principal components analysis of the attribute table were not related to habitat characteristics (NCA1, NCA2; $P > 0.3$) and probably reflected environmental conditions of a wider region (regional filter, Willby *et al.*, 2000). Indeed, both NCA1 and NCA2 possessed an element of competitive ability (e.g. 'large body size', 'free floating surface growth form'), shared with resistance (e.g. 'high root : shoot biomass', 'anchored') or colonisation abilities (e.g. 'vegetative reproduction by stolon or budding', 'very high number of reproductive organs') (Table 3). These combinations of attributes may be the fingerprints of adaptations to conditions encountered in lowland calcareous rivers. A geographically larger plant dataset (e.g. Holmes, Boon & Rowell, 1998) spanning several regions is needed to test this idea (see Willby, 2002). As these

NCA1 and NCA2 were explaining a large part of the attribute variability (see eigenvalue of axis one and two in Fig. 10), it is not surprising that the unconstrained axes of the RDA2 (Fig. 11) explained a large part of the remaining variance.

Inference 4. The most common combinations of life-history characteristics reflect larger scale natural selection processes.

Linking attributes with habitat variables

Life-history characteristics. Based on previous studies, attributes were expected to respond to environmental variables (e.g. substrata, depth; see Bornette *et al.*, 1994), longitudinal connectance ('distance'; see Boedeltje *et al.*, 2003) but not to the different biogeographical areas, represented here by the individual catchments (Wiegleb, 1980, 1984). Overall, very few attributes were linked to any particular catchment (Table 4) except, perhaps, for the River Nar. This is likely to be an artefact relating to site choice: the sites of the River Nar were the least physically altered and the lowest part of the river (which has been straightened and deepened) was not surveyed.

The strongest signals were not limited to particular individual attributes but to internal trends or alternative states within traits (see Table 4, e.g. morphology index, number of reproductive organs year^{-1} individual^{-1}, period of production of reproductive organs were associated with 'distance'; fruit size was related to depth and silt). Amphibious plants were more likely to be found in the upper part of the catchment; free floating submerged species were associated with 'distance' possibly because they are continuously washed down by the water current but also because dispersal from standing waterbodies is more likely in the wider floodplain of the lower part of the rivers. Surveys that include all types of aquatic habitats within a catchment, supported by gene flow studies, are needed to elucidate these effects. Interestingly (see Table 4; Fig. 11), along the 'distance' gradient, the morphology index and mode of reproduction suggest a possible trade-off between intensification of competition and regeneration capacity, the latter also tending to occur during the most favourable period of the year for plant growth in the lower part of the rivers (longest daily insolation in June to July). This possible intensification of competition with

'distance' was also found through analysis of the community structure (Demars, 2002). The change in attribute patterns along the depth and silt gradient were somewhat similar to those seen for 'distance', with a few specific exceptions: rigid leaves were more likely to be present, possibly because of the slow/sluggish flows associated with these habitats; larger fruit size, which may reflect greater allocation of energy to the seedlings (Table 4; Fig. 11).

From patterns to processes. Several biological processes may underpin the observed patterns. The results indicate that the dispersal and regeneration abilities of the plants are likely to be of prime importance in determining the distribution of plants across the landscape as might have been expected (Sculthorpe, 1967; Barrat-Segretain, 1996; Johansson, Nilsson & Nilsson, 1996). Direct evidence for this has also been found in small streams (Butcher, 1933; Boedeltje *et al.*, 2003), across the Rhône floodplain (Bornette *et al.*, 1994; Barrat-Segretain & Amoros, 1996; Cellot, Mouillot & Henry, 1998; Combroux *et al.*, 2001), and also for riparian plants (Nilsson, Gardfjell & Grelsson, 1991; Andersson & Nilsson, 2002; Nilsson *et al.*, 2002). Investigations on nymphaeids have also provided some evidence of the role of environmental factors and colonisation processes at larger scale (Smits, Ruremonde & van der Velde, 1989; Smits, Schmitz & Van der Velde, 1992). Gornall, Hollingsworth & Preston (1998) found indirect evidence of population structure and gene flow in ditches.

Ecophysiology. The problem of the lack of ecophysiological traits in the attribute table of Willby *et al.* (2000) was greatly lessened in this study by the lack of relationship between species distribution and ammonium/phosphate (Table 1) as well as the homogeneity of the area studied in terms of nitrate, carbon availability and minerality (cf. Melzer, 1980; Kohler & Schoen, 1984; Maberly & Madsen, 2002).

Inference 5. Dispersal and regeneration abilities explain the impact of river spatial structure on plant distribution.

Conclusions

Although every aspect of this study has been the subject of individual investigations in the past, this is the first time, for aquatic plants, that the importance of local environmental conditions versus the spatial structure of the river network has been quantified. It suggests that species distribution in lowland rivers is controlled more by species colonisation processes than local environmental conditions. Efforts should be made to enlarge the study to wider geographical areas and a broader range of aquatic habitats to evaluate the generality of this finding.

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