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Algal epiphytes of *Zostera marina*: Variation in assemblage structure from individual leaves to regional scale

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Abstract

Data from a hierarchical study of four *Zostera marina* beds in Wales were used to identify the spatial scales of variation in epiphyte assemblages. There were significant within and among bed differences in assemblage structure. The differences in assemblage structure with spatial scale generally persisted when species identifications were aggregated into functional groups. There was also significant within and among bed variability in *Zostera* density and average length. Local variations in *Zostera* canopy variables at the quadrat scale (total leaf length, average leaf length and leaf density per quadrat) were not related to epiphyte species richness nor to the structure of the assemblage. In contrast, individual leaf length was significantly related to species richness in two of the beds and the structure of epiphyte assemblages was always related to individual leaf lengths. The absence of links between quadrat scale measurements of canopy variables and assemblage structure may reflect the high turnover of individual *Zostera* leaves. Experimental work is required to discriminate further between the potential causes of epiphyte assemblage variation within and between beds. No bed represented a refuge where a rare species was abundant. If a species was uncommon at the bed scale, it was also uncommon in beds where it occurred. The heterogeneous assemblages found in this study suggest that a precautionary approach to conservation is advisable.

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As much of a bed as possible should be retained, both to protect the integrity of local assemblages and to retain rare species at regional scales.

Keywords: Algal; Biodiversity; Heterogeneity; Rarity; Functional group

1. Introduction

Epiphytic algae on submerged macrophytes such as *Zostera marina* L. (eelgrass) have functional significance and they can act as indicators of biodiversity or ecological status. Although estimates of epiphyte productivity are variable, primary production by attached algae has been shown to represent between 22 and 61% of productivity in *Zostera* beds (Hemminga and Duarte, 2000). Epiphytes are potentially important food sources for herbivores associated with seagrass beds (Fry, 1984). Overgrowth by epiphytes can reduce *Zostera* productivity (Sand-Jensen, 1977), potentially leading to degradation and loss of beds. In some cases, the process of overgrowth has been accelerated by eutrophication (Borum, 1985; Hauxwell et al., 2003). Epiphyte communities may therefore act as indicators of anthropogenic environmental impacts (Coleman and Burkholder, 1995).

The ecological and conservation value of *Zostera* beds is widely recognized (Hemminga and Duarte, 2000; Jackson et al., 2001). If beds are lost, the shoreline extent and profile may be altered (Christiansen et al., 1981). Although there is substantial overlap between assemblages associated with seagrass beds and adjacent coastal habitats (Hemminga and Duarte, 2000), some species are restricted to seagrass habitats. Indeed, the first generally recognized extinction of a marine species in recent times was the eelgrass limpet, *Lottia alveus* (Carlton et al., 1991). Examples of *Zostera*-restricted epiphytic algae include *Rhodophysema georgei* and *Leblondiella densa*.

While the conservation value of *Zostera* beds is reflected in the European Union Habitats Directive (Davison and Hughes, 1998), this has created a requirement for scientifically rigorous monitoring and survey programmes. Such approaches rely on detailed information on existing patterns of species diversity. For epiphytes, there have been relatively few studies made of diversity across a range of scales (Jernakoff et al., 1996; Vanderklift and Lavery, 2000; Saunders et al., 2003). More information is available at the scale of individual leaves (e.g., Jacobs et al., 1983; Cebrián et al., 1999). Above the scale of individual leaves, there are contrasting views on the extent of variability between epiphyte assemblages separated by distances of a few metres. Vanderklift and Lavery (2000) found patchiness at metre scales in algal epiphytes of *Posidonia coriacea* to be of the same order as the patchiness over hundreds of metres. Variation in shoot density may have created metre scale environmental variability, leading to the structure in epiphyte assemblages (Vanderklift and Lavery, 2000). In contrast, Saunders et al. (2003) found little variation in epiphyte composition within *Zostera* beds and no relationship between epiphytes and shoot density or total leaf area. The *Zostera* study by Saunders et al. (2003) identified epiphytes at the functional group rather than species level and the authors caution that their results may lack statistical power due to insufficient replication. Nevertheless, observations of comparatively weak within bed structure of epiphyte assemblages may reflect the

demography of *Zostera* leaves. Lavery and Vanderklift (2002) found weaker within bed structure of epiphytes on *P. coriacea* when compared to the patterns on *Amphibolis griffithii*. As turnover of leaves in *Posidonia* is higher than the turnover of leaves and shoots in *Amphibolis*, Lavery and Vanderklift (2002) proposed the hypothesis that there is more scope for distinct small-scale epiphyte assemblages to establish in *Amphibolis* beds. In contrast, high leaf turnover in *Posidonia* will reduce the probability of reproductive epiphytes recruiting to adjacent leaves; weakening small-scale assemblage structure. Individual *Zostera* leaves have a half life of approximately 50 days (Olesen and Sand-Jensen, 1994; Jernakoff et al., 1996). As *Zostera* leaf turnover rates are comparable to *Posidonia* leaf turnover, Lavery and Vanderklift's (2002) hypothesis would predict weak within bed differences in epiphyte assemblages compared to the differences among *Zostera* beds.

We used data from a survey of *Zostera* beds in Wales to characterise spatial variation in algal epiphyte assemblages. The study focused on multicellular epiphytes as a basis for potential monitoring programmes. Epibiotic microorganisms are also found on seagrasses (Sterrenburg et al., 1995) and are less well known than multicellular species (e.g., Aladro-Lubel and Martinez-Murillo, 1999). As new microepiphyte taxa are still being described (De Stefano and Marino, 2001), multicellular species currently seem a more promising candidate for comparative biodiversity studies. Multivariate statistics were used to test the hypothesis that within bed variation in epiphyte assemblages was negligible. The reduction of information when aggregating species into functional groups may mask within bed variability in species assemblages, so we compared species and functional group level analyses. Structure in epiphyte assemblages may be linked to the physical structure of the *Zostera* bed (Cebrián et al., 1999; Vanderklift and Lavery, 2000). We tested for relationships between epiphyte assemblage structure and *Zostera* at the scale of individual leaves (with leaf length as the predictor variable). Variations in epiphyte structure at the quadrat scale were examined with respect to local variations in sample depth and in the *Zostera* canopy (assessed in terms of leaf density, total leaf length and average leaf length per quadrat).

2. Material and methods

2.1. Sample collection and fixation

Zostera was sampled over a 4-week period in July and August 2002 from four beds along the Welsh coastline: Milford Haven (51°44.35′N, 5°16.30′W), Skomer (52°56.6′N, 4°33.8′W), Porth Dinllaen (52°56.6′N, 4°33.8′W) and Criccieth (52°54.9′N, 4°13.1′W). Five sites were randomly selected within the perimeter of each bed. These sites were separated by distances of between 10 and 100 m. At each site, two divers were deployed from a small boat to sample the *Zostera* using 0.0625 m^2 quadrats. A 5 m tape measure was placed on the sea floor, aligned along a randomly selected compass bearing. The divers then sampled from five separate quadrats placed at random distances along the tape measure. All the *Zostera* above the rhizome within each quadrat was collected and placed in labelled plastic mesh bags (mesh size c. 1 mm). Samples were therefore collected as five quadrats per site, with five sites per bed. This resulted in 25 quadrats at each of the 4 beds.

Sample depths were measured with dive computers, subsequently corrected to Chart Datum (the depth of the lowest astronomical tide) using Admiralty Tide Tables. The separate *Zostera* beds were at slightly different depths. The deepest samples were taken from the bed at Skomer (−2.58 m, S.E. 0.01). Average depths for the other samples were −0.59 m (S.E. 0.08) at Milford Haven, −0.79 m (S.E. 0.05) at Criccieth and +0.43 m (S.E. 0.2) at Porth Dinllaen. Sample depth variances were not homogeneous across separate beds (Bartlett's test, $p < 0.01$), with the largest variability at Porth Dinllaen (range +1.1 to −1.1 m). A non-parametric test; however, implied that the median depths sampled in different beds were significantly different (Kruskal Wallis $H = 48.15$, $p < 0.001$).

Initially, it was planned to keep *Zostera* leaves in aerated seawater during transportation back to the laboratory and during analysis. However, the samples deteriorated so quickly during this period (i.e., within 2–3 days) that fixation was necessary. Samples were subsequently fixed after collection in formalin (diluted to between 1 and 5% in seawater).

2.2. Laboratory analysis

Collected material was carefully separated into individual *Zostera* leaves. Each leaf was scored as ''fouled'' (with macroscopic algae, animals or both) or ''clean''. Individual leaf lengths were also measured.

Over 5000 fouled leaves were collected. A random sample of 10 fouled leaves from each quadrat was examined for the presence of algal epiphytes under a dissection microscope. Identified taxa were recorded as present or absent. The sample size was chosen after a preliminary estimate from the species accumulation curve (Kendrick and Lavery, 2001) suggested that 10 leaves were sufficient to detect 90% of the epifloral species in a quadrat. In the few cases where less than 10 fouled leaves were present in a quadrat, all the available leaves were analysed. Algal epiphytes were also classified into functional groups to allow a comparison with previous work on *Zostera* beds (Saunders et al., 2003). Epiphytes were grouped as filamentous, foliose, corticated filamentous, saccate or coralline. An additional functional group of crustose was included to describe the growth form of *Rhodophysema georgei*. Identified taxa are listed in Table 1, with authorities, where appropriate.

2.3. Data analyses

The hierarchical sampling design allows comparisons at a number of spatial scales: within quadrats, between quadrats (1–5 m separation), between sites (>10 m separation) and between beds (>km separation). Not all variables could be compared at the four scales, for example, some variables were derived as a single measurement from each quadrat. Such univariate characteristics of *Zostera* patches at the quadrat scale (number of epiphyte species, average leaf length, percentage of leaves fouled and leaf density) were compared with a nested ANOVA. Data were transformed where necessary to meet the assumption of homogeneity of variances (homogeneity confirmed by non-significant Cochran's tests). Where data were transformed, taking square roots of the original measurements consistently removed heterogeneity. Some of the beds contained bare patches such that no leaves were collected from sample quadrats. A second set of ANOVA examined differences

Table 1
Presence/absence of algal epifloral species found growing on *Zostera marina* leaves at the four Welsh sites: Porth Dinllaen (PD), Criccieth (C), Milford Haven (MH) and Skomer (S)

Taxon	PD	C	MH	S
Audouinella[a] sp.	1	1	1	1
Ceramium diaphanum[c] sensu Harvey	1	1	1	1
Ceramium secundatum[c] Lyngbye	1	1	1	1
Ectocarpus[a] sp.	1	1	1	1
Enteromorpha prolifera[e] (O.F. Müller) J. Agardh	1	1	1	1
Erythrotrichia carnea[a] (Dillwyn) J. Agardh	1	1	1	1
Hypoglossum hypoglossoides[b] (Stackhouse) F.S. Collins and Hervey	1	1	1	1
Lomentaria clavellosa[c] (Turner) Gaillon	1	1	1	1
Polysiphonia fucoides[c] (Hudson) Greville	1	1	1	1
Polysiphonia stricta[c] (Dillwyn) Greville	1	1	1	1
Polysiphonia fibrillosa[c] (Dillwyn) Sprengel	1	1	1	0
Aglaothamnion bipinnatum[a] (P. Crouan and H. Crouan) Feldmann-Mazoyer	1	1	0	1
Sphacelaria[a] sp.	1	1	0	0
Chondria dasyphylla[c] (Woodward) C. Agardh	1	0	1	1
Dictyota dichotoma[b] (Hudson) J.V. Lamouroux	1	0	1	1
Polysiphonia elongata[c] (Hudson) Sprengel	1	0	1	1
Polysiphonia harveyi[c] J. Bailey	1	0	1	1
Rhodophysema georgei[g] Batters	1	0	1	1
Nitophyllum punctatum[b] (Stackhouse) Greville	0	1	1	1
Stylonema alsidii[a] (Zanardini) K. Drew	0	1	1	1
Polysiphonia denudata[c] (Dillwyn) Greville ex Harvey	0	1	1	0
Acrosiphonia[a] sp.	0	1	0	1
Ceramium pallidum[c] (Nägeli ex Kützing) Maggs and Hommersand	0	1	0	1
Palmaria palmata[b] (L.) Kuntze	0	1	0	1
Spermothamnion repens[a] (Dillwyn) Rosenvinge	0	1	0	1
Spermothamnion strictum[a] (C. Agardh) Ardissone	0	1	0	1
Antithamnionella ternifolia[a] (J.D. Hooker and Harvey) Lyle	0	0	1	1
Brongniartella byssoides[c] (Goodenough and Woodward (F. Schmitz)	0	0	1	1
Pterothamnion plumula[a] (J. Ellis) Nägeli	0	0	1	1
Rhodophyllis divaricata[b] (Stackhouse) Papenfuss	0	0	1	1
Total number of taxa	19	23	29	33

Superscripts refer to the functional group classification using the coding of [a]filamentous, [b]foliose, [c]corticated filamentous, [e]saccate, [f]coralline and [g]crustose.
Species restricted to a single bed were: at PD, [c]*Cladosiphon zosterae* (J. Agardh) Kylin; at C, [a]*Aglaothamnion gallicum* (Nägeli) Halos ex Ardré, [c]*Plocamium cartilagineum* (L.) P.S. Dixon; at MH, [a]*Cladophora rupestris* (L.) Kützing, [c]*Cystoclonium purpureum* (Hudson) Batters, [c]*Gelidium* sp., [a]*Griffithsia corallinoides* (L.) Trevisan, [c]*Polysiphonia nigra* (Hudson) Batters, [b]*Ulva* sp.; at S, [b]*Apoglossum ruscifolium* (Turner) J. Agardh, [c]*Bonnemaisonia hamifera* Hariot Trailliella phase, [a]*Callithamnion tetragonum* (Withering) S.F. Gray, [f]*Corallina officinalis* L., [c]*Polysiphonia brodiei* (Dillwyn) Sprengel, [b]*Porphyra* sp.

in *Zostera* where present by omitting the empty quadrats. The separate ANOVA analyses allow comparisons between *Zostera* characteristics (lengths and densities) within a bed perimeter and variability of the same characteristics solely within the areas of continuous canopy. For analyses where all quadrats contained *Zostera*, quadrats were randomly deleted to achieve a balanced design.

Epiphytic algal assemblages were compared among leaves using the Sørenson coefficient, S:

$$S_{ij} = \frac{100 \cdot 2a}{2a + b + c}$$

where S_{ij} is a measure of similarity between the species present on leaves i and j, a the number of species present on both i and j, b the number of species present on i but not on j and c is the number of species present on j but not on i. Due to the multiplication by 100, Sørenson coefficient values range from 0 (no species in common) to 100 (all species in common).

Variation in the epiphyte assemblage structure was compared across the spatial scales sampled using the ANOSIM routine in PRIMER (Clarke and Warwick, 1994). These analyses used a matrix composed of Sørenson coefficients for all the possible pairwise comparisons between separate leaves. ANOSIM tests also examined multivariate variation among quadrats using the variables of depth, total leaf length, average leaf length and leaf density. This is a test of the null hypothesis that there is no spatial variation in a combination of *Zostera* canopy variables and depth within beds (this is a more powerful test of variation in canopy structure, complementing the univariate comparisons carried out using ANOVA).

ANOSIM tests can be used where groups are nested (e.g., quadrats within sites). The procedure is limited to two hierarchical levels (Clarke and Warwick, 1994). To cover the scales sampled in this study, nested ANOSIM tests were carried out for each bed separately (comparing quadrats within sites and sites within a bed). Comparisons between beds were carried out by amalgamating the species lists for separate leaves within a quadrat to produce a single replicate. This facilitated an ANOSIM, where sites within beds and differences among beds could be compared. To standardize the sampling effort for this particular analysis, only quadrats where 10 fouled leaves had been scored were used.

To complement the ANOSIM tests, a hierarchical plot of average dissimilarity between leaves was constructed (e.g., Lavery and Vanderklift, 2002). This allows differences in epiphyte assemblage structure at more than two scales to be compared within the same analysis. The hierarchical plot is based on the same matrix of Sørenson coefficients as used in the ANOSIM tests. Similarities are converted into dissimilarities by subtracting from 100. Hence, a dissimilarity of zero means that leaves have identical epiphyte assemblages. Each number in the matrix of dissimilarities refers to a comparison of leaves at a particular scale (within quadrats, among quadrats within a site, among sites within a bed or among beds). Hence, the matrix of dissimilarities can be summarized with four mean values. If assemblages compared at one scale are no more different than assemblages compared at another scale (the null hypothesis), then the four means will not differ. In contrast, if the differences between leaves increases with spatial scale, then a plot of mean dissimilarity against spatial scale will have a positive slope. The hierarchical dissimilarity plot is an alternative to nMDS (hereafter abbreviated as MDS), which is commonly used to display the structure within a dissimilarity matrix (Clarke and Warwick, 1994). MDS provides a two dimensional representation of the separation between all leaves. The axes of an MDS have arbitrary units with a 'stress' value below 0.2 representing a relatively faithful representation of structure in the underlying matrix of dissimilarities.

In a hierarchical dissimilarity plot, differences between means calculated at different scales can be tested for departure from the null hypothesis of no difference with a randomisation test. This involves calculating a series of differences between two means, each comparison involving random selection of two sets of numbers from the dissimilarity matrix. The size of the randomly selected sets of numbers is constrained to contain the same number of replicates as the means under test. The measured difference between two means can now be compared with a probability distribution of randomly selected differences to test how likely the measured value is to have occurred by chance alone (Manly, 1997). Testing for differences among the four different scales involves an element of multiple hypothesis testing. Hence, the significance level of individual tests was corrected (alpha = 0.0085) to retain an overall Type I error rate of 0.05 using the Dunn–Šidák method (Sokal and Rohlf, 1995).

Relationships between assemblage composition and environmental variables were examined using the RELATE procedure in PRIMER. RELATE is the equivalent of a non-parametric Mantel test (Somerfield et al., 2002). The matrix of similarities between sampled assemblages (based on Sørenson coefficients) is compared with a matrix of the similarity between sites based on environmental parameters. The significance of any correlation between matrices is assessed with a randomisation test. At the scale of individual leaves, variations in assemblage structure were tested against leaf length. When species lists were amalgamated at the quadrat scale, the available environmental variables were sample depth, total leaf length, average leaf length and leaf density. To avoid excessive repetition, subsequent references to *Zostera* canopy variables in this study refer to quadrat scale measurement of total leaf length, average leaf length and leaf density. Correlations and multiple regressions were used to test for associations with species richness at the leaf and quadrat scales. These analyses used the same predictor variables as the multivariate tests. Both RELATE and univariate tests were carried out separately for each bed. This avoids confounding differences among beds with other, unmeasured, variables. For example, a difference between the assemblages of beds A and B may be due to the difference in depth between beds. At regional scales, however, processes such as large-scale variation in propagule supply and climatic tolerances may also cause changes in assemblage structure. These particular sources of variation are not likely to confound within bed analyses.

3. Results

3.1. Comparisons between scales

Densities and lengths of *Zostera* leaves varied significantly within and among beds (Table 2a, Fig. 1). The *Zostera* canopy was patchy within the perimeters of individual beds with 19% of quadrats containing no eelgrass leaves. This patchiness tended to make variances heterogeneous in the analysis. Variance heterogeneity for leaf density could not be removed by (logarithmic or power) transformations, so the significant differences reported must be treated with caution. There were no problems with heterogeneity when restricting the analysis to quadrats where leaves were found (using random selection of

Table 2
Nested ANOVA of average leaf length and leaf density among separate beds and among sites within beds (a) including quadrats with no leaves in the analysis and (b) analysis restricted to quadrats containing *Zostera* leaves

Source of variation	d.f.	Average leaf length		Leaf density	
		Mean square	*F* ratio	Mean square	*F* ratio
(a) All quadrats					
Bed	3	60.82	11.68***	305730	5.11*
Site (bed)	16	5.21	4.03***	59857	18.21***
Residual	80	1.29		3287	
(b) Occupied quadrats only					
Bed	3	1.24	26.96***	249.99	8.78**
Site (bed)	8	0.05	1.67	28.47	3.73**
Residual	24	0.03		7.63	

Variances were homogeneous for average lengths and after square root transformation for leaf density in occupied quadrats and for average length in all quadrats (Cochran's tests, all $p > 0.05$). No suitable transformation was found for leaf density in the analysis with all quadrats. The analysis presented for this variable used untransformed data that had heterogeneous variances.
* Significant probability at $p < 0.05$.
** Significant probability at $p < 0.01$.
*** Significant probability at $p < 0.001$.

occupied quadrats at each site to produce a balanced design). This analysis allows a comparison of canopy structure without the additional variance caused by open spaces within the perimeter of eelgrass beds. When comparing occupied quadrats, the average leaf length differed significantly among beds. However, average leaf lengths in occupied quadrats did not significantly vary within beds. Leaf density in occupied quadrats differed significantly among and within beds (Table 2b).

Variation in the percentage of fouled leaves was significant among sites within beds (Table 3, Fig. 2). In contrast, the average number of epiphyte species did not vary significantly within beds, but there was significant variation among beds.

The spatial structure in epiphyte assemblages can be seen in the MDS and hierarchical dissimilarity plots (Fig. 3). Leaves from different beds tend to be clustered in separate parts of the MDS plot. This is reflected in the hierarchical dissimilarity plot: epiphyte assemblages on leaves from separate beds were far more dissimilar on average than

Table 3
ANOVA comparison of spatial variation in percentage of fouled leaves and number of epiphyte species per quadrat

Source of variation	Percentage leaves fouled			Average number of epiphyte species		
	d.f.	Mean square	*F* ratio	d.f.	Mean square	*F* ratio
Bed	3	2875.5	3.58	2	368.93	77.22***
Site (bed)	8	803.3	5.52**	6	4.78	0.95
Residual	24	145.4		18	5.04	

Variances of untransformed data were homogeneous (Cochran's tests, all $p > 0.05$). The percentage of leaves fouled was calculated for occupied quadrats only. To standardise sampling effort, only quadrats where 10 leaves were scored for epiphytes were used in the analysis of species richness. Surplus quadrats were randomly deleted to produce a balanced design in the separate ANOVA.
** Significant probability at $p < 0.01$.
*** Significant probability at $p < 0.001$.

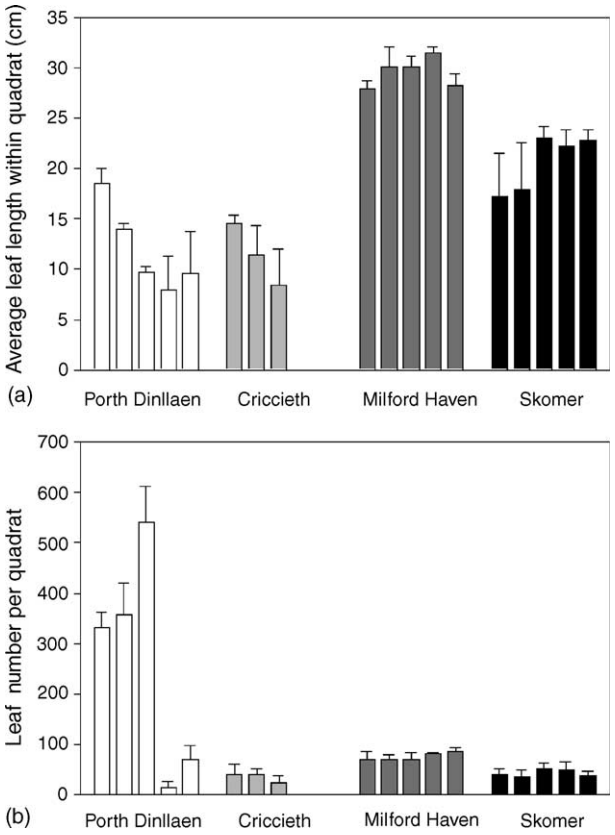

Fig. 1. Average (a) length and (b) density of *Zostera marina* leaves sampled from separate sites within each of the four beds in the survey. Error bars are standard errors.

assemblages on leaves in the same bed. The increase in average dissimilarities when comparing leaves from separate beds was significantly larger than would be expected if there was no pattern (randomisation test, minimum significant difference between averages 2.80% for species data, 2.51% for functional group data). Comparisons between quadrat scale and site scale averages were also significant for both species and functional group data (differences between means of 3 and 3.39%, respectively). Hence, assemblages on leaves within a quadrat were more similar on average than leaves compared between quadrats at the same site. In contrast, the hierarchical plot did not suggest significant changes in average dissimilarity when moving from within site ($<$10 m) to between site ($>$10 m) scales.

There was structure at all spatial scales in the composition of the epiphyte assemblage on *Zostera* (Table 4). There were significant differences in the assemblages on leaves among quadrats and among sites within beds for all areas except Criccieth. Results based on species or functional group composition were similar, with a few small differences in individual beds. Amalgamated species lists for each quadrat suggested that there were significant differences in species assemblages between sites within beds and among beds

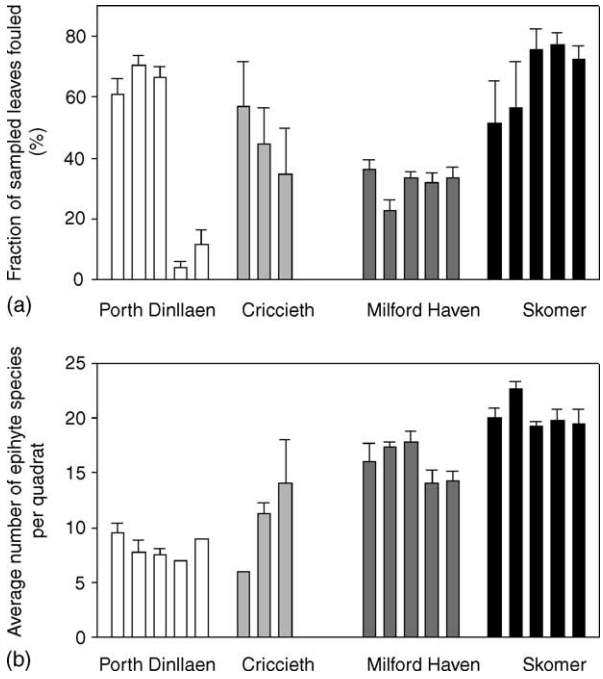

Fig. 2. Average (a) fraction of fouled leaves per quadrat and (b) number of epiphyte species per quadrat in different sites within beds. Shading and error bars as in Fig. 1.

(respective ANOSIM statistics of 0.188, $p < 0.01$ and 0.882, $p < 0.001$). Converting the amalgamated data into functional groups reduced the degree of discrimination between spatially separated assemblages. Differences in functional group assemblages were not significant among sites within beds (ANOSIM statistic of 0.045, not significant). However, separate beds could be discriminated on the basis of functional group composition (ANOSIM statistic of 0.496, $p < 0.001$).

Table 4
ANOSIM test statistics for patterns in epiphyte assemblages based on (a) species and (b) functional groups

Source of variation	Porth Dinllaen	Criccieth	Milford Haven	Skomer
(a) Species data				
Among quadrats within a site	0.184***	0.022	0.123***	0.177***
Among sites	0.294**	0.183	0.265***	0.164*
(b) Functional groups				
Among quadrats within a site	0.131***	0.043	0.039*	0.037*
Among sites	0.279*	0.373**	0.181**	0.042

* Significant probability at $p < 0.05$.
** Significant probability at $p < 0.01$.
*** Significant probability at $p < 0.001$.

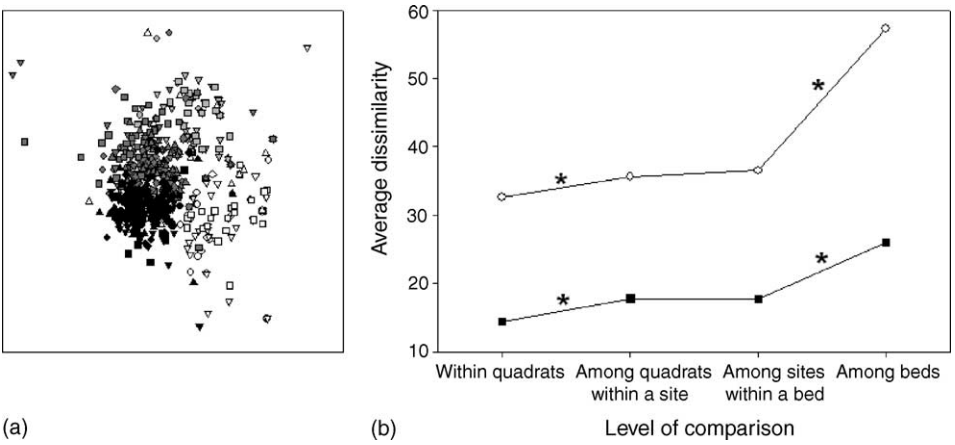

Fig. 3. Spatial variation in epiphyte assemblage structure shown as (a) a MDS ordination (stress = 0.19) and (b) as a hierarchical dissimilarity plot. Points in the MDS represent species assemblages from individual leaves. The different shading of points indicates the bed from which samples were taken (shading levels for each bed shown in Fig. 1. Different symbols indicate separate sites within a bed. The hierarchical plot gives the average dissimilarity between epiphyte assemblages on pairs of leaves compared at different spatial separations. Dissimilarities were calculated using the list of taxa in Table 1 (open symbols) and after reducing data to the functional group level (closed symbols). Asterisks indicate a significant departure from the null hypothesis of no additional assemblage dissimilarity when moving between adjacent scales.

3.2. Associations between variables at leaf and quadrat scales

Epiphyte assemblage structure changed as a function of individual leaf length within each bed. Similarities between assemblages increased as the differences in compared leaf lengths decreased (RELATE test, correlations for Porth Dinllaen 0.408, Criccieth 0.572, Milford Haven 0.472 and for Skomer 0.466, all values significant with $p < 0.001$). The nature of these associations with length varied from bed to bed, partly as the available species pool varied between beds (see ANOSIM results above). The overall species richness of epiphytes increased with individual leaf length at Porth Dinllaen and Criccieth (correlations between species number and leaf length of 0.201 and 0.334, respectively, both significant with $p < 0.01$). Longer leaves did not, however, have larger numbers of epiphyte species at the remaining two *Zostera* beds.

While there was evidence for significant variation in *Zostera* densities and average leaf lengths (per quadrat) within beds (Table 2), these patterns did not relate to epiphyte species

Table 5
RELATE tests of the association between similarity matrices based on species assemblages and similarity matrices based on *Zostera* average length, overall length, density and depth in each quadrat

Zostera bed	Relate test statistic	Significance level, p (999 permutations)
Porth Dinllaen	0.231	0.07
Criccieth	0.371	0.07
Milford Haven	0.066	0.24
Skomer	0.048	0.68

richness. Multiple regressions of species richness using quadrat-scale *Zostera* lengths (average and quadrat total), densities and depths as predictors were not significant. A multivariate analysis using the measured canopy variables and depth suggested that there was significant variation of canopy architecture within separate beds (ANOSIM test statistic for differences between sites 0.261, $p < 0.05$). RELATE tests did not, however, suggest that variation in epiphyte assemblage structure was associated with the multivariate variation in measured canopy architecture within beds (Table 5).

4. Discussion

There was clear variation within and among beds in the measured *Zostera* canopy variables and in epiphyte species assemblages. The percentage of fouled leaves varied among sites within beds and multivariate tests indicated that there was usually significant spatial structure to the epiphyte assemblage within beds (Table 4). Epiphytes within *Zostera* beds were not homogeneously distributed. The caution expressed by Saunders et al. (2003) over their finding of homogeneity within beds near Plymouth seems justified. The Welsh survey analysed in this study used 25 replicate quadrats within a bed, including within bed comparisons at scales >10 m. In comparison, Saunders et al. (2003) collected 12 replicates per bed (all separated by less than 10 m). The analyses presented in this study therefore have greater statistical power to identify the spatial patterns of diversity, both due to the number of replicates involved and perhaps due to the greater spread of spatial scales used. The loss of information when using a functional group approach is illustrated in Fig. 3b. The increase in dissimilarity with spatial scale is more pronounced when using species level data. In contrast to Saunders et al. (2003), however, there was still enough discrimination at the functional group level to distinguish epiphyte assemblages within beds.

Although there was within bed structure to epiphyte assemblages, the species dissimilarity curve in Fig. 3 is strikingly similar to the curve for *Posidonia* in Lavery and Vanderklift (2002). Differences in assemblage structure within beds were far less than the difference between beds (see also Piazzi et al., 2004). This pattern is predicted for seagrass species such as *Zostera*, where leaf turnover is relatively high. The conceptual model of Lavery and Vanderklift (2002) proposes that individual leaves are the fundamental unit of structure for epiphytes. Epiphyte recruitment to seagrass leaves can occur from neighbouring leaves, from elsewhere in the bed or from separate beds or habitats (Van Elven et al., 2004). Recruitment from adjacent leaves will tend to reinforce local structure in the assemblage. Rapid turnover of leaves will, however, reduce the opportunities for local recruitment, weakening small-scale spatial structure of epiphyte assemblages within beds. As an alternative to this model, the structure of the seagrass canopy may alter (or reflect) the local environment at small scales, leading to the recruitment of species favoured by the local conditions. The results from the RELATE tests, showing no relationship between epiphyte assemblages and quadrat scale measurements of canopy structure (density, average length and total leaf length per quadrat) are consistent with Lavery and Vanderklift's model for seagrasses with relatively high leaf turnover. Leaves were the fundamental unit of structure for epiphyte assemblages. If the significant within bed

variation in canopy variables reflected or altered small-scale environmental conditions, these local variations did not affect epiphyte recruitment and survival in a consistent manner (at least for the measured canopy variables). Alternatively, if local variations in environmental conditions directly or indirectly (through the *Zostera* canopy) affect epiphyte assemblages, then such associations are weakened by the turnover of individual leaves. Both the 'no association with local environmental conditions' and the 'associations weakened by leaf turnover' hypotheses are consistent with Lavery and Vanderklift's model. It remains to be seen whether microepiphyte communities are structured in similar ways given that habitat preferences and colonisation mechanisms are likely to differ between multicellular and unicellular organisms.

It is difficult to identify the factors causing variation in canopy structure and epiphyte assemblages among separate beds. *Zostera* beds may be affected by environmental factors such as tidal currents (Schanz and Asmus, 2003), depth (Middelboe et al., 2003) and wave action (Ramage and Schiel, 1999). Epiphytes are also affected by environmental factors (e.g., salinity, Kendrick et al. (1988) and wave exposure, Kendrick and Burt (1997)). Both seagrass and epiphytic algae may be affected by grazing (Hootsmans and Vermaat, 1985). The overall level of herbivory may cause differences in epiphyte biomass among separate beds (Alcoverro et al., 1997). Grazer–epiphyte interactions are, however, complex (Jernakoff et al., 1996) and may be mediated by environmental factors (Schanz et al., 2002). Regional differences in propagule supply, climate and bed history may also shape the local species assemblage in different beds. Given that environmental conditions for each bed are not fully characterised and the limited number of beds in the study, it is difficult to speculate further on the factors associated with spatial variability in epiphyte assemblages and *Zostera* canopy structure. An important result from this paper, however, is that leaf length could confound correlations of epiphyte assemblages with environmental and biotic variables. Such studies need to use a standard leaf length or to include individual leaf lengths as a covariate. Further development of this work would benefit from the inclusion of leaf age as a predictor variable, as length is a less precise measure of the potential colonization period due to the confounding influences of environmentally driven variation in growth rates and leaf breakage.

Some epiphyte species were restricted to a single *Zostera* bed (Table 1). Such rarity was reflected by frequency of occurrence within beds. If a species was restricted to a single bed, it was never common within that bed (average probability of finding rare species was 0.03 per blade in beds where they occurred, compared to a probability of 0.42 for species found in all beds). There was a significant non-parametric correlation between number of beds an epiphyte was found in and the expected probability of finding the species on a fouled leaf from a bed where the species is known to occur (Spearman's $r = 0.747$, $p < 0.001$). The patterns of rarity suggest that a precautionary approach to the conservation of biodiversity is appropriate: there is no spatial 'insurance' for rare species at regional scales, with the loss of a fraction of a single bed potentially leading to regional extinction. It is unclear what traits might be associated with such rarity. More information on comparative reproductive biology and habitat requirements of the epiphytes is needed.

A heterogeneous spatial distribution of epiphytes presumably reflects the influences of limited dispersal distances (Lavery and Vanderklift, 2002), variations in *Zostera* demography, interactions between species on individual leaves and interactions with

environmental conditions and herbivores. Experimental investigations of the effects of leaf demography and spacing, involving artificial mimics (Lethbridge et al., 1988; Kendrick and Lavery, 2001) and translocation of leaves, would lead to definitive tests of Lavery and Vanderklift (2002) hypotheses relating the degree of spatial and temporal heterogeneity in epiphyte assemblages to the turnover of seagrass biomass.

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