Diversity effects beyond species richness: evidence from intertidal macroalgal assemblages

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ABSTRACT: Several recent experimental studies have examined the effects of macroalgal diversity on community functioning using synthetic assemblages. However, their predictive relevance for natural systems remains uncertain. Unlike terrestrial habitats, where observational studies in natural systems are profuse, studies on the relationship between diversity and ecosystem functioning in natural marine ecosystems are scarce. In the present study, we explored how different components of biodiversity influence the performance of macroalgal assemblages in natural communities (intertidal boulders). Specifically, we examined the relationships between biomass, species richness, spatial aggregation and evenness and the productivity of macroalgal assemblages. We found the expected positive relationship for biomass and species richness. Additionally, we found significant statistical relationships between both spatial aggregation and evenness and some of the productivity-related variables analyzed: assemblages with a higher degree of spatial aggregation reduced their light capture and photosynthetic efficiency, while increasing evenness increased maximum net primary productivity. Although these patterns should be further tested using experimental approaches, observational studies may provide valuable insights by revealing patterns usually overlooked by experimental approaches.

KEY WORDS: Biodiversity · Productivity · Spatial aggregation · Evenness · Macroalgae

INTRODUCTION

Over the last decade, global loss of diversity (Pimm & Raven 2000) has fuelled considerable research to investigate the functional consequences of declining diversity in communities. Studies on the existence of a relationship between diversity and ecosystem functioning, particularly productivity, have dominated research in this area of ecology, with a linear or log-linear relationship as the most commonly described patterns (Hooper et al. 2005, Srivastava & Vellend 2005, Balvanera et al. 2006). Biodiversity has often been used as a synonym for species richness, and research to date has focused on species and functional richness effects on ecosystem processes. However, in a broader sense, the definition of biodiversity includes other components such as the relative abundance of species (Hooper et al. 2005). While species richness determines the range of trait variation present in a plant community and thus the possible interspecific interactions that will ultimately generate the diversity effects (Loreau 2000), evenness and other attributes such as density or spatial aggregation of plant communities may influence the net effect of these interspecific interactions by defining their intensity and relative contribution (Kirwan et al. 2007). Empirical studies on the effects of relative abundance of species have found positive effects of evenness on the productivity of plant communities (Wilsey & Potvin 2000, Stevens & Carson 2001, Kirwan et al. 2007). Similarly, the limited existing empirical evidence suggests that spatial aggregation of species may affect the productivity of plant communities (Maestre et al. 2005, Mokany et al. 2008).

In marine communities, the recent profusion of experimental studies performed using artificially constructed assemblages has yielded valuable information

about the functional role of diversity (see Stachowicz et al. 2007 for a recent review), yet the relevance of such simplistic approaches for highly complex natural systems has been questioned (Carpenter 1996). Additionally, experiments have been designed to test specific factors or effects and do not allow a full understanding of the relative importance of other co-occurring factors (Bulling et al. 2006).

As in terrestrial systems, species in marine habitats are not evenly distributed, neither in biomass or spatially. The importance of diversity effects beyond species richness has not been fully explored (Giller et al. 2004), hindering our ability to extrapolate from experimental studies to natural systems. Even the predicted positive effects of species richness have rarely been tested in marine natural assemblages (but see Emmerson & Huxham 2002, Bruno et al. 2006), and observational studies are needed to examine whether the patterns and mechanisms predicted by theories and experiments occur in natural systems (Stachowicz et al. 2007). A good example of the utility of these observational approaches are the unexpected exponential relationships between diversity and ecosystem functioning recently found in deep-sea communities (Danovaro et al. 2008), suggesting that facilitative interactions dominate among benthic species in this environment.

In the present study we explored the relationships between different diversity-related attributes and productivity in natural macroalgal assemblages. Specifically we examined the relationship between standing biomass, species richness, evenness and spatial aggregation with several productivity-related variables. Our model communities were rock-pool boulders with natural algal assemblages dominated by perennial species. These boulders are of a convenient size and present macroalgal communities of different richness. Productivity measurements were carried out in the laboratory under controlled conditions to reduce environmentally induced variability in the responses. Since we used a non-manipulative approach to examine which structural attributes were relevant, we combined multiple regression models with an analytical tool of variance decomposition, the hierarchical partitioning procedure.

MATERIALS AND METHODS

Collection site. Boulders were collected from Praia Norte (Viana do Castelo, Portugal; 41° 41′ 48″ N, 08° 51′ 11″ W), an extensive granitic rocky shore with numerous emergent reefs that partially shelter some areas and create abundant low tide channels. Boulders are abundant in these channels, where they constitute a hard substrate on a predominantly soft bottom area.

Most of these boulders present rich algal assemblages with *Chondrus crispus*, *Lithophyllum incrustans* and *Corallina elongata* as dominant species (together >70% of the total dry biomass).

To perform the incubations with natural assemblages, from November to December 2007, we haphazardly collected 40 boulders of an appropriate size and bearing well-developed algal assemblages. Due to logistic limitations and the time required to measure and process each assemblage, 3 boulders were collected each sampling trip (around 6 boulders per week), carefully brought to the laboratory and immediately rinsed with freshwater to remove swimming grazers and sediment. Other attached animals were removed by hand. Freshwater baths did not affect the seaweeds' photosynthetic performance, as demonstrated by a pilot study using a PAM-chlorophyll fluorometer (Walz® 2000) on the 3 most abundant macroalgal species (*Chondrus crispus*, *Lithophyllum incrustans* and *Corallina elongata*) on the boulders. For the pilot study seaweed fronds were subjected to 20 s freshwater or seawater baths (4 replicates per species and bath type). Quantum efficiency or yield (F_v/F_m) levels were measured the next day after a 40 min dark accommodation period. Despite yield differences among species (ANOVA, $F_{2,2} = 18.97$ p < 0.001), bath type or its interaction with species did not show significant differences ($F_{1,2} = 5.24$, p > 0.05 and $F_{2,18} = 0.54$, p > 0.05 respectively). Boulders were kept in outdoor aerated seawater tanks (16 ± 2°C, SE) for <30 h before the incubations were conducted.

Incubation procedures and estimation of structural predictors. Productivity-irradiance (P-I) curves were estimated by measuring oxygen fluxes inside a sealed incubation chamber at 9 irradiance levels.

The incubation chamber consisted of a 15.2 l Plexiglas chamber partially submersed in a larger, thermostatically controlled 88 l cooling chamber, also Plexiglas. Mean ± SE temperature inside the incubation chamber was 16.4 ± 0.07°C). Water movement inside the incubation chamber was maintained through a submersible pump (1200 l h^{-1}) equipped with a diffuser to reduce turbulence. Oxygen concentration variations were measured using a luminescent dissolved oxygen probe connected to a data-logger (Hach® HQ40) that registered a new measurement every 10 s and was continuously monitored.

Incubations were performed inside a phytoclimatic chamber (Abalab® Fitoclima 750E) with light control facilities which allowed us to measure assemblage productivity at 9 successive and increasing light levels: 0.4 (dark), 32.5, 61.0, 114.5, 259.9, 536.6, 804.3, 1036.3 and 1106.5 µE m^{-2} s^{-1}. Maximum irradiance levels in the chamber were lower than those recorded in the field at sea surface level where, during sunny days in winter,

irradiance can reach >2000 µE m^{-2} s^{-1} (authors' pers. obs. using a scalar quantum sensor). However, the assemblages collected in the low intertidal zone usually experience a much lower irradiance. The light source in the chamber was composed of eighteen 58 W fluorescent tubes (Osram® Cool White). Irradiance inside the chamber was measured continuously using a scalar quantum sensor (Biospherical® QSL-2000 Radiometer) connected to a computer.

For each P-I incubation, the successive irradiance periods lasted between 17 and 20 min, depending on the time necessary for the fluorescent tubes to warm up and the assemblages to reach linear rates of oxygen flux (Migné et al. 2002). The entire set of incubations took around 2:30 to 2:45 h per boulder.

Because oxygen availability is highly dependent on pH levels, variations in pH were continuously monitored inside the incubation chamber using a pH probe connected to a data-logger. Mean (±SE) pH values were 8.3 ± 0.01 with an average increase through the whole incubation of 0.4 ± 0.02. To reduce possible effects of circadian rhythms on algal productivity, incubations were always carried out during daylight hours (between 08:00 and 17:00 h).

Productivity was estimated through the measurement of oxygen fluxes by regressing oxygen concentration in the chamber (measured in µmol) through time (s^{-1}). Fluxes were corrected by seawater volume inside the chamber to take into account the different volumes of the boulders.

Three ecosystem functioning surrogates were determined per boulder assemblage: (1) maximum net primary productivity (max NPP), the maximum productivity (i.e. maximum slope for the oxygen concentration versus time relationship) recorded at any light intensity (µmol O$_2$ s^{-1}); (2) assemblage respiration, the oxygen consumption during the dark period of the incubation (µmol O$_2$ s^{-1}); and (3) photosynthetic efficiency at low irradiance (α), estimated using ordinary least squares (OLS) regressions for the light-limited portion of the curve (µmol O$_2$ µE^{-1} m^{-2}). Linearity of the light-limited portion of the *P-T* curve was high (R^2 > 0.98 in all cases). Regressions were also used to estimate assemblages' compensation irradiance, the irradiance level where each linear model intercepts the *x*-axis (i.e. NPP = 0).

Additionally, we measured light levels inside the algal assemblages as a proxy for resource capture levels by the assemblages. Boulders were submerged in a 15 l aquarium, and light was measured regularly with a 3 × 3 cm grid located on the boulder's upper surface using a scalar quantum sensor (Biospherical® QSL-2000 Radiometer). On average, light was measured at 38.4 ± 1.8 different points per boulder. Light capture is expressed as the average percentage of incident light above canopy for the entire grid. This percentage only reflects the effect of macroalgal species with erect fronds on the light distribution throughout the assemblage. It was not possible to estimate light absorption by encrusting species in the natural assemblage using this methodology.

Once ecosystem functioning surrogates had been measured, the spatial arrangement of the species inhabiting the boulders was mapped by transferring their position onto a transparent plastic sheet. Those maps were then used to create a multi-type (multi-species) spatial point-reference pattern using a 1.5 × 1.5 cm grid, where species presence at the level of the boulder's surface was recorded at every grid intersection point. There is no index to characterize multi-species point-reference spatial data; thus, spatial pattern analyses of single species were conducted using the Clark-Evans (C-E) index for spatial aggregation. Boulder assemblages were frequently dominated by a single species that, on average, represented >50% of the total biomass. Therefore, we used the C-E index of the most abundant species in biomass as an indication of the spatial arrangement for the whole assemblage. Dominant species were different for the different assemblages in terms of biomass. Accordingly, the C-E index for *Lithopyllum incrustans* was calculated in 15 out of 40 boulders, for *Chondrus crispus* in 11 and for *Corallina* elongata in 6 boulders. Furthermore, other dominant species included *Chondracanthus acicularis* (2 boulders) and *Gelidium corneum* (2 boulders). *Chondracanthus teedei*, *Mastocarpus stellatus* (as the encrusting *Petrocelis* phase), *Sargassum muticum* and *Bifurcaria bifurcata* were each the most abundant species on 1 boulder.

The C-E index measures spatial aggregation of a single species by comparing the distances among neighbour points to the expected mean distance if the species was randomly positioned. Values lie between 0 and 2.1491: an index value <1 corresponds to a clustering distribution, values >1 denote regularity, and a completely random pattern would have a value of 1 (Petrere 1985). The C-E index was calculated using the spatstat package (Baddeley & Turner 2005) for R. This spatial analysis package allows fitting the analyzed 2-dimensional spaces to surfaces of different shapes and sizes, as in our boulders.

Finally, seaweeds were scraped from the boulders, rinsed in freshwater, sorted by species and dried at 60°C for 48 h to estimate biomass (g dry weight [DW]). In the case of calcareous species (e.g. *Corallina elongata*, *Lithophyllum incrustans*), seaweeds were decalcified for 48 h in an HCl solution (50 g l^{-1}), rinsed with freshwater and dried as above.

Evenness was estimated using Pielou's index, *J'*, for the biomass data. Pielou's evenness index is the ratio

between the actual Shannon-Wiener information function of the assemblage, H_s, and the theoretical maximum value, H_{max}, if all species in the sample were equally abundant.

Data analysis. To explore how structural attributes might shape assemblage function, we used multiple linear regression models where the additive effects of the independent variables on the functional responses were examined. We fitted linear models for each of the 4 functional measures: respiration, max NPP, α and % light capture. Predictive structural variables included biomass, species richness, evenness and spatial aggregation for the most abundant species. When appropriate, variables were log-transformed in order to guarantee linearity and normality of residual distributions.

We did not intend to seek single predictive models; our goal was to investigate which—and to what extent—structural attributes may influence the functioning of algal assemblages. Mac Nally (1996, 2000) highlighted the differences between both the predictive and the explanatory use of multiple regression models. Predictive models, selected to minimize residuals, do not always identify properly those variables most likely to influence variation in the dependent variable, which is the main goal of the explanatory approach (Mac Nally 2002). Explanatory approaches in multiple regression require an explicit quantification of the relative importance of each predictor for the response (Grömping 2007).

Selection procedures based on significance of regression parameters (i.e. partial correlation coefficients) and sequential selection techniques are often used to choose relevant predictors in multiple regression modelling; however, these techniques have well recognized flaws and drawbacks (Mac Nally 2000, Whittingham et al. 2006). Therefore, we fitted full models, including all predictors for each functional measure, and examined their relative contribution to the total variance explained using the hierarchical partitioning procedure (Chevan & Sutherland 1991). This variance partition procedure compares simultaneously all possible models from a set of predictors and splits the contribution of each variable into 2 components: (1) the independent effect (I), the independent contribution of each predictor variable, calculated by comparing the fit of all models including the variable with their reduced version (i.e. the same model but without the variable) within each hierarchical level and then averaging across levels (the sum of all the independent effects in a model gives the R^2 of the model); and (2) the joint effect (J), the contribution of each predictor in conjunction with other predictors, calculated in a similar way by comparing the effects of combinations of variables with their corresponding reduced models (Mac Nally 1996). Thus, predictors with similar values for both independent and joint effects (i.e. I:J ratio close to 1) indicate that both contributions have a similar strength in the model. The hierarchical partitioning procedure helps to alleviate problems derived from collinearity among predictors by identifying separately both the independent and joint effects of the predictor variables (Chevan & Sutherland 1991, Mac Nally 2000).

The significance of I was calculated by comparing the observed value to the distribution of a population of independent contributions based on 1000 randomizations of the original data matrix. Significance was accepted at the upper 95 % confidence limit (Z-score $\geq$ 1.65). Hierarchical partitioning was conducted using the hier.part package (Walsh & Mac Nally 2008) for R.

After examining the I and J effects of the predictors for all the models calculated, we found that I:J ratios for species richness were close to 1 in all the models analyzed, indicating that both contributions have similar strengths for this predictor. No other significant predictor showed strong joint effects. A detailed analysis of these joint effects via the construction and re-analysis of a new set of models, including interactions between species richness and the other predictors, revealed that the combined effect of biomass and species richness was responsible for two-thirds of these joint effects (data not shown). To carry out this analysis we used the relaimpo package from the R Project, which is able to respect the pre-defined hierarchy existing in linear models where main effects and interactions are included (Grömping 2006). Thus, to test for effects of species richness on assemblage performance after removing the effect of biomass we used ANCOVA (Huitema 1980); differences between groups were tested *a posteriori* using Tukey's tests. Boulders were grouped into 4 categories of replicates of increasing richness (mean no. of species ± SE: low = 4.7 ± 0.4; mid-low = 7.3 ± 0.2; mid-high = 9.6 ± 0.2 and high = 12.75 ± 0.6). To prevent having boulders of the same richness level in different groups, 8 replicates per group were included. Homogeneity of variance was checked using Cochran's test, and transformations were applied when necessary. In the case of variable light capture, heterogeneity was impossible to remove; results should be interpreted with caution despite the robustness of the ANOVA procedures when the number of replicates is high. No differences between within-group regression slopes were detected for any of the analyses performed (p > 0.25 for all cases).

RESULTS

Algal assemblages of each boulder varied in richness and biomass. The boulders were slightly different in size (mean weight ± SE = 2.18 ± 0.14 kg) with an algal
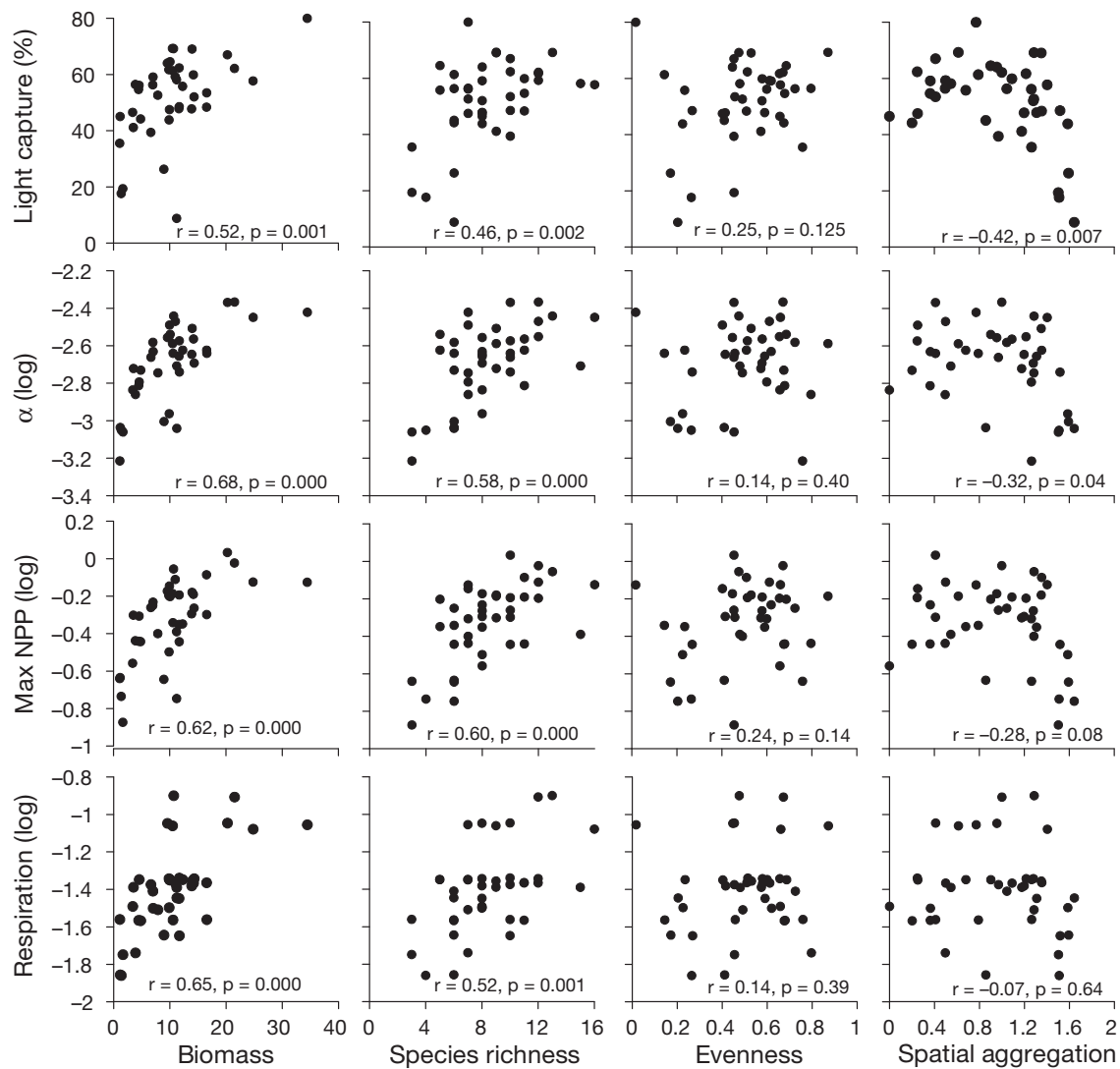
Fig. 1. Bivariate plots for all the variables examined in the present study: biomass, species richness, evenness and spatial aggregation are plotted against % light capture, photosynthetic efficiency at low irradiance (α), maximum net primary productivity (max NPP) and assemblage respiration. Product-moment correlation coefficients (r) and their significance levels are given for each combination shown

cover ranging from 246 to 795 cm^2. Species richness differed from 3 to 16 species (mean ± SE = 8.4 ± 0.4) and total biomass from 1.1 to 34.5 g DW (mean = 10.49 ± 1 g DW). Taking into consideration the differences among boulder sizes, total biomass varied between 1.5 and 36.9 g DW 0.04 m^{-2} (mean = 9.13 ± 1 g DW).

Species richness and total biomass were significantly correlated (r = 0.45, p < 0.001), but no other significant correlation was found among the structural variables. Species abundance distributions were highly skewed, with 3 species accounting for most of the biomass (*Chondrus crispus, Lithophyllum incrustans* and *Corallina* spp. represented 33.3, 30.2 and 10.4% of the overall biomass, respectively). Nevertheless, differences in

assemblage composition meant that *Chondrus crispus* was absent from 12 boulders, *Corallina* spp. from 6 and *Lithophyllum incrustans* from 1.

P-I curves followed the described hyperbolic relationship. In many assemblages (31 out of 40) we found signs of photo-inhibition at the highest irradiance with a mean reduction of 25.9% ± 2 (SE) in NPP.

Fig. 1 shows the bivariate plots for all the variables measured in the present study. Max NPP varied between 0.13 and 1.08 μmol O$_2$ s^{-1} (mean ± SE = 0.52 ± 0.03 μmol O$_2$ s^{-1}), or from 0.016 to 0.21 μmol O$_2$ g DW^{-1} s^{-1} (mean = 0.067 ± 0.006 μmol O$_2$ g DW^{-1} s^{-1}) when corrected by biomass. In terms of productivity per boulder surface area, max NPP ranged from 0.13 to

0.84 µmol O_2 s^{-1} 0.04 m^{-2} (mean = 0.45 ± 0.03 µmol O_2 s^{-1} 0.04 m^{-2}). Values of α varied from 0.00061 to 0.0043 µmol O_2 µE^{-1} m^{-2} (mean = 0.0022 ± 0.0001 µmol O_2 µE^{-1} m^{-2}). Max NPP and α were highly correlated (r = 0.94, p < 0.001). Assemblages' compensation irradiance averaged 17.8 ± 1 µE m^{-2} s^{-1} with a minimum value of 8.2 µE m^{-2} s^{-1} and a maximum of 34.2 µE m^{-2} s^{-1}. Assemblage respiration varied from –0.12 to –0.01 µmol O_2 s^{-1} (mean = –0.045 ± 0.004). Light capture, estimated as the irradiance percentage below algal fronds, varied from 20.0 to 91.1% of the above canopy incident irradiance (mean = 51.7 ± 2.3%). The C-E index for the most abundant species varied from 0 (i.e. most abundant species in a unique clump) to 1.6 (mean = 0.95 ± 0.07). Additionally, when the relationship between the C-E index of the first and the C-E index of the second most abundant, and the second and third most abundant species were examined, we found negative correlations (r = –0.56 and –0.43, respectively, p < 0.05), suggesting that the more spread out is the dominant species of the assemblage the more clumped are the companion species.

Biomass and species richness were positively correlated with all structural variables. Spatial aggregation was negatively correlated with light capture and α. Evenness correlations were not significant for any structural variable (Fig. 1). When all the predictors were combined in multiple regression models, they explained a noticeable proportion of the total variation
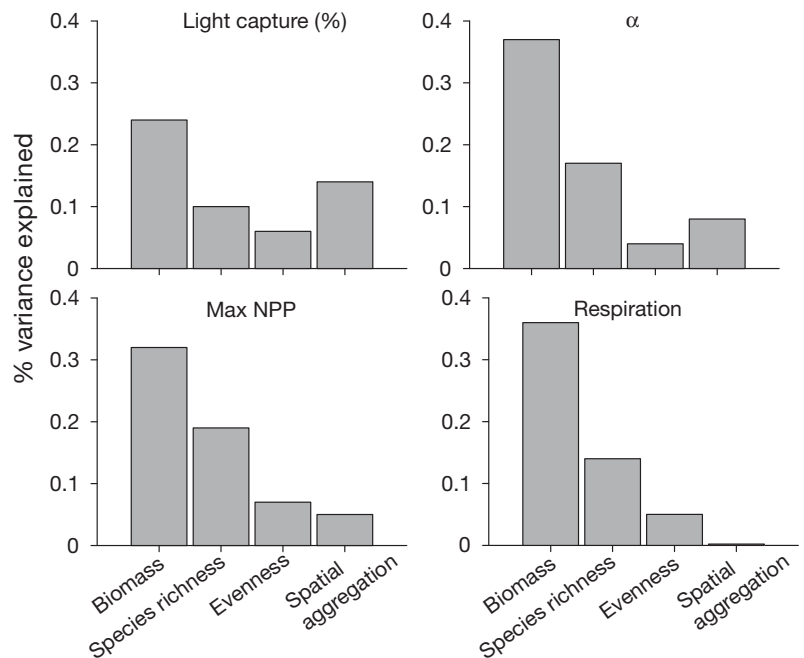

Fig. 2. Contribution to the explained variance of each predictor (biomass, species richness, evenness and spatial aggregation) for all the ecosystem functioning surrogates measured: % light capture, photosynthetic efficiency at low intensity (α), maximum net primary productivity (max NPP) and assemblage respiration

of the functional surrogates (between 54 and 66%). Table 1 shows the OLS multiple regression models and their corresponding variance partitions, I (i.e. effect sizes) and I:J ratios, using the hierarchical partitioning procedure. Biomass, with significant positive regression coefficients for all the responses examined, was the best single predictor as the hierarchical procedure

Table 1. Multiple regression models for the 4 functional measurements analyzed: % light capture, photosynthetic efficiency at low intensity (α), maximum net primary productivity (max NPP) and assemblage respiration. Ordinary least squares (OLS) model columns include coefficients for each predictor and significance levels in the model. Variance partitions (VP) include: (1) the predictor's effect size as the independent contribution to the total variance (I) and significance levels for each predictor (see 'Materials and methods' for the significance determination procedure); and (2) the independent:joint effects ratio (I:J) as an indication of possible interaction among predictors (values close to 1 indicate strong interactions between the predictor and other predictors). VP = variance partition; $^{(\cdot)}$p < 0.1; *p < 0.05; **p < 0.01; ***p < 0.001; ns: not significant

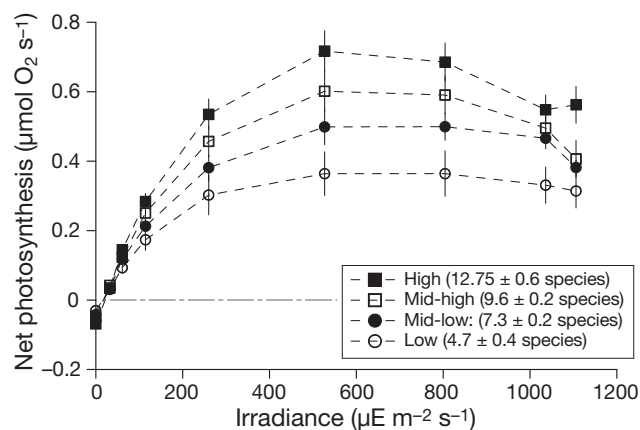| Predictors	Light capture (%)			α (log)			Max NPP (log)			Respiration (log)		
	OLS model	I	I:J	OLS model	I	I:J	OLS model	I	I:J	OLS model	I	I:J
Intercept	33.9***			–3.02***			–0.76***			–1.92***		
Biomass	1.20***	0.24***	94	0.02***	0.37***	8.5	0.02***	0.32***	12.1	0.02***	0.36***	8.6
Species richness	0.57^{ns}	0.11**	1.1	0.01$^{(\cdot)}$	0.17***	1.4	0.02$^{(\cdot)}$	0.19***	1.4	0.01^{ns}	0.14***	1.2
Evenness	21.3*	0.06$^{(\cdot)}$	4.7	0.21^{ns}	0.04^{ns}	1.2	0.32*	0.07*	2.6	0.34*	0.05^{ns}	1.4
Spatial aggregation	–10.7**	0.14***	9.7	–0.11*	0.08*	67	–0.08^{ns}	0.05^{ns}	4.4	0.0^{ns}	0.002^{ns}	0.9
	R^2 = 0.54, $F_{4, 35}$ = 10.4, p < 0.001			R^2 = 0.66, $F_{4, 35}$ = 17.4, p < 0.001			R^2 = 0.63, $F_{4, 35}$ = 15.5, p < 0.001			R^2 = 0.55, $F_{4, 36}$ = 11, p < 0.001		

Fig. 3. Photosynthesis-irradiance curves for the 4 levels of species richness included in the ANCOVA analysis (see Table 2). Mean values (±SE) of 8 assemblages

revealed. Independent contributions of biomass varied from 24% of the total variance for light capture to 37% for α (Fig. 2). Meanwhile, species richness was not a significant predictor in any of the OLS regression models, although it approached significance for α and max NPP. Nevertheless, hierarchical partitioning identified species richness as the second best overall predictor, with significant independent contributions ranging from 11% of the variance for the light capture model to 19% for max NPP (Fig. 2).

Fig. 3 shows the P-I curves for the species richness groups included in the ANCOVA analysis. Despite the observed relationship between species richness and biomass in the assemblages, the positive effects of richness in the performance of the assemblages were not a mere consequence of changes in biomass (Table 2). Assemblages with higher richness showed higher % light capture, α and max NPP. Only in the

Table 2. ANCOVA testing the effect of species richness in the performance of assemblages after removing the effect of biomass. α: photosynthetic efficiancy at low intensity; max NPP: maximum net primary productivity; ns: not significant; *p < 0.05; **p < 0.01; ***p < 0.001

Source	df	MS	F
Light capture (%)[a]	3	563.1	3.84*
Residual	25	146.7	
α (log)[a]	3	0.12	6.36**
Residual	25	0.02	
Max NPP (log)[a]	3	0.15	7.56***
Residual	25	0.02	
Respiration (log)	3	0.06	2.07[ns]
Residual	25	0.03	
[a]Tukey's tests: low < mid-low = mid-high = high			

case of respiration did species richness have no significant effect in the ANCOVA. *A posteriori* Tukey's tests did not find differences among the 3 groups with highest richness, but the assemblages with lowest diversity levels showed a lower productivity performance, suggesting that positive effects of species richness may be restricted to relatively low diversity ranges in these communities.

Spatial aggregation for the most abundant macroalgal species showed a significant negative coefficient for light capture and α, with percentages of explained variance of 14 and 8%, respectively. Finally, OLS models identified evenness as a significant predictor with positive effects for all the responses except α. Its independent contribution was significant for max NPP (7% of variance explained) and close to significant for % light capture (Table 1).

DISCUSSION

Boulder assemblages used in the present study share some properties with natural microcosms as defined by Srivastava et al. (2004). They consist of small habitats with defined boundaries and, because they are naturally assembled and represent realistic species combinations, they provide an opportunity to test whether the processes described in experimental studies may occur in natural systems. Additionally, the use of natural assemblages does not preclude controlled laboratory measurements since, in this case, boulders were easily transported to the laboratory.

Some interesting patterns emerged from these very controlled measurements. Unlike other studied macroalgal communities (Binzer & Sand-Jensen 2002), P-I curves exhibited light saturation, and photo-inhibition was detected in more than 75% of the assemblages examined. Also, the minimum light required to attain positive net production in our assemblages averaged 17.8 $\mu E\ m^{-2}\ s^{-1}$, well below mean compensation points recorded in other natural macroalgal assemblages of 86 $\mu E\ m^{-2}\ s^{-1}$ (Middelboe et al. 2006). These differences probably reflect the low intertidal origin of the boulders and the season of collection, winter.

The present study aimed to explore how different components of diversity might shape the productivity of macroalgal assemblages in natural communities. Despite the impossibility of establishing causation in observational studies, results showed that, beyond species richness, other diversity-related attributes may also shape the relationship between diversity and ecosystem functioning.

Biomass was the best overall predictor for macroalgal assemblage productivity. In most short-term productivity experimental studies, biomass is decided

a priori by the researcher and fixed across diversity treatments. Besides the effects of factors like herbivory and disturbance, in natural communities standing biomass is mostly a cumulative consequence of the productivity of earlier standing biomass. Simultaneously, primary productivity of a community depends on its current standing biomass and the productivity to biomass ratio (Middelboe & Binzer 2004). Therefore, standing biomass and productivity are usually correlated. We used analytical tools such as hierarchical partitioning to separate the potential influence of biomass from the other structural predictors.

Results from the regression models and variance partitioning procedures indicated an overall positive effect of species richness on the performance of natural macroalgal assemblages. Effects independent of species richness explained around 18 % of the total variance for α and 20 % of the total variance of max NPP. Positive relationships between species richness and biomass in natural macroalgal communities were found in previous studies and interpreted as support for the positive effects of richness on productivity (Bruno et al. 2005, 2006). These positive relationships seem to arise from better resource use and, subsequently, higher photosynthetic performance of more diverse communities, allowing them to maintain higher biomass per unit area (Middelboe & Binzer 2004). ANCOVA results supported the positive relationship between richness and most of the productivity surrogates, although the effects of richness were only significant at low levels. Middelboe et al. (2006) found positive relationships between species richness and light capture and α for naturally established communities, but no relationship with max NPP. Positive species richness–ecosystem functioning relationships have also been found in other natural soft-sediment benthic systems (Emmerson & Huxham 2002).

Our findings are also consistent with experimental studies using synthetic algal assemblages. These recent experiments have found positive effects of species richness, irrespective of the response measured: photosynthetic rates (Middelboe & Binzer 2004, Bruno et al. 2005, 2006), biomass accumulation (Bruno et al. 2005, 2006) or nutrient uptake (Bracken & Nielsen 2004, Bracken & Stachowicz 2006, Bracken et al. 2008). Whenever the experimental design allowed disentangling the mechanisms driving this positive relationship, species identity effects were identified as the dominant effect (Bruno et al. 2006).

Despite the fact that the index used to characterize the spatial pattern in the assemblages was based only on the distribution of the one most abundant species, linear models were able to detect significant relationships between the C-E index, % light capture and α. Thus, the spatial aggregation of the dominant species

greatly influenced other species' arrangement in the boulders, which would explain the significant results reported using indexes for a single species.

Not surprisingly, the lowest independent contribution to the C-E index was found for respiration, a light autonomous process. Since a high C-E index for the most abundant species implied more clumped companion species, the negative relationships detected for the C-E index suggest a reduction in both capacity to capture resources and performance under light-limited conditions with increases in spatial aggregation of the community. In intertidal systems, patchy macroalgal assemblages are maintained by different processes such as physical disturbance, successional transitions, predation, spatial heterogeneity, recruitment limitation and differential life histories (Chapman 1990, Benedetti Cecchi & Cinelli 1996, vanTamelen 1996, Menge et al. 2005). Our findings suggest that these factors, mostly considered as diversity-promoting factors, may reduce complementarity or facilitation among species, partially decoupling the relationship between diversity and ecosystem function.

Direct relationships between spatial pattern and ecosystem functioning have been proposed in terrestrial systems, in models (Bolker et al. 2003, De Boeck et al. 2006) as well as in observational studies (Maestre et al. 2005). In sessile organisms such as plants, patterns of intraspecific aggregation change the frequency and intensity of inter- vs. intraspecific interactions (Stoll & Prati 2001), modifying the possibilities of a complementary use of resources between species. The negative relationship between spatial aggregation and complementary resource use has been partially supported by experiments in terrestrial plant communities, although idiosyncratic species-specific effects have sometimes counteracted the effects of aggregation (Mokany et al. 2008). Spatial aggregation could also be acting in experiments as an unexpected effect since, in the substitutive designs usually used, low diversity assemblages would naturally have a higher spatial aggregation than high diversity treatments.

Similar arguments should apply to the positive relationship between evenness and max NPP. Assemblages not dominated by a single, very abundant species would increment their opportunities for complementary resources use among species and, therefore, would exhibit higher productivity rates. Positive effects of evenness on productivity have been recorded in several experimental studies on terrestrial systems (Wilsey & Potvin 2000, Stevens & Carson 2001, Kirwan et al. 2007), although dominance by large species may modify this pattern (Mulder et al. 2004).

In conclusion, the results of the present study give the first evidence of the functional consequences of spatial aggregation and species' relative abundance in

macroalgal assemblages. However, because of the limitations of observational approaches—it is essentially impossible to establish causal relationships and identify underlying mechanisms (Troumbis & Memtsas 2000)—further experiments are needed to fully understand how these structural components of diversity interact to shape the ecosystem function of macroalgal communities.

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