Spatial heterogeneity increases the importance of species richness for an ecosystem process

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The role of biodiversity in mediating ecosystem processes has been the subject of focused theoretical and empirical attention since the mid-1990s. Theory predicts that the balance between species richness and identity effects will critically depend on the degree of environmental heterogeneity, which dictates the extent to which differences between species in patterns of resource use can be expressed. We conducted a mesocosm experiment to explicitly test this hypothesis. We manipulated the richness and identity of intertidal molluscan grazers, as well as the spatial heterogeneity of the substrate upon which they grazed. The magnitude of algal consumption was used as our focal ecosystem process. The grazer treatments consisted of three monocultures and a single polyculture including all three species; heterogeneity was represented as the proportion of topographically complex and flat substrate. Species identity had strong effects on homogeneous substrates, with the identity of the best-performing species dependent on the substrate. On the heterogeneous substrate, suitable conditions for all three species were represented, allowing the expression of spatial complementarity of resource use and the enhancement of total algal consumption. Our findings provide the first explicit experimental evidence that spatial heterogeneity of the physical environment can play a key role in mediating effects of species diversity.

The effect of biodiversity on ecosystem functioning, and by extension ecosystem goods and services, has emerged as a key research priority (Loreau et al. 2001). The role of species richness has been a particular focus of attention. Synthesis of the resultant large body of empirical studies has uncovered a generally positive, but saturating, relationship between species richness and the magnitude of various ecosystem functions (Cardinale et al. 2006, see also Hooper et al. 2005, Balvanera et al. 2006). Importantly however, within these experiments the presence of particular species typically has an effect comparable to – or even greater than – that of species richness per se (Cardinale et al. 2006). Understanding the factors that dictate the relative strength of particular species, species richness and trait diversity is the key to developing an ability to predict the effects of species extinction (or gain) on ecosystem functioning in any given system or context (Finke and Snyder 2008).

Interspecific niche partitioning, or complementarity, is considered one of the principal mechanisms underpinning the effect of species richness on ecosystem processes (Loreau 1998, Duffy 2002). Niche complementarity can occur through the exploitation of different resources (Bracken and Stachowicz 2006, Kahmen et al. 2006, Finke and Snyder 2008), as well as spatial (Albrecht and Gotelli 2001) or temporal (Yachi and Loreau 1999) differences in the use of the same resource. Increasing species richness results in a more complete use of the available spectrum of resources when niche complementarity is evident (Tilman et al. 1997, Loreau 2000), potentially enhancing the aggregate rate of resource uptake (Trenbath 1974, Yachi and Loreau 1999).

Crucially, the expression of interspecific complementarity may depend on the occurrence of a heterogeneous environment or resource space (Cardinale et al. 2004). Under homogeneous conditions, a single species, best-suited to the specific environment or resource, may dominate the acquisition of resources (Straub and Snyder 2006, Råberg and Kautsky 2007). Intuitively, the identity of the dominant species can switch depending on the conditions (Yachi and Loreau 1999, Cardinale et al. 2004, Gamfeldt et al. 2005). Hence the simultaneous consideration of multiple conditions may render multiple species important for the aggregate use of resources (Råberg and Kautsky 2007). The degree of heterogeneity may thus dictate the relative strength of species identity and niche complementarity effects (Tilman and Lehman 2002, Cardinale et al. 2004). It is a common observation that environments are patchy with consequences for both species composition and productivity (Kane and Poulson 1976,
McQuaid and Dower 1990, Downes et al. 1998), and environmental heterogeneity in space and time are thought to be essential ingredients in the mediation of long-term species coexistence (Hutchinson 1961, Chesson 1991). Spatial heterogeneity has only recently, however, begun to be explicitly and experimentally considered in the biodiversity–ecosystem functioning framework (Dyson et al. 2007, Bulling et al. 2008, Weis et al. 2008, Ericson et al. 2009).

Molluscan grazers are important consumers of primary producers in both freshwater (Steinman 1996) and marine (Hillebrand 2002) environments and have the potential to control both the productivity and standing stock of algae (Hawkins and Hartnoll 1983). Many grazing molluscs are microphagous, specializing in the consumption of the microalgal film (often including new macroalgal recruits) which coats any hard surface submerged in water. The distributions of such grazers are often strongly influenced by substrate micro-topography (Downes et al. 1998) since they must graze over the very surfaces they inhabit. On rocky shores, substrate micro-topography is often highly variable over small spatial scales as a result of the interaction between rock type, the erosive power of wave action and sessile biota. In a mesocosm study, we tested the hypothesis that increasing environmental heterogeneity increases the importance of grazer richness for the rate of algal consumption. Environmental heterogeneity was represented as the relative proportion of flat and topographically complex substrates. By virtue of differences in body size and shape, as well as foraging strategy, we predicted that three species of molluscan grazers would vary in their foraging efficiency depending on the type of substrate, generating strong species identity effects on homogeneous substrates. We further hypothesized that an effect of richness would emerge under heterogeneous conditions, when niche complementarity among multiple species could be expressed.

Material and methods

Experimental design

We manipulated the richness and identity of three molluscan grazers (the limpet Patella vulgata, the periwinkle Littorina saxatilis and the topshell Gibbula umbilicalis; hereafter referred to by their genus names only), as well as the form and heterogeneity of the physical substrate on which they grazed. The three species used here are all widespread, abundant and coexisting inhabitants of rocky shores in the southwest of the United Kingdom. The grazer treatments consisted of three separate single species treatments (all possible monocultures), and a mixture of all three species (the polyculture). The physical substrate consisted of three treatments: flat, rough and heterogeneous. We employed a fully-factorial experimental design (Fig. 1), allowing us to examine both the independent and interactive effects of the following two orthogonal factors on the aggregate rate of algal consumption: 1) species composition (four levels: Gibbula, Patella, Littorina, Polyculture) and 2) substrate (three levels: flat, rough, heterogeneous). We also included grazer-free controls for all substrate types, which were sampled both before (following the five-week colonization period) and after the grazing trial. These controls allowed comparisons among substrates in terms of the total biomass and composition of the algal assemblages, in addition to the identification of possible changes in algal biomass through the trial. All treatments were replicated five times. Including controls, the total number of experimental units totaled 90.

Under the standard substitutive approach, the biomass (or number) of organisms is typically equalized across treatments varying in species identity and richness. We modified this design because the study species do not occur at even densities or biomass on the rocky shore (see O’Connor and Crowe 2005 for a similar approach). Species-specific densities are highly variable across the rocky shore habitat mosaic. We therefore selected species densities that were representative of those found on small-scales within favourable habitat patches on the rocky shore; species-specific densities used in both monocultures (Patella = 150 ind. m$^{-2}$, Gibbula =750 ind. m$^{-2}$, Littorina =1000 ind. m$^{-2}$) and polycultures (Patella = 50 ind. m$^{-2}$, Gibbula =250 ind. m$^{-2}$, Littorina = 350 ind. m$^{-2}$) were within the range found within such patches. Consistent with a standard substitutive design, the density of each species in the polyculture was equal to its density in monoculture divided by the number of species in the polyculture (i.e. three in this case). This resulted in a total polyculture density equal to the mean monoculture (Table 1), which is also consistent with a standard substitutive approach and necessary to unambiguously test our hypotheses.

Experimental set-up

We conducted the experiment during May 2008 within the semi-recirculated seawater flow-through facility at the Marine Biological Association Laboratory in Plymouth, UK. The 90 experimental substrates were housed within five adjacent tanks (5625 cm$^2$ area $\times$ 10 cm depth). The substrates were exposed to a natural light–dark regime and sunlight through the glass roof of the seawater facility. Water temperature in the tanks (mean $\pm$ 1SD, 17.07 $\pm$ 0.80°C) was similar to that found within local rock pools at the same time of year (Martins et al. 2007).
Table 1. The numbers of grazers in each treatment (see Methods for rationale). Means are to the nearest whole number (individual grazer).

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<th>Monocultures</th>
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<td>Patella</td>
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<td>Rough</td>
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We constructed the substrates from untreated and unpolished limestone flooring tiles. We created the ‘rough’ substrate by fixing small cubes (1.5 cm$^2$) of two lengths (1.5 and 3 cm) arranged vertically and interspersed with each other, to the flat substrate. Small gaps (∼0.2 cm) were left between the cubes, creating an environment with numerous crevices and only small areas of flat rock surface. The experimental substrates measured 10 × 20 cm in total (Fig. 1).

The heterogeneous substrates consisted of one rough and one flat half, which were initially separated and then glued together prior to the experiment. This allowed us to separate the halves easily at the end of the experiment and therefore to differentiate between grazing rates on the flat and rough halves of the heterogeneous substrates. For consistency all substrate treatments consisted of two halves glued together. Each rough half contained an approximately three times greater surface area than each flat half. Therefore, the rough substrate contained approximately a three-fold greater surface area than the flat; the heterogeneous substrate two-fold greater (the sum of the surface area of the flat and the rough halves). We accounted for this by elevating the total grazer abundance by corresponding amounts, such that for each grazer treatment, grazer abundance per unit area was approximately equilibrated across substrate treatments (Table 1).

Prior to the addition of grazers, the initially bare substrates remained exposed to flowing seawater (and algal propagules within it) for five weeks to allow colonization and growth of epilithic algae. We initially haphazardly divided the substrates among the five separate tanks and subsequently rearranged them within and among tanks every two days throughout the experiment to average out possible tank or positional effects among treatments and replicates.

Grazers were collected by hand from local rocky shores during tidal emersion over a 3-day period and stored within tanks subjected to flowing water prior to the beginning of the experiment on day 4. Individuals of all species were collected on each day and assigned to treatments haphazardly, ensuring no systematic differences between species or treatments in acclimation time. During collection Patella were carefully removed from the rock and immediately transferred to a Perspex sheet to allow re-attachment and to limit physical stress. Gibbula and Littorina are more robust, and were thus simply placed in buckets. Owing to the relatively small size of the mesocosms, we opted to reduce the mean intraspecific body size of organisms relative to the natural mean size on local shores, whilst maintaining a range of body sizes within each grazer species. Specifically, the size ranges were (maximum shell length): Patella = 19–43 mm; Gibbula = 3–7 mm; Littorina = 3–9 mm). Grazers were added to the substrates at random spatially, ensuring that grazers were interspersed over the available substrate at the beginning of the experiment. The experiment ran for six days following addition of grazers.

Measurement and calculation of response variables

As the area-specific biomass of algae differed among the substrate types at the start of the experiment, we had to control for this difference to calculate the absolute magnitude of algal consumption. We simply subtracted the final standing algal biomass within each replicate from the mean biomass recorded in controls of the same substrate type (Supplementary material Appendix 1). This was a valid approach since the variation among control replicates was very low. To generate a comparable index of resource consumption across the disparate experimental substrates, we calculated the relative grazing rate (D) by dividing the difference between algal standing stocks of control (C) and grazer (G) treatments, with the control algal standing stock:

\[ D = \frac{(C - G)}{C} \]

On day three, after the grazers had been given time to move to preferred substrates, we recorded their position (in terms of substrate type) to allow identification of species-specific preferences.

We measured the biomass of algae within each replicate substrate thorough an established technique involving extraction of chlorophyll a in methanol (see Thompson et al. 1999 for a detailed description). We placed each 10 cm$^2$ substrate sector (i.e. half the total substrate) of the experimental substrate in 200 ml of methanol (99.8%) for 15 h to extract chlorophyll a. We then centrifuged 3 ml samples of the resultant solution to remove any particulates, before measuring light absorbance at wavelengths of both 665 and 750 nm in a spectrophotometer. Total biomass per unit of substrate area was then calculated from absorbance levels using known relationships (Thompson et al. 1999). Proportional covers of algal types were estimated visually (Supplementary material Appendix 2). The algal assemblage consisted of a mixture of immature macroalgal (Ecotocarpus sp. Cladophora sp. and Ulva sp.) and microalgal (diatoms and cyanobacteria) forms.

Analysis

Consistent with the experimental design, we used two-way analysis of variance (ANOVA) with grazer species composition and substrate as fixed, orthogonal factors. The relative consumption of algae constituted our focal ecosystem-level response variable, because it provided a comparable measure
across substrate types. We additionally performed the analysis on the absolute rate of algal consumption as a precaution to ensure that key conclusions based on the relative response were robust. Significant treatment effects were elucidated through post-hoc Tukey HSD tests. We validated ANOVAs by checking for heterogeneous variances through both the visual inspection of plots of residuals and Levene’s test. Univariate analyses were performed in SPSS 15.0 (SPSS Inc). Treatment effects on algal diversity (H’), evenness (J’) and composition/relative abundances were tested using PRIMER-6 (PRIMER-E Ltd. Plymouth, UK) (see Supplementary material Appendix 2 for methods).

Non-transgressive overyielding occurs when the magnitude of the ecosystem process in a diverse mixture exceeds that of the average value of component species. We explicitly tested for this form of diversity effect by comparing the mean monoculture with the polyculture using separate planned comparisons for each substrate type (using Supplementary ANOVA). Non-transgressive overyielding can result from the dominance of highly productive species in long-term studies (sensu Petchey 2003). Where biomass remains constant in short-term experiments such as the one presented here, the elevation of the focal ecosystem process above that expected from the monoculture performance of component species measures the net effect of species complementarity (Petchey 2003). Transgressive overyielding occurs when the performance of the polyculture exceeds that of even the best-performing component species in monoculture. We similarly tested for the presence of transgressive overyielding by comparing the best-performing monoculture with the polyculture. Transgressive overyielding is an ‘acid test’ of species complementarity in long-term studies (Loreau 1998). In our short-term study, we tested for this effect to gauge whether species richness effects exceeded that of species identity.

Results

Grazer-free controls: substrate effects

Grazer-free controls showed that substrate treatment affected the accumulated area-specific biomass of algae (Supplementary material Appendix 1; flat > heterogeneous > rough; F2,24 = 32.995, p < 0.001). These controls also indicated that area-specific algal biomass did not change during the six-day experiment in the absence of grazers (Supplementary material Appendix 1; F1,24 = 0.851, p = 0.366). There were also no overall or temporal differences in the composition/relative abundance of algal types (Supplementary material Appendix 1), nor their diversity or evenness (Supplementary material Appendix 1) according to substrate treatment.

Treatment effects on rates of algal consumption

There was no evidence of species richness effects on the focal ecosystem process (mean relative grazing rate [hereafter simply ‘grazing rate(s)’]) on either of the homogeneous substrates; the polyculture did not exceed the respective mean monocultures (planned comparisons; flat: F1,48 = 0.558, p = 0.459; rough: F1,48 = 3.167, p = 0.082). Furthermore, the possibility of transgressive overyielding was ruled out by the fact that the monoculture with the highest grazing rate exceeded the polyculture on both of the homogeneous substrates (Patella on the flat, Gibbula on the rough; Fig. 2). On the heterogeneous substrate the relative rate of grazing was maximized by the polyculture. In this case, the polyculture exceeded that of the mean monoculture (planned comparison; F1,148 = 9.710, p = 0.003) but was not significantly greater than the single species with the highest grazing rate (F1,8 = 2.591, p = 0.146). Absolute rates of algal consumption displayed comparable patterns within substrate types, with non-transgressive overyielding evident only under heterogeneous conditions (Supplementary material Appendix 3).

The effect of grazer species composition on grazing rates was dependent on the substrate type (substrate × grazer interaction, Table 2, Fig. 2). On the rough substrate all treatments differed, with the Gibbula monoculture exhibiting the highest grazing rate, Patella the lowest (Fig. 2; Tukey HSD tests: Gibbula > polyculture > Littorina > Patella). On the flat substrate the rate of grazing exhibited by Patella exceeded that of Littorina, while other treatments were indistinguishable (Tukey HSD tests; Fig. 2). On the
heterogeneous substrate, both *Gibbula* and the polyculture treatments exceeded *Littorina* (Tukey HSD tests; Fig. 2). Separation of the rates of grazing on the flat and rough halves of the heterogeneous substrates revealed species complementarity. *Patella* exhibited a far higher rate of grazing on the flat sector compared to the rough, whereas the opposite applied to *Gibbula*, and to a lesser extent *Littorina*, both of which grazed more effectively on the rough sector (Fig. 3). In the polyculture these interspecific differences were combined to produce a similar rate of algal consumption on both the flat and the rough halves (Fig. 3).

**Patterns of space-use**

Examination of species-specific use of space in both single and multiple species treatments also provided evidence of interspecific complementarity. The three grazers differed markedly in substrate use (Fig. 4). *Patella* occurred almost invariably on the flat substrate when it was available (Fig. 4: 100% on flat [mean ± SE proportion of individuals]; 96.7 ± 3.3% on heterogeneous) but occurred on the sides of the mesocosms where it was unavailable (rough 93.3 ± 6.6%). *Gibbula* was common on both substrates within respective homogeneous treatments (Fig. 4: on flat 87.8 ± 1.9%; on rough 99.5 ± 0.5%), but occurred predominantly on the rough substrate under heterogeneous conditions (Fig. 4: 6.7 ± 1.0% on flat; 89.1 ± 1.0% on rough). A larger proportion of *Littorina* were found attached to the mesocosm sides in the flat (76.4 ± 3.4%) compared to the rough (32.1 ± 3.3%), homogeneous treatment. This apparent preference for rough substrate also occurred under heterogeneous conditions (2.9 ± 1.4% on flat; 39.4 ± 4.8% on rough). Species-specific patterns of substrate use were generally very similar in both monoculture and polyculture within substrate types (Fig. 4). The one exception to this pattern was the reduced proportion of *Littorina* grazing on the rough homogeneous substrate in the polyculture, where it increased in abundance on the mesocosm sides (Fig. 4: 32.1 ± 3.3% in monoculture; 72.7 ± 6.8% in polyculture).

**Treatment effects on algal composition and diversity**

Treatment effects on the taxonomic composition/relative abundances of algae were generally weak. Notably, *Gibbula* grazing left a higher proportion of brown foliose than other treatments under both rough and heterogeneous conditions (Supplementary material Appendix 3, Table 2). Effects on algal diversity and evenness were also relatively weak and variable. Under rough conditions, *Gibbula* reduced algal diversity relative to *Littorina* grazing, whilst under heterogeneous conditions *Patella* and the polyculture reduced algal evenness relative to *Gibbula* and *Littorina* (Supplementary material Appendix 4).

**Discussion**

The results from our model system demonstrate that the spatial heterogeneity of environmental conditions can increase the importance of species richness for the rate of an aggregate ecosystem process. Observations of species-specific patterns of space use and rates of resource consumption support the hypothesis that this effect is primarily mediated through interspecific spatial complementarity.

Previous studies have shown that spatial heterogeneity of resources alone cannot produce effects of biodiversity (Weis et al. 2008, Ericson et al. 2009), emphasizing that heterogeneity must be combined with differences in the relative performance of species across patches. In our experiment, such interspecific differences were evident: particular species maximized grazing rates within homogeneous environments, with the identity of the species with the highest grazing rate dependent on the substrate. Heterogeneous conditions allowed the expression of species complementarity, enhancing the ecosystem process above that expected from a combination of monoculture rates of component species (i.e. non-transgressive overyielding). In effect, niche complementarity can be viewed as a combination of species identity effects across heterogeneous patches (Cardinale et al. 2004).
The context-dependent effects of grazer richness and identity were likely underpinned by differences in functional morphology among the species, which determined spatial complementarity. The effective rasping foraging strategy of *Patella* (Hawkins and Hartnoll 1983), combined with the fact that it has the largest mean body size and standing stock of the three species, renders it a key grazer on northern European rocky shores (Moore et al. 2007, Jenkins et al. 2008). The relatively large foot area and conical shell of *Patella* largely constrained its movements, and thus grazing impact, to areas of flat rock in this experiment. Congruently, this species is most abundant on relatively flat surfaces on local rocky shores (Griffin 2008). With smaller bodies and more maneuverable, slender shells, both *Littorina* and *Gibbula* were able to access algae within the rough substrates; this behaviour is likely adaptive, providing protection from predators and wave-dislodgment under field conditions. Although the mean grazing impact of *Littorina* was relatively low, this species likely increased the spectrum of resources exploited by accessing the algae within crevices that were too small for *Gibbula*. Thus, all three species may have contributed to the observed non-transgressive overyielding within the heterogeneous treatment. Additionally, dietary complementarity within habitat types may have played a subtle role in our experiment. Notably, we found evidence that *Gibbula*, but not the other two species, avoided consuming brown foliose algae (Supplementary material Appendix 2). The presence of all three species may thus have increased the spectrum of algal types consumed.

Despite the evidence of complementarity under heterogeneous conditions, the rate of algal consumption did not significantly exceed the best-performing single species, i.e. transgressive overyielding was not detected. This is a common finding in diversity–function studies (Cardinale et al. 2006), and shows that even under heterogeneous conditions individual species can exert strong influences on functions by efficiently exploiting suitable sectors of resource. There was considerable variability among replicates in this study, probably due to inter-individual differences in grazing performance, and the results suggest that given higher replication, transgressive overyielding may have been detected; statistical power is an important consideration in diversity–function studies (Osler 2002).

Our experiment rigorously tested a key theoretical prediction using the grazing of rocky shore organisms as a model ecosystem process. While model systems such as this can provide a powerful tool to test theory and gain mechanistic insights (Benton et al. 2007), the results need to be interpreted with a caveat in mind: there was an inevitable tradeoff between experimental control and realism. Thus absolute rates of processes and effect sizes should not be directly extrapolated to natural rocky shores. Larger scale and more realistic field experiments are required to demonstrate the importance of the effects reported here under natural conditions.

Several recent experiments have demonstrated that increasing the range of resource types can enhance the effect of species richness on ecosystem processes (Gamfeldt et al. 2005, Bracken and Stachowicz 2006, Räberg and

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**Figure 4.** The proportion of individuals of each grazer species on different substrate types (flat and rough) within each substrate treatment (flat, rough and heterogeneous). Left panels show grazer responses in monocultures, right panels show responses in the polyculture.
Kautsky 2007). Our results add to these studies by showing clearly the importance of spatial heterogeneity of the physical environment; the physical environment mediated the ability of consumers to access the same type of resource distributed across a heterogeneous landscape. It is important to distinguish this form of heterogeneity from the spatial evenness of resource concentration, which may in fact promote dominance of individual species (Maestre and Reynolds 2007).

The hypothesis that species richness will have a greater effect on ecosystem functioning where the environment is heterogeneous is supported by theory (Cardinale et al. 2004) and a recent analysis of observational data (Tylianakis et al. 2008). Our factorial experiment provides explicit empirical support for this hypothesis, and underlines the importance of niche complementarity in mediating this effect. Environmental heterogeneity in space and time, combined with interspecific niche differentiation are thought to be essential ingredients in the mediation of long-term species coexistence (Hutchinson 1961, Chesson 1991). Just as this combination of factors maintains diversity, it renders it important for ecosystem functioning: diverse niches require diverse species to fill them in order to maximize consumption and other ecosystem processes. Experiments performed on small spatial scales, using mesocosms or experimental plots, form a substantial contribution to our current understanding of the functional consequences of biodiversity. If such experiments underestimate the environmental and resource heterogeneity that typically characterizes the natural ecosystems they aim to simulate, they run the danger of underestimating the value of species richness for ecosystem functioning (Ives et al. 2005, Stachowicz et al. 2008a, 2008b). Our study suggests that heterogeneity must be carefully considered before extrapolating experimental findings to broader, landscape contexts.

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