Effects of changes in number, identity and abundance of habitat-forming species on assemblages of rocky seashores

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ABSTRACT: Understanding the relationship between variation in biodiversity and the alteration of ecosystem processes and stability has become a central ecological issue during the last decade. A large number of experimental and theoretical studies have focused on the effects of changes in species richness and identity, while the role of variation in species abundance has received less attention in biodiversity experiments. By using an experimental design that effectively separates the effects of species richness and identity, while controlling for variation in species abundance, we examined the consequences of loss of 2 habitat-forming species (the canopy alga *Cystoseira compressa* and the mussel *Mytilus galloprovincialis*) in Mediterranean low-shore assemblages of algae and invertebrates. Results revealed significant effects associated with changes in number and identity of habitat-forming species on other organisms. The magnitude and direction of these effects, however, changed as a function of the abundance of manipulated species. Our findings indicate how changes in biodiversity of even 2 species can result in complex effects, stressing the importance of investigating non-random loss of habitat-forming species and the need to consider density-dependent effects in biodiversity experiments, particularly when experiments are undertaken in systems like rocky shores, where density-dependent effects are pervasive.

KEY WORDS: Biodiversity · Habitat-forming species · Experimental design · Density-dependence · Rocky shore · Canopy algae · Mussels

INTRODUCTION

Understanding the consequences of alteration in biodiversity for ecosystem processes and society's use of natural resources (Vitousek 1994, Costanza et al. 1997, Chapin et al. 1998), has become one of the main issues in ecology (Hooper et al. 2005). In the last 2 decades, a large number of studies have focused on the importance of changes in number and identity of species in terrestrial habitats (Hooper & Vitousek 1997, Tilman 1997, Loreau & Hector 2001), with the ultimate goal of discriminating between complementarity and sampling effect models (Huston 1997, Loreau et al. 2001). More recently, however, experiments conducted in the marine realm have contributed to this debate, as documented in the review by Stachowicz et al. (2007).

Most studies that stressed a negative effect of loss of diversity on ecosystem processes were conducted in mesocosms or through synthetically assembled experiments, based on the assumption of random loss of species (e.g. Aarssen 1997, Huston 1997, Emmerson et al. 2001, Schmid et al. 2002). A few studies have focused on the selective removal of key species in the field, to simulate non-random changes in patterns of distribution and abundance of organisms (Schläpfer & Schmid 1999, Loreau 2000, Dìaz & Cabido 2001, Dìaz et al. 2003). These studies have emphasized the importance of focusing on species that have a clear functional role on the basis of their contribution to ecosystem processes (Schwartz et al. 2000, Geider et al. 2001, Loreau et al. 2002, Smith & Knapp 2003); from this point of view, habitat-forming species (or ecosystem engineers; Hawkins & Harkin 1985, Jones et al. 1994) warrant particular attention.

In the marine environment, seaweed canopies (Dayton 1975, Menge 1978, Eckman et al. 1989, Bertness et al. 1999, Jenkins et al. 1999, 2004), seagrass (Orth 1977, Irlandi & Peterson 1991), or mussel beds (Suchanek 1985, Witman 1987) may play an important role in structuring assemblages, by modifying the physical features of the habitat through their morphologies. Removal experiments have been historically used to show these effects, but mostly in terms of presence or absence of a single species (Dayton 1975, Santelices & Ojeda 1984, Connolly 1994). Multiple habitat-forming species can, however, coexist in the same place. Low-shore portions of rocky coasts in the north-western Mediterranean, for example, are characterised by mixed stands of the canopy-forming brown alga *Cystoseira compressa* (Esper) Gerloff & Nizamuddin and the mussel *Mytilus galloprovincialis* Lamarck (Benedetti-Cecchi et al. 1996a). Canopy-forming algae and mussels are known to modify levels of light, temperature, water movement and sedimentation (Reed & Foster 1984, Duggins et al. 1990, McCook & Chapman 1991, Seed & Suchanek 1992, Seed 1996, Commito et al. 2005), influencing other members of assemblages either positively, by ameliorating physical conditions and by providing opportunities for colonization, or negatively, by monopolizing resources (mostly space) and thereby preventing colonization (e.g. Dayton 1975, Kanter 1978, Paine & Suchanek 1983, Bertness et al. 1999, Benedetti-Cecchi et al. 2001, Bulleri et al. 2002). Deletion experiments have clarified the roles of individual mussels and canopy algae in influencing the distribution and abundance of other species in these assemblages (Rodríguez-Prieto & Polo 1996, Benedetti-Cecchi et al. 1996a, 2001). How assemblages respond to simultaneous changes in the abundance or presence/absence of these species remains unknown.

In the present paper, we examined the effects of changes in number and identity of habitat-forming species (hereafter HFSs) on assemblages of algae and invertebrates on rocky shores in the north-western Mediterranean, through the selective removal of *Cystoseira compressa* and *Mytilus galloprovincialis*. Although common, these species may undergo drastic fluctuations in abundance and distribution due to natural and anthropogenic processes (Seapy & Littler 1982, Rodríguez-Prieto & Polo 1996, Benedetti-Cecchi et al. 2001). In addition to the presence/absence of target species, we also manipulated their abundances in various combinations to control for density-dependent effects (Benedetti-Cecchi 2004, 2006). Because *C. compressa* and *M. galloprovincialis* can have both positive and negative effects on other members of assemblages, we expected strong interactive effects associated with changes in the number, identity and abundance of these HFSs.

MATERIALS AND METHODS

Study site. The present study was conducted between November 2003 and November 2005, along the rocky coast of Calafuria, 10 km south of Livorno, Italy (43° 30' N, 10° 20' E). Assemblages occurring between 0 and –0.3 m below mean low water level on this coast were characterised by mixed stands of the brown alga *Cystoseira compressa* (Esper) Gerloff & Nizamuddin and the mussel *Mytilus galloprovincialis* Lamarck. Assemblages also included encrusting algae (the coralline *Lithophyllum orbiculatum* [Foslie] Foslie and the brown *Nemoderma tingitanum* Schousboe ex Bornet) and articulated corallines (*Corallina elongata* Ellis and Solander, *Jania rubens* [Linné] Lamouroux and *Haliptilon virgatum* [Zanardini] Garbary & Johansen), filamentous (*Ceramium* spp., *Polysiphonia* spp. and *Cladophora* spp.), coarsely branched (*Laurencia obtusa* [Hudson] Lamouroux, *Chondria boryana* [De Notaris] De Toni and *Gastroclonium clavatum* [Roth] Ardissone) and thin tubular sheet-like algae (*Padina pavonica* [Linnaeus] Thivy, *Dictyota dichotoma* [Hudson] J. V. Lamouroux and *Dictyopteris membranacea* [Stackhouse] Batters). The most common grazers were the limpets *Patella ulyssiponensis* Gmelin and *P. caerulea* Linnè and the topshell *Osilinus turbinatus* Von Born. Sessile invertebrates included the barnacle *Balanus glandula* Darwin and the tube-forming gastropod *Vermetus triqueter* Bivona-Bernardi (Menconi et al. 1999, Benedetti-Cecchi 2001). Organisms were identified to species level if possible and to morphological groups otherwise.

Experimental design. At the beginning of the study, 33 plots of 30 × 30 cm, with a cover of *Cystoseira compressa* and *Mytilus galloprovincialis* of no less than 30 %, were chosen along a stretch of coast of 1 km and marked at their corners with epoxy-putty (Subcoat S, Veneziani). Given the small size of the organisms sampled and the small spatial scales at which most of the variability occurs in these assemblages (Benedetti-Cecchi 2001), the size of the quadrats was considered appropriate to obtain representative estimates of abundance (Andrew & Mapstone 1987). The percentage cover of these and other sessile organisms was estimated using a plastic frame divided into 25 sub-quadrats of 6 × 6 cm, and giving a score from 0 to 4 % for each species in each sub-quadrat. Final cover was obtained by summing the values over the 25 sub-quadrats (Dethier et al. 1993, Benedetti-Cecchi et al. 1996b). The abundance of mobile animals was quantified as the number of individuals per quadrat.

The percentage cover values of *Cystoseira compressa* and of *Mytilus galloprovincialis* were adjusted experimentally to 30 % in 3 randomly selected plots, which were designed as controls (unmanipulated

plots; UP). Ideally, plots in which the 2 co-dominant species each covered 50% of the substratum would have been desirable for the experiment. Unfortunately, *C. compressa* and *M. galloprovincialis* rarely attained such large coverage values when sampled simultaneously at the scale of our plots. In contrast, plots in which the 2 co-dominant species each covered 30 to 40% of the substratum were common, and these were selected for the experiment. The other treatments were obtained by reducing the total cover of HFSs to 50, 40 and 30% through the selective removal of *C. compressa* (P_C; partial removal of *C. compressa*), *M. galloprovincialis* (P_M; partial removal of *M. galloprovincialis*) or both species (P_{CM}; partial removal of *C. compressa* and *M. galloprovincialis*, in equal proportions), resulting in 9 treatment combinations. Reducing the total cover of HFSs to 30% resulted in experimental conditions in which either *C. compressa* or *M. galloprovincialis* were totally eradicated (indicated as T_C and T_M, respectively). Hence, these treatments included only 1 HFS. Finally, there was a treatment in which both *C. compressa* and *M. galloprovincialis* were completely removed, so the cover of HFSs was 0% (T_{CM}) (Table 1).

Treatments were obtained by removing the bases and erect fronds of the brown alga and shells and byssal threads of the mussels with a hammer and chisel, a paint scraper and a knife. Care was taken not to damage the surrounding organisms or to alter the morphology of the substratum by creating cracks and crevices. Abundances of all organisms (as percentage cover or number) were sampled at 7 dates, roughly every 3 to 4 mo. This frequency was chosen to ensure the detection of possible changes on the structure of assemblages given the temporal pattern of variability in abundance of organisms (Menconi et al. 1999, Benedetti-Cecchi 2001).

Analysis of data. Experimental conditions were maintained by visiting the shore approximately every 2 wk. Despite our efforts, however, rough sea prevented the adjustment of treatments as frequently as needed, so that the percentage cover of both *Cystoseira compressa* and *Mytilus galloprovincialis* were, on average, 6% above nominal levels. Importantly, relative differences in abundance among treatments were maintained for the most part over the study period, as shown in Fig. 1. As a consequence, our analyses focused on the average response of the assemblage over the course of the study. For this purpose, we analyzed the data with population averaged–generalized estimating equations (PA-GEEs; Liang & Zeger 1986, Hardin & Hilbe 2002), using the function 'geeglm' in the 'geepack'

Table 1. Experimental design with treatment codes

|		Brief description	Code
Control: Unmanipulated plots	(Cover 60%)	Plots where abundances of both *Cystoseira* and *Mytilus* were adjusted to 30%	UP
Treatments: Cover reduction to 50%:			
1 species removal	–C	Plots where *Cystoseira* was partially removed (to 20%) and *Mytilus* left to 30%	50% P_C
	–M	Plots where *Mytilus* was partially removed (to 20%) and *Cystoseira* left to 30%	50% P_M
2 species removal		Plots where both *Cystoseira* and *Mytilus* were partially removed (to 25%)	50% P_{CM}
to 40%:			
1 species removal	–C	Plots where *Cystoseira* was partially removed (to 10%) and *Mytilus* left to 30%	40% P_C
	–M	Plots where *Mytilus* was partially removed (to 10%) and *Cystoseira* left to 30%	40% P_M
2 species removal		Plots where both *Cystoseira* and *Mytilus* were partially removed (to 20%)	40% P_{CM}
to 30%:			
1 species removal	–C	Plots where *Cystoseira* was totally removed and *Mytilus* left to 30% (1 species present)	30% T_C
	–M	Plots where *Mytilus* was totally removed and *Cystoseira* left to 30% (1 species present)	30% T_M
2 species removal		Plots where both *Cystoseira* and *Mytilus* were partially removed (to 15%) (2 species present)	30% P_{CM}
to 0%: 2 species removal		Plots where both *Cysoseira* and *Mytilus* were totally removed	T_{CM}
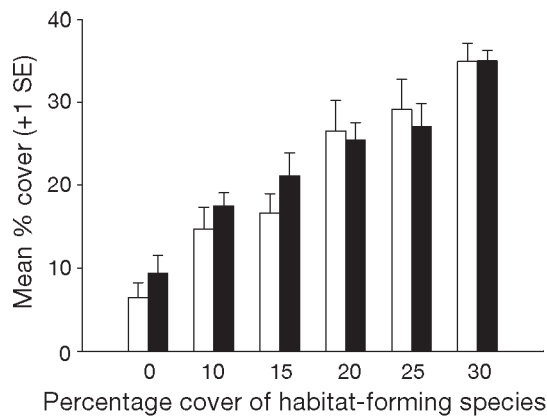
Fig. 1. *Cystoseira compressa* (open bars), *Mytilus galloprovincialis* (filled bars). Mean percentage cover over the study period, for each level of experimental density (there were n = 12 replicate plots for 30%, n = 6 for 20 and 0% and n = 3 replicates for all other experimental levels, averaged over 7 sampling dates over the course of the study; error bars are ±1 SE)

package of R 2.6.1 (R Development Core Team 2003). PA-GEEs allow analyses of correlated data, as in the case of experimental units repeatedly sampled through time, using the within-unit correlation structure to adjust the variance and standard errors of the estimated parameter. We used the first-order autoregressive model AR(1) to model temporal autocorrelation in response variables and fitted PA-GEE models assuming a Poisson distribution of the error terms and a log-link for the number of taxa, whereas a Gaussian distribution with the identity link was used for analyses of abundance data. Plots of standardized residuals versus fitted values and quantile–quantile plots were examined to check for strong deviations from the assumptions of the fitted models.

We examined several treatment contrasts, corresponding to specific hypotheses about the effects of changing the number, identity and cover of HFSs. First, control plots were compared with all the other treatments to examine a general effect due to the manipulation of HFSs (contrast UP vs. Treatments). We then assessed the effect of losing *Cystoseira compressa* and *Mytilus galloprovincialis* simultaneously, by comparing the T_{CM} condition (0% cover) with all the other manipulated treatments (contrast Other treatments vs. T_{CM}). Effects due to changes in number of manipulated species were examined by comparing plots from which both species were removed with plots from which only 1 species was deleted, regardless of its identity (contrast P_{CM} vs. P_C/P_M, where P_C/P_M indicates the removal of either *C. compressa* or *M. galloprovincialis*). To examine identity effects, we contrasted plots from which only *C. compressa* was removed with plots from which only *M. galloprovincialis* was removed (contrast

P_C vs. P_M). Both these contrasts were examined in interaction with changes in cover, to separate the effects of number and identity of manipulated species from those due to changes in their abundance. This first set of tests was limited to plots in which both HFSs were always present, i.e. at covers of 40 and 50%. Further tests were done at the abundance of 30%, and involved comparisons between plots that included both species (P_{CM}) and those that had either *C. compressa* (because mussels were totally removed, T_M) or *M. galloprovincialis* (because *C. compressa* was totally removed, T_C) (contrast P_{CM} vs. T_C/T_M, i.e. 2 vs. 1 species present), and between treatments differing in the identity of the species present (contrast T_C vs. T_M).

While these analyses were based on the orthogonal partitioning of degrees of freedom resulting in independent tests, they only contrasted a limited range of abundances of manipulated species. Additional tests were therefore performed to examine the effect of reducing the cover of 1 species across a wide range of experimental abundances (i.e. reductions in percentage cover values of individual species of 0, 10, 20 and 30%), while holding the cover of the other species at the nominal value of 30%. We examined both linear and quadratic contrasts to test the hypothesis that reductions in cover of HFSs have non-linear effects on response variables, as predicted under the general model that species interactions are not linear (May 1973). These are non-orthogonal tests, and they may have inflated Type I error rates. Nevertheless, we decided to perform these tests at the conventional level of α = 0.05 due to the limited amount of replication.

RESULTS

PA-GEEs on percentage cover of the most abundant taxa identified significant effects associated with changes in number, identity and abundance of HFSs. The effects due to changes in number of manipulated HFSs were revealed by the significant Cover × P_{CM} vs. P_C/P_M interaction for red filamentous algae, *Laurencia* spp. and thin tubular sheet-like algae (Table 2, Fig. 2). Red filamentous algae were more abundant in P_{CM} plots compared to P_C/P_M plots when the cover of HFSs was reduced from 60 to 50%. In contrast, P_C/P_M plots had more filamentous algae than P_{CM} plots when the cover of HFSs was reduced to 40% (Fig. 2). This interaction reflected the positive effect that a reduction in cover of *Cystoseira compressa* to 10%, but not to 20%, had on filamentous algae, as also suggested by a quadratic trend with an estimated coefficient (hereafter E) of 17.82 (SE = 9.97, p < 0.1) associated with the reduction of the brown alga. On the contrary, there was a significant non-linear effect of removing mussels while

Table 2. Analyses of data using population-averaged generalized estimating equations (PA-GEEs). E: estimated coefficient; SE: standard error; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Number of data points for each treatment = 21

	Red filamentous algae		Laurencia spp.		Thin tubular sheet-like algae		Articulated corallines		Encrusting corallines	
	E	SE	E	SE	E	SE	E	SE	E	SE
Intercept	7.810***	0.58	3.823***	0.32	9.140***	0.04	12.966***	1.49	16.988***	1.50
UP vs. Treatments	−0.206	0.18	−0.407***	0.11	0.596	0.83	−0.852	0.50	−1.512*	0.71
T_{CM} vs. Other treatments	−0.170	0.59	−0.539*	0.23	0.426	0.63	1.781	1.63	0.569	1.12
At cover of 40–50%:										
Cover	0.183	0.74	−0.618	0.58	−1.618	0.91	−1.062	1.52	1.875	2.12
P_{CM} vs. P_C/P_M	0.338	0.47	0.220	0.36	0.419	0.54	−0.392	0.98	−0.892	1.51
P_C vs. P_M	2.753**	1.00	1.904*	0.79	1.700	1.27	1.572	2.01	−4.485	2.59
Cover × P_{CM} vs. P_C/P_M	1.463**	0.47	0.999**	0.36	−1.390*	0.54	0.604	0.98	−0.303	1.51
Cover × P_C vs. P_M	−1.321	1.00	−0.588*	0.79	0.405	1.27	−0.010	2.01	0.393	2.59
At cover of 30%:										
P_{CM} vs. T_C/T_M	0.677	0.67	−0.350	0.38	−0.849	0.70	0.322	1.12	1.064	2.61
T_C vs. T_M	−0.451	1.03	0.004	1.08	3.946**	1.51	−3.290*	1.31	4.513	3.74
Correlation parameter	−0.09	0.07	0.29	0.14	0.25	0.09	0.05	0.08	0.69	0.05
Scale parameter	76.93	12.19	19.72	5.23	60.61	7.89	115.11	21.06	178.99	27.37

	Brown encrusting algae		Vermetus triqueter		Patella ulyssiponensis/caerula		Taxa	
	E	SE	E	SE	E	SE	E	SE
Intercept	2.259***	0.40	5.175***	0.54	2.910***	0.36	2.468	0.02
UP vs. Treatments	−0.270	0.16	0.019	0.36	−0.298	0.16	−0.013	0.01
T_{CM} vs. Other treatments	0.848	0.47	1.062**	0.41	−0.077	0.28	−0.005	0.01
At cover of 40–50%:								
Cover	0.180	0.19	−0.873	0.53	0.507	0.52	0.006	0.02
P_{CM} vs. P_C/P_M	0.090	0.14	0.082	0.41	−0.772**	0.28	−0.000	0.01
P_C vs. P_M	−0.350	0.23	1.862**	0.59	−0.957	0.76	0.030	0.03
Cover × P_{CM} vs. P_C/P_M	−0.195	0.14	−0.046	0.41	−0.404	0.28	0.000	0.01
Cover × P_C vs. P_M	−0.350	0.23	−0.087	0.59	−0.004	0.76	−0.010	0.03
At cover of 30%:								
P_{CM} vs. T_C/T_M	0.213	0.15	0.178	0.65	−0.133	0.47	0.019	0.02
T_C vs. T_M	−0.982*	0.42	−1.766**	0.54	1.008	1.02	0.046	0.05
Correlation parameter	0.08	0.09	0.24	0.08	0.62	0.05	0.11	0.06
Scale parameter	7.93	4.21	38.53	6.69	12.39	2.98	0.64	0.06

holding *C. compressa* constant (quadratic contrast: E = 9.392, SE = 3.773, $p < 0.05$), due to a decline in red filamentous algae at intermediate levels of cover of the invertebrate. These identitied effects were also evidenced by the significant P_C versus P_M contrast (Table 2).

The same patterns just described for red filamentous algae were also observed for *Laurencia* spp. (Fig. 2, Table 2). In this case, however, there was a positive linear effect of removing *Cystoseira compressa* on the response variable (linear contrast: E = 14.80, SE = 7.03, $p < 0.05$). In addition, *Laurencia* spp. were generally more abundant in manipulated compared to control plots, as indicated by the significant UP versus Treatments contrast (Table 2).

The significant Cover × P_{CM} versus P_C/P_M interaction observed for thin tubular sheet-like algae reflected the negative effect of reducing to 10% either *Cystoseira compressa* or *Mytilus galloprovincialis* compared to values for plots in which the 2 HFSs were manipulated simultaneously (patterns at 40% in Fig. 2), a trend that was reversed when the cover of either one or the other HFS was reduced to 20% (patterns at 50% in Fig. 2). In this case the removal of *C. compressa* resulted in a larger abundance of thin tubular sheet-like algae compared to that in plots where both HFSs species were manipulated or where only *M. galloprovincialis* was removed. This was evidence of an identity effect that was also present when the cover of HFSs was reduced to 30%, as indicated by the significant T_C versus T_M contrast in Table 2.

A main significant effect of changes in number of manipulated HFSs (P_{CM} vs. P_C/P_M) was observed for *Patella ulyssiponensis/caerula* (Table 2), with an
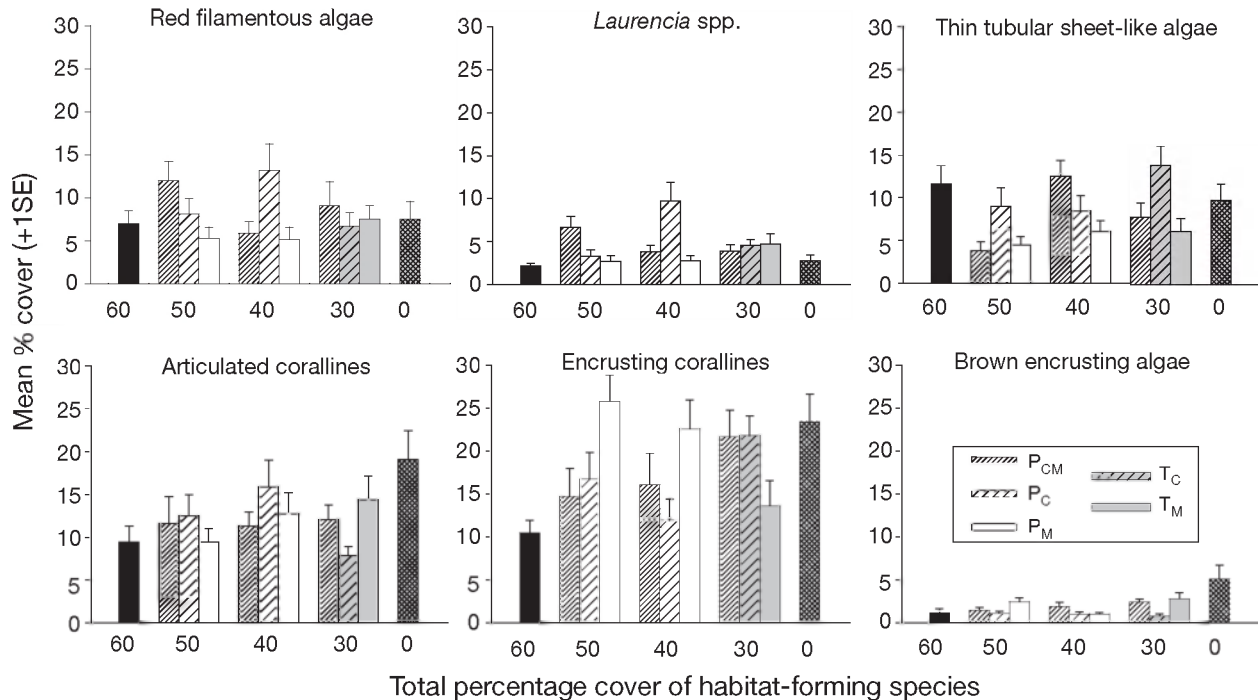
Fig. 2. Mean percentage cover of different macroalgae over the study period for each treatment (n = 3 replicate plots averaged over 7 sampling dates; error bars are ±1 SE). Percentage values on the abscissa refer to the cover of the habitat-forming species *Cystoseira compressa* and *Mytilus galloprovincialis*. Treatments included in linear and quadratic contrasts were the unmanipulated plots (filled bars) and those coded with either subscript C or M (i.e. excluding P_{CM} and the 0% treatment), depending on whether the contrasts examined the effect of removing *C. compressa* or *M. galloprovincialis*. P_C: partial removal of *C. compressa*; P_M: partial removal of *M. galloprovincialis*; P_{CM}: partial removal of both *C. compressa* and *M. galloprovincialis*; T_C: total removal of *C. compressa*; T_M: total removal of *M. galloprovincialis*

increase in number of limpets especially when only mussels were reduced to 10 and 20% (Fig. 3).

The identity effect described for thin tubular sheet-like algae also occurred for articulated coralline algae, but in the opposite direction. In this case, there was a significant reduction in algal cover in plots cleared of *Cystoseira compressa* compared to plots where mussels were totally removed (Fig. 2 and T_C vs. T_M contrast in Table 2). Similar negative effects due to the removal of *C. compressa* were observed on brown encrusting algae (Fig. 2) and the invertebrate *Vermetus triqueter* (Fig. 3). In both cases there was a significant T_C versus T_M contrast (Table 2). The importance of an identity effect was also indicated for the invertebrate by the significant P_C versus P_M contrast (Table 2), i.e. when the cover of HFSs was reduced to 40–50% (Fig. 3). In this case, however, it was the removal of *Mytilus galloprovincialis* that depressed the cover of *V. triqueter*, rather than the removal of *C. compressa* as observed for the T_C versus T_M contrast. When examined across the full range of experimental densities, the effects of mussels was strongly non-linear, with the largest effect observed at the intermediate cover of 40% (quadratic contrast: E = 15.167, SE = 4.964, p < 0.01).

Encrusting coralline algae were more abundant in manipulated than in control plots (UP vs. Treatments contrast; Table 2); in particular, there was a positive linear effect of removing *Cystoseira compressa* on encrusting coralline algae (linear contrast: E = 30.0, SE = 15.1, p < 0.05) (Fig. 2).

Finally, there was a slight, but significant non-linear positive effect of removing *Cystoseira compressa* on mean number of taxa, with larger values of the response variable at 40 and 50% cover of the brown alga (quadratic contrast: E = –0.47, SE = 0.22, p < 0.05) (Fig. 3).

DISCUSSION

The present study pointed out how the simultaneous loss of 2 HFSs can result in complex interactive effects on associated assemblages and emphasized the importance of examining non-random loss of species and the need to control for density-dependent effects in biodiversity experiments.

Past studies conducted in terrestrial and aquatic systems highlighted variable effects of biodiversity on the biomass, productivity and structure of assemblages.
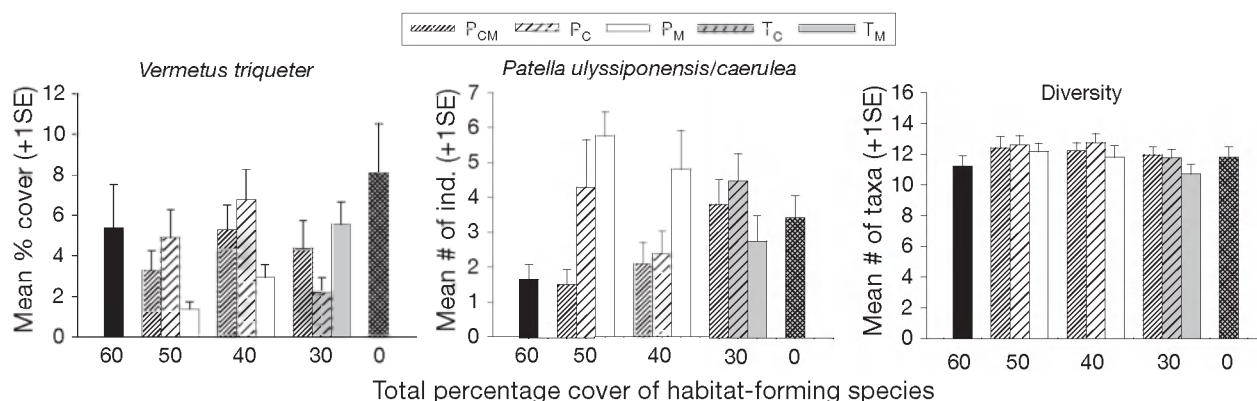
Fig. 3. Mean percentage cover of the tube-forming gastropod *Vermetus triqueter*, mean number of limpets *Patella ulyssiponen-sis/caerulea* and mean diversity over the study period for each treatment (n = 3 replicate plots averaged over 7 sampling dates; error bars are ±1 SE). Percentage values on the abscissa refer to the cover of the habitat-forming species *Cystoseira compressa* and *Mytilus galloprovincialis*. Treatments included in linear and quadratic contrasts were the unmanipulated plots (filled bars) and those coded with either subscript C or M (i.e. excluding P_{CM} and the 0% treatment), depending on whether the contrasts examined the effect of removing *C. compressa* or *M. galloprovincialis*. P_C: partial removal of *C. compressa*; P_M: partial removal of *M. galloprovincialis*; P_{CM}: partial removal of both *C. compressa* and *M. galloprovincialis*; T_C: total removal of *C. compressa*; T_M: total removal of *M. galloprovincialis*

Most terrestrial studies supported the conclusion that the net effect of diversity on biomass resulted from both species-specific selection effects and complementarity (Hooper et al. 2005, Cardinale et al. 2006). Recent studies on aquatic biodiversity, however, have documented strong responses to changes in species composition among experimental treatments, emphasizing the importance of identity effects (e.g. Bruno et al. 2005, O'Connor & Crowe 2005, Moore & Fairweather 2006, Vaughn et al. 2007). Our results have shown how the effect of changing the identity and number of HFSs may vary as a function of the abundance of manipulated species. Abundance, identity and richness effects could be disentangled in the present study because we explicitly manipulated the cover of HFSs as a factor in the experiment.

For several of the response variables analysed, both identity and richness effects (limited to 2 HFSs in the present study) occurred in interaction with the cover of manipulated species. In general, the effect that the removal of a particular HFS had on other organisms changed in magnitude and direction as a function of the relative abundance of the manipulated species. For example, a slight decrease in the cover of HFSs (from 60 to 50%) led to an increase in abundance of some algae (red filamentous algae and the coarsely branched *Laurencia* spp.) and a decrease in others (thin tubular sheet-like algae), but only when *Cystoseira compressa* and *Mytilus galloprovincialis* were both removed (treatment P_{CM}). At lower abundances of manipulated species (40%), however, positive effects were observed only in treatments where *C. compressa* was removed and mussels were left intact. Identity effects still occurred when the cover of HFSs was

reduced to 30%. In this case, however, the complete removal of mussels was important, causing a reduction in cover of thin tubular sheet-like algae and enhancing the abundance of articulated coralline and brown encrusting algae. Identity effects also influenced the percentage cover of the encrusting invertebrate *Vermetus triqueter* and occurred at different abundances of HFSs (50, 40 and 30%).

Our results are in agreement with the outcomes of past studies that have documented both positive and negative responses of species to the removal of either canopy-algae or mussels (Dayton 1975, Kanter 1978, Paine & Suchanek 1983, Bertness et al. 1999, Benedetti-Cecchi et al. 2001, Bulleri et al. 2002, Chapman et al. 2005). By manipulating *Cystoseira compressa* and *Mytilus galloprovincialis* simultaneously, however, we generated complex responses in associated assemblages that likely involved a wide range of direct and indirect effects (Wootton 1994, and references therein). Importantly, in our study, these effects were largely a function of the abundance at which species interacted, highlighting the potential problems that might result from employing substitutive or additive designs that do not control for density-dependent effects in biodiversity experiments (Benedetti-Cecchi 2004, 2006).

The notion that identity effects are important determinants of ecological processes, as emphasized in biodiversity–ecosystem functioning studies, reiterates what ecologists and biologists have discovered in several decades of research on species life histories. Consideration of the morphology and life history of certain species is, therefore, important in order to understand how the removal of these species may have affected

other organisms. Mussel shells, for example, offer a suitable substratum for the colonization of many algae and invertebrates, but they can also prevent colonization of primary space by forming closed beds. Similarly, *Cystoseira compressa* had short fronds, so the understory environment was limited compared to that provided by other congeneric species with larger fronds (Benedetti-Cecchi et al. 2001, Bulleri et al. 2002). Hence, negative effects due to pre-emption of the substratum may have outweighed the positive effects due to the provision of an understory environment. These effects were particularly evident for *Laurencia* spp. and encrusting coralline algae, the cover of which increased linearly with a decrease in abundance of *C. compressa*, as revealed by significant linear contrasts in the analyses.

While the effects of mussels in competitively excluding other invertebrates or macroalgae are well documented (Paine 1966, Dayton 1971, Menge 1976, Paine 1984, Enderlein & Wahl 2004, Miyamoto & Noda 2004), much less is known about how small-canopy algae like *Cystoseira compressa* interact with other species. In terrestrial habitats, the balance of facilitation and competition is known to vary with the life stages and physiologies of interacting species and with indirect interactions involving other organisms (Walker & Vitousek 1991, Chapin et al. 1994, Miller 1994, Callaway et al. 1996). In the aquatic realm, switches from positive to negative effects have been observed along environmental gradients of stress (Bruno et al. 2003), and with different canopy-forming species, along vertical gradients (Hawkins 1983, Jenkins et al. 1999). In our analyses, switches in the direction of the effects of HFSs on other organisms were revealed clearly by the quadratic contrasts. These contrasts examined the effect of reducing the cover of one HFS across a wide range of experimental abundances, while holding the cover of another HFS at the nominal value of 30%. Non-linear effects were common, with mussels maintaining high cover of filamentous algae and *Vermetus* sp. when unmanipulated (30% cover) or when totally eradicated, but not when they were present at intermediate abundances. A similar relationship occurred between *C. compressa* and filamentous algae. Canopy cover was also non-linearly related to the mean number of taxa in quadrats, with the largest diversity observed under intermediate values of canopy abundance. These non-linear interactions indicated that a small change in cover was sufficient to trigger a shift from positive to negative (or negative to positive) effects of the HFSs on other organisms, highlighting the interplay between resource availability and facilitation in these assemblages (Bertness & Leonard 1997, Bertness et al. 1999, Menge 2000, Bulleri et al. 2008).

Changes in cover or presence/absence of HFSs can

potentially influence both primary producers and herbivores. In the investigated system, the most important grazers were limpets. These herbivores were rarely observed on mussel shells, whilst they were common in natural gaps within the canopy of *Cystoseira compressa* (authors' pers. obs.), suggesting a strong identity effect of HFSs on grazers. Our results, in contrast, highlighted a significant effect of the number of manipulated HFSs on the abundance of limpets, with treatments in which only one HFS was removed having larger densities of grazers compared to treatments in which both HFSs were manipulated. A possible explanation is that when present in uneven abundances (especially with the prevalence of the canopy alga), mussels and *C. compressa* would improve the quality of the habitat (in terms of food and/or in terms of substratum attachment).

Recently, some authors have pointed out the importance of considering the effects of biodiversity on different aspects of assemblage structure and ecosystem functioning (Hector & Bagchi 2007). This is particularly applicable to multilayered assemblages of rocky shores. A number of experimental studies have revealed strong responses of mobile macrofauna, including amphipods, isopods and polychaetes, to the removal of canopy algae or mussels (Commito & Dankers 2001, Thrush et al. 2001, Thiel & Ullrich 2002, Goodsell & Connell 2005, Schmidt & Scheibling 2007). Examining the effects of HFSs on these assemblages could, therefore, provide different answers to those obtained by focusing on sessile macro-organisms, the customary approach in this type of study. Our results indicated only a slight effect of HFSs (due to the removal of *Cystoseira compressa*) on diversity, measured at a coarse level of taxonomic resolution as the total number of taxa; a different outcome might have emerged if we had focused on mobile macrofauna, which is an important component of the overall diversity of these assemblages.

Recent biodiversity experiments in marine environments have added important insights to general debate on the role of biodiversity in ecosystem functioning. Here, the relatively small size and fast growing rates of organisms inhabiting assemblages dominated by the mussel *Mytilus galloprovincialis* and the canopy alga *Cystoseira compressa* enabled us to conduct a complex removal experiment to examine the effects associated with changes in number, identity and abundance of HFSs. Our findings highlighted the importance of density-dependent processes in modulating the effects due to the changes in number and identity of HFSs. Because density-dependent processes are pervasive in nature (Barkai & McQuaid 1988, Robinson & Edgemon 1988, Drake 1991, Rand 2003, Griffin et al. 2008), it is desirable that future biodiversity experi-

ments explicitly consider density effects with the appropriate designs (He et al. 2005, Benedetti-Cecchi 2004, O'Connor & Crowe 2005) to foster progress in understanding of biodiversity–ecosystem functioning relationships.

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