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Response of the invader *Sargassum muticum* to variability in nutrient supply

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ABSTRACT: Fluctuations in resource availability are a key factor controlling the susceptibility of an environment to invasion by non-indigenous species. Increases in the availability of resources will facilitate the invasion of habitats and assemblages. In this study, we tested experimentally the combined effects of intensity and temporal variability of nutrient supply on the invasion of a low intertidal macroalgal assemblage along the rocky shore of Galicia (northwest Spain) by the brown seaweed *Sargassum muticum* (Yendo). We estimated invasibility by measuring the number of plants, length of plants and biomass of *S. muticum*. Specifically, we tested the hypothesis that intensity and temporal variation of nutrient addition would affect interactively the invasion of macroalgal assemblages with high temporal variability emphasizing the effects of a high intensity of nutrient addition. Results indicated interactive effects between intensity and temporal variability of nutrient addition on the number and length of plants of *S. muticum*. Moreover, temporal variability of nutrient addition had an effect on biomass of *S. muticum*. In addition, intensity of nutrient enrichment and temporal variability to some extent affected native assemblages, with intensity of nutrients being mainly an important driver of the percentage cover of the most abundant functional groups.

KEY WORDS: *Sargassum muticum* · Nutrient addition · Invasion · Galician coast · Mean intensity · Temporal variability

INTRODUCTION

Along with habitat modification, the introduction of non-indigenous species is a leading cause of the global biodiversity crisis (Wilcove et al. 1998). Invasions may alter composition and diversity of local assemblages (e.g. D'Antonio & Vitousek 1992, Grosholz et al. 2002), dramatically affecting ecosystem functioning and sometimes causing serious consequences for economic and social systems (Vitousek et al. 1996, Pimentel et al. 2000). As a result, there is increasing interest in predicting why, how and where successful invasions occur. The expansion of non-indigenous species may be modulated by a number of factors including the degree of disturbance, propagule pressure and life traits of native and non-indigenous species (Lonsdale 1999). In the context of ecosystem resistance to invasion, one of the most commonly cited hypotheses is the Diversity Resistance Hypothesis (Elton 1958), which predicts that diverse communities are more resistant to invasion because of a more complete use of resources (Loreau 2000). Therefore, any process directly affecting species diversity and/or availability of resources may also indirectly facilitate the success of invaders (Tilman 1997, Kennedy et al. 2002). Recently, the Fluctuating Resources Availability Theory (FRAT, Davis et al. 2000) assigned to fluctuations in resource availability a key role in controlling the susceptibility of an environment to invasions. According to this theory, habitats and assemblages become more susceptible to invasions as heterogeneity in the availability of resources increases. This could be determined by the introduction of new resources, such as nutrient additions, or by reductions in the use of resources used by native species (due, for instance, to a decrease of species biomass). Under this theory, the susceptibility of a

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community to invasion fluctuates over time depending upon the conditions of resource enrichment, with invasibility increasing as the amount of unused resources rises. Moreover, the temporal variability of nutrient addition (i.e. distribution of the disturbance events over the time scale of the study) is likely to influence the invasion process because the settlement of the invasive species may or may not match the timing of disturbance, and the time interval between successive disturbances may also have significant effects on the assemblage response (Hobbs & Huenneke 1992). In fact, several studies have highlighted the importance of frequency and magnitude of disturbance in invasion success in terrestrial, aquatic and marine systems (Moyle & Light 1996, Altman & Whitlatch 2007, Bishop & Kelaher 2007).

The effects of nutrient availability on invasion have been mainly examined on terrestrial plants (Burke & Grime 1996, Gross et al. 2005, Leishman & Thomson 2005). In contrast, few experimental studies in marine systems have dealt with this topic (but see Steen 2003, Sánchez & Fernández 2006). However, most of these studies supported some predictions derived from FRAT, i.e. increasing nutrient availability increases the chance of successful invasion.

Increasing nutrient availability due to human activities is an important ecological factor that strongly affects the productivity and patterns of distribution, abundance and diversity of coastal assemblages on a global scale (Vitousek et al. 1997, Benedetti-Cecchi et al. 2001). In addition, recent models of climate change predict changes in oceanographic and biological processes directly and indirectly linked to nutrient dynamics in marine systems (Denman et al. 1996). Specifically, oceanographic features such as fronts, mixing layers or currents may shift in location and/or strength thereby affecting resources dynamics (e.g. variability in intensity and temporal nutrients supply) and, thus, altering the productivity and functioning of ecosystems (Grime et al. 2000, Soto 2002). Changes in intensity and temporal variability of several biotic and abiotic factors such as grazing (Benedetti-Cecchi et al. 2005), mechanical disturbance (Bertocci et al. 2005), aerial exposure (Benedetti-Cecchi et al. 2006, Bertocci et al. 2007) and sediment scouring (Vaselli et al. 2008) have been reported as important drivers for changes in structure of intertidal macroalgal assemblages. However, the effect of changes in intensity and temporal variability of nutrients in macroalgal assemblages has not yet been tested.

Here, we used a factorial experiment to examine the combined effects of intensity and temporal variability of nutrient supply on the invasion by *Sargassum muticum* (Yendo) of a low intertidal macroalgal assemblage along a rocky shore in northwestern Spain. We estimated invasibility by measuring the success of *S. muticum* (i.e. number of plants, length of plants and biomass), whose propagule pressure was supplied in the experimental plots. We tested the hypothesis that intensity and temporal variation of nutrient addition would affect interactively the invasion of macroalgal assemblages with high temporal variability emphasizing the effect of high intensity of nutrient addition. Thus, intensity of nutrient addition would increase invasibility of native assemblages, but this effect was expected to increase with increasing levels of temporal variability in nutrient addition (i.e. high temporal variability) due to an increase of unused resources.

MATERIALS AND METHODS

Study area. The study was conducted from January 2007 to February 2008 in the low intertidal (0.4 to 0.8 m above the mean low water level [MLWL]) of 2 shores about 10 km apart in northwestern Spain, Cabo Estai (42° 11′ N, 8° 48′ W) (Shore 1) and Monte Lourido (42° 09′ N, 8° 50′ W) (Shore 2). Both shores were invaded by *Sargassum muticum* and the low-shore assemblages were characterised by the leathery alga *Bifurcaria bifurcata* Ross, the articulated calcareus alga *Corallina officinalis* Linnaeus, the corticated alga *Stypocaulon scoparium* (Linnaeus) and the green algae *Ulva rigida* C. Agardh and *U. clathrata* (Roth) C. Agardh (morphofunctional groups, sensu Steneck & Dethier 1994). The most common invertebrates were the limpets *Patella vulgata* Linnaeus and *P. intermedia* Jeffreys, the gastropods *Monodonta lineata* Da Costa, *Gibbula umbilicalis* Da Costa and *G. cineraria* Linnaeus, and the mussel *Mytilus galloprovincialis* Lamarck. Nevertheless, there were quantitative differences in algal assemblages between shores (permutational multivariate analysis of variance [PERMANOVA]; Shore, pseudo-$F_{(1,46)} = 16.18$, $p < 0.001$) mainly due to changes in relative abundance of some taxa rather than in species composition (similarity percentages [SIMPER] analyses). The algae, *C. officinalis* (15.35%), *Laurencia pinnatifida* (Hudson) Lamouroux (10.20%), *Fucus vesiculosus* Linnaeus (9.86%) and *Lithophyllum incrustans* Philippi (8.92%), were the species that contributed most to the observed changes in assemblage structure due to their differences in percentage cover between shores. In particular, *F. vesiculosus* and *L. pinnatifida* were more abundant on Shore 2, whereas *C. officinalis* and *L. incrustans* were more abundant on Shore 1.

Basic information of *Sargassum muticum*. *Sargassum muticum* is a brown alga native to southeast Asia (Yendo 1907) that has successfully colonised a vast part of the Pacific coast of North America and the west coast of Europe (Farham et al. 1973, Norton 1981). *S. muticum* was recorded for the first time in northern Spain in the

1980s (Casares et al. 1987, Fernández et al. 1990). At present, it occurs across a range of wave exposures, from sheltered shores, where it often forms dense monospecific beds (Fernández et al. 1990), to rock pools and emergent substrata at low intertidal and shallow subtidal levels of more exposed shores (Andrew & Viejo 1988). In the north of Spain, *Sargassum* has a spring–summer reproductive period, then main branches arise from the stem during autumn and grow throughout winter and spring until the onset of the reproductive period in late spring (for further details see Arenas & Fernández 1998, 2000, Fernández 1999).

Experimental design. A 3-way factorial experiment was used to test the hypothesis that intensity and temporal variance of nutrient addition interactively influence the invasion of macroalgal assemblages by *Sargassum muticum*. We defined intensity of nutrient addition as the amount of nutrients added to the experimental plots. Temporal variability was defined with reference to the interval of time between successive nutrient additions. The factors were shore (2 levels, corresponding to Shore 1 and Shore 2), intensity of nutrient addition (2 levels, low intensity [LI] versus high intensity [HI]) and temporal variability (2 levels, low variability [LV] versus high variability [HV]) of nutrient addition. Furthermore, there were 4 control plots (C) where nutrients were left at natural levels, and 4 procedural controls (PC) to test for possible confounding effects due to the use of mesh bags. On each shore, a total of 24 plots, 50 × 50 cm in size and at least 3 m apart, were distributed along 200 m of coastline and marked at each corner with epoxy putty for subsequent relocation. At the beginning of the study, both the holdfast and erect fronds of *S. muticum* inside and around each plot (<4 m) were carefully removed. Eight plots were randomly assigned to the HI and 8 different plots to the LI treatments. Within each level of nutrient addition, 4 plots were randomly assigned to each of the LV and HV treatments. The high and low intensity levels of nutrient addition were achieved by placing 3 kg (2 mesh bags containing 1.5 kg each for HI) and 1 kg (2 mesh bags containing 0.5 kg each for LI) of slow, controlled-release fertilizer (Multicote® 4). The mesh bags were placed on 2 sides of the experimental plots, parallel to the shoreline and anchored to the substrate with 1 steel screw and 2 straps on either side of the bags. Procedural controls used similar mesh bags filled with a plastic bag containing sand. We used Multicote® 4 containing a ratio of 15% N (8% NH_4^+, 7% NH_3), 7% P (PO_2), 15% K (K_2O) and 2% MgO. The mesh bags were replaced monthly.

The 2 levels of temporal variability were produced by distributing nutrient additions on 7 occasions and in a different manner during the course of the experiment. The LV treatment was obtained by distributing the nutrient addition over 7 regularly spaced occasions (bad weather prevented visits to the shore at perfectly regular intervals) over the course of the experiment, whereas the HV treatment was obtained by distributing the additions in a more heterogeneous manner (Fig. 1). These arrangements generated an almost null variance of the intervals of time between successive additions of nutrients for the LV treatment that resembled an environmental scenario where the input of nutrients is highly predictable, and a positive variance for the HV treatment that resembled a scenario where input of nutrients is more variable and unpredictable in time. Sampling data were arranged in such a way that the mean interval of time since the final nutrient addition was constant across treatments. This sampling strategy avoided confounding the effect of temporal variability with time since the final nutrient addition. For further details on this experimental approach see Benedetti-Cecchi (2003), Bertocci et al. (2005) and Benedetti-Cecchi et al. (2006).

Experimental invasion. To assure a similar propagule pressure by *Sargassum muticum* in all experimental plots, we conducted an experimental invasion. During the spring tides of May 2007, 96 fertile plants (i.e. with mature receptacles) 30 to 40 cm long were carefully detached from the substratum at an area adjacent to the study sites. Within 1 h after collection, 2 *S. muticum* plants were anchored at each plot using 2 straps attached to screws. Every 15 d and until the end of the reproductive period, i.e. August 2007, we checked the plants and replaced them when necessary.

Sampling procedure. To evaluate the effectiveness of the experimental nutrient addition, plants of *Corallina officinalis* were collected monthly around each plot to analyse nutrient content (N, C and P). We chose this species because it was present on both shores during the whole year. After removing all the epiphytes, *C. officinaliss* plants were rinsed with seawater, dried for 48 h at 60°C and then ground to a fine powder using a pestle. Tissue content of N and C was analysed with a Perkin Elmer CNH analyzer and P tissue content was determined using ICP analysis (Ryle et al. 1981).

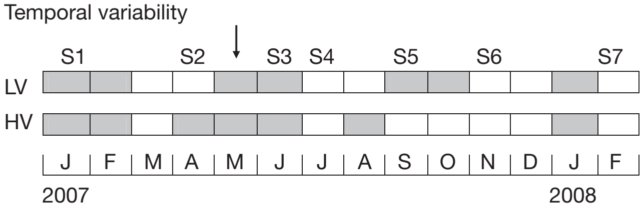

Fig. 1. Experimental time schedule showing the temporal variability (low [LV] versus high [HV]) of nutrient additions (grey rectangles).The arrow indicates the time of the experimental invasion of plots. Times of sampling are shown by S

To evaluate indirect effects of nutrient addition on invasion through changes in native assemblages, the total number of native taxa (a surrogate measure of species diversity) and the percentage cover of macroalgae were measured in each experimental plot at 7 different times (i.e. 2 sampling times before the artificial invasion of plots and 5 times after invasion was imposed, Fig. 1). The percentage cover of macroalgae was estimated using a 50 × 50 cm metallic quadrat divided into 25 sub-quadrats of 10 × 10 cm each, and a score from 0 to 4 % was given to each taxon in each sub-quadrat after visual inspection. Final cover was obtained by summing over the 25 scores obtained in the sub-quadrats (Dethier et al. 1993). Macroalgae were identified to the most detailed level of taxonomic resolution achievable in the field and then aggregated into morpho-functional groups based on similarities in morphology and resource use (encrusting, articulated calcareous, foliose, leathery, filamentous and corticated algae, sensu Steneck & Dethier 1994).

Different variables such as number of plants, length and biomass were used to measure invasion success by *Sargassum muticum*. When plants of *S. muticum* in experimental plots were large enough to be visible to the naked eye, i.e. since October 2007, they were counted monthly. From October 2007 until the end of the experiment (February 2008), the length of plants was also measured monthly. Finally, the biomass (dry weight) of plants in each plot was measured in March 2008.

Data analysis. Data were analysed using Population-Averaged Generalized Estimating Equations (PA-GEEs), an extension of Generalized Linear Models (GLMs) for correlated data (Liang & Zeger 1986). PA-GEEs were useful in this study for 2 reasons. First, our hypothesis applied to the entire duration of the experiment and not to single dates of sampling. Second, each experimental unit (plot) was sampled repeatedly through time, leading to the need to account for correlation between observations through time for the same plot. PA-GEEs were run using the 'geeglm' procedure in the R program, v2.7.0 (R Development Core Team 2003), specifying a link function and an error structure for the residuals. We specified a first order autoregressive model, AR(1), in all the analyses assuming a time dependence for each plot. For data expressed as percentage cover and size (length of plants) of *Sargassum muticum*, we assumed a Gaussian distribution of the error terms and used an identity link to relate the linear predictor with expected values of the response variable. For data expressed as number of *S. muticum* plants and number of native taxa, we assumed a Poisson distribution of the error terms and used a log-link function.

Tests of the hypothesis were based on 'treatment' contrasts and compared the control versus procedural control in the first place. When there were significant differences between the control and procedural control, effects of nutrient addition were examined by contrasting the procedural control with all treatments. The interactions between intensity, temporal variability of nutrient addition and shore were tested using low intensity, low temporal variability and Shore 1 as reference levels. The HV versus LV contrast examined the effect of an increase of temporal variability of nutrient addition. Plots of residuals versus predicted effects were examined after each analysis to check for normality and homogeneity of variance.

Biomass of *Sargassum muticum* was analysed using a 3-way ANOVA with intensity, temporal variability and shore as crossed and fixed factors. The analysis initially included the contrast of 'control versus other treatments.' Before these analyses, a 2-way ANOVA with shore and treatment (control versus procedural control) was used to test for the effect of mesh bags on biomass of *S. muticum*. The homogeneity of variances was tested using Cochran's *C*-test and data were transformed if variances were heterogeneous.

Non-parametric PERMANOVA were used to test hypotheses about differences of native macroalgal assemblages between shores at the beginning of the experiment (January 2007) and to assess responses of native macroalgal assemblages to treatments after the artificial invasion was completed (February 2008). SIMPER analysis was also done to identify macroalgal species most responsible for any observed difference between shores.

RESULTS

Effectiveness of treatments

Nutrients enrichment did not affect significantly the C, P or N tissue content of *Corallina officinalis* (Student's *t*-tests, $p > 0.05$). Although not significant, N tissue concentration showed the strongest response to nutrient enrichment. Nitrogen content tended to be greater in plants collected from high than from low intensity treatments most of the time, although this trend was more evident on Shore 2 than on Shore 1 (average HI and LI [±SE] were 0.69 ± 0.17 % and 0.62 ± 0.15 %, respectively, on Shore 1 and 0.85 ± 0.19 % and 0.76 ± 0.18 %, respectively, on Shore 2; Fig. 2). Despite the weak response of *C. officinalis* to nutrient enrichment we were confident with the effectiveness of treatments for 2 main reasons. First, the release rate of nutrients is only affected by the water temperature, and the range in the study area (from 12.5 to 18.5°C)

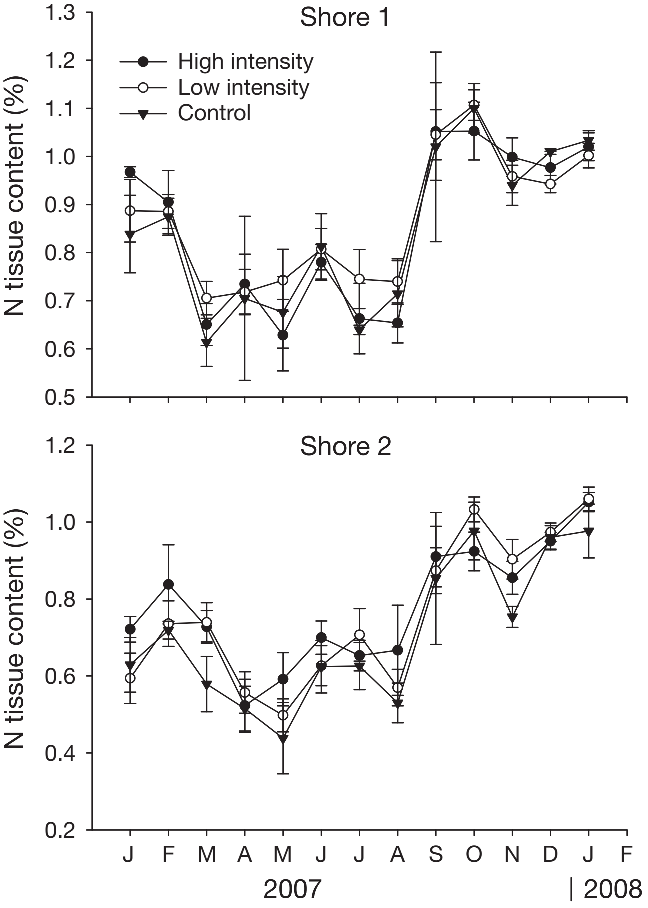

Fig. 2. *Corallina officinalis.* Mean (±SE, n = 4) nitrogen content (% dry weight) of plants collected over time (13 dates) from control, high- and low-intensity nutrient treatments

guaranteed an optimal diffusion of dissolved nutrients (for technical information see Haifa chemical). Second, the frequent visits to the study area (every 15 d) to check mesh bags guaranteed that they remained intact and undamaged during the course of the experiment.

Sargassum muticum

The number of *Sargassum muticum* plants was affected by the intensity and temporal variability of nutrient addition (i.e. significant interaction of Intensity × Temporal Variability; Table 1). On Shore 1, the number of plants was higher in LI than in HI treatments under high temporal variability (Fig. 3). However, when the nutrient addition was made under low temporal variability the number of plants did not differ significantly among levels of nutrient addition (Fig. 3). The opposite pattern was observed on Shore 2. The number of plants was larger in HI combined with LV treatments (Fig. 3). When nutrient addition was made according to the HV treatment, the number of plants did not differ among levels of nutrient addition (Fig. 3). Furthermore, there were differences between Shores 1 and 2. First, the number of plants was higher on Shore 2 than on Shore 1 (i.e. significant effect of Shore; Table 1). Second, the number of plants was affected by intensity of nutrient addition in a different way depending on the shore (i.e. significant interaction of Shore × Intensity; Table 1, Fig. 3). There were more plants in LI treatments on Shore 1, whereas on Shore 2 the number of plants was larger in HI treatments.

The length of plants varied significantly between the control and procedural control on both shores (Table 1), with smaller plants found in procedural control plots than in control plots (Fig. 3). This indicates the occurrence of artefacts due to mesh bags and suggests that the use of mesh bags altered some variables that were important for growth of *Sargassum muticum*. Therefore, results on this response variable must be interpreted with caution.

The length of plants varied significantly with intensity and temporal variability of nutrient addition (i.e. significant interaction of Intensity × Temporal Variability; Table 1), and this variation was consistent between shores. Plants were longer in LI treatments under high temporal variability, whereas under low temporal vari-

Table 1. *Sargassum muticum*. Results of Population-Average Generalized Estimating Equations examining the effects of Shore (S), Intensity (I) and Temporal Variability (V) of nutrients addition on the number and length of plants. E = estimate, SE = standard error, PC = procedural control, HI = high intensity, LI = low intensity, HV= high temporal variability, LV = low temporal variability, *p < 0.05, **p < 0.01, ***p < 0.001

Contrasts	Number of plants		Length of plants	
	E	SE	E	SE
Intercept	1.18***	0.07	16.06***	1.23
Control vs PC	−0.03	0.16	7.34***	1.88
PC vs Treatments	0.04	0.05	1.84**	0.76
HI vs LI = I	0.04	0.08	1.48	1.68
Shore 1 vs Shore 2 = S	−0.28***	0.07	1.60	1.24
HV vs LV = V	0.03	0.08	−1.64	1.68
S × I	−0.24***	0.08	−2.07	1.68
S × V	−0.12	0.08	−5.08**	1.68
I × V	−0.26***	0.08	−3.42*	1.68
Correlation parameter	0.0	0.0	0.0	0.0
Scale parameter	5.60	0.56	588.32	151.07

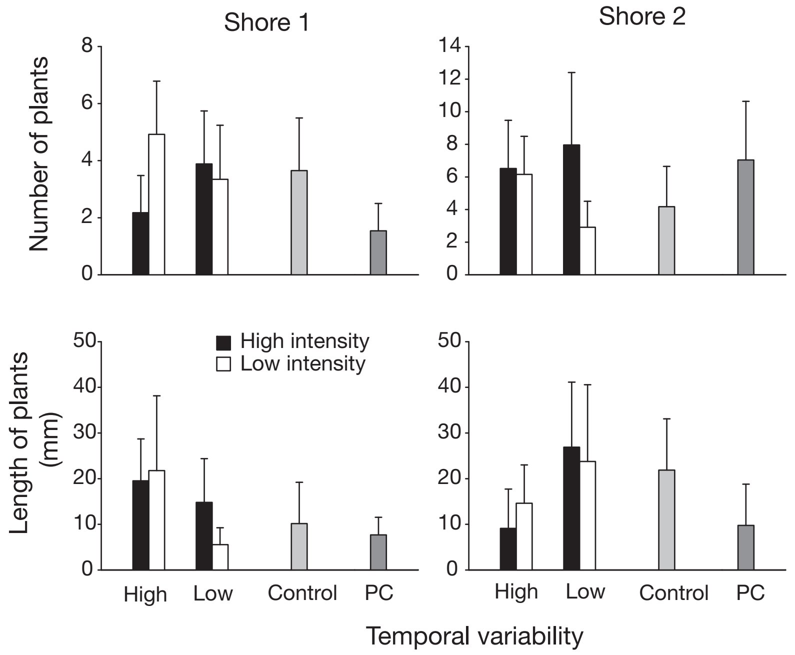

Fig. 3. *Sargassum muticum.* Mean (+SE, n = 28) number and length of plants on Shore 1 and Shore 2, according to intensity and temporal variability of nutrient addition. PC = procedural control. Means are calculated by averaging data from each plot (4) over time (7 dates)

ability plants were longer in HI treatments (Fig. 3). In addition, temporal variability in nutrient addition affected the growth of plants in different ways depending on the shore (i.e. significant interaction of Shore × Temporal Variability; Table 1). Therefore, plants were longer under high temporal variability conditions on Shore 1, whereas on Shore 2 plants were longer under low temporal variability conditions.

Biomass of *Sargassum muticum* did not vary significantly between the control and procedural control ($F_{1,12} = 0.12$, $p > 0.05$), which indicated no effect of mesh bags. Temporal variability of nutrient addition did affect the biomass of *S. muticum* (Table 2). Thus, the low temporal variability treatment was associated with an increase of biomass when combined with HI on both shores (Fig. 4).

Native organisms

Native assemblages were affected by intensity and temporal variability of nutrient addition (PERMANOVA; Treatment, pseudo-$F_{(5,5)} = 1.91$, $p < 0.05$), but specific responses varied depending on the functional group (Table 3). With the exception of corticated and filamentous algae, most groups were affected by the intensity of nutrient addition (either as main effect or in interaction with Shore). In particular, the percentage cover of foliose algae was greater in HI treatments (Fig. 5). In contrast, the percentage cover of leathery (Shore 2), articulated (Shore 1) and encrusting algae (both shores) was adversely affected by the high intensity of nutrient addition (Fig. 5). The percentage cover of leathery algae was also greater in HV than LV treatments (Table 3, Fig. 5). Furthermore, temporal variability of nutrient addition had a significant effect on percentage cover of filamentous algae and the total number of native taxa, although this effect was not consistent between shores (Table 3). Independent of the intensity of nutrient addition, the percentage cover of filamentous algae was greater in LV treatments on Shore 1, whereas on Shore 2 the percentage cover was greater in HV treatments. The number of native taxa was slightly larger under LV than in the HV experimental condition, but only on Shore 2 (Fig. 5).

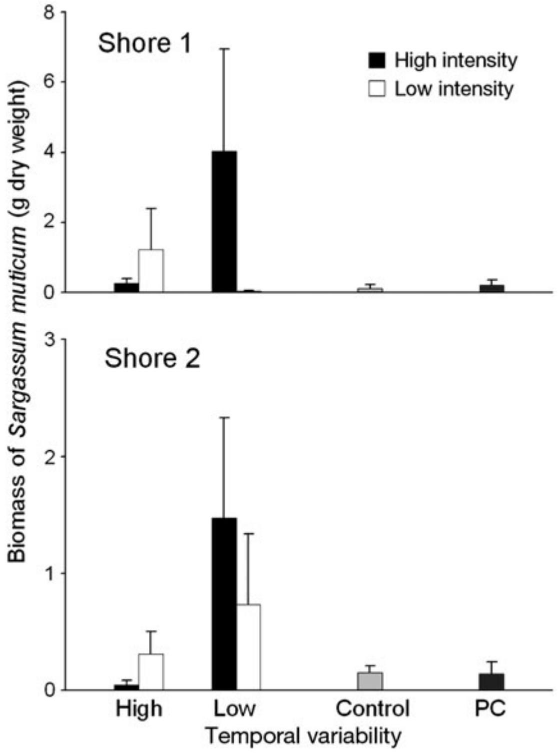

Fig. 4. *Sargassum muticum.* Mean (+SE, n = 28) biomass of plants on Shore 1 and Shore 2, according to intensity and temporal variability of nutrient addition. PC = procedural control. Means are calculated by averaging data from each plot (4) over time (7 dates)

Table 2. *Sargassum muticum.* ANOVA on the effect of Shore (S), Intensity (I) and Temporal Variability (V) on the mean biomass of plants in each plot. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Source of variation	Biomass of *Sargassum muticum* df	MS	*F*
Shore = S	1	384.4	1.02
Control vs Treatments	1	10.55	0.03
Intensity = I	1	124.03	0.38
Temporal Variability = V	1	2064.03	6.35**
I × V	1	3.78	0.02
S × Control vs Treatment	1	434.26	1.34
S × I	1	331.53	1.02
S × V	1	913.78	2.81
I × V × S	1	282.03	0.87
Residual	30	324.89	
Total	39		
Cochran's *C*-test	0.3534		
Transformation	None		

DISCUSSION

This study investigated the role of overall intensity and temporal variability of nutrient addition in modulating the spread of the invasive alga *Sargassum muticum* on intertidal shores. Results indicated interactive effects between intensity and temporal variance of nutrient addition on the number and length of plants. However, for the number of plants the response was not consistent between shores. A larger number of plants occurred in the low intensity and high temporal variability treatments on Shore 1, whereas on Shore 2 the largest number was observed in the high intensity and low variability treatments. Furthermore, plants were longer in the low intensity treatments under high temporal variability, whereas under low temporal variability plants were longer in the high intensity treatments. Caution is needed in interpreting these results due to the presence of artefacts (see 'Results'). However, since plants grew more in control than in procedural controls we are confident with the results, although, we could be underestimating the effect of treatments. On the other hand, mesh bags were used in all experimental treatments and, therefore, potential effects on the growth of plants should have been similar among treatments. Finally, biomass of *S. muticum* was affected by temporal variability of nutrient addition, independently of its intensity. Therefore, the original hypothesis that high intensity combined with high variability of nutrient addition would increase the invasibility of algal assemblages was not supported.

Several studies have shown a higher abundance of invasive species, or a better performance of particular taxa in human-disturbed habitats than in natural ones (Hobbs & Huenneke 1992, Lambrinos 2002), probably

Table 3. Results of Population-Averaged Generalized Estimating Equations examining the effects of Shore (S), Intensity (I) and Temporal Variability (V) of nutrient addition on native assemblages. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Abbreviations as in Table 1

Contrasts	Number of native taxa E	Number of native taxa SE	Encrusting algae E	Encrusting algae SE	Leathery algae E	Leathery algae SE	Corticated algae E	Corticated algae SE	Articulated algae E	Articulated algae SE	Filamentous algae E	Filamentous algae SE	Foliose algae E	Foliose algae SE
Intercept	2.47***	0.01	4.63***	0.28	9.70***	0.50	20.00***	1.02	17.72***	0.78	9.00***	0.52	10.44***	0.69
Control vs PC	−0.05*	0.02	−1.06	0.60	5.34***	1.17	0.22	2.20	−7.88***	1.32	0.80	1.39	0.25	1.74
PC vs Treatments	−0.01	0.01	0.34	0.19	−0.65*	0.33	−0.43	0.71	2.08***	0.50	−0.57	0.40	−0.41	0.53
HI vs LI = I	−0.01*	0.01	−1.51***	0.36	−2.47***	0.65	0.57	1.26	−0.35	1.05	0.86	0.57	2.64***	0.75
Shore 1 vs Shore 2 = S	−0.06***	0.01	2.99***	0.28	−2.42***	0.50	5.76***	1.02	−2.81***	0.78	2.36***	0.52	0.26	0.68
HV vs LV = V	0.03*	0.01	−0.01	0.36	1.24*	0.65	0.57	1.26	−1.53	1.05	−0.08	0.57	0.31	0.75
S × I	−0.01	0.01	−0.29	0.36	1.87**	0.65	−0.13	1.26	−2.06*	1.05	0.29	0.57	0.22	0.75
S × V	−0.03**	0.01	−0.29	0.36	−0.66	0.65	0.56	1.26	−1.67	1.05	−1.20*	0.57	−0.37	0.75
I × V	0.00	0.01	−0.47	0.36	0.25	0.65	−0.96	1.26	0.69	1.05	0.16	0.57	0.69	0.75
Correlation parameter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Scale parameter	0.43	0.03	29.12	3.99	85.67	10.88	349.27	47.88	209.00	18.32	92.17	11.58	159.18	27.61

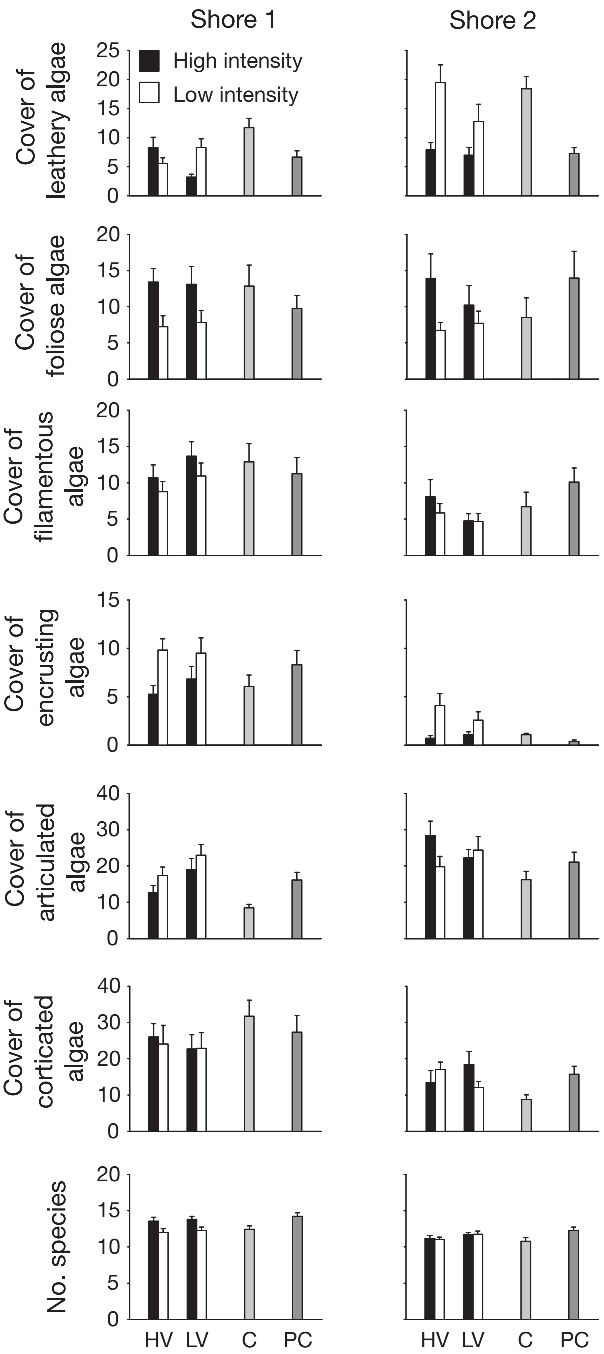

Fig. 5. Mean (+SE, n = 28) percentage cover of leathery, foliose, filamentous, encrusting, articulated and corticated algae and number of native taxa over 7 dates of sampling on Shore 1 and Shore 2, according to intensity and temporal variability of nutrient addition. HV = high temporal variability, LV = low temporal variability, C = control, PC = procedural control. Means are calculated by averaging data from each plot (4) over time (7 dates)

due to advantageous properties (e.g. reproductive strategies, growth rate, life span) of the invader that are lacking in native or pre-existing species (Thompson 1991). Thus, in coastal systems, nutrient enrichment has been reported to play an important role in facilitating the establishment of invasive macroalgae (e.g. Ceccherelli & Cinelli 1997, Steen 2003, Sánchez & Fernández 2006). For example, the establishment of the invasive green alga *Caulerpa taxifolia* was favoured by high nutrient loads in sediments (Ceccherelli & Cinelli 1997), while the spread of *Sargassum muticum* was modulated by interactions between nutrient enrichment and space availability (Sánchez & Fernández 2006). In this study, the most severe combinations of intensity and temporal variability (high intensity and high temporal variability) of nutrient addition were not positively related to the establishment and growth of plants. It is possible that the high intensity–high temporal variability treatment exceeded critical values of disturbance and it might have offset any positive indirect effect resulting from the release of resources, i.e. nutrients, or from facilitative influences by native species. Thus, the high intensity combined with the high temporal variability of nutrient addition might have prevented the settlement and establishment of *S. muticum* propagules. Indirect mechanisms mediated by effects of nutrient addition on other components of native assemblages are difficult to hypothesize on the basis of our findings. Nevertheless, the importance of indirect effects in regulating the establishment and spread of non-indigenous species has been previously suggested (e.g. Duggins et al. 1990, Piazzi et al. 2003). For example, Piazzi et al. (2003) indicated that disturbance can hinder invasion of *Caulerpa racemosa* by removing facilitators like turf-forming algae. In this context, the identity of functional groups of native species has been shown to be a key factor in the invasion process either in terrestrial (e.g. Symstad 2000, Dukes 2001) or marine habitats (Arenas et al. 2006, Britton-Simmons 2006, Sánchez & Fernández 2006).

Nutrient addition has also marked effects in shaping the structure and composition of macroalgal native assemblages (Worm & Sommer 2000, Fong 2003, Korpinen et al. 2007). It is worth noting that macroalgae have diverse strategies to exploit nutrient enrichment depending on their physiological differences (Worm & Sommer 2000). Filamentous algae have a rapid nutrient uptake and fast growth (Rosenberg & Ramus 1984, Hein et al. 1995), whereas canopy-forming species have slower nutrient uptake and growth rates (Pedersen & Borum 1996). In this way, the basic processes operating through the spread of native species are essentially the same as those that facilitate the expansion of invasive species (Davis et al. 2000). Not only is the intensity of nutrient supply important in shaping the structure of native assemblages, but so is the temporal variability; i.e. the interval of time between subsequent events may be a key factor affecting the response of seaweeds in benthic assemblages (Picket & White 1995, Worm & Sommer 2000, Bertocci et al. 2005). In fact, theoretical and empirical studies have indicated that changes in spatial and temporal variability of physical and biological processes can have significant effects on species and structure of assemblages (e.g. Davis et al. 2000, Benedetti-Cecchi et al. 2006).

Intensity of nutrient enrichment and temporal variability to some extent affected native assemblages with the intensity of nutrients being an important driver of the percentage cover of the most abundant functional groups. In general, percentage cover of most functional groups increased with increasing nutrient addition with the exception of articulated, encrusting and leathery algae. It is also interesting to highlight that fact that the effects of intensity and temporal variability of nutrient addition on native assemblages and invasion by *Sargassum multicum* varied between shores. This variation in magnitude and/or direction of effects may indicate intrinsic differences between shores, which may influence the invasion process. Thus, small variations in abiotic and/or biotic factors may affect settlement and survivorship of *S. multicum* recruits. For example, differences in topography of substratum and wave exposure can cause variations on settlement and dispersal rates of *S. multicum* recruits (Engelen et al. 2005, Strong et al. 2006). Differences in grazing pressure and composition of native assemblages, i.e. canopy and understory species, may also affect survivorship and establishment of *S. multicum* (Hawkins & Hartnoll 1983, Arenas et al. 2006, Sánchez & Fernández 2006). For example, differences in percentage cover of the leathery alga *Fucus vesiculosus* (canopy-forming alga) might in part explain different invasion rates on both shores. Therefore, although nutrient fluctuations can increase the invasibility of macroalgal assemblages, the process also depends on the particular species present in the system.

Several experimental and observational studies have highlighted the importance of native seaweed species in shaping patterns of invasion by *Sargassum muticum* (e.g. Andrew & Viejo 1998, Britton-Simmons 2006). These direct interactions are, however, likely to change depending on the habitat and the stage of the invasion. For example, canopy algae, such as *Bifurcaria bifurcata*, and encrusting and turf-forming algae inhibited recruitment of *S. muticum* at intertidal and subtidal sites, respectively (Britton-Simmons 2006, Sánchez & Fernández 2006). However, after settlement, canopy-forming algae seemed to be an environment that enhanced survivorship of *S. muticum* recruits in intertidal sites (Sánchez & Fernández 2006). Our results showed a higher number of *S. muticum* plants when percentage cover of leathery algae was small (Figs. 3 & 5). It is very likely that a large cover of leathery algae could inhibit recruitment of this species although the mechanism underlying this phenomenon is not clear (e.g. sedimentation, shading, space preemption or herbivory by canopy-associated herbivores) (see Sánchez & Fernández 2006). In turn, a small cover of leathery algae could create favourable conditions, such as appropriate moisture and/or protection from direct sunlight, for establishment of *S. muticum* recruits. Nevertheless, once *S. muticum* had recruited into the assemblages, other algae might have affected this variable because different functional groups within the assemblage preempt key limiting resources in different stages of invasion (see Britton-Simmons 2006, Sánchez & Fernández 2006). The number of plants was also higher when the percentage cover of filamentous algae decreased (mainly on Shore 2; Figs. 3 & 5). These results partially agree with previous studies on *S. muticum* that found that recruitment was inhibited by encrusting and turf-forming algae, whereas the survivorship was reduced by canopy and understory algae (Britton-Simmons 2006). In contrast, Sánchez & Fernández (2006) found that in the initial stage of invasion, the canopy species *B. bifurcata* inhibited recruitment by *S. muticum*, whereas understory species (foliose and filamentous algae) did not have a significant effect on invasion success. Results of this study suggest that leathery algae may have played an important role in the invasion by *S. muticum*, since a greater number of plants of the invader were found when percentage cover of leathery algae was small. Filamentous algae might have also exerted a negative although more attenuated effect on the invader.

Elton's (1958) concept of biotic resistance implies a negative relationship between diversity and invasibility through a more efficient use of resources by native species in more diverse assemblages (Loreau 2000). In this study, however, the observed difference in the number of native species in relation to different treatments of intensity and temporal variability of nutrient addition was small. Such a difference could hardly explain, for example, the drastic reduction of *Sargassum muticum* in the high intensity and high temporal variability treatments on Shore 1. The overall resistance of assemblages to invasion might be affected by the additive effects of all functional groups summed over the invader's entire life cycle rather than the functional diversity per se. Alternatively, the lack of an obvious enhancement in the use of resources with increasing functional diversity could explain this lack of relationship between functional diversity and invasibility.

We have provided the first evidence that mean intensity and temporal variance of nutrient addition interactively affected the invasion success of native assemblages by *Sargassum muticum* on intertidal rocky shores. Our results also highlight the need for a better understanding of the requirements of the invader throughout its entire life cycle, as well as the role of different functional groups in shaping the invasion process. Species invasions are one of the main ecological consequences of global changes in climate and land use (e.g. Vitousek et al. 1996, Davis et al. 2000, Pimentel et al. 2000). To respond effectively to this threat, ecologists must start the essential task of transforming the study of invasions into predictive science. Therefore, more experimental studies investigating the relationships between disturbance and invasions are important to eventually predict the consequences of anthropogenic disturbance and global climate change on invasibility of native assemblages and subsequently identify appropriate strategies for environmental management and conservation.

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