THE REGIME OF CLIMATE-RELATED DISTURBANCE AND NUTRIENT ENRICHMENT MODULATE MACROALGAL INVASIONS IN ROCKPOOLS

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Abstract

Patterns of invasion by the seaweeds *Grateloupia turuturu* Yamada and *Sargassum muticum* (Yendo) Fensholt under crossed combinations of the regime (mean intensity and temporal variability) of climate-related mechanical disturbance and constant nutrient enrichment were experimentally examined in rockpools in north Portugal. The cover of both species was larger under high compared to low intensity of disturbance, but this effect was enhanced by events more heterogeneously distributed over a period of nineteen months. The invasion by *G. turuturu* was also larger in enriched pools, but only when disturbance was applied at high intensity. The richness of native taxa was increased by high intensity events of disturbance evenly distributed over time and by low intensity events heterogeneously distributed, while no differences were documented for other treatments. Temporal variability of disturbance and nutrients interactively affected the total cover of native taxa and the availability of bare rock in different directions. Enriched conditions increased the space occupancy by natives and reduced substratum availability only when associated to heterogeneous events of disturbance. At the same time, relatively more variable disturbances caused a reduced cover by native taxa and an increased availability of free space, but only under natural nutrient levels. Present findings contribute to understand the conditions that would be more likely to facilitate the spread of *G. turuturu* and *S. muticum* under current and predicted scenarios of compounded environmental changes and in relation to traits of recipient systems that are considered relevant for the success of invasions, including the native richness and the degree of usage of resources, i.e. primarily space.

Keywords Disturbance - *Grateloupia turuturu* - Mean intensity - Nutrient enrichment - Rocky intertidal - *Sargassum muticum* - Temporal variance

Introduction

Natural systems are directly and indirectly impacted by a range of increasing environmental changes due to human activities (Wilcove et al. 1998; Sala et al. 2000; Halpern et al. 2008). Concerning the marine realm, in particular, anthropogenic activities occurring along the shore or inland can increase inputs of contaminants and nutrients into coastal waters (Vitousek et al. 1997) and subtract natural habitats (Gray 1997; Airoldi and Beck 2007). Sea-based activities can drastically reduce resources, increase pollution and alter biodiversity through the introduction of non-indigenous species (Pauly et al. 2005; Worm et al. 2006; Williams and Smith 2007). Therefore, understanding, and possibly predicting, the effects of such environmental stressors is a major focus of current ecological research as it is key to support a sustainable use of marine resources able to protect ecosystems without jeopardizing the services they provide (Costanza et al. 1997). This goal is complicated by the large variability in patterns of occurrence of concomitant anthropogenic and natural processes (Halpern et al. 2007; Wernberg et al. 2012), which can interact each other and with life-traits of organisms in complex ways (Paine et al. 1998, Bertocci et al. 2005; Pincebourde et al. 2012). Such difficulty is particularly evident in the context of assessing the effects of climate-related environmental stressors, which act in combination (Harley et al. 2006; Darling and Côté 2008), but have been examined individually (e.g. Feely et al. 2004, Bertocci et al 2005; Benedetti-Cecchi et al. 2006; Vaselli et al. 2008; Porzio et al. 2011), with just a few exception (Martin and Gattuso 2009; Russell et al. 2009; Connell and Russell 2010). There is a general lack, in particular, of experimental studies examining the simultaneous and interactive effects of multiple global stressors on biological invasions (but see Vaz-Pinto et al. 2013a), although the spread of non-indigenous species and global (i.e. climate) change are concomitantly considered among the most important threats to marine systems (Stachowicz et al. 2002a; Diez et al. 2012; Hoegh-Guldberg and Bruno 2012). In fact, a vast amount of research has been aimed at exploring diversity-invasibility relationships (Elton 1958; Stachowicz et al. 1999), showing in several cases greater resistance to invasions for more diverse native assemblages (e.g. Stachowicz et al. 1999, 2002b) or the importance of native species identity besides species richness (Crawley et al. 1999; Arenas et al. 2006). The mechanisms of such processes, however, have to be elucidated, with special focus on how biological invasions are modulated by climate-related factors themselves able to affect the regime of disturbances and to alter patterns of abundance and distribution of assemblages (Dukes and Mooney 1999; Bertocci et al. 2005; Benedetti-Cecchi et al. 2006; Vaselli et al. 2008; Widdicombe and Spicer 2008; Molinos and Donohue 2011; Buckley and Kingsolver 2012). Specifically, climate-related disturbances can facilitate biological invasions by increasing the competitive ability of introduced species (Ruiz et al. 1999; Byers 2002; Sorte et al. 2010, Vaz-Pinto et al. 2013a), but very severe conditions could reduce invasion success by directly reducing the abundance and impairing relevant life-history traits of introduced species (Incera et al. 2010). Moreover, considerable variations in rates and spatio-temporal patterns of invasions can be driven by complex interactions of multiple traits of a single stressor or of multiple stressors (e.g. Engelen et al. 2005; Incera et al. 2009, 2010; Vaz-Pinto et al. 2013a, b).

The present study was aimed at experimentally assessing the effects of changes in the regime (i.e. mean intensity and temporal variability) of climate-related mechanical disturbance and nutrient enrichment on the invasion of algal and invertebrate assemblages from rock pools by non-indigenous macroalgae. Empirical observations and climate models indicate changes in patterns of occurrence of extreme meteorological events, such as storms, flood and droughts (e.g. Allen et al. 2000; Muller and Stone 2001; Benestad 2003). Such changes include increases in the mean intensity and the temporal variability, including both the frequency and the variance, of associated disturbance (Muller and Stone 2001; Gutschick and BassiriRad 2003), with potential separate and interactive effects on natural populations and assemblages (Zavaleta et al. 2003; Bertocci et al. 2005; Benedetti-Cecchi et al. 2006, Vaselli et al. 2008; Stuart-Smith et al. 2010; Zimmermann et al. 2010; Molinos and Donohue 2011; Buckley and Kingsolver 2012; Maggi et al. 2012). Stormy waves, for example, can remove organisms from the substratum (e.g. Benedetti-Cecchi 2000) proportionally to the intensity of disturbance, but they can also release resources that can indirectly enhance the local density of populations depending on life-traits of organisms (Connell 1978; Keough 1984; Dayton et al. 1984; Huston 1994; Shumway and Bertness 1994; Bertocci et al. 2005). Analogously, increases of nutrient concentrations are a global phenomenon in coastal areas, mainly due to terrestrial runoff associated to the overuse of land, deforestation and fish farms (Ambasht and Ambasht 2003), which can drive the structure and functioning of benthic assemblages through direct positive effects on primary producers (e.g. Nielsen 2001) or through indirect interactions with biological processes, such as grazing (Worm et al. 2000; Hillebrand 2003). Meteorological events and nutrient inputs to coastal areas are strictly linked, on local to global scales, by increasing coastal erosion and runoff strongly affected by the anthropogenic alteration of the hydrological cycle, particularly of the spatial and temporal patterns and the intensity of precipitation (Vitousek et al. 1997; French 1997). Both physical disturbance and nutrient availability are hypothesized and, in some cases, documented as being able to influence the susceptibility/resistance of natural systems to invasions. For instance, high degrees of disturbance could promote invasions by releasing resources and reducing competition with native species (Burke and Grime 1996; Davis et al. 2000; Prieur-Richard et al. 2000). According to the Fluctuating Resources Availability Theory (FRAT, Davis et al. 2000), increased nutrient inputs could represent a new availability of resources, not fully used by native species, that could facilitate invasions (e.g. Steen 2003; Gross et al. 2005; Leishman and Thomson 2005; Sánchez and Fernández 2006). Such processes can be drastically affected also by the timing of single or repeated disturbances depending on its match with phases of the life cycle of an introduced species that are particularly relevant for the success of invasion (Hobbs and Huenneke 1992; Moyle and Light 1996; Altman and Whitlatch 2007; Bishop and Kelaher 2007). Recent experimental studies have examined the separate or interactive effects of changes in mean intensity and temporal variability of single (e.g. Bertocci et al. 2005; Benedetti-Cecchi et al. 2006, Vaselli et al. 2008; Bulleri et al. 2010; Incera et al. 2010; Molinos and Donohue 2011) or multiple (e.g. Olabarria et al. 2013) sources of physical disturbance on benthic assemblages and invasive macroalgae and those of increased amounts and changing regimes of nutrient inputs alone (Incera et al. 2009) or combined with climate variables (i.e. temperature and CO_2 partial pressure, Vaz-Pinto et al. 2013a) and biological processes (i.e. grazing, Vaz-Pinto et al. 2013b) on the invasibility of rock pool assemblages. To our knowledge, the simultaneous effects of changes in mean intensity and temporal variance of storm-related mechanical disturbance combined with increased availability of nutrients were never experimentally examined before.

Rock pool intertidal assemblages exposed to the invasion by non-indigenous seaweeds (i.e. the red *Grateloupia turuturu* Yamada and the brown *Sargassum muticum* (Yendo) Fensholt) in north Portugal were used as an ideal study system. Intertidal habitats are generally suitable for experimental studies due to their accessibility and critical position at the interface between the terrestrial and the marine realm, thus being exposed to a range of anthropogenic disturbances from both environments. In addition, macroalgae have been shown to represent up to 40% of all the invasive species in marine habitats (Schaffelke et al. 2006), with relevant ecological effects (see Schaffelke and Hewitt 2007; Thomsen et al. 2009 for reviews). *Grateloupia turuturu* and *Sargassum muticum*, in particular, are native Asian seaweeds indicated as important invaders of intertidal and shallow subtidal habitats worldwide. The first species has been recorded in Atlantic Europe, North America and the Mediterranean Sea (Cabioc'h et al. 1997; Villalard-Bohnsack and Harlin 1997) and, more recently, in Australia and New Zealand (Saunders and Withall 2006; D'Archino et al. 2007), the second in Europe and North America (Critchley et al. 1983; Pérez-Cirera et al. 1989; Incera et al. 2011).

We established a 19-months long manipulative experiment in rock pools involving treatments where changes in the regime, i.e. mean intensity and temporal variability (Benedetti-Cecchi 2003; Bertocci et al. 2005), of storm-like mechanical disturbance were crossed with natural vs experimentally increased availability of nutrients. Treatments were compared in terms of cover of *G. turuturu* and *S. muticum* in order to assess which combinations of conditions were associated to a relatively larger susceptibility of local assemblages to the invasion by these species. Such knowledge is key to understand invasion dynamics under current and predicted changes of combined, globally relevant, anthropogenic drivers of patterns of biodiversity on rocky shores.

Materials and methods

Study system

The study was performed between February 2012 and September 2013 in a total of 39 tide pools selected at random out of those located along 1 km of rocky coast in northern Portugal (between 41°42'01"N and 41°42'16"N). Pools ranged from 2.5 m to 7 m in length, from 1.4 m to 4.8 m in width and from 6 m^2 to 33 m^2 in area. A preliminary analysis was done to guarantee that the mean size of replicate rockpools did not statistically differ among each experimental condition (data not shown). This shore is almost rectilinear, oriented from north to south and typically granitic, with semidiurnal tides reaching a maximum amplitude of about 4 m. All sampled pools were located at low-mid intertidal level (between 0.5 m and 1 m above Chart Datum). The coast is exposed to prevailing NW swells associated to severe wave disturbance, particularly between October and March (Dias et al. 2002). Large variations in nutrient concentration are recorded over the year, driven, in particular, by upwelling events that are more intense from April to September (Lemos and Pires 2004). A relaxation of upwelling, however, has been reported in the area during the last years (Lima et al. 2006). Thus, the relative contribution of terrestrial runoff to the coastal concentration of nutrients can be expected to increase in the near future as a consequence of nearby land-based activities, which could also cause a coastal nutrient enrichment more spread over the year. For example, high nutrient levels have been found in the Lima estuary, located about 1.5 km south of the study area (Costa-Dias et al. 2010), and an increase in the concentration of nitrate has been reported for the upstream reservoir of the Douro river (Magalhães et al. 2005), increasing loads to the estuary (about 60 km south of the study area).

At the examined intertidal height, rockpools are characterized by diverse algal and invertebrate assemblages, whose spatial and temporal patterns of distribution, abundance and diversity have been described in detail (Araújo et al. 2011; Rubal et al. 2011; Bertocci et al. 2012), including the occurrence of both *G. turuturu* and *S. muticum* (e.g. Araújo et al. 2011; Cacabelos et al. 2013).

Experimental design and procedures

Before the start of the experiment (January 2012), three out of the 39 rock pools were chosen at random to be left unmanipulated as controls, while three other were allocated to each combination of the following crossed treatments (Fig. 1): intensity of disturbance (low vs. high); temporal variability of disturbance (low vs. large); sequence of disturbance (two arrangements, replicated only within the large level of variability); nutrient availability (natural vs. enriched). Three plots (35 x 35 cm) were established at random in each pool and marked at corners with screws.

Experimental disturbance consisted of striking the substratum of each plot by means of a chisel mounted on a battery hammer (Bertocci et al. 2005). Such disturbance was intended to simulate the mechanical impact of waves during severe storms. In fact, extreme storms are able to remove patches of organisms from intertidal habitats (e.g. Underwood 1998), in most cases generating patches of free space interspersed within intact assemblages in a way and with spatial patterns comparable to our experimental disturbance (e.g. Benedetti-Cecchi and Cinelli 1994). For this goal, the top of the chisel was covered with a plastic plug to produce a mechanical impact in terms of battering rather than scraping and breaking the rock. Levels of intensity were produced by disturbing the whole area of each quadrate once (low, LI) or twice in a row (high, HI).

The temporal variability was manipulated by establishing two experimental levels characterized by a different temporal arrangement of the same total number (i.e. five) of events of disturbance over the period of the experiment (Benedetti-Cecchi 2003; Bertocci et al. 2005). This procedure kept the overall frequency of experimental events the same between the two levels of variability, allowing to separate the possible effects of temporal variability 'per se' from those of the total intensity of disturbance applied over the experiment (Benedetti-Cecchi 2003). In order to establish realistic treatments, the total number of experimental events was chosen with reference to the number of extreme storms (defined as those characterized by maximum wave height larger than 6 m and lasting a minimum of 9 hours) naturally occurred at the study area between 2001 and 2010 (details in Appendix 1). The first level of temporal variability (hereafter indicated as Reg) included events homogenously distributed over time (i.e. one every four months), thus being characterized by a null variance of the intervals of time between consecutive events (Fig. 1). The second level (hereafter indicated as Irreg) was characterized by five events arranged in a way that some of them were clustered in shorter periods compared to the Reg level, separated by prolonged periods without experimental disturbances (Fig. 1). To tease apart possible effects of temporal variability from those of the specific arrangement of events used to produce the desired level of variability (Vaselli et al. 2008), the Irreg treatment was produced according to two, randomly chosen, sequences (S1 and S2) of events, both characterized by the same positive value (i.e. 21 months^2) of the variance of the time intervals between consecutive disturbances (Fig. 1).

Nutrient enrichment was performed using slow-release coated fertilizing pellets (Osmocote® Exact® Standard 15+3.9+9.1+1.5 Mg, corresponding to 7 % nitrate N and 8 % ammoniacal N, 9% P_2O_5, 11% K_2O and 2.5.% MgO, plus microelements) (Worm et al. 2000). A plastic bag (2 x 2 mm mesh size) filled with 200 g of pellets was placed into a cylindrical PVC dispenser (220 mm long, 50 mm diameter) bearing 50 holes (3 mm diameter) evenly distributed over the surface. In February 2012, two replicate dispensers were screwed to the bottom of each replicate pool assigned to the enriched treatment. Empty dispensers filled with 200 g of gravel were placed in all other pools as procedural control. In order to maintain the experimental conditions, all bags were removed every two months and replaced with new ones containing "fresh" pellets (or gravel).

Data collection

To assess the effectiveness of the experimental enrichment, water samples from a randomly chosen set of eight rock pools assigned to each level of the nutrient treatment were collected in each of three bi-monthly periods the fertilizer was applied in the field before its replacement. Water sampling was repeated at the beginning and at the end (first and last two weeks, respectively) of each period in order to assess the maintenance of experimental conditions over the desired time. At each sampling occasion, two 10 ml replicates were collected from each pool with a syringe and put in ice for transportation to the laboratory. There, the concentrations of nitrate (NO_3) and phosphate (PO_4) in each water sample were measured with a colorimetric auto-analyzer (Skalar® SAN Plus Segmented Flow Analyser), using Skalar methods M461-318 (EPA 353.2) and M503-555R (Standard Method IP-450), respectively, and validating the analytical procedures with reference to samples containing known concentrations of each nutrient.

The three plots established in each pool were sampled visually at the end of January 2012, before the start of any experimental manipulation (s0), and at each of ten dates (s1 to s10) between May 2012 and September 2013. Sampling dates were established in a way that the average time elapsed since the previous event of disturbance did not differ between all levels of temporal variability and sequences of disturbance (Fig. 1), otherwise representing a potential confounding factor due to the structure of the experiment (Bertocci et al. 2005). As a consequence, response variables could be examined only integrated over the whole course of the study, but not at single dates of sampling. Specifically, we examined the mean percentage cover of each invasive seaweed, the total cover and richness of individual taxa and the availability of bare rock over the s1 to s10 dates.

Sampling was done using a 30 x 30 cm frame applied in the centre of each plot (to avoid edge effects) and divided into twenty-five 6 x 6 cm sub-quadrates. Percentage cover values of individual taxa and bare rock were visually estimated according to Dethier et al. (1993). The richness of native taxa was quantified as number of taxa in each frame, once organisms were identified to the finest level of taxonomic resolution achievable in the field.

Data analysis

Two-way analyses of variance (ANOVA) were used to compare the concentrations of nitrate and phosphate in the water collected from experimentally enriched and natural pools in each occasion (details in Appendix 2).

Univariate (ANOVA) and multivariate (multivariate analysis of variance based on permutations, PERMANOVA: Anderson 2001) statistical techniques were performed on data collected before any manipulation (s0 sampling). The goal was to assess that the abundance of *G. turuturu* and *S. muticum* and the structure of whole native assemblages, respectively, did not differ among the pools allocated to each subsequent experimental condition, which could have confounded the effects of treatments. Both analyses were based on the same partially asymmetrical design, including a first partitioning of the total variability among all experimental levels into a 'Control vs. Treatments' contrast and the 'Among treatments' variation. The latter was then partitioned into the main effects of 'Temporal variability', 'Intensity' of disturbance and 'Nutrients' and their interactions. Each test involving 'Temporal variability' was further partitioned into a 'Reg vs. Irreg' and a "Between sequences" contrast, examined as main effects or in interaction with the other two factors (see Tab. 1 and Appendix 3). The three plots sampled in each pool were averaged and the three pools assigned to each experimental condition provided the replicates for the analyses. Tests of terms involving sources of variation specific for Reg vs. Irreg treatments were constructed using the natural denominator for that term, instead of the residual mean square. For example, the 'Intensity x (Reg vs. Irreg)' effect was tested over the 'Intensity x Sequences' term.

Cover values of each invasive species, native organisms as a whole and bare rock and the richness of native taxa averaged over ten dates of sampling were analyzed with ANOVA using the same design illustrated for s0 data.

Before each ANOVA, the assumption of homogeneity of variances was checked with Cochran's *C* test. If necessary, data were log-transformed. When this was not possible, untransformed data were analyzed and results considered robust if not significant ($p > 0.05$) or significant at $p < 0.01$, to compensate for the increased probability of Type I error

(Underwood 1997). When relevant, Student-Newman-Keuls (SNK) tests were used for ‘a posteriori’ comparisons of means.

Results

Initial conditions and effectiveness of nutrient enrichment

The analyses of data collected immediately before the application of any experimental treatment (s0: January 2012) indicated no significant main or interactive differences in the structure of the whole native assemblage and in the abundance of both *G. turuturu* and *S. muticum* among rock pools assigned to each experimental condition (details in Appendix 2).

In spite of large variability among individual pools and between sampling occasions, the experimental treatment was, in general, effective to increase the concentration of nutrients, with nitrate increasing, on average, by about 30% and 145% and phosphate by about 20% and 60% in enriched compared to natural pools. The only exception was provided by the samples collected in April 2013, which showed just a non-significant increase in the availability of both nutrients (details in Appendix 3).

Effects of experimental treatments

The invasive species were less abundant in unmanipulated pools than, on average, in treated pools (Tab. 1, Fig. 2 A and B) and were affected in a similar way by the interaction between intensity and temporal variability of disturbance (Tab. 1). Specifically, both *G. turuturu* and *S. muticum* were completely absent in rock pools subject to LI disturbance independently of the temporal arrangement of events, while they were found in HI pools, with cover values relatively larger when events were established heterogeneously over the period of the experiment (Tab. 2, Fig. 2 A and B). In addition, *G. turuturu* responded to the combination of the intensity of disturbance with nutrient enrichment (Tab. 1). Nutrients, in particular, did not obviously exert any effect when disturbance was applied at LI as this species was absent under such treatment, while they increased the cover of *G. turuturu* when combined with HI disturbance. As for the I x V interaction, this pattern also implied that the abundance of *G. turuturu* was always larger in HI than in LI pools, but the entity of this difference was relatively larger under the enriched condition (Tab. 2, Fig. 3).

The interaction of intensity and temporal variability of disturbance affected the total number of native taxa (Tab. 1). When events were evenly distributed, the richness of taxa increased at increasing intensity of disturbance, while no significant differences in this response variable were detected under aggregated events (Tab. 2, Fig. 2 C). Moreover, LI disturbance events irregularly distributed over time were associated to a larger number of taxa compared to regularly distributed ones, while HI events did not exert any significant effect irrespectively of their temporal variability (Tab. 2, Fig. 2 C).

Both the total cover of native taxa and the availability of bare rock differed significantly between the unmanipulated control and the average of experimental treatments and were affected by the temporal variability of disturbance in combination with the availability of nutrients (Tab. 1). Compared with the control, experimental treatments determined an overall reduction of the first variable (Fig. 4 A), while the opposite pattern was displayed by the percentage of bare rock (Fig. 4 B). Both variables did not change with the availability of nutrients under Reg disturbance, while opposite patterns were documented under the Irreg treatment, with the enrichment determining, respectively, larger and lower cover values of native taxa and bare rock compared to the natural condition (Tab. 2, Fig. 4 A and B). Finally, the Irreg treatment was associated to a lower cover of native taxa and a larger availability of bare rock compared to the Reg treatment under natural levels of nutrients, while the temporal variability of disturbance was irrelevant when combined with enrichment (Tab. 2, Fig. 4 A and B).

Discussion

Present findings indicate, in general, the high intensity of mechanical disturbance as the most relevant factor facilitating the invasion by both *G. turuturu* and *S. muticum* in rock pools, particularly when disturbance events were applied heterogeneously over the period of the experiment.

The FRAT (Davis et al. 2000) attributes to the availability of resources and, as a direct consequence, to any processes able to increase it, a key role in promoting biological invasions (e.g. Stachowicz et al. 1999; Naeem et al. 2000; Levine et al. 2004). Physical disturbance, in particular, can remove native organisms and facilitate invasions by reducing the competition for limiting resources (e.g. Hobbs and Huenneke 1992; Prieur-Richard and Lavorel 2000; Bulleri et al. 2010), an effect expectable to be positively related to the intensity of stress (Pausas et al. 2006). At the same time, the temporal variability of disturbance can affect invasion dynamics depending on the match between the timing of the events making resources available and the phases of the life cycle of the invader that are important for colonization, such as the periods of reproduction and recruitment (Dayton et al. 1984; D'Antonio et al. 1999). The observed positive effect of increasing intensity of disturbance events, enhanced by their larger temporal variability, is consistent with these models, but its possible mechanisms of action have to be identified.

The majority of empirical tests on how traits of disturbance modulate invasion dynamics were conducted in terrestrial or freshwater systems (e.g. D'Antonio and Vitousek 1992; Burke and Grime 1996; Lake and Leishman 2004; Kneitel and Perrault 2006). Only one previous study has included crossed manipulations of the mean intensity and the temporal variance of mechanical disturbance (the impact of boulders) to examine storm-related effects on the invasion by the green macroalga *Caulerpa racemosa* var. *cylindracea* in rock pools (Incera et al. 2010). This has reported one of the few cases where increasing intensity of disturbance thwarted seaweed invasions (reviewed by Williams and Smith 2007), likely due to a direct negative influence of very severe disturbance on the invader. The same mechanism did clearly not apply to the type of disturbance and invasive seaweeds examined here. It is acknowledged that different types of disturbance can drive invasions in different ways (Higgins and Richardson 1998; Larson 2003; Hill et al. 2005), but specific responses of *G. turuturu* and *S. muticum* have to be elucidated.

Unfortunately, ecological studies on *G. turuturu* have focused mainly on its patterns of distribution in non-native systems (Araújo et al. 2011 and references therein) and on its possible effects on invaded assemblages (Janiak and Whitlatch 2012), but none have specifically addressed effects of the regime of disturbance on its invasion dynamics. There is evidence, however, that different recruitment modes can allow this species to survive during the periods when environmental conditions are harsher, such as under increased storminess, reduced light and lower temperatures occurring in autumn-winter, and to effectively spread in the following more favourable period (Harlin and Villalard-Bohnsack 2001; Rubal et al. 2011). Moreover, *G. turuturu* has shown a relatively large ability to physiologically acclimatize to oxidative stress caused by chemicals, heavy metals, changes of salinity and extreme high and low temperatures (Liu and Pang 2010). Similarly, a positive relationship between intensity of disturbance and invasion success has been previously reported for *S. muticum*, although in most cases strongly dependent on concomitant factors, such as propagule pressure (Britton-Simmons and Abbott 2008), nutrient enrichment (Sánchez and Fernández 2006; Incera et al. 2009; Vaz-Pinto et al. 2013b) and the source of disturbance (Britton-Simmons and Abbott 2008). Therefore, the present facilitation of invasion under high intensity of disturbance, enhanced by larger temporal variability of events for both species and by nutrient enrichment for *G. turuturu*, is consistent, in general, with predictions from the

FRAT. In fact, our Irreg treatment implied that multiple events of disturbance were clustered in short periods of time (Benedetti-Cecchi 2003), a condition that could reduce the abundance of organisms and, consequently, release resources for relatively longer periods (Bertocci et al. 2005). Analogously, nutrient enrichment represents, particularly for algal organisms, an obvious increase of a resource known as being able to drastically affect patterns of distribution, abundance and diversity of coastal assemblages (Worm et al. 2000; Burkepile and Hay 2006). It is worth noting, however, that a considerable proportion of nutrients available in the water column can be used by benthic and planktonic micro- rather than macroalgae (e.g. Morel 1987; Sigmon and Cahoon 1997; Dalsgaard 2003). This process might have contributed to the relatively smaller effects of experimental nutrient enrichment, compared to those of mechanical disturbance, documented here.

The whole set of present findings, however, prevent the attribution of the above discussed effects to obvious invasion-relevant types of resources likely made available and interactions with traits of native biodiversity. Neither the richness and cover of native taxa nor, in an opposite direction, the availability of bare rock showed patterns of differences related to experimental treatments matching those of *G. turuturu* and *S. muticum* in the most expectable ways. Specifically, the total number of taxa was interactively affected by the intensity and the temporal variability of disturbance, with more severe events determining an increase of this variable only when regularly distributed over time. Taking into account the responses of both invasive macroalgae, this outcome clearly contrasts with the assumption that more diverse native assemblages should be more resistant to invasions due to a more efficient and complete use of resources by sets of native species covering a large range of ecological traits (Elton 1958; Loureau 2000; Stachowicz et al. 1999, 2002b). Some studies, however, have found a negative relationship between native diversity and invasibility (e.g. Lonsdale 1999), while others have shown effects of the identity overwhelming those of the richness of recipient assemblages (Arenas et al. 2006). It has also to be emphasized that a larger diversity is not necessarily paralleled by a larger degree of ecosystem functions, including the resistance to invasions (Mouquet et al. 2002). A positive relationship between diversity and resistance to invasions is likely to occur in systems characterized by niche complementarity among species, that would allow the hypothesized more effective use of resources (Moore et al. 2001). Where, instead, patchy patterns of local assemblages at different stages of succession and of available resources are maintained by a range of processes, such complementarity can fail. This might be the case for rock pool habitats, where abiotic and biological processes, such as physical variables, grazing and competition, can maintain a mosaic-like structure of assemblages independently of niche complementarity among species (e.g. Chapman 1990; Benedetti-Cecchi and Cinelli 1996). Under such circumstances, diversity effects could not occur through a complementary use of resources.

Finally, according to the FRAT, patterns of pre-emption of space by natives and of availability of free substratum negatively or positively, respectively, related to responses of *G. turuturu* and *S. muticum* in the same combinations of treatments could have been expected. Instead, both the cover of native taxa and the availability of bare rock were significantly affected by the interaction of nutrients and temporal variability of events, independently of the intensity of disturbance. These somehow surprising results possibly suggest that complex interactions of abiotic and biological processes might have affected patterns of invasion by *G. turuturu* and *S. muticum* in rock pools besides those specifically examined. For example, propagule pressure is recognized as a key factor potentially facilitating invasion success proportionally to the amount of available propagules (e.g. Grevstad 1999; Vaz-Pinto et al. 2012) and to the proximity to source populations of the invader (Bossenbroek et al. 2001). Grazing can also significantly affect invasions, usually in interaction with concomitant factors such as the availability of nutrients (e.g. Vaz-Pinto et al. 2013b) and propagules (e.g. Britton-

Simmons and Abbott 2008). Specifically addressing these issues was beyond the scope of the present study and a detailed discussion on how such processes might have been affected by experimental treatments would require excessive speculation. Nevertheless, a relevant role of grazing is unlikely in the present case, as the main grazer, i.e. the sea urchin *Paracentrotus lividus*, was always found in very low to null abundances in all experimental rock pools (personal observation), and some species of sea urchins are known to avoid areas invaded by *S. muticum* due to its low palatability (Britton-Simmons 2004).

In conclusion, present study attributes to the high intensity of disturbance a critical role in facilitating the initial invasion by both *G. turuturu* and *S. muticum* in rock pools, possibly enhanced by increased temporal variability of disturbance or nutrient availability for the first or the second species, respectively. Although the specific mechanisms are far from having been elucidated, this finding has relevant implications to understand, and possibly anticipate, invasion dynamics under current and predicted environmental changes directly or indirectly due to anthropogenic activities, with special focus on variations in mean intensity and temporal variance of climate-related variables (Katz and Brown 1992; Easterling et al. 2000; Muller and Stone 2001; Allen and Ingram 2002, Maestre et al. 2012).

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Table 1. Results of ANOVA examining the effects of intensity, temporal variability (Reg = regular treatment; Irreg = irregular treatment) of disturbance and nutrient enrichment on invasive macroalgae, native assemblages and bare rock. * p < 0.05, ** p < 0.01, *** p < 0.001, ns = not significant (p > 0.05).

		Grateloupia turuturu		*Sargassum muticum*		Native taxa richness		Native taxa cover		Bare rock	
Source of variation	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
All exp. levels	12	7.49	38.24***	105.47	33.00***	2.22	1.41	103.51	1.60	85.99	1.92
Control vs. treatments	1	5.78	29.52***	84.36	26.39***	0.68	0.44	268.88	4.15*	182.11	4.07*
Among treatments	11	7.64	39.03***	107.39	33.60***	2.36	1.50	88.47	1.36	77.25	1.73
Temporal variability = V	2	1.41	7.21**	11.67	3.65 *	2.34	1.49	90.33	1.39	132.35	2.96
Reg vs. Irreg	1	2.56	9.56	13.71	1.42	4.65	228.00 *	129.43	2.53	166.02	1.68
Between sequences	1	0.27	1.37	9.63	3.01	0.02	0.01	51.24	0.79	98.69	2.20
Intensity = I	1	75.16	383.72***	1096.64	343.10***	2.10	1.34	0.98	0.02	2.56	0.06
Nutrients = N	1	0.96	4.91 *	9.23	2.89	0.26	0.16	30.62	0.47	61.36	1.37

I x V	2	1.41	7.21**	11.67	3.65 *	5.46	3.48 *	64.49	0.99	42.63	0.95
I x (Reg vs. Irreg)	1	2.56	9.56	13.71	1.42	10.50	25.14	71.73	1.25	60.87	2.49
I x sequences	1	0.27	1.37	9.63	3.01	0.42	0.27	57.25	0.88	24.40	0.55
I x N	1	0.96	4.91 *	9.23	2.89	0.50	0.32	0.03	0.00	2.49	0.06
N x V	2	0.34	1.74	4.89	1.53	3.21	2.05	290.41	4.48*	196.86	4.40*
N x (Reg vs. Irreg)	1	0.29	0.74	9.00	11.50	6.11	19.15	385.49	1.97	259.92	1.94
N x sequences\	1	0.39	2.00	0.78	0.24	0.32	0.20	195.32	3.01	133.80	2.99
I x N x V	2	0.34	1.74	4.89	1.53	0.53	0.34	25.56	0.39	19.85	0.44
I x N x (Reg vs. Irreg)	1	0.29	0.74	9.00	11.50	0.08	0.08	9.34	0.22	16.88	0.74
I x N x sequences	1	0.39	2.00	0.78	0.24	0.97	0.62	41.78	0.64	22.82	0.51
Residual	26	0.20		3.20		1.57		64.83		44.77	
Cochran's test		*C* = 0.990, p<0.01		*C* = 0.514, p<0.01		*C* = 0.298, ns		*C* = 0.214, ns		*C* = 0.282, ns	
Transformation		None		None		None		None		None	

Table 2. Results of SNK tests for *a posteriori* comparisons of means significantly different according to ANOVA reported in Tab. 1. LI = low intensity, HI = high intensity; Nat = natural nutrients, Rich = nutrient enriched; other abbreviations as in Tab. 1.

	Grateloupia turuturu		*Sargassum muticum*	Native taxa richness	Native taxa cover	Bare rock
Test	I x V	I x N	I x V	I x V	N x V	N x V
S.E.	0.188	0.154	0.760	0.521	3.346	2.681
	Reg: HI>LI	LI: Nat=Rich	Reg: HI>LI	Reg: HI>LI	Reg: Nat=Rich	Reg: Rich=Nat
	Irreg: HI>LI	HI: Rich>Nat	Irreg: HI>LI	Irreg: LI=HI	Irreg: Rich>Nat	Irreg: Nat>Rich
	LI: Reg=Irreg	Nat: HI>LI	LI: Reg=Irreg	LI: Irreg>Reg	Nat: Reg>Irreg	Nat: Irreg>Reg
	HI: Irreg>Reg	Rich: HI>LI	HI : Irreg>Reg	HI : Reg=Irreg	Rich: Irreg=Reg	Rich: Reg=Irreg

FIGURE LEGENDS

Fig. 1. Upper panel: schematic representation of the experimental design (note: three control pools not illustrated). Lower panel: schematic representation of the arrangements of events of disturbance (D) over the course of the experiment for each level of temporal variability (Reg = regular treatment; Irreg-S1 =irregular treatment, sequence 1; Irreg-S2 = irregular treatment, sequence 2); s0 to s10 are the dates of sampling.

Fig. 2. Mean (+ SE) percentage cover, calculated over ten dates of sampling, of *G. turuturu* (A) and *S. muticum* (B) and number of native taxa (C) in unmanipulated (Control) rock pools and pools assigned to each combination of intensity (LI = low, HI = high) and temporal variability (Reg = 'Regular', Irreg = 'Irregular') of disturbance (n = 3 for Control and Reg treatments, n = 6 for Irreg treatments). Note that different graphs are on different scales.

Fig. 3. Mean (+ SE) percentage cover, calculated over ten dates of sampling, of *G. turuturu* in unmanipulated (Control) rock pools and pools assigned to each combination of intensity (LI = Low, HI = High) and availability of nutrients (Nat = 'Natural', Rich = 'Enriched') (n = 3 for Control, n = 9 for each treated condition).

Fig. 4. Mean (+ SE) percentage cover, calculated over ten dates of sampling, of native taxa and bare rock in unmanipulated (Control) rock pools and pools assigned to each combination of temporal variability of disturbance and availability of nutrients (abbreviations as in Fig. 2 and Fig. 3) (n = 3 for Control and Reg treatments, n = 6 for Irreg treatments). Note that different graphs are on different scales.

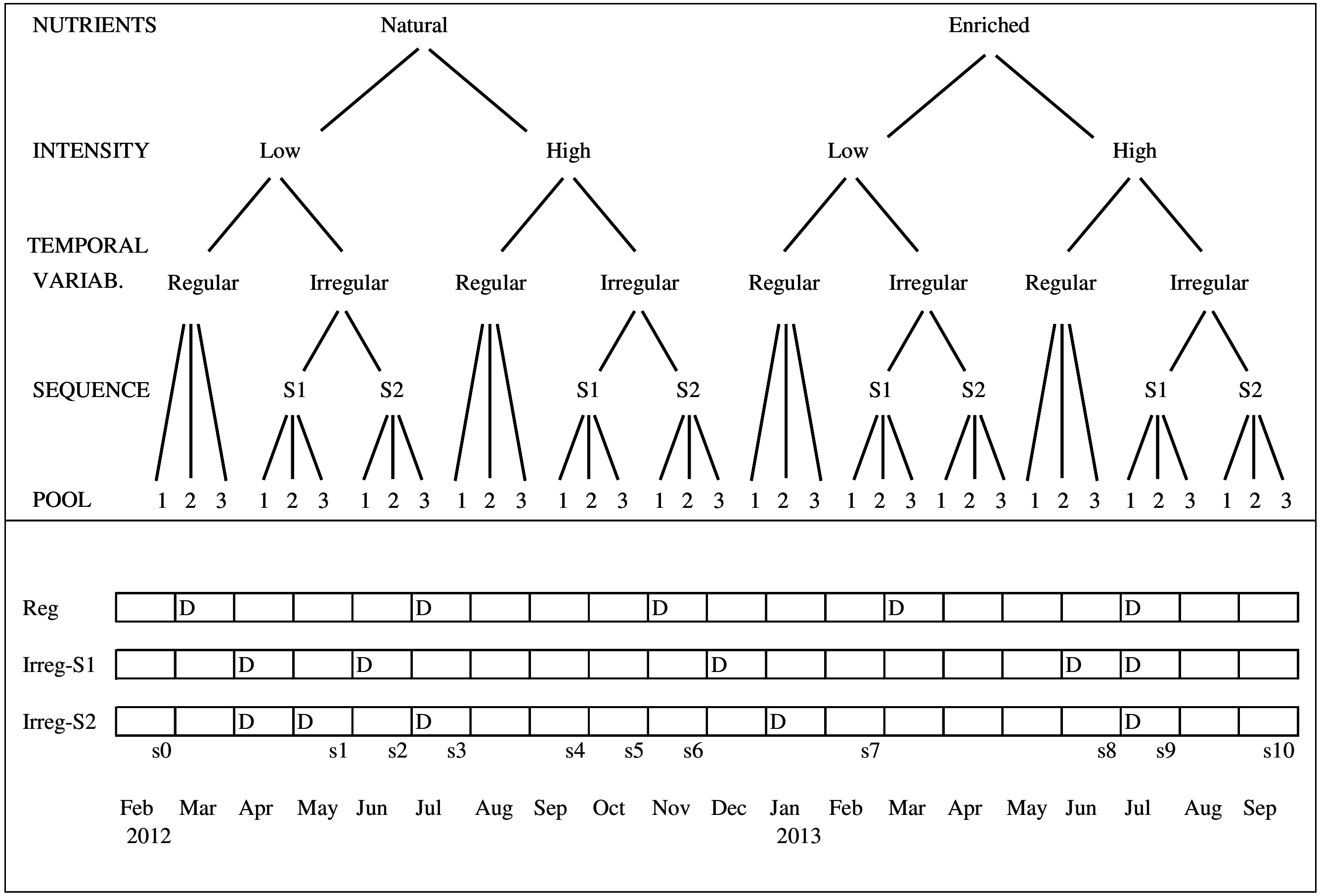
NUTRIENTS
Natural
Enriched
INTENSITY
Low
High
Low
High
TEMPORAL VARIAB.
Regular
Irregular
Regular
Irregular
Regular
Irregular
Regular
Irregular
SEQUENCE
S1
S2
S1
S2
S1
S2
S1
S2
POOL
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
Reg
Irreg-S1
Irreg-S2
D
s0
s1
s2
s3
s4
s5
s6
s7
s8
s9
s10
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
2012
2013

Fig. 1

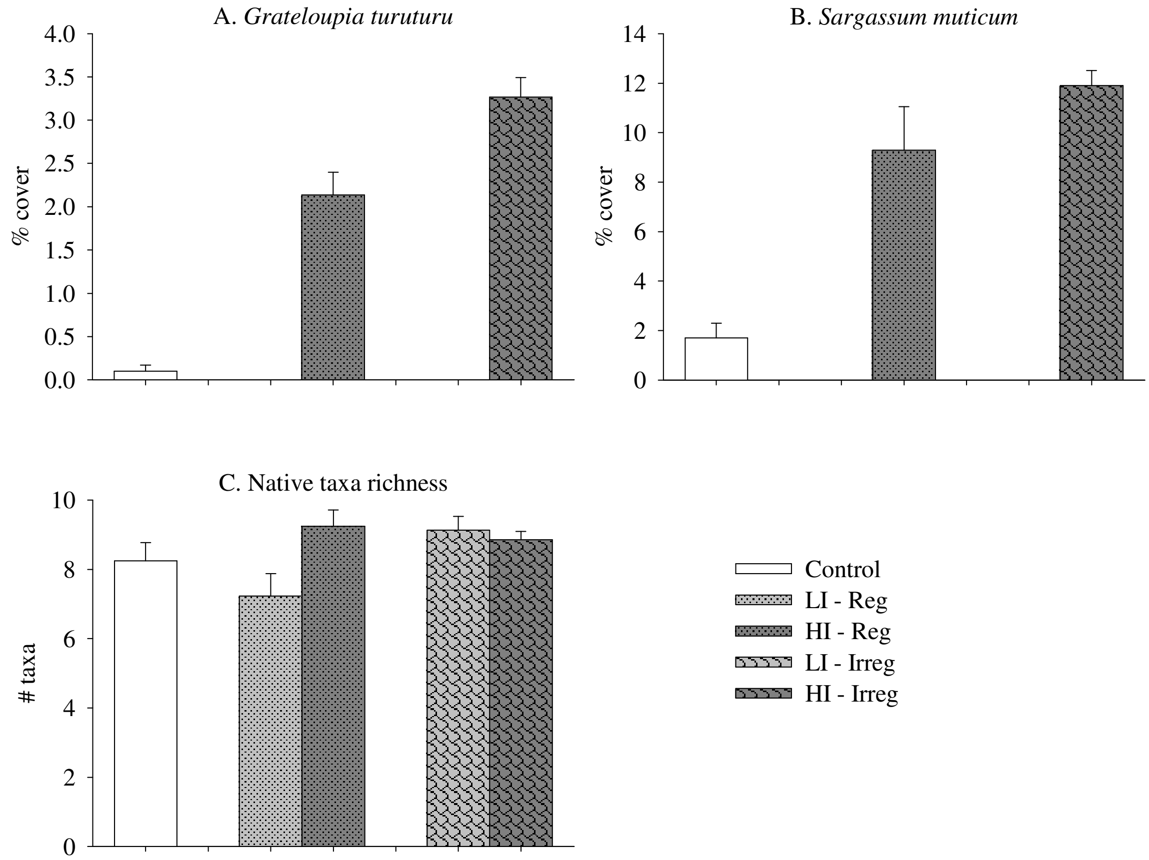
A. *Grateloupia turuturu*
% cover
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
B. *Sargassum muticum*
% cover
14
12
10
8
6
4
2
0
C. Native taxa richness
taxa
10
8
6
4
2
0
Control
LI - Reg
HI - Reg
LI - Irreg
HI - Irreg

Fig. 2

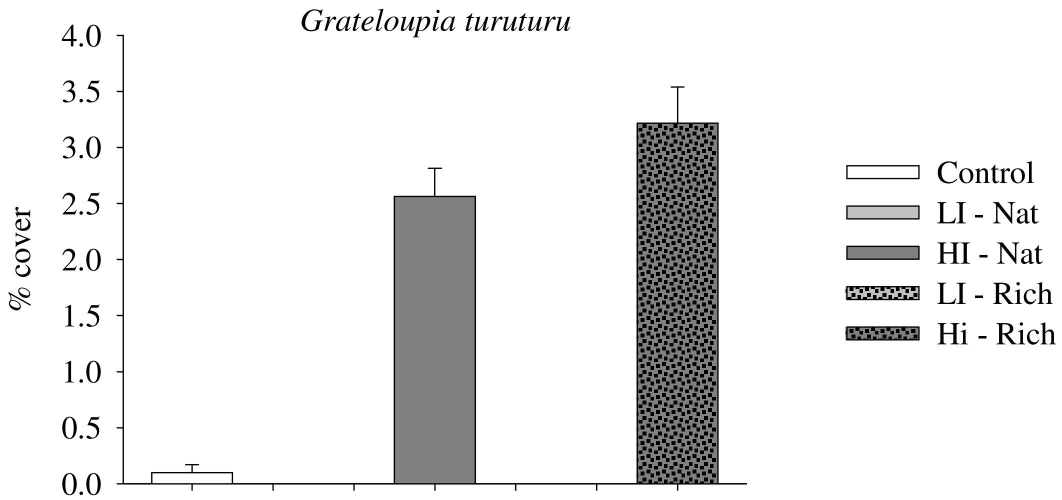
Grateloupia turuturu
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
% cover
Control
LI - Nat
HI - Nat
LI - Rich
Hi - Rich

Fig. 3

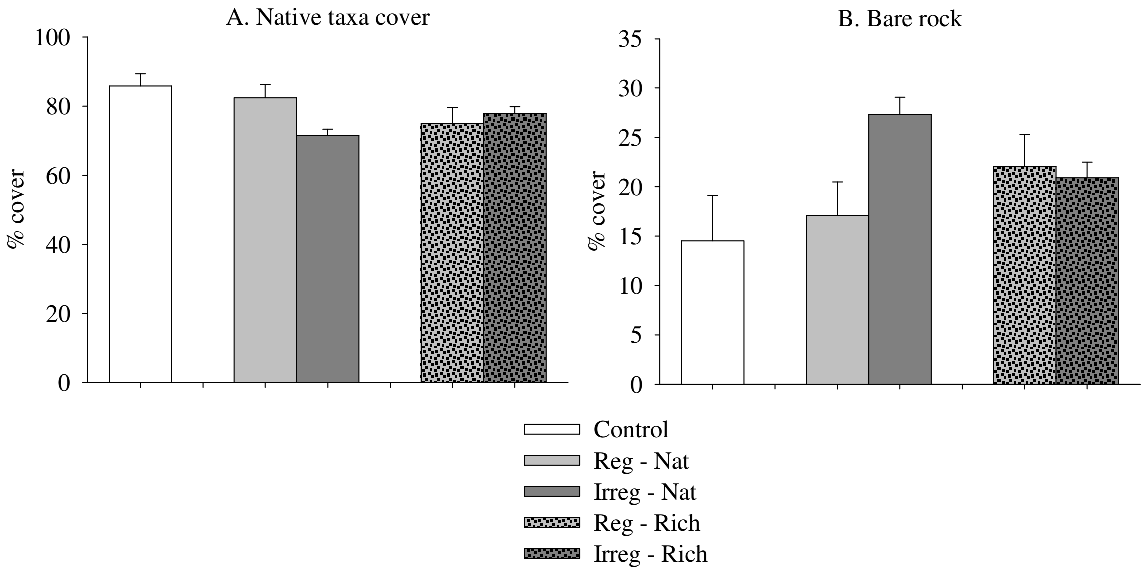
A. Native taxa cover
100
80
60
40
20
0
% cover
B. Bare rock
35
30
25
20
15
10
5
0
% cover
Control
Reg - Nat
Irreg - Nat
Reg - Rich
Irreg - Rich

Fig. 4