

# Patterns of benthic assemblages invaded and non-invaded by *Grateloupia turuturu* in north of Portugal

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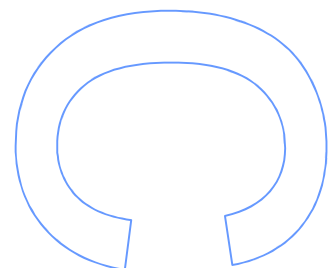
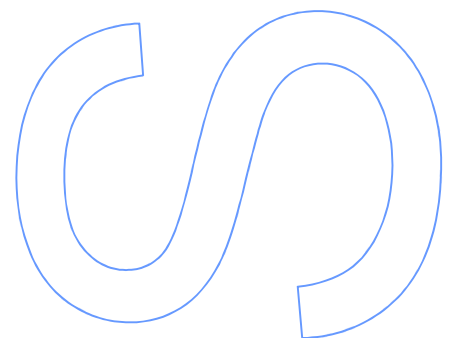
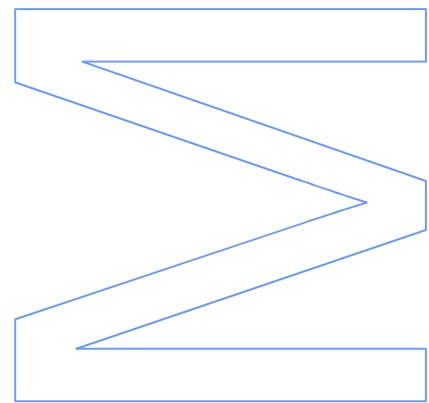
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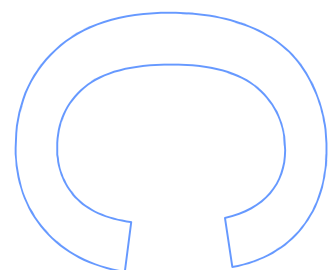
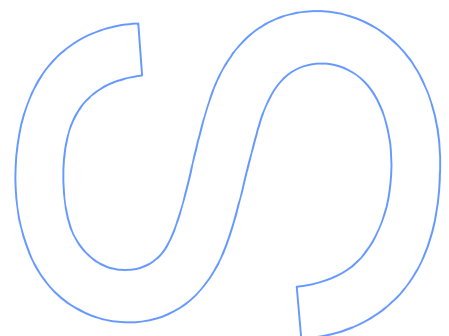
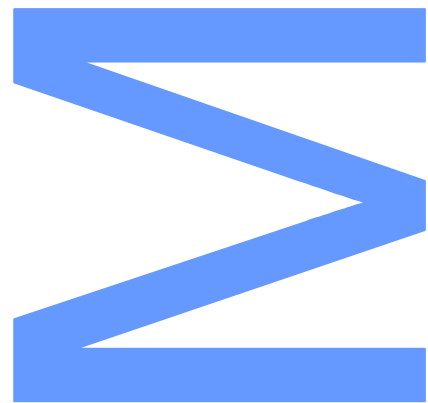




Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, \_\_\_\_/\_\_\_\_/\_\_\_\_



*“It is a curious situation that the sea, from which life first arose, should now be threatened by the activities of one form of that life. But the sea, though changed in a sinister way, will continue to exist; the threat is rather to life itself.”*

Rachel Carson, *The Sea Around Us*

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## Abstract

Biological invasions are a global phenomenon with relevant ecological, economic and social consequences. Understanding the invasion process and its effects on native assemblages is of critical importance for the implementation of conservation and management policies.

In this study we compared intertidal benthic assemblages invaded and non-invaded by the introduced Asian red alga *Grateloupia turuturu* at a rocky shore in the NW coast of Portugal. The structure of whole assemblages, the total richness of taxa and the abundance of individual taxa were examined as response variables at two different habitats (rock pools and emergent rock), two shore levels (low and mid intertidal) and two dates of sampling (June 2013 and June 2014).

Results showed a significant difference in the structure of invaded vs. non-invaded assemblages, independently of all other factors. Multivariate differences were driven by 13 out of 36 taxa identified, which contributed to more than 80% of the total invaded vs. non-invaded dissimilarity. Analyses of variance conducted on 11 of these 13 taxa revealed idiosyncratic responses, in several cases involving differences in abundance of a taxon between invaded and non-invaded assemblages inconsistent between levels of the other experimental factors. Total number of taxa was higher, although with a significantly variable entity of the difference, in invaded compared to non-invaded assemblages for each combination of habitat and shore level.

Patterns of invasion by *G. turuturu* along the Portuguese continental coast have been recently described in terms of its temporal and spatial distribution, but never examined in terms of differences between invaded and non-invaded assemblages. Therefore, the present study provides a new contribution to the understanding of the ecological impacts caused by this species, suggesting that *G. turuturu*'s invasion can be associated to modifications of native benthic assemblages across multiple habitats in north Portugal.

**Keywords:** Biological invasions, invasive algae, *Grateloupia turuturu*, rocky shore, benthic assemblages, Portugal.

## Resumo

Invasões biológicas são um fenómeno de relevantes consequências ecológicas, económicas e sociais. Compreender o processo de invasão e os seus efeitos nas comunidades nativas é de crítica importância para a implementação de políticas de gestão e conservação.

Neste estudo comparamos comunidades bentónicas intertidais invadidas e não-invadidas pela introduzida alga vermelha asiática *Grateloupia turuturu* numa praia rochosa na costa NO de Portugal. A estrutura total das comunidades, a riqueza total de taxa e a abundância individual de taxa foram examinados como variáveis resposta a dois diferentes habitats (poças rochosas e rocha emersa), dois níveis de costa (intertidal baixo e médio) e duas datas de amostragem (junho 2013 e junho 2014).

Resultados demonstraram uma diferença significativa na estrutura total de comunidades invadidas vs. não-invadidas, independentemente de todos os outros fatores. Diferenças na análise multivariada foram conduzidas por 13 dos 36 taxa identificados, que contribuíram para mais de 80% do total da dissimilaridade entre invadida vs. não-invadida. Análises de variância conduzidas em 11 destes 13 taxa revelaram respostas idiossincráticas, em muitos casos envolvendo diferenças de abundância de um taxon entre comunidades invadidas e não-invadidas inconsistentes entre níveis dos outros fatores experimentais. O número total de taxa foi maior, embora com uma entidade de diferença significativamente variável, em comunidades invadidas comparado com não-invadidas para cada combinação de habitat e nível de costa.

Os padrões de invasão de *G. turuturu* ao longo da costa continental Portuguesa em termos da sua distribuição temporal e espacial foram recentemente descritos, mas nunca foram examinados em termos de diferenças entre comunidades invadidas e não-invadidas. Consequentemente, o presente estudo fornece uma nova contribuição para a compreensão dos impactos ecológicos provocados por esta espécie, sugerindo que a invasão de *G. turuturu* pode estar associada a modificações de comunidades bentónicas nativas ao longo de múltiplos habitats no norte de Portugal.

Palavras-chave: Invasões biológicas, algas invasoras, *Grateloupia turuturu*, costa rochosa, comunidades bentónicas, Portugal.

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# 1. Introduction

## 1.1 Generality and definitions of biological invasions

There is evidence that biological invasions have always occurred on Earth as a consequence of slow natural events, such as tectonic activity (Vermeij 1991) or glacial withdraw (Cain et al. 1998), and have been responsible, for example, of the migration of species between biogeographic regions. Over the past few centuries, the rate of biological invasions has exponentially increased and accelerated due to human activities and through the expanding global trade and transport of goods and people (di Castri 1989; Pimentel 2002; Wilson et al. 2009).

Since Charles Darwin's recognition (1859) of biological invasions, these phenomena and their impacts on native communities have been the subject of many ecological studies, particularly in the last century (reviewed by Elton 1958; Lodge 1993; Mack et al. 2000; Callaway and Maron 2006). It is acknowledged, however, that the term "Biological invasion", initially used to describe the spread and persistence of a species outside its natural biogeographic boundaries, has caused great debate due to its extension, in some studies, to other ecological contexts (Davis and Thompson 2000; Colautti and MacIsaac 2004; Colautti and Richardson 2008; Valéry et al. 2008).

At the Convention of Biological Diversity (2002), a new detailed definition of "alien" and "invasive" species was conveyed:

*"An alien species is an organism introduced outside its natural past or present distribution range by human agency, either directly or indirectly. This definition implies an active movement facilitated by humans through a number of different pathways, and covers both intentional and unintentional movements of species.*

*Introductions can in fact be intentional - as in the case of species released to the environment for hunting, angling, aquaculture, forestry, agriculture, horticulture and gardening - and accidental, as in the case of hitchhikers or stowaways, or aquatic species transported through ballast water. Those alien species which cause negative impacts on biodiversity, socio-economy or human health are considered as invasive."*

European Environment Agency - "The impacts of invasive alien species in Europe"

Overall, there are many documented examples of intentionally introduced species, but most of the alien species recorded have been accidentally introduced (Pimentel 2002; Wilson et al. 2009).

Whatever the responsible mechanisms, the spread and total number of alien species is globally increasing worldwide (Levin et al. 2002) in both terrestrial and aquatic (marine and freshwater) systems (Mack et al. 2000, Sala et al. 2000, Lodge 2001, Bax et al. 2003). Despite the fact that only a few of these species eventually become invasive, their potential negative impacts on native communities are great (Crooks 2002).

## 1.2 Stages of biological invasions

Colautti and Mclsaac (2004) proposed the following model of the invasion process, involving a set of stages each with its own set of filters and determinants. This was based on previous invasion models mostly centered on the concept of “propagule pressure” (e.g. Carlton 1985; Williamson and Fitter 1996; Richardson et al. 2000; Kolar and Lodge 2001).

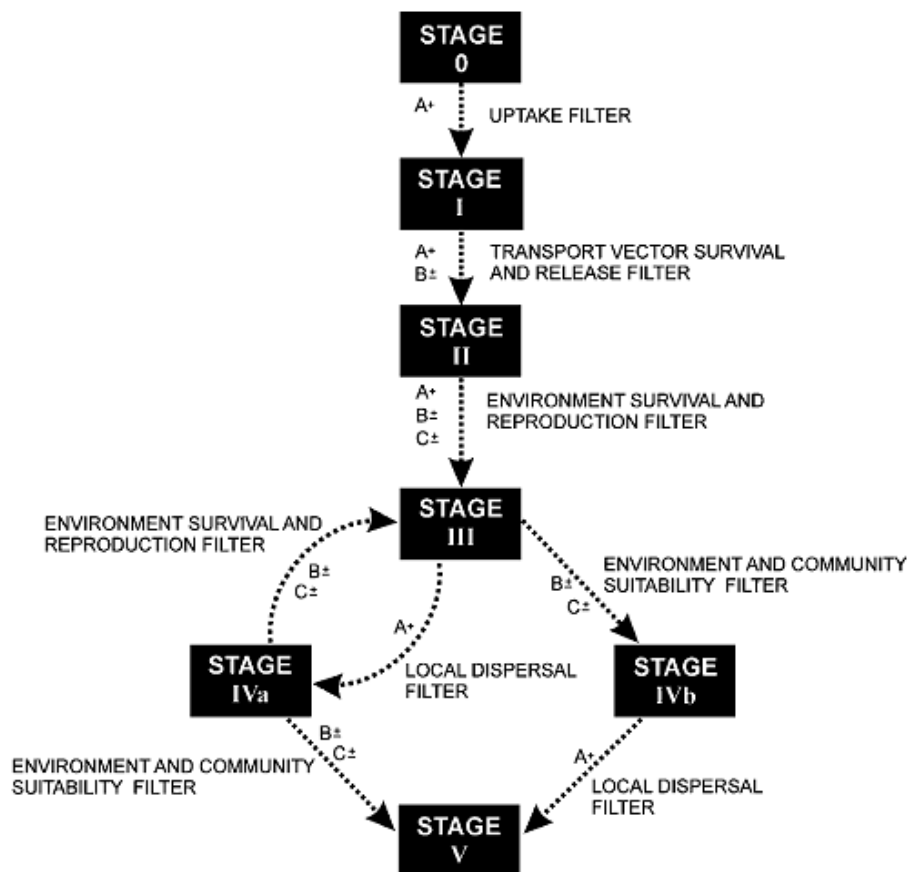


Figure 1– Stages, filters and determinants of a biological invasion according to Colautti and Mclsaac (2004)

According to the Colautti and Mclsaac model, a biological invasion starts at a donor biogeographic region (Stage 0) where available propagules of a local species are taken into a transport vector (Stage I). If propagules are able to survive the transport and consequent release into a new biogeographic region, the species gets introduced (Stage II). The success of these stages is mostly determined by reproduction and survival ability under the new environmental conditions. Such traits may allow the alien species to become established, though normally localized and with low abundance initially, in the new recipient habitat (Stage III). Local dispersion will determine if the species will reach Stage IVa (widespread, but low in abundance), while environmental and community suitability will determine if it reaches Stage IVb (dominant, but still spatially localized). When a species passes through both these filters, it will achieve Stage V (dominant and widespread). The probability of a species to pass through each filter is positively (+) or negatively (-) affected by the following determinants: propagule pressure (A); physicochemical requirements of the potential invader (B); and community interactions (C).

### **1.3 Traits of invaders and invaded habitats**

A number of studies have aimed at identifying common life-traits responsible for the success of an invasive species (Baker 1965; Rejmanek and Richardson 1996; Van Kleunen et al. 2010) and the characteristics that would made native communities more or less vulnerable to invasions (Levine and D'Antonio 1999; Davis et al. 2000; Levine et al. 2004, Dunstan and Johnson's 2007).

Morphological and functional plant traits that might potentially provide an advantage to the invasive success of a species, such as high reproduction and growth rate and large physiological tolerances, were compared between native and introduced (invasive and non-invasive) species (Crawley 1987; Mack et al. 2000; Valentine et al. 2007). Both in terrestrial plants (Crawley 1987; Mack et al. 2000) and algae (Valentine et al. 2007), such studies failed to show consistent traits that could be used to define an invasive species. For example, most invasive algae shared a rapid growth and great dispersion over short and long ranges, but the same traits were documented also for many non-invasive species. Moreover, the scale of observations is important, as the same species could be considered either invasive or non-invasive depending on the examined spatial and temporal extent (e.g., Trowbridge 1998; Chapman 1999). These findings suggest that an invasion might not depend only on the characteristics of the

invasive species, but also on the properties of the recipient habitat (e.g., Levine and D'Antonio 1999, Stachowicz and Tilman 2005, Fridley et al. 2007).

It was hypothesized that systems with a high biodiversity would be less vulnerable to biological invasions. In such diverse systems, resources would be less available due to their relatively more complete and effective use through a range of interspecific relationships, making it harder for a new alien species to become established (Elton 1958; Kennedy et al. 2002). However, such direct inverse relationship between biodiversity and vulnerability to invasions could not be clearly and consistently found (Bezemer and van der Putten 2007; Davis 2009).

Empirical observations and theoretical considerations attributed to the local availability of resources, both in terms of increased overall quantity and variability in space and time, a key role for the success of a biological invasion (Dunstan and Johnson's 2007; Davis et al. 2000; Valentine et al. 2007). For example, it was suggested that several invasive species are not particularly competitive by themselves and depend on disturbances able to eliminate or limit the growth of native species to achieve high densities (deVilléle and Verlaque 1995; Jaubert et al. 2003). Such findings indicate, in general, that disturbance and resource availability (and variability) can be useful predictors of the vulnerability of a habitat to invasion, but, after establishment, the life-traits of the alien species and biotic interactions with natives are crucially important to determine its spread (Colautti et al. 2006).

## 1.4 Impacts of biological invasions

Biological invasions are considered one of the main sources of global change (Vitousek et al. 1996; Chapin et al., 2000; Mack et al. 2000) and the second most important cause of biodiversity loss after habitat destruction (Mack et al. 2000; Levine et al. 2003; Ross et al. 2004).

Introductions of species outside their natural range can drastically alter both ecosystem services and the structure of native communities (Levine et al. 2003; Strayer et al. 2006; Vilà et al. 2011), with increasing concerns on their potential ecological, social and economic impacts (Mack et al. 2000; Levine et al. 2003; Ross et al. 2004).

The range and entity of realized impacts normally depend on the abundance and spread of the introduced species, as more dominant and widespread species are more likely to drive larger modifications of native communities. In general, such modifications

are in most cases in the direction of a decline of native species' abundance and diversity, though increases of the species richness of invaded assemblages compared to non-invaded ones have been observed in some cases (Vitousek et al. 1996; Wilcove et al. 1998; Sax and Gaines 2008; Gurevitch and Padilla 2004). In some extreme cases, invasions can determine the extinction of native species and/or a loss of genetic diversity and biotic homogenization (Rahel 2000; Gurevitch and Padilla 2004).

## **1.5 Macroalgal invasions**

Marine habitats are critically affected by a growing number of alien species. More than 800 introduced species have been recorded just in European seas, mostly in coastal areas (Streftaris et al. 2005). The intensification of international shipping, aquaculture and aquarium activities are listed among the main causes to the increasing number of marine introductions (Verlaque 1994; Ribera and Bouderesque 1995). For example, it has been estimated that thousands of species have been switched between biogeographic regions through ballast water alone (Carlton and Geller 1993, Carlton 1999).

Within all introduced marine species, 277 macroalgae are reported worldwide (Schaffelke et al. 2006; Williams and Smith 2007), in some cases with documented relevant ecological, economic and social losses (Schaffelke et al. 2006)

Introduced marine macroalgae can have varying impacts over the structure and functioning of native systems, with the most important and common represented by modifications of their physical, chemical and biotic properties, the dominance of the available space and alterations of food webs (Carlton and Geller 1993; Thresher 2000; Bax et al. 2001; Schaffelke and Hewitt 2007).

Except for some high profile species, there is usually insufficient information regarding the status and ecology of introduced species in their recipient habitat (Araújo et al. 2011).

## **1.6 The Portuguese coast and *Grateloupia turuturu***

In contrast to other European locations, relatively few introduced macroalgal species (eleven in total) have been recorded along the Portuguese coast (Araújo et al. 2009).

Most of these were described at a small number of locations with very low local abundances, but some, such as *Grateloupia turuturu* Yamada and *Sargassum muticum* (Yendo) Fensholt, are widely distributed (Araújo et al. 2009, 2011).

*Grateloupia turuturu* is a red macroalga of the Halymeniaceae family (Class: Florideophyceae; Order: Halymeniales), native to Japan and Korea (Gavio and Fredericq 2002) and it was first recorded in Portugal only recently (Araújo et al. 2003). In spite of its supposed recent introduction, the number of locations where this species has been recorded has rapidly increased, particularly at areas close to commercial harbors (Araújo et al. 2011).



Figure 2 – Benthic assemblages invaded by the red alga *Grateloupia turuturu* at Foz, NW of Portugal.

*G. turuturu* is one of the biggest macroalgae in its class, with blades reaching 1 m in length and 15 cm in width (Mathieson et al. 2008). It can grow in both sheltered and exposed areas, attaching, by means of a perennial crust, to a range of hard substrates (Simon et al. 2001; Harlin and Villalard-Bohnsack 2001). It is well adapted to eutrophic conditions and tolerates ample variations of salinity and temperature (Villalard-Bohnsack and Harlin 1997; Simon et al. 1999; Harlin and Villalard-Bohnsack 2001; Simon et al. 2001). With a great reproductive potential, *G. turuturu* displays multiple recruiting strategies, with fertile fronds and recruits occurring throughout the entire year (Simon et al. 2001, Araújo et al. 2011). It also demonstrates a high competitive potential by achieving high population densities, with simultaneous increases in both biomass and density up to their maximum values before the occurrence of any intraspecific regulation (Araújo et al. 2011).

Several studies have identified this species as *Grateloupia doryphora*; however, comparative morphology and molecular analysis have shown that these studies were actually referring to misidentified specimens of *G. turuturu* (Gavio and Fredericq 2002).

*G. turuturu* has been reported as an introduced species worldwide (Verlaque et al. 2005; D'Archino et al. 2007; Mathieson et al. 2008). There is evidence attributing the

responsibility of its introductions to human activities, including aquaculture (oyster farming) and ship transportation through fouling and ballast water (Villalard-Bohnsack and Harlin 1997). Short-distance dispersion of *G. turuturu* can also occur by means of detached fertile blades or stone-rafting (Simon et al. 2001).

Despite its worldwide expansion and great invasive ability, most previous ecological studies have aimed at describing spatial and temporal patterns of distribution (Villalard-Bohnsack and Harlin 1997; Harlin and Villalard-Bohnsack 2001; Simon et al. 2001; Bárbara and Cremades 2004; Araújo et al. 2009, 2011), while just a few, and none carried out in Europe, specifically examined the potential impacts of *G. turuturu* on native coastal assemblages (Janiak and Whitlatch 2012).

## 1.7 Objectives of the study

The vast number and range of introductions of marine macroalgae makes assessing the vulnerability of native assemblages to invasions and their modifications in patterns of distribution, abundance and diversity of composing taxa a main goal of ecological research (Rejmánek and Richardson 1996; Williamson and Fitter 1996; Ceccherelli et al. 2000). Such knowledge is a crucial requisite to inform and support environmental management and conservation strategies, as legally recognized, for instance, by the European Water Framework. More generally, macroalgal assemblages are also considered one of the best descriptors for monitoring coastal habitats (Leliaert et al. 2000; Lirman and Biber 2000; Piazzini et al. 2002) as macroalgae can represent valuable indicators of habitat changes caused by human or natural disturbance (Roberts et al. 1998; Piazzini et al. 2001; Díez et al. 2009).

Even though there is a wide number of studies documenting the current distribution and spread of numerous alien species (Carlton and Scanlon 1985; Piazzini et al. 1994; Russell and Balazs 1994; Cohen et al. 1995), studies about their impacts over native communities are relatively scarce and extremely unbalanced, in proportion, towards a few ‘flagship’ species such as *Caulerpa taxifolia*, *Undaria pinnatifida*, *Sargassum muticum* or *Codium fragile* (Schaffelke and Hewitt 2007 and references therein)

*Grateloupia turuturu* is a species with a high invasive potential and worldwide spread (Villalard-Bohnsack and Harlin 1997; Verlaque et al. 2005), largely documented along the Portuguese coast. Somehow surprisingly, however, there are very few studies explicitly addressing how it can modify the structure of native assemblages (Harlin and

Villalard-Bohnsack 2001) and European coasts have never been the focus of such experimental investigations.

The present study was aimed at providing an experimental assessment of differences between assemblages invaded and non-invaded by *G. turuturu* on a rocky shore of NW Portugal across different habitats. Specifically, a multi-factorial sampling design was carried out to test the hypotheses that (1) *G. turuturu* was associated to significant differences in the whole structure (composition and relative abundance of taxa) of invaded and non-invaded assemblages; (2) such differences were consistent across different habitats (rock pools and emergent rock), shore levels (mid and low intertidal environment) and times of sampling over a period of one year (June 2013 and June 2014).

## 2. Materials and methods

### 2.1 Study area

The study was conducted at Foz, a rocky shore some hundreds of m long located close to the city of Porto in NW Portugal (41°09'59.05"N and 08°41'21.86"W, Fig. 3). This location was previously indicated as hosting relatively large and abundant patches of *G. turuturu*, reaching over 25% cover values in at intertidal rock pools (Araújo et al. 2009).

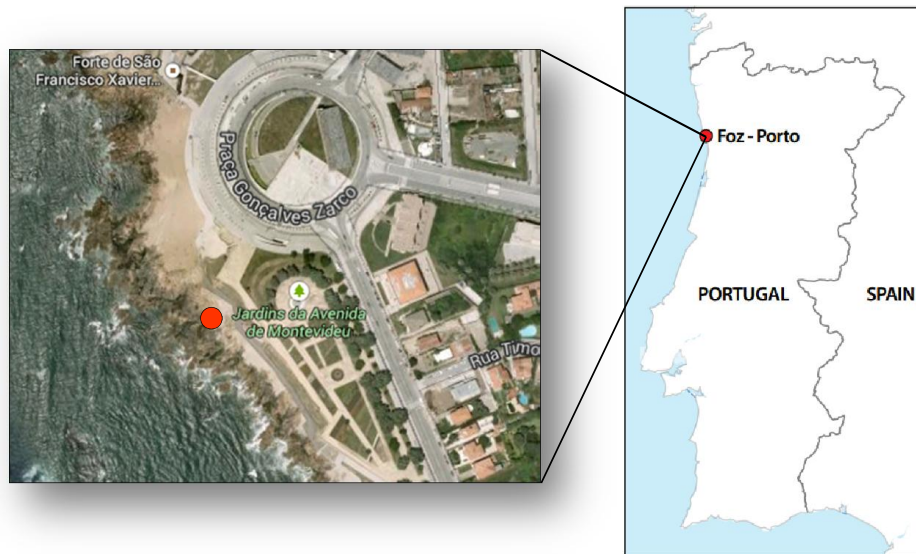


Figure 3 –Geographical location of the study area. Source: Google maps.

The same destructive sampling design was carried out at each of two experimental trials set-up in June 2013 and June 2014, respectively. This time of the year corresponds to the end of the fast-growing spring (in the boreal hemisphere) phase of *G. turuturu*, when populations normally reach their maximum standing biomass, density and size inequality (Araújo et al. 2009).

The studied rocky shore is north-to-south oriented and is mainly characterized by a granitic substratum. The tidal regime is semidiurnal, with the largest spring tides reaching 3.5- 4.0 meters. The mean air temperature historically recorded in the period of the year of the present sampling is around 19 °C, the mean precipitation around 30 mm (<https://www.ipma.pt/pt/oclima/normais.clima>), the sea water temperature around 18 °C and wave height 1-3 m, with periods of 11-13 s. (Dias et al., 2002). The maximum light intensity and photoperiod have been registered in the same period, with the highest concentration of nutrients in the water column associated to upwelling events (Lemos and Pires, 2004).

## 2.2 Sampling design and data collection

Algal and invertebrate benthic assemblages invaded (I) and non-invaded (NI) by *G. turuturu* were sampled from both rock pool and emergent rock habitats occurring both at low and mid tidal level (between 0.1 and 0.5 m and between 0.6 m and 1 m above Chart Datum, respectively). Assemblages were destructively collected from two randomly chosen quadrates (20 x 20 cm) within each of a total of six rock pools and six areas of emergent rock (similar in size to the sampled rock pools) selected at each shore level. Out of the six rock pools and six emergent areas at each height, three were a priori chosen as being invaded and three as being non-invaded (see appendix A for the graphical %cover of *G. turuturu* at each condition across the different factors). Different independent pools/emergent areas and sampling quadrates were chosen at each date of sampling (fig.4). The sampling was performed by hand using a paint scraper, each replicate was put into a separate sealed plastic bag and preserved in 96% ethanol for transportation and next sorting and identification in the laboratory. The abundance of organisms (e.g. encrusting coralline algae) that could not be effectively sampled by this method were visually sampled by superimposing on each quadrate a 20 x 20 cm frame divided into 25 sub-quadrates (4 x 4 cm each). A score from 0 to 4% was given to each taxon in each subquadrate and the final percentage cover was obtained adding up the entire set of 25 scores from each quadrate.

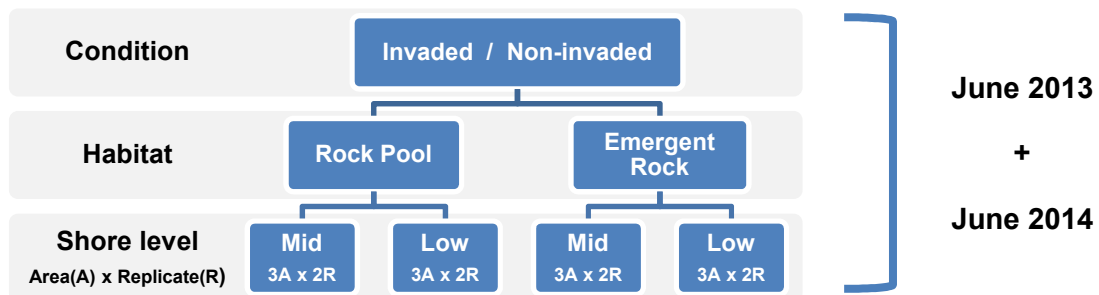


Fig. 4 – Diagram representation of the sampling stratification.

At the lab, all samples were sorted under a magnifying glass and/or a microscope and organisms were identified to the lowest possible taxonomic level (that of species in most cases, genera or morphological groups in the few remaining cases). In order to obtain cover estimates comparable to those visually collected in the field, the abundance of each identified sessile taxon was estimated as the surface occupied by its vertical projection on a 20 x 20 cm grid and expressed as a the total percentage over the whole sampling area (e.g. Piazzini and Balata 2009). Mobile animals were quantified as number of individuals in each replicate.

## 2.3 Data analysis

Multivariate and univariate statistical techniques were used to compare the structure of whole assemblages, the total number of taxa and the abundance of individual taxa between invaded and non-invaded assemblages across dates of sampling, habitats and shore levels.

Differences in whole assemblages (after the removal of *G. turuturu*) were tested with permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) based on Bray-Curtis untransformed dissimilarities. The analysis consisted in a model with 5 factors: “Date” (random, 2 levels: June 2013 and June 2014), “Condition” (fixed, 2 levels: Invaded and Non-Invaded assemblages), “Habitat” (fixed, 2 levels: Pool and Emergent rock), “Shore level” (fixed, 2 levels: Low and Mid) and “Area” (random, 3 levels, nested in all the other 4 factors), with the two quadrates sampled in each area providing the replicates.

The PERMDISP procedure (Anderson 2006) was used to test whether multivariate patterns were driven by significant differences in the dispersion besides the location of centroids.

Non-metric multidimensional scaling (nMDS) plot of centroids of invaded and non-invaded assemblages were used to visualize significant differences detected by PERMANOVA. Because Bray-Curtis is a semi-metric index (Legendre and Anderson 1999), centroids cannot be obtained simply as arithmetic averages of these dissimilarities (Anderson 2001). Thus, principal coordinates were first calculated from the Bray-Curtis dissimilarity matrix of the original dataset. This places observations into an Euclidean space without altering the Bray-Curtis measure, i.e. the distance between any pair of observations based on the principal coordinates is equivalent to the dissimilarity between those observations obtained from the original variables. Centroids were then obtained as arithmetic averages of the principal coordinates over two replicate quadrates, two areas, two dates of sampling and two shore levels (McArdle and Anderson 2001). A matrix of Euclidean distances between each pair of centroids was finally calculated and used as final input for the nMDS.

The SIMPER procedure was used to calculate the contribution ( $\delta_i$ ) of each taxon to the total Bray-Curtis dissimilarity between invaded and non-invaded assemblages. All taxa cumulatively contributing up to 80% dissimilarity were considered important.

The abundance of each individual taxon identified as being important according to the SIMPER results and the total number of taxa were tested with analysis of variance (ANOVA) using the same model described for PERMANOVA. The abundance of *G. turuturu* was also examined with a four-way ANOVA, using the same factors and levels, with the exclusion of “Condition”. Before each ANOVA, Cochran’s C tests were performed to assess the homogeneity of variances. Whenever possible, data were transformed to remove heterogeneity. When this was not possible, untransformed data were analyzed and, to compensate for the increased probability of type I error, results were considered significant at  $p < 0.01$  instead of  $p < 0.05$  (Underwood 1997). Whenever possible, non-significant (at  $p > 0.25$ ) terms were eliminated from the model to obtain more powerful tests for sources of variation involving interactive effects of “Condition”. Student-Newman-Keuls (SNK) tests were used for post-hoc comparisons of significant means.

## 3. Results

### 3.1. Structure of whole assemblages

A total of 36 taxa, including 29 macroalgae (5 Chlorophyta, 4 Ochrophyta and 20 Rhodophyta) and 7 invertebrates were identified over the course of the study.

Despite a large and significant variability between areas, invaded and non-invaded assemblages differed independently of sampling date, habitat and shore level, as indicated by the significant main effect of Condition and by the non-significance of any test involving interactions between this factor and the others (Table 1).

Source	df	MS	Pseudo-F	P(perm)	P(MC)	Denominator
Date = Da	1	7041.6	35.783	<b>0.001</b>	<b>0.002</b>	Area(DaxCoxHaxSI)
Condition = Co	1	16094	75.096	<b>0.001</b>	<b>0.002</b>	DaxCo
Habitat = Ha	1	13622	11.868	0.39	0.394	DaxHa
Shore level = SI	1	23794	43.196	<b>0.009</b>	<b>0.012</b>	DaxSI
DaxCo	1	2143.1	1.089	0.354	0.354	Area(DaxCoxHaxSI)
CoxHa	1	6752	25.049	0.059	0.075	DaxCoxHa
CoxSI	1	5746.9	17.479	0.205	0.19	DaxCoxSI
DaxCoxHa	1	2695.5	13.698	0.192	0.202	Area(DaxCoxHaxSI)
DaxCoxSI	1	3287.8	16.708	0.103	0.112	Area(DaxCoxHaxSI)
CoxHaxSI	1	5792.3	1.912	0.139	0.161	DaxCoxHaxSI
DaxCoxHaxSI	1	3029.4	15.394	0.138	0.128	Area(DaxCoxHaxSI)
Area(DaxCoxHaxSI)	32	1967.8	14.852	<b>0.001</b>	<b>0.002</b>	Res
Residual	48	1325				

Table 1 – Permutational multivariate analysis of variance (PERMANOVA) on benthic assemblages. Significant results are indicated in bold.

Multivariate differences are clearly displayed by the separation of centroids of invaded and non-invaded assemblages in the nMDS plot (Fig. 5). The PERMDISP analysis documented a non-significant multivariate dispersion ( $F=1.53$ ;  $p=0.268$ ), suggesting that invaded vs. non-invaded differences were actually due to differences in the location of centroids.

A total of 13 taxa (11 macroalgae and 2 invertebrates) were indicated by SIMPER as important to contribute more than 80% to the total dissimilarity between invaded and non-invaded assemblages (Table 2).

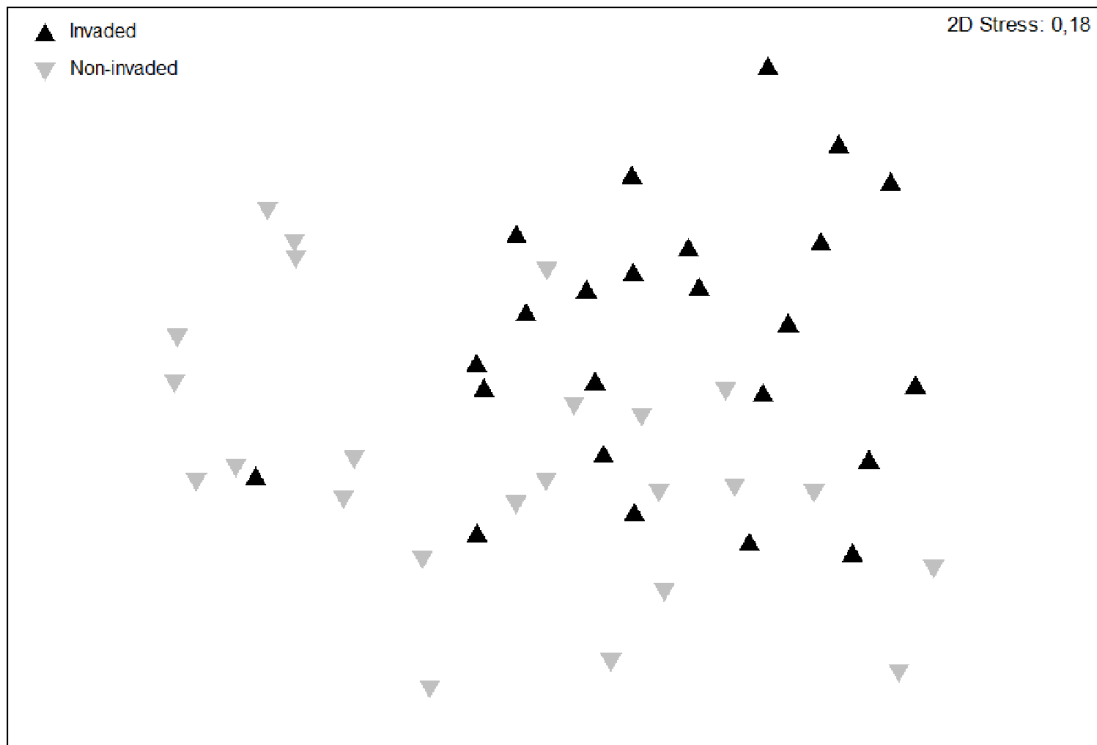


Fig. 5 – nMDS plot of centroids of invaded (black) and non-invaded (grey) assemblages.

Taxon	Average Abundance (%)		$\bar{\delta}_i$	$\bar{\delta}_i\%$	$\bar{\delta}_i/SD(\bar{\delta}_i)$
	Inv	Non-Inv			
<i>Ulva</i> spp.	23.88	37.96	15.96	22.05	1.11
<i>Chondrus crispus</i>	3.72	9.98	5.36	7.40	0.55
<i>Mytilus galloprovincialis</i>	6.66	8.33	5.10	7.05	0.92
<i>Ceramium</i> spp.	3.46	9.01	4.70	6.50	0.80
Filamentous green algae	0.00	7.56	4.44	6.14	0.48
Encrusting coralline algae	5.94	4.85	3.94	5.44	0.60
<i>Osmundea pinnatifida</i>	1.73	6.58	3.84	5.30	0.49
<i>Gibbula</i> spp.	4.58	3.79	3.01	4.16	0.81
<i>Gelidium</i> spp.	4.35	4.28	2.92	4.04	0.94
<i>Corallina</i> spp.	1.66	4.67	2.50	3.45	0.48
<i>Fucus spiralis</i>	0.00	4.21	2.32	3.21	0.25
" <i>Petrocelis</i> " <sup>1</sup>	0.98	3.71	2.19	3.02	0.46
<i>Pterosiphonia complanata</i>	2.08	2.27	1.80	2.48	0.62

Table 2 – Contribution ( $\bar{\delta}_i$ ) of individual taxa to the average Bray-Curtis dissimilarity between invaded (Inv) and non-invaded (Non-Inv) assemblages. Values of  $\bar{\delta}_i/SD(\bar{\delta}_i) \geq 1$  indicate a consistent contribution of a taxon to the percentage dissimilarity among pairwise comparisons of invaded and non-invaded assemblages.

<sup>1</sup>Crustose sporophyte, previously known as *Petrocelis cruenta*, of *Mastocarpus stellatus*.

### 3.2. Individual taxa

Individual taxa selected by SIMPER indicated, in most cases, differences between invaded and non-invaded assemblages, variable depending on levels of the other experimental factors (Table 3 and 4).

Filamentous green algae and the brown alga *Fucus spiralis* Linnaeus were not formally analyzed since they were found only at mid emergent rock of non-invaded assemblages, therefore displaying a graphically obvious pattern (see appendix B).

The abundance of the red alga *Chondrus crispus* Stackhouse (SNK test: Rock pool: NI > I; Emergent rock: I = NI; Fig. 6: B) and of articulated coralline algae of the genus *Corallina* (SNK test: Rock pool: NI > I; Emergent rock: I = NI; Fig. 6: I) showed a significant reduction at invaded compared to non-invaded rock pools, but not on emergent rock, independently of sampling date and shore level. “*Petrocelis*” revealed a decrease in abundance at invaded emergent areas located at mid shore level, independently of date (SNK test: Mid Rock pool: I = NI; Low Rock pool: NI = I; Mid Emergent rock: NI > I; Low Emergent rock: I = NI; Fig. 6: J). The mussel *Mytilus galloprovincialis* Lamarck showed a significant Co x Ha interaction, but the SNK test (Rock pool: NI = I; Emergent rock: I = NI; Fig. 6: C) failed to identify any significant difference between the invaded and non-invaded condition; however, a trend towards an abundance reduction was graphically evident at invaded rock pools, while at invaded emergent areas we could visualize just the opposite. No significant effect, apart from that of Area in some cases, was detected for the other analysed taxa (Fig. 6: A, D, E, F, G; H, and K).

Source	df	<i>Ulva</i> spp.		<i>Chondrus crispus</i>		<i>Mytilus galloprovincialis</i>		<i>Ceramium</i> spp.		Encrusting Corallines		<i>Osmundea pinnatifida</i>	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Co	1	4760.17	10.77	0.41	29.2	0.03	0.07	15.5	8.06	2.52	1.42	1.66	13.86
DaxCo	1	442.04	0.82	0.01	0.01	0.39	0.18	1.92	1.26	1.78	0.62	0.12	0.19
CoxHa	1	1472.67	1.59	13.64	<b>6.14*</b>	11.11	<b>5.25*</b>	0.82	0.49	1.99	7.26	1.78	3.48
CoxSI	1	1093.5	1.18	0.04	0.01	3.29	50.14	4.25	6.44	0	0.11	2.28	27.74
DaxCoxHa	1	925.04	1.71	0.12 <sup>2</sup>	0.05 <sup>2</sup>	2.73 <sup>2</sup>	1.29 <sup>2</sup>	1.68	1.1	0.27	0.1	0.51	0.83
DaxCoxSI	1	925.04	1.71	2.9	1.31	0.06	0.03	0.66	0.43	0.01	0	0.08	0.13
CoxHaxSI	1	1980.17	2.95	2.95	0.62	13.13	5.5	1.92	2.99	1.5	0.53	0.98	22.93
DaxCoxHaxSI	1	672.04	1.24	4.79	2.16	2.39	1.13	0.64	0.42	2.82	0.99	0.04	0.07
Ar(DaxCoxHaxSI)	32	541.79	<b>1.78*</b>	2.22	<b>2.03*</b>	2.12	<b>3.93***</b>	1.52	1.22	2.86	1.55	0.61	1.34
Residual	48	304.54		1.1		0.54		1.25		1.85		0.46	
Cochran's test		C = 0.1035		C = 0.1263		C = 0.1489		C = 0.2360		C = 0.2120		C = 0.2212	
Transformation		None		Ln (X+1)		Ln (X+1)		Sqrt (X+1)		Sqrt (X+1)		Ln (X+1)	

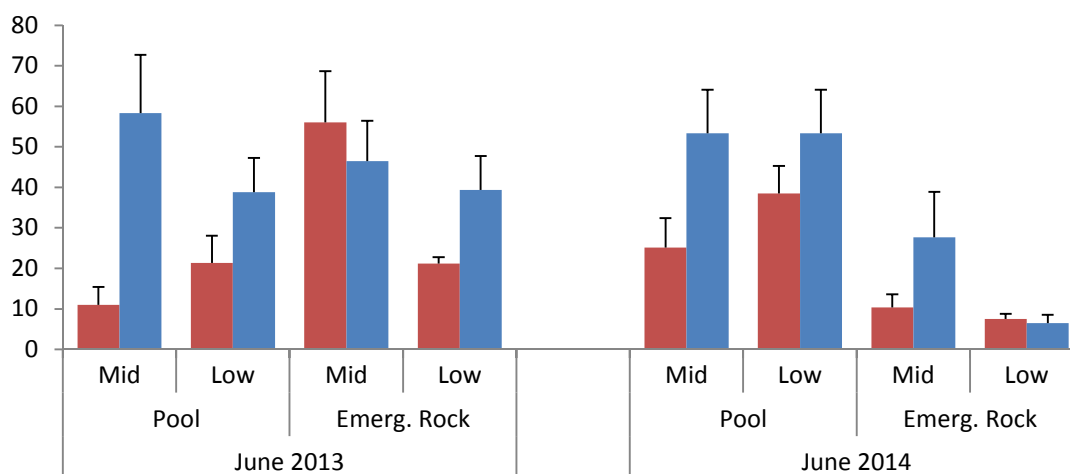
Table 3 – Summary of ANOVAs for individual taxa from benthic assemblages. \* p<0.05; \*\* p<0.01; \*\*\* p<0.001.

<sup>2</sup> The effect of CoxHa was tested over the Ar(DaxCoxHaxSI) mean square after elimination of the DaxCoxHa term that was not significant at  $P > 0.25$ .

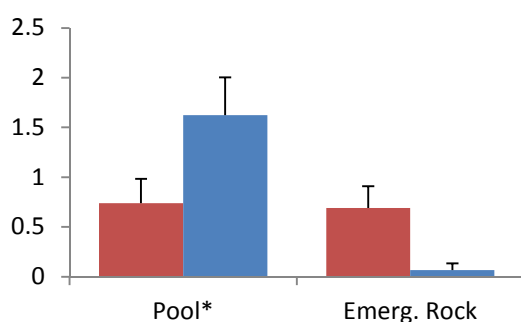
Source	df	<i>Gibbula</i> spp.		<i>Gelidium</i> spp.		<i>Corallina</i> spp.		"Petrocelis"		<i>Pterosiphonia complanata</i>		Total Taxa	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Co	1	15.04	2.98	0.13	0	2.26	1.01	3.77	23.76	0.84	0.08	119.26	10.51
DaxCo	1	5.04	0.1	36.88	0.79	2.25	1.47	0.16	0.09	10.01	1.22	11.34	0.82
CoxHa	1	337.5	3.24	182.88	9.28	4.51	<b>259.95*</b>	1.7	11.55	18.38	17.64	58.59	2.16
CoxSI	1	192.67	3.57	13.13	0.3	2.57	4.11	2.6	<b>205.66*</b>	1.5	0.14	14.26	4.74
DaxCoxHa	1	104.17	2.17	19.71	0.42	0.02	0.01	0.15	0.09	1.04	0.13	27.09	1.95
DaxCoxSI	1	54	1.12	3.56	0.08	0.62	0.41	0.01	0.01	10.67	1.3	3.01	0.22
CoxHaxSI	1	2.04	0.04	91.06	0.51	1.16	22.06	9.33	5.55	14.26	54.76	17.51	<b>1681*</b>
DaxCoxHaxSI	1	51.04	1.06	180.13	3.86	0.05	0.03	0.07 <sup>3</sup>	0.04 <sup>3</sup>	0.26	0.03	0.01	0
Ar(DaxCoxHaxSI)	32	48.08	<b>2.14**</b>	46.62	1.53	1.53	<b>2.4**</b>	1.68	<b>3.31***</b>	8.2	0.49	13.86	<b>2.64***</b>
Residual	48	22.48		30.52		0.64		0.51		16.81		5.26	
Cochran's test		C = 0.2243		C = 0.2307		C = 0.2346		C = 0.1541		<b>C = 0.5576**</b>		C = 0.198	
Transformation		None		None		Sqrt (X+1)		Sqrt (X+1)		None		None	

Table 4 – Summary of ANOVAs for individual taxa and the total number of taxa from benthic assemblages. \* p<0.05; \*\*p<0.01; \*\*\* p<0.001.

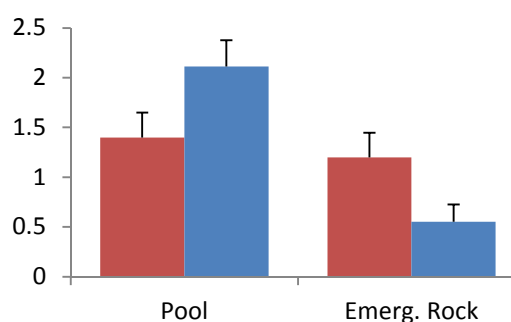
**A - *Ulva* spp.**



**B - *Chondrus crispus***

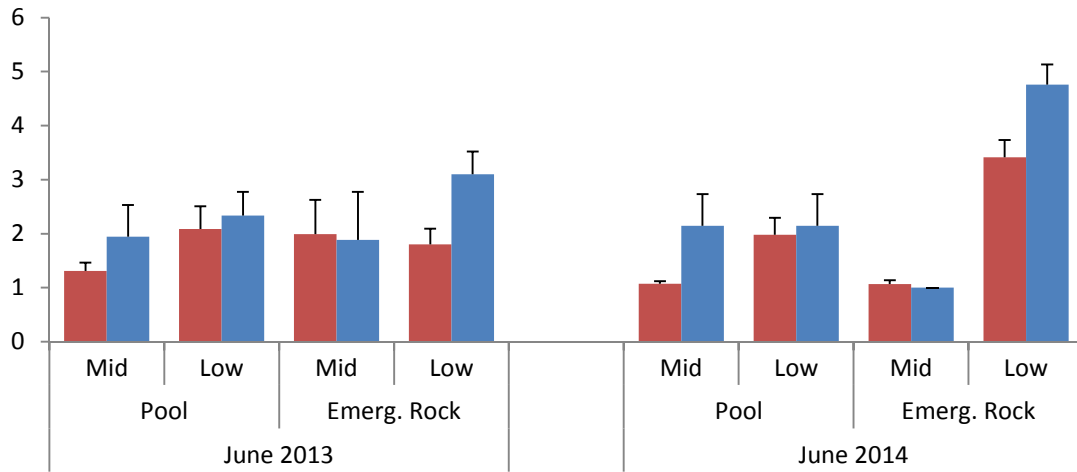


**C - *M. galloprovincialis***

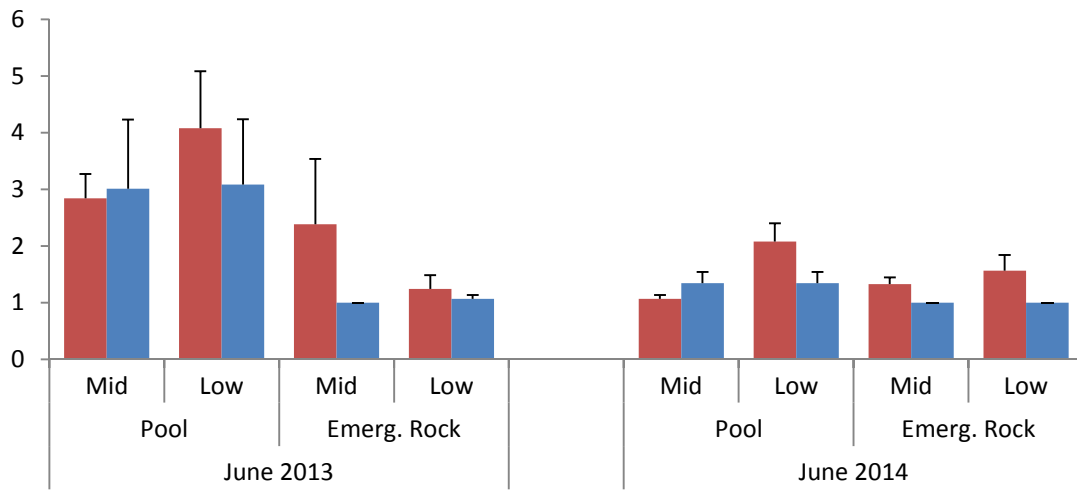


<sup>3</sup> The effect of DaxCoxHaxSI was tested over the Ar(DaxCoxHaxSI) mean square after elimination of the DaxCoxHaxSI term that was not significant at P > 0.25.

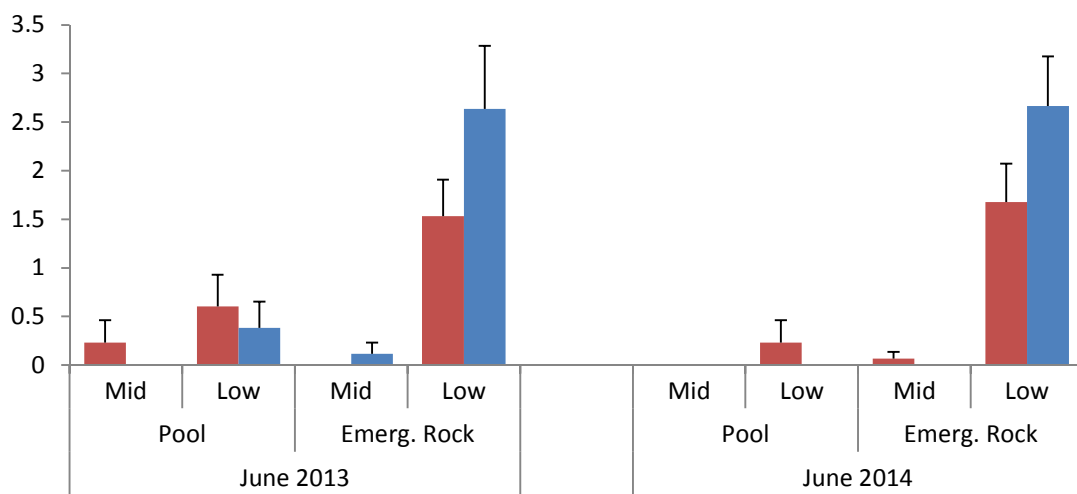
**D - *Ceramium* spp**



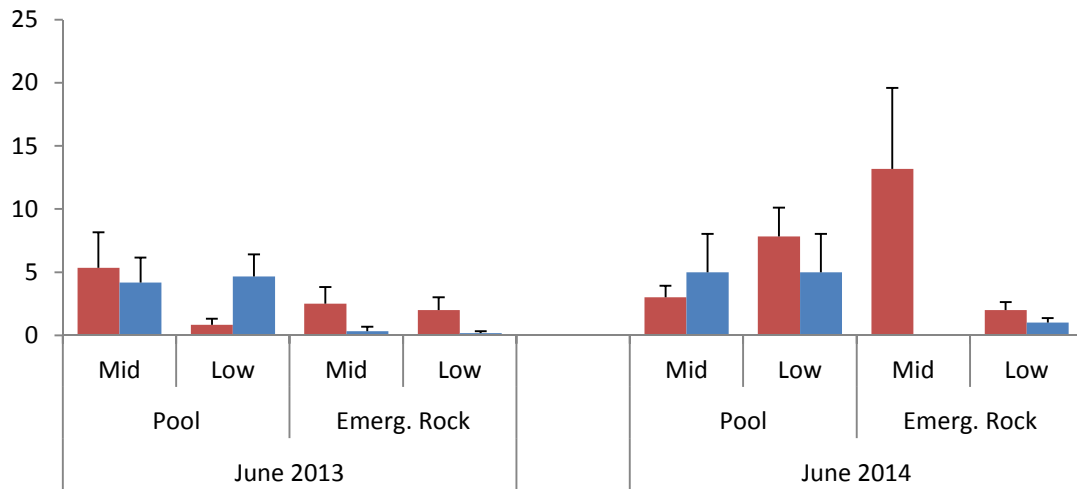
**E - Encrusting Coralline Algae**



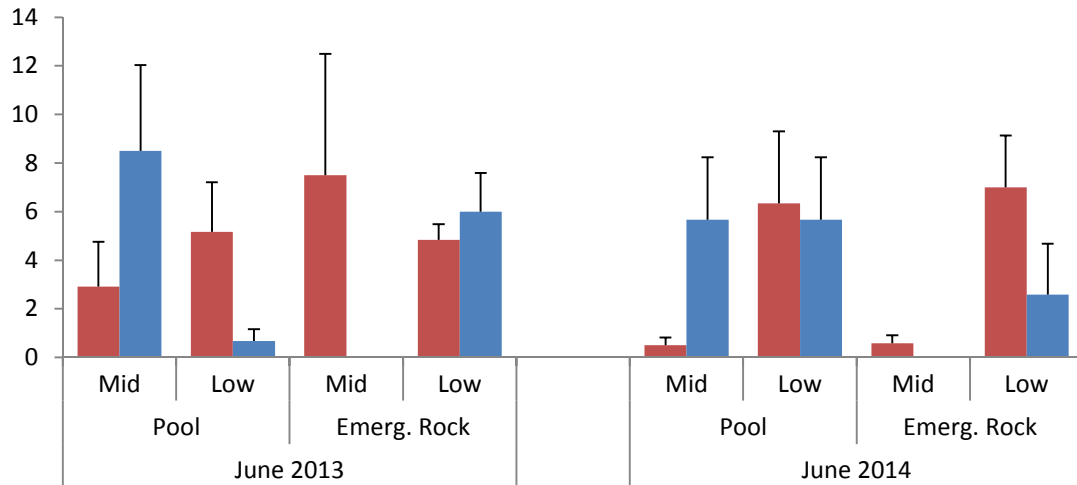
**F - *Osmundea pinnatifida***



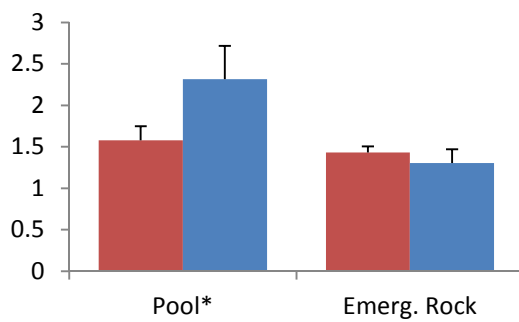
**G - *Gibbula* spp.**



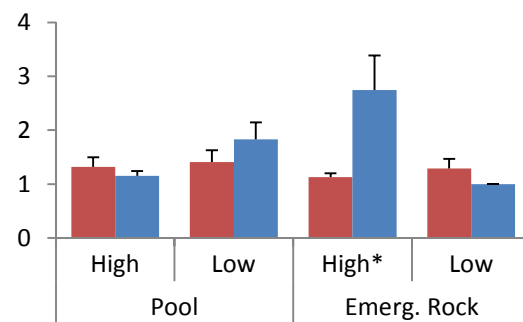
**H - *Gelidium* spp.**



**I - *Corallina* spp.**



**J - "*Petrocelis*"**



***K - Pterosiphonia complanata***

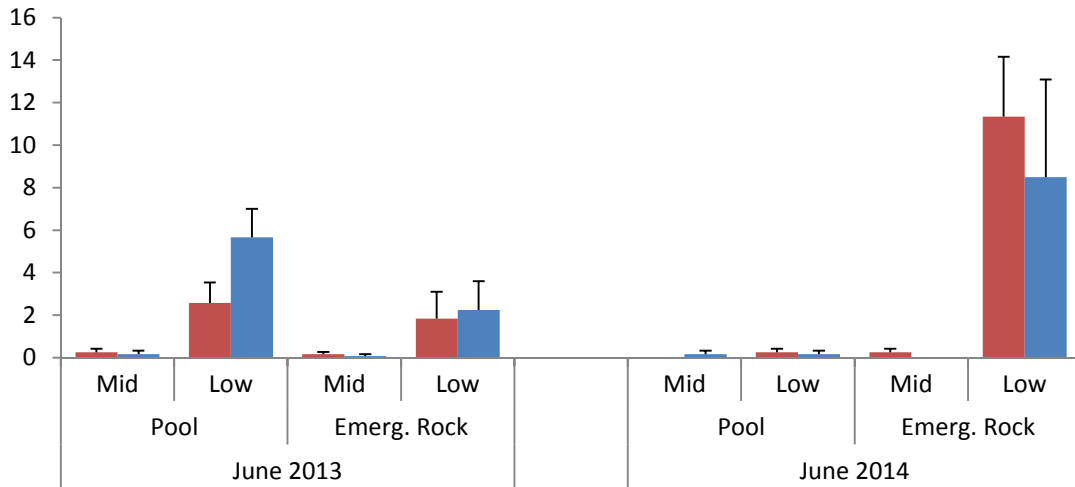


Fig. 6 – Mean (+SE) percentage cover of individual taxa between invaded (red) and non-invaded (blue) assemblages at 2 different dates, habitats and shore levels. Data was averaged over 2 replicates and 3 areas (B, C, I: data averaged additionally over 2 dates and 2 shore levels; J: data averaged additionally over 2 dates), for ease of visualization.

**3.3 Total richness of taxa**

A higher total number of taxa was found at invaded, compared to non-invaded assemblages in each combination of habitat and shore level, although with a significantly variable entity of the difference, independently of sampling date (SNK test: Mid Rock pool: NI > I; Low Rock pool: NI > I; Mid Emergent rock: NI > I; Low Emergent rock: NI > I).

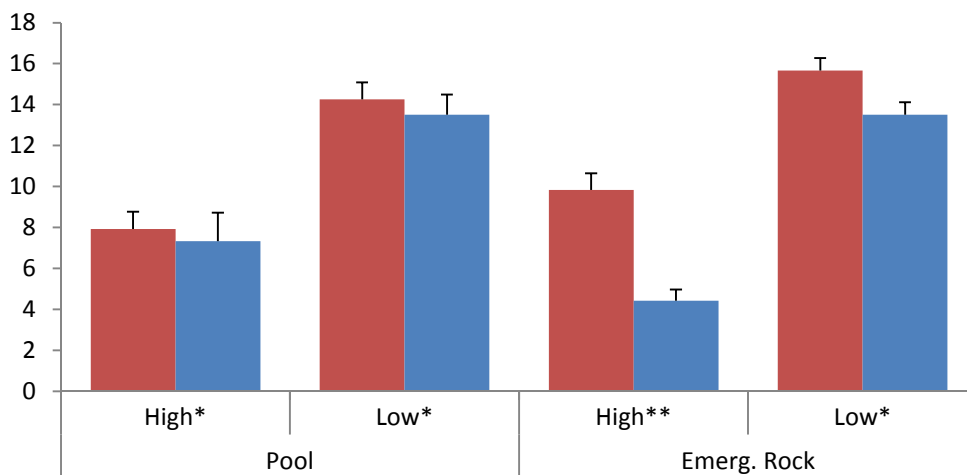


Fig. 7 – Mean (+SE) number of taxa at invaded (red) and non-invaded (blue) assemblages. Data averaged over 2 replicates and 3 areas and 2 dates. Areas averaged for ease of visualization.

## 4. Discussion and conclusions

Invaded and non-invaded benthic assemblages had a significantly different structure and this pattern was independent of date of sampling, habitat and shore level, possibly suggesting a strong and pervasive effect of *G. turuturu* in the studied system.

Several factors and processes were proposed to explain the effects of invasive species on native assemblages (Ambrose and Nelson 1982; Critchley et al. 1990; Vadas et al. 1992; Viejo 1997; Britton-Simmons 2004). Some of these may explain present findings, although cause-effect relationships could not be unambiguously assessed through the adopted descriptive approach and sampling design. Among the main candidate mechanisms, a role might be likely played by the morphological characteristics of *G. turuturu* combined with its ability to compete for resources such as space and light, in agreement with previous findings on assemblages invaded by *G. turuturu* at Long Island Sound (Janiak and Whitlatch 2012) and by *Sargassum muticum* at northern Spain (Sanchez et al. 2005). Janiak and Whitlatch (2012), in particular, explained the lower abundance of several algal species and the higher algal richness in invaded compared to non-invaded assemblages with morphological differences between *G. turuturu* and the native *C. crispus*, which were dominant in the former or in the latter condition, respectively. The long and aggregated blades of *G. turuturu* could not be suitable for sediment accumulation, having negative effects on native invertebrates, but they could provide a higher availability of primary space compared to the shorter and extensively stuffed blades of *C. crispus*. These latter could act as sediment traps suitable for a variety of epiphytes and invertebrates, but making very little primary space available. Similarly, Sanchez (2005) reported a lower abundance of several algal species, such as the dominant *Gelidium spinosum* (S.G. Gmelin) P.C. Silva, at assemblages invaded by *S. muticum*, with associated higher species richness. This was interpreted according to the morphology of *S. muticum*, a large canopy-former likely limiting the availability of light to understory species, which, therefore, could not reach large abundances. Concomitantly, however, the reduction of *G. spinosum* and the subsequent increase in primary space available could have favored an increase of the total number of species. *Grateloupia turuturu* is one of the biggest macroalgae in its class. Its wide and long blades floating above the water column could also modify the quality and quantity of light that reaches the substratum, producing a similar inhibitory effect on understory species colonizers (e.g. Connell 2003; Britton-Simmons 2004; Arenas 2006). Moreover, *G. turuturu* has a high reproductive potential, being able to produce recruits throughout the year and a grazing-resistant perennial crust from which

new blades and crusts regenerate (Harlin and Villalard-Bohnsack 2001). Such traits may give it a competitive advantage in terms of space occupancy compared to native species, especially once free patches were made available by disturbances. Making a direct analogy between such previous findings and present patterns is not straightforward as, in the studied system, differences between invaded and non-invaded assemblages in the abundance of *C. crispus* were inconsistent between habitats, while no significant differences were detected for the abundance of *Gelidium* spp. It is worth noting, however, that foliose green algae *Ulva* spp. were generally more abundant in the non-invaded than in the invaded condition. This genus includes opportunistic species that typically attain large cover values on rocky shores only where the abundance of potential competitors for space is reduced and light and nutrient conditions are suitable (e.g. Denny and Gaines 2007). These ecological mechanisms might have contributed to maintain the cover of such algae relatively lower when associated to the presence of dominant patches of *G. turuturu*.

Biological interactions are considered very important to invasion success. It was hypothesized that systems with low cover and diversity of native species would be relatively more susceptible to invasion and yield higher abundances of alien species (e.g. Gollasch and Leppakoski 1999). Although this view has been recently questioned (Dunstan and Johnson 2007), it was supported by a number of studies (e.g. Ceccherelli and Cinelli 1998; Ceccherelli et al. 2002; Valentine et al. 2007). Additionally, a common effect of biological invasions is the reduction of native species richness and diversity (Abrams 1996; Walker and Kendrick 1998; Bax et al. 2003). These two issues are the core of the recent debate on whether invaders would represent the main cause of ecological change, or opportunistic species taking advantage of already degraded environmental conditions, i.e. the 'driver vs. passenger' dichotomy in invasion ecology (MacDougall and Turkington 2005; Bulleri et al. 2010). The present study could not explicitly test which of these two situations was more likely for *G. turuturu*, but it was interesting to find that invaded assemblages had a higher number of total taxa compared to non-invaded ones. This outcome is not uncommon and has been found in systems where the alien species could provide, for example, a better habitat for epiphytes or a higher availability of primary space through competitive displacement of native species (e.g. Battershill et al. 1998; Sanchez et al. 2005; Thomsen et al. 2006). Based on present results, the mechanism responsible for the detected higher species richness in invaded assemblages might be related to the reduction in abundance of some dominant turf-forming species. As previously discussed, the opportunistic *Ulva* spp. tended to be dominant and more abundant in non-invaded than in invaded

assemblages. A similar pattern was displayed by the red alga *C. crispus* and the articulated coralline algae of the genus *Corallina*, though inconsistent between habitats. Such responses might be due to the aforementioned environmental conditions created by *G. turuturu*, which would negatively affect those algal groups, but indirectly benefit other taxa through the consequent provision of a larger availability of space.

In order to specifically and unambiguously assess the actual impacts of *G. turuturu* over native assemblages, future research will need to: (1) expand the examined spatial and temporal scales with the aim of testing for the consistency of present patterns; (2) include manipulative experiments designed to specifically explore cause-effect relationships between *G. turuturu* invasion and changes in native assemblages and their underlying processes. For example, experimental invasion events into currently non-invaded systems would allow to assess possible changes actually caused by the invasion.

Despite the indicated limitations, however, present findings provide the first empirical evidence to modifications of native intertidal assemblages invaded by *G. turuturu* in Europe, possibly suggesting relevant ecological impacts of this species. This calls for a better understanding of such impacts and their responsible process as, from this point of view, present knowledge of *G. turuturu* is extremely lower compared to other macroalgae (e.g. *S. muticum*) whose invasion's consequences have been historically raised much greater concern and interest.

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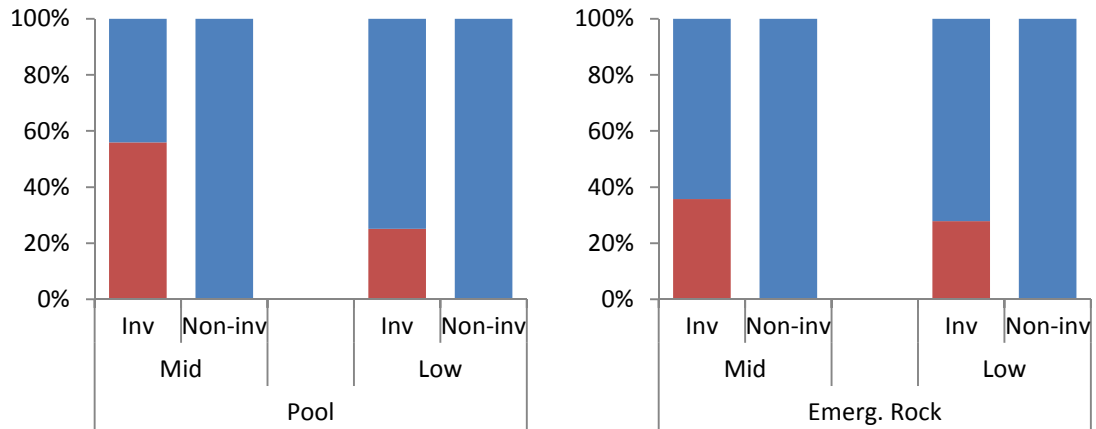
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# Appendices

## Appendix A

June 2013



June 2014

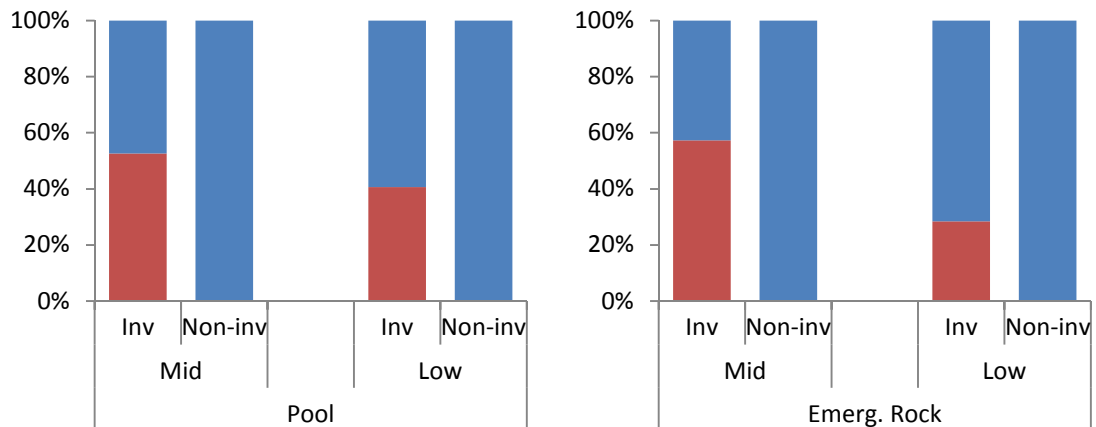


Fig. 1A - Relative mean percentage cover of *G. turuturu* (red) compared to native taxa (blue) at invaded and non-invaded assemblages at 2 different dates, habitats and shore levels. Data averaged over 2 replicates and 3 areas, for ease of visualization.

## Appendix B

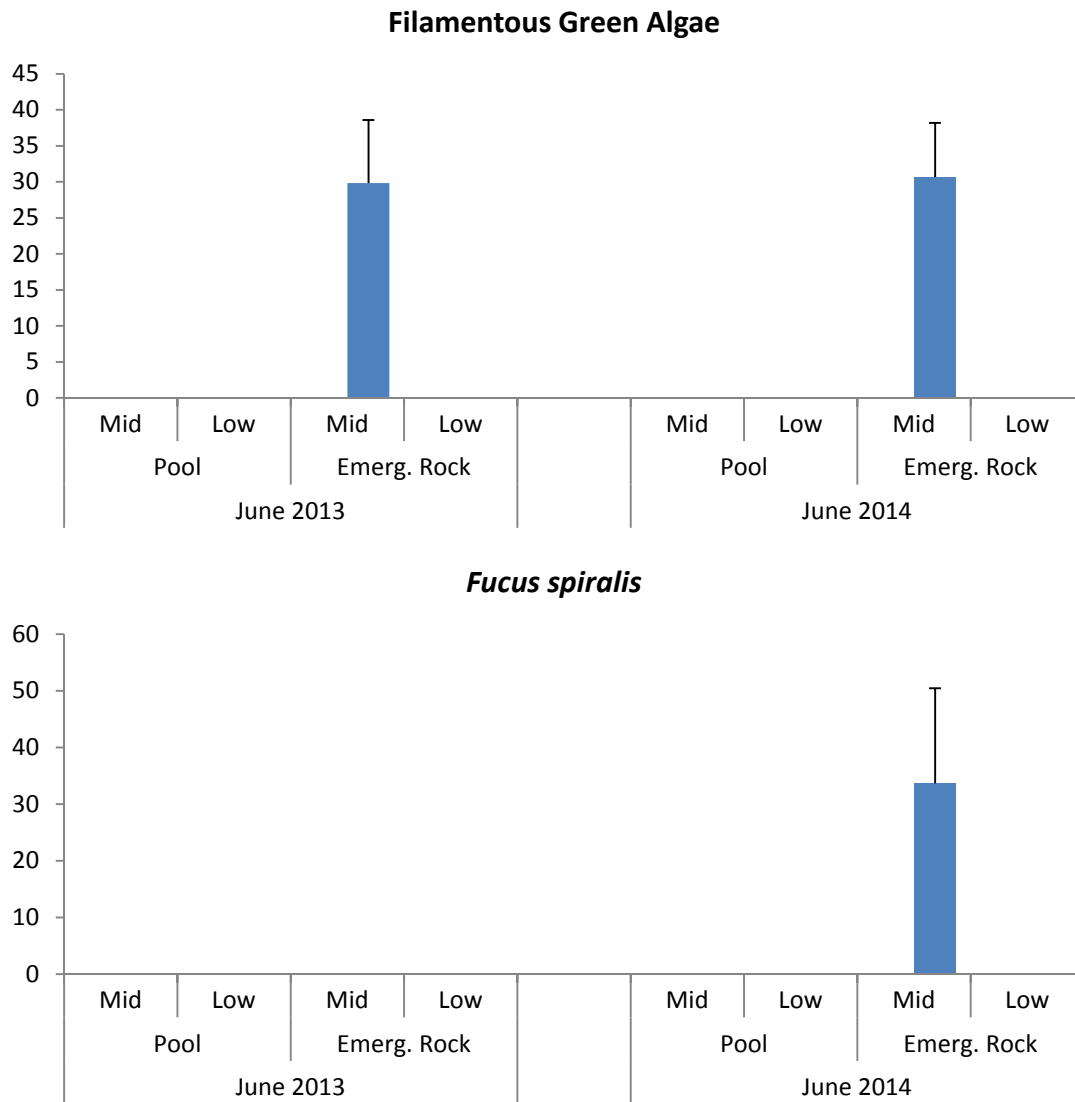


Fig. 1B - Mean (+SE) percentage cover of individual taxa between invaded (red) and non-invaded (blue) assemblages at 2 different dates, habitats and shore levels. Data was averaged over 2 replicates and 3 areas, for ease of visualization.