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Invasive marine macroalgae – understanding community  
invasibility, invasion process, and their ecological role in the  
ecosystem functioning

Fátima de Carvalho Vaz Pinto

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## LEGAL DETAILS

In compliance with what is stated in Decret-Law nº 216/92 of October 13th, it is hereby declared that the author of this thesis participated in the creation and execution of the experimental work leading to the results shown, as well as in their interpretation and the writing of respective manuscripts.

This thesis also includes three scientific papers published in international journals and two submitted articles originating from part of the results obtained in the experimental work referenced to as:

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*“It was the best of times, it was the worst of times,  
it was the age of wisdom, it was the age of foolishness,  
it was the epoch of belief, it was the epoch of incredulity...”*

Charles Dickens

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## Summary

**S**pecies invasions have increased to unprecedented rates due to globalization of human activities around the world. High rates of biodiversity loss and biological invasions put ecosystems under enormous stress. Hence, it is critical not only to understand how the loss of a species influences the stability and function of the ecosystems we rely on, but also the implications of the addition of non-indigenous species. Life in today's seas is changing in an alarming rate. In the present thesis I examine different aspects of the processes governing biological invasions in coastal habitats, using the invasive brown macroalga *Sargassum muticum* Yendo (Fensholt) as a model species. Firstly, I give a general overview of invasion ecology in **Chapter 1**, highlighting registered impacts of non-indigenous species (NIS).

The following two chapters refer to the interaction between the native ecosystem and the invasive macroalgae. **Chapter 2** investigated the interactive effects of functional diversity and propagule pressure on the recruitment success of *S. muticum* through different invasion stages, i.e., settlement, recruitment and colonisation. It is widely accepted that high diverse assemblages are more resistant to invasions because of a more complete or efficient utilization of resources. Invasion success is ultimately determined by the interaction of NIS arrival (propagule pressure) and the availability of resources needed by the NIS to establish. The initial hypothesis was that there is a positive relationship between invasibility of macroalgal assemblages and propagule pressure of the invader, but its shape is determined by the diversity of the recipient assemblages. I found that invasion success was affected by the interaction of environmental factors that covary with species diversity and identity at different invasion stages. **Chapter 3** examined how the availability of nutrients and grazers operate interactively to determine invasion success. Consumers and nutrient availability play a crucial role in the regulation of intertidal habitats. The theory of fluctuating resource availability suggests that the invasibility of a community changes as the amount of unused resources fluctuates. The hypothesis was that a greater availability of resources through an increase in nutrients concentration or an increase in herbivory would affect the invasibility of macroalgal assemblages. Results showed that early recruitment of *S. muticum* was enhanced by low nutrient enrichment, but no effect of grazers was observed. In contrast, at the end of the experiment (9-months after invasion) top-down control, together with the number of non-indigenous species and the percentage cover of ephemerals, was a significant predictor for the invasion success of *S. muticum*.

Species interactions are fundamental in shaping community structure and dynamics. Thus, Chapter 4 and 5 linked the presence of *S. muticum* with competitive interactions and ecosystem-level impacts on native macroalgal assemblages. **Chapter 4** explored the mechanisms involved in the competition between two functionally similar furoid species, the invasive *S. muticum* and the native macroalga *Cystoseira humilis*. Specifically, this chapter intends to explore the effects of neighbors in local interactions. We expected that species would display different resource allocations when subjected to intra- versus interspecific competition and that *S. muticum* would perform better than *C. humilis* when in interaction. Our results showed a negative density-dependent effect on biomass production for *S. muticum*, independently of neighbour identity. Contrary to our expectations, the nutrient uptake experiment revealed a greater competitive response of the native species compared to *S. muticum*. Moreover, our short-term study *C. humilis* showed a greater increase in biomass when in mixed assemblages with *S. muticum* than in monospecific assemblages, suggesting that *S. muticum* neighbours display a positive interaction with *C. humilis* when in mixed assemblages. **Chapter 5** compared the dynamics of respiration and production in native macroalgal assemblages and assemblages invaded by *S. muticum*, to investigate ecosystem-level impacts of macroalgal invasions. Native species with a long history of co-evolution are expected to partition resources among them and promote ecosystem functioning throughout resource use complementary effects. In contrast, newly introduced species, probably enhance ecosystem functioning by sampling effects where the influence of the invader is well beyond its proportion. This chapter aimed to test empirically this hypothesis. I found that *S. muticum* altered ecosystem functioning by increasing assemblage's productivity, although this change was seasonal. Results imply that *S. muticum* was more productive on an area basis than on a biomass-specific basis, suggesting that the impact of *S. muticum* may be related to its high dominance in the invaded assemblages, varying drastically with the season.

Finally, global change drivers are expected to induce higher susceptibility of marine communities to invasions. This hypothesis was tested with intertidal macroalgal assemblages. **Chapter 6** tested the effects of disturbance by climate change-related drivers on early survivorship of *S. muticum* germlings and the legacy effects over recruitment success. Specifically, this chapter examines the combined effects of increase temperature and CO<sub>2</sub> partial pressure and functional diversity in shaping the invasion success of *S. muticum*. Our results showed an interaction of temperature and pCO<sub>2</sub> in the early survivorship of settled germlings (3 and 10 days after invasion). Moreover, after 6 months in the field, legacy effects of laboratory treatments remained, with *S. muticum*

reaching higher cover in most assemblages previously subjected to ambient  $p\text{CO}_2$ , but ephemeral green algae appearing disproportionately after elevated- $p\text{CO}_2$  treatment. Future climatic scenarios of increasing temperature and  $p\text{CO}_2$  may not be beneficial to *S. muticum*.

Lastly, **Chapter 7** provides an overview of the invasion success of *S. muticum* and its implications for biodiversity ecosystem function research in the current and future scenario of global change.

This thesis contributed to a wider understanding of marine macroalgal invasions in general and of the ecology and physiology of the invasive brown alga *S. muticum* in particular.



## Resumo

A introdução de espécies não indígenas tem vindo a aumentar com a intensificação da globalização do planeta. A redução global da biodiversidade e o aumento das invasões biológicas levam a alterações e aumento do stress nos ecossistemas. Neste contexto, torna-se crítico não só entender qual o efeito da perda de espécies na estabilidade e funcionamento dos ecossistemas, mas também quais as implicações da introdução de espécies não indígenas. Especificamente, a vida nos nossos oceanos está a mudar a um ritmo alarmante. Nesta tese de doutoramento foram analisados diferentes aspetos do processo de invasões biológicas em habitats costeiros, usando a alga castanha invasora *Sargassum muticum* Yendo (Fensholt) como espécie modelo. Inicialmente é feita uma revisão sobre a ecologia das invasões biológicas no **Capítulo 1**, descrevendo alguns impactos conhecidos de espécies não indígenas.

Os dois capítulos seguintes integram a relação entre os ecossistemas nativos e as macroalgas invasoras. O **Capítulo 2** investiga a interação entre a diversidade funcional e a pressão de propágulos no recrutamento de *S. muticum* ao longo dos vários estágios do processo de invasão: assentamento, recrutamento e colonização. É amplamente aceite que comunidades com maior diversidade são mais resistentes a invasões devido a uma mais completa e eficiente utilização dos recursos disponíveis. O sucesso de uma invasão biológica é determinado pela interação entre a chegada dos organismos não nativos (pressão de propágulos) e a disponibilidade de recursos necessários para o estabelecimento da espécie não nativa. A hipótese inicial foi que havia uma relação positiva entre a invasibilidade das comunidades de macroalgas e a pressão de propágulos da espécie não nativa, mas o tipo de relação é determinada pela diversidade da comunidade recetora. Os resultados mostram que o sucesso da invasão foi afetado pela interação entre fatores ambientais que co-variam com a riqueza específica e identidade das espécies em diferentes estágios da invasão. O **Capítulo 3** examina como a disponibilidade de nutrientes e a herbivoria interagem no processo de invasão. Estes dois fatores têm um papel crucial na regulação dos habitats intertidais. A teoria da disponibilidade de recursos (*Theory of fluctuating resource availability*) sugere que a invasibilidade de uma comunidade varia com a disponibilidade de recursos não usados. A nossa hipótese foi que uma maior disponibilidade de recursos através de um aumento da concentração de nutrientes ou um aumento na herbivoria afetaria a invasibilidade das comunidades de macroalgas. Os resultados revelaram que o recrutamento inicial de *S. muticum* aumentou com o baixo enriquecimento de nutrientes, mas não foi encontrado efeito de herbivoria. Contrariamente, no final da experiência (9 meses após a invasão) o

fator herbivoria (*top-down control*), o número de espécies não nativas e a percentagem de cobertura de espécies oportunistas afetaram significativamente o processo de invasão de *S. muticum*.

As interações bióticas são processos fundamentais para a estrutura e a dinâmica das comunidades. Assim, o Capítulo 4 e 5 fazem a ligação entre a presença de *S. muticum*, as interações competitivas e os impactos ao nível dos ecossistemas nas comunidades de macroalgas nativas. O **Capítulo 4** explora os mecanismos envolvidos na competição entre duas espécies de fucoides com funções similares no ecossistema, a macroalga não nativa *S. muticum* e a macroalga nativa *Cystoseira humilis* Schousboe ex Kützinger. Em particular, este capítulo explora os efeitos dos vizinhos nas interações bióticas locais. Era esperado que as duas espécies exibissem uma distribuição diferente dos recursos quando sujeitos a competição intra- e interespecífica e que *S. muticum* fosse superior a *C. humilis* quando em comunidades mistas. Os nossos resultados demonstraram um efeito negativo da densidade na produção de biomassa em *S. muticum*, independente da identidade do vizinho. Contrariamente ao esperado, a experiência de absorção de nutrientes revelou uma resposta competitiva superior da espécie nativa em relação a *S. muticum*. Além disso, o estudo de campo de curta duração mostrou um maior crescimento de *C. humilis* quando em comunidades mistas com *S. muticum* do que numa comunidade monoespecífica, o que sugere que uma vizinhança constituída por *S. muticum* produz interações positivas em *C. humilis*. O **Capítulo 5** compara a respiração e produtividade primária em comunidades de macroalgas nativas e comunidades de macroalgas invadidas por *S. muticum*, com o intuito de investigar impactos da invasão de macroalgas ao nível do funcionamento dos ecossistemas. Pensa-se que as espécies nativas, com uma longa história de coevolução, distribuem os recursos existentes entre si e promovem o funcionamento dos ecossistemas através de uma utilização complementar dos recursos. Por outro lado, as espécies não nativas levam a um aumento do funcionamento dos ecossistemas devido a um efeito de amostragem. Este capítulo tem como objetivo testar empiricamente esta hipótese. A presença de *S. muticum* levou a uma alteração do funcionamento do ecossistema que resultou no aumento da produtividade da comunidade. Este aumento foi, no entanto, apenas sazonal. Os resultados apontam para que *S. muticum* foi mais produtivo por área do que por biomassa, o que sugere que o impacto relacionado com *S. muticum* é devido à sua alta dominância nas comunidades invadidas, e varia sazonalmente.

A teoria prediz que agentes causadores de mudanças climáticas podem levar a uma maior suscetibilidade das comunidades marinhas para as invasões biológicas. Esta

hipótese foi testada usando comunidades de macroalgas do intertidal. O **Capítulo 6** pretende testar os efeitos de perturbações relacionadas com mudanças climáticas na sobrevivência inicial dos recrutas de *S. muticum* e possíveis efeitos no sucesso final de colonização. Especificamente, este capítulo examina o efeito conjunto do aumento da temperatura e da pressão parcial de CO<sub>2</sub> ( $p\text{CO}_2$ ) na sobrevivência inicial dos recrutas (3 e 10 dias após a invasão). Adicionalmente, após 6 meses em ambiente natural os efeitos dos tratamentos de laboratório permaneceram e a percentagem de cobertura de *S. muticum* foi maior na maioria das comunidades previamente expostas a tratamentos de  $p\text{CO}_2$  ambiente. As algas verdes efémeras obtiveram uma cobertura altamente desproporcional em comunidades previamente expostas a tratamentos de alto  $p\text{CO}_2$ . Futuras previsões climáticas de aumento de temperatura e  $p\text{CO}_2$  podem não ser favoráveis para *S. muticum*.

Por último, o **Capítulo 7** proporciona uma revisão integrada do processo de invasão de *S. muticum* e as suas implicações para a investigação na área da biodiversidade-funcionamento dos ecossistemas.

Esta tese de doutoramento contribui para um conhecimento mais amplo do processo de invasão das macroalgas marinhas em geral e da ecologia e fisiologia da alga castanha invasora *S. muticum* em particular.



# Part I

## General introduction





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# Chapter 1

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## General Introduction

*“Ecological truth lies at the confluence of  
observation, theory, and experiment”*

Michael Loreau

## *Preface*

This thesis examines different aspects of the invasion success of the macroalga *Sargassum muticum* (Yendo) Fensholt (Phaeophyta, Fucales).

In this general introduction, I define and describe the process of biological invasions, the mechanisms involved and summarise the known impacts of invasion, particularly of introduced marine macroalgae. Then, I make a general overview of interactive effects of global climate change, specifying vulnerability and adaptations of non-indigenous species to those changes. This introduction also provides background information about *S. muticum*: its status, distribution, invasion history and impacts in native assemblages. The thesis chapters have been written as journal articles. Thus, to allow autonomy of each chapter, some repetition of introductory and methodological information was necessary. The general introduction chapter may be seen as an overview where I describe the invasion process, our model organism, *S. muticum*, and our model system, intertidal rock pools.

## **1.1 Invasion ecology**

Since the 19<sup>th</sup> century, pioneering naturalists – namely Charles Darwin, Alphonse De Candolle, Joseph Hooker and Charles Lyell – have mentioned invasive species in their writings (Ludsin and Wolfe, 2001; Richardson and Pysek, 2007), although then invasive species were not perceived as a threat to global biodiversity. Charles Elton book *The Ecology of Invasions by Animals and Plants* (1958) is considered as the seminal work of invasion ecology, classified as one of the first to address biological invaders as key drivers of ecosystem change. More than 50 years after being released, Elton's book is the most cited source in invasion biology (Richardson and Pysek, 2008). Elton's 1958 book most notable arguments are related to the diversity-stability and diversity-invasibility relationships (Richardson and Pysek, 2007). Additionally, its chapter 5 entitled "Changes in the Sea" was the first global overview of marine invasions.

Invasion ecology encompasses the study of the human-mediated introduction of organisms, especially introductions to areas outside the natural range expansions of given organisms (Carlton, 1996b; Mack et al., 2000). Over the last decades the study of patterns and processes behind biological invasions, their interactions with resident organisms in recipient locations and the consideration of costs and benefits from their presence have grown as research topics (Carlton, 1996b; Richardson et al., 2010; Stachowicz et al., 1999). In the beginning, terrestrial and freshwater systems were the most studied

systems, but during the last two decades marine systems have been studied intensely (Grosholz, 2002).

## 1.2. The invasion process

Biological invasions occur when a species enters and spreads into areas beyond its natural range of distribution (Vermeij, 1991). Beyond the changes in species range, which occur constantly in natural communities (Vermeij, 1991), it is the accelerating rate and the magnitude of those changes that pose a threat to global biodiversity (Lodge, 1993). Despite many non-indigenous species (NIS) can arrive into new locations, the development of NIS populations is a dynamic, very complex process and consists of several transitional stages (Williamson, 2006). There is, however, little agreement about how to name these stages, and how many are there. In this research, the definitions were used following two different authors (Colautti and MacIsaac, 2004; Richardson et al., 2000). In particular, Richardson et al. (2000) critically defined the minimum key terms linked to the process of invasion, i.e. introduction, naturalization and invasion. Colautti and MacIsaac (2004) suggested a well defined framework linking the invasion process with different filters that have to be overcome to arrive to subsequent stages. Here, the process of invasion is described as a set of stages, critically defined as follows:

- Non-indigenous species (NIS) are defined as individual(s) of a species that enters a given area outside of its historic or native range, due to human activity (synonyms: alien species, non-native species) (Richardson et al., 2000);

- Introduction (stage II) describes the arrival of individuals (or propagules) to a given area outside of its historic or native range after being transported through human activities. Survival of introduced NIS to adult individuals, establishment, is set as stage III. Stage 0 and stage I correspond to residency in a potential donor region and transportation, respectively.

- Naturalization represents the stage where NIS have a regular reproduction and develop sustainable widespread populations (Stage IVa) that do not necessarily invade recipient ecosystems (stage IVb).

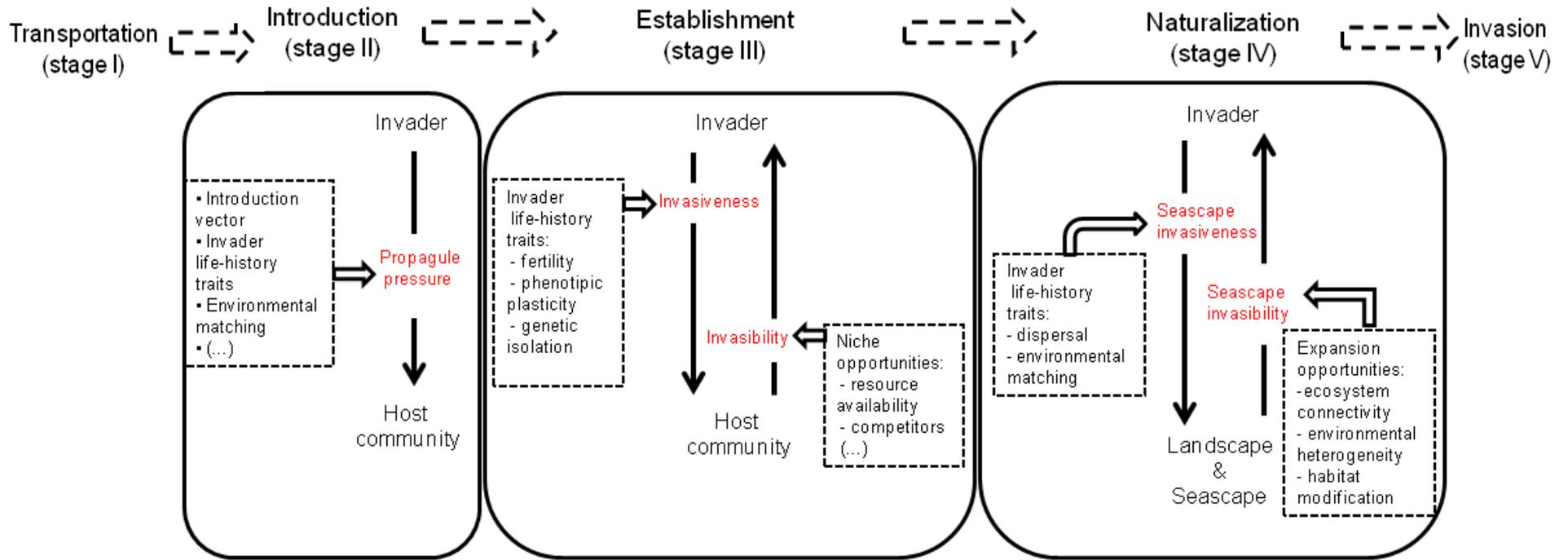
- Invasion requires naturalized NIS to reproduce abundant reproductive offspring also in areas distant from parent plants and thus may be described as widespread and dominant (stage V).

Thus, during the process of invasion, NIS must overcome several ecological barriers at different stages of invasion before they are able to inflict ecological or economic harm (Fig. 1.1). Abiotic conditions may be the main ecological barriers NIS will encounter. Traits such as geographic origin, native range extent and dispersal ability may be particularly related to successful transportation (Goodwin et al., 1999; Theoharides and Dukes, 2007). Additionally, high propagule pressure has been suggested to increase the likelihood that species survive transport (Kolar and Lodge, 2001; Lockwood et al., 2005). Repeated introduction events are also likely to increase the likelihood of a species to arrive at the new location, increasing invasion risks (Drake and Lodge, 2006). Upon arrival, abiotic conditions at the recipient ecosystem, biotic interactions and demographic processes may interfere with NIS establishment. The process of invasion will then be completed by the spread/integration of the NIS within the recipient ecosystem, subjected to ecological and evolutionary processes (e.g. competition, predation, hybridization) of the new ecosystem.

Understanding which factors and to what extent they influence invasion success (Kolar and Lodge, 2001; White and Shurin, 2007) through the different stages is of extreme importance in invasion ecology (Theoharides and Dukes, 2007). This information will allow us to disentangle the interaction between what we know as barriers to invasion and the process of invasion (i.e. number of introduction events, species traits, and ecosystem characteristics).

### **1.2.1. The arrival of introduced species**

Geographic barriers such as oceans, mountains, ice sheets, and river valleys represent boundaries to the movement of individuals between populations of the same species. However, since humans have had the ability to disperse across continents they have accelerated the invasion process by overcoming biogeographical barriers to dispersal (Vermeij, 1991). Associated with human-mediated introductions is its unpredictable character, independent of the natural barriers of space and time. As such, it is estimated that a marine species will be introduced to a new environment on a weekly to daily basis (NOAA, 2012). Possible vectors of marine invasions are shipping, canals, aquaculture, fisheries, ornamental species and live food, marine leisure and research (Minchin et al., 2009). Identifying how a species arrived, however, is not always possible. Nevertheless, shipping and aquaculture are recognized as the most important vectors involved in marine species introductions (Minchin, 2007; Ruiz et al., 2000; Streftaris et al., 2005).



**Fig. 1. 1.** Schematic integrated approach to define the different stages of the invasion process. Potential invaders begin as residents propagules from a donor region (stage 0). The invasion process is described from transportation (stage I) to invasion (stage V) following Colautti and MacIsaac (2004). Potential invaders have to pass through a series of filters that may stop the transition to subsequent stages. We present a set of characteristics that may favour invaders in a particular stage since introduction.

Marine sessile species may be transported by ships both attached to the hull and inside the ship in solid or water ballast (Minchin et al., 2009). In particular, the transport of ballast water provides opportunities for the spread of entire assemblages of marine species (Carlton and Geller, 1993). As an example, it has been estimated that 10000 marine species are transported in ballast water daily (Carlton, 1999). In addition, the accumulation of sediments in the bottom of ballast tanks provides a refuge for infaunal species (Minchin et al., 2009). However, fouling of ship hulls has been recognized as a more important vector for seaweeds than ballast water (Johnson and Chapman, 2007).

Aquaculture-related activities are the second most frequent vector of marine introductions (Minchin, 2007). Many non-native species of fish, invertebrates and seaweeds are cultivated worldwide. For instance, the Japanese oyster, *Crassostrea gigas*, and the Japanese seaweed, *Undaria pinnatifida*, are examples of introduced species with commercial value. Additionally, aquaculture species may unintentionally introduce associated biota, including pests, parasites and diseases (Minchin et al., 2009). This last pathway accounted for 15% of the marine invasions reported by Ruiz et al. (2000) in North America and 19% of the marine invasions in Europe (Streftaris et al., 2005).

A recent review from marine benthic communities has found that the introduction of macroalgae is mainly related to aquaculture while shipping is described as the major introduction vector for the animals (McQuaid and Arenas, 2009).

### **1.2.2. The success of invasions**

Particularly interesting in invasion ecology is the dramatically greater abundance of some NIS in new ecosystems compared to where they are native. Thus, after a general description of the invasion process, we now intend to disentangle the mechanism behind an invasion success and the maintenance of biodiversity.

Overall, three factors are usually cited as determining the fate of invasions: the biology of the introduced species (species invasiveness), number and frequency of introductions (propagule pressure) and the susceptibility of the native community to invasion (community invasibility) (Lonsdale, 1999). Nonetheless, invasion studies are usually focused on only one of these factors (Lonsdale, 1999). Understanding the mechanisms behind resistance to biological invasions is of particular interest due to the

fact that NIS have produced large ecological and economic impacts (Mooney and Drake, 1989; Scalera, 2010; Vitousek et al., 1997).

Taken as a whole, there are many and diverse theories of invasion success (Mitchell et al., 2006; Sakai et al., 2001), although most share the key prediction that successful invaders must be fundamentally different from native species (e.g. Daehler, 2003; Titman, 1976). According to the *Diversity Resistance Hypothesis*, generally credited to Charles Elton (1958), species diversity enhances resistance to biological invasions. This *Hypothesis* suggests that high diversity communities maintain occupied most available niches compared to species-poor communities, believed to contain more unoccupied niches. Through his studies of native and exotic species on oceanic islands, Elton (1958) showed evidence that richer communities were more stable, less vulnerable to disturbances and thus less likely to be invaded. However, an “invasion paradox” has emerged as researchers describe that at regional scales species-rich communities may be more susceptible to invasions (e.g. Lonsdale, 1999; Stohlgren et al., 1999), emphasizing the role of spatial scale in the diversity-invasibility relationship (Fridley et al., 2007; Levine, 2000).

Because competition is regarded as a key biotic resistance mechanism acting upon NIS arrival, special attention has been given to the role of natural enemies, i.e. predators and parasites, in the invasion process. Invasion success of NIS has been related to the *Enemy Release Hypothesis*, which states that the release from natural enemies drives the increase in distribution and abundance of NIS in its new range (Keane and Crawley, 2002), by being unrecognized or unpalatable to native enemies. On the other hand, a key consideration is whether NIS are less vulnerable to native enemies than are native species in its new range. Both hypotheses are not, however, mutually exclusive. Upon arrival into a new region, NIS left behind many of its natural enemies (enemy release), but may also encounter new generalists enemies for which no defenses had been developed (biotic resistance) (Morrison and Hay, 2011; Verhoeven et al., 2009). In the marine realm, however, because marine herbivores are often generalists (Hay, 1991; Morrison and Hay, 2011), the Enemy Release Hypothesis might be of limited use to explain invasion success of marine algae.

The *Fluctuating Resources Theory* (Davis et al., 2000) emerges as an integration of several existing hypotheses regarding community invasibility. This theory suggests that the invasibility of a community changes as the amount of unused resources fluctuates, i.e. a community becomes more susceptible to invasion whenever resource availability (the difference between gross resource supply and resource uptake) increases (Davis

et al., 2000). Availability of resources may fluctuate either due to a pulse in resource supply, a decline in resource uptake, or both which consequently will decrease competition for that resource. Thus, the susceptibility of a community to invasion is not a fixed property and fluctuates in the same way other community properties fluctuate (e.g. nutrient availability, predation intensity, space availability). Experimental studies reinforce this theory, emphasizing resource fluctuation and competition as the proposed mechanisms affecting invasibility (Davis and Pelsor, 2001) and not species richness (Dunstan and Johnson, 2007). Moreover, manipulative field experiments using macroalgal functional groups found that resource availability was mediated by algal species identity (and not species richness), suggesting that a functional group approach may better describe the different mechanisms of species coexistence acting within a community (Arenas et al., 2006b). In addition to biotic and abiotic factors, the relative importance of propagule pressure has been largely acknowledged (see Simberloff, 2009 for a review). Contrasting to species- and community-level particular traits, which remain constant across repeated introductions events separated by some relatively small timeframe, propagule pressure is characteristic of a particular introduction event, differing between introduction events (Lockwood et al., 2005). Thus, propagule pressure could explain some of the idiosyncratic nature of introduction success.

The invasibility of an ecosystem is the outcome of several factors, from ecological interactions between NIS and native species to the region's climate and related interactions, the disturbance regime, unutilized resource availability and propagule supply (Davis et al., 2005; Lonsdale, 1999). Thus, despite many hypotheses have been proposed to explain why some ecosystems are more susceptible to invasion than others, it is unlikely that any single hypothesis will apply to all different environments. Whether biotic resistance is mainly linked with community diversity and associated resource use (e.g. Stachowicz and Byrnes, 2006; Tilman, 1999) or other biotic processes such as competition and facilitation (Crawley et al., 1999) is an open debate in ecology (Occhipinti-Ambrogi, 2007).

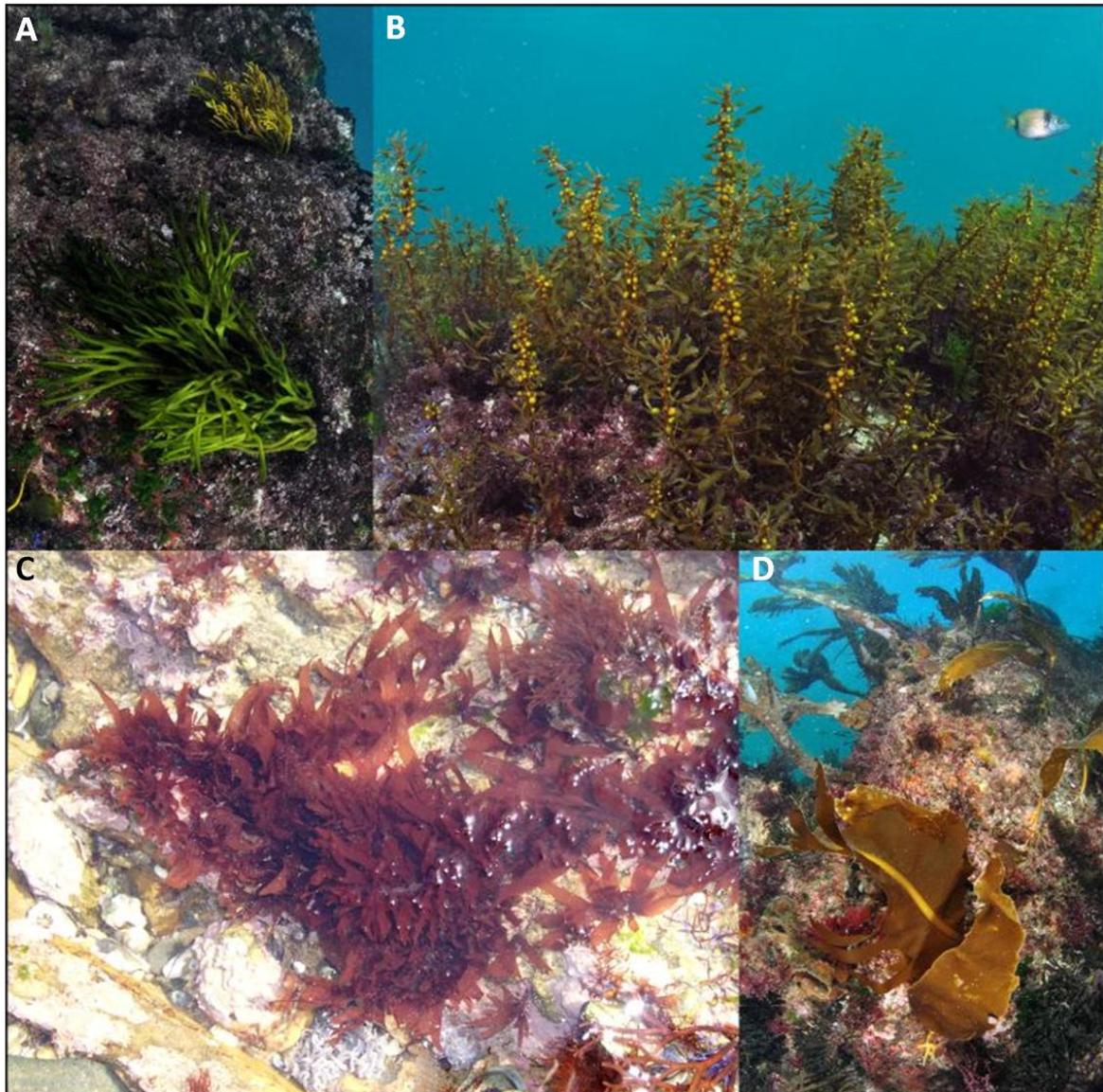
Moreover, recent work has also suggested a varying effect of diversity with life history stage of invader (Britton-Simmons, 2006; White and Shurin, 2007). For example, an experimental study testing the invasion success of the introduced brown macroalga *Sargassum muticum* found that although native algal richness enhanced initial settlement of invaders, an overall negative effect of species richness was observed for the invader abundance (White and Shurin, 2007). Hence, the study of the invasion

process should focus on the mechanisms affecting each stage of the invasion. Upon arrival, the processes that determine species distribution and abundance should be similar for both native and NIS (Davis et al., 2000) where stable coexistence requires competitors to differ in their niches. Thus, species with similar patterns of resource use and habitat requirements (functionally similar species) are expected to increase interspecific competition. Classic niche theory, however, does not explain how NIS without niche differences establish nor why invasive species produce large impacts when their establishment depend on different niche utilization than the native species (MacDougall et al., 2009). For example, it has been described a high invasion success of nitrogen (N) fixers in habitats with only non-N-fixers (Vitousek et al., 1987), supporting the empty niche theory. However, the use of two different niches should facilitate coexistence and not the registered N-fixers impact on community composition (Vitousek et al., 1987). Recently, an integration of the current mechanisms determining the fate of invasions uses *niche differences* versus *fitness differences* as a framework to better understand the invasion success (MacDougall et al., 2009). Fitness differences describe species differences that drive competitive dominance. Thus, while niche differences favour species coexistence, fitness differences drive competitive exclusion (MacDougall et al., 2009). Integrating both aspects of species differences may help to explain small controversies from single theories. For instance, for a NIS to become invasive it must use resources also required by resident species, i.e. little niche difference, but in a superior effective way, i.e. large fitness difference. On the other hand, NIS that become established based on niche differences are unlikely to exert major impacts and coexist with resident species. Therefore, this integrative framework highlights the fact that some NIS do not require niche differences to invade (MacDougall et al., 2009).

### **1.3. Introduced macroalgae and known impacts**

The extent and current rate of species introductions in the marine environment are astonishing. Marine surveys have identified at least 298 introduced algal and invertebrate species in North America (Ruiz et al., 2000), 573 NIS in the Mediterranean Sea (Galil, 2009), 129 NIS in Australian waters (Hayes et al., 2005) and 987 marine NIS in Europe ([www.europe-aliens.org](http://www.europe-aliens.org)). A recent review described 277 records of introduced seaweed, which make up a significant proportion of marine introduced species (Schaffelke et al., 2006; Williams and Smith, 2007). Overall, there are five well-known algae (Fig. 1.2) which are reported to have invaded multiple regions around the world, i.e. *Codium fragile* (Suringar) Hariot spp. *tomentosoides* (Van Goor) Silva,

*Caulerpa taxifolia* (Vahl) C. Agardh, *Grateloupia turuturu* Yamada, *Sargassum muticum* (Yendo) Fensholt and *Undaria pinnatifida* (Harvey) Suringar (e.g. Araújo et al., 2011; Boudouresque and Verlaque, 2002; Critchley et al., 1983; Occhipinti-Ambrogi and Savini, 2003). The macroalgae *Caulerpa taxifolia* and *Undaria pinnatifida* are ranked as two of the World's 100 most invasive species (www.issg.org).



**Fig. 1. 2.** Examples of high-impact invasive species recorded for the NE Atlantic. (A) green alga *Codium fragile* spp. *tomentosoides*, native to the Asian Pacific, <sup>(c)</sup> David Villegas; (B) Japanese wireweed *Sargassum muticum*, native to SE Asia, <sup>(c)</sup> David Villegas; (C) red alga *Grateloupia turuturu*, also native to the Asian Pacific Region <sup>(c)</sup> Fátima Pinto; (D) Wakame *Undaria pinnatifida*, native to Japan, <sup>(c)</sup> David Villegas.

Marine macroalgae, i.e. seaweeds, dominate the rocky intertidal in most oceans, and in temperate and Polar regions cover rock surfaces in the shallow subtidal, describing

particularly important ecological functions. Seaweeds make a substantial contribution to marine primary production (Mann, 1973), and thus form much of the basis for intertidal food webs (Little and Kitching, 1996). Increasing evidence suggests that invasion of macroalgae may cause ecological perturbations in natural communities (Grosholz, 2002). Impacts of non-indigenous macroalgal populations are typically expressed as community dominance through the monopolization of space, and changing competitive relationships in the native assemblage (reviewed by Schaffelke and Hewitt, 2007). Macroalgae can alter light availability to other species, change nutrient cycling, affect herbivory intensity (Britton-Simmons, 2004; Sánchez et al., 2005; Yun and Molis, 2012), modify ecosystem properties and ultimately they may decrease native diversity (Casas et al., 2004). However, some studies describe no significant impact of non-indigenous marine algae in specific areas (e.g. Cecere et al., 2011; Forrest and Taylor, 2002), highlighting unpredictable nature of invasions. For example, a three-year study of sheltered low shore assemblages found little impact from the introduction of *Undaria pinnatifida* (Forrest and Taylor, 2002) while large ecological consequences were described after *U. pinnatifida* introduction in sites without large canopy species (Casas et al., 2004; Forrest and Taylor, 2002). Other seaweed that has spread dramatically during the last century is *Codium fragile* ssp. *tomentosoides*. It has been reported as a fast growing species, growing up to 170 thalli m<sup>-2</sup>, with high impacts on recipient assemblages by damaging and replacing native kelp (*Laminaria* spp.) forests with potential impacts on associated fauna (Levin et al., 2002; Trowbridge, 1995). Moreover, the accumulation of masses of *C. fragile* ssp. *tomentosoides* rotting on beaches of the NW Atlantic, Mediterranean, and New Zealand produces a foul odor that drives away visitors. Despite its known capacity for spreading and replacing indigenous species (Carlton and Scanlon, 1985; Nyberg and Wallentinus, 2005), there are some sites where no impact has been registered for this species, as for example in the Azores (Cardigos et al., 2006) and in the eastern North Atlantic Ocean (Chapman, 1998). So far, no link has been found between the ability of an introduced species to spread rapidly (its invasiveness) and the likelihood that it will have a strong impact on the recipient community (Ricciardi and Cohen, 2007). Thus, it is critical to understand the underlying ecological principles behind successful marine macroalgal invasions.

The North East Atlantic, in particular, has been home to a large number of introductions (Arenas et al., 2006a; Eno, 1996; Rueness, 1989). Recent reviews on introduced marine macroalgae include assessment of introduction vectors as well as mechanisms that may influence invasion success (Hewitt et al., 2007; Schaffelke et al., 2006;

Williams and Smith, 2007). In the context of the present research we will focus on the northwest coast of the Iberian Peninsula (northern Portugal and Galician waters) where the number and distribution range of non-native macroalgal species have increased over the last years (Araújo et al., 2009; Bárbara et al., 2005). The red macroalgae *Grateloupia turuturu*, native to the northwest Pacific (Ribera and Boudouresque, 1995) is a relatively recent introduction to the Portuguese shores (Araújo et al., 2003) while in Galicia the first records are thought to be from the early 90s (Barreiro et al., 2006). Few studies have addressed population dynamics and possible impacts of this species on native assemblages (but see Araújo et al., 2011). The large, broad blades of *G. turuturu* may shade neighbouring organisms and affect local assemblages (Janiak and Whitlatch, 2012; Jones and Thornber, 2010). In Portugal, 28 NIS have been registered in mainland Atlantic waters, 4 in Madeira and 51 in the Azores (www.europe-alien.org). A list of the non-indigenous macroalgal species from this region is provided in Table 1.1.

**Table 1. 1.** List of the non-indigenous macroalgae from the northwest Iberian Peninsula (Araújo et al., 2009; Bárbara et al., 2005; Bárbara et al., 2008). NP, Northern Portugal; G, Galicia.

Phyllum	Species	Region
Chlorophyta	<i>Codium fragile</i> spp. <i>tomentosoides</i>	NP, G
	<i>Ulva pertusa</i> Kjellman	G
Rhodophyta	<i>Anotrichium furcellatum</i> (J. Agardh) Baldock	NP
	<i>Antithamnionella spirographidis</i> (Schiffner) E.M. Wollaston	NP
	<i>Antithamnionella ternifolia</i> (J.D. Hooker & Harvey) Lyle	NP, G
	<i>Antithamnion densum</i> (Suhr) M.A. Howe	NP
	<i>Asparagopsis armata</i> Harvey	NP, G
	<i>Chrysymenia wrightii</i> (Harvey) Yamada	G
	<i>Dasya sessilis</i> Yamada	G
	" <i>Falkenbergia rufolanosa</i> " (stage) (Harvey) F. Schmitz	NP, G
	<i>Grateloupia luxurians</i> (A. Gepp & E.S. Gepp) R.J. Wilkes	G
	<i>Grateloupia subpectinata</i> Holmes	G
	<i>Grateloupia turuturu</i> Yamada	NP, G
	<i>Heterosiphonia japónica</i> Yendo	NP, G
	<i>Lomentaria hakodatensis</i> Yendo	NP, G
	<i>Neosiphonia harveyi</i> (J. Bailey) M.S. Kim, H.-G. Choi, Guiry & G.W. Saunders	NP, G
Ochrophyta	<i>Colpomenia peregrina</i> Sauvageau	NP, G
	<i>Sargassum muticum</i> (Yendo) Fensholt	NP, G
	<i>Undaria pinnatifida</i> (Harvey) Suringar	NP, G

In particular, the brown alga *Sargassum muticum* is the most dominant non-indigenous macroalgal species in this area. On the Iberian Peninsula coast, the presence of *S. muticum* was first reported from Asturias in the 1980s, subsequently it was observed on the Galician coast in 1986 (Péres-Cirera et al., 1989) and it was first recorded in Portugal in 1989 (Rull Lluçh et al., 1994). This species has a particular dispersal mechanism of drifting fertile laterals, which associated with particular traits such as being self-fertile results in a rapid widespread distribution (Critchley et al., 1983; Rueness, 1989). Additionally, drifting germlings have been observed in the plankton up to at least 1.3 km from a fertile individual, possibly representing a viable mechanism for short-range dispersal (Deysher and Norton, 1982). In this context, we have used *S. muticum* as a model species in this research (see section 1.6. for more detailed information).

#### **1.4. NIS and ecosystem functioning in marine ecosystems**

It is worldwide accepted that human activities have a high ecological impact in the world's ecosystems (Chapin III et al., 1998; Halpern et al., 2008). The argument that biodiversity loss will lead to a reduction in global ecosystem functioning, i.e. interactions between biotic assemblages or with their abiotic environment, has emerged as an issue in the early 1990s (Ehrlich and Wilson, 1991; Naeem et al., 1994). The majority of the earlier studies focused on Biodiversity Ecosystem Functioning (BEF) were performed in terrestrial habitats (e.g. Hector et al., 1999; Naeem et al., 1994), and emphasized the importance of niche complementarity and species diversity in governing ecosystem properties, especially productivity (Tilman, 1999). The effect of diversity on efficiency of resource use might influence the fluxes of energy and matter, which are fundamental for other critical ecosystem processes and properties such as productivity, element cycling, invasion resistance, community stability and food web dynamics (Cardinale et al., 2006; Loreau et al., 2001; Stachowicz et al., 2007). Currently, there is growing interest in determining how human-induced change in ecosystem structure will affect ecosystem services (Worm et al., 2006), increasing the ecological research in this particular issue (Hooper et al., 2005; Stachowicz et al., 2007)).

A key question in marine ecology is how invasions can impact marine ecosystem function. Several studies have assessed the issues of biodiversity loss and ecosystem services in general, most of which are reviews of ecological and conservation theory (Naeem and Wright, 2003; Raffaelli, 2006; Tilman, 1999). However, to date, there are virtually no studies focusing on the functional consequences of NIS additions in marine

habitats (Stachowicz and Byrnes, 2006). Although the majority of introduced species develop no major impact on the recipient ecosystem (Sax and Gaines, 2003) some invasions may alter the energy flow between trophic groups, species interactions, productivity (Galil, 2007; Wikström and Hillebrand, 2012), or other aspects of community structure (Carlton, 1996a). Ecosystem level processes are affected by the functional characteristics of the organisms involved (Díaz and Cabido, 2001; Odum, 1969). In particular, special concern has been directed to NIS, following the recognition that large marine ecosystems are losing their biological distinctiveness (Carlton, 1996b; McKinney and Lockwood, 1999; Olden et al., 2004). Due to the fact that different organisms play unique roles in mediating ecosystem performance, there is growing awareness that the worldwide modification of assemblage's structure may influence the performance of ecosystems (Byrnes et al., 2007; Mouillot et al., 2011; Reynolds and Bruno, 2012). Life history features of invaders may be key factors in determining the fate and the impact of invasions. Introduced species often exhibit novel features compared to native species and may have disproportionately high impacts in native ecosystem functioning (Ruesink et al., 2006). However, the consequences of biodiversity change, through changes in species traits, are likely to be idiosyncratic, differing between trophic groups and ecosystems (Emmerson et al., 2001; O'Gorman et al., 2011). In this context, to understand the functioning of Earth systems requires not only understanding biogeochemistry processes, but also the role that biodiversity plays in this complex system (Chapin III et al., 2000; Loreau et al., 2001).

So far, research on the impacts of NIS lack the ability to disentangle the mechanisms involved in the impacts (Ruesink et al., 2006). In particular, no research has compared whether ecosystem impacts vary with the invader's life-history traits and no co-evolution with native species. Nonetheless, the true impact of introduced NIS on ecological and evolutionary processes can still not be predicted, particularly due to the fact that most ecosystem processes are a function of interactions among species, rather than simple presence or absence of species (Chapin III et al., 2000). Native species with a long history of co-evolution are expected to partition resources among them and promote ecosystem functioning throughout resource use complementary effects. In contrast, newly introduced species, probably enhance ecosystem functioning by sampling effects where the influence of the invader is well beyond its proportion (Ruesink et al., 2006).

Nonetheless, changes in species interaction may alter the traits that are expressed by species and consequently the effects of species on ecosystems processes (Chapin III

et al., 2000). Thus, global environmental changes have the potential to exacerbate the ecological and societal impacts of changes in biodiversity (Sala et al., 2000).

### **1.5. Global environmental change: new scenarios**

The increase in marine BEF research in the last decade brought a new vision of the functioning of the ecosystems. The functioning of ecosystems is known to be linked to environmental unpredictability and complexity (e.g. Bulling et al., 2010; Steudel et al., 2012) and so environmental factors should, more often, be incorporated in experiments with its role well defined. Anthropogenic activities not only affect organisms directly but also often alter the abiotic environment in which organisms live. Hence, biodiversity changes, ecosystem functioning, and abiotic factors interactions should be integrated in ecological studies to better understand the “natural” ecosystems.

Climate change is one of the greatest challenges the world is now facing. Global climate has always fluctuated, however, in the last decades we have experienced an accelerated rate of climate change (Hoegh-Guldberg et al., 2007; IPCC, 2007). It is crucial to know the vulnerability and capacity of adaptation of ecosystems to climate change. Changes in ocean temperature, biogeochemistry, salinity, sea level, UV radiation, and current circulation patterns have been identified over the last few decades (IPCC, 2007). Specifically, warmer and more acidic oceanic water (due to the increase of CO<sub>2</sub> in the atmosphere and oceans) are of critical concern (Harley et al., 2006; Kordas et al., 2011). In this context, microalgae and seaweeds have enormous potential and may be actively involved in lowering global warming and climate change (Gao and McKinley, 1994). Seaweeds play significant roles in the normal functioning of atmospheric environments, contributing to about 10% of the total world marine productivity (Israel et al., 2010). Algae, by the process of photosynthesis, absorb carbon dioxide from the atmosphere and release oxygen presenting a great potential for CO<sub>2</sub> bioremediation (Gao and McKinley, 1994). On the other hand, excess atmospheric CO<sub>2</sub> is being absorbed by the oceans, increasing pCO<sub>2</sub> in seawater which would affect physiology of marine macroalgae. Studies on macroalgal responses to different climate change scenarios suggested that macroalgal growth may be enhanced, inhibited, or not affected by increased CO<sub>2</sub> concentration (Diaz-Pulido et al., 2011; Porzio et al., 2011; Suárez-Álvarez et al., 2011). Overall, algal turfs have positive responses, and crustose calcareous algae tended to be negatively affected (Diaz-Pulido et al., 2011; Porzio et al., 2011). In addition, significant changes in macroalgal assemblage structure have also been reported coupled to direct effects of temperature

increase (Pedersen et al., 2008) and both direct and indirect effects due to temperature and ecological interactions, respectively (Schiel et al., 2004).

The recent change in sea temperature is considered as the most pervasive and severe cause of impact in coastal ecosystems worldwide (Halpern et al., 2008), as it affects metabolic rate, growth, reproduction and survival of organisms. For example, ecological disturbance such as severe events of coral bleaching (Hoegh-Guldberg et al., 2007), shifts in the distributional limits of benthic organisms in temperate coastal systems (Helmuth et al., 2006) or shifts in phenology (e.g. Philippart et al., 2003) have been related to temperature alteration. The magnitude of ecological effects of temperature alteration may inherently vary among and even within species, as different species and even different ontogenetic stages may be unequally susceptible to thermal stress (e.g. Kordas and Dudgeon, 2011; Porzio et al., 2011). Global climate change may also alter interactions among biological and physical factors, and recent reviews highlight the need to consider temperature acting in concert with other stressors (Crain et al., 2008). For instance, an experiment on the recruitment of the bloom-forming macroalga *Enteromorpha intestinalis* described strong interacting effects of temperature, nutrient enrichment and grazing (Lotze and Worm, 2002).

Besides the growing concern about the effects of climate change on ecosystems, scientists aim to assess possible interactions between climate change and biological invasions (Hellmann et al., 2008; Stachowicz et al., 2002). Marine NIS are a component of global change in all marine coastal ecosystems and determining interactive effects of anthropogenic agents of disturbance with natural processes should be the focus of ecological research. Warming waters are expected to promote colonisation and spread of NIS (Stachowicz et al., 2002), with smaller propagule size required (Simberloff, 2009). For instance, experimental manipulation of physical factors reduced propagule size required for a high probability of successful establishment of the European grass *Holcus lanatus* in California (Thomsen et al., 2006). Moreover, global climate change is increasing water temperatures in northern-latitude (Hughes, 2000). This may cause seasonally stressful conditions for coldwater-adapted species, but may provide suitable thermal conditions to allow non-native warm-water species to thrive in these habitats (Sharma et al., 2007). Increased range distributions with global warming will increase possible NIS colonisation and interaction with native species, highlighting synergistic effects of climate warming and non-native species. For example, a red seaweed, *Grateloupia turuturu*, native to Japan and Korea has invaded the coasts of New England (Mathieson et al., 2008), although no description has been

made further north. The temperature tolerance of this species is 4-28°C and thus lower sea surface temperature may have stopped its further invasion into Canadian waters.

There has been an increase in the number of case studies showing evidence of ecological impacts of NIS (Walther et al., 2005), although very little is known of the impacts of invasive species in relation to climate change (Sorte et al., 2010). By interacting with biological invasions (Dukes and Mooney, 1999), climate change and other components of global change may have unexpected effects on organisms and ecosystems (Hughes, 2000; Occhipinti-Ambrogi, 2007; Parmesan, 2006).

## **1.6. Model invader species & Model system**

### **1.6.1. *Sargassum muticum*: range distribution & general aspects of its biology**

*Sargassum muticum* (Yendo) Fensholt (Phaeophyceae: Fucales) is native to Southeast Asia (Yendo 1907) where it has been described as a relatively minor component of the native macroalgal flora (Critchley et al., 1983; Norton, 1977). This species has been introduced to the coastlines of several countries in the northern hemisphere in the past 60 years, mainly associated with the transportation of Japanese Oysters (*Crassostrea gigas*) for aquaculture (Scagel, 1956). The first register of an established population of *S. muticum* outside its native range was in British Columbia, Canada in 1945 (Abbott and Hollenberg, 1976) and is now distributed throughout the North America Pacific coast, from SE Alaska to Baja California. Attached individuals were first recorded in Europe in the British Isles in 1973 (Critchley et al., 1983). The invasive seaweed *S. muticum* is now present along most of the Atlantic coast, from Portugal in the south to Norway in the North (Engelen et al., 2008; Rueness, 1989) and in the Mediterranean (Streftaris et al., 2005).

Populations of this species are distributed mainly in sheltered or semi-exposed hard-bottom shores, although it may also attach to hard substrates on soft-bottoms, such as stones or shells (Strong et al., 2006). This species is often visually dominant, forming beds from sublittoral zone (up to 0.8 m above the low tide, LAT) (Arenas et al., 1995) to the mid-intertidal zone. Individuals also appear in rock pools located on exposed intertidal zones, which gives protection from a low tolerance to desiccation (Norton, 1977) and from mechanical stress by wave-action (Viejo et al., 1995). Moreover, *S. muticum* is tolerant to a wide variation in salinity and temperature (Norton, 1977). In northern Portugal and Galicia, *S. muticum* occurs from sublittoral zone to the intertidal,

although in N Portugal intertidal it can only be observed in channels and tidepools (Incera et al., 2011; Monteiro et al., 2009). Longevity estimates of *S. muticum* in Portugal, using a matrix model are of 2.8–8.4 years (Engelen and Santos, 2009).

Intrinsic traits such as being a fast growing species, high fecundity, monoecious, self-fertile and pseudo-perennial life history (Fig. 1.3), among others, have been cited as responsible for the success of *S. muticum* as invader (Norton, 1976). The thallus of *S. muticum* is highly differentiated and has several distinct morphological features, with a marked seasonal variation. It consists of a perennial holdfast from which main axes and annual lateral branches grow. This species has 4 characteristics growth phases, suggesting a highly specialized adaptation to life in a seasonal environment (Fig. 1.3): 1) initial growth phase with large basal leaves which increase photosynthetic surface area; 2) elongation growth where the presence of gas-bladders maintain the thallus erect and closer to the light; 3) reproductive growth (April/May), where energy allocation is now shifted to the production of reproductive tissue (receptacles) and 4) a senescence period (from August onwards) where primary laterals degenerate and float away (Arenas et al., 1995; Deysher, 1984).

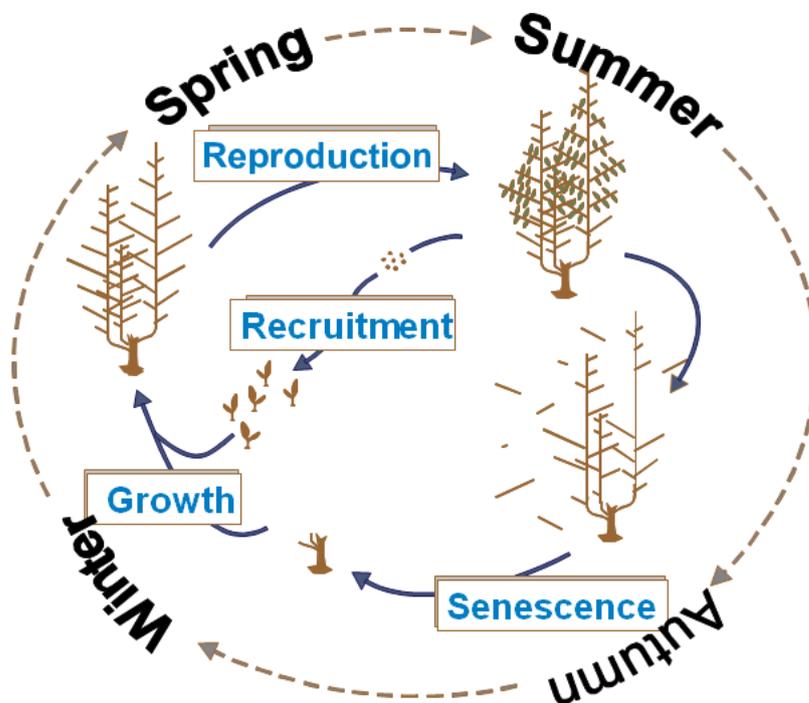


Fig. 1. 3. *Sargassum muticum* life-cycle.

A fertile individual of *S. muticum* bears thousands of receptacles (reproductive structure) and bear both male and female conceptacles, thus self-fertilization is common (Deysher and Norton, 1982). Gamete expulsion and release has a semilunar periodicity, peaking around full and new moon (Monteiro et al., 2009). Fertilized eggs are retained in the external surface of the receptacles until the development of tiny germlings with adhesive rhizoids (Norton, 1981). Embryos are then liberated, they sink and generally reattach to the substratum within a few meters of the parent plant (Deysher and Norton, 1982). The characteristic egg expulsion by pulses, separated of several days (Norton, 1981) can also be seen as an advantageous characteristic of *S. muticum* when facing non-appropriate environmental conditions. However, multiple-range dispersal mechanisms have been cited for this species, including germling settlement and drifting fertile thalli (Norton, 1976). The characteristic air bladders provide a good mode of dispersal for dislodged individuals (Rueness, 1989).

Replacement of native species, increase of filamentous epiphytic algae, changes in composition of flora and fauna, increased sedimentation, interference with coastal fisheries large accumulations of drift algae, blocking of narrow sounds and harbours, and interference with recreational activities are some of the known impacts of *S. muticum* (Critchley et al., 1986; Mack et al., 2000; Pedersen et al., 2005). For example, competition between the giant kelp *Macrocystis pyrifera* and *S. muticum* has shown that *S. muticum* inhibited the recruitment of *M. pyrifera* (Ambrose and Nelson, 1982). Moreover, indirect effects affecting abiotic conditions, such as shading or alteration of the current by the dense canopy during the summer months may affect settlement and recruitment of benthic organisms (Britton-Simmons, 2004; Critchley et al., 1990; Staehr et al., 2000). Because *S. muticum* also undergoes a faster and more complete decomposition than that of the native flora it has replaced, it has been suggested to alter the energy flow, by increasing the turnover rate and regeneration of nutrients (Pedersen et al., 2005; Rossi et al., 2011) and rate of primary production (Cebrián and Duarte, 1995).

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Moreover, indirect effects affecting abiotic conditions, such as shading or alteration of the current by the dense canopy during the summer months may affect settlement and recruitment of benthic organisms (Britton-Simmons, 2004; Critchley et al., 1990; Staehr et al., 2000). Because *S. muticum* also undergoes a faster and more complete decomposition than that of the native flora it has replaced, it has been suggested to alter the energy flow, by increasing the turnover rate and regeneration of nutrients (Pedersen et al., 2005; Rossi et al., 2011) and rate of primary production (Cebrián and Duarte, 1995).

Although non-indigenous species are often linked to negative impacts on the indigenous biota, some positive effects have also been described. In the North Sea, *S. muticum* provides habitats for epibiota otherwise absent in sediments, with consequent strong effects on diversity of soft sediments but not of hard substrata, where native species perform the same function (Buschbaum et al., 2006). Moreover, a recent study in the Galician coast revealed that *S. muticum* offered a suitable habitat for many invertebrates (Gestoso et al., 2012).

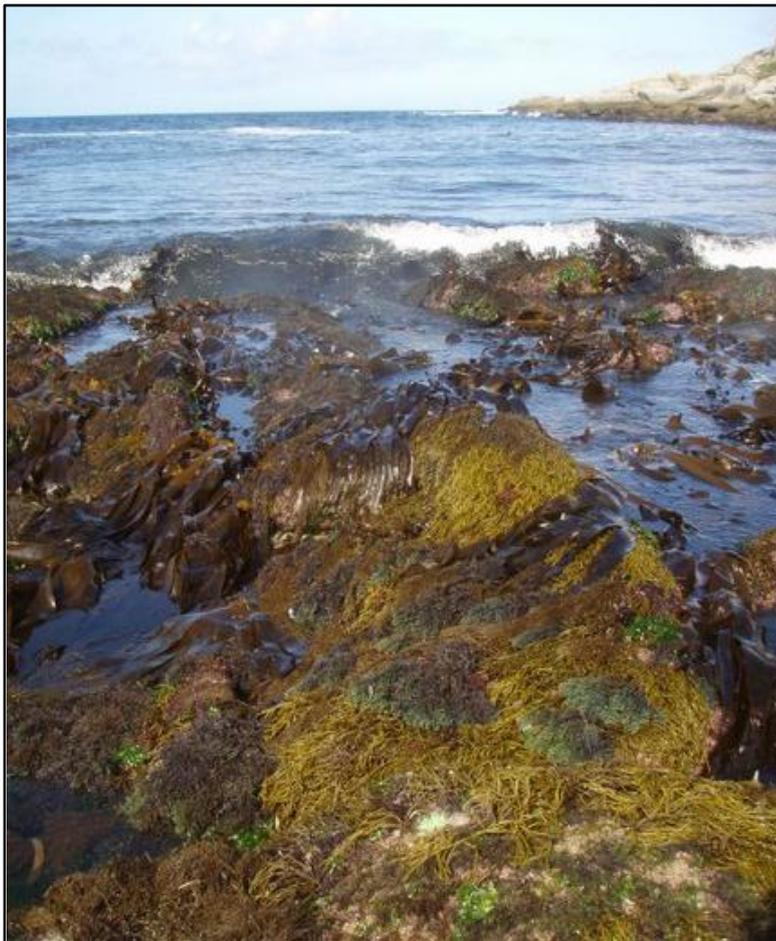
### **1.6.2. Life in intertidal rock pools**

In the NW coast of Portugal and Galicia, *Sargassum muticum* is very abundant in mid- and low-intertidal rock pools and research was mostly conducted in these habitats (Fig. 1.5).

Tidepools are a special habitat on the rocky intertidal platforms, which provide refuge from desiccating conditions during low tide (Metaxas and Scheibling, 1993). It has been suggested that tidepools do not represent an intertidal habitat, due to not being emerged during low tide (Underwood, 1981), nor reflect life in the sublittoral zone, due to dramatic environmental fluctuations in these habitats (Little and Kitching, 1996). Environmental conditions in rock pools are, however, regulated by the tidal cycle (Metaxas and Scheibling, 1993). Each rock pool exhibits specific physic-chemical parameters such as temperature, salinity, oxygen, carbon dioxide and pH (Huggett and Griffiths, 1986), as those vary particularly in relation to height on the shore, but also with pool size, shape and assemblage composition (Metaxas and Scheibling, 1993). However, tidepools not only vary in environmental conditions but also in the availability of food (e.g. Underwood, 1984), level of predation (e.g. Connell, 1970) and availability of recruits (Coleman and Brawley, 2005).

Particularly for macroalgal assemblages, there is evidence that pool depth is of major importance (Martins et al., 2007), probably due to larger fluctuations of physic-chemical parameters in shallow pools (Metaxas and Scheibling, 1993). During daytime, algae absorb carbon dioxide and produce oxygen by photosynthesis whereas at night, when photosynthesis stops, respiration absorbs much of the available oxygen (Morris and Taylor, 1983). This physiological activity provokes changes in the pH of the water, with an inverse relationship with  $p\text{CO}_2$  (Morris and Taylor, 1983). Moreover, the physic-chemical conditions within tidepools are related to the biomass of fauna and flora, and show a marked seasonal variation. Thus, the referred diurnal changes are the result of interactions between physic-chemical and biological processes (Morris and Taylor, 1983).

Overall, like other intertidal habitat pools are very amenable for experimental studies. Also, because manipulations are easy to carry, tidepools can be used as experimental mesocosm providing an ideal system in which to study invasion processes.



**Fig. 1.5.** Intertidal rocky shore in Ria de Vigo (Galicia, Spain). © Eva Cacabelos.

### 1.6.3. The use of synthetic macroalgal assemblages

Some authors argue that certain experimental designs may cause artifacts due to the assumption of random loss of species from the total species pool (Loreau et al., 2001) in experiments such as mesocosms or with synthetic assembled assemblages. It has been demonstrated that non random changes in biodiversity of macroalgal assemblages can have different effects on ecosystem functioning to random changes (Bracken et al., 2008), however, only few studies have focused on the selective removal of key species in the field, to simulate non random changes in patterns of distribution and abundance of organisms (Díaz et al., 2003). Studies based on “natural” ecosystems are scarce (but see Arenas et al., 2009), which leads to a very pertinent point on how comparable are synthetic versus natural assemblages. It is important to recognise that all manipulative experiments are in fact abstractions from reality and all approaches have significant strengths and weaknesses. The great advantage of laboratory experimentation is the opportunity to control the system conditions and to take precise measurements of response variables. Its disadvantages are a measure of its artificiality and possible lack of applicability to the natural world as it is isolated from many of the biotic interactions (e.g. competition, predation, facilitation) that occur in a natural system. On the other hand, the lack of full controlled experimentation in field studies will tolerate possible effects over and above patterns of natural variation, which will represent an interaction closer to reality. It is, however, suggested that biodiversity effects are significantly weaker in less-controlled experimental systems (Hooper et al., 2005; Loreau et al., 2001). Ideally, we should aim to design experiments to test the same hypotheses using the same assemblages in both the laboratory and the field (e.g. Tait and Schiel, 2010).

The intensity of interactions among sessile individuals varies with the spatial arrangement of competitors, and thus, the relative proximity of neighbouring individuals may be an important factor influencing the interactions among seaweeds (Carpenter, 1990; Kim, 2002). Moreover, macroalgal assemblages in tidepools are very patchy systems at very small scales, where extremely small spatial scales of variation seem to be among the most important sources of heterogeneity (Archambault and Bourget, 1996). Thus, synthetic macroalgal assemblages resembling the patchy “tile-like” pattern of intertidal algal assemblages are expected to describe quite realistically the natural system.

## 1.7. Objectives and structure of the thesis

The general objective of this thesis was to provide critical knowledge of the processes governing biological invasions in intertidal rocky shores. Specifically, this PhD Thesis aimed to determine the relevant physical and biological processes affecting the invasion success and potential impacts of the brown macroalga *Sargassum muticum*. The specific objectives were:

i) To assess the interactive effects of functional diversity and propagule limitation on different macroalgal invasion stages;

ii) To study how bottom-up (resources) and top-down (grazers) processes operate interactively to determine invasion success;

iii) To investigate ecosystem-level impacts of macroalgal invasions and discriminate the mechanisms involved behind the impact;

iv) To test the effects of disturbance by climate change-related drivers on early survivorship of *S. muticum* germlings and legacy effects over recruitment success.

The two first objectives, tackled in Chapter 2 and 3, are centered on the interaction between native ecosystem and the invasive success of the macroalga *S. muticum*, employing laboratory and empirical field experiments using intertidal macroalgal assemblages.

The third objective is assessed in Chapter 4 and 5, which investigated the dynamics of production and respiration in native macroalgal assemblages and assemblages invaded by *S. muticum*, and the mechanisms involved in the competition between the native macroalga *Cystoseira humilis* and the invasive macroalga *S. muticum*.

Lastly, Chapter 6 examined the effect of climate change drivers on the invasion success of *S. muticum* and on the susceptibility of marine macroalgal assemblages to invasions.

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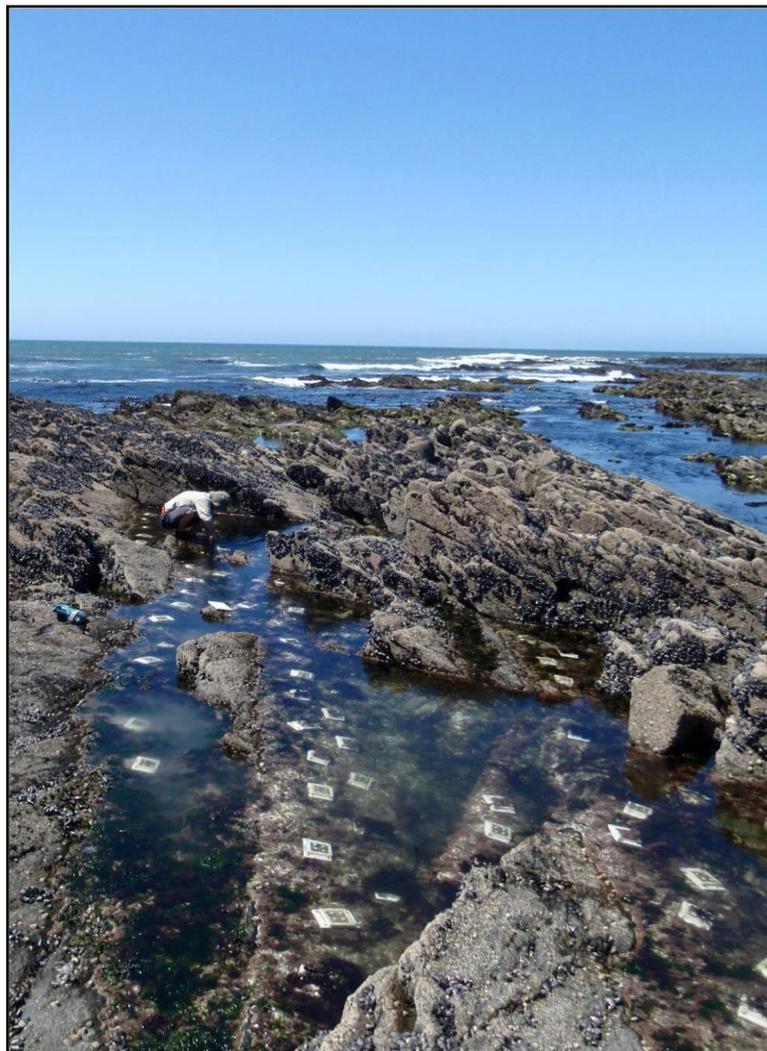
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## Part II

### The process of macroalgal invasions





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## **Chapter 2**

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Propagule pressure and functional diversity:  
interactive effects on a macroalgal invasion process



**Propagule pressure and functional diversity: interactive effects on a macroalgal invasion process**

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## 2.1. Abstract

Invasive species are considered an increasing global threat to the marine ecosystems. Understanding which factors can accelerate or reduce invasion success is one of the main goals of invasion ecology. The present study investigated the interactive effects of propagule pressure and native functional diversity on the invasion success of the invasive alga *Sargassum muticum* (Yendo) Fensholt over a 1-year invasion process (i.e. settlement, recruitment and colonisation). Invasibility of macroalgal assemblages with different functional diversity was assessed under high and low propagule pressure. Synthetic assemblages resembling natural marine macroalgal assemblages were built and used as model systems. Recruitment discs were used to record early settlement at the assemblages. Recruitment and colonisation success were monitored in the field 2 and 10-months after exposure to propagule rain at the laboratory. In addition, availability of resources (space and photosynthetic active radiation, PAR) was also monitored for each assemblage and used as predictive variables in the analyses. Results showed that the effects of propagule pressure and functional diversity varied over the different invasion stages. Contrasting to previous studies on *S. muticum*, space availability did not play a significant role explaining recruitment success of the invader. PAR, however, was a key resource in the invasion success of *S. muticum*. Our findings showed that the invasion success of *S. muticum* over the different invasion stages was influenced differently by recipient assemblage traits. Hence, invasion success may better be explained by the interaction of environmental factors that covary with species diversity and identity at different invasion stages.

**Key words:** invasion success, propagule pressure, resources, functional diversity, *Sargassum muticum*, macroalgae

## 2.2. Introduction

**I**ncreasing intensity of anthropogenic activities is promoting diversity loss and enhancing the introduction and spread of exotic organisms to new areas outside of their native range (Vitousek et al., 1997; Chapin III et al., 2000). Beyond the changes in species range, which occur constantly in nature (Vermeij, 1991), it is the accelerating rate and the magnitude of those changes that pose a threat to global biodiversity (Lodge, 1993). Linked to human-induced modifications, invasions of non-indigenous species (NIS) all over the world are enhancing habitat homogenization (Vitousek et al., 1997; Olden and Poff, 2004), with unknown consequences to Earth's biota and ecosystem functioning (Olden et al., 2004).

The invasion process, however, is a very complex phenomenon and consists of several transitional stages (Williamson, 2006), such as arrival, establishment and success in spreading. Survivorship through each stage of the invasion process is the ultimate fight of any NIS. Understanding which factors and to what extent they influence invasion success through the different stages is of extreme importance in invasion ecology (Kolar and Lodge, 2001; White and Shurin, 2007). Overall, three factors are usually cited as determining the fate of invasions: the biology of the introduced species (species invasiveness), number and frequency of introductions (propagule pressure) and the susceptibility of the native community to invasion (community invasibility) (Lonsdale, 1999). Nonetheless, invasion studies are usually focused on only one of these factors (Lonsdale, 1999).

It is widely accepted that highly diverse communities are more resistant to invasions because of a more complete utilization of resources, the so-called biotic resistance theory (see Elton, 1958; Levine, 2000; Fridley et al., 2007 for a review of diversity-invasibility relationships). For benthic algal communities as sessile photosynthetic organisms, space and light are important limiting resources (Sousa, 1979). For example, lack of space and low light availability negatively affected the invasion success of the brown macroalga *Sargassum muticum* (Staehr et al., 2000; Britton-Simmons, 2006). However, there is some ongoing debate on which component of diversity is more relevant in preventing the communities to be invaded and the mechanisms involved (e.g. Davis et al., 2000, Arenas et al., 2006; Stachowicz et al., 2007). For example, as a component of biodiversity, functional-group diversity has been suggested to contribute to a greater impact on ecosystem processes than the total number of species *per se* (Tilman et al., 1997). Moreover, several studies suggest

that the composition, i.e. identity effect, of a community may determine its susceptibility to invasion (Symstad, 2000; Arenas et al., 2006).

It has been suggested that a large input of propagules is needed for invasion to occur in systems where environmental resistance is strong (D'Antonio et al., 2001). Thus, besides the importance of the biotic resistance on the success or failure of invasions, there is a general consensus on the importance of the propagule pressure (also termed 'introduction effort') as a strong predictor of invasion success (Colautti et al., 2006; Eschtruth and Battles, 2011). Propagule pressure can be defined as the total number of individuals introduced at a given location (Williamson, 1996). This introduction of propagules into a location may occur through several releases. Thus, propagule pressure should include estimates of the number of individuals release in an event, i.e. propagule size, and the number of introduction events, i.e. propagule number (Lockwood et al., 2005). A positive relationship between propagule pressure and invasion success has been suggested (Lonsdale, 1999; Knights et al., 2012), although the true shape of the relationship is poorly studied (Lockwood et al., 2005) and its interaction with other factors (e.g. disturbance, resource availability) mostly unknown (Sakai et al., 2001; Britton-Simmons and Abbott, 2008).

In the present study we explored how the traits of the recipient assemblage, i.e., functional diversity and identity, interact with propagule pressure in shaping the success of an invasion. Our model systems were synthetic macroalgal assemblages with varying levels of functional diversity and as invader we used the Japanese seaweed *Sargassum muticum*. We tested the hypotheses that propagule pressure affects the invasion success of *S. muticum* at different invasion stages, and this effect is shaped by the functional diversity of receiving native assemblages. Invasibility of macroalgal assemblages is expected to decrease with functional diversity (increased biotic resistance) and increase with greater propagule pressure (D'Antonio et al., 2001). In addition, we tested if the availability of resources (space and photosynthetic active radiation) would influence the invasion success.

## **2.3. Materials and Methods**

### **2.3.1. Study area**

Field work was carried out in a large rock pool (11x2 m size, average depth of 35 cm) in the mid-intertidal shore ( $\approx 1.5\text{m}$  above chart datum) at Viana do Castelo (41°42'25.72" N, 8°51'42.71" W, Northwest coast of Portugal). The intertidal zone

along the Portuguese coastline is influenced by a semidiurnal tidal regime. Native algal assemblages from low shore and rock pools are predominantly of two types, a) understory algae dominated by turf-forming algae (e.g. *Corallina* spp., *Chondracanthus* spp., *Gelidium* spp., *Ceramium* spp.) coexisting with encrusting coralline algae like *Lithophyllum incrustans*; and b) subcanopy species like *Bifurcaria bifurcata*, *Mastocarpus stellatus* and *Chondrus crispus* (Araújo et al., 2006). In the study area *Sargassum muticum* is found in mid-intertidal rock-pools, lower intertidal channels, and subtidal zones. Herbivores include a variety of molluscs such as snails (mostly *Gibbula* spp.), limpets (e.g. *Patella* spp.) and the sea hare *Aplysia punctata* Cuvier, 1803 (Monteiro et al., 2009a; Monteiro et al., 2012).

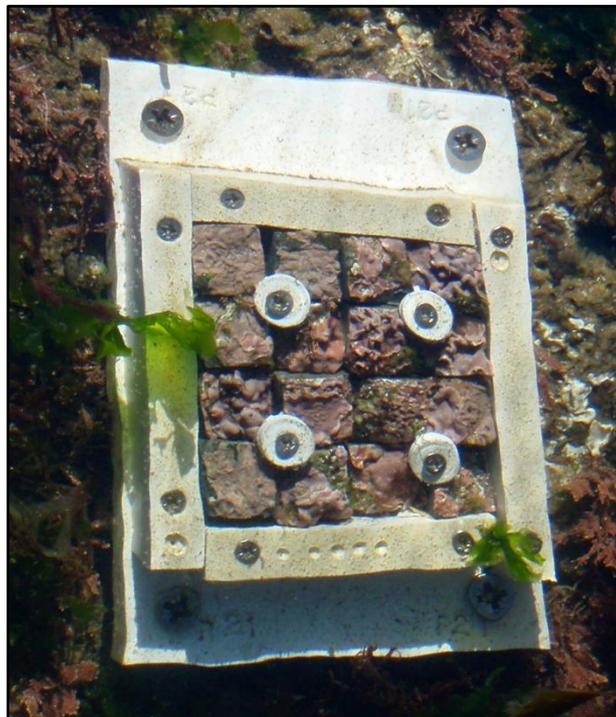
### 2.3.2. The invader, *Sargassum muticum*

*Sargassum muticum* is a marine macroalga native to Southeast Asia (Yendo, 1907). Considered an invasive species all around the world (Norton, 1976; Critchley et al., 1983), it is now one of the dominant seaweeds in many low intertidal and subtidal communities along the Northern Portuguese coast (Monteiro et al., 2009a). This invasive species is monoecious, self-fertile (Norton, 1976) and it is considered a pseudo-perennial alga due to the presence of its lateral branches only for a portion of the year. Reproductive period takes place in spring/summer. In the region of study, peak of reproduction occurs between mid-April and late July, while senescence of lateral branches begins in mid-June (Arenas et al., 1995). Fertilized eggs are attached to the external surface of the receptacles (reproductive structure) and remain there until the development of tiny germlings with adhesive rhizoids (Norton, 1981). After this, the embryo is liberated, sinks and reattaches to the substratum within a few meters of the parent plant (Deysner and Norton, 1982). Adult individuals stay attached to the substratum by a holdfast.

### 2.3.3. Macroalgal assemblages and experimental design

Synthetic assemblages were created in order to manipulate functional group diversity. Based on previous knowledge of macroalgal communities inhabiting rock-pools in the northwest coast of Portugal (Araújo et al., 2006; Monteiro et al., 2009a), we selected three morpho-functional groups (modified from Steneck and Dethier, 1994): a) encrusting coralline species such as *Lithophyllum incrustans*; b) turf-forming species from the genera *Corallina* (usually 5 cm length); and c) subcanopy species like *Chondrus crispus* or *Mastocarpus stellatus* (may reach 20 cm length). Small boulders bearing species of the selected morpho-functional groups were collected in the

intertidal of Viana do Castelo and taken to the laboratory. Using a commercial tile cutter, rock pieces were cut into the correct size (2 x 2 x 2 cm) and were attached to polyvinyl chloride (PVC) plates using fast setting underwater cement and screws. Experimental assemblages consisted of 12 x 17 x 1 cm PVC plates with 16 pieces of rock surrounded by 1cm PVC pieces for support and protection (Fig. 2.1). Each rock piece represented one functional group characterised by a percent cover greater than 50% or, in the case of subcanopy species, the presence of 1 or more adult individuals was considered a representation of that functional group. Although synthetic and relatively small assemblages were used, these assemblages resembled quite realistically the patchy pattern of intertidal algal assemblages, in which extremely small spatial scales of variation (< 20 cm) seem to be among the most important sources of heterogeneity (Archambault and Bourget, 1996).



**Fig. 2. 1.** Illustration of a macroalgal assemblage plate used in the present study, in this case a monospecific assemblage of encrusting species.

Additionally, four recruitment discs (1.13 cm<sup>2</sup> each) were placed in each assemblage plate for assessment of *Sargassum muticum* early settlement. A total of 60 plates (n = 4) were built: 12 plates of only bare rock, 36 plates with only one functional group, and 12 plates with three functional groups. The spatial distribution of the three functional groups within plates was random.

A two-way factorial design was used to examine the interactive effects of functional diversity and propagule pressure on different invasion stages (i.e. settlement, recruitment and colonisation). Both, Functional diversity (FD) and Propagule pressure (PP) were orthogonal fixed factors ( $n = 4$ ). Functional diversity incorporated 4 levels: 1) monospecific assemblages of encrusting species, 2) monospecific assemblages of turf-forming species, 3) monospecific assemblages of subcanopy species, and 4) three functional groups, i.e., high functional diversity. In addition, we also prepared bare rock plates as controls. Propagule pressure had 3 levels: 1) control/none, 2) low density and 3) high density.

### 2.3.4. Invasion procedure

Propagule pressure (PP) level, here regarded as the density of propagule rain from the introduced species used in a single invasion event, was manipulated by placing different amount of fertile individuals of *Sargassum muticum* (high density  $\approx 25\text{kg}$ ; low density  $\approx 13\text{kg}$ ; control – none) floating over the macroalgal assemblages. For that, fertile individuals of *S. muticum* were collected from the field and transported to the laboratory where they were rinsed with fresh water to eliminate grazers. To assure effectiveness of the PP treatment only individuals with receptacles bearing exuded propagules were used. Experimental invasion was programmed to be around the new moon (July 22, 2009) due to the semilunar periodicity of egg expulsion in *S. muticum* around new or full moons (Norton, 1981; Monteiro et al., 2009b). Three tanks of approximately 300L of seawater (i.e., 1 tank per treatment) were used for the experiment. A total of 20 macroalgal assemblages, 4 of each assemblage type plus bare rock plates, were randomly assigned to control, low and high PP treatments. Assemblage plates remained in experimental tanks for 1 week. During invasion procedure, *S. muticum* individuals remained at low PP treatment for three days and at high density treatment for five days. Additionally, ten recruitment discs ( $5.11\text{ cm}^2$ ) placed between assemblages in the tanks were used to assess rain of propagules at each propagule pressure treatment. Low propagule rain consisted of an average ( $n = 10$ ) of  $195 \pm 7.72$  ( $\pm$  SE) germlings  $\text{cm}^{-2}$ , while high density level was approximately  $786 \pm 30.14$  ( $\pm$  SE) germlings  $\text{cm}^{-2}$ .

Assemblages from control treatment were used to check for invasion by *Sargassum muticum* individuals in the field. Synthetic assemblages were transported to Viana do Castelo after laboratory experimental manipulation (control plates at day 6 and plates from PP treatments at day 8). Assemblage plates were randomly placed and screwed to the bottom of a large intertidal rock pool, separated by  $\approx 50\text{ cm}$ , where they

remained for 12 months. The selected rock pool was *S. muticum* free, as all the individuals were ripped off by its holdfast days before the start of the experiment. During the course of the experiment the rock pool was continuously monitored and new individuals of *S. muticum* removed with a scraper.

### 2.3.5. Measurements of invasibility and sampling procedure

Invasion of *Sargassum muticum* was monitored over three stages of invasion; settlement, recruitment and colonisation. Settlement of *S. muticum* germlings was assessed 2 to 4 days after lab exposure to propagule rain, prior to transportation of assemblage plates to the field. Recruitment discs from each assemblage plate were used to record germlings densities with the use of a stereo microscope with additional dimmed light. Plates were then transported to the field. In order to assess the effect of transport, 26 additional recruitment discs (13 per PP treatment) with known densities of *S. muticum* germlings were taken for field transportation and returned to the laboratory where germlings were recounted. Mortality rate ( $\pm$  SE) due to transportation ( $4.3\% \pm 0.91$ ) was insignificant when compared to 2-months field natural mortality rate of  $\approx 99\%$  ( $\pm 0.34$ ) ( $F_{1,50} = 2673.16$ ,  $P < 0.0001$ ). Thus, mortality due to transportation was not taken into account when interpreting results.

Two months after lab exposure to propagule rain (September 2009) recruits were visible to the naked eye and recruitment was assessed by counting *Sargassum muticum* recruits in the assemblage plates at the substratum level. Colonisation success was assessed by quantifying adult *S. muticum* ( $> 20$  cm length) survivorship in assemblage plates from September 2009 to April 2010.

In addition, we also estimated percentage cover of algae (primary and secondary cover) and bare rock surface. Algal cover was estimated by visually dividing each rock cube in 4 and attributing a score from 0 to 4 to the functional groups present and adding up the 16 estimates (Dethier et al., 1993). The above canopy area was measured as secondary cover when subcanopy species were present.

Available resources (space and photosynthetic active radiation, PAR) were also monitored for each macroalgal assemblage prior to the invasion procedure. Space availability was assessed during algae cover monitoring by quantifying percentage of bare rock at the substratum level. PAR penetration (measured as the ratio of PAR under the algal canopy to that above it, or in the case of encrusting species functional group at the substratum level to that 10 cm above it) was registered with a spherical

quantum scalar irradiance sensor (1.9 cm diameter sensor, Mod. QSL–2100 Biospherical Instruments - Inc., USA). PAR availability at substratum level was related to the irradiance intercepted per functional group, and represented a measure of how much PAR reached the substratum. Experimental plates were submerged in a 5-L tank during PAR availability measurements. For each plate, the sensor was moved throughout the substrata (below assemblage canopy) and 10 cm above (or over assemblage canopy) for 30 seconds each.

### 2.3.6. Statistical analyses

Differences in resources availability among functional diversity treatments was assessed with a one-way ANOVA. Functional diversity was a fixed factor with 4 levels (i.e. encrusting, turf, subcanopy and high functional diversity). Both, space and PAR data had homogeneous variances (Cochran's test,  $C = 0.3518$  and  $C = 0.3511$ , respectively;  $P > 0.05$ ).

No *Sargassum muticum* recruitment was recorded in plates from control PP treatment over the 12 months experimental period. All new *S. muticum* recruits counted were assumed to come from the laboratory artificial invasion and thus analyses were only performed for low and high PP treatments. Generalized linear models (GLM) were fitted for settlement and recruitment data to investigate the influence of the explanatory variables over the variation in the number of *S. muticum* germlings and recruits, respectively. For both sets of count data, we assumed a negative binomial distribution (an extension of GLMs) to account for high overdispersion using the `glm.nb` function from the *MASS* package (Venables and Ripley, 2002). Colonisation success was assessed with presence-absence data where 1 represented survivorship of *S. muticum* and 0 represented no *S. muticum* survivorship to adult stage. For presence-absence data we used a binomial GLM and a logit link function. Initial fitted models were built using PP (2 levels, high vs low), FD (5 levels this time: bare rock, monospecific assemblages of encrusting species, monospecific assemblages of turf-forming species, monospecific assemblages of subcanopy species and high functional diversity assemblages) and two continuous variables, PAR and space, as predictors. The continuous variable space availability was not included in the predictive model performed for settlement due to the use of recruitment discs in this stage. We also included biologically reasonable two-way interactions in the initial model.

To obtain the minimum adequate model we performed stepwise variable selection minimizing the Akaike's information criterion (AIC) and then hypothesis testing (Zuur et

al., 2009). *A posteriori* contrasts were performed to assess statistical significance between the various levels of the categorical factor Functional diversity (Crawley, 2009). To assess collinearity between variables we calculated the variance inflation factor (VIF) with `vif` function in the `car` package (Fox and Weisberg, 2011). All VIF values were below 8, indicating low collinearity, so we kept all variables in the analyses. Residuals from the selected model were plotted against the fitted values for model validation.

Rates of instantaneous mortality of the invader were used to test whether or not the mortality rates were constant over the different invasion stages and were calculated following Viejo et al. (1999) as:

$$\text{Ln} [(N_t/N_{t_0}) + 0.0001] / t$$

where  $N_t/N_{t_0}$  is the proportion surviving per macroalgal assemblage and  $t$  is the time after invasion (0-2 months, settlement period; 2-6 months, recruitment period; 6-12, colonisation period). At the end of the experiment 2 out of the 60 experimental plates were lost due to rough weather and, thus colonisation data were analysed with three replicates instead of the initial four in two treatments. The design was balanced by adding the averaged values of the other three replicates to the model and recalculating the  $F$ -values (see Underwood, 1997). Rates of instantaneous mortality were analysed with a three-way ANOVA with PP (2 levels), time (3 levels) and FD (5 levels) as orthogonal fixed factors. Variance was homogeneous (Cochran's Test,  $C = 0.1109$ ;  $P > 0.05$ ).

GLMs were carried out using the R-program 2.12.1 (R Development Core Team, 2010). Analysis of variance for resources availability and instantaneous mortality was performed with WinGMAV 5 (<http://sydney.edu.au/science/bio/eicc>).

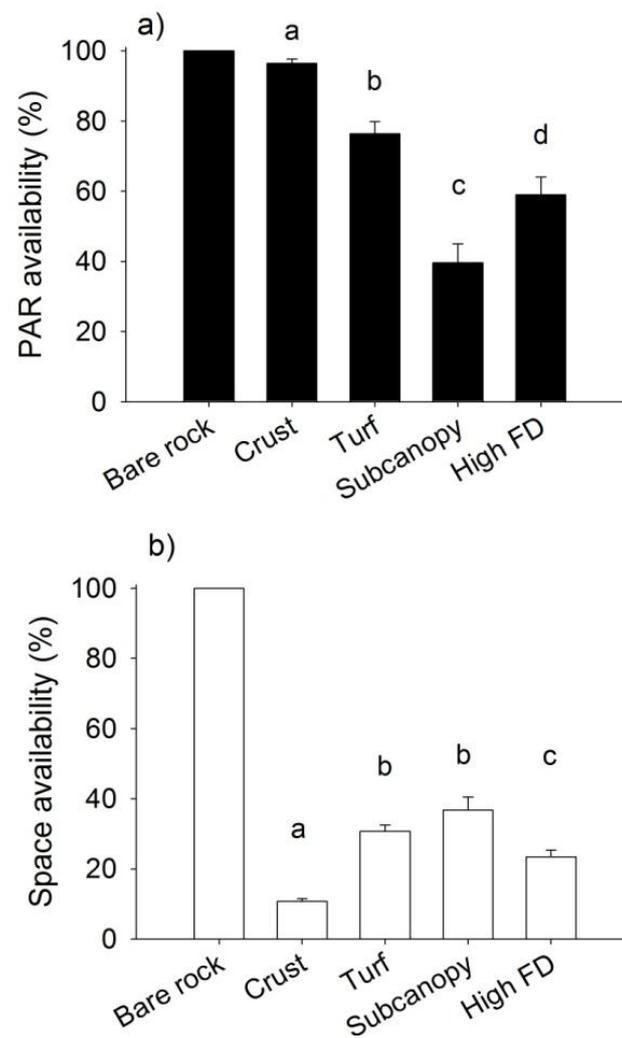
## 2.4. Results

### 2.4.1. Resources availability

PAR availability at substratum level varied significantly among functional diversity treatments (ANOVA,  $F_{3,28} = 35.20$ ,  $P < 0.001$ ). Monospecific assemblages of encrusting species showed the greatest values of PAR reaching the substratum level (Fig. 2a). PAR availability was particularly low at assemblages composed of subcanopy species, i.e. monospecific assemblages of subcanopy species and assemblages of high

functional diversity, whereas assemblages of turf-forming species showed intermediate levels (Fig. 2.2a).

Space availability varied significantly among functional diversity treatments (ANOVA,  $F_{3,28} = 29.52$ ,  $P < 0.0001$ ). Monospecific assemblages of subcanopy species and turf-forming species presented around 40 and 30%, respectively, of bare rock surface while monospecific assemblages of encrusting species exhibit lowest values ( $\approx 9\%$ ). Plates assembled with the three functional groups presented intermediate levels of availability of primary space (Fig. 2.2b).



**Fig. 2. 2.** Availability of resources (mean +SE,  $n = 8$ ) at the substratum level over the different functional diversity treatments, including those made only of bare rock. FD indicates functional diversity. A) Photosynthetic active radiation (PAR) availability; B) space availability as percentage cover of bare rock. Different letters indicate significant differences at  $P < 0.05$ .

### 2.4.2. Invasion success at different invasion stages

Invasion success of *Sargassum muticum* was partially explained by some of the predictive variables included in the models, namely propagule pressure, functional diversity and PAR availability. However, the variables included in the minimum adequate models differed between the three stages of invasion, i.e. settlement, recruitment and colonisation. A different order of inclusion of the variables did not affect the results.

Quantification of *Sargassum muticum* individuals at the different experimental treatments for the different stages of invasion are presented in Fig. 2.3. Settlement of *S. muticum* germlings was interactively influenced by propagule pressure and functional group diversity (Table 2.1a), with much higher settlement at high PP treatment (Fig. 2.3a). Overall, the explanatory variables from the model explained  $\approx 92\%$  of the variability in the number of *S. muticum* settled germlings (Table 2.1a). The interactive effects of PP and FD resulted in contrasting significant numbers of settled germlings between macroalgal assemblages at low and high PP. In the low PP treatment, monospecific assemblages of turf-forming species and high functional diversity assemblages registered significantly more settled germlings compared to the other assemblages (Fig. 2.3a). However, in the high PP treatment an opposite pattern was observed (Fig. 2.3a).

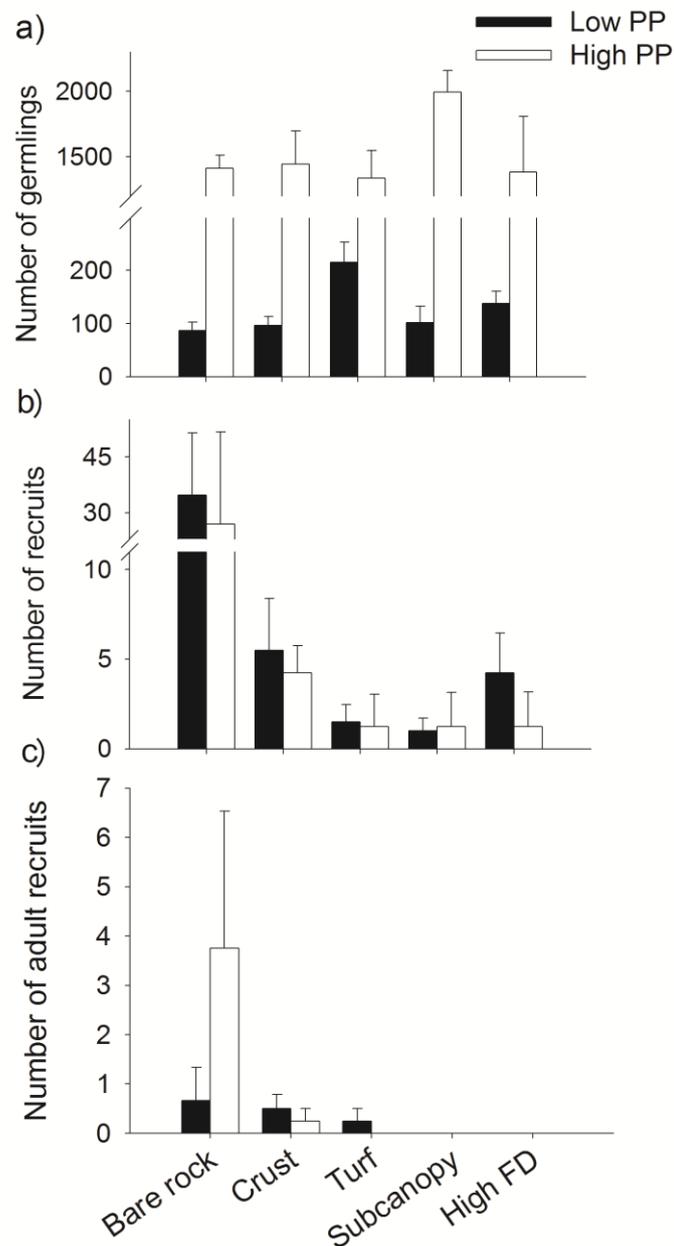
Unlike observed at settlement stage, propagule pressure was not a significant predictor of *Sargassum muticum* recruitment success. Thus, the fitted model for recruitment data, i.e. *S. muticum* juveniles recorded on assemblages at substratum level, included only functional diversity as a significant predictor and explained 52.4% of the variability in the number of *S. muticum* recruits (Table 2.1b). In particular, bare rock assemblages had significantly higher number of recruits than the other assemblage types (Fig. 2.3b).

At the colonisation stage, survivorship of *Sargassum muticum* was mainly influenced by PAR availability at the substratum level (Table 2.1c). Survivorship of *S. muticum* to adult stage was only observed at high PAR availability (Fig. 2.4).

### 2.4.3. Instantaneous mortality rates

The instantaneous mortality rate varied over time, independently from functional diversity of assemblages or propagule pressure (ANOVA,  $P < 0.0001$ ; Table 2.2). *Post-hoc* multiple comparisons showed that instantaneous mortality rates were significantly

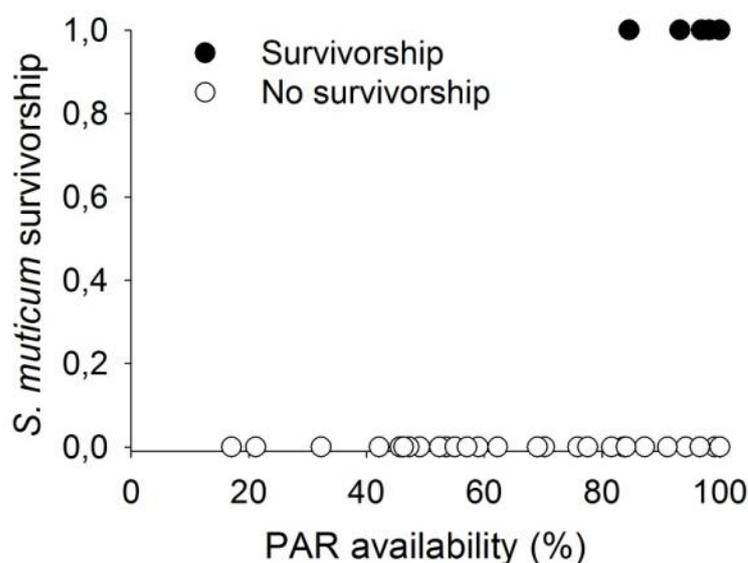
greater during the first two months (mortality of settlers) than over the recruitment period (2 to 12 months after invasion). In addition, instantaneous mortality rates were also greater in the first 4 months (2 to 6 months after invasion) compared to the last 6 months of the experiment (Fig. 2.5).



**Fig. 2. 3.** *Sargassum muticum* over a) settlement (per 2.26 cm<sup>2</sup>), b) recruitment (per 64 cm<sup>2</sup>) and c) colonisation (per 64 cm<sup>2</sup>). Mean (+SE, n = 4 per functional group and bare rock) number of individuals on assemblages of different functional diversity, including those made only of bare rock. FD indicates functional diversity, PP indicates propagule pressure.

**Table 2. 1.** *Sargassum muticum*. Analysis of deviance revealed by generalized linear models fitted to the explanatory variables Propagule pressure (PP), Functional diversity (FD), Photosynthetic active radiation (PAR) and Space availability. Variable's parameters described for the minimum adequate model for the response variable a) number of *S. muticum* germlings, b) number of *S. muticum* recruits and c) presence-absence data for survivorship of *S. muticum* to adult stage.

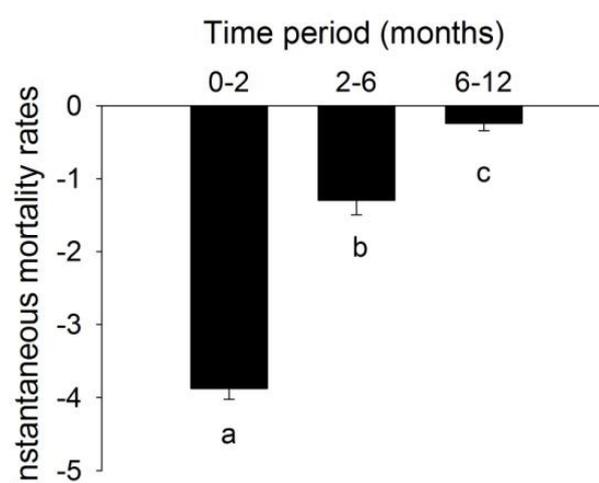
Variables	df	Deviance	Residual df	Residual Deviance	P	AIC
<b>a) Settlement</b>			39	498.68		
Model						
PP	1	434.47	38	64.21	<0.0001	
FD	4	9.12	34	55.09	0.058	
PP:FD	4	14.28	30	40.81	0.006	
Minimum adequate model						530.77
Full model:						531.43
PP*FD + PAR+ PP:PAR + FD:PAR, family = negative.binomial						
<b>b) Recruitment</b>						
Model			39	88.372		
FD	4	46.302	35	42.070	<0.0001	
Minimum adequate model						209.7
Full model:						228.57
PP*FD + PAR + Space + PP:PAR + PP:Space + FD:PAR + FD:Space, family = negative.binomial						
<b>c) Colonisation</b>						
Model			39	42.653		
PAR	1	15.425	38	27.228	<0.0001	
Minimum adequate model						31.29
Full model:						53.44
PP+FD+PAR+Space+PP:FD+FD:PAR, family = binomial						



**Fig. 2. 4.** *Sargassum muticum* colonisation success. Variation in the survivorship of *S. muticum* to adult stage (from 2 to 9 months) with photosynthetic active radiation (PAR) availability at substratum level on macroalgal assemblages.

**Table 2. 2.** *Sargassum muticum*. Results of the three-way ANOVA testing for the effects of Propagule pressure (PP), Time (T) and Functional diversity (FD) on rates of instantaneous mortality. \* Two missing replicates were replaced by the average value of the three replicates remaining in the treatment, and 2 df subtracted from Residuals.

Source	df	MS	F	P
PP	1	0.082	0.11	0.741
T	2	136.747	177.22	<0.0001
FD	4	0.112	0.14	0.967
PP x T	2	0.209	0.27	0.764
PP x FD	4	0.076	0.10	0.982
T x FD	8	0.712	0.92	0.504
PP x T x FD	8	1.433	1.86	0.076
Residuals	88*	0.772		



**Fig. 2. 5.** *Sargassum muticum*. Instantaneous mortality rates (mean ±SE, n = 40) during settlement (0 to 2 months), recruitment (2 to 6 months) and colonisation period (6-12 months after invasion). Different letters indicate significant differences in at P < 0.05.

## 2.5. Discussion

We found that the main and interactive effects of propagule pressure and the functional structure of assemblages varied over the different stages of invasion, i.e. settlement, recruitment and colonisation. Moreover, PAR availability at the substratum level was an important variable explaining the invasion success of *Sargassum muticum*. In contrast to previous studies on *S. muticum* (e.g. Stæhr et al., 2000; Britton-Simmons, 2006), space availability did not play a significant role explaining recruitment success of the invader.

The role of propagule pressure in the invasion process was only relevant over the settlement stage (Clark and Johnston, 2009). Although high percentage cover of canopy, i.e. monospecific assemblages of subcanopy species, may act as increased biotic resistance under low propagule pressure, high propagule pressure seemed to overwhelm biotic resistance to invasion. The swaying of the algal fronds induced by water movement could free the substratum they covered, allowing for propagules to reach the substratum. Here, because of the use of discs for settlement assessment, it was impossible to separate and model the effect of space availability on the number of settled germlings. Over the settlement stage, the observed larger number of *Sargassum muticum* germlings in algal turfs than in areas covered by encrusting species has been also registered for *Cystoseira* spp. (Fucales, Phaeophyta) (Benedetti-Cecchi and Cinelli, 1992). Explanations such as that algal turf might provide a refuge from water displacement (Brawley and Johnson, 1991) or from herbivores (Benedetti-Cecchi and Cinelli, 1992) are not applicable within laboratory artificial invasion. During the process of settlement, propagules have first to locate the substratum and then to establish surface contact (Fletcher and Callow, 1992). Hence, a possible explanation is that the highly compacted algal turfs entrapped the germlings (Fletcher and Callow, 1992) allowing a higher probability of surface contact. Greater biomass of subcanopy species, on the other hand, could either prevent *S. muticum* germlings from reaching the substratum (Deysheer and Norton, 1982) or remove germlings due to scour (Vadas et al., 1992). Nevertheless, results suggested that the positive effect of turf in high diverse assemblages could exceed the negative effect of subcanopy fronds. Despite the possibility of *S. muticum* settling over the fronds (Deysheer and Norton, 1982), that information was not quantified in the present study. However, no recruits were observed over fronds latter on.

The invader, *Sargassum muticum*, presents a highly successful reproductive strategy by releasing large amount of germlings intermittently at intervals of  $\approx 15$  days throughout its fertile period (Fletcher, 1980; Norton, 1981). This strategy will significantly increase the chances of at least one release coinciding with favorable conditions for settlement as suggested by studies manipulating disturbance (Britton-Simmons and Abbott, 2008; Clark and Johnston, 2009). Several studies have reported space and light availability as important resources limiting *Sargassum muticum* recruitment (Andrew and Viejo, 1998; Britton-Simmons, 2006). Results from the present study, however, suggested that the number of *S. muticum* at recruitment stage was not significantly affected by either resource. Nonetheless, the fact that bare rock assemblages showed the greatest number of *S. muticum* recruits suggest that space

availability is determinant for germlings settlement. Encrusting species have been described as suitable settlement substrate in some studies (Ang, 1985; Arenas et al., 2006), while in others they have been suggested to act as an effective barrier to colonisation (Deysher and Norton, 1982). In the present study, monospecific assemblages of encrusting species had the second greatest number of *S. muticum* recruits, highlighting the aptness of encrusting species as a suitable substratum for *S. muticum*. In fact, these results corroborate our field observations that indicate the presence of *S. muticum* adults over crustose substratum. Because the structure of assemblages explained  $\approx 52\%$  of *S. muticum* recruitment, other factors related to post-settlement survivorship such as nutrient availability, herbivory or wave action might be related with the results found.

Colonisation success of *Sargassum muticum* was positively affected by percentage of PAR reaching the substratum, suggesting that the presence of high values of canopy biomass may have a negative effect on the invader survivorship. Canopy may indeed affect significantly the survival of recruits older than 6 months of age (Kendrick, 1994), possibly explaining our non-significant results for 2 months recruits regarding PAR availability.

Although highly diverse communities are expected to achieve a more complete utilization of resources (complementary resource use hypothesis), our experiment based on functional attributes of species provided little support for that. Nonetheless, colonisation of *Sargassum muticum* was more successful in less complex assemblages, i.e. monospecific assemblages of encrusting species, which presented higher levels of both resources, PAR and space availability. Additionally, rate of instantaneous mortality of *S. muticum* was significantly greater over the first two months of age indicating that early post-settlement processes are decisive to the overall invasion success. This fact suggests that post-settlement mortality not related with our predictors (e.g. density dependent mortality) eliminates the effect of propagule pressure in the final success of the invasion.

Overall, our results did not support the theory that more diverse assemblages are less susceptible to invasion by non-native species (Elton, 1958; Lodge, 1993; Naeem et al., 2000). The traits inherent of each functional group have, however, strong effects on specific resources (Symstad, 2000; Britton-Simmons, 2006). This fact and our results suggested that the additive effects of each functional role in high diverse assemblages are important in modifying resources availability and may therefore interfere with community invasibility (Arenas et al., 2006). Our findings showed that the invasion

success of *S. muticum* over the different invasion stages is influenced differently by recipient assemblage traits (Davis et al., 2000; D'Antonio et al., 2001). Throughout the invasion process resource quality may be just as important as resource quantity, as previously implied by Clark and Johnston (2009). Hence, the interaction of environmental factors that covary with species diversity and identity may better explain invasion success (Eschtruth and Battles, 2009).

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## **Chapter 3**

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Role of top-down and bottom-up forces on the  
invasibility of intertidal macroalgal assemblages



**Role of top-down and bottom-up forces on the invasibility of intertidal macroalgal assemblages**

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### 3.1. Abstract

Despite the available information regarding the negative effects of non-indigenous species (NIS) on ecosystem structure and functioning, the mechanisms controlling NIS invasion remain poorly understood. Here, we investigated the relative roles of top-down and bottom-up control on the invasion of intertidal macroalgal assemblages by the macroalga *Sargassum muticum* (Yendo) Fensholt. Using a factorial experiment, nutrient availability and intensity of herbivory were manipulated along an intertidal rocky shore. We found that early recruitment of *S. muticum* was enhanced by low nutrient enrichment but no effect of grazers was observed. In contrast, at the end of the experiment (9-months after invasion) top-down control, together with the number of NIS and the percentage cover of ephemerals, were significant predictors for the invasion success of *S. muticum*. In addition, both top-down and bottom-up forces played a significant role in structuring macroalgal assemblages, which indirectly could have influenced invasion success. Hence, by shaping community structure, main and interactive effects of bottom-up and top down forces may indirectly act on invasion. Our study highlights the importance of the recipient community structure on the invasion process and emphasizes the specific regulation of top-down and bottom-up forces in different stages of *S. muticum* invasion.

**Keywords:** Invasion process; Top-down; Bottom-up; *Sargassum muticum*; NIS

### 3.2. Introduction

Invasions by non-indigenous species (NIS), one of the main human-mediated impacts on ecosystems, can directly and indirectly induce major effects on the structure and functioning of ecosystems (Grosholz, 2002). Understanding which factors influence the susceptibility of communities to invasion has become a central concern in ecology.

Although several factors are thought to play a role in the susceptibility of an ecosystem to invasion (Stachowicz and Byrnes, 2006), the composition and diversity of native species have been considered some of the primary causes (e.g. Arenas et al., 2006; Britton-Simmons, 2006; Elton, 1958). Commonly, reduced resource availability is the most cited mechanism driving community invasibility (e.g. Stachowicz et al., 1999; Britton-Simmons, 2006). Increased diversity of species and functional groups is expected to increase the efficiency of resource use, generating a negative relationship between native diversity and invasion success (Stachowicz and Byrnes, 2006). Despite some controversy, the relationship between native diversity and invasibility is well reported and widely accepted (Davis et al., 2000; Stachowicz et al., 2007). In this context, the Fluctuating Resources Availability Theory (FRAT) suggests that the invasibility of a community may change as the amount of unused resources fluctuates (Davis et al., 2000). Accordingly, the susceptibility of a community to invasion would increase whenever the availability of a limiting resource is increased.

In coastal systems, resource supplies and consumers play a crucial role in the regulation of intertidal habitats, i.e. bottom-up versus top-down control (Menge, 2000; Worm et al., 2000). Specifically, it has been suggested that bottom-up forces, e.g. nutrient levels, are of great importance in regulating species composition while top-down regulation, e.g. herbivory, exert stronger effects over macroalgal biomass (Burkepile and Hay, 2006). Increased availability of resources in an environment can be induced either by introducing resources at a faster rate than the community can sequester them (e.g. nutrient addition) or by reducing resource uptake by the community (e.g. decrease of species biomass) (e.g., Grime, 1977; Davis et al., 2000). Furthermore, complex interactions among herbivory and nutrients levels are also frequent (Worm et al., 1999; Worm et al., 2002; Hillebrand, 2003; Masterson et al., 2008).

Although a large number of studies have addressed the influence of top-down and bottom-up forces in structuring benthic communities (e.g. Worm et al., 2002; Burkepile and Hay, 2006; Bulleri et al., 2012), few experiments have been conducted to address the relative roles of nutrient supply and herbivore processes on invasion success of

macroalgae (but see Vermeij et al., 2009). Roughly 20% of the marine invasive species in the world are macroalgae. These invaders can cause important ecological and economic damage by altering the structure and functioning of recipient ecosystems (Schaffelke et al., 2006). Understanding the mechanisms and processes that allow marine invasive macroalgae to become abundant is crucial for prevention and management of marine invasions in the future.

In the marine environment, macroalgal assemblages depend on resources such as space, nutrients and light (Carpenter, 1990). Thus, the susceptibility to invasion by a macroalgal community is expected to be influenced by the availability of those limiting resources (Arenas et al., 2006). For example, space availability and nutrient enrichment facilitated the establishment and spread of the brown invasive alga *Sargassum muticum* on an intertidal shore (Sánchez and Fernández, 2006). In addition, it has been suggested that susceptibility to invasion should be highest when disturbance is accompanied by nutrient enrichment (Burke and Grime, 1996). The presence of grazers in a habitat can, however, induce disturbance of different kinds either by increasing space availability or by reducing algal biomass. Hence, disturbance by grazers may reduce resource use by decreasing the biomass of algal assemblages (Davis et al., 2000). This phenomenon would increase the amount of resources available and create a favourable time period for new recruitment. Nonetheless, experimental studies addressing the relative roles of herbivory and nutrients in the recruitment of algae indicate complex and possible interactive effects of both factors. For instance, a study in the Great Barrier Reef that simultaneously manipulated intensity of nutrients and herbivory, found that herbivory effects on algal recruitment (density and size) were not consistent among species and were stronger within nutrient-enriched treatments (Diaz-Pulido and McCook, 2003).

The invasion process is characterised by different transitional stages of invasion (Williamson, 2006) and survivorship through each stage is the key to success of invasive NIS. Here, we used a field factorial experiment using intertidal assemblages as a model system to test the individual, combined and potential interactive effects of nutrient enrichment and intensity of herbivory on the invasibility of intertidal macroalgal assemblages by the invasive macroalga *Sargassum muticum* (Yendo) Fensholt. Previous studies on *S. muticum*, both from the intertidal and low subtidal, suggested that invasibility of assemblages was mediated by different resources at different stages of invasion (Britton-Simmons, 2006; Sánchez and Fernández, 2006). Thus, experiments conducted from early life stages throughout adult stages are of great importance in macroalgal invasion ecology (White and Shurin, 2007).

*Sargassum muticum* Yendo (Fensholt) is a brown alga native to East Asia, and it is considered a highly invasive species (Critchley et al., 1983; Norton, 1976). Attached individuals were first recorded in Europe in the British Isles in 1973 (Critchley et al., 1983). The invasive seaweed *S. muticum* arrived to the rocky shores of the northern Spanish coast in the 1980s (Fernández et al., 1990) and is now present from Portugal in the south to Norway in the North. Characteristics such as being a fast growing species, monoecious, self-fertile and pseudo-perennial, among others, have been cited as responsible for the success of this species as an invader (Norton, 1976). A fertile individual of *S. muticum* bears thousands of receptacles (reproductive structure). Fertilized eggs are retained on the external surface of the receptacles until the development of tiny germlings with adhesive rhizoids (Norton, 1981). Embryos are then liberated, they sink and generally reattach to the substratum within a few meters of the parent plant (Deysher and Norton, 1982). Adult plants stay attached to the substratum by a perennial holdfast. Seasonal patterns of growth and reproduction have been reported for this species (Deysher, 1984; Arenas and Fernández, 1998). In the study area, *S. muticum* has a spring-summer reproductive period while a senescence period is observed at the end of the summer (Arenas and Fernández, 1998; Fernández, 1999). Biomass shows a marked seasonality with a period of rapid growth starting around January and a period of decrease in biomass at the end of the reproductive period when fronds detach themselves from the thallus.

By experimental manipulation of nutrients and herbivory levels and by assessing success of the invader after an artificial invasion, we tested the hypothesis that a greater availability of resources, through an increase in herbivory or due to nutrient enrichment, would affect the invasion of macroalgal assemblages. Additionally, we expected an interactive effect between nutrient concentrations and intensity of herbivory, where the magnitude of top-down forces of herbivory should increase with the bottom-up forces of nutrient availability.

### **3.3. Material and methods**

#### **3.3.1. Study site**

The field study was conducted from February 2010 to March 2011 along an intertidal rocky shore in Cabo Estai (42°11' N, 8°48' W), Ria de Vigo (Galicia, Spain). Seawater temperature for the experimental period was about 15°C ( $\pm 0.84$ , mean  $\pm$ SE), while salinity remained stable at around 34.5‰ ([www.meteovigo.es](http://www.meteovigo.es)).

The site can be described as a gently sloping granitic rocky shore, with a wide variability of fauna and flora. Natural assemblages at low intertidal were characterised by a range of

macroalgae with differing morphologies such as the leathery alga *Bifurcaria bifurcata* Ross, articulated calcareous algae such as *Corallina* sp. Linnaeus and *Jania rubens* (Linnaeus) Lamouroux, the corticated alga *Stypocaulon scoparium* Linnaeus, and green filamentous and foliose algae (e.g. *Ulva* spp). NIS such as *Sargassum muticum* (Yendo) Fensholt, *Asparagopsis armata* Harvey, *Grateloupia turuturu* Yamada and *Undaria pinnatifida* (Harvey) Suringar were also present at the site. Invertebrate assemblages were dominated by gastropod grazers, including several topshells from the genera *Gibbula*, the limpet *Patella* spp. and the common topshell *Monodonta lineata* Da Costa.

### 3.3.2. Experimental design and Sampling procedure

Forty-five plots (35 x 35 cm) of natural assemblages were manipulated in the low intertidal (0.4-0.8 m above the lowest astronomical tide) following a two-way factorial design including the intensity of Nutrients and Herbivory as orthogonal fixed factors. All replicate plots were distributed randomly within the study site along 200 m of sea shore, separated by at least 2 m. The corners of the experimental plots were marked with epoxy putty to allow repeated sampling.

To study the effect of increased nutrient concentration, three levels were included in factor Nutrients: "Control", corresponding to no nutrient addition and mesh bags control; "Low nutrients", corresponding to an addition of 1 Kg of nutrient pellets, N+; and "High nutrients", corresponding to an addition of 2 Kg of nutrient pellets, N++. Concentrations of nutrients were manipulated using slow controlled-release fertilizer pellets (Multicote®) in small mesh bags with 1 mm mesh size. Fertilizer pellets consisted of 15% N (8% NH<sub>4</sub><sup>+</sup>, 7% NH<sub>3</sub>), 7% P (PO<sub>2</sub>), 15% K (K<sub>2</sub>O) and 2% MgO. Each experimental plot had 2 mesh bags (500 g each or 1 kg each, for low and high nutrients, respectively) on opposite sides anchored to the substrate with cable ties fasten to two steel screw eyes. Control mesh bags were filled with a plastic bag containing sand. Mesh bags were replaced every 3 months, or before if lost due to rough sea conditions, to ensure continuous delivery of nutrients. Herbivory intensity also had three levels: Natural herbivory, "H+", where herbivores had free access to experimental plots; Low herbivory, "H-", where antifouling paint was used to avoid herbivore access to experimental plots; and Procedural control, "PC", to test for possible artifacts due to the use of antifouling paint, where antifouling paint was only used on two opposite sides of the plots. There were 5 replicates per treatment combination.

Experimental manipulation of nutrients and herbivory was maintained for three months prior to invasion. Prior to the invasion procedure, individuals of *Sargassum muticum* were

removed from the experimental plots and immediate surroundings. To assure a similar propagule pressure by *S. muticum* in all experimental plots, we invaded the plots artificially. Artificial invasion was set on 13<sup>th</sup> of May, 1 day before the new moon, due to the fact that *S. muticum* has a semilunar periodicity of egg expulsion coinciding to 24-48h around new or full moons (Monteiro et al., 2009; Norton, 1981). Fertile individuals of *S. muticum* ( $\approx$  30 cm long) were collected from an intertidal rocky-shore nearby. Approximately 500 g wet weight of algae were fixed to opposite sides in each plot using cable ties and 1 steel screw eye and were left for 1 week.

Algae percentage cover and density of herbivores were recorded immediately before treatment manipulation (at the beginning of the experiment, February 2010) and every 2-3 months thereafter until March 2011. Plots were monitored using a 35 x 35 cm quadrat divided into 100 sub-quadrats of 3.5 x 3.5 cm each. Primary and secondary algal cover was estimated by summing up 1% cover of each taxon individually and adding up the total. By joining primary and secondary algae cover, total cover may greatly exceed 100% within each plot. In addition, percentage cover of sessile invertebrates and bare rock were also assessed adding up to the assemblage structure of the experimental plot. Macroalgae were identified to the most detailed level of taxonomic resolution achievable in the field. Grazers were individually counted and were left in or taken out of the plot depending on the herbivory treatment. Macroalgal species were assigned to different functional groups following Steneck and Dethier (1994). Nutrient enrichment is known to often increase the biomass of opportunistic fast-growing ephemeral species (Kraufvelin et al., 2010). Thus, in order to examine the potential indirect effect of the presence of these particular species, we chose to incorporate together species known as being highly opportunistic into a single category, Ephemerals. We incorporated together annual filamentous and foliose algae belonging to the genera *Ulva*, *Ceramium*, *Pterosiphonia* and *Polysiphonia* (Supplementary data, Table A3.1). Additionally, known NIS were identified and included as a single variable named "NIS" (excluding *S. muticum*). Overall, the macroalgal categories used were: corticated, foliose, leathery macrophytes, crustose, articulated calcareous, ephemerals and NIS.

*S. muticum* individuals were first visible in experimental plots in December 2010. At the end of the experiment (March 2011), recruits of *S. muticum* were scraped from each experimental plot, taken to the laboratory and oven-dried at 60°C for 48 hours to estimate biomass (grams of dry weight). Several variables such as number of recruits, length and biomass were used as estimates of invasion success (Arenas et al., 2006; Britton-Simmons and Abbot, 2008).

### 3.3.3. Statistical analysis

Due to the specific amount of nutrient release in each plot, we assumed dependent repeated measures sampling. In this context, effectiveness of nutrients enrichment was examined using a Repeated measures ANOVA (rmANOVA) with Nutrients as a fixed factor and Month as within-subjects variable. The rm-ANOVA assumption of sphericity was evaluated using Mauchly's criterion. Bonferroni post-hoc comparisons were performed between pairs of variables. Furthermore, effectiveness of herbivory treatment was analysed with a two-way ANOVA with Herbivory intensity (3 levels) as a fixed factor and Month (3 levels) as a random factor ( $n = 15$ ).

Firstly, we tested for differences in assemblage structure between patches assigned to each experimental plot, to assure that any differences found later on were due to experimental treatments. Structure of assemblages (measured as percentage cover of algae, bare rock and sessile invertebrates) was compared through multivariate analysis of variance based on permutations (PERMANOVA). Based on a root-transformed Bray-Curtis similarity matrix, PERMANOVA analysis included 2 fully crossed fixed factors, Nutrients (3 levels) and Herbivory intensity (3 levels) with 5 replicates per factorial combination of treatments. Additionally, PERMANOVA analyses were also performed at the end of the experimental period to compare structure of assemblages at the beginning and at the end of the experiment. For the three-way permanova analysis, we included Nutrients (3 levels), Herbivory intensity (3 levels) and Time (2 levels) as fixed factors. SIMPER analysis was used to identify taxa contributing most to multivariate differences between experimental assemblages.

To identify the main variables that influenced early recruitment and invasion success of *S. muticum* we used generalized linear models (GLMs). For early recruitment, count data expressed as number of recruits was analysed assuming a quasipoisson distribution to account for overdispersion. For final recruitment data we assumed a negative binomial distribution to account for high overdispersion using the `glm.nb` function from the MASS library (Crawley, 2009) in R. We assumed a Gaussian distribution for biomass and length data and used `glm()` function with an identity-link. Predictive variables for both set of analyses were the factors Nutrients and Herbivory intensity, and the variables number of native taxa, number of non-indigenous taxa, percentage cover of morpho-functional groups (crustose, leathery, corticated, articulated calcareous) and percentage cover of ephemeral species.

Generalized Estimating Equations (GEEs), an extension of GLMs for repeated measures data analysis (Liang and Zeger, 1986), were used to assess how the structure of assemblages within each experimental plot varied over the experimental period due to nutrient enrichment and herbivory intensity. The specific response of assemblages was analysed through changes in percentage cover of functional groups, ephemerals and species richness. We used the *geepack* package (Halekoh et al., 2006) and the *geeglm()* function in the R-program. For the analysis, a link function and an error structure for the residuals were specified as in GLM models described above. Hence, for count data we assumed a Poisson distribution and for continuous percentage cover data we assumed a Gaussian distribution. We also specified a first order autoregressive model, using the *corAR1* function, in the whole analysis assuming time dependence for each experimental plot unit.

All univariate analyses were carried out using the R-program 2.14.1 (R Development Core Team, 2011). Multivariate analyses were performed with PERMANOVA + for PRIMER v.6 (Anderson et al., 2008; Clarke and Gorley, 2006).

#### 3.3.4. Effectiveness of treatments

Effectiveness of the nutrient enrichment was assessed by measuring nutrient concentrations in experimental plots in May, August and December 2010. Water samples were collected following low tide, with water 10-15 cm above experimental plots, using acid cleaned 50-ml plastic syringes with microfiber filters (Fisherbrand® MF 300). Immediately after collection, samples were placed on ice, returned to the laboratory, and frozen at -20 °C until analysis (less than three months after). Analyses of  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  were carried out using a continuous-segmented flow *autoanalyzer* (Bran+Luebbe AA3). Analysis revealed marginally significant differences in nitrate availability over the different treatments (rm-ANOVA,  $F_{2,42} = 3.055$ ,  $P = 0.058$ ). Within treatment levels, the Control differed from the High nutrient treatments while the Low nutrient treatment showed intermediate values, not significantly different from the Control or High nutrient treatments. Phosphate concentration was not significantly affected by nutrient enrichment (rm-ANOVA,  $F_{2,42} = 1.443$ ,  $P = 0.248$ ).

To evaluate the effectiveness of the herbivory treatments, the intensity of herbivory in each experimental plot was estimated using wax-discs, as described by Thompson et al. (1997). Three wax discs (1 cm in diameter) were used per experimental plot. Small holes slightly larger than the discs were made with a small drill and filled with epoxy putty where discs were deployed for periods of 10 days every four months. Discs were examined

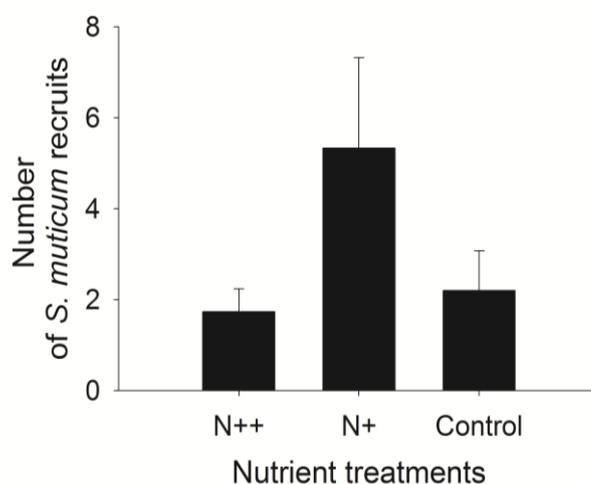
under a dissecting microscope. The percentage area of each disc scraped by radulae was estimated using an acetate grid with twenty evenly spaced holes. Percentage cover of grazing marks was estimated by assessing the number of radular marks observed under these holes. The herbivory treatment was effective because percentage cover of grazing marks varied significantly among treatments, consistently over time (ANOVA,  $F_{2,4} = 7.63$ ,  $P = 0.043$ ). Specifically, High intensity treatments ( $H^+$ ) had larger number of grazers than Low intensity treatments ( $H^-$ ). No significant differences were observed between PC and the other treatments (Student Newman Keuls test,  $P > 0.05$ ).

### 3.4. Results

Results indicated that assemblages from natural patches assigned to each experimental plot did not differ at the beginning of the experimental study (PERMANOVA,  $P > 0.05$  for both studied factors).

#### 3.4.1. Early recruitment of *Sargassum muticum*

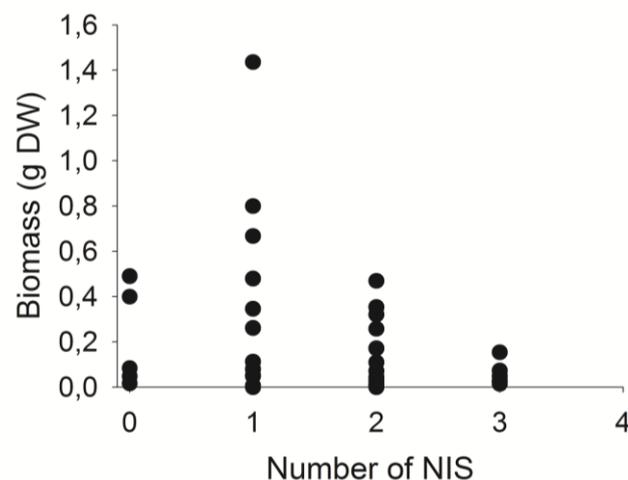
*S. muticum* recruits were not visible during sampling in early September (3 months after induced invasion) as expected. Recruits were first monitored in December 2010, the following sampling date. Number of recruits ranged from 0 to 26 individuals per plot and was significantly affected by nutrients (GLM,  $P = 0.038$ ) with larger number of recruits in low nutrient enriched plots (Fig. 3.1). In contrast, herbivory intensity had no significant effect on early recruitment of *S. muticum*. Length of early recruits was also not related to any of the predicted variables analysed.



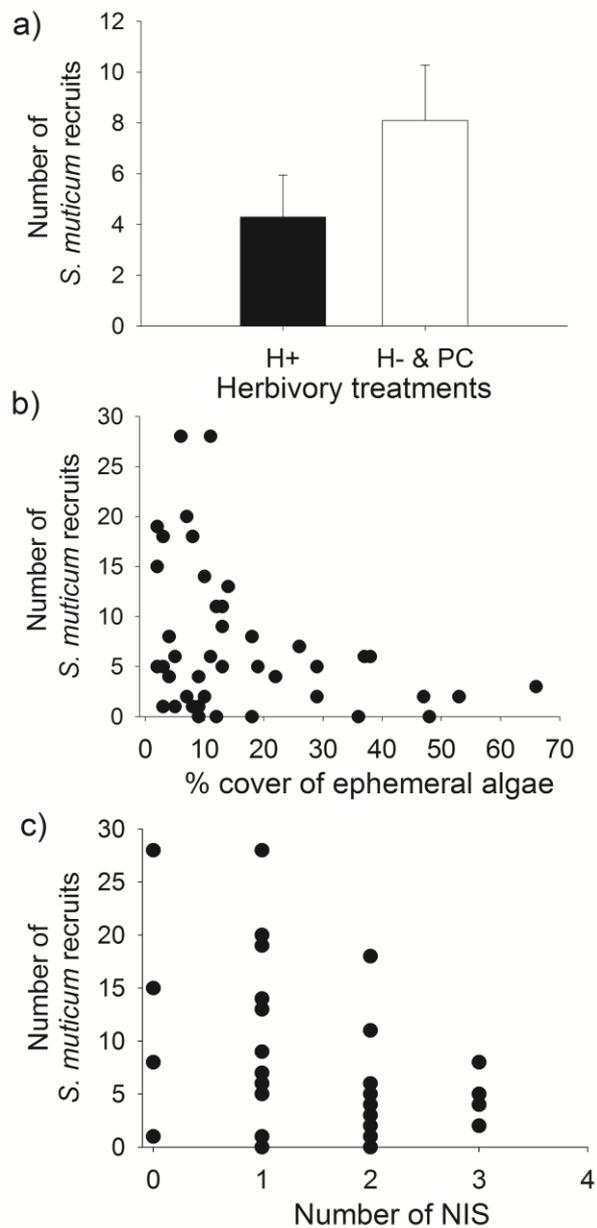
**Fig. 3. 1.** Number of *Sargassum muticum* recruits ( $n = 15$ ) 6 months after invasion (December 2010) over the different nutrient treatments. N++, high nutrient enrichment; N+, low nutrient enrichment; Control, natural nutrient levels.

### 3.4.2. Invasibility of macroalgal assemblages

Invasion success of *S. muticum*, measured as the length, biomass and number of recruits at the end of the experimental period (March 2011), varied in their response to the predictive variables studied. In general, experimental manipulation of nutrients showed no significant effect on the final invasion success of the invader (Table 3.1). Moreover, length of *S. muticum* recruits was not significantly related to any other predictive variable (Table 3.1a). In contrast, for both the biomass and number of recruits, the number of non-indigenous species (NIS) present at the recipient assemblage was a significant predictor for the observed patterns (Table 3.1b, c). Both invasion metrics decreased in relation to larger number of NIS present in experimental plots (Fig. 3.2, 3.3c). In addition, the number of recruits was also significantly affected by herbivory ( $P = 0.046$ ) and marginally affected by ephemeral algae (Table 3.1c). Consistently, higher intensity of herbivory and greater percentage cover of ephemeral algae species led to smaller number of recruits at the end of the experiment (Fig. 3.3a, b).



**Fig. 3. 2.** Relationship between final biomass of *Sargassum muticum* recruits and the number of non-indigenous species (NIS).



**Fig. 3.3.** Invasion success of *Sargassum muticum*. Effect of a) herbivory intensity, b) percentage cover of ephemeral algae and c) number of non-indigenous species (NIS) on the final number of recruits (9 months after invasion). H<sup>+</sup>, natural herbivory; H<sup>-</sup>, herbivory exclusion; PC, procedural control. Data in a) are mean values (+SE, n = 15 per herbivory treatment).

**Table 3. 1.** Results from Generalized Linear Models (GLMs) for a) the length, b) the biomass and c) the number of *S. muticum* recruits at the end of the experimental period. Model fitted to the explanatory variables Nutrients, Herbivory intensity, Number of native taxa (native), Number of non-indigenous species (NIS), Percentage cover of crustose algae (Crustose), Percentage cover of leathery algae (Leathery), Percentage cover of corticated algae (Corticated), Percentage cover of articulated calcareous algae (Artcalcareous), Percentage cover of filamentous and foliose algae (Ephemeral) and biologically meaningful interactions. The NULL (intercept only) model is  $y \sim 1$ , reflecting no dependence of  $y$  on the explanatory variables. Akaike information criterion (AIC). <sup>1,2,3</sup>, indicate the family of distribution applied to the full model.

Variables	df	Deviance	Residual df	Residual Deviance	P	AIC
<i>a) Length of recruits</i> <sup>1</sup>						
NULL			44	3325.8		
NIS	1	251.2	43	3074.6	0.067	
Minimum adequate model						323.80
Full model						360.9
<i>b) Biomass of recruits</i> <sup>2</sup>						
NULL			44	3.290		
NIS	1	0.31	43	2.978	0.039	
Minimum adequate model						11.5
Full model						35.32
<i>c) Number of recruits</i> <sup>3</sup>						
NULL			44	63.517		
Herbivory	1	3.97	43	59.544	0.046	
NIS	1	5.07	42	54.470	0.024	
Ephemeral	1	3.61	41	50.858	0.057	
Minimum adequate model						268.34
Full model:						291.97
Nutrients + Herbivory + Nutrients:Herbivory + native + NIS + Crustose + Leathery + Leathery:Nutrients + Leathery:Herbivory + Corticated + Corticated:Nutrients + Corticated:Herbivory + Artcalcareous + Ephemeral + Ephemeral:Nutrients + Ephemeral:Herbivory,						
<sup>1,2</sup> family = gaussian						
<sup>3</sup> family = negative binomial						

### 3.4.3. Top-down and bottom-up regulation on macroalgal assemblage structure

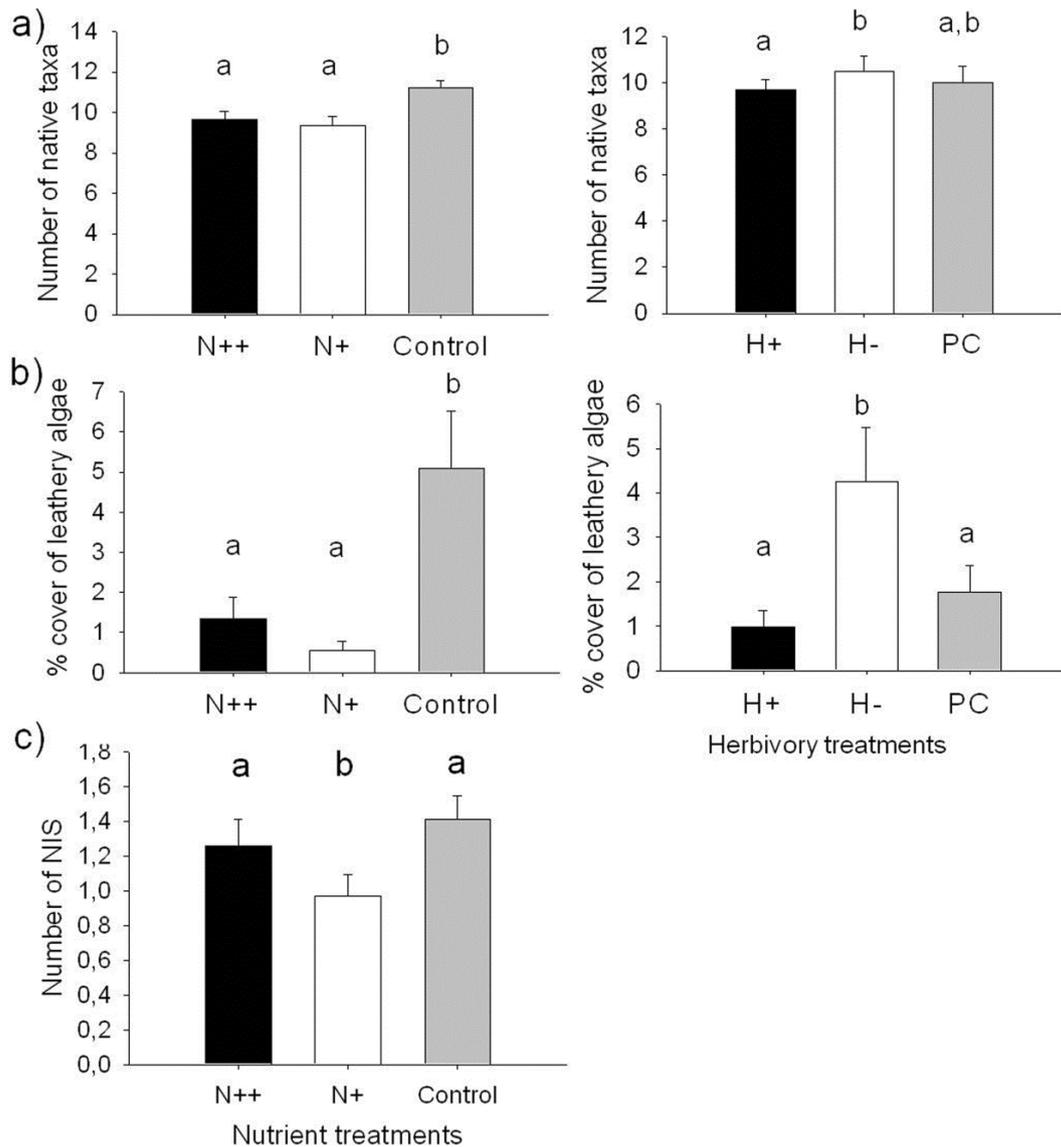
Significant differences between initial and final assemblage structure were evident over the experimental period (Permanova,  $Pseudo-F_{Time} = 15.112$ ,  $P = 0.0001$ ). SIMPER analysis revealed that the average dissimilarity value between initial (pre-treatment) and final (1-year treatment) assemblage structure was 58.97%. These changes were mainly due to a general reduction in dominant species (e.g. *Corallina elongata*, *Bifurcaria bifurcata* and *Stypocaulon scoparium*) and further replacement by species such as *Ulva* spp., *Dictyota dichotoma* and *Sabellaria* sp. (Table 3.2).

**Table 3. 2.** Summary of similarity percentage (SIMPER) analysis. Differences in average percentage cover of species contributing to dissimilarities between initial and final sampling dates (February 2010 and March 2011, respectively). A cut-off of a cumulative percentage dissimilarity of 60 was applied.

Species	Average abundance		Dissimilarity	% contribution
	Feb 2010	March 2011		
<i>Corallina officinalis</i>	6.03	4.03	5.79	9.81
<i>Bifurcaria bifurcata</i>	3.14	2.26	4.06	6.88
<i>Stypocaulon scoparium</i>	3.06	2.22	4.00	6.79
<i>Lithophyllum incrustans</i>	2.73	1.18	3.64	6.17
<i>Ralfsia verrucosa</i>	1.73	0.52	3.13	5.31
<i>Ulva</i> spp.	0.90	2.51	3.04	5.15
<i>Dictyota dichotoma</i>	1.60	2.15	3.02	5.11
Bare rock	1.56	1.13	2.92	4.95
<i>Sabellaria</i> sp.	0.00	1.62	2.90	4.91

Over the experimental period, both top-down and bottom-up regulation had varying influence on the number of native and NIS taxa and the cover of individual morpho-functional groups (Table 3.3). Herbivore exclusion significantly increased the number of native taxa, whereas nutrient enrichment resulted in their decrease (Fig. 3.4a). Similar to native taxa, the number of NIS decreased with nutrient enrichment, although no effect of herbivory was observed (Fig. 3.4c). The percentage cover of large leathery species showed similar variation to the one observed over the number of native species (Fig. 3.4b). In addition, there was a significant interactive effect of grazing and nutrients on the cover of crustose, corticated, articulated calcareous and ephemeral algae species (Table 3.3). In particular, crustose algae showed opposite patterns at natural herbivore densities, depending on the nutrient treatment (Fig. 3.5a). Percentage cover of both corticated and articulated calcareous algae decreased with high nutrient enrichment at natural herbivore

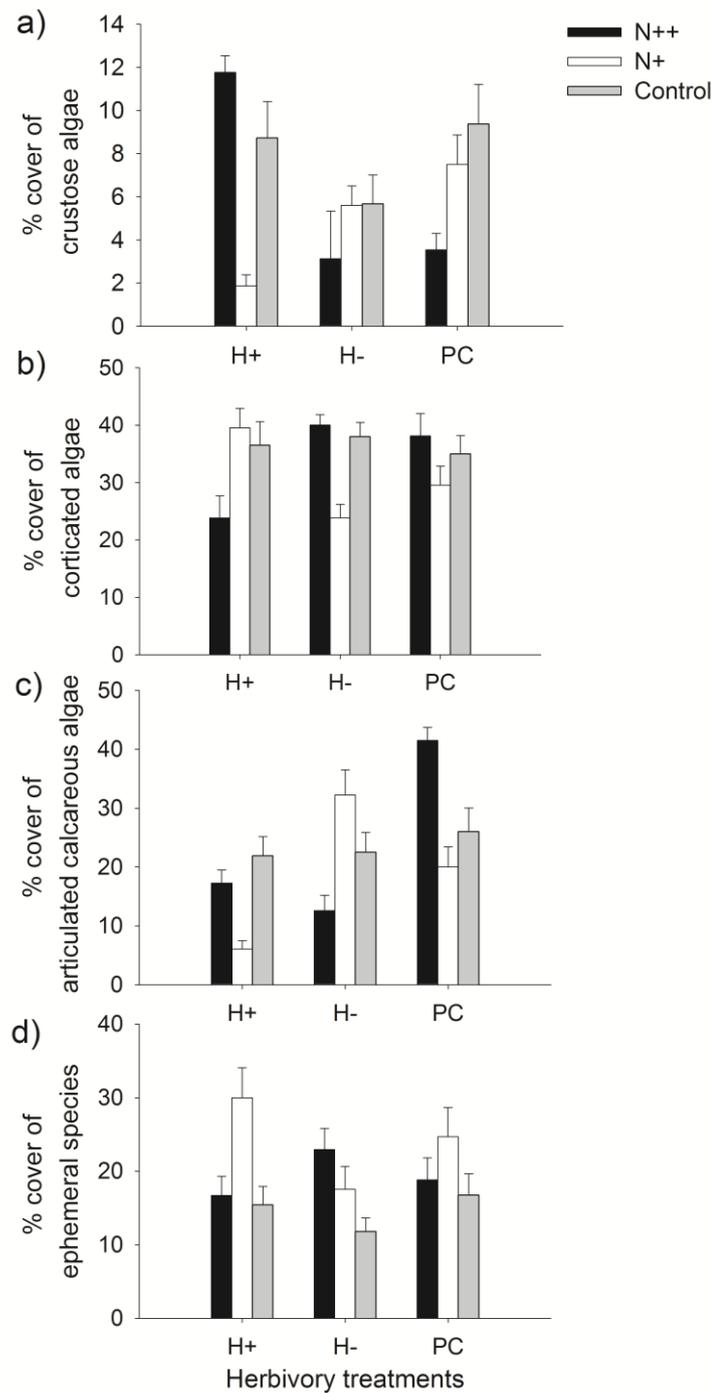
densities. Nonetheless, in herbivore exclusion treatments, both morpho-functional groups showed contrasting patterns, depending on the nutrient experimental treatments (Fig. 3.5b, c). Finally, ephemeral species showed a substantial increase in percentage cover at low nutrient enrichment and natural herbivore densities. Additionally, exclusion of herbivores resulted in a significant increase in percentage cover under nutrient enrichment conditions, i.e., low and high enrichment (Fig. 3.5d).



**Fig. 3. 4.** Structure of assemblages throughout the experimental period (mean  $\pm$ SE,  $n=90$ ). a) Number of native taxa, b) percentage cover of leathery algae across Nutrient and Herbivory intensity treatments and c) non-indigenous species (NIS) across nutrient treatments. Abbreviations as in Figures 1 and 3. Means are calculated by averaging data from each plot unit (15) over time (6). Means with a common letter do not differ from each other based on SNK tests at  $P = 0.05$  level.

**Table 3. 3.** Analysis of Wald statistic table from Generalized Estimating Equations (GEE) examining the main and interactive effects of Nutrient and Herbivory intensity on number of native species, number of non-indigenous species (NIS), Percentage cover of crustose algae, Percentage cover of leathery algae, Percentage cover of corticated algae, Percentage cover of articulated calcareous algae, Percentage cover of ephemeral filamentous and foliose algae (Ephemeral). Data from February 2010 to March 2011.

	Number of native species		Number of NIS		Crustose algae		Leathery algae		Corticated algae		Articulated calcareous		Ephemeral		
	df	$\chi^2$	P	$\chi^2$	P	$\chi^2$	P	$\chi^2$	P	$\chi^2$	P	$\chi^2$	P	$\chi^2$	P
Nutrients (N)	2	34.4	<0.0001	14.45	0.001	5.76	0.056	16.44	0.0003	3.63	0.163	2.1	0.350	14.08	0.001
Herbivory (H)	2	6.5	0.039	2.01	0.365	5.81	0.055	8.73	0.013	14.37	0.001	2.2	0.330	2.17	0.338
N x H	4	2.6	0.635	7.10	0.132	23.63	<0.0001	8.25	0.083	14.12	0.007	48.4	<0.0001	9.84	0.043



**Fig. 3. 5.** Mean (+SE, n = 30) percentage cover of crustose, corticated, articulated calcareous and ephemeral species across Nutrient and Herbivory intensity treatments over 6 sampling dates. Means calculated by averaging data from each plot unit (5) over time (6). Abbreviations as in Figures 3.1 and 3.3.

### 3.5. Discussion

By maintaining similar propagule pressure and disturbance, this study gave evidence of the importance of inherent features of the invasive species (invasiveness) and recipient communities (invasibility) to invasion success. A key finding from our study was that invasion success of *Sargassum muticum* was governed by several variables and the magnitude of each effect depended on the invasion metrics used (i.e. abundance, growth and survivorship of recruits). Additionally, top-down and bottom-up forces induced specific regulation in different stages of the invasion.

Over the initial stage of the invasion, early recruitment of *S. muticum* was positively influenced by low nutrient enrichment whereas high nutrient enrichment and grazers had limited effects. Two justifications could explain the lack of response to high nutrient enrichment during *S. muticum* recruitment. Used to low summer nutrient concentration, *S. muticum* may use nutrients more efficiently at low concentrations than at high concentrations. This fact could explain their greater survival at low nutrient environments. This first explanation is, however, not in agreement with previous manipulative experiments with *S. muticum* in which higher nutrient enrichment played an important role in controlling community invasibility (Sánchez and Fernández, 2006). Another possible explanation is based on the community structure at the time of the invasion. Over the artificial invasion period a substantial increase in the percentage cover of fast-growing ephemeral species can be observed (Supplementary data, Fig. B3.1). The observed bloom of ephemerals could have prevented the settlement and/or early survival of *S. muticum* by reducing available nutrient resources or simply by settling over the germlings preventing their survival. A study along the rocky shores of New England suggested that *Enteromorpha* could have outcompeted the long-lived *Chondrus crispus* by settling on top of its thallus which became bleached and then disappeared (Lubchenco, 1978).

A recent study in northern Portugal (Monteiro et al., 2012) described a varying effect of grazers' exclosure depending on shore-height, suggesting differences in grazing pressure at mid- and low intertidal. Low intertidal habitats showed no effect of grazer exclosure (Monteiro et al., 2012), which could explain our results over the recruitment period. Differences in the assemblage and abundance of herbivores between habitats could be responsible for the patterns found (Monteiro et al., 2012). In contrast, at the end of the experiment the presence of grazers together with specific traits of the recipient assemblage influenced the invasion success of the invader. Here, top-down control, together with number of non-indigenous species and percentage cover of ephemerals were significant predictors for the number of *S. muticum* recruits, a proxy of post-

settlement survival. Additionally, nutrient enrichment might have promoted invasion success of *S. muticum*, though indirectly. Nutrient availability may modify food preference of grazers (Russell and Connell, 2005) and so bottom-up factors may be linked to top-down forces (Menge, 1992). The fast-growing strategy of *S. muticum* linked to its possible rapid nutrient uptake might induce grazing preference over later *S. muticum* recruits. The mechanisms promoting *S. muticum* recruitment are suggested to be the same behind the expansion of native and other non-indigenous species (Davis et al., 2000). Hence, competition for resources may explain our results if we assume NIS in general to be better competitors than natives. Moreover, ephemeral species could act as a barrier to invasion. Accordingly, our results indicated that the cover of ephemerals negatively affected the number of recruits, reinforcing this idea. Furthermore, no significant growth response of *S. muticum* to herbivory was observed in our study. The fact that there was no effect of herbivory on growth when it negatively affected the abundance of recruits could suggest that predation only influences *S. muticum* directly in the germling stage and decreases with growth and maturation. Several studies have indicated that phenolic compounds can act as a chemical defense in marine brown algae (Steinberg, 1985) and suggested that those defensive compounds increase with growth and maturation (Van Alstyne et al., 2001). Another possible explanation could be that the effect of grazers involves consumption of the entire germling, thus affecting abundance but not growth of the remaining individuals (Diaz-Pulido and McCook, 2003). We should also mention that the method used to exclude grazers was effective excluding those grazers that crawl and creep on the rock but was probably not efficient with those species that swim or "jump" from individual to individual.

Strong top-down control in marine habitats may influence species production or biomass (Bracken and Stachowicz, 2007; Jenkins et al., 2008). This control was not evident in our study, where the number of NIS in the recipient community was the key predictor of the overall final *S. muticum* biomass. The presence of NIS has been suggested to create facilitative interactions among invaders, i.e. an "invasion meltdown" process. Invasion meltdown describes positive interactions among invaders with consequent increased probability of survival and/or ecological impact at the community level (Simberloff and Von Holle, 1999). In the present study, however, not only was no facilitation apparent but a negative impact on the final biomass of the invader was found, linked to the presence of NIS on the recipient assemblage. Consistently, the majority of described interactions between NIS are those in which individuals of one species are benefited and those of the other are negatively affected (Simberloff and Von Holle, 1999). Here, the presence of one NIS species seems to be an advantage to *S. muticum* biomass production, whereas the

presence of more than one species had a negative impact. These results suggested mutual interference or competition between NIS, giving evidence of a “biotic resistance” process. The mechanisms supporting such interference or competition were not, however, addressed by the present experimental design. Quantification of population-level impacts of NIS on one another are of great interest and should be addressed in future studies (Parker et al., 1999).

Species coexistence in natural communities is driven by resource partitioning (Chesson, 2000; Schoener, 1974) which is dependent on species biological traits. Over the experimental period leathery algae responded to both manipulated factors independently, whereas all other functional groups showed interactive effects. Surprisingly, there was a substantial increase in leathery cover with herbivorous exclosure. This functional group is expected to be less palatable and more defended than other functional groups such as turf algae (Littler et al., 1983) and so significant top-down effects were not expected. Negative grazing effects on leathery species could also be related to selective herbivory. A recent study suggested that by grazing on specialized tissues, herbivores may reduce N uptake and thus have a greater effect on communities than expected (Bracken and Stachowicz, 2007). Conversely, it has been suggested that by grazing filamentous/turf algae to low biomass levels, herbivores may then be forced to feed on less palatable algae (Burkepile and Hay, 2006). This phenomenon may explain the observed effect of herbivores on leathery cover. In this experiment, there was significantly more grazing (reduction in percentage cover) over corticated algae exposed to elevated nutrients than ambient nutrients concentration. In contrast to corticated algae, crustose algae increased in percentage cover when elevated nutrients and high grazing intensity were present in combination. These results are in agreement with previous experiments in which crustose coralline algae were more abundant under high herbivore pressure (Belliveau and Paul, 2002). Although calcareous macroalgae may be less nutrient limited than fleshy species (Delgado and Lapointe, 1994), the presence of herbivores resulted in higher percent cover probably due to higher consumption of fleshy algae. By limiting algal biomass, grazing promotes patches of bare rock which are then quickly occupied by crustose coralline algae (Wai and Williams, 2005).

In this study, both top-down and bottom-up forces played a significant role in structuring assemblages. Our results suggested that the susceptibility of a community to invasion might be affected by changing specific functional traits of the recipient community, due to potential indirect effects of bottom-up and top-down forces (Leibold et al., 1997). These results may, however, be context-dependent as indicated by a recent study across

regions of contrasting productivity in Australia (Bulleri et al., 2012). Additionally, a study in New Zealand's perennial short tussock grasslands suggested that the characteristics of the resident community were more critical in determining invasion success than fluctuating resource availability (Walker et al., 2005). Although it may appear contradictory, our results agree and reinforce this perspective. The dynamics and structure of the recipient community are key components of an invasion success. Hence, by shaping community structure, main and interactive effects of bottom-up and top down forces may be considered indirect factors acting on invasion.

### **3.6. Acknowledgments**

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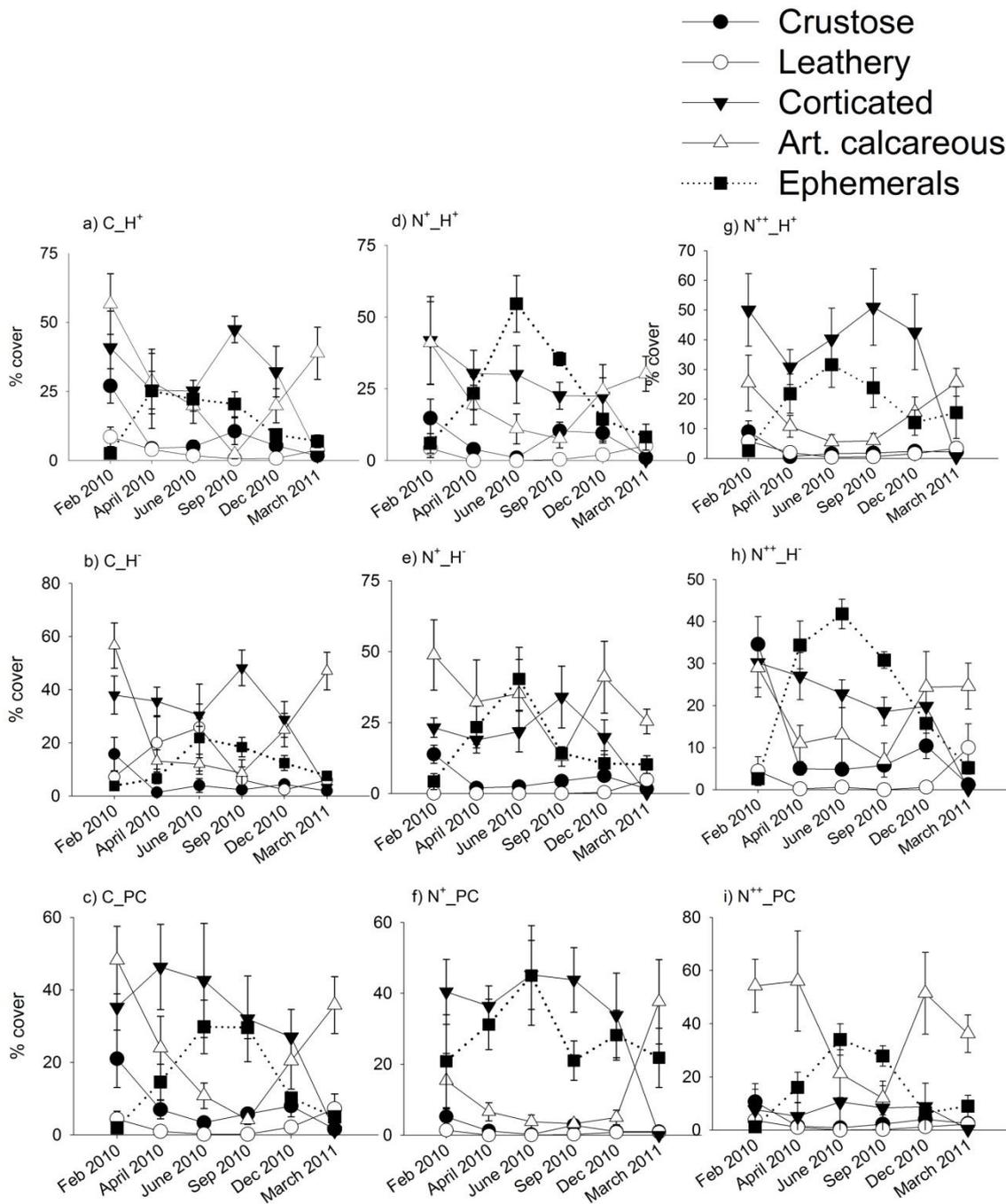
### 3.8. Supplementary data

**Table A3.1.** List of the species registered in experimental patches. Functional groups assigned following Steneck and Dethier (1994).

Phylum	Species	Functional group
Chlorophyta	<i>Codium tomentosum</i> Stackhouse 1797	Corticated
	<i>Ulva intestinalis</i> Linnaeus 1753	Foliose
	<i>Ulva</i> spp.	Foliose
Ochrophyta	<i>Bifurcaria bifurcata</i> R. Ross 1958	Corticated
	<i>Cladostephus spongiosus</i> (Hudson) C. Agardh 1817	Corticated
	<i>Colpomenia peregrina</i> Sauvageau 1927	Corticated
	<i>Cystoseira</i> sp.	Leathery macrophyte
	<i>Cystoseira tamariscifolia</i> (Hudson) Papenfuss 1950	Leathery macrophyte
	<i>Dictyopterus membranacea</i> (Stackhouse) Batters 1902	Corticated
	<i>Dictyota dichotoma</i> (Hudson) J.V. Lamouroux 1809	Corticated
	<i>Fucus</i> sp.	Leathery macrophyte
	<i>Himanthalia elongata</i> (Linnaeus) S.F. Gray 1821	Corticated
	<i>Laminaria</i> sp.	Leathery macrophyte
	<i>Ralfsia verrucosa</i> (Areschoug) Areschoug 1845	Crustose
	<i>Saccorhiza polyschides</i> (Lightfoot) Batters 1902	Leathery macrophyte
	<i>Sargassum muticum</i> (Yendo) Fensholt 1955	Leathery macrophyte
	<i>Stypocaulon scoparium</i> (Linnaeus) Kützing 1843	Corticated
	<i>Undaria pinnatifida</i> (Harvey) Suringar 1873	Leathery macrophyte
	Rhodophyta	<i>Acrosorium ciliolatum</i> (Harvey) Kylin 1924
<i>Ahnfeltiopsis devoniensis</i> (Grev.) P.C. Silva & DeCew 1992		Corticated
<i>Asparagopsis armata</i> Harvey 1855		Corticated
<i>Calliblepharis jubata</i> (Goodenough & Woodward) Kützing 1843		Corticated
<i>Ceramium</i> spp.		Filamentous
<i>Champia parvula</i> (C. Agardh) Harvey 1853		Corticated
<i>Chondracanthus acicularis</i> (Roth) Fredericq 1993		Corticated
<i>Chondracanthus teedii</i> (Mertens ex Roth) Kützing 1843		Corticated
<i>Chondria scintillans</i> G. Feldmann 1964		Corticated
<i>Chondrus crispus</i> Stackhouse 1797		Corticated
<i>Chylocladia verticillata</i> (Lightfoot) Bliding 1928		Corticated
<i>Corallina elongata</i> J. Ellis & Solander 1786		Articulated calcareous
<i>Falkenbergia rufolanosa</i> (stage) (Harvey) F. Schmitz 1897		Filamentous
<i>Gastroclonium ovatum</i> (Hudson) Papenfuss 1944		Corticated
<i>Gelidium</i> spp.		Corticated
<i>Gigartina pistillata</i> (S.G. Gmelin) Stackhouse 1809		Corticated
<i>Grateloupia turuturu</i> Yamada 1941		Corticated
<i>Gymnogongrus crenulatus</i> (Turner) J. Agardh 1851	Corticated	

Table A3.1. (cont.)

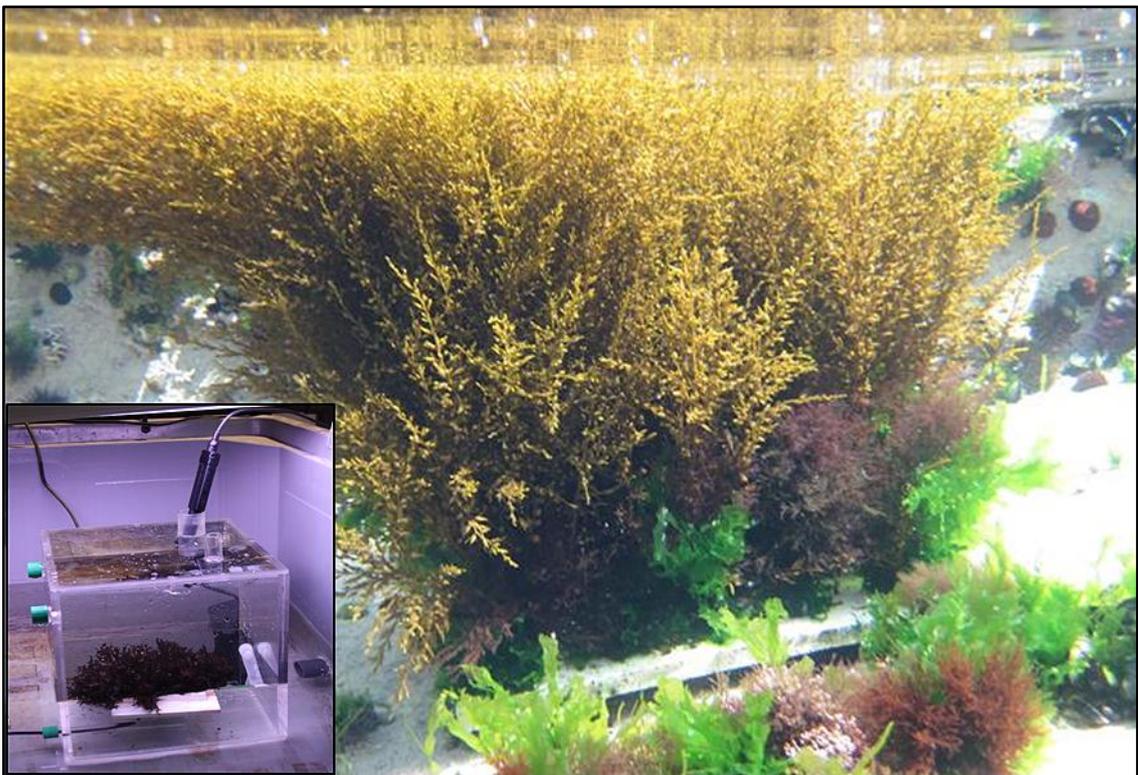
Phylum	Species	Functional group
	<i>Heterosiphonia</i> sp.	Filamentous
	<i>Hildenbrandia rubra</i> (Sommerfelt) Meneghini 1841	Crustose
	<i>Jania rubens</i> (Linnaeus) J.V. Lamouroux 1816	Articulated calcareous
	<i>Laurencia obtusa</i> (Hudson) J.V. Lamouroux 1813	Corticated
	<i>Laurencia</i> sp.	Corticated
	<i>Lithophyllum incrustans</i> R.A. Philippi 1837	Crustose
	<i>Lomentaria articulata</i> (Hudson) Lyngbye 1819	Corticated
	<i>Mastocarpus stellatus</i> (Stackhouse) Guiry 1984	Corticated
	<i>Osmundea pinnatifida</i> (Hudson) Stackhouse 1809	Corticated
	<i>Petrocelis cruenta</i> (stage) J. Agardh 1851	Crustose
	<i>Plocamium cartilagineum</i> (Linnaeus) P.S. Dixon 1967	Corticated
	<i>Polysiphonia</i> sp.	Filamentous
	<i>Pterosiphonia</i> sp.	Filamentous
	<i>Scinaia forcillata</i> Bivona-Bernardi 1822	Corticated



**Fig. B3.1.** Mean ( $\pm$  SE,  $n = 5$ ) percentage cover of the different macroalgal functional groups and ephemeral species throughout the experimental period per treatment combination. a) Control nutrients & presence of herbivores, C\_H<sup>+</sup>; b) Control nutrients & absence of herbivores, C\_H<sup>-</sup>; c) control nutrients & procedural control, C\_PC; d) Low nutrient enrichment & presence of herbivores, N<sup>+</sup>\_H<sup>+</sup>; e) Low nutrient enrichment & absence of herbivores, N<sup>+</sup>\_H<sup>-</sup>; f) Low nutrient enrichment & procedural control, N<sup>+</sup>\_PC; g) high nutrient enrichment & presence of herbivores, N<sup>++</sup>\_H<sup>+</sup>; h) high nutrient enrichment & absence of herbivores, N<sup>++</sup>\_H<sup>-</sup>; i) high nutrient enrichment & procedural control, N<sup>++</sup>\_PC.

## Part III

### Biotic interactions & Ecosystem functioning





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## Chapter 4

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Neighbourhood competition in coexisting macroalgae:  
the native *Cystoseira humilis* vs  
the invasive *Sargassum muticum*

*“It’s not the strongest that survive,  
it’s not the most intelligent that survive;  
it’s those that are best able to adapt”*

Charles Darwin



**Neighbourhood competition in coexisting macroalgae: the native *Cystoseira humilis* vs the invasive *Sargassum muticum***

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#### 4.1. Abstract

The introduction of non-indigenous species (NIS) is expected to have negative effects on native competitors, particularly between similar native and invasive species. We examined the role of species identity and density in the growth of marine macroalgal assemblages at the level of individual neighbourhood (a central, focal individual and its neighbours). A spatial neighbourhood approach was used to quantify intra- and interspecific competition between two similar macroalgal species (Fucales: Sargassaceae), using the invasive *Sargassum muticum* (Yendo) Fensholt and the native *Cystoseira humilis* Schousboe ex Kützinger known to co-occur in intertidal rocky shores from NE Atlantic. A total of 64 plates of monospecific and mixed macroalgal assemblages of *S. muticum* and *C. humilis* with two different densities of neighbours were created. Using either *S. muticum* or *C. humilis* as the focal species, we monitored the focal species' growth responses despite the presence of the neighbours in field conditions and we assessed nutrient uptake rates in laboratory. Our results showed that *C. humilis* grew at a higher rate showing N accumulation if surrounded by *S. muticum*, whereas the invader showed growth and N accumulation at low density conditions. In addition, *C. humilis* presented greater N uptake rates comparing to *S. muticum* suggesting better competitive potential to exploit high N transient pulses. Our results support the fact that because NIS in introduced habitats can be highly abundant, many NIS may have been wrongly considered superior competitors.

**Keywords:** Neighbourhood competition; NIS; macroalgae; *Sargassum muticum*; *Cystoseira humilis*; nutrient uptake rates

## 4.2. Introduction

**B**iological introductions in marine ecosystems are widely reported and have recently increased all around the world, mainly due to human activities such as international shipping, aquaculture and aquarium activity (Bax et al., 2001; Carlton and Geller, 1993). Understanding changes in biodiversity as an impact of species invasions is a major challenge in ecology, particularly in coastal waters which experience a high susceptibility to such events and are amongst the most invaded systems on the planet (Grosholz, 2002). When reaching high densities in a novel community, non-indigenous species (NIS) are expected to have negative effects on native competitors. Particularly, strong competitions arise between ecologically similar native and invasive species (Dudgeon et al., 1999). However, unlike predators and parasites, competitors have rarely been documented to cause extinctions. Thus, when competition occurs it does not necessarily imply that it will have vital consequences for ecological communities (Goldberg and Barton, 1992). Notwithstanding, a review of the mechanisms underlying the impacts of exotic plant invasions revealed that competition is, in most of the cases, suggested to be the process responsible for the registered impact in native communities (Levine et al., 2003). Competition, together with disturbance and physical stress, are key factors in determining distribution and abundance patterns of marine macroalgal assemblages (Paine, 1990). Thus, competitive interactions between NIS and native species need to be investigated in order to predict invasions outcome and the possible consequences on native communities (Brenneis et al., 2010; Staehr et al., 2000; Vermeij, 1991).

Research on biological invasions tried to look for common patterns of species features that increased the likelihood of a successful invasion (Nyberg and Wallentinus, 2005). For instance, species with a wide environmental tolerance, high growth rates and reproductive output are expected to have a greater establishment success while traits such as morphology and habitat effects are linked to ecological impacts (Nyberg and Wallentinus, 2005). Also, the susceptibility of a community to invasion has been suggested to increase with high resource availability (Davis et al., 2000). This pattern may be associated with the previously mentioned traits generally shared by successful NIS that need high supply of resources. However, a recent study in resource-limited habitats registered higher rates of carbon assimilation in invasive species, demonstrating that they can also outperform co-occurring native species in resource use efficiency in habitats of limited resources (Funk and Vitousek, 2007). In the marine environment, macroalgal assemblages depend on resources such as space, nutrients and light (Carpenter, 1990). Nutrients are the primer

resource limiting macroalgal growth in temperate marine ecosystems (Hanisak, 1979) and is an important bottom-up factor controlling the performance and structure of coastal macroalgal communities (Sánchez and Fernández, 2006; Worm and Lotze, 2006). Macroalgal species have developed different ecological strategies to cope with the seasonal nutrient limitation intimately related to their life-cycle. Perennials with slow growth present low uptake rates and develop large nutrient stored pools (Martínez et al., 2012), which allow them to be relatively tolerant to the nutrient summer limitation and dominate in pristine temperate geographic areas (Pedersen et al., 2010). Opportunistic ephemeral macroalgae, on the other hand, with high nutrient uptake rates describe an ecological strategy suited to sites or seasons of high nutrient and light availability to sustain their fast growth and reproductive maturation (Phillips and Hurd, 2003). Other annual species, known as *summer-annuals*, sustain active growth during late spring to summer, achieving their maximum size in these periods (Sears and Wilce, 1975). These species don't present significant nutrient stored pools like previously mentioned for other perennials and they are not bloom-forming species. For example, the summer-annual *Nemalion helminthoides* showed intermediate N content and uptake affinity per unit of biomass compared to the perennial slow-growing strategy and the opportunistic ephemeral strategy, together with a dynamic-inducible activation of nitrate uptake (Martínez et al., 2012). Thus, summer-annuals describe an ecological strategy that takes advantage of light availability in periods of limiting nutrients that prevent the active growth of perennials and the survival of many ephemerals (Sears and Wilce, 1975).

The brown macroalga *Sargassum muticum* (Yendo) Fensholt 1955 is one of the most well studied invasive seaweeds. Commonly distributed throughout the intertidal, *S. muticum* regularly invades the habitats of algal species from the family Cystoseiraceae (Arenas et al., 1995; Fletcher and Fletcher, 1975; Wernberg et al., 2000). Previous studies indicated variable impact of *S. muticum* on native assemblages (Britton-Simmons, 2004; Olabarria et al., 2009). Particularly on the north and northwestern Iberian Peninsula, the impact of the invasive species was evident on three distinct understory morpho-functional groups: filamentous, leathery and foliose algae (Olabarria et al., 2009; Viejo, 1997). These results suggest a higher impact of an invader species over morpho-functional groups with similar resources use. Cystoseiraceae and Sargassaceae are closely related families (Rousseau and De Reviers, 1999) and several studies have investigated differences in life-history strategies between algal species from the family Cystoseiraceae and Sargassaceae (e.g., Arenas et al., 1995; Engelen and Santos, 2009; Engelen et al., 2008). Moreover, it has been suggested that indigenous Cystoseiraceae can be displaced by *S. muticum* (Fletcher and Fletcher, 1975; Viejo, 1997; Engelen and Santos, 2009).

On the semi-exposed rocky shores of southern Portugal, *S. muticum* co-occurs with *Cystoseira humilis* Schousboe ex Kützinger 1860 in intertidal rock pools. Observational results suggested lower population growth of *S. muticum* in pools where the native algae *C. humilis* was dominant, compared to pools where *S. muticum* was dominant (Engelen and Santos, 2009). Mechanisms of these interactions, however, have not been fully studied. Because the intensity of interactions among sessile individuals varies with the spatial arrangement of competitors, the relative proximity of neighbouring individuals may be an important factor influencing the interactions among seaweeds (Carpenter, 1990; Kim, 2002a). Competitive ability of an organism include its ability to deplete resources in detrimental of the neighbours as well as its response to the presence of competitors, growth, survival and reproductive ability in the presence of neighbours (Goldberg, 1987). The morphology of competing individuals, biomass, population density and neighbour distance affect resource competition for light, nutrients and space (Carpenter, 1990; Choi and Norton, 2005; Kim, 2000b).

The present study used both field and laboratory experiments to evaluate the relative competitive abilities of *S. muticum* and *C. humilis* by comparing the species' growth responses and nutrient uptake rates. The specific objectives were to (1) compare the relative effects of intra- and interspecific competition on growth, 2) determine whether density effects influence individual growth, and (3) determine the nutrient uptake kinetics from each species. In general, higher density assemblages are expected to have reduced growth, survivorship and reproductive output compared with those at lower densities. Also, *C. humilis* is expected to show perennial slow-growing strategy and *S. muticum* may be compared to seasonal annuals, with low and high nutrient uptake rates, respectively.

### **4.3. Materials and methods**

#### **4.3.1. Field study site**

This study was carried out in the intertidal of Praia de Moledo (Northern Portugal, 41°50'22" N, 8°52'30" W) from February to August 2011. Moledo is an exposed site with a semi-diurnal tidal regime, with the largest tidal range during spring tides of 3.5-4 m. Sea surface temperatures vary between 13°C and 20°C. The site can be described as a gently sloping granitic rocky shore, with a wide variability of fauna and flora. The experiment was performed in mid-intertidal and in lower-intertidal rock pools.

### 4.3.2. *Sargassum muticum* and *Cystoseira humilis*

*Sargassum muticum* is native to East Asia and it is considered an invasive species all around the world (Critchley et al., 1983; Norton, 1976). In Europe, *S. muticum* was first introduced to the English and French coasts in the early 1970s (Critchley et al., 1983; Druehl, 1973). Its present distribution in Europe range from the Atlantic coast, from Portugal to Norway, to single introductions in the Mediterranean, l'Etang de Thau and the Lagoon of Venice (Curiel et al., 1998; Engelen et al., 2008; Wallentinus, 1999). Populations of this species are distributed mainly in sheltered or semi-exposed rocky shores, although it may also attach to hard substrates on soft-bottoms, such as stones or shells (Strong et al., 2006). The species is monoecious, self-fertile and it is considered a pseudo-perennial species consisting of a perennial basal holdfast and annual main and lateral branches (Arenas and Fernández, 1998; Norton, 1976). Additionally, the presence of gas-bladders maintains the thallus erect and closer to the light in the water column (Arenas et al., 1995). *S. muticum* has a vertical distribution from mid-intertidal pools to the shallow subtidal level.

*Cystoseira humilis* is a member of the family Sargassaceae<sup>1</sup> and can grow up to 150 cm high. It lives attached to the substratum by a compact basal disc. The native brown species are the dominant algae in most intertidal rocky pools along the Atlantic south-west coast of Portugal (Engelen and Santos, 2009). It is described to occur in pools and in shallow standing waters in the eulittoral zone, in moderately wave-exposed sites (Gómez Garreta, 2001). *C. humilis* has a perennial life history (Engelen and Santos, 2009) and is also a monoecious species.

#### *Macroalgal assemblage plates and Experimental design*

Individuals from *S. muticum* and *C. humilis* were collected in the rocky intertidal of Viana do Castelo (41°42'25" N, 8°51'42" W, Northwest coast of Portugal) and Sines (37°53'12" N, 8°47'43" W, Southwest coast of Portugal), respectively, and taken to the laboratory.

Assemblage plates with different species identity and density of both species were created in the laboratory. We constructed experimental plates made of PVC of two different sizes containing either individuals of *S. muticum*, *C. humilis* or both at two different final densities. We set 25 individuals per 500 cm<sup>2</sup> in plates of 27 x 27 cm and 25 individuals per 250 cm<sup>2</sup> in plates of 20 x 20 cm, for low and high density treatments,

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<sup>1</sup> Cystoseiraceae according to traditional classification on morphological grounds (position of receptacles) but transferred to Sargassaceae based on DNA sequence (Draisma et al., 2010).

respectively. This gives final densities of 20 and 40 individuals per 400 cm<sup>2</sup>, respectively. These values mimic those observed in field conditions for the native species (16 to 49 holdfasts per 400 cm<sup>-2</sup> with a mean ( $\pm$ SD) of 31 ( $\pm$ 12), from three scraped quadrates of 20 x 20 cm). Experiments comparing species competition or performance in monoculture and mixed assemblages use additive or substitutive designs depending on whether the density is kept constant or not in the experimental plots (Snaydon, 1991). Here, we used a neighbourhood approach to analyze seaweed species competition by relating the performance of individuals of the focal species to the identity and density of neighbouring species. Using plots with similar size but different density will change the overall biomass of each plot. Thus, to avoid confounding effect among overall plate biomass and density (Underwood, 1997) we changed plate size to achieve different densities but same number of individuals per plate (25 individuals per plate). Thus, monospecific assemblages consisted of 25 individuals of the same species and the one in the center was considered the focal individual, whereas mixed assemblages had 1 focal individual of one species in the center, surrounded by other 24 individuals of the other species. For example, the low density treatment with *S. muticum* as the focal species had 1 individual of this species and 24 of *C. humilis* per 500 cm<sup>2</sup> (Fig. 4.1). The area of individuals' distribution was inferior to the PVC area because a 2 cm border was left intact to allow for plates deployment in the field. A total of 64 assemblage plates were built (n = 8).



**Fig. 4. 1.** Example of a mixed assemblage plate with *Sargassum muticum* as the focal species. It shows 1 individual of *S. muticum* surrounded by 24 individuals of *Cystoseira humilis*.

Algal length, recorded at the time of transplanting, was  $14.96 \pm 0.70$  cm for *S. muticum* individuals and  $8.46 \pm 0.40$  cm for *C. humilis* individuals (mean  $\pm$  SE,  $n = 32$ ). Biomass of both species was estimated following Åberg (1990), from length of primary lateral and perimeter of each alga individual. We used data of dry weight and size from 40 individuals previously collected from each species to construct a regression model that allowed our estimations:

$$S. muticum: DW = 0.4092 + 0.006V (R^2 = 0.98, P < 0.001)$$

$$C. humilis: DW = 0.2636 + 0.007V (R^2 = 0.82, P < 0.001)$$

where DW is dry weight and V is the specimen volume (length x diameter<sup>2</sup>).

After the laboratory experimental manipulation, synthetic assemblages were transported to Praia de Moledo where they remained for 6 months in intertidal rock pools. Assemblage plates were randomly placed and screwed to the bottom of the rock pools. We measured short-term changes in biomass to estimate growth during two sampling events, over a 6-month period.

For biochemical analysis of C and N tissue content, we collected approximately 2-5 cm of non-reproductive tissue from the focal species at each assemblage plate at the end of experimental period. Algal material was dried at 60°C for 48 h and total C and N determined using a Carlo Erba CHNS-O Elemental Analyser (Model EA1108). The percent tissue C and percent tissue N were standardised to algal dry weight (g).

#### 4.3.3. Nutrients over a tidal cycle

To assess the variation of nutrient availability in intertidal rock pools, seawater samples were collected in August from three rock pools over a tidal cycle (5h) in Praia de Moledo. The first water sample was taken as soon as the tide was low enough and water exchange was no longer observed. Then, duplicate water samples were taken every 30 minutes until water exchange was again observed and the tide was high. Water samples were filtered *in situ* using portable microfiber filters (Fisherbrand® MF 300) and acid cleaned 50-ml plastic syringes. Immediately after collection, samples were placed on ice, returned to the laboratory in darkness, and frozen at -20 °C until analysis (less than three months after) of nitrate + nitrite, ammonium and orthophosphate.

#### 4.3.4. Nutrient uptake experiment in the laboratory

##### *Collection of samples and preincubation*

Vegetative individuals of *Sargassum muticum* and *Cystoseira humilis* were collected in the rocky intertidal of Viana do Castelo and Sines, respectively, in April 2012. Collection was made three days before assaying their uptake kinetics. In the laboratory, the material was pre-incubated in 2 L Erlenmeyer flasks filled with artificial seawater (Red sea salt) enriched with Von Stosch's (VSE) medium (Ott, 1965), but the specific nutrient (N for ammonium and nitrate or P for the phosphate uptake experiments) to be assayed. Adequate mixing was assured by bubbling filtered air into the culture medium. Flasks were left in a walk-in culture chamber at 15 °C, with constant photon flux density (approx. 150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and photoperiod (12:12; Light:Dark) until the experiment.

##### *Uptake experiments*

Nutrient uptake kinetics was assessed by measuring the decrease of nutrient concentration at different time intervals in 250 ml Erlenmeyer flasks filled with 200 ml of medium set at different initial substrate concentrations as in Martínez and Rico (2004) and Martínez et al. (2012). Algal fronds of each species, 1 g fresh weight (FW), were incubated in 250 ml Erlenmeyer flasks: 12 flasks with increasing nutrient concentration plus 3 blanks (no seaweed) as controls. In total 30 flasks were used per nutrient experiment (15 per species). Flasks were randomly arranged into a multi-stirrer magnetic plate (IKA-WERKE). Irradiance was provided with fluorescent lamps (cool white F18W/840) to a final value of 425  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and the temperature was controlled inside the walk-in chamber. The nutrient depletion of the medium was determined by nutrient analysis of water samples. Water samples (10 ml) were taken before the addition of the algae and then at 15, 30, 60, 120, 180, 240 and 300 min. After the experiment, algal material was oven-dried at 60 °C to a constant weight (48 h), for determination of dry weight (DW).

Analyses of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{HPO}_4^{2-}$  were carried out using a Skalar Sanplus<sup>++</sup> segmented flow autoanalyser, at CIIMAR, Porto. Blank control flasks registered minor concentration changes and thus no corrections were made to the algal uptake rates. Mean ( $\pm$  SE) differences between initial and final values (0-300 min) in controls were 3.30 ( $\pm$  1.57)  $\mu\text{M}$  for ammonium, 1.17 ( $\pm$  0.67)  $\mu\text{M}$  for nitrate and 0.34 ( $\pm$  0.33)  $\mu\text{M}$  for phosphate experiment.

### Uptake rate calculation

Net uptake rates ( $\mu\text{mol g DW}^{-1} \text{h}^{-1}$ ) for each nutrient source were calculated following the equation:  $V = [(S_i \times \text{Vol}_i) - (S_f \times \text{Vol}_f)] / (\text{DW} \times t)$ , where  $S_i$  and  $S_f$ ,  $\text{vol}_i$  and  $\text{vol}_f$  are the substrate concentration ( $\mu\text{M}$ ) and the water volume (l) at the beginning and at the end of the sample interval,  $t$  is the time of the sampling interval (h), and DW is algal dry weight (g).

No ammonium uptake measurements were displayed after 60 min and 30 min, for *S. muticum* and *C. humilis*, respectively, since nutrients in the flasks were exhausted by algal uptake. The exhaustion of nutrients was also registered at 180-240 min intervals for low nutrient flasks and thus the first 6 uptake measurements are also not displayed for both species. Although all glass material used was washed with 10% HCl to prevent nutrient contamination, anomalous negative uptake rates and outliers suggesting handling contamination, were recorded. These correspond to the 0-15 min intervals for phosphate uptake and 61 wrong uptake measurements out of 504 in the remainder time intervals. These outliers were not used in the calculation of uptake rates but are shown in the figures if within the graph ranges.

#### 4.3.5. Statistical analysis

##### *Field experiment*

Algal growth rate (per month) was only estimated from February to June 2011 due to the senescence state of *S. muticum* in August. A two-way analysis of variance (ANOVA) was used to test main and interactive effects of density of macroalgal assemblages (high and low) and neighbour identity (monospecific or mixed assemblages) on the growth rate of each species individually (*S. muticum* and *C. humilis*).

The same two-way crossed design was used to test the variation of algal tissue C and N content using the density of macroalgal assemblages and neighbour identity as fixed factors.

Some experimental plates were lost in the field due to rough weather conditions and thus the available number of focal species per treatment combination varied between 5 and 8. For statistical analysis we considered  $n = 5$  for macroalgal assemblages with *S. muticum* as focal species and  $n = 6$  for macroalgal assemblages with *C. humilis* as focal species. When more replicates were available per treatment, we randomly chose 5 or 6 assemblage plates, for *S. muticum* and *C. humilis*, respectively.

### *Nutrients over a tidal cycle*

Ordinary linear regression analyses were performed to analyse the relationship between nutrient concentrations in the tidepool over the tidal cycle.

Linear models were carried out using the linear model function (lm) in the R-program 2.15.0 (R Development Core Team, 2012) and analysis of variance were performed in WinGMAV (<http://sydney.edu.au/science/bio/eicc>). Prior to all analyses, the homogeneity of variances was examined using Cochran's C-test. Data were transformed when necessary and in those cases in which transformation did not remove heterogeneity, the level of significance applied was  $P = 0.01$ . Data shown represent mean  $\pm$ SE throughout.

## **4.4. Results**

### **4.4.1. Algal growth rate and tissue C and N**

*Sargassum muticum* growth rate was affected by the density of macroalgal assemblages (ANOVA, Table 4.1a) and C:N nutrient content was affected by both the density of macroalgal assemblage and the neighbour identity (ANOVA, Table 4.2a). Assemblages with high density showed a decrease in biomass and N content while low density ones registered a positive growth rate with time (Fig. 4.2a) and increased N content (Fig. 4.3b). Algal tissue C:N was significantly higher in macroalgal assemblages with high density (Fig. 4.3c). Moreover, monospecific assemblages of *S. muticum* showed a higher algal tissue C:N compared to mix assemblages (Fig. 4.3c). The initial lengths of *S. muticum* focal individuals at low and high density were similar (high density:  $14.54 \pm 1.16$ , low density:  $15.39 \pm 0.80$  cm). Neighbour identity was not a significant factor affecting growth rate of the focal individual (ANOVA,  $P > 0.05$ , Table 4.1a).

The growth rate of *Cystoseira humilis* focal individuals was significantly affected by the neighbour identity, but not by the density of the macroalgal assemblages (Table 4.1b, Fig. 4.2b). Monospecific stands of *C. humilis* showed a biomass decrease of  $0.07 (\pm 0.06)$  g DW month<sup>-1</sup> whereas the presence of *S. muticum* neighbours lead to an increase in biomass of  $0.39 (\pm 0.10)$  g DW month<sup>-1</sup>. The initial lengths of *C. humilis* focal individuals were  $8.50 \pm 0.61$ cm and  $8.43 \pm 0.54$  cm at low and high density, respectively. *C. humilis* algal tissue C:N was not affected by experimental manipulation (Fig. 4.3f; Table 4.2b).

**Table 4. 1.** Two-way analysis of variance (ANOVA) for growth rate (g DW month<sup>-1</sup>) of the focal species, a) *S. muticum* (n = 5) and b) *C. humilis* (n = 6). Density of macroalgal assemblages and neighbour identity were fixed factors.

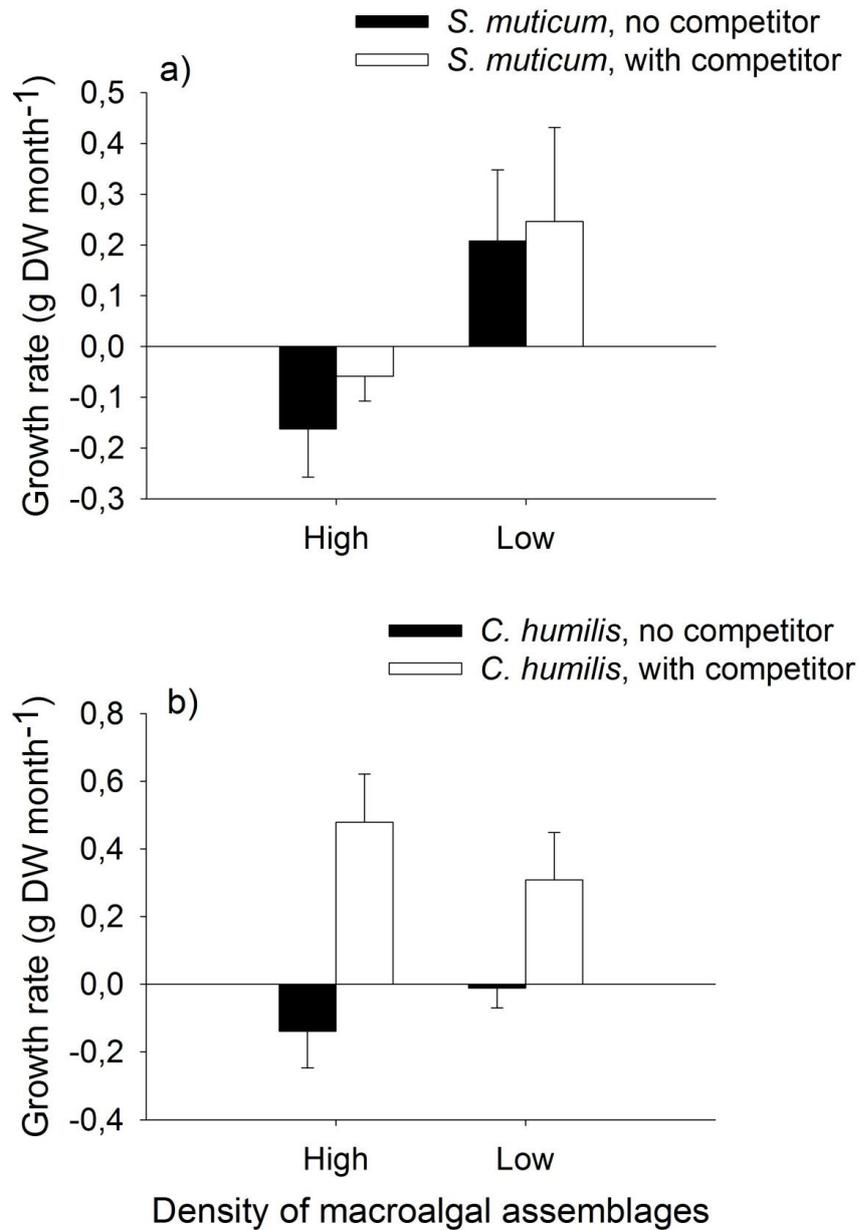
Variables	df	MS	F	P
<i>a) Sargassum muticum</i>				
Density	1	11.57	5.86	0.028
Neighbour	1	0.51	0.26	0.617
Density x Neighbour	1	0.11	0.05	0.818
Residual	16	1.98		
<i>b) Cystoseira humilis</i>				
Density	1	0.06	0.03	0.855
Neighbour	1	26.66	15.98	0.001
Density x Neighbour	1	2.72	1.63	0.217
Residual	20	1.668		

**Table 4. 2.** Two-way analysis of variance (ANOVA) for C:N ratio of the focal species, a) *S. muticum* (n = 5) and b) *C. humilis* (n = 6). Density of macroalgal assemblages and neighbour identity were fixed factors.

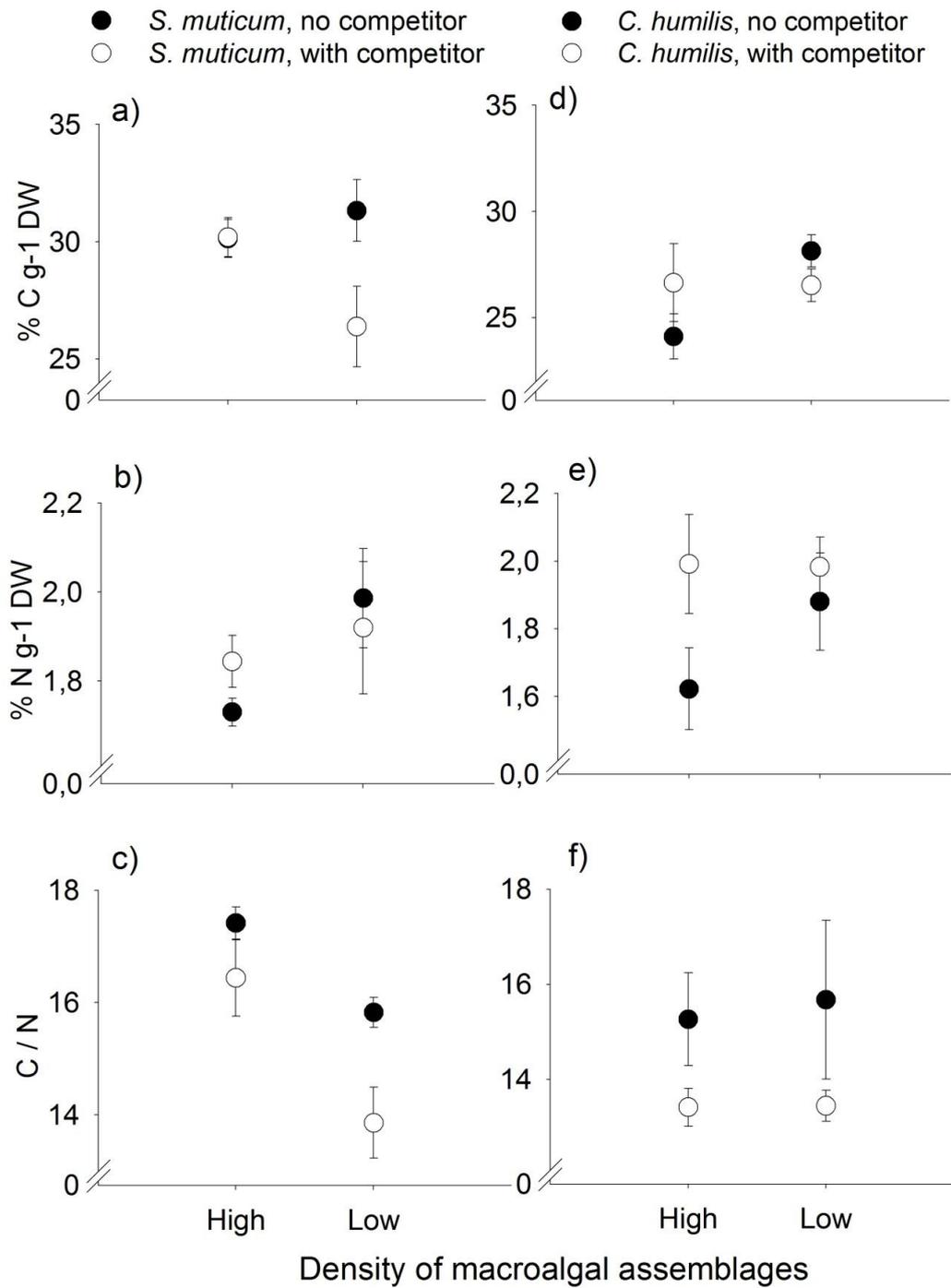
Variables	df	MS	F	P
<i>a) Sargassum muticum</i>				
Density	1	21.69	17.03	0.001
Neighbour	1	10.84	8.51	0.010
Density x Neighbour	1	1.21	0.95	0.343
Residual	16	1.27		
<i>b) Cystoseira humilis</i>				
Density	1	3.35	1.18	0.290
Neighbour	1	8.92	3.15	0.091
Density x Neighbour	1	1.23	0.43	0.518
Residual	20	2.84		

#### 4.4.2. Nutrients over a tidal cycle

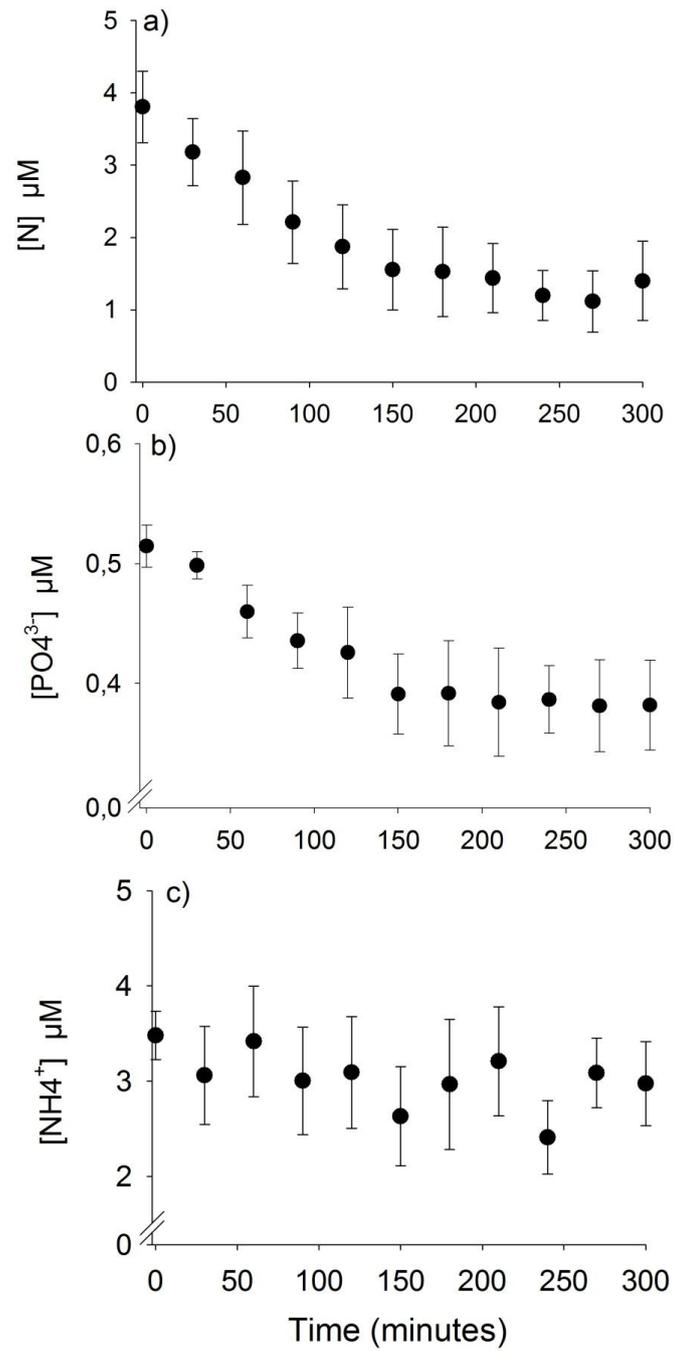
Over a tidal cycle of almost 5 hours, nutrient concentrations (nitrate and phosphate) changed drastically in the first 2h 30min (Fig. 4.4). Overall, there was a significant negative relation between nutrient concentration and time ( $R^2 = 0.84$ ,  $P < 0.0001$ ,  $n = 11$  for both nutrient types). Nitrate concentration varied from 3.8  $\mu\text{M}$  to 1.4  $\mu\text{M}$  (Fig. 4.4a) and phosphate concentration ranged from 0.51  $\mu\text{M}$  to 0.38  $\mu\text{M}$  (Fig. 4.4b). In contrast, ammonium concentration remained quite constant ( $R^2 = 0.26$ ,  $P = 0.112$ ,  $n = 11$ ) at 3.03  $\mu\text{M}$  ( $\pm 0.10$ ) (Fig. 4.4c).



**Fig. 4. 2.** Mean (+SE) variation in the growth rate (gDW.month<sup>-1</sup>) of the focal species between February and June 2011. Monospecific assemblages (with no competitor) and mixed assemblages (with competitor) for both studied species a) *Sargassum muticum* (n = 5) and b) *Cystoseira humilis* (n = 6).



**Fig. 4. 3.** August 2011. Mean changes ( $\pm$ SE) in the content of carbon (a, d), nitrogen (b, e) and C/N ratio (c, f) at high and low density of macroalgal assemblages, for *Sargassum muticum* (n = 5) and *Cystoseira humilis* (n = 6).



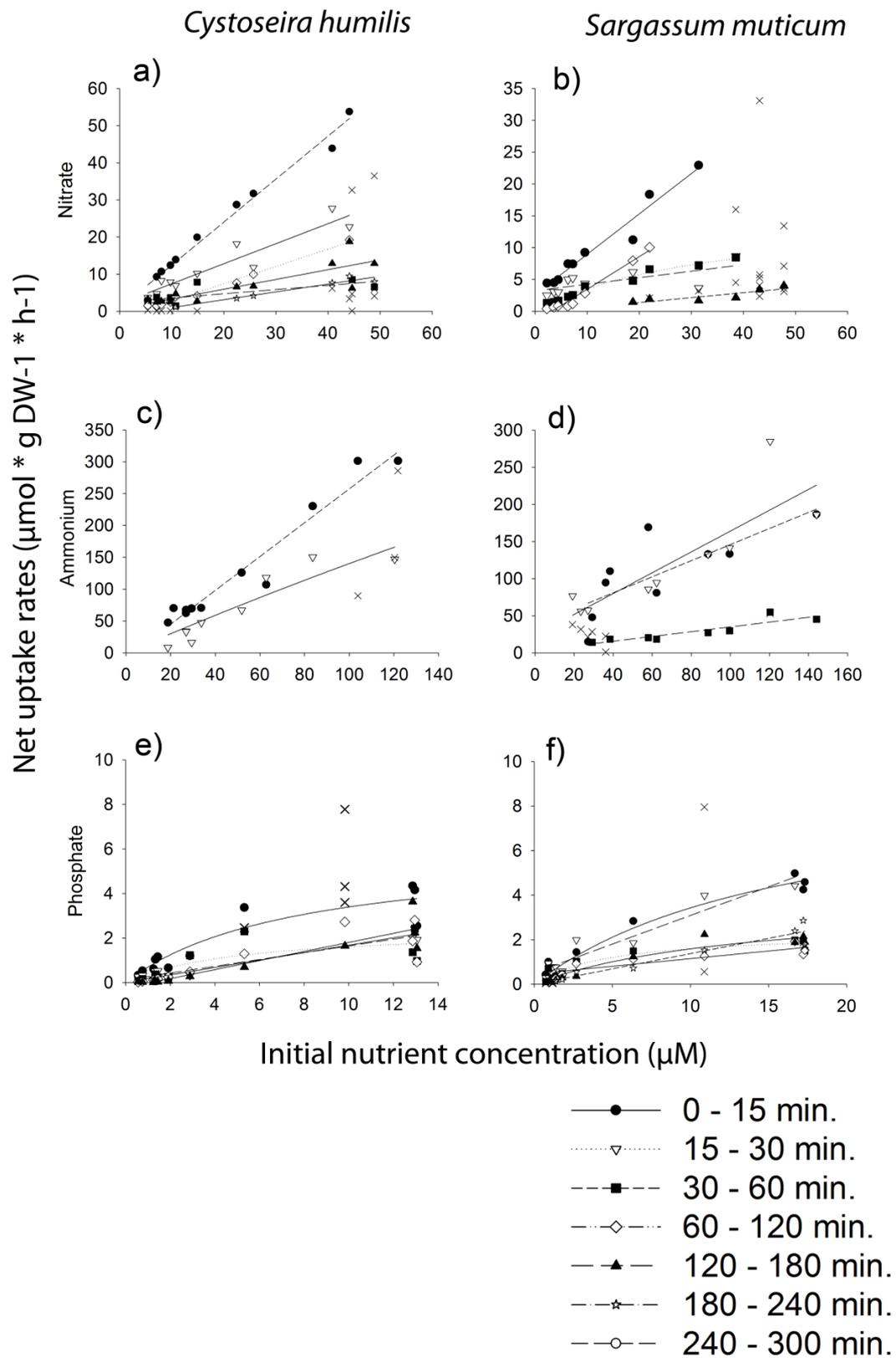
**Fig. 4. 4.** Mean ( $\pm$  SE,  $n = 3$ ) variation in the nutrient concentration in tidepools through a tidal cycle. a) nitrate, b) phosphate and c) ammonium concentration.

#### 4.4.3. Nutrient uptake

The native *C. humilis* showed the greatest uptake rates concerning the N sources (Fig. 4.5). Nitrate uptake increased linearly with substrate concentration for both species, except for *S. muticum* during the period 60-120 min when a saturation response was observed (Fig. 4.5a, b; Table 4.3). Nitrate was exhausted earlier (240 min) by *C. humilis* and at higher rates (compare axes in figures 4.5a, b), suggesting higher nitrate uptake potential of *C. humilis* than *S. muticum*.

The ammonium uptake response was linear for both species (Fig. 4.5c, d; Table 4.3). Ammonium was totally consumed after 30 min by *C. humilis* and 60 minutes later by *S. muticum*. The maximal uptake rates were recorded for *C. humilis* showing higher ammonium uptake potential.

Phosphate uptake rates were similar for both species (Figs. 4.5e, f). Phosphate uptake response for *C. humilis* followed a saturated uptake response at the time intervals 15-30 and 60-120min, and a linear uptake response at 30-60, 120-180 and 180-240 min (Table 4.3). At 240-300 min, no phosphate concentration remained in *C. humilis* experiment. *S. muticum* did not show a complete phosphate uptake over the experimental period (300 min), contrasting with *C. humilis*. Up to the time interval 120-180 min *S. muticum* showed similar, although lower, uptake response pattern as *C. humilis*. The uptake response at 180-240 min described a Michaelis–Menten saturation pattern and the last time interval showed a linear response (Fig 4.5e, f; Table 4.3).



**Fig. 4. 5.** Uptake rates of phosphate (a, b), nitrate (c, d) and ammonium (e, f) for *C. humilis* and *S. muticum*. Kinetics are shown as a function of the substrate concentration at the beginning of 7 different time intervals (as different symbols). X, values not included in the fit.

**Table 4. 3.** Estimations (standard error) of the uptake kinetic parameters for *S. muticum* and *C. humilis* using either linear or Michaelis–Menten (MM) functions.

$V_{\max}$  is expressed in  $\mu\text{mol g DW}^{-1} \text{h}^{-1}$  and  $K_s$  in  $\mu\text{M}$ .

Interval (min)	<i>Sargassum muticum</i>				<i>Cystoseira humilis</i>			
	Slope (linear)	$V_{\max}$ (MM)	$K_s$ (MM)	n	Slope (linear)	$V_{\max}$ (MM)	$K_s$ (MM)	n
<b>Ammonium</b>								
0-15	1.08 (0.34)			9	2.66 (0.21)			12
15-30	1.40 (0.33)			9	1.54 (0.23)			9
30-60	0.32 (0.06)			8	-	-	-	-
60-120	-	-	-	-	-	-	-	-
<b>Nitrate</b>								
0-15	0.32 (0.08)			10	1.16 (0.06)			10
15-30	0.63 (0.05)			9	0.55 (0.07)			10
30-60	0.10 (0.04)			9	0.11 (0.04)			8
60-120		15.74 (2.18)	34.82 (8.08)	10	0.46 (0.02)			9
120-180	0.51 (0.03)			8	0.21 (0.03)			12
180-240	-	-	-	-	1.08 (0.16)			6
240-300	0.08 (0.02)			6	-	-	-	-
<b>Phosphate</b>								
0-15	-	-	-	-	-	-	-	-
15-30		9.16 (2.24)	16.72 (7.50)	10		5.98 (1.57)	7.63 (4.23)	11
30-60	0.26 (0.03)			9	0.15 (0.01)			10
60-120		2.36 (0.31)	4.83 (1.73)	10		2.44 (0.96)	5.19 (4.84)	11
120-180	0.07 (0.01)			8	0.17 (0.03)			12
180-240		3.75 (1.31)	13.61 (9.38)	8	0.20 (0.03)			12
240-300	0.14 (0.02)			8	-	-	-	-

## 4.5. Discussion

Small-scale spatial distributions of individuals belonging to different species have important consequences to the dynamics of the whole community. In particular, sessile organisms compete for space and major resources as nutrients, mainly at the neighbourhood scale (Bonan, 1988; Connell, 1983; Naeem et al., 1999). Moreover, macroalgal assemblages in tidepools are very patchy systems at very small scales, where extremely small spatial scales of variation seem to be among the most important sources of heterogeneity (Archambault and Bourget, 1996). Thus, the growth of a focal organism should be related to the size and identity of the neighbouring organisms. We identified in this study an effect of the identity of the neighbours in the native *Cystoseira humilis* growth and nutritional state whereas the invasive *Sargassum muticum* focal species showed a density-dependent effect unrelated to the identity of the neighbour species. The former grew at a higher rate showing N accumulation if surrounded by *S. muticum*, whereas the later showed growth and N accumulation at low density conditions. In addition, *C. humilis* presented greater N uptake rates comparing to *S. muticum* suggesting better competitive potential to exploit high N transient pulses.

In marine coastal systems, aggregations of predominantly single species of macroalgae, both at the intertidal and at the subtidal, have been reported worldwide (Moore and Seed, 1985). Nevertheless, marine macroalgae are susceptible to competition for space, nutrients and light because those are often in short supply in coastal ecosystems (reviewed in Carpenter, 1990) and vary at small scales. Our results showed a negative density-dependent effect on growth for *S. muticum*, independently of neighbour identity. These results were supported by biochemical analyses that indicated potential growth limitation by nitrogen availability (Corzo and Niell, 1991), i.e. a C/N ratio above 15 (see Hanisak, 1983), in monospecific assemblages and high density assemblages of *S. muticum*. Negative effects of density have already been previously registered for *S. muticum* and other intertidal macroalgae (e.g. Arenas et al., 2002; Viejo and Åberg, 2001). Our study suggests lower accumulation of N at higher densities, limiting growth. Other studies have also registered a positive correlation between individual length and density for fucoids and *Laminaria* spp. (Schiel, 1985). Also, because the present experiment was focused on post-recruitment competition, we exclusive used thalli of similar size ( $\approx 15$  cm). However, natural stands of *S. muticum* include different adult and juvenile stages, i.e. mixture of sizes and biomass (Baer and Stengel, 2010; Strong and Dring, 2011). Thus, it has been suggested that high abnormal densities of *S. muticum* might lead to high rates of frond erosion (Strong and Dring, 2011), possibly decreasing biomass as observed in the

present study. Furthermore, our results showed that density-dependent competition on *S. muticum* focal species can occur between individuals of the same species or among individuals of different species. Thus, our results suggested that density of neighbourhood assemblages was the key mechanism affecting biomass production of *S. muticum* focal species. Contrasting, the present study also revealed that the density of macroalgal assemblages did not represent a negative effect for *C. humilis* biomass production. Our results are in agreement with a previous study which tested for competition in mixed canopies of *S. muticum* and *Saccharina latissima* at subtidal communities in Strangford Lough (Strong and Dring, 2011). A strong intra-specific competition has been reported for *S. muticum* in high densities whereas no density-dependence was observed for the perennial *Saccharina latissima* (Strong and Dring, 2011). As mentioned previously, dense monospecific stands of *C. humilis* are common along the south-west coast of Portugal (Engelen and Santos, 2009), suggesting that density is not a problem for the species.

Moreover, *C. humilis* focal species showed a greater increased in biomass when in mixed assemblages with *S. muticum* than in monospecific assemblages. These results may either suggest that *S. muticum* neighbours display a positive interaction with *C. humilis* when in mixed assemblages or that *C. humilis* presents greater intraspecific competition. Competition between closely related species, e.g. Family: Sargassaceae, is a deterministic factor in natural selection (Darwin, 1875). Positive interactions of neighbouring species on focal species may occur when the presence of one species ameliorates harsh environmental conditions, e.g. by reducing thermal, nutrient, predator or light stress (e.g. Bertness et al., 1999; Brawley and Johnson, 1991). Generally, the presence of algal canopy in rocky intertidal assemblages is known to reduce thermal stress at high tidal heights, whereas at the low intertidal border canopy effects are negative or neutral (Bertness et al., 1999; Bruno et al., 2003). Canopy effects in tidepools are expected to be similar to low intertidal border, as thermal stress is not so evident. Thus, thermal stress reduction may not be related to the positive interaction observed in the present study. Predation and competition are, however, both strong structuring forces in macroalgal assemblage dynamics (Edwards and Connell, 2012; Keane and Crawley, 2002). Nutrient limitation, in particular, may be one of the most important limitation factors for intertidal communities and tide pools due to tides, i.e. lack of nutrient renovation. In the study area, nitrate and phosphate in tide pools showed a linear decrease in concentration over a tidal cycle. Macroalgal productivity is sustained by the acquisition and utilisation of nutrients, particularly nitrogen and phosphorus. Thus, subsidy rates are a key determinant of the intensity of species interactions (Menge et al., 2003), together with net uptake rates capacity. In terms of nutrients, fast-growing opportunistic species show a high nutrient

uptake potential, while slow growing perennials have lower uptake rates and large nutrient store capacity (Lobban and Harrison, 1997; Martínez et al., 2012). Most macroalgae register higher uptake rates for the N form  $\text{NH}_4^+$  than for  $\text{NO}_3^-$ , in accordance with our results (Phillips and Hurd, 2003, 2004; Rees, 2003). Surprisingly, we found remarkably higher nutrient uptake rates for the native perennial *C. humilis*, with values comparable to nutrient uptake potential previously registered for fast-growing opportunistic species (Martínez et al., 2012). A possible explanation may be related to the nutrient availability from the alga recipient region. *C. humilis* individuals were collected from Sines (SW Portugal) while *S. muticum* comes from Viana do Castelo (NW Portugal), with average ( $\pm$ SE)  $\text{NO}_3^-$  concentration in 2011 of  $1.52 \pm 0.46 \mu\text{M}$  and  $4.31 \pm 0.56 \mu\text{M}$ , for Sines and Viana do Castelo, respectively (unpublished data). Generally, nutrient-limited algae show higher uptake rates (Lobban and Harrison, 1997), and thus our results might be explained by the fact that *C. humilis* may be nutrient limited in SW Portugal. Observational field results may reinforce this theory as *C. humilis* showed a maximum length of 44.8 cm in NW Portugal, while the maximum value recorded from SW Portugal was 18.8 cm (pers. obs). *S. muticum*, on the other hand, showed N-uptake rates comparable to summer-annual species (Martínez et al., 2012). The fact that *S. muticum* did not demonstrate opportunistic characteristics in uptake rates is in agreement with a recent study which stated that at a population level, growth of *S. muticum* when invading intertidal rock pool habitats follow K-selected traits (Engelen and Santos, 2009). Also, because light can be absorbed and/or scattered by the macroalgae themselves, both direct and indirect competition with each other may occur (Edwards and Connell, 2012). Indirect effect mediated by grazing can also be an explanation for the observed positive interaction for *C. humilis* in mixed assemblages. Reduced palatability of *C. humilis* has been demonstrated with water-borne cues from grazed *S. muticum* suggesting induced defences (Yun et al., 2012). The same, however, has not been observed for *S. muticum* (Yun et al., 2012). Because induced defences may save metabolic costs, the greater performance of *C. humilis* focal species with *S. muticum* neighbours may be explained by the higher allocation of resources to growth instead of allocated to defence (Agrawal, 2005). Results on nutrient uptake rates obtained in the laboratory may explain the positive effects of *S. muticum* in the growth of *C. humilis* in the field. Reduced uptake rates of *S. muticum* may positively influence the nutrients available for *C. humilis* by decreasing the conditions of nutrient limitation for *C. humilis* individuals. Our results are, however, based on nutrient concentrations far higher than those found in the natural environment. These results can then be used to predict a species response to episodic high nutrient pulses such as those following an upwelling or discharge event (Phillips and Hurd, 2004).

Upwelling off the northern Portuguese coast typically occurs from late spring to late autumn (Lemos and Pires, 2004), thus coinciding with the study period.

Many invasive species are, in fact, weak competitors and its establishment and spread is facilitated by disturbance (Bando, 2006). Nonetheless, the outcome of competitive interactions varies considerably with the age or size at which species interact (Olson and Lubchenco, 1990). Our experiment was a short-term experiment and described the post-recruitment competitive capacity of the native *C. humilis* and the invasive *S. muticum*. In conclusion, our study suggests that the growth of the native *C. humilis* is enhanced by the presence of *S. muticum* as a neighbour, although it remains unclear if this pattern is due to a greater intra- vs interspecific competition in *C. humilis* or due to facilitation by the invader. Moreover, *S. muticum* revealed to be a weaker competitor compared to the native *C. humilis*, supporting the fact that because NIS in introduced habitats can be highly abundant, many NIS have been wrongly considered a superior competitor (Levine et al., 2003).

#### **4.6. Acknowledgements**

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## Chapter 5

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### Ecosystem functioning impacts of the invasive seaweed *Sargassum muticum* (Phaeophyta)

*“The role of large marine algae  
in coastal productivity is far more important  
than has been suspected”*

K. H. Mann



**Ecosystem functioning impacts of the invasive seaweed *Sargassum muticum*  
(Phaeophyta)**

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### 5.1. Abstract

Ongoing changes in natural diversity via species loss or species additions through biological invasions of non-indigenous species (NIS) can alter ecosystem functioning. Particular attention has been given to research over biodiversity loss, and how those changes can affect the functioning of ecosystems, and in a way human welfare. Nonetheless, few studies have addressed how increased diversity due to establishment of NIS may affect ecosystem function in the recipient communities. Marine algae have a highly important role in sustaining nearshore marine ecosystems and are considered a significant component of marine bioinvasions. The present small-scale study examined the patterns of respiration and productivity across macroalgal assemblages with different levels of species richness and evenness. Additionally, we compared our results between native and invaded macroalgal assemblages, using the invasive brown macroalga *Sargassum muticum* (Yendo) Fensholt as a model species. Results showed that the presence of the invader increased the rates of respiration and production as a biomass consequence. This effect disappeared when *S. muticum* lost most of its biomass after senescence. Moreover, predictability-diversity relationships of macroalgal assemblages varied between native and invaded assemblages. Hence, the introduction of an invasive species did influence ecosystem functional responses, suggesting that few high-impact species may trigger major changes in ecosystem functioning. Also, due to their greater canopy height, *S. muticum* was more productive on an area basis than on a biomass-specific basis, suggesting that the impact of *S. muticum* may be related to its high productivity and dominance in the invaded assemblages, varying drastically with season.

**Keywords:** biodiversity; ecosystem functioning; NIS; macroalgae; *Sargassum muticum*

## 5.2. Introduction

Natural diversity is being modified worldwide by changes such as species loss and biological invasions of NIS (Vitousek et al., 1997; Sala et al., 2000). Understanding the consequences of such changes on ecosystem functioning has become a key topic of ecological research (e.g. Worm et al., 2006; Byrnes et al., 2007; Airoldi and Bulleri, 2011). The argument that biodiversity loss could lead to a reduction in global ecosystem functioning, i.e. interactions between biotic assemblages or with their abiotic environment, emerged as an issue in the early 90s (e.g. Ehrlich and Wilson, 1991; Naeem et al., 1994).

Conversely, in some systems local species richness has increased significantly due to recent establishment of NIS, although the long-term consequences of these introductions are still debated (Sax and Gaines, 2003). The spread of NIS has been considered one of the strongest anthropogenic impacts on natural ecosystems by changing abiotic factors, community structure and ecosystem properties (Mack et al., 2000; Byers, 2002; Ruesink et al., 2006). Life history features of invaders may be key factors in determining the fate and the impact of invasions. For instance, invasion by canopy forming macroalgae (e.g. *Sargassum muticum*, *Undaria pinnatifida*) may influence the structure of understory assemblages by modifying levels of light, sedimentation (Airoldi, 2003) or water movement (Eckman et al., 1989). Introduced species often exhibit novel features compared to native species and may have disproportionately high impacts in native ecosystem functioning (Ruesink et al., 2006). Although the majority of the scientific studies on the biodiversity-ecosystem functioning paradigm have been done in terrestrial ecosystems (Hooper et al., 2005), recent studies have also started to investigate ecosystem consequences of biodiversity loss in marine systems (Cardinale et al., 2006; Bracken et al., 2008; Danovaro et al., 2008; Reynolds and Bruno, 2012). A general theoretical framework of marine biodiversity and ecosystem functioning is well described by Boero and Bonsdorff (2007).

Primary production is the most common ecosystem process measured in species richness manipulation experiments (Hooper et al., 2005), also in marine experimental approaches (e.g. Bruno et al., 2005; Power and Cardinal, 2009). Rate of primary production in the ocean plays a key role in global geochemical cycles and transfer of energy through most food webs can be directly linked to the fixation of carbon at the primary producer level (Field et al., 1998). Primary productivity in an ecosystem is closely related to species diversity, although effects of species identity have recently been suggested as an important component on benthic marine community's production (Bruno et al., 2005,

2006). The productivity-diversity relationship, however, is very complex and may present positive, negative, hump-shaped, U-shaped or non-significant patterns (Waide et al., 1999). Additionally, biodiversity has been recognized to regulate ecosystem predictability (McGrady-Steed et al., 1997). As a result, theory predicts a reduction in the temporal or spatial variance of ecosystem properties with increasing biodiversity (Yachi and Loreau, 1999; France and Duffy, 2006). Although macroalgae make up a small proportion of ocean primary production, they are the dominant primary producers of rocky shore ecosystems (Mann, 1973), providing an essential ecological function for aquatic life. In addition, macroalgae are considered foundation species, providing habitat that may modify abiotic and biotic processes essential to overall ecosystem function (Bruno et al., 2003; Dijkstra et al., 2012). Hence, quantifying the primary productivity of these assemblages is essential to our understanding of energetic dynamics in coastal marine systems and the factors affecting it.

Preliminary studies on photosynthesis, growth or nutrient acquisition of macroalgae have been mainly examined in laboratory experiments (Littler and Arnold 1982) using single species (see Sand-Jensen et al., 2007 for a review). More recently, several studies suggested that for a better understanding of the primary production dynamics in macroalgae an assemblage-based approach must be considered (Binzer and Middelboe, 2005; Binzer et al., 2006; Richards et al., 2011). At assemblage level, combining species with different sizes and morphologies allows to account for effects such as canopy-shading, boundary layer and resource partitioning (Middelboe and Binzer, 2004). In particular, canopy structure has been suggested to influence assemblage production by affecting the distribution of light for all photosynthetic tissue in the assemblage and consequent efficiency of light utilization (Binzer and Sand-Jensen, 2002a; Binzer and Sand-Jensen, 2002b). Varying functional trait composition in assemblages is known to directly regulate ecosystem processes (Díaz and Cabido, 2001; McGill et al., 2006) and has been recently incorporated in biodiversity-ecosystem functioning relationships (e.g. Griffin et al., 2009; Roscher et al., 2012) rather than species richness *per se*. Additionally, individual performance of species, i.e. identity effect, has been proposed to affect the magnitude of an ecosystem process in macroalgal assemblages (Arenas et al., 2009; Griffin et al., 2009), indicating a high degree of interspecific variation in macroalgal productivity (Littler and Littler, 1980).

A few laboratory studies have also incorporated an assemblage perspective using natural communities (Arenas et al., 2009; Tait and Schiel, 2011). Examining different components of biodiversity, e.g. biomass, richness, evenness, Arenas et al. (2009) described a positive

relationship for biomass and species richness with productivity on macroalgal assemblages in small boulders bearing intertidal macroalgal assemblages. Recently, experimental studies in marine communities have analysed photosynthesis within intact, *in situ* macroalgal assemblages (e.g. Miller et al., 2009; Noël et al., 2010; Tait and Schiel, 2010). For example, Tait and Schiel (2010) tested for primary production in intertidal macroalgal assemblages dominated by furoid algae and described increased primary productivity of these macroalgal assemblages with a combination of greater biomass and greater numbers of macroalgal species.

Marine coastal ecosystems are strongly affected by invasions of NIS, which together with anthropogenic disturbances can create highly altered habitats. Nonetheless, to date, there are virtually no studies focusing on the ecosystem functional consequences of species richness increase due to the presence of invaders on marine habitats (Stachowicz and Byrnes, 2006; Wikström and Hillebrand, 2012). Marine macroalgae are a significant component of introduced NIS (Schaffelke et al., 2006), highlighting the importance of studies addressing interactions at this level, particularly using strong invaders, *sensu* Ortega and Pearson (2005).

The present study aimed to investigate assemblage-level impacts of macroalgal invasions and discriminate the mechanisms promoting its impact. In particular, we intended to understand the role of a strong invader, *Sargassum muticum* (Yendo) Fensholt, and assemblage structure on the dynamics of respiration and primary productivity of assemblages. We used synthetic assemblages of marine macroalgae, resembling those from intertidal rock pools, with varying levels of functional diversity and invader biomass. Native species with a long history of co-evolution are expected to partition resources among them and promote ecosystem functioning throughout resource use complementary effects. In contrast, newly introduced species, probably enhance ecosystem functioning by sampling effects where the influence of the invader is well beyond its relative abundance in the assemblage (Ruesink et al., 2006). Also, assemblage complexity (number of canopy layers) and diversity is expected to enhance ecosystem properties and predictability of assemblages.

### **5.3. Materials and methods**

#### **5.3.1. Macroalgal assemblages**

Macroalgal assemblages (64 cm<sup>2</sup>) were created from natural boulders collected at Praia Norte (Viana do Castelo, Portugal; 41°41'48" N, 08°51'11" W; see Arenas et al., 2009 for a full description of the collection site and boulders type). Using synthetic assemblages of

macroalgae we manipulated functional diversity by creating assemblages with different number of functional groups. Macroalgae were grouped into functional groups following Arenas et al. (2006), and three morpho-functional groups were selected: a) encrusting coralline species, i.e. *Lithophyllum incrustans*; b) turf-forming species from the genus *Corallina* and c) subcanopy species, i.e. *Chondrus crispus* or *Mastocarpus stellatus*. These species are common in macroalgal assemblages from intertidal rock pools in northern Portugal (Arenas et al., 2009).

Synthetic assemblages consisted of 12 x 17 x 1 cm PVC plates with 16 pieces of rock surrounded by 1cm PVC pieces for support and protection. Boulders were cut into 2 x 2 x 2 cm rock pieces and were sustained in PVC plates using fast setting underwater cement and screws. Individual rock pieces represented one functional group characterized by a percent cover greater than 50% or, in the case of subcanopy species, the presence of 1 or more adult individuals was considered a representation of that functional group. A total of 60 plates were built: 12 plates of only bare-rock, 36 plates with only one functional group, and 12 plates with three functional groups. In the last case, the spatial distribution of the three functional groups within plates was random.

Synthetic macroalgal assemblages were then subjected to an artificial invasion by the brown macroalga *Sargassum muticum*. This was accomplished by collecting fertile individuals of *S. muticum* with receptacles bearing exuded propagules from the field and transporting them to the laboratory where they were rinsed with freshwater to eliminate grazers. Fertile *S. muticum* was then placed floating over the assemblages in tanks of approximately 300L of seawater. To assure different biomass of the invader in the final assemblage composition, propagule pressure was manipulated by suspending different biomass of fertile individuals of *S. muticum* over the macroalgal assemblages (High density  $\approx$  25kg; Low density  $\approx$  13kg; Control – none). Control assemblages were used to assess natural assemblage composition. A total of 20 macroalgal assemblages of combined functional diversity treatments (n = 4) were randomly assigned to each PP treatment (i.e. 1 tank per propagule pressure treatment). Experimental invasion was programmed to be around the new moon (July 22, 2009) due to the semilunar periodicity of egg expulsion in *S. muticum* around new or full moons (Norton, 1981).

To allow for germlings settling, macroalgal assemblages were transported to the field 1 week after artificial invasion. Assemblages were randomly placed and screwed to the bottom of a large rock pool (11 x 2 m size, average depth of 35 cm) in the mid-intertidal shore ( $\approx$  1.5m above chart datum) of Viana do Castelo where they remained for 22 months.

### 5.3.2. Incubation procedures

The present study was performed in the Laboratory of Coastal Biodiversity, at CIIMAR in Oporto. Incubation measurements were carried out in November 2010 and May 2011, to test the generality of the results for low and high biomass of the invader *Sargassum muticum*, respectively. Respiration and productivity measurements were carried out under controlled conditions to reduce environmentally induced variability in the responses.

Assemblage plates were maintained in outdoor aerated seawater tanks for a maximum of 5 days under natural light and temperature conditions before the incubations were done. Nutrients were supplied every 2 days (1ml of nutrient solution per liter of seawater; 42.50 g NaNO<sub>3</sub> l<sup>-1</sup>, 10.75 g Na<sub>2</sub> HOP<sub>4</sub> l<sup>-1</sup>).

Incubations of macroalgal assemblages were carried out inside an experimental chamber, composed of twenty six 18W fluorescent tubes (Osram® Cool White). Inside the experimental chamber, incubations were performed in sealed chambers and comprised measurements of the change in dissolved oxygen concentration during dark and light periods. The irradiance inside the experimental chamber was measured using a spherical scalar quantum sensor connected to a computer (Biospherical® QSL-2000). Productivity-irradiance relationships were estimated at 7 increasing irradiance levels: 0 (dark), 30, 60, 90, 180, 250 and 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  irradiance (i.e. 30 minutes each).

The incubation chambers consisted of a 12.5, 15.5 or 47.5-l transparent Plexiglass chamber, depending on the biomass of the assemblage plate. Incubation chambers were partially submersed in a larger white Plexiglass chamber used as a cooling bath to assure constant temperature during incubations. Mean ( $\pm$  SE) temperature during incubations was  $16.5 \pm 0.006$  °C. Water movement inside the incubation chamber was maintained by submersible pumps with diffusers. Dissolved oxygen concentration and temperature inside the incubation chambers were measured every 30 s using a luminescent dissolved oxygen, LDO, probe connected to a portable oxygen meter (Hach® HQ40). To reduce possible effects of circadian rhythms on algal productivity, incubations were always carried out during daylight hours (between 08:00 and 18:00 h).

Immediately after incubation, macroalgae were scraped from plates, rinsed in freshwater, sorted by species and dried at 60°C for 48 h to estimate biomass (g dry weight, DW). In the case of the encrusting species, we selected 2 x 2 cm rock pieces colonized with encrusting coralline species, oven-dried for 48h at 50°C and weighted for dry-weights. We then placed it in hydrochloric acid (0.5 M HCl) for 48h to remove the calcium carbonate.

Rock pieces were then rinsed with freshwater, oven-dried for 24h and then re-weighted. The difference from the dry-weights, i.e. before and after the HCl treatment, was used to obtain the biomass of the algae per 4 cm<sup>2</sup> and the average of 40 squares allowed us to estimate the biomass in our plates.

### 5.3.3. Ecosystem functioning surrogates

Respiration and productivity were estimated through oxygen fluxes by regressing oxygen concentration ( $\mu\text{mol}$ ) through time ( $\text{s}^{-1}$ ) during dark and light periods of increasing intensities. Estimations were corrected by assemblage surface (64 cm<sup>2</sup>) or biomass (May 2011) and volume of incubation chamber (12, 15 or 47 l, measured with plates inside the chamber). Additionally, estimates of respiration and productivity were also corrected by control blanks (incubations performed simultaneously with only filtered seawater) to control for rates of respiration and production of bacteria and phytoplankton.

The variables Respiration, Maximum net primary productivity (max-NPP), Photosynthetic efficiency at low light irradiance (alpha,  $\alpha$ ) and Light compensation point were measured as surrogates of ecosystem functioning. Respiration of assemblage ( $\mu\text{mol O}_2 \text{ s}^{-1}$ ) corresponded to the oxygen consumption rate during the dark period and max NPP ( $\mu\text{mol O}_2 \text{ s}^{-1}$ ) represented the maximum productivity recorded. Both variables were calculated by plotting oxygen concentration over incubation time and fitting a linear regression line to calculate rates of oxygen change. Photosynthetic efficiency,  $\alpha$  ( $\mu\text{mol O}_2 \mu\text{mol photons m}^{-2}$ ), was estimated as the slope of P-I relationship at light-limited irradiances (up to 87  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), through linear regressions. Regressions were also used to estimate light compensation point of assemblages, the irradiance level at which respiration rate is equal to photosynthetic rate and net oxygen exchange is zero.

### 5.3.4. Data analysis

Linear models were performed to investigate the influence of *Sargassum muticum* (presence or absence) on each the functional responses examined: respiration, max NPP,  $\alpha$  and light compensation point. All response variables were log-transformed in order to guarantee linearity and normality of residual distributions. Homoscedasticity was assessed by graphical examination of the residuals.

We fitted two linear models to each of our functional responses examined. One had *Sargassum muticum* and species richness as predictors, and the second model had *S. muticum* and species evenness as predictors. Species richness describes the number of species in the assemblage, while the evenness takes into account the variation in the

abundance of individuals per species in the assemblage. Species evenness was calculated using the Pielou's evenness index ( $J'$ ). We used two biodiversity components, i.e. species richness and species evenness, as predictive variables to test if the response of ecosystem function measures varied with the component of biodiversity used (see Hooper et al., 2005). Additionally, we re-analysed respiration and max-NPP data after correcting values per assemblage biomass, in order to consider the efficiency of the assemblages.

The coefficient of variation (CV) in assemblage's productivity was quantified for native and invaded assemblages in May, i.e. high biomass of the invader. CV was calculated as the ratio of the standard deviation to the mean,  $CV = \sigma / \mu$ , and was used as a measure of spatial variation.

Linear models were carried out using the linear model function (lm) in the R-program 2.15.0 (R Development Core Team, 2012).

## 5.4. Results

In November 2010, 37 macroalgal assemblage plates were collected from the intertidal pool whereas in May 2011 we were able to locate 55 macroalgal assemblages. Algal assemblages varied in species richness, total biomass and biomass of the invader.

### 5.4.1. November 2010

The presence of *Sargassum muticum* did not affect the relationship between species richness and any functional response of macroalgal assemblages measured (Table 5.1a). Moreover, no significant relationship was found between any functional response of macroalgal assemblages measured and species richness (Table 5.1a). However, when species evenness was considered as predictor variable, the functional response of assemblages in terms of respiration, max NPP and alpha showed a significant negative relationship (Table 5.1b; Fig. 5.1). Respiration and alpha in invaded macroalgal assemblages were not affected by the presence of *S. muticum* ( $P > 0.05$ , Table 5.1b), whereas the slope of max NPP varied between native and invaded macroalgal assemblages ( $F_{3,33} = 9.273$ ,  $P < 0.05$ ,  $R^2 = 0.46$ ). Specifically, native assemblages described a negative relationship between max NPP and evenness ( $F_{1,23} = 23.566$ ,  $P < 0.0001$ ,  $R^2 = 0.51$ ), while in invaded assemblages the slope of the relationship did not differ from 0 ( $F_{1,10} = 4.067$ ,  $P = 0.07$ ,  $R^2 = 0.29$ ; Fig. 5.1b).

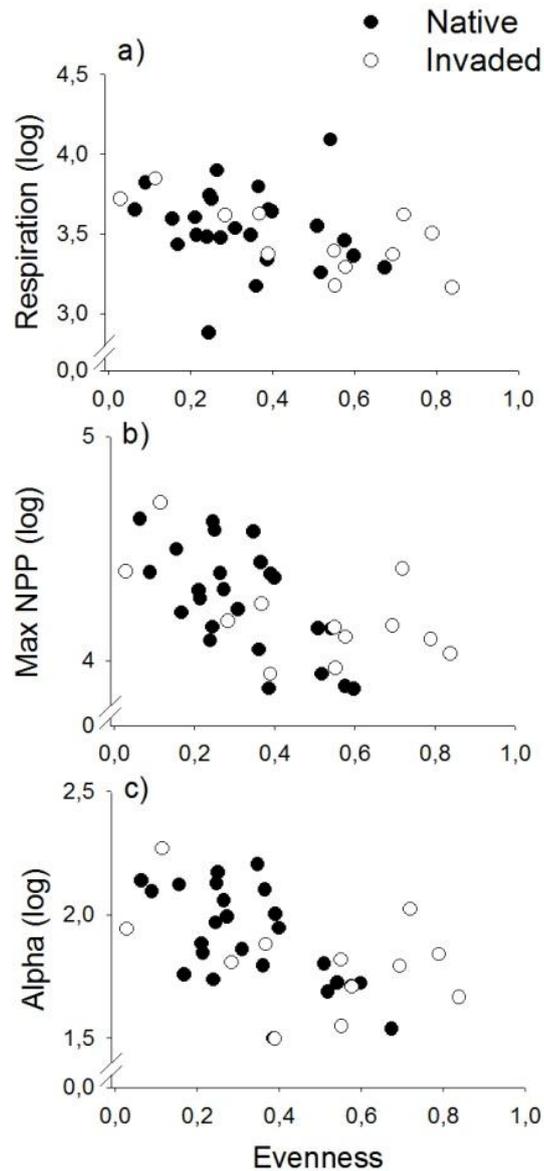
**Table 5. 1.** November 2010. Linear model analysis testing the main and interactive effects of invasion of *Sargassum muticum* and a) species richness or b) species evenness, on the functional responses of macroalgal assemblages (per 64 cm<sup>2</sup>), i.e. Respiration, maximum net primary productivity (Max NPP), and alpha. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; ns: not significant.

		Respiration		Max NPP		Alpha	
a)	df	MS	F	MS	F	MS	F
Richness	1	0.250	0.811 <sup>ns</sup>	0.501	1.583 <sup>ns</sup>	0.606	2.922 <sup>ns</sup>
Invasion	1	0.156	0.509 <sup>ns</sup>	0.074	0.235 <sup>ns</sup>	0.281	1.355 <sup>ns</sup>
Residuals	34	0.308		0.316		0.2075	
b)							
Evenness	1	1.501	5.449 <sup>*</sup>	4.106	22.054 <sup>***</sup>	2.270	13.602 <sup>***</sup>
Invasion	1	0.001	0.003 <sup>ns</sup>	0.213	1.145 <sup>ns</sup>	0.0002	0.001 <sup>ns</sup>
E:Inv	1	-	ns	0.860	4.619 <sup>*</sup>	-	ns
Residuals	33	0.276		0.186		0.1669	

#### 5.4.2. May 2011

In May 2011, the biomass of the invader ranged between 0 and 163.38 g DW ( $3.58 \pm 2.91$  g DW) per 64 cm<sup>2</sup>. Moreover, biomass of *Corallina* spp., *S. muticum* and *Chondrus crispus* accounted for  $\approx 90\%$  of the overall biomass with 38.7, 35.1 and 18 %, respectively. *Corallina* spp. and *Lithophyllum incrustans* were present in all algal assemblages.

Contrasting with results from November, the presence of *Sargassum muticum* affected all functional responses of macroalgal assemblages measured (Table 5.2). Although no significant relationship was observed between respiration and species richness, invaded macroalgal assemblages presented significant higher values (Table 5.2a, Fig. 5.2a). Max NPP and alpha were positively related to species richness and significant higher values were also observed in invaded macroalgal assemblages (Table 5.2a, Fig. 5.2b,c). Interactive effects of *S. muticum* were observed over respiration and max NPP when species evenness was considered (Table 5.2b). Both functional responses showed no relationship with species evenness in invaded macroalgal assemblages, whereas a significant negative relationship was found in native assemblages ( $F_{1,40} = 4.39$ ,  $R^2 = 0.10$ ,  $P = 0.04$  and  $F_{1,40} = 7.95$ ,  $R^2 = 0.17$ ,  $P = 0.007$ , for respiration and max NPP, respectively; Fig. 5.2d,e). Additionally, significant higher alpha values were obtained in invaded macroalgal assemblages (Table 5.2b, Fig. 5.2f).



**Fig. 5. 1.** November 2010. Relationship between the functional responses measures (per 64 cm<sup>2</sup>) and assemblage structure in native and invaded macroalgal assemblages. a) assemblage respiration and species evenness; b) max NPP and species evenness; c) alpha and species evenness.

**Table 5. 2.** May 2011. Linear model analysis testing the main and interactive effects of invasion of *Sargassum muticum* and a) species richness or b) species evenness, on the functional responses of macroalgal assemblages (per 64 cm<sup>2</sup>), i.e. Respiration, maximum net primary productivity (Max NPP) and alpha. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; ns: not significant.

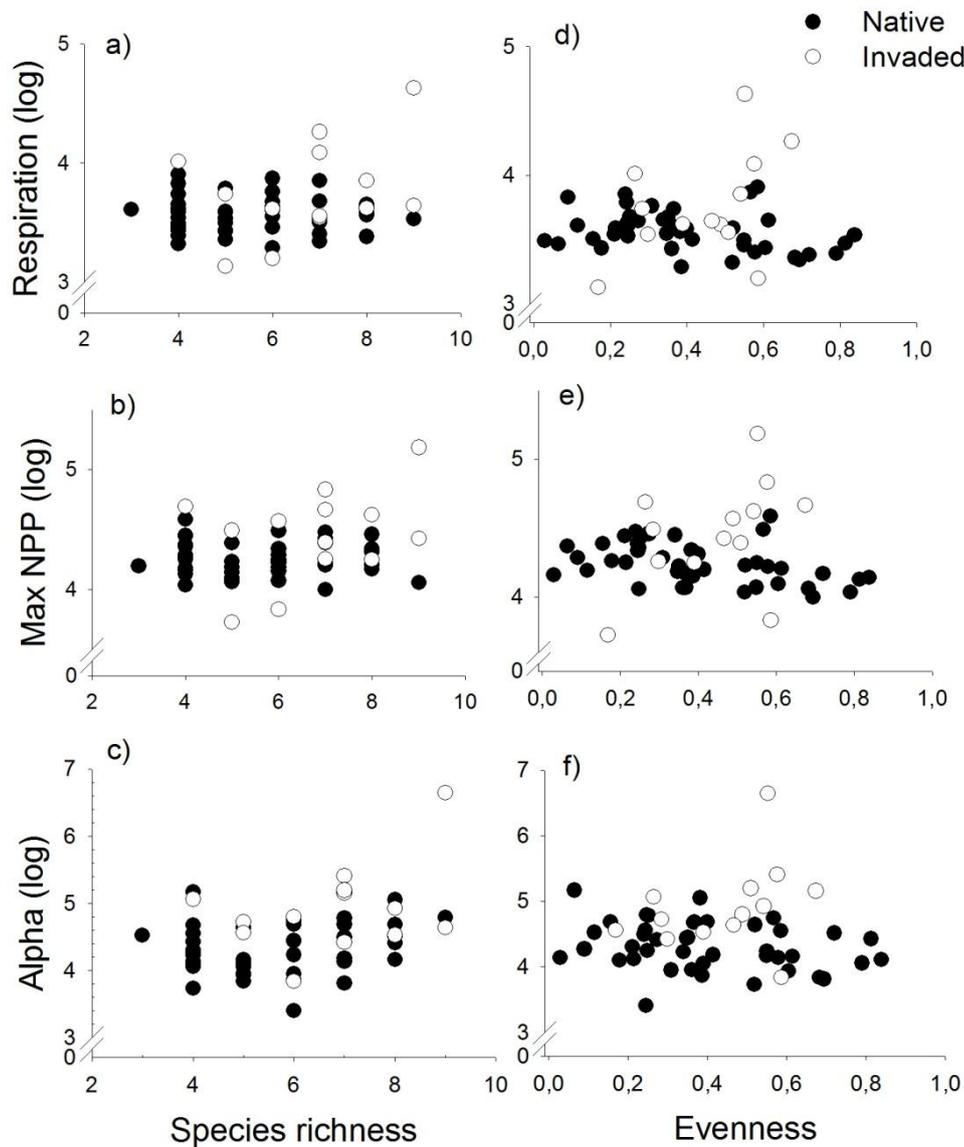
a)	Respiration			Max NPP		Alpha, $\alpha$	
	df	MS	F	MS	F	MS	F
Richness	1	0.876	2.893 <sup>ns</sup>	1.118	4.153 <sup>*</sup>	1.943	10.09 <sup>**</sup>
Invasion	1	1.394	4.606 <sup>*</sup>	1.618	6.010 <sup>*</sup>	2.323	12.07 <sup>**</sup>
Richness: Invasion	1	-	ns	-	ns	-	ns
Residuals	51	0.303		0.269		0.192	
b)							
Evenness	1	0.0001	0.0003 <sup>ns</sup>	0.038	0.164 <sup>ns</sup>	0.013	0.063 <sup>ns</sup>
Invasion	1	2.030	7.736 <sup>**</sup>	2.466	10.592 <sup>**</sup>	3.650	17.885 <sup>***</sup>
Evenness: Invasion	1	2.595	9.889 <sup>**</sup>	2.360	10.136 <sup>**</sup>	-	ns
Residuals	51	0.262		0.233		0.204	

Patterns were, however, quite different when the efficiency of the assemblages was considered. Respiration and max NPP rates per biomass unit, for both native and invaded assemblages, were not significantly related to species richness or evenness (Table 5.3, Fig. 5.3).

The triangular scatter of observations (Fig. 5.3a,b) for native assemblages, suggested that variation among replicates of identical species richness decreased as species richness increased. Predictability-diversity relationships of macroalgal assemblages varied between native and invaded assemblages (Fig. 5.4). We can observe that in native macroalgal assemblages, the coefficient of variation significantly decrease with species richness ( $R^2 = 0.79$ ,  $P = 0.044$ ,  $n = 5$ ). Thus, the variation among replicates of identical species richness declined as species richness increased. Contrasting results were obtained for invaded macroalgal assemblages where no relationship was observed ( $R^2 = 0.003$ ,  $P = 0.933$ ,  $n = 5$ ).

Overall, there was a positive relationship between max NPP (production at high irradiance) and alpha (efficiency at low irradiance) in both experimental periods, November 2010 ( $R^2 = 0.88$ ,  $df = 35$ ,  $P < 0.0001$ ; Fig. 5.5a) and May 2011 ( $R^2 = 0.51$ ,  $df = 53$ ,  $P < 0.0001$ ; Fig. 5.5b). Thus, assemblages that were efficient at utilizing low irradiances were also more efficient at utilizing high irradiances. Also, light compensation

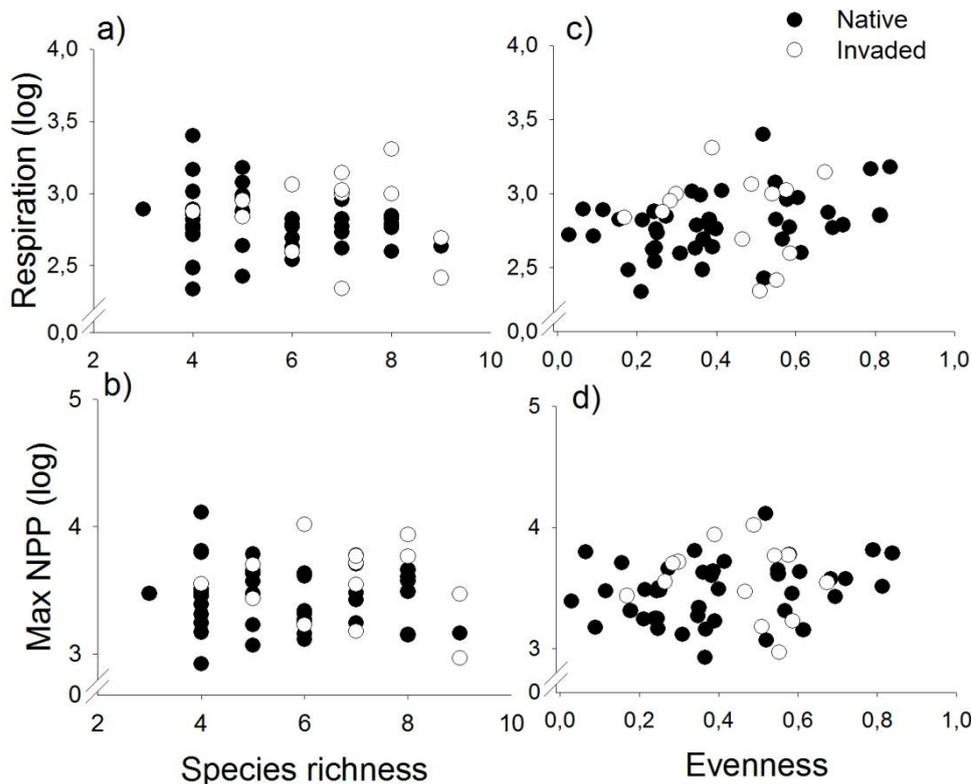
point did not differ between autumn and spring ( $47.07$  and  $48.81 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively). Moreover, light compensation point from macroalgal assemblages was not affected by the presence of *Sargassum muticum* ( $P > 0.05$  for both experimental periods).



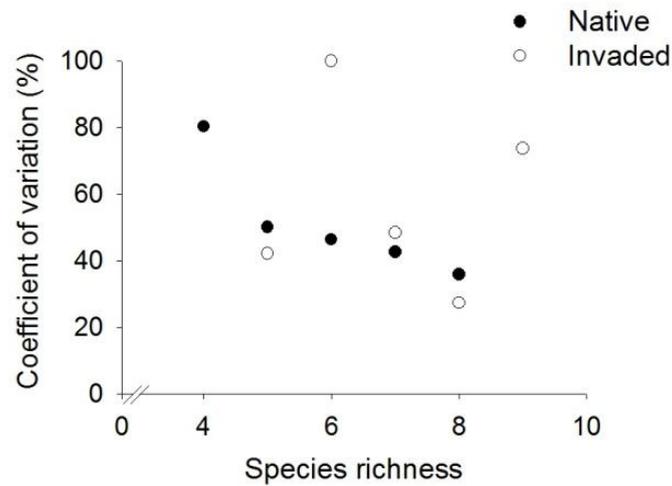
**Fig. 5. 2.** May 2011. Relationship between the functional responses measures (per  $64 \text{ cm}^2$ ) and assemblage structure in native and invaded macroalgal assemblages. a) assemblage respiration and species richness; b) maximum net primary productivity (max NPP) and species richness; c) photosynthetic efficiency at low irradiance (alpha) and species richness; d) assemblage respiration and species evenness; e) max NPP and species evenness; f) alpha and species evenness.

**Table 5. 3.** May 2011. Linear model analysis testing the main and interactive effects of invasion of *Sargassum muticum* and a) species richness or b) species evenness, on the functional responses of macroalgal assemblages, i.e. Respiration, maximum net primary productivity (Max NPP), and alpha. Data was corrected by macroalgal biomass (grams dry weight). <sup>(.)</sup>P < 0.06; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; ns: not significant.

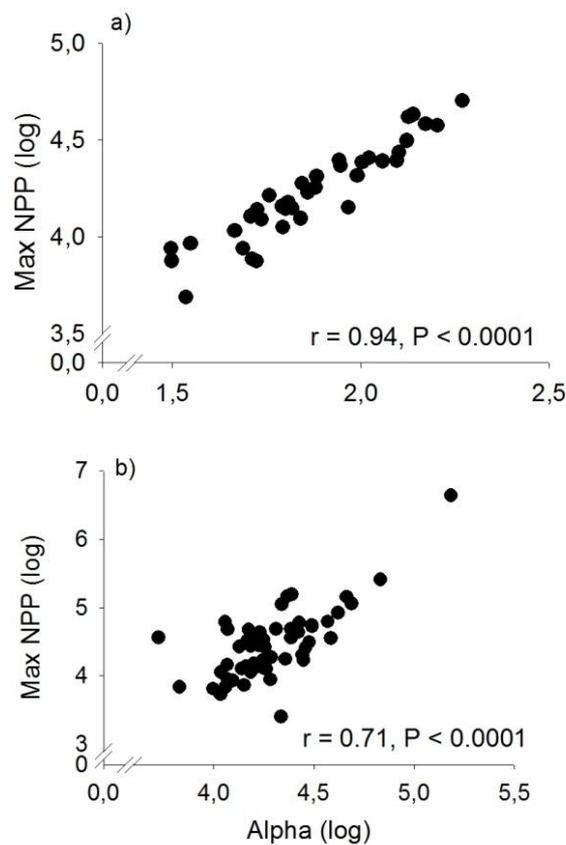
		Respiration		Max NPP	
a)	df	MS	F	MS	F
Richness	1	0.112	0.406 <sup>ns</sup>	0.045	0.122 <sup>ns</sup>
Invasion	1	0.452	1.644 <sup>ns</sup>	0.583	1.574 <sup>ns</sup>
Residuals	52	0.275		0.370	
b)					
Evenness	1	1.010	3.841 <sup>(.)</sup>	0.651	1.790 <sup>ns</sup>
Invasion	1	0.188	0.717 <sup>ns</sup>	0.336	0.923 <sup>ns</sup>
Residuals	52	0.263		0.364	



**Fig. 5. 3.** May 2011. Relationship between the functional responses measures (grams dry weight) and assemblage structure in native and invaded macroalgal assemblages. a) assemblage respiration and species richness; b) maximum net primary productivity (max NPP) and species richness; c) assemblage respiration and species evenness; d) max NPP and species evenness.

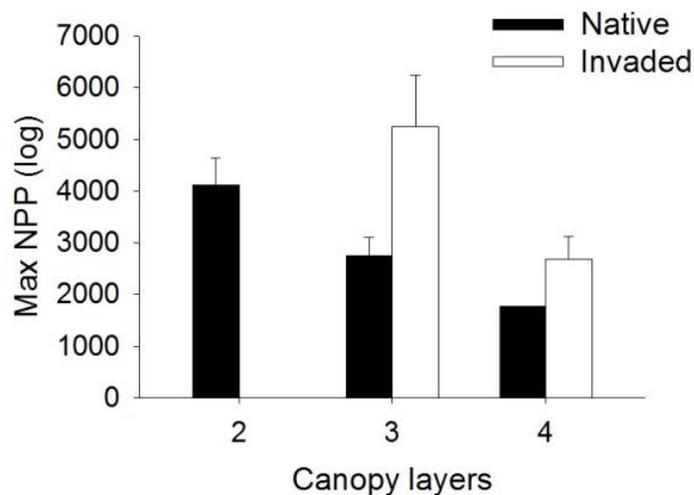


**Fig. 5. 4.** Predictability-diversity relationship. Variability in the coefficient of variation with species richness, regarding maximum net primary productivity (max NPP) per unit biomass, for native and invaded macroalgal assemblages.



**Fig. 5. 5.** Relationship between maximum net primary productivity (max NPP, production at high irradiance) and alpha (photosynthetic efficiency at low irradiance) in macroalgal assemblages (64 cm<sup>2</sup>). a) November 2010; b) May 2011.

There was a significant decrease in max NPP with increasing canopy complexity, which was enhanced in invaded assemblages (Fig. 5.6). Assemblage layers were associated with different functional groups, i.e., crust, turf, subcanopy and canopy, and assemblages were composed of 2, 3 or 4 canopy layers, respectively.



**Fig. 5. 6.** Mean (+SE) effects of assemblage complexity (number of canopy layers) on maximum net primary productivity (max NPP) in native and invaded macroalgal assemblages.

## 5.5. Discussion

Marine algae are photosynthetic organisms, highly important in sustaining nearshore marine ecosystems (Mann, 1973; Bruno et al., 2005; Lilley and Schiel, 2006). The present small-scale study examined the patterns of production across different species richness and evenness levels of macroalgal assemblages of native species versus invaded macroalgal assemblages. Results showed that the presence of *Sargassum muticum* increased the rates of respiration and production. However, this effect was only a biomass consequence and thus it disappeared when *S. muticum* lost most of its biomass after senescence. In addition, the increased predictability between species richness and ecosystem function found in native macroalgal assemblages disappeared in invaded assemblages.

Current research on how marine algae impact nearshore ecosystems addresses how these primary producers influence ecosystem function. However, disturbance such as the loss or addition of species with certain traits can cause unexpected responses. Specifically, introduction of species with traits not found in the recipient assemblages can produce large-scale alterations of ecosystem processes and structure (Ruesink et al.,

2006). When mature, *S. muticum* presents a high structural complexity formed by a holdfast, a stem and large main and lateral bearing branches, leaves, vesicles and receptacles. However, due to the fact that *S. muticum* is a pseudo-perennial species, the main branches are annual and fall off after reaching maturity. Thus, the presence of *S. muticum* and possible impacts should vary drastically with season. In the present study, the invasive species modified respiration and productivity of assemblages, when present in elevated biomass. However, as a habitat modifier, *S. muticum* individuals may also modify a variety of other ecosystem processes (Wallentinus and Nyberg, 2007). *S. muticum* has a high growth capacity and is known to influence assemblages by modifying levels of light (Britton-Simmons, 2004; Strong et al., 2006), water movement and temperature (Strong et al., 2006) within canopy areas. Contrasting results were obtained in autumn, when *S. muticum* had a minimum biomass. Over this experimental period no significant effect of the invader was recorded, with only 1 exception, i.e. the presence of *S. muticum* modified the relationship between max NPP and evenness of the macroalgal assemblages from negative to a no significant relationship. The different morphological forms of the *S. muticum* individuals between seasons may have significant differences in the percentage of photosynthetic tissue, net photosynthesis, and specific growth rate, as previously demonstrated for the red alga *Gracilaria tikvahiae* McLachlan 1979 (Hanisak et al., 1988). Thus, these varying results between seasons are not totally unexpected, although the type of relationship may be. In a previous study using intertidal macroalgal assemblages, also in November/December, correlations with evenness were not significant for any functional variable addressed (Arenas et al., 2009), contrasting with the negative relationship found in the present study.

Considered an invasive species all around the world (Critchley et al., 1983), *S. muticum* varies greatly in its ability to impact native systems due to its seasonal reproduction, followed by a rapid shedding of the reproductive tissues (Arenas and Fernández, 1998). In the autumn, after a substantial loss of biomass, our results suggest that *S. muticum* acts as a weak invader and becomes a minor component of native assemblages. In the spring, due to the elevated biomass, this species becomes dominant at the expense of native species and processes and acts as a strong invader (*sensu* Ortega and Pearson, 2005). Hence, the impact of *S. muticum* can be related to its high productivity and dominance in the invaded assemblages, in agreement with the sampling effect hypothesis, i.e. the increasing probability of selecting a species with a specific property with increasing species richness (Huston, 1997). Accordingly, it has been suggested that native species with a long history of coevolution may influence ecosystem processes through resource

use efficiency whereas NIS effects on the recipient assemblage occurs through sampling effects (Ruesink et al., 2006).

Several studies have revealed that the effect of species diversity on function depends on the system studied and the function measured (Schwartz et al., 2000; Schmid, 2002; Duffy, 2003). Moreover, the effects of species richness on biomass production change through time and are influenced by both selection and multispecies complementarity effects (Cardinale et al., 2007). Specifically, the role of phenology has not been thoroughly assessed in primary productivity studies, although variables such as standing biomass of the species, its functioning (e.g. the outcome of carbon assimilation, nutrient uptake) and phenology have been suggested to influence net primary productivity (Lavorel and Garnier, 2002). In this context, functional traits are considered fundamental for a mechanistic understanding of biodiversity effects (Violle et al., 2007; Roscher et al., 2012). The majority of biodiversity research manipulates species richness with few studies manipulating or measuring species evenness, i.e., the relative contribution of each species to the total biomass or number of individuals (but see Wilsey and Potvin, 2000; Polley et al., 2003; Arenas et al., 2009). Our results showed that species evenness effect on functional measures varied between native and invaded assemblages. However, contrasting with previous results in terrestrial systems (Wilsey and Potvin, 2000; Kirwan et al., 2007), no positive effects of evenness on productivity have been recorded. A negative relationship between evenness and biomass has, however, been observed due to dominance of species with large biomass per unit area (Mulder et al., 2004), reducing evenness and increasing productivity (Nijs and Roy, 2000). In fact, *Corallina* spp. and *Chondrus crispus* were dominant species in native macroalgal assemblages which could explain the negative relationship described in our results. Moreover, the presence of canopy-forming species such as *S. muticum*, has been suggested to modify species evenness, e.g. the removal of canopies promoted evenness (Bulleri et al., 2012). Thus, assemblages with species whose biomass is disproportionately greater than that of associated species, i.e., invaded assemblages, may be related to lower evenness and consequently lower niche complementarity (Nijs and Roy, 2000). However, our results showed no relationship between evenness and functional responses in invaded macroalgal assemblages. This result may be the result of a high variability in *S. muticum* biomass between assemblage plates.

Macroalgal assemblages' productivity was measured as O<sub>2</sub> production, contrasting with general production studies which assess primary and/or secondary production (e.g. Bruno et al., 2005; Cardinale et al., 2006; Reynolds and Bruno, 2012). It has been suggested

that the magnitude of energy transferred to a seaweed from a moving water mass depends on its size and shape (Norton, 1991). Accordingly, after taking in consideration the biomass of the invader, the overall results were entirely different revealing biomass dependent effects (Emmerson and Raffaelli, 2000). A previous study has also described biomass as a key determinant of overall per-area primary productivity (Tait and Schiel, 2011). However, contrary to our results, correcting by biomass revealed that the identity of certain taxa was also important (Tait and Schiel, 2011). Overall, theory describes a positive effect of increasing species richness on assemblage biomass, due to complementarity and sampling effects (Loreau, 2000; Cardinale et al., 2007). The efficiency of macroalgal assemblages after correcting by biomass did not, however, present a species richness or evenness effect on functional processes. Recent findings comparing productivity of subtidal turf and foliose algal assemblages describe foliose assemblages to be more productive due to their greater biomass per unit area and not because of greater production per unit biomass (Miller et al., 2009). The same trend can be described for *Sargassum muticum*. Due to its greater canopy height, *S. muticum* is more productive on an area basis than on a biomass-specific basis. Moreover, when measuring algal assemblage productivity as O<sub>2</sub> production, Bruno et al. (2005) found a species identity effect but no algal species richness effect, in accordance with our results. Theory points out that the positive effect of increasing species richness on assemblage biomass, due to complementarity or sampling effects, could be offset by the negative effects of species interactions including the competitive suppression of highly productive species in diverse polycultures, resulting in weak or no net richness effects (Loreau and Hector, 2001; Hooper and Dukes, 2004). In particular, the invasion of *S. muticum* can be seen as a perturbation that changed species composition and environmental conditions of the invaded assemblages.

Finally, in agreement with the similarity hypothesis (Yachi and Loreau, 1999), the increase in species richness in native macroalgal assemblages increased the predictability of primary production across space. Contrasting results were, however, obtained for invaded assemblages where the presence of *S. muticum* removed the positive relationship between species richness and ecosystem function predictability. The variability of the ecosystem function response found in invaded assemblages suggests that invaded assemblages' dynamics are less predictable than native dynamics. Further interactions between habitat-modifying species can decrease predictability of community-level effects of an invasion, particularly if invasive species show extremely variable cycles over time (Ward and Ricciardi, 2010). The consequences of invasion for the invaded communities, especially with regard to their functioning, have been rarely considered (Pfisterer et al.,

2004). However, the identity of the species being added or removed and its similarity to other species are often of critical importance in determining overall effects (Mooney et al., 1995). Also, it has been suggested that colonization history may alter ecosystem functioning (Zhang and Zhang, 2007). In particular, species arrival order may be a significant factor influencing species interactions and consequent assemblage performance. In native assemblages, the dominant species were perennial algae such as *Chondrus crispus* or *Corallina* spp., while the dominance of the invader varied drastically between seasons. Previous studies have demonstrated a high variability in *S. muticum* productivity and reproductive development between habitats (Baer and Stengel, 2010) and grazing pressure (Plouguerné et al., 2006). Thus, the reduced predictability of high diverse communities when invaded suggested by our results might be related to the very high local variability of the invader. A previous study in experimental multitrophic seagrass ecosystems described that more diverse grazer metacommunities produced greater spatial variability in ecosystem properties such as algal and sessile invertebrate biomass accumulation (France and Duffy, 2006). Thus, it has been suggested that higher diversity does not necessarily increase the predictability of ecosystem functioning in space as it often does in time (France and Duffy, 2006).

Additionally, the negative relationship between assemblage canopy layers and max NPP observed in the present study provides further evidence of the impact of high yield dominants (Thompson et al., 2005). Marine macroalgae present a stunning diversity of life forms and general structure and, composition of macroalgal assemblages includes varying diversity and layering. A recent study described an interaction between canopy layers and irradiance, suggesting that net photosynthesis within canopies of varying complexity depended on the amount of light to which they are exposed (Tait and Schiel, 2011). The fact that the greatest effect of canopy complexity on photosynthesis occurred at  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Tait and Schiel, 2011) suggest that our results were limited by the amount of irradiance employed. Indeed, it has been argued that at high irradiance a multilayered thallus should perform better than a mono-layered one, but at lower irradiances self-shading would become a liability (Norton, 1991; Middelboe and Binzer, 2004; Richards et al., 2011).

Overall, there was a positive relationship between max-NPP (production at high irradiance) and alpha (efficiency at low irradiance). Thus, assemblages that were efficient at utilizing low irradiances were also more efficient at utilizing high irradiances (Binzer et al., 2006). Moreover, macroalgae thalli are known to present adaptations of the light compensation point in winter (King and Schramm, 1976; Middelboe et al., 2006). This

adaptation was not, however, observed in the present study, probably due to the use of assemblages instead of detached thalli.

In conclusion, our results highlight the fact that ecosystem functioning is a function of the biotic ecosystem components (Schläpfer and Schmid, 1999) varying in different ecological contexts, e.g. season. The introduction of an invasive species did influence ecosystem functional responses, suggesting that few high-impact species may trigger major changes in ecosystem functioning (Ruesink et al., 2006). Specifically, the impact of *S. muticum* was related to its high productivity and dominance in the invaded assemblages and varied drastically with season.

## **5.6. Acknowledgments**

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## **Part IV**

### **NIS & Climate change**





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## **Chapter 6**

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Functional diversity and climate change: effects on the  
invasibility of macroalgal assemblages



**Functional diversity and climate change: effects on the invasibility of macroalgal assemblages**

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## 6.1. Abstract

Understanding how climate-driven changes affect the structure and functioning of ecosystems is a central issue in ecological studies. In particular, the response of invasive species to those changes is of special concern. We used a mesocosm experiment to test how increases in temperature and CO<sub>2</sub> partial pressure ( $p\text{CO}_2$ ) interact with functional diversity of resident macroalgal assemblages and affect the invasion success of the non-indigenous macroalga *Sargassum muticum* (Yendo) Fensholt. Early settlement of *S. muticum* germlings was assessed in the laboratory under control ambient conditions. Early survivorship of settled germlings was assessed at different treatment combinations while recruitment was quantified after six months in natural field conditions. Functional diversity was a key driver shaping early settlement of the invader, with significant identity and richness effects. Additionally, early survivorship and recruitment results showed evidence of interactive effects of temperature and  $p\text{CO}_2$  on the invasion success of *S. muticum*. High temperature enhanced the invasiveness of *S. muticum* but only under current  $p\text{CO}_2$  conditions. Future climatic scenarios of increasing temperature and  $p\text{CO}_2$  would not be beneficial to *S. muticum*. Present findings highlight the need to address interactive effects of multiple stressors in ecological studies, particularly when assessing effects of predicted climate change.

**Keywords:** Multiple stressors, Invasibility, Functional diversity, *Sargassum muticum*,  $p\text{CO}_2$ , temperature

## 6.2. Introduction

It is widely accepted that human activities are causing global environmental changes with a high ecological impact on natural systems (Sala et al., 2000; Halpern et al., 2008). Increased anthropogenic CO<sub>2</sub> emissions over the past 200 years have led to greater CO<sub>2</sub> uptake by the oceans (Feely et al., 2004). This ongoing process of atmospheric CO<sub>2</sub> uptake is changing seawater carbon chemistry and is expected to substantially decrease oceanic pH (i.e. pH reduction by 0.7 units based on IPCC scenario for 2000-2100, Caldeira and Wickett, 2003), a phenomenon known as “ocean acidification” (Feely et al., 2004). In addition to ocean acidification, the increase in atmospheric CO<sub>2</sub> also has been reflected in increased global temperatures, with consequent increase of the mean sea-surface temperature of the oceans (IPCC, 2007). Similarly, the introduction of non-indigenous species (NIS) is also recognized as a significant component of global change (Sala et al., 2000; Williams and Smith, 2007), and their impact on native populations, communities and ecosystems have been widely recognized for decades (Elton, 1958; Lodge, 1993).

Currently, ecologists concentrate on investigations examining the effects of single global change agents, yet research on the interactive effects of multiple agents is mostly lacking. Approximately 60% of climate-related research publications in the marine realm are focused on independent effects of temperature (Harley et al., 2006). More recently, several studies examined the independent effect of ocean acidification (e.g. Feely et al., 2004; Porzio et al., 2011), whereas very few studies have examined the interaction of both factors (but see Martin and Gattuso, 2009; Connell and Russell, 2010). Yet, global change agents will not act in isolation and their combination could produce unexpected results (Harley et al., 2006; Darling and Côté, 2008). For example, although ocean acidification has negative effects on calcifying algae (Martin and Gattuso, 2009; Hofmann et al., 2012), there is growing evidence that the combined effect of  $p\text{CO}_2$  with other global-change drivers may also have positive effects on non-calcareous algae (nutrients or temperature, Russell et al., 2009; Connell and Russell, 2010).

Similarly, studies examining the interactions between global change drivers and invasions have been largely ignored. Most experimental work on invasion ecology done to date supports Elton's (1958) ideas in demonstrating greater resistance to invasion in diverse communities (Tilman, 1997; Stachowicz et al., 1999), although patterns are scale-dependent (Fridley et al., 2007), and the mechanism driving such relationship remains unclear. Invasive species can modify ecosystems by displacing native species and/or altering habitat characteristics (Crooks, 2002). Hence, there is increasing concern that

multiple global change drivers of ecological change could interact synergistically to accelerate biodiversity loss. Several experimental studies found a positive relationship between biodiversity and invasion resistance (Stachowicz et al., 1999, 2002a), although others suggested a more important role of species identity over species richness (Crawley et al., 1999; Arenas et al., 2006). Diversity also plays a role in the stability of marine assemblages by reducing the magnitude of changes and increasing resilience of assemblages to environmental stressors (Allison, 2004; Bertocci et al., 2010). Nevertheless, species identity can be even more important than diversity, particularly when species play strong roles in structuring assemblages (Kroeker et al., 2010). Acting simultaneously, ocean warming and acidification are promoting shifts in marine ecosystem through changes in species survival (Fabry et al., 2008) and assemblage composition (Widdicombe and Spicer, 2008). Consequently, by altering disturbance regimes and resource dynamics global-change components can interact with biological invasions (Dukes and Mooney, 1999). In marine systems, NIS are an increasing concern, especially in coastal environments. In particular, macroalgae are a significant component of invasions, representing up to 40% of the total number of introduced NIS (Schaffelke et al., 2006) in a given area. Although the ecological effects of introduced macroalgae have been studied in only 6% of the species (Williams and Smith, 2007), results show mostly negative impacts or changes on the recipient communities (see review in Schaffelke and Hewitt, 2007; Thomsen et al., 2009). The spread of NIS and climate change are indeed pointed as some of the main threats to marine coastal systems (Stachowicz et al., 2002b; Hoegh-Guldberg and Bruno, 2010) that will simultaneously impact marine communities in a cumulative way over time and space. Climate change may interact with NIS and influence colonization, distribution and the main effect of marine invaders (Ruiz et al., 1999). For example, climate-change conditions might increase the invasion success of NIS, in particular by increasing their competitive ability and survival rate (Byers, 2002; Sorte et al., 2010).

There is evidence that the effects of climate change on communities might occur in two ways: 1) Via direct impact on the diversity and abundance of native species and 2) via indirect impact by increasing invasions by NIS (Sorte et al., 2010). To improve our knowledge on the role of functional diversity and global change drivers on the invasibility of marine communities, we conducted an experiment in mesocosm using synthetic assemblages of varying functional diversity that resembled macroalgal assemblages from intertidal rock pools characteristic of the western Atlantic coast of the Iberian Peninsula. There is no single driver of coastal biodiversity impacts, rather a combination of stressors. Rocky intertidal habitats are particularly vulnerable to those stressors as many of the

global change agents acts at the terrestrial-marine interface (IPCC, 2007). For example, the foraging behavior of the keystone predator *Pisaster ochraceus* was related to water temperature (Sanford, 1999) while bivalve species such as *Mytilus* spp. may be affected by both air and water temperature (Harley and Helmuth, 2003). Due to the constantly changing nature of intertidal rock pools, organisms from those systems need to be adapted to deal with those changes. The adaptation of intertidal organisms to short-term changes in their habitat makes them convenient model species to test the effects of climate change over sudden changes in environmental factors. The brown alga *Sargassum muticum* (Yendo) Fensholt 1955, considered one of the most invasive alga in Europe and North America (Norton, 1977), is a good model species for such experimental studies. *S. muticum* inhabits diverse types of habitats from intertidal channels to shallow subtidal being also present throughout the intertidal in rock pools. Native to Southeast Asia (Yendo, 1907), it was first recorded in Europe in 1973 (Critchley et al., 1983) and on the Galician coast in 1986 (see Pérez-Cirera et al., 1989) where its distribution is widespread (Incera et al., 2010). This species is now present from Portugal in the south to Norway in the North.

Ecologists aim to understand whether ecosystems will become more (or less) susceptible to invasion due to global change and its direct consequences on the impacts of invaders (Dukes and Mooney, 1999). In order to assess whether global-change drivers will induce higher susceptibility of marine communities to invasions, it first needs to be demonstrated that survivorship at early stages of invasion will be positively/negatively affected by those drivers. The goal of this study was to examine the combined effects of two climate change-related factors, temperature and CO<sub>2</sub> partial pressure, and diversity erosion in shaping the invasion success of the invasive brown macroalga *Sargassum muticum* (Yendo) Fensholt. For that, we first subjected macroalgal assemblages of varying levels of functional diversity to combined experimental conditions of increased CO<sub>2</sub> partial pressure and temperature for three weeks. After this three-week period, assemblages were seeded with propagules of the invader *S. muticum* at ambient conditions and early settlement of *S. muticum* was evaluated. The invasion of the assemblages was performed under ambient conditions due to the uncertainty of the effects of climate change on the process of propagule release. Survivorship of settled germlings was quantified under the same combined experimental conditions for a period of two weeks. Then assemblages were placed in intertidal rock-pools where *S. muticum* recruitment success and legacy effects of global change disturbance were evaluated after 6 months.

## 6.3. Methods

### 6.3.1. Laboratory mesocosm system

Our mesocosm system was set in 350-L PVC independent tanks supplied with 1 $\mu$ m sand-filtered seawater from the Ria de Vigo (42° 13' 20"N; 8° 49' 28"W, Galician coast, NW Spain), renewed once a week. Each tank contained two submersible pumps (3000 L h<sup>-1</sup>) to insure water movement and a set of 8 cool white fluorescent lamps, (F18W/840; water surface irradiance 140-150  $\mu$ E m<sup>-2</sup> s<sup>-1</sup>) with 12:12 photoperiod (light: dark, h) to resemble natural fluctuations. A nutrient solution (1 ml each nutrient solution per liter; 42.50 g L<sup>-1</sup> NaNO<sub>3</sub> and 10.75 g L<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub>) was added to each tank once a week.

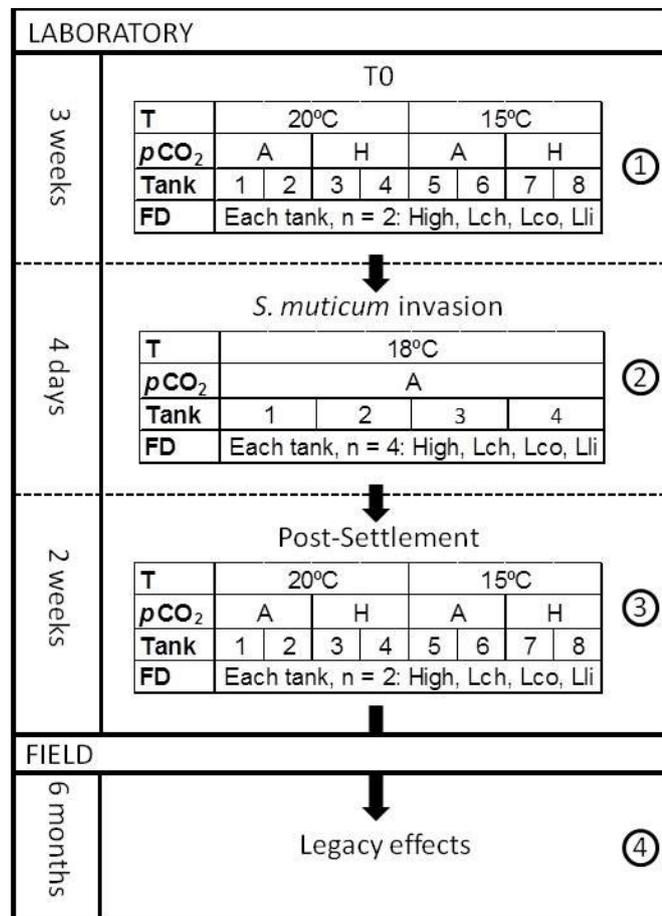
Climate change scenario was achieved by manipulating two climate-change factors, temperature and partial pressure of carbon dioxide ( $p$ CO<sub>2</sub>). The experimental set-up included two temperature levels (average ambient temperature of 15°C and increased temperature of 20°C) and two  $p$ CO<sub>2</sub> levels (ambient 390 ppmv and increased 1000 ppmv) following a 2 x 2 orthogonal design (Fig. 6.1). Average temperature of 15°C corresponds to the average temperature values recorded at the surface of Ria de Vigo for April and May between 2006 and 2010 (14.93°C  $\pm$  0.38, [www.meteogalicia.es](http://www.meteogalicia.es)). IPCC climate change scenario for 2100 (IPCC 2007) shows a possible average increase in the global temperature of 1.4 to 5.8°C and atmospheric  $p$ CO<sub>2</sub> up to 984 ppmv.

Temperature in each tank was controlled with one titanium aquarium chiller with UV sterilizer (TECO-TC15). During the experiment, ambient air from outdoors (ambient  $p$ CO<sub>2</sub> treatments) and CO<sub>2</sub>-rich air (high  $p$ CO<sub>2</sub> treatments) were continuously bubbled to the appropriate tanks. Tanks were covered with plastic caps to prevent gas exchange between them. Thus, by bubbling air with  $\approx$ 1000 ppmv, our experimental design manipulated an increase of 600 ppmv in ambient atmospheric  $p$ CO<sub>2</sub>.

### 6.3.2. Environmental parameters in tanks

Temperature was continuously monitored during the experiment using StowAway Tidbit Data Loggers (Onset Computer Corporation, Pocasset, MA, USA). Salinity and pH were measured in each tank every day around midday with a glass electrode (Cond 340i and pH 340i, respectively, WTW, a Nova Analytics Company, Germany). Total dissolved inorganic carbon (TDIC) was assessed several times a week by filtering water samples through glass microfiber filters (Whatman International Ltdl, Maidstone, UK) into serum vials that were capped without head space. Samples were analysed the same day using an Infrared Gas Analyzer (LiCOR 7000) and TDIC was then partitioned into  $p$ CO<sub>2</sub>,

bicarbonate, carbonate and total alkalinity using the program *csys.m* (Zeebe and Wolf-Gladrow, 2001).



**Fig. 6. 1.** Diagram of experimental design. T0 points out the beginning of the experiment, May 2010, under laboratory conditions, and a time sequence is represented afterwards ending on December 2010 under field conditions. T, temperature; A, ambient pCO<sub>2</sub>; H, high pCO<sub>2</sub>; FD, functional diversity; High, high functional diversity assemblages; Lch, monospecific assemblages of subcanopy species; Lco, monospecific assemblages of turf-forming species; Lli, monospecific assemblages of encrusting species.

### 6.3.3. Synthetic assemblages

Synthetic assemblages were created in order to simulate functional variability/gradients in natural macroalgal assemblages. By relating similarities in morphology and resources use (Arenas et al., 2006), three morpho-functional groups were selected *a priori* (modified from Steneck and Dethier 1994): (a) encrusting coralline species, *Lithophyllum incrustans* (hereafter 'Lli'); (b) turf-forming species from the genus *Corallina* (hereafter 'Lco'); and (c) a mixture of the two morphologically similar subcanopy species, *Chondrus crispus* and *Mastocarpus stellatus* (hereafter 'Lch'). Experimental assemblages consisted of 20 x 20 x

1 cm PVC plates with 14 cubes of rock holding the above referred morpho-functional groups and 2 PVC cubes used to hold two recruitment discs (5.11 cm<sup>2</sup>) to evaluate the early settlement of germlings. The rock and PVC cubes were framed by 4 strips of PVC. To build the experimental assemblages, small boulders bearing the selected morpho-functional groups were collected in intertidal rock-pools. Using a commercial tile cutter, rock pieces were cut into cubes (2 x 2 x 2 cm) which were held in position in the plate using fast setting underwater cement and screws. In total, we constructed 64 synthetic assemblages: 48 with one functional group (16 replicates per functional group, i.e. low diverse assemblages) and 16 with three functional groups (hereafter mentioned as high diverse assemblages, 'High'). Synthetic assemblages were randomly arranged in 8 experimental tanks with the treatment conditions orthogonally applied and were maintained in those conditions for 3 weeks (Fig. 6.1, sequence 1).

#### 6.3.4. Invasion of assemblages

Initial biomass of assemblages was different because of the identity of the species included and changed after 3 weeks of exposition to the environmental factors. To incorporate these differences in biomass as predictor in our statistical analyses of invasion resistance, we estimated biomass before the inoculation of assemblages. Biomass of turf-forming and subcanopy species was estimated following Åberg (1990), from length of primary lateral and perimeter of each alga inside the cubes. We used data of dry weight and size from 60 individuals previously collected from each functional group to construct a regression model that allowed our estimations. In the case of the encrusting species, we selected 2 x 2 cm rock pieces colonized with encrusting coralline species, oven-dried for 48h at 50°C and weighted for dry-weights. We then placed it in hydrochloric acid (0.5 M HCl) for 48h to remove the calcium carbonate. Rock pieces were then rinsed with freshwater, oven-dried for 24h and then re-weighted. The difference from the dry-weights, i.e. before and after the HCl treatment, was used to obtain the biomass of the algae at the square and the average of 40 squares allowed us to estimate the biomass in our plates.

Fertile individuals of *S. muticum* were collected from an intertidal area in the Ria de Vigo. Then, algae were rinsed with freshwater to eliminate epiphytes. Before invasion, assemblages were returned to unmanipulated conditions resembling field environment (18°C and ambient  $p\text{CO}_2$ ), and randomly distributed in 4 aquaria (Fig. 6.1, sequence 2). We took the decision to perform the invasion procedure at 18°C for 2 reasons: 1) it was an average temperature between 15 and 20° and 2) reproduction and embryo liberation is triggered by temperature in *S. muticum* (Deysner, 1984; Arenas and Fernández, 1998). We aimed for the embryos liberation by adult *S. muticum* to be as intense as possible and

thus 18°C was the temperature selected. On 26 May, assemblages were experimentally invaded by suspending fertile *S. muticum* individuals over the 4 tanks for 4 days (average  $\pm$  SD biomass of 5836.90 g FW  $\pm$  30.57). Experimental invasion occurred around the full moon because *S. muticum* has a semilunar periodicity of egg expulsion around new or full moon (Monteiro et al., 2009). For estimating propagule pressure in each aquarium, 7 additional recruitment discs (5.11 cm<sup>2</sup>) were randomly placed at the bottom of the tanks and the number of *S. muticum* germlings was counted using a stereo microscope with additional dimmed light. Propagule pressure, calculated as the number of germlings per 0.5 cm<sup>2</sup>, differed significantly among tanks with a lower propagule pressure in tank 3 (ANOVA,  $F_{3,116} = 20.69$ ,  $P < 0.001$ ; SNK-tests,  $P < 0.05$ ). Invasibility of assemblages was evaluated by counting the number of *S. muticum* germlings in the two recruitment discs installed in each assemblage.

After experimental invasion, assemblages were placed back to the 8 tanks where the experimental conditions were restored for another 2 weeks (Fig. 6.1, sequence 3). Initial success of the invasion was measured as the initial percentage of survivorship and length of settled germlings. Percentage of survivorship was calculated 3 and 10 days after experimental conditions of temperature and  $p\text{CO}_2$  were restored and length of settlers (mm) was measured at day 10. Percentage of survivorship was calculated as  $N_t/N_0 \times 100$ , where  $N_t$  is the number of surviving settlers at day 3 and 10, for  $t = 3$  and  $t = 10$ , respectively, and  $N_0$  is the number of settled germlings just before experimental conditions were restored.

### 6.3.5. Development of assemblages in the field

At the end of June 2010, assemblages were placed in a rocky shore in the Ria de Vigo and were kept until December 2010 (Fig. 6.1, sequence 4). Development of assemblages and recruitment of *S. muticum* were monitored in the field by measuring primary and secondary algal cover (cover of algae on the substratum and overstory canopy, respectively) and number and length (cm) of individuals of *S. muticum*. Primary algal cover was estimated by visually dividing each rock quadrat in 4 and attributing a score from 0 to 4 to the functional groups present, and adding up the 16 estimates (Dethier et al., 1993). Secondary algal percentage cover was determined with a sampling frame (8 x 8 cm) divided by monofilament line into 2 x 2 cm quadrats.

### 6.3.6. Statistical analyses

Differences in  $p\text{CO}_2$  levels among treatments were examined using a Repeated measures ANOVA (rmANOVA) with Temperature and  $p\text{CO}_2$  as fixed factors, Tank as a random

factor and Time as within-subjects variable. The rm-ANOVA assumption of sphericity was evaluated using Mauchly's criterion.

The number of germlings of *S. muticum* settled in each assemblage at time 0, i.e. immediately after invasion under unmanipulated conditions, was analysed by a two-way ANCOVA with Functional diversity as a fixed factor, Tank as a random factor and Biomass of assemblage as a covariate. First we tested for homogeneity among slopes by including the interaction term 'covariate x main factors' in the model. With a non-significant interaction term, homogeneity of slopes can be assumed and the model can be re-run, excluding the interaction (McDonald, 2009). Post-hoc pair-wise comparisons were performed with Tukey's HSD test.

Prior to placement assemblages back into experimental conditions, we have analysed if there was a difference in the number of germlings settled between temperature and  $p\text{CO}_2$  treatments. We performed a 4-way analyses of variance ( $n = 2$ ) with Temperature,  $p\text{CO}_2$  and Functional diversity as fixed factors and Tank as random factor nested within Temperature and  $p\text{CO}_2$ . Then, percentage of survivorship at 3 and 10 days and length of *S. muticum* settled germlings were analysed using a 3-way analyses of variance ( $n = 4$ ). Temperature (low and high),  $p\text{CO}_2$  (ambient and high) and Functional diversity (High, Lch, Lco, Lli) were fixed factors. Previously we had performed a 4-way analyses of variance incorporating Tank as an additional random factor (2 levels), nested within interaction Temperature x  $p\text{CO}_2$  ( $n = 2$ ). There was no tank effect, thus we pooled data from tanks with the same treatment. Post-hoc pair-wise comparisons were performed with Student-Newman-Keuls (SNK) tests.

Recruitment data from field conditions were analysed with a 3-way analysis of variance with Temperature,  $p\text{CO}_2$ , and Functional diversity (3 levels: High, Lch, Lco) as main fixed factors ( $n = 4$ ). We used 3 levels of functional diversity because many monospecific plates of encrusting species were lost in the field. In 8 occasions there was one replicate missing, so the design was balanced by adding the averaged values of the other three replicates to the model and recalculating the  $F$ -values (see Underwood, 1997).

When significant, Functional diversity SS from the ANOVA was partitioned into two orthogonal components in order to separate the effects of species richness and species identity (see McDonald, 2009 for details). Prior to all analyses, the homogeneity of variances was examined using Cochran's  $C$ -test. Data were transformed when necessary and in those cases in which transformation did not remove heterogeneity, the level of significance applied was  $P = 0.01$ .

Changes in structure of assemblages due to experimental treatments were tested with a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001), with Temperature,  $p\text{CO}_2$ , and Functional diversity (High, Lch, Lco, Lli) as main fixed factors ( $n = 4$ ). Due to the fact that PERMANOVA can be applied to unbalanced datasets resulting from missing data (PERMANOVA unbalanced designs, Anderson et al. 2008) we were able to re-incorporate Lli data in our analysis. Pair-wise comparisons were done when significant differences were detected ( $P < 0.05$ ). SIMPER analyses were done to identify the species that contributed most to dissimilarities between treatments.

Multivariate analysis were conducted with PRIMER v6 (Clarke and Gorley, 2006) and univariate analyses were performed with SPSS (PASW 18) statistical software and WinGMAV (<http://sydney.edu.au/science/bio/eicc>).

## 6.4. Results

### 6.4.1. Environmental parameters in tanks

Mean pH and salinity values in tanks over the post-invasion experimental period were  $8.20 (\pm 0.01)$ , all values presented as mean  $\pm$  SE) and  $35.34\text{‰} (\pm 0.09)$ , respectively. Temperature was maintained constant with average values of  $19.87 (\pm 0.06)$  and  $15.16^\circ\text{C} (\pm 0.30)$  for high and low treatments, respectively. Partial pressure of  $\text{CO}_2$  differed between treatments although such differences were marginally non-significant ( $F_{1,3} = 6.17$ ,  $P = 0.069$ ). Marginal non-significance detected might be due to high, somewhat predictable, variability in  $p\text{CO}_2$  concentrations due to interactions between physico-chemical and biological processes (see Morris and Taylor, 1983). Values of  $p\text{CO}_2$  ranged from 301.56 to 513.37 ppmv for ambient and high  $p\text{CO}_2$  levels, respectively.

### 6.4.2. Resistance to invasion and invasiveness of *S. muticum*

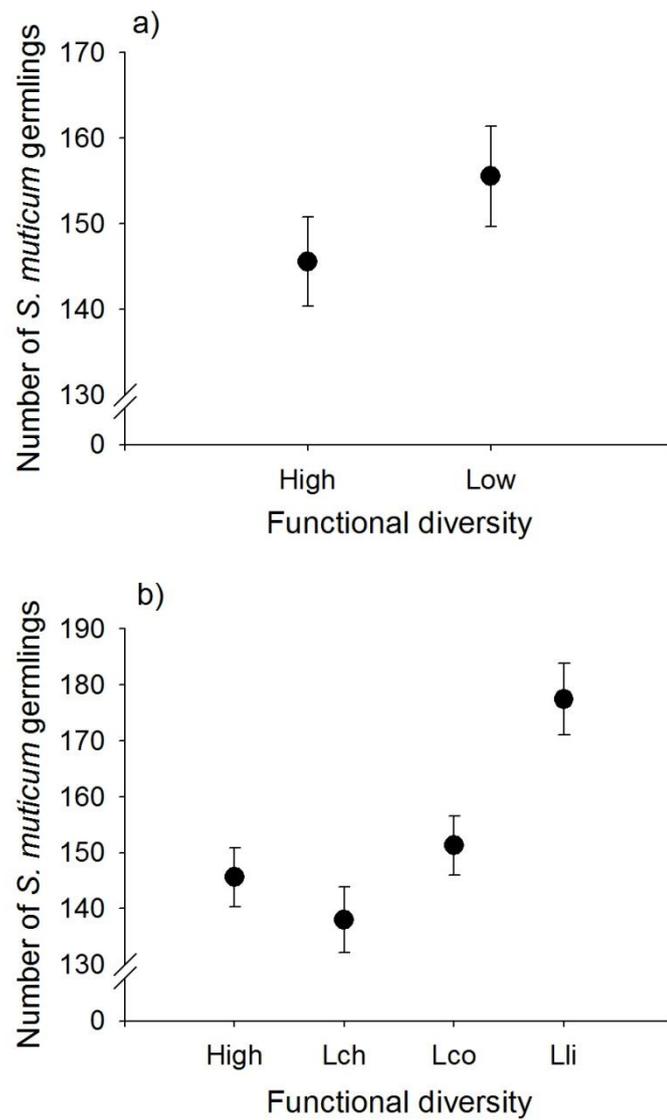
As expected, biomass of assemblages significantly affected the settlement of *S. muticum* germlings (ANCOVA,  $P = 0.032$ ; Table 6.1). The number of germlings also differed significantly depending on functional diversity (ANCOVA,  $P = 0.018$ ; Table 6.1). Both richness and identity effects contributed significantly for this result (Table 6.1). The number of *S. muticum* germlings settled was smaller on high diverse assemblages compared to low diverse assemblages (Richness effect; Fig. 6.2a), mostly driven by a significantly larger number of germlings in monospecific assemblages of encrusting species (Identity effect; Fig. 6.2b). Additionally, we observed no significant differences in the number of germlings between temperature (ANOVA,  $F_{1,4} = 1.63$ ,  $P = 0.271$ ) and  $p\text{CO}_2$

(ANOVA,  $F_{1,4} = 1.34$ ,  $P = 0.312$ ) treatments prior to placement assemblages back into experimental conditions.

**Table 6. 1.** Two-way analysis of covariance (ANCOVA) for the number of *Sargassum muticum* germlings. Functional diversity was a fixed factor and Tank was a random factor, Biomass was a covariate ( $n = 4$ ). Homogeneous variances.

Source	df	Adj MS	F	P
Biomass	1	2059.1	4.87	0.032
Functional diversity (FD)	3	2696.0	5.10	0.018
Richness effect	1	2343.4	5.54	0.023
Identity effect	2	2872.25	6.80	0.002
Tank	3	9570.0	17.69	<0.0001
FD x Tank	9	546.1	1.29	0.267
Residual	47	422.7		

Survivorship of *S. muticum* settlers after 3 days was affected interactively by temperature and  $p\text{CO}_2$  (ANOVA,  $P < 0.05$ ; Table 6.2a). An increase in temperature led to a decrease in the survivorship of *S. muticum* settlers at elevated  $p\text{CO}_2$ , with no significant differences observed at ambient  $p\text{CO}_2$  (SNK-tests,  $P < 0.05$ ; Fig. 6.3a). At low temperature an increase in  $p\text{CO}_2$  led to a significant increase in survivorship (SNK-tests,  $P < 0.01$ ; Fig. 6.3a). Survivorship 10 days after settlement was still affected interactively by temperature and  $p\text{CO}_2$  and there was an additional influence of functional diversity (ANOVA,  $P = 0.007$  and  $P = 0.033$ , respectively; Table 6.2b). Contrasting with previous results from survivorship after 3 days, an increase in temperature led to a decrease in survivorship at ambient  $p\text{CO}_2$  whereas at high temperature treatments survivorship of *S. muticum* germlings was greater at high  $p\text{CO}_2$  (Fig. 6.3b). Survivorship after 10 days was influenced by richness effects rather than identity effects (ANOVA,  $P = 0.006$ ; Table 6.2b). The highest survivorship of settlers was observed in assemblages of high functional diversity (High FD > Lch = Lco with  $65.91 \pm 3.09\%$ ,  $58.02 \pm 3.45\%$  and  $56.53 \pm 2.71\%$ , respectively, while Lli with  $60.17 \pm 2.73\%$  showed similar survivorship with all assemblages).

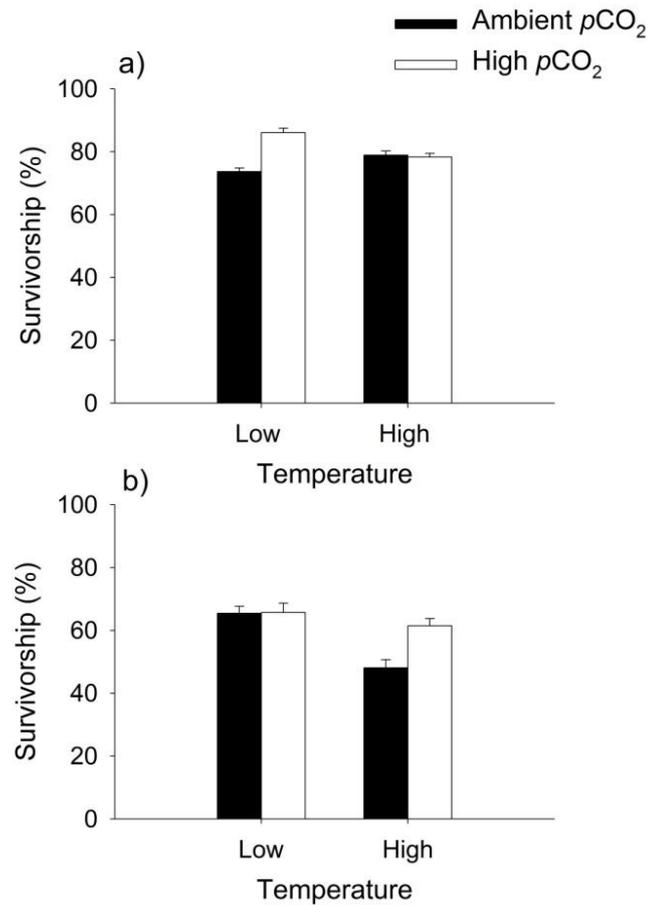


**Fig. 6. 2.** *Sargassum muticum* settled germlings (per 0.5 cm<sup>2</sup>). a) Richness effect; adjusted mean (+SE, n = 16 and n = 48, for high and low functional diversity, respectively); b) Identity effect; adjusted mean (+SE, n = 16). See Fig. 6.1 for functional diversity abbreviations.

**Table 6. 2.** Four-way ANOVA for survivorship of *Sargassum muticum* settlers at a) 3 days and b) 10 days after experimental conditions were imposed (n = 4). Temperature,  $p\text{CO}_2$  and Functional diversity were fixed factors and Tank was a random factor. Homogeneous variances.

Source	df	a) 3 days			b) 10 days		
		MS	F	P	MS	F	P
Temperature (T)	1	25.547	0.26	0.615	1860.519	21.75	<0.0001
$p\text{CO}_2$	1	554.865	5.55	0.023	740.368	8.65	0.005
Functional diversity (FD)	3	70.429	0.70	0.554	271.142	3.17	0.033
Richness effect	1	-	-	-	706.315	8.26	0.006
Identity effect	2	-	-	-	53.555	0.63	0.539
T x $p\text{CO}_2$	1	677.201	6.78	0.012	688.389	8.05	0.007
T x FD	3	223.188	2.23	0.096	56.226	0.66	0.582
$p\text{CO}_2$ x FD	3	201.558	2.02	0.124	224.321	2.62	0.061
T x $p\text{CO}_2$ x FD	3	163.206	1.63	0.195	153.403	1.79	0.161
Residual	48	99.922			85.546		

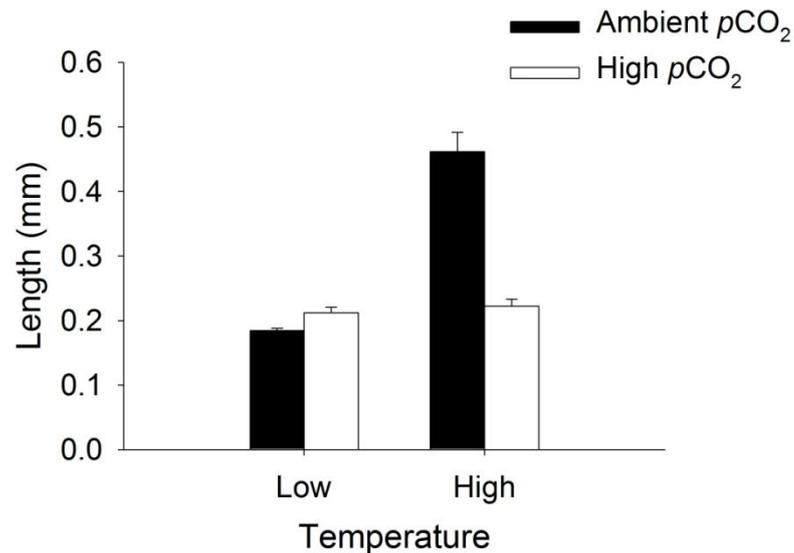
The length of *S. muticum* settlers was influenced at different degree by temperature and  $p\text{CO}_2$  (ANOVA,  $P = 0.003$ ; Table 6.3). Under ambient  $p\text{CO}_2$ , temperature had a positive effect on the length of *S. muticum* germlings. Settlers were longer at ambient  $p\text{CO}_2$  and high temperature and shorter at ambient  $p\text{CO}_2$  and low temperature (SNK-tests,  $P < 0.05$ ; Fig. 6.4). In contrast, there was no effect of temperature under high  $p\text{CO}_2$  (SNK-tests,  $P < 0.05$ ; Fig. 6.4).



**Fig. 6. 3.** Mean (+SE, n = 16) percentage survivorship of *Sargassum muticum* settled germlings. Interaction term Temperature x pCO<sub>2</sub> at a) 3 days after experimental conditions were imposed; b) 10 days after experimental conditions were imposed.

**Table 6. 3.** Four-way analysis of variance (ANOVA) for length (mm) of *Sargassum muticum* settlers (n = 4). Temperature, pCO<sub>2</sub> and Functional Diversity were fixed factors and Tank was a random factor. Variances heterogeneous (Cochran's test, C = 0.2382, P < 0.01). Significance level at P = 0.01.

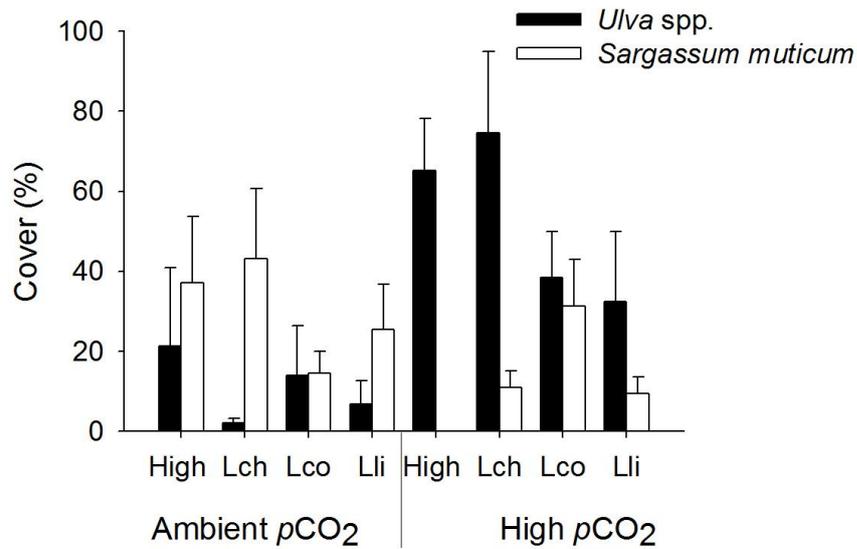
Source	df	MS	F	P
Temperature (T)	1	0.331	95.00	<0.0001
pCO <sub>2</sub>	1	0.176	51.29	<0.0001
Functional diversity (FD)	3	0.006	1.89	0.144
T x pCO <sub>2</sub>	1	0.286	82.24	<0.0001
T x FD	3	0.010	3.00	0.039
pCO <sub>2</sub> x FD	3	0.010	2.83	0.048
T x pCO <sub>2</sub> x FD	3	0.004	1.07	0.370
Residual	48	0.003		



**Fig. 6. 4.** Mean (+SE,  $n = 16$ ) length (mm) of *Sargassum muticum* settled germlings. Interaction term Temperature  $\times$   $p\text{CO}_2$ .

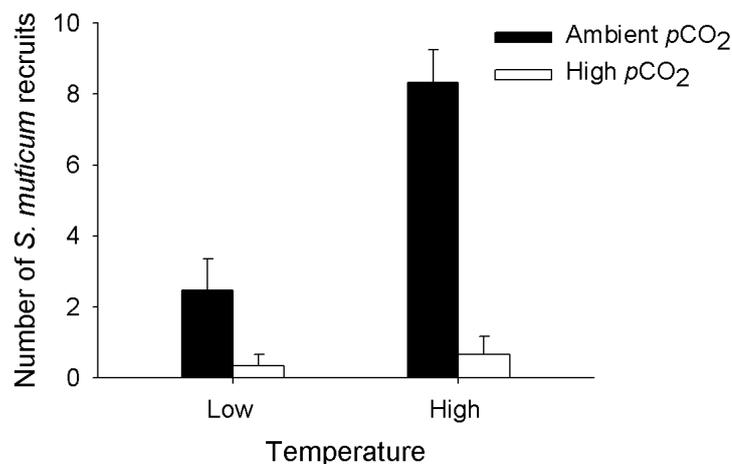
#### 6.4.3. Development of assemblages in the field

Six months after the experimental invasion, the structure of assemblages (i.e. identity and abundance of species) in field conditions was interactively affected by previous experimental conditions (PERMANOVA Temperature  $\times$   $p\text{CO}_2$ ,  $Pseudo-F_{1,34} = 3.70$ ;  $P = 0.010$ ). At ambient  $p\text{CO}_2$  there were significant differences (pair-wise tests,  $P = 0.018$ ) in the structure of assemblages between temperature treatments, not observed at high  $p\text{CO}_2$  (pair-wise tests,  $P = 0.071$ ). Functional diversity also shaped the structure of assemblages, but interactively with  $p\text{CO}_2$  (PERMANOVA  $p\text{CO}_2 \times$  Functional diversity,  $Pseudo-F_{3,34} = 2.22$ ;  $P = 0.015$ ). At high  $p\text{CO}_2$ , the structure of assemblages was similar between functional diversity treatments, with the exception of significant dissimilarities between Lch and Lco (pair-wise tests,  $P = 0.028$ ). In contrast, at ambient  $p\text{CO}_2$  structure of assemblages differed among functional diversity treatments, with the exception of high diverse assemblages and Lli (pair-wise tests,  $P = 0.101$ ). The observed differences were mainly due to *Ulva* spp. and/or *S. muticum*. In fact, *S. muticum* together with the green alga *Ulva* spp. accounted for more than 60% of observed dissimilarity among assemblages of different functional diversity. Invasion in the field by ephemeral green algae was linked to those treatments that underwent high  $p\text{CO}_2$  levels (PERMANOVA,  $Pseudo-F_{1,34} = 5.976$ ;  $P = 0.0001$ ; Fig. 6.5). The invasive *S. muticum* showed an opposite pattern, with greater percentage cover at ambient  $p\text{CO}_2$  (not significant in Lco assemblages). At high  $p\text{CO}_2$ , no individuals of *S. muticum* were recorded in high diverse assemblages (Fig. 6.5).



**Fig. 6. 5.** Mean (+SE, n = 8) percentage cover of *Ulva* spp. and *Sargassum muticum* within Functional diversity in each pCO<sub>2</sub> experimental treatments. See Fig. 6.1 for functional diversity abbreviations.

In addition, recruitment of *S. muticum* in assemblages was affected by environmental conditions experienced in laboratory. Assemblages that were exposed to an increase of temperature at ambient pCO<sub>2</sub> (ANOVA Temperature x pCO<sub>2</sub>,  $F_{1,28} = 13.52$ ;  $P = 0.001$ ), had larger number of recruits, whereas assemblages that experienced high pCO<sub>2</sub> had smaller number of recruits, independently of temperature (SNK-tests,  $P < 0.05$ ; Fig. 6.6). The pCO<sub>2</sub> also affected the length of *S. muticum* recruits (ANOVA,  $F_{1,28} = 39.68$ ;  $P < 0.0001$ ), with longer recruits at ambient pCO<sub>2</sub> ( $3.76 \pm 0.42$  and  $0.31 \pm 0.23$  cm for ambient and high pCO<sub>2</sub>, respectively).



**Fig. 6. 6.** Mean (+SE, n = 16) number of *Sargassum muticum* recruits (64 cm<sup>2</sup>) at each temperature and pCO<sub>2</sub> treatment conditions.

## 6.5. Discussion

Whether or not interactions among climate-related factors will enhance biological invasions is still rather unknown. To our knowledge, the present study is the first to experimentally attempt to identify the interactive effects of enhanced temperature and  $p\text{CO}_2$  on invasibility of marine macroalgal assemblages of different functional diversity. The present study suggested that functional diversity, both richness and identity, was a key driver in the settlement success of *Sargassum muticum* germlings. Additionally, results showed that survival and growth of the invader were conditioned by environmental conditions. High temperature favoured performance of *S. muticum*, but only under the current  $p\text{CO}_2$  levels. Future climatic scenarios of increasing temperature and  $p\text{CO}_2$  would not be beneficial to *S. muticum*.

Our results indicated higher resistance to invader settlement by high diverse assemblages (richness effect), in accordance with Elton's theory. However, individual functional groups also performed differently, suggesting that idiosyncratic traits of each group played an important role over settlement (identity effect). Resistance to invasion by high diverse assemblages was not higher than the best resistant monospecific assemblages (i.e. subcanopy species), suggesting that sampling effect was the mechanism behind the richness effect (Cardinale et al., 2006). Recent experimental studies have also revealed the key role of species identity of macroalgal assemblages in the process of invasion (Crawley et al., 1999; Arenas et al., 2006) and settlement of fucoids (Schiel and Lilley, 2011). In this study, the largest number of *S. muticum* settled germlings was found in monospecific assemblages of encrusting species, a suitable substrate for settlement of new colonizers (Arenas et al., 2006) as well as adult individuals which is able to tolerate overgrowth (Airoldi, 2000). The establishment and spread of *S. muticum* is indeed controlled by space availability (Deysner and Norton, 1982; Fernández, 1999), thus the smallest number of settled germlings in monospecific assemblages of subcanopy species could be explained by the presence of subcanopy fronds, a physical barrier between propagules and hard substrate (Deysner and Norton, 1982).

The relationship between diversity and invasibility varied throughout the invasion process. Functional diversity had a negative effect on early invasibility, no effect on the survivorship of *S. muticum* at 3 days and described a positive effect on the survivorship of *S. muticum* at 10 days. These findings are related to those found by White and Shurin (2007). Using synthetic assemblages and *S. muticum* as the invader species, these authors found a positive effect of diversity on the initial cover of *Sargassum* juveniles 47 days after the invasion. Sequential mortality was also greater in high diverse assemblages (White and

Shurin, 2007). Functional diversity of macroalgal assemblages has been found to affect availability of key resources, i.e., space and light (see Arenas et al., 2006), which are related to survivorship and growth of furoid germlings (Kendrick, 1994). Here, invasion success remained higher in lower diversity assemblages, even though survivorship in high diverse assemblages was highest, suggesting a possible shift in the effects of diversity with development stage (White and Shurin, 2007).

Survivorship in early post-settlement phases is critical to the establishment success of macroalgae populations (Vadas Sr. et al., 1992), as germlings are more susceptible to biological and environmental stress than adults (Schiel and Foster, 2006). In this study, the interactive effects of temperature and  $p\text{CO}_2$  on the survivorship of settlers varied with time. Increased  $p\text{CO}_2$  was beneficial to *S. muticum* short-time survivorship, particularly under low temperature. After 3 days, a positive effect of temperature was observed under ambient  $p\text{CO}_2$  while 10 days after experimental conditions were imposed survivorship was the lowest under that same treatment. These results suggest possible ontogenic-specific differences in susceptibility to mortality (Vadas Sr. et al., 1992). Also, observed lower survivorship after 10 days under high temperature and ambient  $p\text{CO}_2$  could be related to higher growth under those conditions. Density-dependent survivorship and growth have been reported for *S. muticum* recruits (Kendrick, 1994), which could explain our results. It has been suggested that whether interactions between germlings describe competition or facilitation depends on the environmental conditions (Steen and Scrosati, 2004). Studies with furoid germlings have demonstrated stronger intraspecific competition at high temperatures under nutrient enrichment conditions (Steen, 2003; Steen and Scrosati, 2004). Overall, temperature has been positively related to *S. muticum* performance (Norton, 1977; Deysher, 1984). Nevertheless, our results suggest that the magnitude of the temperature effects on the length of *S. muticum* settlers would be considerably lower under high  $p\text{CO}_2$  levels, highlighting interactive effects between manipulated global change drivers.

Additionally, the structure of assemblages in the field latter on was influenced by environmental conditions experienced in laboratory, invasion success and functional diversity of assemblages. Response of macroalgal assemblages to environmental stressors depends strongly on the type and magnitude of the stressor as well as composition of assemblages (Allison, 2004). Assemblages from high  $p\text{CO}_2$  were more homogeneous than those maintained under ambient  $p\text{CO}_2$  6 months after experiencing environmental stress in the laboratory. The persistent shifts in macroalgal assemblages observed 6 months after the source of disturbance disappeared suggests legacy effects of

disturbance by global change drivers. The observed differences were mainly attributed to the dominance of the invader, *S. muticum*, and green ephemerals. *S. muticum* was the most abundant species in assemblages under ambient  $p\text{CO}_2$  whereas *Ulva* spp. was the dominant species in assemblages that experienced high  $p\text{CO}_2$  in laboratory. Green opportunistic algae colonized those assemblages that experienced more stress, i.e., combined increase of temperature and  $p\text{CO}_2$ , and were not successfully colonized by the invader in the laboratory. As many ephemeral species, the opportunistic *Ulva* species, require availability of resources (i.e. space and light) to be competitively superior to perennial or pseudoperennial species (Sousa, 1979). The opportunistic nature in combination with the perennial persistence of *S. muticum* is an unusual feature in canopy forming alga (Rueness, 1989). It is thus very likely that survival and growth of the invader set the stage for posterior competitive interactions (Steen and Scrosati, 2004) and mediated the response of assemblages (Byers, 2002; Sorte et al., 2011). For example, a recent study has described a synergistic positive interaction between future  $\text{CO}_2$  and temperature levels on the abundance of non-calcareous algal turfs enhancing the probability of phase shifts in kelp forests (Connell and Russell, 2010). It would be, however, interestingly to investigate for how long legacy effects of disturbance by global change drivers would persist after disturbance and its impacts. For instance, legacy effects of canopy disturbance have been suggested to still affect macroalgal community structure and primary productivity 8 years after disturbance (Schiel and Lilley, 2011; Tait and Schiel, 2011).

Evidence suggests that contrary to calcified algae (e.g. Martin and Gattuso, 2009) non-calcareous algae can increase in growth and abundance with  $\text{CO}_2$  enrichment (Hall-Spencer et al., 2008; Porzio et al., 2011; Johnson et al., 2012). Previously cited research has been conducted over natural pH gradients, i.e. volcanic vents, not taking into consideration effects over reproductive life cycle of species that recruit into the acidified areas from nearby populations (Porzio et al., 2011). In this context, studies on the microscopic stages of the reproductive life cycle have been mostly overlooked (but see Roleda et al., 2012). The present study highlighted the negative effect of ocean acidification, alone and in combination with temperature, in the recruitment success of the brown canopy macroalga *S. muticum*. When analysed independently we observed a highly positive effect of temperature on invasion success of *S. muticum*, highlighting likely inaccuracies when addressing environmental stressors independently. In addition, many species interactions are under strong abiotic control (O'Connor, 2009), thus it would be interesting to assess the effects of changing conditions on trophic interactions.

In conclusion, we addressed the potential for climate change to facilitate invasions and precipitate shifts in structure of marine macroalgal assemblages by testing effects of increasing temperature and  $p\text{CO}_2$ . This study demonstrated that ocean warming can facilitate invasions. The survival and growth of early life stages of the non-indigenous *S. muticum* increased under increasing temperature but only at current  $p\text{CO}_2$  levels. High  $\text{CO}_2$  conditions seemed to reduce the invasion success of *S. muticum*. Thus, the effect of global environmental change will vary depending on the relative intensity of change of the different environmental factors involved. Our results highlighted the need to consider multiple stressors in combination when addressing the impact of invasions in a climate change scenario.

## **6.6. Acknowledgments**

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## **Part V**

### **General discussion and final remarks**





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## Chapter 7

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### General Discussion

*“That is the essence of science:  
ask an impertinent question,  
and you are on your way to the pertinent answer”*

Jacob Bronowski

**B**iodiversity of natural systems is changing worldwide due to species loss caused by anthropogenic driven extinctions and species gain through intentional and accidental introductions (Sax and Gaines, 2003). In particular, the spread and invasion of non-indigenous species is becoming a serious dangerous reality. Understanding the invasion process can help to identify the role and consequences of NIS to the ecosystems and biodiversity. The aim of this thesis was to better understand the process of invasion of macroalgal assemblages, the factors affecting the distinct invasion stages and possible NIS impacts over native assemblages.

### 7.1. Understanding macroalgal invasions

Marine macroalgae, particularly those in the Order Fucales, as well as sessile animals, have a profound importance in structuring middle to low intertidal zones of temperate shores throughout the world (Chapman, 1995; Dayton, 1975; Schonbeck and Norton, 1978). Although there is increasing evidence supporting the magnitude and potential impacts of marine macroalgal invasions, the mechanisms behind macroalgal invasion success are still poorly understood (Inderjit et al., 2006; Schaffelke et al., 2006).

After introduction, a successful NIS must survive adult hood and then establish a sustainable population. A NIS is then considered an invasive species due to its dominance and spread rate in the new habitat. The first report of *S. muticum* on the Galician coast dates from 1986 (Péres-Cirera et al., 1989), on the northern Portuguese coast dates from 1989 and now it can be observed until the southern Portuguese coast (Engelen et al., 2008; Rull Lluçh et al., 1994). In northern Portugal and Galicia, *S. muticum* established dominant populations and is considered an invasive species (Engelen and Santos, 2009b; Incera et al., 2011). Limited impacts on macroalgal assemblages have been registered for this species in the study area (Olabarria et al., 2009), although in the SW Portuguese coast it has been suggested to displace the native *Cystoseira humilis* (Engelen and Santos, 2009a).

Throughout this thesis, results showed that the invasion of *Sargassum muticum* is a complex phenomenon. My research demonstrated that assemblages vary continuously in their susceptibility to invasion over the different stages of invasion (D'Antonio et al., 2001; Davis et al., 2000) and that interaction with environmental factors that co-vary with species diversity may better explain invasion success (see Eschtruth and Battles, 2009). Results highlighted the fact that the dynamics and structure of the recipient assemblage are key components of an invasion success. Moreover, the role of propagule pressure in

the invasion process revealed greater importance over the settlement period (Clark and Johnston, 2009) while recruitment and colonisation success of *S. muticum* were mainly affected by resources availability, namely light. Different stages of *S. muticum* invasion comprised specific regulation of top-down and bottom-up forces, reinforcing previous results (White and Shurin, 2007). Thus, resource availability may largely explain the invasibility of a habitat, in agreement with the general Elton (1958) theory and other studies (Britton-Simmons, 2006; Davis et al., 2000; Stachowicz et al., 1999).

It seems clear, therefore, that the inherent properties of the introduced species throughout the invasion process and the interaction with the recipient assemblage after introduction determine the invasion success. Moreover, different mechanisms may act in different stages of the invasion process. A review on macroalgal invasions showed that a given species might have very different impacts in different localities (Johnson and Chapman, 2007). Thus, the knowledge of the mechanisms that promote community structure within invaded systems, e.g. interspecific competition, productivity, or environmental heterogeneity (Moore et al., 2001), across a variety of scales (Johnson and Chapman, 2007) is critical to fully understand the process of invasion. This fact anticipates the difficulty in establishing a general theory of invasion. Mostly based on a case-by-case basis, a recent review identified 20 hypotheses linking biotic interactions to NIS invasions (Mitchell et al., 2006).

The consequences of competition between NIS and resident species for invasion success are the basis for most of those hypotheses. In particular, strong competition is expected to arise between functionally similar native and non-indigenous species (Dudgeon et al., 1999). Thus, because NIS in introduced habitats can be highly abundant, many NIS have been wrongly considered a superior competitor (Levine et al., 2003). Instead, mechanisms of community assembly other than competition may help to understand the process of invasion and why a species may be considered an invasive species in one habitat and not in another (Firn et al., 2010; Moore et al., 2001). The entire process of invasion is suggested to be influenced by both niche and fitness differences (Chesson, 2000; MacDougall et al., 2009). Both NIS and their native competitors are influenced by abiotic factors and interactions with resources, competitors, predators, diseases, etc. in the local environment. Understanding the dominance of a species, therefore, requires disentangling the mechanisms behind the abundance of the dominant and those limiting the occurrence of the other species (MacDougall and Turkington, 2005).

My research highlighted the fact that the invader *S. muticum* did not seem to be competitively superior to the native *C. humilis*, which points to mechanisms other than competition to be implicated in the current distribution of the species.

## 7.2. Implications for Biodiversity-Ecosystem Function research

Currently, there is growing interest in determining how human-induced change in ecosystem structure will affect ecosystem services (Worm et al., 2006), increasing the ecological research in this particular issue (see Cardinale et al., 2012 for a recent review). The functioning of an ecosystem is governed by the functional traits of individuals, the distribution and abundance of these individuals, and their biological activity (Naeem and Wright, 2003). Although macroalgae make up a small proportion of ocean primary production, they are the dominant primary producers of rocky shore ecosystems (Mann, 1973), providing an essential ecological function for aquatic life.

Marine macroalgae are characterized by a range of traits including competitive ability, resistance to herbivores, production, and nutrient uptake (Littler and Littler, 1980). Morphological traits such as growth form and thickness and physiological traits such as alternative carbon fixation pathways and nutrient uptake and storage interact to determine resource allocation to competitive interactions or to other plant functions (Carpenter, 1990). For example, a previous study revealed that the native *C. humilis* used water-borne cues to produce induced-defense responses in the presence of grazed *S. muticum* while *S. muticum* showed no differences in resource allocation (Yun et al., 2012). Competition may influence individual survival and reproduction (fitness) with potential evolutionary consequences. Thus, an understanding of how and why the species differ in their competitive abilities is critical to the study of relative abundance of species within assemblages (Goldberg, 1987).

It has been widely documented that there is a current global decline in rocky shore habitat-forming macroalgal species from a wide range of stressors (Airoldi and Beck, 2007). Invasion by foundation species, such as large macroalgae, are considered particularly important and worrying because they may alter both ecosystem structure and function of marine systems (Schaffelke et al., 2006). Several studies have attempted to test the impacts of biodiversity loss on ecosystem function in rocky shore systems (Bruno et al., 2005; Schiel, 2006; Stachowicz et al., 2008), although no study has examined the effect of NIS addition to the BEF of marine macroalgal assemblages. The introduction of *S. muticum* to the recipient assemblages may be seen as an addition of a canopy functional group to the overall system, with a subsequent increase in primary production.

As expected, results showed that the presence of *S. muticum* lead to an increased in assemblages primary productivity, but only due to an increase in biomass. A previous study of the effects of canopy loss on ecosystem functioning in macroalgal assemblages, however, revealed that biomass alone did not entirely explain the loss of productivity and that certain taxa were disproportionately important (Tait and Schiel, 2011), which was not observed in the present study for *S. muticum*. In the study area, the maximum size of *S. muticum* surpasses the size of most of the native species inhabiting intertidal rockpools. Moreover, as observed in the present study it attained a very high productivity on an area basis, which might suggest sampling effects in our results. The sampling effect of diversity is a composition effect that results from a greater chance of including a highly productive species in high-diversity treatments (Huston, 1997). Accordingly, it has been suggested that native species with a long history of coevolution may influence ecosystem processes through resource use efficiency whereas NIS effects on the recipient assemblage occurs through sampling effects (Ruesink et al., 2006).

The consequences of invasion for the invaded assemblages, especially with regard to their functioning, have been rarely considered (Pfisterer et al., 2004). My research with experimental macroalgal assemblages has revealed significant negative impacts linked to the presence of an invader on ecosystem properties and predictability. The variability of the ecosystem function response found in invaded assemblages suggests that invaded assemblages' dynamics are less predictable than native dynamics. Most evidence suggests that the impact of *S. muticum* was related to its high spatial variability (Baer and Stengel, 2010), productivity and dominance in the invaded assemblages, which varied drastically between seasons. Further interactions between habitat-modifying species can decrease predictability of community-level effects of an invasion, particularly if invasive species show extremely variable cycles over time (Ward and Ricciardi, 2010). *S. muticum* is generally observed in highly dense monospecific or mixed-assemblages. The responses of *S. muticum* to high density stands have been suggested to allow the establishment of dense populations with high persistence and resistance to colonization by other species (Arenas et al., 2002).

Theory predicts that the temporal or spatial variance of ecosystem properties decreases with increasing biodiversity and complexity (France and Duffy, 2006; Yachi and Loreau, 1999). Our results highlight the fact that the introduction of *S. muticum* to a system, enhancing the complexity of the system, was not followed by an increase in the function predictability of the system. Thus, our results support the concept that invaders threaten biodiversity values.

### 7.3. Consequences of future climate change

Currently, research is mainly focus on examining the effects of single global change agents and research on the interactive effects of multiple agents is mostly lacking. Dukes and Mooney (1999) emphasized the global dimensions of biological invasions and there is increasing concern that the combined effects of global change and species introductions could interact synergistically to accelerate biodiversity loss with consequent biotic homogenization (Galil, 2007; Olden and Poff, 2004).

In this context, this research provides an integration of two major stressors of ecosystems, namely climate change and invasive species, by considering climate change scenarios, recruitment of NIS and inter-specific biotic interactions. A mesocosm experiment was used to test how increases in temperature and CO<sub>2</sub> partial pressure ( $p\text{CO}_2$ ) interacted with functional diversity of resident macroalgal assemblages and affect the invasion success of *S. muticum*. Evidence from our results suggest that the outcomes of invasion may change at multiple points in the life cycle under higher CO<sub>2</sub> and higher-temperature conditions, in addition to support an important role for intact, functionally diverse assemblages in limiting invader colonization. Overall, final invasion success of *S. muticum* was enhanced by high temperature but only under current  $p\text{CO}_2$  conditions.

However, how this result will be reflected in the distribution of the invasive species remains unknown. Global change effects do not and will not mean the same thing everywhere on the planet and the changes from one area to the other are not well understood. Also, it remains unknown whether non native species life-history traits will facilitate their success in the face of future climate change scenarios. Non-indigenous species like *S. muticum* have already demonstrated their ability to persist in novel and different habitats across their new areas of distribution (Schlaepfer et al., 2005). Although adaption to local conditions may be lower in NIS than in native species, their ability to tolerate a wide range of climatic conditions may be higher which may favour NIS colonisation success.

Additionally, many factors other than climate (e.g. biotic interactions, evolutionary change, and dispersal ability) play an important role determining species distributions (Davis et al., 1998a; Davis et al., 1998b). Thus, because climate change may not only affect species performances but also species interactions, there will always be limitations to predictions of species distribution based only on predicted climate change scenarios (Pearson and Dawson, 2003; Tylianakis et al., 2008). Understanding the relationship between population dynamics (an ecological entity) and the fitness of individuals (evolutionary

entity) may be particularly important to interpret the outcome of species distribution and dominance (Strauss et al., 2006).

#### 7.4. Conclusions and future research

Despite the considerable interest on the problematic of invasive species (Grosholz, 2002), relatively few studies have attempted to examine the overall process of invasion. Several studies focused on establishing those traits that make NIS invasive (Nyberg and Wallentinus, 2005; Rejmanek and Richardson, 1996) while others tried to understand particular characteristics of invaded communities (Davis et al., 2000; Lonsdale, 1999). Although those lines of research are particularly important for our understanding of the invasion process, our research supports the fact that key traits may change as the invasion process proceeds (Sánchez and Fernández, 2006; White and Shurin, 2007; Engelen and Santos, 2009). Moreover, it has been suggested that no species can maximize growth, reproduction and competitive ability across all environments, so the success of invasive species should be habitat-dependent (Funk and Vitousek, 2007).

*S. muticum* has long been described as a highly invasive species (Fletcher and Fletcher, 1975; Paula and Eston, 1987), particularly because it possesses characteristics of both r- and K-selected species (Arenas et al., 1995; Engelen and Santos, 2009a; Rueness, 1989). In particular, it has been described as a species that colonizes rapidly open habitats, often associated with availability of bare rock (e.g. Britton-Simmons, 2006; Britton-Simmons and Abbott, 2008) rather than a species that displaces native species. Accordingly, our results suggest that *S. muticum* is a weak competitor when comparing to the native *Cystoseira humilis*. In fact, it has been suggested that invasive seaweeds are not especially competitive (Bruno et al., 2005; Johnson, 2007). Hence, a NIS between functionally similar species may not be able to increase in abundance unless other mechanisms, such as disturbance or increased propagule pressure, create an opportunity to establishment (Davis et al., 2000; MacDougall and Turkington, 2005) by increasing the NIS fitness (MacDougall et al., 2009).

This research represents an increase in knowledge over the invasion process of macroalgal invasions and reinforces the notion that NIS are affected by different drivers throughout the invasion process (e.g. resource availability, propagule pressure, biotic interactions, climate change). Furthermore, this research gives valuable insight into the effects of *S. muticum* on the primary productivity of intertidal macroalgal assemblages, reinforcing potential threats to this system associated with a lack of predictability-diversity relationship. This study, therefore, provides results which show that the invasion

outcomes may change at multiple points in the life cycle, in addition to supporting a role for functionally diverse assemblages in limiting NIS colonisation.

A very important issue not covered in this research is the possible effect of Global climate change on the reproductive process of both NIS and native species. Evidence shows that over the last decades alterations in species' phenologies have been attributed to climate change (Hughes, 2000; Parmesan and Yohe, 2003). The reproductive process of a species is directly linked to the sustainable subsistence of a population. Thus, phenological changes may be linked to species distributions (Parmesan, 2006) and marginal populations may be particularly affected (Viejo et al., 2011). Predictive models of species distribution should not only use climate change scenarios but also try to incorporate biotic interactions to climate change.

Also, although the generalization of a successful invasion is difficult to formulate, future research should try to understand the ecological mechanism generating the patterns of NIS dominance and distribution. For example, *Codium fragile* is highly invasive in NW Atlantic and although it is registered in NE Atlantic, it is not considered an invasive species there. As discussed in this thesis, particular attention should be given to the different mechanisms of coexistence acting within a community, its effects on invasion resistance (Moore et al., 2001) and the role of niche and fitness differences on the invasion success (MacDougall et al., 2009). Understanding the interplay between ecological dynamics and rapid evolutionary responses like adaption (Schoener, 2011) may help to predict future outcome of invasions in the face of climate change.

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