Neighbourhood competition in coexisting species: The native Cystoseira humilis vs the invasive Sargassum muticum

Fátima Vaz-Pinto a,*, Brezo Martínez b, Celia Olabarri c, Francisco Arenas a

a Laboratory of Coastal Biodiversity, CILMAR – Centro Interdisciplinar de Investigación Marinha e Ambiental, Rua dos Bragas, no 289, Universidade do Porto, 4050-123 Porto, Portugal
b Área de Biodiversidade y Conservación, Universidad Rey Juan Carlos, c/Tulipán s/n, 28933 Móstoles, Madrid, Spain
c Departamento de Ecología y Biología Animal, Facultad de Ciencias del Mar, Universidad de Vigo, 36310 Vigo, Spain

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The introduction of non-indigenous species (NIS) is expected to have negative effects on native competitors, particularly between functionally similar species. Nevertheless, the mechanisms underlying competitive relationships remain poorly studied. Here, a substitutive neighbourhood approach was used to examine the role of assemblage density and neighbour identity in the growth and nutritional strategy of a focal individual. We quantified intra- and interspecific competition between two similar adult macroalgae (Fucales: Sargassaceae), the invasive Sargassum muticum and the native Cystoseira humilis, known to co-occur on intertidal rocky shores from NE Atlantic. Using either S. muticum or C. humilis as the focal species, we monitored the focal individual growth responses and nutrient content in the field, with two different densities of neighbours. Additionally, we quantified the nutrient uptake rates for both species in the laboratory. Our results showed that C. humilis grew at a significantly faster rate showing N accumulation when surrounded by S. muticum, whereas the invader has only showed growth at low density conditions. In addition, C. humilis presented greater uptake rates of nitrate compared to S. muticum suggesting better competitive potential to exploit nitrogen transient pulses of high concentration. Our results suggest a dominant native alga vs the invasive, which is not supported by field observations. This research gives evidence that competition between adult macroalgal individuals may not be the key mechanism linked to the dominance of NIS in introduced habitats.

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1. Introduction

Biological introductions in marine ecosystems are widely reported and have been increasing 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 non-indigenous species (NIS) introductions is a major challenge in ecology, particularly in coastal waters which experience high susceptibility to such events and are among the most invaded ecosystems (Grosholz, 2002). A review of the mechanisms underlying the impacts of non-indigenous 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; Thomsen et al., 2009). Particularly, strong competition is expected to arise between ecologically similar native and invasive species (Dudgeon et al., 1999). Nonetheless, the mechanism underlying competitive relationships remains poorly studied.

An important aspect of successful invasion is the existence of certain life traits or fitness traits (e.g. ecophysiological characteristics), which are frequently associated with invasive species. Introduced invasive species are considered highly efficient organisms using resources, and, as a result, have a more vigorous and faster growth than native or introduced non-invasive species (Pyšek and Richardson, 2007). For example, differences in physiological traits related to C acquisition and N allocation may have direct consequences in growth rates and may indicate a strategy for competition and invasion (Funk et al., 2013; McAlpine et al., 2008; Nyberg and Wallentinus, 2005).

In the marine environment, macroalgal assemblages depend on resources such as space, nutrients and light (Carpenter, 1990). The introduction of NIS will be more successful either in habitats where competition for these resources is reduced, and thus the invader has little or no competition from residents for resources (Davis et al., 2000), or in habitats where competition is very high, whenever an invader has lower maintenance requirements than resident species (Shea and Chesson, 2002). In sessile organisms, differences in morphology and ecophysiology traits of competing individuals, biomass, population density and neighbour distance affect competition for resources (Carpenter, 1990; Kim, 2002). Negative effects of density on survival and growth are common in algal populations (Ang and De Wreede, 1992; Steen and Scrosati, 2004). In some cases, denso-dependent responses include changes in architectures as reported for Sargassum muticum (Arenas et al., 2002; Strong and Dring, 2011). Furthermore, nutrients are the limiting resource for seasonal macroalgal growth in most temperate
marine systems (HANISAK, 1979), and are an important bottom-up factor controlling the performance and structure of coastal macroalgal assemblages (PederSEN and BorUM, 1996; Pedersen et al., 2010). Macroalgal species have developed different ecological strategies to compete for nutrients intimately related to their life-cycle. Perennials are typically slow-growing organisms and present low uptake rates. Nonetheless, perennials develop large nutrient stored pools in winter, particularly N, and use them for growing during spring (Martínez et al., 2012; Pedersen and Borum, 1996). This fact allows them to be relatively tolerant to nutrient seasonal limitation in summer when light levels increase, outcompeting other species in pristine temperate geographic areas (Pedersen et al., 2010). Opportunistic algal forms, on the other hand, have a growth strategy to exploit a pulsed increase in nutrient availability, escaping grazing control and achieving great biomass and dominance in a short time (Pedersen and Borum, 1996). High nutrient uptake rates drive their dominance in sites or seasons, of high nutrient and light availability to sustain their fast growth and reproductive maturation (Phillips and Hurd, 2003). However, these species become rapidly limited if the supply decreases since they do not develop significant storage pools. There are species with strategies that differed from this contrasting response, as for example summer-annuals, which sustain active growth during late spring to summer, achieving their maximum size in these periods of low nutrient supply (Martínez et al., 2012; Sears and Wilce, 1975). Information of how invasive species cope with nutrient limitations is scarce and ambiguous. For instance, research on the brown macroalga Undaria pinnatifida showed nitrogen and light ecophysiological parameters which closely resembled opportunistic algae (Dean and Hurd, 2007). On the other hand, the persistence and dominance of other invasive species were not affected by nutrient enrichment (e.g. Nejrup and Pedersen, 2010; Radford et al., 2010).

The brown macroalga S. muticum (Yendo) Fensholt 1955 is one of the most studied invasive seaweeds. Native to East Asia, it is considered an invasive species almost all around the world (Norton, 1976). 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). Commonly distributed throughout the intertidal, S. muticum regularly invades the habitats of algal species from the genus Cystoseira (Engelen and Santos, 2009; Fletcher and Fletcher, 1975; Otero-Schmitt and Pérez-Cirera, 2002). Previous studies showed varied results regarding the impact of the invasive S. muticum on native assemblages. In northern Spain, S. muticum showed limited impact on native assemblages (Olabarria et al., 2009; Sánchez and Fernández, 2005), whereas in Northern Ireland competitive effects were not apparent between S. muticum and Saccharina latissima (Strong and Dring, 2011). In San Juan Island, USA, however, the presence of S. muticum had a significant impact on native communities at multiple trophic levels (Britton-Simmons, 2004). Overall, despite specific traits of the species, other factors such as space availability and propagule supply, among others, play important roles in the invasion success of this species (Andrew and Viejo, 1998; Vaz-Pinto et al., 2012).

Cystoseira C. Agardh and Sargassum C. Agardh are considered two of the most widely distributed fucacean genera across both hemispheres. Despite their common canopy morphology, these species differ in their life-cycle and nutritional strategy. The native Cystoseira are perennial brown algae, known to dominate complex and diverse assemblages of Mediterranean (Ballesteros et al., 2009) and Atlantic communities (Tuya and Haroun, 2006). The invasive S. muticum is considered a pseudo-perennial species, and its invasive success has been related to the combination of opportunistic traits and perennial persistence (Norton, 1976; Wernberg et al., 2000). In particular, it exhibits fast growth rates under a short favourable season (Norton, 1976), after which senescence is observed. However, little is known about these species nutritional strategy. Moreover, it has been suggested that indigenous Cystoseira can be displaced by S. muticum (Engelen and Santos, 2009; Fletcher and Fletcher, 1975; Viejo, 1997). On the semi-exposed rocky shores of southern Portugal, S. muticum co-occurs with Cystoseira humilis Schousboe ex Kützing 1860 in intertidal rock pools with seasonal nutrient limitation. The native C. humilis (Sargassaceae, Fucales) is the dominant alga in most intertidal rocky pools along the Atlantic southwest coast of Portugal (Engelen and Santos, 2009) and can grow up to 150 cm high. It is described to occur in pools and in shallow standing waters in the eulittoral zone, in moderately wave-exposed sites (Gómez Garreta, 2001). In SW Portugal, observational results suggested lower population growth rates of S. muticum in pools where the native C. humilis was dominant, compared to pools where S. muticum was dominant (Engelen and Santos, 2009). These authors assumed an early phase of invasion in pools dominated by Cystoseira, with slower growth rates of the invader compared to assemblages dominated by the invader i.e., late phase of invasion (Engelen and Santos, 2009). Underlying mechanisms of these competitive interactions, however, remain unknown.

The present study investigated the underlying mechanisms that determine the relative competitive abilities of S. muticum and C. humilis by comparing the species’ growth responses and total nutrient content in field experiments, and nutrient uptake rates in a laboratory experiment. The specific objectives were to (1) compare the relative effects of intra- and inter-specific competition on growth and nutritional content in the field at relevant ecological conditions, 2) determine whether density effects influence in situ individual growth and nutritional state, and (3) determine the characteristic nutrient uptake kinetics from each species, as the first step to determine the species nutritional strategy. We hypothesize that higher density assemblages will have reduced growth and survivorship compared with those at lower densities. In addition, the perennial C. humilis is expected to show slow-growing strategy with reduced uptake rates and richer nutrient tissues whereas S. muticum may present higher nutrient uptake rates and lower storage potential. We propose a novel contribution on the mechanisms involved in neighbouring competition among invasive and native species at relevant ecological conditions.

2. Materials and methods

2.1. Field study site

This study was carried out in the intertidal of Praia de Molelo (Northern Portugal, 41°50′22″ N, 8°52′30″ W) from February to August 2011. Molelo is an exposed site with a semi-diurnal tidal regime, with the largest tidal range of 3.5–4 m during spring tides. Sea Surface Temperatures vary between 13 °C and 20 °C during the year. The experiment was performed in 10 mid-intertidal and low-intertidal rock-pools (~1–1.5 m above chart datum), and the experimental units were randomly displayed among pools. Pools selected were large pools (several square metres) with enough depth to accommodate our assemblages (around 50 cm in the area where the plates were placed). Those pools have a diverse seaweed flora dominated by encrusting and articulate Corallinaceae including species from genus Lithophyllum and Corallina, and non-calcified species like Chondrus crispus, Bifurcaria bifurcata, S. muticum and Cystoseira spp. We placed the plates away from large canopy individuals from these species.

Individuals of 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. Literature data described a gradual north/south trend along the Portuguese continental shelf, with colder and nutrient-richer waters in the north and warmer, nutrient-poorer waters in the south (Peliz and Fiuza, 1999). A recent study in the Portuguese coast (between Viana do Castelo and Sines) also described a year-round latitudinal gradient in several oceanographic patterns (Tuya et al., 2012). In particular, monthly Sea Surface Temperature decreased ~1 to 2 °C with increasing latitude, while chl a increased (Tuya et al., 2012).
2.2. Macroalgal assemblage plates and experimental design

Experimental plates made of PVC were set with 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$^{-2}$ in plates of 27 × 27 cm and 25 individuals per 250 cm$^{-2}$ in plates of 20 × 20 cm, for low and high density treatments, respectively. This gave final densities of 20 and 40 individuals per 400 cm$^2$, respectively. These values mimic those observed in field conditions for the native species (16 to 49 holdfasts per 400 cm$^2$ with a mean (±SD) of 31 (±12), from three scraped quadrates of 20 × 20 cm). Experiments comparing species competition or performance in monospecific 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 substitutive neighbourhood approach to analyse seaweed species competition by relating the performance of a focal individual to the identity and density of the neighbouring individuals. Using plates with similar size, but different density, changes the overall biomass of each plate. Thus, to avoid confounding effect among overall plate biomass and density (Underwood, 1997), we changed plate size to achieve different densities but using the same number of individuals per plate. Thus, monospecific assemblages consisted of 25 individuals of the same species and the one in the centre was considered the focal individual, whereas mixed assemblages had 1 focal individual of one species in the centre, surrounded by 24 individuals of the other species (see Fig. 1A). Individuals were fastened to the plates using a small string that wrapped the holdfast, keeping the main axis vertical in a similar position to natural seaweeds. To do so we made 4 holes in the plate around the holdfast of every single individual and use the string to secure the holdfast in between those holes (Fig. 1B, C). This procedure is an adaption of the one described by Correa et al. (2006) for seaweeds with smaller holdfasts. Most of the individuals transplanted survived. 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).

Algal length, recorded at the time of transplanting, was 14.96 ± 0.70 cm for S. muticum individuals and 8.46 ± 0.40 cm for C. humilis individuals (mean ± SE, n = 32). Dry mass estimations of both species were made from maximum length (L) and maximum circumference (C) of each alga individual, following Åberg (1990). 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.

\[
S.\text{muticum} : \text{DW} = 0.4092 + 0.006 \times V \left( R^2 = 0.98, P < 0.001 \right) \\
C.\text{humilis} : \text{DW} = 0.2636 + 0.007 \times V \left( R^2 = 0.82, P < 0.001 \right)
\]

where DW is dry weight and V is the specimen volume (L$^2$).

After the laboratory manipulation, experimental 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. Algal growth rate (per month) was estimated from February to June 2011, i.e., during spring when maximum growth rates have been found in S. muticum and other Cystoseira species in the same geographical area (Arenas et al., 1995). No net growth was observed from June to August, suggesting senescence of S. muticum in August.

For biochemical analysis of in situ C and N tissue content, we collected approximately 2–5 cm of non-reproductive tissue from the focal individual at each assemblage plate at the end of the experimental period. Algal material was dried at 60 °C for 48 h and total C and N determined using a
To assess the variation of nutrient availability in intertidal rock pools over a tidal cycle, seawater samples were collected in August from three rock pools over a tidal cycle (5 h) 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 min until water exchange was again observed and the tide was high.

To assess the variation in the nutrient concentrations over the experimental period at the sites where algal individuals were collected, seawater samples \((n = 4)\) were collected on November 2010, April/May 2011 and June 2011 from Sines and Viana do Castelo. Also, water samples were collected from Moledo, where the experiment took place.

Water samples were filtered in situ using portable microfiber filters \((\text{Fisherbrand® MF 300)}\) 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 nitrates, ammonium and orthophosphate. Water analyses were carried out using a wet chemistry analyser \((\text{Sanplus}^{+}\text{ System, SKALAR, Breda, The Netherlands})\), at CIIMAR, Porto.

### 2.4. Nutrient uptake experiment in the laboratory

#### 2.4.1. Collection of samples and pre-incubation

Vegetative individuals of \(S. \text{muticum}\) and \(C. \text{humilis}\) were collected in the rocky intertidal of Viana do Castelo \((41°42′25″ \text{N, 8°51′42″} \text{W, Northwest coast of Portugal})\) and Sines \((37°53′12″ \text{N, 8°47′43″} \text{W, Southwest coast of Portugal})\), 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 \((\text{Tropic Marine Zoo Mix, Tropical Marine Centre, U.K.})\) enriched with Von Stosch’s \((\text{VSE})\) medium \((\text{Ott, 1965})\) 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 \((\text{approx. 150} \mu\text{mol photons} \text{m}^{-2} \text{s}^{-1})\) and photoperiod \((12:12; \text{Light: Dark})\) until the beginning of the experiment.

#### 2.4.2. 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 \((\text{see Martinez and Rico, 2004})\). Algal fronds of each species, \(1 \text{g fresh weight (FW)}\), were incubated in 250 ml Erlenmeyer flasks: 12 flasks with increasing initial nutrient concentration \((19.01\) to \(132.26\mu\text{M, 3.85 to 48.35} \mu\text{M, and 0.60 to 15.19} \mu\text{M, for ammonium, nitrate and phosphate, respectively})\); 3 blanks \((\text{no seaweed})\) as controls. In total, 30 flasks were used per nutrient experiment \((15\) per species). Flasks were randomly arranged into a multi-position magnetic stirrer \((\text{IKA-WERKE})\). Irradiance was provided with fluorescent lamps \((\text{cool white F18W/840})\) 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 at \(15 °C\). The nutrient depletion of the medium was determined by nutrient analysis of water samples. Water samples \((10 \text{ml})\) were taken before the addition of the algal fronds and then at \(15, 30, 60, 120, 180, 240\) and \(300 \text{min}\). After the experiment, algal material was oven-dried at \(50 °C\) to a constant weight \((48 \text{h})\), for determination of dry weight \((\text{DW})\).

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

#### 2.4.3. Uptake rate calculation

Net uptake rates \((\mu\text{mol g} \text{DW}^{-1} \text{h}^{-1})\) for each nutrient source were calculated following the equation:

\[
V = (S_i - S_f) \times V_{ol} / (DW \times t),
\]

where \(S_i\) and \(S_f\) 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 \((\text{h})\), and \(DW\) is the algal dry weight \((g)\) \((\text{see Pedersen, 1994})\).

The exhaustion of ammonium in low nutrient flasks was registered for the time intervals \(30–60\) and \(60–120\) min, for \(C. \text{humilis}\) and \(S. \text{muticum}\), respectively. Therefore, the first 6 uptake measurements, i.e. lower nutrient flasks, were not displayed for both species \((n = 6)\). Moreover, no ammonium uptake measurements were displayed after those time intervals, since nutrients in the flasks were exhausted by algal uptake. Although all glass material used was washed with 10% HCl, anomalous negative uptake rates and outliers were recorded, suggesting handling contamination. These corresponded to the 0–15 min intervals for phosphate uptake and 45 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.

#### 2.5. Statistical analysis

A two-way analysis of variance \((\text{ANOVA})\) was used to test main and interactive effects of density of macroalgal assemblages \((\text{high and low})\) and neighbour identity \((\text{monospecific or mixed assemblages})\) on the growth rate of each species individually. 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 analyses we considered an unbalanced design and performed the analysis of variance using type III sums of squares \((\text{SS})\) \((\text{Underwood, 1997})\), separately for each species.

Ordinary linear regression analyses were performed to analyse the relationship between nutrient concentrations in the rock-pool over a tidal cycle. Regarding the uptake laboratory experiment, for each time interval, net uptake rates were plotted against the initial substrate concentration of the current sampling interval. Although nutrient uptake kinetics usually follows a Michaelis–Menten saturation model \((\text{Lobban and Harrison, 1997})\), other alternative linear responses can also be observed \((\text{Martinez et al., 2012; Phillips and Hurd, 2004})\). Thus, the Michaelis–Menten function was only used when the quadratic fit was significant against the corresponding linear fit \((p\text{-value} < 0.05)\); otherwise a linear fit was applied \((\text{see Martinez et al., 2012; Phillips and Hurd, 2004})\). The data was fit using SigmaPlot version 10.0 \((\text{Systat Software, Inc., San Jose California, USA})\).

Analyses of variance with unequal sample sizes were performed using Type III SS using the Anova function from the car package and linear models were carried out using the linear model function \((\text{lm})\) in the R-program \(2.15.0\) \((\text{R Development Core Team, 2012})\). Prior to all analyses, the homogeneity of variances was examined using Cochran’s \(\text{C-test}\). Data were transformed when necessary and in those cases in which transformation did not remove heterogeneity, the level of significance was judged at the more conservative probability of 0.01 \((\text{Underwood, 1997})\). Post-hoc multiple comparisons were obtained using Fisher’s
least significant difference (LSD) test. Data shown represent mean (±SE) throughout.

3. Results

3.1. Nutrient concentrations in the field

Nutrient concentrations varied between algal collection sites and the experimental site over the experimental period. Overall, our data from Sines presented lower values of nitrate and phosphate, whereas Viana do Castelo showed greater nutrient concentrations of ammonium and phosphate (Fig. 2). Moledo, where the experiment took place, showed nitrate values similar to Viana do Castelo and ammonium values closer to Sines (Fig. 2A).

Over a tidal cycle of almost 5 h, nutrient concentrations (nitrate and phosphate) declined drastically in the first 2 h 30 min ($R^2 = 0.84$, $p < 0.0001$, $n = 11$ for both nutrient types, Fig. 3). Nitrate concentration varied from 1.4 μM to 3.8 μM (Fig. 3A) and phosphate concentration ranged from 0.51 μM to 0.38 μM (Fig. 3B). In contrast, ammonium concentration remained quite constant as a recycling N source ($R^2 = 0.26$, $p = 0.112$, $n = 11$) at 3.03 μM (±0.10) (Fig. 3C).

3.2. In situ algal growth rate and tissue C and N

Throughout the field experiment, a greater growth was observed in individuals of C. humilis compared to S. muticum with a mean value (data of all treatments pooled) of 0.15 cm (±0.08, $n = 26$) and 0.002 cm (±0.06, $n = 23$), respectively (Fig. 4). Nonetheless, N content was similar between species, 1.843 (±0.068) and 1.836 (±0.060), for C. humilis and S. muticum, respectively (Anova, $F_{1,48} = 0.23$, $p = 0.634$). High variability within replicates of the same treatments was, however, observed for both studied species and, in particular, for S. muticum.

The growth rate of C. humilis focal individuals was affected interactively by the experimental treatments, i.e., density of macroalgal assemblages and neighbour identity (Table 1a, Fig. 4A). Specifically, there was a significant greater growth rate of C. humilis in assemblages with competitor compared to assemblages without competitor, but only at high macroalgal density (Fisher’s LSD, $p = 0.02$). Monospecific stands of C. humilis at high density showed a slight net biomass loss of 0.14 (±0.11) g DW month$^{-1}$, with only one individual replicate showing some net tissue increment (0.38 g DW month$^{-1}$), whereas the presence of S. muticum neighbours leads to an increase in net biomass of 0.37 (±0.16) g DW month$^{-1}$ with 2 out of 7 replicates showing some tissue loss. Moreover, C. humilis at low density assemblages showed an overall increment of 0.17 (±0.10) g DW month$^{-1}$. The initial lengths of C. humilis focal individuals did not differ significantly at low and high density (Anova, $F_{1,30} = 0.01$, $p = 0.934$). Additionally, the N nutrient content of macroalgal individuals also did not vary between treatments (overall mean ± SE: 25.87 ± 0.72, Table 2a).
content, however, was significantly affected by the neighbour identity (Table 2b). The N nutrient content was greater in mixed assemblages than in monospecific assemblages of C. humilis, 1.96 (±0.09) and 1.70 (±0.09), respectively.

S. muticum growth rate showed high variability of results between macroalgal assemblages and no significant differences were obtained between treatments. Net biomass loss was the main outcome of the experiment in all combination treatments but monospecific stands at low density (Fig. 4B). The initial lengths of S. muticum focal individuals at low and high density were similar, 15.39 (±0.80) cm and 14.54 (±1.16) cm, respectively. C (overall mean ± SE: 29.72 ± 0.48) and N (overall mean ± SE: 1.84 ± 0.06) nutrient content also showed no significant effect of the experimental treatments (Table 2).

3.3. Nutrient uptake

The native C. humilis showed the greatest uptake rates concerning the N sources (Fig. 5). Nitrate uptake increased linearly with substrate concentration, except during the period 30–60 min when a saturation response was observed (Fig. 5A; Table 3). S. muticum, on the other hand, showed mostly saturation kinetics up to 180 min and then linear responses of low slope were observed (Fig. 5B; Table 3). Nitrate was exhausted earlier (240 min) by C. humilis and at greater rates (compare axes in Fig. 5A, B), suggesting greater nitrate uptake potential of C. humilis than S. muticum.

The ammonium uptake response was linear for both species as it passively enters the cells (Fig. 5C, D; Table 3). Ammonium was totally consumed after 60 min by C. humilis and 60 min later by S. muticum. Moreover, maximal uptake rates were recorded for C. humilis, also suggesting greater uptake potential for this nutrient than S. muticum.

Overall, phosphate uptake rates were similar for both species (Fig. 5E, F), although the mechanism varied between species and we found slightly different affinity. Phosphate uptake response for C. humilis followed a linear uptake response throughout the experimental period, whereas S. muticum alternated between linear and saturated responses (Table 3). At 240 min, no phosphate concentration remained in C. humilis experiment, whereas S. muticum did not show a complete phosphate depletion over the experimental period (300 min), thus suggesting somewhat higher affinity for P by the former.

4. Discussion

Marine macroalgae compete for space, nutrients and light because these resources are often in short supply in coastal ecosystems (reviewed in Carpenter, 1990) and vary at spatial and temporal scales (Bonn, 1988; Connell, 1983; Naem et al., 1999). The present study revealed an effect of the identity of the neighbours in the native C. humilis growth and nutritional state. In field conditions, C. humilis grew at a faster rate showing N accumulation if surrounded by S. muticum at high density assemblages. In addition, the invasive species exhibited lower uptake potential. Our results suggest a better nutritional strategy of C. humilis to cope with limiting nutrient conditions of intertidal rock-pools, contrary to the expectations.

The population of C. humilis from the Atlantic Portuguese coast develops on intertidal rock-pools along semi-exposed and exposed shores (Araújo et al., 2009; Engelen and Santos, 2009). Rock-pools are physically stressful environments, and as observed in the present study, nitrate and phosphate showed a strong decrease in concentration over a tidal cycle and thus short-term nutrient limitation may be attained during low tides. Evidence suggest that at low and potentially limiting N availability, slow-growing species, i.e., perennials, should be favoured when compared to fast-growing species, i.e., opportunistic (Pedersen and Borum, 1997). Fast-growing opportunistic species, however, show a great nutrient uptake potential, while slow growing perennials have smaller uptake rates and large nutrient store capacity (Lobban and Harrison, 1997; Martínez et al., 2012). The response obtained in the present study was, however, not the expected for a perennial macroalga.
C. humilis showed elevated growth and nutrient uptake rates. In particular, C. humilis showed a greater increase in biomass when in high density mixed assemblages with S. muticum than in monospecific assemblages, in agreement with its greater nutrient uptake rates. The positive effect of S. muticum at high density could be related to a balance between the fact that S. muticum is less efficient than C. humilis in nutrient uptake and also maybe a positive density effect from protection against waves and other physical perturbations. S. muticum has a very plastic response to density (Arenas et al., 2002), thus changes in the effect of S. muticum on C. humilis at high and low density may also be a consequence of that plasticity. Surprisingly, C. humilis not only showed nutrient uptake rates much greater than those reported for other perennials, but also presented values comparable to the ones reported for ephemeral macroalgae such as Ulva intestinalis and Ceramium sp. (Martínez et al., 2012; Pedersen and Borum, 1997). This result may either be related to the nutrient availability from the alga recipient region, or from its natural habitat, i.e., pools, which, as demonstrated here, are routinely subjected to decreasing nutrients over tidal cycles. Field nutrient measurements revealed lower nutrient concentration in Sines compared to Viana do Castelo. Generally, nutrient-limited algae show greater uptake rates (Lobban and Harrison, 1997). Thus, our results might be explained by the fact that although C. humilis is a highly efficient species, it 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 (unpub. data). Thus, our results are contradictory to the most frequent response of perennial slow-growing species, which are suggested to be better adapted to pristine temperate rocky shores (Pedersen and Borum, 1997), and exhibit low nutrient uptake rates (Martínez et al., 2012).

The invasive species, S. muticum, is known to develop among communities of the genus Cystoseira (e.g. Engelen and Santos, 2009; Otero-Schmitt and Pérez-Cirera, 2002). Despite being dominant throughout the Portuguese coast, our results do not characterize adult S. muticum as a great competitor or with an opportunistic nutritional strategy. The growth of S. muticum was the same regardless of the presence of C. humilis, contrary to our expectations. Also, the lack of significant negative effect of density was quite surprising as negative effects of density have been previously registered for S. muticum and other...
intertidal macroalgae (e.g. Arenas et al., 2002; Viejo and Åberg, 2001). Nonetheless, there was a trend towards the effect of density with great biomass losses within this treatment, as expected. In addition, S. muticum showed N-uptake rates comparable to summer-annual species (Martinez 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 follows K-selected traits (Engelen and Santos, 2009). Our results, however, do not support the fact that S. muticum may displace C. humilis, at least not due to mechanisms related to density or identity of neighbours. This same competitive outcome has been previously reported between S. muticum and the kelp C. latissima (Strong and Dring, 2011). Natural stands of S. muticum comprise different adult and juvenile stages, i.e., mixture of sizes and biomass (Baer and Stengel, 2010; Strong and Dring, 2011). Thus, our findings may be due to the fact that the present experiment was focused on post-recruitment competition and we exclusively used fully grown thalli of similar size (≈15 cm). Density dependent effects may regulate S. muticum populations from embryos and juveniles individuals, where larger densities can be observed (e.g. Arenas et al., 2002). A similar trend has also been registered for Cystoseira spp. settlement and recruitment (Benedetti-Cecchi and Cinelli, 1992).

Table 3

<table>
<thead>
<tr>
<th>Interval (min)</th>
<th>Cystoseira humilis</th>
<th>Sargassum muticum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (linear)</td>
<td>V_max</td>
</tr>
<tr>
<td>Nitrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–15</td>
<td>1.16 (0.06)</td>
<td>0.32 (0.08)</td>
</tr>
<tr>
<td>15–30</td>
<td>0.62 (0.08)</td>
<td>0.56 (0.07)</td>
</tr>
<tr>
<td>30–60</td>
<td>0.13 (0.02)</td>
<td>0.08 (0.02)</td>
</tr>
<tr>
<td>60–120</td>
<td>0.51 (0.02)</td>
<td>8.34 (0.89)</td>
</tr>
<tr>
<td>120–180</td>
<td>15.82 (4.32)</td>
<td>12.06 (8.71)</td>
</tr>
<tr>
<td>180–240</td>
<td>11.16 (2.43)</td>
<td>9.43 (5.33)</td>
</tr>
<tr>
<td>240–300</td>
<td>–</td>
<td>9.57 (0.34)</td>
</tr>
<tr>
<td>Ammonium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–15</td>
<td>2.66 (0.21)</td>
<td>1.03 (0.29)</td>
</tr>
<tr>
<td>15–30</td>
<td>2.60 (0.53)</td>
<td>2.04 (0.22)</td>
</tr>
<tr>
<td>30–60</td>
<td>1.67 (0.51)</td>
<td>0.58 (0.14)</td>
</tr>
<tr>
<td>60–120</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–15</td>
<td>0.29 (0.04)</td>
<td>–</td>
</tr>
<tr>
<td>15–30</td>
<td>–</td>
<td>6.00 (1.57)</td>
</tr>
<tr>
<td>30–60</td>
<td>0.43 (0.04)</td>
<td>0.27 (0.03)</td>
</tr>
<tr>
<td>60–120</td>
<td>0.57 (0.03)</td>
<td>2.41 (0.27)</td>
</tr>
<tr>
<td>120–180</td>
<td>–</td>
<td>1.99 (0.23)</td>
</tr>
<tr>
<td>180–240</td>
<td>0.29 (0.03)</td>
<td>3.15 (0.56)</td>
</tr>
<tr>
<td>240–300</td>
<td>–</td>
<td>0.15 (0.01)</td>
</tr>
</tbody>
</table>

Overall, at the largest density of C. humilis, monospecific assemblages intensify intraspecific interactions, which decrease in mixed assemblages, suggesting that growth in this alga was more sensitive to its own density. Our results in the mixed assemblages suggest that the presence of S. muticum entails that: 1) S. muticum may display a positive interaction with C. humilis by decreasing the conditions of nutrient limitation for C. humilis individuals, or 2) inter-specific competition with S. muticum is lower than intra-specific competition between C. humilis individuals. Because growth rate in high-density mixed assemblages was not greater than at monospecific low-density assemblages, results suggest that this pattern is due to a greater intra- vs interspecific competition in C. humilis. Facilitation by the invader, however, could not be fully investigated with the present experimental design.

Our results, however, are not consistent with field observations where the NIS S. muticum is the dominant macroalga in the intertidal rocky-shore. S. muticum has been previously described as an opportunistic, rapidly colonizing available space rather than displacing native species (Farnham, 1974). Evidence suggests that many invasive species are, in fact, weak competitors and its establishment and spread are facilitated by disturbance (e.g. Bando, 2006; Hierro et al., 2006). Nonetheless, the outcome of competitive interactions varies considerably with the age or size at which species interact (Olson and Lubchenco, 1990). Previous studies have reported physiological differences in Sargassum spp. at germling and adult stages (Choi et al., 2008; Hales and Fletcher, 1989). In this context, our results support the fact that the invasive success of S. muticum may be linked to competitive interactions at the early post-settlement stage (Strong and Dring, 2011).

4.1. Conclusion

The present experiment described the competitive capacity of the native C. humilis and the invasive S. muticum in adulthood. In conclusion, our study suggested that high density of monospecific assemblages leads to increased intra-specific competition, as expected (e.g. Viejo and Åberg, 2001). Moreover, both species differed greatly in their nutritional physiology as well as growth pattern, although contrary to our initial hypotheses. C. humilis presented greater uptake rates of N than S. muticum, suggesting that adult individuals of S. muticum are weaker competitors compared to adult individuals of the native C. humilis. Our results support the fact that competition between adult macroalgal individuals may not be the key mechanism linked to the dominance of NIS in introduced habitats. In accordance, other factors such as disturbance...
have been suggested to enhance the abundance and spread of NIS (Hierro et al., 2006), while invader persistence strongly determines invasion patterns (Spence et al., 2011).

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The funding agencies had no role in the study design, data collection, analysis and interpretation of data or in the writing of this paper.

Author contributions
Planned and designed the experiment: FVP, BM, FA, and CO. Performed the experiment: FVP, FA, and CO. Analysed the data: FVP and BM. Wrote the paper: FVP and BM. All authors have approved the final article.

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