

Research Article

Biotic effects during the settlement process of non-indigenous species in marine benthic communities

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Received: 26 September 2017 / Accepted: 15 January 2018 / Published online: 2 March 2018

Handling editor: Maiju Lehtiniemi

Abstract

Biotic interactions, particularly predation/grazing and competition, are key factors limiting the introduction success of non-indigenous species (NIS). In addition, positive interactions are considered important drivers of community structure, and both positive and negative interactions between native and NIS can determine the ability of communities to resist NIS invasions. This study was conducted in Madeira Island (NE Atlantic) to evaluate how predation and facilitation will affect settlement success of NIS. We manipulated the access of predators to bare and partially pre-invaded PVC settling plates to later be exposed to high propagule pressure of NIS in a marina environment. Results indicate that NIS diversity was greater in pre-invaded treatments, but only on those plates exposed to predators. Our findings suggest that positive interactions between established NIS and incoming settlers may promote the establishment success of newcomer NIS under predation pressure.

Key words: Madeira Island, fouling communities, marina environment, biotic interactions, invasion process

Introduction

Global scale human activities are accelerating and facilitating the movement of a large number of species (Mack et al. 2000; Ricciardi et al. 2017) with dramatic and rapid shifts in species composition which are often long lasting and challenging to reverse (Folke et al. 2004). Non-indigenous species (NIS) interact with all components of the recipient ecosystem, physical and biological, resulting in the eventual success or failure of NIS establishment (Richardson et al. 2000; Kolar and Lodge 2001; Canning-Clode 2016). In general, negative impacts of invasive species prevail and some of them can alter the structure and functioning of native communities (Stæhr et al. 2000; Molnar et al. 2008; Gestoso et al. 2013; Thomsen et al. 2014). However, biological invasions in the marine system provide excellent opportunities

for field experiments to potentially advance our understanding of many ecological questions, and in recent years ecologists and evolutionary biologists have tested theories about factors that structure biodiversity patterns and ecosystem functioning (e.g. Griffen and Delaney 2015; Bellard et al. 2016; Wright et al. 2016). In particular, marine sessile fouling communities are distributed worldwide and are among the most diverse and productive assemblages of macro-organisms, which make them suitable model systems to test ecological theory (Canning-Clode and Wahl 2010). Research focused on these assemblages has evaluated classic paradigms in ecology, such as the Latitudinal Gradient of Species Richness (Canning-Clode et al. 2010), the Intermediate Disturbance Hypothesis (Svensson et al. 2007) and the biotic resistance hypothesis (Stachowicz et al. 1999).

Predicting community invasibility, species invasiveness or identifying factors that make places more or less prone to invasion is one of the most important issues in the study of bioinvasions (Lonsdale 1999). Species interactions such as predation/grazing and competition are key factors limiting the success of established invaders and, together with invader attributes and the environmental context, they can determine the success or failure of an invasion (Elton 1958; Levine et al. 2004; Kimbro et al. 2013). However, the effects of native consumers (i.e. predators or herbivores) are weak on some invasive species (see review in Colautti et al. 2004), and consumer effects can be similar in native and introduced ranges (Maron and Vila 2001). In addition, in some habitats native and invasive species may suffer similar damage from consumers (Agrawal and Kotanen 2003) or invasive species can even suffer greater consumption (Parker and Hay 2005; Pintor and Byers 2015). Moreover, positive interactions are considered as important drivers of community structure (Bulleri 2009) and both positive and negative interactions between native and NIS can determine the ability of communities to resist invasions (Bulleri and Benedetti-Cecchi 2008). In particular, the “Invasional Meltdown” hypothesis suggests that an invasive species facilitates another’s invasion by increasing the likelihood of survival and/or ecological impact, and possibly the magnitude of impact (Simberloff and Holle 1999). In marine systems, experimental evidence has shown how facilitative interactions can promote recruitment of invasive species, for example by reducing environmental stresses (Altieri et al. 2010) or providing physical habitat for recruitment of species (Bulleri and Benedetti-Cecchi 2008). Notably, native habitat-forming species may act as facilitators through their capacity to modify the physical conditions of the resident ecosystem (Wright et al. 2016). However, although direct and indirect interactions contributing to this mechanism are considered significant, evidence confirming that facilitative interactions are more frequent or important than hindering ones is scarce (Simberloff 2006).

The intentional or unintentional transport of marine species to new regions is one of the primary threats to biological diversity and a global issue, since NIS introductions have been detected in all world seas (Reise et al. 1999; Ruiz et al. 2000; Hewitt 2002; Robinson et al. 2005; Wonham and Carlton 2005). Moreover, of marine environments, inland bays, estuaries and harbors suffer more pressure from NIS arrivals, and artificial structures such as seawalls, pilings or floating docks are particularly heavily invaded (Glasby et al. 2007; Ruiz et al. 2009; Bulleri and Chapman 2010; Simkanin et al. 2012). Many

new NIS, particularly sessile invertebrates such as tunicates, bryozoans, sponges or polychaetes, have been detected in the offshore islands of Madeira (NE Atlantic) during recent and ongoing surveys in harbors and marinas (Canning-Clode et al. 2013; Ramalhosa and Canning-Clode 2015; Ramalhosa et al. 2016). These recent NIS occurrences in Madeira are probably explained by the strong anthropogenic and propagule pressure within these port environments that overlap and interact with the invasion process itself (Allen and Williams 2003). The south coast of Madeira Island is especially exposed to intense anthropogenization (e.g. ship trade, coastal urbanization) and artificial habitats probably play a key role in NIS establishment (Cohen and Carlton 1998; Ruiz et al. 2000). The expansion of populations of NIS from already invaded environments to other coastal areas is a matter of particular concern and can have ecological impacts in resident marine communities of the archipelago (Gestoso et al. 2017). These highly impacted human areas (i.e. recreational marinas or harbors) provide an excellent environment (i.e. high NIS propagule pressure) for experiments to test theories concerning mechanisms underlying the invasion process, especially during the settlement and establishment phases.

This study investigated how predation and facilitation interactions could affect NIS settlement success. We manipulated the access of predators in a marina environment (i.e. high NIS propagule pressure conditions), by using cage and uncaged treatments; and used bare versus already invaded settling plates as a proxy for facilitation. In particular, we hypothesized that (1) the exclusion of predators promotes NIS settlement, and (2) the presence of NIS favors the establishment of new NIS settlers. This work provides insights by evaluating the relative importance of these two biotic factors during the first steps of NIS introductions. More importantly, the experimental design allows further understanding of the differences in effects between interactive factors versus these same factors acting in isolation. Finally, the island context makes the present paper a pioneer contribution as to the best of our knowledge little work has examined the interactive effects of predation and facilitation on NIS settlement success in island marine ecosystems.

Material and methods

Study area and experimental setup

This study was conducted at Funchal marina, Madeira Island, Portugal (32°38'N, 16°54'W; Figure 1A), a human impacted location under high NIS pressure

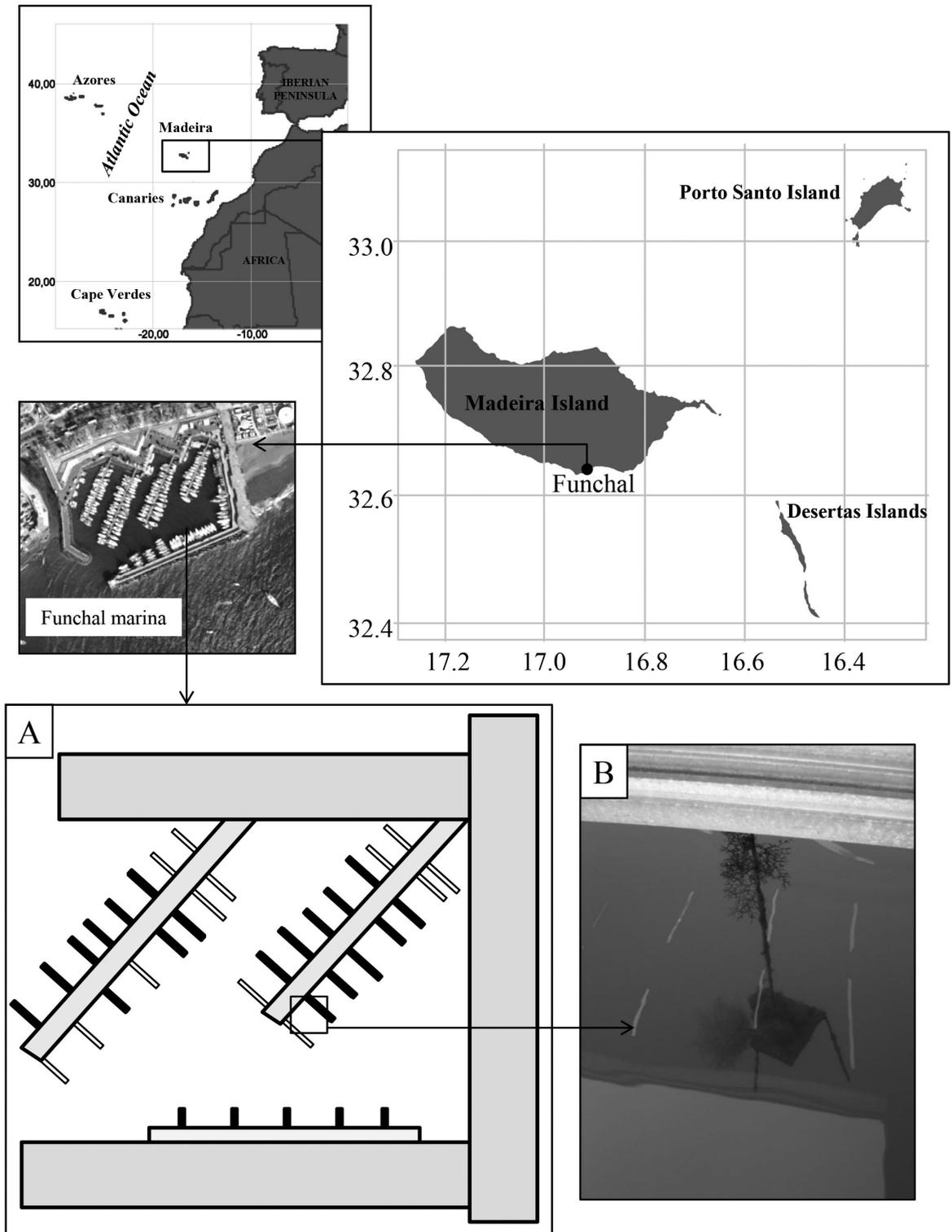


Figure 1. Experimental locations in Funchal marina on the south coast of Madeira Island (Madeira Archipelago, Atlantic Ocean). (A) Schematic of Funchal marina with pontoons selected for the study in black. (B) PVC settling plate (experimental unit) suspended from a rack.

(i.e. heavily invaded system) (Ramalhosa et al. 2016, 2014; Ramalhosa and Canning-Clode 2015). Located on the south coast of Madeira island, Funchal marina is the largest and busiest recreational marina in Madeira Island and is often used as a stopover for yachts and cruises between Europe and the Caribbean. The main potential predators in this area include native generalist predators such as the sea urchin *Arbacia lixula* (Linnaeus, 1758); the decapods *Grapsus grapsus adscensionis* (Osbeck, 1765) and *Pachygrapsus marmoratus* (Fabricius, 1787); the fishes *Chelon labrosus* (Risso, 1827), *Pomadasyus incisus* (Bowdich, 1825) and *Diplodus* sp.; and the polychaete *Hermodice carunculata* (Pallas, 1766) (Canning-Clode et al. 2008; Araujo and Wirtz 2015; Ribeiro et al. 2005). Abundance data for some of these main predators were recently collected during an underwater visual census supporting the existence of predation pressure effects in this area (e.g. *Arbacia lixula* = 12 ind/100 m²; *Hermodice carunculata* = 5 ind/100 m²; *Pomadasyus incisus* = 3 ind/100 m²; *Diplodus* sp. = 3 ind/100 m²; authors' unpublished data).

The experiment lasted 12 months and included an initial recruitment phase (9 months) followed by a manipulation phase (3 months). For the recruitment phase, 10 PVC settling plates (14 × 14 × 0.3 cm), with a smaller plate (10 × 10 × 0.3 cm) placed at the center of each plate to exclude fouling in this central area, were individually attached to a brick in a horizontal orientation facing downwards. In April 2015, bricks were suspended from pontoons in the marina and submerged at approximately 1 m depth. They were left for 9 months to collect NIS fouling communities following current and standard protocols (e.g. Canning-Clode et al. 2011; Ramalhosa and Canning-Clode 2015). After this initial colonization period, all settling plates were collected from the field, the small center plates were detached, and the outer ring communities were photographed (i.e. the larger plates) (Supplementary material Figure S1). Subsequently, in January 2016, the manipulation phase was set up by placing plate fouling communities from the marina and bare plates of two different sizes (i.e. large plates of 14 × 14 × 0.3 cm and small plates of 10 × 10 × 0.3 cm) (Figure 2A, B) in exclusion cages made from 5 × 5 mm² plastic mesh. Two orthogonal fixed factors, “Predation” (P) and “Facilitation” (F) were included in the experimental design ($n = 5$). “Facilitation” (F) included two different treatments: (F⁺) consisted of a small bare plate placed inside a larger NIS-colonized plate (i.e. plates from the recruitment phase which were dominated by NIS); and (F⁻) in which a small plate was placed inside a larger bare plate (i.e. free of NIS) (Figure 2C). To test for predation, both levels of the

factor “Facilitation” were replicated with and without exclusion cages, i.e. caged (C) vs. open (O) (Figure 2C). Simultaneously, one more procedural control (PC) was included to control for potential artifact effects of caging on recruitment of propagules (Figure 2C). This consisted of a bare plate similar to the F⁻ treatment (i.e. small bare plate placed inside a larger bare plate) but protected with a partially open cage with holes on the top and sides (Figure 2A). This allowed predator access and only simulated the potential buffering effect against propagule settlement from the full cage (Figure 2C). Once assembled, all experimental plate units were randomly distributed throughout Funchal marina to be exposed to high levels of NIS propagule pressure for 3 additional months (i.e. January to April 2016). Although the potential role of PVC in possibly biasing the community toward NIS is recognized (Tyrrell and Byers 2007), this material and methodology are standards which have been used in many similar studies (e.g. Crooks et al. 2010; Freestone et al. 2011, 2013; Canning-Clode et al. 2011, 2013).

Sampling

At the end of the experiment, species identity, abundance and biogeographical status (i.e. native/cryptogenic versus NIS) of plate fouling communities on the inner experimental plates (i.e. small plates of 10 × 10 × 0.3 cm, Figure 2B) were examined. Fouling communities collected on settling plates were then analyzed by measuring percent cover of sessile species, including polychaete worms, ascidians, bryozoans, sponges and crustaceans, using the image analysis software Coral Point Count with Excel extensions (CPCe) (Kohler and Gill 2006). Community composition of plates from the initial recruitment phase (outer ring community) was quantified by analyzing 99 randomly distributed points within the outer ring plate area. For plates from the manipulative phase, each image was divided in 10 × 10 cm quadrats and sub-divided into a 3 × 3 grid of 9 cells, with 11 random points per cell resulting in 99 points analyzed per picture. This stratified random sampling method ensured that points were sampled in each region of the image (Kohler and Gill 2006) and has been used previously in similar analyses (e.g. Canning-Clode et al. 2013, 2011). In addition, each plate (and ring area from larger plates) was carefully examined using a dissecting microscope to improve the quantification of the total species pool and to identify organisms to the lowest possible taxonomic group. To quantify NIS abundance, those species categorized as cryptogenic species were pooled with native species for the statistical analysis, as a more conservative approach to evaluate NIS settlement success on experimental plates.

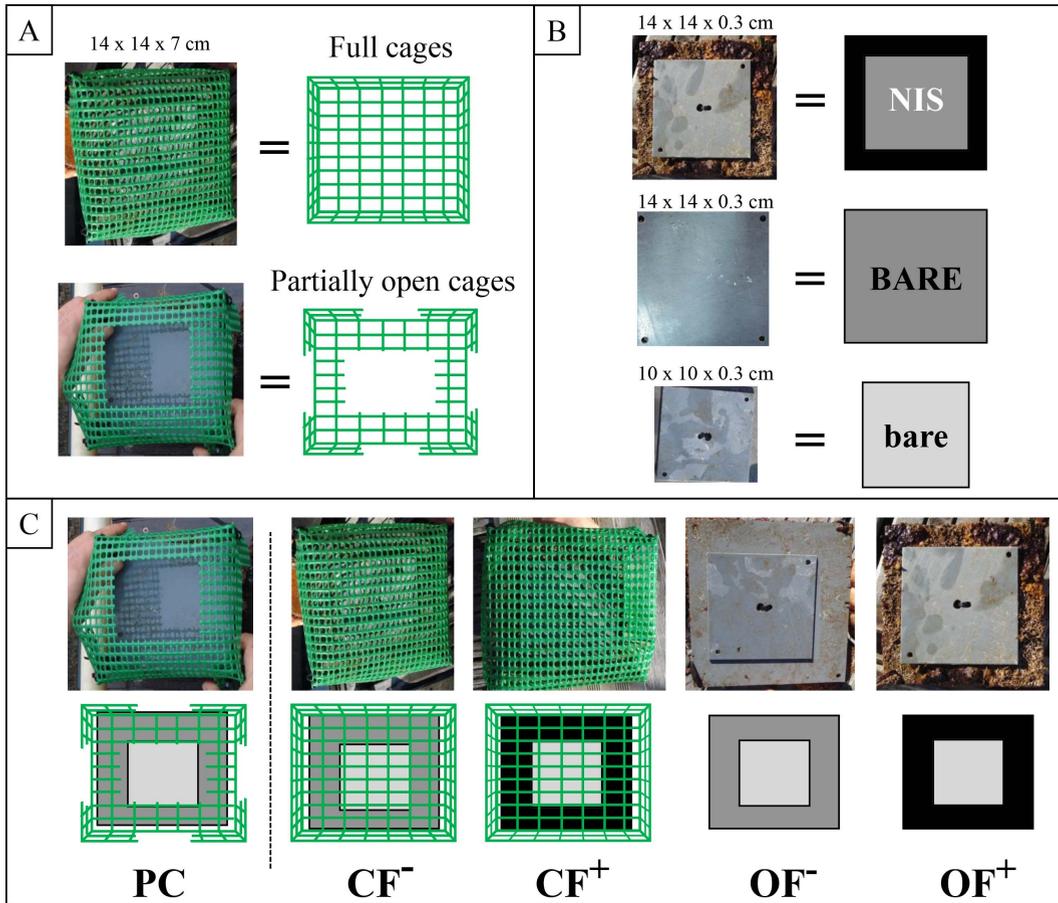


Figure 2. Set up of the experiment. Diagram and photos of (A) exclusion and partially open cages; (B) non-indigenous species (NIS) community and bare plates; and (C) experimental plate treatments used to manipulate biotic interactions of predation pressure and facilitation from non-indigenous species (NIS). PC – Procedural control for the caging effects; CF⁻ – caged with NIS; CF⁺ – caged without presence of NIS; OF⁻ – open with presence of NIS; and OF⁺ – open without presence of NIS.

Statistical analysis

Communities that developed during the 9-month recruitment phase at Funchal marina were analyzed in terms of species composition, native species (pooled with cryptogenic species) and NIS abundance. The percent cover of species was collected from the first 10 plates deployed at Funchal marina after the initial colonization period. SIMPER analysis was employed to evaluate the contribution of NIS to total community abundance and verify the high cover and diversity of NIS on the marina plate communities. Additionally, prior to the analysis of community responses from the manipulative experiment, a 1-way ANOVA was performed to determine the effect of cages against arrival of settlers, by comparing the differences in total bare space on caged (C) and open (O) plates from level F⁻ of the Facilitation factor (i.e. CF⁻ and

OF⁻ treatments) with that of the procedural control treatment (i.e. PC) ($n = 5$).

Both univariate and multivariate analyses of percent cover data were performed to determine the effect of the various treatments on the structure of fouling communities developed on each experimental plate treatment. Differences in percent cover (N), species richness (S) and diversity (H' , Shannon–Wiener index) of both native species and NIS were evaluated separately using a 2-way orthogonal analysis of variance including two fixed factors (i) Predation (P), with 2 levels: caged (C) and open (O), and (ii) Facilitation (F), with 2 levels: with NIS (F⁺), and without NIS (F⁻) ($n = 5$). P -values for the F ratios were considered significant at $P < 0.01$ and marginally significant at $P < 0.07$. When significant differences were found, Student Newman Keuls (SNK) tests were used as a *posteriori* comparison among treatments.

Table 1. Results from analyses of similarities and species contributions (SIMPER) of fouling communities fixed on plates during the initial recruitment phase from Funchal marina. Individual taxa cut-off level was 2% for similarity analyses. Non-indigenous species (NIS) are indicated in bold.

Taxon	Average abundance	Average similitude	Similitude/SD	Contribution %	Cumulative %
<i>Amathia verticillata</i>	26.42	14.65	1,00	32.84	32.84
<i>Schizoporella pungens</i>	23.80	10.73	0.78	24.07	56.91
<i>Cradoscrupocellaria bertholetti</i>	10.79	6.20	1.34	13.89	70.80
<i>Salmacina dysteri</i>	8.34	5.41	1.51	12.12	82.92
<i>Crisia denticulata</i>	3.34	1.36	0.75	3.05	85.98
<i>Sycon ciliatum</i>	2.18	1.14	1.13	2.55	88.53
<i>Mycale senegalensis</i>	4.29	1.10	0.47	2.46	90.98
<i>Symplegma brankenhielmi</i>	9.29	0.99	0.15	2.21	93.20

The homogeneity of variances was examined using Cochran's C-test (Underwood 1997).

Finally, PERMANOVA analysis was run on a Bray-Curtis similarity matrix based on coverage data of communities with the same ANOVA model as above. *P*-values for the pseudo-*F* ratios were calculated from 999 permutations of raw data. Significant effects ($P < 0.05$) were further investigated through pairwise comparisons between treatments. To graphically visualize multivariate patterns of variation among communities associated with plate treatments, nMDS was used to produce an ordination in two dimensions. Taxa that contributed the most to the similarity within fouling community plates were identified using SIMPER analysis (Clarke 1993).

Univariate analyses were performed in the R platform using the statistical GAD package (Sandrini-Neto and Camargo 2010), and multivariate analyses with PRIMER v6 (Clarke and Gorley 2006; Anderson et al. 2008) with the PERMANOVA+ for PRIMER (PRIMER-E Ltd, UK).

Results

Recruitment of non-indigenous species (NIS) and caging effects

Fouling communities collected from Funchal marina during the 9-month recruitment phase were characterized by the presence of NIS (SIMPER, Table 1). In particular, the bryozoans *Schizoporella pungens*, *Amathia verticillata* and *Cradoscrupocellaria bertholetti* showed high abundance values. In addition, the sponge *Mycale senegalensis* and the tunicate *Symplegma brankenhielmi* contributed to almost 75% of the total abundance (SIMPER, Table 1).

The percent cover of bare space did not differ significantly among caged, open and procedural control treatments (ANOVA, $F_{2,12} = 0.244$, $P = 0.229$) suggesting that caging buffer effects against propagule settlement were negligible. Therefore, the experimental

analyses were run without procedural control experimental units.

Manipulative experiment

At the end of the experiment, 32 different taxa were found over all experimental plates. Of these, 19 were categorized as native/cryptogenic and 13 were considered NIS. Although not significant, both native and NIS cover showed a tendency to be affected by predation independent of facilitation interaction (i.e. marginally significant effects, Supplementary material Table S1). However, predation pressure produced opposite effects with greater native species cover on plates from the caged treatment (C), whereas NIS cover was higher in open treatments exposed to predators (O) (Figure 3A). Native species richness was significantly affected by the presence of NIS (F^+), whereas the number of NIS was not significantly affected by any experimental treatment (Table S1). The number of native species was higher without facilitation by NIS but only on caged plates without predation pressure (CF^+) (i.e. marginally significant interaction $P \times F$, Table S1; Figure 3B). In contrast, only the Shannon Index for NIS seemed to be affected by the experimental treatments (i.e. marginally significant interaction $P \times F$, Table S1). Finally, communities fixed on plates exposed to predators and with facilitation of NIS (OF^+) tended to show greater Shannon diversity values (Figure 3C).

Multivariate analysis revealed that both predation and facilitation significantly affected the structure and composition of fouling communities assembled in the different experimental plate treatments (Table S1; Figure 4). However, no interactive effects were detected so both factors influenced communities independently (no significant interaction $P \times F$; Table S1). Fouling communities on plates exposed to predation pressure were significantly different from those protected with cages (Pairwise tests, $P(\text{perm}) = 0.005$), and similarly communities assembled on F^+ plates (i.e.

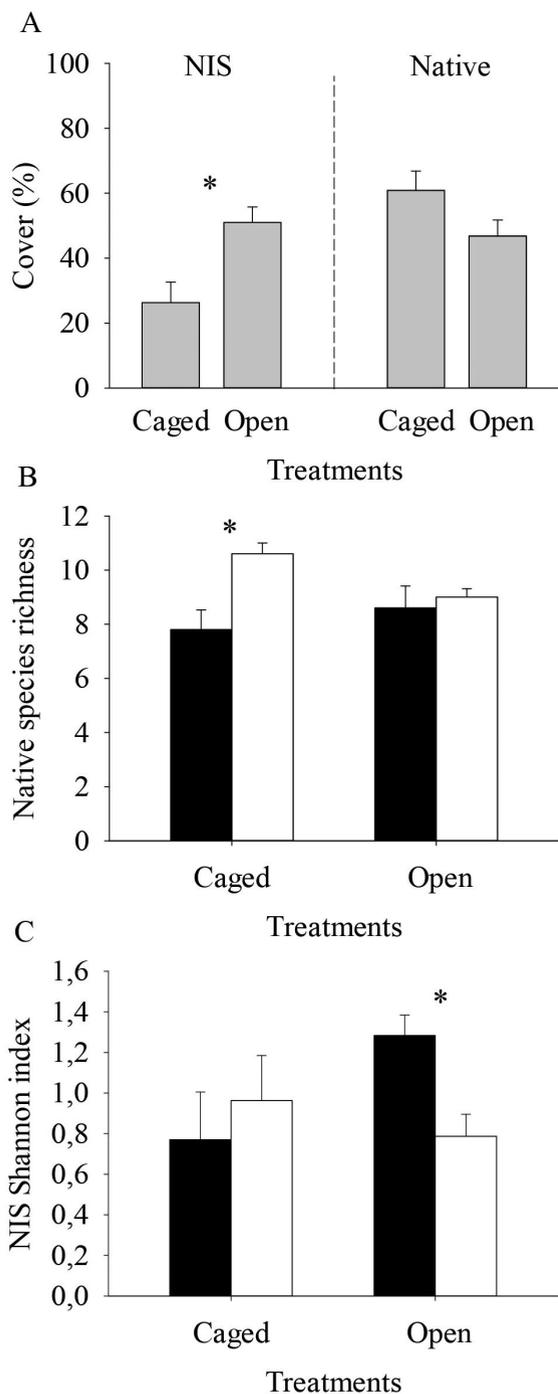


Figure 3. Native and non-indigenous species (NIS) percent cover (A); native species richness (B); and NIS Shannon Index (C) for each experimental treatment combination. Data are presented as mean + S.E., $n = 5$ and significant differences from *posteriori* pairwise tests (SNK) are indicated by the asterisk (*) symbol. Black and white symbols correspond to treatments with NIS (F⁺) and without NIS (F⁻), respectively.

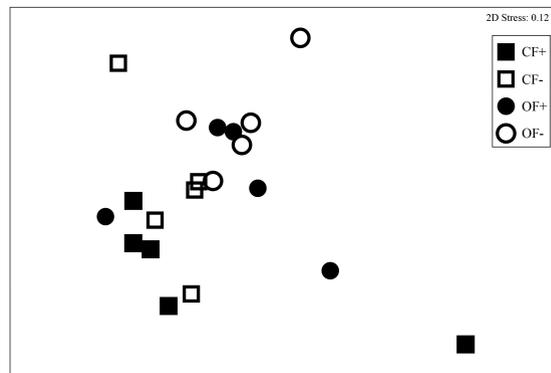


Figure 4. Non metric multidimensional scaling (nMDS) produced with values from percentage cover of fouling species in communities fixed on the inner plates (i.e. $10 \times 10 \times 0.3$ cm) from the four plate treatments. CF⁺ – caged with NIS; CF⁻ – caged without presence of NIS; OF⁺ – open with presence of NIS; and OF⁻ – open without presence of NIS.

with presence of NIS facilitators) differed from those on F⁻ plates (i.e. without presence of NIS facilitators) (Pairwise tests, $P(\text{perm}) = 0.020$).

All treatments contained high abundances of the native amphipod *Monocorophium sextonae* and the polychaetes *Spirorbis* sp., *Spirobranchus triqueter* and *Salmacina dysteri* (SIMPER, Table 2). However, the tunicate *Botryllus schlosseri* was the most abundant NIS in all treatments except on caged plates protected from predators and with presence of NIS facilitators (CF⁺) (SIMPER, Table 2). Furthermore, the non-indigenous bryozoans *Bugula neritina* and *Bugula stolonifera* were especially abundant on plates from the open treatments (O) (SIMPER, Table 2). Interestingly, the non-indigenous bryozoans *Amathia verticillata* and *Cradoscupocellaria bertholetti* were also abundant in treatments with facilitation of NIS (F⁺), although the latter was only found in those plates exposed to predation pressure (i.e. OF⁺). In contrast, the highly resilient non-native bryozoan *Watersipora subtorquata* (Piola and Johnston 2009) was only found in treatments protected from predators and without facilitation of NIS (i.e. CF⁻).

Discussion

In our field experiment, fouling communities colonizing PVC settling plates were used to evaluate the importance of biological factors during the colonization process of NIS in a marina environment. Contrary to our first prediction (i.e. the exclusion of predators will promote NIS settlement), our results suggest that predation pressure could promote percent cover of NIS and would have the opposite effect on the

Table 2. Results from analyses of similarities and species contributions (SIMPER) of fouling communities fixed on each plate treatment. Individual taxa cut-off level was 2% for similarity analyses. Non-indigenous species (NIS) are indicated in bold. CF⁺ – caged with presence of NIS; CF⁻ – caged without presence of NIS; OF⁺ – open with presence of NIS; and OF⁻ – open without presence of NIS.

	Taxon	Average abundance	Average similitude	Similitude/SD	Contribution %	Cumulative %
Treatment CF ⁺	<i>Spirorbis</i> sp.	11.11	10.09	1.15	26.05	26.05
	<i>Spirobranchus triqueter</i>	13.33	9.21	1.62	23.79	49.84
	<i>Monocorophium sextonae</i>	6.87	6.46	2.88	16.67	66.51
	<i>Salmacina dysteri</i>	5.66	3.97	1.63	10.26	76.77
	<i>Amathia verticillata</i>	5.66	3.7	0.76	9.56	86.33
	<i>Diplosoma listerianum</i>	6.06	2.5	0.37	6.45	92.78
	<i>Botryllus schlosseri</i>	2.42	1.27	0.67	3.27	96.05
Treatment CF ⁻	<i>Botryllus schlosseri</i>	17.78	13.81	2.76	24.48	24.48
	<i>Spirorbis</i> sp.	15.15	11.26	6.43	19.96	44.44
	<i>Spirobranchus triqueter</i>	15.96	8.85	1.47	15.68	60.13
	<i>Salmacina dysteri</i>	8.89	6.68	2.00	11.84	71.96
	<i>Sycon ciliatum</i>	4.04	2.61	1.6	4.62	76.58
	<i>Monocorophium sextonae</i>	4.24	2.51	1.39	4.45	81.03
	<i>Trididemnum cereum</i>	4.44	2.11	0.94	3.73	84.76
	<i>Diplosoma listerianum</i>	9.49	2.00	0.99	3.55	88.31
	<i>Aplidium glabrum</i>	2.83	1.78	1.46	3.15	91.46
	<i>Watersipora subtorquata</i>	2.22	1.37	1.12	2.42	93.88
	<i>Crisia denticulata</i>	2.42	1.16	0.85	2.05	95.93
Treatment OF ⁺	<i>Botryllus schlosseri</i>	22.42	14.89	1.88	30.61	30.61
	<i>Spirorbis</i> sp.	14.75	7.64	1.74	15.7	46.31
	<i>Bugula neritina</i>	9.09	5.7	1.47	11.71	58.02
	<i>Monocorophium sextonae</i>	6.26	4.52	1.87	9.3	67.32
	<i>Spirobranchus triqueter</i>	6.06	4.43	2.00	9.11	76.43
	<i>Salmacina dysteri</i>	4.24	2.76	1.76	5.68	82.11
	<i>Cradosrupocellaria bertholetti</i>	4.65	1.87	0.62	3.84	85.95
	<i>Bugula stolonifera</i>	4.85	1.34	0.56	2.76	88.71
	<i>Crisia denticulata</i>	2.83	1.11	0.85	2.29	91.00
	<i>Amathia verticillata</i>	4.04	1.01	0.52	2.08	93.08
	Treatment OF ⁻	<i>Botryllus schlosseri</i>	34.55	31.69	6.18	52.23
<i>Spirobranchus triqueter</i>		9.7	6.77	3.52	11.17	63.4
<i>Salmacina dysteri</i>		8.48	5.95	1.37	9.81	73.21
<i>Spirorbis</i> sp.		10.71	4.81	1.15	7.94	81.15
<i>Monocorophium sextonae</i>		4.04	2.39	1.54	3.94	85.09
<i>Bugula neritina</i>		9.9	2.38	1.54	3.93	89.02
<i>Bugula stolonifera</i>		3.43	1.98	1.57	3.27	92.28
<i>Trididemnum cereum</i>		3.84	1.66	0.62	2.73	95.01

cover of native species. More importantly, our evidence suggests that predation effects can to some extent promote positive ecological interactions between already established NIS and new incoming settlers, partially corroborating our second hypothesis (i.e. the presence of NIS will favor the establishment of new NIS settlers). Nevertheless, rather than a simple explanation, a network of direct and indirect processes seems to be involved in determining the net effect of biological interactions (i.e. predation and facilitation) during NIS settlement within a marina environment.

Predation and herbivore pressures can exert strong effects on communities from low hydrodynamic stress environments, such as the marina environment, and indirectly influence the development and composition

of prey communities (Lavender et al. 2014; Menge and Sutherland 1987). In this context, the “*biotic resistance hypothesis*” predicts that native consumers will confer resistance to resident ecosystems by reducing the abundance and spread of NIS (Elton 1958). Previous predator-exclusion studies developed in harbor environments, like the present study, have shown that consumption by native predators can limit invasion success of sessile invertebrates (Dumont et al. 2011; Rogers et al. 2016; Simkanin et al. 2013); and in tropical areas in particular, where predation pressure is strong, NIS may even be totally excluded (Freestone et al. 2013). This was not the case in our study: our results suggest that predation pressure effects would favor NIS colonization success and

will enhance the cover of NIS on experimental plate fouling communities. For example, high abundances of *B. schlosseri* were found in treatments open to predation pressure although various predator species are able to feed on this non-indigenous tunicate (Cohen 2005). These findings appear to be in accordance with the prediction of the “*Enemy Release Hypothesis (ERH)*” which proposes that specialist enemies of NIS will be absent in areas where it has been introduced and invasive species will also suffer lower predation impact from generalist predators in the new environment than native species (Keane and Crawley 2002). This potential explanation would imply a substantial advantage for NIS over native species, which would have suffered disproportionately from attacks by their native enemies. To the best of our knowledge, few studies have found similar trends in marine environments and most of these were focused on particular predator-prey relationships (e.g. Simoncini and Miller 2007; Veiga et al. 2011). However, a recognized limitation of predator exclusion studies is the inability to separate the effects of predators of different size on sessile community development (Lavender et al. 2014). In theory, meso-predators can influence community structure during the early post-settlement period and larger predators would affect older communities (Lavender et al. 2014; Osman and Whitlatch 2004). Our experimental cages were not constructed to focus on any one predator species, and the $5 \times 5 \text{ mm}^2$ mesh size was used to try to ensure a significant reduction in the net predation pressure from large- to meso- predators. Sea urchins, crabs and fishes were probably the main predators in our field experiment, which as generalist predators may feed on many different organisms of fouling communities (i.e. invertebrates and macroalgae) (Moreira and Creed 2012). Thus, active selection by predators could have played an important role in determining the differences in development of fouling communities between our caged and open plate treatments (Epelbaum et al. 2009). As NIS are new prey in these fouling communities, the potential native predators might have not recognized these new species as food resources and continue to feed mostly on native prey (Cacabelos et al. 2010; López et al. 2010).

Biotic interactions at patch scale (e.g. neighbor growth, trophic group) may be important in explaining the relationship between resident biodiversity and recruitment of NIS (Kennedy et al. 2002; Stachowicz and Byrnes 2006). Traditionally, ecological theory considers competition to be the most important biotic factor, and the main mechanism determining the characteristics of communities (Sih et al. 1985). However, positive interactions are also considered very important drivers and in some cases more

important than hindering ones (Bruno et al. 2003; Bulleri 2009). For example, the presence of adult organisms can promote successful recruitment of new NIS settlers possibly by providing settlement cues or alternative settlement substrates (Marraffini and Geller 2015). In our study, facilitative interactions seem to underlie the settlement success of NIS, however, the effect of such interactions would be indirectly mediated by effects of predation pressure. Open plate treatments with facilitation effects (OF⁺) contained high percent cover of various NIS bryozoans: *Bugula neritina*, *Cradoscrupocellaria bertholetti* and *Amathia verticillata*. Indirect positive effects, i.e. reduction of potential competitors for space and/or food, from predation pressure on other attached native benthic filter feeders (e.g. *Spirobranchus triqueter*, *Sycon ciliatum*; Table 2) seem to be a key aspect in the colonization success of NIS species (Altieri et al. 2010; Harms and Anger 1983; Ruesink 2007). Alternatively, many studies have highlighted the importance of direct positive interactions in benthic communities and resident species can facilitate invasion success via direct or indirect mechanisms (e.g. Altieri et al. 2010; Bell et al. 2014; Bulleri 2009; Leonard 2000). For example, colonies of the NIS bryozoan *Watersipora subtorquata* may facilitate other fouling species by acting as a foundation species, improving their recruitment at an earlier stage of the invasion process (Floerl et al. 2004). Finally, the inclusion of rare species (e.g. *C. bertholetti*, *A. verticillata*) in our data analysis was important as differences in abundance of these species between plates allowed us to detect responses and effects that may not be apparent when examining differences in only abundant species (Cao et al. 2001).

In our study, already established NIS seemed to promote the arrival of additional non-indigenous species, but only under predation pressure. Although the experimental treatments were not specifically designed to disentangle small spatial scale interactions, evidence suggested that initial colonizers could have mechanistically facilitated the colonization of new NIS settlers. Interestingly, bryozoans, like other colonial invertebrates, may possess both chemical and structural defenses against attacks by different predators (Grünbaum 1997; Harvell 1998; McGovern and Hellberg 2003) and these defenses are induced by translocation of information about predation injuries among colonies, even to sites remote from the attack (Harvell 1992, 1984; Iyengar and Harvell 2002). Hypothetically, the presence of encrusted bryozoan colonies previously attacked in OF⁺ experimental plates could have induced the development of defenses in new colonies of NIS bryozoans and hence improved their capacity for successful coloni-

zation under conditions of high predator pressure (Harvell 1984). Thus, it appears that a combination of low cover of native species and the presence of free space in plate communities exposed to predators could have activated positive synergies during the first NIS arrivals, supporting the subsequent high recruitment of new NIS settlers (Nydham and Stachowicz 2007). Further experimental studies will be essential to understand the particular mechanisms underlying these facilitation effects.

The majority of NIS introductions remain rare, with only a fraction becoming widespread, dominating and causing impacts on local communities (Colautti et al. 2014 and references therein). Nevertheless, the arrival and establishment of invasive species can especially damage island communities, particularly if they have the capacity to establish and expand from invaded locations (i.e. ports and marinas) along the coastline (Piazzi et al. 2001; Ricciardi et al. 2013; Ross et al. 2004; Thomsen et al. 2014). The present study contributes to a better understanding of the ecological processes driving colonization success at the local scale of an island system, in this case Madeira Island. Our findings further contribute to unravelling the implications of the multiple interactive biotic factors in the context of a polluted marina environment, and, specifically, it is remarkable how predation effects clearly activated facilitation mechanisms, promoting the recruitment of new NIS settlers.

Conclusion

Strong biotic interactions can be negatively associated with invasion success, particularly at small spatial scales where species interactions strongly occur (Melbourne et al. 2007; Stachowicz et al. 1999). Our findings seem to confirm the importance of biotic interactions during the settlement stages of the invasion process, and their contribution to overall community composition and structure (Freestone et al. 2013, 2011; Osman and Whitlatch 1995a, b). In particular, it appears that the increase in predation pressure negatively affected the cover of native species and indirectly facilitated the recruitment of NIS. This study was conducted in only one site and for a short period (9 + 3 months), which could limit expanding our conclusions to a more general pattern. However, we highlight the particular complexity of mechanisms that modulate the success or failure of invasions and finally, the need for future studies to obtain more empirical support and elucidate the interplay between propagule pressure, ecological interactions and physical disturbance as main drivers implicated in the invasion process.

Acknowledgements

The research was supported by the ARDITI - Regional Agency for Development and Technology through their grants in the scope of the Project with reference M1420-09-5369-000001. I. Gestoso was supported by a postdoctoral grant in the framework of the 2015 ARDITI Grant Programme Madeira 14-20 (Project 002458/2015/132). P. Ramalhosa was partially funded by the Project Observatório Oceânico da Madeira-OOM (M1420-01-0145-FEDER-000001), co-financed by the Madeira Regional Operational Programme (Madeira 14-20), under the Portugal 2020 strategy, through the European Regional Development Fund (ERDF). J. Canning-Clode was supported by a starting grant in the framework of the 2014 FCT Investigator Programme (IF/01606/2014/CP1230/CT0001). This study had the support of Fundação para a Ciência e Tecnologia (FCT), through the strategic project UID/MAR/04292/2013 granted to MARE. This work was partially funded by project MIMAR (MAC/4.6.d/066) INTERREG MAC 2014-2020 Programme. We are grateful to the administration of the Funchal marina and the Nature Park of Madeira for their logistic support during this study. Special thanks go to L. Ferreira for her inestimable help with field set up and sampling.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Photo showing one of the units used to collect the NIS communities with the small inner plate and the bigger one after the 9-month recruitment phase.

Table S1. Results of multivariate (PERMANOVA) and univariate (ANOVA) analyses examining variability in fouling community composition (i.e. percent cover of all sessile species) and differences in native and non-indigenous species (NIS) percent cover, species richness and Shannon Index, respectively (n = 5). *P*-values for the *F* ratios were considered significant at *P* < 0.05, and marginally significant differences were indicated with symbol +; ns = not significant

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