Research Article

Marine fouling communities from artificial and natural habitats: comparison of resistance to chemical and physical disturbances

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Abstract

Assessing the resistance of fouling communities to anthropogenic disturbances is an important goal for the development of effective management and control strategies. In this context, we conducted a manipulative experiment on natural and artificial habitats to examine fouling communities that developed outside and inside a marina on Madeira Island (NE Atlantic Ocean) following the application of two types of stressors frequently observed in coastal habitats, namely chemical and physical disturbances. The tested fouling communities, dominated by native and non-indigenous species respectively, were in general strongly affected by the chemical but not by the physical disturbance applied, and a higher resistance to disturbance was observed in the communities outside the marina. This suggests higher capacities for communities richer in native species to tolerate anthropogenic disturbances, while non-indigenous species did not play a key role. Further research can assess the resilience of natural and artificial fouling communities when exposed to disturbances.

Key words: artificial habitats, mesocosms, antifouling paint, mechanical stress, Madeira, non-indigenous species

Introduction

The composition and structure of fouling communities are dependent on several factors, such as pollution, nutrient availability, sedimentation rate, water flow, turbulence, type of substrate and orientation (Glasby and Connell 1999; Bulleri and Chapman 2004; Dafforn et al. 2015; Simpson et al. 2017). Generally, artificial habitats (e.g., concrete docks and floating pontoons) provide novel hard substrates, a suitable condition for the proliferation and spread of non-indigenous species (NIS; Minchin et al.

2006; Mineur et al. 2012; Airoldi et al. 2015). In contrast, it is typical for fouling native species to dominate natural coastal areas (Glasby et al. 2007; Piola and Johnston 2008), and reflect communities that are mostly local in biodiversity (Lewis 1998; Chapman and Bulleri 2003; Karlson and Osman 2012; Gestoso et al. 2017; Simpson et al. 2017).

Globally, we are witnessing an impressive proliferation of artificial structures, the so-called "coastal urban sprawl", that is considered one of the most important human threats to marine biodiversity and ecosystem services worldwide (Elmqvist et al. 2016; Firth et al. 2016). The loss of natural habitat in favor of coastal urbanization can change the structure and functioning of benthic communities, and studies generally conclude that the new artificial structures cannot be considered surrogates for the natural habitats they replace (e.g., Moschella et al. 2005; Firth et al. 2013). In addition, anthropogenic activities acting along artificial habitats, such as ports and marinas, can increase the connectivity among distant localities, breaking down geographical barriers and facilitating the "stepping-stone" spreading of species (Hewitt et al. 2009; Hulme 2009; Bishop et al. 2017). This in turn can enhance the biotic homogenization of marine fouling communities in artificial habitats (McKinney and Lockwood 1999; Glasby et al. 2007; Bulleri and Chapman 2010; Davidson et al. 2010; Seebens et al. 2013; Airoldi et al. 2015; Ferrario et al. 2017). Artificial fouling communities are usually dominated by highly competitive species (e.g., NIS and cosmopolitan species), which show greater tolerance to variable environmental conditions and are often thought to be more resilient to disturbance than native communities (e.g., Piola and Johnston 2008; Canning-Clode et al. 2011; Crooks et al. 2011).

Both artificial and natural habitats can be affected by anthropogenic disturbance events (either physical or chemical) that can influence resident fouling communities. A disturbance event can alter the structure of an ecosystem, community or population by changing the availability of resources and leading to opportunities for new settlers (Altman and Whitlatch 2007; Lockwood et al. 2007). Physical disturbances (e.g., vessel grounding procedures or maintenance interventions of port structures) can cause abrasions on the substrate itself (Hudson and Goodwin 2001). These types of incidents, caused by small vessels, are mostly unreported although are likely to occur more frequently than by large vessels [e.g., in coral reefs (Lutz 2006)]. Benthic organisms and seagrass beds can also be damaged by disturbances caused by recreational SCUBA diving or snorkeling activities. For example, organisms can be accidentally kicked or buried by fine particles suspended with the fins, or damaged by trampling, anchoring or propeller action in shallow waters (Garrabou et al. 1998; Milazzo et al. 2002; Davenport and Davenport 2006; Huff 2011; Mendez et al. 2017; Renfro and Chadwick 2017).

In addition, heavy metal pollution is considered one of the most significant chemical disturbances in ports and marinas, mostly derived from the use of antifouling paints (Hall et al. 1998; Piola and Johnston 2009; Canning-Clode et al. 2011). These paints have important chemical inhibitors such as metallic biocides (such as zinc oxide, ZnO) that are often used in combination with copper to increase their toxicity and facilitate the leaching process (Guardiola et al. 2012; Watermann et al. 2005). When applied to vessel hulls and to several other artificial fixed structures (e.g., pilings, pontoons, buoys), antifouling paints can inhibit the growth of fouling organisms, slowly release heavy metals and harmful biocides into the water [e.g., copper and zinc (Lewis 1998; Comber et al. 2002; Warnken et al. 2004; Chambers et al. 2006; Thomas and Brooks 2010; Neira et al. 2014), and consequently cause a certain degree of environmental contamination. This can impact local communities (Kinsella and Crowe 2016) leading to losses of sensitive species and reductions in native diversity (Piola and Johnston 2008). In contrast, some widespread fouling species are known to have a certain degree of resistance to antifouling paints, e.g., bryozoans in the genus *Watersipora* or *Bugula neritina* (Linnaeus, 1758) (Wisley 1962; Clark and Johnston 2005; Dafforn et al. 2008; Piola et al. 2009; Crooks et al. 2011; Ramalhosa et al. 2019); and the ascidian *Ascidiella aspersa* (Müller, 1776) (considered a NIS outside Europe; Kenworthy et al. 2018).

Moreover, establishment of NIS may be ameliorated by disturbance events because free niche areas become available for more tolerant colonizers (Occhipinti-Ambrogi and Savini 2003). An example is the successful and rapid colonization of the invasive macroalga *Caulerpa* spp. following the experimental removal of the native seagrass *Posidonia oceanica* (Linnaeus) Delile, 1813 in Mediterranean meadows (e.g., Ceccherelli et al. 2014; Uyà et al. 2018).

Fouling communities have been extensively used in ecological studies as a model system to investigate a variety of general questions of succession, invasion ecology and community resilience (e.g., Svensson et al. 2007; Canning-Clode et al. 2009; Marraffini et al. 2017). Previous research on fouling communities from port habitats have evaluated the responses to different anthropogenic disturbances, e.g., heavy metal pollution and mechanical creation of bare patches (Clark and Johnston 2005; Canning-Clode et al. 2011; Crooks et al. 2011; Ramalhosa et al. 2019). However, most of the previous research has focused on the effects of disturbances acting separately, whereas the interactive effects of disturbances on communities from different habitats (artificial *vs*. natural) remains poorly explored.

In this context, we conducted a manipulative experiment to test the pulse of two types of disturbances on fouling communities composed of native and non-native organisms, in order to assess and compare their responses and resistance to anthropogenic disturbances. In particular, we hypothesize that fouling communities from natural habitats, expected to be

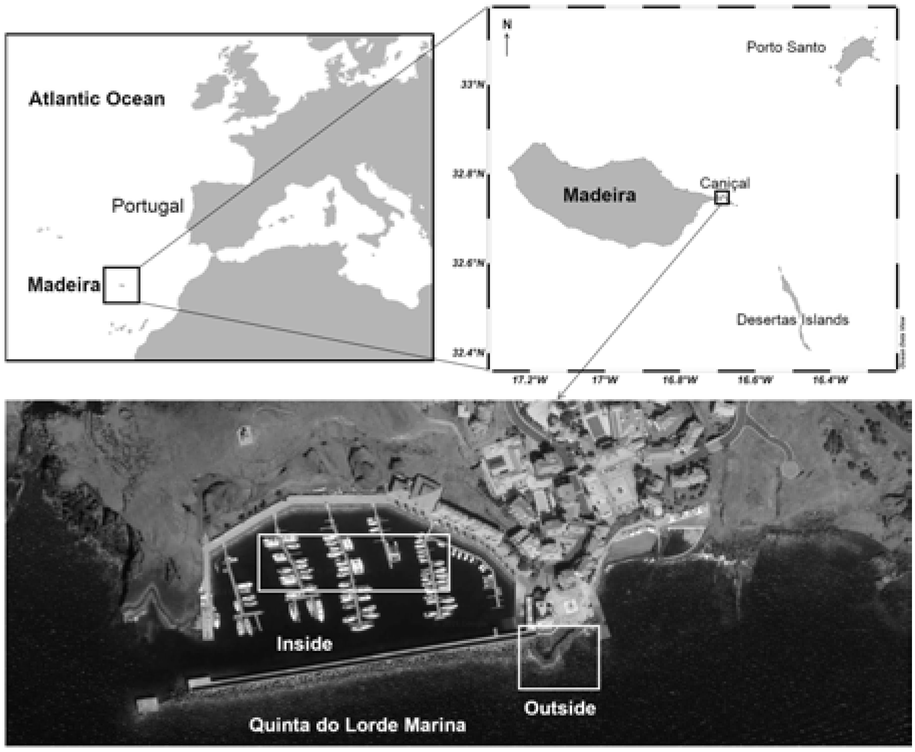

Figure 1. Map of Madeira Archipelago with the location of the marina of Quinta do Lorde (Caniçal) where 24 PVC plates were deployed at 5 meters depth inside and outside the marina.

richer in native species, would be less adapted to anthropogenic disturbances than communities from artificial habitats, whose dominance of NIS should better facilitate resistance to disturbance events.

Materials and methods

Study sites and community recruitment

The Madeira Archipelago is a group of volcanic islands located in the NE Atlantic Ocean 700 km off the Moroccan coast (Figure 1). The recruitment of fouling organisms was conducted in two areas: inside (artificial habitat) and outside (natural rocky habitat) the marina of Quinta do Lorde (Caniçal, Madeira Island: 32.741667N; −16.713333W, Figure 1).

In June 2017, a total of 24 grey polyvinylchloride PVC plates (14 × 14 × 0.3 cm) were deployed for colonization at 5 m depth both inside (12 plates) and outside (12 plates) the marina, known to host NIS (Canning-Clode et al. 2013). We constructed an experimental structure that was composed of a base attached to the rocky bottom (outside the marina) or to a brick (inside the marina), and PVC plates were placed on top, adequately separated from the base (only the side facing down was used for the experiment). The bricks inside the marina were used as a weight and laid approximately 6 cm from the soft bottom. In order to simplify its deployment in the field, plates were grouped in three different units per area, i.e. inside and outside marina (Figure S1). After a 4 month-period, all plates were retrieved and brought back to the laboratory for sampling (mid-October 2017). A preliminary analysis was performed to discard a potential “unit” effect on the plate recruitment before starting the mesocosm experiment (PERMANOVA, non-significant effect of unit: Pseudo-$F_{2,18}$ = 1.975, P(perm) = 0.084).

Mesocosm system and experimental design

A manipulative experiment was designed to examine the resistance of the two fouling communities in response to two types of disturbance (i.e. chemical *vs*. physical). This experiment was conducted inside the mesocosm system and laboratory facilities at the Madeira research unit of MARE – Marine and Environmental Research Centre, located at Quinta do Lorde Marina. Two independent tanks (350 L) were filled with 10 μm filtered seawater in a continuous water flow system of about 30 ml/sec, generating a complete turnover of water every 4 hours. Additionally, constant aeration systems were used in each tank, and artificial light was provided by fluorescent lamps (OSRAM- FH 14W/840 HE Lumilux Cool White 55 cm) under an approximate photoperiod of 12/12 h (light/dark). The use of filtered seawater excluded any possible new recruits, so that we tested the disturbance effects of only the original communities.

In this study, two types of pulse disturbance, *sensu* Glasby and Underwood (1996), were applied: physical and chemical. The "physical disturbance" consisted of pressing the PVC plates with approximately 7 kg weight for 30 seconds, as a proxy for any kind of lighter mechanic disturbances that can affect fouling communities, e.g., vessel docking, diver tourism, anchors (Clark and Johnston 2005). The "chemical disturbance" consisted of exposing the PVC plates to an antifouling paint for 48 hours to simulate the arrival of a boat freshly painted with antifouling coating (Canning-Clode et al. 2011; Crooks et al. 2011; Ramalhosa et al. 2019), considering that paints are designed to release an initial higher amount of biocides, followed by a rapid decline with slow and constant leaching rates (Schiff et al. 2004; Piola and Johnston 2008). Specifically, bare PVC plates were double coated with an antifouling paint (International® YBA067-Trilux 33 Black, containing as main metallic biocide Zinc Oxide 25 < 50%) and placed at the bottom of individual 10 L containers, with the relative manipulated plates hanged from the top. Chemical analysis for heavy metal concentrations of the water inside the containers was carried out in order to verify the effective release of the inhibitors proper of the antifouling paint used. In total, four disturbance types (three replicates per disturbance per area) were applied in this mesocosm experiment: control (C; no disturbance), physical (Phy), chemical (Che) and the combination of both physical and chemical disturbances on the same plates (mixed; Mix). Within the 12 PVC plates from inside and 12 from outside the marina, three plates were randomly selected for each of the four conditions. The manipulative experiment lasted 4 weeks (from October 12th to November 7th, 2017) and the resistance of fouling communities to disturbances was assessed over four scheduled sampling times (Figure 2). On Time_0 the PVC

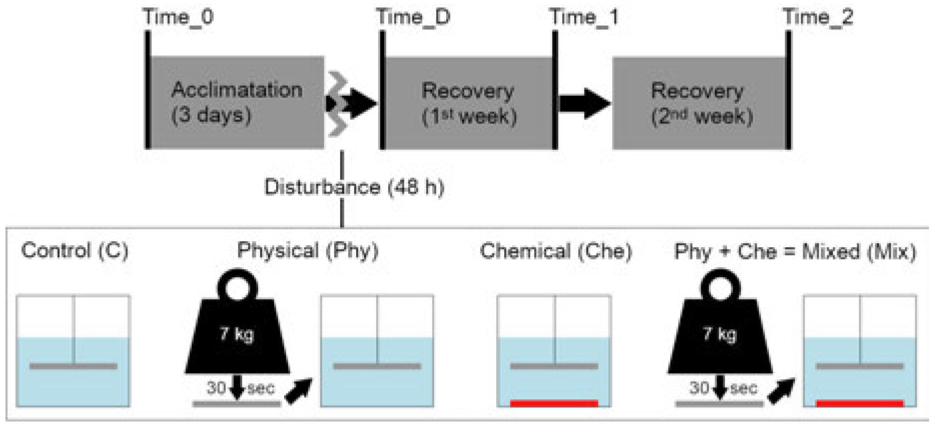

Figure 2. Scheme of the experiment phases (divided in sampling times: Time_0, D, 1, 2) and the type of disturbance used. Grey bars represent the PVC plates inside a single container during the Time_D phase, showing the different disturbances: Control (C: PVC plate not treated and simply moved into a container), Physical (Phy: PVC plate firstly pressed with a weight and then moved into a container), Chemical (Che: PVC plate moved into a container with another PVC plate on bottom previously coated with an antifouling paint, represented in red color) and Mixed (Mix: PVC plates with combined disturbances).

plates were collected in the field, first analyzed and arranged in two tanks of the mesocosm system (from October 12th to 14th, 2017). After an acclimation period of 3 days in the two tanks, a pulse disturbance phase 48h long was carried out (Time_D; "D" stands for disturbance), based on the selected disturbances (on October 20th, 2017). Finally, fouling communities were evaluated after the first recovery week (Time_1; on October 27th, 2017) and the second recovery week (Time_2; on November 7th, 2017). At each sampling event, abundance and structure of the fouling communities were examined. The fouling species were identified under a stereomicroscope (Leica S8APO) using different taxonomic keys, existing literature or taxonomic experts (e.g., Hayward and Ryland 1995; Souto et al. 2015, 2018). During the sampling times Time_D-2, a semi-quantitative index on the "health status" of each species was also considered (values from 1 to 4): value "1" was assigned for totally dead specimens/colonies; value "2" was when the percentage of dead specimens/colonies were higher than live ones; value "3" was when the percentage of live specimens/colonies were higher than dead ones; and value "4" was for totally live specimens/colonies.

All plates per sampling event were photographed using a Olympus Stylus TG-4 camera and images were subsequently analyzed with the software CPCe [Coral Point Count with excel extensions (Kohler and Gill 2006)] to assess the abundance (in percent cover; % cover) of fouling species by considering 50 random points in each plate, and this information was then integrated with the "health status" of each species. Finally, during Time_D, all the plates were moved into single containers for 48-hours and, after each sampling time, all the 24 plates were completely randomized in two experimental tanks (12 plates each) to reduce potential "tank effect".

Chemical-physical analyses

Dissolved oxygen, pH, salinity and temperature of the water were assessed by using a multi-parameter probe (YSI Professional Plus) during the sampling period. Water parameters were assessed i) in the field, during the sampling of the experimental units (inside and outside marina); ii) twice in 4 random containers, during the disturbance period and; iii) 7 times in the two tanks, during the entire duration of the experiment.

Finally, a total of 12 water samples (3 per disturbance level) were collected and analyzed from each container (during Time_D), for heavy metal concentration (zinc and copper), in order to verify water quality differences among conditions and estimate the concentration of these elements. For the water analysis, a total of 9 ml of seawater per sample was collected from the selected containers, with the use of 10 ml sterile syringes (B/Braun Injekt™); then, 1 ml of ultrapure HNO_3 (Trace SELECT®, Sigmae-Aldrich) was added to each sample. Samples were subsequently refrigerated until the chemical analysis. Spiking assays with artificial and natural estuarine water were performed in order to test the accuracy of this method. Heavy metal recovery was always between 90–110% (Duarte et al. 2014).

Heavy metal concentrations were determined by Inductively Coupled Plasma-Atomic Emission Spectrometer [ICP-AES, Horiba Jobin-Yvon, France, Ultima, model equipped with a 40.68 MHz RF generator, Czerny-Turner monochromator with 1.00 m (sequential), autosampler AS500 and CMA (Concomitant Metals Analyzer)]. Blanks were always below the detection limits of ICP-AES (0.33 ppm for Zn and 0.03 ppm for Cu). The accuracy and efficiency of the results were checked by processing reference material CRM 145 and CRM 146, referent to sewage sludge from domestic origin and sludge, respectively. Trace metal concentrations in the reference materials determined by ICP-AES were not statistically different from their certified ones (t-student tests $p = 0.05$), indicating an accurate and total extraction of the heavy metals present in the samples.

Data analyses

Data on fouling abundance (% cover) was integrated with the "health status" visual assessment by multiplying the % cover value of each species with their correspondent "health status" factor; namely 0.1, 0.33, 0.66 and 0.99 factors, related to values "1", "2", "3" and "4" respectively. This integration was applied to limit the observed high % cover of species which, despite dead, maintained their calcareous structure on the plate (e.g., barnacles, encrusting bryozoans and serpulids). In all data analyses, NIS status was attributed only to those species with a confirmed non-indigenous category and verified by specific scientific literature (e.g., Canning-Clode et al. 2013; Chainho et al. 2015; Gestoso et al. 2017). Following a more conservative approach (Marchini et al. 2015) cryptogenic species (i.e. unspecified origin, that is, unknown whether native or introduced; Carlton 1996) were included within the native species category.

Multivariate analyses were performed on the total fouling community structure, whereas univariate analyses were carried out considering the total % cover and species richness on i) the totality of species (total % cover and richness), ii) native/cryptogenic (native % cover and richness) and iii) NIS components (NIS % cover and richness).

First, different one-way permutational analyses of variance (PERMANOVAs) on Time_0 data were performed on the selected variables to compare the initial fouling communities from inside versus outside the marina, with "Area" as a fixed factor (2 levels: inside and outside; fixed, n = 12). A non-metric multidimensional scaling (nMDS) analysis was also used to graphically visualize differences detected between the selected communities. When significant, the structure of the two fouling communities was analyzed through a one-way SIMPER test (Similarity Percentages) in order to assess the main average dissimilarity between areas, and also which species mostly contribute to the similarity within the two areas (with a cut-off level of 90%; only species with a percentage contribution ≥ 2% were reported; Clarke 1993).

As plates were repeatedly sampled, with the aim to assess the potential recovery of the fouling communities, uni- and multivariate PERMANOVA analyses were performed taking into account data from Time_D to Time_2 separately. The use of this approach was preferred to account for non-independence of samples. The design employed for these analyses included two orthogonal factors: "Disturbance" (4 levels: C, Phy, Che and Mix; fixed, orthogonal) and "Area" (2 levels: inside and outside; fixed, orthogonal). When significant differences among factors or their interactions were observed, post-hoc pairwise tests were performed. An additional nMDS was constructed to graphically visualize multivariate patterns of variation in two dimensions, showing the different responses of the communities to the disturbances.

All multivariate tests were run from Bray-Curtis similarity matrices, while for univariate analyses, the Euclidean distances matrices were preferred, using an approach similar to parametric ANOVA (Airoldi et al. 2015). P-values related to the pseudo-F ratios were calculated with 9999 random permutations (Underwood 1997). Whenever there were not enough possible permutations to get a reasonable test, the Monte Carlo p-values were used instead. Prior to analysis, PERMDISP was used to check data for heterogeneity of dispersions, and transformations were applied where necessary.

In order to identify those species with higher resistance capacity to the manipulative experiment, a SIMPER analysis was performed on live species from Time_2, i.e. considering only that ones with "health status" value from "2" to "4". Statistical analyses were performed using the software

Table 1. Zinc (Zn) and copper (Cu) concentration (μg/l) in the water samples collected per condition (average values ± SD, n = 3).

	C	Phy	Che	Mix
Zn	825.56 ± 386.57	733.53 ± 187.15	1322.90 ± 6.37	1386.70 ± 446.70
Cu	27.39 ± 5.54	19.03 ± 1.13	34.31 ± 2.85	36.82 ± 3.12

PRIMER version 6.1.13 (Clarke and Gorley 2006) with the add-on PERMANOVA + version 1.0.3 (Anderson 2005).

Results

Chemical-physical and heavy metal characterization

The chemical-physical parameters showed similar values between inside and outside the marina (in the field), and during all the experimental phases (both in the mesocosm tanks and in the containers used during Time_D; Table S1). The water samples collected in the containers for each condition, showed higher average concentrations (± SD, μg/l) of Cu (35.57 ± 3.00) and Zn (1354.81 ± 284.70) in samples treated with the antifouling paint (chemical and mixed disturbances), compared to the untreated treatments (control and physical disturbances): 23.16 ± 5.77 (Cu) and 779.55 ± 276.27 (Zn). Average values per condition (± SD) are presented in Table 1.

Initial structure of the fouling community inside and outside the marina (Time_0)

After 4 months of colonization, fouling communities inside and outside the marina were composed of a total of 38 and 43 species, respectively (average values ± SD, 14.7 ± 2.4 and 18.2 ± 5.2 species), including 11 NIS inside and 9 NIS outside the marina (4.5 ± 1.3 and 3.7 ± 1.4 NIS) (Table 2; Figure S2). The main taxonomic groups observed on PVC plates inside the marina were ascidians (26%) and bryozoans (21%) while the outside community was dominated by bryozoans (41%).

At the local scale, total % cover and richness displayed significantly higher values outside the marina [PERMANOVAs: Pseudo-$F_{1,22}$ = 5.286, P(perm) = 0.031, PERMDISP-P(perm) = 0.365; Pseudo-$F_{1,22}$ = 4.429, P(perm) = 0.049, PERMDISP-P(perm) = 0.057 (Fourth root transformation), respectively], as well as for native species % cover and richness [PERMANOVAs: Pseudo-$F_{1,22}$ = 59.716, P(perm) = 0.0001, PERMDISP-P(perm) = 0.733; Pseudo-$F_{1,22}$ = 7.283, P(perm) = 0.006, PERMDISP-P(perm) = 0.059 (Square root transformation), respectively]. In contrast, the NIS % cover was significantly higher inside the marina [Pseudo-$F_{1,22}$ = 31.799, P(perm) = 0.0001, PERMDISP-P(perm) = 0.332], while no difference was observed in NIS richness between the two areas [Pseudo-$F_{1,22}$ = 0.538, P(MC) = 0.4683, PERMDISP-P(perm) = 0.294].

Table 2. Non-indigenous species recorded in the areas investigated (inside and outside the marina of Quinta do Lorde) at Time_0

Non-indigenous species	Potential native origin	References	Recorded inside the marina	Recorded outside the marina
Porifera				
Paraleucilla magna Klautau, Monteiro and Borojevic, 2004	Brazil	Klautau et al. 2004; Canning-Clode et al. 2013	•	•
Cnidaria				
Ectopleura crocea (Agassiz, 1862)	North-West Atlantic Ocean	Mills et al. 2007; Wirtz 2007		•
Exaiptasia diaphana (Rapp, 1829)	Mediterranean Sea	Riedl 1991; Canning-Clode et al. 2013	•	•
Polychaeta				
Branchiomma bairdi (McIntosh, 1885)	Gulf of Mexico	McIntosh 1885; Ramalhosa et al. 2014	•	
Crustacea: Cirripeda				
Balanus trigonus Darwin, 1854	Pacific Ocean	Carlton et al. 2011; Pilsbry 1916		•
Bryozoa				
Beania maxilladentata Ramalho, Muricy and Taylor, 2010	Brazil	Ramalho et al. 2008; Souto et al. 2015		•
Celleporaria inaudita Tilbrook, Hayward and Gordon, 2001	Red Sea – Indo-Pacific Ocean	Canning-Clode et al. 2013; Souto et al. 2018	•	
Cradoscrupocellaria bertholletii (Audouin, 1826)	Unknown	Canning-Clode et al. 2013; Vieira et al. 2013	•	
Parasmittina alba Ramalho, Muricy and Taylor, 2011	Brazil	Ramalho et al. 2011; Souto et al. 2018	•	•
Schizoporella pungens (Canu and Bassler, 1928)	Gulf of Mexico	Winston and Maturo 2009; Canning-Clode et al. 2013	•	•
Tricellaria inopinata d'Hondt and Occhipinti Ambrogi, 1985	North-East Pacific Ocean?	Marchini et al. 2007; Cook et al. 2013; Ramalhosa et al. 2019		•
Ascidiacea				
Botrylloides niger Herdman, 1886	Indian Ocean	Millar 1988; Gestoso et al. 2017	•	
Botrylloides violaceus Oka, 1927	North-West Pacific	Oka 1927; Canning-Clode et al. 2013	•	
Botryllus schlosseri (Pallas, 1766)	Unknown	Van Name 1945; Canning-Clode et al. 2008	•	
Distaplia corolla Monniot F., 1974	Caribbean	Monniot 1983; Canning-Clode et al. 2013	•	•

The two fouling communities at Time_0 revealed a significant difference in their structure (PERMANOVA: Pseudo-$F_{1,22}$ = 26.77, P(perm) = 0.0001, PERMDISP-P(perm) = 0.9833; Figure 3A), also confirmed by an average dissimilarity of 82.87% (SIMPER analysis). The taxonomic groups that mostly contributed to the similarity of the community inside the marina were *Parasmittina alba* Ramalho, Muricy and Taylor, 2011 (44.36%) and spirorbids (20.59%); while outside the marina were *Spirobranchus triqueter* (Linnaeus, 1758) (37.07%), *Lithophyllum incrustans* Philippi, 1837 (11.46%) and *Puellina radiata* (Moll, 1803) (11.18%; Table S2).

Mesocosm experiment: effects of the disturbances on the fouling communities

Uni- and multivariate analyses of total richness in Time_1,2, of native richness in Time_1 and of fouling community structure along all times (Time_D-2) detected a significant interaction between the factors "Area" and "Disturbance", revealing how the effect of the disturbance was dependent

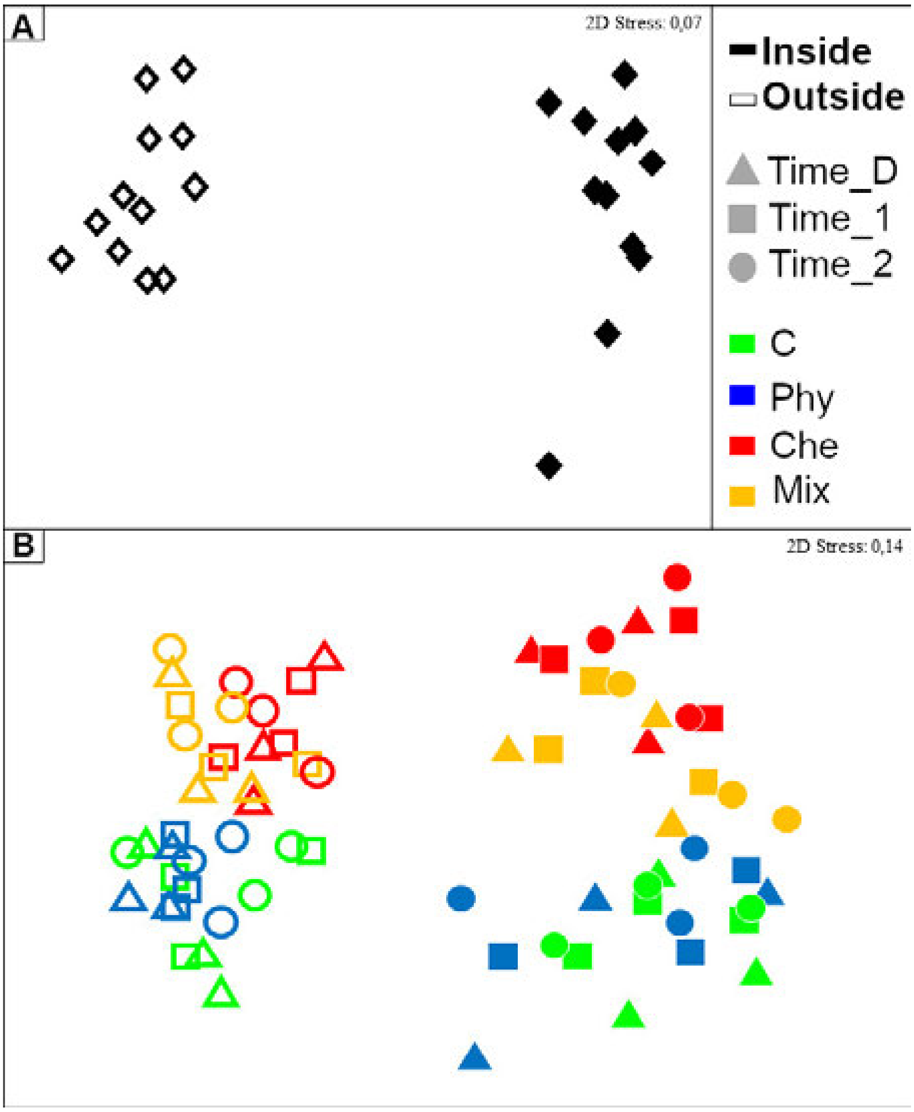

Figure 3. Non-metric multidimensional scaling (nMDS) graphs on fouling communities inside and outside the marina at (A) Time_0 and (B) considering the sampling times from Time_D to Time_2 per each type of disturbance. Filled and empty symbols correspond to fouling community plates placed inside and outside the marina respectively.

on the area (Table S3). Overall, fouling communities from outside the marina maintained greater values of total and native richness than those communities from the inside (Table S4, Figure 4). This also reflects the different abundances of native and NIS in the two communities (Figure 4). The PERMANOVA performed with the other variables (i.e. total, native and NIS cover; and NIS richness) revealed significant effects of the main factor "Disturbance" and/or "Area" (Table S3). A conspicuous decline in total, native and NIS % cover was observed in both communities. Outside the marina, native species recorded higher values of % cover for all tested conditions in comparison with the communities inside the marina; while an opposite trend was observed for NIS % cover, which had higher values inside than outside the marina (Table S3; Figure 4).

Different responses to disturbances were observed between the two communities (Table S4 in Time_D-2) but, generally, while chemical or mixed

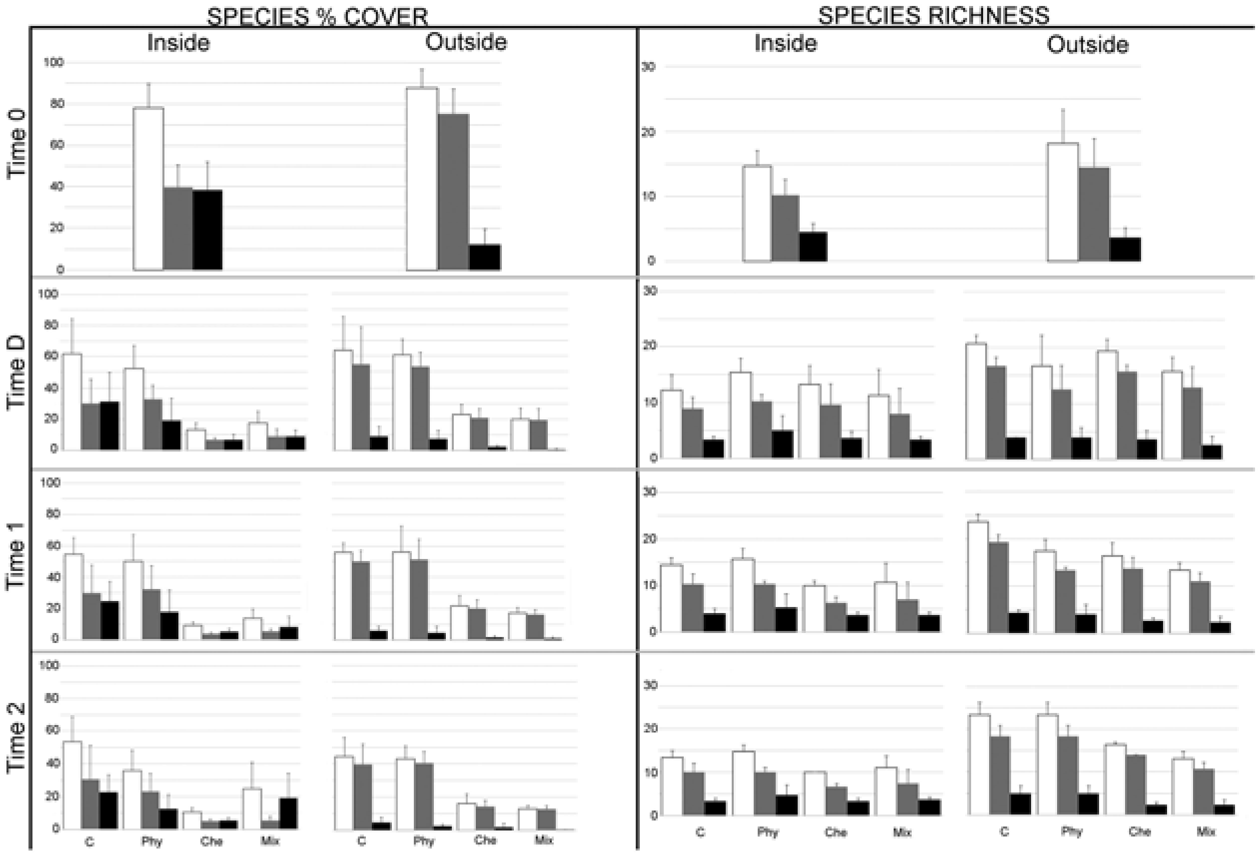

Figure 4. Average number of percent cover and richness, with corresponding SD, on total species (white bars), native/cryptogenic species (grey bars) and non-indigenous species (black bars) divided per experimental times, from inside and outside the marina.

disturbances significantly modified the structure of the communities, the exposure to physical disturbance did not differ from the control plates in both cases (Table S4). Some exceptions from this general trend were observed in the communities outside the marina, where no significant differences were recorded among the different disturbances on the structure of the fouling communities, except for mixed disturbance in Time_1 (Table S4); while cases of significant differences were observed between control and physical disturbances, when considering the total and native richness, due to a slight increase of these values in control from Time_D to Time_1 (Table S4; Figure 4). The nMDS clearly showed the lower effect of the physical disturbance on the fouling communities, while the chemical and mixed disturbances appeared separated from control and physical replicates (Figure 3B).

When considering the live biota from Time_2, algae, bryozoans, sponges and anthozoans were the dominant groups in both fouling communities. Inside the marina, the non-indigenous bryozoan *Celleporaria inaudita* Tilbrook, Hayward and Gordon, 2001 was the species most resistant to chemical disturbance, while algae (*L. incrustans, Nemoderma* sp. and Polysiphonieae) and sponges (*Mycale* sp. and *Sycon* sp.) mostly contributed to the average similarities of all the other conditions, followed by the NIS *Exaiptasia diaphana* (Rapp, 1829) and *P. alba* (the latter only in the control;

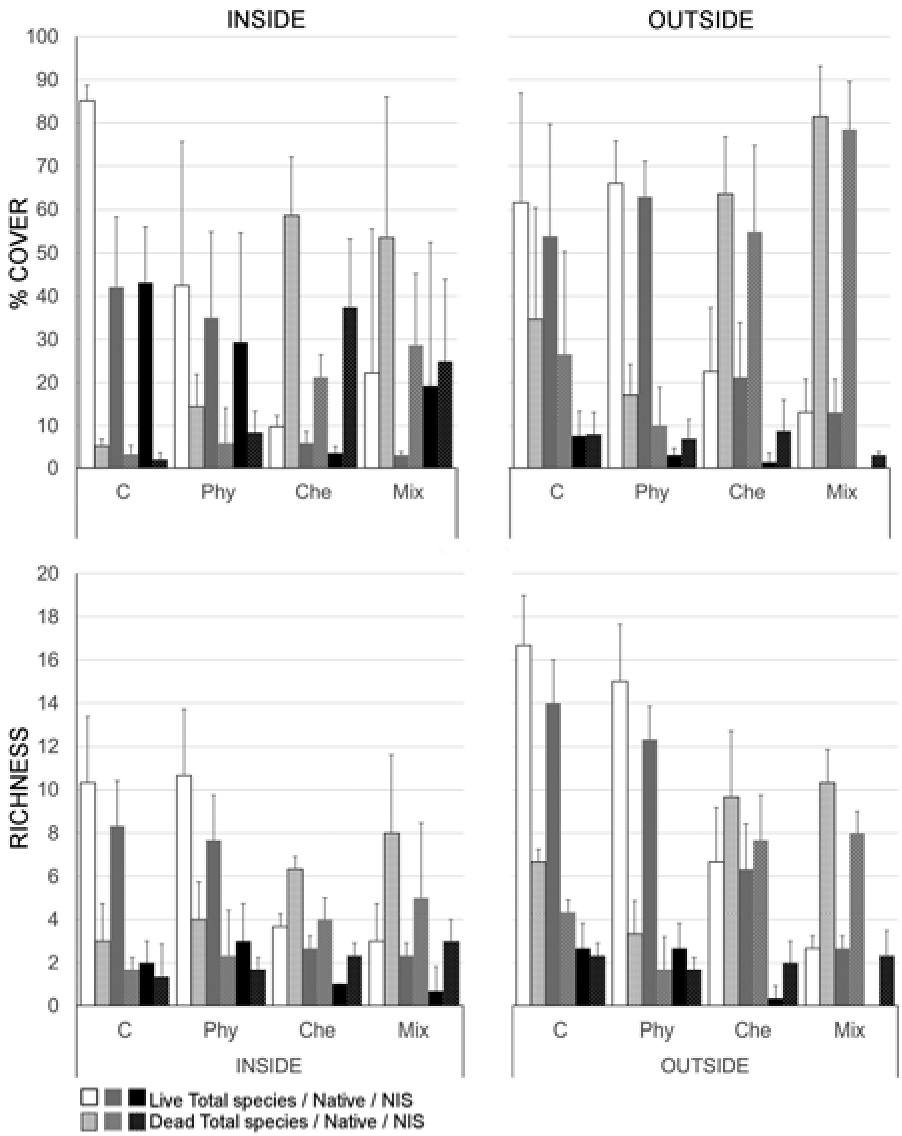

Figure 5. Average number of live/dead percent cover and richness, with corresponding SD, on total species (white bars), native/cryptogenic species (grey bars) and non-indigenous species (black bars) on Time_2, from inside and outside the marina.

Table S5). With regards to plates from outside the marina, the species that mostly contributed to the similarity within different conditions were *E. diaphana, Favosipora purpurea* Souto, Kaufmann and Canning-Clode, 2015, *L. incrustans* and *Nemoderma* sp., followed by sponges (e.g., *Mycale* sp., *Sycon* sp.; Table S5). Finally, a decrease of *P. alba* and other encrusting bryozoans (except for *F. purpurea*), spirorbids and serpulids was observed on fouling communities from both areas affected by physical disturbance, together with a slight increase of sponges and *E. diaphana* (Table S5). The species that were most resistant to the experimental conditions ("health status" from 2 to 4) were natives, with higher values observed outside the marina (Figure 5).

The complete species list per each PVC panel on Time_0, and from Time_D to Time_2 with the relative "health status" assessment for each species, are shown in Table S6 and S7 respectively.

Discussion and conclusions

The present study is a pioneering manipulative experiment to assess the resistance of fouling communities colonizing PVC plates from different areas (namely inside and outside a marina) to selected anthropogenic disturbances. Initially, a comparable number of NIS was recorded in both communities, but the PVC plates from inside the marina showed a dominant cover of NIS, in contrast with a higher native species cover and richness in the plates from the outside. Generally, both fouling communities were strongly affected by the chemical and mixed disturbances, with a small or null effect of the physical disturbance. Despite this general trend, fouling communities outside the marina displayed some exceptions, perhaps due to a slight increase of total/native species richness in the plates associated to control condition. In fact, during the experiment, the random placement of all plates in the tanks could have facilitated new recruitments. Furthermore, differences observed under disturbances between the two fouling communities may depend on their structures, confirming the potential of artificial habitats to modify ecological responses to environmental disturbances (e.g., Connell and Glasby 1999; Bulleri and Chapman 2004; Goodsell et al. 2007; Airoldi et al. 2015), as well as facilitate the settlement, establishment and further spread of NIS (Corriero et al. 2015).

The initial fouling communities experienced a sharp decrease of their most abundant species when treated with the antifouling paint; e.g., the NIS *Parasmittina alba* inside the marina, and the native species *Spirobranchus triqueter* and *Puellina radiata* from outside. Both natives and NIS were drastically reduced in presence and abundance, a trend observed in previous studies (Tait et al. 2018; Ramalhosa et al. 2019). Some species, however, demonstrated a certain degree of tolerance to the selected disturbances (e.g., the NIS *Celleporaria inaudita* and *Exaiptasia diaphana* inside marina; and the native *Favosipora purpurea* from outside). In contrast to previous studies (Clark and Johnston 2005; Piola and Johnston 2008; Piola et al. 2009; Crooks et al. 2011), a higher resistance of the non-indigenous community was not observed at the end of the experiment. As reported by Dafforn et al. (2009), native species also have the potential to survive and re-colonize the substrate after a disturbance event, demonstrating tolerance capabilities similar to NIS.

In this experiment, the impact of physical disturbance was not as strong as the chemical disturbance, but slight effects were recorded in terms of reduction of the hard and fragile encrusting bryozoans (apart to *F. purpurea*) and calcareous tubeworms, while soft and flexible taxa, like anthozoans and sponges, were more resistant (Clark and Johnston 2005). This trend was similar to results found by Clark and Johnston (2005) in a previous experiment using a similar approach, yet in our case, the pressure used was quite lower, i.e. 36 g/cm^2 *vs.* 140 g/cm^2. Other studies on fouling communities manipulated with a physical disturbance were mostly focused on the removal of organisms from the substrata, with the aim to assess the colonization processes and not considering the resistance of fouling organisms to a simulated physical pressure (e.g., Bram et al. 2005; Valdivia et al. 2005; Cifuentes et al. 2007).

Contrary to our initial hypothesis for a higher expected tolerance and resistance to disturbances for NIS (i.e., inside the marina; Piola and Johnston 2008; Canning-Clode et al. 2011; Crooks et al. 2011), fouling communities from outside the marina showed a greater tolerance. Similar to Canning-Clode et al. (2011) and Ramalhosa et al. (2019), the native component exhibited slightly higher levels of resistance, with a higher amount of live native species in most conditions at the end of the experiment. In the same coastal region as the present study, Gestoso et al. (2017) demonstrated a certain degree of biotic resistance of fouling communities from marine protected areas to the NIS settlement rate in port habitats. Nevertheless, the resilient capacity of natural habitats as buffer areas surrounding marinas or ports, e.g., in reducing the stepping-stone of NIS, or the consequences of variation in recruitment across time, is still poorly studied (Glasby et al. 2007; Ruiz et al. 2009; Simkanin et al. 2017).

It is important to note that the experiment conducted here was limited to a single area, lasted 4 months and the manipulated communities were at an early stage of development. However, it represents a starting point in understanding the ecological consequences of anthropogenic disturbances on fouling communities. In summary, and in contrast with expectations, we found evidence that NIS did not play a key role in fouling communities against the selected disturbances. This highlights the need of future studies focused on the response of natural and artificial communities to combined anthropogenic disturbances, simulating long-term consequences and assessing possible solutions against the uninterrupted ocean sprawl phenomenon.

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Authors' contribution

Conceptualization: JF, IG and JCC; Formal analyses: JF, IG, EC; Funding acquisition: JF, IG, JCC; Investigation: JF, IG, PR, EC; Methodology: JF, IG, PR, EC, BD, IC, JCC; Resources: JCC; Supervision: IG, JCC; Visualization: JF, IG; Writing: JF, IG, PR, EC, BD, IC, JCC.

Declaration of interest: none

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Pictures of the experimental units deployed inside (A) and outside (B) the marina.
Figure S2. Pictures of two PVC panels from inside (A) and outside (B) the marina at Time_0.
Table S1. Dissolved oxygen, pH, salinity and temperature values assessed during i) the sampling period, both inside and outside the marina; ii) the experiment period, in the two tanks and iii) the treatment period, in the buckets.
Table S2. Species or taxonomic group average abundances and percentage contributions to similarity within each area derived from SIMPER analysis at Time_0.
Table S3. Non-parametric multivariate and univariate PERMANOVA analyses examining variability in fouling community structure, total percent cover, total richness, native percent cover, native richness, NIS percent cover and NIS richness for the different sampling times.
Table S4. Pairwise tests on significant factors from Table S3 PERMANOVA analyses: A, B) on factor "Area x Disturbance" for pair of levels of "Treatment" and "Area", C) on factor "Disturbance" and D) on "Area" for the different sampling times.
Table S5. Average abundances and percentage contribution to similarity resulting from SIMPER analyses of fouling communities within the four treatments over sampling Time_2.
Table S6. Species list on sampling Time_0 with relative percent cover.
Table S7. Species list from sampling Time_D-2 with relative percent cover and the "health status" category assessments.

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