

The overlooked role of biotic factors in controlling the ecological performance of artificial marine habitats

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Summary

1. Artificial structures are sprawling in marine seascapes as a result of burgeoning coastal populations, increasing development and energy demand, and greater risks from climate change, storm surges and sea level rise. Interest in designing marine developments that maintain vital ecosystems and critical services is growing, but progress requires understanding the factors that influence the ecological performance of these novel artificial habitats.

2. We combined field observations and experiments along 500 km of the North Adriatic coastline to analyse the performance of artificial substrata as habitats to support canopy-forming algae belonging to the genus *Cystoseira*, among the most ecologically relevant foundation species along rocky Mediterranean coastlines. We aimed to: clarify the underlying factors controlling the growth of *Cystoseira* in the artificial habitat; contrast the relative importance of these factors between artificial and natural habitats; and test the generality of the results across different sites and species of *Cystoseira*.

3. We found that: (i) the growth of canopy algae was significantly lower on artificial structures compared to rocky reefs; (ii) such lower growth of canopy algae was not related to less favourable abiotic conditions but to higher biotic disturbance from both consumptive and nonconsumptive interactions on the artificial structures compared to the natural reef; and (iii) this was consistent across different study sites and canopy-forming species.

4. We conclude that biological factors influencing the growth of canopy algae, such as herbivory or other nonconsumptive disturbances, can differ substantially between artificial and natural habitats. The unusually large and previously unreported biotic pressure characterizing many artificial structures can negatively affect their performance as habitats to support ecologically relevant, foundation species.

5. *Synthesis and applications.* While nearly all considerations to improve the ecological performance of hard marine infrastructures focus on abiotic factors (e.g. construction materials, surface texture, habitat complexity or water quality), careful consideration of critical biotic factors is also needed to further progress the green engineering of sprawling marine infrastructures.

Key-words: artificial habitats, biotic disturbance, *Cystoseira*, furoid algae, grazing, Mediterranean sea, plant–herbivore interactions, rocky reefs, urban sprawl

Introduction

Artificial structures (comprising seawalls, breakwaters, dykes, groynes, jetties, pilings, bridges, artificial reefs and offshore installations) are sprawling globally in marine seascapes to protect coastal population and assets, exploit

marine resources and provide alternative energy sources (Dugan *et al.* 2011). The projected escalation in marine urbanization and the growing recognition of severe ecological impacts and habitat loss associated with marine urban sprawl (Airoidi *et al.* 2005a; Airoidi & Beck 2007; Bulleri & Chapman 2010) have raised the need for mitigation (Airoidi & Bulleri 2011; Browne & Chapman 2011; Perkol-Finkel *et al.* 2012; Firth *et al.* 2014). Interest in

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designing marine developments that maintain vital ecosystems and critical services is growing (Adams *et al.* 2014; Dafforn *et al.* 2015), but progress towards green engineering requires understanding the factors influencing the ecological performance of these novel artificial habitats (Chapman & Underwood 2011).

One of the most critical ecological functions to preserve in increasingly urbanized seascapes is the provision of habitat to support native biodiversity (Perkol-Finkel *et al.* 2012; Dafforn *et al.* 2015). Studies consistently show that artificial structures provide habitats that perform poorly compared to natural reefs (Perkol-Finkel, Shashar & Benayahu 2006; Burt *et al.* 2009; Miller *et al.* 2009), and tend to support assemblages with low species and genetic diversity (Fauvelot *et al.* 2009; Airoidi *et al.* 2015) and dominance by opportunistic and invasive species (Bulleri *et al.* 2006; Glasby *et al.* 2007; Airoidi *et al.* 2015). Ecological processes underlying the different performance of artificial habitats compared to natural reefs are still not fully understood (Firth *et al.* 2014). Current knowledge suggests that divergence of benthic assemblages is not simply attributable to incomplete succession, but appears to be a persistent, possibly stable state (Perkol-Finkel, Shashar & Benayahu 2006; Miller *et al.* 2009). Several co-occurring factors could contribute to maintaining these differences, including effects of construction materials (Burt *et al.* 2009), unique habitat characteristics (Vaselli, Bulleri & Benedetti-Cecchi 2008; Browne & Chapman 2011), unnaturally high anthropogenic disturbances (Airoidi & Bulleri 2011), fragmentation and isolation (Airoidi *et al.* 2015). It has also been demonstrated that, whether or not specifically planned for the scope, artificial structures affect the distribution of fish assemblages by providing enhanced nursery grounds, refugia and feeding areas (Brickhill, Lee & Connolly 2005; Clynick, Chapman & Underwood 2007). Therefore, assemblages on artificial structures could be exposed to altered grazing pressures compared to assemblages on natural reefs. Whilst considerable attention has been dedicated to the effects of physical properties of artificial habitats on the colonizing biota (Burt *et al.* 2009; Firth *et al.* 2014; Perkol-Finkel & Sella 2014), the role of biotic factors has received surprisingly little consideration (Bulleri *et al.* 2006; Iveša *et al.* 2010; Marzinelli, Underwood & Coleman 2011; Dafforn, Glasby & Johnston 2012).

We examined the ecological performance of artificial substrata as habitats for native biota along the North Adriatic coastline and experimentally identified the ecological factors influencing the different performance of artificial habitats compared to natural reefs. We worked with canopy-forming macroalgae, the dominant foundation species on many temperate rocky coasts. In the Mediterranean Sea, canopies are mostly comprised of fucales of the genus *Cystoseira*. They form ecosystems functionally analogous to kelps that play a key role in primary production and nutrient cycling, modify both physical and biological factors and sustain rich communities

(Maggi *et al.* 2009). Due to their ecological importance and sensitivity to anthropogenic stressors (Perkol-Finkel & Airoidi 2010; Sales *et al.* 2011), they are a priority conservation habitat (Gianni *et al.* 2013).

We analysed the distribution of canopies of *Cystoseira* spp. (hereafter *Cystoseira*) at a variety of artificial structures and natural reefs in the study region, documenting a consistent scarcity of *Cystoseira* in artificial habitats compared to natural reefs (see Results). Because artificial structures were sometimes very close to the natural reefs (see Study area), therefore allowing for dispersal, and because previous experiments had shown that *Cystoseira* has the potential to successfully settle and grow on artificial substrata even in the absence of an adult canopy (Perkol-Finkel *et al.* 2012), we hypothesized that the scarcity of *Cystoseira* in the artificial habitats would be mainly related to postsettlement factors, including either adverse local environmental conditions (e.g. low water quality) or excess biotic pressure (e.g. grazing or other nonconsumptive disturbance from handling and clipping).

At the natural sites in the study region, *Cystoseira* is mainly limited by poor water quality, high sedimentation rates or degraded habitat characteristics (Irving *et al.* 2009; Perkol-Finkel & Airoidi 2010; Strain *et al.* 2014), while grazing is not a particularly relevant factor. However, previous evidence (Perkol-Finkel *et al.* 2012) lead us to hypothesize that at the artificial sites the biotic pressure could be more important than at the natural sites, exerting proportionally greater control than abiotic factors. We tested this hypothesis by using caging experiments which allowed to: (i) test the relative importance of abiotic vs. biotic factors in influencing the growth of *Cystoseira* at the artificial sites; (ii) identify whether the biotic pressure at the artificial sites was mainly related to mesograzers or macrograzers; (iii) contrast the relative importance of biotic factors in influencing the growth of *Cystoseira* in artificial and natural habitats; and (iv) test the generality of the results across different sites and *Cystoseira* species.

Materials and methods

STUDY AREA

The research was carried out at several sites along 500 km of the north Adriatic Sea (Fig. 1, Table S1 in Supporting Information). The Italian side of the Adriatic is a prevalently sedimentary coastal system and is heavily urbanized. The natural site along the Italian side was located at La Vela (Monte Conero) and was comprised of maerl and limestone rocks extending to a depth of *c.* 8 m. The artificial sites along the Italian side, Marotta, Cesenatico and Punta Marina were located about 40, 120 and 160 km north of La Vela, respectively. These sites were characterized by breakwaters 100–150 m long, deployed 200–300 m from the shore, extending 2–3 m in depth and built with large blocks of quarried rock (1–3 m across). Descriptions of both artificial and natural habitats can be found in Perkol-Finkel & Airoidi (2010) and Perkol-Finkel *et al.* (2012).

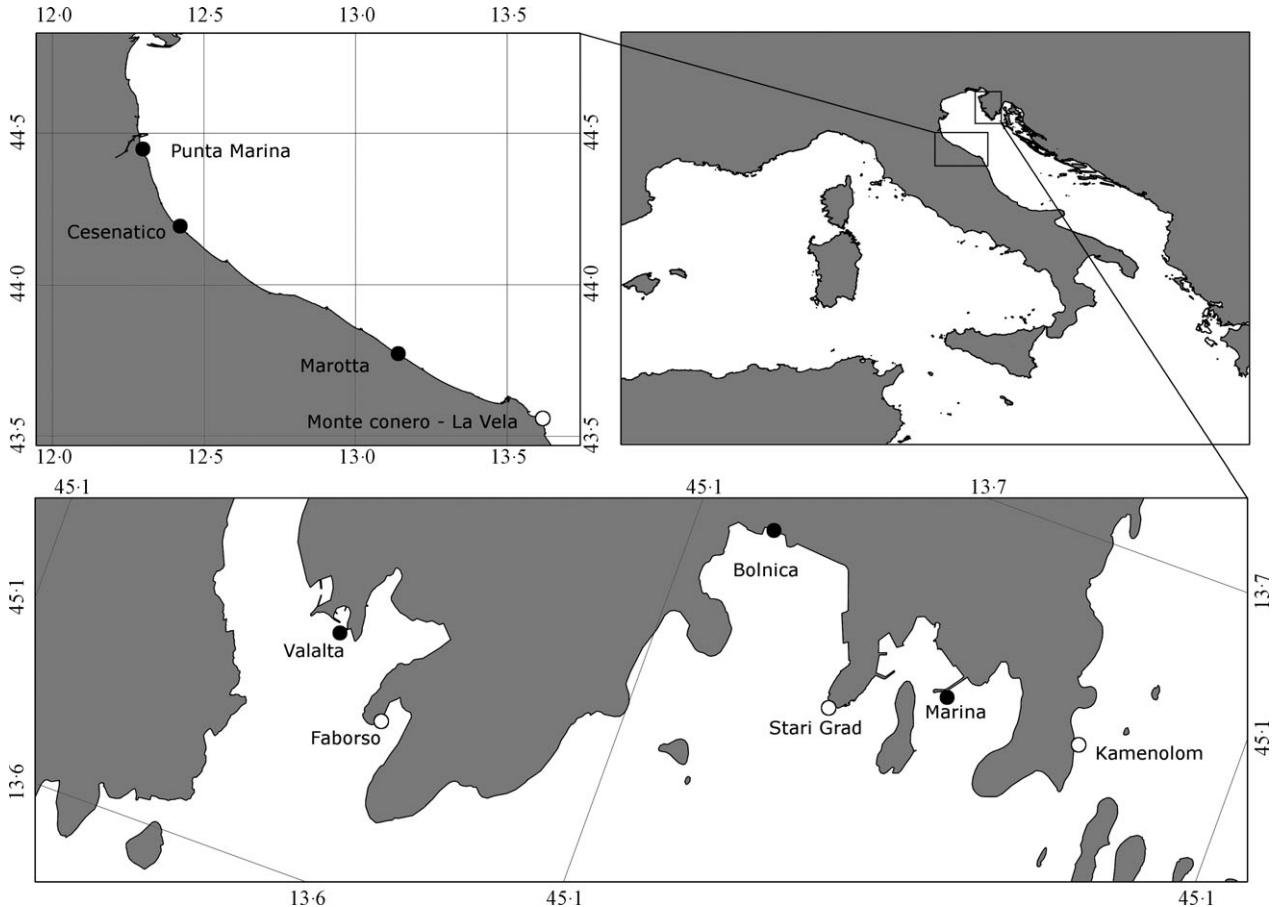


Fig. 1. Location of the study region (Adriatic Sea) and insert maps showing the position of the study sites (○, natural reef; ●, artificial reef) along the Italian (upper left) and Croatian coasts (bottom).

The Croatian side of the Adriatic is a prevalingly rocky limestone coast and compared to the Italian side is far less urbanized. Experiments along the Croatian side were run at three natural rocky sites and three artificial sites (Fig. 1, Table S1, Fig. S1). The natural sites (Faborso, Kamenolom and Stari Grad) were characterized by gently sloping, rocky bottoms densely forested by *Cystoseira* (Iveša, Lyons & Devescovi 2009). The three artificial sites (Valalta, Bolnica and Marina) were interspersed with natural sites, and generally located very close to them. All artificial structures were built with boulders of the same type of quarried rock (Fig. S1).

DISTRIBUTION OF *CYSTOSEIRA* AT ARTIFICIAL AND NATURAL HABITATS

The distribution of species of *Cystoseira* at some artificial and natural sites along the Adriatic Italian coast had been quantified in previous studies (Perkol-Finkel & Airoidi 2010; Perkol-Finkel *et al.* 2012). These observations indicated that *Cystoseira* rarely occurred on artificial substrata in this area. To analyse the generality/consistency of this pattern, in October 2010, we quantified the distribution of *Cystoseira* at the three replicated artificial and natural sites along the Croatian coastline (Fig. 1, Table S1, Fig. S1), where artificial structures were well interspersed with rocky reefs. As typical for that time of the year, *Cystoseira* had started losing branches, but thalli were still fully visible. At each

site, we quantified the percentage cover of *Cystoseira* in five replicated quadrats. We used a grid of 25 squares in a 25 × 25 cm frame; in each square, the coverage of *Cystoseira* was scored from 0 to 4, summing up to 100%.

FACTORS LIMITING THE GROWTH OF *CYSTOSEIRA* IN ARTIFICIAL HABITATS

We tested the hypothesis that the observed limited growth of *Cystoseira* at the artificial sites was related to high biotic pressure (e.g. from grazers or other nonconsumptive disturbance) rather than unfavourable environmental factors by using caging experiments. In these first sets of experiments, we used cages of different mesh size to explore which type of grazers could affect *Cystoseira* at the artificial sites. Since *Cystoseira* is scarce at the artificial sites, we used tiles holding 5–6 juveniles of *C. barbata* from La Vela (Appendix S1; Fig. S2).

The first experiment used cages of 10-mm mesh (hereafter MA) to exclude potential macrograzers and 1-mm mesh (hereafter ME) to also exclude potential mesograzers. Cages (12 × 12 × 25 cm) were built using a plastic-coated iron wire mesh with a 10-mm² grid. ME cages were obtained by further wrapping with a mosquito net. In July 2010, at one of the breakwaters at Marotta, we randomly assigned five replicate experimental tiles to each of three treatments: ME, MA and uncaged (Fig. S3). Both tiles and cages were attached to the rock using epoxy putty. We did not

include a partial-cage control of potential artefacts associated with caging because at this initial stage, the potential grazers on *Cystoseira* were uncertain making it difficult to design a partial control treatment that we could ensure was effective. To minimize any potential artefacts associated with the potential shelter offered by cages on hydrodynamics, the experiment was run during 2 weeks of very calm sea conditions and was set up at the sheltered, landward side of the breakwater. *C. barbata* grows well under sheltered conditions (Perkol-Finkel *et al.* 2012), and we felt that the combination of shelter and calm sea was sufficient to minimize any relevant effects of cages on hydrodynamics.

The experiment lasted 13 days, as responses to treatments were very rapid. We measured the average height of all juveniles at the beginning and at the end of the experiment and calculated the average final height, expressed as the percentage of the average initial height, as the response variable. Data were analysed by one-way permutational ANOVA with factor Exclusion (fixed, 3 levels: MA, ME and uncaged). Three plots were lost during the experiment, and the final number of replicates varied from 3 to 5 per treatment. In this and the following analyses, we used the statistical package PERMANOVA+ for PRIMER (Anderson, Gorley & Clarke 2008) to partition the variability and obtain *F*-statistics on matrices of Euclidean distances calculated from the original data and calculated *P*-values using 9999 random permutations. We used PERMANOVA (as opposed to a classic ANOVA test) due to ease of use with unbalanced design and to avoid the usual normality assumptions. Data had homogeneous variances [Levene' (1960) univariate test run using PERMDISP (Anderson, Gorley & Clarke 2008)], and there was no need for transformation.

In August 2010, we ran a second caging experiment to discriminate between macrograzers of different sizes (the previous experiment had shown no relevant role of mesograzers) and to test for the generality of results at replicated artificial sites. The MA treatment was modified to prevent large macrograzers but allow access by small macrograzers (hereafter LMA: 10-mm mesh as before, with 6 × 7 cm openings cut on each side and on the top of the cage). The ME treatment was unaltered, therefore preventing all grazers. At each of three replicated artificial sites (Marotta, Cesenatico and Punta Marina), we assigned five experimental tiles to each of three treatments (ME, LMA and uncaged) attaching them at the landward side of one breakwater as previously described. After 15 days, we assessed the status of juveniles. Because the losses in uncaged treatments were even more severe than in the previous experiment, we measured responses as percentage survival rather than length of juveniles. Data were analysed by two-way, permutational ANOVA with factors Exclusion (fixed, three levels: M = all grazers excluded; LMA = only small macrograzers allowed; and uncaged = access allowed to all macrograzers) and Site (random, three levels: Marotta, Cesenatico, Punta Marina). Data had homogeneous variances, and there was no need for transformation. The nonsignificant interaction term Exclusion × Site was pooled with residuals following Underwood (1997) and Anderson, Gorley & Clarke (2008).

COMPARISON OF BIOTIC PRESSURE BETWEEN ARTIFICIAL AND NATURAL HABITATS AND DIFFERENT SPECIES OF *CYSTOSEIRA*

In October 2010, we started a larger caging experiment to contrast the relative importance of biotic factors in controlling the

growth of canopy-forming algae in artificial and natural habitats and test the generality of results across different sites and species of *Cystoseira*. The experiment was run in Croatia, where replicated artificial and natural sites were properly interspersed. Here, canopy-forming algae were virtually absent at artificial sites (see 'Results'), but *C. compressa* had been occasionally reported (L. Iveša pers. obs.). We hypothesized that responses might vary among different species of *Cystoseira*, with *C. compressa* showing a greater growth potential in artificial habitats compared to other species.

We used 96 experimental tiles (Appendix S1) colonized by juveniles of either *C. barbata* or *C. compressa*, for a total of 48 independent tiles for each species. We fixed eight tiles for each species at each of three replicated natural rocky sites and three artificial sites. Four random tiles for each species were caged using the same 10-mm mesh size cage described previously (MA) while the remaining four were left uncaged. We used MA cages, as by the start of this experiment, we knew that *Cystoseira* was affected by a pool of macrograzers of different habits and sizes, including fishes, crabs and hermit crabs. Partial control of the caging treatment would have required a cage with so many openings (to allow access to the variety of species involved) to become virtually nonexistent, thus it was not included. As an alternative, we deemed it more effective to quantify differences in biotic interactions with *Cystoseira* at both artificial and natural sites using videos (F. Ferrario, E.M.A. Strain, P. Guidetti and L. Airolidi, unpublished data). We regularly cleaned the cages and removed fouling organisms to minimize any alteration to light or hydrodynamics.

The plots were sampled at the start of the study, in December 2010, April 2011 and October 2011 by using digital photographs. Pictures were analysed in the laboratory by superimposing a digital grid (100 squares) and estimating the percentage cover of either species of *Cystoseira* as the number of quadrats occupied. Due to significant differences in the percentage cover of *C. barbata* and *C. compressa* on the tiles at the start of the experiment, we analysed the data from the two species separately. For each species, we ran a three-way permutational ANOVA including factors Habitat (Fixed, two levels: Artificial vs. Natural), Exclusion (Fixed, two levels: MA vs. uncaged) and Site (Random, three levels, nested in Habitat). Between April 2011 and October 2011, the last two sampling dates, there was a large discontinuity in the data. To highlight this variation, we analysed percentage cover data from both dates. Variances were heterogeneous except for *C. compressa* data in April 2011. Whenever variances were heterogeneous, we used a conservative test, setting the significance level at *P* = 0.01.

Results

DISTRIBUTION OF *CYSTOSEIRA* AT ARTIFICIAL AND NATURAL HABITATS

Along the Croatian coast, stands of *C. barbata*, *C. compressa* and *C. crinita* were present at natural reefs at Faborso (total cover = 24 ± 2.1%, mean ± SE), Stari Grad (total cover 73 ± 4.6%) and Kamenolom (total cover 27.4 ± 5.1%). No *Cystoseira* was detected at the three artificial sites, even if in the past it had been occasionally detected (Iveša pers obs).

Along the Italian coast, only *C. barbata* and *C. compressa* were present on natural rocky reefs at La Vela, covering < 15% of the bedrocks between 2 and 4 m (Perkol-Finkel & Airoidi 2010). During the present study, we never observed *Cystoseira* growing naturally at any artificial Italian study sites, even if *Cystoseira* has been observed at some artificial sites in the region (Perkol-Finkel *et al.* 2012).

FACTORS LIMITING THE GROWTH OF *CYSTOSEIRA* IN ARTIFICIAL HABITATS

At the artificial sites, uncaged juveniles of *C. barbata* showed rapid, severe declines while caged juveniles survived and grew larger, consistent with the prediction of a primary role of biotic factors in limiting the growth of *Cystoseira* at the artificial sites. In the first experiment at Marotta, the length of thalli among uncaged *C. barbata* juveniles declined by 77% within 2 weeks, while length increased in the two caged treatments (Fig. 2a, PERMANOVA: *Pseudo-F*_(d.f. = 2,9) = 79.24, *P* = 0.0003). There were no differences between MA and ME treatments (Fig. 2a, Pairwise test for:

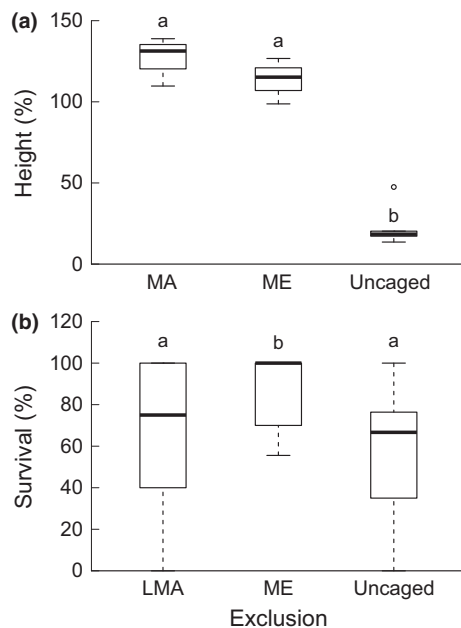


Fig. 2. Caging experiments at artificial habitats. (a) Percentage of initial height of *Cystoseira barbata* juveniles in the treatments: macrograzer exclusion (MA, *n* = 4), mesograzers exclusion (ME, *n* = 3) and Uncaged (*n* = 5) 2 weeks after the start of the experiment in Marotta (July 2010). (b) Percentage survival of *C. barbata* juveniles in the treatments Large macrograzer exclusion (LMA, *n* = 13), Mesograzers exclusion (ME, *n* = 13) and Uncaged (*n* = 15) 2 weeks after the start of the experiment (August 2010). Box plots are drawn for each treatment: (a) from Marotta and (b) from the three sites (Punta Marina, Cesenatico and Marotta). The horizontal line in each box is the median, the boxes define the hinge (25–75% quartile, and the line is 1.5 times the hinge). Points outside this interval are represented as dots. Letters indicate statistically different groups (*P* < 0.05, 9999 permutations).

Uncaged-MA $t_{(d.f. = 1,7)} = 11.8$, *P* = 0.008; Uncaged-ME $t_{(d.f. = 1,6)} = 8.9$, *P* = 0.02; MA-ME $t_{(d.f. = 1,5)} = 1.42$, *P* = 0.22), therefore excluding a relevant role of mesograzers.

Results were confirmed by the second experiment, where survival of juveniles was consistently lower in uncaged treatments at the three study locations (Fig. 2b and Table 1). Two weeks after the start of the experiment, only 55% of uncaged juveniles survived compared to 89% of juveniles in ME plots (Fig. 2b). Survival of juveniles in LMA plots was statistically similar to that measured in uncaged plots (Fig. 2b, Table 1), suggesting an important biotic pressure from organisms of intermediate size. Indeed, we observed several crabs in LMA treatments, in particular at Punta Marina, where we recorded the lowest survival of juveniles in this treatment.

COMPARISONS OF BIOTIC PRESSURE BETWEEN ARTIFICIAL AND NATURAL HABITATS AND DIFFERENT SPECIES OF *CYSTOSEIRA*

Effects of caging were primarily dependent on the habitat nature, suggesting that cages per se did not introduce relevant artefacts (on e.g. light availability or hydrodynamics stress). Juveniles of both species of *Cystoseira* were rapidly lost in uncaged treatments at all artificial sites, where cover decreased from 48 to 6% for *C. barbata* and from 68 to 24% for *C. compressa* in only 2 months (Fig. 3a,b); canopies never recovered in uncaged treatments, while both species of *Cystoseira* grew slightly in caged plots, further increasing the differences between treatments by October 2011. Conversely, at the natural rocky sites, caging did not affect the survival of either species of *Cystoseira* which both persisted during the first 6 months of the experiment without any difference between caged and uncaged treatments (Fig. 3a,b). This

Table 1. Effects of caging on the survival of *Cystoseira barbata* juveniles at three artificial sites (August 2010)

| Source | d.f. | MS [†] | Pseudo-F | <i>P</i> |
|---------------------------|------|-----------------|----------|-------------|
| Exclusion | 2 | 3928 | 3.39 | 0.04 |
| Site | 2 | 15 | 0.01 | 0.99 |
| Residual | 36 | 1158 | | |
| Pairwise within exclusion | | | <i>t</i> | <i>P</i> |
| LMA vs. ME | | | 2.14 | 0.04 |
| Uncaged vs. LMA | | | 0.63 | 0.54 |
| Uncaged vs. ME | | | 2.93 | 0.01 |

[†]Mean square.

Factors are as follows: Exclusion (Fixed with three levels: Mesograzers exclusion – ME; Large Macrograzer exclusion – LMA; no exclusion – Uncaged) and Site (Random with three levels: Marotta, Cesenatico, Punta Marina). Nonsignificant interaction (Exclusion × Site) was pooled with residuals.

Data were analysed by PERMANOVA, with 9999 permutations on a matrix of Euclidean distances.

Significant effects are in bold.

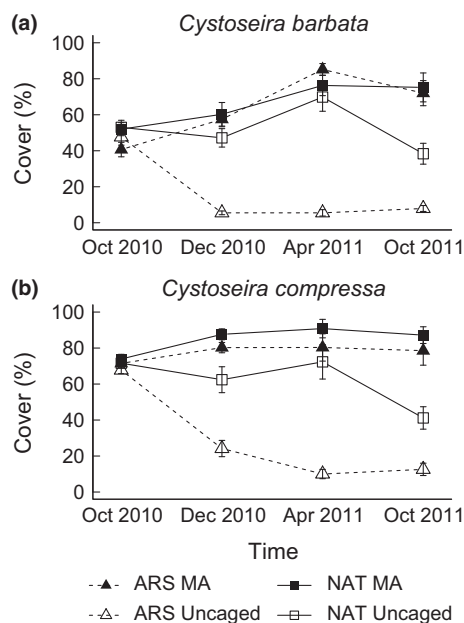


Fig. 3. Caging experiments at artificial and natural habitats. Percentage cover of (a) *Cystoseira barbata* and (b) *C. compressa* in different habitats (artificial = ARS or natural = NAT) and caging treatments (Macrograzer exclusion = MA or Uncaged). Values are averages (± 1 SE) from three sites for each habitat for October 2010, and December, April and October 2011.

difference between habitats was detected by April 2011 as a significant Exclusion by Habitat interaction (Table 2).

Between April and October 2011, both species of *Cystoseira* decreased in uncaged treatments also at natural reef sites. Although by October 2011 the effect of caging was significant at both natural and artificial habitats (Table 2), the difference between caged and uncaged treatments at the artificial sites was twice as large as that at the natural sites, and both species of *Cystoseira* still covered >40% of the space in uncaged reef treatments compared to <10–15% in uncaged artificial treatments.

Discussion

Cystoseira was virtually lacking from all the studied artificial structures, which is consistent with observations from other artificial sites in the Mediterranean Sea, where the presence of canopy-forming algae is hardly reported as an important component of the epibenthos (Falace & Bressan 2002; Vaselli, Bulleri & Benedetti-Cecchi 2008). Previous work had already shown that the poor performance of artificial structures in supporting these ecologically relevant, habitat-forming species was not related to habitat characteristics limiting dispersal or settlement (Perkol-Finkel *et al.* 2012). Here, we further demonstrated that the lack of *Cystoseira* on artificial structures was not even directly related to the poor environmental conditions typical of many marine urban locations (Dugan *et al.* 2011). Indeed, most transplanted juveniles survived perfectly well for longer than a year when protected by cages. Poor

water quality, high nutrient and sediment loads and stagnant conditions can be detrimental for many species of *Cystoseira* (Irving *et al.* 2009; Perkol-Finkel & Airoidi 2010; Strain *et al.* 2014), and it is possible that their effects could have been significant over a time frame longer than the one considered in this study.

Biotic pressure appeared to be the most relevant factor limiting the survival and growth of *Cystoseira* on artificial structures. While biotic pressure had limited to no effects on canopy-forming algae in natural habitats, on artificial structures both *C. barbata* and *C. compressa* persisted only if caging prevented access to grazers. Although we could not include a proper control for artefacts related to caging (i.e. preventing alteration of environmental variables while effectively excluding grazers), we believe that differences observed between caged and uncaged plots in the artificial habitats reflect a true effect of biotic pressure, rather than alterations of other environmental factors. First, on natural rocky reefs, caging had limited to no effect on survival and growth of *Cystoseira*. Secondly, observed responses were consistent across all experiments and sites, thus spanning a variety of hydrodynamic and other environmental conditions. Thirdly, video measures (F. Ferrario, E.M.A. Strain, P. Guidetti and L. Airoidi, unpublished data) clearly showed that *Cystoseira* was subjected to a much greater variety and amount of biotic interactions on artificial structures than on rocky reefs.

Herbivory is known to shape and regulate the benthic algal community in a variety of natural rocky reef systems from temperate to tropical regions (Miller & Hay 1996; Verges, Alcoverro & Ballesteros 2009; Taylor & Schiel 2010), but little is known about its effects on assemblages in artificial habitats (Iveša *et al.* 2010; Marzinelli, Underwood & Coleman 2011). In our experiments, we showed that biotic control on the biota was much stronger in artificial than nearby natural habitats. By using cages of different sizes, we excluded relevant effects by mesograzers (<1 cm), while we observed that both small and large macrograzers were severely limiting the growth of canopy-forming algae. Subsequent video observations clarified that the biotic pressure on *Cystoseira* at the artificial sites resulted from a suite of both 'consumptive' (i.e. a clear feeding behaviour) and 'nonconsumptive' (i.e. algal thalli potentially damaged by handling and clipping without obvious consumption) interactions from a wide range of species, comprising both fishes and crabs of different sizes. Interestingly, most of the interacting species were classified as omnivores rather than as true herbivores (F. Ferrario, E.M.A. Strain, P. Guidetti and L. Airoidi, unpublished data).

The observation that algal assemblages in artificial habitats can be shaped by a stronger biotic pressure than in natural reefs is new, and warrants further investigation with respect to underlying causes. Many of the organisms most frequently interacting with *Cystoseira* on artificial structures were also common at natural rocky sites but exerted different levels of interactions with *Cystoseira* in

Table 2. Relative importance of biotic pressure on the total percentage cover of *Cystoseira barbata* and *C. compressa* in artificial vs. natural habitats

| Source | <i>Cystoseira barbata</i> | | | | | | | | <i>Cystoseira compressa</i> | | | | | | | |
|------------------|---------------------------|-----------------|----------|-------------|--------------|-----------------|----------|-------------|-----------------------------|-----------------|----------|-------------|--------------|-----------------|----------|-------------|
| | April 2011 | | | | October 2011 | | | | April 2011 | | | | October 2011 | | | |
| | d.f. | MS [†] | Pseudo-F | P | d.f. | MS [†] | Pseudo-F | P | d.f. | MS [†] | Pseudo-F | P | d.f. | MS [†] | Pseudo-F | P |
| Habitat = Hab | 1 | 8074 | 16.93 | 0.09 | 1 | 2256 | 4.92 | 0.10 | 1 | 15 507 | 39.22 | 0.00 | 1 | 3341 | 4.23 | 0.11 |
| Exclusion = Exc | 1 | 19 849 | 95.54 | 0.00 | 1 | 26 693 | 26.88 | 0.01 | 1 | 23 062 | 27.17 | 0.01 | 1 | 34 100 | 118.19 | 0.00 |
| Hab × Exc | 1 | 15 440 | 74.32 | 0.00 | 1 | 2758 | 2.78 | 0.18 | 1 | 7826 | 9.22 | 0.04 | 1 | 1186 | 4.11 | 0.12 |
| Site (Hab) | 4 | 480 | 1.59 | 0.20 | 4 | 461 | 1.51 | 0.23 | 4 | 395 | 0.76 | 0.55 | 4 | 796 | 2.22 | 0.09 |
| Site (Hab) × Exc | 4 | 206 | 0.69 | 0.60 | 4 | 1003 | 3.28 | 0.02 | 4 | 849 | 1.64 | 0.18 | 4 | 288 | 0.80 | 0.52 |
| Residual | 34 | 301 | | | 33 | 306 | | | 35 | 517 | | | 32 | 358 | | |

| Pairwise comparison MA vs. Uncaged within levels of Habitat | | | | | | | |
|---|--|--|-------|-------------|------|-------------|--|
| | | | t | P | | | |
| Artificial habitat | | | 13.65 | 0.01 | t | | |
| Natural habitat | | | 0.77 | 0.52 | P | | |
| | | | | | 9.54 | 0.01 | |
| | | | | | 1.19 | 0.36 | |

[†]Mean square.

Factors are as follows: Habitat (Fixed with two levels: Natural vs. Artificial), Exclusion (Fixed with two levels: Macrograzers exclusion = MA vs. no exclusion = Uncaged), Site (Random nested in Hab: three natural rocky sites Faborso, Stari Grad, Kamenolom and three artificial sites at Valalta, Bolnica and Marina). The experiment started in October 2010. The Table reports a separate analysis for April 2011 and October 2011 (see Materials and methods).

Data were analysed by PERMANOVA with 9999 permutations on a Euclidean distance matrix.

Significant effects ($P < 0.05$ for *C. compressa* in April 2011, $P < 0.01$ in all other cases) are in bold.

the two habitats, interacting more frequently at the artificial than at the natural sites (F. Ferrario, E.M.A. Strain, P. Guidetti and L. Airoidi, unpublished data). One possible explanation for these different behaviours is that at natural sites effects of grazing on furoid algae could be mitigated by a larger abundance and variety of more palatable algal species compared to the artificial habitats. Similar hypotheses have been recently proposed to explain the unusually high grazing effects on some algal restoration projects (Campbell *et al.* 2014). Another hypothesis could be that coastal infrastructures represent 'oases' of hard substrata set in a soft bottom environment (Airoidi *et al.* 2005a): such small, isolated, patchy habitats might experience greater pressure from predators than large rocky reefs, similarly to other 'oases' systems such as sea-mounts (Rowden *et al.* 2010). Intense angling/spear fishing on top predators, typical at the artificial structures in this region (Airoidi *et al.* 2005b), could further amplify these effects, leading to greater pressure from herbivores or omnivores. Future work should attempt to quantify differences in grazing pressure at the seascape level, including habitats of different sizes and locations.

Marine artificial structures are becoming extremely common in many coastal areas globally, and they could and should be designed to reduce ecological impacts while maximizing targeted ecosystem services (Dafforn *et al.* 2015). For example, the artificial seeding of canopy-forming algae is a promising technique to enhance the ecological value of artificial structures (Perkol-Finkel *et al.* 2012; Firth *et al.* 2014; Dafforn *et al.*

2015) and for the restoration of damaged canopies (Gianni *et al.* 2013). So far, nearly all ecological considerations in the design of hard marine infrastructures have focused on critical abiotic factors to try to mitigate their impacts and recover lost ecosystem services (Burcharth *et al.* 2007). Different construction materials, surface textures and habitat structural complexities have been tested and are increasingly implemented to help mimic natural habitats, and providing support for desired native biodiversity (Chapman & Underwood 2011; Firth *et al.* 2014). Pools or features mimicking the levels of complexity in natural habitats are used in the design of artificial structures built along rocky coasts to try to facilitate the growth of rich assemblages of species (Martins *et al.* 2010; Firth *et al.* 2013; Coombes *et al.* 2015). In addition to these abiotic factors, we have shown that the ecological performance of artificial structures can be strongly controlled by biotic factors, including biotic pressure but also recruitment and competition, which have also been reported to differ between artificial and natural habitats (Bulleri 2005; Iveša *et al.* 2010; Marzinelli, Underwood & Coleman 2011). For example, the sparse cover of coral species on artificial reefs in Florida was related to a stronger competition by algal turfs than in nearby coral reefs (Miller *et al.* 2009). It is crucial to incorporate such knowledge in the planning, design and operation of current and future marine developments. In our study region, any effort aimed at designing artificial structures to support or restore important ecosystem processes, functions and services, such as those provided by native

populations of canopy-forming algae, should also include appropriate limitation of recreational and artisanal fishery to encourage top predators (Perkol-Finkel *et al.* 2012; Gianni *et al.* 2013), as this would positively cascade down the food web, ultimately facilitating canopy-forming algae. Ecologically sound management of human access and use should, therefore, be considered as one important green engineering tool.

Marine infrastructures are an integral part of urban sprawl. They introduce novel, unique ecosystems in the marine environment (Hobbs, Higgs & Hall 2013), which substantially differ from natural reefs. Ecologically based design of marine infrastructures is possible and necessary, but requires a much deeper understanding of the ecological functioning of these artificial systems and a goal-oriented design (Dafforn *et al.* 2015). Future research should focus on identifying factors, or combinations of factors, that facilitate native, habitat-forming biota and associated ecosystem services. Much greater effort is also needed to identify alternatives to the increasing hardening of marine bottoms. For example, intertidal ecosystems can be integrated in coastal defence, offering more sustainable, cost-effective and ecologically sound alternatives to conventional coastal engineering (Temmerman *et al.* 2013; Ferrario *et al.* 2014).

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Data accessibility

- Study sites location: uploaded as online supporting information
- Experimental data: MERMAID Project Repository at <http://mermaid-madgik.di.uoa.gr/resources/overlooked-role-biotic-factors-controlling-ecological-performance-artificial-marine>

References

- Adams, T.P., Miller, R.G., Aleynik, D. & Burrows, M.T. (2014) Offshore marine renewable energy devices as stepping stones across biogeographical boundaries. *Journal of Applied Ecology*, **51**, 330–338.
- Airoldi, L. & Beck, M.W. (2007) Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology* (eds R.N. Gibson, R.J.A. Atkinson & J.D.M. Gordon), pp. 345–405. Crc Press-Taylor & Francis Group, Boca Raton.
- Airoldi, L. & Bulleri, F. (2011) Anthropogenic disturbance can determine the magnitude of opportunistic species responses on marine urban infrastructures. *PLoS ONE*, **6**, e22985.
- Airoldi, L., Abbiati, M., Beck, M.W., Hawkins, S.J., Jonsson, P.R., Martin, D. *et al.* (2005a) An ecological perspective on the deployment and design of low-crested and other hard coastal defence structures. *Coastal Engineering*, **52**, 1073–1087.
- Airoldi, L., Bacchiocchi, F., Cagliola, C., Bulleri, F. & Abbiati, M. (2005b) Impact of recreational harvesting on assemblages in artificial rocky habitats. *Marine Ecology Progress Series*, **299**, 55–66.
- Airoldi, L., Turon, X., Perkol-Finkel, S. & Rius, M. (2015) Corridors for aliens but not for natives: effects of marine urban sprawl at a regional scale. *Diversity and Distributions*, **21**, 755–768.
- Anderson, M., Gorley, R. & Clarke, K.R. (2008) *PERMANOVA+ for PRIMER. Guide to Software and Statistical Methods*. PRIMER-E, Plymouth.
- Brickhill, M.J., Lee, S.Y. & Connolly, R.M. (2005) Fishes associated with artificial reefs: attributing changes to attraction or production using novel approaches. *Journal of Fish Biology*, **67**, 53–71.
- Browne, M.A. & Chapman, M.G. (2011) Ecologically informed engineering reduces loss of intertidal biodiversity on artificial shorelines. *Environmental Science & Technology*, **45**, 8204–8207.
- Bulleri, F. (2005) Experimental evaluation of early patterns of colonisation of space on rocky shores and seawalls. *Marine Environmental Research*, **60**, 355–374.
- Bulleri, F. & Chapman, M.G. (2010) The introduction of coastal infrastructure as a driver of change in marine environments. *Journal of Applied Ecology*, **47**, 26–35.
- Bulleri, F., Airoldi, L., Branca, G.M. & Abbiati, M. (2006) Positive effects of the introduced green alga, *Codium fragile* ssp *tomentosoides*, on recruitment and survival of mussels. *Marine Biology*, **148**, 1213–1220.
- Burcharth, H.F., Hawkins, S.J., Zanuttigh, B. & Lamberti, A. (2007) *Environmental Design Guidelines for Low Crested Coastal Structures*. Elsevier, Oxford.
- Burt, J., Bartholomew, A., Bauman, A., Saif, A. & Sale, P.F. (2009) Coral recruitment and early benthic community development on several materials used in the construction of artificial reefs and breakwaters. *Journal of Experimental Marine Biology and Ecology*, **373**, 72–78.
- Campbell, A.H., Marzinelli, E.M., Vergés, A., Coleman, M.A. & Steinberg, P.D. (2014) Towards restoration of missing underwater forests. *PLoS ONE*, **9**, e84106.
- Chapman, M.G. & Underwood, A.J. (2011) Evaluation of ecological engineering of “armoured” shorelines to improve their value as habitat. *Journal of Experimental Marine Biology and Ecology*, **400**, 302–313.
- Clynick, B.G., Chapman, M.G. & Underwood, A.J. (2007) Effects of epibiota on assemblages of fish associated with urban structures. *Marine Ecology Progress Series*, **332**, 201–210.
- Coombes, M.A., La Marca, E.C., Naylor, L.A. & Thompson, R.C. (2015) Getting into the groove: Opportunities to enhance the ecological value of hard coastal infrastructure using fine-scale surface textures. *Ecological Engineering*, **77**, 314–323.
- Dafforn, K.A., Glasby, T.M. & Johnston, E.L. (2012) Comparing the invasibility of experimental “reefs” with field observations of natural reefs and artificial structures. *PLoS ONE*, **7**, e38124.
- Dafforn, K.A., Glasby, T.M., Airoldi, L., Rivero, N.K., Mayer-Pinto, M. & Johnston, E.L. (2015) Marine urbanization: an ecological framework for designing multifunctional artificial structures. *Frontiers in Ecology and the Environment*, **13**, 82–90.
- Dugan, J.E., Airoldi, L., Chapman, M.G., Walker, S.J., Schlacher, T., Editors-in-Chief: Eric, W. & Donald, M. (2011) 8.02 - Estuarine and coastal structures: environmental effects, a focus on shore and nearshore structures. *Treatise on Estuarine and Coastal Science* (eds E. Wolanski & D. McLusky), pp. 17–41. Academic Press, Waltham.
- Falace, A. & Bressan, G. (2002) A qualitative and quantitative analysis of the evolution of macroalgal vegetation on an artificial reef with anti-grazing nets (Loano-Ligurian Sea). *Ices Journal of Marine Science*, **59**, S150–S156.
- Fauvelot, C., Bertozzi, F., Costantini, F., Airoldi, L. & Abbiati, M. (2009) Lower genetic diversity in the limpet *Patella caerulea* on urban coastal structures compared to natural rocky habitats. *Marine Biology*, **156**, 2313–2323.
- Ferrario, F., Beck, M.W., Storlazzi, C.D., Micheli, F., Shepard, C.C. & Airoldi, L. (2014) The effectiveness of coral reefs for coastal hazard risk reduction and adaptation. *Nature Communications*, **5**, 3794.
- Firth, L.B., Thompson, R.C., White, F.J., Schofield, M., Skov, M.W., Hoggart, S.P.G., Jackson, J., Knights, A.M. & Hawkins, S.J. (2013) The importance of water-retaining features for biodiversity on artificial intertidal coastal defence structures. *Diversity and Distributions*, **19**, 1275–1283.
- Firth, L.B., Thompson, R.C., Bohn, K., Abbiati, M., Airoldi, L., Bouma, T.J. *et al.* (2014) Between a rock and a hard place: environmental and

- engineering considerations when designing coastal defence structures. *Coastal Engineering*, **87**, 122–135.
- Gianni, F., Bartolini, F., Airolidi, L., Ballesteros, E., Francour, P., Guidetti, P., Meinesz, A., Thibaut, T. & Mangialajo, L. (2013) Conservation and restoration of marine forests in the Mediterranean Sea and the potential role of marine protected areas. *Advances in Oceanography and Limnology*, **4**, 83–101.
- Glasby, T.M., Connell, S.D., Holloway, M.G. & Hewitt, C.L. (2007) Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? *Marine Biology*, **151**, 887–895.
- Hobbs, R.J., Higgs, E.S. & Hall, C. (2013) *Novel Ecosystems: Intervening in the New Ecological World Order*. Wiley-Blackwell, Oxford.
- Irving, A., Balata, D., Colosio, F., Ferrando, G. & Airolidi, L. (2009) Light, sediment, temperature, and the early life-history of the habitat-forming alga *Cystoseira barbata*. *Marine Biology*, **156**, 1223–1231.
- Iveša, L., Lyons, D.M. & Devescovi, M. (2009) Assessment of the ecological status of north-eastern Adriatic coastal waters (Istria, Croatia) using macroalgal assemblages for the European Union Water Framework Directive. *Aquatic Conservation-Marine and Freshwater Ecosystems*, **19**, 14–23.
- Iveša, L., Chapman, M.G., Underwood, A.J. & Murphy, R.J. (2010) Differential patterns of distribution of limpets on intertidal seawalls: experimental investigation of the roles of recruitment, survival and competition. *Marine Ecology Progress Series*, **407**, 55–69.
- Levene, H. (1960) Robust tests for equality of variances. *Contributions to Probability and Statistics* (eds S.G. Ghurye, I. Olkin, W. Hoeffding, W.G. Madow & H.B. Mann), pp. 278–292. Stanford University Press, Stanford, CA.
- Maggi, E., Bertocci, I., Vaselli, S. & Benedetti-Cecchi, L. (2009) Effects of changes in number, identity and abundance of habitat-forming species on assemblages of rocky seashores. *Marine Ecology Progress Series*, **381**, 39–49.
- Martins, G.M., Thompson, R.C., Neto, A.I., Hawkins, S.J. & Jenkins, S.R. (2010) Enhancing stocks of the exploited limpet *Patella candei* d'Orbigny via modifications in coastal engineering. *Biological Conservation*, **143**, 203–211.
- Marzinelli, E.M., Underwood, A.J. & Coleman, R.A. (2011) Modified habitats influence kelp epibiota via direct and indirect effects. *PLoS ONE*, **6**, e21936.
- Miller, M.W. & Hay, M.E. (1996) Coral-seaweed-grazer-nutrient interactions on temperate reefs. *Ecological Monographs*, **66**, 323–344.
- Miller, M.W., Valdivia, A., Kramer, K.L., Mason, B., Williams, D.E. & Johnston, L. (2009) Alternate benthic assemblages on reef restoration structures and cascading effects on coral settlement. *Marine Ecology Progress Series*, **387**, 147–156.
- Perkol-Finkel, S. & Airolidi, L. (2010) Loss and recovery potential of marine habitats: an experimental study of factors maintaining resilience in subtidal algal forests at the Adriatic sea. *PLoS ONE*, **5**, e10791.
- Perkol-Finkel, S. & Sella, I. (2014) Ecologically active concrete for coastal and marine infrastructure: innovative matrices and designs. *From Sea to Shore - Meeting the Challenges of the Sea* (eds W. Allsop & K. Burgess), pp. 1139–1149. ICE Publishing, London.
- Perkol-Finkel, S., Shashar, N. & Benayahu, Y. (2006) Can artificial reefs mimic natural reef communities? The roles of structural features and age. *Marine Environmental Research*, **61**, 121–135.
- Perkol-Finkel, S., Ferrario, F., Nicotera, V. & Airolidi, L. (2012) Conservation challenges in urban seascapes: promoting the growth of threatened species on coastal infrastructures. *Journal of Applied Ecology*, **49**, 1457–1466.
- Rowden, A.A., Schlacher, T.A., Williams, A., Clark, M.R., Stewart, R., Althaus, F. et al. (2010) A test of the seamount oasis hypothesis: seamounts support higher epibenthic megafaunal biomass than adjacent slopes. *Marine Ecology-an Evolutionary Perspective*, **31**, 95–106.
- Sales, M., Cebrian, E., Tomas, F. & Ballesteros, E. (2011) Pollution impacts and recovery potential in three species of the genus *Cystoseira* (Fucales, Heterokontophyta). *Estuarine Coastal and Shelf Science*, **92**, 347–357.
- Strain, E.M.A., Thomson, R.J., Micheli, F., Mancuso, F.P. & Airolidi, L. (2014) Identifying the interacting roles of stressors in driving the global loss of canopy-forming to mat-forming algae in marine ecosystems. *Global Change Biology*, **20**, 3300–3312.
- Taylor, D.I. & Schiel, D.R. (2010) Algal populations controlled by fish herbivory across a wave exposure gradient on southern temperate shores. *Ecology*, **91**, 201–211.
- Temmerman, S., Meire, P., Bouma, T.J., Herman, P.M.J., Ysebaert, T. & De Vriend, H.J. (2013) Ecosystem-based coastal defence in the face of global change. *Nature*, **504**, 79–83.
- Underwood, A.J. (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge.
- Vaselli, S., Bulleri, F. & Benedetti-Cecchi, L. (2008) Hard coastal-defence structures as habitats for native and exotic rocky-bottom species. *Marine Environmental Research*, **66**, 395–403.
- Verges, A., Alcoverro, T. & Ballesteros, E. (2009) Role of fish herbivory in structuring the vertical distribution of canopy algae *Cystoseira* spp. in the Mediterranean Sea. *Marine Ecology Progress Series*, **375**, 1–11.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Details of preparation of experimental tiles.

Table S1. Details of study sites.

Figure S1. Representative photographs of natural rocky reefs and artificial habitats.

Figure S2. Examples of experimental tiles used.

Figure S3. Representative photographs of experimental treatments.