BIODIVERSITY RESEARCH

Corridors for aliens but not for natives: effects of marine urban sprawl at a regional scale

Laura Airoldi[1,2], Xavier Turon[3], Shimrit Perkol-Finkel[1,4] and Marc Rius[5]*

[1]*Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, University of Bologna, Via S. Alberto 163, I-48123 Ravenna, Italy,* [2]*Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950, USA,* [3]*Center for Advanced Studies of Blanes (CEAB-CSIC), Accés a la Cala S. Francesc 14, 17300, Blanes (Girona), Spain,* [4]*SeArc - Ecological Marine Consulting, 13 Namirover St., Tel Aviv 69713, Israel,* [5]*Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton, European Way, SO14 3ZH, UK*

ABSTRACT

Aim The global sprawl of marine hard infrastructure (e.g. breakwaters, sea walls and jetties) can extensively modify coastal seascapes, but the knowledge of such impacts remains limited to local scales. We examined the regional-scale effects of marine artificial habitats on the distribution and abundance of assemblages of ascidians, a key group of ecosystem engineer species in benthic fouling systems.

Location Five hundred kilometers of coastline in the North Adriatic Sea.

Methods We sampled a variety of natural reefs, marine infrastructures and marinas, and tested hypotheses about the role of habitat type and location in influencing the relative distribution and abundance of both native and non-indigenous species.

Results Assemblages differed significantly between natural and artificial habitats and among different types of artificial habitats. Non-indigenous species were 2–3 times more abundant on infrastructures built along sedimentary coastlines than on natural rocky reefs or infrastructures built close to rocky coastlines. Conversely, native species were twice as abundant on natural reefs than on nearby infrastructures and were scarce to virtually absent on infrastructures built along sedimentary coasts. The species composition of assemblages in artificial habitats was more similar to that of marinas than of natural reefs, independently of their location.

Main conclusions Our results show that marine infrastructures along sandy shores disproportionally favour non-indigenous over native hard bottom species, affecting their spread at regional scales. This is particularly concerning for coastal areas that have low natural densities of rocky reef habitats. We discuss design and management options to improve the quality as habitat of marine infrastructures and to favour their preferential use by native species over non-indigenous ones.

*Correspondence: Marc Rius, Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton, European Way, SO14 3ZH, UK.
E-mail: M.Rius@soton.ac.uk

INTRODUCTION

Urban sprawl is one of the most extreme and widespread human impacts (McKinney, 2006) and can lead to species extinctions, severe landscape changes and homogenization of biota at local, regional and global scales (McKinney & Lock-wood, 1999). Sprawl has dramatically expanded across marine seascapes (Airoldi & Beck, 2007; Bulleri & Chapman, 2010). Large coastal and marine areas of Europe, North America, Asia and Australia are nowadays covered by sea walls, dykes, breakwaters, groynes, jetties, pilings, bridges, artificial reefs, offshore platforms and energy installations

(Dugan *et al.*, 2011; Feary *et al.*, 2011). It is expected that constructions will further accelerate as a result of burgeoning coastal populations, greater threats from climate change, storm surges and sea level rise and renewable energy production, which is one of the fastest growing industries on the seafloor (Inger *et al.*, 2009; Witt *et al.*, 2012).

The construction of marine infrastructure typically involves the replacement of natural, most often sedimentary, substrata with harder surfaces such as stone, concrete, asphalt, metal or other artificial material (Airoldi *et al.*, 2009; Witt *et al.*, 2012). These habitat modifications have altered the distribution of a number of species, some of which can thrive on these anthropogenic surfaces. In the Wadden Sea, for example, *c.* 730 km of artificial structures (harbours, causeways, dikes, piers and breakwaters) has introduced *c.* 2–4 km^2 of hard surfaces for colonization of rocky bottom species otherwise rare or absent in such sedimentary environments (Reise, 2005). In the northern Gulf of Mexico, *c.* 4000 oil and gas platforms have enhanced the distribution of coral populations into areas where they were previously absent (Sammarco *et al.*, 2004). For this reason, marine infrastructures are increasingly perceived as an opportunity for habitat enhancement, providing local benefits associated to hard substrata where none previously existed, or potential refugia for rare or threatened native rocky species (Inger *et al.*, 2009; Martins *et al.*, 2010; Langhamer, 2012; Perkol-Finkel *et al.*, 2012). At the same time, the long-term and regional consequences of the extensive sprawl of these artificial habitats are debated (Airoldi *et al.*, 2005b; Feary *et al.*, 2011; Fauvelot *et al.*, 2012; Witt *et al.*, 2012).

The ecological value of artificial structures as habitats for native species can vary in relation to many structural and environmental factors (Moschella *et al.*, 2005; Burt *et al.*, 2009a; Dugan *et al.*, 2011; Perkol-Finkel *et al.*, 2012; Firth *et al.*, 2014). Further, marine infrastructures seem to be particularly susceptible to colonization of non-indigenous species (Bulleri & Airoldi, 2005; Glasby *et al.*, 2007; Vaselli *et al.*, 2008; Dafforn *et al.*, 2012; Mineur *et al.*, 2012). Non-indigenous species may spread out from harbours, marinas or other sources, but the seascape connection between primary entry points and other suitable habitats (either natural or artificial) remains understudied (Floerl *et al.*, 2009; Simkanin *et al.*, 2012), in spite of the fact that these connections may greatly enhance extrarange distribution of introduced species. The capability of rocky bottom species to colonize seascapes increasingly modified by the sprawl of artificial habitats has hardly been assessed at regional scales (Dethier *et al.*, 2003; Bulleri & Airoldi, 2005; Adams *et al.*, 2014). Such knowledge would have enormous management applications in increasingly urbanized marine regions, allowing the adjustment of decisions concerning the design and spatial planning of infrastructures.

In this study, we sampled natural reefs, marine artificial structures (mainly coastal defence structures) and marinas along an extensive coastline to test the hypotheses about the role of habitat type and location in influencing the abundance and distribution of native and non-indigenous species. We focused on assemblages of ascidians, a key ecological group in marine benthic fouling systems (Lambert, 2005; Simkanin *et al.*, 2012); some of these species are invasive and have caused severe ecological and economic impacts in receiving ecosystems either artificial (such as shellfish farms, Ramsay *et al.*, 2008; Rius *et al.*, 2011) or natural, where they have altered native communities (Castilla *et al.*, 2004). We hypothesized that artificial habitats would harbour different, less rich and less diverse assemblages compared with natural reefs and that non-indigenous species, if any, would prevail in artificial habitats, while native species would dominate natural reefs. Artificial infrastructures, such as breakwaters, introduce large amounts of sheltered hard habitats (i.e. at the landward sides), which can be relatively rare in nature compared with exposed habitats and support very different assemblages (Bacchiocchi & Airoldi, 2003; Bulleri & Airoldi, 2005; Burt *et al.*, 2010). Also, the suitability of marine infrastructure to be colonized by rocky bottom species can vary a lot depending on their environmental setting (i.e. whether they are built along a rocky or a sandy coastline) and location (Burt *et al.*, 2009a; Perkol-Finkel *et al.*, 2012). Therefore, we further explored how these two relevant characteristics of the artificial habitats (hereafter exposure and coastline type, respectively) would affect the distribution of both native and non-native species. We finally hypothesized that the species composition of assemblages in artificial habitats would be more similar to that of marinas (i.e. source of non-indigenous propagules) than of natural habitats, independently of their characteristics or location.

METHODS

Study area

The Italian North Adriatic coastline provides an excellent example of a marine system where the extensive construction of artificial infrastructures along predominantly sandy shorelines has modified the relative proportion of artificial versus natural habitats (Bacchiocchi & Airoldi, 2003). The northwestern Adriatic Sea comprises a sandy coastline which extends almost uninterrupted for *c.* 400 Km between the rocky coasts of Croatia and the promontory of Monte Conero (Fig. 1). The area has high seasonal variability, sharp stratification and very high productivity rates (Zavatarelli *et al.*, 1998) and represents a distinct biogeographical region (Bianchi, 2007). It is characterized by moderate exposure to wave action and an average tidal amplitude of about 50–130 cm. Average sea surface temperatures vary between 8 °C in winter and 24 °C in summer, and average salinities vary between 34 and 37 psu (Zavatarelli *et al.*, 1998).

A variety of infrastructures have been built in the past 50 years, for harbours, ports and marinas and for protection of the highly urbanized coastline (Airoldi & Beck, 2007). Nowadays > 200 km of groynes, breakwaters, sea walls and jetties provide the greatest amount of hard substrata in the
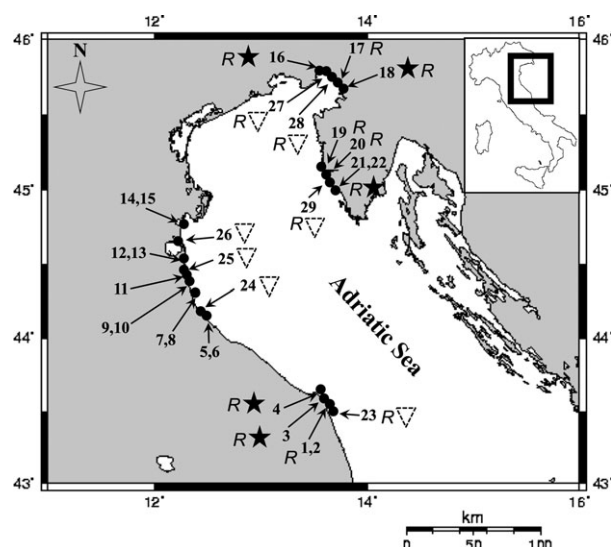
Figure 1 Map of the Northern Adriatic Sea coastline with study sites. Numbers represent site numbers as in Table 1. *Stars* indicate natural reefs, the letter *R* rocky coastlines, *triangles* marinas and *dots without a triangle or a star* artificial habitats.

region. Previous work on these infrastructures has documented the local prevalence of assemblages characterized by low species and genetic diversity (Bacchiocchi & Airoldi, 2003; Fauvelot *et al.*, 2009), comprising opportunistic and non-indigenous species (Bulleri *et al.*, 2005b; Airoldi & Bulleri, 2011), but the changes in species distributions have not been analysed in a broader regional context.

Studied taxa

Ascidians (Tunicata, Chordata) are sessile organisms that are often a key ecological group in marine benthic systems (Gili & Coma, 1998; Castilla *et al.*, 2000). They can become harmful marine invaders (see Castilla *et al.*, 2004; Ramsay *et al.*, 2008; Rius *et al.*, 2011) and thrive in all latitudes world-wide (Shenkar & Swalla, 2011). Introductions of non-indigenous ascidians into harbours in both tropical and temperate waters are common (Cohen *et al.*, 2005; Rius *et al.*, 2014), and ascidians successfully colonize a variety of artificial marine infrastructures (Lambert, 2005; Arenas *et al.*, 2006a; Ordóñez *et al.*, 2013). Ascidians have short-lived lecithotrophic larvae (Svane & Young, 1989), which limit their natural dispersal capabilities. Thus, pre-border dispersal (from overseas source regions *sensu* Forrest *et al.*, 2009) is ensured to be exclusively as a result of human-mediated introductions (Locke, 2009; Pineda *et al.*, 2011; Rius *et al.*, 2012).

Field samplings

In summer 2008, we surveyed assemblages of ascidians at 22 coastal sites (about 200 m of hard substrata) along about 500 km of coastline. The survey was designed to comprise sites on natural reefs or artificial habitats; the latter were set

on structures built either adjacent to natural reefs (hereafter 'artificial rocky') or in sandy areas far from natural reefs (hereafter 'artificial sandy') and comprised either exposed or sheltered artificial habitats (Table 1 and Fig. 1). It was not possible to maintain the number of replicated sites balanced because sites with accessible artificial structures close to natural reefs are infrequent in the region, and they tend to be mainly sea walls, which lack sheltered surfaces. We only examined differences in relation to exposure for artificial habitats, while we did not include a comparison between differently exposed natural and artificial habitats because sheltered natural rocky reefs are virtually absent in the region, and because the exposure of natural reefs is not comparable to that of artificial breakwaters, as these last ones are built specifically to absorb most of the wave energy.

At each site assemblages of ascidians were sampled by SCUBA diving in six replicated 2 m^2 plots (1 m wide × 2 m long) set randomly at depths of about 2 m, except for one site (Punta Marina) where only five plots were sampled. This large plot size was chosen to allow detection of rare species, which is important when trying to describe the distribution of potentially newly arrived, introduced species. To assist the sampling over such large plot surfaces, each plot was subdivided into eight subplots 0.5 × 0.5 m each. The abundance of ascidians in each subplot was quantified visually using a metal frame and ranked as 0 = absent, 1 = scarce (< 10% cover), 2 = common (10–50% cover), 3 = dominant (> 50% cover) as implemented in Perkol-Finkel *et al.* (2008) and Perkol-Finkel & Benayahu (2009). The final species list and abundances for each plot used as data entry for the statistical analyses were obtained by averaging all the counts obtained from the eight subplots. To assist the identification of ascidian species, destructive samples were collected for subsequent analysis in the laboratory, and underwater pictures were taken to record the natural appearance and colour prior to fixation. Collected ascidians were immediately anaesthetized using menthol crystals and were fixed in 4% formaldehyde for subsequent identification.

Ascidians were classified as non-indigenous, cryptogenic or native (Chapman & Carlton, 1991; Carlton, 2009). *Non-indigenous* species (hereafter 'NIS') comprised species for which genetic or biogeographical data are available to support an alien origin. *Cryptogenic* included widely distributed species, generally found abundantly in harbours and suspected to be introduced, but for which there is not enough information to be assigned a given status. *Natives* comprised species identified in the Mediterranean Sea since the first surveys of ascidians were undertaken, which live on natural substrata, and are only found in the Mediterranean Sea or have an Atlanto-Mediterranean distribution. Four taxa that could be only identified to genus level were not given any status as for this classification (i.e. *Unassigned*).

We performed additional surveys at seven marinas in the study region, which again comprised sites close to natural reef areas or far from these (Table 1 and Fig. 1). We did not obtain the authorization to dive or snorkel, but marina -

Table 1 Sampling sites, with details of geographical coordinates, habitat type (natural reef, artificial or marina), type of coastline where the artificial habitats and marinas were built (rocky or sandy) and exposure of the artificial habitats (exposed seaward or sheltered landward). Six replicated 4 m^2 plots were sampled at each site.

Site #	Site name	Latitude(N), Longitude (E)	Habitat	Coastline	Exposure
1	Numana E	43°30′45″, 13°37′33″	Artificial	Rocky	Exposed
2	Numana S	43°30′45″, 13°37′33″	Artificial	Rocky	Sheltered
3	Due Sorelle	43°32′55″, 13°37′38″	Natural		
4	La Vela	43°33′33″, 13°36′34″	Natural		
5	Cesenatico E	44°12′11″, 12°24′43″	Artificial	Sandy	Exposed
6	Cesenatico S	44°12′11″, 12°24′43″	Artificial	Sandy	Sheltered
7	Lido Adriano E	44°25′43″, 12°18′24″	Artificial	Sandy	Exposed
8	Lido Adriano S	44°25′43″, 12°18′24″	Artificial	Sandy	Sheltered
9	Punta Marina E*	44°26′37″, 12°18′01″	Artificial	Sandy	Exposed
10	Punta Marina S*	44°26′37″, 12°18′01″	Artificial	Sandy	Sheltered
11	Marina di Ravenna	44°29′11″, 12°17′16″	Artificial	Sandy	Exposed
12	Casal Borsetti E	44°33′18″, 12°17′9″	Artificial	Sandy	Exposed
13	Casal Borsetti S	44°33′18″, 12°17′9″	Artificial	Sandy	Sheltered
14	Lido delle Nazioni E†	44°44′17″, 12°14′45″	Artificial	Sandy	Exposed
15	Lido delle Nazioni S†	44°44′17″, 12°14′45″	Artificial	Sandy	Sheltered
16	Sistiana	45°46′8″, 13°37′17″	Natural		
17	Miramare breakwater	45°42′22″, 13°42′40″	Artificial	Rocky	Exposed
18	Miramare	45°42′6″, 13°42′48″	Natural		
19	Rovinj fish factory	45°05′11″, 13°38′25″	Artificial	Rocky	Exposed
20	Rovinj marina nuova	45°04′31″, 13°38′0″	Artificial	Rocky	Exposed
21	Rovinj Banjol North	45°04′28″, 13°36′39″	Natural		
22	Rovinj Banjol West	45°04′26″, 13°36′37″	Natural		
23	Numana Porto	43°30′36″, 13°37′29″	Marina	Rocky	
24	Cesenatico Porto Canale	44°12′17″, 12°23′50″	Marina	Sandy	
25	Marina di Ravenna	44°29′24″, 12°17′18″	Marina	Sandy	
26	Porto Garibaldi†	44°40′31″, 12°13′49″	Marina	Sandy	
27	Marina di Sistiana	45°46′07″, 13°37′51″	Marina	Rocky	
28	Marina di Miramare	45°42′22″, 13°42′46″	Marina	Rocky	
29	Marina di Rovinj	45°04′33″, 13°38′06″	Marina	Rocky	

*Only five plots sampled.
†No ascidians found.

owners allowed us to sample these sites from the pontoons using the 'directed-search' method described in Cohen *et al.* (2005) and Grey (2009). In brief, samplings were conducted using scrapers or other hand tools on all available intertidal and subtidal substrates and microhabitats within reach, which included jetties, floating pontoons, pilings and associated supports, as well as buoys, tyres and ropes. Upon collections, we took immediate pictures of the specimens. Sampling was carried out during a period of *c.* 1–2 h (depending on the size of the marina). The samples collected were preserved and identified as described previously.

Data analysis

We analysed differences in the structure and distribution of assemblages of ascidians in relation to habitat type, characteristics and location using mixed-model asymmetrical analyses. These involved partitioning components of variation through two subanalyses (Winer, 1971): the first analysis contrasted the natural reef habitat with the artificial habitats, irrespective of their possible different characteristics, while

the second analysis tested for the effects of the different characteristics (type of coastline and exposure) of the artificial habitats. The analyses included four factors: habitat type (natural rocky reef versus all artificial habitats; fixed factor), coastline type where artificial habitats are built (rocky versus sandy; fixed factor); exposure (seaward exposed versus landward sheltered, fixed) and site (random, nested in each combination of habitat type, coastline type and exposure).

We first tested the differences at the whole assemblage level by running a distance-based permutational multivariate analysis of variance (PERMANOVA) on a Bray–Curtis similarity matrix of all ascidian species (Anderson *et al.*, 2008). As data were semi-quantitative indices of abundance, no transformation was performed prior to analyses (Clarke & Gorley, 2006). For this, and all the following permutational analyses, we used 9999 unrestricted permutations of raw data to generate *P*-values and Type III sums of squares to cope with the unbalanced number of sites (Anderson *et al.*, 2008). The regional distribution of assemblages of ascidians in relation to habitat type was also visually explored using a non-metric multidimensional scaling (NMDS) scatter plot (Clarke

& Gorley, 2006), where we superimposed the species that best correlated with the ordination of the assemblages (Pearson's correlation coefficient $r > 0.25$). Because the sampling design had an unbalanced number of sites, and the NMDS plot had shown an extremely strong effect of coastline type for artificial habitats (which could have driven the significant difference between natural and artificial habitats), we verified the robustness of the results by also running a two-way nested ANOSIM (Clarke & Gorley, 2006) including factors habitat (natural reefs versus artificial rocky versus artificial sandy habitats, fixed factor) and site (random, nested in habitat). In this analysis, the factor exposure was not considered, as all previous analyses had consistently shown non-significant effects of this factor. The results substantially matched those of the PERMANOVA in this and in any of the following analyses (for brevity only the results for the first analysis will be reported).

Subsequently, we tested the hypotheses that artificial habitats would harbour assemblages characterized by lower species richness, lower abundance of native species and a greater abundance of non-indigenous species compared with natural reef habitats. Following Cohen *et al.* (2005), we considered two groups, one with only NIS and another that included both cryptogenic and NIS (hereafter 'NIS+cryptogenic'), which provided low and high estimates, respectively, of the true presence of NIS in the region. We ran four separate asymmetrical PERMANOVAs (same factors as above) on matrices of Euclidean distances calculated from: (1) species richness (number of species per plot); (2) abundance of NIS; (3) abundance of 'NIS+cryptogenic' species; and (4) abundance of native species. Although these tests were purely univariate, we used PERMANOVA instead of classical ANOVA due to ease of use with unbalanced design and to avoid the usual normality assumptions (Anderson *et al.*, 2008).

We finally explored whether assemblages from artificial habitats would be more similar to assemblages in marinas or natural habitats, independently of their characteristics or location. Because we could not obtain quantitative data for marinas, the similarities between marinas, artificial habitats and natural reefs were only explored visually using a NMDS plot built from a Bray–Curtis similarity matrix of the presence/absence data.

All the analyses were performed using PRIMER v. 6.1.11 (Clarke & Gorley, 2006), and PERMANOVA+ v. 1.0.1 (Anderson *et al.*, 2008).

RESULTS

We identified a total of 30 ascidian species (Table 2), which comprised 19 species native to the Mediterranean Sea, four NIS and three cryptogenic species, as well as four taxa that we could only determine to genus level (Table 2). A detailed justification of this classification, with taxonomic remarks, is given in Appendix S1. Although we found many cosmopolitan species that are typically abundant in harbours and marinas world-wide (see Appendix S1), we did not find any of the recent introductions in the Mediterranean Sea (Izquierdo-Muñoz *et al.*, 2009). Very few species were exclusive to either natural or artificial habitats. However, artificial sandy habitats were extremely species poor compared with both natural reefs and artificial rocky habitats: at the 11 artificial sandy sites only four species (the native *Didemnum granulosum*, the non-indigenous *Styela plicata* and the cryptogenic *Botryllus schlosseri* and *Botrylloides leachi*) were found, while 20 and 18 species occurred at least once at the six natural reef sites and at the five artificial rocky sites, respectively. Similarly, only five species (two NIS, two cryptogenic and one unassigned) were found in the three marinas built along sandy coastlines compared with 24 species found in the four marinas built along rocky coastlines. We did not find ascidians in one artificial sandy site (Lido delle Nazioni) and in one marina (Porto Garibaldi); thus, these sites were excluded from the analyses.

Assemblages of ascidians differed significantly between natural and artificial habitats, irrespective of their location or characteristics (Fig. 2 and Table 3). These differences were consistently detected despite the large variability observed between study sites interspersed along 500 km of coastline (Table 3, Appendix S2). The differences between natural and artificial habitats were particularly marked when the latter were located along sandy coastlines, but still there were significant differences also between natural reefs and nearby artificial rocky habitats (Fig. 2, Table 3). Compared with any artificial habitat, natural reefs had significantly larger species richness and abundance of native species (Figs 3 and 4, Table 4), while NIS and cryptogenic species were generally scarce (Fig. 4). On average, species richness was 2–3 times higher, and native species were 2–9 times as abundant in natural reef habitats compared with any artificial habitat (Figs 3 and 4).

As predicted, the suitability of artificial habitats for colonization by ascidians varied significantly in relation to their environmental setting (i.e. whether they were built along a rocky or a sandy coastline), while no differences were found when comparing habitat exposure (Fig. 2, Tables 3 and 4). The artificial sandy habitats had the lowest species richness (Fig. 3), the lowest occurrence of native species and the highest abundance of NIS and cryptogenic species (Fig. 4). Only one native species, *Didemnum granulosum,* was found in artificial sandy sites and at very sparse abundance. Conversely, NIS were 2–3 times more abundant in artificial sandy habitats than in any other habitat (Fig. 4). Even if not statistically significant, a substantially similar trend was observed when cryptogenic species were also accounted for Fig. 4. NIS were twice as abundant at the landward sides of artificial sandy structures compared with the seaward sides, but this difference was not significant due to large variability among sites and plots (Fig. 4 and Table 4).

The species composition of assemblages in artificial habitats was more similar to that of marinas than of natural habitats independently of their characteristics or location (Fig. 5). Most marinas (either located in sandy or rocky

Table 2 List of ascidian species showing status (NIS = non-indigenous, C = cryptogenic, N = native and U = unassigned) and the presence (i.e. ●) of natural reefs, artificial habitats (rocky or sandy, seaward exposed or landward sheltered) and marinas (rocky or sandy). See Table 1 for details of the different habitats and Appendix S1 for a full explanation on assignment of species status.

Status	Species	Natural reef	Artificial rocky	Artificial sandy	Marina sandy	Marina rocky
NIS	*Diplosoma listerianum*	●		●		●
NIS	*Clavelina lepadiformis*					●
NIS	*Ascidiella aspersa*				●	●
NIS	*Styela plicata*	●	●	●	●	●
C	*Ciona intestinalis*	●	●		●	●
C	*Botryllus schlosseri*	●	●	●	●	●
C	*Botrylloides leachi*	●	●	●		●
N	*Aplidium densum*					●
N	*Aplidium elegans*	●				
N	*Aplidium turbinatum*	●				
N	*Didemnum granulosum*		●	●		
N	*Didemnum maculosum*	●	●			●
N	*Trididemnum cereum*	●	●			●
N	*Lissoclinum perforatum*	●	●			
N	*Lissoclinum weigelei*	●	●			
N	*Diplosoma spongiforme*	●	●			●
N	*Clavelina sabbadini*	●				●
N	*Ascidia mentula*					●
N	*Ascidiella scabra*	●	●			●
N	*Phallusia fumigata*	●	●			●
N	*Phallusia ingeria*	●	●			●
N	*Phallusia mammillata*		●			●
N	*Polycarpa pomaria*					●
N	*Microcosmus claudicans*		●			●
N	*Microcosmus polymorphus*					●
N	*Pyura dura*	●	●			●
U	*Didemnum* sp	●	●			
U	*Phallusia* sp	●	●			
U	*Microcosmus* sp	●	●			●
U	*Molgula* sp			●	●	●

areas) were extremely species poor (0–4 species), and they only harboured cryptogenic and NIS. Marinas mostly grouped with artificial sandy habitats despite large variations in geographical location (Fig. 5). Only 2 of the 4 rocky marinas (Marina di Miramare and Marina di Rovinj) harboured native species, but their species composition resembled more artificial habitats than natural reefs.

DISCUSSION

We found profound differences in species composition and diversity between artificial and natural habitats, and artificial structures in sandy environments harboured almost exclusively non-native and cryptogenic species. Our results suggest that marine artificial structures change the relative distribution of non-native versus native rocky coastal species at regional scales. Most native species of ascidians were virtually absent from any artificial habitats built along the extensive sandy coastlines of the North Adriatic Sea. This is despite the fact that many of these infrastructures have been in this region for > 60 years. Even when infrastructures were built along or in close proximity to rocky coasts, they only har-

boured 10–50% of the abundance of native species as compared to nearby natural reefs. At a regional scale, native ascidians remained substantially confined to the natural reef habitats, while artificial infrastructures built along sandy shores provided significant habitat enhancement to NIS and cryptogenic species, which were often the only colonizers on such habitats. Exposure had less prominent effects than predicted in influencing species distributions on artificial structures. On average, the abundance of NIS was twice as large in sheltered than exposed artificial sandy habitats, and native ascidians were on average four times as abundant in exposed than sheltered artificial rocky habitats, but this pattern was not always consistent.

Other work both in the study region and in other geographical areas has consistently documented profound differences between assemblages on artificial structures compared with natural rocky habitats (Bulleri *et al.*, 2005a; Moschella *et al.*, 2005; Perkol-Finkel *et al.*, 2006; Burt *et al.*, 2009b). These included differences in species and genetic diversity (Chapman, 2003; Fauvelot *et al.*, 2009, 2012; Ordóñez *et al.*, 2013), lack of species of particular functional groups, for example large grazers and predators (Chapman, 2003), and
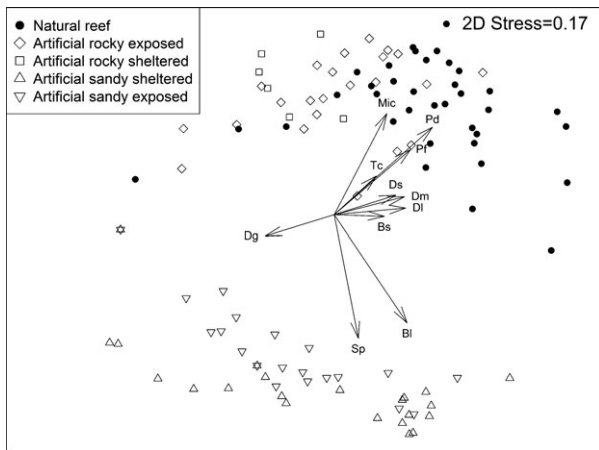
Figure 2 Two-dimensional, non-metric multidimensional scaling (NMDS) plot showing ordination of assemblages of ascidians in relation to habitat type, type of coastline where artificial habitats are built and exposure. The analysis was based on a matrix of Bray–Curtis similarities calculated from semi-quantitative indices of abundance of ascidians. Vectors indicate the taxa that best correlated with the ordination of the assemblages (Pearson's correlation coefficient $r > 0.25$). The direction of vectors indicates the correlation with different groups of sample points, and the length is proportional to the value of the correlation. Codes for taxa: Bl, *Botrylloides leachi*; Bs, *Botryllus schlosseri*; DG, *Didemnum granulosum*; Dl, *Diplosoma listerianum*; Dm, *Didemnum maculosum*; Ds, *Diplosoma spongiforme*; Mic, *Microcosmus* sp.; Pf, *Phallusia fumigata*; Pd, *Pyura dura*; Sp, *Styela plicata*; and Tc, *Trididemnum cereum*.

Table 3 Asymmetrical analysis of the effects of habitat type and type of coastline where artificial habitats are built and exposure on the distribution of ascidian species at 20 sites along 500 km of coastlines (the two sites at Lido delle Nazioni were excluded from the analysis due to the absence of ascidians). Factors are as follows: habitat type (natural reef versus artificial habitats; fixed factor), coastline type (rocky versus sandy; fixed factor), exposure (seaward exposed versus landward sheltered, fixed) and site (random, nested in each combination of habitat type, coastline type and exposure). The analysis consists of two parts, one contrasting natural reef versus artificial habitats and the other examining differences among artificial habitats in relation to coastline type and exposure. We calculated P-values using 9999 random unrestricted permutations of raw data and Type III sums of squares from a matrix of Bray–Curtis similarities. We also report the result of a two-way nested ANOSIM including the factors habitat types (natural reefs versus artificial rocky versus artificial sandy habitats, fixed factor) and site (random, nested in habitat).

Source of variation	d.f.	MS	F
Habitat type = H	4		
Natural versus artificial habitats	1	57,178	4.99**
Among artificial habitats			
Coastline type = C	1	60,408	7.72***
Exposure = E	1	7639	0.98
C × E	1	9433	0.29
Site (H)	15	6898	6.85***
Residual	99	1006	

Results of ANOSIM test	R
Habitat type = H	0.764***
Site (H)	0,547***
Pairwise tests	
Natural versus artificial rocky	0.36*
Natural versus artificial sandy	0.99***
Artificial rocky versus artificial sandy	0.93***

*$0.01 < P < 0.05$, **$0.001 < P < 0.01$, ***$P < 0.001$.

dominance by opportunistic and invasive species (Bulleri & Airoldi, 2005; Glasby *et al.*, 2007; Tyrrell & Byers, 2007; Vaselli *et al.*, 2008; Dafforn *et al.*, 2009; Mineur *et al.*, 2012). These differences are too rarely factored in by practitioners and managers at local scales, let alone at regional scales. Our results show that artificial habitats can act as regional corridors for NIS while not representing adequate substrata for many native species, particularly when built along sandy shores. Considering that marine infrastructures increasingly provide artificial hard habitats along coastlines around the world and influence the proportion of native and NIS abundance at regional scales, there is a need to explore the ultimate effects of marine sprawl for the ecology and functioning of coastal ecosystems.

The observation that artificial habitats do not offer a suitable habitat to many native species while favouring non-indigenous ones warrants further investigation about the underlying causes and implications for management. Marine infrastructures offer atypical substrates for benthic assemblages in terms of orientation, exposure, size, shape, slope, surface composition and texture (Chapman & Underwood, 2011; Dugan *et al.*, 2011), all of which are known to affect the recruitment, survival and growth of a variety of species (Glasby & Connell, 2001; Virgilio *et al.*, 2006; Burt *et al.*, 2009a). For example, many of the native species that were

documented as being absent from sea walls in Sydney Harbour, Australia, required microhabitats that were typically missing in sea walls (Chapman, 2003). Indeed, ecological considerations in the design of hard marine infrastructures tend to focus on construction materials, surface texture and habitat complexity as engineering options to enhance the ecological value of these artificial substrata (Burcharth *et al.*, 2007; Martins *et al.*, 2010; Chapman & Underwood, 2011; Firth *et al.*, 2013, 2014). The type and intensity of biotic interactions can also differ between artificial and natural habitats (Ivesa *et al.*, 2010; Marzinelli *et al.*, 2011, 2012). For example, experiments in the study region have shown that levels of predation particularly large compared with those observed in natural reefs can limit the growth of native canopy forming algae on many marine infrastructure (Perkol-Finkel *et al.*, 2012). Artificial structures are also characterized by unnaturally high levels of disturbance from both natural (e.g. storms and sediment scour) and anthropogenic (e.g.
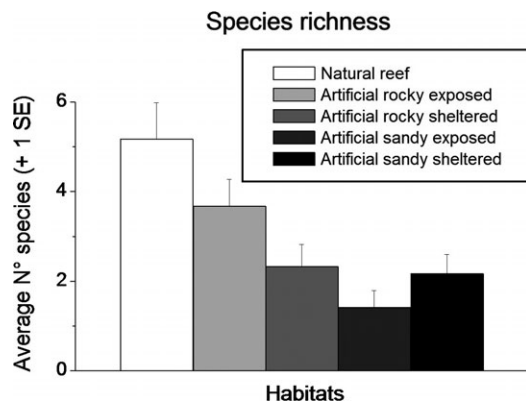
Figure 3 Average species richness (number of species per plot) of ascidians in relation to habitat type, type of coastline where artificial habitats are built and exposure. Data are averages from replicated plots (generally 6) at replicated sites (see Table 1 for details).

harvesting, trampling and maintenance works) sources (Airoldi *et al.*, 2005a; Burcharth *et al.*, 2007). These disturbances damage the native assemblages and offer prolonged availability of unoccupied space or other resources that favour the establishment of species with opportunistic and invasive traits (Airoldi & Bulleri, 2011).

Native and alien ascidian species could have different biological and ecological characteristics, favouring, for example, resilience of the latter to harsh conditions in artificial substrates. We could not, however, substantiate any meaningful trend in this sense. The most profound biological differentiation in ascidians is between solitary and colonial forms, implying different substrate occupation and reproductive strategies (Svane & Young, 1989; Lambert, 2005). The percentage of colonial species, however, was similar across categories (50% in NIS, 66.6% in cryptogenic species, 52.6% in native species). The distributional range of these species is, however, clearly different, with all cryptogenic and NIS being widely distributed or cosmopolitan species, and most native species having an Atlanto-Mediterranean distribution (data in Coll *et al.*, 2010). Cosmopolitanism is likely to indicate high adaptive plasticity to thrive in different habitats, and thus these species may be pre-adapted to occupy particularly unstable environments, while they could be displaced in more stable, natural habitats. There is a dearth of experimental data to support intrinsic biological and ecological differences between native and introduced ascidian species, although some evidence points to differences in traits such as susceptibility to predation, attachment properties or temperature tolerance (Dumont *et al.*, 2011; Zerebecki & Sorte, 2011; Murray *et al.*, 2012; Rius *et al.*, 2014).

Although all artificial habitats differed from natural reefs, those built in sandy environment differed the most: only one native species occurred occasionally, while NIS and cryptogenic species were up to three times as abundant than in rocky reefs. At present, what makes artificial structures built in sandy environments so distinctive remains unknown. Lack
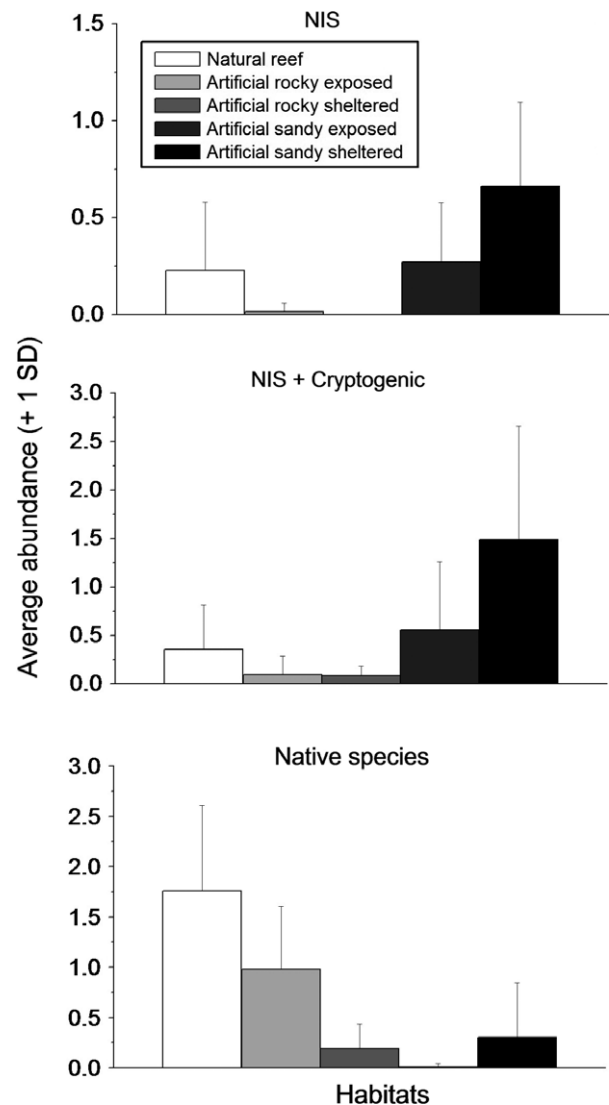

Figure 4 Average abundance (calculated from semi-quantitative indexes of abundance, see methods for details) of non-indigenous species = NIS, NIS + cryptogenic species and native species in relation to habitat type, type of coastline where artificial habitats are built and exposure. Data are averages from replicated plots (generally 6) at replicated sites (see Table 1 for details).

of native species could be related to a greater distance from source populations compared with those artificial structures that are built along rocky coastlines. However, a relatively rich assemblage of ascidian species (also including 4 of the native species in our list, *Phallusia fumigata, P. mammillata, Aplidium densum* and *Clavelina sabbadini*) has been reported at some deeper and isolated natural rocky outcrops just offshore some of the studied sites (Gabriele *et al.*, 1999; Ponti & Mastrototaro, 2006). Size or age of the structures could not cause the differences observed, as also reported in other studies (Perkol-Finkel *et al.*, 2006; Miller *et al.*, 2009): structures were relatively similar in size and depth of deployment (Perkol-Finkel *et al.*, 2006; Miller *et al.*, 2009), and some of

Table 4 Asymmetrical analysis of the effects of habitat type, type of coastline where artificial habitats are built and exposure on: (1) species richness (No species per plot), (2) abundance of non-indigenous species = NIS, (3) abundance of NIS + cryptogenic species and (4) abundance of native species at 20 sites along the studied coastline (the two sites at Lido delle Nazioni were excluded from the analysis due to the absence of ascidians). Factors are the following: habitat type (natural reef versus artificial habitats; fixed factor), coastline type (rocky versus sandy; fixed factor); exposure (seaward exposed versus landward sheltered, fixed) and site (random, nested in each combination of habitat type, coastline type and exposure). The analysis consists of two parts, one contrasting natural reef versus artificial habitats and the other examining differences among artificial habitats in relation to type of coastline and exposure. We calculated P-values using 9999 random unrestricted permutations of raw data and Type III sums of squares from a matrix of Euclidean similarities.

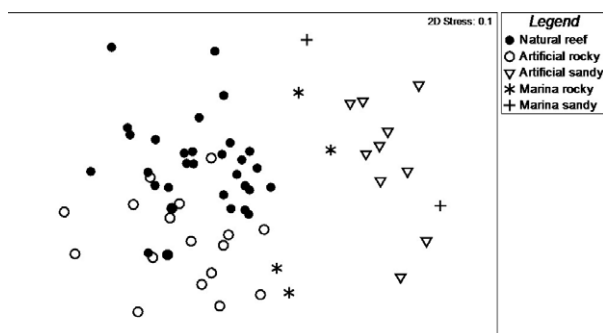| Source of variation	d.f.	Species richness		NIS		NIS+Cryptogenic		Natives	
		MS	F	MS	F	MS	F	MS	F
Habitat type = H	4								
Natural versus artificial habitats	1	200.8	22.3***	0.09	0.17	2.32	0.67	47.4	23.1**
Among artificial habitats									
Coastline type = C	1	20.6	4.29*	2.94	12.72**	12.3	3.86	2.57	2.16
Exposure = E	1	1.18	0.24	0.49	2.14	2.98	0.94	0.87	0.73
C × E	1	15.3	3.19	0.58	2.51	3.12	0.98	4.13	3.47
Site (H)	15	6.2	3.87***	0.24	3.2**	2.32	12.4***	1.58	8.66***
Residual	99	1.6		0.07		0.18		0.18	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Figure 5 Two-dimensional, non-metric multidimensional scaling (NMDS) plot showing ordination of assemblages of ascidians found in different habitats and marinas. The analysis was based on a matrix of Bray–Curtis similarities calculated from the presence/absence data of ascidians.

the artificial infrastructures were > 60 years old. One possible hypothesis, deserving further consideration, is that artificial structures built along sedimentary coastlines could experience greater pressure from both natural predators and humans compared with structures built along rocky coastlines, with ecological effects similar to those reported for oasis or seamounts (Bock et al., 2008; Rowden et al., 2010). Coastal defences built along highly populated sandy coastlines, like those of this study, are extremely popular recreation and harvesting sites due to the lack of nearby rocky reefs (Airoldi et al., 2005a), while those built along rocky coastlines are generally less attractive than the rocky coast itself. The impacts from sediment resuspension, turbidity and potentially associated contaminants are also likely to be greater at the artificial structures built at sandy than rocky sites. Such high concentrations of disturbing factors could negatively affect many native species, including ascidians,

which suffer reduced survival and growth from sedimentation, scour and contaminants (Lohrer et al., 2006; Crooks et al., 2011; Rivero et al., 2013). Further, when disturbance is combined with a potentially higher propagule pressure of NIS (e.g. due to aquaculture activities concentrated in coastal lagoons), the two factors could interact to overcome the biotic resistance of marine invertebrate communities to invasions (Clark & Johnston, 2009), and this in turn could have complex feedbacks on native species (Von Holle & Simberloff, 2005).

Marine artificial structures are becoming spatially significant in many sandy coastal areas globally (Dugan et al., 2011). In many regions, like the one of this study, the process has already been pushed to levels that invert the regional proportion of artificial versus natural hard habitats. We have shown how in these human-made systems, NIS may have an advantage over natives, leading to regional-scale changes in their relative abundances. We have also shown how the value or quality of these emergent novel marine ecosystems can be variable and dependent at least in part on several aspects of seascape and habitat. Attempts at designing artificial habitats to intentionally enhance target native species of recreational, commercial or naturalistic value are increasing (Chapman & Underwood, 2011; Perkol-Finkel et al., 2012; Gianni et al., 2013; Firth et al., 2014). For instance, the likelihood of recruitment of local native species on artificial structures built along rocky coasts can be enhanced by incorporating pools or other features that mimic the levels of complexity of natural habitats (Chapman et al., 2008; Martins et al., 2010; Firth et al., 2013). The settlement and growth of NIS on artificial structures can be limited using materials or coatings that prevent settlement of fouling (Grozea & Walker, 2009), by favouring the design of fixed surfaces rather than floating ones (Dafforn et al.,

2009), by favouring the colonization by native species (Stachowicz *et al.*, 2002; Arenas *et al.*, 2006b) and by minimizing disturbances (Airoldi *et al.*, 2005a; Bulleri *et al.*, 2006). Ecologically informed repair schedules can limit the spread of non-indigenous species by favouring a quicker recovery of the native ones (Airoldi & Bulleri, 2011; Firth *et al.*, 2014). In sandy habitats, recent attempts have also been performed to actively garden ecologically relevant habitat forming species, to contemporaneously enhance native species and deter non-indigenous ones (Perkol-Finkel *et al.*, 2012). The success of these interventions, although extremely encouraging, is still limited by incomplete understanding of the ecological functioning of these systems (Chapman *et al.*, 2008; Chapman & Underwood, 2011; Perkol-Finkel *et al.*, 2012), and we are a long way from the identification of general principles.

Future research should focus on identifying factors, or combinations of factors, that could maintain a high representation and abundance of native species in artificial habitats. Understanding what ecological forces and processes shape biodiversity in increasingly urbanized marine systems would provide predictive power for likely future trends in currently undeveloped seascapes, and better basis for planning, management and design of marine infrastructure. Much greater effort is also needed to identify alternatives to the massive hardening of coastal regions. For example, solutions integrating intertidal ecosystems in coastal defence schemes are increasingly recognized as more sustainable, cost-effective and ecologically sound alternatives to conventional coastal engineering and should be prioritized at a global scale (Temmerman *et al.*, 2013; Bouma *et al.*, 2014; Ferrario *et al.*, 2014).

ACKNOWLEDGEMENTS

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: An Annual Review*, **45**, 345–405.

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., Bacchiocchi, F., Cagliola, C., Bulleri, F. & Abbiati, M. (2005a) Impact of recreational harvesting on assemblages in artificial rocky habitats. *Marine Ecology Progress Series*, **299**, 55–66.

Airoldi, L., Abbiati, M., Beck, M.W., Hawkins, S.J., Jonsson, P.R., Martin, D., Moschella, P.S., Sundelöf, A., Thompson, R.C. & Åberg, P. (2005b) An ecological perspective on the deployment and design of low-crested and other hard coastal defence structures. *Coastal Engineering*, **52**, 1073–1087.

Airoldi, L., Connell, S.D. & Beck, M.W. (2009) The Loss of Natural Habitats and the Addition of Artificial Substrata. *Marine hard bottom communities: patterns, dynamics, diversity, and change* (ed. by M. Wahl), pp. 269–280. Springer, New York, NY.

Anderson, M., Gorley, R. & Clarke, K. (2008) *PERMANOVA+ for PRIMER. Guide to software and statistical methods*. PRIMER-E, Plymouth, UK.

Arenas, F., Bishop, J.D.D., Carlton, J.T., Dyrynda, P.J., Farnham, W.F., Gonzalez, D.J., Jacobs, M.W., Lambert, C., Lambert, G., Nielsen, S.E., Pederson, J.A., Porter, J.S., Ward, S. & Wood, C.A. (2006a) Alien species and other notable records from a rapid assessment survey of marinas on the south coast of England. *Journal of the Marine Biological Association of the United Kingdom*, **86**, 1329–1337.

Arenas, F., Sanchez, I., Hawkins, S.J. & Jenkins, S.R. (2006b) The invasibility of marine algal assemblages: role of functional diversity and identity. *Ecology*, **87**, 2851–2861.

Bacchiocchi, F. & Airoldi, L. (2003) Distribution and dynamics of epibiota on hard structures for coastal protection. *Estuarine, Coastal and Shelf Science*, **56**, 1157–1166.

Bianchi, C.N. (2007) Biodiversity issues for the forthcoming tropical Mediterranean Sea. *Hydrobiologia*, **580**, 7–21.

Bock, C.E., Jones, Z.F. & Bock, J.H. (2008) The oasis effect: response of birds to exurban development in a southwestern savanna. *Ecological Applications*, **18**, 1093–1106.

Bouma, T.J., van Belzen, J., Balke, T. *et al.* (2014) Identifying knowledge gaps hampering application of intertidal habitats in coastal protection: opportunities and steps to take. *Coastal Engineering*, **2014**, 147–157.

Bulleri, F. & Airoldi, L. (2005) Artificial marine structures facilitate the spread of a non-indigenous green alga, *Codium fragile* ssp. *stomentosoides*, in the north Adriatic Sea. *Journal of Applied Ecology*, **42**, 1063–1072.

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., Chapman, M.G. & Underwood, A.J. (2005a) Intertidal assemblages on seawalls and vertical rocky

shores in Sydney Harbour, Australia. *Austral Ecology*, **30**, 655–667.

Bulleri, F., Airoldi, L., Branca, G.M. & Abbiati, M. (2005b) Positive effects of the introduced green alga, *Codium fragile* ssp. *tomentosoides*, on recruitment and survival of mussels. *Marine Biology*, **148**, 1213–1220.

Bulleri, F., Abbiati, M. & Airoldi, L. (2006) The colonisation of human-made structures by the invasive alga *Codium fragile* ssp. *tomentosoides* in the North Adriatic Sea (NE Mediterranean). *Hydrobiologia*, **555**, 263–269.

Burcharth, H.F., Hawkins, S.J., Zanuttigh, B. & Lamberti, A. (2007) *Environmental design guidelines for low crested coastal structures*. Elsevier Science, Amsterdam.

Burt, J., Bartholomew, A., Bauman, A., Saif, A. & Sale, P.F. (2009a) 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.

Burt, J., Bartholomew, A., Usseglio, P., Bauman, A. & Sale, P.F. (2009b) Are artificial reefs surrogates of natural habitats for corals and fish in Dubai, United Arab Emirates? *Coral Reefs*, **28**, 663–675.

Burt, J., Feary, D., Usseglio, P., Bauman, A. & Sale, P.F. (2010) The influence of wave exposure on coral community development on man-made breakwater reefs, with a comparison to a natural reef. *Bulletin of Marine Science*, **86**, 839–859.

Carlton, J.T. (2009) Deep invasion ecology and the assembly of communities in historical time. *Biological Invasions in Marine Ecosystems* (ed. by G. Rilov and J.A. Crooks), pp. 13–56. Springer-Verlag, Berlin, Heidelberg.

Castilla, J.C., Guinez, R., Alvarado, J.L., Pacheco, C. & Varas, M. (2000) Distribution, population structure, population biomass and morphological characteristics of the tunicate *Pyura stolonifera* in the Bay of Antofagasta, Chile. *Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I*, **21**, 161–174.

Castilla, J.C., Guiñez, R., Caro, A.U. & Ortiz, V. (2004) Invasion of a rocky intertidal shore by the tunicate *Pyura praeputialis* in the Bay of Antofagasta, Chile. *Proceedings of the National Academy of Sciences USA*, **101**, 8517–8524.

Chapman, M.G. (2003) Paucity of mobile species on constructed seawalls: effects of urbanization on biodiversity. *Marine Ecology Progress Series*, **264**, 21–29.

Chapman, J.W. & Carlton, J.T. (1991) A test of criteria for introduced species: the global invasion by the isopod *Synidotea laevidorsalis* (Miers, 1881). *Journal of Crustacean Biology*, **11**, 386–400.

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.

Chapman, M.G., Underwood, A.J. & Blockley, D. (2008) Effects of method of deployment of artificial units of habitat on microgastropod colonization. *Marine Ecology Progress Series*, **366**, 49–57.

Clark, G.F. & Johnston, E.L. (2009) Propagule pressure and disturbance interact to overcome biotic resistance of marine invertebrate communities. *Oikos*, **118**, 1679–1686.

Clarke, K.R. & Gorley, R.N. (2006) *PRIMER v.6: user manual/tutorial*. PRIMER-E, Plymouth.

Cohen, A.N., Harris, L.H., Bingham, B.L., Carlton, J.T., Chapman, J.W., Lambert, C.C., Lambert, G., Ljubenkov, J.C., Murray, S.N., Rao, L.C., Reardon, K. & Schwindt, E. (2005) Rapid assessment survey for exotic organisms in southern California bays and harbors, and abundance in port and non-port areas. *Biological Invasions*, **7**, 995–1002.

Coll, M., Piroddi, C., Steenbeck, J. *et al.* (2010) The biodiversity of the Mediterranean Sea: estimates, patterns and threats. *PLoS One*, **5**, e11842.

Crooks, J.A., Chang, A.L. & Ruiz, G.M. (2011) Aquatic pollution increases the relative success of invasive species. *Biological Invasions*, **13**, 165–176.

Dafforn, K.A., Johnston, E.L. & Glasby, T.M. (2009) Shallow moving structures promote marine invader dominance. *Biofouling*, **25**, 277–287.

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.

Dethier, M.N., McDonald, K. & Strathmann, R.R. (2003) Colonization and connectivity of habitat patches for coastal marine species distant from source populations. *Conservation Biology*, **17**, 1024–1035.

Dugan, J.E., Airoldi, L., Chapman, M.G., Walker, S.J. & Schlacher, T. (2011) Estuarine and coastal structures: environmental effects, a focus on shore and nearshore structures. *Treatise on Estuarine and Coastal Science* (ed. by I.E. Wolansk and D.S. Mclusky), pp. 17–41. Academic Press, Waltham.

Dumont, C.P., Gaymer, C.F. & Thiel, M. (2011) Predation contributes to invasion resistance of benthic communities against the non-indigenous tunicate *Ciona intestinalis*. *Biological Invasions*, **13**, 2023–2034.

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.

Fauvelot, C., Costantini, F., Virgilio, M. & Abbiati, M. (2012) Do artificial structures alter marine invertebrate genetic makeup? *Marine Biology*, **159**, 2797–2807.

Feary, D.A., Burt, J.A. & Bartholomew, A. (2011) Artificial marine habitats in the Arabian Gulf: review of current use, benefits and management implications. *Ocean & Coastal Management*, **54**, 742–749.

Ferrario, F., Beck, M.W., Storlazzi, C., 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. & Haw-

kins, 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., Abbiati, M. *et al.* (2014) Between a rock and a hard place: environmental and engineering considerations when designing flood defence structures. *Coastal Engineering*, **2014**, 122–135.

Floerl, O., Inglis, G.J., Dey, K. & Smith, A. (2009) The importance of transport hubs in stepping-stone invasions. *Journal of Applied Ecology*, **46**, 37–45.

Forrest, B.M., Gardner, J.P.A. & Taylor, M.D. (2009) Internal borders for managing invasive marine species. *Journal of Applied Ecology*, **46**, 46–54.

Gabriele, M., Bellot, A., Gallotti, D. & Brunetti, R. (1999) Sublittoral hard substrate communities of the northern Adriatic Sea. *Cahiers De Biologie Marine*, **40**, 65–76.

Gianni, F., Bartolini, F., Airoldi, 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.

Gili, J. & Coma, R. (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends in Ecology and Evolution*, **13**, 316–321.

Glasby, T.M. & Connell, S.D. (2001) Orientation and position of substrata have large effects on epibiotic assemblages. *Marine Ecology Progress Series*, **214**, 127–135.

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.

Grey, E.K. (2009) Do we need to jump in? A comparison of two survey methods of exotic ascidians on docks. *Aquatic Invasions*, **4**, 81–86.

Grozea, C.M. & Walker, G.C. (2009) Approaches in designing non-toxic polymer surfaces to deter marine biofouling. *Soft Matter*, **5**, 4088–4100.

Inger, R., Attrill, M.J., Bearhop, S., Broderick, A.C., Grecian, W.J., Hodgson, D.J., Mills, C., Sheehan, E., Votier, S.C., Witt, M.J. & Godley, B.J. (2009) Marine renewable energy: potential benefits to biodiversity? An urgent call for research. *Journal of Applied Ecology*, **46**, 1145–1153.

Ivesa, 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.

Izquierdo-Muñoz, A., Díaz-Valdés, M. & Ramos-Esplá, A.A. (2009) Recent non-indigenous ascidians in the Mediterranean Sea. *Aquatic Invasions*, **4**, 59–64.

Lambert, G. (2005) Ecology and natural history of the protochordates. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **83**, 34–50.

Langhamer, O. (2012) Artificial reef effect in relation to offshore renewable energy conversion: state of the art. *The Scientific World Journal*, **2012**, 386713, 8 pp.

Locke, A. (2009) A screening procedure for potential tunicate invaders of Atlantic Canada. *Aquatic Invasions*, **4**, 71–79.

Lohrer, A.M., Hewitt, J.E. & Thrush, S.F. (2006) Assessing far-field effects of terrigenous sediment loading in the coastal marine environment. *Marine Ecology Progress Series*, **315**, 13–18.

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.

Marzinelli, E.M., Underwood, A.J. & Coleman, R.A. (2012) Modified habitats change ecological processes affecting a non-indigenous epibiont. *Marine Ecology Progress Series*, **446**, 119–129.

McKinney, M.L. (2006) Urbanization as a major cause of biotic homogenization. *Biological Conservation*, **127**, 247–260.

McKinney, M.L. & Lockwood, J.L. (1999) Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution*, **14**, 450–453.

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.

Mineur, F., Cook, E.J., Minchin, D., Bohn, K., MacLeod, A. & Maggs, C.A. (2012) Changing coasts: marine aliens and artificial structures. *Oceanography and Marine Biology: an Annual Review* (ed. by R.N. Gibson, R.J.A. Atkinson, J.D.M. Gordon and R.N. Hughes), pp. 189–233. Crc Press-Taylor & Francis Group, Boca Raton, FL.

Moschella, P.S., Abbiati, M., Åberg, P., Airoldi, L., Anderson, J.M., Bacchiocchi, F., Bulleri, F., Dinesen, G.E., Frost, M., Gacia, E., Granhag, L., Jonsson, P.R., Satta, M.P., Sundelöf, A., Thompson, R.C. & Hawkins, S.J. (2005) Low-crested coastal defence structures as artificial habitats for marine life: using ecological criteria in design. *Coastal Engineering*, **52**, 1053–1071.

Murray, C.C., Therriault, T.W. & Martone, P.T. (2012) Adapted for invasion? Comparing attachment, drag and dislodgment of native and nonindigenous hull fouling species. *Biological Invasions*, **14**, 1651–1663.

Ordóñez, V., Pascual, M., Rius, M. & Turon, X. (2013) Mixed but not admixed: a spatial analysis of genetic variation of an invasive ascidian on natural and artificial substrates. *Marine Biology*, **160**, 1645–1660.

Perkol-Finkel, S. & Benayahu, Y. (2009) Differential survivorship of two coral species on artificial and natural reefs.

Journal of Experimental Marine Biology and Ecology, **369**, 1–7.

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., Miloh, T., Zilman, G., Sella, I. & Benayahu, Y. (2008) Floating and fixed artificial habitats: spatial and temporal patterns of benthic communities in a coral reef environment. *Estuarine, Coastal and Shelf Science*, **77**, 491–500.

Perkol-Finkel, S., Ferrario, F., Nicotera, V. & Airoldi, L. (2012) Conservation challenges in urban seascapes: promoting the growth of threatened species on coastal infrastructures. *Journal of Applied Ecology*, **49**, 1457–1466.

Pineda, M.C., López-Legentil, S. & Turon, X. (2011) The whereabouts of an ancient wanderer: global phylogeography of the solitary ascidian *Styela plicata*. *PLoS One*, **6**, e25495.

Ponti, M. & Mastrototaro, F. (2006) Ascidians assemblages distribution on the hard bottoms (Tegnue) out of Chioggia (Venice). *Biologia Marina Mediterranea*, **13**, 621–624.

Ramsay, A., Davidson, J., Landry, T. & Arsenault, G. (2008) Process of invasiveness among exotic tunicates in Prince Edward Island, Canada. *Biological Invasions*, **10**, 1311–1316.

Reise, K. (2005) Coast of change: habitat loss and transformations in the Wadden Sea. *Helgoland Marine Research*, **59**, 9–21.

Rius, M., Heasman, K.G. & McQuaid, C.D. (2011) Long-term coexistence of non-indigenous species in aquaculture facilities. *Marine Pollution Bulletin*, **62**, 2395–2403.

Rius, M., Turon, X., Ordóñez, V. & Pascual, M. (2012) Tracking invasion histories in the sea: facing complex scenarios using multilocus data. *PLoS One*, **7**, e35815.

Rius, M., Clusella-Trullas, S., McQuaid, C.D., Navarro, R.A., Griffiths, C.L., Matthee, C.A., von der Heyden, S. & Turon, X. (2014) Range expansions across ecoregions: interactions of climate change, physiology and genetic diversity. *Global Ecology and Biogeography*, **23**, 76–88.

Rivero, N.K., Dafforn, K.A., Coleman, M.A. & Johnston, E.L. (2013) Environmental and ecological changes associated with a marina. *Biofouling*, **29**, 803–815.

Rowden, A.A., Schlacher, T.A., Williams, A., Clark, M.R., Stewart, R., Althaus, F., Bowden, D.A., Consalvey, M., Robinson, W. & Dowdney, J. (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.

Sammarco, P.W., Atchison, A.D. & Boland, G.S. (2004) Expansion of coral communities within the Northern Gulf of Mexico via offshore oil and gas platforms. *Marine Ecology Progress Series*, **280**, 129–143.

Shenkar, N. & Swalla, B.J. (2011) Global diversity of ascidiacea. *PLoS One*, **6**, e20657.

Simkanin, C., Davidson, I.C., Dower, J.F., Jamieson, G. & Therriault, T.W. (2012) Anthropogenic structures and the infiltration of natural benthos by invasive ascidians. *Marine Ecology-an Evolutionary Perspective*, **33**, 499–511.

Stachowicz, J.J., Fried, H., Osman, R.W. & Whitlatch, R.B. (2002) Biodiversity, invasion resistance, and marine ecosystem function: Reconciling pattern and process. *Ecology*, **83**, 2575–2590.

Svane, I. & Young, C.M. (1989) The ecology and behaviour of ascidian larvae. *Oceanography and Marine Biology Annual Review*, **27**, 45–90.

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.

Tyrrell, M.C. & Byers, J.E. (2007) Do artificial substrates favor nonindigenous fouling species over native species? *Journal of Experimental Marine Biology and Ecology*, **342**, 54–60.

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.

Virgilio, M., Airoldi, L. & Abbiati, M. (2006) Spatial and temporal variations of assemblages in a Mediterranean coralligenous reef and relationships with surface orientation. *Coral Reefs*, **25**, 265–272.

Von Holle, B. & Simberloff, D. (2005) Ecological resistance to biological invasion overwhelmed by propagule pressure. *Ecology*, **86**, 3212–3218.

Winer, B.J. (1971) *Statistical principles in experimental design*. McGraw-Hill, New York, NY.

Witt, M.J., Sheehan, E.V., Bearhop, S., Broderick, A.C., Conley, D.C., Cotterell, S.P., Crow, E., Grecian, W.J., Halsband, C., Hodgson, D.J., Hosegood, P., Inger, R., Miller, P.I., Sims, D.W., Thompson, R.C., Vanstaen, K., Votier, S.C., Attrill, M.J. & Godley, B.J. (2012) Assessing wave energy effects on biodiversity: the Wave Hub experience. *Philosophical Transactions of the Royal Society A: Mathematical Physical and Engineering Sciences*, **370**, 502–529.

Zavatarelli, M., Raicich, F., Bregant, D., Russo, A. & Artegiani, A. (1998) Climatological biogeochemical characteristics of the Adriatic Sea. *Journal of Marine Systems*, **18**, 227–263.

Zerebecki, R.A. & Sorte, C.J.B. (2011) Temperature tolerance and stress proteins as mechanisms of invasive species success. *PLoS One*, **6**, e14806.

SUPPORTING INFORMATION

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

Appendix S1 Remarks on taxonomy / group assignment of the species found in the present study.

Appendix S2 Average species richness of ascidians, and average abundance of NIS + cryptogenic species and native spe-

cies at different study sites in relation to habitat type and type of coastline where artificial habitats are built.

BIOSKETCH

Laura Airoldi is an Associated Professor in Ecology at the University of Bologna, Italy. She received a Ph.D. in Marine Science from the University of Genova and carried out research at several institutions in Italy, Australia, USA and UK. She was a Fulbright Research Fellow at Stanford University. Her current research interests are very cross-disciplinary and focus on the consequences of cumulative anthropogenic impacts finalized to design conservation and restoration strategies in increasingly urbanized marine systems. She is Coordinator and/or PI in several (inter) national research projects focusing on the design of sustainable shoreline protection strategies and coastal and offshore infrastructure, including strategies to mitigate the spread of invasive species and preserve critical ecological functions (http://www.ecology.unibo.it/page/laura.htm).

Author contributions: M.R., L.A. and S.P.-F. conceived the ideas; M.R. and S.P.-F. collected the data; M.R. and X.T. identified the field samples, L.A., X.T., M.R. and S.P.-F. analysed the data; L.A. and M.R. led the writing, and S.P.-F. and X.T. contributed to it.

Editor: Reuben Keller