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Reduction of herbivorous fish pressure can facilitate focal algal species forestation on artificial structures



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ARTICLE INFO

Keywords:

Herbivory

Fish

*Cystoseira**Sarpa salpa*

Macroalgae

Infralittoral fringe

Exclusion

ABSTRACT

Coastal areas have been transformed worldwide by urbanization, so that artificial structures are now widespread. Current coastal development locally depletes many native marine species, while offering limited possibilities for their expansion. Eco-engineering interventions intend to identify ways to facilitate the presence of focal species and their associated functions on artificial habitats. An important but overlooked factor controlling restoration operations is overgrazing by herbivores. The aim of this study was to quantify the effects of different potential feeders on *Cystoseira amentacea*, a native canopy-forming alga of the Mediterranean infralittoral fringe, and test whether manipulation of grazing pressure can facilitate the human-guided installation of this focal species on coastal structures. Results of laboratory tests and field experiments revealed that *Sarpa salpa*, the only strictly native herbivorous fish in the Western Mediterranean Sea, can be a very effective grazer of *C. amentacea* in artificial habitats, up to as far as the infralittoral fringe, which is generally considered less accessible to fishes. *S. salpa* can limit the success of forestation operations in artificial novel habitats, causing up to 90% of *Cystoseira* loss after a few days. Other grazers, such as limpets and crabs, had only a moderate impact. Future engineering operations, intended to perform forestation of canopy-forming algae on artificial structures, should consider relevant biotic factors, such as fish overgrazing, identifying cost-effective techniques to limit their impact, as is the usual practice in restoration programmes on land.

1. Introduction

Over the last decades the proportion of coastal areas subjected to the development of infrastructure, such as breakwaters, seawalls, jetties, piers and groynes, has increased (Timmerman and White, 1997; EEA, 2005), causing the extensive regression of marine habitats (Thompson et al., 2002; Bulleri and Chapman, 2010). For example, in Europe about 22,000 km² of the coastline is artificial, mainly in the most highly developed countries (Koike, 1996; Chapman and Bulleri, 2003; Dugan et al., 2011). The same pattern affects the Mediterranean Sea, where infrastructures dominate long stretches of the shoreline (Airoidi and Beck, 2007, and references therein). Current seafront development locally depletes many native marine species, while offering limited possibilities for their expansion (Perkol-Finkel et al., 2012; Airoidi et al., 2015). Most artificial structures are currently characterised by different biotic dynamics, lack of functionally important native taxa (Viola et al., 2018) and have low species and genetic diversity (Chapman, 2003; Fauvelot et al., 2009), being generally dominated by opportunistic and invasive species (Bulleri et al., 2006;

Bracewell et al., 2013; Airoidi et al., 2015). As urbanization progresses, there is a growing need to enhance the ecological performance of artificial structures by facilitating the development of diverse and functional biota on them in order to recreate habitats similar to the natural ones or, at least, to provide the same ecosystem functions (Browne and Chapman, 2011; Chapman and Underwood, 2011; Perkol-Finkel et al., 2012; Firth et al., 2013, 2014; Gianni et al., 2013).

Eco-engineering can improve the design of artificial infrastructures to solve the specific problems associated with hard engineering (e.g. habitat destruction, changes in environmental quality, loss of native biodiversity and ecosystem functionality and spread of non-indigenous species). Microhabitats can be designed or retrofitted to favour the recruitment of different species or to offer refuge from herbivores and/or predators (Hauser et al., 2006; Chapman and Blockley, 2009; Martins et al., 2010; Lapinski et al., 2014; Bouchoucha et al., 2016). Active 'seeding' and transplantation methods, by installing juveniles or adult individuals on artificial structures, are also being tested to favour space pre-emption by focal native habitat-forming species, thereby limiting the space for invasive species and enhancing desired ecosystem

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<https://doi.org/10.1016/j.marenvres.2018.04.007>

Received 18 December 2017; Received in revised form 8 April 2018; Accepted 19 April 2018

Available online 21 April 2018

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functions (Dean and Jung, 2001; Terawaki et al., 2003).

In particular in the Mediterranean Sea, eco-engineering efforts have often focussed on canopy-forming algae, such as *Cystoseira* spp. due to their functional value as habitat engineers, their threatened conservation status and low dispersal ability (Falace et al., 2006; Perkol-Finkel and Airoidi, 2010; Perkol-Finkel et al., 2012; Ferrario et al., 2016). These studies have demonstrated that artificial habitats offer potentially suitable substrata for the installation and growth of canopy-forming algae, but that the ultimate success of the species restoration operations can be extremely variable (Perkol-Finkel et al., 2012). While it is widely acknowledged that abiotic factors can affect the success of these operations (Cacabelos et al., 2016), the role of biotic factors is often neglected, despite recent growing evidence of the potential role of herbivorous fishes in the subtidal zone (Ferrario et al., 2016). In the Mediterranean intertidal zone, the so-called infralittoral fringe, fish herbivory has always been considered as negligible, since this zone is generally assumed to represent a spatial refuge (Vergés et al., 2009). There is, however, growing evidence of the control exerted by herbivorous fish on intertidal rocky shore communities too (Gianni et al., 2017).

The aim of this study was to analyse whether herbivorous fish can affect native canopy-forming species, such as *Cystoseira amentacea*, in the exposed infralittoral fringe of marine infrastructures, and whether limitation of fish access can facilitate the human-guided forestation of this focal species on coastal structures. A combination of field and laboratory experiments was used to: 1) assess whether herbivorous fish inflict greater damage on *C. amentacea* compared to other potential feeders, such as limpets and crabs; 2) assess whether herbivorous fish inflict greater damage on *C. amentacea* than on other fleshy seaweeds coexisting on artificial structures; and 3) test whether reduction of herbivorous fish pressure can limit the loss of *C. amentacea* during forestation operations on an artificial structure.

2. Material and methods

2.1. Study area

The study was carried out in the summer 2013 at the breakwater of Saint Jean Cap Ferrat harbour, French Riviera, North-Western Mediterranean (Fig. 1). The harbour is located in a wave-exposed coastal area and its breakwater, built with large blocks of quarried rock (1–3 m across), extends along the shore for 340 m. The seaward infralittoral fringe of the breakwater (30 cm above/below MSL) is characterised by low diversity of macroalgal communities, dominated by encrusting and articulated Corallinales and other photophilous fleshy algae, mostly turf-forming Ceramiales and Sphacelariales and *Dictyota* spp. Canopy-forming species of the genera *Cystoseira* or *Sargassum* were absent.

2.2. Abundance of herbivores

The density and size of all potential feeders of *Cystoseira amentacea* on and around the breakwater was assessed by visual census twice, in June and July 2013. Sea urchins were absent in the infralittoral fringe (only a few individuals were found at more than 1 m depth on the breakwater). Density and size (carapace length: CL) of crabs (*Eriphia verrucosa* Forskål, 1775 and *Pachygrapsus marmoratus* Fabricius, 1787) were visually estimated by six replicated transects (15 × 3 m) over the mean low water line (Flores and Paula, 2001), carefully checking each crevice of the breakwater. Even if these species of crabs are mostly carnivorous, they also feed on algae occasionally (Cannicci et al., 2002; Silva et al., 2010). Density and size of salema, *Sarpa salpa* (Linnaeus, 1758), the only strictly herbivorous fish in the North-Western Mediterranean Sea, were visually estimated by ten replicated transects (25 × 5 m) (Harmelin-Vivien et al., 1985). Transects were performed parallel and perpendicular to the breakwater over a surface area of

1200 m² characterised by a rocky-sea bottom with 5 m maximum depth. *S. salpa* biomass was calculated by using the length-weight relationship reported on FishBase (www.fishbase.org). Density and shell maximum diameter (SD) of all the species of limpets present on the breakwater (*Patella caerulea* and *Patella ulyssiponensis*) were assessed along six 80 × 20 cm transects, over the mean low water line. Size of limpets was measured by a vernier caliper (precision 0.05 mm).

2.3. Effect of herbivores

Firstly, the effects of the limpet *Patella* spp., the crabs *Pachygrapsus marmoratus* and *Eriphia verrucosa*, and the salema *Sarpa salpa* on *Cystoseira amentacea* were explored with laboratory assays. The limpets and the crabs were collected at the artificial site and transported to the laboratory of the University of Nice in cool boxes filled with seawater and supplied with oxygen by air pumps to reduce stress. Two replicated tanks (about 30 L) were set up for each species. For *Patella* spp. and *Pachygrapsus marmoratus*, four individuals were put in each tank, while only one individual of *E. verrucosa* was put in the tanks to avoid fighting between conspecifics (Rossi and Parisi, 1973). Seawater temperature in the tanks was maintained at 23 °C and a 14L:10D (light:dark cycle) photoperiod was provided by cool-white fluorescent tubes. Concerning *S. salpa*, sub-adult individuals (about 8–10 cm) were caught by fishermen at Saint Jean Cap Ferrat and taken to the Marineland Water Park (Antibes, France) by the park staff, in special bags filled with seawater and pure oxygen (1:3), in order to minimize stress. At the Water Park, two experimental tanks (about 70 L) suitable for keeping *S. salpa* in captivity were set up, each with four *S. salpa* individuals. Fish were acclimatized for one week and fed with lyophilized food before starting the assay. Tanks hosting fish were supplied with continuously renewed seawater (about 20 °C) piped from the sea and exposed to sunlight.

The effects of the four feeders on *C. amentacea* were estimated by placing in each tank four *C. amentacea* primary branches about 12 cm long, fixed with epoxy putty to a concrete tile. Tiles were left in the tanks for one week, since this time was sufficient to observe the herbivore effect, and the animals were not fed with other food during the experiment. Depletion of *C. amentacea* was measured as the relative difference between initial and final algal surface area (in cm²), using ImageJ[®] software (available online at rsb.info.nih.gov/ij/). Weight was assessed in the pilot experiment (data not shown), but since measurements were time consuming, causing desiccation of *Cystoseira* branches, and correlation with surface area was positive (Supplementary Information), only the surface area value was used to assess herbivore consumption.

We also explored whether *S. salpa*, which had been shown to be the most effective grazer in the previous experiment, had higher feeding preferences for *C. amentacea* compared to other fleshy macroalgae common in the infralittoral fringe of the breakwater: *Dictyota fasciola*, *Ellisolandia elongata* and *Padina pavonica*. For each fleshy species, a separate assay was ran, using two replicated tanks with four *S. salpa* individuals. During each assay, *S. salpa* were offered the choice between *C. amentacea* and another alternative target fleshy macroalga: firstly *D. fasciola*, then *E. elongata* and finally *P. pavonica*. A concrete tile was installed in each tank with two branches of *C. amentacea* and two similar quantities of the alternative fleshy alga fixed by epoxy-putty. *C. amentacea* branches were replaced at each feeding preference assay. Each assay lasted seven days and algal consumption was measured as described in the previous experiment.

2.4. Manipulation of herbivore pressure

A field experiment in June and July 2013 was set up on the Saint Jean Cap Ferrat breakwater to quantify herbivory effect on *Cystoseira amentacea* under field conditions and to test whether reduction of grazing could facilitate the installation of such canopy-forming species, otherwise absent from the artificial structure. Breakwaters are quite

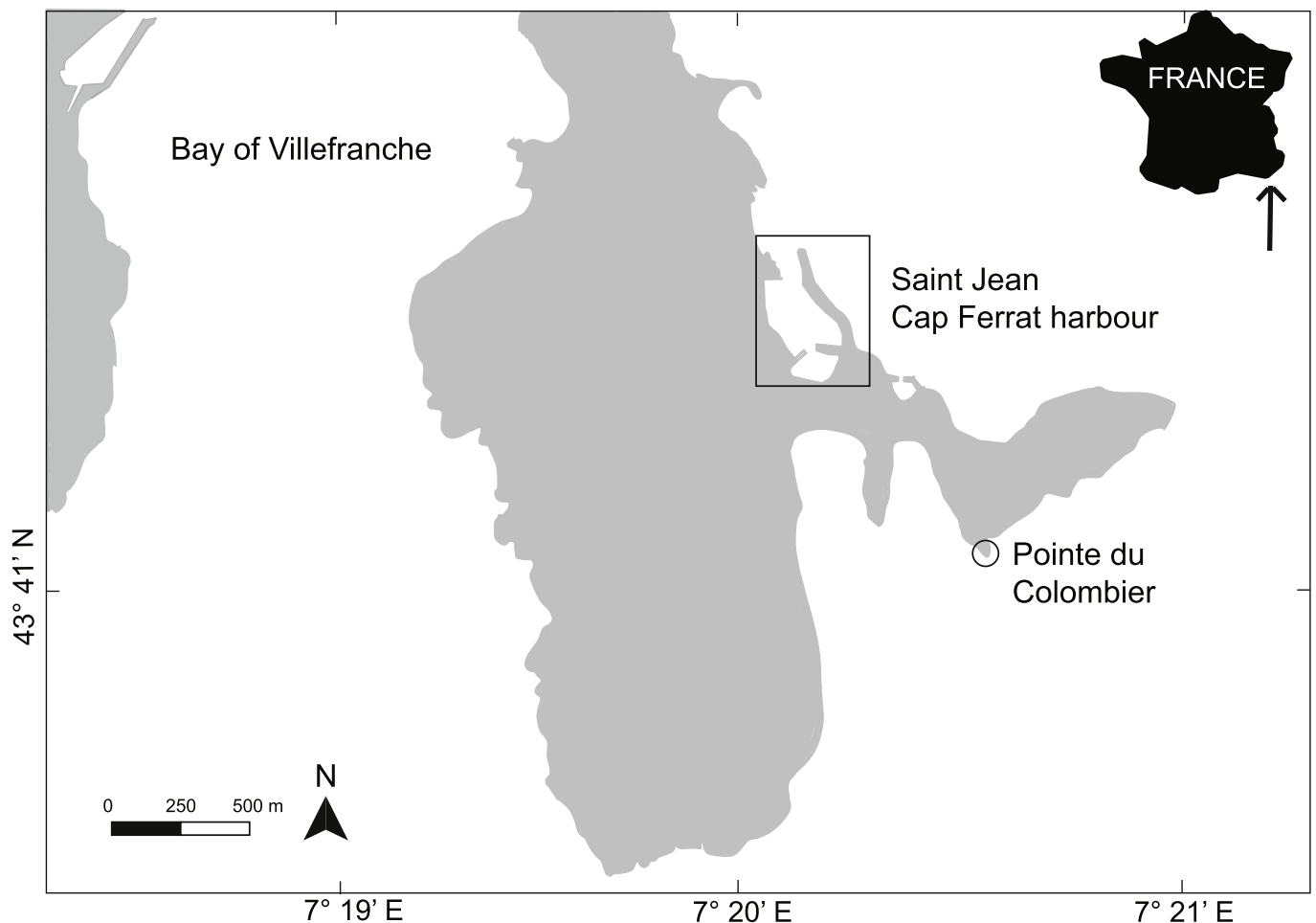


Fig. 1. Map of the study area on the French Riviera (South-East France), close to the Bay of Villefranche and the city of Nice (a city with nearly 400,000 inhabitants, located in an urban area with a population of one million).

common in the region, but the one selected for this study is one of the few artificial structures located very close to natural dense stands of *C. amentacea* on rocky shores (< 300 m). This ensured that local conditions (e.g. water quality, temperature) were broadly suitable for the species and that the potential herbivory effect, the subject of this study, was not affected by other factors.

Twenty-one 30 × 20 cm plots, placed at least 5 m apart, in the infralittoral fringe on the seaward side of the breakwater, were cleared in order to remove all algae and invertebrates. Four *C. amentacea* branches (about 12 cm long) were fixed with epoxy putty in small holes (2 cm) drilled in each plot. The branches were collected at Pointe du Colombier, a nearby natural site with dense fringing belts of *C. amentacea* (Fig. 1). In order to limit any damage to the source population (*C. amentacea* is a priority species, listed in the Barcelona and Bern Conventions), only primary branches were carefully detached from the seaweeds and immediately transferred in a cool box to the artificial structure. Once branches are separated from the parental plant, they are not able to grow further, but this technique was adequate to measure the direct damage from herbivory within a short time-lapse, providing a reasonable proxy of the effects expected to occur during transplantation tests involving adult plants for restoration purposes.

Plastic net cages (about 30 cm large, 20 cm wide, 10 cm high; 1 cm mesh) of different shapes were used to selectively allow access to: All Herbivores, Salema, Limpets, Crabs, Limpets + Crabs or No Herbivores (three randomly allocated replicate plots each, see details of the experimental setup in Table 1). The cages were fastened with cable ties to rods (10 cm high) previously fixed by epoxy putty in holes drilled into

the rock. Care was taken to ensure there were no gaps between complete cages and the rock surface. In addition, three replicate partial cages were fixed on the breakwater to test for a potential cage artefact effect (Cage Effect Treatment). To check whether translocation of *C. amentacea* branches to the artificial structure could have affected the fitness, twelve branches were brought back to the natural site and fixed to the rock, under a completely closed cage, using the same method as on the breakwater. Three separate plots were set up, corresponding to the treatment named Translocation Effect (Table 1). The effectiveness of cages in excluding herbivores was not directly assessed, since numerous studies in the literature have already demonstrated that cages are efficient in controlling fishes, limpets and crabs (e.g. Silva et al., 2004; O'Connor and Crowe, 2005; Tsirintanis et al., 2018). The experiment lasted one week, as the tank experiments, and it was replicated twice in order to verify the consistency of the results. The grazing effects on *C. amentacea* branches in the different treatments were quantified by measuring the relative differences in algal surface area at the start and at the end of the experiment, as described for the laboratory assays. The values of the four branches were averaged for each plot and used in the analyses.

Differences among treatments used in the manipulative experiment were analysed with permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) based on Euclidian distance matrixes of the univariate measure of surface area percent loss. Performing a PERMANOVA on the Euclidean distance of a univariate response variable is equivalent to performing a univariate ANOVA, with the exception that the p-values for a PERMANOVA are calculated using

Table 1

Description of the experimental treatments, named after the herbivores allowed to access the plots.

Treatment name	Herbivores allowed in the plot	Type of treatment	Brief description
All Herbivores	All	No exclusion	No cage
Salema	<i>Sarpa salpa</i> (and potentially other fishes)	Selective exclusion treatment	Plots surrounded by a 10 cm high folded net, preventing crab and limpet access, completely open on top.
Limpets	Only limpets	Selective exclusion treatment	Plots completely protected by a closed cage with four individuals of <i>Patella</i> spp. (3-4 cm shell length) inside.
Crabs	Only crabs (and potentially other fast moving gasteropods)	Selective exclusion treatment	Plots protected by a partially closed cage with openings (13 × 4 cm) at the base. Limpets were manually removed from the surroundings.
Limpets + Crabs	Limpets and crabs	Selective exclusion treatment	Plots protected by a partially closed cage with openings (13 × 4 cm) at the base. Limpets were not removed from the surroundings.
No Herbivores	None	Full exclusion	Plots protected by a completely closed cage on the breakwater.
Cage Effect	All	Artefact control	Plots non-protected, only a partial cage (on one side) was set up.
Translocation Effect	None	Artefact Control (effect of transportation to the artificial site).	Plots completely protected by a closed cage in the original natural site.

permutations rather than tabled p-values, and thus do not assume normality of the data (Anderson et al., 2008). Data met the homogeneity of variance since p-values obtained with the PERMDISP tests were not statistically significant. Treatments were considered as a fixed factor with eight levels, with three replicate plots each. Two separate analyses were performed for each temporal trial due to lack of independence. P-values were obtained by 9999 unrestricted permutations of raw data. Pairwise Monte Carlo tests were done in order to discern potential differences among treatments. All analyses were performed using Primer 6 & PERMANOVA + software package.

2.5. Ethics statement

The manipulative experiment on the breakwater at the Saint Jean Cap Ferrat harbour was authorized by the Municipality and the Saint-Jean-Cap-Ferrat harbour authority.

Even if most *Cystoseira* species are listed in the Bern Convention and in the ASPIM Protocol, at present no conservation measures have been adopted at national and international level. However, in order to reduce damage to natural *Cystoseira amentacea* populations, only primary branches of adult individuals were collected.

The tank experiments involving fish were conducted at the Marineland Water Park of Antibes (France), a certificated Aquarium facility authorized by national legislation for keeping vertebrates in captivity. Care was taken to transport fish to and from the Park, using international standard procedures, and transportation was performed by the specialized staff of the Park. Animals were provided with food and kept in good tank conditions. Fish were not sacrificed and were released at sea after the study.

3. Results

3.1. Abundance of herbivores

At the artificial study site, the estimated densities of all potential herbivores were: 0.2 ± 0.02 ind./m² (mean ± SE) for *Pachygrapsus marmoratus* and 0.02 ± 0.006 ind./m² for *Eriphia verrucosa*; 407 ± 43.1 ind./m² for *Patella* spp.; and 1.5 ± 0.3 ind./m² for *Sarpa salpa*. Size class distributions (Fig. 2) showed that on the breakwater, the limpet population was mainly characterised by small individuals (1-10 and 10-20 mm SD classes, representing about 90% of all individuals). The two species of crabs were represented by different size classes: the *Pachygrapsus marmoratus* population was mainly characterised by individuals between 1 and 2 cm CL, and *E. verrucosa* by larger individuals (> 3 cm CL). In front of the breakwater, large schools of *S. salpa* sub-adult individuals (about 6 cm total length) were observed, representing more than 60% of all individuals, but large individuals (> 16 cm) were also observed. The total biomass of *S. salpa* corresponded to 13.7 ± 2.7 g/m².

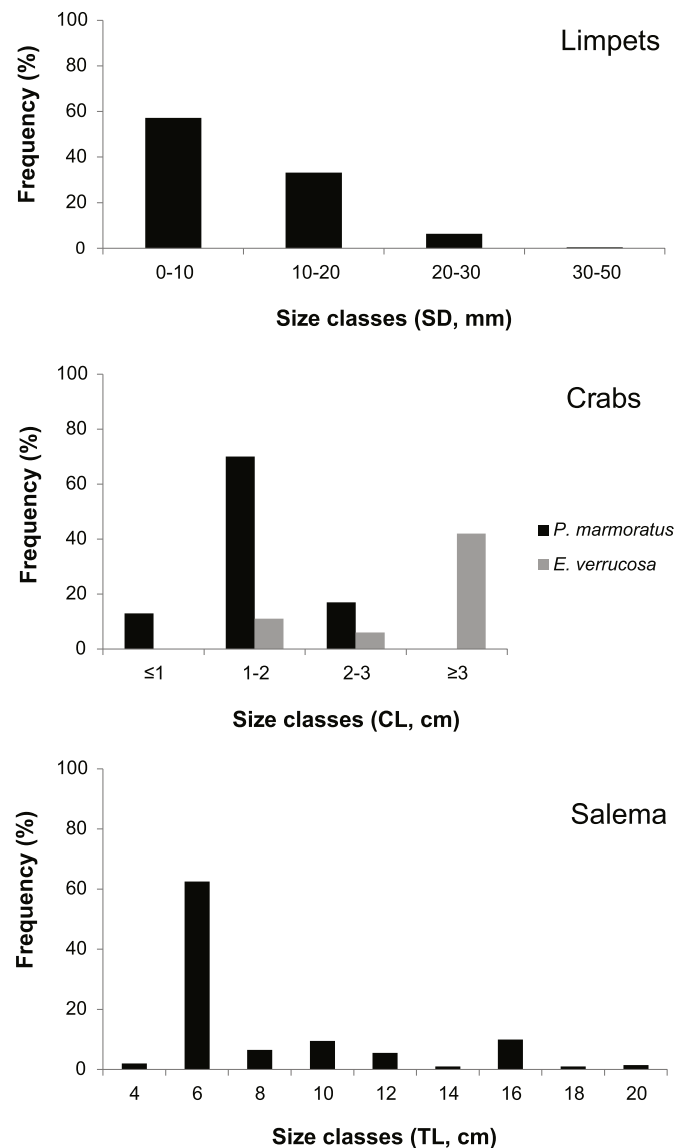


Fig. 2. Size class distributions of the different feeders at the artificial site. SD: shell maximum diameter, CL: carapace length. TL: total length.

3.2. Effect of herbivores

Sarpa salpa was the main herbivore of *Cystoseira amentacea* in the laboratory tank assay, causing more than 80% algal surface loss (Fig. 3). The crab *Pachygrapsus marmoratus*, under food-limitation

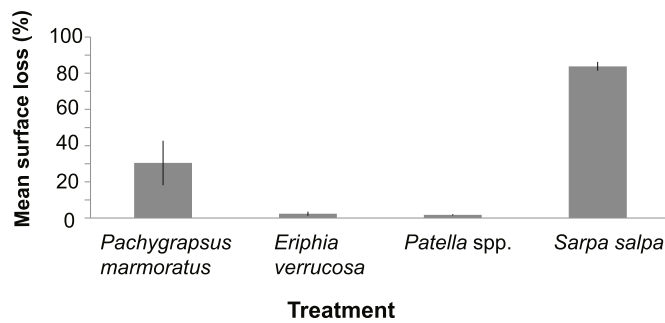


Fig. 3. Percentage of surface area loss of *Cystoseira amentacea* branches due to the different herbivores in the first tank experiment.

conditions, was able to cut and eat only small fragments of *C. amentacea*, while the other grazers (*Eriphia verrucosa* and limpets) did not consume/degrade *C. amentacea* primary branches.

In the feeding preference experiment, *S. salpa* always preferred *C. amentacea* compared to the other common macroalgae of the wave-exposed infralittoral fringe (*Dictyota fasciola*, *Padina pavonica*, *Ellisolandia elongata*), consuming on average more than 60% of *C. amentacea* branches in a few days compared to 30% of *D. fasciola*, 7% of *E. elongata* and 13% of *P. pavonica* (Fig. 4).

3.3. Manipulation of herbivore pressure

At the artificial site, *Cystoseira amentacea* branches were highly consumed (from 70% up to 90%) in all plots where *Sarpa salpa* had access. In treatments where salema were excluded *C. amentacea* loss was significantly reduced (Fig. 5). The analyses of variance (PERMANOVA, $p < 0.01$, Table 2) and pairwise tests (see letters above the bars in Fig. 5) showed significant differences among the treatments accessible to *S. salpa* (All Herbivores, Cage Effect, Salema) and those exclusively accessible to other feeders (Crabs, Limpets, Limpets + Crabs) or completely closed (No Herbivores, Translocation Effect), where *C. amentacea* was not grazed, demonstrating that the grazing pressure of the invertebrates was negligible when compared to that of salema. Cages did not introduce an artefact that compromised the results, as no significant differences were observed between All Herbivores treatment and Cage Effect treatment, even if in this treatment, as well as in Salema treatment, grazing was slightly lower, probably due to the presence of a partial cage that potentially discouraged salema from approaching the plots. In the first trial, an unexpectedly high surface area loss was measured in the Translocation Effect treatment (Fig. 5), probably due to a storm that partially damaged the cages at the natural site, while in the

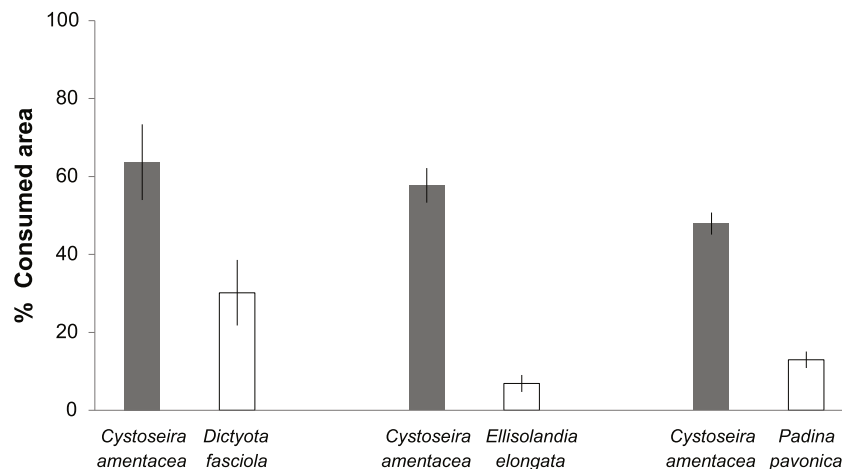


Fig. 4. Feeding preference of *Sarpa salpa* between *Cystoseira amentacea* and other common macroalgae of the infralittoral fringe. Each couple of algae was offered to fish at different times.

second trial no surface loss was observed in the Translocation Effect treatment that was no statistically different from the No Herbivores treatment.

4. Discussion

The spread of coastline urbanization requires mitigation actions, including solutions to restore or revitalise habitats that are increasingly lost, fragmented or degraded (Airoldi et al., 2005a; Bulleri and Chapman, 2010; Firth et al., 2013). Canopy-forming seaweeds are highly sensitive to the effects of coastline urbanization (Airoldi and Beck, 2007; Fowles et al., 2018), so that their conservation and, if necessary restoration, should be prioritized (Gianni et al., 2013). In the Mediterranean Sea, numerous existing coastal defence structures were built on *Cystoseira* habitats and should be used as a scaffold for their restoration, increasing, as far as possible, the connectivity among natural populations (Gianni et al., 2013). However, the results of the present study highlight a paramount role of herbivores, and in particular herbivorous fish, in limiting the success of forestation operations in artificial novel habitats, and suggest the need to exclude/control herbivores during these interventions.

Density of invertebrate herbivores at the breakwater was representative of very shallow artificial structures in the Mediterranean Sea. Limpets were very abundant (about 400 ind./m²), in agreement with the data reported from other artificial structures (10–1100 ind./m²) (Bulleri et al., 2000; Bulleri and Chapman, 2004; Fauvelot et al., 2009). Crab density data for Mediterranean artificial habitats are not available in literature, but the densities measured on the breakwater (0.2 ± 0.02 ind./m² for *Pachygrapsus marmoratus* and 0.02 ± 0.006 ind./m² for *Eriphia verrucosa*) were comparable to the values observed in other studies on natural habitats (*P. marmoratus*: 0.2–2.4 ind./m²; *E. verrucosa*: 0.02–0.05 ind./m²) (Cannicci et al., 1999, 2002; Flores and Paula, 2001). *Sarpa salpa* density estimations in the Mediterranean Sea are very variable (0.0002–0.67 ind./m²) (García-Rubies and Zabala, 1990; Bariche et al., 2004) and biomass may be as high as 12 g/m² (Guidetti et al., 2014). This is probably due to the fact that *S. salpa* is a gregarious species, usually moving in large schools, and its density can change considerably in space and time (Harmelin-Vivien et al., 1985). The values measured in the present study (1.5 ± 0.3 ind./m², 13.7 ± 2.7 g/m²) seem to be representative of high densities and biomass, compared to the other estimates at Mediterranean scale available in literature, using the same fish counting and biomass calculation methods.

The results presented suggest that *Cystoseira* transplantation on artificial structures can be highly impacted by *S. salpa*, which is able to

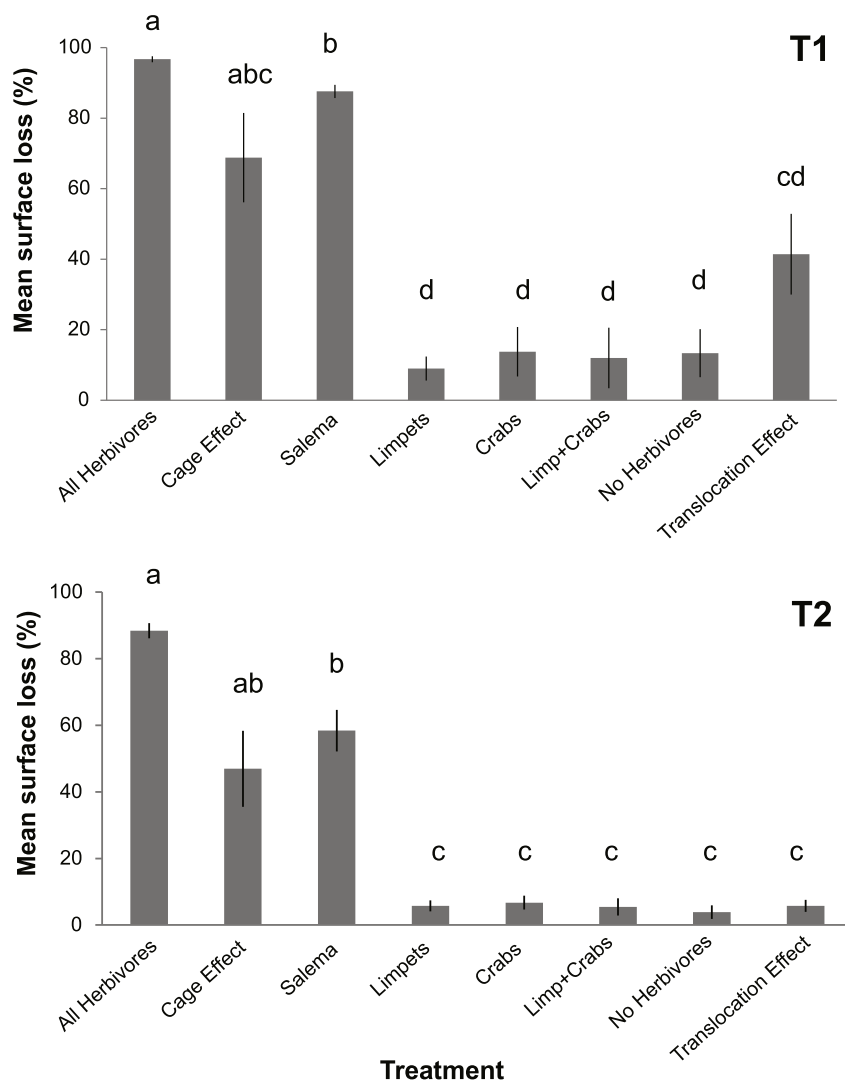


Fig. 5. Percentage of surface area loss of *Cystoseira amentacea* branches in the different treatments (detailed in Table 1) in the two trials of the manipulative experiment (T1: June 2013 and T2: July 2013). Letters indicate significant differences resulting from pairwise-tests.

graze up to the infralittoral fringe, a zone that is generally considered as a spatial refuge from fish herbivory (Vergés et al., 2009), being much of the time exposed to the air with tides and waves. *S. salpa* grazing was far more efficient than that of the other herbivores considered in this study. The fish grazing pressure was sufficient to cause up to 90% of *C. amentacea* surface area loss after a few days, and sometimes even after a few hours, in agreement with the results of a recent study by Gianni et al. (2017). Fish herbivory on the infralittoral fringe seemed to be higher during bad weather conditions, generally associated with low barometric pressure, resulting in a positive variation of the sea-level. Such conditions probably make *C. amentacea* transplants more accessible to fish grazing that, although sporadic, may have a very strong impact on total biomass. It cannot be excluded that other fishes, such as blennids, contributed to the consumption of *C. amentacea* branches;

however, the grazing effect of other fish species on macroalgal communities in the NW Mediterranean is generally considered as limited (Verlaque, 1990, and references therein), and they may not have contributed to the strong grazing rate observed. The distribution range of *Sparisoma cretense* (Linnaeus, 1758), the other native herbivorous fish of this Basin, does not extend to the NW Mediterranean, therefore the potential herbivory effect of this species on the manipulative experiment can be excluded. In addition, tropical herbivorous fishes (*Siganus luridus* Rüppell, 1828 and *Siganus rivulatus* Rüppell, 1828), that are causing *Cystoseira* forest regression in the Eastern Mediterranean Sea (Sala et al., 2011), do not occur along the French Riviera: only two individuals were caught in 2008 in Marseille (Daniel et al., 2009) and a school of several dozens of individuals was observed in 2012 in the Bay of Villefranche (Thibaut et al., 2015). No other observations have been

Table 2

PERMANOVA performed on the surface area loss of *Cystoseira amentacea* branches in the different treatments (detailed in Table 1) in the two trials of the manipulative experiment (T1: June 2013 and T2: July 2013). Statistically significant values are in *italic*. Tr: Treatment.

Source	df	TIME 1			TIME 2		
		MS	Pseudo-F	P (perm)	MS	Pseudo-F	P (perm)
Tr	7	4482.70	23.60	<i>0.0001</i>	3725.50	79.020	<i>0.0001</i>
Residual	15	189.93			47.14		

reported in this area so far, to our knowledge.

The findings from tank experiments were in line with the field observations: *S. salpa* was able to deplete *C. amentacea* branches almost completely within a few days, being the most important feeder among those considered in the study. They also indicated that *C. amentacea* was the preferred species over the other macroalgae tested. This is consistent with past works suggesting that *Cystoseira* can represent up to 60% of the gut content of *S. salpa* (Verlaque, 1990; Tomas et al., 2011) and can reduce biomass up to 86% (Gianni et al., 2017). This preference could be explained by the high nutritional value of *Cystoseira* species, which are notably rich in fatty acids (Durmaz and Duyar, 2008; Vizetto-Duarte et al., 2014). This observation could also partially explain the reason why *Cystoseira* is often absent from artificial structures in many regions, while other algae are present (this work, Bulleri and Chapman, 2004; Vaselli et al., 2008; Ferrario et al., 2016), though the role of abiotic factors, species interaction and propagule dispersal are also crucial in this context (Cacabelos et al., 2016; Ferrario et al., 2016, and references therein).

The results of the present study highlight that crabs are able to damage and possibly ingest *Cystoseira*, but at a lower rate compared to salemma. Previous observations on *Cystoseira barbata* from subtidal artificial habitats showed that different species of crab can contribute to reducing by 50% the survival rate of individuals (Perkol-Finkel et al., 2012; Ferrario et al., 2016). This is not the case for shallow *Cystoseira amentacea* forests. *P. marmoratus* and *E. verrucosa* do not mask themselves with algae, and in general they prefer feeding on small invertebrates and filamentous algae rather than on corticated and leathery macrophytes (Cannicci et al., 2002, 2007; Silva et al., 2010). It is possible that crabs consumed *Cystoseira* in the tank experiments due to the food-limitation experimental conditions. Similarly, limpets did not affect *C. amentacea* branches. Limpets can regulate macroalgal assemblages on intertidal shores, being able to remove mature thalli (Lorenzen, 2007, and references therein), but in the Mediterranean Sea they mostly control early patterns of colonisation, grazing on early life stages of algae (i.e. zygotes and juveniles) rather than on well-developed individuals (Benedetti-Cecchi and Cinelli, 1992; Benedetti-Cecchi et al., 1996). It is worth noting that in the present study, the herbivory rate was assessed solely on adult *C. amentacea* branches; therefore, limpets, crabs and other small herbivores may probably have a more significant role in reducing natural or artificially enhanced settlement of *Cystoseira* propagules, as well as juvenile survival on artificial structures, as already demonstrated in natural habitats (e.g. Benedetti-Cecchi and Cinelli, 1992). More studies are needed to test cumulative effects of different herbivores and their interaction with other factors on restoration success involving *Cystoseira* early-life stages.

In conclusion, the present study demonstrates that fish may be the most important herbivore on artificial structures and may affect the success of restoration/rehabilitation operations for relevant canopy forming species. Future engineering operations aimed at increasing the presence of canopy-forming algae on artificial structures should take into account the effect of biotic factors. Cost-effective approaches are being developed to limit herbivory during restoration, such as the deterrent DeFish device designed by Gianni et al. (2017) to reduce *S. salpa* grazing on shallow *Cystoseira amentacea* forests, the seed-cultivating nets used in Yoon et al. (2014) to restore *Sargassum* beds, or the artificial seagrass leaves used by Tuya et al. (2017) to decrease herbivory-induced mortality on seagrass seedlings. Control of recreational and artisanal fishery, which can be very intensive around some artificial habitats (e.g. Airoldi et al., 2005b), could also be implemented to help the restocking of top predators (Perkol-Finkel et al., 2012; Gianni et al., 2013), as this would positively cascade down the food web, limiting herbivore pressure. Further research would also be worth undertaking to explore density-dependent or interspecific associational resistance to herbivory for transplanted seaweeds. It is known that neighbouring plants can influence the likelihood of damage by herbivores (Kim and Underwood, 2015), but these effects are still poorly known for marine

algal communities (Hoey and Bellwood, 2011), which limits their application in marine restoration operations. Incorporating this knowledge would open up new, effective and cost-effective approaches to enhance the revitalisation of critical habitats and their associated ecosystem services in marine urban environments.

Author contribution

Conceived and performed the experiments: FG, FB, LM. Analysed the data: FG. Wrote the paper: FG, FB, LM, LA.

Declarations of interest

None.

Acknowledgements

The authors wish to thank the Saint Jean Cap Ferrat Municipality and the harbourmaster for allowing the experiments on the breakwater. Special thanks are due to the local fishermen for providing fish and to the Marineland Water Park for providing facilities and help with experiments in tanks. Thank you to all researchers at the ECOMERS laboratory (University of Nice-Sophia Antipolis), to Jean-Michel Cottalorda, Giulia Prato, Eléonore Cambra for their precious help in the field, and to Daniela Catania for the first English revision. The authors wish also to thank the two anonymous reviewers for their useful comments which have helped to improve the manuscript. The work was carried out within the framework of the project MMPA-Training Network for Monitoring Mediterranean Marine Protected Areas, that has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) [grant number 290056].

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.marenvres.2018.04.007>.

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