First *ex situ* outplanting of the habitat former *Cystoseira amentacea* var. *stricta* in a restoration perspective (#34961)

First submission

Editor guidance

Please submit by **9 Mar 2019** for the benefit of the authors (and your $200 publishing discount).

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Raw data check
Review the raw data. Download from the materials page.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files

Download and review all files from the materials page.

7 Figure file(s)
2 Table file(s)
2 Raw data file(s)

Custom checks

Field study

Have you checked the authors field study permits?

Are the field study permits appropriate?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- Data is robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

First *ex situ* outplanting of the habitat former *Cystoseira amentacea* var. *stricta* in a restoration perspective

Gina De La Fuente Corresp., [1] , **Mariachiara Chiantore** [1] , **Valentina Asnaghi** [1] , **Sara Kaleb** [2] , **Annalisa Falace** [2]

[1] Department of Earth, Environment and Life Sciences, University of Genoa, Genoa, Italy

[2] Department of Life Sciences, University of Trieste, Trieste, Italy

Corresponding Author: Gina De La Fuente
Email address: gina.delafuente@edu.unige.it

In the Mediterranean Sea marine forests constituted by brown algae of the genus *Cystoseira* play a valuable role as foundation species. Due to their evidences of regression/loss of these habitats caused by different factors, active restoration techniques are encouraged by European legislation. In particular, non-destructive restoration techniques of threatened species are strongly encouraged, since they avoid depletion of natural donor populations. In the framework of the EU project ROCPOP-Life, the first *ex situ* outplanting experience on *Cystoseira amentacea* var. *stricta* has been implemented in the Cinque Terre Marine Protected Area (North-western Mediterranean). The results provide a well-defined approach of intertidal species outplanting technique in order to maximize the good performance of the restoration action focusing on the different earlier phases: i) laboratory culture; ii) transport; iii) juveniles densities; iv) grazing pressure on the outplanted juveniles.

First *ex situ* outplanting of the habitat former *Cystoseira amentacea* var. *stricta* in a restoration perspective

Gina De La Fuente[1], Mariachiara Chiantore[1], Valentina Asnaghi[1], Sara Kaleb[2], Annalisa Falace[2]

[1] Department of Earth, Environment and Life Sciences, University of Genova, Genova, Italy
[2] Department of Life Sciences, University of Trieste, Trieste, Italy

Corresponding Author:
Gina De La Fuente[1]
Corso Europa 26, Genova, 16132, Italy

Email address: gina.delafuente@edu.unige.it

Abstract

In the Mediterranean Sea marine forests constituted by brown algae of the genus *Cystoseira* play a valuable role as foundation species. Due to their evidences of regression/loss of these habitats caused by different factors, active restoration techniques are encouraged by European legislation. In particular, non-destructive restoration techniques of threatened species are strongly encouraged, since they avoid depletion of natural donor populations. In the framework of the EU project ROCPOP-Life, the first *ex situ* outplanting experience on *Cystoseira amentacea* var. *stricta* has been implemented in the Cinque Terre Marine Protected Area (North-western Mediterranean). The results provide a well-defined approach of individual species outplanting technique in order to maximize the good performance of the restoration action focusing on the different earlier phases: i) laboratory culture; ii) transport; iii) juveniles densities; iv) grazing pressure on the outplanted juveniles.

Introduction

Around thirty-five percent of brown algae species (Fucales and Laminariales; Guiry and Guiry, 2019) contribute to the structure of the coastal landscapes, playing a key role as ecosystem engineers, providing an important value as natural capital. These canopy-forming species form reliable marine forests that provide habitat for many other associated species, affecting the structure, biodiversity and functioning of their habitats (Thompson et al., 1996; Christie et al., 2007; Airoldi et al., 2015). Kelp forests (i.e. *Macrocystis*, *Lessonia* and *Laminaria*) constitute some of the most important habitats, distributed worldwide throughout temperate and polar coastal oceans (Steneck et al., 2002). In the Mediterranean Sea, *Laminaria*, *Sacchoriza*, *Phyllariopsis* and *Sargassum* genera, also play a role as foundation species in some specific

locations but the canopy-forming brown algae of the *Cystoseira* genus are the most important, since they are widespread in this biogeographic region (Rodriguez-Prieto et al., 2). Yet, they are exposed to multiple disturbances that have caused decline in their abundance in many coastal areas (Stene et al., 2002; Airoldi et al., 2008; Connell et al., 2008; Mineur et al., 2015). Main pressures affecting these valuable ecosystems are sedimentation (Perkol-Finkel & Airoldi, 2010), low water quality (Sales et al., 2011), anthropization (Mangialajo et al., 2008), overgrazing (Ve et al., 2014) and harvesting (Zha al., 2008).

In the Mediterranean, several studies have reported about the past and the present distribution and abundance of different canopy-forming species belonging to *Cystoseria* (T aut et al., 2014; Mancuso et al., 2018), detecting regressions or losses caused by the above mentioned factors (Falace et al., 2010; Perkol-Finkel & Airoldi, 2010; Sales et al., 2011; De La Fuente et al., 2018).

In this framework, active marine restoration is strongly encouraged to avoid the loss of these valuables habitats that enhance biodiversity and preserve ecosystem functions and services they provide (i.e. EU Biodiversity Strategy 2020).

As far as *Cystoseira* species, three different restoration techniques have been implemented in the Mediterranean Sea: transplanting juveniles or adults (Falace et al., 2006; Susini et al., 2007), enhancing recruitment potential by fertile receptacles in the target area (*in situ*; Verdura et al, 2018) or by outplanting juveniles, cultured in the laboratory, along the shore (*ex situ*; Sales et al., 2011; Verdura et al. 2018). The latest two techniques are strongly encouraged applied to the restoration of threatened species because avoid the depletion of the natural donor populations (Falace et al., 2018).

Restoration actions have to be addressed depending on the biological traits of the target species (i.e. reproduction strategy) and the environmental features (depth and hydrodynamics). The *in situ* technique is especially recommended for species with high dispersal capacity (e.g.: kelps; Reed et al., 1988; Gaylord et al., 2002), while the *ex situ* technique is more appropriate for species with a low dispersal capacity (e.g. *C. amentacea*; Mangialajo et al., 2012). Both techniques have been recently validated for a shallow subtidal species (*C. barbata*; Verdura et al., 2018), living in calm conditions.

In this study we report the results of the first *ex situ* outplanting of the intertidal *Cystoseira amentacea* var. *stricta* Montagne, (hereafter *C. amentacea*) performed in the framework of the EU project ROCPOP-Life. The results of the restoration action over the first six months are reported as presence and cover percentages of the juveniles outplanted, also pointing out the positive results of the transportation of the cultured juveniles from the laboratory facilities to the ouplanting location. A description of the infauna organisms present in the rocky shores, where juveniles of *C. amentacea* have been outpla , is also provided, in order to assess the potential role of grazing in affecting survival success.

Materials & Methods

Study sites

The *ex situ* outplanting of the ~~intertidal~~ species *C. amentacea* was performed in Summer 2018, through a non-destructive strategy, collecting in June the apical fronds (ca. 3 cm in length) holding mature receptacles, from a healthy population located in the Portofino MPA (donor site). The ~~juveniles, after the laboratory culturing period, were positioned~~ in the Cinque Terre MPA (receiving site; around 80 km apart from the donor site), where this species is presently missing (although its presence in the past is supported by herbaria records; De La Fuente et al., 2018) and is replaced by the cogeneric and more stress tolerant species, *C. compressa*. Both MPAs are located in the Ligurian Sea, North-western Mediterranean (Fig. 1). The sites are characterized by a tide in the range of 30 cm (the barometric tide may dominate the water level) and an average spring temperature of 20℃. After sampling, apices were gently cleaned with tweezers and rinsed with filtered seawater to remove adherent biofouling and detritus. Then, apices wrapped with seawater-wetted towels were delivered within 24h under dark, cold and humid conditions to the laboratory in Trieste (North-eastern Italy; Fig. 1) for culture in environmentally controlled rooms.

Laboratory *ex situ* cultivation

Three apices with mature receptacles (additionally cleaned with a brush and rinsed with autoclaved seawater) were placed on each substrate, constituted by rough clay round tiles (4.5 cm in diameter), with a 0.6 cm hole at the center in order to screw the tile at the rocks. On the next day, the fertile apices were removed and the zygotes attached on the tiles were cultured over a 3 weeks' period (≈ 450 tiles).

Temperature and photoperiod were selected to reflect typical seasonal conditions at the sampling site and according to the optimum conditions tested in the protocol provided in Falace et al. (2018). Von Stosch's enriched filtered seawater (VSE) was used as culture medium to speed up the culturing time and to reach higher size at the time of outplanting. The medium was enriched with antibiotic (Amikacin sulphate 2 µL and Ampicillin sodium 500 µg L-1 of culture medium) and GeO2 (Falace et al., 2006) to avoid bacterial and diatoms growth. The culture medium in the aquaria was renewed every 3 days to minimize any possible effects of nutrient limitation and was aerated, continuously, by bubbling and water pumps to increase oxygenation and hydrodynamics.

After 24 days of controlled growth in the Trieste laboratory, pictures were taken (17th July; 160 random tiles) in order to assess percent cover and juvenile length.

Outplanting in the field and monitoring

On 19th July 2018 the tiles were transported to Cinque Terre MPA. All the tiles were carefully placed into small boxes filled with filtered seawater. They were placed into a large insulated container, which was maintained refrigerated with icepacks. The container was transported by car with air conditioner (around 7 hours trip) to the receiving site, where the boxes were located in an air conditioned room overnight.

On the next day (20th July 2018, time 0), the tiles were carefully transported to the field, using a rubber boat. Eight patches (Fig. S1) were established in the previous weeks: in each patch, 50 holes were drilled and screws were located in advance. So, on the day of the implantation, the tiles were quickly screwed to the rocks. Overall the deployment of 400 tiles was performed in around 5 hours.
The monitoring of the clay tiles started on the same day, by collecting pictures of 20 out of the 50 clay tiles located at each patch, in order to assess the percent cover of the juveniles on the tiles using Image J.
The monitoring of survival was performed on the following 2 months, in order to estimate survival and growth of the juveniles, assessing percent cover of the clay tiles and presence/absence of juveniles on the tiles. The monitoring was performed on July 27th (Time 1), August 6th (Time 2), August 29th (Time 3), September 27th (Time 4).
An additional sampling was performed 4 months later (Time 5). This sampling occurred after a major storm that affected the Ligurian Sea at the end of October 2018 (ANSA, 2018). Since most of the tiles were detached by this unprecedented event, it was not possible to assess quantitatively the restoration performance in terms of percent cover but only in terms of juvenile growth (length).

Grazers abundance

Since one of the obstacles to repopulation success is represented by grazing, grazer density was estimated by collecting 15 cores of infaunal organisms, by way of a stainless steel corer (internal diameter 5 cm; surface area 19.6 cm^2). The samples were preserved in isopropyl alcohol and transferred to the lab, where sorting was performed. All specimens were identified at the finest resolution, then grouped into feeding groups (Grazers, Deposit feeders, Suspensions feeders and Predators) corresponding with their phyla (Mollusca, Crustacea, Annelida, Echinodermata).

Data analysis

One-way ANOVA was applied to assess possible differences between percent cover of the juveniles on the tiles when leaving the laboratory and at the time of the deployment (Time 0), after data transformation according to arcsin and verification of assumptions (normality using Shapiro test and homoscedasticity using Bartlett test).
The effect of percent cover at the time of deployment was assessed using a Generalised Linear Model (GLM) on both the percent cover (family=quasibinomial) and the presence/absence (family=binomial) of juveniles on the tiles at Time 4, using as predictor variable the percent "cover class" of each patch at the start of the experiment. Patches were classified according to 3 "cover classes" (based on percent cover at the start): Low (18.2 ± 1.6, avg ± 1ES; patches 2, 3 and 5), Medium (25.0 ± 2.1, avg ± 1ES; patches 1, 7 and 8), High (32.2 ± 2.5, avg ± 1ES; patches 4 and 6).

Results

The juveniles were 2.65 ± 0.46 mm (average ± std) long when they were transported to the receiving site. The percent cover on the tiles measured at Time 0 was compared with the one at the time of leaving the laboratory (3 days before), in order to assess the transport performance (Fig. 2). Percent cover in laboratory and in outplants was not significantly different (one-way ANOVA; p = 0.327).

At the start of the deployment, average percent cover of the juveniles on tiles was 24.22 % (± 1.24; avg ± 1ES). The percent cover data of the juveniles on tiles in the eight patches along time (Time 0 to Time 4) are reported in Fig. 3. All patches showed a sharp drop from Time 0 to Time 1, due to the expected detachment of some juveniles in the receiving site (not controlled conditions). Some patches clearly showed average lower values of cover at Time 0 and such different cover at the start affected the survival and growth of the juveniles along time (Fig. 3). In the patches characterised by higher cover of juveniles at the start (patches 4, 6, 7 and 8), after the first decline, cover increased in the next sampling dates, because of the growth of the juveniles (Fig. 3 and 4). The GLM on the percent cover on the tiles at Time 4 provided significant differences between the classes (p = 0.0143). The Low class was significantly different from the others while the Medium one did not differ from the High one (Table 1).

The good performance of the outplanting, at least in the early phases, is confirmed by the assessment of the percentage of tiles reporting the presence of juveniles (Fig. 5). In fact, from Time 0 to Time 1, such percentage moved from 100% to 88.13% and was still over 40% after two months (Time 4). Similarly to cover data, also survival was different in the patches characterised by higher cover of juveniles at the start. In fact, the GLM on the presence/absence of juveniles on the tiles at Time 4, using as predictor variable the percent "cover class" of each patch at the start of the experiment, provided significant differences between the classes (p = 0.00161). The Low class was significantly different from the others, while the Medium one did not differ from the High one (Table 1).

The growth over time of the ouplanted juveniles is shown in Figure 6. They grew from 3 mm (Time 0) to 3 - 6 cm (last record, in February 2019) in six months.

Grazing did not clearly affect the outplanted juveniles. During the monitoring and in the collected pictures, no grazers were detected visually crawling on tiles. The densities of the benthic macroinvertebrates are reported in Table 2. On the average the total abundance of potential grazers (mostly crustaceans) was 9.3 ind/19.6 cm^2

Discussion

Outplanting represents an innovative technique for restocking of brown canopy-forming macroalgae (Falace et al., 2018), although this method has to adjust to some fundamental constraints in its different phases of implementation: i) fertile material collection ii) lab juveniles

~~culturing iii) transport of juveniles to the field and iv) attachment of the juveniles on the rocky shore.~~

The lab culture presents issues related to the health of cultured embryos, therefore the reduction of culture time increases the chance of a good performance, other than reduce the costs. *C. amentacea* embryos were cultured in its optimal temperature and light intensity conditions (Falace et al., 2018), which can also unfortunately enhance spores, propagules and bacteria ~~proliferation.~~ The new culture medium formula, the antibacterial mixed solution and the enhanced hydrodynamics preserved the culture in good conditions. In addition, the accurate cleaning of the fertile apices, before and after the transport to the lab facilities, minimized, noticeably, culture contamination, growth of epiphytes and grazing. Moreover, the modified culture medium allowed to obtain in only three weeks healthy juveniles larger (2.65 mm) than in previous *Cystoseira* restoration studies: *C. amentacea* (three weeks - 1.38 mm; Falace et al., 2018) and *C. barbata* (one month -up to 400 µm; Verdura et al., 2018).

Furthermore, the length recorded after one month from the outplanting (5.81 mm; Fig. 6) is similar and even a bit larger than in ~~the~~ previous study on *C. amentacea* (4.73 mm; Falace et al., 2018), since in the present study the outplanting size was also larger.

The transport of early juvenile stages from the laboratory conditions to the receiving site, that may be located at a large distance, as in this case (≈ 520 km), did not affect their health. The results show no significant differences of percent cover of juveniles before and after the transport. The cover of the juveniles on tiles, at the arrival time in the receiving site, was even a little larger (before transport – 21.6%, after transport – 24.2%; Fig. 2).

This result was obtained maintaining a good temperature range (20-22ºC) during the transport and during the attachment of the clay tiles in the field (outplanting action), preserving juveniles in good health conditions. Due to the high numbers, the clay tiles were transported, by rubber boat, in cooled boxes to the rocky shore in several events over the same day in order to avoid the solar heat stress during the attachment.

The attachment technique applied in this study, using screws instead of epoxy putty (generally used both for adults and juveniles; Susini et al., 2007; Whitaker et al., 2010; Perkol-Finkel et al., 2011), increases the effectiveness of the outplanting by reducing the time effort during the attachment.

This method additionally minimizes the impact on the rocky shores and reinforces, remarkably, the attachment avoiding the possible dislodgement of the clay tiles by wave action, as in the case of *Legronia nigrescens* restoration (Vázquez and Tala, 1995). This is particularly important for intertidal species living in high hydrodynamics conditions, as in the case of *C. amentacea*.

In addition to optimization of culture and transport/outplanting techniques, our results stress the relevance of the juvenile covers on the tiles. Higher covers ensure higher growth in terms of percent cover (Table 1; Fig. 4). Intertidal species are exposed to high solar heating and desiccation stress, therefore, more percent cover allows to retain moisture and shading, enhancing the development of the early juveniles (Brawley and Johnson,1991; Dudgeon and Petraitis, 2005).

Another key issue is represented by grazing, which was reported in previous studies, yet without obviously affecting the development of the outplanted juveniles (Yu et al., 2012; Yoon et al., 2014). In this study, the number of the potential grazers was estimated along the rocky shore and, a low number of grazers, below the average for the area (Thrush et al., 2011), was obtained for the receiving site, although the grazer abundances in the region do not seem to potentially exert any relevant pressure on the outplants.

Conclusions

Our findings show that an improved laboratory culture procedure increases growth rate of juveniles and shortens the time to reach the suitable outplanting size. Culture medium, antibacterial mixed solution and accurately fertile material cleaning are relevant elements to guarantee a good culture performance and obtain high densities of healthy embryos, in a short time, ready to be positioned in the field and, consequently, increasing the restoration success. The feasibility of large distance transport from laboratory to the field has been remarkably proved, providing a potential option for replication also on a large scale.
Interestingly, the screw attachment technique avoids dislodgement, particularly in the early stages, when the traditional epoxy putty technique may be strongly affected by wave action, which is particularly relevant for ~~intertidal species~~.
On the other hand, grazing effect cannot be excluded but do not affect the outplants more than other factors (e.g. wave action), especially if outplanting size and high density are assured.
These results are strongly encouraging for the implementation of restoration actions of canopy forming species on a large scale, in light of EU guidelines.

Acknowledgements

We would like to thank the contributions of all colleagues and students who helped in the field and during sample processing: Saul Ciriaco (Shoreline Soc. COOP), Sara Menon (Shoreline Soc. COOP), Marco Segarich (Shoreline Soc. COOP), Massimo Andreoli (Cinque Terre Marine Protected Area), Enrico Agostini (Nemo-Italia), Davide Monteggia (University of Genoa), Maria Paola Ferranti (University of Genoa), Lorenzo Meroni (University of Genoa), Greta Fallanca (University of Genoa), Francesca Piga (University of Genoa).

References

Airoldi L, Balata D, Beck MW. The gray zone: relationships between habitat loss and marine diversity and their applications in conservation. J Exp Mar Bio Ecol. 2008; 366(1-2): 8-15. https://doi.org/10.1016/j.jembe.2008.07.034

Airoldi L, Ballesteros E, Buonuomo R, van Belzen J, Bouma TJ, Cebrian E et al. Marine forests at risk: solutions to halt the loss and promote the recovery of Mediterranean canopy-forming seaweeds. In 5th Mediterranean Symposium on Marine Vegetation. 2015.

ANSA. Storm death toll up to 11, Liguria on its knees. [cited 2018 Oct 30]. Available from: http://www.ansa.it/english/newswire/english_service/2018/10/30/ansa-storm-death-toll-up-to-11-liguria-on-its-knees_0973b7e8-492d-4802-8e16-9820abe65783.html

Brawley SH, Johnson LE. Survival of fucoid embryos in the intertidal zone depends upon development stage and microhabitat. J Phycol. 1991; 27(2): 179-186. https://doi.org/10.1111/j.0022-3646.1991.00179.x

Christie H, Jørgensen NM, Norderhaug KM. Bushy or smooth, high or low; importance of habitat architecture and vertical position for distribution of fauna on kelp. J Sea Res. 2007; 58(3): 198-208. https://doi.org/10.1016/j.seares.2007.03.006

Connell SD, Russell BD, Turner DJ, Shepherd SA, Kildea T, Miller, D et al. Recovering a lost baseline: missing kelp forests from a metropolitan coast. Mar Ecol Prog Ser. 2008; 360: 63-72. https://doi.org/10.3354/meps07526

De La Fuente G, Chiantore M, Gaino F, Asnaghi V. Ecological status improvement over a decade along the Ligurian coast according to a macroalgae based index (CARLIT). PLoS ONE. 2018; 13(12): e0206826. https://doi.org/10.1371/journal.pone.0206826

Dudgeon S, Petraitis PS. First year demography of the foundation species, Ascophyllum nodosum, and its community implications. Oikos. 2005; 109(2): 405-415. https://doi.org/10.1111/j.0030-1299.2005.13782.x

Falace A, Zanelli E, Bressan G. Algal transplantation as a potential tool for artificial reef management and environmental mitigation. Bull Mar Sci. 2006; 8: 161-166.

Falace A, Alongi G, Cormaci M, Furnari G, Curiel D, Cecere, E et al. Changes in the benthic algae along the Adriatic Sea in the last three decades. Chem Ecol. 2010; 26(1): 77-90. https://doi.org/10.1080/02757541003689837

Falace A, Kaleb S, De La Fuente G, Asnaghi V, Chiantore M. Ex situ cultivation protocol for Cystoseira amentacea var. stricta (Fucales, Phaeophyceae) from a restoration perspective. PloS ONE. 2018; 13(2): e0193011. https://doi.org/10.1371/journal.pone.0193011

Gaylord B, Reed DC, Raimondi PT, Washburn L, McLean SR. A physically based model of macroalgal spore dispersal in the wave and current-dominated nearshore. Ecology. 2002; 83(5): 1239-1251. https://doi.org/10.1890/0012-9658(2002)083[1239:APBMOM]2.0.CO;2

Guiry MD, Guiry GM. 2019. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 04 February 2019.

Mangialajo L, Chiantore M, Cattaneo-Vietti R. Loss of fucoid algae along a gradient of urbanisation, and structure of benthic assemblages. Mar Ecol Prog Ser. 2008; 358: 63-74. https://doi.org/10.3354/meps07400

Mangialajo L, Chiantore M, Susini ML, Meinesz A, Cattaneo-Vietti R, Thibaut T. Zonation patterns and interspecific relationships of fucoids in microtidal environments. J Exp Mar Bio Ecol. 2012; 412: 72-80. https://doi.org/10.1016/j.jembe.2011.10.031

Mancuso FP, Strain EMA, Piccioni E, De Clerck O, Sarà G, Airoldi L. Status of vulnerable Cystoseira populations along the Italian infralittoral fringe, and relationships with environmental and anthropogenic variables. Mar Poll Bull. 2018; 129(2): 762-771. https://doi.org/10.1016/j.marpolbul.2017.10.068

Mineur F, Arenas F, Assis J, Davies AJ, Engelen AH, Fernandes F et al. European seaweeds under pressure: Consequences for communities and ecosystem functioning. J Sea Res. 2015; 98: 91-108. https://doi.org/10.1016/j.seares.2014.11.004

Perkol-Finkel S, Airoldi L. Loss and recovery potential of marine habitats: an experimental study of factors maintaining resilience in subtidal algal forests at the Adriatic Sea. PLoS ONE. 2010; 5(5): e10791. https://doi.org/10.1371/journal.pone.0010791

Reed DC, Laur DR, Ebeling AW. Variation in algal dispersal and recruitment: the importance of episodic events. Ecol Monogr. 1988; 58(4): 321-335. https://doi.org/10.2307/1942543

Rodríguez-Prieto C, Ballesteros E, Boisset F, Afonso-Carrillo J. Guía de las macroalgas y fanerógamas marinas del Mediterráneo occidental. Omega. 2013.

Sales M, Cebrian E, Tomas F, Ballesteros E. Pollution impacts and recovery potential in three species of the genus Cystoseira (Fucales, Heterokontophyta). Estuar Coast Shelf Sci. 2011; 92(3): 347-357.

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA et al. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environ Conserv. 2002; 29(4): 436-459. https://doi.org/10.1017/S0376892902000322

Susini ML, Mangialajo L, Thibaut T, Meinesz A. Development of a transplantation technique of Cystoseira amentacea var. stricta and Cystoseira compressa. Hydrobiologia. 2007; 580(1): 241-244. https://doi.org/10.1007/s10750-006-0449-9

Thibaut T, Blanfuné A, Markovic L, Verlaque M, Boudouresque CF, Perret-Boudouresque M et al. Unexpected abundance and long-term relative stability of the brown alga Cystoseira amentacea, hitherto regarded as a threatened species, in the north-western Mediterranean Sea. Mar Poll Bull. 2014; 89(1-2): 305-323. https://doi.org/10.1016/j.marpolbul.2014.09.043

Thompson RC, Wilson BJ, Tobin ML, Hill AS, Hawkins SJ. Biologically generated habitat provision and diversity of rocky shore organisms at a hierarchy of spatial scales. J Exp Mar Biol Ecol. 1996; 202(1): 73-84. https://doi.org/10.1016/0022-0981(96)00032-9

Thrush SF, Chiantore M, Asnaghi V, Hewitt J, Fiorentino D, Cattaneo-Vietti R. Habitat–diversity relationships in rocky shore algal turf infaunal communities. Mar Ecol Prog Ser. 2011; 424: 119-132. https://doi.org/10.3354/meps08960

Vásquez JA, Tala F. Repopulation of intertidal areas with Lessonia nigrescens in northern Chile. J Appl Phycol. 1995; 7(4): 347-349. https://doi.org/10.1007/BF00003791

Verdura J, Sales M, Ballesteros E, Cefalì ME, Cebrian E. Restoration of a canopy-forming alga based on recruitment enhancement: methods and long-term success assessment. Front Plant Sci. 2018; 9: 1832. https://doi.org/10.3389/fpls.2018.01832

Vergés A, Steinberg PD, Hay ME, Poore AG, Campbell AH, Ballesteros E et al. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. Proc R Soc Lond B Biol Sci. 2014; 281(1789): 20140846. https://doi.org/10.1098/rspb.2014.0846

Whitaker SG, Smith JR, Murray SN. Reestablishment of the Southern California rocky intertidal brown alga, Silvetia compressa: an experimental investigation of techniques and abiotic and biotic factors that affect restoration success. Restor Ecol. 2010; 18: 18-26. https://doi.org/10.1111/j.1526-100X.2010.00717.x

Yoon JT, Sun SM, Chung G. Sargassum bed restoration by transplantation of germlings grown under protective mesh cage. J Appl Phycol. 2014; 26(1): 505-509. https://doi.org/10.1007/s10811-013-0058-8

Yu YQ, Zhang QS, Tang YZ, Zhang SB, Lu ZC, Chu SH et al. Establishment of intertidal seaweed beds of Sargassum thunbergii through habitat creation and germling seeding. Ecol Eng. 2012; 44: 10-17. https://doi.org/10.1016/j.ecoleng.2012.03.016

Zhao Z, Zhao F, Yao J, Lu J, Ang PO, Duan D. Early development of germlings of Sargassum thunbergii (Fucales, Phaeophyta) under laboratory conditions. J Appl Phycol. 2008; 20(5): 925. https://doi.org/10.1007/978-1-4020-9619-8_57

Figure 1(on next page)

Position of the culture laboratory facilities and the donor and receiving sites of *Cystoseira amentacea*.


N
University of Trieste facilities
DS-Portofino
RS- Cinque Terre
0
75
150 km
DS - Donor site
RS - Receiving site

Figure 2(on next page)

Percent cover of *C. amentacea* (average ± 1ES) on tiles at the time of leaving the controlled growth conditions and at the time of positioning in the receiving site (after 72 hours).

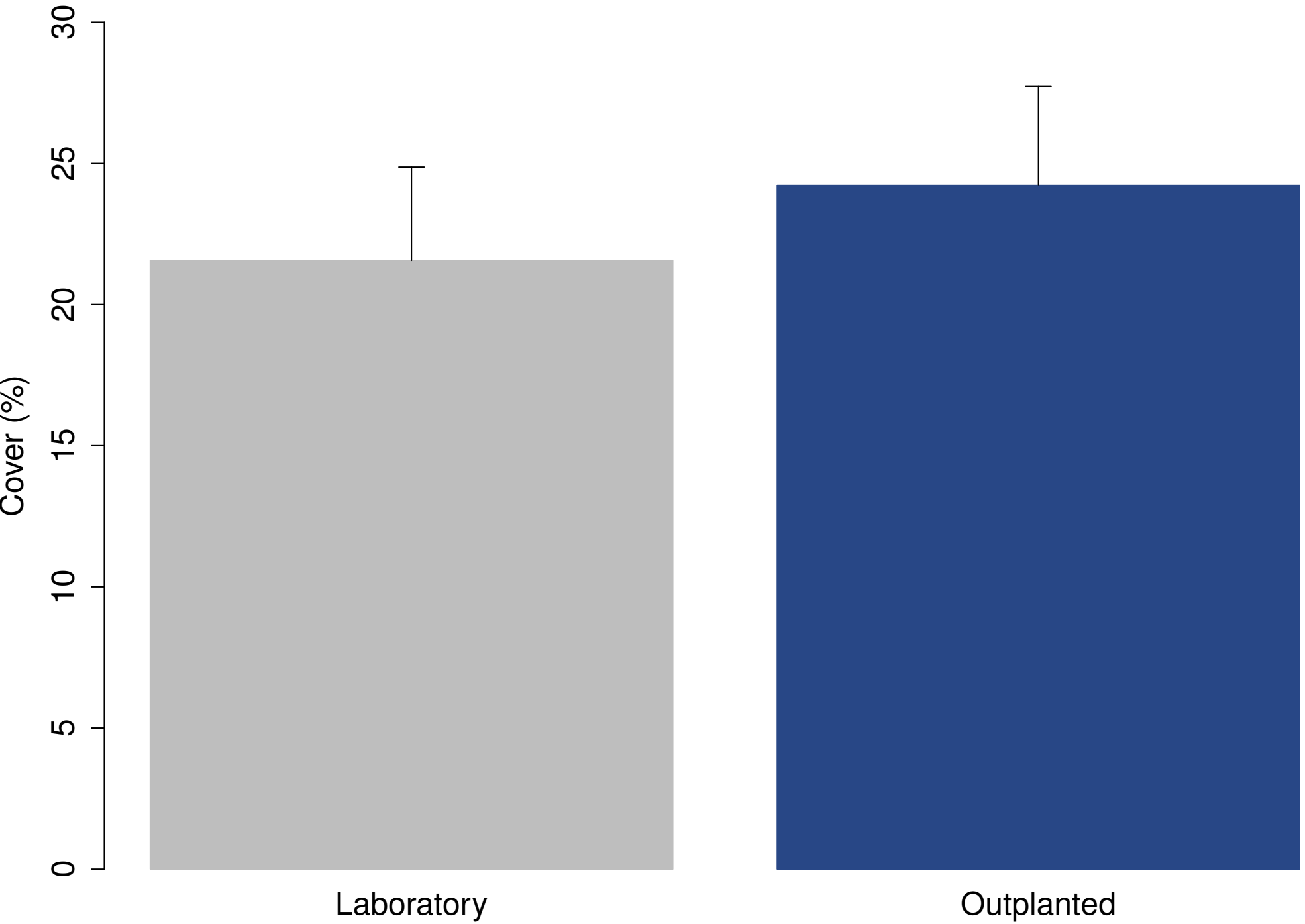

Cover (%)
0
5
10
15
20
25
30
Laboratory
Outplanted

Figure 3(on next page)

Percent cover of *C. amentacea* (average ± 1ES) on the clay tiles over time in the eight patches positioned in the receiving site.

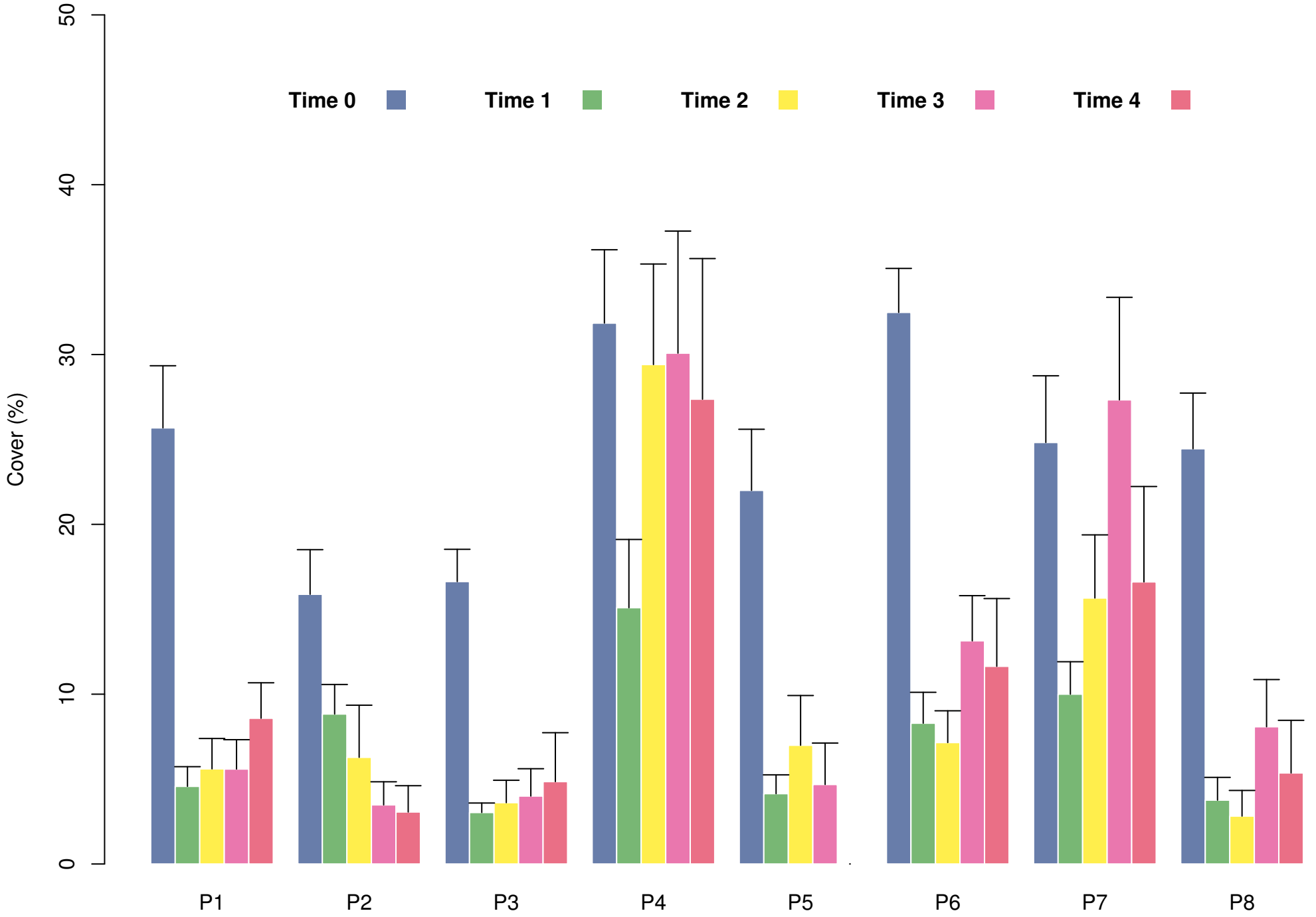

Time 0
Time 1
Time 2
Time 3
Time 4
Cover (%)
0
10
20
30
40
50
P1
P2
P3
P4
P5
P6
P7
P8

Figure 4(on next page)

Percent cover of *C. amentacea* juveniles (average ± 1ES) on the clay tiles over time per each cover class.

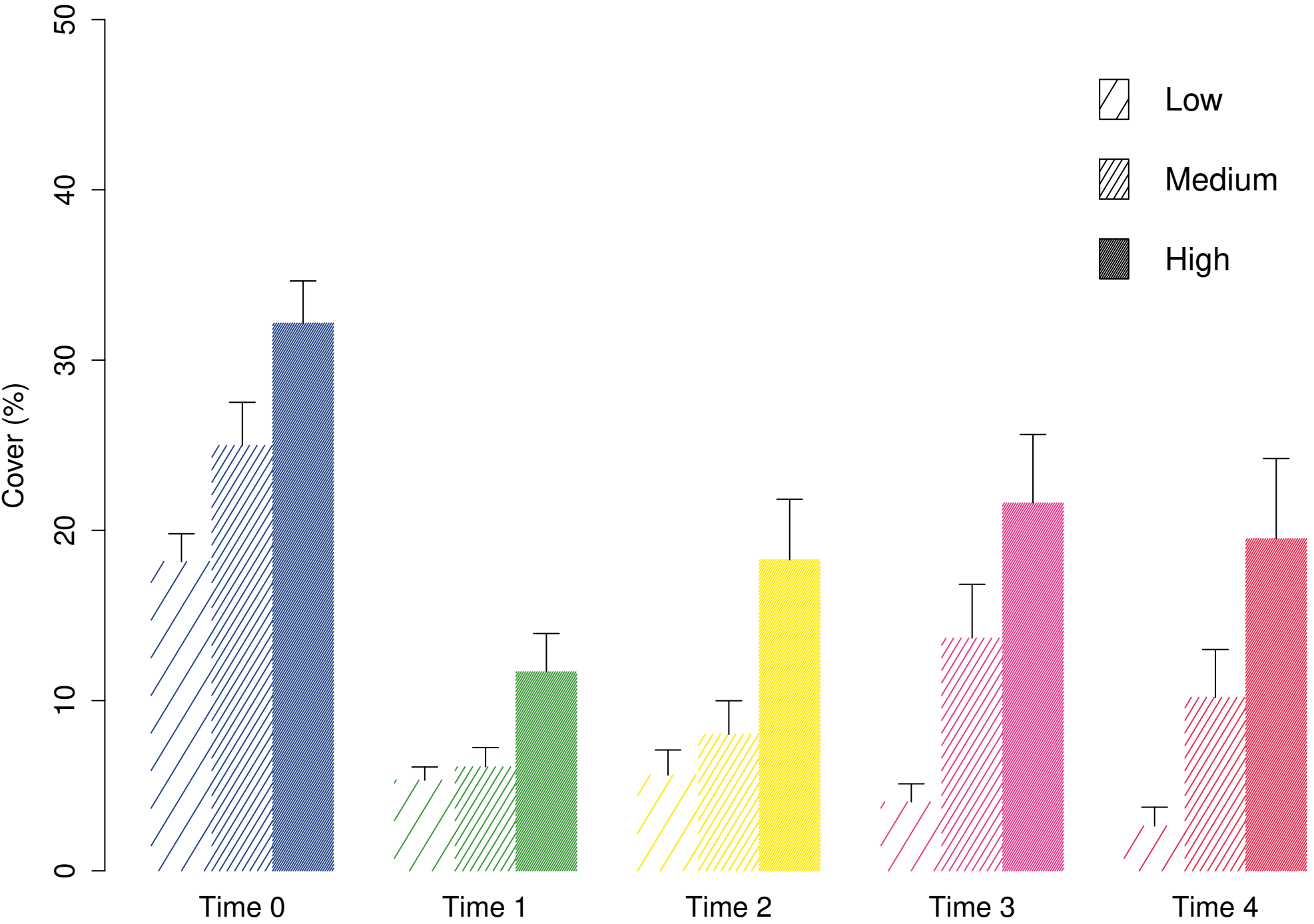

Cover (%)
0
10
20
30
40
50
Time 0
Time 1
Time 2
Time 3
Time 4
Low
Medium
High

Figure 5(on next page)

Percentage of tiles with juveniles over time from presence/absence data on the individual tiles in the eight patches positioned in the receiving site.

Red dots represent average data across the patches (average ± standard error).

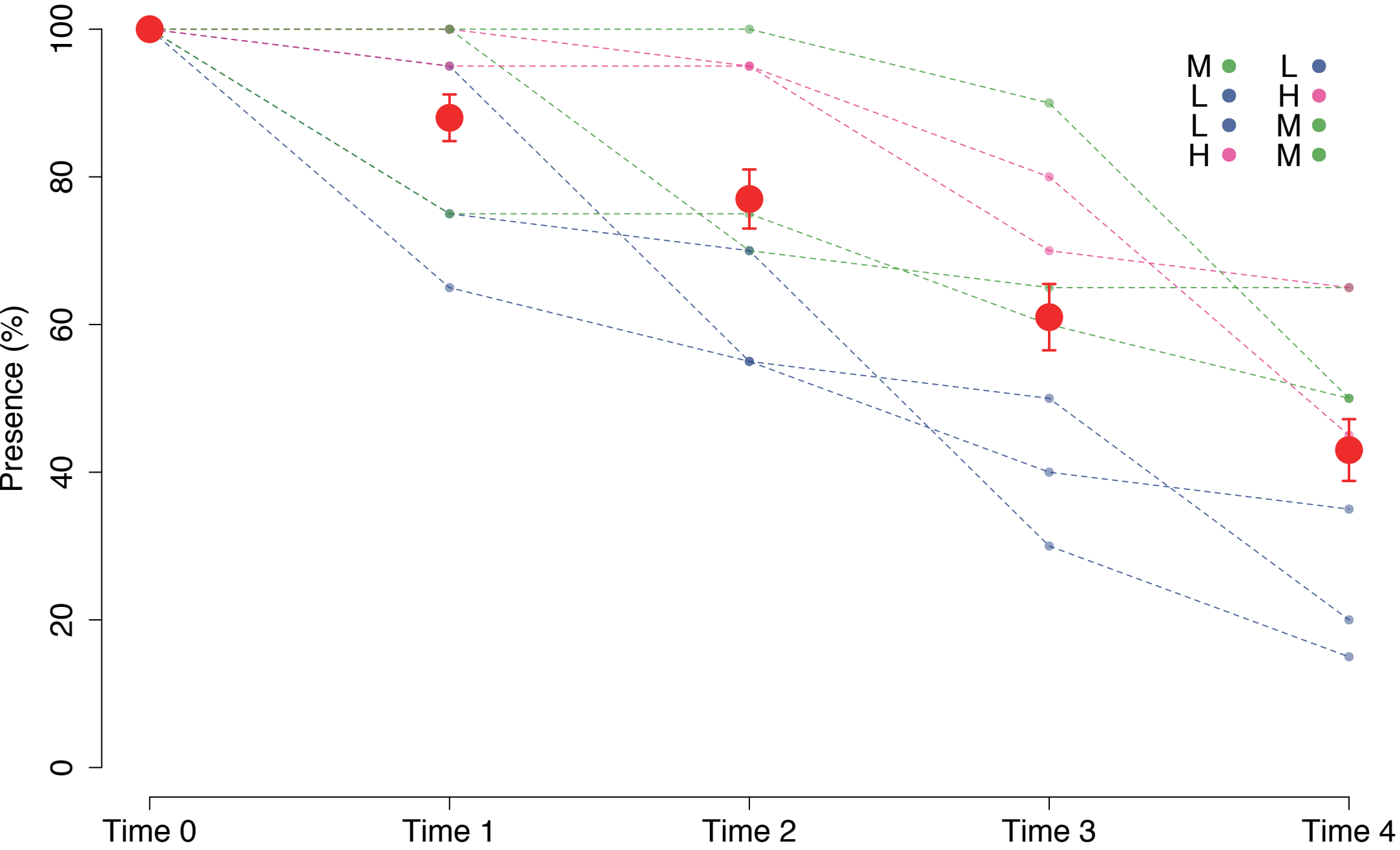
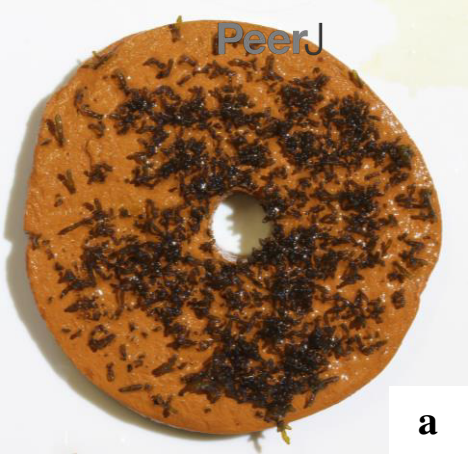

Presence (%)
0
20
40
60
80
100
Time 0
Time 1
Time 2
Time 3
Time 4
M L
L H
L M
H M

Figure 6(on next page)

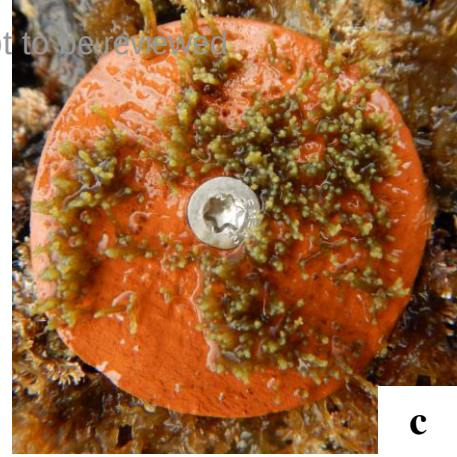
Development of the outplanted juveniles on tiles over time in the receiving site.

a) juveniles leaving the lab facilities (avg: 2.67 mm), b) Time 0 - outplanting day (avg: 3 mm), c) Time 1 – one week (avg: 3.39 mm), d) Time 2 – two weeks (avg: 5.08 mm), e) Time 3 – one month (avg: 5.81 mm), f) Time 4 – two months (avg: 8.32 mm), g-i) juveniles after six months of the outplanting (3 – 6 cm).

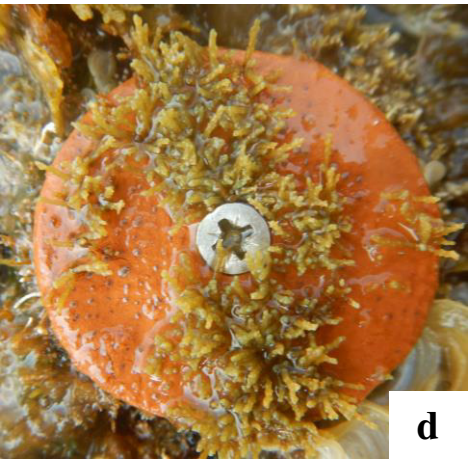


a

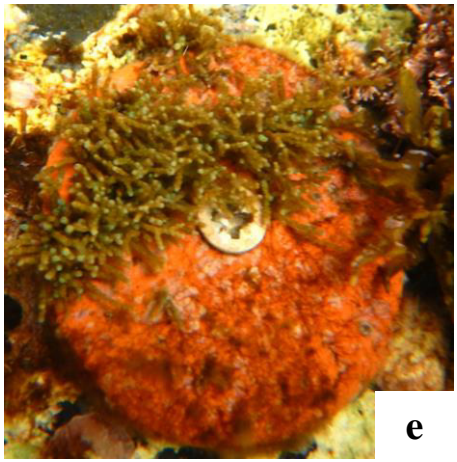
b



c



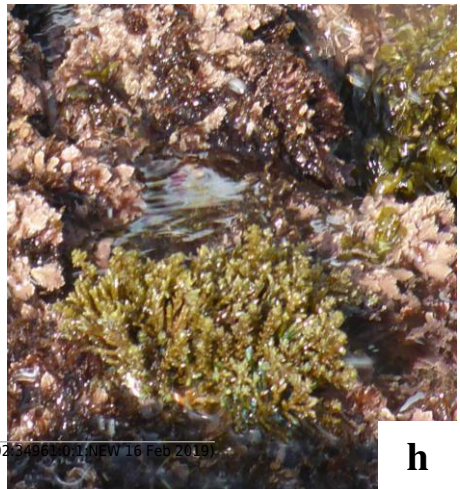
d



e

f

g



h

i

Table 1(on next page)

GLM results of the percent cover class (Low, Medium, High) at the time of the outplanting (Time 0) on the Presence/Absence of juveniles and their percent cover on the tiles at Time 4 (two months later) in the eight patches.

Presence/Absence		Estimate	Std. error	z-value	Pr(>|z|)
	High - Medium	0.05084	0.28864	0.176	0.98288
	High - Low	-0.99550	0.36378	-2.373	**0.01689***
	Medium - Low	1.04634	0.32695	3.200	**0.00387****
Percent cover					
	High - Medium	-0.8179	0.3683	-2.220	0.0652
	High - Low	-2.2942	0.5732	-4.003	**<0.001*****
	Medium - Low	1.4763	0.5642	2.617	**0.0231***

1

Table 2(on next page)

Abundance of the different macroinvertebrates trophic groups at the receiving site.

Data (average ± standard error) refer to the surface of the corer (5 cm internal diameter).

Feeding group	avg ± se (ind/19.6 cm^2)
Mollusca_suspension	23.73 ± 4.71
Mollusca_grazer	0.53 ± 0.27
Echinodermata_deposit	0.20 ± 0.11
Annelida_predator	8.73 ± 1.76
Annelida_deposit	3.80 ± 0.66
Annelida_suspension	0.47 ± 0.17
Crustacea_grazer	9.00 ± 2.28
Crustacea_predator	0.27 ± 0.21
Crustacea_deposit	5.93 ± 2.15
Crustacea_suspension	5.53 ± 1.42
Pycnogonida_predator	0.27 ± 0.21

1