# First ex situ outplanting of the habitat-forming seaweed Cystoseira amentacea var. stricta from a restoration perspective (#34961)

First revision

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# First ex situ outplanting of the habitat-forming seaweed Cystoseira amentacea var. stricta from a restoration perspective

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In the Mediterranean Sea, brown algae belonging to the *Cystoseira* genus play a valuable role as foundation species. Due to evidences of regression/loss of the habitats of these species caused by the interplay of human and climatic disturbances, active restoration measures have been encouraged by EU regulations. In particular, nondestructive restoration techniques, which avoid the depletion of threatened species in donor populations, are strongly recommended. In the framework of the EU project ROCPOP-Life, the first ex situ outplanting experience of Cystoseira amentacea var. stricta has been implemented in the Cinque Terre Marine Protected Area (northwestern Mediterranean). A total of 400 clay tiles, hosting approximately 3 mm-long germlings of C. amentacea, were fixed to the rocky shore with screws: the tiles were monitored for the next two months by photographic sampling, and survival (presence/absence of juveniles on the tiles), cover and growth were assessed. Additional sampling was performed 6 months after tile deployment, after which an unprecedented storm surge severely affected the restoration performance. After two months, over 40% of the tiles were covered with Cystoseira juveniles, which reached approximately 8 mm in total length. The tiles that survived the storm hosted 3-6 cm-long juveniles. The high cover (> 25%), assuring moisture and shading, and the appropriate size of the juveniles, to avert micro-grazing, at time of deployment were key to the survival and growth of the outplanted juveniles, increasing the potential for restoration success. Our findings show that outplanting of midlittoral canopyforming species is a feasible approach for restoration efforts, with particular attention given to the early phases: i) laboratory culture, ii) transport, and iii) juvenile densities. These results are strongly encouraging for the implementation of restoration actions for Cystoseira amentacea on a large scale, in light of EU guidelines.

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# 1 First ex situ outplanting of the habitat-forming

## seaweed Cystoseira amentacea var. stricta from a

## **3 restoration perspective**

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#### **Abstract**

- 17 In the Mediterranean Sea, brown algae belonging to the *Cystoseira* genus play a valuable role as
- 18 foundation species. Due to evidences of regression/loss of the habitats of these species caused by
- 19 the interplay of human and climatic disturbances, active restoration measures have been
- 20 encouraged by EU regulations. In particular, nondestructive restoration techniques, which avoid
- 21 the depletion of threatened species in donor populations, are strongly recommended. In the
- 22 framework of the EU project ROCPOP-Life, the first ex situ outplanting experience of
- 23 Cystoseira amentacea var. stricta has been implemented in the Cinque Terre Marine Protected
- 24 Area (northwestern Mediterranean). A total of 400 clay tiles, hosting approximately 3 mm-long
- 25 germlings of *C. amentacea*, were fixed to the rocky shore with screws: the tiles were monitored
- 26 for the next two months by photographic sampling, and survival (presence/absence of juveniles
- 27 on the tiles), cover and growth were assessed. Additional sampling was performed 6 months after
- 28 tile deployment, after which an unprecedented storm surge severely affected the restoration
- 29 performance. After two months, over 40% of the tiles were covered with *Cystoseira* juveniles,
- 30 which reached approximately 8 mm in total length. The tiles that survived the storm hosted 3-6
- 31 cm-long juveniles.
- 32 The high cover (> 25%), assuring moisture and shading, and the appropriate size of the juveniles,
- 33 to avert micro-grazing, at time of deployment were key to the survival and growth of the
- 34 outplanted juveniles, increasing the potential for restoration success.
- 35 Our findings show that outplanting of midlittoral canopy-forming species is a feasible approach
- 36 for restoration efforts, with particular attention given to the early phases: i) laboratory culture, ii)
- 37 transport, and iii) juvenile densities. These results are strongly encouraging for the
- 38 implementation of restoration actions for *Cystoseira amentacea* on a large scale, in light of EU
- 39 guidelines.



#### Introduction

- 41 Approximately thirty-five percent of brown algae species (Laminariales and Fucales; Guiry and
- 42 Guiry, 2019) play a key role as ecosystem engineers, building kelp forests and fucoid canopies,
- 43 which enhance habitat complexity, biodiversity, ecosystem functioning and the natural capital of
- 44 littoral ecosystems (Thompson et al., 1996; Christie et al., 2007; Airoldi et al., 2015). Kelp
- 45 forests (i.e., Macrocystis, Lessonia and Laminaria) constitute one of the most diverse and
- 46 productive ecosystems distributed worldwide (Steneck et al., 2002). In the Mediterranean Sea,
- 47 some Laminariales (e.g., Laminaria and Saccorhiza) and Fucales (e.g., Sargassum and
- 48 *Cystoseira*) also play a role as foundation species in some specific locations, but the canopy-
- 49 forming brown algae of the *Cystoseira* genus are the most important because they are widespread
- 50 in this biogeographic region (Rodriguez-Prieto et al., 2013). However, they are exposed to
- 51 multiple disturbances that cause a decline in their abundance in many coastal areas (Airoldi et
- 52 al., 2008; Mineur et al., 2015). The main pressures affecting the valuable ecosystems formed by
- 53 Cystoseira are sedimentation (Perkol-Finkel & Airoldi, 2010), low water quality (Arévalo et al.,
- 54 2007; Sales et al., 2011), anthropization (Mangialajo et al., 2008) and overgrazing (Sala et al.,
- 55 1998; Hereu, 2004; Vergés et al., 2014).
- 56 Several studies conducted in the Mediterranean have reported on the past and the present
- 57 distribution and abundance of *Cystoseria* canopies (Thibaut et al., 2014; Mancuso et al., 2018),
- 58 detecting regressions or losses caused by the above mentioned factors (Cormaci and Furnari,
- 59 1999; Thibaut et al., 2005; Falace et al., 2010; De La Fuente et al., 2018). Their natural recovery,
- 60 in the absence of adults, is hampered by the very limited dispersal of *Cystoseira* species due to
- 61 the rapid fertilization of their large eggs and zygote sinking (Falace et al., 2018).
- 62 In this framework, active marine restoration is strongly encouraged to prevent the loss of the
- 63 valuable habitats formed by *Cystoseira* species that enhance biodiversity and preserve ecosystem
- 64 functions and services (i.e., EU Biodiversity Strategy 2020).
- 65 Three different restoration techniques have been implemented in the Mediterranean Sea for
- 66 Cystoseira species: i) transplanting juveniles or adults (Falace et al., 2006; Susini et al., 2007), ii)
- 67 positioning fertile receptacles in the target area (*in situ*; Verdura et al., 2018) or iii) outplanting
- 68 juveniles cultured in the laboratory along the shore (ex situ; Sales et al., 2011; Verdura et al.
- 69 2018). The latter two techniques are strongly recommended for the restoration of threatened
- 70 species to avoid the depletion of natural donor populations (Falace et al., 2018). Active
- 71 restoration actions must be implemented depending on the biological traits of the target species
- 72 (i.e., reproductive strategy) and the environmental characteristics (i.e., depth and
- 73 hydrodynamics). The *in situ* technique seems to be especially suitable for species with high
- 74 dispersal capacity (e.g.,: kelps; Reed et al., 1988; Gaylord et al., 2002), while the ex situ
- 75 technique is more appropriate for species with a low dispersal capacity (e.g., C. amentacea;
- 76 Mangialajo et al., 2012). Both techniques have been recently validated for shallow species (C.
- 77 barbata; Verdura et al., 2018) living in low hydrodynamic conditions, but at present, such
- 78 approaches have never been tested for the midlittoral habitat.



- 79 The aim of this study was to apply the *ex situ* technique (outplanting) to midlittoral *Cystoseira*
- 80 amentacea var. stricta Montagne (hereafter C. amentacea), in the Ligurian Sea (northwestern
- 81 Mediterranean), assessing its survival, cover and growth in the first, most critical, months
- 82 following implementation. The outplanting was performed in the Cinque Terre Marine Protected
- 83 Area (Cinque Terre MPA), where, at present, only the more tolerant congener C. compressa is
- found in continuous and discontinuous belts (Asnaghi et al., 2009; De La Fuente et al., 2018).
- 85 Here, C. amentacea is presently missing as well as across the whole easternmost side of the
- 86 Ligurian coastline starting from Punta Manara, which is approximately 20 km northwest of the
- 87 Cinque Terre MPA (authors' personal observation). This species was recorded in the area until
- 88 the end of the 19th century (authors' personal observation through herbarium records, De La
- 89 Fuente et al., 2018) and its disappearance can be explained by habitat fragmentation due to
- 90 coastal developments, water pollution and high sediment loads. Such stressors were related to the
- 91 intense excavating activities that were carried out to extract construction material (mostly for
- 92 building railways and highways in the area), which occurred in the first half of the 20th century.
- 93 Even though such disruptive activities have largely been reduced in recent decades, with
- 94 significant changes in the riverine basin and sediment load to the sea, and a MPA was
- 95 established (1997), this sensitive species is still absent along these stretches of coastline. This is
- 96 likely due to its above mentioned low dispersal capacity (< 1 m, Johnson & Brawley, 1998;
- 97 Mangialajo et al., 2012) which hampers the natural recovery of this species, in addition to other
- 98 local factors, such as mussel farming (as a result of competition for space with the settled mussel
- 99 juveniles).
- The effectiveness of the *ex situ* approach over the first six months following its implementation,
- was assessed in terms of the presence, cover and growth of the outplanted C. amentacea
- juveniles in the framework of the EU project ROCPOP-Life.

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#### Materials & Methods

#### Study sites

- 106 The ex situ outplanting of the midlittoral species C. amentacea was performed in summer 2018
- 107 following a nondestructive strategy, with apical fronds (ca. 3 cm in length) holding mature
- receptacles collected in June from a healthy population located in the Portofino MPA (donor site)
- along a 200-meters stretch of coastline. After a laboratory culturing period, the juveniles were
- transplanted into the Cinque Terre MPA (receiving site; approximately 80 km from the donor
- site). Both MPAs are located in the Ligurian Sea, northwestern Mediterranean (Fig. 1). The sites
- are characterized by a tide in the range of 30 cm (under these conditions, barometric pressure
- effects may have a dominant effect on the water level) and an average spring temperature of
- 114 20°C. After sampling, the apices were gently cleaned with tweezers and rinsed with filtered
- seawater to remove adherent biofouling and detritus. Then, the apices, which were wrapped in
- seawater-wetted towels, were delivered within 24 hours to the laboratory in Trieste (northeastern
- 117 Italy; Fig. 1) under dark, cold and humid conditions for culturing in environmentally controlled
- 118 rooms.



#### 119 Laboratory ex situ cultivation

- 120 Three apices with mature receptacles (additionally cleaned with a brush and rinsed with
- autoclaved seawater) were placed on each substrate constituted of a rough round tiles composed
- of clay (4.5 cm in diameter) with a 0.6 cm hole at the center to fix the tile onto the rocks with a
- screw. On the next day, the fertile apices were removed, and the zygotes attached to the tiles
- were cultured over a three-week period ( $\approx 450$  tiles).
- 125 Temperature (+20°C) and photoperiod (125 μmoL photons m<sup>-2</sup>s<sup>-1</sup>) were selected according to the
- protocol provided in Falace et al. (2018). Von Stosch's enriched filtered seawater (VSE) was
- used as the culture medium to accelerate the culturing time for the *C. amentacea* individuals to
- reach a greater size at the time of outplanting (Falace et al., 2018). The medium was enriched
- 129 with antibiotics (2 μL of amikacin sulphate and 500 μg of ampicillin sodium L-1 of culture
- medium) and GeO<sub>2</sub> (Falace et al., 2006) to prevent bacterial and diatom growth. The culture
- medium in the aquaria was renewed every 3 days to minimize any possible effect of nutrient
- limitation and was continuously aerated by bubbling and water multifunction pumps ( $\approx 300 \text{ L}$
- hour<sup>-1</sup> flow) to increase oxygenation and hydrodynamics.
- 134 After 24 days of controlled growth in the Trieste laboratory, photographs were taken (17th July;
- 135 160 random tiles) in order to assess the percent cover and juvenile length.

#### Outplanting and monitoring in the field

- On 19th July 2018 the tiles were transported to Cinque Terre MPA. All the tiles were carefully
- placed in small boxes filled with filtered seawater, which were placed in a large insulated
- 140 container that was maintained at a cool temperature with icepacks. The container was transported
- by car with an air conditioner ( $\approx$  7-hours trip, temperature in the range of 20-22°C) to the
- receiving site, where the boxes were stored in an air-conditioned room overnight (at 22°C).
- 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: 50 holes were
- drilled in each patch and screws placed within them in advance. On the day of implementation,
- the tiles were quickly screwed to the rocks. Overall, the deployment of 400 tiles was performed
- in approximately 5 hours. On the days of transport and deployment, the air temperature was in
- the range of 23-27°C and the sea temperature was in the range of 25-26°C.
- Monitoring of the clay tiles started on the same day (Time 0) and continued over the following 2
- months: July 27th (Time 1), August 6th (Time 2), August 29th (Time 3) and September 27th
- 151 (Time 4). During each sampling time, photos were taken of a randomly selected 20 of the 50 clay
- tiles in each patch. The photos were analyzed in the lab using ImageJ to assess percent cover and
- survival (i.e., overall presence/absence of juveniles). Thallus length was measured for 40
- randomly selected individual specimens with ImageJ at each sampling time except Time 5
- 155 (measured in the field).
- 156 Additional sampling was performed 4 months later (Time 5). This sampling occurred after an
- unprecedented storm surge that affected the Ligurian Sea at the end of October 2018 (ANSA).
- 158 2018; The MediTelegraph, 2018), which caused great destruction along the entire Ligurian



coastline, with estimated damages in the range of 2 bur uros. This storm caused the destruction 159 of entire harbors and ships in marinas, particularly along the eastern side of the Ligurian coast. 160 Huge damage was reported among the shallow benthic communities, particularly the *Posidonia* 161 meadows (Il Secolo XIX, 2018). Because most of the tiles were detached by this unprecedented 162 163 event (180 km hour<sup>-1</sup> winds and waves greater than 10 meters high), it was not possible to quantitatively assess restoration performance in terms of percent cover but only in terms of 164 165 juvenile growth (length) on the 16 tiles with C. amentacea juveniles of the approximatively 69 remaining tiles. 166

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#### Data analysis

- One-way ANOVA was applied to assess possible differences in the percent cover of the
- juveniles on the tiles when leaving the laboratory versus at the time of the deployment (Time 0)
- 171 after arcsin transformation of the data and verification of assumptions (normality using the
- 172 Shapiro-Wilk test and homoscedasticity using Bartlett's test).
- 173 The effect of percent cover at the time of deployment was assessed using a generalized linear
- model (GLM) for both the percent cover (family=quasibinomial) and the presence/absence
- 175 (family=binomial) of juveniles on the tiles at Time 4, using the "cover class" of each patch at the
- start of the experiment as the predictor variable. Patches were classified according to 3 "cover
- 177 classes" (based on the percent cover at the start of the experiment): Low (18.2  $\pm$  1.6, avg  $\pm$  SE;
- patches 2, 3 and 5), Medium (25.0  $\pm$  2.1; patches 1, 7 and 8) and High (32.2  $\pm$  2.5; patches 4 and
- 179 6). Statistical analyses were performed with the free software R (R Development Core Team
- 180 2015, version 3.2.3) using the stats package.

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#### Results

- 183 The juveniles were  $2.65 \pm 0.05$  mm (avg  $\pm$  SE) in length when they were transported to the
- receiving site. The percent cover on the tiles measured at Time 0 was compared with that at the
- time they left the laboratory (3 days before) to assess the effect of transport (Fig. 2). The percent
- 186 cover in the laboratory and in outplants at Time 0 did not significantly differ (one-way ANOVA;
- 187 p = 0.327).
- At the start of tile deployment, the average percent cover of the juveniles was  $24.22 (\pm 1.24\%)$ .
- The percent cover data of the juveniles on the tiles in the eight patches over time (Time 0 to
- 190 Time 4) are reported in Fig. 3. All patches showed a sharp decline in percent cover from Time 0
- 191 to Time 1 due to the predictable loss of some juveniles in the field. Some patches clearly showed
- lower average values of cover at Time 0 (ranging from  $15.88 \pm 2.63$  in patch 2 to  $32.48 \pm 2.59$  in
- patch 6; avg  $\pm$  SE), and this difference in cover at the start of the experiment affected the
- survival and growth of the juveniles over time (Fig. 3). In the patches characterized by higher
- 195 cover of juveniles at the beginning of the experiment (patches 4 and 6), after the first decline,
- 196 cover increased on the following sampling dates (reaching  $27.37 \pm 2.63$  and  $11.65 \pm 3.99$  at Time
- 4 in patches 4 and 6, respectively) because of the growth of the juveniles (Fig. 3 and 4), while the
- patches characterized by lower cover showed a general decrease in percent cover (reaching 3.06



 $\pm$  1.55 and 4.85  $\pm$  2.88 at Time 4 in patches 2 and 3 respectively; juveniles lost in patch 5). The 199 GLM for the percent cover on the tiles at Time 4 showed significant differences among the 200 classes (p = 0.0143). The Low class was significantly differed from the others, while the Medium 201 class did not differ from the High class (Table 1). 202 203 The good performance of the outplanting, at least in the early phases, is confirmed by the assessment of the percentage of tiles with the presence of juveniles (Fig. 5). In fact, from Time 0 204 to Time 1, this percentage changed from 100% to 88. 2 and was still over 40% after two 205 months (Time 4). Similar to cover, survival also differed in the patches characterized by higher 206 cover of juveniles at the start of the experiment. In fact, the GLM for the presence/absence of 207 juveniles on the tiles at Time 4 using the cover class of each patch at the start of the experiment 208 as a predictor variable showed significant differences among the classes (p = 0.00161). The Low 209 class significantly differed from the others, while the Medium class did not differ from the High 210 211 class (Table 1): this implies that cover at the start of the experiment greater than 25% is required 212 for a good outplanting performance. The growth of the outplanted juveniles over time is shown in Fig. 6 and 7. The thallus length of 213 the juveniles of C. amentacea was mostly under 6 mm at Time 0 (3.22  $\pm$  0.08, avg  $\pm$  SE), Time 1 214  $(3.73 \pm 0.10)$  and Time 2  $(4.67 \pm 0.13)$ . At Time 3  $(6.02 \pm 0.18)$  and Time 4, juveniles grew to 215 216 up to 11 mm, with a higher number of individuals 8-10 mm in size at Time 4 (8.03  $\pm$  0.22). After six months (Time 5), the outplanted juveniles reached 3-6 cm in length (last record, in February 217 2019). 218

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#### **Discussion**

Outplanting represents an innovative technique for restocking brown canopy-forming macroalgae (Falace et al., 2018), although its implementation consists of a set of delicate steps: i) fertile material collection, ii) culturing of juveniles in the lab, iii) transport of juveniles to the field and iv) attachment of the juveniles to the rocky shore. In addition to the multiple set of phases, this technique needs to be adapted according to target species-specific requirements in the different phases of implementation.

The lab culture process involves several issues related to the wellness of the sensitive embryo

The lab culture process involves several issues related to the wellness of the sensitive embryo stage. The thorough cleaning of the fertile apices before and after transport to the lab facilities to reduce epiphyte outbreaks and the presence of grazers helps to increase the chance of good performance. The *C. amentacea* embryos were cultured under their optimal temperature and light intensity conditions to maximize the yield (Falace et al., 2018), which can also unfortunately enhance spores, propagules and bacteria proliferation. The implemented culture setting (e.g., the addition of antibacterial solutions and enhanced hydrodynamics) preserved the culture in good conditions, with healthy juveniles larger (2.65 mm) than those in previous *Cystoseira* restoration studies being obtained in only three weeks: *C. amentacea* (three weeks - 1.38 mm; Falace et al., 2018) and *C. barbata* (one month 200- 400 µm; Verdura et al., 2018). Furthermore, the length recorded one month after outplanting (6.02 mm; Fig. 6) is similar or even slightly larger than that



238 recorded in a previous study on C. amentacea (4.73 mm; Falace et al., 2018) because the outplanting size was also larger in the present study. 239 The transport of early life stages from the nursery facility to the receiving site, which may be 240 located a large distance away, as in this case ( $\approx 600$  km), was found to not affect their fitness. 241 242 The results show no significant differences in the percent cover of juveniles before and after transport. The cover of the juveniles on tiles at the time of arrival at the receiving site was even 243 slightly greater than that when leaving the nursery facility (before transport -21.6%, after 244 transport – 24.2%; Fig. 2). This result was obtained by maintaining a good temperature range 245 (20-22°C) during transport and during the attachment of the clay tiles in the field (outplanting 246 action). Due to the high numbers, the clay tiles were transported, by rubber boat in cooled boxes 247 to the rocky shore in several times over the course of the same day to prevent solar heat stress 248 during attachment. 249 250 The attachment technique applied in this study, using pre-established screws instead of epoxy 251 putty, which is generally used for both adults and juveniles (Susini et al., 2007; Whitaker et al., 2010; Perkol-Finkel et al., 2012; Verdura et al., 2018), increases the effectiveness of outplanting 252 by reducing the time spent for attachment (the deployment of 400 tiles was performed in 5 hours 253 by 6 people) and the possible dislodgement by wave action on the day of deployment or the 254 255 following days (Susini et al., 2007). This method additionally minimizes the aesthetic and environmental impacts on the rocky shores and is reported to reinforce attachment, preventing 256 the possible dislodgement of the clay tiles by wave action, as in the case of Lessonia nigrescens 257 restoration (Vázquez and Tala, 1995). This is particularly important for midlittoral species living 258 under high hydrodynamic conditions, as in the case of C. amentacea. In our study, we observed 259 260 very positive results over the first 2 months (with the overall dislodgement of approximately 39 tiles). Unfortunately, the experiment was strongly affected by an unprecedented storm surge 261 (winds over 170 km hour<sup>-1</sup> and significant wave of m and maximum waves of 10 m height, over approximately 12 hours). This storm surge caused the destruction of entire harbors and 262 263 264 ships in marinas and caused great damage to the shallow benthic communities. This event caused a large loss of tiles: 69 tiles survived the storm out of the 361 reported at Time 4. This means 265 that, notwithstanding the exceptional strength of the storm, 20% survived the event, although 266 only 16 of them hosted juveniles. Overall, the attachment method used in this study seems to be 267 268 not only more environmentally friendly than the epoxy putty attachment method but also quite resistant to wave action. The screws themselves were not at all removed from the shore. 269 In addition to providing information for the optimization of culture and transport/outplanting 270 techniques, our results stress the relevance of juvenile cover on the tiles. Relatively high cover 271 (greater than 25% of the tile covered at the time of deployment) ensures the survival of the 272 outplanted juveniles (Table 1; Fig. 4). Midlittoral species are exposed to high solar heat and 273 274 desiccation stress; therefore, a higher percentage of cover allows moisture and shading to be retained, enhancing the development of early juveniles (Brawley and Johnson, 1991; Dudgeon 275 and Petraitis, 2005). 276



277 Another key issue regulating outplanting success is grazing, which was reported in previous studies, e.g., on Sargassum species, where did not obviously affect the development of the 278 outplanted juveniles in order to establish population in the restoration site, because of the low 279 density of major grazers (7 individuals/0.25 m<sup>2</sup>, Yu et al., 2012) and the use of cages to protect 280 281 juveniles from grazing, until reaching a suitable grazing free size (Yoon et al., 2014). In this study, we did not formally test the effect of grazing, but we can exclude it having a relevant role. 282 No grazers were observed crawling on the tiles at the time of sampling or in the photographs. In 283 terms of small grazers (mostly small amphipods and isopods), their potential abundance may be 284 estimated from the paper by Thrush et al. (2011), which reports densities of grazing crustaceans 285 in the range of 9 individuals/20 cm<sup>2</sup> in the area, which is likely not high enough to exert a strong 286 grazing effect. Furthermore, the tiles were located relatively high on the shore compared to the 287 mean sea water level, and this could actually prevent grazing by fish and sea urchins, which are 288 generally considered the most relevant herbivores (Ling et al., 2015; Gianni et al., 2017). 289

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#### Conclusions

- Our findings show that outplanting midlittoral canopy-forming species is a feasible approach for 292 restoration efforts. In particular, our study addressed the early steps of restoration and provides information on best practices for the outplanting phases of laboratory culture, transport and reintroduction in the natural environment.
- The laboratory culture phase requires appropriate species-specific protocols to reduce outbreaks 296 of epiphytes, obtain high cover and a large size of the juveniles for field deployment, and 297 increase the potential for restoration success. The use of an appropriate culture medium, the 298 addition of an antibacterial solution and thorough fertile material cleaning are relevant elements 299 300 likely to guarantee good culture performance and obtain high densities of healthy embryos.
- The feasibility of large distance transport from the laboratory to the field has been remarkably 301 shown, providing a potential option for replication also on a large scale. 302
- 303 The applied screw attachment technique was found to be effective in terms of increasing the 304 efficiency of tile deployment and resistance to dislodgement, particularly in the early stages of restoration, when the traditional epoxy putty technique may be strongly affected by wave action, 305 which is particularly relevant for midlittoral species. 306

These results are strongly encouraging for the implementation of restoration actions for canopy-307 forming species on a large scale, in light of EU guidelines. 308

309 310

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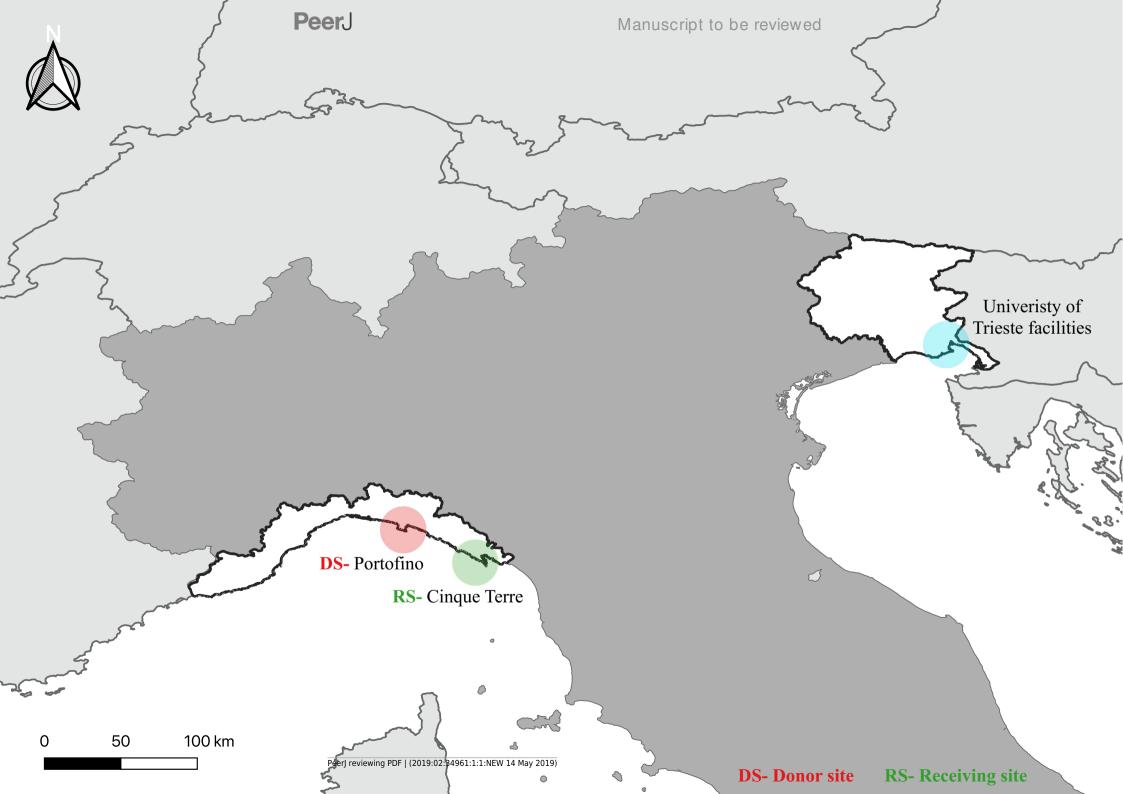
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# Figure 1(on next page)

Map showing the location of the culture laboratory facilities and the donor (DS) and receiving (RS) sites.



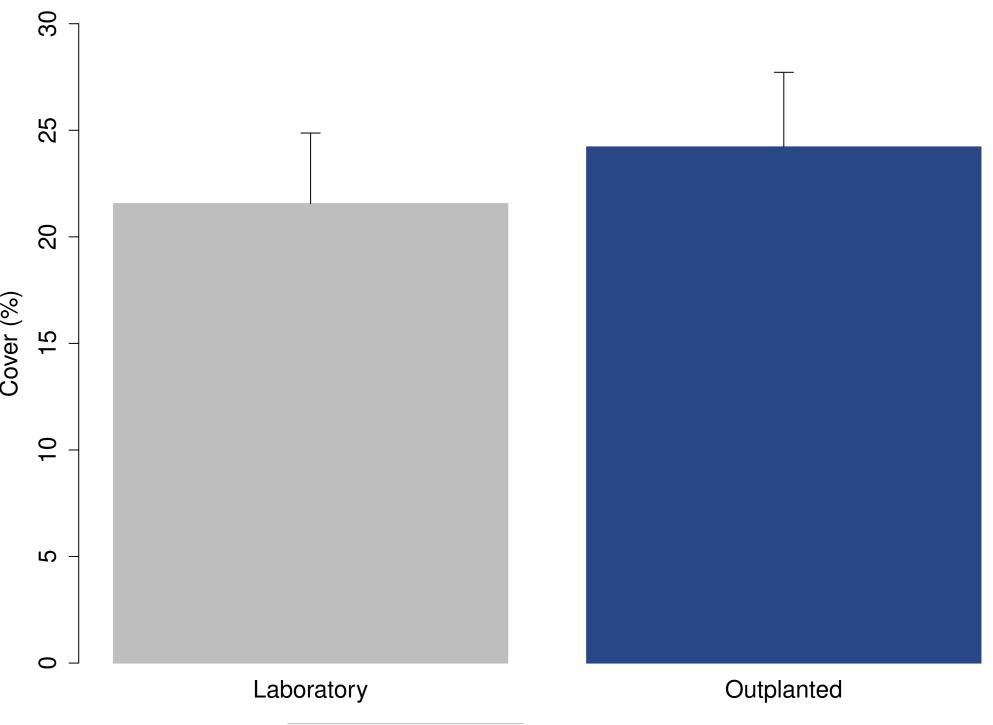


# Figure 2(on next page)

Percent cover of C. amentacea (average + SE) on tiles when leaving the controlled growth conditions (laboratory) and at the time of positioning (outplanting) at the receiving site (72 hours later).



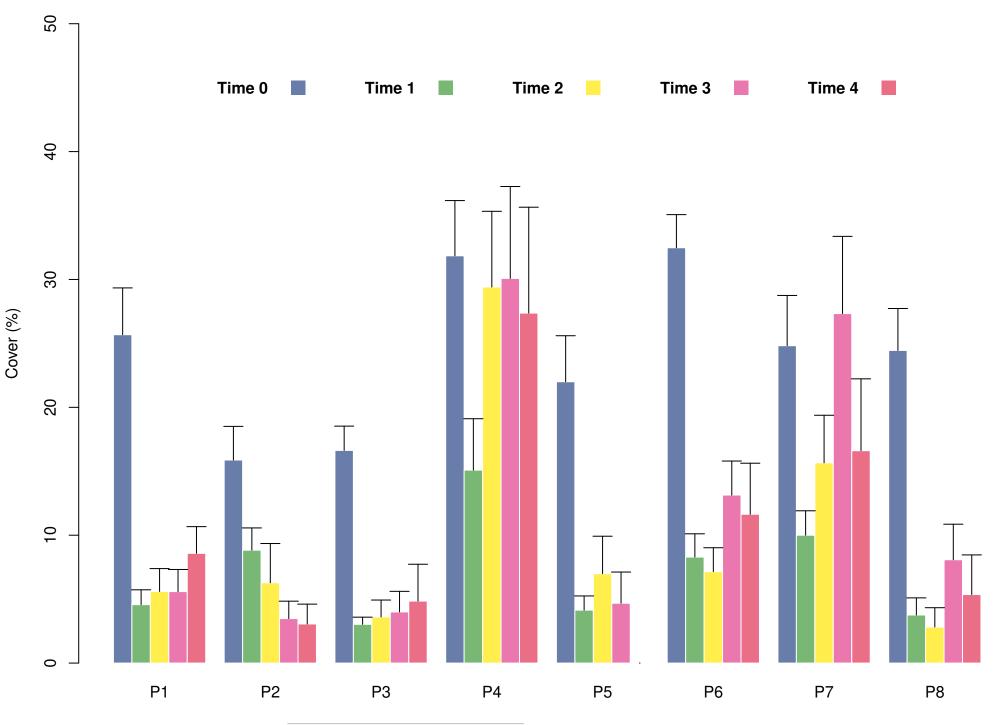
## Manuscript to be reviewed





# Figure 3(on next page)

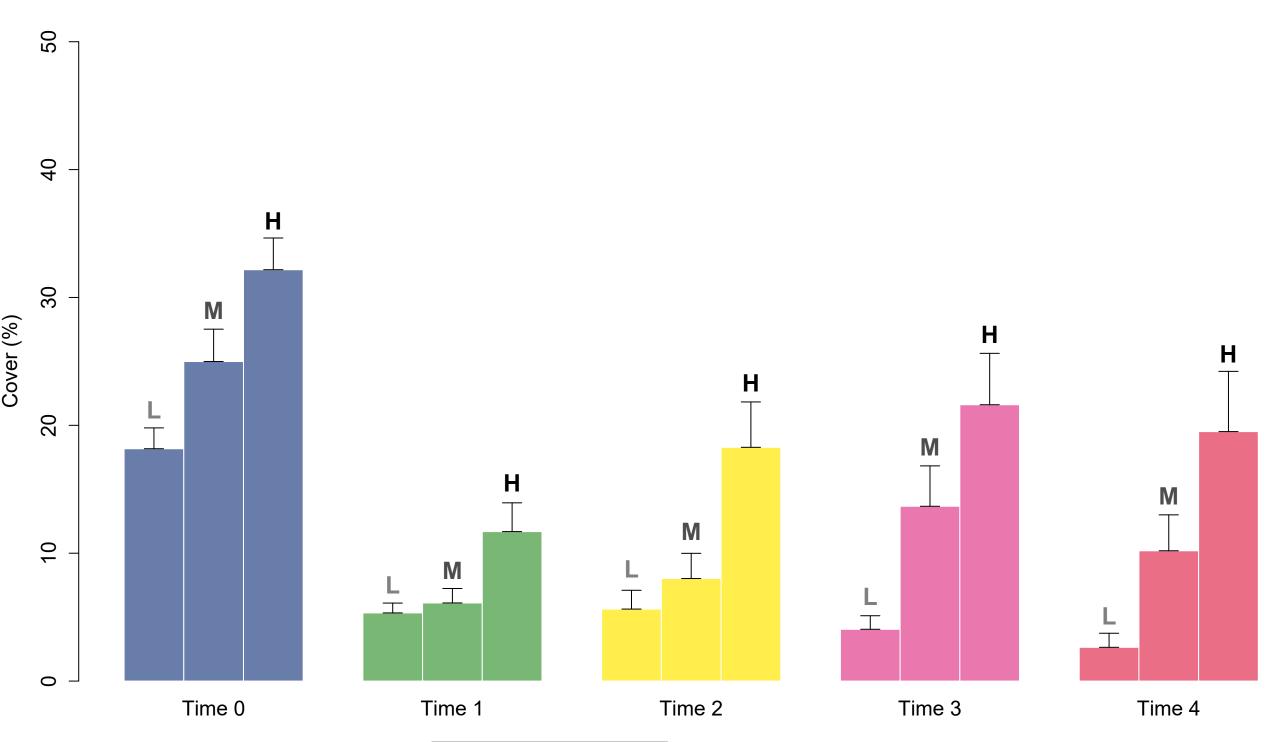
Percent cover of *C. amentacea* (average + SE) on the clay tiles over time (Time 0 to Time 4) in the eight patches at the receiving site.





# Figure 4(on next page)

Percent cover of *C. amentacea* juveniles (average + SE) in each cover class (Low, Medium, High) on the clay tiles over time (Time 0to Time 4) at the start of the experiment.

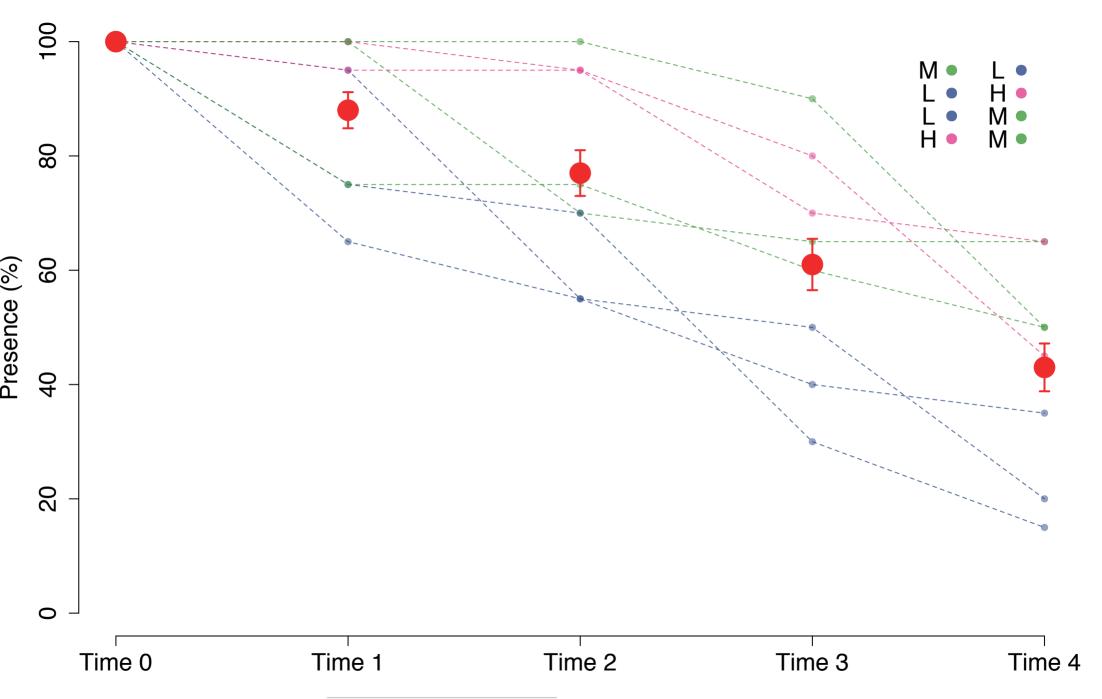




## Figure 5(on next page)

Percentage of tiles with *C. amentacea* juveniles over time (Time 0 to Time 4) according to presence/absence data for the individual tiles in the eight patches at the receiving site.

Eight patches were allocated to the different cover classes. Red dots represent average values across the patches (average  $\pm$  SE).

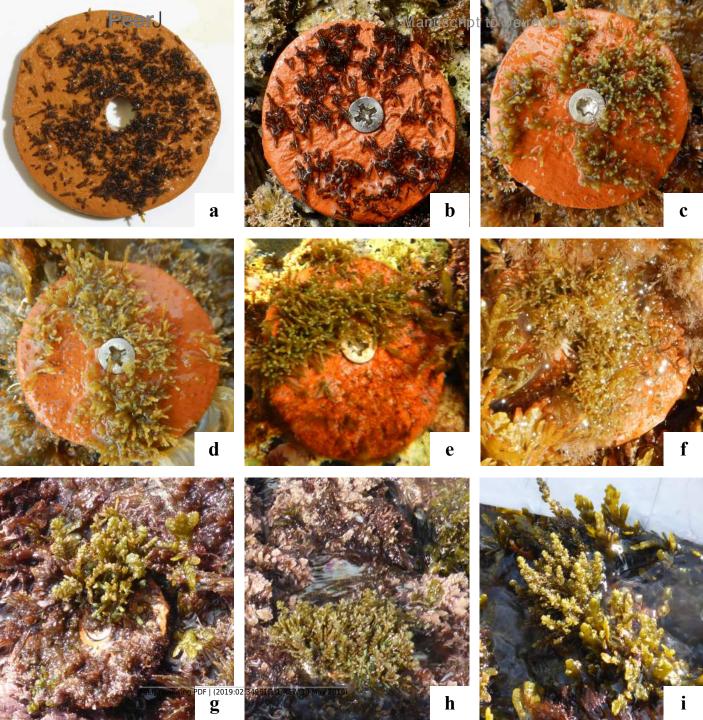




### Figure 6(on next page)

Development of the outplanted juveniles on tiles over time (Time 0 to Time 5) at the receiving site.

a) Juveniles leaving the lab facilities (avg: 2.67 mm), b) Time 0 – outplanting day (avg: 3.22 mm), c) Time 1 – one week (avg: 3.73 mm), d) Time 2 – two weeks (avg: 4.67 mm), e) Time 3 – one month (avg: 6.02 mm), f) Time 4 – two months (avg: 8.03 mm), g-i) juveniles six months after outplanting (3-6 cm).





# Figure 7(on next page)

Size frequency distribution of the outplanted juveniles of *Cystoseira amentacea* over time.



## Table 1(on next page)

Results of the GLMs of the differences in percent cover and presence/absence of juveniles of the cover classes (Low, Medium, High) at Time 4 .



presence/absence		Estimate	Std. error	z-value	<b>Pr(&gt; z )</b>
	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*