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Towards streamlined restoration of macroalgal forests: integrating suspended algaculture and *ex situ* outplanting

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ABSTRACT

Ericaria amentacea, a key habitat-forming brown macroalga on Mediterranean rocky coasts, faces severe decline due to anthropogenic stressors and climate change. Natural recovery is hindered by its limited dispersal and connectivity potential, and active restoration is therefore critical to restocking lost populations. This study evaluates a two-phase restoration approach combining laboratory culture and suspended aquaculture to optimize *E. amentacea* restoration, enabling multiple culture cycles within a single fertility window. Three experimental conditions were tested: (A) 5 days in the laboratory followed by 12 days in suspended aquaculture, (B) 12 days in the laboratory followed by 5 days in suspended aquaculture, and (C) 17 days in the laboratory without suspended aquaculture. Culture performance was assessed in terms of coverage, length of individuals, and photosynthetic efficiency. Results outlined that condition C achieved the best performance at the final time point. Condition B showed intermediate outcomes, with length and photosynthetic efficiency comparable to condition C, but lower coverage, with significant recovery post-outplanting. Condition A exhibited poor resilience, with declining coverage and reduced photosynthetic efficiency. These findings suggest that a moderate reduction in laboratory culture (12 days) may represent an effective trade-off between resource efficiency and germling development. This approach is particularly relevant in the context of climate change, where shorter fertility periods and extreme events challenge restoration efforts. This study highlights the potential of integrated laboratory-aquaculture protocols to enhance the scalability and success of *E. amentacea* restoration, offering a promising strategy for marine habitat recovery.

1. Introduction

Brown macroalgae of the complex *Cystoseira sensu lato* (i.e. *Cystoseira sensu stricto*, *Ericaria*, and *Gongolaria*) are key ecosystem engineers along Mediterranean rocky coasts (Jones et al., 1994), providing habitat, shelter, and food for numerous invertebrate and fish species, and supporting highly biodiverse and structurally complex communities (Ballesteros et al., 1998, 2009; Mineur et al., 2015). Despite their ecological importance, these foundation species are increasingly threatened, experiencing significant range contractions and local extinctions driven by escalating human-induced pressures (Munda, 1974; Verlaque, 1987; Hoffman et al., 1988; Benedetti-Cecchi et al., 2001; Soltan et al., 2001; Strain et al., 2014; Smale, 2020). These declines are mainly driven by eutrophication, habitat destruction, and climate-related stressors such as warming, extreme events, and altered hydrodynamics (Thibaut et al., 2005, 2014; Bevilacqua et al., 2019; Bulleri et al., 2025). Collectively, these factors are contributing to the progressive degradation of *Cystoseira s.l.* populations, undermining their ecological functions and jeopardizing the stability and resilience of the ecosystems they structure.

Recovery of degraded *Cystoseira s.l.* stands is limited by low propagule dispersal and poor connectivity between remaining populations

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(Soltan et al., 2001; Buonomo et al., 2017; Capdevila et al., 2018). These biological limitations greatly reduce the potential for recolonization by neighboring populations (Guern, 1962; Johnson and Brawley, 1998; Gaylord et al., 2002), especially following extensive habitat loss (Soltan et al., 2001; Capdevila et al., 2018). As a result, the self-repair capacity of these ecosystems is inherently limited, thus necessitating the adoption of active restoration strategies. This need is acknowledged in major conservation frameworks, such as the European Biodiversity Strategy to 2030 (EC, 2021) and the United Nations Decade of Ecosystem Restoration (United Nations, 2019), which prioritize the recovery of macroalgal forests as part of broader efforts to counter biodiversity loss and promote ecological resilience.

Restoration strategies have evolved from direct transplantation of thalli to more advanced *ex situ* cultivation techniques (Falace et al., 2006; Falace et al., 2018a; De La Fuente et al., 2019; Orlando-Bonaca et al., 2021, 2022). These approaches typically involve cultivating seedlings under controlled laboratory conditions for a period ranging from 18 to 24 days before outplanting them into natural habitats. This method improves the efficiency and scalability of restoration efforts while reducing pressure on donor populations, providing a more sustainable and ecologically viable pathway for ecosystem recovery.

In recent years, alterations in the reproductive phenology of *Cystoseira s.l.* have been widely documented (Bevilacqua et al., 2019; Savonitto et al., 2019, 2021; Cimini et al., 2024), including a shortened of fertility periods across multiple species. These phenological shifts pose significant challenges for restoration efforts, as they restrict the time available for the collection of fertile material and hinder the feasibility of performing multiple culture cycles within a single reproductive season (Kaleb et al., 2023). Consequently, the scalability and overall efficiency of restoration initiatives are negatively impacted. Potential solutions include inducing fertility under controlled laboratory conditions – so far successfully applied only to *Gongolaria barbata* (Stackhouse) Kuntze (Kaleb et al., 2023) - or shortening the duration of the laboratory phase to allow for multiple culture rounds within the limited reproductive window. Reducing culture time also minimizes the risk of epiphytic overgrowth, a frequent issue associated with prolonged *ex situ* cultivation (Orlando-Bonaca et al., 2021; Clausing et al., 2022; Lardi et al., 2022).

Nonetheless, excessively short cultivation periods may result in underdeveloped germlings lacking the structural and physiological resilience required to withstand post-outplant stressors. Previous studies have shown that seedlings which reach a critical "refuge size" and establish firm attachment to the substrate prior to transplantation exhibit significantly higher survival rates in the field (Savonitto et al., 2021; Malfatti et al., 2023). Achieving this developmental threshold often requires extending the culture period or introducing intermediate cultivation steps. Among these, the use of suspended aquaculture systems - such as lantern nets, commonly adopted in marine aquaculture -has emerged as an effective strategy. In the case of *Gongolaria barbata*, suspended cultivation following a standard three-week laboratory phase allowed seedlings to attain the refuge size, resulting in increased robustness and improved survival upon field deployment (Savonitto et al., 2021; Orlando-Bonaca et al., 2022). Additionally, lantern nets provide enhanced protection against mesograzers -frequent on vegetated rocky substrates - and help reduce desiccation risk for early-stage embryos, particularly in the absence of adult canopy-formers that usually buffer environmental fluctuations. Lastly, suspended cultivation limits the occurrence of bacterial or microalgal overgrowth, which commonly affects prolonged mesocosm culture, thereby improving overall culture stability and survival. However, suspended aquaculture systems may entail specific physiological trade-offs that influence germling performance. To mitigate these risks, such systems should be deployed in sheltered environments to avoid excessive wave exposure and supplemented with nutrients to prevent limitations often occurring in natural waters.

In light of these considerations, we investigated whether it is feasible to shorten the laboratory culture period for *Ericaria amentacea* (C. Agardh) Molinari & Guiry by introducing a subsequent suspended algaculture phase prior to outplanting. Three experimental treatments were tested, each defined by different combinations of laboratory and suspended cultivation durations. The effectiveness of each treatment was assessed through performance indicators, including seedling growth metrics (i.e., surface cover and thallus length) and physiological condition (i.e., photosynthetic efficiency).

We hypothesized that an extremely short laboratory phase (i.e., five days) would result in suboptimal outcomes, with germlings poorly developed to endure environmental stressors. Conversely, a moderate reduction to twelve days was anticipated to strike a balance between resource efficiency and the developmental needs of the germlings, thereby conferring sufficient resilience for successful outplanting. This intermediate approach holds promise for enabling multiple culture cycles within a single reproductive season while maintaining both satisfactory outplanting success and scalability in restoration practices.

2. Materials and methods

2.1. Collection of specimens and culture conditions

Apical fronds of *E. amentacea* bearing mature receptacles, approximately (~3 cm in length), were collected in late June 2024 from a population in the Tyrrhenian Sea, near Civitavecchia, Italy (42°4′15.36″ N, 11°48′30.86 E). About 500 fertile apices were collected randomly, from different and spatially separated individuals (at least a few meters apart). The fronds were transported to the Laboratory of Phycology at the University of Trieste (Italy) under dark conditions and maintained at approximately 5 °C to minimize metabolic activity and prevent receptacle desiccation, thereby preserving reproductive viability.

Upon arrival, apices were cleaned of epiphytes, rinsed with filtered seawater, and stored in aluminum foil at 4 °C in darkness for 24 h to stimulate zygote release (Falace et al., 2018a). The apices were then positioned onto 250 clay tiles (4.5 cm diameter, 0.6 cm central hole for screw anchoring), placed in aquaria filled with 0.22 μm-filtered seawater. The aquaria were kept in controlled-environment rooms under constant conditions: 125 μmol m^{-2} s^{-1} irradiance, 18 °C temperature, and a 15:9 light:dark photoperiod, replicating seasonal field conditions (Falace et al., 2018a). Illumination was provided by LED lamps (AM366, Sicce USA Inc., Knoxville, USA), selected for their spectrum closely matching natural sunlight to optimize photosynthetic performance during culture. Irradiance was measured using a LI-COR LI-190/R photometer (LI-COR Biosciences, Lincoln, NE, USA).

Apices were left undisturbed on the tiles overnight to allow zygote release and attachment. The following day, they were gently removed, and after an additional 24 h, the culture medium was replaced with 0.22 μm-filtered seawater enriched with Von Stosch solution (Von Stosch, 1963; Guiry and Cunningham, 1984).

To ensure adequate gas exchange and nutrient mixing, aeration was provided via bubblers and pumps. The medium was renewed every three days, and aquaria and tile positions were randomly repositioned during each water change to compensate for light gradients under the lamps, thus ensuring uniform growth conditions.

2.2. Experimental design

The experiment was designed to evaluate the effects of three different culture conditions on the growth and performance of *E. amentacea* germlings: (A) five days in the laboratory followed by 12 days in suspended algaculture, (B) 12 days in the laboratory followed by five days in suspended algaculture, and (C) 17 days in the laboratory without subsequent suspended cultivation. The five-day duration was selected based on previous studies showing that *E. amentacea* embryos complete development and establish firm attachment to the substrate within this time span (Falace et al., 2018a). The 12-day period was introduced as an

intermediate condition to assess potential improvements in the cultivation protocol. Finally, the 17-day duration reflects the minimum average time commonly adopted in *Cystoseira* s.l. *ex situ* cultivation, which allows germlings to reach a refuge size considered adequate for successful field transplantation.

After 24 h of culture, 180 out of 250 tiles showed successful embryo attachment and were therefore randomly assigned to nine aquaria, three per condition, and 20 tiles per aquarium. After five days (Time Point 1), the 60 tiles assigned to Condition A were transferred to suspended aquaculture systems in a nursery area in the Gulf of Trieste (45°43′17.88″N, 13°41′19.74″E), using floating supports (Ostriga, Acqua&Co S.r.l., Italy) equipped with slow-release nutrient pellets (Fig. 1).

One week later (Time Point 2), the 60 tiles for Condition B were similarly transferred to the aquaculture system, while the remaining 60 tiles (Condition C) were kept in the laboratory for 17 days until the final time point.

At the end of the culturing phase (in lab and suspended), 32 tiles per experimental condition were randomly selected for the outplanting. The restoration area selected for the outplanting under the RENOVATE Project was the rocky shore of Santa Marinella (42°03′1.25″N 11°49′10.5″E). The area included two sites ~50 m apart to account for environmental heterogeneity. At each site, 16 tiles per experimental condition were securely fixed to the rocky substrate using screws and an underwater drill to ensure stability in the intertidal zone (approximately 0.5 m below the mean sea level). The tiles were arranged in an alternating pattern to minimize spatial bias. Specifically, the tiles were sequentially organized from north to south in a repeating order of eight tiles for each condition A, B, C (for a total of 48 tiles per site), ensuring interspersed positioning of tiles across the study sites.

2.3. Sampling and sample analysis

At each time point during the experiment (5, 12, and 17 days) 20 randomly selected tiles per condition were photographed with a Canon Powershot G9 (Canon Inc., Tokyo, Japan), either in the laboratory or at the aquaculture nursery, depending on the treatment. After image acquisition, all tiles were returned to their original position. To quantify the algal coverage on each tile, photographic samples were processed using ImageJ software (v1.52, National Institutes of Health, USA). The images were analysed by manually thresholding, which involved selecting a colour spectrum corresponding to the tissue of *E. amentacea* to distinguish it from the background and other potential features in the image. This method enabled the accurate identification of pixels representing the algal tissue, and the percentage cover was subsequently calculated based on the proportion of pixels identified (Schneider et al., 2012).

Fig. 1. Floating lantern net with clay tiles hosting germlings of *Ericaria amentacea*.

Following the culturing phase, 32 tiles per condition were randomly selected for outplanting, while the remaining 28 tiles were used to assess the photosynthetic performance and to estimate the length of seedlings at the end of the culturing phase. Photosynthetic performance of seedlings grown under the three different conditions was assessed to evaluate their physiological status and potential resilience in the field. Five randomly selected tiles per condition were dark-adapted in Petri dishes, to ensure that the photosynthetic apparatus of the germlings was in a basal state, allowing accurate measurements of the maximum quantum yield of photosystem II (PSII). The maximum quantum yield (F_v/F_m ratio) was then measured using a PAM-Imaging Fluorometer Open FluorCam (Photon Systems Instruments©, Czech Republic). The device captured high-resolution images of the fluorescence emitted by the germlings, allowing for a precise and quantitative evaluation of their photosynthetic performance. The measured parameter, F_v/F_m, indicates the maximum potential efficiency of PSII when all reaction centres are fully open, providing a reliable metric for assessing the physiological state of photosynthetic organisms. From the remaining tiles, 25 individuals per condition were randomly selected and measured for thallus length using an inverted microscope (DM IL, Leica, Germany).

To evaluate field performance post-outplanting, seedlings of *E. amentacea* were assessed after 18 days. At each of the two experimental sites, 10 tiles per each condition were randomly selected and photographed using a Canon PowerShot G7 X Mark III (Canon Inc., Tokyo, Japan). Photographic samples were processed with ImageJ software to quantify the algal coverage, as previously described. However, due to the presence of other organisms on the tiles, areas containing *E. amentacea* were manually selected to ensure accurate measurements, avoiding overestimation or misidentification of the target species.

2.4. Data analysis

One-way analysis of variance (ANOVA) was conducted to test for potential differences in percent cover of juveniles under the three conditions (A, B, C), separately for each time point of the culturing phase and for post-outplanting. For the culturing phase, three separate ANOVA, one for each time point, were done respectively: (i) to verify the initial homogeneity among treatments at day 5 (Time Point 1); (ii) to monitor the development of seedlings in condition A after the short culture period and placement on the lanternet and to assess the continued comparability of conditions B and C at day 12 (Time Point 2); and (iii) to compare the final performance across all three conditions after 17 days (Time Point 3) and after 18 days in the field, thus evaluating the overall effect of the different culture durations and combinations.

One-way ANOVA was also used to test for differences among conditions in length of individuals and photosynthetic performance at the end of the culturing phase. Prior to analysis, normal distribution of sample means, and variance homogeneity were tested using the Kolmogorov-Smirnov test and the Bartlett's test, respectively. Data were transformed (arcsin square root) to stabilize variance, if required. For two variables, namely length of individuals and photosynthetic performance, Welch's ANOVA was carried out since variance heterogeneity still persisted after data transformation. Tukey's HSD test was used for pairwise post hoc comparisons among the three conditions.

3. Results

For the first two times of the culturing phase, ANOVA did not detect significant differences in percent coverage of experimental tiles among the three conditions (A = 5.48 %, B = 5.76 %, C = 5.39 %, p = 0.875 for Time Point 1, and A = 4.29 %, B = 5.11 %, C = 5.91 %, p = 0.120 for

Time Point 2) (Fig. 2a and b). At the final time point, the mean percent cover instead significantly differed among conditions ($p < 0.001$), with significantly higher values in tiles from condition C (8.75 %) with respect to conditions A (2.39 %) and B (3.09 %) which instead did not differ (Table 1 left, Fig. 2c).

ANOVA also detected significant differences ($p < 0.001$) in the mean percentage cover among conditions on outplanted tiles (18 days since outplanting). In this case, however, the percentage cover differed between all conditions, with higher values for condition C (14.97 %), followed by condition B (9.84 %), and the lowest values for condition A (2.18 %) (Table 1 right, Fig. 2d).

ANOVA on the photosynthetic performance and length of individuals measured at the end of the culturing phase detected significant differences among conditions ($p < 0.001$ in both cases). Post hoc pairwise tests highlighted a signifcantly lower F_v/F_m value for conditions A (0.636 ± 0.012, mean ± SE) if compared to conditions B (0.682 ± 0.005) and C (0.705 ± 0.005), which had comparable higher performance (Table 2 left, Fig. 3). The same pattern of differences between conditions was also detected for the length of individuals, seedlings under condition A were significantly shorter (0.75 ± 0.05 cm), followed by B (1.01 ± 0.06 cm) and C (1.16 ± 0.03 cm) (Table 2 right, Fig. 4).

4. Discussions

Restoration strategies targeting key habitat-forming species such as *Cystoseira s.l.* spp. are urgently needed across a range of Mediterranean ecosystems. However, the success of such interventions is challenged by uncertainties associated with climate change, particularly due to its poorly understood effects on the reproductive biology and phenology of target species, which may hinder progress in unforeseen ways. Temperature, for instance, is known to significantly affect gametogenesis and gamete release in Fucales (Bacon and Vadas, 1991; Vadas et al., 1992; Falace et al., 2018a) and recent studies has documented substantial shifts in the reproductive phenology of *Cystoseira s.l.*, including a marked reduction in fertility duration in various species (Bevilacqua et al., 2019; Savonitto et al., 2019, 2021; Cimini et al., 2024). These phenological constraints hinder restoration by narrowing the timeframe for collecting fertile material and limiting multiple culture cycles per reproductive season, reducing the scalability and efficiency of initiatives. To address these constraints, we tested a protocol designed to shorten the standard three-week laboratory culture period (Falace et al., 2018a; De La Fuente et al., 2019; Savonitto et al., 2021; Orlando-Bonaca et al., 2022), by introducing a suspended algaculture phase, thus enabling multiple culture cycles within a single reproductive season.

To date, germlings of *E. amentacea* have been maintained in mesocosms for a minimum of three-weeks to allow growth to a "refuge size"

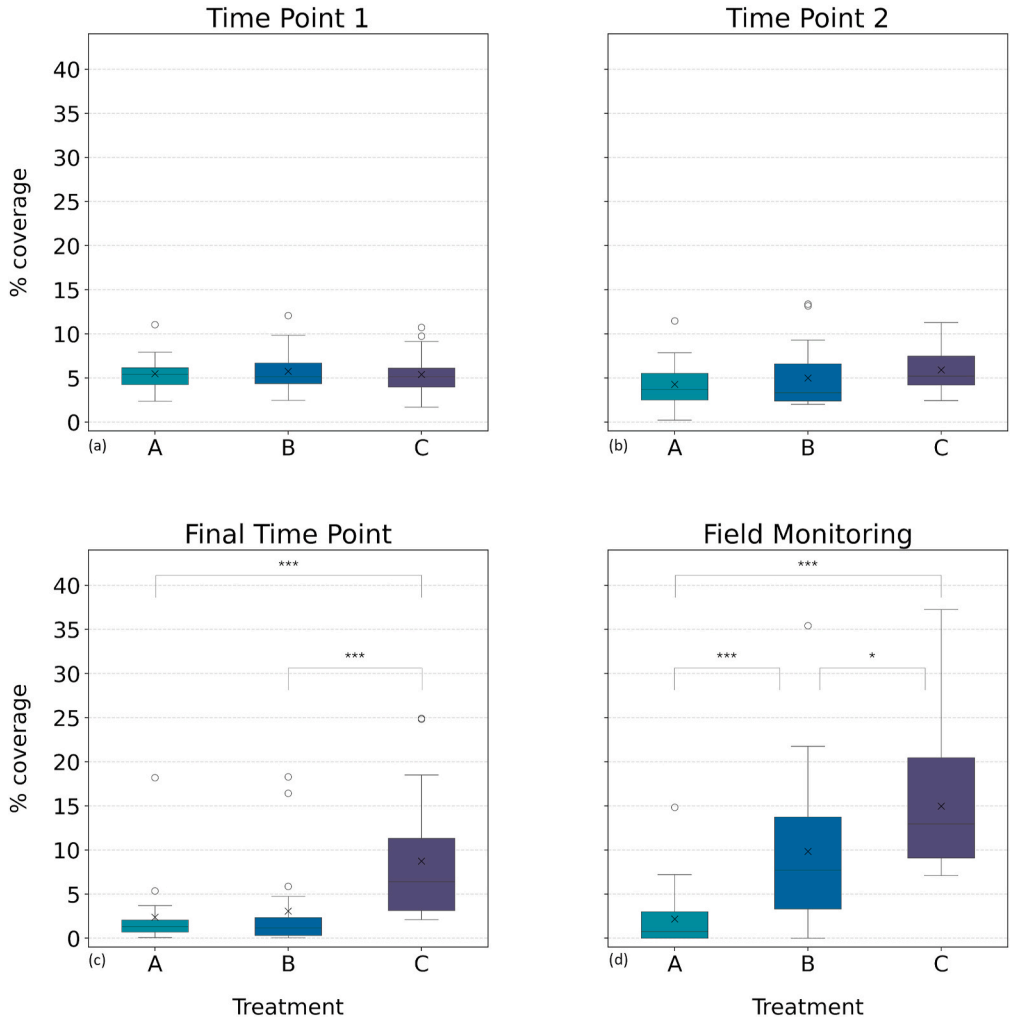

Fig. 2. Boxplots showing the percent cover ($n = 20$) of *E. amentacea* juveniles under treatments A, B, and C at different time points (panels a, b and c) and in the field monitoring (panel d). Boxes represent the interquartile range (IQR), with whiskers extending to 1.5 × IQR from the lower and upper quartiles. Significance levels are indicated by stars: * = $p < 0.05$, *** = $p < 0.001$.

Table 1
Results of Tukey's HSD post hoc test for multiple comparisons of mean percent cover of *E. amentacea* juveniles at the Final Time Point and 18 days after the outplanting.

	Final time point (culture)			Outplanting		
Comparison	*Mean difference*	*p-value*	*Significance*	*Mean difference*	*p-value*	*Significance*
A vs B	0.40	0.9725	ns	10.02	0.0002	***
A vs C	8.53	0.0002	***	16.04	0.0000	***
B vs C	8.08	0.0005	***	6.02	0.0404	*

Table 2
Results of Tukey's HSD post hoc test for multiple comparisons of F_v/F_m ratio and length at the Final Time Point.

	Photosynthetic performance			Length		
Comparison	*Mean difference*	*p-value*	*Significance*	*Mean difference*	*p-value*	*Significance*
A vs B	0.05	0.0006	***	0.26	0.0013	***
A vs C	0.07	0.0000	***	0.41	0.0000	***
B vs C	0.02	0.1254	ns	0.16	0.0769	ns

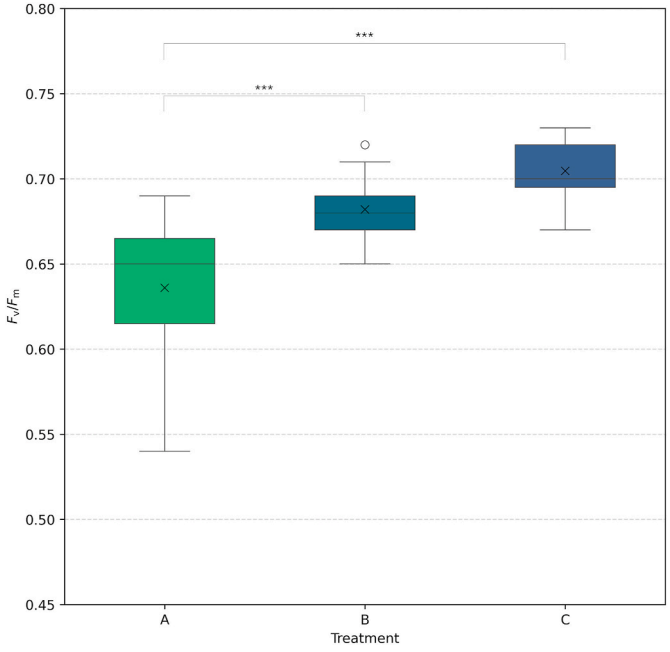

Fig. 3. Boxplot showing the F_v/F_m ratio ($n = 5$) of *E. amentacea* juveniles under treatments A, B, and C at the Final Time Point. IQR shown as boxes; whiskers = 1.5 × IQR. *** = $p < 0.001$.

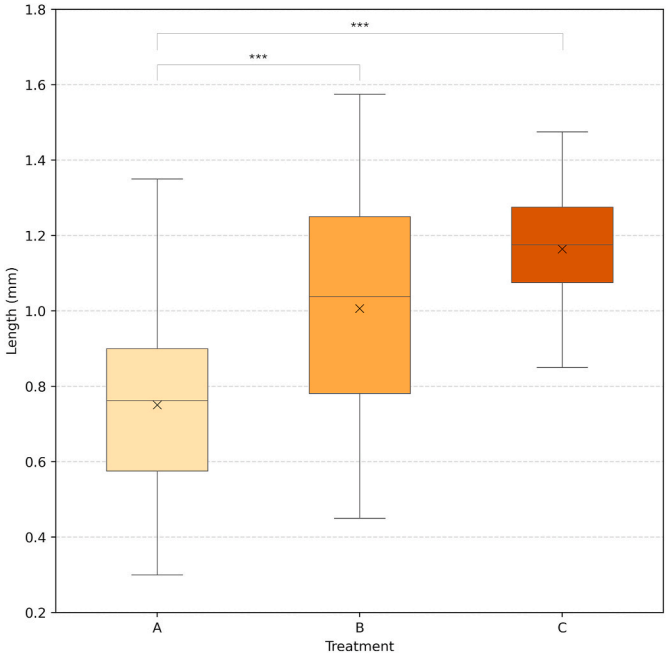

Fig. 4. Boxplot showing the length ($n = 25$) of *E. amentacea* juveniles under treatments A, B, and C at the Final Time Point. IQR shown as boxes; whiskers = 1.5 × IQR. *** = $p < 0.001$.

threshold prior to transplantation, which confers protection against macro- and mesograzers upon outplanting (Vadas et al., 1992; Savonitto et al., 2021). In addition to size, achieving high coverage on the substrate prior to transplantation is also critical for sympodial species as *E. amentacea*, as denser aggregations of germlings promote mutual shading and reduce abiotic stress, leading to increased survival and growth in the intertidal (Clausing et al., 2022, 2024). These factors are particularly important in *ex situ* restoration, where juveniles are transplanted into sites lacking adult conspecifics, which would otherwise buffer them against desiccation, light stress, and grazing pressure (Dayton, 1975; Davison et al., 1993; Cervin et al., 2005; Bulleri et al., 2006; Bulleri, 2009; Capdevila et al., 2019; Duarte et al., 2022).

Our results showed that, in terms of percent cover, both conditions involving a suspended culture phase (A: 5 days in lab + 12 days suspended; B: 12 days in lab + 5 days suspended) underperformed compared to condition C (17 days in lab only) at the final culture time point (Fig. 2c). This could be linked to the prolonged absence of beneficial effects of the Von Stoch culture medium and the heightened vulnerability of seedlings once kept on suspended algaculture system compared to the controlled mesocosm. Indeed, although suspended aquaculture offers certain advantages over immediate outplanting—such as reducing encounters with many of the grazers typically found on rocky shores and mitigating the risk of desiccation to which *E. amentacea* is exposed in the intertidal zone—it remains a less favorable environment compared to the controlled mesocosms. However, while seedlings from condition A – those with the shortest laboratory exposure - failed to recover after fixed on rocky and continued to decline in cover, seedlings from condition B, which remained longer in the controlled laboratory setting, began to recover after 18 days in the field. Notably, the growth curve observed in condition B displayed a slope similar to that of condition C (Fig. 5), suggesting that an intermediate laboratory culture period can still yield favorable outcomes.

Consistently, individuals from condition A also exhibited significantly lower thallus length at the end of the culture phase (Fig. 4). In contrast, germlings from condition B reached comparable sizes to those from condition C, supporting the conclusion that suspended aquaculture can effectively complement *ex situ* laboratory culture if the initial

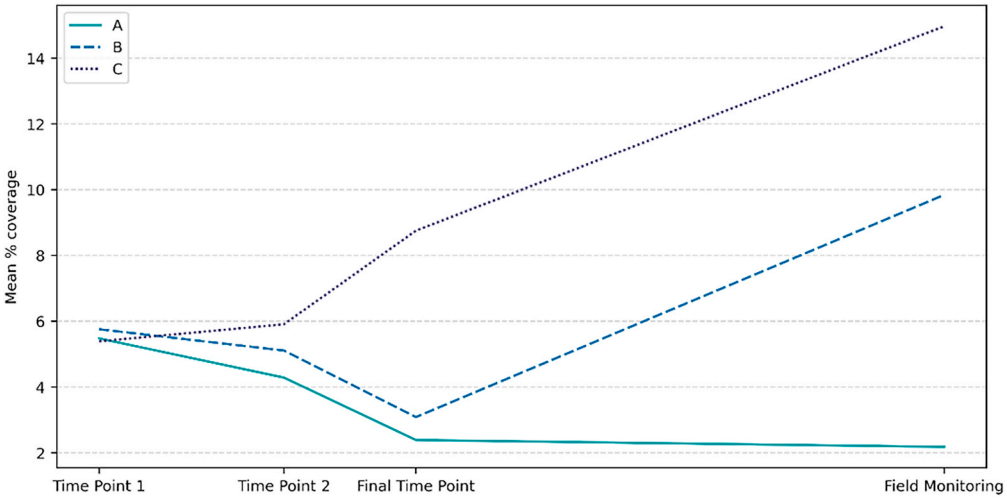

Fig. 5. Line plot showing the temporal changes in percent cover of *E. amentacea* germlings under treatments A, B, and C.

laboratory period is sufficiently long to allow early development and substrate anchoring.

From a physiological standpoint, F_v/F_m measurements further support this finding. Seedlings cultured under condition A showed significantly lower values than those from conditions B and C (Fig. 3), indicating impaired photosynthetic capacity (Falace et al., 2018b; Verdura et al., 2018; Malfatti et al., 2023). No significant differences in F_v/F_m were detected between conditions B and C (Table 2 left), suggesting that a twelve-day laboratory period is adequate to develop basal photosynthetic resilience to environmental stress, which can subsequently support growth and development once the germlings are outplanted in the intertidal.

In conclusion, our study demonstrates that incorporating a suspended algaculture phase can effectively reduce the duration of the laboratory culture period required for *E. amentacea* without compromising seedling performance. A minimum of twelve days in mesocosm conditions was sufficient to attain both refuge size and photosynthetic resilience, enabling subsequent survival and growth after outplanting.

This approach not only increases the potential for multiple culture cycles within a single reproductive season—thus enhancing the scalability and temporal flexibility of restoration protocols—but also significantly reduces the operational costs associated with long-term laboratory maintenance. By limiting the time germlings remain in controlled indoor facilities, this protocol decreases energy consumption, personnel time, and infrastructure occupancy, all of which are critical factors in large-scale or multi-site restoration initiatives.

Overall, these findings provide actionable insights for optimizing *ex situ* cultivation strategies of canopy-forming brown algae, especially under the increasing constraints imposed by climate-driven phenological shifts and limited resource availability. This approach can be adapted to other *Cystoseira s.l.* taxa by adjusting the timing of transfer to aquaculture systems according to the species-specific growth dynamics in mesocosm, and by positioning the lanternets at depths corresponding to the natural habitat of each species. This flexibility may enhance cost-efficiency and makes the protocol potentially suitable for a broader range of taxa with differing habitat preferences and developmental timelines, thus supporting the long-term feasibility and broader implementation of macroalgal forest restoration efforts in the Mediterranean and beyond.

CRediT authorship contribution statement

Sara D'Ambros Burchio: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Stanislao Bevilacqua:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Formal analysis, Conceptualization. **Sofia Comis:** Writing – review & editing, Methodology, Investigation, Data curation. **Marco Marcelli:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Eleonora Amore:** Writing – review & editing, Investigation, Data curation. **Edoardo Batistini:** Writing – review & editing, Investigation, Data curation. **Marco Segarich:** Writing – review & editing, Investigation, Data curation. **Annalisa Falace:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of generative AI in scientific writing

During the preparation of this work the authors used ChatGPT (OpenAI, 2025) in order to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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