# Accounting for environmental stress in restoration of intertidal foundation species

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## Abstract

1. Restoration of foundation species in historical habitat may be difficult if adult facilitation is obligatory for survival of early life stages.
2. On intertidal Mediterranean coasts, large-scale loss of the dominant forest-forming macroalga *Ericaria amentacea* have prompted restoration efforts using recruits. Yet, early life stages may be more susceptible to the abiotic stress that characterizes their habitat.
3. We tested strategies to enhance resilience of lab-cultured juveniles of *E. amentacea* to environmental stress in historical habitat lacking conspecifics. Juveniles were exposed in culture to fluctuations of the dominant physical stressors, irradiance and temperature, and then outplanted in upper and lower zones of their native intertidal range.
4. Without adult canopy, juvenile outplant survival was limited to the lower tidal range, with nearly complete mortality in the upper zone. Survival was also strongly determined by spatial clumping of recruits within the outplant substrate. Longer-term growth in the lower zone was enhanced by fluctuating mild stress in culture, with variable irradiance and concurrent heat pulses increasing post-outplant cover by 40%–60% after 4 months. Clumping also promoted growth across experimental treatments.
5. *Synthesis and applications*. Reliance on self-facilitation feedbacks is a common barrier to foundation species restoration in high-stress habitats. Our results suggest that without adult habitat amelioration, environmental stress limits recruit survival in intertidal algal forest. Yet, exposure to transient, low environmental stress in culture and outplanting of clumped individuals may confer resilience and allow successful establishment of early life stages in zones of reduced abiotic stress, providing a source for gradual colonization of more high-stress areas.

**KEYWORDS**
environmental stress, foundation species, intertidal, macroalgae, marine forest, resilience, restoration, self-facilitation

# 1 | INTRODUCTION

Human impacts have resulted in wide-scale losses of habitat-forming species in ecosystems worldwide (Ellison et al., 2005; Jackson et al., 2001; Lotze et al., 2006; Orth et al., 2006). These habitat-formers are foundation species (sensu Dayton, 1975), providing not only structure and refuge, but also facilitating other organisms, both intra- and inter-specific, by ameliorating the physical environment (Bruno et al., 2003; Bulleri et al., 2016). Thus, loss of these species undermines the diversity, functioning and resilience of the community, as well as its ecosystem service provision (Bruno & Bertness, 2001; Ellison et al., 2005). Recognition of the ecological and economic importance of foundation species has led to calls from the international community to focus restoration on critical habitat-formers (UN, 2019) whose re-establishment is key to whole ecosystem restoration (Byers et al., 2006; Halpern et al., 2007).

Foundation species may be difficult to restore, however, when they depend on the environmental amelioration provided by established conspecifics (hereafter termed 'self-facilitation'; Schotanus, Walles, et al., 2020; Temmink et al., 2020; van Katwijk et al., 2009). Coastal macrophytes such as seagrasses and marsh grasses slow water flow and stabilize the sediment (Gedan et al., 2011; Silliman et al., 2015), while both terrestrial and marine forests moderate light and temperature extremes, among other effects (hemlock: Ellison et al., 2005; kelp: Layton et al., 2019). Moreover, these facilitative effects increase with environmental stress (He & Bertness, 2014). Thus, reducing the inhibiting effects of physical exposure may be necessary to improve restoration potential. Strategies include aggregated or "clumped" outplant design to promote inherent self-facilitative feedbacks (Duggan-Edwards et al., 2019; Halpern et al., 2007; Renzi et al., 2019; Silliman et al., 2015) or manual reduction of stress in the target area using artificial structures or habitats (Schotanus, Capelle, et al., 2020; Stekoll & Deysher, 1996; Yu et al., 2012).

In addition to reduction of physical stress, approaches to enhance the physiological resilience of new transplants or recruits to abiotic extremes may be necessary (Beever et al., 2016; Bulleri et al., 2018). These techniques are important when restoration involves outplanting cultured early-life stages. Outplanting has emerged as a sustainable way to achieve small- to large-scale repopulation without taxing or damaging existing populations, often sourced from protected or remote locations (corals: Pollock et al., 2017; macroalge: De La Fuente et al., 2019; reviewed by Vanderklift et al., 2020). Yet, early life stages may be more susceptible to environmental stress (Cossins & Bowler, 1987; Roleda et al., 2007), and recruits cultured in artificial settings under optimal conditions, such as greenhouses, may have reduced structural allocation (Telewski & Jaffe, 1986) or lack stress-response mechanisms that are critical for survival in the field. Studies have shown, for example, that corals with past exposure to moderate thermal variability are more tolerant of warming (Oliver & Palumbi, 2011).

Thus, it may be critical to explicitly activate stress response gene expression pathways during culture by exposure to environmental conditions relevant to the habitat being restored (see Gilbert, 2001). Such approaches may become essential as increasing climate-related environmental stress tests species' capacities for stress response (Doney et al., 2012; Somero, 2010) and alters their phenology and physiology (Capdevila et al., 2019; Falace et al., 2021). Thus, successful restoration may hinge on life stage-specific knowledge of relevant life-history traits and drivers of outplant mortality (Cebrian et al., 2021; Montero-Serra et al., 2018; Morris et al., 2020).

Coastal marine environments are dynamic and often high-stress ecosystems that are generally dominated by one or several habitat-forming species and are experiencing losses at unprecedented rates (Hoegh-Guldberg & Bruno, 2010; Lotze et al., 2006). To date, restoration in these systems has largely focused on seagrass meadows, salt marshes, and coral reef habitats (Bayraktarov et al., 2016). Active restoration of forest-forming macroalgae (kelps and fucoids), which dominate some of the most dynamic coasts, are still in an early experimental phase (De La Fuente et al., 2019; Layton et al., 2020; Verdura et al., 2021), and to date their restoration success, as evaluated by survival, is low (Basconi et al., 2020).

We tested methods to improve restoration success of the intertidal forest-forming macroalga *Ericaria amentacea* (previously *Cystoseira amentacea var. stricta*) by enhancing resilience of lab-cultured juveniles to environmental stress in the habitat to be restored. We asked if juvenile post-outplant success could be enhanced by repeated exposure to mild stress in culture (fluctuations in irradiance, periodic increases in temperature) combined with methods to reduce physical stress in the target habitat (height on shore, artificial shading). We evaluated direct effects of experimental treatments on survival and growth as well as indirect effects that may result from different degrees of juvenile clumping on the outplant substrate. We hypothesized that reduced stress exposure in the habitat would increase survival, that environmental variability in culture would elevate growth, and that regardless of culture treatment, clumped juveniles would perform better than dispersed juveniles.

# 2 | MATERIALS AND METHODS

## 2.1 | Study species

*Ericaria amentacea* is a canopy-forming fucoid algae that forms the foundation of biodiverse communities in the midlittoral zone of dynamic, exposed rocky coasts throughout the Mediterranean Sea. Due to widespread reductions or loss (Thibaut et al., 2005, 2015), it has become a focus of restoration efforts (reviewed by Cebrian et al., 2021). Yet, high environmental stress caused by strong, seasonally variable fluctuations in temperature and irradiance in its native habitat represent a bottleneck to restoration, particularly for susceptible propagule or juvenile stages during summertime extremes.

## 2.2 | Study sites

In western Italy, the previously dominant *E. amentacea* has been lost along large stretches of the coast (Mancuso et al., 2018; Mangialajo et al., 2008), including Marine Protected Areas (MPAs). The site of ex situ outplanting was located within the most protected zone of the Cinque Terre MPA (44° 8′2.95″N-9°38′10.10″E; Punta Mesco). The disappearance of historical *E. amentacea* forest from Cinque Terre in the early 20th century (De La Fuente et al., 2018) has been attributed to water pollution and sediment loads associated with heavy excavation in the watershed of the largest local riverine outflow (Magra River, ~25 km). The reduction of excavation activities several decades ago has restored water quality, and the establishment of the MPA in 1997 has further removed direct human stressors from the system (see Appendix S1). Nonetheless, the area remains devoid of *E. amentacea* and is dominated by mixed coralline algal turf and fragmented belts of a more tolerant fucoid alga, *Cystoseira compressa*. The donor site consisted of a healthy *E. amentacea* population located along a 200-m stretch of coastline in the Portofino MPA (44°19′21″N-9°08′46″E; Punta Chiappa-Portofino). Authorization for field activities were obtained from Cinque Terre and Portofino MPAs (permit no. 1692/2-1-1).

Both sites are characterized by a small tidal range (<30 cm) that is strongly influenced by barometric pressure, currents and significant swell. The native intertidal habitat of *E. amentacea* is characterized by high physical stress (predominantly light and temperature during the summer recruitment period) that is moderated by wave splash and changes in sea level associated with atmospheric pressure. Work was conducted within the framework of the EU-Life project, ROCPOP-Life (LIFE16 NAT/IT/000816), a restoration initiative for *E. amentacea* based on outplanting of ex situ cultured juveniles.

## 2.3 | Experimental design

The experiment, performed in the summer of 2019, consisted of two phases: (I) laboratory culture of *E. amentacea* germlings under optimal or variable (repeated mild stress) environmental conditions; (II) outplanting of cultured juveniles in the target site under varying physical stress (natural gradient or manual reduction; see Figure 1).

### 2.3.1 | Phase I: Laboratory culture conditions

Fertile apices of *E. amentacea* were collected in the Portofino MPA in June and transported in cool, dark conditions to the culture laboratory at the University of Genova. Apices were rinsed with seawater, gently cleaned of epiphytes, and after 24 h in dark and cold (5°C) conditions, placed on the culture substrate in UV-treated, filtered seawater for gamete release. The substrates were hand-constructed circular clay tiles (4.5 cm in diameter) with a rugose surface to promote attachment. Tiles (*n* ≈ 300) with apices (3–4 per tile) were incubated
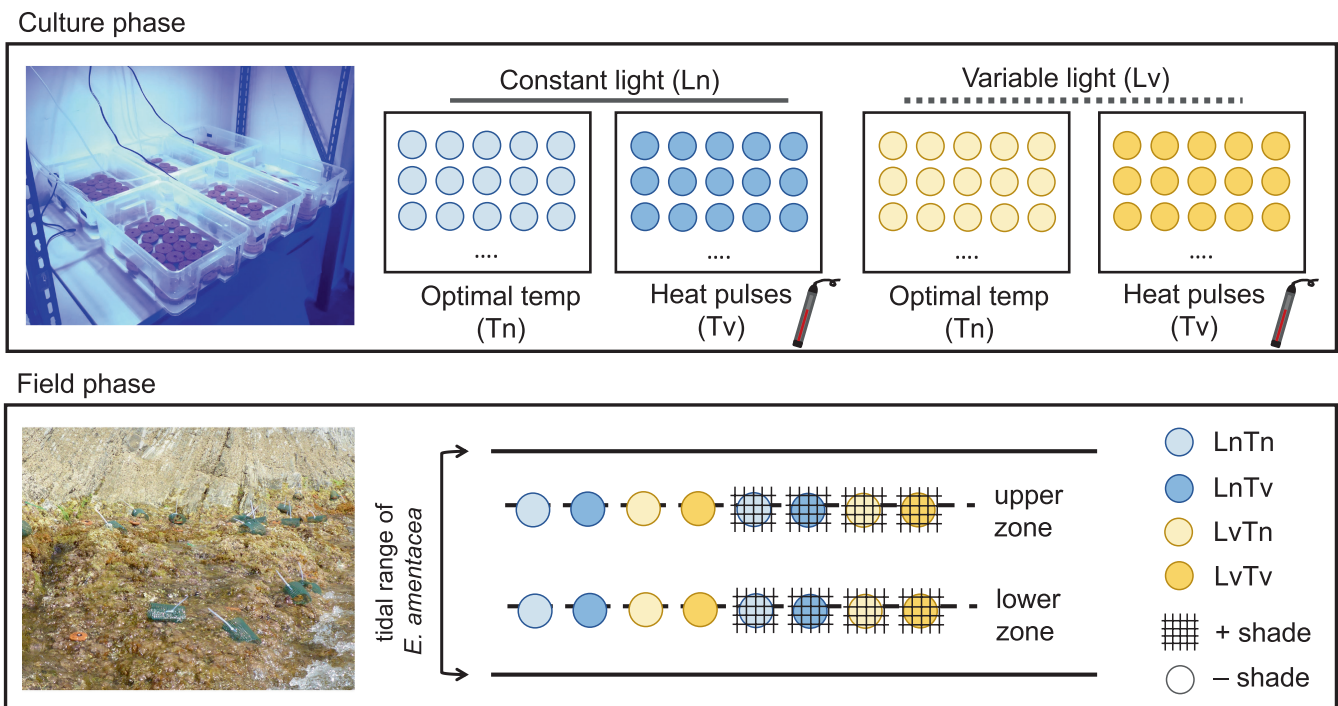


**FIGURE 1** (a) Culture of juvenile *Ericaria amentacea* from spores under constant low light (Ln: 125 μmol photons m$^{-2}$ s$^{-1}$) or fluctuating light (Lv) with an equivalent mean daily intensity, and optimal temperature (Tn: 20°C) or heat pulses (Tv: 25.5°C) every 2–3 days (4 total treatments, *n* = 3 aquaria each). (b) Within culture treatments, tiles were randomly assigned to the *upper* or *lower* zone of the natural tidal range of *E. amentacea* and with or without a shade covering (+/− shade; 4 field treatments). Each of the resulting 16 treatment combinations had *n* ≥ 6 replicate tiles.

overnight at 20°C. The following morning apices were removed, and tiles were left undisturbed for 24 h to ensure embryo attachment.

To assess the role of exposure to variable mild environmental stress during culture on survival and establishment of early stages of *E. amentacea* after field introduction, we implemented culture treatments of fluctuating daytime light intensity and periodic heat pulses (Figure 1a). The control treatment received previously identified optimal light and temperature conditions to maximize growth in culture, following Falace et al. (2018): 20°C (normal/stable temperature: Tn) and 125 µmol photons m$^{-2}$ s$^{-1}$ irradiance (normal/stable light: Ln). The variable light treatment (variable light: Lv) simulated fluctuation in daytime light intensity (but not absolute values) as occurs with intermittent cloud cover, where light intensity changed hourly to random values between 75–200 µmol photons m$^{-2}$ s$^{-1}$ (LED aquaculture lamps: Maxspect RSX 150). To target the effect of variability rather than confound it with higher overall light absorption, the mean daily intensity was maintained constant at 125 µmol photons m$^{-2}$ s$^{-1}$. Falace et al. (2018) previously showed that juvenile growth and development was delayed at 250 µmol photons m$^{-2}$ s$^{-1}$. All treatments were maintained under a light: dark cycle of 14:10 h.

The heat pulse treatment (Tv) simulated periodic daytime warming as occurs in shallow waters. Every 2 to 3 days, resistors warmed the aquaria to 25.5°C over an 8 h period after which the water cooled back to room temperature at 20°C (6 total warming episodes over the 25-day experimental culture period). Rather than simulate extremes experienced in the intertidal environment, treatments were chosen to introduce repeated but temporary mild stress without risking high mortality. Light and temperature treatments were applied randomly to aquaria in a factorial design, giving 4 treatment combinations (LvTv, LvTn, LnTv, LnTn; $n = 3$ aquaria per treatment) and were introduced on the second day of culture. Each aquarium contained 20–25 tiles. All other conditions were equivalent among all treatments and followed the protocols in Falace et al. (2018) and De La Fuente et al. (2019). Treatment aquaria as well as tiles within aquaria were randomly repositioned every 2–3 days during culture medium changes to control for positional variation in light intensity. HOBO® dataloggers were placed in each of the treatments ($n = 1$: LnTn, LvTn; $n = 2$: LnTv, LvTv) to quantify effectiveness of experimental treatments.

Laboratory culture treatments were verified by comparing means and standard deviations of light intensity (daytime) and temperature (during heating/cooling) in aquaria of each treatment (HOBO® dataloggers). Variable light treatments (LvTn, LvTn) had significantly higher standard deviation than normal light treatments (LnTn, LnTv; ANOVA $p < 0.0001$ followed by Tukeys HSD; Table S1), whereas no differences were found within each light treatment (LnTn vs. LnTv and LvTn vs. LvTv; Table S1; Figure S1). Mean daytime light intensity did not vary across treatments ($p = 0.3$), confirming equivalent total light exposure despite variable delivery between Ln and Lv treatments.

Water temperatures in all treatments rose slightly during the day (20.6 ± 0.23°C mean ± SD from 19.4 ± 0.65°C at night; Figure S2).

Heat pulses increased water temperature to 25.1 ± 1.22°C (Tv vs. Tn treatments: $p < 0.0001$, $F_{3,126} = 295.5$; Table S2) with no differences among Tv treatments (LnTv vs. LvTv; $p = 0.57$). Temperatures did not differ among treatments on days without heat pulses (ANOVA $p = 0.2$, $F_{3,284} = 1.5$).

### 2.3.2 | Phase II: Field conditions

To examine if methods to reduce exposure to environmental stress in the native habitat affect juvenile survival and success in the absence of adults, and how patterns vary depending on culture conditions, we outplanted juveniles in conditions of differing physical exposure within the species' natural vertical range. We implemented two treatments: height on shore and artificial shade (Figure 1b). Height on shore included two levels within the infralittoral zone: *upper* ($n = 82$ tiles outplanted) and *lower* ($n = 58$ tiles). The upper level was assumed to have increased temperature and desiccation stress. Though *E. amentacea* can be found throughout this range elsewhere, to date it remains absent in the Cinque Terre MPA and the surrounding area. Uneven replicates between vertical zones reflect greater difficulty to drill holes for attachment in the lower zone. The shade treatment (with or without, +S/–S) was applied to half of the tiles within each zone and consisted of a ~5 cm diameter cylinder of 10-mm plastic mesh with open sides to reduce any effects on flow and allow herbivore access (Figure S3). Herbivore pressure is not considered strong in this zone and, at this site, comes predominantly from mesograzers sheltering in the algal fringe (authors' personal observation). The shade treatment acts to reduce differences in exposure with height on shore, where lower physical stress is one assumed benefit of juvenile recruitment within adult canopy. While simultaneous transplantation of adults into both zones would provide a rigorous way to evaluate positive effects of intact adult canopy on recruits, this was not possible in this study due to protection of the species from collection.

After 27 days in culture, tiles with maximal cover and minimal epiphytization were selected from each treatment ($n = 25+$), labelled with shellfish tags (Hallprint Fish Tags, Australia), photographed, and transported to Cinque Terre MPA for outplant. Tiles from each culture treatment ($2 \times 2$) were allocated randomly to field treatments ($2 \times 2$; 16 total treatments, all with $n \geq 6$). More replicate tiles were outplanted from Lv than Ln treatments because post-culture cover was higher (see 3.1) and attachment sites were limiting. Tiles were affixed to the rock in pre-drilled holes with anchors, screws and neoprene washers. Despite the use of antibiotics and GeO$_2$ in the culture medium, small amounts of epiphytic diatoms were present on tiles, as was some filamentous algae.

After random assignment of tiles to field treatments at outplant, subsequent photo analysis showed that, by chance, % cover was lower on tiles assigned to the shade treatment on the upper shore (*upper* +S, 8.7%) compared to unshaded tiles in the lower zone (*lower* −S, 13.7%; $p < 0.05$). No other treatment pairs differed significantly.

## 2.4 | Juvenile survival and growth and environmental monitoring

Percent cover and the degree of spatial aggregation of *E. amentacea* individuals (hereafter: clumping) on each tile were determined post-culture (*week 0*) by photo analysis (*ImageJ* v1.52, National Institute of Health, USA). Percent cover was measured by manual thresholding to select pixels in the correct colour spectrum that corresponded to the area covered by *E. amentacea* juveniles (Figure S4:A,B). Clumping was assessed by the number and size distribution of polygons (larger than 2 mm$^2$) resulting from aggregated individuals identified in the thresholding process (Figure S4:C,D). Length was determined visually.

After outplant, tiles were monitored at 2–5 week intervals over 4 months (17, 41, 55, 81, 101 days ≅ 3, 6, 8, 12, 15 weeks) as allowed by weather conditions. Survival (presence/absence) was determined by visual inspection in the field, and change in percent cover was assessed by photographic analysis. After 2 weeks, the shade cloth was removed from +S units to avoid potential secondary effects that could result from limited light penetration (epiphyte growth) or damaged mesh (physical abrasion).

Data loggers (HOBO® MX2202) quantified temperature and light exposure in the two outplant zones. Due to logger loss during storms in 2019, measurements were repeated in July–August 2020 and thus reflect relative differences between upper and lower areas of the species vertical range. Daily maximum temperatures were significantly higher in the upper range, including occasional highs over 35°C, while temperatures in the lower range only surpassed 30°C once (Figure 2a; mean daily maximum of 31.8° and 28.0°C, respectively). Light exposure (as average total daily irradiance) was 28.3% higher in the upper experimental zone compared to the lower (Figure 2b).

## 2.5 | Statistical analysis

### 2.5.1 | Juvenile development in culture

Differences in post-culture juvenile percent cover among light and temperature treatments were analysed with factorial ANOVA. Data met assumptions of parametric statistics. Juvenile length after the culture period was assessed with a bootstrapped two-way ANOVA to account for lack of normality and homogeneity of variances. *p*-values were determined by resampling with replacement on de-meaned data (null hypothesis simulation; Manly, 1997; *n* = 10,000).

The polygon data used to assess clumping was binned into 13 size categories of 'clusters' (from 2 mm$^2$ to 200 mm$^2$) based upon exploratory histograms. PERMANOVA, a permutation-based multivariate analysis of variance using distances matrices (*n* = 9999), tested for differences in clumping among laboratory treatments after confirming homogeneity of multivariate dispersions with PERMDISP (*p* = 0.1; Anderson & Walsh, 2013). We visualized these differences in clumping with non-metric multidimensional scaling (nMDS) performed on the Bray-Curtis distance matrix of the binned data. Skewness of the polygon distribution for each tile (unbinned; see Figure S2) was calculated as a univariate measure of the dominance of larger or smaller clumps.

### 2.5.2 | Models of juvenile survival and growth in the field

Survival, a binary outcome defined as the presence (1) or absence (0) of *E. amentacea*, was evaluated across sampling events and treatments using a generalized mixed-effects model (GLMM) with binomial error distribution and logit link. As height on shore was nearly a perfect predictor of survival (quasi-complete separation: 86.8% survival in the lower zone compared to 6.67% in the upper after 15 weeks), model parameters were estimated using a Bayesian framework with Markov Chain Monte Carlo (MCMC). The effects of experimental treatments on post-outplant growth (cover) were assessed over time using a GLMM with a beta distribution and logistic link, which is appropriate for continuous data bounded [0–1] (Douma & Weedon, 2019). Because survival was near zero in the upper zone, analysis of cover was performed on tiles in the lower zone treatment (*n* = 58).

For both models, fixed factors included culture light and temperature treatments and their interactions over time. Initial (week 0) percent cover, length, and clumping measures were modelled as covariates. Where appropriate, continuous predictors were
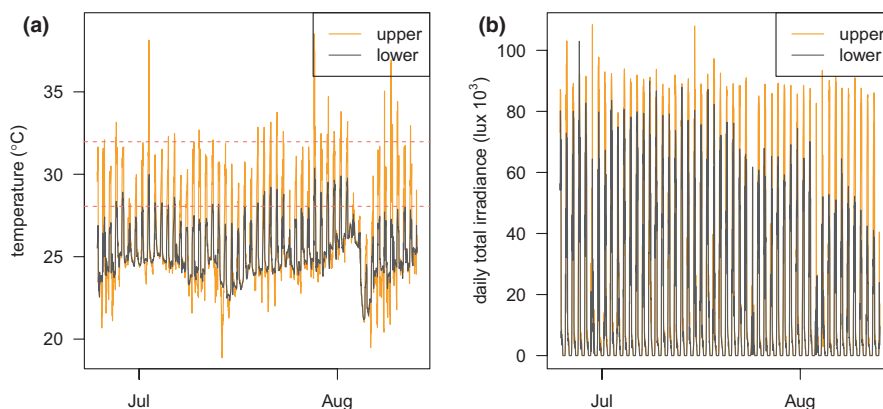


**FIGURE 2** Daily (a) temperature and (b) irradiance in the upper and lower tidal levels of the native range of *E. amentacea* that were chosen for outplanting. Data show a representative area in each zone during the summertime outplant period. Dotted lines in (a) depict the mean daily maximum temperature in the upper and lower levels of outplant.

demeaned. Candidate predictors of clumping included maximum polygon (clump) size, contribution of large clumps ($>30\,mm^2$) to total area, skewness of the clump size distribution and nMDS ordination scores (2.5.1.) across all dimensions. After checking for collinearity using the variance inflation factor (Zuur et al., 2010), the following clumping variables were retained (VIF <3): skewness, nMDS axis 1 (both models), and max clump size (survival only). Tile ID was included as a random factor to account for non-independence associated with repeated observations over time. Best fit models were selected using the Watanabe-Akaike or Akaike information criterion (survival and cover, respectively). Model assumptions were verified with diagnostic plots of scaled residuals (package DHARMa; Hartig, 2021). Shade treatment was not included in either GLMM due to missing replicates in several treatment-time combinations; effects within the lower zone at each time point were evaluated with ANOVA (details in Table S3).

To evaluate the relative contribution of group benefit versus culture treatments to post-outplant growth, we compared marginal coefficients of the full cover model versus a simplified model excluding clumping predictors. Marginal coefficients represent the proportion of model variance explained by fixed effects (Nakagawa & Schielzeth, 2013). This comparison gives an estimate of self-facilitation effects conferred by the aggregation of individuals.

## 2.5.3 | Further investigation of group benefit

Following model results indicating a significant role of group benefit for juvenile establishment, we investigated the development of *E. amentacea* after 4 months as a function of clumping at outplant. First, tiles were divided into 4-month cover classes independent of experimental treatments (0–5%; >5–14%; >14–40%; >40%; all $n \geq 8$, determined a posteriori by natural grouping). Within each 4-month cover class, differences in clumping were then evaluated by the relative abundance of five size classes of polygons at outplant (1: $0$–$10\,mm^2$, 2: $10$–$30\,mm^2$, 3: $30$–$50\,mm^2$, 4: $50$–$100\,mm^2$, 5: $>100\,mm^2$) using PERMANOVA. The contribution of each polygon size class was determined as a proportion of total initial cover (week 0, standardized to 100%), and was thus independent from differences in initial cover. Next, we performed regression analysis of juvenile survival (logistic) and cover (linear) at 4 months as a function of the skewness of the distribution of percent cover (i.e. polygon sizes) at outplant (all culture and field treatments combined), which provides a univariate representation of clumping.

All data processing and statistical analyses were performed in R (R 4.0.4; R Development Core Team, 2021). The GLMM of cover and Bayesian regression model of survival were fit using *glmmTMB* (Brooks et al., 2017) and *rstanarm* (Goodrich et al., 2019), respectively. Model goodness of fit (marginal and conditional $R^2$) were assessed using *performance* (Lüdecke et al., 2021) and *rstanarm*. Multivariate analyses were performed in *vegan* (Oksanen

et al., 2020), and skewness was calculated with *moments* (Komsta & Novomestky, 2015).

No ethical approval was required for this work.

## 3 | RESULTS

### 3.1 | Juvenile development in culture

After the 4-week culture period, juvenile cover and length were significantly higher on tiles exposed to variable light intensities (both $p \leq 0.001$; Table 1, Figure 3). Heat pulses also increased cover marginally but reduced length, and only in the variable light treatment (LvTv: 15.5% cover and 2.10 mm median length vs. LvTn: 12.7% and 2.23 mm compared with Ln: ~6% and 1.79 mm; $p_{interaction} = 0.06$ and 0.033, respectively).

Juvenile clumping was more pronounced on Lv than Ln tiles (Figure 4; ordination analysis, stress = 0.108), as seen by the correlation of larger polygons ($>12\,mm^2$) with more positive nMDS1 scores (larger *x*-axis values), which differentiated Lv and Ln treatments. nMDS axis scores were used as a composite measure of clumping in GLMMs. PERMANOVA of the binned polygon data verified significant effects of both light (pseudo-$F_{1,136} = 38.9$, $p = 0.001$) and temperature (pseudo-$F_{1,136} = 5.8$ and $p = 0.007$) treatments on clumping. After random assignment, patterns of clumping did not differ among field treatments (PERMANOVA height: $F = 2.0$, $p = 0.13$; shade: $F = 0.8$, $p = 0.42$; Figure S5).

### 3.2 | Effects of stress exposure in the field on juvenile survival and growth

Survival in the first month was dictated by the vertical height of outplanting within the species natural range (Table 2). Juveniles in the upper zone experienced rapid mortality (24% survival at 3 weeks) that continued progressively over time (Figure 5a); by 15 weeks, *E. amentacea* was found on only 6% of tiles in the upper zone ($n = 5$), and model predictions for mean chance of survival were <1% (0%–15% CI). In contrast, tiles placed at the lower level showed 98% initial survival with juveniles still present on 87% of tiles at 15 weeks (numbers omit lost tiles; 99% mean predicted chance of survival).

TABLE 1 Two-way ANOVA analysis of percent cover of *E. amentacea* after 4 weeks culture under different laboratory light and temperature treatments

| Treatment | df | SS | MS | F-value | p-value |
|---|---|---|---|---|---|
| light | 1 | 1945 | 1944.5 | 59.853 | <0.001 |
| temp | 1 | 124 | 124.1 | 3.819 | 0.053 |
| light:temp | 1 | 117 | 117.2 | 3.609 | 0.060 |
| residuals | 107 | 3476 | 32.5 | | |

**FIGURE 3** Differences in juvenile development after 4 weeks' culture under treatments of normal (stable) or variable light intensity (Ln and Lv, respectively) combined with constant temperature (Tn) or heat waves (Tv): (a) percent cover of tiles (mean ± SE); (b) length of individuals (mean ± SE). Numbers above bars are replicates.
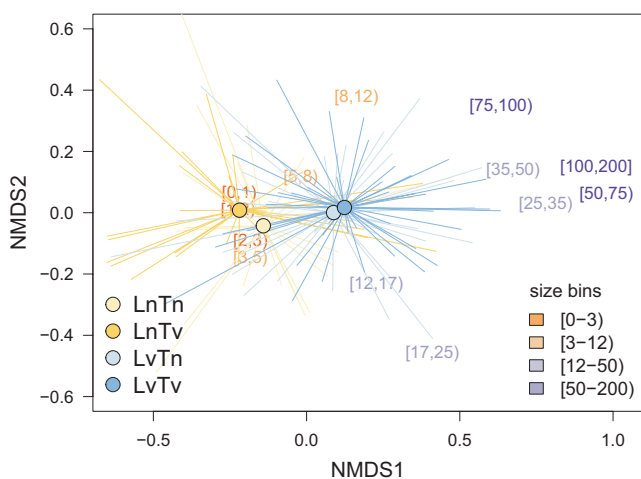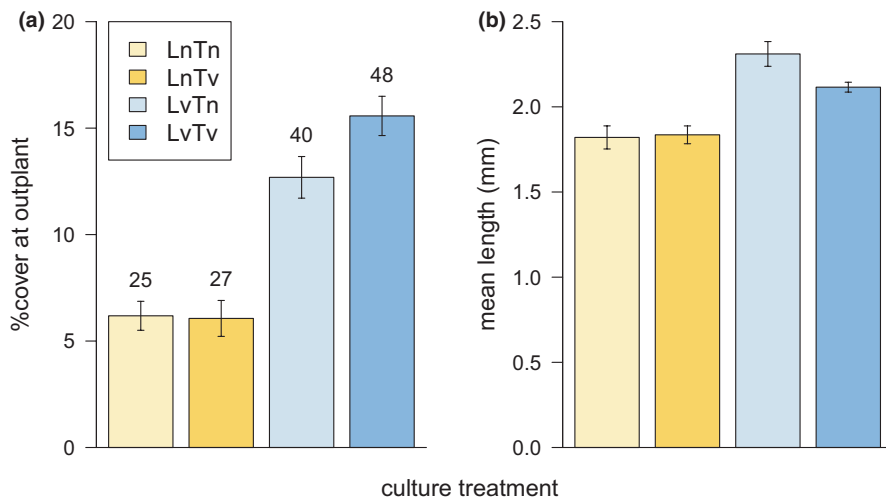


**FIGURE 4** Ordination analysis (nMDS) of the size distribution of juvenile *E. amentacea* aggregations (i.e. degree of clumping) on the tile substrate at the time of experimental outplant. Colours represent culture treatments following Figure 1. Ranges of polygon sizes from image analysis ($n = 12$ bins) are grouped and coloured by size (mm$^2$, $n = 4$).

**TABLE 2** Results of a generalized linear mixed model on survival over time. Means and 95% credible intervals of the model coefficients were determined by simulation with Markov chain Monte Carlo methods. Bold indicates means whose CIs do not include zero. Coefficients are based on a reference of normal light (Ln), normal temperature (Tn), and lower height on shore

| Predictor | Mean | SD | Lower CI | Upper CI |
|---|---|---|---|---|
| intercept | **11.95** | 2.63 | 7.85 | 16.5 |
| light | 0.71 | 2.3 | −2.93 | 4.55 |
| temperature | −4.1 | 2.43 | −8.13 | −0.22 |
| time | **−0.85** | 0.3 | −1.37 | −0.37 |
| height | **−17.04** | 2.46 | −21.2 | −13.23 |
| clumping (nMDS1) | **6.87** | 3.38 | 1.46 | 12.62 |
| light: temperature | **5.75** | 2.88 | 1.02 | 10.36 |

Percent cover also diverged rapidly between zones after outplant (Figure 5b). After 3 weeks, <1% mean cover remained on upper range tiles (including those with 100% mortality); mean cover ranged 1%–6% over the monitoring period on tiles with surviving juveniles. In contrast, in the lower part of its range, *E. amentacea* recovered quickly (<6 weeks) from small initial losses after outplant. By 12 weeks, its cover had doubled from initial losses and by 15 weeks it was threefold (Figure 5b).

Shade, in contrast, had no strong overall effects on cover. Initially, shade increased juvenile cover in LvTv tiles while having negative or minimal impact in all other treatments (shade by temperature interaction, Table S3; Figure S6). These effects, however, disappeared after 8 weeks.

## 3.3 | Effects of stress exposure in culture on post-outplant survival and growth

Culture conditions also altered outplanting success; after 15 weeks, survival across both tidal zones was higher in tiles exposed to variable light in culture (LvTv: 49%; LvTn: 38%) compared to those in constant light treatments (LnTv 8.7%; LnTn: 27%), while heat pulses had lesser, opposing effects depending on the light treatment (+ in Lv, − in Ln; interaction effect: Table 2; Figure S7). These patterns were largely due to differential survival in the upper zone, where tiles from variable light treatments had 1.5 (LvTv) and nearly 2 (LvTn) times higher survival than the control (LnTn, 6%; LnTv, 0% survival after 15 weeks). In the lower zone, survival at 15 weeks was nearly 100% in all treatments except LnTv (40%).

Within the lower zone, cover diverged rapidly among culture treatments (Figure 6; Table 3). LvTv juveniles performed best, with higher cover than the other treatments at each sampling time and minimal initial losses post-outplant. By 15 weeks, LvTv tiles reached 60% cover while all other treatments were <25%. While changes in cover after outplant may have been influenced by initial differences in post-culture cover (Figure 3: ~14% in Lv treatments vs. ~6% in Ln treatments), these patterns diverged over time due to
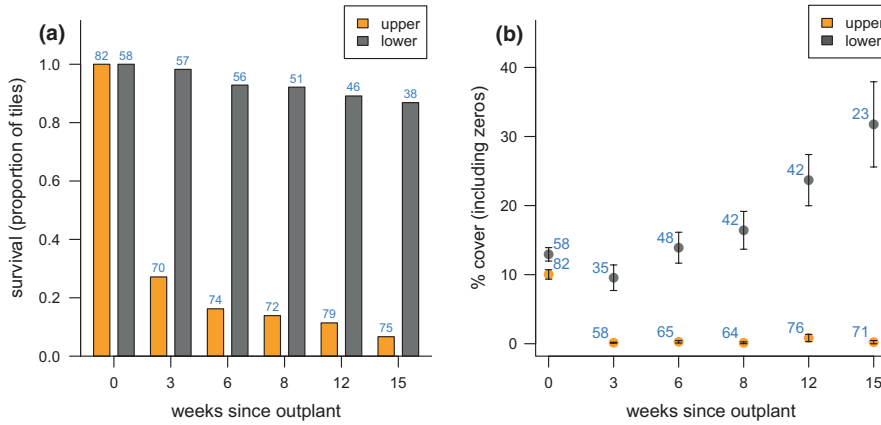
**FIGURE 5** Effects of tidal zone of outplant (upper or lower range of *E. amentacea*) on juvenile (a) survival and (b) percent cover (means ± SE) over time. Week 0 is the initial condition post-culture. Survival is categorical and defined as the presence of at least one individual on the tile. Numbers in blue indicate the number of tiles (a) surveyed or (b) measured for percent cover, where differences between each time point and week 0 in (a) represent tiles not able to be surveyed or lost from the rock.
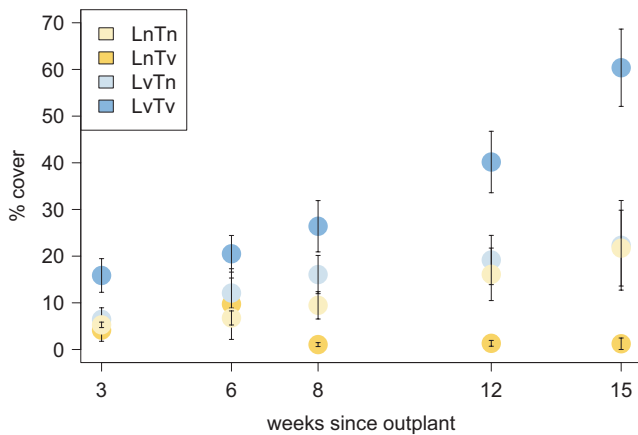


**FIGURE 6** Trends of juvenile percent cover (means ± SE) by culture treatment over time after outplant in the lower vertical zone. Colours and treatment abbreviations follow Figure 1.

**TABLE 3** Results of a generalized linear mixed model on percent cover over time in the low zone (GLMM with beta distribution and a logistic link function). Coefficients are based on the control condition (Ln, Tn). Length is the height of juveniles post-culture (*week 0*)

| Predictor | Estimate | SE | z-value | p-value |
|---|---|---|---|---|
| (Intercept) | −3.85 | 0.49 | −7.93 | **<<0.001** |
| light | 1.25 | 0.63 | 1.96 | **0.049** |
| temp | 0.85 | 0.74 | 1.15 | 0.250 |
| time | 0.46 | 0.08 | 5.42 | **<<0.000** |
| length | −1.13 | 0.46 | −2.43 | **0.014** |
| clumping (nMDS1) | 2.60 | 0.52 | 5.05 | **<<0.000** |
| light:temp | −1.03 | 0.85 | −1.21 | 0.227 |
| light:time | −0.15 | 0.10 | −1.47 | 0.142 |
| temp:time | −0.40 | 0.17 | −2.32 | **0.021** |
| light:temp:time | 0.59 | 0.19 | 3.07 | **0.002** |

differential effects of temperature within the two light treatments (3-way interaction of light, temperature and time, $p = 0.002$). On LvTn tiles, large loss of cover (50%) in the first 3 weeks and slow recovery resulted in convergence with the more rapidly growing LnTn juveniles, despite the initial gap. The LnTv treatment showed the least growth, with cover well under 10% and low survival. Independently of culture treatment, juvenile length at the time of outplant negatively affected subsequent growth (negative model estimate, $p = 0.01$).

## 3.4 | Effects of juvenile clumping on post-outplant survival and growth

Within treatment effects, the degree of juvenile clumping at the time of outplant was also a significant predictor of future success. In both models, positive coefficients for nMDS1 (Tables 2 and 3) indicate that greater juvenile aggregation (more positive nMDS1 values, Figure 4) strongly increase predicted chance of survival and growth. Further, marginal $R^2$ (contribution of fixed effects to model variance) was substantially lower in the simplified model of cover excluding the clumping (nMDS1) predictor (Table 4). The model with culture treatments alone accounted for 35.2% of model variance compared to 57.5% in the full model, indicating 22% of predictive capability is attributed to juvenile clumping.

Among classes of cover at 4 months, the contribution of different polygon sizes to initial cover varied significantly (PERMANOVA, pseudo-$F_{3,39} = 5.46$; $p = 0.001$; Figure 7a). Subsequent univariate analysis revealed that large (30–50 mm$^2$) aggregations were more abundant and made up a greater relative proportion of initial cover on tiles that attained high cover (>40%) by 4 months (Tukeys HSD following significant ANOVA, $p < 0.002$ for comparisons with all classes <40%). This indicates that these larger clumps were a significant predictor for subsequent growth, independent of cover at outplant.

**TABLE 4** Comparison of marginal $R^2$ (i.e. proportion of the model explained by the fixed effects) for models of percent cover with and without clumping (nMDS1) as a fixed factor

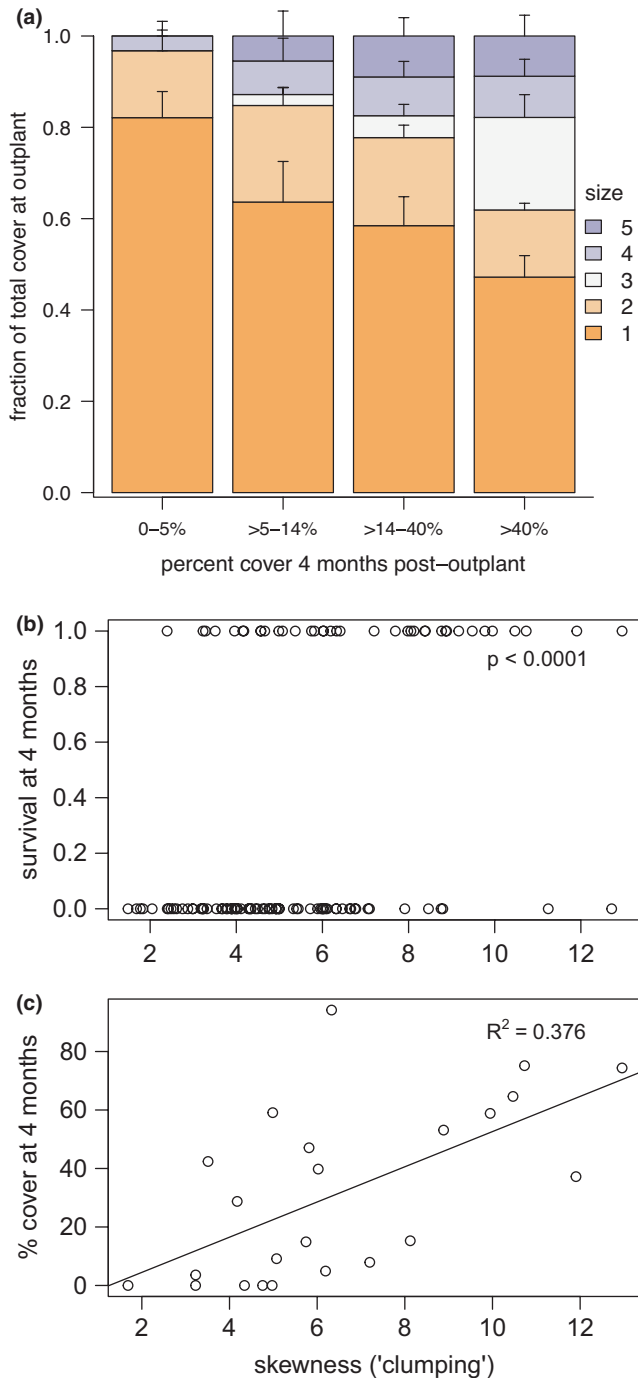| Response | Predictors Fixed | Random | $R^2$ conditional | $R^2$ marginal |
|---|---|---|---|---|
| **cover** (*beta GLMM*) | Culture (light, temp), length, time, clumping | Tile ID | 0.965 | **0.575** |
| | Culture (light, temp), length, time | Tile ID | 0.963 | **0.352** |



**FIGURE 7** (a) Percent cover of *E. amentacea* after 4 months as a function of juvenile clumping at the time of outplanting (week 0). Clumping is evaluated as the mean contribution of five size classes of continuous cover (i.e. polygon size, 1: 0–10 mm$^2$, 2: 10–30 mm$^2$, 3: 30–50 mm$^2$, 4: 50–100 mm$^2$, 5: >100 mm$^2$) to total juvenile cover after culture (*y*-axis; standardized to 100%), and is shown for each of 4 cover classes at 4 months (*x*-axis, classes determined *a posterior* from % cover measured at 4 months post outplant; $n = 8$–11 in each class). Error bars represent SE. (b) Survival and (c) percent cover of *E. amentacea* after 4 months in the field as a function of juvenile clumping at time of outplant (all culture and field treatments combined). Clumping is estimated by the skewness of the distribution of juvenile percent cover (as polygon sizes, see Figure S4) across the tile substrate. Larger values of skewness indicate the presence of aggregated rather than dispersed individuals. (b) all data; (c) lower zone data.

Regression analysis of survival and cover at 4 months by the skewness of the spatial distribution of juveniles at outplant further revealed a strong relationship of clumping with juvenile establishment (Figure 7b,c). Larger values of skewness, which indicate the presence of aggregated rather than dispersed individuals, were associated with both increased survival ($p < 0.001$) and percent cover ($R^2 = 0.376$; $p = 0.002$).

## 4 | DISCUSSION

Understanding the effects of relevant environmental conditions on early life stages is crucial for successful implementation of ex situ restoration measures. We examined how manipulation of environmental stress during laboratory culture and post-outplant (4 months) phases affects restoration of the canopy-forming macroalga, *Ericaria amentacea*.

### 4.1 | Environmental stress drives post-outplant survival, indicating an important role of self-facilitation

We found that juvenile *E. amentacea* were nearly unable to survive in the upper part of the species natural vertical range, regardless of culture conditions, and in the few tiles that did survive, growth was limited and cover never recovered after initial losses. In contrast, juveniles outplanted in the lower range maintained 90% survival through 15 weeks. We suggest that this near-complete mortality in the upper zone may be due to the absence of obligatory facilitatory effects of adults on recruits. Adult canopies create a microhabitat (Keough & Quinn, 1998) that mitigates abiotic extremes and their effects (e.g. heat shock from high irradiance or temperature; Dayton, 1975, Cervin

et al., 2005), potentially extending species' ranges by allowing recruits and early life stages to colonize locations that may otherwise be inhospitable (Bertness, Leonard, Levine, Schmidt, & Ingraham, 1999; Bulleri, 2009; Bulleri et al., 2018). Intraspecific facilitation has been demonstrated in many physically stressful coastal habitats (mussel beds: Bertness & Leonard, 1997; marshes and mangroves: reviewed by Renzi et al., 2019), including intertidal fucoids (Bellgrove et al., 2004, 2017; Bertness & Leonard, 1997; Bertness, Leonard, Levine, Schmidt, & Ingraham, 1999; Brawley & Johnson, 1991; Davison et al., 1993). Without an existing population, we could not perform concurrent outplanting under adult canopy to confirm the role of self-facilitation by adults on recruits. Yet, elsewhere this upper zone hosts thriving populations of *E. amentacea*, suggesting that early life stages survive elevated environmental stress in upper parts of its native niche (see Figure 2) due to positive effects conferred by adults.

These positive feedbacks have been identified as one of the main challenges to marine conservation and restoration (Brooker et al., 2008; Maxwell et al., 2016; Suykerbuyk et al., 2016), particularly for species that modify their physical environment (e.g. Schotanus, Walles, et al., 2020; van Katwijk et al., 2009). As restoration actions are generally implemented in areas devoid of healthy adult populations, other strategies must be used to ensure survival in the critical first days (Suykerbuyk et al., 2016; van Katwijk et al., 2009). Use of artificial structures to reduce effects of high or fluctuating temperatures and UV stress in exposed habitats has shown some success (e.g. mussels on mudflats: Schotanus, Capelle, et al., 2020). While shading structures have been found to improve survival in other exposed habitats (e.g. fucoids: Bertness & Leonard, 1997, Flores et al., 2015; barnacles: Bertness, Leonard, Levine, & Bruno, 1999), our results indicate that restoration in more exposed parts of the native niche may depend on colonization from populations established in areas of lower physical stress.

## 4.2 | Clumping confers facilitative effects that improve post-outplant outcomes

Moving past the historical restoration paradigm to reduce physical exposure in the target habitat, recent studies have provided clear evidence that promoting positive interactions through clumped outplant designs improves restoration potential in high-stress environments (e.g. intertidal systems; Silliman et al., 2015; Duggan-Edwards et al., 2019; reviewed by Renzi et al., 2019). In our study, tiles with more clumped spatial distributions of individuals at outplant performed better, with greater survival and growth over time (4 months), independent of experimental treatments or total initial cover. Theoretical work indicates that facilitative feedbacks increase in importance with stress (Bruno & Bertness, 2001), where negative density dependence dominates when physical stress is low but a critical density is essential for survival in high-stress habitats (e.g. Bennett et al., 2015). Clumping of juveniles within a tile may have buffered or provided resilience to environmental fluctuations, a microscale form of self-facilitation. Such positive density dependence may be specific to certain growth forms, such as caespitose or similar habits (e.g. *E. amentacea*, bunch

grasses) where lateral extension at the base may confer benefits such as reduced substrate overheating or reinforced attachment. While our results are correlative, the strength of the relationship between clumping and success even across grouped culture and field treatments indicates a critical role that requires further experimental investigation.

## 4.3 | Stress exposure in culture enhances resilience and post-outplant growth

Beyond initial survival, successful establishment was strongly differentiated by culture conditions, where juveniles exposed to mild, recurrent stress during the cultivation phase (variable light + heat pulses) were significantly more resilient and productive over the following months. One possible underlying mechanism may be an increased capacity for acclimation in the short term, where fluctuating environmental stress has been shown to invoke a photo-acclimation mechanism in other intertidal fucoids both in situ and in the lab (Celis-Plá, Korbee, et al., 2014; Celis-Plá, Martínez, et al., 2014; Mancuso et al., 2019). In intertidal habitats, light and temperature are highly variable on both short (hour to day) and long (seasonal) timescales. Exposure to changing irradiance may induce more rapid recovery from photoinhibition and reduce its overall extent (Bischof et al., 1999, 2006; King et al., 2018), as regulation of gene expression allows the development of more rapid photo-protective responses or activation of antioxidant production (Feder & Hofmann, 1999; Henkel & Hofmann, 2008 and references therein). Temporary exposures to elevated temperatures have also been shown to increase thermal tolerance of both macroalgae and corals and their algal symbionts by upregulation of genes such as heat shock proteins (Eggert, 2012; Barshis et al., 2013, King et al., 2018) and reduced loss of photosystem II efficiency under stress (Duarte et al., 2018; Oliver & Palumbi, 2011). As early life stages are generally more vulnerable to environmental stress (e.g. temperature, desiccation: Davison et al., 1993, Duarte et al., 2018, Capdevila et al., 2019) than more plastic adults (Falace et al., 2021), an enhanced stress response mechanism may play an important role in recruit resilience and survival in the critical days post-outplant in hot summer months.

Another plausible scenario may be that juveniles in fluctuating culture conditions have undergone selection for more resilient genotypes that are better adapted to resist environmental extremes experienced in the field (Humanes et al., 2021). Laboratory culture under elevated temperature may enhance epigenetic variation in thermal sensitivity of early life stages (intertidal fucoid macroalga: Clark et al., 2013), increasing the potential for thermal plasticity in response to future high temperature events (Duarte et al., 2018). Such selection could prove highly beneficial for restoration efforts (Bulleri et al., 2018; Humanes et al., 2021), reducing costs by increasing the probability that individuals transplanted to the field are fit for the native environment.

Finally, the enhanced success of LvTv tiles may also relate to the higher average cover at outplant (Figure 3). Yet, differences in post-culture cover were not a reliable predictor of growth trajectories across treatments, where LnTn treatments performed better

than LvTn despite tiles having 50% less initial cover. Although bio-chemical and molecular work is needed to uncover the underlying mechanisms, the improved performance of juveniles exposed to concurrent light variability and heat waves in culture indicates that culture methods can be used to increase resilience of early life stages to the environment they repopulate.

## 4.4 | Implications for restoration and conclusions

Overall, our results suggest that successful repopulation and resto-ration of foundation species in high-stress habitats may depend on multi-faceted strategies that promote group benefits and enhance resilience through both lab culture and outplant phases.

First, we found that the absence of adult canopy inhibits recruit re-establishment in the upper tidal zone that undisturbed native stands occupy. This implies that self-facilitation expands the real-ized niche of *E. amentacea* into zones of physical exposure beyond its fundamental niche and that restoration can only be achieved by targeting zones of lower stress for initial repopulation or by effec-tively reducing stress in the target habitat.

Second, the evidence that clustering of *E. amentacea* propagules on the outplant substrate provides self-facilitative benefits for re-population supports calls to integrate positive feedbacks into resto-ration strategies for foundation species (Silliman et al., 2015; Valdez et al., 2020) and implies that consideration of multiple scales may be necessary (adult-recruit, recruit-recruit).

Finally, evidence of enhanced resilience through mild, variable stress exposure in culture highlights the potential for methods of selective breeding to increase adaptation to the target environment (sensu "directional selection" Coleman & Wernberg, 2020).

This study, which employs simple, low-cost methods based on life-stage specific knowledge of the target species ecology and physiology without genetic manipulation (Van Oppen et al., 2015), may provide in-sight for future research and restoration strategies of other foundation species in high-stress habitats (Bulleri et al., 2018; Renzi et al., 2019).

### AUTHOR CONTRIBUTIONS
Rachel Clausing, Mariachiara Chiantore, Gina De La Fuente and Annalisa Falace conceived and designed the research; Gina De La Fuente, Rachel Clausing and Mariachiara Chiantore performed labo-ratory culture; Gina De La Fuente, Rachel Clausing and Mariachiara Chiantore collected the data; Rachel Clausing and Gina De La Fuente analysed the data; Rachel Clausing led manuscript preparation. All authors contributed critically to the drafts and gave final approval for publication.

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