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Feasibility of *ex situ* cultivation and outplanting of the habitat-forming seaweed *Ericaria selaginoides* in an oceanic island: insight for future restoration

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Introduction: Marine forests formed by *Cystoseira* species are undergoing population declines across the Macaronesian archipelagos, threatening the essential ecosystem services they provide. Active restoration strategies, particularly *ex situ* cultivation and outplanting of early stages, are emerging as key tools to recover these habitats. We assessed the feasibility of restoration for the canopy-forming brown alga *Ericaria selaginoides* in the Canary Islands, combining laboratory-based optimization of seedling cultivation with a field outplanting experiment.

Methods: To optimize early development, we conducted a factorial experiment testing the influence of two light intensities (125 and 250 μmol photons $m^{-2}s^{-1}$) and three culture media: filtered seawater (SW), Von Stosch (VS) solution, and a commercial Macrocystis derived bio-stimulant (Algatron Cifo® AL). Then juveniles were outplanted at two tidal heights (i.e., upper and lower intertidal) and under different microhabitat conditions (i.e., inside and outside tidal pools).

Results: Embryos cultured with VS and SW had advanced embryonic development, while those grown under low light and VS medium reached significantly larger sizes at advanced stages, indicating enhanced growth potential. This combination of low light and VS medium was therefore selected to optimize juvenile cultivation. Field monitoring revealed strong environmental filtering. Survival and growth were highest in lower intertidal pools, while individuals in the upper, more exposed, zones experienced high mortality.

Discussion: These findings demonstrate that *ex situ* cultivation of *E. selaginoides*, combined with strategic microhabitat selection, could enhance restoration success. These outcomes provide a scalable and practical solution for the long-term implementation of restoration techniques, offering initial insight into the recovery potential of *Cystoseira* forests in oceanic insular regions that suffered widespread declines.

KEYWORDS

Brown macroalgae, Canary Islands, environmental stress, *ex situ* cultivation, macaronesia, restoration, marine forests

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1 Introduction

Cystoseira sensu lato species, including the genera *Cystoseira* C. Agardh, *Gongolaria* Boehmer and *Ericaria* Stackhouse (Molinari-Novoa and Guiry, 2020), are the major marine forest-forming algae from the Mediterranean and Macaronesian oceanic archipelagos (northeastern Atlantic) (Bernal-Ibáñez et al., 2022; Neiva et al., 2022; Valdazo et al., 2017). These marine forests form dense canopies and enhance the complexity of rocky coastal ecosystems, providing habitat, food and shelter for many other associated species (Cheminée et al., 2013; Galobart et al., 2023; Smith et al., 2023). These forests underpin important ecosystems functions and services (e.g., carbon sequestration, nutrient cycling) (Eger et al., 2023; Moreda et al., 2024; Smith et al., 2023). In addition, *Cystoseira s.l.* provide nursery services for fish stocks, which in turn support commercial and recreational fisheries, thereby delivering both economic and cultural values (Cheminée et al., 2013; Smith et al., 2023). *Cystoseira s.l.* are indicators of high-quality coastal waters (Ballesteros et al., 2007; Bermejo et al., 2013; Blanfuné et al., 2017), according to the criteria of the Water Framework Directive of the European Union (WFD, 2000/60/EC). Moreover, healthy *Cystoseira s.l.* communities are crucial biotic elements for the assessment of "Reefs" (Habitat Type 1,170), which need to be monitored, protected and restored under the EU Habitats Directive 92/43/EEC.

In the last decades, the decline of *Cystoseira s.l.* marine forests from the Macaronesian archipelagos has been documented (Friedlander et al., 2017; Valdazo et al., 2017; Bernal-Ibáñez et al., 2021a; Bernal-Ibáñez et al., 2021b; Martín-García et al., 2022) and has been attributed to several processes, such herbivory by sea urchins, human development and associated local impacts, extreme wave events and marine heat waves (Bernal-Ibáñez et al., 2021a; Bernal-Ibáñez et al., 2021b; Bernal-Ibáñez et al., 2022; Martín-García et al., 2022; Valdazo et al., 2024). However, in other regions, the causes of declines of fucoids are often unclear or multifactorial (Whitaker et al., 2022). As a result, vast underwater marine forests of *Cystoseira s.l.* have gone missing from many coastal areas, being replaced by simpler and less sensitive species, often algal turfs (Mourin et al., 2025). In the 2000s, evidence of extensive range reductions and local *Ericaria selaginoides* loss was recorded in the Canary Islands (Rodríguez et al., 2008). For this reason, this alga is included in the List of Wild Species under Special Protection Regime and the Spanish National Catalogue of Endangered Species (Real Decreto 139/2011, 4 February 2011) and in the regional catalogue of endangered species (Canary Islands Catalogue of Protected Species; Law 4/2010, 4 June 2010), as "vulnerable".

Despite a few populations of *Cystoseira s.l.* exhibiting natural recovery after declines (e.g., Iveša et al., 2016), the natural re-establishment of *Cystoseira* s.l. forests is extremely rare (Smith et al., 2023), hampered by low zygote and embryo dispersal (Clayton, 1990; Perkol-Finkel and Airoldi, 2010; Buonomo et al., 2017). Consequently, active restoration methodologies have become one of the few feasible alternatives to promote the re-establishment of lost *Cystoseira* s.l. forests, following mitigation of the factors responsible for the decline. Restoration is, in turn, increasingly recognized as a useful tool to trigger the overall recovery of degraded marine coastal ecosystems (Abelson et al., 2020, Bayraktarov et al., 2020; Daurte et al., 2020; Eger et al., 2024), as stated by the UN Decade of Ecosystems Restoration (2021-2030; https://www.decadeonrestoration.org/). As a result, the need for restoration of coastal habitats underpinned by macrophytes, such as *Cystoseira* s.l., has been recognized at both the European (Biodiversity Strategy for 2030: EC, 2020; Restoration Law: EC, 2024) and global level (UN Decade of Ecosystems Restoration: UN, 2019).

Along the world, various active restoration methods to reverse the loss of fucoid have been employed with varying degrees of success (Cebrian et al., 2021; Whitaker et al., 2022; Thomsen et al., 2024). These methods include transplanting individuals from the wild, cultivating outplants, constructing artificial habitats or substrates, and seeding with fertile branches or propagules (Campbell et al., 2014; Kautsky et al., 2019; De La Fuente et al., 2019). Several techniques have been advanced for the restoration of *Cystoseira s.l.*, forests in the Mediterranean and Macaronesian, including: transplantation of adult thalli (Falace et al., 2006; Susini et al., 2007; Sales et al., 2011); and *in situ* and *ex situ* recruitment enhancement (Falace et al., 2018; Verdura et al., 2018; Bernal-Ibáñez et al., 2024), both techniques considered non-destructive since they only rely on harvesting a small proportion of reproductive fertile branches of wild donor endangered population (De La Fuente et al., 2019). *In situ* techniques consist in deployment of receptacles at the restoration site to simulate recruitment processes (Verdura et al., 2018; Medrano et al., 2020). *Ex situ* techniques consist in cultivation of juveniles in mesocosms to maximize their recruitment and survival, by setting the optimal culture conditions of temperature, light and nutrients and then outplanting at the restoration site (Falace et al., 2018; Verdura et al., 2018; De La Fuente et al., 2019; Orlando-Bonaca et al., 2021; Savonitto et al., 2021; Lardi et al., 2022; Orlando-Bonaca et al., 2022). In the context of *ex situ* cultivation and restoration efforts, commercial algal biostimulants have been recently utilized to promote fertility and optimize seedlings cultivation (Kaleb et al., 2023; Malfatti et al., 2023).

Restoration knowledge on *Cystoseira s.l.* is now robust but restricted to the Mediterranean Sea (Smith et al., 2023), where oceanographic conditions are different, for example, there is a relatively very small tidal range (<30 cm), relative to the oceanics island that encompass the Macaronesian archipelagos (Tuya et al., 2021), where the tidal range extends to 3 m (Ramírez et al., 2008). Typically, oceanic archipelagos include islands with varying geological and geomorphological histories, which may affect the type and availability of nearshore habitats and, consequently, the abundance and biodiversity of nearshore assemblages, including macroalgae (Tuya and Haroun, 2009; Tuya et al., 2021). Furthermore, the coastal regions of these islands are subjected to varying hydrographic conditions, which predominantly result in algal assemblages characteristic of either swell-protected or swell-exposed areas (Tuya and Haroun, 2006). Distances and depths among adjacent islands, which affect their isolation, can affect successive colonization events by marine biota, particularly for species of limited pelagic dispersal, such as fucoids (Mangialajo et al., 2012; Buonomo et al., 2017; Hachich et al., 2020). In summary, oceanic and isolated islands under large swells and wide tidal ranges represent a challenge in the restoration of *Cystoseira s.l.*, so far developed in the Mediterranean Sea (Smith et al., 2023).

Intertidal rocky zones under large tidal regimes are subjected to considerable environmental stress, experiencing periodic times of emersion during which communities suffer great physiological stress (Harvey and Helmut, 2003). These harsh conditions, when combined with high wave exposure, complicate restoration efforts, particularly for *ex situ* methods employing vulnerable early life stages, which suffer high post-transplant mortality (Schiel and Foster, 2006). Recent studies have concluded that early stages of macroalgae are more susceptible to environmental stress than adults (Verdura et al., 2021; De Caralt., 2023). Therefore, decreasing the inhibitory effect of physical exposure may be necessary to improve the restoration potential (Clausing et al., 2022; De Caralt et al., 2023). Several strategies have been employed to increase the survival of recruits: aggregated outplant designs to promote inherent self-facilitative feedbacks (Clausing et al., 2022; 2024; Renzi et al., 2019; Silliman et al., 2015), or reduction of stress using favourable microhabitats (Clausing et al., 2022; Harvey and Helmut, 2003).

Within this framework, the aims of this study were: (i) to investigate the effects of different culture media on the survival and growth of *Ericaria selaginoides* seedlings; then, we outplanted juveniles in the natural environment (ii) to assess growth and development under varying environmental conditions that may alter stress levels. In brief, the overall objective of this research was to test the feasibility of *Ericaria selaginoides ex situ* cultivation and restoration in Canarian coastal waters, by evaluating the survival and growth of young thalli during the most critical first 3 months post-outplantation, with the intent to optimize sustainable restoration actions in the future.

2 Materials and methods

2.1 Study species and study site

2.1.1 Study species

Ericaria selaginoides (Linnaeus) Molinari & Guiry (formerly known as *Cystoseira/Carpodesmia tamariscifolia*) is a monopodial and perennial species growing in the littoral and sublittoral zones, under moderate and high wave exposure (Roberts, 1970). *E. selaginoides* is distributed throughout the Atlantic, from the British Isles to Mauritania (including the Macaronesia Islands), and in some Mediterranean areas under Atlantic influence, such as southern Spain, Sicily, Morocco, Algeria and Tunisia (Roberts, 1970; Gómez-Garreta, 2000). *E. selaginoides* is one of the most common canopy-forming algal species along the central and eastern islands of the Canary archipelago (Wildpret et al., 1987; Rodríguez et al., 2008). *E. selaginoides* is a monoecious species in which the apical branches of mature receptacles contain conceptacles with both oogonia and antheridia (Roberts, 1970). Reproduction is oogamic (i.e., large non-motile eggs and biflagellate sperm), and fertilization is external (Guern, 1962; Gómez-Garreta, 2000). After fertilization, large and free-living zygotes (~70–100 μm) rapidly sink to the bottom, where they are attached to hard substrate during the first 12–24 h (Verdura et al., 2021). This gives the species a low dispersal ability (<20 cm; Mangialajo et al., 2012). Although this is a perennial species, receptacles are most developed in spring and summer (Gómez-Garreta, 2000).

2.1.2 Study site

On Gran Canaria Island (Canary Islands, eastern Atlantic Ocean, Figure 1A), *E. selaginoides* populations are distributed along the northern coast, which is exposed to substantial swells (Supplementary Figure S1). Historically, populations were found in relatively abundant patches in the western region, which became rare and scattered to the east due to their proximity to areas of intense urban development (Rodríguez et al., 2008; Valdazo et al., 2017; Supplementary Figure S1). Populations of *E. selaginoides* have undergone a marked reduction in both distribution and population size and now predominantly exist as rare and scattered patches. This phenomenon is currently being investigated in an ongoing study (unpublished data), that aims to elucidate the causes of this decline, as has already been documented in Macaronesia (Bernal-Ibáñez et al., 2021a; Martín-García et al., 2022; Valdazo et al., 2024). The environmental parameters, sea and air temperature as well as irradiance, derived from satellite climate data (Supplementary Table S1), exhibit homogeneity across the entire distribution range of *E. selaginodes* (Supplementary Figures S2-S5).

We used the same area as donor and receptor for our restoration pilot experiments, which is located along the northwestern coast of Gran Canaria Island (Figure 1A). The study site at Punta de Gáldar is located within the marine SAC “Costa Sardina del Norte”, where local impacts such as wastewater discharges, eutrophication, pollution and habitat destruction are controlled. This area is dominated by abrupt basaltic cliffs, under significant annual tidal variations (ca. between 1 and 3 m; Ramírez et al., 2008) and wave exposure; sea surface temperature ranges between 18 °C in March and 24 °C in October (Tuya and Haroun, 2006; Valdazo et al., 2017). Fertile material was sourced from Punta de Gáldar (28°10′12.18″N, 15°41′25.46″W; Figures 1A,B), selected because of the observed well-conserved *Cystoseira s.l.* abundant patches in the present study. Historically, this site supported dense populations of *Cystoseira* forests, with species such as *E. selaginoides* coexisting with *Gongolaria abies-marina* (Wildpret et al., 1987; Rodríguez et al., 2008; Valdazo et al., 2017).

2.2 Optimization of *ex situ* cultivation

In April 2024, during the reproductive period of *E. selaginoides*, 300 healthy apical fronds, approximately 3 cm in length and containing mature receptacles (Figure 1E), were collected from the intertidal zone at Punta de Gáldar (Figure 1B), with fronds transported to the laboratory under dark and cold conditions. At the facilities (Figure 1D), receptacles were cleaned of epiphytes under a stereoscopic microscope (OPTIKA, SZP-10, Ponteranica, Italy) using a brush and rinsed with filtered seawater to remove biofouling and detritus. Cleaned receptacles were then stored at 4 °C for 24 h to promote gamete release, as similarly applied in other fucoids (Falace et al., 2018; Verdura et al., 2018).

To replicate seasonal conditions optimal for the reproductive phase of *E. selaginoides* (i.e., late spring to summer), the experimental setup was designed with a 15:9 h light:dark photoperiod at a constant temperature of 20 °C using a temperature-controlled water bath. Two light intensities were applied to simulate natural scenarios: high intensity (L+, 250 μmol photons $m^{-2}s^{-1}$) and low intensity (L-, 125 μmol

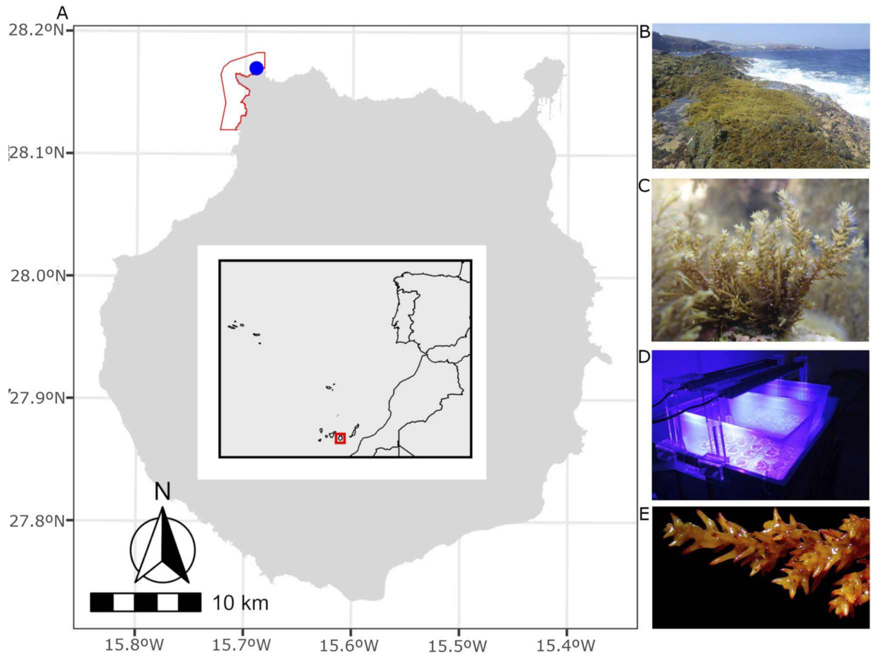

FIGURE 1
Location of the Canary archipelago in the north-eastern Atlantic, including Gran Canaria, with the blue circle denoting location of Punta de Gáldar inside of the Marine SAC "Sardina del Norte", denoted by red line **(A)**, the donor and receiving site of the *Cystoseira* forests, where populations of *E. selaginoides* coexist with *Gongolaria abies-marina* **(B)**, field close-up of *E. selaginoides* **(C)**, laboratory facilities where *ex situ* cultivation of embryos took place **(D)**, and receptacles of *E. selaginoides* **(E)**.

photons m^{-2}s^{-1}) (Falace et al., 2018). Light was provided by LED lamps (Orphek OR4 iCon Sun & Moon Sync), and irradiance was measured using a LI-COR LI-190/R Photometer (LICOR-Biosciences, Lincoln, NE, USAN). A factorial experimental design then combined two light intensity levels (L+ and L-) with three culture media. The control medium consisted of UV- sterilized seawater filtered through 10 and 1 μm membranes (SW). Two enriched media were tested, SW enriched with Von Stosch solution (VS) (Von Stosch, 1963), and SW enriched with Algatron Cifo® (AL) [Cifo S. p.A., San Giorgio de Piano, Bologna, Italy] (Malfatti et al., 2023; Kaleb et al., 2023) at the concentration recommended by the manufacturer (4.5 mL L^{-1}). This resulted in six treatments: L + AL; L + SW; L + VS; L-AL; L-SW; and L-VS. Aquaria (1 L) were filled with 200 mL of the respective medium. The media level was checked daily to ensure it remained constant, and every 4 days it was adjusted by removing 100 mL and adding 100 mL of fresh culture media.

Receptacles were placed on three glass slides (3-4 receptacles per slide) in three replicated aquaria for each treatment (18 aquaria in total). Exposure to temperature shock followed by immersion in the three media rapidly triggered gamete release. In addition to the optimization of *ex situ* cultivation, six aquaria filled with UV-sterilized seawater (filtered through 10 and 1 μm) were used to analyse morphogenesis and were monitored daily during the first 10 days of culture. After 24 h, zygotes were released and receptacles removed, and their dry weight (DW) recorded; this point was considered as the fertilization time (Day 0). Zygote development was monitored using one slide per aquaria at three times: Day 3 (3 days after fertilization), Day 6 (6 days after fertilization), and Day 10 (10 days after fertilization), at which photographs were taken. Zygote development and embryology were observed and photographed using a Leica DM1000 LED microscope (Leica, Wetzlar, Germany) equipped with an incorporated camera; to minimize handling stress, embryo counts were based on image analysis. Measurements from each aquarium were averaged prior to statistical analyses to avoid pseudoreplication. The following data were collected.

2.2.1 Embryo production

The density of live embryos per Gram of receptacle DW (n˚ alive embryos mm^{-2} g^{-1} DW) was used as the dependent variable to compare settlement patterns across treatments at Day 3. By this time, fertilised embryos had already divided and begun rhizoid formation, allowing a clear distinction between viable and non-viable embryos. For each replicated treatment, one glass slide was selected, and live embryos were counted in five 1.50 mm^2 quadrats randomly selected from the photographs. A univariate Generalized Linear Model (GLM) was then fitted to the data (i.e., density of settled live embryos per Gram of receptacle DW), considering "Culture" (three levels) and "Light" (two levels), as fixed factors. The model was fitted using a 'Gamma' distribution and a 'log' link function in the R statistical package, which is suitable for continuous, positive data.

2.2.2 Embryo survival

Counts of living embryos were recorded at Day 3, Day 6 and Day 10, which were conducted by analyzing images of a 1.5 mm^2 area randomly selected from the captured images at each time. The

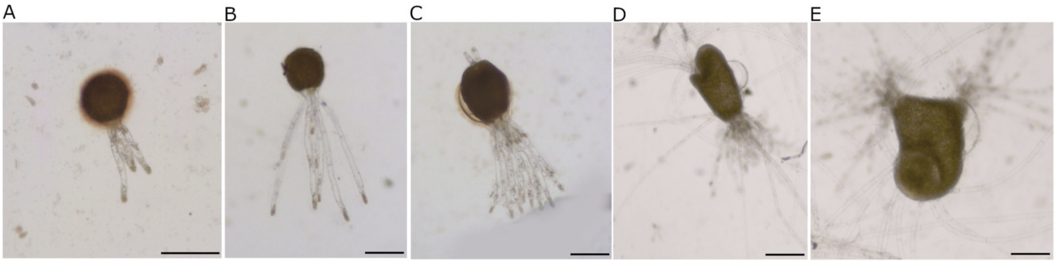

FIGURE 2
Embryonic stages of *E. selaginoides*. I: embryos with four primary rhizoids **(A)**, II: embryos with more than four rhizoids **(B)**, III: elongated embryos with short apical hairs **(C)**, IV: elongated embryos exhibiting branching and long apical hairs **(D)**, and V: irregularly shaped embryos **(E)**. Bar = 100 µm.

density of living embryos was then analyzed using a Generalized Linear Mixed Effect Model (GLMM) with a 'Gaussian' distribution and an 'identity' link function, with "Culture" (three levels) and "Light" (two levels), as fixed factors, and "Time" as a random factor. Models were fitted through the R 'glmmTMB' package (Brooks et al., 2017).

2.2.3 Embryo development

During the culture period, five distinct embryonic stages were observed: (I) embryos with four primary rhizoids (Figure 2A), (II) embryos with more than four rhizoids (Figure 2B), (III) elongated embryos with short apical hairs (Figure 2C), (IV) elongated embryos exhibiting branching and long apical hairs (Figure 2D), and (V) irregularly shaped embryos (Figure 2E). The proportion of embryos at each embryonic stage was then compared at Day 10 through a univariate GLM, considering "Culture" (three levels) and "Light" (two levels), as fixed factors. Models were fitted using the 'betareg' function, implemented via the 'betareg' R package (Cribari-Neto and Zeileis, 2010), which assumes a 'logit' link function and a residual distribution appropriate for continuous, positive, data that are proportions bounded from 0 to 1.

2.2.4 Embryo size

Ten days after fertilization (Day 10), embryos had progressed into the distinguishable developmental stages described above. To assess embryonic size, the area of five randomly selected individuals per embryonic stage was measured by analyzing five randomly selected images of 1.5 mm^2 area (Valdazo et al., 2024). Differences in area per embryonic state were then analyzed using a GLM with a 'Gamma' residual distribution family and a 'log' link function, with "Culture" (three levels) and "Light" (two levels), as fixed factors.

2.3 Field evaluation of juveniles

2.3.1 Laboratory *ex situ* culture

Approximately 500 receptacles (Figure 1E) were harvested on 4 August 2024, following the same transport and release protocol describe above. A total of 40 hexagonal concrete clay tiles (17.50 cm^2 in area, side length of 2.5) with a porous and rough surface were used. The tiles were commercially purchased from Silica Studio, Spain; the rough, porous, texture facilitates embryo adhesion and settlement (Falace et al., 2018; Supplementary Figure S6E–H). Tiles were maintained under culture conditions optimized in the previous phase to promote embryo growth, using Von Stoch culture medium, a 15:9 h light:dark photoperiod, 125 µmol photons $m^{-2}s^{-1}$, and a constant temperature of 20 °C using a temperature-controlled water bath (Figure 1D). Two aquariums (10 L) were used, which were initially filled with 1 L of culture medium for the first 24 h to facilitate embryo settlement. After the receptacle removal, the volume was increased to 8 L per aquarium, with medium renewal every 4 days to mitigate any possible effects of nutrient limitation. Continuous aeration was maintained using air pumps and bubblers to ensure adequate oxygenation.

During laboratory culture, embryo development on tiles was periodically monitored. Twenty-four hours after seeding, receptacles were removed and the dry weight (DW) of the receptacles associated with each tile was measured; this time was considered the fertilization point (Day 0). Release efficiency at Day 0 was calculated as the number of attached embryos per tile divided by the DW of the corresponding receptacles (embryos g^{-1} DW). Release efficiency was obtained through photographic sampling. Each tile was photographed perpendicularly from above using an OM System TG-7 camera (OM Digital Solutions, Tokyo, Japan) mounted on a fixed tripod at a known distance to ensure consistent scale and perspective across images. Image analysis was carried out in ImageJ, and embryos were counted manually by overlaying a grid on each photograph and visually tallying the items within each grid cell (Savonitto et al., 2021).

As development progressed, early juveniles became densely packed and formed aggregated patches on the tiles, preventing individual counts. Therefore, embryo survival was quantified as the percentage of tile area occupied by embryos (cm^2). Survival was measured on days 5 (Day 5) and 12 (Day 12) in 20 randomly selected tiles, and on day 33 (Day 33) for all tiles prior to outplanting. Survival was obtained through photographic sampling using an OM System TG-7 camera (OM Digital Solutions, Tokyo, Japan). Image analysis was carried out in ImageJ to quantify the area occupied by embryos, calculating survival as the proportion of tile surface covered by embryos relative to the total tile area (Schneider et al., 2012; Malfatti et al., 2023). The percentage of tile area occupied by embryos was analysed using a GLM with a 'Gamma' residual distribution and a 'log' link function, with "Time" as a fixed factor with four levels: Day 5, Day 12, Day 33 and the outplanting day (Day 0-OT).

2.3.2 Outplanting conditions

After 33 days in culture (Day 33), tiles were transported to Punta de Gáldar, which is located ca. 50 km away, in refrigerated boxes filled with sea water. To examine how exposure to environmental stress in the donor habitat affected juvenile survival and success, we outplanted juveniles in different physical conditions within the natural vertical distribution of *E. selaginoides*. We implemented two treatments: height on the shore and pool presence. Height on the shore included two levels within the low-intertidal zone: upper and lower (U and L, respectively). The lower level is exposed and accessible for only a few days during spring tides, which occur bi-monthly. Conversely, the upper level is exposed every day at low tide. The upper level was assumed to have increased temperature and desiccation stress (Ramírez et al., 2008; Betancor et al., 2015). The "pool" treatment (inside vs. outside of pools, +P/-P) was applied to half of the tiles within each zone, resulting in four treatment combinations. Each combination included 10 tiles, from a total of 40 initially seeded. We selected four pools with similar physical characteristics, in terms of size and depth (ca. 3 m^2 surface area and 0.5 m deep; Supplementary Figures S6A–D). We verified the presence of *Ericaria selaginoides* in the four pools. Herbivore pressure is not considered locally relevant, predominantly arising from mesograzers sheltering in the algal fringe (authors' personal observation). Seeded tiles were distributed across the four treatments (n = 10 per treatment: P + U, P–U, P + L, and P–L) to ensure homogeneous initial embryo coverage across treatments. Tiles were affixed to the rock using epoxy putty, stainless steel bolts, nuts, washers and screws (Supplementary Figures S6E,F). On the outplanting day (Day 0-OT), measurements were assessed immediately after tiles were attached to the substrate, to account for any potential losses associated with transport and deployment. Tiles were subsequently monitored twice over 3 months, as allowed by weather conditions: at 57 days (Day 57-OT) and 91 days after outplanting (Day 91-OT). Adverse climatic conditions led to the loss of several tiles, reducing the sample size in some treatments, especially in the lower eulittoral treatments. At the lower level, we lost five epoxy bolts at Day 0-OT in both P+ and P− treatments, and an additional two tiles were lost in P− at Day 57-OT (Supplementary Table S2).

At each outplanting monitoring time (Day 0-OT, Day 57-OT and Day 91-OT), several measurements were taken, including: (a) juvenile survival (i.e., % area covered by embryos, cm^2), (b) morphometric analysis, by measuring maximum juvenile length, and (c) juvenile occurrence, calculated as the percentage of tiles in which juveniles were alive. All analysis were done through photographic sampling with an OM System TG-7 camera (OM digital Solutions, Tokyo, Japan). Tiles were photographed perpendicularly from above to estimate survival, and laterally to obtain morphometric data; both orientations included the same scale reference. Image analysis was performed in ImageJ, where survival was calculated as the proportion of juvenile-occupied surface relative to the total tile area, maximum juvenile length was measured from the lateral images and juvenile occurrence was computed as the % of tiles showing juvenile presence (Schneider et al., 2012; Malfatti et al., 2023). We implemented GLMMs with a 'Tweedie' residual distribution family and a 'log' link function to assess the effect of shore level (two levels: Upper and Lower) and pool presence (two levels: absence, P-, and presence, P+), as fixed factors. We used "Time" as a random effect and the percent cover at the time of deployment, as a covariate, to evaluate their effect on independent variables.

All modeling and testing were conducted using R (Rstudio Team, 2022). For all fitted models, diagnosis plots of residuals and Q-Q plots (Supplementary Figures S7-S17) were visually inspected to check the appropriateness of the fitted models (Harrison et al., 2018).

3 Results

3.1 Optimized cultivation

3.1.1 Morphogenesis

Gamete release occurred shortly (0–3 h) after induction, and the release of oospheres and antherozoids was evident by the presence of numerous spherical cells marked by the formation of an orange-colored mound (Figure 3A). Fertilization occurred externally, leading to the formation of a fertilization membrane that facilitated adhesion of the zygotes to the substrate, with a mean diameter of 145.55 μm ± 6.10 (mean ± SD; n = 20). Then, 24 h after fertilization, the zygote cytoplasm, initially homogeneous, became clearly visible along with the nucleus (Figure 3B). Subsequently, cytoplasmic differentiation (polarization) was observed, characterized by the establishment of a vertical growth axis. Approximately 48 h after fertilization, the first division occurred perpendicular to this axis, generating two daughter cells of equal size (Figure 3C). The second division took place 20 h later (Figure 3D), and then a third division (Figure 3E). Between 48 and 72 h post-fertilization, multiple divisions occurred with an increase in the total embryonic volume, leading to the formation of rhizoidal bud protrusions (Figure 3F). After 72 h, nearly all embryos displayed initial rhizoid formation. The rhizoid mother cell divided, giving rise to 4 cells that differentiated into primary rhizoids (Figure 3G). These structures continued to elongate, forming long filamentous projections (Figure 3H). Within 5 days after fertilization, most embryos were detached from the fertilization membrane, coinciding with the emergence of secondary rhizoids (Figure 3I). As development progressed, the embryos exhibited significant elongation and an apical invagination, from which short apical hairs emerged (Figure 3J). By the end of the first week, the embryos had undergone notable morphological changes, measuring 541.91 ± 64.02 μm in length and 260.81 ± 47.81 μm in width (n = 20). Further elongation, the initiation of branching, and the presence of prominent, elongated, apical hairs were observed (Figure 3K), pointing to an advanced stage of early thallus development.

3.1.2 Embryo production

The density of alive embryos (per unit of receptacle mm^{-2} g^{-1} DW) ranged from 192.40 ± 124.10 in L + AL treatment to 377.70 ± 334.70 in L + VS, with intermediate values in the remaining treatments (L–AL: 235.0 ± 148.6; L–SW: 245.1 ± 141.3; L–VS: 263.7 ± 109.3; L + SW: 240.0 ± 126.4; mean ± SD), and did not

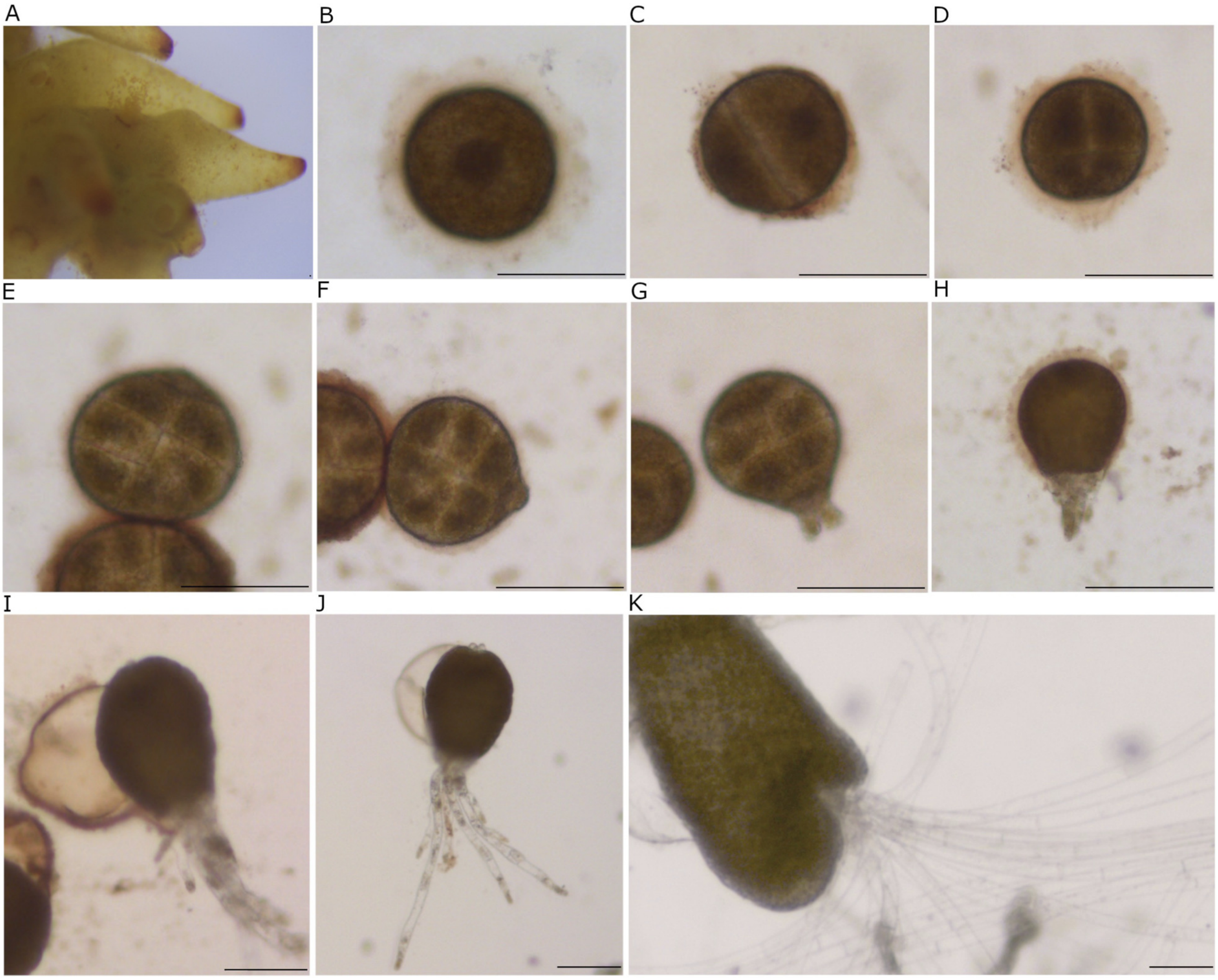

FIGURE 3
Early development of *E. selaginoides*. Release of sexual products through conceptacles **(A)**. Zygote with a central nucleus **(B)**. First zygote division **(C)**. Second zygote division, parallel to the first **(D)**. Third zygote division **(E)**. Embryo with a protuberance (rhizoid buds) **(F)**. Embryo with primary rhizoids **(G)**. Embryo with four secondary rhizoids with the fecundation membrane **(H)**. Embryo with four secondary rhizoids with the fecundation membrane **(I)**. Hyaline hairs growing from the invagination in the apical region of the embryo **(J)**. The initiation of embryo branching **(K)** Bar = 200 µm.

significantly vary across treatments (Figure 4; Supplementary Table S3).

3.1.3 Embryo survival

Embryo density declined over time among all treatments (Figure 5), with no significant differences observed between treatments (Figure 5; Supplementary Table S4).

3.1.4 Embryo development

Significant differences in embryonic developmental stages were observed among treatments on Day 10 after fertilization (Figure 6; Supplementary Table S5). The highest proportions of individuals at the last embryonic stage (stage IV) were achieved in the VS and SW culture media (Figure 6), both showing significantly higher values compared to AL ($p < 0.001$; Supplementary Table S4), with L+

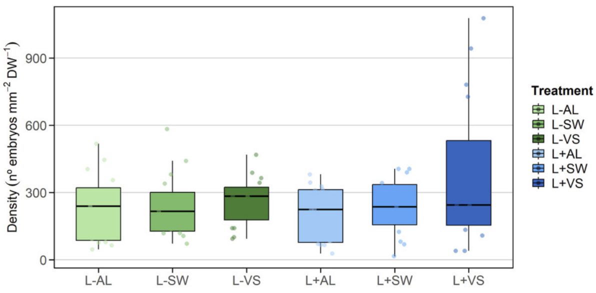

FIGURE 4
Boxplot of embryo production density (N° alive embryos mm^{-2} g^{-1} DW) under different treatments on Day 3 after fertilization. Boxplots denote medians, interquartile ranges, and individual data points. Treatments combine light intensity (L-: low light; L+: high light) and culture media (AL: Algatron Cifo®, SW: seawater, VS: Von Stosch solution).

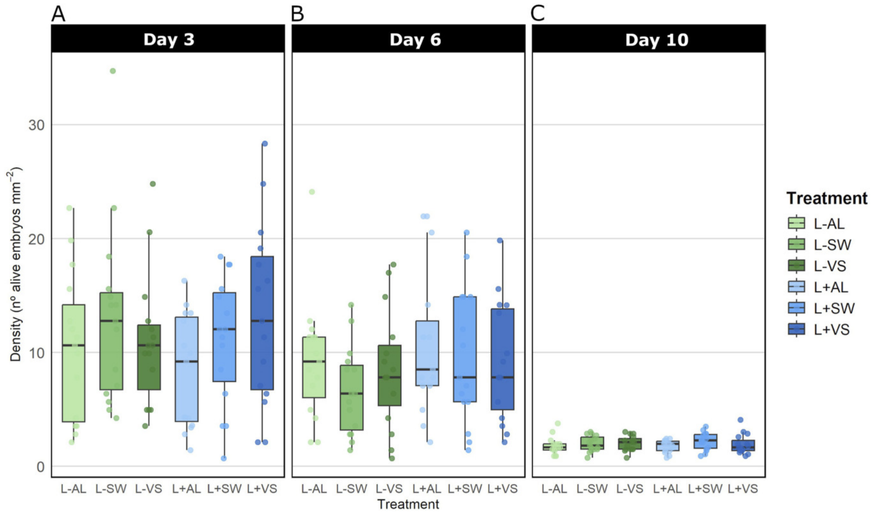

FIGURE 5
Boxplot of embryo density (N° alive embryos mm^{-2}) under different treatments 3 days after fertilization (Day 3; **(A)**), 6 days after fertilization (Day 6, **(B)**) and 10 days after fertilization (Day 10; **(C)**). Boxplots denote medians, interquartile ranges, and individual data points. Treatments combine light intensity (L-: low light; L+: high light) and culture media (AL: Algatron Cifo®, SW: seawater, VS: Von Stosch solution).

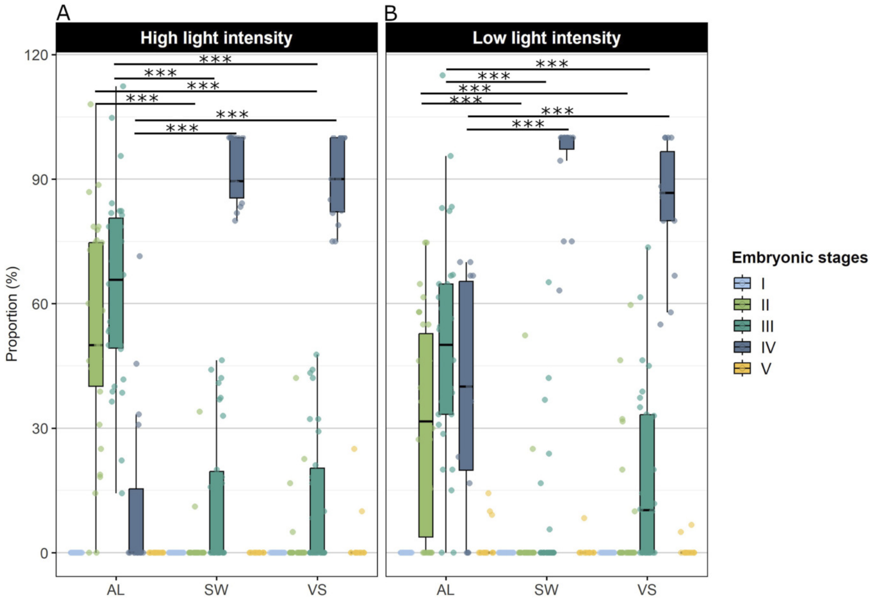

FIGURE 6
Boxplot of the percentage composition of embryonic developmental stages (S1–S5) under different treatments on Day 10 after fertilization, under high light intensity **(A)** and low light intensity **(B)**. Boxplots show medians, interquartile ranges, and individual replicate values. Treatments include culture medium (AL: Algatron Cifo®, SW: seawater, VS: Von Stosch solution). Significant differences between embryonic stages across treatments are indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

conditions showing a negative effect on stage IV ($p < 0.001$; Supplementary Table S5).

3.1.5 Embryo size

On Day 10, embryo size varied across treatments and developmental stages (Figure 7; Supplementary Table S6). At stage II (Figure 7; Supplementary Table S6), no statistically significant effects across treatments were detected. At stage III, the culture media had a significant effect, with VS and SW treatments having smaller areas than the AL treatments ($p = 0.05$ and $p < 0.05$, respectively; Supplementary Table S6). At stage IV, the juvenile area was significantly influenced by the culture media, with an inconsistent effect across light levels (Supplementary Table S6). Embryos under the VS culture showed significantly higher area than both AL and SW

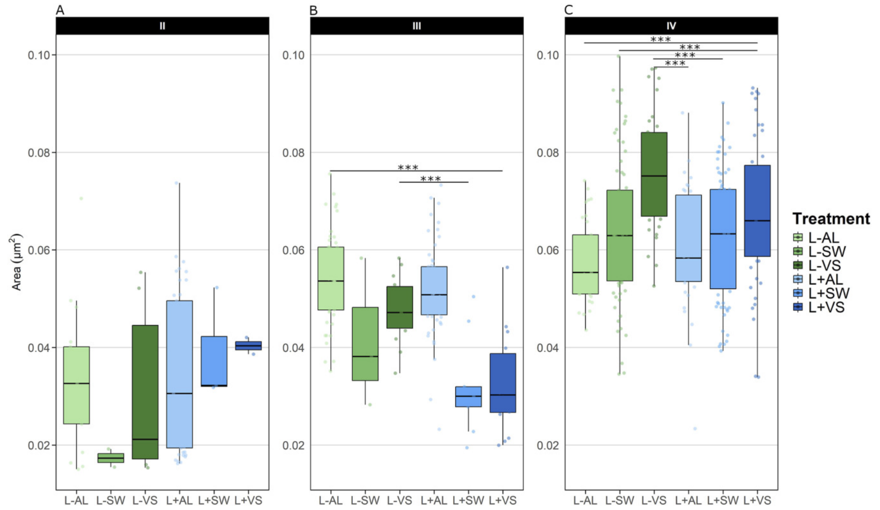

FIGURE 7
Differences in embryo area (µm²) across treatments at developmental stages II **(A)**, III **(B)**, and IV **(C)** on Day 10 after fertilization. Boxplots show medians, interquartile ranges (IQR), and individual data points. Treatments combine light intensity (L: low light; L+: high light) and culture media (AL: Algatron Cifo®, SW: seawater, VS: Von Stosch solution). Significant differences between embryonic stages across treatments are indicated as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

treatments ($p < 0.001$ and $p < 0.01$, respectively; Supplementary Table S6). Interaction effects were also significant, with L–VS showing higher area than both L + AL and L + SW ($p < 0.001$and $p < 0.05$, respectively; Supplementary Table S6), and L + VS showing higher area than L-AL p < 0.01; Supplementary Table S6).

3.2 *Ex situ* cultivation and outplanting

3.2.1 Laboratory *ex situ* cultivation

Zygote release efficiency in culture averaged 3,038.66 ± 2,548.56 (mean ± SD; n = 10) zygotes DW^{-1}. Juvenile cover increased progressively over time, with a mean tile cover of 2.17% ± 1.44% (mean ± SD; n = 10) on Day 5 (Supplementary Figure S18). This value rose to 6.02% ± 4.8 (mean ± SD; n = 10) on Day 12, and further increased to 13.99% ± 4.84% (mean ± SD; n = 10) on Day 33.

3.2.2 Outplanting and monitoring in the field

At the time of outplanting (Day 0-OT), cover stabilized at a mean of 16.61% ± 11.78 (mean ± SD; n = 40), indicating no mortality during transportation (Supplementary Table S7). The average juvenile length at this point was 0.66 ± 0.30 cm (mean ± SD; n = 40). Mean percent cover and juvenile length varied over time. On Day 57-0T, mean cover had decreased to 6.19% ± 9.78, with a mean length of 0.51 ± 0.66 cm (mean ± SD; n = 28). On Day 91-OT, cover slightly increased to 7.76% ± 11.35, while mean juvenile length reached 0.75 ± 0.94 cm (mean ± SD; n = 28). Alive juvenile occurrence varied among treatments (Supplementary Figure S19). Juveniles allocated to the "Lower/P+" treatment exhibited the highest and most stable occurrence of alive juveniles across time. Juveniles from the "Upper/P+" treatment showed an initial mortality but remained relatively stable afterwards. Juveniles from the "Lower/P-" treatment experienced a reduction and maintained a lower but consistent presence, whereas in the "Upper/P-" treatment exhibited a rapid loss of juveniles, leading to total disappearance by Day 57-OT.

Juvenile percentage cover (Figures 8A–C) and length (Figures 8D–F) over time were significantly influenced by pool presence, with individuals in "P+" showing higher values compared to those in "P–" ($p < 0.001$ and $p = 0.001$, respectively, Supplementary Table S8). Cover and length were also significantly higher in the "Lower" compared to the "Upper" zone ($p < 0.001$ both of them, Supplementary Table S8). The covariate (initial cover percentage) had a marginally significant effect on cover ($p = 0.05$, Supplementary Table S8), indicating an influence of initial embryo density on persistence at this stage. However, the effect on length was not significant ($p = 0.20$; Supplementary Table S8).

4 Discussion

This study constitutes the first pilot test of active restoration for endangered populations of habitat-forming brown macroalgae in the Canary Islands, by means of *ex situ* culture techniques. This approach followed the most prevalent strategies for the active restoration of *Cystoseira s.l.* species from the Mediterranean and northeastern Atlantic regions (Falace et al., 2021; Verdura et al., 2018; De La Fuente et al., 2019; Savonitto et al., 2021; Orlando-Bonaca et al., 2021; Lokovšek et al., 2023; Khaya et al., 2024). This approach enables the production of a significant number of viable recruits for reintroduction into the sea with only 500 receptacles and minimal impact on existing donor populations (Clausing et al., 2022; De La Fuente et al., 2019; Falace et al., 2018; Orlando-Bonaca et al., 2022; Savonitto et al., 2019). But, it is crucial to assess the stability of donor populations to determine their ability to support the removal of reproductive biomass from declining and endangered species

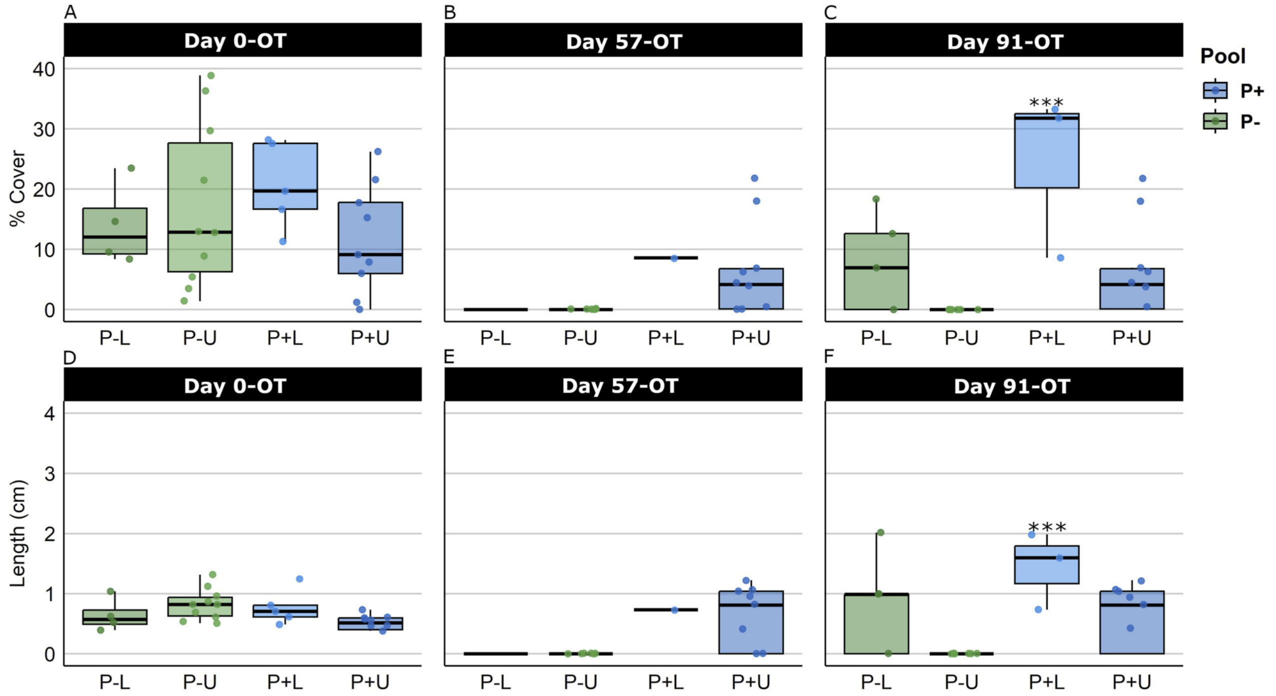

FIGURE 8
Temporal changes in juveniles cover % **(A–C)** and length (cm) **(D–F)** across treatments for *Ericaria selaginoides*. Day 0 of outplanting time (Day 0-OT; A and D), Day 57 of outplanting time (Day 57-OT; B and E), and Day 91 of outplanting time (Day 91-OT; C and F). Treatments include: shore level (U: "Upper" shore, L: "Lower" shore) and the pool presence (P+: present and P-: absence). Boxplots denote medians, interquartile ranges, and individual data points. Significant differences between treatments are indicated as follows: **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

population for future restoration actions (Rindi et al., 2023). Still, this procedure needs to be adapted according to target species-specific requirements in the different phases of implementation (Smith et al., 2023) and the environmental peculiarities of each region.

We described, for the first time, the morphogenesis and embryo development of *E. selaginoides*. Three groups of *Cystoseira s.l.* species have been identified based on zygote segmentation and number of primary rhizoids (Guern, 1962). *E. selaginoides* belongs to the first group, which is characterized by spherical eggs, zygotes that adhere to substrata by the fecundation membrane, and four primary rhizoids (Roberts, 1970; Susini, 2006).

Overall, our *ex situ* culture optimisation showed that culture enrichment and light intensities had no significant effect on embryo settlement and survival over the 10 days of the experiment. Release efficiency was quite variable, likely reflecting the natural variability in propagule release commonly observed among fucoid species (Gunnill, 1980; Reed, 1990; Vadas et al., 1992; Savonitto et al., 2021). Our results showed that the commercial seaweed extract, Algatron Cifo®, did not influence embryo development. Moreover, at the end of experiment (Day 10), the embryos in the AL treatments did not show a larger proportion of embryos in advanced developmental stages (IV), in contrast to embryos from the VS or SW treatments. This result contrasts with those of Malfatti et al. (2023) for *Ericaria amentacea*, a typical Mediterranean species that thrives, as *E. selaginoides*, in the intertidal zone under high wave exposure. The juveniles of *E. amentacea* in Malfatti et al. (2023) showed higher survival and growth in Algatron Cifo® culture at concentrations equal to those used in the present study, highlighting potential species-specific responses to this biostimulant. Moreover, juveniles initially cultured under this medium survived best in the field, while juveniles cultured in unenriched SW had a total failure after 3 weeks in the field. The findings of this study also diverge from those reported by Susini et al. (2006), wherein *E. amentacea* juveniles exhibited reduced growth in SW compared to VS conditions. In contrast, after 10 days of cultivation, the present study observed comparable performance between SW and VS conditions. In the cultivation phase, future work should be conducted with other concentrations or other biostimulants to improve the survival success of transplanted juveniles (Malfatti et al., 2023).

Although both *E. amentacea* and *E. selaginoides* are mainly restricted to oligotrophic waters and exhibit sensitivity to eutrophication (Sales et al., 2011), it is probable that *E. amentacea* is more adapted to assimilating periodic nutrient influxes from river runoff (Malfatti et al., 2023), thereby demonstrating that early stages may have higher nutrient uptake capacity than adult thalli (Sánchez de Pedro et al., 2023). Our experiment to maximize the culture conditions only lasted 10 days, which may have limited the potential effect of the biostimulants; in future experiments, a longer experimental time, coupled with other concentrations of AL and VS, are required to determine the efficacy of these media across a wider range of conditions (Malfatti et al., 2023).

We determined that a lower intensity for irradiance (125 µm mol photons $m^{-2}s^{-1}$) and the VS solution were the best culture setting to accelerate the development of high numbers of healthy and large juveniles. These culture conditions are similar to those used in many restoration studies with *ex situ* techniques (Falace et al., 2018; De La Fuente et al., 2019; Clausing et al., 2022; Lokovšek et al., 2023; Khaya et a., 2024). The implemented culture setting provides healthy juveniles larger (ca. 0.66 cm) than those in previous *Cystoseira* restoration studies obtained around 4 weeks (Falace et al., 2018; Verdura et al., 2018; De La Fuente et al., 2019; Savonitto et al., 2019; Lardi et al., 2022; Khaya et al., 2024). During the 3-month monitoring period, there was no significant increase in juvenile length observed in the field, with measurements ranging from 0.66 to 0.75 cm. This lack of substantial growth is likely attributable to higher allocation of resources and energy to structural components, enhancing its resilience in a highly exposed environment (Gaylord et al., 1994; Hurd, 2000).

We found that juvenile *E. selaginoides* were unable to survive in the upper zone of their vertical distribution range, without the protection offered by mild environmental conditions typical of tidal pools, where the presence of juveniles on the tiles at the end of the study reached ca. 70%. In contrast, juveniles outplanted in the lower zone maintained a ca. 100% presence inside pools and ca. 60% outside them. The results at the lower level should be interpreted with caution, because 12 tiles (ca. 60%) were lost. We suggest that this complete mortality in the upper zone out of pools may be due to the absence of facilitatory/buffer effects of pools on recruits. Pools act as microhabitat that mitigate extreme environmental fluctuations typical of the intertidal, including effects such as heat shock from high irradiance or temperature, desiccation and wave dislodgement (Davison et al., 1993; Harvey and Helmut, 2003). At the lower shore level, juveniles otherwise survived, because of reduced physical stress due to being submerged for longer periods of time. The percentage of initial cover by which the tiles were deployed in the receptor site had a slightly self-facilitative effect on the subsequent survival of juveniles in the field. This self-facilitative effect is consistent with that previously observed in *E. amentacea* (De La Fuente et al., 2019; Clausing et al., 2022; 2024). During the outplanting phase, it is imperative to conduct additional experiments across diverse receiving sites that encompass a comprehensive range of environmental conditions. This environmental variability should be systematically recorded using high-resolution data loggers to quantify *in situ* temperature and light exposure, particularly within various microhabitats, to elucidate the impact of primary environmental stressors on the survival and growth of recruits.

The attachment technique applied in this study, using epoxy putty instead of pre-established screws (De La Fuente et al., 2019; Savonitto et al., 2021; Clausing et al., 2022) seem to have decreased the effectiveness of outplanting, through dislodgement by wave action on the day of deployment or the following days (Susini et al., 2007). For future experiments we suggest trying another attachment technique of fixing the tiles, without using epoxy putty, that improves the resistance to dislodgement, particularly in the early stages of restoration, when epoxy putty technique may be strongly affected by wave action, which is particularly relevant in intertidal species. Using screw attachment technique (De La Fuente et al., 2019; Clausing et al., 2022) can be complicated and dangerous because we work in an environment where the frequency of large swells is high and there is little time to work in the lower intertidal.

Finally, our results suggest that the successful restoration of *Ericaria selaginoides* in high-stress habitats of oceanic islands may depend on a single strategy that promote benefits and enhanced resilience through both mesocosm culture and outplant phases. Furthermore, this study represents the first step toward the active restoration of fucoids in the Canary Islands. Additional research and development are required to enhance the effectiveness of these restoration efforts. In conclusion, we have demonstrated that juveniles successfully cultivated in the laboratory can be effectively outplanting into tidal pools within their vertical distribution range. However, they remain viable only outside of pools at the lower intertidal level. Nonetheless, further research is required to determine whether these juveniles can survive until reproduction in these environments and potentially serve as a foundation for the restoration of *Ericaria selaginodes* forests along the Canary coast.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JV: Writing – original draft, Writing – review and editing. SD-V: Writing – original draft, Writing – review and editing. ED: Writing – review and editing. AF: Writing – review and editing. RH: Writing – review and editing. FO-F: Writing – review and editing. FT: Writing – review and editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JC declared a past co-authorship with the author AF to the handling editor.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2026.1603416/full#supplementary-material

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