Characterization of *Gelidium corneum*'s (Florideophyceae, Rhodophyta) vegetative propagation process under increasing levels of temperature and irradiance

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A B S T R A C T

Climate change is affecting *Gelidium corneum* (Hudson) J.V. Lamouroux fields in the Bay of Biscay by reducing its cover and biomass. Understanding those changes requires a good characterization of the responses of this species to different stressors, particularly the effects on key processes such as the vegetative propagation. Here, we aimed to characterize the interactive effect of temperature (15, 20 and 25 °C) and irradiance (5–10, 55–60 and 95–100 $\mu mol*m^{-2}*s^{-1}$) on two phases of the vegetative propagation process: the re-attachment capacity and the survival of re-attached fragments. The study findings revealed significant effects of both temperature and irradiance in the re-attachment capacity of the species, with higher rates of attachment registered at 20 °C and 5–10 $\mu mol*m^{-2}*s^{-1}$ after 10, 20 and 30 days of culture. However, the interaction effects were not significant at any time interval. At higher or lower temperatures and increasing irradiances, the attachment capacity was reduced. On the other hand, irradiance was demonstrated to be the main factor controlling the survival of rhizoids. In fact, higher levels of irradiance generated severe damage on rhizoids, and thus, conditioned the development of new plants. According to this, it seems clear that the vegetative propagation process of this species is expected to become more vulnerable as both variables are expected to rise due to climate change. An increased vulnerability of this species may have several implications from an ecological and economic perspective, so we encourage to continue exploring the factors and processes controlling its distribution in order to adopt better management actions in the future.

1. Introduction

Climate change is affecting marine ecosystems and the services they provide at different scales (Airoldi and Beck 2007; Gao et al., 2021; Jiang et al., 2022). Canopy-forming macroalgae play an important role in those ecosystems, where they build large biogenic habitats and provide protection for different organisms (Thomsen et al., 2010; Wernberg et al., 2011). Changes in environmental conditions will directly and indirectly affects macroalgae and its distributions at different biogeographical scales (Breeman 1988). Shifts in the vertical zonation patterns and replacement by opportunistic or invasive species, are the most detectable changes at local scales (Harley et al., 2012). Also, the increase in the number and severity of macroalgae blooms (e.g. green tides, *Sargassum* blooms) will have an impact on local communities (Gao et al., 2021). At biogeographical scales, significant population declines and local extinctions have been documented, particularly, for those populations close to their range limits (Airoldi et al., 2014; Borja et al., 2018; Franco et al., 2018; Casado-Amezúa et al., 2019). Another important effect of climate change at global scale is the poleward migration, which has been reported for an increasing number of intertidal and subtidal seaweed species (Assis et al. 2018; de la Hoz et al., 2019).

In recent years, many studies have suggested the rise of ocean temperatures as the major driver of distributional shifts (Wernberg et al., 2011; Díez et al., 2012; Martínez et al., 2012). In fact, macroalgae biogeographical boundaries have been traditionally linked to ocean isotherms, reflecting the conditions that limit physiological processes and reproduction (see Lüning et al., 1990). In Northern Atlantic communities, several canopy-forming species have experienced important losses in its cover and biomass as a consequence of the increasing temperatures (Pehlke and Bartsch 2008; Ramos et al., 2020). These retreats are expected to increase in the following years as the rising trend of

temperatures is projected to continue during the 21st century (IPCC 2019). However, in many cases, distributional shifts are explained by the interaction among temperature and different environmental variables such as light and nutrient availability, acidification or human pressures. Regarding light conditions, an increase in the global surface solar radiation (about 1–4 W/m^2) has been reported in the last 40 years, particularly in the Northern Hemisphere (Wild et al., 2005). Previous research suggests negative effects of this increasing irradiance rates on canopy-forming macrophytes. For example, stressing light conditions can damage photosynthetic pigments (such as chlorophyll *a* and phycobiliproteins) which may result in a reduction of the photosynthetic and growth rates. In morphological terms, high levels of irradiance may lead to frond bleaching and a reduced density of fronds for some species (Quintano et al., 2013).

In the Bay of Biscay, *Gelidium corneum* (Hudson) J.V. Lamouroux, known as "ocle", is a clear example of a species which have experienced important changes in its distribution. This species has experienced a significant reduction in cover and biomass, especially in the eastern part (Basque Country populations) where the estimated stock has been reduced from more than 10.000 t in the 1990s to less than 2.000 t in 2015 (Borja et al., 2018). Several authors have pointed out different variables or combinations of variables to explain this decline. For example, Díez et al. (2012) and Quintano et al. (2013), using field experiments and surveys, found that increasing irradiance levels during summer and the reduction of nutrient concentrations in the last years might be key factors for controlling frond bleaching, density and biomass. Other authors suggested the increasing energy of waves during the last decades to be the main factor limiting its distribution on this area. In that study, the increasing number of wave extreme events seemed to affect the algae by detaching it from the substrata during the growth season more frequently. This process repeated over the years seems to have an accumulative effect avoiding the system to return to the previous state (Borja et al., 2018). In general, these studies (based on field data or field experiments) focused their analysis in the total biomass, cover or in the biochemical or physiological responses of adult plants. However, the effects of the new environmental conditions on the vegetative propagation capacity, which is considered the main mechanism of recruitment (Gorostiaga 1990; Juanes and Borja 1991; Santelices 1991), were not tested.

Vegetative propagation process of *G. corneum* consisted of five sequential phases, starting from the rhizoidal differentiation and finishing with the development of erect fronds (see Juanes and Puente 1993). From those phases, the re-attachment of rhizoidal filaments (probably the most critical part of the process) and the rhizoidal development have been previously analyzed in relation to different environmental conditions. For example, Salinas and Valdés (1993), trying to develop the optimal conditions for the cultivation of this species, found that the highest rhizoidal cluster production was obtained at 16 °C and long-day photoperiods in a factorial experiment combining temperature and photoperiod. On the other hand, Juanes and Puente (1993) tested the differences in the re-attachment capacity among gametophytes and sporophytes in a laboratory experiment with simulated light conditions (high irradiance and short day vs. low irradiance and long day). Their findings revealed higher rates of re-attachment of sporophyte fronds, but no significant differences among light treatments. However, those experiments did not explore extreme conditions which simulate future conditions under climate change scenarios.

Taking all of this into consideration, it seems clear that the shifts registered in *G. corneum*'s distribution cannot be attributed to a single factor. In this sense, the objective of this work was to characterize the interactive effect of temperature and irradiance on the re-attachment capacity and the survival of re-attached fragments of *G. corneum* in a laboratory experiment which simulate present and future climate conditions. We hypothesized a higher re-attachment capacity under typical temperate summer conditions (close to 20 °C) and low irradiance and the inhibition of the process under simulated summer conditions (close to 25 °C). Additionally, we expected increasing irradiance to have a significant negative impact on the survival capacity of re-attached fragments. This research will contribute to clarify the possible interactive effects of increments in temperature and irradiance over *G. corneum* vegetative propagation as a consequence of climate change.

2. Methodology

2.1. Collection of samples and experimental design

Individuals of *Gelidium corneum* were collected from an upper subtidal zone in the Cantabrian Sea (43° 28′ 56″ N, 3° 50′ 18″ W) during August 2021. This area is characterized by an exposed and calcareous rocky sea bottom fully covered with well-developed *G. corneum* fields. The temperature regime at the collection site is shown in Fig. 1. This regime was built with daily sea surface temperature data downloaded from the Operational Sea Surface Temperature and Ice Analysis (OSTIA) database (Donlon et al., 2012). Biased sampling procedures were used to ensure the collection of healthy, highly branched and low epiphyte plants. Samples were carefully removed from the substrata assuring biomass recovery. After collection, fronds were placed inside cool boxes and transported to the laboratory in darkness within 1 h. To avoid desiccation during transport, seawater from the collection site was added when necessary. Once in the laboratory, samples were identified morphologically and epiphytes and non-desired species were removed. Finally, fronds were kept in 60L indoor tanks at 17 °C and low light conditions (10–20 μmol*m^{-2}*s^{-1} on a 12:12h light:dark cycle) for 7 days before the start of the experiment.

The experiment was conducted in an isothermal walk-in growth chamber, with controlled temperature and light conditions. The system consisted of twelve translucent-white plastic boxes (60x40 × 30 cm) supplied with filtered and UV-sterilized seawater. Seawater was collected by a research facility directly from the sea in a location close to the sampling site (<1 km) and provided to us after filtration and sterilization. Three compartments (tanks) were placed inside each box, simulating different light conditions (Fig. 2). Tanks contained three concrete artificial substrata with high limestone content, where the apical fragments of *G. corneum* were assigned. A pumping system controlled by an ARDUINO electronic system was continuously recirculating water from turnover water containers (100L of capacity) in order to assure a continuous flow of water through the tanks. To avoid nutrient depletion, water was changed from the entire system every week.

Experimental design included two factors: Temperature (3 levels: 15 °C, 20 °C and 25 °C) and Photon Flux Density as a proxy of irradiance (PFD: 5–10 μmol*m^{-2}*s^{-1}, 55–60 μmol*m^{-2}*s^{-1} and 95–100 μmol*m^{-2}*s^{-1}). Temperature conditions tried to simulate early spring conditions (15 °C), mean summer conditions (20 °C) and a hypothetical climate change scenario of extreme summer conditions (25 °C). Levels were established using the temperature regime shown in Fig. 1 by calculating the seasonal mean values and adjusting them to the nearest one. Light treatments simulate an increasing gradient of irradiance. Each combination of temperature and photon flux density (referred as treatment from now on) was replicated 4 times.

The cold seawater treatments were determined by the chamber temperature (15 °C), while the other treatments (20 °C and 25 °C) required a heating system consisted of a combination of 150 and 100W auto-regulated aquarium heaters (EHEIM®, Germany). These heaters were placed strategically to avoid heat losses in the different parts of the system. Overhead lighting was provided by a combination of cool-white fluorescents (Sylvania® F30W/835) and Sylvania® Grolux F30W fluorescent lamps. Different light filters applied randomly on each compartment recreated the photon flux density conditions (5–10 μmol*m^{-2}*s^{-1}: Dark-gray filter, 55–60 μmol*m^{-2}*s^{-1}: Translucent-white filter, 95–100 μmol*m^{-2}*s^{-1}: Non-filter treatment). Photoperiod, air temperature and salinity were kept constant for the whole
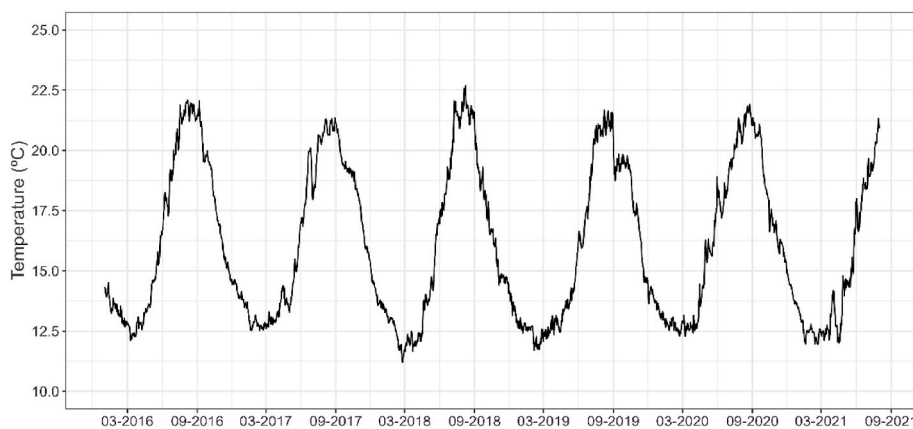
Fig. 1. Sea Surface Temperature (SST) regime at the collection site for the five years prior to the collection date.
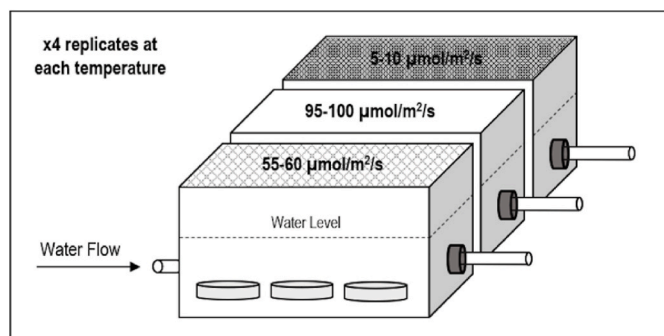

Fig. 2. Schematic representation of the experimental set-up, showing the different irradiance levels. Each temperature (15, 20 and 25 °C) was replicated four times, resulting in 36 water tanks.

experiment (12:12 light:dark cycle, 15 °C and 35‰).

Water parameters were measured every day to control anomalies: Temperature (T): every minute; pH, dissolved oxygen (OD) and Salinity (S): Twice per day. Salinity was adjusted by adding distilled (Milli-Q®) water when required. Registered mean values for the different parameters were as follows: Temperatures (T1 = 14.94 ± 0.10 °C, T2 = 20.18 ± 0.07 °C, T3 = 24.76 ± 0.13 °C), pH (pH at 15 °C = 7.82 ± 0.17, pH at 20 °C = 7.83 ± 0.15, pH at 15 °C = 7.89 ± 0.12), dissolved oxygen (OD at 15 °C = 99.15 ± 1.04%, OD at 20 °C = 100.49 ± 0.91, OD at 25 °C = 100.89 ± 1.38%) and salinity (S at 15 °C = 35.20 ± 0.38‰, S at 20 °C = 35.22 ± 0.91‰, S at 25 °C = 35.60 ± 0.42‰)

A total of 432 apical fragments (2–3 cm in length) were selected among the collected material, washed in the laboratory and then distributed in the different substrata by groups of four. Finally, these substrata were assigned randomly to the experimental treatments. The criteria for the selection tried to avoid fragments with necrotic patches, tissue damages or a high epiphyte load. Also, apical fragments poorly branched were discarded. Concrete substrata were kept in seawater 7 days before the incubation, and once the incubation starts both the biological material and the substrata were covered in a plastic mesh to guarantee the contact between them.

After thirty days of culture, 2 different variables (response variables) were measured: the re-attachment capacity of fronds and the survival of re-attached rhizoids. The former was estimated as the ratio of branches attached to the substrata from the total number of branches in each concrete disc (i.e. the sum of all the branches from the four apical fragments). This variable was also measured at 10 and 20 days. On the other hand, the survival of attached rhizoids was estimated by assigning 0s to those apical fragments with less than a 10% of damaged rhizoids ("alive" state) and 1s to those fragments with more than a 10% showing

damages ("non-alive" state). A damaged rhizoid was considered when it showed symptoms of tissue depigmentation or necrosis. The "alive state" is exemplified in Fig. 3a (completely healthy apical fragment) and 3b (original thalli showing necrotic patches, but less than 10% of damaged rhizoids). The "non-alive" state is exemplified in Fig. 3c, where the whole apical fragment is affected by depigmentation. Those variables try to characterize two phases from the vegetative propagation process (see Juanes and Puente, 1993): the reattachment of rhizoidal filaments and the rhizoidal development.

2.2. Data analysis

The potential effects of the independent variables (temperature and irradiance) and its interaction on the re-attachment capacity and the survival of rhizoids were analyzed by using a mixed modelling approach. For both variables, Generalized Mixed Models (GLMMs; Zuur et al., 2009) were fitted with a binomial distribution of error terms and a logit link function. The possible random effects generated by the culture system configuration were considered by adding Tank as random factor. When overdispersion was detected, an additional observation-level random factor (Substrata) was added to the models. Both kinds of models were applied using the *nlme*, the *lme4* and the *MuMIn* packages (Pinheiro et al., 2012; Bates et al., 2015; Barton 2019) in the R 3.6.2 software (R Core Team 2022).

The full or "beyond optimal" models (see Zuur et al. (2009) for details) included two fixed factors (temperature and irradiance) and the interaction between them. For the re-attachment capacity, 3 models were fitted using the data collected after 10, 20 and 30 days of culture. Only one model was fitted for the survival of rhizoids after 30 days of culture. Model selection was performed in two steps: 1) Selection of the random structure; 2) Selection of the fixed structure. First, the optimal random structure was analyzed using the "beyond optimal" fixed structure. This structure was selected from nested models built with restricted maximum likelihood estimation and by using the hypothesis testing approach (Likelihood ratio test – LRT) (Zuur et al., 2009). Once the optimal random structure was defined, the optimal fixed structure was selected. The different possible combinations of fixed factors and its combinations were compared using the Akaike Information Criteria for small samples (AICc; Burnham and Anderson (2004)). Fixed structure scoring the lowest AICc were selected. Additionally, the differences in AICc ($\Delta_i = AICc_i - AICc_{min}$) with respect to the better model and the Akaike weights (ϖ_i) were also determined. The relative importance of factors could be considered as the sum of all the Akaike weights of the candidate subset of models containing each explanatory variable (Burnham 2015). Candidate models (those with similar performance) were those differing less than 2 units from the model scoring the better AICc. Once the model was selected, post-hoc statistics were applied to test pairwise differences using the R packages *emmeans* (Lenth et al.,
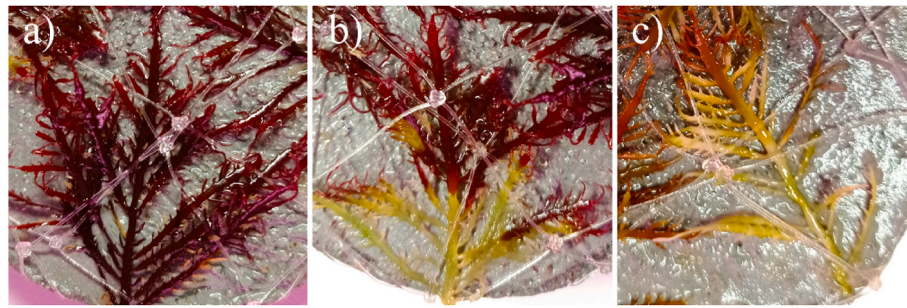
Fig. 3. a) Healthy apical fragment; b) Apical fragment showing depigmentation (necrosis) in the basal part. Rhizoidal filaments not affected; c) Apical fragment showing necrosis all over the thallus. Rhizoidal filaments affected.

2021) and *multcomp* (Hothorn et al., 2008). For all tests, a significance level of 0.05 was considered.

Data normality, homogeneity of variance and data dispersion were analyzed by using both graphical techniques and statistical tests (Kruskal-Wallis' test for normality and Levene's test for homogeneity of variances) and corrected if necessary. For this purpose, R packages *dharma* (Hartig 2021), *car* (Fox and Weisberg 2019), *rcompanion* (Mangiafico 2016) and *Stats* were used.

3. Results

3.1. Re-attachment capacity of apical fragments

After 10 days of culture, the re-attachment capacity was influenced by the additive effects of temperature and irradiance (Table 1a; relative importance of T = 0.710 and L = 0.736). The model including the interaction of temperature and irradiance (T x L) was among the subset of candidate models ($\Delta_i < 2$), suggesting a possible (but marginal) effect on the re-attachment capacity (relative importance of T x L = 0.171). In general terms, more branches were attached to the substrata at 20 °C (regardless of the irradiance level). In fact, only apical fragments growing at 15 °C and low PFD (mean value ± SD: 17.20 ± 9.06%) recorded similar re-attachment capacity to those growing at 20 °C in terms of statistical significance (mean values ± SD: low PFD = 39.8 ± 14.6%, medium PFD = 23.5 ± 13.4% and high PFD = 30.8 ± 7.89%; see Fig. 4a). The number of attached branches to the substrata was particularly low at 25 °C, with all the irradiance treatments showing mean values between 0 and 5% (mean values ± SD: low PFD = 4.07 ± 3.64%, medium PFD = 3.6 ± 3.5% and high PFD = 2.51 ± 2.43%). Values close to 0 were also detected at 15 °C and the highest level of irradiance (mean value ± SD = 2.16 ± 1.69%).

The mean ratio of attached branches to the substrata increased after 20 days of culture for every combination of treatments. Again, this variable was mainly influenced by the additive effect of temperature and irradiance (Table 1b; relative importance of T = 0.582 and L = 0.582). However, contrasting with the results achieved after 10 days, only one model (T + L) was included in the subset of candidates, suggesting a particularly marginal interaction effect. The pattern was similar to that obtained after 10 days (Fig. 4b). Nevertheless, mean values at 15 °C and low PFD were slightly higher than those obtained at 20 °C and medium PFD levels (31.15 ± 14.5% vs 26.6 ± 11.7%), though this difference was not significant. Additionally, at 25 °C mean values of re-attachment started to differ among irradiance levels. In fact, the apical fragments receiving less irradiance attached more branches to the substrata at this temperature (mean values ± SD: low PFD = 20.2 ± 11.50%, medium PFD = 4.46 ± 3.33% and high PFD = 8.02 ± 5.96%), being only significantly higher the comparison among low and medium PFD (Fig. 4b). The re-attachment capacity was also significantly higher at low irradiance when compared to medium levels of PFD at 20 °C (57.30 ± 17.80% vs 26.6 ± 11.7%).

After 30 days of culture, the interquartile range (IQR) at 15 °C was

Table 1

Selection of random and fixed structures of GLMMs for the re-attachment capacity of apical fragments at the different time periods: a) after 10 days of culture, b) after 20 days of culture and c) after 30 days of culture. Selected models are highlighted in bold. Multiplication signs indicate models including individual main factors and all its possible lower order interactions. T: Temperature, L: Irradiance, df: Degrees of freedom, logLik: loglikelihood, AICc: Akaike Information Criteria for small samples, $\Delta_i = AICc_i - AICc_{min}$, ωi: weights.

a) t = 10 days

Random Structure	df	Model Comparison	logLik	χ^2	p-value
R1: T x L	9		−608.66		
R2: T x L + (1 | Tank)	10	R1 vs R2	−367.15	483.01	<0.001
R3: T x L + (1 | Tank) + (1 | Substrata)	**11**	**R2 vs R3**	**−312.12**	**110.07**	**< 0.001**

Fixed Structure	df	AICc	logLik	Δ_i	ω_i
F1: T + L	**7**	**635.5**	**−312.46**	**0**	**0.371**
F2: L	5	636.8	−314.21	1.30	0.194
F3: T x L	11	637.1	−312.12	1.56	0.171
F4: T	5	637.2	−314.36	1.59	0.168
F5: NULL	3	638.2	−316.01	2.72	0.095

b) t = 20 days

Random Structure	df	Model Comparison	logLik	χ^2	p-value
R1: T x L	9		−676.68		
R2: T x L + (1 | Tank)	10	R1 vs R2	−455.13	443.1	<0.001
R3: T x L + (1 | Tank) + (1 | Substrata)	**11**	**R2 vs R3**	**−361.29**	**187.67**	**<0.001**

Fixed Structure	Df	AICc	logLik	Δi	ωi
F1: T + L	**7**	**733.20**	**−361.32**	**0**	**0.582**
F2: T x L	11	735.40	−361.29	2.20	0.194
F3: L	5	735.40	−363.52	2.20	0.193
F4: T	5	739.90	−365.74	6.65	0.021
F5: NULL	3	741.3	−367.53	8.07	0.010

c) t = 30 days

Random Structure	df	Model Comparison	logLik	χ^2	p-value
R1: T x L	9		−793.38		
R2: T x L + (1 | Tank)	10	R1 vs R2	−491.79	603.2	<0.001
R3: T x L + (1 | Tank) + (1 | Substrata)	**11**	**R2 vs R3**	**−378.01**	**227.56**	**<0.001**

Fixed Structure	df	AICc	logLik	Δi	ωi
F1: T + L	**7**	**766.60**	**−378.01**	**0**	**0.547**
F2: T x L	11	768.00	−378.01	1.40	0.272
F3: L	5	768.80	−379.81	2.20	0.179
F4: T	5	779.40	−385.51	12.80	0.001
F5: NULL	3	779.70	−386.73	13.08	0.001

increased especially at low and medium irradiance levels, showing high variability among replicates. Besides, the general pattern keeps quite similar to the one observed after 20 days. Higher increasing rates were
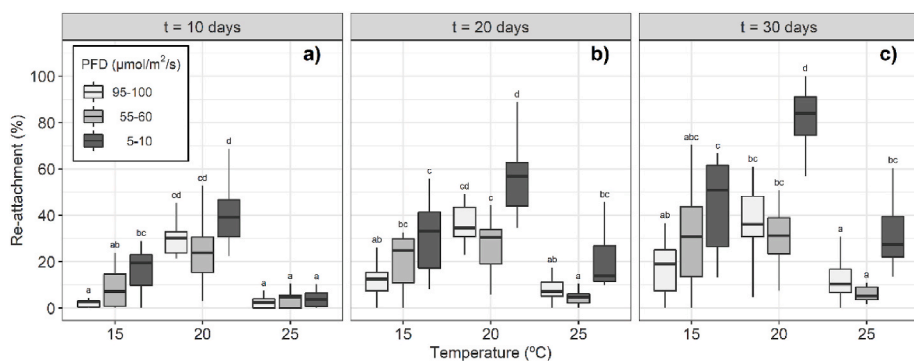
Fig. 4. Boxplot diagram for the re-attachment capacity of apical fragments at the different time periods: a) after 10 days of culture, b) after 20 days of culture and c) after the end of the experiment (30 days). Re-attachment capacity was estimated as the percentage (%) of branches attached to the substrata from the number of branches in each concrete disc ($n_{discs \times treatment} = 12$). Latin letters indicate significant differences ($p < 0.05$) between treatments based on post-hoc analysis. PFD: Photon Flux Density.

recorded at low PFD, staying the rest of irradiance treatments quite constant in terms of re-attachment (Fig. 4b and c). The highest increase was showed at 20 °C and low PFD (from $57.3 \pm 17.8\%$ to $81.7 \pm 13.4\%$), resulting in a significantly higher re-attachment capacity (compared to the other combinations of treatments) (Fig. 4c). Mean values at 25 °C were lower (but not always significantly different) than those registered at 20 °C and 15 °C, when comparing the same irradiance levels. This pattern was also registered after 20 days of culture. Those results were again mainly influenced by the additive effect of temperature and irradiance (Table 1c; relative importance of T = 0.819 and L = 0.819). At this time period, the effect of the interaction between the main terms was also present among the candidate models (relative importance of T x L = 0.272), showing influence in the results.

3.2. Survival capacity of rhizoids

Survival capacity of attached rhizoids seem to be mainly influenced by the irradiance received (Table 2; relative importance L = 0.837). The additive effect of light and temperature was considered in a candidate model, although the effect of temperature in this model seem to have less influence. Conversely, survival capacity decreased when irradiance levels increased. In fact, survival was significantly higher when the apical fragments were exposed to low PFD (with values close to 100%), regardless of the temperature considered (Fig. 5). However, no significant differences were identified between high and medium levels of irradiance at each specific temperature. When comparing the survival capacity among temperatures at each specific irradiance level no significance differences were obtained, which confirms the marginal effect
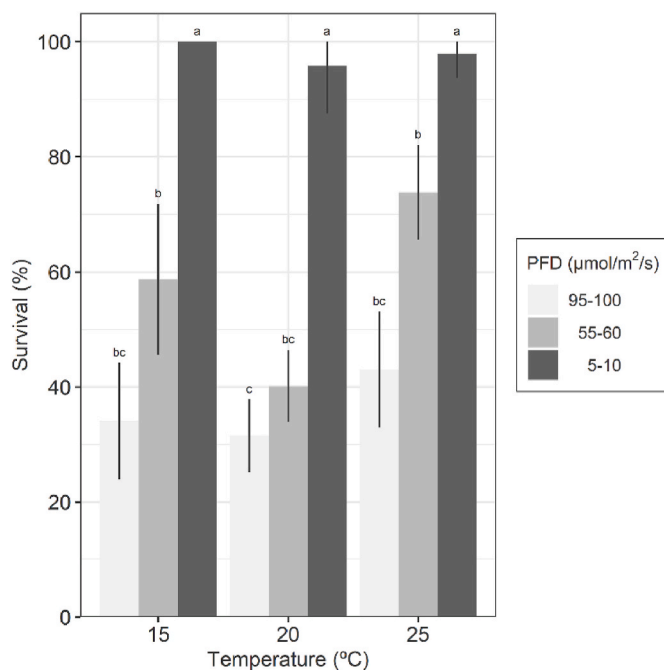

Fig. 5. Survival of re-attached rhizoids (%) in each tank ($n_{tanks \times treatment} = 4$) after 30 days of cultures. Mean values $\pm$ SD are shown. Latin letters indicate significant differences ($p < 0.05$) between treatments based on post-hoc analysis. PFD: Photon Flux Density.

of temperature on the survival of attached rhizoids.

4. Discussion

This study revealed the influence of irradiance and temperature in the re-attachment capacity and the rhizoidal development of *Gelidium corneum*, two stages of the vegetative propagation process considered critical for the maintenance of its populations. Results pointed out the role of irradiance and temperature controlling the secondary attachment of rhizoids, while the development and survival of rhizoids once attached was mainly influenced by irradiance.

Focusing in the re-attachment capacity, the effect of temperature and irradiance was clear at every time interval (after 10, 20 and 30 days). Comparisons at each specific irradiance level showed higher mean re-attachment capacity at 20 °C, being significantly higher in every time period. Similar results were reported by Salinas and Valdés (1993), though, in this research higher rates of attachment at temperatures over 18 °C were generally coupled to the appearance of senescence process all over the apical fragment, including rhizoids. Tissue damages, but limited to the parental thallus have been observed in other species with

Table 2
Selection of random and fixed structures of GLMMs for the survival of re-attached rhizoids after the end of the experiment (30 days). Final selected models are highlighted in bold. Multiplication signs indicate models including individual main factors and all its possible lower order interactions. T: Temperature, L: Irradiance, df: Degrees of freedom, logLik: loglikelihood, AICc: Akaike Information Criteria for small samples, $\Delta i = AICci - AICcmin$, ωi: weights.

Random Structure	df	Model Comparison	logLik	χ^2	p-value
R1: T x L	**9**		**−161.55**		
R2: T x L + (1 | Tank)	10	R1 vs R2	−161.54	0.01	0.908
R2: T x L + (1 | Tank) + (1 | Substrata)	11	R2 vs R3	−161.49	0.10	0.747
R3: T x L + (T | Tank) + (T | Substrata)	21	R2 vs R3	−161.23	0.53	0.970

Fixed Structure	df	AICc	logLik	Δ_i	ω_i
F1: L	**3**	**329.10**	**−162.52**	**0**	**0.474**
F2: T + L	5	329.60	−161.77	0.53	0.363
F3: T x L	9	331.20	−161.55	2.15	0.162
F4: NULL	1	444.80	−221.37	115.68	0.000
F5: T	3	446.70	−221.31	117.59	0.000

reattachment capacity, such as *Chondrocanthus chamissoi*, where vegetative structures remained undamaged and were considered as a mechanism of resistance to unfavorable environmental conditions (Pacheco-Ruíz et al., 2005). Senescence limited to parental tissues was also observed in our analysis but there were no evidences that increasing temperatures generated it. Actually, the appearance of necrotic patches initiated sooner and was higher at 20 °C than at 25 °C (personal observation). However, in *G. corneum,* the spread of necrotic patches and senescence marks over this part of the thallus seemed to be directly linked to the rhizoidal cluster development degree. This makes sense if we considered that the morphological differentiation of apical fragments into rhizoids seemed to be closer to a thigmotropic response used to propagate and perennate rather than a mechanism of resistance to stress. In fact, after the experiment, the system was kept running for another two months until the appearance of new shots was generalized in almost every surviving rhizoid. At this moment, almost every apical fragment with healthy rhizoids had its original thalli fully bleached and necrotized (unpublished data) which support this hypothesis. However, due to the high mortality rates registered as a consequence of failures in the experimental systems, the results were not robust enough to correctly test this hypothesis and further research is needed. Considering all this information, for this species, it is important to differentiate the necrotic patches originated in the parental thalli than those developed in the new structures (rhizoids), which were mainly affected by the effect of light as we will discuss later.

Considering the effect of temperature through time, our findings support that this factor influenced the re-attachment capacity of *G. corneum* during the first 30 days of the vegetative propagation process, showing a different pattern when compared to other studies for different *Gelidium* species. For example, the effect of temperature on the formation of attachment structures stopped after 14 days of culture for *Gelidium lingulatum* (Otaíza et al., 2018). However, it is important to highlight that, in our study, the number of attached branches did not vary too much from 20 to 30 days at intermediate and high irradiance levels, suggesting the stabilization of the attachment rates after the second third of the month. Potential differences may be understood considering the different ways to produce rhizoids. In *G. ligulatum* secondary attachment structures were produced all over the basal parts of the axes, blades and branchlets. However, in *G. corneum* attachment structures were limited to the rhizoids previously differentiated from apical fragment tips. In this sense, the attachment of rhizoids in *G. corneum* require the previous differentiation of this structures before developing the attachment bundle, while the attachment of axes in *G. ligulatum* could be direct if they are in contact with a substratum.

On the other hand, the influence of light on growth and formation of attachment structures has been previously analyzed independently or in combination with other factors (Oliger and Santelices 1981; Macler and Zupan 1991). In general terms, those apical fragments receiving less light registered the higher mean percentages of re-attachment, though differences were not always significant. Furthermore, comparisons between the intermediate and high levels of irradiance showed no significant differences at any time measured. This result suggests a similar limiting effect on the re-attachment capacity for irradiances over 50 μmol*m^{-2}*s^{-1}. Other studies have also reported more secondary attachment structures being produced under low irradiances (D'Antonio and Gibor 1985; Fonck et al., 2007; Otaíza et al., 2018; Rodríguez and Otaíza 2018). In fact, this is supported by Lunning et al. (1981) who stated that germlings of red algae growing under canopy at deep waters achieve its maximum growth rates at very low levels of incident light (approx. 4–5 μmol*m^{-2}*s^{-1}), while growth in blades of adult plants are reported to be favored by higher irradiance levels (Santelices 1991; Perrone and Cecere 1997). Other authors have also explored other components of light, such as photoperiod (Juanes and Puente 1993; Salinas and Valdés 1993). Unlike the findings of Salinas and Valdés (1993) for the interaction effect of photoperiod and temperature, our results suggest weak or marginal interaction effects between

temperature and irradiance after 10 and 30 days of culture and almost inexistent after 20 days. Actually, the influence of irradiance in the re-attachment capacity was similar at every temperature as we have seen before (higher mean values at low level of irradiance, with few differences between intermediate and high levels). Potential differences between studies might be explained if we interpret light effects as daily cumulated energy instead of irradiance or photoperiod. Daily cumulated energy levels in that study were shifted towards low lighting values, being the maximum tested value 560 μmol*m^{-2}*day^{-1} (35 μmol*m^{-2}*s^{-1} and 16:8 light:dark cycle), while our intermediate irradiance treatment resulted of 660–720 μmol*m^{-2}*day^{-1} (55–60 μmol*m^{-2}*s^{-1} and 12:12 light:dark cycle).

After 30 days of culture, the survival of attached rhizoids was totally influenced by irradiance. Appearance of bleaching or senescence signs in the rhizoidal cluster was observed in more than 50% of the apical fragments cultivated under intermediate and high irradiances, limiting the vegetative propagation process. Previous studies have found negative effects of increasing irradiance levels in ecophysiological responses of *G. corneum* adult plants (Quintano et al., 2018, 2019). In these studies, the authors reported higher bleaching when seaweeds were exposed to PFD values ranging from 516.7 to 1070.3 μmol*m^{-2}*s^{-1}. Furthermore, Torres et al. (1991) set photoinhibition irradiance levels at 250 μmol*m^{-2}*s^{-1} for *G. corneum*. This value is 2.5 times higher than the high irradiance considered in our study, suggesting more vulnerability of the photosynthetic apparatus of early-stage structures (rhizoids) to increasing irradiances. In this sense, macroalgae could develop acclimatization strategies, such as reducing the amount of pigments, to protect their structures under irradiance stress, which may cause reversible bleaching effects (Häder and Figueroa 1997; Lüder et al., 2002). However, in our study, after two months of culture, rhizoids showing yellowish colors were not able to recover and develop new shoots (personal observation), even when irradiance was set to lower levels. In fact, when certain irradiance levels are exceeded, photosynthetic pigments (particularly phycobiliproteins) are completely destroyed leading to chronic photoinhibition, photodamage and bleaching (Figueroa and Gómez 2001). However, measuring pigment composition on rhizoids is very challenging due to its small size and to the fact that they are colorless at the tip. The lack of color at the tips does not necessarily mean that tissue damages are present and it is associated to the production of new internal rhizoidal filaments (Santelices and Varela 1994; Perrone and Cecere 1997)

At this point, it seems clear that changes registered in *G. corneum* distribution (particularly those in the Bay of Biscay) are due to a combination of factors and it is easy to find synergies between the results presented in our study and the results presented by other authors to explain the possible effects of climate change. For example, Borja et al. (2018) attributed the reduction of biomass to an increase in the number of extreme events (those with significant wave heights over 5 m), having more impact at shallower depths. On the other hand, Quintano et al. (2017) highlighted the influence of light on biomass, density and bleaching and its influence in *G. corneum* distribution. Also, temperature, in particular heatwaves, may have imposed stressful conditions for those populations. All of these factors are directly linked with the process analyzed in this paper and in combination may explain the declines experienced in the last years. Stressing levels of irradiance and temperature may generate a weakening of the thallus facilitating the dislodgment of *G. corneum* during the extreme events of increased wave energy. Increasing rates of dislodgment could generate important density reductions which could be traduced in higher incidence of light in the understory where vegetative propagation processes occur. As we have demonstrated here, both temperature and light have several implications in the vegetative propagation process which may prevent those dislodged thalli to re-attach again to the substrata under stressful conditions. According to this, those populations located in shallow and exposed areas close to the upper thermal limit will be the more vulnerable to changes.

Taking the future projections of climate change into consideration, it is evident that irradiance levels, temperatures and extreme events will increase towards the 21st century (IPCC 2019). Assuming this situation, it is likely that *G. corneum* populations will face important changes in its distribution, as it has been shown for other species among the Gelidiales order (de la Hoz et al., 2019). This will generate negative consequences in the ecosystem services they provide which is associate to economic losses. In order to reduce those negative consequences, it is important to develop reliable and powerful tools and provide managers and administrations with them. For example, one of the most applied tools to deal with these issues are Species Distribution Models (SDMs), whose applications have increased significantly. SDMs combine occurrence data with environmental information based on a correlative approach to build a representation of the ecological requirements. Recently, it has been shown that these models can be improved by including species' physiological information derived from laboratory or field experiments (e.g. Martínez et al., 2015; Calleja et al., 2019). These models are called Hybrid models as they include environmental information, together with biological data (e.g. survival capacity) or interactions (e.g. competition). However, its application is still uncommon and complex. In this sense, the results achieved in this study may have important implications for the management of *G. corneum* fields in the Atlantic basin, as they can be included in a Hybrid SDM to improve the future mapping of vulnerable areas and to prioritize zones for conservation.

In conclusion, the vegetative propagation process of *G. corneum* is expected to become more vulnerable as temperatures and irradiance levels in the understory are expected to rise due to climate change. However, the future of this species in northern Spain remains an open question as it is still difficult to predict whether the increasing rates of those variables are going to be higher than the ability of the species to cope with them. It seems, according to the published information, that changes may already be happening at rates that exceed acclimatization and adaptive potential of the species particularly in the eastern coasts of the Bay of Biscay. Due to the ecological, cultural and economic value of this species we encourage to continue exploring the factors and processes controlling the distribution of this seaweed in order to anticipate to changes and take actions from a management perspective.

Author statement

SS-V: Conceptualization, Investigation, Data curation, Formal analysis, Writing - original draft, review & editing; BS-A: Investigation, Data curation, Writing - review & editing; AP: Conceptualization, Investigation, Writing - review & editing, Funding acquisition; JJ: Conceptualization, Investigation, Writing - review & editing, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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