# Effect of pretreatment, photoperiod, and light intensity on spore release by *Gracilaria gracilis* from the Moroccan Atlantic coast

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**Abstract**. *Gracilaria gracilis* is an economically important red seaweed with considerable potential for sustainable aquaculture. This study investigates reproductive performance in cystocarps of *G. gracilis* collected from two Moroccan Atlantic sites, Sidi Rahal and Oualidia. The study aimed (i) to quantify the cystocarp abundance per thallus to estimate sporulation rates and identify the most suitable collection period, and (ii) to evaluate the influence of four pretreatments, three photoperiods (8/16, 12/12, 16/8), and three light intensities (15, 40, and 72 µmol photons $m^{-2}$ $s^{-1}$) on spore release. Thalli from Oualidia reached a maximum sporulation rate of 520 cystocarps per thallus in April, whereas those from Sidi Rahal recorded 1,552 cystocarps per thallus during the same period. The optimal collection window extended from March to May. Among the tested treatments, pretreatment C induced the highest sporulation, with 7,113.6 spores released from Sidi Rahal thalli and 2,345.6 spores from Oualidia thalli. Moreover, the 8/16 photoperiod combined with a light intensity of 15 µmol photons $m^{-2}$ $s^{-1}$ promoted rapid and abundant spore release. These results provide a practical basis for optimizing laboratory cultivation conditions of *G. gracilis* and contribute to the development of sustainable seaweed aquaculture in Morocco.
**Keywords**: cystocarps, sporulation, pretreatment, photoperiod, light intensity.

**Introduction**. The red macroalga *Gracilaria gracilis* is widely distributed along temperate and subtropical coastlines, where it plays a significant ecological and economic role. This species is particularly important as a major source of agar, a commercially valuable hydrocolloid extensively used in the food, pharmaceutical, and biotechnology sectors (Armisen, 1995; Bixler & Porse, 2011). In addition to its industrial relevance, *Gracilaria* species are known to produce a wide range of bioactive compounds with potential antioxidant, antimicrobial, and anticancer properties (Rawiwan et al., 2022). From an ecological perspective, these macroalgae contribute to coastal ecosystem functioning by enhancing nutrient uptake and sequestering carbon, particularly in eutrophic environments (Fei, 2004; Spanò et al., 2022). Due to these attributes, *Gracilaria* has been widely cultivated in several regions of the world, including Asia and South America, where large-scale production systems significantly contribute to global biomass supply (Bezerra & Marinho-Soriano, 2010; Bunsom & Prathep, 2012; Buschmann et al., 1995; Hurtado-Ponce et al., 1992; Mantri et al., 2009; Yang et al., 2015).

Despite its widespread cultivation and economic importance, the sustainability of *Gracilaria* aquaculture remains a major concern. The predominant use of vegetative propagation in farming practices has led to a progressive decline in genetic diversity, which in turn affects the resilience and productivity of cultivated populations (Yang et al., 2025). In addition, the increasing pressure on natural stocks has contributed to the depletion of wild resources (Mouedden et al., 2024). These limitations highlight the urgent need to develop alternative propagation strategies that can preserve genetic

variability, improve stress tolerance, and support long-term biomass production. In this context, the use of reproductive spores represents a promising approach for both sustainable aquaculture and ecological restoration.

The carposporophyte is a short-lived diploid phase that remains attached to and nutritionally dependent on the female gametophyte (Wang et al., 2010). The tetrasporophyte produces tetraspores through meiosis, enabling regular sexual reproduction (Kain & Destombe, 1995). This process offers a genetically diverse alternative to clonal propagation, a method commonly used in cultivation (Yu et al., 2013; Zhou et al., 2016). The successful implementation of spore-based cultivation requires a clear understanding of the early developmental stages, from spore release and substrate attachment to basal disc and erect thallus formation (Kain & Destombe, 1995; Kim et al., 2017), all of which are highly sensitive to environmental factors such as temperature, photoperiod, and light intensity (Singh & Singh, 2015; Yu et al., 2016). Environmental influences on spore development have been reported in several cultivated species, including *Gracilaria cornea* (Orduña-Rojas & Robledo, 1999), *Gracilaria pacifica* (Garza-Sánchez et al., 2000), *Gracilaria chilensis* (Guillemin et al., 2013), *G. gracilis* (Hughes et al., 2014; Michetti et al., 2013), and *Gracilaria fisheri* (Yang et al., 2025).

The Moroccan Atlantic coast hosts a rich macroalgal diversity, with over 500 recorded species, including green, brown, and red algae (Grina et al., 2020). Favorable salinity and hydroclimatic conditions have supported the development of a seaweed industry since the 1950s (El Asri et al., 2021). This sector employs more than 500 full-time workers, provides seasonal employment for approximately 9,000 people, and generates around 170 million Moroccan dirhams annually (El Asri et al., 2021). Seaweed harvesting is driven by strong international demand, with export prices (15 MAD $kg^{-1}$) significantly exceeding local processing rates (8 MAD $kg^{-1}$).

This study aimed to evaluate the effects of pretreatment, photoperiod and light intensity, on the early developmental stages of *G. gracilis*. Specifically, it sought to (i) determine the sporulation rate in each sampling area and identify the optimal collection period, (ii) compare the environmental adaptability of *G. gracilis* populations from the two sampling sites (iii) provide a theoretical and practical foundation for optimizing spore seedling production protocols. The findings are expected to advance the understanding of *G. gracilis* ecophysiology and contribute to sustainable and regenerative seaweed aquaculture in Morocco.

## Material and Method

***Physicochemical parameters***. Environmental conditions were monitored at both sampling sites (Sidi Rahal and Oualidia) throughout the study, with measurements conducted every two weeks. Seawater temperature, salinity, and dissolved oxygen were recorded in situ at a depth of approximately 30 cm using a HANNA HI 9829 portable multiparameter instrument equipped with integrated sensors. For nutrient determination, seawater samples were collected in triplicate from each site and transported immediately to the laboratory. Prior to analysis, 10 mL aliquots were filtered through Whatman GF/F glass fiber filters. The concentrations of dissolved inorganic nutrients, including nitrate ($NO_3^-$) and phosphate ($PO_4^{3-}$), were subsequently measured using an auto-analyzer in the Oceanology Laboratory.

***Sampling and acclimatization***. Fronds of *G. gracilis* were collected during low tide from two coastal sites in Morocco: Sidi Rahal (33°28’10.34’’N, 7°59’35.69’’W) and Oualidia (32°44’42’’N, -9°02’50’’W). No strain or clone numbers were assigned, as all samples were collected directly from natural populations. Species identification was confirmed based on morphological characteristics. Samples were transported to the laboratory in dark, cooled containers to minimize stress. Upon arrival, the biomass was carefully washed with filtered seawater collected locally from the Moroccan Atlantic coast and meticulously cleaned to remove epiphytes, debris, necrotic tissues, and other adhering organisms. To assess reproductive activity at each site, the number of cystocarps per thallus was counted in 10 samples (n=10). Representative specimens from both sites

were acclimated under controlled laboratory conditions: artificial cool white light (13±1 µmol photons $m^{-2}$ $s^{-1}$, OSRAM Lumilux Skywhite) and continuously aerated seawater (35±0.5 psu, 20±1°C), maintained in open 10 L plastic containers. Seawater was filtered prior to use. The acclimated biomass was subsequently used for the experimental procedures described below.

***Preparation of biological material***. Following acclimatization, healthy thalli measuring approximately 2–3 cm in length were selected for the cleaning procedure. The thalli were rinsed three times with sterilized seawater and subsequently exposed briefly to sterile distilled water to promote osmotic shock and help reduce contaminating organisms. Individual fragments were then prepared and manually cleaned using sterile cotton swabs. Further decontamination was performed aseptically by gently passing the fragments over 1.0% bacteriological agar prepared with an equal proportion of distilled water and seawater. Throughout the procedure, sterile conditions were maintained. The water and glassware were autoclaved at 121°C for 15 min, with the glassware previously treated with 15% HCl and thoroughly rinsed with distilled water. The cleaning procedure was performed following the approaches described by Redmond et al. (2014) and Freitas et al. (2021).

***Spore release***. Cleaned fragments of female gametophytes were randomly distributed into 20 mL Petri dishes, each containing exactly 20 mature cystocarps (ca. 1.0 mm in diameter), with four dishes per treatment. Four pretreatments were tested to induce cystocarp maturation (Table 1), all conducted in darkness in a controlled chamber maintained at 5°C.

Table 1

Pretreatments applied to induce cystocarp maturation and spore release

| *Pretreatment* | *Substrate* | *Duration* |
|---|---|---|
| Pretreatment A | Moist paper + Plastic | 6 h |
| Pretreatment B | Moist paper + Plastic | 12 h |
| Pretreatment C | GF/2 + Petri dishes | 6 h |
| Pretreatment D | GF/2 + Petri dishes | 12 h |

Treatments A and B were adapted from Kim & Yarish, 2014 for *Gracilaria tikvahiae*. Thalli were wrapped in moist paper towels, sealed in plastic bags, and kept for 6 hours (treatment A) and 12 hours (treatment B). Treatments C and D followed the protocol of Abreu et al. (2011) for *Agarophyton vermiculophyllum* (formerly *Gracilaria vermiculophylla*). In these cases, thallus fragments were placed in Petri dishes containing modified Guillard's F/2 medium (GF/2) formulated for red algae and supplemented with germanium dioxide (GeO2, 1 mL $L^{-1}$) to inhibit diatom growth. Exposure times were 6 hours (treatment C) and 12 hours (treatment D).

Following pretreatments, samples were transferred to a climate-controlled chamber maintained at 20±1°C and subjected to three photoperiod regimes (16/8, 12/12, and 8/16 h light: dark) and three light intensities (15, 40, and 72 µmol photons $m^{-2}$ $s^{-1}$). Light intensity was measured and adjusted using a calibrated quantum PAR sensor (photosynthetically active radiation, µmol photons $m^{-2}$ $s^{-1}$) positioned at the level of the culture vessels inside the experimental chamber. Spore release was assessed one week after the beginning of the experiment. As the density of released spores prevented reliable enumeration of individual spores, spore abundance was estimated indirectly by counting spore aggregates under a stereomicroscope (Stemi 2000-C, Zeiss, Oberkochen, Germany), following the approach previously described by Freitas et al. (2021). A spore aggregate was defined as a visibly distinct cluster of spores that could be recognized as a discrete unit under the stereomicroscope. Based on preliminary observations conducted in our laboratory, each aggregate was estimated to contain approximately 500 spores. Accordingly, the estimated number of released spores was obtained by multiplying the number of counted spore aggregates by 500.

## Results

***Environmental conditions at the study sites***. The environmental parameters recorded at the two study sites, Sidi Rahal and Oualidia, during the morning sampling period (09:00–10:00) from September 2023 to January 2024 are summarized in Tables 2 and 3, respectively.

Table 2

Environmental parameters (mean±SD) recorded at the study site (Sidi Rahal) in the morning between 09:00 and 10:00 during the years 2023-2024

| *Date* | *Temperature (°C)* | *Salinity (PSU)* | *Turbidity (NTU)* | *$PO_4^{3-}$ (µM)* | *$NO_3^-$ (µM)* | *Dissolved oxygen (ppm)* |
|---|---|---|---|---|---|---|
| 01 Sep 2023 | 22.65±0.12 | 36.40±6.53 | 0.47±0.02 | 2.48±0.50 | 17.31±2.21 | 2.36±0.84 |
| 15 Sep 2023 | 21.14±0.10 | 37.17±5.43 | 0.68±0.04 | 3.69±0.45 | 4.62±1.19 | 3.11±1.27 |
| 30 Sep 2023 | 19.13±0.34 | 36.54±6.01 | 0.55±0.01 | 3.46±0.38 | 3.94±0.52 | 2.96±1.46 |
| 01 Oct 2023 | 17.27±0.1 | 36.2±5.22 | 0.72±0.06 | 1.39±0.15 | 10.54±2.08 | 2.76±0.58 |
| 30 Oct 2023 | 17.22±0.23 | 36.37±7.32 | 0.40±0.02 | 0.93±0.07 | 19.47±3.64 | 2.25±0.25 |
| 01 Nov 2023 | 17.28±0.15 | 31.2±4.67 | 0.89±0.03 | 2.07±0.63 | 7.02±1.74 | 5.69±1.41 |
| 30 Nov 2023 | 16.41±0.30 | 37.04±3.89 | 0.52±0.03 | 4.28±1.01 | 4.08±0.95 | 6.91±1.39 |
| 01 Dec 2023 | 17.97±0.13 | 36.92±6.56 | 0.71±0.06 | 0.86±0.08 | 4.57±1.61 | 5.14±1.46 |
| 15 Dec 2023 | 20.35±0.25 | 38.5±6.10 | 0.32±0.04 | 1.69±0.18 | 20.12±5.18 | 4.24±2.01 |
| 30 Dec 2023 | 21.77±0.18 | 37.05±5.63 | 0.43±0.03 | 2.16±0.54 | 6.74±1.71 | 2.45±0.61 |
| 01 Jan 2024 | 21.35±0.29 | 38.01±4.93 | 0.48±0.02 | 1.01±0.11 | 4.57±1.24 | 2.18±0.52 |
| 15 Jan 2024 | 21.9±0.21 | 36.94±6.67 | 0.53±0.04 | 0.94±0.08 | 1.78±0.13 | 2.34±0.14 |

Table 3

Environmental parameters (mean±SD) recorded at the study site (Oualidia) in the morning between 09:00 and 10:00 during the years 2023-2024

| *Date* | *Temperature (°C)* | *Salinity (PSU)* | *Turbidity (NTU)* | *$PO_4^{3-}$ (µM)* | *$NO_3^-$ (µM)* | *Dissolved oxygen (ppm)* |
|---|---|---|---|---|---|---|
| 01 Sep 2023 | 24.18±0.13 | 36.85±6.52 | 0.56±0.03 | 2.72±0.51 | 18.42±2.25 | 2.61±0.82 |
| 15 Sep 2023 | 21.76±0.11 | 37.62±5.68 | 0.72±0.05 | 3.84±0.49 | 5.13±1.28 | 3.28±1.24 |
| 30 Sep 2023 | 19.64±0.33 | 37.15±5.97 | 0.63±0.02 | 3.61±0.43 | 4.46±0.57 | 3.22±1.43 |
| 01 Oct 2023 | 17.82±0.11 | 36.91±5.31 | 0.76±0.06 | 1.58±0.17 | 11.24±2.21 | 2.97±0.63 |
| 30 Oct 2023 | 17.52±0.23 | 37.08±7.21 | 0.47±0.02 | 1.07±0.08 | 20.31±3.68 | 2.48±0.28 |
| 01 Nov 2023 | 17.81±0.16 | 32.26±4.71 | 0.94±0.03 | 2.31±0.61 | 7.64±1.76 | 5.87±1.39 |
| 30 Nov 2023 | 16.93±0.29 | 37.41±4.02 | 0.61±0.03 | 4.43±1.02 | 4.58±0.96 | 7.12±1.37 |
| 01 Dec 2023 | 18.46±0.14 | 37.35±6.71 | 0.81±0.06 | 0.99±0.08 | 5.12±1.64 | 5.51±1.48 |
| 15 Dec 2023 | 20.71±0.25 | 38.74±6.18 | 0.38±0.04 | 1.78±0.20 | 20.83±5.24 | 4.61±2.03 |
| 30 Dec 2023 | 22.34±0.18 | 37.86±5.77 | 0.51±0.03 | 2.37±0.55 | 7.31±1.76 | 2.73±0.64 |
| 01 Jan 2024 | 23.71±0.30 | 38.46±5.08 | 0.54±0.02 | 1.17±0.11 | 4.86±1.29 | 2.42±0.54 |
| 15 Jan 2024 | 22.48±0.22 | 37.31±6.84 | 0.61±0.04 | 1.08±0.09 | 2.06±0.15 | 2.58±0.16 |

Overall, both sites exhibited similar temporal patterns for most environmental variables, although Oualidia generally showed slightly higher values than Sidi Rahal for several parameters. At Sidi Rahal, seawater temperature varied markedly over the sampling period, ranging from 16.41±0.30°C on 30 November 2023 to 22.65±0.12°C on 1 September 2023 (Table 2). Temperatures decreased progressively from September to late November before increasing again during December and January, reaching 21.90±0.21°C by mid-January 2024. A comparable seasonal pattern was observed at Oualidia, where temperature ranged from 16.93±0.29°C to 24.18±0.13°C. The highest temperature was recorded at the beginning of September, whereas the lowest value occurred at the end of November (Table 3). Salinity remained relatively stable throughout the study period at both locations. At Sidi Rahal, values ranged from 31.20±4.67 PSU to 38.50±6.10 PSU, while at Oualidia they ranged from 32.26±4.71 PSU to 38.74±6.18 PSU. The lowest salinity values were recorded on 1 November 2023 at

both sites, whereas the highest values occurred in mid-December. Turbidity showed moderate temporal fluctuations. At Sidi Rahal, turbidity varied between 0.32±0.04 NTU and 0.89±0.03 NTU, with the highest value recorded on 1 November 2023 and the lowest in mid-December. At Oualidia, turbidity ranged from 0.38±0.04 NTU to 0.94±0.03 NTU and followed a similar temporal pattern, with elevated values during early November and reduced values during December. Phosphate concentrations displayed noticeable variability during the sampling period. At Sidi Rahal, $PO_4^{3-}$ concentrations ranged from 0.86±0.08 µM to 4.28±1.01 µM. The highest concentration was observed at the end of November, whereas the lowest was measured at the beginning of December. Similarly, phosphate concentrations at Oualidia ranged from 0.99±0.08 µM to 4.43±1.02 µM, with maximum values also recorded at the end of November. Nitrate concentrations exhibited the greatest temporal variation among the measured nutrients. At Sidi Rahal, $NO_3^-$ values ranged from 1.78±0.13 µM to 20.12±5.18 µM. Elevated concentrations were observed in early September, late October, and mid-December, whereas the lowest concentrations occurred during January. Oualidia showed a similar pattern, with nitrate concentrations ranging from 2.06±0.15 µM to 20.83±5.24 µM. The highest values were recorded in mid-December, while the lowest occurred in mid-January. Dissolved oxygen concentrations also varied considerably throughout the study period. At Sidi Rahal, values ranged from 2.18±0.52 ppm to 6.91±1.39 ppm. The lowest concentrations were measured during January, whereas the highest values occurred at the end of November. At Oualidia, dissolved oxygen ranged from 2.42±0.54 ppm to 7.12±1.37 ppm and showed a similar temporal trend, with maximum concentrations recorded in late November and lower values during January. Overall, the two study sites showed closely comparable seasonal variations in environmental conditions. Oualidia generally exhibited slightly higher temperatures, nutrient concentrations, turbidity, and dissolved oxygen than Sidi Rahal, while salinity values remained within a similar range at both locations throughout the sampling period.

***Number of cystocarps per region***. Data analysis reveals an apparent seasonal variability at both study sites. In Sidi Rahal, the highest values were recorded in spring, peaking in April at 1,552 cystocarps (Figure 1), whereas the lowest values occurred in winter, particularly in December (227.2 cystocarps) and January (238.7 cystocarps).

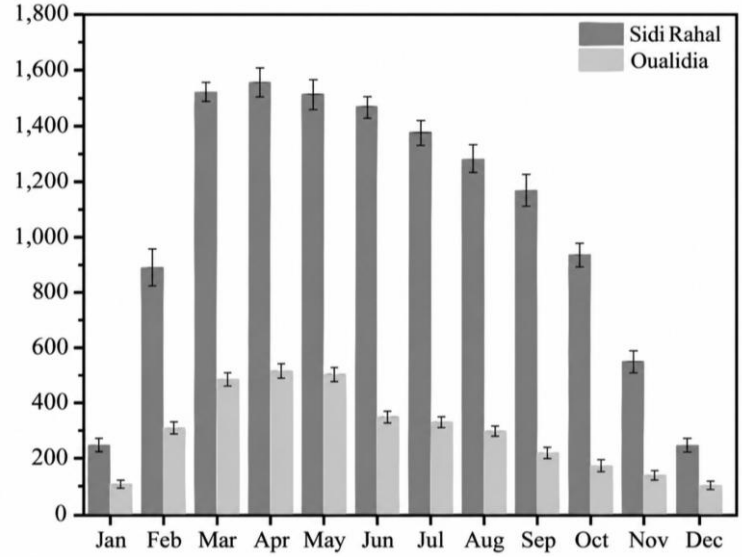


Figure 1. Number of cystocarps at the two sites: Sidi Rahal and Oualidia.

In Oualidia, a similar dynamic was observed, characterized by a gradual increase beginning in February, peaking in April (520.2 cystocarps), followed by a progressive decline to the lowest levels in winter. This seasonal distribution suggests a well-defined annual cycle, with higher reproductive activity in spring. Because the data did not follow a normal distribution, nonparametric statistical analyses were used. At Sidi Rahal, the

Kruskal-Wallis test showed significant variation ($p<0.05$) in cystocarp abundance among months. Post hoc comparisons using Dunn's test with Bonferroni correction, confirmed significant differences between several months. A similar pattern was observed in Oualidia, where the Kruskal-Wallis test ($p<0.05$) also indicated significant monthly variations, further supported by Dunn's post hoc results. The comparison between the two sites, conducted using the Mann-Whitney U test, revealed significant differences in mean cystocarp abundance values between Sidi Rahal and Oualidia, suggesting spatial variability in reproductive activity.

Figure 2 presents the evaluation of the different pretreatments, revealing notable differences between the two sites. In Sidi Rahal, the highest estimated number of released spores was obtained with pretreatment C (7,113.6 spores), followed by pretreatments D (4,360.7 spores) and A (4,229.5 spores), while pretreatment B showed the lowest value (1,016.3 spores). In Oualidia, a similar pattern was observed, with a maximum also recorded for pretreatment C (2,345.6 spores), intermediate values for pretreatments D (1,463.5 spores) and A (1,411.5 spores), and a minimum for pretreatment B (345.6 spores). This distribution highlights a clear hierarchy among the pretreatments, with pretreatment C showing the highest efficiency at both sites, whereas pretreatment B remains significantly less effective. Moreover, the values recorded at Sidi Rahal were consistently higher than those observed at Oualidia. According to the Kruskal-Wallis test ($p<0.05$), a significant difference was found among the pretreatments at Sidi Rahal. Post hoc comparisons were performed using the Duns test with Bonferroni correction, confirming significant differences among pretreatments. Similarly, in Oualidia, the Kruskal-Wallis test ($p<0.05$) indicated a significant difference among the pretreatments, as confirmed by Dunn's post hoc results. Furthermore, the Mann-Whitney U test showed a significant difference in the mean estimated number of spores released between the two sites, confirming that Sidi Rahal exhibited higher sporulation values than Oualidia.

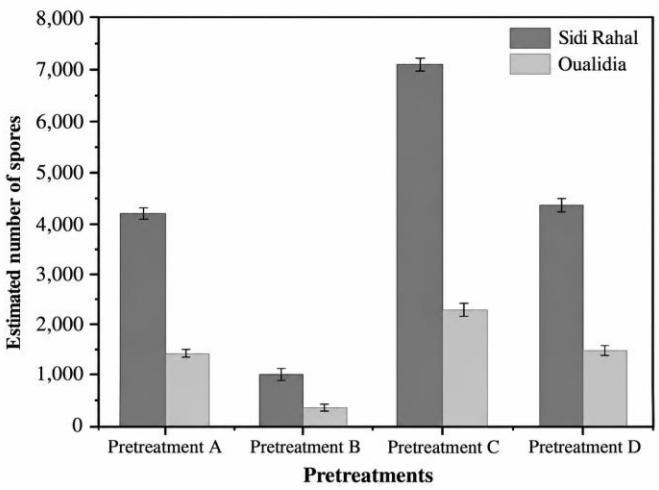



Figure 2. Estimated number of spores released following each pretreatment at Sidi Rahal and Oualidia.

At the Sidi Rahal site (Figure 3), the results highlight the influence of photoperiod and light intensity on spore release. Under the 8/16 photoperiod and low light intensity, the number of released spores increased markedly over time. At low intensity (15 µmol photons $m^{-2}$ $s^{-1}$), values rose from 3,558.2 spores in the first week to 7,488.5 spores in the fourth week. At medium intensity (40 µmol photons $m^{-2}$ $s^{-1}$), the trend was similar but with lower maximum values, reaching 4,069.4 spores in the fourth week. At high light intensity (72 µmol photons $m^{-2}$ $s^{-1}$), spore numbers reached 3,940.6 spores in the fourth week. These results indicate that even under a short photoperiod, moderate light intensities can limit the maximum spore release. In contrast, the combination of a short photoperiod and optimal light intensity promotes a maximal reproductive response.

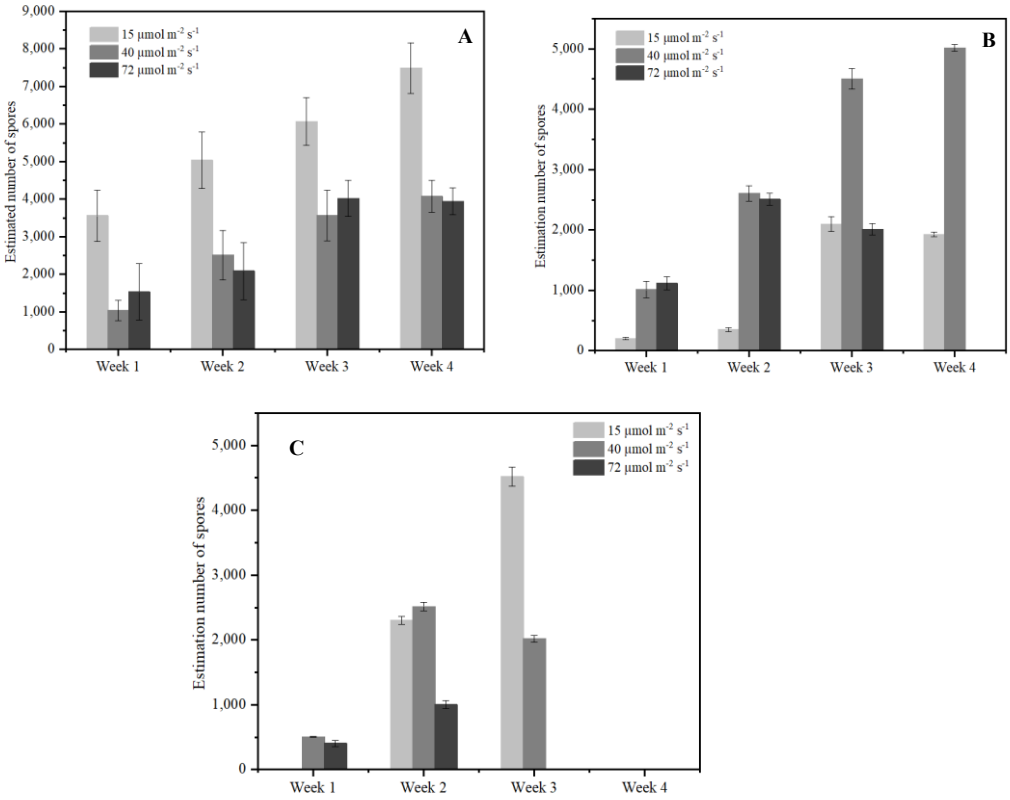



Figure 3. Estimation number of spores released from Sidi Rahal under different photoperiods and light intensities (A: photoperiod 8/16, B: photoperiod 12/12, C: photoperiod 16/8).

Under the 12/12 photoperiod, spore numbers were initially low but increased rapidly, particularly at medium light intensity (40 µmol photons $m^{-2}$ $s^{-1}$), reaching a peak of 5,014.4 spores in the fourth week. At high light intensity (72 µmol photons $m^{-2}$ $s^{-1}$), values remained lower, suggesting that spreading light over a longer duration can moderate the effect of high instantaneous light intensities. Under the long photoperiod (16/8), responses were temporally shifted, with peaks occurring in the second and third weeks, depending on light intensity, such as 2,302.6 and 4,517 spores at 15 µmol photons $m^{-2}$ $s^{-1}$, before dropping to 0 in the fourth week. This indicates that sensitivity to extended light duration is modulated by the average light intensity received.

At Oualidia (Figure 4), the effects were generally less pronounced. Under the short photoperiod (8/16), spore numbers ranged from 1,160.4 to 2,529 spores, depending on the week and light intensity. Higher spore release was generally observed under lower light intensity. Under the medium photoperiod (12/12), values remained relatively low, ranging from 65.5 to 1,667 spores, while under the long photoperiod (16/8), maximum levels were moderate (1,501 spores in the third week). Overall, these results suggest that lower light intensity and shorter photoperiods are associated with greater spore release at Oualidia, although the magnitude of the response varied depending on the experimental conditions. At Sidi Rahal, spore numbers varied significantly across weeks, photoperiods, and light intensities, as indicated by Kruskal-Wallis tests ($p<0.05$). Post hoc comparisons were performed using Dunn's test with Bonferroni correction. A similar pattern was observed at Oualidia, where significant differences were also detected using Kruskal-Wallis tests followed by Dunn's post hoc analyses. Comparisons between the two sites using Mann-Whitney U tests indicated significant differences in spore release between Sidi Rahal and Oualidia.

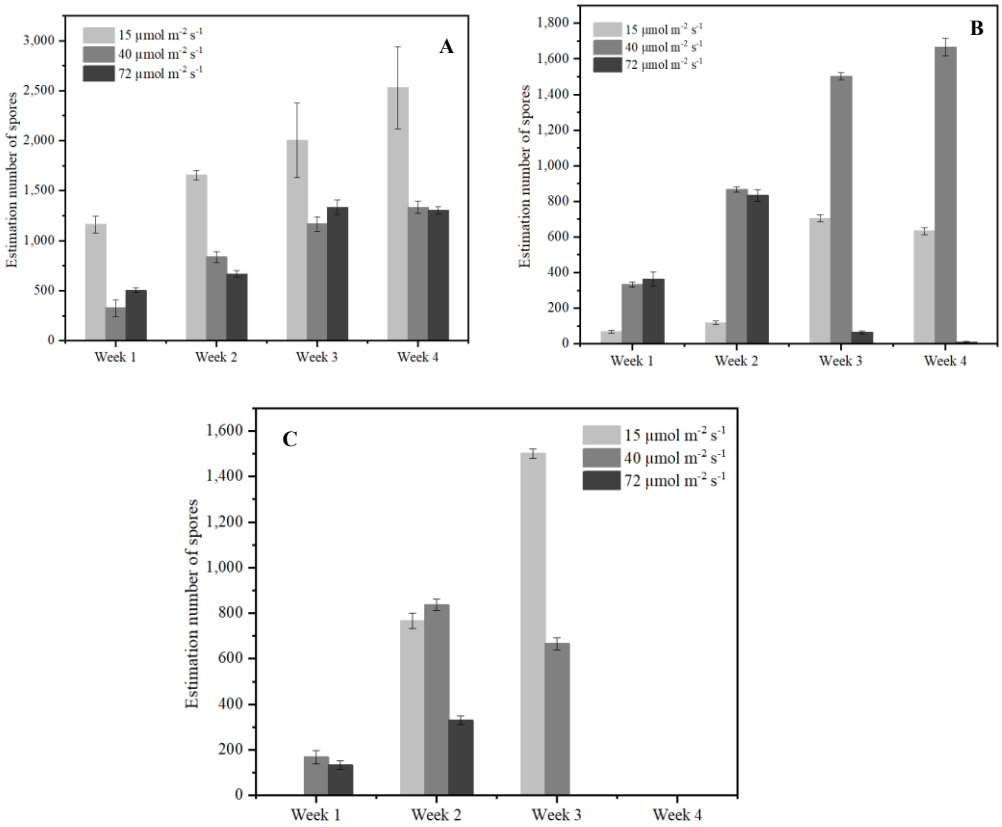



Figure 4. Estimation number of spores released from Oualidia under different photoperiods and light intensities (A: photoperiod 8/16, B: photoperiod 12/12, C: photoperiod 16/8).

**Discussion**. At Sidi Rahal, a clear interaction between photoperiod and light intensity was observed. Under a short photoperiod (8/16), a light intensity of 15 µmol photons $m^{-2}$ $s^{-1}$ promoted the highest spore release (7488.5 spores in the fourth week), whereas higher intensities limited the response. This trend is consistent with the findings of Zhou et al. (2013), who reported that lower light levels can stimulate sporulation, as well as observations on *G. corticata* (Subba Rangaiah, 1983). However, these results differ from those of Kaliaperumal et al. (1986) for *G. corticata var. cylindrica*, in which high light intensities favoured spore release. At Oualidia, the medium photoperiod (12/12) did not induce significant spore release, in contrast to Michetti et al. (2013), who found this condition favorable for *G. gracilis*. Such inter-site differences likely reflect distinct local ecological conditions, including water turbidity, natural irradiance, and the physiological state of the thalli. Boraso de Zaixso (1987) noted that seasonality limits reproductive activity in *G. gracilis* (previously named *Gracilaria verrucosa*), with a maximum of cystocarpic thalli observed in summer and autumn. No effect of irradiance was detected on carpospore release in *G. gracilis*, whereas in other species, such as *Gracilaria foliifera*, low irradiance appeared to stimulate release (Friedlander & Dawes, 1984). In temperate species such as *Gracilaria pacifica*, maximum release occurred at higher temperatures, around 21°C along the northwest coast of Mexico (Garza-Sanchez et al., 2000). Our study also used a temperature of 21°C. However, *G. gracilis* showed the highest proportion of cystocarpic thalli at 13°C (Michetti et al., 2013). These authors further reported a spore potential of approximately 1000 carpospores per cystocarp per day, with prolonged discharge over the first two weeks.

In other Gracilariales, several studies have reported that maximum release occurs during the first three to four days (Garza-Sánchez et al., 2000; Guzmán-Urióstegui & Robledo, 1999; Mantri et al., 2009; Mohan Joseph & Krishnamurthy, 1977), confirming the crucial role of the initial phase in dispersal. For *G. cornea*, Guzmán-Urióstegui and Robledo (1999) showed that a short photoperiod promotes release, whereas Rao and Kaliaperumal (1983) observed the opposite effect in some Gelidiales, revealing

pronounced interspecific plasticity in light responses. Overall, these findings indicate that photoperiod and light intensity interact in complex ways, with this interaction modulated by the ecological history of populations and their acclimation capacity (García-Jiménez & Robaina, 2019). The convergence of our observations with those of Freitas et al. (2021) and López-Campos et al. (2022) underscores the existence of a regular seasonal cycle, as well as the influence of abiotic factors and experimental protocols on modulating release intensity.

Inter-site variability (Sidi Rahal vs. Oualidia) suggests that local hydrological and ecological conditions strongly influence the magnitude of the phenomenon. These results are of applied interest in the context of macroalgal aquaculture development in Morocco (Angriani et al., 2021), where controlling the production of high-quality seed material is a strategic priority and offers promising perspectives for the sustainable development of this industry. Continuous darkness has also been reported to enhance spore release in *G. foliifera* (Friedlander & Dawes, 1984), *G. corticata* (Rao & Subbarangaiah, 1981), and *Gracilariopsis lemaneiformis* (Jayasuriya, 1993). Santelices (1990) noted that the influence of light and darkness on spore release remains uncertain, although low light generally seems to stimulate release. Kain & Destombe (1995) indicated that different *Gracilaria* species exhibit peak release at distinct times. In *G. gracilis*, Lefebvre et al. (1987) observed rhythmic release over approximately one month, whereas Michetti et al. (2013) reported a maximum carpospore release on the seventh day, with nearly 70% of the spores emitted during the first two weeks. Finally, Rao and Kaliaperumal (1983) found that two Gelidiales species released more spores under a more extended photoperiod, suggesting that light duration is a key factor regulating reproductive processes.

This study demonstrates that photoperiod and light intensity exert a combined and site-dependent influence on the reproductive behaviour of *G. gracilis*. At Sidi Rahal, spore release was maximized under a short photoperiod and low intensity. In contrast, at Oualidia, moderate photoperiods elicited limited responses, underscoring the role of local environmental factors, such as turbidity, irradiance, and thallus physiology. The variability observed across sites, together with discrepancies relative to previous reports, underscores the high degree of ecological plasticity within *Gracilaria* species. The contrasting responses to light regimes reported across taxa and geographical regions suggest that reproductive processes in this genus are modulated by both genetic adaptation and acclimation history. Overall, the findings confirm that photoperiod and irradiance are key abiotic drivers regulating spore release, but their effects depend strongly on local ecological contexts. This interaction between environmental cues and physiological responses should be carefully considered when designing controlled culture systems. From an applied perspective, understanding the light-dependent reproductive patterns of *G. gracilis* provides valuable guidance for optimizing spore production and seedling quality in aquaculture settings. These insights inform the development of sustainable *Gracilaria* cultivation strategies in Morocco and other temperate regions, thereby supporting the long-term stability and productivity of the seaweed industry.

**Conclusions**. This study demonstrated that the reproductive performance of *G. gracilis* varies according to season, source population, pretreatment, photoperiod, and light intensity. Cystocarp abundance showed a clear seasonal pattern at both Sidi Rahal and Oualidia, with the highest values recorded in April, indicating March–May as a favourable period for collecting reproductive material. Sidi Rahal consistently exhibited higher cystocarp abundance and higher estimated spore release than Oualidia. Among the tested pretreatments, the 6-h exposure to GF/2 medium under dark conditions (Pretreatment C) produced the highest estimated number of released spores at both sites. Furthermore, at Sidi Rahal, the combination of a short photoperiod (8/16 h light:dark) and low light intensity (15 µmol photons $m^{-2}$ $s^{-1}$) resulted in the highest estimated spore release. These findings highlight the importance of optimizing both the timing of cystocarp collection and the environmental conditions used to induce spore release. The results provide useful information for developing controlled reproductive propagation protocols for *G. gracilis* and may contribute to the development of sustainable spore-based aquaculture practices along the Moroccan Atlantic coast.

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**Data Availability**. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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