

Effect of Light Intensity on the Release and Attachment of *Gracilaria sp.* Spores

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ABSTRACT

The availability of high-quality seedlings remains a major constraint in the sustainable expansion of *Gracilaria* aquaculture, particularly when vegetative propagation dominates seed supply. Spore culture offers an alternative route for producing generative seedlings, but its success depends strongly on environmental conditions during the early reproductive phase. This study evaluated the effect of three light intensities on the release and attachment of *Gracilaria sp.* spores under laboratory conditions. A completely randomized design was applied with three treatments, namely 500 lux, 1000 lux, and 1500 lux, each with three replicates. Fertile thalli in the carpospore stage were acclimatized, sterilized, and cultured in sterile seawater enriched with Grund medium under an 8 h light:16 h dark photoperiod at 25–27°C. Spore release was observed using a Sedgwick–Rafter chamber, whereas attached spores were counted under a stereo microscope. Light intensity significantly affected both response variables (one-way ANOVA, $P < 0.05$). The 1000 lux treatment produced the highest spore release, reaching 720 ± 35 spores/cystocarp on day 4, with the highest mean release of 452 spores/cystocarp. The same treatment also yielded the highest spore attachment, peaking at 520 ± 49 spores/cystocarp on day 3, with a mean of 391 spores/cystocarp. These findings indicate that moderate light intensity provides the most favorable condition for early reproductive performance in *Gracilaria sp.* spore culture and may support the development of more reliable seedling production systems for seaweed aquaculture

INTRODUCTION

Seaweed aquaculture is one of the most important components of coastal bioresource production because it contributes to food systems, hydrocolloid industries, livelihoods, and the emerging blue bioeconomy. Red seaweeds of the genus *Gracilaria* are especially valuable because they are widely cultivated and used as a major raw material for agar production, while also serving as an important commodity in brackishwater and coastal farming systems (McHugh, 2003; Ferdouse et al., 2018). Despite this economic relevance, the continuity of cultivation still depends heavily on the availability of planting material of consistent quality. In many production systems, seed supply remains dominated by vegetative propagation, which can gradually reduce vigor and increase the risk of quality decline across repeated cycles. For this reason, generative propagation through spores has attracted attention as a complementary strategy for improving seed quality and continuity.

Light is a primary environmental factor governing algal physiology because it regulates photosynthesis, carbon assimilation, energy transfer, growth, and reproduction. In red algae, light conditions do not merely sustain metabolism; they also influence reproductive timing, propagule formation, and post-release development (Dawes, 1981; Dwidjoseputro, 1994; Nelson & Smith, 1999). Within this framework, light intensity is a central variable. It determines the amount of energy available for photosynthetic reactions and thereby shapes the physiological status of the thallus and its reproductive structures. Indriani and Sumiarsih (1999) emphasized the importance of light intensity in growth metabolism and photosynthesis, while Susilowati et al. (2019) described light as a limiting factor in photosynthetic performance.

The reproductive biology of marine macroalgae is strongly influenced by environmental signals. Studies on seaweed reproduction have shown that light quality, photoperiod, temperature, and nutrient availability can all modify fertility and propagule release (Dring, 1971; Hoffman, 1987; Charrier et al., 2017). In practical culture systems, this means that reproductive output depends not only on the genetic condition of the broodstock, but also on whether environmental conditions are sufficiently favorable to trigger spore liberation and support subsequent settlement. The first step in successful spore-based cultivation is the release of viable spores from reproductive tissues, followed by rapid attachment to a suitable substrate and continued development into juvenile thalli (Lobban & Harrison, 1994; Massad et al., 2020).

Previous studies have shown that the duration and properties of illumination can influence propagule behavior in macroalgae. Muñoz et al. (2016), for example, reported significant variation in propagule development under different light regimes, indicating that irradiance-related factors deserve closer attention in reproductive studies. Similarly, Hariyati (2008) demonstrated that environmental optimization can affect the number of released spores in red algae, while Hirijal et al. (2024) confirmed the importance of photoperiod in the release and attachment of *Gracilaria* spores. Suryono (2012) also suggested that environmental manipulation can accelerate spore release in *Gracilaria*. However, although these studies support the relevance of light as a regulatory factor,

information remains limited regarding the optimal light intensity for maximizing both spore release and subsequent attachment in *Gracilaria* sp.

This gap is important from both biological and applied perspectives. If the light intensity is too low, photosynthetic activity may be insufficient to support reproductive metabolism and spore liberation. Conversely, if light intensity is too high, physiological stress may occur and reduce reproductive efficiency. Raikar et al. (2001) showed that the performance of *Gracilaria* species is highly responsive to environmental conditions, including light, while Kumar et al. (2018) emphasized that reproductive development in marine algae is sensitive to the optical environment. Such evidence suggests that *Gracilaria* spore culture requires an irradiance range that is high enough to stimulate metabolism but not so high as to impose stress.

The present study was designed to address this issue by testing three light intensities, namely 500, 1000, and 1500 lux, on the release and attachment of *Gracilaria* sp. spores. The working hypothesis was that a moderate light intensity would provide the most favorable condition for both reproductive responses. By identifying a practical irradiance level for spore-based culture, this study aims to contribute to the development of more effective seed production protocols for *Gracilaria* aquaculture. The findings are expected to strengthen the technical basis for generative propagation and thereby support more sustainable and reliable seedling supply systems.

LITERATURE REVIEW

Light as a Regulator of Seaweed Physiology and Reproduction

Light is one of the most fundamental environmental variables governing the biology of marine macroalgae because it directly controls photosynthesis, energy conversion, and carbon assimilation, all of which support vegetative growth and reproductive performance. In seaweeds, light does not act merely as a source of illumination; rather, it functions as a regulatory signal that influences physiological metabolism, morphogenesis, and the timing of reproductive events. Early physiological interpretations emphasized that the photosynthetic apparatus of algae depends strongly on the intensity and duration of light exposure, because these parameters determine the amount of usable energy available for cellular functions (Dawes, 1981; Dwidjoseputro, 1994; Sze, 1993). Through photosynthesis, dissolved inorganic carbon is incorporated into algal tissues, while light-driven electron transport generates the reducing power and ATP required for biosynthesis and development (Dwidjoseputro, 1994; Sze, 1993).

In red algae, the role of light extends beyond general growth to include reproductive differentiation and propagule production. Nelson and Smith (1999) reported that variation in light conditions can alter photosynthetic responses in *Gracilaria*, indicating that irradiance is closely linked to the physiological state of the thallus. Indriani and Sumiarsih (1999) similarly described light intensity as a critical factor in growth metabolism and photosynthetic performance, whereas Susilowati et al. (2019) identified light as a limiting factor in photosynthesis. These observations provide a physiological basis for expecting reproductive output to vary with irradiance.

The reproductive consequences of light have been discussed in classical and modern physiological literature. Dring (1971) argued that increasing irradiance may stimulate spore-related reproductive processes only up to an optimal threshold, after which excess light can become inhibitory. Hoffman (1987) further emphasized that algae, especially red seaweeds, respond to photoperiod and illumination regime in ways that affect propagule production and development. More broadly, Charrier et al. (2017) highlighted that seaweed growth and reproduction are shaped by environmental controls such as light, temperature, and nutrient supply, underscoring that reproductive success is fundamentally an ecophysiological phenomenon rather than a purely genetic one. Taken together, this body of literature shows that light intensity is both a metabolic driver and an ecological signal that can modulate reproductive efficiency in cultured seaweeds.

Spore Release, Attachment, and Early Development in Gracilaria sp.

Spore-based propagation has been recognized as a promising alternative to exclusive reliance on vegetative propagation in commercially cultivated red seaweeds. In *Gracilaria*, the use of spores for seedling production is attractive because it offers a pathway toward more diverse, potentially more vigorous, and more sustainable planting material. Technical guidance for *Gracilaria* seed production has therefore increasingly addressed reproductive material handling, settlement, and nursery-stage management (Lideman et al., 2016). More generally, seaweed industry assessments have emphasized that reliable seed supply remains a strategic issue in seaweed aquaculture development (McHugh, 2003; Ferdouse et al., 2018).

The biological sequence of spore culture consists of at least two critical early events: spore release from reproductive structures and subsequent attachment to a suitable surface. Lobban and Harrison (1994) described attachment as the first crucial requirement after propagules are released, because settled spores form the basis for continued development into juvenile thalli. In applied cultivation systems, this step is decisive, since the number of successfully attached spores determines the pool of individuals available for further growth. Massad et al. (2020) reinforced this interpretation by showing that improved spore seeding and settlement technologies can enhance commercial red-seaweed culture performance.

Several studies have addressed the environmental control of spore release in red seaweeds, including *Gracilaria*. Hariyati (2008) demonstrated that optimization of environmental conditions can influence the number of spores released in red algae, supporting the idea that reproductive output is manageable through culture design. Hartinah et al. (2014) further examined spore release in *Gracilaria* and provided an operational framework for quantifying released spores per cystocarp, which remains relevant for laboratory-based reproductive studies. Suryono (2012) also suggested that environmental shock can accelerate spore release in *Gracilaria*, implying that propagule liberation is highly responsive to external cues.

Beyond release itself, the timing and success of settlement may depend on the physiological status of both the propagules and the surrounding culture environment. Choi and Lee (2020) proposed that reproductive events in marine algae may be associated with rhythmic or synchronized biological control, which could help explain temporal peaks in spore behavior observed during culture. Rao and Rangaswamy (2019) reported that nutrient limitation may reduce spore attachment in red seaweeds, indicating that settlement success is not determined by light alone. Similarly, Kumar et al. (2018) noted that environmental conditions, including the optical environment, influence algal growth and reproduction, while Roleda and Hurd (2019) argued that nutrient enrichment may not fully compensate when light conditions are not favorable. These studies collectively suggest that successful spore culture depends on an integrated balance of light, nutrient availability, and general culture quality.

Muñoz et al. (2016), although working on kelp gametophytes rather than *Gracilaria*, demonstrated that propagule development can vary substantially under different light regimes. This evidence is relevant because it supports the broader principle that reproductive development in macroalgae is highly sensitive to optical conditions. In *Gracilaria*, where propagule release and attachment are essential to generative seedling production, such sensitivity implies that the identification of suitable irradiance conditions is both biologically meaningful and practically necessary.

Environmental Optimization for Gracilaria Spore Culture

Optimization of spore culture requires attention not only to light intensity but also to the wider set of environmental and technical factors that sustain broodstock viability and post-release development. Temperature is one of the most frequently cited co-determinants of reproductive success. Aslan (1998) reported that the favorable temperature range for acclimatization and spore-release processes in marine algae lies between 26 and 33°C, while Raikar et al. (2001) showed that temperatures below 25°C may reduce spore release in *Gracilaria*. These findings indicate that light-response experiments cannot be interpreted independently of thermal conditions.

Nutrient availability and medium quality also play major roles in reproductive culture. Andersen (2005) provided standardized procedures for algal culture media, including nutrient-enriched formulations such as Grund medium, which are commonly applied to support survival and early development in laboratory systems. Gunawan (1987), from a broader tissue-culture perspective, emphasized the importance of aseptic technique and controlled preparation, principles that remain essential in seaweed reproductive work. Culture cleanliness is particularly important during spore release and settlement because contamination may interfere with propagule viability, substrate condition, and observation accuracy.

Photoperiod is another key variable that interacts with light intensity. Hirijal et al. (2024) reported that an 8 h light:16 h dark regime affected the release and attachment of *Gracilaria* spores, indicating that light duration and light quantity should be considered together. This is consistent with earlier reproductive ecology perspectives suggesting that seaweed propagules respond

not simply to the presence of light but to a coordinated regime of irradiance and exposure time (Hoffman, 1987). From a methodological standpoint, the use of a controlled photoperiod helps separate the effects of light intensity from those of fluctuating daily exposure.

The practical importance of environmental optimization becomes clear when considered in relation to aquaculture objectives. A culture system that maximizes spore release but fails to support attachment or early development is of limited value. Conversely, a system that promotes stable attachment but produces few viable spores will also remain inefficient. The relevant literature therefore points toward the need for a balanced reproductive environment in which broodstock condition, light intensity, photoperiod, temperature, nutrient supply, salinity, and substrate quality are mutually supportive (Charrier et al., 2017; Kumar et al., 2018; Roleda & Hurd, 2019). Within this integrated framework, light intensity emerges as a particularly practical variable for manipulation because it can be controlled precisely in hatchery settings and directly influences both metabolic activation and reproductive response.

Overall, the literature indicates that *Gracilaria* spore culture is shaped by a combination of physiological and environmental controls, yet specific evidence on the optimum light intensity for both spore release and attachment remains limited. Existing studies clearly establish the importance of light, photoperiod, temperature, and nutrients, but few directly compare moderate and higher irradiance levels in a focused *Gracilaria* reproductive experiment. This unresolved issue justifies further investigation and supports the need for studies that identify a practical irradiance range for improving spore-based seedling production in seaweed aquaculture.

METHODOLOGY

Study site and experimental period

The study was conducted from May to October 2025 at the Tissue Culture Laboratory and the Marine Hatchery Laboratory, Department of Aquaculture, Politeknik Pertanian Negeri Pangkajene Kepulauan, Indonesia. The work consisted of preliminary preparation followed by the main experiment over two cycles. All culture activities were conducted under controlled laboratory conditions to reduce environmental variability during the reproductive phase.

Broodstock collection and acclimatization

The biological material used in this study was fertile *Gracilaria* sp. in the carpospore stage (carposporophyte). Broodstock was collected from Biringkassi, Pangkep Regency, and transported to the laboratory in seawater-filled seed bags placed inside styrofoam containers. During transport, the temperature was maintained at approximately 25°C. This handling approach was intended to preserve broodstock viability before acclimatization. Aslan (1998) reported that a suitable temperature range for marine algal acclimatization and spore release is 26–33°C, whereas Raikar et al. (2001) noted that temperatures below 25°C may reduce spore release in *Gracilaria*. Based on these considerations, temperature was managed carefully throughout transport and laboratory handling.

Upon arrival, the thalli were rinsed with seawater to remove debris and epiphytes and then acclimatized in aquaria for 2–7 days. This acclimatization period was necessary to stabilize the physiological condition of the broodstock after transfer from the field environment to the laboratory culture system.

Equipment sterilization and medium preparation

Culture vessels and tools, including Petri dishes, Erlenmeyer flasks, measuring pipettes, and beakers, were first washed and dried. The equipment was then wrapped in aluminum foil, secured with rubber bands, and sterilized in an autoclave at 121°C for 20 min under 1 atm pressure. Sterile handling procedures followed standard laboratory practice for plant and algal tissue work (Gunawan, 1987).

Seawater used in the experiment was first settled in a storage tank for 1–2 days. Salinity was then adjusted to 30–33 ppt for broodstock acclimatization and spore-release culture. Before use, the seawater was filtered through 0.45 µm Whatman filter paper and autoclaved at 121°C for 20 min under 1 atm pressure. After cooling, the seawater was used as the culture medium base.

Grund medium was used as the nutrient source for spore culture. Following Andersen (2005), the medium was prepared from 940 mL of filtered sterile natural seawater supplemented aseptically with 10 mL of each stock solution. This nutrient enrichment was intended to support spore survival and early development during the observation period.

Thallus preparation and spore-culture procedure

After acclimatization, fertile thalli were transferred to the tissue culture laboratory for preparation. Sections containing five spore sacs were cut using a sterile scalpel or razor blade while held with forceps on a cutting board. The cut thallus pieces were returned to sterile seawater in plastic Petri dishes.

To minimize contamination, the thallus fragments underwent repeated washing and sterilization. Pieces containing cystocarps were placed in a 100 mL Erlenmeyer flask filled with sterile seawater and shaken to dislodge residual impurities. This procedure was repeated three times. The material was then treated in 100 mL sterile seawater containing one drop of household detergent per 100 mL and 1% betadine, followed by shaking for 2–3 min. The fragments were then drained and rinsed repeatedly until the odor of detergent and iodine disappeared. Rinsing was conducted three times, with sterile seawater sprayed over the material during draining to facilitate cleaning. Finally, the thalli were placed on tissue paper until surface moisture disappeared and were then transferred to culture dishes.

The sterilized thalli were introduced into experimental media according to treatment. During culture, room temperature was maintained at 25–27°C. The photoperiod was set at 8 h light and 16 h dark, following the illumination regime reported by Hirijal et al. (2024). Spores that failed to remain viable after release were identified by pale coloration and a lack of visible cellular development within 24 h.

Experimental design

The experiment employed a completely randomized design with three light-intensity treatments: 500 lux (A), 1000 lux (B), and 1500 lux (C). The selected intensities were based on the reference range used by Hariyati (2008). Each treatment consisted of three replicates.

Table 1. Experimental treatments used in the study

Treatment	Light intensity
A	500 lux
B	1000 lux
C	1500 lux

Observation of spore release and attachment

Observations were divided into two response variables: released spores and attached spores. Released spores were first observed 24 h after thallus placement in culture medium. The number of released spores was measured from the culture medium after homogenization by gentle stirring. A 1 mL sample was transferred to a Sedgwick–Rafter chamber and counted under a microscope across 10 fields of view. The number of released spores per cystocarp was calculated according to Hartinah et al. (2014):

$$[S_c = (S_t / C) V]$$

where: (S_c) is the number of released spores per cystocarp (spores/cystocarp), (S_t) is the number of spores counted in the Sedgwick–Rafter chamber, (C) is the number of cystocarps cultured in the medium (25 cystocarps), and (V) is the culture-medium volume (30 mL).

Attached spores were observed on the base of the Petri dish using a stereo microscope at 10 × 5 magnification with a viewing area of 0.1256 cm². The substrate area was derived from the bottom of a Petri dish with a diameter of 9 cm. The number of attached spores per cystocarp was calculated using the following equation:

$$[S_m = S_a A]$$

where; (S_m) is the number of attached spores on the substrate (spores/cystocarp), (S_a) is the average number of spores observed per field of view, and (A) is the substrate area.

Statistical analysis

Data were tabulated in Microsoft Excel 2019 and analyzed using one-way analysis of variance (ANOVA). When the ANOVA indicated a significant treatment effect, means were compared using the Least Significant Difference test at the 95% confidence level. Statistical analysis was performed in SPSS version 22.

RESEARCH RESULT

Effect of light intensity on spore release

The number of spores released by *Gracilaria* sp. fluctuated over the six-day observation period and differed among light-intensity treatments. On the first day, spore release was still relatively low in all treatments, but the 1000 lux treatment showed the highest initial response, followed by 1500 lux and 500 lux. This early pattern indicates that moderate irradiance was more effective in stimulating the onset of spore release than either lower or higher light exposure.

Spore release increased gradually during the next observation days and reached a clear maximum on day 4. The strongest response was obtained under 1000 lux, which produced 720 ± 35 spores/cystocarp. By comparison, release on day 4 under 500 lux was only 360 ± 40 spores/cystocarp, while the 1500 lux treatment produced 460 ± 104 spores/cystocarp. The mean number of released spores was also highest under 1000 lux, reaching 452 spores/cystocarp. These results demonstrate that the intermediate irradiance level consistently outperformed the lower and higher treatments in terms of both peak and average spore release.

After day 4, the number of released spores declined in all treatments on days 5 and 6. Although the decline was general, the 1000 lux treatment maintained a comparatively higher release level than the other treatments. This temporal pattern suggests that spore liberation was strongest within a limited reproductive window, after which the effective release capacity of the cultured cystocarps declined.

One-way ANOVA indicated that light intensity significantly affected the number of released spores ($P < 0.05$). The statistical result confirms that the observed differences among the three irradiance treatments were not random but reflected a genuine treatment response.

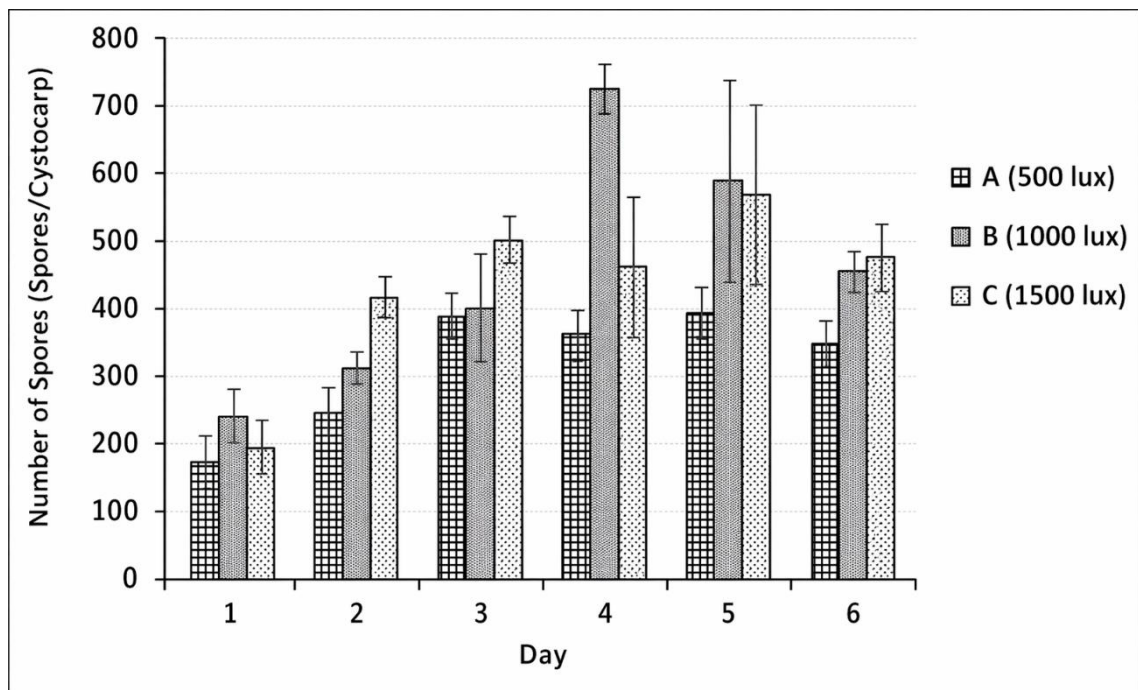


Figure 1. Effect of different light intensities on the number of *Gracilaria* sp. spores released during six days of observation.

Effect of light intensity on spore attachment

Spore attachment also showed a distinct temporal pattern and varied among treatments. During the first day of observation, the number of attached spores remained low across treatments, although the 1500 lux treatment exhibited the highest initial attachment. This early response did not persist, however, and stronger differentiation among treatments emerged during the following days.

A marked increase in spore attachment occurred on days 2 and 3, especially under 1000 lux. The highest attachment value recorded in the entire study was observed on day 3 in treatment B, where the number of attached spores reached 520 ± 49 spores/cystocarp. In addition to achieving the highest peak, the 1000 lux treatment also produced the highest mean attachment value, namely 391 spores/cystocarp. By contrast, the 500 lux treatment consistently generated the lowest attachment throughout the observation period.

Following the day-3 peak, the number of attached spores decreased in all treatments. The reduction after the peak suggests that the most effective settlement phase occurred relatively early and was followed by a decline in the visible number of attached spores or in the persistence of newly settled propagules under the culture conditions used.

The ANOVA result showed that light intensity significantly affected the number of attached spores on the substrate ($P < 0.05$). As in the release analysis, the 1000 lux treatment represented the most favorable condition among the tested irradiance levels.

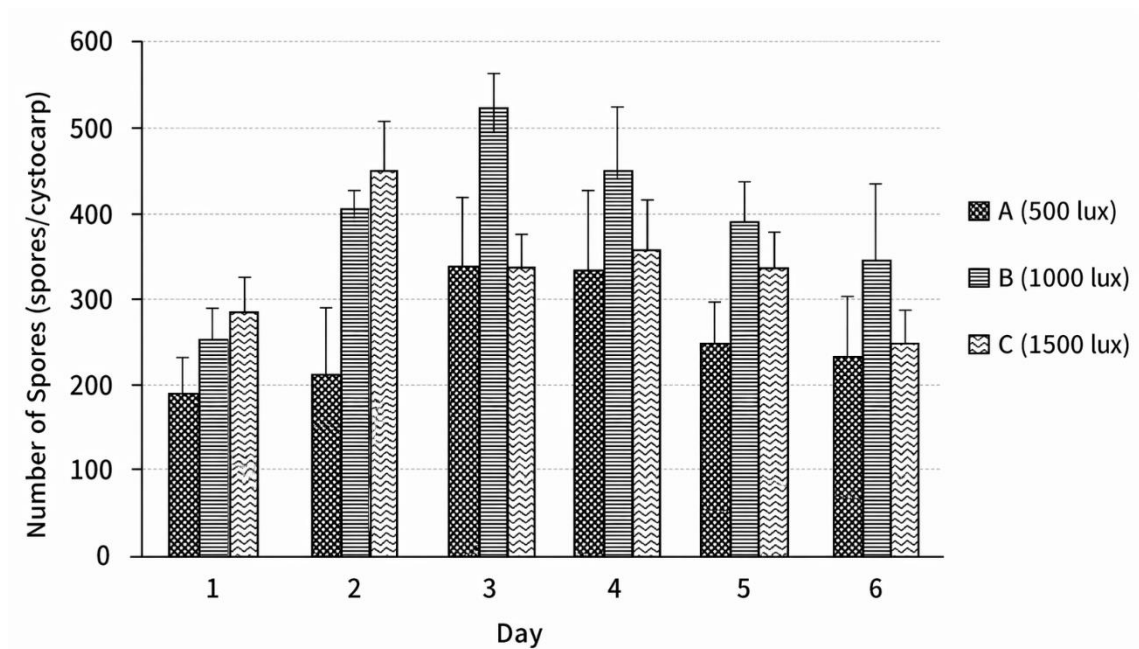


Figure 2. Effect of different light intensities on the number of *Gracilaria* sp. spores attached during six days of observation.

Summary of the main quantitative responses

To facilitate comparison among treatments, the principal quantitative outcomes reported in the experiment are summarized in Table 2.

Table 2. Main quantitative outcomes of spore release and attachment under different light intensities

Response variable	500 lux	1000 lux	1500 lux
Peak released spores (spores/cystocarp)	360 ± 40	720 ± 35	460 ± 104
Day of peak release	4	4	4
Mean released spores (spores/cystocarp)	Not reported	452	Not reported
Peak attached spores (spores/cystocarp)	Not reported	520 ± 49	Not reported
Day of peak attachment	Not reported	3	Not reported
Mean attached spores (spores/cystocarp)	Not reported	391	Not reported

DISCUSSION

The present study demonstrates that light intensity is a decisive factor in the early reproductive performance of *Gracilaria* sp. Under the tested laboratory conditions, 1000 lux produced the strongest response in both spore release and spore attachment, indicating that a moderate irradiance level was more favorable than either 500 lux or 1500 lux. This finding supports the general view that light stimulates reproductive processes only within an optimal physiological range. Dring (1971) proposed that increasing light intensity can enhance spore-related reproductive activity up to a threshold, after which excessive exposure may inhibit the process. The present data fit this pattern well. The 500 lux treatment likely provided insufficient energy to maximize reproductive metabolism, whereas 1500 lux, although not entirely inhibitory, was less effective than 1000 lux and may have imposed a degree of physiological stress.

The role of light in this experiment can be interpreted through its fundamental relationship with photosynthesis. In macroalgae, light energy drives electron transport and carbon fixation, producing ATP and reducing power required for biosynthesis, growth, and reproductive function (Dwidjoseputro, 1994; Sze, 1993). The production of spores and their release from reproductive structures therefore depend on the physiological status of the parent thallus. Dawes (1981) described light as a basic requirement for seaweed growth, and Hariyati (2008) showed that the energy generated through photosynthesis is linked to the development of reproductive organs in algae. The superior performance observed at 1000 lux indicates that this intensity likely provided a favorable balance between metabolic stimulation and cellular stability.

The temporal pattern of release, with a clear peak on day 4 followed by decline, is also biologically meaningful. During the early days of culture, the

cystocarps presumably remained highly responsive and capable of releasing spores under the imposed light regime. Once the peak was reached, the decline may reflect depletion of readily releasable propagules, progressive physiological exhaustion of reproductive tissues, or a reduction in cystocarp viability after continued exposure. The authors' original observation that excessive exposure may reduce reproductive efficiency is consistent with this interpretation. Charrier et al. (2017) emphasized that seaweed fertility and spore release are determined by interacting environmental factors, including light, temperature, and nutrients. Thus, although light intensity was the main experimental variable here, the release trajectory almost certainly reflects a broader physiological process involving the condition of the reproductive thallus over time.

The attachment results complement the release pattern and strengthen the conclusion that 1000 lux was the most suitable treatment. The initial increase in settlement during days 2 and 3 suggests that released propagules needed a short post-release period before maximum attachment became visible. Lobban and Harrison (1994) noted that the first essential step after propagule release is the successful location of and attachment to a surface. In the present study, this process was most successful under the intermediate light treatment. Massad et al. (2020) similarly highlighted that early settlement is a decisive stage because it directly determines the number of individuals that can proceed to subsequent culture phases.

The observation that 1500 lux produced relatively high initial attachment on day 1 but did not sustain the best overall performance is noteworthy. This may indicate that higher irradiance can trigger a rapid short-term response without necessarily supporting longer-term stability of settled spores. By contrast, 1000 lux produced the highest day-3 peak and the highest mean attachment, implying a more favorable environment for both initial settlement and the persistence of attached spores. The decline after the peak may be associated with multiple processes, including reduced nutrient availability, reorganization of newly settled cells, mortality of non-viable propagules, or limitations in the culture microenvironment. Rao and Rangaswamy (2019) linked reduced spore attachment to nutrient limitation in red seaweed culture, while Roleda and Hurd (2019) argued that nutrient effects can remain limited when light conditions are not optimal. Together, these observations suggest that irradiance and nutrient status work in concert during the settlement phase.

The present findings are also consistent with previous work emphasizing the reproductive sensitivity of red algae to optical conditions. Hoffman (1987) described the importance of photoperiod and light-dependent responses in seaweed propagule production, while Muñoz et al. (2016) demonstrated that reproductive development in macroalgae can vary substantially under different light regimes. Kumar et al. (2018) further emphasized that algal growth and reproduction are shaped by optical quality and environmental context. The current study extends this body of knowledge by providing specific experimental evidence that, within the tested range, 1000 lux is the most advantageous light intensity for both release and attachment in *Gracilaria* sp.

From an applied perspective, these results are highly relevant to seedling production. One of the persistent constraints in *Gracilaria* farming is the need for reliable, high-quality seed material that can support stable cultivation performance. Technical manuals and practical work on *Gracilaria* spore culture have long recognized the importance of reproductive handling and environmental control in nursery systems (Lideman et al., 2016). The present study contributes to this applied objective by identifying a concrete light-intensity target that can be adopted or tested further in hatchery-scale operations. Because moderate irradiance enhanced both the liberation of spores and their successful attachment, it may increase the efficiency of generative seed production and reduce dependence on repeated vegetative propagation.

At the same time, the study should be interpreted within its experimental scope. Only three light intensities were tested, and the experiment was conducted under a single photoperiod, temperature range, and salinity condition. The broader culture environment remains important. Aslan (1998) and Raikar et al. (2001) both emphasized the role of temperature in spore-related performance, while Charrier et al. (2017) noted the interaction of light with other environmental variables. Therefore, the superiority of 1000 lux in this study should be understood as the optimum within the tested laboratory conditions rather than as a universal threshold for all *Gracilaria* strains or production systems.

Even so, the practical message is clear. A moderate light level supported the best overall reproductive outcome, indicating that successful spore-based propagation of *Gracilaria* requires balanced irradiance rather than maximal irradiance. Future work should refine this range further, combine light-intensity testing with temperature and nutrient optimization, and evaluate whether the same response is maintained during later developmental stages such as germling growth and juvenile thallus formation. Such efforts would help translate laboratory-scale reproductive success into robust hatchery protocols for commercial seaweed farming.

CONCLUSIONS AND RECOMMENDATIONS

This study confirmed that light intensity significantly influences both the release and attachment of *Gracilaria* sp. spores under controlled laboratory conditions. Among the three tested irradiance levels, 1000 lux consistently produced the best reproductive response. The treatment yielded the highest spore release, reaching 720 ± 35 spores/cystocarp on day 4, and also generated the highest mean release of 452 spores/cystocarp. In the attachment phase, the same treatment achieved the peak value of 520 ± 49 spores/cystocarp on day 3 and the highest mean attachment of 391 spores/cystocarp. These results indicate that a moderate light intensity provides a more favorable physiological environment for early spore release and settlement than either lower irradiance at 500 lux or higher irradiance at 1500 lux.

The findings have direct implications for the development of spore-based seedling production systems in *Gracilaria* aquaculture. By identifying an effective light condition for two critical early reproductive stages, this study contributes practical guidance for improving the reliability of generative

propagation. Such improvement is important for strengthening seed quality, reducing dependence on repeated vegetative propagation, and supporting more sustainable seaweed cultivation. Further studies are recommended to test a narrower irradiance gradient, evaluate interactions with other environmental variables such as temperature and nutrients, and follow the development of attached spores into juvenile thalli under hatchery and semi-commercial conditions.

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