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Brief Report

Optimising Kelp Cultivation to Scale up Habitat Restoration Efforts: Effect of Light Intensity on "Green Gravel" Production

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Abstract: Kelp forests are disappearing worldwide due to climate change and human stressors, highlighting the need for active interventions. A new restoration approach, "green gravel", has been shown to be a potentially effective tool to restore endangered kelp forests. However, green gravel is still a novel technique that needs better experimentation and optimisation at all process stages. Contamination by other algal species is one of the critical factors in early-stage green gravel production because their overgrowth can lead to the loss of the seeded material. In this study, we assessed the effect of light intensity on kelp growth and on the coverage of contaminating algae on green gravel. Our results show that under high lights, kelps displayed faster growth (recruits on average more than three times the size and covering a six-times-larger area in high light intensity than in low light), but there was also a higher percentage of contaminating algae. In contrast, the green gravel cultivated under low lights showed almost no signs of algal contamination, but the area occupied by kelps and the length of the lamina were dramatically lower. Due to the cultivation conditions, opportunistic species can grow fast. This advantage is expected to disappear once the green gravel is deployed. To obtain cleaner cultures and to avoid the risk of losing the cultivated material, we would advise starting rearing under lower light intensity to reduce the risk of contamination but ensure kelp growth and then increasing the light intensity to boost it. Clear and appropriate protocols are absolutely necessary to minimise production costs and times and for the scaling-up of future attempts at marine forest restoration.

Keywords: green gravel; restoration; marine forests; *Laminaria ochroleuca*

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1. Introduction

Kelp forests are complex marine habitats composed of large brown seaweeds that support high levels of local biodiversity [1]. Ecosystem services derived from kelp forests include increased primary and secondary production, protection from coastal erosion, carbon storage, and nursery areas for economically valuable fish species [2,3]. Due to climate change and the increase in human activities, kelp forests are globally declining, with an estimated kelp loss of about 2% per year [4]. In most of the cases, the main effect of these stressors is a persistent shift to less complex habitats (e.g., turf reefs, urchin barrens) [5]. Once kelp forests are replaced by other habitats, it is difficult for them to naturally recover within a reasonable time scale, even after impacts that caused the degradation are removed. In fact, natural recovery is unlikely and can take decades or even centuries [6]. To the present day, degraded kelp forests have not shown signs of natural recovery, and even more worryingly, future projections indicate further losses in many areas [7]. "Passive" conservation measures, which have their main focus on the mitigation or the removal of environmental stressors, may not be enough to ameliorate or reverse the state of depleted marine forests [8]. Recently, the combination of traditional conservation measures with active restoration methods has been proposed as a potential alternative to counteract the abrupt changes in marine ecosystems [9]. To date, several reforestation methods have been tested, but they have been conducted on a very small spatial scale and resulted in short-term improvements compared to the scales of marine forest loss [10,11].

"Green gravel" is a novel marine forest restoration technique [12]. The method consists of sowing kelp spores or gametophytes onto small gravel, rearing them in the laboratory, and later outplanting them at the chosen reforestation site. Green gravel has no impact on natural populations, unlike most traditional transplantation methods, which usually involve the harvesting of individuals from donor populations. For this reason, it has been proposed as an economic and sustainable alternative to current restoration efforts, as most of them demand high investment or are difficult to scale up [12,13].

Green gravel has the potential for scaling up, allowing the recovery of large degraded areas due to its low cost and low maintenance [12]. However, a better implementation of all the phases (from the collection of biological material to cultivation and deployment) is strongly needed to increase the likelihood of success of restoration actions.

Contamination by other algal species is known to be one of the biggest risks in cultivating kelp and other seaweeds in tanks [14]. Epiphytic algae have been identified as one of the primary causes of kelp degeneration and mortality in cultivation tanks [15]. In previous experiments with green gravel, algal contamination affected the cultivation outcomes and the quantity of material available for the deployment. For example, in Alsuwaiyan et al., (2022) [16], after contamination by red algae, approximately 50% of the green gravel was no longer usable. Therefore, maintaining a lower light intensity could be a reasonable approach to reduce the spread of contaminating algae. However, the light availability could be too low to boost the growth of the kelps seeded onto the gravel.

Here, we evaluated the effect of light intensity on the production of green gravel using the golden kelp *Laminaria ochroleuca*. *L. ochroleuca* is a warm-temperate Iberian species that can live in areas ranging from deep intertidal pools to high subtidal pools up to 30 m deep depending on light availability [17]. In Northern Portugal, *L. ochroleuca* usually dominate deep intertidal pools, while forming more scattered patches in the upper subtidal pools [18]. However, in the last decades, this species has already undergone range contractions and declines in abundance along the Iberian Peninsula, mainly in response to anthropogenic stressor and climate change [19]. The loss of this forest-forming species at large spatial scales will have major implications for the associated biodiversity and ecosystem services that they provide [20]. Considering the rapid growth rates of this species but also the potential threat of its global disappearance, *L. ochroleuca* is an ideal candidate for restoration plans.

In our experiment, two different levels of light were tested, in order to assess potential effects on kelp cultivation and develop better cultivation protocols for future reforestation activities.

2. Materials and Methods

Sporogenic tissue was collected from fertile *L. ochroleuca* individuals (n = 12) in Vila Chã (Vila do Conde, Portugal, 41°17′42.8″ N, 8°44′12.1″ W) in May 2022, and spore release was induced in the lab on the same day, as described in Pereira et al., (2011) [21]. The culture was kept under white light (40 μmol m^{-2} s^{-1}) to induce germination. After the spores developed into gametophytes, the stock culture was transferred to low red light (12 μmol m^{-2} s^{-1}) to allow only vegetative growth. To seed the gravel, the healthy gametophyte mass was separated into smaller fractions using a handheld electric blender and then sprayed onto small granite stones (3–5 cm). Before starting the experiment, the gravel was soaked in bleach to remove any contaminant and washed under running water.

Two different levels of light intensity, high (130 μmol m^{-2} s^{-1}) and low (40 μmol m^{-2} s^{-1}), were used to assess the differences in kelp growth and the coverage of both kelps and contaminating algae. The choice of light levels was supported by the existing literature on kelp cultivation. The "high" light intensity had been reported for previous green gravel cultivation experiments with kelp species [16], while the "low" light intensity was chosen to ensure the initial golden kelp growth [22] but reduce the risk of prompting the growth of contaminating algae.

The light was provided by LED lamps (AquaBar Control, AquaRay Horizon), with a photoperiod of 12:12 dark:light, the typical photoperiod for golden kelp recruiting season. Eight tanks (40 × 60 cm, 50 L) were used, with four replicates for each light treatment. Small pumps (Sicce, Syncra Silent) were added to the tanks to ensure water movement. Seawater temperature was controlled by a cooler (Aqua Medic Titan 150) and maintained at 15 °C, the optimum temperature for golden kelp growth. Provasoli's Enriched Seawater (PES) [23] was added only after the first two weeks, as the seawater should have enough nutrients for the low biomass in the tank, thus avoiding boosting the recruitment of opportunistic species. Afterwards, PES was added on a weekly basis to ensure the supply of nutrients from then on. Germanium dioxide (GeO_2) was also added to the tanks to inhibit the growth of contaminating diatoms [24].

When the sporophytes became clearly visible (about 2 months after seeding), their length was analysed through photographic sampling (16 photographs per replicate) to calculate growth. Furthermore, using an 8 × 6 cm frame, coverage of kelp, as well as green and brown (mostly belonging to the genera Ulva and Ectocarpus, respectively) contaminating algae, was assessed (20 replicates for each light treatment) through photographic sampling. All the photographic replicates were analysed using ImageJ software (U. S. National Institutes of Health, Bethesda, ML, USA), a processing program that allows measuring and analysing image data.

Assumptions of normality were assessed using the Shapiro–Wilks test, and data were log-transformed when necessary [25]. To explore potential differences between the two light treatments, sporophyte growth and coverage of kelp and contaminating green and brown algae were analysed using a one-way ANOVA. All the analyses were carried out using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. IBM Corp., Armonk, NY, USA).

3. Results

The one-way ANOVA carried out on the data of lamina lengths highlighted a significant difference ($F_{(1)} = 102.557$, $p < 0.001$) between the two light treatments. The length of the lamina was notably higher (1.70 ± 0.12 cm) in individuals grown under high light than the ones reared under low light (0.45 ± 0.02 cm) (Figure 1a).

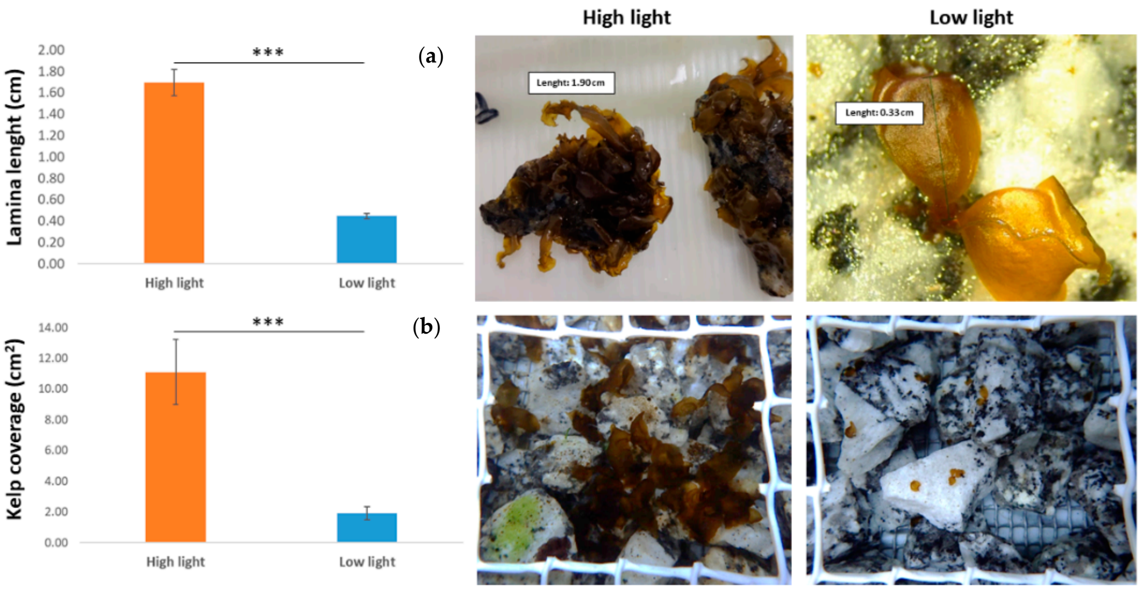

Figure 1. (**a**) Lamina length (mean ± SE) and (**b**) kelp coverage (mean ± SE) varied between different light conditions (high and low light). Significant differences are indicated by ***.

The one-way ANOVA on kelp coverage data followed the same pattern, showing a significant difference between treatments ($F_{(1)}$ = 17.969, $p < 0.001$). In fact, the area covered by kelp was almost sixfold larger under high light (11.10 ± 2.13 cm^2) compared to that with low light (1.91 ± 0.43 cm^2) (Figure 1b).

The area of gravel covered by contaminant algae was also significantly different between the two light treatments, for both brown (ANOVA: $F_{(1)}$ = 23.126, $p < 0.001$) and green species (ANOVA: $F_{(1)}$ = 5.189, $p < 0.05$). Differences in coverage were particularly evident for brown algae, with a wider area observed under high light (9.40 ± 1.92 cm^2) than under low light (0.16 ± 0.05 cm^2). The area colonised by green algae under high light was drastically smaller (0.82 ± 0.36 cm^2), and there was no visible contamination in the green gravel reared under low light.

The percentage of substrate that was not colonised (by either kelps or other algae) was also significantly different between the two light treatments (ANOVA: $F_{(1)}$ = 41.911, $p < 0.001$). In fact, while in high light, only 56% of the surface on average was nude substrate, in low-light conditions, the gravel showed an average of 95% of the surface not colonised (Figure 2).

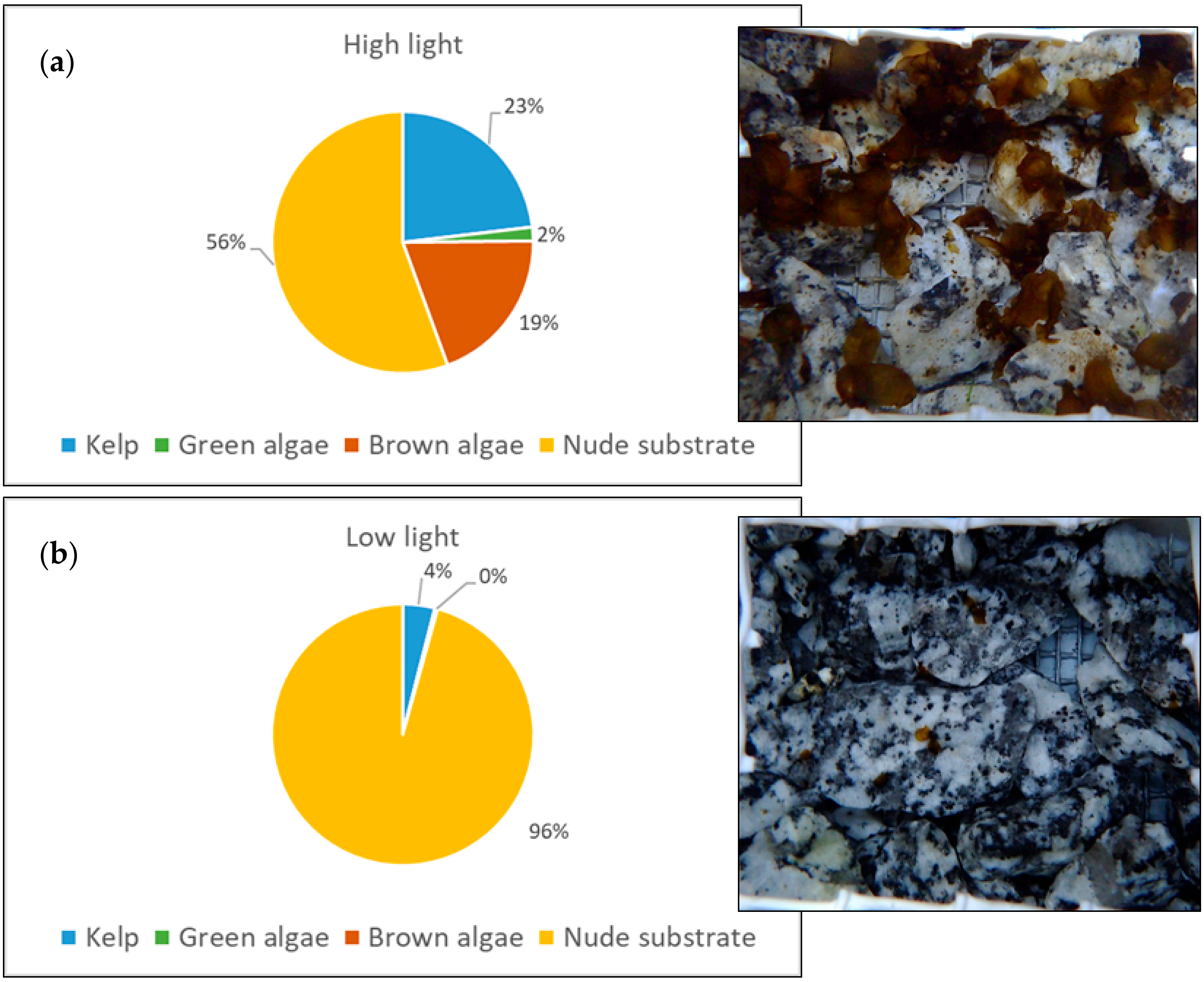

Figure 2. Average coverage (%) of kelps, green algae, brown algae, and nude substrate for the two light treatments, (**a**) high and (**b**) low.

4. Discussion

Two of the major challenges in the Decade of Ecosystem Restoration of the United Nations are marine reforestation and the scaling up of restoration techniques in order to cope with the alarming speed of ecosystem loss [26]. Green gravel has been proposed as a sustainable and economic alternative to traditional restoration efforts, such as herbivore removal, transplantation of adults, and other methods that enhance the natural recruitment of the target species [11]. Unlike other techniques, in fact, the affordability and logistical convenience of green gravel make it a tool with a huge potential for scaling up. Nevertheless, green gravel is still a novel technique that needs optimisation and clear protocols to increase the success of its application. Here, we tested the feasibility of cultivating *Laminaria ochroleuca* gametophytes on gravel and the effect of light intensity on green gravel production.

Due to their slow development, microscopic and juvenile stages of kelps have a reduced ability to compete with opportunistic species, making them extremely sensitive to contamination. Previous experiences have highlighted that this is the main reason for fruitless green gravel production efforts, resulting in economic loss and an inability to move forward with the reforestation activity. It is thus essential to know how to provide an advantage for kelp in relation to opportunistic species, to allow for successful green gravel production. Our results show a significant effect of light intensity on kelp sporophyte growth rate, which resulted in recruits on average more than three times the size in high-light intensity compared to low light. The larger size of the individuals resulted in a six-times-larger area covered by recruits in the gravel reared under high light when compared to that under low light. However, the higher light availability also resulted in considerable contamination by opportunistic algae species, which occupied as much as 21% of the area, in contrast with the neglectable contamination under low light.

Because kelp species are mostly subtidal and the green gravel technique consists in throwing the gravel overboard at the selected site, the conditions the seaweeds will find will vary greatly, particularly in terms of light availability and hydrodynamics. It is thus expected that the conditions that allowed for the growth of opportunistic species will cease to exist. As such, it is expected that the opportunistic species that occupied the gravel will not pose a threat to the kelp recruits after deployment. Taking into account that the production of green gravel has associated costs (energy spent in refrigeration, light, hydrodynamics, and nutrients supply), to make this process more efficient and scalable, we should try to minimise them as much as possible. We thus hypothesize that the best solution for cost-efficient and successful green gravel production would be to maintain lower light until recruitment is visible to the naked eye and increase light exposure afterwards.

Despite its unquestionable convenience, green gravel still presents some potential weaknesses, such as its sensitivity to strong currents that may be able to displace the seeded gravel and complicate subsequent monitoring. Careful preliminary studies for an adequate choice of deployment sites and frequent monitoring to control the acclimatisation of the recruits are therefore essential to ensure the success of restoration attempts using this method.

5. Conclusions

Two light intensities, namely 130 μmol m^{-2} s^{-1}, which has been previously tested for green gravel production with kelp species, and 40 μmol m^{-2} s^{-1}, which is the advised light intensity for *L. ochroleuca* cultivation, have been tested. The main objective was to determine the cultivation conditions that would result in more efficient green gravel production, as costs are a major factor in scaling up reforestation efforts.

Although rearing the green gravel under higher light intensities results in faster growth of the kelp sporophytes, it also results in higher recruitment of opportunistic species, which may jeopardise the survival of kelp recruits due to competition for substrate and nutrients. According to our results, to maximise the likelihood of successful cultivation, the most recommended procedure would be to rear the green gravel at lower light intensity for a short initial period (e.g., the first two weeks, or until recruitment is observed) and increase it afterwards. This would allow obtaining a cleaner green gravel and reduce the probability of losing the reared material, which would also lower the production costs and help scale up future reforestation actions. However, further research is required to improve and refine green gravel cultivation for conservation purposes, such as, for example, testing different types of substrate and cultivation conditions.

Future tests for the deployment phase are also necessary to improve the outplanting process and increase the success of restoration attempts.

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