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Article

Laying the Foundation: How Substrate Choice Influences Kelp Reforestation Success

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Abstract: Over recent decades, widespread declines of kelp forests have been reported along the European coast, prompting the need for effective and scalable restoration strategies. The green gravel technique, in which kelp gametophytes are seeded onto small rocks and cultivated in the lab before being outplanted, has shown promising results. In this study, we tested the effects of four commonly available substrates—granite, limestone, quartz, and schist—on the early development of *Laminaria ochroleuca* recruits under optimal laboratory conditions. All substrates supported gametophyte adhesion and sporophyte development. By week 6, quartz and schist promoted the greatest recruit growth, with quartz and limestone showing the best overall performance by week 7. Final recruit densities were similar across substrates, indicating multiple materials can support early development. While schist performed well initially, its friability raises concerns for field deployment in high-energy environments. Quartz and limestone showed both biological effectiveness and practical advantages, with limestone emerging as the most cost-effective option. Substrate selection should consider not only biological performance but also economic and logistical factors. These findings contribute to refining green gravel protocols and improving the feasibility of large-scale kelp forest restoration, though field validation is necessary to assess long-term outcomes under natural conditions.

Keywords: green gravel; habitat restoration; marine forest; kelp; marine reforestation;

1. Introduction

Kelp forests are suffering a global decline, with some regions facing almost complete local disappearances. This decline is not merely due to range shifts but rather the result of a complex set of factors that vary across different environments [1-3]. These ecosystems are increasingly threatened by anthropogenic and climate-driven factors, including overfishing, eutrophication, pollution, ocean warming, and extreme events such as storms [4,5]. These stressors can have severe consequences for marine ecosystems, and projections indicate further kelp forest losses in the near future [6-8].

Beyond their ecological role, kelp forests play an important role in coastal defence. They can attenuate wave intensity and modify hydrodynamic flow, these effects vary with species and individuals' morphological characteristics, such as height and robustness. By mitigating coastal erosion and stabilizing sediments, kelp forests contribute to maintaining coastal structural integrity [9,10]. Additionally, kelp forests enhance marine biodiversity by modifying local environmental conditions such as temperature, sedimentation, and hydrodynamics. They create complex habitats that support diverse biological communities and facilitate species recruitment [11-13]. Given their ecological significance and growing role in coastal protection, safeguarding kelp forests is essential to ensuring the resilience of marine ecosystems [14,15].

As a response to ongoing declines, restoration efforts have been ongoing with various techniques, including transplanting and non-extractive approaches like green gravel. Seeding offers advantages over transplanting, mainly by reducing pressure on wild populations. However, because kelps experience their highest mortality during early life stages (particularly at the microscopic phase), restoration projects relying on these stages are inherently high-risk [16]. Cultivating kelp in a controlled laboratory setting before outplanting provides a more stable environment, reducing exposure to natural stressors such as grazing, competition, and hydrodynamic forces [17]. This increases the survival rate of juveniles, improving the overall success of restoration efforts. However, deploying recruits at an early developmental stage may enhance their adaptation to strong currents, decreasing the risk of developing fragile stipes that are more prone to breaking under natural hydrodynamic conditions.

Among the seeding-based restoration techniques, the 'green gravel' method has been successfully used to restore kelp forests along the southern coast of Norway [18], on the Portuguese coastline [19], and in Danish waters [20]. This method consists of sowing small rocks with kelp gametophytes and cultivating them in optimal conditions in the laboratory until they reach about 1 cm in length, after which they are outplanted at sea. Several studies have focused on optimizing the green gravel technique to further reduce costs while driving innovation toward sustainable, large-scale, and cost-effective kelp forest restoration [21-23].

Our research focuses on the golden kelp *L. ochroleuca*. This species can be found in Portugal (continental coast and in the seamounts of Azores), in the Northwest coasts of Spain, in Morocco, in the Strait of Messina (Italy), in Brittany (France), the English and Bristol Channels, and, more recently, in Ireland [24,25]. However, ocean warming has been linked to shifts in its distribution range. At its equatorial limit, *L. ochroleuca* is experiencing declines, while at its northern edge, it is expanding and increasingly outcompeting *Laminaria hyperborea* [26]. These shifts highlight the species' ability to persist under warming conditions, particularly at its southern distribution limit, where its forests may be increasingly vulnerable to environmental change. *L. ochroleuca* usually inhabits deep intertidal pools and the subtidal, down to 30m deep. Along the Portuguese coast, they are distributed between lower intertidal pools and 3-23 m deep, with an average depth of about 10 m [27].

The main goal of this study was to optimize the green gravel method by examining how different substrates (granite, limestone, quartz, and schist) can affect kelp recruitment. While most studies testing the green gravel method have used granite, no prior research has explicitly evaluated whether it is the most efficient substrate for this technique. To address this gap, these substrates were selected as they are some of the most common in the intertidal and shallow subtidal areas and are easy to find at an accessible cost in hardware stores.

2. Materials and Methods

Fertile tissue from 12 individuals of *L. ochroleuca* was collected in spring from intertidal pools in Carreço, Northern Portugal (41°44'30.3"N 8°52'38.3"W). Spore release was induced in the laboratory on the same day. This gametophyte solution was sprayed directly onto granite, limestone, quartz, and schist stones (5 cm length) covering the bottom of 50 L tanks. Three tanks were used for each gravel type (n=3). The tanks were filled one hour after spraying to allow them to better fix to the substrate. The system was kept at 15°C, the optimum temperature for this species. Recirculating water was sterilised with TitanUV Steriliser P2 110W. The tanks were illuminated with OSRAM L 58 W /965 Biolux fluorescent lights at 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with a 12:12 h light:dark photoperiod, to match the spring conditions in Portugal, when recruitment usually peaks. Environmental conditions, including dissolved oxygen, pH, salinity, and temperature, were monitored using a Hach HQ40D Portable Multi Meter. To ensure an adequate nutrient supply, Provasoli's Enriched Seawater (PES) was used, and monitoring and water renewal were carried out on a weekly basis. Recruitment and size were monitored every week by randomly selecting 3 stones from each replicate and observed under a Leica EZ4W Stereomicroscope at a 35x magnification. Kelp length and density were

measured using 15 pictures from randomly selected areas, from each of 3 stones per tank, and analysed with ImageJ (US National Institutes of Health, Bethesda, MD, USA).

All statistical analyses were conducted using R software (version 4.4.2). To evaluate differences in kelp size in different substrates (limestone, granite, quartz, and schist) over time, non-parametric methods were employed due to violations of normality and homogeneity of variances, as determined by the Shapiro-Wilk test and Levene's test, respectively. A Kruskal-Wallis test was performed separately for each week to detect significant differences among substrates. Post-hoc comparisons were conducted using Dunn's test with Bonferroni correction to identify specific pairs of substrates with significant differences (with $p < 0.05$ considered significant).

3. Results

3.1. Sporophyte length

The trial started in mid-April and lasted a total of 8 weeks. The first kelp recruitment was observed in the 3rd week. The analysis revealed that time and substrate type are statistically significant factors influencing kelp size ($p < 0.001$) (Figure 1). In week 3, the schist exhibited significantly higher kelp length compared to the other substrates ($p < 0.0001$), while no significant differences were observed among the other three substrates during this period. In week 4, quartz demonstrated significantly higher kelp length compared to granite, limestone, and schist ($p < 0.0001$), while no significant differences were observed among the other three substrates. By week 5, no significant differences were observed among the substrates. In week 6, quartz and schist exhibited the highest kelp length, with no significant differences between the two substrates. Both performed significantly better than granite ($p < 0.0004$) and limestone ($p < 0.0001$), which had the lowest growth during this week. Importantly, no significant differences were observed between quartz and schist, indicating comparable performance under the experimental conditions. Finally, in week 7, limestone outperformed schist ($p = 0.0245$) and granite ($p = 0.0394$) while showing no significant differences compared to quartz. Quartz, schist, and granite exhibited similar growth performance during this period.

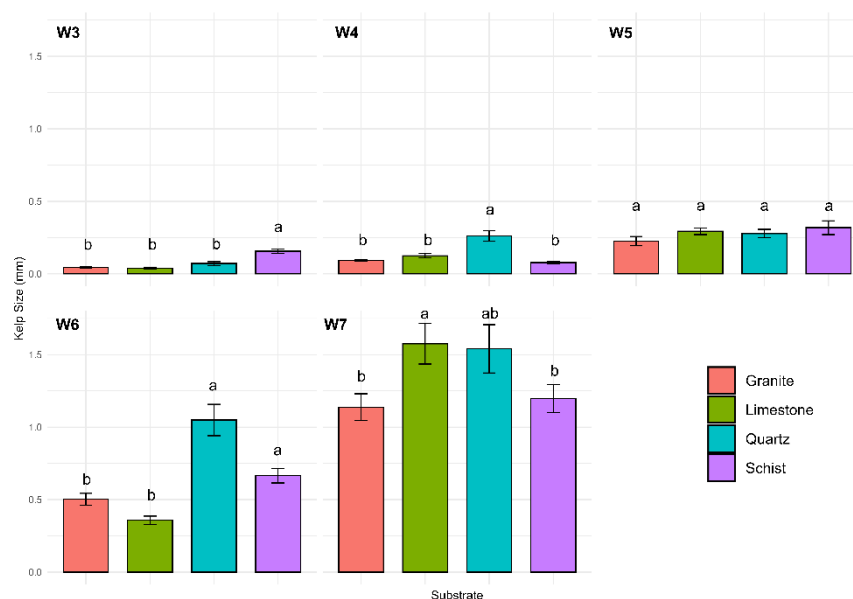


Figure 1. Kelp size variability (mm) across different substrates (granite, limestone, quartz, and schist) for each experimental week (W3 to W7). Bars represent the mean kelp size with error bars indicating the standard error of the mean ($\pm$ SE). Different letters represent significant differences between treatments.

3.2. Sporophyte density

The analysis showed that the interaction between time and substrate type is a statistically significant factor influencing kelp density ($p < 0.001$; Figure 2). In weeks 3, 4, and 6, no significant difference was observed between the substrates. In week 5, significant differences were observed between granite and limestone ($p = 0.0119$) and between limestone and quartz ($p = 0.0002$). However, no significant differences were detected between the remaining substrates. The results indicate that limestone stood out as a substrate with significantly higher densities compared to granite and quartz during this experiment stage. By week 7, however, the only significant difference observed occurred between limestone and schist ($p = 0.0006$), with schist supporting higher densities compared to limestone during the later stage of the experiment.

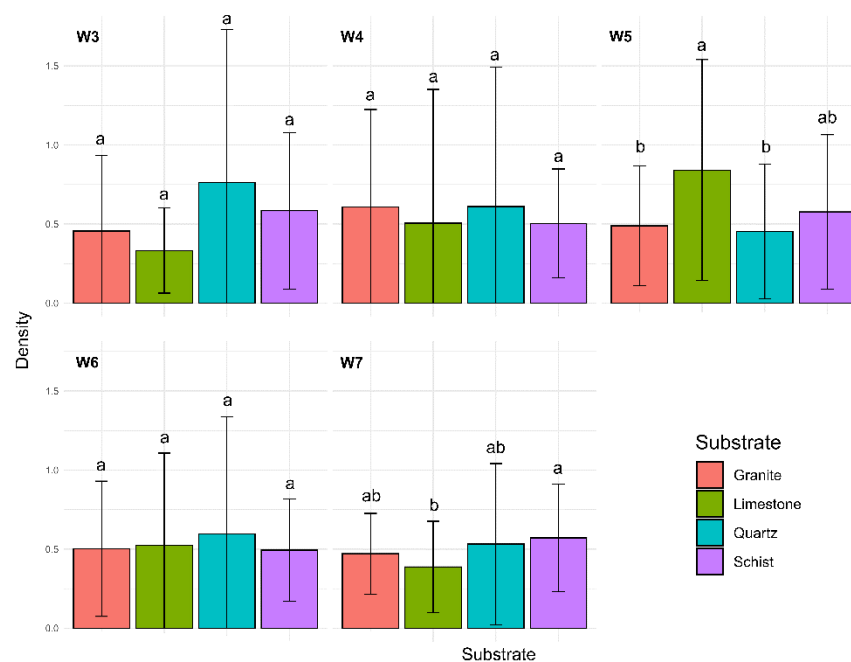


Figure 2. Kelp density in different substrates (granite, limestone, quartz, and schist) for each experimental week (W3 to W7). Bars represent the mean kelp size with error bars indicating the standard error of the mean ($\pm$ SE). Different letters denote significant differences between treatments.

4. Discussion

This study aimed to optimize the green gravel method, focusing on *L. ochroleuca*, by investigating how different substrata (granite, limestone, quartz, and schist) affect recruitment success, particularly in terms of timing, recruit size, and adherence to the substrate.

In terms of recruit density, the overall assessment indicated that substrate type did not have an outstanding effect, suggesting that all tested materials can support *L. ochroleuca* recruitment. However, looking at kelp length, our results demonstrated that the type of substrate plays a significant role in early kelp growth. Among the tested substrates, quartz exhibited the overall highest sporophyte size. Schist initially supported significantly larger sporophytes but did not maintain this advantage over time. By week 6, both quartz and schist emerged as the most favourable substrates for recruitment, outperforming granite and limestone. However, by week 7, limestone demonstrated comparable efficiency, suggesting that multiple substrate types may be suitable for the green gravel technique.

Our findings align with previous research demonstrating that substrate characteristics, such as roughness and colour, play a crucial role in macroalgal recruitment and survival [28]. Past studies on other kelp species have highlighted the importance of surface roughness in enhancing early-stage attachment and survival [29]. Roughened surfaces are believed to strengthen juvenile attachment by

increasing the available area for holdfast anchorage and allowing rhizoids to interlock within microscopic crevices, thereby improving stability [30,31]. Previous research also suggested that colour, through its influence on light absorption and temperature, may affect algal settlement and growth. Darker substrates have been associated with higher settlement rates, potentially due to localized temperature increases that enhance zoospore adhesion [32,33]. While no single substrate had the highest values for both variables, quartz exhibited the most consistent performance overall. This may be attributed to a combination of factors, such as surface texture, mineral composition, and chemical properties, which could create more favourable conditions for attachment and development. These findings highlight the complex interactions between substrate properties and macroalgal recruitment, underscoring the need for further research to better understand the underlying mechanisms.

From a practical and economic perspective, substrate selection is crucial for optimizing large-scale kelp restoration. The green gravel method is recognized as an affordable and scalable technique, reducing reliance on resource-intensive transplantation methods. Since quartz and schist showed promising results by week 6, their use could enable earlier deployment, significantly lowering laboratory cultivation costs. This aligns with the broader goal of making kelp restoration more cost-effective and accessible for conservation initiatives. However, while quartz appears to be a durable and effective option, the friability of schist raises concerns about its suitability in high-energy environments. This is counterbalanced by its flatness, which reduces the likelihood of rolling and breaking recruits. Nevertheless, results from week 7 indicate that limestone may be an equally viable alternative. Quartz and limestone are generally more accessible in hardware stores, though quartz is typically more expensive. Given the unclear differences in suitability between quartz, limestone, and schist, the final choice of substrate will depend on multiple factors, including hydrodynamic conditions, availability, and overall cost. Despite granite being the most commonly used substrate in reforestation efforts [18], our findings do not support its selection and may encourage future studies to explore alternative substrates for a more effective and efficient green gravel protocol. Ultimately, long-term monitoring after deployment will be essential to further inform substrate selection and optimize restoration outcomes.

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