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Textile substrate seeding of *Saccharina latissima* sporophytes using a binder: An effective method for the aquaculture of kelp

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**ABSTRACT**

The macroalga *Saccharina latissima* holds promise as a widespread crop in northern Europe. Currently, seeded lines are cultivated for 1–2 months within a hatchery until they become ≥1 mm sporophytes, which are then outplanted to a farm. Textiles are being developed as a cultivation substrate for macroalgae, however due to their large surface area, these need either direct in-situ seeding at the farm or a very short, high turnover hatchery period. Two materials, Kuralon twine and non-woven textile, were seeded using three *S. latissima* developmental stages: meiospore, gametophyte or juvenile sporophyte. The gametophyte and sporophyte stages were applied using a binder solution to adhere them to the materials. These were outplanted at a seaweed farm in the Sound of Kerrera, UK. After 5 weeks, fronds were significantly larger (45 ± 25 mm) and more abundant (20 ± 8:20 cm⁻¹) when seeded with sporophytes over gametophytes or meiospores (8 ± 10 mm and 2 ± 20 cm⁻¹). This reflects the growth advantage of outplanting juvenile sporophytes, since they are larger and more developed when outplanted. Higher fouling of filamentous algae was seen on the non-woven textile but this did not appear to affect growth. After four months, sporophyte seeded materials had the largest fronds and the greatest fresh mass of 2.1 ± 0.8 kg·20 cm⁻¹, equivalent to simultaneously deployed hatchery reared twine (2.0 ± 0.1 kg·20 cm⁻¹). Gametophyte seeding achieved 0.8 ± 0.6 kg·20 cm⁻¹ while meiospore seeding achieved only 0.1 ± 0.1 kg·20 cm⁻¹. No difference was found between growth on Kuralon twine or non-woven textile (p > 0.05), showing that both are suitable growth substrates. Seeding of juvenile sporophytes onto textile using the binder is demonstrated to be a successful method for the cultivation of *S. latissima*, and may require only 1% of the hatchery tankage, once optimised. It is expected that this method is transferable to the cultivation of other kelp species.

1. Introduction

The macroalga *Saccharina latissima* (Phaeophyceae) is a fast growing subtidal European kelp, which develops from a 8–10 μm meiospore into a 1.5–2 m adult within a single year [1,2]. It is commonly found in shallow, low to moderately wave disturbed subtidal marine environments where it acts as an ecosystem engineer providing a habitat for a range of other species [3,4]. There is continuing research into *S. latissima* cultivation across Europe [5–7], which should limit environmental damage or degradation associated with harvesting of natural beds [4,8]. Cultivated kelp has value as a human food [9], animal feed [10], nutrient absorption and an additional income stream as part of integrated multi-trophic aquaculture [6,11,12]. Kelps are also a focus of research into mass cultivation as a biofuel or for carbon sequestration due to their high carbohydrate content [13–15].

Single-celled haploid meiospores are released from the sorus of adult sporangial tissue of kelp; these settle and develop into either male or female gametophytes. Fertilisation leads to the development of new diploid sporophytes which then grow to adult size [16]. For cultivation, released meiospores or gametophyte cultures are settled or sprayed onto twines which are cultured in indoor hatcheries for 1–2 months [5,17]. This minimises competition and prevents grazing, allowing the successful development of sporophytes for transfer to the open sea (when ≥1 mm) without competition from other settling organisms. Textile sheets have been suggested as growth substrates for the large scale sea cultivation of macroalgae, and are known to be suitable for the attachment of kelp species [18,19]. However, their large size would make the hatchery phase unfeasible. Consequently, for textile sheets to be a viable substrate, either a very short hatchery time or direct in-situ seeding is required.

The aims of this study are:

1. To compare three methods of direct seeding of *S. latissima*; using either meiospores, gametophytes or sporophytes;
2) Determine if textiles are a suitable cultivation substrate for *S. latissima* by comparing growth on both Kuralon twine and a non-woven textile.

# Materials and methods

## 2.1. Material preparation

Braided Kuralon twine (6 mm ø; Tecnored, ES) was cut into 300 mm sections. Non-woven textile (Sioen Industries NV, BE; Sioen Industries NV 2013) was cut into 200 × 45 mm sections, which were folded and sewn along their width (Fig. 1). Both materials were soaked in 5% Decon90 for 24 h to remove manufacturing residues, then rinsed and left to soak in distilled water for 24 h, before finally oven dried at 40 °C.

## 2.2. Culture preparation and seeding of materials

Five fertile *S. latissima* were collected from Seil Sound, UK (56.31724°N, −5.58309°E). The sporangial regions were excised and wiped multiple times with laboratory tissues (Kimtech, UK) and Tyndallised seawater to remove epiphytes [20,21]. The sporangial regions were then left to partially dehydrate overnight in a refrigerator at 4 °C, then rehydrated in 8.5 °C Tyndallised seawater enriched with F/2 medium without silicate (F/2-Si) for 1 h, in the dark to induce meiospore release [17]. The meiospore suspension was filtered through 50 μm mesh, divided into two 2 L flasks and cultured in bubbled F/2-Si at 8.5 °C and 40–50 μmol photon m⁻²s⁻¹, 12:12 L:D. The density of the culture was in the region of 500,000 cell L⁻¹. Germanium dioxide (0.125 mL saturated solution L⁻¹) was also added during the first two weeks of cultivation to prevent diatom contamination [17]. One flask was cultured under red light to prevent gametophyte fertilisation, while the other was cultured under white light to promote fertilisation and sporophyte development. Every 10–14 d, the culture was allowed to settle and half the media decanted and refreshed. Attached gametophytes/sporophytes were gently re-suspended with a sterilised nylon brush.

Once the largest sporophytes reached 1–2 mm (after ca. 6 weeks), the gametophyte flask was transferred into white light and after five days, oogonia formation was evident. Both cultures were brought to the same concentration and mixed with a 1% binder solution (AT–SEA Technologies, BE), creating a concentration of 330 gametophyte/sporophyte·mL⁻¹. 2.75 mL was found to be the maximum volume of this suspension that could be soaked into 30 cm of Kuralon twine. This volume was spread evenly across the surface of the non-woven textile to standardise the seeding density. Both materials were prepared in triplicate and left for 30 min in the air. These were then submerged in 2 L tanks of F/2-Si.

Concurrently, meiospores were extracted from five further pieces of *S. latissima* from the same location. One million extracted meiospores were placed into a 2 L tank of F/2-Si containing both materials, in triplicate. The estimated settlement density was 2500 meiospores·cm⁻². The tanks were then incubated for 3 d at 8.5 °C and 5–10 μmol photon·m⁻²·s⁻¹, 12:12 L:D.

For deployment, the test materials were attached using cable ties onto a polyester rope (8 mm ø; Bexco NV-SA, BE) in a randomised order at 20 cm intervals. The 30 cm sections of Kuralon twine were wrapped around a 20 cm length of rope, the same as the non-woven textile. The line was reeled and wrapped in plastic to prevent dehydration or rain exposure during transport (Fig. 1c). The line was deployed on 28th February 2014 suspended at ca. 1.5 m depth at the Sound of Kerrera experimental seaweed farm, Scotland (56.3820°N, −5.5370°E).

As an additional comparison, 30 m of twine was seeded with meiospores of *S. latissima* and grown using traditional hatchery techniques (Kerrison et al. 2016) until a dense coverage of 1–3 mm sporophytes were present after 8 weeks. These were outplanted at the same time as the experiment above.

## 2.3. Site visits

After five weeks, the lines were inspected. Photographs were taken of a 10 cm section of each substrate. From these photographs, the % cover of fouling filamentous algae was estimated by eye, the number of apparent sporophytes were counted and the length of the five largest fronds were measured using ImageJ software v1.46r. After four months, all the lines were retrieved and all sporophytes were counted, measured and their combined mass recorded.

## 2.4. Statistical analyses

The Anderson-Darling test for normality [22] was applied to all data. Where this was satisfied a two way ANOVA (2wAN) was conducted. If this was not the case a Kruskal Wallis test (KW) was used.
3. Results

3.1. Five week timepoint

The mean length of the five largest fronds (Fig. 2a) was significantly different between the seeding methods (2wAN: $F_{1,2,2,12} = 9.3, p < 0.005$). No significant difference was found between the meiospore and gametophyte treatments ($p > 0.05$), with mean lengths of 3–13 mm. The sporophyte treatment was significantly different to both with 45 ± 25 mm fronds (AN: $F_{1,10} = 14.1–16.0, p < 0.005$). No difference in frond length was seen when comparing the Kuralon and non-woven textile ($p > 0.05$).

The density of apparent sporophytes (Fig. 2b) followed the same pattern as the mean frond length. A significant difference was found between seeding treatments (2wAN: $F_{1,2,2,12} = 21.2, p < 0.0001$). The meiospore and gametophyte treatments had means of 2–3 sporophyte·20 cm$^{-1}$ and were not significantly different ($p > 0.05$). The sporophyte treatment was significantly different to both (AN: $F_{1,10} = 35.4–40.1, p < 0.0001$), with 20 ± 8 sporophyte·20 cm$^{-1}$. Again, no difference was found between the Kuralon and non-woven textile ($p > 0.05$).

The % cover of fouling was significantly different between the two materials (MW: d.f. = 9, $p < 0.05$). Kuralon had a mean fouling coverage of 49 ± 6%, while the non-woven textile had a mean of 81 ± 13%. The fouling cover was not affected by seeding treatment ($p > 0.05$).

3.2. Four month timepoint

After 4 mo, the number of sporophytes was not affected by the substrate material ($p > 0.05$; Fig. 3b) but was significantly affected by the seeding method (2wAN: $F_{1,2,2,12} = 67.3, p < 0.001$). The meiospore seeded treatment had an average of 0.7 ± 0.6 sporophyte·20 cm$^{-1}$ and was significantly different from the other treatments (AN $F_{1,10} = 12.8–31.4, p < 0.01$). The gametophyte and sporophyte treatments were not different ($p > 0.05$) with an average of 45 ± 27 sporophyte·20 cm$^{-1}$.

The mean maximum length of the fronds (Fig. 3a) was also not significantly different between the materials ($p > 0.05$) but was significantly different between seeding methods (2wAN: $F_{1,2,2,12} = 18.2, p < 0.001$), with each combination significantly different from the others (AN $F_{1,10} = 11.4–162, p < 0.001$). The mean maximum length of fronds in each treatment was $3 ± 3, 55 ± 11$ and $87 ± 16$ cm for meiospore, gametophyte and sporophyte, respectively.

The biomass yield of $S$. latissima (Fig. 3c) was not significantly different between the two materials ($p > 0.05$), although it was significantly different between the seeding methods (2wAN: $F_{1,2,2,12} = 18.2, p < 0.0001$) and this was clearly visible on photographs (Fig. 4). The mean yield for the meiospore, gametophyte and sporophyte seeding treatments were 0.1 ± 0.1, 0.8 ± 0.6 and 2.1 ± 0.8 kg·20 cm$^{-1}$, respectively. By comparison, the line seeded using traditional hatchery technique achieved 2.0 ± 0.1 kg·20 cm$^{-1}$, not significantly different from the sporophyte seeded treatment ($p > 0.05$).

The frequency distribution of frond length after 4 months was examined for the gametophyte and sporophyte treatments, and were found to be significantly different (KS: $D_{241/205} = 0.36–0.37, p < 0.0001$; Fig. 5). A more even distribution was seen when sporophyte seeding, due to a greater frequency of larger fronds: When gametophyte seeded, only 2–6% of fronds were ≥80 cm, whereas this was 19–26% when sporophyte seeded. In both cases, the median size class was 20–40 cm, which composed 45% of the population for gametophyte seeded, but only ~30% for sporophyte seeded. The material choice made no difference to the frequency distribution ($p > 0.05$).

4. Discussion

Textiles have been proposed as a substrate for the cultivation of macroalgae as their chemistry and structure can be manipulated to maximise attachment, and they provide versatility to create new structures which may allow optimal sea space usage [23]. Textiles have already been shown to be suitable substrates for the attachment of Laminaria digitata in tanks, with increased rhizoid production due to their microscopic roughness, and the creation of an enmeshed holdfast structure as the rhizoids envelop the fibres [18]. This study has found...
that in the sea, a non-woven textile was just as effective as commercially available Kuralon twine for the cultivation of *S. latissima*. It was also found that in the early stages of growth, fouling by filamentous algae such as *Ectocarpus* spp. was enhanced on the non-woven. However, this did not appear to affect the growth of *S. latissima*.

A primary practical issue impeding the use of textiles for macroalgal cultivation is how to seed them. Generally for kelp cultivation, Kuralon twine is wound onto reels, or other structure, seeded and allowed to grow in a hatchery over many weeks until well attached sporophytes have developed [5]. The twine can then be unfurled onto a length of rope. High surface area textiles sheets cannot be seeded using this hatchery method, without an excessively large areal tankage requirement. For the commercial use of textiles in macroalgal cultivation, they therefore require a method that requires either a very short hatchery time (i.e. days), or allows direct in-situ seeding of the material.

This study has investigated direct seeding using different growth stages of *S. latissima*. A commercially secret binder (AT-SEA Technologies, BE) was used to thicken suspensions of both gametophytes and sporophytes, which were then applied to the test materials, preventing the suspended macroalgae from being washed off before they can attach. Motile meiospores were separately allowed to settle...
onto test materials for 3 d, without the binder. This gave them the best opportunity for adhesion, as using the binder would have reduced the number in contact with the material surface. The binder method can allow seeded materials to be immediately placed in the sea resulting in successful growth (Kerrison et al. in prep). However in this study, a 3 d settlement period was allowed as the authors felt that immediate submersion would have biased the results against the meiospores, washing them away before they could settle.

The results show that binder seeding is a successful method to seed using either gametophytes (20–50 μm) or sporophytes (0.5–2 mm) leading to a sporophyte density of ca. 450 m⁻¹ on both materials, a tenth of the initial seeding density (4540 m⁻¹). However, only 19–25% of these developed into fronds > 80 cm in the sporophyte seeded treatment, with 30% still < 20 cm. This indicates substantial intraspecific competition, with larger fronds suppressing the growth of their smaller compatriots [24]. A lower initial seeding density could lead to a more even population distribution.

Overall, seeding using sporophytes provided a growth advantage over gametophytes: growing larger fronds and achieving twice the biomass after 4 months. This method can therefore be used to A) increase the final biomass achieved and/or B) reduce the cultivation time needed to reach a required size. This is to be expected, given the 2–3-weeks more advanced development of the seedled sporophytes compared to the gametophytes [17]. Binder seeding using sporophytes achieved in similar biomass to the traditional twine method, but with further optimisation, may hold considerable advantages over the traditional method including A) the binder method could be easily automated and applied over large surface areas simultaneously, whereas the twine method requires time-consuming wrapping of individual lines. B) The method may allow fast seeding onto a wide variety of cultivation substrates, including two/three-dimensional structures, as well as traditional 1D ropes. C) The volume of tankage required to produce sporophyte tumble culture is far below that required for a twine hatchery, i.e. the SAMS hatchery produces 1 km of seeded twine in 87 L of tankage, while the cultivation manual of Flavin et al. was calculated to use 150 L [25]. By comparison, sporophyte culture and seeding using the methodology of this study (non-optimised), would require < 2 L of culture to seed 1 km. This means that a hatchery based around sporophyte-binder seeding may be in the order of 100 times as space efficient as a twine-based hatchery.

The purpose of the binder is not to permanently glue the sporophyte/gametophyte onto the substrate, as this could encase them, causing suffocation or preventing growth. Instead, the binder brings them into close enough proximity so that they can develop a holdfast attachment. The high viscosity of the binder solution prevents the sporophyte/gametophyte from being washed away immediately, while the binder itself remains in place over the timescale of more than a week (pers. obs.). Therefore, the binder suspended gametophyte/sporophyte, has this limited time period in which to develop a holdfast attachment to the substrate material. It is expected that a large proportion of the juveniles are washed away at this stage, as their holdfasts are, by chance, oriented so that they are not able to contact the surface before the binder degrades. A rough texture, such as the non-woven textile, may lead to physical entanglement of the macroalgae, increasing the likelihood that they can attach and so reducing the proportion lost, however this effect was not seen in this study since we used an excess seeding density, ten-fold higher than the number of fronds that developed. Experimental manipulation of this seeding density could be used to determine the optimal density and proportion washed away on different seeded materials.

During the attachment period, when the binder is still present, it is highly likely that the prevailing weather conditions will strongly influence the sporophyte/gametophyte attachment success. Higher water motion due to storms will wash the binder solution away more quickly, while low light will limit growth and holdfast development. Further testing of the binder formulation dependent on the prevailing conditions needs to be conducted to test this hypothesis.

5. Conclusions

This study has demonstrated that the sporophyte seeding technique using a binder is an effective method to allow textile substrates to be seeded for macroalgal cultivation. Sporophyte seeding resulted in twice the final biomass yield compared with gametophyte seeding, due to a 2–3 week developmental lag, while meiospore seeding gave very poor results. The non-woven textile tested, had high initial fouling, but gave an equivalent final biomass to when Kuralon twine was either A) seeded with sporophytes or B) seeded and grown within a traditional hatchery. However, the method needs further optimisation and testing to ensure it is reliable.

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