Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case-study

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Abstract

To reach the goal of an industrialised macroalgae industry in Norway and other high-cost countries in the near future, a standardised seedling production method to improve quality control and predictability of cultivated biomass is essential. A total of 11 different treatments for seeding twine or rope with meiospores, gametophytes or juvenile sporophytes from the kelp *Saccharina latissima* were measured for growth (frond length, frond area, biomass yield and density) and protein content after 80 and 120 days at sea. Meiospore- and gametophyte-seeded twines were pre-cultivated in the hatchery for 14–42 days prior to deployment, while juvenile sporophytes of different ages were seeded on ropes directly on the day of deployment using a commercial binder to attach the seedlings. The results showed that seeding with meiospores pre-cultivated in the hatchery for 42 days (S42) before deployment gave significantly longer fronds (77.0 ± 6.7 cm) and a higher biomass yield (7.2 ± 0.1 kg m^{-1}) at sea compared to the other treatments. The poorest growth was measured for the direct seeded sporophytes pre-cultivated in free-floating cultures for 35 days prior to deployment (D35; 34.4 ± 2.4 cm frond length and 1.6 ± 0.4 kg m^{-1}). Image analysis was used to measure the coverage of the twine substrate before deployment, and a correlation was found between substrate coverage and frond length at sea, indicating that this can be used as a tool for quantity and quality control during the hatchery phase and before deployment. The protein content did not reveal any large differences between the treatments after 120 days of cultivation.

Key words: Cultivation strategies; Direct seeding; Kelp aquaculture; Image analysis; Optimising seaweed hatchery; Protein content

Abbreviations: DW, dry weight; IPR, Intellectual Property Rights; K_p, specific nitrogen-to-protein conversion factor; L:D, Light:Dark; OD, Optical density; PES, Provasolis Enriched Seawater; RGR, relative daily growth rate

Introduction

The macroalgae *Saccharina latissima* (Phaeophyceae) is one of the most attractive species for cultivation in the North Atlantic Ocean due to its fast growth and high content of valuable components (Holdt and Kraan 2011; Handå et al. 2013; Peteiro and Freire 2013; Sharma et al. 2018; Bak 2019). In 2017, Europe contributed less than 1000 tons of *S. latissima* to the global macroalgae cultivation of about 30 million tons (Ferdouse et al. 2018), with China and other Asian countries supplying the major part of the biomass and using breeding as a strategy to improve yield and quality (Wu and Guangheng 1987; Zhang et al. 2007). Macroalgae for human consumption accounts for 83–90% of the value of the global market (Wei et al. 2013), and the Western market is expected to increase rapidly due to consumers' desire for new protein sources and healthy food supplements (Kim et al. 2017). This is a key driver for the ongoing development of an industrial macroalgae cultivation in Europe (Cottier-Cook et al. 2016), and high salary costs call for standardised solutions that are easy to scale up.

In aquaculture, seaweeds grow on artificial substrates or under free-floating conditions. Regardless of cultivation methods, land facilities are currently necessary to accommodate the hatchery units and facilities for the processing of harvested biomass. Although most cultivated macroalgae species can be grown through vegetative propagation, the production of seedlings is mandatory for several important commercial species like kelp. *S. latissima* has a diplo-haplontic, heteromorphic life cycle, with alternation between a microscopic haploid (n) gametophyte generation and a macroscopic diploid (2n) sporophyte generation (Kain 1979). During the fertile season, sorus with sporangia develop on the lamina and meiosis produces meiospores (spores) that are released into the surrounding water (Van Patten and Yarish 1993). The spores develop into female and male gametophytes, and fertilisation leads to the development of microscopic sporophytes that grow to adult size (Kain 1979). For any seaweed species grown through sexual reproduction, optimising hatchery production processes is crucial to the success of sea farming. Standardisation of cultivation procedures and strategies is essential to overcome low predictability of production quantity and quality, and to lower the production costs.

There are three main strategies for producing kelp seedlings; seeding the growth substrate with either 1) meiospores, 2) gametophytes or 3) juvenile sporophytes. Seedlings can be kept on a substrate in the hatchery for several weeks before deployment at sea or seeded directly before deployment using a binder.

Using spores for seeding requires fertile sporophytes, which is season-dependent if these are collected in natural habitats. Fertility can also be induced by artificial day rhythm and thus enable

year around access to spores (Pang and Lüning 2004; Forbord et al. 2012). *S. latissima* development can be held in the gametophyte life stage (Zhang et al. 2008) by keeping the cultures in red light under controlled environmental conditions where fertilisation can be induced by changing from red to white light (Lüning and Dring 1972; Cuijuan et al. 2005). These continuous cultures are available for year-through seeding of gametophytes or production of juvenile sporophytes for direct seeding. This method can be advantageous as the use of incubation facilities might be shortened by several weeks or, in the case of direct seeding, omitted completely. The use of a binder to attach spores, gametophytes or sporophytes to the substrate is preferred by several commercial farmers and in research projects (Mols-Mortensen et al. 2017; Bak et al. 2018; Kerrison et al. 2018; Kerrison et al. 2019). Recent experiments have shown that a binder-method of cultivation is not only as effective as traditional methods but can also be 100 times more space-efficient during the laboratory phase (Kerrison et al. 2018). When seeding spores or gametophytes on twine without using a binder, they need to be incubated in a hatchery for several weeks to be able to attach properly to the substrate before being deployed at sea (Xu et al. 2009; Forbord et al. 2018).

This study aimed to compare how three different seeding methods of *S. latissima,* using either meiospores, gametophytes or direct seeding with juvenile sporophytes, and time of hatchery periods affect growth in size (length and area), biomass yield, density and protein content during 80 and 120 days of sea cultivation.

Materials and methods

Preparation of gametophyte cultures. Sporophytes of *S. latissima* were collected by divers in August 2017 for induction of sori under low-light (70 µmol photons m^{-2} s^{-1} at the water surface)/short-day (8 h light:16 h dark) conditions for 6 weeks before maturation according to Forbord et al. (2012). Sorus pieces from around 20 sporophytes were disinfected and dehydrated for 24 hours at 4 °C, and spore release was carried out the following day. The spore solution used for starting gametophyte cultures had a density of 400.000 spores mL^{-1}, which was added to culture flasks with Provasolis Enriched Seawater (PES) (Provasoli 1968) kept at 10°C under constant red light with a light intensity of 30 µmol photons m^{-2} s^{-1} and filtered air provided through silicon tubes for aeration. The cultures were up-scaled and maintained after 4 weeks and then every second week until used in the experiment during January 2018.

Preparation of cultures of juvenile sporophytes for direct seeding. Fertile *S. latissima* sporophytes were collected by divers in December 2017 and stored in tanks with running seawater and low-light

/short-day conditions until used for different seeding trials in the current experiment. Sori tissue from around 20 sporophytes were used to obtain a spore solution with a density of 400.000–470.000 spores mL^{-1} used for starting cultures of free-floating seedlings in aerated flasks containing PES at 10°C in white light (40 µmol photons m^{-2} s^{-1}) and a light regime of 16 h light:8 h dark to promote fertilisation and sporophyte development. Cultures were started 42 (D42), 35 (D35) and 28 (D28) days before they were seeded on ropes using a binder and deployed at sea the same day. The seedlings had an average length of 45–120 µm before seeding and the fraction of sporophytes vs. gametophytes was in the range of 7.4–13.6% where one counted individual was equal to one sporophyte or one gametophyte filament (Table 1). The sporophyte cultures were diluted 50/50 with autoclaved seawater to aim for an equal density as the spore solutions. One culture of gametophytes was induced in white light for 14 days (GF0) before being seeded with the binder directly on ropes on the day of deployment and had an average length of 88 µm and a sporophyte vs gametophyte fraction of 6.4%. A commercial binder (AtSeaNova, BE) was used to thicken the sporophyte/gametophyte cultures, which were applied to the ropes (⌀ 18 mm, braided AlgaeRope), preventing the suspended seedlings from being washed off before they could attach (Kerrison et al. 2018). The protocol for seeding with the binder is IPR of the AT~SEA project partners (http://www.atsea-project.eu/).

Seeding of meiospores and gametophytes on twine in the hatchery. The spore solution used for producing seedlings on twine had a density of 250.000 spores mL^{-1} (Table 1). Gametophytes used for seeding were either taken straight from the red-light conditions or induced for 14 days in white light to make the gametophytes fertile before seeding. The gametophyte densities were measured with optical density at 750 nm (OD_{750}) and diluted to 0.35 mg mL^{-1} (DW) before seeding (OD calculated to DW from standard curves), a density found to give adequate seedling growth on substrate in previous experiments with *Alaria esculenta* (Duarte 2017). Spores and gametophytes were seeded on ⌀ 1.2 mm twisted nylon string coiled around PVC spools. The spools were incubated in 300 L cylinders holding running, nutrient-rich deep water, a light intensity of 70 µmol photons m^{-2} s^{-1} outside the cylinders and a light regime of 16:8 (L:D). To let the propagules settle on the twine, the water was kept stagnant before a water flow of around 2 L min^{-1}, and aeration was turned on three days after seeding. Seeding with spores started 42 (S42), 28 (S28) and 21 (S21) days before deployment, and gametophytes were seeded 28 (G28), 21 (G21) and 14 (G14) days before deployment (Table 1). One culture of gametophytes was induced in white light for 14 days before being seeded on twine and kept in the hatchery for 14 days before deployment (GF14). Details concerning all stages of seedling production are described in Forbord et al. (2018).

Substrate coverage measured by image analysis. To quantify the number of seedlings covering the twine substrate, images were collected two days prior to deployment at sea for later processing. The cylinders with the twine substrate were removed from the incubators into a small water-filled glass tank for depiction. A white LED ring light (Effilux 000 SD P2) was used for even illumination in a brightfield setup with the lens positioned inside the ring light. Each substrate cylinder was depicted at three distinct locations, resulting in three images per substrate (Alver et al. 2018). The working distance was kept identical between images, and the field of view was 30x40mm. Images were collected using a Nikon D800E DSLR and a Sigma AF 105 mm f/2,8 Macro lens. Software was developed in LabVIEW (National Instruments co., Austin, TX, USA), which extracted the saturation colour plane to identify seedlings on the white substrate. The International Commission on Illumination has defined six attributes describing a colour, saturation being one of them, defined as the colourfulness of an area relative to its brightness (CIE Standard S 017/E, 2011). Using the saturation colour plane is a robust method of segmenting the growth from the cultivation substrate. The average pixel intensity from the saturation colour plane of the three images per cylinder is calculated to represent the amount of growth using the developed software. The values presented here were normalised to a percentage of the output range, where 0% is a clean, white substrate and 100% is a substrate completely covered by sporophytes. See Figure 3 for examples of images.

Deployment at *sea, growth measurements and collection of samples for chemical analysis.* The seed lines were entwined onto 6 m long 18 mm carrier ropes using a spinning machine on the day of deployment (Alver et al. 2018; Forbord et al. 2018) and randomly distributed vertically from two longlines in the sea farm Skarvøya in Central Norway (63°39'N 8°39'E) on 13 February 2018 (Figure 1). This area has a mild maritime climate with the coldest season from January to March and the driest season in May to June (Sharma et al. 2018). The farm is sited at a sheltered location. The first registration was done on 4 May 2018 after 80 days at sea and the last one on 13 June 2018 after 120 days at sea. Length and width measurements of 20 sporophytes for all treatments on each of the two longlines were registered in May and June, and biomass and density were measured only in June, all from 1–2 m cultivation depth. Samples of 10 individuals, consisting of frond, stipe and holdfast, from all treatments at both lines, were collected for chemical analysis and kept cold in bags until frozen at -20°C immediately after arriving at the laboratory (Figure 1).

The relative daily growth rate (RGR, day^{-1}) based on increase of mean frond length was calculated as:

$$\mathrm{RGR}\ (day^{-1}) = \frac{\left(\frac{L_1 - L_0}{T}\right)}{L_0} \quad (1)$$

where L_1 represents length (cm) at a given sampling date, L_0 the length (cm) at the previous sampling date, and T is the elapsed time (days) between these sampling days.

The area of the sporophytes was calculated using a factor of 0.75 * length * width (Broch et al. 2013).

Temperature. The temperature was recorded at 2 m depth every 15 minutes using Onset HOBO pendant loggers (Bourne, MA; temperature accuracy ± 0.53 °C, resolution 0.14 °C) situated on a separate rope placed in the middle of the farm. The loggers were cleaned from fouling during the May registration.

Nitrogen analysis. Nitrogen content was analysed for the whole thalli. The samples were frozen at -20°C and later stored at -80 °C until freeze-dried (Hetosicc CD 13-2) at -40 °C for 48 hours. The dried kelp was homogenized into a fine powder, samples of 0.4–1.0 mg freeze-dried kelp were transferred to tin capsules, and nitrogen was analysed in parallels with a Carlo Erba element analyser (model 1106). The nitrogen content was used to calculate the protein content using season and depth-specific nitrogen-to-protein conversion factors (K_p) of 3.6 for May and 4.3 for June (Forbord et al. submitted).

Statistics and data analyses. Independent-samples t-tests were run to assess if there were differences between frond length and width between the two long lines after confirming the assumption of normality (Shapiro-Wilk's test) and homogeneity of variance (Levene's test). Significant differences were not found between any of the 11 treatments on the two lines, and the lines were pooled to get a sample size of n=40. For frond length and sporophyte area, the assumption of homogeneity of variances was violated (Levene's test, $p < 0.001$). The Welsh ANOVA was used to look for significant differences and the Games-Howell post-hoc test to compare all possible combinations of group differences. Linear regression analysis was performed to look for the relationship between density and individual sporophyte weight, and for mean frond length and the substrate coverage before deployment. Data are presented as mean ± standard error (SE) unless otherwise is stated. Significance level was set to 0.05. Statistical analysis was performed using IBM SPSS Statistical software (Version 25) and plots were made using Systat SigmaPlot software (version 14).

Results

Substrate coverage before deployment at sea. The treatment showing the highest substrate coverage was the spore treatment S42 with an average of 84% (Figure 2 and 3). This treatment had the longest incubation time of 42 days in the hatchery before deployment at sea. S28 and S21 had a

coverage of 58% and 25%, respectively. For the gametophyte seeding, the G28 treatment with 28 days of incubation had a substrate coverage of 43% on average compared to G21 and G14 with 25% and 9% coverage, respectively. The GF14 treatment that was induced in white light before seeding and incubated in the hatchery for the same number of days as the G14 treatment had a coverage of 10%.

Temperature. The sea temperature was at its lowest in mid-March with 4.5 °C and at its highest at the beginning of June with 10.2 °C (Figure 4). Average monthly temperatures never reached more than 9.8 °C.

Growth performance at sea. Mean frond lengths of *S. latissima* varied pronouncedly between treatments and a pattern was apparent where the treatments with a long hatchery period had longer fronds than the treatments with a shorter period in the hatchery (Figure 5). The mean maximum frond length in June of 77.0 ± 6.7 cm was found for treatment S42, while the shortest fronds were found for treatment D35 with 34.4 ± 2.4 cm. The S42 sporophytes were significantly longer than the other treatments in both May (Welch's $F_{10,170.2}$=48.7, p<0.001) and June (Welch's $F_{10,171.2}$=20.4, p<0.001).

Generally, the direct seeded treatments (GF0, D42, D35 and D28) showed higher relative growth rates (RGR, day^{-1}) between the two registrations than the spore and gametophyte seeded treatments. The RGR for all treatments fluctuated around 0.02–0.05 a day^{-1} (Figure 5).

The relationship between mean frond length (cm) and the substrate coverage (%) before deployment revealed a strong positive correlation for both May (r=0.84) and June (r=0.90). Linear regression was used to fit straight lines to the data (Figure 6), and the linear association reached statistical significance for both May (R^2=0.7, $F_{1,5}$=11.8, p=0.018) and June (R^2=0.8, $F_{1,5}$=20.7, p=0.006), with a slightly better fit for June than for May.

The highest mean frond area in June was found for treatment S42 with 588.2 ± 52.4 cm^2 and the lowest was measured for D35 with 133.4 ± 11.8 cm^2 (Figure 7). The S42, S28, G28 and G21 sporophytes had a significantly larger area than the other treatments in May (Welch's $F_{10,165.95}$=43.1, p<0.001), and in June the S42 and G28 sporophytes showed a significantly larger area than the other treatments (Welch's $F_{10,170.3}$=19.5, p<0.001).

Biomass yield (kg m^{-1}) and sporophyte density (individuals m^{-1}) were measured in June, and as no fouling by diatoms and filamentous algae was visible, the weight represents only kelp biomass. The mean biomass yield across all treatments was 3.4 ± 0.5 kg m^{-1}, and the range was from 1.6 ± 0.4 for

D35 to 7.2 ± 0.1 kg m^{-1} for the S24 treatment (Figure 8a). The sporophyte density had a mean value across all treatments of 311.1 ± 87.9 individuals m^{-1} and was lowest for the G28 treatment with 175.0 ± 5.0 individuals m^{-1} compared to the highest density of 450.0 ± 35.4 individuals m^{-1} for the D35 treatment (Figure 8b).

The relationship between sporophyte density and the individual sporophyte weight revealed a linear increase of individual weight with decreasing density with a strong positive correlation (r=0.73) (Figure 9). Linear regression was used to fit a straight line to the data, and the linear association reached statistical significance (R^2=0.5, $F_{1,9}$=10.0, p=0.012). The results from the S42 treatment (marked with a circle in Figure 9) deviated strongly from the others, having both a high density (417.0 ± 31.8 individuals m^{-1}) and high individual weight (8.7 ± 0.6 g). With the removal of this treatment from the linear regression, the positive correlation was very strong (r=0.95), with a significant linear association (R^2=0.9, $F_{1,8}$=73.5, p<0.001).

Protein content. The protein content decreased from May to June for all treatments (Figure 10), and the mean content across all treatments was 73.3 ± 2.4 mg g^{-1} DW in May and 57.2 ± 1.5 mg g^{-1} DW in June. In May, the highest protein content was found for the G21 sporophytes with 87.0 ± 5.3 mg g^{-1} DW, and in June the highest content was measured in the G14 treatment with 67.3 ± 2.6 mg g^{-1} DW. Overall, the protein content did not vary a lot between the 11 different treatments over the cultivation period.

Discussion

Building up a full-scale seaweed hatchery can constitute a high investment cost for farmers and might not be manageable for newly established companies. A shortening of the incubation phase, thus enabling several production cycles or a total omittance, might therefore be worth looking into, especially when aiming for large-scale cultivation to reach the prospected production goals of 4 million tons of macroalgae in Norway in 2030 and 20 million tons in 2050 (Olafsen et al. 2012).

Image analysis of substrate coverage before deployment at sea. Standardisation of production methods to improve quality control and predictability of produced quantity of seaweed biomass is needed for upscaling to industrial production volumes of macroalgae in high-cost countries, e.g. in Western Europe. Monitoring, automation and control techniques are needed to replace manpower. In this study, we demonstrate a possible first step through the measurement of the substrate coverage as a form of early-stage control of the seedling quality and expected quantity of produced biomass. The method makes processing of a large number of images possible with little effort, compared to manual counting/analysis of the substrate itself or images of it. However, a weak point

of this method is to separate the growth of target species from that of other contaminant species like diatoms or filamentous algae. If these species have a somewhat similar colour representation, contamination may be hard to distinguish from target species using image processing techniques. Although the measured growth may be correct using the saturation method presented here, it may not be accurately represented for the targeted species if unwanted species are increasingly present. The method may be refined, and one step in that direction would involve the calibration of reflectance from a standard target, as light quality, intensity and sensitivity in the source and camera are due to change between equipment and time. The light-reflecting properties of the substrate influence the definition of zero coverage, and hence this should also be included in the calibration process. A comparison of the method against the manual counting of sporophytes on substrate was not within the scope of the experiment.

Growth performance at sea. All the 11 seeding treatments were cultivated successfully at sea but with significant differences in frond lengths. The overall best performance of all measured variables was obtained by seeding twine with spores and pre-cultivating them in the hatchery for 42 days (S42), a treatment used in previous experiments in Norway (Forbord et al. 2012; Handå et al. 2013; Fossberg et al. 2018; Sharma et al. 2018). Frond lengths in June for the S42 treatment were comparable to previous experiments in the Faroe Island and Norway (Handå et al. 2013; Mols-Mortensen et al. 2017; Bak et al. 2018), and the biomass was well within the range found by other trials in Europe (Peteiro and Freire 2009; Kraan 2013; Mols-Mortensen et al. 2017; Matsson et al. 2019). The D35 treatment, on the contrary, showed the shortest frond lengths and the lowest biomass yield, which was in the same range or higher as found in several Danish cultivation experiments (Marinho et al. 2015; Bruhn et al. 2016). All treatments in the current experiment resulted in shorter fronds than those found in a previous cultivation experiment in the same geographical area but at a more exposed location (Sharma et al. 2018). They found average lengths twice of the best growth in the present experiment after 134 days at sea, suggesting that local environmental conditions have a major impact on the growth at sea.

The frond area showed a similar trend as the length in June with the highest measured values for the S42 treatment and the lowest for D35, indicating that the width of the frond mainly followed the frond length at this sheltered location. Both the area and length of *S. latissima* have been found to give good estimates for the standing biomass (Stagnol et al. 2016; Foldal 2018).

The highest relative growth rate (RGR) for the cultivation period was found for the four direct seeded treatments (GF0, D42, D35, D28), which had the shortest frond lengths compared to the

306 others after 80 and 120 days at sea. This is explained by growth rates becoming reduced with
307 increasing size of the sporophytes (Huges 1973; Kain 1991).

308 Sporophyte density on ropes can affect individual sporophyte growth and total yield (Reed et al. 1991;
309 Creed et al. 1998; Steen and Scrosati 2004; Kerrison et al. 2015; Kerrison et al. 2016). The optimal
310 density for highest achievable biomass yield for *S. latissima* and other kelp species is, however, still
311 unknown (Kerrison et al. 2015). The sporophyte density varied greatly between the treatments in the
312 current experiment with an almost three times higher density for D35 compared with G28, suggesting
313 a better attachment to the substrate (Xu et al. 2009; Kerrison et al. 2018). The sporophyte density had
314 a strong correlation with the individual sporophyte weight, showing that high densities led to low
315 individual weights due to intraspecific competition and resource limitation such as light and nutrients
316 (Kain and Jones 1963). However, the only treatment deviated from this linear trend was the S42 with
317 the highest yield but also one of the highest densities. One possible explanation for this can be that
318 the long incubation period in the hatchery before deployment stimulated the development into
319 sporophytes from a higher number of spores, but the high density obviously did not exceed the
320 optimal density for growth at sea.

321 All 11 treatments were deployed the same day on the same farm and were exposed to the same
322 environmental conditions during sea cultivation. Differences in growth performance were,
323 therefore, most likely attributed to the size and density of the juvenile sporophytes at deployment.
324 The range in temperature measured during the experiment showed that the treatments were
325 cultured within the typical thermal range of 5–15 °C for *S. latissima* (Kerrison et al. 2015) and never
326 encountered temperatures exceeding 17 °C that may cause loss of tissue (Gerard et al. 1987).

327 *Comparing similar seeding methods*. By comparing growth performance at sea for treatments with
328 the same seeding method (spores, gametophytes or juvenile sporophytes), a clear pattern was seen
329 for spores (S42, S28 and S21), with a significantly increased growth at sea with number of days in the
330 hatchery. This was also evident from the substrate coverage before deployment with a decrease
331 from 84% for S42, 58% for S28 and 25% coverage for S21. For seeding with gametophytes (G28, G21,
332 G14 and GF14) on the contrary, no significant differences were found at sea after days in the
333 hatchery. However, a difference in the substrate coverage could be seen with the G28 having 43%
334 coverage, the G21 25% and the G14 and the GF14 9 and 10% coverage, respectively. A clear
335 correlation between the substrate coverage and frond length for all twine seeded treatments was
336 found for both the May and June samplings, suggesting that image analysis can be used as a tool for
337 easy quantification of frond lengths and quality assurance of the seed lines before deployment. The
338 four direct seeded treatments (GF0, D42, D35 and D28) showed the poorest growth at sea for all

measured variables and no significant differences with seedling age. Seedling lengths and share of developed sporophytes compared to gametophytes in the free-floating cultures did not follow an age-specific pattern. This could be due to small and stochastic differences in spore development in the free-floating cultures caused by genetic variation, a different maturation degree of the selected sori, self-shading or minor different physical conditions like light and aeration. However, a clear coherence between the seedling's length at deployment and growth performance at sea was evident for the D35 treatment that had the poorest growth for all measurements in this study, and that deviated most from the robust S42 treatment.

Comparing treatments of similar age. The gametophytes were at a more advanced stage in the development than spores when seeded on twine, but they usually need 8-10 days in white light before reaching fertility (Arbona and Molla 2006) and time to develop rhizoids to attach properly to the substrate in contrast to spores that actively attach (Xu et al. 2009). The substrate coverage before deployment at sea for treatments S28 vs G28 and S21 vs G21, which had the same number of days in the hatchery, was comparable. When comparing these treatments at sea after 120 days of cultivation, they had the same frond lengths and biomass yield, but the spore-seeded twine had a higher density than gametophyte-seeded twine, most likely due to poorer adhesion properties of the gametophytes (Xu et al. 2009; Shan et al. 2013). Loss of propagules after seeding and placement in the incubators could thus be a possible explanation for the gametophyte treatments resulting in the lowest density at sea. Techniques to avoid this can be to disrupt the gametophytes fragments as small as possible for better attachment, increase the period of stagnant water in the incubators (Xu et al. 2009) or use a binder for better attachment (Kerrison et al. 2018). The gametophytes transferred to white light for induction of gametogenesis (GF14) 14 days prior to seeding did not show a significantly better length growth at sea than the gametophytes seeded directly from red-light conditions (G14), indicating that the fertility induction in white light in reality can be omitted for *S. latissima* gametophytes.

Direct seeding. Gluing juvenile sporophytes or gametophytes directly on to the droplines before deployment using a binder saves both time and space by skipping the hatchery incubation and the time-consuming entwining process of the seed string onto the droplines. When cultivating juvenile sporophytes in free-floating cultures, the holdfast of the sporophytes will most likely not develop as rapidly as when pre-cultivated on substrates in a hatchery for several weeks prior to deployment at sea. Mols-Mortensen et al. (2017) explained low yields using a binder-method by a possible detachment of the seedlings shortly after deployment. The sporophyte density of the direct seeded ropes in the current experiment was among the highest in the experiment, suggesting that the direct seeded sporophytes were not washed off the ropes after deployment but rather that extra time was

needed to develop holdfast and a tight attachment to the substrate before the frond elongation could start. The sheltered sea farm and the good light conditions at time of deployment could also have contributed to a successful attachment (Kerrison et al. 2018). A longer cultivation period, by deployment e.g. in autumn, or seeding with lower densities, may have levelled out the growth between the different treatments when harvesting in June. No differences in growth measurements were found when comparing direct seeded gametophytes induced in white light for 14 days to direct seeded sporophytes cultured up to 42 days in white light. This is probably due to the low fraction of developed sporophytes in all treatments before seeding that might be the result of sub-optimal culture conditions for sporophyte development.

The rope structure and material used for seeding have proven to have a large impact on the harvesting yield. Twisted ropes have shown significantly better performance than braided ropes for both spores and juvenile sporophytes seeding (Kerrison et al. 2019). The spores and gametophytes were seeded on a twisted nylon string (⌀ 1.2 mm and wound around ⌀ 18 mm twisted rope when deployed), while the treatments using a binder were seeded directly on a braided rope (⌀ 18 mm, AlgaeRope). This dissimilarity could have impacted the final yield in this experiment and explained some of the differences.

Protein content. A decrease in protein content was measured from the first sampling in early-May of 7.3 ± 2.4% DW to the second sampling in mid-June of 5.7 ± 1.5% of DW on average. The protein content most likely followed the seasonal pattern of ambient nitrate fluctuations in seawater (Rey et al. 2007; Broch et al. 2019), where a higher ambient nitrate concentration is found to result in higher protein content (Harnedy and FitzGerald 2011). Brown seaweeds have been reported to have lower protein content than green and red seaweeds, but the single maximum value found in this study (11% of DW) is within the range of some of the concentrations found in green (10–26% of DW) and red seaweed (5–44% of DW) (Fleurence 1999; Holdt and Kraan 2011). The growth environment is likely to affect the biochemical composition of seaweeds, which may, in turn, affect the quality of the harvested biomass (Kerrison et al. 2015; Schiener et al. 2015). Because the sea cultivation conditions were similar for all treatments, no large differences were expected to be found for the protein content between them. It is worth noting that the protein concentration is calculated from the nitrogen content using season- and depth-specific nitrogen-to-protein conversion factors (K_p) found in a previous experiment from the same geographical area (Forbord et al. submitted). This may both have under- and overestimated the protein concentration at certain points in time (Manns et al. 2017; Mæhre et al. 2018).

Summary and Conclusions

This study has demonstrated that different seeding methods and hatchery periods had high impact on the growth performance of *S. latissima* at sea. Twine seeded with spores pre-cultivated in the hatchery for 42 days gave significantly better growth measurements than any of the other treatments tested in this experiment. A clear coherence was found between days in the hatchery before deployment and growth performance at sea for the spore seeding method. In contrast, no differences were found between the hatchery period and growth at sea for the gametophytes, which indicated that the hatchery period can be reduced down to 14 days and that the induction of fertility in white light before seeding is not crucial. The gametophyte seeding showed the lowest density of all treatments at sea but had larger frond lengths, area and biomass compared to the direct seeded treatments that used a commercial binder to attach juvenile sporophytes before deployment. All measured growth variables were poor for the direct seeded treatments during the relatively short cultivation period of 120 days at sea, but a longer cultivation period might have levelled out the differences between the seeding methods. The reduced costs by skipping the hatchery phase and entwining process may make up for this in a business prospective, but no attempts were made to compare the costs related to the different seeding techniques in this work. Image analysis of substrates before deployment seemed to be a useful tool when assessing frond lengths at sea, but the method needs to be further developed to include predictions about harvesting yields. One source of weakness in this study, which might have affected the comparison of growth measurements between the seeding techniques, is the different methods used to assess seeding density. An important area for further work should be to determine the optimal seeding density for the different life stages of kelp giving the maximum yield at sea and to standardise easy methodology for measurements before deployment.

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Author contributions

JS conceived the idea of this study and SF, KBS, AH and JS conceived and planned the article. SF, KBS, and JS executed the seedling production and SF and JS the cultivation trials and samplings. TS was responsible for the image analysis. SF wrote the first draft of the manuscript, and all authors contributed to the writing/editing of the paper and have approved the final manuscript.

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617 Table 1 Detailed information for the different hatchery treatments before deployment in sea
618 showing their abbreviation (Abb), the starting dates for cultures and for induction of fertility in white
619 light, the culture density, the date of seeding on ropes, the seeding density (spores mL^{-1} for spores,
620 mg mL^{-1} (DW) for gametophytes and individuals mL^{-1} for sporophytes) and the sporophyte lengths at
621 seeding.

622 Table 1:

Hatchery treatment	**Abb**	**Date of culture start/Date of fertility induction**	**Culture density (spores mL^{-1})**	**Date for seeding on ropes**	**Seeding density (spores mL^{-1}, *mg mL^{-1} (DW) or **individuals mL^{-1})**	**Length at seeding (µm)**
Spores 42	S42	-	-	03.01.18	250.000	-
Spores 28	S28	-	-	17.01.18	250.000	-
Spores 21	S21	-	-	24.01.18	250.000	-
Gametophytes 28	G28	06.10.17	400.000	17.01.18	*0.35	-
Gametophytes 21	G21	06.10.17	400.000	24.01.18	*0.35	-
Gametophytes 14	G14	06.10.17	400.000	31.01.18	*0.35	-
Gametophytes Fertile 14	GF14	06.10.17/17.01.18	400.000	31.01.18	*0.35	-
Gametophytes Fertile Direct 0	GF0	06.10.17/31.01.18	400.000	13.02.18	**Gametophytes: 2805 **Spores: 180	87.6
Sporophytes Direct 42	D42	03.01.18	434.000	13.02.18	**Gametophytes: 1740 **Spores: 185	92.4
Sporophytes Direct 35	D35	10.01.18	400.000	13.02.18	**Gametophytes: 5973 **Spores: 440	44.5
Sporophytes Direct 28	D28	17.01.18	467.000	13.02.18	**Gametophytes: 1730 **Spores: 235	120.0

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631 Figure 1 Geographical location of the experimental seaweed farm Skarvøya and the laboratory for
632 producing the seed lines. The region to the right is indicated by a black rectangle in the large-scale
633 map.
634
635 Figure 2 Substrate coverage (%) before deployment of spore and gametophyte treatments on twine
636 (see Table 1) incubated in the hatchery for 14-42 days. Mean ± SD, n=3.

637 Figure 3 Colour images of substrate (top row) and corresponding saturation image planes (bottom
638 row). Treatment S42 (left) had an average substrate coverage of 84%, treatment S28 (mid) with an
639 average substrate coverage of 58% and treatment S21 (right) with an average substrate coverage of
640 25%.

641 Figure 4 Daily average sea temperatures (°C) at 2m depth at the sea farm Skarvøya from deployment
642 in mid-February until harvest in mid-June.

643 Figure 5 Frond length for May and June sampling (left y-axis) and RGR (right y-axis) for the different
644 hatchery treatments (see Table 1), mean ± SE, n=40. Letters above bars denotes significant
645 differences in length between the treatments, lower-case letters for May and capital letters for June.

646 Figure 6 The mean frond length (cm) for May and June as a function of the substrate coverage (%) at
647 deployment in sea in February, with regression lines showing the linear trends.

648 Figure 7 Mean frond area (cm^2) for May and June sampling for the different hatchery treatments
649 (see Table 1), mean ± SE, n=40. Letters above bars denotes significant differences between the
650 treatments, lower-case letters for May and capital letters for June.

651 Figure 8a) Biomass yield (kg m^{-1}) and b) sporophyte density (individuals m^{-1}) for the different
652 hatchery treatments (see Table 1) in June, mean ± SE, n=2.

653 Figure 9 The sporophyte density on the ropes (individuals m^{-1}) as a function of the individual
654 sporophyte weight (g) with regression line showing the linear trend. The S42 treatment deviated
655 from the others with both high density and weight and is marked with a circle.

656 Figure 10 Protein content (mg g^{-1} DW) as [N]*3.6 in May and [N]*4.3 in June in *S. latissima* for
657 different hatchery treatments (see Table 1), mean ± SE, n=2.

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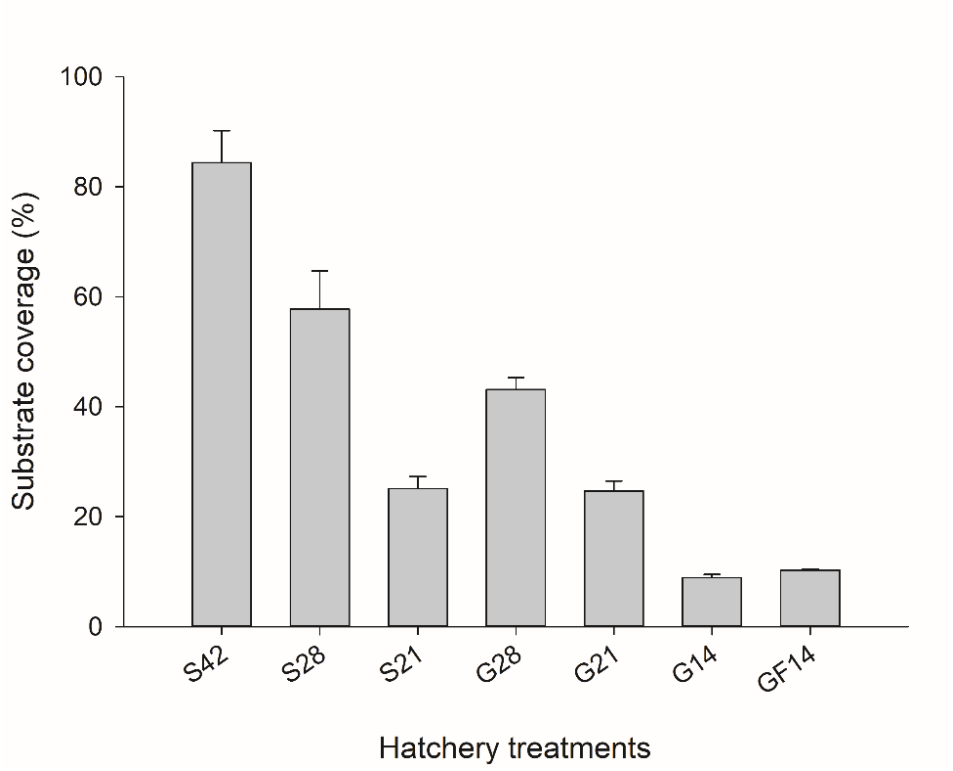
Figure 1:

Figure 2:



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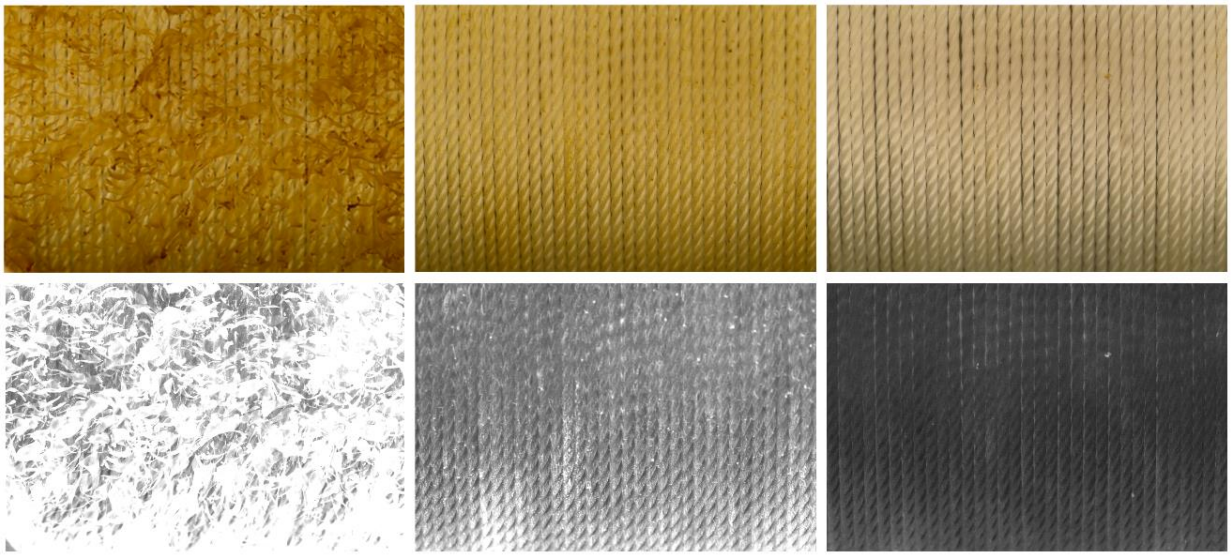

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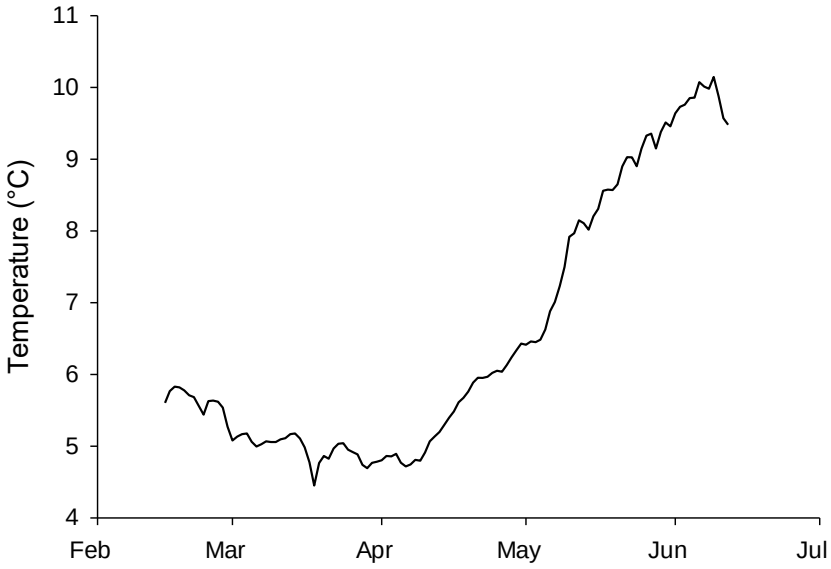

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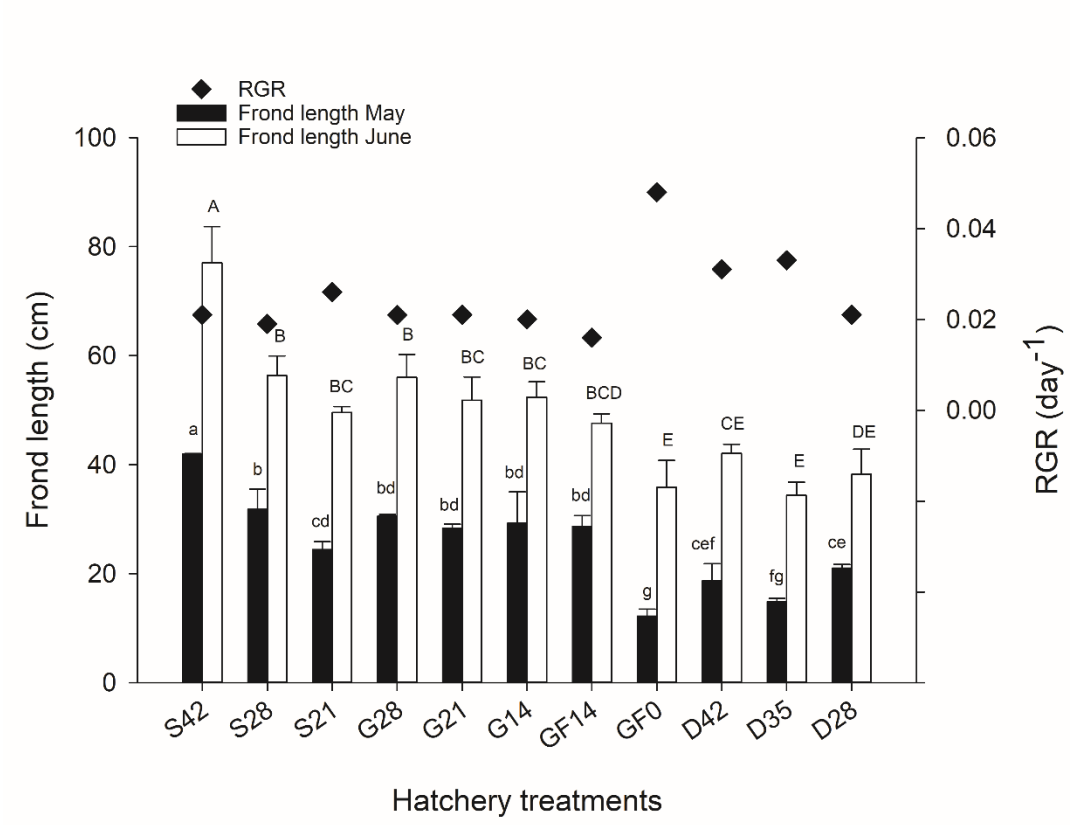
678 Figure 5:



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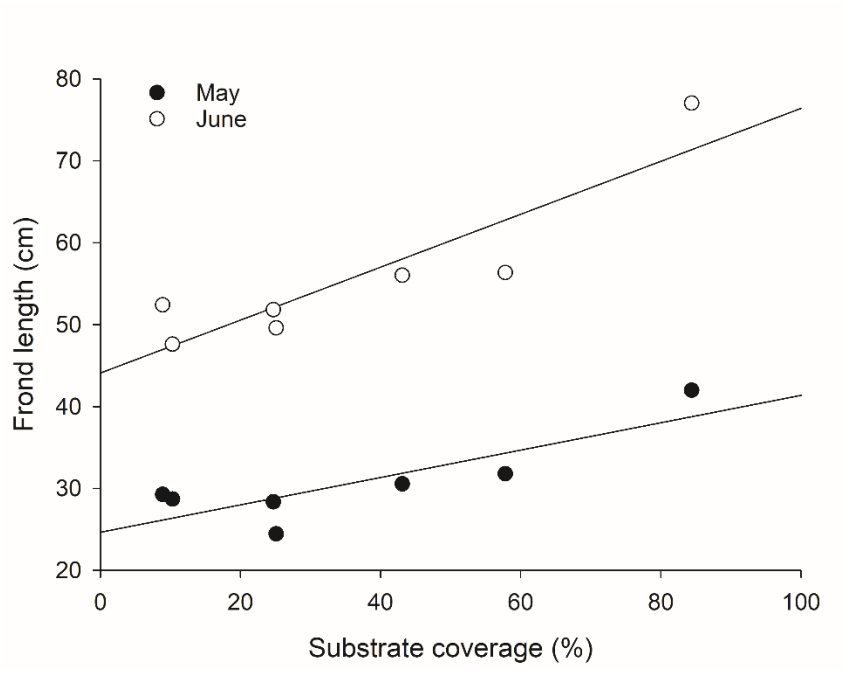
680 Figure 6:



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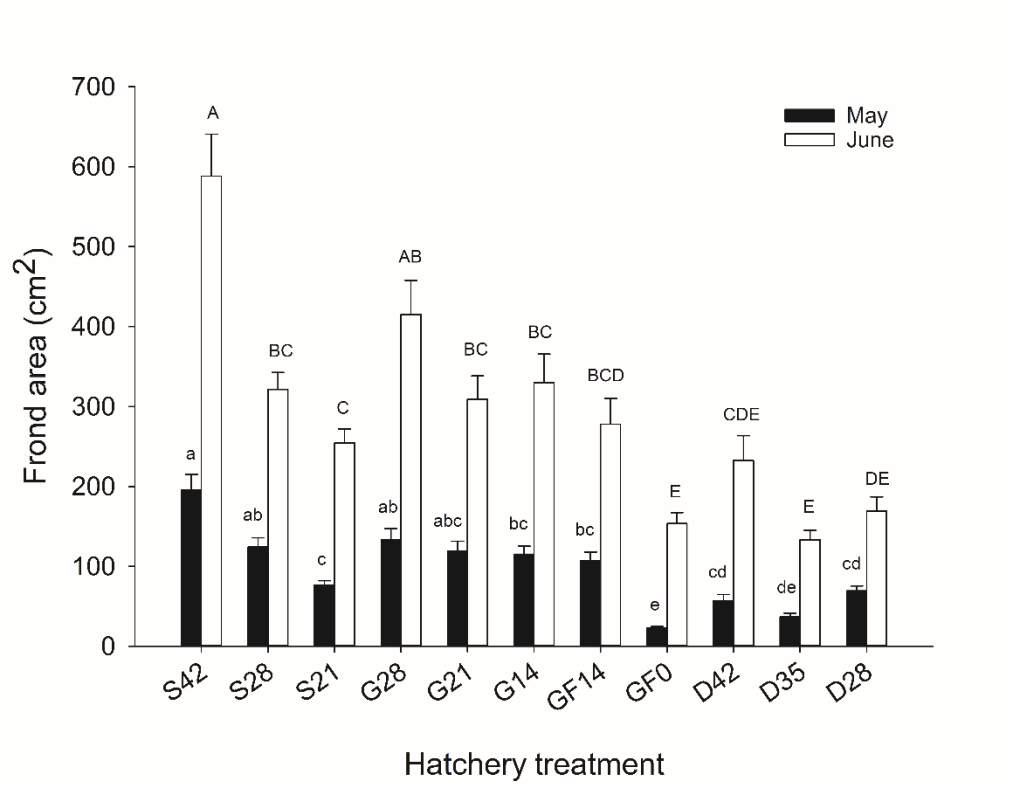
683 Figure 7:



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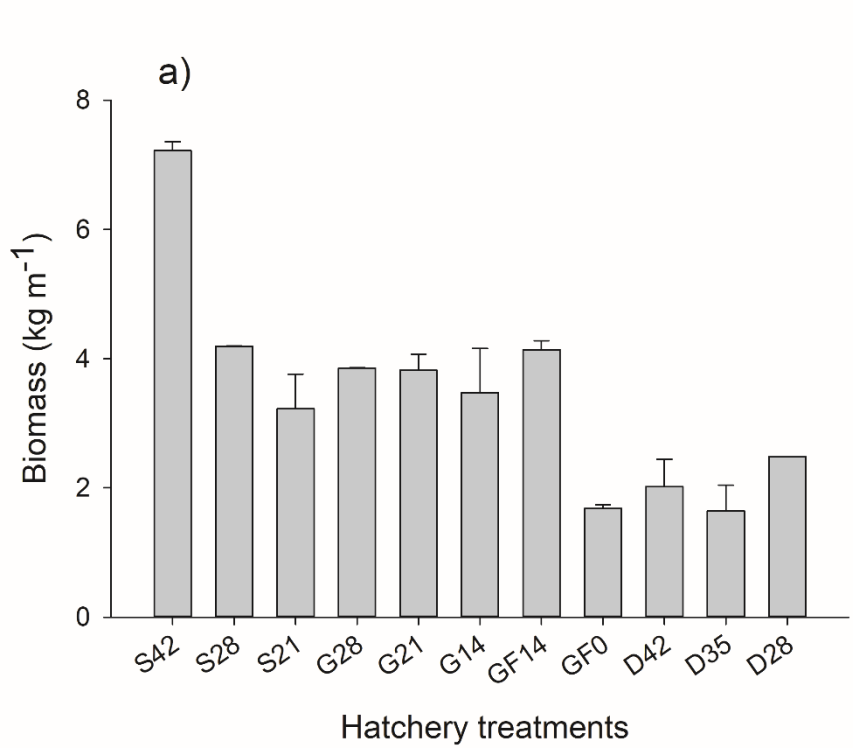

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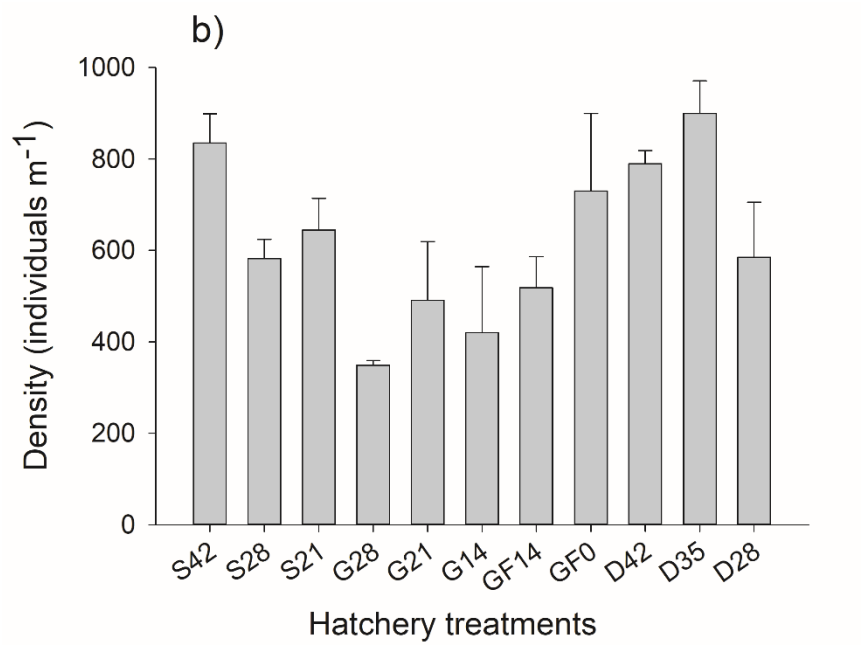

688 Figure 9:

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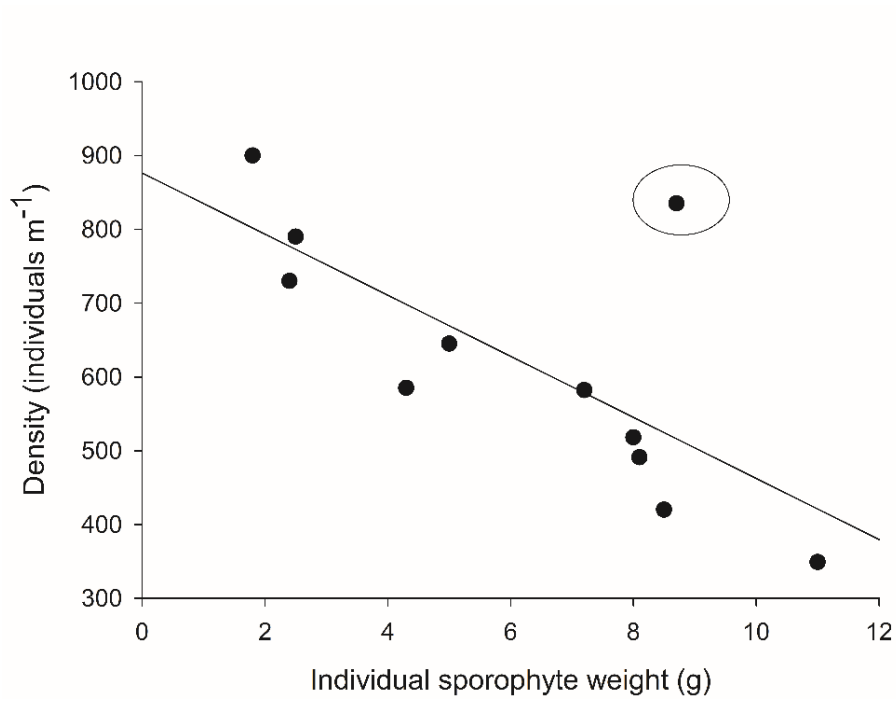

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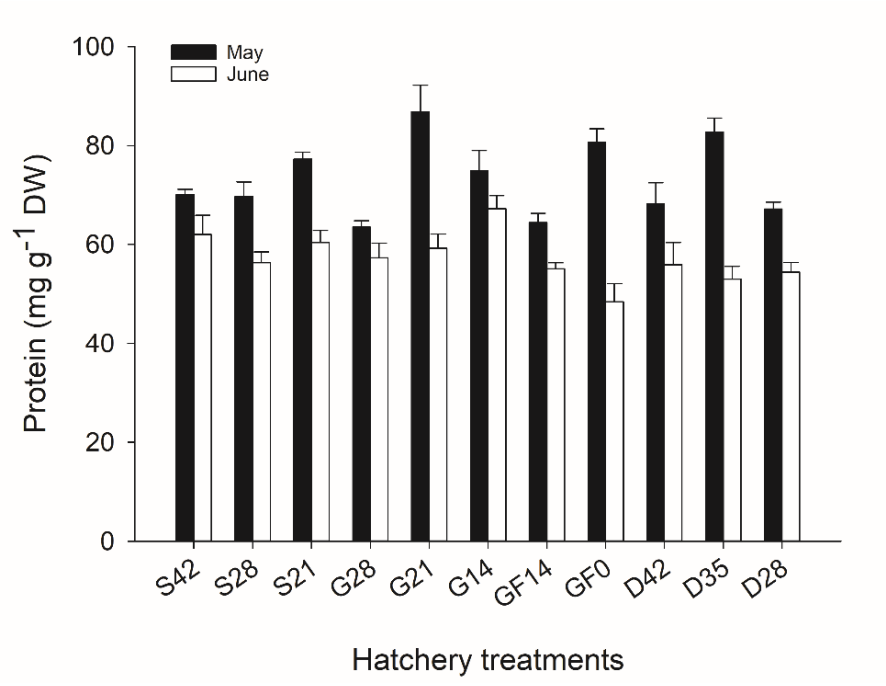

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