



Selecting optimal substrates for ocean-based cultivation of the red seaweed *Asparagopsis taxiformis* (Rhodophyta, Bonnemaisoniales)

Emma J. Theobald^{1,2} · Margaret B. Rule³ · Angela Capper^{1,2} · Guillermo Diaz-Pulido⁴ · Emma L. Jackson^{1,2}

Received: 7 March 2025 / Revised: 24 May 2025 / Accepted: 27 May 2025 / Published online: 13 June 2025
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Abstract

The red seaweed *Asparagopsis taxiformis* has the potential to reduce methane emissions from livestock, but key challenges remain in developing scalable, ocean-based cultivation protocols using a closed-lifecycle approach. A critical milestone in this effort is developing seeding methodologies and identifying effective substrates for the attachment of gametophyte germlings. This study evaluates the suitability of synthetic and natural twine substrates and a sodium alginate binder for *A. taxiformis* germling cultivation under laboratory conditions. Polypropylene, nylon, cotton and sisal twines and a smooth polypropylene control surface were seeded with a germling solution, either directly or within a sodium alginate binder. Germling cover (%) was assessed after six weeks of tank-based cultivation and again following unravelling and exposure to moderate-velocity flow to simulate ocean deployment conditions. Polypropylene twine retained the highest germling cover (104% cover post-exposure), while cotton twine (70% cover) showed promise as a biodegradable alternative. Reduced germling cover was observed on the sodium alginate binder treatments across the majority of substrates, with germling loss particularly high (81% reduction in cover) on the smooth surface of the polypropylene control. By demonstrating a scalable seeding methodology and identifying two effective, widely available substrates for cultivation; polypropylene and cotton twine, this study provides a foundation for developing seeded twine for *A. taxiformis* mariculture. Field trials will be essential to validate this nursery-stage performance at-sea. Ultimately, optimising seeding techniques and cultivation substrates will be key to scaling *A. taxiformis* mariculture and unlocking its potential for sustainable bioproduct applications.

Keywords Aquaculture · Attachment · Gametophyte · Germling · Hatchery · Twine

Introduction

The red macroalgal genus *Asparagopsis* (Bonnemaisoniaceae, Rhodophyta) exhibits a broad, near-global distribution, spanning from tropical to temperate regions across both its native and introduced range (Bonin and Hawkes 1987). Significant global interest in *Asparagopsis* cultivation has emerged, primarily due to its ability to reduce methane

emissions from livestock, including beef steers and dairy cows, through low-levels of dietary inclusion (Roque et al. 2019, 2021). Beyond methane reduction, interest in *Asparagopsis*-derived products is growing in the preservative, nutraceutical, cosmeceutical, and pharmaceutical sectors (Félix et al. 2021). To date, *Asparagopsis* cultivation has focussed primarily on land-based culture of the turf-forming tetrasporophyte life stage (FutureFeed 2023; Rule and Kelly 2023), an approach that enables elevated control over environmental parameters to optimise the synthesis of bioactive metabolites (Zanolla et al. 2022). Compared to the tetrasporophyte, the larger, feathery gametophyte produces significantly greater biomass (Bonin and Hawkes 1987), making gametophyte cultivation a promising avenue for sustainably scaling *Asparagopsis* production. While mature *Asparagopsis* gametophytes do not generally grow well in indoor, land-based systems (Ní Chualáin et al. 2004; Mickelson 2013; Batista 2020; Hall et al. 2025), ocean-based systems have shown promise for gametophyte cultivation and offer

✉ Emma J. Theobald
e.theobald@cqu.edu.au

¹ Coastal Marine Ecosystems Research Centre (CMERC), CQUniversity, Gladstone, QLD 4680, Australia

² School of Health, Medical and Applied Sciences, CQUniversity, Gladstone, QLD 4701, Australia

³ Drift Consulting, 34 Birkett Ave, Perth, WA 6164, Australia

⁴ School of Environment and Science, Coastal and Marine Research Centre, Griffith University, Brisbane, QLD 4111, Australia

significant potential for scaling (Werner et al. 2004; Kraan and Barrington 2005; Wright et al. 2022). Beyond scalability benefits, gametophyte mariculture may also provide nutrient bioremediation services in coastal waters (He et al. 2008; Xu et al. 2023) due to the high nutrient uptake rate of *Asparagopsis* (Mata et al. 2010).

To date, ocean-based cultivation of *Asparagopsis* gametophytes has largely been accomplished via vegetative fragmentation of *Asparagopsis armata* Harvey, by attaching fragmented wild-collected gametophytes to substrates for on-growing (Werner et al. 2004; Kraan and Barrington 2005). The feasibility of this method is due to *A. armata*'s barbed branches, which grip the substrate and are capable of regenerating from fragmented frond tissue (Bonin and Hawkes 1987; Wright et al. 2022). In contrast, *Asparagopsis taxiformis* (Delile) Trevisan lacks these barbed branches, and, at present, no alternative techniques have been developed to enable reliable gametophyte cultivation via vegetative fragmentation. However, cultivating *A. taxiformis* gametophytes from tetraspores and seeding the resultant germlings onto natural or artificial substrates could represent a scalable solution for ocean-based cultivation of this species, as has been achieved in other commercially valuable Rhodophyta (Wang et al. 2020; Freitas et al. 2021). Recently developed hatchery protocols for inducing tetrasporogenesis in *A. taxiformis* (Theobald et al. 2024) and *A. armata* (Mihaila et al. 2023) provide a starting point for developing reliable *Asparagopsis* germling generation. However, identifying suitable substrates and optimising seeding methodologies to maximise germling survival, adherence and growth remains a critical milestone in scaling ocean-based *Asparagopsis* cultivation using a closed-lifecycle approach (Zhu et al. 2021; Visch et al. 2023).

Several artificial substrates have been evaluated for the ocean-based cultivation of *A. armata* gametophytes using vegetative fragmentation of wild-collected specimens. These substrates include fluffy polyethylene rope encased in a degradable sheath and rope composed of discarded twisted monofilament netting, which have been utilised at commercial farms in France and Ireland (Seguin 1996; Kraan and Barrington 2005). Additionally, polypropylene mussel-farming rope has proven effective for promoting attachment of fragmented wild-collected *A. armata* gametophytes in laboratory-scale trials (Wright et al. 2022). While several private companies have conducted in-house trials to assess substrate suitability for the mariculture of nursery-reared *Asparagopsis* germlings, no publicly available literature currently exists on the findings. Therefore, developing protocols for producing germling-seeded substrates remains a key priority for development of the *Asparagopsis* cultivation sector, as identified in a recent review by Rule and Kelly (2023).

Longline systems, whether vertical, horizontal or garland style, are widely used in seaweed mariculture due to their

simplicity, efficiency and scalability (Tullberg et al. 2022). Hatchery-supplied juveniles are typically seeded directly onto culture ropes or first seeded onto a twine or ribbon, which is then attached to culture ropes after a nursery cultivation period (Redmond et al. 2014). Compared to direct seeding, twine seeding represents a reliable cultivation method as the nursery phase enhances the survival of early recruits (Stanley et al. 2019) and may improve subsequent germling retention and mariculture yield (Forbord et al. 2020). Selecting a suitable twine substrate is crucial for maximising germling settlement, survival and attachment tenacity. In a broader context, substrate suitability for macroalgal cultivation varies by species and is determined primarily by polymer surface chemistry and surface texture/topography (Norton 1983; Dimartino et al. 2017; Kerrison et al. 2017, 2018b; Le et al. 2018). Increasingly, natural twine substrates that slowly biodegrade in seawater are being investigated for aquaculture applications, in an attempt to reduce microplastic contamination caused by non-biodegradable plastics (Winger et al. 2015; Le et al. 2018; Kerrison et al. 2019b).

This study examines the suitability of two natural fibre twine substrates (sisal and cotton) and two synthetic fibre twine substrates (nylon and polypropylene) for *A. taxiformis* nursery cultivation, using a germling solution seeding methodology. The germling solution technique, in which young gametophytic thalli are suspended in seawater and allowed to settle onto a substrate, has been successfully applied in the cultivation of several commercially cultivated macroalgae (Aaron-Amper et al. 2020; Forbord et al. 2020; Boderskov et al. 2023). After seeding, this technique requires substrates to be incubated in a nursery for several weeks, to allow the germlings to develop a secure attachment before ocean deployment (Xu et al. 2009; Redmond et al. 2014). Alternatively, using a binder technique, germlings are applied to substrates within a viscous solution, temporarily gluing them in place until a secure attachment is developed. Binder seeding can eliminate the nursery cultivation phase, by enabling direct application of germlings to the cultivation ropes immediately before submersion in the ocean (Forbord et al. 2020; Kerrison et al. 2020). Although the binder approach offers potential time, space, labour and cost benefits, its effectiveness in aiding germling attachment is context-dependent and requires species-specific evaluation (Kerrison et al. 2018a, 2020; Visch et al. 2023). Therefore, this study will also assess the suitability of a sodium alginate binder for improving *A. taxiformis* germling retention.

This study aims to provide a foundation for generating seeded twine for *A. taxiformis* mariculture by identifying suitable substrates and developing seeding methodologies. To achieve this, two sequential experiments were conducted. Experiment 1 assessed the attachment success of germlings applied in solution to a smooth polystyrene surface over a 10-day period, to inform the design of Experiment 2.

Experiment 2 evaluated four twine substrates and the use of sodium alginate binder across three key objectives: (1) to quantify germling density five days after seeding; (2) to assess germling cover after six weeks of nursery cultivation and again following simulated ocean deployment, achieved by unravelling the substrates and exposing them to flow conditions in a laboratory flume; and (3) to measure germling size following deployment simulation.

Materials and methods

Generation of germlings for seeding

Tetrasporophyte cultures of *Asparagopsis taxiformis* (lineage 6) were established from wild-collected gametophytes bearing carposporophytes (Rat Reef, Gladstone, Australia; –23.76557, 151.31753) and maintained for 22 months under laboratory conditions, as described in Theobald et al. (2024). One week prior to experimentation, tetrasporophytes were fragmented into smaller, roughly spherical pieces (4–7 mm in diameter). A total of 160 tetrasporophytes with a combined fresh weight of 5.15 g were used as broodstock and evenly distributed among five 15-cm Petri dishes. To induce tetrasporogenesis, tetrasporophytes were placed under previously identified optimum conditions for the Gladstone isolate (Theobald et al. 2024). Tetrasporophytes were maintained at 22 °C within a temperature-controlled room under 7 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance, provided by LED lights ('all bright' setting, Maxkon; 4000 W Full Spectrum, Buxtehude, Germany) on a 08:16 light–dark cycle. Induction culture media consisted of autoclaved natural seawater (salinity 35 psu) supplemented with silicate-free F/8 media, arsenic trioxide (As_2O_3 ; 98 $\mu\text{g L}^{-1}$), potassium iodide (KI; 166 $\mu\text{g L}^{-1}$) and germanium dioxide (GeO_2 , 0.447 mg L^{-1}), with water changes conducted weekly. Refer to Supplementary Table S1 for nutrient levels in autoclaved seawater. In the second week of culture, a stereo zoom microscope (SMZ745 T; Nikon, Japan) was used to confirm the presence of tetrasporangia and initial tetraspore release.

Experiment 1: Attachment of germlings in solution over ten days

After a three-week induction period, tetrasporophytes were transferred to new Petri dishes to continue tetraspore release to generate gametophytic germlings for Experiment 2. Spores and germlings (0–14 days old; hereafter collectively referred to as germlings) that had settled on the bases of the original Petri dishes were gently dislodged into solution using a soft-bristled paintbrush. The solutions from all dishes were combined into a single beaker and left undisturbed for 20 min, allowing the germlings to settle at the

bottom. Once settled, the supernatant was siphoned off, leaving a 70 mL germling solution (Fig. 1a). The mean density of the solution was quantified at $214 \text{ germlings mL}^{-1} \pm 118$ standard deviation (S.D.) using a Sedgewick-Rafter counting slide.

Germling attachment to a smooth polystyrene surface was assessed over a ten day period using clear, flat-bottomed culture plates (Corning; 3512). Each plate contained six replicate wells filled with culture media, with a separate plate allocated to each day of the experiment. The culture media used was identical to the induction medium, except that F/2 was used in place of F/8 (von Stosch 1963; Codomier et al. 1981). At the start of the experiment, 1 mL of germling solution was added to each well. Germlings were cultured at 22 °C in a temperature-controlled room under 15 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance on a 12:12 light–dark cycle (Mihaila et al. 2024) using LED lighting (Maxkon). Each day, wells from the designated plate were photographed under a stereo zoom microscope (3.35 $\times$ magnification), and germlings were manually counted per well using Image J (Schneider et al. 2012). To assess attachment success, each plate was then inverted for three seconds, re-photographed, and the number of remaining germlings was recorded. Germling attachment for each well was calculated as:

$$\text{Attachment (\%)} = 100 \times \left(\frac{N_2}{N_1} \right) \quad (1)$$

where N_1 is the number of germlings prior to inversion, and N_2 is the number remaining after inversion.

Experiment 2: Assess the suitability of four twine substrates and a sodium alginate binder for maximising germling cover

Twine materials

Four twine substrates were assessed for their suitability for germling seeding: two synthetic fibre twines (nylon and polypropylene) and two natural fibre twines (sisal and cotton). All twines were twisted from three strands, with a total diameter of 6 mm and length of 30 cm. Each length of twine represented a single substrate unit, and six replicate units were used for each substrate type. Twisted twine was selected over braided twine, as this structure has a higher surface area and has been shown to increase biomass in *Saccharina* sp. mariculture (Kerrison et al. 2019b). Nylon, polypropylene, sisal and cotton have been assessed as cultivation substrates for other macroalgae (Hwang et al. 2006; Kerrison et al. 2019b; Lawton and Magnusson 2024) and were selected for this study due to their affordability, durability and widespread availability. Additionally, a control substrate – flat, untextured polypropylene, was included for comparison. To simulate

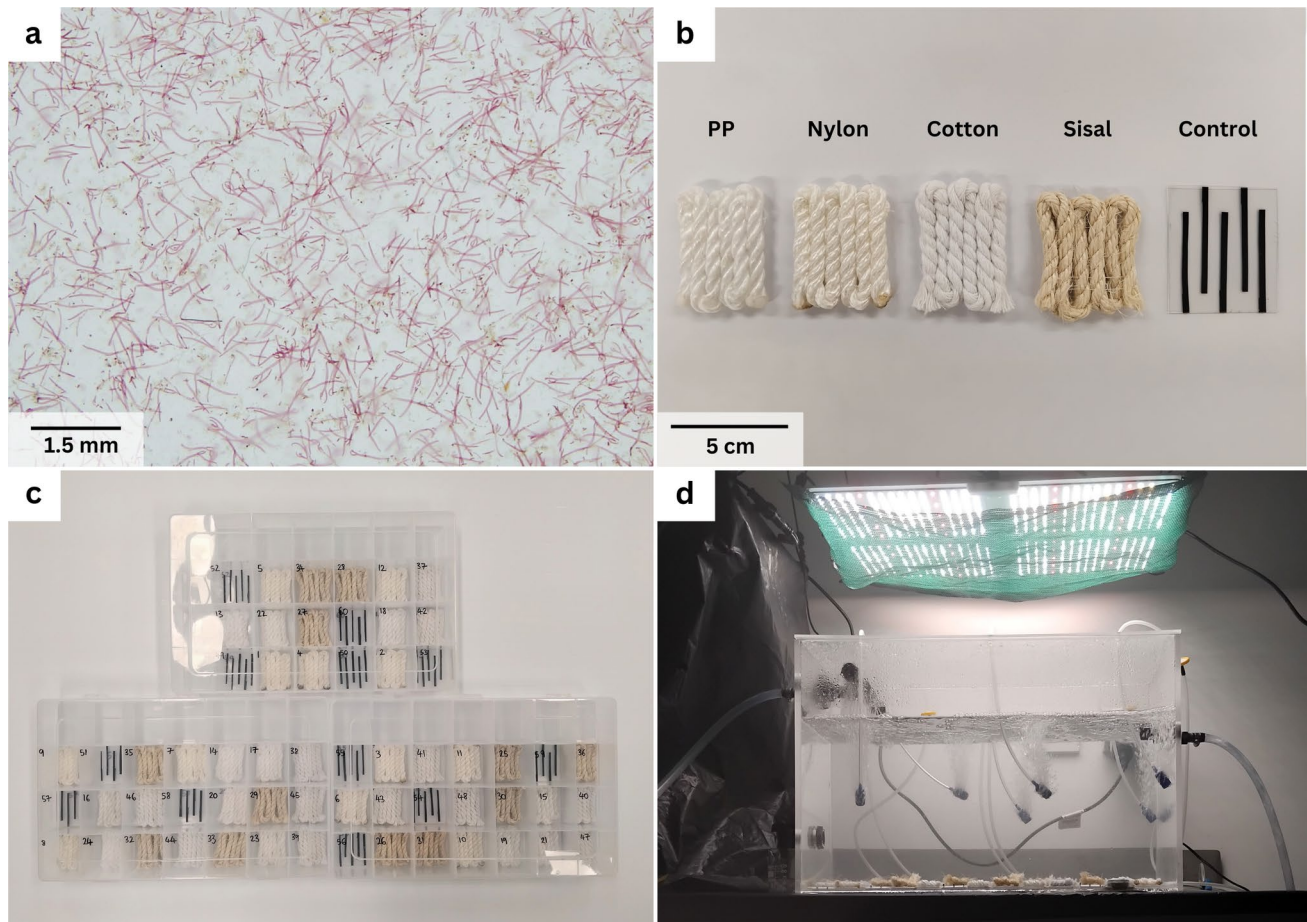


Fig. 1 Experimental setup: **a**) Germling solution ready to be applied to the substrates **b**) The four twine substrates assessed: polypropylene (PP), nylon, cotton and sisal, and the smooth polypropylene control **c**)

Individual tanks used for seeding substrates and for germling adherence (days 1–5) **d**) Communal tank used for germling on-growing (days 6–42)

industrial-scale seeding, twine substrates were folded into rectangular surfaces ($4.0\text{ W} \times 5.1\text{ L cm}$), representing the surface formed when twine is wound onto a reel or spool (Solvang et al. 2021). The folded structures were hand-stitched together with cotton thread, and to ensure negative buoyancy, a porcelain tile ($3.7\text{ W} \times 4.7\text{ L} \times 0.5\text{ H cm}$) was attached to the underside of each substrate using cotton thread. To mimic the unravelling process performed on the twine substrates at the end of the experiment, thin lines of vinyl electrical tape (Temflex 1610; 3 M; Australia) were applied to the control substrate. See Fig. 1b for an image of all substrates. Before use, all substrates were pre-soaked for 48 h in autoclaved natural seawater, followed by 48 h in fresh water, and then dried at $30\text{ }^{\circ}\text{C}$. Prior to seeding, substrates were sterilised under UV light in a laminar flow cabinet for 30 min.

Sodium alginate binder

The effect of a 2% sodium alginate binder (2 g (100 mL)^{-1} autoclaved seawater) on germling adherence was assessed

for each substrate and compared to a no-binder treatment. The 2% concentration was selected based on preliminary experimentation (M. Rule, unpublished data). Six replicate substrate units were used for each level in the crossed-experimental design. As this study was conducted in a laboratory environment, binder-treated substrates could not be deployed into the ocean upon seeding, as done in previous studies (Forbord et al. 2020; Kerrison et al. 2020). Therefore, to enable a direct comparison, binder-treated and untreated substrates were maintained under identical cultivation conditions.

Seeding of substrates with gametophytic germlings and assessing early density

Tetrasporophytes ($n = 160$), previously used to generate germlings in Experiment 1 were maintained under continuous inductive conditions for an additional three weeks to generate germlings for Experiment 2. The resultant germlings (0–21 days old) were transferred into two solutions of equal volume: one composed of culture medium (composition as

described in Experiment 1) and the other containing culture medium + 2% sodium alginate, dissolved to create a binder. The mean germling density in the solutions was 1271 ± 196 (S.D.) germlings mL^{-1} . In the no-binder treatment, substrate units were placed within individual polypropylene tanks ($4.8 \text{ W} \times 5.1 \text{ L} \times 4.5 \text{ H cm}$) filled with 100 mL of culture medium, and 1.5 mL of germling solution was applied evenly to each substrate unit using a pipette. For substrates in the binder treatment, 1.5 mL of germling solution was spread evenly across each dry substrate unit using a soft-bristled paintbrush and left to rest for 15 min before the units were transferred to individual tanks filled with 100 mL of culture medium. All substrate units were maintained in individual tanks for 5 days to allow germling adherence (Fig. 1c) and to minimise germling disturbance, aeration, flow and water changes were not provided during this period. Environmental conditions were maintained as described in Experiment 1 for the duration of Experiment 2. Five days after seeding, substrate units were removed from individual tanks and photographed under a stereo zoom microscope (SMZ745 T; Nikon) at $5\times$ magnification. A series of images was captured to cover the entire upper surface of each substrate unit. The total number of germlings per substrate unit was manually counted from these images using Image J (<https://imagej.net/ij/index.html>), and germling density (per cm^2) was subsequently calculated.

Germling on-growing and assessing germling cover

Following data collection, all substrate units were transferred to a shared acrylic tank ($60 \text{ W} \times 30 \text{ L} \times 40 \text{ H cm}$) containing 49 L of culture medium (Fig. 1d). Substrate unit positioning within the tank was randomised. Recirculating water flow (3.6 L min^{-1}) was delivered via a sprinkler head connected to a submersible water pump (Maxi 104 Powerhead; Aqua One, Australia), housed in a polypropylene sump ($52 \text{ W} \times 30 \text{ L} \times 30 \text{ H cm}$) filled with 34 L of culture medium, bringing the total system volume to 83 L.

A weekly partial water change (10 L) was performed via the sump and aeration was provided via air stones. Germlings were on-grown in the communal tank for 37 days, resulting in a cumulative cultivation time of 6 weeks since substrate seeding. Six weeks after seeding, substrate units were removed from the tank and photographed using a Tough TG-5 camera (Olympus; Australia). Images were analysed using the ‘Threshold Color’ function in Image J to quantitatively determine germling cover (%) on the upper surface of each substrate. Refer to Supplementary Fig. S1 for an example of the image analysis process. Following this, each twine substrate was unravelled by cutting the hand-stitched cotton threads and removing the weighted tile. For the procedural control substrate, the vinyl tape was removed

to replicate the unravelling process undergone by the twine substrates.

Simulating ocean deployment, reassessing germling cover and determining germling size

To simulate the turbulent conditions that germlings experience during ocean-deployment, each substrate unit was individually subjected to moderate-velocity flow (0.392 m s^{-1}) for 30 s within a rectangular laboratory flume ($0.06 \text{ m W} \times 0.62 \text{ m L} \times 0.06 \text{ m H}$) inclined at a 60-degree angle. A submersible water pump (Maxi 104 Powerhead) housed in a header tank dispersed culture media down the flume at a depth of 0.01 m. At the bottom of the flume, the medium was collected in a sump and recirculated back to the header tank via a second pump, ensuring a continuous flow loop. The temperature was maintained at 21°C within a temperature-controlled room. Substrate units were subjected to flow in a randomised order, with the top of each substrate unit positioned 0.18 m from the top of the flume. The flow velocity (v ; m s^{-1}) was calculated from the volumetric flow rate (Q ; $0.00024 \text{ m}^3 \text{ s}^{-1}$) and the cross-sectional area of flow (A ; 0.00060 m^2) using the following equation:

$$v = \frac{Q}{A} \quad (2)$$

To determine residual germling cover (%) after exposure to flow conditions, each substrate unit was rephotographed, and images were analysed in Image J as previously described. To determine the size of the residual germlings, five germlings were randomly sampled from each substrate unit using tweezers and photographed using a Tough TG-5 camera (Olympus). The resultant images were analysed using the ‘Measure’ function in Image J to assess (a) the length of the longest determinate branch, and (b) the cumulative length of all determinate branches, following the method described in Mihaila et al. (2024) for measuring *A. armata* gametophytic germlings after three weeks of culture.

Statistical analysis

Statistical analyses and data visualization were performed using R version 4.4.1 (R Core Team 2024) and RStudio version 2023.12.1.402 (Posit Team 2024). Plots were generated with the ‘ggplot2’ package (Wickham 2016). Statistical significance was assessed at $p < 0.05$ for all tests unless stated otherwise. Image panels were assembled using Canva (<https://www.canva.com/>).

In Experiment 1, significant changes in the mean attachment of germlings over time were identified using the ‘change-point’ package (Killick and Eckley 2014). The changepoint detection algorithm used the Pruned Exact Linear Time

(PELT) method with an asymptotic penalty term, and a low penalty value of 0.03 was applied to enable detection of fine-scale fluctuations.

In Experiment 2, germling density at 5 days post-seeding and germling length at 6 weeks post-seeding were not formally analysed but data is displayed graphically. To assess the relative influence of substrate type and binder application on germling cover (%) per substrate unit at 6 weeks post-seeding, linear models with a normal error distribution were created. Data collected before and after the flume test were analysed separately. In both analyses, germling cover was cube-root transformed to reduce skewness and heteroscedasticity. For the pre-flume test data, a more conservative significance level ($p < 0.01$) was applied, as the residual error did not meet normality assumptions after transformation.

For each analysis, a global model was constructed with the predictor variables of substrate type (nylon, polypropylene, cotton, sisal, control) and binder application (binder, no binder), along with a two-way interaction. From each global model, a set of candidate models was generated, including all possible combinations of predictors and a null model. These models were ranked by goodness of fit using the corrected Akaike's Information Criterion (AIC_c), which penalises complexity based on the number of parameters (Burnham and Anderson 2002). Since smaller AIC_c values indicate a better relative fit, the difference between ranked models (ΔAIC_c) was calculated by subtracting the lowest overall AIC_c from each candidate model's AIC_c . In each analysis, only one model obtained a score of $\Delta AIC_c \leq 2$, so it was selected as optimal and subjected to visual assumption checks. Post-hoc pairwise comparisons of estimated marginal means were performed using the Tukey method in the 'emmeans' package (Lenth 2023).

Results

Experiment 1: Attachment of germlings in solution over ten days

Significant changes in the mean attachment of germlings over time were identified, segmenting the dataset into three time phases, each with a statistically distinct mean. The segmented time-phases were (a) day 0 (mean attachment 18.4%), (b) day 1 (mean attachment 57.0%), and (c) days 2–10 (mean attachment 85.5%) (Fig. 2).

Experiment 2: Assess the suitability of four twine substrates and a sodium alginate binder for maximising germling cover

Germling density five days post-seeding

Five days after seeding, the highest mean germling densities were observed on the polypropylene control (46 germlings

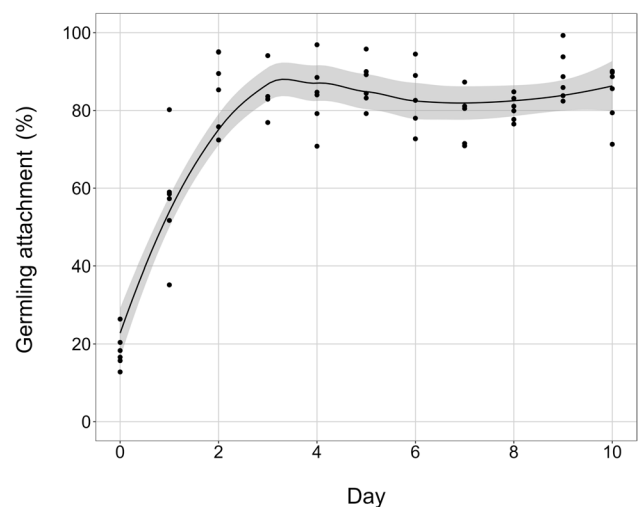


Fig. 2 Proportion of germlings attached (%) to a smooth polystyrene surface over ten days. The black line indicates the line of best fit and the grey area shows confidence interval calculated as standard error. $n = 6$ replicate culture wells per day

cm^{-2}) and polypropylene twine (41 germlings cm^{-2}) substrates without binder (Fig. 3). In contrast, the lowest mean germling densities were observed on sisal twine, both with and without binder, at 4 and 2 germlings cm^{-2} , respectively. Binder application was associated with an increase in germling density only on the cotton and sisal substrates, with respective increases of 16% and 81%.

Germling cover after six weeks of nursery cultivation, before and after deployment simulation

At six weeks post-seeding, the highest mean germling cover was observed on the polypropylene twine (109.8%), polypropylene control (100.5%) and cotton twine (93.7%) substrates without binder (Figs. 4 and 5a). In some cases, germling cover extended beyond the upper surface area of the substrate, resulting in values of greater than 100%. Across all substrates, binder application was associated with a reduction in mean germling cover, except for sisal twine, on which cover remained similar (14.3% with binder, 14.9% without binder). After substrates underwent unravelling and the flume test, mean germling cover decreased across all treatments (Fig. 5b), with the greatest loss (81%) observed on the control substrate with binder applied. The highest mean remaining germling cover was recorded on polypropylene twine (104.0%) and cotton twine (69.5%) without binder (see Fig. 6 for representative images).

The optimal models for predicting germling cover both before deployment simulation (Model 1) and after simulation (Model 6) had the structure: $\sqrt[3]{\text{germling cover}} \sim \text{substrate type} + \text{binder application} + \text{substrate type} * \text{binder application}$; see Supplementary Information Table S2 for

Fig. 3 Germling density per cm^2 at five days post-seeding, according to substrate type and application method. Mean values per group are denoted by a black triangle ($\blacktriangle$) and outliers are denoted by a black circle ($\bullet$). $n = 6$ replicate substrate units per level of the full-factorial design

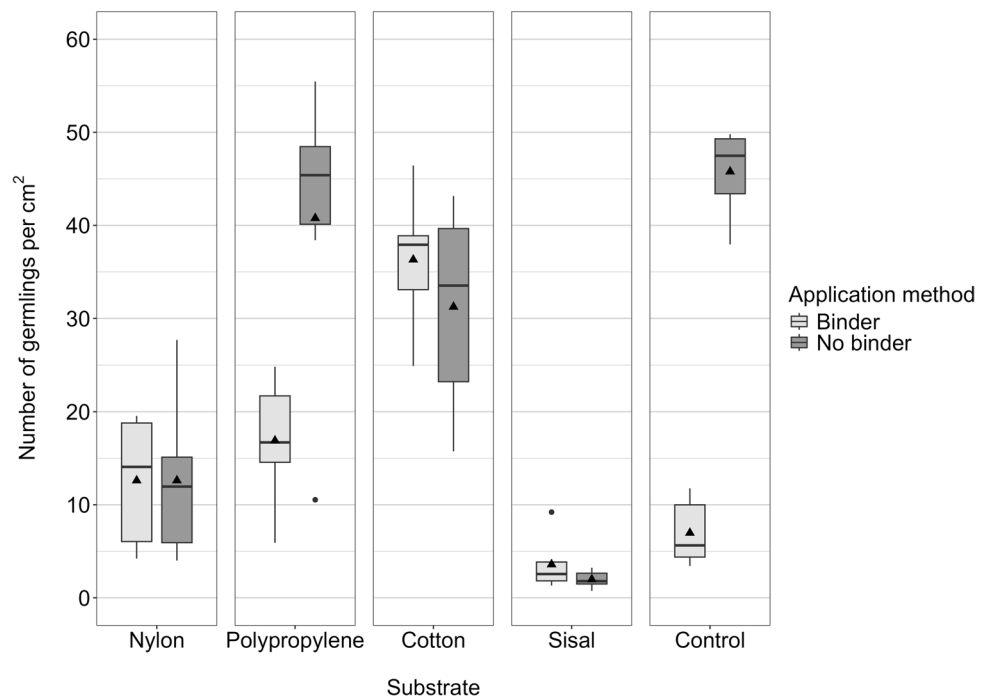
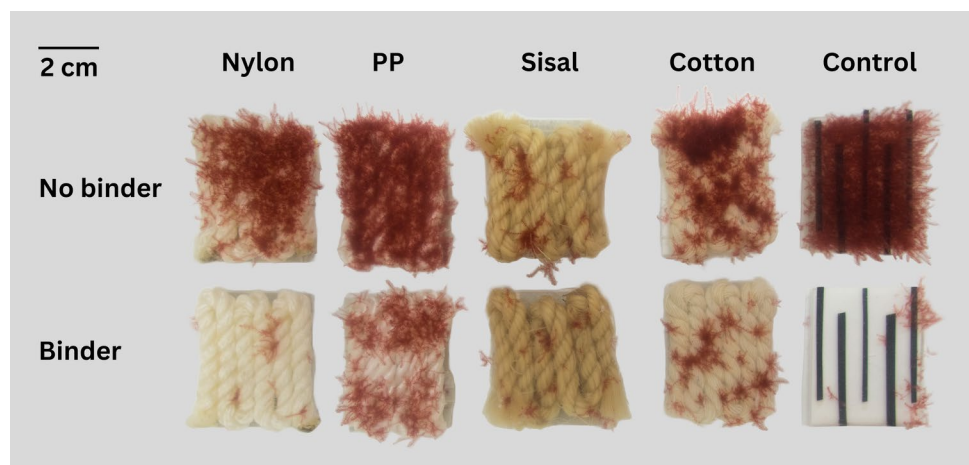


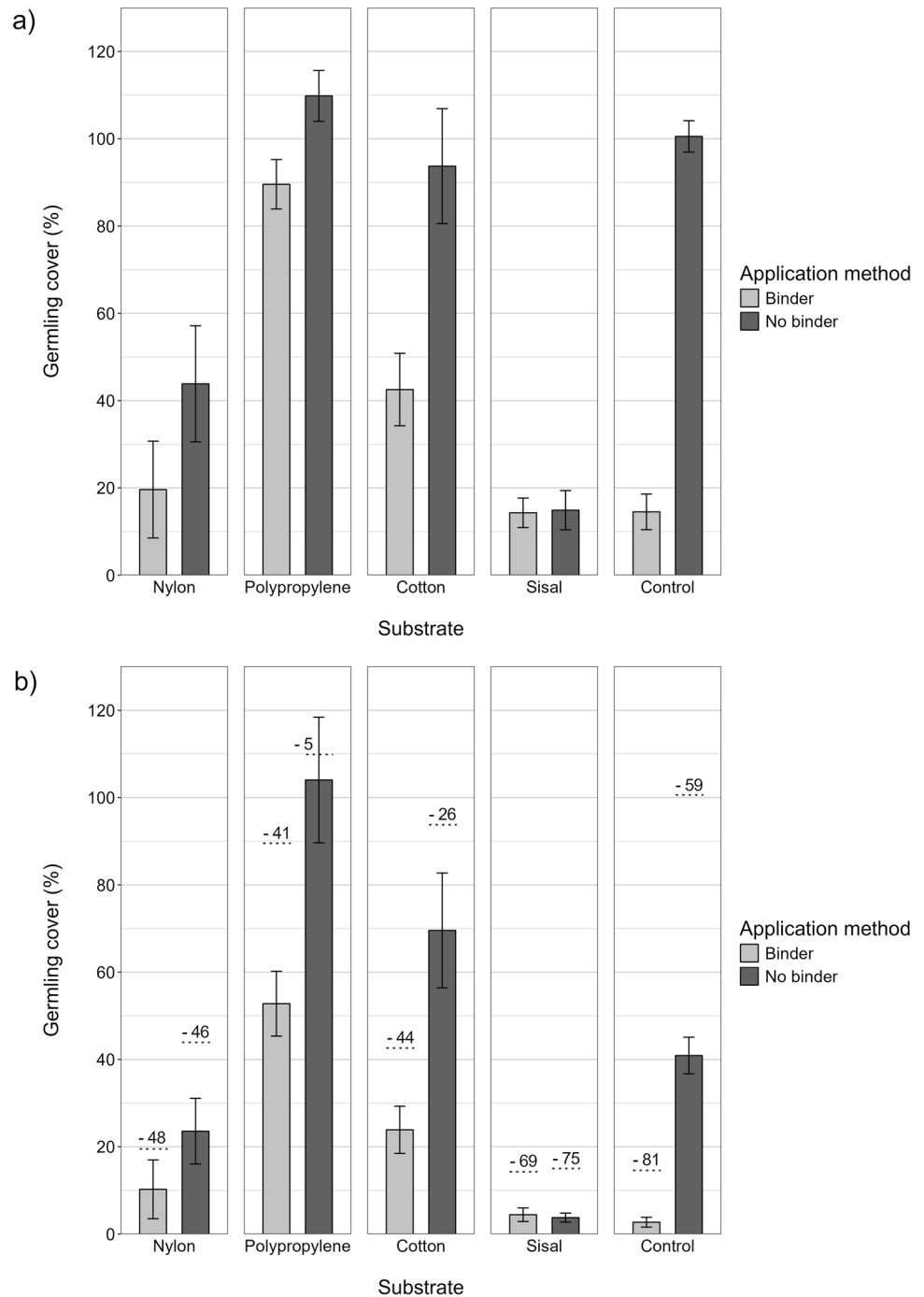
Fig. 4 Representative substrate units at six weeks post-seeding, before being subject to unravelling and the flume test. PP = polypropylene



model selection tables. Significant regression equations were found for Model 1 ($F_{(9,50)} = 15.2$, $p < 0.001$) and Model 6 ($F_{(9,50)} = 19.8$, $p < 0.001$), explaining 73.2% and 78.1% of variation in germling cover, respectively (multiple R^2). Both analyses contained a significant interaction term, with substrate type and binder application significantly influencing germling cover (Table 1; see Supplementary Information Fig. S2 for interaction plot). Estimated marginal means for germling cover, standard error, and confidence limits derived from Model 1 and Model 6 are presented in Supplementary Information Table S3, while p-values for pairwise comparisons of estimated marginal means are displayed in Supplementary Information Table S4.

On substrates without binder, germling cover before the flume test was significantly higher on polypropylene twine compared to nylon ($p = 0.002$) and sisal ($p < 0.001$), and higher on cotton relative to sisal (< 0.001). After the flume test, polypropylene twine retained greater cover than nylon ($p < 0.001$), sisal ($p < 0.001$) and the control ($p = 0.023$), and cotton retained greater cover than nylon ($p = 0.006$) and sisal ($p < 0.001$). On substrates with binder, germling cover before the flume test was higher on polypropylene twine compared to the control, nylon and sisal ($p < 0.001$). After the flume test these trends were still apparent ($p < 0.001$) and additionally, cotton retained significantly greater germling cover than the control ($p < 0.001$). See

Fig. 5 Mean germling cover (%) per substrate unit $\pm$ standard error (S.E.) (**a**) before the flume test, and (**b**) after the flume test, according to substrate type and application method. To aid visualisation of reductions in germling cover between the two time-points, mean germling cover from (**a**) is displayed on (**b**) with a dashed line, with numbers denoting the percentage change. $n = 6$ replicate substrate units per level of the full-factorial design



Supplementary Information Table S4 for additional significant pairwise comparisons.

Germling size after simulating deployment conditions

After being subjected to deployment simulation, the tallest germlings (longest determinate branch) were

observed on the polypropylene twine without binder (mean 10.4 mm) followed by the polypropylene twine with binder (9.8 mm) (Fig. 7a). With respect to the combined length of determinate branches, total germling length was greatest on the polypropylene twine with binder (mean 39.3 mm) followed by the cotton twine without binder (38.5 mm) (Fig. 7b). The effect of binder

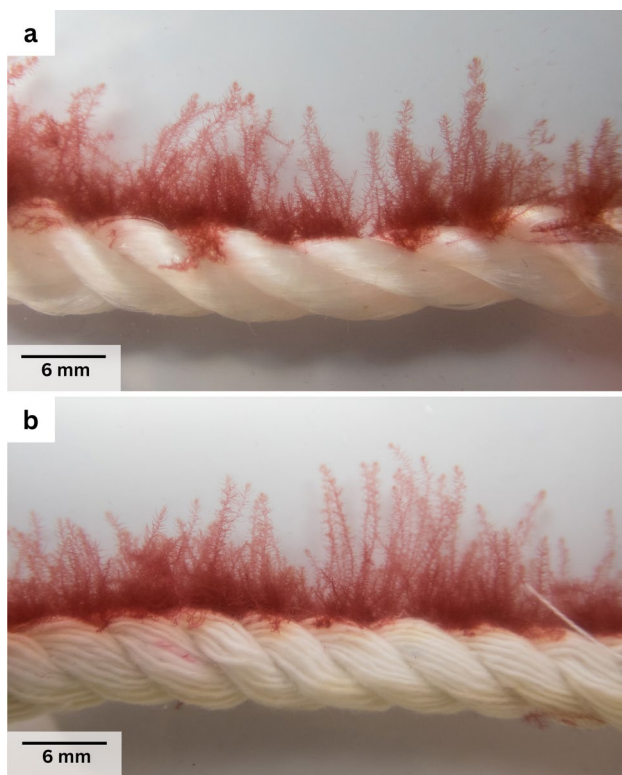


Fig. 6 Representative images of germling cover on the two best performing substrates, (a) polypropylene twine without binder, and (b) cotton twine without binder, after being subjected to unravelling and the flume test

application on germling length was not consistent among substrates.

Discussion

To our knowledge, this study represents the first publicly available evaluation of substrates for *Asparagopsis* germling seeding. We demonstrated that 6 mm polypropylene twine (without binder applied) is a promising substrate for *A. taxiformis* gametophyte cultivation under nursery conditions, retaining an average germling cover of 104% after exposure to simulated deployment conditions. The next best performing substrate, 6 mm cotton twine (without binder applied), retained germling cover of 70% following deployment simulation and may offer a biodegradable alternative to polypropylene. The application of a sodium alginate binder did not enhance germling retention on either substrate. By demonstrating a potentially scalable seeding methodology and identifying promising, widely available synthetic and natural substrates, this study provides a foundation for developing seeded twine for *A. taxiformis* mariculture.

Substrate material

Appropriate substrate material selection is a key component in maximising yield and cost-efficiency in macroalgal mariculture (Kerrison et al. 2017; Le et al. 2018). Polypropylene twine, being both readily available and cost-effective, has been extensively used as a substrate in long-line mariculture for a diverse range of macroalgae (Hwang et al. 2006; Macchiavello et al. 2010; Rößner et al. 2014; Marinho et al. 2015; Pereira et al. 2020). In this study, both the polypropylene twine without binder and the smooth polypropylene control without binder performed extremely well, resulting in a high density of germlings (41 and 46 germlings cm^{-2} , respectively, retaining approximately 44% and 49% of initial germlings) at five days post-seeding and achieving excellent germling coverage (109.8% and 100.5%, respectively) after six weeks of nursery cultivation. These findings suggest that the surface chemistry of polypropylene supports *A. taxiformis* germling adherence, even without a binder (Kerrison et al. 2019a). However, the significant reduction in germling cover (mean 81%) observed on the smooth polypropylene control following the flume test indicates that thigmotactic interaction (attachment through physical contact and mechanical adhesion) is crucial for promoting *A. taxiformis* germling retention, complementing chemical bio-adhesion. Indeed, on the more complex surface structure of the polypropylene twine which only showed a 5% reduction in germling cover following the flume test, we observed mechanical interlocking of the rhizomes and thalli via entanglement with the twine fibres, supporting attachment. Although our study only assessed three-strand twisted twines, germling attachment tenacity may be further optimised by investigating the suitability of polypropylene twines with other structural forms (e.g., different strand numbers, braided vs twisted vs woven structures, and varying twine diameter), which may provide varied surface topographies and increase opportunities for early thigmotactic attachment (Salinas et al. 2006; Kerrison et al. 2019b; Cumming et al. 2020; Sebök et al. 2022). In addition to being the best performing substrate in terms of germling cover, polypropylene twine also retained the largest germlings after substrates were subjected to the flume test. Due to the destructive nature by which germlings were sampled (germlings were removed from the substrates to enable accurate measurement) it was not possible to assess germling size before the flume test. Therefore, the greater germling length observed on polypropylene twine does not necessarily mean that germlings grew larger on this substrate, but it may indicate that larger germlings were more strongly attached to polypropylene twine, enabling a greater proportion of larger germlings to remain adhered when subjected to moderate flow conditions.

Table 1 Germling cover predicted (a) before the flume test by Model 1, and (b) after the flume test by Model 6, according to treatment level. Associated standard error, t-statistic and p-values are displayed.

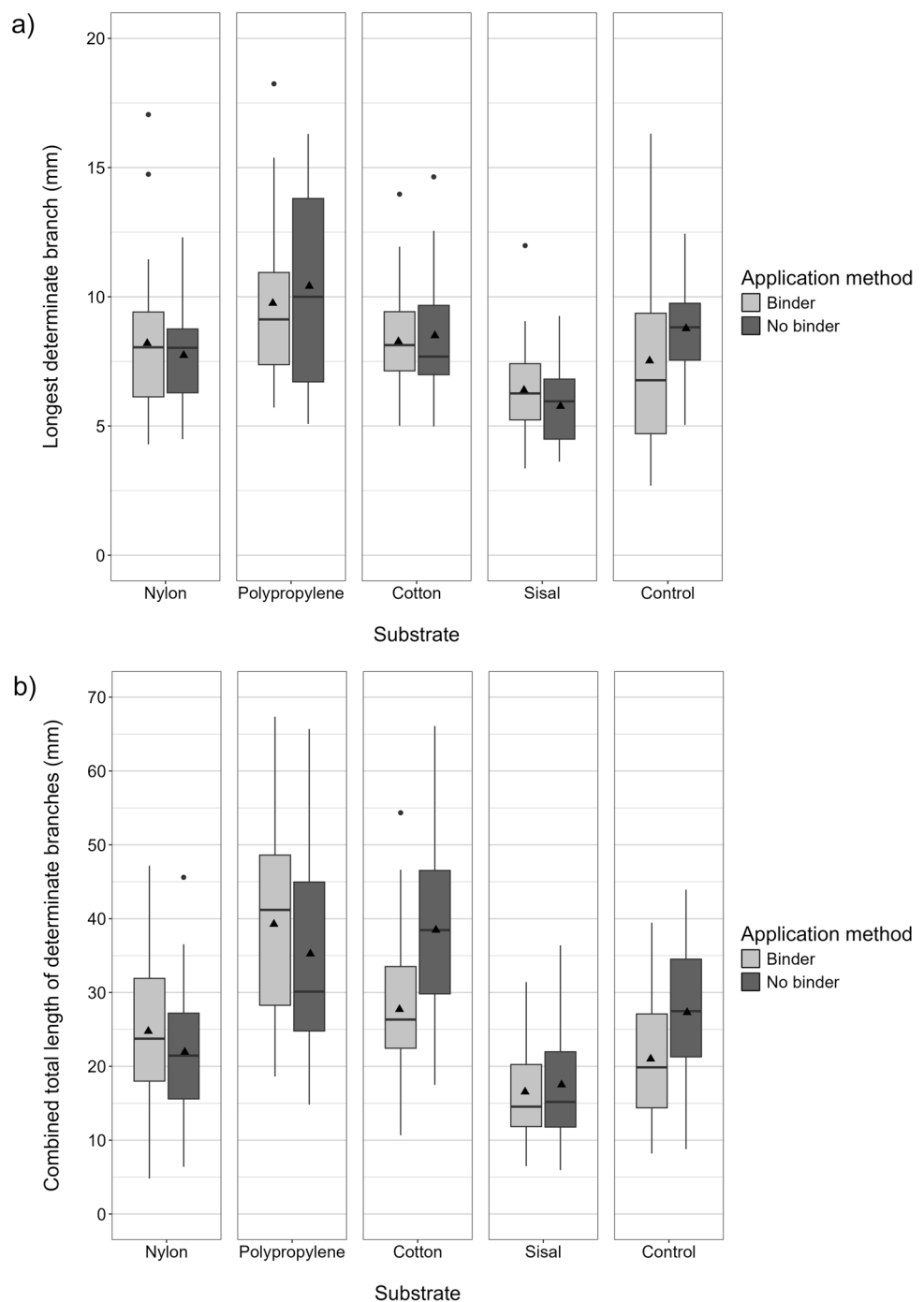
In both models, the treatment levels substrate: control and binder application: binder, were used as the reference for contrasts and are therefore not displayed in the summaries

Coefficients	Estimate: $\sqrt[3]{\text{germling cover}}$	Standard error	t-statistic	p-value
a)				
(Intercept)	2.270	0.276	8.222	< 0.001
substrate: cotton	1.139	0.391	2.917	0.005
substrate: nylon	0.099	0.391	0.255	0.800
substrate: polypropylene	2.194	0.391	5.618	< 0.001
substrate: sisal	0.075	0.391	0.192	0.849
binder application: no binder	2.376	0.391	6.085	< 0.001
substrate: cotton * binder application: no binder	−1.315	0.552	−2.381	0.021
substrate nylon * binder application: no binder	−1.555	0.552	−2.815	0.007
substrate: polypropylene * binder application: no binder	−2.060	0.552	−3.730	< 0.001
substrate: sisal * binder application: no binder	−2.415	0.552	−4.372	< 0.001
b)				
(Intercept)	1.040	0.274	3.794	< 0.001
substrate: cotton	1.764	0.388	4.549	< 0.001
substrate: nylon	0.795	0.388	2.051	0.046
substrate: polypropylene	2.669	0.388	6.883	< 0.001
substrate: sisal	0.471	0.388	1.215	0.230
binder application: no binder	2.381	0.388	6.141	< 0.001
substrate: cotton * binder application: no binder	−1.203	0.548	−2.194	0.032
substrate nylon * binder application: no binder	−1.627	0.548	−2.967	0.005
substrate: polypropylene * binder application: no binder	−1.453	0.548	−2.649	0.011
substrate: sisal * binder application: no binder	−2.422	0.548	−4.418	< 0.001

Although polypropylene twine is widely used as an effective cultivation substrate, its inevitable degradation and fragmentation, exacerbated by abrasion and fouling, is a source of marine microplastic contamination (Welden and Cowie 2017; Feng et al. 2020). Despite the promising performance of polypropylene observed in this study, natural-fibre twines that biodegrade in the marine environment may offer a more sustainable alternative (Lawton and Magnusson 2024). In this study, cotton twine without a binder performed relatively well, resulting in high germling cover (mean 93.7%) after six weeks of nursery cultivation. Although, germling cover decreased by 25.7% following the flume test, indicating that germlings adhered less securely to cotton twine than polypropylene twine, germling length (combined length of determinate branches) was second-highest on cotton twine without binder. Notably, cotton is a component of the twine traditionally used for macroalgal seedling production in China (Pang et al. 2008). Although cotton twine has demonstrated poor performance for *Saccharina latissima* sporophytes in the nursery, during ocean cultivation, cotton twine has been demonstrated to achieve a yield equivalent to that of the best-performing synthetic twine (Kerrison et al. 2019b). Moreover, some brands of

cotton twine have shown remarkable durability in the ocean, exhibiting negligible reductions in breaking strength after 124 days at sea (Winger et al. 2015). In contrast to cotton, the other natural fibre, sisal, performed poorly in our study, negatively impacting germling density within five days of seeding and yielding low germling cover after six weeks of cultivation, both with and without binder application (14.3% with binder, 14.9% without binder). This poor performance could be attributable to species-specific toxicity from compounds leached from the sisal fibre, as reported for *S. latissima* by Kerrison et al. (2019b). Interestingly, on sisal, and, to a lesser extent, on cotton, binder application was associated with an increase in mean germling density five days post-seeding, whereas binder application was associated with reduced mean germling density on all other substrates. The sodium alginate binder may have acted as a buffer, potentially protecting the germlings from the toxic effects of leachates. This phenomenon was also suggested by Kerrison et al. (2019b), who observed that toxicity or developmental inhibition associated with seeding *S. latissima* meiospores onto natural fibres (sisal, jute and cotton) was negated when meiospores were seeded within a hydrocolloid binder.

Fig. 7 Germling length at six weeks post-seeding according to substrate type and application method, after substrates were subjected to unravelling and the flume test, displayed as (a) the length of the longest determinate branch (mm) per germling, (b) the combined length of all determinate branches (mm) per germling. Mean values per group are denoted by a black triangle (▲) and outliers are denoted by a black circle (●). $n = 5$ germlings sampled per substrate unit, with 6 replicate substrate units per level of the full-factorial design



Binder application

Direct *in-situ* binder application could bring significant cost savings by eliminating the nursery phase and reducing space and labour requirements for *Asparagopsis* cultivators (Kerrison et al. 2018a). However, our study found that applying a 2% sodium alginate binder reduced germling cover on nearly all substrates, with the exception of sisal where cover remained consistently low (14.3% with binder, 14.9% without binder). On polypropylene twine, the

best-performing substrate, binder application reduced germling density within five days. Whether this decrease was due to binder-related mortality or reduced adherence rates, perhaps caused by incorrect germling orientation preventing attachment before binder degradation (Kerrison et al. 2018a, 2020), requires further investigation. After six weeks, germling cover on polypropylene twine was 18.4% lower under binder application, a gap that widened to 47.9% after the flume test. This difference would likely have been even greater had the flume test been conducted immediately after

binder application, simulating the rapid deployment typical of this method. In this study, we did not observe any consistent effects of binder on germling length across substrates. Studies on *S. latissima* have observed reduced macroalgal growth rates associated with the binder technique applied by direct-seeding *in-situ*, relative to traditional hatchery methods (Forbord et al. 2020; Visch et al. 2023; Wilding et al. 2025); possibly due to the fact that extra time is required to develop a holdfast before the thallus can elongate. Applying the binder technique *in-situ* using immediate deployment methods is an important next step, to determine whether binder application is associated with reduced growth rates in *A. taxiformis* germlings and to assess binder retention more comprehensively.

An increasing body of recent research demonstrates that the binder technique can yield biomass comparable to or exceeding traditional twine seeding, however, these studies primarily focus on Laminariales cultivation in the Atlantic (Kerrison et al. 2018a, 2020; Bak 2019; Boderkov et al. 2021). Some of this success may be attributable to the relatively smaller size of the juvenile Laminariales sporophytes utilised (largely < 1 mm), relative to the approximately 1.5 mm long germlings used in this study. Furthermore, these successful case studies utilised specifically developed textile substrates, such as non-woven ribbons, which were designed with rough, textured surfaces to enhance algal entanglement and increase bio-adhesion (Kerrison et al. 2018a), in conjunction with proprietary (and commercially confidential) binder formulations. For example, Kuralon, a synthetic polyvinyl alcohol polymer has shown promise in both nursery and at-sea cultivation (Kerrison et al. 2019b; Boderkov et al. 2021; Thomas et al. 2022; Lawton and Magnusson 2024). Although these custom-designed textiles and binders are not yet commercially available, the binder performance of sodium alginate for *Asparagopsis* could be improved by increasing binder viscosity and testing absorbent, textured substrates (for example cotton) with a complex structure and enhanced surface area. However, even with further advancements, the efficacy of binder seeding for *Asparagopsis* may be limited by the environmental conditions at cultivation sites (Boderkov et al. 2021; Visch et al. 2023), with exposed locations or unstable weather potentially resulting in reduced density and unreliable yields (Mols-Mortensen et al. 2017; Stanley et al. 2019; Wilding et al. 2021; 2025).

Protocol development and future research areas

This study has successfully identified promising twine substrates for maximising germling cover; however, significant knowledge gaps remain regarding the optimisation of the nursery phase and ocean-based on-growing to enable large-scale *A. taxiformis* gametophyte cultivation. In this

study, we seeded substrates with a germling solution and determined that a minimum of two days of undisturbed settlement is required to achieve attachment success of 81–90% on a smooth polystyrene surface. This technique could be adapted to a hatchery setting by pouring germlings over a reel of submerged twine, allowing at least two days of undisturbed settlement, and then rotating the reel 180 degrees to seed the opposite side (Boderkov et al. 2023). Alternatively, a direct-seeding approach could involve suspending tetrasporophyte broodstock above the substrate (e.g., resting on a mesh) and allowing the released tetraspores to settle directly onto the substrate below. Both of these approaches are reliant on a consistent supply of tetraspores. Although early hatchery protocols for inducing tetrasporogenesis in *Asparagopsis* have now been developed (Mihaila et al. 2023; Theobald et al. 2024), further protocol refinement will likely be necessary to optimise output according to local conditions and cultivar lineage. Additional research is required to determine whether the reproductive capacity of cultured tetrasporophyte broodstock declines with age. Using transcriptomics, a recent study detected ‘hidden’ senescence in *A. taxiformis* tetrasporophytes cultured at high stocking densities (5 g L⁻¹), though its impact on reproductive output (in the form of tetraspores) remains unexplored (Lang et al. 2025). Optimising culture conditions to accelerate germling growth and reduce the nursery phase duration is a key priority for lowering seaweed hatchery production costs (Coleman et al. 2022). Although Mihaila et al. (2024) recently investigated the effects of temperature, light intensity, photoperiod, nutrient concentration, and flow rates on *A. armata* germling growth, initial seeding density and contamination also significantly affect nursery performance (Pang et al. 2007; Lawton and Magnusson 2024) and require investigation in both *Asparagopsis* species.

Beyond the nursery, ocean-cultivation trials are necessary to determine the optimal germling size and developmental stage for *Asparagopsis* deployment, to balance the trade-off between increased resistance to grazing and fouling conferred by larger biomass versus the costs associated with additional nursery time (Mihaila et al. 2024). Similarly, optimising transportation and deployment techniques to maximise germling viability is crucial for developing scalable ocean-based *Asparagopsis* cultivation (Rule and Kelly 2023). Key considerations to maximise yield and efficiency include identifying the most suitable cultivation depth and selecting appropriate cultivation systems based on site-specific environmental conditions (Nardelli et al. 2024; Wilding et al. 2025). While artificial induction of tetrasporogenesis is feasible year-round in hatchery settings, aligning outplanting schedules with natural growth cycles will be essential to enhance survival, productivity, and the biosynthesis of key bioactive metabolites such as bromoform (Werner et al.

2004; Kraan and Barrington 2005; Forbord et al. 2012; Nardelli et al. 2024).

Conclusions

This study provides a foundation for developing seeded twine for *A. taxiformis* gametophyte mariculture and highlights the importance of material selection in maximising productivity. Following a six week nursery period and after subjecting seeded substrates to deployment conditions, we identified 6 mm polypropylene twine as a promising substrate for *A. taxiformis* gametophyte cultivation, and 6 mm cotton twine as a potential biodegradable alternative. However, nursery performance does not always predict successful at-sea cultivation, so conducting out-planting trials across multiple seasons and locations will be essential to validate our findings. Although sodium alginate binder was associated with reduced germling cover in our study, future trials could explore its performance by direct seeding *in-situ*, exploring absorbent, textured substrates with enhanced surface area and refining the binder composition to increase viscosity. Further research could also explore additional substrate options for seeded twine, such as specially designed polymers. Ultimately, optimising substrates and seeding techniques will be critical for scaling *A. taxiformis* mariculture and maximising its potential for sustainable bioproduct applications.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10811-025-03571-4>.

Acknowledgements This research was gratefully supported by The Great Barrier Reef Foundation, The Australian Seaweed Institute and CQUniversity. The authors respectfully acknowledge the Traditional Custodians of the Land and Sea Country on which this work was conducted; the Bailai, Taribelang Bunda, Gurang, and Gooreng Gooreng Peoples. We thank the editor and reviewer for their constructive feedback and prompt handling of the manuscript.

Author contributions Emma J Theobald: Conceptualization, Methodology, Writing – original draft, review and editing, Investigation, Formal analysis, Visualisation. Margaret B. Rule: Conceptualization, Methodology, Writing – review & editing. Angela Capper: Methodology, Writing – review & editing. Guillermo Diaz-Pulido: Methodology, Writing – review & editing. Emma L. Jackson: Methodology, Writing – review & editing, Funding acquisition.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. This work was supported by a Co-Funded Industry Elevate Scholarship and International Excellence Award, in partnership between CQUniversity and the Australian Seaweed Institute, funded by the Great Barrier Reef Foundation.

Data availability The data supporting this study are available as open data via the CQUniversity online data repository aCQUIRE. <https://doi.org/10.25946/28529264>.

Declarations

Competing interests The authors declare no competing interests.

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