Journal of Applied Phycology (2023) 35:195–200
https://doi.org/10.1007/s10811-022-02860-6

Adherence of kelp (*Saccharina latissima*) gametophytes on ropes with different binder treatments and flow regimes

Wouter Visch[1] · Ann I. Larsson[2] · Per Åberg[3] · Gunilla B. Toth[2]

Received: 29 August 2022 / Revised and accepted: 19 October 2022 / Published online: 8 November 2022

Abstract
The cultivation of kelp typically involves two stages, where an indoor hatchery phase preceedes the grow-out phase at-sea. The in situ adhesion of microscopic propagules onto specifically designed substrates using binders has been proposed as an alternative to conventional hatchery methods where juvenile seaweeds are cultured on seeded twine, aimed at saving resources while increasing productivity as it obviates the hatchery phase. Here we tested how well kelp (*Saccharina latissima*) gametophytes adhere to cultivation ropes using two binder types (agar and κ-carrageenan) and application treatment (separate or mixed application) under two ecologically relevant flow regimes (5 and 15 cm s^{-1}), and a control condition (0 cm s^{-1}) in a laboratory flume. Our findings indicate that the effectiveness of a binder to retain *S. latissima* gametophytes onto cultivation rope, measured by the sporophyte density, was comparable for all binder types in the high flow velocity (15 cm s^{-1}) treatments, including the non-binder control treatment. Sporophyte densities were highest in the low flow velocities (0 and 5 cm s^{-1}) in the absence of a binder compared to all other treatment combinations. In conclusion, our results highlight that the effectiveness of binder assisted seeding of kelp propagules did not differ between the binder and non-binder treatments and was unaffected by flow velocities for the binder treatments. These findings are important in the development of novel methods and further optimisation of existing binder-based methods aimed at retaining seaweed propagules onto cultivation rope.

Keywords Phaeophyceae · Aquaculture · Algal cultivation · Hatchery · Mariculture · Nursery · Laminariales · Seeding

Introduction

Kelps are a group of brown seaweeds of the order Laminariales (Phaeophyceae) and of high global ecological and economic importance (Steneck et al. 2002; Bennett et al. 2015; Chopin and Tacon 2021). Cultivated kelp is used as food (Hu et al. 2021), feed (Troell et al. 2006; Correa et al. 2016), bioactive compounds (Holdt and Kraan 2011; Peteiro 2018), or as extractive component in integrated multi-trophic aquaculture systems (Chopin et al. 2001). Historically kelp aquaculture was primarily in Asia, but there is increasing interest globally (Naylor et al. 2021). Compared to terrestrial crop production, kelp cultivation uses little or no freshwater, arable land, fertiliser, or pesticides. Hence it has become attractive production system in "non-traditional" regions of production, including North America, Australia, and Europe (Stévant et al. 2017; Kim et al. 2019; Kelly 2020).

In traditional kelp (e.g., *Saccharina latissima*) mariculture, thin twine is inoculated with either zoospores or gametophytes and cultured for several weeks or months during an indoor hatchery phase (Camus and Buschmann 2017; Su et al. 2017; Forbord et al. 2018). The thin twine with small (> 1 mm) sporophytes are then transplanted into field cultivation sites and typically left to grow during winter months after which they are harvested in spring/early summer (Visch et al. 2020). Preparing juvenile sporophytes for transplantation includes several cultivation-steps in the laboratory (Forbord et al. 2018) and is a relatively time and labour intensive method. To overcome some of these challenges, seeding methods at the cultivation site are being developed (Sioen Industries 2013; Kerrison et al. 2018). The in situ adhesion of microscopic propagules (i.e. gametophytes, embryonic sporophytes, or a mixture of both) onto specifically designed

✉ Wouter Visch
wouter.visch@utas.edu.au

[1] Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS, Australia

[2] Department of Marine Sciences, Tjärnö Marine Laboratory, University of Gothenburg, 452 96 Strömstad, Sweden

[3] Department of Marine Sciences, University of Gothenburg, Box 461, 405 30 Göteborg, Sweden

substrates using binders has been proposed as an alternative to conventional methods in kelp cultivation, aimed at saving resources while increasing productivity (Kerrison et al. 2018). However, previous studies have highlighted that further research and development is needed if these techniques are to be applied more broadly (Kerrison et al. 2020; Umanzor et al. 2020).

The aim of this study was to test a different method of applying *S. latissima* gametophytes onto ropes that can be transplanted directly for at-sea cultivation without pre-cultivation in the laboratory. We tested if and how well *S. latissima* gametophytes adhere to cultivation ropes using two binder types (agar and κ-carrageenan) and application methods (separate or mixed) under two ecologically relevant flow regimes (5 and 15 cm s^{-1}) and a control condition (0 cm s^{-1}) in a laboratory flume.

Material and methods

Gametophyte culture establishment

Non-fertile *Saccharina latissima* individuals were collected at the Swedish west-coast (58.8363° N, 10.9963° E). Sorus tissue was induced following the methods described in Forbord et al. (2012). In short, tissue from non-fertile individuals was removed by cutting the blades approximately ~ 15 cm above the junction of the stipe and blade. The remaining non-fertile thalli were cultured under flow-through conditions for 10 weeks in ~ 10 °C, white light conditions (~ 100 µmol photons m^{-2} s^{-1}) and short-day photoperiod (8 h light: 16 h dark) provided by fluorescent tubes. Mature sorus tissue was dissected out of the main thallus using a scalpel and sori were thoroughly wiped clean with paper towel to remove any epiphytes and debris then cleaned with paper towel soaked in 10% bleach (v/v) and rinsed successively with autoclaved seawater and kept overnight in the dark at 10 °C in damp paper towel. Spores were released in autoclaved seawater with half strength Provasoli's enriched seawater (PES) and kept in aerated glass flask (5 L) at 12 °C under red light conditions and long-day photoperiod (16 h light: 8 h dark). Under these conditions, the spores were allowed to develop into gametophytes and vegetatively grown for > 6 months with monthly medium changes (Bartsch 2018).

Binder preparation

Binders were prepared by mixing (1) agar or (2) κ-carrageenan with UV-treated filtered seawater (0.2 µm) at a concentration of 0.3%. The mix was heated to boiling in a microwave oven and allowed to cool to room temperature under constant stirring and further cooled to the experimental temperature (14–15 °C) before mixing the gametophytes or inoculating the rope. This experimental temperature was chosen because the seeded lines with kelp are typically deployed when the seawater temperature in Swedish is approximately 14–15 °C. Before the start of the experiment, *S. latissima* gametophyte culture was mixed for 1 min in a kitchen blender. The concentration of gametophytes (defined as a few cells/filaments) was counted under the microscope as follows: 10 µL of the blended gametophyte solution was pipetted onto a microscope slide, and with the aid of a light microscope (magnification 200x) all gametophytes of a transect within the field of view were noted. There were 36,312 ± 1,625 gametophytes mL^{-1} (mean ± SE, n = 8) in the gametophyte cultures used in the experiment.

Rope preparation and binder application

Braided polyamide cultivation ropes (diameter 4 mm) were cut into 50 cm sections and prepared by attaching a labelled cable tie to one end and marking the centre 15 cm with a pen. Directly before deployment in the flume, different binders and *S. latissima* gametophyte cultures were applied onto each of five replicate ropes using three different treatments. See the supplementary material for an illustrative description of the rope preparation, binder treatments, and experimental set-up in the flume. In the first treatment, 1 mL binder solution was first evenly applied over the marked 15 cm in the centre of the rope, followed by application of 1 mL of gametophyte solution on-top of the binder solution, and is referred to as "Agar" or "Carrageenan". In the second treatment, 1 mL each of glue solution and 1 mL gametophyte culture was mixed and then applied to the rope and is referred to as "Agar mixed" or "Carrageenan mixed". In the third treatment, 1 mL of gametophyte culture was applied onto the rope without binder (controls), referred to as "No binder". The binder solutions and culture were applied using 1 mL pipettes and were only applied on one side of the rope.

Flume and flow velocities

The ropes were exposed to three different flow velocities (0, 5 and 15 cm s^{-1}) in the flume for 5 min. The flume was filled to a depth of 20 cm with seawater of salinity 33.5 ppt and the temperature was kept at 14–15 °C. The flow velocity was measured at z = 10 cm above the slate flume floor with an ADV (Nortek) at 25 Hz. The rope was mounted horizontally (slightly tensed) at z = 10 cm with the treated part centred in the flume and with the treated side of the rope facing upwards. The order of the binder treatments and flow velocities were chosen randomly.

Cultivation of rope in tanks

After exposure, the ropes were gently placed into transparent flow-through cultivation tanks (W28 cm x D55 cm x H70 cm, ~ 100 L) where the gametophytes were left to fertilize and resulting sporophytes grew for 4 weeks. The first 3 days there was no aeration, after which the aeration was slowly increased until the tanks were mildly aerated. The cultivation condition in the tanks was UV-treated filtered natural seawater (0.2 µm) at 10 °C with cool white light (35–70 µmol photons m^{-2} s^{-1}) and long-day photoperiod (16 h light: 8 h dark) provided from the side by fluorescence tubes. Every two days the ropes were randomized within the cultivation tank.

Sporophyte density measurements

After 4 weeks of cultivation in the tanks, the sporophyte density on each of five replicate ropes was noted with the aid of a stereo microscope and the mean was taken from three 1-cm sections of a standardized location: between 2–3 cm, 7–8 cm, and 12–13 cm.

Statistical analysis

All statistical analyses were performed using R software (Team RC 2018). The individual and interactive effects of binder type (fixed factor, 5 levels) and flow speed (fixed factor, 3 levels), with 5 replicate ropes per treatment combination, were analysed for the sporophyte density and statistically analysed with analysis of variance (ANOVA) using the *lm* function. When significant, a Student–Newman–Keuls (SNK) post hoc test ($\alpha = 0.05$) was performed, using the *SNK.test* function of the *agricolae* package (de Mendiburu 2020). Data were checked for normality (Shaprio-Wilk test) and homoscedasticity (visually), and the best suited normalizing transformation was estimated using the *bestNormalize* package (Peterson 2017) and data was transformed accordingly.

Results

The mean number of sporophytes growing on the rope was significantly affected by the interactive effects of binder type and flow speed ($p = 0.028$), with significantly more sporophytes when a binder was absent in low flow speeds (0 and 5 cm s^{-1}) compared to the other binder treatments (Fig. 1 and Table 1). The 'No binder' low flow speed (0 and 5 cm s^{-1}) treatment combination resulted in a mean sporophyte density of 44.3 cm^{-1}, compared to a mean sporophyte density of 27.0 cm^{-1} in all other treatments combined. No further differences were noted for the other treatments (Fig. 1 and Table 1), this includes morphological and/or developmental differences in cultured the sporophytes.

Table 1 Number of sporophytes. Summary of two-way ANOVA for the main and interactive effect of binder type and flow speed on the mean number of sporophytes per cm of inoculated line, followed by a Student–Newman–Keuls (SNK) post-hoc test

Source of variation	df	MS	F-value	*p*-value
Binder type	4	15.58	26.80	**< 0.001**
Flow speed	2	3.91	6.73	**0.001**
Binder type x Flow speed	8	1.29	2.21	**0.028**
Residuals	210	0.58		

SNK-tests:

Binder type: No binder > Agar = Agar mixed = Carrageenan = Carrageenan mixed

Flow speed: 0 cm s^{-1} = 5 cm s^{-1} > 15 cm s^{-1}

Interaction: No binder: 0 cm s^{-1} = 5 cm s^{-1} > 15 cm s^{-1}

Agar: 0 cm s^{-1} = 5 cm s^{-1} = 15 cm s^{-1}

Agar mixed: 0 cm s^{-1} = 5 cm s^{-1} = 15 cm s^{-1}

Carrageenan: 0 cm s^{-1} = 5 cm s^{-1} = 15 cm s^{-1}

Carrageenan mixed: 0 cm s^{-1} = 5 cm s^{-1} = 15 cm s^{-1}

Significant differences ($p<0.05$) are denoted in bold

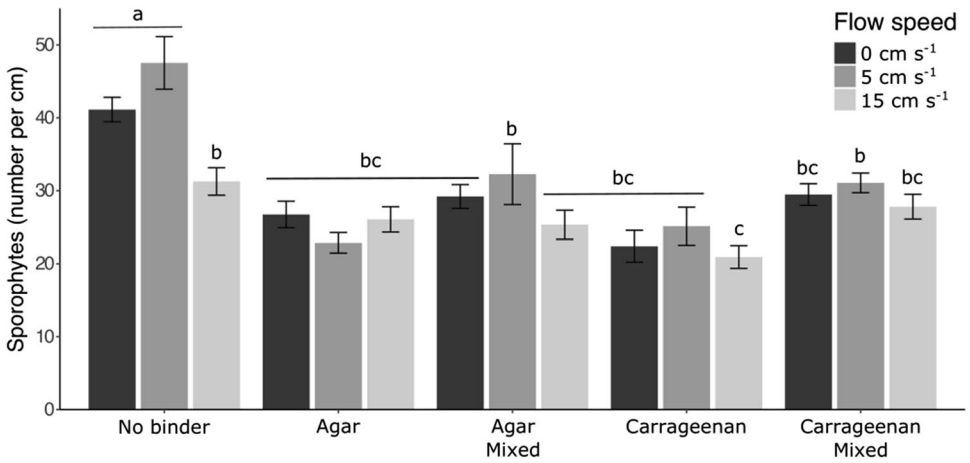

Fig. 1 Mean number of sporophytes cm^{-1} on rope after being exposed to thee flow regimes per binder type. The letters indicate significant differences between mean values per binder treatment and flow speed combination (SNK-test; $p < 0.05$). Error bars show SEM ($n = 5$)

Discussion

Our findings suggest that the effectiveness of a binder to retain *Saccharina latissima* gametophytes onto cultivation rope, measured by the sporophyte density, was comparable in the high flow velocity (15 cm s^{-1}) across all binder treatments, including the non-binder control treatment. The highest sporophyte densities were observed in relatively low flow velocities (0 and 5 cm s^{-1}) in the absence of a binder.

Our results confirm previous studies that found relatively poor growth of *S. latissima* after using the binder technique in situ compared to traditional hatchery methods. Adherence of sporophytes onto substrate was found problematic both in situ (Forbord et al. 2020; Boderskov et al. 2021) as well as under controlled laboratory conditions (Umanzor et al. 2020). Interestingly, a Danish study found the binder method to be only successful at the exposed site compared to more sheltered sites, potentially confounded by fouling on the rope rather than a lack of adherence at the sheltered sites (Boderskov et al. 2021). Finally, Kerrison et al. (2018) found no difference between the traditional hatchery seeding method and using a binder to adhere juvenile sporophytes in situ. Taken together, these results highlight that using currently available substrate and binder combinations failed to yield reliable results. The successful use of a binder to aid adherence of propagules in kelp aquaculture is context dependent and partially determined by the environmental conditions at the cultivation site.

A limitation of our study may be the transferability of a controlled laboratory study with a limited exposure time into a real-world scenario where binders are used in situ. However, Kerrison et al. (2020) found a positive correlation between density of *S. latissima* sporophytes on the cultivation line and growth performance (i.e., yield) when gametophytes were applied using a binder in situ. Thus, confirming the assumption that higher sporophyte densities typically lead to higher yield for this species. However, the inversed relationship (i.e., high density leads to reduced harvested biomass) was noted for the kelp species *Alaria esculenta* (Kerrison et al. 2020). Furthermore, we did not note any differences in sporophyte morphology and/or development as a function of the binder. Both Boderskov et al. (2021) and Forbord et al. (2020) found delayed sporophyte growth when using a binder compared to traditionally seeded string cultured in a hatchery. They hypothesized that this could be because the juvenile sporophytes need time to develop their holdfast for attachment when using a binder, where the embryonic sporophytes are already attached to substrate (i.e. string) in the traditional method. In addition, we did not test the effect of substrate that may accompany and further assist adherence of kelp propagules.

Thus far, most studies have focussed on a limited number of Atlantic kelps (i.e., *S. latissima* and *A. esculenta*). Future work could focus on other commercially interesting species, that have different modes of adherence/settlement depending on the life-stage compared to kelp or are not traditionally cultured. For many of these species or life-stages, adherence onto substate is a major challenge and assisting them may proof beneficial for subsequent large-scale cultivation at-sea. For example, the green species *Ulva* (Steinhagen et al. 2021), *Chaetomorpha* (Gao et al. 2018), *Cladophora* (de Paula Silva et al. 2008), the red species *Asparagopsis* (Zhu et al. 2021) and *Palmaria* (Grote 2019), or species of the order Fucales, such as *Sargassum* (Xie et al. 2013), *Phyllospora* (Cumming et al. 2020), and *Durvillaea* (Velásquez et al. 2020). Furthermore, the use of a binder can be helpful in seaweed restoration projects, where propagules or seeded substrates may need assistance with adherence when they are transplanted into areas with relatively high flow rates or wave action (Morris et al. 2020).

In conclusion, our results highlight that the effectiveness of binder assisted seeding of kelp propagules did not differ between the binder and non-binder treatments and was not affected by flow velocities in a controlled laboratory study. These findings are important in the development of novel methods and further optimisation of existing binder-based methods aimed at retaining seaweed propagules onto cultivation rope.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-022-02860-6.

Acknowledgements The authors wish to thank the Centre of Marine Chemical Ecology (MARICE) at The University of Gothenburg for the support during this project.

Author contributions WV was the lead author, wrote the first draft of the manuscript, and was involved in the laboratory data acquisition. AL, PÅ, and GT were involved in the laboratory data acquisition and contributed to writing of the manuscript through providing feedback on drafts. All authors read and approved the final submitted version.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. This study is supported by The Swedish Foundation for Strategic Environmental Research MISTRA (grant no. 2013/75) and The Swedish Research Council for Sustainable Development Formas (grant no. 213- 2013–92).

Data availability The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Bartsch I (2018) Derivation of clonal stock cultures and hybridization of kelps. In: Charrier B, Wichard T, Reddy CRK (eds) Protocols for Macroalgae Research. CRC Press, Boca Raton, pp 61–78

Bennett S, Wernberg T, Connell SD, Hobday AJ, Johnson CR, Poloczanska ES (2015) The 'Great Southern Reef': social, ecological and economic value of Australia's neglected kelp forests. Mar Freshw Res 67:47–56

Boderskov T, Nielsen MM, Rasmussen MB, Balsby TJS, Macleod A, Holdt SL, Sloth JJ, Bruhn A (2021) Effects of seeding method, timing and site selection on the production and quality of sugar kelp, *Saccharina latissima*: A Danish case study. Algal Res 53:102160

Camus C, Buschmann AH (2017) *Macrocystis pyrifera* aquafarming: Production optimization of rope-seeded juvenile sporophytes. Aquaculture 468:107–114

Chopin T, Tacon AGJ (2021) Importance of seaweeds and extractive species in global aquaculture production. Rev Fish Sci Aquacult 29:139–148

Chopin T, Buschmann AH, Halling C, Troell M, Kautsky N, Neori A, Kraemer GP, Zertuche-González JA, Yarish C, Neefus C (2001) Intergrating seaweeds into marine aquaculture systems: a key toward sustainability. J Phycol 37:975–986

Correa T, Gutiérrez A, Flores R, Buschmann AH, Cornejo P, Bucarey C (2016) Production and economic assessment of giant kelp *Macrocystis pyrifera* cultivation for abalone feed in the south of Chile. Aquacult Res 47:698–707

Cumming EE, Matthews TG, Sanderson JC, Ingram BA, Bellgrove A (2020) Growth and survivorship of *Phyllospora comosa* (Phaeophyceae, Fucales) on different mariculture seeding twines in a hatchery setting. Aquaculture 523:735216

de Mendiburu F (2020) Agricolae: statistical procedures for agricultural research. https://CRAN.R-project.org/package=agricolae

de Paula Silva PH, McBride S, de Nys R, Paul NA (2008) Integrating filamentous 'green tide' algae into tropical pond-based aquaculture. Aquaculture 284:74–80

Forbord S, Skjermo J, Arff J, Handå A, Reitan K, Bjerregaard R, Lüning K (2012) Development of *Saccharina latissima* (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. J Appl Phycol 24:393–399

Forbord S, Steinhovden KB, Rød KK, Handå A, Skjermo J (2018) Cultivation protocol for *Saccharina latissima*. In: Charrier B, Wichard T, Reddy CRK (eds) Protocols for Macroalgae Research. CRC Press, Boca Raton, pp 37–59

Forbord S, Steinhovden KB, Solvang T, Handå A, Skjermo J (2020) Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case study. J Appl Phycol 32:2201–2212

Gao X, Endo H, Agatsuma Y (2018) Comparative study on the physiological differences between three *Chaetomorpha* species from Japan in preparation for cultivation. J Appl Phycol 30:1167–1174

Grote B (2019) Recent developments in aquaculture of *Palmaria palmata* (Linnaeus) (Weber & Mohr 1805): cultivation and uses. Rev Aquacult 11:25–41

Holdt S, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23:543–597

Hu ZM, Shan TF, Zhang J, Zhang QS, Critchley AT, Choi HG, Yotsukura N, Liu FL, Duan DL (2021) Kelp aquaculture in China: a retrospective and future prospects. Rev Aquacult 13:1324–1351

Kelly J (2020) Australian seaweed industry blueprint: a blueprint for growth. Publication No. 20–072. AgriFutures, Australia. https://www.agrifutures.com.au/wp-content/uploads/2020/09/20-072.pdf. Accessed 5 Sept 2022

Kerrison PD, Stanley MS, Hughes AD (2018) Textile substrate seeding of *Saccharina latissima* sporophytes using a binder: An effective method for the aquaculture of kelp. Algal Res 33:352–357

Kerrison PD, Innes M, Macleod A, McCormick E, Elbourne PD, Stanley MS, Hughes AD, Kelly MS (2020) Comparing the effectiveness of twine- and binder-seeding in the Laminariales species *Alaria esculenta* and *Saccharina latissima*. J Appl Phycol 32:2173–2181

Kim J, Stekoll M, Yarish C (2019) Opportunities, challenges and future directions of open-water seaweed aquaculture in the United States. Phycologia 58:446–461

Morris RL, Hale R, Strain EMA, Reeves SE, Vergés A, Marzinelli EM, Layton C, Shelamoff V, Graham TDJ, Chevalier M, Swearer SE (2020) Key principles for managing recovery of kelp forests through restoration. Bioscience 70:688–698

Naylor RL, Hardy RW, Buschmann AH, Bush SR, Cao L, Klinger DH, Little DC, Lubchenco J, Shumway SE, Troell M (2021) A 20-year retrospective review of global aquaculture. Nature 591:551–563

Peteiro C (2018) Alginate production from marine macroalgae, with emphasis on kelp farming. In: Rehm BHA, Moradali MF (eds) Alginates and their biomedical applications. Springer, Dordrecht, pp 27–66

Peterson R (2017) Estimating normalization transformations with bestNormalize. https://cran.r-project.org/web/packages/bestNormalize/bestNormalize.pdf

Sioen Industries N (2013) Drager voor het cultiveren van macro algen. Belgium Patent, BE1021186B1

Steinhagen S, Enge S, Larsson K, Olsson J, Nylund GM, Albers E, Pavia H, Undeland I, Toth GB (2021) Sustainable large-scale aquaculture of the northern hemisphere Sea Lettuce, *Ulva fenestrata*, in an off-shore seafarm. J Mar Sci Eng 9:615

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. Cambridge University Press, Cambridge, pp 436–459

Stévant P, Rebours C, Chapman A (2017) Seaweed aquaculture in Norway: recent industrial developments and future perspectives. Aquacult Int 25:1373–1390

Su L, Pang SJ, Shan TF, Li X (2017) Large-scale hatchery of the kelp *Saccharina japonica*: a case study experience at Lvshun in northern China. J Appl Phycol 29:3003–3013

Team RC (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available online at https://www.R-project.org/

Troell M, Robertson-Andersson D, Anderson RJ, Bolton JJ, Maneveldt G, Halling C, Probyn T (2006) Abalone farming in South Africa: an overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. Aquaculture 257:266–281

Umanzor S, Li Y, Yarish C (2020) Effect of direct "seeding" binders and embryonic sporophyte sizes on the development of the sugar kelp, *Saccharina Latissima*. J Appl Phycol 32:4137–4143

Velásquez M, Fraser CI, Nelson WA, Tala F, Macaya EC (2020) Concise review of the genus *Durvillaea* Bory de Saint-Vincent, 1825. J Appl Phycol 32:3–21

Visch W, Nylund GM, Pavia H (2020) Growth and biofouling in kelp aquaculture (*Saccharina latissima*): the effect of location and wave exposure. J Appl Phycol 32:3199–3209

Xie EY, Liu DC, Jia C, Chen XL, Yang B (2013) Artificial seed production and cultivation of the edible brown alga *Sargassum naozhouense* Tseng et Lu. J Appl Phycol 25:513–522

Zhu P, Li D, Yang Q, Su P, Wang H, Heimann K, Zhang W (2021) Commercial cultivation, industrial application, and potential halocarbon biosynthesis pathway of *Asparagopsis* sp. Algal Res 56:102319