Sustainable cultivation of Dulse, *Palmaria palmata*, (søl) in the North Atlantic (SØLSTAIN)

NORA j.nr.510-213

Second Year Report - SUMMARY

Submitted March 2025

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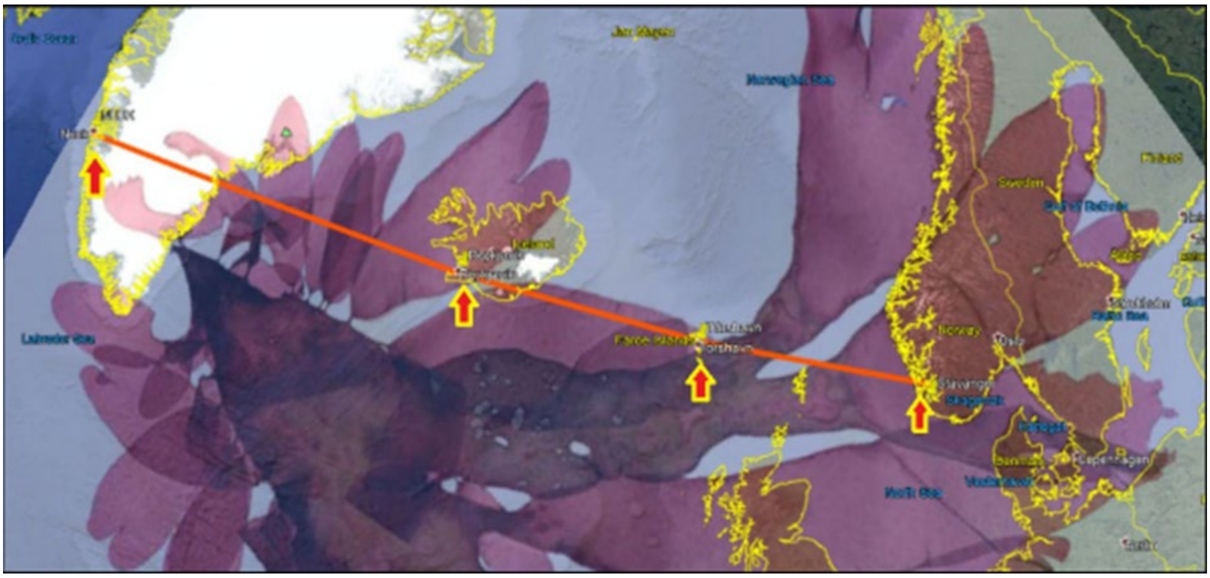

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1. Background

Climate change is the defining crisis of our time, threatening both human livelihoods and ecosystems worldwide. Livestock and land-based agriculture are major contributors to greenhouse gas emissions and deforestation. In this context, transition to plant-based protein sources and sustainable aquaculture are regarded as potent solutions.

Seaweed aquaculture is a booming industry offering food, animal feed and biomedical applications, while contributing to carbon sequestration and climate change mitigation. Many algae contain high amounts of protein and dietary fibres, require little resources and are readily eaten in many cultures. *Palmaria palmata* (also known as dulse and søl) is a low intertidal red seaweed (Rhodophyta) distributed throughout the North Atlantic from Portugal to the Arctic (obis.org, 2021), where it is traditionally harvested and consumed. High protein content with high relative digestibility makes it a strong candidate as a novel, sustainable protein source. Various health benefits are also associated with its consumption, including documented antioxidant and antihypertensive properties. However, current supply comes overwhelmingly from harvesting of natural populations and will not sustain increases in consumption.

Interest in the bioactive potential for pharmaceutical and nutraceutical use of seaweeds is steadily increasing. Specific research on both human gut cells and microbiota is necessary to characterise and quantify such potential properties. Specific bioactivity is relatively trivial to detect, and various *in vitro* assays exist for that purpose. Isolation and characterisation of specific bioactive peptides remains challenging but provide valuable insights into bioactivity mechanisms. Dulse has the potential to be a versatile product, and researched nutraceutical properties will increase its value and encourage the development of commercial cultivation. It may also be combined with other commonly cultivated seaweeds to provide enhanced health benefits, for example as a food supplement. *P. palmata* is a common seaweed offering valuable opportunities to develop sustainable, resilient, sea-based cultures. Despite decades of research, a lack of deep understanding of its life cycle impedes cultivation attempts, while further research into its growth and nutritional and nutraceutical properties will improve the farming cost-efficiency and quality of the product.

P. palmata is characterised by a haplodiplontic life cycle, common in seaweeds. Extreme sexual dimorphism is a particularity of dulse gametophytes. Males grow to macroscopic size, forming thalli and fronds identical to tetrasporophytes, and achieve sexual maturation in 9-12 months. Females are microscopic and become sexually mature a few days after spore release. Sporulation and reproduction are seasonal and happen between January-May depending on latitude and environmental conditions. This represents a significant drawback for aquaculture prospects, hindering the continuous supply of fresh, high-quality product. Various mechanisms have been studied to inhibit and trigger sporulation in sporophyte cultures, including manipulations of photoperiod and light intensity and regeneration of sub-meristematic fragments. Despite partial successes, mechanisms regulating sporulation in *P. palmata* are still unknown. An improved understanding of environmental triggers of sporulation and their effects on seaweed physiology allow the development of consistent methods for year-round production of dulse.

Small-scale cultivation attempts exist, but a lack of sturdy cultivation framework and methods hinders the development of sustainable commercial farms. Therefore, there is a need to improve existing harvesting methods and establish new cultivation methods of the red seaweed species, *P. palmata*, based on geographical specificity of countries.

It is important to support the vision of the Nordic Council of Ministers of making the Nordic region the most sustainable region by 2030 and will target 5 of the United Nations sustainable developmental goals (SDGs) (Figure 1). Enabling the sea to become a part of the future knowledge-based solution itself and to perform seaweed farming is recognized under the United Nations SDG 14 “to conserve and sustainable use the oceans, seas and maritime resources for sustainable development”. Herein, we are addressing the need to develop suitable, economic and feasible methods to upscale the cultivation of dulse in the North Atlantic. The growing public vegetarian movement, that wishes for more climate-friendly food, calls for innovative research to promote the green transition of our food production systems. Primary producers, like seaweeds, are the most climate-friendly food source, making their aquaculture a potentially CO_2 neutral or perhaps even negative production.

Figure 1 The 17 United Nations Sustainable Developmental Goals.

2. Project objectives

The project main objective is to develop reliable, cost-effective, and scalable production methods and protocols for cultivating and harvesting dulse in the different areas based on local/national knowledge and experience as well as existing information. The project plan includes 3 years' activities, and the first-year goals were:

- to strengthen the partner cooperation and extend their network to ensure closer collaborations and develop deeper dedication,
- to provide a list of locations in the partner areas of interest where dulse naturally occurs,
- to provide knowledge of biomass for producers in each of the country, according to each individual business models,
- to discuss optimization of harvesting method to reach the requirement for a sustainable business,
- to start cultivation trials of the species with indication of growth rates and feasibility,
- to suggest method(s) to maintain year-round local strain plants and gametophyte cultures,
- to contribute to the SDG goals.

3. Mapping of *Palmaria palmata* resources

General mapping of dulse resources in Iceland during year 1 found that site accessibility and low demand were the limiting factors in wild harvest of dulse in the country, despite high potential due to widespread populations. This year's objective was to improve the knowledge of currently accessible biomass and explore the potential to expand harvesting operations to other areas. Using reports from various harvesting individuals and companies, including location, depth and method of harvest, as well as harvested biomass, an AI model was used to assess the characteristics common to used harvest sites, which will in turn be used to explore potential new sites and opportunities.

Identification of potential harvest locations in the Faroe Islands was conducted *ad hoc* by TARI during regular commercial harvests and when sourcing material for seeding and cultivation efforts. Current knowledge is focused on the areas around Suðuroy and Streymoy islands, where the company has facilities. However, resources appear plentiful and suggest strong potential for further development of both wild harvest and biomass provision for cultivation.

The size and low population density of Greenland make comprehensive mapping efforts both arduous and likely redundant. However, mapping of wild stocks in fjords and other locations near the capital city Nuuk were carried out, with samples for experiments readily accessible.

For Norway, the mapping of *P. palmata* is comprehensive (Stevant et al., 2023) and provide a suitable platform to transfer the project knowledge to other companies and/or institutions interested in exploring possibilities in their regions.

4. Development of protocols for dulse cultivation in the North Atlantic

Development and evaluation of methods for land-based, small-scale cultivation of dulse in Greenland

Progress from the first year of the project showed mixed results and a low potential for fjord-based cultivation in opaque containers in Greenland. The second-year efforts were thus focused on performing land-based cultivation trials with enriched nutrients. The purpose of the work was to assess the feasibility of cultivation in more controlled conditions using local plants, and potential avenues to boost the growth of these plants, improving yields and enabling year-round production.

Specimen were harvested in a clean area near the harbour in Nuuk in July 2024, cleaned and placed in 16L glass containers with filtered seawater. The containers were placed into a water bath, to maintain a constant 4 ºC temperature throughout the experiment, and lighting was constantly provided to match seasonal atmospheric conditions (Figure 2). Bubbling was provided to the containers to maintain water aeration, and the water was partially changed weekly.

After a week of adaptation, the seaweeds were grown for five weeks with one of five concentrations of plant fertiliser as nutrient enrichment (Control, 5:1000, 1:100, 5:100 and 10:100).

Figure 2: Water bath with 25 (5 treatments x 5 replicates) glass containers containing *Palmaria palmata.*

Weekly wet weight measurements showed growth for all treatments except the highest fertiliser concentration, but there were no significant differences between treatments by the end of the experiment. According to those findings, land-based cultivation of dulse in Greenland is suitable and appears to yield reasonable growth, with regular exchange of seawater. Further attempts in larger units and commercial settings will help refine the potential of this method in Greenland and beyond, while addition of seaweed-specific nutrient mixtures may help boost these growth rates.

Evaluation of methods for cultivation of dulse in Faroe Islands

The development of commercial scale production of *P. palmata* is an important step towards increasing sustainability and resilience in the growing seaweed industry. TARI is aiming to diversify the ongoing commercial production with including *P. palmata* as a cultivar. Following successes with rope seeding and vegetative on-growth during year 1, dulse ropes were deployed and produced a fruitful harvest in 2024, and new ropes were deployed for harvest in Spring/Summer 2025.

Refinement and assessment of seeding methods

The seeding methods developed and tested in year 1 were further refined to allow faster more efficient spore release and seeding. Increased seaweed density during the spore release phase, and the use of aeration to promote rapid release of spores shorten the process from 7 to 4 days, while obtaining seeding densities equal or slightly higher than the previous year (18-22 individuals / 10 cm of rope, against 17 previously). This seeding was performed both in February and May and was successful in both cases. Seeding in February yielded slightly higher densities but was far more variable than in May, suggesting a potential increase in spore viability later in the season. This experiment was successful in reducing the workload and necessary space for spore release and rope seeding, suggesting a viable protocol for cultivation material preparation with potential useability for all partners and simple scaling up or down.

Rope deployment

Following the success of rope seeding, the growth of seeded material was followed regularly until the material was considered large enough for deployment and at-sea growth (Figure 3). Ropes were then deployed at TARI's cultivation site in Kaldbaksfjørður, Streymoy island and grown for 6 months until they reached full size. The length of random individuals was measured before deployment and after harvest, and the yield was systematically measured in kg wet weight per meter of rope. The growth was overall strong, with individuals reaching 24 cm length on average after 6 months from a 1cm average original length. There was high variation between individuals with lengths ranging 12.5-32.5 cm, suggesting potential for improvement of cultivation methods to obtain more homogeneous sizes. Similarly, yields ranged 0.08 – 0.87 kg/m rope, a 10-fold variability highlighting the need for further improvements. The average yield was 0.45 kg/m rope, slightly lower than reported for other at-sea cultivation experiments (Grote, 2019pa). This experiment was a strong first attempt, successfully carrying out an entire cultivation cycle from spore release to harvest and produce clean, quality material which was commercialised by TARI. Further refinements to improve the consistency of seeding densities and yields are in progress and will be carried out during the last year of the project. Additionally, the developed protocols will be shared during the third year of activities with the new partners in Iceland and Greenland to ensure knowledge transfer and development of cultivation trials in these partner areas.

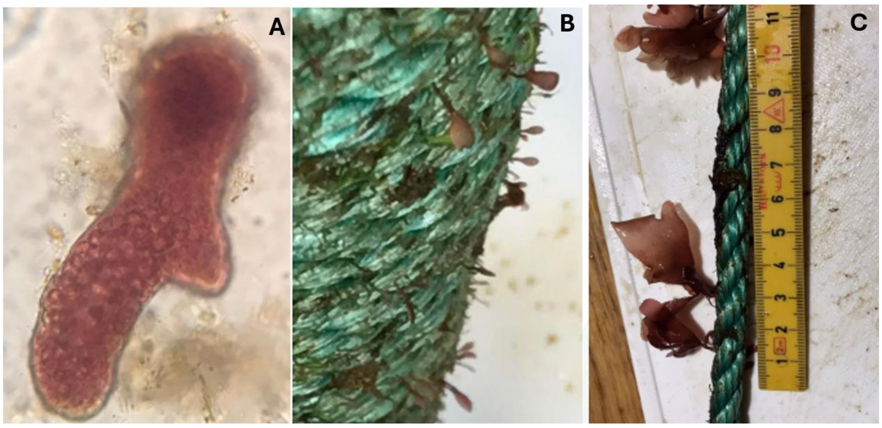

Figure 3: In-hatchery growth of *Palmaria palmata* male gametophytes following seeding on rope. A) after 1 month of growth; B) after 3 months of growth; C) after 6 months of growth, prior to rope deployment at sea.

Epiphyte control using coloured growth light

Epiphyte on-growth is a major issue in seaweed aquaculture, leading to decreased yields and lower product quality and market value (Sahu et al., 2020). It is especially challenging in hatchery settings where rapid growth can quickly lead to shading recently deployed seedling and severely reduce their growth. *P. palmata* presents the advantage of being a red seaweed, undergoing photosynthesis with various pigments in addition to the ubiquitous chlorophyll. The latter does not absorb green light, and it was hypothesised that using green or blue lights, readily absorbed by red pigments, would allow dulse to thrive while decreasing epiphyte growth.

Seaweeds were collected from Froðba, Suðuroy in April. After acclimation, meristematic tips were cut and placed in plastic containers with 650 mL of UV-filtered seawater and air bubbling. Each container with 20 tips was exposed to one of four treatments: white (control), blue and green light (50 µmol.m^{-2}.s^{-1}, 16h:8h L:D) and white light with 4.5mg/L GeO_2, the latter being used for its toxicity to diatoms, a group of common epiphytes. The temperature was kept constant by placing the buckets at the surface of cultivation tanks with constant flow of UV-filtered seawater. The water in the container was changed weekly, at which point the fragments were measured and visually inspected for epiphytes/biofouling. Seeded ropes prepared as described above were also placed in the tanks as an added reference of epiphyte control.

After 64 days, the germanium treatment yielded significantly lower fragment sizes than other treatments. Coloured light treatments did not significantly limit growth, but fragments appeared more discoloured than under control (white) conditions, making them less appetising and suggesting likely decreased nutritional quality, hence hampering the value of such epiphyte control methods. On the other hand, the method was successful at reducing the epiphytic growth on the tanks and ropes, with blue light tanks having little to no epiphyte outgrowth while green light led to patchier contamination than white light (Figure 4).

Overall, due to the potential damage to seaweed material and logistical complications of such a setup, the idea of using coloured lights or germanium oxide as epiphyte control in hatchery was abandoned. However, it might be revisited for fully land-based cultivation, where increased control could help balance potential negative effects.

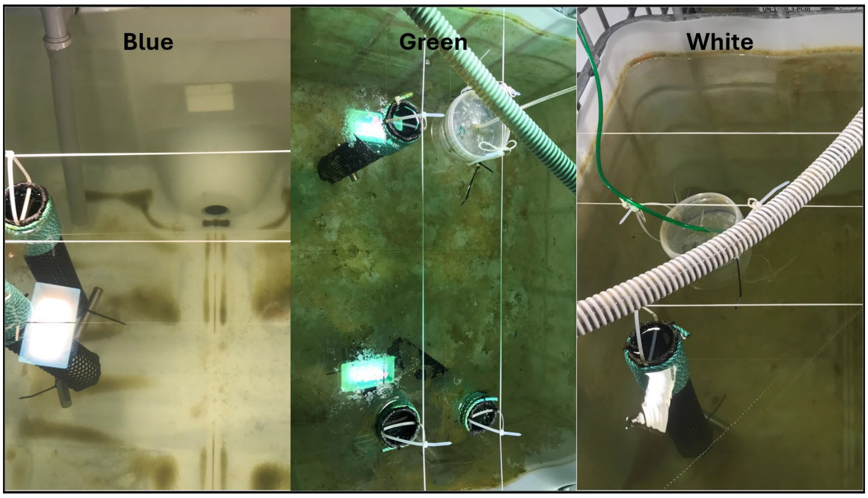

Figure 4: Epiphyte fouling on cultivation tanks after 64 days in epiphyte-control conditions. Labels show the colour of the light the tanks were exposed to.

Evaluation and optimisation of nutritional and nutraceutical potential in *Palmaria palmata*

Dulse is noted for its high protein content and various bioactive properties, including antioxidant and anti-inflammatory effects. Several experiments were carried out at UiS to further measure this potential, assess its natural variability and explore paths to maximising its availability to consumers.

Effects of seasonality and cultivation conditions on the protein content and bioactivity of dulse

Protein content in dulse is known to vary from 7-20% dry weight depending on seasonality, life stage and conditions (Stévant et al., 2023). Such variation is not viable in presenting this species as highly nutritional food and could be damaging to both producers and consumers. The assessment of such variation according to realistic factors in the partner areas was therefore essential to then come up with strategies to maintain consistent, high protein content. Variation in bioactivity, while largely undocumented, required similar assessment. A first step in this direction was performed during year 1 with in-lab cultivation of meristematic fragments from both Southern Norway and the Faroe Islands. This new experiment was split in two parts: a) assessment of seasonal variation; and b) assessment of cultivation conditions.

a) Whole *P. palmata* plants were sampled from Vistnes, Norway (58.983 ºN, 5.569 ºE) approximately every other month from April to March. Plants collected in April were sorted as either reproductive or vegetative.
b) Whole *P. palmata* plants were sampled from Vistnes in January. After acclimation, seaweeds were cut into single-blade fragments and randomly assigned to one of four treatments: a control keeping the adaptation conditions; green light, maintained the same light intensity as the control; aeration only during daytime; and nutrients lowered to 10% of the standard F/2 concentration. The media was changed weekly. After four weeks in these conditions, the fragments were analysed. Meristematic fragments from the year 1 experiment were also used for comparison.

The protein and phenolics contents and antioxidant activity was measured in cell extracts from all samples. Seasonality results showed both protein content and antioxidant activity peaked in April, although reproductive status of the plants had no significant effect. Both protein content and antioxidant activities decreased significantly by June and were lowest during the summer and autumn before increasing again in the winter. These differences were particularly striking for the antioxidant activity where spring values were 2-3-fold higher than in other seasons. These results point to the need to harvest dulse while waters are still cool and nutrient-rich in the early spring. These observations strengthen the current cultivation practices of TARI, which focus on rope deployment at the beginning of the winter and harvest in April-May.

Results from cultivation conditions showed much lower variability than seasonal ones. Only the low nutrient treatment led to significantly lower protein content than in control fragments, although the relative bioactivity in these fragments was the highest. All three other treatments (green light, daytime aeration and meristematic growth) led to lower antioxidant potential but high protein contents. Overall, these results suggest that standard conditions are likely optimal for dulse cultivation, but reduced nutrients or aeration could help reduce costs while having a limited effect on product quality. Furthermore, these results show a consistent potential for the use of *P. palmata* as a strong purveyor of bioactive effects. Using proteomics analyses combined with *in vitro* assay results, a potent bioactive peptide, SLLYSDITRPGGNMYTTR

(SR18) was identified. Part of the pigment allophycocyanin, it may also reveal potential for further development of food supplements using dulse and increasing its valuable uses as a functional food.

Effects of hydrogen peroxide treatment on the antioxidant power

An experiment was designed to test the hypothesis that oxidative conditions led to increased expression of antioxidant potential in dulse. Hydrogen peroxide was selected for its specifically oxidative properties, and is associated with oxidative stress metabolism and antioxidant response in seaweeds (Taenzer et al., 2024). Seaweeds were samples from Vistnes, Norway in November. After acclimation, young thallus fragments were grown at various H_2O_2 concentrations, with their protein content and bioactivity recorded regularly. The results point to a short-term, phenolics-driven increase in antioxidant activity at moderate H_2O_2 concentrations. Protein content was largely unaffected by the treatment. Fragments and cultivation material exposed to higher concentrations of H_2O_2 also had lower epiphyte on-growth by the end of the experiment, which may be an added benefit to the long-term use of mild concentrations of this chemical (Figure 5). These results suggest potential for a short, post-harvesting, pre-processing use of this method to enhance bioactive value in this seaweed which may be tested on cultivated material during year 3 of the project.

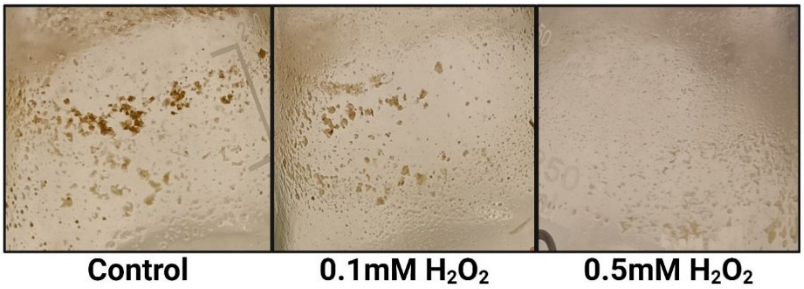

Figure 5: Biofouling on culture flasks after 28 days of *Palmaria palmata* culture with hydrogen peroxide concentrations relevant for promoting antioxidant power.

5. Conclusions

- Wild population mapping efforts were completed in Iceland; they are well underway in relevant areas of the Faroe Islands and Greenland. *P. palmata* wild population mapping in Norway is also available.
- Land-based cultivation attempts in Greenland have demonstrated to viability of this method, but addition of plant fertiliser did not improve growth. The use of seaweed-specific nutrient enrichment may help promote growth and quality (as observed in experiments conducted at UiS), although it might raise cultivation costs.
- An entire seeding and cultivation cycle has been completed by TARI with commercially viable biomass production and high product quality. Methods have been and

continue to be improved to optimise both efficiency and results. These methods will be shared in the third year of the project within the consortium and adapted for the local needs under the leadership of TARI.

- Several experiments attempting to improve cultivation conditions to increase yields, nutritional quality of product and reduce epiphyte growth have led to diverse results. Standard cultivation conditions are likely best suited for general cultivation. However, short treatments post-harvest may provide significant benefits to the seaweed bioactivity potential.
- A strong knowledge and skill base was established in the consortium during the first two years of the project. The last year will focus on implementation of the methods at larger scale and in different locations. Research will focus on better understanding the biology of the species and its response to stressors to encourage the development of sustainable and resilient cultivation methods.

6. Acknowledgments

The consortium is grateful to the support staff at UiS for their support in establishing and maintaining the seaweed laboratory and a good space for the common garden; to bachelor student Astri Kolnes for her contribution to the experimental work. A PhD grant from the UiS allows much of the dissemination of the work at prestigious international conferences; additional funding helped finance the research stay at TARI facilities.

7. References

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