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

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First Year Report - SUMMARY

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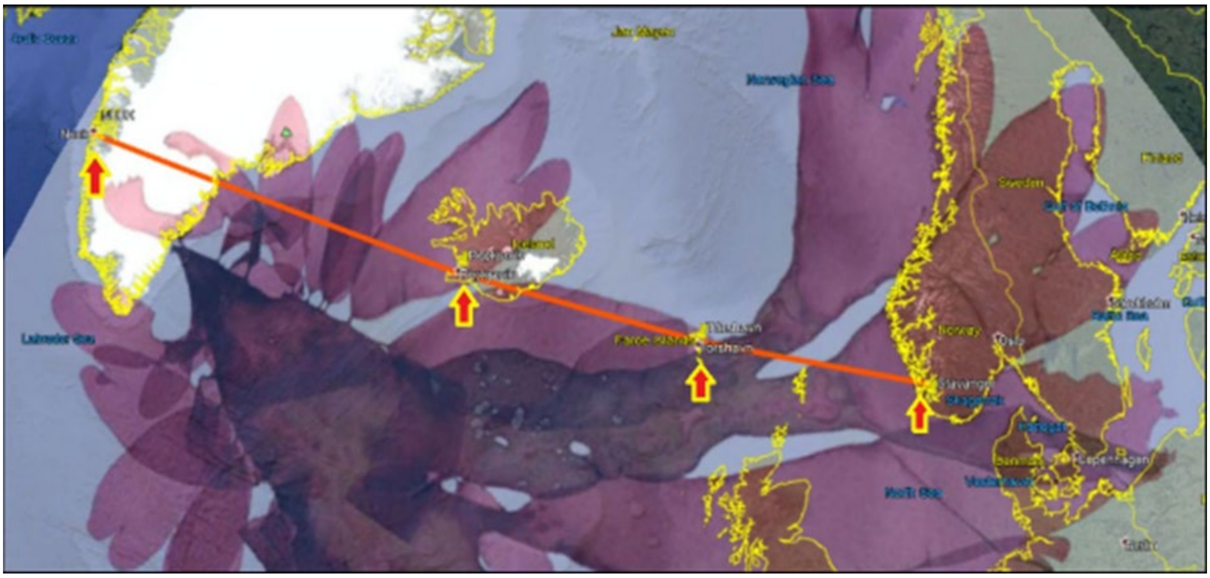

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1. Background

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

At present, scattered efforts can be found when looking for maps of dulse presence on the coast, especially regarding the 4 partner countries, even though harvesting has been carried out over time. In general, the identification of cultivation areas for seaweed can be based on the knowledge of important limiting factors, such as the substrate, the water quality, the light, etc. In addition, a lack of a consistent method for recording data provides an additional challenge. The location of sites is only the first step; several key variables, both biological and local infrastructure, must then be detailed for a life cycle assessment and/or feasibility study. Site accessibility, seaweed bed extension and co-presence of other seaweed species are important criteria to determine to most valuable location for a production/harvesting prospective. After gathering that information, it is critical to integrate it and provide a multi-layer map summarizing all those characteristics, as importance and priority may differ between users.

Dulse is found in cold waters throughout the northern hemisphere, with an optimum temperature of 6°C to 15°C (Bak, 2014). It grows to a maximum depth of 20 m in the lower intertidal and shallow subtidal zones and is most abundant in semi-exposed areas with moderate to strong water currents.

The biomass of *P. palmata* in Norway is still poorly understood and information are patchy. The Ocean Biodiversity Information System (OBIS) (https://obis.org/taxon/145771) and the Global Biodiversity Information Facility (GBIF) (https://www.gbif.org/search?q=palmaria%20palmata) contains valuable databases, which show its occurrence (Figure 2). In the last ten years, several permits for cultivation of seaweeds in the field, some as integrated multi-trophic aquaculture (IMTA) systems (Pereira and Yarish, 2008). Between 2014 and 2016, the surface allocated to seaweed cultivation more than tripled, reaching a total of approximately 277 ha along the entire Norwegian coast, corresponding to a production potential of approximately 16,000 t. (as estimated from the total maximum production by applicants on each cultivation sites). This potential far outweighs the actual production output, as most of the activity were carried out as start-up. In fact, only 10 of the 16 cultivation permit holders were active in 2015. Although licenses for a variety of species have been granted, none of these are currently cultivated at sea. The availability of shores for harvesting seaweed is highly dependent on location. Because dulse thrives in exposed areas, getting to the more productive spots often necessitates the use of a boat. The biomass is also typically found only in the subtidal zone, making harvesting difficult without the assistance of a diver. *P. palmata* is often present as an epiphyte of *Laminaria digitata*, *Laminaria hyperborea*, or *Fucus serratus*, and thrives in the subtidal zone between 1 and 10 m depth, in nutrient rich exposed areas, where it can reach up to 2 kg/m^2.

Figure 2. Occurrence of *Palmaria palmata*, maps from the Ocean Biodiversity Information System web site (https://obis.org/taxon/145771).

In Iceland, the largest biomass of *P. palmata* is found at the South-West and West coast. The substrate is composed of boulders and rocky surfaces which make a perfect substrate for dulse holdfast to attach. In general, dulse resources in Iceland are abundant and spread and the major limitation factor for harvesting is the accessibility of the sites. It has never been commercially grown, but there are sparse initiatives in collecting wild dulse, which are harvested in a very small scale and mostly for local markets.

The mapping on *P. palmata* in the Faroe Islands and in Greenland is still an ongoing activity.

Over the last 15 years, there has been a lot of interest in combining GIS (geographic information system) and multicriteria decision analysis.

Table 1. List of criteria for mapping *Palmaria palmata*.

Category	**Element**	**Comment**
Abiotic factors		
	Wind exposure	Critical element for harvesting seaweed. Specific requirements are introduced, e.g. the use of an adequate boat.
	Co-occurrence of other seaweed species	A high density of other species can affect harvesting efficiency, both for pure collection and by preventing access to the desired spot, especially in shallow waters.
	Seabed depth	For example, shallow waters and/or the slope of the sea floor can provide an advantage when harvesting.
	Tide level	Tidal level knowledge is necessary for site accessibility.
Biotic factors		
	Blade size	To provide the best quality product for the current uses, individuals need to be young and medium size (e.g. most appealing product to customers).
	Appearance	Costumers are attracted by bright and health looking colours and shapes.
	Texture	Harvested individuals should not be too thick, so the product has a good texture.
	Fouling	The presence of epiphytes has a negative impact on the commercial value of seaweed. It is critical to avoid cleaning the harvested individuals (time-consuming and costly). Such procedures may not result in a high-quality product in any case.
	Presence of anthropogenic activities	To make sure there are no high concentrations of chemicals taken up by the individuals, harvesting spots should be far from other anthropogenic activities, e.g. factory or discharge.
Site features		
	Distance to roads	When evaluating harvesting sites, the presence of roads that can facilitate access to the location is regarded as a positive factor.
	Distance to piers	The presence of piers is regarded as positive, as most of the seaweed harvesting is carried out by boats. Although manual collection is also possible.
	Distance from the company production site	This is important in term of direct costs.
	Distance from marketplaces	Due to direct relationship between product quality and transport time, the presence of places selling fresh product and/or transporting harvested individuals is relevant for the classification of a harvesting site.

To localise suitable harvesting locations, a GIS based Multicriteria Evaluation (GIS-MCE) has been chosen as the favourite approach in this project (Malczewski 2006). In brief, the GIS-MCE method can transform and combine geographical data and value judgments to produce appropriate and useful decision-making information. The set of criteria reported in Table 1 are therefore proposed as the mapping's foundation, as they play a role in determining the best harvesting spots.

Elements reported in Table 1 were used to formulate a questionnaire, which was addressed to experts and the collected data were used to generate the weights of each criteria affecting the harvesting possibility through an Analytical Hierarchy Process (AHP) (Saaty 1994). Results from the AHP method showed that the most important criterium when selecting a harvesting spot is the presence of anthropogenic activities nearby, which contributes to 22.3% of the overall weight. This exercise was done considering expertise from all the four countries. The combination of data collected for each country may then be imported into GIS, which will create a map. The resulting map can be a density map in which the colour varies based on the number of criteria found in a certain location and their relative importance.

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

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

There is currently no cultivation of *P. palmata* in Greenland, although wild stocks exist. These wild stocks have only been identified in limited locations close to an urbanised harbour of the capital Nuuk. In this first year of the project, the first attempt to growth *P. palmata* in the fjord (Kobbefjord) was carried out. Specimens were collected twice in May and July 2022, for two separate growing trials, with several plants placed into each of seven opaque plastic containers that served as experimental cultivation units (ECU) (Figure 3). Each month the ECU were collected from the field and weighted. An average increase in mass between July and August was observed, although this was quite variable amongst ECU. Subsequently the combined weight of *P. palmata* in every ECU began to decrease until the experiment was terminated in October when heavy snow began. Along with this reduction in mass, the plants themselves began to lose colour. The results do indicate the importance of timing with growth only achieved until August and the greatest observed increase from May to June. Damages to the ECU meant that data from June to July were missing.

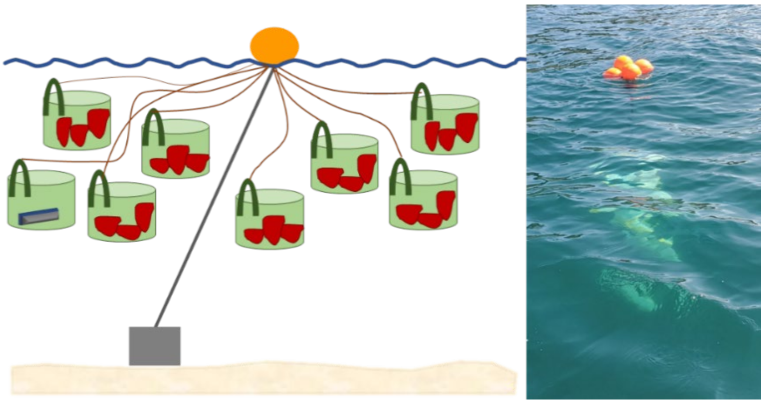

Figure 3. Experimental cultivation units and design of field deployment.

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. The first growth and cultivation experiments were carried out during spring and summer 2022 at TARI's hatchery facility in Fámjin, Faroe Islands with promising preliminary results. Fertile and vegetative individuals of *P. palmata* were collected in Fámjin, Suðuroy in May 2022 and two experiments were carried out: 1) vegetative on-growth, 2) spore release and seeding of rope.

Vegetative on-growth: 1000g wet weight of vegetative *P. palmata* was cut into smaller pieces and put into a tank with flow through seawater, bobbling and 16:8 light:dark cycles. After 19 days, the *P. palmata* biomass was harvested and the total wet weight was recorded. Again, *P. palmata* individuals were cut into smaller pieces and the biomass was divided into two and 732g of *P. palmata* biomass was put into new tanks with the same conditions. After additional 16 days, the total biomass was harvested, and the total wet weight recorded. *P. palmata* increased 464 g in biomass after the first 19 days, and 142 g in biomass after the subsequent 16 days.

Spore release and seeding of rope: Approximately 1000g wet weight of fertile (tetraspore) blades of *P. palmata* were cleaned and dried, in cool room temperature, for about 20 hrs. The dried fertile material was put into a tank with seawater without flow through for rehydration and spore release. After 4 days aggregates of spores were visible on the bottom of the tank. After 5 days, a polysteel rope coiled around cylinders was put on the bottom of the tank with the sporulating material. Seven days after rehydration and initiation of spore release the seeding of the rope was completed.

The cylinders with the seeded rope were placed vertically in another tank with circulating filtered seawater, 16:12 L:D and air bobbling. Another 200 m of rope was put on the bottom of the tank with the sporulating material for a second round of seeding with the same sporulating material. The density of the developing *P. palmata* individuals was observed to see if there was a difference in seeding efficiency between first and second seeding. The first germinating spores were observed on the seeded rope 6 days after seeding. The germinating spores developed into thalli of approx. 1cm in size over a three-month period (Figure 7). The average density of growing *P. palmata* individuals was 17 individuals per 10 cm of rope in the first seeding and 5 individuals per 10 cm of rope in the second seeding. Preliminary data show a relatively large fluctuation in nutrient concentration. Both nitrate and phosphate concentrations fluctuate rapidly over time.

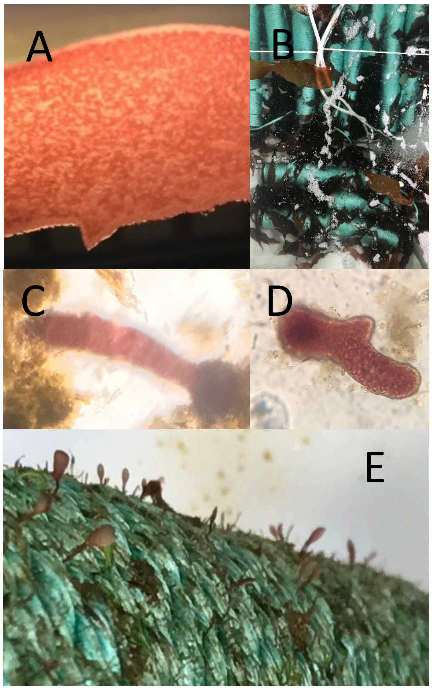

Figure 4. The cultivation process.
A. Fertile P. palmata (tetraspores)
B. Seeding of rope with tetraspores
C. Germinating tetraspore
D. Further growth and development
E. Macroscopic thalli on rope.

Evaluation of growth using apical meristems from different populations

The aim of this experiment was to use the meristematic cells of sporophytes to obtain fragments capable of growing to commercially viable sizes. These cells are mainly located at the apex of the sporophyte blades, where they are responsible for most of the growth (Titlyanov et al., 2006). Such alternative sources of cultivation material can represent a valuable opportunity in developing year-round production of dulse.

Specimen of *P. palmata* sporophytes were collected from two populations, in Southwestern Norway and Faroe Islands, to carry out the experiment. Fragments averaging 1.5 mm^2 in size were cut from the apical meristems and cultivated in F/2 medium, 50 $\mu mol.m^{-2}.s^{-1}$, 12h:12h light:dark cycle at 10ºC for 14 weeks in incubators (Figure 5). From week 4, ~20 mg of fragments was removed from each replicate on even weeks. The total protein content (Lowry assay) and antioxidant activity (Hydroxyl Radical Scavenging activity) of these fragments was measured and compared to values in the mother plants.

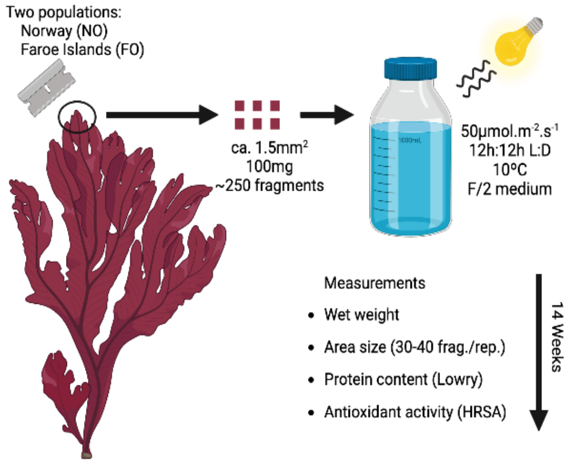

Figure 5. Scheme representing the experimental design.

Results from wet weight measurements showed strong growth, with all replicates reaching over 4.750 g after 14 weeks. Similar growth was observed for both populations, around 5%.day^{-1}, similar to growth rates recorded in juvenile sporophytes (Pang & Lüning, 2004).

Area growth was also similar during the first 10 weeks, but Faroese fragments (FF) grew significantly more than Norwegian fragments (NF) during the last 4 weeks.

This led to an average growth rate of 4.9%.day^{-1} (Norway pop.) against 6.8%.day^{-1} (Faroe pop.) over the whole growth period. FF also reached larger sizes, with the largest fragment measured at 542 mm^2, against 17 9mm^2 for NF. These results suggest that NF grew in thickness more so than in area during the last few weeks.

This may be explained by morphological differences (Figure 6). In general, fragments grew quickly and reached large sizes after 14 weeks in standard cultivation conditions and could represent viable substitutes for sporelings in establishing *P. palmata* cultures. We also suggest lowering nutrient concentrations by 2-10x to reduce epiphytic growth for future research.

Total protein content fluctuated during the experiment, with a marked decrease during the first 8-10 weeks (~4.3% wet weigth) compared to initial values (6% wet weight). During the last 4 weeks, total protein content increased again to reach 90% of original values in FF and up to 120% of original values in NF.

Neither the cultivation method nor the presence of epiphytes seemed to negatively affect protein concentrations in the long term, reinforcing the potential of meristematic fragment cultivation to yield high-quality product. Our results were also in line with protein contents measured for similar extraction method (Mæhre et al., 2016). We also hypothesised that older, thicker tissue may hold more protein than thin, growing tissue. This would explain both the initial decline in meristematic fragments and the higher final protein content in NF compared to FF. Antioxidant activity was different between populations in original plants, but identical in fragments at all time points. Maximum activity was observed between weeks 4-10, with 50-75% of radicals scavenged, which could be linked to either wound healing or growth. Even lower values (30-45% scavenged) obtained in later weeks represent a significant activity and a strong added value to *P. palmata* products.

Figure 6. Morphology and growth of fragments after 7 weeks. Arrows point at growth areas.

5. Conclusions

- The GIS-MCE method is now ready for validation in the cases of Faeroe Island and Greenland, and it is regarded as a useful tool for future *P. palmata* harvesting activity.

- The first experiment carried out in Greenland demonstrated the viability of small-scale cultivation of *P. palmata*, with indications of significant growth, the results were highly variable. The results do highlight the significance of timing, with growth only occurring until August and the greatest observed increase occurring from May to June. As a result, it will be necessary to repeat this experiment with further refinements, as well as to have a complete data set for the first half of the season.

- The first experiment conducted in the Faeroe Islands are promising, and basic knowledge on how to cultivate dulse in this country is available. During the next project year, there will be a continuation of the ongoing experiments and measurements, with the goal of deploying the first *P. palmata*-seeded ropes by autumn/winter.

- Overall, the experiment comparing two populations of *P. palmata* yielded convincing results, paving the way for further research into meristematic fragments as primary sources of material for year-round dulse cultivation. Longer, larger-scale experiments will aid in determining the maximum growth potential of these fragments as well as the viability of this technique for use in sea-based cultivation.

6. Acknowledgments

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7. References

Bak, Urd Grandorf. "Suitable cultivation areas for Palmaria palmata (Rhodophyta) and Saccharina latissima (Phaeophyceae) in the inner Danish waters in relation to variations in light, temperature and salinity. Roskilde University." Roskilde University (2014).

Mæhre, H. K., Jensen, I.-J., & Eilertsen, K.-E. (2016). Enzymatic Pre-Treatment Increases the Protein Bioaccessibility and Extractability in Dulse (Palmaria palmata). Marine Drugs, 14(11), Article 11. https://doi.org/10.3390/md14110196

Malczewski, Jacek. 2006. 'GIS-based multicriteria decision analysis: a survey of the literature', International journal of geographical information science, 20: 703-26.

Pang, S. J., & Lüning, K. (2004). Tank cultivation of the red alga Palmaria palmata: Effects of intermittent light on growth rate, yield and growth kinetics. Journal of Applied Phycology, 16(2), 93–99. https://doi.org/10.1023/B:JAPH.0000044779.30182.d8

Pereira, R., Yarish, C. (2008) Mass production of marine microalgae. In Earth system and environmental science, Encyclopedia of Ecology, 2236-2247.

Saaty, Thomas L. 1994. 'Highlights and critical points in the theory and application of the analytic hierarchy process', European journal of operational research, 74: 426-47.

Titlyanov, E. A., Titlyanova, T. V., Kadel, P., & Lüning, K. (2006). New methods of obtaining plantlets and tetraspores from fragments and cell aggregates of meristematic and submeristematic tissue of the red alga Palmaria palmata. Journal of Experimental Marine Biology and Ecology, 339(1), 55–64. https://doi.org/10.1016/j.jembe.2006.07.014