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ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

Curricular Internship at Seaweedland

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Internship Report for obtaining the Master's Degree in Aquaculture

Internship carried out under the supervision of Imke Meyer and guidance of Specialist in Aquaculture Teresa Baptista

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Abstract

This internship focused on the cultivation, monitoring, and experimental optimization of macroalgae species, namely *Ulva* spp., *Palmaria palmata*, and *Gracilaria* spp., in land-based aquaculture systems in the Netherlands. Activities were centered on raceways and large-capacity tanks, where both production and research tasks were conducted.

The internship has included several trials to test variables such as temperature, light regimes, biomass densities, and nutrient formulations. Based on these results, cultivation protocols were scaled up from laboratory to production systems. Weekly biomass sampling, macroscopic quality assessments, and growth rate analyses (DGR%) were performed, with efforts made to maintain ideal cultivation densities and harvest the surplus biomass for drying and commercialization.

Key contributions during the internship included the development and application of customized nutrient solutions; the management of environmental challenges such as seasonal variations in light and temperature, which triggered events like *Ulva* sporulation or growth decline in *Palmaria palmata*. These challenges were addressed through strategies like artificial lighting, temperature regulation, and customized fertilization schedules. Contamination control protocols were implemented, involving the selection and cleaning of *Gracilaria* spp., removal of epiphytes, and incubation under controlled conditions. Additional activities included participation in innovative seeding techniques such as direct seeding of *Saccharina latissima*, as well as tagging trials for monitoring the growth of *Palmaria palmata* individuals. The technical infrastructure was also a focus, with the installation and maintenance of filtration systems, aeration equipment, light setups, and environmental sensors for monitoring pH, salinity, turbidity, and temperature.

To conclude, this internship was fundamental to deep my understanding of phycology and in developing practical skills related to the design, management, and optimization of land-based cultivation systems specifically adapted for macroalgae production.

Resumo

Este estágio focou-se no cultivo, monitorização e otimização experimental de espécies de macroalgas, nomeadamente *Ulva* spp., *Palmaria palmata* e *Gracilaria* spp., em sistemas de aquacultura terrestre nos Países Baixos. As atividades centraram-se em *raceways* e tanques de maior capacidade, onde foram realizadas tanto tarefas de produção como de investigação.

O estágio incluiu vários ensaios para testar variáveis como temperatura, regimes de luz, densidade de biomassa e formulações de nutrientes. Com base nos resultados obtidos, os protocolos de cultivo foram escalados do laboratório para sistemas de produção. Realizou-se amostragem semanal de biomassa, avaliações macroscópicas da qualidade e análises da taxa de crescimento diário (*DGR*%), com esforços para manter densidades ideais de cultivo, recolhendo o excedente de biomassa para secagem e comercialização.

As principais contribuições durante o estágio incluíram o desenvolvimento e aplicação de soluções nutritivas personalizadas; a gestão de desafios ambientais, como variações sazonais de luz e temperatura, que desencadearam eventos como a esporulação de *Ulva* ou a diminuição do crescimento em *Palmaria palmata*. Estes desafios foram enfrentados com estratégias como iluminação artificial, regulação da temperatura e esquemas de fertilização adaptados. Foram implementados protocolos de controlo de contaminações, incluindo a seleção e limpeza de *Gracilaria* spp., remoção de epífitas e incubação em condições controladas.

Outras atividades incluíram a participação em técnicas inoculação, como *direct seeding* de *Saccharina latissima*, bem como ensaios de marcação para monitorização do crescimento de indivíduos de *Palmaria palmata*. A infraestrutura técnica também foi alvo de atenção, com a instalação e manutenção de sistemas de filtração, equipamentos de arejamento, sistemas de iluminação e sensores ambientais para monitorização de pH, salinidade, turbidez e temperatura.

Para concluir, este estágio foi fundamental para aprofundar o meu conhecimento em ficologia e para o desenvolvimento de competências práticas relacionadas com o design, gestão e otimização de sistemas de cultivo terrestre adaptados especificamente à produção de macroalgas.

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1. Introduction

According to data from the United Nations (2024), the world population is expected to reach 8.5 billion by 2030. This increase implies a growing demand for nutritional resources necessary to ensure human subsistence. The global nutritional disparity is evident, with over 820 million people facing food insecurity, while others consume diets deficient in nutrients but rich in sugar, saturated fats, and salt, factors that contribute to rising obesity rates and related health problems (Silva *et al.,* 2024). This food gap and disparity is likely expanded due to climate change, escalating freshwater demands, and diminishing availability of arable land (Hofmann *et al.,* 2024). Thus, achieving global food security, protecting public health, and promoting environmental sustainability are among the significant challenges posed by the contemporary food system (Silva *et al.,* 2024).

Recently, there has been a noticeable increase in environmental awareness, which has contributed to the rising popularity of vegetarianism and veganism, which have increased demand for organic products and alternative protein sources (Hofmann *et al.,* 2024). The consumption of animal-based products has been criticized due their intensive land use requirements and associations with chronic diseases (Silva *et al.,* 2024).

The ocean, therefore, is expected to play a crucial role in human nutrition. However, marine resources, including marine algae, are under increasing pressure. Overexploitation for farming purposes, along with pests, diseases, and the effects of climate change, has led to a global decline in algae stocks worldwide (FAO, 2024). The intensifying pressure on these resources necessitates sustainable management practices that incorporate environmental, social, and economic awareness (Bond & Morrison-Saunders, 2011). Transitioning from wild harvesting to aquaculture represents a key opportunity to reduce the ecological impact of seaweed extraction and enhance the sustainability of algal production (Burrows *et al.,* 2018; Araújo *et al.,* 2021). Algae-based aquaculture could help meet global protein demands in the coming decades, offering a nutritionally rich food source abundant in essential amino acids and vitamins (Greene *et al.,* 2022).

Algae comprise a highly diverse group of photosynthetic organisms found in a diverse array of life forms. They inhabit various freshwater and marine environments - including rivers, lakes, estuaries, seas, oceans, ponds, and marshes (Ruggiero *et al.,* 2015). Algae play a crucial ecological role in ecosystems, contributing to primary production and delivering key ecosystem services such as food provision, water quality regulation, and biological control (Kuech *et al.,* 2023). They can grow on both soft and hard substrates, from rocky intertidal zones to depths of several hundreds of meters (Spalding *et al.,* 2019). During photosynthesis, algae absorb and sequester atmospheric carbon, positioning them as potential contributors to blue carbon (a term used to describe carbon stored by marine ecosystems) (Krause-Jensen *et al.,* 2018).

2. Theoretical framework

2.1. Global algae aquaculture

In 2022, global aquaculture production, including all cultivated aquatic organisms, reached a record high of 130 million tonnes, representing an increase of over 6% compared to 2020 (FAO, 2024).

Algae production has expanded significantly over the past two decades, more than tripling since 2000. In 2019, wild algae harvested totaled approximately 1.1 million tonnes, while aquaculture production reached 34 million tonnes (FAO, 2021). By 2022, total algae production has risen to 36.5 million tonnes, valued at €15 billion (FAO, 2024). These achievements highlight algae´s growing economic and nutritional relevance. The expansion of algae aquaculture not only benefits global food supply but also represents a key sector in the transition toward more sustainable aquaculture practices. Continued growth of algae production and processing is expected, with the potential to generate sustainable employment and foster small-scale industries, particularly in coastal communities of developing countries (Mirera *et al.,* 2020).

Today, algae are cultivated in over 56 countries, with 99% of global production concentrated in Asia (FAO, 2024; Cottier-Cook *et al.,* 2023). China leads the industry, accounting for 57% of global production, followed by Indonesia (27%) and South Korea (5%) (Figure 1) (Junning & Giulia, 2021).

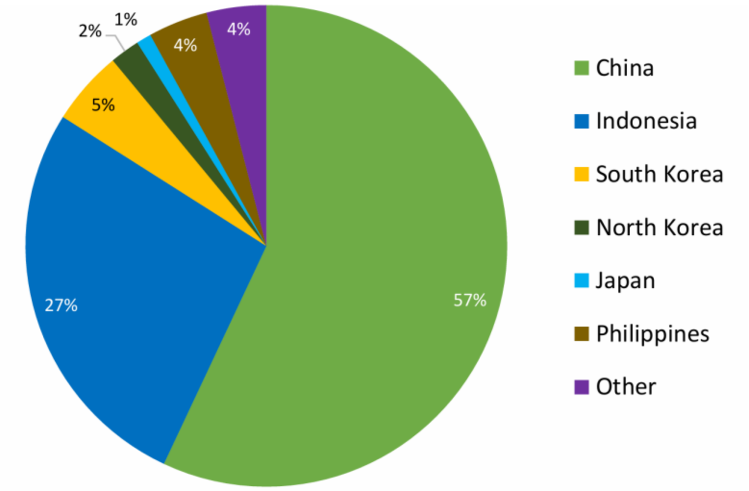

Figure 1 - Global algae production by main producing countries in 2019. (Source: Junning & Giulia, Global seaweeds and microalgae production, FAO 2021).

Although Europe accounts for only 1% of global algae production, the region has witnessed a significant increase in algae-related start-ups, which are actively driving innovation and commercialization within the sector (Figure 2) (Kuech *et al.,* 2023).

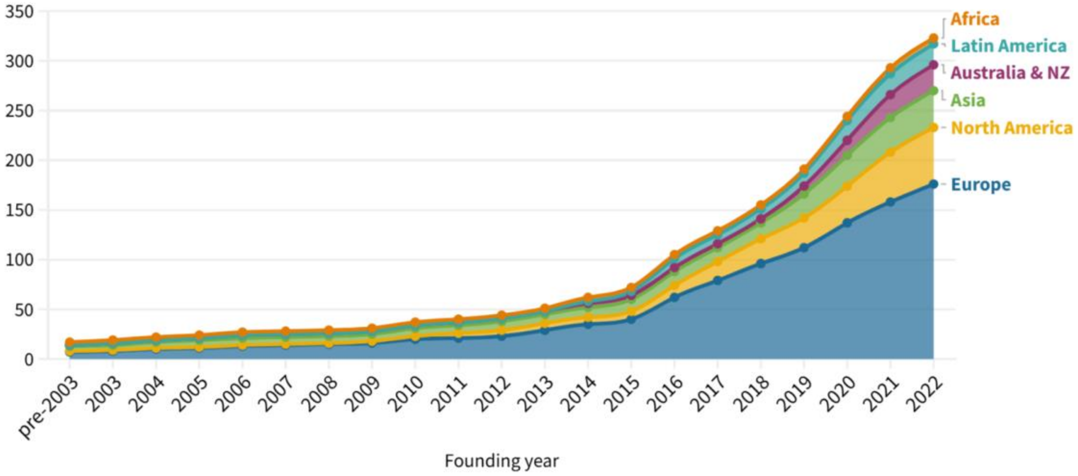

Figure 2 - Global development of algae start-ups by founding year, 2003-2022 (Source: Phyconomy, 2022).

Countries with underdeveloped algae sectors are increasingly implementing strategic measures to enhance production and strengthen their competitiveness in the global market. In the United States, legislative and policy initiatives such as the proposed SEAfood Act and the US National Aquaculture Development Plan aim to stimulate sector growth, particularly in shellfish and seaweed cultivation. These initiatives seek to promote sustainable aquaculture practices by investing in research and development, providing funding for pilot projects, and streamlining regulatory processes (Aquaculture North America, n.d.).

Among cultivated species, Japanese kelp (*Laminaria japonica*) is the most extensively produced, with a reported production of 12 million tonnes in 2019. It is followed by *Eucheuma* spp. at 9.8 million tonnes and *Gracilaria* spp. with 3.6 million tonnes (Figure 3) (FAO, 2021). In Europe, *Laminaria* spp. and *Saccharina latissima* are the main cultivated species (Schiener *et al.,* 2015).

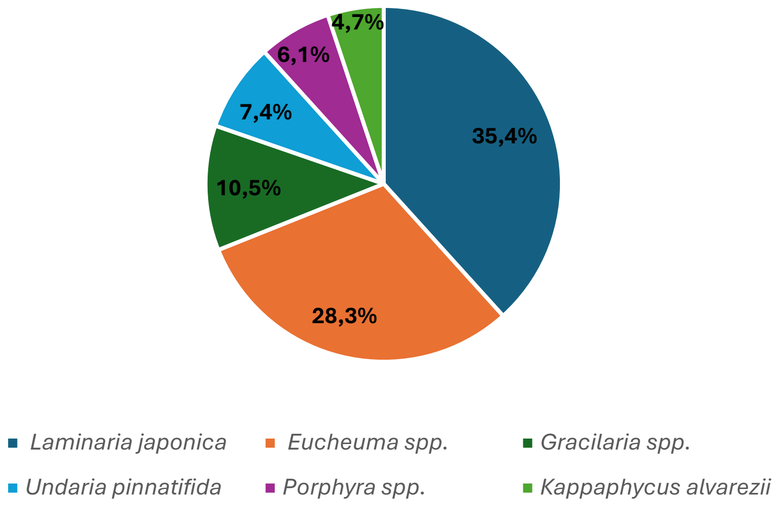

Figure 3 - Species and percentage composition of macroalgal cultivation in 2019 (Source: FAO, 2021).

2.1.1. Challenges of upscaling the macroalgae industry

Scaling up the macroalgae industry presents a range of challenges linked to plant-specific characteristics, economic feasibility, technological limitations, logistics, regulatory barriers, and licensing frameworks.

Limited market demand

The growth in global demand must be accompanied by an expansion in macroalgae production. A significant portion of seaweed is sold as food, generating higher income for seaweed farmers. However, outside of East Asia, consumer exposure to seaweed as a dietary component remains limited. Although some countries outside Asia have begun incorporating seaweed into a broader range of food products. Despite promotional campaigns emphasizing its nutritional benefits, uptake remains slow (van der Werf *et al.,* 2019). Concerns contaminants such as heavy metals, arsenic, and the risk of excessive iodine intake, may further constrain market expansion (Bouga & Combet, 2015).

Industry Fragmentation Outside Asia

In regions beyond Asia, the seaweed industry is still in its early stages, often characterized by fragmented and regionally isolated operations. Initiatives are frequently perceived as disconnected, with limited sharing of best practices. Additionally, pioneering companies frequently face competitive tensions, which can hinder collective progress and innovation (Doumeizel *et al.,* 2020).

Low seedling quality

High-quality seedling production is vital for sustainable and prosperous seaweed aquaculture. Understanding the life cycle of multiple species is critical to develop reliable propagation methods (McHugh, 2003). Techniques, such as hybridization of kelp seedlings, have enabled cultivation in warmer waters and improved yields (Hu *et al.,* 2021). Rising seawater temperatures have made it difficult to rely on natural environments for consistent and high-quality seed stock. As a result, there is a growing dependence on specialized hatcheries that can provide controlled conditions for seed production. While advanced techniques such as selective breeding, strain selection, micropropagation, genetic markers, and hybridization can enhance seed quality, they are technically complex and costly, often requiring public sector support (Cai *et al.,* 2021).

Technological barriers

Despite investments in technology, modeling and automation, further investment is needed to address technological challenges. Species diversity, inter-strain variation, and seasonal fluctuations complicate supply chain optimization and biomass processing (Villares *et al.,* 2013). Real-time monitoring using sensors is essential to assess chemical composition in real time and determine the optimal harvest periods, thereby ensuring consistent quality and economic viability (van der Werf, 2018).

Barriers to integrated farming

Integrating seaweed cultivation with other aquaculture species, such as finfish, faces economic, technical, and institutional obstacles. Finfish farmers may lack the expertise to implement seaweed systems or navigate distinct value chains. Furthermore, commercializing multiple products across different value chains presents significant economic challenges. The technical requirements of integrated aquaculture can increase operating costs, and low-value seaweed species often fail to offer adequate financial incentive to offset increased operational complexity and costs (Cai *et al.,* 2021).

Licensing and regulatory constraints

Marine licensing remains a major obstacle to establishing seaweed farms. Integrating seaweed into marine spatial planning through designated zones and a legislative framework, could facilitate smoother licensing processes (Doumeizel *et al.,* 2020). According to the same authors, the lack of uniform, tailor-made policies and regulations is a major obstacle to scaling the seaweed industry.

Limited awareness of potential applications

The broader potential of macroalgae remains underexplored. For example, seaweed extracts have demonstrated the ability to enhance crop tolerance to salinity, drought, and cold (van der Werf, 2018). However, comprehensive *in vivo* studies are needed to fully evaluate the bioactivity of specific compound. Such research is critical to drawing definitive conclusions about the additional benefits of seaweed extracts and validate additional commercial uses.

2.2. Macroalgae

Photoautotrophic organisms represent a highly diverse group of photosynthetic organisms varying in size, form, and cellular complexity - from microscopic unicellular algae (<3 µm) to large multicellular forms such as kelp exceeding 50 meters in length (Kuech *et al.,* 2023). This diversity includes both prokaryotic and eukaryotic taxa, comprising unicellular or multicellular organisms. In marine ecosystems, macroalgae serve as primary producers at the base of the marine food web, supporting a wide range of aquatic species (Pereira, 2021).

Light availability and the corresponding composition of photosynthetic pigments are key factors shaping macroalgal distribution and classification (Bastos, 2019). Based on pigment profiles, macroalgae are categorized into three major groups: Chlorophyta (green algae), Rhodophyta (red algae), and Ochrophyta with the class Phaeophyceae to brown algae.

Chlorophytes derive their green color from chlorophyll *a* and *b*. These species generally require high light intensity and are predominantly found in shallow, intertidal zones (Barsanti & Gualtieri, 2022; Janeeshma *et al.,* 2022).

The characteristic red color of many Rhodophyta species is primarily due to the presence of phycobiliproteins such as phycoerythrin and phycocyanin, along with chlorophyll a, β-carotene, and xanthophylls in some classes (Che *et al.,* 2022; Dagnino-Leone *et al.,* 2022; Glazer, 1994).

The Phaeophyceae species contain the carotenoid fucoxanthin, that is the primary pigment responsible for brown coloration, along with chlorophylls a, c1 and c2, in addition to carotenoids. Fucoxanthin is notable for its antioxidant properties and has demonstrated potential in inhibiting GOTO neuroblastoma and colon cancer cells (Barsanti & Gualtieri, 2022).

Morphology

The body of macroalgae, known as the thallus, lacks true roots or a vascular structure. The thallus consists of a frond, whose shape and size can vary significantly, and an attachment organ. These morphological variations are often influenced by environmental conditions, used in taxonomic classification (Figure 4) (Pereira, 2021).

Thallus growth can occur via either diffuse or localized mechanisms. In diffuse growth, expansion takes place uniformly in all directions along the thallus. In contrast, localized growth can be further classified as apical or basal (Krumhansl *et al.,* 2015).

Apical growth occurs when new tissues are added to the tips of the blades, promoting the elongation. On the other hand, basal growth takes place in the basal meristem, the region responsible for regeneration and continuous structural development (Krumhansl *et al.,* 2015).

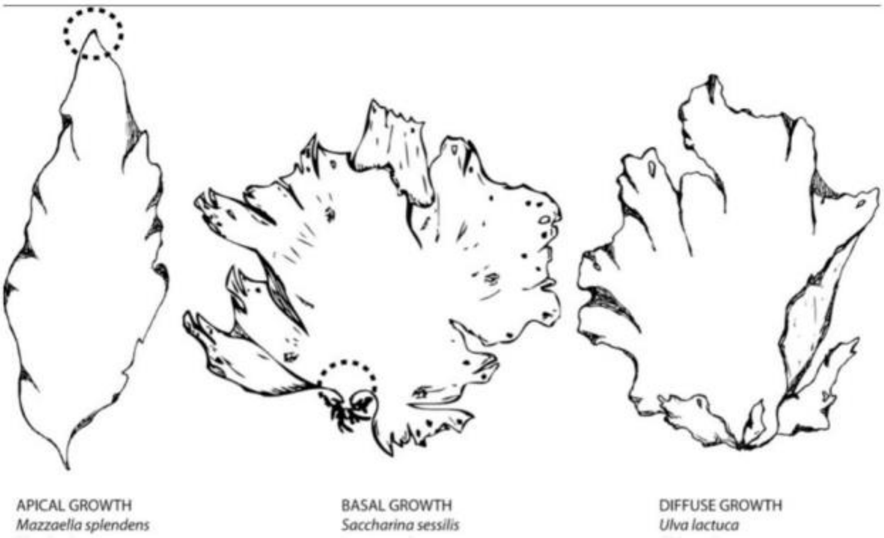

Figure 4 - Schematic illustration of apical growth in a Rhodophyta, basal growth in a Phaeophyceae, and diffuse growth in a Chlorophyta. Dotted circle represents growth areas. Source: Krumhansl *et al.,* (2015).

Reproduction

Successful seaweed domestication relies on a thorough understanding of reproductive biology, alongside ecological and physiological factors (Balar & Mantri, 2020). However, fully closing the life cycle of seaweeds remains challenging due to their complex and often poorly understood reproductive strategies.

Macroalgae reproduce both asexually - without fertilization and through spore production via mitosis - and sexually - via gamete fusion. Many species employ both reproductive strategies during their life cycle (Pereira, 2021). Effective seaweed aquaculture requires propagation methods that reliably generate high-quality propagules. These methods are categorized into vegetative propagation, a type of asexual reproduction based on thallus fragmentation or propagule development, and sexual reproduction, which involves the formation and fusion of gametes, resulting in genetically diverse offspring (Moreira et al., 2022).

Vegetative propagation is a widely used, cost-effective technique in which segments of vegetative tissue are used to produce new individuals (Ask & Azanza, 2002). Typically, younger, healthier branches of the thallus are selected, cut into fragments, and cultivated (Hurtado *et al.,* 2015). However, this approach has limitations: it is labor-intensive on a large scale, it results in clonal propagation, which reduces genetic variability that can impair resilience to environmental stress and disease (Goecke *et al.,* 2020).

Sexual reproduction involves meiosis and gamete fusion, leading to fertilization. The process begins with gametogenesis, culminating in the formation of a zygote, which develops into a new individual. This pathway introduces genetic diversity into populations (Pereira, 2021). Most macroalgae follow a haplodiplontic life cycle, alternating between a haploid gametophyte and a diploid sporophyte (Liu *et al.,* 2017). Haplodiplontic life cycle generally involves the induction of sporulation, leading to the production of male and female gametophytes, fertilization of gametes and development of a diploid zygote, which develops into a sporophyte, completing the cycle (Redmond *et al.,* 2014).

Although sexual reproduction requires a greater expertise and precise cultivation conditions (gamete release, fertilization, and early-stage development), it offers several advantages over vegetative propagation. These include the maintenance of genetic variability, which enhances crop resilience to diseases and environmental changes, the ability to crossbreed strains to improve desirable traits, and the facilitation of large-scale seeding, thereby improving production efficiency (Goecke *et al.,* 2020).

Life Cycle complexity in seaweeds

Seaweeds exhibit alternating genetic stages throughout their life cycle. Specifically, some Rhodophyta (red algae) have a triphasic life cycle, involving three distinct stages: a gametophyte, a carposporophyte (formed after fertilization and dependent on the female gametophyte), and a tetrasporophyte (Balar & Mantri, 2020). This reproductive complexity presents challenges for producers, who aim to genetically develop seaweeds, through breeding programs (FAO, 2024).

There are three main life cycle forms observed in algae (De Bettignies *et al.,* 2018):

1. Haplontic monophasic – Characterized by haploid thalli and zygotic meiosis, this type is more common in unicellular algae and less representative in macroalgae.
2. Diplontic monophasic – Involving diploid thalli with gametic meiosis, more prevalent in brown algae.
3. Diplohaplontic biphasic or triphasic – Featuring alternation of generations between haploid and diploid phases, which is the most common life cycle in macroalgae.

Applications in Industry

Marine macroalgae are a versatile resource with a wide range of applications (Figure 5). They have attracted significant commercial and research interest due to their potential as sources of bioactive compounds and hydrocolloids extracts such as alginate, agar, and carrageenan. These hydrocolloids are widely used in the nutraceutical, pharmaceutical, cosmetic, and agricultural sector. Additionally, they play a role in sustainable food production (FAO, 2024; Silva *et al.,* 2024; Roohinejad *et al.,* 2017).

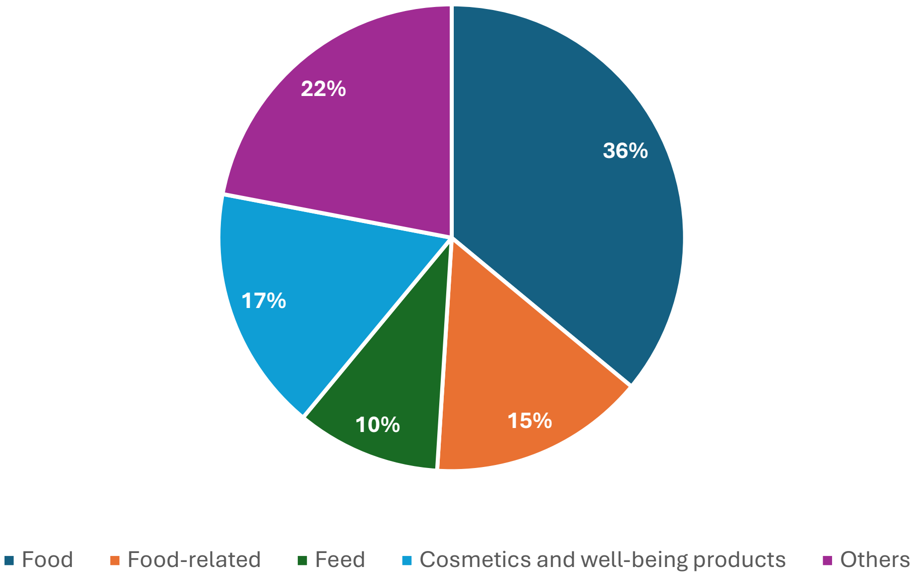

Figure 5 - Applications for macroalgal biomass in Europe. Source: Araújo *et al.,* (2021).

Hydrocolloids interact with water to form colloidal systems, either as gels or sols composed of solubilized particles (Rhein-Knudsen *et al.,* 2015). The cell walls of macroalgae are particularly rich in these polysaccharides, depending on the taxonomic group. Red algae (Rhodophyta) contain mainly carrageenan and agar, brown algae (Phaeophyceae) are known for alginates and fucans, while green algae (Chlorophyta) produce *Ulva*ns (Synytsya *et al.,* 2015). These compounds exhibit notable thickening, emulsifying, and stabilizing properties, which have been exploited across various industries:

- Carrageenan, extracted from red seaweeds, is widely used as a gelling agent and stabilizer in meat products (Glicksman, 2019).
- Agar, also derived from red algae, is employed in the food and medical industries and is currently being explored for the development of biodegradable packaging materials (Leandro *et al.,* 2019).
- Alginates, obtained from brown seaweeds, are regarded as excellent biomaterials for advanced medical applications, including wound dressings and drug delivery systems (Mollah *et al.,* 2021).

Seaweed is also increasingly utilized in animal feed and feed additives, offering a promising alternative to conventional terrestrial feed sources and addressing several challenges within the livestock industry. Livestock production, particularly enteric methane emissions from ruminants, is a significant contributor to global greenhouse gas emissions (Roque *et al.,* 2019). Various studies have demonstrated that incorporating up to 5% of *Asparagopsis* spp. into ruminant diets can reduce enteric methane emissions by as much as 98% (Brooke *et al.,* 2018; Muñoz-Tamayo *et al.,* 2021; Roque *et al.,* 2019). This potent anti-methanogenic effect of *Asparagopsis* is driving increased demand for red seaweed species.

Seaweed based fertilizers present an eco-friendly alternative to fossil fuel-derived fertilizers and pesticides. Although they typically contain lower potassium levels compared to standard NPK (Nitrogen: Phosphorus: Potassium) formulations, they are enriched with a diverse array of minerals, vitamins, enzymes, and bioactive compounds unique to marine environments. These compounds function as growth stimulants, providing benefits not available in standard fertilizer products (Ertani *et al.,* 2018; Hassan *et al.,* 2021).

Beyond the previously mentioned applications, seaweed is also utilized in industries such as cosmetics, renewable energy, bioplastics, textiles and paper. For example:

- In cosmetics, seaweed is valued for its high content of vitamins, minerals, sugars, lipids, amino acids, and other bioactive compounds. Extracted components also function as thickening and water-binding agents (Ariede *et al.,* 2017).
- In renewable energy, seaweed is emerging as a sustainable resource for biofuel production. Technologies such as anaerobic digestion and hydrothermal liquefaction show considerable promise for converting seaweed into renewable fuels (Maneein *et al.,* 2018).

Despite these diverse applications, the largest use of seaweed remains in food or food-related industries, including dietary supplements, thickening agents, and preservatives (Figure 5) (Kuech *et al.,* 2023). Seaweeds contribute to human nutrition due to their exceptional nutritional profile and the bioactive properties of their extracts. These include antioxidant, anti-diabetic, anti-hypertensive, anti-obesity, antimicrobial, anti-cancer, and anti-inflammatory effects (Biris-Dorhoi *et al.,* 2020; Cabrita *et al.,* 2010). Several studies have identified macroalgae as a rich source of protein, highlighting their potential as an alternative to meat (Silva *et al.,* 2024). Given these attributes, seaweed cultivation could play a crucial role in enhancing food security, whether as a direct food source, a dietary supplement, a functional additive, or a complementary ingredient in various food products (FAO, 2024; Hofmann *et al.,* 2024).

2.3. Seaweeds for human consumption

The World Resources Institute (WRI) has identified a 56% food gap between current global food production and the projected demand by 2050 (Searchinger *et al.,* 2019). In this context, algae present a sustainable solution to enhance food and nutrition security, promote resource-efficient food systems, and generate employment opportunities in coastal communities (FAO, 2024). The global plant-based food market is steadily expanding and is expected to account for 8% of the global protein market by 2030 (Bloomberg, 2021).

Historically, seaweeds have been an important component of the human diet in East Asia and are now gaining increased popularity in countries such as China, Korea, Japan, and across much of Southeast Asia (Silva *et al.,* 2024). Various species of seaweeds have been successfully cultivated in tank systems for direct human consumption (Barsanti & Gualtieri, 2022). They provide a highly nutritious, low calorie food source, rich in vitamins, minerals, proteins, dietary fibers, and bioactive polysaccharides, compounds associated with a variety of health benefits (Leandro *et al.,* 2019; Pereira, 2016; Kilinç *et al.,* 2013). Additionally, macroalgae generally contain higher amounts of macro-minerals (Na, K, Ca, Mg) and trace elements (Fe, Zn, Mn, Cu), than those found in land plants (Rupérez, 2002).

The suitability of seaweeds as a food source depends on several factors, including species, physiological characteristics, water quality, harvesting methods and post-harvest processing techniques (FAO, 2024). However, food safety concerns remain a significant barrier to broader consumer acceptance. These concerns primarily relate to the potential presence of harmful microorganisms (e.g., *Salmonella* sp.), heavy metals (principally arsenic and cadmium), excessive iodine levels, and marine biotoxins. Such risks can negatively impact consumer perceptions of seaweed-derived foods products. To enhance consumer trust and encourage adoption, transparency and traceability in supply chain management are essential (Kuech *et al.,* 2023).

Despite growing recognition of their nutritional and health benefits, seaweed remains under-consumed in Europe, primarily due to cultural and economic factors (Silva *et al.,* 2024). Research suggests that food neophobia (the reluctance to try unfamiliar foods) significantly reduces consumer willingness to incorporate seaweeds into their diet (Silva *et al.,* 2024). This indicates that simply promoting seaweeds as healthy and sustainable option may not be enough to shift consumer behavior. Instead, a combination of public education, product innovation, and appealing culinary applications is needed to overcome psychological and cultural barriers. Policy initiatives are supporting this transition. One of the six key priorities of the von der Leyen Commission is the European Green Deal, which aims to build a resource-efficient and sustainable economy. Within this framework, the Farm to Fork Strategy specifically identifies, “seafood based on algae” as an example of “innovative food and feed products” (Kuech *et al.,* 2023).

While seaweed consumption remains uncommon in many regions (Prager, 2017), there is growing evidence to suggest that mainstream global adoption is inevitable. Rising awareness of its nutritional and environmental benefits is expected to continue driving market growth (Chapman *et al.,* 2015).

Moreover, the culinary versatility of macroalgae enhances its appeal. Seaweed can be consumed in various forms, fresh, dried, cooked, fried, flaked or powdered (Table I). This flexibility makes it easier to integrate into diverse cuisines and tailor products to suit different consumer preferences (Silva *et al.,* 2024).

Table I - Preparation techniques and recipes of edible seaweed (From: Freitas *et al.,* 2021; Moss & McSweeney, 2021; Tiitii *et al.,* 2022; Mouritsen *et al.,* 2018; Mouritsen *et al.,* 2013; Pérez-Lloréns, 2019; Chapman *et al.,* 2015).

Preparation techniques	**Recipes**
Fresh/Raw	Raw, incorporated, or consumed as salads; fresh, mixed with butter or lard, and served with potatoes and turnips; consumed with soy sauce and vinegar or marinated with salt, lemon, and vinegar; accompanying dishes of fish, meat, and stews; species such as sea grapes are consumed alongside yams, bananas, breadfruit, fish, and meat.
Cooked	Boiled or cooked in water and used in stews and soups, or as accompaniments to fish and meat; boiled and soaked in vinegar, salt, and lemon or solely vinegar and spices; cooked with milk and added to porridge; cooked together with coconut cream or boiled in coconut milk; cod cheeks in Oarweed broth, fish cakes with Oarweed, pancake with *Saccharina latissima*, haddock with seaweed, flounder with algae crust, cassoulet with algae, tagliatelle pasta with Dulse, brandade with Dulse, and vodka shot with Dulse.
Dehydrated	Consumed between two slices of buttered bread or as a side dish to fish fillets; mixed with butter or lard and served with potatoes and turnips; used in the preparation of teas; nori sheets and sea lettuce are used to make sushi, temaki; dried Dulse consumed as a type of snack or savory.
Flaked	Sprinkled on salads and vegetables; scattered over rice, pasta, or other foods as a substitute for herb salt, as is the case with *Pyropia columbina*; fish pie with *Saccharina latissima* flakes; soups, stews, and casseroles.
Powdered form	Used in the preparation of bread, butter, pies, pizzas, pâtés, hamburgers, vegetables, seasonings, cheeses, pastries, yogurts, and processed meats; preparation of jams; *Osmundea pinnatifida* employed as a seasoning due to its curry-like and peppery flavor; camembert infused with Oarweed and chocolate to create umami algae.
Toasted/Baked/Fried	Toasted dulse serves as a savory snack to complement a dark beer or an appetizer. Cooked in a small amount of oil or butter, serving as a pleasant substitute for fried bacon, for instance, in an omelet.

3. Cultivation of marine macroalgae

Macroalgae production plays a crucial role in advancing the blue bioeconomy and contributes to several United Nations Sustainable Development Goals (SDGs), such as reducing hunger, mitigating climate change, and improving health and well-being (Hofmann *et al.,* 2024). Seaweed cultivation supports ocean regeneration by reducing excess nutrients and preventing eutrophication. Additionally, it serves as a nature-based solution to climate change through carbon sequestration (Racine *et al.,* 2021).

In Europe, seaweed production remains a relatively recent activity, with a significant portion of the harvest coming from wild stocks populations along coastlines (68% of seaweed companies) (Kuech *et al.,* 2023). Most aquaculture operations (76%) are sea-based, with the remaining production conducted in land-based facilities (Araújo *et al.,* 2021).

The European seaweed market is experiencing visible growth, with an annual growth rate of 7-10% (Barbier *et al.,* 2018). Currently, around 163 companies produce seaweed across Europe, with the majority of production concentrated in the North Atlantic region, particularly in France (33 companies), Ireland (29 companies), and Spain (22 companies) (Kuech *et al.,* 2023). Despite this growth, the industry faces several challenges, including limited knowledge of the genetic and phenotypic diversity of cultivated species, underinvestment in breeding programs, and underdeveloped regulatory frameworks (Brakel *et al.,* 2021).

To ensure sustainable development, a comprehensive risk-benefit assessment is necessary (Hofmann *et al.,* 2024). Key considerations include food security in Integrated Multitrophic Aquaculture (IMTA) systems, genetic interactions between wild and cultivated species, ecosystem impacts, and risks such as disease and epiphytism (Rosa *et al.,* 2020; Troell *et al.,* 2023; Stévant *et al.,* 2017). Another concern involves the bioaccumulation of heavy metals (e.g. mercury, cadmium, copper, lead and zinc) which can pose risks to human health when seaweeds are consumed (Silva *et al.,* 2024).

Marine macroalgae can be cultivated in either inshore or offshore environments using structures such as twine ropes or nets (Stévant *et al.,* 2023). Offshore cultivation is limited by environmental factors such as strong waves and currents, which can cause detachment of algae from the substrate. Additionally, growth is highly dependent on abiotic (e.g. temperature, light intensity, salinity, and nutrient availability) and biotic stressors (e.g. epiphytism), which are often unpredictable and variable (Stévant *et al.,* 2023; Cotas *et al.,* 2020). Therefore, understanding the specific environmental requirements of each macroalgae species is crucial for successful cultivation.

In terms of species utilization, kelps (*Laminaria* sp.) are the most important for both aquaculture and wild harvesting. The most commonly cultivated species include sugar kelp (*Saccharina latissima*), winged kelp (*Alaria esculenta*), green sea lettuce (*Ulva* sp.), and dulse (*Palmaria palmata*) (Barbier *et al.,* 2018).

Seaweeds absorb excess nutrients such as nitrogen and phosphorus, as well as carbon dioxide, which they utilize for growth, protein production, and energy storage. To optimize yields, it is essential to maintain optimal salinity, nutrient levels, temperature and light intensity.

3.1.1. Classification of culture systems

Based on their location, macroalgae cultivation systems can be classified into three main categories namely, land-based, sea-based, and mixed systems (combining land- and sea-based approaches) (Pereira *et al.,* 2024).

These systems differ in how they interact with the surrounding environment. Potential interactions may involve nutrient release, impacts on wild populations, pathogen transmission, introduction of invasive species and water flow (Edwards, 2015). Consequently, aquaculture production systems are often classified as open, closed, or semi-closed (Lawson, 1994). Open systems which are fully exposed to the surrounding environment. Closed systems, which isolate farmed organisms from the surrounding environment. Semi-closed systems, which allow limited interaction with the environment and may recycle some nutrients but not fully eliminate all interactions with the surrounding environment. The choice of systems depends on the target species, environmental conditions, and production goals each offering specific advantages and limitations (Lawson, 1994).

Cultivation in open water systems

The most common method of seaweed production is conducted in open-sea coastal environments using suspended lines, rafts, or nets (Redmond *et al.,* 2014). Seaweed cultivation begins with planting materials such as spores, vegetative thalli, or cuttings, tied to or inserted into lines or ropes (Mouedden *et al.,* 2024).

Longline systems are primarily used for extractive species, e.g. blue mussels and macroalgae and can also be adapted for oyster and scallop farming (Buck *et al.,* 2017). The system consists of a main horizontal line, known as the "backbone" anchored at both ends and supported by floating elements, such as barrels or polyethylene pipes (Figure 6) (Goseberg *et al.,* 2017). Buoys are added or removed to adjust buoyancy depending on the biomass weight throughout the growth cycle. A key advantage of polyethylene tubes provides consistently high buoyancy, reducing the frequency of adjustments. Several headlines can also be attached perpendicularly to the main line and connected to vertical dropper lines where the algae grow. Submerged longline systems operate on similar principles but are anchored below the water surface to suit rougher water conditions, making them more suitable for exposed offshore sites (Buck *et al.,* 2017).

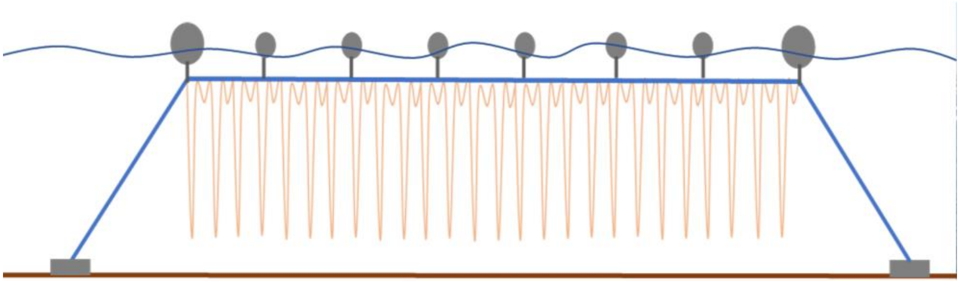

Figure 6 – Illustration of the principal structure of longline systems with a backbone rope supported by floating elements, anchors, and droppers on which the farmed organisms grow. Source: Strand & Suhnel, (2022).

Cultivation in Land-based systems

Land-based seaweed cultivation typically involves controlled environments such as indoor nursery facilities, and outdoor tanks or raceways, where the entire production cycle (from nursery to grow-out phases) takes place on land. This type of cultivation offers enhanced control over critical growth parameters such as light, temperature, salinity, and nutrient levels, making it easier to manage biomass quality and consistency.

One of the main challenges in achieving commercially viable land-based cultivation is maintaining high biomass densities and consistent growth rates while minimizing the presence of epiphytes (Stévant *et al.,* 2023). Epiphytes can significantly hinder growth and lower biomass quality, which is critical for applications in food. Land-based systems, especially those with integrated water monitoring and treatment, are better equipped to control these issues, ensuring a cleaner and higher quality yield.

Raceways

Raceways are elongated, shallow tanks designed to facilitate continuous water flow. They can be constructed from materials such as concrete, plastic, or fiberglass, or excavated directly into the ground to form a continuous, meandering pond. Raceways typically feature flat bottoms and shallow depths, making them particularly suitable for cultivating bottom dwelling organisms (Sompech *et al.,* 2012).

Most raceways operate as flow-through systems, where water enters at one end and exits at the other (Fornshell *et al.,* 2012). Water may be sourced from natural bodies such as rivers, estuaries, or coastal areas and channeled through the raceway before being discharged. Depending on how water is managed, raceways can be classified a open, semi-contained, or completely closed. Treated water may be recirculated within the system or released untreated into the environment.

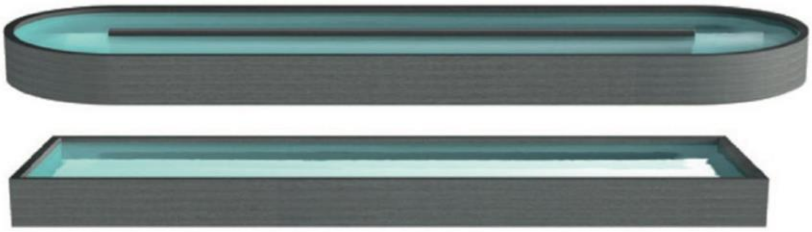

Figure 7 - Examples of raceway design. Top: a D-end system with circulating water, bottom: a linear flow raceway. Source: Strand & Suhnel, (2022).

Mixed cultivation (land- and sea-based)

Both land-based and sea-based macroalgae cultivation systems offer unique advantages and face specific challenges (Table II). A hybrid or mixed cultivation approach, integrating elements from both systems, can harness their complementary benefits, potentially overcoming individual limitations.

Typically, macroalgae cultivation begins with a nursery phase conducted on land, where seedlings are cultivated under controlled indoor conditions. This phase allows for precise management of critical parameters such as temperature, nutrient availability, and pathogen control, which supports the development of robust and healthy seedlings, optimizing their subsequent growth potential. Following this stage, seedlings are transferred to either larger land-based systems or open-sea grow-out installations. The grow-out phase takes advantage of natural environmental conditions, which can support faster biomass production and lower operational costs compared to fully controlled systems.

Table II - Comparison of land-based and sea-based macroalgae cultivation systems.

Cultivation system	Advantages	Disadvantages
Land-based	• **Greater control over production parameters** • **Higher productivity** • **Higher control over epiphytes** • **Allows the cultivation of species not well suited for oceanic farming** • **Year-round production** • **Easier access to the production**	• **Space limited** • **Seawater sourcing needed** • **Higher production cost** • **Higher technical components**
Sea-based	• **Less space limited** • **Higher potential for mass production** • **Lower production cost**	• **Lower control over production.** • **High risk of contamination of epiphytes** • **Access to production limited by weather conditions and requires a boat**

3.2. Characterization of the macroalgae used during the internship

3.2.1. Genus *Ulva*

The genus *Ulva* belongs to the family *Ulva*ceae, order *Ulva*les, class Ulvophyceae, and phylum Chlorophyta (AlgaeBase, 2024). These green macroalgae are of considerable economic importance in aquaculture and human nutrition due to their rapid growth under various conditions and the presence of valuable biochemical compounds, which make them attractive for health-related and commercial applications (Satpati & Pal, 2011).

As opportunistic algae, *Ulva* species are also effective in filtration and bioremediation, helping to remove excess nutrients and prevent eutrophication (Neori *et al.,* 2003). Notably *Ulva lactuca* has demonstrated negative allelopathic effects on harmful red tide microalgae species such as *Heterosigma akashiwo, Alexandrium tamarense,* and *Skeletonema costatum* (Nan *et al.,* 2008), offering a natural strategy to mitigate harmful algal blooms.

Morphological characteristics

Morphologically, *Ulva* species exhibit some laminar, flattened morphology, resembling a leaf, others are tubular such as *Ulva compressa* and *Ulva intestinalis* (Barsanti & Gualtieri, 2022). However, their morphology is highly plastic, varying with environmental conditions and location (Gao *et al.,* 2016). Leskinen *et al.,* (2004) observed that in marine environments, most *U. compressa* specimens were branched, whereas *U. intestinalis* remained unbranched. In brackish water, both forms appeared in both species. Furthermore, Zhang *et al.,* (2013) identified four distinct morphological forms of *U. prolifera* during green tide events in the Yellow Sea: filamentous, tubular, cystic, and folded blades, with filamentous thalli being the predominant morphology.

Nutritional characteristics and Bioactive compounds

Ulva species are consumed fresh in some. Mediterranean coastal regions (Mendes *et al.,* 2022) and are known for their rich profile of bioactive compounds that offer potential health benefits. These compounds, include polysaccharides, phenolic compounds, fatty acids, pigments, minerals, and vitamins, exhibit various biological activities such as antioxidant, anti-inflammatory, immunomodulatory, and cardiovascular health effects (Michalak & Chojnacka, 2015). *Ulva* biochemical diversity enables its use in biorefineries for producing functional foods, skin care formulations, raw material for chemicals and fuels (Hofmann *et al.,* 2024; Putra *et al.,* 2024).

Polysaccharides

Polysaccharides are a major class of bioactive compounds found in *Ulva* spp. The primary types of polysaccharides identified include *Ulva*ns and cellulose (Abou El Azm *et al.,* 2019):

- *Ulva*ns – Rich in rhamnose, xylose, and glucuronic acid (Yaich *et al.,* 2017), exhibit antioxidant, anticoagulant, and immunomodulatory effects (Guidara *et al.,* 2021).
- Cellulose is the most abundant polysaccharide found in nature and serves as a structural component in the cell walls of plants and seaweeds. This polysaccharide is organized into long insoluble microfibrils forming a network within the cell wall, providing algal cells their shape and mechanical support (Putra *et al.,* 2024). In *Ulva* species cellulose provides strength and rigidity to the algal cell walls, contributing to their overall structural integrity (Ghassemi *et al.,* 2021). Cellulose is also known for its high resistance to enzymatic degradation (Putra *et al.,* 2024).

Polysaccharides composition in *Ulva lactuca* varies with environmental conditions, growth stage, and extraction methods (Yaich *et al.,* 2014).

Fatty acids

Fatty acids in *Ulva* spp. contribute to its nutritional value and potential health benefits (Pangestuti *et al.,* 2021). Fatty acids are essential components of lipids and play important roles in human health and metabolism.

The composition and concentration of fatty acids depends on species, geographical location, and environmental conditions. *Ulva lactuca* contains omega-6 fatty acids, such as linoleic acid (LA) and gamma-linolenic acid (GLA) (Gupta & Gupta, 2020). This type of fatty acids are essential in maintaining healthy cell membranes, regulating inflammation, and supporting overall well-being.

Valério Filho *et al.,* (2023) conducted a study of the fatty acid composition in *Ulva lactuca*, revealing significant amounts of various fatty acids:

- Palmitic acid (16:0) is a major component, constituting 21% of the total fatty acid profile;

- Oleic acid (18:1n9c), a monounsaturated fatty acid with a cis configuration (hydrogen atoms on the same side of the double bond), contributes 10 %;
- Other detected fatty acids include linolelaidic acid (18:2n6t), which has a trans configuration (hydrogens on opposite sides of the double bond), α-linolenic acid (18:3n3), and palmitic acid.
- Omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA, 20:5n3) is also present at 1.67%.

This diverse array of fatty acids, encompassing saturated, monounsaturated, and polyunsaturated types, shows nutritional richness and potential health benefits. Omega-3 fatty acids are highly regarded for their health benefits (Putra *et al.,* 2024).

Protein

Ulva species are also rich in protein, with values ranging from 9% to 33% of dry weight, depending on species and environmental conditions (Fleurence *et al.,* 2018). This variability is influenced by several abiotic factors, including seasonality, temperature, irradiance, and nutrient availability, as well as the developmental stage of the seaweed (Bak *et al.,* 2019).

Some studies have shown that *Ulva lactuca* exhibits seasonal fluctuations in protein content. According to Fleurence *et al.* (2018), protein content peaks in August at approximately 32%, while the lowest values occur in April, reaching 8.7%. This variation illustrates the marked influence of seasonal cycles on its biochemical composition.

Species specific differences also influence protein content. For instance, *Ulva pertusa* has been reported to reach values of 26% dry weight, whereas *Ulva lactuca* has demonstrated protein levels of up to 32% (Fleurence *et al.,* 2018).

In terms of protein quality, *Ulva* species are notable for their amino acid composition. A study by Juul *et al.* (2022) reported a total amino acid (TAA) content of 9.3% in freeze-dried *Ulva* sp., underscoring its nutritional relevance. This includes essential amino acids vital for both human and animal nutrition.

Given its high protein content and favorable amino acid profile, *Ulva* spp. represents a promising resource for applications in functional foods, nutraceuticals, aquaculture feed and as a sustainable alternative protein for the growing plant-based food market. Additionally, its cultivation aligns with circular bioeconomy principles, offering a low-impact solution to future nutritional demands.

Environmental parameters and stress factors

Due to their global distribution, *Ulva* species thrive in diverse habitats and exhibit tolerance to various environmental factors such as light, temperature, and salinity (Mantri *et al.,* 2020).

Temperature

Temperature is a crucial abiotic factor influencing plant growth and development, as it affects carbohydrate allocation and photosynthetic productivity (Hatfield & Prueger, 2015).

Species of the genus *Ulva* demonstrate a high thermal tolerance. They can survive extended periods of cold stress, including exposure to sub-zero temperatures (Kamermans *et al.,* 1998). Simultaneously, *Ulva* tolerates elevated temperatures, with optimal growth observed at water temperatures between 20°C and 25°C (Shaojun & Tifeng, 2008). This thermal plasticity renders the genus well-suited for cultivation across a range of climatic regions.

Their ability to thrive in intertidal zones is largely due to physiological adaptations that enable them to withstand frequent and abrupt temperature shifts (Balar & Mantri, 2020). However, temperature plays a key role in regulating metabolic activity. Temperatures exceeding the optimal range induce elevated enzymatic activity, such as nitrate reductase, amylase, invertase, and peroxidase, which may cause metabolic imbalances and consequently reduce growth performance (Kakinuma *et al.,* 2006).

Salinity

Salinity is a key environmental factor affecting the growth of *Ulva* species. These species are generally adapted to the salinity ranges typical of coastal waters, but fluctuations can influence nutrient uptake and overall metabolism. Effective salinity management is therefore essential in aquaculture systems to support healthy growth. Salinity also influences spore release by affecting turgor pressure and sporangial pore diameter (Chávez-Sánchez *et al.,* 2018). Some studies have found that *U. intestinalis* spore biomass was strongly favored at a salinity of 20 ppt and significantly increased at 35 ppt (Sousa *et al.,* 2007; Han *et al.,* 2008). That said, the range of salinity for *Ulva* species can range from 20 – 35 ppt.

Irradiation

Ulva species possess a high capacity to adapt to the highly variable light levels characteristic of intertidal zones in temperate regions (Sand-Jensen, 1988). This adaptability is particularly advantageous in high density cultivation systems where self-shading can occur. Their ability to utilize a wide range of irradiance levels enables sustained photosynthetic efficiency even under fluctuating light conditions (Sand-Jensen, 1998; Malta *et al.,* 2003).

Reproduction and life cycle

Reproductive processes in *Ulva* are influenced by abiotic factors such as light, temperature, and desiccation. However, each factor acts differently and always in combination with others rather than independently (Costa *et al.,* 2024).

Species of the genus *Ulva* exhibit a diplohaplontic isomorphic life cycle (Figure 8) (Balar & Mantri, 2020). In this cycle, sporophytes and gametophytes are morphologically identical (isomorphic) but differ in their functions (Thornber, 2006). Sporophytes reproduce asexually via meiosis, producing quadriflagellated haploid zoospores, while gametophytes engage in sexual reproduction by forming bi-flagellated gametes through mitosis, which fuse during fertilization (Wichard et al., 2015; Balar & Mantri, 2020).

According to Lovlie and Bryhni (1978), haploid gametophytes can be derived from:

1. Haploid zoospores of sporophytes.
2. Zoospores from parthenosporophytes.
3. Unfertilized biflagellate gametes.

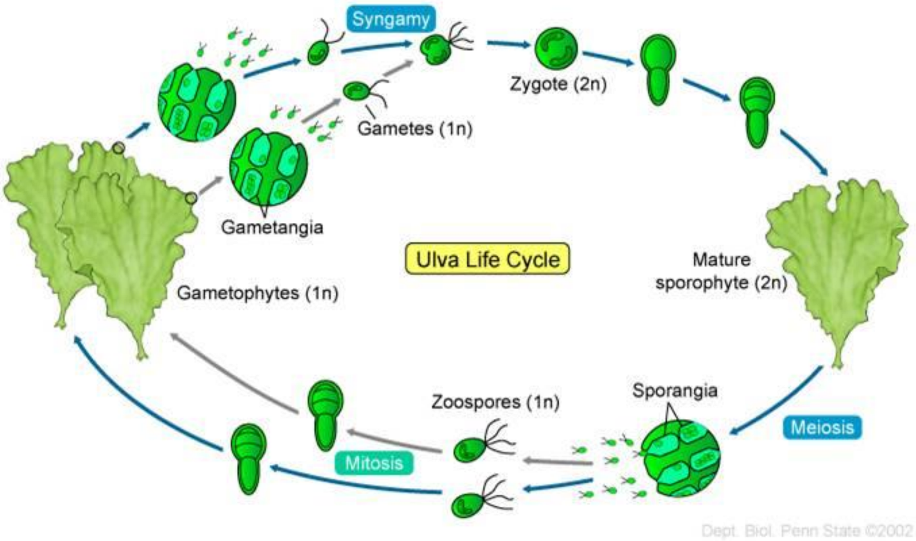

Figure 8 - Life cycle of *Ulva* sp. The gametophyte produces flagellated and identical gametes (isogamy) in gametangia. Gametes fuse and form a diploid zygote, which develops by mitosis to form the sporophyte, diploid generation. The sporophyte develops into sporangia, which produce spores (zoospores) by meiosis. After its release, each zoospore produces, by successive mitoses, a gametophyte. Source: Knowledge Class, (2013)

Induction of reproduction

The formation and release of zoospores through meiosis or gametes through mitosis occur via the transformation of common vegetative cells into these generative cells.

Zoospores formation can take place in any part of the thallus, while gametes are preferentially formed at the frond margins (Lüning *et al.,* 2008). Consequently, the first signs of reproduction induction are observed at the thallus margins (Balar & Mantri, 2020).

In *Ulva* spp., reproduction can be induced by cutting the thallus into small fragments and removing extracellular sporulation inhibitors (SIs) (Vesty *et al.,* 2015). When the thallus is cut, the exposed cells may initiate either sporogenesis (spore formation) or gametogenesis (gamete formation). This process is influenced by the developmental stage of the cells, as only mature cells can undergo reproductive differentiation and the presence of natural sporulation inhibitors, which may be endogenously produced or environmentally present, temporarily suppressing reproductive activation (Balar & Mantri, 2020).

Gametes are formed through bipartite divisions of the protoplast. During this process, the microtubule apparatus is assembled, and the nucleus undergoes division to generate gamete nuclei (Balar & Mantri, 2020). Cytokinesis results in the formation of 16 gametes, which are then ready for release (Katsaros *et al.,* 2017). Zoospore mother cells divide to produce zoospores. Reproductive induction can be visually observed. Before the release of gametes or zoospores, the thallus changes from yellowish green (vegetative state) to dark green (Balar & Mantri, 2020). A white color on the thallus indicates the release of reproductive cells (Stratmann *et al.,* 1996).

Influence of abiotic factors on reproduction induction

Abiotic factors that regulate growth are also responsible inducing and facilitating the release of gametes and zoospores, although their mechanisms of action differ (Rybak & Gąbka, 2018).

Abiotic factors that can trigger sporulation include:

1. Abrupt temperature changes (Mantri *et al.,* 2011).
2. In natural environments, sporulation events are correlate with spring tides, as water movement during these periods dilutes sporulation inhibitors (Smith, 1947).
3. Dehydration and periodic increases in light exposure (Strain *et al.,* 2006).

In addition to abiotic factors, biological influences such as symbiotic microorganisms, plant growth regulators, and secondary hormonal messengers also play crucial roles in promoting maturation (Vesty *et al.,* 2015; Wichard *et al.,* 2015).

Light

Light, as a source of photon energy, is a key factor in inducing reproduction. Variations in light irradiation and spectrum composition provide essential signals to phytochromes, i.e., photoreceptors in macroalgae (Balar & Mantri, 2020). The phytochrome apoprotein is synthesized in the cytoplasm, where it binds to a chromophore. In the absence of light, this apoprotein adopts a stable conformation that absorbs red light, known as Pr. Upon absorbing red light, Pr converts into its far-red light light-absorbing form (Pfr). These phytochrome-like photoreceptors regulate growth and reproductive rhythms via the Pfr/Pr ratio. Consequently, significant changes in light conditions influence the photoconversion from Pr to Pfr, triggering signals that enable vegetative cells to enter the reproductive cycle (Sharrock, 2008).

Periodic increases in light intensity also enhance sporulation, with the optimal intensities for *Ulva* sp. ranging from 40 to 75 µmol photons m^{-2} s^{-1} (Balar & Mantri, 2020).

Temperature

Alongside light and photoperiod, temperature is a key determinant of macroalgae reproduction (Balar & Mantri, 2020). Studies have shown that optimal temperatures for gametes/spores release range between 15-20ºC in species such as *U. prolifera, U. pertusa, U. mutabilis, U. fenestrata, U. spinulosa, U. linza, U. rigida* and *U. fasciata* (Zhang *et al.,* 2016; Balar & Mantri, 2020; Carl *et al.,* 2014). Also, a temperature fluctuation as small as 5ºC can induce reproduction in *Ulva* species (Pettett, 2009).

Fragmentation

Vegetative fragmentation of the thallus is an effective method for inducing reproduction in *Ulva* spp. (Balar & Mantri, 2020). These fragments predominantly undergo asexual reproduction, leading to increased germ cell production (Gao *et al.,* 2010). Other factors, including changes in the culture medium and dehydration, have also been shown to induce reproduction (Smith, 1947; Corradi *et al.,* 2006, Han *et al.,* 2008).

3.2.2. Genus *Gracilaria*

The genus *Gracilaria* belongs to the family Gracilariaceae, order Gracilariales, class Florideophyceae, and phylum Rhodophyta (AlgaeBase, 2024). It comprises over 300 species with high economic value worldwide (Almeida *et al.,* 2011).

There are two primary applications for *Gracilaria* species: phycocolloid extraction, particularly agar, making them valuable agarophytes, and direct human consumption (Mouedden *et al.,* 2024). Additional uses include paper production from agar extraction waste, bioethanol production due to their high carbohydrate content (approximately 45% dry weight), and nutrient bioremediation, which helps mitigate environmental eutrophication (Redmond *et al.,* 2014; Pei *et al.,* 2013; Amanullah *et al.,* 2013). Their nutrient-absorption capacity makes *Gracilaria* a key component in Integrated Multi-trophic Aquaculture (IMTA) systems.

These benefits contribute to a significant commercial value, making *Gracilaria* one of the most promising sources of high-quality industrial agar. In 2020, global *Gracilaria* production reached approximately 5 million tons, accounting for about 15% of the global seaweed production (FAO, 2022). Also, overexploitation of wild populations, driven by high market demand and insufficient crop management, has led to significant decline in natural stocks, underscoring the importance and opportunity of cultivation (Mouedden *et al.,* 2024).

Gracilaria spp.

Gracilaria spp. are benthic macroalgae that typically inhabit sheltered environments such as bays, estuaries, river mouths, and protected rocky areas at mid to sublittoral depths (0 to 20 m) (Gioele *et al.,* 2017). They commonly attach to stones, boulders, bedrocks, and sandy shores using a perennial holdfast (Haddy, 2011). This seaweed is particularly appreciated in Asian countries, particularly in China and Taiwan, where annual production is approximately 1.5 million tons (FAO, 2011).

Morphological characteristics

Species within the genus *Gracilaria* exhibit significant morphological variability, often complicating identification based solely on morphology and necessitating molecular tools for accurate taxonomic classification (Muangmai *et al.,* 2014).

A diverse range of growth forms is observed for the species of the genus. The thallus is typically erect and irregularly branched, ranging from bushy to sparsely branched forms depending on habitat conditions. For instance, thallus shape vary from completely cylindrical (e.g., *Gracilaria gracilis*) to broadly flattened (e.g., *Gracilaria domingensis*) (Torres *et al.,* 2019; Iyer *et al.,* 2004). The holdfast is discoid and well-developed, allowing secure attachment to substrates such as rocks and shell fragments.

Individuals of the same species may also show morphological variation in color, length, branching pattern, and thallus shape. Colors ranges from dark red to brownish red, sometimes turning greenish in shallow, high-light environments (Figure 9) (Bermejo *et al.,* 2020).

Figure 9 - Illustrative figure of *Gracilaria* spp., displaying a vivid reddish coloration. Photo: Paulo Pereira

Nutritional and Bioactive properties

Species of the genus *Gracilaria* have gained attention due to their wide range of applications, including agar extraction, direct human consumption, animal feed supplementation, nutrient bioremediation in Integrated Multi-Trophic Aquaculture (IMTA) systems, and use as feedstock for bioethanol production (Redmond *et al.,* 2014; Amanullah *et al.,* 2013). Cultivation of *Gracilaria* spp. in deeper waters has been shown to enhance the synthesis of bioactive compounds, particularly under environmental stress conditions, which may confer anti-aging and other health promoting benefits (Maehira *et al.,* 2004).

Polysaccharides

Gracilaria spp. are notably rich in carbohydrates, comprising 38% and 63% of dry weight (Rasyid *et al.,* 2019; Freitas *et al.,* 2021). The predominant polysaccharides present are galactans of the agaran group, which form the structural basis for agar production (Torres *et al.,* 2019).

Beyond their industrial value, these polysaccharides exhibit a wide range of bioactivities. Agaran-type polysaccharides have demonstrated antioxidant, antiviral, anticancer, anticoagulant, anti-inflammatory, gastrointestinal, and neuroprotective activities (Wijesekara *et al.,* 2011; Torres *et al.,* 2019). These properties underline the therapeutic potential of the *Gracilaria* spp. and support their increasing use in nutraceuticals and functional food development.

Protein

Red seaweeds, including *Gracilaria* spp., are notable for their high protein content, ranges between 18% and 25% of dry weight. These values are comparable to those found in animal-based sources such as meat (18–25%) and legumes like beans and peas (19–22%) (Rodrigues *et al.,* 2015). This nutritional profile highlights the potential of *Gracilaria* spp. as a promising sustainable alternative for protein supplementation in both human and animal diets.

Lipids

The total lipid content in *Gracilaria* is relatively low, generally, not exceeding 2% of dry weight. Despite their scarcity, lipids serve crucial physiological role, including energy storage, cellular membrane structure, and signaling processes (Eyster, 2007). Reported lipid contents range from 0.3% dry weight in *G. changii* to 0.87% in *G. lemaneiformis* (Chan & Matanjun, 2017; Wen *et al.,* 2006).

Of particular interest is the fatty acid, especially the proportion of polyunsaturated fatty acids (PUFAs), which are essential for human health. On average, the fatty acid profile of *Gracilaria* includes approximately 52% saturated fatty acids (SFAs), 35% polyunsaturated fatty acids (PUFAs), and 13% monounsaturated fatty acids (MUFAs) (Torres *et al.,* 2019).

Environmental parameters and stress factors

Gracilaria spp. is one of the top five most cultivated seaweeds globally due to their fast growth rate, ease of propagation (both asexual and sexual), and high tolerance to environmental fluctuations, particularly in salinity and temperature (Redmond *et al.,* 2014). Growth, life cycle progression, and phycocolloid content depend on several abiotic factors, including temperature, salinity, irradiation and nutrient availability (Gioele *et al.,* 2017).

Temperature

Optimal growth occurs between 19°C to 23°C in general species. Temperatures above 28°C have been associated with stress responses and increased mortality (Gioele *et al.,* 2017; Polifrone *et al.,* 2006). Temperatures below 10°C significantly reduce daily growth rates, although they typically do not induce mortality unless freezing conditions persist (Yang *et al.,* 2015).

Some species , such as *Gracilaria tikvahiae*, exhibit eurythermal properties and can tolerate a wide thermal range from 0°C to 34°C, demonstrating high adaptability (McLachlan & Bird, 1984). This physiological plasticity highlights the potential of selective strain development to enhance thermal resilience in aquaculture practices.

Salinity

Several Gracilaria species exhibit euryhaline characteristics, meaning they can tolerate a wide range of salinity levels without compromising structural integrity or physiological functions. For example, *Gracilaria gracilis* is found in environments ranging from oligohaline conditions (0.5–5 ppt) to fully marine salinities (30–40 ppt) (Pérez-Ruzafa *et al.,* 2011; Wilson & Critchley, 1997). However, optimal growth occurs between 30 and 35 ppt.

Notably, *Gracilaria vermiculophylla* can survive salinities up to 60 ppt for eight weeks (Yokoya *et al.,* 1999). Such resilience underscores the potential for cultivating *Gracilaria* species in variable and suboptimal conditions, making them suitable candidates for aquaculture systems impacted by seasonal salinity fluctuations.

Interestingly, variations in salinity can influence the biochemical profile of *Gracilaria*. Özen *et al.,* (2018) reported that under hypersaline conditions (48 ppt), total protein content in *Gracilaria* spp. increased substantially to 88.47 mg/g wet weight by the second day of exposure, indicating a potential role for osmotic stress in stimulating protein synthesis.

Irradiation

Light is another fundamental factor regulating growth and photosynthetic efficiency in *Gracilaria* spp. Different species and strains have shown different varying levels of light adaptation. A Tunisian strain of *G. gracilis* achieved a high reported daily growth rate (DGR) of 7.24% under red light (620–670 nm) at a low irradiance of 18 µmol photons m^{-2} s^{-1} (Ghedifa *et al.,* 2021).

Studies with *Gracilaria vermiculophylla* has shown exceptional low light adaptability, capable of sustaining growth at irradiance levels below 1 µmol photons m^{-2} s^{-1} (Nejrup *et al.,* 2013). This enables colonization of turbid or deeper environments, where light penetration is limited, thus enhancing its invasive potential and ecological success. These findings indicate that light quality and intensity can be optimized in cultivation systems to improve growth performance, particularly in indoor or high-density cultures.

Nutrients

As phycocolloid rich seaweed and a major biological source of agar, they require specific nutrient conditions to optimize biomass productivity and the quality of the extracted compounds. Nitrogen availability plays a main role in regulating the biosynthesis of agar (Torres *et al.,* 2019). Higher nitrogen concentrations in the culture medium have been shown to enhance both yield and physicochemical quality of extracted agar (Ben Said *et al.,* 2018), likely due to nitrogen's involvement role in protein and polysaccharides synthesis (Lee *et al.,* 2017).

Although, maintaining balanced nutrient ratios, particularly between nitrogen and phosphorus, is critical to avoid nutrient stress that could compromise growth rates and productivity in cultivation.

Reproduction and life cycle

The life cycle of *Gracilaria* follows a *Polysiphonia*-type triphasic cycle, characterized by an isomorphic alternation between haploid and diploid phases, i.e., diploid tetrasporophytes alternate with haploid gametophytes, which are morphologically identical and develop independently (figure 10) (Kain & Destombe, 1995; Polifrone *et al.,* 2006).

Upon fertilization, the zygote develops into a diploid carposporophyte, which produces and releases diploid carpospores. These carpospores give rise to diploid tetrasporophytes, which undergo meiosis to produce and release haploid tetraspores. These tetraspores develop into haploid isomorphic gametophytes. Male gametophytes produce and release non-motile spermatia, which rely on water movement to reach the carpogonium of female gametophytes for fertilization. Once fertilization occurs, a new zygote is formed, initiating a new life cycle (Figure 10) (Kain & Destombe, 1995; Polifrone *et al.,* 2006).

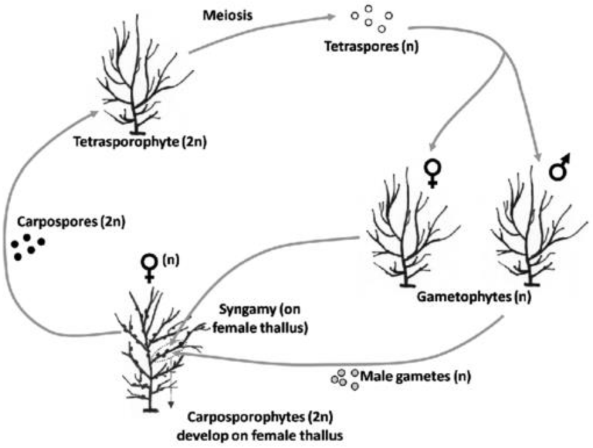

Figure 10 - Typical haploid-diploid life cycle of *Gracilaria* species, with the alternation of meiosis and syngamy connecting tetrasporophytes and gametophytes individual. Source (Vieira *et al.,* 2018).

Induction of reproduction

Sporulation in *Gracilaria* spp. can be induced through several methods, including desiccation, osmotic shock, and changes in abiotic factors such as temperature.

According to Michetti *et al.,* (2013), the following methods successfully induced sporulation:

- Osmotic shock: carpospore production was achieved in *G. gracilis* (of Argentine origin), by exposing individuals to high salinity (60) for 30 minutes, followed by a return to normal salinity (34).
- Desiccation: Fertile fragments were dehydrated in total darkness for 1 hour before being reintroduced into the initial medium.

- Temperature manipulation: Keeping fertile individuals at 13ºC resulted in significantly higher sporulation rates compared to 18ºC.

Freitas *et al.,* (2021) reported successful maturation of cystocarps and subsequent spores release in *G. gracilis* (of Portuguese origin) under the following conditions:

- Darkness – exposing individuals to darkness for 2, 6 or 12 hours.
- Nutrient Supplementation - using Modified Von Stosch Enriched Medium (VSE) with germanium dioxide to prevent diatom spread.
- Low temperature – maintaining individuals at 5ºC.

Another study developed by López-Campos *et al.,* (2022) confirmed that sporulation induction was effective using desiccation followed by hydration, where fertile fragments were kept out of water for 1 hour and then rehydrated.

Maintenance of Spore Cultures

Following successful sporulation, López-Campos *et al.,* (2022) maintained *G. gracilis* spore cultures in controlled conditions, namely:

1. Irradiation: 30 μmol m^{-2} s^{-1}
2. Temperature: ±18 ◦C
3. Photoperiod: 12-hour light/ 12 hours dark cycle
4. Salinity: 34
5. Water changes: weekly

As spores grow into thalli, upscaling cultivation is necessary to increase biomass for large-scale application. This involves transferring individuals to larger culture volumes to support further growth and development.

3.2.3. *Palmaria palmata*

Palmaria palmata, commonly known as Dulse, is a red seaweed increasingly recognized for its sustainability and health promoting benefits in human nutrition. It is widely consumed in Western countries, such as France, Ireland, Canada and the United States. The current market value of dulse estimated between 40€ and 75 €/kg in business-to-business transactions (Silva *et al.,* 2024; Stévant *et al.,* 2023).

Dulse is among the most used seaweed species in food products, appealing due to its high nutritional value and versatility. It is an excellent source of protein, fiber, vitamins, and minerals (Xu *et al.,* 2024; Kuech *et al.,* 2023; Stévant *et al.,* 2023). Highly suitable for direct consumption, it can be incorporated into vegetarian dishes, served alongside fish, or dehydrated and consumed as a savory snack (Silva *et al.,* 2024).

Morphological characteristics

P. palmata can reach lengths of up to 50 cm, although it is typically found in sizes ranging between 10 and 20 cm (Stévant *et al.,* 2023). Its color and texture vary with age and environmental conditions. Younger specimens maintain a fresh red color and tender texture, while older individuals often appear darker and develop a more leathery consistency (Figure 11) (Stévant *et al.,* 2023). Environmental factors such as irradiation and temperature, also influence color. Prolonged exposure to elevated temperatures and intense sunlight may result in a a greenish discoloration of the thallus (Stévant *et al.,* 2023)

Figure 11 - Illustrative image of *Palmaria palmata*. On the left displays a characteristic red coloration, while the individual on the right exhibits a darker coloration and a leathery texture. Photo: Paulo Pereira

Nutritional and Bioactive compounds

Polysaccharides

Unlike most red macroalgae, which predominantly synthesize sulfated galactans, *Palmaria palmata* produces xylan as the primary structural polysaccharide of the cell wall (Xu *et al.,* 2024). Xylan is a hemicellulosic polysaccharide consisting of xylose monomer units, and constitutes the principal water-soluble carbohydrate in this species, followed by galactose and glucose (Xu *et al.,* 2024). Notably, water-soluble xylan extracts derived from *P. palmata* exhibit significant bioactive properties, particularly antiviral activity against herpes simplex virus infections (Xu *et al.,* 2024).

Proteins

In terms of protein content, red macroalgae generally possess higher protein concentrations (ranging from 19% to 47%) compared to green and brown macroalgae (Silva *et al.,* 2024). *Palmaria palmata* typically presents protein levels between 20% and 25%, varying according to factors such as environmental conditions and seasonal fluctuations (Xu *et al.,* 2024; Echave *et al.,* 2021). Indeed, seasonal variation influences protein accumulation in macroalgal tissues (Figure 12). For instance, studies conducted in France revealed higher protein levels during winter and spring (approximately 21.9%) when water temperatures are lower, in contrast to the summer months during which protein content decreased significantly to around 11.9% (Galland-Irmouli *et al.,* 1999).

Recent research has shown the opposite. *Palmaria palmata* harvested during the summer consistently exhibited protein concentrations exceeding 20%, underscoring the species nutritional potential even under higher-temperature regimes (Echave *et al.,* 2021). This information highlights the importance of harvesting time in optimizing the nutritional value of macroalgal biomass for various industrial and nutritional applications.

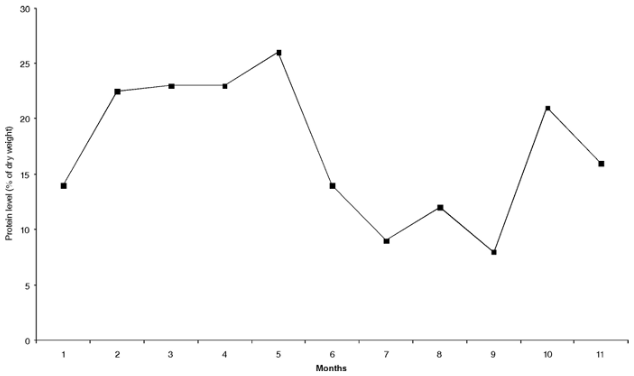

Figure 12 - Seasonal variation of protein content of *Palmaria palmata*. Source: Fleurence *et al.,* (2018).

Lipids

Lipid content in *P. palmata* is relatively low, varying from 0.1 to 2.8% of dry weight (Xu *et al.,* 2024). Despite their low abundance, polyunsaturated fatty acids (PUFA's) make up 50% of the total fatty acids, with eicosapentaenoic acid (EPA) being the most predominant type, with concentrations of 8.3 mg g^{-1} dry weight (Xu *et al.,* 2024; Silva *et al.,* 2024). High EPA values are considered beneficial for reducing the risk of cardiovascular diseases, including thrombosis and arteriosclerosis (Silva *et al.,* 2024).

Environmental parameters and stress factors

Temperature

P. palmata thrives in cold and temperate marine environments, within a temperature range of 0ºC to 20ºC. The optimal growth temperature varies by strain but generally lies between 6ºC and 10ºC (Corey *et al.,* 2012). Growth performance declines above 14ºC, and mortality rates rise significantly when water temperatures exceed 21ºC, particularly during summer (Stévant *et al.,* 2023; Matos *et al.,* 2006).

Salinity

P. palmata prefers stable salinity, levels between 30 and 40 ppt (Stévant *et al.,* 2023). However, wild populations growing in the intertidal zone often experience fluctuations due to precipitation and evaporation. Growth performance decreases at salinity levels around 15 ppt, primarily due to photosynthesis inhibition and increased mortality. Nevertheless, certain ecotypes have demonstrated adaptability to lower salinities, with some individuals exhibiting maximum growth at 15 ppt (Schmedes & Nielsen, 2020).

Irradiation

P. palmata possesses physiological mechanisms that regulate photosynthetic activity in response to daily light variations (Stévant *et al.,* 2023). Photoinhibition acts as a protective mechanism against the formation of reactive oxygen species (ROS), caused by excessive photosynthetic or ultraviolet radiation (Marambio & Bischof, 2021; Hurd *et al.,* 2014). The physiological response includes the breakdown of photosynthetic pigments such as phycoerythrin, phycocyanin, and chlorophyll *a*, which helps dissipate excess energy and facilitates recovery (Stévant *et al.,* 2023). Under cultivation conditions with irradiation levels above 200 µmol photons m^{-2} s^{-1} white staining may appear on the thallus after after 2-3 weeks, often followed by fragmentation (Edwards, 2008).

Nutrients

P. palmata utilizes both ammonium (NH_4^+) and nitrate (NO_3^-) as nitrogen sources, with a higher uptake affinity for ammonium (Grote, 2016). Elements such as nitrogen, phosphorus, and carbon are the basis for growth and, when combined with irradiation, also influence nutrient storage (Stévant *et al.,* 2023). Under conditions of low irradiation and nutrient deficiency, nitrogen storage occurs in the form of the pigment phycoerythrin, ensuring continued photosynthetic activity and growth (Martínez & Rico, 2002).

Reproduction and life cycle

P. palmata exhibits a haplodiplont heteromorphic life cycle (Figure 13), which is unique among Rhodophyta (Stévant *et al.,* 2023). This cycle involves alternating haploid and diploid stages, as described by van der Meer and Todd (1980):

1. Formation of tetraspores: Haploid tetraspores develop via meiosis within diploid tetrasporophytes.
2. Gametophytes development: These haploid tetraspores germinate and differentiate into female or male gametophytes.

3. Fertilization process: The female gametophytes produce oogonia with trichogynes, specialized hair-like structures that capture non-motile spermatia realeased by male gametophytes.
4. Fertilization occurs when spermatia reach the trichogynes, forming a zygote.
5. Tetrasporophytes formation: the zygote matures into a new diploid tetrasporophyte, completing the cycle.

A thorough understanding of this reproductive cycle is key for optimizing cultivation and reproductive induction protocols. While tetrasporophytes may originate from wild populations, only fertile, and epiphyte-free sori (Figure 14) should be selected for propagation to ensure successful cultivation outcomes (Edwards & Dring, 2011).

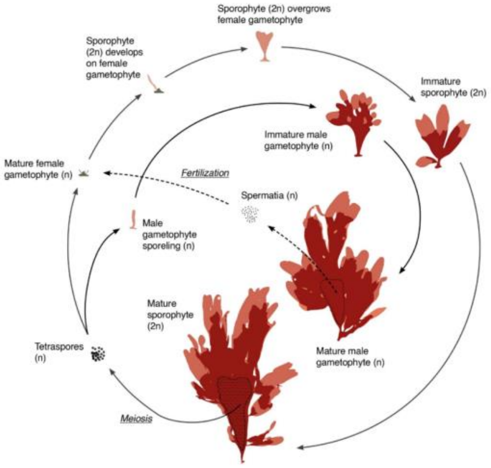

Figure 13 - Life cycle of *Palmaria palmata*, as described by van der Meer and Todd (1980).

Induction of reproduction

As the spores of *P. palmata* are non-motile, breeders are placed in pretreated seawater above an appropriate substrate, onto which the spores can attach (Grote, 2019). To induce spore maturation, and optimize reproduction, it is essential to manipulate the physicochemical water parameters, as these factors directly influence *in situ* reproduction processes.

Spore maturation, particularly the development of tetrasporangia (spore-producing structures in the tetrasporophyte), can be effectively stimulated by maintaining water temperatures at or below 10ºC, coupled with a short photoperiod of 8 hours of light per day (Figure 14) (Stévant *et al.,* 2023). Additionally, under a 12:12h light-dark photoperiod, low irradiation levels (ranging from 5 to 10 µmol photons m^{-2} s^{-1}) further enhance tetrasporangial maturation (Stévant *et al.,* 2023).

Figure 14 – Tetrasporophyte fertile of *Palmaria palmata*. Sori is visible as marble-like structures on the distal part of the frond. (Photo: Peter Schmedes).

Early-stage cultivation

During the early stages of cultivation, spores must be maintained under controlled conditions to ensure successful germination and viability. During the first three days post-release, spores should be kept under low aeration, low temperature (1-10ºC), and low irradiation (Edwards & Dring, 2011; Werner & Dring, 2011). After this period, the spores can be transferred to stirred systems operating a 12:12h (light: dark) photoperiod. Irradiance should initially be maintained at approximately 10 µmol photon m^2 s^{-1}and gradually increased to 40–60 µmol m^2 s^{-1}, after one month (Pang & Lüning, 2006).

To promote optimal growth during this phase, nutrient enrichment is required, ensuring an adequate supply of macronutrients, such as nitrogen, phosphorus, potassium, magnesium, calcium, and sulfur, and micronutrients, including iron, cobalt, manganese, zinc, and essential vitamins (Grote, 2019). Commonly used culture media such as F/2 medium and von Stosch media offer balanced nutrient profiles suitable for early-stage development.

3.3. Interaction of the microbial community and marine macroalgae

The microbial communities associated with marine macroalgae play a crucial role in their physiology, health and overall development of seaweed. The composition of the macroalgae microbiome is highly dynamic and is influenced by geographic location, seasonality, and several abiotic factors, including temperature, salinity, light intensity, and nutrient availability (Simon *et al.,* 2022; Serebryakova *et al.,* 2018). These variations lead to distinct microbial assemblages that impact algae health and growth performance. The interaction between marine macroalgae and microorganisms is also a crucial factor to ensure food safety, extend shelf life and nutritional enhancement (Xu *et al.,* 2024).

In aquaculture systems, where environmental conditions are artificially managed, the microbial profile can differ considerably from that observed in natural environments. Understanding these macroalgae-microbiome interactions is essential, as specific microbial consortia have been shown to facilitate nutrient uptake, enhance resistance to pathogens, and even modulate morphogenesis. For example, some bacteria can trigger cell division or thallus differentiation in *Ulva* (Paix *et al.,* 2020).

Maintaining a beneficial microbiome is critical for successful macroalgal cultivation. Emerging aquaculture strategies may benefit from the deliberate manipulation of the microbial environment, through for example probiotic applications, water conditioning, or co-cultivation approaches to promote algal health, stability, and productivity (Simon *et al.,* 2022).

Disruption of the native microbiota, such as through rinsing *P. palmata* with tap water, may remove beneficial surface-associated microbes and facilitate colonization by spoilage microorganisms (Xu *et al.,* 2024). In addition, pathogenic bacteria such as *Vibrio parahaemolyticus,* can be transmitted via macroalgae, and pose a risk to human health causing gastroenteritis (Mahmud *et al.,* 2007).

Despite these risks, certain epiphytic or symbiotic bacteria offer benefits to red macroalgae, particularly acquiring essential micronutrients. For instance, *P. palmata* relies on external sources for Vitamin B12, which it cannot synthesize due to the absence of relevant biosynthetic genes in its genome (Helliwell, 2017; Watanabe & Bito, 2018).

4. Objectives

This curricular internship, part of the Master's degree in Aquaculture at the School of Tourism and Maritime Technology of the Polytechnic Institute of Leiria, is designed to provide both practical and theoretical knowledge related to pilot-scale seaweed cultivation. The primary aims include:

1. Research on macroalgae;
2. Cultivation techniques;
3. Water quality adjustments;
4. Sampling and monitoring of cultivation;
5. Optimization and maintenance of the cultivation system;
6. Management of a Recirculating Aquaculture System (RAS) adapted for seaweeds;
7. Ensuring sustainable production for future project scalability;
8. Advancing knowledge in phycology.

5. Characterization of the host entity – Seaweedland & Hortimare

5.1. Seaweedland

Founded in 2022, Seaweedland in the Netherlands has been focused on developing a safe, sustainable, and fully traceable cultivation system for marine macroalgae intended for human consumption.

Cultivating macroalgae in offshore environments presents significant challenges, including contamination by fouling organisms and exposure to pollutants and heavy metals, which increase the risk of bioaccumulation. Additionally, offshore cultivation is susceptible to seasonal fluctuations in abiotic factors, impacting macroalgal growth rates and overall survival.

To address these challenges, Seaweedland operates a pilot-scale, land-based cultivation system. This innovative approach enables precise control over environmental conditions, ensuring the production of macroalgae that meet high food safety standards for human nutrition.

Currently in the research and development (R&D) phase, Seaweedland collaborates closely with Hortimare, a key partner specializing in advanced cultivation technologies for various macroalgae species. Through this strategic approach, Seaweedland aims to foster responsible aquaculture practices, reduce pressure on natural marine ecosystems, and contribute to sustainable, fully traceable food production.

5.2. Hortimare

Founded in 2008 and based in Heerhugowaard, Netherlands, Hortimare is a biotechnology driven company that supports marine macroalgae producers, by providing high-quality seed stock, technological solutions, and expertise for large-scale cultivation.

Hortimare focuses on the selective breeding and life cycle completion of multiple macroalgae species, ensuring a reliable and sustainable seed supply for commercial production. Their research includes brown macroalgae such as *Macrocystis pyrifera*, *Saccharina latissima*, *Undaria pinnatifida* and *Alaria esculenta*; green macroalgae *Ulva* spp. and red macroalgae species such as *Palmaria palmata*, *Asparagopsis armata*, *Asparagopsis taxiformis* and *Gracilaria* spp.

Hortimare´s core mission is to achieve full traceability of macroalgal cultivation while minimizing the risk of contamination. By reducing reliance on wild harvesting, Hortimare ensures species preservation in natural habitats.

5.2.1. Hortimare facilities

Hortimare's facilities are specifically designed to support the cultivation, research, and development of marine macroalgae, integrating activities from laboratory research to production level activities. The facilities include specialized laboratories, a hatchery, production site, workshop, office spaces, and meeting/presentations room. This comprehensive setup enables efficient research and optimized production workflows.

Laboratories

The laboratory is organized into several specialized sections to streamline research activities and minimize cross-contamination risks between species. These sections include:

1. *Palmaria palmata* laboratory: Dedicated exclusively to this species for selection and reproduction activities.
2. Brown Macroalgae Laboratory: Designed for species such as *Saccharina latissima* and *Alaria esculenta*.
3. Red macroalgae Laboratory: Supports the cultivation and study of species, from the genus *Gracilaria* and *Asparagopsis*.
4. Contaminated Material Laboratory: Used for screening, cleaning, and selecting incoming material.

Each laboratory is equipped with multiple instruments such as a *hotte*, binocular magnifying glasses, microscopes, high-precision scales, spectrophotometer, and shakers.

Hatchery

This area is intended to ensure the optimal conditions for macroalgae reproduction and early growth of the macroalgae, before scale-up to larger tanks or commercialization. Environmental parameters such as temperature, photoperiod, and light intensity are precisely controlled and tailored to each species.

The hatchery is divided into the following chambers:

1. Low temperature chamber (8ºC): Suitable for species such as *Saccharina latissima*, *Alaria esculenta* and *Palmaria palmata*. It is equipped with 1000-liter cylindrical tanks, 200-liter rectangular tanks, and 10-liter culture bottles.
2. High temperature chamber (20ºC): Used for species such as *Asparagopsis* spp., *Ulva* spp. and *Gracilaria* spp., with smaller capacity tanks.

Production

The production area is equipped with raceways and large-capacity cylindrical tanks (1,000 liters), designed to scaling up macroalgae cultivation from the hatchery stage. Production operations can be conducted either indoors or outdoors, including within greenhouse environments, depending on the species and environmental requirements.

These cultivation systems are critical not only for biomass production but also for applied research and development (R&D). They enable the implementation of experimental protocols aimed at optimizing key cultivation parameters, including:

1. Determining optimal biomass densities to maximize growth rates and nutrient uptake.
2. Evaluating the effectiveness of various fertilization regimens, including frequency and nutrient composition.
3. Assessing the effects of temperature fluctuations on algal physiology and biomass quality.
4. Investigating the effects of photoperiod and light intensity, on photosynthesis and growth performance.
5. Monitoring water quality parameters such as salinity, carbon dioxide ordissolved oxygen, and pH, and their influence on cultivation outcomes.

This production stage is fundamental for ensuring the commercial viability and long-term sustainability of macroalgal farming, while also generating data to inform future scale-up strategies and operational protocols.

Workshop

A workshop is an essential place of any aquaculture operation, functioning as a dedicated space for the storage and maintenance of equipment essential to daily activities. It functions as both a repair and storage center for tools and spare parts, enabling swift resolution of technical issues as they arise

By ensuring operational continuity and reducing downtime, the workshop plays a key role in minimizing production interruptions, thereby reducing costs and preventing associated losses.

6. Tasks performed – Seaweedland

The main objective of this internship was to provide hands-on experience and deepen knowledge regarding the operation of a pilot scale company, dedicated to land-based macroalgae cultivation. Specific tasks were assigned to each trainee based on their academic background and area of interest. In my case, the internship focused on practical experience in cultivation procedures, allowing me to participate in various activities related to maintaining the production of different macroalgae species.

6.1. Water quality monitoring

Abiotic factors such as temperature, pH, salinity, and light intensity are known to influence macroalgae growth and performance. Therefore, continuous monitoring and regulation of these parameters are essential for optimizing production.

Some abiotic variables, such as temperature and light intensity, are impacted by seasonality. In the Netherlands, long photoperiods and elevated temperatures during the summer can lead to excessively high conditions within greenhouse environments, potentially affecting algal growth. Conversely, in winter, low temperatures and reduced sunlight may hinder development, often necessitating the use of artificial lighting systems to compensate.

Although the cultivated species exhibit a degree of tolerance of variations in temperature, salinity and light intensity, environmental adjustments are required when conditions approach limiting thresholds. The first task each day involves monitoring the water parameters in the production tanks, including salinity, temperature and pH (Figure 15). In addition, the oxidation-reduction potential (ORP) is measured in the water storage tank, ensuring that disinfection processes remain within acceptable standards.

Figure 15 - Water quality monitoring. Photo: Paulo Pereira

pH

Seawater naturally has a slightly basic pH, around 8.2. The injection of carbon dioxide into the system lowers the pH increasing water acidity. Monitoring and maintaining pH levels between 7 and 8 is essential to support optimal macroalgae growth. When pH values exceed 9, the availability of inorganic carbon for photosynthesis decreases, which can impair growth and negatively impact algal physiology.

Carbon Dioxide CO_2

The CO_2 injection is controlled by an automated pH control system (Dennerle and Eheim), which adjusts the dosage to maintain the desired pH range. Continuous monitoring is necessary to ensure proper function, and periodic calibration of the pH sensors is required to maintain measurement accuracy.

Temperature

Daily temperature monitoring is a standard practice. Some species require either water cooling or heating systems to maintain optimal conditions. When temperature exceed the upper limit, a chiller tank is activated to reduce the water temperature gradually through a closed cooling circuit. On the other hand, when the temperatures fall below the required threshold, a heating system is activated to raise the temperature. All systems are continuously monitored to ensure efficient and species-specific thermal regulation.

Salinity

Excessive evaporation can increase salinity levels, requiring controlled replenishment of freshwater to prevent stress. When salinity rises above 35 ppt, it may hinder growth due to osmotic stress, leading to cell dehydration and reduced viability. To mitigate this problem, salinity is adjusted by adding demineralized water or through partial water exchange, restoring optimal conditions for cultivation.

Light

Light is one of the most important factors in seaweed cultivation, as it constitutes the main source of energy for photosynthetic cells. Macroalgae shows a differentiated growth in response to the fluctuation of light intensity and quality. Photosynthetic activity increases with light intensity up to a saturation point, beyond further increases do not enhance – and may even inhibit - growth (Bastos, 2019). Optimal light levels, such as intensity and spectrum, vary considerably with the type of crop and the species. Monitoring this factor is crucial to guarantee quality in the production (Figure 16).

During winter months, when natural light drops below 50 µmol photons m^2/s, artificial lighting systems are employed to ensure the maintenance of photosynthetic processes and crop viability.

Excessive light intensity can damage chloroplast receptors and induce photoinhibition, leading to a decrease in growth (Singh & Singh, 2015). Therefore, both light intensity and exposure time must be carefully controlled. An interval timer is typically used to control photoperiods, with a standard setting of 12 hours of light followed by 12 hours of darkness (12:12, L:D) (Redmond *et al.,* 2014). Additionally, shading in high-density cultures must be considered, as it can limit light availability to deeper layers of the biomass.

Figure 16 – Light monitoring in the *Ulva* raceway (left). Infisice Quantum PAR Meter (right). Photo: Paulo Pereira

6.2. Nutrients

Algae require a balanced supply of essential inorganic nutrients to sustain healthy growth and high biomass productivity. These include macronutrients such as nitrogen (N), phosphorus (P), and potassium (K); as well as trace elements such as iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), alongside vitamins (organic nutrients) (Bastos, 2019). The availability and balance of these nutrients are critical factors influencing cellular metabolism, photosynthetic efficiency, and overall productivity in macroalgae cultivation systems.

Nitrogen

Nitrogen is one of the most critical nutrients for macroalgae metabolism, as it is directly involved in the synthesis of essential organic macromolecules, including amino acids, proteins, enzymes, and photosynthetic pigments such as chlorophyll (Leegood *et al.,* 2000). The availability of nitrogen significantly influences both growth rate and productivity.

Nitrogen is available in inorganic forms such as nitrate (NO_3^-) and ammonium (NH_4^+), or organic forms like urea (Roleda & Hurd, 2019). Species-specific preferences have been observed; for instance, *Ulva lactuca* exhibited higher growth rates and higher biomass yields with ammonium (NH_4^+) fertilization compared to nitrate (NO_3^-) (Ale *et al.,* 2011). The reason underlying this difference is because ammonium is readily assimilated into amino acids biosynthesis without requiring energy demanding reduction processes, whereas nitrate must be enzymatically reduced to a bioavailable form (Kumar & Bera, 2020). Nevertheless, this preference is not consistent across all *Ulva* species. For example, *U. rigida* showed greater affinity for nitrate over ammonium (Lavery *et al.,* 1991).

Moreover, nitrogen source can influence not only productivity but also the biochemical composition, of the algae, impacting the nutritional and economic value of the harvested biomass.

Phosphorus

Phosphorus is the second most critical macronutrient and is involved in energy transfer processes and biosynthesis of fundamental cellular components such as phospholipids (integral to cell membrane structure), nucleic acids (DNA and RNA), and essential polysaccharides. Typically, macroalgae cultures maintain a nitrogen and phosphorus ratio of 16N:1P (Suttle & Harrison, 1988). However, variations in nutrient availability or deliberate manipulation of this ratio can induce significant metabolic adjustments, affecting both biomass composition and productivity.

Specifically, altering the N:P ratio serves as a valuable strategy to steer macroalgal metabolic pathways towards increased synthesis of target bioactive compounds. Research indicates that modulating nitrogen and phosphorus availability influences the synthesis of valuable biomolecules such as proteins, polyunsaturated fatty acids (PUFAs), and specialized polysaccharides (Li *et al.,* 2009; Hamed *et al.,* 2015). These biomolecules are highly sought after in sectors including food and feed industries, aquaculture nutrition, pharmaceuticals, and biotechnological applications, underscoring the importance of precise nutrient management strategies in macroalgae cultivation systems.

Tank Fertilization

In *Ulva* spp. systems, routine fertilization ensures constant availability of essential nutrients, crucial for optimal growth and biomass productivity. Typically, fertilization occurs three times per week using F2 medium at a dosage of 1mL per liter.

F2, developed by Guillard and Ryther (1962), is a standardized nutrient medium for both microalgae and macroalgae cultivation. It provides a balanced combination of macronutrients, particularly nitrogen (in the form of nitrate) and phosphorus (in the form of phosphate), along with essential micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and molybdenum (Mo). Additionally, the F2 medium includes essential vitamins, notably thiamine (Vitamin B1), biotin (Vitamin B7), and cobalamin (Vitamin B12), which support the overall metabolic and physiological activities of algal cells (Guillard & Ryther, 1962).

Regular monitoring of nutrient levels and algal uptake is crucial to avoid both nutrient depletion and accumulation, ensuring optimal conditions for sustainable and productive macroalgal growth.

Transition to a custom fertilizer

To enhance and further optimize the cultivation process, the transition from F2 medium to a customized fertilizer was undertaken. This custom formulation was tailored to the specific physiological requirements of the cultivated macroalgae species and the unique conditions of the production system.

The new fertilizer is predominantly based on nitrogen and phosphate sources, allowing for precise adjustment of the (N:P) ratio. Adjusting this nutrient ratio is particularly beneficial, as different species such as *Ulva* spp., *Palmaria palmata*, and *Gracilaria* spp. have distinct nutritional needs.

While the exact formulation remains confidential, its implementation has yielded clear benefits, including improved offering nutrient efficiency, improving growth rates, and greater biomass yields. Additionally, this strategic shift has contributed to a significant reduction in nutrient waste, thereby supporting sustainable production practices and reducing environmental impact.

6.3. Systems assembly

Seaweedland, as a pilot-scale company, continuously adapts and optimizes its production systems. This process involves the periodic reassessment and restructuring of existing systems, to improve efficiency and maintain optimal cultivation conditions.

Restructuring of the production system

One such restructuring initiative was driven by the need to expand the production of *Palmaria palmata* during the summer months. This species requires lower temperatures than *Ulva* spp., necessitating the reallocation and modification of raceway systems, originally used for *Ulva* spp. To meet these specific thermal requirements, a temperature control system was installed to maintain tanks temperatures at approximately 10ºC.

The system involved connecting a chiller unit to a saltwater reservoir maintained at 8ºC. The chilled water from the reservoir was then circulated to the cultivation tanks. To prevent cross-contamination, each tank was equipped with a dedicated piping system. Water was recirculated through a UV disinfection unit before cooled and returned to the tanks, ensuring both thermal regulation and biosecurity.

Installation of automatic sensors

One of Seaweedland's objectives is to increase the efficiency of monitoring the physicochemical parameters of water. To achieve this, an automated system for recording environmental data in the cultivation tanks was implemented. This system includes sensors for pH, temperature, turbidity and salinity, which are installed adjacent to the raceways and interconnected with an Arduino system that collects and transmits data in real time (Figure 17).

The integration of this system with the Adafruit platform allows the remote access to the parameters via mobile devices, thereby optimizing operational control and reducing the need for constant manual monitoring.

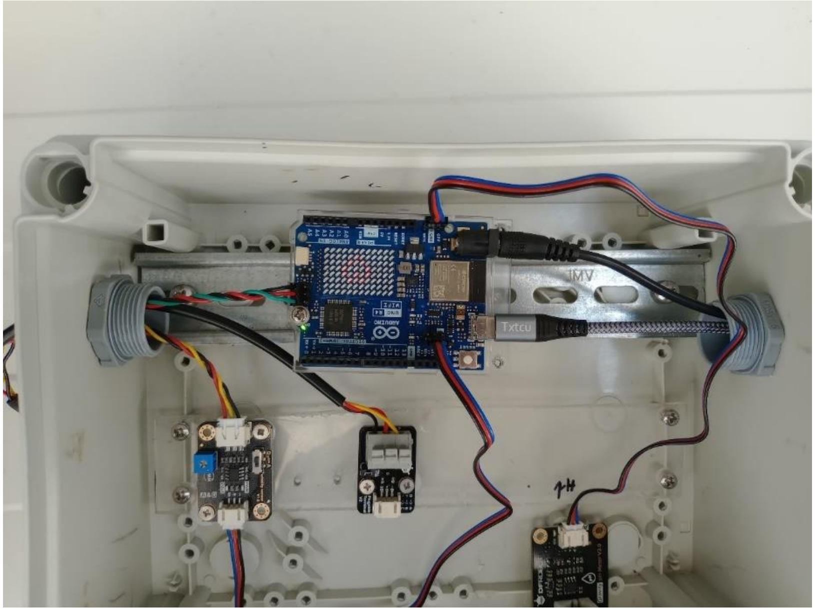

Figure 17 – Hardware installation for the automated monitoring system, including sensors for continuous measurement of pH, temperature, turbidity, and salinity in cultivation tanks. Photo: Paulo Pereira

Installation of artificial lighting

Seasonality imposes significant challenges due to the low availability of natural light during the winter months, which impacts macroalgae growth. To address this limitation, artificial lighting systems were installed in all cultivation tanks (Figure 18).

This process required detailed planning and the installation of multiple light points to ensure a homogeneous distribution of light intensity across the raceways. Different Philips lighting systems were utilized, ranging from white to pink/red spectra (Figure 19).

Figure 18 – Philips white-light illumination systems installed above a raceway for *Ulva* cultivation, providing supplemental lighting to optimize growth conditions. Photo: Paulo Pereira

Figure 19 – Philips red/pink spectrum lighting system, utilized to investigate and enhance growth performance and physiological responses of macroalgae under specific wavelength conditions. Photo: Paulo Pereira

Large raceway filtration system

The implementation of a filtration system in the 10,000 L raceways was essential to maintaining water quality (Figure 20). The process involved the following steps:

1. Planning of the piping and drainage system, with special attention to the outlet drain of the raceways.
2. Installation of recirculation pumps, each with a minimum capacity of 3000 L/h, ensuring high and continuous water flow.
3. Implementation of mechanical filtration, using 10-to-100-micron sock filters to remove suspended particles and sediments.
4. Integration of a UV system to promote water disinfection and eliminate harmful microorganisms (Figure 21).
5. Division of the water flow, directing one portion to the CO_2 injection system and another, with higher flow directly to the cultivation tanks.

Figure 20 – Construction and completion stages of the filtration system designed specifically for the 10,000 L raceways, featuring mechanical filtration to remove particulate matter and ensure optimal water quality. Photo: Paulo Pereira

Figure 21 – UV filtration system utilized to eliminate undesirable microorganisms and maintain water quality within cultivation tanks. Photo: Paulo Pereira

Water Treatment Storage Tank

The saltwater storage tank, with a capacity of 75 m^3, requires continuous treatment to prevent the proliferation of bacteria, microalgae and other potential contaminants. For this, a water treatment system was implemented, consisting of the following components:

1. Two Skimmers, responsible for the removal of sediments and organic matter through foam fractionation.
2. Controlled ozone injection, applied within the skimmers to oxidize organic compounds and pathogens, ensuring an oxidation-reduction potential (ORP) above 600 mV. This parameter is constantly monitored to avoid excessive levels of oxidation.
3. Recirculation pumps, strategically positioned at the end opposite the skimmers, ensuring a continuous water flow and avoiding stagnation zones (Figure 22).
4. Mechanical filtration and UV system, located outside the storage tank, and used during water transfer to the cultivation tanks. This stage reduces impurities and restores ORP balance prior to use in the production systems.

Figure 22 – Recirculation pumps strategically positioned at the opposite end from skimmers, ensuring effective circulation and homogeneous water conditions within the storage reservoir. Photo: Paulo Pereira

6.4. Maintenance, cleaning and conditioning of tanks

The maintenance and cleaning of cultivation systems are fundamental steps to ensure the longevity of the equipment, avoid contamination, and maximize production efficiency. A preventive and corrective maintenance protocol is implemented, which includes the repair of tanks and equipment, cleaning of pipes and reservoirs, and control of unwanted organisms, such as diatoms and other biofouling agents (Figure 23).

Figure 23 – Cleaning process of cultivation tanks following batch completion, focusing on the removal of accumulated diatoms and other biofouling organisms to ensure optimal conditions for subsequent cultures. Photo: Paulo Pereira

Repair of fiberglass tanks

Fiberglass tanks are widely used due to their durability, corrosion resistance, and ease of repair. However, structural damage such as cracks or small leaks can compromise the integrity of the system. Repairs were conducted on several tanks (Figure 24). The repair process follows a specific procedure, which includes:

1. Surface preparation – The damaged area is cleaned and sanded to remove impurities and ensure better adhesion of the repair material.
2. Cutting the fiberglass fabric – The material should fully cover the damaged area, extending about 5 cm beyond its edges. For deeper cracks, multiple layers of fiberglass are applied.
3. Preparation of the resin and hardener – The mixture is prepared using a ratio of 50 mL of resin to 1 mL of hardener, ensuring the appropriate application consistency.
4. Application of the resin and reinforcement - The resin is applied along with multiple layers of fiberglass to reinforce the repair.

Figure 24 – Maintenance and repair work on fiberglass cultivation tanks, involving resurfacing and sealing to prevent leaks and extend tank durability. Photo: Paulo Pereira

Control of biofouling and contamination

Establishing contamination free cultures is a critical step in macroalgae cultivation, as it ensures optimal growth by minimizing the presence of epiphytes and other opportunistic organisms. Diatoms are the most persistent and problematic contaminants, frequently introduced into culture systems via natural seawater or contaminated algal material.

In land-based cultivation systems, diatom colonization is commonly observed on the surfaces of tanks and cultivation equipment. These microorganisms not only compete directly with macroalgae for essential nutrients and light, but can also significantly impair biomass productivity (Rautenberger, 2024).

Decontamination protocols have identified several diatom species commonly associated with macroalgae cultures, including *Navicula* sp., *Nitzschia* sp., and *Sahlingia subintegra* (Fernandes et al., 2011). Preventing and managing their presence is therefore fundamental for maintaining the integrity and performance of macroalgal cultivation systems.

To mitigate this problem, germanium dioxide (GeO_2) is added to the cultivation tanks. This compound selectively inhibits diatoms growth, as diatoms require silicon to construct their silica-based cell walls (frustules) (Maher *et al.,* 2018). Due to its chemical similarity to silicon, germanium is absorbed by diatoms in its place, disrupting cell wall synthesis and ultimately preventing growth (Reid *et al.,* 2021).

Unlike diatoms, macroalgae species such as *Ulva* spp. and *Palmaria palmata* do not depend on silica for cell wall formation. As a result, GeO_2 does not adversely affect their development when used at appropriate concentrations. It is applied at 1–2 mg/L, limited to the initial phases of cultivation. This strategy helps maintain clean culture conditions, reduces competition for light and nutrients, and enhances overall macroalgae performance in aquaculture systems. Additionally, it minimizes the need for manual cleaning of tank surfaces, a time-consuming and costly task (Rautenberger, 2024).

Cleaning and disinfection of cultivation tanks

When a cultivation cycle ends or contamination is detected, cleaning and disinfection of the tank is required. The protocol consists of the following steps:

1. Addition of sodium hypochlorite (5%) to the system, allowing circulation for 24 hours to ensure the elimination of microorganisms, algae and biofilms.
2. Complete drainage of the water, followed by removal of equipment such as recirculation pumps, CO_2 reactors, UV system and piping.
3. Washing of all equipment and tank with freshwater, ensuring complete removal of sodium hypochlorite residues.
4. Drying of the equipment or recirculation with treated salt water to prepare for reuse.

The 5% sodium hypochlorite solution is prepared internally by diluting a 12% sodium hypochlorite solution in distilled water. For a 1,000L tank, 5 liters of 5% sodium hypochlorite are used.

Maintenance of filtration equipment and systems

All cultivation equipment, such as water pumps, CO_2 diffusers, and UV systems, requires periodic maintenance to avoid deterioration due to salt accumulation and other mineral deposits. When not in use, equipment is rinsed with fresh water and properly stored in a dry place.

Maintenance of the filtration system is performed according to a structured schedule or in response to specific operational issues. The procedures are as follows:

1. Water pumps and UV system – thoroughly cleaned monthly to remove accumulated debris (Figure 25).
2. Mechanical filtration – Filters are replaced weekly or whenever they are clogged.
3. Pipes are cleaned biweekly using a steaming machine to avoid biofilm buildup, obstructions and loss of hydraulic efficiency.
4. Cultivation tanks – Evaluated and cleaned based on organic matter levels or when species are changed.

Figure 25 – Maintenance and cleaning of water pumps to prevent the accumulation of organic residues and ensure optimal functioning of the recirculation system. Photo: Paulo Pereira

6.5. Sampling

The implementation of regular sampling protocols is essential for optimizing cultivation parameters and generating scientific data necessary for scaling up the cultivation system.

Sampling to assess growth was carried out weekly (Figure 26). During each event, a portion of the biomass was harvested to maintain optimal cultivation density. This density had previously been identified as yielding the best growth rates. The excess biomass was either sold fresh or transferred to a drying facility for subsequent preparation and commercialization.

Each sampling event also included macroscopic observation of the seaweed to assess visual health indicators such as color, texture, size, and the presence of potential contaminants, epiphytes, or biofouling.

Figure 26 – Weekly biomass sampling from the tanks, used for subsequent analysis of growth rates (DGR%) and assessment of seaweed quality. Photo: Paulo Pereira

Given the size of the large raceway tanks, it was not feasible to harvest the entire biomass for weighing. Instead, sampling was performed by selecting 1-meter sections of the tank (Figure 27). The biomass collected from these representative sections was weighed and the values extrapolated to estimate the total biomass within the entire raceway. This method proved effective in providing accurate biomass estimates while significantly reducing the labor and time required for full harvests.

Figure 27 – Biomass sampling performed by selecting 1-meter sections from the *Palmaria palmata* raceway. Photo: Paulo Pereira

The drying process of *Ulva* spp. and *Palmaria palmata* primarily relied on natural sun-drying, where the seaweed was laid out under direct sunlight until fully dehydrated. Once dried, it was packaged for storage or distribution (Figure 28).

However, during periods of low sunlight intensity and reduced ambient temperatures, an electric dryer was employed to ensure consistent drying conditions and preserve product quality. This approach allowed continuous processing regardless of weather variability, thereby providing a stable supply of dried biomass.

Figure 28 – Drying process of *Ulva* spp. and *Palmaria palmata* using natural sunlight until complete dehydration. Photo: Paulo Pereira

6.6. Cleaning and selection of seaweed

As highlighted by Fernandes *et al.* (2011), one of the biggest challenges in macroalgae cultivation is ensuring that cultures remain free from contamination by other macroalgae, microalgae, cyanobacteria, and small invertebrates. When new cultures are introduced, they must undergo a thorough cleaning procedure to eliminate epiphytes and potential contaminants (Figure 29). These contaminants grow on the surface of the host algae, competing for nutrients and light, thus reducing growth rates and deteriorate crop quality.

Figure 29 - *Gracilaria* spp. contaminated with filamentous green algae. Photo: Paulo Pereira.

To ensure contamination-free crops, it is necessary to implement cleaning protocols, which may include both chemical and physical treatments (Fernandes *et al.,* 2011). While each method independently reduces contamination, combining both tends to yield better results.

1. Chemical treatments: These involve the use of antibiotics, germanium dioxide, and disinfectant solutions such as sodium hypochlorite (Rautenberger, 2024; Fernandes *et al.,* 2011).
2. Physical treatments: Techniques such as ultrasound systems, brushing, and manual removal of contaminats are employed (Fernandes *et al.,* 2011).

Cleaning protocol for *Gracilaria* spp.

For *Gracilaria* spp., a structured cleaning and selection protocol was followed to ensure a contamination-free culture:

Physical Cleaning

1. Washing: thallus segments were rinsed with previously treated saltwater to remove loose particles and contaminants.
2. Manual removal: visible epiphytes were manually removed using scalpel forceps.
3. Distilled water treatment: The cleaned *Gracilaria* was immersed in distilled water for 30 seconds to 1 minute to weaken any remaining contaminants.

Incubation

1. Temperature control: The cleaned biomass was transferred to an incubator maintained at 15ºC. The incubation process lasted 3 months.
2. Monitoring period: Weekly maintenance included weighing the biomass to track daily growth rates (DGR), as well as regular cleaning and removal of emerging contaminants.
3. Precision-based cleaning: Brushing and manual removal of contaminated regions were essential for obtaining clean cultures quickly. However, the effectiveness of this procedure depended on the precision of the operators (Fernandes *et al.,* 2011).

Selection criteria

During the cleaning and selection process, only male individuals of *Gracilaria* spp. were selected for inclusion in the production system. Female *Gracilaria* spp. tend to allocate a portion of their metabolic energy towards cystocarp formation following fertilization, potentially reducing resources available for vegetative growth. In contrast, male individuals are generally favored due to their comparatively higher growth rates and overall ease of cultivation, compared to female or tetrasporic individuals (Figure 30).

The initial batch of *Gracilaria* spp., originating from Portugal, presented high levels of contamination with epiphytic algae. These epiphytes compete intensely with the host species for essential resources such as nutrients and light, potentially reducing growth performance and biomass productivity. Therefore, a rigorous selection and cleaning protocol was implemented, in which male and female individuals were carefully separated. The selected individuals were then maintained at 15ºC with an F/4 fertilizer solution. The F/4 formulation provides essential nutrients and trace elements tailored for maintaining cultures, rather than promoting rapid growth. F/4 medium is essentially a diluted version of F/2, containing half the nutrient concentrations found in F/2 medium.

Figure 30 - Female *Gracilaria* spp. with clearly visible cystocarps. Cystocarps are reproductive structures containing the carpospores. Photo: Paulo Pereira

A selection process for tetrasporic individuals (tetraspores) was initiated, involving their cultivation under two distinct temperature regimes (15ºC and 20ºC). Over a period of approximately two months, these tetraspores underwent weekly cleaning and inspection procedures aimed at consistently removing any emergent contamination. This meticulous protocol ensured the establishment of a backup stock of *Gracilaria* spp. that was free from contaminants, thereby safeguarding future cultivation cycles.

The implementation of such a selection and cleaning process is essential, as contamination by epiphytic macroalgae or other competitive organisms can significantly impair macroalgal growth and productivity (Figure 31). Although this protocol required considerable time and effort, establishing a clean and contamination-free initial batch is a crucial step toward the long term success and sustainability of macroalgae production systems.

Figure 31 – Epiphytic organisms surrounding *Gracilaria* spp. Photo: Paulo Pereira

7. R&D Laboratory to production

A series of laboratory trials were conducted to gather data for optimizing the macroalgae cultivation process. These experimental studies served as a foundation for scaling-up the cultivation to larger production tanks.

Although the specific protocols and experimental data remain confidential, the trials systematically evaluated key parameters such as daily growth rate (DGR %), surface area coverage, seaweed quality (e.g., color, texture), and overall biomass productivity.

The trials focused on refining and validating several essential aspects of macroalgal cultivation for the target species, including:

- Determination of optimal stock density.
- Determination of optimal temperature.
- Evaluation of alternative fertilizer formulations to enhance nutrient efficiency and reduce costs.
- Supplementation trials with additional elements, such as magnesium.
- Comparative analysis of water sources, analyzing the effects of different water qualities from potential expansion sites.

Insights gained from these laboratory-scale experiments provided a solid framework for informed decision-making during the transition to large-scale production.

8. Production

***Ulva* spp.**

The cultivation of *Ulva* spp. primarily relies on vegetative propagation, aiming to maintain consistent and long-term biomass production. Cultivation systems such as raceways or tumbling tanks, are managed to maintain optimal biomass density, enabling periodic harvesting of excess material in response to market demands.

Environmental challenges, particularly those associated with seasonal variations, significantly affect *Ulva* spp. cultivation. Abrupt temperature fluctuations and reductions in photoperiod can lead to sporulation - an asexual reproductive event characterized by fragmentation of thalli, consequently reducing the overall growth rates.

Interestingly, post-sporulation of residual biomass within cultivation systems often results in a substantial increase in subsequent growth rates. This may reflect a recovery or adaptation mechanism, including the germination and growth of spores.

The occurrence of sporulation highlights the importance of environmental management strategies aimed at maintaining stable cultivation conditions. The use of artificial illumination to mitigate reductions in natural photoperiod and application of thermal regulation devices such as chillers and heaters to control temperature fluctuations have proven to be effective. Such interventions not only reduce the negative impacts of seasonal variability but also significantly improve productivity.

Induction of sporulation in *Ulva* spp.

Gametogenesis in *Ulva* spp. was achieved through physical stimulation via fragmentation washing of the thalli. The protocol involved cutting the thalli into smaller fragments, followed by three sequential five-minute washes using sterile artificial seawater. The cleaned fragments were then transferred to Petri dishes containing fresh artificial seawater.

Environmental conditions were carefully and rigorously controlled: fragments were incubated at a constant temperature of 15°C under a photoperiod of 16 hours of light and 8 hours dark.

Direct microscopic observation confirmed spore release, validating the success of the induction protocol. This method represents a practical approach for controlled reproduction, supporting optimized cultivation and scale-up for commercial production.

Spontaneous sporulation in raceway cultivation

A spontaneous sporulation event was observed in 1,000L raceway systems cultivating *Ulva* spp., at the onset of winter, coinciding with significant environmental shifts, including drop in temperature and reduction in natural light intensity.

This seasonal transition triggered widespread sporulation, underscoring the sensitivity of *Ulva* spp. to abrupt environmental fluctuations. The sporulation event resulted in a considerable reduction in biomass and growth rates.

However, after the implementation of artificial lighting, substantial recovery in biomass growth was observed. The supplementary lighting compensated for reduced natural illumination, providing adequate photosynthetic stimulus and continuous growth, even under water temperatures below 10°C.

This occurrence emphasizes the necessity of continuous monitoring and proactive management of environmental parameters in aquaculture systems. Detailed understanding of macroalgal responses to environmental variations is crucial for developing effective cultivation strategies, minimizing productivity losses from spontaneous sporulation, and ensuring consistent biomass yield.

Palmaria palmata

Following the selection and nursery propagation of *Palmaria palmata*, production scale-up is conducted outdoors during the colder months, specifically when water temperatures fall below 10ºC. Under these favorable environmental conditions, biomass growth is effectively carried out in both 1000-liter raceways and tumbling tanks. When seasonal fluctuations result in water temperatures exceeding 13–15ºC, cultivation is either relocated to a temperature-controlled hatchery facility or managed externally with the integration of a chiller to maintain optimal conditions.

Strict biosecurity protocols are crucial in this cultivation system to prevent cross-contamination, especially from *Ulva* spp., which can rapidly proliferate rapidly and compromise *Palmaria* cultures. To mitigate this risk, specific measures are implemented, including the use of dedicated handling nets for each tank and the mandatory use of disposable gloves when manipulating algal biomass. These rigorous practices are essential for maintaining the integrity and purity of the cultivated *Palmaria palmata*, thereby ensuring consistent production quality.

Commercialization

Seaweeds can be commercialized either fresh or dried form, depending on market demand and the intended application. For fresh seaweed, the biomass is packaged in insulated polystyrene containers with ice packs to preserve its quality and freshness, and to prevent degradation during transportation.

In the case of dried seaweed, the complete removal of moisture is critical to avoid enzymatic activity and fungal growth, both which can negatively impact the product's nutritional and commercial value. After dehydration, seaweed can be processed and packaged in various formats, including flakes or finely ground powder, in accordance with consumer preferences and industrial requirements (Figure 32).

Figure 32 – Packaged of *Ulva* flakes after dehydration. Photo: Paulo Pereira

Scale-up production to raceways of 10,000 liters

Preparation activities for the installation of the two raceways began in August, starting with ground clearance and leveling at the designated tank installation site (Figure 33).

Figure 33 – Works on the ground of the raceway. Photo: Paulo Pereira

The construction phase for scaling up production using two PVC-lined raceways, each measuring 15 m by 2 m and with an approximate capacity of approximately 10,000 liters, started in September. Each raceway was equipped with a 90 cm diameter paddlewheel and two strategically positioned drainage outlets. One located near the front of the tank and the other adjacent to the paddlewheel. This configuration ensured efficient water circulation, preventing algal accumulation within the raceways.

Production of *Palmaria palmata* and *Ulva* spp. started in January. The initial weeks were critical for fine-tuning and optimizing the cultivation systems, enabling the identification and improvement of key operational aspects to enhance overall productivity and system performance (Figure 34).

Figure 34 – On the left, a photo of the raceway after completion, filled with water for leak testing. On the right, the raceway after successful testing, with *Ulva* spp. introduced to initiate production. Photo: Paulo Pereira

9. Cultivation procedures

9.1. Cultivation typology

Hatchery for cold water species

The hatchery provides a highly controlled environment that supports the initial growth of seaweeds while minimizing the influence of external factors such as contamination, temperature fluctuations, and light variations (Figure 35).

An example of a species cultivated in the hatchery is *Palmaria palmata*, which is maintained at 8ºC until the scale-up to larger outdoor tanks. Cultivation is carried out in five 1000L tumbling tanks, each representing a distinct growth stage. Culture density is maintained at a maximum of 5 g/L, with controlled temperature, Ultraviolet filtration system, and a 600 L/hour recirculation pump. No CO_2 injection is applied. Daily monitoring of pH and salinity ensures values remain below 8.5 and 35, respectively. The fertilization regimen consisted of the addition of 100 mL of F2 medium three times per week. Light intensity was adjusted to 80 µmol photons m^{-2} s^{-1} to optimize photosynthetic efficiency.

Figure 35 – Production of *Palmaria palmata* gametophytes in a 10-L glass bottle within the hatchery. The system includes aeration, visible by the tubing generating bubbles, which maintains water circulation. Photo: Paulo Pereira.

Hatchery for warm water species

The cultivation of *Gracilaria* spp. was maintained in an incubator at 15ºC. Despite the implementation of cleaning and selection protocols, challenges persist, including the emergence of green filamentous algae contaminations, which compromises biomass quality, and episodes of whitish water, potentially associated with microbiological factors.

Greenhouse – Raceways

The greenhouse environment reduces external environmental impacts, such as rainfall-induced salinity fluctuations and contamination. However, during summer, air temperatures can reach up to 40ºC, requiring cooling strategies to maintain optimal growth conditions for the cultivated species.

The maximum tolerated temperatures for species cultivated greenhouse raceways are 14ºC for *Palmaria palmata* and 20ºC for species of the genus *Ulva*.

To maintain these conditions, a 15,000 liters water tank equipped with a chiller was used for temperature regulation.

Cultivation was conducted in four 1000L raceways, each equipped with a CO_2 injection system, pH control to maintain values below 8, a UV filtration system, and a 600 liters/hour water recirculation pump (Figure 36).

Figure 36 – Water recirculation system equipped with a 600 liters/hour recirculation pump and UV sterilization unit. Photo: Paulo Pereira.

Following the initial cultivation phases, production was expanded with the addition of two 15,000 liters raceways. These new systems were equipped with:

- Recirculation pumps with a capacity of up to 10,000 litres/hour.
- 50-micron mechanical filtration to effectively remove suspended particles.
- UV disinfection system (AquaForte's), with a minimum treatment capacity of 15,000 liters.
- CO_2 injection system to support optimal carbon availability.
- Artificial lighting system used during winter months when natural photoperiods are reduced.

Greenhouse – Tumbling tanks

During winter, ambient temperatures can drop to 0ºC and water temperatures to 5-6ºC. These conditions are favorable for the cultivation of *Palmaria palmata*, allowing its transfer from the hatchery to the greenhouse while maintaining previous operating conditions.

9.2. Cultivation technical components

Controlled macroalgae cultivation requires a range of specialized technical components to ensure process efficiency. Key components include ultraviolet filtration systems, CO_2 injection, cooling equipment, mechanical filtration, ozonation systems, and various pumps to guarantee effective water recirculation.

Software & Monitoring

Water quality monitoring was initially conducted with AdaFruit software, later replaced by OxyGuard for enhanced precision. For production management, Rubisko software was used to analyze data and optimize water usage and system performance.

UV filtration system

The ultraviolet filtration system is essential for eliminating microorganisms in the cultivation environment. Each tank is fitted with and individual UV system tailored to its volume: 200L tanks - XClear UV-C Economy 9W, 230V System,1000L tanks - AquaMedic Helix Max 2.0 SSW UV system, 220-240V, 15,000 L raceways - AquaForte 75W, with a minimum filtration capacity of 15,000L.

Aeration and water motion

Seaweeds require efficient carbon supplementation and water movement to facilitate gas exchange and nutrient absorption (Harrison & Hurd, 2001). Water circulation is achieved through paddlewheels in raceways, air pumps connected to diffuser stones or perforated pipes, ensuring constant movement of algae biomass.

Aeration is provided by AquaForte Model V-60 air pumps (220-240V, 35W). However, during warmer months, the use of aeration is challenging, as the ambient air temperature may exceed 30ºC, thereby increasing water temperature and necessitating additional energy for cooling.

Water supply and treatment

Saltwater is supplied to the cultivation facility via tanker trucks ensuring continuous and controlled input. Upon arrival, the water is stored in a 70 m^3 reservoir, passing through a 1 μm and 0.2 μm mechanical filtration system, to remove suspended particles.

To maintain optimal water quality, treatment includes ozonation and particle removal via a protein skimmer. This process eliminates harmful microorganisms, reduces dissolved organic matter, and minimizes the risk of macroalgae contamination.

When water is required for cultivation, specific pumps direct the flow through a mechanical filtration unit followed by ultraviolet (UV) sterilization. The UV system further reduces microbial load and degrades any residual ozone, ensuring the cultivation tanks is free from harmful pathogens and oxidative compounds.

Ozonization

Ozone treatment is conducted using two AquaMedic Ozone 400 generators, which inject ozone into the water within the skimmer (Figure 37). The primary objectives are the elimination of opportunistic bacteria, microalgae, and other potential contaminants.

The ozone concentration is monitored with a Milwaukee digital ORP controller, ensuring oxidation-reduction potential (ORP) is maintained at 600 mV, the threshold required for effective disinfection.

Before being introduced into the cultivation systems, ozonated water undergoes UV filtration to neutralize any residual ozone. Final ORP values are manually monitored to ensure the safety and suitability of the treated water.

Figure 37 – Monitoring system showing the Milwaukee Digital Controller (left) and the AquaMedic Ozone 400 system (right). Photo: Paulo Pereira.

Mechanical filtration

In larger raceways, mechanical filtration plays a critical role in removing suspended particles, organic debris associated with macroalgae, and other sediments that accumulate in the tanks (Figure 38).

Each mechanical filter is fitted with filter socks ranging from 10 to 100 micron, ensuring efficient removal of impurities while maintaining adequate water flow throughout the system. The replacement and routine cleaning of filters socks are essential to sustain filtration efficiency. In the *Palmaria palmata* system, filters were replaced twice weekly, whereas in *Ulva* spp. cultures, more replacement was required due to higher organic load.

Figure 38 – Mechanical filtration system in 10,000 L raceways, showing the housing unit and filter socks ranging from 10 to 100 microns. Photo: Paulo Pereira

Skimmer

To assist in the removal of dissolved organic matter and suspended particles, two AquaMedic Turboflotor 5000 single 6.0 skimmers were installed in the water reservoir tank (Figure 39).

Each skimmer is equipped with an AquaMedic DC Runner 600 pump, which includes a brush wheel mechanism designated to enhance ozone diffusion in the water and facilitate the separation of solid particles, through foam formation. The pump speed is adjustable, allowing for the optimization of foam formation and, consequently, more efficient removal of suspended solids from the water column.

Figure 39 – AquaMedic Turboflotor 5000 skimmer installed in the reservoir tank for particulate matter removal and ozone injection. Photo: Paulo Pereira

Carbon Dioxide (CO_2) injection System

Carbon dioxide (CO_2) plays an essential role in macroalgae cultivation, as algal tissue consists of approximately 45-50% carbon. The presence of CO_2 in the culture medium directly affects the availability of dissolved inorganic carbon in the water and, consequently, affects algal photosynthesis of algae growth (Leegood *et al.,* 2000).

A pertinent aspect in macroalgal cultivation systems is maintaining a stable pH, with carbon dioxide injection serving as an important tool for pH regulation. When CO_2 dissolves in water, it forms carbonic acid, thus lowering the pH. As macroalgae assimilate carbon, the pH tends to rise due to carbon uptake. Therefore, by controlling CO_2 injection, it is possible to regulate both carbon availability and the chemical stability of the culture environment.

Carbon dioxide is added through a Sera Flore CO_2 active reactor 1000) (Figure 40), and its injection is regulated by an automated Dennerle pH control system, which activates CO_2 release when the pH exceeds 7.30.

Flow regulation is managed by an electronic solenoid valve, which responds to electrical signals to open or close the gas passage. The system includes a CO_2 cylinder pressurized to 50 bar, with an average consumption of one bottle every 40 days for four 1000L raceways (Figure 40).

Beyond supporting photosynthesis, CO_2 injection also helps mitigate the effects of elevated dissolved oxygen concentrations, which have linked to photo-oxidative damage to chlorophyll, potentially reducing photosynthetic efficiency in macroalgae (Ledford & Niyogi, 2005).

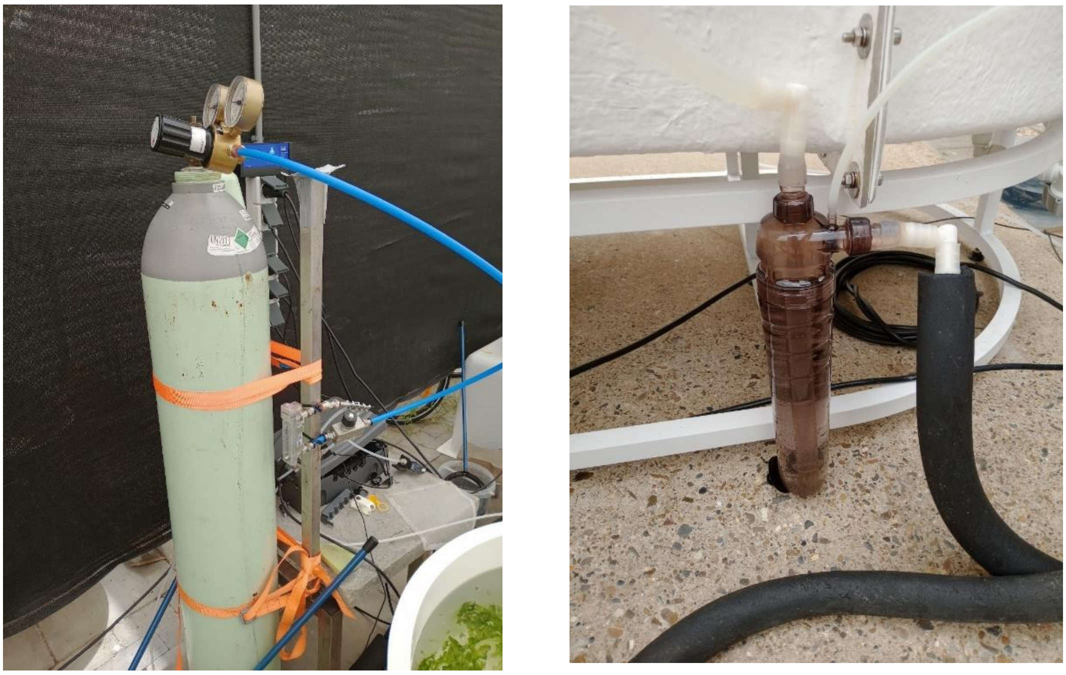

Figure 40 – Carbon dioxide cylinder (left) and Sera Flore CO_2 Active Reactor 1000 (right). Photo: Paulo Pereira

Lighting system

During the winter months, natural light incidence is insufficient to support the optimal growth of the cultivated macroalgae. To compensate for this limitation, supplementary lighting is employed in the cultivation tanks. Natural luminous intensity, typically around 50 μmol photons $m^{-2}s^{-1}$, is increased to 150-200 μmol photons m^{-2-1}s using Philips lighting systems.

Water renewal rate

The water renewal rate is a determining factor for maintaining optimal water quality. Each 1000L raceway has a continuous renewal flow of approximately 600 L/hour, ensuring a good filtration rate by the UV system.

In 15,000L raceways, the renovation is carried out by means of a pump with a maximum capacity of 10,000 L/hour, ensuring efficient circulation.

Chiller tank

Temperature is one of the abiotic factors that directly influences the growth, reproduction, and overall performance of macroalgae (Stévant *et al.,* 2023). Because the cultivation systems are located within a greenhouse, water temperature can increase significantly, particularly during warmer months.

To prevent undesirable temperature fluctuations and ensuring optimal growing conditions, an adjacent tank equipped with a Cool Cube C3 Khione Cold Therapy chiller is used. This system maintains water temperatures between 6-8ºC, which is essential for the successful cultivation of cold-water species such as *Palmaria palmata*, which require lower temperatures for optimal growth.

10. Other tasks

Inoculation of Seaweed Seedlings onto ropes

Seeding refers to the process of inoculating substrates, such as ropes, with macroalgae propagules or gametophytes prior to their deployment at sea or use in research. Effective macroalgae cultivation in marine environments requires adequate seedling density on these substrates. A thorough understanding of the macroalgal life cycle, particularly in kelp species, is crucial for successful seeding and selective breeding strategies.

Kelps exhibit a biphasic life cycle consisting of two distinct stages: a macroscopic sporophyte phase and a microscopic gametophyte phase (Figure 41). The fronds commonly observed in natural environments constitute the sporophyte generation. Upon reaching maturity, these sporophytes develop specialized reproductive tissues called sori, which produce and release billions of haploid, bi-flagellated zoospores (meiospores) into the surrounding water. These zoospores exhibit limited motility and must successfully attach to a suitable substrate within approximately 48 hours of release to ensure further development (Redmond *et al.,* 2014).

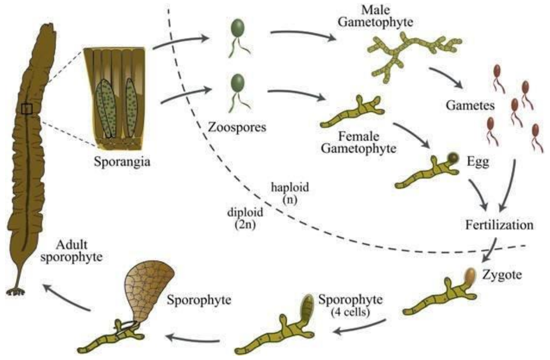

Figure 41 – Life cycle of kelp. Source: Visch *et al.,* 2019

Upon settlement onto a substrate, zoospores shed their flagella and enter the microscopic gametophyte phase. Initially, the spores develop a germ tube and translocate their cellular contents into a primary cell, forming a structure commonly referred to as the "dumb-bell stage." This critical developmental transition typically occurs within 48 hours post-settlement. Subsequently, gametophytes enter a vegetative growth phase characterized by cellular expansion and the formation of microscopic filaments, which may range from only a few to several hundred cells in length. After approximately one to two weeks of culture, morphological differentiation into male and female gametophytes becomes evident (Redmond *et al.,* 2014).

Gametophyte maturation progresses under favorable environmental conditions, including optimal light intensity, sufficient nutrient availability, an appropriate photoperiod, and controlled temperature. Mature female gametophytes develop oogonia containing eggs, while mature male gametophytes form antheridia that release spermatozoids. During fertilization, female gametophytes emit a pheromone known as lamoxirene, which acts as a chemical signal to attract the motile spermatozoids (Maier & Muller, 1986). Following successful fertilization, a diploid zygote is formed. This zygote undergoes longitudinal division and gives rise to a juvenile sporophyte that develops directly on the female gametophyte (Figure 42). Concurrently, small rhizoids emerge and eventually differentiate into a holdfast structure, facilitating the attachment of the juvenile sporophyte to the substrate (Redmond *et al.,* 2014).

Figure 42 – Development of three sporophytes emerging from a fertilized female gametophyte. Photo: Alexander Ebbing

An advanced understanding of the kelp life cycle stages is essential for laboratory manipulation during the seeding process, particularly in controlled cultivation and selective breeding practices. Two primary methods are employed for seeding kelp onto substrates: twine seeding and direct seeding. Both approaches require reliable spore availability, which necessitates the controlled induction of sporogenesis. In laboratory settings, sporogenesis is initiated by excising meristematic tissues from mature sporophytes and maintaining them under controlled environmental conditions (typically 10 °C, an 8:16-hour Light:Dark photoperiod, and a light intensity of 80–130 µmol photons m^{-2} s^{-1}). Regular seawater exchange (twice weekly) and the maintenance of optimal nutrient concentrations (e.g., 200 µM NO_3^{-}-N) are critical factors to stimulate efficient spore production. Spores are subsequently harvested from these induced, sori-containing sporophytes and inoculated onto the chosen substrates (Boderskov *et al.,* 2021).

Twine Seeding

Twine seeding, commonly known as spore seeding, represents a conventional and widely used method in macroalgae aquaculture, particularly for kelp cultivation (Figure 43). In this method, prepared coils of kuralon twine are initially arranged horizontally in containers filled with artificial seawater. These containers are maintained under rigorously controlled environmental conditions, typically a stable temperature of 10 °C, a light intensity of approximately 60 µmol photons m^{-2} s^{-1} at the water surface, and a photoperiod of 16 hours of light and 8 hours of dark. Spore extracts derived from mature sorus tissues are evenly dispersed over the twine coils at a concentration of 7,500 spores mL^{-1} of seawater. To ensure uniform spore settlement, the coils are rotated regularly during the initial incubation period.

After an initial incubation of 48 hours, the coils are repositioned vertically in larger nursery tanks, improving light exposure and promoting homogenous juvenile sporophyte development around the entire circumference of the twine. To support continuous growth during the nursery phase, seawater is exchanged biweekly, and nutrient levels are rigorously monitored and maintained to support continuous growth. The nursery stage typically spans at least six weeks, during which the spores develop into juvenile sporophytes measuring approximately 1-2 mm in length. At this developmental stage, the twine with the attached juvenile sporophytes is ready for transplantation onto longlines positioned in open-sea environments for grow out (Boderskov *et al.,* 2021; Redmond *et al.,* 2014).

Although the twine seeding technique generally results in higher biomass yields compared to direct seeding, it also incurs greater operational costs and requires substantial hatchery space and resources.

Figure 43 – Twine seeding method and subsequent deployment of kelp lines at sea. Source: Nautical Farms

Direct seeding

In contrast to twine seeding, direct seeding begins with the establishment of gametophyte cultures under controlled environments. Spores are introduced into culture vessels that contain multiple plates, which facilitate effective attachment and proliferation of gametophytes. In this approach, male and female gametophytes are co-cultured, allowing natural gametogenesis without physical separation.

Under continuous red-light exposure, the gametophytes undergo vegetative proliferation, increasing in both biomass and density. Fertility induction is triggered by switching the light regime to white light for three weeks prior to the planned seeding operation. This photoperiod shift stimulates sexual reproduction, resulting in cultures containing a mixture of fertile gametophytes and newly formed juvenile sporophytes. Following the induction phase, the mixed culture is filtered to significantly reduce the number of gametophytes. The resulting suspension, now a uniform population of juvenile sporophytes, is then blended with a binder solution to facilitate adhesion to cultivation substrates. The final seeding suspension is adjusted to achieve a final density ranging between 165 to 404 sporophytes per milliliter of binder solution (Figure 44). The seeded ropes are immediately placed into plastic bags and stored in the dark at 10 °C to preserve viability. These are typically deployed into open-sea cultivation systems on the following day (Boderskov *et al.,* 2021).

A significant advantage of the direct seeding is the elimination of the prolonged nursery phase, thereby substantially reducing hatchery space requirements and associated operational costs. Additionally, direct seeding facilitates more effective selective breeding, enhances quality control, and supports increased biomass productivity through the cultivation of genetically superior strains (Boderskov *et al.,* 2021).

Figure 44 – Direct seeding technique using algae binder.

Selective Breeding

Selective breeding is an advanced aquaculture technique that facilitates cost-efficiency in macroalgae farming through the enhancement of specific traits, such as biomass yield, chemical composition, stress tolerance, and delayed maturity (Huang *et al.,* 2022). The core objective of selective breeding programs is the sustained genetic improvement of seaweed stocks across successive generations. Through controlled selection, beneficial traits accumulate incrementally with each new generation, building on the genetic improvements achieved from preceding generations (Robinson *et al.,* 2013).

Once superior parental gametophyte strains are identified and isolated, large-scale propagation of these selected genotypes can be initiated. This practice significantly boosts the quality and productivity of seaweed cultivation, ensuring a consistent, year-round supply while reducing dependency on wild stocks and alleviating environmental pressure. Moreover, selective breeding promotes stock uniformity and enables predictable production outcomes (Robinson *et al.,* 2013).

The success of any selective breeding program hinges on the careful selection of the base population, as these initial parental lines form the genetic foundation for future breeding cycles. Effective genetic improvement also requires the species complete life cycle to be controlled in laboratory settings. Synchronization of gamete release is equally critical to allow deliberate pairing of desirable genetic combinations. Throughout the program, it is essential to manage genetic diversity carefully to avoid excessive inbreeding, which can compromise the breeding population´s resilience and limit long-term genetic gain. Without proper evaluation of genetic relationships among breeding candidates, the risk of reduced genetic variation increases, along with heightened vulnerability to diseases and environmental stress (Goecke *et al.,* 2020; Robinson *et al.,* 2013).

The incorporation of genomics, proteomics, transcriptomics, and metabolomics technologies has significantly advanced selective breeding. Metabolomic studies help identify critical biochemical pathways influencing growth, resilience, and biochemical profiles. Metagenomic analyses provide valuable insights into the microbial communities associated with macroalgae and their potential roles in host physiology, growth enhancement, and stress resistance. With increasing access to affordable multi-omics technologies, there is substantial potential to accelerate the domestication, optimization, and sustainable production of economically and ecologically important macroalgae species through selective breeding (DeWeese & Osborne, 2021).

Direct Seeding Activity at Hortimare

A direct seeding procedure for *Saccharina latissima* was carried out at Hortimare, following a previously established protocol to ensure consistency and optimal spore adhesion.

The process began quantifying the number of sporophytes per milliliter, an essential step to guarantee even and effective inoculation. A standard culture to rope ratio was applied to maintain uniform sporophyte density along the seeded ropes.

The rope was placed into a designated seeding structure. The seeding process involved the simultaneous application of the spore suspension and passage through a pre-prepared Algae Binder solution, designed to enhance spore adhesion to the substrate. This combination ensures effective anchorage of spores to the rope surface.

Following inoculation, the seeded ropes were incubated at 8°C for 30 minutes to allow for initial attachment. Subsequently, they were transferred to the designated nursery or grow-out facility to initiate the early sporophyte development phase under controlled conditions.

This standardized direct seeding method is essential for ensuring reliable germination, uniform development, and the overall scalability and efficiency of kelp cultivation.

Palmaria palmata Tagging Experiment at Hortimare

An identification and monitoring experiment was conducted involving tagged individuals of *Palmaria palmata* to assess growth performance and morphological variation. As shown in Figure 45, physical tags were applied to individual thalli, allowing the tracking of specific specimens throughout the trial.

Following tagging, biweekly samplings were carried out to evaluate growth rates and determine whether morphologically distinct individuals exhibited superior growth performance. Parameters such as thallus length, width, pigmentation, and texture were observed and recorded at each sampling point.

The experiment lasted for one month. This type of phenotypic monitoring provides critical data for selecting elite genotypes for future cultivation and breeding programs, particularly when aiming to enhance productivity and stress tolerance under controlled aquaculture conditions.

Figure 45 – Morphologically distinct individuals of *Palmaria palmata* tagged for subsequent growth rate analysis. Photo: Paulo Pereira

11. Challenges

Several challenges arose during the course of the internship, each requiring adaptative strategies to ensure the continued success of macroalgae cultivation:

- Seasonal growth decline in *Palmaria palmata* - A marked decrease in the growth rate of *Palmaria palmata* was observed during the summer months. This reduction coincided with increased solar radiation and water temperatures, conditions unfavorable for optimal growth. As a mitigation strategy, the affected biomass was transferred to indoor nursery tanks maintained at 8°C, where stable and controlled conditions supported recovery and sustained development.
- Spontaneous sporulation of *Ulva* - During the seasonal transition from summer to autumn/winter, *Ulva* exhibited spontaneous sporulation triggered by declining light intensity and lower temperatures. This reproductive shift led to a significant reduction in biomass yield. To address this artificial lighting systems were implemented to stabilize photoperiod and light intensity, successfully minimizing the environmental conditions that triggered sporulation.
- Temperature fluctuations – Sudden and unpredictable variations in temperature presented a continuous challenge throughout the cultivation period. These fluctuations risked inducing stress responses in the macroalgae, potentially impairing growth and reproduction. As a response, close real time monitoring and continuous adjustments of system parameters were necessary to maintain thermal stability and prevent stress-induced decline.
- Pigmentation anomaly in *P. palmata* - A monitoring experiment was conducted using tagged individuals of *P. palmata* that exhibited atypical pigmentation (Figure 46). The purpose was to investigate the correlation between discoloration and growth performance over time.

Figure 46 - Monitoring of *Palmaria palmata* individuals with an unusual pigmentation. Photo: Paulo Pereira

- White "bloom-like" episodes in *P. palmata* raceway - Occasional occurrences of white, bloom-like formations were recorded. These occurrences are hypothesized to result from a combination of abiotic stressors, including sudden temperature and pH fluctuations, decreased oxygen levels, and nutrient imbalances.
- Green filamentous algal contamination – The proliferation epiphytes (Figure 47) emerged as a significant challenge, particularly in systems where nutrient levels and light intensity were less tightly regulated. To mitigate this problem, several preventive measures were evaluated, including the reducing of light intensity, the implementation of enhanced cleaning protocols.

Figure 47 - Contamination with green algae in a *Gracilaria* spp. individual. Photo: Paulo Pereira

- Mechanical disruptions in *Ulva* raceway systems – Mechanical issues also posed operational challenges particularly in the large *Ulva* raceway (Figure 48) where a blocked drainage system was recorded. The blockage was caused by the accumulation of small *Ulva* fragments which clog the filtration system. This obstruction interfered with the CO_2 injection system, leading to fluctuations in water pH and adversely affecting overall cultivation performance. Prompt manual removal were required to restore system functionality.

Figure 48 - Blocked drain in *Ulva* Raceway. Photo: Paulo Pereira.

12. Presentations and knowledge sharing

One of the most enriching aspects of the internship was the opportunity to attend a variety of presentations, including experimental trials conducted in the company, collaborative academic research projects with Hortimare, and innovative entrepreneurial pitches focused on the macroalgae sector. The open culture of knowledge sharing, and scientific exchange fostered a highly stimulating work environment that supported both learning and professional development. The collaborative atmosphere encourages critical thinking, dialogue, and innovation, contributing significantly to the overall value of the internship experience.

13. Critical analysis and suggestions

Direct involvement in cultivation systems, coupled with routine monitoring and experimentation, enabled the identification of several key insights and recommendations for future research and optimization of macroalgal production systems:

- Post-sporulation recovery dynamics: An unexpected yet consistent observation was the accelerated growth rates of *Ulva* spp. following natural sporulation events. This response suggests a physiological reset or rejuvenation mechanism, potentially linked to improved stress resilience or nutrient uptake. Controlled studies are recommended to investigate biochemical and cellular changes occurring during this post-sporulation phase, which could be strategically harnessed to optimize productivity cycles and improve biomass yield.
- Sporulation induction in red macroalgae: Given the success of red-light stimulation in *Ulva* similar techniques could be explored for red macroalgae such as *Palmaria palmata*. Targeted investigations into wavelength- specific photoperiod manipulation may uncover methods to induce reproductive processes in Rhodophyta. This could support genetic diversity management, biomass renewal, and enhanced lifecycle control, ultimately increasing the scalability of red macroalgae production systems.
- Seasonal fertilization strategies: The development of liquid fertilization protocols, tailored to seasonal environmental variables such as temperature and light availability represents a promising area for refinement. By synchronizing nutrient input with physiological demand, and environmental conditions, it is possible to improve uptake efficiency, reduce nutrient losses, and minimize eutrophication risks or contamination events, thereby promoting more sustainable and cost-effective production.
- Water reuse and treatment systems: exploring closed-loop water reuse strategies could significantly reduce the environmental impact and operational costs of macroalgae farming. Investigative trials employing ozonation, UV disinfection, activated carbon filtration, or biofiltration with microbial communities may enable safe recirculation of water removing excess nutrients, contaminants, or pathogens. Integrating such treatment systems could support circular economy principles and enhance sustainability profile of the aquaculture practices.

14. Conclusions

This internship provided a comprehensive immersion into the field of seaweed aquaculture, particularly in the cultivation of *Ulva* spp., *Gracilaria* spp. and *Palmaria palmata*. Active participation in both land-based and pilot-scale cultivation systems, offered in-depth insights to the biological, environmental, and technical factors that influence the successful production of macroalgae.

Key aspects of the internship included the optimization of cultivation protocols, the development and application of tailored fertilization strategies, and the implementation of routine biomass monitoring and health assessments. Emphasis was placed on maintaining biomass health and quality through regular cleaning, microbial management, and the mitigation of abiotic stressors, such as temperature and photoperiod fluctuations. Additionally, hands-on experience with system maintenance (e.g., filtration units, UV sterilization, CO_2 dosing), combined with problem solving responses to real-time issues, such as epiphytic blooms, spontaneous sporulation, and nutrient imbalances, greatly enriched the learning outcomes.

Experimental trials conducted in both laboratory and production environments were fundamental in defining species-specific needs, ideal growth conditions, and nutrient ratios for each species, contributing not only to improved productivity, but also to more sustainable and cost-effective cultivation practices. The transition from experimental to scalable systems underscored the importance of bridging R&D with commercial feasibility.

Furthermore, the involvement in innovation-focused activities, such as the formulation of custom fertilizers, selection of ideal densities, and microbial interaction studies, highlighted the necessity of continuous adaptation and multidisciplinary approaches. The importance of meticulous data collection, ranging from growth rate assessments to environmental monitoring, proved essential for optimizing production and informing future investment and upscaling decisions.

Overall, this internship represented a crucial step in my professional development within the seaweed sector. It fostered critical thinking, technical skills, and a deeper understanding of the biological and operational complexities of macroalgal aquaculture. The knowledge gained will serve as a strong foundation for future contributions in research, development, or industry initiatives aimed at advancing sustainable marine bioresources.

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