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Evaluation of growth performance and nutrient uptake of dulse *(Palmaria palmata)* cultivated in commercial Atlantic salmon Recirculating Aquaculture System (RAS)

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Masters in Aquatic Food Production- Safety and Quality

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Contents

List of Tables

List of Figures

Foreword

This study was carried out to complete the prerequisites for a master's degree at the Faculty of Science and Technology (REALTEK) at the Norwegian University of Life Sciences (NMBU). NMBU covered the cost for experiments in the water laboratory while NOFIMA covered the cost to travel to Tromso. The comprehensive nature of the experiment would not have been possible without this generous funding.

The goal of the thesis was to produce dulse from the effluent of RAS. Production of dulse using effluent water of RAS can reduce the effects of effluent discharge with help of bioremediation. However, more research is required to fully understand dulse's development, chemical composition, and nutrient utilization.

In my thesis, I acknowledge the use of ChatGPT and other AI tools like Quillbot to help with sentence structure and language improvement. These resources guaranteed the uniqueness and integrity of my study material while assisting me in efficiently and concisely communicating my ideas.

I have a deep gratitude for my supervisor Vasco C. Mota, whose constant encouragement, support, and direction have been crucial to my thesis project. His knowledge, helpful criticism, and patience have greatly impacted my studies and enhanced my educational process. I am incredibly grateful for his guidance and the information he has imparted. His insightful suggestions, comments, and way of teaching helped me greatly in enhancing my analytical skills.

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Finally, I am profoundly grateful to my parents for their constant prayers, guidance, and endless support, my late brother Ubaid, whose love and memory have inspired me throughout this journey, and my sister Varda and brother Umer for their constant encouragement, belief in me, and standing by me in every step of life.

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Abstract

The nutrients discharged from the effluent of recirculating aquaculture systems (RAS) cause significant environmental risks such as eutrophication, degradation of water quality, and biodiversity loss highlighting the need for innovative strategies for the reuse of the nutrients in a sustainable way. The major nutrients in RAS effluent water are ammonium (NH_4-N), nitrate (NO_3-N), and phosphate (PO_4-P). Thus, these nutrients are part of the analysis in the current study. This study evaluates the potential of dulse *(Palmaria palmata),* a red macroalgae to utilize the effluent from RAS as a source of nutrients for sustainable biomass production. A single experiment was performed to examine the growth performance of dulse, under the same environment conditions, involving three treatments with 10 replicates each: Treatment A as control (100% seawater), Treatment B (25% RAS effluent water + 75% seawater), and Treatment C (100% F2 Solution). Each replicate contained a single dulse plant in a 1L glass flask. The total duration of the experiment was 64 days, divided into three growth periods, each lasting about 21 days. The rationale behind this approach was to completely renew 1 liter of water every 21 days to ensure no nutrient deficiencies occurred. At the beginning and end of each growth period, water samples (15 ml) were collected for ammonium (NH_4-N), nitrate (NO_3-N), and phosphate (PO_4-P) analysis. At the beginning of each growth period and the end of the last one, ie. day 0, day 21 (T1), day 42 (T2), and day 64 (T3), each dulse plant's wet weight, frond length and width, and three cluster lengths per plant were recorded. This study found a significant difference in growth across treatments, with RAS effluent water treatment showing better growth and retention of color. The biomass of dulse in RAS increased from 2.08g initially to 5.06g by the end of the 9-week experiment. In contrast, dulse in seawater treatment and F2 solution treatment died after 3 weeks. The removal efficiency of NO_3-N from RAS, which is also the primary nutrient in RAS, was highest (332%) compared to NH_4-N (48%) and PO_4-P (23%) by the end of the experiment, indicating that dulse effectively contributed to the removal of this primary nutrient from the RAS effluent water. This suggests that growing dulse in RAS is possible and is an effective method for bioremediation of nutrients. This finding highlights the potential for integrating macroalgal cultivation into aquaculture systems to reduce environmental impacts and effective resource utilization while creating high-value products.

Graphical Abstract

Figure 1 illustrates a graphical abstract to visualize and summarize the study of this master thesis.

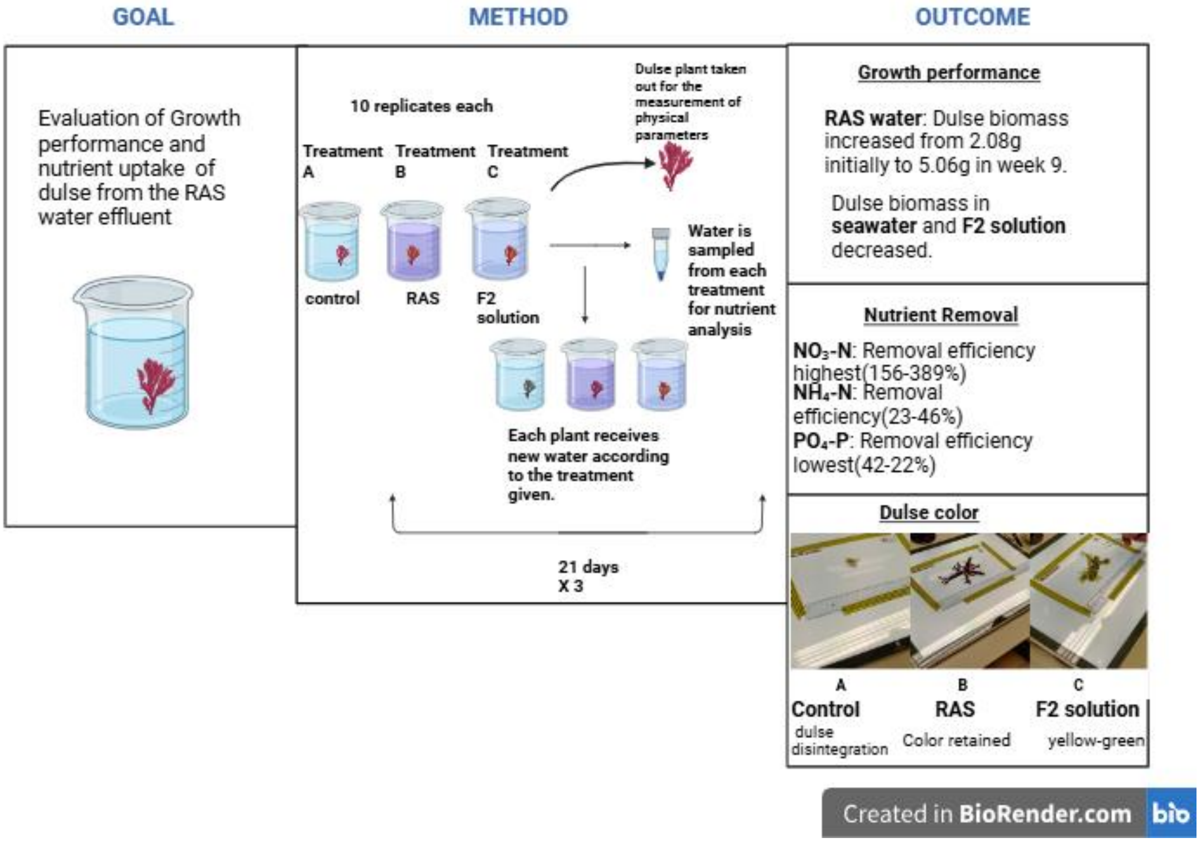


Figure 1: Graphical abstract representing the goal, methods, and outcome of the trial. Created with BioRender.com

1. Introduction

Macroalgae also known as seaweeds are macroscopic algae that can grow to many meters in length. Macroalgae are primary producers that support various communities of herbivorous creatures (vertebrates such as herbivorous fish, and invertebrates like some sea urchins and/or gastropods) at the base of the marine food chain (Pereira, 2016, 2021). Macroalgae have significant economic importance, play vital roles in the ecosystem, and provide substantial benefits for not only humans and animals but also the environment (Kurniawan & Anouzla). These organisms provide a wide range of bioactive compounds with potential medicinal uses and significant nutrition in terms of proteins, fibers, vitamins, and minerals (Moga et al., 2021). About eighty percent of the oxygen in the environment is produced by macroalgae. In many parts of the world, macroalgae are a perfect source of medicine and food due to their special nutritional as well as bioactive qualities (Adarshan et al., 2023). Macroalgae are found in brackish or saltwater environments and require adequate light for photosynthesis. Marine algae most frequently grow on coastal areas on rocky sea fronts, instead of sand or gravel, due to another common requirement: a fixation point (Pereira, 2021). Phaeophyceae (brown algae), Rhodophyta (red algae), and Chlorophyta (green algae) are the taxonomic groups of macroalgae (Spalding et al., 2019). Each phylum has distinct qualities and bioactive compounds (Adarshan et al., 2023). The phylum Chlorophyta contains green macroalgae, which have pigmentation similar to vascular plants' (carotenoids and chlorophylls a and b). Phycobilins, chlorophyll a, and some carotenoids are examples of photosynthetic pigments found in red macroalgae, which belong to the phylum Rhodophyta. The pigments that give brown macroalgae their brownish color are chlorophylls a and c and carotenoids, of which fucoxanthin is the main pigment. Brown macroalgae belong to the Ochrophyta phylum (Pereira, 2021).

When compared with other types of algae, red algae are known as the most significant source of numerous physiologically active compounds (El Gamal, 2010). Antiviral properties are found in several polysaccharides of red algae, including naviculan, agarans, laminaran, carrageenan, fucan, and alginate (Toubane et al., 2024). Moreover, there is an abundance of soluble fibers in the carrageenans and sulfated polysaccharides of red algae that may have anticancer properties. Antioxidants like ascorbic acid, phlorotannins, tocopherols, and carotenoids from red algae are toxic to certain cancer cells without having negative consequences. It was found that mosquito larvae are susceptible to insecticides from *Laurencia nipponica* which is a type of red algae (Toubane et al., 2024). Fibers from red algae are crucial for purgative and laxative properties (Aziz et al., 2021). Red algae's biomass yields a large amount of bioenergy. Numerous red algae species, including *Gelidium amansii*, have been studied or used as feedstock to produce bioethanol (Wang et al., 2020). Red algae are considered to be nutrient-dense and low-calorie food. The protein content of red algae is higher than brown algae, with a protein content of 18.8 g/100 g on average (Gamero-Vega et al., 2020). Furthermore, red algae have a low fat content but they have fatty acids of high quality. They contain fatty acids like DHA, EPA, oleic acids, arachidonic acid, EPA, and DHA. The low value of omega-6/omega-3 ratio (median = 0.8) in red algae has benefits for human health. Red algae have also high value of soluble fibers. It also has significant quantities of minerals, with sodium and iodine being particularly prominent (Gamero-Vega et al., 2020). Most red seaweed species are potential candidates for the IMTA system as well (Wang et al., 2020).

The goal of our study was to evaluate the growth performance and nutrient uptake of Dulse (*Palmaria palmata*),a red algae, from the effluent water of RAS. Dulse is a highly valued red algae as a nutritious food, and a potential source of bioactive compounds for use in nutraceuticals, aquaculture feed, and cosmetics (Schmedes & Nielsen, 2020). Because of its potential useful properties, the demand for dulse has increased and led to various studies on the development and growth of dulse. The growth of dulse depends on several water quality parameters. One of the most important factors that directly affects dulse growth, reproduction, and photosynthetic efficiency is temperature. The temperature range for optimal development of dulse is recorded to be between 6-12°C depending upon the strain. It is also recorded that growth decreases above 14 °C and biomass mortality increases at 21 °C (Stévant et al., 2023). Salinity is another parameter for the growth of dulse. Dulse is frequently found in environments with comparatively constant salinity levels ranging from 30–40 PSU (Stévant et al., 2023). However, Dulse from Spitsbergen, Norway exhibited substantial inhibition of photosynthetic processes and mortality at 15 PSU (Karsten et al., 2003). Like other plants exposed to high amounts of UV radiations and Photosynthetically Active Radiation (PAR) , dulse can adjust its photosynthetic activity due to fluctuations in light levels throughout the day (Stévant et al., 2023).

To cultivate dulse at sea, substrates which are spore-seeded are dispersed with sporelings to create harvestable biomass. The growth is primarily determined by natural variations in environmental conditions, with minimal control over the quality of the biomass produced. After deployment, sporelings are vulnerable to fouling and may endure detrimental irradiance levels, nutrient deficiencies, and stressful variations in salinity. Studies have indicated that tissue quality can be harmed when dulse is grown near salmon farms which are sea-based to address insufficient availability of nutrients. This indicates that ephemeral epiphytes or the deposition of depris macroalgae may be the cause of the surface layer's contamination (Schmedes & Nielsen, 2020). In contrast, land-based farming provides strategies to counteract epiphyte growth and is a scalable means of producing biomass of the required quality by managing environmental elements including light, nutrients, and salinity. For example, usage of relatively low light intensities and pulse additions of nitrate or ammonium in tank trials resulted in reduced growth of epiphytic ephemeral algae and high production in related species. Land-based cultivation may be able to guarantee high clean macroalgae production, which is necessary for the application of biomass. Numerous investigations have used regulated environmental factors to examine the significance of stocking density and scale in culture systems concerning macroalgae yield and nitrogen elimination (Schmedes & Nielsen, 2020).

The biggest problem faced by the aquaculture industry is maintaining the water quality and reducing the amount of nitrogen and phosphorus released into the environment (Islam, 2005). A marine fish farm may lose to the environment 52–95% of its nitrogen intake, 85% of its phosphorus input, and 80–88% of its carbon input through feed waste, fish excretion, and respiration. Ammonium ion (NH_4^+) and unionized ammonia (NH_3) make up total ammonia nitrogen which can also be called TAN (Ramli et al., 2020). The nitrification process may cause the pH of the water in RAS to drop. TAN is converted into nitrite (NO_2) and nitrite into nitrate (NO_3) during nitrification. Nitrite is toxic at levels more than 1 mg/L (Ramli et al., 2020). Nitrate is proven to be hazardous for many freshwater fish species at concentrations above 1,000 mg/L and for marine species at concentrations over 500 mg/L (Colt, 2006). The primary cause of ortho-phosphate accumulation in RAS is applied feed (van Bussel et al., 2013). Cultivated fish cannot absorb all of the phosphates in the feed in recirculating aquaculture systems. The culture water and the sludge that is produced contain excessive phosphate (Schleyken et al., 2024). The common concentration of ortho-phosphate in intensive RAS is 25-45 mg/L (van Bussel et al., 2013). Fish can excrete P, however, elevated levels of ortho-P can cause hyperphosphatemia in fish which can lead to death (van Bussel et al., 2013). Alternatively, these nutrients can be transformed into a useful product by combining macroalgae with land-based marine finfish cultivation (Corey et al., 2014). This problem can be solved by integrating dulse with nearby sea farms or land-based marine fish farms. Several red algae are proposed as an ideal option to boost the utilization of nitrogen resources. This process is known as IMTA or integrated multi-trophic aquaculture (Rößner et al., 2014). The concept of IMTA was coined in 2004 and it means the incorporation of species from different trophic or nutritional levels in the same system (Chopin & Robinson, 2004). Dulse absorbs nutrients from its surroundings by uptake throughout its thallus. Dulse has a higher affinity for ammonium (NH_4^+) than for nitrate (NO_3^-) however, both ammonium (NH_4^+) and nitrate (NO_3^-) are important nitrogen sources for the development and growth of dulse. Lubsch and Timmermans (2020) observed a synchronized pulse-like pattern of phosphate (PO_4^{3-}) and NO_3^- uptake with intervals of around 7 days under controlled settings. It can store carbon, phosphorus, and nitrogen to promote growth in nutrient-limited environments. Irradiance is crucial for the storage of nutrients in dulse in addition to nutrient availability (Stévant et al., 2023).

Numerous studies have shown that a wide variety of macroalgal species will absorb nitrogen as ammonium, and some macroalgae even exhibit a preference for ammonium over Nitrate which is the more readily available form of nitrogen necessary for plant growth (Sanderson et al., 2012). Macroalgae like dulse have a high nutrient content due to their high capacity to uptake nutrients to produce nutrient compounds (Schmedes & Nielsen, 2020). Dulse can take up nutrients like nitrate-N, nitrite-N, ammonium-N, and Orthophosphate from the RAS as it is required for the ideal growth of dulse and helps reduce these nutrients from harming the fish in the tanks. However, more study and research is required for the understanding of dulse's nutrient uptake and development.

2. Literature Review

2.1 Macroalgae

Macroalgae also known as seaweeds are those algae that can be observed through the naked eye and are said to be benthic which means that they can adhere themselves to the seabed

(Mouritsen, 2013). Macroalgae are divided into three classes according to their pigmentation: Red algae (Rhodophyta), Brown algae (Phaeophyta), and Green algae (Chlorophyta) (Chan et al., 2006). The major criteria that are used to divide macroalgae into these classifications or phyla are photosynthetic pigments, cell wall components, cell structure, and flagella (Sahoo & Seckbach, 2015). Macroalgae are macroscopic, but every type of macroalgae remains unicellular at some stage as spores or zygotes (Lobban & Harrison, 1994). It is further known that most species of macroalgae show seasonal differences in their growth pattern in response to external factors like light, availability of nutrients, and water temperature. Because of that, they might appear larger at one time of the year and smaller at another (Norton et al., 1981). They can be found in many ecological habitats such as sandy bottoms, shallow coral reefs, inter-reefal zones, mangrove roots, and seagrass meadows. They are also found in intertidal zones across all coastal zones (El-Manaway & Rashedy, 2022).

2.1.1 Macroalgae as food

Macroalgae typically contain micronutrients like minerals and vitamins and macronutrients like essential amino acids, proteins, lipids (n-3 and n-6 essential fatty acids), and dietary fibers. These ingredients have demonstrated several health benefits for humans like anti-cancer, antibacterial, , anti-obesity, and anti-inflammatory characteristics (Ms et al., 2022). Apart from macronutrients, some red algae, including Gelidium, Gracilaria, and Neopyropia, are utilized to produce agar and carrageenan. In contrast, brown algae are employed in the production of alginate, which finds widespread applications in food additives, cosmetics, pharmaceutical products , and ingredients present in the functional food (Rogel-Castillo et al., 2023).

Aquatic food webs in the ocean depend heavily on macroalgae as primary producers. They are abundant in vital trace elements, minerals, and raw materials used in the cosmetics and pharmaceutical industries (Chapman, 1970). The abundance of minerals, certain vitamins, and polysaccharides found in macroalgae is well-known. Brown algae have comparatively low protein content, while most red algae have a significant protein content. In general, there is low lipid content (Mabeau & Fleurence, 1993). In addition, they include numerous additional bioactive compounds that have antiviral, antibacterial, and antifungal qualities.

Macroalgae that are edible are eaten raw, such as when added to salads, soups, and sushi wraps. Therefore, per Regulation (EU) 2015/2283, they can be categorized as "novel food" based on their nutritional makeup (Parliament & Union, 2015). Edible macroalgae are generally thought to have a lower environmental impact and provide many health advantages when eaten raw or added to meals (Demarco et al., 2022). According to the United Nations (UN) , the production of macroalgae, which is a potential meat alternative, will increase to 75% in the year 2050 (Rogel-Castillo et al., 2023). Potential food substitutes like macroalgae could reduce the overconsumption of animal protein and hence solve the environmental harm that comes with meat production (Gullón et al., 2021). Edible macroalgae can be eaten independently or as a component of cooked meals. These edible forms include fresh macroalgae, whole, fermented, dried, frozen, granules, flakes, and powders. Macroalgae is used in some food products, such as beverages, pasta, bread, and pastries (Mendes et al., 2022). Generally speaking, about 70% of macroalgae are consumed for food, with the remaining 30% being used, among other things, for feed and fertilizer (Poblete-Castro et al., 2020).

In general, the meat business makes use of a broad variety of components with particular technological qualities to enhance the nutritional content, flavor, texture, and appearance of goods. Thanks to its ability to bind water and fat, macroalgae which have low-calories, dietary fibers, high amounts of essential minerals, and health-promoting substances can help solve some of the technological issues related to these reformulated meat products (Rogel-Castillo et al., 2023). For example, the effectiveness of Undaria sp. as a functional ingredient in pig patties with reduced fat content was assessed by Nagai et al. (2022). The product's fatty acid profiles and fat content were unaffected by including macroalgae in the patties. Consequently, it might be a good technique for creating beef products with enhanced features and antioxidant qualities (Nagai et al., 2022). The fatty acid profile was primarily composed of a high proportion of monounsaturated (38.2%) and saturated (48.8%) fatty acids, followed by PUFA or polyunsaturated fatty acids (13%) with higher abundances of oleic (C18:1n-9; 34.4%) and palmitic (C16:0; 36.9%) acids (Nagai et al., 2022). The number of double bonds determines whether an unsaturated fatty acid is categorized as mono- or polyunsaturated. The main structural components of cell membranes, polyunsaturated fatty acids (PUFAs) are essential nutrients for treating autoimmune diseases, non-alcoholic fatty liver, and other chronic conditions (Kapoor et al., 2021).

In addition to being consumed as food, macroalgae can be utilised as food additives, which are compounds added to food to preserve or enhance its flavour, texture, freshness, safety, or appearance (Rogel-Castillo et al., 2023). Food additives, according to the World Health Organization (WHO) and FAO also known as the Food and Agriculture Organization of WHO (Demarco et al., 2022), involve enzymes, flavoring of food, and additives used for various purposes such as coloring, sweetening, or preservation. They assist in preventing contamination, which can result in foodborne illnesses, in addition to preserving the quality of food. In this regard, using macroalgal extracts and powders to prevent oxidative stress in target tissues and lipid oxidation in food appears promising (Zhang et al., 2022). Macroalgae are emerging as a unique source of naturally occurring lipophilic pigments, which are important food ingredients. Colorants are another type of food additive that is frequently employed in food preparation. 20 brown algae and 4 red algae from the northern Spanish coast were the subject of a study that assessed the lipophilic pigment content of these macroalgae and concentrated on the carotenoid concentration of fucoxanthin or chlorophylls. These could be employed as natural food colorings because the results indicated that *Undaria pinnatifida* and members of the order Fucales had the highest level of lipophilic pigment (Rogel-Castillo et al., 2023).

The primary obstacles facing this developing market are the lack of knowledge regarding the health advantages of adding macroalgae to food and the absence of laws governing the harvesting and growing of macroalgae from natural macroalgal beds for the large-scale manufacturing of products using macroalgae (Rogel-Castillo et al., 2023). A customer's decision to consume macroalgae may be influenced by several factors, including availability, cost, and sensory aspects including taste, smell, and texture as well as information on the hazards and advantages to health. For example, 46% of participants were unaware of the health benefits of macroalgae according to a study (Redway et al., 2022).

2.1.2 Pharmaceutical and Medicinal Properties of Macroalgae

In addition to providing nutritional support, its antioxidant, antibacterial, antifungal, antiviral, antiallergic, anticancer, anticoagulant, antifouling, and antioxidant properties (See **Figure 2)** have been employed in treating various biological disorders (Pooja, 2014). *Sargassum wightii*, a marine brown alga, possesses antibacterial, antioxidant, anti-inflammatory, and anti-tumor properties (Yuvaraj & Arul, 2014). The crude extract of the Chinese brown algae *Sargassum* *naozhouense* is being used to cure fever, infections, laryngitis, and other illnesses (Wang et al., 2010).

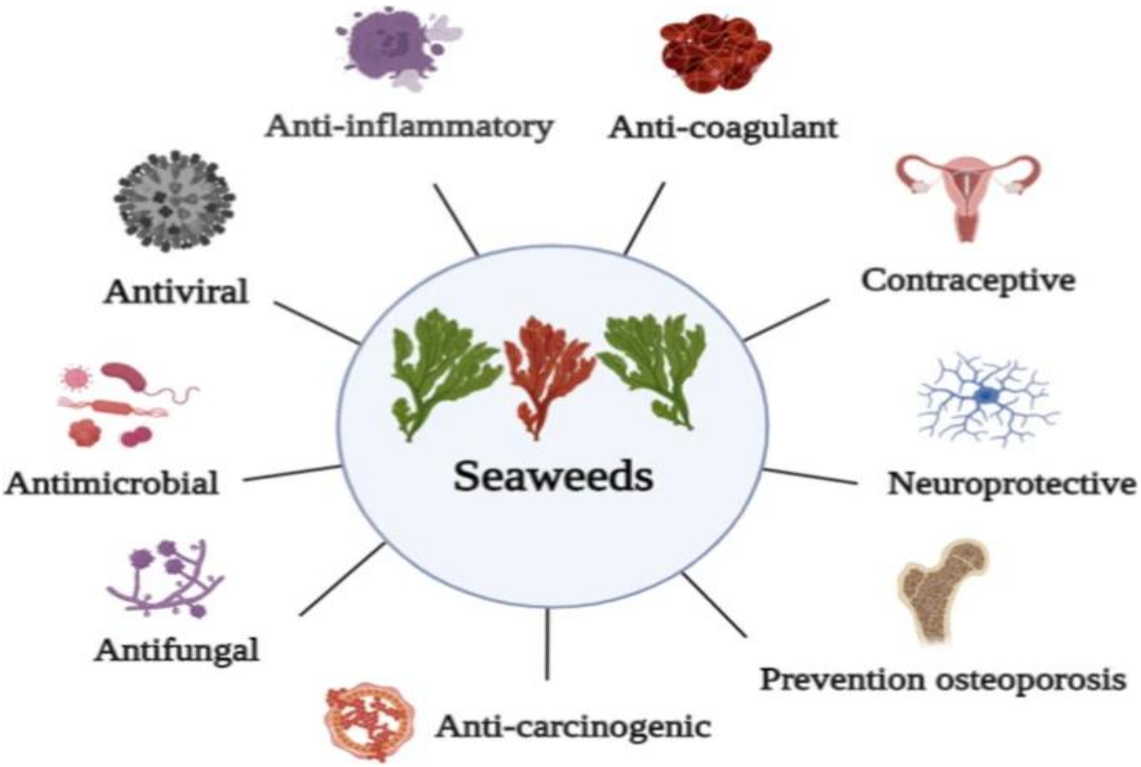


Figure 2: Biological activity of macroalgae (Seaweed). Macroalgal extracts can be used in biological processes as antimicrobial, antifungal, antioxidant, anticoagulant, anti-inflammatory, and mosquitocidal agents, to name a few. Macroalgal-derived secondary metabolites, including caulerpin, sulfoquinovosildiacyl-glycerols, and the polysaccharide fucoidan display numerous biological functions (Farghali et al., 2023).

Numerous secondary biomolecules present in macroalgae like caulerpin, sulfoquinovosildiacyl-glycerols, and the polysaccharide fucoidan, have a wide range of biological activities, including antibacterial activity (Farghali et al., 2023). Most of the antimicrobial chemicals in macroalgae are part of the natural defense system that protects them from invasive diseases (Bhowmick et al., 2020; Polat et al., 2023). Furthermore, studies on macroalgae extracts have demonstrated antibacterial properties against certain infections, including Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* (Asharaf et al., 2022). This may prove to be a promising discovery in the fight against infections and in reducing antibiotic use in the poultry industry, where antibiotic resistance is common (Farghali et al., 2023).

The immune system uses inflammation as a crucial host defense mechanism to shield the body from dangerous stimuli such as invading viruses, damaged cells, and irritants (Lee et al., 2017). However, new research indicates that regulating excessive inflammation is crucial for maintaining human health, as persistent or excessive inflammation frequently causes inflammatory illnesses and age-related diseases (Iwalewa et al., 2007). It has been studied that diet can play a major role in keeping inflammation in check (Wu & Schauss, 2012). Phycocyanin, phycoerythrin, and phycobiliproteins are the main components of dulse(red algae) proteins (Gantt et al., 1979). These pigment-proteins are light-harvesting and can be found in a water-soluble protein complex called a phycobilisome, linked to the chlorophyll-containing Photosystem II (Lee et al., 2017). As previously mentioned, phycobiliproteins and chlorophylls have been demonstrated to have anti-inflammatory properties. Therefore, efficiently utilizing these physiologically active substances could aid in the development of value-added applications for dulse resources.

Macroalgae's metabolites can act as antiviral agents by enhancing the immune system of the host or preventing the replication of viruses before they enter host cells (Lomartire & Gonçalves, 2022). Macroalgae can target a variety of viruses, including coronaviruses, lentivirus, influenza viruses, and herpes viruses (Lomartire & Gonçalves, 2022; Wei et al., 2022). Sulfated polysaccharides have a virucidal impact because they prevent the virus from adhering to the negatively charged surface of the host cell at first. Negatively charged sulphated polysaccharides and positively charged viral glycoproteins may function collectively to prevent the virus from infecting the target cell (Lomartire & Gonçalves, 2022). For example, it has been discovered that sulfated polysaccharides that are derived from macroalgae, including calcium spirulan, alginate, naviculan, fucoidan, galactan, and carrageenan, have inhibitory actions against cell damage caused by a variety of viruses (Wei et al., 2022). Particularly concerning are the chemicals derived from macroalgae that have been shown to have mosquitocidal properties against mosquitoes, which are known to carry nearly all viruses (Farghali et al., 2023). With a mortality rate of over 91% at 50 parts per million, halogenated sesquiterpene-elatol produced from *Laurencia dendroidea,* a red algae, demonstrated significant larvicidal effects against *Aedes aegypti* (Yu et al., 2014).

Strong antioxidant systems have been shown in macroalgae in extremely oxidative environments. Because macroalgae is a photosynthetic organism, it is exposed to a lot of light and oxygen, which promotes the production of free radicals and other potent oxidants (Peñalver et al., 2020). Algal antioxidant metabolites are categorized into five groups: phenolic molecules, which include flavonoids, phlorotannins, and bromophenols; nitrogenous compounds, which include peptides; terpenoids, which include steroids and carotenoids; pigments derived from chlorophyll and alkaloids; and carbohydrates and polysaccharides (Tziveleka et al., 2021). The most important types of antioxidants are fucoidans, phlorotannins, carotenoids, and secondary metabolites found in macroalgae. Phlorotannins can be utilized in food refineries as a useful substitute for synthetic antioxidants (Hermund, 2018). Moreover, macroalgae contain certain bioactive polyphenols that may affect the expression of certain genes (Hoseinifar et al., 2022). Furthermore, macroalgae contain certain bioactive polyphenols that may affect the expression of certain genes (Peñalver et al., 2020; Tziveleka et al., 2021). The mechanisms of action of widely recognised lipid- and water-soluble antioxidants, like ascorbic acid and tocopherols, as well as antioxidants derived from herbs, spices, oilseeds, green and black teas, grapes, citrus fruits, and alcoholic beverages have recently been studied. This is because natural sources of antioxidant molecules are of interest to the food and beverage and beauty industries (Yuan et al., 2005). The quest for naturally occurring substances with antioxidant activity as substitutes for manufactured goods has gained traction in recent years. Natural antioxidants can also be found in abundance in aquatic plants (Duan et al., 2006). Previous research has demonstrated that dulse includes l-ascorbic acid, glutathione (GSH), polyphenols, and MAAs (Mycosporine-like Amino Acids), among other types of hydrophilic antioxidant components (Yuan et al., 2009).

Apart from essential oils, other components linked to the anti-inflammatory characteristics of macroalgae include fucoxanthin, sulfated polysaccharides, polyunsaturated fatty acids, alkaloids, and astaxanthin (Farghali et al., 2023). The author further stated that macroalgae may have anticancer properties because of the existence of sulfated polysaccharides and carotenoid fucoxanthin as their constituents. Numerous research studies have demonstrated a robust association between the anticancer activities of macroalgae and their phenolic antioxidant capacity. In a dose-dependent way, polysaccharides isolated from *Sargassum wightii*, a brown algae, significantly reduced the proliferation of human breast cancer cell lines (Vaikundamoorthy et al., 2018).

According to some accounts, polysaccharides from the macroalgae may have anticoagulant properties and be devoid of prions or harmful viruses that are known to contaminate commercial heparins (Faggio et al., 2016). The author further reported that after using the prothrombin time and partial thromboplastin time assays, sulfated polysaccharides from *Agardhiella subulata* and *Ulva fasciata* extended the coagulation time activities on human blood.

Diverse macroalgae species showed different contraceptive qualities. For instance, *Gracilaria corticata* and *Gelidiella acerosa*, two red algae that were obtained from Sri Lanka's coastal waters, demonstrated strong post-copulatory contraceptive potential in female rats without causing any negative side effects (Dolui, 2021). The author further reported that ethanolic extracts from *Gracilaria edulis* demonstrated a 100% reduction of sperm motility, indicating the presence of a spermicidal chemical that damages sperm plasma membranes.

2.1.3 Use of Macroalgae in Cosmetics

Many research advised against using synthetic-material-based cosmetics, emphasized their toxicity in terms of an increase in unfavorable consequences after application, and declared them to be hazardous items for consumers' health (Kalasariya et al., 2021). The cosmetics industry has recently included natural bioactive elements during the production of their products due to the ineffectiveness of synthetics and a change in consumer attitudes towards goods derived from natural sources (Jesumani et al., 2019). In particular, macroalgae can be utilized as organic colors, bioactive ingredients, texturing stabilizers or emulsifiers, and sources of biomolecules related to skincare in the cosmetics sector (Pimentel et al., 2017).

Macroalgae can be employed as photoprotective elements in sunscreen because, as photosynthetic organisms, they frequently create secondary metabolites that shield the cells and organelles from ultraviolet light (Farghali et al., 2023). The author further reported that macroalgae can be added to formulations for skin whitening, anti-aging, and anti-pigmentation. The main purpose of macroalgae, specifically Fucus, Laminaria, and Chondrus, is to hydrate and nourish the skin (Jesumani et al., 2019). Laminarin, fucoidan, mycosporine, carrageenan, amino acids, and fucoxanthin are examples of macroalgae bioactive components with distinct functional qualities that are frequently utilized in cosmetics due to their anti-photoaging qualities (Pangestuti et al., 2021). Fucoidan has the potential to be used as an anti-aging treatment since it can increase cell hydration and flexibility by encouraging the synthesis of the heparin-growth factor, which enhances tissue and cell growth (Dolui, 2021; Pangestuti et al., 2021).

2.1.4 Uses of Macroalgae in livestock feed

A vital source of nutrients for livestock is animal protein. For livestock farming to be sustainable, high-protein and nutritional sources must be maintained immediately. Livestock production uses one billion tonnes of feed worldwide, and in 2016 this yielded a $400 billion profit (Farghali et al., 2023). The same author stated that macroalgae are widely distributed in Europe and have long been used as animal feed because of their high fat and protein content in comparison to cereals and legumes.

Most studies on the possible benefits of macroalgae for ruminant nutrition have concentrated on introducing trace amounts of different macroalgal species into animal diets and assessing the health, performance, and quality of the resultant products (Farghali et al., 2023). In particular, adding 1–5% Ascophyllum nodosum brown algae to sheep's daily ration could reduce the amount of *Escherichia coli* and balance the ruminal microbiota (Zhou et al., 2017). Red algae have gained more interest, especially for ruminant feed. For instance, 0.5 g/kg feed with 70% concentrated *Phymatolithon calcareum*, a red algae, extract buffered the pH of the rumen but had no effect on rumen fermentation or fiber digestion (Farghali et al., 2023).

Macroalage has the potential to be a special poultry feed because of its high content of macro and microelements, which will improve the growth and performance of poultry as well as the quality of their eggs and meat (Farghali et al., 2023). Furthermore, probiotic bioactivities of the polysaccharides in macroalgae may enhance the health, productivity, and egg quality of chickens. Macroalgae may also enhance the flavor of chicken meat and raise the amount of omega-3 fatty acids in eggs (Zewei et al., 2019).

Since soybeans do not fully meet the nutritional demands of fish, macroalage can be utilized as a nutritionally and financially advantageous replacement for soybeans in fish meals (Chirapart & Ruangchuay, 2022). Sugar kelp or brown algae(*Saccharina latissimi*) could improve fish farming and increase fish resilience to oxidative stress by being added to feed (Kamunde et al., 2019). The author stated that to improve intake, growth performance, plasma antioxidant capacity, and mitochondrial respiration in salmon-fed macroalgae, macroalgae can mitigate temperature rises in the atmosphere. They also mentioned that salmon smolts showed no negative effects from reducing crude protein and minerals when 10% of their diet was made up of macroalgae. Thus, incorporating brown algae into aquafeeds may offer an economical and ideal solution that benefits the aquaculture sector. In summary, there is a chance that fish farms could double their productivity by employing macroalgae. There could be financial gain, a faster development rate, resistance to disease, and preservation of the environment (Farghali et al., 2023).

2.1.5 Environmental Benefits of Macroalgae Production

Climate change has led to the development of the "blue carbon" paradigm, which suggests using carbon sequestration, carbon sinking, and carbon harvesting to derive food and fuel from aquatic habitats instead of fossil sources (Yong et al., 2022). Macroalgae have the potential to be a carbon sink and a renewable energy source. As seen in **Figure 3**, macroalgae may be important in mitigating the effects of climate change (Farghali et al., 2023).

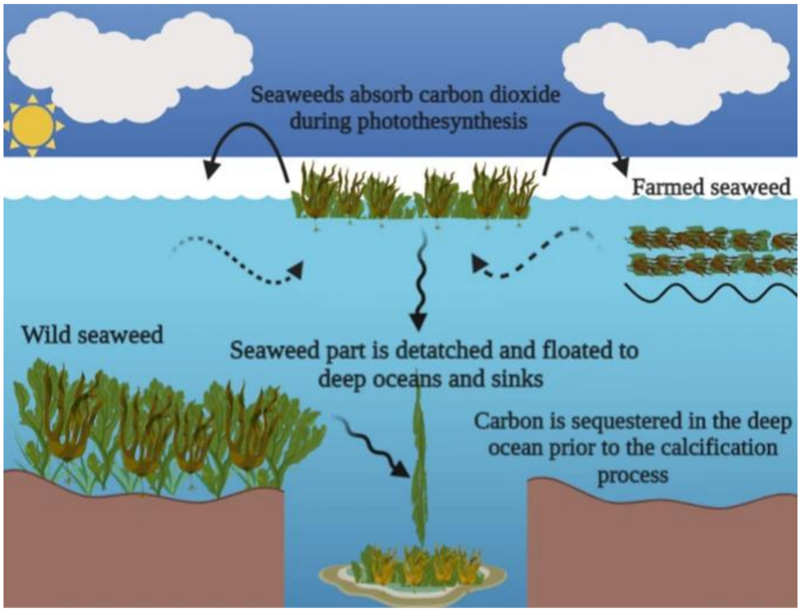


Figure 3: The function of macroalgae(seaweed) in deep ocean carbon sequestration, an efficient method of sequestering carbon. Macroalgae can absorb atmospheric carbon dioxide. Then, macroalgae are transported to the ocean's sediment and depths by two different processes: the sinking of negatively floating macroalgae debris and the drift of macroalgae particles through marine canyons. In total, macroalgae have an average annual storage capacity of 173 teragrams of carbon adapted from (Farghali et al., 2023).

In particular, macroalgae can absorb a large quantity of carbon dioxide from the aquatic ecosystem and provide various ecological benefits, including habitat for other marine animals and the cleanup of toxins along the shore (Duarte et al., 2017; Macreadie et al., 2019). Macroalgae has the potential to absorb around half of the carbon in the atmosphere. Furthermore, macroalgae can balance out half of the world's bioenergy, suggesting that macroalgae could be a useful tool for lowering greenhouse gas emissions (Farghali et al., 2023). The potential for macroalgae aquaculture to trap roughly 1500 tons of carbon dioxide per square kilometer is comparable to the annual carbon dioxide emissions of about 300 Chinese people (Duarte et al., 2017).

2.2 Green Algae

Eukaryotic photosynthetic organisms, green algae have plastids that are double membrane-bound and contain chlorophyll a and b. They also have a unique stellate structure in the flagellar base that connects nine pairs of microtubules, as well as accessory pigments like beta carotene and xanthophylls that are present in embryophytes (Lewis & McCourt, 2004). The plastid stores starch, and when cell walls are present, they are often made of cellulose. (Graham et al., 1991)

A class of macroscopic marine algae known as green algae(Chlorophyta) is distinguished by their green hue because of the pigment called chlorophyll (Abbott et al., 1992). One of the main groupings of oxygenic photosynthetic eukaryotes is the green lineage (Viridiplantae), which is made up of green algae and its progeny, land plants. According to current theories, two distinct clades split out early from an original green flagellate. The early diverging prasinophytes that gave rise to the core chlorophytes are grouped in one clade, the Chlorophyta. The terrestrial plants descended from charophyte green algae, which belong to the other clade, the Streptophyta (Leliaert et al., 2012).

2.2.1 General Morphology

The green algae, which are primarily microscopic and rarely more than a meter, compensate for their smaller size with a variety of development habits and intricate cellular architecture details. The size and habits of the body (thallus) vary from microscopic swimming or nonmotile forms (such as benthos, lichen phycobionts, or nanoplankton) to macroscopic (benthic attached forms). From swimming and nonmotile unicells to filaments, colonies, and different tissue organization levels (pseudo-parenchymatous, parenchymatous, or thalloid) and branching morphologies, thallus structure spans the spectrum of complexity. Unicells range in shape from spherical to elongated, and they may or may not have scales, flagella, wall layers, or other coverings (like loricas). Although chains of unevenly shaped cells are known, filaments typically include cylindrical cells placed end-to-end. There are branching (Draparnaldia) and unbranched (Oedogonium) forms, and many branching forms have terminal filament tips that are attenuated

(Chaetophora). colonies come in a variety of sizes from pairs of cells (Euastropsis) to thousands (Hydrodiction). Colonies of cells may have a common parental wall or be connected by gelatinous strands. The shape of colonies varies, ranging from the aggregation of thousands of swimming cells (Volvox) to tiny sarcinoid packets (nonlinear clusters of cells; Chlorokybus). Some branching forms reach a complexity that can be referred to as tissuelike, while others are straightforward bifurcating or reticulating networks of filaments (Nitella). Both uninucleate and coenocytic cells are possible; in the former case, the cytoplasm of so-called gigantic cells (Caulerpa) contains several nuclei. (Lewis & McCourt, 2004)

2.2.2 Habitat

Marine and brackish coastal habitats rely heavily on green macroalgae, mostly represented by the Ulvophyceae, the main multicellular branch of the Chlorophyceae family. Some of the most common species are ulva, or sea lettuce, which is found worldwide in coastal benthic habitats (Wichard et al., 2015). Marine green algal sequences, especially those found in polar seas, are dominated by groups such as the Mamiellophyceae (Micromonas, Bathycoccus, and Ostreococcus). They give many species with habitat as well as food for a variety of marine critters. A few green algae, such as Ulva (sea lettuce), can counteract eutrophication by taking up surplus nutrients from the surrounding water (Tragin et al., 2016).

Freshwater environments such as lakes, streams, and ponds are rich in green algae.
They are substantial contributors to the food web in these settings and key primary producers. Spirogyra is one type of green algae that can cause harmful blooms in eutrophic freshwater environments (NASELLI FLORES & Barone, 2009).

Numerous lineages of green algae have adapted to survive in terrestrial environments such as rocks, bark, soil, and other substrates. Terrestrial green algae are important for soil formation, nitrogen cycling, and pioneering communities. Extreme circumstances including desiccation, intense UV rays, and temperature swings are situations they can withstand (Rindi et al., 2011).

2.2.3 Application of Green algae

Being the most prevalent type of macroalgae, green algae is a valuable marine biological resource. It is abundant in several fatty acids, amino acids, dietary fibers, polysaccharides, polyphenols, pigments, and other active ingredients vital to many biological processes, including immunoregulation, antioxidant activity, and anti-inflammatory response. The exploration and use of green algae for increased economic value has intensified in recent years due to increased attention to marine resources (Xu et al., 2023).

2.2.3.1 Food and nutrition

Compared to terrestrial plants, green algae species have higher nutritional content because they use less energy to create their reproductive organs, leaves, roots, circulatory systems, and stems. As a result, more fats, proteins, and phytonutrients can be preserved (Anis et al., 2017).

The species of green algae that are commonly used as food sources are Ulva sp., Enteromorpha sp., Monostroma sp., Caulerpa sp., and Codium sp. Dried fronds of edible Monostroma sp. and Enteromorpha sp. are known as "aonori-green laver-ele ele-lulua-lumi boso" in Asian nations, particularly in Japan. Humans can consume these algae raw, dried, or cooked. They are employed in the "nori-jam" soup preparation (Kılınç et al., 2013).

2.2.3.2 Cosmetics

The species of green algae has been shown to contain several bioactive and nutritious substances, including lipids, proteins, polysaccharides, natural pigments (NPs), and polyunsaturated fatty acids (PUFAs) (Khalid et al., 2018). These commercial bioactive substances are thought to have a wide range of potential health benefits, which the nutraceutical and cosmeceutical businesses may eventually use (Khalid et al., 2018).

Antioxidant substances found in green algae, such as vitamins, carotenoids, and polyphenols, can help stop oxidative stress-related cell damage and premature skin aging. Because of their antioxidant and anti-wrinkle qualities, extracts from green algae can be employed in anti-aging cosmetic products (López-Hortas et al., 2021).

Cosmeceutical businesses have been using macroalgal species like green algae to create a new range of cosmetic products that will serve as thickening, gelling, and many other functions. Adding macroalgal species generally increases neocollagenesis, which in turn promotes the creation of extracellular tissue matrix (ETM), improving consumer youth and well-being as well as skin replacement (Pimentel et al., 2017). In addition, the antioxidants found in green algae can be used as sources of ingredients for anti-inflammatory, anti-aging, anti-photoaging, colorants, and radical scavengers in the cosmetic sector (Christaki et al., 2013). For example, beta-carotene can help skin seem younger, fight against aging, and lower the chance of developing skin cancer in those who use it (Joshi et al., 2018).

Apart from polysaccharides and pigment, U. lactuca has been widely used in the beauty industry as an anti-wrinkle agent because of its exceptional concentration of several vitamins and minerals, including iron, magnesium, calcium, and amino acids (Łęska et al., 2018).

2.2.3.3 Nutricinal Applications

Biochemical components of green algae provide a host of nutraceutical advantages. For example, carbohydrates, lipids, and amino acids are highly concentrated in U. facciata and C. racemosa (Magdugo et al., 2020). Also, carotenoids from green algae have been used in medicines and nutraceuticals equally. Carotenoids have the potential to function as powerful antioxidants, which would be advantageous for human health. When it comes to nutraceuticals, several food products have used carotenoids like tocopherol as a food preservative. It has also been observed that astaxanthin derived from H. pluvialis reduces oxidative stress, and inflammation, and strengthens the immune system in individuals with cardiovascular disease (Shah et al., 2020).

In addition to the previously noted nutraceutical benefits of green algal species, dried green algae (Enteromorpha) have been predicted to serve as a vitamin B12 substitute, particularly for individuals following a certain diet. Furthermore, the U. lactuca species is an essential source of vitamin B (MacArtain et al., 2007). Due to the galactans and fucans present, U. fasciata also demonstrated an anticoagulant effect (Ruocco et al., 2016).

2.3 Brown Algae

In many coastal parts of the world, brown algae, also known as brown seaweeds, are the predominant organisms and are primarily responsible for most primary production. They frequently form huge undersea forests that support a high degree of biodiversity (Cock et al., 2011). The brown algae in the Phaeophyceae class range in size from small filamentous forms to large, complex macroalgae (Sarma et al., 2024). Due to the scarcity of freshwater brown algae worldwide and ongoing research, less than 1 percent of the estimated 1,836 species in 285 genera are found in freshwater habitats (Wehr, 2015).

Approximately 2000 species of brown algae (Phaeophyceae) have been described; they are among the few eukaryotic lineages to have achieved complex multicellularity (Bringloe et al., 2020). Brown algae have several essential traits that have allowed them to flourish as macroscopic organisms, along with other multicellular groups like metazoans, fungi, and green plants. These traits include cell-to-cell adhesion and communication, tissue differentiation, internal transport of sugars, and the ability to grow in three dimensions (Bringloe et al., 2020). Apart from clonal plants, these characteristics have helped the world's largest marine autotrophs (such as Laminariales and Fucales) develop and diversify (Arnaud-Haond et al., 2012).

2.3.1 General Morphology

There are only multicellular filamentous species observed in brown algae; no reports of unicellular or colonial organisms have been discovered yet (Bold et al., 1987). Brown algae have thalli that can range in size from microscopic to several meters long and branching. Typically, the macroscopic thallus can be distinguished into holdfast, stipe, and blade (see **Figure 4**). Though all the groups thought to be the closest relatives of the browns comprise single-celled or colony form, this divergence of the brown algae thallus may be the product of taxonomy rather than evolution (Lee, 2008).

The characteristic brown color of brown algae is caused by the carotenoid pigment fucoxanthin, which is found in their chloroplasts and, in certain species, in certain phaeophycean tannins. Chlorophylls A, C1, and C2, β-carotene, diatoxanthin, violaxanthin, and significant levels of fucoxanthin are also present in their chloroplasts (Verma et al., 2015).

2.3.2 Habitat

Brackish seas and marine habitats are the main habitats for brown algae. There are additional freshwater species of brown algae, according to (Lee, 2008) and (Wehr et al., 2015). Freshwater phaeophytes are primarily found in rivers, streams, and the lakeshore regions of lakes. Although species like Heribaudiella fluviatilis may become dominant in river flora, little is known about their biology (Wehr et al., 2015).

2.3.3 Reproduction

Brown algae reproduce in three ways namely asexual reproduction, sexual reproduction and vegetative reproduction.

2.3.3.1 Asexual Reproduction

Asexual reproduction in ectocarpales and spherocarpales takes place through biflagellate zoospores, which grow into reproductive organs termed sporangia. These organs can be many cells plurilocular, as seen in Hinskia mitchelliae Fig., or unilocular (one-celled). Additionally, gametes can divide parthenogenetically to create asexual progenies, as in the case of Ectocarpus (Peters et al., 2004).

2.3.3.2 Sexual Reproduction

In Phaeophyceae, gametangia contain flagellate gametes, which are generated during sexual reproduction. Only a small percentage of brown algae produce multicellular gametangia (Chapman, 1970). The process of sexual reproduction involves the fusing of big, non-flagellated female gametes with flagellated male gametes or vice versa (Fritsch, 1977). The haploid and diploid organisms' generations are altered in the brown algal life cycle. Through mitosis, haploid gametophytes (n) produce haploid gametes. After the fusing of male and female gametes (n), a zygote (2n) is produced, which develops into a diploid sporophyte. Through meiosis, the sporophyte (2n) generates meiospores (n), which germinate and form haploid gametophytes. The life cycle of brown algae can be diplontic, heteromorphic, or isomorphic (Verma et al., 2015).

2.3.3.3 Vegetative Reproduction

Numerous brown algal species exhibit fragmentation-based vegetative reproduction. Propagules are found in members of the sphaecelariales family (Kumar & Singh, 1979).

2.3.4 Application of Brown Algae

Sulfurated laminarans derived from brown algae (*Saccharina japonica*, *Saccharina cichorioides*, and *Fucus evanescens*) have demonstrated a variety of anticancer properties that inhibited the migration of breast adenocarcinoma by blocking the activities of the metalloproteinases 9 and 2 matrixes (Malyarenko et al., 2017). Several types of brown algae that exhibit antioxidant, anti-inflammatory, and anticancer properties are commonly found to contain fucoxanthin (Farghali et al., 2023). Polysaccharides found in brown algae, including fucoidans, laminarans, and alginic acid, have strong antioxidant properties (Afonso et al., 2019).

Consumers and the food industry are becoming more interested in incorporating macroalgae, such as Phaeophytae, into Western diets because they are thought to be a rich and balanced source of nutrients and bioactive compounds. New products are being introduced to the European market at a rapid pace (Afonso et al., 2019).

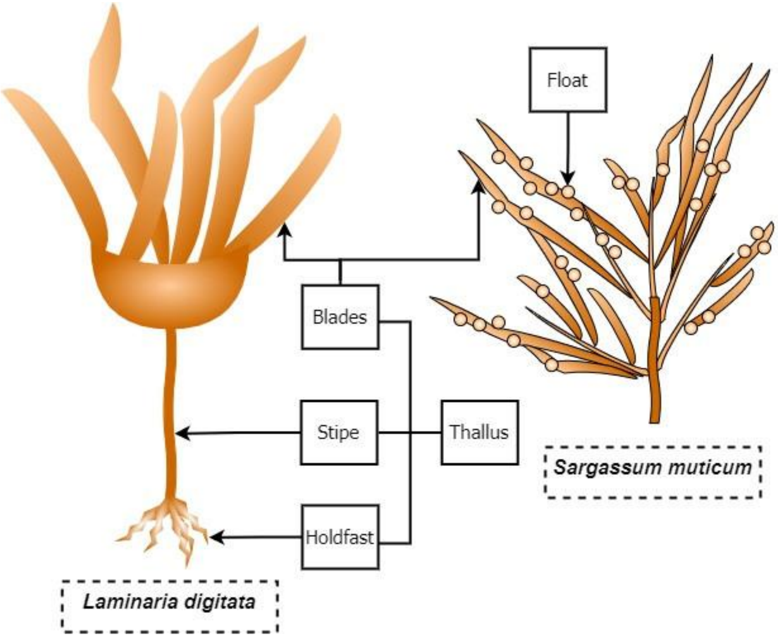


Figure 4: General morphology of brown algae (Zhao et al., 2022).

2.4 Red Algae

Red algae, also known as Rhodophyta, are a diverse group of photoautotrophic plants ranging from unicellular to multicellular forms. They exhibit a variety of morphologies and simple anatomical structures, and their life cycles display considerable diversity (see **Figure 5**). Following fertilisation and development, the red-pigmented female haploid thallus was observed to have a green diploid tetrasporophyte attached to it (Stévant et al., 2023). Through meiosis, haploid tetraspores grow inside the sori of diploid tetrasporophytes' fronds. Both blade-like male and tiny female gametophytes that resemble diploid tetraspo-rophytes are produced when the haploid tetraspores germinate (van derMeer & Todd, 1980). To capture spermatia, or non-motile male gametes, discharged from a mature male gametophyte (at least one year old), the female gametophyte grows oogonia containing trichogynes which are hair-like structures. The encrusting female gametophyte, which is outgrown early on when the tetrasporophyte develops its own discoid holdfast, gives rise to a diploid tetrasporophyte from the zygote produced when a spermatium fertilises a female gamete (Stévant et al., 2023). Approximately 98% of red algae species live in marine environments, and the rest 2% are found in freshwater habitats (C. F. D. Gurgel & J. Lopez-Bautista, 2007).

Red algae have several characteristics which set them apart from other algal groups like complete absence of flagella and centrioles, phycobilisomes, unstacked thylakoids within the chloroplast, the lack of parenchyma, and incomplete cytokinesis (C. Gurgel & J. Lopez-Bautista, 2007). The characteristic red color of red algae is due to the presence of photosynthetic pigments(for example, chlorophyll and carotenoids) and phycobilisomes which are water-soluble protein complexes composed of three main classes: phycoerythrin(red), phycocyanin(blue), and allophycocyanin which is blue-greenish (Grossman et al., 1993).

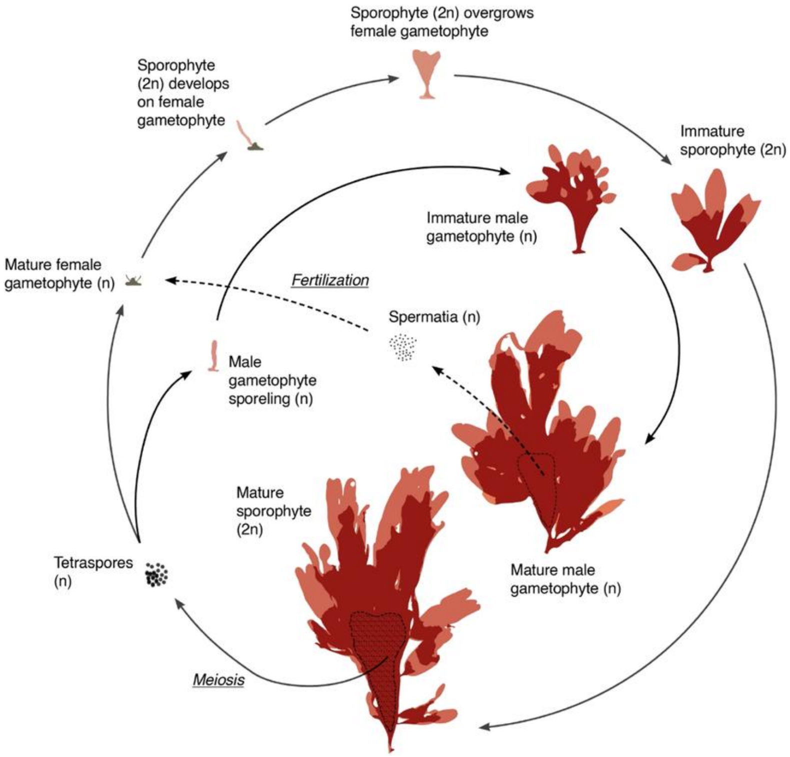


Figure 5: Life cycle of Red Algae (Mayanglambam & Sahoo, 2015).

2.4.1 Dulse

Dulse is a red macroalgae that is widely recognized and highly valued. It is found in the North Atlantic, mostly between 40 and 80 °N in latitude (Stévant et al., 2023). This species holds significant commercial value, as evidenced by several centuries of its use as food. Today, handpicked dulse are harvested from the shoreline in Western Europe, Canada (New Brunswick and Nova Scotia), and the United States (Stévant et al., 2023).

2.4.2 Dulse Morphology

The name dulse refers to the plant's distinctive form, which comprises oblong, flattened lobes extending from the center of the frond to resemble a hand or palm. As seen in **Figure 6,** the fronds emerge from a disc-shaped holdfast that is extended by a short stipe (Stévant et al., 2023). The individual plant can grow up to the size of 50cm in length but the typical usual length is around 10 to 20 cm (Sears, 2002). In aquaculture, When grown with continuously flowing and spinning water, it can take on an almost isotropic shape, as demonstrated by the specimen photograph in **Figure 7** (Mouritsen et al., 2013).

Figure 6: Photographic black-and-white image shows a dulse specimen raised in a seawater pool in Horsens, Denmark (Mouritsen et al., 2013).

Figure 7: Two morphotypes of Dulse herbarium samples, found in Western Brittany, France. Top: common plant, gathered at Plougastel-Daoulas (France, Britanny). Bottom: Plant found near Le Conquet (Brittany, France) that is thought to be a member of the sarniensis-sobolifera complex (Stévant et al., 2023).

Fronds have a leathery or membranous texture. Moreover, the fronds emerge from a tiny discoid holdfast that can be attached to the substrate singly or in clusters. Stipes are thin, frequently measuring less than 5 mm. Marginal outgrowths of fresh fronds from the parent blade are frequently seen in older plants (**Figure 7**). The morphology might vary greatly based on the surrounding circumstances (Werner & Dring, 2011).

It has a thin, delicate, disk-shaped holdfast and is found in cold, turbulent seas in the wild on the substrates of rocks or other huge macroalgae, such as kelps like *Laminaría hyperborea*. After drying and bleaching in the sun, the wet fronds' colors change to pinky-red from purple, crimson, or brownish-red (Mouritsen et al., 2013).

2.4.3 Importance of Dulse

In the West, dulse is one of the more widely consumed macroalgal species for human nutrition. It has been used historically and currently as a snack, nutritional supplement, and ingredient in many different recipes in Ireland, Brittany (France), Iceland, Maine (USA), and Nova Scotia in Canada (Mouritsen et al., 2013). The author further reported that dulse has a good chance of becoming commercialized as a complete food as well as an ingredient because it may be gathered in the wild and grown in pools on land and in the sea. It's interesting to note that dulse is among the few macroalgae species that has a history of being consumed by humans in Europe spanning decades, if not millennia.

2.4.4 Chemical and Nutrient Composition of Dulse

The chemical and nutrient composition of dulse have been discussed in this section. See **Table 1.** Like most macroalgal species, dulse has a very high moisture content, which can make up to 88% of its fresh biomass. The moisture content of dulse is maximum in the winter and spring, as is typically the case with macroalgae (Stévant et al., 2023).

The majority of minerals are present in high amounts in dulse, especially sodium, potassium, and chlorine. Additionally, the fresh weight and dry weight of the food are quite rich in calcium, iron, and magnesium, and the high iodine content means that less than 1 g of dulse is enough to meet an adult's daily needs for this element (Morgan et al., 1980).

Table 1: Comparison of mineral content of dulse with other food (mg per 100 g of edible portion) (Morgan et al., 1980).

	Na	K	Ca	Mg	Mn	Fe
Dulse						
Dried	1740	7000	560	450	4.5	50
Fresh	295	12000	95	75	0.8	0.5
Apples						
Dried	1		24			
Fresh		116	6	6	0.08	0.07
Oranges	0.3	170	33	10	0.03	0.08
Carrots	89	311	41	17	0.25	0.11
Peas	75	380	22	27	0.41	0.23
Potatoes	87	880	14	27	0.17	0.16
Peanuts	5	740	740	167	1.51	0.27

2.4.4.1 Carbohydrates

Up to 74% of dulse’s dry weight (DW) comprises carbohydrates (**Figure 8**). The main constituent of this species' cell wall is xylans (Stévant et al., 2023). The previous author went on to say that because xylans are indigestible, they are thought to be Dulse’s primary source of dietary fiber. They can be found in both water-soluble and insoluble forms. As structural carbohydrates, cellulose (i.e., insoluble glucans) is also present in trace amounts (approximately 3% DW). Less than 5% of the DW is because of Floridoside in the winter and up to 25% in the summer (Rødde et al., 2004). Floridoside can stimulate the immune system in response to antigens, which may have therapeutic implications (Courtois et al., 2008).

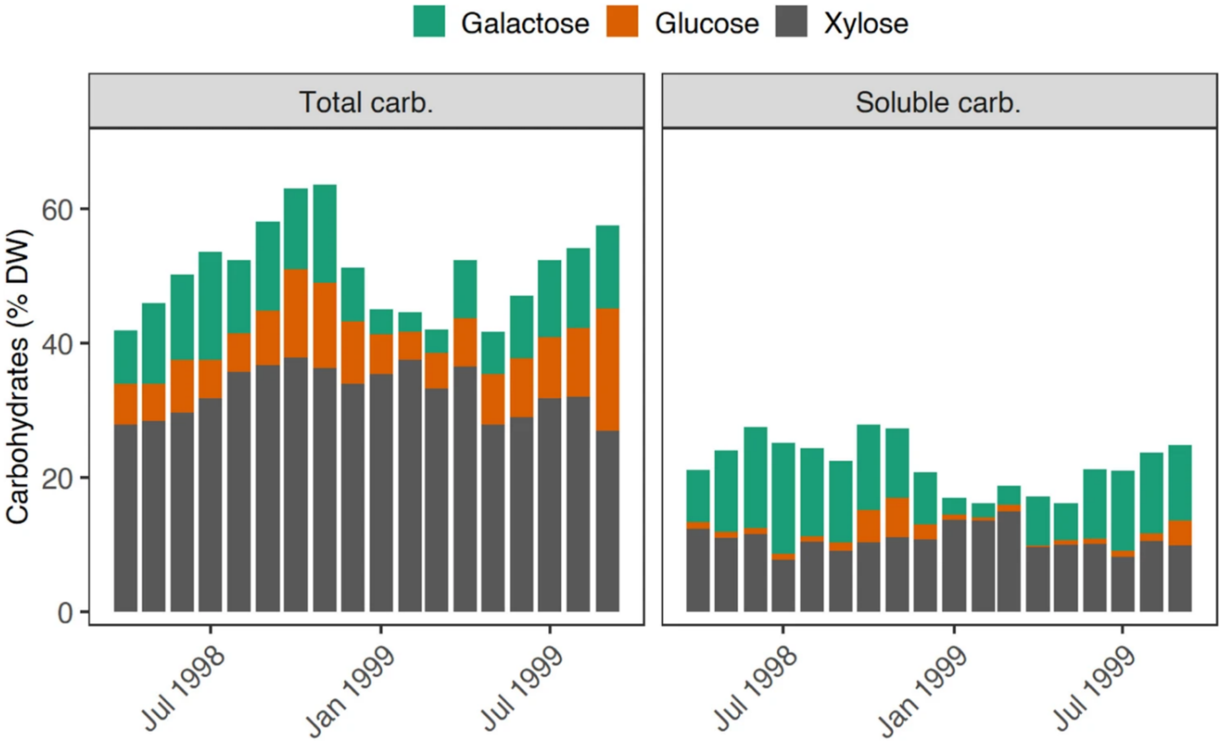


Figure 8: Variation in the carbohydrate content of Dulse collected in Trondheim fjord, Norway (Stévant et al., 2023).

2.4.4.2 Proteins and Amino Acids

Dulse has a high protein content (around 10% DW) in comparison to most other edible brown species, such as *Undaria pinnatifida* and S. latissima (Jard et al., 2013; Mæhre et al., 2014; Stévant, 2019). Although the level is less than Pyropia sp. (33–47% DW) levels and similar to those observed in Ulva sp. which is 10–26% DW (Fleurence et al., 2018).

In dulse, glutamate and aspartate are the most prevalent amino acids. Together with EAA(Essential Amino Acids) that are frequently limited in plant food sources, such as methionine, lysine, threonine, and tryptophan, the species also includes significant levels of arginine, glycine, serine, leucine, and valine (Stévant et al., 2023). It should be noted that free amino acids make up around 10% of dulse's total amino acid composition (Aasen et al., 2022; Maehre et al., 2016). Peptides and monomeric aromatic acids (MAAs) from dulse have been proposed as natural antioxidants in various commercial applications based on their documented bioactivity in scientific literature (Harnedy et al., 2017; Yuan et al., 2009).

The protein composition of eleven specimens of dulse that were collected monthly in Belle Ile in 1996 was examined by (Galland-Irmouli et al., 1999). Based on the level of nitrogen, the protein content of these specimens ranged from 9.7 to 25.5% of the dry mass. See **Figure 9.**

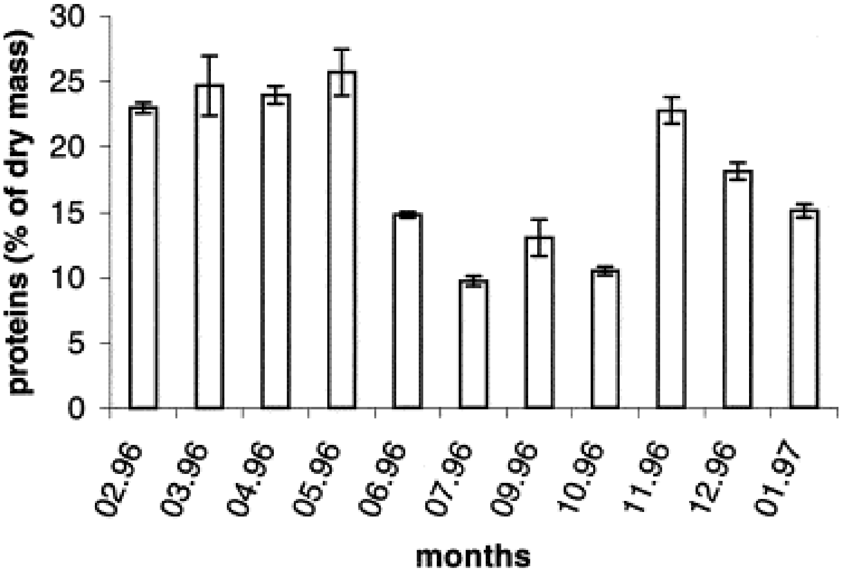


Figure 9: Seasonal fluctuations in dulse's protein concentration. Each specimen's mean values and standard deviation of measurements (n = 3) are illustrated in the bar graph (Galland-Irmouli et al., 1999).

The specimens of dulse were collected in various months throughout 1996 during the same study, and the amino acid content of their proteins was ascertained (see **Table 2**). Aspartic acid and glycine were found in significant concentrations in all 10 samples, while glutamic acid, leucine, and valine were found in smaller amounts. Dulse proteins were likewise low in methionine, hydroxyproline, proline, and histidine, and had reduced levels of alanine, serine, arginine, and phenylalanine. Cystine wasn't available. The majority of amino acids were acidic (28.5 ± 8.7% of total amino acids) as opposed to basic (7.8 ± 6.2%). With an average of 35.8%, the essential amino acids in dulse proteins made up 26.1 to 50.0% of the total amino acids.

Table 2: Seasonal fluctuation in essential amino acid composition from dulse proteins (g/100 g amino acids) (Galland-Irmouli et al., 1999).

Amino acids	02/96	04/96	05/96	06/96	07/96	09/96	10/96	11/96	12/96
Ile	4.0	3.6	1.9	4.4	6.4	0.0	3.1	4.6	4.1
Leu	7.1	9.4	8.2	0.0	13.6	0.0	6.7	9.0	8.3
Lys	5.3	0.0	0.0	0.0	0.0	6.1	4.3	5.9	5.5
Met	2.0	3.7	2.5	3.4	3.8	1.8	1.6	2.5	2.7
Cys	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Met+Cys	2.0	3.7	2.5	3.4	3.8	1.8	1.6	2.5	2.7
Phe	4.1	0.0	6.6	3.7	10.0	0.0	5.0	6.8	6.8
Tyr	3.9	0.0	0.0	4.5	6.4	0.0	0.0	5.4	6.4
Phe+Tyr	8.0	0.0	6.6	8.2	16.4	0.0	5.0	12.2	13.2
Thr	5.4	6.9	8.7	0.0	0.0	6.7	8.0	0.0	0.0
Val	7.1	7.9	0.0	10.2	9.8	5.7	5.2	8.1	7.0
Total	38.9	31.5	27.9	26.2	50.0	20.3	33.9	44.3	40.8
His	1.2	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.1
Asp	12.7	15.7	18.6	19.8	10.3	15.8	12.2	26.8	27.9
Glu	12.1	20.7	17.6	19.8	0.0	15.6	13.5	0.0	0.0
hPro	0.0	0.0	0.0	0.0	8.4	0.0	0.0	4.5	4.9
Pro	6.6	0.0	0.0	0.0	0.0	6.9	4.7	0.0	0.0
Ser	7.4	10.7	18.3	12.7	0.0	7.7	5.7	0.0	0.0
Gly	6.5	9.3	8.7	8.4	22.5	15.3	9.0	19.1	18.6
Ala	8.7	12.2	8.9	13.3	0.0	11.1	12.6	0.0	0.0
Arg	6.1	0.0	0.0	0.0	8.9	7.4	8.4	6.1	6.9

Dulse has comparatively high protein levels when compared to the majority of macroalgae that are traditionally eaten in Japan except for *Porphyra tenera* **(Table 3).** The higher protein concentrations of dulse (21.9 ± 3.5%) during the winter-spring season are equivalent to those of high-protein vegetables, especially leguminous plants like soy (25% of proteins) (Galland-Irmouli et al., 1999).

Table 3: The protein content of some algae compared with dulse (Galland-Irmouli et al., 1999).

Algae	**Protein (% of frond)**
Porphyra tenera	47.5
Analipus japonicus	23.7
Grateloupia turuturu	20.0
Dulse (Palmaria palmata)	18.3
Ulva pertusa	17.5
Laminaria japonica	15.6
Codium fragile	15.6
Eisenia bicyclis	13.1
Undaria pinnatifida	12.5

2.4.4.3 Lipids

In dulse, lipids are broken down into three categories: neutral, polar, and free fatty acids (FA), which make up 36%, 37.5%, and 26.5% of the total FA, respectively (Foseid et al., 2020). Long-chain ω-3 PUFA, specifically eicosapentaenoic acid (EPA), is abundant in dulse's lipid fraction and may make up more than 50% of the total lipids (Lopes et al., 2019; Mouritsen et al., 2013). Because dulse does not contain any appreciable levels of docosahexaenoic acid (DHA), it is a promising raw material for the manufacturing of very pure EPA concentrates (Mishra et al., 1993). Additionally, the EPA-rich polar lipid fraction from dulse was shown to have antioxidant activity (Lopes et al., 2019). Dulse is unique in that it produces desmosterol as a primary C27 sterol instead of cholesterol, which is the principal sterol of the unsaponifiable lipid fraction of the majority of red algae (Morgan et al., 1980). The author further reported that dulse has trace amounts of hydrocarbons, which make up 0.003 and 0.009% of the dry weight in two samples taken from the US east coast and 0.019% in samples taken from the Bay of Fundy.

2.4.4.4 Ash Content

The ash content of the dulse indicates that it is a rich source of macro- and microelements (e.g., Fe, I, Mn) as well as macro-elements (Na, K, Ca, and Mg) (Stévant et al., 2023). Generally, the ash content is highest in the winter and spring and lowest in the summer and fall (Rødde et al., 2004). **Figure 10** illustrates that ash and dry matter content was highest from December to May and lowest the rest of the year. From a nutritional standpoint, (Stévant et al., 2023) wrote that it's interesting that dulse has higher amounts of K than Na, as diets high in Na are linked to health hazards like high blood pressure and cardiovascular disorders. The average values of the iodine content of dulse are lower than those of commercial kelp species, and consuming a few meals containing dulse each week won't expose the consumer to excessive iodine intakes, even though it could reach up to 790 mg kg−1 DW, which is the range of values found in brown macroalgae based on screening 26 samples taken throughout Norway (Duinker et al., 2020).

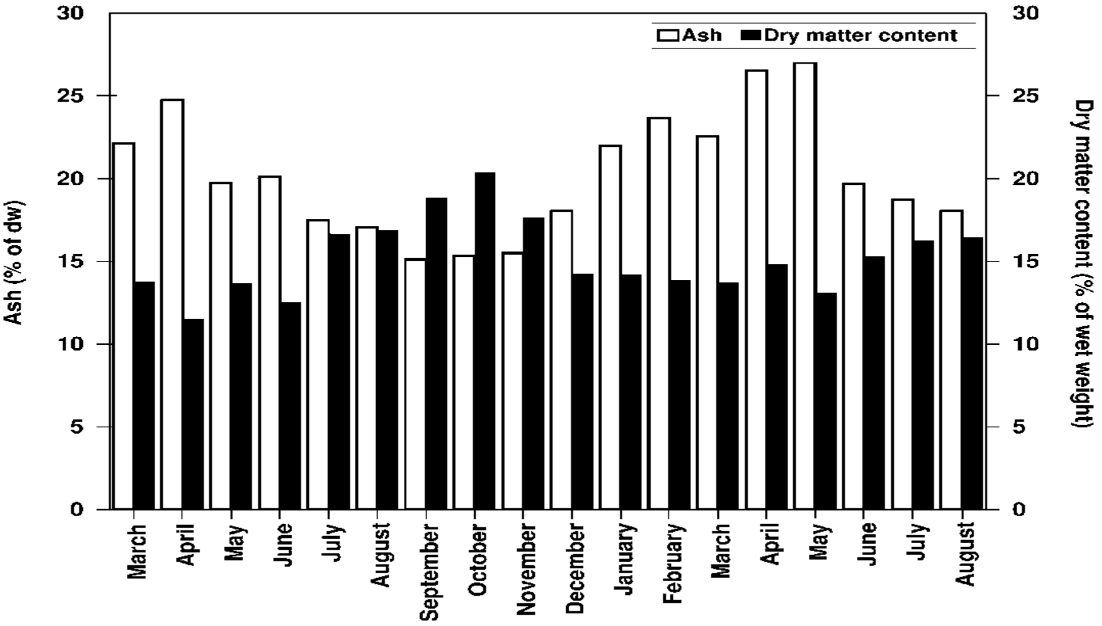


Figure 10: Seasonal variation of dulse in the dry matter content (given as % of wet weight) and ash content (given as % of dry weight, mean values given wns5, subsamples, not true replicates) in plants from the Trondheim fjord, Norway (Rødde et al., 2004).

2.4.4.5 Vitamins

The majority of species, including dulse, are not rich sources of vitamin C when compared to other plants (such as rosehip, parsley, and broccoli), and daily consumption will not significantly increase the recommended nutrient intake for this compound (Nielsen et al., 2021). Conversely, dulse seems to be an excellent source of vitamin B, specifically B12 (cobalamin), B2 (riboflavin), and B1 (thiamine) (Kraan, 2013; MacArtain et al., 2007). 10% of the World Health Organization's recommended daily nutritional intake of 2.4 μg day−1 can be met by consuming 1 g of dried material (WHO, 2004). Out of all the fat-soluble vitamins (A, D, E, and K), dulse is rich in β-carotene (Kraan, 2013). β-carotene, also referred to as provitamin A, is a precursor to both vitamin K and vitamin A (Mouritsen et al., 2013).

While vitamin C content of dulse typically makes up more than 75% of the value of oranges, vitamin A is almost 50% that of carrots. Dulse's drying and storage, however, most likely eliminates a large portion of its vitamin C content and negatively impacts the majority of B vitamins. Despite this, dried dulse is a poor source of vitamin B6 and biotin but has average levels of thiamine, riboflavin, and niacin compared to other foods (Morgan et al., 1980).

2.4.4.6 Nutritional Value of Proteins

Red algae is widely known for having a high protein content. Particularly high concentrations are present in the fronds of P.tenera and dulse, where they can be found in as much as 47.5% and 30%, respectively. The nutritional value of dulse should be evaluated by comparing its amino acid content to the requirements of humans (Galland-Irmouli et al., 1999). According to Dupin et al. (1992) and Friedman (1996), the ratio of essential amino acids, the balance of essential amino acids relative to the reference protein egg, and the essential relative amino acid content are the three key elements. The essential amino acid profile of dulse proteins was found to be similar to that of egg protein according to the study conducted by Galland-Irmouli et al. (1999), indicating that the necessary amino acids constituted between 26 and 50% of the total amino acid content, indicating the good quality of these proteins.

Dulse is known for its abundance of acidic amino acids (see **Table 2**) relative to basic amino acids (Munda & Gubenšek, 1976). The most abundant amino acids in dulse are aspartic acids and glutamic acids (Friedman, 1996). According to reports, Dulse proteins are also low in histidine, tryptophane, cystine, and methionine and high in glycine, alanine, leucine, valine, and arginine (Coulson, 1955).

2.5 Dulse Growth

2.5.1 Dulse Growth Factors

2.5.1.1 Temperature

Temperature is one of the most important abiotic variables that directly affects growth, reproduction, and photosynthetic efficiency. With varying thermic amplitudes and sea temperatures ranging from 0 °C to over 20 °C, dulse is found in Arctic and warm temperate regions (Stévant et al., 2023). Cultivation studies of material gathered from the wild indicate that a temperature range of 6 to 12 °C is ideal for growth (Corey et al., 2012; Morgan & Simpson, 1981a). Above 14 °C, lower productivity is noted by Corey et al. (2013) whereas, increased biomass mortality occured at 21 °C (Matos et al., 2006).

2.5.1.2 Salinity

Dulse is frequently found in environments with somewhat stable salinity levels (30–40 PSU). It is also found in the intertidal zone, though, where precipitation may expose it to abrupt changes in salinity. Some populations are subject to a seasonal drop in ocean salinity as a result of things like significant ice-melting water discharges from arctic and boreal regions (Stévant et al., 2023). Under controlled conditions, dulse from Spitzbergen was found to have significant photosynthetic inhibition and death at 15 PSU (Stévant et al., 2023). On the other hand, individual fronds grown in tanks from Danish inner waters showed a maximum specific growth rate of 15 PSU, as opposed to 25 and 35 PSU. This indicates that dulse ecotypes are suited to a broad variety of salt levels (Schmedes & Nielsen, 2020).

2.5.1.3 Nutrients

Through absorption throughout the thallus area, macroalgae absorb nutrients from their surroundings. Although dulse has a higher affinity for NH_4^+, both ammonium (NH_4^+) and nitrate (NO_3^-) are nitrogen sources for macroalgal development (Morgan & Simpson, 1981a) (Corey et al., 2013). Dulse can store carbon, phosphorus, and nitrogen (it can utilize both CO_2 and HCO_3) to promote growth in nutrient-limited environments (Stévant et al., 2023). According to a study conducted by Morgan and Simpson (1981a), Higher growth rates were promoted by NO_3^- than by similar quantities of $NH4^+$. Lower yields were achieved when $NH4^+$ was added four times a week as opposed to once or twice a week, whereas somewhat greater yields were obtained when NO_3^- was added four times or twice a week instead of once a week. Plants that received 0.5 mM NO_3^- four times a week produced over 100% more biomass by the end of the fifth week than plants that received 0.5 mM NH_4^+ four times a week (See **Table 4)**. Throughout the experiment, dulse's total nitrogen content rose. Plants receiving NH_4^+ had greater amounts than plants getting NO_3^-. When plants were given NH_4^+ four times a week, extremely high nitrogen levels (5.9% of dry weight) were observed. The study further stated that NH_4^+ was somewhat toxic, even though NH_4^+ had a higher nitrogen absorption and accumulation than NO_3^-. Unionized ammonia may limit photosynthetic carbon metabolism, leading to this toxicity.

The nutrient need for dulse was also explained by (Stévant et al., 2023) as they cultivated dulse using optimal nutrient needs for the production of dulse. They described the nutrients needed for dulse production as an addition of 300uM NO_3^- and/or preferably 300uM NH_4^+ twice a week as a nitrogen source. Additionally, PO_4^{3+} at a molar N:P ratio of 10:1 (or higher) is required.

Table 4: Impact of fertilization regime on dulse's total nitrogen content (as a percentage of dry weight). Plants' initial total nitrogen concentration was 1.65% of their dry weight (Morgan & Simpson, 1981a).

Nitrate			
Week	**0.5 mM x4**	**1mM x2**	**2mM x1**
1	2.55	2.0	1.75
3	2.70	2.5	1.50
5	3.05	2.35	1.80
Ammonium			
Week	**0.5 mM x4**	**1mM x2**	**2mM x1**
1	2.50	2.5	2.10
3	2.90	2.90	2.10
5	3.70	3.70	2.95

The nutrient need for dulse was also explained by Stévant et al. (2023) as they cultivated dulse using optimal nutrient needs for the production of dulse. They described the nutrients needed for dulse production as an addition of 300uM NO_3^- and/or preferably 300uM NH_4^+ twice a week as a nitrogen source. Additionally, PO_4^{3+} at a molar N:P ratio of 10:1 (or higher) is required.

2.5.1.4 Irradiance and Nutrient Absorption

Dulse can adjust its photosynthetic activity in response to changes in light levels throughout the day, just like other plants exposed to high levels of Photosynthetic Active Radiation(PAR)and Ultra Violet (UV)radiation (Stévant et al., 2023). Similar to other macroalgal species, photosynthetic activity in dulse is primarily inhibited by UV-B wavelengths (Karsten et al., 2001; Pakker et al., 2000). The physiological response to photooxidative stress is characterized by reversible damage to pigments that harvest light, such as phycocyanin, phycoerythrin, and chlorophyll a. This process dissipates the excess energy absorbed in the form of heat and facilitates a swift recovery following the cessation of stressful conditions (Hanelt & Nultsch, 1995). Chronic photoinhibition results in DNA (Deoxyribo Nucleic Acid) damage and alteration of a crucial component of photosystem II (i.e. D1 protein) requiring De novo synthesis and a longer recovery period (Pakker et al., 2000). The plant's growth and physiological processes may be adversely impacted by such damage (Stévant et al., 2023).

Dulse stores nutrients in large part as a result of irradiance. Comparing plants growing under high irradiation to individuals acclimated to low irradiance, the latter showed lower soluble carbohydrate contents but higher amounts of phosphate and nitrogen (Martínez & Rico, 2002; Morgan & Simpson, 1981b). Phycoerythrin, which is involved in light absorption, stores some nitrogen and is used to boost photosynthesis in low irradiance settings and to promote growth in optimal nutritional environments (Martínez & Rico, 2002).

In general, a marine fish farm may lose 52–95% of its nitrogen, 85% of its phosphorus, and 80–88% of its carbon input to the environment through respiration, feed waste, fish excretion, and excrement production (Wu, 1995). This is equal to 95 to 102 kg of nitrogen and 9.5 kg of phosphorus per tonne of fish produced (Black, 2001; Hall et al., 1992; Subandar et al., 1993). Since they are most likely to limit growth rate under "natural" conditions, nitrogen(N) and phosphorus(P) are the two most critical components in algal metabolism in coastal waters. (Sanderson et al., 2012) Since phosphorus(P) is relatively abundant in the open ocean, nitrogen(N) and light are the main variables limiting algal production (Dring, 1992; Lobban & Harrison, 1994).

A few studies have looked at the productivity and efficacy of dulse bioremediation in land-based tank systems and pilot-scale offshore cultivation, for instance, near fish farms. Most of these investigations have concentrated on the effectiveness and yield of eliminating nitrogenous chemicals from the water column, such as nitrate (NO_3^-) and ammonium (NH_4^+) (Lubsch & Timmermans, 2020). By observing the uptake rates of DIN(Dissolved Inorganic Nitrogen) and DIP(Dissolved Inorganic Phosphate) in dulse sporophytes over approximately six hours, (Martínez & Rico, 2004) were able to demonstrate a biphasic nutrient uptake for both species. Nutrient-starved macroalgae have been reported to exhibit a biphasic nutrient uptake (Dy & Yap, 2001; Fujita, 1985). This includes an internal or metabolic uptake (VM), which is regarded as equivalent to the rate of assimilation, and a surge uptake (VS), which describes the filling of internal nutrient pools, uncoupled from growth (Barr et al., 2004; Taylor & Rees, 1999).

Dulse and other perennial macroalgae depend on stored N and P, which they acquire in the fall and winter when nutrient availability is high. During spring and summer, it benefits from this internal storage when there are more daylight hours, higher temperatures, and often less availability of nutrients (Martínez & Rico, 2002).

2.6 Recirculating Aquaculture System

For the production of dulse, we are also using water samples from different sections of RAS. Thus, a better understanding of RAS is required in this regard. There is a great concern about sustainability during aquaculture processes and the discharge of aquaculture waste material into the environment, which can be hazardous for aquatic animals and other animals and humans (Shitu et al., 2024). RAS (Recirculating aquaculture system) is designed particularly for this concern in which water is re-circulated and re-used after certain treatments (Martins et al., 2010). According to the same authors, the RAS is developed to reduce water usage, the proper management of waste, the recycling of nutrients, and proper disease management. Moreover, the utilization of RAS will help in the production of a variety of seafood products in the future (Masser et al., 2000).

The RAS works on the basic premise of diverting the water supply through ponds or tanks to recirculate the water through flow-through fish farms. Recirculation, on the other hand, entails treating all or part of the discharge water before returning it to the fish-rearing system (Aich et al., 2020). See **Figure 11** for the visual figure of a typical RAS system.

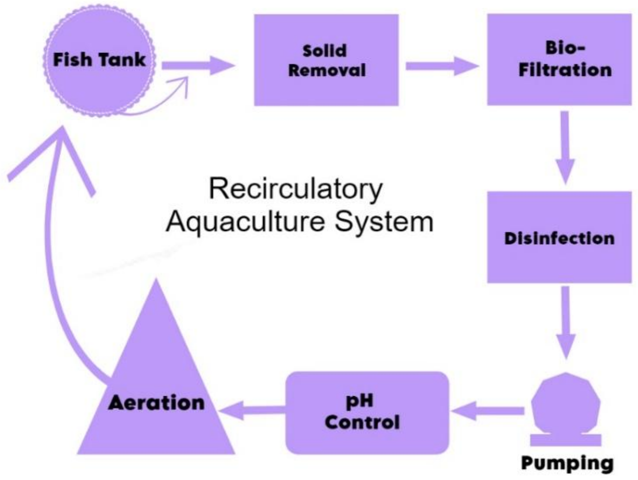


Figure 11: The diagram showing different compartments of a typical RAS.

2.6.1 Solids Removal

The removal of solids from the water reaching fish tanks is necessary as they have adverse effects on the fish health (Chapman et al., 1987). The presence of solids in finfish aquaculture systems has numerous detrimental effects on fish as it can cause potential harm to fish gills, increase biochemical oxygen demand, impair biofilter nitrification process, and also the escalation of nitrification process in the aquaculture system (Chapman et al., 1987). Removal of solids is considered the most crucial step in aquaculture systems (Summerfelt, 1996).

The introduction of feed to fish regulated the generation of solid particles in the system, encompassing feces and feed that is not consumed. These constituents are one of the primary contributors to carbonaceous oxygen demand and nutrient influx into the water, particularly upon degradation within the system. The part of the feed which is not metabolized well by the fish is excreted as organic waste which consists of fecal solids, while the leftover feed which is not consumed by the fish triggers the production of Total Ammonia Nitrogen (TAN) as it is broken down by bacteria present in the system (Timmons & Ebeling, 2007).

2.6.2 Biofiltration

Biofilters in RAS use specially and specifically designed substrates called media (Wafula et al., 2023). The media is used to proliferate a wide variety of microorganisms and encourages the formation of biofilms (Gutierrez-Wing & Malone, 2006). Furthermore, the bio-filters are categorized into aerobic filters and anaerobic filters based on their mechanism of operation. Aerobic filters use aerobic bacteria as they can convert ammonia into nitrate through the process of nitrification (Xiao et al., 2019). The nitrifying bacteria grow on media and convert ammonia into nitrite and then into nitrate. The filtered water is then returned to tanks or ponds (Wafula et al., 2023).

Anaerobic bio-filters utilize anaerobic bacteria which converts nitrate to nitrogen gas. Although these kinds of filters are less common in RAS, anaerobic filters can serve to remove surplus organic material from the water (Wafula et al., 2023). Biofilters can be made from different materials such as wood, chips, gravel, beads, and activated charcoal (Diver & Rinehart, 2000).

2.6.3 Disinfection

All the information regarding disinfection in RAS(Recirculating Aquaculture System) is taken from (Malone, 2013). According to the author, the requirement for internal disinfection has essentially been removed thanks to source water control (mostly groundwater) and other external disease prevention techniques. UV light and sonic disinfection are the two most popular types along with ozone therapy. UV light is mostly utilized extensively because of how simple it is to set up and utilize. Ozone is used in larger establishments if the expense can be justified. Initial setup and management by skilled technician personnel.

Regarding disinfection through UV light, the author stated that UV light serves as the main internal disinfection tool. The concentrated light spectrum that these lights emit is in the UV wavelength range, which is lethal to microbes. Generally speaking, disinfection rates are correlated with light intensity, which is determined by the wattage of the utilized light bulb and the flow rate that is treated.

The author further reported that Ozone is the second, less popular choice for internal disinfection. Ozone is a particularly potent oxidant that functions similarly to chlorine but with greater potency. Many devices can produce it, but corona discharge designs are usually acknowledged as the most potent. The amount of oxygen in the air that the corona discharge ozone generator is supplied regulates how well it works. The air's humidity level has a significant impact on how long the unit lasts. Gases that are supplied into corona discharge units are often enhanced in oxygen and go through an air-drying machine before being fed into the unit. Ozone can be dosed using a packed column after it has been produced. More often than not, ozone is dosed using a straightforward venturi that draws air into the recirculating line as tiny bubbles using the energy from the pump. Most recirculating waters quickly absorb ozone, which kills bacteria, viruses, and eventually protozoan organisms after first eliminating dissolved organics.

2.6.4 pH Control

In RAS, pH must be closely watched and managed. Fish and bacteria produce carbon dioxide (CO_2) through respiration, and this gas dissolves in water to make carbonic acid, which further lowers pH levels (Wright, 2011).

Acid exposure can have various effects on the gills, including raising mucus production, drawing in leukocytes, and boosting the number and turnover of ion-transporting cells, also called ionocytes (Kwong et al., 2014). Additionally, fish exposed to significantly acidic water (pH 2.0–3.5) could suffer gill breakdown and suffocate as a result of mucus deposition (Packer & Dunson, 1972).

2.6.5 Aeration

For healthy living of aquaculture species especially fish, the concentration of dissolved oxygen (DO) is really important. Generally, for the effective culture of fish, the concentration of DO should be at least 5mg/l. Its level below 3mg/l causes stress in aquatic organisms and can lead to significant fish mortality (Roy et al., 2021). The amount of dissolved oxygen (DO) in the water has a direct impact on fish and other aquatic animal growth (Roy et al., 2021). When the concentration of DO is extremely low, the utilization of feed is significantly diminished (Eltawil & ElSbaay, 2016). A culture system that has low DO may not be the direct cause of fish death but it can cause the deterioration of water quality, which in return imparts a high level of stress on the fish, reduces their feed consumption, and ultimately results in their increased mortality rate (Roy et al., 2021).

The aeration process involves transferring oxygen into the water which is rapidly consumed by both fish and bacteria. During periods of high activity, the RAS aeration section should be able to replenish all oxygen in the system every 20-30 minutes to keep up with the peak feeding rates (Malone, 2013). Most aquaculture systems use the method of either blown air or delivery of pure oxygen to maintain an adequate level of oxygen.

Blown air systems are simple because they add oxygen and remove carbon dioxide at similar rates. Conversely, pure oxygen systems can sustain higher dissolved oxygen levels and are cost-effective depending on the operation and cost of pure oxygen (Malone, 2013).

An airlift pump is another system that provides aeration while continuously flowing water. The air-lift pumps operate on the principle of buoyancy in which the gas is injected into a submerged pipe within a liquid medium. The resulting gas-liquid mixture, having a lower density than the surrounding liquid, generates a lifting effect that propels the liquid along with the mixture. This technology offers several advantages over the conventional pump system, including low maintenance requirements, simplicity, and cost-effectiveness (Mohammed et al., 2018).

2.6.6 Discharge of Nutrients into the Environment

The maximum carrying capacity of systems is determined by controlling factors such as water quality limits, which are essential for designing flow rates and water treatment units (Colt & Orwicz, 1991). **Table 5** shows the water quality limits of different parameters like NH_4-N, NO3-N, NO_2-N, and PO_4-P for different species in RAS.

During operation, aquaculture production units are loaded with large amounts of nutrients. It is necessary to remove these nutrients in order to preserve water quality unless the system is very extensive (Verdegem, 2013). The fish is fed feed, and it produce fecal waste, urine and other toxic nutrients like ammonia (NH_3) which is converted into either nitrate (NO_3-N) or nitrite (NO_2-N) through nitrification process (Maigual-Enriquez et al., 2019). As discussed above, fish release NH_3 through their gills as part of their metabolism. NH_3 is mostly transformed into the comparatively non-toxic NH_4^+ at the normal operating RAS pH levels. According to Eq (1), reduction in the level of pH shifts TAN toward NH_4^+ and vice versa. (Gendel & Lahav, 2013)

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad pKa = 9.24 \text{ (Eq 1)}$$

The primary source of phosphorus (P) in RAS is feed. Fish excrete indigestible phosphorus (P) as particulates un their faeces, while digested P is mainly eliminated in fish urine as inorganic orthophosphate (PO_4-P) (Huang et al., 2023).

Reusing treated water and requiring less water volume are two benefits of RAS overflow and semi-flow-through systems (Jakhwal et al., 2024). Furthermore, RAS offers biosecurity and a controlled environment for fish production. For example, before water is released into the environment, it would be treated to remove any antibiotics and appropriate disinfection procedures would be put in place (Lazado & Good, 2021; Ramli et al., 2020). Nitrate accumulation is one of the primary issues with high-density aquaculture in RAS, despite its advantages. In RAS, ammonia is produced either by excretion or by the deamination of protein that is available in fish feed. Since ammonia is hazardous to aquatic life even in small amounts, biofilters are an important component of the RAS system (Jakhwal et al., 2024). Biofilters provide a surface for the growth of nitrifying bacteria and help to reduce high ammonia levels, which oxidize ammonia to nitrite and then convert nitrite to nitrate (Crab et al., 2007). The nitrification process causes nitrate accumulation in RAS, and a high concentration of nitrate (>200–500 mg/L) in RAS is hazardous for aquatic species like shrimps and fish (Jakhwal et al., 2024). The author went on to say that water that is high in nutrients, such as phosphate and nitrogen, needs to be treated before being discharged into the environment since it can otherwise eutrophicate nearby water bodies.

Table 5: The water quality limits of different RAS species' different parameters.

Parameters	**Concentration (mg/L)**	**Specie**	**Reference**
NH4-N	0.99727*	Salmon	(Hurtado & Cancino-Madariaga, 2014)
NO3-N	<100	African Catfish	(Eding & Van Weerd, 1999)
NO2-N	<0.1	Trout	(Timmons, 2002)
PO4-P	<1.3	Juvenile Rainbow trout	(Huang, 2024)

**Only 0.273%, or 0.00273 mg/L NH3, equates to 1 mg N/L TAN at 15 °C and pH 7, or standard circumstances. This is an acceptable number for salmon, a species that is sensitive to ammonia. The remaining 1 mg N/L is equivalent to NH_4^+ (Hurtado & Cancino-Madariaga, 2014).*

2.6.7 Nutrients Re-use

Effluent water from many aquaculture systems is produced in large quantities and contains elements like total nitrogen, total phosphorus, and suspended particles (Turcios & Papenbrock, 2014). See **Table 6**. Due to the toxicity of ammonia and nitrite as well as the possibility of nitrate-induced environmental hyper-eutrophication, the breakdown, and recycling of these nitrogenous compounds is particularly crucial in aquaculture that uses recirculation systems (Brown et al., 1999). Fish excrete dissolved nitrogen mostly as urea and ammonia, with teleost fish such as salmonids and rainbow trout being the primary emitters of ammonia (Altinok & Grizzle, 2004; Wright & Land, 1998). Nitrite serves as an intermediary during the nitrification process of ammonia to nitrate. During anoxic denitrification, facultative heterotrophic bacteria use the energy and electrons they obtain from biodegradable organic matter to convert nitrate and nitrite to nitrogen gas. These bacteria utilize oxygen for the oxidation of organic materials more effectively in an aerobic environment (Wik et al., 2009).

Utilizing production methods with the least negative ecological effects is the only way to meet sustainable aquaculture's dual goals of producing food and protecting natural resources. Recirculating aquaculture systems, or RAS, offer ways to recycle nutrients, manage waste better, and use less water (Martins et al., 2010). The biggest problem facing aquaculture in the area of water quality is lowering the amount of nitrogen and phosphorus released into the environment (Islam, 2005). Most of the nitrogen in RAS effluents is found as nitrate, which is nevertheless a contaminant of surface waterways although not as hazardous as ammonia (Kazakis et al., 2020). If nutrient-rich water is not properly treated before being returned to the fish in recirculating aquaculture systems, it may negatively affect their growth and performance (Corey et al., 2013). Ammonium (NH_4^+) and ammonia (NH_3) make up soluble nitrogenous waste, with the unionized moiety hazardous to marine finfish at quantities of about 0.05 mg/L (Foss et al., 2009; Person-Le Ruyet et al., 1997). The most popular technique for changing poisonous ammonia into comparatively non-toxic nitrate (NO_3^-) is using bacterial biofilters (Timmons, 2002). A fluidized sand bed biofilter, on the other hand, is expensive to operate, consumes oxygen, and produces no useful secondary byproduct (Corey et al., 2013). The authors further suggested that macroalgae produces marketable biomass, is a net oxygen producer during the day, and is an alternate way to deal with waste nitrogen, carbon, and phosphate in the system. By diversifying the products produced, the integration of macroalgae in land-based aquaculture systems can lower nutrient concentrations in effluent, boost operating income, and improve water quality for finfish farming.

Dulse has been confirmed to be a feasible species for aquaculture integration due to its fast growth rate, robust biomass, and high rates of nitrogen uptake (Shaojun & Tifeng, 2008). Finfish and macroalgae cooperate to make effective use of the water and nutrient cycles, rather than competing for farming resources. When these aquaculture methods are integrated, the overall net productivity of land-based aquaculture systems is increased (Corey, 2012).

Table 6: Overview of the rearing unit and water quality parameters of the six sampled RAS (Mota et al., 2014).

Parameters	**RAS 1**	**RAS 2**	**RAS 3**	**RAS 4**	**RAS 5**	**RAS 6**
Species	*Solea solea*	*Anguilla anguilla*	*Psetta maxima*	*Stizostedion lucioperca*	*Clarias gariepinus*	*Oreochromis niloticus*
Fish Tanks	Raceways	Circular	Raceways	Circular	Rectangular	Rectangular
Standing Stock(kg)	20000		65000	7500	6000	4750
Stocking density (kg/m^3)	104	175	59	43	162	68
Feed load(kg/d)	60	300	331	48	100	48
TAN (mg/L)	5.7	63.5	0.3	1.4	48.8	5.9
NO_3-N	64.5	92.3	27.0	91.1	53.5	72.3
PO_4-P	4.9	21.6	2.1	7.1	13.1	6.5
NO_2-N	0.14	0.15	0.05	0.22	4.6	1.3

3. Study objectives, research question, and hypothesis

3.1 Objectives

The main objective of this thesis is to evaluate the feasibility of growing dulse in effluent water from a commercial recirculating aquaculture system for Atlantic salmon (*Salmo salar*).

Specific objective 1: To analyze the growth rate and biomass yield of dulse in water sourced from RAS effluent, F2 solution, and seawater (control).

Specific objective 2: To estimate the orthophosphate (PO4), ammonium (NH4), and nitrate (NO3) uptake rates of dulce from RAS effluent, F2 solution, and seawater (control).

3.2 Research Questions

R1: To what extent does the growth performance of dulse differ between cultivation in RAS effluent and F2 solution, and is there a significant relationship between water source and biomass yield?

R2: Does the nutrient uptake of dulse vary depending on whether the water is sourced from RAS effluent or F2 solution (a nutrient-spiked water)?

3.3 Hypothesis

$H_0$1: The growth performance of dulse cultivated in RAS effluent is not different from that in the F2 solution.

$H_0$2: The uptake rates of orthophosphate (PO_4-P), ammonium (NH_4-N), and nitrate (NO_3-N) by dulse do not vary between the RAS effluent water and the F2 solution treatment.

4. Material and Methods

The in-vitro growth of dulse was tested and the growth performance and nutrient uptake rate of dulse was examined in this experiment. The effluent water from the RAS facility was gathered and brought to the Nofima facilities. The In-vitro dulse growth in RAS effluent was examined along with the rate of nutrient uptake.

4.1 Experimental Design

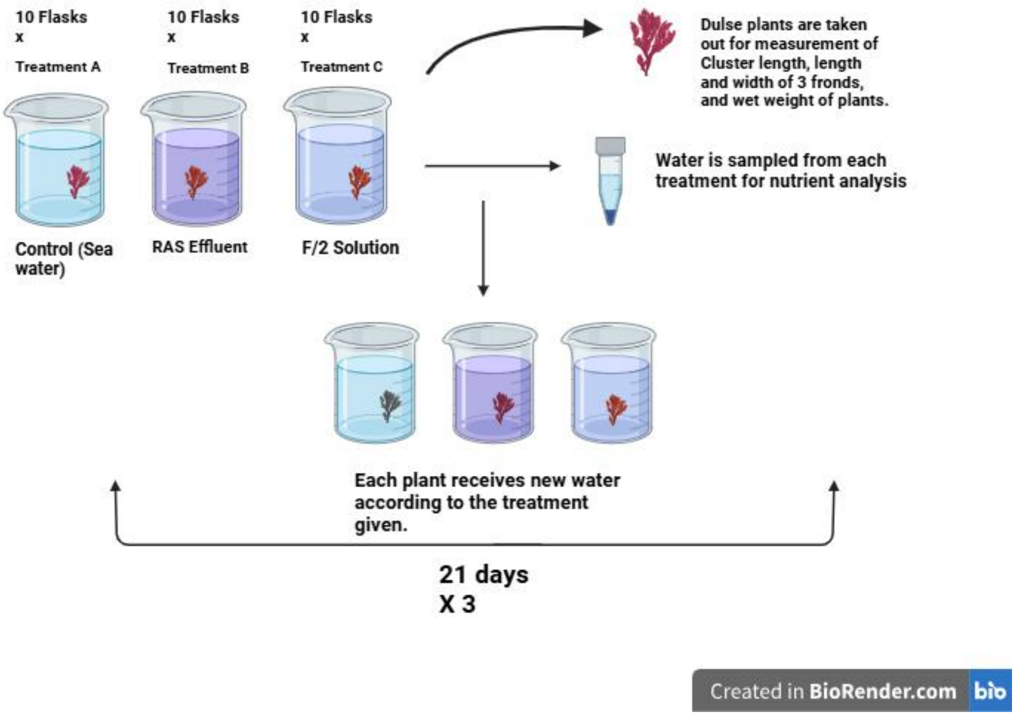


Figure 12: The illustration of the experimental design showing three treatments with replicates and experimental method over the course of 64 days. (Created in BioRender.com)

The experimental trial was conducted at Nofima in Tromso, Norway, for 64 days from July to September (see **Figure 12**). During this trial, dulse was placed in the container and three different treatments were applied to the water to evaluate the effect of nutrient sources on the growth of dulse. The light intensity, light regime, temperature of the room, and salinity of each water treatment were controlled. Treatment A served as the control and contained seawater. Treatment B received water from the Recirculating Aquaculture System (RAS). Treatment C included F2 Solution which is a nutrient-spiked water. F2 Guillard's solution (G0154, Sigma-Aldrich) is the standard nutrient medium used for the growth of algae and it contains a balanced mixture of micro and macronutrients (see **Table 7**). The experimental trial lasted for 9 weeks. This was called census in our thesis. The 9-week experiment was divided into three Censuses. Census 1 was from the initial setup to week 3. Census 2 was from week 3 to week 6, and Census 3 was from week 6 to week 9. The initial setup was on 4th July 2024. Census 1 was conducted on 25th July 2024, Census 2 on 14th August 2024, and Census 3 on 6th September 2024. Each plant was weighed and measured before placement in the experimental setup. After every census, the wet weight, cluster length, frond length, and frond width of the dulse were measured. In each treatment, water was taken at the start of the experiment acting as the sample from the initial setup. After every Census (every 3 weeks), the water was replaced according to the treatment given, and the previous water was sampled in falcon tubes for transport to analyse the water of samples. The water samples taken for the water quality analysis were transported to me at the Norwegian University of Life Sciences (NMBU) in Ås. The water quality analysis was then performed using a Spectroquant Prove 100 spectrophotometer (Merck, Darmstadt, Germany).

Table 7: A chemical composition of Guillard's F2 solution including the concentration of its content (Monkonsit et al., 2011)

Nutrient	**Amount**
Solution A: Nitrate and Phosphate stock solution (1L)	
$NaNO_3$	84.15 g
$Na_2HPO_4 \cdot H_2O$	6.0 g
$FeCl_3 \cdot 6H_2O$	2.90 g
$Na_2EDTA \cdot 2H_2O$	10.0 g
Solution B: Silicate stock solution (1L)	
$Na_2SiO_3 \cdot 9H_2O$	33.0 g
Solution C: Trace metal stock solution (1L)	
$CuSO_4 \cdot 5H_2O$	1.96 g
$ZnSO_4 \cdot 7H_2O$	4.40
$Na_2MoO_4 \cdot 2H_2O$	1.26
$MnCl_2 \cdot 4H_2O$	36.0
$CoCl_2 \cdot 6H_2O$	2.0
Solution D: Vitamin stock solution (1L)	
Vitamin B1	0.4g
Vitamin B12	0.002mg
Biotin	0.10mg

4.2 Dulse and Water Source

The dulse samples were sourced from the littoral zone in Hamna on the North-Western side of Tromsø island in Norway.

The water for the experiment was sourced from the RAS of the Salmar facility in Dåfjorden, Norway.

4.3 Experimental Systems

The dulse samples taken were provided three water treatments and each treatment had 10 replicates. These samples were placed in a 1L flask for the experiment. The salinity of all three water treatments was adjusted to 34 ppt. Treatment A included 100% seawater, Treatment B included 25% RAS effluent water from the Salmar facility in Dafjorden and 75% seawater, and Treatment C was given 100% F2 solution (see **Figure 13)** for the illustration of the solution). The RAS water was boiled in a big boiler for 30 minutes and then chilled. The boiling of the RAS water was performed to kill bacteria present in the water. The temperature of the experiment room was set to 10 degrees Celsius. The light intensity was measured and set using the iPhone's “Photone” app. PAR (Photosynthetically Active Radiation) measurement showed 40 PPFD (Photosynthetic Photon Flux Density) umol/m2/sec. The same meter set to Lux showed 2600. The light regime for the dulse plant was 18 hours of light and 6 hours of dark.

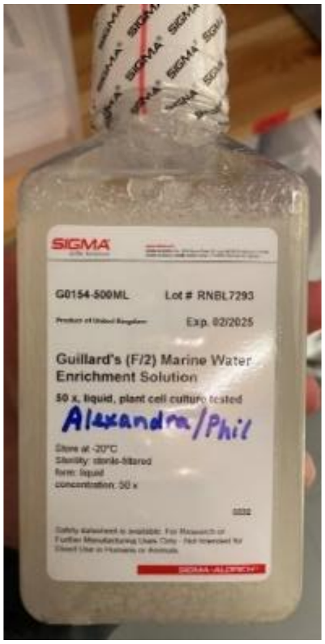


Figure 13: The illustration of the F2 solution bottle as a reference.

4.4 Dulse Analysis

The growth of the dulse was monitored every 3 weeks by measuring the wet weight (WW), the length and width of 3 dulse sample's fronds, the cluster length of the dulse, and the color changes in all treatments. These parameters were measured at T_0 which is the initial setup time. The plants were taken collectively from each treatment after each Census (every 3 weeks) and the water sample in a 15mL falcon tube for water analysis was taken. The plants were placed on a strainer for 5 minutes to strain the extra water, and labeled as A, B, and C as seen in **Figure 14**. After 5 minutes, the WW of each dulse sample was measured and noted with the help of a weighing machine. The length and width of each frond were measured with the help of the vernier caliper. The three fronds which showed better growth than other fronds were generally taken for measurement and pinched so that it could be recognized during the next census. The dulse samples were classified into different categories as pretty good in shape, disintegrated, highly disintegrated, or completely disintegrated. The dulse samples were also classified based on color retention. After that, the dulse samples were weighed again, placed it in the container, and added the desired treatment. We did the same procedure for T_1 which is census 1 (3 weeks or 21 days) after initial setup, T_2 (census 2), and T_3 (Census 3).

Figure 14: Dulse samples in a strainer labeled as A, B, and C representing the three treatments.

4.5 Water Sampling

The water for the trial was sampled from the effluent of the Salmar facility in Dafjorden, Norway. The samples were collected in 15ml falcon tubes and the pipette tip was exchanged between each container and then frozen at -20 Degree Celsius. The samples were then shipped with overnight delivery mail to NMBU packed in a Styrofoam box with ice packs. Upon delivery, samples were thawed 24 hours before being tested for water quality analysis. The samples from Nofima in Tromso to NMBU included samples from the initial setup which was on 4th July 2024, then before water exchange and samples for after water exchange from Census 1 which was on 25th July 2024, before and after water exchange samples from Census 2 conducted on 14th August 2024, and samples from Census 3 done on 6th September 2024, making it total of 6 samples with 30 replicates each.

To explain it in more detail, The water was sampled at the time of the initial setup and after 3 weeks (21 days) we sampled before changing the water which we called “before water exchange” and after changing the water which we called “after water exchange”. We repeated the same process after 3 weeks (20 days) and again after another 3 weeks (23 days).

4.6 Water Quality Analysis

The Spectro-quant Prove 100 was used for water quality analysis (see **Figure 15**). According to (Merck, 2024a), it can quickly, simply, and reliably compute the amounts of COD (Chemical Oxygen Demand), BOD (Biological Oxygen Demand), TOC (Total Organic Carbon), phosphate, nitrate, nitrite, ammonium, total nitrogen, chromate, lead, volatile organic acids, and many more substances. It does this by using the spectrophotometry principle. The same website states that a cell test port is required to determine the type of chemical that must be calculated in the water sample. The Spectroquant system will instantly recognize the test method, lot number, expiration date, and calibration updates from the QR code on the cell test as it is inserted. Zero error correction is required following the insertion and removal of the cell test. A cuvette tube for this purpose is required which is filled with distilled water before inserting into the Spectroquant. See **Figure 16**. After zero correction is processed, the sample solution to the tube is added and then the tube is placed into the Spectroquant. The precise concentration of the nutrients or chemicals included in the sample are provided by the self-running system of the Spectroquant.

Figure 15: Spectro-quant Prove 100

Figure 16: Visual image of 10mm, 20mm and 50mm cuvette cell (Merck, 2024b)

4.7.1 Ammonium (NH_4-N)

The Spectroquant Prove 100 was employed to examine the amount of nitrogen in ammonium (NH_4-N) in our water samples using the kit number 114752 with the measuring range from 0.05 to 3.00 mg/L in 10mm cuvette cell and 0.010 mg/L to 0.500 mg/L in 50mm cuvette cell. The amount of ammonia was calculated after pipetting 5 mL of our water sample into a test tube and adding 0.60 mL of NH_4-1 from the ammonium kit. After the solution was combined, the test tube was filled with a one-level blue microspoon of NH_4-2 from the kit. The test tube was given a good shake to dissolve the solids and about 5 minutes of the rest was provided to the solution. After 5 minutes, 4 drops of NH_4-3 from the kit were introduced to the solution. Once more, the solution was provided 5 minutes to react. **Figure 17** illustrates the solution of NH_4-N to measure. Lastly, to measure and examine the amount of NH_4-N in a sample, the solution was transferred into the cuvette cell tube, which was then fixed inside the spectroquant's cell compartment. The spectroquant used the principle of spectrophotometry to measure the accurate value of the concentration of NH_4-N present in our sample.

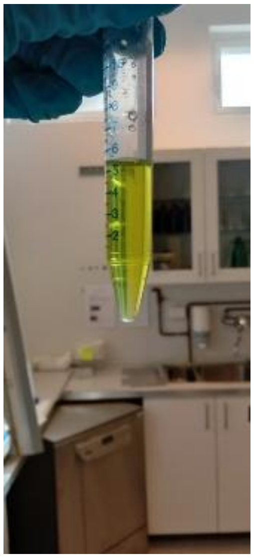


Figure 17: Ammonium solution for testing

4.7.2 Nitrate (NO_3-N)

The Spectroquant Prove 100 was used for the determination of NO_3-N in the sample using kit number 114773 with the measuring range from 0.5 mg/L to 20.0 mg/L in a 10mm cuvette cell. The test tube was filled with one micro-spoon of NO_3-1 from the kit and a pipette was used to add 5 mL of NO_3-2 to the tube and thoroughly stirred for a minute. The 1.5 mL of the sample was slowly poured into the tube. The solution was cautiously mixed as the tube was heating due to the reaction and given 10 minutes of reaction time. After the 10-minute reaction time, the solution was transferred into the cuvette tube and the accurate value of NO_3-N was determined. **Figure 18** represents the solution for the analysis of NO_3-N.

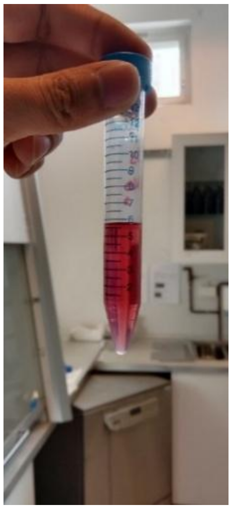


Figure 18: Nitrate solution for testing

4.7.3 Ortho-phosphate (PO4-P)

The Spectro-quant Prove 100 was utilized to measure the PO_4-P level in our samples using the kit number 114848 with the measuring range from 0.05 mg/L to 5.0 mg/L in 10mm cuvette cell and 0.005 mg/L to 1.000 mg/L in 50mm cuvette cell. Similar to the procedure described above, 5 mL of the sample and 5 drops of PO_4-1 solution from the test kit were added to the test tube. After mixing it thoroughly, one level of blue micro-spoon of PO_4-2 was added to the test tube. The solution was thoroughly mixed once again to completely dissolve any remaining solid materials. The solution was given a 5-minute reaction time. Following that, the solution was added to the cuvette and examined using a spectrophotometer.

4.8 Calculation

The removal efficiency of nutrients was calculated by using eq 2.

$$Removal\ efficiency\ (\%) = \frac{Initial\ concentration - final\ concentration}{Initial\ concentraytion} \times 100$$

4.9 Statistics Analysis

Microsoft Excel was used to calculate mean and SD values for all the data collected. For statistical analysis, the program JMP pro 18 (SAS Institute, USA) was used. Each data set obtained from trials was expressed as mean ± SD and checked for homogeneity using Levene's test. One-way ANOVA analysis was used to compare the wet weight of dulse between different treatments at a time point. The analysis for cluster length and frond area of dulse couldn't be performed due to limited time. Turkey's post-hoc test for equal variances was then used to compare the variations among the treatments. To ascertain whether sampling points differed from one another and whether sampling and treatment for the wet weight interacted, a two-way ANOVA analysis was employed. To meet the homogeneity of variances assumption, data in percentages (nutrient removal efficiency) were arcsine-transformed before ANOVA. Log transformation was used for the data that did not meet the homogeneity of variances assumption. The results were deemed statistically significant when $p < 0.05$. Non-parametric test like the Kruskal-Wallis test was used to compare the difference in wet weight at each time point if $p<0.05$ even after the log-transformation of the data. A two-tailed t-test was used to check the significant difference between the two nutrients in Census 2 and Census 3.

5. Results

5.1 Color and Condition of Dulse

The results revealed distinct variations in the retention of color by dulse and structural integrity in treatments A (seawater), B (RAS effluent water), and C (F2 solution) as seen in **Figure 19**. In RAS treatment(B), dulse showed characteristic red coloration and well-developed fronds, indicating healthy pigmentation and optimal growth environment. The dulse samples in F2 solution (C) had robustly developed fronds. However, the dulse did not retain the color and instead showed a yellowish-green color. The opposite condition was seen in seawater treatment (A) where the dulse displayed complete disintegration, lacking structural integrity and pigmentation.

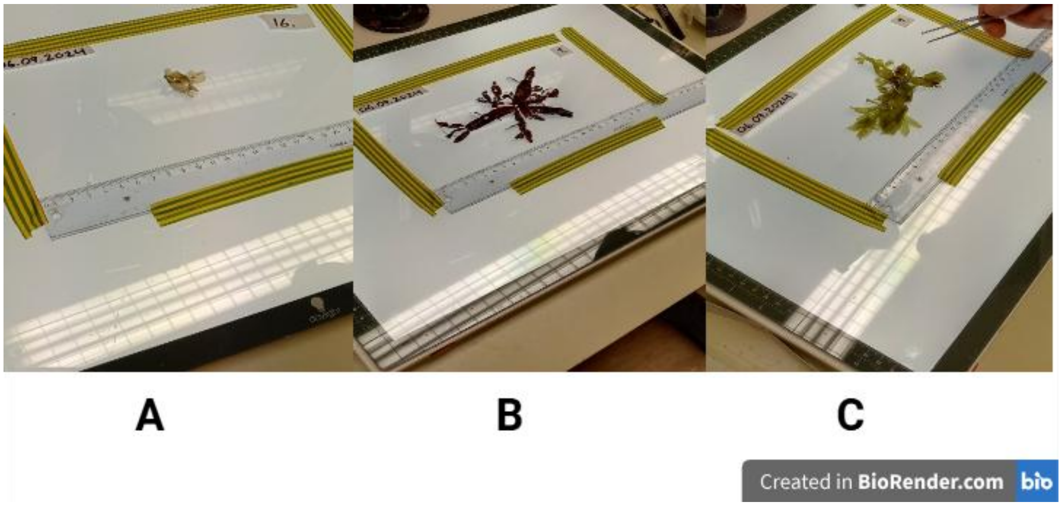


Figure 19: Color retention and condition of dulse across all three treatments. 'A' represents seawater treatment as control, 'B' represents RAS effluent water treatment, and 'C' represents F2 solution treatment. Created by Biorender.com

5.2 Growth performance of dulse

5.2.1 Dulse Biomass Increase

Figure 20 illustrates the biomass or wet weight increase of the dulse across seawater, RAS, and F2 solution treatments during 9 weeks. The biomass in seawater and F2 solution increased until 3 weeks after which it declined whereas biomass in RAS water increased constantly throughout the experiment.

Initially, the average biomass increase of dulse in seawater was 2.07g (±0.81), which increased to 2.51g (±1.37) after 3 weeks. At the end of the 6th and 9th weeks, the average biomass of dulse was significantly decreased to 1.96g (±1.72) and 1.86g (±2.31) respectively. The dulse in the F2 solution in the initial setup had a biomass of 2.74g (±1.08) which was increased significantly to 3.70g (±1.43) in week 3 and decreased by the end of week 6, at 3.57g (±2.36), and further reduced to 3.13g (±2.06). The dulse in RAS treatment showed a stable growth pattern. The initial average biomass measured was 2.08g (±0.70), which increased significantly to 3.30 (±0.98) in week 3 and further to 4.12g (±1.32). By the end of week 9, the average biomass increased to a striking 5.60g (±1.41).

The Kruskal-Wallis test revealed a significant difference between treatments at week 6 (p=0.03) and week 9 (p=0.002). Turkey's post-hoc test shows that RAS treatment had significantly higher biomass than seawater treatment at week 6 (p=0.01) and week 9 (p=0.0005). No significant difference was found at the initial setup and week 3 i.e. p>0.05.

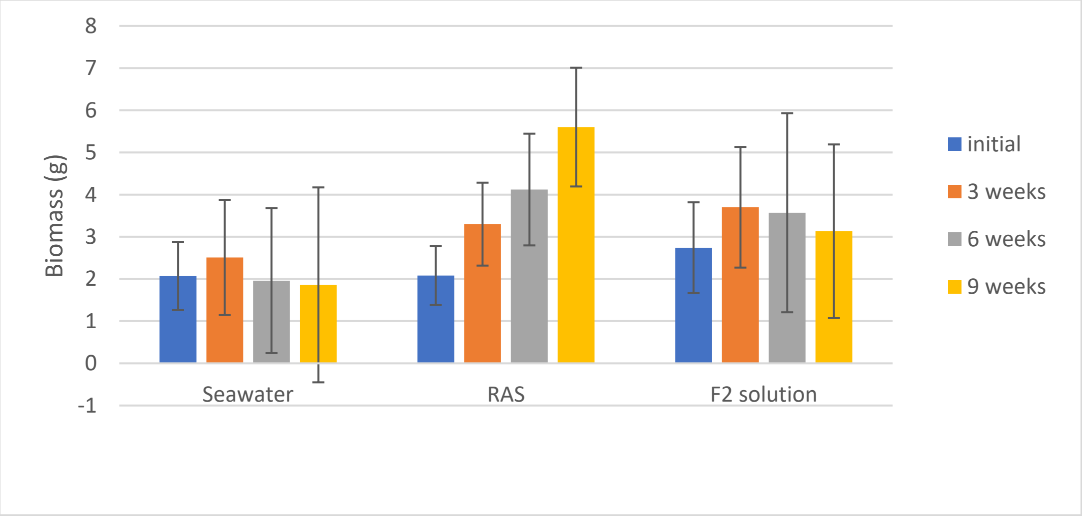


Figure 20: Bar graph. Biomass increase of dulse (g) in all three treatments and comparison of average biomass increase between initial setup, week 3, week 6, and week 9. Values are given as treatment group mean ± S.D. (N =3 to 10).

5.2.2 Dulse Frond Area Increase

The increase in the Frond area of dulse over 9 weeks across 3 different treatments is illustrated in **Figure 21**. Dulse present in the seawater and F2 solution treatment exhibited a similar trend in the growth of the frond area. The frond area at the initial point was 452mm^2 (±135) and 461mm^2 (±199) respectively. After 3 weeks, the frond area in the seawater was increased to 493mm^2(±139), while the F2 solution showed a negligible change, at 464mm^2 (±163) which is somewhat equal to the size initially. By the end of 9 weeks, the decline was observed, the frond area was reduced to 351mm^2 (±351) in the seawater and 327mm^2 (±327) in the F2 solution.

In contrast, the samples in RAS effluent treatment showed a more stable pattern where all the values for the frond area demonstrated minimal fluctuations throughout 9 weeks. Initially, the frond area measured was 420mm^2 (±119), followed by a slight increase in frond area after 3 weeks, at 437mm^2 (±137). The frond area at the end of the 9-week mark was slightly reduced, measuring 427mm^2.

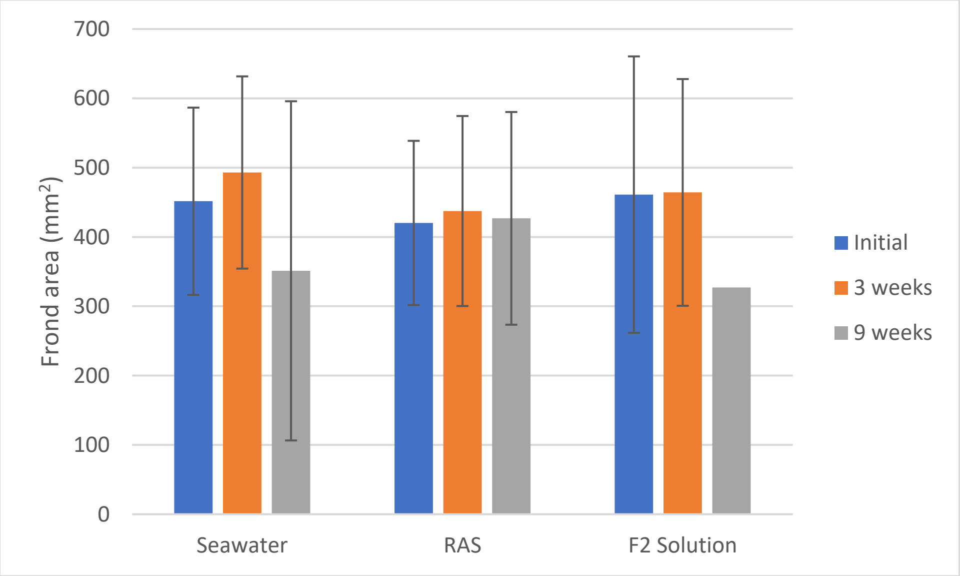


Figure 21: The Bar Graph illustrates the average dulse frond area (mm^2) increase in all three treatments compared with the initial setup, weeks 3 and 9. Values are given as treatment group mean ± S.D (N = 3 to 10).

5.2.3 Dulse Cluster Length Increase

Figure 22 represents the increase in the cluster length of the dulse across three different treatments over nine weeks. Dulse in the RAS effluent water and the F2 solution exhibited a comparable pattern in the cluster length development. In contrast, dulse in the seawater treatment showed distinct differences.

Initially, the average cluster length measured in seawater was 92mm (±15.4) similar to the initial measurements in the RAS water treatment (88 mm±11.7) and F2 solution treatments (88.2 mm±22.3). After 3 weeks, the cluster length reduced slightly in the seawater, measuring 83mm (±19.5). In contrast, the RAS treatment showed no change, maintaining an average cluster length at 88mm (±12.3). A slight reduction (86mm±24.4) was observed in dulse cluster length in the F2 solution.

By the end of 9 weeks, a slight decrease in the cluster length of dulse was observed in seawater treatment, dropping to 36mm (±9.6). Conversely, the RAS treatment showed a slight increase in the cluster length, reaching 91mm (±21.0), while the dulse in the F2 solution treatment demonstrated a slight decrease, with the dulse cluster length measuring 71mm (±21.7).

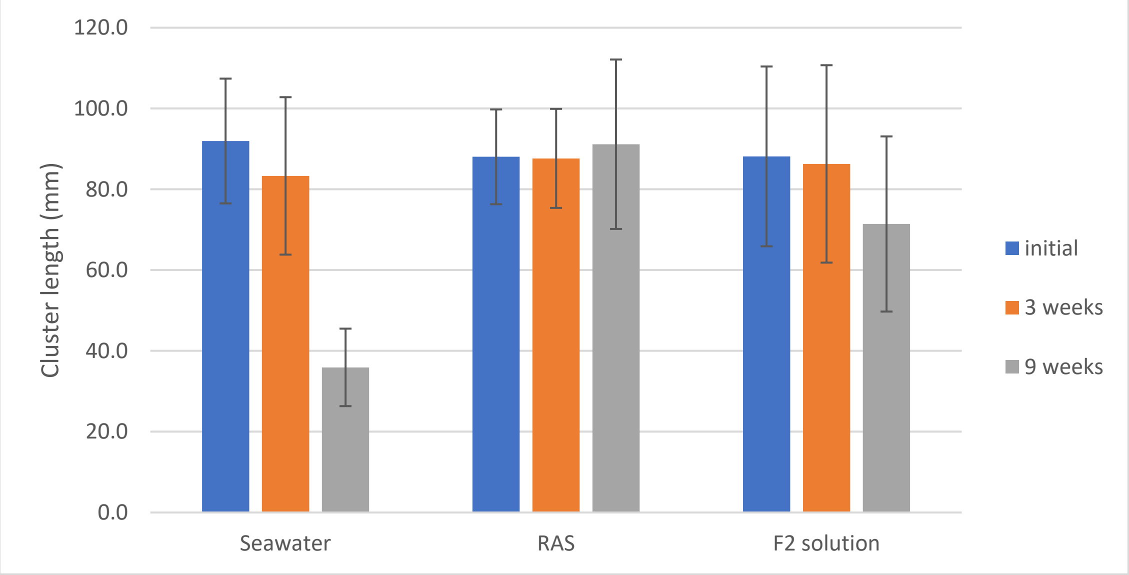


Figure 22: The bar graph illustrates the dulse average Cluster length (mm) increase in all three treatments and compares it with the initial setup, week 3, and week 9 increase. Values are given as treatment group mean ± S.D. (N = 3 to 10).

5.3 Efficiency of Nutrient Removal in RAS

Figure 23 illustrates the nutrient removal efficiency for NO_3-N, PO_4-P, and NH_4-N in Census 1, Census 2, and Census 3. The results indicate significant variations in the nutrient removal efficiency in RAS throughout the experiment. The data for the removal efficiency of PO_4-P in Census 2 and NH_4-N in Census 3 was unavailable.

The removal efficiency of NO3-N was the highest in each census compared to other nutrients. In Census 1, the removal efficiency of NO3-N was 156±53 %. The removal efficiency increased significantly in Census 2 and Census 3, at 274±223% and 389±104%, respectively. The removal efficiency of NH_4-N during Census 1 was 23±14% which doubled to 46±10% in Census 2. In contrast, the removal efficiency of PO_4-P in RAS was 42±18% during Census 1 and decreased significantly by almost one-half to 22±4% in Census 3.

There was no significant difference observed by one-way ANOVA in nutrient removal efficiency in Census 1 (p=0.65). The two-tailed t-test revealed a significant difference between the removal efficiency of NH_4-N and NO3-N by dulse during census 2 (p=0.03). Similarly, there was a significant difference found between NO3-N and PO_4-P in Census 3 by a two-tailed t-test (p=0.01).

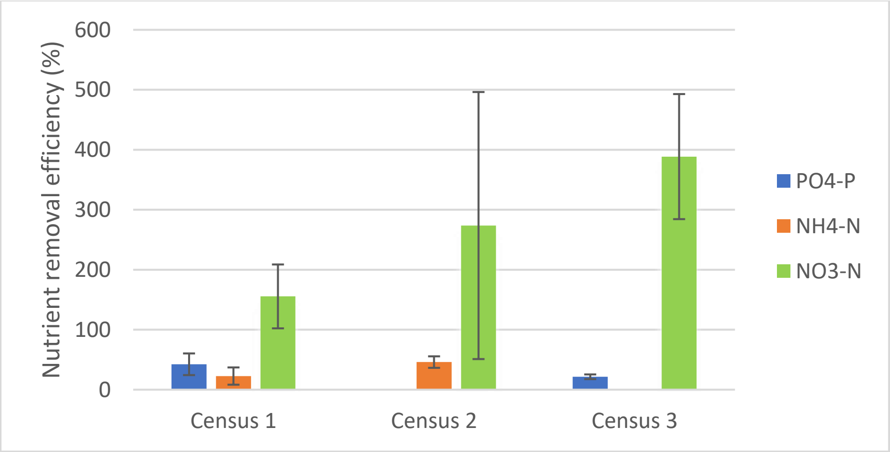
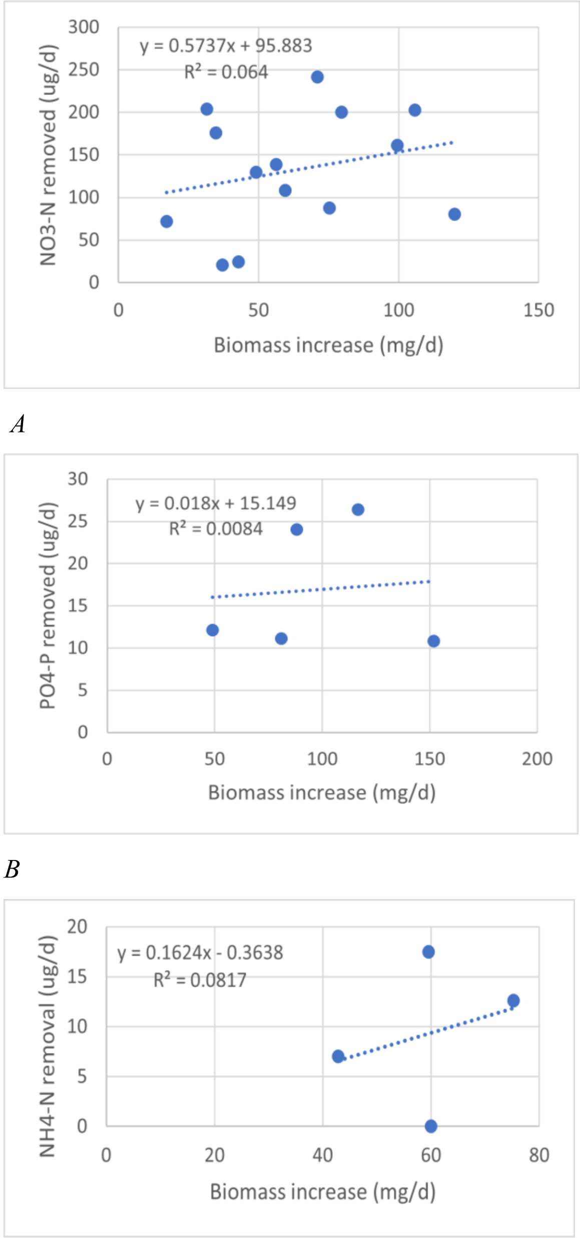


Figure 23: Bar graph. The removal efficiency (%) of PO4-P, NH4-N, and NO3-N from RAS water in Census 1, Census 2, and Census 3. Absolute Values are given as treatment group (RAS) mean ± S.D. (N =2 to 8).

5.4 Relation between Dulse growth and Nutrient removal

5.4.1 Biomass as an indicator of Nutrient removal

The scattered plot (**Figure 24**) represents the relationship between biomass increase (mg/d) and the removal rate (ug/d) of NO_3-N (A), PO_4-P (B), and NH_4-N (C) where d stands for days. A linear regression was performed and the R^2 value calculated was extremely low which shows a very weak positive relationship. It shows that as biomass increases, there is a slight tendency for NO_3-N, PO_4-P, and NH_4-N removal to increase.



A

B

C

Figure 24: Scattered plot graph illustrates NO3-N (A), PO4-P (B), and NH4-N (C) removed (ug/d) per biomass increase (mg/d). N=1 to 6.

The bar graph in **Figure 25** illustrates the NO_3-N removal efficiency of dulse expressed as the amount of removed per unit increase in the biomass of dulse (ug/mg). From census 1 to census 2, the efficiency of NO_3-Nremoval per unit increase in biomass of dulse was significantly increased from 0.8ug/mg±0.3 to 3.7 ug/mg±1.8, followed by a decline in the efficiency during census 3, at 2.4ug/mg±1.5.

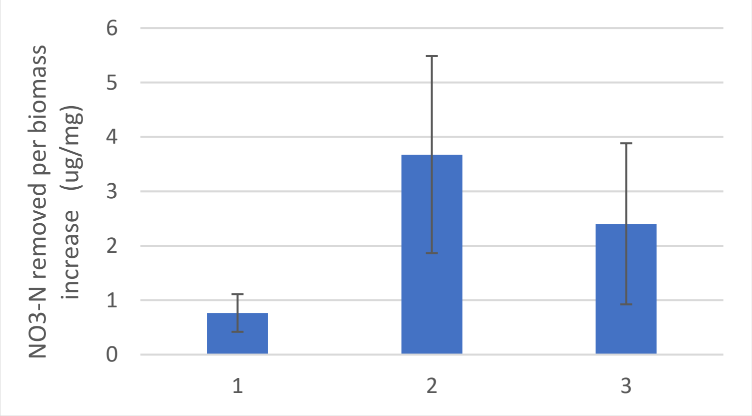


Figure 25: Bar graph. Comparison of NO3-N removed per biomass increase(ug/mg) in each of the censuses. N=3 to 6.

The bar graph in **Figure 26** shows the PO_4-P removal efficiency of dulse expressed as the amount of NO3-N removed per unit increase in the biomass of dulse (ug/mg). The data for Census 2 was unavailable. A slight decrease in PO_4-P removal efficiency was observed from census 1 (0.21ug/mg±0.07) to census 3 (0.16ug/mg±0.12).

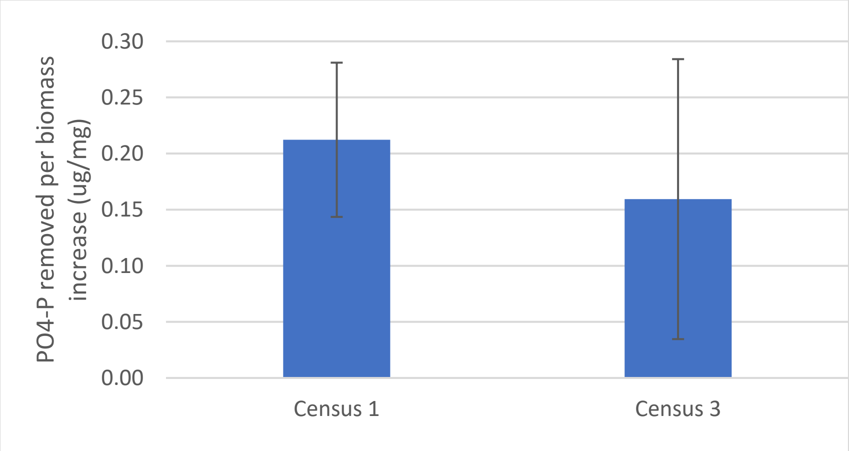


Figure 26: Bar Graph. Comparison of PO4-P removed per biomass increase(ug/mg) between census 1 and census 3. N=2 to 3.

The bar graph in **Figure 27** represents the dulse NH_4-N removal efficiency, expressed as the amount of NH_4-N removed per unit increase in the dulse biomass (ug/mg). The data for Census 3 were unavailable. A slight rise in NH_4-N removal efficiency was observed from census 1 (0.24 ug/mg±0.16) to census 2 (0.30 ug/mg).

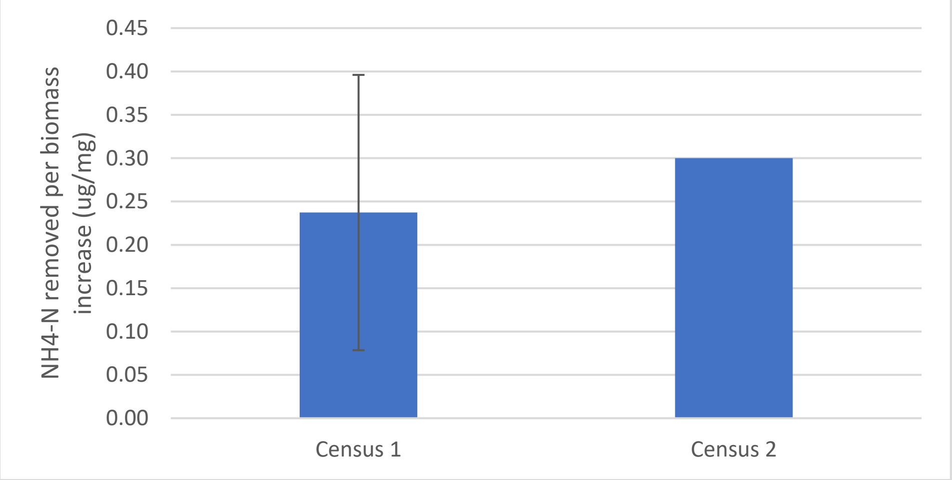
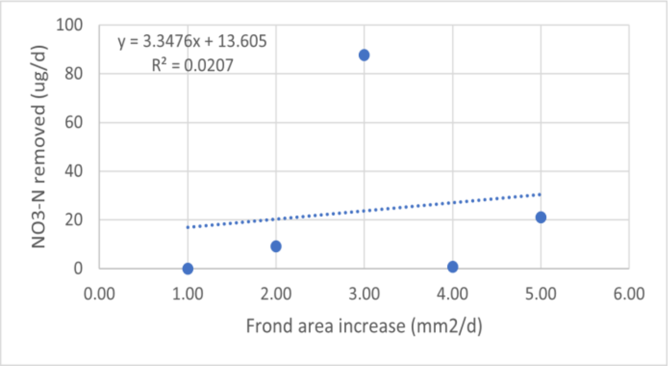


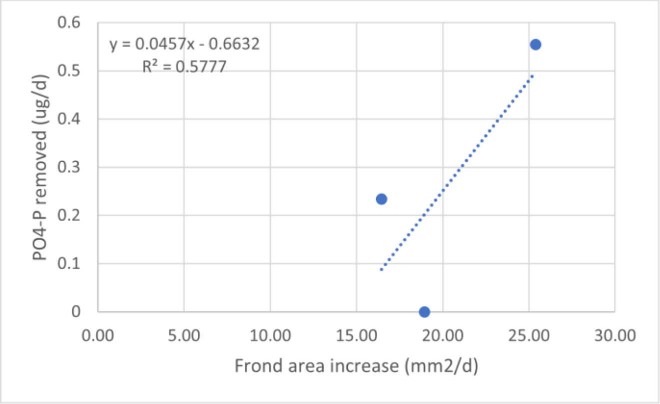
Figure 27: Comparison of NH4-N removed per biomass increase(ug/mg) between Census 1 and Census 2. N= 1 to 3.

5.4.2 Dulse Frond Area and Nutrient Removal Efficiency

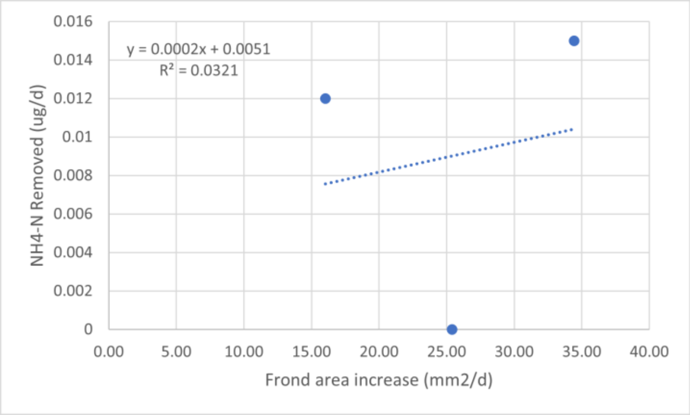
The scattered plot in **Figure 28** illustrates the relationship between the dulse frond area increase (mm^2/d) and the removal rate (ug/d) of NO_3-N (A), PO_4-P (B), and NH_4-N (C) where d stands for days. A linear regression was conducted and as a result, we got the value of R^2. The R^2 value was between 0.5 to 0.02 which is significantly low and shows a very weak positive relation between the dulse frond area increase and the removal efficiency of NO_3-N, PO_4-P, and NH_4-N from the RAS by dulse.



A



B



C

Figure 28: Scattered Plot Graph illustrates the relation between NO3-N (A), PO4-P (B), and NH4-N (C) removed (ug/d) per Frond area increase (mm2/d). N=3-4

The bar graph (**Figure 29**) illustrates the nutrient removal efficiency of PO_4-P, NH4-N, and NO_3-N per increase in a unit area of the dulse frond (ug/mm2) during census 1. The data for census 2 and census 3 were not available. The PO_4-P removal per frond area increase was recorded at 1ug/mm^2±0.15, whereas the removal rate of NH_4-N per frond area increase was the lowest among the other two nutrients, at 0.03 ug/mm^2±0.01. In contrast, the NO_3-N removal per frond area increase was the highest compared to PO_4-P and NH_4-N, at 14.4 ug/mm^2±12.2.

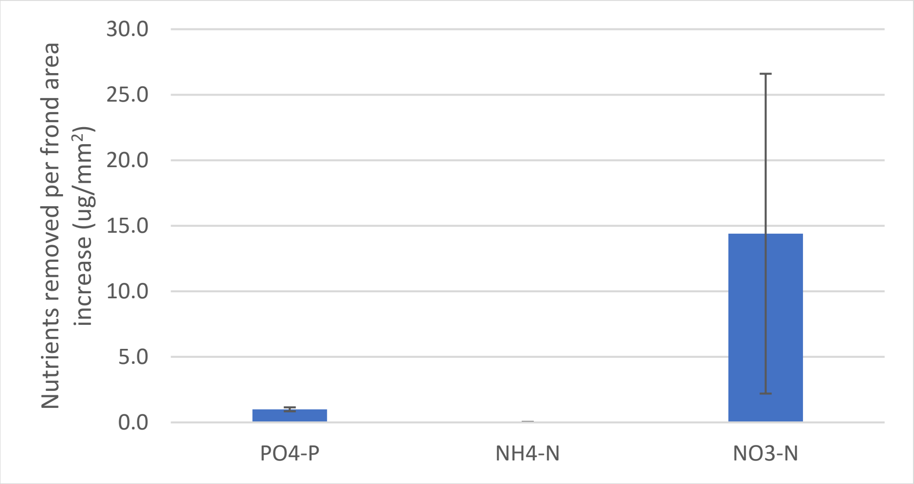


Figure 29: Bar graph. Comparison of PO4-P. NH4-N, and NO3-N removed per Frond area increase during Census 1. N=3 to 4.

5.4.3 Cluster Length and its Impact on Nutrient Dynamics

The scattered plot in **Figure 30** illustrates the relationship between the cluster length (mm/d) of the dulse and the removal rate (ug/d) of NO_3-N (A), PO_4-P (B), and NH_4-N (C) where d stands for days. A linear regression was conducted and as a result we got the value of R^2. The R^2 value was 0.06, 0.4, and 0.64 respectively which is significantly low and shows a weak positive relation between the dulse cluster length increase and the removal efficiency of NO_3-N, PO_4-P, and NH_4-N from the RAS by dulse.

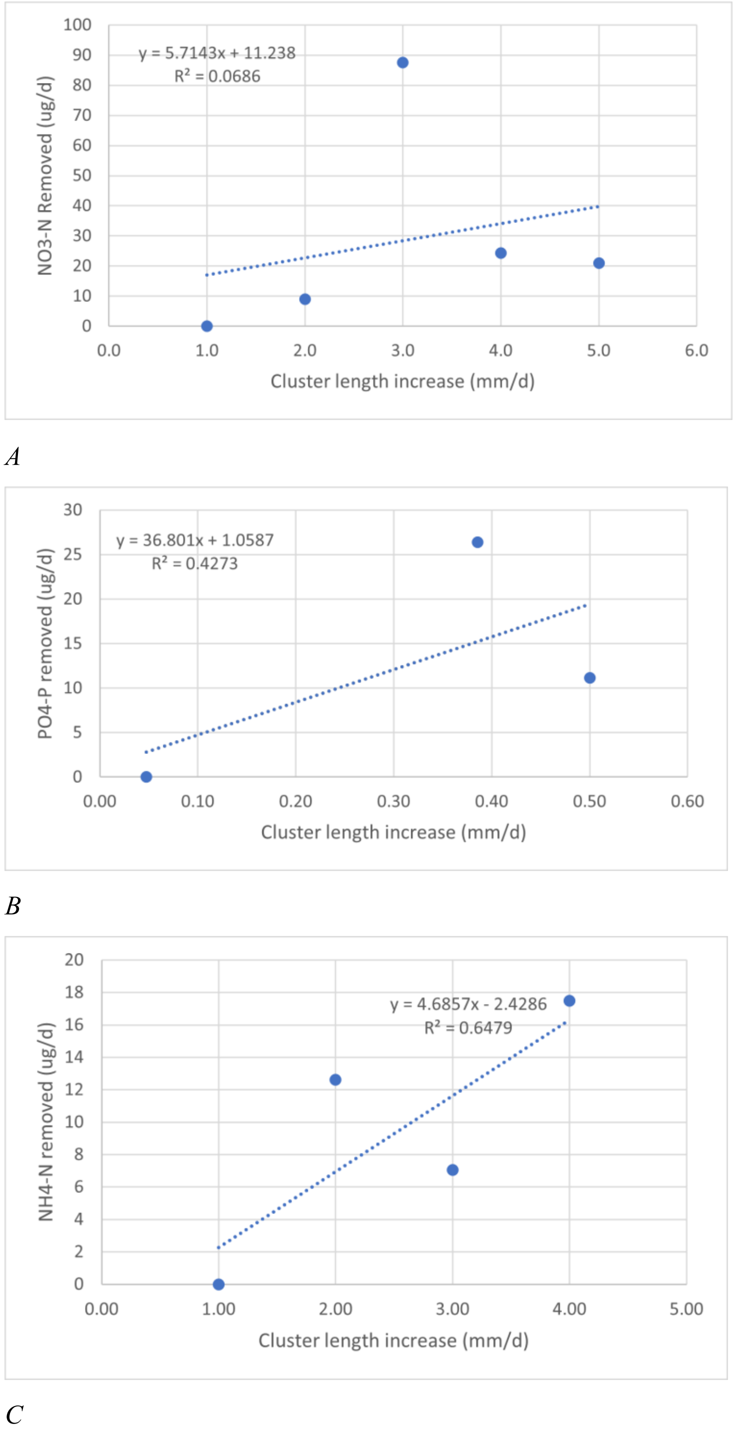


Figure 30: Scattered Plot Graph. Correlation between NO3-N (A), PO4-P (B), and NH4-N (C) removed (ug/d) per Cluster length increase (mm/d). N=3 to 4.

The bar graph (**Figure 31**) illustrates the nutrient removal efficiency of PO_4-P, NH_4-N, and NO_3-N per increase in the cluster length of the dulse (ug/mm) during census 1. The data for census 2 and census 3 were not available. The PO_4-P removal per cluster length increase was recorded at 116.4ug/mm±102, whereas the removal rate of NH4-N per cluster length increase was almost equal to that of PO_4-P, at 108.8 ug/mm±132.8. However, the NO_3-N removal per cluster length increase was the highest compared to PO_4-P and NH_4-N, at 211.7 ug/mm±193.

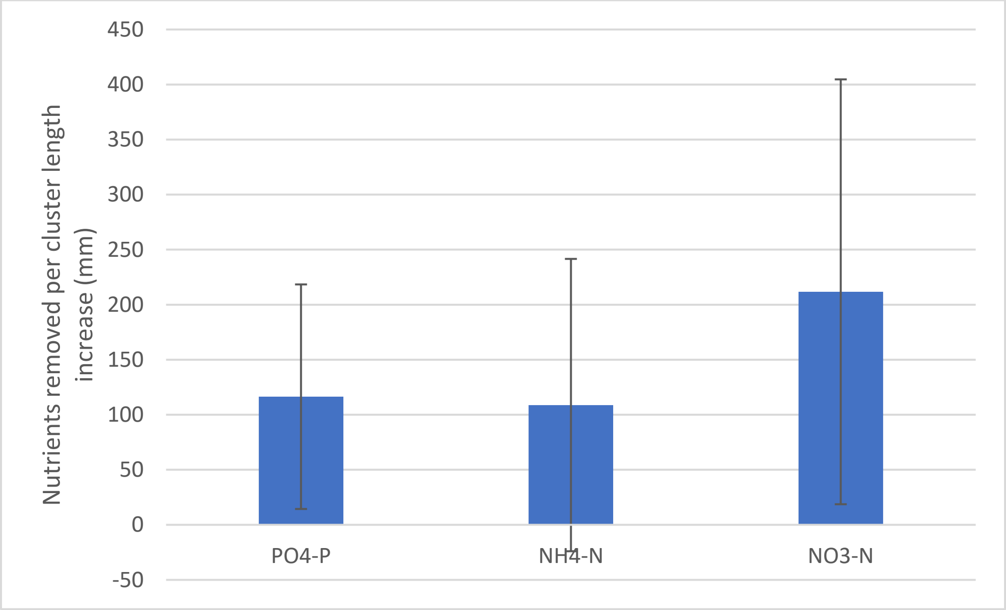


Figure 31: Bar graph. Comparison of PO4-P. NH4-N and NO3-N removal per Cluster length increase during Census 1. N=3 to 4.

6. Discussion

This thesis addresses the growth performance of dulse using effluent water from RAS. The experiment investigated the effect of different water treatments like Seawater, RAS, and F2 solution on the growth performance of dulse. The water nutrients like NH_4-N, NO_3-N, and PO_4-P were analyzed to check their effect on the growth dynamics of the dulse. Nutrient removal efficiency and the growth performance of the dulse were followed for 9 weeks after the initial setup. The growth performance of dulse in different treatments and nutrient removal in RAS as well as the relation between nutrient removal and the growth performance of the dulse are discussed in this section.

6.1 Effect of water treatments on color and growth performance of dulse

A study by Vasconcelos et al. (2022) investigated the effect of environmental conditions on the composition and coloration of wild and cultivated dulse. They found that dulse samples displayed intense coloration in June, possibly due to higher protein content observed during this month. They further reported reduced protein content in dulse in autumn, attributing this reduction to the protein nature of phycoerythrin (PE) which is the primary pigment in dulse. An experiment by Ramus (1983) demonstrated that insufficient nitrogen availability to meet the growth demands of dulse resulted in a reduction in the pigment concentration, resulting in the dulse’s color pales. Similarly, Parjikolaei et al. (2013) found that the concentration of pigment decreases and the color of the macroalgae pales if there is not enough nitrogen available to meet growth demands. In the current study, the dulse in the RAS effluent water retained their coloration, whereas, dulse in seawater and F2 solution treatment did not retain their coloration. This might suggest that RAS water provided enough nitrogen to enhance the protein content in the dulse for the retention of the color, while other treatments lacked enough nitrogen to enhance protein content to sustain pigmentation. These results highlight the importance of protein and nitrogen availability in preserving dulse's composition and color. Further research is needed to assess the dulse's protein content to better understand the mechanism behind color retention in the dulse under these water treatments.

Most of the research on dulse cultivation has primarily aimed at enhancing biomass through nutrient enrichment of the seawater, adjustments of the salinity, and light manipulation under controlled conditions on land (Schmedes & Nielsen, 2020). In a study, the effect of nutrient medium like culture medium or F2 solution on the length and growth rate of dulse sporelings grown in a lab setting at varying irradiances (5, 10, 25, and 50 μmol photons m−2 s−1) was examined by Edwards and Dring (2011). They found that adding vitamins (like vitamins B1, B12, and H) to F2 media significantly increases the sporeling length of dulse under low irradiance (5 and 10 μmol photons m−2 s−1). This demonstrates the potential for optimized nutrient formulations, which could be one of the reasons for the limited growth performance of dulse in the F2 treatment of the current study without added vitamins. However, Chatfield (2023) reported the opposite results. His study investigated the effects of water-change frequencies (weekly, fortnightly, and every three weeks) and nutrient treatments (f/2, f/2 without vitamins, and macronutrients without vitamins or trace metals) on dulse. Nutrients were added weekly, and for treatments involving water change, nutrients were added immediately following the water change. The results showed that, as long as nutrients were regularly restored, growth rates were essentially insensitive to the frequency of water changes. When compared to dulse grown using unmodified f/2, dulse produced using Guillard's f/2 media with the vitamin component removed did not exhibit a significant change in growth rate. Additionally, growth rates were noticeably higher in f/2 without vitamins than in macronutrients and macronutrients with EDTA alone. This implies that when nutrient availability is not a limiting issue, dulse is adaptable to a variety of water-change regimes. These findings support the significance of weekly nutrient additions in preserving ideal growth conditions and are consistent with a larger understanding of nutrient dynamics in dulse farming.

The present study discussed the dulse average increase in biomass and other growth parameters like frond area and cluster length. The biomass of the dulse in the F2 solution treatment increased until 3 weeks. Then it decreased constantly by the end of the experimental period suggesting that nutrients especially nitrate and ammonium might have been depleted after 3 weeks. Dulse in seawater treatment showed a decline in biomass over an experiment of 9 weeks. It suggests that dulse cannot sustain growth under these conditions. The average biomass was highest in RAS water where the initial value of biomass was 2.08g and the final value after 9 weeks was 5.60g. The consistent biomass increase in the RAS effluent water suggests a sustained nutrient supply, possibly due to the higher initial concentration of nutrients compared to seawater and F2 solution treatment.

A study by Martínez et al. (2006) demonstrated dulse cultivation success in the open sea using culture ropes, with frond length increasing significantly during the spring culture period. While losses were noticeable (90.6% in summer and 76.1% in spring), their findings contrast with the current study's land-based cultivation experimental setup, where frond area and cluster length illustrated more stable growth in the RAS effluent water treatment.

In conclusion, the concentration of NH_4-N, NO_3-N, and PO_4-P was significantly lower in seawater and F2 solution treatment to support the growth of dulse. Moreover, supplementing the F2 solution treatment with vitamins could enhance the growth performance (biomass, frond area, and cluster length) of dulse. However, providing the high concentration of nutrients in the initial setup in F2 and seawater treatments might have given different results with biomass, cluster length, and frond area increasing over time. Dulse grown in the RAS effluent water treatment showed better growth performance, potentially due to sustained nutrient availability and higher initial nutrient concentration. However, further research on nutrient dynamics in the RAS effluent water and its impact on other growth parameters like protein or pigment content will assist in fully understanding the potential of RAS as a medium to grow dulse.

6.2 Nutrient removal efficiency from RAS Effluent Water

In this section of the discussion, the removal efficiency of NH_4-N, NO_3-N, and PO_4-P from the RAS effluent water treatment will be discussed. The concentration of nutrients in F2 solution and seawater treatment was significantly low and hence the analysis of nutrient removal efficiency in these treatments could not be performed. However, the opposite was observed in RAS effluent water treatment where the initial nutrient concentration was markedly higher.

6.2.1 Ammonium and Nitrate removal from the RAS effluent water

The nitrogen uptake dynamics of dulse have historically been explored using individual nitrogen sources such as NO_3^- or NH_4^+. Morgan and Simpson (1981a) provided dulse with 28 mg/L (2 mM) nitrogen weekly in the form of NO_3^- or NH_4^+. They found that dulse grew faster when supplied with NO_3^-. In a separate study, Grote (2016) evaluated nitrogen uptake for 3 hours at various NO_3^- and NH_4^+ concentrations and ratios. Dulse exhibited a higher affinity for NH_4^+ but demonstrated greater NO_3^- uptake in treatments with single nitrogen sources. Similarly, Corey et al. (2013) investigated five combinations of NH_4-N and NO_3-N while maintaining a total nitrogen concentration of 300 μM. They found that the highest growth rates (8.9% per day) and total nitrogen intake were achieved at 300 µM NH_4^+. Dulse removed 91–100% of NH_4-N and 23–37% of NO_3-N over 24 hours, with NH_4-N being absorbed 1.5–7 times more quickly than NO_3^-.

In contrast to these studies, the present study analyzed NO_3-N and NH_4-N together in the RAS effluent water. The current study found that dulse showed higher NO_3-N removal efficiency and consumption rates in the RAS effluent water treatment compared to NH_4-N. The removal efficiency of NO_3-N varied from 156% to 389%, with consumption rates between 19.3 µg/day to 148.9 µg/day. Although the removal efficiency of NH_4-N increased by 100% from Census 1 (23%) to Census 2 (46%), it remained significantly lower overall. This suggests that the concentration of NH_4-N was lower in RAS water and hence dulse absorbed all the available NH_4-N present in the RAS effluent water. The Data for Census 3 (week 9) was unavailable limiting the ability to assess the long-term trends of the current study related to NH_4-N.

The dominance of NO_3-N in the RAS water treatment in our study could be attributed to the higher initial concentration of NO_3-N in RAS water. However, further research is needed to confirm the uptake dynamics of dulse when both NO_3-N and NH_4-N are present in the water.

6.2.2 Phosphate removal from the RAS effluent water

Compared to nitrogen dynamics, phosphate (PO_4-P) removal by dulse has received less attention. Corey et al. (2013) assessed PO4-P uptake while cultivating dulse for 4 weeks at 10 °C in 1L flasks with a nitrogen-to-phosphorus molar ratio of 10:1. Despite the initial nutritional enrichment, the PO_4-P removal was negligible during weeks 3 and 4, with uptake rates of only 0.73 and 0.75 mg PO_4-P gDW^{-1} day^{-1} respectively. According to the statistical analysis, there were no significant variations in PO_4-P uptake over these weeks. A study by Lubsch and Timmermans (2020) involved growing dulse (n = 49) in a range of dissolved inorganic phosphate (DIP) concentrations (0.0–6.0 µmol L^{-1}) under carefully monitored laboratory conditions. The uptake dynamics of DIP by the dulse were measured using a "pulse-and-chase" method for five weeks. They found that the sporophytes of dulse exposed to nominal DIP concentrations of 0.2, 0.4, and 0.8 µmol L^{-1} consistently removed all available DIP during the daily 24-hour sampling period throughout the experiment. Nyheim (2023) extended this research by exposing dulse biomass to a phosphate gradient during a nutrient uptake experiment to determine whether there was any effect on the tissue content and uptake rate of phosphorus by the dulse. They observed a significant difference in the reduction of DIP after 180 minutes for the lowest concentrations (0.25, 0.5, 1.0 & 2.0 µM P, demonstrating the ability of dulse to effectively uptake phosphorus even at low ambient concentrations. A study by Demetropoulos and Langdon (2004) involving Pacific dulse *(Palmaria mollis)* found that moderate to high load of PO_4^- (125–500 µM d-1 provided as NaH_2PO_4) depressed the SGR of dulse. Above 83 µM d-1, there was an inverse relationship between tissue P concentrations and dulse growth. Providing the correct level of P is essential for generating high yields of dulse since tissue P concentrations >0.70% [dw] were positively connected with daily P loads and inversely connected with growth rates.

In contrast, the current study found a decreasing trend in the removal efficiency of PO_4-P from the effluent of the RAS even though the concentration of PO_4-P in RAS water treatment was significantly low. In particular, the removal efficiency decreased by about 47%, from 42% in Census 1 to 22% in Census 3. This decreased removal efficiency could be explained by the initial low concentration of PO_4-P in the RAS effluent water.

Previous studies have demonstrated the ability of dulse to effectively remove phosphate at low concentrations. However, the current study suggests that the low concentration of PO_4-P in the RAS effluent water may reduce the removal efficiency of PO_4-P by dulse. Future studies should aim at finding the optimum concentration of PO_4-P to maintain growth in RAS.

6.3 The relation between nutrient removal and the growth of the dulse.

Studies have shown that nutrient availability significantly affects the growth performance of dulse. Schmedes and Nielsen (2020) examined how differences in nutrient phases and environmental conditions impact the productivity and the Specific Growth Rate (SGR) of the dulse. They found that growth was slowed and pigmentation was lost during low-nutrient periods; however, switching to a high-nutrient culture not only recovered pigmentation but also SGR and nutrient composition of the dulse. This emphasizes the importance of sufficient nutrient availability for the optimal growth of the dulse. Another study by Morgan and Simpson (1981a) involved growing dulse in flowing seawater enriched with 0.5-2.0 mM concentrations of NO_3^- or NH_4^+. They found that NO_3^- was a better source of nitrogen for growth than NH_4^+. However, plants given NH_4^+ accumulated more nitrogen than plants supplied with NO_3^-. The study also revealed that NH_4^+ was somewhat toxic even though nitrogen uptake and accumulation were greater with NH_4^+ than with NO_3^-. Toxicity may be due to the inhibition of photosynthetic carbon metabolism by unionized ammonia. Rizzo et al. (2024) fed juvenile red abalone *(Haliotis rufescens)* of two different size classes in ambient water conditions with the seaweed dulse *(Devaleraea mollis)* while being conditioned at 13°C, 15°C, and 17°C for 92 days. Nutritional analysis revealed that the dulse culture at 17°C had a much higher protein and nitrogen content than the cultures at 15°C and 13°C, although there were no significant differences in the amount of carbohydrates. This suggests that higher temperatures lead to higher protein and nitrogen content compared to lower temperatures.

The current study aligns with these observations by revealing a positive relation between dulse growth performance and the removal of nutrients from the RAS effluent water. The current study showed that the removal rate of NO_3-N from the RAS water treatment was higher as compared to NH_4-N, reflecting its significance as the primary source of nitrogen for the dulse in the present experiment. However, dulse in the experiment demonstrated increased growth with the increasing removal rates of all nutrients (NO_3-N, NH_4-N, and PO_4-P) from the RAS effluent water. In contrast to the previous studies, the current experiment involved a single dulse sample in 1L flasks of the RAS effluent water with replicates in lab settings, focusing on the nutrient removal from the RAS effluent water rather than the nutrient composition within the dulse tissue.

Further research on the tissue content of dulse would be ideal for investigating the nutrient uptake of the dulse. Long-term studies investigating the relation between nutrient availability and dulse growth, particularly in a variety of environmental circumstances, should be the main focus of future studies. It would be helpful to know the ideal salinity, light, and temperature for growth and the removal of nutrients.

7. Conclusion

The primary goal of the study was to assess the practicality of growing dulse in effluent water from a Recirculating Aquaculture System (RAS) for Atlantic salmon (*Salmo salar).* The study focused on evaluating the growth performance of dulse and its ability to remove nutrients from the effluent water of the RAS. The findings from the present study demonstrated that dulse cultivated in the RAS effluent water treatment retained its color. In contrast, the dulse grown in F2 solution (nutrient-spiked water) and seawater (control) treatments did not retain color. The growth performance of the dulse was highest in the RAS water treatment compared to other treatments. However, the dulse in the seawater and F2 solution showed initial growth until 3 weeks after which the growth declined resulting in the mortality of dulse by the end of the experiment. Regarding nutrient removal from the RAS water, the current study established that dulse effectively removed NO_3-N from RAS and had the highest removal efficiency among the nutrients analyzed in the experiment. While the removal efficiency of NH_4-N improved over time from Census 1 to Census 3, the removal efficiency of PO_4-P declined. These results suggest that the growth performance of the dulse is related to its ability to remove nutrients, particularly NO_3-N from the effluent water of RAS.

Research Question 1

To what extent does the growth performance of dulse differ between cultivation in RAS effluent water and F2 solution (nutrient-spiked water), and is there a significant relationship between water source and biomass yield?

The study concluded that the dulse showed a significant growth performance in RAS water compared to the F2 solution. The values of NO_3-N, NH_4-N, and PO_4-P were too low in the F2 solution treatment to support growth which resulted in the disintegration of the dulse by the end of the experiment. In contrast, dulse in the RAS effluent water treatment increased biomass yield proportionally alongside higher nutrient removal rates. This highlights a positive relationship between nutrient availability in RAS and biomass yield.

Research Question 2

Does the nutrient uptake of dulse vary depending on whether the water is sourced from RAS effluent or the F2 solution?

The current study revealed significant differences in nutrient uptake between the two nutrient sources. Dulse grown in the RAS effluent water demonstrated higher nutrient uptake compared to the F2 solution where the nutrient values were too low for accurate analysis. This emphasizes the suitability of the RAS effluent water as a nutrient source for the cultivation of dulse.

Overall, the present study highlights the challenges of growing dulse in laboratory conditions, primarily due to the incapacity of the laboratory environment to replicate the natural parameters necessary for the optimal growth of dulse. Moreover, the disintegration of dulse in the F2 solution and seawater treatments represents the limitations of these treatments. Furthermore, the removal of NO_3-N, NH_4-N, and PO_4-P was analyzed in this study. The removal efficiency of NO_3-N from RAS was particularly noteworthy as it is the main nutrient of the RAS. These findings concluded that dulse has the potential for use in nutrient bioremediation, and confirms that dulse can be cultured in the effluent water of RAS. The current study establishes a clear trend that higher nutrient removal from the RAS effluent water leads to an increase in the biomass of dulse.

8. Study limitations and future recommendations

This section discusses the limitations that occurred in the current study and provides recommendations for future studies.

8.1 Study limitations

The experiment was conducted under controlled laboratory conditions with constant temperature, salinity, and light regimes throughout the experiment, enabling us to examine how nutrient availability affects dulse growth and nutrient removal from RAS. However, these conditions do not replicate the dynamic environmental conditions of large-scale aquaculture or natural ecosystems.

The low concentration of PO_4-P and NH_4-N in the F2 solution and seawater was another limitation of the present study. The values were below the detection limit of our spectroquant. Thus, it hinders the accurate measurement of the nutrients. This limitation made it impossible to evaluate the nutrient dynamics in these treatments.

Dulce tissue analysis was not performed in the current study, making it difficult to understand the composition of micronutrients (vitamins and minerals) and macronutrients (protein, carbohydrates, and lipids) before and after the experiment.

8.2 Future Recommendations

The main focus of future research should be on conducting experiments under different environmental conditions. This includes variable light intensity, temperature, and salinity to understand their effect on the growth and nutrient removal efficiency of dulse.

Future studies should use higher concentrations of PO_4-P and NH_4-N in seawater and F2 solution treatments to measure the nutrient concentrations accurately.

Additionally, future research should analyze the role of microorganisms, such as nitrifying bacteria, in the nutrient dynamics of dulse. The microbial influence might have impacted the nutrient dynamics observed in the present study.

Furthermore, the evaluation of the possibility of dulse cultivation for the treatment of wastewater and the effectiveness of integrated aquaculture systems to maximize nutrient utilization should be investigated.

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