Assessing The Feasibility and Impacts of Introducing Seaweed Cultivation in Shrimp Farming Ghers/Ponds in Coastal Regions of Bangladesh

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August, 2025

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Abstract

To assess the feasibility and ecological impacts of introducing seaweed cultivation into shrimp farming systems, a comparative cultivation trial of *Gracilaria* sp. was conducted from October 2023 to March 2024 across pond and nearshore environments in Cox's Bazar, Bangladesh. The study employed a total of 18 experimental plots—9 in shrimp ponds (ghers) and 9 in nearshore waters—spanning both surface and bottom layers. In the nearshore environment, cultivation was conducted using floating long-line systems at the surface and off-bottom long-line and off-bottom net systems at the bottom, whereas in pond conditions, floating long-line and floating net methods were applied at the surface, with a broadcast technique used for bottom-layer cultivation. A two-way ANOVA revealed that the biomass production was significantly influenced by both cultivation environment and layer, with nearshore environments yielding substantially higher values (1.47 ± 0.68 kg m^{-2}) than ponds (0.19 ± 0.09 kg m^{-2}), and greater productivity at the surface (1.04 ± 0.98 kg m^{-2}) compared to the bottom layer (0.62 ± 0.52 kg m^{-2}). The proximate composition of *Gracilaria* sp. was also significantly affected by environment and layer, with nearshore and surface-grown seaweeds exhibiting notably higher protein, lipid, ash, and fiber contents compared to pond-cultivated seaweeds, which consistently showed lower nutritional values across all parameters. The amino acid concentrations of *Gracilaria* sp. were also substantially higher in nearshore grown seaweeds, whereas the effect of cultivation layer remained relatively minor across most amino acids. A total of 50 distinct phytochemical compounds were identified through GC-MS analysis in both pond and nearshore cultivated *Gracilaria*, with their concentrations showing clear variations based on cultivation environment and layer, reflecting different accumulation patterns under different culture conditions. Among the 13 essential minerals detected, Na, K, Fe, Co, and Cu were more abundant in nearshore-cultivated *Gracilaria*, whereas Ca, Mn, Se, and Rb were predominantly enriched in pond-grown seaweeds. Among the four identified heavy metals, Cr and Pb showed significantly higher accumulation in nearshore- and pond-grown *Gracilaria*, respectively, while Cd and Hg levels remained relatively stable across all cultivation conditions. The study also revealed that although the antioxidant levels in *Gracilaria* remained consistent across cultivation environments, flavonoid content was markedly elevated in nearshore-cultivated seaweeds (192.13 ± 35.50) compared to those grown in ponds (44.07 ± 15.50). The

findings of the study demonstrated that surface based floating net systems could be a promising culture technology for the production of *Gracilaria* sp. in shrimp farming ghers/ponds in coastal regions of Bangladesh.

Keywords: *Gracilaria* sp.; Shrimp Gher/pond; Growth performance; Biochemical composition; Heavy metals

CHAPTER I

INTRODUCTION

1.1 Background

Seaweeds, commonly referred to as marine algae, are evolutionarily primitive, non-flowering, and photosynthetic macrophytes that primarily inhabit the intertidal zones of seas and oceans ecosystem, that span around 71% of the Earth's surface and are considered an important source of renewable biological resources (Vroom, 2001; Jueterbock et al., 2013; Rao et al., 2018). They are often regarded as plants of the new millennium due to their wide-ranging social, economic, and ecological contributions, which are observed across coastal regions globally, from warm tropical areas to the cold Arctic zones (McHugh, 2003; Sarkar et al., 2016). Seaweeds possess a valuable nutritional profile, characterized by a high carbohydrate content (reaching up to 60%), moderate to substantial protein levels (ranging from 10% to 47%), and low lipid concentrations (around 1–3%), along with mineral ash content that can vary between 7% and 38% (Dominguez and Loret, 2019; Pappou et al., 2022). These marine macroalgae are a rich source of vitamins such as A, B1, B12, C, D, and E, along with riboflavin, niacin, pantothenic acid, and folic acid (Ortiz et al., 2006; Besada et al., 2009), while also supply vital minerals including calcium (Ca), phosphorus (P), sodium (Na), and potassium (K) (Dhargalkar and Pereira, 2005). Moreover, they offer an abundance of a variety of over 54 trace elements crucial for human physiological functions, surpassing the amounts found in vegetables and terrestrial plants (Dhargalkar and Pereira, 2005). Therefore, the worldwide demand of seaweed biomass for the production of food and bio-active compounds is steadily rising; hence, improving seaweed farming practices is essential and has become a vital priority (Camus et al., 2019; Shannon and Abu-Ghannam, 2019).

Globally, an estimated 10,100 seaweed species have been identified across both marine and freshwater ecosystems (O'Sullivan et al., 2010; Morais et al., 2020). These include around 2,200 green algae, 6,100 red algae, and 1,800 brown algae species (Morais et al., 2020). Of these, approximately 221 species are commercially exploited—comprising 125 red, 64 brown, and 32 green seaweeds (Nayar and Bott, 2014). Among them, 101 species are utilized for hydrocolloid extraction, while 145 are processed into various food products. Globally, about 34 distinct seaweed types are actively cultivated

(Chowdhury et al., 2022), with species like *Eucheuma*, *Laminaria*, *Gracilaria*, *Kappaphycus*, and *Porphyra* dominating the industry—collectively representing around 95% of total cultured seaweed output (Chowdhury et al., 2022). Covering over 48 million km² and involving 132 countries, seaweed aquaculture has emerged as one of the fastest-growing sectors globally, with consistent production reported from 37 to 44 nations (Froehlich et al., 2019; FAO, 2021). By 2019, farmed seaweed production had surged to 35.8 million tonnes—constituting 97% of global supply and valued at US$11.8 billion—while harvests from wild stocks remained stable at 1.1 million tonnes over the past 50 years (FAO, 2021). During this timeframe, production of red seaweed experienced a significant increase, climbing from 1 million to 18.3 million tonnes, while brown seaweed output rose from 3.1 million to 16.4 million tonnes; in contrast, green seaweed cultivation saw a slight decline, dropping from 31,000 to 17,000 tonnes (FAO, 2021). The vast majority of farmed seaweed is produced in East and Southeast Asia, where China, Indonesia, Korea, and Japan collectively accounted for over 90% of global output in 2019 (FAO, 2021).

Bangladesh is going to enter its third decade of seaweed aquaculture development, following the initial farming trials conducted in the early 2000s (Chowdhury et al., 2022). The first decade (2001–2010) marked the exploratory phase, focusing on identifying cultivable species and their suitability for farming under local coastal conditions. Initial trials were conducted with *Hypnea* spp. (Rhodophyta) and *Caulerpa* spp. (Chlorophyta) in the intertidal zones of the southeastern coast (Zafar, 2004, 2005, 2007). The second decade (2011–2020) saw an expansion to pilot-scale culture trials, with community-level demonstrations aimed at building local capacity and increasing farmer participation. The government of Bangladesh has incorporated seaweed aquaculture into the 7th Five-Year Plan (BFRI, 2019) and launched cultivation programs through the Palli Karma-Sahayak Foundation (PKSF), Bangladesh Fisheries Research Institute (BFRI), and Bangladesh Agricultural Research Council (BARC) in collaboration with different development partners like FAO and USAID to promote the activity (Chowdhury et al., 2022). Entering the third decade, seaweed farming is gaining wider interest, particularly along the protected intertidal areas of Cox's Bazar, including Nuniarchara, Inani, Rezu Khal, the Bakkhali river estuary, and Saint Martin's Island (Chowdhury et al., 2022). Recent scientific efforts have focused on optimizing farming systems, with several studies assessing the growth performance of various

seaweed species under different culture methods, while simultaneously evaluating how specific cultivation practices influence the biochemical composition and food safety attributes of seaweed reinforcing its potential in food and nutraceutical applications (Banik et al., 2023; Mohiuddin et al., 2024; Chamily et al., 2025). In parallel, a Generalized Additive Modelling (GAM) framework has been applied to develop predictive site suitability maps for seaweed aquaculture across Bangladesh's coastal and marine ecosystems, offering strategic insights for spatial planning and sustainable sectoral expansion (Tasnim et al., 2024).

Seaweed cultivation, traditionally, involves various methods such as long lines, bottom nets, raft/hanging lines, bamboo poles, and planting/sowing techniques in coastal regions (Aktar et al., 2020). Seaweed cultivation techniques in the coastal waters of Bangladesh mainly involve the long line method in the intertidal zone, the bottom net method, and the raft/hanging line method in the lower intertidal and subtidal zones (Banik et al., 2023; Hossain et al., 2020). However, these traditional onshore methods are not recommended throughout the year because fluctuating seawater conditions impact growth and annual turnover (Suthar et al., 2019). Furthermore, due to its geographic location within the low-lying Ganges Delta, Bangladesh's coastal regions are highly vulnerable to frequent flooding, cyclonic storms, and intense wave action-factors that pose significant threats to the structural integrity and sustainability of seaweed cultivation systems (Islam et al., 2016; Rezaie et al., 2019). These problems necessitate a standardized land-based cultivation system to ensure consistent yield and quality of seaweed, which ocean-based methods struggle to provide. Despite the absence of a universally acknowledged global legal definition, land-based seaweed farming entails the cultivation of marine algae in controlled designated areas (pond/tanks), usually positioned at a specific minimum distance from the coastline (García-Poza et al., 2020). In addition to enhanced biomass productivity per unit area, land-based cultivation provides better control over biomass quantity and quality, traceability, and security compared to conventional methods, and also enables year-round culture (Hafting et al., 2012; Ladner et al., 2018).

Bangladesh's coastal districts host over 55,000 marine shrimp farms across approximately 170,000 hectares (Karim, 2019). Many of these shrimp farms have salinity levels exceeding 10 ppt during the seaweed culture season (October to March) and often remain unused due to widespread viral disease outbreaks (Hossain, 2004).

These conditions suggest that seaweed farming could be feasible in these ponds, potentially providing mutual benefits for both fed aquaculture species, like shrimp, and extractive aquaculture species, such as seaweed (Kim et al., 2022). For instance, incorporating *Gracilaria* seaweed into conventional aquaculture ponds has been shown to enhance shrimp production in Indonesia (Andayani et al., 2016). In fact, this integrated approach maximizes the efficient use of land and resources while offering mutual benefits to co-cultured species (e.g., fish, shrimp, shellfish) by improving nutrient bioremediation competence, and offers economic diversification through the production of value-added aquaculture crops, thereby increasing unit farm productivity and profitability (Anaya-Rosas et al., 2019; Nauta et al., 2024). Seaweeds absorb excess nutrients and pollutants, thus improving water quality and creating healthier conditions for shrimp farming (Sarkar et al., 2019; Widowati et al., 2020). This symbiotic relationship reduces the need for chemical inputs and lowers the environmental impact of aquaculture (Sukhdhane et al., 2018). Economically, this method also diversifies the income sources for farmers, increasing resilience against market fluctuations and environmental challenges.

Gracilaria, a prominent genus within the red algae group *Rhodophyta*, ranks as the second largest in the phylum, encompassing over 185 recognized species with a global distribution (Meena et al., 2006; Brodie and Lewis, 2007). Its high commercial significance stems primarily from its abundant agar content, which typically ranges between 19.0% and 30.0% of its dry matter (Marinho-Soriano and Bourret, 2003). Species belonging to this genus are responsible for producing approximately 66% to 80% of the agar used worldwide (Pereira and Yarish, 2008; Bixler and Porse, 2011). Beyond agar, *Gracilaria* is also valued as a rich source of bioactive polysaccharides (Xu et al., 2017), and it contains considerable amounts of chlorophyll and carotenoids, which act as natural antioxidants (Stengel et al., 2011; Asih et al., 2019). Furthermore, this genus has various culinary and agricultural applications—it is consumed as a sea vegetable (Haglund and Pedersen, 1993; Tseng and Xia, 1999), used in fresh salads (Phang, 2006), and incorporated into animal feed (FAO, 2021). *Gracilaria* species are well adapted to diverse coastal habitats, spanning temperate zones (such as Morocco, Spain, and Portugal), tropical regions (like the Philippines and Indonesia), and even colder waters (e.g., Chile and Canada) (McHugh, 2003; Du et al., 2016; Hernandez, 2017). In terms of global production, *Gracilaria* ranked third among seaweed genera

in 2019—following *Laminaria* and *Eucheuma*—with an output of 3.6 million tonnes, representing 10.5% of total aquaculture seaweed production and an estimated market value of USD 2 billion (FAO, 2021). One of the major advantages of this genus is its ease of reproduction, both sexually and asexually, coupled with its rapid growth rates (Abreu et al., 2011; Kim et al., 2017). As a euryhaline species, *Gracilaria* is capable of enduring a wide salinity spectrum ranging from 10 to 40 psu, although optimal growth typically occurs within the 25–33 psu range (Yokoya et al., 1999; Kim et al., 2017; Vanderklift et al., 2020). Although optimal growth occurs between 20°C and 28°C, *Gracilaria* is capable of surviving in temperature ranges as low as 0°C and up to 35°C. (Yokoya et al., 1999; Kim et al., 2017). This resilience to fluctuating environmental conditions—especially salinity and temperature—makes *Gracilaria* particularly suitable for integration into pond-based aquaculture systems. The ability to tolerate fluctuations in water quality parameters makes *Gracilaria* a promising candidate for pond-based cultivation systems, especially in dynamic and semi-controlled aquaculture environments. For these reasons, several researchers have selected various species of *Gracilaria* for their pond-based cultivation trials (Hendri et al., 2018; Brito et al., 2014; Anh et al., 2019; Diatin et al., 2020).

In the context of pond-based seaweed cultivation, researchers across various studies have pursued diverse objectives. For instance, Hendri et al. (2018) explored seaweed farming as a means to generate alternative income through efficient utilization of available pond space. Diatin et al. (2020) focused on integrating seaweed with finfish and shellfish to develop a successful and economically viable polyculture system. Others, such as Seema et al. (2005), introduced seaweed into shrimp culture systems with the aim of improving water quality by effectively removing ammoniacal nitrogen from the rearing environment. Similarly, Anh et al. (2019) and Brito et al. (2014) incorporated seaweed into shrimp ponds to evaluate its impact on shrimp growth performance. In each case, the outcomes were positive, demonstrating the multifaceted benefits of seaweed integration in pond aquaculture. Given the extensive area of coastal ponds and shrimp ghers in Bangladesh, adopting pond-based seaweed cultivation could offer significant socio-economic advantages for coastal farmers by enhancing productivity, environmental quality, and income diversification.

Differences in cultivation environments and methods exert a profound influence on the biochemical composition of seaweeds (Anburaj et al., 2024; Anwar et al., 2014).

Environmental parameters such as nutrient availability, salinity, light intensity, temperature, and hydrodynamics vary substantially between land-based ponds, nearshore areas, and open-sea systems, thereby shaping the metabolic profile of cultivated biomass (Kaladharan et al., 1996; Barr et al., 2008). Similarly, differences in culture methods, such as surface versus bottom layering, or monoculture versus integrated multi-trophic aquaculture (IMTA), can alter biochemical traits including proximate composition, amino acid profiles, fatty acid distribution, and antioxidant potential (Kumar et al., 2008; Lomartire et al., 2022). These variations not only reflect physiological responses to the surrounding culture conditions but also determine the nutritional, industrial, and pharmaceutical value of the harvested seaweed biomass (Munier et al., 2013).

1.2 Justification of the study

Several studies have examined the growth performance of various seaweed species, including *Gracilaria*, under different culture systems deployed in the nearshore waters along the coast of Bangladesh (Bhuyan et al., 2023; Banik et al., 2023; Mohiuddin et al., 2024; Chamily et al., 2025). Although pond-based seaweed cultivation offers multiple advantages, no comprehensive research has yet been conducted in the coastal ponds or shrimp ghers of Bangladesh to explore its feasibility and potential benefits. In addition, although the correlation between environmental parameters and seaweed growth has been extensively studied in open-water (nearshore) cultivation along the southeastern coast of Bangladesh, the correlation between water quality variables and the growth dynamics of seaweed within coastal ponds or shrimp gher environments remains unexplored. While variations in the nutritional composition (proximate composition, amino acids, and minerals) of seaweeds cultured through different methods in nearshore environments have been relatively well studied (Banik et al., 2023, Mohibbullah et al., 2023; Ullah et al., 2025), comparative research evaluating whether such differences exist between pond and nearshore cultivated seaweeds remains notably scarce. In addition, while several studies have addressed the antioxidant and flavonoid profiles and amino acid composition of seaweeds grown in open-water systems, such biochemical attributes of pond-cultivated *Gracilaria* remain poorly understood. Although heavy metal accumulation in seaweeds from nearshore environments has been studied, investigations comparing heavy metal content between

seaweeds cultivated in ponds and those grown in nearshore waters are still lacking, which raises critical concerns regarding the food safety of farmed seaweed.

1.3 Objectives of the Study

In this study, we aimed to achieve the following objectives,

1. To explore the feasibility of cultivating *Gracilaria* sp. into shrimp farming ponds (*ghers*) through a comparative experiment conducted in pond and nearshore environments.
2. Analyze the effects of cultivation conditions, including cultivation environment (pond vs. nearshore) and cultivation depth (surface vs. bottom), on the growth performance and biomass yield of *Gracilaria* sp.
3. To analyze the intricate relationships between ecological factors and their regulatory effect on the growth dynamics of *Gracilaria* sp. within both pond-based and nearshore cultivation systems.
4. To evaluate how cultivation environment (pond vs. nearshore) and cultivation depth (surface vs. bottom) influence the nutritional composition and food safety aspects of *Gracilaria* sp. seaweed.

CHAPTER II

REVIEW OF LITERATURE

2.1 Ecological Role and Structural Characteristics of Seaweeds

Seaweeds are large, multicellular, photosynthetic organisms that form a vital component of coastal marine ecosystems, contributing significantly to oceanic primary productivity, carbon fixation, and habitat complexity (Cotas et al., 2023; Anburaj et al., 2024). Unlike terrestrial plants, seaweeds lack true roots, stems, and leaves, and their plant body is termed a thallus, typically composed of a holdfast, stipe, and blade (Jayaprakash et al., 2017). The holdfast functions solely as a structural anchor to the substrate, lacking any role in nutrient absorption, thereby differentiating seaweeds from vascular plants (Kasimala et al., 2015). Seaweeds function predominantly as autotrophs, using sunlight to convert inorganic nutrients such as nitrogen, phosphates, water, and carbon dioxide into organic matter through photosynthesis (Lee et al., 1999). While seaweeds themselves are not heterotrophic, they frequently harbor diverse communities of surface-associated heterotrophic bacteria, which contribute to their microenvironment and may produce bioactive compounds of pharmaceutical interest (Egan et al., 2013; Mahajan et al., 2025). Seaweeds exhibit a wide array of reproductive strategies that contribute to their ecological success, population resilience, and wide distribution across diverse marine environments (Liu et al., 2017). Several seaweed species reproduce asexually by means of thallus fragmentation, formation of vegetative propagules, or the release of spores from specialized structures known as sporocysts (Pereira, 2022). In contrast, sexual reproduction involves the fusion of male and female gametes, with many species exhibiting a life cycle that alternates between a haploid gametophyte and a diploid sporophyte phase (Sangtong et al., 2020). Red algae often follow a more complex triphasic life cycle that includes an additional carposporophyte stage, whereas brown and green algae typically follow a simpler biphasic pattern (Yoon et al., 2016). The algae widely vary in size that includes many bacteria-sized (1-5 μm) (e.g. *Micromonas* sp*p* and *Chlorella* sp*p* in the Chlorophyta) and also the largest protists in the entire world, including kelps like *Macrocystis* species, which can grow to a length of 30 m. (Bhattacharya and Medlin, 1998).

Pereira and Neto (2014) proposed a classification scheme that categorizes seaweeds into three major groups: Chlorophyta (green algae), Phaeophyta (brown algae), and

Rhodophyta (red algae), based primarily on differences in photosynthetic pigments, and supported by biochemical, physiological, and electron microscopic research. Brown seaweeds, display a characteristic brown or olive-green hue, resulting from the combined presence of green pigment chlorophyll and the yellow pigment xanthophylls (Kasimala et al., 2015). They exhibit considerable morphological diversity, ranging from microscopic filamentous forms such as *Ectocarpus* to large, structurally complex species like *Laminaria* (kelp) (Dayton et al., 1999). Many brown algae are valued for their high iodine content and are widely exploited for the extraction of alginates, particularly from genera such as *Laminaria*, which are commonly used in food, pharmaceutical, and industrial applications (Kasimala et al., 2015). Green seaweeds, are photosynthetic eukaryotes characterized by double membrane-bound plastids containing chlorophylls a and b, along with accessory carotenoids such as beta-carotene and xanthophylls (Garcia-Poza et al., 2020; Khotimchenko et al., 1993). These algae typically inhabit well-lit intertidal and shallow subtidal zones, often attaching to rocky substrates near the ocean surface where sunlight is abundant (Kasimala et al., 2015). Their cells store starch inside plastids and have cell walls made of cellulose, reflecting structural traits that are also found in land plants and highlighting their evolutionary connection (Graham and Wilcox, 2000). Red algae, are among the most taxonomically diverse marine macroalgae, particularly in tropical regions (Maggs et al., 2007). They are eukaryotic, predominantly macroscopic, and photosynthetic organisms that inhabit rocky substrates across intertidal and subtidal zones (Kasimala et al., 2015). Their red appearance comes from accessory pigments such as phycoerythrin and phycocyanin, in addition to chlorophyll a, beta-carotene, and various xanthophylls to optimize light absorption in deeper or shaded marine environments (Nisizawa, 2006). The cell walls of red algae are primarily composed of cellulose, agar, and carrageenan, which are long-chained polysaccharides of significant commercial value widely used in food, pharmaceutical, and cosmetic industries (Kasimala et al., 2015).

2.2 Red Seaweed (*Gracilaria* sp.)

Gracilaria, also known as irish moss or ogonori, a genus of red algae within the family Gracilariaceae, is economically significant as an agarophyte due to its widespread use in agar production and is also utilized as a nutritional resource for both human consumption and shellfish feed (Oliveira et al., 2000; Nguyen, 2015). The genus *Gracilaria* was established by Greville in 1830 with 4 species, which Agardh expanded

to 23 species in 1852 and later to 61 species by 1901. Since then, the number of reported *Gracilaria* species has increased to approximately 150 worldwide, with 24 species recorded in China (Lewmanomont, 1995). *Gracilaria* exhibits erect thalli originating from a discoid holdfast, with species-specific variations in thallus form (cylindrical, compressed, or blade-shaped), branching patterns, and apex-base morphology. Internally, the structure consists of a cortex and medulla, where species differ in the number of cortical layers, size and arrangement of medullary cells, and the transition between these tissues (Othman et al., 2018; Arbit et al., 2019). *Gracilaria* species are globally distributed in warm marine waters, with seasonal occurrences in temperate zones, and are present in all major oceans except the Arctic (Muller et al., 2009). The Western Pacific region represents the center of their biodiversity and has historically served as a key area for *Gracilaria* cultivation, primarily for the commercial extraction of agar (Torres et al., 2019). However, *Gracilaria* is traditionally consumed in several Asian and Caribbean countries, where it is known by different local names, such as *gulaman* in the Philippines, *ogonori* in Japan, *kkosiraegi* in Korea, and is used in traditional dishes like soups and jellies in Sri Lanka (Trono & Ganzon-Fortes, 1981; Jayasuriya, 1989). Moreover, sulfated oligosaccharides (with a polymerization degree of 6) derived from agar-rich *Gracilaria* polysaccharides through agarase digestion, have demonstrated significant prophylactic efficacy against Japanese encephalitis virus under both laboratory and animal testing conditions (Kazlowski et al., 2012). Due to its wide range of applications in food, pharmaceuticals, and industrial uses, *Gracilaria* holds significant economic value globally, leading to increased interest and investment in its cultivation across various regions (Mouedden et al., 2024).

The taxonomic position of *Gracilaria* sp. is outlined below,

Phylum Rhodophyta

Sub-phylum Eurhodophytina

Class Florideophyceae

Subclass Rhodymeniophycidae

Order *Gracilaria*les

Family *Gracilaria*cae

Genus *Gracilaria*

Species *Gracilaria* sp. (Guiry and Guiry, 2010)

Fig 1: Red Seaweed (*Gracilaria* sp.)

2.3 Evolution of Shrimp Aquaculture and Scope for Seaweed Co-Cultivation in Bangladesh

Shrimp farming in Bangladesh began in the early 1970s in the southeastern coastal region, particularly in Cox's Bazar, where traditional earthen enclosures known as ghers, were adapted for shrimp and prawn cultivation (Islam et al., 2005; Ahmed et al., 2008; Kabir and Hossain, 2021). Over time, with the rise of global demand and favorable environmental conditions, shrimp farming expanded rapidly throughout the southwestern coastal districts of Khulna, Satkhira, and Bagerhat, as well as parts of Noakhali, Barishal, and Patuakhali. The culture area increased from 26,315 ha in 1979–80 (Haque, 1984) to 62,756 ha in 1984, and reached 108,280 ha by 1990–91. By 2007–08, the area had nearly doubled to 217,877 ha (DoF, 2009), and by 2016, it expanded further to 275,509 ha (Kabir & Hossain, 2021). Currently, Bangladesh's coastal districts support over 55,000 marine shrimp farms spread across approximately 170,000 hectares of land, among which about 135,000 hectares are located in Khulna, Satkhira, and Bagerhat, and 35,000 hectares in Cox's Bazar within the Chittagong region (DoF, 2024).

However, shrimp cultivation in Bangladesh, despite its significant growth, has been increasingly affected by a range of biophysical and disease-related challenges, leading to the gradual decline or abandonment of many ghers. As highlighted by Hossain et al.

(2013), disease outbreaks such as white spot syndrome, black gill, and tail rot have become increasingly common in shrimp ghers, particularly during the winter months, often forcing farmers to suspend or halt cultivation during that period. According to Washim et al. (2020), the water quality in these shrimp ghers of Bangladesh typically shows salinity between 10 and 17 ppt, temperature ranging from 30 to 33°C, alkalinity of 140 to 160 mg/l, dissolved oxygen between 4.0 and 5.9 mg/l, and pH from 8.5 to 9.2. These parameters are well-suited for the growth of various commercially important seaweed species, such as *Gracilaria* sp., making the ghers potentially suitable for seaweed cultivation. Introducing seaweed cultivation into these ghers could offer multiple benefits, including nutrient absorption that improves water quality, reduced risk of eutrophication, enhanced biodiversity, and an alternative source of income for farmers—especially in ghers where shrimp production has declined due to environmental or disease-related constraints.

2.4 Historical Progression of Seaweed Cultivation in Shrimp Pond Ecosystems

The earliest record of seaweed farming in coastal ponds began in the Philippines during the early 1950s, when *Caulerpa lentillifera* was accidentally introduced into milkfish and shrimp ponds in Mactan Island, and later became a primary crop due to its fast growth and market demand (Trono, 1990). In 1962, Taiwan initiated pond farming of *Gracilaria*, particularly *G. verrucosa*, due to its ecological adaptability and high quality agar yield, marking a significant advancement in the country's seaweed aquaculture development (Chiang, 1981). By the early 2000s, Brazil integrated *Gracilaria* cultivation into shrimp pond effluents for nutrient recycling (Marinho-Soriano et al., 2002), followed by experiments showing how *Gracilaria birdiae* growth varied with depth and environmental parameters (de Oliveira et al., 2012). In 2006, Japan's contributions were rooted in ecological integration, where *Ulva* and other seaweeds were used as biofilters in shrimp systems, helping to reduce ammonia and control nutrient levels (Shimoda et al., 2006; Sato et al., 2006). By 2010, researchers in China introduced *Sargassum hemiphyllum* to shrimp ponds during winter via a simple long-line system to counteract overharvesting and environmental degradation (Zonghe et al., 2013). That same year, Indonesia explored *Gracilaria* production in acid sulfate soil ponds in South Sulawesi, identifying key environmental challenges but also showing that production could be improved with proper management (Mustafa & Sammut, 2010). In 2016, Malaysia studied how *Gelidium elegans* could help to clean shrimp

hatchery wastewater (Rabiei et al., 2016), and by 2018, it began using *Gracilaria edulis* and *G. changii* in outdoor systems, which proved effective in removing nutrients and improving shrimp survival (Mawi, 2019).

In 2018, Indonesia took a step forward in refining pond-based seaweed farming by testing floating net and long-line methods for cultivating *Gracilaria* in traditional shrimp ponds, focusing on different planting distances to optimize space and yield (Hendri et al., 2018; Samidjan & Rachmawati, 2018). In 2019, India initiated experimental cultivation of *Gracilaria tenuistipitata* in tide-fed brackishwater ponds in the Sundarbans, aiming to understand how depth and initial density affect its growth potential (Sarkar et al., 2019). The same year, Indonesia further expanded its research by evaluating the impact of *Gracilaria* stocking density on the growth and performance of whiteleg shrimp (*Litopenaeus vannamei*) in polyculture settings, highlighting its potential for co-culture integration (Samidjan et al., 2019). This progression continued into 2020, as both Vietnam and Indonesia embraced integrated multi-trophic aquaculture (IMTA) models that combined *Gracilaria*, shrimp, mud crab, and tilapia, with the goal of enhancing water quality and overall pond productivity (Pantjara et al., 2020; Anh et al., 2020; Verdian et al., 2020). The most recent advancements came in 2024–2025, when Vietnam conducted a series of advanced polyculture trials integrating *Gracilaria tenuistipitata* and *Caulerpa lentillifera* with *Penaeus monodon* and *L. vannamei*, demonstrating clear improvements in water quality, feed efficiency, and shrimp resilience against disease, and showcasing how decades of research have shaped a more sustainable and effective approach to seaweed cultivation in the region (Anh et al., 2024; Huong et al., 2025).

2.5 Species Suitability Assessment for Seaweed–Shrimp Integrated Cultivation

Various researchers collaborated on studying different types of seaweed species to determine their suitability for cultivation within shrimp farming systems. For example, In Muttukadu, Soumyabrata et al., (2021) conducted a study to assess the viability of cultivating red seaweed (*Agarophyton tenuistipitatum*) in conjunction with white leg shrimp (*Litopenaeus vannamei*) within an integrated approach. Yu et al. (2013) studied the seaweed *Sargassum hemiphyllum* to develop an effective way to grow it in shrimp ponds, aiming to support its growth and check its nutritional quality, as its natural supply has been decreasing due to overuse and pollution. In a separate investigation

carried out by Seema and Reeta in 2005, they introduced the green seaweed Ulva reticulata into the shrimp culture system with the intention of effectively eliminating ammonical nitrogen from the culture environment. Syahrir (2023) conducted a study in South Sulawesi, Indonesia, using three seaweed species: *Gracilaria verrucosa*, *Eucheuma spinosum* and *Kappaphycus alvarezii* to assess their potential in nutrient waste bioremediation, aiming to identify effective candidates for improving water quality in shrimp pond systems. Mashoreng et al. (2019) conducted a study on the pond-based cultivation of *Gracilaria verrucosa* and *Caulerpa racemosa* to assess their capacity for carbon sequestration, aiming to explore their potential role in climate change mitigation and sustainable integration within aquaculture systems.

In South Sumatra, Hendri et al., (2018) conducted an experiment to evaluate water parameters and assess the growth rate of *Gracilaria* sp seaweed within shrimp ghers, aiming to optimize pond space utilization. In Indonesia, Diatin et al., (2020) employed *Gracilaria* sp. in combination with milkfish (*Chanos chanos*) and black tiger shrimp (*Penaeus monodon*) to develop a successful and economically beneficial polyculture arrangement. In an experiment, Nguyen et al., (2019) introduced red seaweed (*Gracilaria tenuistipitata*) during the nursery phase of white leg shrimp (*Litopenaeus vannamei*) to investigate the impact on water quality and the performance of the shrimp. On the other hand, Luis et al., (2018) implemented *Gracilaria birdiae* in a similar manner for the white leg shrimp. In a recent study, Naskar et al., (2023) conducted an integration experiment involving the green seaweed (*Enteromorpha intestinalis*) and the Pacific white-leg shrimp (*Penaeus vannamei*) to assess the impact on various growth parameters.

2.6 Culture Systems Employed in Pond-Based Seaweed Cultivation

Various techniques have been employed for cultivating seaweeds within shrimp farming ghers. These methods range from raft-based systems and submerged lines to mesh structures, showcasing the versatility of approaches to integrate seaweed cultivation effectively alongside shrimp farming activities. For instance, Nelson et al. (2001) evaluated a novel method for cultivating the *Gracilaria* seaweed and this technique involves utilizing effluents from shrimp farms as a fertilizing source and employing floating cage culture for the seaweed's growth. This two-step approach has been successfully implemented in Molokai, Hawaii, resulting in the production of high-

quality products suitable for direct human consumption. In the cage-culture system, the average relative growth rates (RGRs) of *Gracilaria parvispora* thalli, that were enriched with effluents, ranged from 8.8% to 10.4% per day. This growth rate increase was significant compared to thalli fertilized using traditional inorganic methods, which exhibited a growth rate of 4.6% per day.

Hendri et al., (2018) used the Floating Net method (MFN) to cultivate *Gracilaria* sp seaweed in shrimp ghers. Results indicated notable growth patterns over a six-week period and the methods (MFN) showed DGR results below 2% per day, indicating favorable conditions for *Gracilaria* sp cultivation. Andayani et al. (2016) also carried out an experiment and the findings revealed that incorporating *Gracilaria verucosa* as a biofilter agent in aquaculture ponds (using bottom methods) can significantly enhance Vannamei shrimp yields. The study yielded 5 tons/ha of seaweed from an initial density of 1 ton/ha within a 3-month cultivation period, displaying a daily growth rate of 4.18%. Vannamei shrimp exhibited growth from 0.5 grams to 16.7 grams per shrimp, elevating production from 1 ton/ha to 3.5 tons/ha. The study conducted in the Minicoy lagoon by Kaladharan et al. (1996) found that floating raft culture methods were suitable and economically viable for large-scale production of *Gracilaria edulis* seaweed. After conducting experimental cultivation of *Gracilaria dura*, Mantri et al. (2017) deduced that raft culture of seaweed offers advantages such as quicker seeding, protection against drifting of seedlings, and the flexibility to adjust planting depth based on location and season.

2.7 Constraints and Limitations of Seaweed Cultivation in Coastal Ghers

Buschmann et al. (2001) highlighted that the success of seaweed cultivation in shrimp ponds largely depends on multiple environmental and management factors such as nutrient availability, water movement, and culture techniques. However, Marinho-Soriano et al. (2002) and Buschmann et al. (2008) reported that, despite increasing scientific knowledge, integrating *Gracilaria* cultivation into pond systems remains challenging both theoretically and operationally. Nauta et al. (2024) mentioned that while old or abandoned ponds demonstrated successful outcomes in seaweed–shrimp co-culture, the suitability of such degraded ponds is not universal, as effective use often

requires site-specific improvements like enhancing water flow and removing accumulated sludge.

Marinho-Soriano et al. (2009) observed that turbidity in shrimp ponds, caused by suspended solids from aquaculture waste, reduces light penetration in the water column, thereby limiting photosynthetic activity and ultimately restricting seaweed growth. Oliveira et al. (2012), while culturing seaweed in ponds, found that surface-grown thalli suffered biomass loss due to dehydration and excessive light exposure. This was attributed to the partial emergence of seaweed above the water surface due to evaporation and wind-driven water level fluctuations. A similar constraint was reported by Msuya and Neori (2002), who noted poor performance of *Ulva reticulata* when cultured in fish ponds, due to exposure-related stress.

Ryder et al. (2004) further emphasized that insufficient water movement significantly reduced the growth rate of *Gracilaria parvispora* in pond environments, suggesting that hydrodynamics play a crucial role in nutrient uptake and thallus expansion. In contrast, Hendri et al. (2018) noted that compared to open water bodies or nearshore environments, the limited water flow in pond systems leads to greater accumulation of sediments and epiphytic organisms on the seaweed thallus, which can obstruct light penetration, hinder photosynthesis, and consequently reduce biomass productivity. Additionally, Yu and Yang (2008) demonstrated that while seaweeds generally respond positively to nitrogen- and phosphorus-rich environments, prolonged exposure to such high nutrient concentrations can surpass their physiological tolerance, ultimately leading to reduced growth performance over time.

2.8 Biochemical Compositions of *Gracilaria* sp.

Seaweed's chemical composition varies depending on the species, cultivation techniques, environmental factors, geographic location, season, and the availability of various nutrients (Marinho-Soriano and Bourret, 2005; Kumar et al., 2008). Despite having a low-calorie count, seaweeds are considered nutritious since they are full of vital elements like protein, vital nutrients, important amino acids, vitamins, and a few bioactive substances (Debbarama et al., 2016).

2.8.1 Crude Protein Content

Red macroalgae generally exhibit higher protein content than both green and brown macroalgae, making them a particularly valuable source of marine-derived proteins (Castro-Gonzáles et al., 1996; Galland-Irmouli et al., 1999; Dawczynski et al., 2007). Within this group, *Gracilaria* species have shown a wide range of protein concentrations, typically ranging from 5.6% to 30% of dry weight, depending on species, season, and cultivation conditions (McDermid, 2003; Marrion et al., 2005; Renaud and Luong-Van, 2006). For instance, Francavilla et al. (2013) reported that *Gracilaria gracilis* cultivated in the Mediterranean exhibited substantial seasonal variation in protein content, reaching 45% (dry weight) in January, decreasing to around 41% in April and October, and dropping to 31% in July, indicating winter and early spring as the most favorable periods for harvesting protein-rich biomass. Similarly, Sambhwani et al. (2022) found that cultivated *Gracilaria dura* showed significantly higher protein levels in February (22.38% dry weight) than wild populations, and when assessed on a fresh weight basis, the protein content ranged from 0.97 ± 0.02 to 1.20 ± 0.10% in wild samples and from 0.84 ± 0.03 to 1.25 ± 0.08% in cultivated ones, suggesting that both seasonal factors and cultivation methods substantially influence the protein yield of *Gracilaria* species. Aspartic acid, alanine, glutamic acid, and glutamine are among the dominant amino acids contributing to the protein profile of *Gracilaria*. These amino acids tend to accumulate under stress conditions and are also responsible for imparting the characteristic flavor to the algae (Sakthivel and Devi, 2015).

2.8.2 Crude Lipid Content

Although *Gracilaria* generally contains a low overall lipid content (Debbarama et al., 2016), it is notable for possessing docosahexaenoic acid (DHA)—a key omega-3 polyunsaturated fatty acid (PUFA) known for its role in reducing cardiovascular disease risk (Benjama and Masniyom, 2012). Omega-3 PUFAs also exhibit strong antioxidant properties, contributing to cellular membrane stability, tissue regeneration, improved cardiovascular function, and even cancer prevention (Syad et al., 2013). The lipid composition of *Gracilaria* varies notably across developmental stages and cultivation conditions, as demonstrated by Khotimchenko (2006), who found that although the

main lipid classes (glycolipids, phospholipids, and neutral lipids) were present throughout all stages in *Gracilaria*, their proportions shifted significantly, with phospholipids, especially phosphatidylcholine, being most abundant in shoots, glycolipids predominating in juvenile and mature reproductive stages, and mature thalli containing 2–3 times more storage lipids (triacylglycerols) than shoots and young algae, likely due to increased energy demands and cellular restructuring during development. Cultivation condition causes clear differences in the lipid content of *Gracilaria* compared to wild plants, as Sambhwani et al. (2022) found that cultivated samples had a higher lipid percentage (25% dry weight) than wild ones (lipid levels ranged from 0.2 ± 0.02 to 0.27 ± 0.01% in wild samples and 0.21 ± 0.01 to 0.26 ± 0.03% in cultivated samples); additionally, fatty acid analysis revealed that polyunsaturated fatty acids (PUFAs) were 2.71–3.11 times more abundant than saturated fatty acids (SFAs) in wild specimens and 1.92–2.86 times higher in cultivated samples, highlighting that cultivation practices can enhance the lipid yield and nutritional quality of *Gracilaria*.

2.8.3 Crude Fiber Composition

Despite not being classified as nutrients, dietary fibers play a crucial role in maintaining health and are associated with a lower risk of conditions like colon cancer, cardiovascular diseases, diabetes, gallstones, hemorrhoids, and hiatal hernia (Rimm et al., 1996; Leontowicz et al., 2002; Schneeman, 1987; Santoso et al., 2006). *Gracilaria* species are rich in both soluble dietary fiber (SDF) and insoluble dietary fiber (IDF), positioning them as viable alternatives to cereal-based fibers, especially in Western diets (Debbarama et al., 2016). SDF in *Gracilaria*, mainly in the form of sulfated galactans, enhances intestinal viscosity, reduces blood glucose and cholesterol levels, and slows nutrient absorption, while IDF increases fecal bulk and reduces intestinal transit time, thereby supporting gastrointestinal health and reducing the risk of obesity and colorectal cancer (Schneeman, 1987; Wong and Cheung, 2000; Elleuch et al., 2011). Rasyid et al. (2019) conducted a comparative analysis of dietary fiber content across several *Gracilaria* species and found that *G. gracilis* contained 27.48% total dietary fiber, which was higher than that of *G. arcuata* (10.90%), *G. salicornia* (10.4–12.52%), and *G. verrucosa* (8.35%), but slightly lower than *G. cornea* (29.08%) and *G. salicornia* as reported by McDermid et al. (2005) (35.8%). Furthermore, *G. fisheri* and *G. tenuistipitata* from Pattani Bay in Thailand exhibited exceptionally high fiber contents, with SDF ranging from 15.6–18.8%, IDF from 38.9–45.2%, and total dietary

fiber between 57.5–64.0% of dry weight—values that exceed those found in most terrestrial plant sources such as whole wheat (44.5%), beans (36.5%), and onions (16.9%) (Benjama and Masniyom, 2012; Lahaye, 1991; Prosky et al., 1992). A near 1:2 ratio of soluble to insoluble dietary fiber in these species indicates a well-balanced fiber profile, which is considered optimal for supporting digestive health, regulating metabolism, and maximizing the overall health benefits of dietary fiber intake (Figuerola et al., 2005).

2.9 Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

(GC/MS) combines two analytical tools, the separation power of gas chromatography with the detection capabilities of mass spectrometry, and is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and for the determination of thermodynamic properties (Marston, 2007; Al-Rubaye et al., 2017). The notable work was done by Izzreen and Ratnam (2011), who applied SPME-GCMS to profile volatile compounds in *Kappaphycus alvarezii*, *Caulerpa lentillifera*, and *Sargassum polycystum*, successfully identifying 223 volatile compounds and highlighting hydrocarbons as the dominant group. Later, Deepak et al. (2017) used GC-MS and FTIR to analyze brown seaweeds *Sargassum wightii* and *Stoechospermum marginatum*, detecting key compounds such as hexadecenoic acid methyl ester, which were linked to antioxidant, antibacterial, and antidiabetic properties. In a comparative phytochemical study, Arulkumar et al. (2018) investigated *Gracilaria corticata* and *G. edulis*, identifying eugenol, phthalic acid, and other phenolic compounds using GC-MS, which contributed to the seaweeds' antioxidant and antibacterial activities. That same year, Deepak et al. (2018) again explored bioactive metabolites in *S. wightii*, *C. racemosa*, and *A. spicifera*, finding heptadecanoic acid in ethyl acetate extracts, which may explain their strong enzyme-inhibitory (antidiabetic) and antioxidant effects. In Egypt, Hassan and Shobier (2018) applied GC-MS to characterize seaweed extracts from *Cystoseira sinuosa* and *C. officinalis*, revealing compounds like eicosanol and nonadecane with effective antibacterial effects. Focusing specifically on *G. corticata*, Ragunathan et al. (2019) conducted a GC-MS analysis that identified a high abundance of fatty acids, especially n-hexadecanoic acid (74.2%), which supported the alga's potential for antimicrobial and antioxidant applications. More recently, Palaniyappan et al. (2023) profiled methanolic extracts of *Caulerpa racemosa* using GC-MS and identified compounds like phthalic acid and 3-hexadecene, which corresponded to

significant antioxidant and antibacterial properties against aquatic pathogens. Finally, a significant advancement came from Bajwa et al. (2024), who introduced a methylation-GC-MS-based linkage analysis to study unfractionated polysaccharides in brown seaweeds, revealing diverse glycosidic linkages and improving the structural understanding of fucose-containing sulfated polysaccharides and alginates.

2.10 Minerals and Heavy Metal Contents and Food Safety Concerns of *Gracilaria* sp.

The mineral composition of seaweed is heavily influenced by environmental conditions such as salinity, water movement, nutrient availability, and anthropogenic inputs. Similarly, the accumulation of heavy metals within the cellular structure of algae is influenced by a range of factors, including species, age, and physiological state, alongside environmental aspects like pollution level, salinity, pH, temperature, and seasonal changes (Kaimoussi et al., 2004). Rapid urbanization and industrialization have significantly increased heavy metal contamination in aquatic systems, primarily due to anthropogenic sources (such as mining, petrochemical processing, electronics manufacturing, and municipal waste discharge), and then these toxic metals can enter marine environments directly or through estuarine transport, where they are readily absorbed by seaweeds (Saraee et al., 2011; Santawamaitre et al., 2011; Wang et al., 2013).

Seaweeds are known to contain higher mineral content than land plants and animal products (Ito & Hori, 1989; Ortega-Calvo et al., 1993), a fact further supported by Rupérez (2002), who analyzed several edible brown (*Fucus vesiculosus*, *Laminaria digitata*, *Undaria pinnatifida*) and red (*Chondrus crispus*, *Porphyra tenera*) seaweeds, revealing through atomic absorption spectrophotometry that these species possessed significantly higher levels of macrominerals (Na, K, Ca, Mg: 8,083–17,875 mg/100 g) and trace elements (Fe, Zn, Mn, Cu: 5.1–15.2 mg/100 g) compared to commonly consumed land plants. To expand this understanding, Premarathna et al. (2022) highlighted the species-specific mineral distribution among seaweeds, reporting that Cu, Zn, and Co were more concentrated in green algae, Mg and Sr in brown algae, and Na in red algae, indicating that mineral accumulation patterns vary distinctly across algal groups.

Since the onset of commercial seaweed farming, several studies across different regions have compared wild and cultivated seaweeds to assess variations in mineral and heavy metal content. In a comparative study across the NE Atlantic, Roleda et al. (2019) found that although *Saccharina latissima*, *Alaria esculenta*, and *Palmaria palmata* contained species-specific levels of polyphenols and heavy metals (As, Cd, Hg, Pb), the concentrations remained below European food safety limits in both wild and cultivated samples, with only minor seasonal fluctuations. Similarly, Cardoso et al. (2023) evaluated wild and farmed *Saccorhiza polyschides* and observed that while wild samples had slightly higher nutritional value and a better ω3/ω6 fatty acid ratio, cultivated forms showed higher selenium content; however, both sources (wild and farmed seaweeds) exhibited considerable iodine and arsenic bioaccessibility, suggesting the need for consumption monitoring to avoid excessive intake and potential health risks. Several studies have also explored how seaweeds cultivated in shrimp and fish ponds, accumulate heavy metals. In Indonesia, Perryman et al. (2017) examined *Caulerpa* species cultivated in traditional coastal ponds and found that while most metal levels were within national food safety limits, arsenic (0.7 mg/kg) and lead (0.35 mg/kg) were close to the maximum allowable levels. These variations were linked to site-specific factors, particularly acid sulfate soils (ASS), highlighting the importance of considering pond soil conditions when planning seaweed cultivation. Similarly, Gosavi et al. (2004) found that macroalgae like *Ulva*, *Enteromorpha*, and *Chaetomorpha* grown in shrimp ponds with acid sulfate soils (ASS) accumulated high levels of metals such as Fe, Al, Zn, Cu, Cd, As, and Pb, indicating that maintaining pond pH above 5 is not enough to prevent metal uptake, as dissolved metals remain available for absorption even under standard management conditions.

In Bangladesh context, Bhuyan et al. (2024) analyzed *Hypnea musciformes* and *Gracilaria lemaneiformis* from the Cox's Bazar coast of Bangladesh and reported that mineral and heavy metal concentrations, particularly Mg, Fe, Zn, Cu, and Ni in *H. musciformes*, and Ca, Pb, and Cr in *G. lemaneiformis*, varied by species but did not exceed health risk thresholds. To better understand how cultivation depth and site conditions affect seaweed quality, Banik et al. (2023) and Chamily et al. (2025) investigated nearshore seaweed farming along the southeast coast of Bangladesh. Banik et al. (2023) cultivated *Gracilaria sp.* using three different farming systems and found that seaweeds grown in floating long-line systems near the surface accumulated

significantly lower levels of heavy metals (Cr, Cd, Pb, As) and higher levels of essential minerals (Ca, K, Zn) compared to those grown in off-bottom systems. Similarly, Chamily et al. (2025) observed that *Ulva lactuca* cultured in surface-floating setups contained elevated Na and K but reduced Pb, Cr, Co, and Fe, reinforcing that both the vertical position in the water column and local site conditions can strongly influence mineral uptake and heavy metal accumulation in cultivated seaweeds. While such insights are available for nearshore environments, similar evaluations focusing on pond-based seaweed cultivation in Bangladesh, particularly regarding mineral and heavy metal composition, are yet to be explored in detail, highlighting an important area for future research.

CHAPTER III

MATERIALS AND METHODS

3.1 Study Area

The experiment was carried out between October 2023 to March 2024 at three separate coastal locations, along the southeastern coast of Cox's Bazar, Bangladesh, to compare the growth performance and nutritional composition of *Gracilaria* sp. across different cultivation environments (pond vs nearshore) and cultivation layers (surface vs bottom). For pond-based seaweed cultivation, three shrimp farming ghers were selected in Sonapara (21°17'22.2"N, 92°03'48.7"E) region due to its ideal water depth and clear water conditions suitable for *Gracilaria* sp. cultivation. For nearshore based seaweed cultivation, Khurushkhul (21º 30' 40.13" N, 91º 59' 53.3" E) and Nuniachora (21º 28' 26.6" N, 91º 57' 56.4" E) were selected for their favorable water quality parameters (Zafar, 2005) and the presence of extensive tidal flats with sandy subtrates, which are well-suited for employing both floating (floating long-line) and semi-floating (off-bottom long-lines and off-bottom net) culture systems (Banik et al., 2023; Mohiuddin et al., 2024).

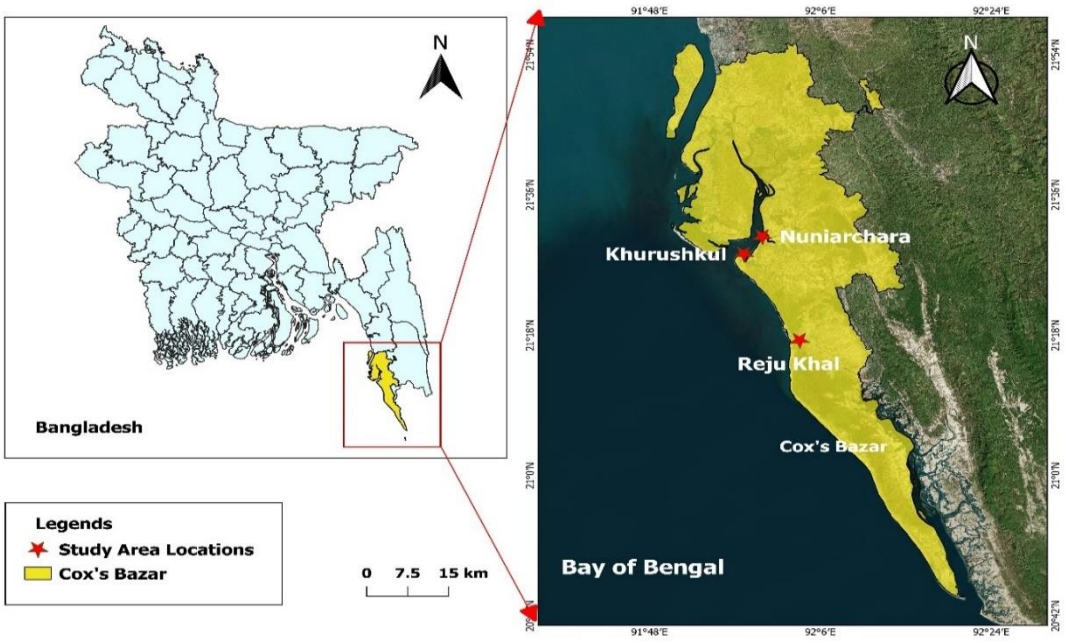

Figure 2. The map showing the locations of different cultivation systems of red seaweed (*Gracilaria* sp.) along the southeastern coast of the Bay of Bengal, Bangladesh, with Khurushkhul and Nuniachora representing nearshore cultivation sites and Sonapara representing the pond-based seaweed cultivation site in the Cox's Bazar district.

3.2 Construction and Installation of Seaweed Culture Systems in Shrimp Gher/Pond

In this study, three distinct seaweed farming methods were implemented in shrimp farming gher: floating long-line, floating net, and broadcast methods. Total 9 seaweed culture plots (3 floating long-line, 3 floating net, and 3 broadcast systems) were established in 3 different shrimp farming pond (gher), and each shrimp farming pond (gher) contained one culture plot from each farming method- resulting in three different culture plots per pond. The floating long-line and floating net systems were installed at the water surface, whereas the broadcast method was applied to the bottom of the ponds. No fertilizers, insecticides, or pesticides were applied at any stage of the seaweed cultivation process.

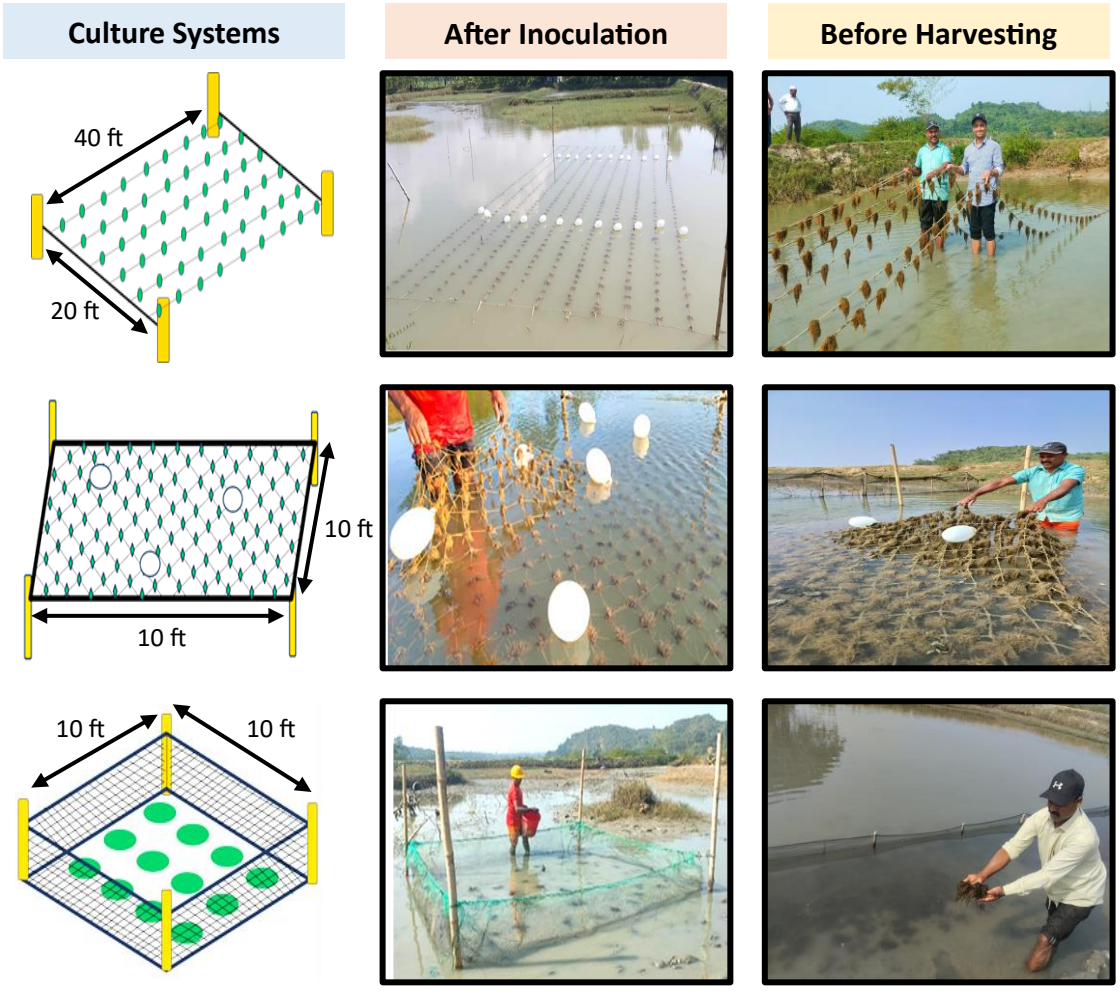

Figure 3: Layout and photographic visualization of various cultivation systems of red seaweed (*Gracilaria* sp.) after the first inoculation and before the harvesting period in shrimp farming pond (ghers). (A-B) Floating Long Line System; (C-D) Floating Net System; (E-F) Broadcast System.

3.2.1 Installation of Floating Long Line Culture System in Shrimp Pond

To set up a floating long-line culture plot, at first 2 bamboo poles were posted 20 feet apart in parallel position into the bottom of the shrimp farming pond. Another two bamboo poles were posted on the opposite side at a 40 feet distance, forming a rectangular area of 800 square feet (40 ft × 20 ft) for each plot. The bamboo poles on both sides were then connected horizontally with nylon ropes to create the main support line. Total ten seaweed cultivation ropes were hung vertically from these horizontal lines at 2 feet intervals. Two plastic floats were attached to each cultivation rope to ensure continuous flotation in the water.

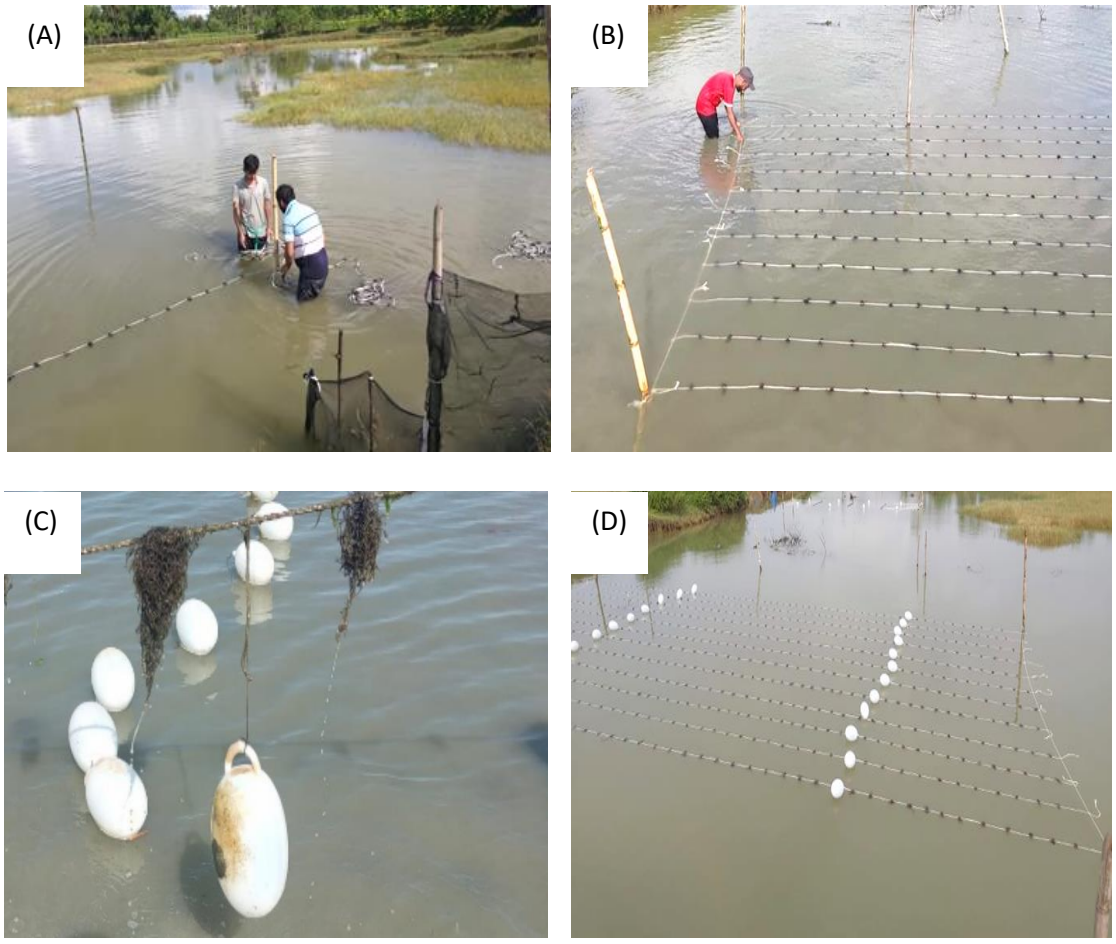

Figure 4: Steps in the Formation of a Floating Long-Line Culture Plot in shrimp farming pond in the Sonarpara region. (A) Attachment of cultivation ropes to the main horizontal support line. (B) Maintenance of adequate spacing between individual cultivation ropes. (C) Attachment of buoyancy floats to each cultivation rope to ensure flotation. (D) Fully established floating long-line seaweed culture plot.

3.2.2 Installation of Floating Net Culture System in Shrimp Pond

For the installation of a floating net culture plot, a nylon net measuring 10ft × 10ft with a mesh size of 15 cm was used. The net was firmly tied to bamboo poles that were placed vertically into the pond bottom at all four corners, ensuring stable positioning within the pond. To maintain consistent buoyancy and keep the net afloat at the water surface, approximately eight plastic floats were evenly distributed and attached along the central area of the net.

Figure 5: Steps in the Establishment of a Floating Net Culture Plot in shrimp farming pond in the Sonarpara region. (A) Shaping the cultivation net into the desired rectangular form. (B) Post-inoculation stage showing distributed seaweed on the net. (C) Attachment of the cultivation net to the bamboo poles for structural support. (D) Fully established floating net seaweed culture plot.

3.2.3 Installation of Broadcast Culture System in Shrimp Pond

For the installation of a broadcast culture plot, initially six bamboo poles measuring 3 feet in length were prepared and inserted vertically into the pond bottom to form a square framework with dimensions of 10 × 10 feet. A fine-meshed net, with a mesh size of 1 cm, was then placed around the perimeter of the square to prevent the cultured seaweed from dispersing outside the designated plot area during the cultivation period.

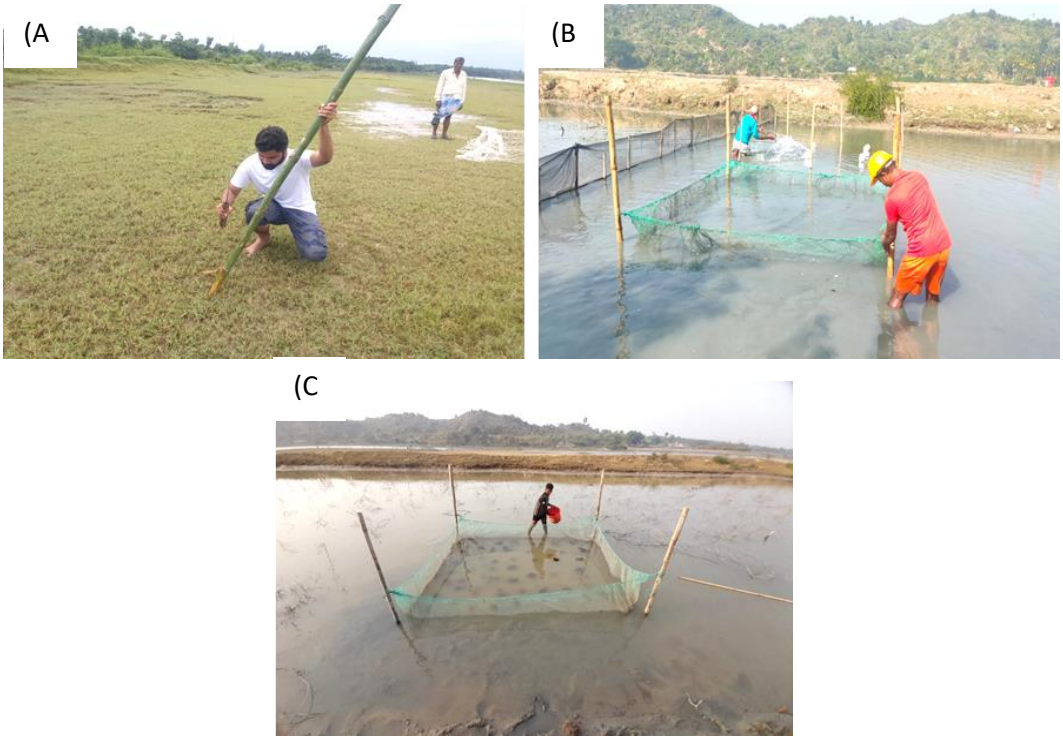

Figure 6: Steps in the Establishment of a Broadcast Culture Plot in shrimp farming pond in the Sonarpara region. (A) Preparation and placement of bamboo poles for anchoring the culture structure. (B) Enclosure of the defined square perimeter using a fine-meshed net. (C) Fully established broadcast seaweed culture plot.

3.3 Construction and Installation of Seaweed Culture Systems in Nearshore Environments

Nearshore-based seaweed cultivation was also carried out using three distinct farming techniques: off-bottom net, off-bottom long-line, and floating long-line systems. The off-bottom net and off-bottom long-line systems were deployed on sandy bottoms within the intertidal zone, while the floating long-line system was positioned in the

subtidal region at an average depth of 3 meters (Figure 7). The semi-floating off-bottom culture systems remained partially underwater during high tide and settled on the exposed sandy flats when the tide ebbed. In contrast, the raft-based floating long-line system remained submerged throughout both high and low tides. A total of 9 experimental plots were established- specifically the 3 floating long-line plots were installed at the Khurushkhul site, while the 3 off-bottom net and 3 off-bottom long-line plots were positioned in the Nuniarchora site. Similar with the pond-based cultivation approach, no chemical inputs, such as fertilizers, insecticides, or pesticides, were applied at any stage of the farming process.

For the installation of an off-bottom long-line culture plot, 10 bamboo poles were initially installed vertically into the sandy-mud substrate, spaced 50 cm apart in a parallel arrangement. Another set of 10 poles was positioned directly opposite at a distance of 14 meters, forming the 14m × 5m plot layout. Each pole on one side was connected to its counterpart using polypropylene ropes, and each rope was fitted with three plastic floats to ensure buoyancy during high tide conditions. An off-bottom net culture plot was constructed using a nylon rope net measuring 1.8 m × 3.5 m, featuring a 15 cm mesh size. The net was firmly tied to bamboo poles at each of the four corners. To enhance buoyancy, 3 plastic floats were positioned along the central section of the net. To set up a floating long-line culture plot, a rectangular raft frame (measuring 10m × 3m) was built by attaching two horizontal bamboo poles (each 10 meters long) to five vertical bamboo poles (each 3 meters long), forming a strong and stable structure. Beneath the frame, ten recycled plastic drums were affixed to provide continuous buoyancy. Each plot supported six parallel nylon ropes, with three plastic floats attached to each rope to maintain flotation. The entire raft structure was anchored in place using two iron-bar weights, each weighing approximately 25 kg, to ensure stability in the water column.

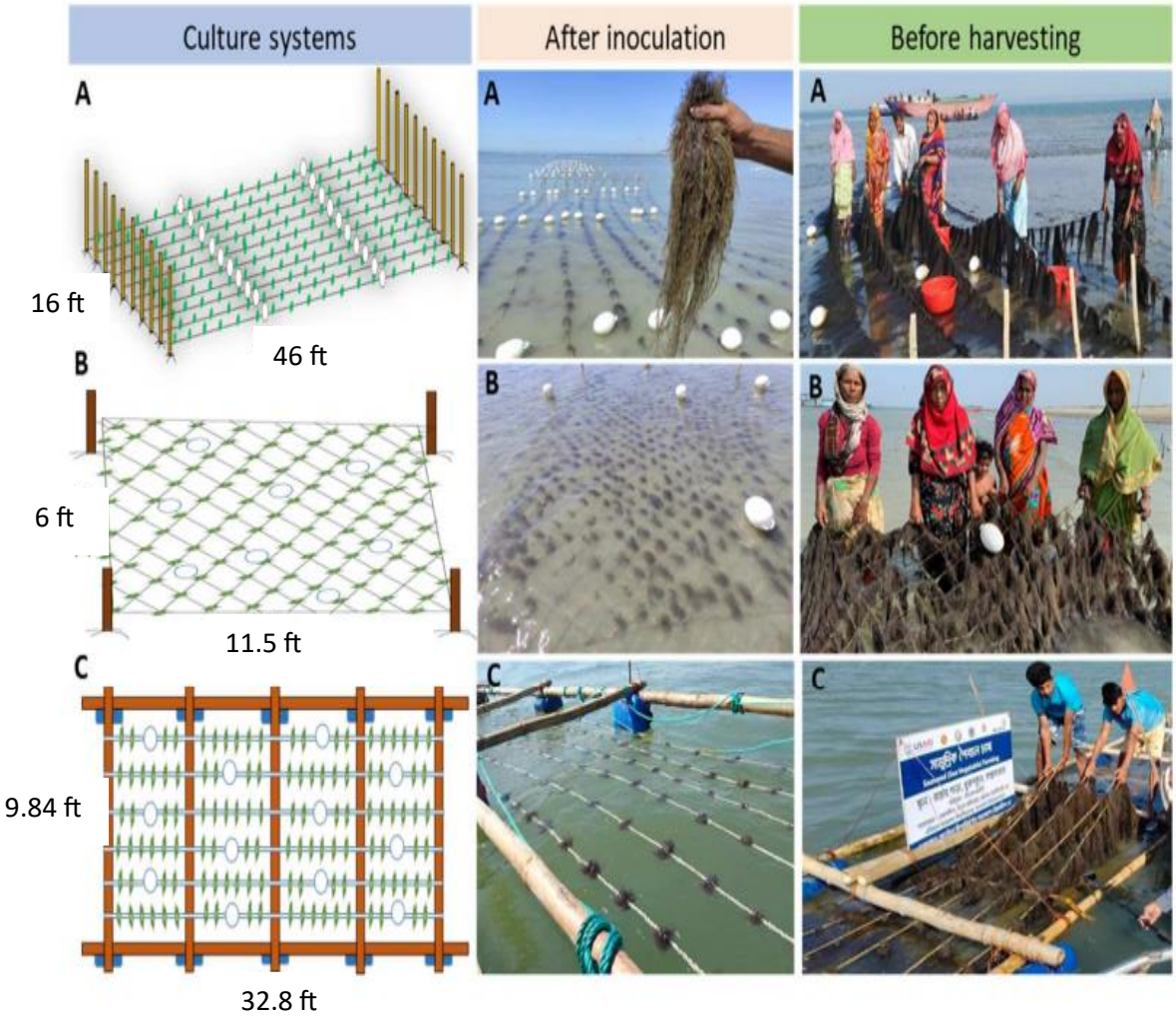

Figure 7: Layout and photographic visualization of various cultivation systems of red seaweed (*Gracilaria* sp.) after the first inoculation and before the harvesting period in nearshore environments. (A) Off-bottom long line system; (B) Off-bottom net system; (C) Floating long-line system.

3.4 Collection of Seed and Inoculation in the Culture Systems

In October, healthy and rapidly growing fragments of *Gracilaria sp.* were gathered from Saint Martin's Island (92º28′40.12″E and 20º65′51.43″N), which is a natural habitat for many seaweed species in Bangladesh. The collection was done about 12 hours before using them as seed for cultivation. Healthy and strong branches were carefully selected from the middle and tips of the plants using sharp stainless-steel knives to ensure smooth, clean cuts that help the seaweed grow better. In pond environments, for the floating long-line method, small pieces of seaweed (called propagules) were placed into holes made by untwisting the ropes. Each rope needed

about 500 g of seed (around 63 propagules, each weighing 8 g), and one culture plot with 10 ropes required about 5 kg of seed. In the floating net method, propagules were tied to each knot of a 10 ft × 10 ft net, using around 2 kg of seed per plot. In the broadcast method, about 2 kg of seed was evenly spread by hand across the pond bottom within a 100 square feet area. For nearshore cultivation, the same seed collection method was followed. In both off-bottom and floating long-line systems, seaweed fragments (about 6 g each) were inserted into the rope holes, with each rope holding 34 propagules (about 200 g). So, a plot with 10 ropes in the off-bottom setup used around 2 kg of seed, while a floating long-line plot with 6 ropes required 1.2 kg. In the off-bottom net method, small pieces of seaweed (5–6 g) were attached to each knot of a 1.8 m × 3.5 m net, and each net plot required about 6.2 kg of seed. These different techniques helped ensure proper and consistent planting in both pond and nearshore areas.

Figure 8: Comparative Overview of Seed Inoculation Techniques Across Seaweed Culture Systems. (A–B) Seed inoculation process in the floating long-line system. (C–D) Seed inoculation process in the floating net system. (E–F) Seed inoculation process in the broadcast system.

3.5 Water Quality Monitoring

In both cultivation environments, water quality parameters were recorded separately for each culture method to capture system-specific variations. Both physical and chemical variables (water quality parameters) were observed and recorded from each culture plot at the experimental site on monthly basis. The physical characteristics encompass water temperature, pH level, dissolved oxygen concentration, salinity, and turbidity. The water temperature was measured using a Celsius thermometer, pH levels were recorded using a digital pH meter (EcoSence pH10A by YSI), dissolved oxygen levels were monitored using a digital DO meter (Lutron PDO-519), salinity was measured using a Bellingham and Stanley E-Line Refractometer (Xylen), and turbidity was recorded using a digital turbidity meter (Turb430 IR by WTW). For chemical analysis, water samples were taken from each culture plot to measure nutrient concentrations, including phosphate (PO_4–P), nitrate (NO_3–N), nitrite (NO_2–N), and ammonia (NH_3–N). These parameters were determined using a portable photometer (pHotoFlex STD, WTW).

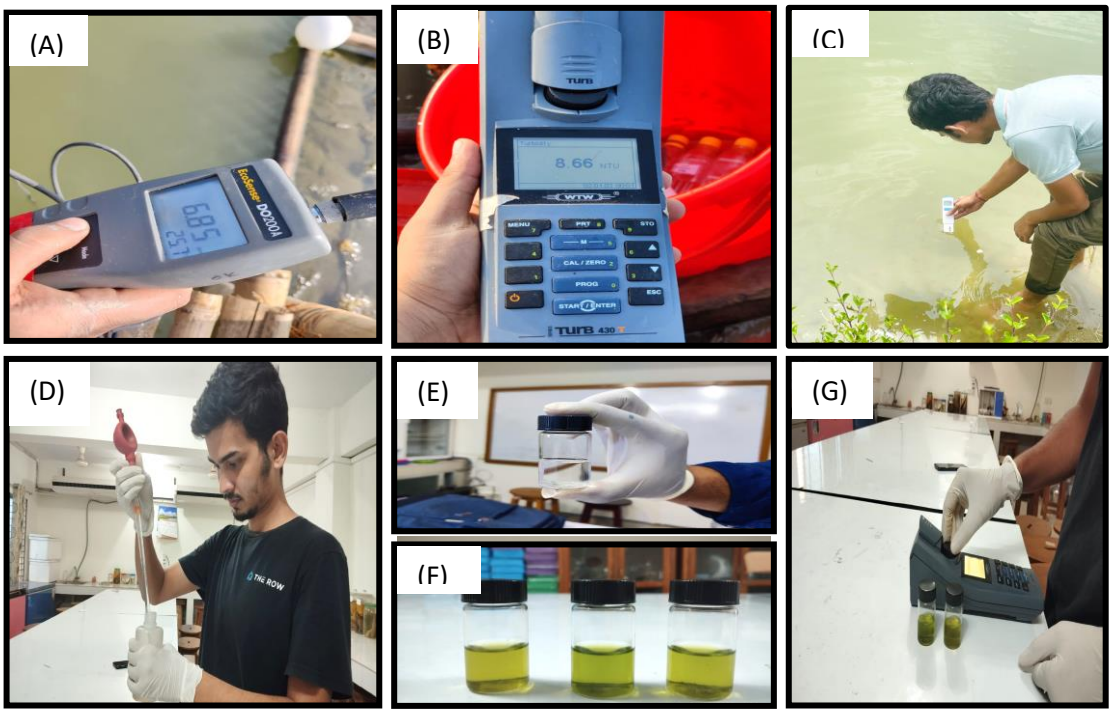

Figure 9: Assessment of Water Quality Parameters (physical and chemical) at the Experimental Site and in the CVASU Oceanography Laboratory. (A–C) On-site measurement of physical parameters (temperature, salinity, DO, and pH). (D–G) Determination of chemical parameters (nitrite, nitrate, ammonia, and phosphate) in the laboratory.

3.6 Harvesting Strategies of Seaweed

Partial harvesting was conducted at regular 30-day intervals throughout the cultivation period (October to March) across all culture methods in both pond and nearshore environments. In the long-line and net systems, seaweed fronds were trimmed during each harvest, leaving approximately 6 cm of the basal portion attached to the cultivation rope for further growth, while the remaining upper portions were harvested. For the broadcast method, about 2 kg of seaweed was retained within the defined plot during each harvest to support further growth, and the excess biomass was removed. Freshly harvested seaweed from each plot was collected separately, weighed using a digital balance to assess growth performance, and subsequently processed according to the respective culture method for biochemical analysis.

Figure 10: Photographic representation of *Gracilaria* sp. harvesting by participating coastal farmers from pond-based culture systems in the Sonapara coastal region, Bangladesh. (A–C) Harvesting of *Gracilaria* sp. from the floating long-line system. (D–E) Harvesting from the floating net system. (G–J) Harvesting from the broadcast culture system.

3.7 Growth Performance Estimation

The daily growth rate, specific growth rate, and the amount of biomass yielded for *Gracilaria* sp. seaweed were calculated every month after the harvesting. These growth factors were calculated separately for each culture system based on the total amount of seaweed produced in each plot.

Daily Growth Rate (DGR, % day^{-1}) was calculated by following the formula of Hung et al., (2009).

$$DGR = [(W_t-W_0)^{1/t} -1] \times 100\% \text{ day}^{-1}$$

Where,

W_0 = Initial wet weight,

W_t = Final wet weight after day (t),

t = Duration of culture in days.

Specific Growth Rate (SGR, % day^{-1}) was calculated by following the formula of Bhatia et al., (2015).

$$SGR = [(\ln W_t - \ln W_0)/t] \times 100 \text{ \% day}^{-1}$$

Where,

W_0 = Initial wet weight,

W_t = Final wet weight after day (t),

t = Duration of culture in days.

Net Weight Biomass (NWB, kg/m^2) of seaweed was calculated using the formula of Doty, (1986).

$$NWB = (W_t - W_0)/A$$

Where,

W_0 = Initial weight,

W_t = Weight at day (t),

A = Area of a culture plot (m^2).

3.8 Sample Preparation for Biochemical Analysis

During the peak growth period (December to February), approximately 1 kg of fresh *Gracilaria sp.* was manually harvested from each culture plot by using a knife to cut it. These collected seaweeds were thoroughly rinsed with seawater two to three times to eliminate any biofouling organisms and surface impurities. In the laboratory, the samples underwent an additional rinsing with clean water and then air-dried for three days. Afterward, they were freeze-dried at -20ºC until a constant weight was achieved. The dried material was then ground into a fine powder using a blender (Kiam, BL-100, 750 watts, Bangladesh). Powdered samples from three replicate plots within each culture method were pooled to form a representative replicated sample. Ultimately, three replicated samples were prepared per culture system for subsequent biochemical analyses.

3.9 Proximate Composition Analysis

The proximate composition analysis was carried out at the Nutrition and Processing Laboratory, Faculty of Fisheries, CVASU. The protein, lipid, fiber, and ash contents of *Gracilaria* sp. were measured separately for samples taken from each culture system in both pond and nearshore conditions.

3.9.1 Protein Analysis

The protein content of *Gracilaria sp.* was measured using the Kjeldahl method as described by Guebel et al. (1991). For this analysis, 0.5 g of dried and powdered seaweed was placed into a digestion tube along with 10 ml of concentrated sulfuric acid (H_2SO_4) and 4 g of a digestion catalyst. The mixture was then heated in a digestion unit at 420°C for 45 minutes. After allowing the tube to cool for about 30 minutes, 50 ml of distilled water was added to the digested mixture. In the distillation step, 20 ml of a mixed indicator solution was added to the conical flask. Finally, the amount of nitrogen in the sample was determined by titrating the solution with 0.2N hydrochloric acid (HCl). The crude protein content was then calculated by converting the measured nitrogen value using a standard factor of 5.85 (AOAC, 2000). The percentages of nitrogen and protein were calculated using the following formulas-

$$\% \text{ N} = [(\text{ml of HCl} \times 0.02 \times 0.014)/\text{sample weight}] \times 100$$

$$\% \text{ of Protein} = \% \text{ N} \times 5.85$$

3.9.2 Lipid Analysis

The lipid content was measured using the Soxhlet extraction technique in accordance with the AOAC (2005) guidelines. First, clean empty beakers were sterilized in a hot air oven. After cooling, each beaker was labeled and weighed. Then, 2 grams of powdered sample from each replicate were wrapped in three separate thimble papers. These thimbles were placed beneath the magnetic holder inside the Soxhlet apparatus. Next, 70 ml of diethyl ether was poured into each labeled beaker. The stopcock was opened in an upright position, and the beakers were secured under the condenser containing the solvent. The thimbles were then lowered into the beakers, and the entire setup was placed on a burner. The machine was turned on and heated until the temperature reached 100°C, allowing the extraction process to continue for 20 minutes. After that, the thimbles were lifted, and the reflux stopcock was closed after waiting for another 20 minutes. An additional 10–15 minutes were allowed for the solvent to fully evaporate. Finally, the beakers were then transferred to a hot air oven and heated at 105°C for 30 minutes. Once cooled, the beakers were weighed again. The total lipid content (%) was calculated using the following formula-

$$\% \text{ of lipid} = (\text{weight of lipid}/\text{weight of sample}) \times 100$$

3.9.3 Fiber Analysis

Crude fiber content was measured following the AOAC (2000) guidelines, using a raw fiber extraction unit (Model: FIWE3, VELP Scientifica). To begin, 1 gram of dried sample was placed in a crucible, labeled as F_0, and mounted onto the fiber analyzer. The sample was first boiled in 150 ml of 1.25% sulfuric acid (H_2SO_4) for 30 minutes. After cooling, it was rinsed 3 times with hot distilled water. Then, it was boiled again in 150 ml of 1.25% sodium hydroxide (NaOH) for another 30 minutes. This step was followed by three more washes with hot distilled water and one final rinse with normal distilled water. Next, the samples were washed three times with 25 ml of acetone to remove any remaining impurities. After this, all the crucibles were placed in a hot air oven at 105°C for one hour. Once cooled, they were weighed again and recorded as F_1. Finally, the crucibles were subsequently placed in a muffle furnace and incinerated at 550°C for a duration of 3 hours. After cooling, the final weight (F_2) was taken. The percentage of crude fiber was then calculated using a standard formula-

$$\% \text{ of Fibre} = [(F_1\text{-}F_2)/F0] \times 100$$

3.9.4 Ash Content Analysis

Ash content in *Gracilaria* sp. was measured using the incineration method as described by AOAC (2000). First, clean empty crucibles were sterilized in a hot air oven and then weighed. After cooling, about 3 grams of dried and finely ground sample was placed into each crucible. The crucibles were then placed in a muffle furnace and heated at 550°C for 5 hours, which burned off all organic matter, leaving only the mineral ash. After incineration, the crucibles were transferred to a desiccator to cool down. Once cooled, the crucibles were weighed again. Subsequently, ash content was computed using the provided formula.

$$\% \text{ of Ash} = (\text{Ash weight} / \text{ sample weight}) \times 100$$

3.10 GC-MS Analysis

Gas Chromatography–Mass Spectrometry (GC-MS) analysis was conducted using a Perkin Elmer Turbo Mass Spectrophotometer integrated with an Auto Sampler XLGC. Separation of compounds was achieved using a Perkin Elmer Elite-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, 95% dimethyl polysiloxane). High-purity helium was employed as the carrier gas at a constant flow rate of 0.5 ml/min. A 1 µl sample was introduced in split mode, with the injector temperature maintained at 250°C. The oven temperature was initially held at 110°C for 4 minutes, then gradually increased to 240°C, and finally raised to 280°C at a rate of 20°C per minute, where it was held for 5 minutes. The total runtime was 90 minutes. The MS transfer line and ion source temperatures were maintained at 200°C and 180°C, respectively. Electron impact ionization was carried out at 70 eV. Data were recorded as total ion chromatograms (TIC) for identifying and quantifying compounds. The resulting mass spectra were compared with a reference library of known compounds. From the matches, the name, molecular weight, and chemical structure of each compound in the sample were determined. Peak area measurement and data processing were done using Turbo-Mass OCPTVS-Demo SPL software.

3.11 Amino Acids Analysis

The amino acid composition of *Gracilaria sp.* from each culture system was analyzed using the AccQ-Tag Amino Acid Analysis method at the Centre for Sophisticated Instrumentation and Research Laboratory, JUST. For acid hydrolysis, 2 grams of a

combined seaweed sample were mixed with 20 ml of 6N hydrochloric acid (HCl) in a sealed test tube. The tube was shaken for 20 minutes and then placed in an oven at 105°C for 24 hours. After hydrolysis, 20 ml of deionized water was added, and the solution was filtered first through Whatman No. 1 filter paper and then through a 0.2 µm nylon cellulose acetate membrane filter. The filtered sample was neutralized using 0.1N sodium hydroxide (NaOH) or ammonium hydroxide (NH_3OH). For tryptophan analysis, alkaline hydrolysis was carried out using 20 ml of 4.3N lithium hydroxide (LiOH), and the sample was heated in an oven at 160°C for 16 hours. After that, 9 ml of 6N HCl and deionized water were added to bring the final volume to 100 ml. The pH of the solution was adjusted to 4.5 using HCl, and it was filtered again through Whatman No. 1 paper and a 0.2 µm nylon membrane filter. The hydrolyzed samples were then derivatized using an AccQ-Fluor reagent kit (WAT052875, Waters). During the HPLC (High-Performance Liquid Chromatography) run, acid-hydrolyzed samples were analyzed at a column temperature of 37°C, while alkaline-hydrolyzed samples were analyzed at room temperature. A 10 µl portion of each derivatized sample was injected into the HPLC system (e2695, Waters) equipped with a C18 reverse-phase column (3.9 × 150 mm), a fluorescence detector (2475, Waters), and run for 60 minutes with a flow rate of 1 ml/min. Identification and quantification of amino acids were based on comparison of retention times and peak areas with a known standard mixture (WAT088122, Waters). UV detection was used at 248 nm for identifying peaks, while the fluorescence detector was set to an excitation wavelength of 250 nm and an emission wavelength of 395 nm for quantification.

3.12 Antioxidant Content Analysis

The antioxidant content of *Gracilaria* sp. was evaluated through the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. In this assay, 100 µL of the seaweed extract was mixed with 900 µL of ethanol and vortexed thoroughly. This solution was then blended with 6 mL of ethanol and 2 mL of a freshly prepared DPPH solution (0.1 mM in ethanol). To avoid degradation by light, the reaction mixture was incubated at room temperature in the dark for 30 minutes. Following incubation, absorbance was recorded at 517 nm using a UV-Vis spectrophotometer. A reduction in absorbance signified the ability of the extract's antioxidants to neutralize DPPH radicals. The free radical scavenging activity was then calculated and presented as the percentage of DPPH inhibition, indicating the sample's antioxidant capacity.

3.13 Flavonoid Content Analysis

The total flavonoid content (TFC) in *Gracilaria* sp. was measured using a modified aluminum chloride ($AlCl_3$) colorimetric method. For extract preparation, 1 g of dried seaweed was soaked in 10 mL of ethanol and kept in the dark for 72 hours to facilitate effective extraction of flavonoid compounds. The extract was then filtered and used for the assay. To assess TFC, 500 µL of the filtered extract was sequentially combined with 5 mL of ethanol, 100 µL of 10% aluminum chloride, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water, with gentle vortexing after each addition. The mixture was incubated at room temperature in the dark for 30 minutes. After incubation, absorbance was recorded at 415 nm using a UV-Vis spectrophotometer. Flavonoid content was quantified as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW), using a standard calibration curve generated from quercetin.

3.14 Minerals and Heavy Metal Analysis

The levels of thirteen essential minerals (Na, P, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Se, and Rb) and four heavy metals (Cr, Hg, Pb, and Cd) in the seaweed samples were determined using flame atomic absorption spectrophotometry (AAS) following microwave-assisted digestion. For sample preparation, 0.50 g of powdered seaweed was weighed into clean, dry Teflon digestion vessels and treated with 8 mL of nitric acid (HNO_3). Digestion was performed at 200°C for 10 minutes using a microwave digester. After digestion, the solutions were filtered, transferred into 50 mL volumetric flasks, and diluted with distilled water. The clear digests were then placed in falcon tubes for elemental analysis using an atomic absorption spectrophotometer (Model: AA-6300, Shimadzu, Kyoto, Japan) equipped with appropriate hollow cathode lamps for each element. A blank sample (without seaweed) was also processed to ensure measurement accuracy. Element concentrations were quantified using calibration curves with correlation coefficients (r^2) greater than 0.998. Final results were expressed in milligrams per kilogram (mg/kg) on a dry weight basis, and quality assurance/quality control (QA/QC) protocols were strictly followed to ensure data reliability.

3.15 Statistical Analysis

The statistical analyses were carried out using SPSS (version 26) and R software (version 4.0.5). Before analysis, percentage data were transformed using the arcsine square root method to meet statistical assumptions. The Shapiro-Wilk test was used to check whether the data followed a normal distribution, and Levene's test (via the 'onewaytests' package; Dag et al., 2018) was used to ensure that the variances across groups were equal. One-way ANOVA was performed using both SPSS and the "car" package in R (Fox and Weisberg, 2011) to compare differences among the different culture systems. When significant differences were found, Tukey's post hoc test was applied using SPSS and the "multcomp" package in R (Hothorn et al., 2008) to identify which groups differed from each other. A 95% confidence level was used for determining statistical significance. Additionally, the "PerformanceAnalytics" package in R (Peterson and Carl, 2020) was used to explore and visualize the relationships between variables.

CHAPTER IV

RESULTS

4.1 Growth and Production Performance of *Gracilaria* sp.

The variability of growth rate (daily growth rate and specific growth rate) and biomass yield among the three culture systems applied in both pond and nearshore environments is shown by box and whiskers plots (Fig 11). A two-way ANOVA was performed to evaluate the effects of cultivation environment (pond vs. nearshore), cultivation layer (surface vs. bottom), and their interaction on the growth performance of *Gracilaria* sp. The analysis revealed that all the three growth parameters- DGR, SGR, and NWB were significantly influenced by both the cultivation environment and the cultivation layer, with a statistically significant interaction between the two factors. The mean DGR and SGR were substantially higher in the nearshore environment ($13.62 \pm 2.00\%$ day^{-1} and $12.76 \pm 1.77\%$ day^{-1}, respectively) compared to the pond conditions ($2.34 \pm 0.93\%$ day^{-1} and $2.31 \pm 0.91\%$ day^{-1}; $p < 0.001$). Moreover, both parameters were significantly elevated at the surface layer ($8.71 \pm 6.35\%$ day^{-1} for DGR and $8.19 \pm 5.84\%$ day^{-1} for SGR) compare to the bottom layer ($7.26 \pm 5.37\%$ day^{-1} and $6.88 \pm 5.00\%$ day^{-1}; $p < 0.001$). A highly significant interaction effect was observed for DGR ($p < 0.001$), while the interaction for SGR was moderately significant ($p < 0.01$), indicating that the combined influence of environment and depth further modulated growth outcomes. The NWB accumulation was also markedly greater in the nearshore environment (1.47 ± 0.68 kg m^{-2}) compared to pond conditions (0.19 ± 0.09 kg m^{-2}), and higher at the surface layer (1.04 ± 0.98 kg m^{-2}) than the bottom (0.62 ± 0.52 kg m^{-2}), with all effects showing strong statistical significance ($p < 0.001$).

Table 1: Effect of cultivation environment and cultivation layer combinations on growth performance in cultivated seaweed based on two-way ANOVA. Values are expressed as mean ± standard deviation for each treatment group. The statistical significance levels (p) are denoted by asterisks (* < 0.05, ** < 0.01, *** < 0.001).

Growth Parameters	Cultivation Environment (CE)		Cultivation layer (CL)		ANOVA Significance P-value		
	Pond	Nearshore	Surface	Bottom	CE	CL	CE*CL
DGR (%/day)	2.34± 0.93	13.62±2.00	8.71±6.35	7.26±5.37	***	***	***
SGR (%/day)	2.31±0.91	12.76±1.77	8.19±5.84	6.88±5.00	***	***	**
NWB (kg/m^2)	0.19±0.09	1.47±0.68	1.04±0.98	0.62±0.52	***	***	***

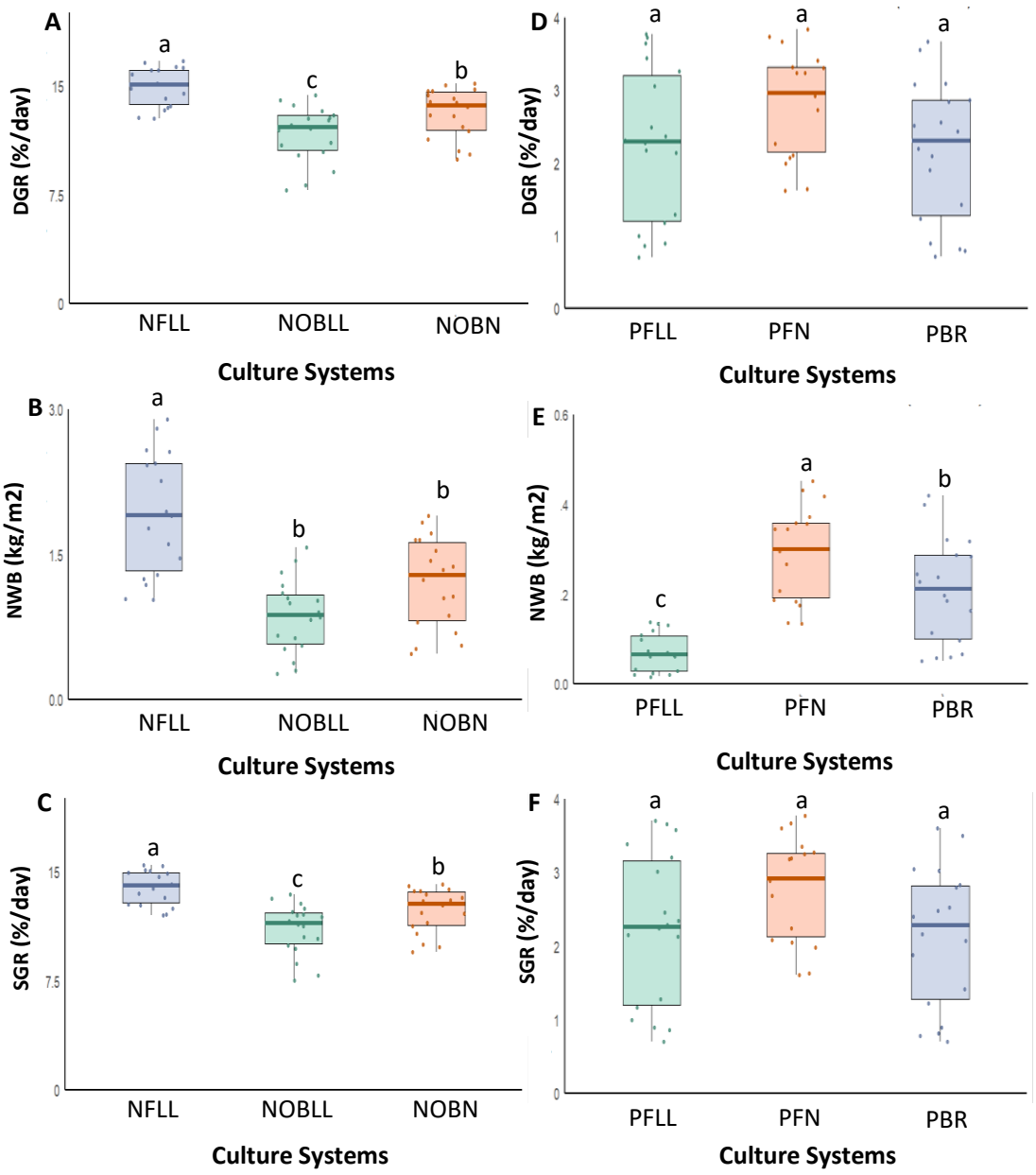

Figure 11: Comparison of growth performance of *Gracilaria* sp. seaweed cultured in shrimp farming ghers and nearshore environments along the southeastern coast of the Bay of Bengal, Bangladesh, in terms of Daily Growth Rate (DGR), Net Weight Biomass (NWB), and Specific Growth Rate (SGR). The Box and Whisker plots illustrate growth performance across three nearshore (A-C) and pond (D-F) based culture methods. Here the variables full forms are: NFLL- nearshore based floating long-line, NOBLL- nearshore based off-bottom long-line, NOBN- nearshore based off-bottom net, PFLL- pond based floating long-line, PFN- pond based floating net, and PBR- pond based broadcast method. Distinct superscript letters indicate statistically significant differences ($p < 0.05$) among culture methods within each environment.

Table 2: Monthly variation in production and growth performance parameters of *Gracilaria* sp. cultivated under different culture systems in shrimp farming ghers along the southeast coast of the Bay of Bengal, Bangladesh. All the values are expressed as mean ± standard deviation (SD) from three replicated culture units for each system: Floating Long Line, Floating Net, and Broadcast. Different superscript letters within a row represent statistically significant differences among months based on Tukey's post hoc test ($p < 0.05$). Here the variables full forms are: PFLL- pond based floating long-line, PFN- pond based floating net, and PBR- pond based broadcast method.

Culture system	Growth Parameter	Month						F value	P value
		Oct	Nov	Dec	Jan	Feb	Mar		
PFLL	SGR	0.95±0.24^{c}	2.28±0.15^{b}	3.65±0.06^{a}	3.20±0.19^{a}	2.25±0.11^{b}	1.01±0.23^{c}	116.917	.000***
	NWB	0.02±0.01^{d}	0.07±0.01^{c}	0.13±0.01^{a}	0.11±0.01^{b}	0.06±0.01^{c}	0.02±0.01^{d}	140.295	.000***
	DGR	0.96±0.24^{c}	2.31±0.16^{b}	3.71±0.07^{a}	3.25±0.19^{a}	2.27±0.12^{b}	1.01±0.24^{c}	118.040	.000***
PFN	SGR	1.97±0.33^{b}	2.93±0.25^{a}	3.54±0.26^{a}	3.43±0.21^{a}	2.99±0.16^{a}	1.89±0.24^{b}	24.532	.000***
	NWB	0.17±0.04^{c}	0.30±0.04^{b}	0.41±0.05^{a}	0.39±0.04^{ab}	0.31±0.03^{ab}	0.17±0.03^{c}	23.230	.000***
	DGR	1.99±0.33^{b}	2.98±0.26^{a}	3.61±0.27^{a}	3.49±0.22^{a}	3.04±0.17^{a}	1.92±0.24^{b}	24.539	.000***
PBR	SGR	0.91±0.28^{c}	2.68±0.30^{ab}	3.38±0.29^{a}	2.67±0.24^{ab}	2.04±0.14^{b}	1.04±0.33^{c}	39.136	.000***
	NWB	0.07±0.02^{c}	0.27±0.04^{b}	0.38±0.05^{a}	0.26±0.03^{b}	0.18±0.02^{b}	0.08±0.03^{c}	34.397	.000***
	DGR	0.91±0.28^{c}	2.71±0.31^{ab}	3.44±0.31^{a}	2.71±0.24^{ab}	2.06±0.15^{b}	1.04±0.34^{c}	39.087	.000***

Table 3: Monthly variation in production and growth performance parameters of *Gracilaria* sp. cultivated under different culture systems in nearshore environments located along the southeast coast of the Bay of Bengal, Bangladesh. All the values are expressed as mean ± standard deviation (SD) from three replicated culture units for each system: Floating Long Line, Floating Net, and Broadcast. Different superscript letters within a row represent statistically significant differences among months based on Tukey's post hoc test ($p < 0.05$). Here the variables full forms are: NFLL- nearshore based floating long-line, NOBLL- nearshore based off-bottom long-line, NOBN- nearshore based off-bottom net.

Culture system	Growth Parameter	Month						F value	P value
		Oct	Nov	Dec	Jan	Feb	Mar		
NFLL	SGR	12.16±0.26^{c}	14.04±0.57^{b}	15.26±0.21^{a}	14.92±0.11^{a}	13.98±0.17^{b}	12.84±0.28^{c}	46.382	.000***
	NWB	1.08±0.08^{c}	1.92±0.35^{b}	2.75±0.17^{a}	2.48±0.08^{a}	1.87±0.09^{b}	1.33±0.11^{c}	43.075	.000***
	DGR	12.93±0.29^{c}	15.08±0.65^{b}	16.49±0.24^{a}	16.09±0.13^{a}	15.01±0.19^{b}	13.70±0.32^{c}	46.352	.000***
NOBLL	SGR	7.99±0.58^{e}	11.03±0.46^{cd}	12.15±0.28^{ab}	13.09±0.30^{a}	11.84±0.26^{bc}	9.99±0.33^{d}	66.342	.000***
	NWB	0.31±0.06^{e}	0.78±0.10^{cd}	1.09±0.09^{b}	1.44±0.13^{a}	0.99±0.07^{bc}	0.57±0.06^{d}	59.897	.000***
	DGR	8.32±0.63^{e}	11.67±0.51^{cd}	12.93±0.32^{ab}	13.98±0.34^{a}	12.56±0.29^{bc}	10.50±0.36^{d}	66.815	.000***
NOBN	SGR	9.74±0.27^{e}	12.64±0.53^{c}	13.77±0.17^{a}	13.69±0.35^{ab}	12.69±0.47^{bc}	11.12±0.39^{d}	49.787	.000***
	NWB	0.52±0.04^{c}	1.24±0.19^{b}	1.73±0.09^{a}	1.69±0.18^{a}	1.26±0.17^{b}	0.78±0.09^{c}	35.272	.000***
	DGR	10.23±0.29^{e}	13.47±0.60^{c}	14.76±0.19^{a}	14.67±0.41^{ab}	13.53±0.53^{bc}	11.76±0.44^{d}	49.284	.000***

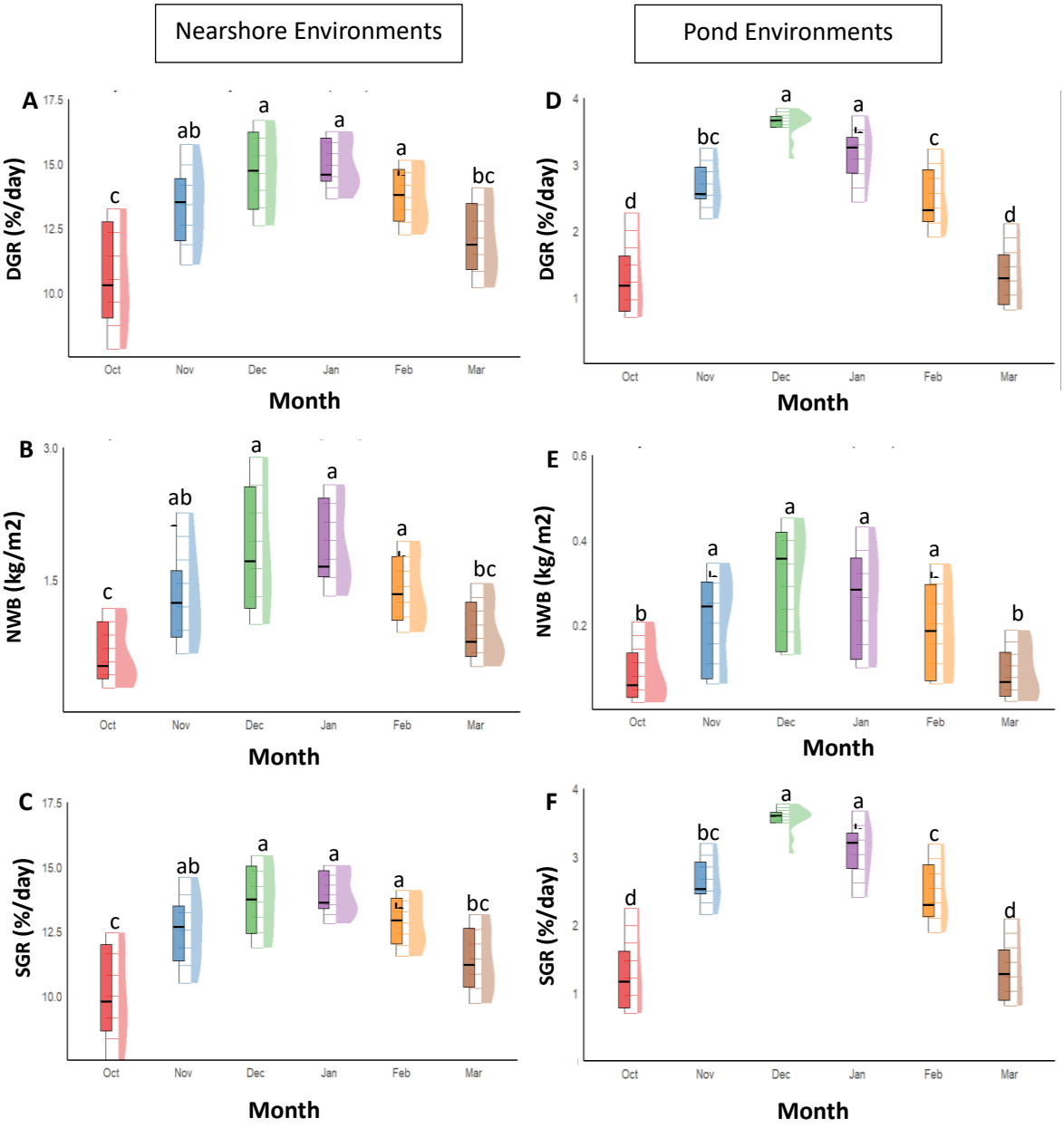

Figure 12: Comparison of monthly production performance in terms of Daily Growth Rate (DGR), Net Weight Biomass (NWB) and Specific Growth Rate (SGR) of *Gracilaria* sp. cultured in shrimp farming ghers and nearshore environments along the southeastern coast of the Bay of Bengal, Bangladesh, from October 2023 to March 2024. The raincloud plots illustrate monthly growth trends across nearshore (A–C) and pond-based (D–F) systems. Different superscript letters indicate statistically significant differences ($p < 0.05$) among culture methods within each environment. Raincloud plots represent an illustration of half-density distribution combined with individual data points. The analysis is based on monthly datasets from 9 seaweed culture plots for each environment (pond vs nearshore).

4.2 Ecological Factor and Growth Performance of *Gracilaria* sp. Seaweed

Variations of water quality parameters across different culture systems established in pond and nearshore environments are presented in Tables 4 and 5. The correlation analysis between ecological factors and the production performance of *Gracilaria* sp. cultured in shrimp farming ghers and nearshore environment (Figure 13 and 14) revealed distinct patterns in each system. In both environments, temperature was negatively correlated with seaweed production parameters, showing moderate negative correlations in the pond system with biomass yield ($r = –0.27$, $p < 0.05$) and growth rate ($r = –0.32$, $p < 0.05$), while in the nearshore system, stronger negative correlations were observed for growth rate ($r = –0.66$, $p < 0.001$) and biomass yield ($r = –0.65$, $p < 0.001$). Turbidity also showed a consistently negative relationship with growth rate in ponds ($r = –0.37$, $p < 0.001$) and with both growth rate ($r = –0.63$, $p < 0.001$) and biomass yield ($r = –0.59$, $p < 0.001$) in nearshore environments. Dissolved oxygen showed a positive correlation with growth rate and biomass in both environments, with stronger associations observed in the pond system (DGR: $r = 0.77$, $p < 0.001$; NWB: $r = 0.43$, $p < 0.01$) compared to moderate correlations in the nearshore system (DGR: $r = 0.43$, $p < 0.001$; biomass: $r = 0.45$, $p < 0.001$). Salinity and pH had no significant influence on pond-based production, whereas in the nearshore system, both were positively correlated with DGR ($r = 0.54$, $p < 0.001$ and $r = 0.31$, $p < 0.001$, respectively) and biomass yield ($r = 0.52$, $p < 0.001$ and $r = 0.25$, $p < 0.001$, respectively).

Among the nutrients, nitrate (NO_3–N) and nitrite (NO_2–N) positively influenced growth in both environments. In ponds, they were significantly correlated with DGR and SGR ($r = 0.30$, $p<0.05$; $r= 0.35$, $p< 0.01$; respectively), but not with NWB, while in the nearshore system, they were positively correlated with both DGR ($r = 0.51$, $p<0.001$; $r= 0.39$, $p< 0.001$; respectively) and biomass yield ($r = 0.47$, $p<0.001$; $r=0.41$, $p<0.001$; respectively). Ammonia (NH_3–N) showed a strong positive correlation with all production metrics in ponds (DGR: $r = 0.78$, $p<0.001$; SGR: $r=0.78$, $p<0.001$; and NWB: $r=0.58$, $p < 0.001$; respectively), but was not significantly correlated in the nearshore environment. In contrast, phosphate (PO_4–P) was positively associated with both DGR and NWB in both systems—stronger in the nearshore system (DGR: $r = 0.67$, $p<0.001$; NWB: $r = 0.71$; $p < 0.001$) than in ponds (DGR: $r = 0.64$, $p<0.001$; NWB: $r = 0.31$; $p < 0.05$) (Figure 13 and 14).

Table 4: Water quality parameters across various culture systems of *Gracilaria* sp. cultivated in shrimp farming ghers along the southeastern coast of the Bay of Bengal, Bangladesh. Values are presented as mean ± standard deviation. Here, the variables' full forms are: PFLL- pond based floating long-line, PFN- pond based floating net, and PBR- pond based broadcast method. Within each row, differing superscript letters indicate statistically significant differences among culture methods, determined by Tukey's post hoc test ($p < 0.05$).

Water Quality Parameters	Culture Systems			F Value	P-value
	PFLL	PFN	PBR		
Temperature (°C)	28.69±3.74[a]	28.1±3.82[a]	27.26±3.55[a]	0.68	0.511 NS
Salinity (PPT)	23.1±2.62[a]	23.04±2.65[a]	23.13±2.82[a]	0.006	0.994 NS
pH	7.93±0.37[a]	7.98±0.33[a]	8.1±0.33[a]	1.188	0.313 NS
Turbidity (PSU)	24.77±5.21[a]	24.85±5.27[a]	26.03±6.23[a]	.288	0.751 NS
DO (mg/L)	5.43±0.49[a]	5.43±0.53[a]	5.03±0.47[b]	3.86	0.027*
Nitrate (mg/L)	1.14±0.40[a]	1.14±0.39[a]	0.89±0.33[a]	2.568	0.087 NS
Nitrite (mg/L)	0.13±0.04[a]	0.12±0.04[a]	0.13±0.04[a]	.113	.893 NS
Ammonia(mg/L)	0.44±0.06[a]	0.44±0.07[a]	0.44±0.08[a]	.037	.963 NS
Phosphate (mg/L)	0.75±0.07[a]	0.73±0.07[a]	0.68±0.07[b]	4.530	0.015*

Table 5: Water quality parameters across various culture systems of *Gracilaria* sp. cultivated in nearshore environments along the southeast coast of the Bay of Bengal, Bangladesh. Values are presented as the mean ± standard deviation. Here, the variables' full forms are: NFLL- nearshore based floating long-line, NOBLL- nearshore based off-bottom long-line, NOBN- nearshore based off-bottom net. Within each row, differing superscript letters indicate statistically significant differences among culture methods, determined by Tukey's post hoc test ($p < 0.05$).

Water Quality Parameters	Culture Systems			F Value	P-value
	NFLL	NOBN	NOBLL		
Temperature (°C)	27.70±3.75[a]	28.48±3.57[a]	28.40±3.39[a]	1.292	0.277 NS
Salinity (PPT)	29.87±3.15[a]	30.43±3.36[a]	30.15±3.44[a]	0.640	0.528 NS
pH	7.62±0.37[b]	7.78±0.44[a]	7.78±0.42[a]	4.806	0.009**
Turbidity (PSU)	61.57±84.42[a]	63.92±89.35[a]	64.56±90.17[a]	0.029	0.972 NS
DO (mg/L)	6.20±0.96[a]	5.67±0.66[b]	5.70±0.62[b]	13.815	0.000***
Nitrate (mg/L)	0.97±0.88[a]	0.59±0.72[b]	0.55±0.58[b]	8.731	0.000***
Nitrite (mg/L)	0.04±0.03[a]	0.03±0.02[a]	0.04±0.07[a]	0.557	0.574 NS
Ammonia(mg/L)	0.34±0.15[a]	0.26±0.13[b]	0.23±0.06[b]	20.237	0.000***
Phosphate (mg/L)	0.38±0.23[a]	0.34±0.15[a]	0.34±0.17[a]	1.450	0.237 NS

Figure 13: Interrelationship between the growth and production parameters of *Gracilaria* sp. and the water quality variables in shrimp farming gher culture units along the southeastern coast of the Bay of Bengal, Bangladesh. The values displayed along each axis represent the range of measured unit values for individual parameters. Correlation coefficients (r) are shown numerically, and statistical significance is indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The analysis is based on the monthly sampling of production performances and water quality parameters of 9 seaweed culture plots ((3 floating long-line, 3 floating net, and 3 broadcast unit) for 6 months (October 2023 to March 2024).

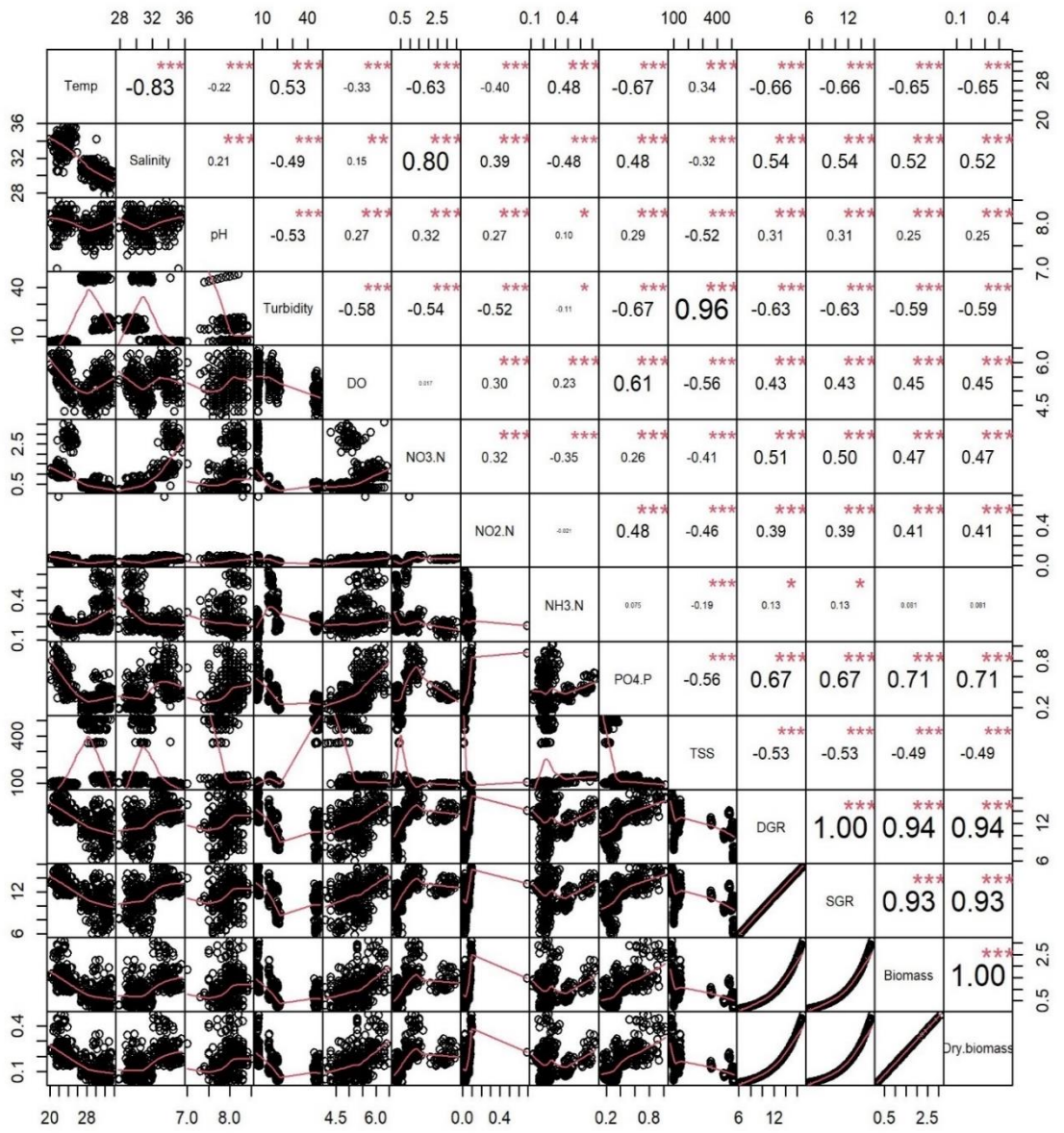

Figure 14: Interrelationship among the growth and production parameters of *Gracilaria* sp. seaweed and the water quality variables in nearshore culture units along the southeastern coast of the Bay of Bengal, Bangladesh. The values surrounding each axis indicate the range of measured unit values for the corresponding parameters. Correlation coefficients (r) are presented as numeric values, with larger font sizes reflecting stronger correlation strength. Statistical significance levels are denoted by asterisks (* for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$). This analysis is based on monthly sampling of growth performance and water quality parameters across nine seaweed culture plots—comprising three each of floating long-line, off-bottom net, and off-bottom long-line systems—conducted over a six-month period from October 2023 to March 2024.

The Multivariate Principal Component Analysis (PCA) was employed to comprehensively examine the complex interrelationships between seasonal fluctuations, environmental parameters, and the production performance of *Gracilaria* sp. cultivated in two distinct environments—shrimp farming ghers and nearshore waters—along the southeastern coast of the Bay of Bengal, Bangladesh (Figure 15). In the pond-based system, the first two principal components (PC1 and PC2) explained 79.7% of the total variance, with PC1 accounting for 43.9%, while in the nearshore system, PC1 and PC2 together explained 68.5% of the variability, with PC1 contributing 53.3%. Across both environments, PC1 was primarily associated with key growth parameters (Daily Growth rate, DGR; Specific Growth Rate, SGR; and Net weight Biomass, NWB), which were positively correlated with several environmental factors, including salinity, dissolved oxygen (DO), pH, and nutrient concentrations (such as nitrate, nitrite, and phosphate), and negatively correlated with temperature and turbidity. Seasonal dynamics revealed that from December to February, elevated levels of nutrients (NO_3-N, NO_2-N, and PO_4-P) promoted higher growth rates and biomass yield in both systems. In contrast, the periods from October to November and March to April were characterized by increased temperature and turbidity, which were associated with suppressed growth performance of *Gracilaria* sp.

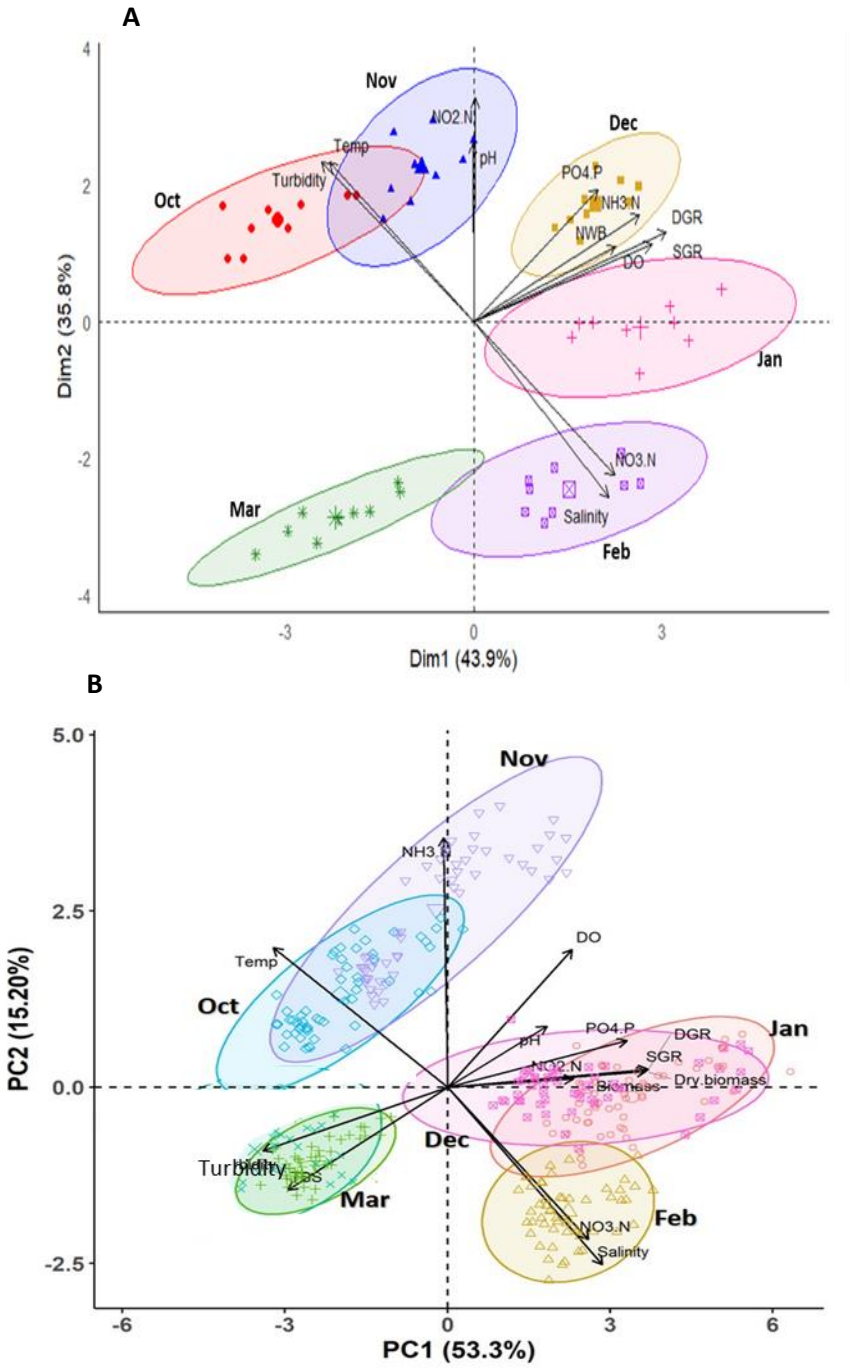

Figure 15: Biplots of principal component analysis (PCA) illustrating the monthly variation in growth parameters of *Gracilaria* sp. in relation to associated water quality variables over a six-month cultivation period (October 2023 to March 2024). **(A)** PCA based on datasets from 9 pond-based seaweed culture plots (3 floating long-line, 3 floating net, and 3 broadcast systems) established in shrimp ghers (n = 54). **(B)** PCA based on datasets from 9 nearshore seaweed culture plots (3 off-bottom long-line, 3 off-bottom net, and 3 floating long-line systems) (n = 54).

4.3 Chemical Properties Comparison of *Gracilaria* sp.

4.3.1 Proximate Composition

A two-way ANOVA was conducted to evaluate the effects of cultivation environment (CE) and cultivation layer (CL), as well as their interaction (CE × CL), on the proximate composition of *Gracilaria* sp., specifically focusing on variations in protein, lipid, ash, and fiber contents. The results revealed that the cultivation environment had a highly significant effect ($p < 0.001$) on all proximate parameters of *Gracilaria* sp., with pond-cultivated seaweeds showing lower values of protein, lipid, ash, and fiber ($15.82 \pm 1.49\%$, $0.31 \pm 0.09\%$, $11.21 \pm 1.21\%$, and $5.86 \pm 0.80\%$, respectively), while nearshore-cultivated seaweeds exhibited elevated levels of these components ($22.82 \pm 1.06\%$, $0.69 \pm 0.19\%$, $15.77 \pm 0.74\%$, and $8.71 \pm 0.33\%$, respectively). Cultivation layer also markedly influenced the proximate composition of *a*sp., with surface-grown seaweed exhibiting significantly higher protein ($20.38 \pm 3.61\%$), lipid ($0.62 \pm 0.26\%$), and ash ($14.30 \pm 2.25\%$) contents compared to bottom-grown counterparts ($18.26 \pm 4.11\%$, $0.37 \pm 0.17\%$, and $12.67 \pm 2.79\%$, respectively) ($p < 0.001$), while fiber content was slightly but significantly elevated in surface-cultivated seaweed, recorded at $7.54 \pm 1.10\%$ compared to $7.03 \pm 2.06\%$ in those grown at the bottom layer ($p < 0.05$). The interaction between cultivation environment and layer (CE × CL) exhibited parameter-specific significance: it was non-significant for protein ($p > 0.05$), significant for lipid ($p < 0.05$), marginally significant for ash ($0.05 < p < 0.1$), and highly significant for fiber ($p < 0.001$), indicating that fiber content was strongly affected by the combined influence of environment and layer, while the effect of layer on lipid varied depending on the environment.

Table 6: Effect of cultivation environment and cultivation layer combinations on proximate composition in cultivated seaweed based on two-way ANOVA. Environment and Cultivation Layer. Values are presented as mean ± standard deviation for each treatment combination. The significance levels (p) are denoted by asterisks (* < 0.05, ** < 0.01, *** < 0.001).

Proximate Composition (%)	Cultivation Environment (CE)		Cultivation layer (CL)		ANOVA Significance P-value		
	Pond	Nearshore	Surface	Bottom	CE	CL	CE*CL
Protein	15.82± 1.49	22.82±1.06	20.38±3.61	18.26±4.11	***	***	NS
Lipid	0.31±0.09	0.69±0.19	0.62±0.26	0.37±0.17	***	***	*
Ash	11.21±1.21	15.77±0.74	14.30±2.25	12.67±2.79	***	***	-
Fiber	5.86±0.80	8.71±0.33	7.54±1.10	7.03±2.06	***	*	***

4.3.2 Amino Acid Content

Gracilaria sp. was found to be rich in a diverse profile of amino acids, comprising both essential (EAA) and non-essential (NEAA) types. The amino acid content of *Gracilaria* sp. is listed in Table 7. A total of 16 different amino acids were detected, of which 9 were classified as essential amino acids (EAA) and 7 as non-essential amino acids (NEAA). A two-way ANOVA was conducted to evaluate the influence of cultivation environment (CE: pond vs. nearshore) and cultivation layer (CL: surface vs. bottom), as well as their interaction (CE×CL), on the concentrations of these amino acids. Significant effects of the cultivation environment (CE) were observed for all essential and non-essential amino acids ($p < 0.05$), with nearshore-cultivated seaweeds generally exhibiting higher concentrations than those grown in ponds, except for the non-essential amino acid serine, which showed a significantly higher content in pond-cultivated samples (13.19 ± 0.67 mg/g) compared to those from the nearshore environment (10.35 ± 0.89 mg/g).

In contrast to the cultivation environment, the effect of the cultivation layer (CL) was relatively weaker, as no statistically significant variation was observed in 8 out of the 16 detected amino acids. These included 4 essential amino acids (namely arginine, methionine, isoleucine, and valine), and 4 non-essential amino acids (namely aspartic acid, alanine, proline, and serine), indicating that vertical positioning within the water column had minimal influence on their accumulation. Among the remaining amino acids, leucine (EAA) and tyrosine (NEAA) showed marginal significance, with leucine being slightly higher in bottom-cultivated samples (13.24 ± 1.77 mg/g) compared to surface ones (12.55 ± 5.61 mg/g), while tyrosine exhibited a slightly higher concentration at the surface (7.32 ± 3.13 mg/g) than at the bottom (6.85 ± 2.20 mg/g). Statistically significant differences ($p < 0.05$) were also observed for lysine, threonine (both EAA), and glycine (NEAA). Lysine content was higher at the surface (9.78 ± 5.15 mg/g) than at the bottom (9.13 ± 3.40 mg/g), while threonine and glycine showed the opposite trend, being higher in bottom layers (9.72 ± 1.22 mg/g and 8.29 ± 1.85 mg/g, respectively) than at the surface (8.48 ± 2.93 mg/g and 6.93 ± 3.96 mg/g, respectively). More pronounced differences ($p < 0.01$) were found in phenylalanine (EAA) and glutamic acid (NEAA), with both exhibiting higher concentrations in bottom-cultivated samples (8.47 ± 1.14 mg/g and 17.53 ± 3.68 mg/g, respectively) compared to surface ones (6.25 ± 3.34 mg/g and 20.04 ± 4.61 mg/g, respectively). Notably, histidine (EAA)

was the only amino acid to show a highly significant variation ($p < 0.001$), with higher accumulation in the surface layer (5.53 ± 3.15 mg/g) than in the bottom layer (4.38 ± 2.07 mg/g).

Of the 16 amino acids analyzed, only methionine (EAA) and glutamic acid (NEAA) showed no significant interaction effect (CE×CL), indicating their concentrations remained stable across combined cultivation conditions. Both NEAA and TAA concentrations were higher in nearshore samples (114.55 ± 56.43 mg/g and 247.56 ± 121.96 mg/g, respectively) than in pond-cultivated samples (60.09 ± 5.96 mg/g and 119.08 ± 15.65 mg/g, respectively). No significant effects of cultivation layer or CE×CL interaction were detected for either total NEAA or TAA.

Table 7: Effect of cultivation environment and cultivation layer combinations on amino acid concentrations in cultivated seaweed based on two-way ANOVA. All the values are presented as mean ± standard deviation for each treatment combination. The significance levels (p) are denoted by asterisks (* < 0.05, ** < 0.01, *** < 0.001).

Amino Acids (mg/g of dry weight)	Cultivation Environment (CE)		Cultivation layer (CL)		ANOVA Significance P-value		
	Pond	Nearshore	Surface	Bottom	CE	CL	CE*CL
Arginine	8.01 ± 1.01	17.28 ± 2.73	13.20 ± 6.65	12.08 ± 3.88	***	NS	**
Histidine	2.59 ± 0.33	7.31 ± 1.23	5.53 ± 3.15	4.38 ± 2.07	***	***	**
Leucine	9.58 ± 2.45	16.20 ± 1.61	12.55 ± 5.61	13.24 ± 1.77	***	-	***
Lycine	5.58 ± 0.66	13.33 ± 1.32	9.78 ± 5.15	9.13 ± 3.40	***	*	***
Methionine	1.97 ± 0.14	5.07 ± 0.55	3.67 ± 1.83	3.36 ± 1.63	***	NS	NS
Phynylalanine	5.68 ± 2.81	9.04 ± 0.88	6.25 ± 3.34	8.47 ± 1.14	***	**	***
Threonine	7.36 ± 1.74	10.84 ± 0.84	8.48 ± 2.93	9.72 ± 1.22	***	*	**
Isoleucine	7.22 ± 0.75	12.23 ± 0.97	9.98 ± 3.43	9.47 ± 2.15	***	NS	**
Valine	7.25 ± 1.08	13.03 ± 1.50	10.31 ± 4.35	9.97 ± 2.10	***	NS	***
EAA	58.99 ± 9.91	132.89 ± 65.47	81.50 ± 34.43	110.38 ± 77.30	*	NS	NS
Aspertic acid	14.01 ± 2.10	17.18 ± 1.36	15.36 ± 3.36	15.83 ± 0.92	***	NS	***
Glutamic Acid	15.15 ± 1.60	22.42 ± 2.10	20.04 ± 4.61	17.53 ± 3.68	***	**	NS
Alanine	5.07 ± 1.19	12.33 ± 0.96	8.58 ± 5.01	8.83 ± 2.97	***	NS	***
Glycine	5.08 ± 1.95	10.14 ± 1.03	6.93 ± 3.96	8.29 ± 1.85	***	*	**
Proline	2.91 ± 1.16	9.21 ± 0.81	5.82 ± 4.31	6.30 ± 2.66	***	NS	***
Tyrocine	4.67 ± 0.53	9.50 ± 0.71	7.32 ± 3.13	6.85 ± 2.20	***	-	**
Serine	13.19 ± 0.67	10.35 ± 0.89	11.82 ± 1.20	11.72 ± 2.16	***	NS	-
NEAA	60.09 ± 5.96	114.55 ± 56.43	75.88 ± 23.13	98.76 ± 64.43	-	NS	NS
Total AA	119.08 ± 15.65	247.56 ± 121.96	157.43 ± 57.59	209.20 ± 141.77	*	NS	NS

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4.3.3 Phytochemical Content

Gas Chromatography–Mass Spectrometry (GC-MS) analysis was employed to comprehensively identify and quantify the phytochemical constituents of cultivated *Gracilaria* sp. A total of 50 distinct phytochemical compounds were detected. The detailed composition is presented in Table 8. A two-way ANOVA was performed to assess the effects of cultivation environment (CE: pond vs. nearshore), cultivation layer (CL: surface vs. bottom), and their interaction (CE×CL) on the concentration levels of the identified phytochemical compounds. Among the identified compounds, only $C_{16}H_{32}O_2$ (RT 31.46 min) and $C_{27}H_{46}O$ (RT 45.95 min) showed no significant variation in terms of cultivation environments ($p > 0.05$), and even so, these two compounds were the most abundant in both pond and nearshore cultivated *Gracilaria*, highlighting their dominant presence regardless of environmental differences. In contrast, the cultivation layer had a significant effect on both compounds, with the first compound ($C_{16}H_{32}O_2$) showing higher concentration in the surface layer ($p < 0.05$), while the second ($C_{27}H_{46}O$) was markedly more abundant in the bottom layer ($\boldsymbol{p} < 0.001$). Other dominant compounds in pond-cultivated samples included $C_{14}H_{28}O_2$ (RT 26.75), C_8H_{10} (RT 3.73), $C_{17}H_{34}O_2$ (RT 30.31), $C_{22}H_{42}O_4$ (RT 39.55), and $C_{27}H_{44}O$ (RT 50.92), each showing comparatively higher concentrations than their corresponding levels in nearshore samples ($p < 0.001$, < 0.001, < 0.001, < 0.01, < 0.001, respectively). Conversely, nearshore-cultivated *Gracilaria* exhibited significantly greater accumulation of $C_{17}H_{34}O_2$ (RT 31.77), $C_{19}H_{38}O_4$ (RT 41.45), and $C_{21}H_{46}O_3Si_2$ (RT 43.62) ($p < 0.001$, < 0.001, < 0.001, respectively), highlighting notable statistical differences between cultivation environments for these compounds. However, around seven compounds, C_8H_{10} (RT 3.73 min), $C_{17}H_{34}$ (RT 24.86 min), $C_{34}H_{66}O_2$ (RT 29.35 min), $C_{20}H_{37}O_4N$ (RT 37.45 min), $C_{17}H_{18}ON_2$ (RT 40.92 min), $C_{19}H_{38}O_4$ (RT 41.45 min), and $C_{21}H_{46}O_3Si_2$ (RT 43.62 min), showed no significant differences in relation to cultivation layer, suggesting a more uniform distribution across surface and bottom conditions. In terms of the interaction between cultivation environment and cultivation layer (CE × CL), out of the 50 identified compounds, 11 compounds—C_8H_{10} (RT 3.73 min), $C_{12}H_{36}O_6Si_6$ (RT 15.35 min), $C_{17}H_{34}$ (RT 24.86 min), $C_{14}H_{28}O_2$ (RT 26.75 min), $C_{20}H_{38}$ (RT 28.40 min), $C_{34}H_{66}O_2$ (RT 29.35 min), $C_{17}H_{34}O_2$ (RT 30.31 min), $C_{20}H_{37}O_4N$ (RT 37.45 min), $C_{17}H_{18}ON_2$ (RT 40.92 min), $C_{21}H_{46}O_3Si_2$ (RT 43.62 min), and $C_{27}H_{46}O$ (RT 45.95 min)—showed no significant variation ($p > 0.05$). The

remaining compounds were significantly affected by the CE × CL interaction, with 21 displaying highly significant variation ($p < 0.001$), 9 demonstrating significant effects ($p < 0.01$), 5 showing weak but significant differences ($p < 0.05$), and 4 indicating marginal significance ($p \approx 0.05$), reflecting the interactive influence of environment and cultivation depth on phytochemical profiles.

Table 8: Effect of cultivation environments and cultivation layer combinations on phytochemical compound concentrations in cultivated seaweed based on two-way ANOVA. All the values are presented as mean ± standard deviation for each treatment combination. The significance levels (p) are denoted by asterisks (* < 0.05, ** < 0.01, *** < 0.001).

Retention time	Compound name	Cultivation Environment		Cultivation Layer		ANOVA Significance P-value		
		Pond	Nearshore	Surface	Bottom	CE	CL	CE*CL
3.73	C_8H_{10}	4.21 ± 0.76	0.01 ± 0.01	2.03 ± 2.29	2.19 ± 2.43	***	NS	NS
3.74	C_8H_{10}	2.20 ± 2.47	3.07 ± 0.91	4.14 ± 0.64	1.13 ± 1.24	*	***	***
11.06	$C_9H_{18}O_2Si$	0.01 ± 0.01	0.44 ± 0.47	0.01 ± 0.01	0.44 ± 0.48	***	***	***
12.79	$C_7H_9O_2N$	0.16 ± 0.08	0.01 ± 0.01	0.05 ± 0.05	0.12 ± 0.13	***	**	**
14.96	$C_5H_{10}ON_2$	0.02 ± 0.02	0.93 ± 1.02	0.01 ± 0.01	0.95 ± 1.00	***	***	***
15.35	$C_{12}H_{36}O_6Si_6$	0.27 ± 0.15	0.14 ± 0.11	0.31 ± 0.11	0.10 ± 0.06	**	***	NS
16.55	$C_{10}H_{20}O_2$	0.20 ± 0.12	0.04 ± 0.03	0.15 ± 0.17	0.09 ± 0.02	***	-	**
17.21	$C_{13}H_{23}O_2Cl_3$	0.03 ± 0.03	0.30 ± 0.19	0.07 ± 0.08	0.26 ± 0.23	***	***	**
20.14	$C_{12}H_{36}O_4Si_5$	0.22 ± 0.04	0.07 ± 0.07	0.16 ± 0.06	0.12 ± 0.13	***	*	**
20.52	$C_{14}H_{22}O$	0.01 ± 0.01	0.16 ± 0.09	0.04 ± 0.04	0.13 ± 0.13	***	***	***
20.89	$C_{11}H_{20}$	0.04 ± 0.02	0.11 ± 0.06	0.09 ± 0.08	0.06 ± 0.01	***	**	***
24.86	$C_{17}H_{34}$	0.31 ± 0.05	0.01 ± 0.01	0.17 ± 0.18	0.15 ± 0.15	***	NS	NS
25.15	$C_{31}H_{64}$	1.27 ± 0.58	0.38 ± 0.14	1.10 ± 0.74	0.55 ± 0.32	***	**	-
25.78	$C_{15}H_{30}O_2$	0.41 ± 0.19	0.03 ± 0.03	0.30 ± 0.30	0.14 ± 0.15	***	*	-
26.2	$C_{15}H_{26}O$	0.01 ± 0.01	0.32 ± 0.28	0.29 ± 0.32	0.03 ± 0.04	***	***	***
26.75	$C_{14}H_{28}O_2$	4.32 ± 1.00	2.57 ± 0.76	4.18 ± 1.02	2.70 ± 1.02	***	***	NS
28.4	$C_{20}H_{38}$	2.08 ± 0.95	2.89 ± 0.32	2.88 ± 0.72	2.09 ± 0.70	*	*	NS
28.55	$C_{10}H_{20}$	0.01 ± 0.01	0.35 ± 0.13	0.24 ± 0.25	0.12 ± 0.13	***	***	***
28.94	$C_{37}H_{70}O$	0.54 ± 0.40	0.24 ± 0.21	0.47 ± 0.48	0.31 ± 0.13	**	*	***
28.95	$C_{36}H_{70}O_2$	0.17 ± 0.18	0.70 ± 0.32	0.50 ± 0.54	0.37 ± 0.07	***	**	***
29.35	$C_{34}H_{66}O_2$	0.03 ± 0.02	1.25 ± 0.13	0.63 ± 0.69	0.65 ± 0.66	***	NS	NS
29.84	$C_{22}H_{44}O$	0.25 ± 0.29	0.01 ± 0.01	0.25 ± 0.29	0.01 ± 0.01	**	**	**
29.86	$C_{17}H_{32}O_2$	0.21 ± 0.22	0.03 ± 0.03	0.01 ± 0.01	0.24 ± 0.19	***	***	***
30.31	$C_{17}H_{34}O_2$	3.79 ± 1.24	1.67 ± 0.46	3.33 ± 1.61	2.13 ± 0.99	***	*	NS
31.46	$C_{16}H_{32}O_2$	48.99 ± 6.17	46.34 ± 11.86	51.86 ± 7.83	43.47 ± 8.91	NS	*	***
31.77	$C_{17}H_{34}O_2$	0.01 ± 0.01	4.86 ± 2.32	1.38 ± 1.52	3.49 ± 3.82	***	***	***
31.79	$C_{19}H_{38}O_2$	3.64 ± 3.98	0.01 ± 0.01	3.61 ± 4.02	0.03 ± 0.04	***	***	***
33.57	$C_{38}H_{68}O_8$	0.01 ± 0.01	0.14 ± 0.12	0.02 ± 0.01	0.13 ± 0.13	***	***	***
33.94	$C_{20}H_{40}O_2$	0.01 ± 0.01	0.15 ± 0.11	0.13 ± 0.13	0.03 ± 0.03	***	***	***
34.17	$C_{20}H_{40}O$	0.17 ± 0.11	1.22 ± 0.46	0.95 ± 0.75	0.45 ± 0.41	***	***	***
34.76	$C_{18}H_{34}O_2$	0.01 ± 0.01	1.55 ± 1.87	1.56 ± 1.86	0.01 ± 0.01	**	**	**
35.24	$C_{18}H_{36}O_2$	2.43 ± 0.44	0.63 ± 0.70	1.39 ± 1.52	1.68 ± 0.51	***	*	***
35.52	$C_{38}H_{68}O_8$	0.01 ± 0.01	0.54 ± 0.52	0.51 ± 0.55	0.04 ± 0.04	***	***	***
35.53	$C_{18}H_{37}ON$	0.04 ± 0.04	0.88 ± 1.03	0.01 ± 0.01	0.91 ± 1.00	**	***	**
37	$C_{21}H_{34}O_2$	0.04 ± 0.03	0.86 ± 0.48	0.62 ± 0.68	0.28 ± 0.31	***	*	*

37.45	$C_{20}H_{37}O_4N$	0.01 ± 0.01	1.66 ± 0.16	0.83 ± 0.92	0.83 ± 0.91	***	NS	NS
37.68	$C_{19}H_{36}O_3$	0.01 ± 0.01	1.10 ± 0.37	0.69 ± 0.77	0.43 ± 0.47	***	*	-
39.55	$C_{22}H_{42}O_4$	3.26 ± 0.71	2.52 ± 0.29	3.28 ± 0.68	2.50 ± 0.31	**	**	-
40.91	$C_{22}H_{46}O_3Si$	0.36 ± 0.35	0.05 ± 0.02	0.06 ± 0.01	0.35 ± 0.36	***	***	***
40.92	$C_{17}H_{18}ON_2$	0.02 ± 0.02	1.96 ± 0.27	1.03 ± 1.14	0.96 ± 1.02	***	NS	NS
41.45	$C_{19}H_{38}O_4$	0.40 ± 0.51	3.26 ± 0.74	1.87 ± 2.09	1.80 ± 1.14	***	NS	*
41.56	$C_{22}H_{42}O_2$	0.62 ± 0.69	0.01 ± 0.01	0.62 ± 0.69	0.01 ± 0.01	***	***	***
41.83	$C_{40}H_{82}O_2$	0.48 ± 0.11	0.01 ± 0.01	0.28 ± 0.31	0.20 ± 0.22	***	*	*
43.43	$C_{24}H_{39}O_4N$	0.02 ± 0.02	0.73 ± 0.09	0.35 ± 0.34	0.40 ± 0.44	***	-	**
43.62	$C_{21}H_{46}O_3Si_2$	0.01 ± 0.01	2.14 ± 0.09	1.07 ± 1.17	1.08 ± 1.17	***	NS	NS
43.63	$C_{10}H_{20}O_2S$	2.13 ± 0.83	0.03 ± 0.02	1.38 ± 1.58	0.78 ± 0.81	***	*	*
45.95	$C_{27}H_{46}O$	10.75 ± 12.05	12.85 ± 14.06	0.04 ± 0.02	23.56 ± 3.67	NS	***	NS
47.90	$C_{15}H_{24}O_2F_6$	0.04 ± 0.04	0.33 ± 0.27	0.29 ± 0.32	0.08 ± 0.02	***	***	***
50.92	$C_{27}H_{44}O$	2.94 ± 2.12	0.43 ± 0.47	2.75 ± 2.31	0.62 ± 0.68	***	**	*
51.88	$C_{27}H_{42}O$	2.85 ± 1.50	0.04 ± 0.02	2.05 ± 2.31	0.84 ± 0.87	***	**	**

4.3.4 Antioxidant and Flavonoid Content

A two-way ANOVA analysis revealed distinct patterns in antioxidant and flavonoid content based on cultivation environment (CE) and cultivation layer (CL) as well as their interaction (CE*CL). While antioxidant activity did not vary significantly with the cultivation environment ($p > 0.05$), a highly significant effect was observed for the cultivation layer ($p < 0.001$), with seaweeds from the bottom layer exhibiting markedly higher antioxidant activity (2.88 ± 0.22) compared to those from the surface (1.96 ± 0.19). In contrast, flavonoid content was significantly influenced by both cultivation environment and cultivation layer ($p < 0.001$ in both cases), with nearshore-cultivated seaweeds showing substantially higher flavonoid levels (192.13 ± 35.50) than those from pond systems (44.07 ± 15.50), while bottom-layer seaweeds exhibited higher flavonoid content (141.07 ± 91.21) than surface-layer ones (95.13 ± 71.18). Moreover, the interaction between CE and CL (CE × CL) was not significant for antioxidant content, but showed a highly significant effect ($p < 0.001$) for flavonoids, highlighting that the combined influence of environmental and positional factors is particularly critical for flavonoid biosynthesis.

Table 9: Effect of cultivation environment and cultivation layer combinations on anti-oxidants and flavonoids concentrations in cultivated seaweed based on two-way ANOVA. All the values are presented as mean ± standard deviation for each treatment combination. The significance levels (p) are denoted by asterisks (* < 0.05, ** < 0.01, *** < 0.001).

Measured Parameters	Cultivation Environment (CE)		Cultivation layer (CL)		ANOVA Significance P-value		
	Pond	Nearshore	Surface	Bottom	CE	CL	CE*CL
Anti-oxidant	2.41 ± 0.54	2.44 ±0.55	1.96 ±0.19	2.88 ±0.22	NS	***	NS
Flavonoid	44.07 ±15.50	192.13 ±35.50	95.13 ±71.18	141.07 ±91.21	***	***	***

4.3.5 Minerals and Heavy Metal Content

Table 10 outlines the elemental composition of *Gracilaria* sp., revealing the presence of 13 essential minerals (Na, P, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Se, and Rb) and four heavy metals (Cr, Cd, Hg, and Pb), detected across pond- and nearshore-grown samples. A two-way ANOVA was employed to assess the effects of cultivation environment (CE: pond vs. nearshore) and cultivation layer (CL: surface vs. bottom), as well as their interaction (CE×CL), on the concentrations of these elements. Significant effects of the cultivation environment (CE) were observed for nearly all essential minerals ($p < 0.05$), with the exception of phosphorus (P) and nickel (Ni), which showed no significant variation between environments ($p > 0.05$). Among the elements with significant differences, sodium (Na, $p < 0.001$), potassium (K, $p < 0.001$), iron (Fe, $p < 0.01$), cobalt (Co, $p < 0.001$), and copper (Cu, $p < 0.001$) exhibited markedly higher concentrations in nearshore-cultivated *Gracilaria* compared to pond-grown samples. Conversely, calcium (Ca, $p < 0.001$), vanadium (V, $p < 0.05$), manganese (Mn, $p < 0.001$), zinc (Zn, $p < 0.05$), selenium (Se, $p < 0.001$), and rubidium (Rb, $p < 0.001$) were significantly more enriched in pond-cultivated samples. In terms of cultivation layer, no significant differences were observed for potassium (K), calcium (Ca), vanadium (V), nickel (Ni), and zinc (Zn) ($p > 0.05$). Among the elements exhibiting significant variation, sodium (Na, $p < 0.001$), phosphorus (P, $p < 0.001$), and cobalt (Co, $p \approx 0.05$; marginally significant) showed higher concentrations in bottom-cultivated *Gracilaria* compared to surface-grown samples. In contrast, manganese (Mn, $p < 0.001$), iron (Fe, $p < 0.01$), copper (Cu, $p < 0.01$), selenium (Se, $p < 0.001$), and

rubidium (Rb, $p < 0.001$) were significantly more concentrated in surface-cultivated samples than in those from the bottom layer. In terms of the interaction between cultivation environment and cultivation layer (CE × CL), only four essential minerals—potassium (K), calcium (Ca), vanadium (V), and nickel (Ni)—showed no significant interaction effects ($p > 0.05$). In contrast, significant CE × CL interactions were observed for sodium ($p < 0.001$), phosphorus ($p < 0.01$), manganese ($p < 0.001$), iron ($p < 0.05$), cobalt ($p < 0.01$), copper ($p < 0.001$), zinc ($p < 0.001$), selenium ($p < 0.001$), and rubidium ($p < 0.001$), indicating that the combined influence of environment and cultivation depth plays a critical role in shaping their accumulation patterns.

Among the four identified heavy metals, chromium (Cr, $p < 0.01$) exhibited significantly higher concentrations in nearshore-cultivated *Gracilaria*, whereas lead (Pb, $p < 0.001$) was more abundant in pond-grown samples, indicating contrasting accumulation patterns between environments. In terms of cultivation layer, both Cr ($p < 0.05$) and Pb ($p < 0.001$) showed greater concentrations in bottom-layer cultivation compared to the surface. Furthermore, both elements demonstrated highly significant interaction effects between cultivation environment and layer (CE × CL, $p < 0.001$), indicating that their accumulation is jointly shaped by cultivation environment and depth. In contrast, cadmium (Cd) and mercury (Hg) showed no significant variation in relation to cultivation environment, layer, or their interaction ($p > 0.05$), reflecting a relatively stable presence across conditions.

Table 10: Effect of cultivation environment and cultivation layer combinations on minerals and heavy metals concentrations in cultivated seaweed based on two-way ANOVA. All the values are presented as mean ± standard deviation for each treatment combination. The significance levels (p) are denoted by asterisks (* < 0.05, ** < 0.01, *** < 0.001).

Minerals (mg/kg)	**Cultivation Environment (CE)**		**Cultivation layer (CL)**		ANOVA Significance P-value		
	Pond	Nearshore	Surface	Bottom	CE	CL	CE*CL
Na	436.25 ± 61.65	2169.33 ± 864.49	927.75 ± 542.98	1677.83 ± 1385.96	***	***	***
P	3347.00 ± 589.97	3318.00 ± 273.69	3004.00 ± 257.87	3661.00 ± 312.34	NS	***	**
K	7436.00 ± 678.70	26740.00 ± 1265.03	17310.00 ± 10275.67	16870.00 ± 10955.93	***	NS	NS
Ca	1137.58 ± 88.80	370.67 ± 32.20	768.58 ± 450.64	739.67 ± 397.80	***	NS	NS
V	0.80 ± 0.10	0.62 ± 0.16	0.66 ± 0.16	0.75 ± 0.16	*	NS	NS
Cr	1.66 ± 0.64	2.12 ± 0.90	1.78 ± 0.53	2.00 ± 1.01	**	*	***
Mn	1510.00 ± 833.64	484.50 ± 385.67	1140.00 ± 123.08	421.17 ± 374.22	***	***	***
Fe	133.00 ± 65.92	158.00 ± 65.73	157.00 ± 97.94	134.00 ± 67.39	**	**	*
Co	0.14 ± 0.06	0.38 ± 0.14	0.22 ± 0.08	0.30 ± 0.22	***	-	**
Ni	1.41 ± 0.13	1.47 ± 0.09	1.39 ± 0.12	1.49 ± 0.08	NS	NS	NS
Cu	9.72 ± 1.67	14.28 ± 0.66	12.59 ± 1.66	11.42 ± 3.49	***	**	***
Zn	28.50 ± 8.51	22.91 ± 4.69	24.01 ± 3.59	27.41 ± 9.66	*	NS	***
Se	0.19 ± 0.12	0.05 ± 0.02	0.17 ± 0.14	0.07 ± 0.02	***	***	***
Rb	5.87 ± 2.97	4.13 ± 1.24	5.79 ± 3.06	4.22 ± 1.15	***	***	***
Cd	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	NS	NS	NS
Hg	0.15 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	0.15 ± 0.02	NS	NS	NS
Pb	13.20 ± 2.10	7.33 ± 1.57	6.75 ± 1.08	14.27 ± 2.58	***	***	***

CHAPTER V

DISCUSSION

The present study highlights how biomass yield, biochemical composition, and nutrient assimilation in *Gracilaria* sp. vary significantly with both the cultivation environment (pond vs nearshore) and cultivation layer (surface vs bottom). These findings present both challenges and opportunities for scaling up *Gracilaria* cultivation.

5.1 Production and Growth Performance of *Gracilaria* sp. in Different Culture Systems

The differential growth performance of *Gracilaria* sp. across pond and nearshore environments in this study underscores the complex interplay between environmental conditions and cultivation techniques. The consistently higher productivity observed in nearshore systems aligns with previous studies that report the favorable influence of open-water dynamics, such as enhanced nutrient availability, stable salinity, and continuous water movement on seaweed growth (Ganesan et al., 2011; Nelson et al., 2001). These natural factors help reduce sediment deposition and improve gas exchange on the seaweed thalli, thereby stimulating photosynthetic efficiency (Kim et al., 2019). Conversely, pond-based systems often experience constraints such as limited water exchange, and organic waste accumulation from shrimp farming activities, all of which impair light penetration and create suboptimal growing conditions (Marinho-Soriano et al., 2009; Boyd, 2017).

In nearshore systems, floating long-line cultivation offered a distinct advantage over off-bottom net and line systems, primarily due to its reduced exposure to tidal desiccation. While off-bottom systems in intertidal zones often become partially or fully exposed during low tide, floating long-line structures remain submerged, shielding the thalli from desiccation stress. Exposure to desiccation has been demonstrated to significantly impair photosynthetic efficiency, reduce nutrient uptake, and cause physiological stress in various seaweed species, including *Pyropia yezoensis* and *Gracilaria* spp., ultimately leading to diminished growth and productivity (Hurd et al., 2014; Wang et al., 2016; Li et al., 2021).

Even in pond environments, the use of floating-based systems—particularly the floating net— demonstrated improved performance, highlighting their effectiveness

despite the confined conditions. Several other studies have also demonstrated the effectiveness of floating net or cage-based systems in pond environments for seaweed cultivation. For example, Hendri et al. (2018) applied the Floating Net Method (FNM) to grow *Gracilaria* sp. in shrimp ponds and observed steady growth over six weeks, with DGR values below 2% per day. Similarly, Nelson et al. (2001) employed a floating cage culture system for cultivating *Gracilaria parvispora* using nutrient-rich shrimp farm effluents, which led to a significantly higher relative growth rate (RGR) of 8.8% to 10.4% per day, compared to only 4.6% per day in thalli fertilized with traditional inorganic sources, still indicating a favorable environment for seaweed development in pond-based systems. This enhanced performance can be attributed to the net's ability to reduce wave action and tidal currents, thereby minimizing seeding loss in the culture setup (Banik et al., 2023). The use of a horizontally positioned square net provides additional structural support and protection against wave-induced damage (Paramasivam et al., 1987). Moreover, the expanded surface area of the square net may have enhanced the formation of new holdfasts, which supports stable attachment and promote growth (Paramasivam et al., 1987).

Regardless of whether in pond or nearshore systems, surface cultivation may provide advantages such as greater exposure to sunlight, improved water flow, and reduced sedimentation, which are likely to influence photosynthetic activity and nutrient assimilation (Putra and Aryawati, 2011; Chapman et al., 2002). This finding of superior surface-layer productivity aligns with the results of Marinho-Soriano et al. (2009), who highlighted light limitation and sediment load as major inhibitors of bottom-based seaweed growth, particularly in nutrient-rich aquaculture effluents. Seaweeds in bottom layers are prone to low oxygen levels and harmful compounds such as hydrogen sulfide, making them less conducive to growth (Prema, 2013). Moreover, bottom culture methods—such as the broadcast technique or off-bottom setups—are more vulnerable to epiphytic fouling, which inhibits seaweed growth (Andayani et al., 2016; Airoldi, 2003), and these limitations tend to be even more pronounced in pond systems due to their confined nature.

5.2 Seasonality, Environmental Parameters, and Production Performance of Gracilaria sp.

The growth and photosynthetic efficiency of seaweed are strongly shaped by environmental factors like water temperature, salinity levels, water clarity, and the presence of essential nutrients (Radiarta et al., 2014). According to prior research, seaweed farming in the coastal regions of Bangladesh is most viable during a five-month period, generally from November to March (Islam et al., 2019; Hossain et al., 2021; Mohiuddin et al., 2023). In the present study, growth rates peaked during the winter and declined sharply during the warmer months (October-November and February-March), with total die-off observed in April across both pond and nearshore cultivation systems as temperatures exceeded 30°C. These findings are consistent with the temperature preferences of *Gracilaria* species, which thrive between 20–27°C (Mojumdar, 1979; Bird and McLachlan, 1984) and show reduced growth or mortality beyond 30°C (Wang et al., 1984; Chirapart and Lewmanomont, 2004). The improved growth of *Gracilaria* sp. during the winter months is largely driven by favorable conditions in the Bay of Bengal, where low rainfall and increased evaporation from November to January elevate nutrient concentrations (Asaduzzaman et al., 2019; Noor et al., 2021; Mohiuddin et al., 2023). This seasonal nutrient enrichment creates optimal conditions for seaweed development, resulting in enhanced biomass and growth performance.

In the present study, water temperature varied between 25–32°C in pond systems and 22–32°C in nearshore environments, showing a negative correlation with seaweed growth performance (Figure 13 and 14). Elevated temperatures may disrupt metabolic functions and nutrient uptake in seaweeds (Ravan and Geider, 1988) and inhibit enzymatic activity, thereby lowering growth performance (Piazzi et al., 2001). Temperature-related constraints on *Gracilaria* cultivation have also been reported in other regions; for example, Chirapart and Lewmanomont (2004) observed a sharp decline in the growth and productivity of *Gracilaria fisheri* and *Gracilaria tenuistipitata* cultured in shrimp pond effluent during the summer months (March–July) despite the addition of seawater, where extreme temperatures severely hindered seaweed development.

The present study also identified a significant negative correlation between turbidity and seaweed growth. High turbidity obstructs light penetration, reducing photosynthetic activity and limiting seaweed growth (Asaduzzaman et al., 2019; Banik et al., 2023). In pond systems, turbidity is often associated with suspended solids generated by shrimp farming operations, which further restrict sunlight transmission into the water column (Marinho-Soriano et al., 2009). This phenomenon likely explains the reduced growth rates observed in shrimp gher/pond-based *Gracilaria* cultivation in our study area. Similar trends were observed in nearshore waters during late winter and early spring when sediment resuspension and runoff increased particulate load, exacerbating light limitation (Chaoyuan et al., 1993; Marinho-Soriano et al., 2009).

Regarding pH, previous research has shown that *Gracilaria* growth is favored in slightly alkaline conditions, particularly within the range of 7–8.5 (Wisnu Ariyati et al., 2016; Banik et al., 2023). In this study, seaweed growth was found to be optimal within a pH range of 7.56–8.43 in pond systems and 7.6–8.0 in nearshore environments, indicating that slightly alkaline conditions in both settings supports healthy biomass production of *Gracilaria* sp.. Dissolved oxygen (DO) levels also played a crucial role in this investigation, showing a positive correlation with growth parameters. Susilowati et al. (2014) emphasized the importance of oxygen availability in shrimp pond-based seaweed cultivation systems, where *Gracilaria verrucosa* was grown alongside *Litopenaeus vannamei* at high stocking densities (75 prawns m^{-2}); since prawns exhibit high oxygen demand at night while seaweeds contribute oxygen only during daylight via photosynthesis, the strategic use of aeration and sunlight was necessary to sustain optimal DO levels, ultimately supporting the enhanced survival and growth of both organisms. Moreover, salinity levels ranging from 15 to 44 ppt have been reported to favor the growth and development of *Gracilaria* sp. (Choi et al., 2006). In this experiment, moderate to high salinity levels (ranging between 21–26 ppt in ponds and 29–34 ppt nearshore) during the winter months positively correlated with *Gracilaria* growth.

In addition to physical characteristics, the presence of various nutrients at the culture site also significantly influences the growth of seaweed (Masyahoro and Mappiratu, 2012). Among these, nitrate (NO_3-N) and phosphate (PO_4-P) are recognized as the primary nutrients essential for seaweed growth and reproduction (Masyahoro and Mappiratu, 2010), with nitrate (NO_3-N) functioning as a vital macronutrient that drives

primary productivity. According to Effendi (2003), if the water nitrate (NO3-N) level reaches 0.2 mg/L, the aquatic plant grows rapidly. In this study, elevated concentrations of nitrate and other nutrients were observed during the winter months (December–January) across both cultivation environments, which coincided with the highest growth rates and biomass yields, suggesting that nutrient enrichment during this period significantly contributed to optimal seaweed production.

5.3 Biochemical Composition of *Gracilaria* sp.

5.3.1 Proximate Composition

Nearshore-cultivated *Gracilaria* sp. exhibited significantly higher protein, lipid, ash, and crude fiber contents compared to pond-cultivated samples, highlighting the influence of cultivation environment on nutritional quality. Geographical location, and environmental conditions vary between pond and nearshore systems, leading to differences in water temperature and nutrient availability, which in turn contribute to variations in the proximate composition of seaweeds (Benjama and Mashiyom, 2012; Nazni and Deepa, 2015). Seaweed's basic metabolic processes are governed by temperature and the concentration of vital nutrients in the water around them (Nazni and Deepa, 2015).

5.3.1.1 Protein Content

Red macroalgae, particularly *Gracilaria* species, are recognized as a valuable source of marine-derived proteins, generally exhibiting higher protein content than green and brown macroalgae, with concentrations ranging from 5.6% to 30% of dry weight depending on species, season, and cultivation conditions (Castro-Gonzáles et al., 1996; Galland-Irmouli et al., 1999; Dawczynski et al., 2007; McDermid, 2003; Marrion et al., 2005; Renaud and Luong-Van, 2006). Among the various nutrients absorbed by seaweeds, nitrogen, particularly in the form of nitrate (NO_3-N) and ammonium (NH3-N), plays the most critical role in enhancing the protein content of seaweed, as it serves as a fundamental precursor for amino acid and protein synthesis (Harrison et al., 2001; Roleda et al., 2019). According to findings from the present study, *Gracilaria* cultivated in nearshore environments exhibited approximately 44.25% higher protein content compared to those grown in pond systems, highlighting the strong influence of environmental conditions on the biochemical composition of seaweed. This marked difference can be attributed to the continuous hydrodynamic activity in open water

bodies, where tidal and wave-driven mixing ensures a steady and naturally replenished supply of dissolved inorganic nitrogen (DIN) from the surrounding environment (Anwar et al., 2014; Fu et al., 2023). During winter, when nitrate (NO_3^-N) concentrations in coastal waters typically peak, ranging from 5 to 20 μM depending on geographic location (Roleda & Hurd, 2019). Such dynamic conditions not only enhance nutrient availability but also prevent local nutrient depletion near the thalli, thereby promoting sustained nitrogen uptake and protein synthesis. In contrast, pond environments generally experience limited water exchange and higher turbidity, which restricts light penetration and reduces photosynthetic efficiency (Marinho-Soriano et al., 2009; Sutherland et al., 2015). These factors collectively impair nutrient flux and nitrogen assimilation, ultimately leading to lower protein accumulation in pond-cultivated seaweed.

5.3.1.2 Lipid Content

The lipid content of seaweeds is generally low, typically comprising only 1–5% of dry matter (Chamily et al., 2025), with most species exhibiting concentrations below 4% (Herbetreau et al., 1997; Ortiz et al., 2006). Despite their minor proportion, lipids in seaweeds are of nutritional interest due to their bioactive properties and fatty acid composition. Cultivation conditions have been shown to significantly influence lipid accumulation in *Gracilaria*. For instance, Sambhwani et al. (2022) reported that lipid content in cultivated *Gracilaria* was notably higher (0.21 ± 0.01 to 0.26 ± 0.03% dry weight) compared to wild samples (0.2 ± 0.02 to 0.27 ± 0.01% dry weight), suggesting that environmental and physiological factors associated with cultivation can affect biochemical profiles. These differences likely reflect enhanced nutrient availability and light penetration in open water settings, which may promote greater metabolic activity and lipid biosynthesis (Guschina & Harwood, 2009). A similar result was reported by Banik et al. (2023), who found that lipid content in nearshore-cultivated *Gracilaria sp.* ranged from 0.47 ± 0.1% to 0.83 ± 0.1% of dry weight, which is closely aligned with the values observed in the present study. This consistency across studies highlights the strong influence of environmental factors, such as water movement, nutrient availability, and light penetration, on lipid accumulation in *Gracilaria*.

5.3.1.3 Fiber content

Gracilaria species are well-recognized for their significant content of both soluble dietary fiber (SDF) and insoluble dietary fiber (IDF), which positions them as promising alternatives to conventional cereal-based fibers in human diets (Wong and Cheung, 2000; Elleuch et al., 2011; Debbarama et al., 2016). In the present study, *Gracilaria* cultivated in pond environments exhibited a fiber content of 5.86 ± 0.80% (dry weight), which is lower than values reported by Nunraksa et al. (2018) for pond-based *Gracilaria fisheri* (8.73 ± 0.44%) and G. *tenuistipitata* (8.50 ± 0.08%). However, it aligns more closely with the crude fiber yield of *G. tenuistipitata* (4.96%) reported by Chirapart et al. (2014). In contrast, nearshore-cultivated *Gracilaria* in the current study showed a considerably higher fiber content of 8.71 ± 0.33% (dry weight), closely matching the findings of Banik et al. (2023), who reported fiber contents ranging from 8.99 ± 0.5% to 8.78 ± 0.4%. These values are also consistent with previous studies on other *Gracilaria* species, such as *G. arcuata* (10.90%) and *G. salicornia* (10.4–12.52%) (Mwalugha et al., 2015; Tabarsa et al., 2012), *G. verrucosa* (8.35%) (Nazni and Deepa, 2015), and *G. edulis* (8.9%) (Sakthivel and Devi, 2015). Such differences may be attributed to species-specific variation and the different methods used to quantify total vs. crude dietary fiber.

5.3.1.4 Ash Content

Variations in ash content among seaweeds are largely influenced by species differences, environmental settings, and habitat characteristics (McDermid and Stuercke 2003; Robic et al. 2009; Munier et al. 2013). In the present study, *Gracilaria* cultivated in pond systems exhibited an ash content of 11.21 ± 1.21% (dry weight), which was lower than the nearshore-cultivated samples (15.77 ± 0.74% dry weight). Similar trends have been reported in previous literature. For instance, Nunraksa et al. (2018) observed ash contents of 7.62 ± 0.20% in *G. fisheri* and 5.83 ± 0.08% in *G. tenuistipitata*, both grown under pond-based conditions. These values are notably lower than those reported by Benjama and Masniyom (2012) and Chirapart et al. (2014) for *G. tenuistipitata*, which reached 17.00% and 22.91%, respectively. Similarly, ash values reported for nearshore-cultivated *Gracilaria* in other studies show close agreement with the values observed in the present study. Benjama and Masniyom (2011) reported an ash content of 17 ± 1.1% in *Gracilaria* sp., while Gressler et al. (2010) found even higher values in *G.*

domingensis (23.8%) and *G. birdiae* (22.5%). Conversely, *G. cervicornis* exhibited relatively lower ash content (10.51%) in the study by Marinho-Soriano et al. (2006), highlighting species-specific differences. The higher ash content observed in nearshore-cultivated seaweed may be attributed to stronger wave action and greater exposure to mineral-rich seawater, which enhances the uptake and accumulation of inorganic components compared to more static and nutrient-limited pond environments (Barr et al., 2008; Benes & Bracken 2016).

5.3.2 Amino Acid Content

The present study revealed that *Gracilaria* sp. contains a rich and diverse profile of amino acids, encompassing both essential amino acids (EAAs) and non-essential amino acids (NEAAs), with a total of 16 different amino acids identified. This aligns with findings from earlier studies that underscore the nutritional potential of red seaweeds, particularly *Gracilaria*, as a source of high-quality protein and bioavailable amino acids (Norziah & Ching, 2000; Lumbessy et al., 2019; Kazir et al., 2019). The presence of this Essential Amino Acid (EAA) makes *Gracilaria* sp. much more valuable because among the EAA, arginine was notably abundant and holds particular physiological importance during periods of rapid growth, metabolic stress, illness, and especially during the first month of a newborn, where it is considered conditionally essential (Saini et al., 2013). For the Non-Essential Amino Acid (NEAA), *Gracilaria* sp. showed a higher amount of Glutamic acid (Glutamine), Aspartic acid (Aspartate) and Serine (Rosemary et al., 2019). Aspartic acid and Glutamic acid are vital from an organoleptic point of view as it was found that, these two NEAAs are responsible for the special flavour and taste of seaweeds (Matanjun et al., 2009).

A remarkable observation from the current study was the relatively minor influence of the cultivation layer (surface vs. bottom) on the total amino acid (∑TAA) content, in contrast to the pronounced effects exerted by cultivation environment (e.g., pond vs. nearshore). Similar outcomes were reported by Banik et al. (2023), who found no statistically significant difference in total amino acid between surface (158.3 ± 2.1 mg/g dry weight) and bottom (152.5 ± 5.3 mg/g dry weight) cultured *Gracilaria* sp. in a nearshore setting.

When compared to other red and green macroalgal species, *Gracilaria* spp. generally exhibited lower ∑TAA values. For example, Machado et al. (2020) reported ∑TAA in

Gracilaria vermiculophylla as 106.62 mg/g dry sample (ds), which was notably lower than in *Porphyra dioica* (203.99 ± 8.20 mg/g ds) and *Porphyra umbilicalis* (193.34 ± 2.16 mg/g ds). These differences were statistically significant ($p < 0.05$), underscoring the superior amino acid density in *Porphyra* species. Within the genus *Gracilaria*, similar ∑TAA levels have been observed across different species: *Gracilaria changii* (91.90 mg/g dw) (Chan & Matanjun, 2017) and *Gracilaria birdiae* (91 mg/g dw) (Gressler et al., 2010), aligning closely with the values reported for *G. vermiculophylla*. Such interspecies variation in ∑TAA may reflect inherent biochemical differences driven by genetic factors, environmental conditions (e.g., salinity, light, nutrient availability), harvest season, and geographic location (Astorga-España et al., 2016; Paiva et al., 2014). Taken together, these findings suggest that while *Gracilaria* offers a moderately high-quality protein source relative to green seaweeds like *Ulva rigida* (92.22 mg/g ds), it remains inferior to certain red macroalgae such as *Porphyra* in terms of amino acid density (Machado et al.,2020).

5.3.3 Phytochemical Content

Izzreen and Ratnam (2011) applied SPME-GCMS to profile volatile compounds in *Kappaphycus alvarezii*, *Caulerpa lentillifera*, and *Sargassum polycystum*, successfully identifying 223 volatile compounds and highlighting hydrocarbons as the dominant group. The present GC-MS analysis also revealed a diverse phytochemical profile in *Gracilaria* sp., most exhibited significant variation in concentration across cultivation environments and depths. Despite being the most abundant metabolites identified, C16H32O2 (Palmitic acid) and C27H46O (Cholestrol) showed no significant variation between pond and nearshore cultivation systems, suggesting that their biosynthesis is governed by intrinsic metabolic stability rather than external environmental factors. This finding aligns with previous studies where n-hexadecanoic acid (palmitic acid) was consistently reported as a dominant compound in various *Gracilaria* species regardless of geographical origin or extraction method. For instance, Ragunathan et al. (2019) identified it as the most abundant fatty acid (74.2%) in methanolic extracts of G. corticate. Similarly, Cyriac and Eswaran (2015) detected n-hexadecanoic acid as a dominant component in *G. dura*, and Akbar and Hasan (2024) confirmed its presence in *G. edulis* collected from Indonesian coastal waters. The repeated detection of such

core lipids across studies suggests their conserved biochemical role in red seaweeds and their potential as stable chemical markers. This phenomenon was also observed in the work of Arulkumar et al. (2018), where certain phenolic and fatty acid derivatives in *G. corticata* and *G. edulis* were unaffected by the nature of extraction or ecological origin, yet still contributed significantly to antioxidant and antibacterial activities. However, the present study also identified certain unique compounds in cultivated *Gracilaria* sp., such as C34H66O2 (Phytyl Tetradecanoate), C17H34 (8-Heptadecene) that showed no significant variation between cultivation layers, pointing to a more uniform distribution across vertical positions. This uniformity could reflect structural or constitutive roles of these metabolites that are less sensitive to light or nutrient gradients. These bioactive compounds are known for their pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities (Holdt & Kraan, 2011; Lomartire & Goncalves, 2022; Pradhan et al., 2022).

5.3.4 Antioxidant and Flavonoid Content

Seaweeds are increasingly recognized for their rich antioxidant and polyphenolic profiles, which contribute to their nutraceutical and functional food potential (Gomez-Zavaglia et al., 2019; Kumar et al., 2021; Coelho et al., 2023). In this study, seaweeds grown at the bottom layer showed notably higher antioxidant activity (2.88 ± 0.22) than those from the surface layer (1.96 ± 0.19). This suggests that bottom environments, with reduced exposure to UV radiation and more stable temperatures, may reduce stress and allow the seaweed to produce more antioxidant compounds. These results are in line with Xu et al. (2018), who reported that seaweeds inhabiting intertidal zones characterized by low salinity, reduced dissolved oxygen, and nutrient-rich waters exhibited markedly elevated antioxidant capacities, such as *Caloglossa nanaoensis* (18.6 ± 0.9 mg GAE/g) and *Ulva conglobata* (13.9 ± 1.0 mg GAE/g), with strong correlations observed between antioxidant activity and abiotic factors like salinity, DO, and ammonium levels ($R > 0.91$, $p < 0.05$). A study by Ramdani et al. (2017) reported that *Gracilaria bursa-pastoris* collected from the Nador lagoon in Morocco exhibited a very high antioxidant potential, with total phenolic content reaching up to 142.26 mg GAE/g extract and a strong DPPH radical scavenging activity with an EC_{50} value of 0.085 mg/mL. This highlights the natural antioxidant richness of *Gracilaria* species grown in nutrient-rich lagoon environments, aligning with the elevated antioxidant levels observed in the bottom-layer samples in the present study. However, Chan et al.

(2015) showed that the antioxidant activity of *Gracilaria* sp. can vary greatly depending on the extraction method used, and found that ethyl acetate extract had the strongest antioxidant effect, showing the highest DPPH radical scavenging activity (EC_{50} = 0.51 ± 0.09 mg/mL) and the strongest ferric reducing power, while water extract was the least effective, with the weakest DPPH activity (EC_{50} = 7.80 ± 0.57 mg/mL). These results clearly indicate that solvent type has a major impact on how much antioxidant potential is extracted from seaweed, and using more efficient solvents like ethyl acetate can significantly improve the measured antioxidant activity.

The present investigation revealed that flavonoid accumulation in *Gracilaria* is strongly influenced by both cultivation environment and vertical positioning, with seaweeds from nearshore and bottom-layer conditions yielding the highest flavonoid concentrations. A closer match to this results was observed by Akhter et al. (2024), who investigated *Gracilariopsis longissima* from the Bay of Bengal and found a flavonoid content of 75.06 mg QE/g in acetone extracts. Despite species differences, their study supports our conclusion that environmental factors in coastal Bangladesh, such as nutrient fluxes and tidal exposure, can enhance flavonoid biosynthesis in red seaweeds. On the other hand, much lower values were reported by Hidayati et al. (2023), who found only 2.26 mg QE/g flavonoids in *Gracilaria* sp. from Bintan Island, Indonesia. Heffernan et al. (2015) investigated *Gracilaria gracilis* from the coastal waters of Ireland and, although exact total flavonoid content (TFC) values were not specified, the flavonoid levels were comparatively lower than those observed in brown seaweeds such as *Fucus serratus*, indicating a relatively limited flavonoid profile in *G. gracilis* under temperate conditions. Moreover, Tanna et al. (2019) detected several flavonoids including quercetin and kaempferol in *Gracilaria corticata* from the Arabian Sea coast. Although quantitative values were not the focus, their results confirm the presence of flavonoid-rich profiles in red seaweeds from tropical regions, supporting the present finding that nearshore environments, potentially rich in dissolved nutrients, may enhance flavonoid production. Flavonoids derived from seaweeds are valued for their potent antioxidant, anti-inflammatory, and cytoprotective properties, making them promising candidates for use in functional foods, pharmaceuticals, and nutraceutical formulations aimed at preventing oxidative stress-related diseases (Pradhan et al., 2020; Catarino et al., 2023).

5.3.5 Minerals and Heavy Metal Contents

Seaweeds are recognized as a notable reservoir of minerals because they can assimilate inorganic substances from their surroundings (Mišurcová, 2011). This capability is linked to the existence of polysaccharides within seaweed cell walls, allowing these organisms to store minerals in various parts of their tissues (Küpper et al., 1998; Hashim and Chu, 2004; Mišurcová, 2011). Diverse seaweed groups exhibit varied mineral uptake capacities owing to the presence of distinct polysaccharides in their cell walls (Mišurcová, 2011). In the present study, cultivated *Gracilaria* sp. also demonstrated diverse mineral accumulation patterns significantly influenced by both cultivation environment and cultivation layer, aligning with previous findings that environmental factors, such as water movement, nutrient flux, salinity, and light availability, can substantially affect mineral uptake (Sánchez-Rodríguez et al., 2001; Bocanegra et al., 2009). In this study, among the macrominerals, Na, K, Fe, Co, and Cu were found to be significantly higher in nearshore-grown samples compared to pond-grown ones. This observation supports earlier work by Chan and Matanjun (2017) on *G. changii*, which showed high Fe and K concentrations in wild seaweed, and by Tabarsa et al. (2012), who reported *G. salicornia* to contain exceptionally high levels of K (up to 11,380 mg/100 g d.w.) and Fe. Similarly, Norziah and Ching (2000) also documented that *Gracilaria changii* contained notably high amounts of calcium (651 mg/100 g) and iron (95.6 mg/100 g), which closely resemble the concentrations observed in the nearshore-grown *Gracilaria* in the present study. The enhanced accumulation in nearshore environments is likely attributed to better water circulation, nutrient replenishment, and naturally occurring upwelling zones, which collectively promote higher bioavailability of minerals (Fu et al., 2023; Banik et al., 2023).

In terms of vertical distribution, the present findings showed that surface-cultivated *Gracilaria* exhibited significantly higher levels of Fe, Mn, Cu, Se, and Rb, whereas bottom-cultivated forms were more enriched in Na, P, and Co. This stratified mineral pattern is likely influenced by light intensity and oxygen availability, factors generally higher near the surface, which can enhance metabolic activity and facilitate the uptake of certain nutrients (Banik et al., 2023; Chamily et al., 2025). Rosemary et al. (2019) also noted high Fe and Mg contents in *G. corticata*, aligning with the elevated Fe levels recorded in surface-grown *Gracilaria* in this study. The elevated levels of redox-sensitive elements such as Fe and Cu in surface-grown *Gracilaria* may be explained by

light-driven metabolic regulation tied to photosynthetic processes, while increased concentrations of Na and P at the bottom may result from ionic exchange with pond sediments or slower diffusion rates in deeper layers. However, it is also essential to contextualize the variability in mineral content with respect to interspecific and environmental factors. For example, while G. lemaneiformis was reported to be rich in iodine and phosphorus (Wen et al., 2006), the present study found no significant differences in P content between pond and nearshore environments. This suggests that P uptake in *Gracilaria* may be less influenced by environmental gradients compared to other elements.

Heavy metals are particularly concerning due to their toxicity, persistence, and potential for bioaccumulation, contributing significantly to environmental pollution (Dauvalter and Rognerud, 2001; Yaich et al., 2011). Seaweed can accumulate dissolved heavy metals from seawater due to some molecular groups that are present in their tissues (Veroy et al., 1980). The present study revealed that *Gracilaria* sp. cultivated in pond and nearshore environments in coastal Bangladesh accumulated measurable levels of heavy metals including lead (Pb), cadmium (Cd), chromium (Cr), and arsenic (As). Notably, the concentration of Pb was significantly higher in pond-grown samples compared to nearshore ones ($p < 0.001$), with the bottom-layer samples exhibiting greater Pb accumulation than those from the surface layer ($p < 0.001$). This trend aligns with the findings of Nguyen et al. (2018), who reported considerably higher Pb levels (4.79–6.60 μg/g) in *G. fisheri* cultivated in ponds environments in Thailand.

Consistent with the present findings, Banik et al. (2023) also observed that in nearshore-based seaweed cultivation systems, metal accumulation was lower in surface-layer cultivated *Gracilaria* compared to those grown in off-bottom setups. A similar trend was also reported by Chamily et al. (2025) in *Ulva* species, where seaweeds positioned closer to the surface accumulated fewer heavy metals than those cultivated near the seabed, further highlighting the influence of vertical cultivation position on metal uptake. Pond environments, especially those with limited water exchange and potential legacy contamination from fertilizers or feeds, appear more susceptible to heavy metal buildup, particularly for Pb and Cr. These observations echo earlier studies showing that macroalgae grown in acid sulfate soil-affected ponds exhibited higher metal concentrations (Gosavi et al., 2004). Moreover, post-harvest management of *Gracilaria* biomass is critical, as studies by Luo et al. (2022, 2023) found that decomposing

seaweed litter released 77.7% of previously absorbed Pb, 75.1% Cr, and 37.4% Cd back into the surrounding environment, thereby posing risks of secondary pollution in aquaculture ponds or coastal areas. Although heavy metal concentrations in *Gracilaria* from the present study fall within acceptable safety limits, the significant effects of cultivation site and depth, particularly for Pb and Cr, suggest that seaweed farming practices must consider both environmental quality and system design.

CHAPTER VI

CONCLUSION

Seaweeds are widely recognized as valuable marine bioresources due to their diverse applications in food, pharmaceuticals, environmental management, and blue economy initiatives. This study revealed that nearshore cultivation of *Gracilaria* sp. resulted in significantly better growth performance compared to pond-based systems; however, in both environments, surface-layer cultivation consistently produced higher biomass than bottom-layer setups. The study also found that the most appropriate time for cultivating *Gracilaria* in the coastal region of Bangladesh is from November to March, when environmental conditions are most favorable. The study also revealed that variations in cultivation site (pond vs. nearshore) and position (surface vs. bottom) significantly affected the biochemical composition of *Gracilaria* sp., including its proximate content, amino acids, phytochemicals, antioxidants, flavonoids, and mineral accumulation. These findings suggest that environment- and layer-specific cultivation strategies can enhance not only growth performance but also the nutritional and commercial value of the biomass. Optimizing these factors could allow farmers to tailor cultivation practices for desired biochemical outcomes, improving both market potential and sustainability in integrated aquaculture systems. However, despite the comparatively lower growth performance in shrimp ponds, seaweed cultivation in these systems can still offer important benefits. It can help improve water quality through nutrient uptake, reduce harmful algal blooms by shading, contribute to carbon sequestration, and promote crop diversification with minimal resource input. Moreover, pond-based systems are more accessible and manageable for small-scale farmers, making them a practical option for integrated farming. These findings provide critical insights for policymakers, researchers, and coastal farmers aiming to strengthen the blue economy through ecosystem-based aquaculture innovations. However, further research on large-scale economic feasibility, long-term ecological impacts, and farmer-level adaptation mechanisms is essential to support the broader adoption and scaling of seaweed-shrimp co-culture systems across the coastal landscape of Bangladesh.

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