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Research Article

Comparative Study on Three Selected Culture Methods for *Padina antillarum*, on the Coast of Mannar Island, Sri Lanka

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Abstract

Brown seaweed of Padina antillarum is a raw material for many industries worldwide. There is a tremendous potential to utilize this resource in various sectors in Sri Lanka, however, the naturally accessible stocks are insufficient for industrial use, and the cultivation methods of this species have not been researched. The aim of this research was the comparative assessment of the cultivation of P. antillarum using commonly practiced three cultivation methods in Sri Lanka. Raft culture, longline culture, and bag culture methods were used in the study with three replicates in an open sea area of Mannar Island. A wet weight of 100 g P. antillarum was stocked in each replicate and maintained for 42 days measuring water quality and environmental parameters. The culture systems were monitored and cleaned by removing epiphytes and debris every day. Weekly Growth Rate (WGR) and Absolute Growth Rate (AGR) were calculated and data were analyzed by one-way ANOVA. It indicated a significant difference between the three culture methods ($P<0.05$) in WGR and AGR. The highest mean WGR showed in the raft culture with 11.11±2.82 gweek^{-1} and the lowest mean WGR was shown in the bag culture as 5.19±1.67 gweek^{-1}. The highest AGR showed in raft culture was 65.69±0.89 g week^{-1} and the lowest in bag culture was 31.39±057 gweek^{-1}. The present study showed that raft culture is the best method out of the experimented three culture methods which can be practiced in future farming activities on the coast of Mannar Island, Sri Lanka.

***Keywords**: Culture methods, growth rate performance, Mannar Island, Padina antillarum, seaweed cultivation*

1. Introduction

Seaweeds are macroalgae, and there is a growing trend to use them as a key component in fertilizer, pharmaceuticals, paper manufacturing, food for humans and animals, and other significant commercial applications (Arioli *et al*., 2015; Ansari *et al.*, 2019; Purcell-Meyerink, 2021; Priyadarshini, and Rajauria, 2021). As a result, over the past 25 years, the demand for seaweed has dramatically increased on a global basis (Tullberg *et al*., 2022). The two primary sources of fresh seaweed supply for varied uses are wild stocks and aquaculture (Nayar, 2014; FAO, 2020). The total volume of seaweed harvested globally between 2000 and 2019 including that from aquaculture and the wild rose from 118,000 tons to 358,200 tons (FAO, 2020; Zhang *et al*., 2022). Asia was the major producer with 99.1% of global seaweed production in 2019 (Zhang *et al*., 2022). The attention on scaling up existing efforts, and expanding seaweed production is increasing (Buschmann *et al.*, 2017).

Padina species have properties including antimicrobial, insecticidal, antioxidant, antibiotic, anti-inflammatory, and antidiabetic activities. Therefore, *Padina* species are utilized for direct human consumption or processed into food additives, nutraceuticals, feeds, fodder, bio-fertilizer, biofuels, plant growth promoters, cosmetics, and medicines (Ansari *et al*., 2019; Babahan *et al*., 2019). *P. antillarum*, a tropical brown seaweed, can be observed in a range of habitats (Barrow *et al*., 2015). It is a fan-shaped perennial seaweed and shows its occurrence during unfavorable seasons in the form of rhizoid, filamentous thallus, or sporelings (Uddin *et al.*, 2015). They grow either epiphytically on some larger seaweeds, such as *Sargassum* spp., or attach to solid substrates (Geraldino *et al*., 2005; Win *et al*., 2013; Ansari *et al*., 2019). They are more prevalent in the months with full sunshine, widely dispersed across the tropics, and simple to identify due to their structure, which is similar to a peacock's tail. They are also quite common in South America, the tropics with cool temperate waters, and Southeast Asia (Geraldino *et al*., 2005; Ansari *et al*., 2019).

Due to the substantial demand for seaweed, aquaculture methods for growing seaweed have significantly improved throughout Asia, but there are still numerous issues that need to be resolved (Chirapart and Ruangchuay, 2022). The most popular seaweed culture methods were reported as line culture (longline, off-bottom culture, and submerged hanging culture), net culture, floating raft culture, tank culture, and pond culture (Radulovich *et al*., 2015). In addition, polythene bag culture and net bag culture methods were considered as recently developed seaweed culture techniques. These culture methods were determined based on habitat, depth of cultivation site, season and planting methods (tie-tie/direct insertion into ropes) (Veeragurunathan *et al*., 2021).

Seaweed cultivation in Sri Lanka has gained popularity as an important economic activity, particularly in the coastal regions. The country has a total coastline of approximately 1,700 km, providing suitable conditions for seaweed farming (Sutharsan *et al*., 2014).

Nearly 400 types of seaweed, including brown seaweed *P. antillarum*, are naturally present in Sri Lanka along the coastline (Coejans *et al*., 2009). However, the commercially cultivated seaweed species in the country is the exotic carrageenophyte *Kaaphycus alvarezii*, primarily cultivated on the northern and northwestern coasts (Gamage *et al*., 2021).

The Northern Province of Sri Lanka is particularly well-suited for seaweed cultivation due to favorable factors such as land suitability, suitable seasons, availability of seedlings, and favorable conditions for post-harvest practices (Ginigaddara *et al*., 2018a; Ginigaddara *et al*., 2018b). Consequently, seaweed farming has experienced significant growth in recent years, particularly in the Northern coastal areas.

While seaweed aquaculture technologies have developed considerably in Sri Lanka, challenges still need to be addressed. These challenges can vary depending on the species being cultivated and the specific techniques employed in the cultivation process. Overcoming these challenges is crucial for sustainable and successful seaweed farming in the country.

Although this species has a lot of potential for use in a variety of industrial applications, Sri Lankan stock levels of it in the natural environment are insufficient, making it an underutilized resource. Despite being a tropical island with ideal environmental conditions for seaweed cultivation in Sri Lanka, no recorded previous culture experiments on this particular brown seaweed species have been conducted. In order to allow commercial scale production and improve the standard of living in the coastal population of Sri Lanka, the current study concentrated on examining an appropriate culture method of *P. antillarum* using common seaweed culture methods.

2. Materials and Methods

2.1. Collection of Seaweed

P. antillarum was collected from Kilinochchi district of Sri Lanka. The collected seaweed samples were identified based on their morphological features, following the identification methods outlined by Coejans *et al*. (2009). Fresh seaweeds were carefully washed with seawater to remove sand and other impurities. After cleaning, the seaweeds were packed in low-density polyethylene bags along with seawater to maintain their freshness, and samples were then transported to the study site.

2.2. Study Site

Experimental culture sites of *P. antillarum* were set up on intertidal zones (Wichachucherd *et al*., 2010) of the Mannar Sea basin in Sri Lanka. The Mannar sea basin is located in the Northern Province of Sri Lanka, specifically in the Mannar District. The selected study site is directly exposed to the Gulf of Mannar and possesses moderate wave action, average wind speed, and a diverse ecosystem that includes seaweeds, salt marshes, sea grasses, and some mangrove vegetation environmental conditions such as temperature (Baghdadli *et al*., 1990; Chung *et al*., 2007; Agarwal, 2009) and salinity (Zou *et al*., 2017; Ding *et al*., 2013) were in the optimal range.

The study was conducted over a duration of 42 days, from March to April, 2021.

2.3 Experimented culture methods

Three different culture methods were employed in the study; Raft culture, Longline culture, and bag culture. Each method had its specific characteristics and procedures.

2.3.1. Raft Culture

In raft culture, floating rafts were used to support the growth of *P. antillarum*. The floating PVC rafts used had dimensions of 3 m × 3 m (Ganesan *et al*., 2019). They consisted of a PVC frame with a diameter of 2 inches. Underneath the PVC frame, a fishing net with a thickness of 0.5 mm and a mesh size of 1 mm x 1 mm was mounted (Seth and Shanmugam, 2016). This net served the purpose of preventing fish grazing and protecting the seaweed. The seaweed specimens were attached to the rafts, allowing them to grow in the water column (Figure 1). Three rafts were used as replicates in the experiment. Each raft was equipped with five planting lines made of 3 mm polypropylene rope. The lines were fixed to the raft and served as a substrate for attaching the seaweed seedlings. Along the length of each rope, five seedlings were fixed with a space of 50 cm apart (Mantri *et al*., 2017). In each raft, the average fresh weight of the planted seaweed seedlings was 100 ± 1 g.

The rafts were positioned at the seawater surface, and due to the PVC frame, they were able to float without additional floats. The weight of the raft was utilized to withstand the strong water currents.

After a cultivation period of 42 days, the entire biomass of seaweed from each raft was harvested. The harvested seaweed biomass was then weighed to determine its weight.

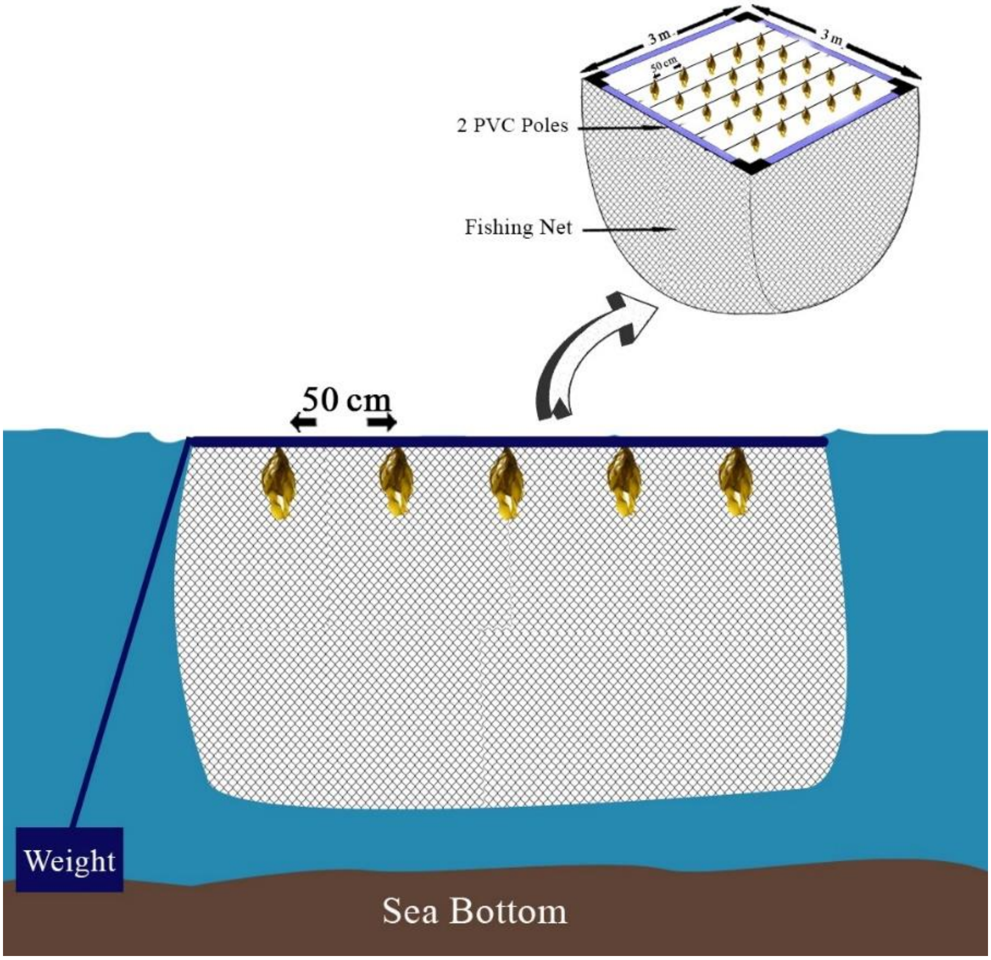

Figure 1: Raft culture

2.3.2. Longline Culture

In this particular study, an 8 mm thick and 4 m long polypropylene rope was used as the longline. Along the length of the longline rope, seaweed fragments weighing 100 ± 1g were tied. The fragments were spaced at intervals of 50 cm along the rope. This spacing allowed for the growth and development of the seaweed along the long line. Casuarina poles, weights, and empty plastic bottles (floats) were used to provide stability in the water column (Johnson *et al*., 2020; Kasim *et al*., 2020; Tullberg *et al*., 2022).

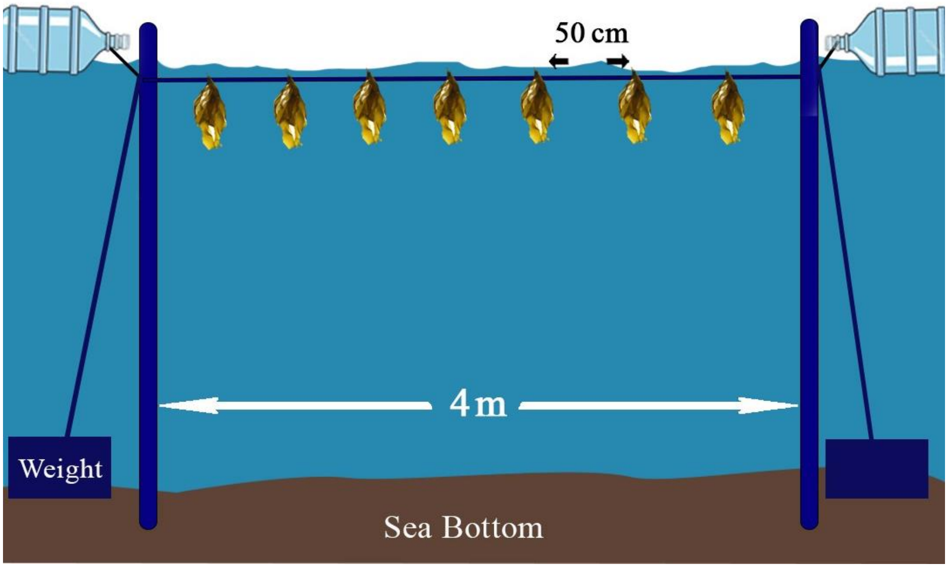

Figure 2: Longline culture

Three longlines were fixed along a line in the open sea and after a cultivation period of 42 days, the entire biomass of seaweed along the longlines was harvested. The harvested seaweed biomass was then weighed to determine its weight.

2.3.3. Bag culture method

In bag culture, bags were prepared using a fish net with a mesh size of 20 mm. The fish net with the specified mesh size ensured proper water circulation while holding the seaweed fragments within the bag. Each bag was used to contain seaweed fragments weighing 100±1g (Figure 3). Three culture bags were fixed to a 4 m long polypropylene rope with a thickness of 8 mm which was used as the main line and suspended in the water. The bags were securely attached to the main line and the space between each pair of bags was maintained at 50 cm ensuring stability and proper spacing between them. Casuarina poles, empty plastic bottles, and weights were used to stabilize the main line (Johnson *et al*., 2020; Kasim *et al*., 2020).

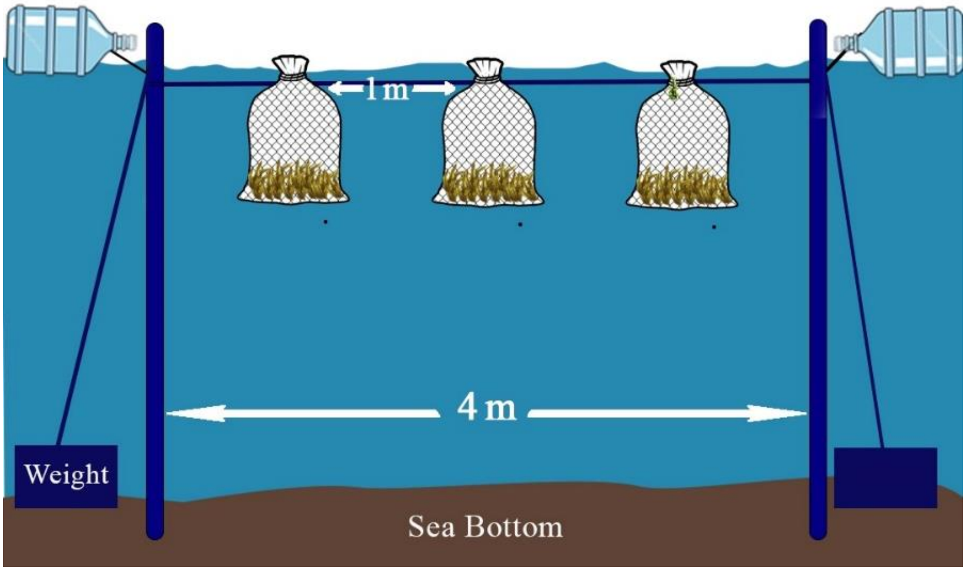

Figure 3: Bag Culture

After a cultivation period of 42 days, the entire biomass of seaweed from the three bags was harvested. The harvested seaweed biomass was then weighed to determine its weight.

2.4. Maintenance Activities

Daily farming operations were carried out to ensure the appropriate maintenance of the culture systems throughout the 42-day culture period. Fixing loose knots, and clearing dirt and weeds from culture structures were among the activities. These maintenance procedures were critical in keeping the seaweed cultures healthy and productive.

2.5. Environmental parameters

Seawater parameters were measured *in situ* using a thermometer for temperature, a refractometer for salinity, and a portable pH meter (HANNA, Model HI98190) for pH. Each measurement was performed once a week during the daytime.

2.6. Growth Parameters

2.6.1. Weekly Growth Rate (WGR)

To evaluate the growth performances of each culture system, the Weekly Growth Rate (WGR) was calculated using the following equation.

$$WGR = Wa(g) - Wb(g)$$ (Hendri *et al*. 2018)

Where:

W_a= the final weight after one week (g)

W_b = the initial weight (g)

2.6.2. Absolute Growth Rate (AGR)

The absolute Growth Rate in gd^{-1} of seaweed was measured with the following formula

$$AGR = \frac{Wt2 - Wt1}{t2 - t1}$$ (Masyahoro and Mairatu, 2010).

Where:

Wt1 = the weight of seaweed on day t1 (g)

Wt2 = the weight of seaweed on day t2 (g)

t1 = the time of observation of the first sample (day)

t2 = for the time of observation of the second sample (day)

2.7. Statistical analysis

P. antillarum wet weight was calculated in terms of mean ± standard deviation. One-way analysis of variance ANOVA was utilized to determine the significance of each parameter among different treatments. Multiple regression analysis was conducted to assess the effect of water quality parameters on the growth of the seaweed under different culture methods. All statistical analyses were performed using Microsoft Excel 2016 and Minitab 17 software. The experimental data were computed and analyzed using standard statistical techniques, with a significance level of 5%.

3. Results and Discussion

3.1. Weekly Growth Rate

Table 1 presents the weekly mean growth rate of each culture method. The weekly growth rates were found to be highly significant for all three culture methods. Statistical analysis revealed significant differences in the weekly growth rate among the three culture methods ($p<0.05$).

The highest Weekly Growth Rate (WGR) was recorded in the raft culture method, while the lowest weekly growth rate was observed in the bag culture method (Table 1). The raft culture method exhibited the highest mean WGR of 11.11±2.82 $gweek^{-1}$, whereas the bag culture method displayed the lowest mean WGR of 5.19±1.67 g/week. The availability of space in the raft culture and longline methods is greater compared to the limited space in the bag culture method. The restricted space in the bag culture method may be the main reason for its lower growth rate (Wijayanto *et al.*, 2020). Furthermore, predators have a significant impact on seaweed growth. In the research site, fish and turtles were natural predators of seaweed (Beer *et al.*, 2014). When we compare the longline culture, raft culture method, and bag culture method, the raft culture method had relatively higher protection from predators, and the space availability was higher, encouraging seaweed growth. These may potentially be the reasons for higher growth rates in raft culture.

The mean WGR of the longline method was 7.83±1.46 $g/week^{-1}$, which was lower than that of the raft culture. The increased growth rate may be due to the protection from herbivores provided in raft culture, which is not provided for longline technique. The weekly growth rates of each method gradually increased until week 3, followed by a slight decrease in weeks 6 and 7 (Figure 4). The variation in weekly growth rates of *P. antillarum* among the three different culture methods may be attributed to differences in one or more environmental factors at the study sites. In our study, the seaweeds were grown throughout the entire period without partial harvest. Self-shading can reduce nutrient uptake by seaweed, resulting in a decline in growth rate (Bambaranda *et al.*, 2019). Therefore, self-shading may be a possible reason for the reduction in growth rates observed in each culture method after week four.

Table 1. The weekly mean growth rate of *P.antillarum*

Time/Week	Raft culture (g)	StDev	Longline (g)	StDev	Bag culture	StDev.
0	100.00	1.12	100.00	1.54	100.00	1.38
1	109.36	1.96	108.00	1.45	102.70	1.13
2	121.92	2.13	117.57	2.00	109.41	3.18
3	135.78	2.86	125.73	3.09	116.42	3.51
4	150.14	2.12	134.79	1.81	122.50	3.12
5	158.44	3.10	140.99	2.64	126.71	0.77
6	166.64	3.82	146.99	3.04	131.16	3.25

This study was conducted during March and April, and previous studies have indicated that January, February, and March are most suitable for the growth and development of *Padina* species (Uddin *et al*., 2015). The favorable season of March and April in our study positively impacted the optimum growth of *Padina* within a four-week duration. The decreasing trend in the Weekly Growth Rate of each culture method may be attributed to environmental impacts.

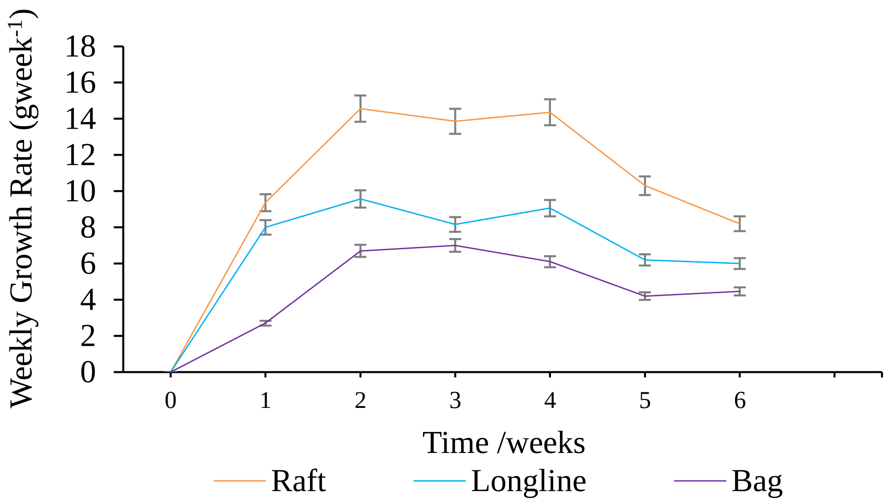

Figure 4: Comparison of weekly growth rate of *padina antillarum* in three culture methods

Regarding the culture methods, the bag and longline method is likely to cause a decrease in seaweed growth rate compared to the simple longline method. This is because it restricts water circulation in the bag culture method, which can negatively impact the growth of seaweed.

3.2. Absolute Growth Rate

The highest Absolute Growth Rate (AGR) was observed in the raft culture method with a value of 1.58 ± 0.40 gd^{-1}, while the lowest AGR was recorded in the bag culture method with a value of 0.74 ± 0.24 gd^{-1}. The longline culture method had an AGR of 1.11 ± 0.22 gd^{-1} (Figure 5).

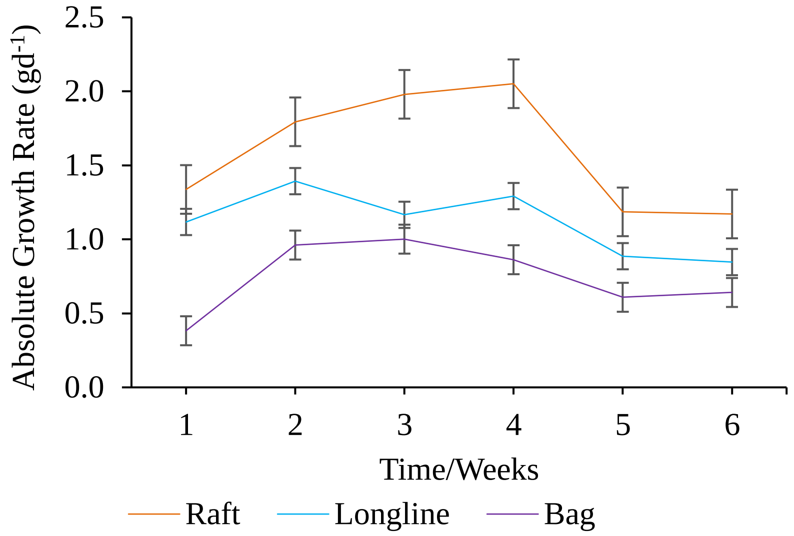

Figure 5: Comparison of absolute growth rate variations during the study period

Based on the results of the One-Way ANOVA, there was a significant difference among the three culture methods in terms of their Weekly Growth Rates and Absolute Growth Rates ($p<0.05$).

3.3. Environmental factors

The effect of environmental factors on the growth of different species of seaweed can vary. Seaweed growth can be impacted by factors such as water depth, nutrient availability, and sunlight intensity (Susilowati *et al*., 2012; Beer *et al*., 2014). However, it is generally observed that favorable levels of salinity, temperature, light, and sufficient nutrients have a direct impact on the growth and development of seaweeds (Kerrison *et al*., 2015).

3.3.1. Seawater Temperature

Figure 6 depicts the variation of seawater temperature in the study site, which ranges from 20°C to 30°C. Temperature is a crucial factor that regulates seasonal changes in the growth, development, survival, and reproduction of seaweed (Baghdadli *et al.*, 1990; Chung *et al.*, 2007; Agarwal, 2009).

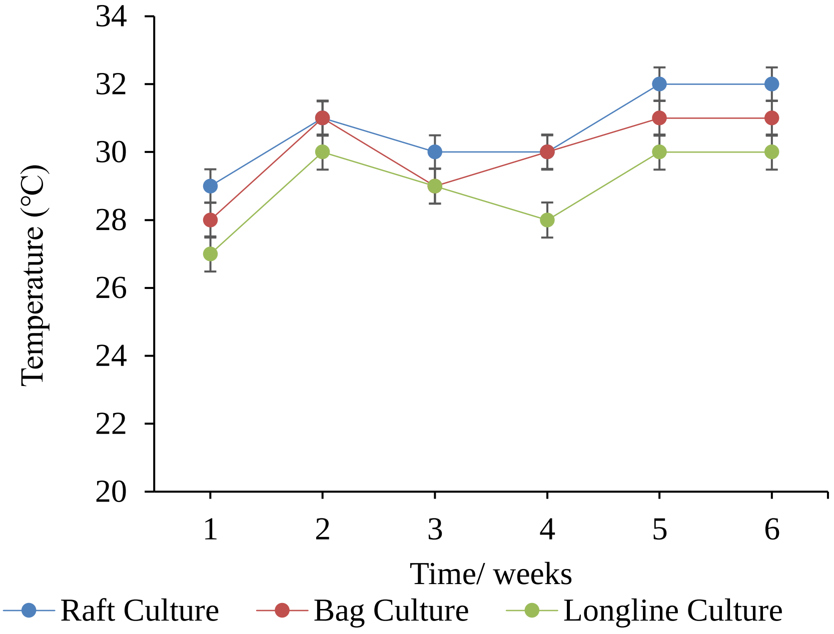

Figure 6: Sea water temperature variation in three different culture methods

Almost all seaweed species exhibit seasonal cycles of growth, development, reproduction, senescence, and dieback. Seaweed growth can be influenced by the season (Uddin *et al.*, 2015; Wijayanto *et al.*, 2020). The winter season is the most suitable period for the growth and development of *Padina*, while the spring season marks a decline in the life cycle of *Padina* following its peak season in winter (Uddin *et al.*, 2015). Although Sri Lanka may not have distinct seasons, temperature fluctuations can still have an influence on the growth of *Padina* species. In tropical regions with cool temperate seas, *P. antillarum* is dominant (Geraldino *et al.*, 2005; Ansari *et al.*, 2019). Growth of *P. antillarum* is correlated with seawater temperature and relatively higher temperatures were observed during week 6 in our study, which coincided with a lower weekly growth rate during the same period.

The temperature range of 20°C to 30°C has been reported as favorable for the growth and development of brown seaweed (Baghdadli *et al*., 1990). Uddin *et al*. (2015) also highlighted the significant role of seawater temperature in influencing the growth of *Padina* species. According to their study, a decrease in seawater temperature is more suitable for the growth and development of *Padina* species. However, it should be noted that information specifically regarding the effect of seawater temperature on the growth or development of *P. antillarum* is scarce.

Uddin *et al*. (2015), it was found that seawater temperature did not have a significant effect on any biological parameter of *P. pavonica*. However, previous study observed that a decrease in seawater temperature was favorable for the growth and development of *Padina* species in general.

Overall, while the specific effects of seawater temperature on *P. antillarum* may not be well-documented, the available records and this study suggested that a decrease in temperature can be beneficial for the growth and development of *Padina* species.

3.3.2. Salinity

Salinity is one of the important environmental factors that influence the survival and growth of organisms in the ocean. The sensitivity and tolerance levels of salinity for various seaweed species differ significantly (De Cruz, 2021). Seaweed biomass yield was negatively correlated to water salinity (Islam *et al*., 2021). The optimum salinity range for seaweed growth was recorded as 26-32 ppt (Zou *et al*., 2017). Figure 7 shows the salinity variations during the study period.

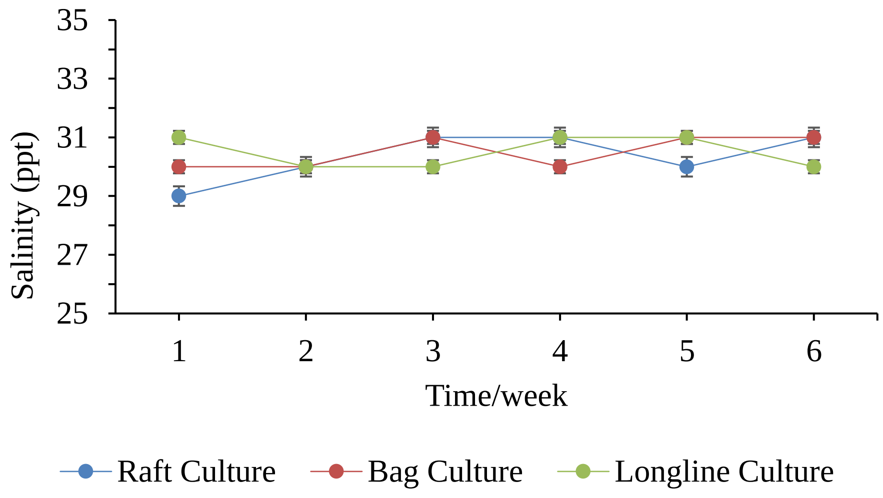

Figure 7: Salinity variation in three different culture methods

It was found that a salinity range of 25-35 ppt is suitable for brown seaweed cultivation (Ding *et al*., 2013). In our study, it was observed that the salinity ranges lay between 29 ppt to 31 ppt, and there were no significant differences among the water salinity levels of the three culture methods during the study period ($p > 0.05$). The water salinity levels of our experimental sites were at the optimum level, which is favorable for seaweed growth (Zou *et al*., 2017; Ding *et al*., 2013). However, according to Uddin *et al*. (2015), there was no significant effect of salinity on any biological parameter of *Padina tetrastromatica*.

3.3.3. pH

The pH variations of three different culture methods are shown in Figure 8, with a pH range of 7.4 to 7.9. No significant differences were found among the three culture methods of *P. antillarum* ($p > 0.05$). The uptake of nutrient compounds by seaweeds depends on the pH of the growing medium (Arthur *et al*., 2013). The metabolic processes of marine organisms are impacted by pH, which is critical for seaweed growth (Visviki and Palladino, 2001).

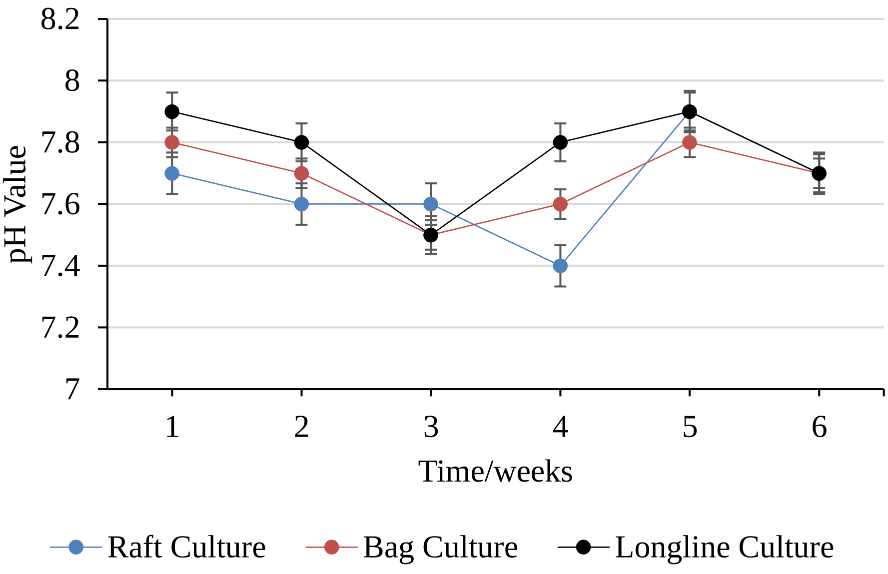

Figure 8: pH variation in three different culture methods

The three distinct culture methods did not significantly differ in the environmental parameters. Previous research carried out by Sahoo and Yarish, 2005; Kasim *et al*., 2020 have shown that, the culture method and the environmental conditions have a direct impact on the quality and productivity of seaweed. When compared to shallow water and deep water farming, the highest productivity and quality were recorded in deep water farming (Msuya, 2015).

Even though the bag culture method in our study had somewhat slow growth rates with compare to other two methods, other studies have found that it is more productive and needs less investment to produce one unit of seaweed than raft culture methods (Ganesan *et al*., 2019). Bag culture also offers the benefit of being resilient to typhoons and preventing losses during adverse weather. However, bag culture is mainly restricted to places that encounter environmental risks like typhoons and is not very well known throughout the world.

During the maintenance period, the net bags must be shaken every day or two to minimize the growth of epiphytes and accumulation of sediment, which requires more labor compared to the raft and longline methods (Selvavinayagam and Dharmar, 2017). These maintenance activities may have an impact on reduced growth compared to the other two culture methods. In our study, there was a significant difference among the three culture methods in terms of Weekly Growth Rates ($p<0.05$). The Average Growth Rate (AGR) of *Padina antillarum* was 65.69±0.89 in the raft culture method, 46.94±0.06 in the longline method, and 31.39±0.57 in the bag culture method. These findings are consistent with previous research which have conducted to compare these three culture methods, the productivity of red seaweed species (*Eucheuma denticulatum* and *Kaaphycus alvarezii*) with the highest seaweed growth in raft culture and the lowest growth in net bag culture (Sahoo and Yarish, 2005; Kasim *et al.*, 2020; Mantri, 2022).

Grazing by herbivores is a common problem in every seaweed cultivation site (Kasim *et al*., 2020). Herbivory is a key factor affecting seaweed growth rates (Yahya *et al*., 2020). In our study for each raft a fish net was mounted underneath to prevent fish grazing, whereas no such method was employed in the other two culture methods. This difference may be a key factor contributing to the relatively higher growth rates observed in raft culture (Yahya *et al*., 2020).

Overall, our findings support the notion that the raft culture method, despite being placed in shallow water, can lead to higher growth rates compared to other culture methods, and this may be attributed to the inclusion of measures to protect against herbivory.

4. Conclusions

In comparison to the other two culture methods, the raft culture approach showed greater growth performance for *Padina antillarum*. Raft culture can provide space and good protection for seaweed planting materials to thrive while avoiding from herbivores or predators.

Further research should be done to determine how these culturing techniques are affected by water current patterns and other environmental conditions. It may be possible to improve seaweed productivity overall and optimize farming methods by being aware of these extra elements.

Based on the results of this study, *P. antillarum* cultivation can begin in the coastal waters of Mannar Island in Sri Lanka. The findings offer useful information for developing and putting into practice appropriate seaweed cultivation strategies in the area.

5. Acknowledgments

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