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Specific growth rate and biomass production of seaweeds Gracilaria (*Gracilaria* spp.)

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Abstract

Gracilaria is the most promising seaweed in the fishery industry nowadays and can tolerate different environmental conditions. This study aimed to evaluate the specific growth rate and biomass production of *Gracilaria* spp. in marine water environments using different culture techniques like vertical rope, floating monoline bamboo, plastic bottles, and the half-side bamboo technique. The experiment was laid out in a randomized complete block design (RCBD), where each treatment had three replications. A one-way ANOVA was used to test if there was a significant difference among the four treatments. Results have shown that all the seaweed seedlings had 100% survival across the different treatments. The specific growth from floating-monoline bamboo techniques is 83.73 g/day (v/v) and the width of clusters is 16.78 cm/day. The vertical rope technique got the highest result in length (15.50 cm/day). The result is significant since the computed F-value was greater than the tabular value. Results showed that the floating-monoline bamboo technique was the best among four techniques used in culturing Gracilaria (*Gracilaria* spp.) in marine water environments. The results of the study provide highlights on the possible adoption of culture techniques to cater to the fast-growing seaweed production among fish farmers and might be supported by other future studies.

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Introduction

Biomass production has been the most important topic recently in some seaweed species, particularly in the Gracilaria culture. The Gracilaria are marine species of seaweed belonging to the division Rhodophyta of the Gracilariaceae family. Their appearance is characterized by an erect, fleshy, and succulent thallus, which consists of a small discoid holdfast with lateral branches. It is bushy, branching seaweed comprised of rounded branches. Blades of gracilaria are usually red but can be brownish, green, or almost black. Growth occurs in the apical meristem, located at the tip of each branch. It has the ability to regenerate, where each time a fragment is broken off, it can grow and develop into a new individual. So far, hundreds of species have been found around the world, many of which are wild harvested and cultivated for their important uses.

In the Philippines, gracilaria is locally known as "*gulaman*". It is fast-growing seaweed that has a wide range of tolerance for changing environmental conditions. It is an ideal candidate for aquaculture due to its warm-water growing season, quality and relative ease of propagation, fast growth rates, existing and potential commercial applications, and good agar yield (Buschmann *et al*., 2009). Gracilaria (*Gracilaria* spp.*)* is characterized by an erect, fleshy, and succulent thallus that consists of a small discoid holdfast with lateral branches. The thalli of most species are cylindrical; some are compressed, and some are foliose. It has a variable morphology or structure, which differs by species, strain, and growing conditions. It is bushy, branching seaweed comprised of rounded branches. Blades are usually red but can be brownish, green, or almost black. Blades grow from a flattened disc that is formed from Many of these species are found in Asia, particularly China, Japan, Taiwan, Indonesia, and the Philippines. Gracilaria reproduces sexually by means of fertilization of the male thallus, which produces spermatangia, and the female thallus, which produces carpogonia. It can also reproduce asexually through "vegetative propagation". A single individual has the capacity to become hundreds or thousands of individuals through a process called continual fragmentation. Each time a fragment is broken off, it can grow and develop into a new individual. This is possible because growth occurs in the apical meristem, where the seaweed grows from the tips of its branches. Each tip has the capacity to grow and branch into its own blade. Vegetative propagation is the most common means of culture since it is quicker, easier, and more efficient than starting from spores, and it allows for consistency as all blades in a culture are genetically identical. This is very important if the blades are being grown for a specific characteristic, such as agar consistency, specific morphology, or favorable growth rates and biomass yields.

Gracilaria has a wide range of tolerance for changing environmental conditions. It is commonly found in estuaries or bays, often in intertidal or shallow subtidal areas less than 1 meter deep, either attached to rocks or free floating. It is often found in eutrophic areas, which are environments rich in nutrients but low in oxygen. *Gracilaria* species are euryhaline, which means they can tolerate a wide range of salinities, from about 10 ppt to 40 ppt, though they grow best in the 25 ppt to 33 ppt range. They can survive temperature ranges from 10°C to 35°C, but have an optimal range of 24°C to 30°C. The Bureau of Fisheries and Aquatic Resources Region 2 (BFAR) explain the value of cultivating *Gracilaria*. According to BFAR (1998) National Seaweed Coordinator Salvacion Ferrer, the by-product of *Gracilaria* is more expensive in the international market compared to *Eucheuma,* which is the top seaweed variety being produced and exported by the country.

The principal product of Gracilaria is agar. Industrial applications are dominated by three quality grades, a) sugar reactive agar, b) standard agar and c) food grade agar. In the sugar reactive agar, the gels are stronger as a function of sugar concentration. Standard agar is recognized because the gel has the temperature, consistency and structure for microbiological purposes. The food grade agar designates any kind of agar not meeting the requirements for sugar-reactive or bacteriological agar. It is extracted from a wide variety of *Gracilaria* species.

Market demands for species of *Gracilaria* to produce agar have increased markedly in the past decade (1999–2009). Table 2 summarizes several important market parameters. Global agar production increased from 7 500 to 9 600 tons, with sale prices of USD 17/kg increasing, on average, to USD 18/kg. The world agar sale value increased, therefore, from USD 128 million in 1999 to USD 173 million in 2009. In 1999, about 63 percent of the total agar production was produced by *Gracilaria*. In 2009, the relative importance of *Gracilaria* had increased to 80 percent of the total agar production. These data indicate that although the industrial agar growth has been modest, it has been enough to generate the cash flows necessary to support the overheads needed for regulatory reform and the capital investment needed to improve plants and equipment. Selling price increases have generally been adequate to offset seaweed, energy, and chemical costs. In addition, the cultivation of *Gracilaria* has provided enough raw materials to support expansion. In fact, *Gracilaria* has grown in importance for extracting agar between 1999 and 2009, while Gelidium is declining in importance.

The demand for seaweeds on the world market is increasing, so the development of this commodity is encouraged. It is a welcome economic venture for the coastal communities, as this will ease pressure on the fast depletion of coastal and marine fisheries. It provides better income opportunities and an additional source of income to improve the quality of life of fishermen. Furthermore, the development of the seaweed industry will boost export earnings and contribute to the economic stability of the country.

Furthermore, *Gracilaria* is highly valued seaweed. Their market acceptance has increased because of their important uses. According to BFAR, the by-product of *Gracilaria* is more expensive in the international market compared to Eucheuma, which is the top seaweed variety being produced and exported by the country. Moreover, it is known in other countries as a popular ingredient in salads, usually sold fresh or salted in some restaurants.

It becomes more popular in the aquaculture world because it is fast-growing seaweed and can tolerate different environmental conditions.

Different cultivation techniques had given some awareness to fishermen, particularly in the culture of *Gracilaria,* knowing their previous uses and applications. The culture of *Gracilaria* is through its vegetative propagation, a process of planting *Gracilaria* using an apportion of their branches, which is the most common means of culture since it is quicker, easier, and more efficient than starting from spores. There have been several studies that have documented that in order to attain sustainable production, proper site selection is important to consider, and effective measures must also be adopted in order to get a good result. The information gives assurance that culturing seaweeds, especially Gracilaria, is one of the fastest-growing industries in the Philippines and in other foreign countries and needs more attention from all people because of the important uses mentioned. This will help in the improvement of the economic condition of the country as well as impart knowledge to the fishermen about culturing *Gracilaria*. According to McHugh (1991), large beds of *Gracilaria* usually grow best where there is rapid water movement in the eulittoral zone or just below it in the beginning of the sublittoral, on sandy or muddy sediments that are protected from waves (FAO, 2016).

Nowadays, some recent changes are being employed for some fish farmers in order to improve the production and among of these techniques are bottom culture, raft culture, stake-rope culture and pond culture. Pond culture can be divided into two systems, monoculture and polyculture with shrimp and other species. The varieties of culture methods can be adapted for different areas. Fixed bottom monoline method is the technique being used in the culture of seaweeds. It has many advantages over the other methods used in the past *e.g., the* net method. It is cheaper and easier to install and maintain, although it is not as intensive as the net method. In this method, the stakes, which are driven deep into the substrate, are spaced 10 m apart at a one-meter interval in rows. Another is the raft or floating bamboo method, which is applicable in areas where space requirements for the fixed bottom method are not available or where this method does not seem to work due to associated problems such as intense grazing, seasonality in growth, disease, and changes in the degree of water movement brought about by monsoons. The advantage of this method is that grazing by the bottom and associated animals are minimized or eliminated because the plants are raised way out of reach of the grazers, and the plants, being near the surface of the water column, are exposed to more moderate water movement caused by waves.

To contextualize the importance of *gracilaria* as a sea farming commodity, the National Coordinators of the Regional Sea Farming Project recommended the dissemination of its culture and processing technology through a regional training and demonstration activity as a means to further increase the opportunities to develop the sea farming industry of the region.

But in spite of the challenges mentioned, some fishermen showed some eagerness to learn how to increase biomass production in a more efficient, effective, and cheaper way. Indeed, the willingness that has been shown is a promise to be used to discover how the vertical rope, floating monoline bamboo, indigenous material, plastic bottle, and half-side bamboo techniques are performed.

Generally, this study aimed to determine the specific growth rate and biomass production of Gracilaria *(Gracilaria sp.)* using different culture techniques with the following indices: a) survival rate; b) specific growth rate; c) total biomass in the four treatments; and d) statistical difference of the four treatments, namely: T_0-Vertical Rope Technique, T_1-Floating Monoline-Bamboo Technique, T_2-Plastic Bottle Technique, and T_3-side Bamboo Technique.

Materials and methods

Research design

The experiment was laid out in a randomized Block Design (RCBD). The study was conducted in a marine environment using different culture techniques, such as vertical rope, floating monoline method, plastic bottle, and half-side bamboo technique, with three (3) replications. Each method contains 10 samples, for a total of 120 *Gracilaria* seedlings.

Research environment

The study was conducted in the marine water environment, which was located at Kahayag, Pangangan, Calape, Bohol, situated 350 meters away from the shoreline without the reach of changing tides at a depth of 1.0 meter. The area was surrounded by aquatic trees. The area is suitable for cultivating *Gracilaria* seaweed since it is free from pollution and any disturbances. The marine water environment is composed of sandy bottom soil that is very suitable for the cultivation of Gracilaria *(Gracilaria* spp.*).*

Research subject

The genus *Gracilaria* belongs to the family *Gracilariaceae,* order *Gracilariales*, in the division Rhodophyta. It is one of the most productive varieties of seaweed grown in the Philippines. It is characterized by an erect, fleshy, and succulent thallus, which will consist of small discoid holdfasts with lateral branches. The thalli of most species are cylindrical; some are compressed, and some are foliose. Gracilaria is found on the tidal mudflats, in sandy, muddy basins with scattered rocks, where it is rarely out of the water. It occurs in the sea at depths of up to 25 meters, although 98% of the population is found between 0.5 and 10 meters, with an optimum depth of 3 to 4 meters. *Gracilaria* species are euryhaline, which means they can tolerate a wide range of salinities, from about 10 ppt to 40 ppt, though they grow best in the 25 ppt to 33 ppt range. They can survive temperature ranges of 10–35 °C, but have an optimal range of 24–30 °C. Light intensity does not appear to be a basis factor since they can survive with very low lightning. As *Gracilaria* spp. lives preferentially in sandy areas; it needs to be with great variations in turbidity, which limit photosynthesis by reducing light intensity. They can survive an entire day out of the water, but in sunlight, they resist for no more than an hour.

Research materials

In the study, the researchers used the following materials: 120 cuttings or seedlings of *Gracilaria* seaweeds, which served as the population; one roll of rope used in constructing vertical rope and floating-monoline bamboo techniques; one roll of straw used to tie *Gracilaria* seedlings; bamboos used in constructing floating-monoline bamboo and half-side bamboo techniques; forty-five (45) 1.5 L empty bottles used for plastic bottle technique; plastic floaters and buoys used as floaters; and nails, hammer, saw, and knife were used in constructing the techniques. Researchers also used litmus paper in testing the acidity of the water; a refractometer in measuring the salinity of the water; a thermometer in getting the temperature of the water; a caliper and ruler in measuring the length and width of Gracilaria; a*racilaria; a* plastic basin in handling the samples; and recording sheets (a notebook) and writing pens in gathering data on water parameters and measurements of the samples.

Research procedures

During site selection, a suitable site for cultivating *Gracilaria* is composed of sandy bottom soil, away from strong waves, pollution, predators, and any disturbances. The recommended water parameters must have a salinity range of 25 ppt to 35 ppt and a temperature range of 24°C to 30 °C. The preparation of planting materials for the construction of the four techniques and gathering and choosing ideal *Gracilaria* seedlings to be cultured were done.

For vertical rope technique

The 2m-long ropes suspended in the bottom of the seabed were anchored with the use of pointed steel on each side, where the opposite side of the rope contained a buoy to raise it upward. Two (2) ropes, 1m each, were attached to the suspended rope, with a floater leading it to stand vertically. Five (5) seedlings of *Gracilaria* were attached and tied using plastic straw to each vertical rope at a distance of six inches apart.

For floating-monoline bamboo technique

A 2x1 m bamboo cage was constructed (5 inches in diameter) as floaters with two ropes measuring 2 meters each arranged horizontally at a distance of 10 inches apart. There were five seedlings of *Gracilaria* seaweed attached to each rope, tied using straw, and arranged at a distance of six (6) inches apart. Anchors are used to keep the bamboo cage in place.

For plastic bottle technique

A 1.5-liter plastic bottle was used with two small holes at the center where the straw is inserted. This served as the anchor for the seaweed seedlings. Seedlings of *Gracilaria* were then attached and tied to the straw inserted in the bottle. A plastic bottle contains only one seedling, and there were ten seedlings per replication, arranged well with a distance of one foot between each. The researchers have erected two-pointed bamboo sticks (12 inches) alternately on the bottle to prevent it from moving away from its place.

For half-side bamboo technique

A 3 meters of bamboo was used (5 inches in diameter), which was cut into half and contained ten pairs of holes with a distance of six inches each where straw was inserted and submerged in the seabed. This has been used as an anchor for the cultivation of seedlings. Ten seedlings were attached and tied to the straw, which was then placed in the bamboo.

Gathering of water parameter

The measurement of water parameters was done using the corresponding measuring instrument. It was done twice a day, early in the morning and late in the afternoon. The litmus paper used to test the acidity of the water; a refractometer was used to measure the salinity; and a thermometer for water temperature. This was done to determine the sudden change in the water parameters during the conduct of the study that may affect the growth and survival of *Gracilaria*.

Sampling

The study lasted for 45 days. Sampling of *Gracilaria* was done only once during the low tide early in the morning at the last day or the termination period of the study to determine the growth and survival of the cultured *Gracilaria* using four techniques. Plastic basin has also been used to handle samples so as to avoid damages. Samples were measured individually using measuring instrument. Caliper and ruler were used to measure the length and width of the samples and digital weighing scale was used to measure its weight. After measuring the samples, data were recorded.

Care and maintenance

Cleaning of ropes, bamboo cages, plastic bottles, and floaters, as well as the environment, was done every five days by means of brushing and removing barnacles, nuisance species, and excess leaves attached to the techniques and samples to ensure good growth.

Statistical treatment

To test if there was a significant difference among the treatments, a one-way ANOVA was used, and the results were analyzed using SPSS, or Statistical Packages for the Social Sciences.

Results and discussion

Percent survival

The percent survival of *Gracilaria* was recorded to determine how many percent of the samples survived during the course of the study. It was done during the termination of the experiment. It was found out that all *Gracilaria* samples cultured in vertical rope, floating monoline bamboo, plastic bottles, and half-side bamboo techniques survived from the beginning of the experiment until the termination period, with a percent survival rate of 100%. The result showed that *Gracilaria* can survive in different culture techniques since it is fast-growing seaweed and can survive in different environmental conditions. Culture techniques used in the study, such as vertical rope, floating monoline, plastic bottle, and half-side bamboo, have shown effectiveness during the conduct of the experiment since all of the samples survived, which could be beneficial in culturing *Gracilaria*. The cultivation of *Gracilaria* utilizes the marine environment. However, recent studies have stated that artificial cultivation of *Gracilaria* is carried out by taking into account its biological characteristics. Suitable sites must be selected and effective measures must be adopted in order to get good results (FAO, 2016).

Water parameters

Environmental parameters such as salinity and temperature were gathered and recorded every morning and afternoon. Researchers used litmus paper to test the acidity of water; a refractometer has also been used to measure water salinity; and a thermometer to measure the temperature of the water. A tolerable range of environmental parameters must be observed because they can possibly affect the growth and survival of *Gracilaria*. The standard water parameters of *Gracilaria* are a salinity of 25 ppt to 35 ppt and a temperature of 24 °C to 30°C. Environmental parameters changed every week during the duration of the study. The water parameters increased to 35.4 ppt. during the fourth week. Also, the temperature slightly increased to 30.3°C. There was a slight increase in the water parameters, yet *Gracilaria* grew and survived. The sudden increase in water parameters was tolerable for the growth and survival of Gracilaria. However, it is said that *Gracilaria* has a wide range of tolerance for changing environmental conditions (Marine Aquaculture, 2019). It can be cultured easily since it is fast-growing seaweed and can survive at different water parameter ranges.

Specific growth rate

The specific growth rate of the *Gracilaria* was evaluated in terms of length, width, and weight. In terms of length, the *Gracilaria* in the vertical rope gained the highest specific growth rate of 15.50%/day, while the floating-monoline bamboo technique gained 12.27%/day. *Gracilaria* in the plastic bottle technique gained 3.47% per day. Those using the half-side bamboo technique gained the lowest specific growth rate of 3.25% per day. In terms of width, the *Gracilaria* in the floating-monoline bamboo technique gained the highest specific growth rate of 16.78%/day, while the vertical rope technique gained 16.57%/day. Gracilaria in the plastic bottle technique gained 5.15%/day. Those using the half-side bamboo technique gained the lowest specific growth rate of 5.03% per day. In terms of weight, the *Gracilaria* in the floating-monoline bamboo gained the highest specific growth rate of 83.73%/day, while in the vertical rope technique it gained 56.88%/day.

Table 1. The biomass production (growth increment) *Gracilaria* using different culture techniques

Treatment	Replication total			Replication mean		
	I	II	III	I	II	III
	Length(cm)	Width (cm)	Weight (g)	Length (cm)	Width (cm)	Weight (g)
T_0-Vertical rope	20.92	22.37	76.79	6.97^{a}	7.46 ^{b}	25.60 b
T_1-Floating-Monoline bamboo	16.57	22.66	113.03	5.52 ^{b}	7.55 ^{a}	37.68 ^{a}
T_2-Plastic bottle	3.45	6.95	31.48	1.15 ^{c}	2.32 ^{d}	10.49 ^{c}
T_3-Half-side bamboo	4.39	6.79	32.72	1.46 ^{d}	2.26 ^{c}	10.91 ^{d}
Standard deviation	8.75	9.03	39.17	2.92	3.01	13.06
CV	0.77	0.61	0.62	0.77	0.61	0.62

@0.05level of significance

Table 2. Test of hypothesis

Source Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F-value	Tabular F(0.5)	Decision	Interpretation
			Weight				
Between	7577.24	3	2525.75	19.39	4.07	Reject Ho	Significant
Within	1,042.26	8	130.28				
			Length				
Between	347.76	3	115.92	44.76	4.07	Reject Ho	Significant
Within	20.75	8	2.59				
			Width				
Between	402.83	3	134.28	59.95	4.07	Reject Ho	Significant
Within	17.95	8	2.24				

Gracilaria in the half-side bamboo technique gained 24.24% per day. Those using the plastic bottle technique gained the lowest specific growth rate of 23.31% per day. This is because the samples cultured under the techniques situated at the bottom of the sea are prone to predators and other disturbances that affect the growth of the samples compared to those situated along water with moderate water movement and away from any disturbances. The specific growth rates of *Gracilaria* in terms of length, width, and weight in the four techniques were inversely proportional to each other.

Biomass production

In terms of length, the *Gracilaria* in the vertical rope gained the highest growth increment of 6.97 cm, while the floating-monoline bamboo technique gained 5.52 cm (Table 1). Gracilaria in the half-side bamboo technique gained 1.46 cm. Those using the plastic bottle technique gained the lowest growth increment of 1.15 cm. In terms of width, the *Gracilaria* in the floating-monoline bamboo technique gained the highest growth increment of 7.55 cm, while the vertical rope technique gained 7.46 cm. *Gracilaria* in the plastic bottle technique gained 2.32 cm. Those using the half-side bamboo technique gained the lowest growth increment of only 2.26 cm. In terms of weight, the Gracilaria in the floating-monoline bamboo gained the highest growth rate of 37.68 g, while the vertical rope technique gained 25.60 g. *Gracilaria* in the half-side bamboo technique gained 10.91 g. Those using the plastic bottle technique gained the lowest growth increment of 10.49 g. The growth increments of Gracilaria in terms of length, width, and weight in the four techniques were inversely proportional to each other. Samples cultured in the techniques situated at the bottom of the sea are more susceptible to any disturbances that affect the growth of the samples compared to those situated along the water surface with moderate water movement and away from any disturbances. However, all of the techniques showed an increase in the termination period. According to McHugh (1991), large beds of *Gracilaria* usually grow in the eulittoral zone, or just below it in the beginning of the sublittoral, on sandy or muddy sediments that are protected from waves. On the other hand, FAO (2016) said that it grows in a rapid movement of water where the rate of water exchange is good. In relation to this, the success of farming *Gracilaria* is highly dependent on the selection of an appropriate site since *Gracilaria* species have different environmental preferences (Trono, 1988).

Sustainable sites must be selected and effective measures must be adopted in order to get good results (FAO, 2016).

Test of hypothesis

At the 5% level of significance with 3 and 8 degrees of freedom, the tabulated F-value is 4.07 (Table 2). Since the computed F-value of 19.93 in terms of length is greater than the tabulated F-value, the null hypothesis was rejected. In terms of significance, at a 5% level of significance with 3 and 8 degrees of freedom, the tabulated F-value is 4.07. Since the computed F-value of 44.76 is greater than the tabulated F-value, the null hypothesis was rejected. In terms of weight, at a 5% level of significance with 3 and 8 degrees of freedom, the tabulated F-value is 4.07. Since the computed F-value of 59.95 is greater than the tabulated F-value, the null hypothesis was rejected. This implies that there is a significant difference among the four (4) culture techniques in the growth increment of the Gracilaria (*Gracilaria spp.*) in terms of length, width, and weight. Results showed that using the floating-monoline bamboo technique gained the highest growth increment.

Conclusion

The *Gracilaria* seaweeds that are cultured in the floating-monoline bamboo technique gained the highest increment in terms of weight and width, while those also cultured in the vertical rope technique got the highest increment in terms of length. Those situated in the sea bottom, such as plastic bottles and half-side bamboo techniques, were not applicable because they were very prone to predators and other disturbances. However, it was also found out that all samples cultured using the four techniques survived. The results imply that all of the techniques, including floating monoline, have the potential for adoption and mass production.

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