

Growth, Survival, and Colloidal Properties on the Cultivation Trial of *Halymenia durvillei* Bory De Saint-Vincent, 1828

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ABSTRACT

Halymenia durvillei is a marine red alga that is a carrageenophyte species and a potential alternative source of carrageenan. The cultivation trial of *H. durvillei* was conducted in Pandong Bato Marine Sanctuary Puente, Carmen, Cebu to compare its growth, survival, and colloidal properties using the modified fixed-off bottom method and cage culture method. Both methods showed increased growth rates ranging from 13.6-28.7% and 311-718%, respectively, after a forty-five days culture period. The cage culture method showed higher growth than the modified fixed-off bottom method. Results showed a significant difference in growth rate using t-test at a 5% level of significance (0.0033, $p>0.05$). The *H. durvillei* had adapted to the culture site, and the two methods had a 100% survival rate. The colloidal properties of *H. durvillei* extract have a moisture content, carrageenan, gel strength, and viscosity of $87.72\pm0.85\%$, $22.52\pm4.13\%$, $55.67\pm1.63\text{g cm}^{-2}$, $53.67\pm3.01\text{cP}$, respectively. The water parameters recorded in the culture site during low tide and high tide were salinity, temperature, pH, current, dissolved oxygen, nitrates, and phosphates which ranged from 30-35ppt, 25-30°C, 6-23-9.39cm/s, 2.65-4.98ppm, 0.21-0.25mg/l, and 0.01-0.02mg/l, respectively. Results suggest that this red seaweed could potentially be used as raw material for carrageenan production aside from *Kappaphycus alvarezii*.

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1. Introduction.

The *Halymenia durvillei*, commonly known as “red sea lettuce,” is one of the targeted seaweed species for cultivation and resource development in the Philippines. This red alga is known locally as buwak-saang, lablabig, gayong-gayong, gargarnatis, gamet, aragantilek, and guraman. It is one of the favorite seaweeds consumed directly or used as add-ons to “tinuwa/tinula,” a boiled fish with spices as a soup by the Filipinos. This red seaweed was first described by Bory de Saint-Vincent (1828) from New Ireland, Papua New Guinea, and has been reported from various localities around the Pacific and the Indian Oceans (Guiry et al. 2003). It is characterized by large, bushy thalli that may grow up to 35cm.

It is soft, cartilaginous, and slimy when fresh and found in the upper subtidal attached to the rocky substrate by its discoid holdfast (Trono and Largo, 2019).

In the Philippines, Fantonalgo (2018) conducted preliminary study on biogeography and diversity of red alga *Halymenia* in Manila Bay and found few species of *Halymenia durvillei* in Brgy. Bucana, Ternate, Cavite with density of 0.0048n / 2500m² and relative density of 0.2500d/Dx100. This red alga is also abundantly found in Sta. Fe, Bantayan Island, Cebu (Torrevillas, Pers. Comm. 2014) and Brgy. Tubigan, Initao, Misamis Oriental (Kho et al., 2016). Trono (2013) conducted pilot-scaled production of *Halymenia durvillei* in land-based facility using vegetative propagules and spores. The findings led to conclusion that the study is technically feasible through the optimization of parameters such as light to increase yield and the establishment of an open sea support system for grow-out of carpospores cultures.

Halymenia durvillei is one of the seven emerging red seaweeds from the Philippines as a potential alternative source

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of lambda-carrageenan and r-phycoerythrin (Trono and Largo 2019; Hurtado et al. 2020). Phycoerythrin is an important food and cosmetic colorant, a therapeutic agent owing to its immunomodulating and anti-cancer activity, and a fluorescent agent, among others (Bermejo Román et al. 2002; Spolaore 2006). Lambda-carrageenan is widely used in the food industry as an emulsifier (Fenoradosoa et al. 2009), but it can also act as an allergy suppressant against certain food products (Tsuji et al. 2003). In terms of its carrageenan and biochemical composition, Kho et al. (2016) obtained $28.41 \pm 2.77\%$ for carrageenan yield, $12.40 \pm 0.05\%$ for moisture content, $13.02 \pm 0.33\%$ for protein, 1.29 ± 0.06 for fat, 53.65 ± 0.07 for carbohydrate, and 39.54 ± 0.52 for ash of *H. durvillei*. As far as literature cited, no study was found about its culture in the open field in Cebu Islands.

Seaweed culture ranks no.1 in terms of raw dry production among the marine-based products in the country (Hurtado et al., 2001). In addition, PSA (2020) data shows that seaweed is the main commodity produced, followed by milkfish and tilapia in the aquaculture fisheries subsector. Furthermore, seaweed came 2nd on export value which went up from US\$ 207million (13%) in 2018 to US\$ 250 million in 2019, which translates to a 22% share of the total export earnings for that year. Seaweed farming is presently one of the most productive of the varied mariculture activities undertaken in the Philippines. The fixed-off bottom method is a widely used method of seaweed farming for *Kappaphycus alvarezii* in the Philippines (Trono, 1993). The Fixed off-bottom monoline method is used in the shallow sub-tidal waters of one-foot depth at the lowest tide and generally considered to be environmentally benign compared to other forms of mariculture (Bryceson, 2002).

Due to its economic importance as human food, as a possible source of lambda-carrageenan, medicinal, cosmetics, and other industrial uses, hence there is a need to study the propagation technique of this seaweed, *H. durvillei*, to sustain the demand for this seaweed as raw material for its wider scope uses. The study on the cultivation trial of *H. durvillei* in the open field will be conducted since there was no study yet in this aspect. This study could contribute to the lack of information on its growth rate and natural standing stock availability and can be an alternative for *Kappaphycus* culture. Furthermore, it can provide livelihood options to the fisherman and other prospective individuals and groups interested in the cultural technology of this species.

2. Materials and Methods.

2.1. Culture Site.

The mariculture trial of *Halymenia durvillei* was conducted in Pandong Bato Marine Sanctuary, Puente, Carmen, Cebu (Latitude: $10^{\circ}37'00.2''N$, Longitude: $124^{\circ}01'43.1''E$). The sanctuary lies on the Northeastern coast of Cebu Province which is bounded in the North by the Municipality of Catmon; on the South by Danao City; on the West by the Municipality of Tuburan, and on the East by the Camotes Sea. The culture site was 200 meters away from the shoreline (Figure 1). The water depth ranged from 2 meters to 5 meters during low tide and high tide,

respectively. The substrates are sandy-rocky with patches of coral heads and some species of seaweeds (*Halophila ovalis*, *Sargassum* sp., *Enhalus acoroides*, etc.).

Figure 1: The culture site of *Halymenia durvillei* at Pandong Bato Marine Sanctuary Puente, Carmen, Cebu (Latitude: $10^{\circ}37'00.2''N$, Longitude: $124^{\circ}01'43.1''E$).



Source: Authors.

2.2. Research Materials.

The materials used in the study were *Halymenia durvillei*, ice bucket, polypropylene rope no. 16, net bags, sinkers, bamboo, plastic screen (3 x ½), cable tie no.10, Shark Monoline Nylon 40mm, and hand tools such as hammer, knife, scissors, handsaw. The devices used for sampling and monitoring the water parameters were the D.O. meter, refractometer, pH meter, thermometer, surface drogue, amber bottles, digital weighing balance, and slit board.

2.3. Procurement of Plant Material.

The *Halymenia durvillei* were collected from Sta Fe. Bantayan Island, Cebu. The area where the seaweeds were collected is clear and free from the source of pollution. The salinity ranges from 30 to 33 ppt, and the temperature ranges from 26 to 28 °C. The bottom substrates are firm, rocky or sandy and well protected from strong waves and winds, and dotted by coral reefs, inhabited by hundreds of different kinds of fishes and invertebrates. The *H. durvillei* are attached to rocky substrates in shallow areas 2 to 5 meters deep. Collected red seaweeds were placed in an ice bucket and transported in a closed van to the culture site.

2.4. Cultivation Methods.

Two culture methods were used for this study: the modified fixed-off bottom method and the cage culture method.

2.4.1. Modified Fixed-off Bottom Method.

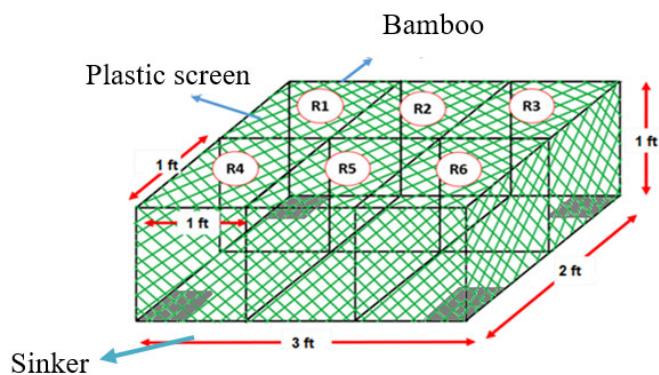
The traditional fixed-off bottom method was constructed and modified. The *Halymenia durvillei* were placed inside a net bag instead of tying directly to the cultivation line. The method

has a three-meter cultivation line using polyethylene rope No. 16 and net bags were placed thirty-five meters apart to ensure free movement. The cultivation line is 0.5 meters away from the bottom to keep the net bags from hanging and to avoid them from settling down.

2.4.2. Cage Culture Method.

The cage culture method is a new method for the cultivation of *Halymenia durvillei* (Figure 2 and 3). The cage was constructed and designed based on the external features of *Halymenia durvillei* with branched thalli, toothed margins, spinose proliferations on the blade, and a firm gelatinous texture. The materials used were bamboo, plastic screen (3 x ½), cable tie no.10, Shark Monoline Nylon 40mm, and other hand tools. It has a surface area of twenty-two (22) feet with six compartments measuring one square foot. Moreover, the cage culture method is submerged in the ocean considering the biological characteristics of the cultured seaweed.

Figure 2: Diagram of the cage culture method.



Source: Authors.

Figure 3: Cage culture method at Pandong Bato Marine Sanctuary Carmen, Cebu.



Source: Authors.

2.5. Extraction of Colloidal Properties.

2.5.1. Moisture Content.

Raw seaweed was prepared by sun drying the fresh *H. durvillei* collected from cultivation. Sun-dried *H. durvillei* was washed

thoroughly with distilled water for two reasons; to remove contaminants and debris of the seaweed. Wet *H. durvillei* was then dried in the oven at 60°C for 15 to 16 hours to remove the excess moisture. To determine the moisture content, the following formula was used;

$$\% \text{Moisture} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.5.2. Carrageenan.

The extraction process started with sample cleaning and was dipped in water for 24 hours. Five grams of the sample was boiled at 70 to 90°C using 100ml of aqueous NaOH at pH 5 to 9 for three hours. The extract was filtered in the hot condition and was precipitated with ethanol. The extracted carrageenan was dried in an oven at 60°C for 24 hours, and then it was weighed. The carrageenan content was determined using the formula by Winarno, (1990) as follows:

$$\% \text{carrageenan} = \frac{\text{carrageenan weight}}{\text{sample weight}} \times 100$$

2.5.3. Gel Strength.

Dry carrageenan was dissolved in aquadest at a concentration of 15% through heating. To obtain the gel strength, 10 ml of 1.5% carrageenan solution was poured into three cm-diameter glass with solution height ranging from 1.2 to 1.4 cm, set aside for one night at room temperature, after which the glass was put on a balance. A stainless cylindrical rod (cross-sectional area of 0.786 cm²) was laid on the sample and then pressed with hand until the gel was broken and the weight was recorded. Three replications were used for the same sample. Gel strength is the difference between the gel weight before broken and the weight after broken divided by the cross-sectional area of the stainless cylinder (Kreckhoff et al., 2015).

2.5.4. Viscosity.

Extract solution of 1.5% concentration was heated in boiling water while regularly stirred until the temperature reached 75°C. The viscosity was measured with Viscometer Brookfield. The spindle position was set in the hot solution. The viscometer was activated and the solution temperature was measured. At the solution temperature of 75°C, the viscosity values appeared at the scale of the viscometer. Reading was done after 1 minute of 2 full rotations of spindle no.1 (Kreckhoff et al., 2015).

2.6. Water Quality.

Physico-chemical parameters at the culture site were measured as supporting data to check the physical environment suitability for the cultured species. D.O. meter was used to measure the dissolved oxygen, a refractometer was used to measure the salinity, a pH meter was used to measure the pH of the water, and a thermometer was used to measure the temperature and surface drogue to measure the current velocity. The water sample for the nitrate and phosphate nutrients were collected and taken to the laboratory for analysis.

2.6.1. Data Analysis.

The initial weight and harvested weight of *Halymenia durvillei* were recorded for the analysis of data. The growth rate of the *H. durvillei* was measured using the Dawes et al, 1993 equation:

$$GR = \left(\frac{W_t}{W_{in}} - 1 \right) \times 100$$

where:

W_t and W_{in} are the final and initial weights in (%), respectively.

The survival rate of the *H. durvillei* was measured using the Karim, 2007 equation:

$$SR = \frac{N_t}{N_0} \times 100\%$$

where:

N_t and N₀ are the number of individuals in sampling days and the initial number of individuals in (%), respectively.

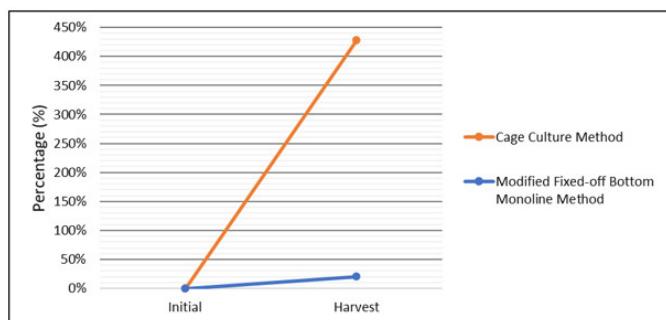
Mega statistic software was used for the analysis of data with confidence level was set to 95% (p<0.05) to test the effects of the means \pm of growth in weight in different culture trials of *Halymenia durvillei*.

3. Results.

3.1. Growth and Survival Rate.

The growth of *Halymenia durvillei* for the forty-five days' cultivation period differed in the two culture methods (Figure 4). The growth rate of *H. durvillei* cultivated in the modified fixed-off bottom method ranged from 13.6-28.7%, but on cage culture method, the growth rate ranged from 278.4-703.9%. The wide difference in growth rate between the two methods shows a significant difference using t-test at a 5% level of significance (0.0033, p>0.05).

Figure 4: Average growth rate of *Halymenia durvillei* using different culture methods.



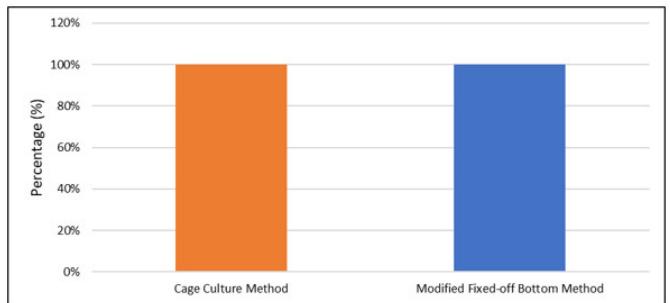
Source: Authors.

3.2. Survival Rate.

In this study, both culture methods had a 100% survival rate (Figure 5). In the modified fixed-off bottom method, fragmented thalli and bites were observed while the cultured seaweed was freely moving inside the cage culture method. The

cultured *H. durvillei* was observed to have thalli discoloration after five culture days and regained their original reddish color after ten days in both methods.

Figure 5: Survival rate of the cultured *Halymenia durvillei* using the two methods.

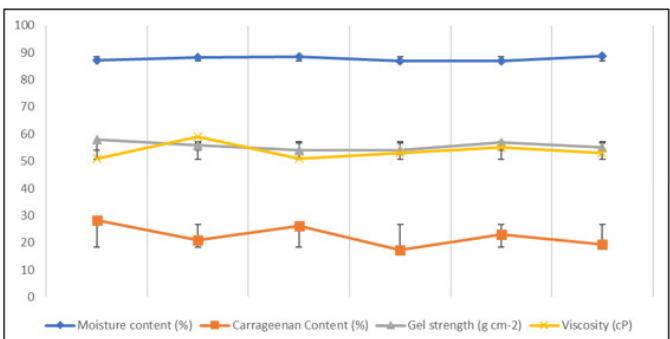


Source: Authors.

3.3. Colloidal Properties of *Halymenia durvillei* extract.

The colloidal properties of *Halymenia durvillei* determined in this study were moisture content, carrageenan, gel strength, and viscosity (Figure 6). In this study, the red seaweed contains $87.72 \pm 0.85\%$ which exceeded the criterion of 35-39% moisture content most stable seaweed condition and falls to the category that undergoes degradation during storage. In terms of carrageenan content, result shows $22.52 \pm 4.13\%$ and the value was within the commercial requirement needed for commercial crops. In terms of gel strength and viscosity, result for this study shows $55.67 \pm 1.63 \text{ g cm}^{-2}$ and $53.67 \pm 3.01 \text{ cP}$, respectively.

Figure 6: Colloidal properties of *Halymenia durvillei* extract (mean \pm SD).



Source: Authors.

3.4. Water Quality.

In this study, the water quality data from the culture site indicated conditions suitable for the culture of *Halymenia durvillei*. Salinity ranged from 30-35 ppt, water temperature from 23-30°C, pH from 6.23-9.39, water current from 6-46 cm/s, and DO from 2.65-4.98 ppm (Table 1). Higher values were mostly recorded during high tides. Nitrate values ranged from 0.21 to 0.25 mg/L, and phosphate ranged from 0.01 to 0.02 mg/L throughout the culture cycle. Although turbidity levels were

not measured in this study, turbidity may likely also impact the growth and product quality of *H. durvillei*.

Table 1: Water quality data in the culture site.

Parameters	Lowest Tide	Highest Tide
Salinity(ppt)	30-35	32-35
Temperature(°C)	25-30	23-30
pH	7.03-9.23	6.23-9.39
Current(cm/s)	6-42	12.46
Dissolved Oxygen(ppm)	2.65-4.56	3.12-4.98
Nitrate (mg/L)	0.21 mg/L to 0.25 mg/L	
Phosphate mg/L	0.01 mg/L to 0.02 mg/L	

Source: Authors.

4. Discussion.

The cultivation trial of *Halymenia durvillei* conducted at Pandong Bato Marine Sanctuary in Carmen, Cebu, showed positive results using the modified fixed-off bottom method and cage culture method. The cage culture method showed a higher growth rate of 311-718% than the modified fixed-off bottom method of 13.6-28.7%. In the modified fixed-off bottom method, the main reason for the lesser weight increase was that some of the branched thalli of *H. durvillei* came out on the meshes of the net bags resulting in the grazing of herbivorous fishes. In contrast, the cage culture method protected the seaweeds from grazing animals and lessened the impact of strong water current.

The cultured red seaweed in both methods acquired green pigmentation after five culture days but regained their original reddish color after ten days. Due to photo-destruction of the phycoerythrin, many red algae do not appear reddish at all, and a full range of pigment is exhibited (Wyne and Bold, 1985). This ability of red algae to alternate their proportion of pigment in response to differing quantities of incident light is referred to as chromatic adaptation (Crosset et al., 1965). The presence of green pigmentation indicates that the stock *Halymenia durvillei* adopted the new environment in the cultured site. This can be attributed to its adaption trait that resulted to 100% survival rate.

Moisture content is an essential criterion in determining the shelf-life and quality of processed seaweed meals, as high moisture may hasten the growth of microorganisms (Rohani - Ghadikolaei et al., 2012). It is preferably not more than 40% (McHugh, 2006) however the recommended value is not more than 35% (Pelinggon and Tito, 2009). In this study, the seaweed contains $87.72 \pm 0.85\%$, exceeding the criterion of 35-39% moisture content most stable seaweed condition and falls to the category that undergoes degradation during storage.

In terms of carrageenan content, the result shows $22.52 \pm 4.13\%$, and the value was within the commercial requirement needed for commercial crops. Kho et al. (2016) obtained a higher mean carrageenan content, 28.41 ± 2.77 , of *H. durvillaea*,

and the value was comparable to *Kappaphycus alvarezii* (22.62-79.62%) cultivated in Kolambungan, Lanao del Norte, Mindanao (Orbita, 2013).

The gel strength is the main physical properties of carrageenan because the strength of the gel shows the ability of carrageenan in gel formation (Wenno 2009). The cultured *H. durvillei* in this study obtained 55.67 ± 1.63 g cm⁻² gel strength, which significantly differ from *Kappaphycus alvarezii* with 503-1105 g cm⁻² (Hung et al., 2009), 850-2000 g cm⁻² (Ohno et al., 1994), 94.5-152.3 (Manuhara et al., 2016), and 20.3-80.3 g cm⁻² (Harun et al., 2013). Variations in the gel strength may differ from area to area.

Viscosity is one of the essential physical characteristics of carrageenan. The viscosity values for this study were 53.67 ± 3.01 cP, which is within the range of the values recommended by FAO, which is 5-800 cP. The study results were comparable to the value of *Kappaphycus alvarezii* (55-85 cP) cultivated in North Gorontalo, Sulawesi, Indonesia (Harun et al., 2013).

The environmental factors such as temperature, salinity, pH, dissolved oxygen, current and, nutrients proved to be the important factors for the growth of *Halymenia durvillei*. (Trono and Ohno, 1989) reported the rapid growth and high biomass at the temperature ranged from 25-30°C. Similarly, the higher growth rate and biomass were reported at seawater temperature (25.4°C-26.9°C) by Subba Rao et al., 2008. The results of the present study are in agreement with the aforementioned observation. No significant variation in the salinity was observed in the present study, and it was more than 30ppt, which is optimum growth for the seaweeds.

Conclusions.

In this study, the field experiment has adequately demonstrated the feasibility of farming of *Halymenia durvillei* in Pandong Bato, Marine Sanctuary, Carmen, Cebu. It is concluded that the cage culture method was an ideal and effective method to be used for the cultivation of *H. durvillei*. Considering that the cultivars showed positive growth, they can be considered adequate for commercial farming.

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