

Growth, Biomass Yield, and Proximate Composition of Sea Vegetable, *Caulerpa macrodisca* (Bryopsidales, Chlorophyta) Cultured in Tank

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Caulerpa macrodisca Decaisne, 1842 is a siphonous green macroalgae that has been reported to be distributed around southwest Asia (Sri Lanka); South China Sea; southeast Asia (Indonesia, Philippines, Singapore, and Vietnam); and Pacific islands (Samoan archipelago). However, the cultivation and consumption of *C. macrodisca* in the reported region were very uncommon due to the limited research on this green seaweed species. Thus, this study reported the growth rate, biomass yield, and proximate composition of *C. macrodisca* cultured in the tank. The *C. macrodisca* was successfully grown in the tank within 40 d under the following conditions: salinity (30.29–32.18 ppt), temperature (28.03–31.42 °C), dissolved oxygen level (DO; 4.75–5.21 mg L⁻¹), pH (7.62–8.06), and light intensity (50.77–87.55 μmol photons m⁻² s⁻¹). The average specific growth rate of *C. macrodisca* in the tank was as high as 5.13 ± 0.06 % g day⁻¹ – as indicated by average mass increments in terms of fresh weight (from 16.56 ± 0.17 g to 129.30 ± 2.83 g), disc portion (from 30–60 discs to >100 discs in each tank), disc diameter (from 0.24 ± 0.33 cm to 2.42 ± 0.18 cm), and frond length (from 0.82 ± 0.14 cm to 14.51 ± 0.27 cm). The average biomass yield of *C. macrodisca* in the tank was 114.58 ± 0.67 g m⁻² day⁻¹. A proximate analysis was performed on the harvested *C. macrodisca* – with values (%) of 20.84 ± 0.41, 19.74 ± 0.24, 1.34 ± 0.05, 21.79 ± 0.08, and 93.35 ± 0.13 for crude protein, crude fibre, crude lipid, crude ash, and moisture content, respectively – comparable to the proximate content of wild *C. macrodisca* except for the slightly lower crude fiber content and higher ash content. This study suggested that *C. macrodisca* would perform equally well in tank with good proximate composition. In conclusion, this study is significant to provide a baseline data of an alternative *Caulerpa* species (*C. macrodisca*) for cultivation.

Keywords: *Caulerpa macrodisca*, growth rate, proximate composition, seaweed culture

INTRODUCTION

Limited research is done on the cultivation of *Caulerpa* seaweed species. The ones that are commonly studied for their cultivation performance are *C. lentillifera*, *C. racemosa*, and *C. taxifolia*. The genus *Caulerpa* J.V. Lamouroux (1809) is a green macroalga that belongs to the Group Chlorophyta, Order Bryopsidales, and the Family

Caulerpaceae (De Gaillande *et al.* 2016). The thallus of *Caulerpa* consists of creeping tube-like stolons (horizontal runners) with upright photosynthetic “shoots” (also known as assimilators or fronds) being varied in form – leaf-like, feathery, and so forth – that arise from them. The fronds also have multiple ramuli around them, which can also exist in different forms such as disc-shaped, cup-shaped, or grape-like. In addition, the stolons of *Caulerpa* are anchored by bundles of rhizoids. Each thallus of *Caulerpa*

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consists of single immense cell, which is multinucleate and contains one to several central vacuoles and thin peripheral layer of protoplasm (van den Hoek *et al.* 1995). In many *Caulerpa* species, stolons grow through the surface layers of sandy or muddy sediment, forming dense swards on the bottom of tropical lagoons (van den Hoek *et al.* 1995).

Caulerpa seaweed in general is able to grow in both marine and brackish water where the water is clear with slow current. The thallus of *Caulerpa* is greenish and brownish depending on the quality of water and soil (sediment). *Caulerpa* sp. naturally inhabits a wide range of substrate consisting of rubbles to over 50 m deep, under surfaces of overhanging rocks, and also around the sand on reef flats and shallow, muddy lagoons that form beds and meadows (Tanduyan *et al.* 2013). Recently, another species of *Caulerpa* were found abundant in the coastal area of Menumbok, Sabah, Malaysia – within a silt sea surface of 1.4–2.7 m in depth around the coastal waters. The *Caulerpa* seaweed is identified as *C. macrodisca* with a wider and thinner disc-shaped ramuli (0.2–2.7 cm in diameter) that grow out at many angles from the frequent upright frond. The stolon diameter ranged 2.0–5.0 mm, with the frond length averaging 3.2–10.5 cm. This species, known as “Lathuh,” is collected seasonally from the wild and consumed as fresh salad by the local people in Menumbok area. The locals believe that *C. macrodisca* tastes better than *C. lentillifera* due to its distinctive peppery taste.

This species is originally claimed to be distributed around Vietnam, Australia, New Zealand, Republic of Palau, Sri Lanka, South China Sea, Indonesia, Philippines, Singapore, and Samoan archipelago (Guiry 2016). In fact, Phang *et al.* (2016) recently reported that *C. macrodisca* can only be found in the coastal area of Singapore, Thailand, and Vietnam. None have properly reported the existence of *C. macrodisca* in Malaysia. *C. macrodisca* – previously known as *C. racemosa* J. Agardh var. *macrodisca* and *C. peltata* J.V. Lamouroux var. *macrodisca* – was first introduced by Decaisne in 1842 as localized around Anambas Island, Indonesia (Belton *et al.* 2014). This species is a type of green macroalga that can be used as a source of natural food and dietary fiber without toxic and harmful effects no humans (Movahhedini *et al.* 2014). *C. macrodisca* is locally known as “eaba-eaba” around Iloilo, Philippines whereby locals collect this sea vegetable seasonally from the wild, sold in the local markets, and consumed as fresh salads.

In addition, *Caulerpa* in general can also be used as animal feed and as an ingredient in folk medicine to reduce blood pressure and treat rheumatism (Novaczek 2001). *Caulerpa* also contains a type of alkaloid known as caulerpin that exhibit antinociceptive and anti-inflammatory activities. Alkaloids stand out as one of major importance in the development of new drugs as they possess a wide variety

of chemical structures that have been identified to be responsible for many of the pharmacological properties of medicinal plants (De Gaillande *et al.* 2016).

MATERIALS AND METHODS

Algal Fragments Preparation and Experimental Design

The fragments of *C. macrodisca* were collected from the sampling site (5°18.933’N, 115°22.399’E) along the coastal area of Menumbok, Sabah and brought to the BMRI Shrimp Hatchery. The depth in the coordinate was 2.7 m and the sediments around the area were mostly medium-silted sand. The algal fragments were collected during low tide, placed in polyethylene bags, and stored in a polystyrene icebox until transportation to the hatchery. The fragments were cleaned from other benthic materials and washed thoroughly before being placed in a 1000 L storage tank. A conventional flow-through tank culture system (FTS) with continuous filtered seawater supply – with inlet and outlet water flow rates kept at 0.5 L/min and 0.8 L/min, respectively – was applied in the experiment. Nine (9) replicates of 40 L acrylic aquarium tank were used for the culture system (n = 9). All the aquariums were placed in an open space covered with transparent rooftop at the hatchery. A customized 0.2 m x 0.2 m net tray was used as a culture platform and placed in each aquarium tank.

Physico-chemical Water Quality Analysis

The physical water quality parameters – namely pH, dissolved oxygen (DO) level, temperature, salinity, and light intensity – were monitored daily in every culture tank using YSI multiparameter equipment. The chemical analysis of seawater nutrient content – nitrate, nitrite, and ammonia – were also tested using the colorimetric analysis, applying the methods described by Hansen and Koroleff (1999) within 10-day intervals during the 40-day culture period.

Growth Rate and Biomass Yield Analysis

The *C. macrodisca* fragments were enclosed in a customized net tray (0.2 m x 0.2 m, 30 cm deep) for 40 d, with 10-day interval weight measurements for growth rate measurement. The growth and biomass yield of *C. macrodisca* were determined using the following formulae (Al-Hafedh *et al.* 2015):

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

where W_t = final fresh weight at Day t (g), W_0 = initial fresh weight at Day 0 (g),

t = number of culture days; and:

$$\text{Biomass Yield (g m}^{-2} \text{ day}^{-1}) = [(W_t - W_0) / t] / \text{SA} \quad (2)$$

where W_t = final fresh weight at Day t (g), W_0 = initial fresh weight at Day 0 (g),

t = number of culture days, and SA = surface area of tank.

Each fragment of *C. macrodisca* in the tray consisted of basal portion (stolons), rhizomes, and 8–10 erect axes (fronds) with over 30 disc-shaped ramuli ranging 0.2–0.4 cm in diameter. The selected initial stocking density of *C. macrodisca* was 0.4 kg m⁻² in each tray within 40 d of culture period. The initial and final values in terms of number of ramuli (discs), disc diameter, and stolon and frond length were also measured and recorded randomly – whereas the initial values were 40 discs and 20 fronds in each tray (Figure 1).



Figure 1. The standard disc diameter and frond length measurements of *C. macrodisca*.

Proximate Analysis

C. macrodisca biomass was harvested from all the nine tanks after 40 d. The cleaned harvested seaweeds were oven-dried at 50 °C for 48 h. The dried seaweeds were then grounded into fine powder and used for proximate analysis. Proximate composition of the tank-cultured *C. macrodisca* was determined – following the standard AOAC (2000) method for moisture, ash, crude lipid, crude fibre, and crude protein content. Fresh samples of *C. macrodisca* were collected from the wild for proximate data comparison. Samples were also oven-dried and ground into fine powder for proximate analysis. A secondary data from Matanjun *et al.* (2009) was used for *C. lentillifera* proximate data comparison with the tank-cultured and wild *C. macrodisca*.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to test for differences on the growth rate and biomass yield of the tank-cultured *C. macrodisca*, as well as the proximate

composition of the tank-cultured and wild *C. macrodisca*. When significant differences were observed, Tukey's Honest Significant Difference (HSD) test was used to determine which replicate were significantly different from one another. Data was analyzed using SPSS 20.0 software. A significance level of $p < 0.05$ was chosen for all statistical analyses.

RESULTS

Growth Rate and Biomass Yield of *C. macrodisca*

The average specific growth rate of the tank-cultured *C. macrodisca* was 5.13 ± 0.06 % g day⁻¹ and the biomass yield was 114.58 ± 0.67 g m⁻² day⁻¹ within 40 d of culture period (Figure 2). There is no significant difference ($p < 0.05$) between the growth rate and biomass yield of *C. macrodisca* for all tank replicates. At Day 40, mass increment of disc portion (from an average of 60 discs to >100 discs) with enlarged diameter were observed in every *C. macrodisca* thallus in the net tray (from 0.24 ± 0.33 cm to 2.42 ± 0.18 cm); the same was true for the increment of frond portion with increased length (from 0.82 ± 0.14 cm to 14.51 ± 0.27 cm) after 40 d (Figure 3 and Table 1).

Water Quality

C. macrodisca was successfully grown in the tank for 40 d under the following conditions: salinity (30.29–32.18 ppt), temperature (28.03–31.42 °C), DO level (4.75–5.21

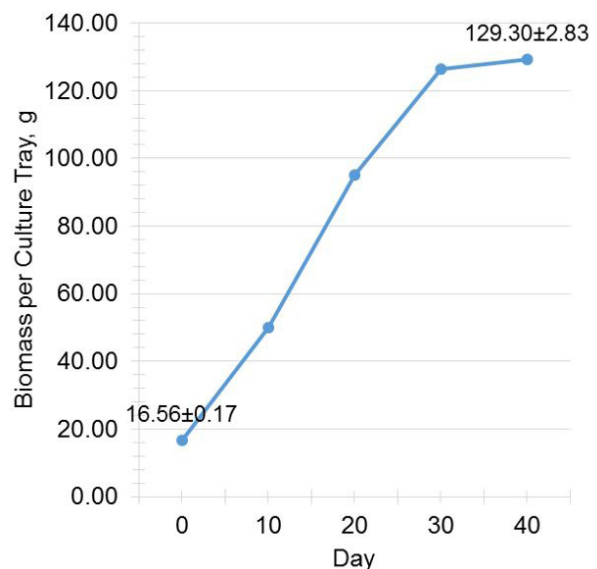


Figure 2. The weight increment (mean ± SD) of *C. macrodisca* cultured within 40 days in the aquarium tank at the Borneo Marine Research Institute, Universiti Malaysia Sabah. Values are expressed as mean ± SD of n = 9 tanks throughout the culture period.

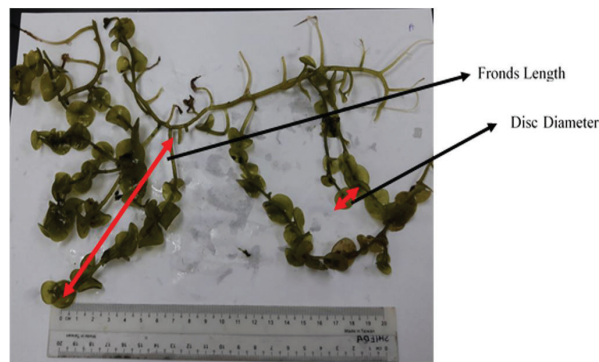


Figure 3. A) The *C. macrodisca* cultured in the tank at Day 10 and B) the mass addition of new discs with bigger diameter and longer fronds of *C. macrodisca* from the similar tank after 20 d, as shown in the red circles.

Table 1. Average range of discs diameter and fronds length and growth rate of *C. macrodisca* cultured in tank within 40 d.

Characteristics		Range/Values
Disc Diameter	Day 0	0.24 ± 0.33 cm
	Day 40	2.42 ± 0.18 cm
Disc Portion	Day 0	30–60 discs
	Day 40	>100 discs
Froned Length	Day 0	0.82 ± 0.14 cm
	Day 40	14.51 ± 0.27 cm
Growth Rate		5.13 ± 0.06 % g day ⁻¹
Biomass Yield		114.58 ± 0.67 g m ⁻² day ⁻¹

Note: Values are expressed as mean ± SD, n = 9.

Table 2. Water quality parameters recorded daily in the *C. macrodisca* culture tank.

Water Quality Parameters	Range
pH	7.62–8.06
DO Level (mg/L)	4.75–5.21
Salinity (ppt)	30.29–32.18
Temperature (°C)	28.03–31.42
Irradiance (μmol photons m ⁻² s ⁻¹)	50.77–87.55

mg L⁻¹), pH (7.62–8.06), and irradiance (50.77–87.55 μmol photons m⁻² s⁻¹) – as shown in Table 2.

Proximate Composition

The proximate composition of *C. macrodisca* cultured in the tank was comparable (analyzed using one-way ANOVA test where n = 3 for every proximate analysis) to the proximate content of wild *C. macrodisca*, except for the crude fiber and ash content – with values of 20.84 ± 0.41 %, 19.74 ± 0.24 %, 1.34 ± 0.05 %, 21.79 ± 0.08 %, and 93.35 ± 0.13 % for crude protein, crude fibre, crude lipid, ash, and moisture content, respectively (Table 4). To our knowledge, this is the first report on the proximate content of *C. macrodisca*.

Table 3. Water nutrient content (mean ± SD, n = 3) measured weekly in the *C. macrodisca* culture tank.

Water Nutrient Type	Concentrations (mgL ⁻¹)
Nitrate	0.02 ± 0.01
Nitrite	0.006 ± 0.01
Ammonia	0.04 ± 0.02

Table 4. Proximate composition (mean ± SD, n = 3) of *C. macrodisca* after 40 d of culture in comparison with wild specimens of *C. macrodisca* and *C. lentillifera* recorded by Matanjun *et al.* (2009).

Seaweed Species (Dry Basis)	Wild <i>C. macrodisca</i>	Tank-cultured <i>C. macrodisca</i>	<i>C. lentillifera</i> (Matanjun <i>et al.</i> 2009)
Protein (%)	21.52 ± 0.15 ^a	20.84 ± 0.41 ^a	10.41 ± 0.26
Fiber (%)	23.85 ± 0.09 ^a	19.74 ± 0.24 ^b	1.91 ± 0.00
Ash (%)	13.08 ± 0.05 ^a	21.79 ± 0.08 ^b	37.15 ± 0.64
Lipid (%)	1.09 ± 0.07 ^a	1.34 ± 0.05 ^a	1.11 ± 0.05
Moisture (%) Fresh Sample	97.01 ± 0.02 ^a	93.35 ± 0.13 ^a	38.66 ± 0.96

Notes:

i. Values are expressed as mean ± SD, n = 3.

ii. Values in the same row with different superscripts letters are significantly different ($p > 0.05$).

and 93.35 ± 0.13 % for crude protein, crude fibre, crude lipid, ash, and moisture content, respectively (Table 4). To our knowledge, this is the first report on the proximate content of *C. macrodisca*.

DISCUSSION

Growth Performance of *C. macrodisca* in Captivity

Limited research work was found on the growth performance of *C. macrodisca*. Indeed, to our knowledge, none have reported the growth rate of *C. macrodisca* in captivity. The commonly studied and highly cultivated *Caulerpa* species in the southeast Asian region were *C. lentillifera* and *C. racemosa*. Paul *et al.* (2014) reported that the average biomass yield of *C. lentillifera* and *C. racemosa* cultivated using tray culture method within a six-week period were 1.5 kg wk⁻¹ and 0.1 kg wk⁻¹, respectively. In addition, Rabia (2016) also reported that the specific growth rate of *C. lentillifera* cultivated using sowing method was 3.85 % g day⁻¹ after 30 days of culture.

The specific growth rate of *C. macrodisca* (5.13 ± 0.06 % g day⁻¹) in captivity in the present study was higher compared to *C. lentillifera* reported by Rabia (2016) and

Paul *et al.* (2014). The data indicates that *C. macrodisca* could be easily cultivated with a good growth performance and faster growth rate within 40 d. The increment of frond length was claimed to be one of the growth indicators for *Caulerpa* sp., as suggested by Paul *et al.* (2014). The data suggests that *C. macrodisca* could be harvested as early as 40 d when cultured in captivity.

The Sea Water Quality in the Tank Culture System

The growth of *Caulerpa* seaweed varied due to the differences in environmental factors and farming techniques such as off-bottom culture, tray, and sowing methods (Tanduyan *et al.* 2013, Rabia 2016). Among the environmental and physical factors that could influence the growth of *Caulerpa* sp. are water depth, water temperature, irradiance, salinity, sedimentation levels, wave action, pH, oxygen content, seasonal variation, geographical difference, and stocking density (Fernández-García *et al.* 2011).

According to Rabia (2016), *Caulerpa* sp. in general is sensitive to changes in salinity as it is stenohaline – salinities lower than 30 ppt might result to poor growth and lower than 25 ppt might cause mortality. The recorded seawater quality parameters around the sampling site of Menumbok waters were as follows: salinity (23.63–24.48 ppt), temperature (31.10–32.00 °C), DO level (4.40–5.50 mg L⁻¹), and pH (7.30–7.72). Surprisingly, salinity around the sampling site was lower than 25 ppt since the area was close to a mangrove marsh. *C. macrodisca* managed to survive at the sampling site where salinity is lower than 25 ppt. The present study suggests that *C. macrodisca* might be able to tolerate a wider range of salinities; however, further experiments need to be done to confirm its salinity tolerance.

Guo *et al.* (2015) claimed that the optimum temperatures and irradiances for growth of *Caulerpa* sp. varied depending on the species. Temperature and irradiance of 15–39 °C and 30–60 μmol photons m⁻² s⁻¹ were respectively reported to be able to support good growth of *Caulerpa* sp. in captivity. Irradiance was claimed to have a strong influence on the seaweed growth rate through the activity of the pigment levels that could indirectly affect the photosynthetic ability of the seaweed (Soriano 2012). In this study, the temperature readings in the tank fall within the values mentioned in the literature, except for the higher irradiance that suggests *C. macrodisca* could tolerate a wider irradiance range. Nevertheless, further research needs to be done to prove the irradiance tolerance of *C. macrodisca*. Meanwhile, the average concentrations of nitrite, nitrate, and ammonia in the tank were below 0.1 mg L⁻¹ – indicating good water quality as shown in Table 3.

The Comparison on the Proximate Composition of Tank-Cultured and Wild *C. macrodisca* with Commonly Consumed *C. lentillifera*

Most of the proximate studies were focused on common *Caulerpa* sp. such as *C. lentillifera*, *C. racemosa*, and *C. peltata* (Matanjun *et al.* 2009, Ratana-Arporn and Chirapart 2006, Movahhedin *et al.* 2014, Nagappan and Vairappan 2014). The crude fiber content of tank-cultured *C. macrodisca* was slightly lower while the ash content was slightly higher than the wild; however, the values were still within the recommended values stated by De Gaillande *et al.* (2016). The proximate content of edible *Caulerpa* sp. ranged 3.6–83.2% carbohydrate (making it an alternative dietary fiber source), 0.6–20.8% crude protein, 0.1–7.2% crude lipid, 2.9–55.1% of ash, and 74.7–94.3% of moisture based on dry matter (De Gaillande *et al.* 2016).

Nagappan and Vairappan (2014) stated that fiber content had always been an important parameter in nutritional evaluation of any food ingredient or organic food. The proximate data in this study shows higher fiber and protein contents of both tank-cultured and wild *C. macrodisca* compared to the commonly consumed *C. lentillifera* (Table 4), which indicates that this seaweed could be used as a kind of natural food and fiber source for humans. Further study on its nutritional composition – in terms of fatty acids, amino acids, minerals, and so forth – warrant a thorough investigation.

CONCLUSIONS

The data suggests that *C. macrodisca* offers good biomass production and promising nutrient content when harvested in captivity. In conclusion, the *C. macrodisca* holds high potential to be commercialized in the local and global market due to its high growth rate and promising nutrient composition – as proven in this study.

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