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Growth, biomass and productivity of green seaweed *Caulerpa lentillifera* (J. Agardh) at different stocking densities

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Abstract

Caulerpa lentillifera has been the focus of research in recent years. However, its production is still insufficient and does not meet the demand which might be due to lack of additional information in its culture method. Therefore, this study was conducted to provide information to determine the effect of stocking densities on the growth, biomass and productivity of *C. lentillifera* and evaluate how the following environmental factors such as water temperature, salinity, water flow and nutrients would affect its growth, biomass and productivity in Casul Bay. Following the bottom culture method, a total of 144 plots were established in the sampling area. Each plot was planted with the amount of seed stock from 50 to 450 grams m^{-2}, harvested after 7 days, weighed and the final weight was recorded. Some environmental factors such as water temperature, salinity, water flow and nutrients (phosphate and nitrate) were also noted. Results showed that the lowest stocking density (50 g m^{-2}) produced the highest daily growth rate and productivity while the highest stocking density produced the highest biomass of *C. lentillifera*. Its growth rate, biomass and productivity showed a significant negative correlation with water temperature but a positive significant correlation with salinity. It was concluded that the best stocking density was 50 g $m^{-2}$to yield the highest growth rate and productivity attained at lower water temperature and higher salinities.

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Introduction

*Caulerpalentillifera*is green seaweed which belongs to the Family Caulerpaceae, Order Caulerpales in the Division Chlorophyta (Trono, 1997). This species has grape-like shape, soft and succulent nature. It can grow on sand and rock bottoms in the upper sublittoral zone of tropical coral reefs (Horstmann, 1983; Mao *et al.,* 2011). It is naturally distributed in tropical and sub-tropical regions, suchas South China Sea, Southeast Asia, Japan, Okinawa, Taiwan and Oceania (Paul *et al.,* 2014). Based on several literatures, this green sea weed was firstly documented on Red Seacoast (Agardh, 1837), andthen was observed at many other locations, especiallyin Indo-Pacificregion (Hackett, 1977; Taylor, 1977; Meñez and Calumpong, 1982; Coppejans and Beeckman, 1990; Phillips *et al.,* 1999; Schils and Coppejans, 2003; Titlyanov *et al.,* 2012). This species is known to have high protein, carbohydrates, minerals, dietary fibers, Vitamin A and Vitamin Ccontent (Ratana-arporn and Chirapart, 2006; Matanjun *et al.,* 2009) and have several essential polyunsaturated fatty acids or PUFAs (Saito *et al.,* 2010) and has low levels of lipids (Niwano *et al.,* 2009).Because of its good taste, *C. lentillifera* is often cooked as salad in some Asian countries like China, Japan, Korea and Philippines (Dawes, 1998). In addition, this species is also used as food for livestock and aquaculture fish. With its blooming success, there has been increasing demand and rising market prices for *C. lentillifera*in some Asian countries. Although this alga is widely cultivated in the Philippines (Zemkewhite and Ohno, 1999), Okinawa (Kurashima *et al.,* 2003), TaiwanIsland (Shi, 2008), FujianandHainanprovincesin China (Wang, 2011), the commercial-scale production of *C. lentillifera*is still not sufficient, and its productivity does not meet the demand. It might be due to lack of additional information in the culture method of this green seaweed either in pond or open sea cultivation. To this end therefore, this study was done in order to know the effect of stocking densities on the growth, biomass and productivity of *Caulerpalentillifera* and to evaluate how the following environmental factors (water temperature, salinity, water flow and nutrients) would affect the growth, biomass and productivity of this species.

Materials and methods

Study area

The study was conducted in Barangay Caluya, Sapang Dalaga, Misamis Occidental 8°35'59"N 123°34'57"E (Figure 1), Mindanao. The area is located in Casul Bay, a semi-enclosed body of seawater which is connected to Murcielagos Bay. The water is generally calm, hence suitable for *Caulerpa*farming. The area has been farmed by small scale entrepreneur with one variety of *Caulerpa*, *Caulerpalentillifera* in the open sea. The seaweed farmers used the off bottom culture method at a depth of less than 1 meter below the water surface in a muddy type of substratum.

In situ cultivation of C. lentillifera with varying stocking densities

There were one hundred forty-four (144) plots that were established in the sampling area. The plots were divided into two sets; the first set was for daily growth rate (DGR) and the second set was for biomass determination. Each set which contained seventy-two (72) plots were planted with different amount of seedstock such as 50, 100, 150, 200, 250, 300, 350, 400 and 450 grams m^{-2} (with 8 replicates per plot).

The seedlings were planted 2 - 4 feet below the water surface following the traditional bottom culture method and left to grow undisturbed for a period of 7 days (Guo *et al.,* 2015) after which the plants were harvested, placed in an individual net bag properly labelled. Each sample was weighed using a weighing balance, taking note of the final weight.

Measurement of daily growth rate (DGR), biomass and productivity

The average daily growth rate (DGR = % day^{-1}) of seaweed after 7 days of culture was calculated and expressed as percent increase in wet weight (Yong *et al.*, 2013):

$$DGR = \left[\left(\frac{W_t}{W_0} \right)^{\frac{1}{t}} - 1 \right] x\ 100\%$$

Where:
W_o = initial wet weight
W_t = weight after t days
t = time interval (days)

For biomass data, the seaweeds that were collected in each plot were rinsed and cleaned thoroughly with freshwater. After cleaning, the seaweed samples were dried in the oven at 70°C to constant weight to obtain dry weight (DW). The data on biomass was expressed in terms of production per unit area (g DW m^{-2}) while data on productivity was expressed as rate of production per unit area (g day $^{-1}$ m^{-2}).

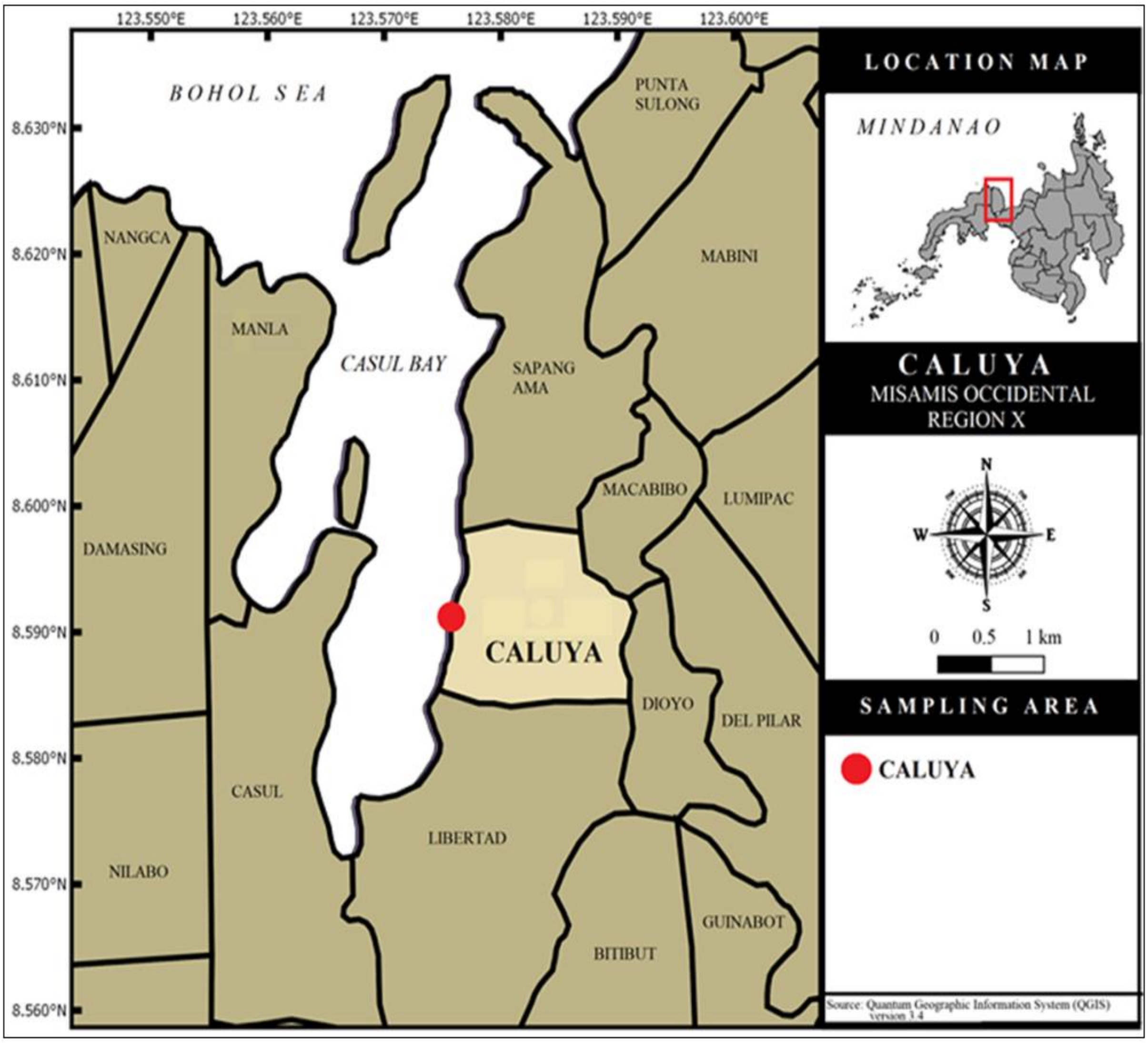

Fig. 1. Location of the sampling area in Barangay Casul, Sapang Dalaga, Misamis Occidental, Mindanao (Source: QGIS version 3.4).

Environmental parameters

Measurements of the environmental parameters were done randomly within the cultured area ofC. *lentillifera* from June to July 2016during the southwest monsoon or "*habagat*". Water temperature was measured using an ordinary mercury thermometer while salinity was measured using a handheld refract meter. Water flow was estimated using clod cards (Doty, 1971) and the calculation of water flow (cm^{-2}) was done based on the method of Anzai (2001). Nutrient analysis was carried out following the method found in APHA (1995).

Statistical analysis

Data were determined by Analysis of Variance (One-Way ANOVA, level of significance, *P* of 0.05) in SPSS (version 21).) Pearson's correlation analysis was used to determine the correlation coefficients between environmental factors with growth rates, biomass and productivity (PPMCC, level of significance, *P* of 0.05)

Results and discussion

Daily growth rate (DGR), biomass and productivity of C. lentillifera

The three dependent variables (growth rate, biomass and productivity) vary significantly with stocking densities (p = 0.000, Table 1). The lowest stocking density (50 g m^{-2}) produced the highest daily growth rate and productivity (Figure 2). Several authors suggested that the high growth rate of seaweeds in low stocking density is due to low competition in space and environmental resources such as nutrients, amount of carbon, light, temperature and water movement (Bidwell *et al.,* 1985;Santelices 1999; Msuya, 2013; Al-Hafedh *et al.,* 2013; Manriquez-Hernandez, 2013; Yong *et al.,* 2014).

Similar result was observed by Trono and Denila (1987) on the same species that was cultured in BalongBato, Calatagan, Batangas.

Table 1. Statistical analysis (One-Way ANOVA) of the daily growth rate, biomass and productivity of *C. lentillifera* with varying stocking densities.

Source	d.f.	*F*-statistics	*p*	Analysis
Growth rate	8	9.238	0.000	Significant
Biomass	8	51.650	0.000	Significant
Productivity	8	9.238	0.000	Significant

Table 2. The environmental parameters measured in *C. lentillifera* cultured farm site (mean ± SD).

Parameter	This Study	Suggested Range Value of Environmental Parameters for the Culture of *Caulerpa*	Source
Temperature (°C)	28.67±3.17	25-30	McHugh, 2003
Salinity (‰)	34.67±1.67	>30	Trono, 1988
Phosphate (mg/L)	0.03±0.01	0.01-0.4	Guo*et al.*(2015)
Nitrate (mg/L)	0.05±0.01	0.05-0.5	Guo*et al.*(2015)
Waterflow (cm s^{-1})	2.35±1.13	-	-

On the other hand, Hurtado *et al.* (2008), Athithan (2014) and Wenno *et al.* (2015) observed the same result on *Kappaphycus striatum* and *Kappaphycusalvarezii* with varying stocking densities. In contrast, the highest stocking density produced the highest biomass of *C. lentillifera*.

Table 3. Correlation coefficients (R) between growth rate, biomass and productivity of *C. lentillifera* with environmental parameters. *DGR* is daily growth rate, BM is biomass, PRD is Productivity, *Temp* is temperature, *Sal* is salinity.

	DGR	BM	PRD	Temp	Sal	pH	Phosphate	Nitrate	Water flow
DGR	1	-0.234	0.387	-0.563*	0.507*	0.044	-0.023	0.043	-0.345
BM	-0.234	1	-0.267	-0.413*	0.433*	-0.057	-0.027	-0.063	-0.434
PRD	0.376	0.267	1	-0.441*	0.457*	0.065	0.023	-0.138	-0.387
Temp	-0.563*	-0.413*	-0.441*	1	-0.931	0.005	0.051	0.048	0.218
Sal	0.507*	0.433*	0.457*	-0.931	1	0.045	-0.123	-0.176	0.191
pH	0.044	-0.057	0.065	0.005	0.045	1	0.894	0.239	0.627
Phosphate	-0.023	-0.027	0.023	0.051	-0.123	0.894	1	0.154	0.003
Nitrate	0.043	-0.063	-0.138	0.048	-0.176	0.239	0.154	1	0.508
Waterflow	-0.345	-0.434	-0.387	0.218	0.191	0.627	0.003	0.508	1

*represents significant differences at p<0.05.

This result is in agreement with the study conducted by Paul *et al.* (2013) where the biomass of *C.lentillifera*increases with stocking densities. *C. lentillifera* has higher density of fronds (edible portion) when compared to other species of *Caulerpa* (Paul *et al.,* 2013) which might have contributed to its high biomass. Some authors mentioned that the advantage of seaweeds grown in high stocking density would be high resistance tohydrodynamism, less vulnerable to grazing, not easily removed from its substrate and less epiphyte growth (Schiel and Choat, 1980; Hay, 1981; Padilla, 1984; Reed, 1990).

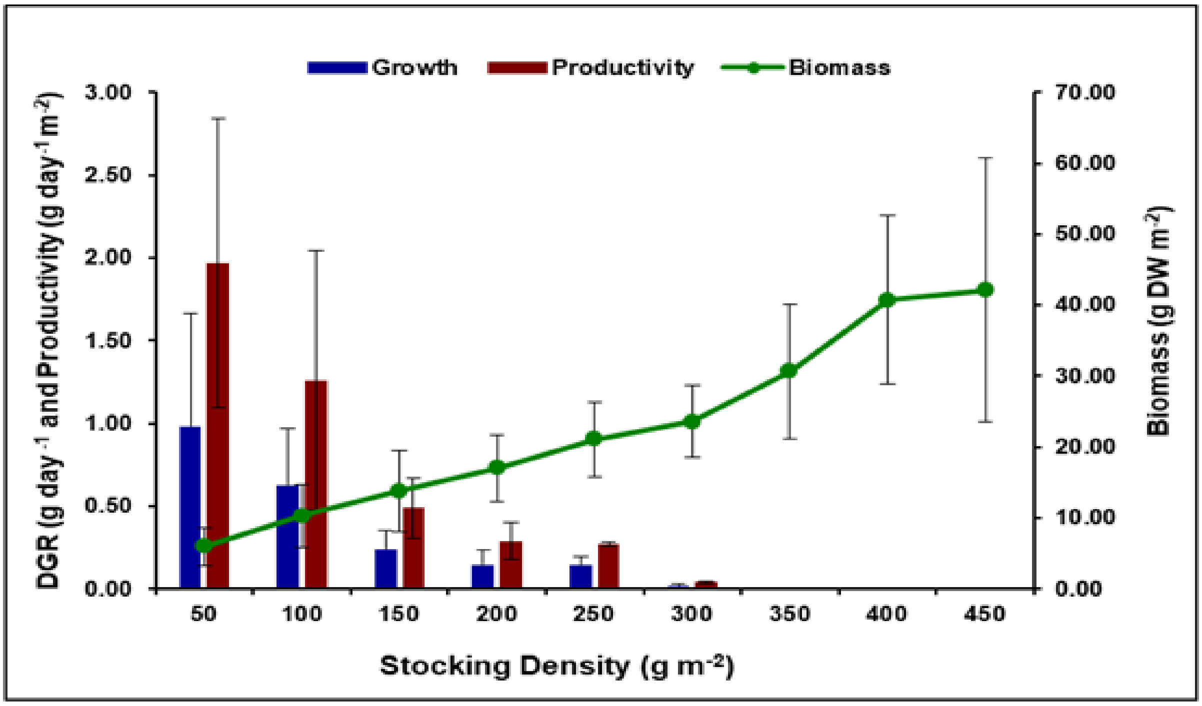

Fig. 2. Growth rate, biomass and productivity of C. *lentillifera* with varying stocking densities (mean ± SO).

Correlations in environmental parameters with daily growth rate, biomass and productivity of C. lentillifera

Within the duration of the experiment, the environmental parameters recorded were within the favorable range for *Caulerpa* culture (Table 2). The growth rate, biomass and productivity of *C. lentillifera* showed a significant negative correlation with water temperature indicating that *C. lentillifera* has low capability to survive in changing temperature (Table 3). The growth rate, biomass and productivity of *C. lentillifera* showed a significant negative correlation with water temperature indicating that *C. lentillifera* has low capability to survive in changing temperature. Temperature profoundly influences the survival, recruitment, growth and reproduction of seaweeds (Breeman, 1988). A temperature above 30˚C would already affect the growth in *Caulerpa*species (McHugh, 2003; Li *et al.,* 2009). A negative correlation between temperature and growth rate of *K. alvarezii* has also been reported (Glenn and Doty 1992; Muñoz *et al* 2004; Orbita, 2013) seemingly consistent with the negative correlation between temperature and growth rate in this study. On the other hand, *C. lentillifera* showed a positive significant correlation with salinity. This means that an increase in salinity would stimulate the growth, biomass and productivity of *C. lentillifera* and vice versa. This is true because *Caulerpa* is purely marine stenohaline alga and will die even in slightly brackish water. The salinity value (34.67±1.67) observed in this study was within the range (>30‰) specified by Trono (1988) for normal growth of *Caulerpa*species.

Moreover, salinity is one of the most important abiotic environmental factors to influence algal growth and distribution (Lobban and Harrison, 1994). Many studies have documented the effects of salinity on several *Caulerpa* species, including *C. lentillifera* (Guo*et al.,* 2015), *C. paspaloides* (O'Neil and Prince, 1988) and *C. taxifolia* (Theil *et al.,* 2007; West and West, 2007).

Conclusion

The present study suggests that *C. lentillifera* that is cultured in Casul Bay is best grown at a stocking density of 50 g m^{-2} in order to yield the highest growth rate and productivity. Also, the maximum growth rate, biomass and productivity correspond to lower water temperature and vice versa, whereas higher growth rate, biomass and productivity can be attained at higher salinities since *Caulerpa* is purely marine stenohaline alga. This result should be considered in establishing and managing *C. lentillifera* farming in the area because this seaweed is a significant source of food and produces other potential natural products in the global market.

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