STANDING-STOCK BIOMASS AND DIVERSITY OF *Caulerpa* (Chlorophyta) IN SOLONG-ON, SIQUIJOR ISLAND, PHILIPPINES

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ABSTRAK

Spesies *Caulerpa* yang tumbuh di perairan dangkal sampai dilaut dalam dianggap sebagai gangguan ekonomi dan penting. Meskipun invasif di daerah beriklim sedang, spesies *Caulerpa* terutama *C. racemosa* dan *C. lentillifera* (lato) dipanen dan dikonsumsi sebagai makanan, terutama di Filipina. Komunitas Caulerpa di Solong-on, Siquijor adalah salah satu sumber untuk ekspor ke kota tetangga. Penelitian ini bertujuan untuk menentukan stok tegakan biomassa saat ini, prosentase dan keragaman Caulerpa di Solong-on, serta untuk menentukan parameter lingkungan yang dapat mempengaruhi kelimpahan dan keragamannya. Hasil penelitian menunjukkan total delapan spesies *Caulerpa* diidentifikasi di komunitas *Caulerpa* dangkal di Solong-on, Siquijor. Biomasa yang memiliki stok tertinggi diperoleh oleh *C. cupressoides* dengan biomassa rata-rata 26,16 ± 2,44 (SE) g berat kering/m^{-2}, sedangkan *C. microphysa* memiliki biomassa stok yang berdiri paling sedikit dengan rata-rata 9,16 ± 0,26 g berat kering/m^{-2}. Secara umum, total biomassa stok berdiri untuk semua spesies yang diperoleh selama periode sampling adalah 132,57 ± 2,06 g berat kering/m^{-2}. Indeks keragaman Shannon-Wiener (H ') berkisar dari 1,7-2 yang menunjukkan komunitas Caulerpa yang relatif beragam. Analisis korespondensi kanonik (CCA) mengungkapkan bahwa suhu dan jenis substrat adalah pendorong utama biomassa *Caulerpa*.
Kata Kunci: biomassa, *Caulerpa*, tutupan, makroalga, parameter fisika-kimia

ABSTRACT

Caulerpa species growing on shallow to deep waters are considered to be both economically nuisance and important. Although invasive in the temperate regions, *Caulerpa* species mainly *C. racemosa* and *C. lentillifera* (lato) are harvested and consumed as food, especially in the Philippines. *Caulerpa* communities in Solong-on, Siquijor are one of the sources for export to neighbouring municipalities. This study aimed to determine the present standing stock biomass, percent cover and diversity of *Caulerpa* in Solong-on, as well as to determine the environmental parameters that may affect its abundance and diversity. The results showed a total of eight species of *Caulerpa* were identified in the shallow *Caulerpa* community in Solong-on, Siquijor. The highest standing stock biomass was obtained by *C. cupressoides* with mean biomass of 26.16±2.44 (SE) g dry wt m^{-2}, whereas *C. microphysa* has the least standing stock biomass with an average mean of 9.16±0.26 g dry wt m^{-2}. In general, the total standing stock biomass for all species obtained throughout the sampling period was 132.57±2.06 g dry wt m^{-2}. Shannon-Wiener diversity index (H') ranged from 1.7-2 suggesting a relatively diverse *Caulerpa* community. Canonical correspondence analysis (CCA) revealed that temperature and type of substrate were the main drivers of *Caulerpa* biomass.
Key Words: biomass, *Caulerpa*, cover, macroalgae, physic-chemical parameters

I. INTRODUCTION

The genus *Caulerpa* (Family Caulerpaceae) of Chlorophyta is distributed worldwide from the tropical to temperate marine habitats with about 97 species, of which 63 species identified in the Philippines (Guiry & Guiry 2018). They grow on sandy rock bottoms in the upper sublittoral zone of tropical coral reefs (Nagappan & Vairappan 2013).

Although certain *Caulerpa* species (e.g., *C. taxifolia*, *C. racemosa*) became invasive in the Mediterranean Sea (Boudouresque et al 1995; Jousson et al 1998; Thibaut et al 2004; Klein & Verlaque 2008; Cebrian & Ballesteros 2009), some species (e.g. *C. racemosa* and *C. lentillifera*) are native and considered delicacies in the Philippines, Indonesia, Malaysia, and Japan (Nagappan & Vairappan 2013). *Caulerpa*, together with other macroalgae, promise to yield economic benefit once maricultured intensively in the Philippines (Cordero 1990). Due to its demand, culture of these varieties in shallow tidal ponds was initiated over years ago in Mactan Island, Cebu and distributed widely in the Philippines, even exported to Japan and Taiwan (Nagappan & Vairappan 2013). In Siquijor Island, *C. racemosa* (locally known as lato) has been harvested and supplied to Dumaguete City since the 1980s (Calumpong 1984). However, there was a decline in the local harvest of *C. racemosa* based on the 1984 baseline study of Wagey & Bucol (2014).

Many studies have been conducted to determine the abundance and species diversity of *Caulerpa* around the world (Terrados & Ros 1995; Thibaut et al 2004; Verlaque et al 2004; Schembri et al 2015; Aplikioti et al 2016). Moreover, the focus on these studies were mainly on the invasiveness of the species (Cebrian & Ballesteros 2009; Bouiadjra et al 2010; Cevik et al 2007; Schembri et al 2015; Aplikioti et al 2016). Apart from an initial study on the standing stock of *Caulerpa racemosa* at Siquijor Island (Calumpong 1984), no similar study has been done.

This study generally aimed to determine the standing stock biomass, percent cover and diversity of *Caulerpa* species present in Solong-on, Siquijor, Philippines. In particular, this research aimed to: (1) determine the standing stock biomass (g dry wt. m^{-2}) and percent cover of *Caulerpa* species; (2) determine the diversity of *Caulerpa* species; and (3) determine the environmental parameters (e.g. salinity, pH, water temperature, and types of substrate) in the study area that may have influenced the abundance and diversity of *Caulerpa*.

II. MATERIAL AND METHOD

2.1. Description of the study area

The study was carried out in the intertidal and upper subtidal zone of Solong-on (9°11'55" N, 123°27'38" E), Municipality of Siquijor, Siquijor Island, Philippines. The total land area of the municipality is 90.70 km2 while the overall shallow marine ecosystem (below 30 m deep) has an estimated area of 1,035 ha. This marine ecosystem comprised of 175 ha of coral reef (slope and crest combined), 636 ha of seagrass beds, and about 90 ha of *Caulerpa racemosa* (lato) communities. Eleven species of *Caulerpa* grow abundantly on northwestern side, particularly in the adjacent barangays of Pasihagon, Tambisan, and Cang-alwang, where the substrate is generally muddy. Substrate also includes limestone platform, broken limestone with mud and rubble, mud, and fine silty sand extending to the seagrass community. Mangrove community is also present dominated by the reforested *Rhizophora* species.

2.1. Sampling Design and Sampling Procedures

Systematic sampling design was adapted in this study because three transect lines were established at equal intervals. Furthermore, a total of ten quadrats were established in each transect lines at equal intervals. Sampling covered three months, once every month, starting from November 2017 until January 2018. One day was allotted for the collection of samples. Transect-quadrat method was used to provide both destructive and non-destructive way of quantifying the biomass and percent cover, as well as the qualitative and quantitative description of the species of *Caulerpa* along the intertidal and upper subtidal zone of Solong-on, Siquijor. The survey was guided by the general procedure for the establishment of transect line by English et al (1997). Three replicate transects were established with a nylon transect line measuring 200-m perpendicular to the mangrove line. Each transect had a 150-m intervals parallel to each other. A 25-cm x 50-cm quadrat was laid along each transect lines with an interval of 20 m (Calumpong 1984). Furthermore, a bamboo marker was placed in each quadrat.

After establishment of transect lines and quadrats, the survey on percent cover was conducted using the Braun-Blanquet (B-B) cover abundance scale (Mueller-Dombois & Ellenberg 1974; Short & Coles 2001) (Table 1). The coverage of all species of *Caulerpa* within the quadrat was observed directly from above and was roughly estimated. After scoring, the values were converted into abundance using the formula:

Abundance = Sum of B-B scale values/number of occupied quadrats

Figure 1. Map of the sampling area showing the transect lines in Solong-on, Barangay Tambisan, Municipality of Siquijor, Siquijor Island. Source: openstreetmap.org

Table 1. Braun-Blanquet scale (adopted from Short & Coles 2001)

Class	Cover of the Quadrat
5	More than 75%
4	50-75%
3	25-50%
2	5-25%
1	Numerous, but less than 5% cover or scattered with cover up to 5%
0.5 or +	Few, with small cover
0.1 or r	Solitary, with small cover

Caulerpa species were identified based on available field guides and taxonomic references: "*Field Guide and Atlas of the Seaweed Resources of the Philippines*" by Trono (1997) and "*The Genus Caulerpa from Central Visayas, Philippines*" by Meñez & Calumpong (1982). Identification of samples was solely based on its morphological features. In addition, photographs of representative, fresh, *Caulerpa* samples were sent to Dr. Stefano Giovanni Angelo Draisma of the Faculty of Science, Prince of Songkla University for confirmation.

Within each quadrat, above-ground samples were carefully removed using a pair of scissors or knife, excluding the substrate. The samples were placed inside pre-labeled net bags. The samples were transported at the Biology Laboratory of Negros Oriental State University (NORSU), Main Campus I in Dumaguete City. Collected samples were later sorted to species. Samples were cleaned of all debris, epiphytes, and sediments (Thibaut et al 2004; Manas et al 2015). The samples were air dried for 3-5 days, then placed in labeled aluminum foils and placed inside an oven for drying at 80°C for 8 hours. The samples were kept inside the oven overnight. The biomass of each *Caulerpa* species was expressed as g dry wt. m^{-2} (Terrados & Ros 1995; Thibaut et al 2004; Iveŝa et al 2006; Qari 2017). Above-ground biomass represents the standing crop biomass (Calumpong 1984). The dried samples were weighed using an analytical balance at the nearest five decimal places. The dry weight biomass was then expressed as g dry wt m-2.
The following physico-chemical parameters were measured every sampling: salinity was measured using a hand-held refractometer, salinity for each transect lines was recorded. A drop of about 1 mL was placed on the daylight plate of the refractometer; pH using a Vertigrow LCD Digital pH Water Meter Tester Pen Type; water temperature was recorded using a glass thermometer (with three readings); and types of substrate using standard grab collection with scoop was adopted for the collection of surface sediments (Taft & Jones 2001; U.S. Army Corps of Engineers Jacksonville District 2009). The scoop was placed into the sediment for about 6 inches deep and about 150 g of sediment samples were collected and transferred inside pre-labeled plastic bags. The samples were processed for the determination of grain size. Prior to sieving, samples were air dried for 4-5 days, or until completely dried. Sieving took place using different available mesh sieve sizes. The collected sieved samples were identified based on its particle size range using the Wentworth Grade Scale as described by English et al (1997).

2.3. Data Analysis

Abundance (Standing Stock Biomass)

Differences between the biomass between transect and sampling months were statistically tested using Two-Way Analysis of Variance with repeated measures (RM-ANOVA) in PAST3 (v3.1) software (Hammer et al 2001). Prior to the analysis, using the packages nortest, car and MASS in R Studio (v1.1.419) (RStudio Team 2015), preliminary tests were performed to check for normality of data (Anderson-Darling normality test and QQ-plot) as well as evaluating homoscedasticity and assessing outliers. Values were log(x+1) transformed to meet parametric assumptions of ANOVA.

Diversity of Caulerpa species

Species diversity for each transect was calculated using the Shannon-Wiener Diversity Index (H'):

$$H' = -\sum_{i=1}^{s} \frac{ni}{N} \ln \frac{ni}{N}$$

Where, H' = value of Shannon-Wiener Diversity Index, ni = biomass in the ith species, N = total biomass, ln = natural logarithm and s = number of species in community

Environmental Parameters in Relation to Abundance and Diversity

To determine the influence of the environmental parameters (water temperature, salinity, pH and types of substrate) on the biomass of *Caulerpa* species, the multivariate Canonical Correspondence Analysis (CCA) using the PAST3 (v3.1) was used. This multivariate method was a direct gradient analysis that provided: (1) simultaneous representation of sampling sites, environmental variables and species centroids in a reduced ordination space of orthogonal axes, and (2) an integrated description of species-environment relationships by assuming a common response (as a unimodal distribution) of all species to a set of underlying environmental gradients (Ter Braak 1986).

III. RESULTS

A total of eight species of *Caulerpa* were observed in the intertidal and upper subtidal zone of Solong-on, Siquijor throughout the sampling period. As shown in Figure 2, the species obtaining the highest standing stock biomass of *Caulerpa* was *C. cupressoides* with mean biomass of 26.16±2.44 (SE) g dry wt m^{-2}, whereas C. microphysa has the least standing stock biomass with an average mean of 9.16±0.26 g dry wt m^{-2} (Figure 2). In general, the total standing stock biomass for all species obtained throughout the sampling period was 132.57±2.06 g dry wt m^{-2}.

Abundance, Diversity, and Physico-Chemical Parameters

Figure 3 showed that B-B abundance scale values were consistent throughout the three-month sampling. Using the B-B Abundance Scale, percent cover was converted into abundance. Overall, abundance of *Caulerpa* was highest in January 2018 (4.07) and lowest in December 2017 (3.7). One-way ANOVA (F=7.099, d.f.= 2, p=0.5288) revealed no significant difference between months. This suggests that percent cover does not depend on months of sampling. However, the limited time of sampling may have contributed to this result.

Throughout the study, water temperature ranged from 34.17 to 35.67 °C (mean: 35.11 °C) and Welch's ANOVA (p=0.9382) revealed no significant difference between months (November to January). Salinity, on the other hand, ranged from 34.8 to 35.1 ppt (mean: 34.99 ppt) and Welch's ANOVA (p=0.0713) revealed no significant difference between months. Likewise, pH range from 8.03 to 8.07 (mean: 8.06) and Welch's ANOVA (p=0.1484) revealed no significant difference between months (Table 2). Welch's ANOVA was performed since the monthly data for the environmental variables were of small sample size. Overall, there was no variation found in water temperature, salinity and pH on the sampling area throughout the sampling period.

Five types of substrates were classified according to the Wenthworth Grade Scale: gravel (14.5%), coarse sand (23.9%), medium sand (20.1%), fine sand (20.6%), and very fine sand (21.1%). Coarse sand obtained the highest mean percentage composition for transect 1 (T1), transect 2 (T2) and transect 3 (T3) with values 25.16, 23.47 and 23.05, respectively throughout the sampling period (Figure 4). Meanwhile, gravel had the least mean percentage composition for T1, T2 and T3 with values 13.79, 14.36, and 15.26, respectively.

Using the Shannon-Wiener Diversity Index (H'), the diversity for each transect throughout the sampling period was calculated. Both transect 1 (T1) and transect 3 (3) obtained almost the same H' values throughout the sampling period. However, T3 in the month of November obtained the highest H' value of 2.03. Transect 2 (T2), on the other hand, has the lowest H' value obtained all throughout the sampling period. T2 obtained the lowest H' value of 1.77 in the month of November. Overall, the *Caulerpa* in the study site were relatively diverse (Figure 5).

Table 2. Summary of water temperature, salinity, and pH in the study area in three months of sampling.

Month	Water Temperature (°C)	Salinity (ppt)	pH
November-2017	35.50±0.58	34.8±0.44	8.07±0.03
December-2017	34.17±0.44	35.1±0.15	8.07±0.03
January-2018	35.67±0.44	35.0±0.06	8.03±0.07

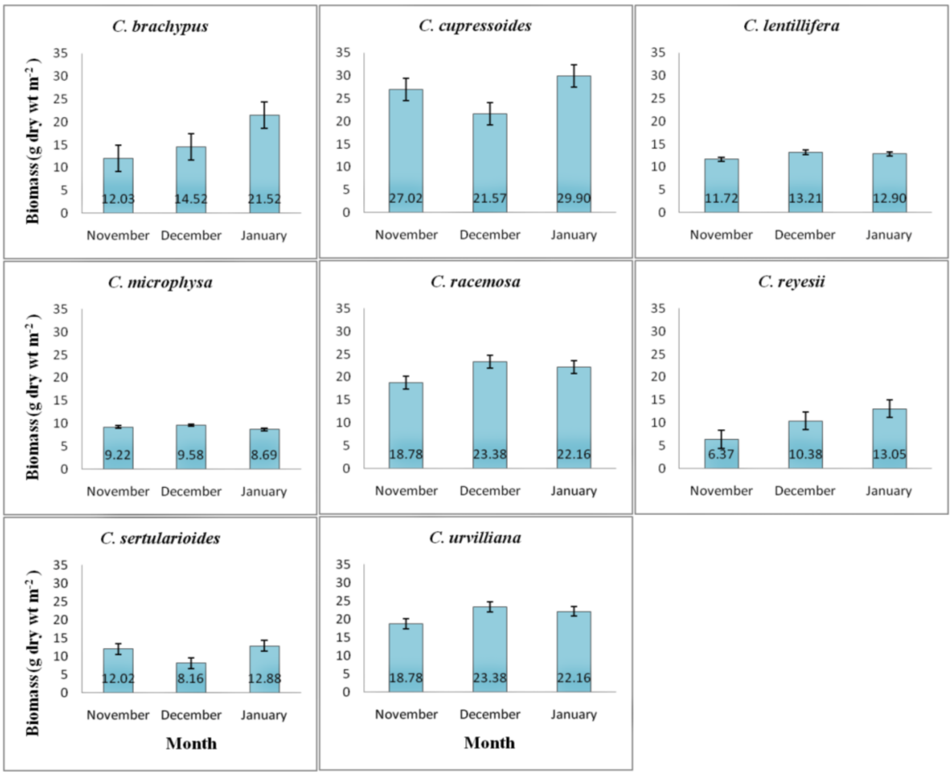

Figure 2. Mean±SE standing stock biomass of each *Caulerpa* species in the study site during the three-month sampling period. A. *C. brachypus*, B. *C. cupressoides*, C. *C. lentillifera*, D. *C. microphysa*, E. *C. racemosa*, F. *C. reyesii*, G. *C. sertularioides*, H. *C. urvilliana*.

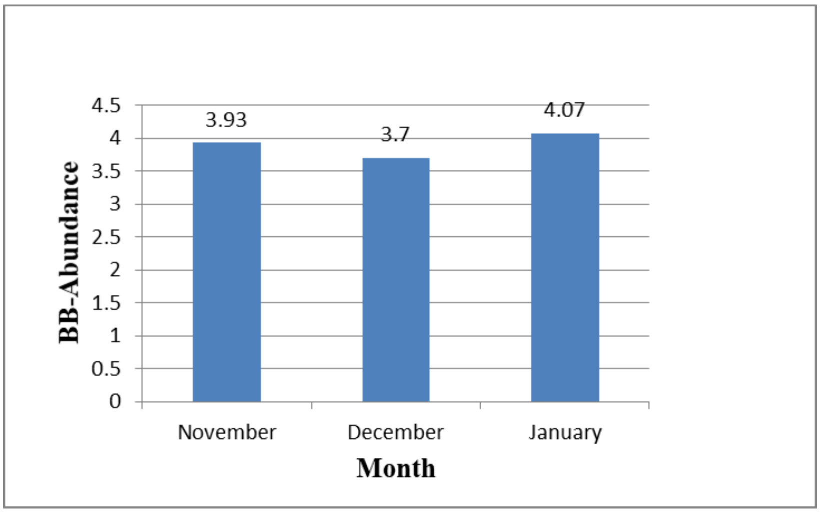

Figure 3. Mean (±SE) of B-B abundance scores of *Caulerpa* (overall) between sampling months (November 2016-January 2017)

The relationship between biomass of *Caulerpa* species and the eight environmental variables was assessed using the multivariate Canonical Correspondence Analysis (CCA) following ter Braak & Verdonschot (1995). Based on the CCA biplot, Axis 1 explained 52.4% of the variation in the abundance of the eight species, whereas Axis 2 explained 34.59%. However, an eigenvalue of 0.025 in Axis 1 indicate a relatively low gradient, with Axis 2 much weaker (0.016). It can be seen that of the eight environmental variables, four of which are positively correlated with the first axis (horizontal): pH, water temperature, medium sand and gravel. The rest are negatively correlated with the first axis: coarse sand, fine sand, very fine sand, and salinity. *C. cupressoides* and *C. racemosa* were the most abundant during the study. It can be inferred that these species having scores of 0.174 and 0.302 in Axis 1, respectively, were influenced by pH, water temperature, and two types of substrate grain size, gravel and medium sand (Figure 6).

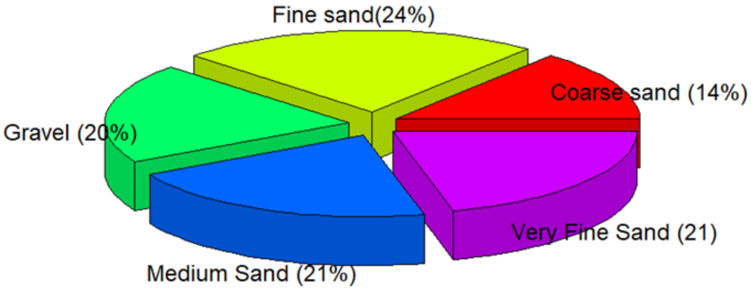

Figure 4. Type of substrates in the sampling sites

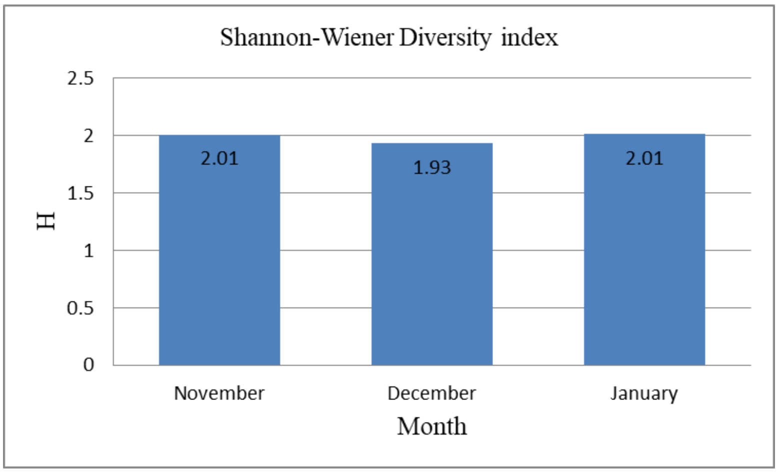

Figure 5. Shannon-Weiner Diversity Index between sampling months.

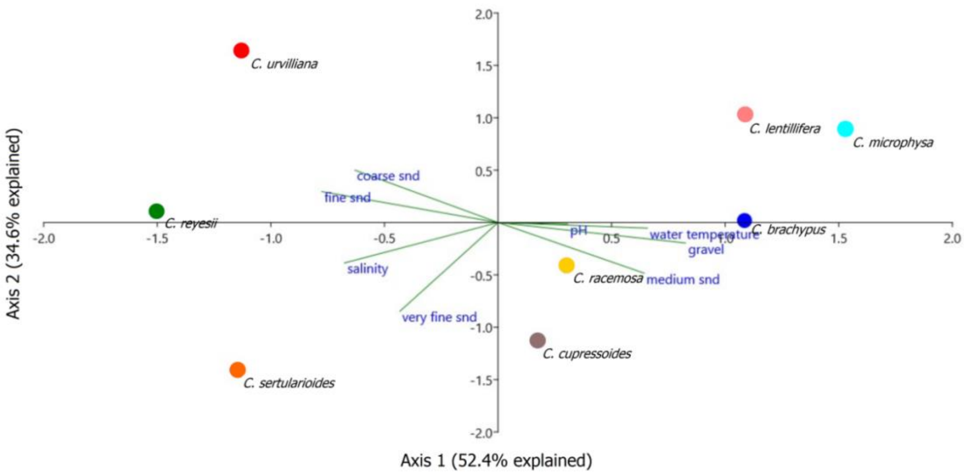

Figure 6. Canonical Correspondence Analysis (CCA) biplot showing the relationship of *Caulerpa* species and environmental parameters.

IV. DISCUSSION

After three months of sampling, eight species of *Caulerpa* were identified. These were *C. brachypus*, *C. cupressoides*, *C. lentillifera*, *C. microphysa*, *C. racemosa*, *C. reyesii*, *C. sertularoides*, and C. *urvilliana* (Plate 1). According to Meñez and Calumpong (1982), these species were commonly found in Siquijor, Philippines. The present study has fewer species count than the previous study by Calumpong (1984) which mentioned eleven species of *Caulerpa*, including *C. taxifolia* which may be found in deeper and sandy areas (Belsher & Meinesz 1995; Thibaut et al 2004; Aplikioti et al 2016) while the present study focused only in the shallow areas. Moreover, there was limitation of the length of transect line used. For example, Calumpong (1984) extended up to 600 m from the mangrove-lined shoreline. Given the limited sampling period, the following interpretations on standing stock biomass, cover, and diversity should be treated with caution.

The overall standing stock biomass in this study was relatively lower than the study conducted in the Mediterranean Sea by Thibaut et al. (2004). It has been considered that these macroalgal species were invasive in these areas due to their highly proliferative abilities (Boudouresque et al 1995; Klein & Verlaque 2008; Cebrian & Ballesteros 2009; Ruiz et al 2011; Papini et al 2013). In contrast, Calumpong (1984) reported a relatively lower standing stock biomass for *C. racemosa*. However, the present study obtained much higher values in the standing stock biomass. It has been reported that a decline in the harvest of *C. racemosa* occurred (Wagey & Bucol 2014). This decline in harvest, driven by local price of lato, may have contributed to increased standing stock biomass. Moreover, their proliferative abilities may have also contributed to the observed increase. According to the locals, when *Caulerpa* thalli are carefully cut, this would lead to an abrupt increase in the abundance. This observation needs further investigation. According to Klein and Verlaque (2008), one way of vegetative multiplication by *Caulerpa* is through fragmentation. Fragmentation caused by these anthropogenic disturbances may able to survive for several days and may re-establish on a suitable substrate (Ceccherelli & Piazzi 2001).

Braun-Blanquet cover abundance in this study showed that the genus *Caulerpa* occurred with the highest abundance throughout the sampling period. This means that *Caulerpa* were the dominant algal species present in the study site, although seagrasses (e.g. *Enhalus acoroides*) and sponges were also observed. This result is in agreement with the studies by Gab-Alla (2007) and Baldacconi and Corriero (2009) wherein *Caulerpa* dominates over *Halophila stipulacea* and sponge assemblages, respectively. This may be due to the competitive success of *Caulerpa* which is related to its specific characteristics which are its dispersal abilities (Klein & Verlaque 2008; Prathep et al 2011), its tolerance to the lack of severe nutrient limitation (Delgado et al 1994) and its tolerance to wide temperature (Gacia et al 1996).

This study also showed a relatively strong correlation of the types of substrate or sediment that may have contributed to the distribution and abundance of *Caulerpa* (Fernández-García et al 2012; Sahayaraj et al 2014; Dean et al 2015). The *Caulerpa* species collected in the publication by Meñez and Calumpong (1982) include sand, mud, coral rubbles, and rocky substrate. This was also true according to the study by Fernández-García et al. (2012). However, there was preference over the different types of substrates (Infantes et al 2011; Pinna et al, 2011) with varying growth rates (Katsanevakis et al 2010). According to Piazzi et al. (2007), these sediments are considered as structural constituents of *Caulerpa* that could play a role in their proliferation. In the present study, *C. cupressoides* was the most abundant in sandy substrates. According to Williams (1984), *C. cupressoides* was able to take up nutrients from these types of sediments as an adaptation to nutrient-limited waters.

Throughout the sampling period, a consistent H' index was observed. Although sampling was done for only three months. In contrast to other studies done elsewhere, which showed temporal variation (Tribollet & Vroom, 2007; Mendoza & Soliman, 2013; Kokabi et al 2016), the present study do not. The homogeneity of the environmental variables may have also accounted for this result.

Meanwhile, since there is no available data dealing with the molecular identification of C. reyesii at present, it would be an essential subject matter to consider for future studies. This species was first identified from the sampling area in 1978 and identification through molecular phylogeny would possibly update the taxonomic classification of *Caulerpa* (Belton et al. 2014).

V. CONCLUSIONS

. A total of eight species of *Caulerpa* was identified throughout the sampling period. This study has also shown that the standing stock biomass of *Caulerpa* was the same throughout the sampling period. The highest standing stock biomass was obtained by *C. cupressoides*. *Caulerpa* community was also relatively diverse (H' index = 1.7 – 2). Tropical shallow environments apparently provide all the requirements for a relatively diverse and abundant *Caulerpa* species. Water temperature, in particular, has greatly influenced the biomass and abundance of *Caulerpa*. Thus, when culturing these macroalgal species, water temperature as well as the percentage composition of sediments must be monitored well.

Recommendations

The increase in the standing stock biomass between the study in 1984 and the present study may have illustrated the prolific character of *Caulerpa*. However, more studies are still needed to fully understand these characteristics especially in the tropical regions. Other environmental parameters which are vital to the abundance and distribution of *Caulerpa* species were not included in this study. Thus, for future studies, the following environmental parameters (e.g. light intensity, wave exposure, and sediment and water nutrient content) must be included. It is also worthy to mention the effects between the competition of *Caulerpa* and seagrasses in the study site. The duration of the study should also be extended to at least one-year to cover temporal variation in the biomass and abundance of *Caulerpa*. Extending of transect lines or different zones must also be considered when sampling these *Caulerpa* community.

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