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Chlorophyll content of *Thalassia hemprichii* (Ehrenberg) Ascherson leaves in some coastal areas of Cebu Island, Central Philippines

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ABSTRACT

Variations in the chlorophyll concentrations in seagrass leaves can be used to indicate biomass, productivity and overall ecosystem condition in coastal areas. In this study, we determined the chlorophyll content (ie chl *a* and *b*) of *Thalassia hemprichii* from selected coastal areas in Cebu Island, Central Philippines to determine if there were spatial variations between intertidal (0m) and subtidal (1.5m) sections, and in the five geographically separate coastal areas of the island. Pigment extraction was done using 95% acetone, the solution analyzed via spectrophotometry and quantified using the equation of Ritchie (2006). The values were statistically compared using nonparametric tests. Mean chlorophyll content and chlorophyll *a/b* ratio of *T. hemprichii* showed no significant variations between intertidal and subtidal sections: $F_{(4,45)}$ =27.75, *p=0.192* for mean chlorophyll content and $F_{(4,45)}$ =18.28, *p=0.116* for chlorophyll *a/b* ratio. However, there was significant difference between geographic areas (*p=0.000*). Although *T. hemprichii* tend to display a physiological response to shading and light limitation, the difference in depth (≈ 1.5m) between intertidal and subtidal sections did not produce a statistically meaningful difference. However, the significant differences between sites for the chlorophyll content and chlorophyll *a/b* ratio of *T. hemprichii* could guide future decision in seagrass transplantation of specific localities.

Keywords: chlorophyll *a/b* ratios, seagrass health, bioindicator of coastal pollution, spatial variation, Philippine reefs

INTRODUCTION

Seagrass meadows have an annual production between 975 to 3,614 g DW m^{-2} $year^{-1}$ in the tropics, making them one of the most productive ecosystems in the world (Rasheed et al 2008, Wirachwong & Holmer 2010). Seagrasses are utilized for medical, nutritional, aesthetic, and household purposes (Newmaster et al 2011, Smithsonian Ocean 2018). Among the 50 seagrass species worldwide

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(Den Hartog 1970), there are 12 species in the Philippines, of which, eight are found in the Visayas region (Meñez et al 1983). *Thalassia hemprichii* (Ehrenberg) Ascherson is one of the most common seagrass species in Philippine waters, coexisting with *Cymodocea rotundata* and *Enhalus acoroides* (Meñez et al 1983). Distribution of *T. hemprichii* is primarily influenced by daylength and acquired light regimes (Fortes & Santos 2004). Furthermore, it also has the ability to grow optimally despite decreased oxygen conditions due to its unique metabolic strategies, enabling it to successfully colonize shallow-water marine habitats. The ability to vary the amount of chlorophyll *a* in the leaves of *T. hemprichii* may, among others, confer some competitive advantage. *Thalassia* species are good candidates in laboratory and field transplantations (Fuss & Kelly 1969, Kelly et al 1971) and are also considered climax species (Fortes & Santos 2004).

Chlorophyll is vital to photosynthesis because it allows plants to acquire energy from light. Measuring chlorophyll content becomes an important tool in estimating photosynthetic efficiency (Wagey 2013) and detecting light stresses in seagrass communities (Dawes 1998). In seagrasses, significant fluctuation in chlorophyll content in terms of chlorophyll *a:b* ratios is affected by light regime and temperature (Abal et al 1994, Dennison & Alberte 1982, 1985, Lee & Dunton 1997, Macauley et al 1988, Wiginton & McMillan 1979). Since chlorophyll concentration is easy to analyze, interests were directed to its application as indicator of productivity and ecosystem condition. In the past, chlorophyll was used as an index of biomass (Cullen 1982). But the same parameter could also be used for other interpretations (Ralph & Burchett 1995). Dale and Causton (1992) stressed the use of chlorophyll content and chlorophyll *a/b* ratio as a form of bioassay and the potential use of leaf chlorophyll ratio as a rapid and simple approach to evaluate seagrass health (Kettenring et al 2014). In tropical islands, the development of beach resorts to attract local and foreign tourists may have led to localized changes in the marine coastal environment where stands of *T. hemprichii* are naturally growing. There is thus, a need to study photosynthetic efficiency of the seagrasses through measurement of the chlorophyll content of the leaves. In this study, we determined the chlorophyll content and chlorophyll *a:b* ratios of *T. hemprichii* leaves in the intertidal and subtidal sections of five seagrass sites in Cebu, Central Philippines.

MATERIALS AND METHODS

Collection Sites

The collection was conducted from December 4, 2017 to January 22, 2018 in five seagrass communities in Cebu Island, Central Philippines. These collection sites were strategically selected according to where they are particularly located in the island. The collection sites were Maribago, Lapu-lapu (10.2840°N, 123.0003°E) and Cordova, Mactan, Central East Cebu (10.2397°N, 123.9479°E), Poblacion, Dalaguete, Southern Cebu (9.7540°N, 123.5316°E), Aloguinsan, Western Cebu (10.2403°N, 123.5610°E), and Medellin, Northern Cebu (11.1207°N, 124.0217°E) (Figure 1). Leaf samples were collected during low tide from the intertidal (0 to 0.1m) and subtidal (1 to 1.5m) sections in each site. The physicochemical features of the collection sites were not measured.

The leaf samples were placed in re-sealable plastic bags, wrapped with

Figure 1. Location of Cebu and the collection sites (●) showing their relative distance from each other. (Maps courtesy of PhilGIS)

aluminum foil to avoid chlorophyll degradation, stored in an insulated ice box with ice slurry and were immediately transported to the Bioprocessing Laboratory of the University of San Carlos - Talamban Campus for analysis. All samples were frozen prior to chlorophyll extraction.

Chlorophyll Extraction and Analysis

Frozen seagrass leaves were thawed, the epiphytes removed by wiping off the leaf blades with delicate task wipers. All grinding and extraction procedures were done under dark and cool (23°C) room conditions. About 0.50 to 0.51g fresh weight (FW) of the leaf blade samples were crushed using a chilled mortar and pestle. Chlorophyll was extracted by adding 10ml of 90% acetone. The test tubes were wrapped in aluminum foil and placed in a refrigerator overnight to complete the chlorophyll extraction. The samples were centrifuged the next day at 1,000rpm for 5min. A 1-ml supernatant was carefully pipetted and was further diluted by adding another 9ml of the extraction solvent. The final extract was analyzed spectrophotometrically using a 1-cm cuvette. The absorbance of the extract was read at 647, 664, and 750nm (Biber 2007, Granger & Lizumi 2001, Jeffrey & Humphrey 1975, Wagey 2013). Chlorophyll concentration was determined using the equation of Ritchie (2006):

$$Chl\ a\ (mg/g\ DW) = -1.7858 \times A_{647} + 11.8668 \times A_{664} \qquad \text{Equation 1}$$

$$Chl\ b\ (mg/g\ DW) = 18.9775 \times A_{647} - 4.8950 \times A_{664} \qquad \text{Equation 2}$$

where A is the absorbance of a particular wavelength. The total chlorophyll of each sample was computed and the value multiplied by a dilution factor. The total chlorophyll was standardized to per unit weight by dividing the value with the dry weight derived from equation 3. The dry weight (DW) was determined by linear regression of DW with FW using the equation:

$$Dry\ weight\ (DW) = 0.0001 + (0.027 \times FW) \qquad \text{Equation 3}$$

A nonparametric Wilcoxon Signed Rank test was used to examine significant differences of chlorophyll content and chlorophyll *a/*b ratio of leaves collected between intertidal and subtidal section since data was not amenable to transformation. Kruskal-Wallis test was used to determine significant difference in chlorophyll content and ratio across sites. Dunn's nonparametric comparison was used as a post hoc test should there be a significant difference in the Kruskal-Wallis test result. The significant level was set at 95%.

RESULTS AND DISCUSSION

Thalassia hemprichii leaves collected from the intertidal section had chlorophyll values of 4.3 ± 2.5mg/g DW (mean ± SD) while the subtidal section had 3.5 ± 2.3mg/g DW. There was no significant difference (*p=0.192*) in the mean There was no significant difference (*p=0.192*) in the mean chlorophyll content between the two sections (Figure 2A). The chlorophyll *a/b* ratio of *T. hemprichii* leaves did not also vary significantly between sections (2.5 ± 3.2 for intertidal section and 4.5 ± 8.3 for subtidal section) (Figure 2B).

The comparison of the mean chlorophyll content and chlorophyll *a/b* ratio shows the adaptive limits of optical plasticity in *T. hemprichii* leaves (Wagey 2013). However, significant variation in chlorophyll content and chlorophyll *a/b* ratio was not observed in *T. hemprichii* leaves in relation to where they are particularly located (ie, intertidal vs subtidal) suggesting that small variations in depth did not significantly contribute to chlorophyll concentration. The ambient water irradiance between the collection areas must have been relatively uniform.

The mean chlorophyll content of *T. hemprichii* ranged from 1.0 ± 0.3 to 6.7 ± 1.9mg/g DW in the five seagrass sites. Highest chlorophyll content was found in Medellin (6.7 ± 1.9mg/g DW), followed by Dalaguete (5.7 ± 1.3mg/g DW), then Maribago (3.3 ± 1.9mg/g DW), Cordova (2.9 ± 0.2mg/g DW), and lowest at Aloguinsan (1.0 ± 0.3mg/g DW). There was a significant difference (*p=0.000*) in the chlorophyll contents of *T. hemprichii* leaves. The mean chlorophyll content in Aloguinsan was significantly lower than that of Dalaguete, Cordova and Medellin (*p=0.000*) (Figure 2C).

Similarly, chlorophyll *a/b* ratios showed significant differences (*p=0.000*) across collection sites. Maribago contained the highest chlorophyll *a/b* ratio (13.2 ± 9.1), followed by Medellin (2.5 ± 0.2), then Aloguinsan (0.7 ± 0.4), Cordova (0.5 ± 0.0), and lowest at Dalaguete (0.5 ± 0.0). The mean chlorophyll *a/b* ratio in Maribago was significantly higher than those in the other sites (Figure 2D). In general, chlorophyll concentration is affected by light conditions (Collier et al 2012, Enríquez 2005, Ralph et al 2007) and the volume of epibionts (Fong et al 2000, Fyfe 2003, Sand-Jensen 1977). Our results suggest that localized environmental conditions could have affected the seagrass chlorophyll concentration. The intrinsic reaction of increasing chlorophyll *a* than *b* values is an adaptation to develop larger harvesting complexes resulting to the increased number of chlorophyll concentration. The different chlorophyll content listed in Table 1 seemed to indicate the lack of standardization in reporting chlorophyll values and units. Standardization will facilitate geographic comparison of chlorophyll parameters of *T. hemprichii*. The need to survey more *T. hemprichii* areas and determine their chlorophyll concentration should be interesting to the rehabilitation effort of denuded coastal zones in the tropics.

The relatively low chlorophyll *a/b* ratio in Dalaguete, Cordova, Medellin and Aloguinsan indicates a different type of adaptation to local conditions. Collier et al (2012) observed that seagrasses under low light conditions exhibited low chlorophyll *a/b* ratios as an adaptation to improve light capture by accessing the longer wavelengths or the blue-violet lights, which are known to be proportionally more dominant in low light situations. The presence/absence of cloud cover as well as the volume of epibionts covering the leaves of *T. hemprichii* could have affected the chlorophyll *a/b* ratios. Observable volume of epibionts, including microalgae, foraminiferans and sessile invertebrates, were also found to encrust *T. hemprichii* samples from Medellin, Dalaguete and Maribago. These epibionts could have masked the *T. hemprichii* leaves to some degree and could have significantly reduced light absorption at the green peak of the light spectrum (Fyfe 2003). In principle, chlorophyll *a* concentration decreases when the amount of sunlight becomes limited, hence increasing chlorophyll *a/b* ratio (Garrigue 1998). Under localized scenario, it may also not be energetically worthwhile for these *T. hemprichii* populations to boost chlorophyll concentration. This adjustment may be economically better strategy for *T. hemprichii*. Among our sites, the coastal embayment of Maribago is considered the most disturbed, due to the presence of beach resorts including high frequency of tourist activities (eg, swimming, scuba diving, snorkeling, gleaning, boat parking, jet skiing, etc). The development of this particular area accelerated within the past 15 years, as contrasted to the other sites that remained relatively unexploited. This high human presence might have increased disturbance to the seagrass assemblages in the intertidal flat.

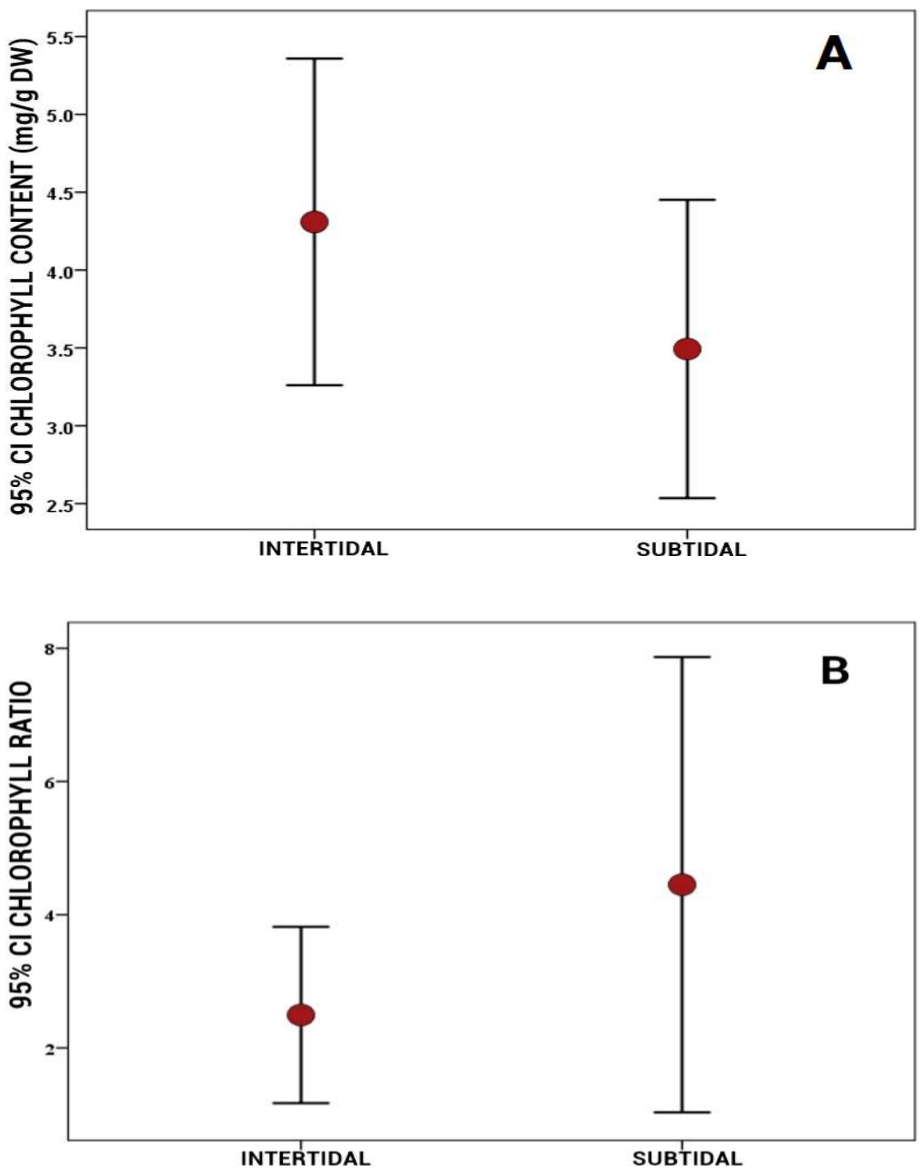

Figure 2. Chlorophyll content (Subfigures A, C) and chlorophyll ratio (Subfigures B, D) of *T. hemprichii* respectively across sections and collection sites. Values are mean ± 95% CI

The chlorophyll content

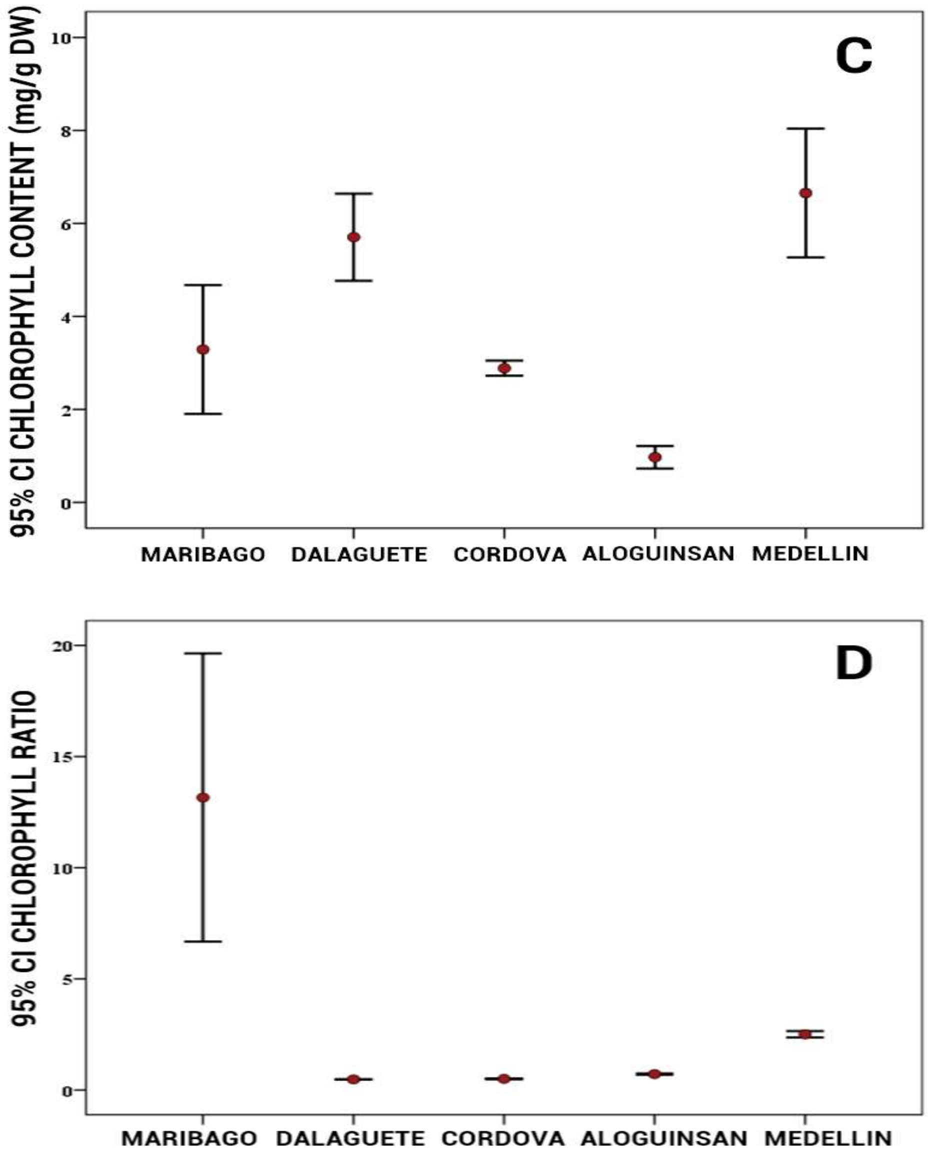

Figure 2 Continued

Table 1. Chlorophyll content of T. *hemprichii* from other areas

Locality	Chlorophyll content	Reference
Hainan Island, South China	1.75 mg FW g^{-1}	Zhang et al 2014
St. John's Island, Singapore	8.5 mg FW g^{-1}	Ali et al 2012
Talim Point, Lian, Batangas	54.13 mg l^{-1}	Clores and Carandang 2013
South Mantuod, Lian, Batangas	1.72 mg l^{-1}	Clores and Carandang 2013
Shields Marine Station, Lian, Batangas	1.79 mg l^{-1}	Clores and Carandang 2013
Kayreyna, Lian, Batangas	9.05 mg l^{-1}	Clores and Carandang 2013
Medellin, Northern Cebu	6.7 mg DW g^{-1}	This study
Dalaguete, Southern Cebu	5.7 mg DW g^{-1}	
Maribago, Mactan Island	3.3 mg DW g^{-1}	
Cordova, Mactan Island	2.9 mg DW g^{-1}	
Aloguinsan, Western Cebu	1.2 mg DW g^{-1}	

CONCLUSION

In retrospect, *T. hemprichii* from selected coastal areas of Cebu exhibited differences in chlorophyll content and ratios, and need further ecophysiological investigations to determine their practical applications. The idea of using chlorophyll concentration in assessing seagrass communities can, in the future, explain how local environmental conditions could alter physiological seagrass responses. The chlorophyll parameters could also be used as proxy indicator to monitor seagrass health.

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