



Morphometric analysis of congeneric seagrasses (*Cymodocea rotundata* and *Cymodocea serrulata*) in the coastal areas of Bunaken National Park, North Sulawesi, Indonesia

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Abstract. This study compared the morphometric variations of two seagrass species, *Cymodocea rotundata* and *C. serrulata*, from two sampling locations (with subsites: Bunaken A & B, Wawontulap A & B) within the Bunaken National Park, North Sulawesi, Indonesia. Based on the multivariate ordination (Principal Component Analysis, PCA), certain characters (leaf frontal area, leaf cross section, and leaf width) characterized *C. serrulata* while leaf length, rhizome and total production characterized *C. rotundata* samples. These observations roughly coincide with what is known about the morphological characteristics of these two species. At the location level, there was no direct relationship with seagrass morphometric and specific sampling locations, with the only possible exception is on above-ground and below-ground biomasses, which appear highly correlated with Wawontulap. Two-Way Analysis of Variance (ANOVA) revealed that the two species, *C. rotundata* and *C. serrulata*, statistically differ in terms of leaf length, rhizome, and both above-ground and below-ground biomasses. On the other hand, *C. serrulata* showed higher values in terms of leaf width, leaf cross-section area, and leaf front area. Wawontulap had consistently and significantly higher above-ground and below-ground biomass for the two species than Bunaken. The observed morphometric differences may be due in part to their inherent phenotypic characters but the influence of abiotic and biotic (e.g. grazing) stressors cannot be underestimated. It is recommended that a long-term temporal monitoring of both above-ground and below-ground biomass in relation to environmental (both biotic and abiotic) parameters be conducted in the study area.

Key Words: biomass, ecosystem, productivity, morphology seagrass, leaves.

Introduction. Seagrass is one of the most productive ecosystems in the shallow marine environment that have a vital part of life at sea. Ecologically, the existence of seagrass in the coastal areas provides substantial contributions to nutrient for the fertility of coastal waters and marine environment, in general. Seagrass meadows have high biological productivity, comparable to other ecosystems (Azkab 2006). They also function as primary producers, recycling nutrients, stabilizers bottom waters, sediment traps and barriers to erosion (Tomascik et al 1997; Nobil et al 2012). Seagrass beds are as important as other ecosystems in the shallow waters such as coral reefs and mangrove ecosystems. Seagrass are the classic ecosystems and highly productive habitat because it is a habitat for many types of biota such as macroalgae, sponges, coral, various types of shells, and various types of sessile invertebrates that are essential sources of food and income for coastal inhabitants (Duffy 2006).

Research on the seagrass ecosystems in areas impacted by anthropogenic (human) activities remain very limited, especially in developing countries. With this research, it is hoped that an overview of how morphological characters of closely-related seagrass species may be influenced by environmental factors at the local scale and also to serve as baseline information of these parameters with particular emphasis on the present status of primary production (expressed in terms of standing crop biomass) of the seagrass beds within the Bunaken National Park (BNP), a UNESCO Heritage site.

Specifically, this study aims to compare the morphometric variation of two seagrass species (*Cymodocea rotundata* and *Cymodocea serrulata*) in relation to environmental characteristics in Bunaken National Park, North Sulawesi, Indonesia.

Material and Method. This research was conducted from June to October 2017 in the coastal waters of Bunaken National Park (BNP), North Sulawesi, Indonesia, specifically in Wawontulap, the District of Tatapaan, South Minahasa (1.3128°N, 124.5145°E) and Bunaken Islands (1.5873°N, 124.7810°E), Manado City (Figure 1). Wawontulap is ~43.8 km to the southwest of the sampling site in Bunaken. In each sampling site (Wawontulap and Bunaken), subsites (A & B) were designated. The entire BNP has a total area of 79,056 hectares (land and marine areas combined). Since its establishment in 1991 by the Ministry of Environment and Forestry, Bunaken National Park (Taman Nasional Bunaken) is well-known as a major tourist destination in Southeast Asia. In 2006 alone, a total of 32,000 visitors (10,229 foreign) were reported with total revenue of Rp 1,395,158.00 (Davis 2005).

Primary data was collected with simple random sampling. Measurements of seagrass morphometric were done at the Laboratory of Marine Biology, Sam Ratulangi University, Manado. The Image-J software was applied to measure the leaves (length, wide, rhizome, leaf cross-section area, and leaf front area). For physical environment data, HOBO® pendant temp/light loggers (UA-002-08; #2254901 and #2245954) were used to measure sub-surface light intensity and temperature. Data logging was set at an hourly interval. Sampling method of seagrass used a quadrat (50 x 50 cm) with transect lines based on Seagrass Net protocol, i.e., stretched perpendicular line of the coastline in an offshore direction as far as 100 m (depending on conditions of the seafloor). These transects were placed 20 m from each other (McKenzie et al 2009).

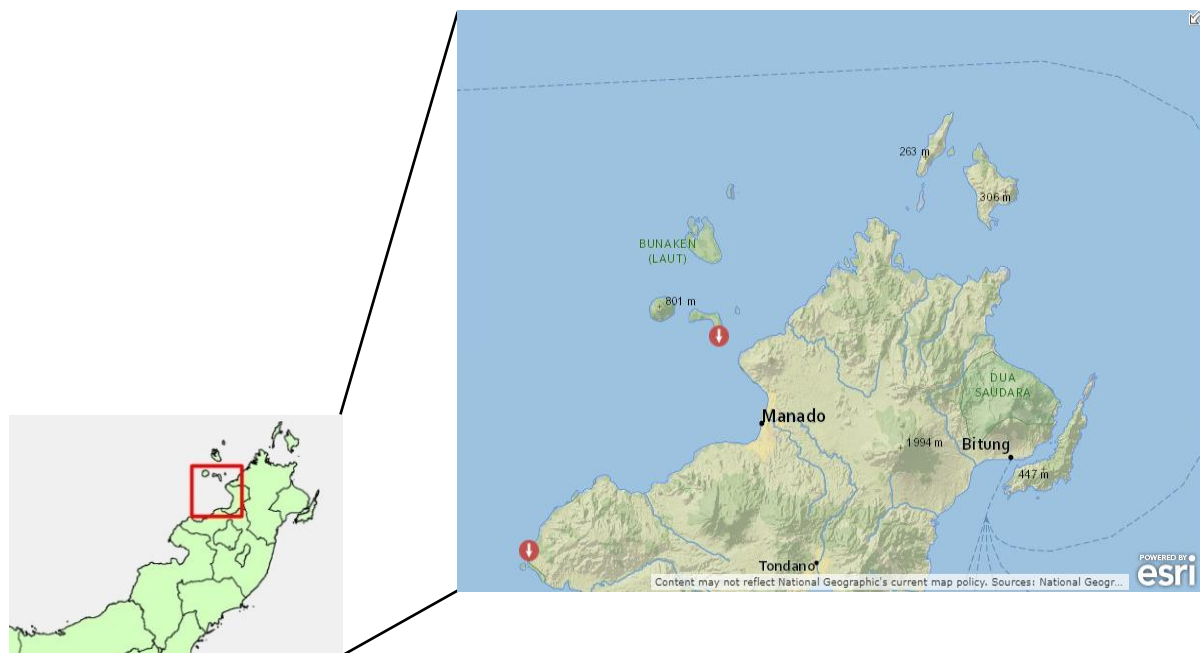


Figure 1. Map of Bunaken National Park showing the sampling locations (Wawontulap, left red dot; Bunaken, middle red dot).

Data analyses. Morphometric data on the two species of seagrasses, *C. serrulata* and *C. rotundata*, were visualized using the Principal Component Analysis (PCA) following the procedures described by Abdi & Williams (2010). PCA plot was generated using the *ggfortify* package in R platform (R Core Team 2013; RStudio 2015). Interpretation of PCA was based on eigenvalues and projected inertia (%) using the “ade4” package (Dray & Dufour 2007).

A Two-way ANOVA (using the *avov* function in R) was used to compare morphological data across the survey locations (Bunaken A, Bunaken B, Wawontulap A, Wawontulap B) and between the two species (*C. serrulata* and *C. rotundata*). Analyses were carried out in either PAST3 (Hammer 2013) and RStudio 3.2.5 (R Core Team 2013) softwares. Prior to ANOVAs, data were checked to conform to parametric assumptions (normality, homogeneity of variance, outliers) using the *car* and *norstest* packages in R. The data on morphometrics conformed to the parametric assumptions, thus log transformation was not needed (Zar 1999). A post hoc test (Tukey's Honest Significant Difference, Tukey HSD function) using Tukey multiple comparisons of means 95% family-wise confidence level was performed when significant differences were revealed in ANOVA.

Results. PCA (plotted using *ggfortify* in R) output shows correlations among *Cymodocea* morphometric variables and locations (Figure 2). Based on the eigenvalue of 2.48, Axis 1 (or PC1) explained 41.33% (projected inertia) of the observed variation while Axis 2 (PC2) explained 22.4% of the observed variation. Based on the correlation with PC1 and the variables (loadings), leaf width had the lowest loadings. Leaf width was highly related to PC1 (along the horizontal axis) can be interpreted as highly variable. Leaf frontal area, appeared moderately correlated with PC2, while leaf length and biomass (above-ground and below-ground) were of moderate correlation with PC1 and PC2. The arrows indicate the direction and range of increasing value for the environmental variables. Longer vectors (arrows) signify a greater range of variation in the observed values for the variable. In this case, leaf frontal area, leaf length, and biomass values showed high ranges while leaf width and root length appeared to have less variability. More acute angles between vectors and axes indicate a stronger correlation. It can be shown in the graph above that the characters leaf frontal area, rhizome, leaf length and leaf width characterized *C. serrulata* while leaf cross-section and biomass (above-ground and below-ground) characterized *C. rotundata* samples. These observations fit well with what is known about the morphological characteristics of these two species (Wagey; Sakey et al 2015).

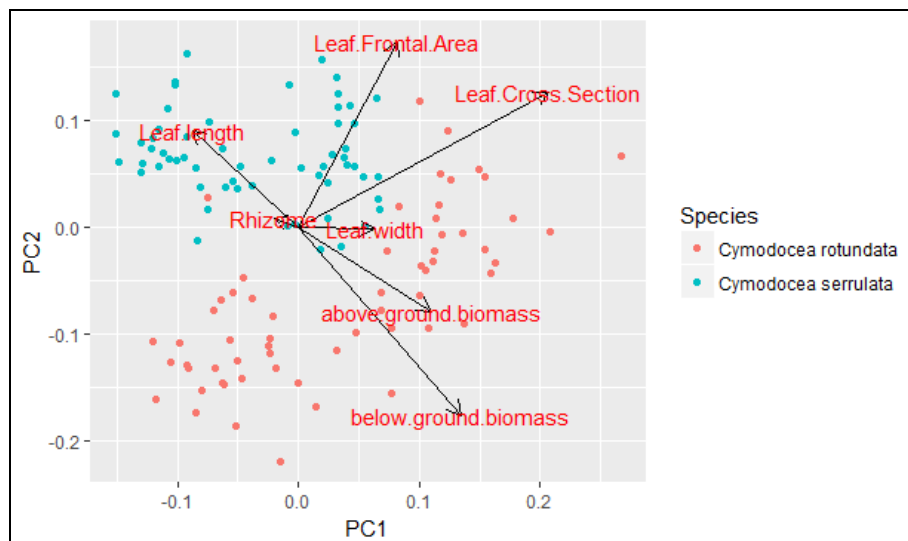


Figure 2. Principal component analysis (PCA) biplot showing relationship between seagrass morphometric data of the two species and locations. Data were log-transformed to standardize prior to analysis.

Using the same morphological characters of the two seagrass species, PCA biplot showed no relationship with any of the seagrass morphology as indicated by a scattered and overlapping specific sampling locations (Figure 3). This means that there is a reason to believe that there was no direct relationship with seagrass morphometrics and locations, unlike what was shown above on species. The only possible exception is on above-ground biomass and below-ground biomass, which appear highly correlated with Wawontulap sites.

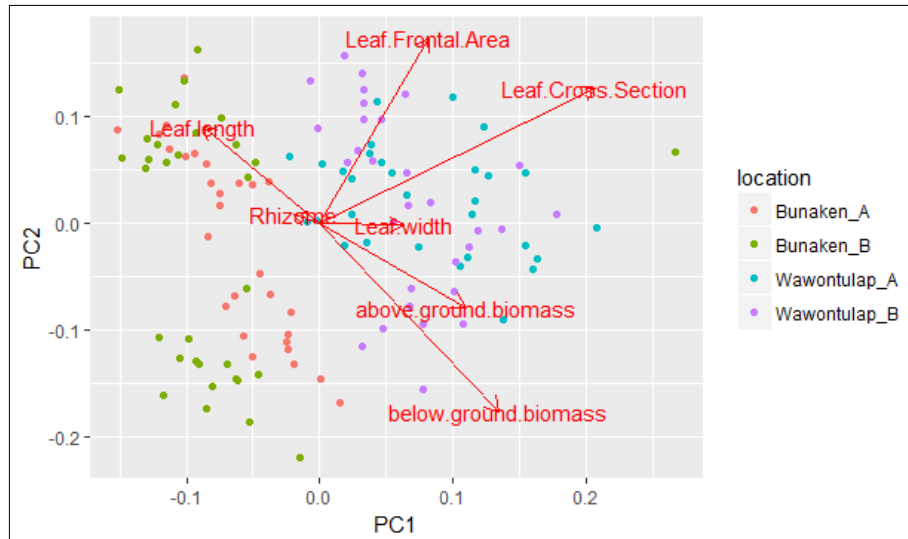


Figure 3. Principal component analysis (PCA) biplot showing relationship between seagrass morphometric data and location. Data were log-transformed to standardize prior to analysis.

As shown in Figure 4, leaf length (measured in cm) for *C. rotundata* had a mean value of 10.3-10.78±0.4 SE in Bunaken while *C. serrulata* are generally shorter (with only 9.3-9.7 cm) than *C. rotundata*. In Wawontulap sites, *C. rotundata* grew at 13.4 to 15.23 cm±0.3-0.5. Leaf width (mm) of the two species did not differ in all sites (3.6 to 4.36 mm±0.07 to 0.1). In terms of leaf frontal area (cm²), lowest values (4.5 to 5.5 cm²) were observed in Bunaken for *C. rotundata* while the values in Wawontulap for this species are comparable to that of *C. serrulata* in Bunaken and Wawontulap sites (8-9 cm²). Leaf cross-section (mm²) was generally a little higher in *C. serrulata* compared to *C. rotundata*. Interestingly, the pattern for rhizome appears a slight opposite, i.e., *C. rotundata* has slightly higher values compared to *C. serrulata*. Finally, in terms of the biomasses (i.e. g.dw/m²), *C. rotundata* consistently had higher mean biomass values compared to *C. serrulata*, with Wawontulap A and B, contributing to the observed significant differences. The mean above-ground biomass of *C. rotundata* ranged from 12.3±0.49 (S.E. althroughout) g.dw/m² in Bunaken B to 17.13±0.49 g.dw/m² in Wawontulap B. Below-ground biomass of *C. rotundata*, on the other hand, which are consistently lower than above-ground biomass values (on average above-ground biomass 1.5 times higher than below-ground biomass), ranged from 8.89±0.63 g.dw/m² in Bunaken A to 11.16±0.63 g.dw/m² in Wawontulap B. Mean above-ground biomasses of *C. serrulata* ranged from 9.17±0.3 g.dw/m² in Bunaken A to 12.09±0.5 g.dw/m² in Wawontulap B. Mean below-ground biomasses of this species ranged from 5.47±0.25 g.dw/m² in Bunaken B to 8.68±0.29 g.dw/m² in Wawontulap A.

When considered as entire sites as Bunaken and Wawontulap, overall trend in terms of mean total biomass (above-ground and below-ground biomasses combined), was still observed, i.e., each site and species would have a mean total biomass of 23.56±0.66 g.dw/m² and 28.04±0.33 g.dw/m² (*C. rotundata*) and 15.78±0.25 g.dw/m² and 19.29±0.27 g.dw/m² (*C. serrulata*), respectively.

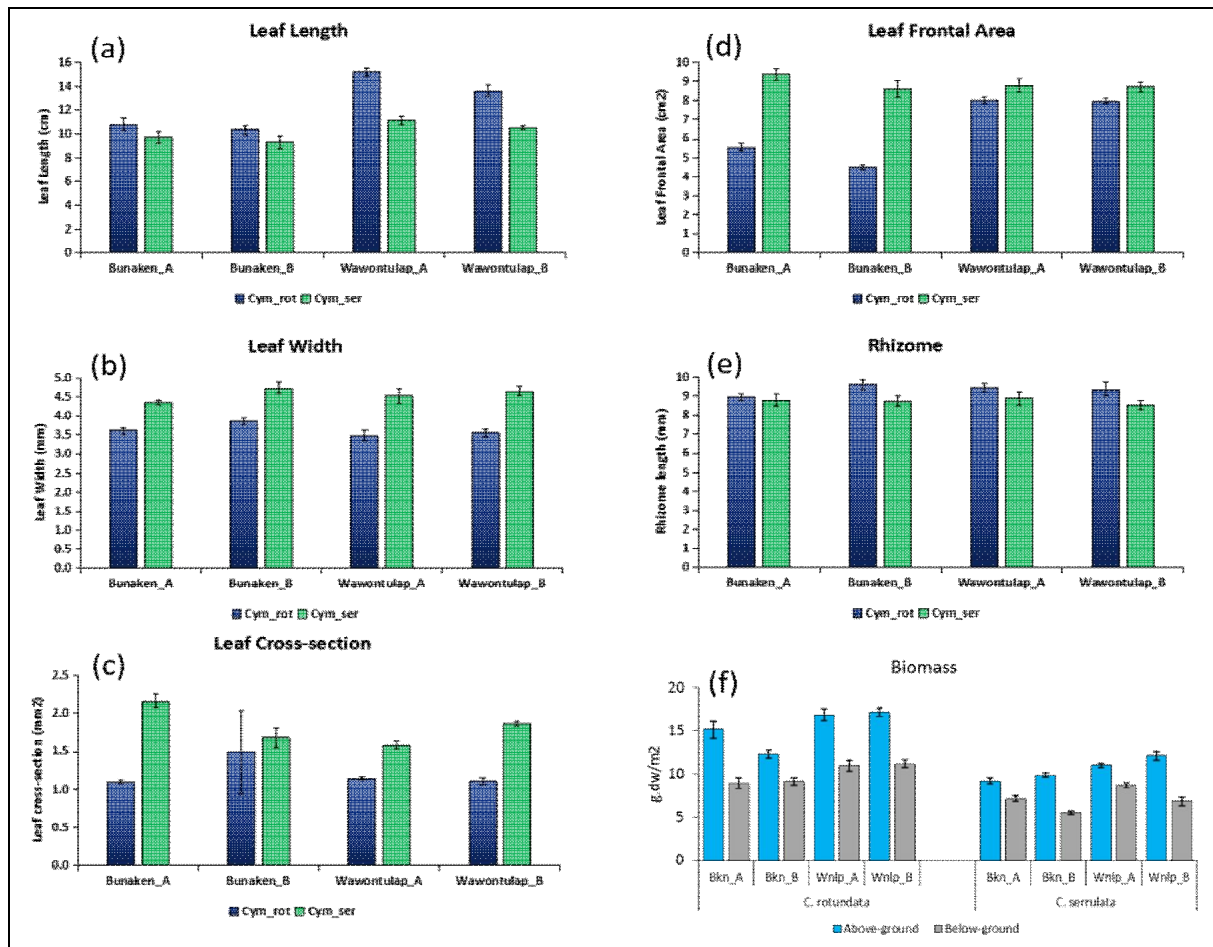


Figure 4. Mean \pm SE of morphometrics of the seagrasses *Cymodocea rotundata* and *C. serrulata* compared between sites (Bunaken A, Bunaken B, Wawontulap A, and Wawontulap).

Two-way ANOVA revealed significant differences between sites in all morphometric variables between species ($p < 0.001$; Table 1). Subsequent Tukey's HSD tests revealed further that the following comparisons caused the observed significant differences: for seagrass length (Wawontulap A & B > Bunaken A & B); seagrass width (no significant differences between locations); leaf cross-section (no significant differences between locations); leaf frontal area (no significant differences between locations); frontal area (Wawontulap A & B > Bunaken A & B); and rhizome (no significant differences between locations); and total production (Wawontulap A & B > Bunaken A & B). Between the two species, *C. rotundata* had statistically significant leaf length, rhizome, and both above-ground and below-ground biomasses compared to *C. serrulata*. On the other hand, *C. serrulata* showed higher values in terms of leaf width, leaf cross-section area, and leaf front area. In terms of above-ground and below-ground biomass, Wawontulap sites were consistently and significantly higher than the sites in Bunaken ($p < 0.001$; Tukeys HSD test revealed: Wawontulap A, B > Bunaken A, B) (Table 1). These findings parallels with the patterns observed using bar plot method (Figure 4).

Table 1

Summary of Two-Way ANOVA Results with post hoc (Tukey's HSD) comparisons

Variable	Source	Df	Sum squares	Mean squares	F value	P-value	S C	Tukey's HSD test
Leaf length	Species	1	161.68	161.68	60.043	< 0.001	***	<i>C. rotundata</i> > <i>C. serrulata</i>
	Location	3	227.7	75.9	28.187	< 0.001	***	Wawontulap A&B > Bunaken A&B
	Species x location	3	51.92	17.31	6.428	< 0.001	***	
	Residuals	112	301.59	2.69				
Leaf width	Species	1	26.292	26.292	108.379	< 0.001	***	<i>C. serrulata</i> > <i>C. rotundata</i>
	Location	3	1.835	0.612	2.521	0.0615	n.s.	
	Species x location	3	0.605	0.202	0.831	0.4795	n.s.	
	Residuals	112	27.171	0.243				
Leaf cross-section	Species	1	11.39	11.39	18.485	< 0.001	***	<i>C. serrulata</i> > <i>C. rotundata</i>
	Location	3	1.3	0.432	0.702	0.553	n.s.	
	Species x location	3	3.18	1.061	1.722	0.167	n.s.	
	Residuals	112	69.01	0.616				
Leaf frontal area	Species	1	168.98	168.98	153.77	< 0.001	***	<i>C. serrulata</i> > <i>C. rotundata</i>
	Location	3	68.04	22.68	20.64	< 0.001	***	Wawontulap A&B > Bunaken A&B
	Species x location	3	78.21	26.07	23.72	< 0.001	***	
	Residuals	112	123.08	1.1				
Rhizome	Species	1	10.97	10.969	8.723	< 0.001	**	<i>C. rotundata</i> > <i>C. serrulata</i>
	Location	3	2.4	0.801	0.637	0.59276	n.s.	
	Species x location	3	2.31	0.769	0.611	0.60896	n.s.	
	Residuals	112	140.83	1.257				
Above-ground biomass	Species	1	676.4	676.4	155.539	< 0.001	***	<i>C. rotundata</i> > <i>C. serrulata</i>
	Location	3	239	79.7	18.32	< 0.001	***	Wawontulap A&B > Bunaken A&B
	Species x location	3	61.7	20.6	4.73	< 0.01	**	
	Residuals	112	487.1	4.3				
Below-ground biomass	Species	1	276.9	276.9	85.92	< 0.001	***	<i>C. rotundata</i> > <i>C. serrulata</i>
	Location	3	40.7	36.9	11.45	< 0.001	***	Wawontulap A&B > Bunaken A&B
	Species x location	3	32.1	10.69	3.32	< 0.05	*	
	Residuals	112	361	3.22				

SC = Significance codes: *** = p < 0.001; ** = p < 0.01; * = p < 0.05; n.s. = not significant (p > 0.05).

The temperature readings based on submerged data loggers in both Bunaken and Wawontulap waters differed with time of the day, generally lower right after midnight at ~27.04°C with stable readings until 03h00 to 06h00, gradually increasing to 28-29°C right before noon then immediately peaked at > 30°C until 18h00 then gradually decline to 28-29°C at 19h00 until reaching ~27-28°C at 21h00 (Figure 5). This temperature cycle appears consistent throughout the observation period and the previous study in November 2001 (Wagey, unpublished data) when temperature readings peaked at 31.74±0.52°C and 31.55±0.27°C from 13h00-13h50 hours. Whereas, light intensity in Bunaken and Wawontulap coastal waters areas peaked consistently at around 13h00 at 20,000±4,279.07 Lux and 19,966.73±3,448.54 Lux, respectively (Figure 6).

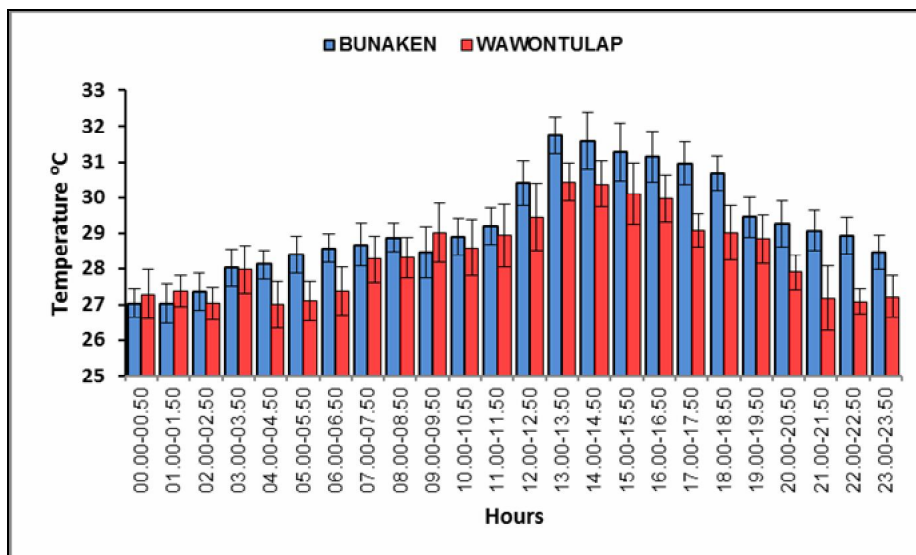


Figure 5. Mean±SD temperature readings on October 2017 in Bunaken and Wawontulap coastal areas (N = 6 days).

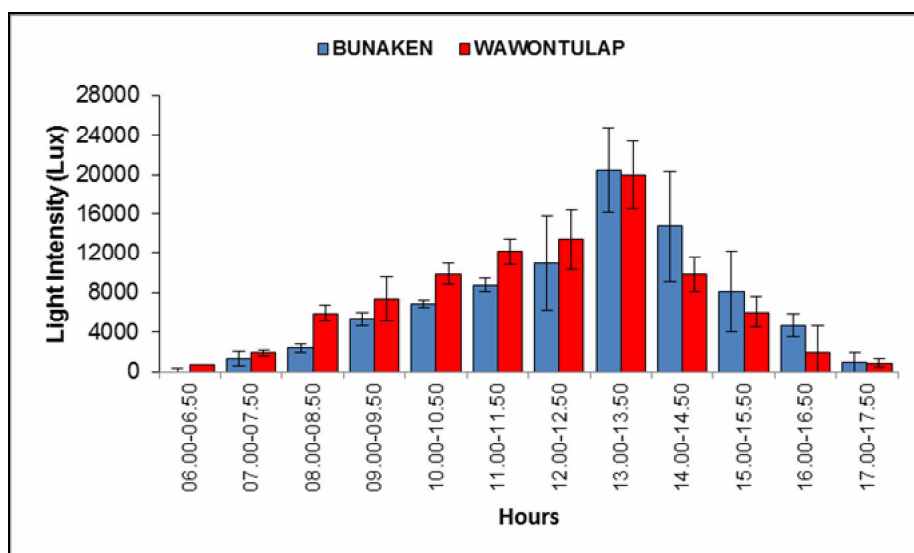


Figure 6. Mean±SD light intensity reading on October 2017 in Bunaken and Wawontulap coastal areas (N = 6 days).

Discussion. Seagrasses have been known to show morphometric plasticity to respond to abiotic stressors (La Nafie 2016). This study compared morphometrics of two seagrass species (*C. rotundata* and *C. serrulata*) from two locations within the Bunaken National Park. In a related study (Wagey 2013), there was found no significant difference among morphological characters of *C. serrulata* between sites in Negros Oriental, central Philippines. Variations depend upon prevailing biophysical conditions. For example, variation in size of the seagrass *C. serrulata* between locations (e.g. intertidal vs subtidal) has been described by a number of authors, including Sakey et al (2015) in their study in North Sulawesi, Indonesia.

Seagrasses can be affected by various ecological factors, including sediment burial and erosion (Cabaco et al 2008). It should be noted that in terms of sensitivity to siltation, *C. rotundata* is more sensitive than *C. serrulata* (Terrados et al 1998). As described by Terrados et al (1998), when siltation increases, i.e., silt and clay content exceeded 15%, leaf biomass of seagrass community sharply decline. Other factors may also affect the observed morphometric differences reported in this study. For example, Peralta et al (2006) found a direct effect of current velocity and seagrass morphology, growth, and architecture of the seagrass *Zostera marina*. Kiswara et al (2009) found out

that root architecture, including rhizome elongation, may respond to eutrophication gradient.

Another potential factor that may affect seagrass morphology and biomass includes grazing. For example, Kuiper-Linley et al (2007) found a significant decline in total standing biomass of *C. serrulata* in grazed plots. Although other physico-chemical factors such as light, temperature, and light may also affect certain morphological characters such as leaf width (Wagey 2015). Ahmad-Khamil et al (2013) examined the effects of water temperature on seagrass cover in Lawas, East Malaysia. It is noteworthy that morphometrics, biomass, and production of other species of seagrass belonging to the genus *Cymodocea* (e.g. *C. nodosa*), has been known to differ with season (Sfriso & Ghetti 1998). Similarly, Lanyon & Marsh (1995) found monthly fluctuations in the above-ground and below ground biomass of *C. serrulata* in the intertidal area of North Queensland, Australia.

Conclusions. This study focused on the morphometric differences of two congeneric species (*Cymodocea rotundata* and *C. serrulata*) between two localities. It can be deduced that the observed morphometric differences may be due in part to their inherent phenotypic characters but the influence of abiotic (substrate, N and P) and biotic (e.g. grazing) stressors cannot be ruled out. It is hoped that future studies should include these stressors in their investigations. The influence of nutrient enrichment (eutrophication), especially in highly populated coastal communities within the Bunaken National Park, should also be monitored. Moreover, long-term temporal monitoring of both above-ground and below-ground biomass in relation to environmental (both biotic and abiotic) parameters is highly recommended.

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