

Ecology, diversity and biomass of seagrass species in Manado Tua Island, North Sulawesi, Indonesia

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Abstract. Seagrass meadows are among the most important marine ecosystems but threatened by anthropogenic activities. They are economically important, providing food and shelters for a wide array of marine organisms, including commercially important species. In this study, the researchers gathered preliminary but important baseline information on density and biomass (wet and dry), and carbon stock for each seagrass species around Manado Tua Island in North Sulawesi, Indonesia. Baseline data on physico-chemical parameters and nutrient levels (nitrate and phosphate) are also presented. A total of five species was identified in two months (August and October, 2019). *Cymodocea rotundata* had the highest mean density (99 ± 4.09 shoots m^{-2} (S.E.) in August and 85.2 ± 4.45 shoots m^{-2} in October). Mean per cent cover showed no significant difference between the sampling months while statistically significant difference was observed between species, with *Thalassia hemprichii* having the highest per cent cover (67.8-68%). The eelgrass *Enhalus acoroides* had the highest biomass (wet and dry) and consequently in terms of carbon stock (44.7 ± 4.2 g C m^{-2}) while the rest of the species have carbon stocks below 10 g C m^{-2} .

Key Words: carbon stock, nursery-ground, seagrass, meadows, Manado, North Sulawesi.

Introduction. Seagrass beds are highly valuable ecosystems (Fortes 1990), and are well known as habitat for diverse marine flora and fauna. Globally, seagrass ecosystems are considered major blue carbon sinks that help mitigate the effects of climate change (Gullström et al 2018). They also provide food and shelters, such as nursery grounds for commercially important species of fish and macroinvertebrates (Jackson et al 2001). Unfortunately, seagrass meadows around the globe have been declining rapidly (Adams et al 2020) due to combination of natural and anthropogenic factors, such as climate change (Short & Neckles 1999), pollution (Neverauskas 1987; Todd et al 2010), sedimentation (Madsen et al 2001), and destructive fishing (Jackson et al 2001).

Alongi (2018) compiled data on carbon sequestration around the globe and showed that only 12 observations have been made in Indonesia, with carbon sequestration estimates ranging from -1434 to 77 g C_{org} m^{-2} $year^{-1}$ (mean = -578.5). However, carbon sequestration estimates are likely underestimated as seagrasses export a substantial portion of primary production, both in particulate and dissolved forms (Alongi 2018). In terms of data on carbon stocks worldwide, as compiled by Fourqurean et al (2012a, b), most of the organic carbon are stored in soil. Alongi (2018) further emphasized the difficulty in discerning geographic trends due to the scarcity of data in some parts of the world, including Indonesia where estuarine and marine wetlands (including seagrass meadows) comprised about 17% of the world's blue carbon (Alongi et al 2016).

Wagey (2018) reviewed the status of researches done on the ecology of seagrasses in Indonesia, with emphasis on the Northern Sulawesi. His review revealed that only a few studies have dealt with the carbon stocks of seagrasses in Indonesian waters (Rustam et al 2017; Kondoy 2017) and previous studies dealt mainly with seagrass morphometrics (Sakey et al 2015; Wagey et al 2016; Wagey 2017) or

community structure (Merly et al 2013). Recently, Fortes et al (2018) noted that research output on seagrasses, especially in relation to climate change and carbon stocks in Southeast Asia remain scarce although there is an increasing trend. The main goal of this paper is to provide baseline information on the ecological (e.g. physico-chemical and density and cover) aspects and carbon stock of seagrasses in Manado Tua Island in North Sulawesi.

Material and Method

Description of the study site. This study was conducted at Manado Tua Island, North Sulawesi, Indonesia. Manado Tua Island is part of the Bunaken National Park (Figure 1). The seagrass meadows surveyed (August and October, 2019) were all in the shallow areas.

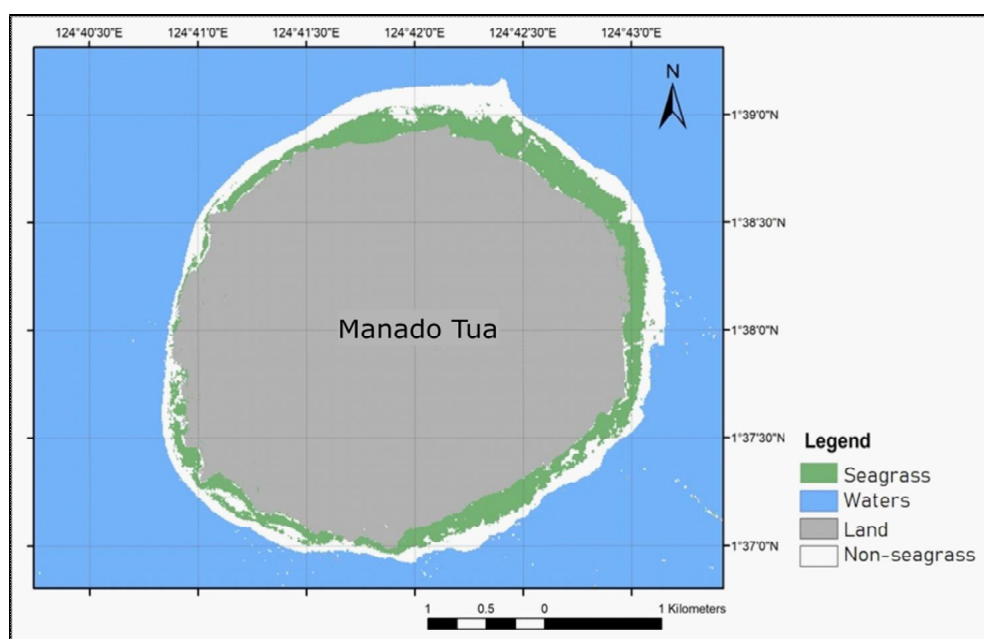


Figure 1. The location of the seagrass beds surrounding Manado Tua Island, North Sulawesi, Indonesia.

Physico-chemical parameters. Prior to sampling, the following physico-chemical parameters were measured in situ: 1) salinity using a portable salinometer with probe (Horiba U-50); 2) water temperature using a data logger (HOBO® Pendant UA-002-08); 3) water current was expressed as the average distance (m) traveled by a float (pingpong ball) per sec; and 4) wave length measured using a meter roll.

A 1-L seawater sample was collected and immediately transported to a regional testing laboratory (Baristand Industri Manado) for analysis of nitrate and phosphate levels. This laboratory followed standard water quality analysis protocols (e.g. American Public Health Association 1999).

Nutrient level. A 1-L seawater sample was collected and immediately transported to a regional testing laboratory (Baristand Industri Manado) for analysis of nitrate and phosphate levels. This laboratory followed standard water quality analysis protocols (e.g. American Public Health Association 1999).

Seagrass sampling. The seagrass sampling procedures described by McKenzie (2003) were followed. In each site, 50-m transect lines were laid perpendicular to the shoreline. The geographic coordinates of each transect were determined and marked by a GPS (Geographic Positioning System) unit (Garmin®). A total of 6 quadrats (each 1m x 1m) were randomly positioned in each transect. Within each quadrat, percent (%) cover of each species was visually estimated and the number of shoots recorded to determine

shoot density. Photographs were also taken as part of photo-documentation and for later verification of species identification. Species identification was based on available taxonomic references (Waycott et al 2004).

All seagrass samples were collected from each quadrat, cleaned of sediments and debris, sorted to species, then components were segregated as to above-ground and below-ground parts for further laboratory processing and analyses. Wet-weight (nearest grams) were immediately determined using a portable electronic balance (OEM[®], Model: TopMall-BN291).

Determination of biomass and carbon stock. Only the samples collected in August were subjected to biomass and carbon stock determination. Upon arrival at the laboratory, samples from each quadrat (segregated as to above-ground and below-ground samples) were air-dried for at least 5 days then later oven-dried (-60°C) for 24-hours to constant weight. Oven-dried samples were weighed using an analytical weighing scale (Merk Matrix, Model: DJ203A, 200g x 0.001 g) for determination of biomass. To quantify carbon stock (g C m^{-2}), the lost on ignition (LOI) method was used as described by Kondoy (2017) and Hulopi et al (2017).

Statistical analysis. All data were tested for assumptions of normality (Anderson-Darling Test), QQ-Plot, and homoscedasticity (Non-constant Variance Score Test) using the nortest package in R (R Core Team 2017), and were log-transformed when required. Variations between sampling stations were compared using one-way and two-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test. Significance of differences was defined at $p < 0.01$. Statistical analyses were performed using RStudio.

Results

Physico-chemical parameters. Mean salinity readings of the water during the sampling in the seagrass beds of Manado Tua was determined at 31.17 ± 0.48 to $32.33 \pm 0.76\text{‰}$, in August and October 2019, respectively. Mean water temperature ($^{\circ}\text{C}$) readings were 29.50 ± 0.43 and 30.83 ± 0.31 during the survey months. Water current (m second^{-1}) was consistently slow at 0.02-0.03 while mean wave length (cm) ranged from 13.73 ± 2.31 to 21.28 ± 3.46 . These parameters did not differ significantly between the two months, with the exception of wave length which was higher in August, coinciding the southwest monsoon period.

Nutrient level. Figure 2 shows the levels of nutrients (nitrate and phosphate) in sediment and water column in the seagrass beds of Manado Tua Island. Nitrate level in the sediment (Figure 2A) ranged from 0.97 to 1.88 mg kg^{-1} (mean = 1.88 ± 0.21 SE) while phosphate ranged from 9.98 to 12.4 mg kg^{-1} (mean = 11.30 ± 0.56). In the water column (Figure 2B), nitrate level was slightly elevated compared to that of the sediment with values ranging from 0.78 to 2.36 mg L^{-1} (mean = 1.45 ± 0.37) while phosphate level was lower compared to that of the sediment, ranging from 0.43 to 72 mg L^{-1} (mean = 0.61 ± 0.06). At present, temporal and spatial comparisons cannot be made.

Seagrass density and cover. A total of five species of seagrasses was recorded from the sampling sites. In both sampling occasions (August and October, 2019), seagrass density were comparable. *Cymodocea rotundata* had the highest mean density (99 ± 4.09 shoots m^{-2} (S.E.) in August and 85.2 ± 4.45 shoots m^{-2} in October), followed by *Thalassia hemprichii* (98.2 ± 7 shoots m^{-2} in August and 101.8 ± 4.6 shoots m^{-2} in October, respectively), *Enhalus acoroides* (65.5 ± 6.6 and 51.2 ± 5.1 shoots m^{-2} in the same months), while lower densities were observed in *Halophila ovalis* (37.85 ± 2.38 and 41.5 ± 4.7 shoots m^{-2}) and *Syringodium isoetifolium* (28 to 34.5 shoots m^{-2}), in that order (Figure 3). Two-Way ANOVA revealed no significant difference between both sampling occasions ($p > 0.05$) but statistically significant between species ($p < 0.01$). Subsequent Tukey's HSD test further showed that the observed significant differences between species was attributable to pair-wise comparisons between *E. acoroides* and the rest of

the four species as well as between with *T. hemprichii* and *S. isoetifolium* and *H. ovalis*, and *C. rotundata* against *S. isoetifolium* and *H. ovalis* with all $p < 0.01$.

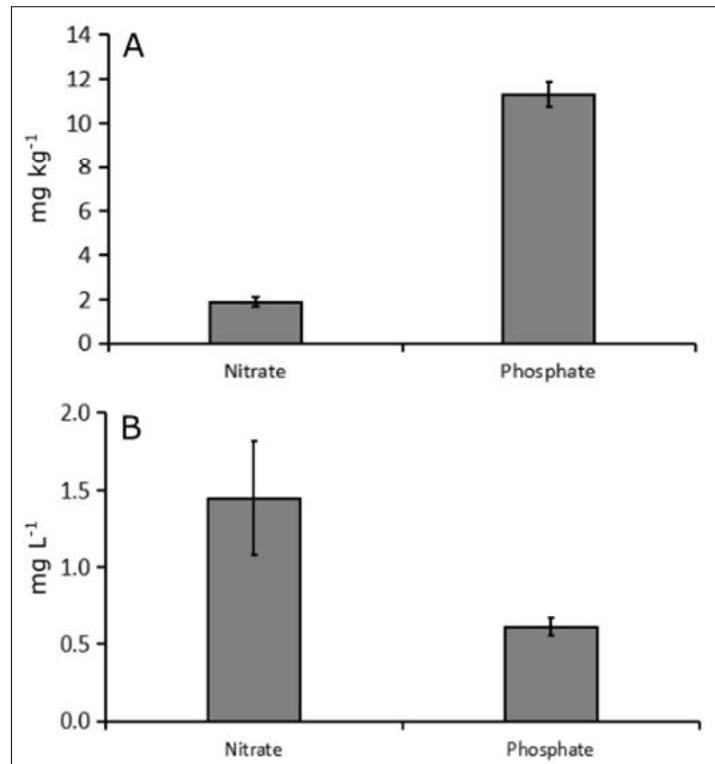


Figure 2. Nutrient (nitrate and phosphate) mean levels in the sediment (A) and water column (B) during the study.

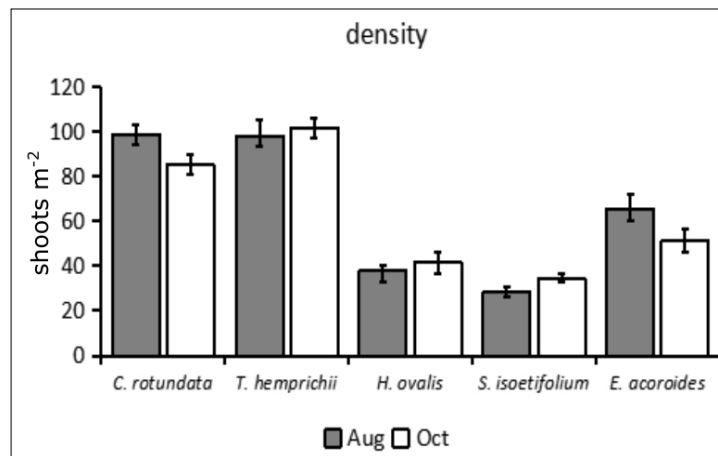


Figure 3. Mean density of seagrass species in August and October, 2019. Error bars indicate standard error values.

Mean per cent cover (Figure 4) showed no significant difference between August and October based on the Two-Way ANOVA ($p > 0.05$). Between species, however, *T. hemprichii* had the highest per cent cover (67.8-68%), followed by *C. rotundata* (37-44%), *E. acoroides* (17-18.2%), *H. ovalis* (10-12%) and *S. isoetifolium* (10-12.5%). ANOVA revealed that there was a significant difference in cover between species ($p < 0.01$).

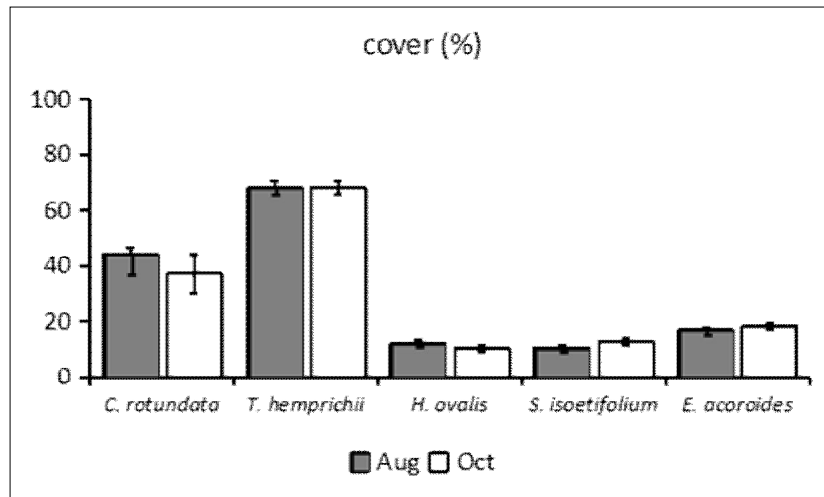


Figure 4. Mean percent cover (%) of seagrass species in August and October, 2019. Error bars indicate standard error values.

In terms of fresh (wet) weight of the seagrass species (Figure 5), *E. acoroides* was found to have the highest wet values (above-ground: $492 \pm 73.3 \text{ g m}^{-2}$; below ground: $419 \pm 42.2 \text{ g m}^{-2}$), followed by *T. hemprichii* (above-ground: $302 \pm 11.9 \text{ g m}^{-2}$; below ground: $225.8 \pm 8.95 \text{ g m}^{-2}$), *C. rotundata* (above-ground: $226 \pm 17 \text{ g m}^{-2}$; below ground: $208 \pm 17 \text{ g m}^{-2}$), *S. isoetifolium* (above-ground: $70.5 \pm 6.8 \text{ g m}^{-2}$; below ground: $43 \pm 2.6 \text{ g m}^{-2}$) and while *H. ovalis* (above-ground: $32.5 \pm 4.2 \text{ g m}^{-2}$; below ground: $30 \pm 4.34 \text{ g m}^{-2}$) had the lowest wet weight values. Two-Way ANOVA showed wet biomass differ between above-ground and below-ground biomass ($p < 0.05$) but differences were more pronounced between species ($p < 0.001$).

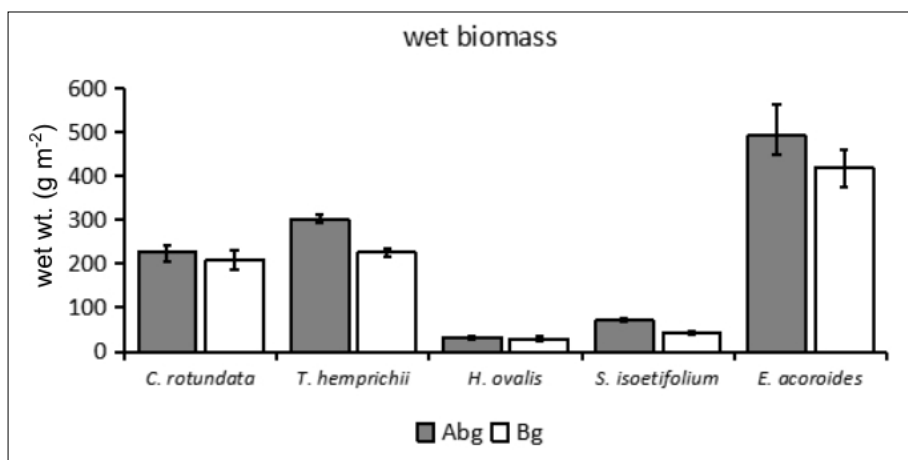


Figure 5. Mean wet weight (g m^{-2}) of seagrass species at Manado Tua in August 2019. Error bars indicate standard error values. Abg = above-ground biomass; Bg = below-ground biomass.

Mean biomass (grams dry weight m^{-2}) of seagrass species (Figure 6) appear consistent with the trend observed using wet weight values. *E. acoroides* was found to have the highest biomass (above-ground: $126 \pm 14.4 \text{ g m}^{-2}$; below ground: $135 \pm 18.1 \text{ g m}^{-2}$) followed by *T. hemprichii* (above-ground: $109.9 \pm 6 \text{ g m}^{-2}$; below ground: $80.5 \pm 4.25 \text{ g m}^{-2}$) and *C. rotundata* (above-ground: $79 \pm 2.4 \text{ g m}^{-2}$; below ground: $70 \pm 6.3 \text{ g m}^{-2}$), *S. isoetifolium* (above-ground: $17.5 \pm 1.43 \text{ g m}^{-2}$; below ground: $21 \pm 4.41 \text{ g m}^{-2}$) while *H. ovalis* had the lowest dry weight biomass values (above-ground: $14 \pm 1.6 \text{ g m}^{-2}$; below ground: $11.3 \pm 1.5 \text{ g m}^{-2}$). Two-Way ANOVA showed no significant difference between above-ground and below-ground dry biomass ($p > 0.05$) but significant differences were detected between species ($p < 0.01$).

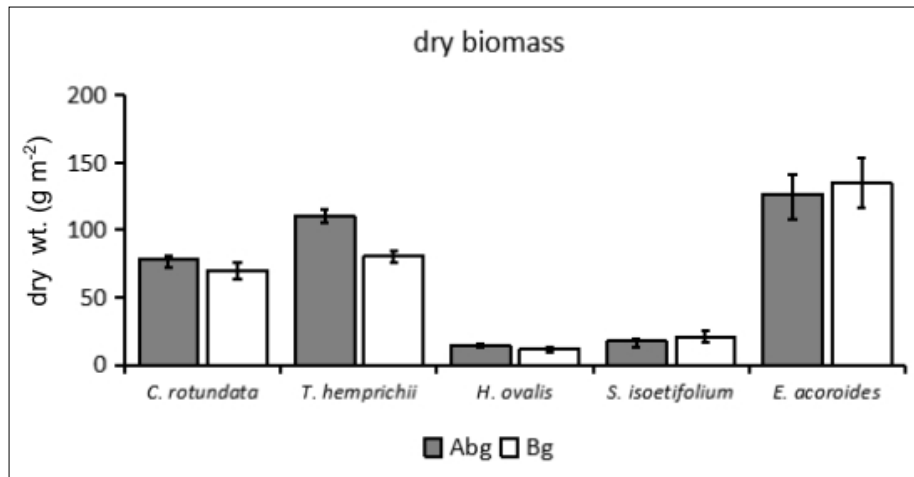


Figure 6. Mean biomass (dry weight in g m⁻²) of seagrass species at Manado Tua Island. Error bars indicate standard error values. Abg = above-ground biomass; Bg = below-ground biomass.

As already pointed out, carbon stock (Figure 7) was, thus far, determined and compared between species. As expected, *E. acoroides* had the highest carbon stock (44.7±4.2 g C m⁻²) while the rest of the species were all below 10 g C m⁻². One-Way ANOVA showed significant difference in carbon stock between species ($p < 0.001$). Tukey's HSD test confirmed that the observed significant difference was due to *E. acoroides* carbon stock when compared to the rest of the species ($p < 0.01$).

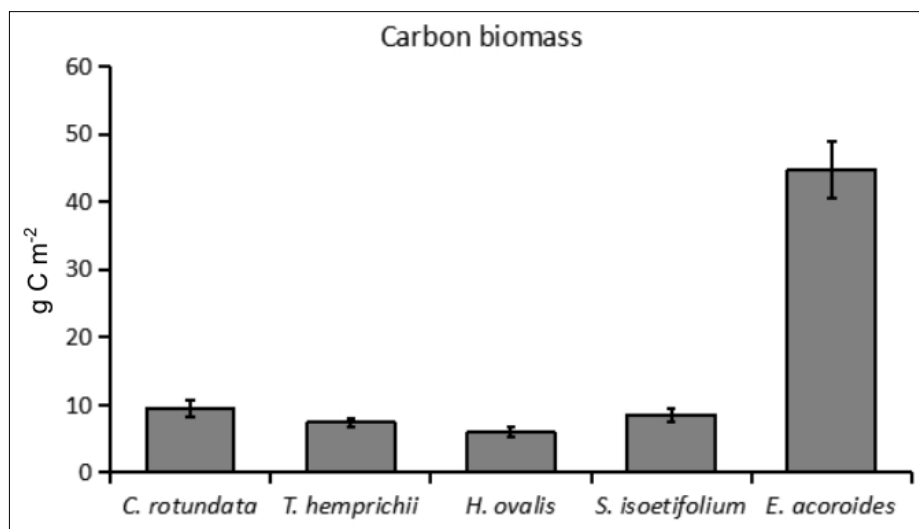


Figure 7. Carbon stock (g C m⁻²) of seagrass species in Manado Tua Island. Error bars indicate standard error values.

Discussion. This study quantified seagrass density, per cent cover, biomass (wet and dry), and carbon stocks of five species found in Manado Tua Island in North Sulawesi, Indonesia. The results showed that density and cover were mainly influenced by two species, *T. hemprichii* and *C. rotundata* (consistent to earlier findings by Kondoy (2017)). *E. acoroides* had the highest contribution in terms of biomasses and carbon stocks, despite the latter species having a sparse distribution and lower density and cover values. The results may have some implications as to the GIS-mapping and subsequently on the management of seagrass beds. For example, when seagrass cover derived from satellite imagery will be used as a proxy data to infer carbon stocks, there is a tendency of underestimating the actual carbon stocks if the seagrass beds are dominated by species with lower carbon stocks such as *T. hemprichii* and *C. rotundata*.

The carbon stock estimates presented in this study do not support the earlier study by Kondoy (2017) wherein *T. hemprichii* had the highest carbon stock in North

Sulawesi. While in this study, the seagrass *E. acoroides* had the highest carbon stock. Differences might be attributable to variances in terms of the placement of the sampling sites. Kondoy (2017), however, only showed carbon stock percentages and not actual values, making direct comparisons not feasible at this stage. On the other hand, the high carbon stock of *E. acoroides* tend to parallel the findings of Hulopi et al (2017) in Galala and Tanjung Tiram waters around Ambon Island, Indonesia. Similarly, large-sized species such as *E. acoroides* and *T. hemprichii* were also found to have the highest carbon stock in Spermonde Archipelago, South Sulawesi, Indonesia (Rustam et al 2017). It is noteworthy that *E. acoroides* is the largest among the species and abundant throughout its range in the Indo-Pacific, making this species a blue carbon source. Likewise, Alongi et al (2016) compiled information on carbon stocks of seagrasses (extrapolated to per hectare values) in selected sites in Indonesia (no data in North Sulawesi), with *E. acoroides* as dominant species (in terms of biomass) in at least 6 sites.

Carbon stocks of seagrass species are expected to vary between locations, especially influenced by variations in depths (Lavery et al 2013). Depth may not be a factor affecting possible variability in the sampling sites in Manado Tua. However, other factors should also be taken into consideration such as the effect of substrate (Katuuk et al 2018) on the variability of biomass and carbon storage. This study did not include soil carbon content and should be included in the next phases of the project.

Conclusions. This study has documented the baseline data for carbon stocks of five seagrass species sampled around Manado Tua Island, North Sulawesi. Pertinent data on seagrass density, cover, biomass (both wet and dry), and carbon stock were described and compared with existing information in the literature. Among the five species, the eelgrass *E. acoroides* had the highest biomass and carbon stock values, suggesting that this species as a potential blue carbon source.

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