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Using morphometric characters to estimate leaf productivity of seagrass *Halophila major*

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Abstract. *Darus RF, Bengen DG, Zamani NP, Ismet MS. 2024. Using morphometric characters to estimate leaf productivity of seagrass* Halophila major. *Biodiversitas 25: 4994-5004. Halophila major* is a seagrass species with new records in Indonesia. This species was described based on morphometric characteristics and DNA sequencing. In this study, we expand our understanding about this species by looking at its leaf productivity. This study aims to determine the productivity of *H. major* leaves collected across various locations of Indonesian waters and analyses its relationship with morphometric characters. Morphometric characters (Leaf Length-LL, Leaf Width-LW, and Leaf Area-LA) were used to see the correlation with leaf productivity parameters, namely Leaf Dry Weight (LDW), Specific Leaf Area (SLA), Biomass (BIO), and Carbon Concentration (CS) estimated using allometric methods. The result of this study indicates that the biomass of *H. major* is greater than *H. ovalis*, its closest relative. LL and LW were more strongly correlated with LDW compared to LA. Biomass was also greatly influenced by LL and LDW. Leaf productivity variation is affected by the leaf size, suggesting that leaf productivity can be used to distinguish seagrass species under the similar genus. This study showed that allometric equations can be used quickly to assess the biomass of small seagrasses. However, more data is needed for knowing and interpreting a character in new seagrass species. Hence, this study must be replicated for other seagrass species.

Keywords: *Halophila*, Indonesia, marine plant, morphology, rediscovery

INTRODUCTION

Seagrass is a marine angiosperm that plays an important role in delivering various ecosystem services. It serves as feeding sources of various fishes (Marimba et al. 2019; Ambo-Rappe 2020; Milchakova 2020), traps sediment and nutrients (Jiang et al. 2019; Surinati et al. 2023), reduces wave strength and current speed (Manousakas et al. 2022; Muna et al. 2023), provides habitat of numerous marine organisms (Syukur et al. 2021), has aesthetic and recreation value and is important for water filters, coastal protection, bioindicators and carbon storage (McKenzie et al. 2021a, b). Indonesia is an important region of seagrass with a 2,935 km² extent (Rahmawati et al. 2022). Indonesia is also a seagrass distribution area with high species diversity, namely 15 species (Spalding et al. 2007; Fortes et al. 2018). Recently, two seagrass species in Indonesia has been added, namely *Halophila major* (Zoll.) Miq. and *Ruppia brevipendunculata* Shuo Yu & Hartog (Kurniawan et al. 2020, et al. 2024).

Halophila major is a species in the genus *Halophila*. It is often called "Big Ovalis" (Tuntiprapas et al. 2015), referring to its similarity to *Halophila ovalis* (R. Brown) J.D. Hooker, 1858, but *H. major* has a greater size (Kuo et al. 2006). Recently, *H. major* has been recorded in Indonesian waters (Kurniawan et al. 2020) based on the description using morphological, molecular, and reproductive characteristics (Kuo et al. 2006; Kuo 2020). This species spread in Wallacea Region namely Malala Bay-Tolitoli, Sindhu Beach-Bali, Sekotong Beach-Lombok, Tayando Island-Tual, Weduar Kei Besar Beach-Tual, Kambing Island Bima-Sumbawa (Uchimura et al. 2008; Kurniawan et al. 2020).

Seagrass growth is strongly influenced by physical, chemical, and biological factors of the environment where it grows (Najdek et al. 2020). For example, light availability will affect photosynthesis (Lapointe et al. 2020), thus reduced light will induce the morphology of some seagrass species to become larger or taller, impacting some pioneer seagrass species such as the genus *Halophila* (Wong et al. 2019; Choon 2023). Beside for taxonomical identification, physiological characteristics are often used to investigate seagrass responses to environmental changes (Deguette et al. 2022; Wong and Dowd 2023). Nonetheless, among various physiological characteristics, leaf productivity is rarely investigated, including in *H. major,* since it is newly described. Assessing leaf productivity requires long period and costly laboratory observation (Echavarría-Heras et al.

2011). It is crucial to note that environmental factors strongly influence leaf productivity of seagrass species, resulting in a broad range of growth rate from fast to slow (Echavarría-Heras et al. 2006).

Leaf productivity affects biomass, which has important functions in marine organisms, especially seagrass (Duarte and Chiscano 1999). Biomass determines the amount of carbon in seagrass leaves and that released into the water, which is then utilized by others biota. Thus, changes in biomass will have an impact on the existence of biota around seagrass beds (Echavarría-Heras et al. 2011). Surface temperature and geographic factor influence leaf dynamics, which eventually affect biomass (Solana-Arellano et al. 1997; 2009). For example, maximum biomass of *Zostera japonica* Asch. & Graebn. correlates with temperature and latitude (Ito et al. 2021). Temperature in tropical region is warmer than in other regions, this might cause different responses to seagrass morphology. Beside temperature, depth and light availability also correlate with Leaf Area Index (LAI) and shoot density which eventually influence leaves morphology and seagrass cover (Misbari and Hashim 2015; Gaubert-Boussarie et al. 2021; Wong and Dowd 2023).

Traditionally, biomass assessment is carried out destructively and requires time to assess the dry weight in the laboratory. However, several studies used non-destructive methods, such as the use of hyperspectral reflectance (Costa et al. 2021), radar, optical satellite imagery, and combining both with machine learning (Ha et al. 2021). Some studies estimated above-ground biomass using leaf dry weight and allometric approach. For example, Duarte (1991) demonstrated an allometric method related to the growth of seagrass species. Using a non-destructive method, Echavarría-Heras et al. (2010) conducted an allometric study to assess the relationship between leaf area and biomass of *Zostera marina* L. Leaf area can be determined accurately using electronic devices (Echavarría-Heras et al. 2011).

Considering the limited information of a newly recorded seagrass species, this study aimed to investigate the leaf productivity of *H. major* based on morphometric characteristics. In this study, we used the allometric method for assessing Leaf Dry Weight (LDW), Biomass (BIO), Specific Leaf Area (SLA), and Carbon Concentration (CS) as additional descriptive characters of *H. major*. We also tested the relationship between the leaf morphometric (Leaf Length/LL, Leaf Width/LW, and Leaf Area/LA) (LL, LW, LA) and productivity (LDW, SLA, BIO, and CS) of this species. We expected the results of this study might be useful for. describing new species based on leaf productivity characteristics. Moreover, the results of this study also contribute to the development of allometric biomass and carbon concentration in seagrass.

MATERIALS AND METHODS

Plant materials

Halophila major "Big Ovalis" samples were collected from nine locations in Eastern Indonesian waters, including Sekotong Beach, Lombok (SBL) (8° 44' 21.53″ S, 116° 01' 30.18″ E), Malala Bay, Tolitoli (MBT) (0° 45' 48.86″ N, 120° 34' 15.09″ E), Tayando Island, Tual (TIT) (5° 32′ 48.93″ S, 132° 21′ 25.51″ E), Weduar Beach, Tual (WBT) (5° 57′ 29.88″ S, 132° 51′19.69″ E), Terora Beach, Bali (TBB) (8°46'36.14"S, 115°13'31.33"E), Bungin Island, Sumbawa (BIS) (8° 28' 28.79" S, 116° 59' 32.21" E), Arefi Island, Raja Ampat (AIR) (0° 47' 39.67" S, 130° 42' 16.56" E), Yensawai Beach, Raja Ampat (YBR) (0° 48' 2.69" S, 130° 40' 38.26" E), and Marandanweser Beach, Raja Ampat (MBR) (0° 47' 55.45" S, 130° 34' 35.09" E) (Figure 1). All locations were chosen based on reporting from a preliminary study of seagrass ecology and local communities.

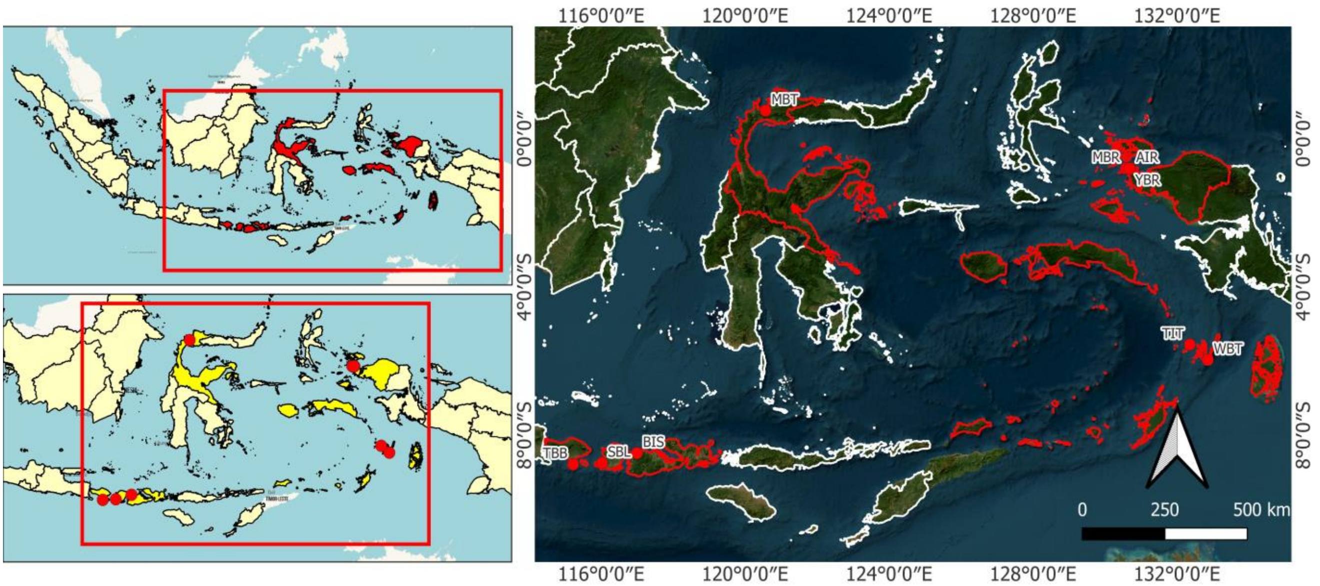

Figure 1. Sample collection sites in Eastern Indonesian waters. A. TIT: Tayando Island, Tual; B. WBT: Weduar Beach, Tual; C. SBL: Sekotong Beach, Lombok; D. MBT: Malala Bay, Tolitoli; E. TBB: Terora Beach, Bali; F. BIS: Bungin Island, Sumbawa; G. AIR: Arefi Island, Raja Ampat; H. YBR: Yensawai Beach, Raja Ampat; and I. MBR: Marandanweser Beach, Raja Ampat

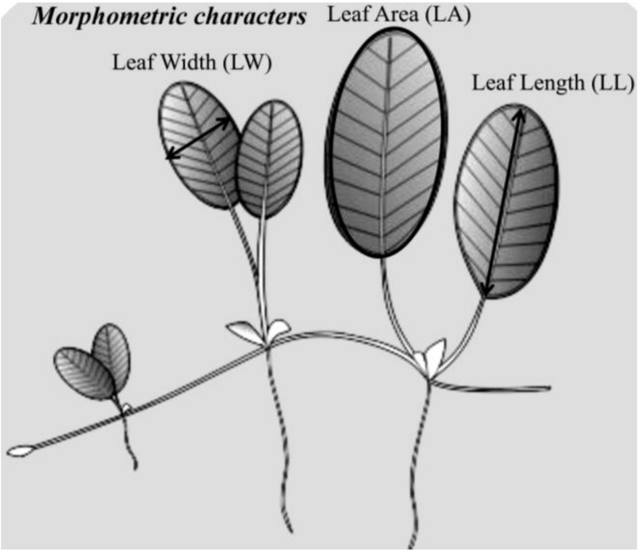

Figure 2. Morphometric characters of *Halophila major* measured in this study

Fresh specimens (leaves, rhizomes, and roots) of *H. major* were collected at 2-4 m depth using snorkeling. Furthermore, wet specimens collected from the field were preserved using wet wipes and stored in a cool box for transfer to the laboratory. Wet specimens were first labeled, photographed with a scale bar, and identified. The number of paired and branched leaf veins is used as a key to the morphological identification of *H. major* (Kuo et al. 2006; Nguyen et al. 2013, et al. 2021; Tuntiprapas et al. 2015; Kurniawan et al. 2020). We only used digital specimens for leaves, while rhizomes and roots were used for different research purposes. The leaf length, width, and area of 362 digital specimens of *H. major* were measured, and the leaf productivity was estimated using an allometric approach.

Morphometric measurements of seagrass leaves

In this study, morphometrics of seagrass leaves were not differentiated between young and mature leaves. Morphometric measurements were carried out on Leaf Length (LL), Leaf Width (LW), and Leaf Area (LA) using ImageJ software (Figure 2). LL and LW measurements were performed by importing digital specimens into the ImageJ software. Digital specimens were standardized by setting the scale with the scale bar on the set scale menu on the analyze menu bar. Drawing lines on the width and length of the leaf used a freehand line, then re-selected the analyze menu bar and clicked measure to display the measurement results. Meanwhile, the LA measurement applied different stages from the LL and LW measurements. The calculation scale on the digital image was adjusted by setting the set scale tools. The digital specimens were set to 8-bit image type. The color was modified with the color threshold tool to adjust the red color to fill the seagrass leaves. The wand tool selected the leaves whose area will be measured. Previously, the ROI tool manager was displayed to add the leaves to be measured, then click measure to show the measurement value. Furthermore, LL, LW, and LA measurement results were analyzed to estimate Leaf Dry Weight (LDW), Specific Leaf Area (SLA), Biomass (BIO), and Carbon Concentration (CS) using an allometric approach.

Productivity of seagrass leaves

The Leaf Dry Weight (LDW) estimation used the equation developed by Hamburg and Homann (1986). The allometric equation for dry leaf weight is as follows:

$$B = 0.00143 \times L \times W^{1.3}$$

Where:
B : Dry weight of the leaf (mg)
L : Length of the leaf (mm)
W : Width of the leaf (mm)

Leaf Area (LA) was converted to Specific Leaf Area (SLA), where SLA is the ratio between area and leaf dry weight (Dingkuhn et al. 2001; Vile et al. 2005). The allometric equation can assume leaf biomass using the following equation (Echavarría-Heras et al. 2012, 2013b):

$$w = \alpha\, l^{b}$$

Where:
α and b: Positive constants
w : Biomass
l : Leaf length of *H. major*

The carbon concentration of *H. major* leaves was calculated using the equation below (Rahmawati et al. 2019):

Carbon concentration = Biomass × 0.336

Statistical analysis

Analysis of Variance (ANOVA) was carried out to see the difference in morphometric characteristics (LL, LW, and LA) and leaf productivity (LDW, SLA, BIO, CS) across the nine locations. These data were standardized first before being analyzed. Duncan's advanced test was applied if there was an indication of a difference at $\rho<0.05$. Pearson's correlation examined the relationship between morphometrics and leaf productivity. Regression analysis tested the correlation between morphometric variables and productivity at each location. All statistical analyses were processed with the help of IBM Statistics SPSS 27 software, R free software, and PAST 4.03 (Hammer et al. 2001).

RESULTS AND DISCUSSION

Leaf length, leaf width, and leaf area

The average Leaf Length (LL) of *H. major* ranged between 18.27 and 27.43 mm, with the highest leaf length being Sekotong Beach, Lombok (Table 1). The widest average Leaf Width (LW) was the sample from Tayando Island, Tual, with variations in width ranging from 9.66-13.48 mm (Table 1). The highest Leaf Area (LA) was found in Tayando Island, Tual, where the average LA value ranged from 129.27 to 282.60 mm² (Table 1). The significant differences in LL were found in samples from Sekotong Beach, Lombok (Table 1). Meanwhile, LW and LA had little differences across the nine locations.

Leaf dry weight and specific leaf area

Leaf dry weight was significantly higher in samples from Tayando Island Tual (1.12±0.58 mg), while specific leaf area was highest in those from Bungin Island Sumbawa (294.84±105.05 mm²/mg, Table 1). The highest biomass and carbon concentration was in the leaves from Sekotong Beach Lombok (Table 1). The range of leaf biomass was between 0.68 and 1.15 g/leaf, while the carbon concentration in the leaves was 0.23 and 0.38 gC/leaf. A significant difference ($\rho<0.05$) in biomass and carbon concentration was only found in leaves from Sekotong Beach.

Morphometric relationship with leaf productivity

Pearson test illustrates the relationship between morphometrics (LL, LW, and LA) and productivity (LDW, SLA, BIO, CS) of *H. major* leaves (Figure 3). Several morphometric variables were significantly related to leaf productivity. LDW was negatively correlated with SLA and strongly affected by LL, LW, BIO, and CS. A positive relationship only occurred between SLA and LA. BIO and CS were positively correlated with leaf morphometrics and strongly associated with LDW. LA did not affect LDW, BIO, and CS because it had a small correlation value (<0.60).

The results of the regression analysis support the Pearson correlation, which showed a strong correlation between LLvsLDW, LLvsBIO, LLvsCS, LWvsLDW, LDWvsBIO, LDWvsCS, and BIOvsCS (Figures 4-10). This indicates that LL and LW influence leaf productivity. In contrast, LA had no impact on increasing leaf dry weight, biomass, and carbon concentration in seagrass leaves. The strongest relationship occured between LL with BIO and CS for all locations with an R^2 value of 1 (Figures 5 and 6).

Discussion

This study provides alternative characters to describe seagrass species, especially new species or records, using leaf productivity. Besides, rapid assessment of leaf productivity can generate biomass using a limited number of samples and for small seagrass. This study describes the morphometric relationship with the productivity of *H. major* leaves in Indonesian waters. This result shows a significant relationship between leaf length and width with dry weight, biomass, and carbon concentration using an allometric approach. These results support non-destructive biomass estimation and can be combined with shoot density to understand biomass and carbon concentration in unit areas.

This pioneering study is the first to estimate biomass and carbon concentration for *H. major*. The estimated biomass of *H. major*, ranging from 0.68-1.15 mg/leaf and the carbon concentration of 0.23-0.38 mg C/leaf (Table 1), marks a significant advancement in our understanding. The biomass of *H. major* outstrips that of *H. ovalis*, which is 0.03 g (Duarte and Chiscano 1999). Similarly, the carbon concentration is akin to *H. ovalis* at 0.27 g dry weight (Rahmawati et al. 2019). *Halophila ovalis*, a synonym of *H. major*, has smaller leaf morphometrics than *H. major*, which contributes to the latter's greater biomass and carbon concentration values. Table 1 demonstrates that the highest leaf length and width of *H. major* also correspond to high biomass and carbon concentration values. However, leaf area and specific leaf area do not influence biomass production and carbon concentration.

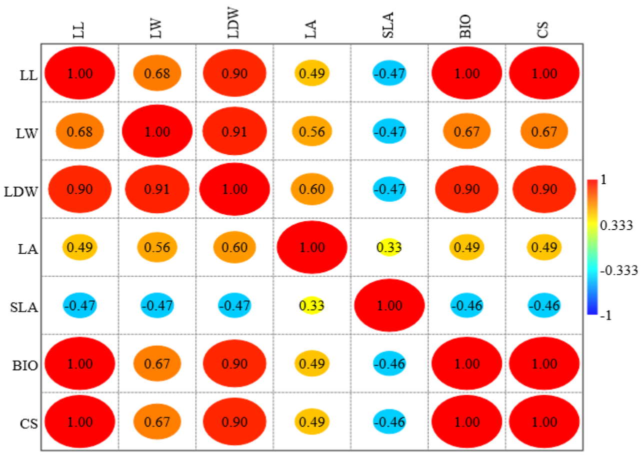

Figure 3. Pearson correlation of all seagrass growth variables. The red color and large circles depict a strong correlation. LL: Leaf Length; LW: Leaf Width; LDW: Leaf Dry Weight; LA: Leaf Area; SLA: Specific Leaf Area; BIO: Biomass, and CS: Carbon Concentration

Table 1. Comparison of morphometrics and productivity of *Halophila major* seagrass leaves from each sampled location

Location	Variable						
	LL (mm)	**LW (mm)**	**LDW (mg)**	**LA (mm²)**	**SLA (mm²/mg)**	**BIO (g/leaf)**	**CS (g C/leaf)**
Terora Beach, Bali (n = 38)	20.18±3.18^{a,b}	9.78±1.49^{a}	0.58±0.21^{a}	138.93±47.66^{a,b}	281.02±152.49^{a}	0.77±0.16^{a}	0.26±0.54^{a}
Sekotong Beach, Lombok (n = 43)	27.43±4.31^{e}	12.89±1.53^{d}	1.09±0.27^{c}	249.82±58.05^{d}	241.39±89.02^{a}	1.15±0.24^{d}	0.38±0.08^{d}
Bungin Island, Sumbawa (n = 60)	19.51±2.66^{a,b}	9.66±0.99^{a}	0.54±0.13^{a}	150.16±31.59^{a,b}	294.84±105.05^{a}	0.73±0.13^{a}	0.24±0.04^{a}
Arefi Island, Raja Ampat (n = 81)	19.16±1.99^{a,b}	11.18±1.35^{b,c}	0.64±0.15^{a}	165.77±39.36^{a,b}	268.22±73.24^{a}	0.71±0.09^{a}	0.24±0.03^{a}
Yensawai Beach, Raja Ampat (n = 61)	18.27±3.91^{a}	10.18±1.56^{a,b}	0.55±0.22^{a}	129.27±50.08^{a}	266.94±149.54^{a}	0.68±0.19^{a}	0.23±0.06^{a}
Marandanweser Beach, Raja Ampat (n = 32)	20.19±2.71^{a,b}	10.87±1.53^{a,b}	0.66±0.19^{a}	174.29±47.95^{b,c}	281.87±96.65^{a}	0.77±0.14^{a}	0.26±0.05^{a}
Malala Bay, Tolitoli (n = 36)	20.89±2.88^{b,c}	12.59±2.26^{d}	0.83±0.28^{b}	204.21±51.08^{c}	266.42±90.48^{a}	0.80±0.15^{a,b}	0.27±0.05^{a,b}
Weduar Beach, Tual (n = 3)	22.84±0.91^{c,d}	12.26±0.19^{c,d}	0.85±0.05^{b}	247.83±19.95^{d}	292.77±34.97^{a}	0.89±0.05^{b}	0.29±0.02^{b,c}
Tayando Island, Tual (n = 8)	25.04±4.88^{d}	13.48±3.25^{d}	1.12±0.58^{c}	282.60±128.31^{d}	259.51±16.50^{a}	1.02±0.27^{c}	0.34±0.88^{c}

Note: Values represent the mean±standard deviation. LL: Leaf Length; LW: Leaf Width; LDW: Leaf Dry Weight; LA: Leaf Area; SLA: Specific Leaf Area; BIO: Biomass; and CS: Carbon Concentration. Different superscript letters on each row indicate significant differences at $\rho<0.05$, which was tested using Duncan's test

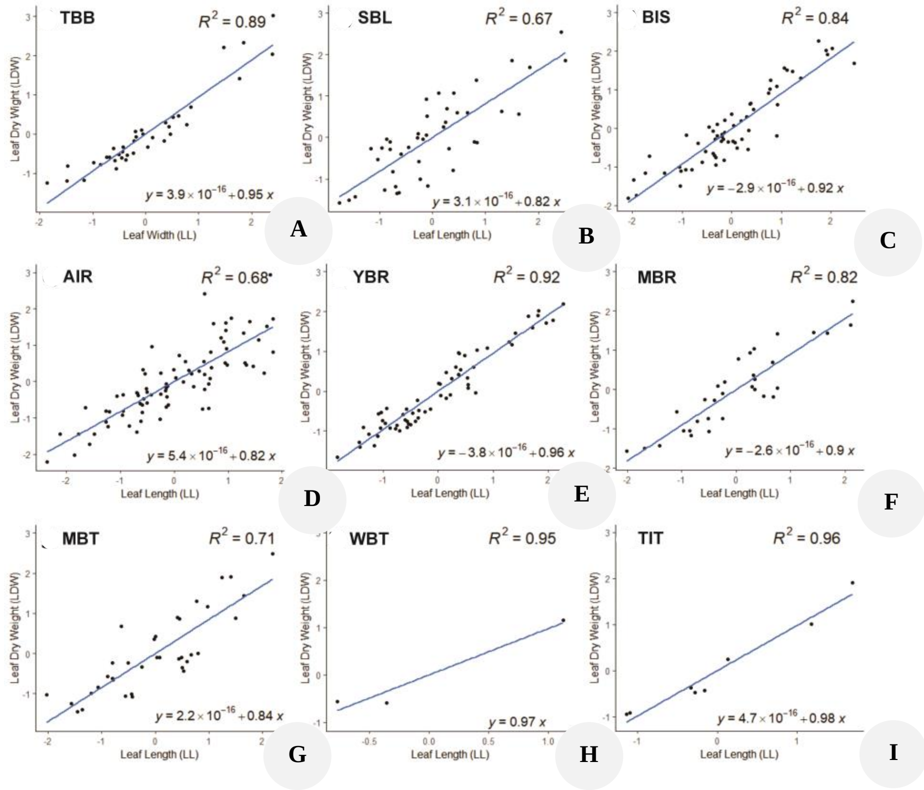

Figure 4. Regression analysis of the relationship between Leaf Length (LL) and Leaf Dry Weight (LDW). A. TBB: Terora Beach, Bali; B. SBL: Sekotong Beach, Lombok; C. BIS: Bungin Island, Sumbawa; D. AIR: Arefi Island, Raja Ampat; E. YBR: Yensawai Beach, Raja Ampat; F. MBR: Marandanweser Beach, Raja Ampat; G. MBT: Malala Bay, Tolitoli; H. WBT: Weduar Beach, Tual; and I. TIT: Tayando Island, Tual

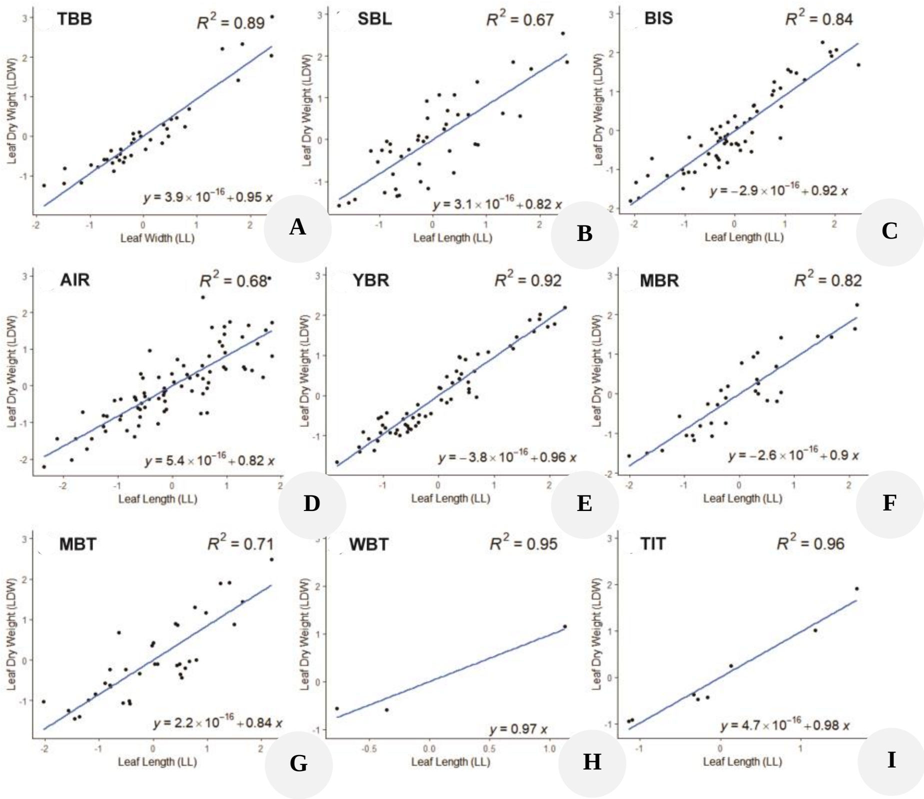

Figure 5. Regression analysis of the relationship between Leaf Length (LL) and Biomass (BIO). A. TBB: Terora Beach, Bali; B. SBL: Sekotong Beach, Lombok; C. BIS: Bungin Island, Sumbawa; D. AIR: Arefi Island, Raja Ampat; E. YBR: Yensawai Beach, Raja Ampat; F. MBR: Marandanweser Beach, Raja Ampat; G. MBT: Malala Bay, Tolitoli; H. WBT: Weduar Beach, Tual; and I. TIT: Tayando Island, Tual

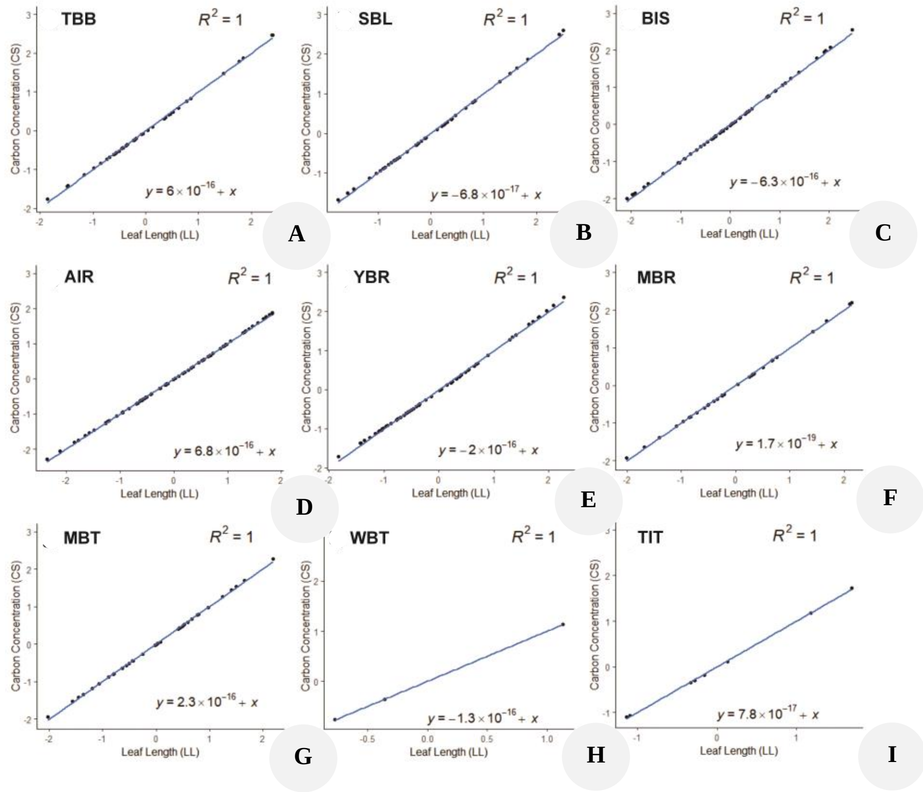

Figure 6. Regression analysis of the relationship between Leaf Length (LL) and Carbon Concentration (CS). A. TBB: Terora Beach, Bali; B. SBL: Sekotong Beach, Lombok; C. BIS: Bungin Island, Sumbawa; D. AIR: Arefi Island, Raja Ampat; E. YBR: Yensawai Beach, Raja Ampat; F. MBR: Marandanweser Beach, Raja Ampat; G. MBT: Malala Bay, Tolitoli; H. WBT: Weduar Beach, Tual; and I. TIT: Tayando Island, Tual

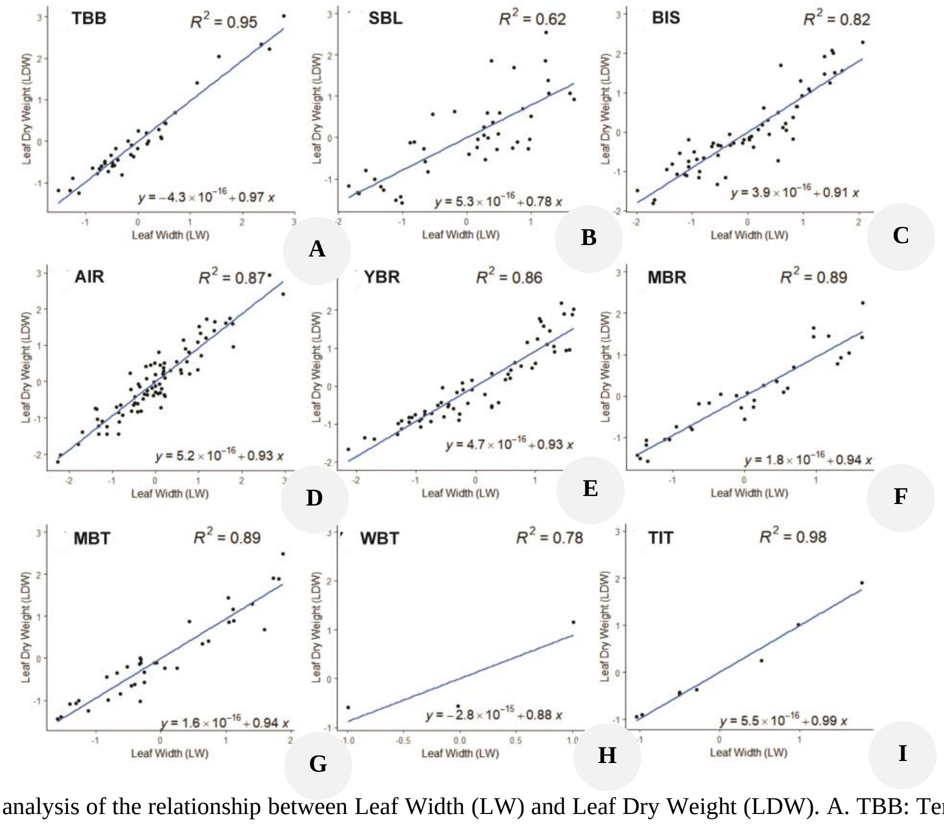

Figure 7. Regression analysis of the relationship between Leaf Width (LW) and Leaf Dry Weight (LDW). A. TBB: Terora Beach, Bali; B. SBL: Sekotong Beach, Lombok; C. BIS: Bungin Island, Sumbawa; D. AIR: Arefi Island, Raja Ampat; E. YBR: Yensawai Beach, Raja Ampat; F. MBR: Marandanweser Beach, Raja Ampat; G. MBT: Malala Bay, Tolitoli; H. WBT: Weduar Beach, Tual; and I. TIT: Tayando Island, Tual

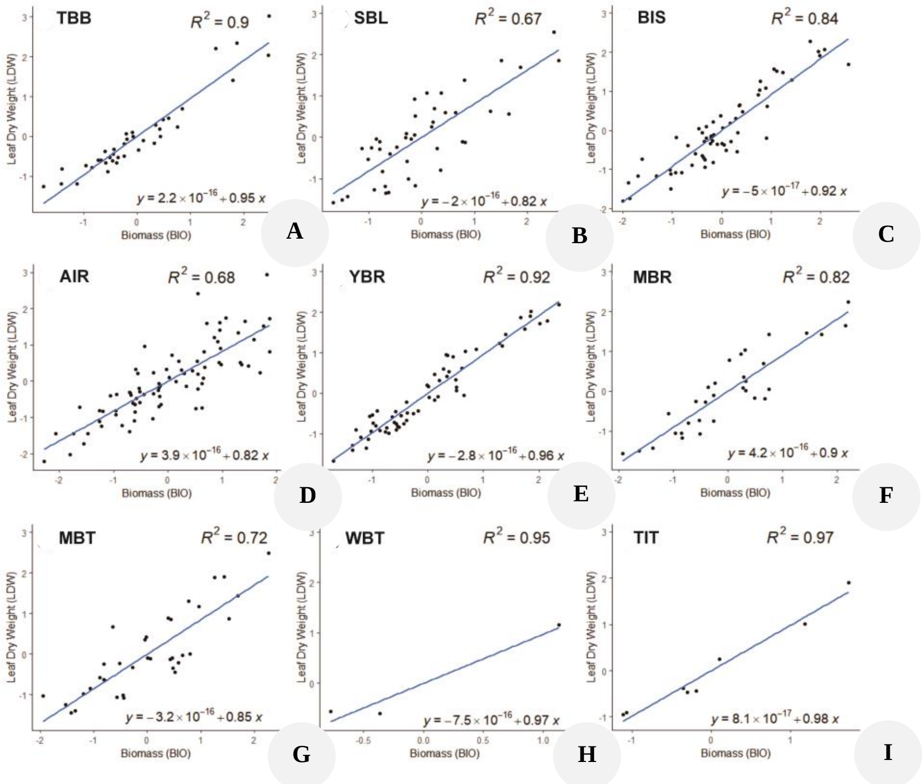

Figure 8. Regression analysis of the relationship between Biomass (BIO) and Leaf Dry Weight (LDW). A. TBB: Terora Beach, Bali; B. SBL: Sekotong Beach, Lombok; C. BIS: Bungin Island, Sumbawa; D. AIR: Arefi Island, Raja Ampat; E. YBR: Yensawai Beach, Raja Ampat; F. MBR: Marandanweser Beach, Raja Ampat; G. MBT: Malala Bay, Tolitoli; H. WBT: Weduar Beach, Tual; and I. TIT: Tayando Island, Tual

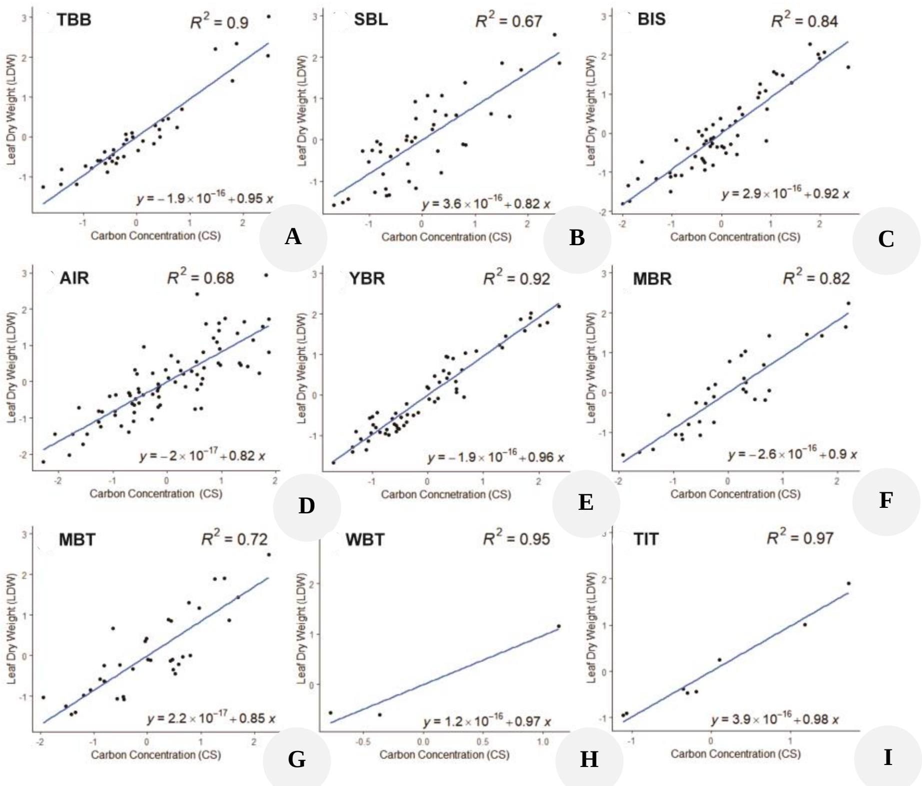

Figure 9. Regression analysis of the relationship between Carbon Concentration (CS) and Leaf Dry Weight (LDW). A. TBB: Terora Beach, Bali; B. SBL: Sekotong Beach, Lombok; C. BIS: Bungin Island, Sumbawa; D. AIR: Arefi Island, Raja Ampat; E. YBR: Yensawai Beach, Raja Ampat; F. MBR: Marandanweser Beach, Raja Ampat; G. MBT: Malala Bay, Tolitoli; H. WBT: Weduar Beach, Tual; and I. TIT: Tayando Island, Tual

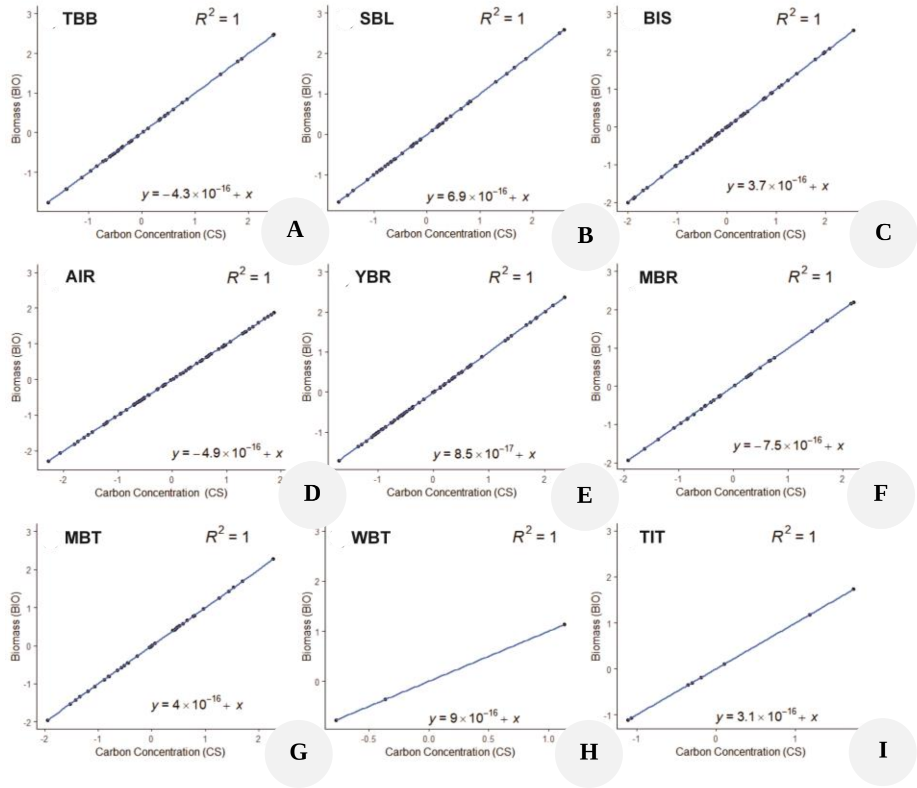

Figure 10. Regression analysis of the relationship between Carbon Concentration (CS) and Biomass (BIO). A. TBB: Terora Beach, Bali; B. SBL: Sekotong Beach, Lombok; C. BIS: Bungin Island, Sumbawa; D. AIR: Arefi Island, Raja Ampat; E. YBR: Yensawai Beach, Raja Ampat; F. MBR: Marandanweser Beach, Raja Ampat; G. MBT: Malala Bay, Tolitoli; H. WBT: Weduar Beach, Tual; and I. TIT: Tayando Island, Tual

The large leaf of *H. major* is an adaptation to the environment. Habitat strongly correlates with leaf length, leaf width, and petiole length (Kaewsrikhaw and Prathep 2014). The availability of light and substrate influences this morphology (Kaewsrikhaw and Prathep 2014; Horinouchi et al. 2016). The availability of little sunlight has an impact on widening the cross-sectional area of seagrass leaves (Kaewsrikhaw et al. 2016). Kurniawan et al. (2020) stated that *H. major* lives on fine sand and rocky sand substrates, making morphological adaptations to obtain energy to grow. Seagrasses with flooded habitats always have large morphology (Kaewsrikhaw and Prathep 2014). *Halophila major* is found at a 2-6 m depth in a flooded habitat. Meanwhile, *H. ovalis* can be found at 0.5-1 m depth and is often exposed to the sun at the lowest tide. Depth is closely related to light availability, thus impacting differences in leaf morphology (Gaubert-Boussarie et al. 2021). This condition causes the morphology of *H. ovalis* to be smaller than *H. major*.

Non-destructive biomass assessment has been proven to have consistent values between leaf length and biomass (Echavarría-Heras et al. 2012). Echavarría-Heras et al. (2013b) have evaluated the relationship between leaf length and leaf weight and can influence biomass using an allometric approach. Thus, the leaf size used in assessing leaf biomass first influences the leaf's dry weight. Because leaf dry weight was analyzed using an allometric model of leaf length and width (Hamburg and Homann 1986). Remote sensing using satellite imagery has been widely used to estimate above-ground biomass as there are many advantages to using this approach, such as being able to capture large seagrass biomass (AGB) (Hartoko et al. 2021; Wicaksono et al. 2021). However, it still has limitations, namely image resolution, water clarity, and accuracy (Veettil et al. 2020). Field surveys are therefore needed to ensure accuracy.

Using different allometric models to estimate biomass, this study showed that leaf length and leaf weight were strongly correlated, as has been proven by Solana-Arellano et al. (2012). In contrast, biomass and carbon concentration were not influenced by leaf area. Leaf area was only moderately correlated with leaf dry weight (Figure 3). The relationship between biomass and leaf area is unique and supports several previous studies. Weraduwage et al. (2015) showed that the relationship between leaf area and plant biomass is not linear. Thus, these results prove that photosynthesis can influence leaf growth but not leaf area. Meanwhile, Leaf Area Index (LAI) significantly influences biomass (Bartelink 1997; Chen et al. 2009; Forrester et al. 2017; de Almeida et al. 2022). LAI can be defined as the total area of leaves per unit area or land area (Gholz et al. 1976) and differs from the leaf area definition for each leaf. A linear relationship between LAI and biomass was carried out in seagrass studies on *Z. marina* (Solana-Arellano et al. 2003). LAI also correlated significantly with the dry weight of seagrass leaves of *Thalassia testudinum* Banks & Sol. ex

K.D.Koenig, *Syrigodium filiforme* Kütz., and *Halodule wrightii* Asch. (de Almeida et al. 2022; Hill et al. 2023). The linear model shows consistency, so the model can be a powerful tool for estimating biomass without destroying seagrass.

Biomass and carbon concentration assessments using non-destructive approaches in seagrasses have developed so far. Duarte (1991) predicted seagrass productivity using allometrics. The same method was used to estimate seagrass leaf biomass production (Echavarría-Heras et al. 2010, 2011, 2019) and leaf growth (Echavarría-Heras et al. 2013a, b). The accuracy, consistency, and sensitivity of allometric models of seagrass growth and biomass continue to be tested for different species (Echavarría-Heras et al. 2015, 2018). In the future, allometric models will be widely used to reduce the massive destruction of seagrass in nature for various seagrass species and sizes.

This study revealed biomass and carbon concentration as supporting characters to describe new seagrass species. Variations in biomass and carbon concentrations influenced by seagrass leaf morphometrics and the environment are the reasons for using these characters to describe seagrass species. For example, the number of chromosomes distinguishes *Ruppia maritima* L., and *Ruppia cirrhosa* (Petagna) Grande (Dandy 2005). The monoecious character of the seagrass *H. sulawesii* J.Kuo makes it a new species in the genus *Halophila* (Kuo 2007). Principally, morphology and phylogenetics are the main analyses for identifying and describing seagrass species in the genus *Halophila* (Kuo 2020). In the future, research into the biomass and carbon concentration of seagrass leaves can be considered as characters to differentiate seagrass species.

The main goals of this work are more than just descriptive characterization of the species, assessing biomass, and calculating carbon concentration in seagrass leaves. These results can be used as a standard for the value of *H. major* leaf biomass, providing practical application for the research. Furthermore, the biomass value is combined with the shoot density to calculate the biomass in certain areas, offering a practical and efficient solution that cuts the time and cost of the laboratory process. In the future, the biomass model of seagrass species found in the sampling area can be accurately calculated based on species diversity, providing a practical tool for future research. These results allow for a correction factor for biomass assessment using satellite imagery. In addition, this leaf biomass can be continued to estimate the below-ground biomass in other seagrass species, further expanding the practical implications of the research. Furthermore, further research can build an allometric model of the below-ground biomass of each seagrass species and the dynamics of biomass and seagrass density (Vieira et al. 2019). The developed allometric models can be a powerful tool for monitoring seagrass health from growth or productivity indicators, underlining the practical relevance of the work.

In conclusion, this study revealed the variation of leaf dry weight, specific leaf area, biomass, and carbon concentration of *H. major* and their relationship with morphometric traits. Nonetheless, Leaf Area (LA) did not strongly correlate with leaf productivity (Leaf Dry Weight-LDW, Biomass-BIO, and Carbon Concentration-CS). The allometric approach for estimating biomass and carbon concentration emerged as a crucial tool to avoid damaging seagrass abundance and enable rapid assessment. This result suggests considering the leaf productivity as the variable for describing new seagrass species. A comprehensive study is needed to estimate leaf productivity in other seagrass species using mesocosm, allometric, or correlation approaches. The potential for a new model to be generated and developed for assessing above-ground and below-ground biomass and carbon stock is a promising prospect for the future of this research.

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