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Research Article

Influence of Water Depth on the Morphology Structure of Seagrass from the Southern of Peninsular Malaysia

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ABSTRACT

Globally, seagrass meadows have declined due to environmental factors and human activities, particularly by limiting light to seagrass in turbid coastal waters. Furthermore, publications of seagrass research findings from the Southeast Asia region are scarce, making understanding these habitats difficult despite their ecological and economic importance. This research aimed to provide mean and standard deviation of seagrass morphology, as well as to examine the morphology structures in response to water depth. Samples of two species of seagrass, *Halodule uninervis* and *Halophila ovalis*, were collected by using random sampling in a line transect at Pulau Besar and Pulau Tinggi in Johor, southern Malaysia, in September 2013 and April 2014. Six morphological features of each seagrass species were measured physically using CPCe software and the relationship of water depth to seagrass were evaluated using Pearson's Correlation. The result highlights that the leaf and root morphology is larger in Pulau Tinggi because it is nearer to the Johor mainland, where the introduction of nutrients from economic activity positively influence seagrass growth. The overall morphology structures of both species in both islands are greater in 2013 than 2014. For the relationship with water depth, it had greater positive relationship to H. univervis leaf width (r = 0.7532), internode width (r = 0.6722), leaf length (r = 0.5739); whereas for H. ovalis, water depth was correlated strongly with leaf width (r = 0.6697) and leaf surface area (r = 0.6313). The morphology of seagrass species varies depending on habitat conditions, this study can fill knowledge gaps, but more fundamental research on seagrass meadows is required particularly for the seagrasses in the Southeast Asia marine region.

Keywords: Halodule; Halophila; Morphology; Seagrass; Water depth

Introduction

Seagrass is a productive ecosystem in the world (Lin et al., 2018). They provide food for herbivores (Inoue et al., 2021), habitat for juvenile and aquatic ecosystems (Nakaoka & Supanwanich, 2000, Unsworth et al., 2019; Johan et al, 2020), and stabilize sediment of seabed (Christianen et al., 2013). Furthermore, seagrass offers nutritional and pharmaceutical values to local communities (Wisespongpand et al., 2022), as well as support local economic development (Praisankul & Nabangchang-Srisawalak, 2016).

Many previous studies indicated that the seagrass morphology is subject to the environmental parameters of different water depth (Short et al., 2001; van der Heide et al., 2010). Water depth and sunlight penetration are related to seagrass growth in terms of photosynthetic function (Duarte, 1991; Kenworthy & Fonseca, 1996; Enríquez et al., 2019). The maximum depth at which seagrass can grow was determined by the growth compensation irradiance (Hemminga & Duarte, 2000) and low amount of light penetration can also limit the efficiency of photosynthesis efficiency of seagrass (Short et al., 2001). For example, tropical seagrasses are adaptable to water depth of less than 10 meters (Short et al., 2001). In Malaysia, Ooi et al. (2011a) recorded seagrass meadows below 10 meters of water depth. In northeastern Queensland, there are 13 seagrass species under 6 meters and four seagrass species above 20 meters (Lee Long et al., 1996).

Morphological variation of seagrass, including leaf area, length and width and number of leaves per shoot, associated to differences of water depths have been observed (Short, 1983, Collier et al., 2007). A recent study of *Halophila stipulacea* shows that significantly larger (11 %) rhizome internodes and longer (19 %) and wider (15 %) leaves were observed in deeper plants compared to those inhabiting shallow meadows. In contrast, shoot and internode formation rates in shallow plants were markedly higher than in deep-adapted plants (Azcárate-García et al., 2020). Additionally, smaller leaves and lower leaf area index of *Halophila stipulacea* were found at shallower (1.8–2.0m depth) than deeper depth (3.0–4.0m) in Palinuro harbour, Mediterranean Sea (Di Genio et al., 2021).

In addition, the amount of light a seagrass species can tolerate varies greatly among species. For examples, *Halophila ovalis* has a low tolerance to light deprivation (Longstaff et al., 1999) whereas *Thalassia testudinum* can survive more than 5 months without sufficient sunlight (e.g., Lee & Dunton, 1997).

In Malaysia, there are 16 seagrass species with distinctive morphological features in leaves, rhizomes, and roots (Bujang et al., 2018). Loss of approximately 50.7 kg seagrass from 2009–2013 was reported in Merambong, Straits of Johor (Misbari & Hashim, 2016). Due to continuing decline of seagrass meadows, fundamental research on seagrass biology, ecology and conservation is needed for future assessment of marine community and restoration planning (Sievers et al., 2019). However, research is few in seagrass and their environment interaction, especially the relationship between morphology and environment parameters (Fortes et al., 2018).

Along the Johor offshore of southern Malaysia, two seagrass species, *Halodule uninervis* and *Halophila ovalis* are relatively common. Currently, the seagrass bed in this area has been greatly affected by coastal reclamation and garbage dumping (Unswoth et al., 2018; Huang et al., 2020). Accordingly, the habitat changes could potentially affect the morphology and ecology of seagrass. This present study aims to observe the relationship between water depth and morphology of *H. uninervis* and *H. ovalis*, which could provide fundamental information for future assessment and restoration programmes of Malaysian seagrass bed.

Materials and Methods

Study area

The present study was conducted in the seagrass meadows of Pulau Besar (Besar Island) (2°25’25”-2°26’00”N; 103°58’22”-103°59’16”E) and Pulau Tinggi (Tinggi Island) (2°16’55”-2°17’32”N; 104°06’10”-104°07’22”E), Johor, Malaysia (**Figure 1**), known as part of the Sultan Iskandar Marine Park. These islands are annually affected by monsoon seasons. Pulau Besar is about 16.49 km north off Pulau Tinggi. Pulau Tinggi is located 12 km off the southeast coast of Peninsula Malaysia and around 30 km southeast of Mersing (Azman et al., 2008). The distribution of seagrass in Pulau Tinggi is limited to depths of less than 10 m and the mean wave height around this island is 0.9 ± 0.2 m (Ooi et al., 2011a). The water in the study area is relatively shallow, with a mean depth of 5.0 m. Both islands receive a lot of rain each year (an average of 2500 mm), and the climate and seagrass meadows are both influenced by two tropical monsoons: the wet Northeast monsoon from November to March, and the dry Southwest monsoon from May to September (monthly average 120 mm) (Malaysian Meteorological Department 2016). It can be assumed that the water conditions for both islands are similar to other nearby islands within the marine park. Both islands have relatively high turbidity probably due to proximity to the mainland (Mohamed et al., 2015). The water parameters of Johor Marine Parks were within the standard range of the Malaysian Marine Quality Criteria and Standards. Although Pulau Tinggi and Pulau Besar are located about 8.0 km away from each other, the water temperature of this area was constantly between 28.0–31.6°C. The pH value was between 8.0–8.2 and salinity was between 26.12–33.10 ppt

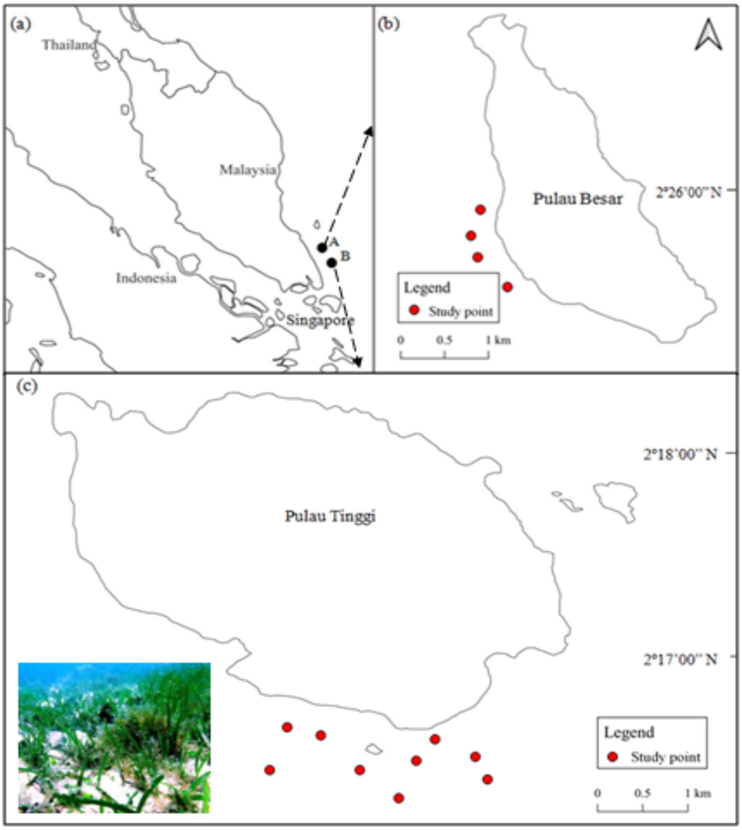

Figure 1. (a) Approximate location of Pulau Besar (A) and Pulau Tinggi (B) in Peninsular Malaysia, location of study points at Pulau Besar (b) and Pulau Tinggi (c), and the image of seagrass meadows in the study areas.

(Mohamed et al., 2015). The Marine Water Quality Status for Island (MDQI) value for Pulau Tinggi and Pulau Besar were 89.51 and 87.65 respectively (MDQI: 0-100; the study areas are in relatively good condition) (Department of Environment, 2015).

Pulau Tinggi and Pulau Besar are mainly tourist attractions and have less than 400 inhabitants and Mohamed et al. (2015), suggested that domestic waste and sewage contributed to the issue of heavy metals pollution in the marine waters, and the high turbidity of the study sites. From personal observation, the seabed is characterized as sandy and muddy. The sandy seabed of both islands has a high variation of seagrass species. Pulau Tinggi was reported to have high seagrass diversity between 3 and 6 m, covering of two dominant species, *H. uninervis* and *H. ovalis* (Ooi et al., 2011a).

Field sampling

The samples of *H. uninervis* and *H. ovalis* were collected by hand on 8–14 September 2013 and on 6–12 April 2014. Seagrass samples were collected at 4 sampling points at Pulau Besar and 9 sampling points at Pulau Tinggi, Johor. Seagrasses were collected by snorkeling during low tide or by SCUBA diving during high tide. Water depth was recorded. The sampling method was random sampling in a line transect perpendicular to shoreline for each study point. One line transect was placed in each sampling point. Five samples were collected for *H. uninervis* and *H. ovalis* in every transect. Each seagrass sample consisted of five shoots linked on a rhizome, beginning with the youngest shoots. The sediment and epiphytes attached to the samples were removed *in situ* with sea water prior to the physical measurement (Rattanachot et al., 2020).

Seagrass measurement

For every five-linked shoots seagrass, seagrass morphology was measured on the fourth shoot using vernier caliper. The morphology included in this study: 1. leaf length, 2. leaf width, 3. root length, 4. internodes length, 5. internodes width, and 6. leaf surface area (**Figure 2**). For leaf surface, the complete leaf without being grazed or broken was taken into account, the average value of leaf surface area for both species was taken if there were two or more leaves on the third shoot of seagrass. For both species, leaf length was measured from the tip to the end of the leaf blade without taking petiole into account, while leaf width was measured from the left to the right of the widest part of the leaf horizontally. The longest seagrass root was measured for each sample. Internode length was measured from the connecting point of shoot, rhizome and root of one shoot to the next shoot. Rhizome and internode are interchangeable for the present study. Internode diameter was measured on the middle part of each internode. The average values of both third and fourth internode length and rhizome width were calculated. The leaf images of the third shoot on samples were recorded digitally using camera. The leaf surface area was generated by using the digitized sample pictures in the CPCe (Coral Point Count with Excel extension) software (Kohler & Gill, 2006). The measurements were saved for further analyses to explore the relationship between water depths and the morphology of the studied seagrass. The water depths of sampling points were corrected according to the Tide Tables Malaysia 2014 (Nichols & Williams, 2009). Readings of water depth was obtained in situ by using a dive computer with recorded time and time for each sampling point. Water depths of sampling points were corrected according to the Tide Tables Malaysia. These tables provide information on heights and times of maximum and minimum water levels (Nichols, 2009).

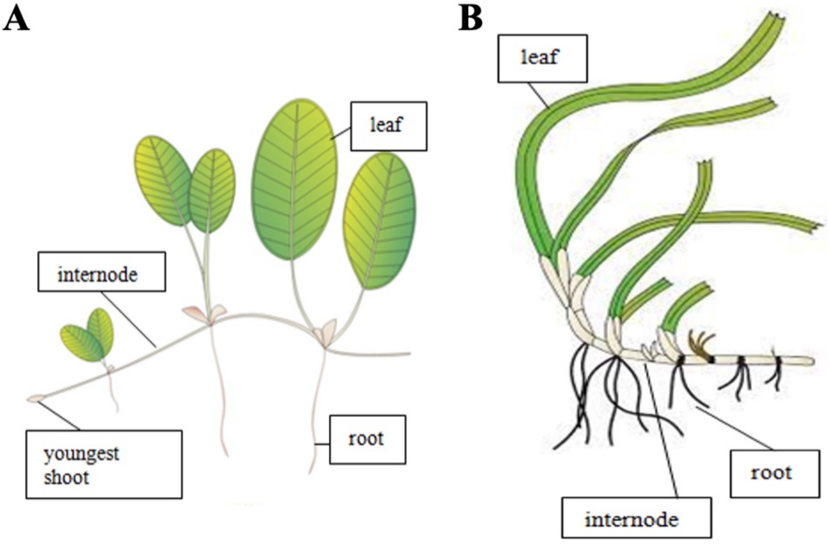

Figure 2. Measurement of seagrass (A) *Halophila ovalis* and (B) *Halodule uninervis* (adapted from Lelian et al., 2008).

Data analysis

The data were analyzed statistically using Pearson Correlation analysis in SAS/PC 9.3 software (Wang et al., 2017) to examine the relationship between water depth and morphological characteristics of seagrass samples. The tests were considered significant at p-value < 0.05.

Table 1. Interpretation of correlation coefficient and relationship level for this study (adapted from Wahyuni & Purwanto, 2020).

Correlation Coefficient (r)	Relationship Level
0.00-0.19	Negligible correlation
0.20-0.39	Weak correlation
0.40-0.59	Moderate correlation
0.60-0.79	Strong correlation
0.80-1.00	Very strong correlation

The correlation coefficient was interpreted using the standard shown in **Table 1.** The level of relationship between water depth and seagrass morphology has been classified into five categories: negligible correlation, weak correlation, moderate correlation, strong correlation, and very strong correlation for positive relationship and vice versa.

Results and Discussions

The physical characteristics of seagrass is presented in **Table 2**, the overall leaf and root structures of *H. uninervis* and *H. ovali*s were greater in Pulau Tinggi than Pulau Besar, whereas the internode structures of seagrass were higher in Pulau Besar.

Table 2 Mean and standard deviation of morphology of *H. uninervis* (*Hu*) and *H. ovalis* (*Ho*) from Pulau Tinggi and Pulau Besar between 2013 and 2014 (The greater value of morphology is highlighted in grey by comparing both island in the same year).

Morphological features (mean value)	2013				2014			
	Pulau Tinggi		Pulau Besar		Pulau Tinggi		Pulau Besar	
	Hu (n=35)	*Ho* (n=35)	*Hu* (n=35)	*Ho* (n=35)	*Hu* (n=35)	*Ho* (n=35)	*Hu* (n=35)	*Ho* (n=35)
Leaf length (mm)	68.86 ± 8.16	18.69 ± 1.56	47.98 ± 3.14	14.70 ± 0.76	59.68 ± 8.56	18.27 ± 1.55	41.78 ± 4.12	14.30 ± 0.42
Leaf width (mm)	3.98 ± 0.14	12.93 ± 0.51	2.67 ± 0.31	9.70 ± 0.69	3.58 ± 0.13	12.58 ± 0.48	2.27 ± 0.27	9.50 ± 0.52
Leaf surface area (mm^2)	4.45 ± 0.39	1.54 ± 0.16	3.36 ± 0.26	1.37 ± 0.15	4.05 ± 0.27	1.30 ± 0.10	2.76 ± 0.37	1.17 ± 0.12
Internode length (mm)	27.50 ± 2.73	28.93 ± 7.02	28.77 ± 4.25	31.44 ± 5.36	26.50 ± 2.23	27.91 ± 5.06	26.57 ± 4.14	31.04 ± 5.36
Internode width (mm)	1.58 ± 0.20	1.24 ± 0.14	1.63 ± 0.16	1.36 ± 0.15	1.18 ± 0.20	1.14 ± 0.14	1.33 ± 0.16	1.16 ± 0.15
Root length (mm)	50.99 ± 1.60	26.59 ± 3.48	48.08 ± 5.59	24.97 ± 2.78	47.85 ± 1.60	26.15 ± 3.48	46.95 ± 5.59	24.77 ± 2.78

Seagrass meadows were found in the water depth from 3.0 to 8.3m, which is within the range of <1.00–10.72m (Ooi et al., 2011a). Both species in Pulau Tinggi had greater leaf structures and root length than Pulau Besar. Pulau Tinggi is nearer to the Mersing mainland compared to Pulau Besar. The larger leaf and root of seagrass maybe due to the anthropogenic discharges of the industrial and domestic wastewater that causes high nutrition values for the plant growth in Pulau Tinggi marine area. Most internode structures of both species in Pulau Besar were greater than those collated in Pulau Tinggi except for internode width for *H. uninvervis*.

Relationship of water depth and seagrass morphology

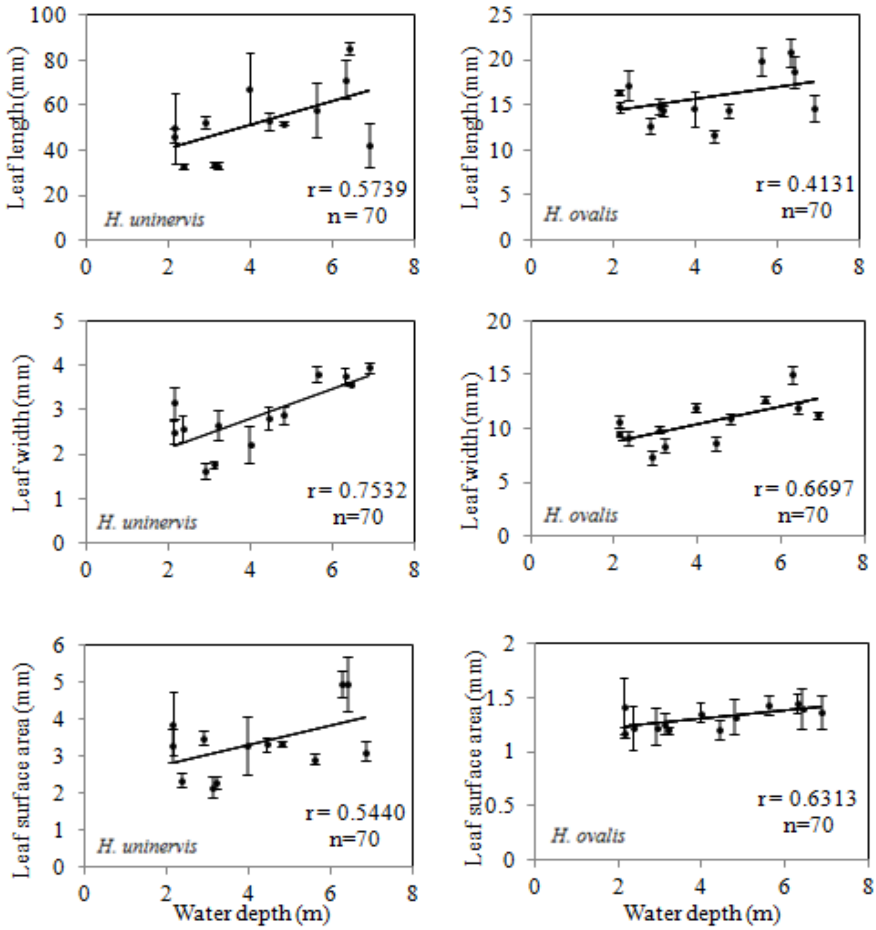

Figure 3 Pearson correlation of water depth and leaf morphology for *Halodule uninervis* and *Halophila ovalis*. The tests were considered significant at p-value < 0.05.

Internode morphology

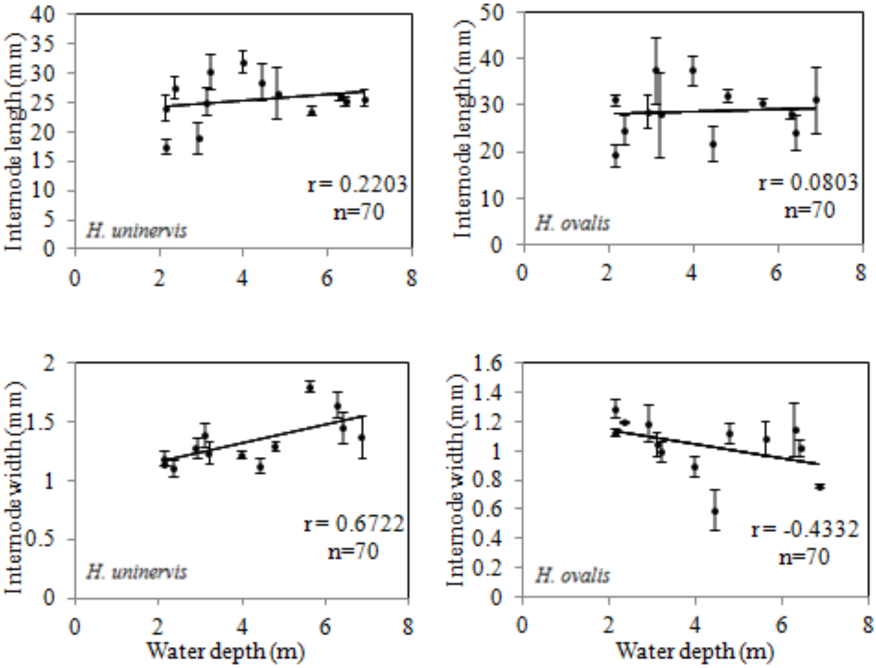

Figure 4. Pearson correlation of internode morphology for *Halodule uninervis* and *Halophila ovalis*. The tests were considered significant at p-value < 0.05.

Root morphology

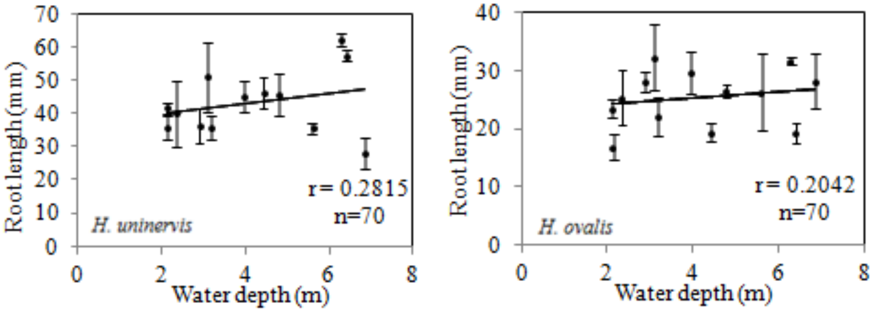

Figure 5. Pearson correlation of root morphology for *Halodule uninervis* and *Halophila ovalis*. The tests were considered significant at p-value < 0.05.

For *H. uninervis*, water depth had positive relationship to leaf length (r = 0.5739) and leaf width (r = 0.7532) (**Figure 3**), internode width (r = 0.6722) (**Figure 4**) and leaf surface area (r = 0.5440) (**Figure 3**); but it was less correlated to root length (0.2815) (**Figure 5**) and internode length (0.2203) (**Figure 4**). There was a negative correlation between water depth and seagrass decline in earlier research, but there were few fundamental research on the relationship between water depth and seagrass morphology, especially in Malaysia (Zhang et al., 2020).

For *H. ovalis*, water depth had strong relationship with leaf width (r = 0.6697) and leaf surface area (r = 0.6313) (**Figure 3**). Water depth had moderate relationship with leaf length (r = 0.4131) (**Figure 3**) and internode width (r = -0.4332) (**Figure 4**). Water depth had weak correlation with root length (r = 0.2042) (**Figure 5**) and no correlation with internode length (r = 0.0803) (**Figure 4**).

In the study area, Ooi et al. (2011) reported that H. *ovalis* (mean shoot density 1454.6 ± 145.1 m^{-2}) and *H. uninervis* (mean shoot density 861.7 ± 372.0 m^{-2}) growing in relatively low light conditions at 3 meter water depth. Water depth had a positive relationship with all leaf structures (leaf length, leaf width, leaf surface area) and internode width, but a weak relationship with internode length and root length in *H. uninervis*. For *H. ovalis*, water depth had a strong relationship with leaf width and surface area; a moderate relationship with leaf length and internode width (negative relationship); a weak relationship with root length; and no correlation with internode length. Water depth had a moderate relationship with leaf surface area for both *H. uninervis* and *H. ovalis*.

In the present study, seagrass morphology varied to water depth. The growth of seagrass required light for photosynthesis. This process was also affected by many environmental factors that were not included in this study, such as light intensity, surrounding temperature, salinity, and nutrient quality (Longstaff & Dennison, 1999). Previous research suggested that habitat conditions and seasons greatly impacted the seagrass morphological variation (Vermaat & Verhagen, 1996), but a recent study on *H. ovalis* shows reduction of about 32% in leaf surface area under high shading (68%) treatment (Kong et al., 2019). For example, the width of the leaves of *Thalassia testudinum* which decreased 26.6 % as depth increases in response to 41 % light reduction (Lee & Dunton, 1997). The average leaf length, rhizome elongation and photosynthetic rate of *H. ovalis* were higher in seawater with higher salinity level (S30) compared to river water with lower salinity level (S20) (Lamit & Tanaka, 2021). The morphological characteristics of seagrass are also greatly affected by human activities. Increasing turbidity level of 87.6% caused increased leaves length of 46.1%, leaf width of 27.5%, rhizome diameter of 36.8 % and root length of 12.6% for H. ovalis in Pulau Bintan, Indonesia. The concentration of nutrient such as nitrate and phosphate is higher near to the shore areas where domestic dumping is relatively higher than in the deeper water area which is less disturbed by anthropogenic factors. The increasing nutrient levels in the water caused by human activities lead to higher turbidity levels, high suspended sediment concentration and decreasing dissolved oxygen levels in the water. This is likely to cause regression of seagrass (Nugraha et al., 2020). Preliminary laboratory studies suggest that currents between 2 and 50 cm s^{-1} affect leaf production of *Zostera marina L.* under light-saturated conditions (Fonseca & Kenworthy, 1987).

Significant variations of leaf width of *H. ovalis* were found at different seasons and locations (Hedge et al., 2009). Environmental parameters such as salinity, disturbance, intertidal conditions, and water depth were also affected seagrass morphology. Furthermore, the study of seagrass leaf morphology revealed significant differences among populations (Bujang et al., 2006; Vy et al., 2013), but they are in the range of morphological parameters concluded by den Hartog (1970).

For *H. uninervis*, there was high correlation between water depth with leaf width and internode width, which showed that larger leaf surface was beneficial to obtain more sunlight in the deeper water. This result is in contrast to the hypothesis that the energy and nutritional costs of producing leaves are lower along a continuum of water depth. In this instance, the leaf surface area was therefore used as an indicator of the distribution of nutrients and energy as it grows larger in deeper sea beds (Sinden-Hempstead & Killingbeck, 1996).

In addition, water depth was weakly correlated to root length and internode length shows that these parts are less affected by light attenuation. However, the growth and morphology of these below ground structures are more affected by sediment composition and sedimental burial (Ooi et al., 2011b). Seagrass meadows receive organic inputs of various origins, and this organic matter forms a complicated mix of labile and more refractory compounds (Hemminga & Duarte, 2000). Therefore, the high complexity of sediment compounds leads to a weak relationship between water depth and internode length, which is often buried under the sand.

For *H. ovalis*, there was also high correlation between water depth and leaf width; water depth and leaf surface area whereas root length was weak correlated to the water depth. Water depth showed the variability best for leaf width, followed by leaf surface area, internode width, leaf length, root length, and internode length. This partially agreed with the findings on *Halodule wrightii* in Rio de Janeiro, where each seagrass morphological parameter varied depending on location and water depth. Similar to the reported study, seagrass variation in some characteristics was high within a single population (Creed, 1997).

Only the rhizome width of this species had a negative relationship with water depth. The complexity of nutrient contents in soil composition and wave movement at the study sites are factors that explain the negative relationship between water depth and internode width of *H. ovalis*. The leaf morphology of small and fast growing Halophila species varies greatly between study sites and is highly dependent on various environmental conditions (Emmclan et al., 2022), with substrate conditions significantly affected the growth of seagrass compared to other habitat variables (Zabarte-Maeztu et al., 2020).

Additionally, there was a moderate correlation between leaf surface area and water depth because the amount and quality of light available for photosynthesis determines how much seagrass can grow (Ziemen & Wetzel, 1980). Therefore, decreases in underwater lighting in the deep sea frequently cause widespread seagrass die-off (Short & Wyllie-Echeverria, 1996). As a result, the leaves of this species grew bigger at deeper water depths to catch more light for photosynthesis. The biggest leaf size of *H. ovalis* was noticed in the lower intertidal zone among three zones of upper intertidal, lower intertidal, and subtidal zones (Kaewsrikhaw et al., 2016). Masini and Manning (1997) found that certain plants had higher photosynthesis rate and lower light requirements in deeper water.

Seagrasses were also attached with epiphytes and the roots were tangled in the soil, the seagrass roots for these small species are fragile and easily broken when sampling and cleaning, so the removal of epiphytes and soils on the samples had to be done gently to prevent broken samples. Overgrazing activities are popular for the Johor Marine Park Islands due to high density of seagrass and coral habitats. Seagrasses of these study sites are popular for overgrazing other aquatic herbivores, so many samples were observed to have broken leaves when sampling which agreed with the previous observations (Bujang et al., 2006).

The result of this study is important for understanding the environmental factors that influence the growth of marine plants. For this study, only water depth was selected to study its relationship with the different morphology of two dominant seagrass species at Johor Marine Park islands. This study also aims to close the knowledge gaps in seagrass research and conservation in Southeast Asian countries, which are important for many tropical marine animals and which also provide significant economic value to island residents.

This study has several limitations. The sample is small and not representative of the broader seagrass population in the study area. Additionally, parameters of sunlight and wave movement aren't included in the study due to limited facilities and funding at the time of field survey. We are currently developing a more comprehensive and long-term study on the seagrass meadows of the study area. Additionally, the parameters of other variables will also be taken into account in the future study to provide an ongoing seagrass survey for the study area. As a result, future research on variations in seagrass morphology is required to fill knowledge gaps in the study area.

Conclusion

The overall leaf structures of *H. uninervis* and *H. ovalis* were greater in Pulau Tinggi than Pulau Besar in the same year. In terms of morphological structures, water depth had positive relationship to the morphological properties of *H. uninervis* (e.g., leaf length, leaf width, internode length and leaf surface area) whereas other structures had weak relationships with different water depth (e.g., root length and internode length). The ecological and biological functions of seagrass meadows are often underrated in Malaysia, therefore, understanding how seagrass morphology responds to water depth is essential for providing fundamental information in effective restoration and management of seagrass meadows.

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