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Morphological characteristics, shoot density and biomass variability of *Halophila* sp. in a coastal lagoon of the east coast of Malaysia

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Abstract — This study in a coastal lagoon of Merchang Terengganu, east coast of Peninsular Malaysia evaluated the morphological characteristics, shoot density and biomass for *Halophila* in pure and mixed population with *Halodule pinifolia* (Miki) den Hartog. Both species inhabited the silt and sand substrates at depth of about 1.9 m to 2.0 m. They are well adapted and tolerated a range of micro-ecology; pH of 6.57–7.32, wide salinity differences of 9.42–34.47 psu, conductivity 16.14–52.27 ms/cm, and light availability of 446.63–624.1 lux. Morphologically, there are two forms for *Halophila* (a) small-leaved in pure population and those mixed with the short-leaved, (b) big-leaved with the long-leaved *Halodule pinifolia*. Both forms have variable leaf shapes, a respond to the wide and frequent fluctuation in water salinity. Leaves possessed red or purplish spots or blotches with more spots and blotches in leaves of *Halophila* in pure population. These spots or blotches are believed to be UV-blocking pigments for protection of plants exposed directly to strong sun-light during low tides. Shoot density of 79.08±38.02 shoots/100 cm²; is comparatively higher in pure *Halophila* population compared with 26.33±13.20 shoots/100 cm² and 64.00±17.09 shoots/100 cm² for small-leaved and big-leaved *Halophila* sp. respectively. *Halophila* biomass (AG and BG) exhibit similar trend as those observed for shoot density. In pure or mixed *Halophila* population the majority of the biomasses (63–77% of the total) were in the below-ground parts (rhizome and roots). Although *Halophila* sp. is a smaller size seagrass, for propagation they would need extensive rhizome networks buried in the substrates.

Key words: Seagrass, Halophila, morphology, shoot density, biomass, Malaysia

Introduction

Seagrass is considered as productive in tropical ecosystem (Bronwyn 2006) and act as shelter and food for shore fisheries, marine reptiles and mammals. They influenced the physical, chemical and biological environment in which they grow by acting as ecological engineer (McKenzie 2008). The distribution and abundance of seagrasses is controlled by range of environmental conditions including light availability (Dennison and Alberte 1985, Dennison 1987), nutrient availability (Short 1987), water motion (Fonseca and Kenworthy 1987) and grazing (Longstaff and Dennison 1999). Light availability controlled the depth to which seagrasses can grow (Abal and Dennison 1996). The reduced availability of light can also be caused by bigger and taller seagrass species shading the understory species e.g. in a mixed seagrass population of *Enhalus acoroides* (L.f.) Royle, *Halophila ovalis* (R.Br.) Hook. f. and Cymodocea serrulata (R.Br.) Aschers. & Magnus (Japar Sidik et al. 2001a). A considerable part of morphological variability is due to environmental circumstances and therefore merely phenotypical. However, one can often find in the same habitat two morphologically quite distinct forms growing together. In such a case the differences might be genotypically determined. Seagrass plants in mixed population e.g. in the Merchang lagoon, depending on their habits of growth forms, tall versus short or those growing overlying the substrates under the canopy of other seagrass species may have different light requirement and simultaneously compete for space and nutrients. The response to low light and acclimatization towards such conditions may be expressed in the plant morphology e.g. the leaf dimension, the nervation (leaf cross veins number in Halophila), the length of the petiole and the rhizome length and biomasses. This present study in a coastal lagoon of Merchang Terengganu, east coast of Peninsular Malaysia evaluate the morphological

characteristics, shoot density and biomass for *Halophila* pure population and those growing in association with *Halodule pinifolia*.

Materials and Methods

The *Halophila* plants were sampled during low tides, on 13th September 2007 and 8th November 2007 from different sites in Merchang coastal lagoon (Lat. 5°02'15.0"N, Long. 103°17'53.0"E, Fig. 1), Terengganu, east coast of Malaysia. At the same site, water temperature, salinity, depth, pH where possible, were recorded using Hydrolab Surveyor 4a or SCT meter and light availability using Li-Cor model 250 Quantum Light Meter.

Halophila plants were sampled from pure population and those in association with Halodule pinifolia within 5 $0.5 \text{ m} \times 0.5 \text{ m}$ quadrat with sub-divisions of 25 units. Both plants and substrates were taken within the three sub-division of each quadrat. Samples were carefully sieved using soil sieve with a mesh size of 0.45 mm (which retained seed c. 0.5 mm). The number of shoot (for Halophila in Merchang, a shoot is a pair of leaves) in each sample was recorded. The plants were placed in a labeled plastic bag and kept in an ice chest before processing in the laboratory. In the laboratory each sample was then rinsed in 5% v/v o-phosphoric acid for 3-4 minutes (Parthasarathy et al. 1988) to remove calcareous epiphytes and washed with distilled water and carefully observed for leaf length, width, petiole length, distance between intra-marginal vein to leaf edge, paired cross vein number and distance between cross-veins that were used for taxonomic identification of Halophila ovalis (den Hartog 1970) and H. minor (Zoll.) den Hartog (Kuo 2000). In addition the Halophila plants' rhizome length was recorded. All plant dimensions were measured using Mitutoyo Digimatic Vernier Caliper (measured to two decimal points) and plant habits were recorded digitally using Nikon Coolpix 995 Digital

Camera. After plant dimensions and characteristics were recorded they were then separated into leaves, male and female flowers and fruits (assigned as above ground, AG), rhizomes and roots (assigned as below ground, BG). Seagrass fractions were dried at 80°C in an air-circulating oven to constant weight (approximately 5 days). Biomasses of AG, BG and T (total) biomass were determined by weighing the material on a Sartorius chemical balance. Analysis of variance (ANOVA, p<0.05) and post-hoc Duncan's Multiple Range Test (DMRT, p<0.05) were used to compare the vegetative parts dimensions, shoot density and biomasses of *Halophila* between stands.

Results and Discussion

Habitat type

A coastal lagoon comprising of pure and mixed patches of two seagrass species, the dominant *Halodule pinifolia* interspersed with *Halophila* sp. The *Halophila* sp. based on leaf dimension (leaf length and leaf width), the number of cross-veins and the ascending angles from cross-vein to the mid-vein, showed overlapping characters with most of the descriptive morphology used in the taxonomy of *Halophila ovalis* (den Hartog 1970) and *Halophila minor* (Kuo 2000). They inhabited the silt and sand substrates at depth of about 1.9 m to 2.0 m from the highest high water level. Both species are well-adapted and tolerated a range of micro-ecology; pH of 6.57–7.32, wide salinity differences of 9.42–34.47 psu, conductivity 16.14–52.27 ms/cm, and light availability of 446.63–624.1 lux.

Leaf and rhizome dimension

There are two categories for *Halophila* (a) small-leaved as pure population and those mixed with the short-leaved *Halodule* species (b) big-leaved mixed with the long-leaved *Halodule pinifolia*. Leaf dimensions varied in pure and



Fig. 1. Merchang coastal lagoon during low tide. Site A-a mixed population, *Halophila* with long-leaved *Halodule pinifolia*, Site B-pure population of short-leaved *Halodule pinifolia*, Site C-a mixed population of *Halophila* with short-leaved *Halodule pinifolia* and Site D-a pure population of *Halophila*.

			Halophila	ophila population			
Vegetative dimension and characteristic Site	Pure			Mixed with short-leaved Halodule		Mixed with long-leaved	
	Area D-1	Area D-2	Area D-3	Area C1	Area C2	Area A	
Leaf dimension	Mean±s.d	Mean±s.d	Mean±s.d	Mean±s.d	Mean±s.d	Mean±s.d	
	Range (mm)	Range (mm)	Range (mm)	Range (mm)	Range (mm)	Range (mm)	
	N	N	N	N	N	N	
Leaf length	9.36±2.13 ^{bc}	8.91±1.76 ^{ab}	10.05±2.58 ^{cd}	10.41±1.47 ^d	8.35±2.26ª	14.59±1.14 ^e	
	4.56–14.02	5.74–12.82	5.90–16.20	6.90–14.06	4.90–14.30	12.00-16.30	
	82	79	74	43	97	18	
Leaf width	4.33±1.24 ^b	4.42±1.19 ^b	4.72±1.47 ^b	4.44±1.07 ^b	3.73±1.14ª	6.40±0.42°	
	1.55–7.40	1.95–7.43	1.70–8.40	1.58–7.00	1.60-7.00	5.60-7.20	
	82	79	74	43	97	18	
Petiole length	12.95±3.86 ^{ab}	10.86±4.01ª	14.52±5.33 ^{bc}	16.21±5.84°	12.07±6.02ª	26.33±8.08 ^d	
	3.28–21.71	4.24–19.36	4.10–26.00	3.21–28.19	3.00–33.00	9.30–36.90	
	82	79	74	43	95	18	
Rhizome length	10.09±5.09ª	11.17±5.75ª	10.30±6.29ª	9.83±2.74ª	9.27±3.47ª	13.22±3.92 ^b	
	1.98–22.95	2.53–23.80	2.60–33.80	4.47–15.78	3.20–20.20	5.90–19.60	
	66	76	209	76	106	27	
A-distance between cross-vein	1.15±5.24ª	0.57±0.25ª	1.31±2.56ª	0.91±4.47ª	1.15±0.43ª	1.38±0.40ª	
	0.30–81	0.16–1.32	0.64–1.92	0.10–79.00	0.43–2.38	0.50–2.55	
	347	223	50	309	42	227	
B-distance between	0.26±0.07ª	0.56±0.25°	0.69±0.07 ^d	0.67±0.33 ^d	0.44±0.07 ^b	0.47±0.01 ^b	
intra-marginal vein	0.10–0.47	0.18−1.47	0.61–0.88	0.19–1.63	0.35–0.58	0.23–0.76	
to leaf edge	160	115	14	154	21	125	
Number of cross-vein	8.94±1.76 ^{ab}	10.60±1.75°	10.61±1.71°	9.59±1.79 ^b	8.33±1.90ª	10.97±1.19°	
	3–14	6–13	7–14	6–13	4–13	9–13	
	122	40	72	39	72	32	

 Table 1.
 Leaf and rhizome dimensions of Halophila plants from Merchang, Terengganu. Means with the same alphabet (a–d) are not significant different (ANOVA, p<0.05).</td>

mixed population. Bigger leaves, longer petioles and rhizomes were observed particularly in Halophila growing with long-leaved Halodule pinifolia (Table 1, Site A, Fig. 1). Morphological variability occurred among Halophila associated with the short-leaved and long-leaved Halodule pinifolia, and those as a pure populaton. Halodule pinifolia being common, a larger and taller seagrass have its leaves raised or extended above that of smaller understory seagrass, Halophila. The canopy formed by Halodule pinifolia caused shading of the understory species. Halophila has to compete for light and habitat because leaves as well as roots are position at similar level in the water column and sediment respectively (Duarte 1991). The understory Halophila responded or acclimatized to shading by morphological responses through altered growth pattern of leaf size by having longer leaf length, petiole length and horizontal rhizome length as opposed to the one in pure population. The morphology differences in the forms of Halophila sp. have selective significance in adapting to habitats of varying light (shaded or unshaded) availability. The big-leaved and long-petiolate *Halophila* has selective advantage in habitats with low light e.g. created by *Halodule pinifolia*, can also survive in turbid water and subtidal zone (Japar Sidik et al. 2001b). The short-petiolate *Halophila* sp. may be confined to unshaded sites or higher light conditions.

Leaf shape and characteristic

Leaf variants occurred in pure or mixed populations (Site A-a mixed population, *Halophila* associated with longleaved *Halodule pinifolia*, Site C-a mixed population of *Halophila* with short-leaved *Halodule pinifolia* and Site D-a pure population of *Halophila*, Fig. 2). Den Hartog (1970) regarded such observations as the respond to environmental modifications. The variability in *Halophila*'s leaf shapes in Merchang lagoon is regarded as a respond to the wide (9.42 to 34.47 psu) and frequent fluctuation in water salinity.

Irrespective of pure or mixed population, *Halophila* leaves possessed red or purplish spots or blotches with more



Fig. 2. Red or purplish spots or blotches were more in leaves of (a)-Halophila in pure populations, compared with leaves of Halophila in population mixed with (b)-short-leaved Halodule and (c)-long-leaved Halodule.

Table 2. Shoot density and biomass in an area of 100 cm² (N=15) for the three *Halophila* populations.

Halophila	Shoot density	Biomass (gram dry weight)			
population	Shoot density	AG	BG	Total	
Pure	79.08±38.02	0.0956±0.0404	0.3241±0.3636	0.4197±0.3670	
	(41–155)	(0.0490-0.1745)	(0.0865-1.2955)	(0.1355-1.4060)	
Mixed with short-leaved	26.33±13.20	0.03±0.0128	0.0512±0.0356	0.0813±0.0477	
<i>Halodule</i>	(12–38)	0.0153-0.0375	(0.0128-0.0830)	(0.0281–0.1205)	
Mixed with long-leaved	64.00±17.09	0.0873±0.0179	0.1829±0.0823	0.2702±0.0821	
Halodule	(48–82)	(0.0680–0.1034)	(0.1152-0.2745)	(0.2186-0.3649)	

spots and blotches in leaves of *Halophila* in pure population (Sites A, C, D, Fig. 2). We believed these spots or blotches are UV-blocking pigments (Hemminga and Duarte 2000) formed as a response for protection of pure stand *Halophila* to direct exposure to strong sun-light during the low tides. *Halophila* in mixed populations have less spots in their leaves as they were partially protected by shading afforded by the *Halodule pinifolia*.

Shoot density and biomass

Shoot density is comparatively higher in pure *Halophila* population. In pure population plants propagated without competing for light, space (substrate) and nutrients. Shoot density was 79.08 ± 38.02 shoots/100 cm² (Table 2). *Halophila* plants in mixed populations required more energy

for propagation as they were competing for light, space and nutrient under the canopy of *Halodule pinifolia* hence less shoot density compared with those from pure population.

Halophila biomass (AG and BG) exhibit similar trend as those observed for shoot density. Leaves of *Halophila* is the part which up-take the nutrient in the sea-water. The highest level in up-taking the nutrient from the environment was from plants that have higher shoot density. These would definitely contribute to the significant contribution to higher above ground biomass. In pure or mixed *Halophila* population, the majority of the biomasses (63–77% of the total) were in the below ground parts (rhizome and roots). Although *Halophila* sp. is a smaller seagrass species, for propagation *Halophila* sp. would need prostate stems or extensive rhizome networks buried in substrate similar to those observed for larger seagrasses such as *Thalassia hemprichii* (Ehrenb.) Aschers., *Halodule uninervis* (Forssk.) Aschers. *Cymodocea serrulata*, and *Syringodium isoetifolium* (Aschers.) Dandy (Norhadi 1993, Japar Sidik et al. 1996).

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