UNIVERSITI PUTRA MALAYSIA

BIOLOGY OF THE SEAGRASSES, Halophila ovalis (R.Br.) Hook.f. AND Halophila beccarii Ascherson UNDER LABORATORY CONDITIONS

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Chairman : Japar Sidik Bujang, PhD
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Two tropical seagrasses, *Halophila ovalis* (R.Br.) Hook.f. and *H. beccarii* Ascherson, in Malaysia were selected for this study. Seagrasses play an important role in Malaysian marine ecosystems. Little were known of their morphology variations in the natural habitats and these variations could be attributed to environmental factors. To understand these effects, both plants species responses were assessed under laboratory cultures conditions. These were archived via experiments conducted to investigate effects of different substrates, salinity, light availability and nutrient enrichment to the plants' morphology i.e., leaf length, leaf width, petiole length and no. of paired cross veins (*H. ovalis*) or no. of leaf per shoot (*H. beccarii*). Both plants species were collected and observed at their natural habitats, from two study sites, Punang-Sari-Lawas, Sarawak and Merambong Shoal, Johor. At the natural habitats, *H. ovalis* grew on substrates from sandy to loamy sand. *Halophila beccarii* which was only observed at Punang-Sari-Lawas, grew on muddy substrate. Both plants species variability in leaf morphology at their natural habitats were compared with the plants cultured under laboratory condition. Both plants species were tested under different substrates for eight weeks of growing, i.e., substrate from a river, medium sand and substrates from beaches, fine sand, loamy medium sand, coarse sand and mixed sand (mixture between loamy medium sand and coarse sand). *Halophila ovalis* showed better growth performance in medium sand and *H. beccarii* in both loamy medium sand and mixed sand in term of leaf morphology changes. With respect to salinity ranges, both plants species were tested under decreasing and increasing salinity. *Halophila ovalis* tolerated low salinity of 5 psu to as high as 55 psu, while *H. beccarii* tolerated salinity range of 0 psu (freshwater) to as high as 80 psu. Both plants species showed better growth performance in the salinity range of 20-25 psu. In the light availability experiment which corresponded with four different water depths (10 cm, 20 cm, 30 cm, and 40 cm) for indoor and outdoor were

tested. *Halophila ovalis* and *H. beccarii* showed significant morphology changes under indoor and outdoor light conditions, on leaf length, leaf width, petiole length and shoot density with all different light availability. Both plants species responded to reduced light intensity by producing bigger leaf and longer petiole but with less leaves density and vice versa when light intensity increased. Selected nutrient e.g., Ammonium chloride, Sodium nitrite, N:P:K (15:15:15) and slow release fertilizer tablet enrichments were used to evaluate both plants species responses to fluctuation of nutrient in water on their morphology. Relatively in solid fertilizers, *Halophila ovalis* and *H. beccarii* showed better growth responses with slow release fertilizer tablet. In soluble fertilizers, both plant species showed better growth responses with Ammonium chloride.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Sarjana Sains

BIOLOGI RUMPUT LAUT, *Halophila ovalis* (R.Br.) Hook.f. DAN *Halophila beccarii* Ascherson DI BAWAH KONDISI MAKMAL

Oleh

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Pengerusi : Japar Sidik Bujang, PhD
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Dua rumput laut tropika, *Halophila ovalis* (R.Br.) Hook.f. dan *H. beccarii* Ascherson di Malaysia telah dipilih untuk kajian ini. Rumput laut memainkan peranan penting dalam ekosistem marin Malaysia. Sedikit telah diketahui tentang variasi morfologi pada habitat semulajadi dan variasi morfologi ini mungkin diakibatkan oleh faktor alam sekitar. Dalam memahami kesan ini, tindak balas kedua spesies tumbuhan diuji di bawah keadaan kultur. Ini boleh dicapai melalui eksperimen untuk mengkaji kesan substrat, kemasinan, kesediaan cahaya dan pengkayaan nutrien yang berbeza terhadap morfologi tumbuhan iaitu panjang daun, lebar daun, panjang tangkai daun dan bilangan urat daun berpasang (*H. ovalis*) dan bilangan daun per tunas (*H. beccarii*). Kedua-dua spesies tumbuhan telah dikumpul dan diperhatikan daripada dua tempat kajian di habitat semulajadi mereka, Punang-Sari-Lawas, Sarawak dan Beting Merambong, Johor. Pada keadaan habitat semulajadi, *H. ovalis* tumbuh di atas substrat daripada berpasir ke pasir berloam. *Halophila beccarii* pula hanya dijumpai di Punang-Sari-Lawas, tumbuh di atas substrat yang berlumpur. Kedua-dua spesies pada habitat semulajadi yang mempunyai variasi di dalam morfologi daun telah dibandingkan dengan tumbuhan yang dikultur di bawah keadaan makmal. Kedua-dua spesies tumbuhan telah diuji pada substrat yang berbeza selama lapan minggu pertumbuhan, iaitu substrat daripada sungai, pasir medium dan substrat daripada pantai, pasir halus, pasir loam medium, pasir kasar dan campuran (pasir loam medium dan kasar). *Halophila ovalis* menunjukan pertumbuhan yang baik di atas pasir medium dan *H. beccarii* di atas pasir berloam medium dan pasir campuran dari segi perubahan morfologi daun. Merujuk kepada julat kemasinan, kedua-dua spesies tumbuhan telah diuji di bawah kemasinan menaik dan menurun. *Halophila ovalis* toleransi kepada kemasinan serendah 5 psu dan kemasinan tinggi sehingga 55 psu, manakala *H. beccarii* toleransi kemasinan daripada 0 psu (air tawar) kepada yang tertinggi 80 psu. Kedua-dua spesies tumbuhan menunjukkan prestasi pertumbuhan yang baik pada julat kemasinan 20-25 psu. Di dalam keamatan cahaya yang

merujuk kepada empat kedalaman air yang berbeza telah diuji (10 cm, 20 cm, 30 cm dan 40 cm) dalam keadaan cahaya pada persekitaran terbuka dan tertutup. *Halophila ovalis* dan *H. beccarii* telah menunjukkan perubahan morfologi yang ketara di bawah keadaan terbuka dan tertutup, pada panjang daun, lebar daun, panjang tangkai daun dan kepadatan daun pada kesemua keamatan cahaya berbeza. Kedua-dua spesies tumbuhan bertindak balas kepada penurunan keamatan cahaya dengan menghasilkan daun yang lebih besar dan tangkai daun yang panjang tetapi kepadatan daun berkurang dan tindak balas sebaliknya apabila keamatan cahaya meningkat. Nutrien yang dipilih iaitu, pengkayaan Ammonium klorida, Natrium nitrit, N:P:K (15:15:15) dan baja tablet larut resap digunakan untuk menilai tindak balas kedua-dua spesies tumbuhan terhadap naik turun nutrien di dalam air terhadap morfologi mereka. Secara relatifnya dalam baja pejal, *Halophila ovalis* and *H. beccarii* menunjukkan tindak balas pertumbuhan yang baik dengan baja larut resap. Dalam baja larutan, kedua spesies tumbuhan menunjukkan tindak balas pertumbuhan yang baik dengan Ammonium klorida.

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LIST OF ABBREVIATIONS

psu	Practical salinity unit
NTU	Nephelometric turbidity units

CHAPTER 1

INTRODUCTION

The term "Seagrasses" is collectively used to describe 12 genera of flowering aquatic plants (*Zostera, Phyllospadix, Heterozostera, Posidonia, Halodule, Cymodocea, Syringodium, Thalassodendron, Amphibolis, Enhalus, Thalassia* and *Halophila*) which grow under marine environment as one group and they are not necessarily related to each other. The plants are grouped based on their ability to grow fully submerged in a saline environment (obligate halophyte), completely become specialize to grow and completed their life cycle under saline environment (Arber, 1920). The important characteristic of seagrasses was that the plants must be able to perform hydrophilous pollination (den Hartog, 1970). The 12 genera mentioned above satisfy the characteristics requirement and grouped under seagrasses, while others namely *Zannichellia, Lepilaena, Althenia, Ruppia* and some species of *Potamogeton* apparently satisfy all listed characteristics but not included in seagrasses group due to their wide tolerance range to salinity and not truly marine as they were primarily found in brackish water. Hence the artificial term seagrasses were used collectively to describe a group of marine plants that selectively fulfills the characteristic listed (den Hartog and Kuo, 2006). There are 72 species known worldwide from six families (Short *et al.*, 2011). Seagrasses plants are often confused with seaweeds or macroalgae due to the morphology and habitat that they grow are similar to seagrasses. Seagrasses possesses complex tissue to carry out specific task. In contrast, macroalgae are more primitive with no specialize organ such as leaves, roots and stems to carry out specific task. The main difference between seagrass and macroalgae is via reproductions. Seagrasses is the only flowering plants that are able to reproduce under marine and submerge environment (den Hartog and Kuo, 2006), while macroalgae reproduce by spores which are more primitive. A current accepted hypothesis was that they evolved from terrestrial plants into marine aquatic plants. Seagrasses lack strong supportive stem to withstand strong waves and water currents, and this is overcome with natural buoyancy to remain flexible.

Seagrasses are also known for their important role in ecology closely related to coastal zone economic, due to highly primary and secondary productive tropical ecosystem (Hemminga and Duarte, 2000; Bronwyn, 2006). Huge extensive meadow is always vital to near shore fisheries, acting as natural retreat, source of food and shelter for juvenile fishes, marine invertebrates, marine reptiles and mammals. It is well accepted that seagrasses affects significantly the chemical, physical and biological environment and therefore is considered as "ecological engineer"

(McKenzie, 2008). As fragile ecosystem, continuous lost worldwide are caused mainly by human activities (anthropogenic) and some natural occurrences. Polidoro *et al.* (2010) reported human activities effect fragile seagrass ecosystem through pollution and habitats destructions. Seagrass bed found nearby estuaries is frequently exposed to those pollution compared to coastal seagrass bed (Ralph *et al.*, 2006). The impact of pollution and disturbance are interconnected when influx of nutrient in water column (de Boer, 2007) causing eutrophication which increases water turbidity (Burkholder *et al.*, 2007) and sedimentation (Erskine and Koch, 2000) causing reduction of light availability to the plants (Lee *et al.*, 2007). Once the important value of seagrass meadow become more understood, it is important to restore our seagrasses area from further deteriorating by human activities. Various studies conducted in the last three decades brought great information for understanding the complexity of seagrasses and their behavior. For example the effect of light (Terrados, 1997), substrate conditions (Terrados *et al.*, 1997), photosynthesis response (Ralph, 1998), replanting of seagrasses (Kelly *et al.*, 1971), and biology, ecology and their conservation (Larkum *et al.*, 2006). However, few studies have been conducted on seagrass behavior in tropical areas especially Malaysia. Temperate seagrass may act differently on natural factors to determine their behavior compared to tropical area. Studies to culture them in laboratory conditions are needed to further understand how seagrasses react to variability in factors such as substrate, salinity and light. For this purpose, *Halophila* species were selected as they are known for variations of morphology and shapes (Short *et al.*, 2010). These variations are often thought to be influenced by environment factors such as shade (Japar Sidik *et al.*, 2001), salinity (Vermaat *et al.*, 2000) and substrate (Bradley and Stolt, 2005). Two fast growing seagrasses, *H. ovalis* and *H. beccarii* were chosen based on their distribution and morphology, where *H. ovalis* is a common seagrass (paired leaf blades) and *H. beccarii* is a rare seagrass (rosette leaves). The variations on morphology of both plant species in diverse natural habitats could be due to adaptation to different environment from geographical distribution or merely genetic plasticity. Culture studies of *H. ovalis* and *H. beccarii* would give information on the plants responses in term of morphological changes to different sets of parameters e.g., substrate, salinity, and light conditions. Therefore the objectives of this study are:

1. To assess *Halophila ovalis* and *Halophila beccarii* morphological variation in natural habitats and under laboratory conditions.
2. To determine effect of different substrate, light intensity and salinity to *Halophila ovalis* and *Halophila beccarii* morphology.
3. To determine effect of selected nutrient enrichment for sustaining plants growth and survival under laboratory conditions.

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