Malaysian Journal of Science **24**: 23 - 29 (2005)

Shading Responses of the Seagrass, *Halophila ovalis*: A Preliminary Comparison between Cultures and Naturally Growing Plants

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Received 9 June 2005; accepted in revised form 30 June 2005

ABSTRACT The seagrass, *Halophila ovalis* was grown in culture to investigate its responses to artificial shading. *H. ovalis* was collected from a low water level seabed off Teluk Kemang coast (2° 30'N, 101 ° 45'E) in Negri Sembilan, Malaysia. Live plant portions were transplanted indoors providing natural sunlight, substrate and seawater. Shade screens providing 10-15 percent of incident light, resulting in approximately 150-250 μmol m^{-2} s^{-1} of light intensity, was imposed. Observations after three months revealed morphological responses of the plant towards both indoor culturing as well as towards shading Plants in cultures exhibited two leaf size variations- both larger and smaller leaf sizes. In comparison, *H. ovalis* in the wild showed little variations in leaf size. As such, this study can provide insights to the plant's responses to photoacclimational conditions as well as to culturing conditions.

ABSTRAK Rumput laut, *Halophila ovalis* telah dikultur untuk menyiasat perkembangannya terhadap peneduhan. *H. ovalis* diperoleh dari kawasan aras air cetek di persisir Teluk Kemang (2 ° 30'N, 101 ° 45'E), Negeri Sembilan, Malaysia. Bhagian tumbuhan hidup dikultur secara tertutup dengan bekalan cahaya matahari, substrak dan air laut. Skrin teduh digunakan untuk pendedahan cahaya matahari kepada purata 10-15 peratus intensiti cahaya persekitaran (150-250 μmol m^{-2} s^{-1} intensiti cahaya). Pemerhatian selepas tiga bulan menunjukkan respons morfologi tumbuhan pada keadaan kultur dan peneduhan. *H. ovalis* di bawah keadaan peneduhan mempunya dua variasi saiz daun, iaitu besar dan kecil. *H. ovalis* yang tumbuh di dalam habitat semulajadi tidak menunjukkan perbezaan saiz daun yang ketara. Oleh itu, kajian ini dapat memberikan gambaran tentang respons rumput laut ini terhadap keadaan fotoaklimatasi sewaktu dikultur.

(Seagrass, *Halophila ovalis*, Shading, Morphological plasticity)

INTRODUCTION

Marine angiosperms, collectively named "Seagrasses" have a worldwide distribution [1]. This global distribution is encompassed by a limited number of species - comprising a group of about 50 species of closely-related angiosperms, all belonging to two families of monocots, and containing 12 genera [2]. Despite having small species diversity, a single seagrass species may traverse extensive global geographical regions, leading to a number of species variations according to its locality. This ability to colonise diverse marine habitats is well documented [3, 4 and 5]. As such, seagrass species such as *Cymodocea serrulata* [6, 7], *Cymodocea nodosa* [8], and *Halodule wrightii* [9], exhibit instances of morphological plasticity resulting from inherent environmental habitat conditions. Presence of these ecomorphs may even deceive researchers into recording these growth forms as separate species.

Seagrasses respond to their habitats comparable, if not similar to terrestrial plants. With the exception of the factors concerning its marine environment, seagrass physiology is similarly affected by inputs of light, inorganic carbon, nutrients, adequate rooting substrate and water. Light attenuation in terms of shading elicits natural responses from the plant whether in a natural or artificial environment. And especially to the latter, culturing conditions are essentially man-altered plant responses. With respect to

shading studies, both culture and field experimental approaches have been utilised to elucidate the biology of seagrasses further. The temperate seagrass *Zostera marina* responded to a shaded environment by altering allocation patterns such as the reduced formation of below ground biomass with reduced light availability [10]. The results of the shading could be quite apparent such that different patches of the same species exposed to different degrees of light intensities showed significant morphological differences. The study on *Halophila ovalis* and *Cymodocea serrulata* growing in the shade of *Enhalus acoroides* is a demonstration of this ability [11]. *Zostera capricorni* grown in culture produced larger shoots and had greener leaves than those exposed to 100% sunlight [12]. On the anatomical level, by lowering light exposures from high to low regimes, there was a decrease in lacunal leaf areas from *Syringodium isoetifolium, Zostera caopricorni, Halodule uninervis, Cymodocea serrulata* and *Halophila spinulosa*[13]. Contrastingly, artificial shading screens placed upon *Posidonia sinuosa* reduced the density of leaf-bearing shoots, leaf density, leaf length, primary productivity and the leaf production per shoot [14] as well as leaf growth rates and biomass [15].

Seagrass beds are important breeding grounds for a multitude of organisms ranging from commercially important molluscs to a variety of fish species as well as crustaceans. Despite its small and seemingly fragile infrastructure, *Halophila ovalis* covers extensive areas and is one of the most important seagrass species in Malaysia. Knowledge in this area may have potentially wide implications for future marine resources.

This study is aimed to observe the initial outcomes of transplanting *H. ovalis* to indoor cultures as well as the effects of lowering light exposure to the transplants in the new artificial environment. Insights from this study, however small, may provide leads for potential innovative fisheries and open up new fronts in aqua-agriculture.

MATERIALS AND METHODS

H. ovalis sprigs (planting units on a single rhizome strand with intact leaves and roots) were collected from Teluk Kemang coast (2°30'N, 101°45'E) in October 2004. This location is in the vicinity of the Universiti Putra Malaysia Marine Biology Research Station. The sprigs, having a range of approximately 3 to 10 paired leaves per sprig, were transplanted into 25.5 cm x 20.5 cm sized baskets with each basket containing one sprig. Sets of 8 of these baskets were then placed into separate, 200 litre-capacity indoor tanks in the station within the same day of collection. Natural aerated seawater having a salinity of 30 practical salinity units (psu) and sunlight having a maximum light intensity of 500 μmol m^{-2} s^{-1} according to normal daily regimes were supplied. Natural sandy-muddy seagrass substrates originating from Merambong shoal, Johore, Malaysia, were used. A layer of clean beach sand was introduced to stifle algal growth.

Commercially available shade screens having a mesh size of 1.5 mm x 10 mm were used. The shading resulted in light intensity attenuated to about 150-250 μmol m^{-2} s^{-1} providing approximately about 10-15% of total light intensity outdoors. Maintenance of the tanks included maintaining the water salinity as close to 30 psu and removal of slime (algal) growth. This removal is not more of reducing nutrient loss to the algae, but rather to prevent the slime from forming extensive layers over the substrate and leaves. These layers would directly contribute to more light attenuation onto the leaves as well as an expansion of more energy to break through the relatively cohesive slime layer. Apart from this, slime coating the leaves would also reduce the leaf-air diffusion boundary resulting in compromised exchange of expired and transpired gases.

Leaf form measurements, were made from mature senescent leaves. This forms the maximal growth size attained by the plant. Living leaves were not used since it was intended that minimal disturbance be inflicted upon the plants to promote its growth. Two aspects of the leaf form were recorded- the leaf shape and the number of paired cross-veins per leaf. The former constituted the length and width of the leaf measured end to end. For number of paired cross-veins per leaf, only leaves displaying clear lines of the cross-veins were taken.

For chlorophyll content, live leaves were harvested. The fresh weight (FWg^{-1}) was taken and the approximate leaf surface area recorded by

tracing on a graph paper. Single leaf blades were ground and the mass soaked in 5 ml of 90% acetone. Absorbances of the extracts were then measured in a spectrophotometer at 725, 663 and 645 nm [see 16]. Chlorophyll *a* and *b* concentrations were calculated from these absorbances using the appropriate equations [17].

A comparison was then made between leaves from culture and those growing naturally in Teluk Kemang. The field samples were collected during low tide in midday approximately after four months of having cultured *H. ovalis* indoors.

RESULTS AND DISCUSSION

Plants in cultures

All explants survived through the transplanting with varying degrees of lushness. Plants having longer rhizomes and number of shoots- hence more nutrient storage- were not necessarily observed to have better growth than those with shorter rhizomes. Leaf production was relatively slow- approximately 1-2 new paired leaves per week. It was faster in those rhizome strands having a growing leaf apical meristem. For those rhizomes planted without the leaf apical meristems, new meristems grew from the rhizome from the base of the leaf petiole.

Leaf growth showed extremes in dimensions. The leaf form from small to large exhibited a gradual but continuous progression. On a single rhizome strand, this progression was very much evident – with the smallest leaf being the oldest and thus the earliest new leaves formed after transplantation. When a certain possible stabilisation in adequate acclimatization has taken place, the plants showed consistency in leaf sizes, and this was usually the larger form.

Despite the stark variations in leaf sizes, leaves from culture displayed a consistent and distinct leaf shape. Both small and large leaves appear to elongate linearly, resulting in the spatulate shape.

Plants in the field

There was a relatively small range in dimensions for *H. ovalis* leaves collected from its naturally growing site. This trend is exemplified from leaves growing along the same rhizome strand. The leaves were very similar in size along the same sprig. The oldest leaf, usually densely covered by epiphytes, on the rhizome strand was minimally larger than the second youngest leaf or, in some samples, even from the youngest leaf. As with leaves in culture displaying a consistency in shape, leaves from the wild exhibited a similarity in shape, where the leaves tended to be very much ovate.

Figure 1 shows a comparison between cultured leaved with that of wild *H. ovalis* leaves. Table 1 summarises the disjunctions between leaf lengths and widths between leaves of *H. ovalis* in culture and from field collections.

Chlorophyll content

Chlorophyll content analyses show typical shade-unshaded leaf chlorophyll responses. Shaded leaves from the cultures tended to produce more chlorophyll and a decreased chlorophyll *a* amount to chlorophyll *b* amount ratio. Table 2 summarizes the chlorophyll analyses done on cultured and wild *H. ovalis* leaves.

The results collated reveal only partially on *H. ovalis*' response to a light reduced environment since only leaf morphology and chlorophyll content is being investigated. The observations firstly illustrates that *H. ovalis* is able to be maintained in cultures since all the plants survived through the transplanting. Apart from this, morphological plasticity of the plant is able to be induced by manipulating light availability in a relatively limited length of time.

Using the wild collected plants as a control group, the plants in culture showed shading responses by varying leaf form and chlorophyll content; the most indicative being the latter. Chlorophyll content between cultured and wild collected leaves proves from a biochemical standpoint that the responses from the shaded cultures were in fact due to reducing the light source rather than due to "healthier" growth from the bigger leaves. This is shown by the higher chlorophyll content and lowered chlorophyll *a* to *b* ratio in shaded cultures compared to wild leaves. An account for this chlorophyll differences is adequately described elsewhere [18].

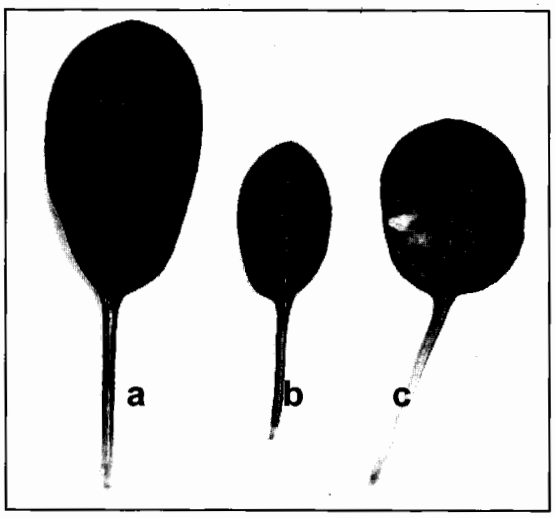

Figure 1. A comparison of *Halophila ovalis* leaves originating from the same area:
a. 3 weeks old leaf from cultures (length 29.04 mm; width 11.19 mm), cv - 15;
b. 4 weeks old leaf from cultures (length 16.14 mm; width 6.38 mm), cv - 15;
c. Wild leaf from Teluk Kemang (length 18.43 mm; width 10.71 mm), cv - 15.
Bar represents 10mm
cv – Number of leaf paired cross veins

The light conditions in culture had induced a stark and obvious difference in leaf morphology to that of wild *H. ovalis*. Table 1 illustrates clearly that the leaf tended to elongate in the shaded cultures rather than widen in size and have an increased leaf length to width ratio. This response consequently would increase the canopy height of the plant to intercept more light. Various terrestrial plants respond in the same way [see 19]. The difference would be that canopy extensions in land plants are very much more considerable than canopy extensions of the relatively miniscule *H. ovalis*. As could be inferred from the results, productivity of the seagrass itself may be lowered due to lowered photosynthesis by the reduction of light despite the bigger surface area that may be attained by the seagrass. But in consequence to a bigger surface area, there will be a larger area for epiphytic interactions. Even more so when *H. ovalis*, despite its small leaf size, form extensive subterranean meadows. As such, an overall higher productivity may be affected from the various interactions between the seagrass and other marine organisms.

It has to be noted that the leaf tends to elongate in the shaded cultures rather than attain a larger size. Figure 1 illustrates this point. The leaves "a" and "b" were from the same *H. ovalis* clone and removed from the plant at the same time. Leaf "a" was approximately three weeks old and leaf "b" four weeks old. Leaf size discrepancy as a result of the leaf's age factor may thus be negated in this sense.

From Table 1, the range between the lengths of the biggest leaf and the smallest leaf from the wild was 8.21 mm. Comparatively; the range for cultures was 14.74 cm. This shows that wild *H. ovalis* leaves exhibit a relatively small range in leaf length. As such, the extremes between leaf lengths in cultures show that it is very much possibly a direct consequence to transplanting rather than to shading.

The probable explanation to this occurrence is that this small size occured when the first new leaves grew from newly transplanted *H. ovalis*. As such, this could be a direct result of initial transplanting. There were also similar observations in other transplants. And as a cause and consequence to that, it could also be that there were minimal resources available from the rhizome strand to produce bigger leaves. The priority would then be to hasten production of smaller leaves and this in turn, may possibly initiate productivity in the shortest time possible.

Table 1. Observations between *H. ovalis* leaves of plants from shaded cultures and leaves from the wild. *P < 0.05 and **P < 0.01 represent significant differences between cultured leaf samples and wild leaf samples

Leaf form parameter	Plants from shaded cultures	Plants from wild
Smallest Leaf (mm)	16.81(length)	13.57 (length)
	8.98 (width)	7.42 (width)
Largest leaf size (mm)	31.55 (length)	21.78 (length)
	12.49 (width)	12.16 (width)
Mean Length (mm) **	22.75 ± 0.50	18.21 ± 0.21
	(*n* = 58)	(*n* =67)
Mean Width (mm)	9.84 ± 0.18	9.75 ± 0.13
	(n =58)	(*n* =67)
Mean leaf length : width ratio **	2.31 ± 0.02	1.87 ± 0.02
	(*n* = 58)	(*n* = 67)
Number of paired cross veins per leaf	Between 10 and 20	Between 13 and 17
	(*n* = 25)	(*n* = 38)
Mean number of paired cross veins *	15.40 ± 0.48	14.50 ± 0.19
	(*n* =25)	(*n* =38)
Leaf shape	More linearly spatulate. Half- diamond-like tapering from leaf base towards the leaf centre	Leaf form ovate with a rounded leaf base

Table 2. Chlorophyll analyses by acetone extractions. Means all present significant differences at P < 0.05 between cultured leaf samples and wild leaf samples

Mean analysis	Plants from shaded cultures		Plants from wild	
Chl *a* content:				
(μgChlcm^{-2})	1.50 ± 0.10	(*n* = 18)	1.07 ± 0.06	(*n* = 21)
(μgChlFW^{-1}g)	178.59 ± 14.19	(*n* = 18)	146.45 ± 9.25	(*n* = 21)
Chl *b* content:				
(μgChlcm^{-2})	0.98 ± 0.07	(*n* = 18)	0.53 ± 0.04	(*n* = 21)
(μgChlFW^{-1}g)	117.09 ± 9.98	(*n* = 18)	73.75 ± 5.24	(*n* = 21)
Total Chl content :				
(μgChlcm^{-2})	2.49 ± 0.17	(*n* = 18)	1.61 ± 0.10	(*n* = 21)
(μgChlFW^{-1}g)	295.68 ± 24.16	(*n* = 18)	220.20 ± 14.46	(*n* = 21)
Chl *a* - Chl *b* ratio	1.54 ± 0.01	(*n* = 18)	1.99 ± 0.02	(*n* = 20)

Chlorophyll content analyses showed that leaves in culture had a higher chlorophyll level compared to wild leaves. Chlorophyll *a* to *b* ratio was also lower for shaded cultures. These chlorophyll quantitative measurements follow the similar trend from chlorophyll analyses done elsewhere showing responses of plants to a shaded environment [20].

Chlorophyll production is directly proportional to the amount of light a plant receives up to its saturation point where beyond this, photodamage of the internal tissue ensues. The comparison for chlorophyll content in this study show that as a whole, *H. ovalis* in the shaded cultures show responses which are in fact due to the reduction in light source rather than to transplantation. And with comparing to wild *H. ovalis* as a benchmark, these responses are much more apparent.

This study represents a preliminary investigation into the shading responses of *H. ovalis* for both

cultured and wild growing forms. A more complete picture can be observed by measuring other parameters like biomass, photosynthesis rates and carbohydrate content.

Acknowledgements We would like to thank Vice-Chancellor, Universiti Putra Malaysia, for encouragement and facilities. This research is funded by the Ministry of Science, Technology and Environment Malaysia, under the 'Intensification of Research in Priority Areas' programme entitled "Seagrass taxonomy, biology and habitat characteristics: EA-001-09-02-04-0679" and some financial and travel supports from Japan Society for the Promotion of Science (JSPS) are acknowledged.

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