

## LABORATORY CULTURE OF THE SEAGRASS, *HALOPHILA OVALIS* (R.Br.) HOOKER f.

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### ABSTRACT

A small-culture system for growing of *Halophila ovalis* was developed. Plugs of explants were successfully grown in the native substrate and under the light regime of  $\sim 200 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ . The culturing of *H. ovalis* in the laboratory permitted observations on (i) the sustain growth and the development of the population, (ii) the reproductive biology (flowering and fruiting) and (iii) the pattern of seedling development from seeds to mature plants. Plants increased in density via vegetative propagation and sexual reproduction. Plants produced male, female flowers and fruits. The presence of viable seeds and seedlings demonstrated the successful pollination and sexual reproduction of *H. ovalis* in culture. The morphology of progressive development of *H. ovalis* seedlings to juvenile or young plants for *H. ovalis* in culture is described.

**Keywords:** Seagrass, *Halophila ovalis*, Laboratory culture, Phenology

### INTRODUCTION

Growing of seagrasses had been attempted by many biologists. The attempt had been conducted as early as 1959 (Wood, 1959). Various methods had been employed on various species of seagrasses in order to create a small-scale marine system that simulates the environmental conditions of the natural habitats accordingly to species and locality. Among the successful one is for example at Austin, Texas where representatives of 9 out of 12 genera of seagrasses; *Thalassia*, *Halodule*, *Halophila*, *Posidonia*, *Zostera*, *Cymodocea*, *Syringodium*, *Enhalus* and *Thalassodendron* have been successfully cultured in synthetic seawater and under controlled environmental conditions by McMillan (1976, 1978, 1980a, 1980b). Using the culture system, seagrasses were maintained that facilitated studies involving the biological, ecological and phenological aspects of seagrasses (McMillan, 1980a; McMillan 1980b; McMillan *et al.*, 1981). Although such cultures have been developed they

were only suitable for a particular type of seagrasses and their conditions of growth under studied (Hillman *et al.*, 1995; Ralph, 1998; Longstaff *et al.*, 1999; Short, 1985). In addition, the origin, biology, distribution and the habitat of seagrasses used in the studies were not similar, for example, *Halophila ovalis* (R. Br.) Hook f. that live in estuaries can tolerate lower salinities than that of deep sea *H. ovalis* because they have to adapt to low salinities caused by freshwater runoff and wet monsoon season (Benjamin *et al.*, 1999). In Malaysia, there are 14 species of seagrasses of which 5 species belong to *Halophila* and *Halophila ovalis*, being the most common occurred in lagoons, along the shallow inter-tidal coast and sub-tidal areas. Although common, its biology and phenology have not been examined (Japar Sidik *et al.*, 2006). The aim of this present study is to develop a culture system that could assess the growth and development of Malaysian *H. ovalis* under condition favorable to the plants. This culture study could be then form the basis for examining; (i) the sustain growth and development

of the *H. ovalis* population, (ii) the reproductive biology (flowering and fruiting) and (iii) the pattern of seedling development from seeds to mature plants.

## MATERIALS AND METHODS

A small-scale culture system for growing *H. ovalis* comprises 0.42 m x 0.42 m x 1.22 m glass aquarium, provided with 3-4 cm thick of substrate and flooded with 45 liters of 30 psu (practical salinity unit) of Instant Ocean synthetic seawater. The culture system is fitted with an external filter system and a submersible pump to provide filtration and circulation of water inside the aquarium. The substrate was flooded with distilled water for a month to reduce the nutrients in the *H. ovalis* natural substrate before the actual experiments were performed. The aquarium was then filled with 45 liters of 30 psu (practical salinity unit) of Instant Ocean synthetic seawater. The Instant Ocean synthetic seawater provide the nutrients to sustain growth and development of *H. ovalis* (Fig. 1). Plugs of *H. ovalis* explants collected from Merambong shoal (Lat. 1° 19' 50.9" N, Long. 103° 36' 45.0" E), Johore were planted randomly in the aquarium covering approximately about 7% of the substrate's surface area. Experiments were performed separately in two types of substrate; *H. ovalis* native substrate of calcareous sandy mud from Merambong shoal and commercial artificial sand, each under separate light regimes of  $\sim 90 \mu\text{mol}/\text{m}^2/\text{sec}$  (9% of the ambient light in

the field) and  $\sim 200 \mu\text{mol}/\text{m}^2/\text{sec}$  (20% of the ambient light in the field) supplied by Phillips Natural Daylight lighting tubes with 12 hours of photoperiod. Water level in each of the aquarium was maintained by adding distilled water. To maintain the water clarity, the external filter was cleaned with distilled water bimonthly. The algal growths covering the substrate were removed manually when necessary. After six months, 25% by volume of the water was replaced to replenish the nutrients and to improve the water quality of the aquarium. Light, temperature, pH and salinity were recorded daily. Observations on the growth and development of *H. ovalis* population were recorded from February 2004 to May 2005.

## RESULTS AND DISCUSSION

### Observations on growth development and reproductive biology

Plugs of explants were successfully grown in natural substrate and under both light regimes of  $\sim 90 \mu\text{mol m}^{-2}\text{s}^{-1}$  and  $\sim 200 \mu\text{mol m}^{-2}\text{s}^{-1}$ . The sequence of events on the growth and development, and reproductive biology of *H. ovalis* plug (Fig. 2) to the formation of population (Fig. 3a) is described below. Better growth performance of plugs was observed when grown in natural substrate and under the light regime of  $\sim 200 \mu\text{mol m}^{-2}\text{s}^{-1}$ . By the first week after initial planting in February, plants in plugs began to multiply through vegetative propagation by producing new

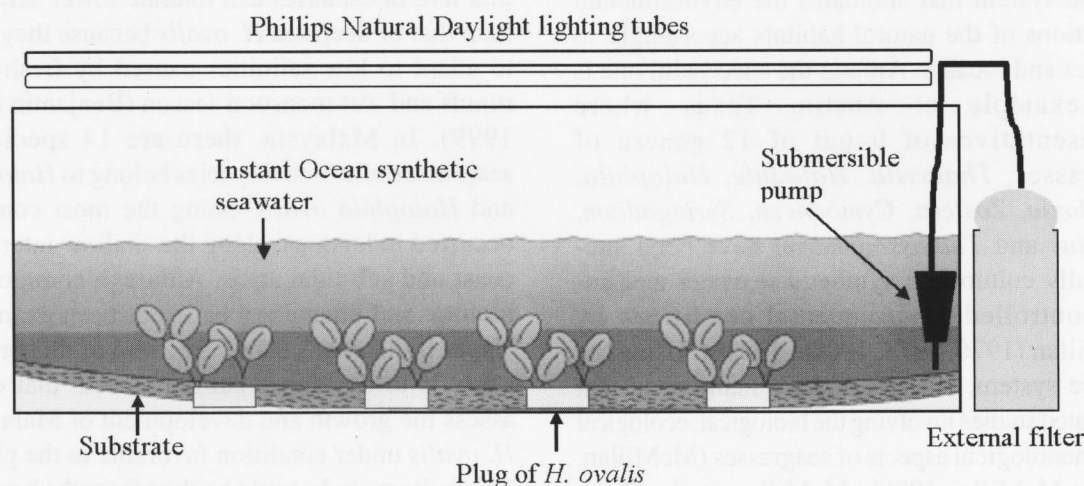


Figure 1. The culture system for growing of *Halophila ovalis* in the laboratory

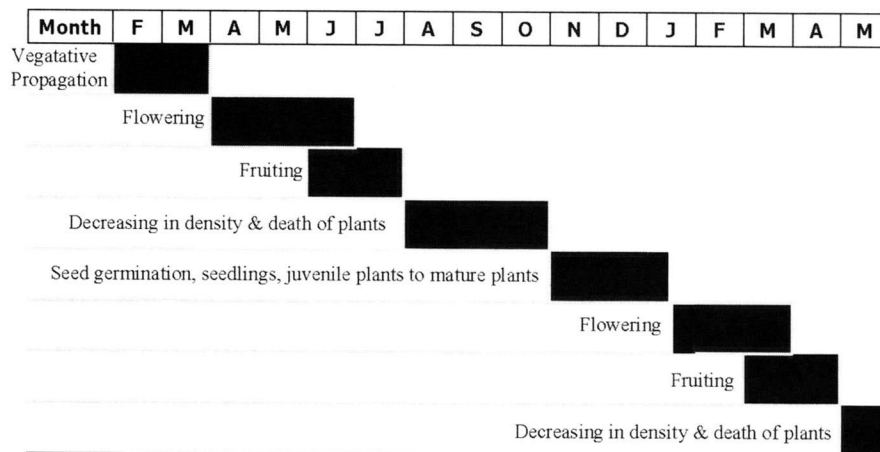


Figure 2. The growth development and reproductive biology of *H. ovalis* plug to the formation of population.

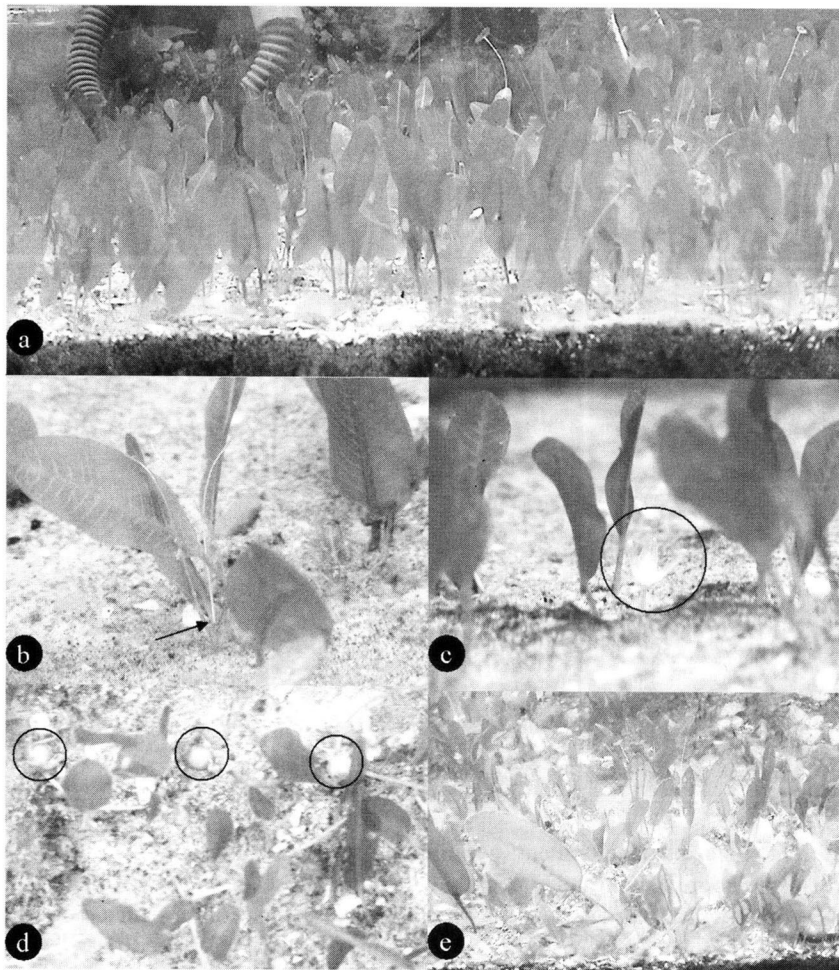
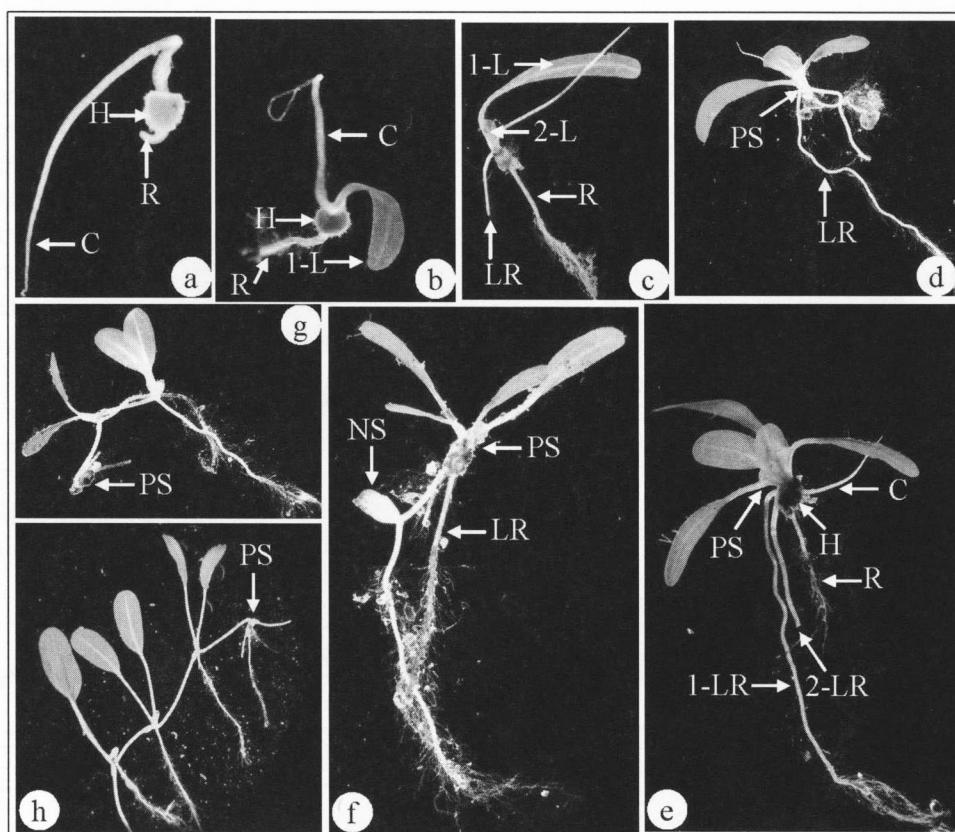


Figure 3. The various developmental stages of *H. ovalis* in culture. (a) plugs forming plant population are able to adapt and exhibit continuous growth development in natural substrate and under the ~20% of the ambient light in the field, (b) a shoot possessing a female flower as shown by arrow, (c) a shoot with an open male flower shown in circle, (d) successful pollination of female flowers produce fruits shown in circles and (d) after producing fruits, plants shading their leaves thus showing symptom of decreasing in shoot density.

shoots. A new shoot was produced after every two days. By the second months some plants propagated inside the plug areas, thus making the plugs became dense. As the plugs were grown randomly, vegetative propagation occurred all around the side of the plugs colonizing the bare adjacent substrate areas. After two months of planting, plants produced female (Fig. 3b) and male (Fig. 3c) flowers for three months from April to July. Fruits (Fig. 3d) were only detected in June to July. This event was followed by plants shading their leaves thus showing symptom of decreasing in shoot density (Fig. 3e) in August to September

and absence of living plants in October. Based on the above observations after eight months of the initial planting, the plants had covered almost 75-80% of the substrates surface area (Fig. 3a). This showed that plugs were able to adapt and exhibit continuous growth and development in the laboratory condition even under the 20% of the ambient light in the field. Sprouting of new plants occurred in early November with the germination of seeds forming seedlings. The presence of viable seeds and seedlings demonstrated the successful pollination and sexual reproduction of *H. ovalis* in the culture. New plants profusely propagate new



**Figure 4.** The different stages of development; germinating seeds, seedlings to juvenile plants. (a) germinating seed with a cotyledon, radicle and hypocotyl with hairs, (b) seedling with a pioneer shoot having a single leaf, (c) seedling with a pioneer shoot having two leaves, (d) seedling with a pioneer shoot having three leaves, (e) seedling with a pioneer shoot possessing five leaves, (f) seedling with a pioneer shoot that continued to propagate producing a new rhizome with a new developing shoot, (g) seedling with two shoots although the hypocotyl still intact but pioneer shoot leaves have dropped off and, (h) seedling still maintain shrunken hypocotyls bore a total of 3 shoots. The seedling at this stage is referred to as young or juvenile plant. Other than the seedling stage having more than a pair of leaves, a mature plant has a pair of leaves ( $23.5 \pm 0.18$  mm in length,  $13.8 \pm 0.11$  in width) at each rhizome node. C-cotyledon, H-hypocotyl, R-radicle, 1-L-first leaf of pioneer shoot, 2-L-second leaf of pioneer shoot, LR-lateral root, 1-LR-first lateral root, 2-LR-second lateral root, PS-pioneer shoot, NS-new shoot.

shoots in December and started to produce flowers from mid January onwards. Symptoms of plants shading their leaves and decreasing in shoot density started again in early March.

### Seedling morphology and development

The representative samples of germinating seeds, seedlings and juvenile plants from the culture were collected from early November to mid December. Seedlings exhibited progressive development as indicated by propagation of rhizomes and the number of shoots produced, and development stages can be distinguished into eight stages as illustrated in Figure 4; (a) germinating seed with a cotyledon, radicle and hypocotyl with hairs, (b) seedling with a pioneer shoot having a single leaf, (c) seedling with a pioneer shoot having two leaves. The second leaf was just starting to develop. A lateral root with root hairs was formed opposite to base of the cotyledon, (d) seedling with a pioneer shoot having three leaves. The lateral root continued to elongate. Seedlings up to the protrusion of the third leaf essentially showed the same development as described for *H. spinulosa* (Birch, 1981), *H. engelmannii* (McMillan, 1987), *H. decipiens* (McMillan, 1988), *H. ovalis* (Kuo and Kirkman, 1992), *H. tricostata* (Kuo *et al.*, 1993) and *H. beccarii* (Muta Harah *et al.*, 2002), (e) seedling with a pioneer shoot possessing five leaves. In the case of this seedling, a second lateral root developed next to the first lateral root. The cotyledon still persisted up to this stage. In other *Halophila* species, pioneer shoot possessed six leaves in *H. decipiens* (McMillan, 1987) and *H. decipiens* (McMillan, 1988) and, eight leaves in *H. beccarii* (Muta Harah *et al.*, 2002), (f) seedling with a pioneer shoot that continued to propagate producing a new rhizome with a new developing shoot, (g) seedling with two shoots although the hypocotyl was intact but pioneer shoot leaves have dropped off and, (h) seedlings at this stage bore a total of three shoots. The hypocotyl though present was shrunken. The seedling at this stage is referred to as young or juvenile plant.

### CONCLUSIONS

A small-scale marine system had been successfully set up to simulate the natural environmental conditions of the habitat of *H.*

*ovalis*. The culture system established, permitted observations on growth and development, the phenological cycle and seedlings development from seeds to mature plants of *H. ovalis*. The vegetative propagation expands and maintains the *H. ovalis* population, and simultaneously, plants flower and fruit, disseminating seeds until they disappear. The presence of viable seeds and seedlings demonstrated the successful pollination and sexual reproduction of *H. ovalis* in a laboratory culture.

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