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**The Effect of Shoot Density on Growth, Recruitment and Reproduction of  
*Enhalus acoroides* (L.f.) Royle at Haad Chao Mai National Park,  
Trang Province, Thailand**

**Ekkalak Rattanachot**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of  
Master of Science in Ecology (International Program)**

**Prince of Songkla University**

**2008**

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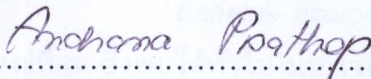
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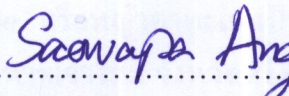
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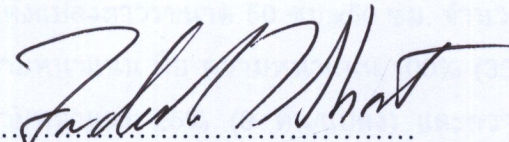
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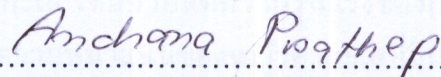
  
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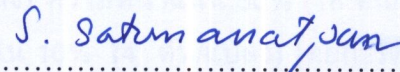
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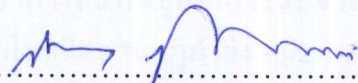
  
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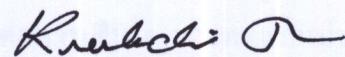
  
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ชื่อวิทยานิพนธ์	ผลของความหนาแน่นต่อการเติบโต การทดแทนประชากร และการสืบพันธุ์ ของหญ้าทะเลชนิด <i>Enhalus acoroides</i> (L.f.)
ผู้เขียน	นายเอกลักษณ์ รัตนโชติ
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### บทคัดย่อ

*Enhalus acoroides* (L. f.) Royle หรือหญ้าคาทะเล เป็นหญ้าทะเลที่มีขนาดใหญ่ที่สุด ซึ่งพบได้ทั่วไปในบริเวณอุทยานแห่งชาติหาดเจ้าไหม จังหวัดตรัง พบเจริญเติบโตเป็นแนวหญ้าทะเลชนิดเดียวที่มีความหนาแน่นสูงถึง  $141.0 \pm 8.7$  ต้น/ตร. ม. ดังนั้น อาจส่งผลให้เกิดการแข่งขันภายในชนิดและการบดบังแสงกันเอง อย่างไรก็ตาม ในบริเวณที่มีความหนาแน่นสูงอาจช่วยเพิ่มโอกาสในการถ่ายเรณูของหญ้าทะเล โดยช่วยลดความรุนแรงของกระแสน้ำ จุดประสงค์ของการวิจัยครั้งนี้ เพื่อศึกษาผลของความหนาแน่นของหญ้าคาทะเลที่มีผลต่อการเติบโต การทดแทนประชากร และการสืบพันธุ์ในแนวหญ้าคาทะเล ซึ่งทำการทดลองโดยการติดตั้งแปลงถาวรขนาด 50 ซม. x 50 ซม. จำนวน 10 แปลง ในแต่ละความหนาแน่น ซึ่งมีทั้งสิ้น 4 ความหนาแน่น คือ ความหนาแน่น 100% (35 ต้น/แปลง) ความหนาแน่น 50% (18 ต้น/แปลง) ความหนาแน่น 25% (9 ต้น/แปลง) และความหนาแน่น 10% (4 ต้น/แปลง) โดยการตัดส่วนเนื้อเยื่อเจริญกลุ่มใบออกไป จากนั้นทำการศึกษาการเจริญเติบโตโดยประยุกต์จากวิธีพลาสติกโครน (plastochrone technique) ศึกษาการทดแทนประชากร โดยการคำนวณอัตราการทดแทนประชากรจากสมการเอกซ์โพเนนเชียล และศึกษาการสืบพันธุ์โดยการนับจำนวนดอกและผลจากผลการศึกษา พบว่าไม่เกิดการแข่งขันขึ้นภายในชนิดตามกฎกำลัง  $-3/2$  ( $-3/2$  power rule) แต่มีความแตกต่างกันอย่างมีนัยสำคัญ ( $P < 0.05$ ) ของพื้นที่ผิวใบ, น้ำหนักเนื้อผิวดินของต้น, อัตราการทดแทนประชากร จำนวนดอกเพศเมีย และจำนวนดอกรวม โดยพบว่ามีค่าต่ำในชุดการทดลองที่มีความหนาแน่นสูง ดังนี้  $199.5 \pm 12.4$  ตร.ซม./ต้น,  $0.899 \pm 0.099$  ก. น้ำหนักแห้ง/ต้น,  $0.010 \pm 0.001$  ต้นใหม่/ต้น/วัน,  $0.001 \pm 0.001$  ดอกเพศเมีย/ต้นทั้งหมด และ  $0.002 \pm 0.001$  ดอก/ต้นทั้งหมด ตามลำดับ แต่มีเพียงดัชนีพื้นที่ผิวใบเท่านั้นที่มีค่าสูงในชุดการทดลองที่มีความหนาแน่นสูง ( $27.9 \pm 1.7$  ตร.ม. ใบ/ตร. ม.) จากผลการศึกษาชี้ให้เห็นว่า หญ้าคาทะเลได้รับผลกระทบจากการบดบังแสงกันเอง และมีความต้องการแสงในแนวหญ้าคาทะเลที่มีความหนาแน่นสูง ดังนั้น ความเข้มแสงอาจเป็นปัจจัยสำคัญต่อการเติบโต การทดแทนประชากร และการสืบพันธุ์ของหญ้าคาทะเล *E. acoroides*

<b>Thesis Title</b>	The Effect of Shoot Density on Growth, Recruitment and Reproduction of <i>Enhalus acoroides</i> (L.f.) Royle at Haad Chao Mai National Park, Trang Province, Thailand.
<b>Author</b>	Mr. Ekkalak Rattanachot
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## Abstract

*Enhalus acoroides* (L.f.) Royle is the largest species of seagrass common at Haad Chao Mai National Park, Trang Province. *E. acoroides* grows in monospecific meadows in a high density of  $141.0 \pm 8.7$  shoots/  $m^2$ , which may lead to intraspecific competition and self shading. However, a canopy in a dense meadow seems also to increase the chance of successive pollination by reducing the water motion. The aim of this research is to study the effect of shoot density of *E. acoroides* on growth, recruitment and reproduction in a monospecific meadow. The project consisted of a series of experiments carried out in an *E. acoroides* meadow by placing ten permanent quadrats (50 cmx50 cm) in each of four densities designated as follows: 100% density (35 shoots/quadrat), 50% density (18 shoots/quadrat), 25% density (9 shoots/quadrat) and 10% density (4 shoots/quadrat). The plants were manipulated by clipping the shoots at the leaf bundle meristem. The growth study was modified from the plastochrone technique. Recruitment was assessed by calculating the rate using an exponential equation. Reproduction potential was also assessed by analyzing the data of numbers of flowers and fruit. The results showed that intraspecific competition did not occur at this site according the  $-3/2$  power rule but that leaf surface area, above ground shoot weight, recruitment rate and female and total flower production were significantly different among densities ( $P < 0.05$ ). Only  $199.5 \pm 12.4$   $cm^2$ / shoot,  $0.899 \pm 0.099$  g dw/ shoot,  $0.010 \pm 0.001$  new shoots/ shoot/ day,  $0.001 \pm 0.001$  female flowers/ total shoot and  $0.002 \pm 0.001$  flowers/ total shoot were observed in high density treatments but the Leaf Area Index (LAI) was high in high density treatments at  $27.9 \pm 1.7$   $m^2$  leaves/  $m^2$ . The results suggested that *E. acoroides* was affected by



shelf shading and light availability in a dense meadow. Therefore, light intensity may be a crucial factor on *E. acoroides* growth, recruitment and reproduction.

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# CHAPTER 1

## INTRODUCTION

Seagrasses are a group of marine angiosperms adapted to grow and reproduce in the sea. Seagrass beds are an important coastal community because they provide food, habitat, and nursery grounds for many forms of marine life. Widely distributed in both tropical and temperate coastal regions (Green and Short, 2003), seagrasses are distributed from the high intertidal to deep water where light can penetrate to the sea bottom. For example, *Halophila decipiens* Ostenfeld has been reported from 15-58 m depth (Carruthers *et al.*, 2002). Seagrasses are clonal plants which reproduce asexually by means of branches that remain attached to the parent during their establishment (Hemminga and Duarte, 2000). Only clonal species are able to directly share resources by transporting water, carbohydrate and mineral nutrients between the basic units called ramets through connecting rhizomes (Aplert, 1999).

The largest, long-lived, dioecious seagrass *Enhalus acoroides* (L.f.) Royle is abundant in Southeast Asia seagrass meadows. In Thailand, this species is common in 11 provinces of both in the Gulf of Thailand (5 provinces) and Andaman sea (6 provinces) (Poovachiranon *et al.*, 2006). The largest (ca.18 km<sup>2</sup>) and perhaps most important seagrass bed of Thailand is in Haad Chao Mai National Park, Trang Province (Supanwanid and Lewmanomont, 2003). *E. acoroides* may be found in variable densities in monospecific and multispecific meadows (Terrados *et al.*, 1998). In a dense meadow intraspecific competition might occur and lead to reduction in survival, growth and reproduction because the plants require and compete for the same resources (i.e. light, nutrients, and space).

*Intraspecific competition has been investigated in terrestrial plants and interpreted by the self thinning or -3/2 power rule (Yoda et al., 1963), well known in plant competition studies that are analyzed by plotting the logarithm of plant density (plants/m<sup>2</sup>) against the logarithm of average plant dry weight (g/plant). The competitive interaction is determined by the slope of a straight line derived from this relationship which is -3/2 (Yoda et al., 1963). However direct experimentation to test*

intraspecific competition in seagrasses has not been carried out. Light availability is reduced by self shading in dense meadows. This undoubtedly affects the population dynamics (Dalla Via *et al.*, 1998; Collier, 2006). The indirect effect of high coverage suggests that there is greater opportunity for pollen to be trapped by the canopy, which increases reproductive output (Vermaat *et al.*, 2004). Therefore, seagrasses that grow in dense meadows might be at both a disadvantage (i.e. inhibit survival, growth and reproduction) and an advantage (increased pollination rates).

The study of interspecific competition in mutispecific seagrass meadows by Duarte *et al.* (2000) showed that when *Thalassia hemprichii* (Ehrenberg) Ascherson was removed, the density of *E. acoroides* decreased. This might due to oxygen release by the *T. hemprichii* roots which could improve the soil conditions. Thus, co-occurrence would benefit both species. On the other hand, little is known in this respect about intraspecific competition of seagrass, with respect to growth rate, reproduction and succession.

The results from intraspecific and interspecific competition, not only provides basic knowledge of the effects of density in a dense seagrass meadow but it also provides important information for seagrass management and restoration. For example, we could estimate the optimal seagrass density in order to avoid intraspecific competition at the beginning phase of transplantation or, what species should be transplanted or restored together.

The main purpose of this study is to investigate the effect of shoot density on growth, recruitment and reproduction of *E. acoroides* at Haad Chao Mai National park, Thailand.

## Review of literature

Classification of *Enhalus acoroides* (L.f) Royle following Phillips and Meñez (1998)

Kingdom	Plantae
Division	Anthophyta (Magnoliophyta)
Class	Monocotyledoneae
Order	Helobiae
Family	Hydrocharitaceae
Genus	<i>Enhalus</i>
Species	<i>Enhalus acoroides</i> (L.f.) Royle

## The characteristics of *Enhalus acoroides* (L.f.) Royle

*E. acoroides* is the only species of *Enhalus*, the largest tropical seagrass genus. Its leaves grow from 30 –150 cm in length, 1.25 – 1.75 cm width (Phillips and Meñez, 1998), have up to 30 longitudinal veins, and their apices are round. It can be identified by the enrolled or recurved edges of the leaves making one surface of the leaf feel as if it has a rim. The rhizome is ca. 1.5 cm in diameter, is surrounded by a dense, persistent fibrous stand of decayed leaves and has numerous pale colored and unbranched roots 10 – 20 cm long, 3 – 5 mm wide (Figure 1).

## The ecology of *Enhalus acoroides* (L.f.) Royle

*E. acoroides* usually occurs in large sheltered bays or areas sheltered by mangrove stands and forms extensive dense meadows with a closed canopy providing an important habitat for other species. It is found on sandy and muddy bottoms and grows best just above the level of mean low water and but also grows to ca. 4 m depth. Flowering occurs where plants are uncovered briefly during spring low tides or where the flowers can reach the water surface (Phillips and Meñez, 1998). It grows in mixed meadows with other tropical seagrasses and also forms single species beds (Terrados *et al.*, 1998). Numerous animals inhabit the rhizome mat that this seagrass forms. The long leaves of this species are usually obvious in the shallow waters it inhabits (Waycott *et al.*, 2004).

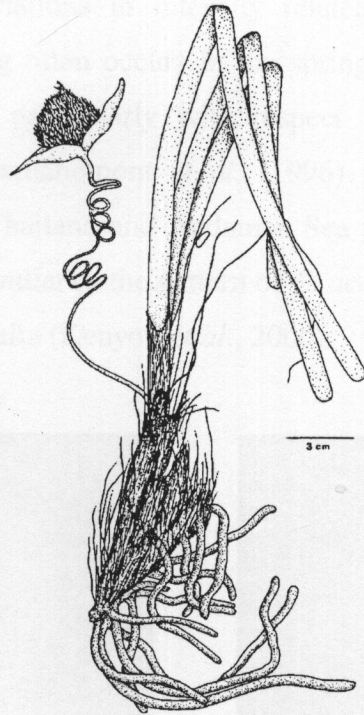


Figure 1. *Enhalus acoroides* (L.f) Royle with fruit (Phillips and Meñez, 1998)

### The reproduction in *Enhalus acoroides* (L.f.) Royle

Seagrasses are the only flowering plants that grow submerged in the marine habitat. Almost all seagrasses have underwater pollination and use the flower itself for pollen dispersal. *E. acoroides* is the only species of seagrass that has water surface pollination, occurring on ebbing or low tides. It has separate male and female plants (dioecious). The female flower forms on a peduncle 40–50 cm long, coiled and contracted after anthesis (Figure 3A and 3B), while the male flower forms on a peduncle 5–10 cm long (Figure 2A), is submerged and produces spherical pollen grains ca. 175  $\mu\text{m}$  in diameter (Figure 2C). Male flowers are released and float to the water surface (Figure 2B) and the female flower is raised to surface on its stalk and opens forming a cup into which male flowers become trapped and the pollen is then released. A large fruit (5–10 cm long) forms on the end of the stalk with a spiky texture on the outside (Figure 3C). Each fruit contains several seeds that germinate on release (Figure 3D); no seed bank is maintained (Waycott *et al.*, 2004).

The extent and timing of flowering in seagrasses worldwide is variable both between species and location. In general, flowering in the tropics is a year round



phenomenon but with variations in intensity related to location. In contrast, in temperate regions flowering often occurs in the spring, but the timing of the whole reproductive cycle varies, particularly with respect to both species and location (Walker *et al.*, 2001). Lewmanomont *et al.* (1996) reported that flowering of *E. acoroides* in The Gulf of Thailand and Andaman Sea produced flowers and formed fruits throughout the year similar to the pattern of *E. acoroides* growing in the western Gulf of Carpentaria, Australia (Kenyon *et al.*, 2000).

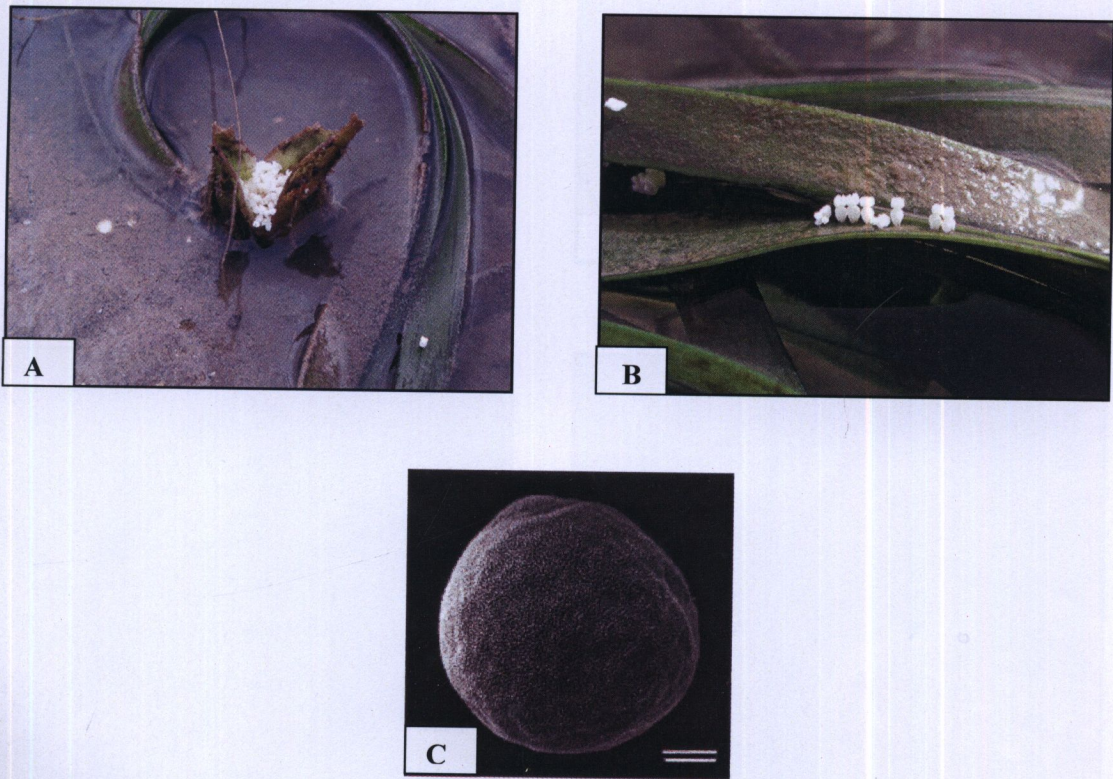


Figure 2. (A) inflorescence of male flowers , (B) male flowers after released, (C) pollen grain; bar = 25  $\mu\text{m}$  (Tanaka *et al.*, 2004).



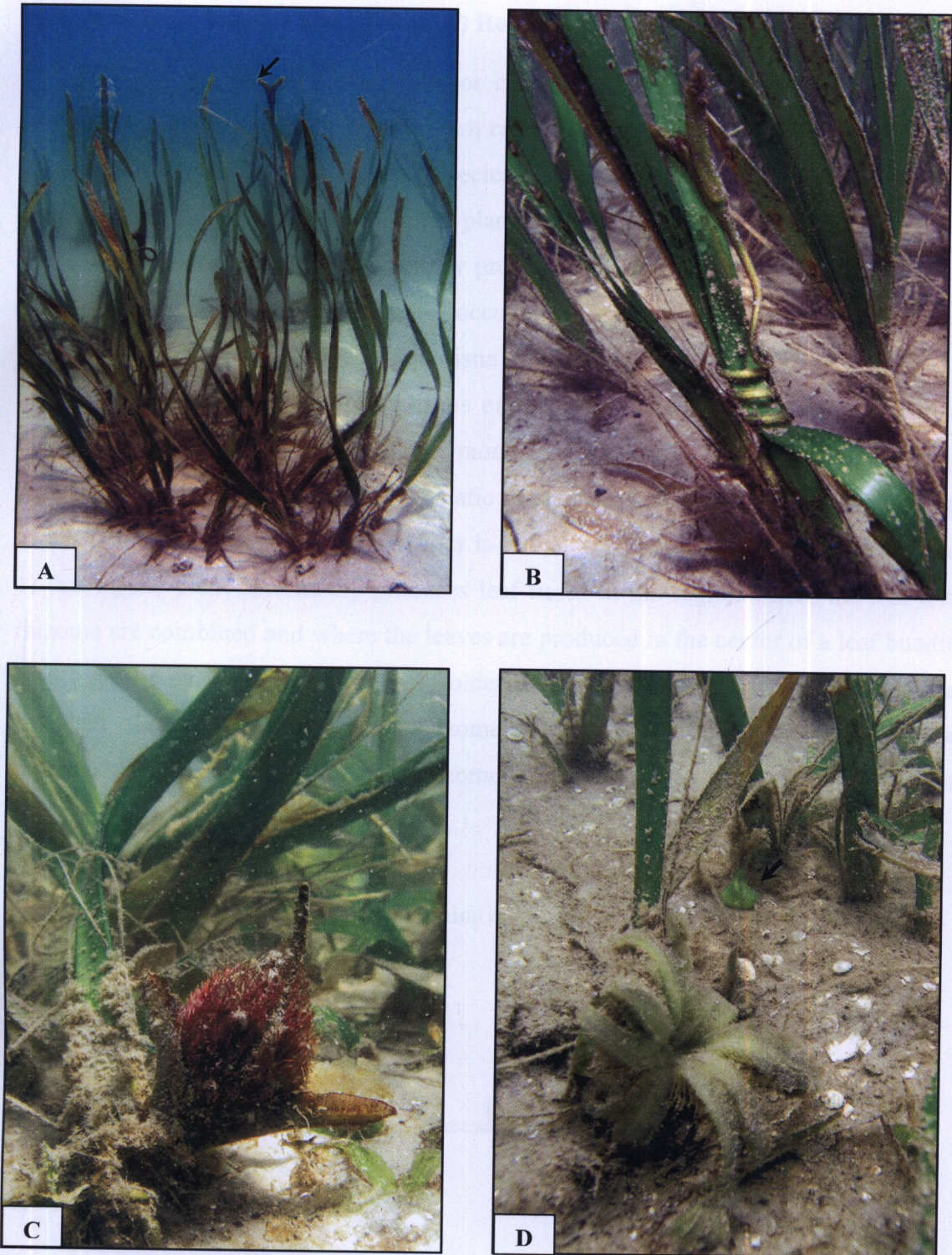


Figure 3. (A) female flower (arrow) before fertilized, (B) female flower after fertilized (C) fruit and (D) fruit after release seeds and released seed (arrow).



### The growth of *Enhalus acoroides* (L.f.) Royle

Seagrasses are modular or clonal plants. Each clone is composed of many units called ramets. In general each ramet of a seagrass is composed of 3 main parts which vary somewhat between species: leaves, rhizome (stem), both horizontal and vertical, and roots. The modular plant can reproduce asexually by means of branches of the rhizome and continually produce new shoots. The production of new shoots is an important factor for space occupation, especially during the colonization of new habitats on their recovery from disturbance (Hemminga and Duarte, 2000).

There are 4 growth forms of seagrasses based on generation within a leaf cluster (Short and Duarte, 2000): mono-meristematic and di-meristematic leaf-replacing forms, and mono-meristematic and di-meristematic non-leaf-replacing forms. The growth form of *E. acoroides* is mono-meristematic leaf-replacing (Figure 4) that is, the plant continually produces leaf tissue at the region where the leaf and rhizome are combined and where the leaves are produced in the center of a leaf bundle held together by the sheath portion of older leaves. Every time the plant produces a new leaf, the production of a new rhizome segment occurs. New lateral shoots are produced on alternate sides of the rhizome and grow away from the rhizome of the terminal shoot.

Mono-meristematic Leaf-replacing

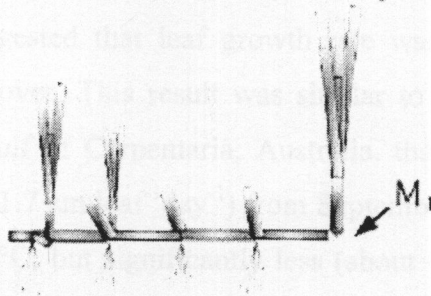


Figure 4. Rhizome fragment and shoots of mono-meristematic leaf-replacing growth form,  $M_1$  indicates the meristem area of growth. (Short and Duarte, 2001).

### *Enhalus acoroides* (L.f.) Royle studies

*E. acoroides* is widespread from the east coast of Africa to northern Australia (Phillips and Meñez, 1998; Waycott *et al.*, 2004). In Thailand the species is found both along the coasts of the Gulf of Thailand and the Andaman Sea (Lewmanomont *et al.*, 1996). It is present in both monospecific and multispecific meadows. It is a robust seagrass and is the only seagrass species which grows in sediments with high silt and clay content (Terrados *et al.*, 1998; Gacia *et al.*, 2003) and maintains a normal shoot density when there is high sediment deposition (Duarte *et al.*, 1997).

Agawin *et al.* (2001) studied temporal changes in the abundance, growth and photosynthesis of three co-occurring seagrasses (including *E. acoroides*) at Cape Bolinao, Northwest Philippines. They found that *E. acoroides* shoot density was  $20.1 \pm 2.6$  shoots  $\text{m}^{-2}$ , leaf biomass was  $15.5 \pm 2.4$  g DW  $\text{m}^{-2}$ , leaf growth was  $3.78 \pm 0.37$   $\text{cm}^2$  shoot $^{-1}$  day $^{-1}$  (or approx. 1.94 cm. shoot $^{-1}$  day $^{-1}$ ), leaf appearance rate was  $0.030 \pm 0.004$  leaves shoot $^{-1}$  day $^{-1}$  (the estimated annual leaf appearance rate was  $12 \pm 1.5$  leaves shoot $^{-1}$  day $^{-1}$ ), leaf area was  $124.5 \pm 11.5$   $\text{cm}^2$  shoot $^{-1}$ , maximum photosynthetic rate was  $4.08 \pm 0.62$  mg O<sub>2</sub> g DW $^{-1}$  h $^{-1}$ . Photosynthetic efficiency was  $0.06 \pm 0.02$  mg O<sub>2</sub> g DW $^{-1}$  hr $^{-1}$  /  $\mu$  mol photons  $\text{m}^{-2}$  s $^{-1}$ , respiration rate was  $0.74 \pm 0.31$  mg O<sub>2</sub> g DW $^{-1}$  h $^{-1}$  and Chlorophyll content a+b was  $4.19 \pm 1.04$  mg g DW $^{-1}$ . *E. acoroides* leaf growth was highest in late September and lowest between November and February. They suggested that leaf growth rate was positively correlated with temperature and cloud cover. This result was similar to the study of Kenyon *et al.* (1997) at the western Gulf of Carpentaria, Australia, that showed *E. acoroides* leaf growth was highest (1.3–1.7 cm leaf $^{-1}$  day $^{-1}$ ) from September to March, when the water temperature exceeded 30°C, but significantly less (about 0.6 cm. leaf $^{-1}$  day $^{-1}$ ) in June and July, when water temperatures were 23–24°C. In addition, *E. acoroides* showed high shoot density between August and October, positively correlated with air temperature, water turbulence and total daily photosynthetically active radiation (PAR). They suggested that water turbulence induces mixing of the water column and reduction of the water boundary layer around the plant. Therefore, the CO<sub>2</sub> and nutrient uptake rate at the leaf surface increased and promoted the vegetative development of *E. acoroides*.

## The studies of plant competition

Competition is an interaction between individuals that live together and share the requirements for resources that lead to reduction in growth, survivorship, and reproduction (Begon *et al.*, 1996). Competition has been divided into 2 types: intraspecific or the competition between individuals of the same species and interspecific competition between individuals of different species (Begon *et al.*, 1996). Grime (2001) defined the competition of plants as the tendency of neighboring plants to utilize the same quantum of light, ions of mineral nutrients, molecules of water, or volume of space concluding that these are the important resources for plant survival.

Competition in plants is a little different than it is many animals. Many plants are clones, so if some stems die, others of the same individual may live on. For this reason, competition in plants is usually examined by studying the change in biomass of competitors, rather than their numbers. Being rooted in the ground, plants may suffer severe competition for water, nutrients, or light because they cannot pick up their roots and escape from competitors. In 1963, Yoda described competition between plants by a  $-3/2$  power rule, sometimes called Yoda's law or the self-thinning rule (Begon *et al.*, 1996). The rule describes the rate at which the biomass of individual plants increases as the number of plant competitors decrease mathematically:

$$\text{Log } w = -3/2 (\log N) + \log c$$

Or

$$w = c N^{3/2}$$

Where  $w$  is the mean plant weight (grams/plant),  $N$  is the plant density (per  $\text{m}^2$ ), and  $c$  is a constant.

As plants grow, they require more resources. If resources remain constant, then some plants die or are outcompeted, while the survivors continue to grow. White (1980) showed how information from 31 data sets supported the  $-3/2$  power rule (Figure 5). Plant weight increased faster than density decreased, and a

steady increase in biomass resulted. Of course, this increase is not infinite, and the slope changes to  $-1$  when the maximum growth of species in the environment has been reached (Smith and Smith, 2001).

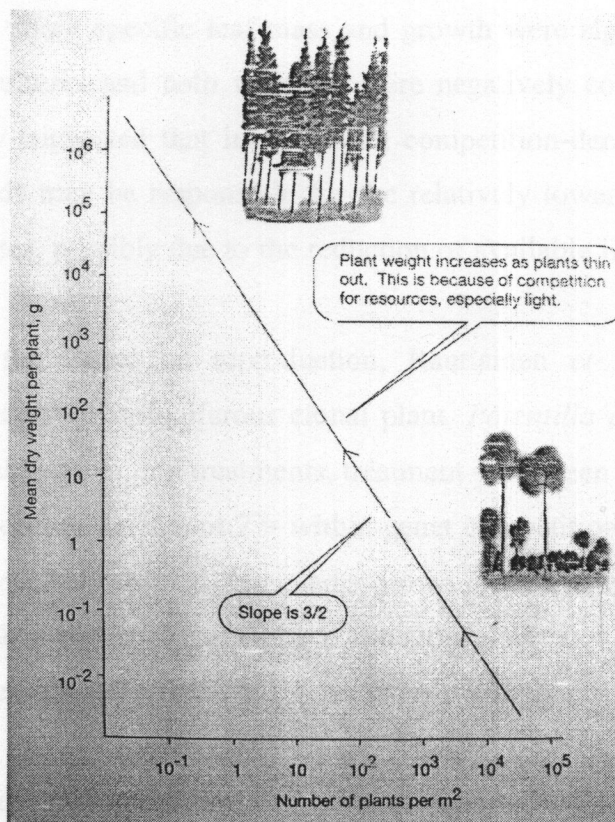


Figure 5.  $-3/2$  power rule in plants as a result of intraspecific competition (Smith and Smith, 2001).

In 2005, Wang *et al.* grew the clonal salt marsh species *Atriplex prostrata* Boucher in different densities (2, 4, 8 and 16 plant pot<sup>-1</sup>) and found that dry mass production decreased significantly in the higher density treatment. However, self-thinning was not observed at the highest density. It is possible that the densities examined were not high enough for self thinning to occur.

Viejo and Åberg (2001) studied the effects of density on modules of the brown seaweed *Ascophyllum nodosum* (L.) Le Jol in nature between 2 sites (Tjärnö and Isle of Man) by manipulating both increased and decreased density. The results showed that the relative change in dry mass/plot in low density plots were higher than in mid and high density plots at both sites. In the same studies, Viejo and Åberg



(2001), found that the number of new shoots (new recruits) at the Isle of Man was higher in low density than at mid and high density.

Rose and Dawes (1999) studied the effect of intraspecific competition on the seagrass *Thalassia testudinum* Banks ex König at Cockroach Bay, Florida. The results showed that shoot specific leaf mass and growth were significantly higher in low density monocultures and both variables were negatively correlated with short-shoot density. They suggested that intraspecific, competition-density effects in high density seagrass beds may be responsible for the relatively lower shoot specific leaf mass and growth rates, possibly due to the reduction of available light from dense leaf canopies.

For the effect on reproduction, Rautiainen *et al.* (2004) studied competition in a perennial stoloniferous clonal plant, *Potentilla anserine* (L.) Rydb. spp. *Egedii* (Wormsk) grown in 3 treatments, treatment 1 = between genet (a genetically unified plant) competition, treatment 2 = within-genet competition, and treatment 3 = no competition. The result showed that plants grown alone (treatment 3) performed consistently better than the plants in either competition treatment. Both biomass and the number of flowers also decreased in the presence of competition (treatments 1 and 2).

However, the opposite results were reported by Van Kleunen *et al.*, (2001) in the study of the reproductive allocation in a clonal plant *Ranunculus reptans* L. The results showed that sexual reproduction (proportion of flowering rosettes) was higher when the population of *R. reptans* grew in the high density treatment than in the low density treatment. In addition, seed mass and germination percentages were also higher for seeds of *R. reptans* produced in the high density treatment than for seeds produced in the low density treatment.

In addition, there is only one published study on meadow fragmentation and reproductive output of *E. acoroides* which was done in the Philippines (Vermatt *et al.*, 2004). The results showed that the female flowers of *E. acoroides* that developed to fruits increased sharply as overall seagrass cover at around 50%. They suggested that a possible mechanism was the increase in efficiency of trapping the pollen with increasing canopy density.

Duarte *et al.* (2000) in their study of interspecific competition of Southeast Asian seagrasses found that when *Thalassia hemprichii* was removed from plots of *E. acoroides* the density of *E. acorodes* decreased. They suggested that this negative response might involve the release of oxygen by the roots of *Thalassia* which enhanced the growth of *E. acorodes*. So, co-occurrence of seagrass species might benefit both.

### Research question

1. Is there intraspecific competition in the *E. acoroides* meadow?
2. Do shoot densities of *E. acoroides* have an effect on their growth (leaf elongation rate, leaf plastochrone interval, number of leaves, new leaf production, leaf surface area and Leaf Area Index)? How?
3. Do shoot densities of *E. acoroides* have an effect on their recruitment rate How?
4. Do shoot densities of *E. acoroides* have an effect on their sexual reproduction (flower and fruit production)? How?

### Hypotheses

#### *Null hypotheses*

$H_0$  : There is no intraspecific competition in *E. acoroides* meadows

$H_{0A}$  : there is no relationship between log shoot density and log mean shoot weight and -3/2 power rule does not apply.

$H_{0B}$  : there is no significant difference between shoot density and growth variables (leaf elongation rate, leaf plastochrone interval, number of leaves, new leaf production, leaf surface area and leaf area index).

$H_{0C}$  : there is no significant difference between recruitment rates between density treatments.

$H_{0D}$  : there is no significant difference between numbers of flowers and fruits.

### *Alternative hypotheses*

H<sub>0</sub>: There is intraspecific competition in *E. acoroides* meadow.

H<sub>0A</sub>: there is a relationship between log shoot density and log mean shoot weight and the -3/2 power rule applies.

H<sub>0B</sub>: there is a significant difference between shoot density and growth variables (leaf elongation rate, number of leaves, new leaf production, leaf surface area and leaf area index). The growth variables in high density treatments will be lower than in low density treatments.

H<sub>0C</sub>: there is a significant difference between recruitment rates. The recruitment rate in high density treatments will be lower than in low density treatments.

H<sub>0D</sub>: there is a significant difference between numbers of flowers and fruits. The number of male and female flowers and fruit in high density treatments will be lower than in low density treatments.

### **Objectives**

1. To investigate intraspecific competition in *E. acoroides* meadow at Haad Chao Mai National Park, Trang Province.
2. To study the effect of shoot density on growth in terms of leaf elongation rate, leaf plantochrone interval (PL), new leaf production, number of leaves, leaf surface area and Leaf Area Index (LAI) of *E. acoroides*.
3. To study the effect of shoot density on the recruitment rate of *E. acoroides*.
4. To study the effect of shoot density on sexual reproduction in *E. acoroides*.

## CHAPTER 2

### MATERIALS AND METHODS

#### Study site

The study was carried out at Laem Yong Lam (7° 23' N, 99°20' E) in Haad Chao Mai National Park, where the largest seagrass bed in Thailand covers an area of 18.0 square kilometers. Nine of the twelve species reported from Thailand, the highest diversity of seagrasses (Supanwanid and Lewmanomont, 2003; Lewmanomont and Supanwanid, 2000; Nakaoka and Supanwanid, 2000), may be found there. Laem Yong Lam is located on the coastline of Trang Province, Southwest of Thailand and connected with the Andaman Sea, Indian Ocean (Figure 6). Kuan Tung Ku estuary is nearby the study area, about 2 kilometers away, allowing high nutrient input into the site. Mook Island, situated on the opposite side, is the site of local fishery villages, resorts and travel activity. However only a little human activity was observed at the study site. This region is affected by the monsoon wind. There are two dominant seasons: a monsoon season dominated by the Southwest Monsoon (May to October) and the dry season dominated by the Northeast Monsoon (November to April).

Seagrass beds at Laem Yong Lam are distributed from the intertidal zone where they are exposed to air during at least some low tides, to the subtidal zone where the meadows are submerged all the time. In this study area, many seagrass species are found but the dominant species are *Halophila ovalis* (R. Brown) Hooker f., *Thalassia hemprichii* (Ehrenberg) Ascherson and *Enhalus acoroides* (L.f.) Royle. The plants grow on the clayey sand with shell substrate. There is zonation of the seagrass species. For example, near shore *H. ovalis* is the dominant species while *E. acoroides* is dominant around 300 m from the shore. *E. acoroides* is found both in monospecific meadows and multispecific meadows in this area (mostly with *H. ovalis* and *T. hemprichii*). This experiment was carried out in a monospecific meadow of *E. acoroides* in the intertidal zone (Figure 7).



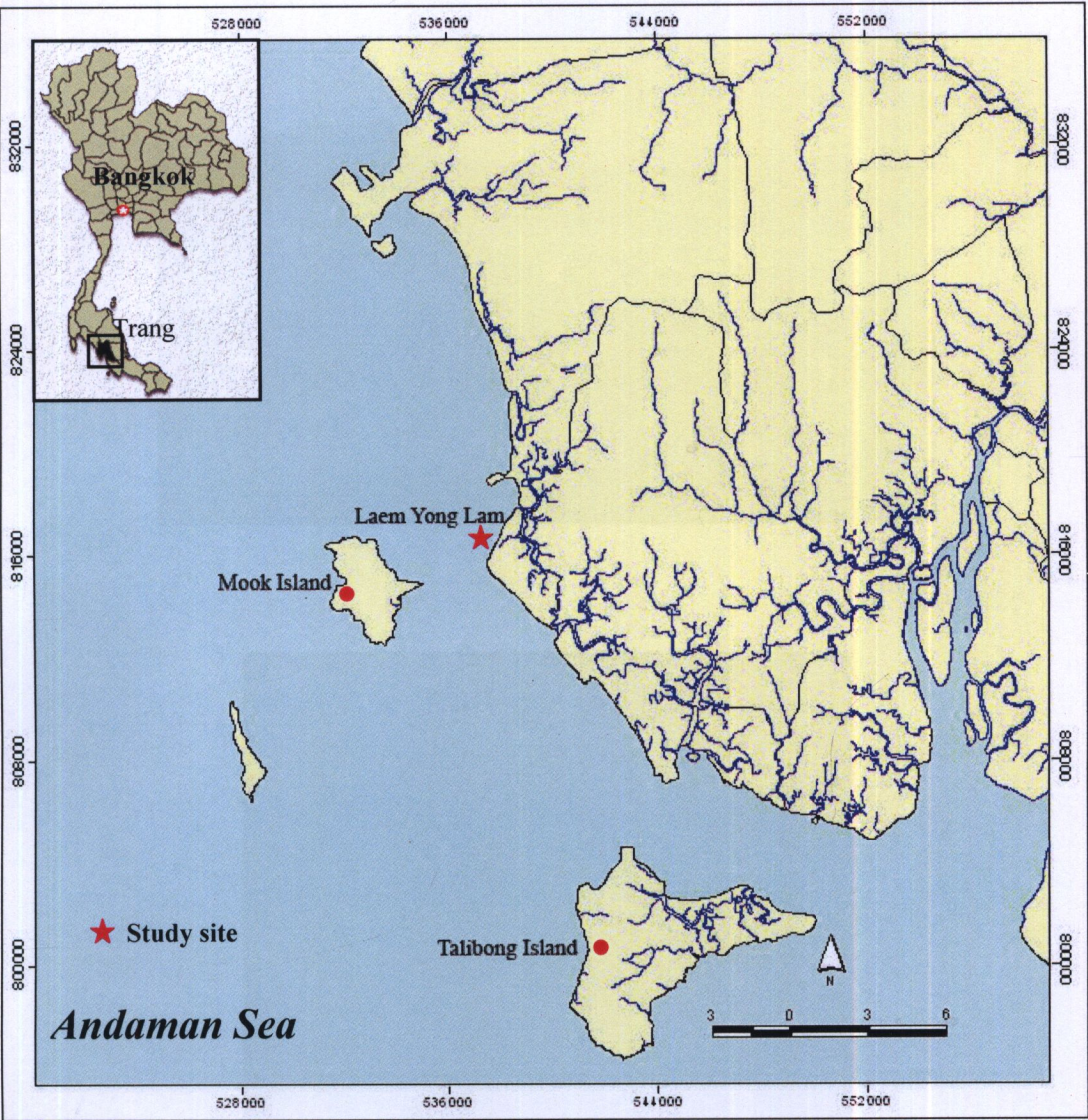


Figure 6. Map of the study site at Laem Yong Lam, Trang Province, Thailand



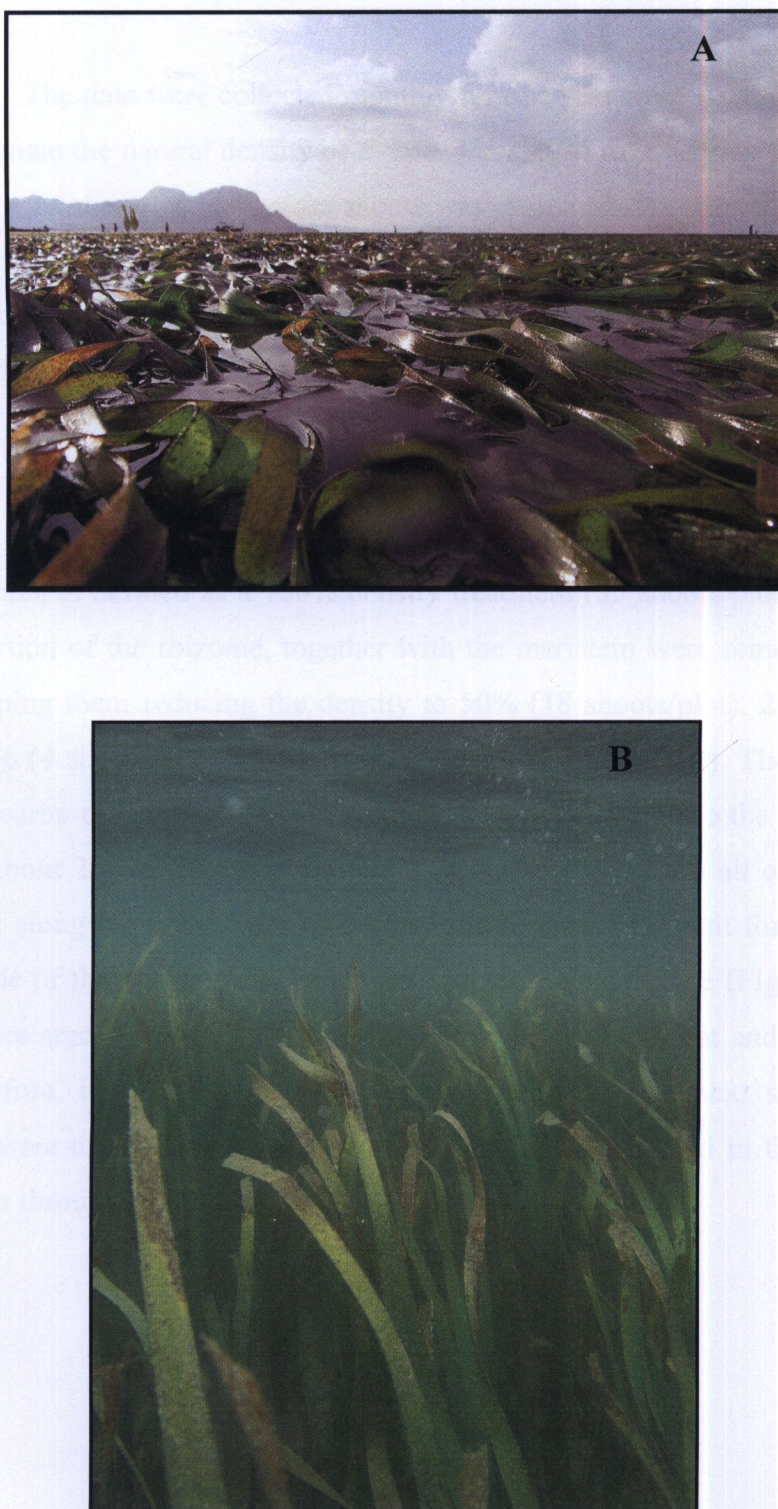


Figure 7. *E. acoroides* meadow at study site (A) low tide (B) high tide.

## Methods

The data were collected monthly for one year from August 2006 to July 2007. To estimate the natural density of *E. acoroides*, 0.25 m<sup>2</sup> quadrats were randomly placed and the number of *E. acoroides* shoots was estimated. Preliminary observations showed that the natural shoot density of *E. acoroides* at Laem Yong Lam was  $35.2 \pm 2.2$  shoots/quadrat (or  $141.0 \pm 8.7$  shoots/m<sup>2</sup>, Mean  $\pm$  SE). Forty permanent quadrats (4 treatments x 10 replicates) of 0.5 m x 0.5 m made from PVC pipe (~1.5 cm in diameter) were placed over a visually homogenous section in an area of similar density (about 35 shoots/plot) at a distance of at least 2 m from each other (Figure 9).

The experiment was composed of 4 treatments including a control treatment, which is defined as a 100% density treatment (35 shoots/plot). Leaves and the upper portion of the rhizome, together with the meristem were removed from the plots by clipping them reducing the density to 50% (18 shoots/plot), 25% (9 shoots/plot) and 10% (4 shoots/plot), respectively (Figures 10-Figures 13). Then the density of seagrass nearby the quadrat was reduced to the density similar to the density inside the plot for about 25 cm, which is defined as a buffer zone. Then all of the seagrass was removed along the boundaries of buffer zone of each treatment for about 25 cm from each side of the buffer zone, which is defined as a clear zone (Figures 8). After that poles were stuck into the bottom at the corner of each quadrat and tied with the buoys. Therefore, it was easy to re-locate the quadrat for the next sampling. The experiments were divided into 3 sections, which were investigated in the field using the same plots throughout the study.

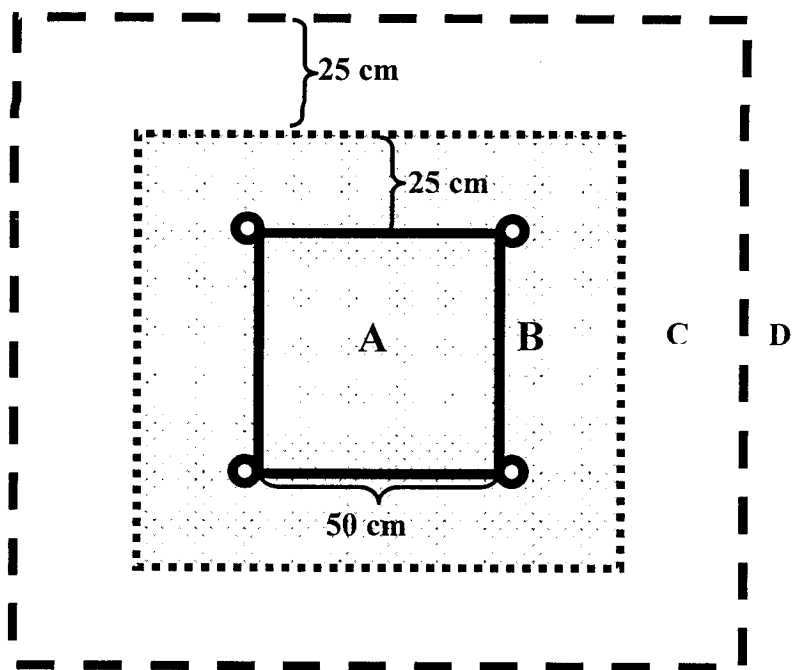


Figure 8. Diagram of the study plot, A; experimental area (manipulated density), B; buffer zone (density similar to area A) C; Cleared zone (without seagrass), D; natural density (without manipulation), solid line; boundary of quadrat, dotted line; buffer zone boundary, dash line; clear zone boundary and circle; poles.

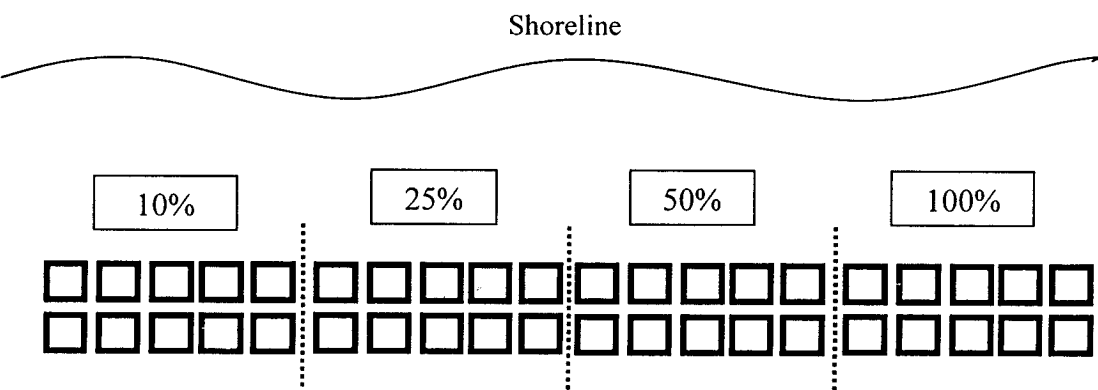


Figure 9. Diagram of placement the forty study plots (the space between plots was at least 2 m). The experiment was setup around 300 m from the shore.



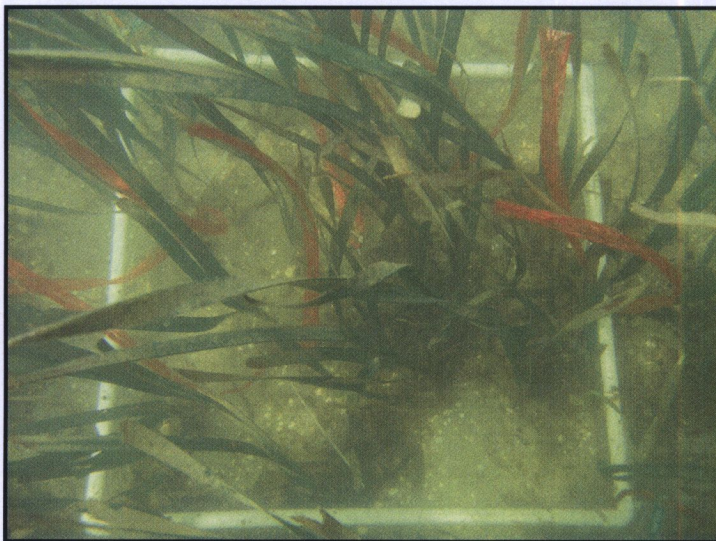


Figure 10. 100% density plots (35 shoots/plot), defined as natural density

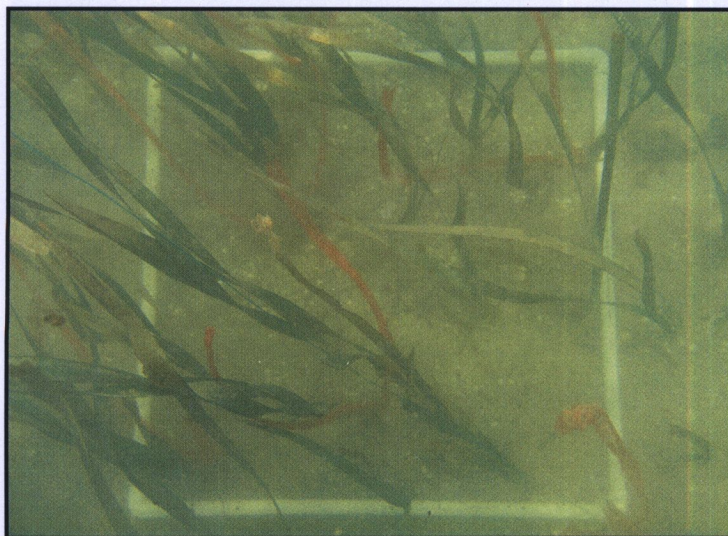


Figure 11. 50% density plots (18 shoots/plot)



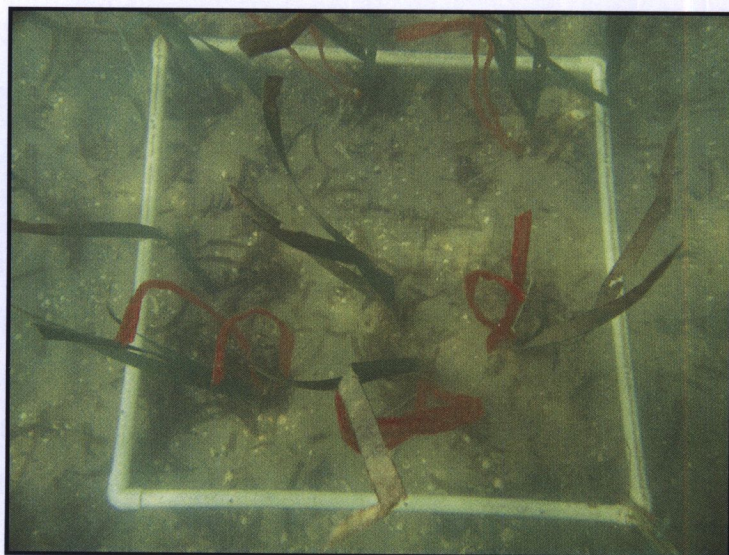


Figure 12. 50% density plots (9 shoots/plot)



Figure 13. 10% density plots (4 shoots/plot)

## 1. Growth study

Measurements of leaf elongation to estimate the growth of *E. acoroide* were accomplished by a modification of the plastochrone technique (Short and Duarte, 2001). Ten permanent plots were created in each treatment, then 3-5 shoots were marked in each treatment by making two big pin holes (approx. 0.3 mm diameter) through the bundle of leaves at the 5 and 6 cm level above the top of the leaf sheath (or ligula) to create a scar on the leaf tissue, and the marked shoots were tied firmly but not too tightly with plastic cable tie. After 1 month, marked shoots were measured at in the field. The following variables were determined:

- New leaf production (number of new leaves/shoot) is the count of new leaves produced (leaves without leaf scars from pinning at the center of leaf bundle) since the original mark.
- Leaf plastochrone interval,  $P_L$  (days) is the number of days since marking began divided by new leaf production and is the time interval between initiation of new leaves.
- Leaf area ( $\text{cm}^2/\text{shoot}$ ) is the sum of every leaf area (length x width) within the shoot (3 shoots per plot were measured).
- Leaf elongation rate ( $\text{cm}/\text{shoot}/\text{day}$ ) is the leaf length divided by  $P_L$ .
- Number of leaves (number of leaves/shoot) is the total number of leaves within a shoot (3 shoots per plot were measured.)
- Leaf Area Index (LAI) ( $\text{m}^2 \text{ leaves}/\text{m}^2$ ) is the total leaf surface area ( $\text{m}^2$ ) divided by experimental area ( $\text{m}^2$ ) that was estimated by mean leaf surface area ( $\text{m}^2/\text{shoot}$ ) multiplied by shoot density in each treatment then divided by experimental area ( $0.25 \text{ m}^2$ )



Note:

Leaf length (cm) was measured using the youngest fully mature seagrass leaf (fully grown, often the second or third youngest) from the base to the leaf tip (broken or grazed leaf tips were not used ).

Table 1. Summary of variables in the growth study

Variables	
New leaf production, N	Number of new leaves within shoot
Leaf plastochrone Interval, P <sub>L</sub> (days)	Interval time/ N
Leaf elongation rate (cm/ shoot/ day)	Leaf length / P <sub>L</sub>
Leaf area (cm <sup>2</sup> /shoot)	(Leaf area) <sub>first leaf</sub> + (Leaf area) <sub>second leaf</sub> + (Leaf area) <sub>third leaf</sub> +.....+ (Leaf area) <sub>last leaf</sub>
Number of leaves (number of leaves/ shoot)	Total number of leaves/ shoot
Leaf Area Index, LAI (m <sup>2</sup> leaves/ m <sup>2</sup> )	(mean leaves surface area per shoot x shoot density)/quadrat area

At the end of the experiment, *E. acoroides* was removed from the plots (three plots from each treatment); then specimens were kept in dark, cool containers and transported to the laboratory. Then specimens were washed with fresh water, epiphytes were removed by a brush and razor blade. Above ground parts (leaves and leaf sheath) of each shoot were cut then dried at 70 °C until a constant weight was



obtained. After that they were weighed and calculated to the above substrate weight/shoot.

## 2. Recruitment study

The recruitment study was carried out in the growth study plots by counting the number of new shoots produced in each plot. All of the new shoots were cut and removed to maintain the density as in the first set up. The recruitment rate for each month was calculated using the exponential equation below (Short and Duarte, 2000), this study was continued for 1 year.

$$R = \frac{(\ln(N_0 + N_{new}) - \ln N_0)}{t}$$

$N_0$  is the number of shoots present at the beginning of each observation period,  $N_{new}$  is the number of new shoots that appeared during the observation period, and  $t$  is the duration of the observation period in days. The unit of  $R$  is number of new shoots/ shoot/ day or  $\text{day}^{-1}$

## 3. Reproduction study

The reproduction studies were done in the growth and recruitment study plots by counting both male and female flowers and fruiting shoots every month for 1 year. The results are presented as male flowers/female flowers and total flower production (number of flowers produced/ total shoot) and fruit production (number of fruits produced/ total shoot).

#### 4. Environmental measurements

Air and water temperature were measured. A salinity refractometer (ATC, 0-100 ppt, XHO RHS-10ATC, ATACO, China) was used to measure salinity. At the end of the experiment, light intensity within the canopy of *E. acoroides* was measured using a light meter (Li-Cor, LI-250A, LI-COR Inc., USA) with an under water  $4\pi$  sensor. The sensor measured the light intensity at approximately 10 cm above the substrate, 5 plots of each density were random measured at high tide around noon in July 2008. The exposure duration was calculated from the tide tables of the Hydrographic Department Royal Thai Navy (2006, 2007).

The data of ambient underwater light intensity in each month were provided by the Marine National Park Education Center, Trang Province (May 2006, June 2006, July 2006, October 2006, December 2006 and January 2007) and Tuntiprapas (personal communication) from January – March 2008. Light intensity was recorded by Hobo light data logger that was set near the study area at Laem Yong Lam, the data logger was recorded in Lumens per square foot ( $L/ft^2$ ) and the light intensity was analyzed only 12 hours during day time around 7 am. to 6 pm.

The data of sunshine period were provided by The Thai Meteorological Department (personal communication) from the Phuket International Airport which is the nearest station. The data are presented from January 2005 to October 2007, however some months are missing.

#### 5. Statistical Analyses

SPSS version 13.0 for Windows was used to analyze the data; significance levels of 95% were used. The data were mainly analyzed for both the short term observations, the monthly observations, and the long term observations which were the annual mean of each variable in order to understand the effect of shoot density in the long term.

The data (and data transformed with  $\text{Log}(X)$ ,  $\text{Log}(X+1)$  or square root( $X$ )) of the short term observations were tested for normal distribution by Levene's Test. If the data were normally distributed, a parametric one way repeated measures ANOVA was used to test for differences of within subjects (month) and between subject (density) and interaction. If not, a non-parametric Kruskal Wallis Test was

used to test for differences in months and density and the Friedman Test was employed to test the different of interaction of densities and months. In addition the Kruskal Wallis Test was also used to test the effect of month on environmental parameters.

The data from the long term observations were analyzed using one way analysis of variance (ANOVA) to test the effect of shoot density on variables in each study. Where the ANOVA showed significant treatment effects, a least significant difference test (LSD) was used for pairwise comparison of treatments ( $P < 0.05$ ). Linear regression was employed to test the relationship between LAI and light intensity and shoot density and above ground weight.

## CHAPTER 3

### RESULTS

Leam Yong Lam, located in the area of Haad Chao Mai National Park, is the site of large seagrass bed of Thailand. There are several seagrass species in this area from the small species *Halophila ovalis* R. Br. Hook f. to the large species *Enhalus acoroides* (L.f.) Royle. At Leam Yong Lam, monospecific meadows of *E. acoroides* were normally found at the outer edge of the seagrass bed where they grow in a high density of ca.  $141.0 \pm 8.7$  shoots/m<sup>2</sup>.

A rich diversity of marine organisms are found in this area. For example, Goby fish together with snapping shrimp nest at the bottom, pen shells, swimming crabs among others are characteristic. Dugongs (*Dugong dogon*) inhabit the area also.

#### Environmental parameters

There were significant differences in air temperature, water temperature and salinity between months ( $P < 0.05$ , Table 2). The average air temperature, water temperature and salinity were  $29.1 \pm 0.8$  °C,  $30.2 \pm 0.6$  °C and  $32.6 \pm 0.5$  ‰ respectively (Figure 14 and Figure 15). Low temperature were occurred from August 2006 to February 2007 and high temperature from March to July 2007.

Table 2. The differences in environmental parameter between months.

Source of variation			
Between subjects	$\chi^2$	d.f.	P
Months			
Air Temperature	29.422	11	0.002
Water temperature	29.154	11	0.002
Salinity	23.932	11	0.013

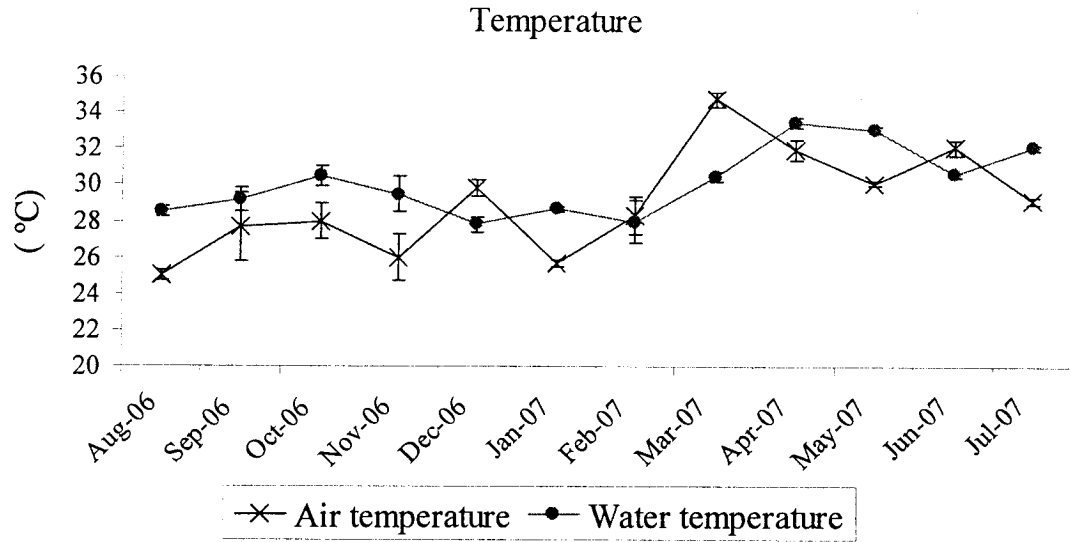


Figure 14. Seasonal changes in temperature from August 2006 to July 2007.



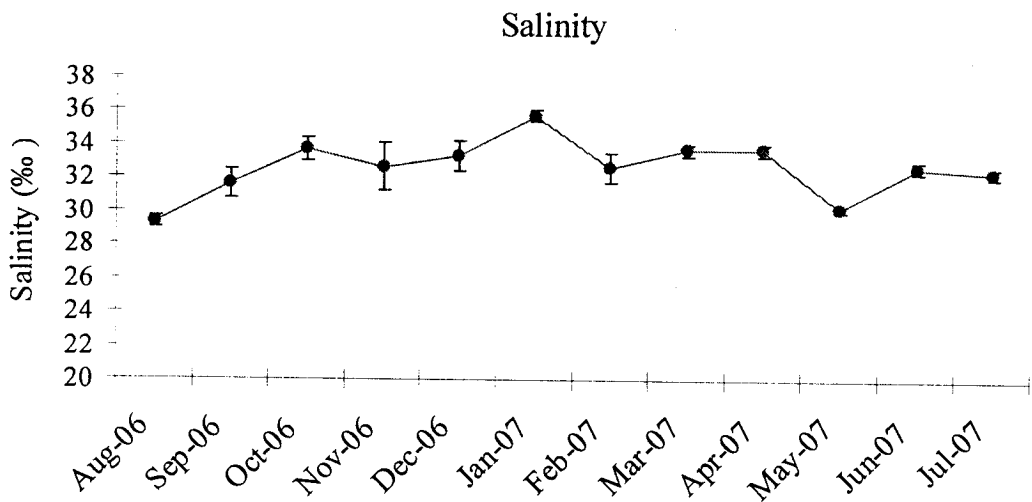


Figure 15. Seasonal changes in salinity from August 2006 to July 2007.

**Exposure time**

The tide at the study area is semi-diurnal tide, with the low tides normally occurring in the morning and evening during spring tides. There was variation in exposure time throughout the year. The long exposure hours were found in dry season from January to April 2007, the longest exposure time was found in February 2007 ( $4.7 \pm 0.3$  hrs./day). The short exposure hours were found in monsoon season from August to November 2006 and June to July 2008, the shortest exposure time was found in June 2007 ( $1.0 \pm 0.0$  hr./day) (Figure 16).

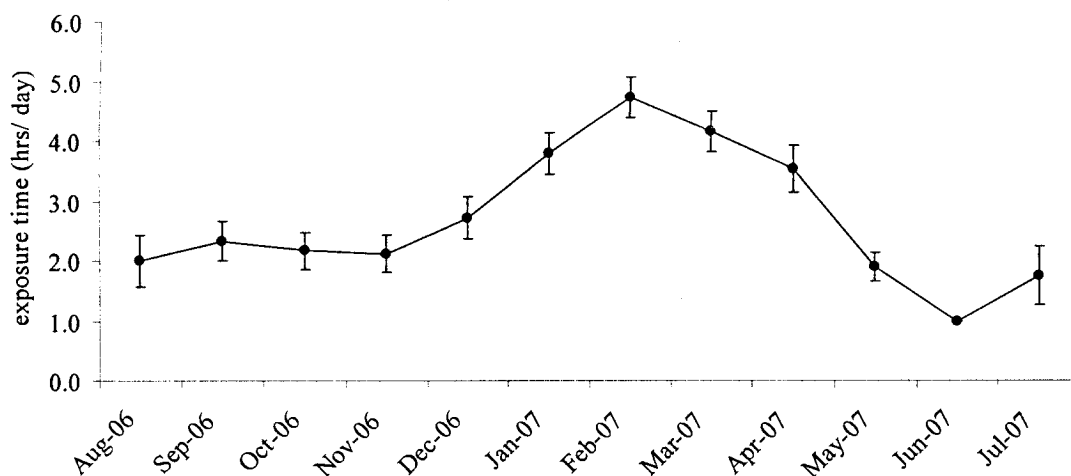


Figure 16. Seasonal changes in exposure time from August 2006 to July 2007.

#### Ambient underwater light intensity

Light intensity show similar patterns from May 2006 to March 2008. During the study period the light intensity was lowest in October 2006 ( $9.62 \pm 2.10$   $L/f^2$ ) and highest in January 2008 ( $62.30 \pm 3.90$   $L/f^2$ ). The same trend of light intensity was found, high light intensity occurred during January-March 2008 ( $59.29 \pm 4.99$   $L/f^2$  to  $85.01 \pm 5.24$   $L/f^2$ ) while low light intensity occurred during May – June 2006 ( $10.03 \pm 5.84$   $L/f^2$  to  $55.24 \pm 6.02$   $L/f^2$ ) (Figure 17).

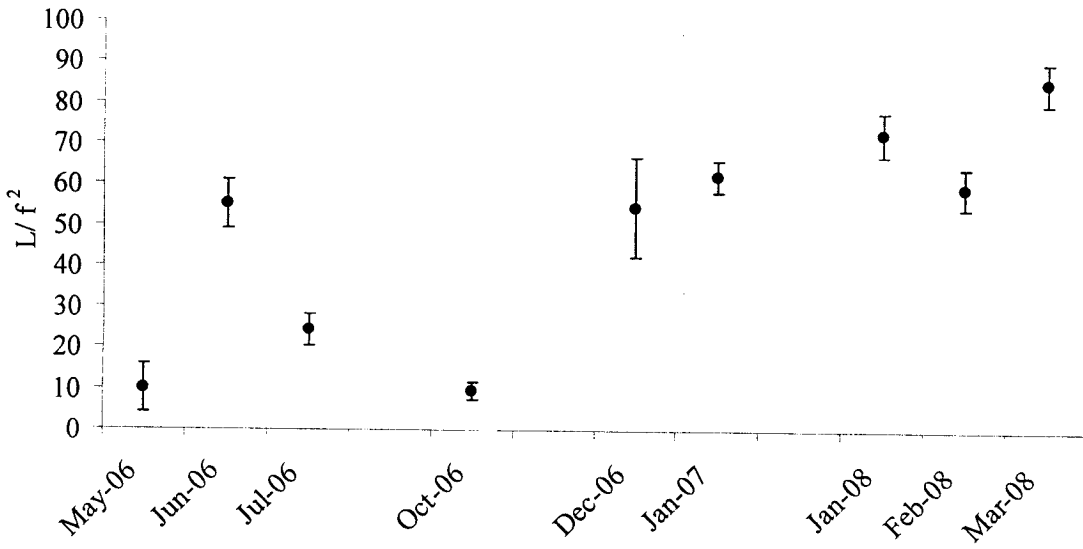


Figure 17. Ambient underwater light intensity from May 2006 to March 2008.

### Sunshine period

Although there were no data of the sunshine period during the study year the data from 2 previous years showed similar trends. The longest sunshine period was observed during January to March and the short period during May to August. Therefore, it is likely that the sunshine period in the study year would be the same (Figure 18).

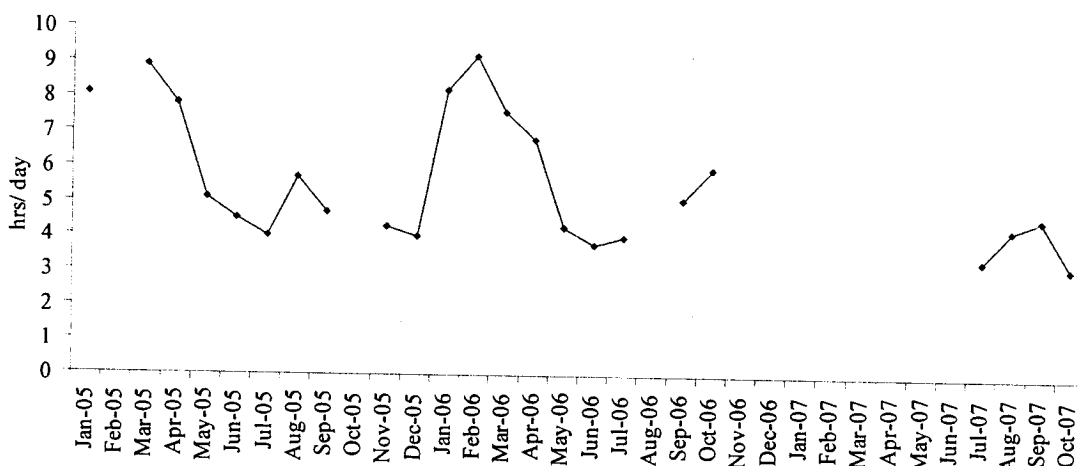


Figure 18. Seasonal changes in sunshine period from January 2005 to October 2007.

### Under canopy light intensity

There was a significant difference in under canopy light intensity between densities ( $P < 0.01$ ) (Table 3). The light intensities in the canopy of *E. acoroides* in the low density plots were  $987.38 \pm 17.10 \mu\text{mole/s/m}^2$  in 10% density plots and  $894.38 \pm 36.11 \mu\text{mole/s/m}^2$  in 25% density plots. There were lower light intensities in the high density plots,  $493.71 \pm 56.22 \mu\text{mole/s/m}^2$  in 50% plots and  $408.13 \pm 40.11 \mu\text{mole/s/m}^2$  in 100% plots (Figure 19).

Table 3. The differences of under canopy light intensity between densities

Source of Variation	df	MS	F	P
Between groups	3	330718.910	51.947	0.000
Within groups	12	6366.436		
Total	15			



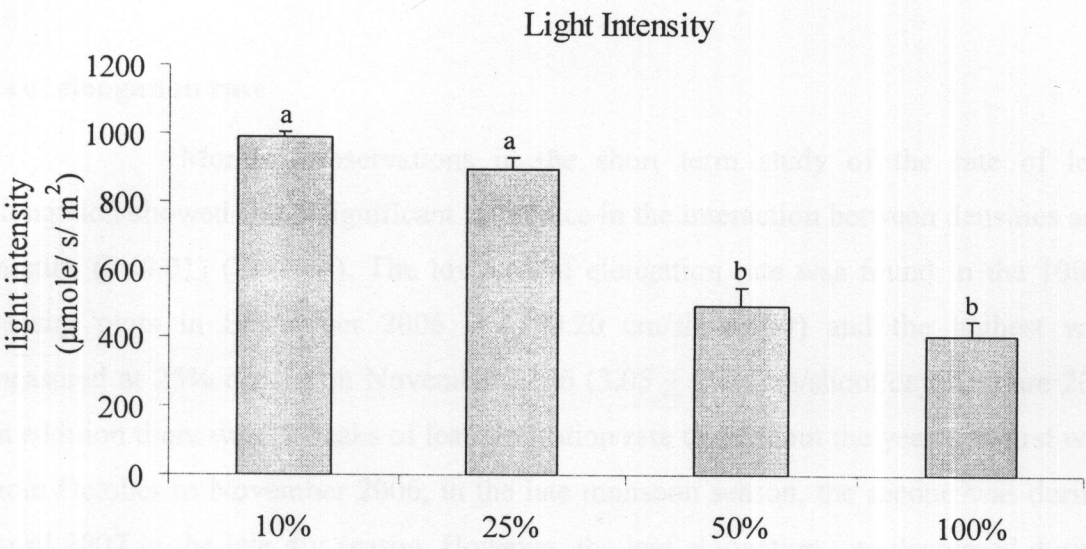


Figure 19. The difference of under canopy light intensity of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other.

## **The effect of shoot density on growth**

### **Leaf elongation rate**

Monthly observations in the short term study of the rate of leaf elongation showed that a significant difference in the interaction between densities and months ( $P < 0.01$ ) (Table 4). The lowest leaf elongation rate was found in the 100% density plots in September 2006 ( $1.47 \pm 0.20$  cm/shoot/day) and the highest was measured at 25% density in November 2006 ( $3.05 \pm 0.26$  cm/shoot/day) (Figure 20). In addition there were 2 peaks of leaf elongation rate throughout the year; the first was from October to November 2006, in the late monsoon season, the second was during April 2007 in the late dry season. However, the leaf elongation rate decreased during January and February 2007 and during August and September 2006 and June- July 2007 in the mid monsoon season.

The long term observations (annual mean leaf elongation rate), showed no significant differences among densities ( $P > 0.05$ ) (Table 5). The highest of the annual mean leaf elongation rate was found at 10% density ( $2.40 \pm 0.12$  cm/shoot/day), while the lowest rate was found at 100% density ( $2.07 \pm 0.01$  cm/shoot/day) (Figure 21).

Table 4. The difference in mean leaf elongation rate of *E. acoroides* between densities and months.

Source of Variation	df	MS	F	P
<b>Between subjects</b>				
Density	3	1.179	2.567	0.070
Error	36	0.459		
<b>Within subjects</b>				
Month	7	4.929	11.534	0.000
Month x Density	22	0.891	2.084	0.003
Error	268	0.427		

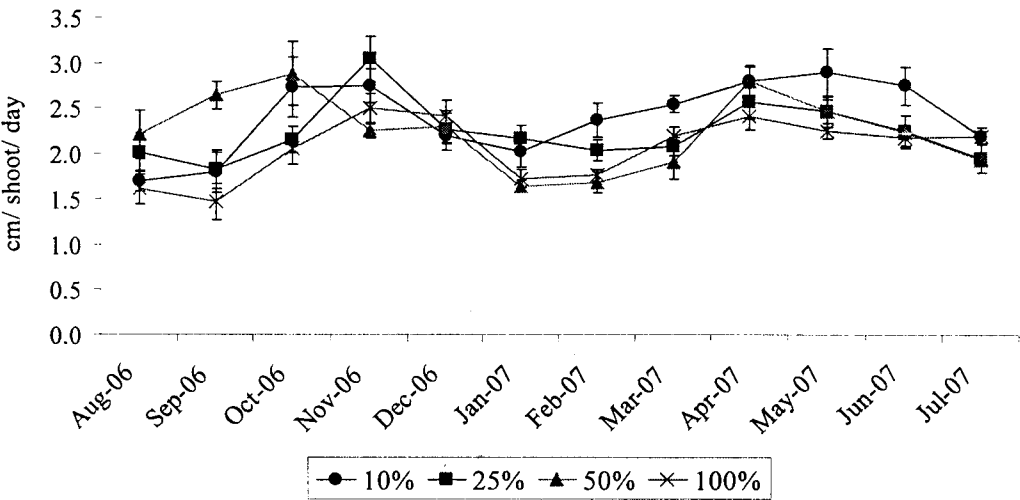


Figure 20. Mean leaf elongation rate of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 5. The difference of annual mean leaf elongation rate of *E. acoroides* in each density.

Source of Variation	df	MS	F	P
Between groups	3	0.222	1.575	0.209
Within groups	44	0.141		
Total	47			

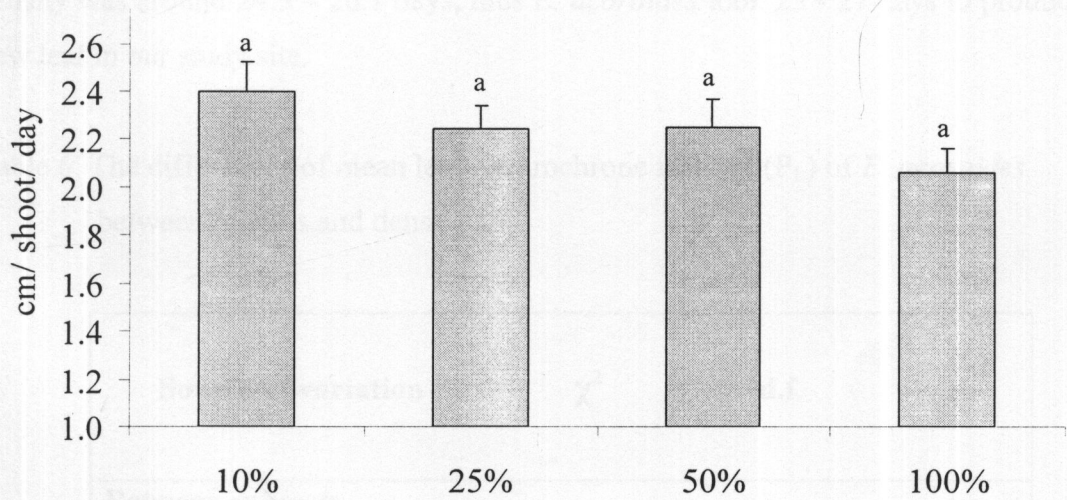


Figure 21. Annual mean leaf elongation rate of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other.



### Leaf Plastochrone Interval ( $P_L$ )

The Leaf Plastochrone Interval ( $P_L$ ) represents the number of days that plants take to produce a new leaf. The results of  $P_L$  in the short term study showed that there was significant difference in the interactions between densities and months ( $\chi^2 = 155.495$ ,  $P < 0.01$ ) (Table 6). There was seasonal variation on  $P_L$  with the highest  $P_L$  in January 2007 in 50% density plots ( $31.0 \pm 0.0$  days), and the lowest in September 2006 and April 2007 at the same density ( $18.5 \pm 1.05$  days and  $19.9 \pm 1.72$  days respectively) (Figure 22).

In long term study, there was no significant difference between mean annual  $P_L$  at each density ( $P > 0.05$ , Table 7). The low mean annual  $P_L$  was found at low density treatments,  $24.5 \pm 0.7$  days in 10% density plots and  $25.4 \pm 0.8$  days in 25% density plots. The higher  $P_L$  was found in high density treatments,  $25.9 \pm 1.2$  days in 50% density plots and  $26.1 \pm 0.8$  days in 100% density plots (Figure 23). The  $P_L$  of all density was around 24.5 – 26.1 days, thus *E. acoroides* took 25 - 27 days to produce a new leaf in our study site.

Table 6. The differences of mean leaf plastochrone interval ( $P_L$ ) of *E. acoroides* between months and densities.

Source of variation	$\chi^2$	d.f.	P
<b>Between subjects</b>			
Density	2.978	3	0.395
Month	115.988	11	0.000
Month $\times$ Density	155.495	47	0.000

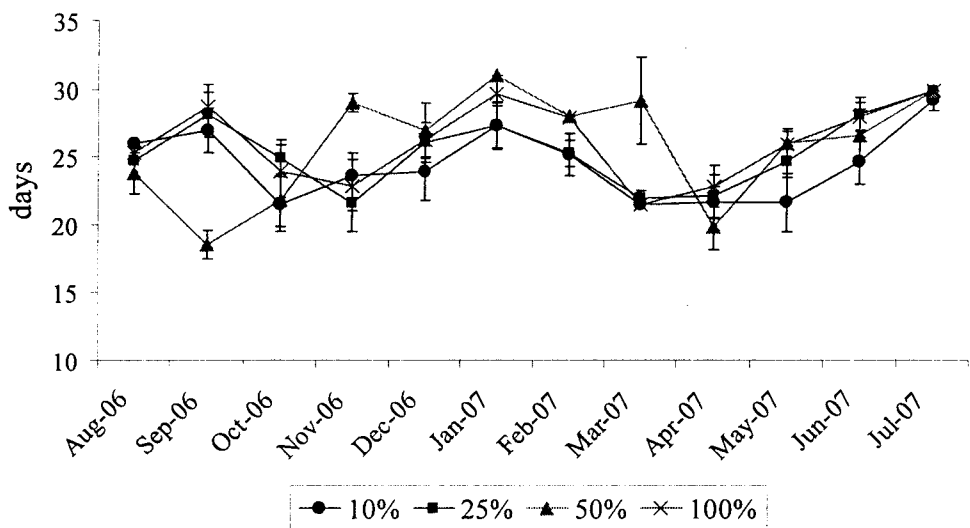


Figure 22. Mean leaf plastochrone interval ( $P_L$ ) of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 7. The difference of annual mean leaf plastochrone interval ( $P_L$ ) of *E. acoroides* at each density.

Source of Variation	df	MS	F	P
Between groups	3	6.435	0.671	0.574
Within groups	44	9.586		
Total	47			

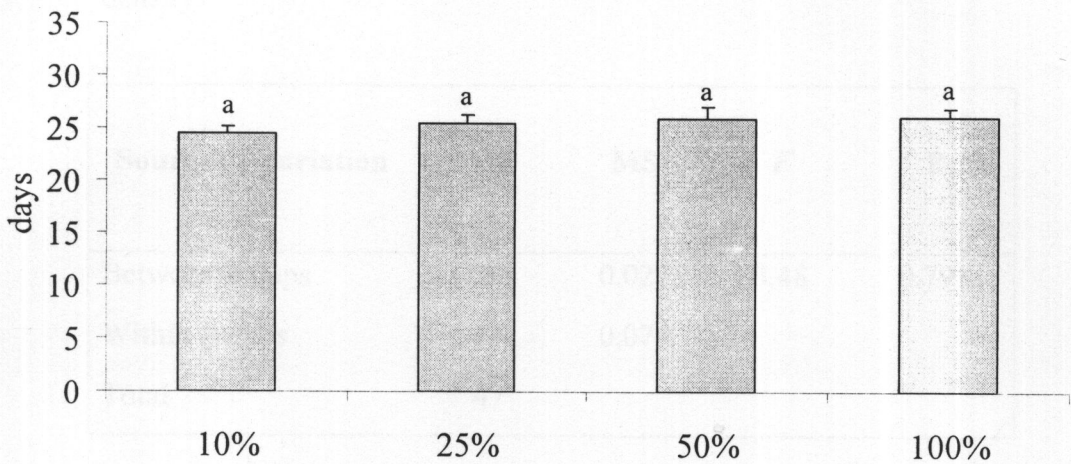


Figure 23. Annual mean leaf plastochrone interval (P<sub>L</sub>) of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other

**New leaves production**

There were no significant differences between annual mean new leaf production and cumulative new leaf production ( $P>0.05$ ) (Table 8 and Table 9) among densities. The annual mean new leaf production was in the range of 1.3-1.4 leaves/shoot (Figure 24). The cumulative new leaf production was similar at both low densities (10%=16.5±0.3 leaves/shoot/year, 25%=15.8±0.3 leaves/shoot/year) and high densities (50%=15.5±0.5 leaves/shoot/year, 100%=15.2±0.3 leaves/shoot/year) (Figure 25). The growth form of *E. acoroides* is the mono-meristematic leaf-replacing type, that is, every time the plants produce a new leaf, a new node was produced at their rhizomes. Therefore the cumulative new leaf production revealed the number of total nodes that they produced in a year (~ 15 – 17 nodes/year).

Table 8. The difference of mean annual new leaves production of *E. acoroides* in each density.

Source of Variation	df	MS	F	P
Between groups	3	0.027	3.48	0.791
Within groups	44	0.079		
Total	47			

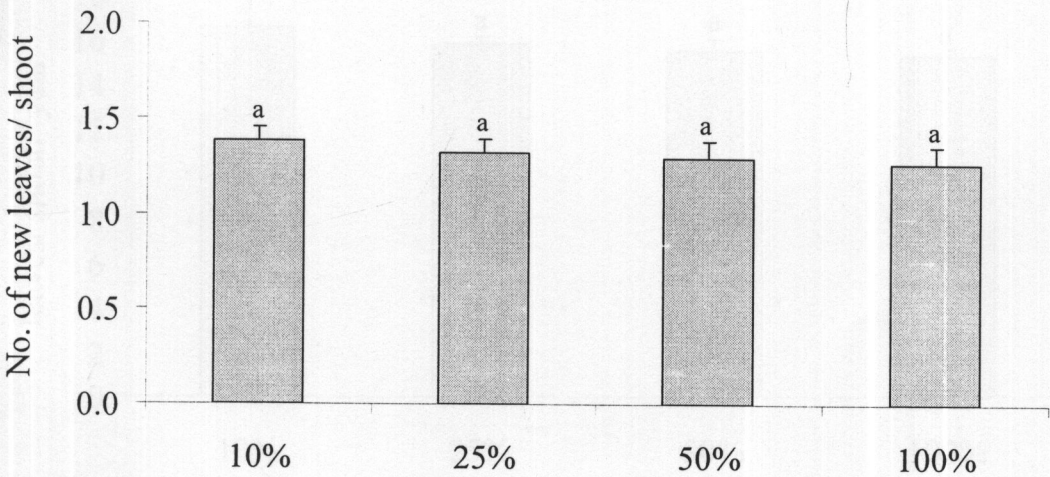


Figure 24. Annual mean new leaf production of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other.

Table 9. The difference of cumulative new leaves production of *E. acoroides* at each density.

Source of Variation	df	MS	F	P
Between groups	3	3.247	2.665	0.062
Within groups	36	1.218		
Total	39			

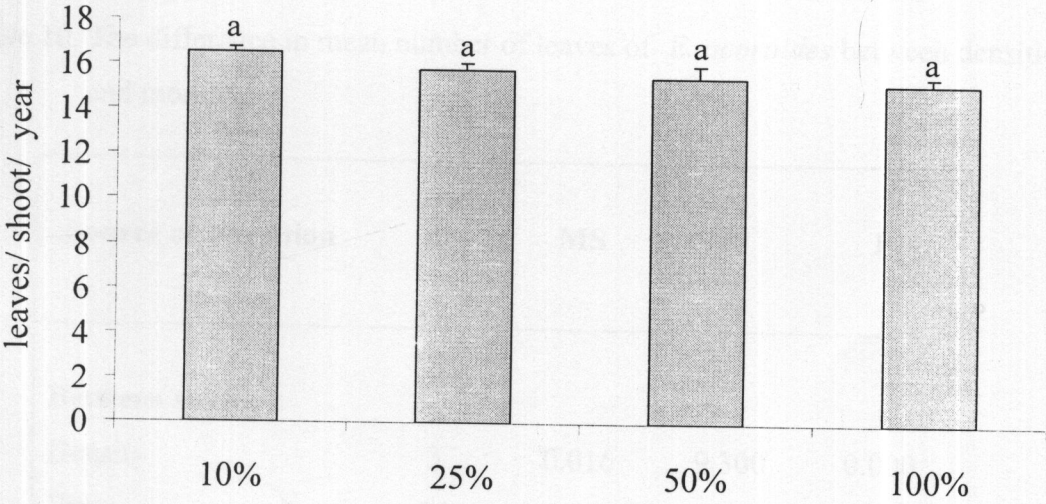


Figure 25. Cumulative new leaf production of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error. Densities that share the same letter are not significantly different from each other.



### Number of leaves

The short term experiment (monthly observation) of number of leaves showed that there were significant differences in the interaction between months and densities ( $P < 0.01$ ) (Table 10). The highest number of leaves was found in February 2007 at 25% density ( $4.8 \pm 0.1$  leaves/shoot), while the lowest was found in August 2006 100% and 50% density plots (3.1 leaves/shoot) (Figure 26). Moreover, the number of leaves was high from November 2006 to March 2007 when it was the dry season and low in the monsoon season (August – October 2006 and May – July 2007).

However, there was no significant difference in annual mean number of leaves among densities ( $P > 0.05$ ) (Table 11). The number of leaves at each density was similar, the high value was found at low densities (25% =  $4.1 \pm 0.1$  leaves/shoot and 10% =  $4.0 \pm 0.1$  leaves/shoot), and the low value at high densities (50% =  $3.8 \pm 0.1$  leaves/shoot and 100% =  $3.9 \pm 0.1$  leaves/shoot) (Figure 27).

Table 10. The difference in mean number of leaves of *E. acoroides* between densities and months.

Source of Variation	df	MS	F	P
<b>Between subjects</b>				
Density	3	0.016	9.300	0.000
Error	36	0.002		
<b>Within subjects</b>				
Month	11	0.045	64.356	0.000
Month x Density	33	0.002	2.339	0.000
Error	396	0.001		

Note: Analysis performed on  $\text{Log}(x+1)$  transformed data.

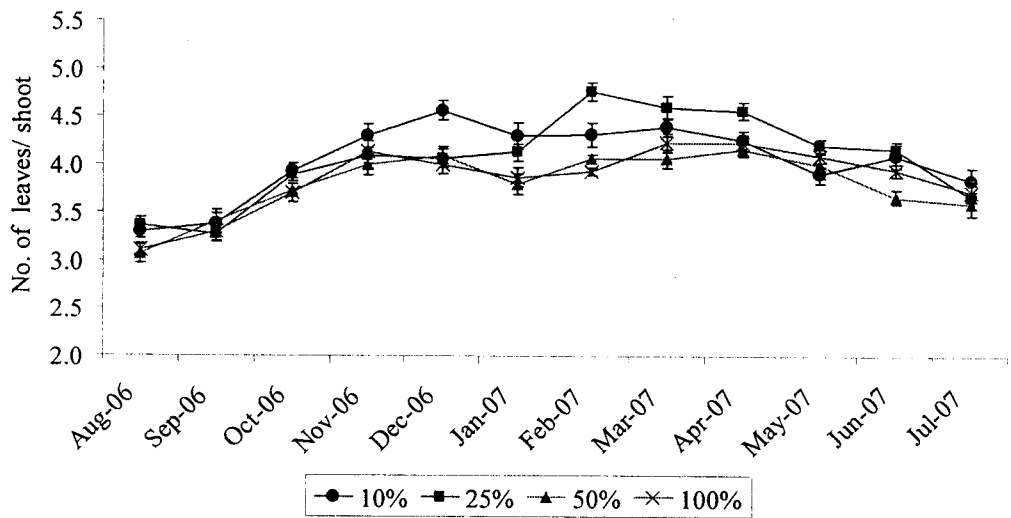


Figure 26. Mean number of leaves of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 11. The difference of annual mean number of leaves of *E. acoroides* in each density.

Source of Variation	df	MS	F	P
Between groups	3	0.214	1.405	0.254
Within groups	44	0.152		
Total	47			

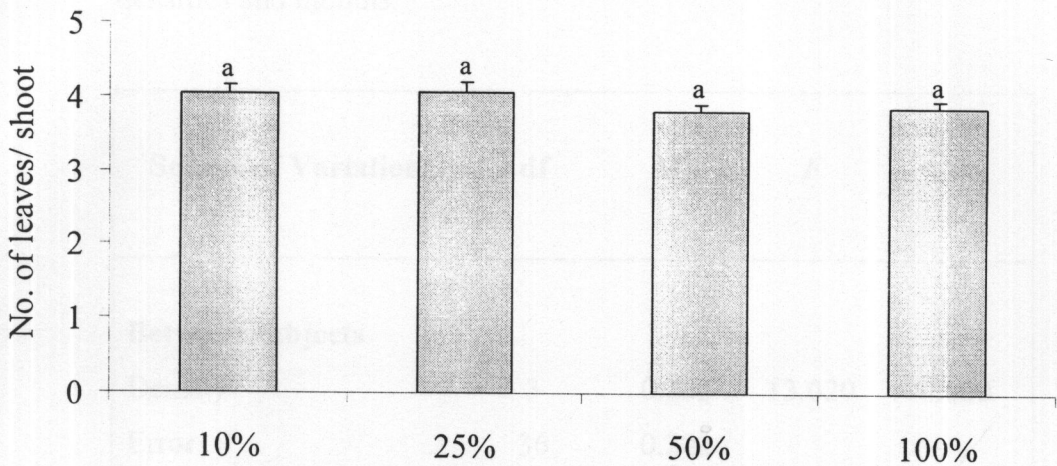


Figure 27. Annual mean number of leaves of *E. acoroides* in each density . The error bars are standard error. Densities that share the same letter are not significantly different from each other.

### Leaf surface area

In short term study (monthly observation) of leaf surface area of *E. acoroides*, the result showed that there was a significant difference in the interaction between months and densities ( $P < 0.01$ ) (Table 12). The highest value was in March 2007 in the 10% density plots ( $307.2 \pm 11.6 \text{ cm}^2/\text{shoot}$ ), while the lowest value was in September 2006 in the 100% density plots ( $123.7 \pm 8.3 \text{ cm}^2/\text{shoot}$ ) (Figure 28). In addition, there were 2 peaks of high leaf surface area. The first was from November to December 2006, a transitional season between the late monsoon and early summer seasons. The second peak was from March to May 2007, late summer.

The results of the long term observations (annual mean leaf surface area) showed that there was a significant difference among densities ( $P < 0.05$ ) (Table 13) especially between 10% density plots ( $255.6 \pm 14.8 \text{ cm}^2/\text{shoot}$ ) and 100% density plots ( $199.5 \pm 12.4 \text{ cm}^2/\text{shoot}$ ) (Figure 29). In addition, I observed that leaf blades of *E. acoroides* at low densities were wider (max. 1.8 cm in low densities to 1.6 cm in high densities) and stronger than at high densities.

Table 12. The difference of mean leaf surface area of *E. acoroides* between densities and months.

Source of Variation	df	MS	F	P
<b>Between subjects</b>				
Density	3	0.262	13.020	0.000
Error	36	0.202		
<b>Within subjects</b>				
Month	7	0.549	79.173	0.000
Month x Density	22	0.020	2.951	0.000
Error	259	0.007		

Note: Analysis performed on Log(x+1) transformed data

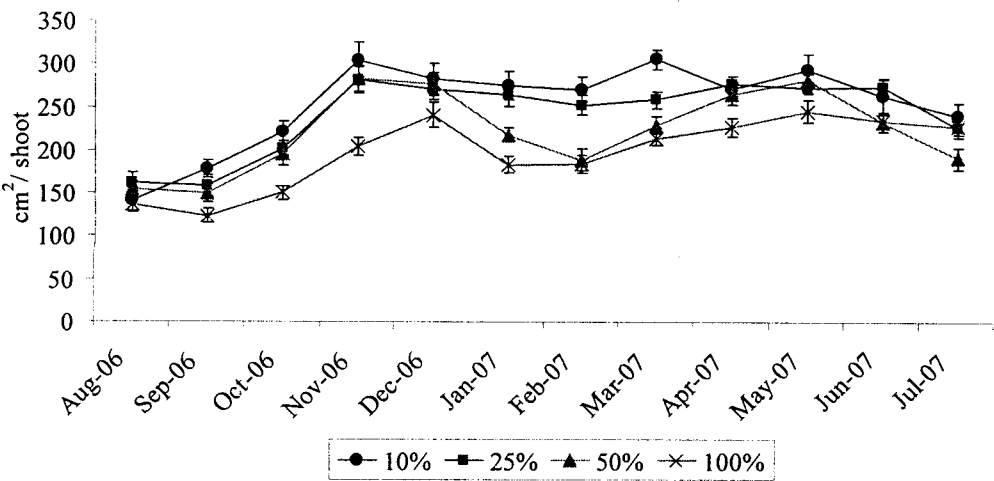


Figure 28. Mean leaf surface area of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 13. The difference of annual mean leaf surface area of *E. acoroides* in each density.

Source of Variation	df	MS	<i>F</i>	P
Between groups	3	7170.277	3.270	0.030
Within groups	36	2192.579		
Total	47			



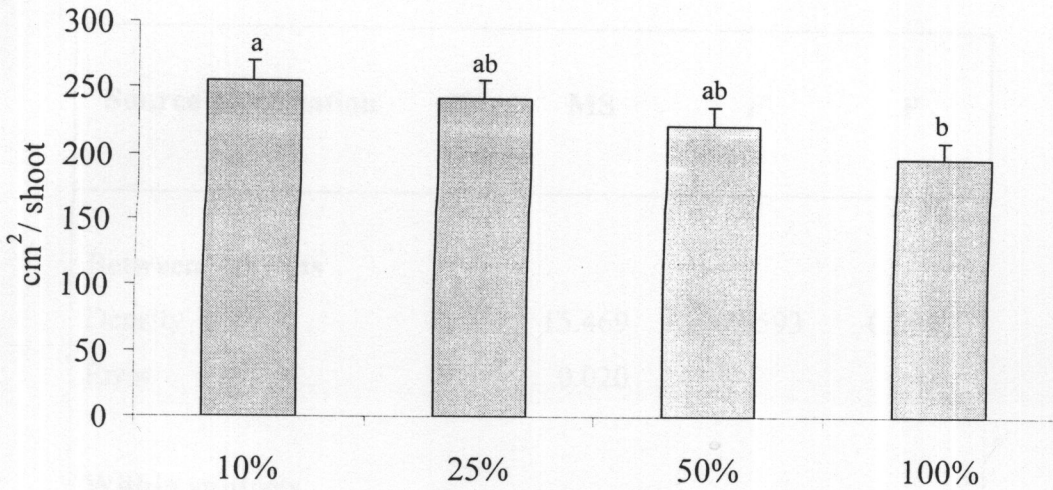


Figure 29. Annual mean leaf surface area of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other.

### Leaf Area Index (LAI)

During the short term observations of Leaf Area Index (LAI), the result showed that there was a significant difference in the interaction between months and densities ( $P < 0.01$ ) (Table 14). The highest value was measured in December 2006 in the 100% density plots ( $33.9 \pm 2.0 \text{ m}^2 \text{ leaves/m}^2$ ), while the lowest was found in August 2006 in the 10% density plots ( $2.3 \pm 0.2 \text{ m}^2 \text{ leaves/m}^2$ ) (Figure 30). In addition, there were 2 peaks of LAI, the first peak was found in December 2006 in early summer and the second was in May 2007 in late summer. There was the effect of seasonal variation on LAI at high densities more than at low densities.

In addition, there was a significant difference in annual mean LAI among densities ( $P < 0.05$ ) (Table 15). The mean annual LAI was highest at 100% density ( $27.9 \pm 1.7 \text{ m}^2 \text{ leaves/m}^2$ ) and lowest at 10% density ( $4.1 \pm 0.2 \text{ m}^2 \text{ leaves/m}^2$ ) (Figure 31).

Table 14. The difference in mean LAI of *E. acoroides* between densities and months

Source of Variation	df	MS	F	P
<b>Between subjects</b>				
Density	3	15.469	762.593	0.000
Error	36	0.020		
<b>Within subjects</b>				
Month	7	0.554	79.201	0.000
Month x Density	22	0.021	2.950	0.000
Error	259	0.007		

Note: Analysis performed on Log(x) transformed data.

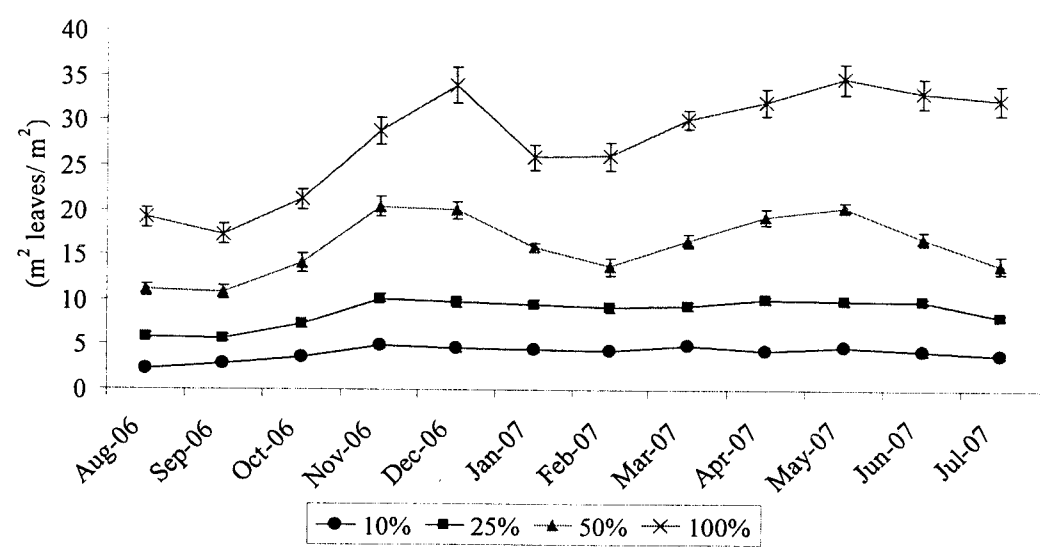


Figure 30. Mean LAI of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 15. The difference of annual mean LAI of *E. acoroides* in each density.

Source of Variation	df	MS	F	P
Between groups	3	1.539	159.613	0.000
Within groups	44	0.010		
Total	47			

Note: Analysis performed on Log(x) transformed data.

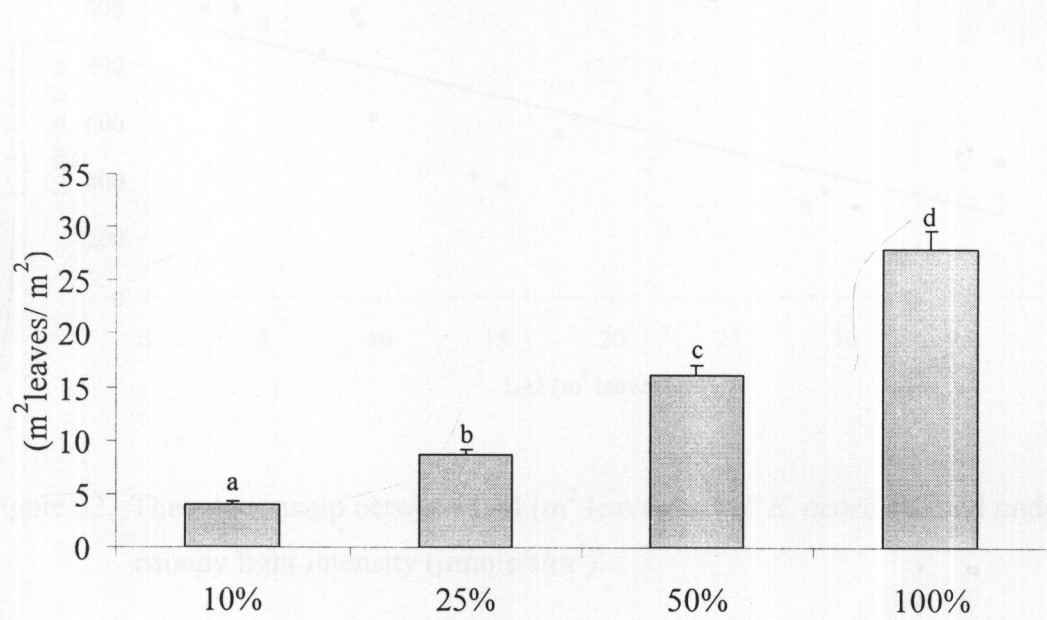


Figure 31. Annual mean LAI of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other.

**Relationship between Leaf Area Index of *Enhalus acoroides* (L.f.) Royle on the under canopy light intensity**

There was inverse relationship between LAI and under canopy light intensity ( $R^2= 0.65$ ,  $P<0.01$ ), when the LAI increased, the light intensity decreased (Figure 32).

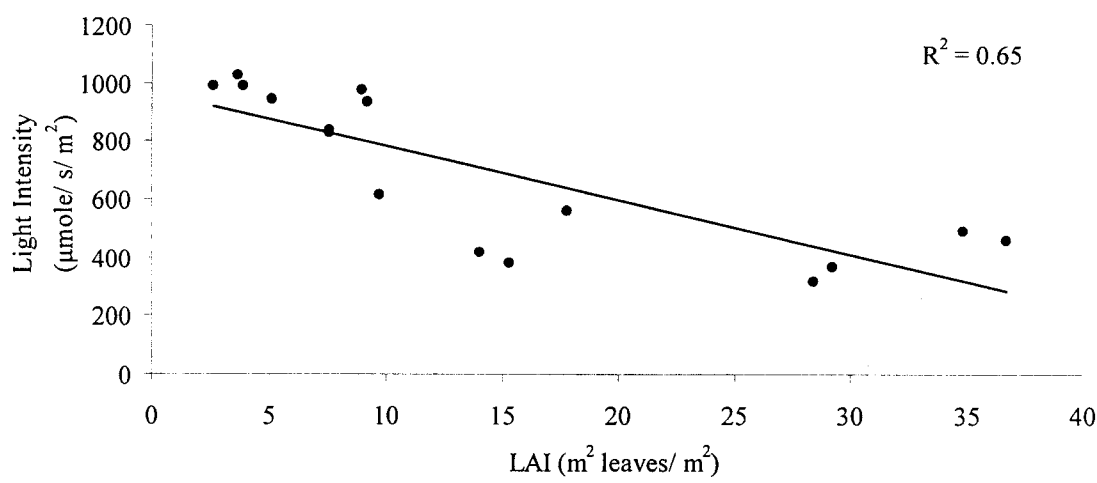


Figure 32. The relationship between LAI (m² leaves/m²) of *E. acoroides* and under canopy light intensity (µmole/s/m²).

**Relationship between shoot density of *Enhalus acoroides* (L.f.) Royle on above ground weight**

There was a significant difference in above ground weight between shoot densities of *E. acoroides* ( $P<0.01$ ) (Table 16). The highest value was found at 10% density plots ( $1.471\pm0.06$  g dw/shoot), while the lowest was found at 25% ( $1.024\pm0.064$  g dw/shoot), 50% ( $0.954\pm0.041$  g dw/shoot) and 100% densities ( $0.899\pm0.099$  g dw/shoot), respectively (Figure 33). In addition, a linear regression between shoot density and above ground shoot weight showed that there was a relationship between shoot density on shoot dry weight ( $R^2=0.479$ ,  $P<0.05$ ) (Figure 34). Moreover, to test the self thinning rule, a linear regression between log shoot

density and log above ground weight was plotted. There was a negative relationship between both variables ( $R^2 = 0.692$ ,  $P < 0.05$ ), the linear equation was fitted to  $Y = -0.224X + 0.404$  and the slope was  $-0.224$  (Figure 35).

Table 16. The difference of above ground weight of *E. acoroides* in each density.

Source of Variation	df	MS	F	P
Between groups	3	0.205	15.445	0.001
Within groups	8	0.013		
Total	11			

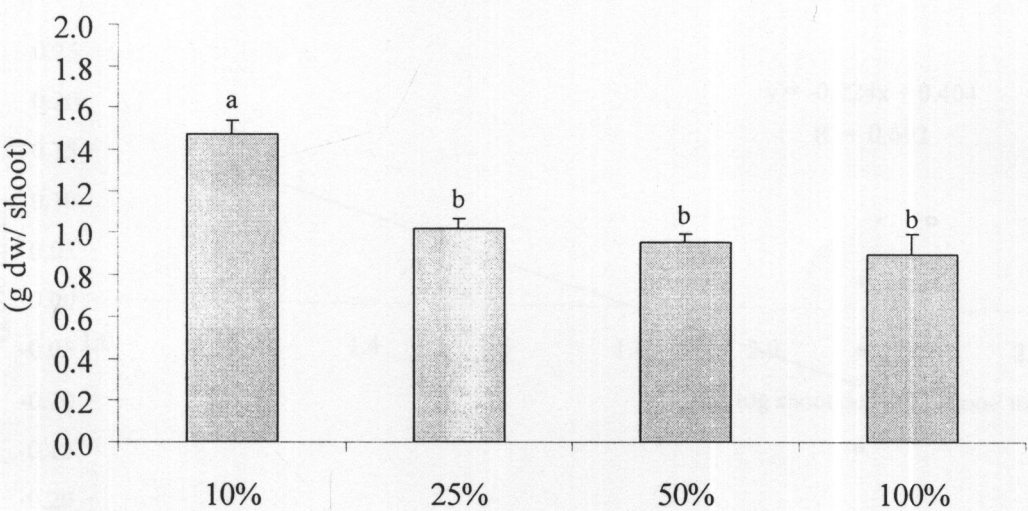


Figure 33. Mean above ground dry weight of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other.



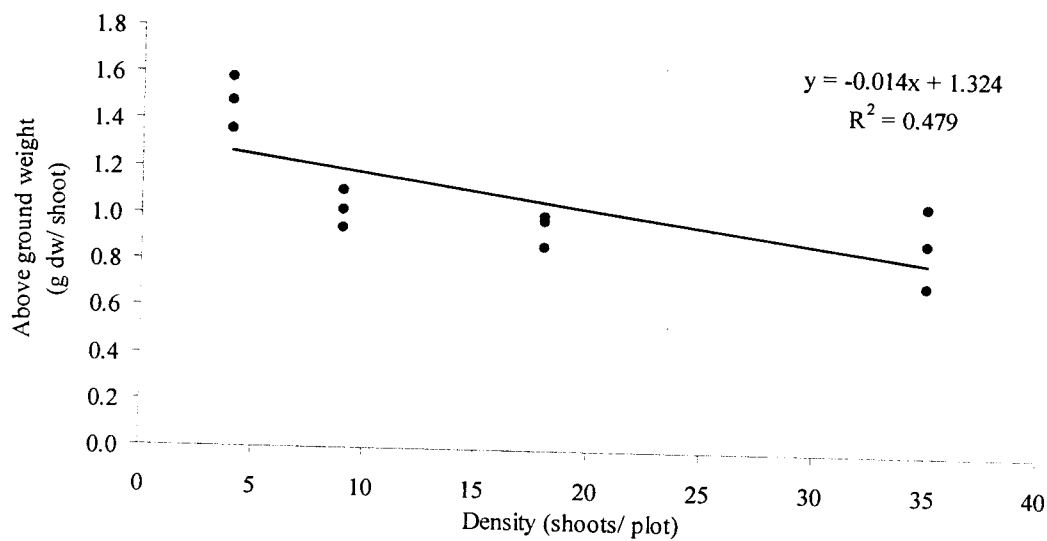


Figure 34. The relationship between shoot density (shoots/plot) and above ground weight (g dw/ shoot) of *E. acoroides*.

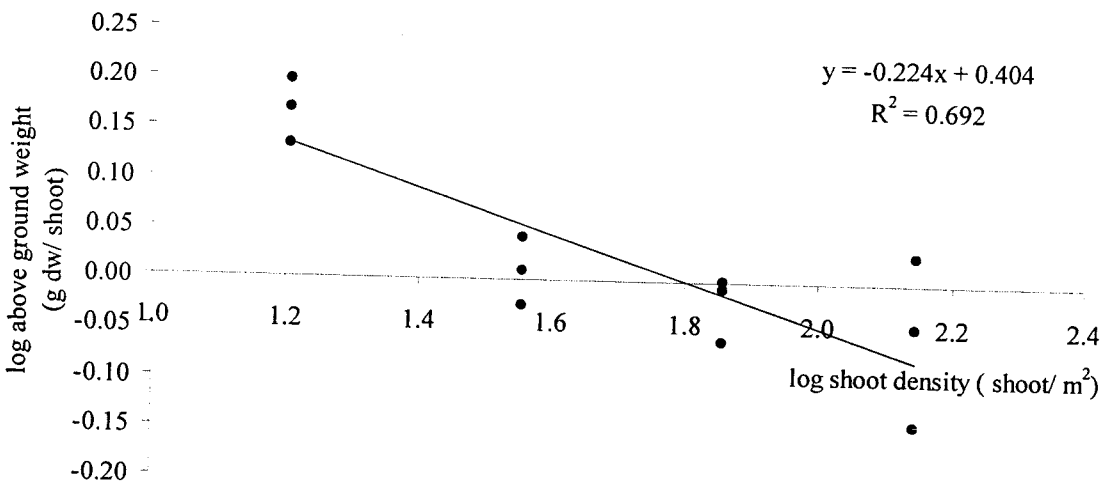


Figure 35. The relationship between log shoot density (shoots/m<sup>2</sup>) and log above ground weight (g dw shoot) of *E. acoroides*.

### Effect of shoot density on recruitment

After a year of study it was clear that most of the new shoots in the experimental plots had re-grown from the old rhizomes which had been cut each month. Only two seedlings were found in the 25% density plots in February 2007. The results in the recruitment study showed that the recruitment rate of *E. acoroides* was significantly different among densities ( $\chi^2=321.664$ ,  $P<0.01$ ), months ( $\chi^2=50.144$ ,  $P<0.01$ ) and the interactions between densities and months ( $\chi^2=385.652$ ,  $P<0.01$ ) (Table 17.). The highest recruitment rate was found in the 10% density plots in August 2006 ( $0.051 \pm 0.005$  new shoots/shoot/day) and the lowest rate was found in the 100% density plots in March 2007 ( $0.007 \pm 0.001$  new shoots/shoot/day). The recruitment rate of all densities dropped in September 2006 during the monsoon season (Figure 36). In addition, lots of seagrass leaf litter was found in the study site and in some experimental plots the rhizomes of *E. acoroides* were emerged. A large amount of *E. acoroides* shoots were washed up on the shore. In March 2007, during mid-summer, the recruitment rate of all densities decreased. Some *Enhalus* shoots turned brownish and dry when exposed during the low tide. The annual mean recruitment rate of *E. acoroides* was significantly different between densities ( $P<0.01$ ) (Table 18). The highest rate was found in the 10% density plots ( $0.040 \pm 0.002$  new shoots/shoot/day) and the lower rate were found in the 25% plots ( $0.028 \pm 0.002$  new shoots/shoot/day), 50% plots ( $0.020 \pm 0.002$  new shoots/shoot/day) and 100% density plots ( $0.010 \pm 0.001$  new shoots/shoot/day) respectively (Figure 37).

In addition throughout the one year of study there were other organisms that were recruited and grew inside the experimental plots, especially in the low density plot. For example Spoon Grass (*Halophila ovalis*), young thalli of green algae such as *Avrainvillia* sp., and Goby Fish together with Snapping Shrimp that made big nests (ca. 30 cm. in diameter and 10 cm. depth) were observed.

Table 17. The difference of mean recruitment rate of *E. acoroides* between densities and months.

Source of variation	$\chi^2$	d.f.	P
<b>Between subjects</b>			
Density	321.664	3	0.000
Month	50.144	11	0.000
Month $\times$ Density	385.652	46	0.000

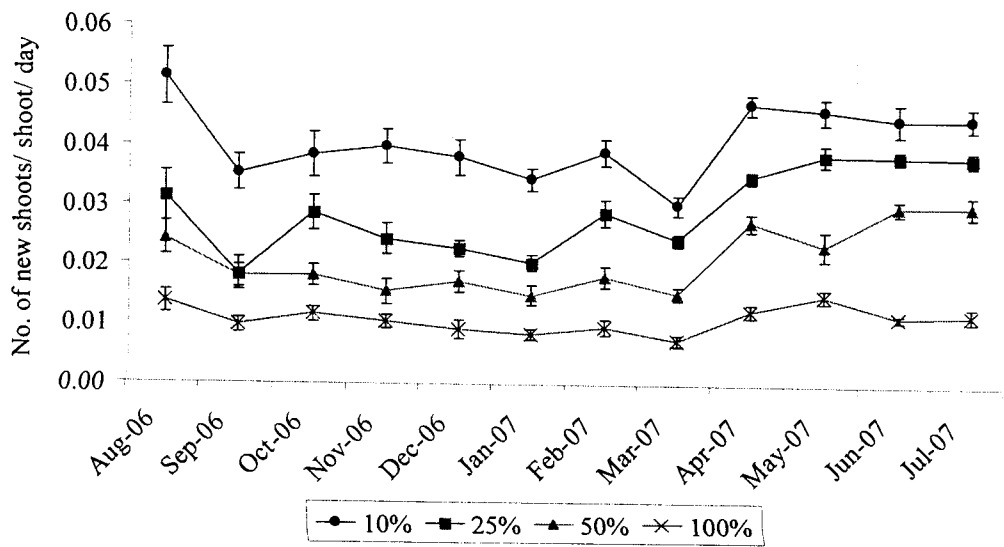


Figure 36. Mean recruitment rate of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 18. The difference of annual mean recruitment rate of *E. acoroides* in each density.

Source of Variation	df	MS	F	P
Between groups	3	1.539	159.513	0.000
Within groups	44	0.010		
Total	47			

Note: Analysis performed on Log(x) transformed data.

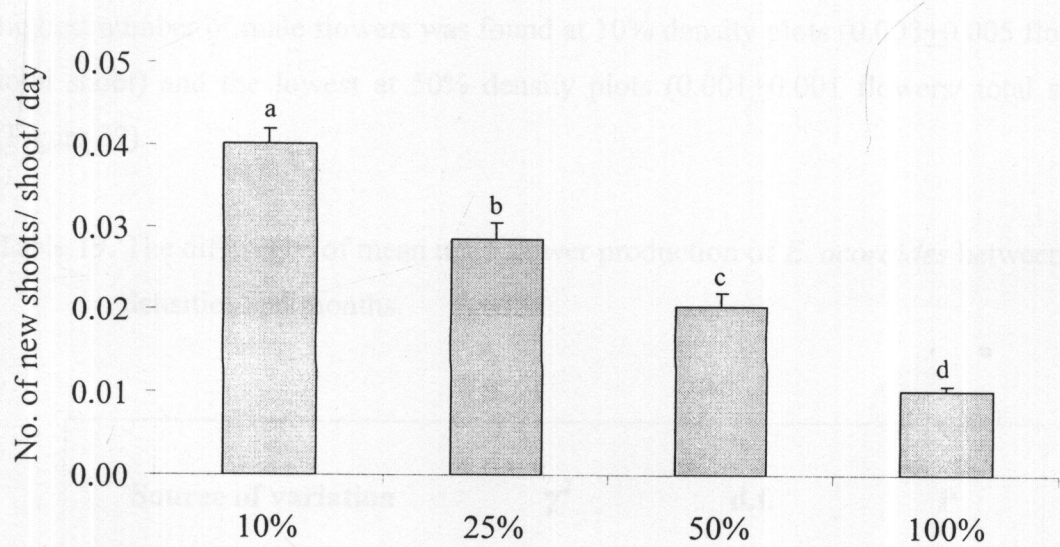


Figure 37. Annual mean recruitment rate of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other.

# Effect of shoot density on sexual reproduction

## Male flower production

There were no significant differences in male flower production among months ( $\chi^2=6.835$ ,  $P>0.05$ ), densities ( $\chi^2=7.413$ ,  $P>0.05$ ) and interactions between months and densities ( $\chi^2=54.032$ ,  $P>0.05$ ) (Table 19). However, more male flowers were found in the low density plots than in the high. The highest number of male flowers was found at 10% density plots on November 2006 ( $0.050\pm0.047$  flowers/total shoot) (Figure 38). Male flowers seemed to be produced throughout the year (except in March and July 2007). The highest frequency of male flowering was found in the 25% density plots (5 from 12 months), while the lowest frequency was found in the 50% density plots (1 of 12 months, on December 2006). There was no significant difference in annual mean male flower production between densities ( $P>0.05$ , Table 20). The highest number of male flowers was found at 10% density plots ( $0.008\pm0.005$  flowers/total shoot) and the lowest at 50% density plots ( $0.001\pm0.001$  flowers/total shoot) (Figure 39).

Table 19. The difference of mean male flower production of *E. acoroides* between densities and months.

Source of variation	$\chi^2$	d.f.	P
<b>Between subjects</b>			
Density	7.413	3	0.060
Month	6.835	11	0.812
Month $\times$ Density	54.032	47	0.224



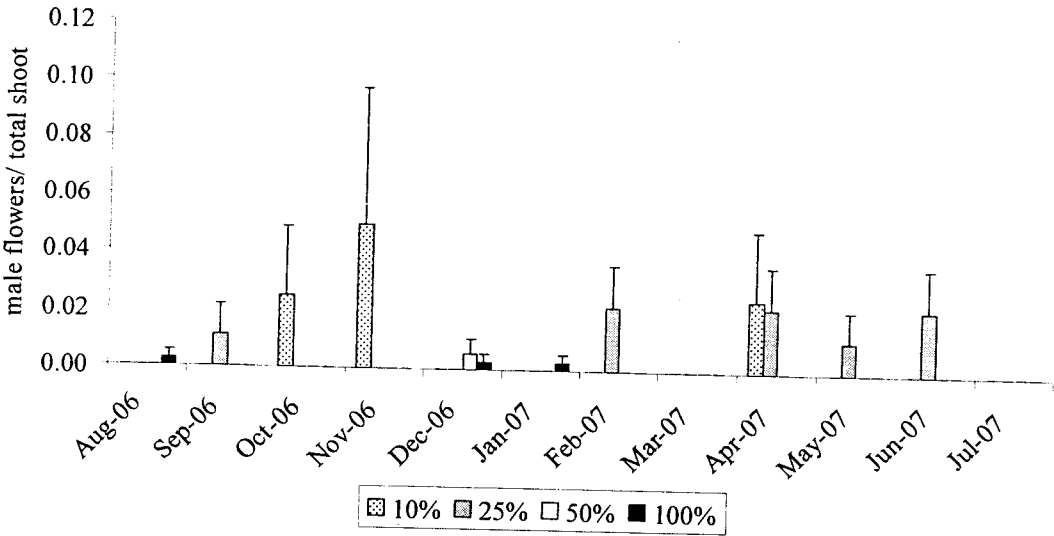


Figure 38. Mean male flower production of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 20. The difference of annual mean male flowers production of *E. acoroides* between densities.

Source of variation	$\chi^2$	d.f.	P
Between subjects			
Density	7.649	3	0.054

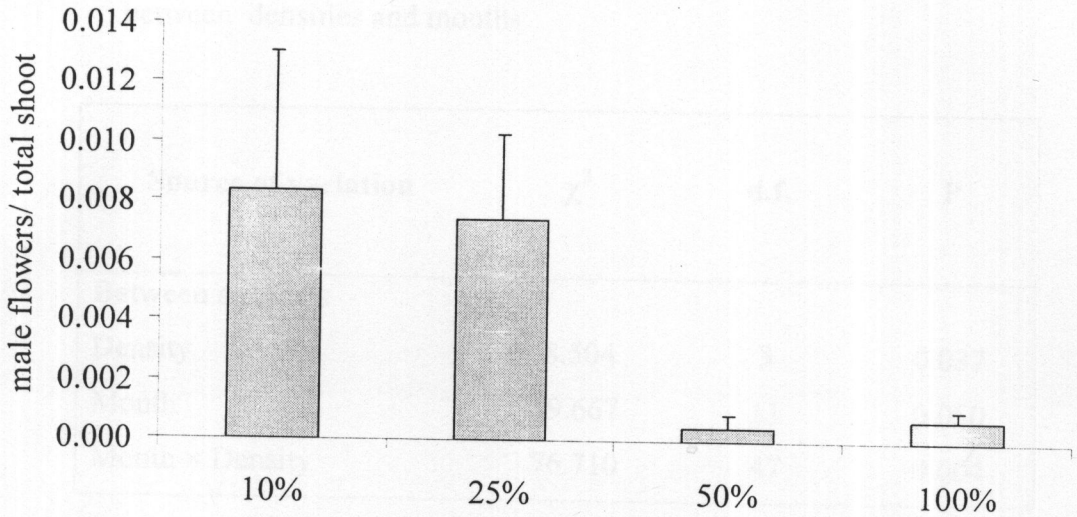


Figure 39. Annual mean of male flowers production of *E. acoroides* in each density. The error bars are standard error.

### Female flower production

There were significant differences in female flower production among densities ( $\chi^2=8.504$ ,  $P<0.05$ ), months ( $\chi^2=8.504$ ,  $P\leq 0.05$ ), and interactions between densities and months ( $\chi^2=76.710$ ,  $P<0.01$ ) (Table 21.). *E. acoroides* produced female flowers all year round. The highest number of female flowers was found in the 10% density plots in April 2007 ( $0.125\pm 0.053$  flowers/total shoot). The highest frequency of female flowers was found in the 25% density plots (9 of 12 months), while the lowest was found in the 100% density plots (2 from 12 months). However, female flowers were not found in September 2006 (Figure 40). The annual mean of female flower production was significantly different between densities ( $P<0.01$ ) (Table 22). The highest value was found at 10% density ( $0.025\pm 0.011$  flowers/total shoot) and the lowest was found at 100% density ( $0.001\pm 0.001$  flowers/total shoot) (Figure 41).

Table 21. The difference of mean female flower production of *E. acoroides* between densities and months.

Source of variation	$\chi^2$	d.f.	P
<b>Between subjects</b>			
Density	8.504	3	0.037
Month	19.667	11	0.050
Month $\times$ Density	76.710	47	0.004

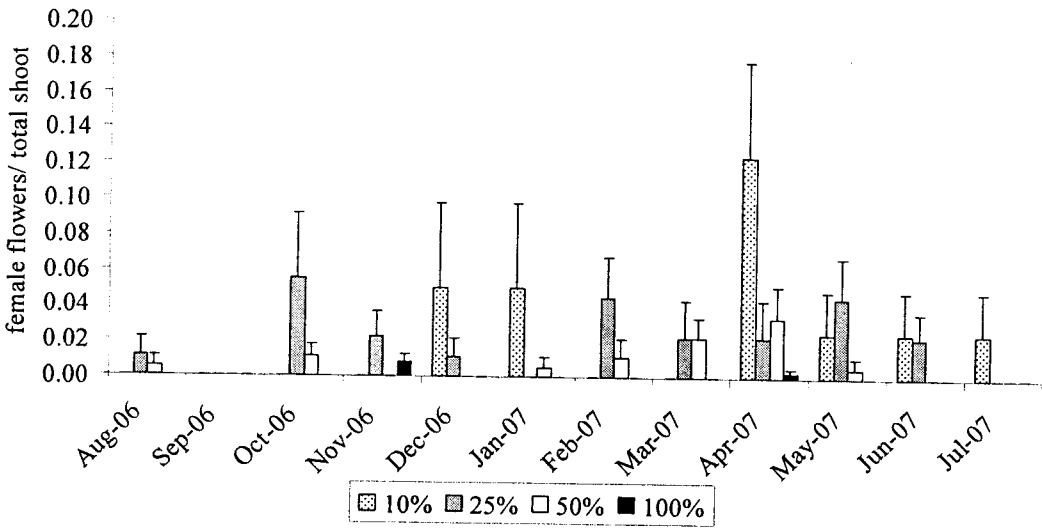


Figure 40. Mean female flowerproduction of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 22. The difference of annual mean female flowers production of *E. acoroides* between densities.

Source of variation	$\chi^2$	d.f.	P
Between subjects			
Density	11.715	3	0.008

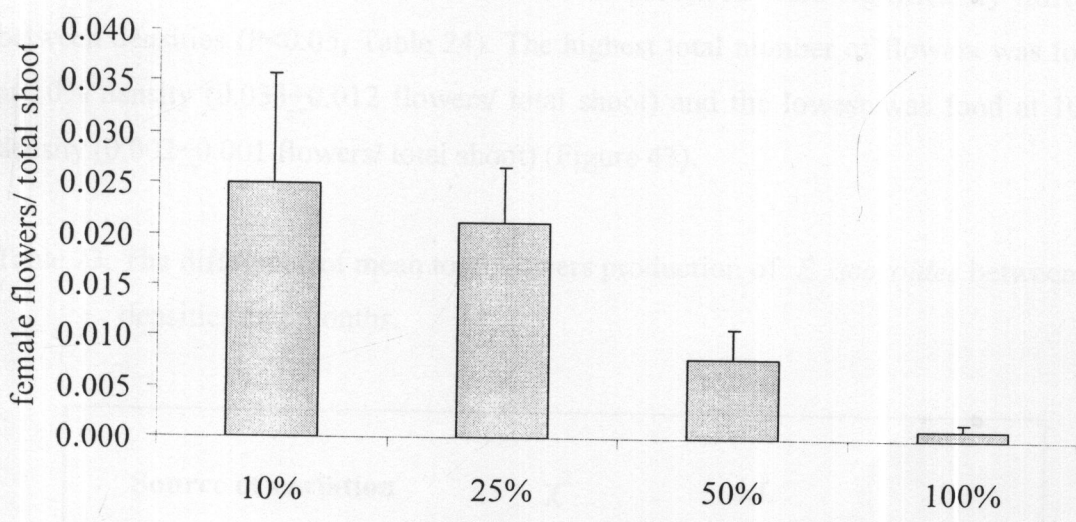


Figure 41. Annual mean female flowers production of *E. acoroides* in each density. The error bars are standard error.

**Total flowers production**

There were significant differences in the total number of flowers produced (the sum of male flowers and female flowers) in densities ( $\chi^2= 12.562$ ,  $P<0.01$ ), months ( $\chi^2= 20.085$ ,  $P<0.05$ ) and interactions between these 2 factors ( $\chi^2= 78.221$ ,  $P<0.01$ ) (Table 23). The flowers of *E. acoroides* were produced throughout the year and peaked in April 2007, but the least flowering occurred from August to September 2006. The most flowers were produced at low densities (10% and 25% density) more than at high densities (50% and 100% density). The highest total number of flowers was found at 10% density in April 2007 ( $0.150\pm0.076$  flowers/ total shoot). The highest frequency of flowering was found at 25% density (10 from 12 months), while the lowest frequency was found at 100% density (5 of 12 months) (Figure 42) The annual mean of total flowers produced were significantly different between densities ( $P<0.05$ , Table 24). The highest total number of flowers was found at 10% density ( $0.033\pm0.012$  flowers/ total shoot) and the lowest was found at 100% density ( $0.002\pm0.001$  flowers/ total shoot) (Figure 43).

Table 23. The difference of mean total flowers production of *E. acoroides* between densities and months.

Source of variation	$\chi^2$	d.f.	P
<b>Between subjects</b>			
Density	12.562	3	0.006
Month	20.085	11	0.044
Month $\times$ Density	78.221	47	0.003



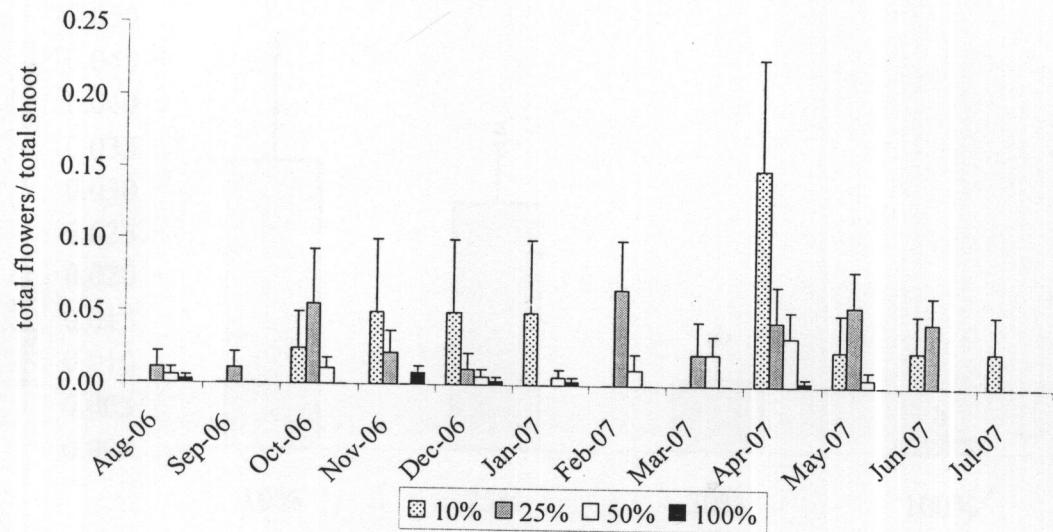


Figure 42. Mean total flower production of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 24. The difference of annual mean total flower production of *E. acoroides* in each density.

Source of Variation	df	MS	F	P
Between groups	3	0.050	7.882	0.000
Within groups	44	0.006		
Total	47			

Note: Analysis performed on Square root(x) transformed data.

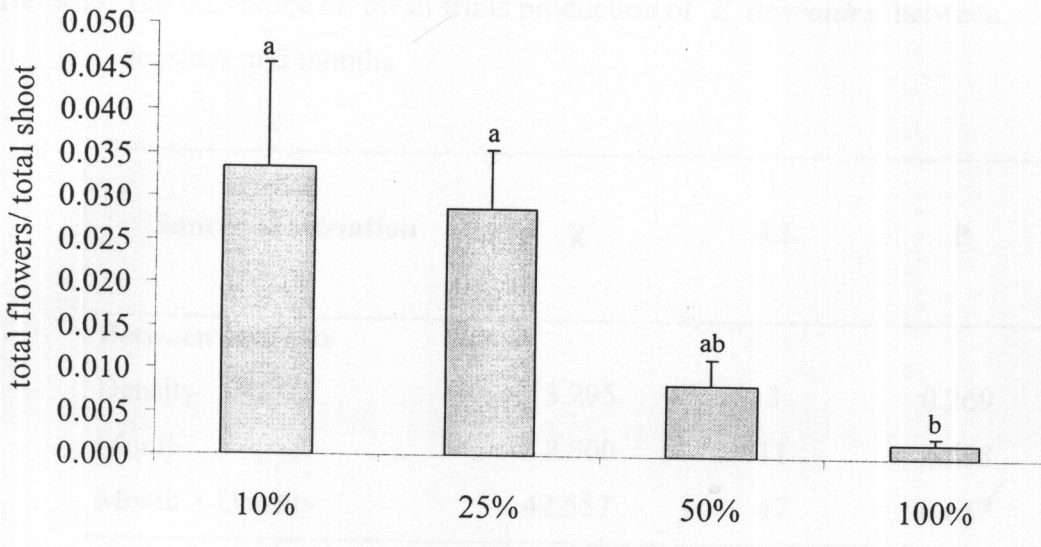


Figure 43. Annual mean total flowers production of *E. acoroides* in each density. The error bars are standard error. Densities that share the same letter are not significantly different from each other.

### Fruit production

The number of fruits was not significantly different among densities ( $\chi^2=3.929$ ,  $P>0.05$ ), months ( $\chi^2=8.500$ ,  $P>0.05$ ) and interactions between months and densities ( $\chi^2= 42.557$ ,  $P>0.05$ ) (Table 25). Although in this study *E. acoroides* produced flowers throughout the year, the fruits were found inside the experimental plots only from February to June 2007. The highest number of fruit was found at 10% density in February 2007 ( $0.050 \pm 0.047$  fruits/total shoot), while the lowest was at 50% density in March and May 2007 ( $0.006 \pm 0.005$  fruits/total shoot) (Figure 44). There was no significant difference in annual mean fruit production among densities ( $\chi^2= 4.232$ ,  $P>0.05$ ) (Table 26). The highest number of fruits was found at 10% density and no fruits were found at 100% density plots (Figure 45).

Table 25. The difference of mean fruits production of *E. acoroides* between densities and months

Source of variation	$\chi^2$	d.f.	P
<b>Between subjects</b>			
Density	3.295	3	0.269
Month	8.500	11	0.668
Month $\times$ Density	42.557	47	0.657

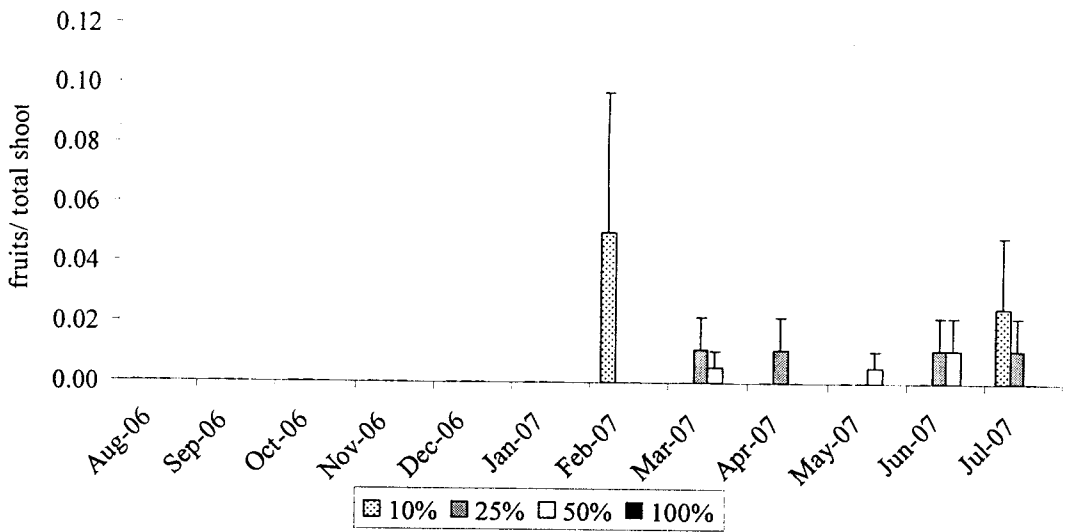


Figure 44. Mean fruit production of *E. acoroides* in each density from August 2006 to July 2007. The error bars are standard error.

Table 26. The difference of annual mean fruits production of *E. acoroides* between densities

Source of variation	$\chi^2$	d.f.	P
Between subjects			
Density	4.232	3	0.237

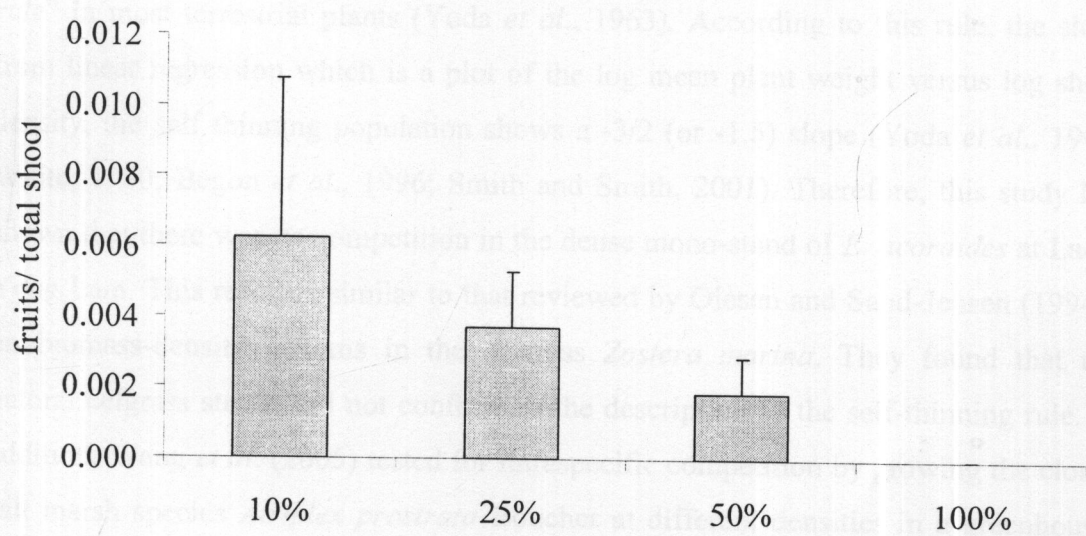


Figure 45. Annual mean fruit production of *E. acoroides* in each density. The error bars are standard error.

## CHAPTER 4

### DISCUSSION

#### Intraspecific competition in *Enhalus acoroides* (L.f.) Royle meadow

The linear regression between log shoot density and log above ground shoot weight of *E. acoroides* in this study shows an inverse relationship between the two factors ( $R^2 = 0.692$ ,  $P < 0.01$ ). The slope from the linear equation ( $Y = -0.224X + 0.269$ ), was  $-0.224$ . This slope of the linear equation is usually indicative of the occurrence of competition, this is true and well-known as “self-thinning or  $-3/2$  power rule” in most terrestrial plants (Yoda *et al.*, 1963). According to this rule, the slope from linear regression which is a plot of the log mean plant weight versus log shoot density, the self thinning population shows a  $-3/2$  (or  $-1.5$ ) slope (Yoda *et al.*, 1963; White, 1980; Begon *et al.*, 1996; Smith and Smith, 2001). Therefore, this study has shown that there was no competition in the dense mono-stand of *E. acoroides* at Laem Yong Lam. This result is similar to that reviewed by Olesen and Sand-Jensen (1994a) on biomass-density patterns in the eelgrass *Zostera marina*. They found that the natural eelgrass stands did not conform to the description of the self-thinning rule. In addition, Wang *et al.* (2005) tested for intraspecific competition by growing the clonal salt marsh species *Atriplex prostrata* Boucher at different densities in a greenhouse. Their results show that self-thinning was not observed at the highest density. They suggested that maybe the densities examined were not high enough for self thinning to occur. Although, at the Laem Yong Lam study site competition was not expressed in an *E. acoroides* bed but other evidence supported that the difference in shoot density influenced some characteristics of *E. acoroides*. For example, leaf surface area, recruitment rate and the production of flowers.

Grime (2001) defined the competition of plants as the tendency of neighboring plants to utilize the same quantum of light, ions of mineral nutrients, molecules of water, or volume of space. However, since seagrasses live in a marine habitat, water is not considered to be a main competitive resource. Nutrients are one of



the important resources for seagrass growth to supply the biochemical pathways, such as photosynthesis and carbon fixation. However, the importance of nutrient limitation as a constraint to seagrass growth is still a subject of controversy (e.g. Short, 1987; Duarte, 1990; Worm and Reusch, 2000). In some seagrass meadows, nutrient limitation has been observed (Agawin *et al.*, 1996, Terrados *et al.*, 1990a). Although in some seagrass beds there is no evidence of nutrient limitation (Erftemeijer *et al.*, 1994; Worm and Reusch, 2000). The Laem Yong Lam study site is approximately 2 km from the mouth of the Kuan Tung Ku Canal and Mook island is also approximately 2 km in front of the study site and both are considered to be nutrient loading sources. The large seagrass bed surrounding the study site may supply nutrients as well. The natural density of *E. acoroides* in this study ( $141.0 \pm 8.7$  shoots/m<sup>2</sup>) increased approx. 15% from that reported by Koike *et al.* in 1999 ( $125$  shoots/m<sup>2</sup>) near the same area. The high density in this year long study may be an effect of nutrient loading from coastal development by human activity during these 9 years. This expectation was supported by the study of Tuntiprapas (personal communication) in 2008 at Laem Yong Lam. He found that nutrients in water column (Nitrate,  $0.49 \mu\text{M}$  and Phosphate,  $0.45 \mu\text{M}$ ) were higher than the previous study in 1990 by Umezawa *et al.* (Nitrate,  $<0.01$  and Phosphate,  $0.04 \mu\text{M}$ ). However, it was lower than the average nutrient concentration in water column of seagrass meadow world wide (Nitrate,  $2.7 \mu\text{M}$ , Phosphate,  $1.0 \mu\text{M}$ ) (Hemminga, 1998) In addition, during my one year observation at Laem Young Lam there were no plankton blooms nor serious overgrowth of epiphytes on *E. acoroides* which implies that nutrients at this site did not exceed what was necessary for seagrass growth. In addition, the lack of significant difference of the leaf elongation rate between densities supports the idea that nutrient limitation did not occur. Therefore, at this site nutrients may not be a major factor to depress *E. acoroides* growth. Other resources light and space, however, might be the key factors influencing *E. acoroides* growth.

### **The effect of shoot density on growth of *Enhalus acoroides* (L.f.) Royle**

The results of the growth study show that for the short term the number of leaves, the leaf surface area and leaf area index (LAI) were significantly different between densities, while for the long term only the leaf surface area and LAI were different. The change in leaf morphology of *E. acoroides* might be due to reduced light within the canopy or from self-shading (Collier, 2006), that is, light intensity at high densities was lower than at low densities. Ralph *et al.* (2007) mentioned that seagrasses are particularly sensitive to reduction in light availability, where small decreases can cause significant declines in growth. Seagrasses have shown varied levels of change because of light reduction, including leaf changes, within-canopy changes and meadow changes (e.g. Dennison, 1987; Dalla Via *et al.*, 1998; Olesen *et al.*, 2002; Ralph *et al.*, 2007)

Leaf level changes were reported by many investigators (e. g. Gordon *et al.*, 1994; Dalla Via *et al.*, 1998; Collier, 2006). Various leaf characteristics respond to light reduction as for examples, leaf width (Dalla Via *et al.*, 1998), leaf length (Gordon *et al.*, 1994; Longstuauff and Dennison, 1999) and leaf area (Campbell and Miller, 2002). Gordon *et al.* (1994) mentioned that leaf size of *Posidonia sinuosa* Cambridge et Kuo usually decreases under low light conditions. Campbell and Miller (2002) interpreted the response as that which reduces the respiratory demand of the shoot and also decreases the photosynthetic capacity of leaves. However, the opposite can also occur (Longstuauff and Dennison, 1999; Dalla Via *et al.*, 1998). Ralph *et al.* (2007) mentioned that large genera in the Functional Form model usually adjust leaf width to both acute and chronic light reduction, while small species increase leaf length in response to light reduction. This is corroborated by the present study with *E. acoroides* in which the maximum leaf width at low densities was 1.8 cm while at high densities it was 1.6 cm.

On the canopy level the leaf area index (LAI) generally decreases with light limitation, primarily because of reduced shoot density. Increases in shoot size (total area/shoot) with light reduction (in the case of depth ) has been attributed to a relaxation of intraspecific competition for light as shoot densities decline and may show an increase in shoot leafiness or increase in leaf length (Ralph *et al.*, 2007). Although, my results for the LAI were different from other reports which may be due

to the fact that the density was controlled (by clipping) throughout the year. The change in shoot density did not occur naturally over the year as reported in other studies. However, the discussion of the results from recruitment study will give more detail on this subject.

The minimum light requirement for seagrass growth is usually used to determine the effect of light limitation on the distribution of seagrasses. It is often expressed as percent of surface irradiance (%SI; Dennison *et al.*, 1993). Theoretically, the minimum light requirements for growth of seagrasses have been estimated at 11% of surface irradiance (Duarte, 1991a). However, Dennison *et al.* (1993) reported values between 5% to 25% of surface irradiance, while Lee *et al.* (2007) reported the minimum light requirement for tropical/subtropical seagrass species in the range of 4.4% (in *Halophila decipiens*) to 37% (in *Halodule wrightii*). Although, there was no measurement of percent of surface irradiance in this study the results implied that the light intensity at 100% density ( $408.13 \pm 40.11 \mu\text{mole/s/m}^2$ ) decreased about 59% from the 10% density ( $987.37 \pm 17.10 \mu\text{mole/s/m}^2$ ).

Agawin *et al.* (2001) reported that the compensation irradiance of *E. acoroides* in the Philippines was in the range of 2.5-26.0  $\mu\text{mole/s/m}^2$  (mean  $14.1 \pm 3.1 \mu\text{mole/s/m}^2$ ) which was rather low for tropical and subtropical seagrasses ( $5.7 \pm 0.5 \mu\text{mole/s/m}^2$  in *Halophila johnsonii* to  $89.5 \pm 11.3 \mu\text{mole/s/m}^2$  in *Halodule wrightii*) (Lee *et al.*, 2007). In addition, Campbell *et al.* (2007) reported that the saturating irradiance of *E. acoroides* at Orman reef, Australia, at a depth of 2 m was  $365.5 \pm 52.7 \mu\text{mole/s/m}^2$ . Their analysis was that *E. acoroides* at their study site grew under light saturated conditions. Compared with my results, the lowest light intensity was  $408.13 \pm 40.11 \mu\text{mole/s/m}^2$  at 100% density (natural density), which was higher than the reported by Campbell *et al.* (2007) and Agawin *et al.* (2001). The light intensity in this study was measured around noon when there was the highest light intensity. Thus, the light intensity in the morning and evening should be lower than the saturating irradiance ( $365.5 \pm 52.7 \mu\text{mole/s/m}^2$ ). In addition, *E. acoroides* has long leaves, which often overlay each other, thus reducing the light transmission to the photosynthetic tissue that promoted the self-shading effect. Therefore, it is likely that *E. acoroides* at the study site might encounter light limiting conditions.

In this study, LAI and leaf surface area were considered as good variables to determine growth since they significantly responded to light reduction. Moreover, these variables could reveal growth of seagrasses on the larger scale, which is more useful than an individual level. LAI ( $\text{m}^2$  leaves/ $\text{m}^2$ ) was calculated from the shoot density, leave surface area and by unit area. These three factors could well reflect growth of seagrass meadow in monitoring site. New leaf production, leaf elongation rate and leaf plastochrone interval did not show significantly response in growth among treatments since they might have energy transport from neighbor ramets, which is well known in seagrasses and clonal plants (Aplert, 1996; Aplert, 1999; Hemminga and Duarte, 2000)

### **The effect of shoot density on recruitment of *Enhalus acoroides* (L.f.) Royle**

Seagrasses are clonal plants that occupy an area by horizontal growth. They employ various physiological strategies when competing for resources. These strategies have been described as *guerilla* and *phalanx* strategies (Lovett-Doust, 1981). The term *guerilla* is defined as the fast growing plant, that exploits resources quickly. In general they are small species and are considered to be opportunistic species. In contrast, the term *phalanx* defines slow growing plants most *phalanx* species are large and long-lived (Ralph *et al.*, 2007).

According to the above definition, *E. acoroides* would be considered to be a *phalanx* species because it is the largest species (nearly 1 m in high), has high weight (7.4 g DW/ramet, Terrados *et al.*, 1999b) and has the slowest rhizome elongation rate of all the other seagrasses (0.9 cm/year; Duarte, 1991b). Moreover, the horizontal branching angle is less wide (57 degree) compared with the small species such as *Halophila ovalis* (72 degree) (Marbà and Duarte, 1998) which is considered to be an opportunistic species. These characters also suggest that *E. acoroides* has a lower potential to occupy a wide space in a short time compared with the smaller species. Although space does not seem to be a limiting factor according to my results, I calculated *percent rhizome occupation* as a rough estimate of the total volume of new rhizome segments produced in one year divided by the total volume of the experimental plot (see appendix). In the 100% density treatment the percent rhizome occupation was estimated to be 0.83% (based on 10 cm of rhizome depth; total

internodes, 15 internodes/year; rhizome diameter, 1.0 cm and density, 35 shoots/plot). Therefore, from this estimation I found that in 1 year new segments of *E. acoroides* rhizomes occupied only 0.83% of the experimental plot. This information implies that space was not a limiting factor for large species as *E. acoroides*.

However, the present results show a significant difference in recruitment rate between densities. This might be due to light attenuation within the canopy. Compared with seagrasses that grow at a deep site with low light intensity (such as *P. sinuosa*, Collier, 2006), the population was less dense which reduces the effect of self-shading, compensating for the gradient of light availability at the top of the canopy (Collier, 2006) and decrease respiratory demand as well (Campbell and Miller, 2002). Therefore, the low recruitment rate at high densities must be assumed to reduce self-shading. The results of the present study was similar those of Viejo and Åberg (2001) on modules of the brown seaweed *Ascophyllum nodosum* (L.) Le Jol at the Isle of Man, Northeastern Atlantic Ocean, in which populations were manipulated to have 3 densities (low, mid and high), and the results show that the number of new shoots in the low density treatment was higher than in the mid and high density as well. They mentioned that small adult plants growing beneath the canopy might suffer from reduction of light.

In addition, there was an effect of environmental factors on recruitment rate of *E. acoroides* as indicated by the fact that at all densities it dropped in September 2006, and had a constant low rate to January 2007. These drops might have been due to the effect of high wave motion during the monsoon season as indicated by a large quantity of leaf litter on the ground and rhizomes in some experimental plots were emerged. Recruitment dropped again in March 2006, which might have been the chronic effect of long exposure to the air when low tides were during the day time.

However, temperature might be another factor that affects production of new shoots as indicated by a study on *E. acoroides* in the western Gulf of Carpentaria, Australia, by Kenyon *et al.* (1997). They reported that the smallest number of new shoots was found between January and June when water temperature were high but declining (33- 24 °C) while, the greatest was found in September and November when water temperatures were high and increasing (30-32 °C). Compared with this present study, the low recruitment rate was found from September 2006 to



January 2007, when water temperatures were low (27.8- 30.5 °C) and the rate of increase of new shoots was found from April to June when water temperatures were high (30.7-33.5 °C). Therefore, temperature variation throughout the year seems to effect the recruitment rate of *E. acoroides*.

### **The effect of shoot density on sexual reproduction of *Enhalus acoroides* (L.f.) Royle**

Total flower and female flower production of *E. acoroides* were different between densities, while the male flower production was not different. Therefore the difference of total flower production is due to the high number of female flowers. The results clearly show that total flower and female flowers in low density treatments were higher than in high density treatments and the tendency of male flower production as well. These results were similar to a previous study in *Zostera marina* L. that showed the percentage of reproductive shoots of most low density populations were higher than in high density populations (17% in 162 shoot/m<sup>2</sup>, (Bayer, 1979); 2% in 600-800 shoots/m<sup>2</sup>, (Olesen and Sand-Jensen, 1994b); 4% in <1,000 – 1,800 shoots/m<sup>2</sup>, (Sand-Jensen, 1975); 2% in 550-1,400 shoots/m<sup>2</sup>, (Wium-Anderson and Brorum, 1984); 5% in 279-929 shoots/m<sup>2</sup>, (Poumian-Tapia and Ibarra-Obanda)). This result might be due to the effect of light availability in the canopy of *E. acoroides*. In general flowering intensities of *E. acoroides* were low in dark environments (at depths and/ or turbid waters). The research of Rollón *et al.* (2003) at 5 sites in different environments in the northwest Philippines shows that the intensity of flowering correlated positively with light level and they suggested that light provided sufficient energy to induce flowering and in addition, light level may be a trigger for male flower release due to oxygen production in their tissue. Therefore male flower discharge is most likely to occur from noon until late afternoon when oxygen production is high and gas is saturated inside the tissue. No releases were found in the early morning. However, my result is in contrast with that reported by Vermaat *et al.* (2004) based on their study of the effect of meadow fragmentation and reproductive output of *E. acoroides* in the multispecific seagrass meadow at Santiago Island, Philippines. It seems clear that the abundance of male and possibly also female inflorescences of *E. acoroides* increases with overall seagrass cover. This is an

expected consequence of higher abundance of plants which is characteristic of the less fragmented seagrass meadow.

Although, fruit production was not significantly different between densities, the trend showed that the number of fruits at low densities was higher probably due to the difference of female flower production in each density. However, the study of Vermaat *et al.* (2004) showed that the proportion of female flowers that developed fruits increase sharply when overall seagrass cover was around 50%. They suggested that the possible mechanism was the influence of seagrass canopies on water motion, that is, the increasing seagrass canopy density might increase the opportunity to trap the surface-dispersed pollen. This is similar to the observation of Brouns and Heijs (1986) at Papua New Guinea where they found that male flowers of *E. acoroides* were trapped and conglomerated between the brown seaweeds *Padina tenuis* (Bory) Wom & Bali and *Padina australis* Hauck.

In addition, I observed that *E. acoroides* produced flowers all year round which is similar to previous studies in the Andaman sea and the Gulf of Thailand, (Lewmanomont *et al.*, 1996); Papua New Guinea, (Bronus and Heijs, 1986); and Australia, (Kenyon *et al.*, 1997). Surprisingly, fruit production was observed only during the last half year of the study (February 2007 – July 2007). This may be due to the high wave motion and low light intensity during the monsoon season causing damage to the fruits and flowers since it was observed that the total number of flowers was low. In fact, female flowers were absent in September 2006. These results were similar to those of the recruitment study which showed low recruitment rates in September 2006 as well.

## CHAPTER 5

### CONCLUSIONS

From this study, it can be conclude that:

Intraspecific competition did not occur in the monospecific meadow of *Enhalus acoroides* at Laem Yong Lam according to the self thinning rule. However, there was a negative relationship between shoot density and shoot weight of this species. Shoot density of *E. acoroides* affected the light intensity within the canopy by self shading.

With respect to growth, the interaction between density and month affected all growth variables in short term observations, but for long term observations shoot density affected only the leaf surface area and leaf area index.

The interaction between density and season influenced the recruitment rate of *E. acoroides* in both the short and long term studies. Moreover, asexual reproduction supported formation of new shoots more than sexual reproduction.

The interaction between density and season affected only female flowers and total flower production. However, both male and female flowers were produced all year round but fewer male flowers were produced than female. Fruits were found only from the summer to the early monsoon season (February to July 2007).

Further investigations are required to answer the questions:

1.1 How do seagrasses adapt their physiology, such as rates of nutrient up take, photosynthesis and respiration, to live in high density populations?

1.2 Is there intraspecific competition with other seagrasses especially the opportunistic and rapid growth species such as *Halophila* spp.?

## REFERENCES

- Agawin, N. S. R., Duarte, C. M. and Fortes, M. D. 1996. Nutrient Limitation of Philippines Seagrasses (Cape Bolinao, NW Philippines): In situ Experimental Evidence. *Marine Ecology Progress Series*. 138: 233-243.
- Agawin, N. S. R., Duarte, C. M., Fortes, M. D., Uri, J. S. and Vermaat, J. E. 2001. Temporal Changes in the Abundance, Leaf Growth and Photosynthesis of Three Co-occurring Philippines Seagrasses. *Journal of Experimental Marine Biology and Ecology*. 260: 217-239.
- Alpert, P. 1996. Nutrient Sharing in Clonal Fragments of *Fragaria chiloensis*. *Journal of Ecology*. 84: 395-406.
- Alpert, P. 1999. Clonal Integration in *Fragaria chiloensis* Differs Between Populations: Ramets from Grassland are Selfish. *Oecologia*. 120: 69-76.
- Bayer, R. D. 1979. Intertidal Zonation of *Zostera marina* in the Yaquina Estuary, Oregon. *Syesis*. 12: 147-153.
- Begon, M., Harper, J. L. and Townsend, C. R. 1996. *Ecology: Individuals, Populations and Communities*. 3<sup>rd</sup> Edition. Blackwell Science. Oxford.
- Brouns, J. J. W. M. and Heijls, F. M. L. 1986. Production and Biomass of the Seagrass *Enhalus acoroides* (L.f.) Royle and Its Epiphytes. *Aquatic Botany*. 25: 21-45.
- Campbell, S. J. and Miller, C. J. 2002. Shoot and Abundance Characteristics of the Seagrass *Heterozostera tasmanica* in Westernport Estuary (South-Eastern Australia). *Aquatic Botany*. 73: 33-46.
- Campbell, S. J., McKenzie, L. J., Kerville, S. P. and Bité. 2007. Patterns in Tropic Seagrass Photosynthesis in Relation to Light, Depth and Habitat. *Estuarine, Coastal and Shelf Science*. 73: 551-562.
- Carruthers, T. J. B., Dennison, W. C., Longstaff, B. J., Waycott, M., Abal, E. G., McKenzie, L. J. and Lee Long, W. J. 2002. Seagrass Habitats of Northeast Australia: Models of Key Processes and Controls. *Bulletin of Marine Science*. 71(3): 1153 – 1169.

- Collier, C. J. 2006. Characterising Responses of the Seagrass *Posidonia sinuosa* to Changes in Light Availability. Ph. D. Thesis, Faculty of Computing, Health and Science. Edith Cowan University.
- Dalla Via, J., Sturmbauer, C., Schönweger, G., Sötz, E., Mathekowitsch, S., Stifter, M. and Rieger, R. 1998. Light gradients and Meadow Structure in *Posidonia oceanica*: Ecomorphological and Function Correlates. Marine Ecology Progress Series. 163: 267-278.
- Dennison, W. C. 1987. Effects of Light on Seagrass Photosynthesis, Growth and Depth Distribution. Aquatic Botany. 27: 15-26.
- Dennison, W. C., Orth, R. J., Moore, K. A., Stevenson, J. C., Carter, V., Kollar, S., Bergstrom, P. W. and Batiuk, R. A. 1993. Assessing Water Quality with Submersed Aquatic Vegetation. BioScience. 43(2): 86-94.
- Duarte, C. M. 1990. Seagrass Nutrient Content. Marine Ecology Progress Series. 62: 201-207.
- Duarte, C. M. 1991a. Seagrass Depth Limits. Aquatic Botany. 40: 363-377.
- Duarte, C. M. 1991b. Allometric Scaling of Seagrass Form and Productivity. Marine Ecology Progress Series. 77: 289-300.
- Duarte, C. M., Terrados, J., Agawin, N. S. R., Fortes, M. D., Bach, S. and Kenworthy, W. J. 1997. Response of a Mixed Philippines Seagrass Meadow to Experiment Burial. Marine Ecology Progress Series. 147: 285-294.
- Duarte, C. M., Terrados, J., Agawin, N. S. R. and Fortes, M. D. 2000. An Experimental Test of Occurrence of Competitive Interaction Among SE Asian Seagrasses. Marine Ecology Progress Series. 197: 231-240.
- Erftemeijer, P. L. A., Stapel, J., Smekens, M. J. E. and Drossaert, W. M. E. 1994. The Limited Effect of in situ Phosphorus and Nitrogen additions to Seagrass Beds on Carbonate and Terrigenous Sediments in South Sulawesi, Indonesia. Journal of Experimental Marine Biology and Ecology. 182: 123-140.
- Hydrographic Department Royal Thai Navy. 2006. Tide Tables Thai Waters Mae Nam Chaophraya-Gulf of Thailand and Andaman Sea. Hydrographic Department: Bangkok.



- Hydrographic Department Royal Thai Navy. 2007. Tide Tables Thai Waters Mae Nam Chaophraya-Gulf of Thailand and Andaman Sea. Hydrographic Department: Bangkok.
- Gacia, E., Duarte, C. M., Matbà, N., Terrados, J., Kennedy, H., Fortes, M. D. And Tri, N. H. 2003. Sediment Deposition and Production in SE-Asia Seagrass Meadow. *Estuarine, Coastal and Shelf Science*. 56: 909-919.
- Gordon, D. M., Grey, K. A. Chase, S. C. and Simpson C. J. 1994. Changes to the Structure and Productivity of a *Posidonia sinuosa* meadow During and After Imposed Shading. *Aquatic Botany*. 47: 265-275.
- Green, E. P. and Short, F. T. 2003. World Atlas of Seagrasses. University of California Press. Berkeley. USA.
- Grime, J. P. 2001. Plant Strategies, Vegetation Processes, and Ecosystem Properties. 2<sup>nd</sup> Edition. John Wiley & Sons, LTD.UK.
- Hemminga, M. A. 1998. The Root/ Rhizome System of Seagrass: an Asset and a Burden. *Journal of Sea Research*. 39: 183-196.
- Hemminga, M. A. and Duarte, C. M. 2000. Seagrass Ecology. Cambridge University Press. UK.
- Kenyon, R. A., Conacher, C. A. and Poiner, I. R. 1997. Seasonal Growth and Reproduction of *Enhalus acoroides* (L.f.) Royle in a Shallow Bay in the Western Gulf of Carpentaria, Australia. *Marine Freshwater Research*. 48: 335-342.
- Koike, I., Nakaoka, M., Iizumi, H., Umezawa, Y., Kuramoto, T., Komatsu, T., Yamamura, M., Kogure, K., Supawanid, C. and Lewmanomont, K. 1999. Environmental Factors Controlling Biomass and Production of Seagrasses of Southern Thailand. *In: Grazing and Disturbance by Dugongs and Turtles on Tropical Seagrass Ecosystem*. Ocean Research Institute. The University of Tokyo. 66-81.
- Lee, K.-S., Park, S. R. and Kim, Y. K. 2007. Effects of Irradiance, Temperature, and Nutrients on Growth Dynamics of Seagrasses: A review. *Journal of Experimental Marine Biology and Ecology*. 350: 144-175.

- Lewmanomont, K., Deetae, S. And Srimanobhas, V. 1996. Seagrass of Thailand. In: Kuo, J., Phillips, R., Walker, D. I. and Kirman, H. (eds.). Seagrass Biology: Proceeding of and International Workshop, Rottnest Island, Western Australia, 25-29<sup>th</sup> January 1996. Faculty of Science, University of Western Australia. 21-26.
- Lewmanomont, K. and Supanwanid, C. 2000. Species Composition of Seagrass at Haad Chao Mai National Park, Trang Province, Thailand. Kasetsart University Fishery Research Bulletin. 22: 1-9.
- Longstaff, B. J. and Dennison, W. C. 1999. Seagrass Survival During Pulsed Turbidity Events: The Effects of Light Deprivation on the Seagrasses *Halodule pinifolia* and *Halophila ovalis*. Aquatic Botany. 65: 101-121.
- Lovett-Doust, L. 1981. Population Dynamics and Local Specialization in a Clonal Perennial (*Ranunculus repens*) I. The Dynamics of Ramets in Contrasting Habitats. Journal of Ecology. 69: 742-755.
- Marbà, N. and Duarte, C. M. 1998. Rhizome Elongation and Seagrass Clonal Growth. Marine Ecology Progress Series. 174: 269-280.
- Nakaoka, M. and Supanwanid, C. 2000. Quantitative Estimation of the Distribution and Biomass of Seagrasses at Haad Chao Mai National Park, Trang Province, Thailand. Kasetsart University Fishery Research Bulletin. 22: 10-22.
- Olesen, B. and Sand-Jensen, K. 1994a. Biomass-Density Patterns in the Temperate Seagrass *Zostera marina*. Marine Ecology Progress Series. 109: 283-291.
- Olesen, B. and Sand-Jensen, K. 1994b. Demography of Shallow Eelgrass (*Zostera marina*) Populations Shoot Dynamics and Biomass Development. Journal of Ecology. 82: 379-390.
- Olesen, B., Enríquez, S., Duarte, C. M. and Sand-Jensen, K. 2002. Depth-Acclimation of Photosynthesis, Morphology and Deography of *Posidonia oceanica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. Marine Ecology Progress Series. 236: 89-97.
- Phillips, R. C. and Meñez, E. G. 1998. Seagrass. Smithsonian Institution press. Washington D. C., USA.

- Poovachiranon, S., Adulyanukosol, K., Saelim, K., Charoenpornwattana, A. Yaem-arunchai, C. and Wuththivorawong, C. 2006. Seagrasses in Thai Waters. Phuket Marine Biological Center : Phuket (In Thai).
- Poumian-Tapia, M. and Ibarra-Obanda, S. E. 1999. Demography and Biomass of the Seagrass *Zostera marina* in a Mexican Coastal Lagoon. *Estuarine*. 22(4): 837-847.
- Ralph, P. J., Durako, M. J., Enríquez, S., Collier, C. J. and Doblin, M. A. 2007. Impact of Light Limitation on Seagrasses. *Journal of Experimental Marine Biology and Ecology*. 350: 176-193.
- Rautiainen, P., Koivula, K. and Hyvärinen, M. 2004. The Effect of Within-Genet and Between-Genet competition on Sexual Reproduction and Vegetative Spread in *Potentilla anserine* spp. *egedii*. *Journal of Ecology*. 92: 505-511.
- Rollón, R., de Ruyter van Sterveninck, E. D. and van Viersse, W. 2003. Spatio-Temporal variation in Sexual Reproduction of the Tropic Seagrass *Enhalus acoroides* (L.f.) Royle in Cape Bolinao, NW Philippines. *Aquatic Botany*. 76: 339-354.
- Rose, C. D. and Dawes, C. J. 1999. Effects of Community Structure on the Seagrass *Thalassia testudinum*. *Marine Ecology Progress Series*. 184: 83-95.
- Sand-Jensen, K. 1975. Biomass, Net Production, and Growth Dynamics in an Eelgrass (*Zostera marina* L.) Population in Vellerup Vig, Denmark. *Ophelia*. 14: 185- 201.
- Short, F. T. 1987. Effects of Sediment Nutrients on Seagrasses: Literature Review and Mesocosm Experiment. *Aquatic Botany*. 27: 41-57.
- Short, F. T. and Duarte, C. M. 2000. Method for the Measurement of Seagrass Growth and Production. In: Short, F. T., Coles, R. G. and Short, C. A. (eds.), *Global Seagrass Research Methods*. Elsevier Science B. V., Amsterdam. 155-182.
- Smith, R.L. and Smith, T. M. 2001. *Ecology and Field Biology*. 6<sup>th</sup> Edition. Addison Wesley Longman, Inc. USA.
- Supanwanid, C. and Lewmanomont, K. 2003. The Seagrasses of Thailand. In: Green, E. P. and Short, F. T. (eds.), *World Atlas of Seagrasses*. University of California Press. Berkeley. USA. 144-151.

- Tanaka, N., Uehara, K. And Murata, J. 2004. Correlation Between Pollen Morphology and Pollination Mechanisms in the Hydrocharitaceae. *Journal of Plant Research*. 117: 265-276.
- Terrados, J., Duarte, C. M., Fortes, M. D., Borum, J., Agawin, N. S. R., Bach, S., Thampanya, U., Kamp - Nielsen, L., Kenworthy, W. J., Geertz – Hansen, O. and Vermaat, J. 1998. Changes in Community Structure and Biomass of Seagrass Communities along Gradients of Siltation in SE Asia. *Estuarine, Coastal and Shelf Science*. 46 : 757 – 768.
- Terrados, J., Agawin, N. S. R., Duarte, C. M., Fortes, M. D., Kamp - Nielsen, L. and Borum, J. 1999a. Nutrient Limitation of the Tropic seagrass *Enhalus acoroides* (L.) Royle. In Cape Bolinao, NW Philippines. *Aquatic Botany*. 65 : 123-139.
- Terrados, J., Borum, J., Duarte, C. M., Fortes, M. D., Kamp - Nielsen, L. Agawin, N. S. R. and Kenworthy, W. J. 1999b. Nutrient and Mass Allocation of South-East Asian Seagrass. *Aquatic Botany*. 63: 203-217.
- Umezawa, Y., Koike, I. and Lewmanomont, K. 1999. Evolution of Nitrogen Environment at Coastal Waters of Southern Thailand from Chemical Composition and Species of Macroalgae. *In: Grazing and Disturbance by Dugongs and Turtles on Tropical Seagrass Ecosystem*. Ocean Research Institute. The University of Tokyo. 1-16.
- Van Kleunen, M. Fischer, M and Schmid, B. 2001. Effects of Intraspecific Competition on Size Variation and Reproductive Allocation in a Clonal Plant. *Oikos*. 94: 515-524.
- Vermaat, J. E., Rollon, R. N., Lacap, C. D., Billot, C., Alberto, F., Nacorda, H. M. E., Wiegman, F. and Terrados, J. 2004. Meadow Fragmentation and Reproductive Output of the SE Asian Seagrass *Enhalus acoroides*. *Journal of Sea Research*. 52: 312-328.
- Viejo, R. M. and Åberg, P. 2001. Effects of Density on Vital rates of a Modular Seaweed. *Marine Ecology Progress Series*. 221: 105 – 115.
- Walker, D. I., Olesen, B. and Phillips, R. C. 2001. Reproduction and Phynology in Seagrasses. *In : Short, F. T., Cloes, R. G. and Short, C. A.(eds.). Global Seagrass Research Methods*. Elsevier Science B. V., Amsterdam. 59 – 78.

- Wang, L. W., Showalter, A., Ungar, I. A. 2005. Effects of Intraspecific Competition on Growth and Photosynthesis of *Atriplex prostrata*. *Aquatic Botany*. 83: 187 - 192
- Waycott, M., McMahon, K., Mellors, J., Calladine, A. and Kleine, D. 2004. A Guide to Tropical Seagrasses of the Indo-West Pacific. James Cook University. Queensland, Australia.
- White, J. 1980. Demographic factors in Populations of Plant. *In*: Solbrig, O. T. (ed.). *Demography and Dynamics of Plant Populations*. Blackwell, Oxford. 21-48.
- Wium-Anderson, S. and Brorum, J. 1984. Biomass Variation and Autotrophic Production of an Epiphyte-Macrophyte Community in a Coastal Danish Area: I. Eelgrass (*Zostera marina* L.) Biomass and Net Production. *Ophelia*. 23: 33-46.
- Worm, B. and Reusch, T. B. H. 2000. Do Nutrient Availability and Plant Density Limit Seagrass Colonization in the Baltic Sea. *Marine Ecology Progress Series*. 200: 159-166.
- Yoda, K., Kira, T., Ogawa, H. and Hozumi, K. 1963. Self-thinning in Overcrowded Pure Stands Under Cultivated and Natural Conditions (Intraspecific Competition Among Higher Plants XI). *Journal of the Institute of Polytechnics, Osaka City University, Series D*. 14: 107-129.



## Appendix

# Appendix 1. The calculation of percent rhizome occupation

$$\text{Percent rhizome occupation} = 100 \times (V_r / V_{\text{exp}})$$

When  $V_r$  = total volume of new rhizome segment produced in a year ( $\text{cm}^3$ )

$V_{\text{exp}}$  = total volume of experimental plot ( $\text{cm}^3$ )

$$V_{\text{exp}} = (W_p) \times (L_p) \times (D_r)$$

When  $W_p$  = the width of experimental plot (cm)

$L_p$  = the length of experimental plot (cm)

$D_r$  = the depth of rhizome level (cm)

$$V_o = \pi r^2 L D$$

When  $r$  = radius of rhizome (cm)

$L$  = the total length of rhizomes produced in a year (cm/ shoot/ plot/year)

$D$  = shoot density in experimental plot (shoot/ plot)

$\pi$  ~ 22/ 7 or 3.14

$$L = N_i \times I_l$$

When  $N_i$  = total number of internodes produce in a year (# internode/ plot/ year)

$I_l$  = internode length (cm/ internode)

Note. In case of *E. acoroides*,  $N_i$  can be estimated by

$$N_i = N_n - 1$$

When  $N_n$  = Total number of nodes produced in a year (# node/ plot/ year)

In the case of *E. acoroides*,  $N_n$  can be estimated by the total number of new leaves produced in a year.

***For example***

In 1 year of observation of *Enhalus acoroides* (L.f.) Royle in 100% density treatment (35 shoots/ plot) sixteen new leaves/ shoot were produced, the average internode length was 0.5 cm, rhizome diameter was 1 cm, the rhizomes of *E. acoroides* were found at 10 cm depth and the quadrat size was 50 x 50 cm.

So:

$$N_i = (N_n) - 1$$

In *E. acoroides*,  $N_i$  was estimated from total number of new leaves in a year

$$\begin{aligned} N_i &= (16 \text{ nodes/ plot/ year}) - 1 \\ &= 15 \text{ internodes/ plot/ year} \end{aligned}$$

Then:

$$\begin{aligned} L &= (N_i) \times (I_i) \\ L &= (15 \text{ internodes/ shoot/ plot/year}) \times (0.5 \text{ cm/ internode}) \\ &= 7.5 \text{ cm/ shoot/ plot/ year} \end{aligned}$$

Then:

$$\begin{aligned} V_r &= \pi r^2 L D \\ V_r &= \pi (0.5 \text{ cm})^2 \times (7.5 \text{ cm/ shoot/ plot/ year}) \times (35 \text{ shoot/ plot}) \\ &= 206.25 \text{ cm}^3 / \text{year} \end{aligned}$$

Then:

$$\begin{aligned} V_{\text{exp}} &= (W_p) \times (L_p) \times (D_r) \\ V_{\text{exp}} &= 50 \text{ cm} \times 50 \text{ cm} \times 10 \text{ cm} \\ &= 25,000 \text{ cm}^3 \end{aligned}$$

Therefore:

$$\begin{aligned} \text{Percent rhizome occupation} &= 100 \times (V_r / V_{\text{exp}}) \\ \text{Percent rhizome occupation} &= 100 \times (206.25 / 25,000) \\ &= 0.83 \% \end{aligned}$$

Appendix 2. Summary of statistical analysis of variable in short term study, \*  $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; ns = non significant.

Variables	Density	Month	Density x Month
<b>Growth study</b>			
- Leaf elongation rate	0.070 <sup>ns</sup>	0.000**	0.003**
- Leaf plastochrone interval (PL)	0.395 <sup>ns</sup>	0.000**	0.000**
- Number of leaf	0.000**	0.000**	0.000**
- Leaf surface area	0.000**	0.000**	0.000**
- Leaf Area Index (LAI)	0.000**	0.000**	0.000**
<b>Recruitment study</b>			
- Recruitment rate	0.000**	0.000**	0.000**
<b>Sexual Reproduction study</b>			
- Male flower production	0.060 <sup>ns</sup>	0.812 <sup>ns</sup>	0.224 <sup>ns</sup>
- Female flower production	0.037*	0.050*	0.004**
- Total flower production	0.006**	0.044*	0.003**
- Fruit production	0.269 <sup>ns</sup>	0.668 <sup>ns</sup>	0.657 <sup>ns</sup>

Appendix 3. Summary of statistical analysis of variable in long term study, \*  $P \leq 0.05$ ;

\*\* $P \leq 0.01$  ; ns = non significant.

Variables	P -value
<b>Growth study</b>	
- Leaf elongation rate	0.209 <sup>ns</sup>
- Leaf plastochrone interval (PL)	0.574 <sup>ns</sup>
- New leaf production	0.791 <sup>ns</sup>
- Cumulative new leaf production	0.062 <sup>ns</sup>
- Number of leaf	0.254 <sup>ns</sup>
- Leaf surface area	0.030*
- Leaf Area Index (LAI)	0.000**
- Above ground weight	0.001**
<b>Recruitment study</b>	
- Recruitment rate	0.000**
<b>Sexual Reproduction study</b>	
- Male flower production	0.054 <sup>ns</sup>
- Female flower production	0.008**
- Total flower production	0.000**
- Fruit production	0.237 <sup>ns</sup>



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B. Sc. (Aquatic Science) (First Class Honors)	Prince of Songkla University	2005

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### List of Publication and Proceeding

- Rattanachot, E., Tantiprapas, P., Wichachucherd, B., Nokkate, N. and Prathep, A. 2008. Seasonal variation in coverage, canopy height, fruit density and biomass of subtidal seagrass *Halophila decipiens* Ostenfeld in Chumphon Coast, The Gulf of Thailand. Proceeding of The 6<sup>th</sup> Regional IMT-GT UNINET Conference 2008. The Gurney Hotel, Penang, Malaysia, 28-30 August 2008.
- Tuntiprapas, P., Rattanachot, E., Pongparadon, S., Promdam, R., Raungprataungsuk, K., Phatthnarhatcharoen, C., Panumpun, K., Greenberg, D. B. and Prathep, A. 2008. The effect of seagrass coverage on species diversity and abundance of swimming crabs (Portunidae) at Had Khanom - Moo Koh Talay Tai National Park, Thailand. Proceeding of The 6<sup>th</sup> Regional IMT-GT UNINET Conference 2008. The Gurney Hotel, Penang, Malaysia, 28-30 August 2008.
- Rattanachot, E., Short, F. T. and Prathep, A. The effect of shoot density on growth, recruitment and Reproduction of *Enhalus acoroides* (L. f.) Royle at Haad Chao Mai National Park, Trang Province, Thailand. (In preparation).