Effect of Seasonal Variation on Growth and Sexual Reproduction of *Thalassia hemprichii* (Ehrenb.) Asch. in Haad Chao Mai National Park, Trang Province, Thailand

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
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Thesis Title   Effect of Seasonal Variation on Growth and Sexual
Reproduction of *Thalassia hemprichii* (Ehrenb.) Asch. in
Haad Chao Mai National Park, Trang Province, Thailand

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ชื่อวิทยานิพนธ์   ผลจากความแปรผันของฤดูกาลต่อการเจริญเติบโต และการสืบพันธุ์
แบบอาศัยเพศของหญ้าทะเล *Thalassia hemprichii* (Ehrenb.)
Asch. ในบริเวณอุทยานแห่งชาติหาดเจ้าไหม จังหวัดตรัง

ผู้เขียน   นายปิยะลาภ ตันติประภาส

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บทคัดย่อ

การเปลี่ยนแปลงตามฤดูกาล ส่งผลต่อการเปลี่ยนแปลงของปัจจัยสิ่งแวดล้อมต่างๆ นั้น มี
ผลต่อการเจริญเติบโต และการสืบพันธุ์ของหญ้าทะเล การศึกษาครั้งนี้ได้ศึกษาความสัมพันธ์
ระหว่างปัจจัยสิ่งแวดล้อมต่างๆ ได้แก่ ความเข้มแสง (LI)   อุณหภูมิ (T) ความเค็ม (S)
สารอาหารในมวลน้ำ (N_{water}) สารอาหารในดิน ($N_{sediment}$) จำนวนเวลาในช่วงที่แนวหญ้าทะเล
โผล่พ้นน้ำ (H) และ จำนวนความหนาแน่นของกุ้งดีดขัน (B) ต่อ การเติบโตและการสืบพันธุ์
ของหญ้าทะเล *Thalassia hemprichii* (Ehrenb.) Asch. ในบริเวณอุทยานแห่งชาติหาดเจ้า
ไหม จังหวัดตรัง ประเทศไทย ในระหว่างเดือน กุมภาพันธ์ 2551 ถึง มกราคม 2552 โดยทำการ
หาค่าของ การเติบโตของใบ (อัตราการยาวของใบ (L_E) การเพิ่มมวลชีวภาพของใบ (L_G) และ
ช่วงเวลาในการสร้างใบใหม่ (P_L)) และ   การเติบโตของผืนหญ้า (มวลชีวภาพส่วนบนดิน มวล
ชีวภาพส่วนใต้ดิน และ น้ำหนักทุกส่วนของหญ้าทะเล   (ใบ ลำต้นใต้ดิน และ ราก) ตลอดจน
ความหนาแน่นของต้นหญ้า ซึ่งทำการนับในแปลงถาวร (0.25 ม x 0.25 ม) รวมถึงติดตามการ
ออกดอก ออกผล ของหญ้าทะเลในแปลงถาวร (1 ม x 1 ม) ด้วยเช่นกัน โดยผลจากการทดสอบ
ทางสถิติด้วย One-way ANOVA พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติของ LI, T, S,
H, และ B รวมถึงการเติบโตของใบ และบางค่าของการเติบโตของผืนหญ้าทะเล (ลำต้นใต้ดิน
ราก มวลชีวภาพส่วนใต้ดิน และความหนาแน่น) การศึกษาครั้งนี้พบว่าจำนวนเวลาในช่วงที่แนว
หญ้าทะเลโผล่พ้นน้ำ และความหนาแน่นของกุ้งดีดขัน (Alpheidae) น่าจะเป็นปัจจัยหลักในการ
ควบคุมการเติบโตของหญ้าทะล และการออกดอกและผลในผืนหญ้าทะเล *T. hemprichii*

Thesis Title   Effect of seasonal variation on growth and sexual reproduction of *Thalassia hemprichii* (Ehrenb.) Asch. in Haad Chao Mai National Park, Trang Province, Thailand.

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Abstract

Temporal variation influences environmental parameters, affects growth and reproduction of seagrasses. This study examined the relationship between environmental parameters i.e., light intensity (LI), temperature(T), salinity(S), nutrients in seawater (N_{water}) and sediment ($N_{sediment}$), exposure hour (H) and burrowing shrimp density (B) on growth and reproduction of *Thalassia hemprichii* (Ehrenb.) Asch. at the Haad Chao Mai National Park, Trang province, Thailand between February 2008-January 2009. Leaf growth characters (Leaf elongation rate (L_E), leaf growth (L_G) and leaf plastochrone interval (P_L)), and meadow characters (above ground, below ground biomass per area, leaf biomass, rhizome and root) were investigated; the shoot density were also counted in the permanent quadrate (0.25 m x 0.25 m). The phenology of flowering and fruiting were also observed in the plot (1 m x 1 m). One-way ANOVA revealed that there were significant differences in LI, T, S, H, and B, and all leaf growth characters and some of meadow growth characters (rhizome, root, below ground biomass and shoot density) among months. The study revealed that exposure hours influenced leaf growth characters; and density of burrowing shrimp (Alpheidae) seemed to be the main driving force on growth and reproduction in *Thalassia* bed.

ACKNOWLEDEMENTS

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CHAPTER 1

INTRODUCTION

Seagrasses are marine flowering plants that play an important role in marine ecosystem, they filter estuarine and coastal waters of nutrients, contaminants, and sediments, and are closely linked to other communities e.g. coral reef and mangrove systems (Hemminga and Duarte, 2000; Nybakken, 2001). They are primary producers providing food and also habitat for many organisms such as benthic fauna, epifauna, nekton (Bell *et al*., 2001; Hovel and Lipcius, 2001; Hovel 2003, Connolly and Hindell, 2006; Bostrom *et al*., 2006) and large marine animals, that are endangered species (e.g. sea turtle and dugong), which use seagrass as their most important food source (Marsh *et al*., 2002).

Except on the Antarctic shore, seagrass occurs in most coastal area of the world (Hemminga and Duarte, 2000, Green and Short, 2003). About 60 seagrass species are found in the world, with 24 species being the most diverse flora, in the Indo-Pacific region (Short *et al*., 2001). In Thailand, 12 species of seagrass have been reported (Poovachiranon *et al*., 1994; Lewmanomont and Ogawa, 1995; Supanwanid and Lewmanomont, 2003; Poovachiranon *et al*., 2006). Seagrass beds in Haad Chao Mai National Park are mix beds which contain up to 10 species of seagrass (Poovachiranon, 2000; Lewmanomont and Supanwanid, 2000). In this area, Nakaoka and Supanwanid (2000) found that *Enhalus acoroides* (L. f.) Royle*, Halophila ovalis* (R. Br.) Hook. f. and *Thalassia hemprichii* (Ehrenb.) Asch. are most dominant. Not only in Thailand but also throughout SE Asia, *T. hemprichii* is the dominant species in mix seagrass beds (Vermaat *et al*., 1995; Terrados *et al*., 1998; Lacap *et al*., 2002). In Thailand, most seagrass studies investigated the composition and distribution of seagrass (Poovachiranon *et al*., 1994; Changsang and Poovachiranon, 1994; Poovachiranon and Changsang, 1994; Lewmanomont *et al*., 1996; Meesawat *et al*., 1999; Nakaoka and Supanwanid, 2000), but biology, phenology and ecology studies of seagrass are still very limited.

Basic requirement of seagrass for growth are similar to terrestrial angiosperms. However, seagrass in the marine realm is exposing to many different environmental conditions from terrestrial habitat. The environmental conditions such as light, temperature, salinity and nutrient had effect on the seagrass beds and seem to be the important major conditions (Hemminga and Duarte, 2000; Lee *et al.*, 2007). Light is the important source for photosynthesis mechanism that makes energy to seagrass growth and functioning. The penetration of light through the natural water is less than through the air. Furthermore, light intensity is rapidly decreased with water depth and contribution of absorption light by particulate in the water that make an attenuation of light in water column (Hemminga and Duarte, 2000). So, seagrass is found the limit of distribution in shallow water that is sufficient light to growth (Dennison, 1987). Also, water temperature has a strong influence on plant metabolism and photosynthesis (Bulthuis, 1987). It also seems to be the major factor controlling the seasonal growth, flowering and senescence of some seagrass species such as *Zostera marina* (Setchell, 1929 cited by Bulthuis, 1987). Nutrient is one of the environmental conditions that can limit the growth of seagrass. Ammonium, nitrate and phosphate as ambient source of nitrogen and phosphorus that used by seagrass, these compounds are found in water column and sediment pore water. Nutrients in the water column are less than the sediment pore water (Hemminga and Duarte, 2000; Romero *et al.*, 2006).

The consumption of the seagrass by the herbivores could affect seasonal growth and production of seagrass (Valentine and Heck Jr., 1999; Heck Jr. and Valentine, 2006), including the mega herbivore i.e., Dugong (Preen, 1995). The largest of dugong population in Thailand were found in the seagrass beds between Haad Chao Mai National Park and Libong Island, Trang province (Hines *et al.*, 2005), which might also affect growth and production of seagrass. Burrowing shrimp (Alpheidae), another herbivore, had collected and stored seagrass leaf in their hole (Griffis and Suchanek, 1991; Stapel and Erftemeijer, 2000; Vonk *et al.*, 2008).

Because of the decline of many seagrass community and seagrass are known to be threatened by various human activities such as anchoring from boat (Creed and Filho, 1999), increasing of sediment in the water column from coastal developments, deforestation and erosion. These can cause dramatic changes to seagrasses and other marine communities (e.g. Airoldi *et al*.,1996; Duarte, 2001). The ecological information in population growth, coverage rate, net population growth rate, and environmental setting are needed to providing the restoration guidance (Fonseca *et al.,* 2000). Although the study of seagrass are increased in the last decade, but the knowledge concerning the foundation of seagrass community is still insufficient, and basic research into process and interaction, which allow seagrass to cover large coastal areas, are essential for conservation (van Tussenbroek *et al*., 2006). The main purpose of this study is to investigate the effect of seasonal variation on growth and sexual reproduction of *T. hemprichii* in the Haad Chao Mai National Park, Thailand.

Review of literature

Classification of *Thalassia hemprichii* (Ehrenberg) Ascherson following USDA-NRCS Nationnal Plant Data Center (2010)

Kingdom Plantae

Subkingdom Tracheobionta

Superdivision Spermatophyta

Division Magnoliophyta

Class Liliopsida

Subclass Alismatidae

Order Hydrocharitales

Family Hydrocharitaceae

Genus *Thalassia* Banks & Sol. ex K.D. Koenig

Species *Thalassia hemprichii* (Ehrenb.) Asch.

The characteristic of *Thalassia hemprichii*

The genus *Thalassia* is composing of two species, *Thalassia testudinum* Banks ex König and *Thalassia hemprichii* (Ehrenb.) Asch. that are considered to be 'twin species'(van Tussenbroek *et al*., 2006). Both species are very similar morphologically (but genetically the two species show divergence). On the macro scale, they can only be separated on the basis of genetic counts and dimensions of styles and stamens of the flowers (van Tussenbroek *et al*., 2006). *T. hemprichii* is dioecious plant and a perennial herb. Leaf blades are 10-40 cm long, 4-11 mm wide, with 10-17 longitudinal veins and leaf entries margins. Male peduncle ca. 3 cm long; pedicels 2-3 cm long; tepal 7-8 mm long; stamens 3-12, anther oblong, 7-11 mm long. Female flower have 6 styles that each with 2 stigmas, 10-15 mm long. Fruits globose, roughly echinate, 2-2.5 cm long, 1.75-3.25 mm wide, bursting open into 5-8 irregular valves; beak 4-7 mm (Haynes, 2001; Figure 1, 2)
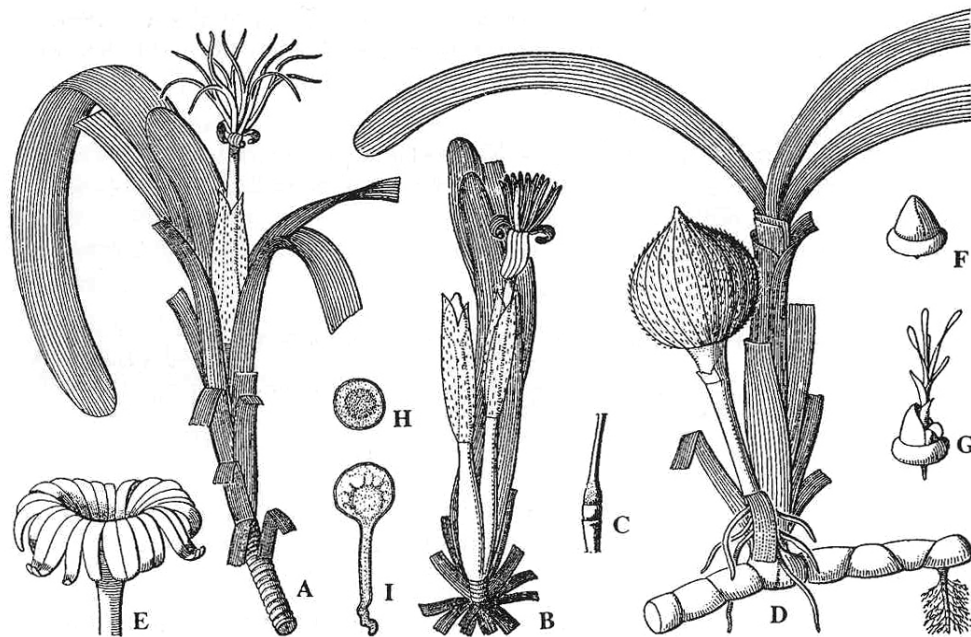
Figure 1. *Thalassia hemprichii* (Ehrenb.) Asch. : A. habit female flower plant, B. habit male flower, one in anthesis, C. ovary after fertilization, D. female plant with mature fruit, F. seed, G. seeding 3 weeks old, H. mature pollen gain, I. germinate pollen grain (den Hartog, 1970).

The reproductive and phenology of *Thalassia hemprichii*

Seagrass can reproduce new shoot in 2 ways. First is asexual reproduction where the plant rapidly grows new short shoots and extends rhizome to adjacent area. The asexual reproduction is a well known way of increasing seagrass beds, and many large area of seagrass bed can be formed through this vegetative reproduction. However, this could also be catastrophic to the plant, for example, in the case of a lethal epidemic disease, the lack of genetic variations of the plants could prevent recovery. Second, sexual reproduction where female flowers are fertilized by pollen form male flowers, and fruits and seed are produced. Sexual reproduction is an important process for genetic material exchange, which keeps the genetic diversity of population high. Also recruitment, by seeds is the main source of first generation new shoots, which is critical for dispersal in *T. hemprichii* (Lacap *et al*, 2002). However, very little is known about reproduction of Thai seagrasses.

Seagrass are marine flowering plants that can grow and reproduce submerged in the sea. Most seagrass have hydrophilous pollination, so that they use water as a vector in transportation of pollen (Cox, 1988). While in *Thalassia*, the pollen grains drift through the water and arrive at the stigma completely underwater. The flowering of *T. testudinum* coincides in the spring tides. The flowers are raised only in a few cm above the substrate. The male flowers open at night, pollen are dispersed in negatively buoyant strings of mucilaginous slime that glide along the substrate surface. Pollination occurs by collision with the stiff, papillate stigma of the female flowers (Cox, 1988). This could be similar in *T. hemprichii*, but is little known about flowering biology in this species. *T. hemprichii* have no seed dormancy, they germinate rapidly after being released from the fruit (den Hartog, 1970; Kuo *et al*., 1991). Lacap *et al*., (2002) reported the fruit and seed of *T. hemprichii* traveled at 0.47 km/h and the flotation time of them was 55 h and < 0.5 h respectively.

The timing of flowering in seagrass worldwide is varied among species and location (Walker *et al*., 2001). In Thailand, Lewmanomont *et al*., (1996) reported the timing of flowering of *E. acoroides* in the Gulf of Thailand and the Andaman Sea; they produced flower and formed fruit throughout the year and *T. hemprichii* could produced fruit all year round. However, further investigation on quality and quantity of reproductive output and phenology of the seagrass would allow us to understand more about the seagrass species. This could provide baseline information for further management of the seagrass which are threatened in many places in Thailand and in SE Asian regions.
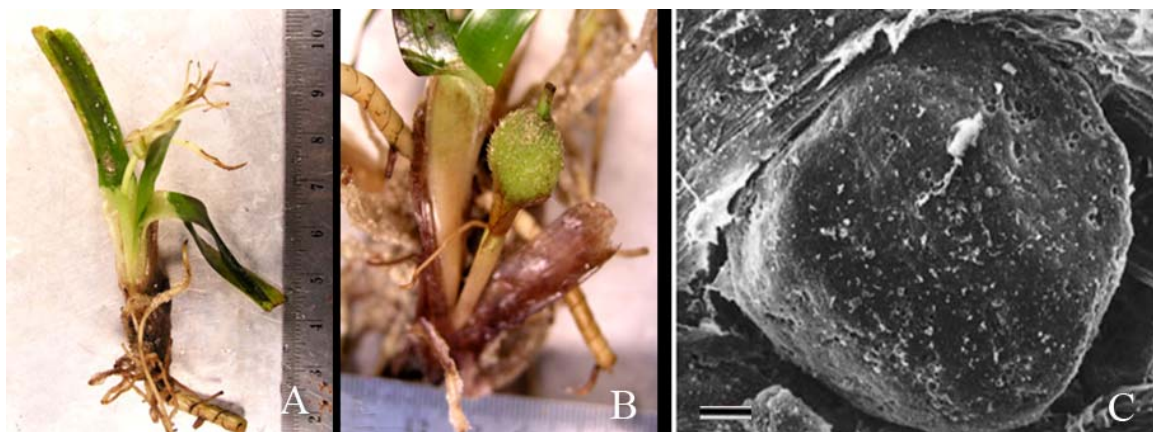
Figure 2. *Thalassia hemprichii*: A. female flower, B. fruit, C. pollen grain and bar =10 µm. Source: C. Tanaka *et al.*, (2004)

The basic environmental parameters requirement for *Thalassia hemprichii*

Various study had been carried out to investigate the effected of dissolved in organic nitrogen and phosphorus on growth and productivity of *T. hemprichii* (Erftemeijer *et al*., 1994; Agawin *et al*., 1996, Stapel *et al*., 1996; Stapel *et al*., 1997; Evrard *et al*., 2005). Although, *T. hemprichii* can uptake nutrient by leaf and root parts (Stapel *et al.*, 1996), nutrient limitation still occurred, and varied from sites to sites, which could depress growth and production (Agawin *et al*., 1996). In addition, *T. hemprichii* can grow in wide range of temperature, 24 – 33 ^{o}C (Agawin *et al*., 2001) and can neither tolerate prolong exposure to high temperature nor long term desiccation on intertidal area (Brouns, 1985; Stapel *et al*., 1997).

T. hemprichii are well studied in the Philippines (e.g., Rollon *et al*., 1998; Rollon *et al*., 2001; Rollon *et al*., 2003; Agawin *et al*., 2001; Duarte *et al*., 2000; Vermaat, *et al*., 1995) and Indonesia (Evrard *et al*., 2005; Stapel *et al*., 1996; Stapel *et al*., 1997; Stapel *et al*., 2001; Erftemeijer and Herman, 1994; Erftemeijer, *et al*., 1994). This study seems to be the first study on growth and reproduction of *T. hemprichii* in Thailand, however similar studies on *E. acoroieds* (Rattanachot, 2008) and *Halophila decipiens* Ostenf. (Rattanachot *et al*., 2008) have recently been

reported. Such studies would be useful for seagrass restoration program, since it has become popular to the public; and recently introduced (Lawrence, *et al*., 2007). The limitation of seagrass biology, ecology and insufficient data for seagrass restoration made the restoration failed, there was only less than 10% survival of the donor plants as seen at Tha Kham, Trang province (personal observation). Thus, this is an urgent issue to tackle before ruining the seagrass beds and coastline without knowing.

Research question

1. Is growth of *Thalassia hemprichii* (Ehrenb.) Asch. affected by seasonal variation? How ?

2. What are the phenology-cycles of *Thalassia hemprichii* (Ehrenb.) Asch. in Haad Chao Mai National Park, Thailand?

Hypotheses:

1. Is growth of *Thalassia hemprichii* affected by seasonal variation?, How?

H_0**:** There are no differences in growth of *T. hemprichii*   in different seasons.

> *$H_{0\,1}$***:** All leaf plastochronee intervals of *T. hemprichii* are the same in different seasons.

> *$H_{0\,2}$***:** Leaf growth rates of *T. hemprichii* are the same in different seasons.

> *$H_{0\,3}$***:** Leaf elongation rates of *T. hemprichii* are the same in different seasons.

> *$H_{0\,4}$***:** The above ground and below ground biomass of *T. hemprichii* are the same in different seasons.

H$_1$: There are differences in growth of *T. hemprichii* in different seasons.

H$_{1\,1}$: Leaf plastochronee intervals of *T. hemprichii* are difference in different seasons.

H$_{1\,2}$: Leaf growth rates of *T. hemprichii* are difference in different seasons.

H$_{1\,3}$: Leaf elongation rates of *T. hemprichii* are difference in different seasons.

H$_{14}$: The above ground and below ground biomass of *T. hemprichii* are difference in different seasons.

2. What are the phenology-cycles of *Thalassia hemprichii* in Haad Chao Mai National Park, Thailand?

H$_0$: There is no seasonal cycle in the reproductive phenology of *T. hemprichii.*

H$_{0\,1b}$: There is no seasonal cycle in the flowering of *T. hemprichii.*

H$_{0\,2b}$: There is no seasonal cycle in the fruiting of *T. hemprichii.*

H$_1$: There is seasonal cycle in the reproductive phenology of *T. hemprichii.*

H$_{1\,1b}$: There is seasonal cycle in the flowering of *T. hemprichii.*

H$_{1\,2b}$: There is seasonal cycle in the fruiting of *T. hemprichii.*

Objectives:

1. To study the effects of seasonal variation on growth and sexual reproduction of *Thalassia hemprichii.*

2. To study phenology of flowering and fruiting in *Thalassia hemprichii.*

CHAPTER 2

MATERIALS AND METHODS

Study Site

This study was carried out in the seagrass beds at Laem Yong Lam (7°23′ N, 99°20′ E) in Haad Chao Mai National Park, Trang Province (Figure 3). On the Andaman Sea coast of Southwest Thailand, this region is affected by two dominant seasons: a rainy season most influenced by the Southwest Monsoon (May to October) and a dry season influenced by the Northeast Monsoon (November to April). The site is subjected to a semi-diurnal tide (Poovachiranon and Chansang, 1994).

Laem Yong Lam is located between the river mount (Figure 3), surrounded by mangrove forest which along the river. This area is located opposite the Muk Island that provides shelter during the monsoon season for seagrass. This site supports a high diversity in which the following nine of the twelve seagrass species reported in Thailand are found: *Halodule pinifolia* Hartog, *Halodule uninervis* (Forssk.) Asch*., Cymodocea rotundata* Asch. & Schweinf*., Cymodocea serrulata* (R.Br.) Asch. & Magnus, *Syringodium isoetifolium* (Asch.) Dandy, *Thalassia hemprichii* (Ehrenb.) Asch., *Enhalus acoroides* (L. f.) Royle and *Halophila ovalis* (R. Br.) Hook. f. (Lewmanomont and Supanwanid, 2000). This study was setup in the mid-intertidal zone of the *T. hemprichii* bed which is surrounded by *E. acoroides* (Figure 4*)*. This *T. hemprichii* bed is situated at 0.5 ± 0.1 m above low tide when compares with the tidal cycle data on Pak Num Trang (Hydrographic Department Royal Thai Navy, 2008 and 2009) the nearest reference point. This bed is in the shallow tide pool during the lowest tide, thus there is some water covered the plants. There are some burrows form the burrowing shrimp in this area, which rather abundance. The substrate is sandy with some sea shells and there is sediment cover on seagrass leaf which might come from the nearby river mouth, nevertheless the water is clear; there is low turbidity during the non monsoon season.

Methods

Data on the growth and reproductive phenology of *T. hemprichii* were investigated monthly throughout a year along with measurements of environmental parameters (light intensity, temperature, salinity, nutrients in seawater/or water column, nutrients in sediment, exposure hour and density of burrowing shrimp). The growth study was separated into leaf growth characters measurement (i.e., leaf plastochrone interval, leaf elongation rate and leaf growth) and meadow growth characters measurement (i.e., leaf biomass, sheath biomass, rhizome biomass, root biomass, above ground biomass, below ground biomass and shoot density), while the time of flowering and fruit were observed monthly for the phenology study.
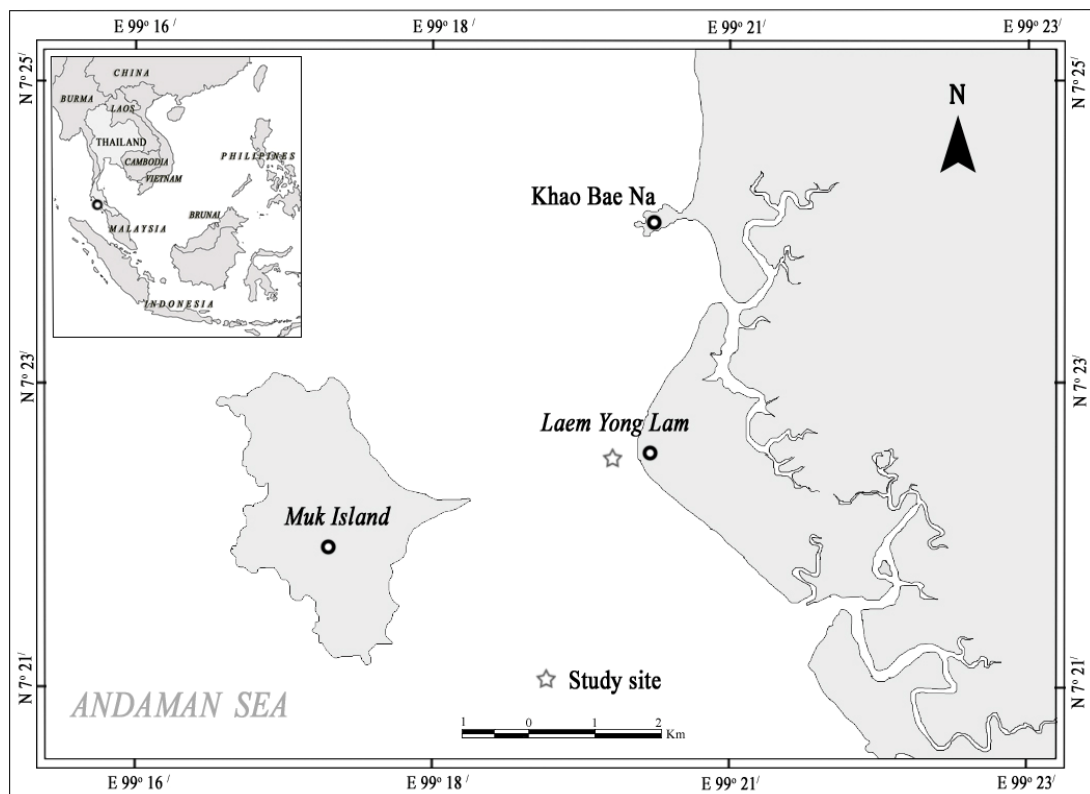
Figure 3. Leam Yong Lum, the large seagrass bed, in Trang province, southern part of Thailand.

Figure 4. The pure *Thalassia hemprichii* bed.

Thalassia hemprichii growth study

Leaf growth characters measurement

Leaf growth was monitored in ten permanent 1m x 1m plots in the *T. hemprichii* bed. Leaf growth characters were measured by the plastochrone method (Short and Duarte, 2001; Figure 5). Holes approximately 2 mm in diameter were punched in the middle part of the sheath using a crochet hook, 20 shoots from each plot. These plants were marked with a string. After 10-15 days, marked plants were collected and kept at 0- 4°C in the dark for further study in laboratory.

In the laboratory, each leaf was cleaned and separated from each shoot. The number of new leafs that had no hole was counted. Then the length in cm of the youngest fully mature leaf was measured. Leaf length was measured from the base to the leaf tip that was not used the broken or grazed leaf tips (Figure 6). The dry weight biomass (mg) was obtained by desiccation at 60 °C until the weight was in constant. The following variables were determined:

- *Leaf plastochrone interval*, P_L (days) is the time in days to produce one new leaf.

- *Leaf elongation rate* (cm/shoot/day) is a rate of leaf elongation. It was calculated by dividing the leaf length by P_L.

-*Leaf growth* (mg/shoot/day) is the rate of biomass that a leaf can produce in a day. It was calculated by dividing the leaf biomass by P_L.

Meadow growth characters measurement

Cylindrical PVC tubes, 10 cm in diameter were used to collect the biomass of all *T. hemprichii* plant parts. Five cores were made randomly each month outside the permanent plots. The sample was taken down to 15 cm depth, over the rhizome (Stapel *et al*., 1996). The samples were kept at 0- 4 ^{o} C in the dark for further study in laboratory. Moreover, shoot density is the density of the plant that was counted in permanent quadrate (25 cm x 25 cm).

In the laboratory, the samples were washed; sediments, epiphytes and associated animals were removed, and then separated into 4 parts: the leaf, the sheath, the rhizome and root. All parts were dried at 60 ^{o} C until the weight was constant. The following variables were determined:

- *Leaf biomass* (g/m^2) is the dry weight of the leaf.

- *Sheath biomass* (g/m^2) is the dry weight of the sheath.

- *Rhizome biomass* (g/m^2) is the dry weight of the rhizome.

- *Root biomass* (g/m^2) is the dry weight of the cleaned root.

- *Above ground Biomass* (g/m^2) is the dry weight of the leaf and sheath parts that above the soil level.

- *Below ground biomass* (g/m^2) is the dry weight of the rhizome and root from under the soil level.

Note: The entire plant was used to measure and collect biomass; incomplete specimens were excluded from this study. The fully youngest mature leaf is the leaf that the punched-hole appears near to the leaf tip and mostly is the leaf in No. 2, No. 3 or No. 4 representing the growth during the interval time (Figure 6).
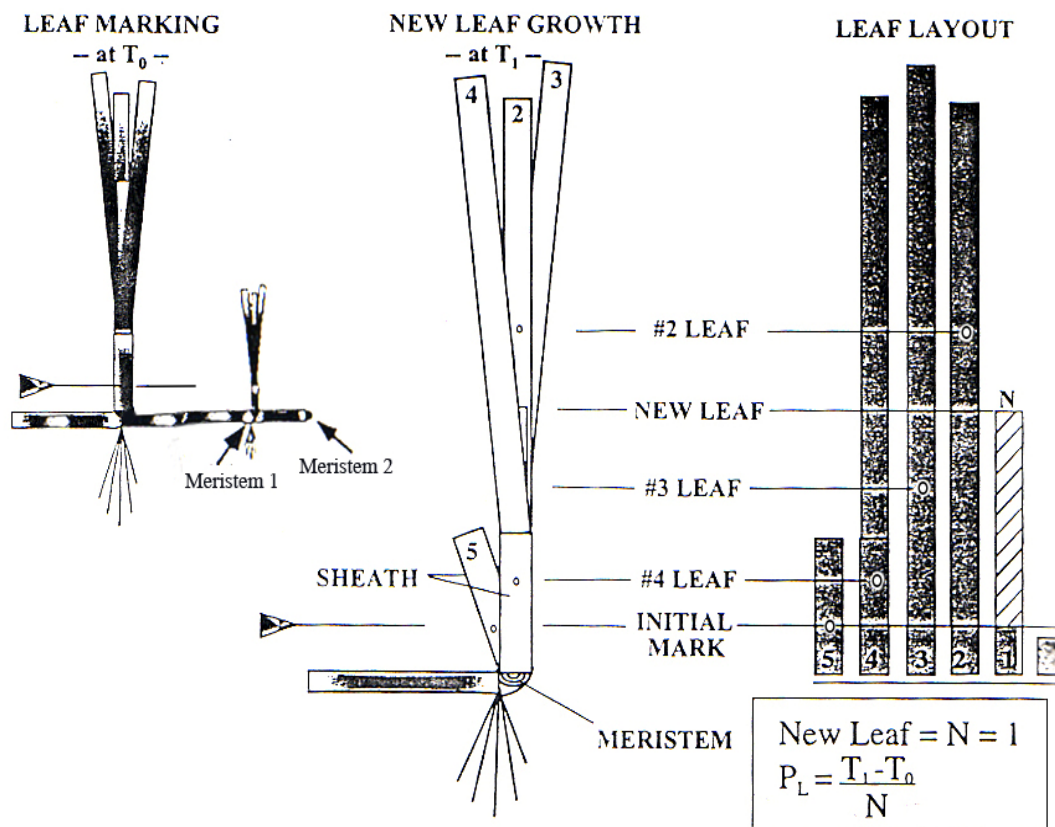
Figure 5. Leaf marking to determine the leaf plastochrone interval, P_L on *T. hemprichii* (Picture adapted from Short and Duarte, 2001).
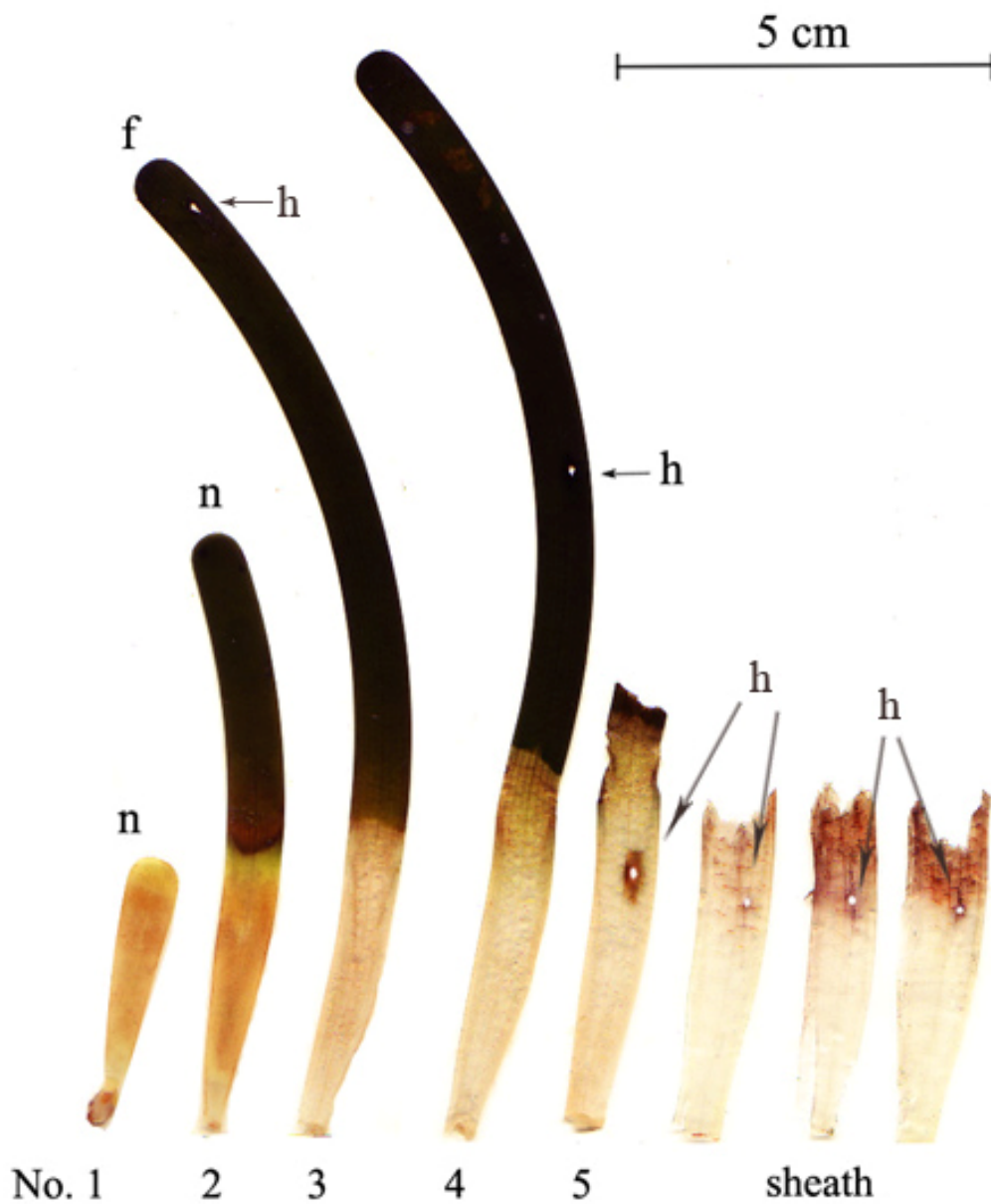
Figure 6. Marking leaf of *T. hemprichii* in this study: the new leaf (n) is the leaf with no hole, fully youngest mature leaf (f) is the leaf with hole near the tip and marking hole (h) and sheath. Each No. 1, 2, 3, 4 and 5 was the number of leaf, where No. 1 represented the newest leaf and No. 5 represented the oldest.

Phenology study

Flower and fruit were observed monthly in the 10 monitoring plots. Young flowering bud plants were collected from the nearby plots, if any, and put into aquaria to observe the development until the flowers become mature. The sand was set as substrate for seagrass in aquaria (24″ x 14.5″x 12 ″, two of 18 W fluorescence lamps; Sylvania Aqua star and Phillip TLD) were set above the tank and turn on during 6.00-18.00 hours.

Environmental parameters measurement

In this study, the environmental parameters were collected monthly throughout the year.

- *Light intensity* (lumens/square foot) was measured using the Onset-Hobo® LI light logger. A data logger was set up about 10 cm above the ground level to avoid shading by the seagrass leaves; data were collected every hour. The data from the logger (Lumens/foot^2) were transformed to $\mu mol \cdot m^{-2} \cdot s^{-1}$ following the equation form calibration with light meter 4π sensor (Li-Cor, LI-250A, LI-COR Inc., USA); (Figure.7).

$$\mu mol \cdot m^{-2} \cdot s^{-1} = 7.178 \text{ (lumens/sf)} + 803.978 \qquad ; R^2 = 0.811$$

- *Temperature* (°C) was measured using the iButton® that logs temperatures over a range of -40 °C to 85 °C, data were collected every 60 minutes.

- *Salinity* (ppt) was measured using a refractometer (ATC, 0-100 ppt, XHO RHS-10ATC, ATACO, China).

- *Nutrients in seawater* (μM of NO_3^- and μM of PO_4^{3-}): The water was collected within the bed when seagrass was submerged. This water was filtered through a GF/C filter in the field. The water was stored in the dark at 4 °C and sent to

the laboratory. Nutrient concentrations were analyzed using a Hach[®] DR/890 data logging colorimeter (Hach company, 2004).
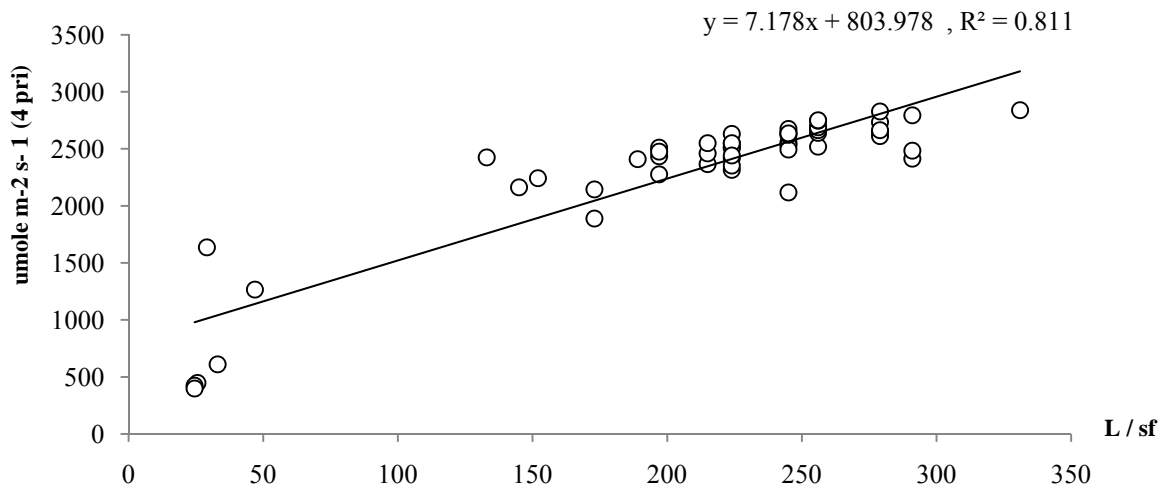
Figure 7. Linear correlation between lumens/sf and $\mu mole \cdot m^{-2} \cdot s^{-1}$ and the correlation equation (p<0.05). When y is $\mu mole \cdot m^{-2} \cdot s^{-1}$ and x is lumens/f^2.

- *Nutrients in the sediment* ($\mu mole\ NO_3^-$ / kg dry weight sediment, $\mu mole\ PO_4^{3-}$ / kg dry weight sediment): Sediment was collected during the lowest tide to reduce error from the nutrients in the seawater. The sediment sample was obtained using a 7 cm diameter, 15 cm deep corer in the *T. hemprichii* zone (Stapel *et al.*, 1996). Sediment was analyzed for nitrate and phosphate using Chan and Sugahara (1994) as cited by La-ongsiriwong and Intramontree (2003). Nutrient concentrations were analyzed using a Hach[®] DR/890 data logging colorimeter according (Hach company, 2004).

- *Meteological data*: The data on rainy days, total rainfall and air temperature during January 2006 to January 2009 were provided by the Trang Meteological station (personal communication).

- *Exposure hour*: The amount of exposure hour in each month was estimated and calculated using the data on tidal cycle of Pak Nam Trang in Tide Tables Thai Waters Mae Nam Chaophraya-Gulf of Thailand and Andaman Sea from Hydrographic Department, Royal Thai Navy (2008 and 2009).

- *Density of burrowing shrimp*: The density of burrowing shrimp is not feasible to observe, because of time limitation in the field and burrowing shrimp is a vigilant animal. Thus, the densities of burrowing shrimp in each month were collected by counting the number of burrow in the same 10 monitoring permanent plots of leaf growth characters measurement's plots.

Statistical analysis

Significance level of 95 % interval was used in all statistical analyses for this study. One way ANOVA was employed to test the differences in nitrate and phosphate in the sediment among months. The data for phosphate in seawater was transformed to log (data + 1) to meet the assumption of ANOVA. The differences among months of nitrate in seawater, salinity, temperature, and light intensity did not fit the parametric test, thus Kruskal Wallis Test was employed.

The differences among months of leaf, root and below ground biomass were tested using one way ANOVA. However, the data of leaf elongation rate, leaf growth, above ground biomass and shoot density were transformed with log, while the data of sheath-stem biomass and rhizome biomass were transformed with 1/ log (data) to meet the assumption of ANOVA. The leaf plastochrone interval data (P_L) were not normally distributed after the transformation, thus the Kruskal Wallis Test was employed.

The relationships between environmental parameters and growth characters were tested using step-wise multiple regressions. Leaf elongation rate, leaf growth, rhizome biomass and below ground biomass were transformed with log (data) to meet the assumption of multiple regression. The delay effect of environmental parameters on growth characters was also tested, the correlation between the previous month data of environmental parameters (30 days) and current month of growth characters were investigated.

Figure 8. Field set up: A. Plant was punched and marked with a string, B. Sediment was collected by plastic core for nutrients in the sediment analysis, C. Data logger set on the permanent plot at the same height of the *T. hemprichii* tip, about 10 cm from substrate.

CHAPTER 3

RESULTS

Environmental parameters

Meteorological data

The repeatable seasonal trend of the number of rainy days and total rainfall were observed during year 2006-2008 (Figure 9). They were both high from May to October each year during the monsoon season. A low number of rainy days and total rainfall was regularly recorded from November through April, the non-monsoon season on Andaman Sea coast. The air temperature was decreased on the late of monsoon season.

Light intensity

There was significant difference in light intensity among months ($p < 0.05$, Table 1). Low light intensity occurred during the 2008 monsoon season, May to October, an average of 1149.21 ± 9.14 $\mu mol \cdot m^{-2} \cdot s^{-1}$. High light intensity occurred during the non-monsoon season, January to April 2008, and November 2008 to January 2009, an average of 1673.66 ± 23.64 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figure 10).

Temperature

There was significant difference in temperature (24h) among months ($p < 0.05$, Table 1). The highest temperature was recorded from April to October 2008, 29.53 ± 0.09 ^{o}C to 30.23 ± 0.07 ^{o}C, and the lowest temperatures was found in December 2008, 27.57 ± 0.05 ^{o}C (Figure 11).
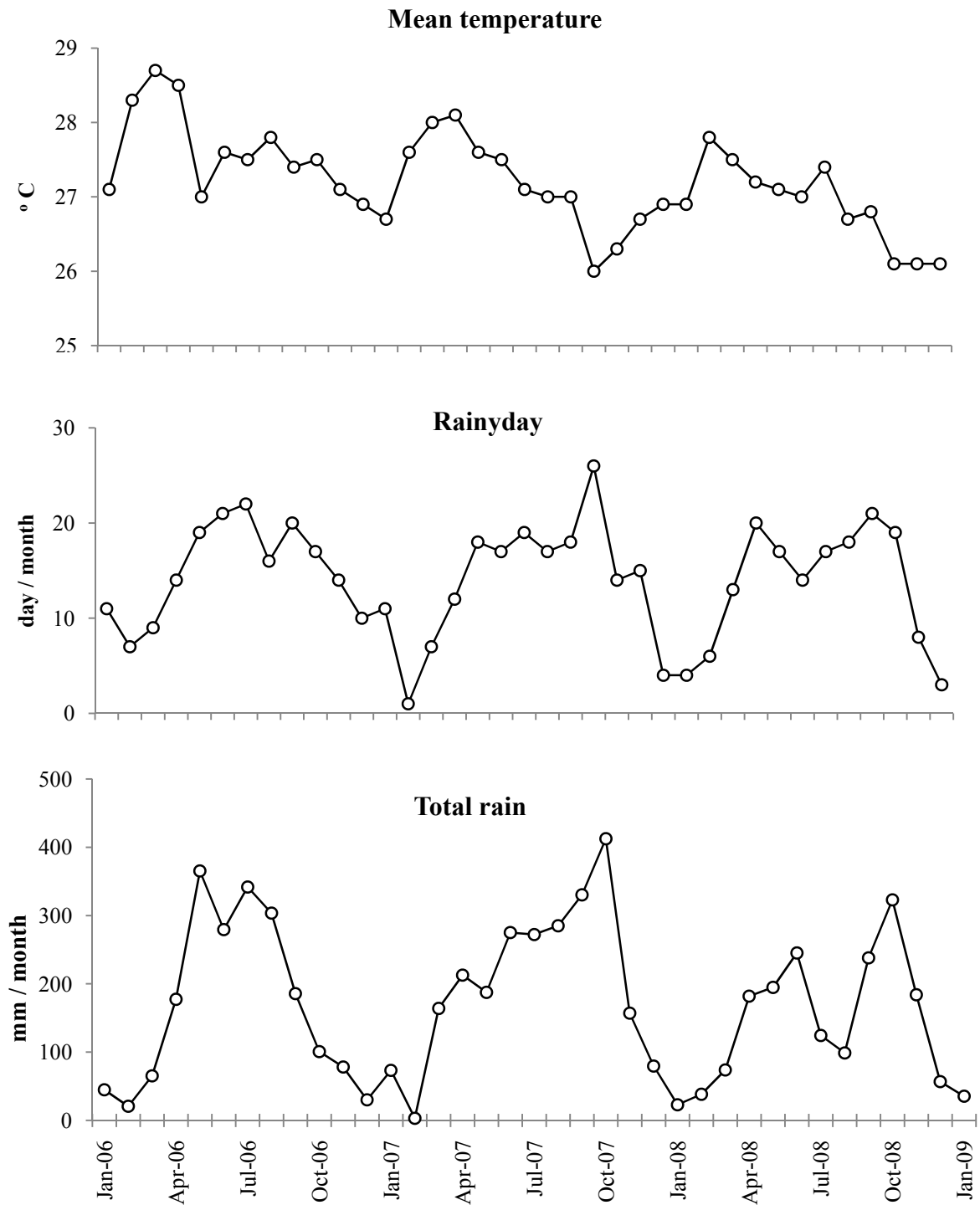
Figure 9. Meteorological data of mean air temperature, rainy days and total rain in each month during January 2006-January 2009.
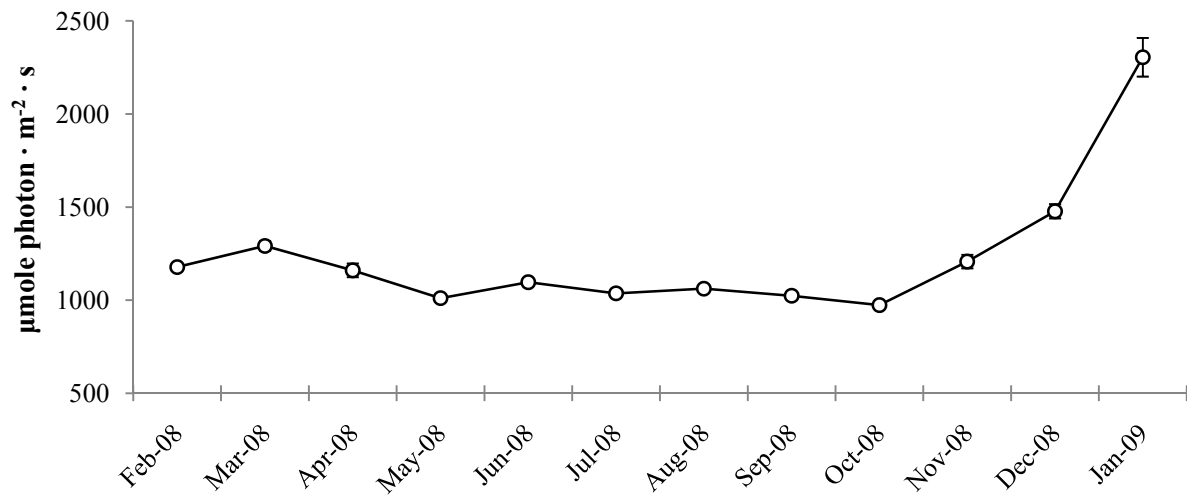
Figure 10. Average light intensity between 8.00-16.00 hours in each month during February 2008 to January 2009. The error bars are standard error.
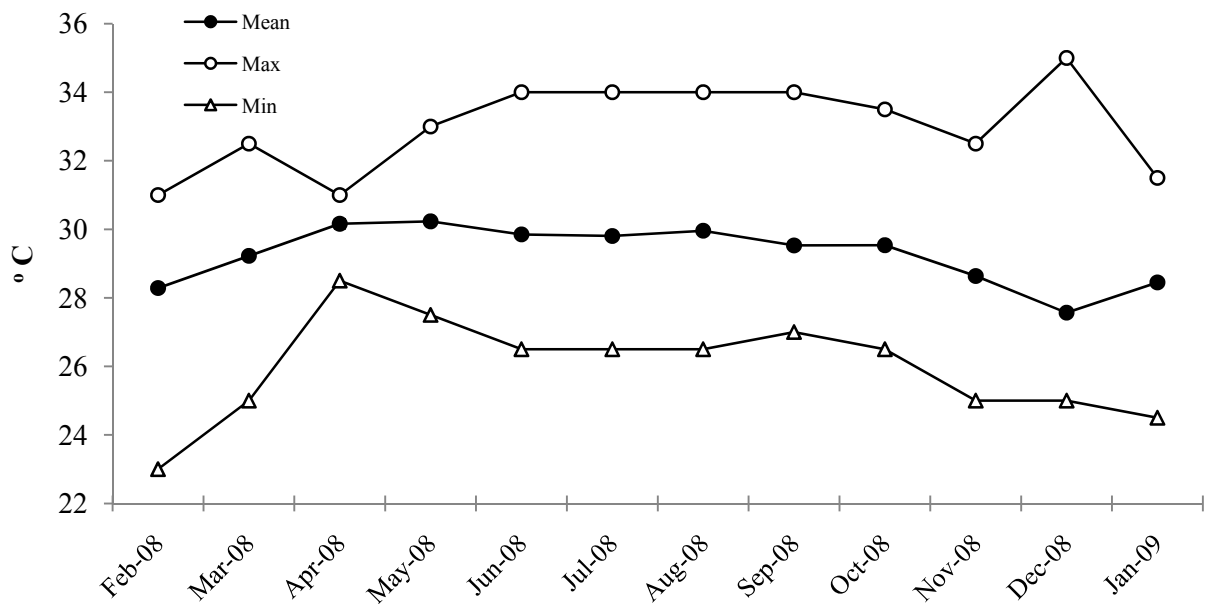
Figure 11. Maximum, minimum and average data of temperatures (24 h) in monthly during February 2008 to January 2009. The error bars are standard error.

Nutrients in the sediment

A lower concentration of Phosphate (PO_4^{3-}) than Nitrate (NO_3^-) was found in the sediments every month. There were no significant differences in nitrate and phosphate among months ($p > 0.05$, Table 1, Figure 12). However, high amounts of nitrate were found in March and August 2008 (1292.90 ± 111.34 and 1275.70 ± 88.61 μmole NO_3^- / kg dry weight sediment, respectively). The lowest amount of nitrate was found in February 2008 (709.98 ± 127.32 μmole NO_3^- / kg dry weight sediment). The highest amount of phosphate was found in January 2009 and the lowest in February 2008 (177.25 ± 23.77 and 102.78 ± 13.41 μmole PO_4^{3-} / kg dry weight sediment, respectively).
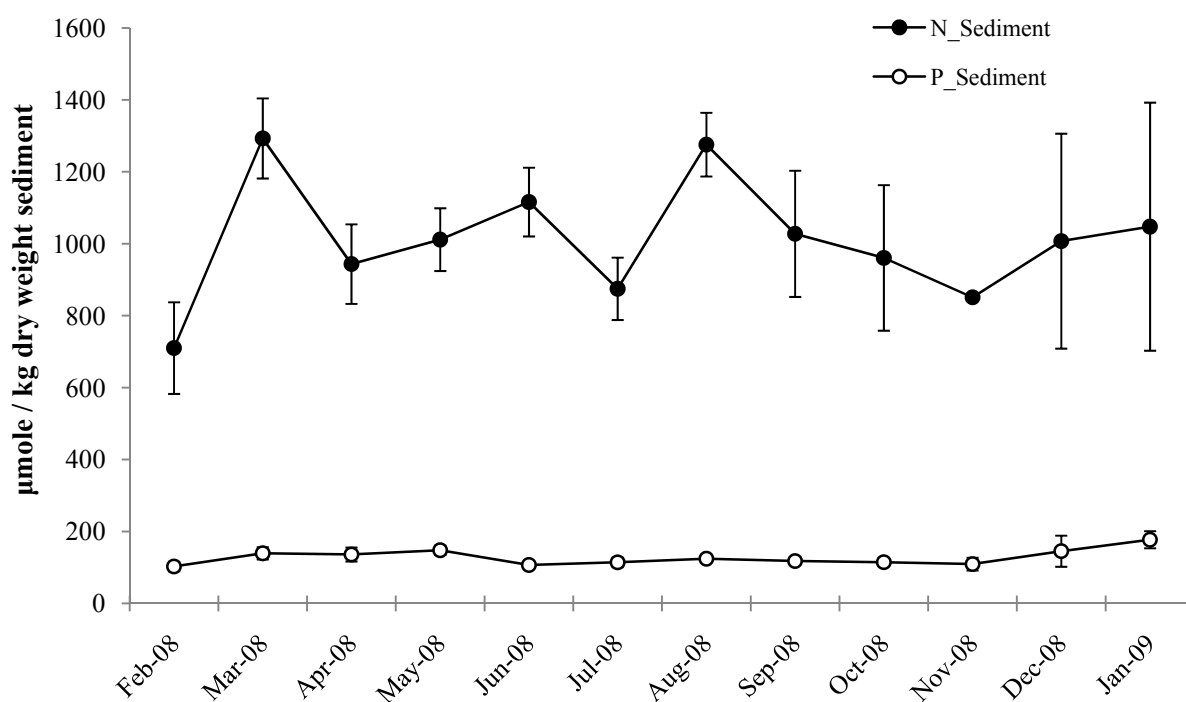
Figure 12. Average nutrient levels in sediments showing nitrates (NO_3^-) higher than phosphates (PO_4^{3-}). The bars represent standard error, (N=6).

Nutrients in sea water

    The nutrient levels in seawater showed the opposite trend of nitrate and phosphate to those in the sediments. Lower amounts of nitrate (NO_3^-) than phosphate (PO_4^{3-}) were found every month, excepted in April 2008. Nitrate was not significantly different among months (p>0.05, Table 1). However, nitrate decreased in July 2008 to the lowest level, 0.1554 ± 0.0983 μM NO_3^-, (Figure 13). Phosphate was significantly different among months (p<0.05, Table 1) and the highest in October 2008, 2.6534 ± 0.61612 μM PO_4^{3-}, (Figure 13), suggesting that there might have nutrients run off during rainy season.
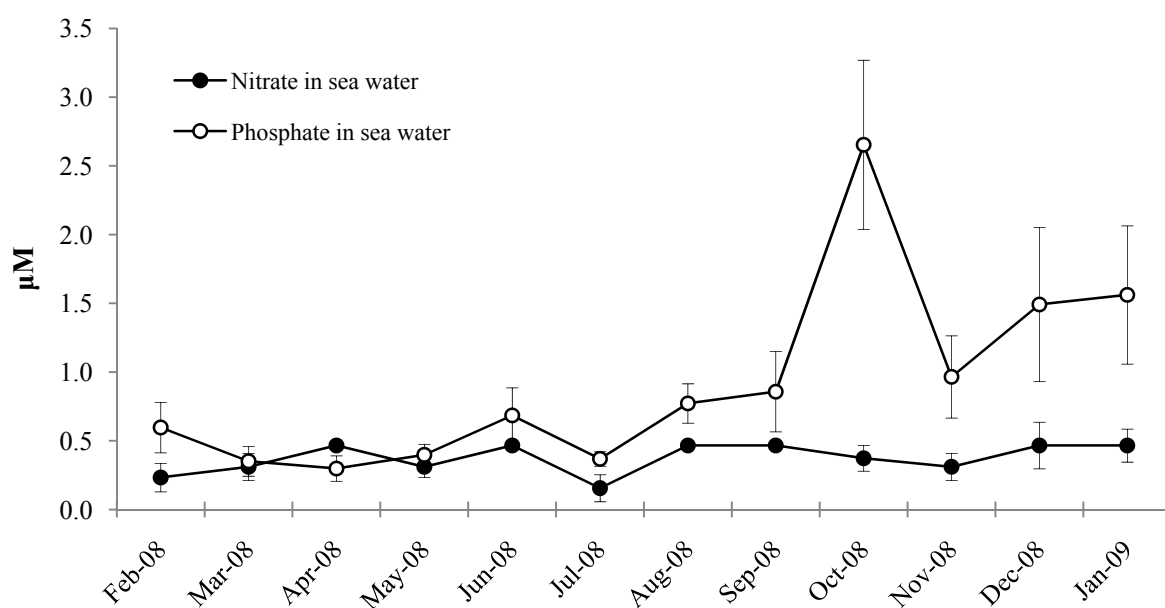
Figure 13. Average nutrient levels in seawater showing that phosphate (PO_4^{3-}) was higher than nitrate (NO_3^-). The bars represent standard error, (N = 6).

Salinity

      The salinity varied between 27–33 ppt. There was significant difference in salinity among months ($p < 0.05$, Table 1). The highest, 35 ppt, was found in January 2009, during the beginning of summer and the lowest was found in August 2008, 27.33 ± 1.48 ppt (Figure 14).
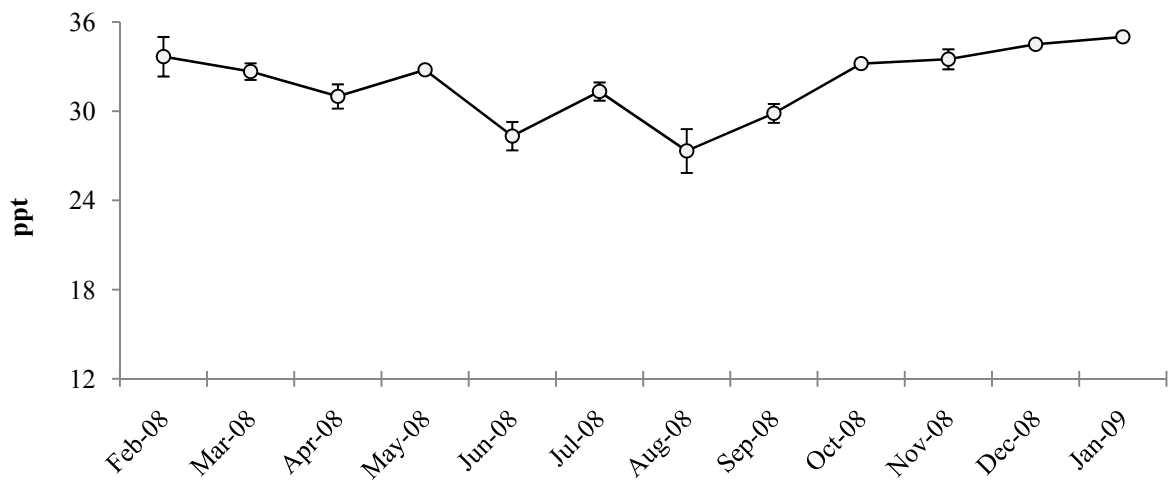
Figure 14. The salinity of seawater from February 2008 to January 2009. The bars represent standard error, (N=6).

Exposure hours

        The highest exposure hour (36 h/month) was found in the February 2008 (Figure 15, Table 1). The tide was out in the early morning (05.00-09.00 hour) and the evening (18.00-20.00 hour; Figure 16). Seagrasses were not exposed during the monsoon season and the beginning of non-monsoon season, May-October and November 2008, respectively.
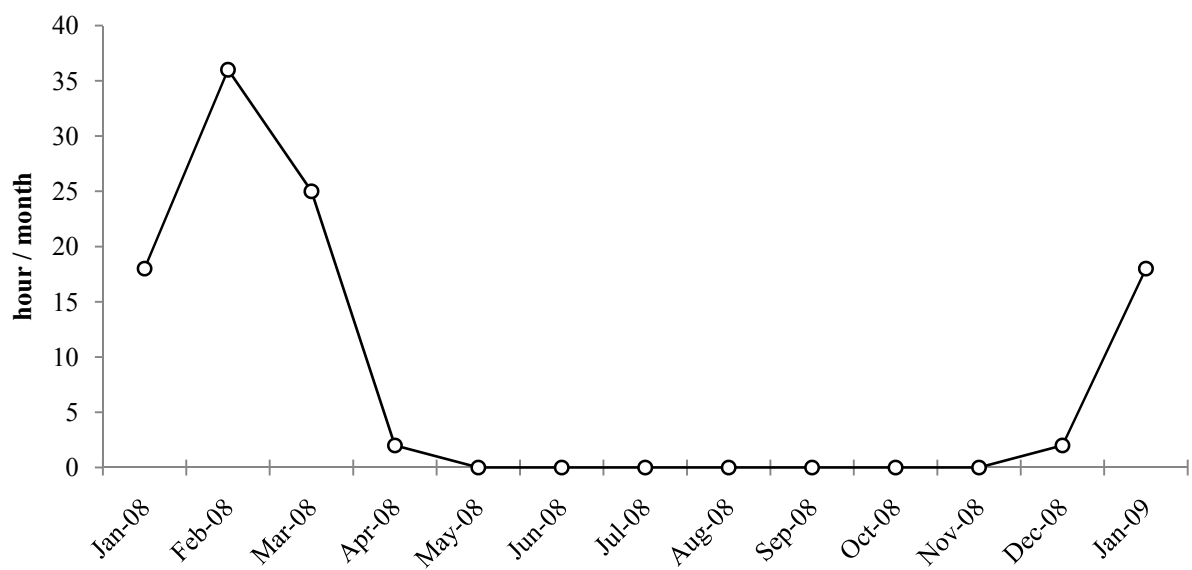
Figure 15. The exposure hours in the study site from January 2008 to January 2009.
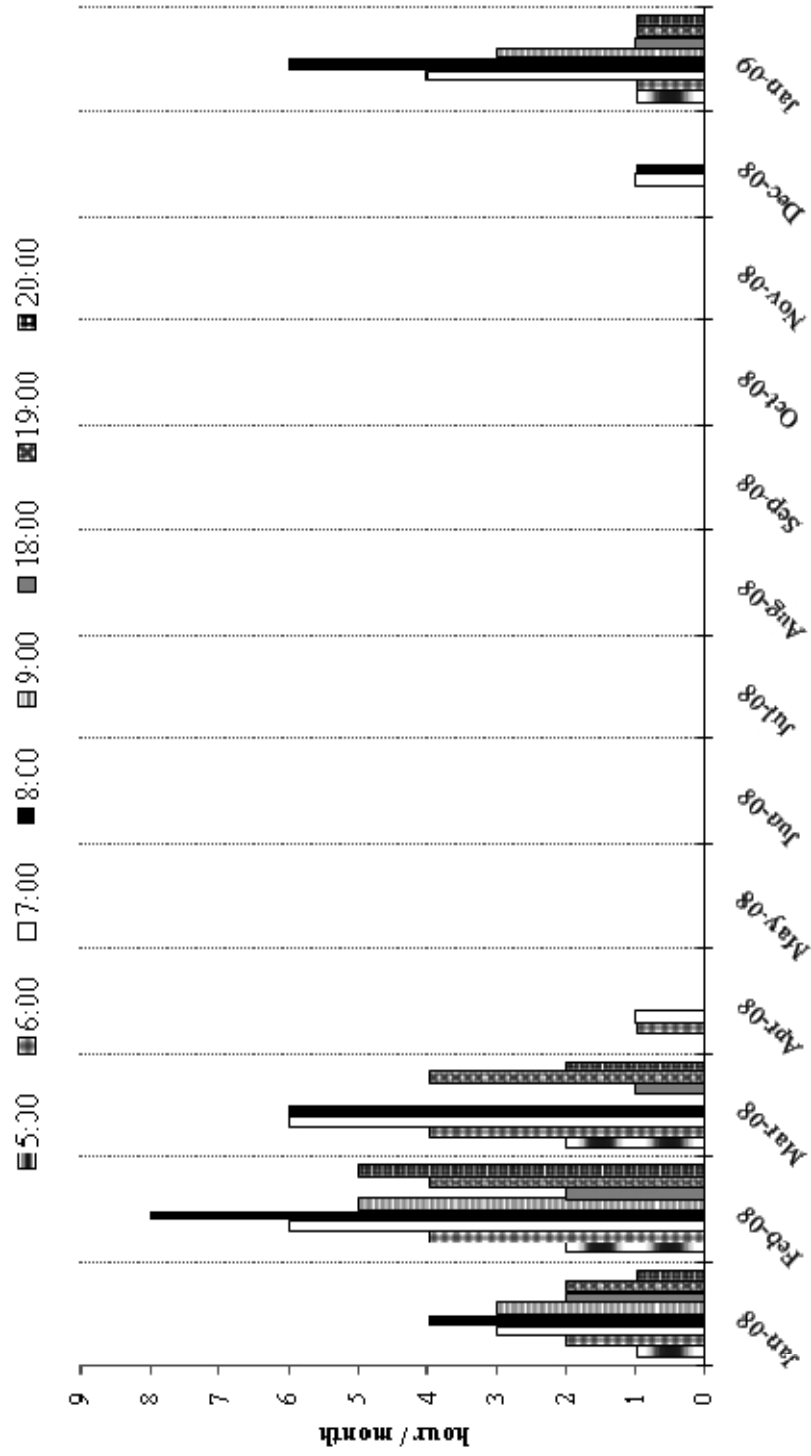
Figure 16. The exposure hour and time that this area were exposured from January 2008 to January 2009

Burrowing shrimp and goby density

The burrowing shrimps (Family: Alpheidae) associated with goby fish (Family: *Gobiidae*) were observed in the study area (Figure 17: A-C). This shrimp made a hole and cut the seagrass leaves, and stored in their burrow (Figure 17: D; personal observation). There was significant difference among months ($p < 0.05$, Table 1). The highest burrow density was found in August and the lowest was found in June on year 2008 (Figure 18). Unfortunately, the burrow density was not observed in May 2008 because of the strong wind and surf from the monsoon during the time for data collection.

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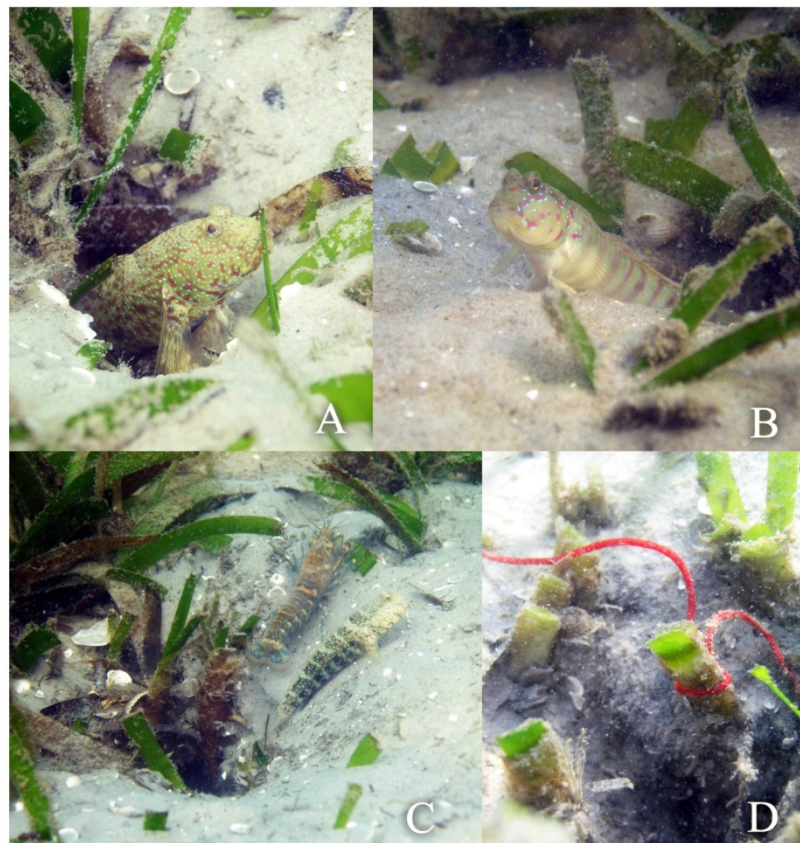
Figure 17. The burrowing shrimp (Alpheidae) and associated goby fish (Gobiidae) in *T. hemprichii* meadow. A-B. Goby fish; C. goby and shrimp; D. shoot of *T. hemprichii* cut by shrimp.
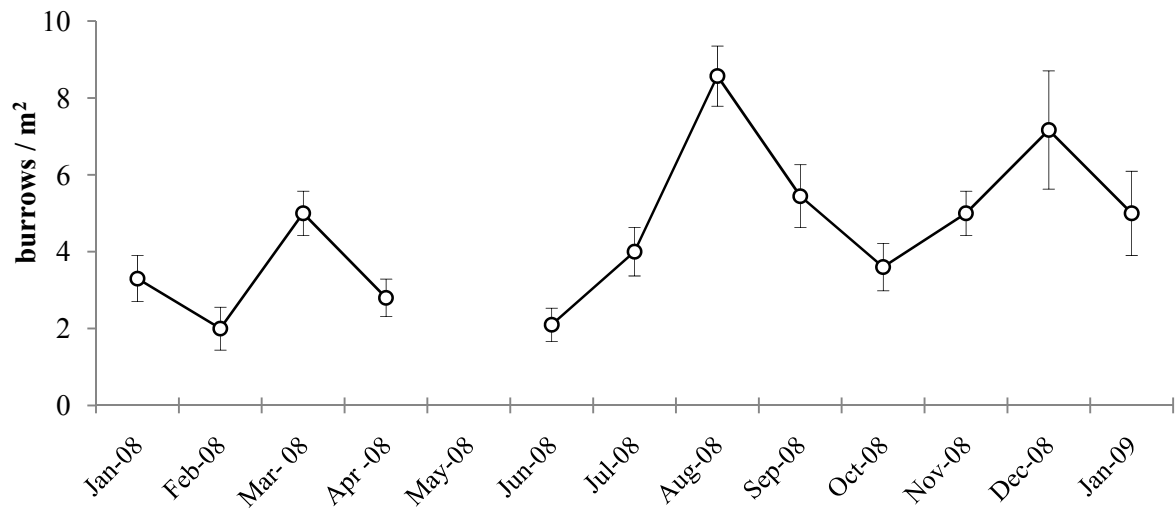
Figure 18. The burrowing shrimp density in *T. hemprichii* bed from January 2008 to January 2009. The bars represent standard error and ** is no data, (N = 7).

Table 1. Average environmental parameters in each month between Febuary 2008 to January 2009 and the statistical value to show the significantly different among month. * represent the 95 % significant differences; and – = No data.

Environmental parameters	Months												Annual average	Statistic value			
	Feb-08	Mar-08	Apr-08	May-08	Jun-08	Jul-08	Aug-08	Sep-08	Oct-08	Nov-08	Dec-08	Jan-09		N	df	F	Sig
Light Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	1177.90	1291.40	1160.20	1010.80	1096.50	1036.60	1062.00	1023.70	973.48	1207.30	1477.40	2304.60	1313.59	3705	12	451.38	0.00*
SE	13.99	26.46	37.11	19.70	18.69	15.26	13.69	20.99	15.87	36.30	38.21	103.82	16.94				
Temp 24 h (^{o}C)	28.29	29.22	30.16	30.23	29.85	29.81	29.96	29.53	29.54	28.64	27.57	28.45	29.20	5277	11	1421.62	0.00*
SE	0.06	0.05	0.14	0.07	0.06	0.04	0.06	0.09	0.07	0.06	0.05	0.06	0.03				
N_Sea water (μM (NO^{3-}))	0.23	0.31	0.47	0.31	0.47	0.16	0.47	0.47	0.37	0.31	0.47	0.47	0.37	75	11	15.49	0.161
SE	0.10	0.10	0.00	0.08	0.00	0.10	0.00	0.00	0.09	0.10	0.17	0.12	0.03				
Salinity (ppt)	33.67	32.67	31.00	32.78	28.33	31.33	27.33	29.86	33.20	33.50	34.50	35.00	31.85	72	11	52.77	0.00*
SE	1.33	0.56	0.82	0.22	0.96	0.62	1.48	0.63	0.20	0.67	0.22	0.00	0.33				
N_Sediment (umole (NO^{3-}) / kg dry sed)	709.98	1292.90	943.59	1011.60	1116.10	874.88	1275.70	1027.70	960.68	851.03	1007.40	1047.60	1047.63	72	11	1.32	0.237
SE	127.32	111.34	110.59	87.12	95.60	86.68	88.61	175.52	202.35	1.56	298.72	344.92	53.21				
P_Sediment ($\mu mole$ (PO_4^{3-}) / kg dry sed)	102.78	139.49	136.12	147.94	106.91	114.37	124.30	118.00	114.73	109.41	145.36	177.25	128.05	72	11	1.52	0.148
SE	13.41	16.98	19.26	13.06	10.13	13.31	5.99	10.57	11.31	17.82	43.30	23.77	5.61				
P_Sea water (μM (PO_4^{3-}))	0.60	0.35	0.30	0.40	0.68	0.37	0.77	0.86	2.65	0.97	1.49	1.56	0.87	75	11	4.43	0.00*
SE	0.18	0.11	0.09	0.08	0.20	0.05	0.14	0.29	0.62	0.30	0.56	0.50	0.11				
Burrowing shrimp density ($hole \cdot m^{-2}$)	2.00	5.57	2.43	-	1.71	3.29	8.57	4.14	4.00	5.00	6.14	3.57	4.13	84	11	7.14	0.00*
SE	0.58	0.65	0.37	-	0.36	0.52	0.78	1.08	0.72	0.76	1.65	1.19	0.31				

Leaf growth characters study

Leaf Plastochronee Interval (P_L)

The Leaf Plastochronee Interval (P_L) is the number of days required for a plant to produce a new leaf. The mean P_L throughout the year was 10.80 days/leaf; there was significant difference in P_L among months ($p<0.05$, Table 2, 3). The lowest P_L was found in October 2008 and the highest in February 2008, 8.89 ± 0.41 and 13.35 ± 0.37 days/leaf, respectively (Figure 19). In the non-monsoon season, P_L was higher than in the monsoon season, 11 ± 0.13 and 10.39 ± 0.16 day/leaf, respectively.
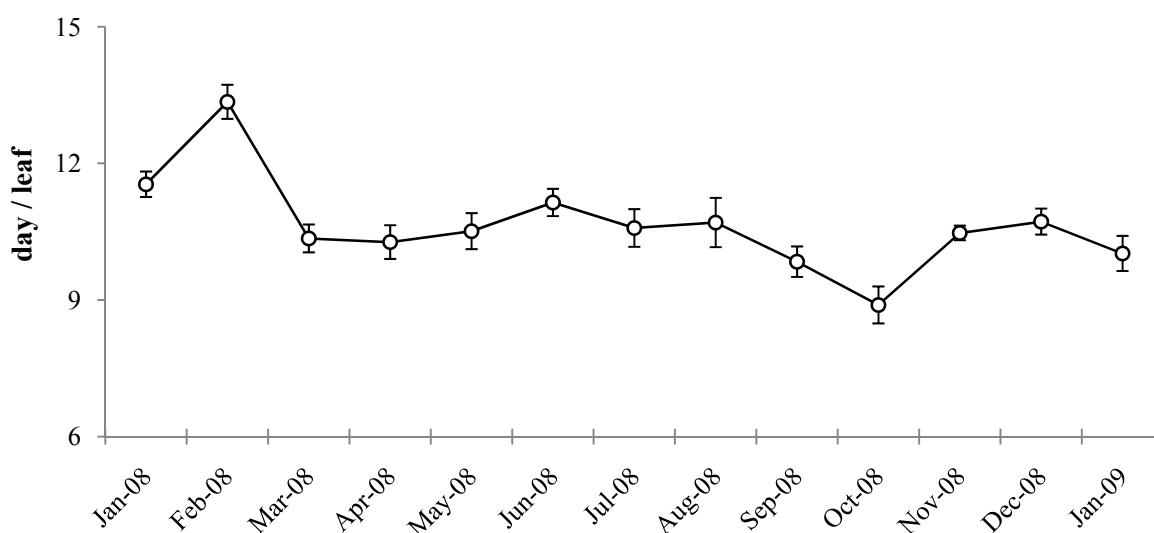
Figure 19. Leaf plastochronee intervals in each month from January 2008 to January 2009. The bars represent standard error. (N: Jan 08 = 145, Feb 08 = 109, Mar 08 =141, Apr 08 = 116, May 08 = 80, Jun 08 = 87, Jul 08 = 57, Aug 08 = 43, Sep 08 = 75, Oct 08 = 38, Nov 08 = 98, Dec 08 = 75, Jan 09 = 84)

Leaf elongation rate

Leaf elongation rate was significantly different among months (p<0.05; Table 2, 3). The mean of leaf elongation rate for the entire year was 1.21 ± 0.02 cm/shoot·day and the lowest was in February 2008, 0.90 ± 0.04 cm/shoot·day; and the highest was in October 2008, 1.77 ± 0.09 cm/shoot·day (Figure 20). Leaf elongation rates were 1.20 ± 0.02 and 1.24 ± 0.03 cm/shoot·day in the non-monsoon and monsoon seasons, respectively. There was significant correlation between leaf elongation rate with temperature, salinity, phosphate (PO_4^{3-}) in seawater and in sediment (Table 4).
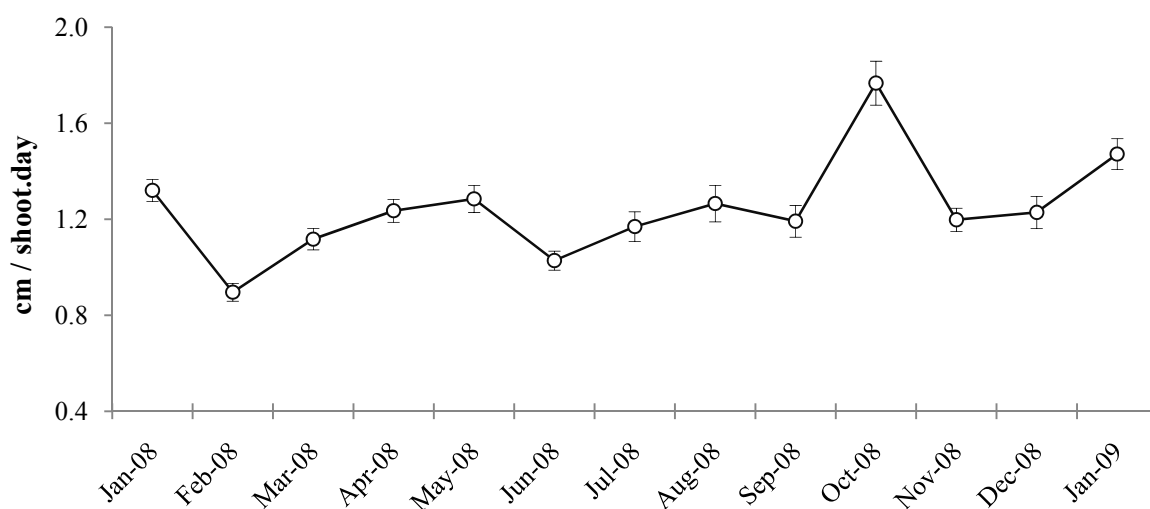
Figure 20. Leaf elongation rate in each month from January 2008 to January 2009. The bars represent standard error. (N : Jan 08 = 145, Feb 08 = 108, Mar 08 = 139, Apr 08 = 116, May 08 = 80, Jun 08 = 87, Jul 08 = 57, Aug 08 = 43, Sep 08 = 75, Oct 08 = 38, Nov 08 = 98, Dec 08 =75, Jan 09 = 84)

Leaf growth

The lowest leaf growth was in February and the highest was in October 2008, 0.16 ± 0.01 and 0.33 ± 0.02 mg/shoot·day, respectively (Figure 21). Leaf growth was significantly different among months ($p < 0.05$; Table 1, 5). Annual mean was 0.21 ± 0.01 mg/shoot·day. The growths of leaf were 0.21 ± 0.01 and 0.22 ± 0.01 mg/shoot·day in non-monsoon and monsoon seasons, respectively. There was significant relationship between leaf growth and temperature, nitrate (NO_3^-) in sediment, phosphate (PO_4^{3-}) in sea water and in sediment (Table 2).
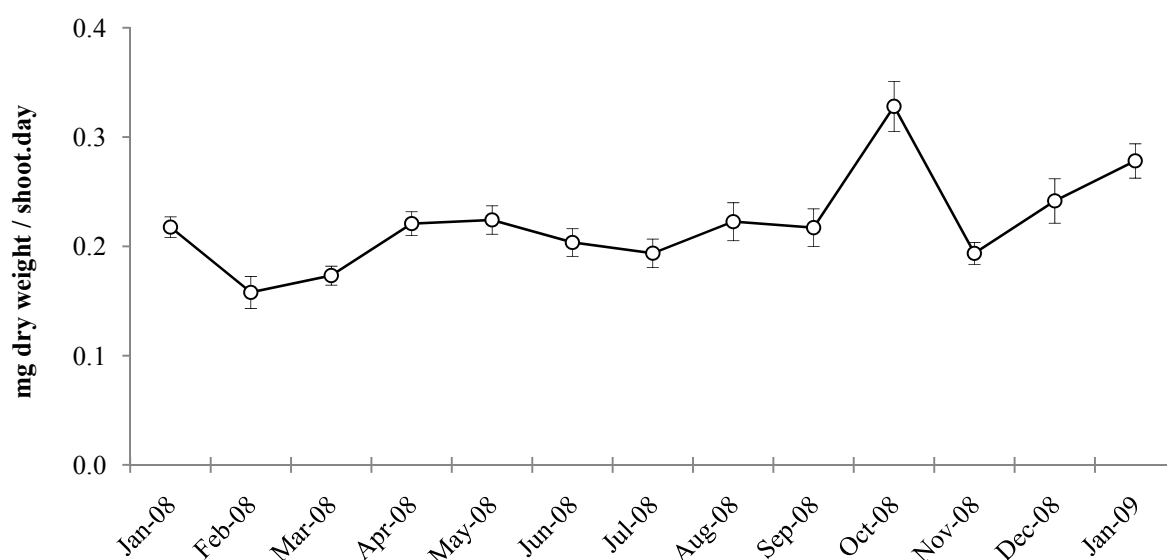
Figure 21. Leaf growth in each month from January 2008 to January 2009. The bars represent standard error. (N: Jan 08 = 145, Feb 08 = 108, Mar 08 = 139, Apr 08 = 116, May 08 = 80, Jun 08 = 87, Jul 08 = 57, Aug 08 = 43, Sep 08 = 75, Oct 08 = 38, Nov 08 = 98, Dec 08 = 75, Jan 09 = 84).

Meadow characters Study

Leaf biomass

Leaf biomass was not significantly different among months (p>0.05; Table 2). The lowest biomass was found in September 2008 and the highest biomass was in March 2008, 29.18 ± 6.43 and 52.93 ± 7.12 g/m^2, respectively (Figure 22). The mean leaf biomass throughout the year was 38.89 ± 2.13 g/m^2 and the biomass was 37.28 ± 2.69 and 40.52 ± 3.33 g/m^2 in non-monsoon and monsoon seasons, respectively.
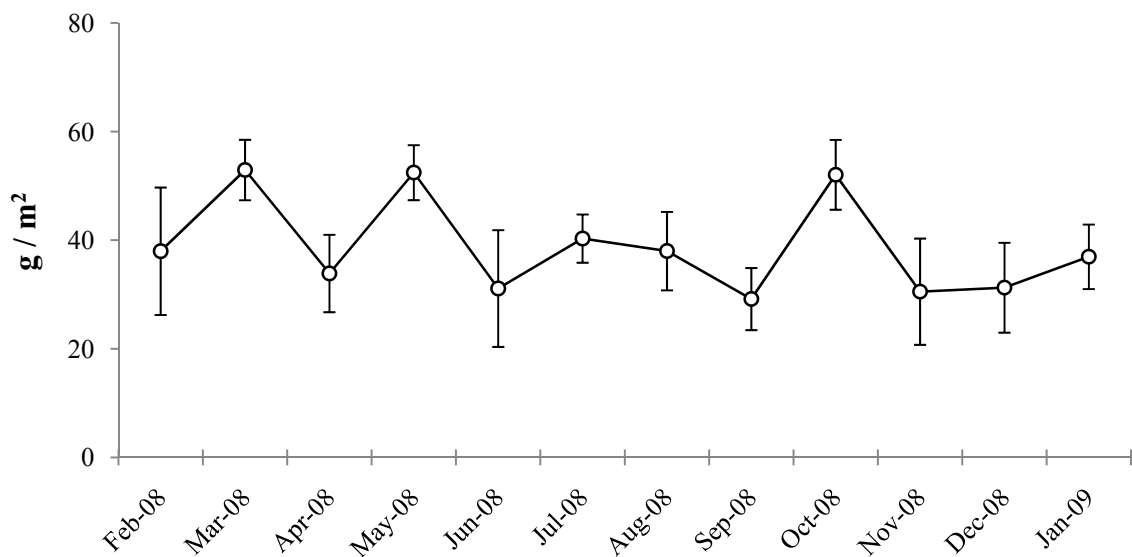
Figure 22. Leaf biomass in each month from February 2008 to January 2009. The bars represent standard error, (N = 5).

Sheath biomass

Sheath and stem biomass were not significantly different among months (p>0.005; Table 2). These biomasses were lowest in January 2009 and highest in September 2008, 21.51 ± 5.57 and 50.09 ± 14.84 g/m^2, respectively (Figure 23). The annual mean of sheath and stem biomass was 33.55 ± 2.78 g/m^2 and the sheath and stem biomass in non-monsoon and monsoon seasons were 1.38 ± 0.05 and 1.51 ± 0.05 g/m^2, respectively.
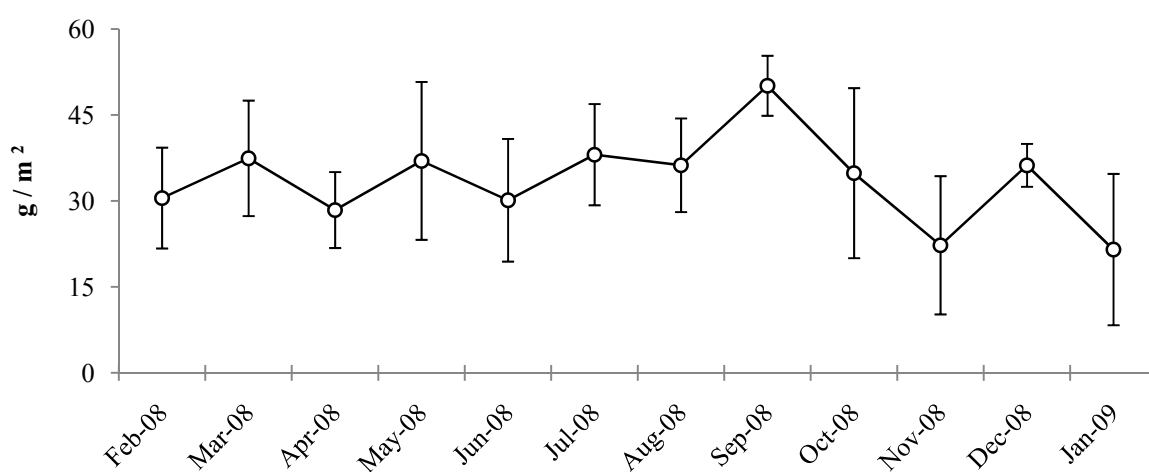
Figure 23. Sheath and stem biomass in each month during February 2008 to January 2009. The error bars are standard error, (N = 5).

Rhizome biomass

Rhizome biomass was significantly different among months (p<0.05; Table 2, 3). The lowest rhizome biomass was found in February 2008 and the highest in May 2008, 7.34 ± 0.98 and 133.21 ± 34.88 g/m^2, respectively (Figure 24). They were 53.50 ± 5.96 and 56.78 ± 11.07 g/m^2 in non-monsoon season and monsoon seasons, respectively. There was a positive correlation between rhizome biomass and nitrate (NO_3^-) levels in sediment, phosphate (PO_4^{3-}) levels in sediment, salinity and total rain, while there was a negative correlation with light intensity (Table 4).
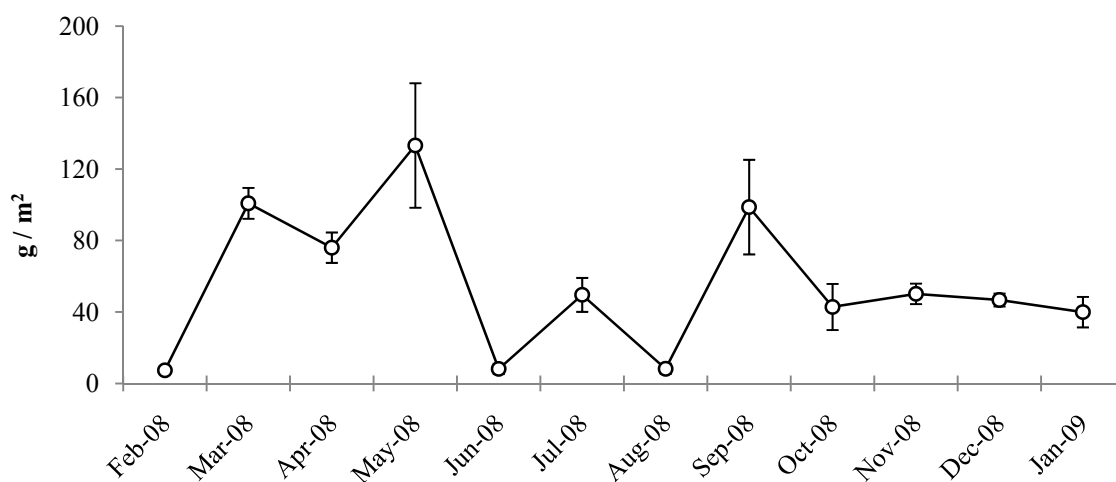
Figure 24. Rhizome biomass in each month during February 2008 to January 2009. The error bars are standard error, (N = 5).

Root biomass

Root biomass was significantly different among months (p<0.05; Table 2, 3). The lowest biomass was found in January 2009 and the highest was in May 2008, 9.31 ± 2.96 and 84.36 ± 14.41 g/m^2, respectively (Figure 25). The annual mean of root biomass was 33.74 ± 4.31 g/m^2. It was higher in the monsoon season than in the non-monsoon season, 40.58 ± 6.83 and 26.89 ± 55.08 g/m^2, respectively.
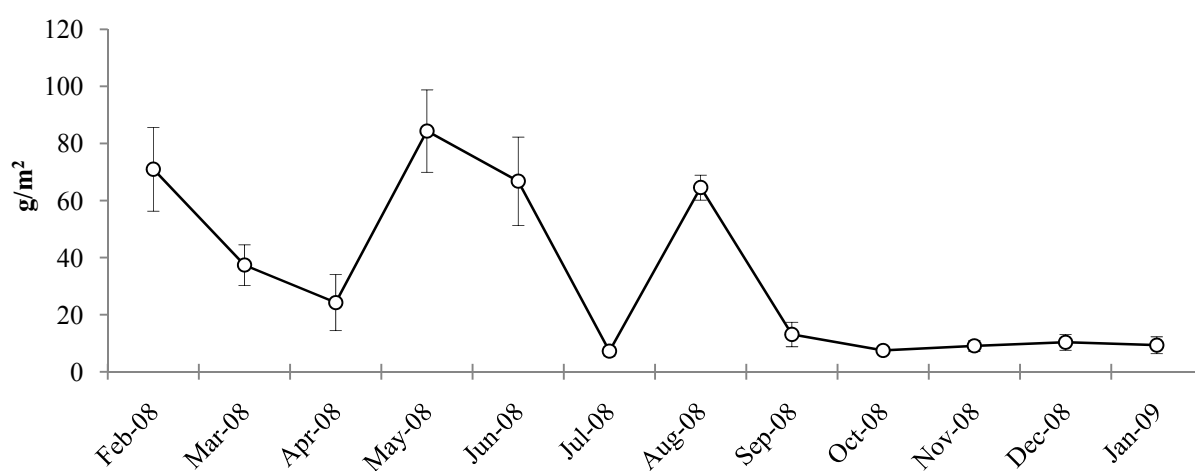
Figure 25. Root biomass in each month during January 2008 to January 2009. The error bars are standard error, (N = 5).

Above ground and below ground biomass

Above ground biomass was not significantly different among months (p>0.05), but there was significant difference in below ground biomass (p<0.05; Table 2, 3; Figure 26). The annual below ground biomass was higher than the above ground biomass, 88.87 ± 7.86 and 72.44 ± 4.42 g/m^2, respectively. The above ground biomass was also lower than the below ground biomass during the monsoon season than during the non-monsoon season, above ground: 78.24 ± 6.63 and 66.64 ±5.77 g/m^2, respectively and below ground: 97.36 ± 7.86 and 80.38 ± 7.36 g/m^2, respectively. In addition, the stepwise multiple regression revealed that there was a negative correlation between below ground biomass with nitrate (NO_3^-) in both sediment and water column, light intensity and density of burrowing shrimp, 1 month delayed effect (Table 4).
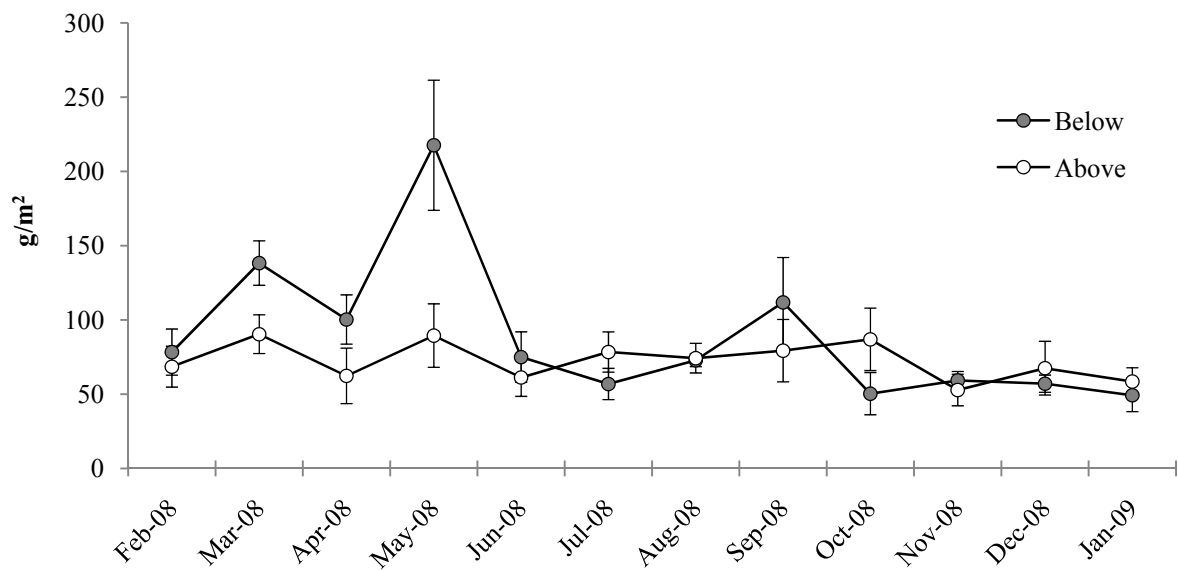
Figure 26. Above ground and below ground biomass in each month during February   2008 to January 2009. The error bars are standard error, (N = 5).

Shoot density

There was significant difference in shoot density among months ($p<0.05$; Table 2, 3). The lowest was found in December 2008 and the highest in January 2008, 522.67 ± 35.88 and 1099.43 ± 30.14 shoot/m^2, respectively (Figure 27). The annual mean density was 821.74 ± 17.46 shoot/m^2. The shoot densities in non-monsoon season and monsoon season were 865.86 ± 23.32 and 758.29 ± 23.60 shoot/m^2, respectively.
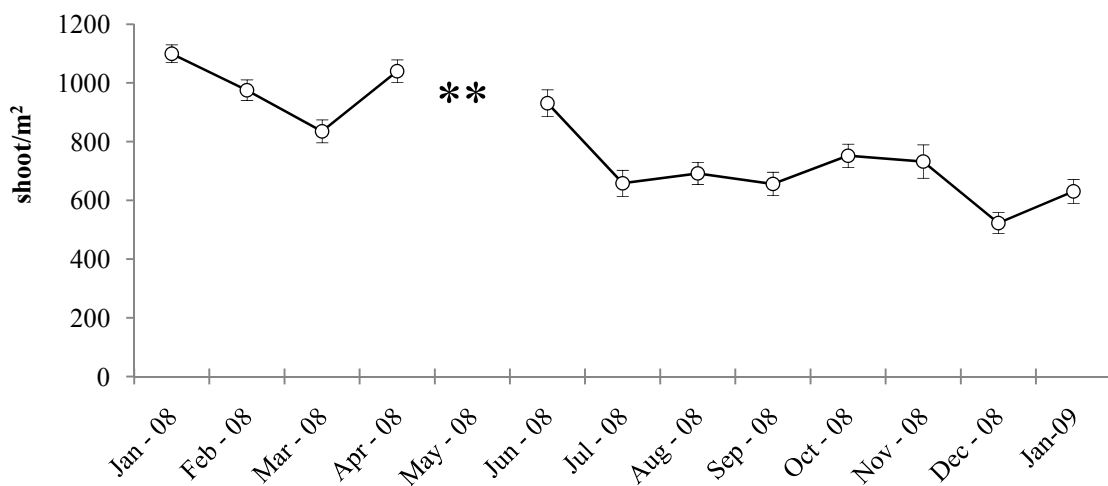
Figure 27. Shoot density in each month during January 2008 to January 2009. The error bars are standard error. ** is no data collection. (N = 5).

Table 2. The difference in means of PL, leaf elongation rate, leaf growth, leaf biomass, sheath and stem biomass, rhizome biomass, above and below ground biomass and shoot density in each month, and seasons between January 2008-January 2009. * represent the 95 % significant differences; and – = No data. Non Monsoon season is the time during NW monsoon and Monsoon season is the time during SE monsoon

Variables	Mean													N	Non-Monsoon season	Monsoon season	Annual mean	Difference between month		
	Jan-08	Feb-08	Mar-08	Apr-08	May-08	Jun-08	Jul-08	Aug-08	Sep-08	Oct-08	Nov-08	Dec-08	Jan-09					df	F	Sig
P_L (day/leaf)	11.54	13.35	10.35	10.27	10.51	11.14	10.58	10.70	9.84	8.89	10.47	10.72	10.02	1142	11.00	10.39	10.80	12	115.05	0.00*
SE	0.28	0.37	0.31	0.37	0.40	0.30	0.41	0.54	0.34	0.41	0.16	0.29	0.39		0.13	0.16	0.10			
Leaf Elongation Rate (cm/shoot·day)	1.32	0.90	1.12	1.24	1.28	1.03	1.17	1.27	1.19	1.77	1.20	1.23	1.47	1142	1.20	1.24	1.21	12	11.51	0.00*
SE	0.05	0.04	0.04	0.05	0.06	0.04	0.06	0.08	0.07	0.09	0.05	0.07	0.06		0.02	0.03	0.02			
Leaf Growth (mg/shoot·day)	0.22	0.16	0.17	0.22	0.22	0.20	0.19	0.22	0.22	0.33	0.19	0.24	0.28	1142	0.21	0.22	0.21	12	10.80	0.00*
SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.02	0.02		0.00	0.01	0.00			
Leaf Biomass (g/m^2)	-	37.98	52.93	33.89	52.45	31.12	40.31	38.01	29.18	52.06	30.54	31.27	36.96	60	37.26	40.52	38.89	11	1.61	0.13
SE	-	5.56	7.12	5.06	10.75	4.44	7.22	5.72	6.43	9.79	8.27	5.93	4.49		2.69	3.33	2.13			
Sheath Biomass (g/m^2)	-	30.49	37.43	28.40	36.98	30.11	38.07	36.22	50.09	34.84	22.25	36.20	21.51	60	1.38	1.51	33.55	11	0.95	0.51
SE	-	10.08	6.63	13.78	10.71	8.83	8.17	5.24	14.84	12.06	3.74	13.20	5.57		0.05	0.05	2.78			
Rhizome Biomass (g/m^2)	-	7.34	100.82	76.01	133.21	8.15	49.60	8.21	98.69	42.84	50.16	46.71	39.94	60	53.50	56.78	55.14	11	12.70	0.00*
SE	-	0.98	8.64	8.51	34.86	2.87	9.49	2.85	26.47	12.90	5.74	3.62	8.54		5.96	11.07	6.24			
Root Biomass (g/m^2)	-	70.97	37.40	24.24	84.36	66.78	7.23	64.57	13.10	7.47	9.08	10.33	9.31	60	26.89	40.58	33.74	11	12.10	0.00
SE	-	14.64	7.09	9.81	14.41	15.52	1.64	4.38	4.31	1.41	1.88	2.74	2.96		5.08	6.83	4.31			
Above ground Biomass(g/m^2)	-	68.47	90.36	62.29	89.43	61.22	78.37	74.22	79.28	86.90	52.79	67.47	58.47	60	66.64	78.24	72.44	11	0.69	0.74
SE	-	13.74	13.02	18.70	21.35	12.74	13.52	9.98	21.01	21.05	10.64	18.10	9.27		5.77	6.63	4.42			
Below ground Biomass (g/m^2)	-	78.31	138.23	100.24	217.57	74.93	56.83	72.77	111.79	50.30	59.24	57.04	49.26	60	80.38	97.36	88.87	11	6.52	0.00*
SE	-	15.54	14.94	16.55	43.83	16.98	10.51	4.31	30.19	14.20	6.04	5.79	11.07		7.36	13.86	7.86			
Shoot Density (shoot/m^2)	1099.43	975.24	835.05	1040.00	-	931.05	658.29	691.81	656.00	752.00	732.19	522.67	630.59	209	865.86	758.29	821.74	9	20.22	0.00*
SE	30.14	35.01	39.15	38.25	-	45.34	44.35	37.70	40.02	39.81	56.74	35.88	40.35		23.32	23.60	17.46			

Table 3. Multiple comparison of P_L, leaf elongation rate, leaf growth, rhizome biomass, root biomass, below ground biomass and shoot density among months (1 = Jan 08, 2 = Feb 08, 3 = Mar 08, 4 = Apr 08, 5 = May 08, 6 = Jun 08, 7 = Jul 08, 8 = Aug 08, 9 = Sep 08, 10 = Oct 08, 11 = Nov 08, 12 = Dec 08, 13 = Jan 09 and NS = Not significant).

Variables	Multiple Comparison
P_L (day/leaf)	(1,2),(1,3),(1,5),(1,6),(1,7),(1,8),(1,9),(1,10),(1,11),(1,12),(1,13),(2,3),(2,4),(2,5), (2,6),(2,7),(2,8),(2,9),(2,10),(2,11),(2,12),(2,13),(3,4),(3,5),(3,7),(3,8),(3,9),(3,12), (3,13),(4,11),(5,7),(6,9),(6,10),(6,11),(6,12),(7,8),(7,9),(7,10),(7,11),(7,12),(8,9), (9,10),(9,11),(10,11),(10,12),(11,12),(11,13)
Leaf Elongation Rate (cm/shoot.day)	(1,2),(1,3),(1,6),(1,10),(2,3),(2,4),(2,5),(2,7),(2,8),(2,9),(2,10),(2,11),(2,12),(2,13) (3,10),(3,13),(4,10),(5,10),(6,10),(6,13),(7,10),(8,10),(9,10),(10,11),(10,12)
Leaf Growth (mg/shoot .day)	(1,2),(1,3),(1,10),(2,4),(2,5),(2,6),(2,7),(2,8),(2,9),(2,10),(2,11),(2,12),(2,13) (3,4),(3,5),(3,10),(3,12),(3,13),(4,10),(5,10),(6,10),(6,13),(7,10),(7,13),(8,10) (9,10),(10,11),(10,12),(10,13),(11,13)
Leaf Biomass (g)	NS
Sheat and Stem (g)	NS
Rhizome (g)	(2,3),(2,4),(2,5),(2,7),(2,9),(2,10),(2,11),(2,12),(2,13),(3,6),(3,8),(4,6),(4,8),(5,6), (5,8),(6,7),(6,9),(6,10),(6,11),(6,12),(6,13),(7,8),(8,9),(8,10),(8,11),(8,12),(8,13)
Root Biomass (g)	(2,4),(2,7),(2,9),(2,10),(2,11),(2,12),(2,13),(3,5),(4,5),(4,6),(5,7),(5,9),(5,10), (5,11),(5,12),(5,13),(6,7),(6,9),(6,10),(6,11),(6,12),(6,13),(7,8),(8,9),(8,10) (8,11),(8,12),(8,13)
Above ground (g)	NS
Below ground (g)	(2,5),(4,5),(5,6),(5,7),(5,8),(5,9),(5,10),(5,11),(5,12),(5,13)
Shoot Density;Quadrat: (shoot/m2)	(1,3),(1,7),(1,8),(1,10),(1,11),(1,12),(2,7),(2,8),(2,10),(2,11),(2,12),(3,4),(3,12), (4,7),(4,8),(4,10),(4,11),(4,12),(6,7),(6,8),(6,11),(6,12)

Table 4. Partial correlation coefficients of environmental parameters on leaf elongation rate, leaf growth, and below ground biomass both on direct and delayed (1month) effects.

| Effect | Characters | Partial correlations coefficients | | | | | | | | | | F | sig | R square |
		Light L/Sf	Temp °C	Salinity ppt	Total Rain mm.	Exposure hours	Burrowing shrimp	Sea water uM (PO_4^{3-})	Sea water uM (NO_3^-)	Sediment umole (PO_4^{3-})/kg dry sed	Sediment umole (NO_3^-)/kg dry sed			equation
Directly effect	Leaf Elongation Rate (cm/shoot.day)	-	0.19	0.11	-	-	0.07	0.24	-	0.06	-	19.29	0.00*	0.10
Directly effect	Leaf Growth (g/shoot .day)	-	0.22	-	-	-	-	0.29	-	0.14	-	38.59	0.00*	0.11
Directly effect	Below ground (g/m^2)	-	-	-	-	-	-	-	-0.34	-	-	6.74	0.01*	0.11
Delay effect	*Below ground (g/m^2)*	*-0.36*	-	-	-	-	*-0.41*	-	*-0.59*	-	*-0.55*	*9.93*	*0.00**	*0.50*

Phenology study

A few flowers and fruits of *T. hemprichii* were found throughout the year (Figure 28, 29). Both male and female inflorescences (long bifid stigmas) were found only in October 2008. Fruits, however, were found in February and July 2008. A change in the character of male inflorescences (n = 3) was observed in the aquarium tank on October 2008. Unfortunately, a female inflorescence was observed in October 2008 and disappeared in November 2008. So, the development of female inflorescence to fruit could not be observed.
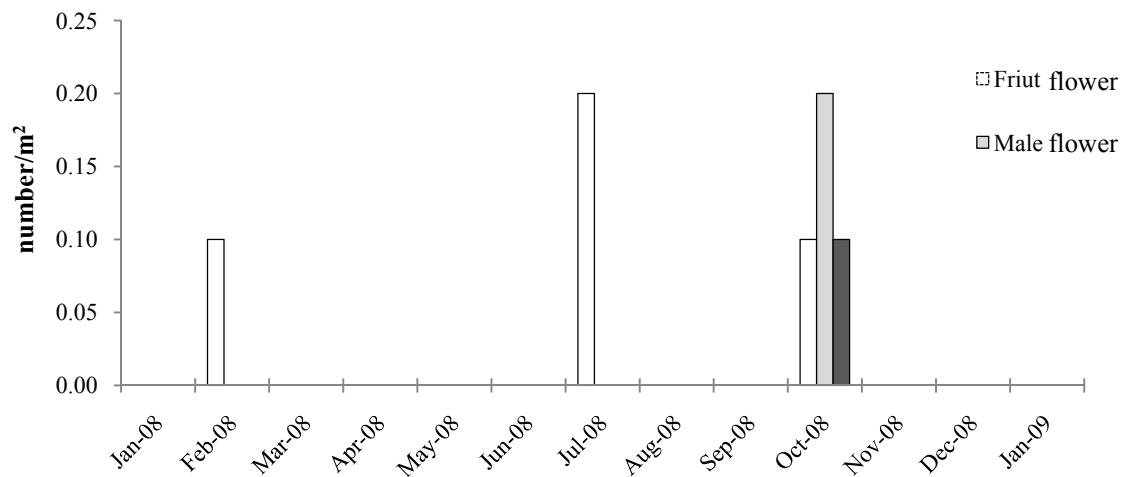
Figure 28. Number of flowers and fruits of *T. hemprichii* from January 2008 – January 2009.

Thalassia hemprichii produced the two perianth male inflorescences per shoot (n = 3), which flowering in different times. The young inflorescence was developed in the enclosed leaf sheath. Until it matured the peduncles that bearing the inflorescence was developed and extended outside the enclosed leaf sheath. Perianth part was open and stamen was appeared. Pollen is spherical shape that cohort and mucilage pollen gains were observed (Figure 30).
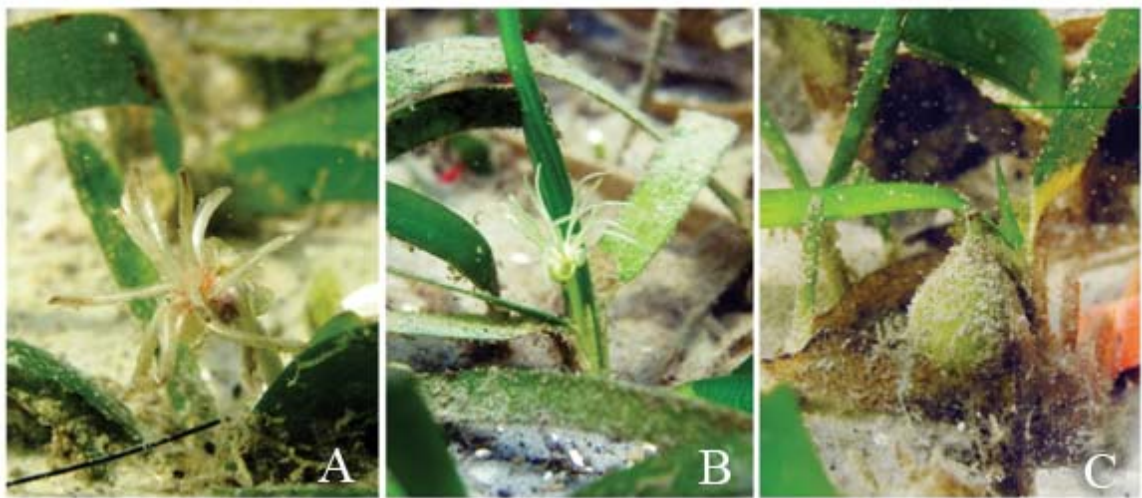
Figure 29. Flower and fruit of *T. hemprichii* found on the study site, A. male flower that pollen were released, B. female flower that had long bifid stigmas and C. fruit.
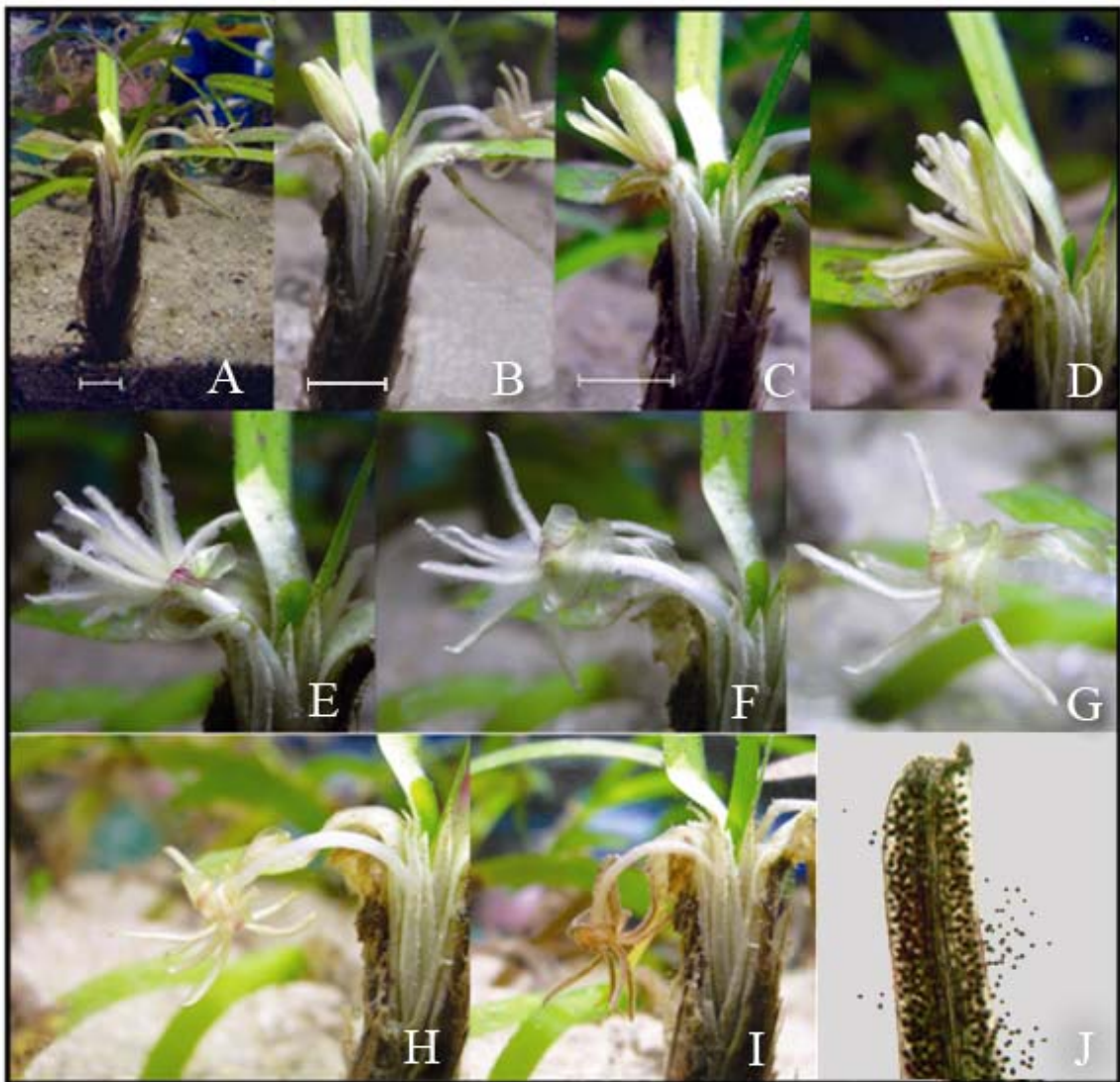
Figure 30. Male inflorescence of *T. hemprichii* was observed in the aquarium tank (N = 5); A. young inflorescence stage, B.-F. flowering of male inflorescence and pollen are releasing, G.-I. = inflorescence after released pollen, J. Pollen gain in the anther and bar = 1 cm.

Summary of data

The environmental conditions showed seasonal pattern throughout the year and influenced the growth parameters as well as the meadow. Here is the summary of all results, which would allow us to understand the relationship between environmental condition on growth and reproductions of *T. hemprichii* clearer (Figure 31 – 35).

In leaf growth characters, P_L reflected growth, higher P_L value suggested that longer days for plants to produce new leaf than the lower PL value. Thus, PL value showed opposite trend with leaf elongation rate and leaf growth rate. There was a similar trend between leaf growth and leaf elongation rate (Figure 31). In addition, leaf growth rate had correlation with phosphate (PO_4^{3-}) in seawater and shown the negative correlation with the exposure hour (Figure 32).

In meadow growth characters, the negative effect between shoot density and the burrow density were observed, low shoot density occurred when high burrow density was observed (Figure 33). Moreover, the below ground biomass had the negative correlation with the burrow density that shown the delayed (1 month) effected (Figure 34). While, the number of flowering and fruit had a negative correlation with the burrowing shrimp density, flowers and fruits were less with the increasing of burrowing density (Figure 35).
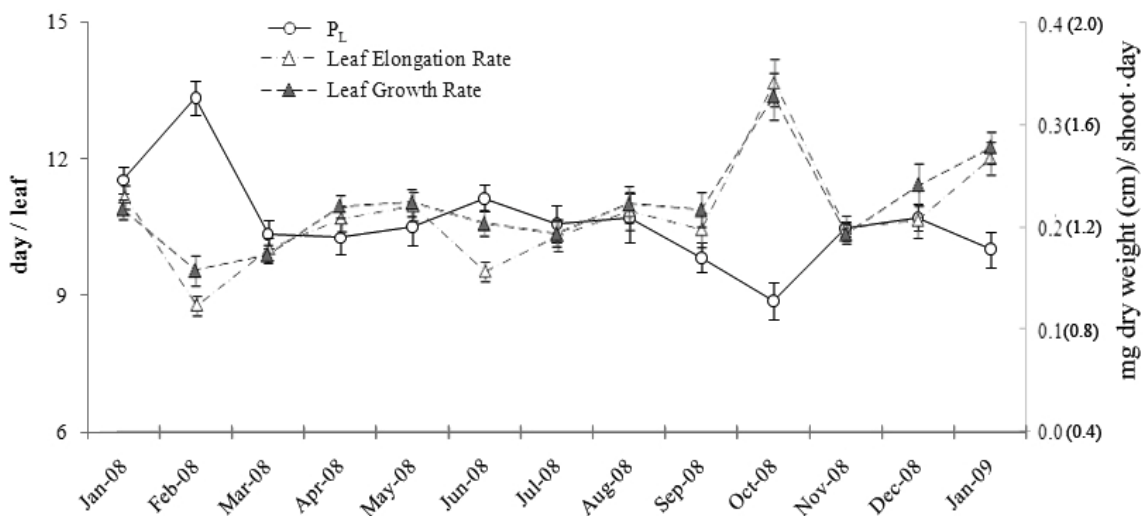
Figure 31. Temporal variation of all growth and meadow characters throughout the year. The error bars are standard error.
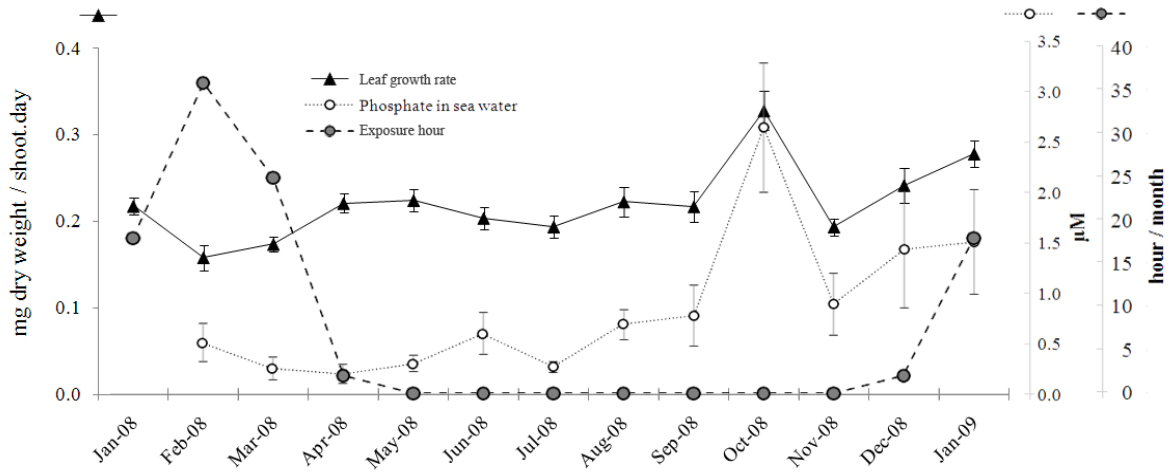
Figure 32. Relationship between leaf growth rate, phosphate in seawater and exposure hour throughout the year. The error bars are standard error.
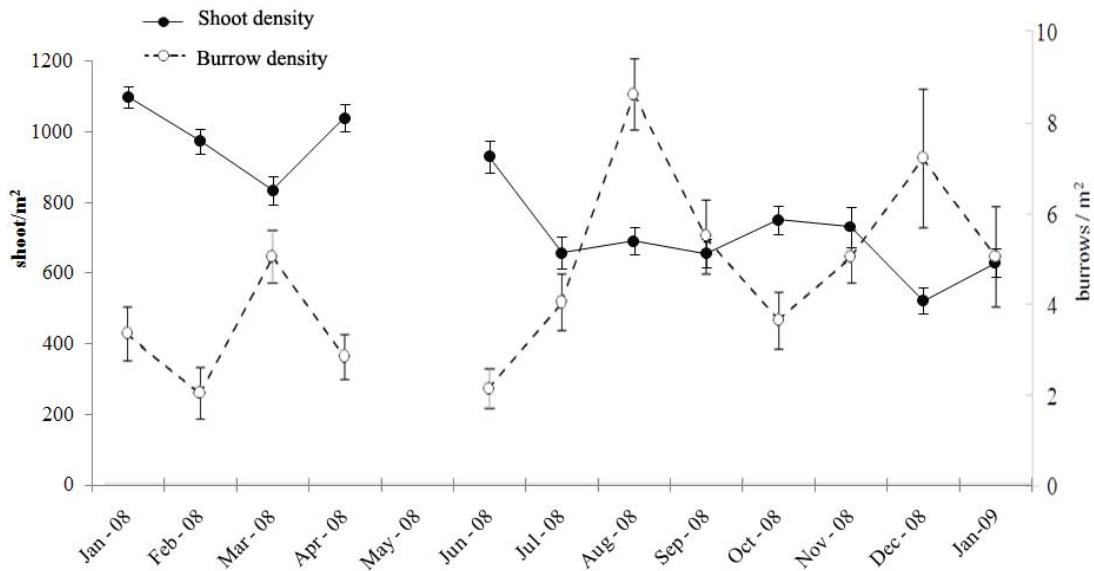
Figure 33. Relationship between shoot density and burrow density throughout the year. The error bars are standard error.
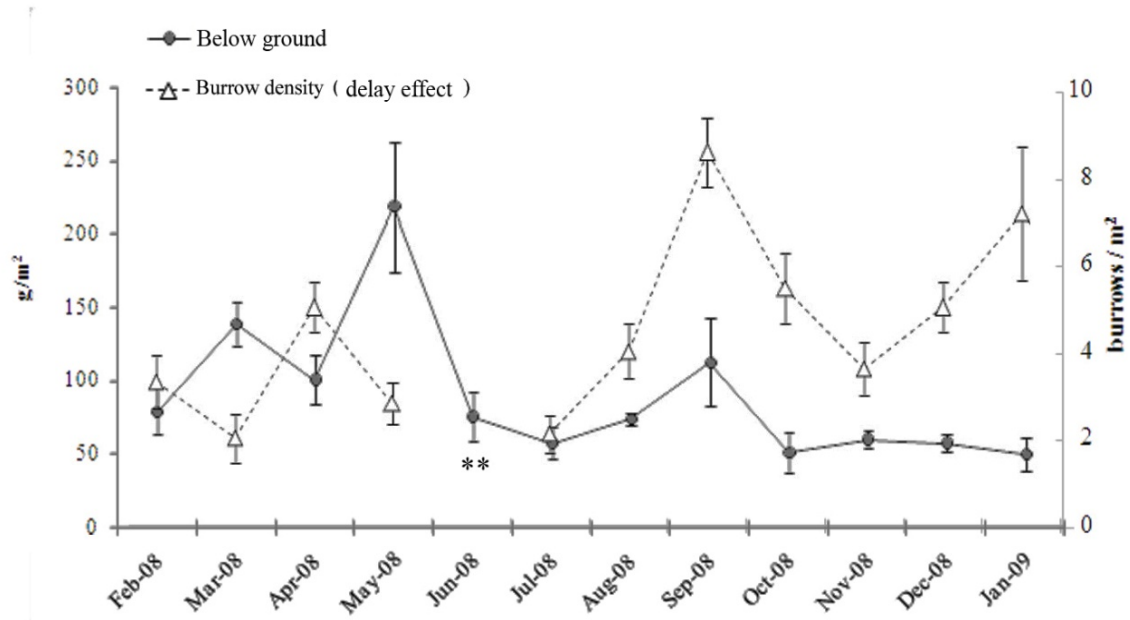
Figure 34. Relationship between below ground and burrow density (delay effect) throughout the year. The error bars are standard error.
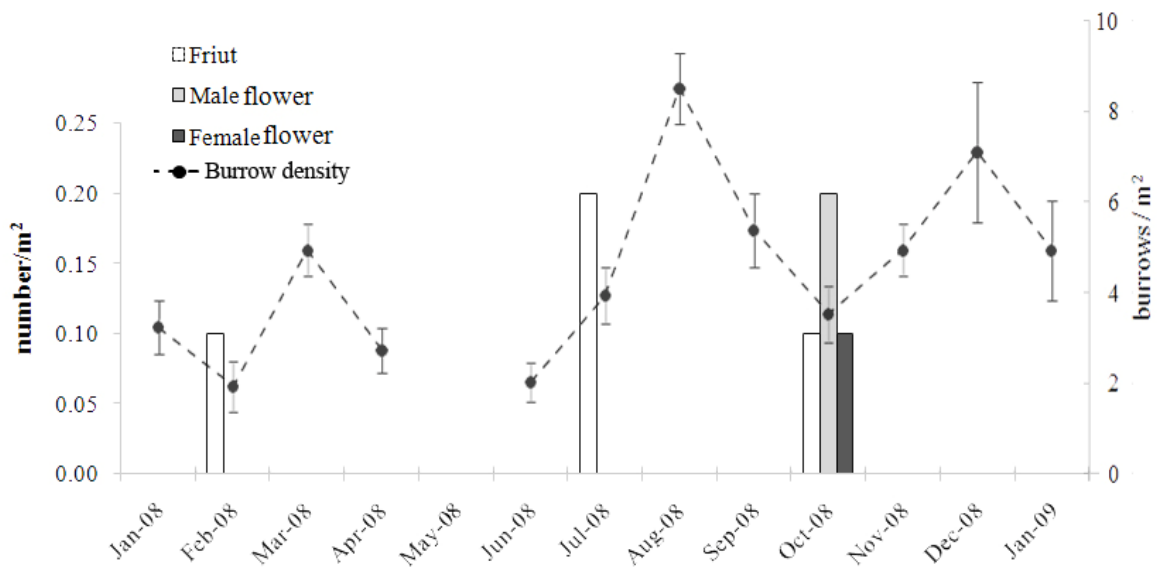
Figure 35. Relationship between fruit, male and female flowers and burrow density throughout the year. The error bars are standard error.

CHAPTER 4

DISCUSSION

This study had demonstrated the variations of individual leaf growth characters (i.e. leaf elongation rate, leaf growth and leaf plastrochone interval) and some meadow characters (i.e. shoot density, rhizome biomass, root biomass and below ground biomass) of *Thalassia hemprichii* throughout the year. It is known that seasonal variation in light, day length and temperature have strongly affected seagrass growth and reproduction in the temperate (Marbà *et al.*, 1996; Alcoverro *et al.*, 2004). Seagrasses in the tropical, on the other hand, are influenced by nutrient, desiccation and temperature (Erftemeijer and Herman, 1994; Stapel *et al.* 1997; Lin and Shao, 1998). Here, leaf growth characters and meadow production of *T. hemprichii* were influenced by amount of exposure hour, nutrients, light intensity and density of burrowing shrimp.

Leaf elongation rate (L_E), leaf growth (L_G) and leaf plastrochone interval (P_L), all growth variables, of *T. hemprichii* had the same pattern throughout the year. The lowest leaf growth productions (lowest L_E and L_G and highest on P_L) were found in February 2008, during summer months when plants exposed longest hours to air during the low tide (36 hours in this month). The loss of seagrass bed during the lowest tide has also been documented by Ertemeijer and Herman (1994). They have found that *T. hemprichii* and *Enhalus acoroides* above ground biomass were reduced by 80-90% on the seagrass bed in South Sulawesi when plant exposed to longer exposure hours during daylight. This co-occurred with intense insolation and high water temperature in the small tidal pool during mid-day. The negative effects of desiccation during the extreme low tide on the intertidal seagrass were also observed i.e., *T. hemprichii* in Papua New Guinea (Brouns, 1985) and also *Zostera noltii*, a subtropical species in Mauretania (Van Lent *et al.*, 1991). While the leaf growth

productions (L_E, L_G and P_L) in this study were decreased during the month of lowest tide, the meadow characters (i.e., leaf biomass per area and above ground biomass per area ($g \cdot m^{-2}$)) were constant throughout the year. This suggested that the lowest tide in this area was not severe when compared with seagrass bed in South Sulawesi, because the lowest tides occurred in the morning (5:00-9:00 hours) and the evening (18.00-20.00 hours); thus plants would not have to cope with high temperature. In addition, *T. hemprichii* have a strategies for coping with desiccation during the lowest tide by having a capacity to recover the photosynthesis ability close to original photosynthesis ability after losing the water up to 85% in the desiccation when re-submergence (Björk *et al*., 1999) and they also have a large sheath to prevent water loss from the meristem during low tide (Tanaka and Nakaoka, 2004). Thus, high growth rate and dense meadow are expected.

Seagrass beds normally are known to occur in an oligotrophic condition, thus growth and production of seagrasses increased by supplying N and/or P into the sediment (Bulthuis *et al*., 1992; Agawin *et al*., 1996; Lee and Dunton, 2000). However, this is not always true, Erftemeijer *et al*. (1994) shown that nutrients at Barang Lompo and Palanro in Indonesia had no nutrient limitation even increasing the nutrient in sediment that 100 times for Nitrogen and 2000 time of phosphorus. Here, nutrients in sediment seem to be very high on NO_3^- and PO_4^{3-} throughout the year. Although, the unit of nutrients in sediment on this study (μmole NO_3^- or PO_4^{3-} per kg dry weight of sediment) was different from the other studies (μmole of nutrient in 1 litre of pore water; μM). The roughly estimation of nutrients in sediment could be converted to 1047.63 μM on NO_3^- and 128.05 μM on PO_4^{3-} with the estimated total volume of pore water (litre) in sediment 1 kg dry weight sediment contained l liter of pore water. Here, there were 14 times higher of phosphate and 200 times higher of nitrate when compare with Palanro, Indonesia; thus nutrients in the sediment were not limited in this study site comparing with previous studies (Table 5). The increasing of leaf growth productions (L_E, L_G and P_L) coincidentally with increasing of PO_4^{3-} in water column during October 2008 (late monsoon season); tissue nutrients should be further investigated to provide a better understanding on nutrients and growth of *T. hemprichii*, since nutrients do not seem to be a limiting factor in this seagrass bed.

Normally, the above ground biomass is correlated with shoot density; decreasing shoot density would decrease the above ground biomass. Surprisingly, the above ground and leaf biomass were rather consistence throughout the year, although the environmental conditions greatly varied. The environmental parameters (i.e., light intensity, temperature, and nutrients) at the site seemed to provide an optimal condition for growth of *T. hemprichii*.

Light intensity was in range of light saturation for photosynthesis, 1000 $\mu mole.m^{-2}.s^{-1}$ and more than the 2000 $\mu mole.m^{-2}.s^{-1}$ for photo inhibition, respectively (Agawin *et al.,* 1996; Abu Hena *et al.,* 2001; see Appendix 1), while average light intensity on this study were in range of 973.48 - 2304.60 $\mu mole \cdot m^{-2} \cdot s^{-1}$ (Table 1). Also, nutrients concentrations seem to be sufficient especially nutrients in the sediment that contain high value of phosphate and nitrate comparing with the non nutrients limited area (Table 3). The temperatures also varied in the range of the optimum temperature (25-35 ^{o} C) for seagrass growth (Bulthuis, 1987; Lee *et al.*, 2007). Thus, *T. hemprichii* occurred in an optimal condition, had rather high production in this study, the above ground biomass, 72.44 $gDW \cdot m^{-2}$ were nearly the maximum value, 86.9 $gDW \cdot m^{-2}$ (Duarte and Chiscano, 1999). However, the below ground biomass was much less than 2.36 times; these lower below ground biomass might be because of the burrowing shrimp, limiting the below ground production due to the space limitation.

The phenomena that burrowing shrimp cut leafs or shoots of seagrass and stored them in their burrow were observed; and this seems to greatly influence the below ground biomass. The association of thalassinidean and alpheid shrimp is common in seagrass (Griffis and Suchanek, 1991, Stapel and Erftemeijer, 2000). Vonk *et al.*, (2008) found that the total amount of seagrass leaf collection by *Neaxius acanthus* and *Alpheus macellarius* shrimp (density of burrow openings were 0.71-1.84 burrow $\cdot m^{-2}$ and 0.03-0.09 burrow $\cdot m^{-2}$, respectively) was 1.2 - 3.1 g dry weight $\cdot m^{-2}$ $\cdot day^{-1}$, 50 - 63 % of leaf production in the seagrass meadow on Spermonde Archipelago, Sulawesi, Indonesia. The high density of burrow were 1.71-8.57 burrow $\cdot m^{-2}$ in this study; and indeed this could decrease leaf and above ground biomass of *T. hemprichii*, especially the below ground production. The recent study

reported that the burrow of burrowing shrimp *Neaxius acanthus* and *Alpheus macellarius* could be as deep as 50 cm (Vonk *et al*., 2008); while the below ground parts (i.e. rhizome and root) of *T. hemprichii* occupied 0-15 cm underground (personal observation). These suggested that the process of burrowing shrimp by making the hole might influence the below ground part of *T. hemprichii* losing their below ground biomass.

Leaf biomass and above ground biomass, however, were not significantly different among months throughout the year. This might be a result from energy translocation from below ground parts to produce the new leaf and new shoot that seagrass are known to be able to transfer the energy from the rhizome (Eklöf *et al*., 2008) and/or the neighbouring shoot in the same ramet or clone (Marbà *et al*., 2002). This translocation of reserves energy, indeed, affected the below ground biomass that shown the fluctuation throughout the year. On the others hand, the production of leaf growth and above ground biomass, which were cut by the burrowing shrimp have increased the N and P assimilation of *T. hemprichii*. This assimilation process requires energy and carbon skeleton from rhizome (Invers *et al*., 2004), which could also affect the below ground biomass and caused the delayed effect. Moreover, this might affect the other productions such as flower and fruit since these uses more energy to produce and maintain.

Seventeen percent of *T. hemprichii* shoots in the Philippines produced flowers (Duarte *et al*., 1997) and 30 % in Pag-asa Island (Rollon *et al*., 2001). However, only less than 1 % of shoot produced flowers in this study. This might be because of the insufficient energy for producing the flower/fruit and fluctuating of below ground parts which was disturbed by the burrowing shrimp. Furthermore, the burrowing shrimp had limited the development of above ground shoots by cutting it away. This would not allow living shoot to become mature and reproduce. *T. testudinum* in New Guinea reproduced at shoot ages between 275 days to 1050 days (Cox and Tomlinson, 1988); and *T. hemprichii* population showed high percentage producing flowers in Pag-asa Island at 328-363 days (Rollon *et al*., 2001). In this study, the shoot age were mostly about 100-200 days, *T. hemprichii* plants need at least a year to become mature before producing flowers and fruit (Duarte *et al*., 1997),

thus this could also limit the flowering plants in the site. The burrowing shrimp could be a major drive limiting growth and also reproduction of *T. hemprichii* in this study.

Few shoots of the maturing age (more than 365 days), however, were found, but the flowers or fruits were still rare. This might be other factors influence the flowers and fruit production. Water temperature has been proposed as the primary factor controlling flowering of tropical seagrass (Philips *et al.*, 1981; McMillan, 1982; Pettitt, 1984). The previous study shown that the low water temperature could induce the flowers of *T. hemprichii* (McMillan, 1980); and day-lengths plays a minor role in reproductive periodicity of tropical seagrass (McMillan, 1982). It is reported that only low flowering frequency of *T. hemprichii* observed (Duarte *et al*. 1997) in Silaqui Island, Philippines; and *T. hemprichii* produced fruits throughout the year in the Gulf of Thailand and Andaman Sea (Lewmanomont *et al.*, 1996). However, only a few flowers and fruits were found in this study, further investigations are needed also in a larger scale, since some flowers and fruits were observed on the upper shore during January 2009 (personal observation)

Although, burrowing shrimps play an important role to restrict the seagrass growth and reproduction by their burrow and behavior by cutting and storing the seagrass leaf. The burrowing and storage of seagrass leaf by this burrowing shrimp could affect biogeochemical of sediment, nutrient exchange, and organic composition as shown in ghost shrimp (Ziebis *et al.*, 1996; Webb and Eyre, 2004). The presence of burrowing shrimp could also increase the O_2 in to the sediment (Webb and Eyre, 2004; Vonk *et al.*, 2008) that enhancement of oxidation reaction in the sediment such as the nitrification, which could increase nutrients around their burrow, as also seen and had promoted growth in this study. Moreover, the presence of burrowing shrimp might increase the survival potential of seagrass by providing greater O_2 for below ground respiration and decreasing sulphide in sediment, which could make seagrass less vulnerability in low light or during the night time condition (Hemminga, 1998).

In conclusion, this study demonstrated that there were variations in leaf growth characters and below ground biomass of *T. hemprichii* among months. The environmental parameters provide an optimum condition for growth, but the exposure hours could affect the leaf growth characters during the summer months; and burrowing shrimp was a major factor driving on growth and reproduction of *T. hemprichii* in this area.

Further study on interaction between burrowing shrimp and *T. hemprichii* would be interesting since only a few studies have been carried out although they seem to strongly influence *T. hemprichii* population, In addition, a larger scale monitoring should be investigated, this would allow us to understand the phenomenon of *T. hemprichii* population throughout the landscape.

Table 5. Nutrients comparison between water column and sediment at various seagrass bed in Southeast Asia. The abbreviation are a = μmole PO_4^{3-} per kg dry weight sediment, b = μmole NO_3^- per kg dry weight of sediment, and * = NO_3^- only.

Site	Sediment Type	Enrichment	Pore water (μM)			Water column (μM)			Nutrient limited		Refference
			PO_4^{3-}	NH_4^{3-}	$NO_3^- + NO_2^-$	PO_4^{3-}	NH_4^{3-}	$NO_3^- + NO_2^-$	P	N	
Barang Lompo, Indonesia	Coral sand	None	7.5	82.2	3.4	0.8	1.4	0.9	No	No	Ertemeijer et all., 1994
		N+P	13	109	3.4	0.8	1.4	0.9			
Kudingareng Lompo, Indonesia	Coral sand	None	7.3	50.9	-	-	-	-			
		N+P	-12	-	-	-	-	-			
Palanro, Indonesia	Terrigenous	None	8.7	23.0	5.2	3.3	bld	1.5			
		N+P	-14	-	5.2	3.3	bld	1.5			
Silaqui, Philippines	Coral sand	None	0.9	8.3	1.4	0.2	1.8	0.6	Yes	Yes	Agawin et all, 1996
		N+P	55.9	111.3	2.3	0.2	1.8	0.6			
Lucero, Cape Bolinao, Philippines	Coral sand	None	0.6	10.2	1.5	0.1	1.7	0.5			
		N+P	35.7	40.1	1.5	0.1	1.9	0.6			
Kho Bae Na, Trang, Thailand	sand with shell	None	5 - 15	20 - 50	*nd*	nd	0.08	0.04	-	-	Yamamuro et all, 2001
Leam Yong Lum, Trang, Thailand	sand with shell	None	128.1[a]	-	1009.93[b]	0.92	-	0.37*	-	-	This study

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Appendix
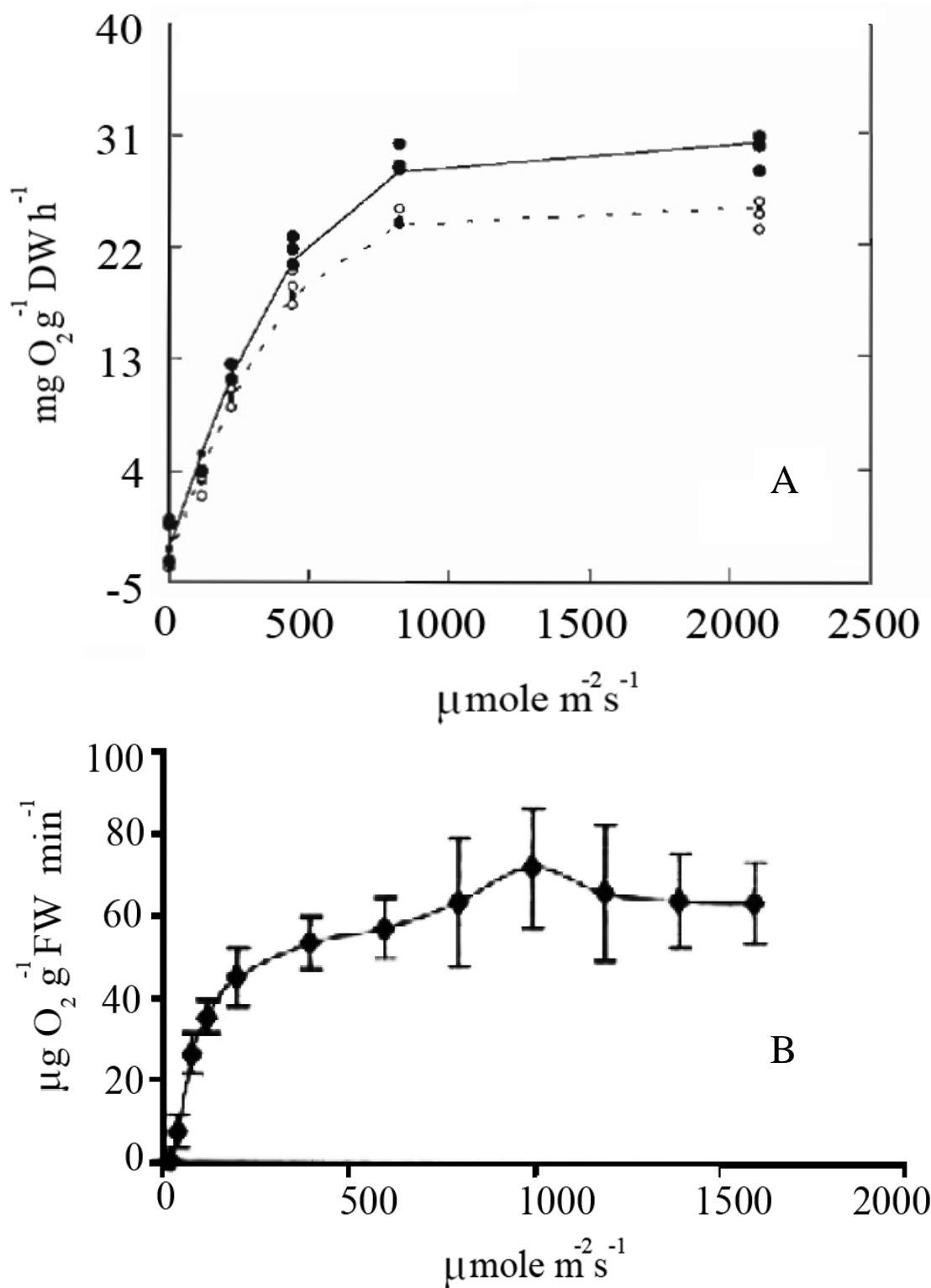
Appendix 1. The Photosynthetic rate of *Thalassia hemprichii* in the previously stdies: A. results from Agawin *et al.,* 1996; B. results from Abu Hena *et al.,* 2001. FW = fresh weight, DW = dry weight

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