Seasonal Variation in Distribution, Density, and Life Stage of *Halimeda macroloba* Decaisne at Tangkhen Bay, Phuket Province, Thailand

Sutinee Sinutok

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Ecology (International Program)

Prince of Songkla University

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ชื่อวิทยานิพนธ์ ความแปรผันตามฤดูกาลของการกระจายตัว ความหนาแน่น และช่วงชีวิตของสาหร่าย *Halimeda macroloba* Decaisne บริเวณอ่าวตังเข็น จังหวัดภูเก็ต

ผู้เขียน นางสาวสุธินี สินุธก

สาขาวิชา นิเวศวิทยา (นานาชาติ)

ปีการศึกษา 2551

บทคัดย่อ

สาหร่ายสกุล *Halimeda* มีการกระจายทั่วโลกทั้งในเขตร้อนและเขตกึ่งร้อน สาหร่าย *Halimeda macroloba* Decaisne ประกอบด้วยชิ้นส่วนที่สะสมแคลเซียมคาร์บอเนตยึดต่อกันเป็นทัลลัสที่มีลักษณะแบนและตั้งตรง ยึดเกาะพื้นทรายด้วยโฮลด์ฟาสต์รูปร่างทรงกระบอกที่ประกอบด้วยตะกอนทรายจากพื้นผิวที่สาหร่ายยึดเกาะ งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาความแปรผันตามฤดูกาลของการกระจายตัว ความหนาแน่น และช่วงชีวิตของสาหร่าย *H. macroloba* บริเวณอ่าวตังเข็น จังหวัดภูเก็ต และศึกษาความสัมพันธ์ระหว่างองค์ประกอบของตะกอนทรายบริเวณโฮลด์ฟาสต์และพื้นผิวยึดเกาะ พื้นที่ผิวของใบสาหร่ายและปริมาตรของโฮลด์ฟาสต์ และความสัมพันธ์ของคลื่นลมและพื้นที่ผิวของใบสาหร่ายและปริมาตรของโฮลด์ฟาสต์ สำหรับการศึกษาการกระจายตัวและความหนาแน่นของสาหร่าย ได้มีการเก็บตัวอย่างทุกเดือนเป็นเวลา 1 ปี ระหว่างเดือนสิงหาคม 2549 ถึงเดือนกรกฎาคม 2550 โดยสุ่มตัวอย่างจากควอแดรตขนาด 0.25 ตารางเมตร การศึกษาช่วงอายุและช่วงชีวิตของสาหร่าย ได้ศึกษาในพลอตถาวรขนาด 0.25 ตารางเมตรจำนวน 21 พลอต นอกจากนี้ได้เก็บตัวอย่างสาหร่ายทุกๆ 2 เดือนเพื่อมาศึกษาองค์ประกอบของตะกอนทรายในโฮลด์ฟาสต์ พื้นที่ผิวของใบและปริมาตรของโฮลด์ฟาสต์ นอกจากนี้ได้เก็บข้อมูลค่าความแรงของคลื่น อุณหภูมิน้ำและอากาศ ความเค็มของน้ำทะเล ตัวอย่างน้ำและตะกอนทรายในพื้นที่ยึดเกาะ ผลการศึกษาพบว่ามีความแปรผันของการกระจายตัว ความหนาแน่น และช่วงชีวิตของสาหร่ายในแต่ละเดือนซึ่งเป็นผลมาจากการกระทำของคลื่นลมที่ส่งผลให้เกิดการสืบพันธุ์แบบไม่อาศัยเพศจากการแตกหักของทัลลัส ปริมาณน้ำฝนที่ส่งผลเพิ่มตะกอนปกคลุมสาหร่าย ลักษณะของพื้นที่ยึดเกาะ และการรุกรานของหญ้าทะเล การศึกษาช่วงชีวิตพบว่าสาหร่ายมีช่วงอายุ 8-12 เดือน การศึกษาองค์ประกอบของตะกอนทรายในโฮลด์ฟาสต์พบว่า สาหร่ายมีกลไกการเลือกสัดส่วนองค์ประกอบของตะกอนทราย ซึ่งอาจเพิ่ม

ประสิทธิภาพในการยึดเกาะ การดูดซึมสารอาหาร และลดการขาดน้ำ นอกจากนี้ ยังพบว่า มีความสัมพันธ์กันน้อยระหว่างขนาดพื้นที่ผิวของใบและปริมาตรของโฮลด์ฟาสต์ และไม่มีความสัมพันธ์ระหว่างความแรงของคลื่นลมกับขนาดพื้นที่ผิวของใบและปริมาตรของโฮลด์ฟาสต์ ซึ่งอาจเป็นผลมาจากลักษณะของต้นที่ยืดหยุ่นไปตามแรงกระทำของคลื่น สามารถลดแรงกระทำจากคลื่นลมได้

Thesis Title Seasonal Variation in Distribution, Density, and Life Stage of *Halimeda macroloba* Decaisne at Tangkhen Bay, Phuket Province, Thailand.

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ABSTRACT

The genus *Halimeda*, a green alga (Chlorophyta), is widely distributed in the tropical to sub-tropical marine environment. *Halimeda macroloba* Decaisne has several calcified segments, an erect and flat thallus and a massive bulbous holdfast attached in the sandy bottom. This bulbous holdfast may adhere to fine particles of loose substrate. The purpose of this study is to investigate the seasonal variation in distribution, density, and life stage of *H. macroloba* Decaisne at Tangkhen Bay, Phuket Province, Thailand, and to investigate the relationship between sediment size fractions in the holdfast and in the study area, blade surface area and holdfast volume, and wave motion and blade and holdfast size. To study distribution and population density, data were collected every month for a year during August 2006-July 2007 using 0.25 m^2 quadrat placed along three transect lines. Twenty one of 0.25 m^2 permanent plots were used to monitor life stage and life span of *H. macroloba*. *Halimeda* thalli were collected to investigate the sediment size fractions in the holdfast, blade surface area and holdfast volume. Wave motion, air and water temperature, salinity, water samples and sediment samples were collected. The results showed that there were seasonal variation in distribution, density, and life stage of *H. macroloba* resulting from wave action which contributes to an asexual reproduction by vegetative fragmentation, rainfall which increases sedimentation, sediment size fraction in substrate, and the invasion of seagrasses. The life stage study showed that the life span of *H. macroloba* at Tangkhen Bay was 8-12 months. The sediment study showed that the sediment accumulation in the holdfast of *H. macroloba* might be a progress of sediment selection for the advantages in increasing holdfast strength (holdfast tenacity), nutrient uptake and decreasing desiccation. There was a positive relationship between holdfast volume and blade surface area in *H. macroloba*. However, there was no relationship between water velocity and both holdfast volume and blade surface area of *H. macroloba* at Thangkhen Bay. This might result from its flexible morphology which can reduce the drag force imposed on the algae by reconforming with flow.

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CHAPTER 1

INTRODUCTION

Seaweeds are marine algae attached or fixed to the substratum by a holdfast. Most seaweeds are either green, brown or red. They are found between the top of the intertidal zone and the maximum depth to which adequate light for growth can penetrate. They interact with other marine organisms and the marine environment. The major environmental factors affecting seaweeds are light, temperature, salinity, water motion, and nutrient availability. Seaweeds are very important ecologically as primary producers. Moreover, they are used as food by humans, as fertilizer, and to obtain chemical extracts (Dawson, 1966; Lobban *et al*., 1985).

The genus *Halimeda*, a green alga (Chlorophyta), is widely distributed in the tropical to sub-tropical marine environment. *Halimeda* consists of articulated, plate-like and calcified segments. These are joined together by small, uncalcified nodes into branching chains, to produce a more or less bushy plant. They attach to the substratum by a holdfast. *Halimeda* may grow in depths to 100-150 meters, where light levels are calculated to be only 0.05-0.08 percent of the surface intensity (Hillis-Colinvaux, 1980). They are known as an important contributor of sand to mud-size carbonate sediments (Drew, 1983), primary producers (Bach, 1979), and as a provider of shelter and nursery grounds for a number of invertebrates (Hillis-Collinvaux, 1980). Species of *Halimeda* have been classified according to the shape, size and internal structure of their segments. Each species of *Halimeda* has different habitats and growth forms. Several species are attached by a single, small holdfast and grow on rock surfaces or hang as drapes from rocks. The second group of species sprawls across rock, sand or coarse algal and coral debris. The third group grows in sandy substrate and forms a large holdfast attached in sand (Verbruggen, 2005).

Halimeda macroloba Decaisne has several calcified segments, an erect and flat thallus and a massive bulbous holdfast attached in the sandy bottom. This bulbous holdfast, which might be 13 cm or more in length, may adhere to fine particles of loose substrate (Blaxter *et al.,* 1980). This unique bulbous holdfast, as found in *H. incrassata*, *Avrainvillea*, *Penicillus* and *Udotea*, provides anchorage in the sandy substratum and has an ability to obtain nutrients directly from sediments (Williams, 1984; Fong *et al.*, 2003; Littler *et al.*, 2004) which may help explain its wide distribution and rapid growth (Multer and Clavijo, 1989).

A study of *Halimeda tuna* (Ellis and Solander) Lamouroux in the Florida Keys showed that densities of *H. tuna* fluctuated because of environmental and seasonal parameters. There is some evidence that both biotic and abiotic factors, for example, nutrient concentration, light intensity, water motion, herbivores and epiphytes, influence *Halimeda* populations in the subtidal such as growth, biomass, survivorship, recruitment and segment loss (Ballesteros, 1991; Beach *et al.*, 2003; Vroom *et al.*, 2003). However, there is little information on *Halimeda* populations in the intertidal zone.

H. macroloba holdfast morphology is unique. It is similar to other coenocytic algae growing in sediment such as *Penicillus* J.B. de Lamarck and *Udotea* J.V.F. Lamouroux. Research on coenocytes has yield insights on their biology and ecology, but less is known of their biomechanical properties (DeWreede, 2006). The relationship between morphological and biomechanical properties has been little studied, for examples between blade surface area and holdfast volume, between blade surface area and force to remove, and between removal force and holdfast volume. Moreover, their environmental factors, e.g. wave motion and substrate has been little studied.

The purpose of this study is to investigate the seasonal variation in distribution, density, and life stage of *H. macroloba* Decaisne at Tangkhen Bay, Phuket Province, Thailand. The biomechanical properties in *H. macroloba* holdfast and thallus are included.

Review of literature

The characteristic of the genus *Halimeda*

Classification of *Halimeda* (Bold and Wynne, 1978).

Kingdom	Protista
Division (Phylum)	Chlorophyta
Class	Chlorophyceae
Order	Caulerpales
Family	Udoteaceae
Genus	*Halimeda*
Species	*Halimeda macroloba* Decaisne

Halimeda macroloba Decaisne plants are erect, flat and somewhat bushy, to 23 cm tall, excluding the bulbous holdfast which is usually well developed and may extend to 5 cm in length (Figure 1A). Segments are flat and fan-shaped. The basal segments are commonly compressed-cylindrical to trapezoidal. Other segments are compressed-cylindrical but more commonly subcuneate, discoidal or subreniform. The margin of segments is entire, undulating or lobed. Calcification moderate to rather light throughout the thallus. Thallus color is green when fresh and cream or greenish when dried. Cortex occasionally of two, but more commonly of three to four layers of utricles; outermost utricles separating on decalcification or remaining slightly attached, their lateral and peripheral margins occasionally thicken, 23-49 µm in surface diameter, usually four or occasionally two supported by each secondary utricles. Nodal medullary filaments uniting as a single group for a distance of approximately 44-80(-115) µm, the adjacent filaments communicating by pores; walls in this region thickened and pigmented (Figure 1C). The plant grows in mud or sandy flats, from above low-tide line to 12 meters deep. However, it is commonly located in shallow water. Moreover, it often grows in quiet water (Blaxter *et al.*, 1980; Lewmanomont *et al.*, 1995; Lee, 1999).

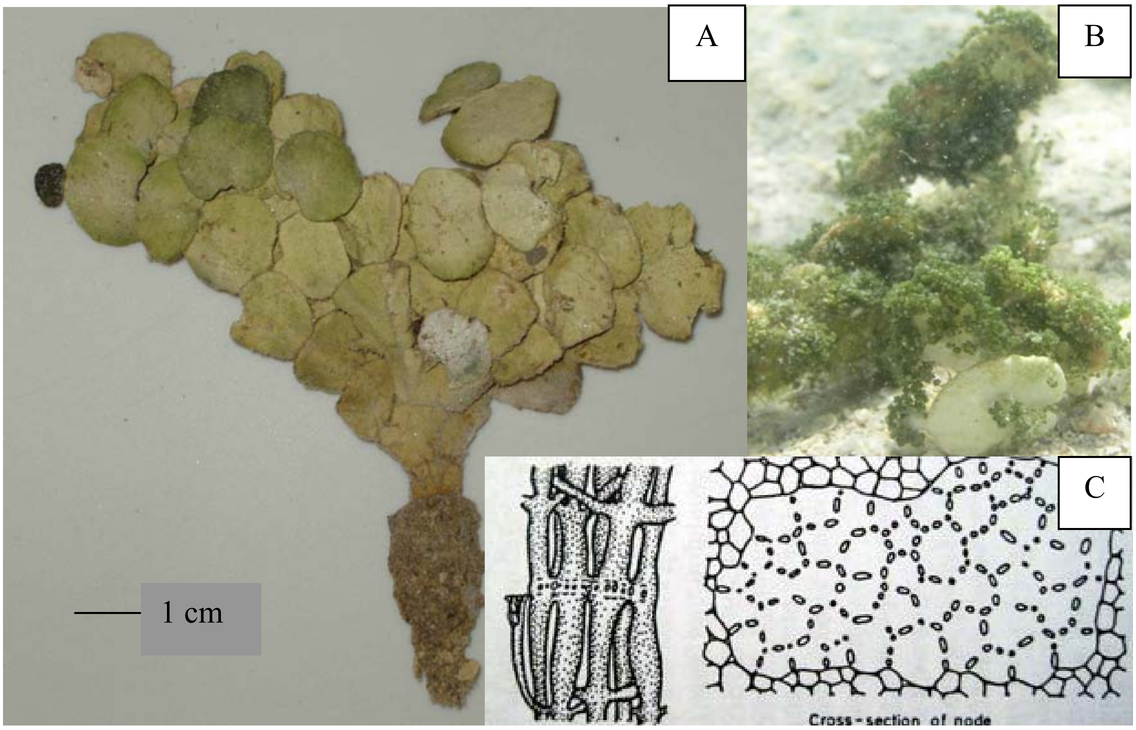

Figure 1. *Halimeda macroloba* Decaisne plant from Tangkhen Bay, Phuket Province, Thailand. A= The whole thallus, B= Fertile plant with reproductive organ, C= Pattern of nodal medullary filaments which come together essentially in a single unit (Blaxter *et al*., 1980).

Growth and calcification

Growth of *Halimeda* involves the development of new segments which begin as a white, conical lobe from the apex of the last segment. Within 24 hours, this white lobe has grown into a fairly complete and greenish, segment. Calcification of the new segment begins after approximately 36 hours. Length and width of the segment is fixed within the first few days. The calcification results in some changes in segment thickness which depends on the species and the segment's location within the thallus. It can be seen that the oldest segments will be found closest to the holdfast. New segments may form daily. The rapid growth of *Halimeda* results in large amounts of sediments when *Halimeda* die (Multer and Clavijo, 1989). Sediment production ranges from 4.2 g of calcium carbonate m^{-2} $year^{-1}$ in Florida (Bach, 1979) to 2,234-3,000 g of calcium carbonate m^{-2} $year^{-1}$ on the Great Barrier Reef (Drew, 1983).

Reproduction of *Halimeda* species

Halimeda is dioecious, the male and female gametes are born on different plants. The gamete is biflagellate. The macrogamete is bigger in size and has eye spot while the microgamete is smaller and has no eye spot. We can distinguish between the macrogametangia and microgametangia by eye spots because they are different in color; macrogametangia is brown-dark green and microgametangia is yellow-like green (Figure 2) (Clifton and Clifton, 1999).

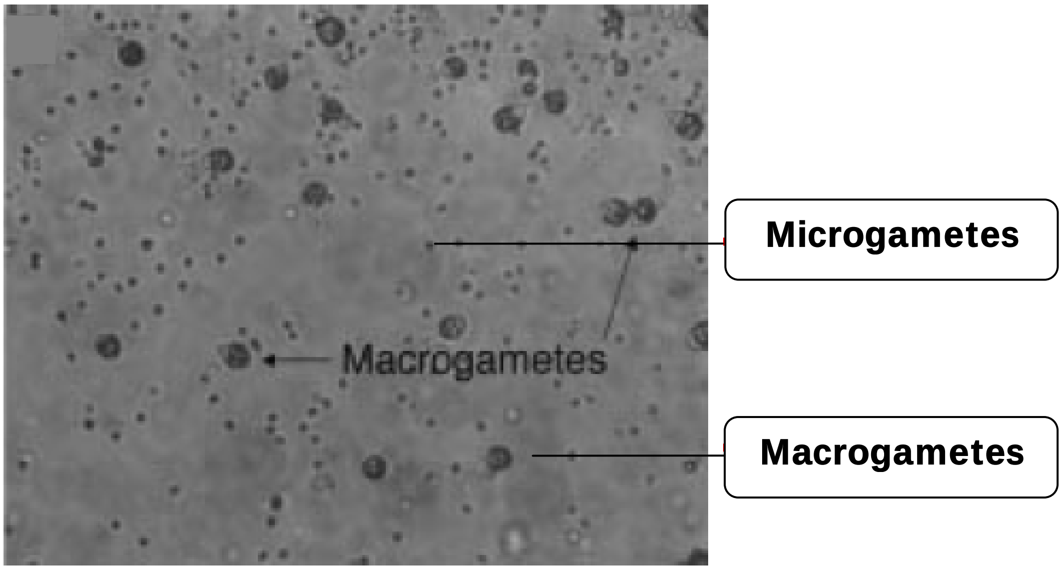

Figure 2. Macrogametes and microgametes of *Halimeda*.

In sexual reproduction (Figure 3) which is rarely seen in *Halimeda*, gametangia (which are many green dots produced at the tips of *Halimeda* segments) become white overnight as gametes are released (Figure 1B). Mature macrogametes and microgametes are released in the morning and fuse in the water. The process is brief, perhaps 36 hours from start to finish, and after spawning the thallus completely disintegrates. The dead segments are shed, within two or three days, the plant has gone and the carbonate segments become part of the surface sediments. The sexual reproduction of *Halimeda* is synchronized. Many individuals in a population may become fertile in a few days, and sometimes on the same day (Clifton, 1997; Clifton and Clifton, 1999). Mass spawning is the unique character in siphonous green algae (*Halimeda*, *Caulerpa, Penicillus, Rhipocephalus,* and *Udotea*). The study of Clifton (1997) showed that each species spawn in the same morning but did so at different time. Moreover, there was no apparent relation to lunar, tidal, or water temperature condition but sunrise. It can be concluded that mass spawning is seasonal but not related to tidal and the environmental factors triggering sexual reproduction are still unclear.

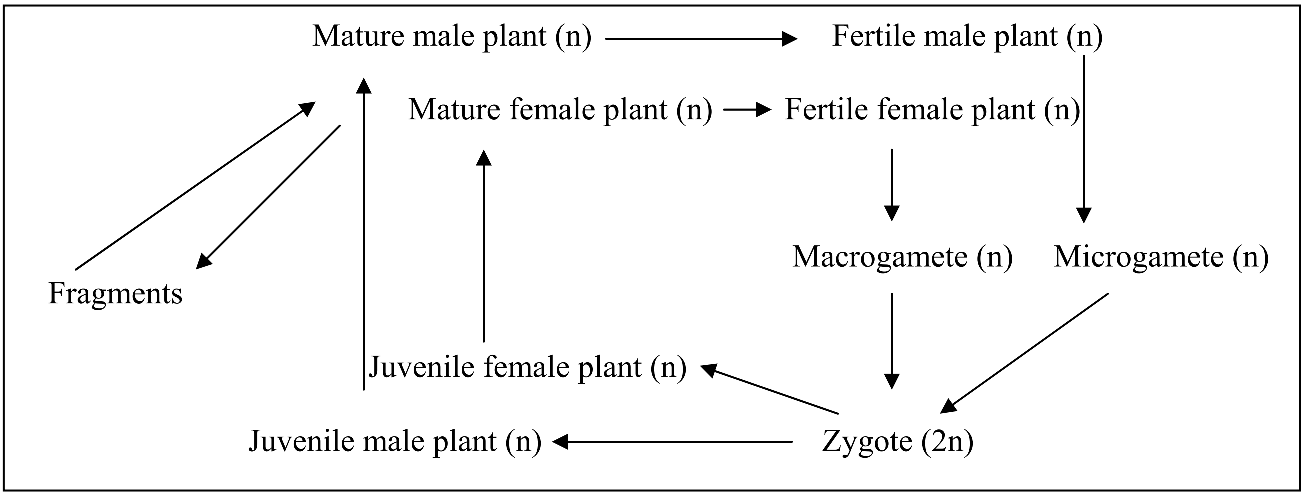

Figure 3. Diagram of the life cycle of *Halimeda* (Blaxter *et al.,* 1980).

Asexual reproduction in *Halimeda* is by vegetative fragmentation, or by development of new thalli at ends of uncorticated siphons growing out either from segments or from filaments of the holdfast, which occurs in *Halimeda* plants that grow in sand reproduce by runners of filament. These filaments were at least 20 cm long and spreaded laterally through the substrate from the main holdfast (Blaxter *et. al,* 1980). In the sprawling lithophytic species of the section Opuntia, the development of rhizoids from segments results in the formation of multiple holdfasts which may be followed by thallus fragmentation (Drew and Abel, 1988).

In asexual reproduction by vegetative fragmentation, fragmentation may be the result of endogenous part of the life history or exogenous processes, such as predation, boring organisms or physical disturbance events (e.g. fish grazing and storm damage). Moreover, it might be the result of a combination of endogenous and exogenous events. Fragments that are produced as an integral part of an organism's life history are predicted to have high survivorship, whereas fragments produced by biotic or abiotic factors may or may not be able to survive and grow clonally (Walter *et al.*, 2002).

In *Halimeda discoidea*, Walters and Smith (1994) found that 56-100% of fragments produced by fish grazing and 100% of fragments generated by Hurricane Iniki survived and produced rhizoid. Damage that occurred between segments (nodes) or within segments resulted in either a few fast-growing or many slower-growing rhizoids.

The advantages of asexual reproduction are: (1) extension of the distribution of genets and the species, (2) increases in the abundance of the organism and individual biomass, and (3) colonization of areas where sexual propagules are unable to settle or early postsettlement mortality rate are high. On the other hand, there is a disadvantage of asexual reproduction in reducing in a variety of species. However, if fertilization and recruitment of sexual reproduction are not successful, asexual reproduction via vegetative fragmentation may contribute substantially to populations.

Walter and Smith (1994) studied on a mechanism for survival after separation from adult *H. discoidea* thalli. They found that all *H. discoidea* fragment rapidly produced new attachment rhizoid, regardless of the source of fragmentation (fish, storm, razor blade), location where fragments were held (field, lab), fragment amount (0.5-4.0 segments), location along the original plant axis, and breakage orientation. However, vertically-cut segments produce rhizoid significantly earlier than horizontally-cut segments or node-cut segment. This study showed that vegetative fragmentation can increase many plant numbers by clonally propagating nearby adults in reef habitats.

Walter *et al.* (2002) investigated the importance of vegetative fragmentation and the potential for fragment dispersal in *H. tuna, H. opuntia* and *H. goreaui* commonly found in the Florida Keys reef tract. They found that fragment survival and production of attachment rhizoids depend on fragment size and depth of water. The larger fragments were significantly more successful than small segments in producing rhizoid because they have more chance to survive from sedimentation. It can be concluded that vegetative fragmentation contributes to the abundance of this genus on coral reef.

Biomechanical properties of *Halimeda*

Halimeda is a coenocytic alga, multinucleate organisms lacking transverse walls. Coenocytic construction is uncommon in algae; however, it occurs in *Halimeda* which is found in shallow subtidal sediments associated with coral reefs. The investigation of some biomechanical properties (force to remove, force to break, and strength) of *Halimeda* showed that in more than 95% of cases when these species are so stressed, they detach whole rather than break. This contrasts with the response of most multicellular algae test which frequently break within the thallus. The study of biomechanical properties and holdfast morphology of coenocytic algae by Anderson *et al.* (2006) revealed that no significant correlation between holdfast volume and force-to-remove. It might be because holdfast tenacity is determined by a combination of factors such as sediment shear strength, holdfast surface area, and rhizoid strength. Thus, localized compaction of sediments may result in a smaller holdfast plug but one with similar tenacity as a larger volume holdfast in looser sediment. However, they found a significant positive correlation between the blade surface area and the force required to remove an individual from the substratum, for *H. gracilis*.

Environmental factors

Many biological and physical factors influence *Halimeda* population. Beach *et al.* (2003) found the negative impacts of epiphytic *Dictyota* on the metabolic rates of *H. tuna* by shading, limiting nutrients and releasing some chemical substance. The calcareous nature of *Halimeda* and the ability to synthesize noxious and potentially toxic secondary metabolites makes them less appetizing meal to grazing fish such as surgeon and parrot fishes than more succulent algae (Hay *et al.,* 1994). Moreover, the strategy in producing new buds at night when herbivorous fishes are inactive can prevent *Halimeda* from herbivore (Ganesan *et al.,* 2006).

Seagrass and rhizophytic macroalgae utilize the same resources such as, substrate, light, and nutrients. A study of the competition between *H. incrassata* and the seagrass *Thalassia testudinum* Banks ex Konig at Florida Keys revealed that *T. testudinum*, was a superior competitor over *H. incrassata* (Davis and Fourqurean, 2001).

A study of *H. tuna* at Florida Keys revealed that densities of *H. tuna* fluctuated because of environmental and seasonal parameters, e.g. light intensity and nutrients concentration. Moreover, it was found that an average growth rate of *H. tuna* at two locations (Shallow Conch (0.012 g · plant · day^{-1}) and Pinnacle (0.025 g · plant · day^{-1})) was highest under summer conditions of longer photoperiods and at the highest temperature (Vroom *et al.*, 2003). A study testing the effects of nutrients on growth in *H. tuna* at Conch Reef showed that plants respond positively to elevated nutrient concentrations (Smith *et al.,* 2004).

Many studies showed that calcification process in *Halimeda* is influenced by plant age, growth rate, photosynthesis, respiration, light, concentration of Ca^{2+} and CO_3^{2-} ion and CO_2, phosphate concentration, and herbivory (Simkiss, 1964, Stark *et al.*, 1969; Borowitzka and Larkum, 1976, Borowitzka, 1977, Blaxter *et al.*, 1980, Paul and Van Alstyne, 1988; Lobban and Harrison, 1994, Kangwe, 2006).

Wave can impose large hydrodynamic forces on benthic organisms (Denny, 2006). Adult plants interact to water motion by two major aspects: tolerance to wave action and morphological adaptations to reduce the effect of wave force. To withstand wave force, seaweed must be tough enough and the strength of holdfast seems to be related to the wave force experienced (Lobban *et al.*, 1985). On the other hand, the flexibility and its ability to adjust morphology in intertidal macroalgae reduce wave force. For example, the kelp *Laminaria saccharina* typically has thinner and longer blade at more wave-exposed site (Denny, 2006).

Hypotheses

1. The patterns of density, distribution, life stage and life span determine the population structure of *Halimeda macroloba* Decaisne in natural habitats, and also explain the temporal variation.
2. Null hypotheses 1

   H_0: Sediment accumulation in the holdfast does not occur by a progress of selection.

   - H_{0A}: There will not be a significant difference between type of sediment in holdfast and sediment in that area.
   - H_{0B}: There will not be a significant difference between size of sediment in holdfast and sediment in that area.

   Alternative hypotheses 1

   H_1: Sediment accumulation in the holdfast occurs by a progress of selection.

   - H_{1A}: There will be a significant difference between type of sediment in holdfast and sediment in that area.
   - H_{1B}: There will be a significant difference between size of sediment in holdfast and sediment in that area.
3. Null hypotheses 2

   H_0: There is no relationship between thallus size and holdfast size.

   - H_{0A}: Thallus size will not correlate with holdfast size.

   Alternative hypotheses 2

   H_1: There is relationship between thallus size and holdfast size.

   - H_{1A}: Large thallus will have large holdfast.
   - H_{1B}: Small thallus will have small holdfast.
4. Null hypotheses 3

   H_0: There is no relationship between wave motion and holdfast and thallus size.

   - H_{0A}: In high wave motion, there will not be correlation between holdfast size.
   - H_{0B}: In high wave motion, there will not be correlation between thallus size.

H_{0C}: In low wave motion, there will not be correlation between holdfast size.

H_{0D}: In low wave motion, there will not be correlation between thallus size.

Alternative hypotheses 3

H_1: There is relationship between wave motion and holdfast and thallus size.

H_{1A}: In high wave motion, there will be positive correlation between wave motion and holdfast size.

H_{1B}: In high wave motion, there will be negative correlation between wave motion and thallus size.

H_{1C}: In low wave motion, there will be negative correlation between wave motion and holdfast size.

H_{1D}: In low wave motion, there will be positive correlation between wave motion and holdfast size.

Objectives

1. To study the distribution and population density of *Halimeda macroloba* Decaisne at Tangkhen Bay, Phuket.
2. To investigate the life stage and life span of *Halimeda macroloba* Decaisne at Tangkhen Bay, Phuket.
3. To investigate the relationship between sediment size fraction from the study area and from the holdfast.
4. To investigate the relationship between thallus size and holdfast size.
5. To investigate the relationship between wave motion and holdfast and thallus size.

Research questions

An experiment was designed to address the following questions:

1. Is there seasonal variation in distribution, density and life stage of *H. macroloba* Decaisne? If so, what are these variations?
2. How long is the life span of *H. macroloba* Decaisne?
3. Is sediment size fraction in environment related to sediment size fraction in holdfast? How?
4. Is holdfast size related to thallus size? How?
5. Is holdfast and thallus size related to wave motion? How?

CHAPTER 2

MATERIALS AND METHODS

Study site

The study site is located at Tangkhen Bay (7°48’N, 98°24’E), a sheltered bay on Panwa Cape, Phuket province, Southern Thailand (Figure 4). This bay covers an area of 0.42 square kilometers on the southeast of Phuket Island. There are two dominant seasons; a monsoon season dominated by the southwest monsoon (May to October) and a dry season dominated by the northeast monsoon (November to April). The wind speed is greatest in the monsoon season. This bay is composed of various marine habitats, e.g., sandy beach, rocky shore, seagrass bed, and coral reef. There are four seagrass species inhabiting this area, i.e., *Cymodocea rotundata* Ehrenb. & Hempr. ex. Aschers, *Halophila minor* Zoll, *Halodule pinifolia* (Miki) Hartog and *Halophila ovalis* R.Br. Hook.f (Poovachiranon *et al*., 2006). *H. ovalis* is dominant in this area. There are many macroalgae inhabiting this area, i.e., *Cladophora* sp., *Ulva* sp., *Laurencia* sp., *Padina spp.*, *Halimeda macroloba* Decaisne and *H. opuntia* (Linnaeus) Lamouroux. Two species of *Halimeda* occur in the south of the bay, *H. opuntia* attaches on a hard substrate such as coral reefs while *H. macroloba* attaches on a sandy substrate. *H. macroloba* is found 200-400 meters from the shoreline (Figure 5, 6).

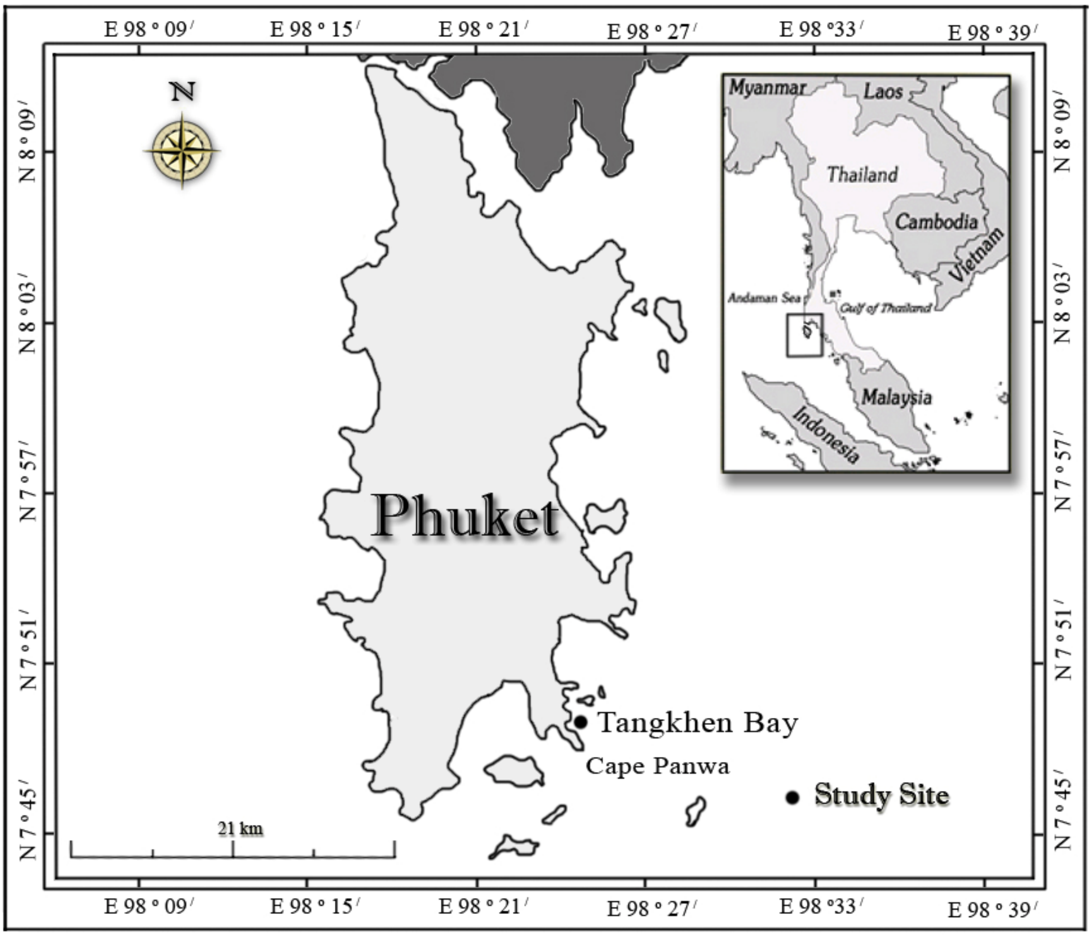

Figure 4. Map of the sampling site at Tangkhen Bay, Phuket Province, Thailand.

Fugure 5. Tangkhen Bay, Phuket Province, Thailand at low tide.

Figure 6. *Halimeda macroloba* Decaisne*, Halimeda opuntia* (Linnaeus) Lamouroux and *Halophila ovalis* R.Br. Hook.f at Tangkhen Bay, Phuket Province, Thailand.

Methods

Population structure, distribution, population density, life stage distribution, and life span, of *Halimeda macroloba* Decaisne were studied. The samples were collected every month for one year from the southeast of Tangkhen Bay, Phuket province. Moreover, one hundred and thirty of *H. macroloba* thalli were collected bimonthly for a year for using in the relationship between sediment size fraction from the study area and from the holdfast, thallus size and holdfast size, and wave motion and holdfast and thallus size studies. The thalli were cleaned, dried. Holdfast and blade of each thallus was separated and kept in the dry plastic bag.

Information on wave velocity, air and water temperature, salinity, water samples (for studying nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentration), and sediment samples for studying sediment size fraction, were collected.

Water velocity was measured using an Image Processing Technique. The motion of objects in 20 cm distance was recorded by the video camera. The time of the motion was noted. The velocity was calculated using the distance between the beginning and end of the object and the time difference between the beginning and end time as shown in the formula below.

$$velocity = \frac{distance}{end \quad time - beginning \quad time}$$

Air and water temperature were measured using a thermometer. Salinity refractometer (ATC, 0-100 ppt, XHO RHS-10ATC, ATACO, China) was used to measure salinity. Portable calorimeter (DR/890, Hach, USA) was used in studying nitrate and phosphate concentration. Sediments were collected from upper (200 meter from the shoreline) and mid level (300 meter from the shoreline) of the shore to study sediment size fraction (grain size). The sediments were classified into four size class, i.e. <212 µm, 212-350 µm, 350-500 µm, and 500-1,000 µm) using sieve analysis (Wankhow *et al*., 2001).

1. Distribution and population density study

Line transects were used to study the distribution and population density of *Halimeda* at each shore level. Three line transects, each 50 m long were placed randomly over approximately 200 to 400 meters of shoreline-where *Halimeda* is present. The shoreline was separated into three intervals, i.e. the sandy substrate at 200-260 meter from the shoreline (upper shore level), the sandy substrate with high amount of large grain size at 260-340 meter from the shoreline (mid shore level), and the dead coral at 340-400 meter from the shoreline (lower shore level). The density of *H. macroloba* (the number of *H. macroloba* per quadrat) was quantified in three 0.25 m^2 quadrats at 10-meter intervals along the transect line. There were one hundred and eighty quadrats placed in this study (Figure. 7).

Figure 7. Three transect lines for distribution study.

2. Life stage and life span study

Twenty one 0.25 m^2 permanent plots in sandy substrate were used to study life stage and life span of *Halimeda macroloba* at 200 to 340 meters from the shoreline which is sandy substrate (Figure 8) on the second line of the distribution and density study (Figure 7). Each plant of *Halimeda* was tagged when it was first found (except for Stage 1 and 6, see below). The number of each life stage of *H. macroloba* per 0.25 m^2 permanent plot were quantified every month from July 2006 to July 2007 as follows:

Stage 1: newly recruited plant; unable to be tagged.
Stage 2: young plant; non-calcified plant.
Stage 3: partially-calcified plant.
Stage 4: mature plant; calcified plant.
Stage 5: fertile plant; small dark green dots on the surface of each plant segment
Stage 6: dead plant (Figure 9).

The data reveal life stage and life span of *H. macroloba* at Tangkhen Bay, over a one year time-span. There were three hundred and five thalli which were tagged in this study.

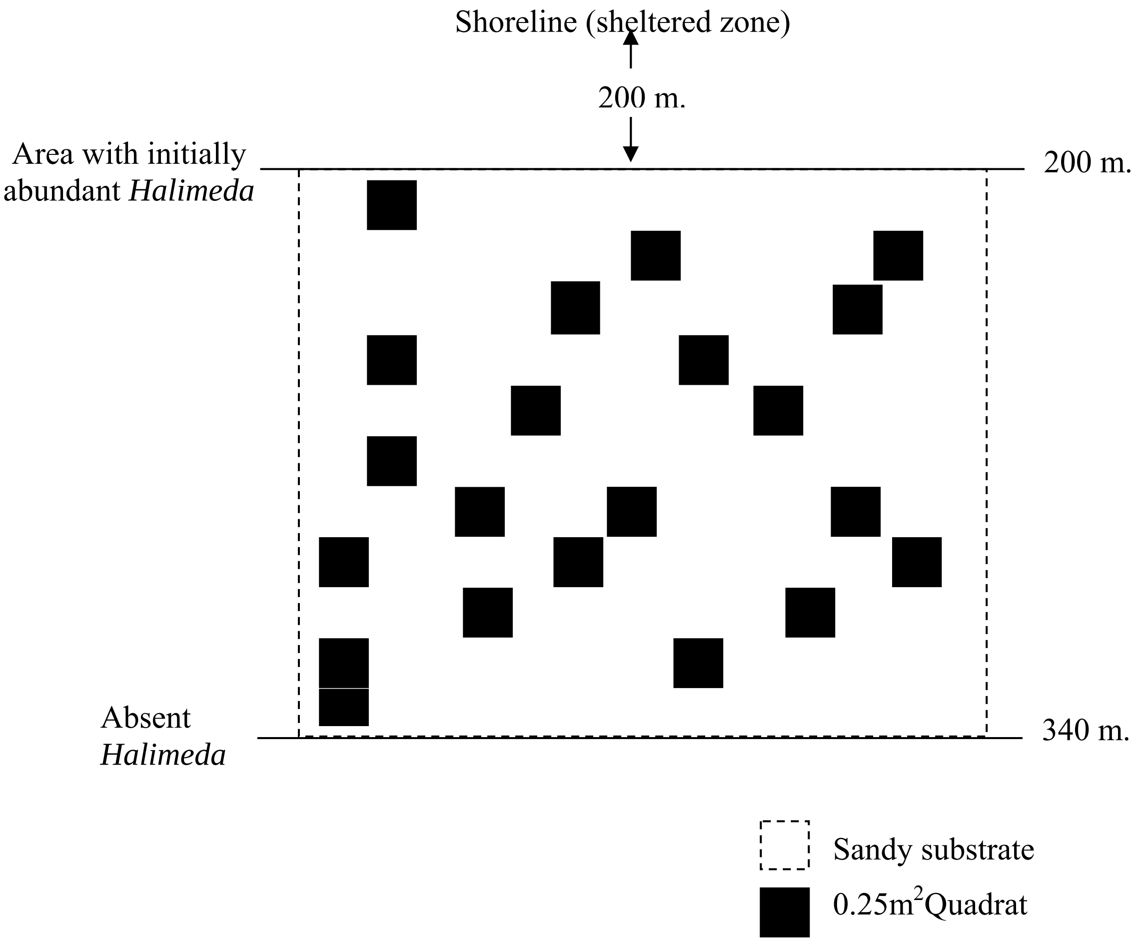

Figure 8. Twenty one permanent plots for life stage and life span study.

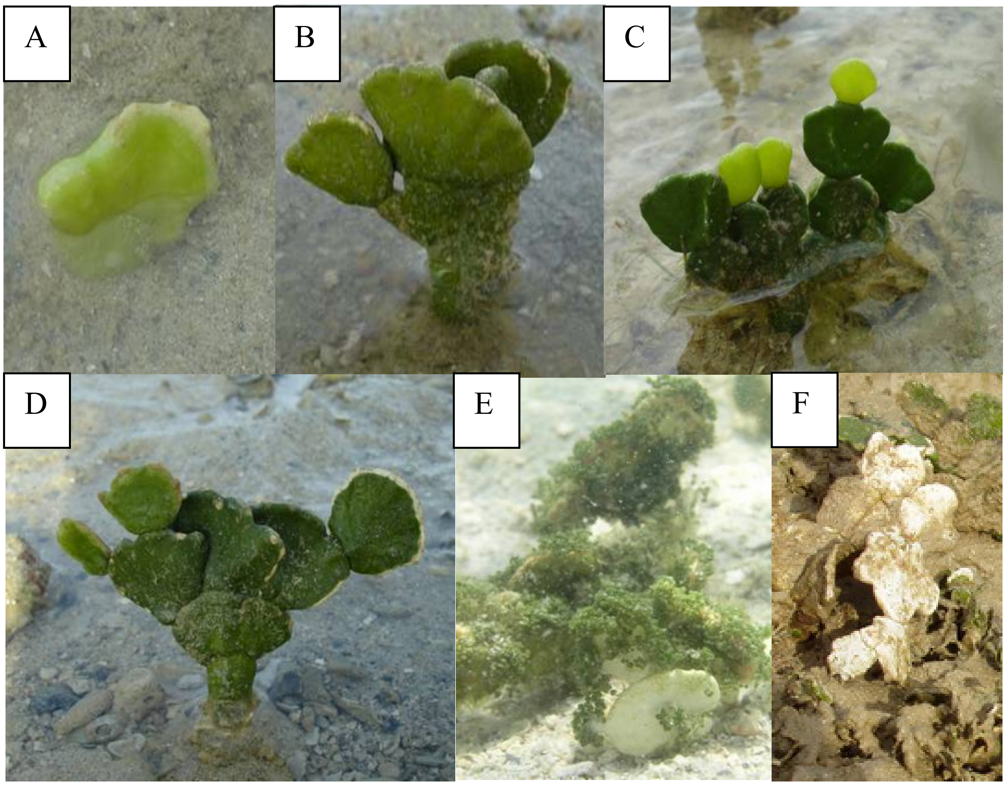

Figure 9. Six stages of *Halimeda macroloba* Decaisne. A= stage 1, B = stage 2, C = stage 3, D = stage 4, E = stage 5, F = stage 6.

3. Relationship between sediment size fraction from the study area and from the holdfast

Thirty six samples of sediment were collected from mid shore level (300 meter from the shoreline) to study sediment size fraction (Grain size) in study area. Sediments in one hundred and thirty holdfasts and sediments in studied area were classified into four size class, i.e. <212 µm, 212-350 µm, 350-500 µm, and 500-1,000 µm) using sieve analysis (Wankhow *et al*., 2001). The proportions of each sediment size from the study area and from the holdfast were correlated.

4. Relationship between thallus size and holdfast size

H. macroloba thalli were collected bimonthly for a year. The thalli were cleaned, dried. Holdfast and blade of each thallus was separated and kept in the dry plastic bag. The dry weights of thallus and holdfast were investigated. The blade surface area was measured using image processing technique by Scion Image for Windows (Scion Corporation, USA). The reliability of Scion Image for Windows is 98.86%. Holdfast volume was measured using the water displacement technique. The 2-dimensional blade surface area and holdfast volume of one hundred and thirty thalli of *H. macroloba* were correlated (Figure 10).

5. Relationship between wave motion and holdfast and thallus size

The thalli from the fourth experiment were used in this study. The 2-dimensional blade surface area and holdfast volume of one hundred and thirty thalli of *H. macroloba* were correlated with the level of water velocity each month (Figure 10).

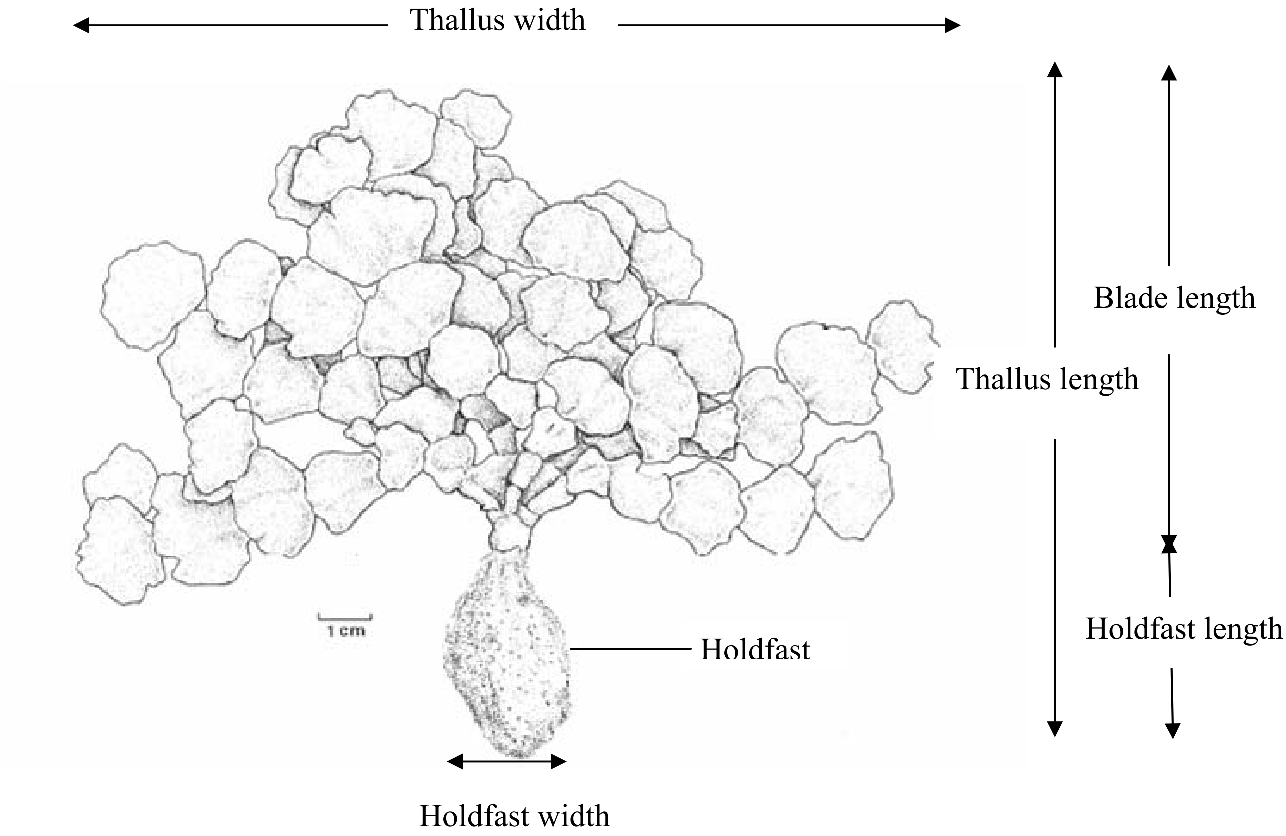

Figure 10. *Halimeda* plant (Bianchi *et al.*,1999)

Statistical analyses

SPSS version 13.0 for Windows was used to analyze data; significance levels of 95% were used. Because the data (and data transformed with logarithm and square root) had a non-normal distribution as tested with Levene's test, a non-parametric Kruskal Wallis Test was used to test for differences in environmental parameters (water and air temperature, salinity, nitrate and phosphate concentration and sediment size fractions) and density of *H. macroloba* between months. The Friedman Test was employed to test density of *H. macroloba* against different shore levels and months and to test the difference of density of each life stage of *H. macroloba* between months.

Because the data were normally distributed, a parametric, 1-way ANOVA, was used to test for differences of water velocity between months. Z-test was used to test the relationship between sediment proportions from the study area and the holdfast. Linear regression was employed to test the relationship between thallus size and holdfast size, and wave motion and holdfast and thallus size.

CHAPTER 3

RESULTS

Tangkhen Bay has various marine habitats i.e. rocky shore, coral reef, seagrass bed and sandy beach. There were dynamic in the population of seaweeds, *Padina* spp., and *Ulva* sp. and the seagrass, *Halophila ovalis* R.Br. Hook.f among twelve months sampling period. *H. macroloba* was found attached to the sandy substrate at 200-400 meters from the shoreline with the seagrass *Halophila ovalis*. *Halimeda opuntia* attaches on hard substrate at the higher distance (300 meters) from.

Environmental parameters

There were significant differences in air and water temperature, salinity, nitrate, phosphate, and water velocity between months ($P<0.05$). The average air temperature, water temperature, salinity, nitrate (NO_3^-), phosphate (PO_4^{3-}), and water velocity were 31.14±0.72 °C, 32.80±0.54 °C, 31.50±0.67%o, 0.13±0.02 mg/l, 0.74±0.17 mg/l, and 0.17±0.01 m/s, respectively (Figure 11). There were no significant differences in rainfall between months ($P>0.05$) (Table 1, 2).

The study in sediment size showed that there were no significant differences between months and shore level, but there were significant differences between size fractions (Table 3). The average percent of each sediment size fraction (<212: 212-350: 350-500: 500-1,000 µm) in upper and mid shore levels was 23.17: 27.78: 40.47: 7.58.and 20.78: 26.67: 27.95: 24.60, respectively (Figure 12).

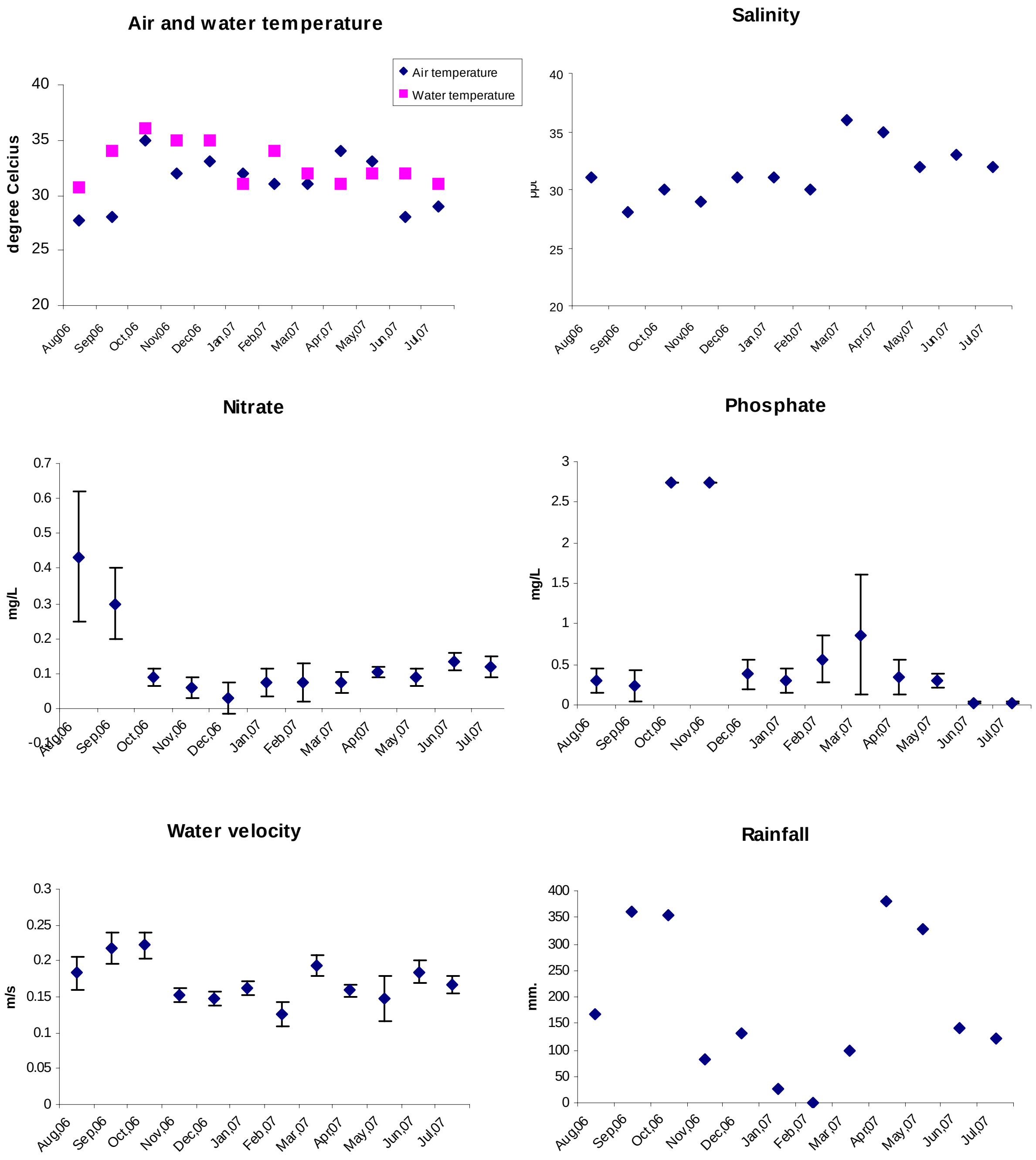

Figure 11. Seasonal changes of environmental parameters during August 2006 – July 2007.

Table 1. The differences of each environmental parameter between months.

Source of variation Between subjects Month	Chi-Square	df	P
Air temperature	35	11	0.00
Water temperature	35	11	0.00
Salinity	35	11	0.00
Nitrate	20.41	11	0.04
Phosphate	20.01	11	0.05
Water velocity	3.38	11	0.01
Rainfall	11	11	0.44

Table 2. The difference of water velocity between months.

Source of variation	df	MS	F	P
Between group	11	0.01	3.378	0.00
Within group	56	0.00		
Total	67			

Table 3. The difference of sediment size fraction between months and shore levels.

Source of variation	Chi-Square	df	P
Between subjects			
Month	0.20	11	1.00
Shore level	0.09	1	0.76
Size	272.65	3	0.00
Month*Shore level*Size	271.08	95	0.00

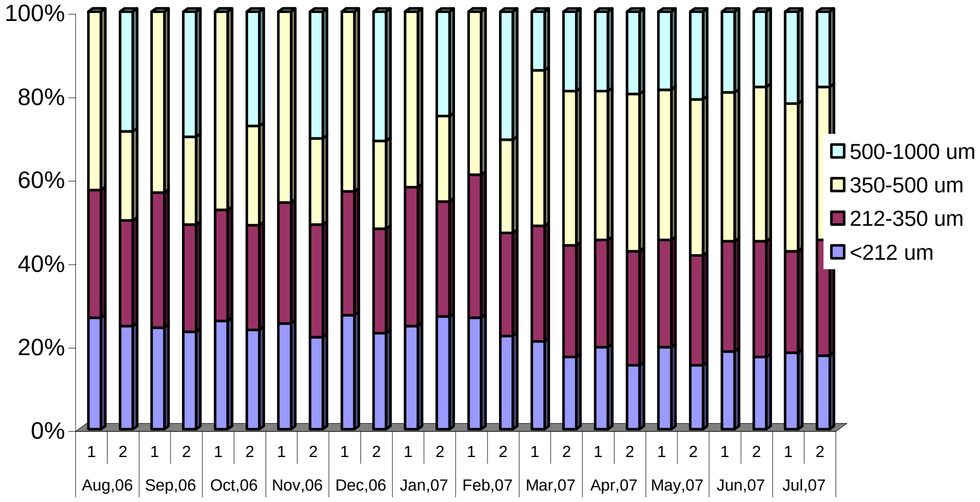

Figure 12. Proportion of sediments in each size class (< 212, 212-350, 350-500, and 500-1,000 μm) and shore level (1 = upper shore level, 200 m from shoreline; 2 = mid shore level, 300 m from shoreline).

Distribution and population density

The study using one-hundred and eighty random quadrats from three lines transect method showed that *H. macroloba* plants were found on the south of the bay at around 200-400 meters from the shoreline. Density of *H. macroloba* varied among months ($P < 0.05$) (Table 4). The highest density at 17.16±3.79 thalli/m^2 was in September 2006, while the lowest at 2.31±0.72 thalli/m^2 was found in October 2006 (Figure 13). In October 2006, large amounts of *H. macroloba* plants covered by sediments were observed (Figure 14). Moreover, *H. macroloba* thalli often had white band at the tip of their segments (Figure 15), also in October.

The study of distribution on the shore found that density of *H. macroloba* varied among months and shore levels ($P < 0.05$) (Table 4). *H. macroloba* plants were most frequently found in the mid shore level (260-340 meters from the shoreline). The highest density at 34.21±2.23 thalli/m^2 was in September 2006 in the mid shore level (260-340 meters from the shoreline), while the lowest (at 0.15±0.15 thalli/m^2) was found the upper shore level (200-260 meters from the shoreline) in October 2006. The density of *H. macroloba* in the lower shore level (340-400 meters from the shoreline) was significantly higher later in the sampling period (Figure 16). Large amounts of seagrass in the permanent plots later in the sampling period (during the last five months) (Figure 17).

Table 4. The difference of *Halimeda macroloba* Decaisne density between months and shore levels.

Source of variation	Chi-Square	df	P
Between subjects			
Month	57.85	11	0.00
Shore level	203.38	1	0.00
Month*Shore level	270.74	35	0.00

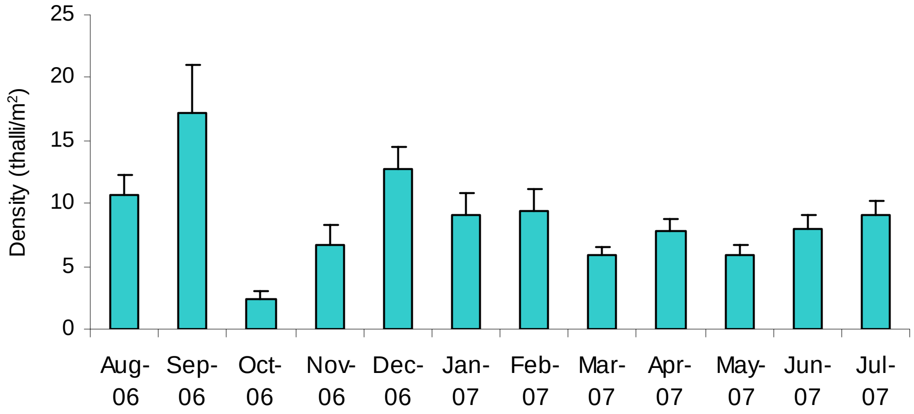

Figure 13. The average density of *Halimeda macroloba* Decaisne from three lines transect from one-hundred and eighty random quadrats during August 2006 to July 2007 at Tangkhen Bay, Phuket Province, Thailand. The error bars are standard error.

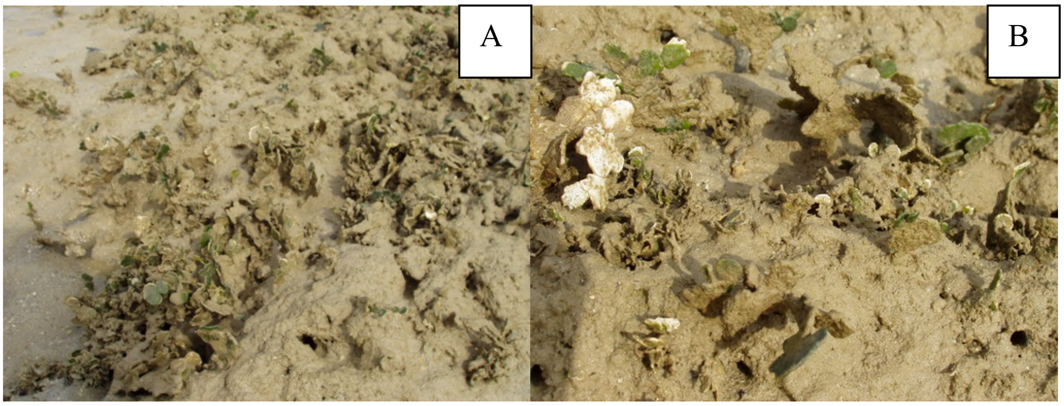

Figure 14. The large amounts of *Halimeda macroloba* Decaisne plants covered by sediments. A = Natural habitat, B = Close up.

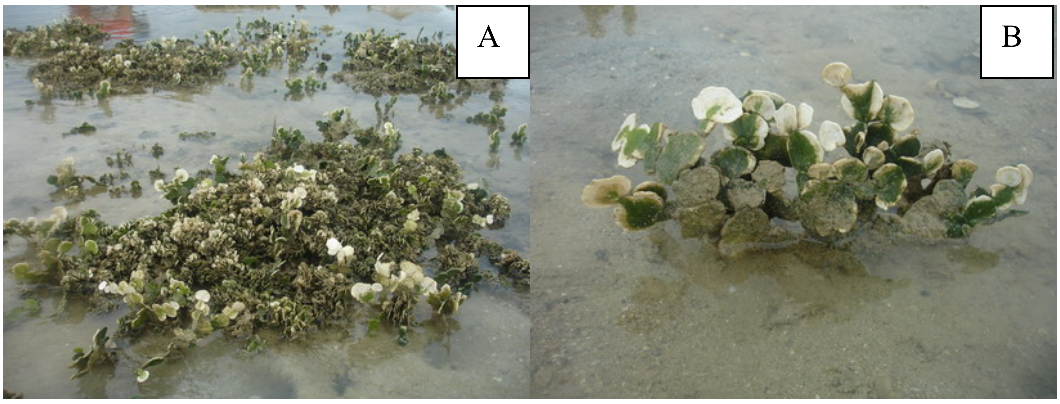

Figure 15. The *Halimeda macroloba* Decaisne thalli with white band at the tip of their segments. A = Natural habitat, B = Close up.

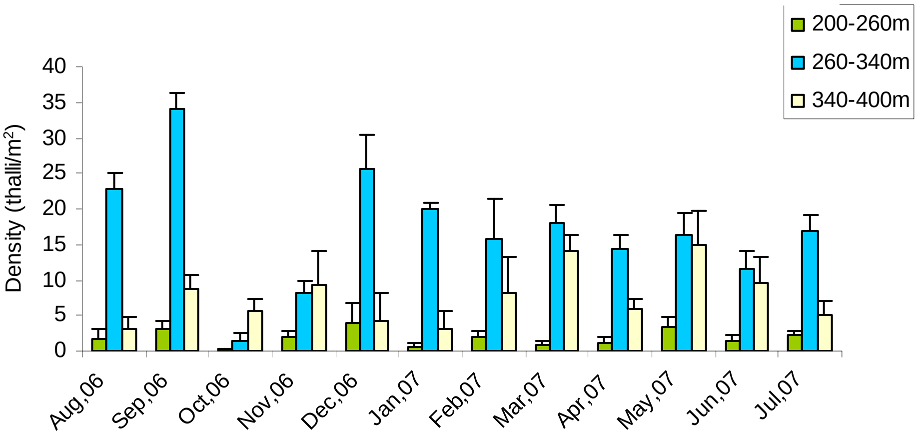

Figure 16. Density of *Halimeda macroloba* Decaisne in each shore level (200-260, 260-340, and 340-400 meter from the shoreline) during August 2006 to July 2007 at Tangkhen Bay, Phuket Province, Thailand. The error bars are standard error.

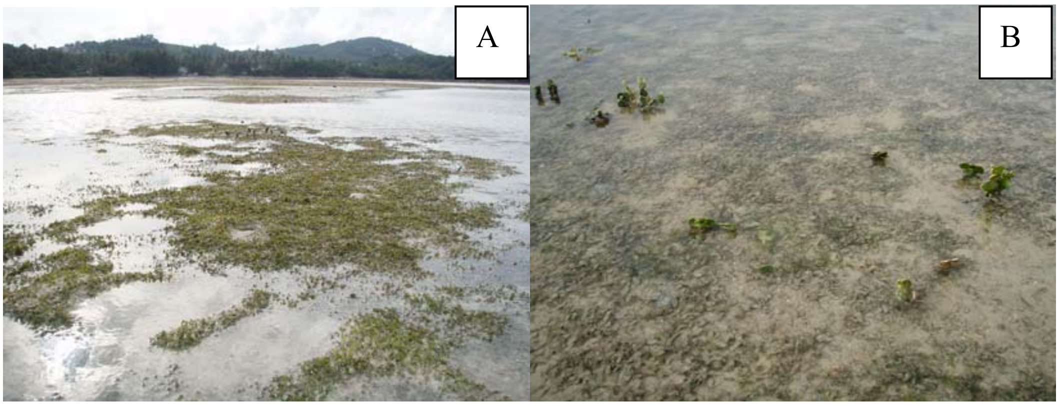

Figure 17. The large amounts of seagrass in the plots later in the sampling period (during the last five months). A = Natural habitat, B = Close up.

Life stage and life span

From the study on the twenty-one permanent plots using 305 thalli showed, there were 214 thalli which were monitored until die (70.16% of the start). The density of each life stage of *H. macroloba* varied among months ($P<0.05$) (Table 5). The highest density at 9.90±4.15 and 9.71±3.75 thalli/m^2 was of the mature stage found in July and August 2006, respectively. New recruits were found only in December 2006. Dead plants were most dense in September 2006 at 8±2.55 thalli/m^2. No fertile plants were found in this study (Figure 18). The large amounts of seagrass in the permanent plots were found later in the sampling period (last five months).

The interval of the development from new recruited plant to partially calcified plant was less than one month. It took 1-4 months to develop from partially calcified plant to mature plant. The longest time require to reach mature plant was 8 months. The estimated life span of *H. macroloba* from this study was 8-12 months.

Table 5. The difference of density in each life stage of *Halimeda macroloba* Decaisne between months.

Source of variation	Chi-Square	df	P
Between subjects			
Month	79.58	12	0.00
Life stage	119.13	5	0.00
Month*life stage	484.66	77	0.00

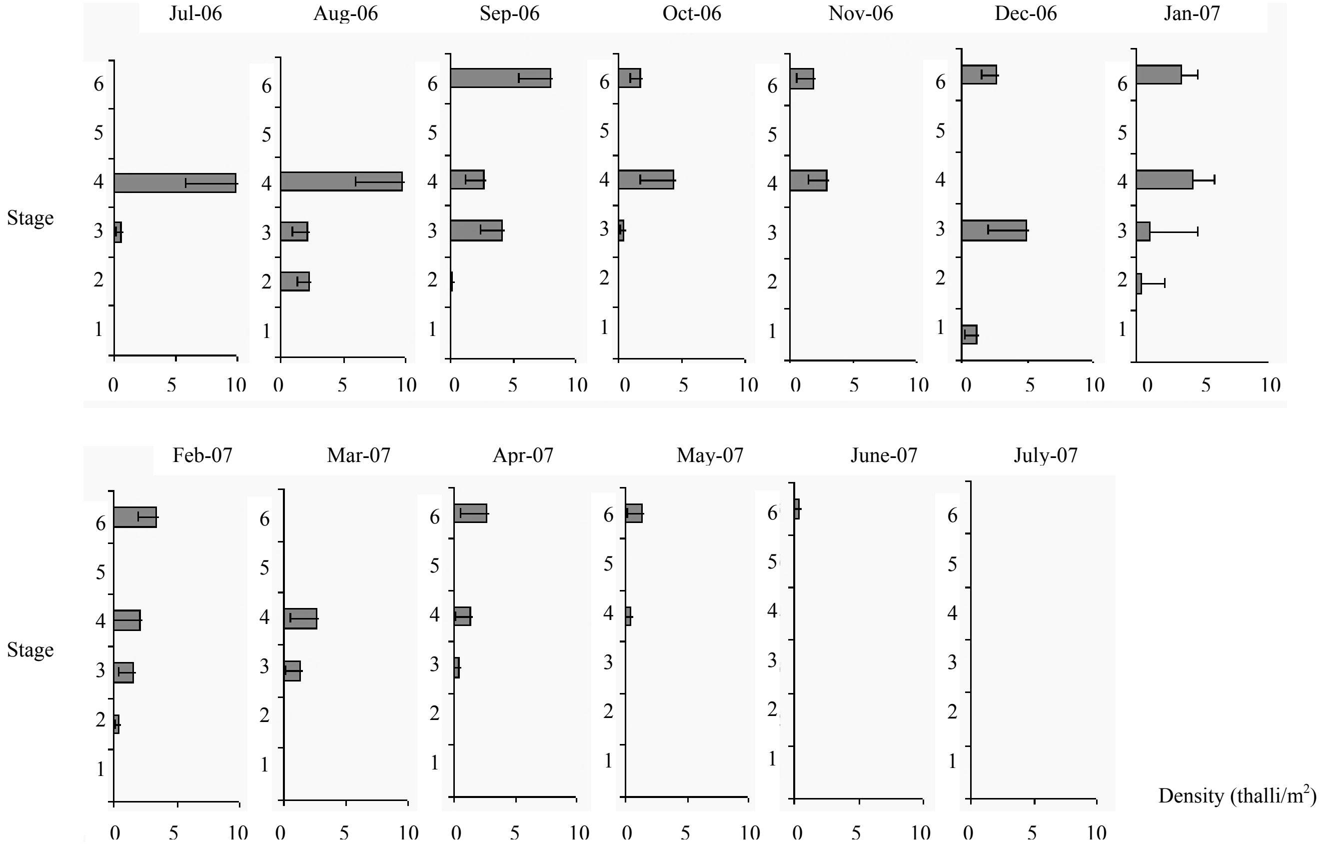

Figure 18. Density of each life stage of *Halimeda macroloba* Decaisne from twenty-one permanent plots during July 2006 to July 2007 at Tangkhen Bay, Phuket Province, Thailand. Stage 1 = New recruit, Stage 2 = Uncalcified, Stage 3 = Partially calcified, Stage 4 = Mature, Stage 5 = Fertile, Stage 6 = Dead. The error bars are standard error.

Relationship between the environmental parameters on the density of *Halimeda macroloba* Decaisne

Linear regressions of all parameters showed that there was no relationship between these factors (air temperature, water temperature, salinity, nitrate, phosphate, water velocity, rainfall, and each sediment size fraction) and the density of *H. macroloba* ($P>0.05$) (Table 6, 7).

Table 6. Summary of the density of *Halimeda macroloba* Decaisne in relation to water and air temperature, salinity, nitrate, phosphate, water velocity, and rainfall during August 2006 – July 2007 at Tangkhen Bay, Phuket province, Thailand.

	N	Air temperature (°C)		Water temperature (°C)		Salinity (ppt)		Nitrate (mg/l)		Phosphate (mg/l)		Water velocity (m/s)		Rainfall (mm.)	
		r^2	P	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P
Density	720	0.32	0.06	0.00	0.87	0.15	0.21	0.22	0.12	0.31	0.06	0.00	0.99	0.00	0.94

Table 7. Summary of the density of *Halimeda macroloba* Decaisne in relation to each sediment size fraction during August 2006 – July 2007 at Tangkhen Bay, Phuket province, Thailand.

	N	Sediment size (µm)							
		< 212		212-350		350-500		500-1000	
		r^2	P	r^2	P	r^2	P	r^2	P
Density	720	0.09	0.33	0.04	0.49	0.14	0.23	0.15	0.21

Relationship between the sediment size fraction and the density of *Halimeda macroloba* Decaisne in each shore levels

Linear regressions showed that there was no relationship between the sediment size fractions and the density of *H. macroloba* in upper and mid shore levels ($P>0.05$) (Table 8). This suggested that there was no preference in each sediment size.

Table 8. Summary of the density of *Halimeda macroloba* Decaisne in upper and mid shore levels in relation to each sediment size fraction during August 2006 – July 2007 at Tangkhen Bay, Phuket province, Thailand.

Density	N	Sediment size (μm)							
		< 212		212-350		350-500		500-1000	
		r^2	P	r^2	P	r^2	P	r^2	P
Upper shore level	216	0.00	0.82	0.00	0.85	0.01	0.75	0.00	0.95
Mid shore level	288	0.04	0.52	0.04	0.52	0.05	0.49	0.05	0.49

Relationship between the environmental parameters on the density of each life stage of *Halimeda macroloba* Decaisne

Linear regressions of all parameters showed that there were relationships between nitrate concentration and the density of uncalcified and mature stage of *H. macroloba* ($P<0.05$) (Table 9), sediment size fraction 1 (< 212 µm) and the density of mature stage, and sediment size fraction 2 (212-350 µm) and 3 (500-1000 µm) and the density of partially calcified stage of *H. macroloba* ($P<0.05$) (Table 10).

Table 9. Summary of the density of each life stage of *Halimeda macroloba* Decaisne in relation to water and air temperature, salinity, nitrate, phosphate, water velocity, and rainfall during August 2006 – July 2007 at Tangkhen Bay, Phuket province, Thailand.

	N	Air temperature (°C)		Water temperature (°C)		Salinity (ppt)		Nitrate (mg/l)		Phosphate (mg/l)		Water velocity (m/s)		Rainfall (mm.)	
		r^2	P	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P
Stage 1	21	0.06	0.46	0.14	0.24	0.01	0.83	0.08	0.38	0.01	0.72	0.07	0.42	0.01	0.72
Stage 2	21	0.22	0.13	0.13	0.25	0.02	0.63	0.66	0.00	0.04	0.55	0.00	0.86	0.02	0.69
Stage 3	21	0.03	0.5	0.19	0.32	0.11	0.29	0.08	0.39	0.07	0.41	0.01	0.74	0.00	0.94
Stage 4	21	0.04	0.53	0.02	0.69	0.05	0.47	0.47	0.01	0.05	0.49	0.11	0.31	0.00	0.91
Stage 5	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stage 6	21	0.00	0.98	0.13	0.25	0.33	0.06	0.01	0.71	0.01	0.83	0.02	0.69	0.08	0.39

Table 10. Summary of the density of each life stage of *Halimeda macroloba* Decaisne in relation to each sediment size fraction during August 2006 – July 2007 at Tangkhen Bay, Phuket province, Thailand.

	N	Sediment size (µm)							
		< 212		212-350		350-500		500-1000	
		r^2	P	r^2	P	r^2	P	r^2	P
Stage 1	21	0.03	0.58	0.09	0.33	0.07	0.39	0.13	0.25
Stage 2	21	0.19	0.16	0.13	0.26	0.14	0.24	0.09	0.34
Stage 3	21	0.20	0.14	0.34	0.05	0.28	0.08	0.35	0.04
Stage 4	21	0.37	0.04	0.13	0.25	0.27	0.08	0.15	0.07
Stage 5	21	-	-	-	-	-	-	-	-
Stage 6	21	0.15	0.22	0.09	0.34	0.24	0.10	0.27	0.08

Relationship between sediment size fraction from the study area and from the holdfast

Two proportion z-test showed that there was a difference in sediment size fraction in the study area (substrate) (1: 1.2: 1.3: 1.1) and the holdfast (4: 1: 1.7: 2.5) (P<0.05) (Figure 19). This suggested that there was sediment selection in holdfast.

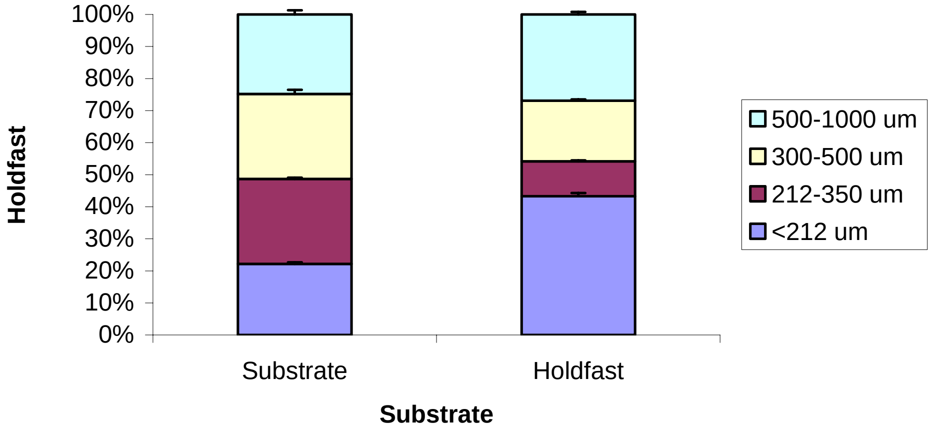

Figure 19. The proportion of each sediment size from substrate (study area) and holdfast of *Halimeda macroloba* Decaisne during July 2006 to May 2007 at Tangkhen Bay, Phuket Province, Thailand. The error bars are standard error.

Relationship between thallus size and holdfast size

The thallus of *H. macroloba* oriented perpendicular to the wave direction (to the shore). The average dry weight of thallus, blade and holdfast of *H. macroloba* from Tangkhen Bay were 21.30, 2.75, and 18.55 g, respectively. The average holdfast volume and blade surface area of *H. macroloba* were 16.02 cm^3 and 30.94 cm^2, respectively. Linear regression showed that there was a relationship between holdfast and blade size ($r^2 = 0.08$, $P<0.05$) (Figure 20).

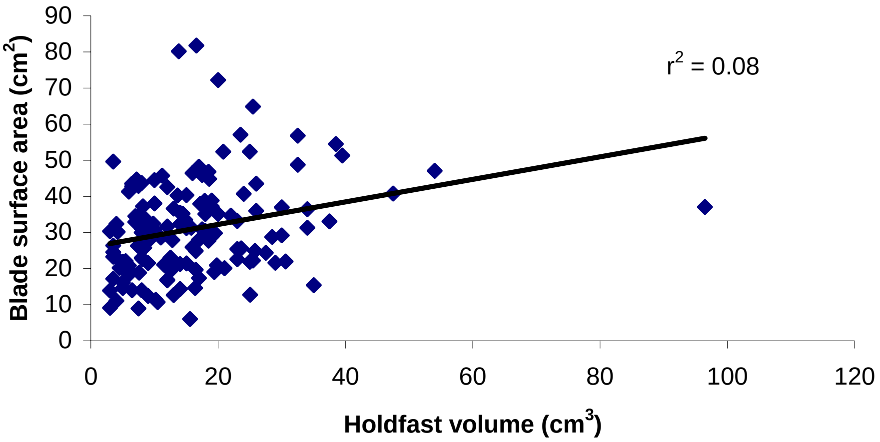

Figure 20. Relationship between holdfast volume (cm^3) and two-dimensional blade surface area (cm^2) of *Halimeda macroloba* Decaisne during July 2006 to May 2007 at Tangkhen Bay, Phuket Province, Thailand.

Relationship between wave motion and holdfast and thallus size

Linear regression showed that there was no relationship between water velocity and holdfast size ($r^2 = 0.38$, $P>0.05$) and water velocity and blade size ($r^2 = 0.07$, $P>0.05$) (Figure 21).

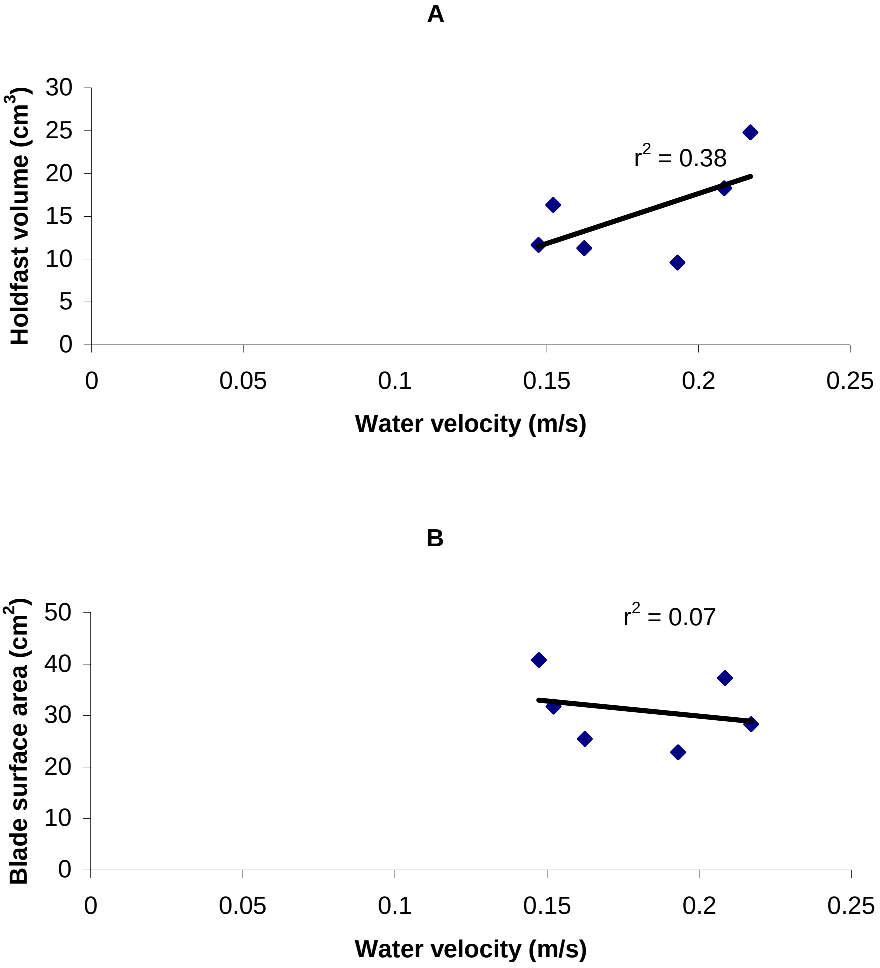

Figure 21. Relationship between water velocity (m/s) and holdfast volume (cm^3) (A) and water velocity (m/s) and blade surface area (cm^2) (B) of *Halimeda macroloba* Decaisne during July 2006 to May 2007 at Tangkhen Bay, Phuket Province, Thailand.

CHAPTER 4

DISCUSSION

Density and life history of *Halimeda macroloba* Decaisne

Halimeda populations were affected by both biotic and abiotic factors, for example, nutrient concentration, temperature, light intensity, water motion, herbivore and epiphytes. These factors influence growth, biomass, survivorship, recruitment and segment loss (Drew, 1983; Ballesteros 1991, Beach *et al.*, 2003, Vroom *et al.,* 2003; Smith *et al.,* 2004; Collado-Vides *et al.,* 2005). *Halimeda* can propagate very successfully by vegetative fragmentation due to disturbance from animals, waves or storms. Many species can produce runners or filaments which can attach and extend over the substrate, and then form new segments (Blaxter *et al.,* 1980). The study of Walters *et al.* (2002) showed that asexual propagation of fragments of *H. opuntia, H. tuna,* and *H. goreaui* is an important component of the life-history of these species and vegetative fragmentation contributes to the abundance of these species on coral reefs. This study showed the variation in density of H. macroloba between months, the highest density was found in September 2006. The shift of density within one month might be a result from asexual reproduction. The sexual reproduction, the length of time required for the development of zygotes into creeping filaments takes more than seven months (Blaxter et al., 1980). Thus it is likely that the large amounts of H. macroloba in September 2006 were from an asexual reproduction via vegetative fragmentation triggered from high wave motion in during August and early September.

However, the *H. macroloba* density dramatically dropped in October 2006. This might result from the effects of sedimentation. We found that a great number of *H. macroloba* plants were covered by large amounts of sediment in this month. High loads of suspended and settling inorganic particles are known to decrease the rates of recruitment, growth, survival and vegetative regeneration (Umar *et al*., 1998; Airoldi, 2003; Wichachucherd, 2008). The high rainfall in September and

October 2006 might increase the sediment load from the land by surface runoff. However, the recovery of the *H. macroloba* population at Tangkhen Bay was quick according to the higher density in November and December 2006 because of the low rainfall.

I found that *H. macroloba* segments at Tangkhen Bay often bleach without any evidence of sexual reproduction. Verbruggen (personal communication, 2006) has seen this on many occasions in species growing in very shallow water (e.g. *H. macroloba*, *H. discoidea*, and *H. opuntia*). It is likely that exposure to extreme drought and insolation (for example at spring low tides) causes the bleaching. Moreover, *Halimeda* plants usually recover from such bleaching. It is possible that the bleach portions of *Halimeda* recuperately obtaining organelles from the healthier part. It was suggested that the chloroplasts from the exposed plant parts were transported to the unexposed parts before the extreme stress took place (Verbruggen, personal communication, 2006).

The results of the life stage and life span of *H. macroloba* at Tangkhen Bay from the permanent plots showed that the time from new recruited plant to partially calcified plant and partially calcified plant to mature plant were less than one month and 1-4 months, respectively. The longest time that the mature plant takes was 8 months. The estimated life span of *H. macroloba* from this study was 12 months. Each species of *Halimeda* has a different life span. Multer (1988) found that the life spans of *H. incrassata* and *H. monile* in Antigua were 3 months. The life span of *H. opuntia* at St. Croix ranges from 6 to 12 months (Multer, 1988). Williams (1988 in Multer and Clavijo, 1989) found that the life span of *H. incrassata* and *H. monile* in St. Croix varied according to locations; 1-4 months at shallow areas of Tague Bay and 4-8 months at deeper Salt River Canyon. The life span *H. macroloba* at Tangkhen Bay (12 months) was much longer than other species in this genus in other locations. The long life span of *H. macroloba* might contribute to its abundance at Tangkhen Bay.

Effect of substrates and sediment size fraction on the holdfast

Substrate is known to influence the distribution of marine benthic organism such as coral, seagrass and seaweed (Lobban *et al.,* 1985; Nybakken and Bertness, 2005). Types and properties of substratum influence settlement, growth and survival of marine organisms (McGuinness, 1989; Diaz-Pulido and McCook, 2004). In *Halimeda,* each species has different habitats and growth forms. One group of species is attached by a single, small holdfast and grows on rock surfaces or hangs as drapes from rocks. The second group of species sprawls across rock, sand or coarse algal and coral debris. The third group grows in sandy substrate and forms a large holdfast attached in sand. *H. macroloba* plants were found in the south of the bay at around 200-400 meters from the shoreline; the most abundant plants were found at the mid shore level (260-340 meters from the shoreline) which is a sandy substrate. Higher proportions of large size granules were found in mid shore level substrate sediment (<212: 212-350: 350-500: 500-1,000 µm = 1: 1.2: 1.3: 1.1) while higher proportions of small size granules were found in upper shore level substrate sediment (3.1: 3.7: 5.3: 1). Anderson *et al.* (2006) suggested that localized compaction of sediments may result in a smaller holdfast plug but one with similar tenacity as a larger volume holdfast in looser sediment. Moreover, the recruitment of algae was generally higher on the larger size granules and rougher textured sediments (Harlin and Lindbergh, 1977; Diaz-Pulido and McCook, 2004). Thus, sediment size fraction in mid shore level (e.g. grain size) might be more compact and suitable for the attachment of *H. macroloba* at Tangkhen Bay than sediments in the upper shore level.

Seagrass and rhizophytic macroalgae are known to utilize the same resources such as substrate, light, and nutrients (Davis and Fourqurean, 2001). Davis and Fourqurean (2001) indicated that the seagrass *Thalassia testudinum*, a late successional species was the competitive dominant over the rhizophytic macroalgae *H. incrassata*, an early successional species. *H. macroloba* is a rhizophytic alga which is commonly found in many seagrass beds in Thailand such as, Koh Tharai, Koh Tan and Koh Mudsum in Khanom-Mu Koh Talay Tai Marine National Park (personal observation). At Tangkhen Bay, there were four species of seagrass inhabiting the same area as *H. macroloba.* My results showed that density of *H. macroloba* in the lower shore level, which was dominated by dead coral (340-400 meter from the shoreline), was significantly higher later in the sampling period (5 months). I also observed a wider expense of seagrass at Tangkhen Bay later in the sampling period at around 200-340 meter from the shoreline. Thus, I hypothesize that the reduced distribution of *H. macroloba* at Tangkhen Bay at the latter sampling period might result from the invasion of the seagrasses. However, further studies on the competition between seagrasses and *H. macroloba* at Tangkhen Bay are needed.

Sediment size fraction from the study area (substrate) and holdfast was different. Similar proportions of each size fraction were found in substrate sediment (1: 1.2: 1.3: 1.1) while different proportions were found in holdfast sediment (4: 1: 1.7: 2.5). Thus, the sediment accumulation in the holdfast of *H. macroloba* might be a process of sediment selection. Selection is also found in many organisms such as juvenile polychaete *Aranicola marina*, juvenile sea scallops *Placopecten magellanicu*s (Gmelin), juvenile plaice *Pleuronectes platessa*, the burrowing mayfly nymphs *Ephemera simulans* Walker and *Hexagenia limbat* and Texas Wildrice *Zizania texana*. Sediment selection has advantages as a refuge for survival of the juvenile fish by avoiding predator by burying, a presence of food in enrichment sediment with organic materials, and a consumption of oxygen (Eriksen, 1963; Power, 1996; Hardege *et al.,* 1998; Gibson and Robb, 2000; Wong *et al.,* 2006).

H. macroloba holdfasts are composed of a high proportion of the very fine grains (<212 μm) and of coarse grains (500-1000 μm). *H. macroloba* has a massive bulbous holdfast consisting of a mass of rhizoids attaching to sand particles (Verbruggen, 2005). This unique bulbous holdfast provides anchorage in the sandy substratum and has an ability to obtain nutrients directly from sediments (Williams, 1984; Fong *et al*, 2003; Littler *et al*, 2004). The fine sediment holds much water in its interstices after the tide has retreated, protecting the seaweed from desiccation in at least its basal parts (Nybakken and Bertness, 2004), and the fine sediment has high nutrient concentrations because organic matter has a high affinity for fine-grained sediment (Levinton, 2001). In contrast, coarse sediment might prevent anoxia in the substratum which might be harmful to the holdfast. Thus, the largest and smallest grain size (500-1000 μm and <212 μm), which were abundant in the holdfast, might be an advantage in increasing holdfast strength (holdfast tenacity), nutrient uptake and decreasing desiccation.

Wave motion and thallus and holdfast size

There was a positive relationship between holdfast volume and blade surface area in *H. macroloba* ($R^2 = 0.08$, $P<0.05$). Our finding agrees with the study of Milligan and DeWreede (2000) and Wernberg and Thomsen (2005) who found that larger holdfasts supported greater thallus size in *Hedophyllum sessile* (C. Agardh) Setchell and *Ecklonia radiata* (C. Agardh) J. Agardh, respectively. Our result also supports the expectation that as blade surface area increases holdfast volume will increase to compensate in coenocytic algae (DeWreede, 2006).

Surprisingly, there was no relationship between water velocity and both holdfast volume and blade surface area of *H. macroloba* at Thangkhen Bay. Generally, seaweeds adapt by increasing holdfast size and decreasing blade size when they exposed to greater degree of wave exposure, such as *Laminuria longicruris* de la Pylaie, *Fucus gardneri* Silva, *Egregia menziesii* (Turner) Areschoug, *Laminaria japonica* Areschoug, Avrainvillea spp., *Ecklonia radiata* (C. Agardh) J. Agardh (Gerard and Mann, 1979; Blanchette, 1997; Koehl, 1999; Kawamata, 2001; Littler *et al.*, 2004; Blanchette *et al.*, 2002; Fowler-Walker *et al.*, 2006). Here I found that *Halimeda* blades are oriented perpendicular to wave direction. This appears to be conveying an advantage in coping with wave action. *H. macroloba* thalli consists of articulated, plate-like and calcified segments which are joined together by small, uncalcified nodes into branching chains. This structure leads to a flexible morphology which can reduce the drag force imposed on the algae by reconforming with flow (Denny *et al.,* 1998; Denny and Gaylord, 2002; Stewart, 2004; Boller and Carrington, 2006; Stewart, 2006). This characteristic is also found in other coenocytic algae such as *Penicillus dumetosus* (J.V. Lamouroux) Blainville and *P. pyriformes* A. Gepp & E.S. Gepp which are flattened perpendicular to the direction of incoming waves (DeWreede, 2006).

CHAPTER 5

CONCLUSIONS

From this study, it can be concluded that:

1. There was variation in density of *H. macroloba* between months influenced by wave motion, sedimentation and rainfall.
2. The estimate life span of *H. macroloba* at Tangkhen Bay was 8-12 months.
3. The abundance of *H. macroloba* at Tangkhen Bay might maintained by asexual reproduction and its long life span.
4. The distribution of *H. macroloba* on the shore was influenced by sediment composition and interspecific competition with seagrass.
5. The sediment size fraction in the holdfast of *H. macroloba* differed from those in surrounding sediments and holdfast size fraction may be advantaged in increasing holdfast strength (holdfast tenacity), nutrient uptake and decreasing desiccation.
6. There was a positive significant relationship between holdfast volume and blade surface area in *H. macroloba*.
7. There was no significant relationship between water velocity and both holdfast volume and blade surface area of *H. macroloba* at Thangkhen Bay.

Further investigations are required to answer the questions:

1. Is there competition between *H. macroloba* and seagrass at Tangkhen Bay?
2. What is the mechanism by which sediment size fraction occurs in *H. macroloba* holdfast?

CHAPTER 6

REFERENCES

Airoldi, L. 2003. The Effects of Sedimentation on Rocky Coast Assemblages. Oceanography and Marine Biology: an Annual Review. 41, 161–236.

Anderson, K., Close, L., DeWreede, R.E., Lynch, B.J., Ormond, C. and Walker, M. 2006. Biomechanical Properties and Holdfast Morphology of Coenocytic Algae (Halimedales, Chlorophyta) in Bocas del Toro, Panama. Journal of Experimental Marine Biology and Ecology. 328, 155-167.

Bach, S.D. 1979. Standing Crop, Growth and Production of Calcareous Siphonales (Chlorophyta) in a South Florida Lagoon. Bulletin of Marine Science. 29, 191-201.

Ballesteros, E. 1991. Seasonality of Growth and Production of a Deep-Water Population of *Halimeda tuna (*Chlorophyceae, Caulerpales) in the North-Western Mediterranean. Botanica Marina. 34 (4), 291-301.

Beach, K., Walters, L., Borgeas, H., Smith, C., Coyer, J. and Vroom, P. 2003. The impact of *Dictyota* spp. on *Halimeda* populations of Conch Reef, Florida Keys. Journal of Experimental Marine Biology and Ecology. 297, 141– 159

Bianchi, G., Carpenter, K.E., Roux, J.P., Molloy, F.J., Boyer, D. and Boyer, H.J. 1999. FAO Species Identification Field Guide for Fishery Purposes: The Living Marine Resources of Namibia. FAO: Rome.

Blanchette, C.A. 1997. Size and Survival of Intertidal Plants in Response to Wave Action: A Case Study with *Fucus gardneri*. Ecology. 78(5), 1563-1578.

Blanchette, C.A., Miner, B.G. and Gaines, S.D. 2002. Geographic Variability in Form, Size and Survival of *Egregia menziesii* around Point Conception, California. Marine Ecology Progress Series. 239, 69-82.

Blaxter, J.H.S., Russel, F.S. and Young, M. 1980. Advances in Marine Biology Volume 17. Academic Press: New York.

Bold, H.C. and Wynne, M.J. 1978. Introduction to the Algae. Prentice-Hall, Inc., Englewood Cliffs: New Jersey.

Boller, M.L. and Carrington, E. 2006. The Hydrodynamic Effects of Shape and Size Change during Reconfiguration of a Flexible Macroalga. The Journal of Experimental Biology. 209, 1894-1903.

Borowitzka, M. A. 1977. Algal Calcification. Oceanography and Marine Biology: an Annual Review. 15, 189-223.

Borowitzka, M. A. and Larkum, A.W.D. 1976. Calcification in the Green Alga *Halimeda.* I. an Ultrastructure Study of Thallus Development. Journal of Phycology. 13, 6-16.

Clifton, K. E. 1997. Mass Spawning by Green Algae on Coral Reefs. Science. 275, 1116-1118.

Clifton, K.E. and Clifton, L.M. 1999. The Phenology of Sexual Reproduction by Green Algae (Bryopsidales) on Caribbean Coral Reef. Journal of Phycology. 35, 24–34.

Collado-Vides, L., Rutten, L.M. and Fourqurean, J.W. 2005. Spatiotemporal Variation of the Abundance of Calcareous Green Macroalgae in the Florida Keys: a Study of Synchrony within a Macroalgal Functional Form Group. Journal of Phycology. 41, 742-752.

Davis, B.C. and Fourqurean, J.W. 2001. Competition between the Tropical Alga, *Halimeda incrassata,* and the Seagrass, *Thalassia testudinum*. Aquatic Botany. 71, 217-232.

Dawson, E.Y. 1966. Marine Botany: an Introduction. Holt, Rinehart and Winston, Inc: New York.

Denny, M.W. 2006. Ocean Waves, Nearshore Ecology, and Natural Selection. Aquatic Ecology. 40, 439-461.

Denny, M. and Gaylord, B. 2002. The Mechanics of Wave-Swept Algae. The Journal of Experimental Biology. 205, 1355-1362.

Denny, M., Gaylord, B., Helmuth. B. and Daniel, T. 1998. The Menace of Momentum: Dynamic Forces on Flexible Organisms. Limnology and Oceanography. 43(5), 955-968.

DeWreede, R.E. 2006. Biomechanical Properties of Coenocytic Algae (Chlorophyta, Caulerpales). ScienceAsia. 32(1), 57-62.

Diaz-Pulido, G. and McCook, L.J. 2004. Effects of Live Coral, Epilithic Algal Communities and Substrate Type on Algal Recruitment. Coral Reefs. 23(2), 225-233.

Drew, E.A. 1983. *Halimeda* Biomass, Growth Rates and Sediment Generation on Reefs in the Central Great Barrier Reef Province. Coral Reefs. 2, 101-110.

Drew, E.A. and Abel, K.M. 1988. Studies on *Halimeda*: II. Reproduction, Particularly the Seasonality of Gametangia Formation, in a Number of Species from the Great Barrier Reef Province. Coral Reefs. 6, 207-218.

Eriksen, C.H. 1963. The Relation of Oxygen Consumption to Substrate Particle Size in two Burrowing Mayflies. Journal of Experimental Biology. 40, 447-453.

Fong, P., Boyer, K.E., Kamer, K. and Boyle, K. 2003. Influence of Initial Tissue Nutrient Status of Tropical Marine Algae on Response to Nitrogen and Phosphorus Additions. Marine Ecology Progress Series. 262, 111-123.

Fowler-Walker, M.J., Wernberg, T. and Connell, S.D. 2006. Differences in Kelp Morphology between Wave Sheltered and Exposed Localities: Morphologically Plastic or Fixed Traits? Marine Biology. 148, 755-767.

Ganesan, M., Thiruppathi, S., Sahu, N., Rengarajan, N., Veeragurunathan, V. and Jha, B. 2006. *In Situ* Observations on Preferential Grazing of Seaweeds by Some Herbivores. Current Science. 91(9), 1256-1260.

Gerard, V.A. and Mann, K.H. 1979. Growth and Production of *Laminaria longicruris* (Phaeophyta) Population Exposed to Different Intensities of Water Movement. Journal of Phycology. 15, 33-41.

Gibson, R.N. and Robb, L. 2000. Sediment Selection in Juvenile Plaice and Its Behavioural Basis. Journal of Fish Biology. 56, 1258-1275.

Hardege, J.D., Bentley, M.G. and Snape, L. 1998. Sediment Selection by Juvenile *Arenicola marina*. Marine Ecology Progress Series. 166, 187-195.

Harlin, M.M. and Lindbergh, J.M. 1977. Selection of Substrata by Seaweed: Optimal Surface Relief. Marine Biology. 40(1), 33-40.

Hay, M.E., Kappel, Q.E. and Fenical, W. 1994. Synergisms in Plant Defenses Against Herbivores: Interactions of Chemistry, Calcification, and Plant Quality. Ecology. 75(6), 1714-1726.

Hillis-Collinvaux, L. 1980. Ecology and taxonomy of *Halimeda*: primary producer of coral reefs. Advances in Marine Biology, v. 17.

Kangwe, J. W. 2006. Calcareous Algae of a Tropical Lagoon: Primary Productivity, Calcification and Carbonate Production. Ph.D. Thesis, Department of botany, Stockholm University, Stockholm, Sweden.

Kawamata, S. 2001. Adaptive Mechanical Tolerance and Dislodgement Velocity of the Kelp *Laminaria japonica* in Wave-Induced Water Motion. Marine Ecology Progress Series. 211, 89-104.

Koehl, M.A.R. 1999. Ecological Biomechanics of Benthic Organisms: Life History, Mechanical Design and Temporal Patterns of Mechanical Stress. The Journal of Experimental Biology. 202, 3469–3476.

Lee, R.W. 1999. Phycology. Cambridge University Press: Cambridge.

Levinton, J.S. 2001. Marine Biology: Function, Biodiversity, Ecology. 2nd ed. Oxford University Press: New York.

Littler, M.M., Littler, D.S. and Brooks, B.L. 2004. Extraordinary Mound-Building Forms of *Avrainvillea* (Bryopsidales, Chlorophyta): Their Experimental Taxonomy, Comparative Functional Morphology and Ecological Strategies. Atoll Research Bulletin. 515, 1-26.

Lewmanomont, K., Wongrat, L. and Supanwanid, C. (1995). Algae in Thailand. Office of Environmental Policy and Planning. Bangkok.

Lobban, C. S. and Harrison, P. J. 1994. Seaweed Ecology and Physiology. Cambridge University Press: Cambridge.

Lobban, C.S., Harrison, P.J. and Duncan, M.J. 1985. The Physiological Ecology of Seaweeds. Cambridge University Press: Cambridge.

McGuinness, K.A. 1989. Effects of Some Natural and Artificial Substrata on Sessile Marine Organisms at Galeta Reef, Panama. Marine Ecology Progress Series. 52, 201-208.

Milligan, K.L.D. and DeWreede, R.E. 2000. Variations in Holdfast Attachment Mechanics with Developmental Stage, Substratum-Type, Season, and Wave-Exposure for the Intertidal Kelp Species *Hedophyllum sessile* (C. Agardh) Setchell. Journal of Experimental Marine Biology and Ecology. 254, 189-209.

Multer, H.G. 1988. Growth Rate, Ultrastructure and Sediment Contribution of *Halimeda incrassata* and *Halimeda monile,* Nonsuch and Falmouth Bays, Antigua, W.I. Coral Reefs. 6, 179-186.

Multer, H.G. and Clavijo, I.E. 1989. *Halimeda* Investigations: Progress and Problems. 12th Carribean Geological Conference. 117-127.

Nybakken, J.W. and Bertness, M.D. 2005. Marine Biology: an Ecological Approach. 6th ed. Pearson Education: San Francisco.

Paul, V. J. and Van Alstyne, K. L. 1988. Chemical Defense and Chemical Variation in Some Tropical Pacific Species of *Halimeda* (Halimedaceae; Chlorophyta). Coral Reefs. 6, 263-269.

Poovachiranon, S., Adulyanukosol. K., Salim, P., Charoenpornwattana, A., Yaem-arunchai, C. and Wutthivarawong, C. 2006. Seagrasses in Thai Waters. Phuket Marine Biological Center: Phuket (In Thai).

Power, P. 1996. Growth of Texas Wildrice (*Zizania texana*) in Three Sediments from the San Marcos River. Journal of Aquatic Plant Management. 34, 21-23.

Simkiss, K. 1964. Phosphates as Crystal Poisons of Calcification. Biological Reviews. 39, 487-505.

Smith, J.E., Smith, C.M., Vroom, P.S., Beach, K.L. and Miller, S. 2004. Nutrient and Growth Dynamics of *Halimeda tuna* on Conch Reef, Florida Keys: Possible Influence of Internal Tides on Nutrient Status and Physiology. Limnology and Oceanography. 49(6), 1923-1936.

Stark, L. M., Almodovar*,* L. and Krauss, R.W. 1969. Factors Affecting the Rate of Calcification in *Halimeda opuntia* (L.) Lamouroux and *Halimeda discoidea* Decaisne. Journal of Phycology. 5(4), 305-312.

Stewart, H.L. 2004. Hydrodynamic Consequences of Maintaining an Upright Posture by Different Magnitudes of Stiffness and Buoyancy in the Tropical Alga *Turbinaria ornata*. Journal of Marine Systems. 49, 157-167.

Stewart, H.L. 2006. Hydrodynamic Consequences of Flexural Stiffness and Buoyancy for Seaweeds: a Study using Physical Models. The Journal of Experimental Biology. 209, 2170-2181.

Umar, M.J., McCook, L.J. and Price, I.R. 1998. Effects of Sediment Deposition on the Seaweed *Sargassum* on a Fringing Coral Reef. 17(2), 169-177.

Verbruggen, H. 2005. Resegmenting *Halimeda*: Molecular and Morphometric Studies of Species Boundaries within a Green Algal Genus. Ph.D. Thesis, Ghent University, Ghent, Belgium.

Vroom, P.S., Smith, C.M., Coyer, J.A., Walters, L.J., Hunter, C.L., Beach. K.S. and Smith, J.E. 2003. Field Biology of *Halimeda tuna* (Bryopsidales, Chlorophyta) Across a Depth Gradient: Comparative Growth, Survivorship, Recruitment, and Reproduction. Hydrobiologia. 501, 149-166.

Walter, L. J. and Smith, C. M. 1994. Rapid Rhizoid Production in *Halimeda descoidea* Decaisne (Chlorophyta, Caulerpaler) Fragments: a Mechanism for Survival after Separation from Adult Thalli. Journal of Experimental Marine Biology and Ecology. 175, 105-120.

Walter, L.J., Smith, C.M., Coyer, J.A., Hunter, C.L., Beach, K.S. and Vroom, P.S. 2002. Asexual Propagation in the Coral Reef Macroalga *Halimeda* (Chlorophyta, Bryopsidales): Production, Dispersal and Attachment of Small Fragments. Journal of Phycology. 278, 47-65.

Wankhow, L., Udomsilp, L. and Janyakorn, S. 2001. Soil Mechanics: Theory and Workshop. Department of Geotechnology, Khon Kaen University. Khon Kaen, Thailand.

Wernberg, T. and Thomsen, M.S. 2005. The Effect of Wave Exposure on the Morphology of *Ecklonia radiata*. Aquatic Botany. 83, 61-70.

Wichachucherd, B. 2008. Population Structure of *Padina boryana* (Dictyotales, Heterokontophyta) in Two Locations in Phuket Province in Southern Thailand. M.Sc. Thesis, Prince of Songkla University, Songkhla, Thailand.

Williams, S.L. 1984. Uptake of Sediment Ammonium and Translocation in a Marine Green Macroalga *Caulerpa cupressoides*. Limnology and Oceanography. 29(2), 374-379.

Wong, M.C., Wright, L.D. and Barbeau, M.A. 2006. Sediment Selection by Juvenile Sea Scallops (*Placopecten magellanicus*), Sea Stars (*Asterias vulgaris*) and Rock Crabs (*Cancer irroratus*). Journal of Shellfish Research. 25(3), 813-821.

APPENDIX

Appendix 1. Phosphate and nitrate concentration, air and water temperature at Tangkhen Bay from August 2006 to July 2007.

	Phosphate (mg/L)		Nitrate (mg/L)		Air temperature (ºC)		Water temperature (ºC)	
Month	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Aug-06	0.30	0.10	0.43	0.19	27.67	0.58	30.67	0.43
Sep-06	0.23	0.60	0.30	0.10	28.00	0.00	34.00	0.00
Oct-06	2.75	2.75	0.09	0.03	35.00	0.00	36.00	0.00
Nov-06	2.75	2.75	0.06	0.03	32.00	0.00	35.00	0.00
Dec-06	0.38	0.39	0.03	0.04	33.00	0.00	35.00	0.00
Jan-07	0.30	0.44	0.07	0.04	32.00	0.00	31.00	0.00
Feb-07	0.56	0.70	0.07	0.05	31.00	0.00	34.00	0.00
Mar-07	0.87	0.16	0.07	0.03	31.00	0.00	32.00	0.00
Apr-07	0.35	0.18	0.10	0.01	34.00	0.00	31.00	0.00
May-07	0.31	0.33	0.09	0.03	33.00	0.00	32.00	0.00
Jun-07	0.03	0.02	0.13	0.03	28.00	0.00	32.00	0.00
Jul-07	0.03	0.02	0.12	0.03	29.00	0.00	31.00	0.00

Appendix 2. Salinity, water velocity and rainfall at Tangkhen Bay from August 2006 to July 2007.

	Salinity (ppt)		Water velocity (m/s)		Rainfall (mm) *	
Month	Mean	SE	Mean	SE	Mean	SE
Aug-06	31	0	0.18	0.02	168.8	-
Sep-06	28	0	0.22	0.02	360.1	-
Oct-06	30	0	0.22	0.02	354.6	-
Nov-06	29	0	0.15	0.01	82.4	-
Dec-06	31	0	0.15	0.01	132.3	-
Jan-07	31	0	0.16	0.01	27.8	-
Feb-07	30	0	0.13	0.02	0.1	-
Mar-07	36	0	0.19	0.01	98.9	-
Apr-07	35	0	0.16	0.01	379.2	-
May-07	32	0	0.15	0.03	328	-
Jun-07	33	0	0.18	0.02	142.3	-
Jul-07	32	0	0.17	0.01	122	-

* Data from The Thai Meteorological Department

Appendix 3. Sediment size fraction (%) in each shore level (upper and mid shore level) at Tangkhen Bay from August 2006 to July 2007.

	Sediment size (μm)							
	<212		212-350		350-500		500-1000	
Month	Upper	Mid	Upper	Mid	Upper	Mid	Upper	Mid
Aug-06	26.67	24.67	30.67	25.33	42.67	21.33	0.00	28.67
Sep-06	24.33	23.33	32.33	25.67	43.33	21.00	0.00	30.00
Oct-06	25.67	24.00	26.33	25.33	47.00	24.00	0.00	27.67
Nov-06	25.33	22.00	29.00	27.00	45.67	20.67	0.00	30.33
Dec-06	27.33	23.00	29.67	25.00	43.00	21.00	0.00	31.00
Jan-07	24.67	27.00	33.33	27.50	42.00	20.50	0.00	25.00
Feb-07	26.67	22.33	34.33	24.67	39.00	22.33	0.00	30.67
Mar-07	21.00	17.33	27.67	26.67	37.33	37.00	14.00	19.00
Apr-07	19.67	15.33	25.67	27.33	35.67	37.67	19.00	19.67
May-07	19.67	15.33	25.67	26.33	36.00	37.33	18.67	21.00
Jun-07	18.67	17.33	26.33	27.67	35.67	37.00	19.33	18.00
Jul-07	18.33	17.67	24.33	27.67	35.33	36.67	22.00	18.00

Appendix 4. Density of *Halimeda macroloba* Decaisne at Tangkhen Bay from August 2006 to July 2007.

Month	Density (thalli/m^2)				
	1	2	3	Mean	SE
Aug-06	10.47	9.00	12.47	10.64	1.65
Sep-06	17.18	17.56	16.75	17.16	3.79
Oct-06	2.73	0.80	3.40	2.31	0.72
Nov-06	2.93	5.73	11.27	6.64	1.62
Dec-06	11.60	14.07	12.40	12.69	1.81
Jan-07	6.60	8.20	12.60	9.13	1.64
Feb-07	12.93	9.47	5.73	9.38	1.70
Mar-07	5.33	7.93	4.27	5.84	0.71
Apr-07	7.20	9.07	7.33	7.87	0.92
May-07	6.73	5.87	4.93	5.84	0.89
Jun-07	6.60	6.07	11.00	7.89	1.22
Jul-07	14.20	6.67	6.20	9.02	1.11

Appendix 5. Density of *Halimeda macroloba* Decaisne in each shore level at Tangkhen Bay from August 2006 to July 2007.

Month	Density (thalli/m^2)					
	200-260 m		260-340 m		340-400 m	
	Mean	SE	Mean	SE	Mean	SE
Aug-06	1.78	1.29	22.89	2.05	3.19	1.58
Sep-06	2.96	1.13	34.21	2.23	8.64	2.13
Oct-06	0.15	0.15	1.44	0.99	5.63	1.78
Nov-06	1.85	1.03	8.28	1.56	9.26	4.78
Dec-06	3.85	2.96	25.72	4.79	4.15	4.15
Jan-07	0.59	0.52	20.00	0.78	3.19	2.39
Feb-07	2.00	0.86	15.78	5.60	8.22	5.01
Mar-07	0.74	0.59	18.00	2.63	14.22	2.02
Apr-07	1.26	0.69	14.39	2.06	5.78	1.43
May-07	3.33	1.47	16.44	3.09	14.81	4.96
Jun-07	1.33	0.82	11.44	2.53	9.70	3.52
Jul-07	2.30	0.65	17.00	2.28	5.11	1.82

Appendix 6. Density of *Halimeda macroloba* Decaisne in each life stage at Tangkhen Bay from August 2006 to July 2007.

Month	Density (thalli/m^2) ± SE					
	New recruit	uncalcified	partially calcified	Mature	fertile	Die
Jul-06	0	0	0.57±0.39	9.9±4.15	0	0
Aug-06	0	2.28±0.99	2.09±1.16	9.71±3.74	0	0
Sep-06	0	0.19±0.19	4.19+1.82	2.67±1.51	0	8±2.54
Oct-06	0	0	0.38±0.24	4.38±2.72	0	1.71±0.80
Nov-06	0	0	0	2.6±1.43	0	1.90±1.36
Dec-06	1.14±0.94	0	4.95±2.84	0	0	2.66±1.10
Jan-07	0	0.38±1.71	0.95±3.68	4.19±1.69	0	3.42±1.21
Feb-07	0	0.38±0.26	1.52±1.08	2.09±2.09	0	3.42±1.52
Mar-07	0	0	1.33±1.14	2.67±2.14	0	0
Apr-07	0	0	0.38±0.38	1.33±1.14	0	2.66±2.14
May-07	0	0	0	0.38±0.38	0	1.33±1.14
Jun-07	0	0	0	0	0	0.38±0.38
Jul-07	0	0	0	0	0	0

Appendix 7. Sediment size fraction in substrate and holdfast of *Halimeda macroloba* Decaisne from August 2006 to July 2007.

	Sediment size (μm)							
	<212		212-350		300-500		500-1000	
Source	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Substrate	22.17	0.57	26.54	0.39	26.51	1.25	24.78	1.29
Holdfast	43.38	0.94	10.88	0.24	18.87	0.38	26.94	0.78

Appendix 8. Holdfast volume and blade surface area of *Halimeda macroloba* Decaisne from August 2006 to July 2007.

	Holdfast volume (cm^3)		Blade surface area (cm^2)	
Month	Mean	SE	Mean	SE
Jul-06	18.29	1.80	37.32	2.79
Sep-06	24.82	3.87	28.32	2.80
Nov-06	16.34	1.71	31.73	2.26
Jan-07	11.29	1.18	25.45	1.88
Mar-07	9.61	1.11	22.86	1.90
May-07	11.64	1.93	40.77	3.64
Mean	16.02	1.93	30.94	2.54

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- Scholarship of Development and Promotion of Science and Technology Talents Project; DPST from Thai Government.
- Endeavour Research Fellowship, Department of Education, Employment and Workplace Relations, Australian Government.

List of Publication and Proceeding

- Prathep, A., Darakrai, A., Tantiprapas, P., Mayakun, J., Thongroy, P., Wichachucherd, B. and Sinutok, S. 2007. Diversity and community structure of macroalgae at Koh Taen, Haas Khanom-Mu Koh Tale Tai, Marine National Park, Nakhon Si Thammarat Province, Thailand. Marine Research Indonesia. 32(2), 153-162.
- Sinutok. S. and Prathep A. A comparison of the growth form of *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho between sheltered and exposed shores in Songkhla province, Thailand. Journal of Applied Phycology. (In submission)
- Sinutok, S., Pongparadorn, S. and Prathep, A. Seasonal variation in density, growth rate and calcium carbonate accumulation of *Halimeda macroloba* Decaisne at Tangkhen Bay, Phuket Province, Thailand. Malaysian Journal of Science. (In submission)

- Sinutok, S., DeWreede, R.E. and Prathep, A. Seasonal variation in distribution, density, and life Stage of *Halimeda macroloba* Decaisne at Tangkhen Bay, Phuket Province, Thailand. Marine Biology. (In preparation)