博士論文

Ecology of *Sargassum* species distributed in the northeast coast of the Gulf of Thailand

(タイランド湾北東部沿岸に分布する
ホンダワラ類の生態)

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Chapter 1

General Introduction

1.1 Genus *Sargassum* in Thai waters

The genus *Sargassum* was established by C. Agardh in 1820 when he made the foundation of a new classification system of the algae. J. Agardh (1848, 1889) revised the classification system of C. Agardh and published a new arrangement of the species, according to his particular idea of evolution within the genus. He divided *Sargassum* into five subgenera based upon the ideas of the evolution of the frond and the vesicles, together with the shape and arrangement of the receptacles. The five subgenera are: *Phyllotrichia*, *Schizophycus*, *Bactrophycus*, *Arthrophycus* and *Eusargassum*. This system was followed by Grunow (1915, 1916) and Setchell (1931a, 1931b, 1933, 1935, 1936). Of the five subgenera, subgenus *Bactrophycus* is known only from the east Asian region (Yoshida, 1983; Tseng *et al.*, 1985). The subgeneric name *Sargassum* was substituted for *Eusargassum*, according to the Article 21.3 of the International Code of Botanical Nomenclature which requires that names of subdivisions of a genus not carry the prefix *Eu*-.

Sargassum, with nearly 400 species, is widely distributed in warm and temperate waters, especially in the Indo-west Pacific region and Australia (Tseng *et al.*, 1985). Along the coasts of the East Asian countries such as China, Korea or Japan, the plants of this genus are very conspicuous, growing in the sublittoral zone to a length of about six meters. They may remain attached to rocky substrata to form "*Sargassum* beds" or may be frequently free-floating, often drifted along the coasts or far out into the open sea.

In Thailand, the first record of *Sargassum* was made by Reinbold in 1901 in "Flora of Koh Chang" on the basis of the specimens collected by Schmidt during the Danish Expedition to Siam from 1899-1900. The collections were made in Koh Chang and adjacent islands in Trat Province, the east coast of the Gulf of Thailand. Only one species, *S. polycystum* C. Agardh was reported. The second record was made by Egerod in 1974 on the specimens collected in the Fifth Thai-Danish Expedition of 1966. The expedition was promoted along the Andaman Sea from the Burmese border in the north to the Malaysian border in the south. Only one species, *S. grevillei* J. Agardh was reported from Koh Ra, Ranong Province Ajisaka and Lewmanomont (2004) believe that *S. grevillei* reported by Egerod could be *S. stolonifolium* Phang et Yoshida. Nateewathana *et al.* (1981) reported *S. crassifolium* J. Agardh based on the specimens deposited in the reference collection of the Phuket Marine Biological Center.

Lewmanomont (1988) found 2 species from the coral reef of the Andaman Sea: *S. polycystum* C. Agardh *and S. crassifolium* J. Agardh. Lewmanomont and Ogawa (1995) reported 3 species of *Sargassum*: *S. crassifolium* J. Agardh and *S. oligocystum* Montagne from Phuket and Phangnga, the Andaman Sae and *S. polycystum* C. Agardh from Rayong, the east coast of the Gulf of Thailand. Aungtonya and Liao (2002) revised and updated the list in the reference collection of the Phuket Marine Biological Center (PMBC) adding 3 species of *Sargassum*. The three species are: *S. crassifolium* J. Agardh from Leamka Singha Beach, Phuket; *S. granuliferum* C. Agardh from Takuapa, Phang-nga Province; and *S. siliquosum* J. Agardh from Takuapa, Phang-nga Province and Patong Beach, Phuket Province. Ajisaka and Lewmanomont (2004) reported *S. stolonifolium* Phang et Yoshida from several localities along the Andaman Sea coast. Noiraksar *et.al.* (2006) reported four species of *Sargassum* are reported from the east coast of the Gulf of Thailand. They are *S. baccularia* (Mertens) C.A. Agardh, *S. binderi* Sonder, *S. oligocystum* Montagne, and *S. polycystum* C.A. Agardh. The common species are *S. binderi*, *S. oligocystum* and *S. polycystum* distributed in all the study areas while *S. baccularia* has been found only in Chantaburi and Trat Province. Ajisaka *et al.*, (2007) reported thirteen species of *Sargassum* were found in Thai waters. Nine species were from the west coast of the Gulf of Thailand, five species from the east coast of the Gulf of Thailand, and six species from the Andaman Sea. They are: *S. baccularia* (Mertens) C.A. Agardh, *S. binderi* Sonder, *S. cinereum* J. Agardh, *S. crassifolium* J.G. Agardh, *S. cristaefolium* C. A. Agardh, *S. longifructum* Tseng et Lu, *S. oligocystum* Montagne, *S. polycystum* C.A. Agardh, *S. siliquosum* J.G. Agardh, *S. stolonifolium* Phang et Yoshida, *S. swartzii* (Turner) C.A. Agardh and two unidentified species. Noiraksar and Ajisaka (2008) reported ten species of *Sargassum* (Sargassaceae, Phaeophyceae) were found along the Gulf of Thailand. Morphological characteristics of *S. baccularia* (Mertens) C.A. Agardh, *S. binderi* Sonder, *S. cinereum* J.G. Agardh, *S.crassifolium* J.G. Agardh, *S. longifructum* Tseng et Lu, *S. oligocystum* Montagne, *S. polycystum* C.A. Agardh, *S. siliquosum* J.G. Agardh, *S. swartzii* (Turner) C.A. Agardh and one unidentified species were examined and are described in detail. The most common species were *S. polycystum* distributed widely in almost all the study sites, *S. crassifolium* restricted to Prachuap Khirikhan Province, *S. longifructum* restricted to Chumphon Province, *S. siliquosum* restricted to Surat Thani Province and one unidentified species restricted to Songkhla Province. Three species (*S. cinereum, S. longifructum* and S. *swartzii*) are new records for the algal flora of Thailand. Tsutsui *et. al.*, (2012) reported ten species of *Sargassum* from Samui Island, Surat Thani Province. They are *S. denticarpum* Ajisaka, *S. oligocystum* Montagne, and *S. polycystum* C.A. Agardh, *S. swartzii* (Turner) C.A.

Agardh and one unidentified species. There are fifteen species of *Sargassum* in Thai waters. Eight species have been recorded from the Andaman Sea, and twelve species have been found in the Gulf of Thailand.

1.2 *Sargassum* in the eastern coast of the Gulf of Thailand

Five species of *Sargassum* were recorded, they are *S. aquifolium* (as report *S. binderi*), *S. baccularia, , S. oligocystum, S. polycystum* and *S. swartzii*. All the five species commonly grow on submerged substrate particularly on rocks and dead corals in littoral and sublittoral zones. The plants are more abundant in Chanthaburi and Trat than Chon Buri and Rayong (Fig.1). The common species are *S. aquifolium*, *S. baccularia, , S. oligocystum, S. polycystum and S. swartzii* distributed along the ortheast area.

Key to the species of *Sargassum* from the eastern coast of the Gulf of Thailand

1. Secondary holdfasts present transformed from stolons; main branches terete,
   with warts or spines. ....……………………………*S. polycystum* (Fig. 5)
1. Secondary holdfasts lacking; main branches terete to flattened, without
spine. 2
   2. Primary branches terete; plants dioecious. …..............…........…   *S. baccularia* (Fig. 3)
   2. Primary branches compressed or flattened; plant monoecious......................….............3
3. Receptacles cymosely arranged ……………………………………….. *S. swartzii* (Fig. 6)
3. Receptacles racemosely arranged ………………………………………...…..………. 4
4. Receptacles flattened, often twisted with dentate magins, vesicle stalks
   flattened. …...............……………………...………………………..… *S. aquifolium* (Fig. 2)
4. Receptacles compressed, not twisted, with apical spines, vesicle stalks
   terete. ………..................…………………………………………….. *S. oligocystum* (Fig. 4)

1.3 Aims and scopes of this thesis

On these backgrounds were mentioned by reviewing genus *Sargassum* in Thai waters and classification system of genus *Sargassum* in the eastern coast of the Gulf of Thailand, the objective of the present study is to totally clarify (1) the distribution of *Sargassum* plants using satellite remote sensing; (2) growth and reproductive pattern in natural habitat of *Sargassum* species; (3) life history and growth in laboratory of *Sargassum* species. The results would greatly contribute to the better understanding of the *Sargassum* species in Thailand waters, and also to the fishery science.

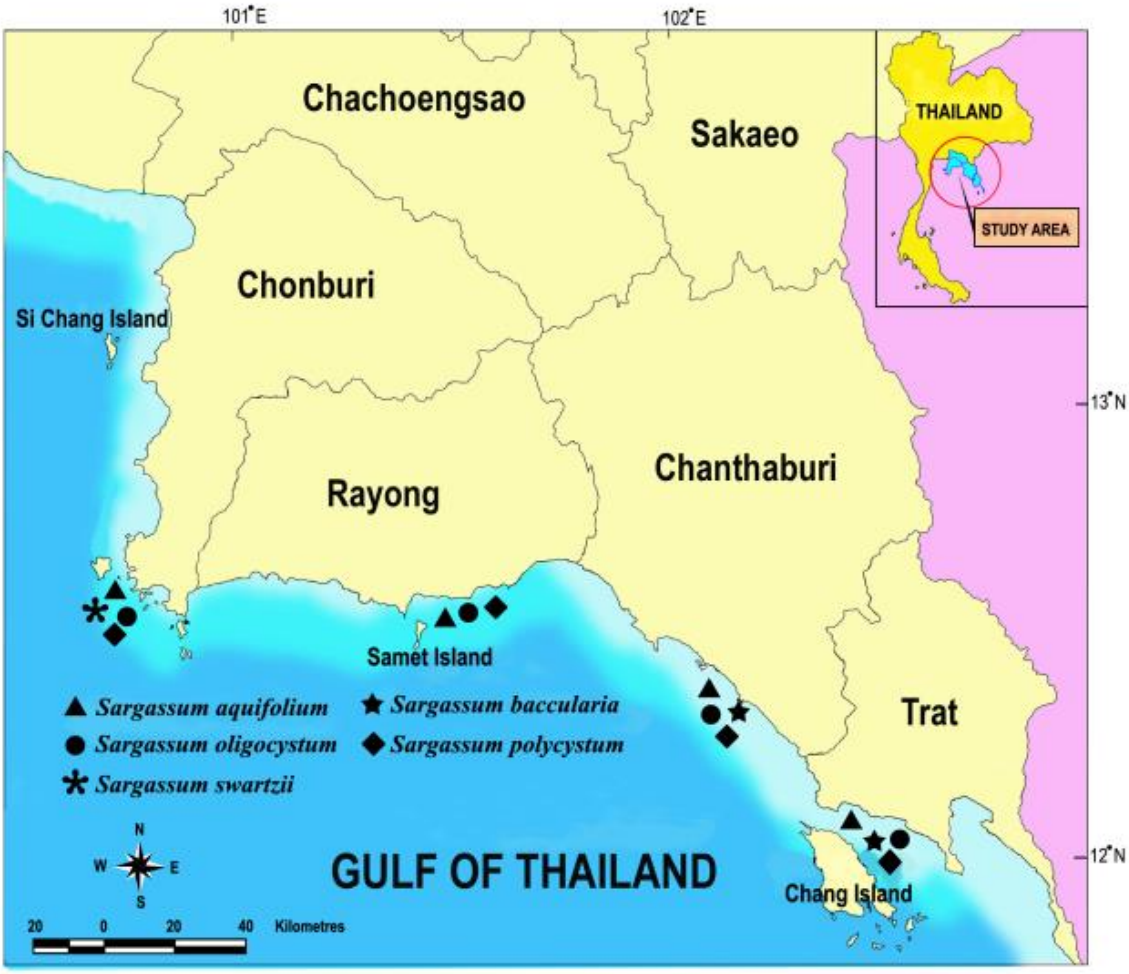


Fig. 1 Distribution of *Sargassum* species along the east coast of the Gulf of Thailand.

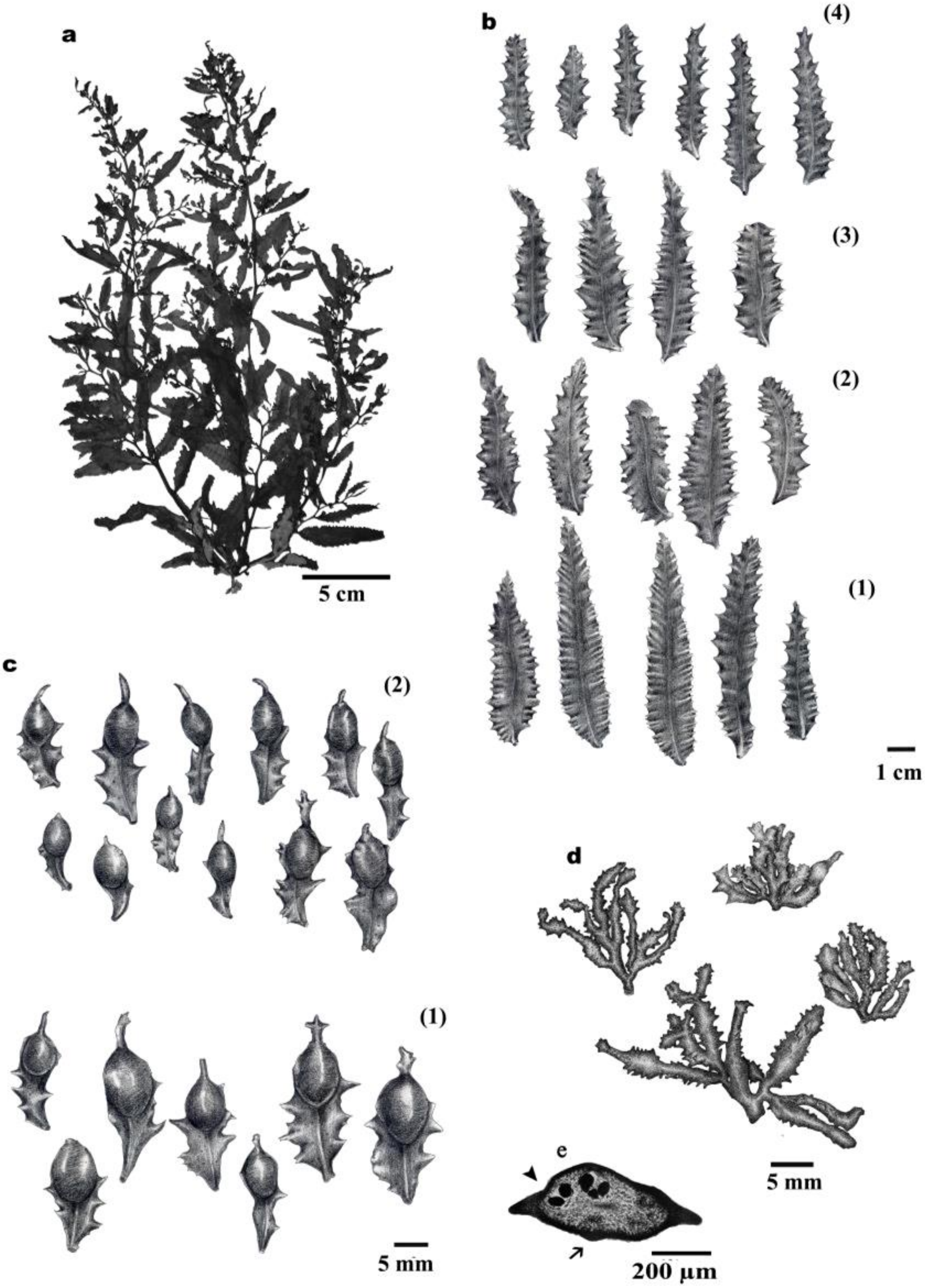


Fig. 2 *Sargassum aquifolium* (Turner) C.Agardh: a. Habit, b. Leaves: (1) primary leaves of lower part, (2) primary leaves of upper part, (3) secondary leaves of lower part, (4) Secondary leaves of upper part, c. Vesicles: (1) of primary branch, (2) of secondary branch, d. Androgynous receptacles, e. Transverse section of receptacle showing male conceptacle (arrow) and female conceptacle (arrowhead).

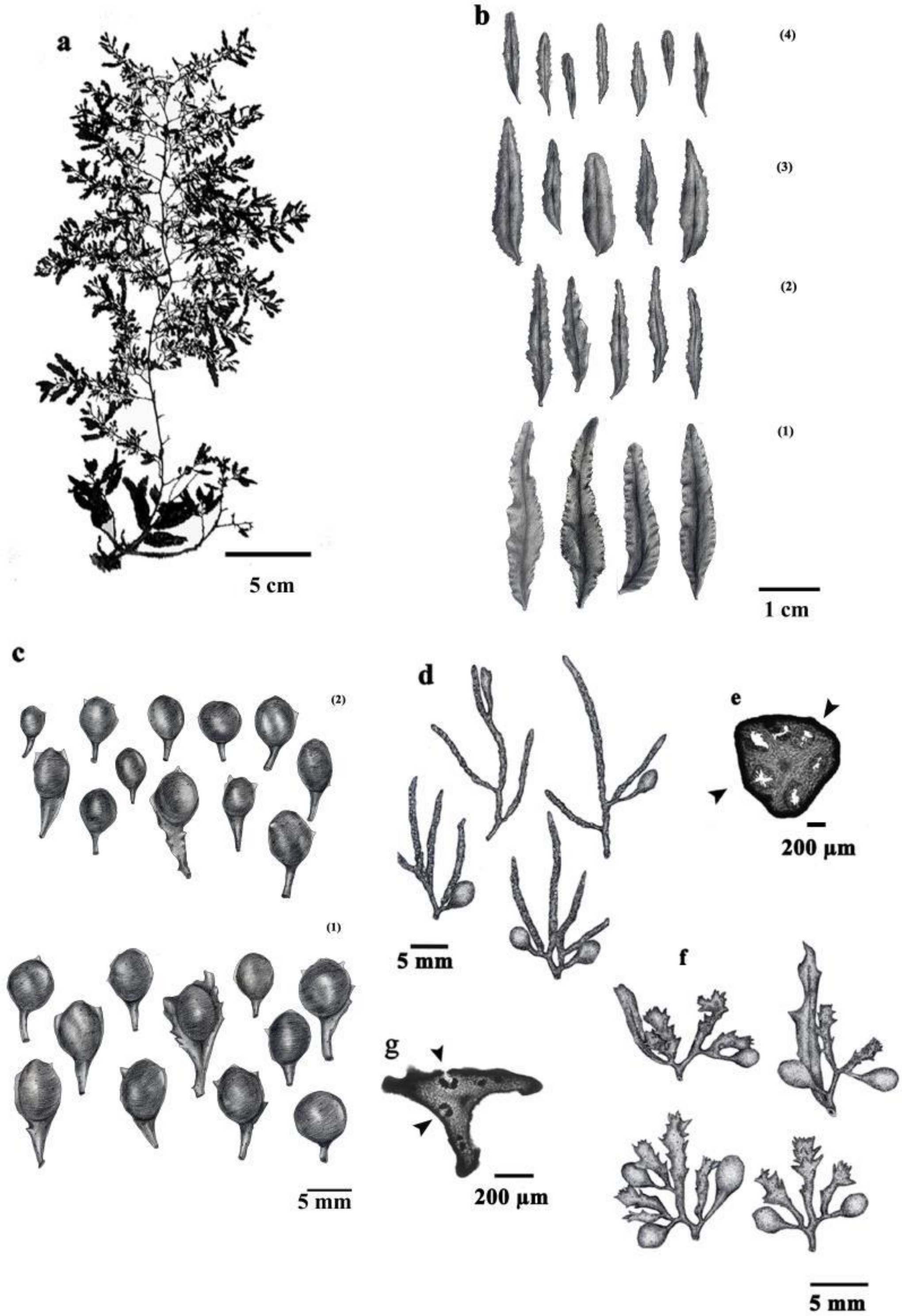


Fig. 3 *Sargassum baccularia* (Mertens) C.A. Agardh: a. Habit, b. Leaves: (1) Primary leaves of lower part, (2) Primary leaves of upper part, (3) Secondary leaves of lower part, (4) Secondary leaves of upper part, c. Vesicles: (1) on primary branch, (2) on secondary branch, d. Male receptacles, e. Transverse section of male receptacle showing male conceptacles (arrowhead), f. Female receptacles, g. Transverse section of female receptacle showing female conceptacles (arrowhead).

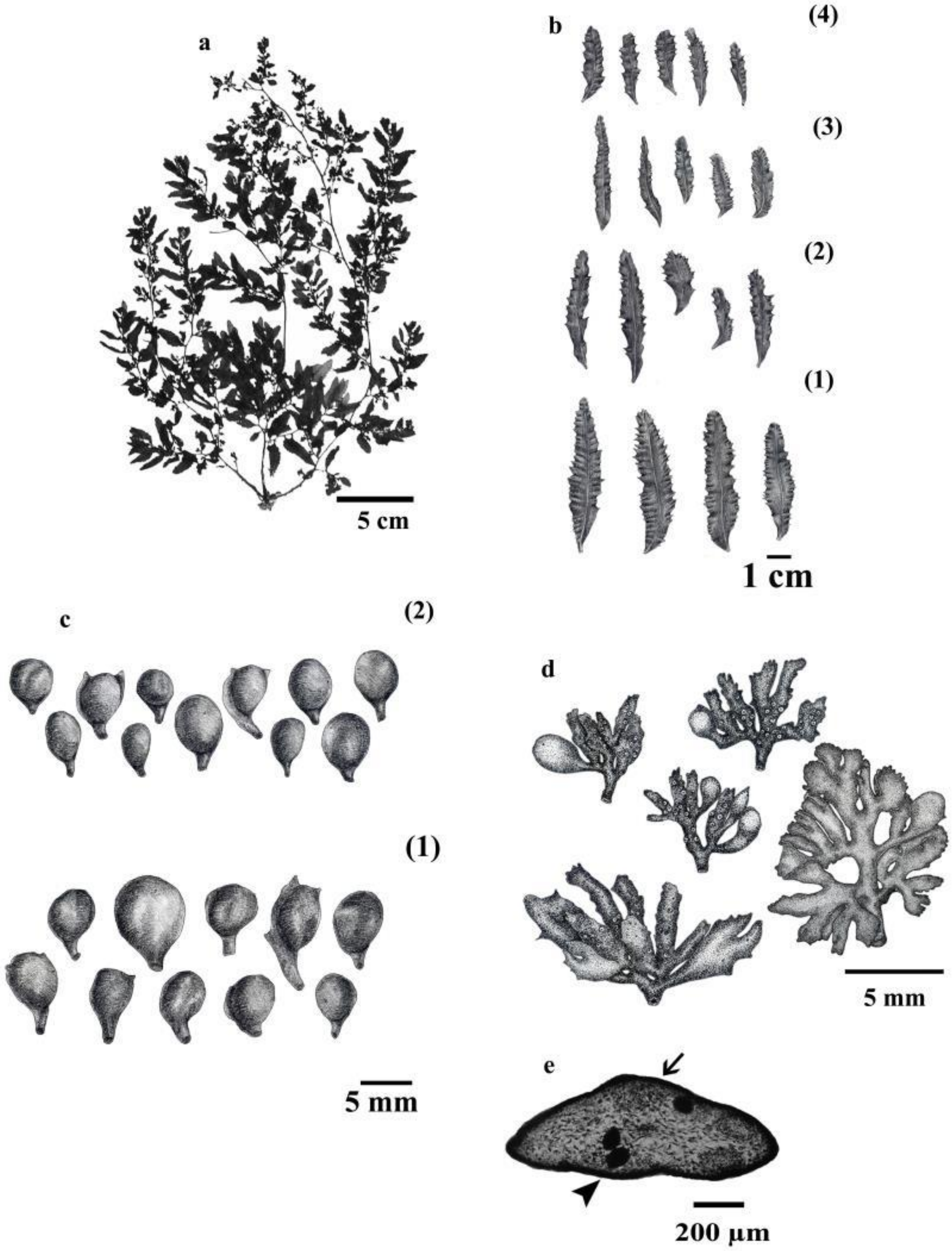


Fig. 4 *Sargassum oligocystum* Montagne: a. Habit, b. Leaves: (1) Primary leaves of lower part, (2) Primary leaves of upper part, (3) Secondary leaves of lower part, (4) Secondary leaves of upper part, c. Vesicles: (1) on primary branch, (2) on econdary branch, d. Androgynous receptacles, e. Transverse section of receptacle showing male conceptacle (arrow) and female conceptacle (arrowhead).

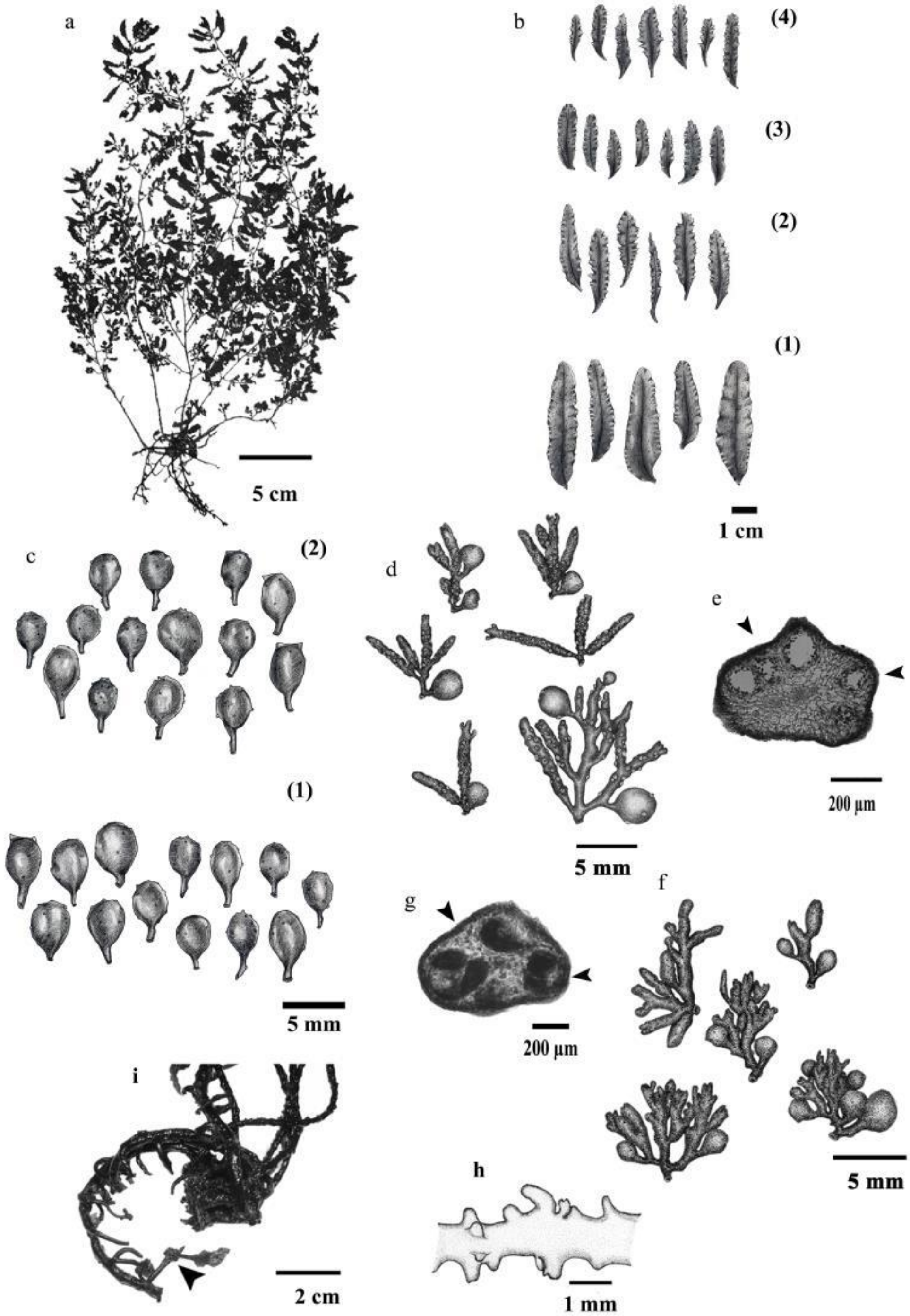


Fig. 5 *Sargassum polycystum* C.A. Agardh: a. Habit, b. Leaves: (1) Primary leaves of lower part, (2) Primary leaves of upper part, (3) Secondary leaves of lower part, (4) Secondary leaves of upper part, c. Vesicles: (1) on primary branch, (2) on secondary branch, d. Male receptacles, e. Transverse section of male receptacle showing male conceptacles (arrowhead)., f. Female receptacles, g. Transverse section of female receptacle showing female conceptacles (arrowhead), h. Spines on primary branch, i. Secondary holdfast (arrowhead).

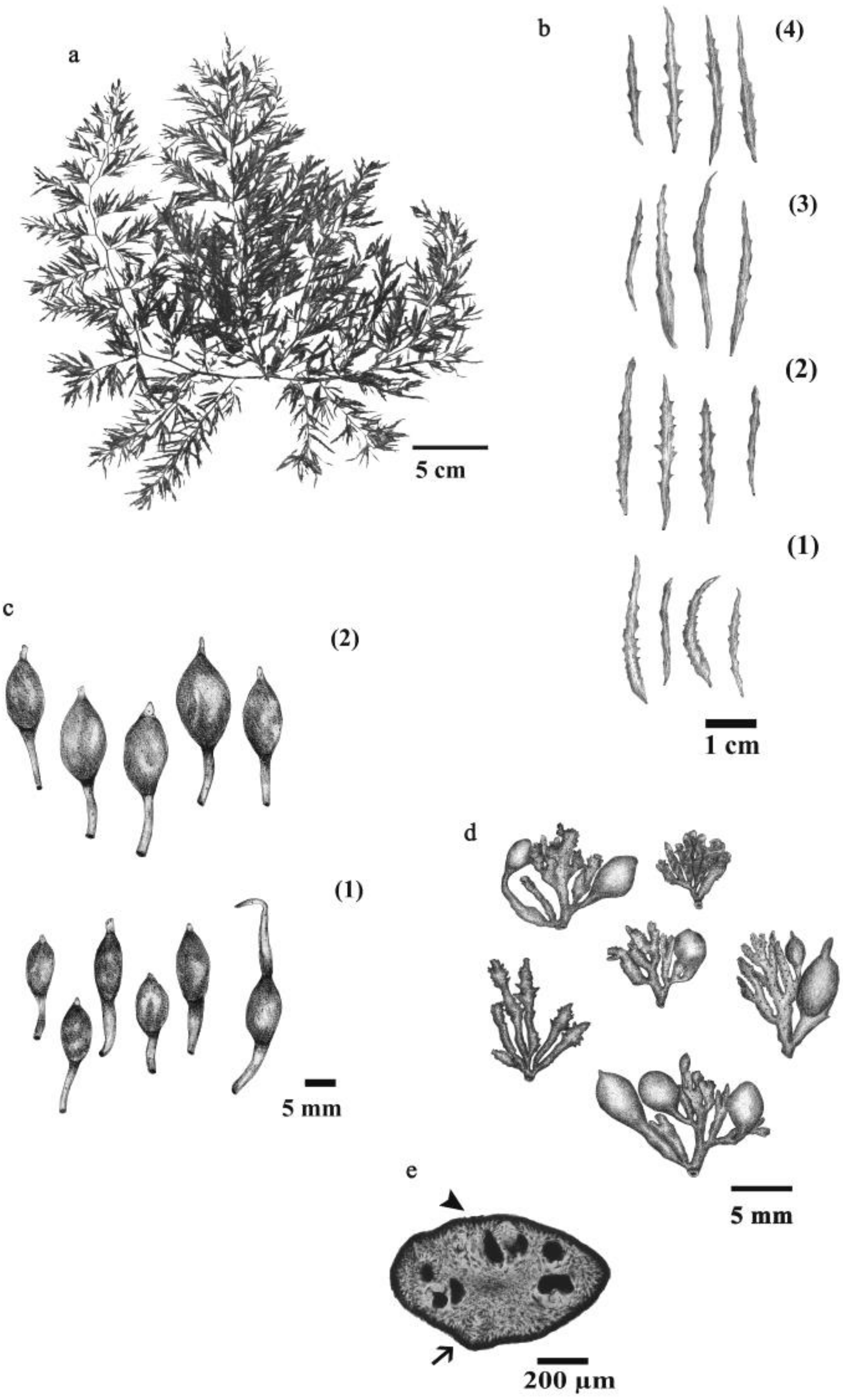


Fig. 6 *Sargassum swartzii* (Turner) C.A. Agardh: a. Habit, b. Leaves: (1) Primary leaves of lower part, (2) Primary leaves of upper part, (3) Secondary leaves of lower part, (4) Secondary leaves of upper part, c. Vesicles: (1) on primary branch, (2) on secondary branch, d. Androgynous receptacles, e. Transverse section of receptacles showing male conceptacle (arrow) and female conceptacle (arrowhead).

Chapter 2

Distribution of *Saragssum* beds off Sattahip, Chonburi Province, in the northeast coast of the Gulf of Thailand

2.1 Introduction

Seaweed beds are typically found in coastal waters, growing on a range of shallow rocky or hard substrates. They serve not only as materials for human consumption and for chemical industries (e.g. alginic acid), but also as spawning, feeding and nursery grounds for a diverse range of fish and molluscs. Among coastal seaweed beds, *Sargassum* is one of the most productive seaweeds in temperate and tropical coastal waters. Many commercially important species spawn within *Sargassum* beds (e.g. sea urchins, abalones, cuttlefish), with the larvae and juveniles using the beds as nursery grounds. *Sargassum* species detaching from the substratum form drifting seaweed mats that serve as a habitat for fish and other aquatic animals that attach to it (Komatsu *et al*., 2007, 2008). Providing an important habitat for aquatic animals, *Sargassum* beds support a rich diversity of species in both coastal and offshore waters. *Sargassum* beds create unique environments equivalent to forests on land due to their luxuriant growth. They influence downward radiation from the sun through their canopy (Komatsu *et. al*., 1990), and profiles of water temperature inside the *Sargassum* forest (Komatsu, 1985; Komatsu *et. al*., 1982, 1995). Their stipes and fronds buffer water motion inside the forest (Komatsu and Murakami, 1994) in the same way as in seagrass beds (Komatsu, 1996; Komatsu and Yamano, 2000; Komatsu *et. al*,. 2004). Seaweed beds, including those of *Sargassum*, however, are as susceptible as seagrass beds to the damaging effects of man's activities such as reclamation, aquaculture, trawling and marine pollution (Komatsu, 1997; Sagawa *et. al*., 2010).

Increased sea floor reclamation, together with increased industrial and agricultural pollution resulting from economic development, have had a detrimental impact on coastal habitats including mangrove forests, seagrass beds, *Sargassum* beds and coral reefs. In Japan, large areas of seagrass beds that were present in coastal zones before the 1960s have since disappeared (Komatsu, 1997), whilst the total area of South East Asian mangrove forest has also decreased dramatically since the 1970s (Valiela *et. al*., 2001). Much of the impact on Thai mangroves was the consequence of shrimp aquaculture, which began its development in the 1970s. Excess feed and waste water emanating from the aquaculture ponds resulted in eutrophication and a decrease in water transparency. In South East Asia, not only mangroves but also beds of *Sargassum* species are subject to and threatened by a range of anthropogenic activities (Kantachumpoo *et. al*., 2014). For the sustainable development of coastal waters, it is imperative that *Sargassum* beds are conserved, since they play an important role in marine ecosystems.

To conserve existing *Sargassum* beds or to restore impacted sites, it is necessary to establish their current status and distribution. Dive surveys and /or observations made from a boat are usually used for mapping seabed habitats, but these methods are laborious and time consuming (Komatsu *et. al*., 2002, 2003). It is therefore, appropriate, to develop alternative, efficient systems for mapping and monitoring *Sargassum* beds.

There has been active development of satellite remote sensing for land use studies in forestry and agriculture, but not so far for the aquatic environment. Only a limited number of studies using satellite remote sensing have been conducted for the mapping and area estimation of brown algal coverage (e.g. Guillaumont *et al*., 1993; Deysher, 1993; Andréfouët *et al*., 2004; McGonigle *et al*., 2011). Only a few studies, however, have mapped *Sargassum* forests in sub-tidal zones and most of the studies that have been conducted have focused on single-species *Sargassum* forests. For example, using SPOT satellite images, Belsher *et al*. (1990) studied the distribution of *Sargassum muticum*, an invasive species in European waters since the 1980s. One possible explanation for the limited number of studies may be that the non-commercial or inexpensive satellite images have low spatial resolution, which limits their utility within image analysis based studies of this nature. For example, LANDSAT and SPOT have spatial resolutions of 30 m and 15 m, respectively (Jensen and Cowen, 1999). These images, unfortunately, are not suitable for seaweed mapping because they are not sufficiently detailed to detect seaweed and seagrass patches, which, in many cases, range from several metres to several tens of metres in coverage. Although commercial satellite images with high spatial resolution such as IKONOS (spatial resolution, 4 m), Worldview 2 and Geoeye etc. are available, they are quite expensive. Mattio *et. al*. (2008) used optical images from LANDSAT 7 (30 m resolution) and Quickbird (2.4 m resolution) on the South West Lagoon, New Caledonia to determine the coverage of *Sargassum* beds. This latter study, however, did not evaluate the differences in overall accuracy between the two image types. In 2006, JAXA launched the ALOS satellite, which had an on-board multispectral sensor “AVNIR-2” with a spatial resolution of 10 m and a panchromatic sensor “PRISM” with a spatial resolution of 2.5 m. These sensors, which have a higher spatial resolution than those of LANDSAT 7 ETM, may permit the various ecosystems in coastal areas to be accurately mapped. ALOS AVNIR-2 has, for example, been effectively used for mapping both coral reefs (e.g. Kakuta *et. al*. 2010; Suyarso *et. al*., 2011) and seagrass beds (Komatsu *et. al*. 2012). There are, however, no reports of the use of these on seaweed beds. There is, therefore, a need to ascertain the current area covered by *Sargassum* beds in tropical waters by analysing archived images from ALOS AVNIR-2 and monitoring changes in these over time. This study, therefore, set out to examine the utility of ALOS AVNIR-2 images for mapping *Sargassum* beds in the tropical waters of Thailand by using a site off the coast of Sattahip, Chon Buri Province for assessment.

2.2 Materials and methods

2.2.1Study site

In Thailand, *Sargassum* beds are distributed in the shallow coastal waters of the Andaman Sea and the Gulf of Thailand. One area where *Sargassum* beds are widely found is in the coastal waters of Chon Buri Province, which is south-east of Bangkok, and is influenced by the open sea of the Gulf of Thailand (Fig. 7). The coastal zone around Sattahip is protected and serves as a natural marine park reserve. This area was selected as the study site for the current investigation because human activities such as fisheries or reclamation are not permitted within the natural park.

2.2.2 Field observation

Sea-truthing for verification of seabed substrate types was conducted on 27-28 February and 4 October 2012. A diver with an underwater camera was towed with a rope from a boat, and periodically photographed the bottom substrates every few seconds. The point at which each photograph was taken was recorded using GPS (m-241, Holux). The substrate types were subsequently identified from the diver's photographs. These photographs produced continuous data of bottom types along the tracks of the boat in an area of 400 × 800 m. They were resampled at a spatial distance of more than 20 m to avoid an overlap of pixels on AVNIR-2 satellite images with a spatial resolution of 10 m. Several substrate types were identified from the photographs and a selection of these are shown in Figure 2. Since the objective of the study was to map *Sargassum* beds, here we used two classes of substrate type: "*Sargassum*" and "others".

2.2.3 *Sargassum* biomass

Sampling was conducted during the 2009 growth season, i.e., January, February, March and December. Divers with SCUBA verified the *Sargassum* species present, their densities and distributions and took images of the various substrate types (Fig. 8). *Sargassum* biomass was determined by quadrat sampling using 50 × 50 cm grids by diving in *Sargassum* beds randomly selected at two depths of 1 m and 1.5-2 m below mean sea level (MSL). Bottom depths were standardised to depth from MSL based on tide tables published by the Maritime Agency of Thailand (Hydrographic Department, 2009). Samples of *Sargassum* were harvested and preserved in plastic bags with sodium chloride (dry salt). In the laboratory, each sample was identified to species level using relevant taxonomic literature (i.e., Pham, 1969; Noro *et. al*., 1994; Silva *et. al*., 1996; Noiraksar and Ajisaka, 2008; Mattio *et. al*., 2009; Sun *et. al*., 2012), and then rinsed in fresh water and cleaned of sand and shells. Plant density and stipe length were measured in the collected samples. Any epiphytic plants and or aquatic animals within each sample were removed before the wet weight of the *Sargassum* was determined prior to drying in a hot air oven (Termaks, TS8000) at 60 $^{\circ}$C for 48 h. Dry weights of the samples were then obtained and used to calculate biomass which is expressed as the dry weight (g) per unit area (i.e., g dw m^{-2}).

2.2.4 Satellite image and analysis methods

ALOS AVNIR-2 data taken on 15 January 2008 were used for the current analysis (Figure 1). Image analysis software ENVI Ver. 5.0 (EXELIS) provided by the Academic Center for Computing and Media Studies, Kyoto University, was used to analyse the captured images. Prior to calculating these processes, the digital number (DN) value of the ALOS AVNIR-2 data was converted to a physical quantity, radiance, as follows:

$$L_i = \mathrm{a}_i \cdot DN_i + \mathrm{b}_i \qquad (1)$$

where

i represents spectral band i.

L is the radiance value recorded by the AVNIR-2 sensor.

a and b are coefficients provided by JAXA.

The image processing procedure is described in Fig. 9. First, the land area was masked using near-infrared band data following a geometrical correction. A Depth Invariant Index (DII) was then applied which removed light scattering and absorption effects within both the atmosphere and the water body (Lyzenga, 1981). The DII is expressed by the following equation:

$$DII_{ij} = \frac{K_j \ln(L_i - L_{si}) - K_i \ln(L_j - L_{sj})}{\sqrt{K_i^2 + K_j^2}} \qquad (2)$$

where

i and *j* represent spectral bands i and j, respectively.

L_i and L_j are the radiance values calculated by equation (1).

L_{si} and L_{sj} are the radiance values calculated by equation (1) recorded over deep water (signals consisting of only external reflection from the water surface and scattering in the atmosphere).

K_i and K_j are the effective attenuation coefficients of the water (m^{-1}).

Equation (2) is transformed to equation (3).

$$DII_{ij} = \frac{\ln(L_i - L_{si})}{\sqrt{K_i^2 / K_j^2 + 1}} - \frac{\ln(L_j - L_{sj})}{\sqrt{1 + K_j^2 / K_i^2}} \qquad (3)$$

The ratio of K_i/K_j is obtained with a biplot of L_i-L_{si} and L_j-L_{sj} of which L_i and L_j are radiances on the sand bed according to Mumby and Edwards (2000). The DII is useful for mapping the seabed substrate types when seabed depth distributions are not available.

2.2.5 Classification procedure

The land area was masked by using near-infra red after geometrical correction because near-infra red is readily absorbed by the sea surface and reaches low values over the sea surface. Three classification methods were used to map the *Sargassum* and these are presented in Fig. 9. The first method used supervised classification of a maximum likelihood method based on red, green and blue bands (left hand column). The maximum likelihood classification has been used for supervised classification (e.g. Fuller *et al*. 1994), which assumes that the statistics for each class in each band are normally distributed and calculates the probability that a given pixel belongs to a specific class (Richards, 1999). Unless a probability threshold is selected, all pixels are classified. Each pixel is assigned to the class that has the highest probability (i.e., the maximum likelihood). The second method used supervised classification of a minimum distance method based on red, green and blue bands (centre column). The minimum distance method has also been used for supervised classification (e.g., Hodgson *et al*., 1987). The minimum distance classification uses the mean vectors of each region of interest and calculates the Euclidean distance from each unknown pixel to the mean vector for each class (Richards, 1999). The third method used supervised classification of a minimum distance method based on DII of green and blue bands (right hand column). Supervised classifications assign seabed substrate types into either the class of *Sargassum* beds or the class of "other" seabed substrate types. Supervised classification used 158 pixels identified by sea-truthing for training of the *Sargassum* class and 195 pixels for training of the "other" class, and another 80 pixels for validating the classification results (Fig. 10). Story and Congalton (1986) suggested that at least 50 pixels per map class should be used in the training process. The number of pixels used for training in this study, therefore, conforms to the recommended standard. The datasets used for training were different to those used for validation.

2.3 Results

2.3.1 *Sargassum* biomass

Sargassum aquifolium and *S. oligocystum* were found to be abundant in the Sattahip study area, and were seen to be growing close together in beds, which were distributed parallel to the coast. Several other algal species, including *Turbinaria conoides, Lobophora asiatica, Padina australis* and *Amphiroa anceps,* were also observed but were not abundant. The mean biomass of *S. aquifolium* at depths of 1 and 1.5-2 m below MSL were 7.73 and 92.75 g dw m^{-2} respectively, whilst that of *S. oligocystum* at the same depths were 44.05 and 87.97 g dw m^{-2} (Table 1).

2.3.2 Image analysis of the *Sargassum* beds

The blue band image showed a dark area with low radiance that was distributed parallel to the coast (Fig. 11a). The green band image also showed a dark area with low radiance that was parallel to the coast that also extended into offshore waters (Fig. 11b). An area of low reflectance in the red band was observed, however, not only close to the shore, as was seen for the blue and green bands, but also in areas of deep water (Fig. 11c). The depth invariant index image indicated that the low DII areas were parallel to the coast. These corresponded with the *Sargassum* beds, the presence and position of which were verified by the ground truth data (Fig. 12a).

The results of the classification using the minimum distance method with the red, green and blue bands were not realistic because all the *Sargassum* beds were distributed in offshore waters (Fig. 12b; Table 2). On the other hand, the results from the maximum likelihood method on the red, green and blue bands and the minimum distance method of the DII on the green and blue bands closely corresponded to the distribution pattern of the *Sargassum* beds found along the shore (Fig. 12c and 12d). There was a difference in the mapping results generated by the maximum likelihood method using the red, green and blue bands, and the minimum distance method using the DII of the green and blue bands in determining the distribution of *Sargassum* beds, with the accuracy of the latter method being superior to that of the former (Tables 3 and 4).

2.4 Discussion

The mean biomasses of *S. aquifolium* and *S. oligocystum* during their growth season, found at a depth of 1 m below MSL, were 7 and 44 g dw m^{-2}, respectively, whilst at depths of 1.5-2 m below MSL they were 87 and 92 g dw m^{-2}. In the Philippines, Trono and Lluisma (1990) reported that the highest biomass of *S. oligocystum* was attained at Bolinao in December (282 g ww m^{-2}), which is equivalent to nearly 30 g dw m^{-2}. Ohno *et. al.* (1995) surveyed the *Sargassum* communities around the Philippine Islands and found that the *Sargassum* beds extended from a few metres off shore to the shallow sub-tidal zone, typically to a depth of 3 m or to depths of 5-6 m where some patches of thalli were observed on rocks, and the growth of *S. aquifolium* (reported as *S. binderi*) in Cordova, Mactan Island had a biomass value of 3.4 kg ww m^{-2}, which is equivalent to nearly 340 g dw m^{-2}. In Malaysia, Wong and Phang (2004) reported on species of *Sargassum* at Cape Rachado, and found that the highest biomass of *S. aquifolium* was obtained in July 1995 when it was 54.30 g dw m^{-2} and then in April 1996 when a biomass of 80.81 g dw m^{-2} was found. Later, Yeong and Wong (2012) reported the biomass of the same species in October 2008 as being >350 g dw m^{-2}. The biomasses of *S. aquifolium* and *S. oligocystum* beds in waters off Sattahip are similar to those in the Philippines and Malaysia.

The overall accuracies of supervised classification with the maximum likelihood method and the minimum distance method using the data of red, green and blue bands were

66.9-68.8% (Tables 2 and 3). Maximum likelihood generally gives better results than the minimum distance classifier because the covariance of the data is taken into account (Ozesmi and Bauer, 2002). The overall accuracy and kappa coefficient of the maximum likelihood method are inferior to those of the minimum distance method using the DII (Table 4). The classification result from the minimum distance method using DII from the green and blue bands gave the best classification performance. This method indicated less *Sargassum* beds in offshore waters than suggested by the maximum likelihood method using data from the red, green and blue bands. This means that the radiance from the sea bottom in offshore waters was reduced by the water column. The radiance of sand beds in offshore waters decreases and is similar to that of the *Sargassum* beds. The user accuracy of *Sargassum* beds from supervised classification of the maximum likelihood method using data of red, green and blue bands is higher than that of minimum distance method using DII of green and blue bands, while the kappa coefficient of the maximum likelihood method is lower than that of the minimum distance method. This means that the maximum likelihood method using data of red, green and blue bands overestimates *Sargassum* beds, indicating that the *Sargassum* beds are distributed in offshore waters. DII, however, can successfully compensate for reduction by the water column. In this way, the radiometric corrections, including atmospheric and water column corrections, are the key processes having an influence on mapping accuracy (Sagawa *et al*., 2008). On the other hand, Andréfouët *et. al*., (2004) pointed out that bathymetric correction is not always ameliorated when they analysed a Tahitian coral reef using IKONOS satellite images. This is true for analysing habitats in a narrow depth range between 0 and 3 m deep as was the case for the Tahitian coral reef. DII is useful when the depth range is as wide as in this study.

Small differences in the overall accuracies between the methods are caused by the ground truth data concentrated around the *Sargassum* beds near the shore. Another reason for low accuracy may also be caused by the class of other bottom substrates including sand, dead corals and a small quantity of live corals. The reflectance of live corals though is lower than that of sand (Nurdin *et. al*., 2012). A mixel effect results when a pixel covers plural bottom types and shows mixed reflectance of these bottom types. Thus, it is estimated that the mixel effect by live coral patches on sand beds results in misclassification. Mikami *et. al*., (2007) used aerial photographs to map the *Sargassum* beds in a small cove facing the Pacific Ocean. In this latter study, the authors were able to classify the *Sargassum* beds, the *Ecklonia* and *Eisenia* beds, sand beds, and rocks both below and above the sea surface with an overall accuracy of about 93%. The authors pointed out that high accuracy is partially attributable to the high spatial resolution of the aerial photography, which allows objects as small as 21 cm to be resolved. This spatial resolution of the pixels may correspond to one individual seaweed plant such as fronds of *Sargassum* species or *Ecklonia cava*. Thus, high spatial resolution is very important to detect *Sargassum* beds when the *Sargassum* species form a patchy distribution on rock or dead coral scattered at a horizontal scale within several metres. As the images generated by the ALOS AVNIR-2 have a spatial resolution of 10 m, it is not easy to avoid mixel effects. Also, there is a time difference between the capture of the ALOS AVNIR-2 images and the ground truthing. *Sargassum* species that were distributed in Sattahip are perennial species (Noiraksar *et. al*., 2006). Although mature stipes detach from their holdfasts, their holdfasts remain with smaller sized stipes. Once these *Sargassum* beds cover a broad area they are less subject to abrupt changes as a consequence of human interference.

Using aerial data on the *Sargassum* beds in the coastal waters off Sattahip, it is possible to estimate the biomass of the *Sargassum* species within the study site. The *Sargassum* beds can be divided into two areas, those that are in shallow waters with a depth of 1 m, and those that are in waters deeper than 1 m. In this study, the areas of *Sargassum* identified by remote sensing are divided into shallow and deep areas for biomass estimation. Here, it is assumed that shallow and deep areas are covered with mean biomasses of *Sargassum* obtained from separate quadrat samples at depths of 1 m, and 1.5-2 m. The mean biomass in the shallow area was 25 g dw m^{-2}, whilst that in the deeper area was 89 g dw m^{-2}. Since the area of *Sargassum* beds in front of the natural park of Sattahip is about 129,600 m^2, the total biomass in December 2009 was estimated to be about 7.4 t dw. When the monsoon season starts in Thailand in May, after the *Sargassum* have matured, they become detached by wave action. Thus it can be estimated that more than 7.4 t dw of *Sargassum* species become drifting seaweeds or stranded seaweeds at that time. They serve as habitats for other species on the sea surface and on the beach.

This study demonstrates that images from ALOS AVNIR-2 can serve as a practical tool for mapping *Sargassum* beds in tropical waters when coupled with depth invariant indices. If non-commercial satellite optical images with higher spatial resolutions are available, then it will be possible to map the *Sargassum* beds and estimate biomass with a greater degree of accuracy.

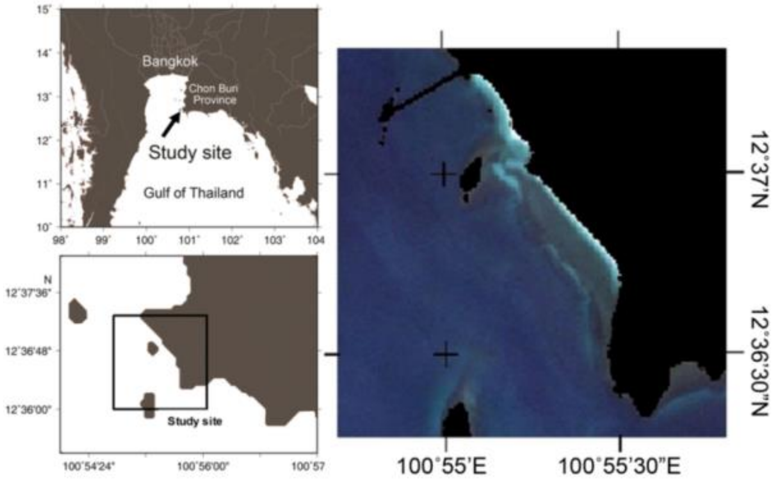


Fig. 7 Maps showing the Gulf of Thailand and the location of the study site relative to Bangkok (left upper map) and within the Sattahip coastal zone of Chon Buri Province (left lower map), and an ALOS AVNIR-2 true color image of study site taken on January 15, 2008 (right).

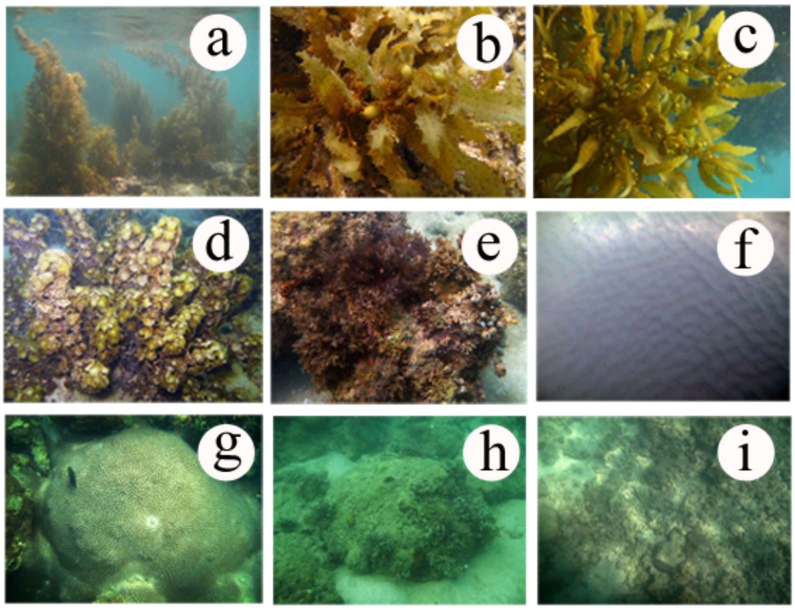


Fig. 8 Images of habitats taken in the study site near Sattahip during the ground surveys. (a) *Sargassum* bed, (b) *Sargassum aquifolium*, (c) *Sargassum oligocystum*, (d) *Turbinaria conoides*, (e) red algae, (f) sand, (g) live coral, (Hh) dead coral, (i) coral rubble.

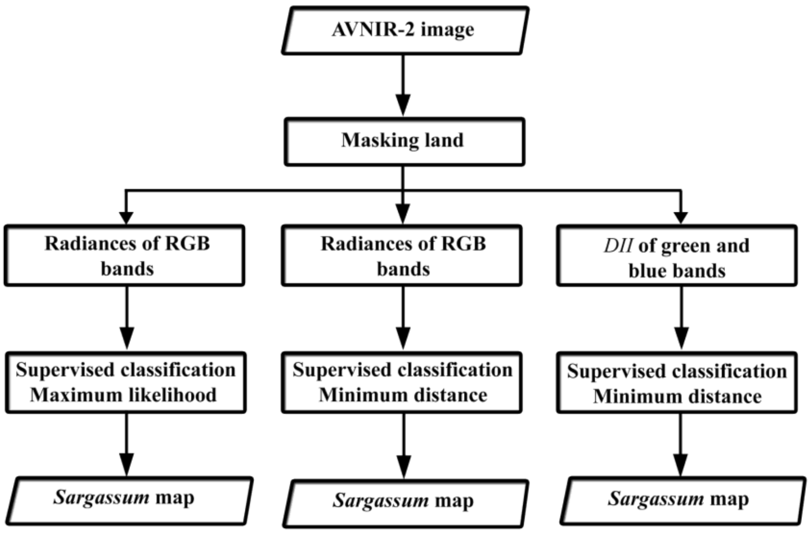


Fig. 9 Flow chart illustrating the data process. The land area was masked by using near infrared after geometrical correction. Three classification methods were applied to map the *Sargassum* beds: classification using maximum likelihood (left-hand column) and minimum distance (center column) methods based on red, green, and blue (RGB) bands and supervised classification using minimum distance methods based on depth invariant indices (*DII*) of green and blue bands (right hand column).

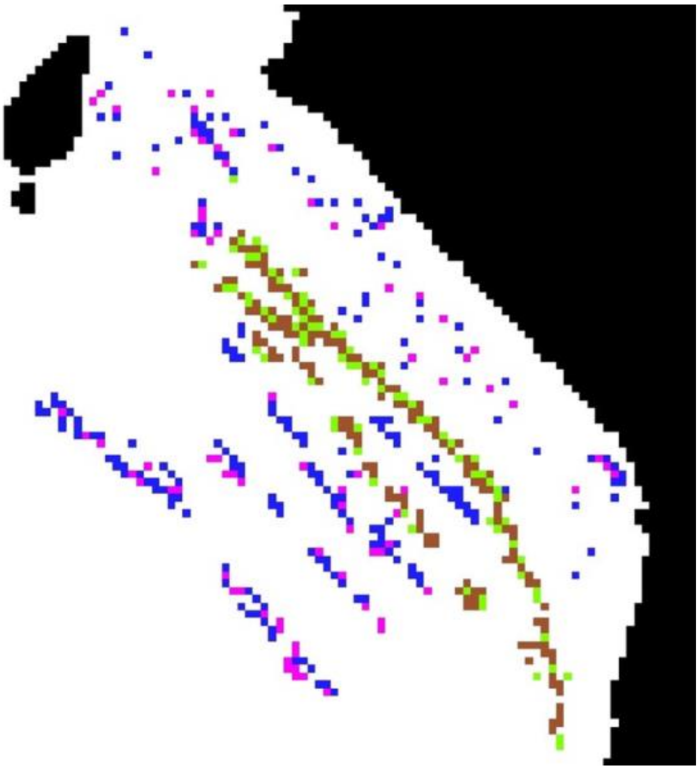


Fig. 10 Distribution of training and validating datasets obtained by sea truthing in an area of 400 m × 800 m off Sattahip. Brown and blue pixels are training datasets of *Sargassum* and "other" seabed types, respectively. Green and pink pixels are validating datasets of *Sargassum* and "other", respectively.

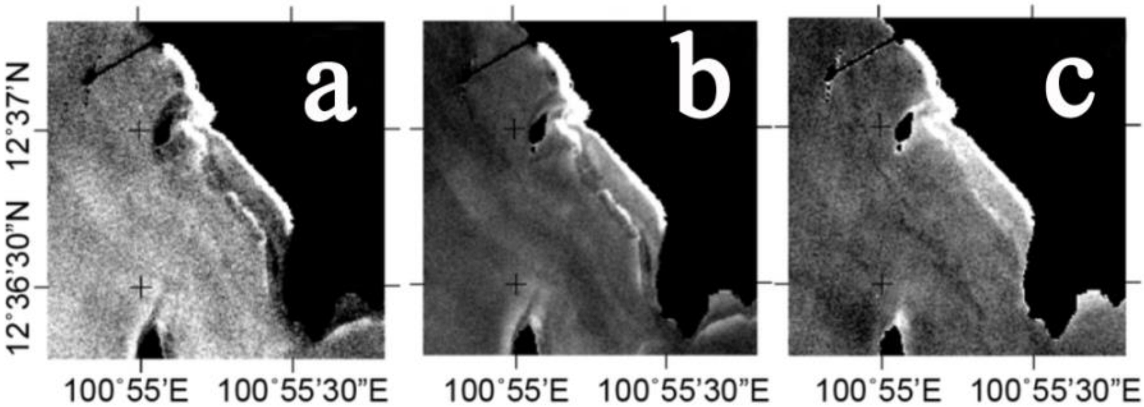


Fig. 11 Images of the Sattahip coastline, Chon Buri Province, Thailand, captured by the satellite ALOS AVNIR-2. (a) Blue band, (b) green band, (c) red band.

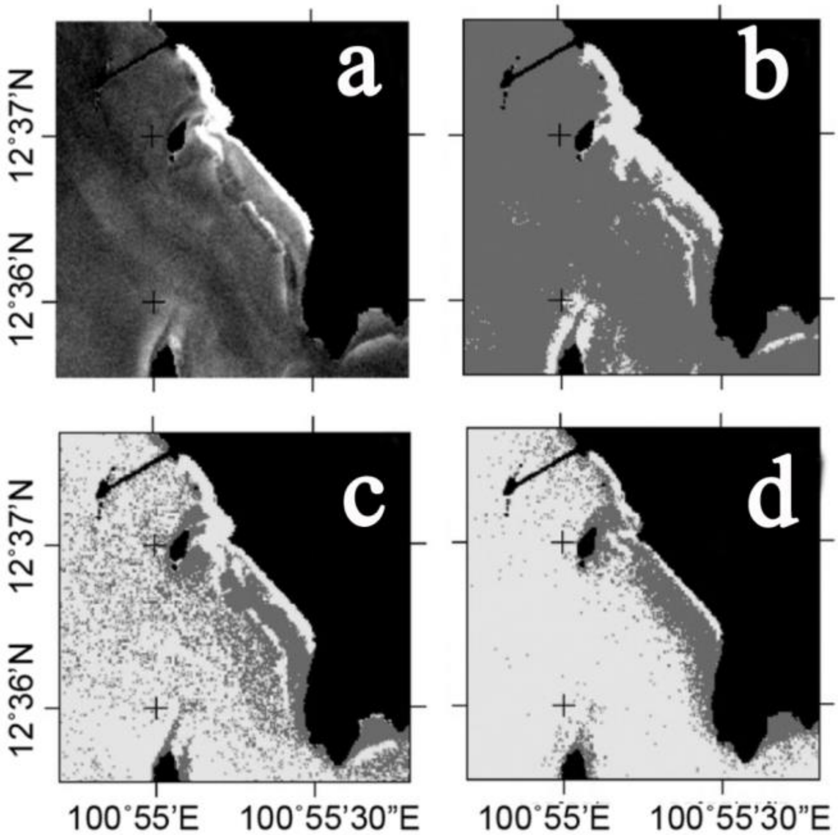


Fig. 12 Images of the Sattahip coastline. (a) Depth invariant index image based on blue and green bands; (b) *Sargassum* area (gray area in the sea) and the "other substrates" area (brighter area in the sea) extracted by supervised classification using the minimum distance method from radiances of the red, green, and blue bands; (c) *Sargassum* area and the "other substrates" area extracted by supervised classification of maximum likelihood method from radiances of red, green, and blue bands; (d) *Sargassum* area and the "other substrates" area extracted by supervised classification of minimum distance method from depth invariant index based on green and blue bands.

Table 1 Biomass of *Sargassum aquifolium* and *S. oligocystum* obtained by sampling 20 quadrats at each depth in the Sattahip study area in January-March and December 2009. Mean values ± SE are shown. MSL, mean sea level

Species	Biomass (gm^{-2}) 1 m below MSL (n = 20)		Biomass (gm^{-2}) 1.5-2 m below MSL (n = 20)	
	Wet weight	Dry weight	Wet weight	Dry weight
S. aquifolium	50.82 ± 24.76	7.73 ± 3.77	608.35 ± 100.69	92.75 ± 15.32
S. oligocystum	292.91± 105.63	44.05 ± 15.89	584.96 ± 257.55	87.97 ± 38.74

Table 2 Error classification matrix generated by the minimum distance method applied to data from the red, green and blue bands.

	Class	Ground truth data			User accuracy
		Sargassum	Other	Total	
Classification	*Sargassum*	71	44	115	61.7
	Other	9	36	45	80.0
	Unclassified	0	0	0	
	Total	80	80	160	
	Producer accuracy	90.0	45.0		
	Overall accuracy				66.9
	Kappa coefficient				0.338

Table 3 Error classification matrix generated by the maximum likelihood method applied to data from the red, green and blue bands.

	Class	Ground truth data			User accuracy
		Sargassum	Other	Total	
Classification	*Sargassum*	51	21	72	70.8
	Other	29	59	88	67.0
	Unclassified	0	0	0	
	Total	80	80	160	
	Producer accuracy	63.8	73.8		
	Overall accuracy				68.8
	Kappa coefficient				0.375

Table 4 Error classification matrix generated by the minimum distance method applied to the depth invariant indices derived from the green and blue bands.

	Class	Ground truth data			User accuracy
		Sargassum	Other	Total	
Classification	*Sargassum*	74	34	108	68.5
	Other	6	46	52	88.5
	Unclassified	0	0	0	
	Total	80	80	160	
	Producer accuracy	92.5	57.5		
	Overall accuracy				75.0
	Kappa coefficient				0.500

Chapter 3

Seasonal changes in biomass of *Sargassum* species off Sattahip

3.1 Introduction

There are about 6,000 seaweed species in the world. They are classified into three major groups: green (chlorophytes), red (rhodophytes) and brown (phaeophytes). Brown seaweeds are the group with the largest production, contributing approximately to 59% of the total production in 2006, followed by red (40%) and green (<1%) seaweeds (D'Orazio *et. al.,* 2012). Seaweed beds are typically found in coastal waters, growing on a range of shallow rocky or hard substrates. They serve not only as materials for human consumption and for chemical industries (e.g. alginic acid), but also as spawning, feeding and nursery grounds for a diverse range of fish and molluscs. Among coastal seaweed beds, *Sargassum* is one of the most productive seaweeds in temperate and tropical coastal waters. Many commercially important species spawn within *Sargassum* beds (e.g. sea urchins, abalones, cuttlefish), with the larvae and juveniles using the beds as nursery grounds. *Sargassum* species detaching from the substratum form drifting seaweed mats that serve as a habitat for fish and other aquatic animals that attach to it (Komatsu *et al*., 2007, 2008). Providing an important habitat for aquatic animals, *Sargassum* beds support a rich diversity of species in both coastal and offshore waters. *Sargassum* beds create unique environments equivalent to forests on land due to their luxuriant growth. They influence downward radiation from the sun through their canopy (Komatsu *et. al*., 1990), and profiles of water temperature inside the *Sargassum* forest (Komatsu, 1985; Komatsu *et. al*., 1982, 1995). Their stipes and fronds buffer water motion inside the forest (Komatsu and Murakami, 1994) in the same way as in seagrass beds (Komatsu, 1996; Komatsu and Yamano, 2000; Komatsu *et. al*,. 2004). Seaweed beds, including those of *Sargassum*, however, are as susceptible as seagrass beds to the damaging effects of man's activities such as reclamation, aquaculture, trawling and marine pollution (Komatsu, 1997; Sagawa *et. al*., 2010).

On these backgrounds, the objective of the present study is to totally clarify the seasonal changes in grwth of *Sargassum* species in Thai waters. The results would greatly contribute to the better understanding of the *Sargassum* species in whole Thailand, and also to the fishery science.

3.2 Materials and methods

3.2.1 Study site

Nang Rong Beach, Chon Buri Province (12°36'46.7"N, 100°55'19.8"E) is a large intertidal flat, the substratum is composed of dead coral fragments with fine to coarse sand and a subtidal coral reef, the tidal range is 1.0 m mean sea level (Fig.13). This intertidal flat is exposed during low tide in the day time for about 4-6 hours during the month of April and May. The average seawater temperature is 29.74°C, salinity 30.8 psu and wind magnitude 5.6 m s^{-1}. This area is rich in marine algal flora. Two species of *Sargassum*, *S. aquifolium* (as reported *S. binderi*) and *S. oligocystum* are the most dominant species growing amoung other bentic marine algae such as *Turbinaria conoides*, *Padina australis, Padina santae-crucis*, *Lobophora variegata, Amphiroa anceps, Bryopsis pennata, Gelidiella acerosa*, *Chondrophycus cartilagineus*, etc.

3.2.2 Sampling design

Samples were collected every two months interval from January 2006 to January 2007. Four transect lines were placed across the *Sargassum* bed in a direction perpendicular to the shore. The quadrats (0.5x0.5 m^2) were laid on the transect lines from the shoreward to the seaward. The quadrat samples were placed at 50 m interval. The distribution, percentage cover, average branch length and reproductive period of *Sargassum* were investigated.

3.2.3 Statistical analysis

A one-way ANOVA was applied to examine differences among characteristics of *S. aquifolium* and *S. oligocystum* by month. Before the ANOVA, standing crop and thallus length were transformed using logarithm transformation, while percent cover and percent fertility were transformed using arcsine transformations. We examined relationships among percent cover, thallus length, standing crop and percent fertility with the environmental parameters using Spearman's rank correlation analysis.

3.3 Results

3.3.1 *Sargassum aquifolium*

The distribution of *S. aquifolium* at Nangrong beach, Chon Buri Province was from intertidal to subtidal areas at the depth 0.5-6.2 m. The occurrence of plants was from 70 m from shore to 300 m seaward. Some plants were exposed in the air during low tide (Fig. 14). Water temperature increased from 28 ºC in January 2006 to 32 ºC in November 2006 (Fig.

15). The salinity during January 2006 to January 2007 ranged between 32.0-33.3 psu.The highest salinity occurred in March 2006 (the dry season), and lowest in November 2006 and January 2007 (Fig. 15). In this study, the samples were collected only 5 times, due to heavy storm and strong waves of southwest monsoon during July to September 2006.

The peak of percentage cover was occurred in March 2006 (37.85±8.7 %) The lowest percent cover occurred in May 2006 (0.3±0.2 %) (Fig. 16). Results of one-way ANOVA (95% confidence level) indicated monthly differences in percent cover of *S. aquifolium* per unit area were significant (Table 5). The percentage cover was not significantly correlated with water temperature and salinity.

The peak average of branch length was occurred in March 2006 (18.27±2.7 cm). The lowest average of branch length occurred in November 2006 (6.47±2.3 cm) (Fig. 16). Results of one-way ANOVA (95% confidence level) indicated monthly differences in average of branch length of *S. aquifolium* were significant (Table 5). The average of branch length was not significantly correlated with water temperature and salinity.

The peak of standing crop was occurred in March 2006 (12.57±2.7 g dw $0.25m^{-1}$). The lowest of standing crop occurred in May 2006 (0.09±0.05 g dw $0.25m^{-1}$) (Fig. 16). Results of one-way ANOVA (95% confidence level) indicated monthly differences in standing crop of *S. aquifolium* were significant (Table 5). The standing crop was not significantly correlated with water temperature and salinity.

The peak of percentage of fertile plants with receptacles was occurred in March 2006 (80.0±10.32 %). The lowest was occurred in January and May 2006 (20.0±10.32 %) (Fig. 16). Results of one-way ANOVA (95% confidence level) indicated monthly differences in percentage of fertile of *S. aquifolium* were significant (Table 5). The percentage of fertile was not significantly correlated with water temperature and salinity.

These receptacles were examined by transverse sectioning to see the fertility of receptacles whether they were in the young or mature stage. The mature plants occurred in March 2006 with some released eggs on the outside of female receptacles. In January and November 2006 the eggs were smaller than in March 2006. In May 2006 the receptacles were found to be in young developing stage with small eggs. No receptacles were found in January 2007 (Fig. 17).

3.3.2 *Sargassum oligocystum*

The distribution of *S. oligocystum* at Nangrong beach, Chon Buri Province was from intertidal to subtidal areas at the depth from 0.5-3.4 m. The occurrence of plants was from 50 m from shore to 300 m seaward. Some plants were exposed in the air during low tide (Fig. 18). Water temperature increased from 28 ºC in January 2006 to 32 ºC in November 2006 (Fig. 15). The salinity during January 2006 to January 2007 ranged between 32.0-33.3 psu. The highest salinity occurred in March 2006 (the dry season), and lowest in November 2006 and January 2007 (Fig. 15). In this study, the samples were collected only 5 times, due to heavy storm and strong waves of southwest monsoon during July to September 2006.

The peak of percentage cover was occurred in January 2006 (20.85±5.8 %) The lowest percent cover occurred in March 2006 (1.55±0.53 %) (Fig. 16). Results of one-way ANOVA (95% confidence level) indicated monthly differences in percent cover of *S. oligocystum* per unit area were significant (Table 5). The percentage cover was not significantly correlated with water temperature and salinity.

The peak average of branch length was occurred in March 2006 (13.71±1.9 cm). The lowest average of branch length occurred in November 2006 (4.6±0.29 cm). Results of one-way ANOVA (95% confidence level) indicated monthly differences in average of branch length of *S. oligocystum* per unit area were significant (Table 5). The average of branch length was not significantly correlated with water temperature and salinity.

The peak of standing crop was occurred in January 2006 (12.52±2.94 g dw $0.25m^{-1}$). The lowest of standing crop occurred in March 2006 (0.37±0.27 g dw $0.25m^{-1}$) (Fig. 16). Results of one-way ANOVA (95% confidence level) indicated monthly differences in standing crop of *S. oligocystum* were significant (Table 5). The standing crop was not significantly correlated with water temperature and salinity.

The peak of percentage of fertile plants with receptacles was occurred in March 2006 (100±0.0 %). The lowest was occurred in January 2006 (40.0±12.65 %). Results of one-way ANOVA (95% confidence level) indicated monthly differences in standing crop of *S. oligocystum* were significant (Table 5). The percentage of fertile plants was not significantly correlated with water temperature and salinity.

These receptacles were examined by transverse sectioning to see the fertility of receptacles whether they were in young or mature stage. The mature plants occurred in January and March 2006 with some release eggs on the outside of the receptacles. In May and November 2006 the receptacles were small and found to be in young developing stage with small eggs. The receptacle was not found in January 2007 (Fig. 19).

3.4 Discussion

Two species of *Sargassum* (*S. aquifolium, and S. oligocystum*) were monitored the distribution, percentage cover, average branch length, standing crop and percentage of fertile plants with receptacles at Chon Buri from January 2006 to January 2007.

From statistical analysis of percent cover, thallus length, standing crop and percent fertility were significantly different with months ($P<0.05$), but were not significantly correlated with water temperature and salinity.

All the species of *Sargassum* collected from nine provinces along the Gulf of Thailand from 2005 to 2008 having the reproductive stage during the period of March to June and showed the highest peak in April in almost all the collection sites. Only some species were found also in November to February.

The reproductive period of *Sargassum* species in Thailand compared to other neighbouring countries. Wong and Phang (2004) reported two species of *Sargassum* from Cape Rachado, Malaysia. *S. baccularia* was found the reproductive period in July 1995 and April 1996, with very low percentage fertility (<1%), and that of *S. aquifolium* was fertile throughout the year. The reproductive period of *Sargassum* in the Philippines varied in various localities. Trono and Lluisma (1990) reported *S. oligocystum* at Bolinao, attained highest fertility in February 1988 and January 1989, whereas Calumpong *et. al.* (1999) reported the reproductive stage of the same species in Negro Island from March to April 1993. For *S. polycystum*, Largo *et. al.* (1994) reported the reproductive period at Cebu was in February to May 1989; and Calumpong *et. al.*(1999) reported in Negro Island from March to April 1993.

It can be concluded that the reproductive period of tropical *Sargassum* growing in Thailand, Malaysia and Philippines usually occurs during the period from January to May, only few species occur throughout the year.

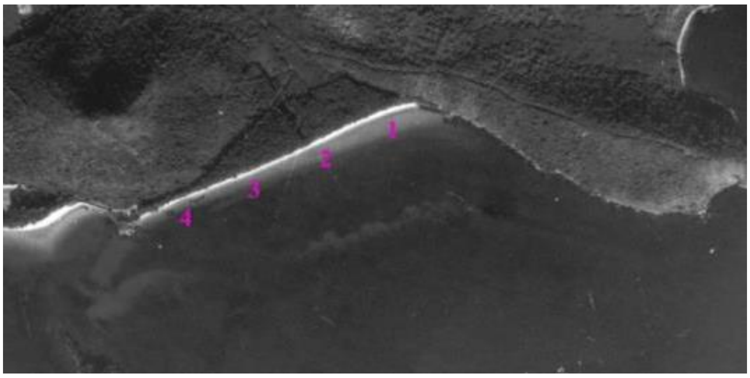


Fig. 13 The study site at Nang Rong Beach, Chon Buri Province showing the transect line positions.

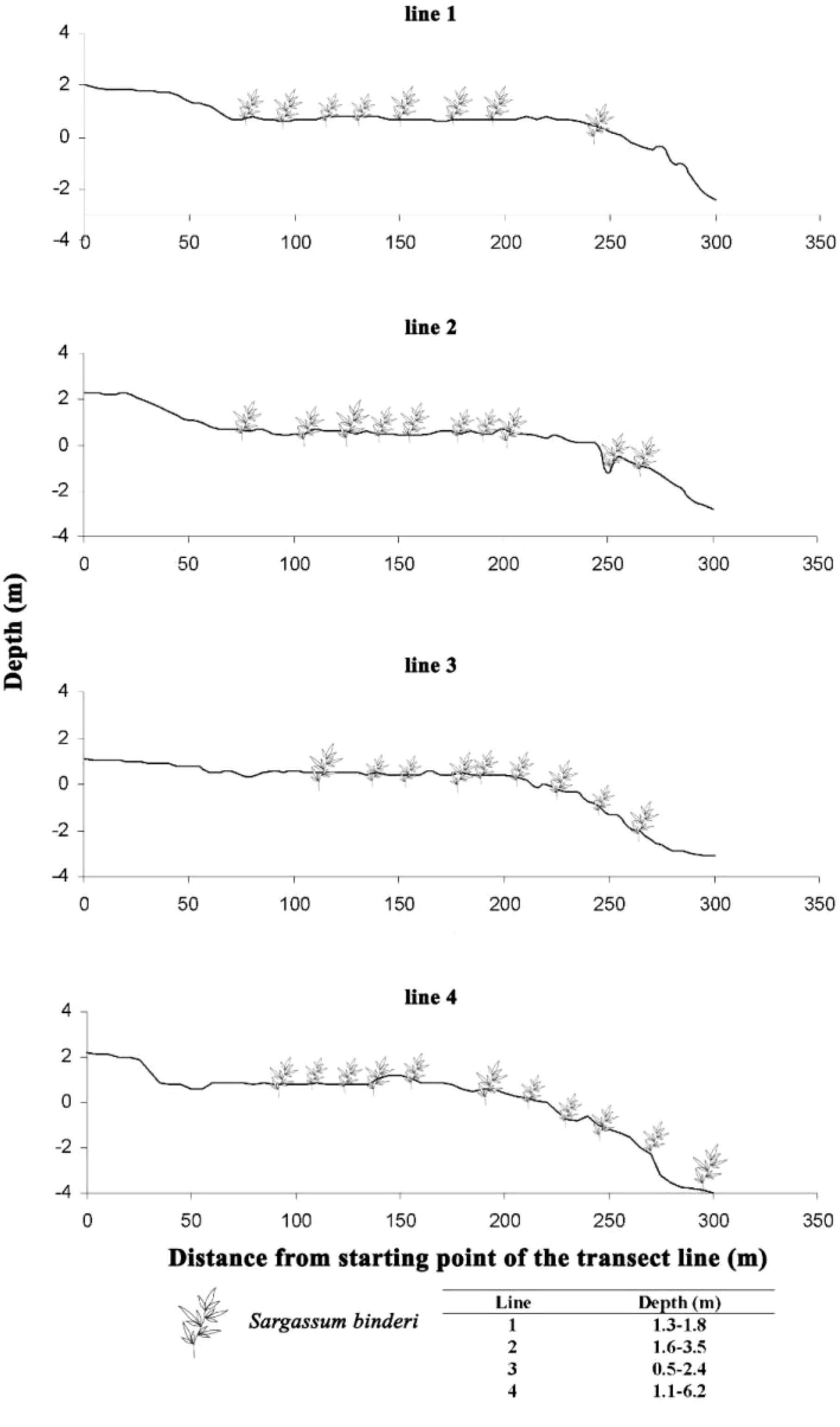


Fig. 14 Zonation and distribution of *Sargassum aquifolium* at Chon Buri Province.

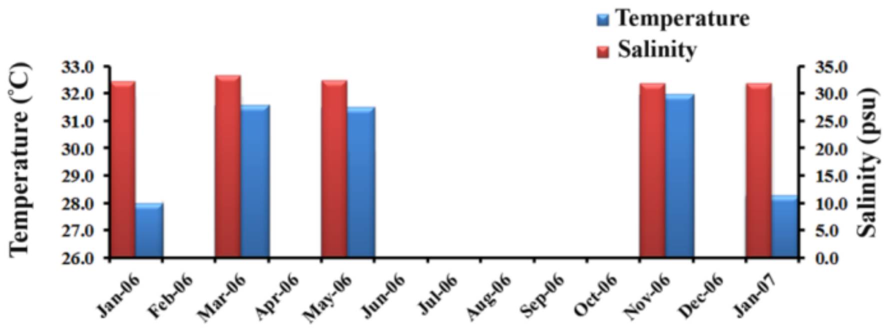


Fig. 15 Water temperatures and salinity at Nangrong beach, Chon Buri Province from January 2006 – January 2007.

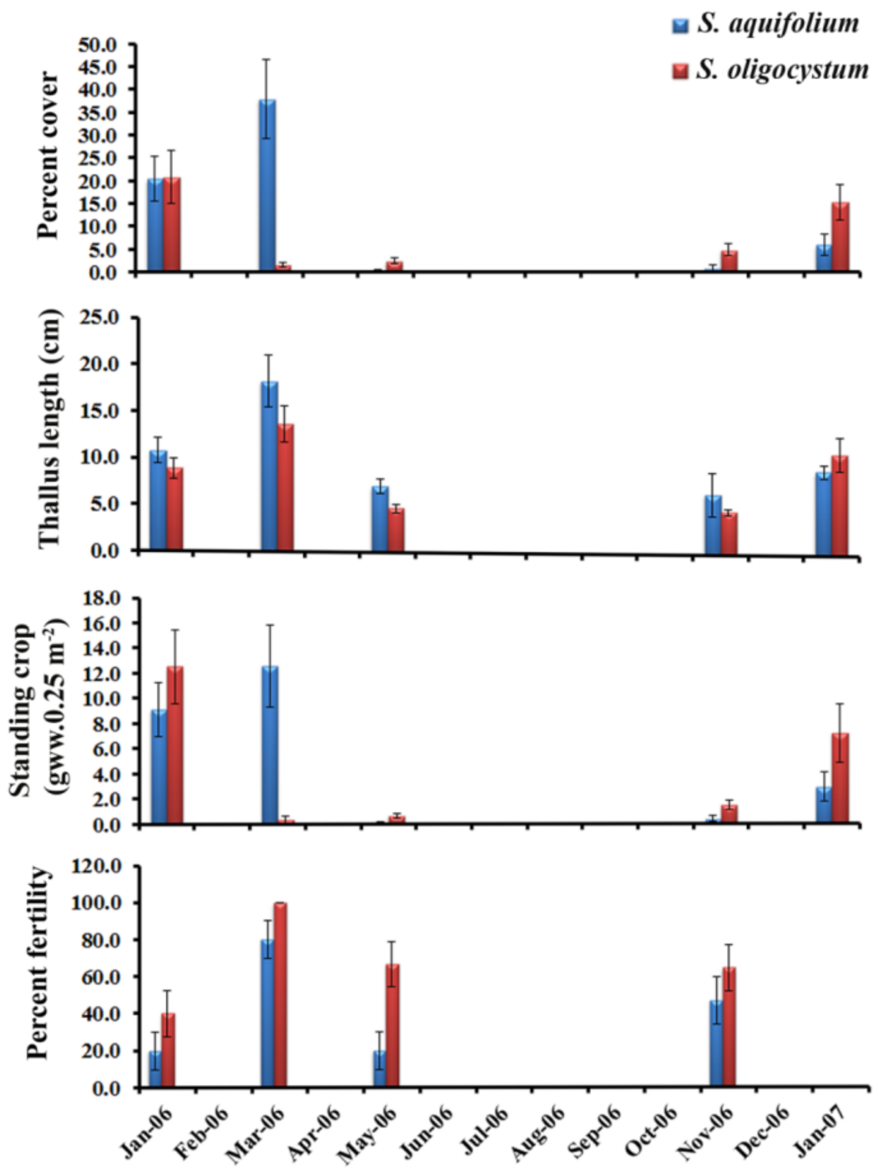


Fig. 16 Percent cover, thallus length, standing crop and percent fertility of *Sargassum aquifolium and S. oligocystum* (mean±standard error) at Nangrong Beach, Chon Buri Province from January 2006 – January 2007.

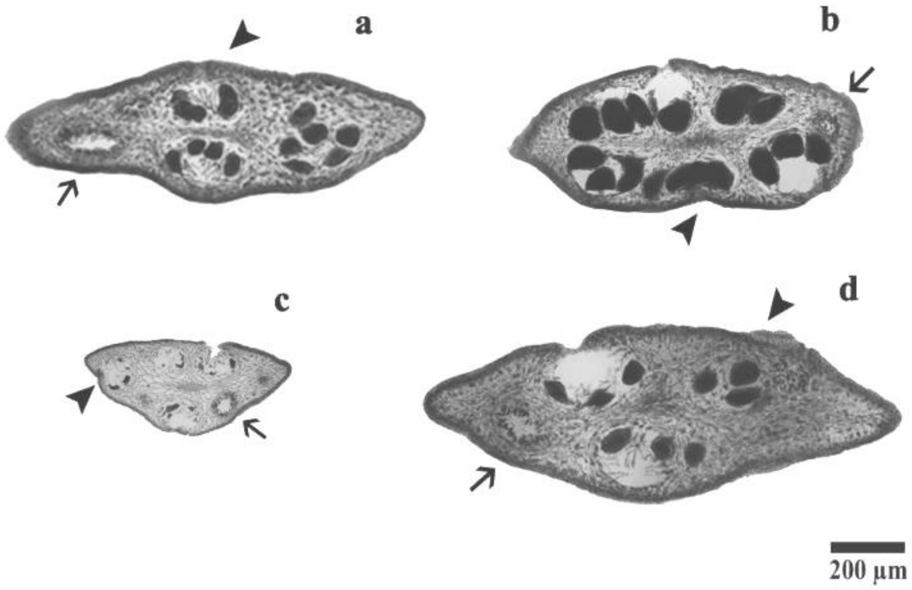
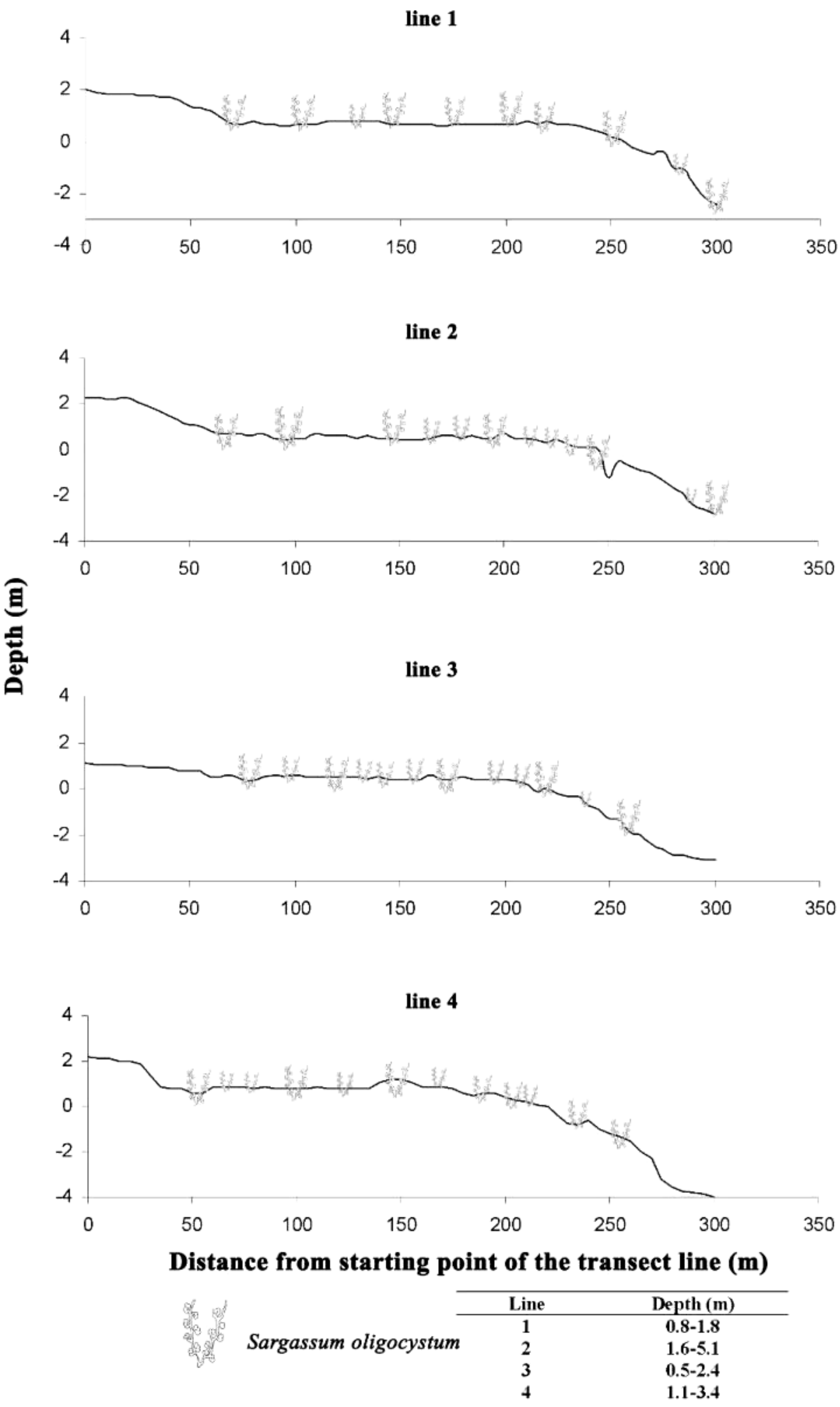


Fig. 17 Transverse sections of the receptacles *Sargassum aquifolium* at Nangrong beach, Chon Buri Province: a. Jan 2006, b. Mar 2006, c. May 2006, d. Nov 2006. (arrow = male conceptacle, arrowhead = female conceptacle)



Line	Depth (m)
1	0.8-1.8
2	1.6-5.1
3	0.5-2.4
4	1.1-3.4

Fig. 18 Zonation and distribution of *Sargassum oligocystum* at Chon Buri Province

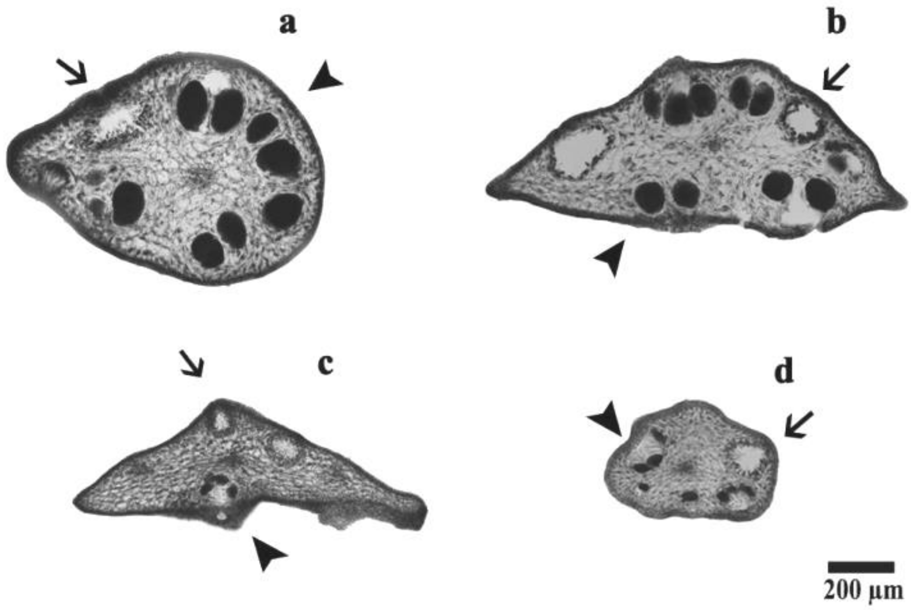


Fig. 19 Transverse sections of the receptacles *Sargassum oligocystum* at Nangrong beach, Chon Buri Province: a. Jan 2006, b. Mar 2006, c. May 2006, d. Nov 2006. (arrow = male conceptacle, arrowhead = female conceptacle)

Table 5 Results of ANOVA testing effects of month and *Sargassum species* on percent cover, thallus length, standing crop and percent fertility.

	Percent coverage of *S. aquifolium*				Percent coverage of *S.oligocystum*			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Month	4	2.652	7.067	.000	4	3.763	8.094	.000
Error	95	.375			95	.465		
	Thallus length of *S. aquifolium*				Thallus length of *S. oligocystum*			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Month	4	.600	7.958	.000	4	.544	13.973	.000
Error	70	.075			70	.039		
	Standing crop of *S. aquifolium*				Standing crop of *S. oligocystum*			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Month	4	1.384	3.249	.023	4	4.843	8.042	.000
Error	35	.426			56	.602		
	Percent fertility of *S. aquifolium*				Percent fertility of *S. oligocystum*			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Month	4	2.652	7.067	.000	4	3.763	8.094	.000
Error	95	.375			95	.465		

Chapter 4

Seasonal Changes of *Sargassum polycystum* in Saemaern Island in northeast of the Gulf of Thailand

4.1 Introduction

Genus**,** *Sargassum* C. Agardh (Sargassaceae, Phaeophyceae), is the largest genus of the brown algae which is the most ecologically abundant and economically important. *Sargassum* species are distributed in the tropical to temperate regions all over the world (Yoshida, 1983). *Sargassum* beds absorb CO_2 and produce O_2 through photosynthesis. Thus, they influence dissolved oxygen content in sea waters (Mikami *et al*. 2007) and consequently pH distributions through changing equilibrium of carbonate in sea waters by absorption of CO_2 (Komatsu, 1989; Komatsu and Kawai, 1989). They influence downward radiation from the sun through their canopy (Komatsu *et al*., 1990), and water temperature distributions inside the *Sargassum* forest (Komatsu *et al*., 1982; Komatsu, 1985, Komatsu *et al*., 1995). Their stipes and fronds buffer water motion inside the forest (Komatsu and Murakami, 1994). Many commercially important species spawn in *Sargassum* beds (e.g. sea urchins, abalones, cuttlefish); larvae and juveniles use the beds as nursery grounds. Detached *Sargassum* species from the substrates forms drifting seaweeds providing habitats for fishes and attached animals (Komatsu *et al*., 2007; Komatsu *et al*., 2008). Thus, *Sargassum* beds support biodiversity and are an important habitat for marine animals.

In Thailand, *Sargassum* species were recorded by Reinbold in "Flora of Koh Chang" from the specimens collected by Schmidt (1900) during the Danish Expedition to Siam. *S. polycystum* was reported from Koh Kahdat, Trat Province situated on the east coast of the north Gulf of Thailand for the first time. *S. polycystum* has distributed widely along the Gulf of Thailand (Lewmanomont, 1988; Noiraksar *et al*., 2006; Noiraksar and Ajisaka, 2008). *Sargassum polycystum* C. Agardh has secondary holdfasts that transformed from a stolon and heavily muricate on main branches (Chiang *et al*., 1992; Ajisaka *et al*., 1995, 1999) (Fig. 20). Normally recruitment of *S. polycystum* populations are mainly maintained by sexual reproduction. Nevertheless, recruits are produced by secondary holdfasts (Noiraksar and Ajisaka, 2008). Although there are some reports about distributions and ecology of *S. polycystum*, its ecology of Thailand hasn't been fully examined. To conserve *S. polycystum* in Thailand, it is necessary to understand its ecology. This study aims to elucidate growth and reproductive patterns in a natural habitat.

4.2 Materials and methods

4.2.1 Study site

Samaesarn Island, Chon Buri Province (12°31'21.37"N, 100°57'25.12"E) is a large intertidal flat, the substratum is composed of rock and dead coral with fine to coarse sand and a subtidal coral reef. This intertidal flat is exposed during low tide in the day time for about 4-6 hours during the month of April and May (Fig. 21). This island is the conservation area, and rich in marine algal flora. Four species of *Sargassum* such as *S. aquifolium*, *S. oligocystum*, *S. polycystum* and *S. swartzii*. Whereas *S. polycystum* is the most dominant species among bentic marine algae growing there such as *Turbinaria conoides*, *Padina australis*, *Padina santae-crucis*, *Lobophora asiatica*, *L. pachyventera*, *Amphiroa anceps*, *Bryopsis pennata*, *Gelidiella acerosa*, *Chondrophycus cartilagineus*, etc.

4.2.2 Quadrat sampling of seaweeds and measurements of environmental parameters

Three belt transect, 30 m apart parallel to the shore were set in the *Sargassum* bed (Fig. 2). Quadrats (0.5x0.5 m^2) were placed at 10 m intervals along each line. Thirty-six quadrats were monitored in each month for a period of 24 months from January 2014 to December 2015. We measured percentage cover of all quadrats and collected seaweeds on three quadrats per line in each month. Collected seaweeds were preserved in plastic bags with sodium chloride and brought back to the laboratory of the Institute of Marine Science, Burapha University. The samples were rinsed in fresh water and cleaned of sand and shells. Plant density such as juvenile plant, immature plant, mature plant and holdfast were counted. Thus, plant density included juvenile plants, immature plants, mature plants and holdfasts of *S. polycystum* per 0.5 x 0.5 m A stipe length of individual *S. polycystum* was measured. Epiphytic plants and aquatic animals attached to an individual was removed before wet weight of *Sargassum* was weighed prior to drying in a hot air oven (TS8000 Termaks, Bergen, Norway) at 60°C for 48 h. Dry weight of each sample was then obtained (CP3202S, Sartorius, Goettingen, Germany) and used for calculating standing crop, which is expressed as the dry weight (g) per unit area.

Three environmental parameters such as water temperature, salinity and DO were recorded at the time of sample collection with a portable multiparameter measuring instrument (YSI 556 MPS, Ohio, USA). Water current was measured by current meters (Valeport Model-105, Valeport Limited, UK) deployed at study site. Water sample were collected for the analysis of nutrients: phosphate, nitrate and silicate, and using spectrophotometer (HACH DR 2500, Colorado, USA). Nitrate was measured using cadmium reduction and diazotization method. Phosphate was analyzed by ascorbic acid method. Silicate was analyzed by molybdosilicate method (Strickland and Parsons, 1972)

4.2.3 Statistical analysis

A two-way ANOVA was applied to examine differences among characteristics of *S. polucystum* by month and year. Before the ANOVA, standing crop, plant density and thallus length per unit area were transformed using logarithm transformation, while percent cover, percentage of juvenile plant, percentage of immature plants, percentage of mature plants and percentage of holdfasts were transformed using arcsine transformations. We examined relationships among percent cover, thallus length, standing crop, plant density, percentage of juvenile plants, percentage of immature plants, percentage of mature plants and percentage of holdfasts of *S. polycystum* per unit area with the eight environmental parameters using Spearman's rank correlation analysis.

4.3 Results

4.3.1 Seasonal growth pattern of *Sargassum polycystum*

S. polysystum was monthly found throughout the year for a period of two years. The maximum percent cover of *S. polysystum* per unit area was obtained in January 2014 (66.30 ± 5.44%) and January 2015 (69 ± 7.95 %) during the northeast monsoon season from November to February corresponding to the dry season of winter months in the east coast of Gulf of Thailand. The minimum percent cover of *S. polysystum* per unit area occurred in June 2014 (4.25 ± 1.43%) and July 2015 (4.50 ± 0.75%) during the southwest monsoon season from May to September corresponding to the rainy season of summer months in the east coast of Gulf of Thailand (Fig. 22). Results of two-way ANOVA (95% confidence level) indicated monthly differences in percent cover of *S. polycystum* per unit area were significant although its interaction of year and months was significant (Table 6).

The maximum thallus length was obtained in December 2014 (17.95 ± 3.24 cm) and February 2015 (17.99 ± 2.78 cm) during the dry season. The minimum thallus length occurred in July 2014 (1.57 ± 0.40 cm) and July 2015 (1.06 ± 1.06 cm) during the rainy season (Fig. 22). Results of two-way ANOVA indicated difference in monthly thallus length of *S. polycystum* was significant although its interaction of year and months was significant (Table 6).

The maximum standing crop was obtained in January 2014 (58.23 ± 13.41 g dw. 0.25 m^{-2}) and February 2015 (63.15 ± 9.25 g dw. 0.25 m^{-2}) during the dry season. The minimum standing crop was in July 2014 (3.59 ± 0.95 g dw. 0.25 m^{-2}) and July 2015 (5.21 ± 1.09 g dw. 0.25 m^{-2}) during the rainy season (Fig. 22). Results of two-way ANOVA indicated difference in monthly standing crop of *S. polycystum* was significant although its interaction of year and months was significant (Table 6).

The maximum plant density (included juvenile plants, immature plants, mature plants and only holdfasts) was obtained in August 2014 (533.22 ± 148.93 no.0.25 m^{-2}) and August 2015 (387.00 ± 78.29 no.0.25 m^{-2}) during the rainy season. The minimum plant density occurred in February 2014 (102.22 ± 35.94 no.0.25 m^{-2}) during the dry season and June 2015 (108.78 ± 21.90 no.0.25 m^{-2}) during the rainy season (Fig. 22). Results of two-way ANOVA indicated difference in monthly plant density of *S. polycystum* was significant although its interaction of year and months was significant (Table 1).

The maximum percentage of juvenile plants was obtained in August 2014 (62.87 ± 15.74%) and July 2015 (86.29 ± 2.85 %) during the rainy season. The minimum percentage of juvenile plant occurred in February 2014 (3.39 ± 1.35%) and February 2015 (15.30 ± 4.65%) during the dry season (Fig. 23). Results of two-way ANOVA indicated difference in monthly percentage of juvenile plants of *S. polycystum* was significant (Table 6).

The maximum percentage of immature plants was obtained in January 2014 (87.94 ± 2.16%) and February 2015 (61.09 ± 5.36 %) during the dry season. The minimum percentage of immature plant occurred in August 2014 (3.22 ± 1.09%) and July 2015 (0.46 ± 0.46%) during the rainy season (Fig. 23). Results of two-way ANOVA indicated difference in monthly percentage of immature plants of *S. polycystum* was significant although its interaction of year and months was significant (Table 6).

The maximum percentage of mature plants was obtained in February 2014 (13.59 ± 4.97%) and January 2015 (13.44 ± 6.12 %) during the dry season (Fig. 23). There was a low percentage of mature plant between March and November every year. Results of two-way ANOVA, indicated difference in monthly percentage of mature plants of *S. polycystum* was significant (Table 6).

The maximum percentage of holdfasts was obtained in March 2014 (23.36 ± 7.45%) during the first inter-monsoon and December 2015 (24.48 ± 10.02 %) during the dry season. There was a low percentage of holdfasts during the rainy season (Fig. 23). Results of two-way ANOVA, indicated differences between year and among month were significant although its interaction of year and months was significant (Table 6).

4.3.2 Relationships between the features of plant and environmental variables

The monthly average measurements of environmental parameters were shown in Fig. 24. Water temperature was the highest in May 2014 (31.2°C) and May 2015 (31.0°C) during the rainy season and was the lowest in December 2014 (27.4 $^{\circ}$C) and December 2015 (27.2 $^{\circ}$C) during the dry season. Salinity was the highest in October 2014 (35.1) during the second inter-monsoon and July 2015 (35.3) during the rainy season and was the lowest in November 2014 (32.0) and January 2015 (30.4) during the dry season. DO was the highest in December 2014 (7.3 mg l^{-1}) and February 2015 (8.21 mg l^{-1}) during the dry season and was the lowest in July 2014 (4.22 mg l^{-1}) and July 2015 (4.13 mg l^{-1}) during the rainy season. Phosphate was the highest in October 2014 (0.12 mg l^{-1}) during the second inter-monsoon season and July 2015 (0.21 mg l^{-1}) during the rainy season and was the lowest in March 2014 (0.02 mg l^{-1}) during the first inter-monsoon season and May 2015 (0.03 mg l^{-1}) during the rainy season. Nitrate was the highest in July 2014 (1.15 mg l^{-1}) and July 2015 (1.53 mg l^{-1}) during the rainy season and was the lowest in April 2014 (0.73 mg l^{-1}) during the first inter-monsoon season and August 2015 (0.6 mg l^{-1}) during the rainy season. Silicate was the highest in June 2014 (4.73 mg l^{-1}) and July 2015 (5.53 mg l^{-1}) during the rainy season and was the lowest in January 2014 (1 mg l^{-1}) and December 2015 (1.1 mg l^{-1}) during the dry season. In general, silicate and phosphate were increased during the rainy season except December 2014 for phosphate. Water current was the highest in July 2014 (38.3 cm s^{-1}) and September 2015 (33.1 cm s^{-1}) during the rainy season and was the lowest in March 2014 (14.2 cm s^{-1}) during the first inter-monsoon season and July 2015 (10.8 cm s^{-1}) during the rainy season.

A spearman's rank-order correlation was run to determine the relationship between the features of *S. polycystum* and environmental variables (Table 7). There was a significant negative correlation between temperature, percent cover (r_s = 0.569, p = 0.004), thallus length (r_s = 0.630, p = 0.001), standing crop (r_s = 0.583, p = 0.003), percentage of immature plants (r_s = 0.469, p = 0.021) and percentage of mature plants (r_s = 0.496, p = 0.014), while it was positively correlated with percentage of juvenile plants (r_s = 0.496, p = 0.014). Negative correlation was significant between salinity and percent cover (r_s = 0.424, p = 0.039), standing crop (r_s = 0.463, p = 0.023), percentage of immature plants (r_s = 0.522, p = 0.009) or percentage of mature plants (r_s = 0.429, p = 0.036. There were significant positive correlations between DO and percent cover (r_s = 0.539, p = 0.007), thallus length (r_s = 0.602, p = 0.002), standing crop (r_s = 0.504, p = 0.012), percentage of immature plants (r_s = 0.431, p = 0.035) or percentage of mature plants (r_s = 0.518, p = 0.009), while there was a negative correlation between DO and percentage of juvenile plants (r_s = 0.470, p = 0.020). There were significant positive correlations between phosphate and plant density (r_s = 0.416, p = 0.043) or percentage of juvenile plants (r_s = 0.482, p = 0.017). There was a significant negative correlation between current speed with percentage of holdfast*s* *(r_s = 0.790, p = 0.002).*

4.4 Discussion

Populations of *S. polycystum* existed throughout the year in Samaesarn Island, Chon Buri Province, included in the Gulf of Thailand as in the Philippines (Trono and Lluisma, 1990; Calumpong *et. al*., 1999), Malaysia (May-Lin and Ching-Lee, 2013) and India (Rao, 2002; Padal *et al.*, 2014). This means that *S. polycystum* regenerates new shoots from a persistent rhizoidal holdfast and *S. polycystum* is a perennial species. Many young *S. polycystum* consisting of juvenile and immature plants constituted population.

Plant density, percentage of juvenile plants were the maximum during the rainy season from May to September. The maximum plant density (included juvenile plant, immature plant, mature plant and holdfast) was obtained in the rainy months (August 2014 and 2015). This is explained by recruitment of juvenile plants spawn in January to February during the dry season.

The growth and reproductive period of *S. polycystum* in Thailand compared to other neighbouring countries. In the Philippines; Trono and Lluisma (1990) reported the biomass of *Sargassum* populations at Santiago Island, Bolinao. *S. polycystum* attained highest in December (447 g wet wt m^{-2}) and highest fertility in January or February, before the sudden reduction in standing crop; Trono and Tolentino (1993) studied on the management of *Saragssum* bed in Bolinao. The maximum standing crop was obtained in October to December or January for both intertidal and subtidal parts of the bed, and the reproductive period during the cold months of November to January; Largo *et. al.* (1994) reported the seasonal changes in the growth and reproduction of S*argassum* species from Liloan, Cebu. The maximum thallus length of *S. polycystum* was obtained in January (30.0±11.4 cm), and the maximum biomass was recorded in July, and the reproductive period was produced from February to May and again, from December to January; Ohno *et. al*. (1995) reported *S. polycystum* at Danajon Reef, the Central Visayas area had a mean standing crop of 4.3 kg m^{-2} with the maximum one of 9.6 kg m^{-2} in December. Calumpong *et. al.* (1999) reported the maximum standing crop and percent cover of *S. polycystum* in Negro Island were in May (11.3±0.5 g m^{-2}, 10-15%) and lowest in October (3.6±0.2 g m^{-2}), and the reproductive stage was found from March to May. In Taiwan; Hwang *et. al*. (2004) reported *S. polycystum* on coral reef of Nanwan Bay had the maximum percent cover in late November through June, and biomass in April-May and January-April. In Malaysia; MAY-LIN and Ching-Lee (2013) studied *S. polycystum* at Teluk Kemang, Port Dickson, and reported the pattern of mean thallus length (MTL) in September 2009 (MTL = 76.43 mm, largest length class within 200-299 mm); February 2010 (MTL = 127 mm, largest length class within 500-599 mm) and July 2010 that was highest (MTL = 228 mm, largest length class within 800-899 mm). The maximum fertilities were in October 2009 (13%); February 2010 (14%) and August 2010 (17%). In India; Rao (2002) reported seasonal the growth and reproductive at Visakhapatnam coast, attained highest mean length were in December 1995 (15.9 cm); December 1995 (16.9 cm) and October 1996 (7.1 cm).

The plants bearing receptacles were found in November 1994, all plants possessed receptacles in December 1994 and the percentage of reproductive decreased in February 1995. Padal *et al.* (2014) reported the same species and same area, and also agrees with the results of Rao (2002). It can be concluded that the reproductive period of tropical *S. polycystum* growing in Thailand, Philippines, Taiwan, Malaysia and India usually occurs during the period from November to May. Typical growth cycle of *Sargassum* species is characterized by presence of a slow growth phase, a rapid growth phase, and a reproductive phase that is followed by senescence and dieback (Ang, 2006). In the present study, we can summarize phenology of *S. polycystum* to three periods of growth, reproduction and degeneration in a year as indicated in Table 3.

Percent cover, thallus length, standing crop, percentage of immature plants and percentage of mature plants of *S. polycystum* were the maximum during the dry season from November to February. They showed a significant negative correlation with water temperature ($P<0.05$). Water temperature is one of the most critical factors in affecting the phenological patterns of *Sargassum* species (Ang, 2006). High water temperature decreases growth of *S. polycsytum* and low water temperature promotes maturation and reproduction in the Philippines, India and Taiwan (Largo *et. al*., 1994; Rao, 2002; Hwang *et. al*., 2004). This is also true for the case of *S. polycystum* in Samaesarn Island in the north Gulf of Thailand because water temperature is high in the rainy season from May to September and low in the dry season from November to February. After maturation in January to February, juvenile plants spawn in the dry season and also regenerated juvenile plants from holdfasts are recruited to the *Sargassum* forest in the rainy season. Thus, the total number of individuals shows the maximum several months after February corresponding to the months during the rainy season and a positive correlation to the water temperature.

Percentage of holdfasts was low in the rainy season and showed a significant and negative correlation with current speed ($P<0.05$). In general, main branches and stipes of *Sargassum* species are damaged by strong waves in the monsoon season and remained only holdfasts. The *Sargassum* plants were damaged by the strong water motion (Largo *et. al*., 1994). The study area is affected by the southwest monsoon around May-October. In northeast of Gulf of Thailand, southwest monsoon produces greater waves with fetch longer than in northeast monsoon season. Therefore, the onset of southeast monsoon removes large thallus after the luxuriant growth in February. DO is also influenced by stratification of surface layer. Since southwest monsoon brings rain and warm water in northeast Gulf of Thailand, it is possible that the stratification is strengthened and eventually DO is decreased. Therefore, DO was higher in the dry season and lower in the rainy season. This phenomenon coincides with higher percent cover, thallus length, standing crop, percentage of immature plants and percentage of mature plants of *S. polycystum* during the dry season from November to February. Therefore, they are apparently correlated to DO. Positive relations between DO, and percent cover, thallus length, standing crop, percentage of immature plant or percentage of mature plant of *S. binderi* were reported in Malaysia (Yeong and Wong, 2012).

For reproduction of *S. polycystum*, thallus need to become longest in a year. Longer the thallus length is, stronger the drag force posed by waves is (Xu and Komatsu, in press). Therefore, maturation period must be under a calm condition. In the study area, the northeast monsoon season from November to February and the first inter-monsoon season from March to April are calm sea condition. Since the northeast monsoon season is dry season, the solar radiation is sufficient for photosynthesis of *S. polycystum* to acquire energy to prepare reproduction with elongate thallus.

Phosphate and nitrate might be increased with increase in discharge from the river to the Gulf of Thailand during the rainy season in east Gulf of Thailand. Hwang *et al*. (2004) stated that phosphate limits growth of *Sargassum* germlings. This hypothesis can be applied to a positive relation between phosphate, and plant density or percentage of juvenile plants. However, it is not corresponding to reports from the Philippines, India and Malaysia (Trono and Lluisma, 1990; Rao, 2002; May-Lin and Ching-Lee, 2013).

Present study shows the variations of environmental factors and growth patterns of *S. polycystum* from the eastern cost of Thailand. In the northeast Gulf of Thailand, monsoon drives environmental variables and also seasonal variations of growth and reproduction of *S. polycyctum*, which are also influenced by the environmental variables. Reproduction of *S.*

polycystum occurs under the calm condition during the dry season. In this way, seasonal growth and reproduction are controlled by the monsoon in the northeast Gulf of Thailand.

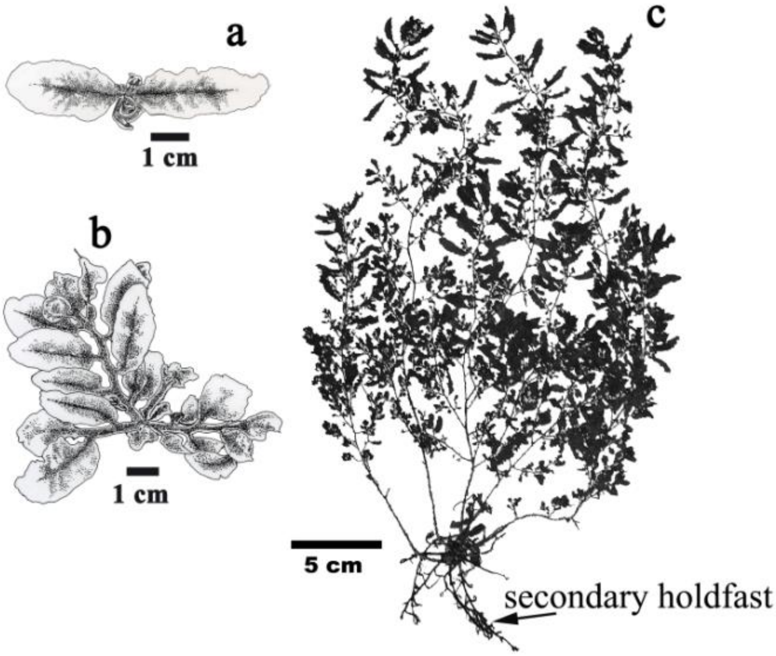


Fig. 20 Thallus stages of *Sargassum polycystum*. **a** juvenile plant, **b** immature plant, and **c** mature plant.

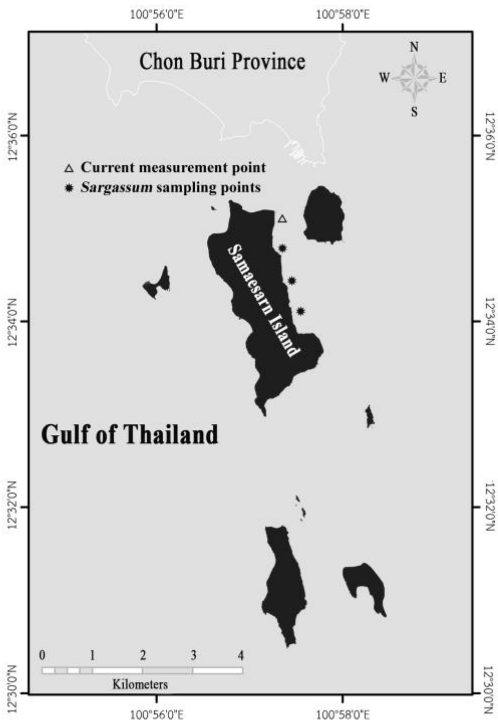


Fig. 21 The study site, on the eastern coast of Samaesarn Island, Chon Buri Province.

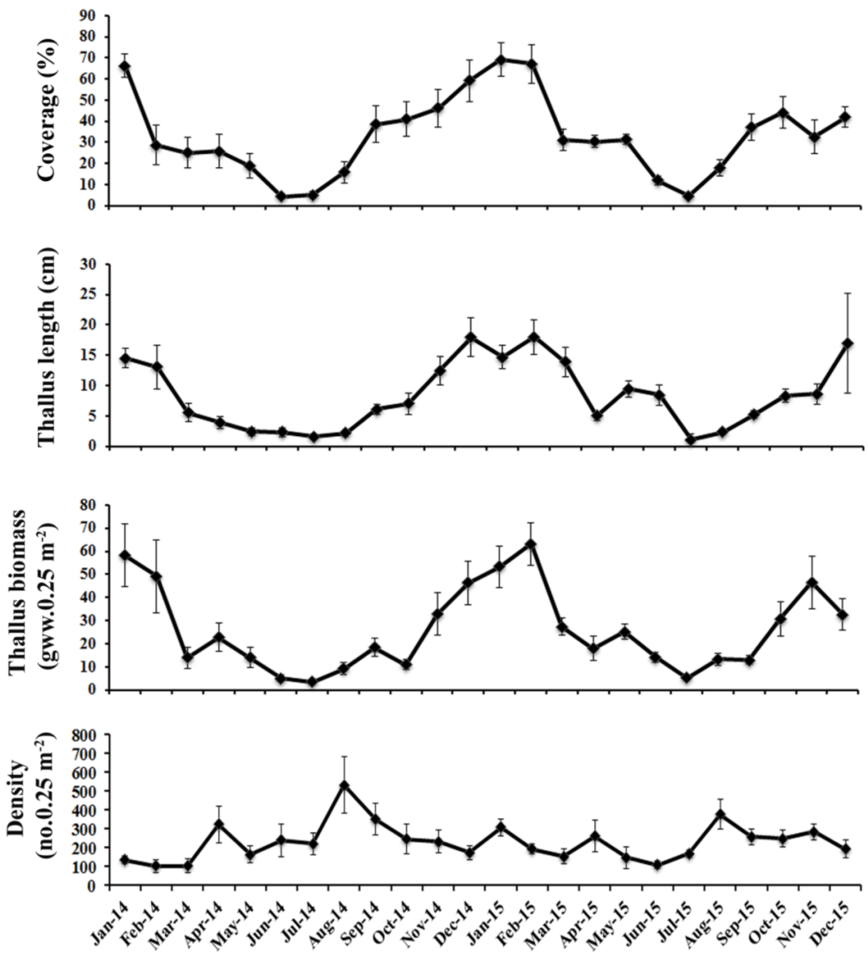


Fig. 22 Percentage cover, thallus length, thallus biomass and density of *Sargassum polycystum* (mean±standard error) at Samaesarn Island, Chon Buri Province from January 2014-December 2015.

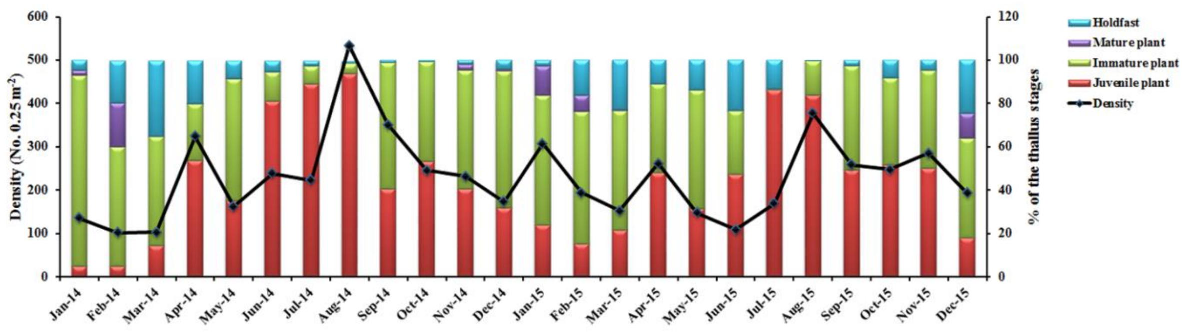


Fig. 23 Percentage plant stages and density of *Sargassum polycystum* at Samaesarn Island, Chon Buri Province from January 2014-December 2015

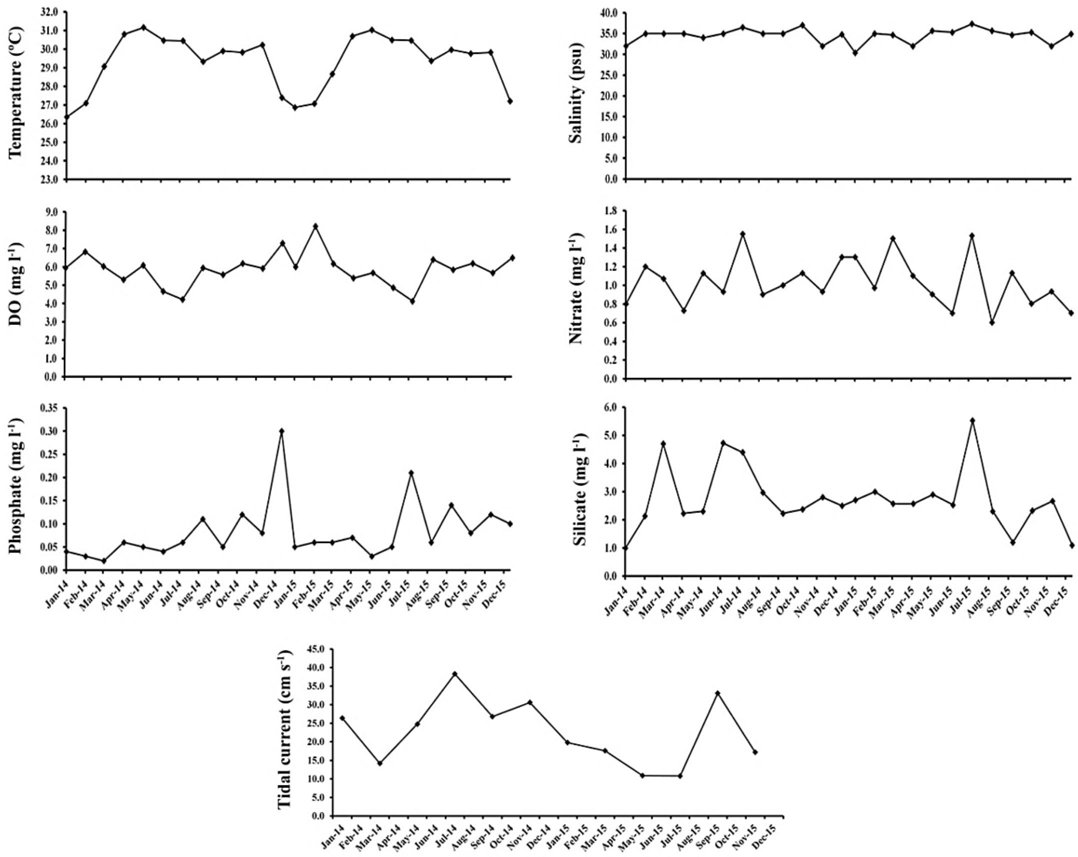


Fig. 24 The environmental parameters of seawater on the eastern coast of Samaesarn Island from January 2014-December 2015.

Table 6 Results of ANOVA testing effects of year and month on thallus coverage, thallus length, thallus biomass, thallus density, percentage of juvenile plant, percentage of immature plant, percentage of mature plant and percentage of hold fast of *Sargassum polycystum*.

Source of variation	Coverage				Length			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Year (Y)	1	0.099	1.906	0.169	1	0.188	4.206	0.042
Month (M)	11	0.815	15.667	0.000	11	0.818	18.268	0.000
YXM	11	0.141	2.707	0.003	11	0.182	4.056	0.000
Error term	192	.052			163	0.045		
	Biomass				**Density**			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Year (Y)	1	0.012	0.138	0.711	1	0.443	2.844	0.04
Month (M)	11	1.415	16.315	0.000	11	0.417	2.740	0.003
YXM	11	0.221	2.551	0.005	11	0.415	2.725	0.003
Error term	171	0.087			171	0.152		
	Juvenile plant				**Immature plant**			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Year (Y)	1	0.498	6.618	0.011	1	0.045	0.954	0.330
Month (M)	11	1.373	18.257	0.000	11	1.004	21.160	0.000
YXM	11	0.085	1.133	0.338	11	0.224	4.725	0.000
Error term	192	0.075			192	0.047		
	Mature plant				**Holdfast**			
	df	MS	*F*	*p*	df	MS	*F*	*p*
Year (Y)	1	0.015	1.058	0.305	1	0.195	15.641	0.000
Month (M)	11	0.031	2.179	0.017	11	0.086	6.887	0.000
YXM	11	0.018	1.248	0.258	11	0.031	2.520	0.005
Error term	192	0.014			192	0.012		

Table 7 Significant of Spearman's rank correlation between analysis between environmental parameters and the features of *Sargassum* plant.

S. polycystum	**Temperature (°C)**		**Salinity (psu)**		**DO (mg l^{-1})**		**Phosphate (mg l^{-1})**		**Water current (cm s^{-1})**	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
Coverage	-0.569	0.004**	-0.476	0.019*	0.539	0.007**	0.185	0.387	0.315	0.319
Length	-0.630	0.001**	-0.427	0.038*	0.602	0.002**	-0.048	0.823	-0.028	0.931
Biomass	-0.583	0.003**	-0.537	0.007**	0.504	0.012*	-0.103	0.631	-0.119	0.713
Density	0.091	0.671	-0.095	0.658	-0.144	0.501	0.416	0.043*	0.392	0.208
Juvenile plant	0.444	0.030*	0.436	0.033*	-0.470	0.020*	0.482	0.017*	0.126	0.697
Immature plant	-0.469	0.021*	-0.538	0.007**	0.431	0.035*	0.014	0.948	0.224	0.484
Mature plant	-0.496	0.014*	-0.431	0.036*	0.518	0.009**	-0.200	0.348	0.037	0.908
Holdfast	0.015	0.944	-0.011	0.959	0.074	0.731	-0.232	0.274	-0.790	0.002**

** $p = 0.01$
* $p = 0.05$

Table 8 Periods of *S. polycystum* growth, reproduction and degeneration phases at Samaesarn Island from January 2014 to December 2015

Plant stages	**Timing patterns**
Growth 1	January 2014
Reproduction 1	January-February 2014
Degeneration 1	February-July 2014
Growth 2	August 2014-February 2015
Reproduction 2	November 2014-Februaty 2015
Degeneration 2	March-July 2015
Growth 3	August-December 2015
Reproduction 3	December 2015
Degeneration 3	December 2015

Chapter 5

Life history of *Sargassum oligocystum*

5.1 Introduction

The genus, *Sargassum* C. Agardh, is one of the largest genus of the brown algae and is the most important seaweeds in ecology and economy. The plants of *Sargassum* distribute all over the world, especially in tropical to temperate regions (Yoshida, 1983). *Sargassum* beds give an influence on the dissolved oxygen content in sea water through photosynthesis (Muraoka, 2004; Mikami *et al*., 2007) and consequently pH value by CO_2 absorption (Komatsu and Kawai, 1986). They support biodiversity and habitat for the marine organisms (Komatsu *et al*., 1982; Komatsu, 1985, Komatsu *et al*., 1990; Komatsu and Murakami, 1994; Komatsu *et al*., 1995; Komatsu *et al*., 2007; Komatsu *et al*., 2008). *Sargassum* species comprise bioactive compounds such as vitamins, carotenoids, dietary fibers, proteins, and minerals, and biologically active compounds, like terpenoids, flavonoids, sterols, sulfated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol and pheophytine (e.g. Lucas and Southgate, 2012). *Sargassum* species are used as human food, especially by the people living in coastal areas (e.g. Kirimura, 2007). There are many reports on the bioactive substances extracted from seaweeds, like antibacterial, antifungal, antiviral, anti-inflammatory, anti-diabetic, antioxidant and cytotoxic substances (Zandi *et al*., 2010; Tajbakhsh *et al*., 2011; Yendo *et al*., 2014; Mehdinezhad *et al*., 2015). *Sargassum* species play also an effective bio-absorption role to remove not only nutrients (e.g. Fei, 2004) but also heavy metals such as cadmium ions (Cd^{2+}), copper ions (Cu^{2+}), and mercuric ion (Hg^{2+}) dissolving in seawater. Therefore, this function of *Sargassum* species is focused from environmental and economic viewpoints (Ramavandi *et al*., 2015; Delshab *et al*., 2016).

There were many reports about early development stages of *Sargassum* species such as in *S. micracanthum* and *S. ringgoldianum* (Ogawa, 1974); *S. muticum* (Norton, 1977; Hales and Fletcher, 1989; Uchida *et. al*., 1991; Kerrison and Le, 2016); *S. horneri* (Nanba, 1993; Uchida, 1993;Yoshida *et. al*., 1995, Yoshida *et. al*., 1999, Choi *et. al*., 2008); *S. filicinum* (Yoshida *et. al*., 1999); *S. confusum* (Kawagoe *et. al*., 2005); *S. vachellianum* (Yan and Zhang, 2013) ; *S. thunbergii* (Ziguo *et. al*., 2008; Yongzheng *et. al*., 2015); *S. echinocarpum* (Hamza *et. al*., 2016); *S. swartzii* (Kavale and Veeragurunathan, 2016). An addition to development the techniques for artificial seed production such as in *S. fulvellum* (Hwang *et.*

al., 2006, 2007); *S. thunbergii* (Zhang *et. al*., 2012). However, there is no report on embryo release and early development of *S. oligocystum*.

There were many researches about fertilizers were applied in seaweed cultivation (Amano and Noda, 1987, Brault and Quéguiner, 1989; Phillips and Hurd, 2003; Tyler *et.al*., 2005; Mansilla *et.al*., 2007; Kim and Yarish, 2014; Miki *et al*., 2016). Urea is an organic compound with the chemical formula of $CO(NH_2)_2$ produced as an important raw material by chemical industries. Dissolved in water, it is neither acidic nor alkaline, practically non-toxic, and widely used in fertilizers as a source of nitrogen. Urea has the highest nitrogen content of all solid nitrogenous fertilizers in common use. Therefore, a cost of its use is very low. The standard crop-nutrient rating (NPK rating) of urea is 46-0-0. Urea is also used in many multi-component solid fertilizer formulations (Wikipedia, 2016). However, effect of urea on growth of *S. oligocystum* is unknow

Development of seaweed cultivation techniques has led researchers to publish numerous reports on early development of seaweeds. On the other hand, no detail studies on Thai *Sargasum* species have appeared until now. The objective of the present study is to present early development of *S. oligocystum* from mature thalli to juvenile thalli through embryo release with use of culture in laboratory and quantify growth rates of juvenile thalli cultured in outdoor tanks filled with seawater and seawater mixed with urea.

5.2 Materials and methods

5.2.1 Embryo culture in laboratory

The mature plants of *S. oligocystum* were collected on March-April 2014 (Fig. 25), in an intertidal zone of Samaesarn Island, Chon Buri Province, Thailand (12°31'21.37"N, 100°57'25.12"E). The plants were cleaned to eliminate surface epiphytes and rinsed thoroughly with sterilized seawater. We examined whether receptacles had released germlings attached on their surface. We removed germlings from the receptacles by brush and rinsed several times with sterile seawater when recptacles had germlings. Plant Nutrition+ liquid was used as a culture medium (Tropica, AQUACARE). The culture medium was renewed once a week. Culture conditions were as follows: a salinity of 30, a water temperature of 25 ºC, photosynthetic active radiation (PAR) of 85 μmol photons $m^{-2}s^{-1}$ with use of cool daylight fluorescent tubes (Philips, TL-D 18W/54-765 1SL, Thailand) for a12:12 h (L:D) (Fig. 26a, b). PAR was measured with a light meter (LI-250A LI-COR®, USA). We observed growth and development of germling from embryo stage to juvenile thalli stage for 90 d. The juvenile thalli on 90^{th} day were used for outdoor tank culture.

5.2.2 Juvenile thalli growth experiments in outdoor tank culture

Three-month-old juvenile thalli of *S. oligocystum* were reared in an outdoor tank of 500 L made from fiberglass under a roof with translucent plastic windows (Fig. 26c). Three hundred juvenile thalli were cultured in a tank filled with seawater or seawater in which urea fertilizer of 4 g ton^{-1} was dissolved (hereafter, we call it as seawater mixed with urea for simplicity) (Fig. 26d). The culture mediums were renewed once a week. Two replicates were used for each treatment. At intervals of 7 d during 35 d of cultivation, fifteen young thalli were randomly selected to measure a diameter of juvenile thalli under each treatment for examination of their growth (Fig. 26e). Eight environmental parameters such as water temperature and PAR in a tank under an outside condition were recorded by data loggers (HOBO Pendant UA-002-64, USA). pH was recorded by pH meter (Metter Toledo pH Five Go, Switzerland). Salinity was measured with a hand refractometer (ATAGO 508 IIW, Japan).

5.2.3 Growth rate and data analyses

Growth rate was estimated from increase in a diameter of thallus. A specific growth rate of *S. oligocystum* was obtained with the formula proposed by Luhan and Sollesta (2010):

$$SGR = \frac{100(\ln Wt - \ln W0)}{t}, \quad (1)$$

where SGR, t, W_0 and W_t are specific growth rate, time in day after the start of outdoor culture, an initial diameter of thallus (mm) on the first day of culture and a diameter of thallus (mm) at t. The first day t and a diameter W_t on the first day of each week were set as 0 and w_0 because measurements were conducted at intervals of 7 d for 35 d. We examined differences in specific growth rates of *S. oligocystum* per week between those cultured in seawater or urea dissolution seawater by measuring with the eight environmental parameters.

5.3 Results

5.3.1 Field observation and embryo culture in a laboratory

Receptacle formation of *S. oligocystum* was observed from February-June around Samaesarn Island, Chon Buri Province, Thailand. Fertilized eggs released from receptacles were attached on the surface of receptacles. After verifying start of germination, zygotes were isolated to containers filled with culture medium. A germling continuously divided transversely into a large and a small cell. The latter gradually developed into rhizoids after several divisions. An end where the rhizoids grew out became a base of the germling, and the other became an apex in 1 d (Fig. 27a). Embryos produced many rhizoids in 3 d (Fig. 27b). Germlings developed the first cauline leaf in 7 d (Fig. 3c) and four cauline leaves in 30 d (Fig. 27d). They became juvenile thalli producing spatulate to lanceolate cauline leaves in 60 d (Fig. 27e) and producing broad spatulate cauline leaves in 90 d (Fig. 27f).

In 7^{th} week of culture, juvenile thalli of *S. oligocystum* developed the primary branch (Fig. 28c). the average number of branches and the average length of branches were 2.3-2.4 branches and 8.3-10.9 mm in 9^{th} week to 10^{th} week, 2.5-3.0 branches and 12.6-13.7 mm between 11^{st} and 12^{nd} week and 2.9-3.5 branches and 13.0-13.3 mm between 13^{rd} and 14^{nd} week (Figs. 28d and 29a).

5.3.2 Growth rate of juvenile thalli in outdoor tank culture

Three-months-old juvenile plants of *S. oligocystum* (hereafter we call them as young thalli) were used for a growth experiment. Young thalli of *S. oligocystum* under the two different mediums showed different responses of their growth. At the end of experiment on 35^{th} day, the greatest increase in diameter of thallus was obtained with seawater 18.71±0.29 mm whilst that culture in seawater mix urea fertilizer was 13.69±0.19 mm. The highest growth rates of young thalli in seawater culture and seawater mixed with urea were 2.68±0.17% d^{-1} on 14^{th} day and 1.26±0.18% d^{-1} on 7^{th} day during the culture, respectively (Fig. 30). Growth rates of juvenile thalli of *S. oligocystum* cultured in seawater mixed with urea were decreased by cover of blue green algae attached on thalli (Fig. 28b), whilst those in seawater weren't covered so much with blue green algae (Fig. 28a).

The average measurements of environmental parameters were shown in Fig. 31. Water temperature was ranged from 30.4 to 30.9 $^{\circ}$C. Photosynthetic active radiation was from 21.7 to 40.5 μmol photons $m^{-2}s^{-1}$. Salinity in seawater and seawater mixed with urea were from 32 to 33.9 and from 31.5 to 35.5, respectively. pH in seawater and seawater mixed with urea were from 8.1 to 8.3 from 8.2 to 8.4, respectively. Differences in environmental parameters between tanks filled with seawater and seawater mixed with urea were very little.

5.4 Discussion

The germling development of *S. oligocystum* is similar to those of tropical species such as *S. confusum, S. horneri, S. thunbergii*, *S. swartzii* and *S. vachellianum* (Uchida, 1993; Kawagoe *et. al.*, 2005; Zhao *et. al*, 2008; Yan and Zhang, 2013; Kavale and Veeragurunathan, 2016). The development of embryonic germlings of this species follows the classic "8 nuclei 1 egg" type described for Sargassaceae. Fertilized eggs developed in to embryo at primary-rhizoid stage in 24 hours, secondary-rhizoid stage in 3 d. The first leaflet of the germling formed and had a cylindrical shape in 7 d. Cues on egg release and early germling growth of seaweeds were water temperature, irradiance, photoperiod, day length, nutrient, desiccation, thermal and osmotic stress (Norton, 1977; Uchida *et. al*., 1991; Nanba, and Okuda, 1993; Yoshida *et. al*., 1995, 1999; Steen, 2004; Steen and Rueness, 2004, Hwang *et. al*., 2006; Choi *et. al*., 2008; Chu *et.al*., 2012; Yongzheng, *et. al*., 2015). Our observation can't identify the cues.

The growth rates of the juvenile thalli of *S. oligocystum* cultured in seawater were higher than in seawater mixed with urea. These differences may be due to the chemical composition of the nutrient solutions used. Urea is an excellent nitrogen source for some seaweeds (e.g., kelp), but other seaweeds show reduced growth on urea. For example, Brault and Quéguiner (1989) studied on the effect of inorganic and organic nitrogen sources on growth of *Ulva gigantean* and found that ammonium was the best nitrogen rather than urea and nitrate. Phillips and Hurd (2003) reported the nitrogen ecophysiology of four intertidal seaweeds (*Stictosiphonia arbuscula*, *Apophlaea lyallii*, *Scytothamnus australis*, *Xiphophora gladiata)* from southeastern New Zealand and reported that a relative preference indices of nitrogen sources in winter were in the order of $NH4^+ > NO3^- >$ urea, while those in summer were the order of $NH4^+ = NO3^- >$ urea. Mansilla *et.al*. (2007) reported that *Gigartina skottsbergii* germlings grew more rapidly when cultivated in a solution of Bayfoland 250 SL and Provasoli, while growth rates in urea and superphosphate were significantly lower. Nitrogen and phosphorus are two nutrients that limit seaweeds growth and yields in most natural environments. The physiological and biological factors, such as inter-seaweed variability, nutritional history, type of tissue, life history stage, age, the surface area to volume ratio of the thallus, and morphology will influence growth and uptake of nutrients. (Harrison and Hurd, 2001). In the present study, the growth rates of juvenile thalli of *S. oligocystum* in seawater mixed with urea were decreased by blue green algae contamination. Harrison and Hurd (2001) mentioned that epiphytes can also controlled by starving the seaweed of N for several days until the tissue N (i.e. stored N) decrease to a critical level. Thus, it is estimated that *S. oligocystum* may adapt to nutrient level of tropical water in east coast of the Gulf of Thailand.

Fig. 25 Mature thalli of *Sargassum oligocystum* around Samaesarn Island.

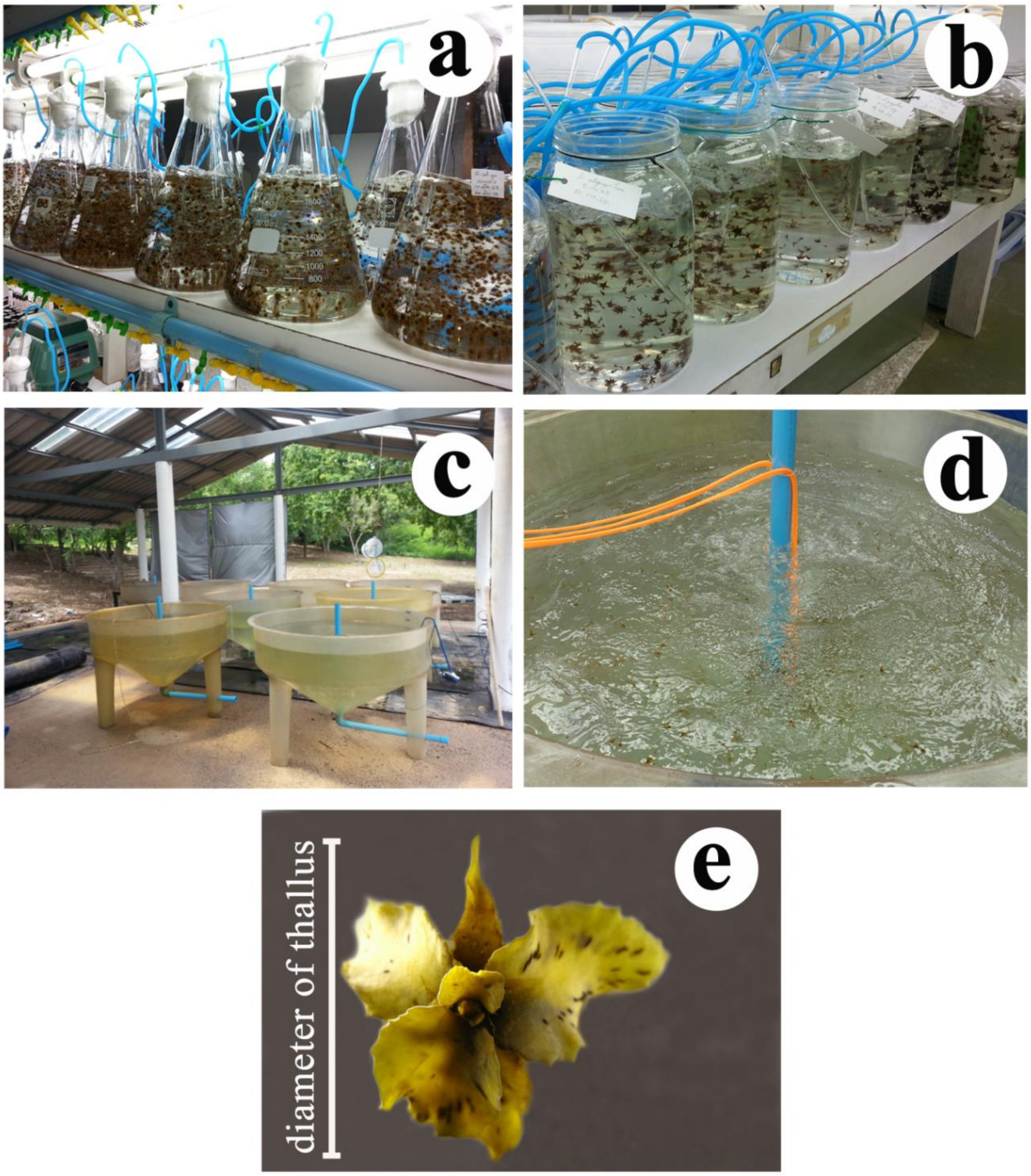


Fig. 26 Pictures showing cultures of *Sargassum oligocystum* germlings in a laboratory (a and b); those of juvenile thalli in outdoor tanks (c and d) in Samaesarn Island and diameter of thallus measurement (e).

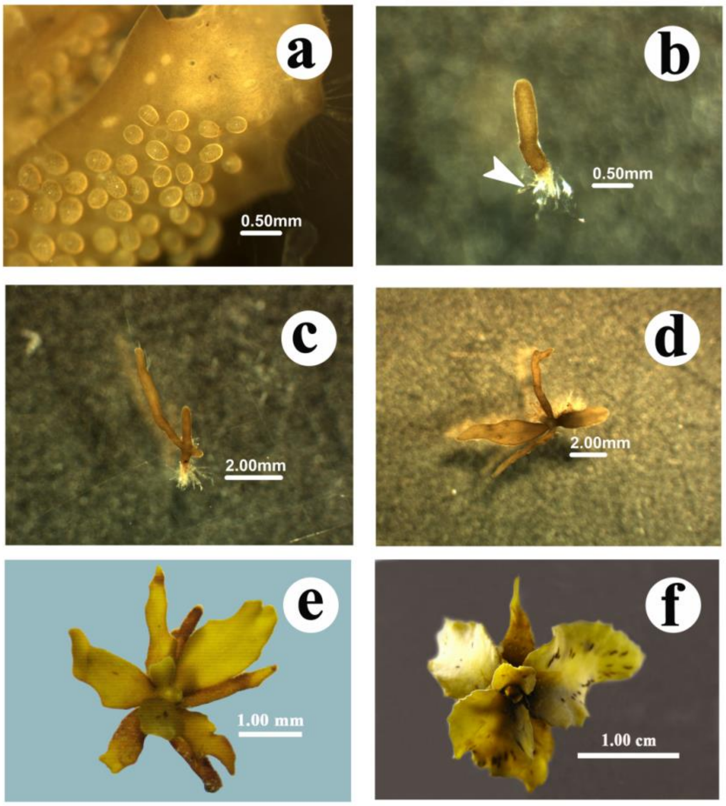


Fig. 27 Embryo development of *Sargassum oligocystum* in a container filled with culture medium of Plant Nutrition+ liquid (Tropica® AQUACARE) under a salinity of 30, a water temperature of 25 °C and PAR of 85μmol photons $m^{-2}s^{-1}$**:** Pictures of embryos on 1st day (a), 3rd day with an arrow showing rhizoidal cell (b), 7th day (c), 30th day (d), 60th day (e) and 90th day (f).

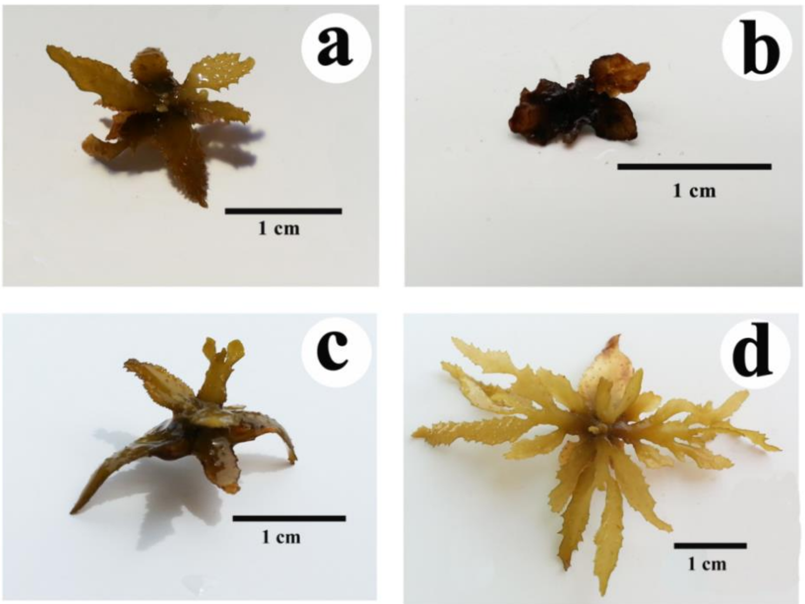


Fig. 28 Juvenile thalli of *S. oligocystum* in seawater (a) and seawater mix urea 4 g ton^{-1} (b) on 5th day, that with a lateral branches in seawater on 7th day (c) in seawater on 14th day and (d) from the start of outdoor tank culture.

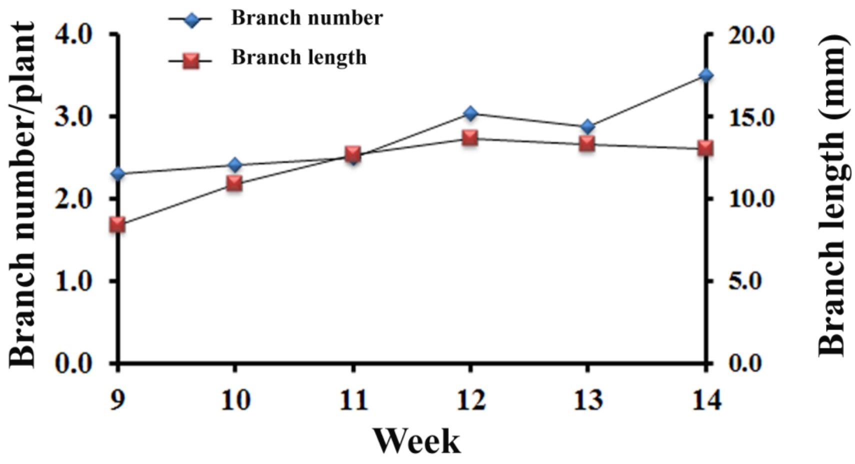


Fig. 29 Branches development of *Sargassum oligocystum*; tank culture from 9th week to 14th week.

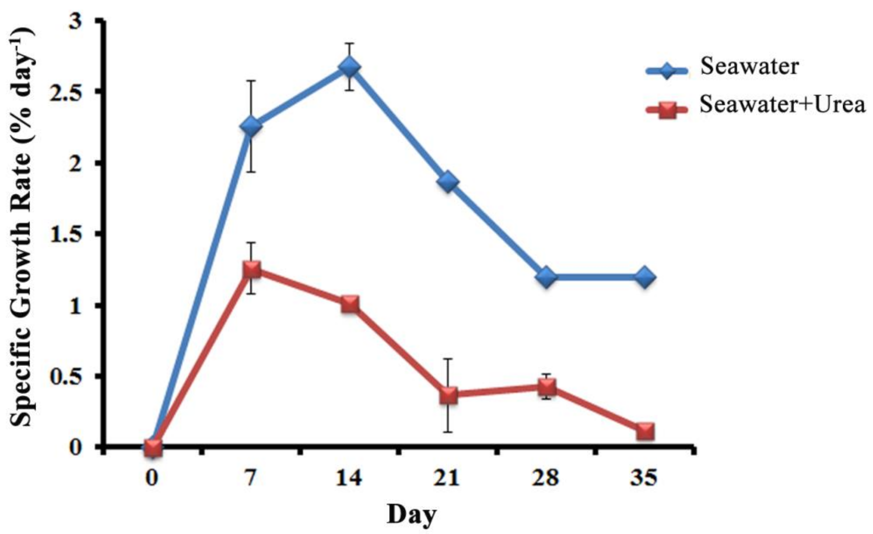


Fig. 30 Growth rates of juvenile thalli *of Sargassum oligocystum* (mean± standard error) at intervals of 7 d cultured in outdoor tanks filled with seawater (blue) and seawater mixed with urea (red) for 35 d.

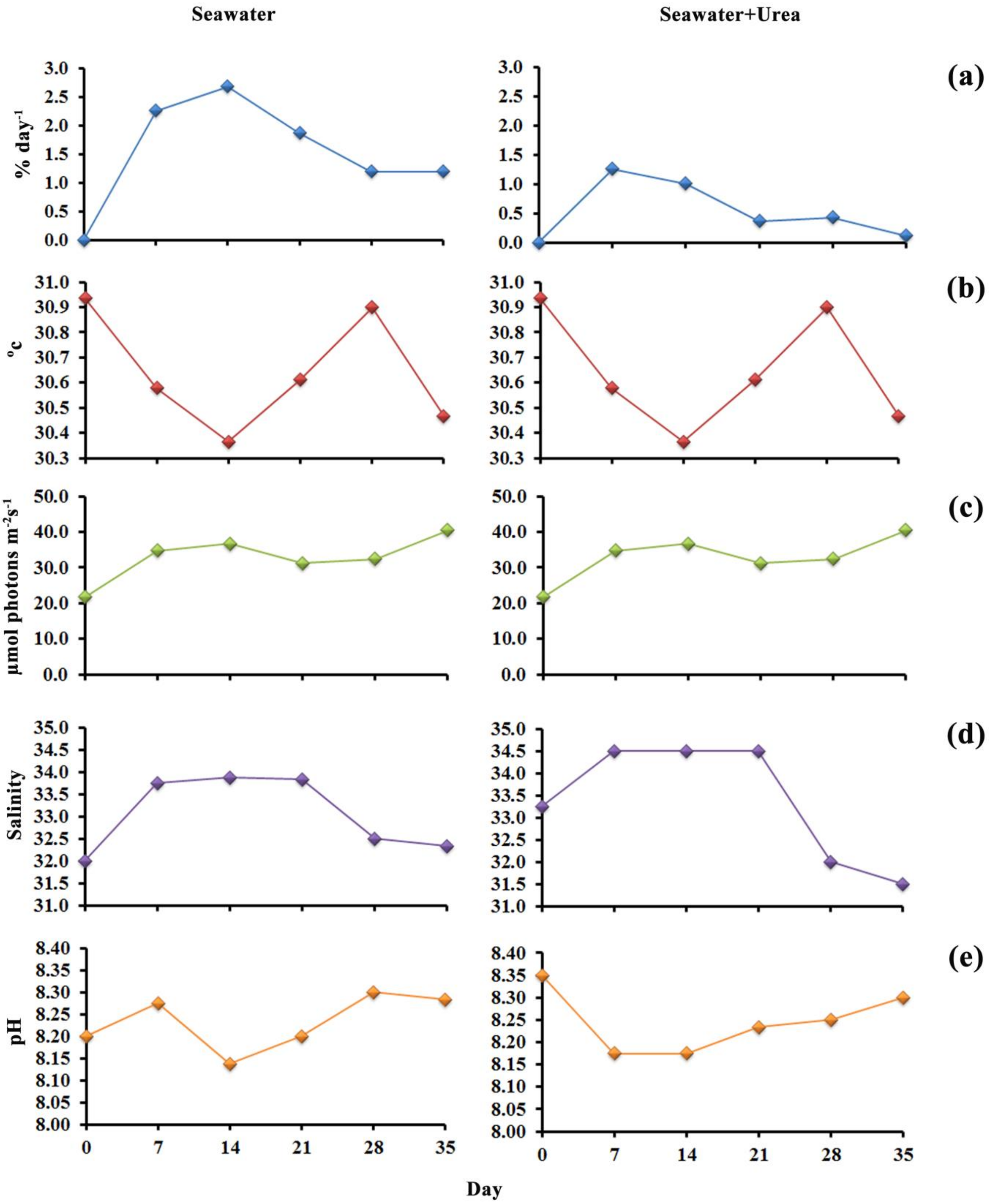


Fig. 31 Effect of environmental factors on growth rate in tank culture of juvenile plants *Sargassum oligocystum* with seawater and seawater mix urea 4 g ton^{-1}; (a) growth rate, (b) temperature, (c) irradiance, (d) salinity, (e) pH.

Chapter 6

General Discussion

6.1 Genus *Sargassum* in Thai waters

From the previous reports of Thai *Sargassum*, there are fifteen species of *Sargassum* in Thai waters. Eight species have been recorded from the Andaman Sea, and twelve species have been found in the Gulf of Thailand. The most common species was *S. polycystum* distributed widely in almost all the collection sites (Reinbold, 1901; Lewmanomont, 1988; Lewmanomont and Ogawa, 1995; Noiraksar *et.a.l,* 2006; Ajisaka *et al*., 2007; Noiraksar and Ajisaka, 2008; Tsutsui *et. al.*, 2012). In addition, for the eastern coast of the Gulf of Thailand the five taxa was performed and descriptions of each species was written in details: three taxa of *Sargassum* with compressed primary branches, *S. aquifolium*, *S. oligocystum* and *S. swartzii* have been reported from the Gulf of Thailand. The distinction among these three taxa has clearly shown and discussed. *S. aquifolium* has slender lanceolate leaves, dentate margins, elliptical vesicles with crown leaf sometimes mucronate at the apex, the compressed stalk with dentate margin , receptacles flattened with spines along the whole margin. *S. oligocystum* has broader lanceolate leaves with acute to rounded apex, serrate margins, spherical vesicles, stalk terete, receptacles slightly compressed with few spines on the margin. *S. swartzii* has linear lanceolate leaves, elliptical vesicles with mucronate sometimes with crown leaf at the apex, stalk terete, receptacles cylindrical with few spines near the apex. Juvinile plants of *S. binderi* and *S. oligocystum* from Thailand are rather similar in spatulate to lanceolate leaves with brownish stripes on both sides of the midrib.They are different in colors and characters of leaf margins. *S. aquifolium* is blackish brown in color and the leaf margins showed some small dentation, while *S. oligocystum* is yellowish brown in color and the leaf margins with fine serration throughout the whole margin. *S. swartzii* is yellowish brown in color, leaves elongated lanceolate to narrow lanceolate, and no brownish stripes on both sides of the midrib.

S. baccularia is similar to *S. polycystum*, both with terete to subterete primary branches. *S. baccularia* has no spines on branches while those of *S. polycystum* muricate with simple or forked spines, some primary branches transformed into stolons and secondary holdfasts. The most important character to separate the two species is form of female receptacles. In *S. baccularia,* female receptacles are triquetrous, while those of *S. polycystum* are terete to slightly compressed. Leaves of juvinile plants, both cauline and primary leaves, of *S. baccularia* have small cryptostomata conspicuously scattered on both sides of the midrib, while those of *S. polycystum* have small cryptostomata scattered near both sides of the margins and not as dense as in *S. baccularia* (Noiraksar *et.a.l*., 2006; Noiraksar and Ajisaka, 2008).

6.2 Distribution of *Sargassum* beds

Seaweed beds are typically found in coastal waters, growing on a range of shallow rocky or hard substrates. In Thailand *Sargassum* beds were found along the Gulf of Thailand and the Andaman Sea. We applied a combination of remote sensing and in situ methods applied to the waters off the coast of Sattahip, Chonburi Province. These beds can be mapped using the noncommercial satellite, ALOS, which can provide multiband images using high spatial resolution optical sensors (sensitive to 10 m^{-2} plots), AVNIR-2. These images are of sufficient quality for examining coastal ecosystems. Biomass data were obtained in January, February, March, and December 2009 from quadrat sampling. The biomass of *Sargassum aquifolium* at a bottom depth of 1 m and 1.5–2 m was determined to be 7.73 and 92.75 g dw m^{-2}, while that of *Sargassum oligocystum* was found to be 44.05 and 87.97 g dw m^{-2}, respectively. By applying this methodology, the *Sargassum* beds off Sattahip can be detected with an accuracy of about 70%. It is estimated that the error is caused by mixel effects of the bottom substrates in individual pixels, each of which covers an area of 10 × 10 m. Small differences in the overall accuracies betweenthe methods are caused by the ground truth data concentrated around the *Sargassum* beds near the shore. Another reason for low accuracy may also be caused by the class of other bottom substrates including sand, dead corals, and a small quantity of live corals. The reflectance of live corals though is lower than that of sand (Nurdin *et al*., 2012). A mixel effect results when a pixel covers plural bottom types and shows mixed reflectance of these bottom types. Thus, it is estimated that the mixel effect by live coral patches on sand beds results in misclassification. Our results indicate that the images captured by ALOS AVNIR-2 are informative and useful for mapping the *Sargassum* beds in Southeast Asia

6.3 Seasonal changes in growth of *Sargassum* species

Sargassum forests form important habitats in tropic coastal waters. *Sargassum aquifolium*, *S. oligocystum* and *S. polycystum* are the dominant species consisting of *Sargassum* forests and distributed widely in the Gulf of Thailand. We studied seasonal variations of *Sargassum* species (*S. aquifolium*, *S. oligocystum* and *S. polycystum*) on the reef flats in the east coast of the north Gulf of Thailand and measurements of environmental. Percent cover, thallus length, standing crop, and percent fertility of *Sargassum* species were the maximum during the dry season from November to May. Plant density was the maximum during the rainy season from May to September. This is explained by recruitment of juvenile plants spawn in January to February during the dry season.

The growth and reproductive period of Thai *Sargassum* species can be concluded that the reproductive period of tropical *Sargassum* species growing in Thailand, Philippines, Taiwan, Malaysia and India usually occurs during the period from November to May (Trono and Lluisma,1990; Calumpong *et. al*., 1999), (May-Lin and Ching-Lee, 2013) and (Rao, 2002; Padal *et al.*, 2014). Typical growth cycle of *Sargassum* species is characterized by presence of a slow growth phase, a rapid growth phase, and a reproductive phase that is followed by senescence and dieback (ANG, 2006). All the species of Thai *Sargassum* collected from nine provinces along the Gulf of Thailand from 2005 to 2008 having the reproductive stage during the period of March to June and showed the highest peak in April in almost all the collection sites. Only some species were found also in November to February (Noiraksar, no publication).

For our study shows the variations of environmental factors and growth patterns of *Sargassum* species from the eastern cost of Thailand. In the northeast Gulf of Thailand, monsoon drives environmental variables and also seasonal variations of growth and reproduction of *Sargassum* species, which are also influenced by the environmental variables. Reproduction of *Sargassum* species occurs under the calm condition during the dry season. In this way, seasonal growth and reproduction are controlled by the monsoon in the northeast Gulf of Thailand

6.4 Life history of *Sargassum*

Reproduction in *Sargassum*: the reproduction of *Sargassum* takes place by vegetative and sexual methods. Vegetative Reproduction in S*argassum*: *Sargassum* multiplies profusely by vegetative fragmentation. The thallus breaks into fragments due to mechanical injury or death and decay of older parts. The species like *S. hystrix* and *S. natans* growing in Sargasso sea are completely sterile as they do not form any reproductive structures. In these species the fragmentation is the only method of multiplication. Sexual Reproduction in *Sargassum*: sexual reproduction in *Sargassum* is oogamous. The male sex organs are called antheridia and the female oogonia. The sex organs develop in special flask shaped cavity called conceptacle. These conceptacles are present is specially modified laterals called receptacles. The male and female sex organs develop in separate conceptacles (*Sharma*, 2017). For the present study, the development of embryonic germlings of *S. oligocystum* follows the classic "8 nuclei 1 egg" type described for Sargassaceae. Fertilized eggs developed in to embryo at primary-rhizoid stage in 24 hours, secondary-rhizoid stage in 3 d. The first leaflet of the germling formed and had a cylindrical shape in 7 d. Nevertheless, the germling development of *S. oligocystum* is similar to that in related tropical species such as *S. confusum*, *S. horneri, S. thunbergii, S. swartzii* and *S. vachellianum* (Uchida, 1993; Kawagoe *et. al*., 2005; Zhao *et. al,* 2008; Yan and Zhang, 2013; Kavale and Veeragurunathan, 2016).

The growth rates of the juvenile thalli of *S. oligocystum* cultured in seawater were higher than in seawater mixed with urea. These differences may be due to the chemical composition of the nutrient solutions used. Urea is an excellent nitrogen source for some seaweeds (e.g., kelp), but other seaweeds show reduced growth on urea. For example, *Ulva gigantean* (Brault and Quéguiner, 1989); *Stictosiphonia arbuscula*, *Apophlaea lyallii*, *Scytothamnus australis*, *Xiphophora gladiate (*Phillips and Hurd, 2003); *Gigartina skottsbergii* (Mansilla *et.al*., 2007): Nitrogen and phosphorus are two nutrients that limit seaweeds growth and yields in most natural environments. The physiological and biological factors, such as inter-seaweed variability, nutritional history, type of tissue, life history stage, age, the surface area to volume ratio of the thallus, and morphology will influence growth and uptake of nutrients. (Harrison and Hurd, 2001). In the present study, the growth rates of juvenile thalli of *S. oligocystum* in seawater mixed with urea were decreased by blue green algae contamination. Harrison and Hurd (2001) mentioned that epiphytes can also controlled by starving the seaweed of N for several days until the tissue N (i.e. stored N) decrease to a critical level. Thus, it is estimated that *S. oligocystum* may adapt to nutrient level of tropical water in east coast of the Gulf of Thailand.

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