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Original Article

Growth rate and calcium carbonate accumulation of *Halimeda macroloba* Decaisne (Chlorophyta: Halimedaceae) in Thai waters

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Abstract

Halimeda macroloba Decaisne can utilize the CO_2 used for carbon fixation in photosynthesis and use bicarbonate as the main carbon source for calcification. Although *Halimeda* has been recognized as a carbon sink species, the calcium accumulation of *Halimeda* species in Thai waters remain poorly understood. In this study, the highest density of *H. macroloba* was 26 thalli/m^2 and *Halimeda* quickly produced 1-2 new segments/thallus/day or 20.1 mg dry weight/thallus/day. Its calcium carbonate accumulation rate was 16.6 mg $CaCO_3$/thallus/day, or 82.46 % per thallus. In Thailand, however, only three scientific papers of growth rate and $CaCO_3$ accumulation rate of *H. macroloba* have been found and collected. Of these records, the mean density was 26-104 thalli/m^2. The growth rate of *H. macroloba* was around 1-2 mg dry weight/day and the $CaCO_3$ accumulation rate varied around 41-91%. Thus, *Halimeda* has a great potential to decrease the carbon dioxide concentration in the ocean.

Keywords: calcification, CO_2, carbon sink, growth rate, *Halimeda macroloba*

1. Introduction

Over the past decade, atmospheric CO_2 concentrations have been rising by anthropogenic activities such as industrialization and burning of fossil fuels. Around 30% of anthropogenic CO_2 emissions were removed by oceanic calcification and photosynthesis. Seaweed can utilize the CO_2 used for carbon fixation in photosynthesis and also can use dissolved inorganic carbon; bicarbonate as the main carbon source for calcification. Calcifying green alga, *Halimeda*, is the major producers of calcium carbonate by precipitation from Ca^{2+} and HCO_3^- ions in solution and can deposit calcium carbonate in the form of aragonite in the intercellular spaces (Borowitzka and Larkum, 1976; Brownlee and Taylor, 2002; Robbins *et al*., 2009). *Halimeda* contributes to mass of carbonate sediment in many reefs (Hillis-Colinvaux, 1980; Drew and Abel, 1988; Beach *et al*., 2003; Vroom and Smith, 2003; van Tussenbroek and van Dijk, 2007). Around 8% of the total world carbonate production has been contributed by *Halimeda* species varying among species, 50-2,323 g $CaCO_3$ m^{-2} y^{-1} for the psammophytic or sand-dwelling species and 28-2,234 g $CaCO_3$ m^{-2} y^{-1} for lithophytic species (van Tussenbroek and van Dijk, 2007). In a Caribbean reef lagoon, *Halimeda incrassata* produced carbonate around 815 g $CaCO_3$ m^{-2} y^{-1} and had a turnover of 30 days (van Tussenbroek and van Dijk, 2007). Drew (1983) showed that *Halimeda opuntia* produced new segments every six days and *Halimeda copiosa* and *H. opuntia* produced around 2,234 g $CaCO_3$ m^{-2} y^{-1}. In addition, Payri (1988) measured a production by *H. opuntia* in Tahiti that was about 2,300 g $CaCO_3$ m^{-2} y^{-1}. *Halimeda* has been recognized as a carbon sink species that can help decrease CO_2. However, the estimates of the $CaCO_3$ production rates vary depending upon the spatial and temporal variability (van Tussenbroek and van Dijk,

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2007), plant age, photosynthesis, respiration, pH, two orders of magnitude, herbivory, and growth rate (Paul and Van Alstyne, 1988, 1992; Lobban and Harrison, 1994; De Beer and Larkum, 2001).

In recent years, *Halimeda* has become a dominant component of many reefs in the tropical regions of the Pacific, Atlantic, and Indian Ocean and especially in Thai waters (Lapointe and Thacker, 2002; Beach *et al*., 2003; Vroom *et al*., 2003; Mayakun *et al*., 2012a,b). Although *Halimeda* has been recognized as a carbon sink species, the biological and ecological parameters of *Halimeda* species in Thailand remain poorly understood. Few studies have examined the growth rate, reproduction, and responses of *Halimeda* in Thai waters (Mayakun, 2010; Mayakun *et al*., 2012a,b). For the $CaCO_3$ accumulation of *Halimeda*, it was first determined in 2008 at Phuket, Andaman Sea (Sinutok *et al*., 2008), but no other studies have been reported. Thus, the $CaCO_3$ accumulation rates are still needed to confirm the potential of *Halimeda* as a carbon sink species. The objectives of the present study were 1) to determine the growth rate and calcium carbonate accumulation of *H. macroloba* at Koh Mut Sum, Mu Ko Thale Tai National Park, Gulf of Thailand, and 2) to assemble the data of growth rate and calcium carbonate accumulation of *H. macroloba* in Thai waters from the literature.

2. Materials and Methods

2.1 Study site

This study was carried out in the intertidal zone at Ko Mat Sum, Mu Ko Thale Tai National Park, Gulf of Thailand, East coast of Southern Thailand. The study site was a sand plain in the shallow zone (1-2 m in depth). The climate and seasons of this study site were described in Mayakun *et al.* (2012b). The site was visited during April-May 2011.

2.2 Physical and chemical parameters

Water temperature and salinity were measured at the site using thermometer and salinity refractometer. Water samples were collected and kept in a dark and cold box and then sent to the Central Laboratory of the Faculty of Science, Prince of Songkla University, for calcium, nitrate, and phosphate analyses.

2.3 Density of *H. macroloba*

The natural density of *H. macroloba* was measured using two transect lines of 100 m length and a 50 cm × 50 cm quadrat. Two line transects were placed in the *Halimeda* patch. Thalli were counted in three quadrats at 10 m intervals along the transect line. There were 66 quadrats in total.

2.4 Growth rate and calcium carbonate accumulation

Alizarin Red-S marking technique was used for growth assessment. 50 thalli of *H. macroloba* were randomly selected and marked with Alizarin Red-S. Thalli were covered with transparent bags (volume ~ 500 mL) and attached the base of thallus with wire ribbon. A concentrated solution of Alizarin Red (1 g per 100 mL of seawater) was injected into the enclosures and checked for leakage of the coloring agent. The bags were removed 24 hrs later. The Alizarin Red-S marking technique is quite difficult to carry out in the field where the tide is high or the water depth is around 2-5 meters. Snorkeling or scuba diving is necessary to dive and dye algal thalli. Also, the thallus of *Halimeda* is relatively fragile. After 18 days, the dyed thalli were collected and bought back to the laboratory. The collected thalli were cleaned of epiphytes and bleached in a 5-10 % sodium hypochloride solution for 20-30 minutes until thalli lost its green color. The segments were separated into stained (old segments) and unstained segments (new segments) (Figure 1). The number of segments in each part were counted and dried in a drying oven at 65°C to constant dry weight. The dried segments were weighed and recorded. The dry weights of the unstained segments (new segments) were used to calculate the growth rate (mg dry weight day^{-1}) as mg dry weight/18 days. After weighing, the segments were placed in 5% hydrochloric acid for 30 min to 1 hr, and then rinsed with freshwater to remove all traces of acid. Thalli were placed in a drying oven at 65°C to constant dry weight and then weighed to determine their somatic weight. The $CaCO_3$ content was calculated from the difference in dry weight of the calcified thalli and the somatic weight (van Tussenbroek and van Dijk, 2007; Sinutok *et al*., 2008).

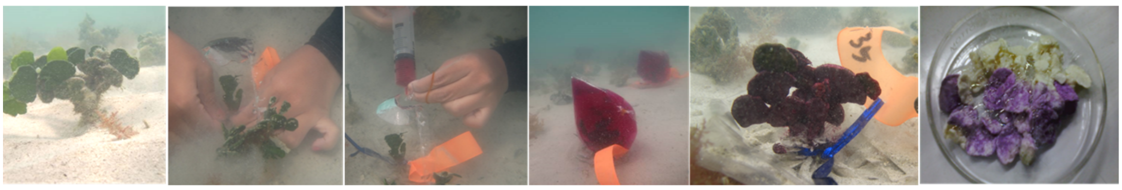

Figure 1. Alizarin Red–S marking technique.

2.5 The information of growth rate and calcium carbonate accumulation

The data of growth rate and calcium carbonate accumulation of *H. macroloba* in Thai waters were assembled from literatures in order to assess the importance of *H. macroloba* as a carbon sink.

3. Results

3.1 Physical and chemical parameters:

Water temperature, salinity, calcium, nitrate, and phosphate at the study site were 28-29°C, 30-32 psu, 176.4 mg/L, 1.73 mg/L, and 0.31 mg/L, respectively.

3.2 Density

The mean natural density of *H. macroloba* in the intertidal zone (1-2 m in depth) at Ko Mat Sum, Mu Ko Thale Tai National Park, was 11.70±1.77 thalli/m^2 (n = 66 quadrats) during April-May 2011. The highest density was 26.83±10.1 thalli/m^2 and the high density was found at 10-40 meter from the shore (Figure 2). At the site, the substrate was sand plain and the depth was 1-2 m. *H. macroloba* grows in a sandy bottom, anchoraging by a large bulbous holdfast.

3.3 Growth rate and calcium carbonate accumulation

The average growth rate of *H. macroloba* was 20.1 mg dry weight/thallus/day. New segments were produced around 1-2 segments/thallus/day. Calcium carbonate accumulation rate of *H. macroloba* was 16.6 mg $CaCO_3$/thallus/day or 82.46% per thallus.

3.4 Information of growth rate and calcium carbonate accumulation of *H. macroloba*:

Only three scientific papers of growth rate and $CaCO_3$ accumulation rate have been found and collected (Table 1). Of these records, the growth rate of *H. macroloba* in Thai waters was around 1-2 mg dry weight/day (Mayakun *et al*., 2012 a,b) and the $CaCO_3$ accumulation rate varied around 41-91% (Sinutok *et al*., 2008).

4. Discussion

Our results showed that the average growth rate of *H. macroloba* in the intertidal zone at Ko Mat Sum, Mu Ko Thale Tai National Park, Gulf of Thailand, was 20.1 mg dry weight/thallus/day or it produced 1-2 new segments per thallus per day. Similarity in *Halimeda tuna*, its growth rate varied from 6 to 25 mg dry weight day^{-1}(Vroom *et al*., 2003). In contrast to Sinutok *et al*. (2008), *H. macroloba* in Tangkhen Bay, Phuket Province, Andaman Sea, produced around 1.51±0.34 to 2.23±0.38 mg dry weight/thallus/day. The growth rate of *H. macroloba* in Ko Mat Sum was ten times greater than *Halimeda* in Thangkhen Bay. It might be because of the nutrient concentrations that may influence the growth rate. Mayakun *et al*. (2012b) found that the elevated nutrient concentration might increase the growth rate of *H. macroloba* at Ko Rab, Mu Ko Thale Tai National Park, Gulf of Thailand. Its growth rate was around two times higher after nutrient enrichment. The growth rates of *Halimeda* have been reported from many reefs such as the Great Barrier Reefs, Caribbean reefs, and Thai waters (Drew, 1983; van Tussenbroek and van Dijk, 2007; Mayakun *et al*., 2012 a,b).

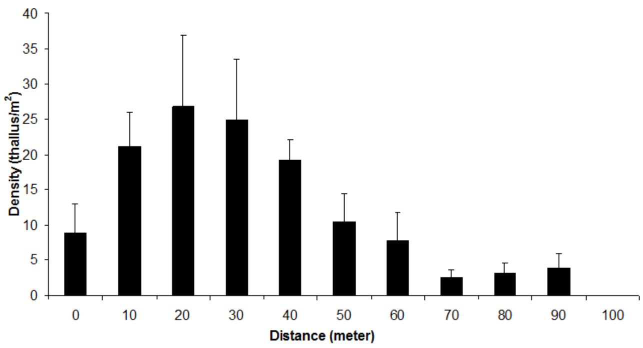

Figure 2. Density of *H. macroloba* in the intertidal zone at Ko Mat Sum, Mu Ko Thale Tai National Park, Thailand, during April-May 2011 (mean±SE).

Table 1. Data of growth rate and calcium carbonate accumulation of *H. macroloba* in Thai waters.

	Halimeda macroloba			
Reference	Location	Density (Thalli/m^2)	Growth rate (segments/thallus/day or mg dry weight/thallus/day)	$CaCO_3$ accumulation (mg $CaCO_3$/thallus/day or %)
Sinutok *et al.* (2008)	Tangkhen Bay, Phuket Province, Andaman Sea	18.72±1.68 (summer) 5.02±0.66 (rainy season)	2.23±0.38 (summer) 1.51±0.34 (rainy season) mg dry weight/thallus/day	80.88±1.02% (summer) 67.87±1.82% (rainy season)
Mayakun *et al.* (2012 a,b)	Ko Rab, Gulf of Thailand	104±5	1-2 new segments	-

These studies revealed that the growth rates varied among species; *Halimeda opuntia* and *H. copiosa* produced one new segment every six days (Drew, 1983), while *H. incrassata* has a mean turnover of 30 days (van Tussenbroek and van Dijk, 2007). The growth rates were affected by biotic and abiotic factors, such as light intensity, nutrient concentration, and herbivore (Beach *et al*., 2003; Vroom *et al*., 2003).

The highest density of *H. macroloba* in this study was 26.83±10.1 thalli/m^2 that is similar to the density of *H. macroloba* in Thangkhen Bay with 24 thalli/m^2. However, in Thai waters, the highest density of *H. macroloba* was found at Ko Rab in the Gulf of Thailand, with around 104 thalli/m^2, and >200 thalli/m^2 was recorded at Lidee Island in the Andaman Sea (Mayakun *et al*., 2012a). The relatively high density and abundance of this species might be due to its reproduction by both sexual and asexual ways.

For the calcium carbonate accumulation in Thailand, the calcium carbonate accumulation rate of *H. macroloba* was 16.6 mg $CaCO_3$/thallus/day or 82.46 % per thallus. Once the plants die, the carbon is still stored in the sediment. The $CaCO_3$ accumulation rates differ by thallus age, growth rate, photosynthesis, concentration of Ca^{2+}, CO_3^{2-}, CO_2, and phosphate concentration (Borowitzka and Larkum, 1976; Borowitzka, 1977). When compared to Sinutok *et al*. (2008), the $CaCO_3$ accumulation rate and calcium concentration in the present study were higher than in their study. The phosphate concentration in their study (2.09±0.65 mg/L) was 6.7 times higher than in our study. Low phosphate and high calcium concentrations in our study site could be the factors that can influence calcium carbonate precipitation, showing the high accumulation rate in our study. Björk *et al*. (1995) reported that increased phosphate concentrations affected calcification of the coralline algae. High concentrations of phosphate may inhibit biomineralization and can act as crystal poison, inhibiting the $CaCO_3$ precipitation and interfering with the formation of the crystal lattice (Delgado and Lapointe, 1994; Björk *et al*., 1995; Demes *et al*., 2009). However, the direct effect of added phosphate concentration on algal calcification is still unclear and further experimental studies are needed to investigate this effect.

The results are believed to be useful as a database and reference sources for the growth rate and calcium carbonate accumulation of *H. macroloba* that is known as a "carbon sink". However, much less is known about how much CO_2 is used and accumulated in those processes. So, there are the interesting and challenging questions that will allow us to get a better understanding of $CaCO_3$ accumulation and the great potential of *Halimeda* to decrease carbon dioxide.

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