

Original Article

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The standing stock and CaCO₃ contribution of *Halimeda macroloba* in the tropical seagrass-dominated ecosystem in Dongsha Island, the main island of Dongsha Atoll, South China Sea

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Abstract

Calcareous green algae in the genus *Halimeda* are important contributors to the marine carbonate budget. Dongsha Island is located in the northernmost South China Sea and is a seagrass-dominated ecosystem with intermixed *Halimeda macroloba* patches, making it an excellent system to better examine the extent of carbonate contribution by *H. macroloba* in such an ecosystem. To this end, we examined the standing stock and actual CaCO₃ contribution of *H. macroloba* in the seagrass-dominated ecosystem (herein Dongsha Island) and compared them with those in *Halimeda*-dominated ecosystems. The density, growth rate, calcification rate and CaCO₃ content of *H. macroloba* at four life stages were investigated. The mean density of *H. macroloba* was around 8.82 ± 1.57 thalli m⁻² and the estimated standing stock was 61,740 to 72,730 thalli. Thalli produced 1 to 2 new segments day⁻¹, giving a growth rate of 0.003 ± 0.001 g dry weight thallus⁻¹ day⁻¹. Calculated algal biomass and annual areal production were 0.03 g m⁻² and 9.66 g m⁻² year⁻¹. In each square metre of this area, *H. macroloba* produced 8.82 to 17.64 new segments day⁻¹, accumulating 0.002 ± 0.001 g CaCO₃ thallus⁻¹ day⁻¹ or around 6.44 g CaCO₃ m⁻² year⁻¹. Mean CaCO₃ content was 0.32 ± 0.05 g thallus⁻¹. As expected, the growth rate and CaCO₃ production of *H. macroloba* in Dongsha Island were lower than in other studies from *Halimeda* tropical ecosystems. Overall, this work provides the baseline of carbonate production of *H. macroloba* in Dongsha Island and relevant systems where the ecosystem is dominated by seagrasses.

Introduction

The calcareous green algae *Halimeda* is widely distributed across the tropics and subtropics (Verbruggen & Kooistra, 2004; Dijoux *et al.*, 2012; Cremen *et al.*, 2016). It can utilize bicarbonate (HCO₃⁻) for calcification and precipitates aragonite crystals in its intercellular spaces (Borowitzka & Larkum, 1976). It is one of the main calcium carbonate (CaCO₃) producers that contribute to the marine carbonate budget in tropical reefs and lagoons (Ree *et al.*, 2007; Mayakun *et al.*, 2014; Carneiro & Morais, 2016; Kaewsrikhaw *et al.*, 2016; McNeil *et al.*, 2016; Perry *et al.*, 2016; Carneiro *et al.*, 2018; Prathep *et al.*, 2018; Mayakun & Prathep, 2019). For instance, global estimates showed that *Halimeda* species contributed around 2 kg CaCO₃ m⁻² year⁻¹ (Drew & Abel, 1985; van Tussenbroek & van Dijk, 2007; Perry *et al.*, 2016) or accumulated around 0.15 Gt CaCO₃ year⁻¹ (Milliman, 1993), resulting in ~8% of total world carbonate production (Hillis, 1997). When only tropical reefs are considered, *Halimeda* can produce ~54.37 g m⁻² year⁻¹ of carbonate sediments (Carneiro *et al.*, 2018), equivalent to at least 13,050.14 kg year⁻¹ or around 42% of total sedimentary organic carbon (Tuntiprapas *et al.*, 2019). Studies have shown that the carbonate production of *Halimeda* can vary due to abiotic and biotic factors. For abiotic factors, the CaCO₃ production varied depending on local, environmental parameters such as temperature, light (Kaewsrikhaw *et al.*, 2016; Ortégón-Aznar *et al.*, 2017) or seawater inorganic carbon (C_i) (Peach *et al.*, 2017). For biotic factors, CaCO₃ production and sediment generation varied within and among *Halimeda* species because of differences in growth rates, calcification rates, segment sizes, turnover rates and life stages (Hillis-Colinvaux, 1980; Perry *et al.*, 2016; Mayakun & Prathep, 2019). However, the effect of biotic interactions that regulate *Halimeda* dominance is less commonly studied (but see Davis & Fourqurean, 2001; Barry *et al.*, 2013).

In the Indo-Pacific Ocean, *H. macroloba* is a common widespread *Halimeda* species ranging from the tropics to subtropics (Pongparadon *et al.*, 2017). Its abundance has been reported in shallow subtidal and intertidal zones at population densities from 24–200 thalli m⁻² in seagrass-free or seagrass-poor ecosystems (Sinutok *et al.*, 2008; Mayakun *et al.*, 2014; Mayakun & Prathep, 2019). Producing one to two new segments daily, thalli grew by 0.021 g dry weight thallus⁻¹ day⁻¹. Total CaCO₃ production by *H. macroloba* was 291.94–908.11 g m⁻² y⁻¹ and the CaCO₃ content increased with age (Mayakun & Prathep, 2019). Due to its fast growth, and high CaCO₃ productivity, this alga is a significant contributor to carbonate budgets (Mayakun *et al.*, 2012a, 2012b, 2014; Mayakun & Prathep, 2019).



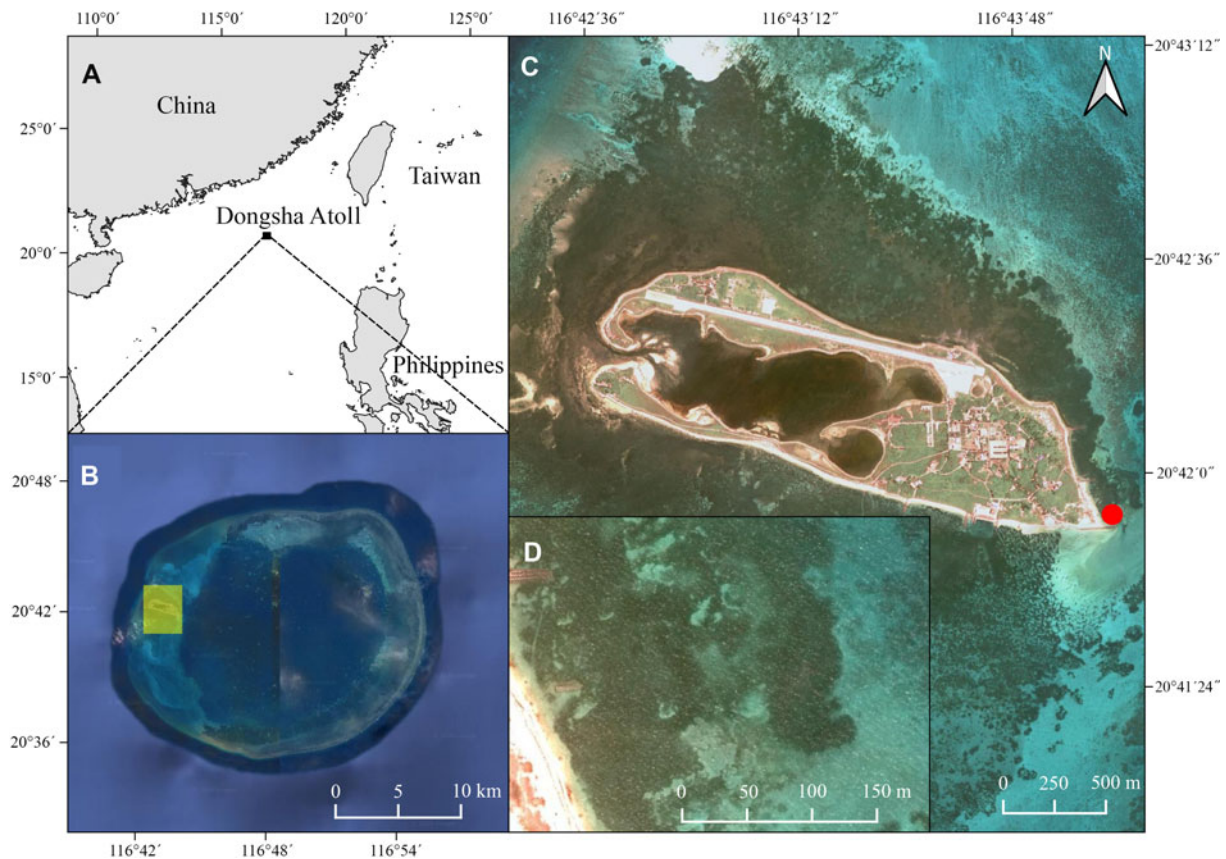


Fig. 1. Study site: (A) Location of Dongsha Island in the South China Sea; (B) Location of the main island (Dongsha Island) in the atoll; (C) Location of our surveyed site (labelled as the red dot) on the Dongsha Island; (D) The dark area surrounding the Dongsha Island is the seagrass bed.

Halimeda macroloba is a common and abundant species in seagrass beds in the shallow subtidal zone of Dongsha Island (also known as Pratas Island), South China Sea. Despite some fishing disturbances around the margins of Dongsha Atoll, a no-take reinforcement in the Dongsha Island (i.e. with total fishing restrictions, see the definition in Rolim *et al.*, 2019) is implemented via the establishment of Dongsha Atoll National Park under Taiwan governance as a marine protected area since January 2007. Thus, the island, as well as the atoll, is currently considered a less disturbed spot for marine biodiversity in South China Sea (Huang *et al.*, 2015). Due to the highly sandy environments, Dongsha Island harbours various seagrass species, resulting in a seagrass-dominated ecosystem (Lin *et al.*, 2005). The estimation of carbonate contribution by *H. macroloba* has primarily focused on *Halimeda*-dominated areas in tropical ecosystems (Mayakun *et al.*, 2014; Mayakun & Prathep, 2019). However, estimation of the carbon contribution by *H. macroloba* remains unexplored for most tropical seagrass-dominated areas where seagrass seems to both facilitate and compete with *H. macroloba*. So, the CaCO_3 contribution by *H. macroloba* in the seagrass-dominated area should be different from that in the *Halimeda*-dominated area. Due to its diverse macrophyte community, Dongsha Island is an ideal research site to investigate the growth rate, number of new segments, standing stock and CaCO_3 content of this carbonate sediment producer in seagrass-dominated settings. Therefore, understanding the standing stock of *H. macroloba* and its actual CaCO_3 contribution in Dongsha Island would provide a valuable comparative insight into the carbonate contribution of *Halimeda* in the global context. The aim of this study was to estimate the standing stock and CaCO_3 content of this species in a seagrass-dominated ecosystem and provide a better understanding of the potential role of *H. macroloba* as a CaCO_3

producer in comparison to other *Halimeda*-dominated ecosystems. The outcome of this study will also benefit efforts to produce a better global estimate of carbonate production by *H. macroloba*.

Materials and methods

Study site

The Dongsha Atoll is located on the northern part of the South China Sea and is 25 km in diameter (Figure 1A, B). This study was carried out at Dongsha or Pratas Island, located on the western margin of the Dongsha Atoll (Figure 1B). Dongsha Island is around 2.80 km long and 0.87 km wide. This site has two seasons: a winter season dominated by the north-east monsoon (October–April) and a summer season dominated by the south-west monsoon (May–September). The marine environment around Dongsha Island and its atoll provides different types of habitat, including seagrass beds, coral reef and a mixture of both. *Halimeda macroloba* and *Caulerpa* spp. (including *C. cupressoides*, *C. oligophylla* and *C. sertularioides*) are abundant seaweeds in the seagrass beds of Dongsha Island and its atoll (our own observations) (Supplementary Figure S1). Dongsha Island protects the lagoon from much of the disturbance caused by ocean waves. In addition, the water inside the lagoon is exchanged daily with water from outside the lagoon through north-western and south-western inlets, where the coral reefs are relatively less extensive with a mixture of corals and sandy substrates that allow the development of seagrass beds. Seven seagrass species have been identified around Dongsha Island: *Cymodocea rotundata*, *Cymodocea serrulata*, *Halodule uninervis*, *Halophila ovalis*, *Syringodium isoetifolium*, *Thalassia hemprichii* and *Thalassodendron ciliatum* (Chou

et al., 2018). This study was conducted in the seagrass beds on the east coast of Dongsha Island (Figure 1C) on 23 May–20 June 2019. The study site has sandy substrates and over 70% of the area was covered by seagrasses (Figure 1D). The seagrass meadows are broadly distributed in the shallow subtidal zones, ranging between 1.5 and 3 m in depth at low tide based on depth gauges during scuba diving. Nitrate and phosphate concentrations where we conducted our survey were roughly 0.16 and 0.11 $\mu\text{mol l}^{-1}$ according to point measurements made over the course of our survey period ($N=3$). Additionally, the water temperature was measured using HOBO Pendant Temperature/Light Data Loggers (Onset Computer Corporation, USA), averaging 30.9°C over the course of our survey.

Density of macrophytes

The density of macrophytes (i.e. *H. macroloba*, *Caulerpa* spp. and seagrasses) were measured using eight line transects of 50 m. Line transects were laid perpendicular to the shoreline, each 20 m apart. Two quadrats (50 × 50 cm) were placed at 5 m intervals along each line transect to estimate the density of macrophytes based on percentage cover of seaweeds and shoot density of seagrasses, resulting in a total of 176 quadrats in the surveyed 7000 m² area.

Growth rate and calcium carbonate accumulation rate

To investigate the growth rate and calcification rate, 15 *H. macroloba* specimens with no epiphytes were collected from the site where *Cymodocea rotundata* and *Syringodium isoetifolium* were the dominant seagrass species. *Halimeda macroloba* thalli were stained with Alizarin Red-S (a concentrated solution = 1 g per 100 ml of seawater) for 10–12 h. Then, each sample was tied to a wooden stick and transplanted back into the field. After 7 days, the dyed thalli were collected and taken back to the laboratory at Tunghai University to measure growth and calcification rates. All thalli without their basal holdfast were cleaned of epiphytes and bleached in a 5–10% sodium hypochloride solution for 20–30 min until the thalli lost their green colour. The segments were separated into (1) the stained segments or old segments and (2) the unstained segments or new segments. The numbers 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 weight of the unstained segments (new segments) was used to calculate the growth rate ($\text{g DW thallus}^{-1} \text{ day}^{-1}$). After the determination of the dry weight of the calcified segments, the new segments were decalcified in 5% hydrochloric acid for 10–15 min then rinsed with fresh water. These decalcified segments were placed in a drying oven at 65°C to achieve constant dry weight and then weighed to determine their somatic weight. The CaCO_3 accumulation rate was calculated based on the difference in the dry weight of the calcified new segments and their somatic weight produced per thallus and per day ($\text{g CaCO}_3 \text{ thallus}^{-1} \text{ day}^{-1}$) (van Tussenbroek & van Dijk, 2007; Mayakun *et al.*, 2014; Mayakun & Prathep, 2019). The mean value was expressed in terms of per thallus because it can convert to annual CaCO_3 production rate ($\text{g CaCO}_3 \text{ m}^{-2} \text{ year}^{-1}$) by multiplying $\text{CaCO}_3 \text{ thallus}^{-1} \text{ day}^{-1}$ by mean density and 365 days.

Segment CaCO_3 content and number of aragonite crystals of life stages

To examine whether segment CaCO_3 content increased with age, we measured CaCO_3 content, number and width of aragonite crystals of each life stage of *H. macroloba*. In this study, four

developmental stages were categorically defined in our *H. macroloba* samples as follows: stage 1: newly recruited plant; stage 2: young plant with 3–4 new segments and 1–2 levels of segment; stage 3: partially calcified plant with 3–4 levels of segment; and stage 4: mature calcified plant. We followed such developmental categorization according to the definition of Mayakun & Prathep (2019). Unfortunately, stage 5: fertile plant with reproductive cells and stage 6: dead plant, were rarely found and could not be collected. For the segment CaCO_3 content, 210 thalli were haphazardly selected and at least 10 thalli of each stage of *H. macroloba* were collected. All thalli without their basal holdfast were cleaned and brought back to the laboratory, Tunghai University. The number of both old and new segments in each thallus and stage was counted, and dried at 65°C to determine their calcified dry weight. Then, the segments were decalcified by placing in 5% hydrochloric acid for 10–15 min then rinsed with fresh water for several times. These decalcified segments were dried at 65°C and then weighed afterward to determine their somatic weight. The segment CaCO_3 content per segment of each life stage was derived from the difference in the dry weight of the calcified segments and their somatic weight ($\text{g CaCO}_3 \text{ segment}^{-1}$). Dry segments of each stage were brought back to Prince of Songkla University, Thailand to examine the number and the width of aragonite crystals. For number of aragonite crystals, images of aragonite crystals were examined among the four life stages to calculate crystal numbers using a scanning electron microscope (SEM-Quanta) operating at 20 kV. Specimens at each of the four life stages were mounted on aluminium stubs using double-sided carbon adhesive tape, and sputter-coated with gold. An area of 1 μm^2 was selected ($N=10$ per stage), the aragonite crystals were counted ($\text{number } \mu\text{m}^{-2}$) and the width of aragonite needles was measured.

Statistical analyses

Density of *H. macroloba* (number of thalli per quadrat; $N=88$) was fitted using a general linear regression. We subsequently included principal coordinates of neighbour matrices (PCNM) eigenvectors to de-trend the spatial pattern of the density of *H. macroloba* (number of thalli per quadrat) and then included polynomial terms (degree of 1 and 2) of seagrass density. Each PCNM eigenvector and polynomial term of seagrass density were imported for the downstream analyses only if P -value was smaller than 0.05 and its inclusion increased the adjusted R^2 . We partitioned the variation of the *H. macroloba* density into the seagrass spatial pattern effect and the seagrass density effect (Borcard *et al.*, 1992) and performed type III F-tests to examine the marginal effects. One-way analysis of variance (ANOVA) was used to test for differences in the number and the width of aragonite crystals and CaCO_3 content among stages of *H. macroloba*. Tukey's multiple comparisons were used. All data were analysed using R statistical package and the software SPSS v. 13.0.

Results

The field survey revealed that *Cymodocea rotundata* and *Syringodium isoetifolium* were common and dominant species with 626.36 ± 26.58 and 290.93 ± 18.56 shoots m^{-2} , respectively. Three common *Caulerpa* species were found in the seagrass beds in this area: *C. cupressoides*, *C. oligophylla*, *C. sertularioides*, with cover around $0.09 \pm 0.06\%$, $7.93 \pm 0.99\%$, $0.19 \pm 0.11\%$, respectively. Coral cover was around $3.51 \pm 0.90\%$. The mean density of *H. macroloba* was 8.82 ± 1.57 thalli m^{-2} . Given that our surveyed area is 7000 m², the standing stock of this species was estimated at 61,740–72,730 thalli (calculated as mean density multiplied by the area of occurrence). Our PCNM analyses

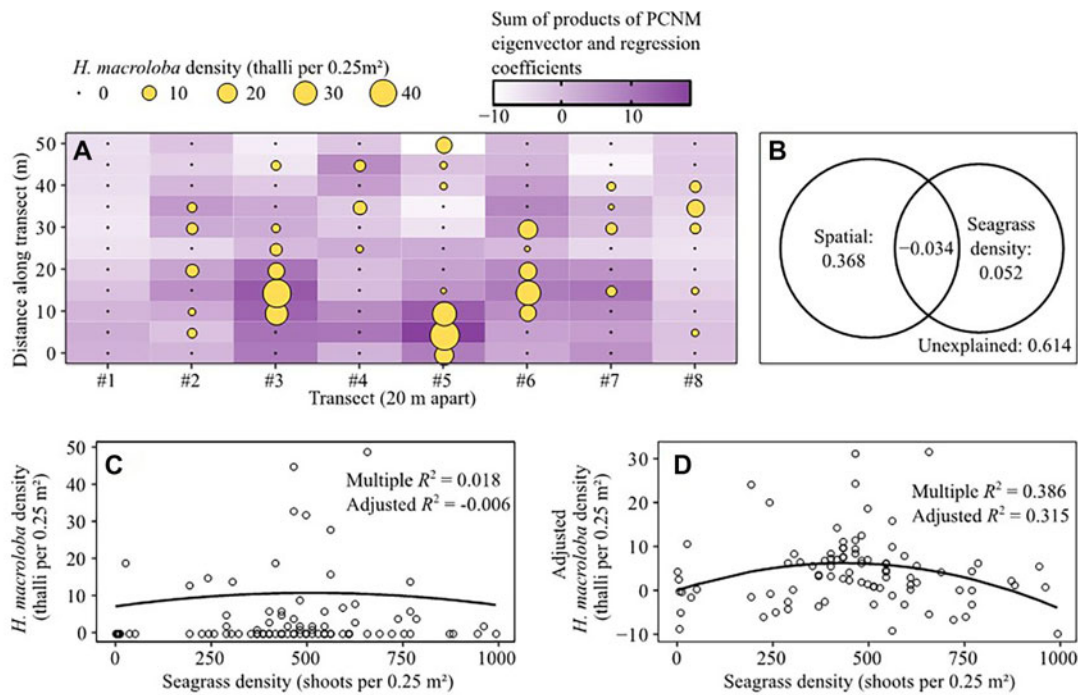


Fig. 2. (A) Principal coordinates of neighbour matrices (PCNM) results showing the spatial distribution of *H. macroloba* in our surveyed area; (B) relative effects of the spatial structure and seagrass shoot density on *H. macroloba* distribution; (C) the relationship between *H. macroloba* density and seagrass density before; (D) after the spatial de-trend.

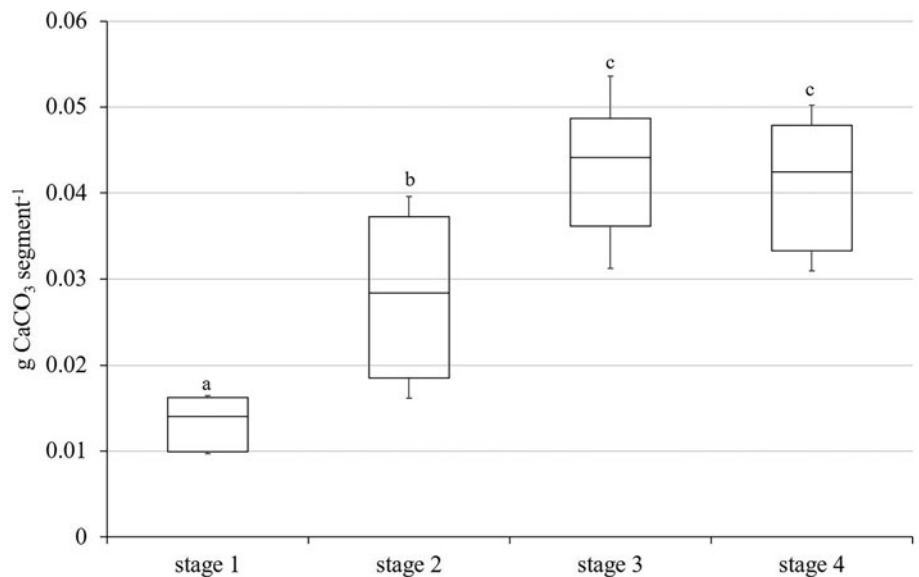


Fig. 3. CaCO_3 content at each studied life stage of *H. macroloba*: Boxes illustrate the 25th, 50th and 75th percentiles. Whiskers indicate the 10th and 90th percentiles. Different letters (a, b, c) indicate significant differences among groups (Tukey, $P < 0.05$).

revealed that the distribution of *H. macroloba* is spatially clustered in certain regions in our surveyed area (Figure 2A). A total of seven different spatial clusters of *H. macroloba* were extracted by the PCNM analyses (Supplementary Figure S2). The variation partitioning analysis revealed that the spatial clustering of seagrass contributed to about 37% ($F = 6.686$, $df = 7, 78$; $P < 0.001$) of total variance in terms of the distribution pattern of *H. macroloba*, followed by the seagrass density contributing 5% ($F = 3.287$, $df = 2, 78$; $P = 0.043$) of the total variance on the *H. macroloba* distribution (Figure 2B). As expected, without removing the effect of spatial factor that is likely associated with other unmeasured abiotic and biotic factors, we did not observe a significant relationship between *H. macroloba* density and seagrass density (total $R^2 = 0.018$, adjusted $R^2 = -0.006$, $F = 0.762$, $df = 2, 85$; $P = 0.470$; Figure 2C). However, after de-trending the spatial factor (treated

as a confounding factor herein given that we want to explore whether or not the seagrass competition (or density) might have any influences on the density of *H. macroloba*), the seagrass density (i.e. the main biotic factor measured in this study) and its quadratic term were entered to increase the goodness of fit (multiple $R^2 = 0.386$, adjusted $R^2 = 0.315$; Figure 2D), and we observed a trend that *H. macroloba* was more dominant and associated with seagrass at densities of 150–400 shoots m^{-2} and decreased when it occurred with seagrass densities below 100 or above 400 shoots m^{-2} (Figure 2D).

Thalli of *H. macroloba* produced 1–2 new segments day^{-1} , giving a daily dry weight growth rate per thallus of 0.003 ± 0.001 g. Multiplying mean density by daily growth rate, the daily increase of algal biomass was calculated to be 0.03 g DW m^{-2} day^{-1} or 9.66 g DW m^{-2} year^{-1} . It was estimated that in each square

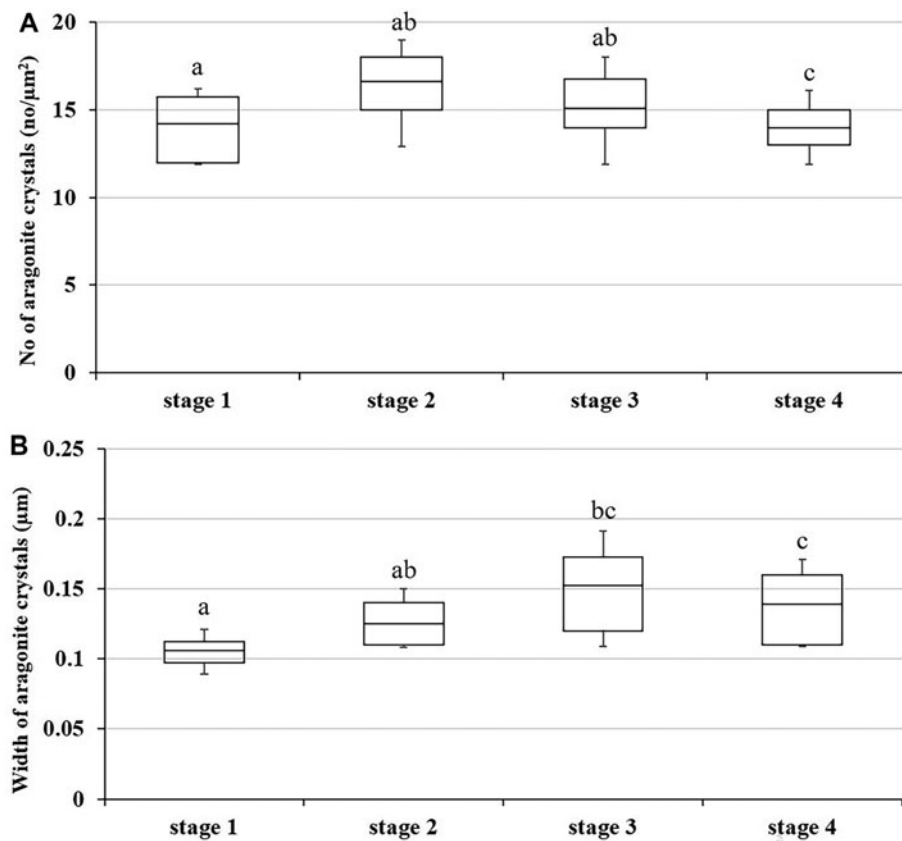


Fig. 4. (A) Number and (B) width of aragonite crystals in each studied life stage of *H. macroloba*: Box plots illustrate the 25th, 50th and 75th percentiles. Whiskers indicate the 10th and 90th percentiles. Different letters (a, b, c) indicate significant differences among groups (Tukey's post hoc test, $P < 0.05$).

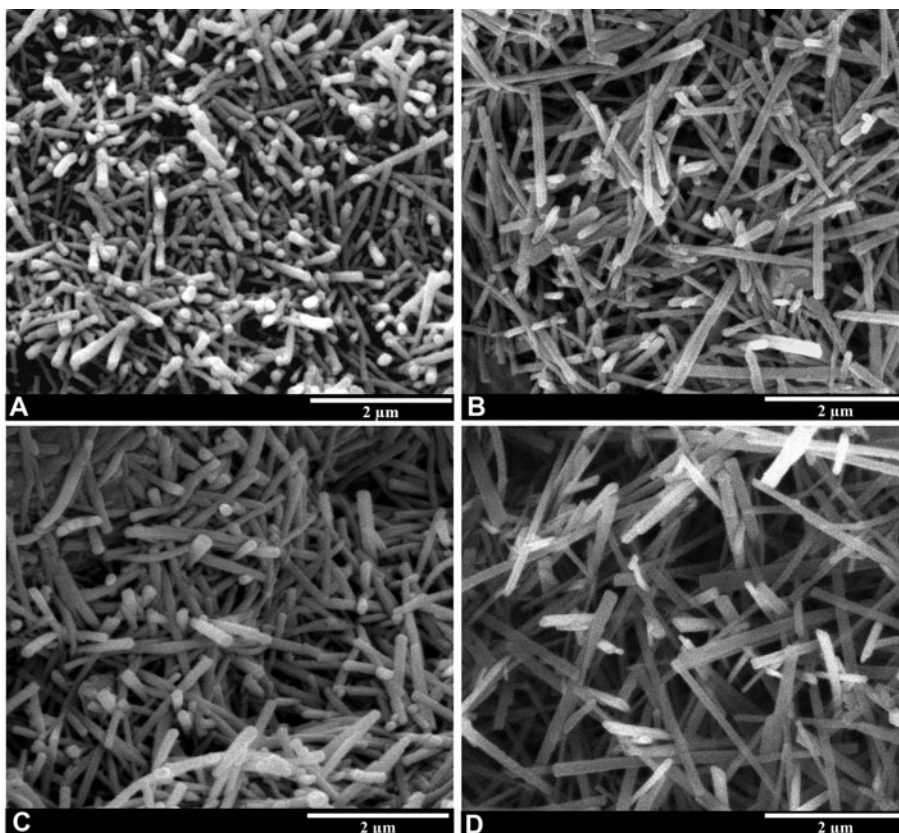


Fig. 5. Scanning electron microscope (SEM) photographs show aragonite crystals from specimens of *H. macroloba* at four stages of development: (A) newly recruited plant; (B) young plant with 3–4 new segments and 1–2 levels of the segment; (C) partially calcified plant with 3–4 levels of the segment; (D) mature and calcified plant. Scale bar = 2 μm .

metre *H. macroloba* produced around 8.82–17.64 new segments per day.

For the CaCO_3 accumulation rate, *H. macroloba* accumulates CaCO_3 around $0.002 \pm 0.001 \text{ g CaCO}_3 \text{ thallus}^{-1} \text{ day}^{-1}$ or 6.44 g

$\text{CaCO}_3 \text{ m}^{-2} \text{ year}^{-1}$. The mean CaCO_3 content of *H. macroloba* was around $0.32 \pm 0.05 \text{ g CaCO}_3 \text{ thallus}^{-1}$. For the segment CaCO_3 content of each stage, the segment CaCO_3 content was assessed from a total of 1301 segments and 59 individual thalli.

The segment CaCO₃ content increased with age and *H. macroloba* accumulated less CaCO₃ in stage 1 than in stages 2, 3 and 4 (Figure 3). The lowest CaCO₃ content was found in stage 1 and the highest content was found in stage 3 (0.014 ± 0.004 and 0.044 ± 0.004 g CaCO₃ segment⁻¹, respectively). There was a significant difference in CaCO₃ content among the 4 stages ($P < 0.05$).

For the aragonite crystals, the highest number of crystals was found in stage 2 with 16.65 ± 0.57 crystals μm^{-2} and the lowest number of crystals was found in stage 4 with 14.00 ± 0.58 crystals μm^{-2} (Figure 4A). There was a significant difference in the number of aragonite crystals between the four stages of *H. macroloba* ($P = 0.013$). The number of aragonite crystals was significantly greater in stage 2 than in stages 1 ($P = 0.01$) and 4 ($P = 0.005$). The crystals of stage 3 were the widest at 0.15 ± 0.01 μm and the crystals in stage 1 were the thinnest at 0.11 ± 0.00 μm . There was a significant difference in the width of crystals between the four stages ($P < 0.001$) (Figure 4B). SEM photos of aragonite crystals showed that thin and short aragonite needles presented in stage 1 specimens and wider aragonite needles presented in specimens from stages 2, 3 and 4 (Figure 5).

Discussion

This study is the first to estimate the standing stock, growth rate, CaCO₃ accumulation rate, aragonite crystal numbers and CaCO₃ content of *H. macroloba* in the rarely studied Dongsha Island in the South China Sea. This study provides baseline data and an understanding of the potential role of *H. macroloba* as a CaCO₃ contributor to the marine carbonate budget and in comparison to other *Halimeda*-dominated ecosystems. Such baseline data can improve the rough global estimate of carbonate production in *H. macroloba*. We observed that *H. macroloba* grows fast, producing 1–2 new segments day⁻¹. Overall, annual biomass and CaCO₃ production were low in Dongsha Island because these are determined by *H. macroloba* density, which can be influenced by many abiotic and biotic factors. However, in this study, we found that seagrass density is not the main driver regulating *H. macroloba* density although our statistical analysis reveals that seagrass density can play a role in regulating *H. macroloba* density. Thus, the low annual biomass and CaCO₃ production in the seagrass-dominated ecosystem in Dongsha Island might be largely controlled by other factors, instead of seagrass competition. The growth rate of *H. macroloba* is estimated in the literature to be 0.0015 – 0.021 g thallus⁻¹ day⁻¹, implying the production of 1–2 new segments per day, and CaCO₃ production ranging between 1.87 (the lowest estimate) and 908.11 (the highest estimate) g m⁻² year⁻¹ (Sinutok et al., 2008; Mayakun et al., 2014; Mayakun & Prathep, 2019). Our growth rate and annual CaCO₃ production estimation (0.003 g thallus⁻¹ day⁻¹ and 6.44 g m⁻² year⁻¹, respectively) fell within the lower limits of studies conducted in mono-specific patches of *H. macroloba* in the tropical intertidal ecosystem (Table 1). Davis & Fourqurean (2001) showed that the interspecific competition among seagrass, *Thalassia testudinum* (400–800 short-shoots m⁻²) and *Halimeda incrustata* (100 thalli m⁻²) in mature seagrass bed decreased the growth rate and size of *Halimeda* thalli by competing for nitrogen resources. Other than nutrients, seagrass is also a superior competitor for space and light after its establishment and can decrease algal abundance (Barry et al., 2013). Thus, the low production of *H. macroloba* estimated in this study is partially because *H. macroloba* in Dongsha Island coexists with the large seagrass species – *Cymodocea* and *Syringodium* – that impose an interspecific competition for resources such as space, nutrients and light. In results comparable to this study, Sinutok et al. (2008) and Sinutok (2008) reported a low growth rate and CaCO₃ production

for *H. macroloba* at Tangkhen Bay, Phuket, Thailand (Table 1). In their study site, *H. macroloba*, however, experienced very low interspecific competition from the seagrass, *Halophila ovalis*, which grew primarily underneath *H. macroloba*. Thus, the low growth rate and CaCO₃ production of *Halimeda* may be explained by other factors in their study. Personal communications with the authors indicated that high sedimentation and strong wave action are present at the Tangkhen Bay site. These two physical disturbances could explain the unexpectedly low growth and carbonate accumulation in *Halimeda*. Without seagrass competition, high sedimentation, and strong waves, Mayakun et al. (2014) and Mayakun & Prathep (2019) showed much higher density, growth rate and CaCO₃ production of *H. macroloba* at Ko Mat Sum, in the Gulf of Thailand (with mean density of 11.70 ± 1.77 thalli m⁻²) and Lidee Island in the Andaman Sea (with mean density of 44.42 ± 13.95 thalli m⁻²), respectively.

Seagrass has been shown to compete with *Halimeda* for space, light and nutrients (Davis & Fourqurean, 2001; Barry et al., 2013) and limit the *Halimeda* establishment at higher seagrass densities. Thus, we expect to observe a negatively correlated relationship between *Halimeda* density and seagrass density if they indeed compete with each other for resources. From our field survey, we unexpectedly observed that the density of *H. macroloba* seems to reach a peak when the seagrass densities range between 150–400 shoots m⁻². In other words, the density of *H. macroloba* is lower when the shoot density of seagrasses is below 100 or above 400 shoots m⁻². Harney & Fletcher (2003) showed that the low or moderate density of seagrasses can benefit the recruitment of algal germlings by providing space, reducing strong wave action and trapping and stabilizing sediments. But, if the seagrass density is too high, seagrass would over-compete *H. macroloba*. Thus, our observation might partially support the ‘resource competition’ point of view when the seagrass density is not too high. In contrast, we observed a trend that *H. macroloba* density is low when seagrass density is also low. Additionally, we often observed bite marks on segments of *H. macroloba* in low seagrass density. A follow-up field study using a video camera revealed that *H. macroloba* was quickly consumed by herbivorous fishes such as parrotfishes within 1–2 days when we placed *H. macroloba* in bare sands whereas those placed in the seagrass beds were not (data not shown). According to these observations, we hypothesize that the moderate density of seagrasses might benefit *H. macroloba* by providing it with a refuge and reducing the grazing impact from herbivorous fishes. Thus, *H. macroloba* density that reaches a peak between low and high density of seagrasses could possibly be the outcome of the ecological trade-off between herbivorous grazing and seagrass competition. Alternatively, the sand size or microhabitat type at the edge of the seagrass bed in which the ecosystem transits from a seagrass-dominated to a coral-dominated area might not be suitable for the growth of both seagrasses and *H. macroloba* in our surveyed area. The relative contribution of unmeasured biotic (e.g. grazing) and abiotic (e.g. microhabitat type) factors on the spatial distribution of *H. macroloba* await further examination.

In this study, we found that CaCO₃ production significantly varied with different age in *H. macroloba*. The CaCO₃ content and number of crystals increased with age, being highest in stages 2, 3 and 4. Small and short aragonite needles were found in new segments in early stages and wider aragonite needles were found in later stages. The trend that emerged from the present study was similar to that reported from Thailand by Mayakun & Prathep (2019) who found the lowest number of crystals and short needles in stages 1 and 2 while small, long needles were found in stages 3 to 6, indicating that aragonite deposition increased with age. Borowitzka & Larkum (1977) suggested that the calcification of

Table 1. Comparison of CaCO₃ production for *Halimeda in situ*

Species	Habitat	Location	Density (individuals m ⁻²)	CaCO ₃ (g CaCO ₃ m ⁻² year ⁻¹)	New segments (segment thallus ⁻¹ day ⁻¹)	Growth rate (g thallus ⁻¹ day ⁻¹)	Study
<i>H. tuna</i>	Florida Keys	Shallow Conch (5 m in depth)	70.61	77.62	0.54–1.21	nr	Vroom <i>et al.</i> (2003)
<i>H. macroloba</i>	Intertidal zone, coexist with very low-density seagrass, <i>Halophila ovalis</i>	Tangkhen Bay, Phuket, Andaman Sea	18.72 (dry) 5.02 (rainy)	11.05 (dry) 1.87 (rainy)	nr	0.0020 (dry) 0.0015 (rainy)	Sinutok <i>et al.</i> (2008)
<i>H. macroloba</i>	Intertidal zone, mono-specific patch	Ko Mat Sum, Gulf of Thailand	11.70 (mean density) 26.83 (the highest density)	72.60 (mean density) 166.48 (the highest density)	1.00–2.00	0.0201	Mayakun <i>et al.</i> (2014)
<i>H. macroloba</i>	Intertidal zone, mono-specific patch	Lidee Island, Satun, Andaman Sea	44.42 (mean density) 138.22 (the highest density)	291.84 (mean density) 908.11 (the highest density)	1.00–2.00	0.0210	Mayakun & Prathep (2019)
<i>H. macroloba</i>	Shallow subtidal zone, coexist with high density seagrasses; <i>Cymodocea</i> and <i>Syringodium</i>	The main island of Dongsha Island, South China Sea	8.82	6.44	1.00–2.00	0.0030	This study
<i>H. incrassata</i>	Reef lagoon, coexist with seagrasses <i>Thalassia testudinum</i> and <i>Syringodium filiforme</i>	Puerto Morelos, Yucatan Peninsula, Mexican Caribbean	99.00	815.00	~1.17	nr	van Tussenbroek & van Dijk (2007)
<i>H. incrassata</i>	Sand banks, Sand dunes	The continental shelf of eastern equatorial South America, Brazil	174.00	1190.00	0–15.17	nr	Carneiro & Morais (2016)
<i>H. macrophysa</i> <i>H. micronesica</i>	Reef flat and reef slope	Kandahalagala Island, Maldives	32.00–39.00	~70.00	nr	nr	Perry <i>et al.</i> (2016)

nr, not reported.

Halimeda coincides with growth and development, peripheral utricles fusion and photosynthesis. Once CaCO₃ was precipitated, the crystals began as small granules and then the deposition spread over the entire intercellular space. Overall, these observations raise an interesting question as to whether or not the CaCO₃ production of *Halimeda* under different developmental stages can also be regulated by the seagrass density. Although it is beyond the scope of this study, the effects of the ecological trade-off between seagrass competition and grazing pressure on CaCO₃ production in *Halimeda* would be an interesting topic for further examination.

This study supported the view that *H. macroloba* has a role in CaCO₃ production. Although untested, our preliminary observations suggested that shading by seagrass might be detrimental to the growth rate and CaCO₃ production of *H. macroloba* in this area. Qualitative and quantitative studies based on population dynamics, standing stock, reproduction and accurate measurement of growth rate and CaCO₃ production are very important and useful in monitoring of *Halimeda* populations and estimation of CaCO₃ production by the species. Unlike the *Halimeda*-dominated ecosystems observed in tropical areas such as Thailand, competition with seagrasses might impose an adverse condition for the occurrence of *H. macroloba* in the seagrass bed in this tropical area. Nevertheless, the co-occurrence of *H. macroloba* and seagrasses is a common ecological phenomenon in Dongsha Island in comparison to tropical areas where these two benthic organisms rarely co-occur abundantly. Based on our field observations about the grazing pressure on the *H. macroloba* populations in low density seagrass beds or bare sands at the edge of seagrass beds, we hypothesize that the moderate density of seagrasses might serve as a refuge to relieve *H. macroloba* from the grazers (i.e. the seagrass-refuge hypothesis). Dongsha Island is located in an area of the South China Sea that is predicted to be highly impacted by climate change (Tkachenko & Soong, 2017). Thus, the co-occurrence of *H. macroloba* and seagrass in Dongsha Island provides an interesting ecological setting to further explore the trade-off between interspecific competition and grazing and the effect of changes in unmeasured factors (e.g. microhabitat types) on their spatial distribution.

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