The production of calcareous sediment by *Halimeda incrassata* in the Puerto Morelos Lagoon, Mexican Caribbean

By Korjent van Dijk

Supervised by: Brigitta I. van Tussenbroek
Estación Puerto Morelos,
Instituto de Ciencias del Mar y Limnología
Universidad Nacional Autónoma de México
and Anneke Breeman
Rijksuniversiteit Groningen
September 1997 - May 1998
Study thesis for:
Department Marine Biology
University of Groningen

D 652

Contents

	Page
Summary	iii
Introduction	1 - 3
Halimeda	3 - 6
Study area	7 - 8
Material and Methods	9 - 14
Results	14 - 24
Discussion	25 – 31
Acknowledgements	32
References	33 - 35

...it Groningen
Bibliotheek Biologisch Centrum
Kerklaan 30 — Postbus 14
9750 AA HAREN

Summary

Halimeda incrassata is the most abundant of Halimeda species growing in the Puerto Morelos reef lagoon (Mexico). Somatic plus calcareous dry biomass was 120.32 g m^{-2}, 117.99 g m^{-2} and 77.90 g m^{-2} at the Reef-, Lagoon-, and Beach-station respectively. Growth was measured during a period of two weeks in the months November, December (1997) and January (1998) using Alizarin Red S as colouring agent. Different variables were measured to establish a time sparing methodology. The dry weight resulted to be the best parameter. For each month and station, regressions of the somatic calcareous dry weight of the thallus vs. the somatic calcareous dry weight of the new segments were computed. Based on previous data, yearly production of *Halimeda incrassata* was estimated at 224.0 g and 140.5 g somatic calcareous dry weight m^{-2} y^{-1} for the Reef- and the Lagoon-station respectively. Variations in growth between sampling months were too large at the Beach-station to deduce yearly production. The contribution of new plants to the production was small, the highest value being 13.3% of total production of the Beach-station in January. Maximum number of new visible thalli was 12 in two weeks. Mean percentage $CaCO_3$ of the total somatic calcareous dry weight was 86.1%. Calcium content of new and old segments was similar at the Reef-station, but at the Lagoon- and Beach-station the percentage of Calcium in new segments was lower than that in the old segments. Percentage of $CaCO_3$ of the thalli did not differ throughout the three months at the Reef-station (86.46%) and Lagoon-station (86.24%), however differences were encountered at the Beach-station in sampling periods. This study also revealed that production results in some other studies were probably overestimated due to the use of other growth parameters. The present work will be used as a pilot study for further analyses of somatic calcareous production of *Halimeda incrassata*.

Introduction

Halimeda is a primary producer of organic matter and Calcium Carbonate to sand- and mud-size Carbonate sediments in tropical shallow water reef ecosystems. Drew and Able (1985) found that segments comprise up to 99% of the calcareous sediments of algal bioherms, which are comparable to coral reef structures, but their contribution is normally lower and differs from place to place. A deposition of 1 m sediment every 1892 years was found for the Davies Reef, Australia, halimeda contributing 25% (Drew 1983). Not only segments of *Halimeda* spp. contributed to calcareous sediments but also calcified algae, corals, molluscs, bryozoa and foraminifers are of great importance. At the San Blas Archipelago, Panama ≈89% of the sediment by weight was calcareous; of the >125µm fraction of the surface sediment, 25% was of Halimeda origin and 27% of coral origin (Freile & Hillis 1997).

The growth rate of *Halimeda* spp. is very high. A fast growing tip can produce one segment a day, but not all tips of a thallus grow simultaneously. A production of 356 segments out of one growing tip in 68 days has been measured for *H. opuntia* at the Davies Reef, Australia (Drew 1983). A daily growth average for *H. incrassata* of 3.30 ± 2.18 segments d^{-1} per plant was measured at the Tiahura Reef, Tahiti (Payri 1988). But while some fronds of the thallus are actively growing, others are losing segments at the same time. Dying segments loose their colour, turn white, possibly attract epiphytes and then fall off the thallus (Hillis-Colinvaux 1980). A halimeda thallus is capable of producing many times its own weight in one year; a turnover rate of seven crops a year was reported for *H. incrassata* at Bermuda (Wefer 1980). The resulting halimeda litter is responsible for the large amount of sediment. The contribution of halimedas to the total carbonate production of the world has been estimated to be 10% (Hillis 1997). This is a crude figure and has been calculated with the scarce available information on this subject at the moment. Freile *et al.* (1995) estimated a production of 2400 g $CaCO_3$ m^{-2} y^{-1} for *Halimeda* spp. on Great Bahama Bank using visual density observations and the data of Hillis-Colinvaux (1980). A similar production of 2323 g $CaCO_3$ m^{-2} y^{-1} for *H. incrassata* was estimated by Freile & Hillis (1997) at the Pico Feo lagoon, Panama. They used 177 individuals over a period of 3,7,10,14,18 and 23 day intervals, to estimate the production of the lagoon. Payri (1988) measured the production of *H. incrassata* f. ovata using 328 individuals for a period of 6 months at Tiahura reef, Tahiti and estimated 2300 g $CaCO_3$ m^{-2} y^{-1}. Drew (1983) found 2234 g dry calcareous weight m^{-2} y^{-1} for *H. opuntia* and *H. copiosa* at the Davies Reef lagoon, Australia. The last study only concerned lithophytic species, which mostly grow faster than psamophytic ones. A

problem with these lithophytics is their patchy distribution, making estimates of contribution of these species to calcareous sediments for a whole area difficult.

Biomass of *Halimeda* spp. can also be very high. Drew & Able (1985) found an average calcareous biomass of 503 g $CaCO_3$ m^{-2}, with a maximum of 4637 g $CaCO_3$ m^{-2} in one sample (*Halimeda* spp) for meadows of the Great Barrier Reef. This was measured with a "van Veen" grab sampler at seven different stations (five samples per station). Biomass of *Halimeda* spp. generally changes little throughout the whole year, which means that the new growth must be equal to the loss of older segments (Drew 1983).

The contribution of calcareous sediment by psamophytic halimeda species in lagoons of the Caribbean must be considerable. Even if the relative biomass is low compared to bioherms and reefs, which are inhabited by lithophytic species, the overall contribution is high, considering the large areas they inhabit. Reef lagoons in the Caribbean are mainly inhabited by *H. incrassata* and *H. monile*, which normally have a lower biomass, compared with halimedas in the Pacific reef lagoons. (Table 10)

The subject of the present study is *Halimeda incrassata*, because it is the most abundant halimeda species in the Puerto Morelos lagoon. It is also the easiest halimeda species to handle and colour with Alizarin Red S. Wefer (1980), estimated a production of 50 g $CaCO_3$ m^{-2} y^{-1} for *H. incrassata* (155 ind.) Bermuda and Multer (1988) of 97.1 g $CaCO_3$ m^{-2} y^{-1} for *H. incrassata* and *H. monile* (>1800 ind.) in Antigua: in both studies Alizarin Red S was used as a time marker. This is the first study on the halimeda production in the Puerto Morelos reef lagoon (Mexico).

This thesis concerns three months (November, December (1997) and January (1998)). The short duration of the study is due to logistic reasons. This study is part of a larger project concerning *Halimeda incrassata.*

Objectives of this project are:

1. To obtain a figure of the contribution of *Halimeda* spp. to calcareous sand production in the Puerto Morelos Reef lagoon.
2. To obtain a more detailed view of the variability (seasonal and spatial) of the growth and biomass of rhipsalian *Halimeda incrassata* in a tropical reef lagoon.

Aims of this study are:

1. To determine the biomass of *Halimeda incrassata* at three stations in the Puerto Morelos reef lagoon.
2. To determine the growth rate, carbonate percentage, production of *Halimeda incrassata* in the Puerto Morelos lagoon during a period of three months, to evaluate possible temporal variations.
3. To establish procedures for further monitoring.

Halimeda

The genus *Halimeda* is first recorded in the Cretaceous, but is supposed to date from the Middle Jurassic (Elliott 1960, Johnson 1969). Fossil *Halimeda* spp. has been found in limestone facies of the Tethyan region (Elliott 1960), Mexico and Texas (Johnson 1969).

The genus is currently placed in the order Caulerpales (Wynne 1986) of the Chlorophyta (i.e. green algae) and are amongst the largest and most complex genera of the green algae. Thirty species are distinguished according to shape, size and internal structure of their segments (Drew 1983). Most species can be found at depths of 0 - 50 m (Hillis-Colinvaux 1980), but some halimeda species have been found on deep-reef slopes in the Bahamas at a depth of 150 m (Blair & Norris 1988). The geographical distribution of halimeda is limited to tropical seas. They are found wherever there is sufficient light and appropriate substrate. The usual temperature at which halimeda grows is approximately 25°C (Hillis-Colinvaux 1980) and has an optimum temperature between 27°C - 29°C. *H. tuna* and *H. cuneata* also grow in the Mediterranean and tolerate colder temperatures, approximately 20°C (Hillis 1959). Halimeda vegetations are particularly well developed in the neighbourhood of coral reefs. In these reef communities, different species may form either sand dwelling (psamophytic) communities, anchored by substantial rhizodal holdfasts, or lithophytic communities on dead coral, crustalline red algae, or other hard substrata (Hillis-Colinvaux 1974).

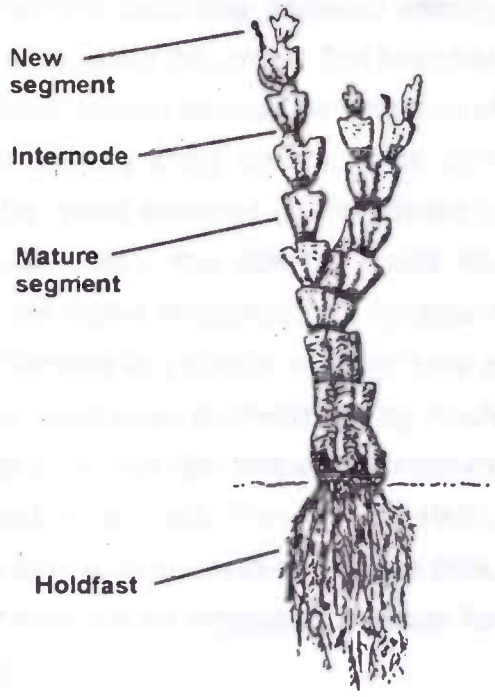


Figure 1
Schematic picture of *Halimeda sp.*.
(Picture from Multer 1988)

Halimeda is easy to recognise; it looks like an underwater cactus (Hillis-Colinvaux 1980), with many segments. The plant can reach heights of 25 cm if erect, or sprawl to over 1 m length. The thallus is formed by a holdfast and a series of calcified segments (Figure 1), which are connected by small uncalcified nodes. The constructional units of the entire alga, both holdfast and segments, are the parallel non-septated filaments, which have a diameter of approximately 0.05-0.1 mm. The filament itself is unusual compared to filaments of other plants. It lacks crosswalls, which would divide the filament into a linear row of cells. A halimeda thallus is constructed out of a mass of these filaments. This filament without crosswalls, or coenocytic filament, occurs also in many other algae belonging to the order of the Caulerpales (*Penicillus*, *Tydemania*, *Udotea* and *Caulerpa*) (Hillis-Colinvaux 1980), and few genera belonging to other families of the Chlorophyta (Lobban & Wynne 1981). The holdfast, which anchors the plant, can be a few loose filaments, to a truly massive production, which may extend 13 cm or more in the substrate (Hillis-Colinvaux 1980). The holdfast is formed by pigmentless uncalcified rhizoids (Walters 1994). These are not organised into a regular shape such as the segments, but generally branch irregularly to form a mass of threads (Hillis-Colinvaux 1980). The segments show, in contrast to the holdfast, a definite pattern of organisation. In most species several central, so-called medullary, filaments run the length of the segments and so the entire length of the branches, "stringing" the segments together. These filaments form the core or medulla. The filaments generally branch trichotomously, with the resultant branches becoming displaced laterally and rebranching one to three times. This branch system is called the cortex. The branches themselves, called utricles, are relatively short and become shorter towards the periphery of the segments. The outermost or peripheral branches called primary utricles, touch their peripheral edges and adhere in mature segments. The space enclosed by the utricles is called the interutricular-space and is completely closed from the outside. Inside the siphoneous filament the organelles are not uniformly distributed throughout the cytoplasm although the construction is coenocytic. The medullar filaments in a mature segment have a thin peripheral layer of cytoplasm with few chloroplasts, while outer filaments occupy much of the volume and the vacuole thereby being less extensive. The disc-shaped chloroplasts are very abundant and migrate internally at night (Stark *et al.* 1969, Drew & Able 1990). Looking at the utricles on surface view, they appear to form a honeycomb-structure of cells, although they are the tips of the filaments. These utricles are an important character for determining the different species (Hillis-Colinvaux 1980).

Deposition of Calcium takes place in the interutricular-spaces of the cortex and medulla, (Askenasy 1888, Wilbur *et al.* 1969, Borowitzka and Larkun, 1976a, b). All species of halimeda deposit Calcium Carbonate ($CaCO_3$). Calcification begins when the new

segment is ≈36 hours old (Wilbur *et al.* 1969), even the small young thalli, that appear flatulent and green are calcified (Hillis-Colinvaux 1980). Calcification only occurs in the form of aragonite; the crystals are generally needle-shaped, reaching about 10 µm in length 0.08-0.60 µm in width and 0.01 µm or less in thickness (Wilbur *et al.* 1969, Marzelek 1971, Borowitzka *et al.* 1974). The size and number of crystals vary with the age of the segments, with the species and to some extent from specimen to specimen of the same species. Carbonate deposition seems to be an important function of the metabolism of halimeda. In its simplest form, the reaction of calcification is the following:

$$\mathbf{Ca^{2+} + 2HCO_3^- \Leftrightarrow CaCO_3 \downarrow + H_2O + CO_2}$$

The deposition of Aragonite is coupled to photosynthesis: In daylight uptake of CO_2 for photosynthesis out of the interutricular-space results in a drop of $[CO_2]$. The $[CO_2]$ can not be quickly replenished by the seawater due to diffusion barrier formed by the utricles resulting in a rise of pH and $[CO_3^{2-}]$. This rise of $[CO_3^{2-}]$ with Ca^{2+} exchanged out of the seawater, cellwall and cell stimulate the deposition of Aragonite (Borowitzka 1977, Borowitzka & Larkum 1976c, Borowitzka 1982). Thus formation of Aragonite can best be seen as a by-product of the photosynthesis. Deposition begins at the outer surface of the lateral walls of the peripheral utricles and soon spreads over the entire space between them (Askenasy 1888). The crystals keep growing until almost the complete interutricular-space is filled (Borowitzka 1977, Borowitzka and Larkum 1977, Hillis-Colinvaux 1980).

Sexual reproduction is infrequent and occurs via holocarpy; i.e. is the transfer of free organic matter of the thallus, including reserves, to the gametangia (Hillis-Colinvaux 1980). The gametangia have the shape of small dark green globules and appear overnight at the tips of the segments, leaving the whole plants white. The following day, the entire protoplastic content of the segments is explosively released as gametes. These gametes are biflagellates and differ in size, female plants release large gametes and smaller ones are released by the male plants. The parent plants die and disintegrate within hours (Drew & Abel 1988, Hillis-Colinvaux 1980, Clifton 1997). Gametes remain motile for 40 to 60 min after release. Gamete movements stop after fusion, resulting in a sinking zygote (Clifton 1997). The zygote remains at the bottom for seven months growing from 3 µm to 100-150 µm. The next five months a cluster of filaments is formed, followed by the formation of a new plant (Meinesz 1988). Vegetative reproduction also occurs in halimeda and is probably the most common means of reproduction. Different types of reproduction are known. The first is reproduction by creeping rhizoids. Long runners are formed (at least 20 cm), thin threadlike bundles of filament coming out of the holdfast (Colinvaux *et al.* 1965, Colinvaux 1968a,b, Hillis-Colinvaux 1972, 1973). These runners form a tight clump or cone-like mass of

filamentous material, out of which a new segment is formed. A second way of reproduction is the production of rhizoids between the segments of the thallus. These rhizoid filaments first give more anchorage, and permit the division of the alga into two separate thalli. A third mechanism for vegetative reproduction is the growth of a new thallus out of a partly buried thallus or out of broken fragment of a thallus of halimeda, which can be caused by for example heavy weather. The buried thallus or fragment develops a new holdfast-system and produces a new thallus. (Hillis-Colinvaux 1972, 1973).

Halimeda incrassata

Subject of this study will be *Halimeda incrassata* (Figure 2), which is one of the most common rhipsalian species of halimeda. *H. incrassata* is normally an erect and compact alga up to 24 cm tall, without the holdfast. Holdfast can extend to 9 cm in length. The calcification is heavy at the base, becoming moderate to light in the middle and upper parts. The branching is mainly di- to tetrachotomous, the lower part often remaining simple. Basal segments sometimes can consolidate laterally forming a massive fan-shaped structure. The first segments are cylindrical and the other segments flat, ribbed or keeled. Shape can be variable, from cylindrical to reniform. The outer margin is entire, undulating or deeply lobed. Segments are about 10 mm long, 14 mm wide and 0.75-1.00 mm thick. *H. incrassata* grows in sand, mud or other unconsolidated substrate. It grows from the low-tide line to about 65 m of depth. In the Caribbean, it forms very dense stands with *H. monile* and is frequently associated with seagrasses. Its geographic distribution is Pantropical and includes the eastern and western Indian Ocean; the western Pacific and the western Atlantic. This species is best known from the Caribbean, where it appears to be the most common of the rhipsalian taxa and often forms extensive populations in the shallow sandy regions of the reef (Hillis-Colinvaux 1980).

Figure 2

Halimeda incrassata, foto taken in the Puerto Morelos reef lagoon.

The study area

The study area, the Puerto Morelos reef forms part of an extensive barrier-fringing reef tract, that runs from Belize to the Yucatan Channel (Jordan *et al.* 1981). It is situated between 20°40' and 21°12' North Latitude and 86°47' and 86°58' West Longitude. Puerto Morelos is a small fishing village, situated on the north-eastern coast of the Yucatan Peninsula, Mexico. The peninsula is a large, mostly flat, heavily karstified limestone platform. The climate of this region is characterised as warm, sub-humid with rainy seasons. It has an annual average maximum temperature of 29.4°C and an average minimum temperature of 24.4°C (measured in 1992). Seasonal south-eastern trade winds dominate, although strong north winds (with a duration of 3-10 days) can be important during winter months. Hurricanes may reach the region from June to October and likely to occur in September (Merino & Otero 1991). Because of the lack of rivers hardly any terrestrial influence exists in the study area. The only discharge of fresh and brackish water is caused by submarine springs in the coastal zone. These have an estimated average of 8.6 10^6 m^3 water km^{-1} coast y^{-1} over a coastline of app. 1100 km. These waters are low of nutrients and have minimum influence on the ecosystem (Back 1985). The surface currents flow mainly northward, caused by the Yucatan Current (precursor of the Gulf Stream), flowing parallel to the continental shelf of Puerto Morelos. Circulation in the lagoon is mainly parallel to the coast. This is caused by the Yucatan Current, winds, wave spillage over the reef and the location of surge channels. Waves in the lagoon are low with a measured average height of 0.14 m. The tidal influence is very small, maximal sea level variability is 0,68 m. Salinity is 35.7 $^0/_{00}$.

Used values were measured from March 1982 through July 1983 (Ruis-Renteria *et al.* in press).

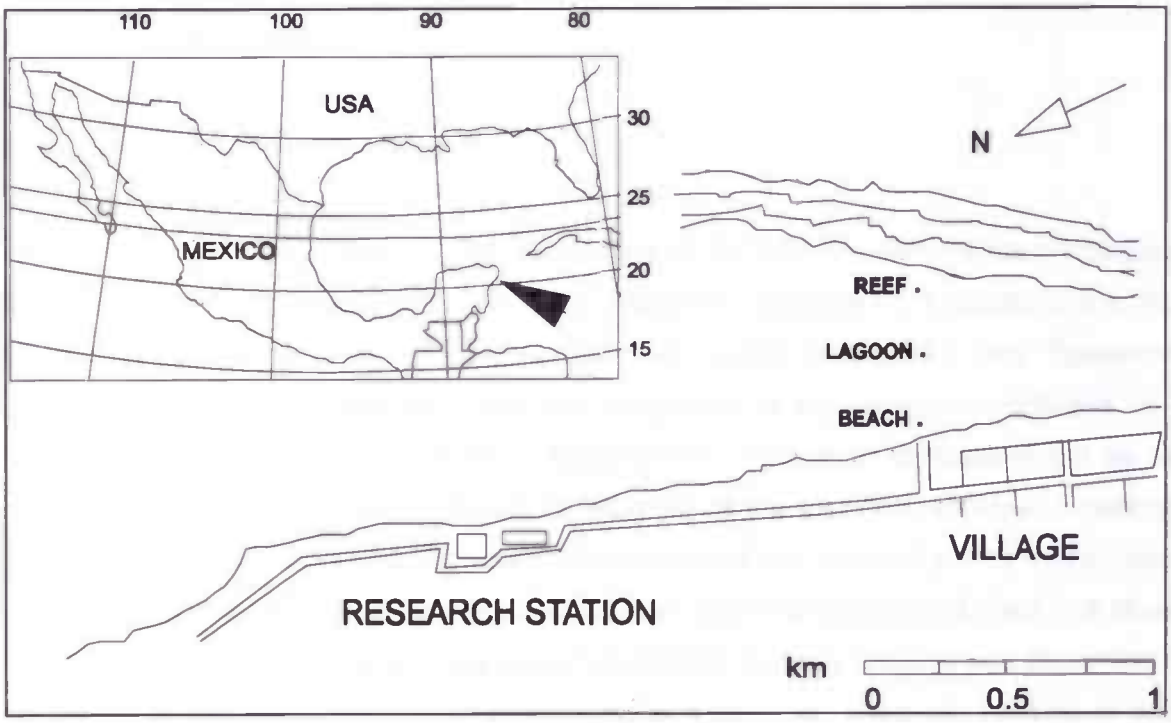


Figure 3: Map of Puerto Morelos with locations of the stations.

The preliminary study was carried out in the reef lagoon, in front of the Hotel Ojo de Agua, near the village of Puerto Morelos (Figure 3). The water depth is 1.5m to 5m and the bottom is mainly covered by seagrasses, *Halimeda* spp. and many other algae. It is a typical Caribbean seagrass vegetation as described by Brasier (1975). Three stations where chosen in front of the hotel. The Reef-station: located at approximately 50 m from the first coral formations, the Lagoon-station; halfway reef and beach, and finally the Beach-station; located at approximately 50 m from the beach. The locations Lagoon and Beach have similar vegetation structure, long slim halimedas with few epiphytes. The vegetation structure at the Reef has smaller, more bush-like thalli and has more and bigger epiphytes. The Lagoon station is situated at a depth of 3.1 m, the Beach station at a depth of 4.9 m, and the Reef station at a depth of 2.4 m. Locations were marked with a submerged buoy, the stations could be found by a three point line gauging with locations at shore. At each of these stations a site was chosen with a average distribution of halimeda. The locations of the stations were chosen, because they were subject of earlier studies.

Materials and Methods

Biomass

For biomass assessment, eight quadrates of 30 X 30 cm were randomly placed on the bottom of every station and all halimedas inside the quadrate were collected in a plastic bag. Collections were done in October 1997. In earlier studies B.I. van Tussenbroek (personal comment) showed that with five quadrates a representative estimate of the biomass of all species per station could be obtained. Thus eight samples would be more than sufficient to obtain a reliable figure for biomass at the sampling stations. If necessary epiphytes were removed with a toothbrush. The height of the collected plants was measured and all the plants of one quadrat were placed on an aluminium plate and dried in a stove at 60°C for at least 24 h. $DW_{calcified}$ (=dry weight of somatic tissue + $CaCO_3$) was determined for all plants and finally total $DW_{calcified}$ of all individuals together per quadrate. With these results the calcified biomass ($DW_{calcified}$ m^{-2}) at each station was estimated, with their mean value per quadrate and SD. With a One-way ANOVA was tested if there was a significant difference between the biomass of the different stations. Total biomass per station was used in further analysis.

Growth

Figure 4

Seringe containing Alizarine Red S, attached to the seringe is a small hose.

Alizarin Red S was used as a time marker to allow measurement of incremental growth of *Halimeda incrassata*. Alizarin Red S stains organisms, which incorporate $CaCO_3$ into their internal or external skeleton. This colouring agent left a fixed red colour on the segments (Figure 7) of the halimedas after an incubation of 24 hours. The Calcium incorporated in the segments after staining was white thus newly grown segments could be recognised by their white colour after bleaching (Wefer 1980, Hudson 1986, Multer 1988, Payri

1988, Freile & Hillis 1997).

At three stations, in total 24 loops (eight per station) were placed to measure halimeda growth. The loops (25 cm diameter) were made with steel rods (∅ 3/8 inch) and three steel legs of 15 cm were attached to the loops. In the laboratory, concentrated Alizarin solution was prepared with seawater: per sample 0.247 g of Alizarin Red S (SIGMA C.I.58005; Alizarin Red) was ground in a mortar with a little seawater, making sure that all the Alizarin was dissolved. This was deluded in seawater to a final volume of 50ml. A solution for sufficient experiments was made and collected in a dark bottle (Alizarin is damaged by light), one day before placing the loops. At the stations, the loops where placed where halimeda abundance was high. Plastic bags were used to make a tent, enclosing the algae. A line was drawn on the bag where the content of the bag was 13 l. On the line of the bag a hole was punched with the legs of the loop. The loops with bag were pushed into the sediment, to seal the algae off from the surroundings. A chain of lead was placed on the edge of the bag to secure it, and to avoid leakage of the colouring agent. Finally sand was placed on the edges of the bag to close small holes (Figure 5). With a syringe equipped with a small curved hose, 50 ml of the concentrated Alizarin solution was extracted (Figure 4). The hose was stuck in the sand at the border of the loop and the Alizarin solution was injected into the enclosed area. A concentration of ≈19 mg l^{-1} was the final concentration in the enclosed area. During preliminary tests this concentration was sufficient for colouring the segments. Wefer (1980) used a concentration of 1.5 mg l^{-1}, but no good results were obtained with this concentration during preliminary trials, so the concentration was raised. The eight loops were placed at each station (Reef, Lagoon and Beach) each loop being on a distance of app. 2 m from each other. This was done early in the morning at sunrise, so the Alizarin could be incorporated into the algae all day, as Alizarin binds to $CaCO_3$ formed during the period in which algae are photosyntheticly active. The bags were removed next morning leaving the loops in the sediment, the loop-locations were called plots. On each loop a cork with a rope was attached, to facilitate

Figure 5

Metal loop with plastic bag enclosing an area containing Alizarine Red S. A chain of lead is put on the edges of the loop to close the smallest holes.

relocation. The algae were left to grow for two weeks. This time length was chosen to avoid possible impact of grazing and the falling off of new grown segments. In these two weeks, halimeda grew sufficient new segments for an adequate analysis. After two weeks, all halimedas were collected in numbered bags and stored in a freezer. The experiments were repeated every month. From the 30th of October until the 14th of November, the 7th of December till the 21st of December and the 8th of January till the 22d of January. All fieldwork was done by SCUBA.

Laboratory work

Figure 6

Aluminum tray containing dried not bleached *Halimeda incrassata* plants.

If necessary thalli were cleaned from epiphytes with a toothbrush. The height (H) of each plant was measured and the total number of segments (TT), the number of terminal segments and the number of new segments (NS) were counted. The number of terminal segments (TS) was defined as all the segments, which were at the end of a branch or were single segments. Plants of one quadrat (or loop) were placed in an aluminium tray (Figure 6), numbered and dried in a stove at 60°C for at least 24 h. For all plants, the $DW_{calcified}$ was determined and the plants were bleached in a 20% sodium hypochlorite (Clorox) solution during 30 minutes until plants coloured white (Multer 1988). Plants were placed in the stove (60°C) for at least 24 h. From each dried and bleached plant (Figure 7), the new segments were cut off with a sharp razor.

Figure 7

Close up of new and stained segments.

Unstained plants were discarded. Small stainless plants were included (these were new plants). The new segments (NS) and the new terminal segments (NTS) were counted, and the $DW_{calcified}$ of the new segments (WNS) was determined. Finally the total $DW_{calcified}$ of the old segments (number of segments, without new ones) per quadrat was determined. New segments and old segments were decalcified separately in a solution of 10% phosphoric acid. The $DW_{somatic}$ (dry weight decalcified somatic tissue) were filtered out of the acid with a plastic wire-netting and placed in clean water for several hours to avoid burning of the algae by the acid. The decalcified algae were filtered again, rinsed with fresh water and dried in the stove (60°C) for at least 24 h. Finally the decalcified algae were weighed.

Statistical Analyses

To check if bleaching affected original $DW_{calcified}$ of the new segments, the total $DW_{calcified}$ of the bleached plants (old segments plus new ones), per plot, were divided by the total $DW_{calcified}$. The percentage of $CaCO_3$ was calculated separately of the whole plant and in the new segments respectively. This was done to evaluate whether $CaCO_3$ content was different in the new segments.

The data were transposed to the STATISTICAL package and further analysis was realised with this package. Plants with only new segments were selected and analysed separately. Contribution of the $DW_{calcified}$ of the new plants to the total production was evaluated. Also the number of new plants was determined at each station.

The remainder of analysis was realised with the thalli with new and old segments. The $DW_{calcified}$ of new segments was divided by $DW_{calcified}$ of the same plant. This was repeated for the number of new segments (NS) dividing it with the total number of segments (TT). With these data it could be determined if the growth was equal for plants of different sizes and to decide if any transformation was necessary. The latter was realised by plotting these data. If the inclination of the correlation line was zero, no transformation was needed. Outliners were selected with a residual analysis, using the equation $DW_{calcified}$ new segments/ $DW_{calcified}$ (NS/TT) and $DW_{calcified}$ (TT) as variables. All data with a residual greater than (-)+2 where discarded.

Many parameters were measured (H, TT, NTS, $DW_{calcified}$ whole thallus, NNS, NNTS, $DW_{calcified}$ new segments), this was done to determine what parameters were the best too obtain a good growth and production estimator. Other authors have used the number of new segments as growth and production estimator and multiplied this with the mean weight of a segment. This study also looked at other parameters to try to obtain a methodology that would take the least time. First the regression lines of $DW_{calcified}$ whole thallus vs. $DW_{calcified}$ new segments was set out, this represented the real growth line and the production

established in 14 days. This regression line was compared to the regression line of TT vs. NNS estimating growth and production this way safes time and was used by other authors to estimate the yearly production. Determining the $DW_{calcified}$ which is the real production takes app. 4 days in order to obtain the final result. Estimating the $DW_{calcified}$ by counting segments takes only one day of work and is therefor preferable. The comparison of the different regression lines lead to the choice of $DW_{calcified}$ as estimator for growth and production, other estimators were dropped.

To evaluate spatial variation in halimeda growth, the regression slopes of $DW_{calcified}$ against $DW_{calcified}$ new segments of the different stations (Reef, Lagoon and Beach) for every month were analysed with a comparison of slopes (Zar 1974). To see if temporal variation exists, the slopes $DW_{calcified}$ against $DW_{calcified}$ new segments of all months at each station (Reef, Lagoon and Beach) were compared with the same analysis. If for a station similar regressions were found it was assumed that there is no temporal variation and the production per year was estimated.

Production at each station was calculated using the total production in 14 days and standardising this value using the biomass values of this study. The $DW_{calcified}$ of the plants at each station and month per m^2 was divided by the biomass of each station. This value was multiplied with the growth per m^2 resulting in the standardised production per 14 days at each station. To calculate yearly production, only stations which had no significant difference in growth between the three months, could be used (Reef- and Lagoon-station). First the mean production per day of the three months was calculated and multiplied by 365 too obtain an estimate of the yearly production.

Production per plot

The production per plot was also estimated, summing the values ($DW_{calcified}$ of the new grown segments and of the total $DW_{calcified}$) of all the plants without the new plants per plot. A regression of the total $DW_{calcified}$ of the new growth vs the total $DW_{calcified}$ of the plants was plotted. This to evaluate if, the summed values per plot were a reliable estimator for the total calcified production of a station. This way a lot of time would be saved.

Percentage of $CaCO_3$

The percentage of $CaCO_3$ is defined as the weight of the $CaCO_3$ (dry weight minus decalcified weight) divided by the dry weight (DW) of the plant. A "2*arcsin x^{-2}" transformation was applied to normalise the proportional data (Howell 1997). To evaluate possible differences in the $CaCO_3$ percentage between the new segments and whole plant a t-test of dependent values per station was realised, using the mean percentages of $CaCO_3$ per plot.

Also the percentage $CaCO_3$ of the halimedas at the three stations (Reef, Lagoon and Beach) at each month was compared using a One-way ANOVA. The transformed percentage of $CaCO_3$ of the whole plants at each plot (eight per station, except November at Lagoon-station) were used.

Results

Biomass

The results of biomass measurements are shown in Table 1. Biomass values of *H. incrassata* were highest on the Reef-station and lowest on the Beach-station.

Table 1
$DW_{calcified}$ of *Halimeda incrassata* thalli per plot (30 x 30 cm) and total $DW_{calcified}$ all quadrates, mean weight per plot, SD and biomass ($DW_{calcified}$ m^{-2}) at the stations Reef, Lagoon and Beach in Puerto Morelos reef lagoon. Measurements were done in October 1997.

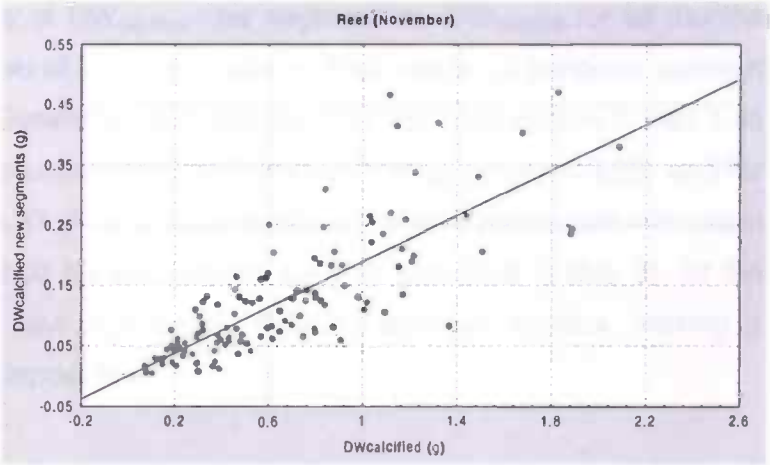
Nr. of quadrate	Reef $DW_{calcified}$ per quadrate (30x30 cm).	Lagoon $DW_{calcified}$ per quadrate (30x30 cm).	Beach $DW_{calcified}$ per quadrate (30x30 cm).
1	6.087	13.446	5.637
2	15.941	10.948	4.914
3	15.109	11.239	14.175
4	11.655	13.705	4.230
5	3.309	4.678	6.129
6	12.816	4.028	6.432
7	14.500	19.000	5.095
8	7.2152	7.908	9.476
Total	86.632	84.952	56.088
Mean	10.829	10.619	7.011
SD	4.7003	4.990	3.299
Biomass ($DW_{calcified}$ m^{-2})	**120.32**	**117.99**	**77.9**

No significant differences were found between the different $DW_{calcified}$ at the different stations, when tested with a One-way ANOVA, ($F=1.910$, $p= 0.173$, $df=2$)

Growth

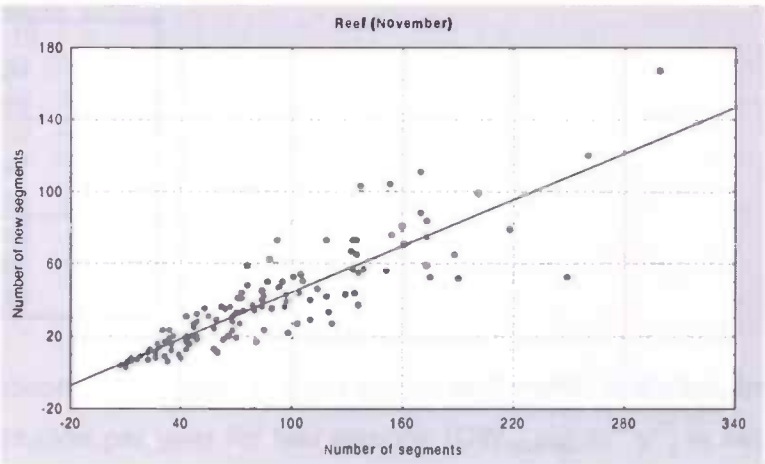
In Graph 1 the regression line of $DW_{calcified}$ whole thallus vs. $DW_{calcified}$ new segments of the Reef-station in November is set out. This station and month are an example for all the other stations and months. This graph represents the real production in $DW_{calcified}$ in 14 days and the growth rate during these days. This results in a production of 0.2 g $DW_{calcified}$ of new segments per gram of $DW_{calcified}$ of thallus. This means that 20 % of the $DW_{calcified}$ of the thallus after 14 days is newly formed. With this results a turnover rate of app. 4-5 crops a year was estimated for The Reef-station in November. In Graph 2 the regression line of total number of segments per thallus (TT) vs. number of new segments per thallus (NNS) of the Reef station in November is shown. This represents the number of newly produced

segments in 14 days and the growth rate of these segments. This results in a production of 50 new segments for every 100 segments. This means that app. 50 % of the segments of the thallus was newly formed, according to these parameters. This leads to a turnover of app. 12 crops a year. Due to these findings $DW_{calcified}$ of the thallus and the $DW_{calcified}$ of the new segments have been chosen to be the best parameters to estimate production and growth.



Graph 1

Regression line of $DW_{calcifid}$ whole thallus vs. $DW_{calcified}$ new segments. Each point represents one plant at the Reef-Station in November (1997).



Graph 2

Regression line of # segments whole thallus vs. # new segments. Each point represents one plant at the Reef-Station in November (1997).

In Graph 3 the regression lines of $DW_{calcified}$ new segment vs. $DW_{calcified}$ of the different stations at each month are plotted representing the spatial variation and in Graph 4 the regression lines of $DW_{calcified}$ new segment vs. $DW_{calcified}$ for the months at each station are plotted representing the temporal variation. Both graphs are shown with the corresponding scatterplot. The r^2 is highest at the Beach-station in November and lowest at the Beach-station in December.

Regression lines of $DW_{calcified}$ new segment vs. $DW_{calcified}$ were significantly different between the sampling stations during the three sampling times. For November F was 17.46 ($F_{0.05\ (1),\ 2,300}$= 3.03), for December F was 6.57 ($F_{0.05\ (1),\ 2,300}$=3.03) and for January F was 67.76 ($F_{0.05.\ (1),\ 2,200}$ = 3.04), so there was spatial variation between the three stations.

The regression lines of $DW_{calcified}$ new segment vs. $DW_{calcified}$ for all months per station were equal for two of the three stations. This means no temporal variation in the months November, December and January. For the Reef-station F was 1.45 ($F_{0.05\ (1),\ 2,300} = 3.03$), for the Lagoon-station F was 0.69 ($F_{0.05\ (1),\ 2,300} \doteq 3.03$) and for the Beach-station F was 95.37 ($F_{0.05\ (1),\ 2,200} = 3.04$). A tentative production estimation per year could be established for the stations Lagoon and Reef (Table 3). At the Beach-station, the growth was significantly different between months, making a yearly production estimate impossible.

Table 2

Production of *Halimeda incrassata* per 14 days ($DW_{calcified}\ m^{-2}$) at the different stations (Reef, Lagoon and Beach) during the different months. These values were measured during the months November, December (1997) and January (1998), in the Puerto Morelos reef lagoon.

Station/month		Production per 14 days ($DW_{calcified}\ m^{-2}$)
Reef	Nov.	14.13
Lagoon	Nov.	4.34
Beach	Nov.	3.48
Reef	Dec.	7.22
Lagoon	Dec.	6.97
Beach	Dec.	2.84
Reef	Jan.	4.43
Lagoon	Jan.	4.85
Beach	Jan.	2.42

In Table 2 the production per 14 days at each station and month is shown. In Table 3 the production estimation per year for two stations ($DW_{calcified}\ m^{-2}\ y^{-1}$) is set out. First mean daily production in the three months was calculated and the multiplication with 365 resulted in the tentative estimate for annual production for the Reef- and Lagoon-station. The production is the $DW_{calcified}$ (g) of the somatic tissue plus the amount of fixed $CaCO_3$.

Table 3

Yearly production ($DW_{calcified}\ m^{-2}\ y^{-1}$) estimates of *Halimeda incrassata* at the Reef- and Lagoon-station at the Puerto Morelos reef lagoon. Values were measured during the months November, December (1997) and January (1998).

Station	Mean production per day ($DW_{calcified}\ m^{-2}\ d^{-1}$).	Mean production per year ($DW_{calcified}\ m^{-2}\ y^{-1}$).
Reef	0.61	224.0
Lagoon	0.38	140.5

Growth per quadrat

The results of the regression analyses (r^2, F, significance of F and df) of the total $DW_{calcified}$ and $DW_{calcified}$ of the new segments, per quadrat are shown in Table 4.

Table 4
Parameters of the regression analyses of the $DW_{calcified}$ vs. $DW_{calcified}$ of the new segments of quadrats at each station for each month. Values measured for *Halimeda incrassata* in the months November, December (1997) and January (1998), in the Puerto Morelos reef lagoon.

Month	Station	r^2	F	Sig. F	DF
Nov.	Reef	.821	27.511	.0019	6
	Lagoon	.824	23.346	.0047	5
	Beach	.579	8.256	.0283	6
Dec.	Reef	.925	74.456	.0001	6
	Lagoon	.437	4.659	.0742	6
	Beach	.273	2.249	.184	6
Jan.	Reef	.589	8.608	.0262	6
	Lagoon	.576	8.142	.0291	6
	Beach	.479	5.523	.0571	6

The F value was greater than 0.05 in 3 of the 9 cases. This means that the fit of the regression lines in these 3 cases was not significant (Table 4). No further analysis was done using the growth per plot as a production estimator.

Percentage CaCO3

The mean percentages of $CaCO_3$ in the plants and the new segments of these plants of each plot at each month are shown in Table 5.

Table 6
Percentages of $CaCO_3$ of the whole thalli and the new segments of *Halimeda incrassata* at the different stations (Reef, Lagoon and Beach) during the different months. Values measured in the months November, December (1997) and January (1998), in the Puerto Morelos reef lagoon.

Station/month	% $CaCO_3$ whole thallus	% $CaCO_3$ new segments
Reef Nov.	84.89	85.90
Lagoon Nov.	85.32	81.23
Beach Nov.	83.52	79.63
Reef Dec.	86.57	86.04
Lagoon Dec.	85.90	80.92
Beach Dec.	87.28	83.46
Reef Jan.	87.92	85.30
Lagoon Jan.	87.38	81.54
Beach Jan.	86.45	83.16

Results of the t-test with dependent values of the difference between the transformed (2*arcsin x^2) percentage of $CaCO_3$ of the whole plants and the new segments at the different stations are shown in Table 6.

Table 6
Results of the t-test with dependent values, with transformed $CaCO_3$ percentage of whole thalli of *Halimeda incrassata* and transformed $CaCO_3$ percentage of new segments as variables. The analyses were realised for the three different stations at the Puerto Morelos reef lagoon (Reef, Lagoon and Beach). Mean percentage of entire plants and new segments with their standard deviations are given. Also n, df, t and the correspondent p value are given. Measurements were done during November, December (1997) and January (1998).

Station	Mean $CaCO_3$% plant	SD plant	Mean $CaCO_3$% new seg.	SD new seg.	n	df	t	P
Reef	86.46	2.731	85.74	2.105	24	23	1.458	.1583
Lagoon	86.24	2.011	81.23	4.851	23	22	6.842	.0000
Beach	85.75	3.354	82.08	4.654	24	23	3.568	.0018

Table 6 shows the Reef station with a $p>0.05$. This means that the $CaCO_3$ percentage in the old plants and in the new segments was not different. At the Lagoon- and Beach-station, the difference between new and old segments was significant ($p<0.05$).

In Table 7 the results of the comparison of $CaCO_3$ percentage between the different months at each station are shown. There was no significant difference in $CaCO_3$ percentage between the different months at the Reef- and Lagoon-stations using a One-way ANOVA ($p>0.05$). The percentage of $CaCO_3$ at the Reef-station was 86.46% and at the Lagoon-station was 86.24%. At the Beach-station ($p<0.05$), there was a significant difference in the percentages at the different months.

Table 7
Results of the One-way ANOVA, comparing the different transformed $CaCO_3$ percentages of *Halimeda incrassata* at the three stations at the Puerto Morelos reef lagoon (Reef, Lagoon and Beach) over the different months (November, December (1997) and January (1998)), using the values of the entire plant.

Station	df	F	p-level
Reef	2	2.894	0.0776
Lagoon	2	2.436	0.1130
Beach	2	3.502	0.0487

Contribution new thalli

New thalli contributed little to total areal production of *Halimeda incrassata* (Table 8).

Table 8
Total $DW_{calcified}$ of new segments and total $DW_{calcified}$ of new thalli of each station at the Puerto Morelos reef lagoon (Reef, Lagoon and Beach) and each month (November, December (1997) and January (1998)) and the percentage contributed by the new thalli to the total $DW_{calcified}$.

Station/month	$DW_{calcified}$ new segments	$DW_{calcified}$ new thalli	% new seg. /new seg.+ new thalli
Reef Nov.	16.10	0.12	0.72
Lagoon Nov.	7.92	0.14	1.76
Beach Nov.	6.47	0.10	1.49
Reef Dec.	11.55	0.94	7.55
Lagoon Dec.	8.09	0.00	0.00
Beach Dec.	4.07	0.12	2.84
Reef Jan.	9.02	0.13	1.39
Lagoon Jan.	7.42	0.06	0.78
Beach Jan.	4.50	0.69	13.30

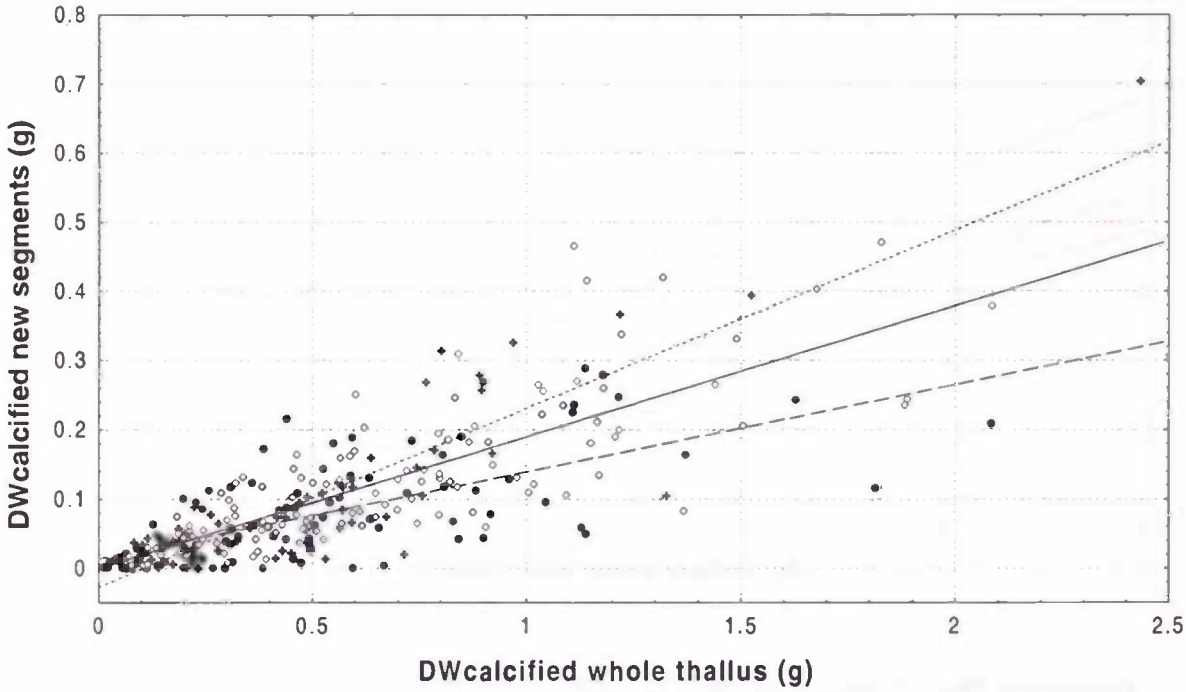
The Beach-station contributed the maximum percentage of $DW_{calcified}$ by the new plants, 13.30% in January. New plants were not used to determine the regression lines for the comparison of growth. Their contribution was usually small.

Few new thalli were produced at each station and month (Table 9); a maximum of 12 thalli was found at the Lagoon-station in November.

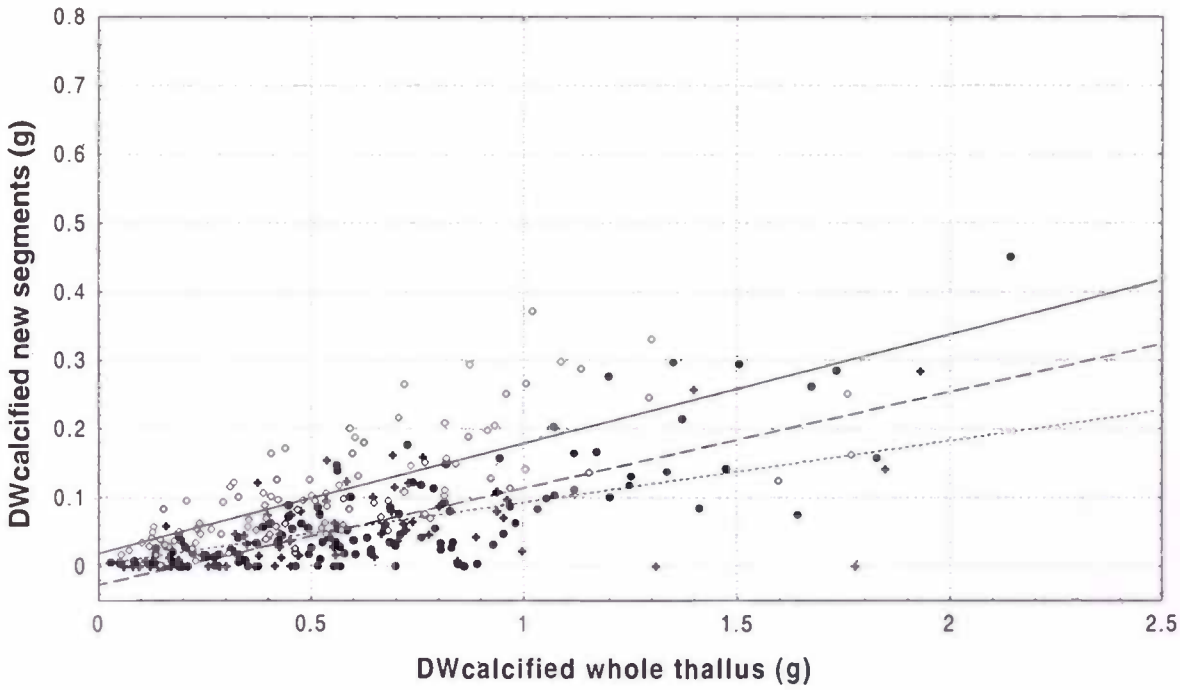
Table 9
Number of new *Halimeda incrassata* thalli at each station at the Puerto Morelos reef lagoon with the maximum and minimum amount of thalli per quadrat. The values were measured during the months November, December (1997) and January (1998).

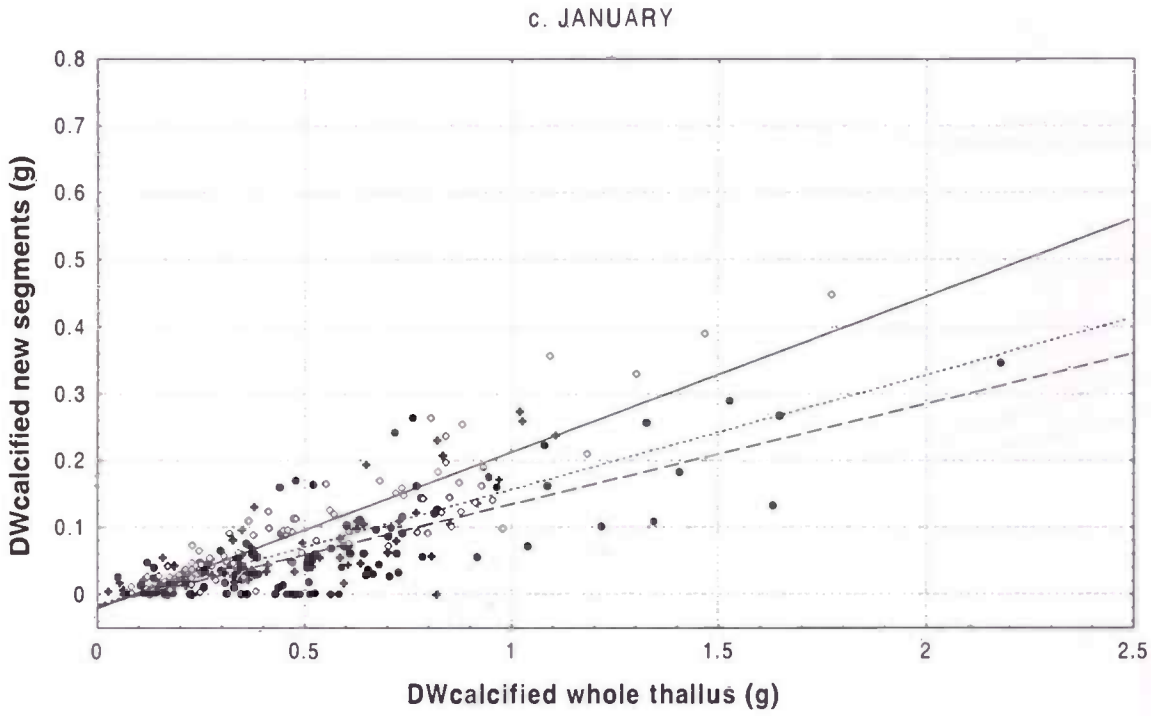
Station/month	# new plants station	Minimum per quadrat	Maximum per quadrat
Reef Nov.	2	0	1
Lagoon Nov.	12	0	5
Beach Nov.	1	0	1
Reef Dec.	3	0	1
Lagoon Dec.	0	0	0
Beach Dec.	1	0	1
Reef Jan.	1	0	1
Lagoon Jan.	4	0	1
Beach Jan.	8	0	3

a. NOVEMBER



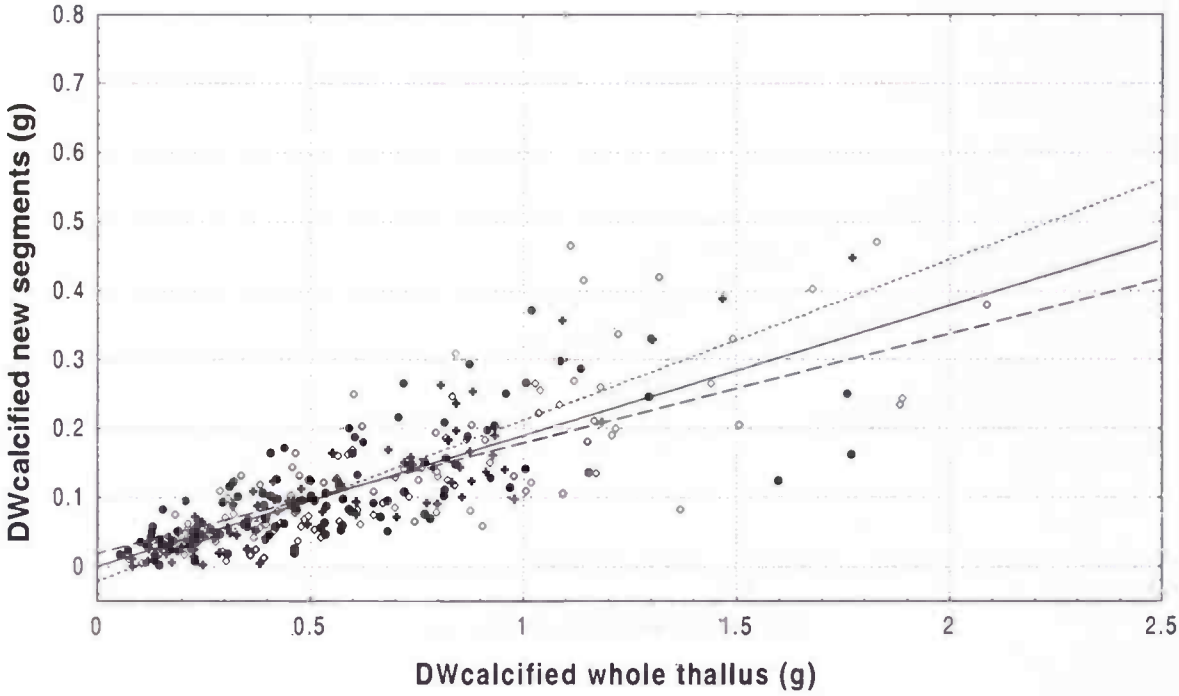
b. DECEMBER



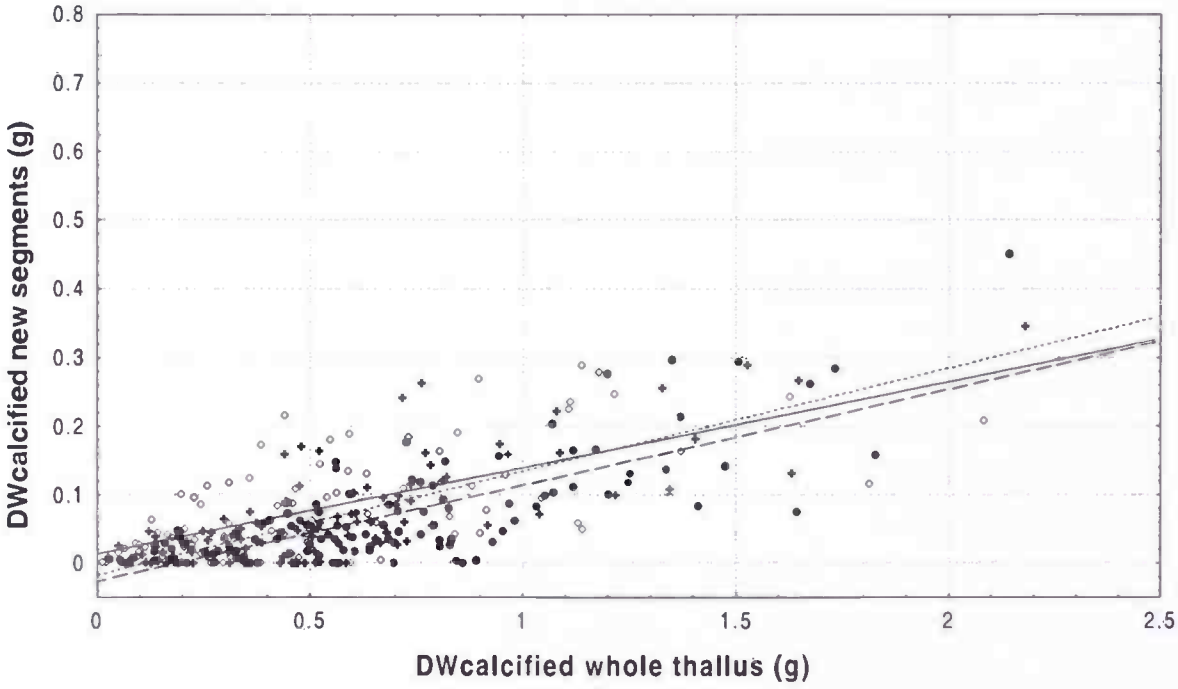


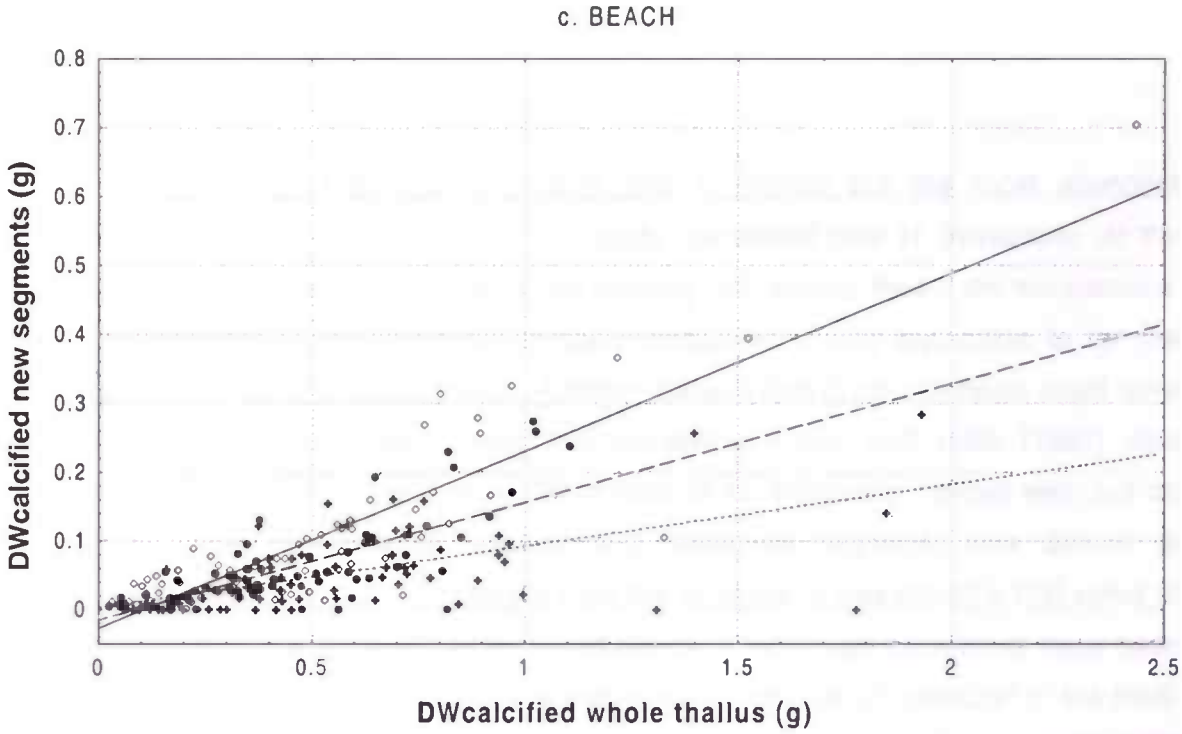
Graph 3 a. b. c.
Scatterplot of $DW_{calcified}$ whole thallus (g) vs. $DW_{calcified}$ new segments (g) with regression lines for *Halimeda incrassata* for each sampling month. + Reef (full line), ↑ Lagoon (spaced line), • Beach (dotted line).

a. REEF



b. LAGOON





Graph 4 a. b. c.
Scatterplot of $DW_{calcified}$ whole thallus (g) vs. $DW_{calcified}$ new segments (g), with regression lines for *Halimeda incrassata* for each station at all sampling months. ↑ November (full line), ● December (spaced line), + January (dotted line).

Discussion

Methodology

In the Caribbean *Halimeda incrassata* and *H. monile* are the most abundant sanddwelling halimeda species. However this study concerned only *H. incrassata*. At the start of the project, *H. monile* was included, but staining with Alizarin Red S did not succeed. *H. monile* stained, but very weakly and in some occasions it was impossible to tell the difference between new and old segments. A higher Alizarin Red S concentration could have been used, but the scarceness limited this possibility. Freile and Hillis (1997) also encountered this problem with *H. monile*. Most thalli of *H. incrassata* stained well, but on occasions, the difference between stained and unstained segments was difficult to differentiate. These individuals were omitted from the analysis. It was possible that some of the thalli did not stain, because of sediment turbulence. Thalli could sometimes have been buried during the 24 hours of staining and reappear at the moment of collection of the thalli. Another reason the thalli did not stain well could have been the concentration of agent in the bags. It was difficult to seal the bags and separate the surroundings without leaks. Some thalli at the edge of the quadrate were excluded from staining, because the plastic bag covered over these thalli. One bag was not removed the day after colouring at the Lagoon-station in November 1997. Plants from this bag were left out of the analysis.

The allowed growing time was two weeks because all thalli could grow enough segments, to have a good estimation of new segment formation. The growing time was as short as possible, minimising the influence of the falling off of new segments due to old age, storm damage, or grazing.

After collection, thalli were cleaned with a toothbrush if necessary. This cleaning could have had some influence on the dry weight of the thallus. The cleaning removed not only the epiphytes, but also some old uncoloured dead segments, which still were connected to the thallus. The weight of the bleached thalli was slightly different from the thalli without bleaching. A correction was made to estimate the original weight of the bleached segments.

$DW_{calcified}$ (dry weight somatic + calcareous tissue) of the thallus and $DW_{calcified}$ of new segments were chosen to be the best estimators for production and growth. These variables also represent the real production established by the plants. In Graph 1 can be seen that 20 % of the total dry weight of a *H. incrassata* stand is newly formed during the 14 days of measurement at this station. The Reef-station in November has been chosen as example and represents the rest of the stations. The r^2 in the regression analysis of $DW_{calcified}$ of the thallus vs. $DW_{calcified}$ of new segments is not very high, but represents the real growth.

Many other parameters were determined, this to establish a methodology that would be less time consuming than determining the dry weight. Other authors (Wefer 1980, Freile & Hillis 1997 and Multer 1988) used the number of segments to determine the turnover rate. A possibility in this study would have been to determine the total number of segments or weight by just counting the number of terminal segments and so estimating the dry weight of the thallus. To do this a good relationship was necessary between the regression lines of the $DW_{calcified}$ and the number of segments. In the results was shown that for every 100 segments app. 50 were newly formed. This is a new-segment growth of 50 % what would lead to a turnover of app. 12 crops a year in this example. Using these values to estimate production and growth an overestimation would be the result. Freile and Hillis (1997) used this value and multiplied the number of crops with the biomass, resulting in a production of 2323 g $CaCO_3$ m^{-2} y^{-1}. A possibility would be to use the mean dry weight of a newly formed segment and multiplying this with the number of new segments produced. This only would implicate that the growing time of new segments should be equal at each month and that no seasonal variation existed. If this were not the case the mean weight of newly formed segments would change during the different experiments and an over- or underestimation would be realised. Therefor the best way to determine the production and growth without mistakes is to compare the $DW_{calcified}$ of the thallus and $DW_{calcified}$ of new segments. This way seasonal variation can also be monitored more accurately. The rest of the parameters were not used in further analysis.

Growth was highest on the Beach-station in November and lowest on the Beach-station in December. The Beach-station was the deepest station.

In the spatial variation analysis all stations differed. This was expected because of the different depths of the three stations. In the temporal variation analysis, the Beach-station is the only station for which the regression lines of $DW_{calcified}$ whole thallus vs. $DW_{calcified}$ of new segments, was not equal for the different sampling months. A possible explanation is that the number of thalli on the Beach-station was smaller and some extreme values had a big influence on the regression lines. Graph 4c indicates that the January regression line had a few points, representing plants, which weighed rather heavy, but had no new grown segments.

Production was also estimated by using the values of summed $DW_{calcified}$ of all plants at each plot, this to save time in future experiments. However the F significance of the regression lines obtained by setting summed total $DW_{calcified}$ of the whole thallus vs. $DW_{calcified}$ of new segments, was too low. This low significance was due to small sample size.

The real percentages of $CaCO_3$ of the *H. incrassata* thalli were transformed with a "2*arcsin x^2" transformation to establish percentage values that could be analysed in the different calculations. This is a transformation for partial results involving percentages.

A difference in $CaCO_3$ percentage was found between the different sampling months at the Beach-station using a One-way ANOVA where the $CaCO_3$ percentages between different months for all stations were compared. This difference was very small, and was possibly caused by the smaller number of thalli collected at this station.

The newly formed juvenile plants were treated separately, because it was hard to determine the time they had grown. New plants could have started growing at the beginning or the end of the experimental period, therefore making it impossible to estimate the time a new plant had grown. Another possibility is that small new plants were buried during the colouring period and that they reappeared afterwards. Multer (1988) also mentioned that juvenile plants are in different growth phase than the older plants, this is another reason to leave the small plants out.

The contribution of the new plants to the total $DW_{calcified}$ was very small, with a maximum value of 13.3 % of the total $DW_{calcified}$ of collected halimedas at the Beach-station in January. The numbers of the new plants varied per plot and per station (Table 9). In order to study recruitment, a larger area should be analysed with enough plants available to give a representative recruitment estimation. The examined plants should be tagged as well and counted on regular intervals in a specified area this to observe if plants really get buried by sediment turbulence or that these plants are newly formed plants.

One of the aims of the present study was to establish the methodology of an efficient monitoring program of *H. incrassata* productivity in the Puerto Morelos reef lagoon. Recommendations for after this study are:

1. To get a better and more accurate estimation more thalli could be used collected over a bigger area. 318 thalli were used in this study for the Reef-station, 328 for the Lagoon-station and 223 for the Beach-station, this adds up to a total of 869 thalli for the three months.
2. To make future sampling less time consuming, it only would be necessary to determine the somatic calcareous dry weight ($DW_{calcified}$) of the whole thallus and the $DW_{calcified}$ of the newly formed segments. Numbering of thalli could than be done after bleaching, this saves a lot of time.

This monitoring methodology will probably lead to regression lines with a better fit, and laboratory and statistical work will be less time consuming.

Results

The biomasses found in this study, 77.9 g $DW_{calcified}$ m^{-2} at the Beach-station, 118.0 g $DW_{calcified}$ m^{-2} at the Lagoon-station and 120 g $DW_{calcified}$ m^{-2} at the Reef-station are

comparable with results found for the Puerto Morelos lagoon in a previous study. Van Tussenbroek (unpublished) found a biomass for the Beach-station of 41.7 g $DW_{calcified}$ m^{-2} (min.) - 104.5 g $DW_{calcified}$ m^{-2} (max.), for the Lagoon-station 44.2 g $DW_{calcified}$ m^{-2} (min.) - 242.4 g $DW_{calcified}$ m^{-2} (max.) and for the Reef-station 78.4 g $DW_{calcified}$ m^{-2} (min.) - 113.4 g $DW_{calcified}$ m^{-2} (max.), these measurements were done in February and August ('93 -'98). In this study mean of 3 corer samples of 20 cm Ø each month was taken.

The biomass found for *H. incrassata* was high compared to results found in some other studies. Wefer (1988) found a biomass 6.7 g $CaCO_3$ m^{-2} for *H. incrassata* in Bermuda, and Payri (1988) 7.7 g m^{-2} for *H. incrassata f. ovata* in Moorea, Tahiti (Table 10). Comparing the biomass of *H. incrassata* with the results of Freile & Hillis (1997), 211.15 g m^{-2} in the Pico Feo lagoon, San Blas Panama, the values are similar.

In the Puerto Morelos reef lagoon only the sanddwelling *H. incrassata* was observed. The other present halimeda species in the lagoon were not used. These species were in a minority and would probably not contribute a lot to the total lagoon biomass. Sanddwelling species, in general produce less $CaCO_3$ than lithophytic ones (Drew 1983, Payri 1988, Table 10). Biomass attained by sprawling lithophytic species can be very high: Drew (1983) recorded an average biomass of 111.1 to 748.9 dry g m^{-2} for lithophytic reef dwelling species of the Great Barrier Reef. Even higher biomasses have been reported (> 4 dry kg m^{-2}) by Drew and Able (1985, 1988) for reef meadows on the outer shelf of the Great Barrier Reef which were dominated by sprawling species of the Opuntia section of the halimedas. These high biomasses can not be attained by sanddwelling species. Consequently, max. values of $CaCO_3$ depositions of halimeda communities dominated by sanddwelling species can be as high as those reported for sprawling or draping lithophytic species.

Production estimations of 224.0 g $DW_{calcified}$ m^{-2} y^{-1} and 140.5 g $DW_{calcified}$ m^{-2} y^{-1} were found for the Reef- and Lagoon-station respectively, this corresponds with a Calcium production of 192.9 g dry $CaCO_3$ m^{-2} y^{-1} and 120.9 g dry $CaCO_3$ m^{-2} y^{-1}, considering a Calcium percentage of 86.1%. Results of *H. incrassata* in earlier studies are; 74.5 g $CaCO_3$ m^{-2} y^{-1} for *H. incrassata f. ovata* in French Ploynesia (Payri 1988), 50 g $CaCO_3$ m^{-2} y^{-1} for *H. incrassata* in Bermuda (Wefer 1980) and 2323 g $CaCO_3$ m^{-2} y^{-1} for *H. incrassata* at the Pico Feo lagoon, San Blas Panama (Freile & Hillis 1997), Table 10. Comparing the first two studies the production in this study was rather high. A probable explanation is the higher biomass of *H. incrassata* in the Puerto Morelos reef lagoon resulting in a higher production. However the result of Freile and Hillis (1997) is an order of magnitude higher than the one found in this study. Freile and Hillis found a rather high biomass for *H. incrassata* (211.15 g m^{-2}) and a turnover of 11 crops a year (obtained by counting the newly formed segments). This leads to a high yearly production. If the yearly production in this study had been

estimated by counting the segments the result would have been in the same order as the yearly production found by Freile and Hillis. For the example in Graph 2 a turnover of app. 12 crops was found, multiplying this with the biomass leads to a production of app. 1440 g $DW_{calcified}$ m^{-2} y^{-1}. So it is possible that the results found by Freile and Hillis are too high.

The production of contributions of other halimeda species like: *H. monile* and *H. tuna* in the Puerto Morelos reef ecosystem (reef + lagoon) was not measured, so the overall production at Puerto Morelos reef is not known. In other studies considering whole reef ecosystem productions of 2400 g $CaCO_3$ m^{-2} y^{-1} for *Halimeda* spp. at the Great Bahama Bank (Freile 1995) and 2234 g $CaCO_3$ m^{-2} y^{-1} for *Halimeda* spp. at the Great Barrier Reef (Drew 1983) were found. It was difficult to determine the methodology of most studies, but if the number of new segments was used as turnover rate, these values are probably too high.

Halimeda incrassata in the Puerto Morelos lagoon has a turnover rate of ca. two crops a year calculated with the tentative yearly production values and biomass values. Hillis (1991) estimated a range of 3 -12 crops a year for most halimeda species and environments, so yearly turnover of the present study does not fit this estimation of Hillis. But how this range was estimated is unknown, by counting the segments or by determining the dry weight.

During the time of the experiments (November 1997 till January 1998) the water temperature was rather low, due to a high frequency of cold north winds. The temperature of the seawater was sometimes about 25°C. Because of Puerto Morelos rather high latitude, there is a clear temperature difference between winter and summer. The optimum growth temperature for Halimeda is between 27°C and 29°C (Hillis-Colinvaux 1980). In summer the growth is probably higher and this would lead to a higher yearly production. The experiment should be repeated during the whole year to see what the seasonal variation is and to obtain a better production estimate.

The percentages of $CaCO_3$ for *Halimeda incrassata* found in the Puerto Morelos reef lagoon were not different compared with others studies (Table 10). Mean $CaCO_3$ percentage for all thalli at all stations and months was 86.1%. Multer (1988) found a percentage of 88%, Payri (1988) a percentage of 86.5% and Freile and Hillis (1997) a percentage of 81%, these figures are not significantly different compared to this study. No difference in $CaCO_3$ contents was found between the new and old segments of each thallus at the Reef-station, but a significant difference was found at the Lagoon- and the Beach-station between the new and old segments.

Possible explanations are:

1. The turbulence is higher at the Reef-station due to the wave activity, resulting in a higher availability of nutrients in the vicinity of the reef.
2. The Reef-station is the shallowest of all the stations and so having a higher photosynthesis. Because the calcification is a process mediated by photosynthesis, this could result in a higher and faster deposition of $CaCO_3$ in the intercellular-spaces.

Halimedas reproduce sexually by production of gametes, which are released in the water. Such sporulation was also observed at the Beach-station in the last two weeks of January for *Halimeda incrassata*. Plants were found with the gametangia still in the process of ripening, but also dead individuals were found with the gametangia empty and the thallus totally white. These were easy to recognise. In two occasions thalli, which had not sporulated, were taken to the lab, put in an aquarium and were monitored during the day and the night. Sporulation occurred at 7:30 am the next day. Sporulation took place in less than 30 min, leaving the aquarium with a green dense cloud of spores and the thallus totally white. Many juvenile plants were observed in July (personal comment) of the same year, suggesting a development period of 15 months, considering these plants the result of the gamete release of the past year. This is a somewhat longer period than found by Meinesz (1988) who estimated that a zygote took 12 months to develop into a plant. The contribution of the sexual production to population maintenance is not known and is an interesting topic for future studies.

Figure 8

Halimeda incrassata with gametangia. Photograph taken in the Puerto Morelos reef lagoon.

Table 10

Summary of results of other authors who directly have measured the growth, turnover or productivity of Halimeda spp. If not mentioned otherwise direct techniques, such as staining with Alizarin Red S or marking of branching tips were used. The values presented are averages over the sampling period: more than one value indicates spatial differences in the study area. The sample size is for the measurement of growth, production or turnover, and expressed as number of plants if not mentioned otherwise

Species	Biomass (g $CaCO_3$ m^{-2})	Density (ind. m^{-2})	Production (g $CaCO_3$ m^{-2} yr^{-1})	[$CaCO_3$] (% dry weight)	Turnover (d)	Sample Size. No. plants	Sample dates	Duration of growth period	Location	Source
Rihpsalian spp.										
H. macroloba	135	-	404.9	-	120	-	-	-	Guam	Merten 1971
H. incrassata	*1 0.1 - 21.7 dry	-	0.1 – 62.3	-	-	1 m^2 samples	Sep. - Dec. '73 & March - July '74	8 weeks	South Florida	Bach 1979
H. monile	0 - 7.1 dry	-	0.1 - 62.3	-	-	Idem	Idem	Idem	Idem	Bach 1979
H.incrassata.	6.7	-	50.0	-	52	155	Aug. & Sep. '78	3 - 27 d	Bermuda	Wefer 1980
H.monile, H.incrassata.	-	26 - 36	60.7 - 114.3	88	39	1866	Apr. - Jul. '80 & '83	5 - 40 d	Antigua, Leeward. Isl.	Multer 1988
H.incrassata f. *ovata*	*3 24.6	-	*3 74.5	86.5	*4 30	328 ind.	Aug. '84 - Jul. '85	9 - 169 d	French Ploynesia	Payri 1988
H.incrassata	221.2	88	2323.0	81	32	177	Sep - Nov '93	3 - 23 d	San Blas, Panama	Frelle & Hillis 1997
H. incrassata	**67.1 – 103.6**	-	**121.5 – 193.8**	**86.1**	***4 199**	**869**	**Nov. '97 - Jan. '98**	**14 d**	**Puerto Morelos, Mexico**	**Present Study**
Lithophytic spp.										
H. coplosa & H. opuntia	*1 145.5	-	2234	90	-	1685 branch tips	'81	*9 40 - 73 d	Great Barrier Reef, Australia	Drew 1983
H. opuntia	*3 180	-	*3, 5 2300	-	*4 29	-	Aug. '84 - July '85	-	French Polynesia	Payri 1988
H. discoidea	*3 4.6	-	*3, 5 28	-	*5 57	-	Aug. '85	-	idem	Payri 1988

*1 Data only for stations or sites which were used for growth measurements.
*2 This is an estimated turnover by the author.
*3 Values compensated for areas where the Halimeda species occurred.
*4 Turnover calculated from P/B ratio.
*5 Production measured by oxygen respirometry (24 hour incubation).

Acknowledgments

I would like to thank the director of 'Instituto de Ciencias del Mar y Limnologia' of the 'Universidad Nacional Autonoma de México' and specially the head of the station in Puerto Morelos to give me the opportunity to realize my study project. Also I would like to thank Brigitta van Tussenbroek for accepting me as her student and for all the help and supervision. I Thank Briggita van Tussenbroek and Luz Maria Arguelles for their presence in the early morning and the assistance with fieldwork. I would like to thank all the staff of the station in Puerto Morelos, for all the help and good times. I am grateful to Anneke Breeman for her support as my supervisor from the University of Groningen. I would like to thanks Iris Hendriks for her mental support throughout the whole period and thanks to my parents who helped me realizing this project. Also I would like to thank the "Marco Polo" foundation, "Alberta Magna" foundation and the "Groninger Universiteit Fonds" for the financial support.

Gracias a todos los estudiantes que me acompañaron en la temporada que yo pasé en Puerto Morelos. Sin ustedes yo no hubiera pasado un tiempo tan inolvidable.

Gracias!

References:

Askenasy E (1888) Algen. In "Die Forschungsreise S.M.S. Gazelle 1874 bis 1876 unter Kommando.Freiherrn von Schleinits" Th. 4, Bot ., 58 pp., Berlin

Bach SD (1979) Standing crop. Growth and production of calcareous Siphonales (Chlorophyta) in a South Florida Lagoon. Bull. Mar. Sci. 29:191-201

Back W (1985) Hydrogeology of Yucatán. Geology and Hydrogeology of Northeastern Yucatán and Quarternary Geology of Northeastern Yucatán: 99-124

Blair SM, Norris JN (1988) The deep-water species of *Halimeda Lamouroux* (Halimedaceae, Chlorophyta) from San Salvador Island, Bahamas: species composition, distribution and depth records. Coral Reefs 6:227-236

Borowitzka MA (1977) Algal calcification. Oceanography and Marine Biology, Anual Revieuw 15:189- 223

Borowitzka MA (1982) Mechanisms in algal cacification. Progress in Phycological Research 1:137-177

Borowitzka MA, Larkum AWD, Nockolds CE (1974) A scanning electron microscope study of the structure and organization of the Calcium Carbonate deposits of algae. Phycologia 13(3):195-203

Borowitzka MA, Larkum AWD (1976a) Calcification in the green alga *Halimeda*. II. The exchange of Ca^{2+} and the occurence of age gradients in calcification and photosynthesis. Journal of Experimental Botany 27(100):864-878

Borowitzka MA, Larkum AWD (1976b) Calcification in the green alga *Halimeda*. III. The sources of inorganic carbon for photosynthesis and calcification and a model of the mechanism of calcification. Journal of Experimental Botany 27(100):879-893

Borowitzka MA, Larkum AWD (1976c) Calcification in the green alga *Halimeda*. IV. The action of metabolic inhibitors on photosynthesis and calcification. Journal of Experimental Botany 27(100):894-907

Borowitzka MA, Larkum AWD (1977) Calcification in the green alga *Halimeda*. V. An ultrastructure study of the thallus development. Journal of Phycology 13(1):6-16

Brasier MD (1975) An outline history of seagrass communities. Palacontology, Vol.18 Part. 4:681-702

Clifton KE (1997) Mass spawning by green algae on coral reefs. Vol 275:1116-1118

Colivaux L Hillis, Wilbur KM, Watabe N (1965) Tropical marine algae: growth in laboratoy culture. Journal of Phycology 1(2):69-78

Colivaux L Hillis (1968a) New species of *Halimeda*: a taxonomic reappraisal. Journal of Phycology 4:30-35

Colivaux L Hillis (1968b) Vegetative reproduction of *Halimeda* and related Siphonales in laboratory culture. Journal of Phycology 4 (suppl.), 4.

Drew AD (1983) *Halimeda* biomass, growth rates and sediment generation on reefs in the Central Great Barrier Reef Province. Coral Reefs 2:101-110

Drew AD, Able KM (1988) Studies on *Halimeda*. I. The distribution and species composition of *Halimeda* meadows throughout the Great Barrier Reef Province. Coral Reefs 6:195- 205

Drew AD, Able KM (1988) Studies on *Halimeda*. II. Reproduction, particulary the seasonnality of gametangia formation, in a number of species from the Great Barrier Reef Province. Coral Reefs 6:207-218

Drew AD, Able KM (1985) Biology, sedimentology and geography of the vast inter-reefal *Halimeda* meadow within the Great Barrier Reef province. Proc 5th Int Coral Reefs Sym:15-20

Drew AD, Able KM (1990) Studies on *Halimeda*, III A daily cycle of chloroplast migration within segments. Botanica Marina 33:31-45

Elliott GF (1960) Fossil calcareous algal floras of the Middle East with a note on a Cretaceous problamaticum, *Hensonella cylindrica* gen. et sp. nov. Quarterly Journal of the Geological Society of London, CXV:217-232

Freile D (1995) Leeward bank margin *Halimeda* meadows and draperies and their sedimentary importance on the Western Great Bahama Bank slope. Coral Reefs 14:27-33

Freile D, Hillis L (1997) Carbonate productivity by *Halimeda incrassata* in a land proximal lagoon, Pico Feo, San Blas, Panama. Proc. 8th int. Coral Reef sym 1:767-772

Gonzáles J (1987) Las algás de México. Ciencias 16-25.

Hillis L (1959) A revision of the genus *Halimeda* (order Siphonales). Publications of the Institue of Marine Science, University of Texas 6:321-403

Hillis L (1991) Recent calcified Halimedaceae. In: Riding , R (ed) Calcareous Algae and Stromatolites. Springer-Verlag, Berlin 167-188

Hillis L (1997) Coralgal reefs from a calcareous green alga perspective, and a first carbonate budget. Proc 8th int. Coral Reef sym 1:761-766

Hillis-Colinvaux L (1972) "Research on and culture and calcareous Green Algae", Ohio State University Research Foundation, Report to Department of the Navy, Office of Naval Research, Washington, DC. ???

Hillis-Colinvaux L (1973) Reproduction in the calcareous green algae of coral reefs. Journal of Marine Biological Association of India 14(2): 328-334

Hillis-Colinvaux L (1974) Productivity of the coral reef alga *Halimeda* (Siphonales). Proc. 2nd Int. Coral Reefs sym. 1:35-42

Hillis-Colinvaux L (1980) Ecology and taxonomy of *Halimeda*: Primary producer of coral reefs. In :Blaxter JHS, Russel FS, Yonge M (eds) Adv Mar Biol, Academic Press, London New York,17:vii, viii + 327pp

Howell DC (1997) Statistical methods for psychology. Duxbury press, USA 4th Edition, 724pp

Hudson JH (1985(6)) Growth rate and Carbonate production in *Halimeda opuntia* : Marquesas Keys, Florida. In: Toomey, DF and Nitecki, MH, Paneoalgology: Contemporary Research and Applications. Springer-Verslag: 257-263

Johnson JH (1969) A review of the Lower Cretaceous algae. Professional Contribution of the Colorado School of Mines 6:180 pp, Golden Colorado

Jordán E, Merino M, Moreno M, Martin E (1981) Community structure of coral reefs in the Mexican Caribean. Proc. 4th Int. Colral Reefs sym. 2:303-308

Litter MM, Litter DS, Lapointe BE (1988) A comparison of nutrient- and light-limeted photosynthesis in psammophytic versus epilithic forms of *Halimeda* (Caulerpales, Halimedaceae) from the Bahamas. Coral Reefs 6:219-225

Lobban MJ, Wynne MJ (1981) The biology of seaweeds. Botanical monographs 17. Backwell scientific publications, Great Brittan. 786pp.

Marzelek DS (1971) Skeletal ultrastructure of sediment producing green algae. Proc. 4th Annual Scanning Electron Microscope sym.: 273-280

Meinesz A (1988) Connaissances actuelles et contribution à l'étude de la reproduction et du cycle des Udotéacées (Caulerpales, Chlorophytes). Phycologia 19(2):110-138

Merino M, Otero L (1991) Atlas Ambiental Costero, Puerto Morelos - Quintana Roo.: 1-80

Multer HG (1988) Growth rate, ultrastructure and sediment contribution of *Halimeda incrassata* and *Halimeda monile*, Nonsuch and Falmouth Bays, Antigua, W.I.. Coral Reefs 6:179-186

Payri CE (1988) *Halimeda* contribution to organic and inorganic production in a Thahitian reef system. Coral Reefs 6:251-262

Ruíz-Rentería F, van Tussenbroek BI, Jordán-Dahlgren E (in press) Characterization of the Puerto Morelos (Mexico) CARICOMP site. In: Kjerfve B (editor), Caribbean Coastal Marine Productivity (CARICOMP): Coralreef, Seagrass and Mangrove Site Characteristics. UNESCO, Paris.

Stark LM, Almodovar L, Krauss RW (1969) Factors affecting the rate of calcification in *Halimeda opuntia (L.) Lamouroux* and *Halimeda discoidea Decaisne*. Journal of Phycology 5(4):305-312

Walters LJ, Smith CM (1994) Rapid rhizoid production in *Halimeda discoidea Decaisne* (Chlorophyta, Caulerpales) fragments: a mechanism for survival after separation from adult thalli. J. Exp. Mar. Biol. Ecol. 175:105-120

Wefer G (1980) Carbonate production by algae *Halimeda, Penicillus* and *Padina*. Nature Vol. 285:323-324

Wilbur KM, Hillis-Colinvaux L, Watabe N (1969) Electron microscope study of calcification in the alga *Halimeda* (order Siphonales). Phycologia 8(1):27-35

Wynne MJ (1986) A checklist of benthic marine algae of the tropical and subtropical Western Atlantic. Canad. J. Bot. 64: 2239-2281

Zar JH (1982) Biostatistical analysis. 2nd Edn., Prentice Hall, Englewood Cliffs NJ. 718pp