Deep-Sea Research, Vol. 32, No. 4, pp. 451 to 464, 1985.
Printed in Great Britain.

0198–0149/85 $3.00 + 0.00

Photosynthesis and calcification in four deep-water *Halimeda* species (Chlorophyceae, Caulerpales)

PAUL R. JENSEN,* ROBERT A. GIBSON,* MARK M. LITTLER† and DIANE S. LITTLER†

(*Received* 31 *July* 1984; *in revised form* 30 *October* 1984; *accepted* 5 *November* 1984)

Abstract—Measurements of organic and inorganic carbon production were made for deep-water (76 m) specimens of *Halimeda discoidea* Descaisne, *H. cryptica* L. H. Colinvaux and Graham, *H. copiosa* Goreau and Graham and *H. lacrimosa* Howe. Photosynthetic and calcification rates ($CaCO_3$ production) were estimated on shore at simulated *in situ* light intensities (20 $\mu E\ m^{-2}\ s^{-1}$) and temperatures (27°C). Photosynthetic rates, as measured by both oxygen evolution and carbon-14 incorporation, agreed well and showed efficient light utilization, ranging from 0.04 to 0.24 mg organic C g dry $wt^{-1}\ h^{-1}$. Calcification rates, measured by ^{14}C incorporation, ranged from 0.06 to 0.16 mg inorganic C g dry $wt^{-1}\ h^{-1}$ and were positively correlated ($R^2 = 77$) with photosynthesis, implying a physiological mediation of the depositional process. Of the four species, only *H. cryptica* exhibited significant ($P < 0.05$) interspecific differences in calcification and photosynthetic rates (both rates being higher) due, in part, to its greater organic content. The carbon incorporation rates and associated algal cover suggest deep-growing *Halimeda* species to be important producers of carbonaceous materials.

INTRODUCTION

THE calcareous green alga *Halimeda* has been noted as a major element of tropical Atlantic reefs (GINSBERG, 1956; MILLMAN, 1974; NEUMANN and LAND, 1969, 1975; STEARN *et al.*, 1977; HILLIS-COLINVAUX, 1980, 1982). It is the single most abundant component of Atlantic reef sands, dominating both reef and nearshore algal banks (LITTLER, 1976). Skeletal sand-size components from some lagoonal and peripheral reef sediments are comprised of up to 77 and 44% *Halimeda* fragments, respectively [MILLMAN (1974) and ORME (1977) as cited in HILLIS-COLINVAUX (1980)]. It was estimated that, perhaps for all the Bahama Banks, calcareous green algae have produced more aragonitic lime mud during the past 5500 years than could be accommodated on the bank tops (about 24 g $CaCO_3\ m^{-2}\ y^{-1}$, NEUMANN and LAND, 1975). This significant production of unconsolidated carbonate sediments, in terms of total carbonate production, appears to be quantitatively more important than the carbonate produced by the reef framework itself (MILLIMAN, 1974). Thus, at times, *Halimeda* is the most important contributor to the geomorphology of reef structures (HILLIS-COLINVAUX, 1980).

To date, all studies of *Halimeda* production have been with shallow-water samples. Extensive populations of deep-water *Halimeda* are known to exist, but estimates of their contributions of both organic and carbonate materials are purely speculative (HILLIS-COLINVAUX,

* Division of Marine Sciences, Harbor Branch Foundation, Inc., Rt. 1, Box 196, Ft. Pierce, FL 33450, U.S.A.
† Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A.

1980). Quantification of the numerical importance of the deep fore-reef *Halimeda* to biogenic carbonaceous accumulations is essential to understanding reef biogenesis, deep-sea sedimentary processes, and the geological record of the tropics.

The deposition of $CaCO_3$ in most calcareous macroalgae is a physiological process (BOROWITZKA, 1982a) in intimate association with the utricles or thallus surface (BOROWITZKA, 1982b). The two proposed mechanisms of skeletal deposition are: (1) cellular metabolic processes (not yet elucidated) and (2) photosynthetic precipitation, i.e., the removal of carbon dioxide and bicarbonate during photosynthesis, thereby raising the pH to levels where $CaCO_3$ precipitation occurs. Photosynthetic precipitation within intercellular spaces is the mechanism suggested to explain aragonite deposition in *Halimeda* (BOROWITZKA, 1982b). LEWIN (1962) proposed that carbon dioxide uptake alone is insufficient to explain calcification because of the fact that calcified and non-calcified photosynthetic algae live side-by-side in nature. A second point is the presence of uncalcified photosynthetic joints or flex-points in *Halimeda*. This is difficult to explain if photosynthetic precipitation is solely the mechanism. Thirdly, algal calcification in the absence of light has been documented (GOREAU, 1963; STARK *et al.*, 1969; SMITH, 1973). However, the presence of extracellular organic inhibitors of calcification could explain the lack of calcification by photosynthetic cells (BOROWITZKA, 1982a), whereas dark calcification might result from strictly non-metabolic physical precipitation. These considerations indicate that calcification is more than a fortuitous by-product of photosynthesis; yet a relationship between the two processes exists and has been demonstrated in *Halimeda* (GOREAU, 1963; STARK *et al.*, 1969; BOROWITZKA and LARKUM, 1976a) and in other algae (IKEMORI, 1970; PEARSE, 1972; PENTECOST, 1978; BOROWITZKA, 1981).

This study examined four of the dominant species from the '*Halimeda* zone' off San Salvador Island in the Bahama Islands (24°10′N, 74°30′W). The objectives were to quantify rates of photosynthesis and calcification, determine if interspecific variations in these rates occur and assess the interactions between the two processes. This report provides the first recorded production rates for deep-water (> 50 m) siphonaceous algae, contributes to the pool of knowledge accumulating on the relationship between calcification and photosynthesis in marine algae and is the initial step in our ongoing assessment of the contributions made by calcareous algae in the '*Halimeda* zone' to production and sedimentary processes in the tropics.

The San Salvador region is characterized by vertical algal zonation and a prominent submarine geomorphology. A deep fore-reef exists in this area starting at depths from 20 to 40 m. Extending from the top of the deep fore-reef to below 100 m is a zone dominated by the genus *Halimeda*. Personal observations made via SCUBA and submersible diving off San Salvador indicate the '*Halimeda* zone' to exhibit the highest algal biomass of any deep fore-reef algal zone encountered. *Halimeda* species attain cover values as high as 21% at 76 m but reach maximum cover still deeper (44% at 110 m, LITTLER *et al.*, 1985).

MATERIALS AND METHODS

Four deep-water *Halimeda* species (*Halimeda discoidea, H. cryptica, H. copiosa,* and *H. lacrimosa*) were collected from San Salvador Island. Collections were made at 76 m using the JOHNSON-SEA-LINK four-man submersible and its Petersen-type manipulator grab sampler. This device removes biota and substrata without undue pulling or tearing and, consequently, minimizes physical injury. Samples were kept shaded to prevent light shock and

taken to the College Center of the Finger Lakes, San Salvador Island. In preparation for photosynthetic and calcification rate measurements, samples were placed in trays of ambient seawater, carefully sorted, hand cleaned of epiphytic material, and held overnight in mesh dive bags anchored offshore. Material selected was 'healthy' in appearance, i.e., characteristically pigmented, uninjured, and free of epiphytes. Excess material was subsequently preserved in 5% buffered seawater Formalin as voucher specimens.

Photosynthetic and calcification rate determinations were performed on samples concurrently using both O_2 and ^{14}C techniques. Incubations were conducted in 11, wide-mouth, glass canning jars between 0900 and 1400 EST (avoiding the problem of diel periodicity) on 15 October 1983. Jars were cleaned and aged with seawater prior to the start of experiments. Incubation water, collected before the experiments, was filtered through a nanoplankton net (10 µm pore size) then vigorously poured from bucket to bucket to bring the O_2 level to ambient saturation. Single whole organisms minus holdfasts where incubated for 4 h using four replicate light bottles for each species. Dark bottles were not used as respiration cannot be estimated using the ^{14}C technique. Bottles were put in clear plastic trays in groups of four, with each bottle receiving a teflon-coated stir bar. Trays were placed on specially constructed four-unit magnetic stir motors powered by a foot pump from an inflatable boat kit and stirred at *ca.* 10 min intervals. This mixing disrupts any metabolically induced diffusion gradients that may form. Incubation light intensities, as measured by a Li-Cor LI-550 integrating light meter with a 190S cosine response sensor, were maintained at collection intensities (*ca.* 20 µE m^{-2} s^{-1}, 1 to 2% surface irradiance). The light source was clear, blue skies (not direct sunlight) with multiple layers of neutral density screening used to reduce intensities. Water temperature was maintained at the collection temperature (27°C), by constant flushing of the incubation bath. Water samples were taken *in situ* for pH, alkalinity, and salinity determinations to estimate amounts of available, soluble inorganic carbon as described in Strickland and Parsons (1972).

Oxygen technique

Care was taken to follow the recommendations of Littler (1979, 1980) and Littler and Arnold (1980) concerning proper thallus to volume ratios, handling of algae, and incubation and O_2 analysis. Oxygen concentrations were recorded at the start and finish of all incubations using an Orion digital O_2 analyzer (Model 801). Changes in O_2 concentration were converted to mg C fixed g dry wt^{-1} h^{-1} by standard methods (Strickland, 1960), assuming a photosynthetic quotient of 1.00.

Carbon-14 technique

The isotope technique was modified from that of Paasche (1963) which measures calcification and photosynthesis simultaneously. This method reduces the error associated with polysaccaride binding when ^{45}Ca is used. Incubation procedures involved the addition of 11 µCi (407 kBq) $NaH^{14}CO_3$ (Amersham Corp.) to the incubation vessel prior to the initial O_2 reading. Carbon-14 experiments were terminated, after final O_2 measurements were made, by rinsing the thalli with filtered seawater and freezing. Plant material was oven-dried at 80°C for 12 h and subsequently powdered using a mortar and pestle, producing a homogeneous sample. Powdered samples yield an average rate measurement for the entire thallus, as metabolic activities vary from proximal to distal segments in *Halimeda* (Borowitzka and Larkum, 1976a). Laboratory processing separated incorporated ^{14}C into two fractions—organic and inorganic—enabling distinctions to be made between carbon

uptake via photosynthesis and calcification (see below). For each fraction, triplicate subsamples of every replicate were weighed and processed (when subsample standard deviations were $\geqslant$20%, the procedure was repeated and the results combined). Samples were counted in 10 ml ACS liquid scintillation cocktail (Amersham Corp.) using a Searle Mark III liquid scintillation counter. All counts were corrected for background at 75 to 90% counting efficiencies using the samples channels ratio method.

Fraction 1: *photosynthetic rate.* Approximately 15 µg of powered thallus material were weighed into scintillation vials and 500 µl of 1.0 N HCl were added to remove inorganic ^{14}C. Samples were allowed to dry at room temperature and digested following a procedure modified from LOBBAN (1974) for the solubilization of algal material: 500 µl of 30% H_2O_2 were added to each sample and reacted at 60°C overnight with the vials capped; 200 µl of 70% $HClO_4$ were then added and the samples digested at room temperature with lids recapped for 1.0 h; scintillation cocktail was added and the samples counted. Photosynthetic rates represent mg organic carbon incorporated per gram dry tissue per hour as described in UNESCO (1973).

Fraction 2: *calcification rate.* Ten to 20 µg of powdered thallus material were weighed into a stoppered 20/100 mm Pyrex side arm test tube—the reaction flask. This tube was connected via surgical tubing to a stoppered 10 ml scintillation vial containing 4 ml of the CO_2 trapping agent ethanolamine (Amersham Corp.). A steady flow of nitrogen gas was passed serially through the reaction flask and into the scintillation vial where it was bubbled into the ethanolamine with a Pasteur pipette. Decalcification was initiated by the addition of an excess (4 ml) of 1.0 N HCl by syringe into the reaction flask. The acid reaction proceeded for 2.0 h, at which time the gas flow was terminated and the entire volume of ethanolamine counted. Calcification rates represent inorganic carbon incorporated per gram dry tissue per hour as calculated for organic carbon (UNESCO, 1973).

The 'photosynthetic' and 'calcification' rates measured using radioisotope tracer techniques are estimates and include certain inherent errors [see BOROWITZKA (1977) and WETZEL (1974) for reviews of macroalgal calcification and photosynthetic rate determinations]. Errors in measuring photosynthesis may, in part, be ascribed to the loss of intermediary photosynthates (simple sugar precurserates) during acid hydrolysis or solubilization. Direct loss of recently photosynthetically fixed ^{14}C can occur through either active excretion (as well as wounding) or from respiratory processes that occur in the light. On a theoretical basis, gross or 'real' photosynthesis will be measured by the ^{14}C method only if the following conditions are met (THOMAS, 1963): (1) ^{14}C is assimilated at the same rate as ^{12}C; (2) no ^{14}C is incorporated into cellular material by non-metabolic processes; (3) no ^{14}C is lost by dark respiration and/or photorespiration (BURRIS, 1977) which may accompany photosynthesis; and (4) no ^{14}C is lost by excretion. In reality, none of these conditions are completely satisfied, therefore the ^{14}C method measures something between net and gross photosynthesis (PETERSON, 1980). The amount of non-metabolic ^{14}C uptake via adsorption, absorption, and isotopic exchange is thought to be small and no correction was made for it in this study. In the past, values of dark carbon fixation have been either subtracted from the light carbon fixation rates, recorded separately, or omitted entirely.

Calcification rates have been measured in calcareous macroalgae using both ^{14}C and ^{45}Ca techniques (GOREAU, 1963; STARK *et al.*, 1969; PEARSE, 1972; LITTLER, 1973; BOROWITZKA and LARKUM, 1976a, b; BOROWITZKA, 1977, 1979; BOHM, 1978; PENTECOST, 1978). Results of the ^{45}Ca method are complicated by the existence of a large, readily exchangeable pool of calcium in the thallus, isotopic exchange on the aragonite crystal surface, and the binding of

calcium to cell wall mucilages and polysaccharides (BOROWITZKA and LARKUM, 1976a). This incorporation of non-calcium carbonate ^{45}Ca into the thallus and the exchange of labeled with non-labeled calcium in the aragonite crystal results in a significant overestimation in calcification rates when the ^{45}Ca technique is used (BOROWITZKA and LARKUM, 1976a).

The ^{14}C technique has been used successfully in measuring both calcification and photosynthesis rates simultaneously in coccolithophores (PAASCHE, 1963), coralline algae (PENTECOST, 1978), and in *Halimeda* (BOROWITZKA and LARKUM, 1976a). Carbon-14 does not bind to cell walls or mucilages (BOROWITZKA and LARKUM, 1976a) and the free intercellular inorganic ^{14}C species are largely lost during the drying and grinding processes. This appears to be the method of choice for measuring algal calcification rates, although the following assumptions still must be made: (1) rinsing removes all externally adsorbed $NaH^{14}CO_3$, (2) organic species remain intact during acid hydrolysis, and (3) all label released as CO_2 was in the $CaCO_3$ form. The length of experiments employed here was assumed to be of sufficient duration to have eliminated any effects of initial curvilinear uptake (BOHM, 1978) due to isotopic exchange with the internal carbonate pool and intercellular absorption (BOROWITZKA, 1982a).

Ash-free dry weight

Samples used in the oxygen determinations were dried at 80°C for 12.0 h, weighed, ashed at 500°C for 6.0 h, and reweighed. The difference in weights was thus reported as ash-free dry weight and used to calculate percent organic weight.

Efficiency of techniques

A series of methodological tests revealed that trapping efficiency exceeded 95%. The recovery of inorganic label ($NaH^{14}CO_3$) in the trap and organic label (^{14}C-sucrose) in the reaction flask, after 1 N HCl decalcification, averaged 94 and 98%, respectively.

Recovery of organic label after HCl decalcification and H_2O_2-$HClO_4$ solubilization. A known activity of ^{14}C-labeled sucrose was added to powdered thallus material in the reaction flask. The tissue was decalcified and digested following the technique given in the methods section on photosynthetic rates using the apparatus described in the section on calcification rates. This digestion, when performed under a nitrogen gas flow, showed a recovery rate in the reaction flask of 33% while trap 1 showed only background. The same test without the gas flow (actual experimental conditions) indicated that 98% of the activity remained in the acid solution (reaction flask). It is suggested that the concentrated perchloric acid hydrolyzed the disaccharide sucrose into glucose and fructose which were then dehydrated yielding furfurals, an aldehyde derivative of furan (LEHNINGER, 1975). These ring structures, under positive gas flow, left the acid solution and passed through the traps. These findings show that concentrated acids are not required for the decalcification process and their use, in conjunction with a gas flow trapping system, will result in hydrolysis of photosynthates and a likely underestimation of photosynthetic production.

RESULTS

Irrespective of measurement method, photosynthetic rates of the four species showed a six-fold range, between 0.04 and 0.24 mg organic C g dry wt^{-1} h^{-1} (Fig. 1). Analyses of variance (all statistical analyses were tested at the $P = 0.05$ level) showed that interspecific differences exist; Student–Newman–Keuls (SNK) multiple range tests indicated that

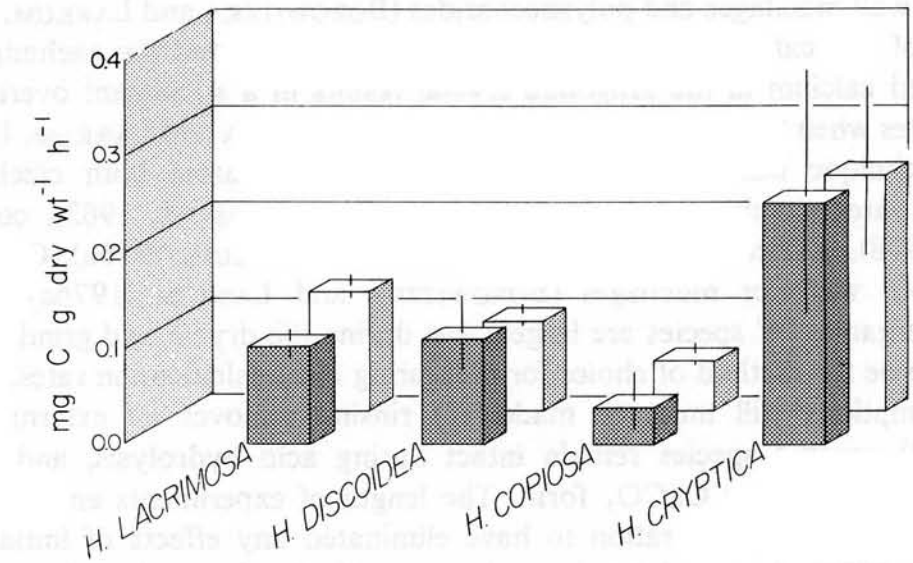

Fig. 1. Mean photosynthetic rates per dry weight (± 1 s.d.) of four deep-water *Halimeda* species as measured by the ^{14}C (front row) and O_2 (back row) techniques.

Halimeda copiosa, H. discoidea, and *H. lacrimosa* produced at essentially equal rates while *H. cryptica* produced at a significantly higher rate on a gravimetric basis.

The following percent ash-free dry weights (organic contents) were measured: *Halimeda lacrimosa,* 7.24 ± 0.40%; *H. discoidea,* 7.55 ± 1.10%; *H. copiosa,* 4.58 ± 0.20%; and *H. cryptica,* 15.80 ± 2.87. Normalizing photosynthesis to ash-free dry weights (expressed as mg C g ash-free dry wt^{-1} h^{-1}) reduced interspecific differences to an undiscernible level (SNK test) for both the O_2 and ^{14}C data (Fig. 2).

Rates of photosynthesis as measured by the O_2 and ^{14}C techniques, were quite similar (Figs 1 and 2). Results indicate the rates for *Halimeda copiosa, H. cryptica,* and *H. discoidea* were not significantly different between the two techniques. Rates for *H. lacrimosa* were different, however by <18%. This difference was small and could be associated with the precision of the experimental techniques (i.e., coefficients of variation) and/or the estimate made

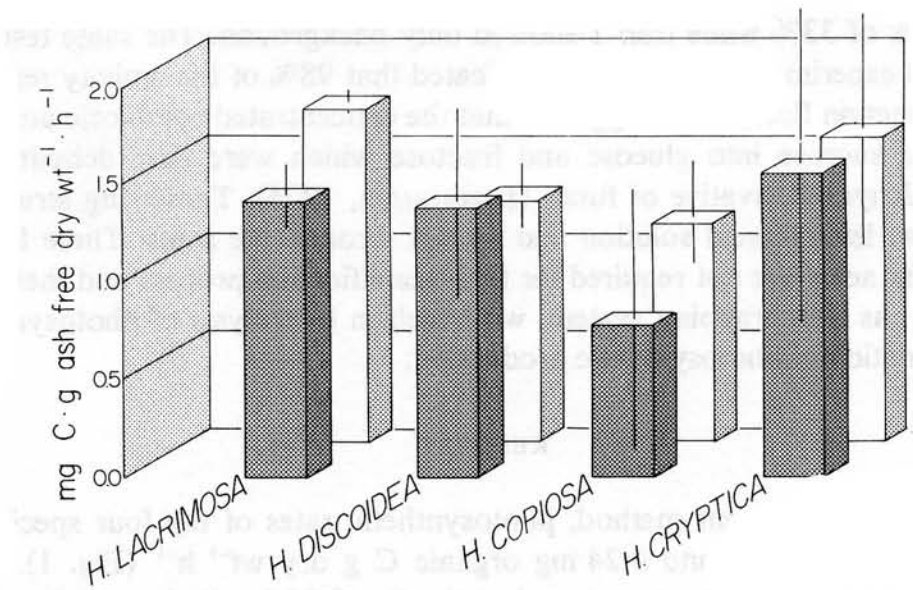

Fig. 2. Mean photosynthetic rates per organic weight (± 1 s.d.) of four deep-water *Halimeda* species as measured by the ^{14}C (front row) and O_2 (back row) techniques.

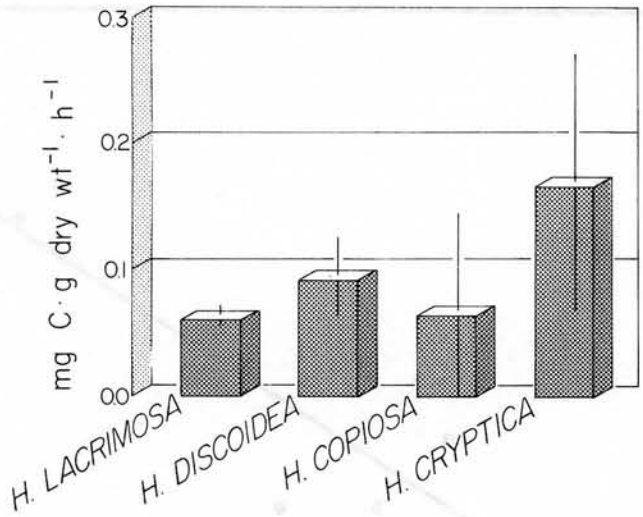

Fig. 3. Mean calcification rates per dry weight (± 1 s.d.) of four deep-water *Halimeda* species as measured by the ^{14}C technique.

in converting from O_2 to carbon production. The general agreement in methodologies adds validity to the use of ^{14}C in measuring photosynthetic processes.

Average calcification rates (Fig. 3) ranged between 0.06 and 0.16 mg inorganic C g dry wt^{-1} h^{-1}. The species with the lowest percent inorganic material, *Halimeda cryptica*, produced inorganic material at the greatest rate. Analyses of variance indicated that interspecific calcification rates were significantly different; a SNK test indicated all species produced at equal rates except for *H. cryptica* which was significantly higher. Calcification rates were expressed as mg inorganic C g ash-free dry wt^{-1} h^{-1} (Fig. 4). This conversion reduced interspecific differences to an undiscernible level (SNK test).

Trends of photosynthesis and calcification (viz. Figs 1 and 3) are similar and suggest a linear relationship between these systems (Fig. 5). Regression analysis indicated that 77% of the variance in the data is attributed to the relationship between photosynthesis and calcification. In terms of carbon fixed, average species production to calcification ratios ranged from 0.93 to 1.76.

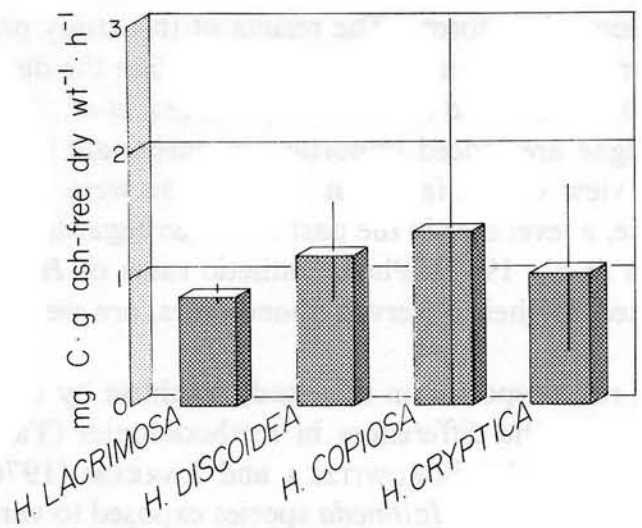

Fig. 4. Mean calcification rates per organic weight (± 1 s.d.) of four deep-water *Halimeda* species as measured by the ^{14}C technique.

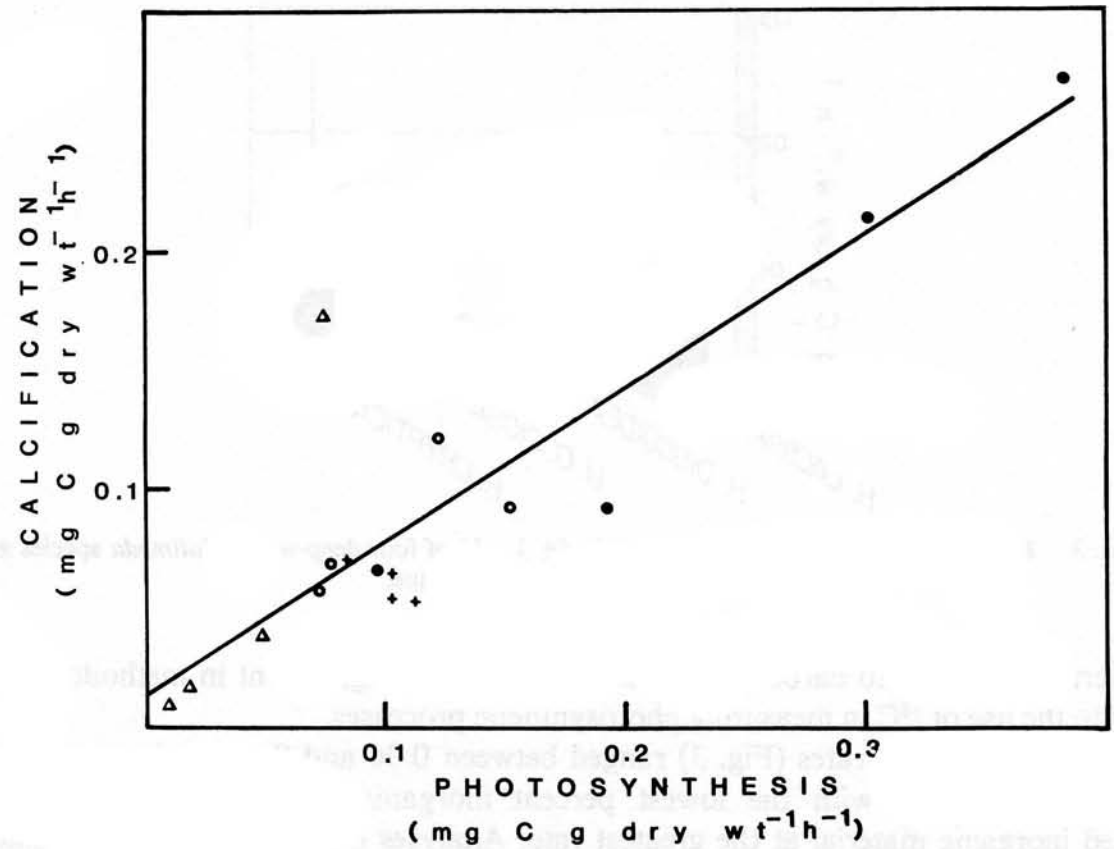

Fig. 5. The relationship between photosynthesis and calcification rates per dry weight, as measured by the ^{14}C technique for *H. lacrimosa* (+), *H. discoidea* (o), *H. copiosa* (Δ), and *H. cryptica* (●). The line is the linear regression through the data points. The regression equation is: calcification = 0.64 (photosynthesis) + 0.0135; ($R^2 = 76.9\%$).

DISCUSSION

The numerical importance of the deep fore-reef inhabiting *Halimeda* species as contributors of carbonaceous sediments has been inferred (GOREAU, 1963). Yet, estimates of this production have been, at best, speculative (HILLIS-COLINVAUX, 1980), since all work to date has been with shallow-water forms. The results of this study provide the first recorded production rates for four *Halimeda* species found inhabiting the deep fore-reef. Given these rates, and the percent cover observed (up to 44%, LITTLER *et al.*, 1985), it can be concluded that these deep-water algae are indeed important to carbonate production. This finding is especially important in view of the fact that incubations were conducted at *ca.* 1 to 2% typical surface irradiance, a level that in the past has been regarded as the lowest limit of the euphotic zone (PARSONS *et al.*, 1977). Photosynthetic rates of *Halimeda* species, as documented here and indicated by their observed abundances, are well above the compensation point at these intensities.

A comparison of the rates reported in this study to those by other investigators reveals remarkable similarities given the differences in methodologies (Table 1). The rates are in agreement with those reported by BOROWITZKA and LARKUM (1976a), BUESA (1977), and LITTLER *et al.* (1983) for a variety of *Halimeda* species exposed to varied light intensities. The rates for the jointed calcareous-group, which includes all *Halimeda* species, on a gram dry weight basis are lower than those reported for many other primary producers. It is the high

Table 1. *Rates of photosynthesis* (mg C g dry $wt^{-1}h^{-1}$) *in various marine systems*

System	Location	Production	Method	Light ($\mu E\ m^{-2}s^{-1}$)	Reference
Marine algae (functional form-groupings)					
Sheet-group	Belize	5.06	O_2	500–2100	LITTLER *et al.* (1983)
Thick leathery-group	Belize	0.88	O_2	500–2100	LITTLER *et al.* (1983)
Jointed calcareous-group	Belize	0.18	O_2	500–2100	LITTLER *et al.* (1983)
Halimeda copiosa	Bahamas	0.05	O_2	20	This paper
	Bahamas	0.04	^{14}C	20	This paper
H. cryptica	Bahamas	0.24	O_2	20	This paper
	Bahamas	0.24	^{14}C	20	This paper
H. discoidea	Hawaii	0.61	O_2	0.04*	DOTY (1971)†
	Belize	0.20	O_2	500–2100	LITTLER *et al.* (1983)
	Bahamas	0.09	O_2	20	This paper
	Bahamas	0.11	^{14}C	20	This paper
H. gracilis	Cuba	0.61	O_2	0.21*	BUESA (1977)
H. incrassata	Cuba	0.48	O_2	0.14*	BUESA (1977)
H. lacrimosa	Bahamas	0.12	O_2	20	This paper
	Bahamas	0.10	O_2	20	This paper
H. monile	Belize	0.30	O_2	500–2100	LITTLER *et al.* (1983)
	Cuba	0.20	O_2	0.20*	BUESA (1977)
H. opuntia	Cuba	0.24	O_2	0.20*	BUESA (1977)
H. simulans	Cuba	0.37	O_2	0.20*	BUESA (1977)
H. tuna	Australia	0.11–0.35	^{14}C	24‡	BOROWITZKA and LARKHAM (1976a)
Seagrasses					
Thalassia testudinum	Florida	0.36–1.30	O_2	–	POMEROY (1960)§
Zostera marina	Alaska	0.55	O_2	–	MCROY (1966)§

* kJ $m^{-2}s^{-1}$.
† From BUESA (1977).
‡ Converted from 1200 lux.
§ From MCROY and MCMILLAN (1977).

percent cover and associated high algal biomass that makes these algae significant producers of organic carbon and carbonates on an areal basis.

The prolific growth by *Halimeda* at low light intensities suggests an efficient utilization of available light energies. The agreement of reported photosynthetic rates for *Halimeda* under varied light conditions suggests a low saturation light intensity and a low photosynthetic maximum. A potential problem still exists in our productivity measurements in that incubations were conducted at *in situ* photosynthetically active light energies but not light qualities. The neutral density screening used to reduce intensity does so without preference for wavelength, unlike seawater (Jerlov type I) which absorbs preferentially in the red and ultra violet ends of the spectrum. Any effects of this experimental artifact can be debated as DRING (1981) and RAMUS (1983) concluded that reductions in light quantity have the same effect on photosynthesis as increasing depth. In addition, the light source was clear, blue skies (not direct sunlight), light was measured as photosynthetically active radiation (400 to 700 nm) and quality does not effect quantity when PAR is compared, i.e., PAR at depth equals PAR used in incubations.

The results of both the carbon-14 and oxygen photosynthetic rate determinations indicated that *Halimeda cryptica,* on a gravimetric basis, was more than twice as productive as the other three species. When photosynthetic rates were normalized to gram ash-free dry weight, this difference was eliminated, suggesting the increase in photosynthesis was due to a higher ratio of organic matter. From this it is concluded that metabolic activity is correlated with the amount of organic tissue present, independent of the species involved.

The calcification rates per gram dry weight revealed, as did the photosynthetic rates, that *H. cryptica* calcified at a significantly higher rate and displayed more scatter about the mean than the other three species. This also is attributed to the higher organic content of this species. Normalizing the data to percent organic content resulted in no significant ($P > 0.05$) interspecific differences. Calcification rates obtained by BOROWITZKA and LARKUM (1976a) are higher (Table 2), the difference is likely due to either metabolic differences in the physiological ecology of the different species from deep vs shallow habitats or differences in experimental techniques. Calcification rates given by BOROWITZKA (1981) and PENTECOST (1978) for two species of coralline algae are similar to the rates we report (Table 2). The rates reported using ^{45}Ca are similar, but generally higher than those determined using ^{14}C, as might be expected from the findings of BOHM and GOREAU (1973) and BOROWITZKA and LARKUM (1976a). The relationship between inorganic and organic carbon incorporation rates reported by this study supports the concept of a photosynthetic mediation of the calcification process. The physiological mechanism responsible for this biological mediation remains undetermined but must be, at least in part, related to photosynthesis.

The photosynthesis : calcification ratios are lower than anticipated, considering that in *Halimeda,* 1 mole of $CaCO_3$ is precipitated for every 4 to 8 moles of CO_2 fixed in photosynthesis (BOROWITZKA and LARKUM, 1976b). Perhaps carbon uptake in calcification is a separate step from precipitation, as STARK *et al.* (1969) found it to be for calcium uptake in *H. opuntia* and *H. discoidea.* If so, this could explain the low ratios calculated. The ratio of photosynthetic ^{14}C uptake to ^{14}C incorporation into calcite of 1.3 reported for *Corallina officinalis* (PENTECOST, 1978) is, however, well within our range.

The adaptive significance of skeletal carbonate production to marine algae, to date, is a subject not completely understood (LITTLER, 1976). Further experimentation is needed to better understand calcification and the physiological ecology of the deep-water algae. Future studies should compare the inorganic content of shallow vs deep-water *Halimeda* species,

Table 2. Calcification rates (mg C g dry $wt^{-1}h^{-1}$) *recorded using* ^{14}C or ^{45}Ca* *techniques for various marine algae*

Species	Location	Calcification	Method	Light ($\mu E\ m^{-2}s^{-1}$)	Reference
Amphiroa officinalis	Australia	0.05	^{14}C	600	BOROWITZKA (1981)
Bossiella orbigniana	California	0.27	^{45}Ca	6†	PEARSE (1972)
Corallina officinalis	Wales	0.02–0.03	^{14}C	98–293‡	PENTECOST (1978)
Halimeda discoidea	Puerto Rico	0.20	^{45}Ca	1§	STARK *et al.* (1969)
	Bahamas	0.09	^{14}C	20	This paper
H. copiosa	Bahamas	0.06	^{14}C	20	This paper
H. cryptica	Bahamas	0.16	^{14}C	20	This paper
H. lacrimosa	Bahamas	0.06	^{14}C	20	This paper
H. optunia	Puerto Rico	0.26	^{45}Ca	1§	STARK *et al.* (1969)
	Jamaica	0.07	^{45}Ca	—	BOHM and GOREAU (1973)
H. tuna	Australia	0.14–0.50	^{14}C	24‖	BOROWITZKA and LARKHAM (1976a)

* Calcium-45 incorporation rates were multiplied by 0.30, the weight ratio of carbon to calcium in $CaCO_3$. This converts from mg Ca g dry $wt^{-1}\ h^{-1}$, as reported by reference listed, to mg C g dry $wt^{-1}\ h^{-1}$.

† Converted from 1.3×10^3 ergs $cm^{-2}s^{-1}$.

‡ Converted from 5 to 15 klux.

§ Converted from 600 ft.c.

‖ Converted from 1200 lux.

along with associated calcification rates, in an attempt to substantiate GOREAU's (1963) statement that *Halimeda* species are more heavily calcified in deep than in shallow water, and an areal production estimate should be made to quantify the contribution by the '*Halimeda* zone' to carbonate production.

CONCLUSIONS

The photosynthetic and calcification rates recorded for four prominent deep-water inhabiting *Halimeda* species indicate the potential of the genus as a significant contributor to carbonate deposition, despite the low light energy environment they inhabit. Relatively minor interspecific differences exist between photosynthetic and calcification rates. The rates varied with the organic content of the species tested. Photosynthetic rates are in agreement with those reported by BOROWITZKA and LARKUM (1976a) for *H. tuna* incubated at similar light intensities and for a variety of *Halimeda* species studied by LITTLER *et al.* (1983) incubated at surface light intensities. The relationship between photosynthesis and calcification was linear, supporting the concept that calcification is, in part, a biologically mediated process.

Acknowledgements—The authors thank Dennis Hanisak (Harbor Branch Foundation) and Stephen Zeeman (Harbor Branch Institution) for reviewing the manuscript and providing helpful suggestions for improving the contents. We also thank Steve Blair (Harbor Branch Foundation) and Jim Norris (National Museum of Natural History, Smithsonian Institution) for their technical assistance in the field. Special appreciation is extended to Donald Gerace (College Center of the Finger Lakes) for making available the facilities at the CCFL field station.

This paper represents Contribution No. 396 of the Harbor Branch Foundation, Contribution No. 140 of the Smithsonian Marine Station at Link Port and Contribution No. B122 of the College Center of the Finger Lakes.

REFERENCES

BOHM E. L. (1978) Application of the ^{45}Ca tracer method for determination of calcification rates in calcareous algae: effect of calcium exchange and differential saturation of algal calcium pools. *Marine Biology*, **47**, 9–14.

BOHM E. L. and T. F. GOREAU (1973) Rates of turnover and net accretion of calcium and the role of calcium binding polysaccharides during calcification in the calcareous alga *Halimeda opuntia* (L.). *International Review der gesamten Hydrobiologie*, **58**, 723–740.

BOROWITZKA M. A. (1977) Algal calcification. *Oceanography and Marine Biology Annual Review*, **15**, 189–223.

BOROWITZKA M. A. (1979) Calcium exchange and the measurement of calcification rates in the calcareous coralline red alga *Amphiroa foliacea*. *Marine Biology*, **50**, 339–347.

BOROWITZKA M. A. (1981) Photosynthesis and calcification in the articulated coralline red algae *Amphiro anceps* and *A. foliacea*. *Marine Biology*, **62**, 17–23.

BOROWITZKA M. A. (1982a) Mechanisms in algal calcification. In: *Progress in phycological research*, Vol. 1, F. E. ROUND and D. J. CHAPMAN, editors, Elsevier Biomedical Press, Amsterdam, pp. 139–177.

BOROWITZKA M. A. (1982b) Morphological and cytological aspects of algal calcification. *International Review of Cytology*, **74**, 127–162.

BOROWITZKA M. A. and A. W. D. LARKUM (1976a) Calcification in the green alga *Halimeda*. II. The exchange of Ca^{2+} and the occurrence of age gradients in calcification and photosynthesis. *Journal of Experimental Botany*, **27**, 864–878.

BOROWITZKA M. A. and A. W. D. LARKUM (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**, 879–893.

BUESA R. J. (1977) Photosynthesis and respiration of some tropical marine plants. *Aquatic Botany*, **3**, 203–216.

BURRIS J. E. (1977) Photosynthesis, photorespiration, and dark respiration in eight species of algae. *Marine Biology*, **39**, 371–379.

DOTY M. S. (1971) The productivity of benthic frondose algae at Waikiki beach, 1967–1968, University of Hawaii. *Botanical Science Paper*, **22**, 1–119.

DRING M. J. (1981) Chromatic adaptation of photosynthesis in benthic marine algae: an examination of its ecological significance using a theoretical model. *Limnology and Oceanography*, **26**, 271–284.

GINSBERG R. N. (1956) Environmental relationships of grain size and constituent particles in some south Florida carbonate sediments. *Bulletin of the American Association of Petroleum Geology,* **40**, 2384–2427.

GOREAU T. F. (1963) Calcium carbonate deposition by coralline algae and corals in relation to their roles as reef-builders. *New York Academy of Sciences. Annals,* **109**, 127–167.

HILLIS-COLINVAUX L. (1980) Ecology and taxonomy of *Halimeda*: primary producer of coral reefs. *Advances in Marine Biology,* **17**, 1–327.

HILLIS-COLINVAUX L. (1982) Submersible study of the calcareous green algae of Enewetak Atoll, Marshall Islands. 21st Northeast Algal Symposium, Woods Hole, MA, p. 9 (Abstract only).

IKEMORI M. (1970) Relation of calcium uptake to photosynthetic activity as a factor controlling calcification in marine algae. *Botanical Magazine of Tokyo,* **83**, 152–162.

LEHNINGER A. L., editor (1975) *Biochemistry,* 2nd edition, Worth Publishers, New York, 1104 pp.

LEWIN J. C., editor (1962) *Physiology and biochemistry of algae,* Academic Press, New York, 929 pp.

LITTLER M. M. (1973) The productivity of Hawaiian fringing-reef crustose Corallinaceae and an experimental evaluation of the production methodology. *Limnology and Oceanography,* **18**, 946–952.

LITTLER M. M. (1976) Calcification and its role among the macroalgae. *Micronesica,* **12**, 27–41.

LITTLER M. M. (1979) The effects of bottle volume, thallus weight, oxygen saturation levels, and water movement on apparent photosynthetic rates in marine algae. *Aquatic Botany,* **7**, 21–34.

LITTLER M. M. (1980) Morphological and photosynthetic performances of marine macroalgae: tests of a fundamental/form hypothesis. *Botanica Marina,* **20**, 161–165.

LITTLER M. M. and K. E. ARNOLD (1980) Sources of variability in macroalgal primary productivity: sampling and interpretative problems. *Aquatic Botany,* **8**, 141–156.

LITTLER M. M., D. S. LITTLER and P. R. TAYLOR (1983) Evolutionary strategies in a tropical barrier reef system: functional-form groups of marine macroalgae. *Journal of Phycology,* **19**, 229–237.

LITTLER M. M., D. S. LITTLER, S. M. BLAIR and J. N. NORRIS (1985) The deepest known plant life on earth is discovered on an uncharted seamount. *Science, Wash.,* in press.

LOBBAN C. S. (1974) A simple, rapid method of solubilizing algal tissue for scintillation counting. *Limnology and Oceanography,* **19**, 356–359.

MCROY C. P. (1966) Standing stock and ecology of eelgrass (*Zostera marina* L.) in Izembek Lagoon, Alaska. MSc Thesis, University of Washington, 138 pp.

MCROY C. P. and C. MCMILLAN (1977) Production ecology and physiology of seagrasses. In: *Seagrass ecosystems: a scientific perspective,* C. P. MCROY and C. HELFFERICH, editors, Marcel Dekker, pp. 53–87.

MILLIMAN J. D. (1974) *Recent sedimentary carbonates. Marine carbonates,* Part I, Springer-Verlag, New York, 375 pp.

NEUMANN A. C. and L. S. LAND (1969) Algal production and lime mud deposition in the Bight of Abaco: a budget. *Geological Society of America Special Paper,* **121**, 1–219.

NEUMANN A. C. and L. S. LAND (1975) Lime mud deposition and calcareous algae in the Bight of Abaco, Bahamas: a budget. *Journal of Sedimentary Petrology,* **45**, 763–786.

ORME G. R. (1977) Aspects of sedimentation in the coral reef environment. In: *Biology and geology of coral reefs,* Vol. IV, Geology 2, O. A. JONES and R. ENDEAN, editors, Academic Press, New York, pp. 129–182.

PAASCHE E. (1963) The adaptation of the carbon-14 method for the measurement of coccolith production in *Coccolithus huxleyi. Physiologia Plantrum,* **16**, 186–200.

PARSONS T. R., M. TAKAHASHI and B. HARGRAVE, editors (1977) *Biological oceanographic processes,* 2nd edition, Pergamon Press, New York, 332 pp.

PEARSE V. B. (1972) Radioisotope study of calcification in the articulated coralline alga *Bossiella orbigniana. Journal of Phycology,* **5**, 305–312.

PENTECOST A. (1978) Calcification and photosynthesis in *Corallina officinalis* L. using the $^{14}CO_2$ method. *British Phycological Journal,* **13**, 383–390.

PETERSON B. J. (1980) Aquatic primary productivity and the ^{14}C–CO_2 method. A history of the productivity problem. *Annual Review of Ecological Systems,* **11**, 359–385.

POMEROY L. R. (1960) Primary production of Boca Ciega Bay, Florida. *Bulletin of Marine Science of the Gulf and Caribbean,* **10**, 1–10.

RAMUS J. (1983) A physiological test of the theory of complimentary chromatic adaption. II. Brown, green and red seaweeds. *Journal of Phycology,* **19**, 173–178.

SMITH S. V. (1973) Carbon dioxide dynamics: a record of organic carbon production, respiration and calcification in the Eniwetok reef flat community. *Limnology and Oceanography,* **18**, 106–120.

STARK L. M., L. ALMODOVAR and R. W. KRAUSS (1969) Factors affecting the rate of calcification in *Halimeda opuntia* (L.) Lamouroux and *Halimeda discoidea* Descaisne. *Journal of Phycology,* **5**, 305–312.

STEARN C. W., T. P. SCOFFIN and W. MARTINDALE (1977) Calcium carbonate budget of a fringing reef on the west coast of Barbados. Part I—Zonation and productivity. *Bulletin of Marine Science,* **27**, 479–510.

STRICKLAND J. D. H. (1960) Measuring the production of marine phytoplankton. *Bulletin of Fisheries Research Board of Canada,* **122**, 1–172.

STRICKLAND J. D. H. and T. R. PARSONS (1972) A practical handbook of seawater analysis, 2nd edition, *Bulletin of the Fisheries Research Board of Canada,* **167**, 1–310.

THOMAS W. H. (1963) Physiological factors affecting the interpretation of phytoplankton measurements. In: *Proceedings of the Conference on Primary Productivity Measurements, Marine and Freshwater,* M. S. DOTY, editor, U.S. Atomic Energy Commission, Washington, D.C., pp. 147–162.

UNESCO (1973) Primary production of smaller seaweeds using the ^{14}C-technique. In: *A guide to the measurement of marine primary production under some special conditions,* UNESCO, 73 pp.

WETZEL R. G. (1974) Techniques and problems of primary productivity measurements in higher aquatic plants and periphyton. In: *Primary productivity in aquatic environments,* C. R. GOLDMAN, editor, University of California Press, pp. 249–267.