

INCREASED PRODUCTION OF CALCITE AND SLOWER GROWTH FOR THE MAJOR SEDIMENT-PRODUCING ALGA *HALIMEDA* AS THE Mg/Ca RATIO OF SEAWATER IS LOWERED TO A “CALCITE SEA” LEVEL

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ABSTRACT: Laboratory experiments were conducted to subject the calcareous alga *Halimeda*, a prolific producer of aragonite in the modern ocean, to a variety of ambient Mg/Ca ratios. These experiments were based on three predictions: (1) that *Halimeda* would calcify at a lower rate when the ambient Mg/Ca ratio was reduced from its high level in modern seawater (5.2); (2) that this alga would grow more slowly under these conditions because its calcification yields as a byproduct CO₂, which is available for photosynthesis; and (3) that the calcite content of *Halimeda*'s skeleton would increase as a percentage of total CaCO₃. As predicted, reduction of the Mg/Ca ratio of ambient artificial seawater from its modern level to estimated early Oligocene and early Eocene levels (2.5 and 1.5, respectively) resulted in progressively lower rates of calcification, production of organic matter, and linear growth for *Halimeda incrassata* colonies. The ambient Mg/Ca ratio exerted a stronger influence over the rate of calcification than [Ca²⁺], which in the geologic past has risen in seawater whenever the ambient Mg/Ca ratio has declined. Thus, although elevation of [Ca²⁺] at such times would have promoted the calcification and growth of *Halimeda*, the concomitant decline of the Mg/Ca ratio would have had a stronger negative effect. In addition, the percentage of skeletal material consisting of calcite rose from 8% in modern-day seawater to 16% in artificial early Oligocene seawater and 46% in artificial early Eocene seawater. Magnesium was incorporated into this calcite as a function of the ambient Mg/Ca ratio with a fractionation pattern differing only slightly from that of nonskeletal calcite.

Halimeda exerts less biological control over its biomineralization than the related genera *Penicillus* and *Udotea*, which in previous experiments produced only 22–25% of skeletal CaCO₃ as calcite in seawater having a very low Mg/Ca ratio (1.0). Nonetheless, it is likely that many taxa of green algae that produce little or no calcite today would have produced a substantial proportion of skeletal low-Mg CaCO₃ as calcite in ancient seas, such as those of the Cretaceous, whose Mg/Ca ratios were close to unity.

INTRODUCTION

Halimeda is a major producer of carbonate sediment in the modern ocean. Like other extant genera of calcareous green algae, it produces skeletal material consisting largely of aragonite. This paper presents results of laboratory experiments designed to investigate whether *Halimeda*'s skeletal mineralogy and productivity may have varied over geologic time in response to secular changes in seawater chemistry.

Concentrations of Mg²⁺ and Ca²⁺ in seawater have varied during the Phanerozoic Eon, with the result that the molar Mg/Ca ratio (hereafter abbreviated as Mg/Ca) of seawater has oscillated markedly, ranging from > 5 to ~ 0.5 (Fig. 1). The importance of this oscillation is indicated by laboratory experiments on inorganic precipitation showing that low-Mg calcite (Mg/Ca < 4%) forms at seawater Mg/Ca < 1, marginally high magnesium calcite forms at Mg/Ca = 1–2, and high-Mg calcite (Mg/Ca > 4 mol %) or aragonite forms at Mg/Ca = 2–5.2 (Füchtbauer and Hardie 1976, 1980). Since early in the Paleozoic, secular variation in the Mg/Ca ratio of seawater subjected calcifying organisms to transitions

between three intervals favoring the precipitation of aragonite and high-Mg calcite and two intervening intervals favoring the precipitation of low-Mg calcite (“aragonite seas,” and “calcite seas,” respectively) (Fig. 1).

Along mid-ocean ridges and in areas of oceanic plateau formation, hydrothermal activity removes Mg from seawater and releases Ca into it. Thus, when overall rates of seafloor formation rise to high levels, the Mg/Ca ratio of seawater declines to a relatively low level; this effect is quite powerful because the numerator and denominator of the ratio simultaneously shift in opposite directions. At such times, sea level rises because igneous activity increases the volume of mid-ocean ridges or oceanic plateaus. Conversely, when rates of seafloor formation and sea level are low (as at the present), [Ca²⁺] is relatively low and Mg/Ca is relatively high in seawater. Thus, Hardie (1996) used rates of oceanic crust production that Gaffin (1987) estimated from the first-order eustatic sea-level curve, along with river water fluxes, to calculate the history of the Mg/Ca ratio of seawater. Hardie's calculations, modified slightly by Demicco et al. (2005) via an improved calculation method, precisely predict the observed transitions between aragonite and calcite seas (Fig. 1). Similar calculations also accurately predict transitions between

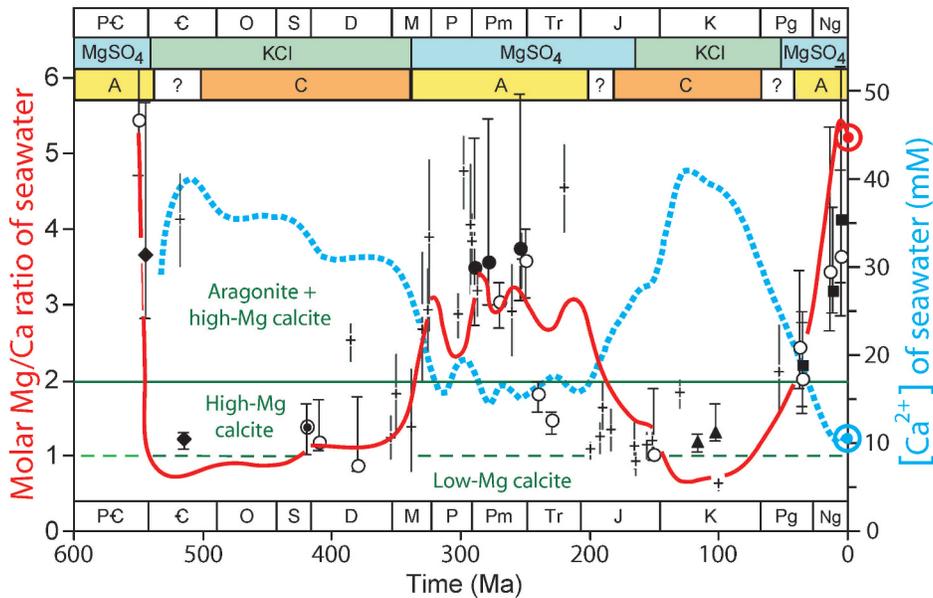


FIG. 1.—Empirical estimates and model results for secular variation in Mg/Ca and $[Ca^{2+}]$ of seawater throughout Phanerozoic time, showing three intervals of nonskeletal precipitation of aragonite + high-Mg calcite in shallow seas and two intervals of nonskeletal precipitation of calcite. Red curve represents the molar Mg/Ca ratio of seawater, and dotted blue curve represents $[Ca^{2+}]$ of seawater (Demicco et al. 2005), both modeled from river water contributions and from varying rates of global ocean crust production (Gaffin 1987) that were estimated from the stratigraphic record of eustatic sea-level change. Empirical estimates of Mg/Ca for seawater from fluid inclusions in primary marine halite (Brennan 2002 [squares], Timofeeff et al. 2006 [triangles], Lowenstein et al. 2005 [large solid circles], Brennan and Lowenstein 2002 [circumscribed small solid circle], Brennan et al. 2004 [diamonds], and Horita et al. 2002 [open circles]). Red and blue encircled points represent modern seawater (Mg/Ca = 5.2, $[Ca^{2+}]$ = 10.2). Solid green line separates calcite and aragonite + high-Mg calcite (Mg/Ca > 2) nucleation fields, and dashed green line separates low-Mg and high-Mg nucleation fields, for seawater at 25°C. Colored horizontal bars depict intervals when abiogenic precipitates were aragonite (A) or calcite (C) (Sandburg 1983; Wilkinson et al. 1985) and intervals when $MgSO_4$ or KCl formed as marine evaporites (Hardie 1996).

times of precipitation of KCl and $MgSO_4$ evaporites along ocean margins (Fig. 1), because potassium is released by submarine hydrothermal reactions along with calcium, and sulfate is consumed along with magnesium (Hardie 1996). Studies of fluid inclusions in halite of various ages have confirmed that the secular changes in the mineralogy of nonskeletal carbonates have generally corresponded to changes in the Mg/Ca ratio of seawater (Fig. 1).

The Mg/Ca ratio of seawater exerts a strong influence over carbonate biomineralization. Throughout Phanerozoic time, dominant reef builders and sediment producers have for the most part been forms that secrete low-Mg calcite in calcite seas and aragonite or high-Mg calcite in aragonite seas (Stanley and Hardie 1998, 1999).

Halimeda did not become a major sediment producer until the latter part of the Cenozoic. It has been proposed that the emergence of *Halimeda* in this role resulted from the substantial rise of the Mg/Ca ratio of seawater that was occurring simultaneously (Stanley and Hardie 1998, 1999). Experiments described below support this hypothesis and, more generally, show that the Mg/Ca ratio of seawater influences the mineralogy, calcification, primary production, and linear growth of the extant species *Halimeda incrassata* and that ambient $[Ca^{2+}]$ also influences the latter three variables. This work complements previous studies of conspicuous effects of seawater chemistry on calcification in marine algae: the effect of ambient Mg/Ca on calcitic coralline red algae (Stanley et al. 2002; Ries 2006b), calcitic coccolithophores (Stanley et al. 2005), and the aragonitic calcareous green algae *Penicillus* (Ries 2005) and *Udotea* (Ries 2006a).

BIOLOGICAL TRAITS OF HALIMEDA

Anatomy

Halimeda is an upright, branching green alga with a thallus consisting of linear arrays of segments less than a centimeter in maximum dimension (Fig. 2A). It is coenocytic, meaning that its thallus is not divided into discrete cells by cell walls. Thus, it functions as a single, huge multinucleate cell (Hillis-Colinvaux 1980). Flexible medullar filaments extend the length of the thallus, connecting the segments. The thallus

grows upward from a fibrous holdfast. Linear extension of the thallus occurs by addition of new segments and enlargement of existing segments. The thallus branches when a segment gives rise to two or more new segments. *Halimeda* can rapidly colonize a broad area of substratum via vegetative reproduction. In this process, a rhizoid grows laterally from an existing thallus (often beneath the sediment) and sprouts a new thallus.

The medullar filaments of *Halimeda* branch laterally to form utricles, which ramify to form the cortex, where at the margin of the alga they effectively seal off an extracellular internal space (hereafter referred to as the *intertricular space*); this space becomes filled with $CaCO_3$, which surrounds the utricles (Fig. 2B).

Ecology and Biogeography

Halimeda is globally distributed in tropical and subtropical seas, attaching to skeletal debris, rocky surfaces, or reefs. It tolerates a variety of hydrodynamic regimes and ranges from the intertidal zone to fore-reef depths greater than 150 m (Moore et al. 1976; Hillis-Colinvaux 1980; Jensen et al. 1985; Blair and Norris 1988).

General Phylogenetic Relationships

Recent molecular phylogenetic analyses assign *Halimeda*, along with *Penicillus* and *Udotea*, which are two other heavily calcifying genera of green algae (phylum Chlorophyta), to the suborder Halimedineae of the order Bryopsidales; within this suborder, *Halimeda* is assigned to the family Halimedaceae, whereas *Penicillus* and *Udotea* are assigned to the family Udoteaceae (Lam and Zechman 2006). Molecular phylogenetic analysis has also revealed five so-called sections (the equivalents of subgenera) of the genus *Halimeda* (Verbruggen and Kooistra 2004).

GEOLOGICAL SIGNIFICANCE OF HALIMEDA

Carbonate Production in Modern Seas

Upon death, the *Halimeda* thallus rapidly disaggregates into its component segments, which constitute conspicuous (1–10 mm) particles.

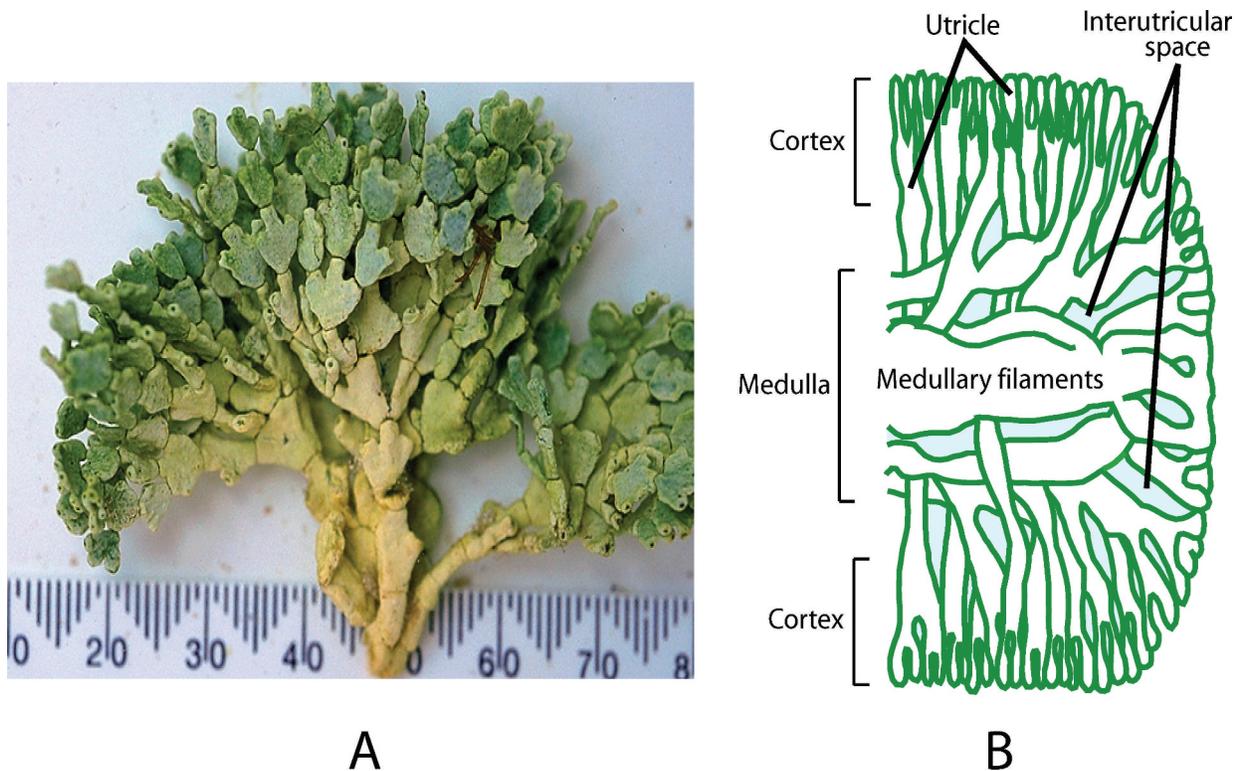


FIG. 2.—Anatomy of *Halimeda incrassata*. **A**) Thallus of a full-grown alga of the Pacific cryptic form named *H. incrassata* 1b by Verbruggen et al. (2005); it is morphologically indistinguishable but genetically distinct from Atlantic populations (Gerald McCormack [2007] Cook Islands Biodiversity Database, Version 2007.2. Cook Islands Natural Heritage Trust, Rarotonga. Online at <http://cookislands.bishopmuseum.org>). **B**) Cartoon of a cross section of the tip of a thallus. The cortex forms where the utricles bend at right angles and coalesce (Hillis-Colinvaux 1980).

The genus contributes sediment from shallow back-reef environments (Ginsburg 1956) to deep fore-reef environments (Goreau and Goreau 1973). When present, it typically contributes 25–80% of carbonate sediments on shallow tropical seafloors (Emery et al. 1954; Neumann and Land 1975; Drew 1983; Payri 1988; Rees et al. 2007). It generally produces carbonate sediment at rates in excess of 2,000 g/m²/yr (Drew 1983; Freile et al. 1995; Freile and Hillis 1997). Accumulation rates from sediment production range from 0.18 to 5.9 m /1000 yr (Neumann and Land 1975; Marshall and Davies 1988; Orme and Salama 1988; Roberts et al. 1988; Searle and Semeniuk 1988; Hillis 1991; Freile et al. 1995).

Today *Halimeda* locally forms massive reeflike structures, some of them tens of meters tall and many kilometers long (Martin et al. 1997). These structures, which consist almost entirely of *Halimeda* segments cemented and bound by microbially precipitated micrite and marine cement, occur both in the Caribbean (Hine et al. 1988) and in many Pacific regions (Orme et al. 1978; Marshall and Davies 1988; Orme and Salama 1988; Phipps and Roberts 1988; Roberts et al. 1988; Purnachandra Rao et al. 1994; Harris et al. 1996; Granier et al. 1996); *Halimeda* bioherms on the shelf of Australia's Great Barrier Reef may contain a volume of carbonate at least as large as that of the neighboring coral barrier reefs (Rees et al. 2007).

Geologic History of *Halimeda* and Its Sediment Production

The oldest species assigned to *Halimeda*, *H. soltanensis* (Poncet 1989), dates to the Permian, but probably should be assigned to a different genus. Several workers have summarized the fossil record of *Halimeda* and its close relatives (Elliott 1981, 1984; Bassoullet et al. 1983; Flügel

and Kahler 1988; Hillis 2001; Dragastan et al. 2002). Two of these relatives flourished during the Mesozoic: *Boueina* (Upper Triassic–Upper Cretaceous) was locally a major sediment producer during the Late Triassic, and this genus and *Arabicodium* (Middle Jurassic–early Cenozoic) were widespread during the Cretaceous, though they were not as productive as *Halimeda* is today. *Halimeda* was rare in the Early Cretaceous and became more common in the Late Cretaceous. It did not become widely distributed until early in the Cenozoic, however, and only in the late Miocene–Pliocene interval did it emerge as a major sediment producer and reef builder. At ~ 6 Ma in what is now southeastern Spain it formed numerous microbially bound reefs resembling those of modern seas (Martin et al. 1997).

Owing to relatively poor preservation, the fossil record of *Halimeda* has failed to provide a clear picture of the temporal occurrences of its five sections. Dragastan and Herbig (2007) nonetheless concluded from fossil data that all five sections arose in the Eocene. However, two applications of molecular clocks have led to the conclusion that all five sections arose within the past 15 Myr (and possibly even within the past 5 Myr) or, alternatively, in the Early Cretaceous (Verbruggen et al. 2009). *Halimeda* appears to have undergone a burst of speciation in the Holocene (Hillis 2001), although this apparent phenomenon may simply reflect the relatively high quality of the Holocene sedimentary record.

Some calcareous green algae have flourished during calcite-sea intervals. However, uncertainty as to original mineralogy and biological affinities of these taxa makes it difficult to employ them to test the influence of seawater chemistry on algal productivity far back in geologic time. The present study addresses the well-documented emergence of *Halimeda* as a prolific sediment producer late in Neogene time.

OCCURRENCE OF CaCO₃ WITHIN HALIMEDA SEGMENTS

Crystals of CaCO₃ (for the most part aragonite) occupy the intertricular space of both the medullar and cortical regions of *Halimeda*. The degree of calcification increases with the age of a segment, and mature segments contain up to 88 wt% CaCO₃ (Multer 1988).

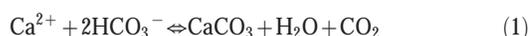
Calcification stiffens the thallus of heavily calcified bryopsidalean algae, causing them to remain relatively upright in the face of strong water movements and thus increasing drag and the likelihood of dislodgement (Kooistra 2002). This disadvantage of calcification is apparently offset by its well-documented deterrence of predatory grazers. Calcification makes the algae tougher and more difficult to bite into, unpalatable if fed upon, and of low nutritional value or even harmful if consumed (reviews by Hay et al. 1994; Schupp and Paul 1994).

A variety of morphologies and growth patterns characterize CaCO₃ precipitated within *Halimeda*. Crystals are initially produced within the pilose layer. This is a thin mat of fibrils that faces the intertricular space and overlies the cuticle, which in turn overlies the cell wall; CaCO₃ then progressively fills the intertricular space (Borowitzka and Larkum 1977).

Several workers have asserted that precipitation of aragonite by *Halimeda* commences with the nucleation of submicron, randomly oriented, equant-anhedral crystals adjacent to the alga's external cell wall and that the intertricular space is later filled with the larger (3–10 μm), acicular-euhedral crystals (Wilbur et al. 1969; Marszalek 1971; Borowitzka and Larkum 1977; Flajs 1977; Multer 1988). Macintyre and Reid (1995), however, concluded that aragonite within the intertricular space of *H. incrassata* initially takes the form of aragonite needles in the 1–3 μm size range that are subsequently altered to equant, anhedral, submicron-size crystals, perhaps by way of respiration-induced dissolution. This *in vivo* alteration is accompanied by the precipitation of larger, euhedral aragonite needles that may attain lengths of 25 μm. The proportion of equant, submicron-size aragonite crystals increases with the age of the segment (Macintyre and Reid 1995), such that only 25–40% of the CaCO₃ within mature segments exhibits an acicular morphology (Macintyre and Reid 1992).

CHEMISTRY OF PHOTOSYNTHESIS AND CaCO₃ PRECIPITATION

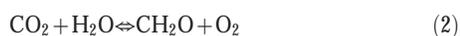
The net reaction for the production of CaCO₃ by calcareous organisms is



(Sikes et al. 1980; Erez 2003; Mackenzie and Lerman 2006, p. 146). Experiments have shown Ca²⁺ to be passively transported to *Halimeda*'s intertricular space in seawater that leaks into it; [Ca²⁺] declines slightly from the center of this space toward the zone of calcification (de Beer and Larkum 2001). The rate and location of calcification, however, are governed chiefly by changes in pH that shift the equilibrium conditions of the carbonate system within the intertricular space.

Photosynthesis as a Promoter of Calcification

The following reaction depicts photosynthesis in simplified form:



Inorganic carbon leaks into the intertricular space along with Ca²⁺. It is likely that much of the CO₂ used in photosynthesis is derived from HCO₃⁻ because of the latter's abundance in seawater. Carbonic anhydrase converts HCO₃⁻ to CO₂ and thus promotes photosynthesis in *Halimeda* (Borowitzka and Larkum 1976c). Addition of reactions (1) and (2) gives



This equation presents the net result of calcification and photosynthesis by

various organisms in the photic zone of the ocean (Mackenzie and Lerman 2006, p. 149). The interior of a *Halimeda* thallus can be thought of as a microcosm of the pelagic system, with calcium carbonate and carbohydrate being produced by the same net reaction.

That photosynthesis enhances calcification by elevating the pH of the intertricular space is demonstrated by three facts: (1) calcification is initiated in a *Halimeda* segment only after its chloroplasts reach functional maturity, (2) calcification is restricted to daylight hours (Borowitzka and Larkum 1976a, 1976b; de Beer and Larkum 2001), and (3) calcification is drastically reduced when a chemical blocker is introduced to inhibit the functioning of carbonic anhydrase (de Beer and Larkum 2001). Water-soluble acid polysaccharides known to bind calcium are present in the cell walls of *Halimeda* (Böhm 1972) and may also play a role in calcification (Borowitzka and Larkum 1976b).

Calcification as a Promoter of Photosynthesis

The inorganic carbon released by the calcification of some coccolithophores (Reaction 1) is thought to be employed in their photosynthesis (Reactions 2 and 3) (Sikes et al. 1980). Calcification would not be expected to provide all the inorganic carbon required for photosynthesis because many other taxa of red algae prosper without calcifying. Nonetheless, two kinds of evidence indicate that enhancement of photosynthesis by calcification should be inevitable in *Halimeda*. First, because consumption of CO₂ by photosynthesis in this genus has been shown to promote calcification, and respiration has been shown to suppress it (Borowitzka and Larkum 1976a), there must be efficient movement of chemical species containing inorganic carbon back and forth across the cell wall between the cytosol and chloroplasts on one side and the intertricular space on the other side, where calcification takes place. Carbon-dioxide-concentrating mechanisms facilitate the transport of inorganic carbon here (Raven and Beardall 2003). Second, because ~ 70% of the total cell-wall surface area of *Halimeda* faces the intertricular space (Borowitzka and Larkum 1976a), the inorganic carbon obtained for photosynthesis from seawater that has leaked into the intertricular space must be pooled with CO₂ generated there by calcification; the latter is therefore equally available for photosynthesis.

Enhancement of photosynthesis by calcification amounts to a positive feedback: photosynthesis promotes calcification by consuming CO₂, but in return, calcification provides additional inorganic carbon for photosynthesis. Thus, increased production of calcium carbonate should accelerate organic growth. Laboratory experiments were undertaken on *Halimeda incrassata* to test the prediction that changes in the Mg/Ca ratio and [Ca²⁺] of seawater would affect this species' rate of calcification and have a similar effect on its production of organic material.

EXPERIMENTS ON THE EFFECTS OF SEAWATER CHEMISTRY ON BIOMINERALIZATION AND GROWTH

The growth-rate experiments conducted on *Halimeda* resembled those previously undertaken on *Penicillus* (Ries 2005) and *Udotea* (Ries 2006a), but went further in making it possible to assess the relative importance of the Mg/Ca ratio and [Ca²⁺] of ambient seawater, as was done for coccolithophores (Stanley et al. 2005). The design of these experiments also made it possible to test the possibility that poisoning by high cation concentrations in unfamiliar seawater influenced the outcome of the experiments. As in previous studies, the effects of seawater chemistry on skeletal mineralogy were also investigated.

The individuals employed in the present study were Atlantic representatives of a globally distributed group of populations traditionally assigned to the species *Halimeda incrassata*. However, recent molecular phylogenetic analyses have shown that Hawaiian populations previously assigned to this species actually represent a distinct cryptic species (Verbruggen et al. 2005) and that Indo-Pacific populations

constitute a second cryptic species (Kooistra 2002). The type specimen for *Halimeda incrassata*, though now lost, was from Jamaica (Hillis-Colinvaux 1980), so the specific name resides with the Atlantic population and thus applies to the specimens employed in the present study.

Mineralogical Analysis

It has been suggested that the cation-binding properties of a particular polysaccharide within *Halimeda* determine what polymorph of CaCO_3 precipitates within it (Borowitzka and Larkum 1977). It has also been suggested that the number of nucleation sites on the algal organic matter may be the rate-limiting factor for calcification (Borowitzka 1982). Experiments described below indicate that these propositions cannot be entirely valid.

To investigate how changes in the Mg/Ca ratio of seawater might influence the mineralogy of *Halimeda*, thalli of *H. incrassata* were grown for 90 days in each of three aquaria whose artificial seawaters differed in their Mg/Ca ratios. One treatment mimicked modern seawater (Mg/Ca = 5.2, Ca^{2+} = 10.2 mM); the second had the composition calculated for the early Oligocene, shortly after the global shift to the present aragonite sea (Mg/Ca = 2.5, Ca^{2+} = 18.1 mM); and the third had a composition calculated for the early Eocene (Mg/Ca = 1.5, Ca^{2+} = 25.3 mM) (Fig. 1). The sum of the molar concentrations of $[\text{Mg}^{2+}]$ and $[\text{Ca}^{2+}]$ was the same in all three treatments (the modern value). By way of rhizoids, the thalli soon produced offspring, which were harvested for mineralogical analysis and employed in growth-rate studies.

Precipitation of Calcite in Addition to Aragonite in All Seawater Treatments

Backscatter electron images of offspring from algae in each of our seawater treatments revealed patterns of intertricular CaCO_3 precipitation (Fig. 3). Some of this CaCO_3 consisted of euhedral, acicular aragonite needles that were generally 1–10 μm in length (Figs. 2B, 3). Randomly interspersed among these needles were subhedral, rhombic crystals that powder X-ray diffraction showed to be calcite. Quantitative powder X-ray diffraction confirmed the evidence of electron imaging that the percentage of calcite in the skeletal material increased markedly with a decrease in the Mg/Ca ratio of the ambient seawater, exhibiting considerable variation in each treatment but reaching an average of 46% in imputed early Eocene seawater (Figs. 4, 5).

Mg Content of Calcite as a Function of the Mg/Ca of Seawater

As expected, the Mg/Ca ratio of the calcite produced by the experimental algae increased with the ambient Mg/Ca ratio. X-ray diffractometry, confirmed with EDS microprobe spot analysis, revealed that the calcite in the early Eocene, early Oligocene, and modern seawater treatments, respectively, had percentages of MgCO_3 of 6.0, 9.8, and 16.4 (Fig. 6). This observation, combined with fractionation patterns for the incorporation of Mg in calcite by coralline algae (Stanley et al. 2002; Ries 2006b), coccolithophores (Stanley et al. 2005), and several animal taxa (Ries 2005; Ries et al. 2006) strongly suggests that if members of any higher taxon produce high-Mg skeletal calcite in modern seawater, then calcite produced by any members of this same higher taxon living in Cretaceous seas would have been low-Mg calcite. Fractionation patterns for the incorporation of Mg in skeletal calcite as a function of the ambient Mg ratio vary among taxa, but for *Halimeda* the pattern is similar to that for nonskeletal calcite (Fig. 6).

RATES OF GROWTH AND CALCIFICATION

An important aspect of *Halimeda*'s calcification is that, as noted earlier, this alga does not pump Ca^{2+} into its intertricular space; Ca^{2+}

and Mg^{2+} enter at their ambient concentrations in the seawater that leaks into this space. Thus, changes in the concentration of these ions in seawater can be expected to have a direct effect on *Halimeda*'s calcification. An upward or downward trend in the Mg/Ca ratio of seawater always entails an inverse trend for $[\text{Ca}^{2+}]$ (Fig. 1). Thus, although the Mg/Ca ratio was lower during the Eocene than today, inhibiting precipitation of aragonite, $[\text{Ca}^{2+}]$ was higher, increasing the saturation state of aragonite as well as calcite, and thus promoting the precipitation of both minerals (Reaction 1). As in previous experiments with coccolithophores (Stanley et al. 2005), the relative importance of these opposing factors for *Halimeda* were tested via experiments in which Mg/Ca was held constant while $[\text{Ca}^{2+}]$ was varied, and vice versa.

Relative Effects of the Mg/Ca Ratio and $[\text{Ca}^{2+}]$ of Seawater

Nine seawater treatments, each within a separate aquarium (Fig. 7), were employed in the experimental design. Three of these were the ones used to investigate mineralogical changes, and these were designated "natural" seawaters because each had the composition of seawater calculated for a particular time in earth history. The other six treatments entailed Mg/Ca ratios that never have existed during the Phanerozoic. These "non-natural" treatments made it possible to examine the effects of varying either $[\text{Ca}^{2+}]$ or Mg/Ca while the other was held constant. Each of the two non-natural seawaters for a given Mg/Ca ratio had a level of $[\text{Ca}^{2+}]$ equal to one of the natural seawaters. This arrangement made it possible to compare such scenarios as (1) the effect of lowering the Mg/Ca ratio of modern seawater to the early Eocene level while leaving $[\text{Ca}^{2+}]$ at its modern level and (2) the effect of elevating $[\text{Ca}^{2+}]$ to the early Eocene level while leaving Mg/Ca at its modern level. The salinity of modern seawater was maintained in all non-natural seawaters by varying $[\text{Na}^+]$.

Fifty *H. incrassata* thalli of similar size were employed in each of the seawater treatments in order to investigate growth rates. Those grown in seawaters of unfamiliar chemistry were moved to these treatments in stages, to avoid shock. Throughout the experiments, most of the parent algae produced descendant thalli via rhizoids; because the parents were of similar size, they provided similar sustenance for their offspring before rhizoids atrophied. Offspring were employed to measure rates of growth and calcification. The date of appearance of each offspring was recorded, and its linear growth was measured from photographs taken every seven days. After 90 days, offspring were harvested and their average linear growth rates were calculated for intervals over which they exhibited measurable extension. The specimens employed to measure linear growth rates were also used to measure rates of production of carbonate and organic matter. The organic content of a thallus was calculated as the difference between its original dry weight and the weight of its CaCO_3 content following combustion. Division of the weight of CaCO_3 and dry organic matter of a thallus by its age gave rates of calcification and primary production.

As predicted for the controlled experiments, rates of calcification, organic-matter production, and linear growth were all positively correlated ($p < 0.05$) with increases in both the Mg/Ca ratio and $[\text{Ca}^{2+}]$ of seawater (Fig. 7).

Absence of Cation Poisoning in Unfamiliar Seawaters

Previous experiments on the effects of seawater chemistry on coccolithophores (Stanley et al. 2005) were robust in the sense that these unicellular forms multiplied and calcified more rapidly as the ambient Mg/Ca was lowered from that of the modern ocean to unaccustomed Cretaceous levels. However, in the present study and in similar studies of *Penicillus* and *Udotea* (Ries 2005, 2006b) it was in unfamiliar seawaters that rates of productivity and calcification were reduced. The experiments on *Halimeda*, however, demonstrated that unfamiliar seawater had no adverse effect on its rates of calcification and growth, except via lowering

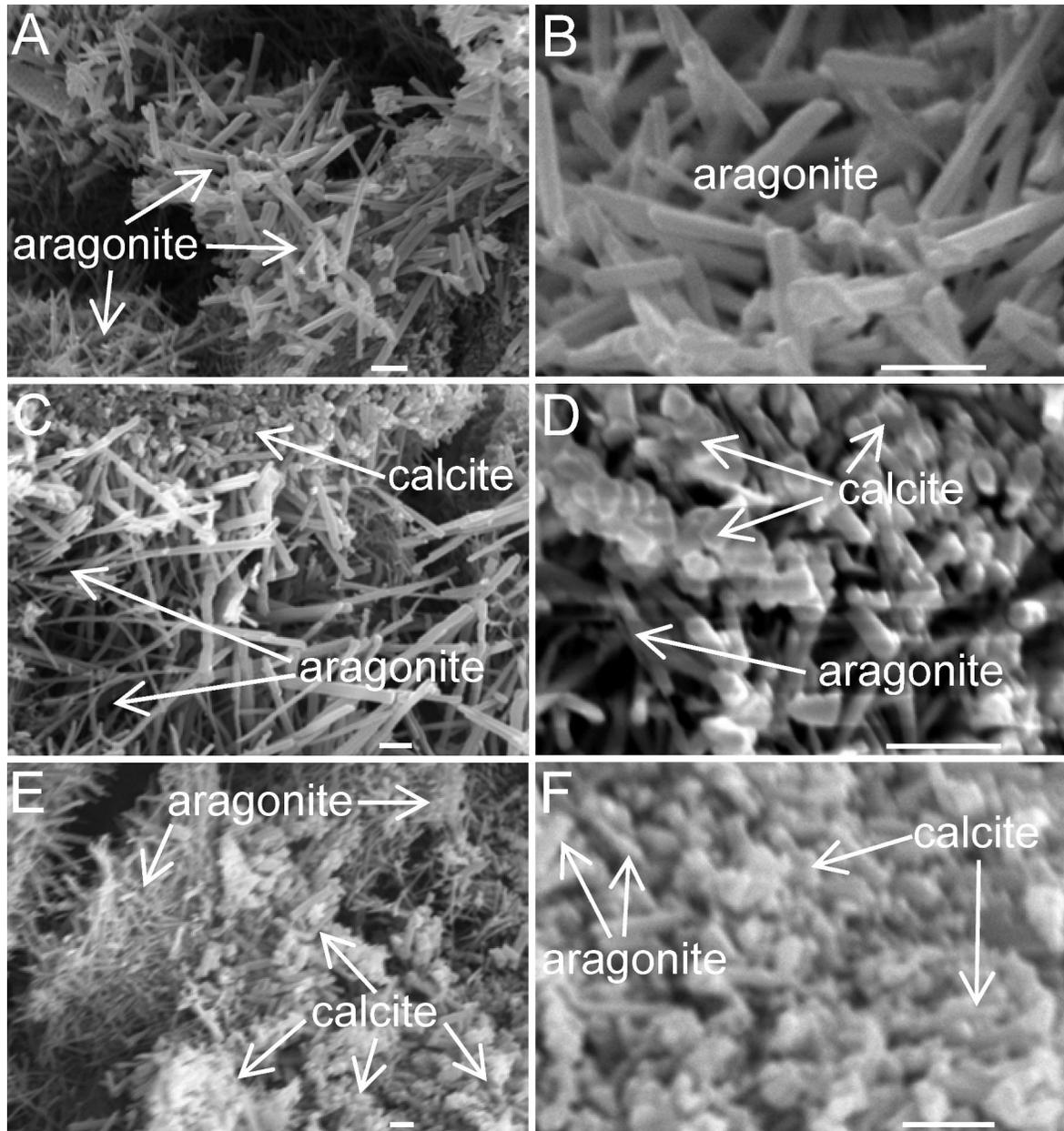


FIG. 3.—Backscatter electron images showing the occurrences of aragonite and calcite precipitated within the interutricular space of segments comprising the thalli of *H. incrassata* algae grown in experimental seawaters with A, B) Mg/Ca = 5.2 and $[Ca^{2+}] = 10.2$ mM (modern seawater). C, D) Results for Mg/Ca = 2.5, and $[Ca^{2+}] = 18.1$ mM (imputed early Oligocene seawater). E, F) Results for Mg/Ca = 1.5 and $[Ca^{2+}] = 25.3$ mM (imputed early Eocene seawater). Scale bars are $1\mu m$.

of the Mg/Ca ratio. For each Mg/Ca ratio, the highest rates of calcification and growth were for the highest level of $[Ca^{2+}]$. Calcium poisoning, which is well known to occur in plants (White and Broadley 2003), was therefore not an issue. The highest level of $[Ca^{2+}]$ for each Mg/Ca ratio was necessarily accompanied by the highest $[Mg^{2+}]$ level, so high levels of this cation also had no deleterious effect. Because of the high $[Na^+]$ of seawater, adjustments of this ion to keep salinity constant represented only minor fractional changes.

Quantification of the Effects of the Mg/Ca Ratio and $[Ca^{2+}]$

For calcification, primary productivity, and linear growth, rates relative to those in modern seawater declined, respectively, by 37%,

41%, and 32% for the early Oligocene treatment and 52%, 55%, and 49% for the early Eocene treatment (Fig. 7). These declines resulted from the reduction of the ambient Mg/Ca ratio, which militated against aragonite precipitation, but they occurred in the face of a progressive increase in ambient $[Ca^{2+}]$ from 10.2 to 18.1 to 25.3 mM, which favored both aragonite and calcite precipitation. Thus, for the natural experimental seawaters, differences in Mg/Ca had a greater effect than differences in $[Ca^{2+}]$ on rates of calcification and growth.

The relative influences of the Mg/Ca ratio and $[Ca^{2+}]$ (Fig. 7) were quantified by taking into account the rates for the non-natural seawater treatments. For example, lowering the Mg/Ca ratio from that of modern seawater (5.2) to the early Eocene level (1.5), while maintaining $[Ca^{2+}]$ at its modern level of 10.2 mM, reduced rates for calcification, productivity,

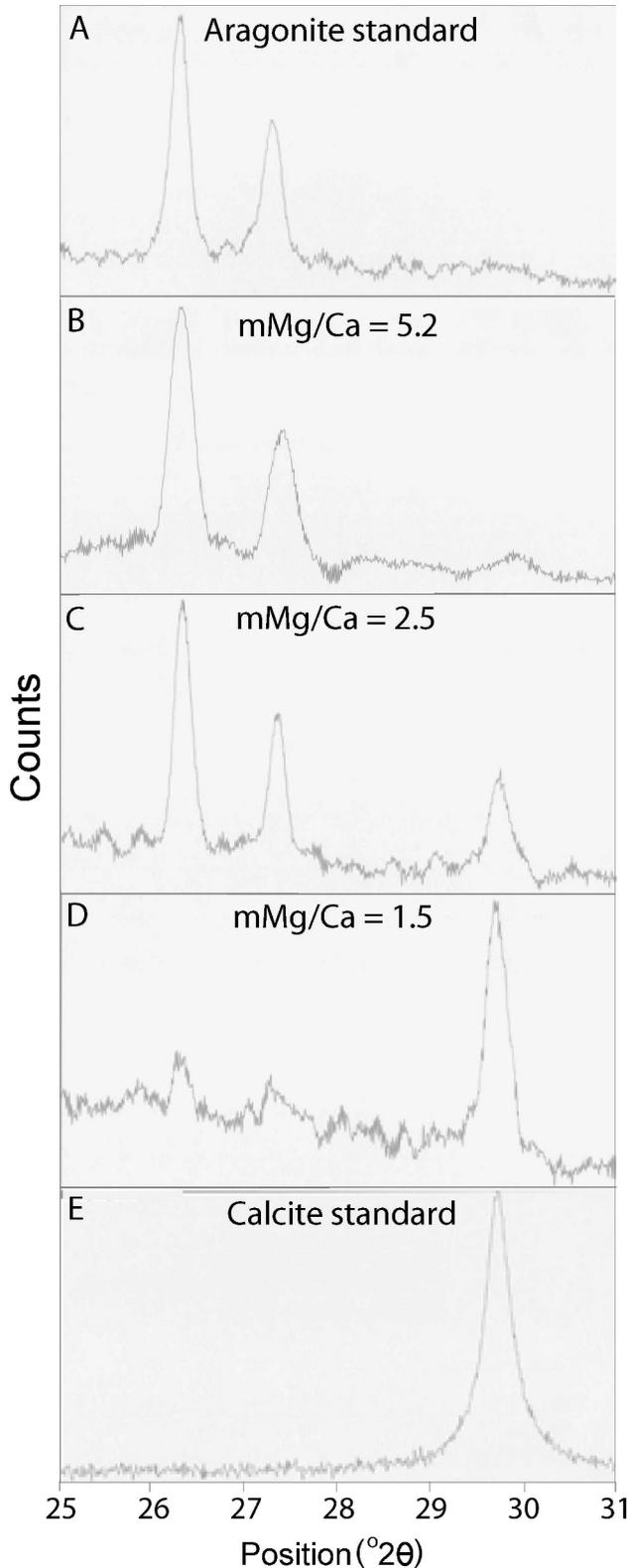


FIG. 4.—X-ray diffraction patterns for polymorphs of CaCO_3 . A) Pattern for pure aragonite, revealing primary aragonite peak [$d(111)$] at $2\theta = 26.2$ (3.40 Å). B) Results for modern seawater ($\text{Mg}/\text{Ca} = 5.2$; $[\text{Ca}^{2+}] = 10.2$ mM), which yielded mean values of 92% aragonite and 8% calcite. C) Results for imputed early Oligocene seawater ($\text{Mg}/\text{Ca} = 2.5$; $[\text{Ca}^{2+}] = 18.1$ mM), which yielded average values of 84% aragonite and 16% calcite. D) Results for imputed early Eocene

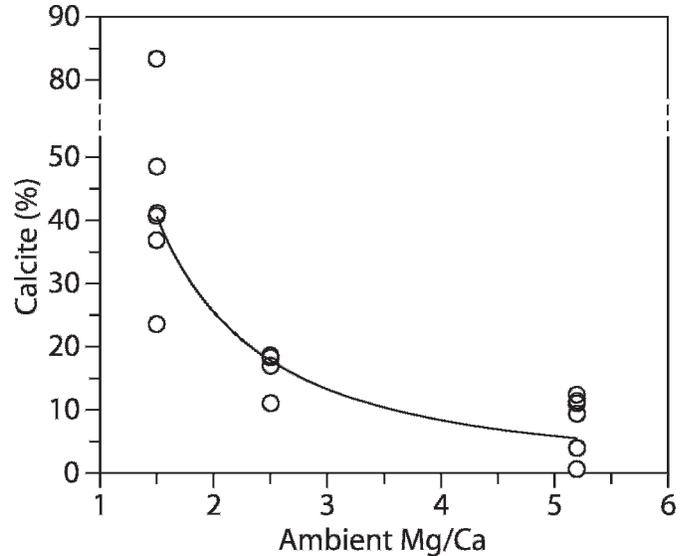


FIG. 5.—Relative amount (mol%) of calcite precipitated within *H. incrassata* in experimental seawaters, as determined by powder X-ray diffraction. Data are fitted to a power function ($R^2 = 0.595$).

and linear growth by 95%, 95%, and 68%, respectively. In contrast, elevating $[\text{Ca}^{2+}]$ from its level in modern seawater (10.2 mM) to its early Eocene level (25.3), while holding the Mg/Ca ratio at its modern level of 5.2, elevated the same set of rates by only 43%, 40%, and 54%, respectively. If these results, along with the more limited ones of Ries (2005, 2006a), can be extrapolated to other taxa, it can be concluded that the ambient Mg/Ca ratio has always exerted a stronger influence than $[\text{Ca}^{2+}]$ over rates of growth and calcification for green algae of the kinds that produce aragonite in the modern ocean. Experiments on coccolithophores (Stanley et al. 2005) yielded the same result with respect to the relative impacts of Mg/Ca and $[\text{Ca}^{2+}]$.

Effects of Secular Changes of Mineralogy on Growth Rates

Complicating the influence of seawater chemistry on rates of thallus growth and calcification for *Halimeda* is the inverse relationship between the amount of calcite formed within the alga's skeletal material and the ambient Mg/Ca ratio. The fact that the fractionation pattern for incorporation of Mg into this calcite resembles that for nonskeletal calcite (Fig. 6) means that the composition of calcite formed by *Halimeda* is compatible with ambient seawater regardless of this seawater's Mg/Ca ratio. Therefore, the relative increase in the percentage of calcite produced as the ambient Mg/Ca ratio is lowered must, like the concomitant elevation of $[\text{Ca}^{2+}]$, partly offset the negative effect of a reduced ambient Mg/Ca ratio on aragonite production. Presumably this beneficial effect of calcite secretion is damped by an additional factor, however, at least for ambient seawaters in the 1.5–2.0 range. In these seawaters, the alga secretes a moderate amount of calcite, but this calcite contains a substantial amount of Mg and incorporation of magnesium retards the growth rate of calcite (Davis et al. 2000).

← seawater ($\text{Mg}/\text{Ca} = .5$; $[\text{Ca}^{2+}] = 25.3$ mM), which yielded average values of 54% aragonite and 46% calcite. E) Pattern for pure calcite, revealing primary calcite peak [$d(104)$; 3.03–3.04 Å; $2\theta = 29.4$ –29.5°].

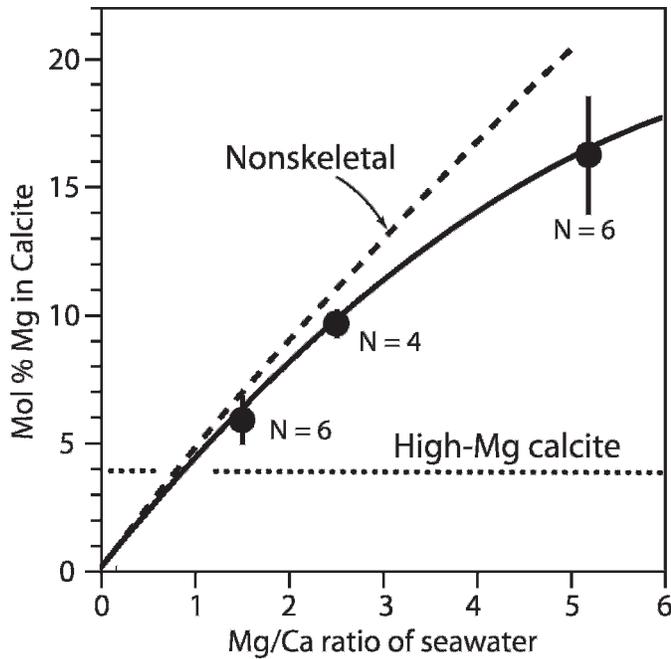


FIG. 6.— Molar Mg/Ca of calcite precipitated by *H. incrassata* algae in experimental seawaters, as determined by powder X-ray diffraction and energy-dispersive spectrometry. Mg-fractionation algorithm (solid curve) is calculated using a power function ($R^2 = 0.99$). Dashed curve is Mg-fractionation curve for calcite precipitated abiotically from seawater at 25°C ($y = 0.0482x^{0.898}$, $R^2 = 0.930$ (Füchtbauer and Hardie 1976, 1980). Error bars represent instrument error and variation within and between specimens.

Halimeda from the Early Oligocene to the Present: the Influences of Atmospheric pCO_2 and the Mg/Ca Ratio of Seawater

Four environmental factors are likely to have influenced *Halimeda*'s productivity over the course of geologic time: (1) the Mg/Ca ratio of seawater, (2) $[Ca^{2+}]$ of seawater, (3) the influence of atmospheric pCO_2 on the saturation state of $CaCO_3$, and (4) the influence of atmospheric pCO_2 on *Halimeda*'s rate of photosynthesis. Secular changes in nutrient levels in seawater are unlikely to have played a significant role here because bryopsidalean algae subjected to low nutrient levels are known to employ stolons to obtain nutrients from sediment. (Littler et al. 1988; Malta et al. 2005). Secular changes in solar irradiance are unlikely to have played a significant role because the optimal light intensity for *Halimeda* occurs well below the sea surface (Littler et al. 1988), and over time, algal populations can shift with the depth of this optimal level.

Did changes documented for atmospheric pCO_2 in the geologic past influence rates of the calcification and growth of *Halimeda*? Elevated atmospheric pCO_2 depresses the saturation state of seawater with respect to $CaCO_3$. Therefore, any past increase in atmospheric pCO_2 would have adversely affected *Halimeda*'s calcification and the resulting provision of CO_2 to photosynthesis. On the other hand, increased pCO_2 has been shown to enhance photosynthesis by noncalcifying algae (Zou 2005; Zou et al. 2007), and it must therefore also tend to promote total calcification of taxa like *Halimeda* by increasing their organic growth rate. The net effect of these opposing factors is not immediately apparent, but geologic evidence described below suggests that changes in atmospheric pCO_2 have not strongly influenced the productivity of *Halimeda*.

Studies of carbon isotopes in alkenones produced by coccolithophores and preserved in deep-sea sediments have revealed that a sudden drop in atmospheric pCO_2 from more than 1000 ppmv to about 500 ppmv occurred near the beginning of Oligocene time, slightly before 30 Ma

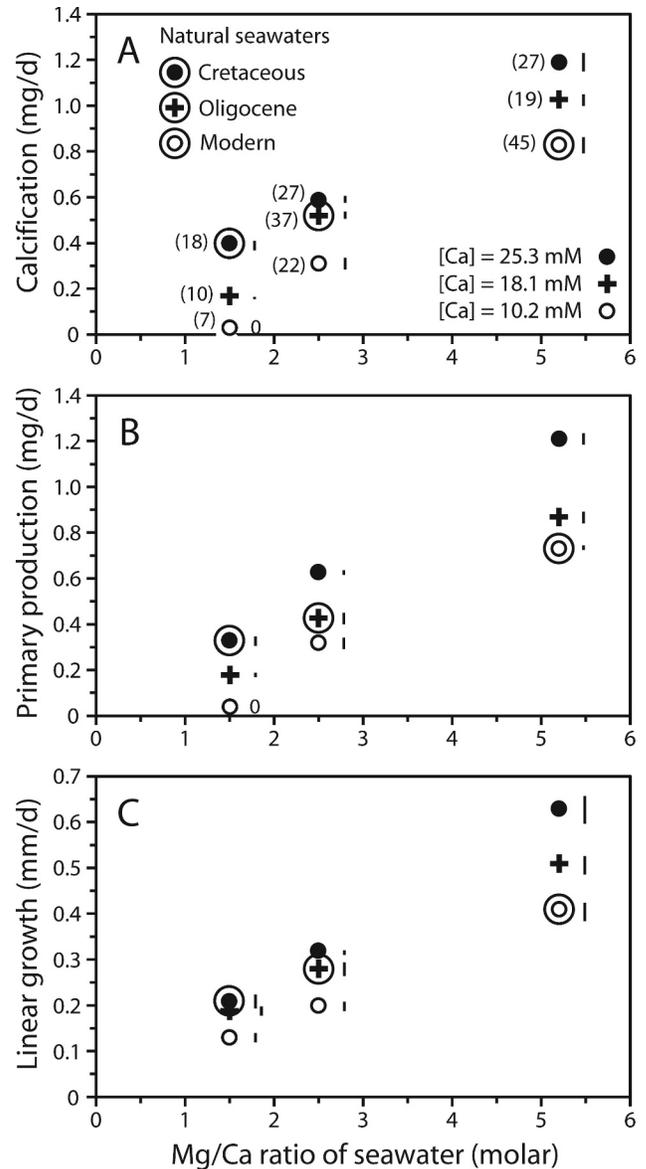


FIG. 7.— Rates of increase for *H. incrassata* grown in nine experimental seawaters A) Calcification (numbers in parentheses are sample sizes [numbers of experimental thalli]). B) Primary production. C) Linear growth. All rates increase significantly ($p < 0.05$) for both natural and non-natural seawaters with an increase in ambient Mg/Ca ($[Ca^{2+}]$ fixed) and with an increase in ambient $[Ca^{2+}]$ (Mg/Ca fixed). Bars beside symbols depict standard errors.

(Pagani et al. 2005). There was, however, no substantial effect of this change on the productivity of *Halimeda*; throughout the Oligocene it remained a relatively minor contributor of carbonate sediments on a global scale. This pCO_2 decline, occurring within ~ 2 Myr, was too sudden for any change in the Mg/Ca ratio or $[Ca^{2+}]$ of seawater to have simultaneously exerted a significant influence on the growth or calcification of *Halimeda*. On the other hand, during late Cenozoic time, when *Halimeda*'s marked increase in productivity occurred, there were only minor changes in the level of atmospheric pCO_2 (Pagani et al. 2005) but a dramatic increase in the Mg/Ca ratio of seawater (Fig. 1). The experiments reported on here suggest that this cationic change in seawater chemistry is likely to have contributed to the late Cenozoic flourishing of *Halimeda*.

It is theoretically possible, of course, that in the geologic past *Halimeda* was genetically programmed to precipitate all of its CaCO_3 as calcite in ancient calcite seas, in which case seawater chemistry would have favored its calcification and growth. This seems unlikely because it is evident that *Halimeda* did not flourish during the Cretaceous or early Mesozoic to the degree that it has during the late Neogene.

RELATIVELY WEAK BIOLOGICAL CONTROL OF MINERALOGY

A long-standing debate concerns the degree to which various biomineralizing organisms govern the mineralogy of their skeletons, as opposed to having it influenced, or even dictated, by environmental conditions (Lowenstam and Weiner 1989, p. 26–27; Mann 2001, p. 24–27; Weiner and Dove 2003). Although it has been suggested that *Halimeda* employs polysaccharides to induce precipitation of aragonite rather than calcite (Borowitzka and Larkum 1976b), experiments of the present study indicate that this genus exerts only partial control over its skeletal mineralogy.

In previous experiments, the calcareous green algae *Penicillus capitatus* and *Udotea flabellum* precipitated, on average, 22 (± 3) and 25 (± 3) wt% of their CaCO_3 as the calcite polymorph, respectively, in artificial Cretaceous seawater (Mg/Ca = 1.0), and they produced no calcite when the ambient Mg/Ca was 5.2 or 2.5 (Ries 2005, 2006b). In the present study, the very high average percentage of 43 wt% was found in *Halimeda* grown in seawater having a Mg/Ca ratio of 1.5. Relatively weak biomineralogical control by *Halimeda* is further evidenced by the surprising observation that the *H. incrassata* specimens produced a significant portion of their CaCO_3 as calcite even in the Oligocene treatment [16.2 (± 1.8) wt%] and the modern treatment [8.1 (± 1.9) wt%] (Fig. 5). *Penicillus* and *Udotea*, in contrast, precipitate exclusively aragonite under these conditions.

The spatial heterogeneity of the aragonite and calcite precipitated within the *H. incrassata* grown in the experimental calcite seawater is of a scale comparable to that of the purported acid and alkaline zones within this alga (de Beer and Larkum 2001). Both conditions may reflect the alga's spatially limited control over the chemistry of its intertricular fluid.

Penicillus and *Udotea* calcify partly within the cell wall, but this cannot explain their relatively strong control over their skeletal mineralogy because, unlike *H. incrassata*, in experiments they did not produce calcite in intertricular space when the ambient Mg/Ca ratio was 5.2 or 2.5 (Ries 2005, 2006b). Furthermore, in *Udotea flabellum* (the species studied by Ries 2006a) cell-wall calcification is restricted to narrow zones within the thin cortical region. In fact, in analyzing the anatomy of *U. flabellum*, Böhm (1978) supported earlier workers' findings in concluding, "the calcification pattern of *U. flabellum* and *Halimeda* ... [are] indeed similar." Presumably the relatively strong control of biomineralization by *Penicillus* and *Udotea* results not from their anatomy but from some physiological trait that they share as members of the same family.

CONCLUSIONS

1. *Halimeda incrassata* today produces skeletal material consisting of aragonite and a small amount of high-Mg calcite (~ 8% of skeletal CaCO_3), both of which are also produced by abiotic precipitation in the modern ocean because of the high Mg/Ca ratio (5.2) of modern seawater. Because previous studies have suggested that CO_2 liberated by the calcification of *Halimeda* is used in this alga's photosynthesis, thus enhancing growth, it was hypothesized that the high Mg/Ca ratio of modern seawater, by favoring aragonite precipitation, contributes to *Halimeda*'s high rates of organic productivity and sediment production in the modern ocean.

2. As predicted, in laboratory experiments rates of production of organic matter and also linear growth for *Halimeda* declined progressively along with rate of calcification as the Mg/Ca ratio of ambient seawater was reduced from its present level of 5.2 to 2.5 (an imputed early Oligocene level) and 1.5 (an imputed early Eocene level).
3. Because secular changes in $[\text{Mg}^{2+}]$ and $[\text{Ca}^{2+}]$ of seawater are inversely related, experimental reductions of the ambient Mg/Ca were accompanied by increases of ambient $[\text{Ca}^{2+}]$. The increases of $[\text{Ca}^{2+}]$ raised the saturation state of CaCO_3 , thus favoring the precipitation of aragonite, in opposition to the effect of reductions of the Mg/Ca ratio. As was predicted, in controlled experiments rates of calcification and growth decreased with a reduction of ambient Mg/Ca but increased with an elevation of ambient $[\text{Ca}^{2+}]$. Nonetheless, the Mg/Ca ratio exerted a much stronger influence than $[\text{Ca}^{2+}]$. This disparity accounts for the decreased rates of calcification and growth rates as the ratio was lowered in the experiments.
4. With a reduction of the Mg/Ca ratio in experimental treatments, the mean percentage of calcite in the *Halimeda* skeleton increased from ~ 8% for modern seawater to ~ 16% for imputed early Oligocene seawater (Mg = 2.5) and ~ 46% for imputed early Eocene seawater (Mg = 1.5).
5. The magnesium content of calcite in *Halimeda* skeletons increased with the ambient Mg/Ca ratio of experimental seawaters, following a fractionation pattern similar to that for abiotic calcite at the same temperature.
6. The fact that *Halimeda* produced skeletons containing, on average, 54% aragonite in artificial seawater with Mg/Ca = 1.5, which favored calcite, indicates that the genus exerts some control over its skeletal mineralogy. Nonetheless, this control is weaker than that of *Penicillus* and *Udotea*, which are more closely related to one another phylogenetically than either is to *Halimeda*. It is nonetheless likely that all bryopsidalean algae produced a substantial amount of calcite at times in the Cretaceous when the Mg/Ca ratio of seawater was close to unity.
7. *Halimeda* failed to respond with any significant change in productivity when a sharp decline of atmospheric $p\text{CO}_2$ occurred early in Oligocene time. It did, however, diversify markedly and greatly increase its global productivity in late Neogene time, probably in response to the dramatic increase in the Mg/Ca ratio of seawater.

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APPENDIX: METHODS

Ten-gallon glass aquaria were each filled with 30 L of experimental seawater formulated identically at normal modern marine values (Bidwell and Spotte 1985), except for variations in [Mg²⁺] and [Ca²⁺], and also for [Na⁺] in experiments in which the molar sum of [Mg²⁺] + [Ca²⁺] was required to differ from that of modern seawater. Salinity was maintained at the modern value of 35‰. Ionic strengths were also maintained at the modern value of 0.7. Mg/Ca ratios remained within 5% of their initial values for the duration of the experiments. The aquaria were fertilized with 20.0 mg/L NaNO₃, 1.3 mg/L NaH₂PO₄H₂O, and 0.025 mL/L EDTA, all within the normal range for shallow tropical marine waters (Spotte 1979). The aquaria were maintained at 25 ± 1° C using 50-watt electric heaters, continuously filtered with Millenium 2000 Wet-Dry Multi-filters (rate of filtration = 600 L/hour), and illuminated with identical irradiance (19 watts) for 10 hours/day.

Specimens of young (~ 4 cm) *H. incrassata* were collected for mineralogical studies from Florida Bay and for growth rate experiments from Roatán and the back-reef lagoon of Caye Caulker, Belize. Upon arrival at the laboratory, the algae were placed in a holding tank filled with normal seawater for two weeks and then, if they were to be studied in non-normal seawater, moved over a one-month interval to seawater compositions that were increasingly different from modern seawater. Offspring that they produced at their final locations were employed in the experiments.

Specimens harvested to measure primary production were gently rinsed for 20 seconds with 95% ethyl alcohol and then oven-dried at 100° C for 24 hours, after which their dry weight was determined. The algae were then baked at 450° C for four hours to combust organic matter. The residual CaCO₃ material was weighed and recorded as the algae's total calcification.

Specimens grown for mineralogical analysis were gently rinsed for 20 seconds with 95% ethyl alcohol to remove any seawater that might have precipitated salts upon drying. Five thallus segments were removed from each of the nine offspring algae, sectioned parallel to their long axes, and dried in an oven for 24 hours at 100° C. Sectioned segments were secured to cylindrical carbon SEM mounts, which were then carbon-coated for 1–2 min. The *H. incrassata* specimens were thoroughly examined in a JEOL 8600 scanning electron microscope for carbonate minerals. Back-scatter electron images of CaCO₃ within segments were obtained. The five segments from each of the nine offspring algae were gently ground to a fine powder, mixed with 95% ethyl alcohol to create a suspension that was injected into a 1 cm × 1 cm × 10 mm reservoir on a glass slide and allowed to dry overnight. The even layer of CaCO₃ crystals that resulted was analyzed for polymorph mineralogy using powder X-ray diffraction. The proportion of aragonite to calcite was calculated from the X-ray diffraction patterns as the ratio of the area under the primary aragonite peak [*d*(111): 3.40 Å; 2θ = 26.2°] to the area under the primary calcite peak [*d*(104): 3.03–3.04 Å; 2θ = 29.4–29.5°]. The Mg/Ca ratio of calcite was determined by the *d* spacing of the calcite crystal lattice and confirmed by EDS microprobe spot analysis in the JEOL 8600 SEM.