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Community and environmental influences on reef coral calcification

Abstract—Reef corals calcify faster in the presence of noncalcareous algae. Ratios of calcification to photosynthesis appear to be affected by the ratio of alkalinity to acidity, which controls how efficiently the protons from calcification convert bicarbonate to carbon dioxide. By forcing calcifiers to calcify faster, algal proliferation on nutrient-enriched reefs may adversely affect the reef-builders.

Reef corals and calcareous algae usually calcify fastest during the daytime and often fix carbon into calcium carbonate and biomass at similar rates. Light-enhanced calcification has long been recognized (Kawaguti and Sakumoto 1949; Goreau 1959) but still poses a chicken-and-egg dilemma: Does photosynthesis stimulate calcification by raising $CO_3^=$ concentrations and the $CaCO_3$ saturation state of the water? Does calcification counteract CO_2 depletion and thereby stimulate photosynthesis? Does photosynthesis provide metabolic energy to power calcification? We examine these questions and their extension from the organismal to the community level.

CO_2 diffusion through the boundary layer surrounding an aquatic autotroph (Fig. 1A) can support photosynthetic rates of about 0.2 μmol m^{-2} s^{-1}, calculated using Fick's first law, with a dissolved molecular CO_2 concentration of 10 μMol kg^{-1}, a 50-μm-thick boundary layer, and 50% depletion of CO_2 level at the autotroph's absorptive surface. Algae and corals often have photosynthetic rates that are several times that fast, indicating that they use mainly bicarbonate. However, bicarbonate utilization requires additional protons ($H^+ + HCO_3^- = CH_2O + O_2$). The protons may derive from H_2O and HCO_3^-, with a corresponding efflux of OH^- and $CO_3^=$ (Fig. 1B). Large OH^- and $CO_3^=$ effluxes imply alkalinization and therefore CO_2 depletion at the absorptive surface. This process reduces photosynthetic efficiency because of the Michaelis kinetics of CO_2 fixation by the enzyme Rubisco (ribulose bisphosphate carboxylase oxygenase) (Fig. 1E). Calcification (Fig. 1C) provides an alternative proton source and potentially allows autotrophs to avoid most of the alkalinization and CO_2 depletion that otherwise accompanies HCO_3^- utilization.

Figure 2A illustrates how photosynthesis and calcification affect the aragonite saturation state (Ω) and molecular CO_2 content of tropical surface seawater. Block arrows labeled "C" and "NC" represent hypothetical calcareous and noncalcareous autotrophs that photosynthetically remove 20% of the dissolved inorganic carbon (DIC) initially found in the seawater. The calcifier has a molar ratio of calcification to net photosynthesis ($C:P_n$) of 1.3, which is about average for reef corals (Gattuso et al. 1999). Although the calcifier and noncalcifier have equal photosynthetic rates, the calcifier depletes molecular CO_2 about one-half as much—by 43%, compared to the 81% rate associated with the noncalcifier. If both obey the same Michaelis curve for CO_2 fixation (Fig. 1E), the calcifier should fix carbon almost twice as efficiently. Figure 2B and C explore how photosynthesis, calcification, and diffusion interact to control chemistry at the autotroph's absorptive surface. With reduced diffusion problems, calcareous plants and algae–invertebrate symbioses often adopt massive morphologies.

Gattuso et al. (2000) argued that calcification does not help photosynthesis, based on observations that a coral incubated at reduced Ca^{2+} concentrations (2.85 mMol kg^{-1}) maintained normal photosynthesis but calcified less. Calcium transport inhibitors and Ca^{2+} concentrations below 0.5 mM have, however, inhibited photosynthesis in other experiments (reviewed by McConnaughey and Whelan 1997). The disparity may depend on how strongly the treatment inhibits the proton flux from the calcifying space. This H^+ flux appears to be coupled to Ca^{2+} fluxes through $Ca^{2+}/2H^+$ exchange catalyzed by the enzyme Ca^{2+} adenosine triphosphatase (ATPase), as illustrated in Fig. 1C. Gattuso's Ca^{2+} concentrations were adequate to support this coupling; hence, H^+ fluxes probably continued, supported temporarily by OH^- and $CO_3^=$ accumulation within the calcifying space. Gattuso's experiments therefore demonstrate that photosynthesis does not depend on $CaCO_3$ precipitation, but they do not rule out stimulation by the H^+ (and Ca^{2+}) fluxes that produce calcification under normal conditions. In light of typical Michaelis curves for photosynthesis (Fig. 1E) and calcification's ability to counteract CO_2 depletion (Fig. 2), it would be surprising if calcification did not sometimes benefit photosynthesis.

Calcification and photosynthesis become coupled through the reactions:

Calcification:

$$Ca^{2+} + CO_2 + H_2O = CaCO_3 + 2H^+ \quad (1)$$

Bicarbonate conversion:

$$2H^+ + 2HCO_3^- = 2CO_2 + 2H_2O \quad (2)$$

Photosynthesis:

$$CO_2 + H_2O = \text{“}CH_2O\text{”} + O_2 \quad (3)$$

Net:

$$Ca^{2+} + 2HCO_3^- = CaCO_3 + \text{“}CH_2O\text{”} + O_2 \quad (4)$$

The use of molecular CO_2 in biological calcification (reaction 1) was originally postulated to account for ^{18}O and ^{13}C deficiencies in coral skeletons, which appeared to be caused by kinetic discrimination against the heavy isotopes during CO_2 hydroxylation and hydration reactions (McConnaughey 1989). The conversion of HCO_3^- to CO_2 (reaction 2) is often catalyzed and can theoretically occur either within the extracellular boundary layer or intracellularly after importation of HCO_3^-, H^+ combinations. Alkaline and acidic conditions favor reactions 1 and 2, respectively, and most calcareous organisms develop recognizable, highly alkaline calcareous zones that contrast with the noncalcareous, absorptive regions in which HCO_3^- assimilation occurs.

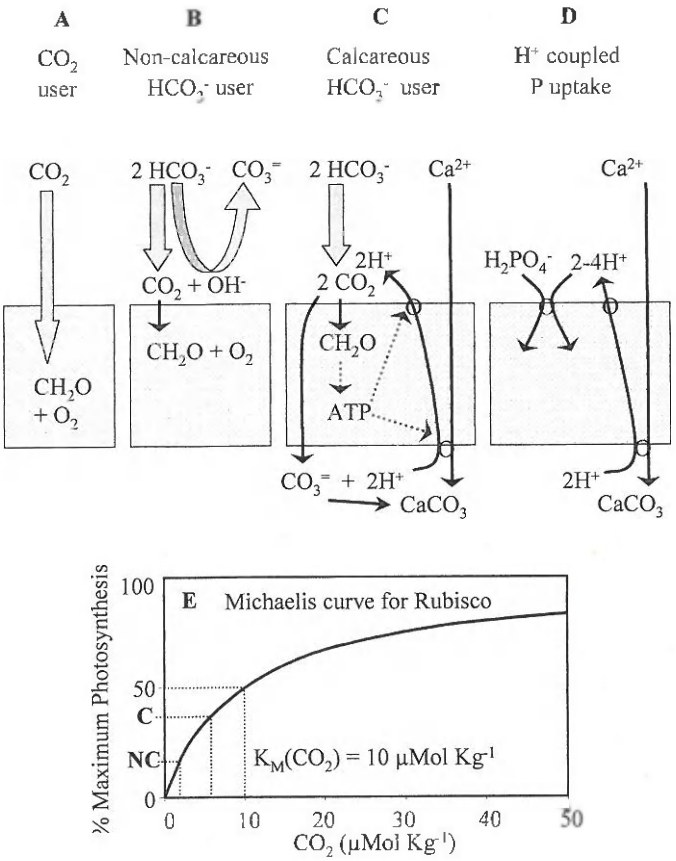

Fig. 1. Carbon assimilation strategies. (A) CO_2 uptake. (B) HCO_3^- assimilation using proton equivalents from solution. (C) HCO_3^- assimilation using protons from calcification. (D) Phosphate-proton cotransport. (E) Michaelis curve, illustrating kinetics of CO_2 fixation by the enzyme Rubisco. "C" and "NC" refer to the hypothetical calcifier and noncalcifier, respectively, in Fig. 2A.

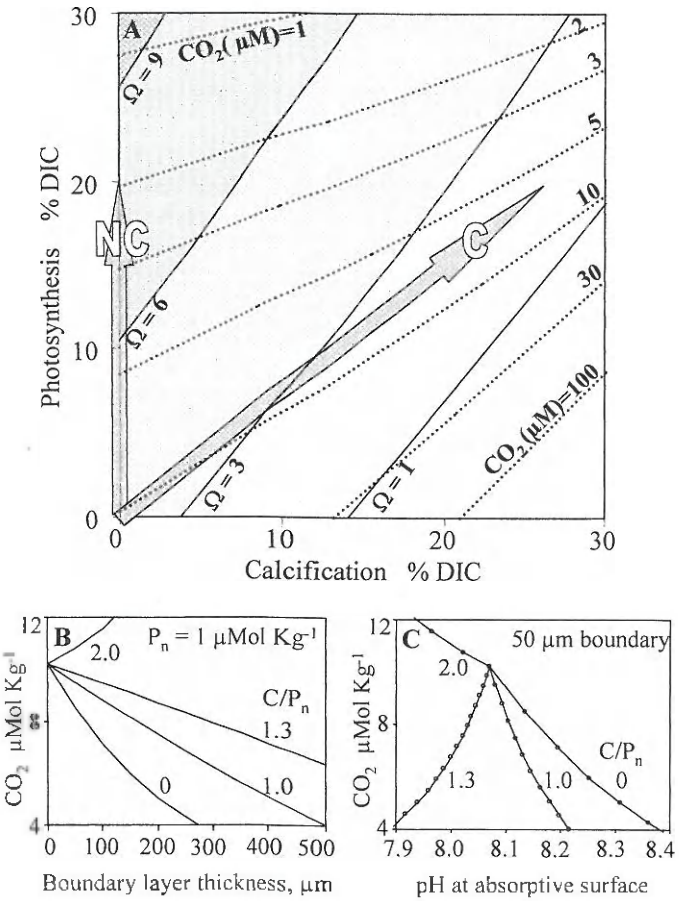

Fig. 2. Photosynthesis, calcification, and seawater chemistry. (A) Effects of photosynthesis (Y axis) and calcification (X axis) on molecular CO_2 concentrations (dotted lines, in μMol kg^{-1}) and the aragonite saturation state (Ω, solid lines with shading) of tropical surface seawater. Initial conditions at origin are described in methods. Block arrows "C" and "NC" represent hypothetical calcareous and noncalcareous autotrophs, respectively. (B) CO_2 concentrations at an autotroph's absorptive surface for $C:P_n$ ratios of 0, 1.0, 1.3, and 2.0, plotted as a function of the boundary-layer thickness. P_n = 1 μMol C m^{-2} s^{-1}. (C) CO_2 and pH at the autotroph's absorptive surface for a boundary-layer thickness of 50 μm. Multiples of P_n = 1 μMol C m^{-2} s^{-1} are plotted as dots along the curves.

These are represented by the lower and upper surfaces, respectively, in Fig. 1C.

To successfully couple reactions 1–4, the autotroph balances the photosynthetic benefits of higher CO_2 concentrations (Fig. 1E) against the costs of calcification. Calcification costs about 2 ATP per $CaCO_3$ unit precipitated (McConnaughey and Whelan 1997). Each "CH_2O" unit produced during photosynthesis generates about 6 ATP upon respiration, so the cost : benefit ratio of coupling calcification and gross photosynthesis is about $(C \times 2)/(P_g \times 6) = 1/3$ $C:P_g$. $C:P_g$ ratios above 3 appear to be energetically unproductive.

As the protons from calcification are discharged into ambient waters (or the boundary layer adjacent to the absorptive surface; Fig. 1C), not all react with HCO_3^- to produce CO_2. Some protons leak from the absorptive to the calcifying surface, and more importantly, some are soaked up by bases such as OH^-, $CO_3^=$, and $B(OH)_3O^-$ rather than HCO_3^-. The alkalinity of solution, which measures the concentration of all bases, therefore affects how much CO_2 can be produced through calcification. Conversely, acids in solution supplement the protons from calcification. Community metabolism affects seawater alkalinity and acidity and potentially influences calcification rates by individual organisms that stimulate photosynthesis through calcification.

Materials and methods—Experiments were carried out in a coral reef microcosm located at the Marine Systems Laboratory of the Smithsonian Institution of Natural History (Adey 1983; Small et al. 1998). The microcosm's main tank held 400 liters, and associated algal turf scrubbers contained an additional 1,280 liters. Natural sunshine supplied about one-third of the total light, and metal halide bulbs provided the remainder. Photosynthetic calcifiers included green algae, coralline reds, foraminifera, stony corals, and giant clams. Calcium and bicarbonate were replenished daily to maintain $Ca^{2+} > 10.5$ mM L^{-1} and total alkalinity > 2.40 mEq L^{-1} during the period when measurements were made in the microcosm.

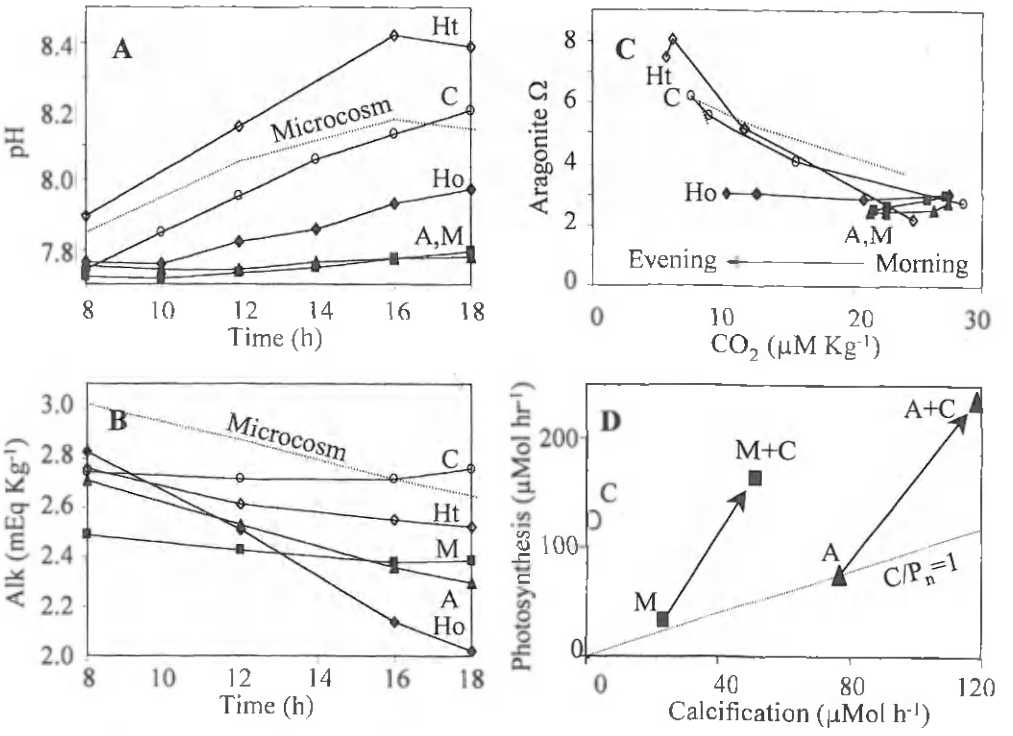

Fig. 3. Changes in water chemistry during daytime incubations of algae and corals. (A) pH. (B) Total alkalinity. (C) Calculated changes in CO_2 and aragonite saturation states. (D) Coral + *Chondria* incubations. Dashed lines, microcosm; M = *Montipora,* squares; A = *Acropora,* triangles; C = *Chondria,* circles; Ht and Ho = *Halimeda tuna* (open diamonds) and *H. opuntia* (filled diamonds), respectively. n = 5 Replicates, except *Chondria* and *H. tuna,* for which n = 2; standard deviations are somewhat larger than markers.

For various experiments, corals and algae were placed in 1- and 3-liter chambers that were suspended within the microcosm. Water-filled chambers provided controls. Pumps circulated water within the chambers. Incubations lasted 10 h, with sampling for O_2 and pH every 2 h and sampling for alkalinity every 4 h. Five replicates were conducted for *Acropora, Montipora,* and *Halimeda tuna* and for combinations of *Acropora* or *Montipora* with *Chondria.* Three replicates were conducted with *Chondria* and two were conducted for *Halimeda opuntia.*

Dissolved oxygen was measured by Winkler titrations, with a precision based on two to three titrations per sample of ± 0.94 μM L^{-1} (n = 15). Titration alkalinity was determined with a precision for three replicates of ± 0.03 mEq L^{-1} (n = 15). Prior to obtaining measurements, pH electrodes were calibrated in Fisher-brand buffers (pH, 7.00 and 10.00; NIST scale) with a precision of ± 0.02, and pH electrodes were later recalibrated to the seawater scale (Millero 1995) through measurements of a seawater carbon standard provided by Andrew Dixon (Scripps Institution of Oceanography). DIC, CO_2 concentration, and aragonite saturation state were calculated from pH and alkalinity data.

Calcification was estimated as one-half of alkalinity change, and photosynthesis was estimated as the DIC change minus calcification (Smith and Kinsey 1978). Calculations based on microcosm experiments used observed temperatures (t $\sim$ 28°C) and salinities (S $\sim$ 36‰). Borate and calcium concentrations were assigned seawater values (B_T = 416 × S/35; Ca^{2+} = 10280 × S/35 μM kg^{-1}) (Millero 1995). Chemical modeling not tied to the microcosm assumed that t = 25°C and S = 35‰, initially with 10.2 μM kg^{-1} dissolved molecular CO_2; 1,962 μM kg^{-1} total DIC; 2,300 μEq kg^{-1} alkalinity; and pH 8.0697. Millero's (1995) carbonate system thermodynamics were used throughout.

Results—The noncalcareous alga *Chondria* sp. increased pH but did not change alkalinity, whereas highly calcareous species reduced in terms of alkalinity and did not elevate pH as much (Fig. 3A,B). *Chondria* reduced molecular CO_2 concentrations by 73% and increased the aragonite saturation state from $\Omega \approx 3$ to 6.2 (Fig. 3C). The corals *Acropora* and *Montipora* reduced CO_2 by only 22 and 21%, respectively, and reduced Ω by 11 and 17%, respectively. The lightly calcareous *H. tuna* behaved somewhat like the noncalcareous *Chondria,* whereas the more calcareous *H. opuntia* behaved more like the corals.

Calcification and photosynthesis generally correlated over the course of the day. *Acropora* and sometimes *H. opuntia* achieved ratios of calcification to net photosynthesis ($C:P_n$) as high as 1. Calcification and photosynthesis generally declined during the afternoon, with photosynthesis declining more sharply. The higher photosynthetic rates in the morning coincided with higher CO_2 concentrations and lower pH and O_2 concentrations. Decreased calcification in the afternoon

occurred despite higher Ω, which was, for example, 40% higher during the afternoon in the microcosm. Net calcification in the microcosm essentially ceased at night, even though the water remained supersaturated with respect to aragonite. Calcification and aragonite supersaturation were thus only loosely correlated. The noncalcareous *Chondria* and lightly calcareous *H. tuna* raised Ω most strongly, whereas highly calcareous corals and *H. opuntia* left Ω unchanged (or even lowered Ω) after several hours.

When *Chondria* and corals were incubated together, pH increased as it did with *Chondria* alone, but alkalinity dropped rapidly. Calcification rates increased 60% (*Acropora*) and 130% (*Montipora*) compared with incubations of corals alone (Fig. 3D) (Student's *t*-test, $P = 0.002$ and $P = 0.00002$, respectively; $n = 5$ in both cases.) Thus, the noncalcareous alga apparently stimulated calcification in nearby corals. Photosynthetic rates in combined incubations were also somewhat higher than were the sums from separate incubations, by values of 7% (*Acropora*) and 18% (*Montipora*).

Discussion—Autotrophs balance benefits and costs in various ways. *Chondria* and other fleshy algae reduce metabolic costs by not calcifying. (Photosynthesis by such organisms alkalinizes the water and raises Ω, but this does not cause much calcification.) Corals and *H. opuntia* invest heavily in calcification and enjoy higher CO_2 levels. The lightly calcified *H. tuna* uses an intermediate strategy. The corals calcify faster when incubated together with noncalcareous algae under more alkaline, CO_2-depleted conditions, indicating an active effort to forestall CO_2 depletion.

Ambient chemistry affects how much CO_2 is generated by a particular amount of calcification. In reaction 4, HCO_3^- supplies the autotroph with both carbon and protons, and $C:P_n$ ratios = 1. However, dissolved bases, including OH^-, $B(OH)_3O^-$, and $CO_3^=$, compete with HCO_3^- for protonation at the organism's absorptive surface. This increases the amount of calcification required to produce CO_2 (reaction 2) by the factor [alkalinity]/[HCO_3^-]. Dissolved acids, including CO_2 and $B(OH)_4$, conversely supplement the protons from calcification, reducing the amount of calcification required by the factor [HCO_3^-]/[acidity]. The $C:P_n = 1$ ratio of reaction 4 therefore becomes:

$$C/P_n = \text{alkalinity/acidity} \quad (5)$$

Figure 4 plots this predictor of $C:P_n$ ratio as a function of pH. Further adjustments related to the different diffusivities of various ions have only a small effect. Within the normal pH range of reef seawater (7.9–8.3), the $C:P_n$ predictor lies between 1.0 and 1.3, a value that is similar to those values common among highly calcareous corals and algae. Seawater pH does not change when calcification and photosynthesis occur in a 1.2 ratio, which, according to Eq. 5, should occur at a pH of 8.2. Higher pH raises the predicted $C:P_n$ ratio and should stimulate calcification, nudging pH back down. This probably helps to maintain reef pH around 8.2.

Calcifiers tend to isolate their calcification sites, as beneath the aboral epithelium of corals, and then raise Ω locally through ion transport. pH often exceeds 10 at the calcification site of the alga *Chara*, and $CO_3^=$ accumulates to high levels as CO_2 diffuses and ionizes (reviewed by McConnaughey and Whelan 1997). Even nonphotosynthetic tissues can calcify through such physiologies. The ability of calcifiers to locally elevate Ω well beyond ambient values contributes to the loose correlations between ambient Ω and calcification rates observed in the present experiments.

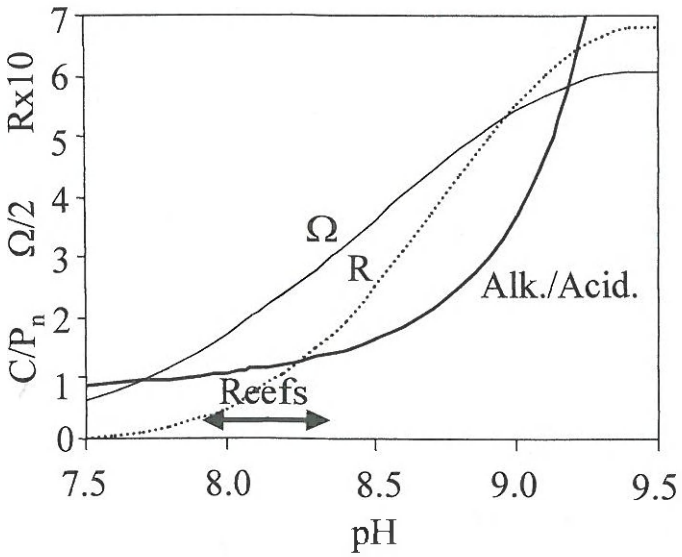

Fig. 4. Projected ratio of calcification to photosynthesis ($C:P_n$ = alkalinity : acidity; heavy line), aragonite saturation levels (Ω; solid line), and inorganic aragonite precipitation rate (dotted line, in μmol $CaCO_3$ m^{-2} s^{-1}, calculated from kinetics of Burton and Walter [1987]) in seawater as a function of pH (25°C, 35‰, constant total alkalinity = 2,300 μEq kg^{-1}).

Inorganic aragonite precipitation from seawater obeys rate equations such as $R = k(\Omega - 1)^{1.7}$ (Burton and Walter 1987) (Fig. 4). The inorganic calcification rate doubles between pH 8.0 and 8.2, whereas the $C:P_n$ ratio projected from the alkalinity : acidity ratio increases only 14%. (This assumes constant alkalinity. A constant DIC scenario produces similar results, whereas calcification increases more strongly with pH under a constant CO_2 scenario.) Calcification rates predicted from ambient Ω and the alkalinity : acidity ratio are therefore quite different.

Nutrients may also affect reef calcification rates. Highly calcareous autotrophs such as corals (e.g., Marubini and Davies 1996) and coccolithophorids (e.g., Paasche and Brubak 1994) calcify faster when nutrients are scarce and thrive in nutrient-deficient waters. McConnaughey and Whelan (1997) therefore suggested that calcification assists nutrient uptake. Nutrient-proton cotransport (Fig. 1D) provides one likely mechanism. NO_3^- and $H_2PO_4^-$ uptake in various studies appear to involve cotransport of at least $1H^+$ and $2–4H^+$, respectively (Sakano 1990; Wollenweber 1997). Energetically, an ion entering a cell performs the chemical work RT ln a_i/a_o, where R is the gas constant, T is Kelvin temperature, and a_i and a_o are the activities of the chemical inside and outside of the cell. The electrical work is ZFV, where Z is the charge on the ion, F is the Faraday constant, and V is the cell's membrane potential. Adding these terms for a nutrient ion plus "n" protons and rearranging yields

$$\log(a_i/a_o) = n(pH_i - pH_o) + (n + Z)FV/2.3RT + E/2.3RT \quad (6)$$

The final term accommodates additional energy terms such as ATP hydrolysis. V and E are shown as positive so that all terms are additive. If a cell increases (pH_i-pH_o) by 0.5 pH units through calcification, it might therefore accumulate NO_3^- and $H_2PO_4^-$ at least three and 10–100 times more strongly. Extracellular acidification should also improve NH_4^+ uptake slightly and slow Fe^{2+} oxidation, aiding in assimilation. Calcification's potential for improving nutrient assimilation therefore appears to be substantial. Moreover, seawater pH and alkalinity should affect this physiology much as they affect HCO_3^- assimilation. Photosynthesis and calcification may therefore become correlated, as will calcification and pH, even if the autotroph calcifies mainly to obtain nutrients.

People increase nutrient inputs to many reefs through agricultural runoff, sewage disposal, increased erosion, and airborne nutrient transport. Nutrients may directly suppress calcification, as was observed, for example, by Marubini and Davies (1996), and nutrients may encourage fleshy algae that compete with the calcifiers and feed their predators. Through such mechanisms, nutrients may reduce reef calcification. On the other hand, nutrients stimulate photosynthesis by both calcareous and noncalcareous autotrophs. By raising pH and the alkalinity : acidity ratio, reef calcification may be stimulated, as was observed in the present coral + *Chondria* experiments. This represents a metabolic cost to the calcifiers and may reduce their competitiveness in nutrient-rich situations. Nutrients probably have a greater potential for affecting reef calcification rates than does anthropogenic CO_2, which will decrease the alkalinity : acidity ratio by only 10% as CO_2 levels double. Industrial nitrogen fixation has already doubled the global rate of nitrogen fixation.

Returning to the introductory chicken-and-egg question, calcification appears quite capable of stimulating photosynthesis, but this process is not metabolically "free." Photosynthesis can also stimulate calcification. Photosynthesis increases ambient Ω, but this only slightly stimulates biological calcification. More importantly, photosynthesis increases the alkalinity : acidity ratio, which reduces how efficiently calcification generates CO_2. More calcification is therefore needed to obtain a particular photosynthetic benefit. Fleshy algae can thereby stimulate calcification in nearby corals. Coral calcification likewise counteracts CO_2 depletion and may stimulate photosynthesis in nearby algae. Nutrient levels meanwhile affect the competitiveness of calcareous and noncalcareous autotrophs differently. Interactions between calcifiers and noncalcifiers on reefs are thus complex.

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