Calcification Rates in Corals

The report "Calcification in hermatypic and ahermatypic corals" by A. T. Marshall (2 Feb., p. 637) sheds light on the poorly understood relationship between photosynthesis and calcification in reef corals, but it does not provide a strong test of whether calcification proceeds at higher rates in hermatypic (reef-building) species compared with that in ahermatypic species. This hypothesis, if true, would validate the pivotal role of coral-algal symbiosis in the development of coral reefs in tropical waters.

There are two reasons why I am not convinced. First, as Goreau and Goreau (*1*) and others have found, calcification rates can vary by as much as a factor of 10 between hermatypic species. Thus, hypotheses concerning calcification rates in hermatypic and ahermatypic corals are dealing with statistical phenomena and therefore require a sample size greater than one. Second, the use of the term (Marshall's included) "reef-building coral" is problematic. While all hermatypic corals are termed "reef-building," it is unlikely that all hermatypic corals make significant contributions to the construction of coral reefs. The steps between organismal-level calcification and the incorporation of this material in reef growth are complex (*2*) and poorly understood. However, a few species (that is, framework species) may make disproportionate contributions to reef accretion. Marshall says he chose the hermatype *Galaxea fascicularis* for his experiments because it has polyps of a size similar to those of the ahermatype *Tubastrea faulkneri*. It would also be useful to compare calcification rates in known framework species and in ahermatypes.

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REFERENCES

1. T. F. Goreau and N. I. Goreau, *Biol. Bull.* **117**, 239 (1959).
2. D. K. Hubbard, A. I. Miller, D. Scaturo, *J. Sediment. Petrol.* **60**, 335 (1990).

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Marshall's conclusions are in disagreement with a large existing literature. One study (*1*) found no notable differences in calcification rate between ahermatypes in the light and in the dark; that hermatypes calcify about 3.58 times faster in the light than in the dark; that hermatypic corals calcify about 5.27 times faster than ahermatypes (daily average); and that there is a positive correlation between daily calcium deposition and specific primary productivity. They can be spatially and temporally separated as the result of differing biochemical mechanisms. This study (*1*) used two different methods and three species each, as opposed to one method and one species each used in Marshall's study.

Marshall's conclusions apparently result in part from inappropriate normalization of his ^{45}Ca uptake data by the weight of skeleton. Coral calcification is limited to the basal ectoderm, so the layer of skeleton deposited during measurements is a fraction of a millimeter. Coral species differ greatly in skeletal micro-architecture, and corals with porous skeletons will have high apparent calcification rates by Marshall's calculation. *Tubastrea faullkneri* forms an exceptionally porous and friable skeleton. Only *Tubastrea micrantha* is capable of forming tall structures, and only in highly protected habitats where its faster growing competitors have been eliminated by *Acanthaster* predation (*2*). In contrast, *Galaxea fascicularis* forms extremely hard, dense, and massive basal skeletons, despite having exsert calices that superficially resemble those of *Tubastrea*. More appropriate measures by which to contrast experimental physiological uptake rates are those of tissue biomass, protein, or nitrogen content (*3*, *4*).

Marshall's proposal that symbiotic algae suppress calcification in the dark rather than stimulating it in the light appears to misinterpret the acid-base reactions that link photosynthesis and calcification through use of a common bicarbonate pool (*1*, *3*, *5*, *6*). The mechanism originally proposed predicts that removal of CO_2 during photosynthesis, mediated by carbonic anhydrase, increases alkalinity in the light. The high carbonic anhydrase levels predicted have been found (*1*, *5*, *8*). The increase of alkalinity stimulates supersaturation and catalyzes nucleation of calcium carbonate (*7*). Marshall suggests that carbonate ion removal by calcification releases CO_2 for photosynthesis, which would make carbonic anhydrase superfluous and drive cellular pH acidic. But cellular pH measurements by microelectrode show that internal pH is highly alkaline in the daytime and acidic at night (*9*), refuting the mechanism Marshall proposes.

Marshall's measurements are neither inconsistent with the existing data nor do they provide a basis for the radical reinterpretation of the role of symbiotic algae in coral reefs that he proposes.

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REFERENCES

1. T. F. Goreau, in *The Biology of Hydra*, H. M. Lenhoff and W. F. Loomis, Eds. (Univ. of Miami Press, Miami, FL, 1961), pp. 269–285.
2. G. M. Wellington and R. K. Trench, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2432 (1985).
3. T. F. Goreau, thesis, Yale University, 1956.
4. _____ and N. I. Goreau, *Biol. Bull.* **118**, 419 (1960).
5. T. F. Goreau, *Endeavour* **20**, 32 (1961).
6. T. J. Goreau, *Proc. R. Soc. London Ser. B* **196**, 291 (1977); *Proc. Int. Symp. Coral Reefs* **2**, 395 (1977).
7. R. L. Hayes and N. I. Goreau, *Biol. Bull.* **152**, 26 (1977).
8. V. M. Weis, G. J. Smith, L. Muscatine, *Mar. Biol.* **100**, 195 (1989).
9. M. Kuhl, Y. Cohen, T. Dalsgaard, B. B. Jorgensen, N. P. Revsbech, *Mar. Ecol. Progr. Ser.* **117**, 159 (1995).

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Response: Carlon is not convinced that my experiments on *Galaxea* and *Tubastrea* provide a strong test of the hypothesis that calcification proceeds at higher rates in hermatypic as compared with that in ahermatypic species. My results show that short-term calcification rates (strictly accretion rates) can be similar in a tropical zooxanthellate and a tropical azooxanthellate coral. This suggests that light-enhanced calcification may not be a real phenomenon and that zooxanthellae may repress calcification in the dark. As a corollary, I suggested that the development of coral reefs is not attributable to increased calcification rates that result from the direct activities of zooxanthellae. This does not necessarily mean that long-term accretion rates are not greater in hermatypic corals than in tropical ahermatypic corals.

There must certainly be a range of calcification rates for zooxanthellate corals and for azooxanthellate corals. My main point, however, is that they have now been shown to overlap. This raises the possibility that the relationship between the algae and the host is not one of direct calcification enhancement.

Because the calcification rate of *Galaxea* appears to be comparable to, or even greater

than, that of some framework corals (for example, *Montastrea annulares*), the conclusions made for *Galaxea* should be equally valid for at least those same corals. If this is so, the comparison with the azooxanthellate coral *Tubastrea* should also be valid.

While my results are from experiments on "one species each" (hermatype and ahermatype), rather than two of each (the third species were hydrozoans, not scleractinian corals), as in Goreau's paper of 1961 (*2*), they are statistically documented with respect to replication. I compared polyps with polyps of similar size, not polyps with colonies, or colonies with colonies of different polyp sizes and unspecified dimensions, as did Goreau (*2*).

With regard to calcification rates, *Acropora pulchra* was shown to calcify faster than *Tubastrea micrantha* (*9*). However, if values for *Tubastrea micrantha* are converted from protein to nitrogen units by assuming that the nitrogen content of protein is 16% (*3*), then a calcification rate of 104 μg of calcium per milligram of nitrogen per hour is obtained, which compares well with the values given for *Acropora cervicornis* by Goreau and Goreau (*4*) (61 to 74 μg of calcium per milligram of nitrogen per hour) and Goreau (*5*) (63 to 134 μg of calcium per milligram of nitrogen per hour). If the methods are sufficiently reproducible to permit comparisons, then the comparisons suggest that the azooxanthellate *T. micrantha* calcifies at rates similar to those of the fast growing zooxanthellate *A. cervicornis* [which itself calcifies at rates up to 11 times faster than other zooxanthellate corals (*4*)]. Contrary to being found in "highly protected habitats," *T. micrantha* "is designed well to withstand hydrodynamic attacks and to colonize a current exposed habitat" (*10*).

Goreau *et al.* state that "Marshall's conclusions result apparently in part from inappropriate normalization of his ^{45}Ca uptake data by the weight of skeleton." They imply that *Galaxea* has a more massive and dense skeleton than *Tubastrea*. Superficially, this appears to be so. Comparisons of similar sized corallites (in diameter of the open or mouth end of the corallite and lengths), however, reveal that the skeletal mass of *Tubastrea* is about 70 to 80% that of *Galaxea* and that the skeletal densities are similar (*Tubastrea*, SD = 2.32 ± 0.61, n = 5; *Galaxea*, SD = 2.14 ± 0.24, n = 4).

Goreau *et al.* suggest that more appropriate measurements for the normalization of ^{45}Ca incorporation data are "tissue biomass protein, or nitrogen content." In fact, we gave normalized data using wet tissue mass and showed that, in these terms, the rate of ^{45}Ca incorporation in *Tubastrea* is approximately half that in *Galaxea*. I pointed out, however, that the mass ratio of tissue to skeleton in *Tubastrea* is almost 2.5 times that of *Galaxea*. This example illustrates the difficulty of using tissue mass protein, or nitrogen content for normalization when between-species comparisons are made. The underlying assumption is that these parameters are a measure of the surface area of the skeletogenic epithelium, that is, the calicoblastic (basal) ectoderm. This cell layer is highly attenuated, being only 1 to 3 μm thick in most cases, while the other three cell layers (aboral endoderm, oral endoderm, and oral ectoderm) vary in thickness up to approximately 50 μm, and the types of cells vary considerably between layers, between regions of a polyp, and particularly between species (*6*, *7*). Clausen and Roth (*3*) have suggested that "particularly in perforate corals, tissue content (and organic nitrogen) may be better correlated with volume than with surface area."

Even if the true surface area of the calicoblastic ectoderm could be measured, the information is not useful unless the sites of ^{45}Ca deposition during the incubation are known. Deposition in *Galaxea* occurs, in light, on the wall of the corallite, but not on the costae, and on the centrad regions of the septa. In this case, normalizing by the total surface area of the calicoblastic ectoderm (which covers the surface of the septa as well as the other parts of the corallite) would give inaccurate results. Preliminary results for *Tubastrea* indicate that short term (3 to 5 hours) of ^{45}Ca deposition may also not be uniform. (*8*).

In the light of the foregoing, it seems that calcification in terms of accretion (mass of new calcium deposited per total mass of calcium) as defined and used by Goreau (*5*) would be the most appropriate method for interspecific comparisons. A slightly modified version of this (mass of new calcium deposited per total mass of skeleton) is the method I used in my report.

I have suggested that my data on the mechanisms of calcification are not inconsistent with the model proposed by McConnaughey (*11*), who suggests that calcification may be viewed as a by-product of a CO_2-generating process for algal photosynthesis. Far from being a "misinterpretation of the acid-base reactions," this seems to be a well-considered proposal. The conversion of extracellular bicarbonate to CO_2 in the model may well involve carbonic anhydrase. In terms of this model, it makes sense that zooxanthellae may repress calcification in the dark when CO_2 is not required. Goreau *et al.* say that "cellular pH measurements by microelectrode" refute "the mechanism Marshall proposes." Actually, it is McConnaughey's proposal (*11*), and Kuhl *et al.* (*12*) do not state that their measurements are intracellular.

I agree that my measurements are not inconsistent with the existing literature because the only sufficiently detailed data are those on *T. micrantha* (*9*), which appear to support my proposal that azooxanthellate corals may calcify at rates similar to those of some zooxanthellate corals.

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REFERENCES

1. D. J. Barnes and B. E. Chalker, in *Coral Reefs*, Z. Dubinsky, Ed. (Elsevier, Amsterdam, 1990), pp. 109–131.
2. T. F. Goreau, in *The Biology of Hydra and Some Other Coelenterates*, H. M. Lenhoff and W. F. Loomis, Eds. (Univ. of Miami Press, Miami, FL, 1961), pp. 269–285.
3. C. D. Clausen and A. A. Roth, *Mar. Biol.* **33**, 85 (1975).
4. T. F. Goreau and N. I. Goreau, *Biol. Bull.* **117**, 239 (1959).
5. T. F. Goreau, *Ann. N.Y. Acad. Sci.* **109**, 127 (1963).
6. A. T. Marshall and O. P. Wright, *Cell Tiss. Res.* **272**, 533 (1993).
7. ———, *Microbeam Anal.* **4**, 305 (1995).
8. A. T. Marshall and A. Wright, unpublished data.
9. G. M. Wellington and R. K. Trench, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2432 (1985).
10. H. Schumacher, *Mar. Ecol. Progr. Ser.* **20**, 93 (1984).
11. T. McConnaughey, in *Origin, Evolution and Modern Aspects of Biomineralization in Plants and Animals*, R. E. Crick, Ed. (Plenum, New York, 1989), pp. 57–73.
12. M. Kuhl, Y. Cohen, T. Dalsgaard, B. B. Jorgensen, N. P. Revsbech, *Mar. Ecol. Progr. Ser.* **117**, 159 (1995).

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Imaging Substrate-Mediated Interactions

Surface defects, such as steps and adsorbed atoms and molecules, perturb the electronic structure of the surrounding surface. These perturbations greatly affect adsorbate structures, dynamics, and chemistry. We have now observed these perturbations directly with the use of scanning tunneling microscopy and have shown that they determine the structure and dynamics for benzene on Cu{111} that we had previously found (*1*). These substrate-mediated interactions have important implications for the atomic-scale mechanisms of film growth and heterogeneous selective catalysis.

There are three ways in which the electronic structure of a surface can be perturbed. First, the electron distributions at the steps are smoothed by a charge transfer from the top to the bottom of the step edge, the so-called Smoluchowski effect (*2*). Sec-