

Misunderstandings in the Aquarium Literature

A Response to “Calcification Part 2: Are We Changing Natural Processes?” (Borneman and Small 2000)

By Craig Bingman

Several individuals have independently contacted me and asked me to write a response to an article that appeared in the June 2000 issue of Freshwater and Marine Aquarium (FAMA) magazine entitled “Calcification Part 2: Are We Changing Natural Processes?” by Eric Borneman and Allegra Small. Given the number of requests, I agreed to do this, but with some reluctance.

From my perspective, the perils are twofold. First, such a response can become impossible to follow unless the reader is also looking at a copy of the manuscript in question. The second peril is that the authors of the other article may be offended by this column, which I’m hopeful will not be the case. I will try to make this as constructive as possible, and make it readable for people who haven’t yet seen the FAMA article.

Let me begin with a brief summary of the article. Borneman provides us with some intriguing observations on his algal turf scrubber (ATS) system as a function of time and differences in husbandry practices. The authors then advance their hypothesis that coral calcification is somehow altered by the use of limewater, and some interesting experiments on coral photosynthesis and calcification conducted by Small and Adey (1998) are discussed. Let us consider each in turn.

First, Borneman writes about his experiences with his ATS system. The dominant part of the narrative is his observation that at the time he started to use limewater, he noticed a change in the amount of macroalgae growth in the system. In particular, macroalgae growth decreased after increased amounts of limewater were used. Increased amounts of limewater were used after the system was uncovered and fans were installed that blew air across the surface of the water.

I certainly don’t doubt that dramatic changes in the system happened, and it is likely that the use of limewater did influence the dominant photosynthetic organisms in his tank. In general, however, it is difficult to separate correlation from causation in simple time-series data from one aquarium.

Borneman and Small have been influenced by Ted McConnaughey’s model (1996) of how calcification can give photosynthetic organisms an adaptive advantage. The idea is that calcification running in parallel with photosynthesis provides protons that can be used to convert bicarbonate ions into carbon dioxide. The carbon dioxide is then used for photosynthesis. This idea is often informally called the “proton hypothesis.”

I regard the proton hypothesis to be one of the most significant advances in our understanding of how and why coral reefs form, so it is nice to see this idea covered by Borneman and Small in their article. And, although it is really impossible to be certain based on a sample size of one, I agree with Borneman that the use of limewater probably had a powerful influence on the types of photosynthetic organisms dominating his reef aquarium.

In fairness, however, there are other possible explanations for this. Perhaps the system ran short of a critical nutrient that was supporting the growth of macroalgae in the system. Perhaps there was an expansion of organisms that ate the macroalgae in the system or perhaps something else happened that I won’t be able to guess.

As for the notion that the pool of available nutrients changed, Borneman’s idea is that the amount of available carbon dioxide changed and that this was chiefly responsible for the differences in the photosynthetic community before and after limewater additions. I agree that this is a possibility. Unfortunately, no details about the carbon dioxide or pH of that system as a function of time were presented in the article. I would also point out that at high pH values, phosphate, iron and a number of other essential transition elements become less soluble. Perhaps changes in their availability also had an influence on the “phase change” Borneman observed in his system. Limewater would also be involved, but other nutrients besides carbon dioxide might also have been important.

An additional factor that was largely unexplored by the authors might also have been significant. The aquarium was not covered at the same time limewater use increased. I would imagine the dynamics of gas exchange between the aquarium and its surroundings might have been substantially affected. In particular, an uncovered aquarium will have a more difficult time holding carbon dioxide than a more closed system.

These observations prompted an extended period of communication between Borneman and Small as to what might be responsible for the phase change he observed in his system. The authors then go on to advance a number of ideas about how calcification in limewater systems might be different from calcification in systems with natural seawater. It is at this point where I must politely disagree with the authors.

There are a number of problems with the discussion of carbonate chemistry in this section of the article, and these misunderstandings propagate through the rest of their discussion. Rather than offer a line by line refutation, I'll try to group the misunderstandings, because some of them are surprisingly widespread in the popular aquarium literature and the "collective consciousness of aquarists."

First, the authors seem to believe that the hydroxide ions added by limewater persist for some considerable period of time in the water as hydroxide ions, and further that this will cause errors in total alkalinity tests. This is the most significant problem with the article, and it is the root of almost all of the non-trivial errors subsequent to it. It is also such a common misunderstanding about how limewater works that it is worth considering here in detail.

Limewater is a solution of lime (calcium hydroxide or calcium oxide) in water. When lime is dissolved in purified water, one gets a solution of mainly calcium ions and hydroxide ions. This lime solution is frequently dripped into aquaria slowly to replace both the calcium and the alkalinity depleted from the water by calcification in the aquarium. It is what I have called a "balanced calcium/alkalinity additive" in the past because it adds calcium and alkalinity in the 1:2 molar ratio depleted by the formation of calcium carbonate.

There isn't much magic to this. Lime is produced by "burning" calcium carbonate to drive off the carbon dioxide in the carbonate ions. One is left with calcium oxide. Hydration of calcium oxide with water gives calcium hydroxide. When that solution is dripped gradually into the aquarium, the hydroxide ions help the system to trap carbon dioxide. If you wish, you may think of the aquarium as recapturing the carbon dioxide driven off in the process of making the lime. One is then left with calcium carbonate after calcification by organisms in the aquarium. Altogether, this is a very neat process. If the demand for calcium carbonate isn't too high, dripping limewater is the least expensive and best way to meet the calcium carbonate demand for the system. That said, there are still a number of misunderstandings about the process.

First, people have this notion that the hydroxide ions from dripping limewater lurk around in the system for extended periods of time. They don't. The rate constants for the interconversion of dissolved carbon dioxide into carbonic acid, carbonic acid into bicarbonate and bicarbonate into carbonate are well known. The only significantly slow step in this process is the hydration of carbon dioxide to form carbonic acid or the back reaction of carbonic acid to form carbon dioxide and water.

To keep the discussion simple, these reactions take on the order of a second to happen at everyday temperatures and concentrations. Biological systems have found it useful to develop catalysts to accelerate these rates, and the enzymatic activity is called carbonic anhydrase. Pretty much all complex organisms have it, and it helps them to both get rid of carbon dioxide and take it up from the atmosphere.

The other two reactions, interconversion of carbonic acid and bicarbonate ions and the interconversion of bicarbonate and carbonate ions, are extremely rapid in comparison. Both involve the simple loss or uptake of a single proton. Proton transfer reactions for simple ions like these are almost always very quick in water and these two are no exceptions. They reach equilibrium in the blink of an eye. By contrast, the hydration of carbon dioxide to form carbonic acid involves the formation of a chemical bond

When you drip limewater into your aquarium, the primary reaction is that the hydroxide ions remove a proton from a bicarbonate ion and form a carbonate ion and water. Equilibrium is re-established pretty much as rapidly as you can mix the solutions. This raises the pH of the system. The amount of dissolved carbon dioxide goes down some seconds later, after the carbonic acid/dissolved carbon dioxide populations relax to equilibrium values.

Let's imagine that the system was previously at equilibrium with atmosphere. When we drip limewater into the system, the pH goes up and the dissolved carbon dioxide concentration in the water goes down. At the air/water interfaces in the system, there is a constant exchange of carbon dioxide from the water to the atmosphere and from the atmosphere into the water. When the carbon dioxide concentration goes down in the water, fewer carbon dioxide molecules go from the tank to the atmosphere. The rate at which they are entering the water from the atmosphere is unchanged, so the system tends to accumulate carbon dioxide.

Unless you had the pleasure of years of college-level chemistry education, there is a good possibility that this description is

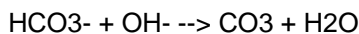
somewhat at odds with the mental image you had of the process. You may have thought you were suddenly asking the aquarium to take up carbon dioxide from the atmosphere when you dripped limewater. Maybe this is difficult for the system? In fact, what happens in there is just less dissolved carbon dioxide in the water, so less of it escapes from the water into the atmosphere.

I suspect the reason this is difficult for people to understand is that the realities of equilibrium reactions are somewhat at odds with our macroscopic experience (with our unaided eyes) of the processes. When a glass of water evaporates, there is the temptation to believe that the only thing that is happening is that water is leaving the surface and escaping into the atmosphere.

As an example of how human experience can lead us to think about chemical processes incorrectly, human experience tells us that water evaporates more slowly in humid air than in dry air. Take two glasses of water, at identical temperatures. Put them into two environmental chambers where the temperature, pressure, airflow and all other variables, aside from the humidity of the air, are held constant. You know that the (net) evaporation rate will be higher for the glass in the low humidity chamber, so you may form the mental image that water molecules are leaving the water faster under the low humidity conditions. Unfortunately, this is absolutely backwards.

If the two glasses of water are at the same temperature, water molecules will be exiting from the surface of the water to the atmosphere at exactly the same rates. What will be different is that water molecules will be entering the water from the atmosphere at a lower rate in the low humidity chamber. They are entering the water in fewer numbers simply because there are fewer water molecules dissolved in low humidity air than at higher humidities. So the image you might have of the process from your human experience is totally at odds with what is happening on a microscopic level at the surface of the water. And this is what trips most people up when they try to think about equilibrium reactions.

Another misunderstanding most aquarists have about limewater is their impression that adding hydroxide doesn't immediately increase the carbonate alkalinity of the water. Carbonate alkalinity is the weighted sum of the concentrations of carbonate and bicarbonate ions in the water. This misunderstanding seems to exist explicitly in the Borneman and Small manuscript as well. The belief is that somehow the system needs to trap carbon dioxide from the atmosphere before the carbonate alkalinity increases. This is incorrect. The carbonate alkalinity of the system increases as soon as the hydroxide ions deprotonate bicarbonate ions. It increases because the carbonate alkalinity isn't simply the sum of the molar concentrations of carbonate and bicarbonate ions in seawater. Rather, it is the weighted sum of these two concentrations, with carbonate ion having twice the weight as a bicarbonate ion. So in the reaction



the carbonate alkalinity is one on the left side of the equation and the hydroxide alkalinity is also one. On the right side, after the reaction, the carbonate alkalinity is two and the hydroxide alkalinity as written is 0. So, the carbonate alkalinity of the system goes up as soon as you add limewater.

This isn't the "problem." The problem, to the extent that it actually is a problem, is that limewater doesn't add any inorganic carbon to the system to replace the inorganic carbon removed by calcification. This comes either from the atmosphere or the system nets additional carbon dioxide because it leaves the system at a lower rate when limewater is added and the system pH is elevated.

Borneman and Small state that "the pH of natural seawater is 8.0-8.1 and the alkalinity is approximately 2.8 milliequivalents per liter." Unfortunately, we find a snare here for unwary authors, and it seems that even experienced aquarists sometimes fall victim to it. The snare is the fact that there are a number of pH scales in use by oceanographers. And, the really tricky part is that all of their common pH scales are different than the pH scale that pretty much everyone else uses.

Oceanographers presently use three different scales: the so called "total," "free" and "seawater" pH scales. (There is a detailed description of the various pH scales in the documentation for the CO2SYS program that I mentioned in a previous column, and there is also a very good discussion of the different scales in Millero 1996.) Most of the rest of us use the NIST (formerly NBS) pH scale.

The pH buffers used to calibrate your pH electrodes are NIST buffers. So all of you will be working on that pH scale. Electrodes calibrated in NIST buffers read approximately 0.2 pH units higher than electrodes calibrated on one of the pH scales commonly used by oceanographers. So, although there isn't a single "typical" pH value for ocean surface seawater, we do know that we need to add about 0.2 pH units to get from the pH scales that oceanographers use to our scale. So, the pH values of 8.0 and 8.1 reported by Borneman and Small translate to pH values of 8.2 to 8.3, the approximate

values that you would measure if you dropped your aquarium's pH electrode into water at the surface of the ocean. Thus, the typical ocean surface pH values and the pH values we observe in our reef aquaria aren't really all that different. Total alkalinity in seawater also isn't a conservative parameter, so it varies somewhat from place to place. Looking at the t-alk versus latitude slices presented in Millero 1996, one would say that it can vary from 2.25 to 2.45 milliequivalents per kilogram or 2.3 to 2.5 milliequivalents per liter (mEq/liter).

Borneman and Small also state that "the OH⁻ groups of kalkwasser (aka limewater) tend to obscure true carbonate alkalinity in test kits." No, they don't. Most of the components of the carbon dioxide system in seawater are in very rapid equilibrium. The only slow component will relax as quickly as you can get the sample to a pH meter or take a sample for a total alkalinity measurement. Limewater absolutely, definitively, does not interfere with measurements of total alkalinity nor does it present any obstacles to extracting the parameters of the carbon dioxide system from measurements of pH and total alkalinity.

From this somewhat shaky foundation, the authors propose that the mechanism of limewater calcification is somehow different than natural calcification in the ocean, or calcification when alkalinity is added to the aquarium as bicarbonate or carbonate ions. There is a diagram of the proposed differences that features calcium hydroxide being neutralized in the gastric cavity of a coral polyp. I suspect it was this aspect of the manuscript that prompted several people to contact me for an opinion, and more than one request to publish an opinion on the paper.

My first observation must be that the authors have not even discussed the mechanism of coral calcification, except in the most peripheral fashion. What is discussed are possible differences in the state of an aquarium based on different management practices. From the perspective of the source of inorganic carbon, limewater is somewhat different than a calcium carbonate reactor or the liquid two-part solutions, or sodium bicarbonate for that matter. In the latter methodologies, the supplement added has inorganic carbon. Limewater does not, and it relies on either net uptake from the atmosphere or less net loss of carbon dioxide to the atmosphere.

From the perspective of an individual coral polyp calcifying, it can't much matter whether the carbon is coming from a bottle of aquarium supplement or it is trapped indirectly, as long as the concentrations are the same in the two systems. Hydroxide ions from limewater are not persistent entities in the water. The deprotonation of bicarbonate happens in the blink of an eye.

The authors suggest that because limewater is converting bicarbonate to carbonate ions, the mechanism of calcification is somehow changed to one that is more dependent on carbon dioxide. There is some discussion of how paradoxical this is, and how one would expect that if the system were really depending on carbon dioxide, the organisms that use carbon dioxide more extensively would flourish, rather than (mainly) bicarbonate consumers like the common calcifying organisms in our systems.

References Borneman, E. and A. Small. 2000. Calcification. Part 2: Are we changing natural processes? *Freshwater and Marine Aquarium* 23(6):198-206.

Millero, F. J. 1996. *Chemical Oceanography*, Second Edition. CRC Press, Boca Raton, FL.

Lewis, E. and D. W. R. Wallace. 1998. Program developed for CO₂ system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.

Really the best way to work our way through the set of problems presented by this manuscript is to perform some quantitative calculations on the carbon dioxide system in seawater and see if these quantitative calculations match the qualitative statements in the manuscript. Accordingly, I've used CO₂SYN to calculate the amounts of the various species of carbon dioxide at different pH values.

First, let's quantitatively examine the notion that limewater is going to interfere with a total alkalinity test kit because of high concentrations of hydroxide ions. The concentration of protons and hydroxide ions in water is linked through the autoionization constant of water. It is convenient for me to use an oceanographic pH scale for the calculations and then to translate that back to our typical pH scales. It is uncommon for an aquarist to measure pH values (using an NIST calibrated electrode) in a reef system that are much higher than 8.5. As previously indicated, to go to a seawater pH scale, we subtract about 0.2 from that value. This is approximately pH 8.3. It is about 0.2 to 0.3 of a pH unit higher than one might expect to find at the surface of the ocean. Is there any truth to the assertion that hydroxide ions will somehow

throw off a total alkalinity measurement with a test kit? What is the concentration of hydroxide ions at this rather high pH value?

At this point, we must also take into account that oceanographers use a modified value of Kw to describe seawater. The sulfate and magnesium ions present at high concentrations in seawater affect the activities of the hydrogen ion and the hydroxide ion, respectively. According to Millero (1996) "this causes the free H+ and OH- concentrations to decrease by 84 and 33 percent, respectively" (compared to a freshwater system). The value of pKw* in seawater (the * indicates that it is corrected for this effect) is 13.19 at salinity (S) = 35 parts per thousand (ppt), 25 degrees Celsius. Many of you will remember a value of pKw of 14 from high school chemistry. At pH 8.3 on one of the oceanographic pH scales, the concentration of hydroxide ions is approximately $10^{(13.19-8.3)} = 104.89 = 0.013$ mEq/L, a much smaller amount than you can measure with any alkalinity test kit in the aquarium hobby. So, the contribution of hydroxide ion to our total alkalinity measurements is absolutely negligible. You don't need to worry about this effect ever again if you are testing water from a reef aquarium, contrary to any suggestion you might read in the popular literature.

The authors are similarly concerned that calcifying organisms will be running low on bicarbonate ions because limewater additions have converted much of the inorganic carbon in the system to carbonate ions. These values were calculated using CO2SYS with the NIST pH scale, total alkalinity of 2.2 mEq/L, S = 35 and 25

Table I

pH _{NIST}	[bicarbonate] (μmoles/kg)	[carbonate] (μmoles/kg)	pCO ₂ (μatm*)	[CO ₂] (μmoles/kg)	8.7	1058.4	460.0	73.5	2.1	8.6	1187.0
409.8	103.7	3.0	8.5	1312.9	360.0	144.5	4.1	8.4			
1433.1	312.2	198.5	5.7	8.3	1544.9	267.3	269.4	7.7			
8.2	1646.5	226.3	361.5	10.3	8.1	1736.9	189.6	480.1			
13.7	8.0	1816.0	157.5	631.8	18.0						

*atm = atmospheric partial pressure degrees Celsius, and setting the relevant nutrient concentrations equal to 0 micromoles per kilogram. I've chosen to express most of these values in micromoles per kilogram. To convert to millimoles per kilogram, simply divide them by 1000.

The atmospheric partial pressure of carbon dioxide is around 360 microatmospheres (although that number may be a bit low because carbon dioxide generated by human activities increases it constantly). You can see that the carbonate system for this case is at equilibrium with the atmosphere at about pH 8.2 because the partial pressure of carbon dioxide in the water matches the partial pressure of carbon dioxide in the atmosphere at around this pH value.

These values also provide additional confirmation that the ocean surface pH values reported by Borneman and Small were not corrected to the NIST pH scale that the vast majority of aquarists use. Let's take pH_{NIST} = 8.2 to be our baseline value for comparisons. As I've noted above, a pH_{NIST} = 8.5 is quite high for a limewater-managed system, and you probably won't ever see real pH values higher than this in a working reef system. At pH_{NIST} = 8.5, the concentration of dissolved carbon dioxide has fallen to only 40 percent of its concentration at pH_{NIST} = 8.2 (the carbon dioxide concentration has decreased by 60 percent). In contrast, the concentration of bicarbonate ion has fallen to 80 percent of its baseline value. This, in a nutshell, shows you how organisms that can utilize bicarbonate (e.g., coralline algae and corals) achieve a competitive advantage over organisms that can only utilize carbon dioxide at high pH values.

It is also worth noting that the bicarbonate concentration at this very high pH value has only decreased 20 percent from our baseline value at pH_{NIST} = 8.2. Assertions of Borneman and Small to the contrary, your corals are not in any danger of running out of bicarbonate ions when you drip limewater...provided you get enough limewater into your system to maintain a reasonable target alkalinity value.

Borneman also noted that once he started adding limewater to his system, the daily alkalinity drop actually increased. This is not exactly surprising. Additions of limewater promote rapid calcification. Unfortunately, very little quantitative information on the system is presented (apparent calcification rates before and after opening up the canopy, typical minimum and maximum pH values for the system before and after the change and so on), so it really isn't possible to say much more about this time-series data.

The final portion of the paper describes some experiments conducted by Small and Adey (1998) on coral fragments. Results from these experiments were taken to support the somewhat vague notion that calcification by corals has a different mechanism in limewater-managed systems than natural ocean water. The experiments are interesting, but I believe they are pointing to something else entirely.

The experiments went something like this: A chamber was filled with water from a mesocosm (perhaps the one at the Smithsonian) and placed in the mesocosm. I presume it was at the surface of the water and could do gas exchange with the atmosphere, but this isn't explicitly noted in the somewhat abbreviated experimental description in the article. Either nothing (the control,) a fragment of *Montipora digitata* or a fragment of *Acropora* sp. was added to the chamber. The pH value within the experimental chamber was kept at the pH value of the aquarium through addition of sodium hydroxide solution.

There were multiple runs, only one of which for each case seems to be reported in the article. At the end of the experiment, the water in the experimental chambers was analyzed for calcium concentration and total alkalinity, and compared to the starting conditions. The authors chose to report their results as differences between these values and they are reproduced in the first two lines in the table below.

	Alkalinity			Control			
Montipora digitata (mEq/L)	+0.25	+0.02	-0.34	Calcium (mg/L)	-4.0	-47.0	-43.0
Acropora sp. Calcium (mmoles/L)	-0.1	-1.17	-1.07	Expected alkalinity drop	-0.2	-2.35	

The authors interpret these results in terms of their model for altered calcification when limewater is used. Rather than pointing to differences in the mechanism of calcification when limewater is used, I believe the differences between the two species of corals are more accurately interpreted in terms of a difference in the relative amounts of carbon sequestered in calcium carbonate and the amount of inorganic carbon converted to organic compounds by photosynthesis. If only calcification were occurring in the system, then a large amount of sodium hydroxide would need to be added to maintain pH. (Calcification at surface seawater conditions releases about 0.8 moles of carbon dioxide per mole of calcium carbonate formed.) If more photosynthesis is occurring, then the net carbon dioxide uptake from photosynthesis will tend to keep the pH value higher, and less sodium hydroxide will be required to maintain constant pH. So, based on the experimental results presented above, one could hypothesize that the photosynthesis/calcification ratio is higher for the *Acropora* fragments under these experimental conditions than the *Montipora digitata* fragments. This is rather interesting, but unfortunately, the authors of the article didn't advance this hypothesis.

It is always fun to discuss calcification in reef aquaria and I particularly enjoy discussing limewater chemistry. The part that wasn't fun about writing this column was the need to point out numerous errors in another article. I give Borneman and Small a lot of credit for discussing the proton hypothesis in the popular aquarium literature, and credit for some hard work and presenting results from intriguing experiments. I'm hopeful future discussions of calcification in this column can take place under happier circumstances.