Alkalinity and Hardness: Critical but Elusive Concepts in Aquaculture

Article *in* Journal of the World Aquaculture Society · February 2016

DOI: 10.1111/jwas.12241

CITATIONS
49

READS
5,264

3 authors, including:

Claude E. Boyd
Auburn University
514 PUBLICATIONS **15,842** CITATIONS
SEE PROFILE

Benjaporn Somridhivej
Auburn University
6 PUBLICATIONS **64** CITATIONS
SEE PROFILE

JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 47, No. 1
February, 2016
doi: 10.1111/jwas.12241

Alkalinity and Hardness: Critical but Elusive Concepts in Aquaculture

Claude E. Boyd[1]

School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, Alabama, 36849, USA

Craig S. Tucker

USDA-ARS Warmwater Aquaculture Research Unit, Stoneville, Mississippi, 38776, USA

Benjaporn Somridhivej

Department of Fisheries, Inland Aquatic Health Institute, Chatuchak, Bangkok 10900, Thailand

Abstract

Total alkalinity and total hardness are familiar variables in aquatic animal production. Aquaculturists – both scientists and practitioners alike – have some understanding of the two variables and of methods for adjusting their concentrations. The chemistry and the biological effects of alkalinity and hardness, however, are more complex than generally realized or depicted in the aquaculture literature. Moreover, the discussions of alkalinity and hardness – alkalinity in particular – found in water chemistry texts are presented in a rigorous manner and without explanation of how the two variables relate to aquaculture. This review provides a thorough but less rigorous discussion of alkalinity and hardness specifically oriented toward aquaculture. Alkalinity and hardness are defined, their sources identified, and analytical methods explained. This is followed by a discussion of the roles of the two variables in aquaculture, including their relationships with carbon dioxide, pH, atmospheric pollution, ammonia, and other inorganic nitrogen compounds, phytoplankton communities, trace metals, animal physiology, and clay turbidity. Liming and other practices to manage alkalinity and hardness are explained. Changes in alkalinity and hardness concentrations that occur over time in aquaculture systems are discussed. Emphasis is placed on interactions among alkalinity, hardness, water quality, and aquacultural production.

Total alkalinity and total hardness are common water quality variables important to water supply and use, productivity of aquatic ecosystems, and aquaculture production. Together with data on a water's total dissolved solids concentration (or salinity), information on alkalinity and hardness can be used to make important inferences about the usefulness of water for many purposes. The role of these two variables in most water uses is thoroughly elucidated and put to practical use in many applications. For example, civil engineers use information on the alkalinity and hardness of water supplies to predict whether water will be corrosive or encrustive (forming a scale that clogs pipes) in water-distribution systems. That information can also be used in simple models to determine treatments needed to condition the water for use – such as calculating amounts of chemicals needed for water softening. But many aquaculturists do not understand the concepts of total alkalinity and total hardness adequately to meaningfully interpret measured concentrations and respond accordingly. This may result because total alkalinity and total hardness are indices rather than actual concentrations of solutes, but there are other reasons. In freshwater, the two variables tend to be of common origin and are often of similar concentration. The system used to express alkalinity and hardness concentrations in the United States is somewhat unusual and is identical for both variables. This sometimes leads to confusion regarding what

[1] Correspondence to: boydce1@auburn.edu

is actually being measured. Total alkalinity and total hardness influence chemical and biological processes in different manners, and effects often are not easily discerned. Moreover, the chemistry associated with these two variables is complex, and discussions are often presented in an extremely rigorous format and without reference to aquaculture.

Aquaculturists should understand the concepts of total alkalinity and total hardness, the various systems of expressing concentration, effects of these variables on other aspects of water quality and culture species, and methods for maintaining concentrations within satisfactory ranges. Boyd et al. (2011) presented a discussion of pH, acidity, and total alkalinity in relation to aquaculture, but that report focused mainly on pH. The purpose of this report is to provide a thorough discussion of total alkalinity and total hardness as they influence aquaculture applications. We will use a level of chemistry that should be understandable to biologists with a basic, college-level understanding of chemistry.

Definitions

Total Alkalinity

Total alkalinity is the concentration of titratable bases in water. A base will react to neutralize a hydrogen ion (H^+), for example, in the reaction $H^+ + OH^- = H_2O$, OH^- (hydroxyl ion) is the base. Several common substances in water react with H^+ such as

Hydroxide ($OH^- + H^+ = H_2O$)
Carbonate ($CO_3^{2-} + H^+ = HCO_3^-$)
Bicarbonate ($HCO_3^- + H^+ = H_2O + CO_2$)
Ammonia ($NH_3 + H^+ = NH_4^+$)
Phosphate ($PO_4^{3-} + H^+ = HPO_4^{2-}$; $HPO_4^- + H^+ = H_2PO_4^-$)
Borate ($H_2BO_4^- + H^+ = H_3BO_4$)
Silicate ($H_3SiO_4^- + H^+ = H_4SiO_4$)
Organic acids ($RCOO^- + H^+ = RCOOH$)

The word "total" is added to alkalinity because the contribution of different ions to total alkalinity may sometimes be reported separately, for example, hydroxide alkalinity. However, in this report, we will drop the adjective "total" and simply refer to alkalinity unless otherwise specified. In most natural waters, nearly all of the alkalinity will derive from HCO_3^-, CO_3^{2-}, and OH^-. Therefore, alkalinity is described sometimes as

$$\text{Alkalinity} = \left[HCO_3^-\right] + 2\left[CO_3^{2-}\right] + [OH^-] - \left[H^+\right] \quad (1)$$

where the brackets indicate measured molar concentrations. Suppose that water contains 61 mg/L HCO_3^-, 2.81 mg/L CO_3^{2-}, and pH = 9. Because pH = − log[H^+], [H^+] = 10^{-9} M at pH 9, and [H^+][OH^-] = $K_w = 10^{-14}$ at 25 C, [OH^-] = 10^{-5}. The respective molar weights of HCO_3^- and CO_3^{2-} are 61 and 60 g/mole; thus, (HCO_3^-) = 0.001 M and (CO_3^{2-}) = 0.000047 M. By substitution into Eq. 1,

$$\begin{aligned}\text{Alkalinity} &= 0.001\,\text{M} + 2\,(0.000047\,\text{M}) \\ &\quad + 0.00001\,\text{M} - 0.000000001\,\text{M} \\ &= 0.0011\,\text{M}.\end{aligned}$$

Thus, 0.0011 moles of H^+ would be necessary to neutralize the alkalinity in 1 L of the water.

Moles and equivalents of H^+ necessary to neutralize alkalinity are equal, but it is inconvenient to express alkalinity as molarity or normality even though they clearly show chemical relationships. Those making practical use of alkalinity data are more familiar with concentration expressed on a weight/volume basis, and the molar of normal concentrations often would be small decimal fractions. In the United States, alkalinity traditionally has been expressed as milligrams per liter of calcium carbonate ($CaCO_3$). This practice no doubt originated because a major source of alkalinity is limestone that often is mostly $CaCO_3$ and because $CaCO_3$ precipitates from some waters during use. In some countries, and especially in European ones, alkalinity may be expressed in milliequivalents per liter of $CaCO_3$ or calcium oxide (CaO) (1 meq/L = 50 mg/L of $CaCO_3$ or 28 mg/L of CaO).

The equivalent weight of $CaCO_3$ is half of its formula weight of 100.08 or 50.04

because it consists of divalent ions. The alkalinity of the water sample referred to above is 0.0011 eq/L $H^+ \times 50.04$ g $CaCO_3$/eq $\times$ 10^3 mg/g = 55.04 mg/L as $CaCO_3$. Equation 1 is seldom used for estimating alkalinity. Instead, the alkalinity is determined by acidimetry as will be discussed later.

Total Hardness

Total hardness is the concentration of divalent cations in water also expressed as $CaCO_3$. It is important to note that both alkalinity and hardness are expressed in the same units (mg/L as $CaCO_3$) even though they refer to distinctly different properties of water. The most abundant divalent cations in natural waters are calcium (Ca^{2+}) and magnesium (Mg^{2+}). Some waters contain small amounts of strontium (Sr^{2+}), and anaerobic water or highly acidic water may contain measureable concentrations of ferrous iron (Fe^{2+}) and manganous manganese (Mn^{2+}). The hardness of water can be expressed as the contribution of individual ions, for example, calcium hardness, and the combined contribution of all ions is called total hardness. But, as with alkalinity, in this report, the word hardness will mean total hardness unless otherwise specified.

The expression water hardness apparently resulted from the observation that $CaCO_3$ precipitates when water with appreciable concentrations of HCO_3^- and Ca^{2+} is heated

$$Ca^{2+} + 2HCO_3^- \xrightarrow[\Delta]{} CaCO_3 \downarrow + CO_2 \uparrow + H_2O$$

This phenomenon is particularly troublesome in boilers and conduits conveying heated water, because the precipitate forms hard deposits – often called boiler scale – reducing efficiency of boilers and clogging pipes.

The concentration of a divalent cation multiplied by the ratio $CaCO_3$:atomic weight of the divalent cation is the contribution of that ion to hardness; for example, Ca^{2+} concentration multiplied by $CaCO_3$:Ca^{2+} (100.08:40.08) or 2.5 gives the calcium hardness. Factors for converting other divalent cation concentrations to a $CaCO_3$ basis can be used in the following equation to calculate hardness:

$$\begin{aligned}\text{Hardness } (\text{mg/L as } CaCO_3) &= (Ca^{2+} \times 2.5) + (Mg^{2+} \times 4.12) \\ &\quad + (Sr^{2+} \times 1.14) + (Fe^{2+} \times 1.79) \\ &\quad + (Mn^{2+} \times 1.82) \end{aligned} \quad (2)$$

where Ca^{2+}, Mg^{2+}, Sr^{2+}, Fe^{2+}, and Mn^{2+} = measured concentrations (mg/L). In most water samples, only Ca^{2+} and Mg^{2+} will contribute significantly to hardness. In a sample with 20 mg/L Ca^{2+} and 4 mg/L Mg^{2+}, hardness calculated by Eq. 2 would be 66.48 mg/L as $CaCO_3$. Hardness also can be measured directly by chemical analysis as discussed later.

Although reporting hardness in milligrams per liter as $CaCO_3$ is most common, there are other ways of expressing this variable. These include milliequivalents per liter of $CaCO_3$ or CaO, the German hardness degree or dH (1 dH = 17.85 mg/L as $CaCO_3$), the French hardness degree or °f (1°f = 10 mg/L as $CaCO_3$), and the US grain per gallon or gr/gal (1 gr/gal = 17.1 mg/L as $CaCO_3$) (http://dardel.info/1x/water-analysis.html).

Sources of Alkalinity and Hardness

Limestone is a major source of alkalinity and hardness. This substance varies in composition ranging from $CaCO_3$ (calcite) to $MgCO_3 \cdot CaCO_3$ (dolomite), but most limestone is a mixture of $CaCO_3$ and $MgCO_3$ in which $CaCO_3$ is most abundant (Bowles 1956). The dissolution of limestone in nature is highly dependent upon the dissolved CO_2 concentration, so the discussion of limestone solubility begins with the dissolution of gaseous CO_2 in water.

Reaction of CO_2 in Water

Carbon dioxide enters water from the atmosphere and from respiration of aquatic organisms. The solubility of CO_2 in water depends on atmospheric pressure, the concentration of CO_2 in the atmosphere, water temperature, and salinity. The current atmospheric CO_2 concentration is roughly 400 ppm

(http://www.esrl.noaa.gov/gmd/ccgg/trends/), and the equilibrium CO_2 concentration between air and pure water is 0.57 mg/L at sea level and 25 C. Boyd and Tucker (2014) explained how to calculate CO_2 solubility, and the solubility of CO_2 from moist air at an atmospheric pressure of 760 mm Hg and different temperatures and salinities is provided in Table 1.

The small amount of the CO_2 that dissolves reacts to yield carbonic acid (H_2CO_3)

$$CO_2 + H_2O = H_2CO_3 \quad K = 10^{-2.75} \qquad (3)$$

where K = the equilibrium constant. The amount of H_2CO_3 formed in relation to dissolved CO_2 is minute; the molar ratio is

$$\frac{(H_2CO_3)}{(CO_2)} = 10^{-2.75} = 0.00178.$$

This relationship shows that there is only one H_2CO_3 molecule for every 562 CO_2 molecules at equilibrium.

Carbonic acid dissociates in two steps

$$H_2CO_3 = H^+ + HCO_3^- \quad K = 10^{-3.6} \qquad (4)$$

$$HCO_3^- = H^+ + CO_3^{2-} \quad K = 10^{-10.33} \qquad (5)$$

The second dissociation (Eq. 5) may be ignored in acidic solution, and the amount of H_2CO_3 is very small and also can be ignored. Equations 3 and 4 can be combined by addition to obtain the apparent reaction of CO_2 with water

$$\begin{aligned} CO_2 + H_2O &= H_2CO_3 \\ + H_2CO_3 &= H^+ + HCO_3^- \\ \hline CO_2 + H_2O &= H^+ + HCO_3 \end{aligned} \qquad (6)$$

The K values for apparent reactions can be estimated from the Gibbs free energy of reaction (ΔG^o) or derived by algebraic manipulation of the mass action forms of the apparent reactions (Boyd 2015). Multiplying mass action expressions for Eqs. 3 and 4 together gives the mass action form of Eq. 6

$$\frac{(H_2CO_3)}{(CO_2)} \times \frac{(HCO_3^-)(H^+)}{(H_2CO_3)} = 10^{-2.75} \times 10^{-3.6}$$

$$\frac{(HCO_3^-)(H^+)}{(CO_2)} = 10^{-6.35}. \qquad (7)$$

The K derived above agrees well with the K of $10^{-6.366}$ determined experimentally by Larson and Buswell (1942), and the K of $10^{-6.356}$ that we calculated using ΔG^o of the apparent reaction. Values of K at different temperatures for Eq. 6 are given (Table 2). Equation 6 allows use of dissolved CO_2 concentration in equilibrium calculations, and it also reveals that CO_2 reacts with water to form HCO_3^- and H^+ in equal amounts. The alkalinity from HCO_3^- is offset by the acidity of H^+, and CO_2 alone is not a source of alkalinity. The fact that changes in dissolved CO_2 concentration do not affect alkalinity concentration in samples is an important concept in water quality, because dissolved CO_2 concentration may change during sampling and, as explained later, throughout the course of the day. Addition and removal of dissolved CO_2 affects the form of alkalinity, but it does not affect overall alkalinity concentration.

Solubility of $CaCO_3$

In general discussions of limestone solubility, $CaCO_3$ typically is used as a model to explain the reactions involved, even though, as noted above, natural limestones often contain variable amounts of $MgCO_3$. Limestones that are mixtures of $CaCO_3$ and $MgCO_3$ dissolve in the same manner as shown below for $CaCO_3$, but the equilibrium constants for the mixtures of $CaCO_3$ and $MgCO_3$ will differ from those for pure $CaCO_3$.

The solubility of $CaCO_3$ is depicted in general chemistry texts as

$$CaCO_3 = Ca^{2+} + CO_3^{2-} \qquad (8)$$

Various values for the solubility product constant (K_{sp}) of $CaCO_3$ have been determined experimentally; the value of $10^{-8.3}$ (Akin and Lagerwerff 1965) will be used here. The equilibrium

TABLE 1. *Solubility of carbon dioxide (mg/L) in water at different temperatures and salinities exposed to moist air containing 0.04% carbon dioxide at a total air pressure of 760 mm Hg. Source: Boyd and Tucker (2014).*

Temperature	Salinity ppt								
(C)	0	5	10	15	20	25	30	35	40
0	1.34	1.31	1.28	1.24	1.21	1.18	1.15	1.12	1.09
5	1.10	1.08	1.06	1.03	1.01	0.98	0.96	0.93	0.89
10	0.93	0.91	0.87	0.85	0.83	0.81	0.79	0.77	0.75
15	0.78	0.77	0.75	0.73	0.70	0.68	0.66	0.65	0.64
20	0.67	0.65	0.63	0.62	0.61	0.60	0.58	0.57	0.56
25	0.57	0.56	0.54	0.53	0.52	0.51	0.50	0.49	0.48
30	0.50	0.49	0.48	0.47	0.46	0.45	0.44	0.43	0.42
35	0.44	0.43	0.42	0.41	0.40	0.39	0.39	0.38	0.37
40	0.39	0.38	0.37	0.36	0.36	0.35	0.35	0.34	0.33

TABLE 2. *Apparent equilibrium constants (K) for reaction of carbon dioxide and water ($CO_2 + H_2O = H^+ + HCO_3^-$) at different temperatures. Source: Larson and Buswell (1942).*

Temperature (C)	K
5	$10^{-6.583}$
10	$10^{-6.476}$
15	$10^{-6.432}$
20	$10^{-6.393}$
25	$10^{-6.366}$
30	$10^{-6.345}$
35	$10^{-6.326}$
40	$10^{-6.314}$

concentrations of Ca^{2+} and CO_3^{2-} are calculated below using Eq. 8:

$$\left(Ca^{2+}\right)\left(CO_3^{2-}\right) = 10^{-8.3};$$
$$\left(Ca^{2+}\right) = \left(CO_3^{2-}\right) = X;\ (X)(X) = 10^{-8.3}$$
$$X = 10^{-4.15}\,\text{M}.$$

Because $X = (Ca^{2+}) = (CO_3^{2-}) = 10^{-4.15}$ M (7.08×10^{-2} mM), multiplying by 60 mg CO_3^{2-}/mmole gives 4.25 mg/L CO_3^{2-} and by 40.08 mg Ca/mmole gives 2.84 mg Ca^{2+}/L or 7.1 mg/L hardness as $CaCO_3$. In terms of alkalinity, CO_3^{2-} reacts with $2H^+$ ($CO_3^{2-} + H^+ = HCO_3^-$; $HCO_3^- + H^+ = CO_2 + H_2O$), and the ratio of alkalinity:CO_3^{2-} is 50 g $CaCO_3$/eq:30 g CO_3^{2-}/eq or 1.67. Alkalinity at equilibrium also would be 7.1 mg/L as $CaCO_3$.

The calculation above is not accurate, because CO_3^{2-} hydrolyzes

$$CO_3^{2-} + H_2O = OH^- + HCO_3^- \qquad (9)$$

Hydrolysis removes CO_3^{2-} from solution and allows more $CaCO_3$ to dissolve.

The exercise in the preceding paragraph shows that relatively little alkalinity and hardness are produced when pure calcite dissolves in water in the absence of dissolved carbon dioxide. Further discussion of the solubility of $CaCO_3$ in absence of dissolved CO_2 is meaningless, because natural systems are open to the atmosphere and typically contain dissolved CO_2, which greatly increases the solubility of limestones.

Reaction of CO_2 and $CaCO_3$

Carbonic acid in water reacts with $CaCO_3$ to cause dissolution, but it is common to present an apparent reaction in which CO_2 reacts with $CaCO_3$ as follows:

$$CaCO_3 + CO_2 + H_2O = Ca^{2+} + 2HCO_3^- \qquad (10)$$

Limnology and water quality tests often present Eq. 10, but we have not seen a K for the reaction. The K was derived by inverting the mass action form of Eq. 5 (in order to have H^+ and CO_3^{2-} in the denominator) and multiplying it with the mass action forms of Eqs. 6 and 8

$$\frac{\left(HCO_3^-\right)}{\left(H^+\right)\left(CO_3^{2-}\right)} \times \frac{\left(H^+\right)\left(HCO_3^-\right)}{\left(CO_2\right)} \times \left(Ca^{2+}\right)\left(CO_3^{2-}\right) = \frac{1}{10^{-10.33}} \times 10^{-6.35} \times 10^{-8.3}$$

which reduces to

$$\frac{\left(Ca^{2+}\right)\left(HCO_3^-\right)^2}{\left(CO_2\right)} = 10^{-4.32}$$

One mole Ca^{2+} and 2 moles HCO_3^- result when 1 mole $CaCO_3$ dissolves in a system open to the atmosphere (Eq. 10). The molar concentration of CO_2 in water exposed to the atmosphere is $10^{-4.89}$ M; substituting $X = (Ca^{2+})$, $2X = (HCO_3^-)$, and $CO_2 = 10^{-4.89}$ M into the mass action form gives

$$\frac{(X)\left(2X^2\right)}{10^{-4.89}} = 10^{-4.32};\ 4X^3 = 6.17 \times 10^{-10};$$

$$X = 5.37 \times 10^{-4}\,\text{M}.$$

Thus, $Ca^{2+} = 21.5$ mg/L (53.7 mg/L hardness) and $HCO_3^- = 65.5$ mg/L (53.7 mg/L alkalinity). These concentrations agree reasonably well with experimentally determined concentrations of 22.4 mg/L Ca^{2+} (56 mg/L hardness) and 67.1 mg/L HCO_3^- (55 mg/L alkalinity) at a CO_2 concentration of about 400 ppm (Frear and Johnston 1929). These solubility estimates are for calcite, but limestone varies in composition and solubility (Bowles 1956). For example, we placed 0.5 g samples of food grade $CaCO_3$ in 250 mL distilled water and gently agitated on a shaker until a constant total alkalinity of 62.4 ± 0.87 (SD) mg/L was achieved – considerably greater than measured by Frear and Johnson for pure calcite.

Half of the carbon (C) in HCO_3^- is from $CaCO_3$ and half is from dissolved CO_2, but the amount of $CaCO_3$ that dissolves in an open system is more than twice the amount that will dissolve in a closed system. This is because removal of dissolved CO_2 by reaction with $CaCO_3$ allows more atmospheric CO_2 to enter the water and react with $CaCO_3$ until equilibrium is reached.

At this point it is important to summarize the discussion above and emphasize the effect that even a small amount of dissolved carbon dioxide has on the solubility of $CaCO_3$. When dissolved CO_2 is absent, $CaCO_3$ dissolves to produce hardness and alkalinity concentrations less than 10 mg/L as $CaCO_3$. When water is equilibrated with the atmosphere, the dissolved CO_2 concentration is about 0.6 mg/L, and that small amount of CO_2 increases the solubility of $CaCO_3$ to produce alkalinity and hardness concentrations of more than 50 mg/L.

Increasing the dissolved CO_2 concentration above that possible from atmospheric CO_2 increases the solubility of $CaCO_3$ even further. Water infiltrating soil and other formations accumulates CO_2 from root respiration and microbial respiration, and in saturated underground formations, hydrostatic pressure increases with depth allowing water to hold more CO_2. Groundwater from formations containing limestone may have higher alkalinity than commonly found in surface waters. Carbon dioxide also may accumulate in surface waters because of decomposition of organic matter. Equation 10 can proceed in either direction, and removal of CO_2 from water can cause $CaCO_3$ to precipitate.

Solubility product constants are based on ionic activities rather than measured ionic concentrations (Adams 1971, 1974). At great dilution, electrical charges on ions are uninfluenced by charges on other ions and the activity of the ions equals their measured molar concentrations. As ion concentration (ionic strength) increases, electrostatic interactions among ions increase neutralizing a portion of the ionic charges causing ions to react less efficiently. The Debye-Hückel equation – which will not be presented – can be used to calculate single ion activity coefficients (Chang 2005). Activity coefficients are 1.0 only when ionic strength is very low – a condition often referred to as infinite dilution. The activity of an ion equals the measured molar concentration of the ion times its activity coefficient; thus, measured molar concentrations must increase in a solution of greater ionic strength in order to maintain K. This results in the solubility of $CaCO_3$ and other minerals increasing as the ionic strength of the aqueous phase increases.

The ocean has a much greater concentration of ions than found in freshwater. Equilibrium constants given for reactions among water, dissolved CO_2, $CaCO_3$, HCO_3^-, and CO_3^{2-} in freshwater cannot be used for these reactions in ocean

water. For example, at 15 C in freshwater, the K for Eq. 5 is $10^{-10.43}$ while that of Eq. 6 is $10^{-6.42}$ (Snoeyink and Jenkins 1980); respective values in seawater are $10^{-9.13}$ and $10^{-5.94}$ (Prieto and Millero 2001).

Reaction of $CaCO_3$ with a Strong Acid

The solubility of $CaCO_3$ as explained above is for the normal situation in which dissolved CO_2 – a weak acid – is the source of acidity. Some waters contain a strong acid such as H_2SO_4 from oxidation of sulfur dioxide in the atmosphere (Boyd and McNevin 2015) or oxidation of iron pyrite contained in certain soils or other formations (Dent 1986). Strong acids react directly with $CaCO_3$

$$CaCO_3 + H_2SO_4 = Ca^{2+} + SO_4^{2-} + CO_2 + H_2O$$

resulting in hardness but no alkalinity.

Other Sources of Alkalinity and Hardness

Many discussions of alkalinity and hardness leave the impression that limestone is the only natural source of this variable. Dissolved CO_2 also reacts with calcium silicate and feldspars such as olivine, orthoclase, and several others. The reactions are

Calcium silicate

$$CaSiO_3 + 2CO_2 + 3H_2O \rightarrow$$
$$Ca^{2+} + 2HCO_3^- + H_4SiO_3$$

Olivine

$$Mg_2SiO_4 + 4CO_2 + 4H_2O \rightarrow$$
$$2Mg^{2+} + 4HCO_3^- + H_4SiO_3$$

Orthoclase

$$KAlSi_3O_8 + 2CO_2 + 11H_2O \rightarrow$$
$$Al_2Si_2O_5(OH)_4 + 4H_4SiO_4 + 2K^+ = 2HCO_3^-.$$

Calcium silicate is a major source of alkalinity in natural waters (Ittekkot 2003), and feldspars also are important sources in areas with acidic soils that do not contain limestone or calcium silicate. Calcium silicate and some feldspars also are sources of hardness, but as can be seen in the example above, some feldspars such as orthoclase do not provide hardness when they dissolve.

Silicic acid dissolved in water hydrolyzes as follows:

$$H_4SiO_4 = H^+ + H_3SiO_4^-$$

The K for dissociation is about $10^{-9.5}$ (Seward 1974). Thus, in water of pH above 9 that contains appreciable silicic acid, there is a significant amount of $H_3SiO_4^-$ that will titrate as alkalinity. Snoeyink and Jenkins (1980) presented an example from a major water supply to the Bay Area in California where 20% of the alkalinity is contributed by silicate. The water had a total alkalinity of 20 mg/L as $CaCO_3$, pH was 9.65, and the silica concentration was 8 mg/L as SiO_2. The water is derived from the Sierra Nevada mountain range where rock formations are rich in silicate minerals.

Gypsum ($CaSO_4 \cdot 2H_2O$) is a common mineral formed when seawater or lake water evaporates. It is often found in layered beds with limestones and other sedimentary rocks or in surface deposits formed in dry lake beds in arid regions. Dissolution of gypsum is a source of calcium hardness but does not add to alkalinity.

Analysis of Alkalinity

The protocol for determining alkalinity by standard methodology is presented by Eaton et al. (2005). The traditional procedure for alkalinity is to measure how much H^+ is required to titrate a sample to the methyl orange endpoint (about pH 4.5). The pH at the titration endpoint corresponds approximately to the point where an amount of H^+ has been added to react with all the OH^-, CO_3^{2-}, and HCO_3^- in the sample to produce CO_2 and H_2O. The milliequivalents of H^+ used in the titration multiplied by 50.04 mg $CaCO_3$/meq is the alkalinity. For example, if titration of a 100-mL sample consumes 10.0 mL of 0.02 N acid, the alkalinity is 100.08 mg/L: [(10.0 mL acid)(0.02 meq H^+/mL acid)(50.04 mg $CaCO_3$/meq)(1000 mL/L)] ÷ 100 mL sample volume = 100.08 mg/L as $CaCO_3$.

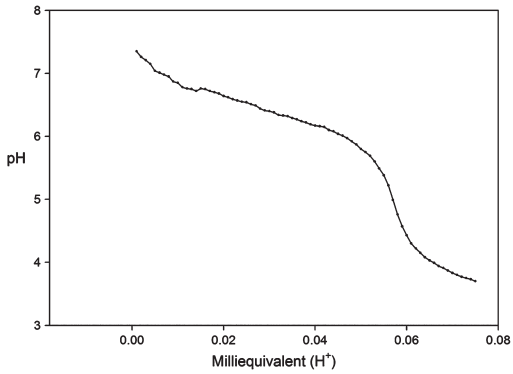

FIGURE 1. *Titration curve for a 100-mL sample of spring water that contained about 30 mg/L total alkalinity.*

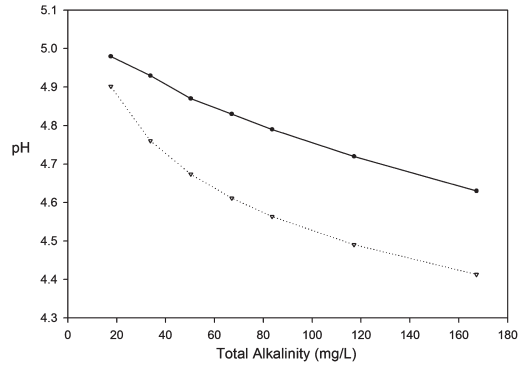

FIGURE 2. *The pH of inflection points for titration of water samples of different alkalinity concentrations based on steepest portions of inflections in titration curves (solid line) and pH of endpoints of the titrations if all carbon dioxide released during the titration remained in the sample.*

The Endpoint Dilemma

Titration of alkalinity is not as simple as it appears in the explanation above. The pH declines gradually as the titration progresses, and the inflection point in the titration curve at the endpoint is not sharp (Fig. 1) as illustrated for titration of a sample of about 30 mg/L alkalinity. A titration curve is not made in routine determinations; the titration is stopped at a predetermined pH marked by the color change of an indicator or the response of a pH electrode.

The theoretical endpoint for titration of HCO_3^- would seem to be at the same pH as the pH of a sample of freshwater in equilibrium with atmospheric CO_2. At equilibrium, the dissolved CO_2 concentration at 25 C is 0.57 mg/L ($10^{-4.89}$ M), the concentrations of HCO_3^- and H^+ will be equal, and H^+ can be substituted for HCO_3^- into Eq. 7 giving

$$\frac{(H^+)(H^+)}{(10^{-4.89})} = 10^{-6.35};\ (H^+)^2 = 10^{-11.24};$$

$$(H^+) = 10^{-5.62};\ \text{pH} = 5.62.$$

However, this endpoint pH is too great, because CO_2 is produced in the sample as HCO_3^- is neutralized

$$HCO_3^- + H^+ = CO_2 + H_2O$$

The dilemma is that the titration endpoint – and the sharpness of the endpoint – depends on the dissolved CO_2 concentration in the sample as the endpoint is approached. If dissolved CO_2 produced during the titration is rapidly removed by, say, vigorously bubbling CO_2-free gas through the sample during the titration, the endpoint pH will be sharp and near pH 5.6 as described above. But under usual laboratory conditions, dissolved CO_2 is produced faster during the titration than it is lost by diffusion from the sample to the atmosphere, and CO_2 accumulates in the sample and shifts the apparent endpoint pH down.

Cooper (1941) titrated sodium carbonate (Na_2CO_3)-sodium bicarbonate ($NaHCO_3$) solutions of different concentrations and estimated endpoints from the steepest portion of the inflections in the titration curves. We used those data to show the relationship between alkalinity concentration and pH at endpoints (Fig. 2); as alkalinity increased from 17.56 to 167.24 mg/L, amounts of CO_2 released during titrations increased and lowered pH of endpoints from 4.98 to 4.63. We also used Eq. 7 to calculate endpoints that would have resulted if all of the CO_2 had remained (Fig. 2). These pHs are considerably lower than pHs estimated from inflections in the titration curves. Although CO_2 is lost from samples during titration, the amount lost varies with alkalinity concentration, stirring method, and duration of the titration.

Cooper (1941) recommended using bromocresol green-methyl red indicator in total alkalinity titrations – this indicator is still commonly used today. The color of this indicator with respect to pH follows: ≥5.2, blue with trace of green; 5.0, light blue with lavender gray; 4.8, light pink gray with cast of blue; 4.6, light pink, <4.6, pink or rose. Discerning the colors is difficult, and analysts vary in their ability to perceive these hues. One of us (CST) is color-blind and has never been able to see either the methyl orange or bromocresol green-methyl red endpoints; for 40 yr he has used a pH meter to detect the apparent equivalency point of the titration. Likewise, some analysts titrate samples more quickly than do others and different stirring methods may be used during titration; thus, the amount of dissolved CO_2 remaining at the endpoint for a particular alkalinity concentration no doubt varies greatly. Moreover, some samples have alkalinity from ions other than HCO_3^- and CO_3^{2-} that do not release CO_2 when neutralized. It is not surprising that recommendation of endpoint pHs for different total alkalinity concentrations has been simplified over the years. Eaton et al. (2005) gave the following alkalinity-pH endpoint recommendation: 30 mg/L, pH 4.9; 150 mg/L, pH 4.6; 500 mg/L, pH 4.3. They also suggested pH 4.5 as the endpoint pH for routine analyses irrespective of sample alkalinity.

Although alkalinity is determined frequently in aquaculture water quality investigations, the details of endpoint detection seldom are reported. Titration curves for Na_2CO_3 – $NaHCO_2$ solutions with calculated alkalinities ranging from 17.56 to 167.24 mg/L (Cooper 1941) reveal that the differences in milliequivalents of acidity for titration to pH 5.0 as compared to pH 4.5 equated to about 2 mg/L alkalinity at the lowest pH and 10 mg/L alkalinity at the greatest pH. We prepared standard Na_2CO_3 solutions representing different alkalinity concentrations and titrated them to endpoints of 4.5 and 5.0 (Table 3). The accuracies of titrations to the two endpoints were estimated from the relative error (Boyd and Tucker 1992). Relative errors ranged from 1.72 to 4.24% (average = 3.73%) for titration to pH 5.0, and from 0.09 to 2.16% (average = 1.25%) for titration to pH 4.5. The relative difference between the two endpoints at each alkalinity concentration ranged from 1.83% at 300 mg/L to 3.88% at 100 mg/L with an average difference of 2.63%. Such differences in alkalinity concentration caused by endpoint selection likely would not affect management decisions in aquaculture, but they could possibly lead to erroneous interpretation of research data. For example, if a person was interested in the effects of increasing atmospheric CO_2 concentrations on the dissolution of minerals in a watershed, the relatively minor changes in stream and lake alkalinity that should result from that effect could be obscured by analytical errors associated with titration method and choice of endpoint. This issue deserves further study that could provide specific recommendations for alkalinity determination in aquaculture applications.

TABLE 3. *Effects of two endpoint pHs (4.5 and 5.0) on measured total alkalinity concentration.*

Alkalinity equivalent of standard sodium carbonate solution (mg/L as $CaCO_3$)	Measured alkalinity (mg/L as $CaCO_3$)	
	pH 4.5	pH 5.0
25	24.62	23.94
50	51.08	49.14
100	99.07	96.28
150	151.52	147.42
300	295.48	291.48

Forms of Alkalinity

A water sample with a pH above 8.3 contains both HCO_3^- and CO_3^{2-}, and some samples of very high pH may contain measurable alkalinity from OH^-. The alkalinity titration may be made in two steps. The first step takes the sample pH from the initial sample pH to 8.3. That endpoint is usually indicated by the color change of phenolphthalein indicator from pink at pH values above 8.3 and colorless below. This part of the titration is called the phenolphthalein alkalinity; the reactions are $OH^- + H^+ = H_2O$ if the pH is very high and $CO_3^{2-} + H^+ = HCO_3^-$. Bicarbonate initially in the sample is not titrated until the pH falls below 8.3. The second part of the

titration takes the sample from pH 8.3 to pH 4.5 (or other selected endpoint) for completing the neutralization of HCO_3^-. In the second step of the titration, both HCO_3^- formed in the first step and HCO_3^- initially present in the sample will be titrated. The phenolphthalein alkalinity is estimated from the titration volume in the first step, and the total alkalinity is estimated from the entire titration volume as usual. The two-step titration allows estimation of alkalinity resulting from HCO_3^-, CO_3^{2-}, and OH^- (Eaton et al. 2005). In aquaculture, it is seldom necessary to determine phenolphthalein alkalinity because the form of alkalinity changes throughout the day in response to diurnal changes in rates of dissolved CO_2 removal during photosynthesis and release in respiration. Interpretation of these changes in the form of alkalinity is usually meaningless for aquaculture purposes.

Analysis of Hardness

Divalent cations form insoluble salts with the fatty acids in soaps and historically the accepted analytical technique for measuring hardness was to add a standard soap solution to a sample until a persistent lather was obtained when the sample was vigorously shaken. Beginning in about 1950, a more precise method was developed using a standard solution of ethylenediaminetetraacetic acid (EDTA) as the titrating agent.

Hardness is determined by titrating a sample with 0.01 M EDTA to form complexes with divalent cations (Eaton et al. 2005). The endpoint of the titration usually is marked by the color change of the indicator eriochrome black-T. Eriochrome black-T is a dye that is red when complexed with divalent cations. During the titration, sufficient EDTA is added to chelate the metal ions, which removes them from the dye-cation complex. The uncomplexed indicator is blue, which signals the endpoint of the titration. Divalent cations and EDTA react in a 1:1 ratio, and the millimoles of EDTA used in the titration equal the millimoles of divalent ions in the sample. To illustrate, if a 100-mL sample requires 10.0 mL of 0.01 EDTA to complex the divalent cations, the total hardness is 100.08 mg/L: [(10.0 mL EDTA)(0.01 mmole EDTA/mL)(100.08 mg $CaCO_3$/mmole)(1000 mL/L)] ÷ 100 mL sample volume = 100.08 mg/L as $CaCO_3$.

The Ca hardness of a sample can be determined separately if sample pH is increased to 12 or 13 to precipitate Mg^{2+} as magnesium hydroxide. The sample is titrated with standard EDTA to complex Ca^{2+}, but a different indicator, murexide, is necessary because eriochrome black-T is not stable at such a high pH. Calcium hardness is calculated from the titration volume exactly as done for calculation of hardness. Most waters also have Mg hardness: Mg hardness = total hardness – Ca hardness.

Alkalinity and Hardness Kits

Alkalinity and hardness also can be measured with several brands of water-analysis kits. One of the earlier kits that had a method for doing titrations with a small buret was reasonably accurate (Boyd 1977), but another kit by the same manufacturer that relied on counting drops to measure titration volumes did not give accurate results – especially for samples of low alkalinity (Boyd 1976). Some modern kits have digital titrators that increase the accuracy of titrant volume measurements. These kits should provide results similar to those obtained by standard laboratory procedures, but this opinion should be verified.

Concentrations of Alkalinity and Hardness

Alkalinity and hardness of surface waters depend on watershed geology, climate, and weather. Concentrations range from less than 5 to more than 500 mg/L as $CaCO_3$ and often vary together because of the common origin of the carbonate bases and alkaline earth metals in limestones. There are many exceptions to the general rule and many waters exist with wide differences in alkalinity and hardness. Values are often higher in groundwaters because they are enriched with dissolved CO_2 which increases the water's dissolving power and supports higher concentrations of alkalinity and hardness in solution. Again, there are many exceptions to this second generality; waters in aquifers composed of sands or silicate rocks will

have low alkalinities and even lower hardness values. The alkalinity of estuarine waters is affected by mixing of river inflow and seawater. Seawater has an alkalinity of about 120 mg/L and a hardness of about 6300 mg/L as $CaCO_3$.

Freshwater ponds filled by surface water tend to have low alkalinities and hardnesses in humid areas where precipitation exceeds evaporation and soils are highly leached and acidic. In humid areas with more fertile soil – especially where soils contain carbonate – pond waters have greater alkalinities and hardnesses. Soils in the Piedmont Plateau area of Alabama are highly leached and acidic, and average alkalinities and hardnesses of farm ponds were 11.6 and 12.9 mg/L, respectively. But, in the Blackland Prairie region of Alabama where soils contain limestone, farm ponds averaged 51.1 mg/L alkalinity and 55.5 mg/L hardness (Arce and Boyd 1980). These concentrations are similar to alkalinity and hardness concentrations expected in a freshwater at equilibrium with atmospheric CO_2 and $CaCO_3$ as discussed earlier.

Catfish production ponds in the Blackland Prairie of Alabama had average alkalinities and hardnesses of 107 mg/L and 103 mg/L, respectively (Silapajarn et al. 2004). Higher alkalinities and hardnesses in the catfish production ponds than in farm ponds likely resulted from catfish ponds receiving large organic matter input in feed that led to greater availability of CO_2 and enhanced dissolution of limestone in bottom soil.

Stream water often has greater alkalinity and hardness than pond water (Boyd and Walley 1975). Streams from four physiographic provinces (Piedmont Plateau, Ridge, and Valley, Appalachian Plateau, and Interior Low Plateau) in Alabama had averages of 70.5 mg/L alkalinity and 70.6 mg/L hardness; corresponding averages for ponds were 25.4 and 24.0 mg/L, respectively. This phenomenon is a result of a large portion of stream flow – especially during dry weather – being groundwater inflow that often is more mineralized than surface runoff. Ponds filled from streams may – at least initially – have higher alkalinity and hardness than those filled from surface runoff.

Groundwater is even more variable in alkalinity and hardness than is surface water. For example, four wells located within a 50-km radius in the Blackland Prairie of Alabama had wide ranges in alkalinity and hardness as well as large differences between the concentrations of the two variables in the same sample (Table 4).

TABLE 4. *Total alkalinity and total hardness of waters from four wells located within a 50-km radius in west-central Alabama (Boyd and Brown 1990).*

Well	Total alkalinity (mg/L)	Total hardness (mg/L)
1	11.0	10.1
2	71.4	27.0
3	106.6	633.2
4	260.1	4.8

While water stands in ponds, cations in water can exchange with cations in bottom soils, acidity in bottom soil can neutralize alkalinity, limestone in bottom soil may dissolve, or $CaCO_3$ may precipitate from the water. Alkalinity and hardness concentrations in ponds may be quite different from those of the water source (Li et al. 2013).

Sample 4 in Table 4 represents a type of water found in some aquifers in coastal plain regions that have undergone natural softening (Renick 1925; Hem 1985). In these areas during earlier geological periods, aquifers contained seawater, but because of gradual uplifting of the land, seawater in aquifers was replaced by freshwater. However, the aquifer solids still contain much Na^+. If the land above such an aquifer contains limestone, water infiltrating into the aquifer will contain appreciable HCO_3^- and Ca^{2+}. Calcium in infiltrating water will exchange for Na^+ on aquifer solids, and the groundwater will have a high alkalinity but a low hardness. For example, the water supply for a fish hatchery at Meridian, Mississippi contained 136 mg/L alkalinity and 22 mg/L hardness, while a well supplying a pond in Wiggins, South Carolina had 313 mg/L alkalinity and 37 mg/L hardness (Boyd et al. 1978). Ponds supplied by the well had pH of 10–11 in the afternoon as a result of photosynthesis as will be explained later.

Saline aquifers also are common in many countries. Such an aquifer in west-central Alabama is a water source for culturing marine shrimp. Well water at one Alabama shrimp farm has alkalinity of around 275 mg/L and hardness

of about 325 mg/L, but the average alkalinity and hardness of five ponds about 1 mo after filling from the well were 120 mg/L and 168 mg/L, respectively (McNevin et al. 2004). Well water discharged into the ponds had a pH of 7.9 and was supersaturated with CO_2; upon equilibrium with atmospheric CO_2, $CaCO_3$ precipitated reducing hardness and alkalinity.

In arid regions, alkalinity and hardness usually exceed 100 mg/L and greater concentrations are common. To illustrate, aquaculture ponds filled by surface water in arid Faisalabad, Pakistan had an average of 355 mg/L alkalinity and 236 mg/L hardness (Ahmed et al. 2013). Calcium carbonate saturation may occur because of evaporation or increased pH as a result of photosynthesis in arid-region ponds, and $CaCO_3$ precipitation may lessen alkalinity. Hardness usually will continue to increase because there are abundant noncarbonate sources of divalent cations in arid regions. Hardness often greatly exceeds alkalinity in arid regions; hardness cations are balanced by chloride and sulfate rather than HCO_3^- and CO_3^{2-}.

Ocean water has a relatively constant composition of major ions – average concentrations are 142 mg/L HCO_3^-, 412 mg/L Ca^{2+}, and 1290 mg/L Mg^{2+} (Brown et al. 1989). These concentrations equate to 116 mg/L alkalinity and 6345 mg/L hardness. The concentrations of alkalinity and hardness vary with region and depth within the ocean, but the ocean has a moderate alkalinity concentration – less than many freshwaters – while hardness is extremely high.

In estuaries, the concentration of hardness tends to decline in rough proportion to salinity, because the ocean contains more hardness than inflowing rivers. Of course, alkalinity in estuaries is influenced mainly by the alkalinities of inflowing rivers which may have more or less alkalinity than found in ocean water.

Role of Alkalinity and Hardness in Aquaculture

Alkalinity, CO_2, and pH

The initial pH of most freshwaters (before they are impacted by interaction with bottom muds, biological activity, acidic precipitation, and pollution) is determined primarily by the ratio of base (HCO_3^-) to acid (dissolved CO_2 derived from diffusion from the atmosphere). That pH can be estimated using the equilibrium expression in Eq. 7. Solving Eq. 7 for a series of bicarbonate concentrations (alkalinities) gives the following relationship between alkalinity and water pH: alkalinity = 0 mg/L as $CaCO_3$, pH = 5.6; alkalinity = 1 mg/L, pH = 6.6; alkalinity = 5 mg/L, pH = 7.3; alkalinity = 10 mg/L, pH = 7.6; alkalinity = 50 mg/L, pH = 8.3. This shows that initial water pH increases as more base is added (i.e., as alkalinity increases). Also note that the pH of pure water in equilibrium with CO_2 in the air is not pH 7 as some may assume, but is acidic (~pH 5.6) because of the carbonic acid formed when CO_2 dissolves in water.

Several processes important in aquaculture will add acids or bases to the water (i.e., cause alkalinity to change) or cause CO_2 concentrations to change, and changes in either variable will cause pH to change. The first of these processes we will discuss is the effect of photosynthesis on water pH.

Dissolved CO_2 concentration in waters at equilibrium with atmospheric CO_2 is presented in Table 1. However, natural waters very seldom are at equilibrium with the atmosphere and this point deserves comment. Various biogeochemical processes remove or add CO_2 from water and concentrations range from 0 to more than 1000 mg/L. In most surface waters, concentrations range from 0 to 20 mg/L and are affected primarily by underwater biological activity. Respiration by aquatic animals, plants, and bacteria often adds CO_2 to water faster than it is removed, resulting in CO_2 supersaturation. Likewise, photosynthesis by underwater plants often removes CO_2 faster than it can be replenished, resulting in CO_2 undersaturation.

Equation 7 relates dissolved CO_2, H^+, and HCO_3^- concentrations through an equilibrium constant. The amount of dissolved CO_2 that must be held in the water to maintain the equilibrium constant increases with greater bicarbonate concentration (alkalinity) and varies with pH. For example, equilibrium conditions for Eq. 7 would be achieved at a pH around 5.62 for a dissolved

CO_2 concentration of 0.57 mg/L in freshwater containing no alkalinity and in equilibrium with atmospheric CO_2 at 25 C (Table 1). In a water with 10^{-3} M bicarbonate (50 mg/L alkalinity) the pH would be about 8.23 at the same dissolved CO_2 concentration and temperature. This should not be surprising because the pH of a solution in equilibrium with $CaCO_3$ and atmospheric CO_2 is about 8.3. But, many natural waters of 50 mg/L alkalinity may have pH values in the range of 7–8 because they are supersaturated with CO_2 despite being in equilibrium with respect to Eq. 7.

Daily and seasonal changes in biological activity cause dissolved CO_2 concentration to change and, through its relationship with the bicarbonate alkalinity system, those changes cause pH to change. As explained previously, CO_2 reacts with water to produce H_2CO_3 which lowers water pH. So, during periods when respiration exceeds photosynthesis (at night, e.g.), CO_2 accumulates and pH decreases. The extent of pH change as CO_2 is added depends on the water's alkalinity, as explained in the next section.

Removing dissolved CO_2 from water increases pH. Water at pH 7, 25 C, with 61 mg/L HCO_3^- (50 mg/L alkalinity) holds 9.85 mg/L CO_2, but if phytoplankton remove half of the CO_2, pH will rise to 7.3. Removing half of the remaining CO_2 will cause a pH of 7.6.

Water containing HCO_3^- or CO_3^{2-} contains traces of dissolved CO_2 because HCO_3^- is in equilibrium with CO_2 and CO_3^{2-} simultaneously

$$CO_2 + H_2O = HCO_3^- + H^+;$$
$$HCO_3^- = CO_3^{2-} + H^+.$$

When HCO_3^- concentration is maximum, both CO_2 and CO_3^{2-} will be at minimum concentration. By combining the two expressions above and their Ks we get

$$\frac{\left(H^+\right)\left(HCO_3^-\right)}{\left(CO_2\right)} \times \frac{\left(H^+\right)\left(CO_3^{2-}\right)}{\left(HCO_3^-\right)} = 10^{-6.35} \times 10^{-10.33}.$$

In the two mass action expressions, HCO_3^- cancels because it is at the same concentration in numerator and denominator, while CO_2 and CO_3^{2-} are at minimum concentration and can be ignored. As a result, we have

$$\left(H^+\right)^2 = 10^{-16.68}; \left(H^+\right) = 10^{-8.34}; \text{pH} = 8.34$$

This calculation reveals that when pH exceeds 8.3, CO_2 concentration will be negligible.

Many submersed aquatic plants (perhaps half of all species) (Spence and Maberly 1985) and most freshwater phytoplankton (Raven et al. 2012) can use either dissolved CO_2 or HCO_3^- as a carbon source for photosynthesis. Species that can use only dissolved CO_2 are generally restricted to waters of low alkalinity, because species that can use either dissolved CO_2 or HCO_3^- have a growth advantage in waters with abundant HCO_3^-. The ecological implications of carbon use in phytoplankton are discussed later. The water quality effects of HCO_3^- use by underwater plants can be explained by adding the reverse reaction of Eq. 6 in which HCO_3^- reacts with H^+ to release CO_2 and the dissociation of HCO_3^- to give CO_3^{2-} and H^+ (Eq. 5).

$$HCO_3^- + H^+ = CO_2 + H_2O$$
$$HCO_3^- = H^+ + CO_3^{2-}$$

to obtain

$$2HCO_3^- = CO_2 + CO_3^{2-} + H_2O \qquad (11)$$

Carbonate released when aquatic plants remove HCO_3^- hydrolyzes (Eq. 5) causing pH to rise. Two HCO_3^- are removed for each CO_3^{2-} formed (Eq. 11), and only a small portion of CO_3^{2-} hydrolyzes. Thus, HCO_3^- decreases while CO_3^{2-} and OH^- increase causing pH to rise as photosynthesis proceeds. The presence of Ca^{2+} limits pH rise by precipitating CO_3^{2-} as $CaCO_3$. Plants that tolerate especially high pH can drive the pH to 12 or more by removing HCO_3^- for photosynthesis (Ruttner 1963). The greatest pH values result in waters where alkalinity anions are balanced mostly by Na^+ and K^+ rather than Ca^{2+} and Mg^{2+} (Mandal and Boyd 1980).

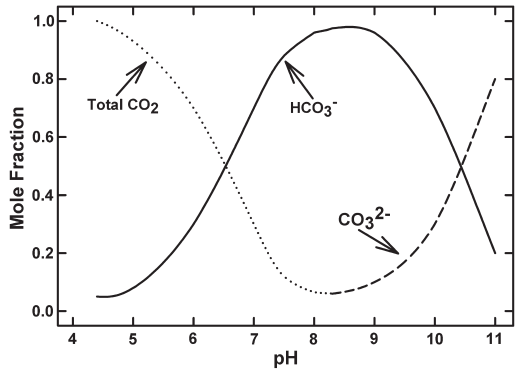

FIGURE 3. *Relationship between pH and mole fraction of the different sources of inorganic carbon in water.*

Precipitation of $CaCO_3$ when plants remove dissolved CO_2 causes a phenomenon called "whiting" or "whitening" in productive water bodies with moderate to high alkalinity and hardness (Thompson et al. 1997). The minute (<1 μm) $CaCO_3$ crystals remain suspended in the water giving it a milky appearance. The milkiness often disappears at night when CO_2 produced in respiration re-dissolves the $CaCO_3$ crystals or during winter when photosynthesis decreases with seasonal changes in light and temperature.

The relationship of pH to the forms of inorganic C (CO_2, HCO_3^-, and CO_3^{2-}) is illustrated in Figure 3. Above pH 4.5, water contains HCO_3^- and CO_2; CO_2 is nil for practical purposes at pH 8.3, and essentially all inorganic C is in HCO_3^-. As pH climbs above 8.3, water contains HCO_3^- and CO_3^{2-}, and at pH 10.33, HCO_3^- and CO_3^{2-} reach equal concentrations.

Apart from explaining and quantifying the effects of CO_2 addition and removal on pH, the relationship between CO_2, alkalinity, and pH also are commonly used to measure CO_2 concentrations in water (Eaton et al. 2005). Measurements of the water's pH, bicarbonate concentration (estimated as total alkalinity for most waters), temperature, and total dissolved solids concentration can be used in a computer program or a nomograph to calculate CO_2 concentrations. For practical use in most fresh waters, simplifying assumptions allow dissolved carbon dioxide to be estimated from pH, water temperature, and total alkalinity using values in Table 5.

TABLE 5. *Factors for estimating the concentration of carbon dioxide available for photosynthesis from water temperature, pH, and total alkalinity.*[a,b]

	Temperature (°C)							
pH	5	10	15	20	25	30	35	40
6.0	3.143	2.46	2.219	2.028	1.907	1.815	1.738	1.690
6.2	1.984	1.547	1.401	1.280	1.203	1.144	1.096	1.067
6.4	1.250	0.979	0.883	0.807	0.759	0.722	0.693	0.675
6.6	0.788	0.502	0.557	0.510	0.481	0.455	0.436	0.425
6.8	0.499	0.389	0.352	0.323	0.301	0.286	0.275	0.268
7.0	0.315	0.246	0.224	0.202	0.191	0.183	0.172	0.169
7.2	0.198	0.154	0.139	0.128	0.121	0.114	0.110	0.106
7.4	0.125	0.099	0.088	0.081	0.077	0.073	0.070	0.066
7.6	0.081	0.062	0.055	0.051	0.048	0.044	0.044	0.044
7.8	0.051	0.040	0.037	0.033	0.029	0.029	0.029	0.026
8.0	0.033	0.026	0.002	0.022	0.018	0.018	0.018	0.018
8.2	0.018	0.015	0.015	0.011	0.011	0.011	0.011	0.011

[a]Factors were calculated with the equation $CO_2 + H_2O = HCO_3^- + H^+$ using K values from Larson and Buswell (1942).

[b]Total alkalinity (mg/L as $CaCO_3$) × factor = CO_2 (mg/L).

Buffering

The amplitudes of daily excursions of pH in aquaculture ponds resulting for additions and removals of CO_2 by biological processes and the effect on pH of natural sources of acidity depend upon the alkalinity concentration (Fig. 4). The water of higher alkalinity has a lesser pH amplitude because it has a greater buffering capacity. A buffer consists of a mixture of a weak acid and its conjugate base (salt) or a weak base and its conjugate acid. For example, an acidic buffer can be made from acetic acid and its conjugate base sodium acetate, while an alkaline buffer can be made from ammonium hydroxide and its conjugate acid ammonium chloride.

The pH of a buffer is calculated with an equation derived from the equilibrium expression of the weak acid or base as illustrated below for a weak acid:

$$HA = H^+ + A^-$$

where HA = a weak acid and A^- = the conjugate base. The mass action expression for the

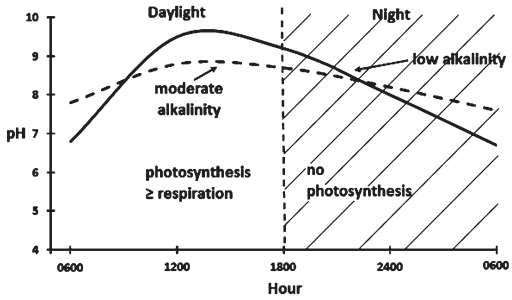

FIGURE 4. *Effect of time of day on pH in water of low and moderate total alkalinity.*

dissociation of the weak acid is

$$K = \frac{(H^+)\,(A^-)}{(HA)}.$$

The conjugate base is completely soluble. Taking the negative logarithm of both sides of the mass action expression gives

$$-\log K = -\log\left[\frac{(H^+)\,(A^-)}{(HA)}\right]$$

which may be rearranged as

$$-\log K = -\log(H^+) - \log\left[\frac{(A^-)}{(HA)}\right].$$

Of course, $-\log K = pK$ and $-\log (H^+) = pH$ giving

$$pK = pH - \log\left[\frac{(A^-)}{(HA)}\right]$$

$$\text{or} \quad pK + \log\left[\frac{(A^-)}{(HA)}\right] = pH$$

which may be rearranged to give

$$pH = pK + \log_{10}\left[\frac{(A^-)}{(HA)}\right]. \qquad (12)$$

Equation 12 is known as the Henderson-Hasselbalch equation.

Dissolved CO_2, HCO_3^-, and CO_3^{2-} buffer waters against sudden pH changes. Water with low alkalinity will exhibit greater pH fluctuation during a 24-h period as a result of fluctuations in dissolved CO_2 concentration caused by photosynthesis and respiration than will water of greater alkalinity. At pH below 8.3, if H^+ is added, it reacts with HCO_3^- to form dissolved CO_2 and water so that the pH changes only slightly. A small addition of OH^- will reduce the H^+ concentration, but dissolved CO_2 and water react to form more H^+, thereby minimizing change in pH.

The buffer system in natural water for pH below 8.3 may be expressed in the Henderson-Hasselbalch equation form as follows:

$$pH = 6.35 + \log_{10}\frac{(HCO_3^-)}{(CO_2)} \qquad (13)$$

where 6.35 is the pK for Eq. 6, and in terms of buffers, CO_2 is the acid and the HCO_3^- is the salt or conjugate base.

Above pH 8.34, added H^+ reacts with CO_3^{2-} to form HCO_3^-, while added OH^- reacts with HCO_3^- to form CO_3^{2-} and water. Putting Eq. 5 into the Henderson-Hasselbalch equation form gives

$$pH = 10.33 + \log_{10}\frac{(CO_3^{2-})}{(HCO_3^-)}$$

Alkalinity is an indicator of buffering capacity, and aquaculture pond waters of low alkalinity exhibit a large daily fluctuation in pH as a result of photosynthesis and respiration, while the fluctuation is less in waters of moderate to high alkalinity. Nevertheless, the pH measured in the morning when photosynthesis rate is low tends to be greater at higher alkalinity.

Alkalinity, Hardness, and Phytoplankton Communities

Phytoplankton is the predominant plant community found in most aquaculture ponds. Phytoplankton is the base of the food chain in aquatic animal cultures that rely on autochthonous food production rather than manufactured feeds. As such, phytoplankton growth rate controls animal production. Certain phytoplankton communities also are either more or less desirable

in aquaculture ponds (Boyd and Tucker 1998). In particular, some species of blue-green algae (cyanobacteria) cause problems that impact animal health or reduce profitability. Blue-green algae are subject to sudden, massive mortality that can negatively impact water quality, under some conditions certain species produce toxins, and some blue-green algae produce odorous compounds that impart off-flavor to culture animals (Paerl and Tucker 1995; Boyd and Tucker 2014).

Overall phytoplankton community productivity depends on availability of light, carbon, and other nutrients. Success of individual species (or groups of species) depends on the ability to gather those resources better than competing species (Reynolds 1984). Availability of inorganic carbon can be an important regulator of phytoplankton productivity and of the taxonomic makeup of phytoplankton communities. Calcium and magnesium are themselves essential plant nutrients, and calcium may also affect availability of phosphorus – a key plant nutrient.

Most aquaculture ponds are enriched with key plant nutrients – either intentionally from fertilization practices or as an unintentional byproduct of feeding practices – and contain abundant phytoplankton that require large amounts of inorganic carbon for growth. Gross carbon uptake by phytoplankton in highly productive waters may exceed 10 g C/m^2 per day, which is 20 times more than the amount of CO_2 in water 1-m deep at equilibrium with the atmosphere. Of course, CO_2 is replenished from the atmosphere as it is removed from water, but the rate of replenishment is slow. Atmospheric CO_2 flux into water during periods of light to moderate winds is less than 0.3 g C/m^2 per day (Pruder 1983; Reynolds 1997). Clearly, sources of inorganic carbon other than atmospheric CO_2 must be available to support rapid rates of photosynthesis in aquaculture ponds.

The alkalinity system is one potential source of inorganic carbon to supplement atmospheric supplies. Alkalinity (specifically HCO_3^-) increases inorganic carbon supply in two ways. First, as CO_2 is removed, HCO_3^- dehydrates to form more CO_2 and cause pH to increase (Eq. 11). Second, many phytoplankton can assimilate HCO_3^- directly, catalytically dehydrate it within the cell via the enzyme carbonic anhydrase, and concentrate the resulting CO_2 near the cell's photosynthetic apparatus (Raven et al. 2012).

Although bicarbonate offers a significant reserve of inorganic carbon for plant growth in waters of high total alkalinity, the supply in most waters remains insufficient to meet the needs of plants for more than a few days when rates of primary production are high. Schroeder (1987) estimated that carbon available from HCO_3^- in fish ponds having total alkalinities of about 250 mg/L (a rather high value) was sufficient to meet the demands of phytoplankton gross primary production for only 3 d.

Often, the most important source of inorganic carbon in productive water bodies is CO_2 produced as organic matter decomposes. The organic matter may be produced outside the water body and transported in (leaves from trees surrounding a pond is a simple example) or, more important in productive waters, the organic matter is produced by aquatic plants (usually phytoplankton) in the water body. That is, a portion of the inorganic carbon initially fixed into organic matter by plants is recycled back into the water when CO_2 is produced in cellular respiration or during decomposition when plants die. The CO_2 is then available for reassimilation by plants.

Phytoplankton species (and even strains within species) differ in their relative abilities to use inorganic carbon substrates, and this has led to speculation about the role of inorganic carbon supply (or alkalinity) as a factor that might give a growth advantage to one species (or a group of species) over another in different environments.

Freshwater phytoplankton in the classes Chrysophyceae and Synurophyceae, and some species in the Chlorophyceae, rely on diffusive entry of CO_2 and cannot directly use HCO_3^- (Raven et al. 2012). These algae are often found in low-pH, low-alkalinity waters where dissolved CO_2 is the sole source (or nearly so) of inorganic carbon. On the other hand, most other phytoplankton can assimilate either CO_2 or HCO_3^- for use in photosynthesis. Blue-green algae are known to have highly effective carbon-concentrating mechanisms for both CO_2 and HCO_3^- (Raven et al. 2012) and are therefore

particularly adept at obtaining inorganic carbon when supplies are low (Shapiro 1990). As explained above, dissolved CO_2 concentrations can become vanishingly low when rapid CO_2 removal during phytoplankton photosynthesis drives pH upward and HCO_3^- becomes the only inorganic carbon source. King (1970), Talling (1976), and many others have demonstrated that blue-green algae are more competitive than other algae for inorganic carbon where pH is high and dissolved CO_2 concentrations are low. Total inorganic carbon availability may be limited in low-alkalinity waters and the observation that channel catfish ponds with low-alkalinity water (10–15 mg/L) tended to have greater abundance of blue-green algae than channel catfish ponds with water of 70–150 mg/L alkalinity (Boyd et al. 1983) seems to agree that low carbon availability favors blue-green algae under some conditions.

It is likely, however, that alkalinity is relatively less important in shaping phytoplankton community composition in aquaculture ponds with very high nutrient loading because other ecological forcing factors come into play (Paerl and Tucker 1995). For example, when abundant phytoplankton communities develop in response to high-nutrient loading rates, the resulting turbidity restricts light penetration to shallow depths. Some blue-green algae possess traits that give them advantages over other groups under low-light conditions. These traits include possession of unique accessory pigments that gather light energy in wavelengths not used by other algae and the ability to change cell density and float toward the surface where light is greater. The ability of bloom-forming blue-green algae (notably species of *Microcystis*, *Anabaena*, and *Planktothrix*) to outcompete other algal groups for limited light is probably more important than carbon resources in explaining their frequent occurrence and dominance in nutrient-rich environments (Scheffer et al. 1997).

Both Ca^{2+} and Mg^{2+} are essential nutrients for all organisms (Pais and Jones 1997). Concentrations of these two ions necessary for optimum growth of phytoplankton – the dominant plants in aquaculture ponds – are low. According to Gerloff and Fishbeck (1969), optimum concentrations of Ca^{2+} and Mg^{2+} for six species of algae ranged from 0.01 to 0.95 mg/L and 0.08 to 1.56 mg/L, respectively. Interestingly, the optimum Mg^{2+} concentration usually is greater than the Ca^{2+}. Calcium has numerous functions in plants, but it is particularly important as a component of cell walls (White and Broadley 2003). Magnesium also has varied functions, but it is especially important because it is a part of the chlorophyll molecule (Bose et al. 2011). Phytoplankton are buoyed up by the water and do not require rigid cell walls like higher plants, and phytoplankton have large amounts of chlorophyll. This likely is the reason that phytoplankton have a greater need for Mg^{2+} relative to Ca^{2+} than higher plants. Most waters contain adequate Ca^{2+} and Mg^{2+} to meet the needs of phytoplankton, and their availability seldom limits primary productivity in nature (Reynolds 1984).

Atmospheric Pollution, pH, and Alkalinity

Alkalinity and hardness cannot be manipulated in most other types of aquaculture as it can be in ponds and intensive water reuse systems. Still, factors affecting the alkalinity and hardness of sources of water used in culture systems – although uncontrollable – are of interest, because they affect water quality in culture systems. Acidic rainfall obviously can lessen the alkalinity of water bodies over time (Haines 1981). There are instances in the eastern United States where low-pH rainfall has resulted in excessively low pH in stream water used in trout raceways (Boyd and Tucker 1998).

Recent research suggests that acid rain has accelerated on catchments weathering leading to greater alkalinity in rivers in many regions – particularly where there is limestone (Kaushal et al. 2013). This observation does not initially seem reasonable, because increased acidity would be expected to decrease alkalinity. However, the effect of acidity causes the limestone to break into smaller pieces to increase surface area and accelerate weathering. There is not enough known about the process to speculate upon its effect on water quality in aquaculture ponds.

Increasing CO_2 concentration in the atmosphere increases $CaCO_3$ solubility. At the estimated, pre-industrial revolution atmospheric CO_2 concentration of 280 ppm, the calculated solubility of CO_2 in pure water at 25 C and 760 mm Hg is 0.37 mg/L ($10^{-5.07}$ M). The equilibrium alkalinity at 280 ppm calculated from Eq. 10 is 46.7 mg/L as compared to a calculated value of 53.7 mg/L at the current atmospheric CO_2 concentration of about 400 ppm. The continued rise in atmospheric CO_2 predicted for the future may increase the solubility of limestone slightly, but small increases of alkalinity are not of significance in freshwater aquaculture – especially when liming is practiced.

The CO_2 concentration in the ocean is increasing because of greater atmospheric CO_2, and a decline in the pH of the ocean is occurring. According to Caldeira and Wickett (2003), the average pH of ocean surface water has diminished by 0.1 pH unit since the industrial revolution, and another drop of 0.3–0.4 units by 2100 has been predicted (Feely et al. 2004). Reduction in ocean pH causes $CaCO_3$ saturation to decline, and this increases the solubilities of aragonite and calcite that comprise shells of marine organisms. Thinner shells will affect survival and growth of these organisms (Orr et al. 2005). Obviously, ocean acidification is a potential threat to molluscan aquaculture.

Alkalinity, Hardness, and Aquaculture Production

Phytoplankton productivity and fish production in natural waters tend to increase at greater alkalinities (Moyle 1946; Ball 1948; Geagan and Allen 1960; Turner 1960; Hayes and Anthony 1964). The positive relationships between alkalinity and fish production were observed in natural waters that were relatively unpolluted and dissolved inorganic substances – including nutrients – were mainly of natural origin. Moyle (1946) pointed out that water with higher alkalinity has a greater concentration of ions (and plant nutrients) than does water of lower alkalinity. Thus, the greater productivity of waters of higher alkalinity is not entirely the effect of increasing carbon availability. This hypothesis is supported by the fact that in aquaculture ponds without feed input, primary production and fish production increase in response to fertilization across a wide range of alkalinity and hardness, but the response differs with the levels of alkalinity and hardness (Boyd and Tucker 1998). Phytoplankton in fertilized ponds with low-alkalinity and acidic bottom soil may be limited by a shortage of inorganic C (Boyd 1972), microbial decomposition of matter may be inhibited (Boyd and Pipoppinyo 1994), and wide daily fluctuations in pH may occur because of low buffering capacity. In addition, acidic bottom soils strongly adsorb phosphate (Boyd and Tucker 2014). Liming can increase alkalinity and hardness and improve fish production in acidic ponds (Hickling 1962; Arce and Boyd 1975). Thomaston and Zeller (1961) recommended that sportfish ponds in the southeastern United States with less than 20 mg/L hardness (and alkalinity) be limed, but Viriyatum and Boyd (2011) suggested that sportfish production in fertilized ponds likely increased up to alkalinities of about 40 mg/L.

Fertilized food fish ponds have greater fish biomass than sportfish ponds, and alkalinities and hardnesses above 40 mg/L would seem to be desirable. This opinion is supported by data from tilapia-carp culture in Bangladesh in which average alkalinities of 34, 51, 65, and 94 mg/L were established in replicate ponds ($n = 9$), and mean fish production of 4199, 4643, 5339, and 5175 kg/ha, respectively, was achieved (Boyd and Tucker 1998). These data suggest that around 60–70 mg/L is an optimal target alkalinity when liming food fish ponds. But, farmers often lime routinely without regard for alkalinity. In tilapia ponds in central Thailand – where soils are naturally acidic – are typically limed during each crop. Alkalinities and hardnesses in these ponds usually are above 100 mg/L and some pond bottoms contain free carbonates from undissolved $CaCO_3$ (Thunjai et al. 2004; Wudtisin and Boyd 2006).

Natural productivity is not as important in ponds with feeding as in fertilized ponds – except possibly soon after stocking fingerlings or postlarvae that cannot use pelleted

feed efficiently (Boyd and Tucker 2014). For example, different alkalinities were maintained in sunfish and channel catfish ponds through periodic applications of sulfuric acid (Murad and Boyd 1991). In fertilized sunfish ponds, pH and carbon dioxide concentrations were lower at dawn at alkalinities below 10 mg/L than in the 32.8 mg/L alkalinity treatment, but phosphorus concentrations were similar in all ponds (Table 6). Greater primary productivity in ponds of the greatest alkalinity apparently resulted from more available inorganic carbon for photosynthesis, greater primary productivity, and higher fish production. Alkalinities of 2.1, 5.8, and 46.2 mg/L were maintained in ponds with feeding, but channel catfish production averaged 3700, 3940, and 3960 kg/ha, respectively. Although natural productivity also was greater in the higher alkalinity treatment than in the two treatments with low alkalinity, catfish production was not influenced by low natural productivity because fish were fed a pelleted ration. The importance of hardness could not be assessed, because all ponds had similar hardness (30–45 mg/L) at initiation of the study, and addition of sulfuric acid did not alter hardness.

Hardness can be excessive in ponds; the combination of high pH and elevated Ca^{2+} concentration causes precipitation of phosphorus from fertilizer (Hepher 1958, 1966). In Israel where pond waters had high hardness (>300 mg/L), Hepher (1962) found it necessary to apply phosphate fertilizer at 11 kg/ha P_2O_5 every 2 wk to maintain the same level of phytoplankton productivity achieved by Swingle and Smith (1947) using monthly application of 9 kg/ha P_2O_5 in Alabama ponds with lower pH and <10 mg/L Ca^{2+} – over twice as much phosphate fertilizer was required in Israel.

Alkalinity, pH, and Inorganic Nitrogen

Nitrogen is a major plant nutrient and ponds often are fertilized with nitrogen-containing materials to increase primary production. In cultures provided with manufactured feed, nitrogen is a constituent of feed protein, and large amounts of nitrogenous waste are produced as a byproduct of feeding. The principal forms of inorganic nitrogen found in aquaculture systems are nitrogen gas (N_2), ammonia (either NH_4^+ or NH_3 depending on pH and temperature), nitrite (NO_2^-), and nitrate (NO_3^-). The ammonium-ammonia acid–base pair has a pKa of 9.3 and can contribute directly to alkalinity if the water's pH is high. However, total ammonia-nitrogen concentrations are low in aquaculture systems (otherwise animals would die – see the next section) so the contribution of ammonia to alkalinity is trivial. The other forms of inorganic nitrogen do not contribute directly to alkalinity; however, biological processes that transform one form of nitrogen to another may produce or consume protons (hydrogen ions), and thereby affect alkalinity (Ebeling et al. 2006; Wolf-Gladow et al. 2007).

Most metabolic ammonia produced by aquatic animals is excreted passively as NH_3 across the gills. Some fraction may also be excreted as NH_4^+, especially by marine fishes (Ip and Chew 2010). Un-ionized ammonia excreted from gills reacts with water to produce NH_4^+ and OH^-, which contributes to alkalinity:

$$NH_3 + H_2O = NH_4^+ + OH^- \qquad (14)$$

The maximum amount of alkalinity produced by ammonia excretion occurs when all ammonia is excreted as NH_3 and the water's pH is relatively low so that all NH_3 excreted ionizes after leaving the gills. Under those conditions, each equivalent of ammonia excreted will produce 1 equivalent of alkalinity.

Ammonia excreted by animals in aquaculture systems may be immediately flushed from the culture unit (in flow-through systems), lost into the atmosphere by diffusion (Weiler 1979; Gross et al. 1999), adsorbed on cation exchange sites in sediment (Armstrong et al. 2011) of ponds and lakes for cage culture, assimilated by underwater plants and algae (in ponds and cage culture), or oxidized to nitrate by bacteria in the nitrification process (in ponds, cage culture, and water-recirculating systems). Algae and other aquatic plants assimilate ammonia as NH_4^+. To maintain charge balance, NH_4^+ removed from water is – in effect – replaced by H^+, which destroys alkalinity (Wolf-Gladow et al. 2007).

TABLE 6. *Average (n = 3) values for limnological variables and net sunfish production in ponds with different concentrations of total alkalinity. Source: Murad and Boyd (1991).*

Total alkalinity (mg/L as $CaCO_3$)[a]	pH at dawn	Available carbon dioxide at dawn (mg/L)	Gross primary productivity (g C/m^2/d)	Phosphorus (mg/L)	Net sunfish production (kg/ha)
1.6a	5.7a	2.3a	0.53a	0.35a	174a
4.2b	6.3a	2.3a	0.72a	0.29a	267a
9.6c	6.7a	3.1a	0.97a	0.28a	297a
32.8d	7.9b	7.8b	1.42b	0.32a	412b

[a]Means indicated by the same letter did not differ significantly at the 5% probability level by Duncan's new multiple-range test (vertical comparisons only).

Approximately one equivalent of alkalinity is lost for each equivalent of NH_4^+ assimilated by plants. Likewise, when NO_3^- is assimilated by plants, the charge balance is maintained by the release of OH^- into the water (Uusitalo 1996). Each equivalent of nitrate assimilated increases the alkalinity by about one equivalent.

Large amounts of nitrogen-containing organic detritus are produced in aquaculture. Depending on the type of aquaculture system, organic detritus consists of dead plants and algae, feces, uneaten feed, and associated detrital bacterial communities. Decomposition of organic matter produces alkalinity in much the same way as alkalinity is produced when ammonia is excreted by fish. Proteins liberated during decomposition are broken down into amino acids. Enzymatic oxidation of amino acids liberates ammonia in the process called ammonification:

$$\text{R-CH}(\text{NH}_2)\text{-COOH} + \frac{1}{2}\text{O}_2 \rightarrow \text{R-CO-COOH} + \text{NH}_3$$

The NH_3 hydrolyzes according to Eq. 14, potentially producing one equivalent of alkalinity for each equivalent of NH_3 produced in ammonification (Conyers et al. 1995).

Nitrification is a bacteria-mediated process whereby NH_4^+ is oxidized to NO_3^-:

$$\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$$

In biological nitrification two equivalents of H^+ (100 mg $CaCO_3$ equivalent) result from oxidation of one equivalent of NH_4^+ to NO_3^-. This is a weight ratio of 100 mg $CaCO_3$:14 mg N or 7.14 mg/L. Each milligram of NH_4^+-N oxidized can lead to neutralization of 7.14 mg/L alkalinity. Brewer and Goldman (1976) and Schindler et al. (1985) reported that nitrification could cause acidification in lakes.

Most nitrogen-containing fertilizers – including manures – are acid-forming because of nitrification. In a laboratory study, the reduction in alkalinity caused per milligram per liter of chemical fertilizer ranged from 0.87 mg/L for monoammonium phosphate to 1.67 mg/L for urea (Hunt and Boyd 1981). The potential acidities of some common pond fertilizers are provided (Table 7).

The influence of acid-forming fertilizers can be significant in ponds with low-alkalinity water. Rainwater collected during 1984 at Auburn, Alabama ranged in pH from 3.52 to 5.61 with an average of 4.51, but the potential acidity of nitrogen fertilizers applied to sportfish ponds annually was 15–30 times greater than the input of acidity in rainfall (Boyd 1985a).

Feed also is acid-forming, because of ammonia nitrogen resulting from fish metabolism and decomposition of feces and uneaten feed. The potential acidity from feeds is relatively great ranging from about 0.3 to 0.5 kg $CaCO_3$/kg feed (Boyd and Tucker 2014) and can be estimated from the equation

$$\text{LR}_{\text{feed}} = \left[\text{N}_\text{f} - \left(\text{FCE} \times \text{N}_\text{a}\right)\right] \times 7.14$$

where LR_{feed} is the lime requirement of feed (kg $CaCO_3$/kg feed); FCE is the feed conversion efficiency (kg animal growth ÷ kg feed); N_f and Na are the decimal fractions of nitrogen in feed and culture species, respectively; 7.14 is the ratio of $CaCO_3$:ammonia nitrogen oxidized in nitrification.

TABLE 7. *Potential acidities of some common pond fertilizers.*

Fertilizer	Potential acidity[a] (kg $CaCO_3$/kg)	Manure	Potential acidity[b] (kg $CaCO_3$/kg dry weight)
Urea	161	Dairy cattle	24
Ammonium sulfate	151	Beef cattle	33
Ammonium nitrate	118	Poultry	31
Diammonium phosphate	97	Fresh cut grass	19
Monoammonium phosphate	79		
Ammonium polyphosphate	72		

[a]Source: Boyd and Tucker (1998).
[b]Calculated from nitrogen content.

Relationships among alkalinity, dissolved CO_2, pH, and acid produced by nitrification are especially important in recirculating aquaculture systems, because small water volumes coupled with large feed additions and high levels of biological activity can lead to rapid and dangerous changes in pH. Carbon dioxide produced by fish and microorganisms can depress pH to dangerous levels (< pH 6) in waters of low alkalinity, but this can be avoided by maintaining adequate alkalinity levels (50–100 mg/L) and by stripping dissolved CO_2 from recycled water using vigorous aeration. Ammonia produced by the culture species is removed from recycled water using biofilters that rapidly nitrify ammonia to nontoxic nitrate. Alkalinity is constantly destroyed by acid produced in nitrification, and when the water's buffering capacity is exhausted, pH will fall rapidly to levels that will kill fish or shrimp. Alkalinity in recirculating systems must be continually restored from outside sources – either from alkalinity in the makeup water (which is rarely adequate to offset total losses) or by frequent addition of bases, such as sodium bicarbonate (Loyless and Malone 1997).

Assimilation of nitrate (NO_3^-) or nitrite (NO_2^-) by aquatic plants and denitrification of NO_3^- by bacteria cause alkalinity to increase. Denitrification is the reduction of NO_3^- to dinitrogen gas (N_2) or other nitrogen gases (Boyd and Tucker 2014). The process occurs when dissolved oxygen concentrations are very low and common heterotrophic bacteria use NO_3^- instead of oxygen as a terminal electron acceptor in respiration. Although the two processes are very different in function, they both involve the chemical reduction of nitrogen which consumes one equivalent of H^+ for every equivalent of NO_3^- lost. The net effect is the production of approximately one equivalent of OH^- for every equivalent of NO_3^- lost. Or, stated another way, 1 mg/L of NO_3-N assimilated or denitrified produces approximately 3.5 mg/L alkalinity expressed as $CaCO_3$ (Ebeling et al. 2006). When nitrification and denitrification are coupled, as they often are in aquatic and aquaculture systems, denitrification can potentially restore about half the alkalinity destroyed by acid produced in nitrification.

Several other biological processes affect alkalinity. One of these processes – bacterial sulfur oxidation in acid-sulfate soils – is discussed later, in the section on liming. Sulfur oxidation can have dramatic effects on alkalinity and pH. Other processes are relatively unimportant in aquaculture systems, although they can have large effects on the long-term, global alkalinity balance (Wolf-Gladow et al. 2007). Examples of other processes that can affect alkalinity include phosphate assimilation by plants and algae (increases alkalinity by 1 mole per mole of P assimilated), release of phosphate during decomposition and mineralization of organic matter (decreases alkalinity by 1 mole per mole of P released), sulfate assimilation by plants and algae (increases alkalinity by 2 moles per mole of S assimilated), and release of sulfate during decomposition and mineralization of organic matter (decreases alkalinity by 2 moles per mole of S released).

Alkalinity, pH, and Ammonia Toxicity

Ponds to which feed is applied typically develop dense phytoplankton blooms, and

where alkalinity is low, wide daily swings in pH occur (Boyd and Tucker 2014). This is undesirable because high pH stresses fish. Ponds to which feed is applied also may have elevated total ammonia nitrogen (TAN) concentration (Tucker et al. 1984; Zhou and Boyd 2014). At elevated pH, a high proportion of the ammonia nitrogen is in the toxic, ammonia (NH_3) form as opposed to the less toxic ammonium (NH_4^+) form

$$NH_3 + H^+ = NH_4^+$$

Ponds with higher alkalinity are better buffered against extreme pH swings, but aquaculture ponds often have high rates of photosynthesis. Zhou and Boyd (2014) reported that NH_3 concentrations during the afternoon in Alabama ictalurid catfish ponds that had alkalinities of 100–150 mg/L often were above levels that have been reported to lessen fish growth in laboratory studies. Nevertheless, the situation would be worse if ponds had been of low alkalinity.

The design and carrying capacity of flow-through culture systems (raceways) also is affected by interactions between alkalinity and ammonia (Colt and Orwicz 1991; Colt et al. 2009). The number of times that water can be reused in a series of raceways and the ultimate carrying capacity of a flow-through culture facility is initially constrained by dissolved oxygen availability. If that limitation is overcome by providing aeration or oxygenation, fish production is eventually limited either by accumulation of dissolved CO_2 or ammonia. If the source water has low total alkalinity (<10 mg/L), CO_2 produced by fish can quickly reduce pH to levels that limit carrying capacity unless the gas is efficiently removed in the aeration process. If the source water has high total alkalinity (>100 mg/L), the initial pH will be poised above 8.3 and the water will be well-buffered against drops in pH as dissolved CO_2 increases. The high initial pH will cause a large proportion of the ammonia produced by fish to exist in the more toxic un-ionized form, and the end result will be that ammonia accumulation limits carrying capacity.

Interactions with Dissolved Trace Metals

Alkalinity influences the concentrations of trace metal in water. One reason is that the solubility of trace metal ions increase as pH declines, and low-alkalinity water often has a lower pH. Another reason is that metal ions in solution form soluble ion pairs with HCO_3^-, CO_3^{2-}, and OH^- (Schindler 1967; Clever and Johnston 1980; Boyd 2015) as illustrated for Cu^{2+} below:

$$Cu^{2+} + HCO_3^- = CuHCO_3^+$$
$$Cu^{2+} + CO_3^{2-} = CuCO_3^0$$
$$Cu^{2+} + 2CO_3^{2-} = Cu(CO_3)_2^{2-}$$
$$Cu^{2+} + OH^- = CuOH^+$$
$$Cu^{2+} + 2OH = Cu(OH)_2^0$$

The amount of dissolved copper (or other trace metals) bound in ion pairs typically exceeds the concentration of the free metal ion, for example, Cu^{2+}. Of course, metal ions also form complexes with dissolved organic matter, and dissolved organic matter often is at elevated concentration in acidic waters as compared to more alkaline waters. Aquatic plants can obtain copper and other nutrient metals from ion pairs and organic complexes, because when they remove the ion from the water, equilibrium will be disrupted and the ion pair will dissociate (Fig. 5). Ion pairs and organic complexes also protect organisms from metal ion toxicity, because the metal ion usually is more toxic than other dissolved forms of the metal (Davies et al. 1976). Moreover, calcium ion also protects against metal ion toxicity because it interferes in the uptake of the metal ion across the gill (Barron and Albeke 2000). The toxicity of metals tends to decrease as alkalinity and hardness increase – a water chemistry influence of alkalinity and a biological influence of hardness. But, the situation is even more complex because several other water quality variables can modify metal toxicity. For example, the copper criteria for aquatic life published by EPA in 1984 (USEPA 1985) considered only the effect of hardness on copper toxicity. The Environmental Protection Agency revised their criteria for copper (USEPA 2007)

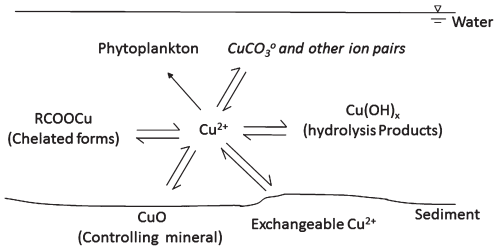

FIGURE 5. *Schematic showing the relationships among dissolved copper ion (Cu^{2+}) and other forms of copper in water and sediment.*

and currently use a model that requires input of ten water quality variables: temperature, pH, dissolved organic carbon, calcium, magnesium, sodium, potassium, sulfate, chloride, and alkalinity. This model is far too complex for everyday use in aquaculture and simpler approaches have been developed.

There are many data on the 96-h LC50s of metals to fish in waters of different hardness and alkalinities, but two examples will suffice. Inglis and Davis (1972) reported a 96-h LC50 of copper to bluegill of 0.25 mg/L at 52 mg/L hardness, 0.44 mg/L at 280 mg/L hardness, and 0.64 mg/L at 365 mg/L hardness. Straus and Tucker (1993) found 96-h LC50 values of copper to channel catfish of 0.051–0.065 mg/L at 16 mg/L hardness and 1.04–1.88 mg/L at 287 mg/L hardness. In both of these studies, the alkalinities and hardnesses were roughly equal as in most other studies. But Wurts and Perschbacher (1994) reported that the toxicity of equal total Cu concentrations decreased with greater alkalinity, and mortality at a constant alkalinity tended to decrease as Ca^{2+} concentration increased – these findings suggested that the water chemistry influence of alkalinity on toxicity exceeded the biological influence of Ca^{2+} on toxicity. Perschbacher and Wurts (1999) also conducted studies in which Mg^{2+} replaced Ca^{2+} as the source of hardness, and Mg hardness did not lessen the toxicity at equal total Cu concentrations. Laurén and McDonald (1986), however, reported that in rainbow trout, sodium loss was the main cause of mortality in fish exposed to elevated Cu concentration, but they found that increasing the alkalinity – but not the hardness – lessened sodium loss.

In catfish culture in the United States, copper sulfate is commonly used to control blue-green algae responsible for off-flavor, and this chemical also is used as a treatment for some fish parasites (Tucker 1996; Boyd and McNevin 2015). The application rate of copper sulfate must be adjusted for the alkalinity and hardness of water, because as explained above, copper is more toxic to fish in soft, acidic water than in harder, more alkaline water. The usual application rate of copper sulfate in channel catfish ponds is 0.01 times the alkalinity concentration (Boyd and Tucker 1998).

Hardness and Aquatic Animal Physiology

Aquaculture species have a fairly large amount of Ca in their bodies – fish in particular, because bone is made mainly of calcium phosphate. The amount of Mg is less than for Ca (Davis and Boyd 1978; Boyd and Teichert-Coddington 1995). It is difficult to assess the minimum concentration of Ca and Mg needed in water for aquaculture species, because they obtain these two nutrients from both the water and their diet (Wurts and Stickney 1989; Lovell 1998). In fact, Lovell (1998) contends that in aquaculture systems with feeding, the animals can get all of their Ca (and presumably Mg) from the water. However, Wurts and Stickney (1989) found that the red drum – a marine species that can be cultured in freshwater – needed at least 9 mg/L Ca^{2+} when cultured in freshwater. Moreover, Howells et al. (1983) concluded that natural fish populations required a Ca^{2+} concentration around 1 mg/L to survive.

Calcium is important in the development and hatching of fish eggs. Freshly fertilized eggs of brown trout need a minimum of 10 mg/L Ca^{2+} to survive and hatch (Brown and Lynam 1981). Tucker and Steeby (1993) suggested that 4 mg/L Ca^{2+} was the minimum acceptable concentration in water supplied for channel catfish hatcheries. However, Chatakondi and Torrans (2012) found that hatching success for channel catfish eggs was greater at a Ca^{2+} concentration of 30 mg/L than at 10 and 20 mg/L. Magnesium also is essential at all life stages of aquatic animals. But, studies of Mg^{2+} similar to those reported above for Ca^{2+} could not be found.

Fish hatchery water supplies with low pH and Ca^{2+} concentration have been remediated with various degrees of success by metering liming material into the inflow with various devices (Boyd 1990). At Auburn University, the hatchery for the catfish breeding program is supplied by a small stream that originally had average alkalinity and hardness of about 10 and 12 mg/L, respectively. A truckload (≅25 t) of agricultural limestone has been dumped into a small pond at the headwaters of the stream annually for over a decade, and the stream water now averages about 35 mg/L alkalinity and hardness (Soongsawang and Boyd 2012), but heavy rainfall dilutes Ca^{2+} concentration in the stream. At times, it is necessary to treat water entering the hatchery with a solution of calcium chloride. Sibrell et al. (2006) developed a limestone fluidized bed system for treatment of acid-impacted water at the Craig Brook National Fish Hatchery in East Orland, Maine in which CO_2 is metered into the fluidized bed to enhance solution of the limestone.

Hardness and Flocculation of Suspended Clay Particles

Water in aquaculture ponds often may become turbid with suspended soil particles – mainly colloidal clay particles that remain in suspension because their negative charges repel, preventing flocculation, and settling. Dissolved divalent ions tend to neutralize the charge on the particles allowing flocculation and settling thereby lessening turbidity (Boyd and Tucker 2014). Alkalinity has no direct effect on settling of particles, but hardness does and harder waters tend to be clearer than softer waters. To illustrate this, water samples with different concentrations and alkalinity:hardness ratios were mixed with pond sediment to achieve a turbidity of 85–90 NTU. The samples were allowed to stand in sedimentation cylinders for 12 h and the percentages of initial turbidity remaining after 12 h were measured (Table 8). At roughly equal concentrations of alkalinity and hardness, sedimentation increased with greater amounts of either variable. However, increasing alkalinity concentration had no effect upon the extent of sedimentation, but hardness did.

TABLE 8. *Effect of concentrations and ratios of alkalinity and hardness on settling of clay particles from water samples.*

Concentration (mg/L)		Turbidity remaining after 12 h
Alkalinity	Hardness	(% initial)
38	36	35
138	136	17
238	236	9
138	36	37
238	36	36
38	136	15
38	236	8

Source: Modified from Saengrungruang (2012).

Liming sometimes will result in the precipitation of suspended clay particles from water by increasing the concentration of divalent ions to coagulate colloidal clay (Boyd and Tucker 2014). However, a more effective way of removing turbidity is to apply gypsum (calcium sulfate). Gypsum application rates necessary to clear water of turbidity typically are 500–1500 mg/L. Gypsum also is sometimes applied to waters where the Ca^{2+} concentration is considered too low for the culture species.

Hardness and High pH in Ponds

Ponds with low hardness and high alkalinity often have high pH. As described above, this phenomenon is related to production of CO_3^{2-} when CO_2 is removed rapidly from water by plants during photosynthesis coupled with the high solubility of monovalent cation salts of CO_3^{2-}. One way to moderate the effect of photosynthesis on pH is to increase calcium concentrations by applying calcium sulfate. The usual treatment rate is to apply enough calcium sulfate to increase the hardness to the same concentration as the alkalinity. The ratio of pure gypsum to hardness is 1.72:1, but agricultural gypsum is not 100% $CaSO_4 \cdot 2H_2O$, and a ratio of 2:1 generally is used.

Removal of CO_2 from Culture Systems

Aquaculturists realize that pond waters tend to be supersaturated with dissolved oxygen during daytime and undersaturated with this gas at

night. However, it is not generally understood that pond water may be depleted of CO_2 in daytime and extremely supersaturated with CO_2 at night. For example, in a freshwater pond at 30 C the dissolved oxygen concentration might be 18 mg/L (239% saturation) in the afternoon but only 3 mg/L (38% saturation) at dawn. In the same pond, CO_2 concentration might be depleted (0% saturation) in the afternoon but at 12 mg/L (2400% saturation) at dawn. Carbon dioxide does not diffuse rapidly into the air even from water highly saturated with CO_2 (Moore and Boyd 1984). Thus, high morning concentrations of CO_2 tend to persist until phytoplankton remove CO_2 (and release O_2) through photosynthesis.

The high level of CO_2 at dawn interferes with oxygen uptake by fish and other aquatic animals. Thus, in the early days of feed-based aquaculture, aquaculturists often applied lime [$Ca(OH)_2$ or CaO)] to ponds in the early morning on days when dissolved oxygen concentration was especially low in order to remove CO_2 and allow more efficient use of the dissolved oxygen present.

If CaO is applied, it is converted to $Ca(OH)_2$ immediately [$CaO + H_2O = Ca(OH)_2$], and the reaction between CO_2 and $Ca(OH)_2$ occurs in two steps.

$$CO_2 + Ca(OH)_2 = CaCO_3 + H_2O$$

$$CaCO_3 + CO_2 + H_2O = Ca^{2+} + 2HCO_3^-$$

The second step in the reaction is slow, and the treatment rate is based on the first step – 1.7 mg/L of $Ca(OH)_2$ for each milligram per liter of CO_2. This treatment is seldom used today, because mechanical aeration is used to prevent low dissolved oxygen concentration in ponds.

Dissolved carbon dioxide is difficult to strip from water because the gas is very soluble in water and its partial pressure in the normal atmosphere is low. Dissolved carbon dioxide concentrations may be several thousand percent of the concentration for water in equilibrium with the atmosphere, so relatively large amounts of gas can be lost from water during aeration. When carbon dioxide-supersaturated water droplets pass through air or when bubbles pass through water, gas lost from the water accumulates in the gas phase and carbon dioxide partial pressure can quickly rise far above that in normal air, especially if the volume of the gas phase is low compared to the volume of the liquid phase. When this happens, the carbon dioxide partial pressure gradient between water and air disappears and no more gas can be stripped from the water. To be effective at dissolved CO_2 removal, aerators must operate so that large air volumes are in contact with small water volumes (engineers call this a "high gas–liquid ratio"). This requires breaking water up into a fine spray or vigorously bubbling large air volumes through the water. Most aerators used in pond aquaculture operate at intermediate gas–liquid ratios. They provide adequate dissolved oxygen to fish by adding modest amounts of oxygen to large volumes of water. Because gas–liquid ratios are not high, aerators typically used in pond aquaculture are not very effective at removing dissolved CO_2 (Eshchar et al. 2003). Carbon dioxide accumulation is a common problem in intensive flow-through and recirculating aquaculture systems because of high rates of respiration in those systems. The most common CO_2 degassing devices used in intensive aquaculture systems are packed-column aerators in which water is broken up into a thin film that trickles over a packing media having a large surface area. Water flows through the column by gravity and forced, counter-current air ventilation is used to rapidly remove CO_2 that is degassed from the water.

Liming

The importance of alkalinity and hardness in aquaculture should be evident from the preceding discussions. In fact, managing alkalinity, hardness, or both are common management practices in aquaculture. Some of these practices were described above. Some examples were adding sodium bicarbonate to recirculating aquaculture systems water to offset alkalinity losses to nitrification, adding calcium chloride to hatchery waters to improve fish egg hatchability, and adding gypsum to flocculate clay or reduce pH. However, liming ponds is the most common alkalinity and hardness management practice in

aquaculture. In fact, it is probably the most common of all water quality management practices.

Acidity in ponds is neutralized and alkalinity and hardness increased by liming – just as done in agriculture to remediate acidic soils. The most common liming material is agricultural limestone (finely crushed limestone), but lime also is used. Lime is made by burning limestone in a kiln to produce CaO – commonly called burnt lime

$$CaCO_3 \xrightarrow[\Delta]{} CaO + CO_2 \uparrow$$

Burnt lime can be hydrated (slaked) to give calcium hydroxide [$Ca(OH)_2$] known as hydrated or slaked lime. Calcium silicate and $NaHCO_3$ also are occasionally used for liming. The neutralizing capacity of liming materials is based on the amount of acidity that they will neutralize as compared to pure $CaCO_3$ to which a neutralizing value of 100% is assigned. Neutralizing values of other common liming compounds are: $NaHCO_3$, 59%; $CaSiO_3$, 86%; $CaCO_3 \cdot MgCO_3$, 109%; $Ca(OH)_2$, 135%; CaO, 179%. Because $CaCO_3 \cdot MgCO_3$ has a neutralizing value of 109%, it will require only 100/109 of 0.917 g of $CaCO_3 \cdot MgCO_3$ to neutralize as much acidity as 1.0 g $CaCO_3$, etc. Commercial liming materials are not pure compounds, and the neutralizing value will be slightly lower than for pure compounds and must be measured by chemical analysis (Boyd and Tucker 1998).

Agricultural limestone and other liming materials – except $NaHCO_3$ that is highly soluble – settle quickly to the pond bottom where they dissolve slowly and react with soil acidity. To clearly illustrate this fact, we measured the dissolution rate of agricultural limestone by placing 10 g in 4 L of distilled water, mixing gently with a small air stone supplied by an aquarium air pump, and measuring total alkalinity for 15 wk (Fig. 6). Alkalinity reached equilibrium of about 60 mg/L after 11 wk. Agricultural limestone comes from many sources, and there likely is variability in the solubility of material from different sources – an issue that deserves further consideration.

Soil acidity typically results from exchangeable aluminum ion (Al^{3+}) held by negative

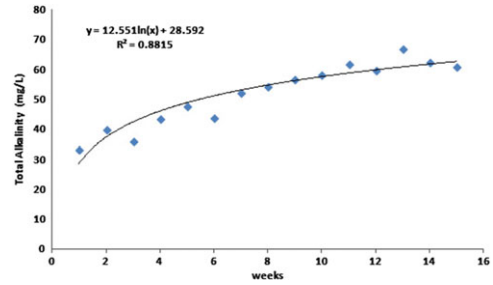

FIGURE 6. *Dissolution rate of a calcium carbonate sample in freshwater at 25 C.*

charges (cation exchange sites) on soil colloids – clay and fine particles of organic matter. Equilibrium exists between exchangeable cations in soil and Al^{3+} in the surrounding water, but aluminum ion hydrolyzes and precipitates as aluminum hydroxide [$Al(OH_3)$] producing acidity. Liming materials neutralize acidity in the aqueous phase causing the release of more exchangeable Al^{3+}. Calcium ion from reaction of liming materials with acidity replace Al^{3+} on cation exchange sites to increase soil pH. These reactions are summarized below:

$$\text{Soil-Al} = Al^{3+} + H_2O = Al(OH)_3\downarrow + \boxed{3H^+}$$

$$1.5H_2O + 1.5CO_2 + \boxed{1.5Ca^{2+}} = 1.5CaCO_3.$$

More liming material than necessary to neutralize soil acidity must be applied to have a residual that can dissolve and increase the alkalinity. In other words, subtracting alkalinity of water from the target alkalinity will not provide a reliable liming rate. For example, to raise alkalinity from 10 to 40 mg/L in a 1-ha pond of 1 m average depth represents an alkalinity increase of 30 mg/L or 300 kg $CaCO_3$. In ponds in Alabama, such an increase in alkalinity would require from 1000 to 4000 kg/ha of $CaCO_3$. Of course, there are sources of acidity in ponds, and liming typically must be repeated at 1- to 3-yr intervals.

Liming rates often are based on experience or "trial and error," but methods based on bottom soil analyses allow determination of the amount of liming material necessary in a

particular pond (Pillai and Boyd 1985; Han et al. 2014). Difficulty often is encountered in attempting to increase alkalinity and hardness above 50–60 mg/L by liming because of the low solubility of limestone. Organic matter sometimes is applied to ponds at the time of liming to increase the amount of dissolved CO_2 and enhance limestone solubility (Boyd and Tucker 2014), but the effectiveness of this technique has not been carefully studied. In ponds with feeding, there is a large dissolved CO_2 input from fish respiration and the decomposition of feces and uneaten feed; it seems logical that this also would favor the solubility of liming materials.

Acid sulfate soils – those containing iron pyrite (FeS_2) have exchangeable acidity from Al^{3+} cation exchange sites, but they also have acidity from oxidation of pyrite. Pyrite acidity can be great, often equal to 20–100 t/ha of $CaCO_3$ (Boyd and Tucker 1998). Liming materials will not react the same way in such soils as they do in non-acid-sulfate soils. Acidity from pyrite can be measured by hydrogen peroxide oxidation (Boyd and Tucker 1998), but in the soil, acidity from iron pyrite cannot be neutralized until pyrite oxidizes – a gradual process. Applying large amounts of agricultural limestone or lime will neutralize exchangeable acidity and active pyrite acidity. But the residual limestone particles will be coated with ferric hydroxide and lose efficiency for reacting with acid, while residual lime particles will be converted to $CaCO_3$ by reaction with dissolved CO_2. Boyd and Tucker (1998) discuss methods for managing acid-sulfate ponds to avoid low alkalinity and pH.

Declining alkalinity and pH are especially troublesome in indoor water-recirculating systems with nitrification biofilters (Timmons and Ebeling 2010) and in plastic-lined ponds without soil that often are used in highly intensive culture (Avnimelech 2012). Because of slow dissolution of traditional liming materials, sodium bicarbonate often is added to neutralize acidity.

Shrimp ponds are filled with estuarine or ocean water. Ocean water usually is near saturation with $CaCO_3$; but in areas with plankton blooms waters may be supersaturated with $CaCO_3$ and other areas may be undersaturated (Chave and Suess 1970; Chierici and Fransson 2009). However, once put into shrimp ponds, the pH of the water often increases because of phytoplankton photosynthesis leading to $CaCO_3$-saturation. Marine shrimp ponds typically are limed routinely without regard for total alkalinity concentration, but we suspect that liming materials added to shrimp ponds often do not dissolve, because the water already is saturated with $CaCO_3$. Studies to determine the relationships between alkalinity and the dissolution of liming materials in shrimp ponds would be beneficial.

Calcium hydroxide and oxide are more soluble than limestone, but they cause a marked increase in pH initially and cannot be used in large amounts where fish or other culture animals are present. Also, if applied to bottom soil between crops, the two compounds react with dissolved CO_2 forming $CaCO_3$.

Variation with Time

Alkalinity and hardness in aquaculture systems may consistently increase or decrease, fluctuate, or remain essentially constant over time. Alkalinity and hardness (especially calcium hardness) are called "non-conservative" water quality variables because their concentrations are affected not only by dilution and concentration (which affect "conservative" variables parameters, such as sodium), but also by myriad biological and chemical processes. It is important to note that observed or expected changes often depend on the system's state relative to the solubility product of $CaCO_3$. Waters with low alkalinity and calcium hardness (i.e., waters very undersaturated with respect to solid $CaCO_3$; $[Ca^{2+}][CO_3^{2-}] < K_{sp}$) may behave differently than waters near or at saturation with $CaCO_3$.

Interactions among alkalinity, hardness and the biotic and chemical environment vary from simple to extraordinarily complex depending on the type of aquaculture system. In flow-through systems, CO_2 produced in fish respiration decreases pH as water flows through the system, but does not affect alkalinity or hardness concentrations. Water exchange in most flow-through systems is too rapid for other chemical and biological processes to have significant effects.

Changes in alkalinity are more complex in recirculating aquaculture systems, mainly because acid produced in nitrification biofilters destroys alkalinity over time (but has no effect on hardness) and addition of makeup water may either increase or decrease alkalinity or hardness concentrations. Basic chemicals are periodically added to restore alkalinity lost to acid produced in nitrification; the effect of base addition on hardness depends on whether the base contains calcium [e.g., $Ca(OH)_2$] or not (e.g., $NaHCO_3$). Some recirculating aquaculture systems incorporate a denitrification process to remove nitrate and partially offset alkalinity lost during nitrification.

Changes in alkalinity and hardness in ponds are much more complex than in other aquaculture systems. Several processes that may change alkalinity or hardness concentrations operate concurrently and those processes are affected by weather, solar radiation, and other environmental conditions that vary hourly, daily, and seasonally.

During a period when evaporation exceeds rainfall, alkalinity and hardness concentrations will tend to increase – especially in ponds with low nitrogen inputs. As an illustration, evaporation typically exceeds precipitation during warm months at Auburn, Alabama (Boyd 1985b), and the concentration of alkalinity and hardness normally increases in ponds with low to moderate nitrogen inputs (Fig. 7). When rainfall is greater than evaporation, the opposite tendency will occur. Rainfall also can cause fluctuations in alkalinity over relatively short periods and especially in ponds with low alkalinity and hardness. Ponds supplied by watershed runoff may have especially large changes in alkalinity and hardness following heavy rainfall events causing large amounts of overflow. In coastal ponds filled from estuaries, alkalinity and hardness of source water may vary greatly during the year depending on the amount of freshwater inflow into the estuary.

Rainfall may also affect alkalinity and hardness through direct chemical reactions. Atmospheric CO_2 makes precipitation acidic, with dramatic effects on the dissolution of rocks and minerals at the earth's surface. Increasing atmospheric CO_2 levels will increase the solubility of carbonate solids, which may have long-term consequences for marine aquaculture. As explained previously, precipitation made acidic by atmospheric pollutants may either decrease or increase alkalinity depending on the geology of the catchment basin.

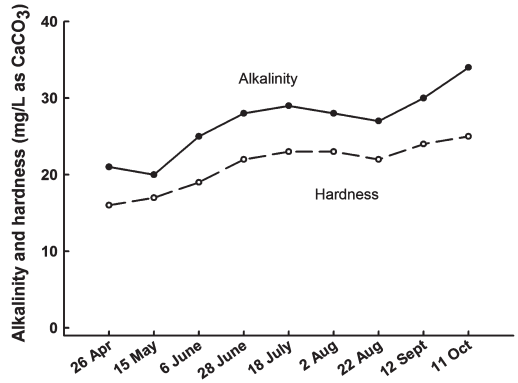

FIGURE 7. *Increase in alkalinity and hardness during the growing season in ponds with small feed inputs at Auburn, Alabama. Source: Boyd (1974).*

Where pond bottom soils contain limestone and water used to fill ponds is of low alkalinity and hardness, concentrations in ponds will increase until equilibrium is reached. On the other hand, if water used to fill ponds is groundwater with elevated concentrations of carbon dioxide, alkalinity, and hardness, carbon dioxide will diffuse into the atmosphere and calcium carbonate will precipitate. Alkalinity and hardness concentrations will decrease until equilibrium is attained. Alternatively, pond bottom soils may be acidic, and the alkalinity will decline as it reacts with bottom soil acidity (Li et al. 2013). This phenomenon is especially prominent in ponds constructed on acid-sulfate soils.

The effect of aquatic plant photosynthesis on CO_2 concentrations, pH, and alkalinity and hardness was discussed at length above. Net removal of CO_2 or HCO_3^- during photosynthesis causes the forms of alkalinity to change (carbonate alkalinity increases as bicarbonate alkalinity decreases) and pH to rise. At night or during cloudy weather, net CO_2 addition causes pH to decline and bicarbonate alkalinity increases as carbonate is protonated. In dilute fresh waters that are undersaturated with respect to $CaCO_3$,

the daily cycle of net carbon uptake and release by plants has no direct effect on either total alkalinity or hardness. But, in waters with high alkalinity and calcium hardness concentrations, the increased CO_3^{2-} concentration that results when CO_2 and HCO_3^- are removed in photosynthesis may cause precipitation of $CaCO_3$ and decreased alkalinity and hardness concentrations. Alkalinity and hardness may be restored at night or at some later date when CO_2 levels increase and $CaCO_3$ redissolves.

Photosynthesis by phytoplankton is a shallow-water phenomenon because light energy is rapidly attenuated in water. If a hard-water lake or pond is thermally stratified, $CaCO_3$ formed during periods of intense photosynthesis may sink from the surface water (epilimnion) and redissolve in deep water (hypolimnion) where CO_2 concentrations are elevated because of decomposition processes in deep sediments. Over time, alkalinity and hardness in surface waters decrease in a process called "epilimnetic decalcification" while concentrations in the hypolimnion increase. When waters of the stratified water body mix – as may happen during the autumnal turnover in deep, temperate-climate lakes and ponds – much of the alkalinity and hardness in the epilimnion is restored.

As discussed earlier, some processes increase alkalinity and hardness, while others function to decrease concentrations of these two variables. In natural water bodies – except for highly eutrophic or polluted ones (Abril and Frankignoulle 2001) – the opposing processes tend to prevent large changes in alkalinity and hardness. Alkalinity and hardness usually will be rather constant other than for season changes associated with the amount of evaporation relative to the quantity of rainfall that occur in most climates as discussed above.

Alkalinity and hardness concentrations in extensive aquaculture ponds with low nitrogen inputs tend to follow the same tendency as natural water bodies. More intensive aquaculture systems receive large inputs of ammonia nitrogen in fertilizer, from decomposition of feces and uneaten feed, and from metabolic excretions by the culture species and other aquatic organisms. Uptake of NH_4^+ and NO_3^- by phytoplankton and other aquatic plants may diminish or increase alkalinity, respectively, but the combined effects of nitrification and denitrification on alkalinity usually are greater. As discussed earlier, nitrification potentially removes two equivalents of alkalinity for each equivalent of ammonia nitrogen oxidized, while denitrification can replace only one equivalent of OH^- (alkalinity) for each equivalent of nitrate reduced to gaseous nitrogen. In a pond with a large input of ammonia nitrogen, the overall effect of the two processes is a loss of alkalinity even if all the nitrate from nitrification is denitrified, that is, nitrification and denitrification are perfectly linked (Hargreaves 1998). Of course, if a pond receives a large input of nitrate fertilizer – an uncommon practice – there would be much more denitrification than nitrification and the alkalinity would tend to rise.

The processes affecting alkalinity in an intensive aquaculture pond in which the main input of ammonia nitrogen is excretion by fish or ammonification during organic matter decomposition are summarized in Figure 8. This simplified model shows the delicate balance among processes that produce alkalinity and those that destroy alkalinity. Hypothetically, the net effect on alkalinity would be zero no matter which pathway is selected. For example, if one equivalent of NH_3 is excreted across fish gills, completely hydrolyzed, nitrified to NO_3^-, and denitrified, the net effect on alkalinity is $(+1)+(-2)+(+1)=0$ equivalent. The reaction of ammonia nitrogen to yield alkalinity depends upon whether NH_3 or NH_4^+ is added. Fish and organisms decomposing organic matter excrete NH_3 that results in an alkalinity increase because of hydrolysis ($NH_3 + H_2O = NH_4^+ + OH^-$). On the other hand, NH_4^+ does not hydrolyze to produce alkalinity. Ammonia added to water through excretion or ammonification results in less loss of alkalinity per unit of nitrogen than would addition of NH_4^+, for example, application of ammonium sulfate fertilizer (a scenario not depicted in Fig. 8). Of course, the pH and temperature of the water determines the ratio NH_3:NH_4^+ regardless of the form of ammonia nitrogen added, and this also will influence the amount of NH_3 available to hydrolyze.

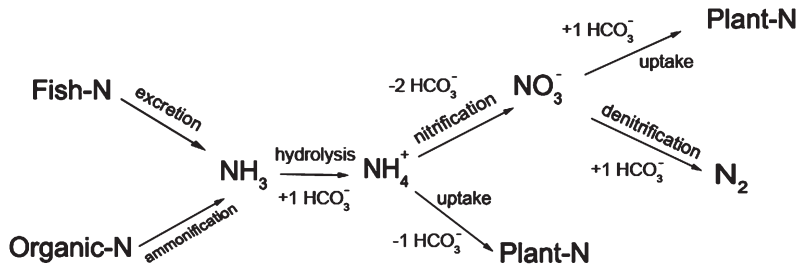

FIGURE 8. *A simple model of major nitrogen transformations that affect alkalinity concentrations in aquaculture ponds. Organic N in the model refers to nitrogen in plants and decomposing organic matter. The number of equivalents of alkalinity formed or destroyed per equivalent of nitrogen are given for each step.*

Changes in the ratio of equivalents of nitrogen transformed to equivalents of alkalinity either produced or destroyed can result in an increase or a decrease in alkalinity concentration. The assumption that each equivalent of waste NH_3 produces one equivalent of alkalinity (Fig. 8) is an oversimplification necessary in a simple model. For example, not all ammonia nitrogen is excreted as NH_3 and not all of the NH_3 excreted is ionized at normal pH values for ponds. Also, not all NO_3^- from nitrification is assimilated by plants or denitrified. These and other deviations from the simplified model usually result in a net loss of alkalinity because of the large acidifying potential of nitrification. The decline in alkalinity is especially rapid and great in plastic-lined heterotrophic biofloc production units without water exchange where the inputs of NH_3 are particularly large.

The influence of nitrogen additions on alkalinity can be illustrated by results from a pond fertilization study. Tepe and Boyd (2002) measured alkalinity in unfertilized control ponds and ponds fertilized with ammonium sulfate (with a large potential acidity from nitrification) or sodium nitrate (with potential basicity from denitrification). In the control ponds, alkalinity increased only slightly (26–31 mg/L) between mid-April and early August because of concentration of dissolved solids by evaporation in excess of rainfall. In the ammonium sulfate treatment, alkalinity decreased from 20 mg/L to 14 mg/L during the same period, but in the sodium nitrate treatment, alkalinity rose for 26–40 mg/L (Fig. 9). Alkalinity values were not reported in Figure 9 for the entire study period,

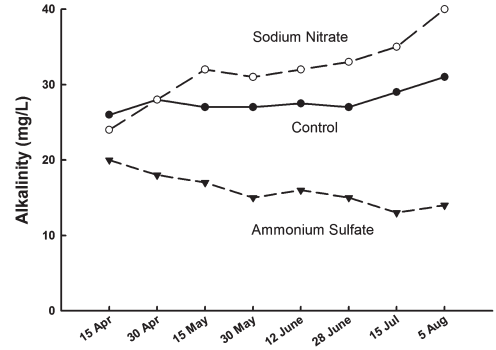

FIGURE 9. *Changes in alkalinity in ponds without fertilization and in ponds treated at 9 kg N/ha every 2 wk with ammonium sulfate or with sodium nitrate. Source: Tepe and Boyd (2002).*

because the ponds treated with ammonium sulfate were limed in August to restore the alkalinity loss.

Reference to Figure 8 also shows qualitatively why recirculating aquaculture systems require addition of basic chemicals to maintain alkalinity. In recirculating systems, waste ammonia is excreted by fish, which is an alkalinity-producing reaction. Ammonia nitrogen is then nitrified in biofilters to prevent toxic accumulation of ammonia. Acid produced in nitrification destroys at least twice the amount of alkalinity produced during ammonia excretion. Unlike in ponds, however, additional alkalinity is not produced during NO_3^- uptake by plants or in denitrification. Lack of those alkalinity-producing processes in recirculating systems means that there is net alkalinity destruction, which must be compensated by addition of $NaHCO_3$ or other basic chemicals.

Alkalinity and hardness in aquaculture ponds also are affected by chemical additions. Liming materials, of course, are intentionally added to make large increases in alkalinity and hardness. Likewise, gypsum may be added to increase calcium hardness. Calcium enters pond water in several fertilizers – especially triple superphosphate that contains a large amount of monocalcium phosphate [$Ca(H_2PO_4)_2 \cdot H_2O$]. Manures and feeds contain both calcium and magnesium. These possible sources of hardness in ponds have not been studied, but some rough calculations made below revealed that both phosphate fertilizer and feed potentially can increase hardness by a relevant amount – especially in waters of low hardness. Phosphate fertilization rates in tilapia ponds vary, but an application of 50 kg/ha per month would not be large (Boyd and Tucker 1998). Triple superphosphate contains 15% calcium (Jones 1979), and application of 50 kg/ha of this fertilizer per month over an 8-mo grow-out period in a tilapia pond would supply 6 mg/L Ca^{2+} (15 mg/L hardness). Tilapia contain about 1.30% calcium (Boyd and Green 1998), and tilapia feeds have an average calcium concentration of 1.82% (Chatvijitkul and Boyd unpublished data). At a feed conversion ratio of 1.75, production of 6000 kg/ha of tilapia containing 78 kg calcium in a 10,000 m^3 pond would require an input of 10,500 kg feed containing 191 kg calcium. The input of calcium to the pond in unassimilated feed calcium would equal 113 kg/10,000 m^3 or 11.3 mg/L (28.2 mg/L hardness) assuming it all dissolved in the water.

The amount and application frequency for liming materials in ponds also is influenced by processes causing fluctuations in alkalinity and hardness concentrations. Ponds with high flushing rates caused by runoff from watersheds with acidic soil and ponds with especially high rates of nitrification typically need the largest and most frequent applications of liming material.

Conclusion

Biological productivity may be limited by low availability of inorganic C for photosynthesis in some waters – especially those of low alkalinity (Verspagen et al. 2014). Alkalinity buffers water against wide daily pH swings related to photosynthesis and respiration rates, but early morning pH of waters tends to increase with greater alkalinity. Bicarbonate and CO_3^{2-} responsible for alkalinity form ion pairs with metal ions increasing the concentrations possible at equilibrium between ions and their controlling minerals. This increases the availability of trace metals for aquatic plants. Also, higher concentrations of alkalinity protect aquatic animals from toxicity of trace metal ions added to water intentionally or through pollution, because the ionic forms of metals are most toxic.

The main effect of hardness seems to result from Ca^{2+}, despite both Ca^{2+} and Mg^{2+} being hardness cations. Calcium precipitates CO_3^{2-} as $CaCO_3$ to minimize pH rise in waters of pH above 8.3 when photosynthesis is progressing rapidly. Calcium also tends to block trace metal uptake by fish thereby lessening toxicity. In fish hatcheries, Ca^{2+} concentration is important to egg development and hatching. Greater hardness also facilitates the flocculation and precipitation of suspended clay that causes turbidity.

Alkalinity and hardness in freshwater ponds for sportfish should be 40 mg/L or more, but in food fish ponds alkalinity should be 60 mg/L or more. In culture of estuarine or marine species, alkalinity should be above 100 mg/L. Hardness in estuarine or marine water is much greater than in freshwater and seldom will affect aquaculture production.

Low alkalinity and hardness in ponds usually are increased by additions of agricultural limestone or other liming materials. Enough liming material must be used to neutralize bottom soil acidity and provide a residual to dissolve and increase alkalinity and hardness. Nitrification of ammonia from fertilizers, excretion by fish, and organic matter decomposition is a major source of acidity in ponds, and liming must be repeated at 1- to 3-yr intervals. In highly intensive aquaculture in plastic-lined ponds or other culture units, alkalinity may fall rapidly because of nitrification. Thus, frequent applications of sodium bicarbonate – a highly soluble compound – is used in place of agricultural limestone or other common materials that dissolve more slowly.

Lime can be applied to water to lessen CO_2 concentrations, and gypsum may be used to increase Ca^{2+} concentration and hardness above concentrations possible with liming materials.

Literature Cited

Abril, G. and M. Frankignoulle. 2001. Nitrogen-alkalinity interactions in the highly polluted Scheldt Basin (Belgium). Water Research 35(3):844–850.

Adams, F. 1971. Ionic concentrations and activities in soil solutions. Soil Science Society American Proceedings 35:420–426.

Adams, F. 1974. Soil solution. Pages 441–481 in W. E. Carson, editor. The plant root and its environment. University Press of Virginia, Charlottesville, Virginia, USA.

Ahmed, I., M. Ashraf, S. Abbas, and P. Akhtar. 2013. Effect of poultry droppings on water quality parameters in Indian major carp ponds. Journal of Animal and Plant Sciences 23(1):68–75.

Akin, G. W. and J. V. Lagerwerff. 1965. Calcium carbonate equilibria in aqueous solutions open to the air. I. The solubility of calcite in relation to ionic strength. Geochimica et Cosmochimica Acta 29:343–352.

Arce, R. G. and C. E. Boyd. 1975. Effects of agricultural limestone on water chemistry, phytoplankton productivity, and fish production in soft-water ponds. Transactions of the American Fisheries Society 104:308–312.

Arce, R. G. and C. E. Boyd. 1980. Water chemistry of Alabama ponds. Bulletin 522, Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama, USA.

Armstrong, S., D. Smith, P. Owens, B. Joern, A. Leytem, C. Huang, and O. Adeola. 2011. Phosphorus and nitrogen loading depth in fluvial sediment following manure spill simulations. Canadian Journal of Soil Science 91:427–436.

Avnimelech, Y. 2012. Biofloc technology, a practical handbook, 2nd edition. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Ball, R. C. 1948. Fertilization of natural lakes in Michigan. Transactions of the American Fisheries Society 78:145–155.

Barron, M. G. and S. Albeke. 2000. Calcium control of zinc uptake in rainbow trout. Aquatic Toxicology 50:257–264.

Bose, J., O. Babourina, and Z. Rengel. 2011. Role of magnesium in alleviation of aluminum toxicity in plants. Journal of Experimental Botany 62(7):2251–2264.

Bowles, O. 1956. Limestone and dolomite. Bureau of Mines Information Circular 7738, U.S. Department of the Interior, Washington, District of Columbia, USA.

Boyd, C. E. 1972. Sources of carbon dioxide for nuisance blooms of algae. Weed Science 20:492–497.

Boyd, C. E. 1974. Water quality in catfish ponds. Journal of Marine Science of Alabama 2(3):19–30.

Boyd, C. E. 1976. An evaluation of a water analysis kit. Leaflet 88, Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama, USA.

Boyd, C. E. 1977. Evaluation of a water analysis kit. Journal of Environmental Quality 6:381–384.

Boyd, C. E. 1985a. Effects of acid rain on surface waters in Alabama. Alabama Agricultural Experiment Station, Highlights of Agricultural Research 32(3):7.

Boyd, C. E. 1985b. Pond evaporation. Transactions of the American Fisheries Society 114:299–303.

Boyd, C. E. 1990. Water quality in ponds for aquaculture. Auburn, Alabama, USA, Alabama Agricultural Experiment Station, Auburn University.

Boyd, C. E. 2015. Water quality, an introduction, 2nd edition. Springer, New York, New York, USA.

Boyd, C. E. and S. W. Brown. 1990. Quality of water from wells in the major catfish farming area of Alabama. Pages 195–206 in Proceedings 50th anniversary symposium. Department of Fisheries and Allied Aquacultures, Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama, USA.

Boyd, C. E., S. W. Brown, and D. R. Bayne. 1983. Phytoplankton communities in channel catfish ponds. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 37:401–407.

Boyd, C. E. and B. W. Green. 1998. Dry matter, ash, and elemental composition of pond-cultured tilapia *Oreochromis aureus* and *O. niloticus*. Journal of the World Aquaculture Society 29:125–128.

Boyd, C. E. and A. A. McNevin. 2015. Aquaculture, resource use, and the environment. Wiley Blackwell, Hoboken, New Jersey, USA.

Boyd, C. E. and S. Pipoppinyo. 1994. Factors affecting respiration in dry pond bottoms. Aquaculture 120:283–293.

Boyd, C. E., J. W. Preacher, and L. Justice. 1978. Hardness, alkalinity, pH, and pond fertilization. Proceedings of the Annual Conference of Southeastern Association of Game and Fish Commissioners 107:605–611.

Boyd, C. E. and D. Teichert-Coddington. 1995. Dry matter, ash, and elemental composition of pond-cultured *Penaeus vannamei* and *P. stylirostis*. Journal of the World Aquaculture Society 26:88–92.

Boyd, C. E. and C. S. Tucker. 1992. Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama, USA.

Boyd, C. E. and C. S. Tucker. 1998. Pond aquaculture water quality management. Kluwer Academic Publishers, Boston, Massachusetts, USA.

Boyd, C. E. and C. S. Tucker. 2014. Handbook for aquaculture water quality. Craftmaster Printers, Inc., Auburn, Alabama, USA.

Boyd, C. E., C. S. Tucker, and R. Viriyatum. 2011. Interpretation of pH, acidity and alkalinity in aquaculture and fisheries. North American Journal of Aquaculture 73(4):403–408.

Boyd, C. E. and W. W. Walley. 1975. Total alkalinity and hardness of surface waters in Alabama and Mississippi.

Bulletin 465, Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama, USA.

Brewer, P. G. and J. C. Goldman. 1976. Alkalinity changes generated by phytoplankton growth. Limnology and Oceanography 21:108–117.

Brown, J., A. Colling, D. Park, J. Phillips, D. Rothery, and J. Wright. 1989. Seawater: its composition, properties, and behavior. Pergamon Press, Oxford, UK.

Brown, J. A. and S. Lynam. 1981. The effect of sodium and calcium concentrations on the hatching of eggs and the survival of the yolk sac of brown trout, *Salmo trutta* L. at low pH. Journal of Fish Biology 19(2):205–211.

Caldeira, K. and M. E. Wickett. 2003. Anthropogenic carbon and ocean pH. Nature 425:365.

Chang, R. 2005. Physical chemistry for the biosciences. University Science Books, Sausalito, California, USA.

Chatakondi, N. G. and E. L. Torrans. 2012. The effect of calcium hardness on hatching success of channel catfish × blue catfish hybrid catfish eggs. North American Journal of Aquaculture 74:306–309.

Chave, K. E. and E. Suess. 1970. Calcium carbonate saturation in seawater: effects of dissolved organic matter. American Society of Limnology and Oceanography 15(4):633–638.

Chierici, M. and A. Fransson. 2009. Calcium carbonate saturation in the surface water of the Arctic Ocean: undersaturation in freshwater influenced shelves. Biogeosciences 6:2421–2432.

Clever, H. L. and F. J. Johnston. 1980. The solubility of some sparingly soluble lead salts: an evaluation of the solubility in water and aqueous electrolyte solution. Journal of Physical Chemistry 9(3):751–784.

Colt, J. and K. Orwicz. 1991. Modeling production capacity of aquatic culture systems under freshwater conditions. Aquacultural Engineering 10:1–29.

Colt, J., B. Watten, and M. Rust. 2009. Modeling carbon dioxide, pH, and un-ionized ammonia relationships in serial reuse systems. Aquacultural Engineering 40:28–44.

Conyers, M. K., N. C. Uren, and K. R. Helyer. 1995. Causes of changes in pH in acidic mineral soils. Soil Biology and Biochemistry 27:1383–1392.

Cooper, S. S. 1941. The mixed indicator bromocresol green-methyl red for carbonates in water. Industrial and Engineering Chemistry 13(7):466–470.

Davies, P. H., J. P. Goetti Jr.**, J. R. Sinley, and N. F. Smith.** 1976. Acute and chronic toxicity of lead to rainbow trout, *Salmo gairdneri*, in hard and soft water. Water Research 10:199–206.

Davis, J. A. and C. E. Boyd. 1978. Concentrations of selected elements and ash in bluegill (*Lepomis macrochirus*) and certain other freshwater fish. Transactions of the American Fisheries Society 107:862–867.

Dent, D. 1986. Acid sulfate soils: a baseline for research and development. Publication 39, International Institute of Land Reclamation and Improvement, Wageningen, The Netherlands.

Eaton, A. D., L. S. Clesceri, E. W. Rice, and A. E. Greenberg, editors. 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, Washington, District of Columbia, USA.

Ebeling, J. M., M. B. Timmons, and J. J. Bisogni. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257:346–358.

Eshchar, M., N. Mozes, and M. Fediuk. 2003. Carbon dioxide removal rate by aeration devices in intensive sea bream tanks. Bamidgeh 55:79–85.

Feely, R. A., C. L. Sabine, K. Lee, W. Berelson, J. Kleypas, V. J. Fabry, and F. J. Millero. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the ocean. Science 305:362–366.

Frear, G. L. and J. Johnston. 1929. The solubility of calcium carbonate (calcite) in certain aqueous solutions at 25°. American Chemical Society 51:2082–2093.

Geagan, D. W. and T. D. Allen. 1960. An ecological survey of factors affecting fish production in Louisiana waters. Louisiana Wildlife and Fisheries Commission, Baton Rouge, Louisiana, USA.

Gerloff, G. C. and K. A. Fishbeck. 1969. Quantitative cation requirements of several green and blue-green algae. Journal of Phycology 5:109–114.

Gross, A., C. E. Boyd, and C. W. Wood. 1999. Ammonia volatilization from freshwater fish ponds. Journal of Environmental Quality 28:793–797.

Haines, T. A. 1981. Acid precipitation and its consequences for aquatic ecosystems: a review. Transactions of the American Fisheries Society 110:669–707.

Han, Y., C. E. Boyd, and R. Viriyatum. 2014. A bicarbonate titration method for lime requirement to neutralize exchangeable acidity of pond bottom soils. Aquaculture 434:282–287.

Hargreaves, J. A. 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 116:181–212.

Hayes, F. R. and E. H. Anthony. 1964. Productive capacity of North American lakes as related to the quantity and the trophic level of fish, the lake dimensions, and the water chemistry. Transactions of the American Fisheries Society 93:53–57.

Hepher, B. 1958. On the dynamics of phosphorus added to fishponds in Israel. Limnology and Oceanography 3:84–100.

Hepher, B. 1962. Ten years of research in fish pond fertilization in Israel. I. The effect of fertilization on fish yields. Bamidgeh 14:29–38.

Hepher, B. 1966. Some aspects of the phosphorus cycle in fishponds. Verhandlungen des Internationalen Verein Limnologie 16:1293–1297.

Hem, J. D. 1985. Study and interpretation of the chemical characteristics of natural water. Water Supply Paper 2254, U.S. Geological Survey, U. S. Government Printing Office, Washington, District of Columbia, USA.

Hickling, C. F. 1962. Fish cultures. Faber and Faber, London, UK.

Howells, G. D., D. J. A. Brown, and K. Sadler. 1983. Effects of acidity, calcium, and aluminum on fish survival and productivity – a review. Journal of the Science of Food and Agriculture 34(6):559–570.

Hunt, D. and C. E. Boyd. 1981. Alkalinity losses resulting from ammonium fertilizers used in fish ponds. Transactions of the American Fisheries Society 110:81–85.

Inglis, A. and E. L. Davis. 1972. Effects of water hardness on the toxicity of several organic and inorganic herbicides to fish. Technical Paper 67, U. S. Fish and Wildlife Service, Washington, District of Columbia, USA.

Ip, Y. K. and S. F. Chew. 2010. Ammonia production, excretion, toxicity, and defense in fish: a review. Frontiers in Physiology 1:1–20.

Ittekkot, V. 2003. A new story from the Ol' Man River. Science 301:56–58.

Jones, U. S. 1979. Fertilizers and soil fertility. Reston Publishing Company, Reston, Virginia, USA.

Kaushal, S. S., G. E. Likens, R. M. Utz, M. L. Pace, M. Grese, and M. Yepsen. 2013. Increased river alkalinization in the eastern U.S. Environmental Science and Technology 47:10302–10311.

King, D. L. 1970. The role of carbon in eutrophication. Journal of the Water Pollution Control Federation 42:2035–2051.

Larson, T. E. and A. M. Buswell. 1942. Calcium carbonate saturation index and alkalinity interpretations. Journal of the American Water Works Association 34(11):1667–1684.

Laurén, D. J. and D. G. McDonald. 1986. Influence of water hardness, pH and alkalinity on the mechanisms of copper toxicity in juvenile rainbow trout, *Salmo gairdneri*. Canadian Journal of Fisheries and Aquatic Sciences 43:1488–1496.

Li, L., S. Dong, X. Tian, and C. E. Boyd. 2013. Equilibrium concentrations of major cations and total alkalinity in laboratory soil-water systems. Journal of Applied Aquaculture 25(1):50–65.

Lovell, R. T. 1998. Nutrition and feeding of fish, 2nd edition. Springer Science and Business Media, New York, New York, USA.

Loyless, J. C. and R. F. Malone. 1997. A sodium bicarbonate dosing methodology for pH management in freshwater recirculating systems. Progressive Fish-Culturist 59:198–205.

Mandal, B. K. and C. E. Boyd. 1980. The reduction of pH in water of high total alkalinity and low total hardness. Progressive Fish-Culturist 42:183–185.

McNevin, A., C. E. Boyd, O. Silapajarn, and K. Silapajarn. 2004. Ionic supplementation of pond waters for inland culture of marine shrimp. Journal of the World Aquaculture Society 35:460–467.

Moore, J. M. and C. E. Boyd. 1984. Comparison of devices for aerating inflow of pipes. Aquaculture 38:89–96.

Moyle, J. B. 1946. Some indices of lake productivity. Transactions of the American Fisheries Society 76:322–334.

Murad, H. and C. E. Boyd. 1991. Production of sunfish (*Lepomis* spp.) and channel catfish (*Ictalurus punctatus*) in acidified ponds. Aquaculture 94:381–388.

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R. M. Key, K. Lindsay, E. Maier-Reimer, R. Matear, P. Monfray, A. Mouchet, R. G. Najjar, G. Plattner, K. B. Rodgers, C. L. Sabine, J. L. Sarmiento, R. Schlitzer, R. D. Slater, I. J. Totterdell, M. Weirig, Y. Yamanaka, and A. Yool. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–686.

Pais, I. and J. B. Jones Jr.**.** 1997. The handbook of trace elements. Saint Lucie Press, Boca Raton, Florida, USA.

Paerl, H. W. and C. S. Tucker. 1995. Ecology of blue-green algae in aquaculture ponds. Journal of the World Aquaculture Society 26:109–131.

Perschbacher, P. W. and W. A. Wurts. 1999. Effects of calcium and magnesium hardness on acute copper toxicity to juvenile channel catfish Ictalurus punctatus. Aquaculture 172:275–280.

Pillai, V. K. and C. E. Boyd. 1985. A simple method for calculating liming rates for fish ponds. Aquaculture 46:157–162.

Prieto, F. J. M. and F. J. Millero. 2001. The values of pK_1 and pK_2 for the dissociation of carbonic acid in seawater. Geochimica et Cosmochimica Acta 66(14):2529–2540.

Pruder, G. 1983. Biological control of gas exchange in intensive aquatic production systems. Oceans 83:1002–1004.

Raven, J. A., M. Giordano, J. Beardall, and S. C. Maberly. 2012. Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. Philosophical Transactions of the Royal Society B 367:493–507.

Renick, B. C. 1925. Base exchange in ground water by silicates as illustrated in Montana. Water Supply Paper 520-D, U.S. Geological Survey, U.S. Government Printing Office, Washington, District of Columbia, USA.

Reynolds, C. S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge, UK.

Reynolds, C. S. 1997. Vegetation processes in the pelagic: a model for ecosystem theory. Ecology Institute, Oldendorf/Luhe, Germany.

Ruttner, F. 1963. Fundamentals of limnology. University of Toronto Press, Toronto, Canada.

Saengrungruang, P. 2012. Erosion control and removal of suspended soil particles in ponds: Evaluation of geofabric liners and chemical coagulants. Ph.D. dissertation, Auburn University, Auburn, Alabama, USA.

Scheffer, M., S. Rinaldi, A. Gragnani, L. R. Mur, and E. H. Van Nes. 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology 78:272–282.

Schindler, P. W. 1967. Heterogeneous equilibria involving oxides, hydroxides, carbonates, and hydroxide carbonates. Pages 196–221 *in* R. F. Gould, editor. Equilibrium concepts in natural water systems. Advanced Chemistry

Series 67. American Chemical Society, Washington, District of Columbia, USA.

Schindler, D. W., M. A. Turner, and R. H. Hesslein. 1985. Acidification and alkalinization of lakes by experimental addition of nitrogen compounds. Biogeochemistry 1:117–133.

Schroeder, G. L. 1987. Carbon and nitrogen budgets in manured fish ponds on Israel's coastal plain. Aquaculture 62:259–279.

Seward, T. M. 1974. Determination of the first ionization constant of silicic acid from quartz solubility in borate buffer solutions to 350°C. Geochimica and Cosmochimica Acta 38:1651–1664.

Shapiro, J. 1990. Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 24:38–54.

Sibrell, P. L., B. J. Watten, T. A. Haines, and B. W. Spaulding. 2006. Limestone fluidized bed treatment of acid-impacted water at Craig Brook National Fish Hatchery, Maine, USA. Aquacultural Engineering 34(2):61–71.

Silapajarn, K., C. E. Boyd, and O. Silapajarn. 2004. Physical and chemical characteristics of pond water and bottom soil in channel catfish ponds in west-central Alabama. Bulletin 655, Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama, USA.

Snoeyink, V. L. and D. Jenkins. 1980. Water chemistry. John Wiley and Sons, New York, New York, USA.

Soongsawang, S. and C. E. Boyd. 2012. Effects of effluents from a fisheries station on stream water quality. North American Journal of Aquaculture 74:73–79.

Spence, D. H. N. and S. C. Maberly. 1985. Occurrence and ecological importance of HCO_3-use among aquatic higher plants. Pages 125–143 *in* W. J. Lucas and J. Berry, editors. Inorganic carbon uptake by aquatic photosynthetic organisms. American Society of Plant Physiologists, Rockville, Maryland, USA.

Straus, D. L. and C. S. Tucker. 1993. Acute toxicity of copper sulfate and chelated copper to channel catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society 24:390–395.

Swingle, H. S. and E. V. Smith. 1947. Management of farm fish ponds. Bulletin 254, Alabama Agricultural Experiment Station, Alabama Polytechnical Institute, Auburn, Alabama, USA.

Talling, J. F. 1976. The depletion of carbon dioxide from lake water by phytoplankton. Journal of Ecology 64:79–121.

Tepe, Y. and C. E. Boyd. 2002. Nitrogen fertilization of golden shiner ponds. North American Journal of Aquaculture 64:284–289.

Thompson, J. B., S. Schultzepam, T. J. Beveridge, and D. J. DesMarais. 1997. Whiting events: biogenic origin due to the photosynthetic activity of cyanobacterial picoplankton. Limnology and Oceanography 42:133–141.

Thomaston, W. W. and H. D. Zeller. 1961. Results of a six-year investigation of chemical soil and water analysis and lime treatment in Georgia fish ponds. Proceedings Annual Conference of the Southeastern Association Game and Fish Commissioners 15:236–245.

Thunjai, T., C. E. Boyd, and M. Boonyaratpalin. 2004. Bottom soil quality in tilapia ponds of different age in Thailand. Aquaculture Research 35:698–705.

Timmons, M. B. and J. M. Ebeling. 2010. Recirculating aquaculture, 2nd edition. Cayuga Aqua Ventures, Ithaca, New York, USA.

Tucker, C. S. 1996. The ecology of channel catfish ponds in northwest Mississippi. Reviews in Fisheries Science 4:1–55.

Tucker, C. S., S. W. Lloyd, and R. L. Busch. 1984. Relationships between phytoplankton periodicity and the concentrations of total and un-ionized ammonia in channel catfish ponds. Hydrobiologia 111:75–79.

Tucker, C. S. and J. A. Steeby. 1993. A practical calcium hardness criterion for channel catfish hatchery water supplies. Journal of the World Aquaculture Society 24(3):396–401.

Turner, W. R. 1960. Standing crop of fishes in Kentucky farm ponds. Transactions of the American Fisheries Society 89:333–337.

U.S. Environmental Protection Agency. 1985. Ambient water quality criteria for copper – 1984. EPA 440/5-84-031, U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, District of Columbia, USA.

U.S. Environmental Protection Agency. 2007. Aquatic life ambient freshwater quality criteria for copper. EPA-822-R-07-001, U.S. Environmental Protection Agency, Office of Water, Washington, District of Columbia, USA.

Uusitalo, J. 1996. Algal carbon uptake and the differences between alkalinity and high pH ("alkalization") exemplified with a pH drift experiment. Scientica Marina 60:129–134.

Verspagen, J. M. H., D. B. Van de Wall, J. F. Finke, P. M. Visser, E. Van Donk, and J. Huisman. 2014. Rising CO_2 levels will intensify phytoplankton blooms in eutrophic and hypertrophic lakes. PLoS ONE 9(8):e104325. DOI: 10.1371/journal/pone.0104325.

Viriyatum, R. and C. E. Boyd. 2011. Re-evaluation of potassium fertilization of bluegill, *Lepomis macrochirus*, ponds. Journal of the World Aquaculture Society 42:332–338.

Weiler, R. R. 1979. Rate of loss of ammonia from water to the atmosphere. Journal of the Fisheries Research Board of Canada 36:685–689.

White, P. J. and M. R. Broadley. 2003. Calcium in plants. Annals of Botany 92:487–511.

Wolf-Gladow, D. A., R. E. Zeebe, C. Klass, A. Körtzinger, and A. G. Dickson. 2007. Total alkalinity: the explicit conservative expression and its application to biogeochemical processes. Marine Chemistry 106:287–300.

Wudtisin, I. and C. E. Boyd. 2006. Physical and chemical characteristics of sediments in catfish, freshwater prawn

and carp ponds in Thailand. Aquaculture Research 37:1202–1214.

Wurts, W. A. and P. W. Perschbacher. 1994. Effects of bicarbonate alkalinity and calcium on the acute toxicity of copper to juvenile channel catfish (*Ictalurus punctatus*). Aquaculture 125:73–79.

Wurts, W. A. and R. R. Stickney. 1989. Responses of red drum (*Sciaenops ocellatus*) to calcium and magnesium concentrations in fresh and salt water. Aquaculture 76:21–35.

Zhou, L. and C. E. Boyd. 2014. Total ammonia nitrogen removal from aqueous solutions by the natural zeolite, mordenite: a laboratory test and experimental study. Aquaculture 432:252–257.