Investigation of Several Factors Affecting Total Alkalinity Concentration and Its Measurement

by

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Abstract

The optimal endpoint pH for total alkalinity titrations decreased from 5.0 at 10 mg/L alkalinity to 4.2 for 300 mg/L alkalinity or more. The appropriate color changes for bromocresol green-methyl red (BG-MR) and methyl orange (MO) indicators also varied with the initial total alkalinity of samples. Despite differences in pH at endpoints for samples of different alkalinities, when the best endpoint pH , best color of BG-MR and MO, or the endpoint of methyl purple were used in titrations of standard solutions, there were few differences between measured alkalinities and standard alkalinities – the accuracy was better than ±3 mg/L. Results of spike and recovery tests on aquaculture pond water samples also revealed that an accuracy of ±3 mg/L alkalinity could be achieved on either unfiltered or filtered samples by all four methods of acceptable endpoint detection. Although precision of measurements could not be consistently maintained below ±1 mg/L, coefficients of variation for repeated measurements usually were less than 5% for all methods of endpoint detection. Nevertheless, this degree of precision was adequate to achieve good accuracy that is the major concern in water analysis. Variations in alkalinity measurement that could result from improper selection of endpoint pH (or color) were rather small – usually not more than ±5 mg/L. In an inter-laboratory comparison of alkalinity determinations on standard solutions, most laboratories reported inaccurate alkalinities. These inaccuracies were greater than possible endpoint variations. It was clear that most of the participating laboratories did not have a satisfactory method of quality control.

Total alkalinity and total hardness concentrations in small streams in Alabama have increased from 41.56 mg/L and 43.42 mg/L, respectively, in 1973 to 47.19 mg/L and 52.28 mg/L at present (2014-15). These findings are in agreement with two other studies reporting an increase in the alkalinity of major rivers in the eastern United States. Possible explanations for the increase were given in one study as greater weathering of limestone by acidic deposition, and in the other study, as the result of streams recovering from past acidification. Observations and calculations made in the present study suggest that the dissolution of limestone, calcium silicate, and feldspars has increased in recent years because of rising atmospheric carbon dioxide concentrations resulted in greater alkalinity and hardness. The estimated solubility increase is great enough to explain a major portion of the rise in stream alkalinity and hardness that has occurred in small streams in Alabama since 1973.

Large applications (~20t/year) of agricultural limestone in pond FP-14, a large watershed reservoir on the research station, have roughly doubled average annual conductivity and concentration of total alkalinity, total hardness, and calcium hardness as compared to concentrations of these variables in control water sources. The pH of FP-14 discharge is not much greater than for the control, which is an un-limed pond, but pH was never below 6.65 and had an annual average of 7.12 in water released from FP-14. The concentration of water quality variables decreased following large rainfall events and during periods of prolonged rainfall. In general, concentrations were lowest in winter when rainfall tended to be high and evapotranspiration low favoring greater runoff. Because of the effect of rainfall on water quality, calcium hardness concentrations were sometimes so low that they would be expected to adversely impact fish egg survival and hatching at the Fish Genetic Unit (FGU) hatchery. The discharge of FP-14 ultimately reaches pond FP-11 that is the water supply for the Production Research Unit (PRU). Despite having been diluted by considerable runoff from the Research Station Creek (RSC) catchment below FP-14, the total alkalinity of water in FP-11 was about 6-7 mg/L higher than in the control. This should reduce the amount of liming required for the small ponds of the PRU.

The pH, conductivity, and concentrations of total alkalinity, total hardness, and calcium hardness in Alabama streams tended to be lowest in the Coastal Plain and Piedmont Plateau, intermediate in the Ridge and Valley, Appalachian Plateau, and Highland Rim, and highest in the Blackland Prairie. Streams seldom had pH below 6, and most pH values ranged between 7 and 8. There were few instances with alkalinity and hardness below 5 mg/L or above 100 mg/L. Conductivity usually was below 500 μmho/cm.

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List of Abbreviations

°f	French hardness degree
ΔG^o	Gibbs free energy
All	Samples combined
AP	Appalachian Plateau
BG-MR	Bromocresol green-methyl red
BP	Blackland Prairie
C	Calcareous areas
C	Carbon
Ca^{2+}	Calcium
$CaCO_3$	Calcium carbonate/Calcite
CaO	Calcium oxide
$CaSO_4 \cdot 2H_2O$	Gypsum
cm	Centimeter
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate
CP	Coastal Plain
dH	German hardness degree
EDTA	Ethylenediaminetetraacetic acid
Eq	Equation
eq	Equivalent

Fe^{2+}	Ferrous iron
FGU	Fish Genetic Unit
g	Gram
gal	Gallon
GPS	Global positioning system
gr	Grain
H^+	Hydrogen ion
$H_2BO_4^-$	Borate
H_2CO_3	Carbonic acid
H_2O	Water vapor
H_2SO_4	Sulfuric acid
$H_3SiO_4^-$	Silicate
H_4SiO_4	Silicic acid
Ha	Hactare
HCO_3^-	Bicarbonate
HR	Highland Rim
hr	Hour
HSD	Honest Significant Difference
IQR	Interquartile range
K	Equilibrium constant
K^+	Potassium
$KAlSi_3O_8$	Orthoclase
Km	Kilometer

K_{sp}	Solubility product constant
M	Mole
meq	Milliequivalent
mg	Milligram
Mg^{2+}	Magnesium
Mg_2SiO_4	Olivine
$MgCO_3 \cdot CaCO_3$	Dolomite
mL	Milliliter
mM	Millimole
Mn^{2+}	Manganous manganese
MO	Methyl orange
MP	Methyl purple
N	Normality
Na^+	Sodium
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NC	Non-calcareous areas
NH_3	Ammonia
OH^-	Hydroxyl ion
PP	Piedmont Plateau
ppt	Part per thousand
PRU	Production Research Unit

R	Correlation coefficients
R^2	Coefficients of determination
RSC	Research Station Creek
RV	Ridge and Valley
SD	Standard deviation
SFG	E. W. Shell Fisheries Center
Sr^{2+}	Strontium
TA	Total alkalinity
TH	Total hardness
US	United States
V	Volume
wk	Week
XC-MO	Xylene cyanol-methyl orange
yr	Year

INTRODUCTION

The pH, alkalinity and hardness are commonly measured in aquaculture, because they influence the suitability of water for fish culture (Boyd and Tucker 1998, 2014). These variables are complex, and they often are poorly understood by those attempting to manage them through various techniques. A paper on interpretation of pH measurements in aquaculture and fisheries was prepared for the *North American Journal of Aquacultur*e by request of the journal's editor. The resulting essay (Boyd et al. 2011) has been number one on that journal's most read list ever since, and it was recently promoted to all involved in aquaculture and fisheries in the magazine *Fisheries* by Schaeffer (2015). The board of World Aquaculture Society asked for a similar paper to print as a review article in the *Journal of the World Aquaculture Society*. The result was the soon to be published manuscript "Total alkalinity and total hardness in aquaculture: critical but elusive concepts" (Boyd et al. in press).

During preparation of the alkalinity and hardness review – of which this Ph.D. candidate was a coauthor – several important questions arose as follows:

1. How much are the results of total alkalinity determinations affected by the method of titration endpoint used?

2. A recent study by Kaushal et al. (2013) claimed that stream waters in the eastern United States – including Alabama – are increasing in alkalinity because of accelerated weathering of watershed minerals by acid rain. Thus, has the alkalinity and hardness of

Alabama streams increased above concentrations reported in the early 1970s in a survey by Boyd and Walley (1975)

3. If alkalinity concentrations have increased, is this increase the result of the weathering effects of acidic rain or the influence of greater atmospheric carbon dioxide concentration?

4. Alkalinity is closely entwined with several other variables: pH, total hardness, calcium hardness, and conductivity. Are these variables interrelated to the extent that one can be a good predictor of the others in Alabama streams?

5. Can periodic application of agricultural limestone be effective in remediating acidic water and low calcium concentrations found in water supplies for some fish hatcheries?

The research for this dissertation was conducted to provide better answers to these questions.

LITERATURE REVIEW

Total Alkalinity

Total alkalinity is the concentration of titratable bases in water. A base will react to neutralize a hydrogen ion (H^+), e.g., 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, e.g., hydroxide alkalinity. However, in this report, the adjective “total” will be dropped and alkalinity will be used. 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

$$\text{Alkalinity} = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] – [H^+] \tag{1}$$

where the brackets indicate measured molar concentrations. Suppose that a 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,

$$\text{Alkalinity} = 0.001 \text{ M} + 2(0.000047 \text{ M}) + 0.00001 \text{ M} – 0.000000001 \text{ M} = 0.0011 \text{ M}.$$

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 (Eq.) 1 is not useful for estimating alkalinity because the concentrations of bicarbonate and carbonate are impossible to measure as discrete substances. The method for measuring alkalinity 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, e.g., 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, e.g., 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:

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

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 United States 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)

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

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

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

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

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 to obtain the apparent reaction of CO_2 with water

$$\begin{array}{ll} & CO_2 + H_2O = H_2CO_3 \\ + & \underline{H_2CO_3 = H^+ + HCO_3^-} \\ & CO_2 + H_2O = H^+ + HCO_3^- \end{array} \tag{6}$$

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 2000). Multiplying mass action expressions for Eqs. 3 and 4 together gives the mass action form of Eq. 6

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

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

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

$$(Ca^{2+})(CO_3^{2-}) = 10^{-8.3}; \quad (Ca^{2+}) = (CO_3^{2-}) = X; \quad (X)(X) = 10^{-8.3}$$

$$X = 10^{-4.15}\ M.$$

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 the 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 they do not give 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

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

which reduces to

$$\frac{(\mathrm{Ca}^{2+})(\mathrm{HCO}_3^{2-})^2}{(\mathrm{CO}_2)} = 10^{-4.32}$$

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

$$\frac{(\mathrm{X})(2\mathrm{X}^2)}{10^{-4.89}} = 10^{-4.32};\ 4\mathrm{X}^3 = 6.17 \times 10^{-10};\ \mathrm{X} = 5.37 \times 10^{-4}\ \mathrm{M}.$$

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, 0.5 g samples of food grade $CaCO_3$ were placed 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 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 $10^{-5.94}$ and $10^{-9.13}$ (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

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

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

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

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 it dissolves.

Silicic acid dissolved in water ionizes 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 sea water 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 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)(1,000 mL/L)] ÷ 100 mL sample volume = 100.08 mg/L as $CaCO_3$.

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

$$\frac{(H^+)(H^+)}{(10^{-4.89})} = 10^{-6.35}; \quad (H^+)^2 = 10^{-11.24}; \quad (H^+) = 10^{-5.62}; \quad pH = 5.62.$$

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. Those data sets are used 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. The Eq. 7 is also used to calculate endpoints that would have resulted if all of the CO_2 had remained (Fig. 2). These pH values are considerably lower than pH values estimated from inflections in the titration curves. Considerable CO_2 is lost from samples during titration, but 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. A colorblind impaired analyst cannot see the color-changes of the indicators and most use 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 can vary 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 recommendations of endpoint pH for different total alkalinity concentrations have 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. Standard Na_2CO_3 solutions are prepared for representing different alkalinity concentrations and titrated 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.

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.

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 ethylenediaminetetraacetic acid (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 equals 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)(1,000 mL/L)] ÷ 100 mL sample volume = 100.08 mg/L as $CaCO_3$.

The calcium 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 = 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 ground waters 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 6,300 mg/L as $CaCO_3$.

Freshwater ponds filled by surface water tend to have low alkalinities and hardness 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 hardness. Soils in the Piedmont Plateau area of Alabama are highly leached and acidic, and average alkalinity and hardness 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 fresh water 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 hardness of 107 mg/L and 103 mg/L, respectively (Silapajarn et al. 2004). Higher alkalinity and hardness 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 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 5).

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 5 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 fresh water. 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 to 11 in the afternoon.

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 month 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 non-carbonate 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 1,290 mg/L Mg^{2+} (Brown et al. 1989). These concentrations equate to 116 mg/L alkalinity and 6,345 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 fresh waters – 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 rivers that flow into it. Of course, alkalinity in estuaries is influenced mainly by the alkalinities of inflowing rivers, which may have more or less 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 (that is, 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 that will be discussed is the effect of photosynthesis on water pH.

Dissolved CO_2 concentration in water 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 1,000 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 under-saturation.

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 to 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 their 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, for example), 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

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

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

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

In the left-hand terms, 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

$$(H^+)^2 = 10^{-16.68}; \quad (H^+) = 10^{-8.34}; \quad pH = 8.34.$$

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

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

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).

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 milky appearance 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 Fig. 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 a pH of 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.

Buffering

The amplitudes of daily excursions of pH in aquaculture ponds resulting from additions and removal 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 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:

$$\mathrm{pH} = 6.35 + \log_{10}\frac{(\mathrm{HCO}_3^-)}{(\mathrm{CO}_2)} \tag{13}$$

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

$$\mathrm{pH} = 10.33 + \log_{10}\frac{(\mathrm{CO}_3^{2-})}{(\mathrm{HCO}_3^-)} \tag{14}$$

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 make up 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 1m 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 three days.

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 re-assimilation 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 actors 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 towards the surface where light is greater. The ability of bloom-forming blue-green algae (notably species of Microcystis, Anabaena, and Planktothrix) to out-compete 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).

Calcium 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.

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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	$10^{-6.314}$

Table 3. Effects of two endpoint pH values (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

Table 4. Total alkalinity and total hardness of waters from four wells locates 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

Table 5. Factors for estimating the concentrations of carbon dioxide available for photosynthesis from water temperature, pH, and total alkalinity.[1,2]

	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

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

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

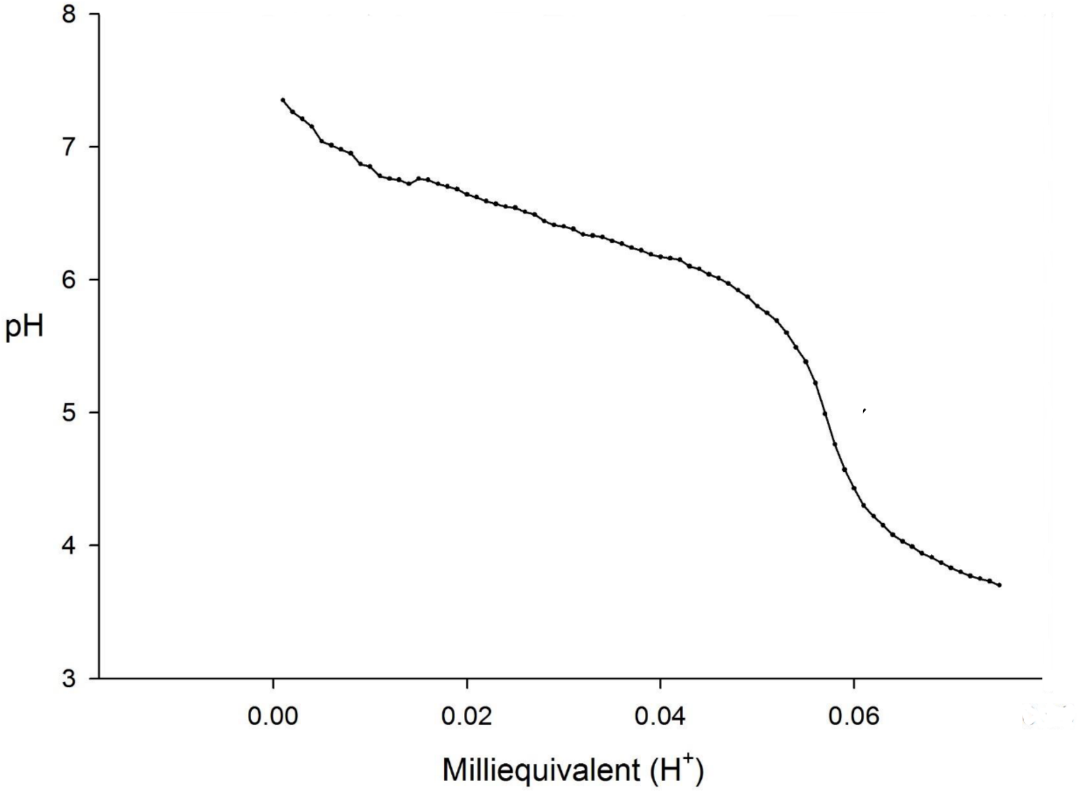

Figure 1. Titration curve for a sodium bicarbonate solution in which standard sulfuric acid was added in equal consecutive increments.

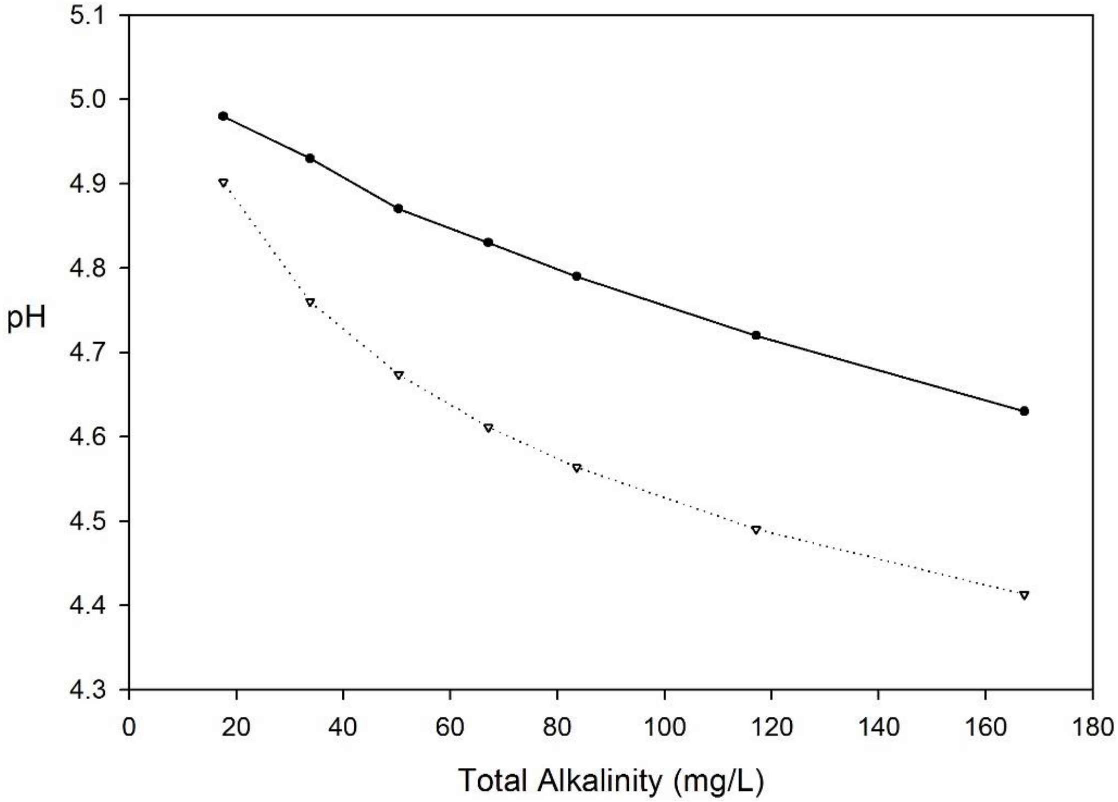

Figure 2. The pH of inflection points for titration of water samples of different alkalinity concentrations based on steepest portions of inflections in titration curves (solid line) and pH of endpoints of the titrations if all carbon dioxide released during the titration remained in the sample.

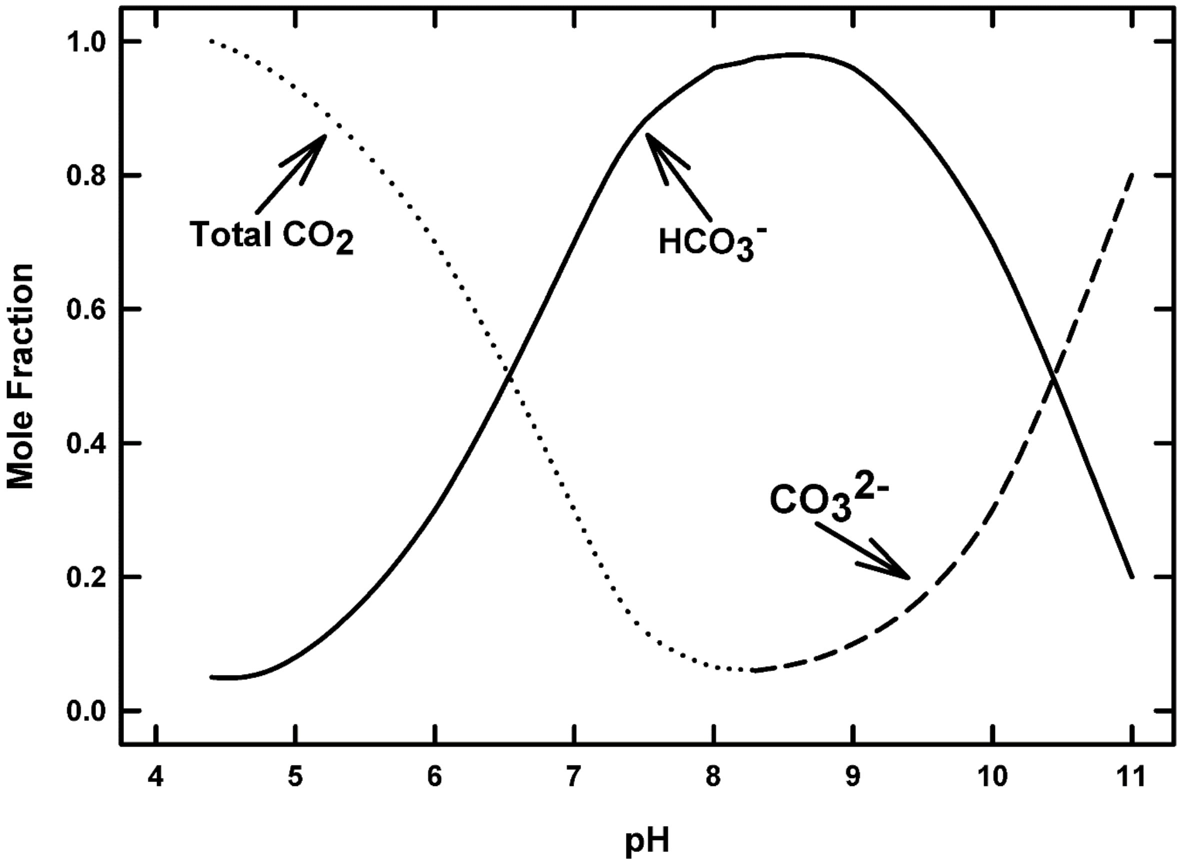

Figure 3. Relationship between pH and mole fraction of the different sources of inorganic carbon in water.

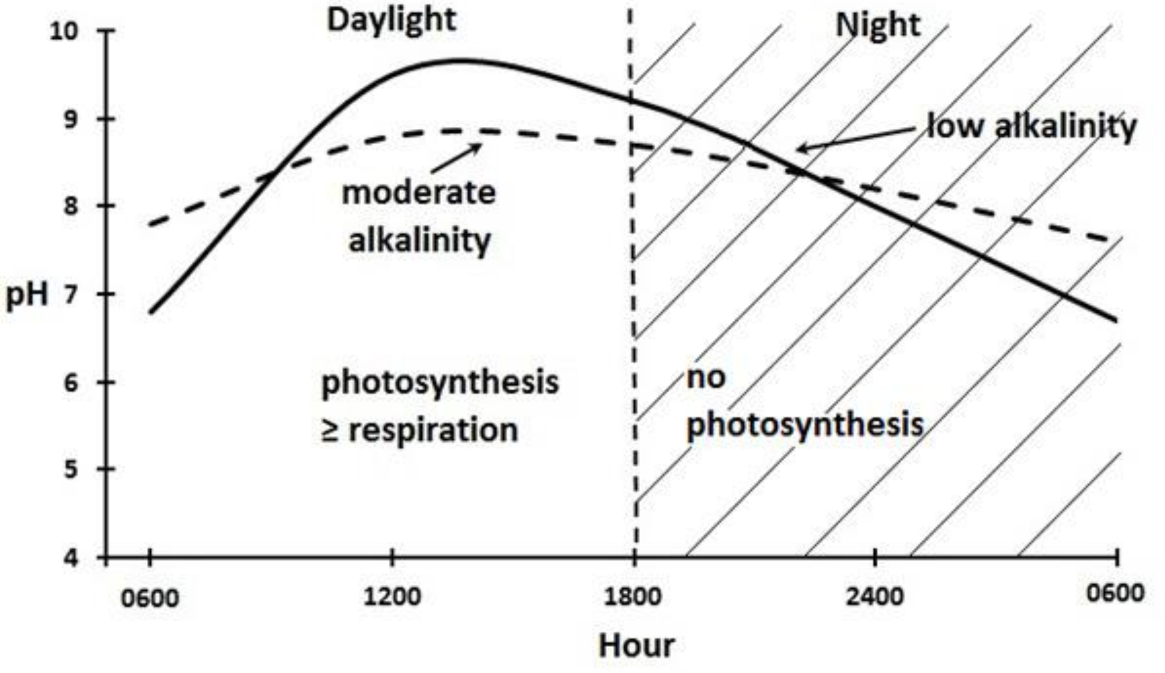

Figure 4. Effect of time of day on pH in water of low and moderate total alkalinity.

An Assessment of Factors Affecting the Reliability of the Total Alkalinity Measurement

Introduction

Total alkalinity of water in aquaculture systems often is measured, because the buffering capacity of water and the availability of inorganic carbon for photosynthesis by aquatic plants are related closely to this variable (Boyd et al. 2015). The alkalinity concentration is important for deciding whether to lime aquaculture ponds and other production units, for determining treatment rates for copper algaecides and coagulants, and for several other purposes.

The total alkalinity is defined as the total concentration of bases in water. It is determined by titrating a water sample with standard sulfuric or hydrochloric acid to neutralize the bases, and expressing the alkalinity as the calcium carbonate equivalence of the acid required in the titration. The common bases in natural waters are hydroxide, carbonate, bicarbonate, silicate, borate, phosphate, and ammonia. But, in most natural waters, and especially in waters of aquaculture systems, bicarbonate contributes most of the alkalinity at pH ≤8.3, while carbonate, and sometimes hydroxide, contribute to alkalinity at greater pH (Eaton et al. 2005).

It would be extremely rare to find a natural water in which hydroxide is the only base. During the titration of alkalinity in such a water, the pH would drop precipitously upon neutralization of the hydroxide from around 10 to about 4. The endpoint can be detected with phenolphthalein indicator that changes from pink to clear at pH 8.3. In a sample that contains both hydroxide and carbonate alkalinity, acid added during titration will react with hydroxide ($OH^- + H^+ = H_2O$) before it reacts with carbonate. After neutralization of hydroxide alkalinity, further addition of acid converts carbonate to bicarbonate:

$CO_3^{2-} + H^+ = HCO_3^-$ (1)

Bicarbonate resulting from titration of carbonate is indistinguishable from bicarbonate originally in the sample, and after carbonate has been titrated (pH = 8.3), bicarbonate reacts with acid to form carbon dioxide and water. The endpoint of nearly all samples being titrated for total alkalinity occurs at the completion of the following reaction:

$HCO_3^- + H^+ = CO_2 + H_2O \qquad K = 10^{-6.35}$ (2)

However, because carbon dioxide reacts with water (reverse reaction in Equation 2), a small, but untitratable, amount of bicarbonate remains at the endpoint.

The titration curve for total alkalinity (Fig. 5) reveals a gradual decrease in pH to around 5.5. The pH then drops faster (see circled portion of the titration curve), but not precipitously as in the titration of a strong base with a strong acid, e.g., titration of NaOH with H_2SO_4. Beyond around pH 4, the pH declines more slowly in response to continued addition of acid. The point at which a unit of acid causes the greatest pH drop – see arrow in Fig. 5 – is called the inflection point and corresponds to the endpoint of the total alkalinity titration.

According to Colt (2012), water at 20°C in equilibrium with atmospheric carbon dioxide contains 0.65 mg/L ($10^{-4.83}$ M) of this gas. The apparent reaction of carbon dioxide with water is

$CO_2 + H_2O = H^+ + HCO_3^- \qquad K = 10^{-6.35}$. (3)

The pH of carbon dioxide saturated water calculated from the expression above is 5.6. At this pH, H^+ and HCO_3^- concentrations are equal and the water does not contain measurable alkalinity. Therefore, pH 5.6 should be the endpoint of the alkalinity titration. But, the titration of alkalinity releases carbon dioxide into the water (Equation 2) and depresses pH. The amount of carbon dioxide present in a sample as the endpoint is approached depends to a great extent upon the amount of alkalinity originally present in the sample, because carbon dioxide does not diffuse from the sample as fast as it is produced in the titration. Cooper (1941) illustrated how the pH of the inflection point decreases as alkalinity increases by use of titration curves for samples of different alkalinities.

The degree of stirring during titration often differs greatly among analysts. Some may gently swirl the titration vessel or mix its contents with a stirring rod during the titration. Others put the vessel on a magnetic stirrer during titration, and the size of the magnetic stirring bar and the stirring speed may vary. Greater stirring or longer duration of titrations both favor diffusion of carbon dioxide from the sample and thereby would be expected to raise the endpoint pH to some extent. The endpoint for total alkalinity determination has traditionally been signaled with methyl orange indicator that often is said to change from yellow to orange at pH 4.5 (Nollet and Nollet 2000), but the pH for methyl orange is 3.7 and the color change range is pH 3.1-4.4 (http://chemwiki.ucdavis.edu/Physical_Chemistry/Equilibria/Acid-Base_Equilibria/6._Acid-Base_Indicators). It is generally agreed that the distinct orange endpoint pH of methyl orange is too low for samples with a low to moderate alkalinity. Methyl orange does not change color abruptly, and it is possible to consistently detect a faint orange color at a higher pH than the pH for the distinct orange color, and a few analysts use this endpoint (Boyd and Tucker 1992).

Bromocresol green-methyl red indicator goes through several color changes beginning at around pH 5.2 and continuing to about pH 4.5, and it can be used as an alternative to methyl orange. Cooper (1941) gave the following color hues and approximate endpoint pH values for bromocresol green-methyl red indicator: pH ≥ 5.2, blue with trace of green; pH 5.0, light blue with lavender gray; pH 4.8, light pink gray with cast of blue, pH 4.6, light pink; > 4.6, pink or rose. Using this indicator, endpoints can be selected based on the total alkalinity concentration of the sample.

Less common indicators for alkalinity determination are methyl purple (Hesselton 2005; Wilson 2010) and xylene cyanol (Brooke 1953; Swingle 1969). Methyl purple changes from green to purple between pH 5.4 and 4.8 (Wilson 2010). According to Swingle (1969), xylene cyanol-methyl orange changes from red to gray at a pH below 4.5. Garratt (1964) reported that the endpoint for xylene cyanol-methyl orange was at pH 3.7.

Some analysts may not assign the same endpoint hues to indicators as described above, and it is not uncommon for some analysts to have difficulty detecting the hues and color changes. Complications with indicators have led many analysts to use a pH electrode to follow pH during titration of alkalinity eliminating the need for an indicator (Eaton et al. 2005). Of course, this approach requires a decision on the proper pH of the endpoint. Eaton et al. (2005) recommended endpoint pH values for total alkalinity of 4.9 for 30 mg/L, 4.6 for 150 mg/L, and 4.3 for 500 mg/L; but, they also suggested an endpoint pH of 4.5 for routine titrations of total alkalinity. Rounds (2012) recommended determination of the inflection point as the endpoint of the total alkalinity titration, but also stated that the inflection point is around pH 4.5 in most total alkalinity titrations. Taras et al. (1971) concluded that in the alkalinity range of 10 to 500 mg/L, an accuracy of ±3 mg/L and a precision of ±1 mg/L is achievable for total alkalinity concentrations measured by reasonably good technique. These authors did not elaborate on which method of endpoint detection to use for obtaining such accuracy and precision.

Despite the importance of alkalinity and other water quality variables in production systems for fish, shrimp, and other aquatic animals, little effort has been made to standardize procedures for alkalinity titrations, or for that matter, other water quality measurements in aquaculture and fisheries. According to C.E. Boyd (personal communication) all methods of endpoint detection described above – other than the inflection point – are in rather common use for total alkalinity measurements for aquaculture. The method used to detect the endpoint of the total alkalinity titration affects the measured concentration (Copper 1941; Eaton et al. 2005), but there is little information on the differences in concentrations that may result from endpoint detection choices. If these differences are large enough to affect the interpretation of alkalinity data for aquaculture and fisheries purposes, recommendations on endpoints would be helpful.

The present study was initiated to determine how much variation may occur in total alkalinity measurements based on the endpoint detection method selected and to ascertain if one or more of the methods might be superior to the others. In addition, variation in results of total alkalinity determinations among laboratories and analysts was investigated.

Materials and Methods

In order to assess the rate of carbon dioxide loss during titration, 50-mL samples containing 30, 150, and 500 mg/L alkalinity made with sodium carbonate and distilled water were placed in 100-mL beakers. An amount of sulfuric acid calculated to neutralize the alkalinity was added quickly from a pipet into a sample, and the pH of the sample was measured at 1- to 5-

min intervals for 30 min. This was done for two stirring rates of a magnetic stirrer that had a stirring control dial ranging from 0 to 10. A setting of 2 on the dial – the stirring rate normally used in the water quality laboratory at Auburn University – was compared with a setting of 6 for each of the three concentrations of alkalinity. Each standard concentration and mixing rate was done separately so that the duration of pH measurements did not differ with respect to time after acid addition.

Alkalinity titrations were made with sulfuric acid standardized against 5.00 mL of 0.020 N sodium carbonate prepared with distilled water that had been freshly boiled to expel carbon dioxide and cooled to room temperature (Boyd and Tucker 1992). The aliquots of sodium carbonate were diluted to about 50 mL with carbon dioxide-free water before being titrated to pH 4.5. Samples containing total alkalinity concentrations of 10, 20, 30, 50, 80, 120, 150, 300, and 500 mg/L as $CaCO_3$ were prepared from sodium carbonate and carbon dioxide-free distilled water. Triplicate aliquots of each standard alkalinity concentration were titrated with standard sulfuric acid that always had a known normality near 0.020 N. The titrations were made with a microburet on 50-mL aliquots to the following pH endpoints: 5.2, 5.0, 4.8, 4.6, 4.5, 4.4, and 4.2 as detected with a glass electrode (Orion 3 Star, pH Benchtop, Thermo Scientific, Singapore); to the five endpoints of bromocresol green-methyl red indicator (Cooper 1941); to the faint orange and distinct orange endpoints of methyl orange indicator; to the purple and gray endpoints of methyl purple and xylene cyanol-methyl orange indicators, respectively. The faint orange endpoint of methyl orange can be detected by observing when the addition of a single drop of standard acid causes a very faint orange hue to persist in the water being titrated (Boyd and Tucker 1992).

The titrations were conducted in a room where temperature varied from 22-24°C. The aliquots were stirred with a magnetic stirrer at a setting of 2 on the speed regulator dial and titrations were completed in 2 to 8 min – the higher the alkalinity, the longer the time necessary to complete the titration. For detecting the endpoint with the pH electrode, when the target pH was reached, more acid was added if the pH increased after 20 sec. This step was repeated until the pH remained stable for 20 sec. With the indicators, the same procedure was followed in reaching the desired endpoint color. Once the desired endpoint was reached for the indicators, the pH of samples was measured immediately. Total alkalinity was calculated by the equation:

$$\mathrm{TA} = \frac{(\mathrm{V})(\mathrm{N})(50)(1{,}000)}{\mathrm{V}} \tag{4}$$

where TA = total alkalinity (mg/L as $CaCO_3$); V = volume of acid used in the titration (mL); 50 = mg $CaCO_3$/meq; 1,000 = mL/L; and V = sample volume (mL).

Errors were calculated as follows:

$$\text{Absolute error (mg/L)} = \left|\text{Alkalinity of standard – measured alkalinity}\right| \tag{5}$$

$$\text{Relative error (\%)} = \frac{\text{Absolute error}}{\text{Alkalinity of standard}} \times 100 \tag{6}$$

Standards (20 L) containing 20, 80, and 240 mg/L total alkalinity were made from sodium carbonate and distilled water. As suggested by the United States Environmental Protection Agency (1977), seven replicates of each standard were analyzed for total alkalinity by the different methods of endpoint detection in order to obtain reliable estimates of precision and accuracy for results obtained by the different methods of detecting endpoints.

An inter-laboratory comparison of the reliability of alkalinity analyses was conducted. A portion (500 mL) of each of the 20, 80, and 240 mg/L alkalinity standards was sent to 18 outside laboratories located in seven states of the United States for analysis by the specific procedure used at each laboratory. These laboratories included one state “Water Watch” program, one water and soil testing laboratory, two municipal water treatment plants, two university aquatic biology laboratories, and 12 research or extension laboratories in universities and governmental agencies that are involved in aquaculture. The concentrations were not revealed beforehand to the analysts and each laboratory analyzed three replications of each sample.

Seven graduate students in the aquaculture water quality laboratory at the Auburn University School of Fisheries, Aquaculture and Aquatic Sciences also analyzed three replicates of each of the three standard alkalinity samples by the pH method as well as the two commonly-used indicators – bromocresol green-methyl red and methyl orange – applying the appropriate pH and indicator color hues that had been previously determined by conducting the studies of endpoint pH described above. In the graduate student titrations, the same pH meter, indicator solutions, and standard sulfuric acid were used for conducting titrations with the same microburet. The graduate students were instructed as to the pH or indicator color hue to use for endpoint detection for each of the standards, but the students were unaware of the alkalinity concentrations. Data from the titrations by the different laboratories and students were used to calculate the mean, standard deviation of the individual measurements, coefficient of variation (relative standard deviation), and relative error from each standard by each method of endpoint detection.

Fifteen samples of water from aquaculture research ponds at the Auburn University E. W. Shell Fisheries Center and from commercial, ictalurid catfish ponds in Hale County Alabama were obtained. Each water sample was divided into two portions, one filtered through a Whatman No. 42 filter paper and one not filtered. The initial alkalinity was determined in triplicate on each sample using the best pH and colors of BG-MR and MO found after conducting the studies of endpoint pH, and the MP endpoint for titrations. A 1,500 mg/L total alkalinity standard was made with sodium carbonate and distilled water. Exactly 10.0 mL of the sodium carbonate solution were pipetted into two, 1,000-mL volumetric flasks and each flask made to volume with either the filtered or the unfiltered portion of a sample. This provided a 15 mg/L alkalinity spike, but diluted the alkalinity of the original sample by 10 mL or 1%. The percentage recovery of the spike, an estimate of accuracy (Boyd and Tucker 1992) was calculated as follows:

$$\text{Spike and recovery (\%)} = \frac{\text{Final alkalinity}}{(\text{Initial alkalinity } \times 0.99) + 15\ \text{mg/L}} \qquad (7)$$

Results

Evaluation of methods of endpoint detection

The change in pH with time following sudden neutralization of alkalinity in standards (Fig. 6) was rather slow revealing that carbon dioxide is not lost quickly from samples during titration. Of course, pH changed more quickly at the higher than at the lower stirring rate, but during the first 5 to 10 min of stirring, the change was not great in either case.

Measured alkalinity standards increased linearly (R^2 values of 0.958 to 0.995) as the titration of standards progressed from pH 5.2 to 4.2 (Fig. 7). The endpoint pH resulting in the nearest estimate of the standard concentration (Table 6) decreased with greater alkalinity. The pH values that would result from the quantities of carbon dioxide released during titration at different alkalinity concentrations – assuming no loss of carbon dioxide during titrations – were calculated with the mass action form of Equation 3 and found to be similar to pH values observed to give the best accuracies in titrations of standards (Table 6).

Because pH 4.5 often is used as the endpoint for titration of any alkalinity concentration, the differences between measured concentrations by titration to pH 4.5 and the best pH were compared and relative errors for the two methods calculated (Table 6). Compared to the best pH for the endpoint, titration to pH 4.5 gave higher results (1.39 to 1.89 mg/L) up to 50 mg/L alkalinity, was the best pH at 80 to 150 mg/L alkalinity, and gave lower results (-0.81 to -4.67 mg/L) at higher alkalinity. The relative errors, therefore, were usually lower for the best pH than for pH 4.5.

The colors of the several endpoints of BG-MR indicator were described by different names than those by Cooper (1941). The color was initially green, the first color change was to blue, the second to gray with a purple cast, the third to purple, the fourth to light pink, and the final to rose. The averages and standard deviations of pH values measured at each endpoint of BG-MR for the standards were blue, 5.15 ± 0.029; gray with purple cast, 4.83 ± 0.037; purple, 4.69 ± 0.034; light pink, 4.51 ± 0.032; rose, 4.35 ± 0.038. The variation in pH at endpoints resulted because the changes from one color to the next were subtle and difficult to detect. As a result, titrations could not be stopped at exactly the same pH in each sample of a particular concentration.

The endpoint color of BG-MR indicator resulting in best agreement between measured concentrations and standards varied with the alkalinity concentration (Table 7). For example, at 10 mg/L alkalinity, the first color change to blue gave the best results, while at 300 and 500 mg/L alkalinity, the final color change to rose provided the best outcome. The relative errors tended to be slightly larger than for the best pH, but only at 300 and 500 mg/L alkalinity was the error slightly over or near 1.0%, respectively.

The faint orange endpoint of MO provided closer estimates of the standard concentrations than did the distinct orange endpoint up to 80 mg/L alkalinity. The converse was true at higher alkalinity (Table 8). Relative errors for measurements using MO indicator were less than 1.0% between 80 and 300 mg/L. The pH of the faint orange endpoint was 4.58 ± 0.087 while that of the distinct orange endpoint was 4.31 ± 0.077. It should be noted that the distinct orange endpoint commonly is considered to have a pH of 4.5.

The methyl purple (MP) and xylene cyanol-methyl orange (XC-MO) indicators each had single and fairly distinct color changes. The endpoint pH values averaged 4.65 ± 0.119 for MP and 3.54 ± 0.127 for XC-MO. Alkalinity measured with MP agreed reasonably well with those of the standards, and the range in relative errors was 0.03% to 4.30% (Table 9). Because of its low endpoint pH, use of XC-MO indicator resulted in substantial overestimates of standard concentrations. The relative errors for this indicator varied from 5.35% to 190.1%.

The results of titrations to the best endpoint pH, best color for BG-MR and MO, and the endpoints of MP and XC-MO (Fig. 8) clearly show that all methods of endpoint detection other than XC-MO gave fairly close estimates of standard concentrations. Only for the 20 mg/L and 500 mg/L standards were differences ($P<0.05$) found by the Tukey HSD test among the four acceptable methods of endpoint detection. These differences were not large and all estimates –

excluding the ones made with XC-MO – were within ±3 mg/L of the standard alkalinity between 10 and 150 mg/L. At alkalinity of 300 and 500 mg/L, only those results obtained for the best pH were within ±3 mg/L of the standards. Of course, use of XC-MO indicator gave higher estimates of all standard alkalinity than did the other four methods.

Accuracy and precision

Seven replicate analyses of standards with 20 mg/L, 80 mg/L, and 240 mg/L total alkalinity were made using the pH and the indicator color that gave the best accuracy for each alkalinity. At a total alkalinity of 20 mg/L, the best pH and BG-MR indicator methods had similar means ($P>0.05$) and low relative errors (Table 10). The MO and MP indicators gave a higher mean ($P<0.05$) than the pH and BG-MR methods with relative errors over 10%. The XC-MO indicator had a greater mean and relative error than the other four procedures. All methods but the XC-MO indicator had similar means (P>0.05) and low relative errors at 80 mg/L total alkalinity. At 240 mg/L alkalinity, the results for the pH and BG-MR indicator methods were similar and of low relative error. The MO indicator technique had a higher mean and relative error, while the MP indicator resulted in a lower mean, but the relative error was similar to that for MO. The XC-MO indicator gave higher results and relative error than the other four methods.

Nine of the laboratories that participated in this study reported that they used standard methods for titrations (Eaton et al. 2005) for making the analyses, while the other nine used Hach water analysis kits (Hach Chemical Company, Loveland, Colorado, USA). Of the laboratories using standard titrations, five used BG-MR indicator, one used MO, one used MP, and five used pH 4.5 to signal endpoints. One laboratory did the analyses by both kits and standard methods resulting in 19 sets of results. The findings reported by the different laboratories were in general surprisingly inaccurate (Fig. 9), and the inaccuracies were mostly greater than would be expected from differences among endpoint detection methods. Analysis by t-tests indicated that the water analysis kits gave a higher average estimate for the 20 mg/L standard ($P<0.05$) than did the standard method, but differences in means did not occur for the other two standards. The average absolute errors for estimating standard concentrations were 5.85, 13.5, and 26.08 mg/L and average relative errors were 29.2, 16.9, and 11.0% for low, medium, and high alkalinity, respectively, for laboratories using standard protocol. For water analysis kits, the respective average absolute errors were 10.19, 12.60, and 38.27 mg/L and average relative errors were 51.0, 15.8, and 15.9%.

Precision was usually much better than accuracy, and most laboratories had coefficients of variation below 5% for the three replicate analyses of each standard alkalinity concentration. For all laboratories and standards, there were only six instances in which the coefficient of variation exceeded 5%. The average coefficients of variation were in order of increasing alkalinity 2.95, 1.52, and 1.13% for the standard method and 1.92, 3.15, and 1.09% for the water analysis kits.

The graduate students obtained remarkably better results for the alkalinity standards than did the participating laboratories (Fig. 9). Considering all the endpoint detection methods, the means for the 20 mg/l standard ranged from 18.16 to 25.91 mg/L. Corresponding ranges for 80 mg/L and 240 mg/L alkalinity standards were 80.45 to 85.35 mg/L and 220.34 to 247.43 mg/L, respectively. The smallest range in means was obtained using BG-MR at 20 mg/L alkalinity, but for the 80 mg/L and 240 mg/L alkalinity standards, the best pH endpoint resulted in the best results. The widest range in means was for MO indicator for all three standards. Average relative errors were below 5% in all instances other than for 20 mg/L alkalinity standard titrated to the MO endpoint.

The students also reported better precision than obtained by most of the laboratories. The coefficients of variation – all endpoint detection methods included – ranged from 0.57 to 25.47% (average = 4.64%) for the 20 mg/L standard, from 0.14 to 6.49% (average = 0.83%) for the 80 mg/L standard, and from 0.10 to 8.54% (average = 1.11%) for the 240 mg/L standard. Precision was similar among the three methods, but it was better for the medium and high alkalinity standards than for the low alkalinity standard.

Filtration of samples

Initial alkalinities of the 15 pond water samples measured before filtration ranged from a minimum concentration of 12.00 to 13.13 mg/L to a maximum concentration of 376.80 to 391.27 mg/L depending upon which method of endpoint detection was used. The corresponding alkalinity in the filtered portions of the samples ranged from a minimum of 11.80 to 13.29 mg/L to a maximum of from 369.73 to 392.67 mg/L. The mean coefficients of variation for triplicate analyses of unfiltered portions of samples ranged from 0.78% for the best pH to 1.55% for MP indicator, while the range for the filtered portions were from 0.58% for best pH to 1.98% for MP. According to Tukey's HSD test, the means of coefficients of variation for the analyses of the 15 samples did not differ ($P>0.05$) among the methods.

The differences between the mean total alkalinity of unfiltered and filtered portions of each sample were determined, and they were averaged to give the grand mean of the differences for each method (Table 10). There was a wide range in the differences obtained between the unfiltered and filtered portions of individual samples, but the differences tended to be in both directions. The number of samples for which the unfiltered sample had the higher measured alkalinity than did the filtered sample for the different methods for detecting endpoints were: best pH, 7; BG-MR, 8; MO, 10; MP, 10. Thus, there was a general tendency for measurements of a higher alkalinity in the unfiltered samples other than when using pH to detect the endpoint. Nevertheless, the means of the differences were determined by the Tukey's HSD test to be similar ($P>0.05$) for the four methods. Moreover, the coefficients of variation for the mean differences also were similar among the four methods of endpoint detection (Table 11). Overall, filtration did not have a discernable effect upon measured alkalinities of samples.

Spike and recovery

Variation among spike and recovery estimates for the different pond water samples was not great and similar among the methods of endpoint detection. The ranges in spike and recovery for unfiltered and filtered portions of samples were, respectively, as follows: best pH, 93.75-100.42% and 96.63-100.19%; BG-MR, 96.45-102.06% and 95.62-103.29%; MO, 95.18-102.95% and 96.1-104.2%; MP, 94.37-102.55% and 94.83-102.84%. Thus, in the worst instances, the estimates were as much as 6% less or 4% more than perfect recovery (100%).

Mean spike and recoveries (Table 12) were quite good, being below but within 3% of complete recovery for unfiltered and filtered samples for all methods of detecting endpoints. The estimates of spike and recovery did not differ by t-tests ($P>0.05$) for unfiltered or filtered samples. Analysis by Tukey's HSD test also did not reveal differences in spike and recovery means among the four methods of endpoint detection on either unfiltered or filtered samples. The coefficients of variation were small ranging from 1.11 to 2.93% when all variations in the method of spike and recovery were considered.

An attempt was made to obtain correlation coefficients between initial sample total alkalinity (X) with either percentage recovery or the absolute difference of the percentage spike and recovery minus 100% as (Y). In both arrangements of the data, the SAS program would not fit a line or give a correlation coefficient. This was taken as an indication that no correlation existed between the initial alkalinity and the amount of the spike that could be recovered (or accuracy of alkalinity determinations). It appears, that overall, filtration does not improve spike and recovery results, and each of the four methods of endpoint detection are comparable with respect to accuracy as estimated by spike and recovery.

Discussion

The endpoint pH used for the total alkalinity determination can affect the accuracy of results. As can be seen from Fig. 6, a titration stopped short of the optimum endpoint pH will underestimate total alkalinity, while one continued beyond the optimum endpoint pH will overestimate total alkalinity. The same applies for titrations using indicators – stopping the titration before the correct hue or passing the correct hue will underestimate and overestimate alkalinity, respectively. Of course, the cause of the variation in pH endpoints is carbon dioxide released during neutralization of alkalinity.

Carbon dioxide also is produced when sulfuric or hydrochloric acid are standardized against sodium carbonate. The 0.020 N sodium carbonate solution used to standardize sulfuric acid in this study was equivalent to about 100 mg/L of alkalinity (0.02 meq/mL × 5.0 mL × 50 mg $CaCO_3$/meq = 5 mg $CaCO_3$; 5 mg $CaCO_3$ in 50 mL = 100 mg $CaCO_3$/L). Thus, the endpoint of 4.5 used in the standardizations was appropriate, because pH 4.5 was shown to be the best endpoint pH for alkalinity standards of 80 to 150 mg/L. Further verification of the standardization procedure was provided by standardizing sodium hydroxide solution against potassium acid and using it to standardize a sulfuric acid solution without carbon dioxide being released. The sulfuric acid that was 0.020 N against sodium hydroxide also was 0.020 N against the sodium carbonate standard. The important point is that when standardizing sulfuric acid against sodium carbonate, the endpoint pH should be appropriate for the alkalinity equivalent of the sodium carbonate standard.

The sodium carbonate used as a standard for ascertaining the normality of sulfuric acid was dried in an oven at 140°C to assure that it did not contain moisture absorbed during storage. Such moisture would cause less sulfuric acid to be required to reach the endpoint, and thereby result in overestimation of the normality of the acid. The necessity for drying is illustrated by the fact that sulfuric acid standardized against sodium carbonate that had not been dried was found to be 0.0191 N while against oven-dried sodium carbonate, it was 0.0189 N ($P<0.05$ for t-test). However, when the distilled water for making the sodium carbonate standard was un-boiled, no difference ($P>0.05$) could be detected in three separate trials for standardizing sulfuric acid against standard sodium carbonate solutions made with either previously boiled or un-boiled distilled water. This suggests that the traditional boiling step is unnecessary.

The pH endpoints and colors of BG-MR and MO indicators providing the greatest accuracy in analyses of total alkalinity standards varied with the concentrations of the standards. The estimates of standard concentrations obtained by these three methods of endpoint detection and with MP indicator (Fig. 7) usually did not differ, and the few differences were quite small. Moreover, at concentrations of 150 mg/L alkalinity and less, all methods gave an accuracy better than ±3 mg/L. But, at higher pH, such accuracy was achieved only by the pH method.

Analyses of aquaculture pond waters indicated that filtration did not affect the measured alkalinity concentration in samples. Means of the absolute differences between measured alkalinities were not significant when compared between unfiltered and filtered portions. But, the differences were not consistently in the same direction thereby accounting for the lack of difference between the absolute means. Although filtration does not appear necessary for obtaining reliable results for alkalinity, it does remove turbidity. Clearer samples are easier to titrate when using indicators to detect endpoints, because true indicator color is more likely to be expressed making the color change at the endpoint less difficult to discern. The spike and recovery estimates did not improve when filtered samples instead of unfiltered ones were used, and all four methods of endpoint detection provided a similar mean percentage recovery of the spike. Based on spike and recovery estimates, it should be possible to maintain an average accuracy of ±3 mg/L, but for some samples, a greater error could be expected.

The comment by Taras et al. (1973) that a precision of ±1 mg/L in total alkalinity determinations is possible with good laboratory technique does not appear from the present study to be a reasonable expectation. Such precision was not consistently obtained using any of the endpoint detection methods on alkalinity standards or pond water samples. Nevertheless, in most cases, the repeated measurements of alkalinity on individual standards using all four of the methods of endpoint detection had coefficients of variation less than 3%. Good precision is desirable, and a coefficient of variation of 3% is quite acceptable for most analytical procedures (Boyd and Tucker 1992). Moreover, maintaining accuracy is the primary goal; if precision is sufficient to obtain satisfactory accuracy, then it should be acceptable.

The pH probably provides the easiest way of attaining the desired endpoint in alkalinity titrations done on a laboratory bench. Of course, this method requires data on the initial sample alkalinity, but the analyst can estimate it when the solution being titrated reaches a pH of about 5.2 by reference to the titration volume. The titration can then be continued to the optimal endpoint pH. This procedure is facilitated by knowing the specific factor that should be multiplied by the titration volume of the acid being used to provide the total alkalinity concentration. Of course, the same general technique can be followed with indicators – the alkalinity can be estimated at the first color change of BG-MR and MO indicators, and the titration stopped or continued accordingly. The detection of the endpoint color is more challenging to recognize than is the measured pH for the endpoint. Of course, for alkalinity titrations done in the field, an indicator usually is the only feasible method of endpoint detection.

Despite the best pH method giving the best overall results when standards of 10 to 500 mg/L alkalinity are considered, it did not provide superior spike and recovery compared to that achieved by three indicators (BG-MR, MO, and MP) for water samples from aquaculture ponds. Because spike and recovery is an estimate of accuracy, best pH, the appropriate endpoint colors of any of the three indicators may be used with satisfactory results in aquaculture provided alkalinity determinations are made according to good technique. Of course, the traditional pH 4.5 endpoint was the best endpoint pH for standard alkalinities of 80 to 150 mg/L, but it gave high estimates of the standard concentration at lower pH and vice-versa. For those desiring a single endpoint to simplify the alkalinity procedure, MP is probably a better choice than pH 4.5 and especially for alkalinities below 300 mg/L.

The differences in total alkalinity concentration that can result from using different pH endpoints or different indicators do not seem of utmost concern considering the variation among alkalinity concentrations obtained on standards in the inter-laboratory comparison of alkalinity analyses. The likelihood of sending a sample to a laboratory at random and obtaining a result close to the actual total alkalinity concentration appears to be quite low, and this problem results from more serious issues than the method applied for endpoint detection.

Only one of seven non-aquaculture laboratories reported results from a water analysis kit, while eight of the aquaculture laboratories reported results acquired with water analysis kits. Thus, poor performance by the aquaculture laboratories – especially for samples of low alkalinity – may be partially explained by the use of water analysis kits. Nevertheless, previous studies (Boyd 1977, 1980a, b, and Boyd and Daniels 1988) showed that some kits – especially kits with small burets or digital titration devices – could provide accurate total alkalinity measurements on low alkalinity samples.

The graduate students generally provided more accurate results than did the participating laboratories. A combination of factors contributed to the students generally achieving greater accuracy than the participating laboratories. The students had all taken a class on water quality analysis no more than 2 years before. They were provided a standard sulfuric acid for which the normality had been recently and carefully determined; they used the same appropriately-sized graduated cylinder for measuring samples of alkalinity standards for titration, and they titrated samples with the same microburet while stirring them at the same speed with the same magnetic stirrer. Moreover, the students were instructed to stop the titrations at a particular endpoint – but, like the participating laboratories they were unaware of the alkalinity concentrations of the standards. In essence, analytical quality control was inherent in the students' efforts.

The comparison of accuracy between the participating laboratories and graduate students suggested a general failure to use good technique and to maintain quality control. Water quality analysts – especially those in aquaculture – often have limited training in analytical chemistry. This may be the reason that they tend to confuse accuracy (nearness to the true value) and precision (the repeatability of replicate measurements). The emphasis in most participating laboratories appeared to be on the latter. Samples were analyzed in triplicate, and similar results were taken as an indication of reliable work (both precision and accuracy). Apparently, only a few of the laboratories routinely analyzed samples of known alkalinity (standards) to assure these concentrations could be obtained by analysis. This issue was discussed with several analysts, and the usual attitude was that standardized titrating agents and prepared indicators were purchased, and duplicate analyses were conducted. For example, one participating laboratory that reported especially erroneous results was offered the opportunity to repeat the analyses. The response was that new indicator solution and standardized acid were purchased and the triplicate analyses of each sample provided almost identical results. The individual did not desire to do the sample again and was confident in the results. When another laboratory was informed of their poor performance, they responded that the standards provided them were likely incorrect and did not desire to re-analyze them.

Many water quality laboratories use macroburets that cannot be read with as great a precision and accuracy as possible with the microburet used in the current study. Some water analysis kits rely on drop counting for estimating titration volumes of the standard acid. These means of determining titration volumes favor high precision, but detract from accuracy. Each drop of acid is equivalent to several milligrams per liter of alkalinity. Thus, for a low alkalinity sample, it usually takes the same number of drops in each replicate analyzed for the sample. Likewise, samples of different but low alkalinity may require the same number of drops of acid. Of course, some water analyses kits employ a digital titration device that can dispense small volumes of titrating agents as accurately and precisely as can be done with a microburet.

The traditional rule in analytical chemistry (Griffin 1954; Boyd and Tucker 1992) is that where possible measurements should be precise and accurate enough to maintain the relative error within ± one part per thousand (±1 ppt). A buret calibrated with 0.1-mL graduations can be read to three decimal places, e.g., 10.1, but the level of the meniscus between two graduation marks can be estimated. Although this estimate incurs an error of judgement, e.g., a reading of 10.14 might actually be 10.13 or 10.15, the estimated value is considered a significant figure. To maintain the buret reading error at ±1 ppt or less, the buret reading in this example should be at least 10.00 mL. This volume of 0.02 N sulfuric acid – the normality often used in alkalinity titrations – is equivalent to 10 mg of alkalinity as calcium carbonate equivalent (10 mL × 0.02 meq H^+/mL × 50 mg $CaCO_3$/meq). A water sample for titration must contain at least 100 mg/L of alkalinity [(1,000 mL/L ÷ 100 mL) × 10 mg], if an error of ±1 ppt is obtained when reading a buret calibrated in 0.1-mL graduations. Of course, one may adjust either sample size, normality of acid, or use a microburet to avoid excessive error.

Measurement of sample volume is equally important. The sample should be measured with a device that results in error less than ±1 ppt. A 100-mL graduated cylinder calibrated to deliver 100 mL (not just to contain 100 mL) and having 1.0-mL graduations can be filled to 100.0 ±0.1 mL. This represents an error of ±1 ppt. It is important to measure samples properly and to use an adequate volume to achieve an acceptable error of measurement. Samples smaller than 100 mL can be pipetted with a volumetric pipet to assure a measurement with an error of ±1 ppt or less. One flagrant breech of proper technique is to use the printed volume calibrations often found on beakers or Erlenmeyer flasks to measure sample volume. Samples measured by the devices will be several milliliters greater or lesser than the graduation mark suggests, because the devices are not calibrated closely by the manufacturer.

The normality of sulfuric acid (or hydrochloric acid) used in alkalinity titrations – including those purchased as pre-standardized – should be verified initially and periodically. Where more than a single standard acid is used in a laboratory for titrating samples, e.g., 0.01 N for low alkalinity, 0.02 N for medium alkalinity, and 0.04 N for high alkalinity samples, care must be taken to assure that the different normalities are not confused for titrating samples or for calculating results.

There are several places where errors can be made in alkalinity measurements, and the best procedure is to have a system of quality control. It is suspected that the only method of quality control in most of the participating laboratories in the present study was to check duplicate analyses on samples – a practice that only verifies precision. Elaborate systems, such as Shewart quality control charts (United States Environmental Protection Agency 1977), may be used to verify precision and accuracy. However, in small laboratories that only conduct a few types of analyses and do not conduct analyses on a regular schedule, simpler approaches to quality control are possible.

A simple way of assuring the reliability of alkalinity analyses is to maintain one or more waters of known alkalinity concentration and make duplicate alkalinity determinations at weekly intervals. This procedure is illustrated in Fig. 10 with actual data collected over time in the aquaculture water quality laboratory at Auburn University. This graph represents a routine quality control effort not associated with the present study. The mean alkalinities represented by the line in the graph reveals that average alkalinity concentrations were consistently within ±1 mg/L of the actual concentration. The two dots on the graph for each week reveal that there usually was excellent agreement between duplicate analyses. A departure from the observed pattern signals that a problem exists within the procedure, and the source of error must be located and accuracy and precision restored.

In laboratories that do not make analyses on a regular basis, it may be more practical to check the reliability of titrations against a sodium carbonate standard whenever analyses are made. The spike and recovery technique used in the present study is also a method for ascertaining the reliability of analyses that does not require charting performance over time. Based on the results of this study, it would appear that a spike and recovery of no less than 97 to 98% would be a reasonable expectation.

Conclusion

The optimum endpoint pH for total alkalinity titrations decreased from 5.0 at 10 mg/L alkalinity to 4.2 for 300 mg/L alkalinity or more. The appropriate color changes for BG-MR and MO indicators – which are affected by pH – also varied with the initial total alkalinity of samples. Despite differences in pH values at endpoints for samples of different alkalinities, when the best endpoint pH, best color of BG-MR and MO, or the endpoint of MP were used in titrations of standard solutions, there were few differences between measured alkalinities and standard alkalinities – the accuracy was almost always better than ±3 mg/L. The exception was for XC-MO indicator that has a color change at such a low pH that it badly overestimated all alkalinity standards. Results of spike and recovery tests on aquaculture pond water samples also revealed that an accuracy of ±3 mg/L alkalinity could be achieved on either unfiltered or filtered samples by all four methods of acceptable endpoint detection. Although precision of measurements could not be consistently maintained below ±1 mg/L, coefficients of variation for repeated measurements usually were less than 5% for all methods of endpoint detection. Nevertheless, this degree of precision was adequate to achieve good accuracy that is the major concern in water analysis.

Variations in alkalinity measurement that could result from improper selection of endpoint pH (or color) were rather small – usually not more than ±5 mg/L. However, in the inter-laboratory comparison of the results of alkalinity determinations on standard solutions, most participating laboratories reported inaccurate alkalinities. These inaccuracies were much greater than could be expected because of issues related to endpoint variations. Nevertheless, the laboratories provided precise results and apparently confused precision with accuracy. It was clear that most of the participating laboratories did not have a satisfactory method of quality control.

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Table 6. Comparisons of calculated pH at endpoint versus best pH in titration for total alkalinity standards, and of differences and relative errors for titrations to pH 4.5 or to the best observed pH.

Standard alkalinity (mg/L)	Endpoint pH		Alkalinity difference (mg/L) (pH 4.5 – best pH)	Relative error	
	Titration	Calculated		pH 4.5	Best pH
10	5.0	5.02	+1.43	14.0	0.30
20	4.8	4.88	+1.39	7.15	0.20
30	4.8	4.78	+1.59	5.43	0.13
50	4.8	4.68	+1.89	3.70	0.01
80	4.5	4.58	0.0	0.04	0.04
120	4.5	4.48	0.0	0.48	0.48
150	4.5	4.44	-0.81	0.98	0.44
300	4.2	4.28	-3.77	1.92	0.67
500	4.2	4.18	-4.67	0.99	0.05

Table 7. Measured alkalinities obtained with the different endpoint colors of bromocresol green-methyl red indicators for titration of alkalinity standards and relative error of the best endpoint color for each standard.

Standard alkalinity	Measured alkalinity (mg/L)					Relative error
(mg/L)	Blue	Gray with purple cast	Purple	Light pink	Rose	for best color (%)
10	(9.92)	10.43	10.84	11.37	12.31	0.80
20	18.95	(19.89)	20.37	20.94	21.59	0.55
30	28.73	29.60	(30.14)	30.85	31.62	0.47
50	48.08	49.66	(50.23)	51.23	52.08	0.50
80	78.85	78.51	78.95	(79.89)	80.85	0.14
120	114.64	117.63	118.76	(120.10)	121.27	0.08
150	143.05	146.82	149.08	(150.51)	151.91	0.34
300	282.30	290.33	292.74	294.66	(296.79)	1.07
500	475.74	485.71	488.95	492.75	(495.17)	0.99

Table 8. Total alkalinity concentrations measured for alkalinity standards using faint orange and distinct orange endpoints of methyl orange indicator. Parentheses around means designate best agreement with the standard concentration.

Alkalinity standard (mg/L)	Measured alkalinity (mg/L)		Relative error for best color (%)
	Faint orange	Distinct orange	
10	(10.71)	12.03	7.10
20	(20.98)	25.04	4.90
30	(30.94)	32.23	3.13
50	(50.57)	52.34	1.14
80	(80.18)	82.42	0.08
120	118.98	(120.62)	0.52
150	147.40	(150.05)	0.03
300	295.00	(297.83)	0.72
500	490.72	(493.36)	1.33

Table 9. Total alkalinity concentrations measured for alkalinity standards using methyl purple and xylene cyanol-methyl orange as indicators.

	Methyl purple		Xylene cyanol-methyl orange	
Total alkalinity standard (mg/L)	Measured alkalinity (mg/L)	Relative error (%)	Measured alkalinity (mg/L)	Relative error (%)
10	10.43	4.30	29.01	190.10
20	20.23	1.15	32.20	61.00
30	30.41	1.37	39.00	63.33
50	50.41	0.08	58.60	17.20
80	79.73	0.03	97.00	21.25
120	118.52	1.23	140.67	17.22
150	151.09	0.73	170.63	13.75
300	295.80	1.40	323.93	7.98
500	488.93	2.21	526.73	5.35

Table 10. Ranges of individual analyses, means, standard deviation, coefficient of variation, absolute errors, and relative errors for analyses of seven replicates of three total alkalinity standards using different methods of endpoint detection.

	Endpoint detection method[1]				
Statistic	Best pH	BG-MR	MO	MP	XC-MO
	20 mg/L alkalinity standard				
Mean[2]	20.11 a	19.47 a	23.56 b	22.96 b	36.31 c
Minimum	19.80	19.18	22.77	20.0	35.2
Maximum	20.79	19.69	23.76	22.8	37.4
Standard deviation (mg/L)	0.36	0.15	0.36	0.893	0.770
Coefficient of variation (%)	1.79	0.77	1.53	4.26	2.12
Absolute errors (mg/L)	0.11	0.53	0.44	2.96	16.31
Relative error (%)	0.56	2.63	17.81	14.80	81.55
	80 mg/L alkalinity standard				
Mean[2]	79.37 a	79.41 a	80.13 a	80.08 a	96.61 b
Minimum	78.76	74.38	79.37	78.80	95.4
Maximum	79.68	80.73	80.79	82.80	98.4
Standard deviation (mg/L)	0.33	2.23	0.57	1.338	1.150
Coefficient of variation (%)	0.41	2.81	0.71	1.67	1.19
Absolute errors (mg/L)	0.63	0.59	0.13	0.08	16.61
Relative error (%)	0.79	0.74	0.16	0.10	20.76
	240 mg/L alkalinity standard				
Mean[2]	238.61 a	239.05 a	244.64 b	234.62 c	259.06 d
Minimum	237.92	237.51	238.32	232.9	254.8
Maximum	240.35	239.95	251.80	237.2	261.0
Standard deviation (mg/L)	1.07	2.23	0.57	1.661	2.440
Coefficient of variation (%)	1.78	2.81	0.71	0.71	0.94
Absolute errors (mg/L)	1.39	0.95	4.64	5.38	9.06
Relative error (%)	0.58	0.40	1.93	2.24	7.94

[1] BG-MR = bromocresol green-methyl red; MO = methyl orange; MP = methyl purple; XC-MO = xylene cyanol-methyl orange.

[2] Means represented by the same letter did not differ (P>0.05) as determined by the Tukey Ad Hoc Test.

Table 11. Evaluation of differences between total alkalinity concentrations measured by four methods of endpoint detection on triplicate unfiltered and filtered portions of 15 aquaculture pond water samples.

	Method of endpoint detection[1,2]			
Comparison	Best pH	BG-MR	MO	MP
Range of differences in mean alkalinity concentrations between filtered and unfiltered portions of individual samples (mg/L)	0.0-13.60	0.16-8.00	0.0-13.80	0-14.40
Grand mean of differences (mg/L)	2.14 a	2.11 a	2.72 a	2.38 a
Range of CVs of differences between filtered and unfiltered portions of individual samples (%)	0.0-2.93	0.01-6.10	0.0-7.28	0.03-5.26
Grand mean of CVs of differences (%)	0.44 a	1.45 a	1.32 a	1.43 a

[1] BG-MR = bromocresol green-methyl red; MO = methyl orange; MP = methyl purple.

[2] Grand means designated by the same letter did not differ at P = 0.05 as determined by Tukey's HSD test. Horizontal comparisons only.

Table 12. Means, standard errors, and coefficients of variation for spike and recovery estimates on unfiltered and filtered water samples from 15 aquaculture ponds.

Endpoint detection method[1]	Mean ± standard deviation[2,3]		Coefficients of variation	
	Unfiltered	Filtered	Unfiltered	Filtered
Best pH	98.90 ± 2.10	98.84 ± 1.10	2.12	1.11
BG-MR	97.29 ± 2.01	98.50 ± 2.66	1.42	2.70
MO	99.66 ± 2.04	98.79 ± 2.89	2.05	2.93
MP	99.58 ± 2.17	97.05 ± 1.42	2.18	1.42

[1] BG-MR = bromocresol green-methyl red; MO = methyl orange; MP = methyl purple.

[2] Differences between means for raw and filtered portions of samples did not differ for each method of endpoint detection (P>0.05) as determined by t-tests.

[3] Differences among methods of endpoint detection for raw and filtered portions of samples did not differ (P>0.05) as ascertained by Tukey's HSD range tests.

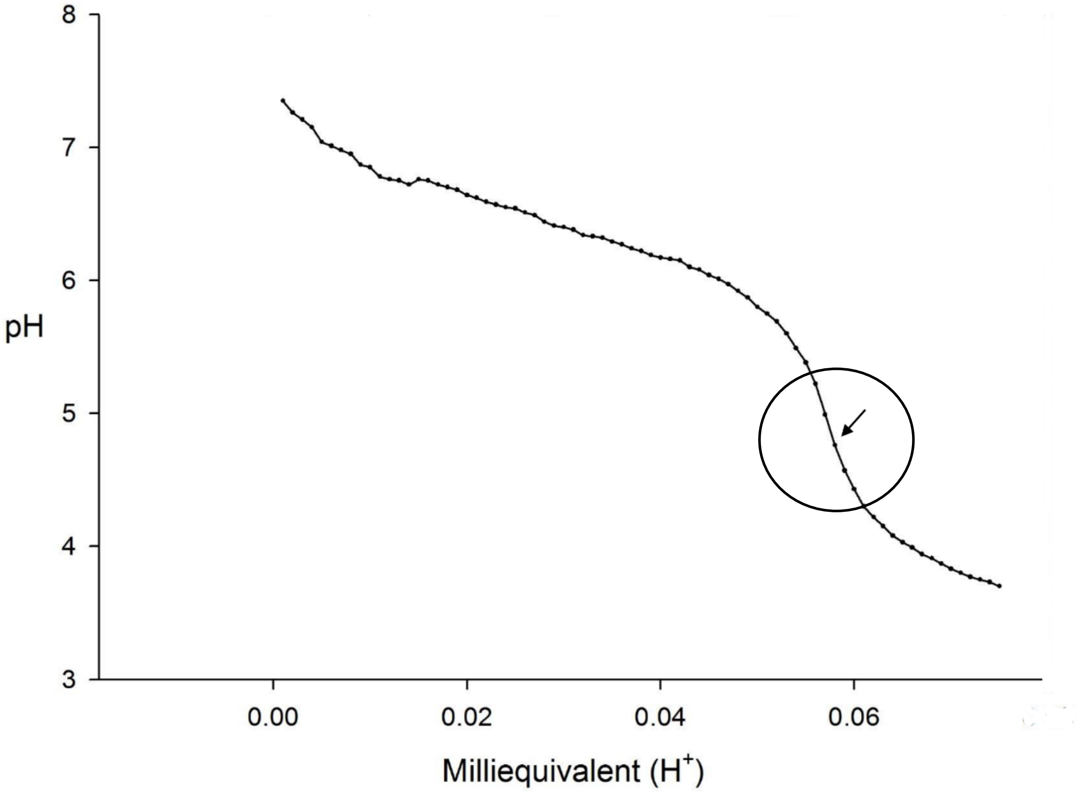

Figure 5. Titration curve for a sodium bicarbonate solution in which standard sulfuric acid was added in equal consecutive increments. The inflection point is marked with the arrow (see circle portion of curve).

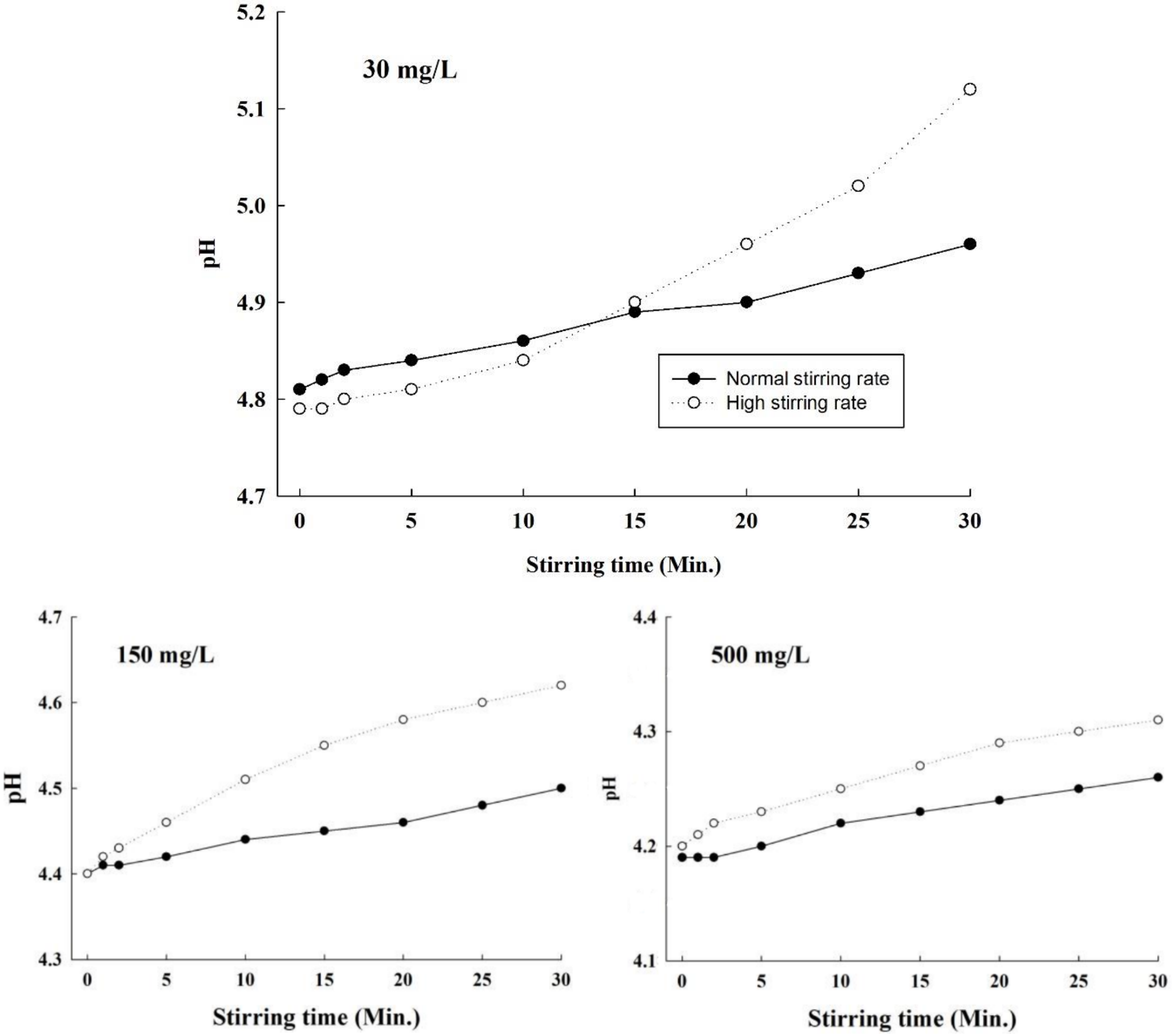

Figure 6. Change in pH over time following the sudden addition of enough acid to neutral the alkalinity in standards containing 30, 150, and 500 mg/L alkalinity. A normal and a high stirring rate was compared.

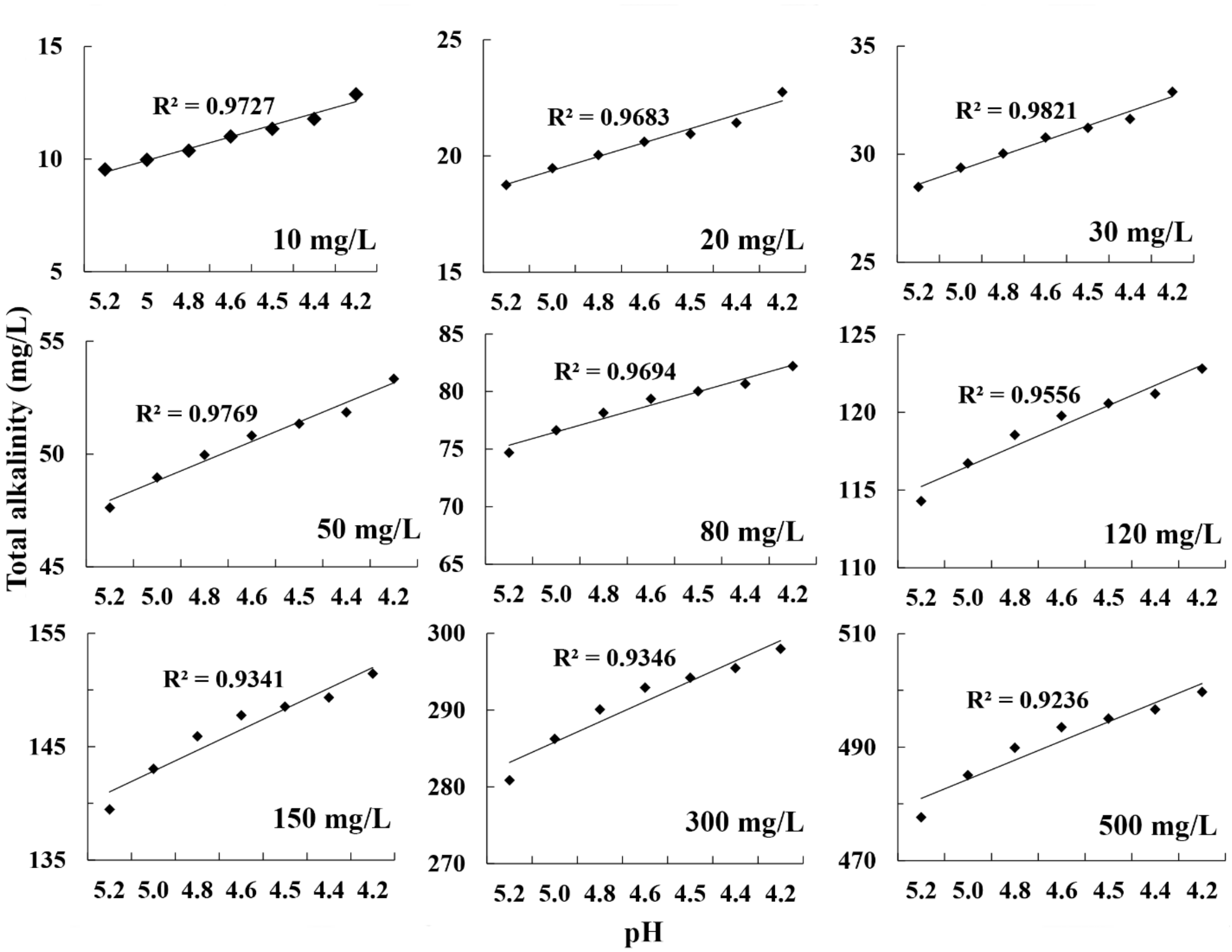

Figure 7. Measured concentrations for total alkalinity standard is based on different pH values as titration endpoints.

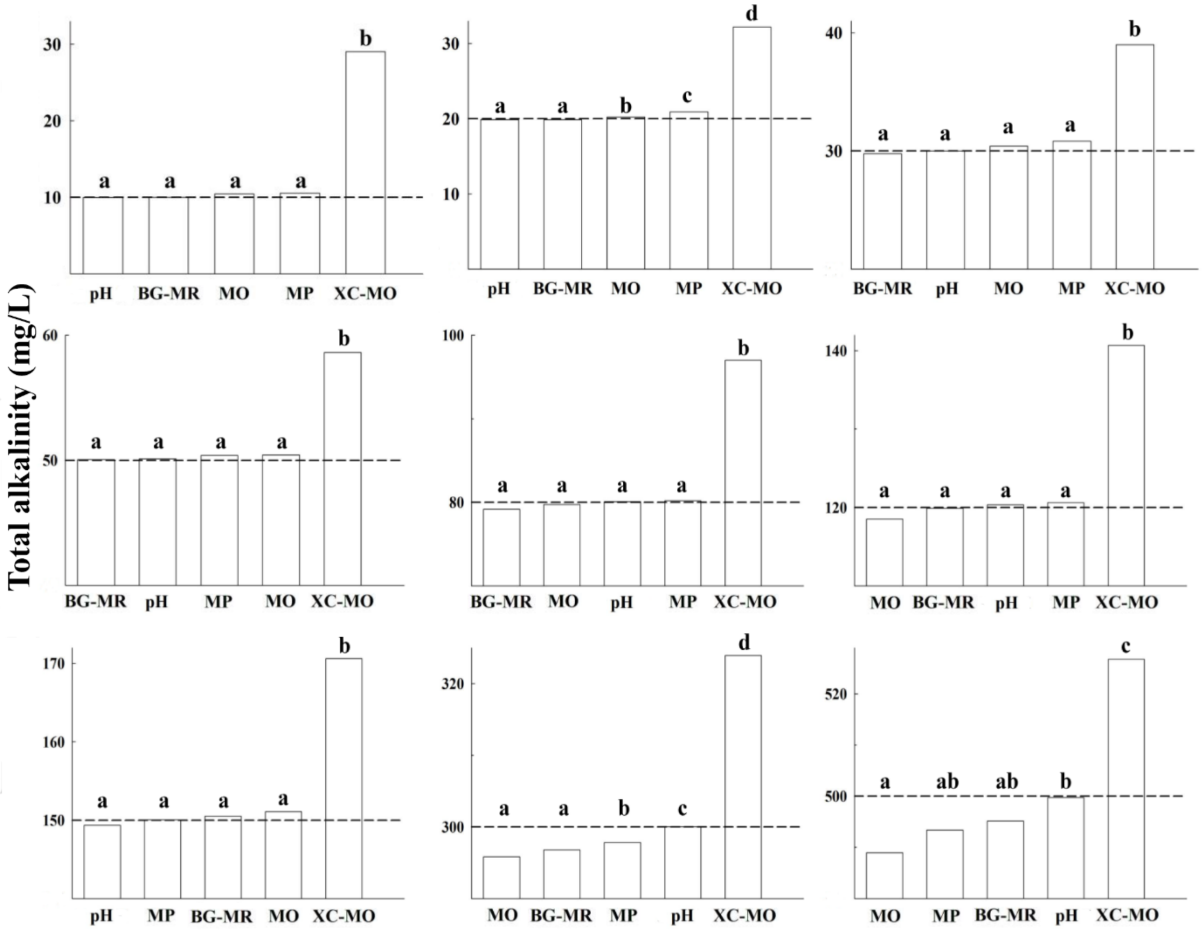

Figure 8. Measured concentrations obtained for total alkalinity standards at the best pH or indicator color for endpoints. The concentrations of standards are indicated by the dashed lines. Abbreviations: BG-MO= Bromocresol green-methyl red; MO= Methyl orange; MP= Methyl purple; XC-MO= Methyl orange -Xylene cyanol.

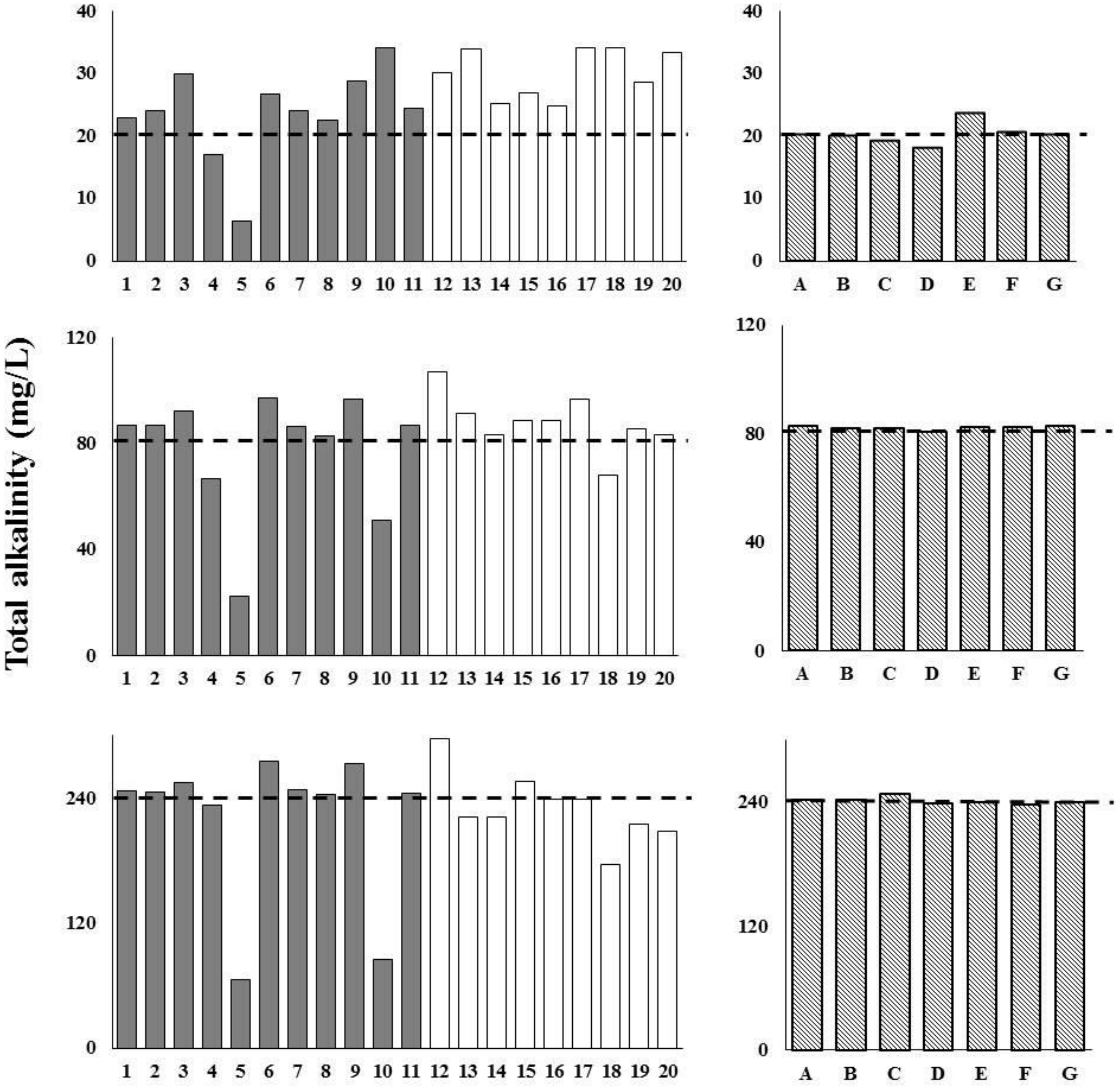

Figure 9. Range is concentrations of alkalinity concentrations reported by different laboratories for 30 mg/L, 80 mg/L, and 240 mg/L alkalinity standards.

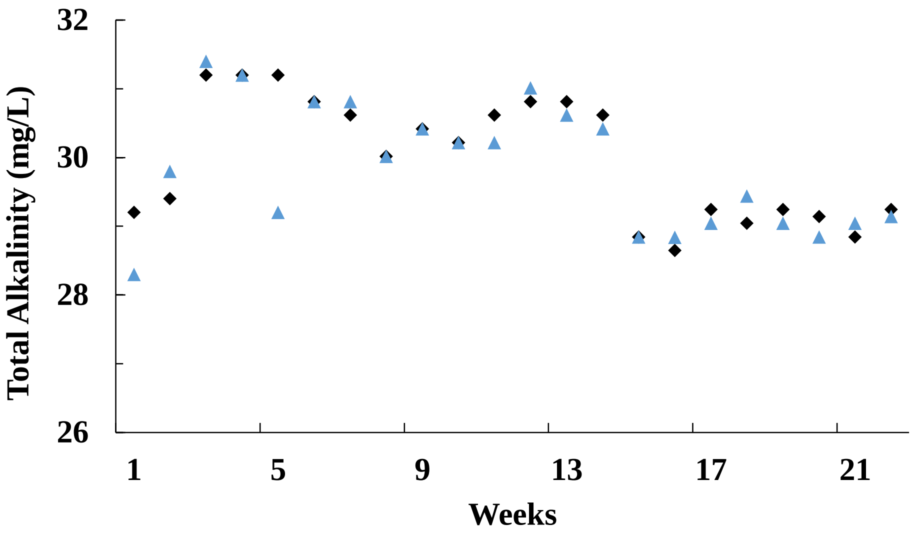

Figure 10. Result of a simple quality control that prepared from weekly, duplicate analyses of an alkalinity standards.

Alkalinity and Hardness Concentrations of Streams in Major Physiographic Areas of Alabama and Contiguous Areas in Mississippi: Are they increasing?

Introduction

A recent study (Kaushal et al. 2013) reported that alkalinity concentrations in many streams of the eastern United States (US) are increasing and especially in those draining areas where limestone is common. This conclusion was based mainly on Theil-Sen slope estimates (Theil 1950; Sen 1968) of trends in alkalinity concentration over the past 30 to 50 years for several major rivers. Stream alkalinization was attributed mainly to an increase in chemical weathering caused by acidic atmospheric deposition (acid rain) particularly in regions with abundant limestone and in urban areas with large amounts of exposed concrete. According to Kaushal et al. (2013), chemical weathering fractures limestone and removes small particles from concrete. These processes create additional and fresh surface area allowing greater dissolution of limestone and concrete particles, thereby neutralizing the acidity and also increasing the alkalinity of the water.

The usual response to acid rain is a decrease in the alkalinity of water (Haines 1981) as illustrated below for the neutralization of the anions bicarbonate and carbonate that usually are responsible for most of the alkalinity in water:

$$CO_3^- + H^+ = HCO_3^- \qquad (1)$$

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

In areas with abundant rainfall, and especially in calcareous areas, alkalinity anions are largely balanced by the cations calcium and magnesium – these two cations are the main source of total hardness in water (Boyd et al. 2015). Neutralization of alkalinity does not affect hardness, and stream acidification typically decreases the alkalinity: hardness ratio (Boyd et al. 2015).

A possible link between acid rain and alkalinization is a new and somewhat surprising hypothesis. Of course, if the Theil-Sen slope line analysis by Kaushal et al. (2013) is not considered, visual inspection of scatter diagrams of alkalinity concentrations over time for nine major rivers reveals tremendous variations, but a less convincing trend of increase. Moreover, Stets et al. (2014) suggested that stream alkalinization was resulting from diverse processes: decrease in acidic deposition, changes in agricultural production methods including greater use of agricultural limestone, less land devoted to agriculture in many areas of the US, and fewer but more responsible hydrologic modifications of stream catchments. Those authors basically considered alkalinization to be a recovery of streams from acidification in the past. Although stream acidification is decreasing in many areas of the US, there are areas in the US and in other countries where stream acidification is still occurring (Stet et al. 2014; Chen et al. 2005).

The increasing carbon dioxide concentration in the atmosphere has resulted in a greater equilibrium concentration of carbon dioxide in water (Boyd et al. 2015). Reactions of carbon dioxide with the minerals that produce alkalinity are illustrated below using calcium carbonate to represent limestone, calcium silicate, and two representative feldspars, the sodium calcium feldspar oligoclase and the potassium feldspar orthoclase:

$$CaCO_3 + CO_2 + H_2O = Ca^{2+} + 2HCO_3^-. \tag{3}$$

$$CaSiO_3 + 2CO_2 + 3H_2O = Ca^{2+} + 2HCO_3^- + H_4SiO_4. \tag{4}$$

$Na_{0.7}Ca_{0.5}Al_{1.3}Si_{2.7}O_8 + 1.3CO_2 + 1.96H_2O = 0.65Al_2Si_2O_5(OH)_4 + 0.7Na^+ + 0.3Ca^{2+} +1.4SiO_2$

$1.3HCO_3^-$. (5)

$KAlSi_3O_8 + CO_2 + 5.52O = K^+ + HCO_3^- + 2H_4SiO_4 + 0.5Al_2Si_2O_5(OH)_4$. (6)

Thus, increasing atmospheric carbon dioxide concentration also should contribute to stream alkalinization. Of course, dissolution of minerals containing calcium or magnesium are sources of hardness.

Two rivers in Alabama were among the major US rivers used as examples by Kaushal et al. (2013) for making the case for stream alkalinization. Of course, if alkalinization is occurring in large rivers, one would expect the alkalinity of tributary streams to be increasing as well. Results of a survey in 1973 of total alkalinity and total hardness concentrations in fish ponds and streams of Alabama and Mississippi were presented by Boyd and Walley (1975). Thus, the present study was conducted to compare current alkalinity and hardness concentrations in Alabama streams in different physiographic areas with those reported in the 1973 study. In addition, rainfall pH was monitored at Auburn, Alabama, to allow the present day average pH and range of pH for different storms to be compared with those reported for 1984 at the same location (Boyd 1985).

Background Information

Physiographic Regions

The five physiographic provinces of Alabama are depicted in Fig. 1. The geology of these areas is described in the Encyclopedia of Alabama (http://www.encyclopediaofalamaba.org) and in several other references such as Adams et al. (1926), Fenneman (1938), and Hunt (1967). The largest physiographical area in Alabama is the eastern section of the Gulf Coastal Plain. This region is relatively young geologically, and it was derived primarily from deposition of material eroded from geologically-older, physiographic provinces located north and east of it and at higher elevation. The Coastal Plain of Alabama consists mainly of formations made up of various mixtures of gravel, sand, silt, and clay, and massive formations of limestone are not common in most areas. The Piedmont Plateau developed from metamorphic rock, such as slate, phyllite, marble, quartzite, mica, amphibolite, and gneiss. There also are few deposits of limestone of significant expanse in this province. The Ridge and Valley developed on folded and faulted layers of sandstone, shale, limestone and dolomite and presents steep-sided valleys between ridges. The section of Alabama located in the Appalachian Plateaus – often called the Cumberland Plateau – has flat-topped plateaus separated by steep-sided valleys that cut through shale, limestone, and dolomite. The part of Alabama located in the Interior Low Plateaus is called the Highland Rim. It consists of ridges of sandstone with valleys cut through limestone formations.

Each of the physiographic provinces contains subdivisions that differ in edaphic and geological features. The Alabama Coastal Plain has been subdivided by different authorities and for various purposes to contain from five to eleven subdivisions. The Blackland Prairie that developed mainly on Selma chalk is the most unique of these subdivisions. It has many areas with outcrops of Selma chalk, and the soils typically are black and clayey and contain free carbonate (Dixon and Nash 1968). The edaphic and geological differences among the subdivisions of the other four physiographic provinces that extend into Alabama are not great. Therefore, only the major physiographic provinces and the Black Prairie area of the Coastal Plain were delineated in Fig. 11.

The Earlier Survey and Data

Sportfish ponds have been popular in Alabama and other southeastern states since the 1930s, and considerable research has been devoted to their management (Boyd 2014). With respect to alkalinity and hardness concentrations, Thomaston and Zeller (1961) demonstrated that ponds with <20 mg/L of these two variables should be limed to improve the effectiveness of nitrogen and phosphorus fertilization in enhancing phytoplankton productivity and fish production. In response to this finding, Boyd (1974) developed a method for estimating the lime requirement of bottom soil samples from ponds with low alkalinity for use by soil-testing laboratories in making recommendations on agricultural limestone treatment rates necessary to increase alkalinity and hardness above 20 mg/L in sportfish ponds. The survey of surface water alkalinity and hardness by Boyd and Walley (1975) – referred to as the 1973 study or data hereafter – was conducted primarily to determine the areas of Alabama and Mississippi in which ponds were most likely to require liming. Streams were included in the 1973 study mainly as a matter of interest to the investigators. The original sample log and sheets containing the results of the laboratory analyses from the 1973 study were lost as the result of a fire. Although the locations of the streams sampled were marked on maps in the resulting publication, it is not possible to ascertain their exact locations because of the small scale of the maps.

The largest part of both Alabama and Mississippi lies in the Gulf Coastal Plain, and on some physiographic maps, Mississippi is shown to be entirely in the Gulf Coastal Plain. But, a small area in Tishomingo County, Mississippi that borders on Tennessee in the north and Alabama in the east drains to the Tennessee River (McGregor et al. 2006). On some physiographic maps, this small area of Mississippi is shown as being in the Highland Rim. The flood plain of the Mississippi River forms a long crescent-shaped area beginning just north of Tunica, Mississippi, near the Tennessee border and extending southward reaching its greatest width slightly west of Holcomb and Carroltown, Mississippi, and then narrowing as it continues southward to Vicksburg, Mississippi. This area often is called the Yazoo Basin or the Mississippi Delta, and it is in part of the Mississippi Alluvial Valley section of the Gulf Coastal Plain.

The 1973 study included the Yazoo Basin as well as other subdivisions of the Gulf Coastal Plain in Mississippi. As a means of improving the delineation on maps of areas where ponds often need to be limed, the samples from the Gulf Coastal Plain of both states were segregated according to the physiographic subdivisions outlined by Hunt (1967) as follows: Fall Line Hills, Blackland Prairie, Flatwoods, Red Hills, Jackson Prairie, Southern Pine Hills, Pine Hills, Pine Meadows, Loess Hills, and Yazoo Basin. Three of these subdivisions – Jackson Prairie, Loess Hills, and Yazoo Basin – occur only in Mississippi, but the others are contiguous between the two states. The Blackland Prairie and Jackson Prairie developed on limestone formations (Hunt 1967; Dixon and Nash 1968), and the soils of the Yazoo Basin are deep, slightly alkaline, and high in calcium content (Pettry and Switzer 1996). Streams in these areas typically had alkalinity and hardness >40 mg/L. Alkalinity and hardness concentrations were often <20 mg/L in the other subdivisions of the Gulf Coastal Plain. Alkalinity and hardness concentrations were particularly low in the Southern Pine Hills and Pine Meadows – frequently <10 mg/L.

In the other physiographic provinces that occur in Alabama, the Piedmont Plateau often had surface waters with alkalinity and hardness concentrations <20 mg/L, but concentrations of these two variables tended to be considerably higher – often >40 mg/L – in the Ridge and Valley, Appalachian Plateau, and Highland Rim. No pattern in alkalinity and hardness could be discerned among the subdivisions within these physiographic provinces, and the samples were not assigned to physiographic subdivisions as was done for the Coastal Plain. No water samples were collected from the small area in Tishomingo County, Mississippi, that is sometimes shown as belonging to the Highland Rim.

Fish ponds and streams were designated in the 1973 study as open and solid dots, respectively, on four maps showing locations where alkalinity and hardness were <10 mg/L, 10-20 mg/L, 20-40 mg/L, and >40 mg/L. Streams had greater alkalinity and hardness concentrations than ponds when compared within sampling areas. The number of samples, means, and standard deviations for alkalinity and hardness for each of the subdivisions of the Coastal Plain and 0.each of the other four major physiographic provinces also were provided. However, no differences in the ranges in alkalinity and hardness concentrations were obvious between states for the contiguous subdivisions of the Coastal Plain, and the samples from the two states were combined for calculating averages and standard deviations. Of course, samples for the Piedmont Plateau, Ridge and Valley, Appalachian Plateau, and Highland Rim were only from Alabama.

Constructed fish ponds are extremely abundant across Alabama (Chaney et al. 2012), and they were the main focus of the 1973 study. They were not included in the present study, because these water bodies are filled primarily by storm runoff, and their water chemistry is strongly influenced by the chemical composition of watershed soils (Boyd and Walley 1975). Many fish ponds in Alabama also are limed routinely to increase alkalinity and hardness and improve the response of ponds to fertilizers applied to enhance primary productivity, the base of the food web culminating in sportfish production (Boyd 2014). Moreover, at normal or low flow, water in streams consists primarily of inflow from shallow aquifers – usually referred to as base flow (Yoo and Boyd 1994). This water percolated through the soil into underlying formations containing aquifers. Water in aquifers has relatively prolonged and intimate contact with minerals of soils and underlying geological formations. Non-flood-stage stream flow is more likely to exhibit differences in water quality resulting from the geological factors that define physiographic provinces and their subdivisions than is storm runoff and water stored in ponds.

The original data on the pH of rainfall collected at Auburn, Alabama, during 1984 also were lost as a result of the fire. However, the annual average pH was 4.51 and the pH range was from 3.52 to 5.61 (Boyd 1985). Water collected from daily rainfall amounts less than 1 cm were not included, because water from such events tended to be highly contaminated.

Materials and Methods

Stream Survey

The survey of Alabama streams described here was conducted mostly in 2014, but some samples were taken in 2015. This survey will be referred to as the present study. The approximate locations of the 1973 sampling locations were revisited as nearly as possible by reference to the small maps in Boyd and Walley (1975) and the memory of C.E. Boyd (personal communication).

Streams were sampled during periods of dry weather in spring and summer when flow was considered normal – neither near flood stage nor stagnant. Streams that appeared polluted and those with visible sources of possible pollution were not sampled. The majority of streams were in woodland, but some were in pasture and near row-crop agriculture. Of course, there may have been upstream pollution sources not visible from the sampling sites. This was the same approach followed in selecting streams for sampling in the 1973 study.

The GPS coordinates were obtained for each sampling site, and stream names (if available) and approximate highway locations also were recorded. Samples were taken from bridges using a plastic sampler attached to a rope. The samples were confined in 500-mL plastic bottles, placed on ice in an insulated chest, and transported to Auburn University for analyses that were made no more than 72-hr after collection. The number of samples for each physiographic sampling area is listed in Table 13. Each stream was sampled at a single location, so the number of samples equals the number of streams from which water was collected.

The water samples were analyzed for total alkalinity by titration to pH 4.5 with sulfuric acid of a known normality (always near 0.020 N), and for total hardness by titration with ethylenediaminetetraacetic acid (EDTA) of a known normality (always near 0.010 M) to the eriochrome black-T endpoint as recommended by Eaton et al. (2005). These were the same analytical procedures used in the 1973 study. Samples also were analyzed for pH with a glass electrode (Orion 3 Star, pH Benchtop, Thermo Scientific, Singapore) – a measurement not made in the 1973 study.

Other than in the Blackland Prairie, streams of the entire Coastal Plain area of Alabama had comparatively low concentrations of alkalinity and hardness in the 1973 study. Thus, for the comparison of data from the 1973 study with data collected in the present study, it was decided to combine the Coastal Plain data from all subdivisions other than the Blackland Prairie. This resulted in six physiographic sampling areas – those delineated in Fig. 11.

Averages and standard deviations for the 1973 data from the individual subdivisions of the Alabama and Mississippi Coastal Plain contiguous between the two states – excluding the Blackland Prairie – were combined for comparison with data collected from the same areas in the present study. This was done by multiplying both means and standard deviations for each subdivision by the corresponding number of samples, summing the products, and dividing the sums by the total number of samples (both states) for all subdivisions. The 1973 data from the Blackland Prairie were combined separately from the other contiguous subdivisions of the Coastal Plain in the same manner. Of course, the data from the 1973 study combined in this manner provided average alkalinity and hardness concentrations for the contiguous sampling areas of the two states.

In order to ascertain if there were differences in the average alkalinity and hardness concentrations of the contiguous sampling areas between the two states – a comparison not tested statistically in the 1973 study – samples also were collected from Mississippi in August 2015. The original sampling sites were revisited as nearly as possible and additional samples were collected (Table 13) and analyzed as described above.

Limestone Solubility

A sample of agricultural limestone from the Martin-Marietta Quarry, Auburn, Alabama, and a sample of food-grade limestone (Duda Energy, Decatur, Alabama) were obtained. The agricultural limestone was screened to pass 0.053-mm apertures, and the food-grade limestone previously had been processed to particles smaller than 0.037-mm. Fifty-gram aliquots of each of the limestone products were placed individually in 4,000-mL Erlenmeyer flasks filled with distilled water, the flasks were covered with parafilm, and allowed to stand on a laboratory bench where temperature was 23 to 27 ^{0}C. Gentle mixing was maintained in each flask with a small, aquarium air pump and air stone. At weekly intervals, 50-mL water samples were removed and analyzed for total alkalinity.

Rainfall pH

Rainfall was measured every 24 hr for a 1-yr period beginning on 20 June 2014 with a standard US Weather Bureau rain gauge (Ben Meadows, Atlanta, Georgia) mounted on a wooden platform and installed in an open, mowed, grassy area at the E. W. Shell Fisheries

Center, Auburn, Alabama. Rainwater for pH analysis was collected in a plastic container into which a large funnel was inserted. The container was checked daily to assure that it was free of contamination and washed with distilled water as necessary. Rainfall samples for daily rainfall greater than 1 cm were collected when the rain gauge was checked and analyzed within 1 hr for pH by glass electrode. Rainwater from smaller daily rainfall events was discarded, and the container washed with distilled water before being remounted in its holder. As done by Boyd (1985), direct pH averaging was used to obtain the annual mean pH. Direct pH averaging often is criticized; but, as explained by Boyd et al. (2011), this method is more appropriate for most studies of pH in nature than is transforming pH to $[H^+]$, averaging $[H^+]$, and calculating average pH.

Statistical Analyses

Calculations for means, standard deviations, coefficients of variation, and Tukey's Post Hoc range test were conducted with SAS programs (SAS Institute, Inc. 2004). As already mentioned, sample sizes, means, and standard deviations were presented for alkalinity and hardness of streams in each physiographic sampling areas of the 1973 study. Thus, it was possible to calculate variances for the 1973 sampling categories so that the means of those data could be compared with those of the present study by use of Tukey's Post Hoc range test and Welch's modification of Student's t-test (Welch 1947).

Results

Samples

Sampling locations in Alabama for the present study were plotted according to physiographic sampling areas (Fig. 11). The GPS coordinates for the 1973 study sampling locations were, of course, unavailable, and it was not considered feasible to enter their approximate locations on Fig. 11 by reference to dots on the small maps of Boyd and Walley (1975). The sampling locations for streams of the contiguous areas of the Coastal Plain and Blackland Prairie in Mississippi in August 2015 are not shown, but these samples were taken from the same general locations as those obtained in the 1973 study.

The average area represented by each sample was 758 km^2 in the 1973 study and 700 km^2 in the present study. The average area per sample also was fairly similar between Alabama and the contiguous sampling areas in Mississippi for the 1973 and present sampling efforts. Samples for the 1973 and present study did not, however, represent exactly the same locations in many instances. The samples were not evenly distributed over each sampling area, because of the arrangement of the highways and the distribution of streams. Moreover, the number of samples from a given physiographic sampling area was not in direct proportion to the land area of the sampling area in either study (Table 14). In the 1973 study, the Ridge and Valley and Highland Rim regions were the most overrepresented (312 and 404 km^2/sample, respectively), while the Appalachian Plateau was the most underrepresented (1,120 km^2/sample). The Appalachian Plateau and Coastal Plain were the most underrepresented in the present study while the Piedmont Plateau was the most overrepresented. Slightly more samples were taken in the present study than in the 1973 study (316 versus 292, respectively). Although this may appear to be a large sample size, there are about 70,000 km of perennial streams in Alabama (http://www.encyclopediaofalabama.org) and much additional stream distance occurs in the contiguous sampling area in Mississippi. Based on the assessments of stream length and order by Downing et al. (2012), there are probably a few thousand first or greater order perennial streams in the sampling areas. Thus, the sample sizes for the two sampling efforts were not particularly large.

Alkalinity and Hardness Comparisons

Samples collected in the present study from the Alabama Coastal Plain and Blackland Prairie did not differ in average alkalinity and hardness concentrations from samples collected from the contiguous areas in Mississippi (Table 13). Nevertheless, to avoid comparing data collected in the present study from Alabama with 1973 data that included Coastal Plain and Blackland Prairie sampling areas contiguous in Alabama and Mississippi, the Mississippi data collected in 2015 (Table 13) were combined with the Alabama data of the present study.

The average concentrations of total alkalinity for the six physiographic sampling areas for the 1973 data (Fig. 12) fell into three groups as determined by Tukey’s Post Hoc range test ($P = 0.05$) as follows: Coastal Plain and Piedmont Plateau (low); Ridge and Valley and Highland Rim (intermediate); Blackland Prairie and Appalachian Plateau (high). In the present study, the Blackland Prairie had greater average alkalinity than the other sampling areas, while the Appalachian Plateau was in the intermediate alkalinity group. The non-calcareous areas (Coastal

Plain and Piedmont Plateau) clearly had lower alkalinity than the other sampling areas that were calcareous.

The numerical values of mean alkalinity in the present study were slightly higher in the Coastal Plain and Blackland Prairie, but slightly lower in the other sampling areas than in the 1973 study. However, when compared by individual sampling area with Welch's t-test, the probability of no increase in alkalinity was 0.088 for the Coastal Plain, while the probabilities of no difference in means for the other sampling areas ranged from 0.284 to 0.849.

The 1973 data on hardness fell into two concentration categories with respect to sampling areas: Coastal Plain and Piedmont Plateau (low) and the other sampling areas (high). In the present study, the Coastal Plain and Piedmont Plateau had similar but low hardness. In the areas with high hardness, the Ridge and Valley, Appalachian Plateau, and Highland Rim had similar hardness, while the Blackland Prairie was higher in hardness than all other areas except the Appalachian Plateau. As with alkalinity, hardness was clearly lower for the non-calcareous areas than for the calcareous ones.

The numerical values for hardness means increased in the Coastal Plain, Piedmont Plateau, Appalachian Plateau, and Blackland Prairie, but decreased in the other two sampling areas, between 1973 and the present (Fig. 12). The P value for the t-tests was 0.001 for no increase in hardness for the Coastal Plain streams. The probability of no decrease of hardness in Highland Rim streams was 0.051. The other P-values fell between 0.366 and 0.525.

There was considerable variation in alkalinity and hardness of different streams within each sampling area as indicated by the wide standard deviations (Fig. 12). The coefficients of variation for alkalinity from the different physiographic sampling areas ranged from 52.5 to 96.0% (average = 66.4%) in the 1973 study and from 50.4 to 97.7% (average = 73.6%) in the present study. The variation in total hardness was: 17.8 to 95.6% (average = 57.4%) and 39.1 to 111.5% (average = 72.8%), respectively.

Because of the large amount of variation and the relatively small number of samples in some sampling areas, the average alkalinity and hardness concentrations were compared between 1973 and the present for calcareous areas, non-calcareous areas, and all samples combined (Fig. 13). The numerical values for averages of both alkalinity and hardness concentrations were slightly greater for the present than for the 1973 study for the three categories of sampling area combinations. The probability of no difference in alkalinity between 1973 and the present was less for the non-calcareous areas combined than for the calcareous ones combined (P of 0.098 versus 0.409). The probability of no increase in alkalinity over time was 0.116 of all sampling areas combined. The probabilities for an increase in hardness since 1973 were greater for hardness than for alkalinity. In fact, the probabilities of no increase in hardness for the non-calcareous areas combined and for all sampling areas were <0.05.

The ratio total alkalinity: total hardness in both the 1973 and present study (Fig. 14) did not reveal large differences among physiographic sampling areas other than for an elevated ratio in the Piedmont Plateau in the 1973 study, and a particularly low ratio in the Appalachian Plateau in the present study. The alkalinity: hardness ratio tended to be slightly higher in 1973 than at present for the non-calcareous areas, calcareous areas, and for all samples combined (Fig. 14). A statistical test of means for the alkalinity: hardness ratios could not be made, because Boyd and Walley (1975) did not provide standard deviations for means of the alkalinity: hardness ratio for the different sampling areas.

Stream pH

None of the streams sampled in the present study were highly acidic, pH below 6.0 was recorded in only nine streams, and the lowest pH was 4.21. The pH averages ranged from 7.08 on the Coastal Plain to 8.00 in the Blackland Prairie (Fig. 15). Coastal Plain and Piedmont Plateau streams had similar average pH, while streams of the other four sampling areas were of similar but higher pH.

Limestone Solubility

The equilibrium alkalinity for both limestone products was about 62 mg/L (Fig.16). Equilibrium was reached after about 8 weeks for the food-grade calcium carbonate and after about 11 weeks for the agricultural limestone. The faster acquisition of equilibrium by the food-grade limestone was no doubt the result of its finer particle size.

Rainfall pH

The pH of rainwater at Auburn, Alabama, fluctuated over time (Fig. 17) and ranged from 4.14 to 6.69. The lowest pH values typically were associated with the larger daily rainfall totals. The average, annual pH was 5.14 with a standard deviation of ±0.622. Rainfall of 141 cm for the 12-month period was very close to the normal annual rainfall of 144 cm at Auburn, Alabama (Boyd et al. 2009).

Discussion

Averaged across Alabama and contiguous physiographic areas in Mississippi, the alkalinity concentration in streams was 41.56 mg/L in the 1973 survey and 47.19 mg/L in the present study; the corresponding average hardness concentrations were 43.42 mg/L and 52.28 mg/L (Fig. 13). The probability of no difference between these means was 0.116 for alkalinity and 0.025 for hardness. These findings support the conclusion of increasing alkalinity in streams of the eastern US (Kaushal et al. 2013; Stets et al. 2014). The apparent increase in alkalinity observed in the present study was less than the increases in alkalinity estimated by Kaushal et al. (2013) from trends in river water alkalinity data extending back to the 1960s and 1970s. For example, graphs presented by those authors suggest that since 1970, the alkalinity of the Tombigbee River increased by about 16 mg/L at Coffeeville, Alabama, and that of the Alabama River rose by around 20 mg/L at Claiborne, Alabama.

The greater increase for hardness relative to alkalinity between 1973 and the present indicates that sources of anthropogenic acidity currently neutralize a portion of the alkalinity in stream water without causing a corresponding decrease in hardness. Thus, acidity – most likely from precipitation – lessens the alkalinity concentrations below those that would be expected provided carbon dioxide was the only source of acidity. This does not necessarily conflict with the Kaushal et al. (2013) hypothesis of acid deposition causing an increase in the solubility of limestone and a rise in stream alkalinity. Neither does it dispel the hypothesis of recovery of streams from past acidification (Stets et al. 2014) or of a combined influence of the two processes.

There is, however, a third possible reason for stream alkalinization. The carbon dioxide concentration of the atmosphere has increased from about 320 ppm in 1965 to around 400 ppm in 2015 at Mauna Loa Observatory, Hawaii (www.esrl.noaa.gov/gmd/ccgg/trends/). A rising atmospheric carbon dioxide concentration increases the equilibrium carbon dioxide concentration in water and should accelerate the dissolution of limestone and other mineral sources of alkalinity. The carbon dioxide concentration at saturation in water at 25°C was reported as 0.46 mg/L in the early 1980s (Colt 1984) and 0.57 mg/L in 2012 (Colt 2012). Boyd et al. (2015) calculated the equilibrium concentration of alkalinity from calcite in distilled water containing 0.46 mg/L carbon dioxide to be 50.0 mg/L, while at 0.57 mg/L carbon dioxide, the concentration of alkalinity would be 53.7 mg/L. The calculated estimate of 53.7 mg/L alkalinity for 0.57 mg/L of carbon dioxide is similar to the alkalinity concentration of 55.0 mg/L measured experimentally by Frear and Johnston (1929) for a calcite-water system in equilibrium with air containing 390 ppm carbon dioxide – about the same as the atmospheric carbon dioxide concentration in 2012. However, most limestone is not calcite and solubilities of limestone from various sources may differ slightly (Zo and Mbarawa 2009).

Boyd and Hollerman (1982) found the equilibrium alkalinity of a sample of agricultural limestone from the Martin-Marietta Quarry near Auburn, Alabama to be 57 mg/L in 1980. The equilibrium alkalinity measured for agricultural limestone from the same quarry and by the same method was 62 mg/L in 2014 (Fig. 5). The solubility of this limestone increased by 8.8% over the 34-yr period as the result of increased atmospheric carbon dioxide. This increase is similar to the calculated increase of 7.4% in equilibrium alkalinity in a calcite-water system that should result because of the increase in carbon dioxide concentration at saturation from 0.46 mg/L in the early 1980s to around 0.57 at present. Hardness was not calculated or measured in the alkalinity-carbon dioxide assessments mentioned above, but it would have increased by the same amount as alkalinity.

The concentrations of alkalinity and hardness in streams of calcareous areas often exceeded the equilibrium alkalinity of a limestone-water system open to the atmosphere. This can result because surface water often becomes supersaturated with carbon dioxide as a result of metabolic activities of aquatic organisms, and water infiltrating through the soil accumulates carbon dioxide from respiratory processes of soil organisms. Supersaturation of water with carbon dioxide accelerates its reaction with limestone allowing alkalinity concentrations far above those of a limestone-water system open to the air. Of course, the proceeding scenario assumes that the atmosphere is the only source of carbon dioxide to dissolve limestone. Carbon dioxide also is derived from organic matter decomposition and greater pressure in ground water allows a lighter equilibrium concentration of carbon dioxide (Boyd et al. 2015).

The increase in alkalinity in streams of this study averaged 13.5% across all sampling areas; 16.2% for non-calcareous areas combined, and 8.3% for calcareous areas combined. These increases are relatively small and subject to uncertainty, because of the limited number of samples and high degree of variability among streams. Nevertheless, the increase in the solubility of limestone and other mineral sources of alkalinity as a result of increasing atmospheric carbon dioxide concentration seems to be a plausible explanation for at least a portion of the apparent increases in alkalinity and hardness observed over the sampling area of the 1973 and present studies – certainly this explanation should be included as another possible cause of stream alkalinization.

The smaller percentage increase in average alkalinity for the calcareous areas likely is related to the equilibrium alkalinity concentration being attained. Moreover, water from limestone aquifers often becomes supersaturated with alkalinity when it enters streams because of equilibration with the air and other natural sources of carbon dioxide to the stream. This results in the precipitation of calcium carbonate and a decline in the alkalinity and hardness concentration. The larger percentage increase observed in the non-calcareous areas likely was a result of the alkalinity concentrations not reaching the equilibrium level for the mineral sources present and carbon dioxide availability. Of course, the observation of a greater percentage increase in average alkalinity for the non-calcareous areas did not result in a greater absolute alkalinity increase in streams of these areas. The increase in average alkalinity between 1973 and the present was 3.35 mg/L in non-calcareous areas and 7.37 mg/L in the calcareous areas. This is in agreement with the conclusion of Kaushal et al. (2013) that the greatest increases in alkalinization are occurring on catchments with abundant limestone lithology.

Efforts to control emissions of sulfur dioxide into the atmosphere have been relatively successful decreasing global sulfur emissions since the late 1970s (Smith et al. 2011). As a result, there has been a reduction in acidic, atmospheric deposition, and many ecosystems are recovering from past acidification (Brimblecombe et al. 2007). The pH of rainfall at Auburn, Alabama – and presumably throughout the area of the present study – has increased markedly above the pH values recorded at Auburn, Alabama, in 1984 (Boyd 1985). For example, the increase in rainfall pH at Auburn between 1984 and the present was 0.63 pH units. Nevertheless, the greater apparent increase in hardness than in alkalinity and the resulting decline in the alkalinity: hardness ratio between 1973 and the present (Figs. 13 and 14) suggest that anthropogenic sources of acidity still affect alkalinity and hardness concentrations.

Alkalinity: hardness ratios were near 1.0 in all sampling areas other than the Piedmont Plateau in 1973. This suggests that much of the alkalinity in the streams of the Piedmont Plateau derived from feldspars that had relatively large proportions of sodium and potassium relative to calcium and magnesium. In the Coastal Plains sampling area, the feldspars apparently contained mostly calcium and magnesium. The alkalinity: hardness ratio near unity in the calcareous areas observed in the 1973 study obviously resulted from limestone being the main source of both variables.

Particularly large declines in the alkalinity: hardness ratio that have occurred in the Coastal Plains, Piedmont Plateau, and Appalachian Plateau sampling areas since 1973 deserve mention. Alkalinity concentrations in streams of the Coastal Plains and Piedmont Plateau are naturally lower than those in streams of the calcareous sampling areas (Fig. 12). This may be the reason that the alkalinity: hardness ratio exhibited a decline in response to inputs of acidity in the Coastal Plains and Piedmont Plateau. Of course, this supposition also implies that acid deposition is greater at present than it was in 1973 despite the decrease in acidic deposition since the mid-1980s (Brimblecombe et al. 2007), and as confirmed for one location in Alabama in the present study.

The large decline in the alkalinity: hardness ratio in the Appalachian Plateau contributed strongly to the decline of the average alkalinity: hardness ratio in the calcareous areas combined (Fig. 14). Because no marked decline in the alkalinity: hardness ratio occurred in the other calcareous areas, the large decline in the Appalachian Plateau apparently was the result of a greater source of acidity than acidic deposition. There is considerable coal mining in the Appalachian Plateau in the Birmingham vicinity. Mine drainage may be the reason for particularly low total alkalinity: total hardness ratios in a few samples collected from streams in the coal-producing area that were mainly responsible for the low average ratio for the Appalachian Plateau. Of course, liming material must be applied to exposed mine spoil, mine-out areas, and discharge from other mines to avoid low pH and alkalinity prohibited by wastewater discharge permits. Neutralization of acidic discharge from coal mining operations result in an effluent with higher concentrations of hardness than of alkalinity, and this would be reflected in the receiving streams.

Conclusion

On average, alkalinity and hardness concentrations appear to have increased somewhat in small streams of Alabama between 1973 and the present. But, the increase in alkalinity is not as great as that reported for two larger rivers in Alabama by Kaushal et al. (2013). Moreover, rainwater pH at Auburn, Alabama has increased considerably since 1984. These observations do not disprove Kaushal et al.'s hypothesis of stream alkalinization being caused by weathering of limestone and certain other minerals in river catchments and of concrete in urban areas by acid rain, or Stets et al. (2014) assessment that streams are recovering from acidification of the past. The present study does, however, provide a third hypothesis for at least a portion of the observed increases in stream alkalinity. The rising atmospheric carbon dioxide concentration is increasing the carbon dioxide concentration in water, which, in turn, accelerates weathering of limestone and other minerals.

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Table 13. Surface areas, number of samples, and area per sample for physiographic sampling areas for streams in Alabama and contiguous reaches of sampling areas in Mississippi.

Physiographic sampling area	Land area[a] (km^2)	1973 (n)	1973 (km^2/sample)	2014-15 (n)	2014-15 (km^2/sample)
Coastal Plain (excluding Blackland Prairie):					
Alabama	72,920	98	744	88	829
Contiguous area in Mississippi	79,632	84	948	86	915
Combined	152,552	182	838	174	877
Blackland Prairie:					
Alabama	11,947	12	996	24	498
Contiguous area in Mississippi	5,946	9	661	13	457
Combined	17,893	21	852	37	483
Piedmont Plateau[b]	12,450	16	778	42	296
Ridge and Valley[b]	10,603	34	312	22	482
Appalachian Plateau[b]	19,048	17	1,120	20	952
Highland Rim of Interior Low Plateau[c]	8,893	22	404	21	423
Entire sampling area:					
Alabama	135,765	199	682	217	626
Contiguous area in Mississippi	85,578	93	920	99	864
Combined	221,343	292	758	316	700

[a] Determined by planimetry of a physiographic map.

[b] These physiographic regions do not extend into Mississippi.

[c] Small area of this region in Mississippi was not sampled.

Table 14. Averages and two standard errors for total alkalinity and total hardness concentrations for stream water samples taken in 2014-2015 from two physiographic areas in Alabama and their contiguous reaches in Mississippi.

State	n	Total alkalinity (mg/L)	Total Hardness (mg/L)
		Coastal Plain[a]	
Alabama	88	25.3 ± 5.28	31.6 ± 5.60
Mississippi	86	23.3 ± 4.46	28.9 ± 5.26
		Blackland Prairie[a]	
Alabama	23	146.1 ± 42.68	137.0 ± 40.76
Mississippi	13	110.0 ± 19.22	133.7 ± 27.00

[a] None of the means for alkalinity and hardness differed between states ($P>0.05$) as determined by t-tests.

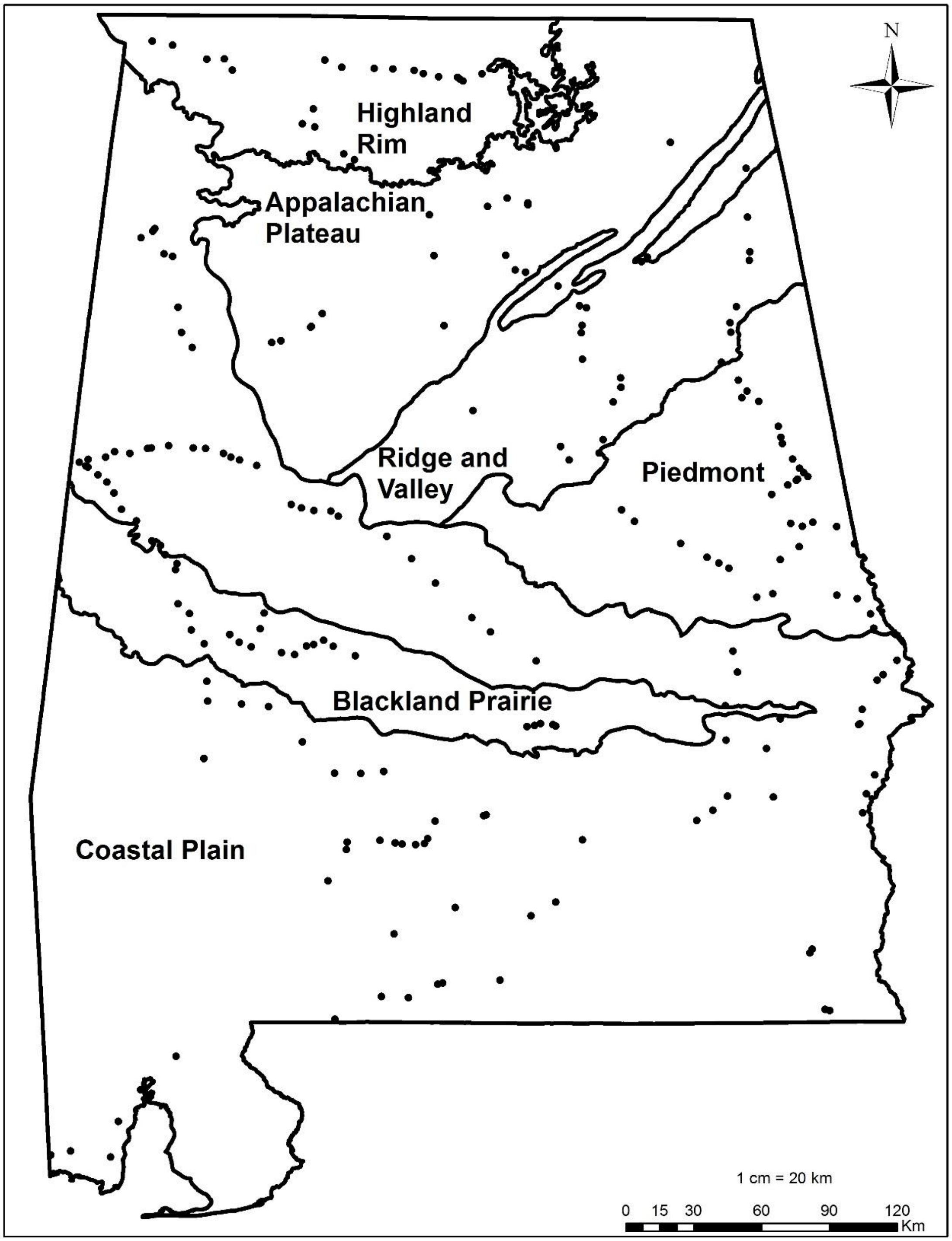

Figure 11. Physiographic sampling areas for assessing total alkalinity and total hardness concentrations in Alabama streams in 2014-2015. The dots represent the GPS coordinates of sampling locations.

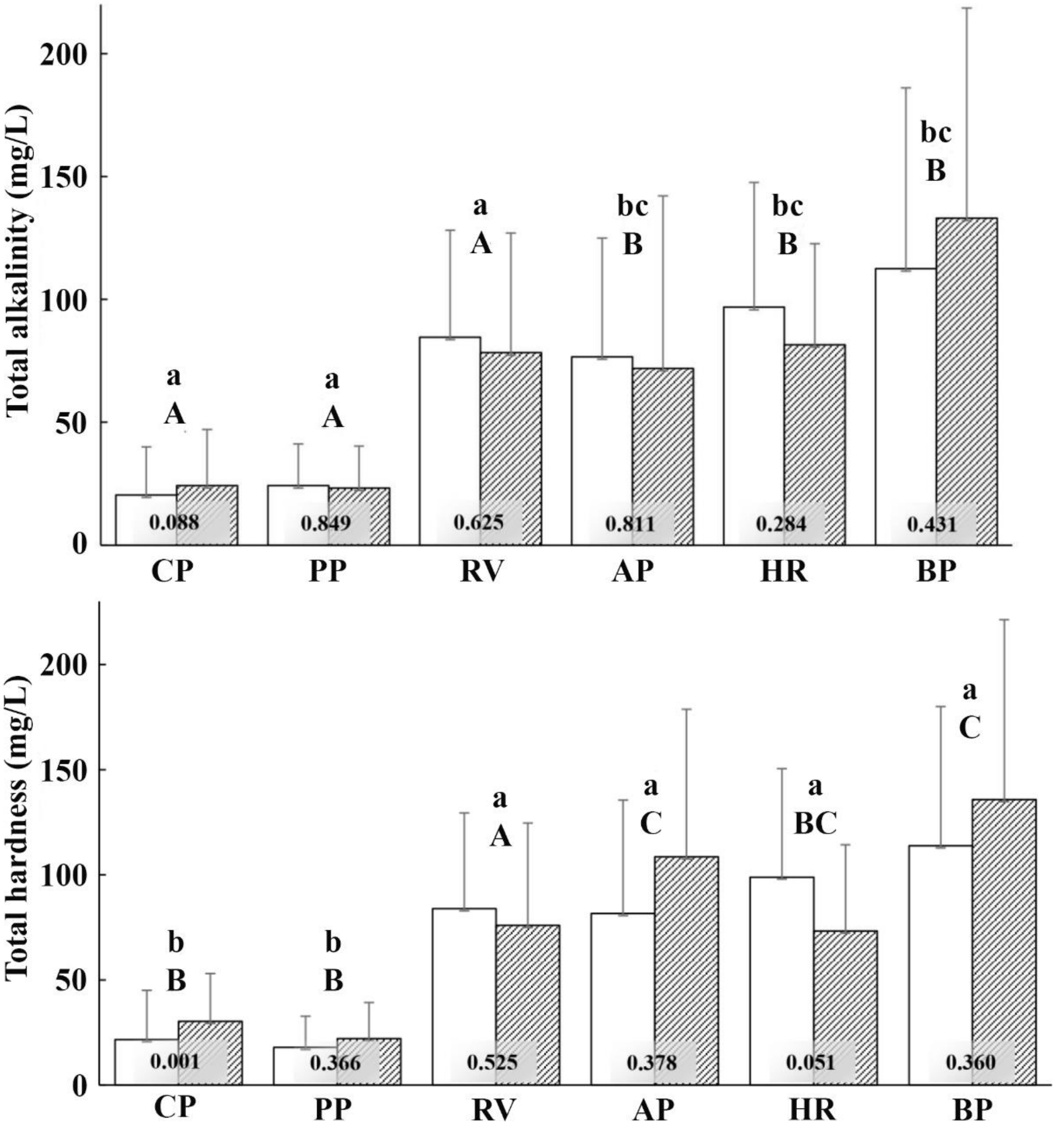

Figure 12. Average concentrations of total alkalinity and total hardness in Alabama streams in 1973 (clear bars) and 2014 (shaded bars). Vertical lines in ends of bars donate standard deviations. Abbreviations: CP=Coastal Plain (excluding Blackland Prairie); PP= Piedmont Plateau; BP= Blackland Prairie; RV= Ridge and Valley; AP= Appalachain Plateau; HR= Highland Rim. The results of a Tukey's Post Hoc multiple comparison test of means for difference at P=0.05 are indicated by lowercase letter (1973) and uppercase letters (2014-2015). The numbers inside the bars for each category are the probabilities of differences between the two sampling periods as determined by the Welch's t-test.

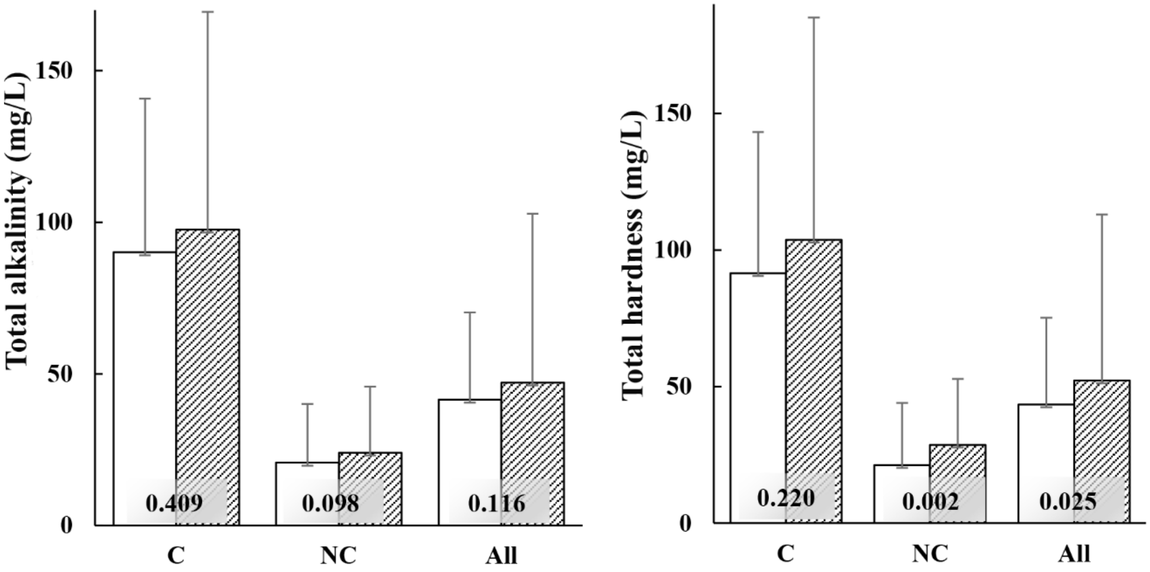

Figure 13. Average concentrations of total alkalinity and total hardness in Alabama streams in 1973 (open bars) and 2014-2015 (shaded bars) for calcareous areas (C), non-calcareous areas (NC), and all samples combined (All). Vertical lines in ends of bars denote standard deviations. The numbers inside the bars for each category are the probabilities of differences between the two sampling periods as determined by the Welch's t-test.

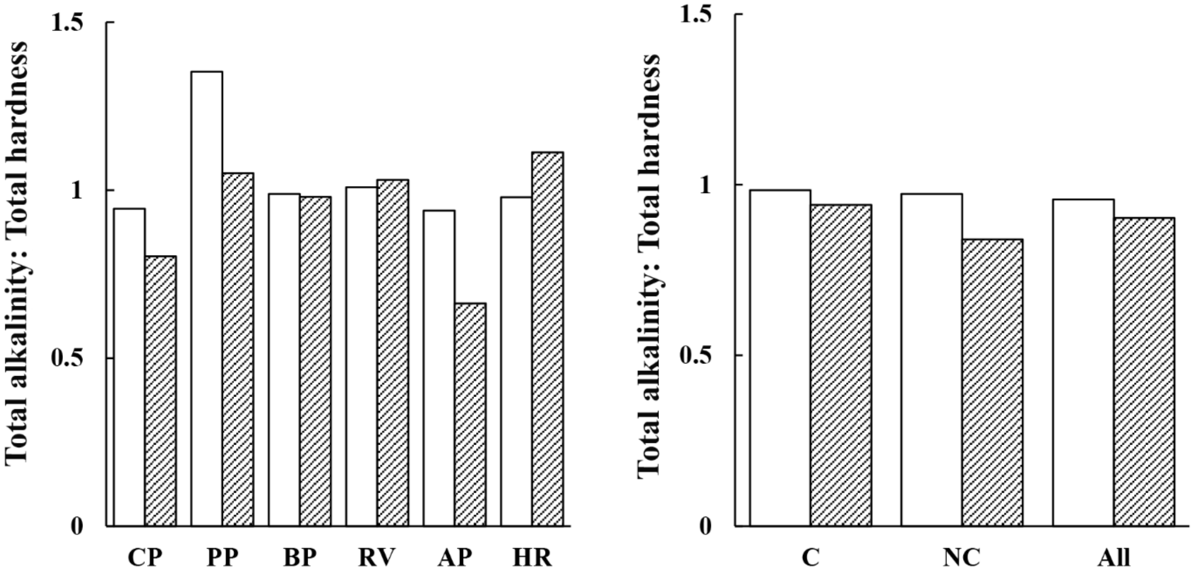

Figure 14. Ratios of total alkalinity: total hardness for Alabama streams in 1973 (open bars) and 2014-2015 (shaded bars) from different physiographic sampling areas (left) and calcareous areas (C), non-calcareous areas (NC), and all samples combined (All). Abbreviations: CP=Coastal Plain (excluding Blackland Prairie); BP= Blackland Prairie; PP= Piedmont Plateau; RV= Ridge and Valley; AP= Appalachian Plateau; HR= Highland Rim); Note: The CP and BP sampling areas included continuous areas in Mississippi.

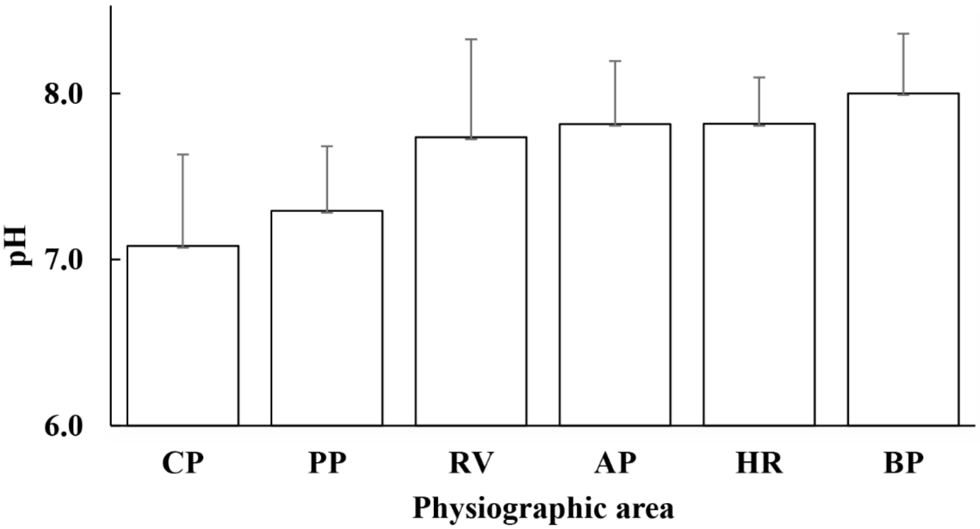

Figure 15. Average standard deviations for stream water pH in different physiographic sampling areas in Alabama (CP=Coastal Plain excluding Blackland Prairie; BP= Blackland Prairie; PP= Piedmont Plateau; RV= Ridge and Valley; AP= Appalachain Plateau; HR= Highland Rim)

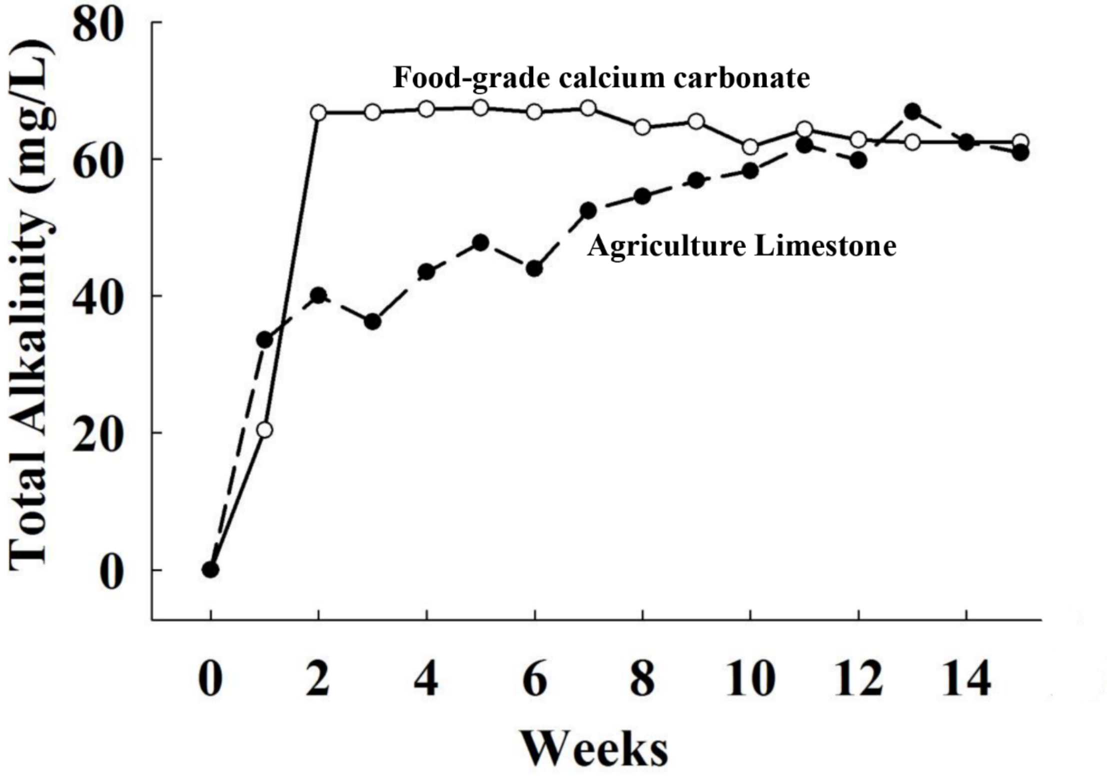

Figure 16. Total alkalinity changes over time for samples of calcitic agricultural limestone and food-grade calcium carbonate placed in open systems containing distilled water and allowed to attain equilibrium alkalinity.

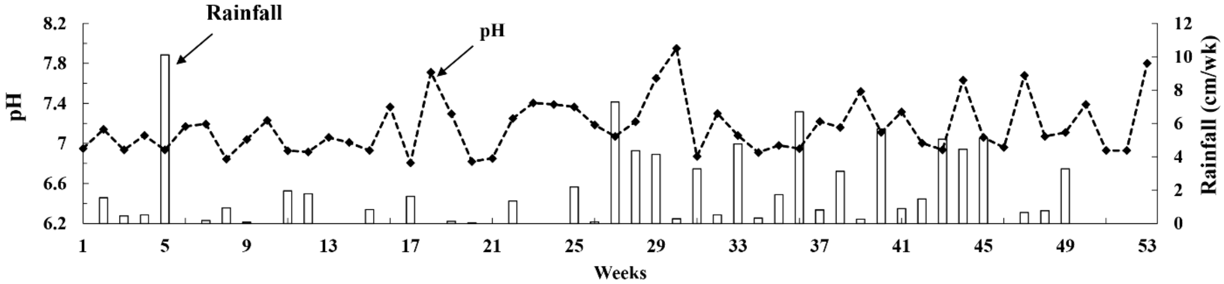

Figure 17. The pH of daily rainfall totals greater than 1 cm at Auburn, Alabama for a 1-year period beginning 20 June 2014.

Effects of Liming on Quality of Water Discharged from the Supply Reservoir at a Fisheries Research Station

Introduction

Acidic waters with low concentrations of total alkalinity and hardness are undesirable for fish culture. In fish hatcheries, pH below 5 greatly diminishes hatchability of eggs, and pH around 7 appears to be the best pH for hatchability (Mount 1973, Menendez 1976, Reynalte-Tataje 2015). Calcium also is important in the development and hatching of fish eggs. Minimum calcium requirements of 4 mg/L (10 mg/L calcium hardness) have been reported for channel catfish *Ictalurus punctatus* (Tucker and Steeby 1993) and of 10 mg/L (25 mg/L calcium carbonate) for brown trout *Salmo trutta*, respectively (Brown and Lynam 1981). However, Chatakondi and Torrans (2012) found channel catfish egg hatching success to be better at 30 mg/L calcium (75 mg/L calcium hardness) than at 10 or 20 mg/L of this cation (25 or 50 mg/L calcium hardness).

In pond culture of most warmwater species, a pH range of 6.5 to 8.5 is considered optimal, and total alkalinity and total hardness concentration should be 30 to 40 mg/L or more (Boyd and Tucker 2014). Alkalinity is especially important in ponds, because photosynthesis can cause afternoon pH above 9 in low alkalinity, poorly-buffered waters. Calcium and magnesium are essential for all life stages of fish. Calcium concentrations in the water of 1 or 2 mg/L and even lower concentrations of magnesium are adequate for freshwater fish, because they also can obtain these cations from food (Howells et al. 1983; Lovell 1998). Nevertheless, calcium concentration is important in ponds. When aquatic plants deplete the dissolved carbon dioxide, most species can use bicarbonate as a source of carbon for photosynthesis (Spence and Maberly 1985; Raven et al. 2012), resulting in the release of carbonate that hydrolyzes to increase pH. Calcium precipitates carbonate as calcium carbonate and limits the rise in pH.

Soft, acidic water can be remediated by treatment with agricultural limestone or other types of liming materials. Various types of liming mills have been used to monitor liming materials into water for fish hatcheries (Boyd et al. 2015). Sibrell et al. (2006) developed a fluidized limestone bed into which carbon dioxide was monitored to enhance the solubility of the limestone for treating acidic water for a fish hatchery. Ponds with low concentrations of alkalinity and hardness typically are treated with agricultural limestone (Boyd and Tucker 2014). At research stations where ponds are drained annually or more often, liming material usually must be applied each year, or even more frequently, to avoid low alkalinity, hardness, and wide daily shifts in pH.

An attempt is made to simplify the remediation of the acidic water supply for the ponds and hatchery at the Fish Genetic Unit (FGU) of Auburn University's E. W. Shell Fisheries Center (SFC). The reservoir through which all the source water passes before entering the pipe that delivers it to the FGU is treated annually with a truck load (18,000-22,000 kg) of agricultural limestone. This practice has been relatively successful by improving alkalinity and hardness in ponds and hatchery. But, calcium chloride solution must sometimes be dripped into the water after it enters the hatchery, and some ponds still must be limed.

The pH, alkalinity, and hardness of the water released from the pond treated with agricultural limestone has not been monitored, and little is known about the extent of fluctuations in these variables with season or following rainfall events. The present study was conducted to acquire this information and allow a better assessment of the effectiveness of the practice.

Hydrology of Study Area

The water supply for the FGU derives from the runoff of a catchment with an estimated 67.3 ha of land area and 5.01 ha of pond water surface (Fig. 18). The runoff from the catchment either flows into pond FP-12 or pond FP-14 or into a small unnamed stream, called Research Station Creek (RSC), which was dammed to make the two ponds. In addition, water from adjacent catchments may be conveyed via pipes by gravity flow from pond S-28 and pond S-29 to FP-14 and from pond S-30 to FP-12 that discharges into FP-14. The full pool surface areas and volumes of the five ponds are given in Table 15.

Water from FP-14 is transferred to the FGU via a pipeline. The water that overflows from S-28 and S-29 and the runoff from the area below these ponds, including the small area south and east of FP-14, is intercepted by the bed of the highway and directed into the RSC. This stream then passes through the FGU in a long culvert. After passing through the culvert, RSC receives overflow and effluents from the hatchery and ponds of the FGU and continues in its natural channel until entering FP-11 that is the reservoir for the many small ponds of the Production Research Unit (PRU) of the SFC. The catchment of RSC contains 242 ha in addition to the area that supplies water to FP-14.

The stream that flows from FP-14 is not gauged and water meters were not installed in any of the pipes that convey water to FP-12 and FP-14 or from FP-14 to the FRU. However, runoff for catchments in the Piedmont Plateau – in which the SFC is located – is 48.8 cm based on average stream discharge and 51.4 cm when calculated as the difference between average rainfall and average annual potential evapotranspiration (Boyd et al. 2009). Using an average of the two runoff estimates for the Piedmont Plateau of 50.1 cm/yr, the total runoff from the catchment land area for FP-14 should average around 337,000 m^3/yr. Annual rainfall averages 143 cm at Auburn, Alabama, while annual evaporation from pond surfaces averages 113 cm (Boyd et al. 2009). Thus, the two ponds store an additional 15,030 m^3 of water from rainfall. Water seeps from ponds, but much of the seepage passes under or through the dam (Yoo and Boyd 1994). The part seeping through the bottom of ponds enters the shallow water table from which it can infiltrate into streams.

On an average year about half of the water stored in the three ponds and adjacent catchments is transferred to FP-12 or FP-14. Thus, an estimated average of 459,649 m^3 of water enters FP-14 annually. Of course, FP-14 usually is maintained at full pool and much of the water overflows into RSC that also has input of runoff from other areas to RSC below FP-14. The amount of water diverted from FP-14 to the FGU is not monitored.

Materials and Methods

The FGU began operations in 1990, and with the exception of 2 year since, between 18,000 and 22,000 kg of agricultural limestone have been applied over the surface of FP-14 in the deep water area near the overflow structure and intake for the FGU. The average liming rate is an estimated 20,500 kg/yr for the 13 year for which agricultural limestone has been applied (Karen Veverica, Director, SFC, personal communication).

Discharge from FP-14 (Fig. 18, point 1) and two small streams not affected by agricultural limestone treatment (Fig. 18, points 2 and 3) were sampled between 20 June 2014 and 26 June 2015. Samples were taken weekly, but a rain gauge at the SFC was monitored daily, and samples also were taken in FP-14 discharge (point 1) after each day with measurable rainfall. Samples at point 1 represented discharge of FP-14 before it was diluted by water from sources not affected by the agricultural limestone treatment. The samples from points 2 and 3 served as the control as these streams were not affected by agricultural limestone treatment. Samples were dipped with plastic bottles and transported to the laboratory on the SFC for immediate analysis.

Analyses were made for total alkalinity (titration with standard acid to pH 4.5), total hardness ([titration with standard ethylenediaminetetraacetic acid (EDTA) to the eriochrome black-T endpoint], calcium hardness (titration with standard EDTA to the murexide endpoint), pH (Orion 3 Star, pH Benchtop, Thermo Scientific, Singapore), and conductivity (Orion 3 Star, Conductivity Benchtop, Thermo Scientific, Singapore) according to standard protocol (Eaton et al. 2005).

Results

There was little difference among the concentrations of the water quality variables in the two control streams, and the data for points 2 and 3 were averaged for each date. The annual means for the discharge of FP-14 and the control are presented in Table 16. Agricultural limestone resulted in higher concentrations ($P<0.01$) of all variables as compared to the concentrations in the control. Although the increase in pH as a result of liming in FP-14 was modest, the conductivity and concentrations of alkalinity, total hardness, and calcium hardness roughly doubled.

There was considerable variation in pH, conductivity, alkalinity, and the two forms of hardness – especially in FP-14 discharge – as indicated by the rather large standard deviations and the wide range in minimum and maximum values (Table 16). Therefore, the measured values of the variables in FP-14 discharge were plotted versus time and rainfall amounts (Figs. 19 and 20). The lower values tended to be associated with large amounts of daily rainfall or periods with frequent rainfall. Alkalinity and total hardness concentrations sometimes fell below 20 mg/L, and calcium hardness was occasionally below 10 mg/L. The pH did not fall below 6.5, but it was below 7.0 on numerous sampling dates. The conductivity was below 75 mg/L fairly often.

The seasonal averages and standard deviations for FP-14 discharge were as follows: pH, 6.97 ± 0.17 (spring) to 7.23 ± 0.29 (fall); total alkalinity, 33.47 ± 9.02 mg/L (winter) to 47.77 ± 6.41 mg/L (fall); total hardness, 38.21 ± 12.82 mg/L (spring) to 54.77 ± 8.60 mg/L (fall); calcium hardness, 16.94 ± 4.81 mg/L (spring) to 26.43 ± 5.57 mg/L (fall); conductivity 95.9 ± 17.7 μmho/cm (winter) to 130.3 ± 9.4 μmho/cm (fall). Range tests revealed that the seasons with the lowest and highest averages differed ($P<0.05$), but there were no differences between the values for the two seasons with intermediate averages. Moreover, the intermediate averages were sometimes not different from either the highest or the lowest average.

Rainfall for the 12-month sampling period was 141 cm or approximately equal to the normal rainfall of 143 cm at Auburn, Alabama. The seasonal trend in rainfall also was typical for Auburn with the highest occurring in winter and spring, intermediate amounts in the summer, and least in the fall.

Discussion

Total alkalinity in RSC was measured several times in the 1970s, because it was the water source for ponds in liming experiments. Typical concentrations ranged from 14 to 18 mg/L (Arce and Boyd 1975). The control streams have an average alkalinity of 19.09 mg/L (Table 16). The increase in alkalinity since the 1970s is small and could result from the rising atmosphere carbon dioxide concentration increasing the solubility of mineral source of alkalinity in soils and other geological formations on catchments (Boyd et al. 2015). Thus, the control streams appear to represent the natural baseline for total alkalinity and other variables that could be expected in RSC and in FP-14 without liming. The application of agricultural limestone to FP-14 caused concentrations of total alkalinity, total hardness, calcium hardness, and conductivity in the discharge of this pond to roughly double as compared to the control. But, there was less influence on the pH of the discharge other than for some instances of pH above 7.5 not noted in the control. The pH of water bodies depends more upon the carbon dioxide concentration than upon the alkalinity concentration (Boyd et al. 2015). The higher pH values in FP-14 discharge were thought to be related to elevated phytoplankton photosynthesis that withdrew carbon dioxide from the water. Despite the large increases in alkalinity, total and calcium hardness, and conductivity in FP-14 discharge, the concentrations of these variables were highly sensitive to rainfall events and sometimes diminished considerably after the larger rainfall events.

The most critical issue for the channel catfish hatchery at the FGU is calcium hardness, and concentrations below 10 mg/L should be avoided (Tucker and Steeby 1993). The hatchery typically has eggs in hatching containers from mid-May through late July. During this period, the pH of FP-14 discharge ranged from 6.7 to 7.7 and was acceptable for hatching eggs. However, the calcium hardness was below 10 mg/L on three sampling dates, and it was often below 15 mg/L (Fig. 19). Liming of FP-14 should be expected to minimize the number of episodes of low calcium concentration and lessens their duration, but liming alone is not adequate to avoid low calcium hardness entirely. The water entering the hatchery should be monitored for calcium hardness (or calcium ion) concentration, and calcium chloride solution or other calcium source added to the water.

The dilution effect of rainfall does not detract from the value of the agricultural limestone application for improving the discharge of FP-14 for use in earthen ponds at the FGU – provided that the ponds are not filled during a rainy period or soon after a large rainfall event.

Alkalinity can be neutralized by natural sources of acidity in FP-14, but hardness cannot. The difference of 24.08 mg/L between the average, annual total hardness of FP-14 and that of the control allows an estimate of the amount of limestone that dissolves annually. The water contained in FP-14 plus the annual discharge of this pond was estimated earlier as approximately 460,000 m^3/yr, while the agricultural limestone input is about 20,500 kg/yr. This amounts to about 11,000 kg of agricultural limestone (460,000 $m^3 \times 24.08$ mg/L $\times 10^{-6}$ kg/mg) suggesting that an amount of agricultural limestone equal to about half of that applied to FP-14 each year dissolves. It would seem that the residual agricultural limestone from previous years might allow the application rate to be decreased. But, there is a possibility that the deposition of sediment in the pond gradually buries the residual limestone, and iron oxide and other substances may coat the particles of residual limestone armoring them against dissolution (Boyd and Tucker 1998). It is unlikely that applying larger amounts of limestone each year would lessen the declines in variables associated with rainfall events.

The equilibrium alkalinity in a distilled water system open to the atmosphere and containing agricultural limestone from the same source as for that applied to FP-14 was 62 mg/L (Boyd et al. 2015). Thus, the discharge of FP-14 was seldom near saturation with alkalinity, and the average alkalinity of the discharge was well below saturation. Periodic application of organic matter to FP-14 to increase the availability of carbon dioxide for dissolution of agricultural limestone could possibly increase the alkalinity and both forms of hardness in the discharge.

Below sampling point 1, RSC receives effluent from the FGU and runoff from 242 ha of catchment below FP-14 before entering a 8.13-ha pond (FP-11) that is the water supply for the small research ponds on the PRU. Seasonal concentrations of total alkalinity measured at the overflow structure of FP-11 between spring 2006 and winter 2008 ranged from 21.46 to 31.35 with an average of 26.41± 3.41 mg/L (Soongsawang and Boyd 2012). Alkalinity concentrations in January and June 2015 at the FP-11 overflow structure were similar to those determined earlier, averaging 25.8 mg/L. The concentration of alkalinity in FP-11 appears to be about 6 to 7 mg/L higher than the average alkalinity of the control of 19.03 mg/L. Thus, some benefit of liming in FP-14 is realized as an improvement in alkalinity (and presumably other water quality variables) in FP-11 that supplies water to the PRU.

Conclusions

Large applications of agricultural limestone in FP-14 have roughly doubled average annual conductivity and concentration of total alkalinity, total hardness, and calcium hardness as compared to concentrations of these variables in control water sources. The pH of FP-14 discharge is not much greater than for the control, but pH was never below 6.65 and had an annual average of 7.12 in water released from FP-14.

The concentration of water quality variables decreased following large rainfall events and during periods of prolonged rainfall. In general, concentrations were lowest in winter when rainfall tended to be high and evapotranspiration low favoring greater runoff. Because of the effect of rainfall on water quality, calcium hardness concentrations were sometimes so low that they would be expected to adversely impact fish egg survival and hatching at the FGU hatchery. The decrease in alkalinity and hardness following rainfall events does not lessen the benefit of liming on the quality of FP-14 discharge for use in hatchery ponds.

The discharge of FP-14 ultimately reaches FP-11 that is the water supply for the PRU. Despite having been diluted by considerable runoff from the RSC catchment below FP-14, the total alkalinity of water in FP-11 was about 6-7 mg/L higher than in the control. This should reduce the amount of liming required for the small ponds of the PRU.

The results of this study show that liming of the water supply reservoir can be beneficial in lessening the need for liming in ponds at a research station. Although, it will not completely negate the possibility of excessively low calcium concentration, it will prevent dangerously low pH in fish hatcheries.

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Table 15. Areas and volumes of ponds that could contribute water to stream below sampling point 1.

Pond	Area (ha)	Volume (m^3)
FP-12	1.81	43,731
FP-14	3.20	84,132
S-28	1.92	60,397
S-29	2.93	63,900
S-30	5.34	88,942

Table 16. Mean annual concentrations, standard deviations, and ranges of selected water quality variables in discharge from a limed water supply pond and in un-limed, control streams.

	FP-14	Control	t-value
	pH		
Mean	7.12	6.69	8.27**
Standard deviation	0.31	0.30	
Range	6.65-8.46	5.0-7.30	
	Total alkalinity (mg/L)		
Mean	39.99	19.09	13.64**
Standard deviation	10.41	2.81	
Minimum Maximum	14.80-61.56	13.80-25.20	
	Total hardness (mg/L)		
Mean	43.11	19.03	11.68**
Standard deviation	13.51	3.79	
Range	16.02-72.07	12.0-30.0	
	Calcium hardness (mg/L)		
Mean	21.65	8.57	14.11**
Standard deviation	13.51	1.64	
Range	4.80-38.44	5.0-14.0	
	Conductivity (μmho/cm)		
Mean	10.99	57.0	15.12**
Standard deviation	23.6	5.6	
Range	57.2-158.3	47.9-73.4	

**t-value significant at P = 0.001.

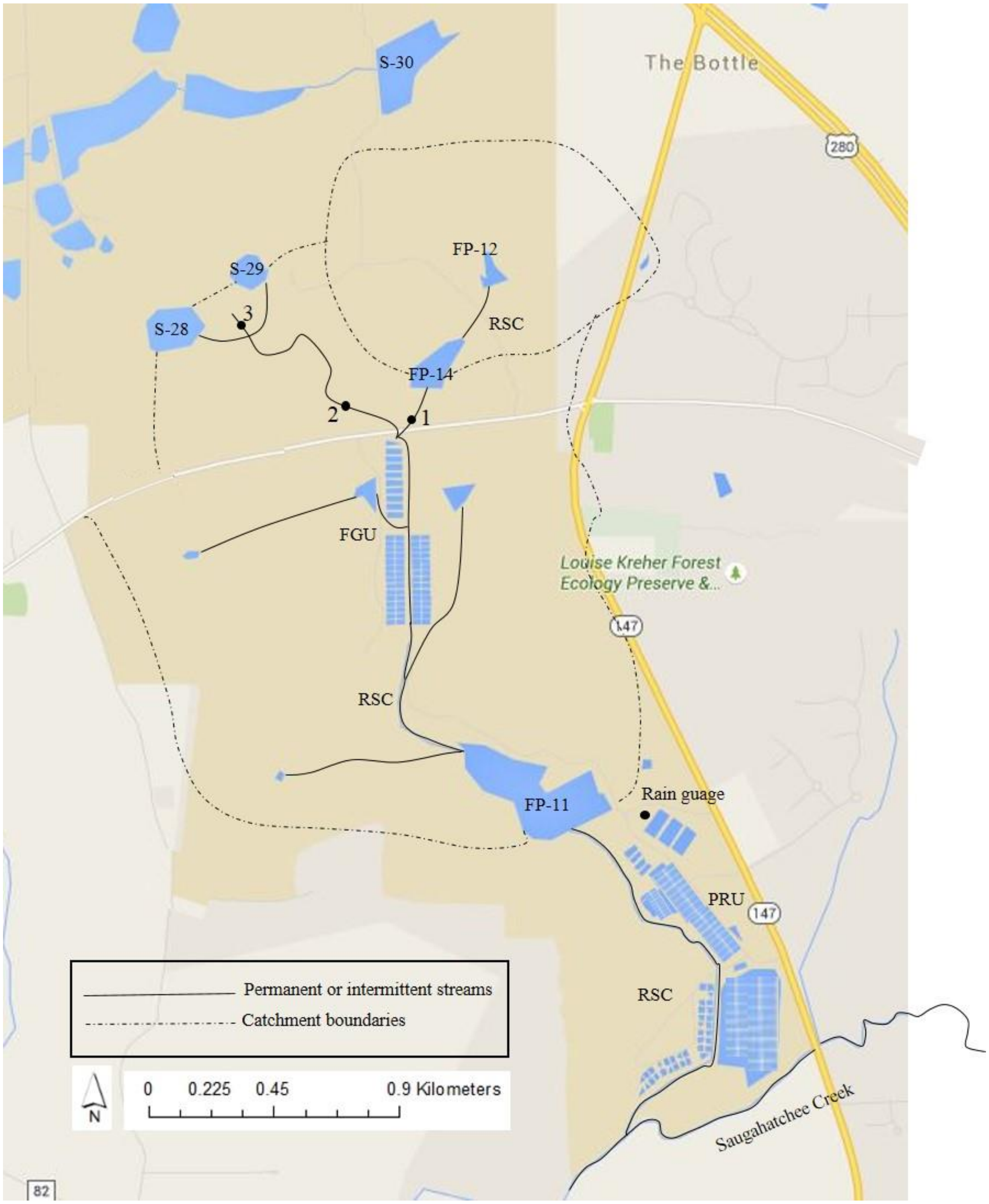

Figure 18. Map of sampling area showings FP-14, other selected ponds, catchments, and sampling locations for the study of the effect of limestone application in FP-14 on total alkalinity and total hardness at the E.W. Shell Fisheries Center, Auburn University, Auburn, Alabama.

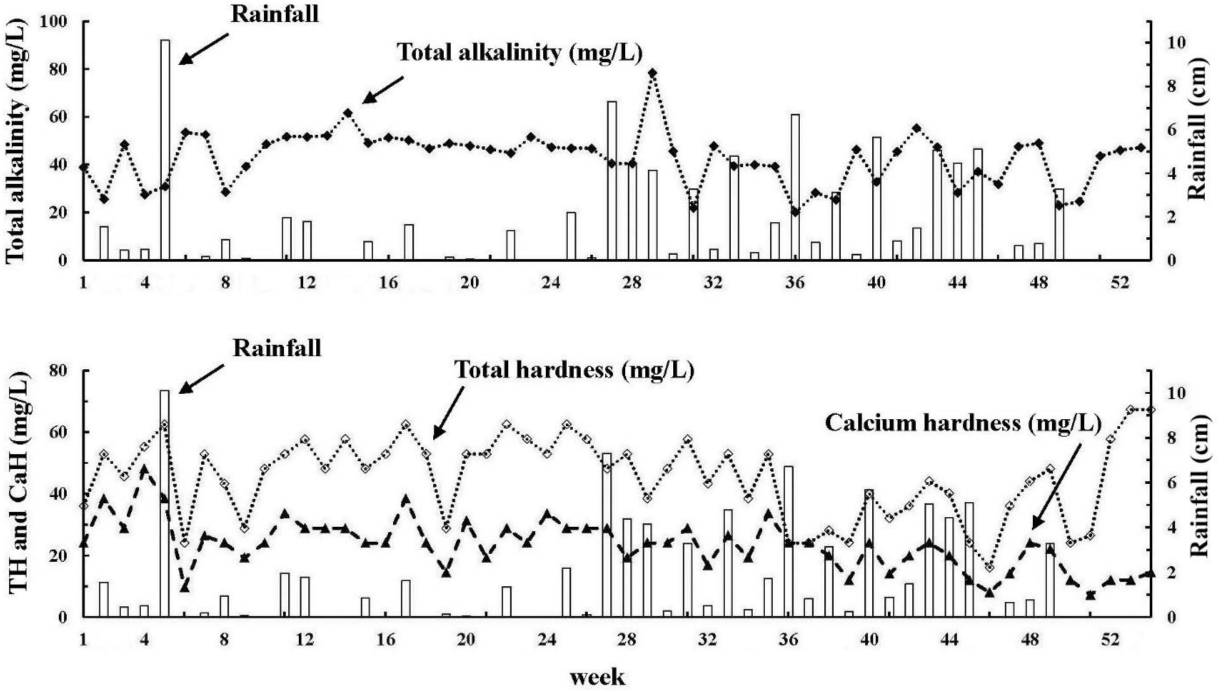

Figure 19. The total alkalinity (upper), total hardness, and calcium hardness (lower) of discharge from FP-14 in relation to weekly rainfall amounts between 29 May 2014 and 30 June 2015.

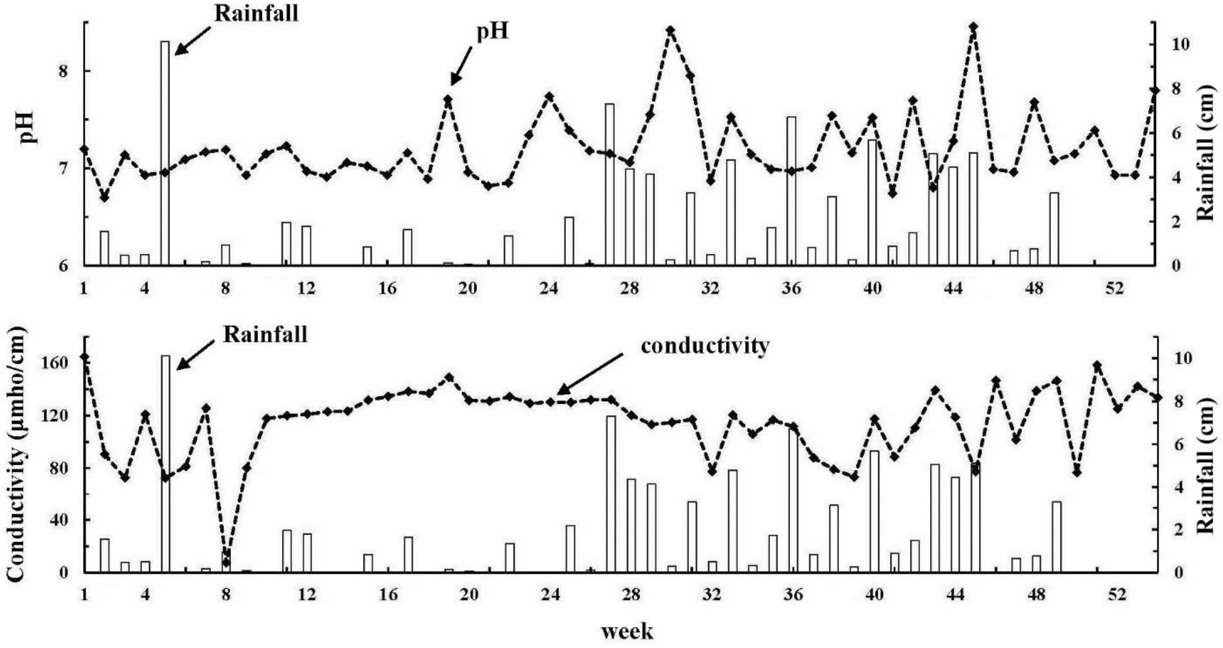

Figure 20. The pH (upper) and specific conductance (lower) of discharge from FP-14 in relation to weekly rainfall amounts between 29 May 2014 and 30 June 2015.

An Overview of pH, Conductivity, Alkalinity and Hardness in Alabama Stream Waters

Introduction

A recent investigation suggested that total alkalinity concentrations are increasing in streams in the eastern United States, and alkalinity increases in two Alabama rivers (The Alabama and the Tombigbee) were among the examples presented as evidence (Kaushal et al. 2013). Boyd and Walley (1975) made a survey of alkalinity and hardness concentration in Alabama streams in 1973. This earlier study allowed the opportunity to repeat the Alabama stream survey and to compare current alkalinity and hardness concentrations to those obtained in 1973.

The comparison of the results of the 2014 survey with those of the earlier study is the subject of another report. However, the 2014 survey resulted in extensive data on five common water quality variables – pH, conductivity, total alkalinity, total hardness, and calcium hardness – in streams of the major physiographic regions of Alabama. In the present report, the 2014 data will be assessed independently of the comparison of alkalinity and hardness concentrations between 1973 and 2014. This was done because many individuals concerned with the quality of surface waters in Alabama should find an assessment of the current status of these five water quality variables in Alabama streams interesting and possibly useful.

Materials and Methods

Samples were collected from the major physiographic regions of Alabama, and locations of sampling sites are depicted in Fig. 21. Streams were identified to physiographic sampling areas by visual observation and by reference to physiographic and highway maps, and GPS coordinates were determined at each sampling site. Streams located near obvious or possible sources of pollution were not selected for sampling. Most sampling locations were in wooded or pastured rural areas, but some were in areas with row crops.

Samples were taken from bridges using a water sampler attached to a rope, placed in plastic bottles, and held during transport in an insulated chest with ice. The samples were analyzed within 72 hour after collection by standard protocol (Eaton et al. 2005) for pH (Orion 3 Star, pH Benchtop, Thermo Scientific, Singapore), electrical conductance (Orion 3 Star Conductivity Benchtop, Thermo Scientific, Singapore), total alkalinity (acidimetry to pH 4.5 endpoint), total hardness (versenate titration to eriochrome black-T endpoint), and calcium hardness (precipitation of magnesium followed by versenate titration to the murexide endpoint).

Statistical analyses included calculations of averages, preparation of frequency distribution histograms and box and whisker plots, Tukey’s Honest Significant Difference (HSD) tests, simple linear, polynomial, and multiple regression. Statistical analyses including box and whisker plots were done with SAS programs (SAS Institute, Inc. 2004). The histograms were prepared using Microsoft Excel.

Results

Alabama has five major physiographic provinces as described in the Encyclopedia of Alabama (http://www.encyclopediaofalabama.org) and many other sources, such as Adams et al. (1926) and Hunt (1967). The Coastal Plain (CP) is the largest of these regions and comprises around 60% of the state's surface area. The CP typically is separated into six sub-regions: Fall Line Hills, Blackland Prairie, Flatwoods, Red Hills, Southern Pine Hills, and Pine Meadows (Hunt 1967). Geological features are fairly similar among five of these sub-regions, but the Blackland Prairie (BP) developed mainly on limestone formations making it quite different from the remainder of the CP. The four other physiographic provinces of Alabama – Piedmont Plateau (PP), Ridge and Valley (RV), Appalachian Plateau (AP), and Highland Rim (HR) of the Interior Low Plateaus Province – also have sub-regions. The differences among sub-regions in these provinces are not usually great, and the BP was the only sub-region of a physiological province that was considered unique enough to be classified as a separate sampling area for this study (Fig. 1). The physiographic sampling areas also were combined into a calcareous area where limestone deposits are common and a non-calcareous area. The calcareous area of Alabama includes the BP, RV, AP, and HR; the non-calcareous area consists of the CP (excluding the BP) and the PP.

Box and whisker plots of the water quality data are provided in Figs. 22 to 24. The line through the middle of each box is the median value of the measurements, the bottom of the box begins the second quartile of measurements (Q_2), and the top of the box ends the third quartile (Q_3) of the measurements. The length of the box represents 50% of the measurements and provides an estimate of variation among measurements and is called the interquartile range (IQR). According to the program used (SAS Institute, Inc. 2004), the lower whiskers in the box plots were calculated as $Q_1 - [1.58(IQR/\sqrt{n})]$; the upper whiskers were calculated as $Q_2 + [1.58(IQR)/\sqrt{n})]$. Values falling outside the whiskers are outliers and indicated as small circles. The diamond in each box indicates the mean, and an imaginary horizontal line bisecting the angles of the diamond strikes the Y axis at the mean.

The pH was above 6.0 for all samples (except the outliers) in all sampling areas, and average pH values were greater than 7.0. Streams of the CP and PP were lower in pH than those of the BP, RV, AP, and HR ($P<0.05$), but there were no differences in pH within the two groupings. Variation in pH within sampling areas was lowest for the HR and the PP compared to the other sampling areas.

The CP and PP streams were of similar total alkalinity and lower in mean alkalinity than streams of the other areas ($P<0.05$). Streams of the RV, AP, and HR did not differ in average pH, but the Blackland Prairie streams had greater mean alkalinity than did those of other sampling areas. Variability in alkalinity was lowest in the PP and CP despite several outliers. The greatest variation in alkalinity, as indicated by the interquartile ranges, was noted in the BP.

The same statements made for total alkalinity apply equally well for total hardness. However, in the case of calcium hardness, there were no differences in this variable between the CP and RV; but, the CP and HR did not differ from each other in calcium hardness, and the RV, AP, and HR did not differ among themselves. Of course, streams of the BP had the greatest average calcium hardness concentration.

Electrical conductivity was more variable in streams of the BP and AP than in streams of the other areas. Variation in electrical conductivity was especially low in the PP despite a few outliers. Mean electrical conductivity did not differ between the BP and AP, and the AP did not differ from the HR. Moreover, there were no differences in mean electrical conductivity between CP, PP, HR, and RV.

The total alkalinity: total hardness ratios of the samples were as follows: CP, 0.73; PP, 1.05; BP, 1.07; RV, 1.03; AP, 0.66; HR, 1.12. The average ratio for all samples was 0.93. The magnesium hardness of a sample was obtained by subtracting calcium hardness from total hardness. Calcium concentration was calculated as calcium hardness divided by 2.5, and magnesium concentration was obtained by dividing magnesium hardness by 4.12 (Boyd et al. 2015). The calcium and magnesium concentrations revealed that the calcium: magnesium ratios in the streams were as follows: CP, 3.18; PP, 3.55; BP, 12.93; RV, 2.34; AP, 1.43; HR, 4.34.

Frequency distribution histograms for each variable were plotted for data from all sampling areas combined (Figs. 25-27). The vast majority of samples (95.6%) had pH between 6.5 and 8.5 – the range usually considered best for fish populations (Boyd 2015). Only 1.4% of samples had pH below 6, and none had pH above 9.

Electrical conductivity was less than 250 µmho/cm in 81.1% of streams, and 48.3% of streams had values less than 100 µmho/cm. Conductivity values greater than 500 µmho/cm were found in only 6.9% of samples, and the highest measured value was 1,661 µmho/cm.

Alkalinity, total hardness, and calcium hardness concentrations tended to be mostly below 50 mg/L, and especially in the non-calcareous areas. In the calcareous areas, there were higher concentrations of these three variables. A few alkalinity and hardness concentrations in excess of 200 mg/L were measured in calcareous areas, but values above 400 mg/L were not observed. It should be noted that a few streams with low concentrations of alkalinity and of total and calcium hardness were found in calcareous areas, because limestone formations do not occur throughout an entire calcareous sampling area. Moreover, in non-calcareous provinces, there are some places where limestone formations occur. High concentrations of alkalinity and hardness in a few of the streams in the CP and PP apparently were the result of limestone formations. The range in concentrations of alkalinity, total hardness, and calcium hardness were much less in the CP and PP than in other provinces. The BP had the highest concentrations by far of these three variables.

Streams with especially low alkalinity were encountered most frequently in the CP where 6.9% of samples had less than 5 mg/L alkalinity, and 11.5% had between 5 and 10 mg/L. None of the samples from the BP or AP had alkalinity below 10 mg/L. Overall, 49.6% of streams from non-calcareous areas had total alkalinity below 20 mg/L as compared to 9.3% of streams in calcareous areas. Total hardness and calcium hardness concentrations also were consistently lower in areas where limestone formations are uncommon.

Correlation coefficients (R) were calculated for each measured water quality variable versus each of the other measured water quality variables using both polynomial regression to obtain the line of best fit and simple, linear regression. The polynomial regressions had slightly greater correlation coefficients in all cases. But, the polynomial regression lines for pH, alkalinity, and hardness, in some cases, did not appear reasonable based upon the principles of water chemistry, and apparently resulted from the effort of the statistical program to achieve the line of best fit. There were no negative correlations, so the R values were squared to give R^2 values (coefficients of determination) for use in the correlation matrix (Table 1). This is convenient, because R^2 values give a direct estimate of the proportion of the variation shared between two variables in a simple, linear regression, i.e., for $R^2 = 0.915$, 91.5% of the variation is shared between the two variables. Because of the rather large sample sizes, many R^2 values were significant even at $P = 0.01$. There were 20 R^2s in each matrix. Of these, the numbers of significant correlations were: CP, 20; PP, 16; BP, 18; RV, 20; AP, 12; HR, 12. The R^2 values that were not significant involved pH, electrical conductivity, or both. All combinations of variables were significant for regressions on all data combined.

Multiple regressions were run separately so that each variable was Y and the remaining variables the X (Table18). Excluding pH, all of the multiple correlations were significant ($P<0.001$). The significant R^2 values from the four variables ranged from 0.590 to 0.961. Of the 24 combinations possible after excluding pH, 13 R^2s exceeded 0.900, and 18 R^2s exceeded 0.800 for the individual sampling areas. The R^2 for all samples combined (but excluding the pH data) ranged between 0.846 and 0.884. The pH was significantly correlated with the other variables in the CP, RV, and for all data combined. But, the R^2 values for pH were low. For individual provinces, R^2s ranged from 0.043 to 0.442, and for all samples R^2 was 0.390.

Discussion

The means for alkalinity, hardness, and calcium hardness, not surprisingly, were higher for streams in calcareous areas as compared to those in non-calcareous areas. Moreover, the electrical conductivity and pH also tended to be greater in the calcareous areas. Most streams in Alabama appear to have adequate pH, alkalinity, and hardness to support good fish populations. Moreover, the range in pH values for Alabama streams suggests that acidification or alkalinization would rarely result from natural causes, but suggest pollution. Only a few streams had highly elevated electrical conductivity, and only one had a conductivity greater than typically found in fresh water. This value of 1,661 μmho/cm was recorded for a stream in the

BP. It is suspected that water from an underground, saline aquifer that occurs in the BP (Cook 1993; Boyd et al. 2009) enters that stream from an uncapped artesian well or from groundwater intrusion from the saline aquifer. Stream waters in Alabama seldom would have high conductivity unless they received elevated concentrations of total dissolved solids in surface runoff or point source discharge as a result of pollution.

Several other factors also influence relationships among the variables. The total alkalinity: total hardness ratio is influenced by the source of alkalinity. If limestone is the only source of alkalinity and hardness, then the two variables should have chemically equivalent concentrations – at least at the time of dissolution – as illustrated for calcite and dolomite:

$$CaCO_3 + CO_2 + H_2O = Ca^{2+} + 2HCO_3^- \qquad (1)$$

$$CaMg(CO_3)_2 + 2CO_2 + 2H_2O = Ca^{2+} + Mg^{2+} + 4HCO_3^- \qquad (2)$$

Of course, acidity can neutralize alkalinity anions, but leave hardness cations.

The same reasoning for limestone applies to the dissolution of calcium silicate, a common source of alkalinity (Ittekkot 2003).

$$CaSiO_3 + 2CO_2 + 3H_2O = Ca^{2+} + 2HCO_3^- + H_4SiO_4 \qquad (3)$$

Alkalinity also can be derived from dissolved silicate (Snoeyink and Jenkins 1980), because orthosilicic acid resulting from the dissolution of calcium silicate can ionize to yield $H_3SiO_3^-$

$$H_4SiO_4 = H^+ + H_3SiO_3^- \qquad K = 10^{-9.84} \tag{4}$$

The $H_3SiO_3^-$ ions react with H^+ during titration with acid and is thereby measured as alkalinity. At the highest pH recorded for Alabama streams (8.61), only 5.6% of the orthosilicic acid would be ionized. In the typical pH range (7-8) found in Alabama streams, only 0.14% of the orthosilicic acid would be ionized at pH 7 and 1.4% at pH 8. Thus, for practical purposes, the alkalinity: hardness ratio is about 1.0 for dissolution of calcium silicate.

Feldspars also dissolve to yield alkalinity, but the hardness yield is usually less than that for alkalinity. Plagioclase feldspars contain various proportions of sodium and calcium, but usually more sodium than calcium, while orthoclase contains potassium and no sodium (https://www.esci.umn.edu/courses/1001/minerals/plagioclase_feldspar.shtml). Thus, where such feldspars are the major source of alkalinity, their dissolution would result in more alkalinity than hardness. A sodium feldspar such as albite provides no hardness upon dissolution:

$$2NaAlS_3O_3 + CO_2 + 11H_2O \rightarrow 2Na^+ + 2HCO_3^- + 4H_4SiO_4 + Al_2Si_2O_5(OH)_4 \tag{5}$$

The alkalinity: hardness ratio will usually be more than 1.0 when feldspars are the major source of these two variables. Moreover, feldspars tend to be less soluble than limestone or calcium silicate, and they do not cause high alkalinity (Boyd et al. 2015).

Acid rain, acidic effluents, nitrification, and certain other physical, chemical, and biological phenomena remove alkalinity from water. Some processes also can remove hardness cations without affecting alkalinity, e.g., plant uptake or adsorption by sediment. In arid climates in particular, minerals such as gypsum (calcium sulfate) dissolved in water supplying hardness cations but no alkalinity anions.

Average total alkalinity: total hardness ratios were typically less than 1.0 in streams of the CP. It is suspected that acidification from various natural processes is mainly the result of the lower alkalinity: hardness ratio in this area where limestone is uncommon. The relatively high alkalinity: hardness ratio in streams of the PP likely is the result of a substantial portion of the alkalinity deriving from feldspars that do not contain appreciable calcium and magnesium. The alkalinity: hardness ratio is near 1.0 in the RV and HR where calcareous conditions are common on watersheds. Despite the common occurrence of limestone in the AP, the alkalinity: hardness ratio is low. This is likely the result of acidification resulting from coal mining activities in the AP region around Birmingham. The findings of this survey suggest that stream acidification generally is avoided, because limestone is present naturally and mine spoils and wastewater from mining areas are treated with liming material in order to comply with wastewater discharge permits. Nevertheless, neutralization of the acidity would produce runoff or point source effluent higher in hardness than in alkalinity.

Pure calcitic limestone ($CaCO_3$) does not yield magnesium upon dissolution, but the ratio of calcium: magnesium is 1.64 following dissolution of pure dolomite ($CaCO_3 \cdot MgCO_3$). According to Smith and McCalley (1904), the Selma chalk of the BP has an average of 74.8% calcium carbonate and 1.53% magnesium carbonate. Because of this, it is not surprising that streams of the BP had a calcium: magnesium ratio of 12.73. The other calcareous areas had calcium: magnesium ratios of 1.43 (AP) to 4.34 (HR). This suggests the presence of considerable magnesium – probably in dolomitic limestone formations. The especially low calcium: magnesium ratio in the AP is puzzling, because limestone with appreciably more magnesium carbonate than calcium carbonate apparently does not exist in the AP (Smith and McCalley 1904; Prouty 1916) or elsewhere. Magnesium carbonate is more soluble than calcium carbonate; possibly, the low calcium: magnesium ratio in AP streams resulted from calcium carbonate precipitation in some of these streams.

The calcium: magnesium ratios were usually between 3 and 4 in CP and PP streams. This indicates that there is more calcium than magnesium available from the alkalinity sources – mainly feldspars and possibly calcium silicate. Of course, calcium and magnesium also are available from some other minerals, decaying organic matter, and soil.

The five water quality variables measured in this study are interrelated. Alkalinity and hardness originate mainly from the same sources. The alkalinity of water is a measure of the total concentration of titratable bases usually expressed as calcium carbonate equivalence. The ions main anions of alkalinity, bicarbonate and carbonate, in conjunction with dissolved carbon dioxide, comprise a buffer system that moderates pH change as a result of biological activity in water or the addition of acidic or basic pollutants (Boyd et al. 2015). In freshwater – especially in humid areas such as Alabama – alkalinity anions and hardness cations often comprise two-thirds or more of the total dissolved ions in water (Boyd 2015). Therefore, the electrical conductivity that increases as the concentration of dissolved ions rises also is highly influenced by alkalinity and hardness. Because of the interactions mentioned above, it is understandable why so many significant correlations occurred in the data.

The correlations involving total alkalinity, total hardness, and calcium hardness tended to share a greater percentage of the variation between the various combinations than was achieved in correlations between these variables and pH or electrical conductivity. Nevertheless, the correlations among alkalinity, hardness, and calcium hardness tended to have greater R^2s for the

CP and BP than for the other sampling areas. The PP and RV tended to have lower R^2s than the CP and BP, but greater ones than obtained in the HR and AP. The correlations between electrical conductivity and other variables usually were stronger than those obtained with pH. Multiple regression analyses further confirmed the strong relationship among the five variables and also showed that the correlation between pH and the other variables was the lowest for the five variables.

There seems to be a general assumption that the pH of a water body increases as the alkalinity increases. This likely would be correct if all water bodies contained similar amounts of carbon dioxide, because the pH of water is strongly influenced by the dissolved carbon dioxide concentration when pH is below 8.3 (Boyd et al. 2015). Higher pHs are regulated mainly by the relative concentrations of bicarbonate and carbonate with pH increasing as carbonate concentration rises (Boyd 2015). The main factor affecting carbon dioxide concentration in water is biological activity and especially the relative rates of photosynthesis and respiration that remove or add carbon dioxide, respectively. At pH above 8.3, carbon dioxide is not present, and aquatic plants obtain carbon for photosynthesis from bicarbonate (Spence and Maberly 1985; Raven et al. 2012), but in the process, they release carbonate into the water. Carbonate hydrolyzes increasing the pH, but calcium carbonate often precipitates removing a portion of the carbonate and moderating the rise in pH (Boyd 2015). When respiration exceeds photosynthesis in waters with pH above 8.3, the carbon dioxide from respiration reacts with OH^- to increase the bicarbonate concentration and lower pH. As a result of these complex relationships, the correlation between alkalinity and pH is not as strong as often believed. Likewise, because carbon dioxide is the main factor regulating pH, there is not a high correlation between pH and the other four variables.

Regression equations for the relationships among alkalinity and the two forms of hardness are of little interest for predictive purposes, because in many water quality surveys these variables are not measured. However, pH and electrical conductivity can be measured quickly in the field, and these two variables are measured much more frequently than alkalinity or either form of hardness. Although none of the correlations were high enough to allow estimations of the other variables from pH, electrical conductivity tended to have stronger correlations with other variables. Equations for estimating total alkalinity and hardness from electrical conductivity potentially could be useful in some stream water quality studies. Thus, polynomial equations for predicting alkalinity and hardness from electrical conductivity are provided (Table 19), because they had higher R^2 values than did the linear regression equations. The polynomial regression lines were of expected shapes with respect to water chemistry principles as illustrated in Fig. 28.

Conclusions

The pH, conductivity, and concentrations of total alkalinity, total hardness, and calcium hardness in Alabama streams tended to be lowest in the CP and PP, intermediate in the RV, AP, and HR, and highest in the BP. Alabama streams seldom had pH below 6, and most pH values ranged between 7 and 8. There were few instances with alkalinity and hardness below 5 mg/L or above 100 mg/L. Conductivity usually was below 500 μmho/cm.

There were many significant R^2 values among the variables for both simple, linear and multiple regressions, but the correlations tended to be much weaker in regressions involving pH than for the other variables. The pH of water is regulated mainly by the carbon dioxide concentration rather than the alkalinity. The only regression equations that might be of practical value are those that allow alkalinity or hardness to be estimated from conductivity.

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Table 17. Matrices for correlations of determination (R^2) for five water quality variables from streams in six physiographic sampling areas[1] of Alabama.

	Coastal Plain (n = 88; P 0.01 = 0.072)					Piedmont Plateau (n = 42; P 0.01 = 0.154)				
	TA	TH	CH	pH	EC	TA	TH	CH	pH	EC
TA		0.896	0.845	0.446	0.654		0.779	0.637	0.246	0.561
TH	0.896		0.822	0.423	0.71	0.780		0.724	0.213	0.780
CH	0.845	0.822		0.383	0.608	0.637	0.724		0.082	0.597
pH	0.446	0.423	0.383		0.232	0.246	0.213	0.082		0.0118
EC	0.654	0.707	0.566	0.232		0.561	0.800	0.597	0.118	
	Blackland Prairie (n = 24; P 0.01 = 0.265)					Ridge and Valley (n = 22; P 0.01 = 0.288)				
TA		0.945	0.968	0.393	0.940		0.883	0.777	0.385	0.825
TH	0.945		0.958	0.335	0.305	0.883		0.883	0.329	0.891
CH	0.968	0.958		0.373	0.331	0.777	0.883		0.350	0.868
pH	0.310	0.180	0.199		0.296	0.385	0.329	0.350		0.392
EC	0.940	0.305	0.331	0.418		0.825	0.891	0.868	0.392	
	Appalachian Plateau (n = 20; P 0.01 = 0.314)					Highland Rim (n = 21; P 0.01 = 0.301)				
TA		0.529	0.339	0.173	0.683		0.550	0.393	0.004	0.388
TH	0.528		0.851	0.211	0.879	0.550		0.816	0.008	0.811
CH	0.400	0.851		0.201	0.678	0.393	0.816		0.016	0.471
pH	0.173	0.211	0.208		0.179	0.004	0.008	0.016		0.009
EC	0.683	0.879	0.678	0.179		0.388	0.811	0.471	0.009	
	All data pooled (n = 217; P 0.01 = 0.033)									
TA		0.782	0.808	0.378	0.748					
TH	0.782		0.793	0.313	0.729					
CH	0.808	0.793		0.294	0.678					
pH	0.379	0.313	0.294		0.270					
EC	0.749	0.729	0.678	0.270						

[1] TA = total alkalinity, TH = total hardness, CH = calcium hardness, and EC = electrical conductivity.

Table 18. Adjusted R^2 for multiple regression of the Y variables versus total alkalinity (TA), total hardness (TH), calcium hardness (CH), pH, and electrical conductivity (EC).

	Physiographic sampling area[1]						
Y-variable	CP	PP	BP	RV	AP	HR	All pooled
TA	0.917	0.787	0.965	0.871	0.636	0.591	0.843
TH	0.917	0.908	0.961	0.936	0.938	0.976	0.884
CH	0.852	0.729	0.956	0.880	0.830	0.905	0.877
pH	0.442	0.243	0.176	0.306	0.043	0.168	0.390
EC	0.705	0.791	0.956	0.896	0.911	0.922	0.846

[1] CP = Coastal Plain (excluding Blackland Prairie), PP = Piedmont Plateau, BP = Blackland Prairie, RV = Ridge and Valley, AP = Appalachian Plateau, and HR = Highland Rim.

Table 19. Equations for estimating total alkalinity (TA) and total hardness (TH) concentrations (mg/L as $CaCO_3$) in Alabama streams from electrical conductivity (μmho/cm) readings.

Area and Y variable	Equation	R^2
Coastal Plain		
TA	$-0.0003X^2 + 0.3416X – 4.616$	0.693
TH	$-0.0003X^2 + 0.337X – 2.758$	0.765
Piedmont Plateau		
TA	$0.0005X^2 + 0.1252X + 9.677$	0.569
TH	$0.0013X^2 – 0.0531X + 15.238$	0.858
Blackland Prairie		
TA	$-0.00009X^2 + 0.4253X – 13.618$	0.955
TH	$-0.0005X^2 + 0.7574X – 52.552$	0.751
Ridge and Valley		
TA	$-0.0003X^2 + 0.5581X – 10.985$	0.829
TH	$-0.0002X^2 + 0.3624X – 2.656$	0.894
Appalachian Plateau		
TA	$-0.00005X^2 + 0.0699X + 34.602$	0.704
TH	$-0.0002X^2 + 0.533X – 30.264$	0.950
Highland Rim		
TA	$-0.0017X^2 + 1.0418X – 53.037$	0.442
TH	$0.00002X^2 + 0.3709X – 2.0311$	0.811
All areas		
TA	$0.0001X^2 + 0.3861X – 4.3894$	0.768
TH	$0.0001X^2 + 0.4371X – 8.6927$	0.922

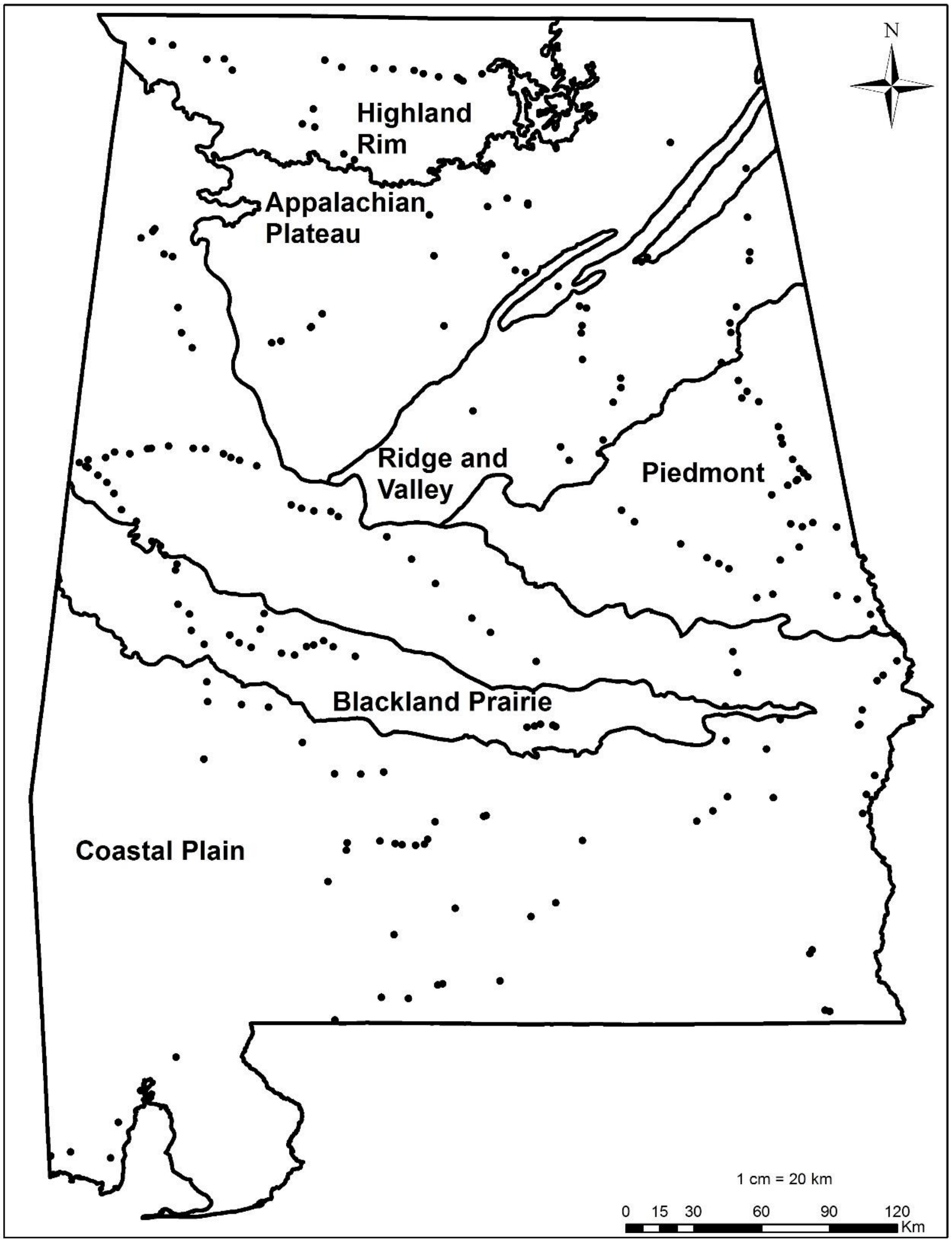

Figure 21. Physiographic sampling areas for assessing total alkalinity and total hardness concentrations in Alabama streams in 2014-2015. The dots represent the GPS coordinates of sampling locations.

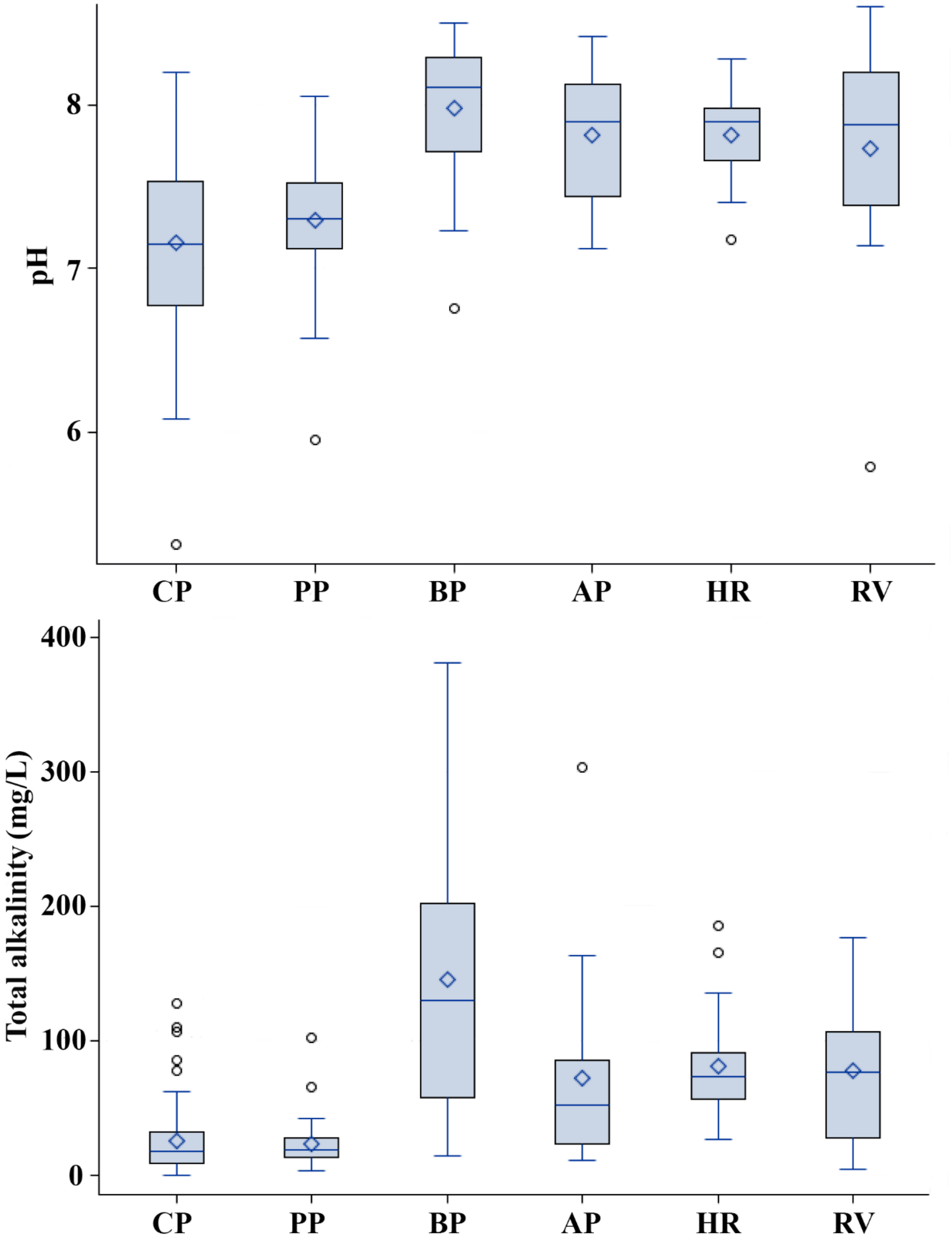

Figure 22. Boxplots for pH and total alkalinity concentrations for Alabama streams in different physiographic sampling areas. Abbreviations: CP= Coastal Plain; PP= Piedmont Plateau; BP= Blackland Prairie; AP= Appalachian Plateau; HR= Highland Rim; RV= Ridge and Valley.

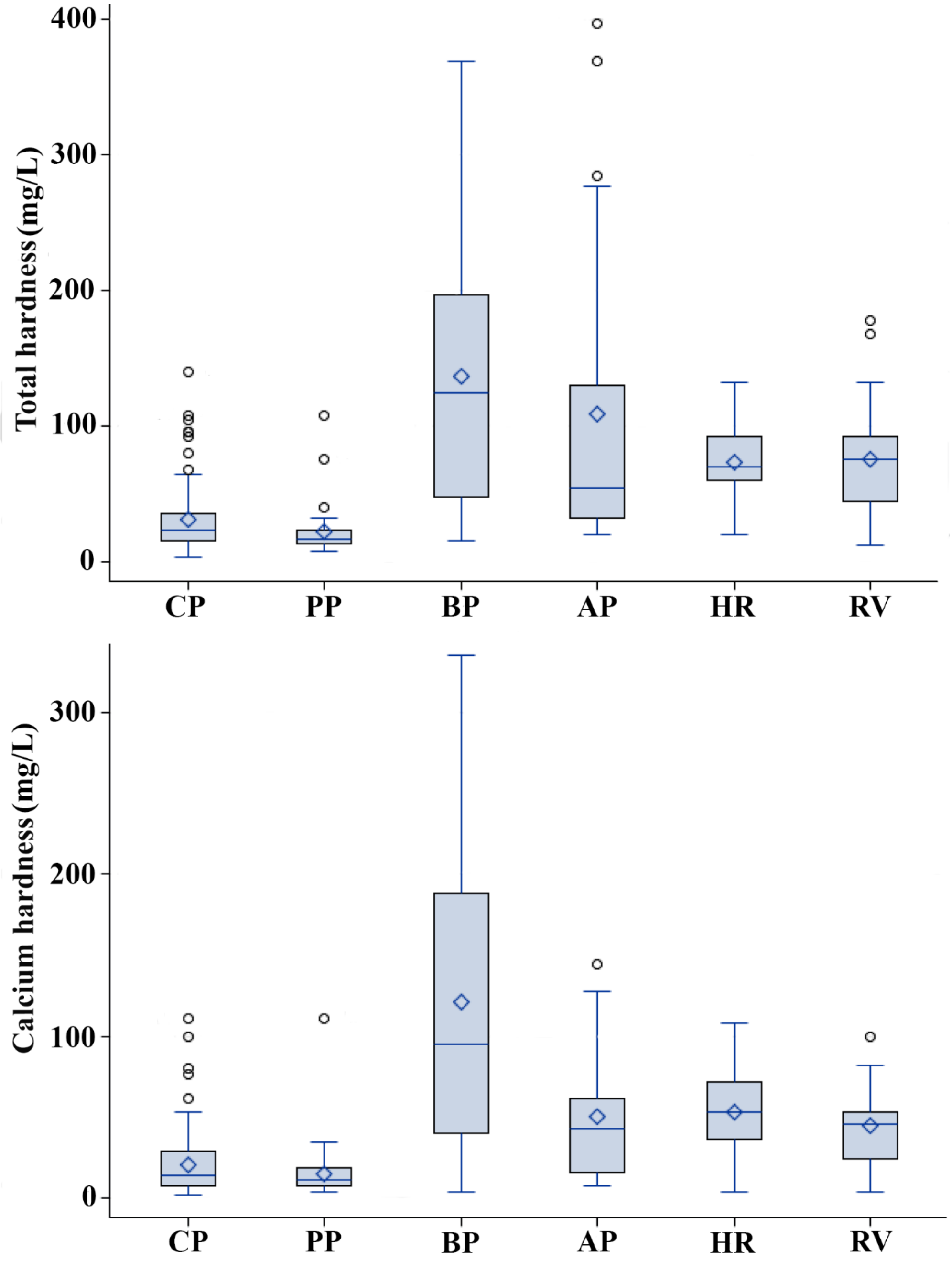

Figure 23. Boxplots for TH (total hardness) and CaH (calcium hardness) concentrations for Alabama streams in different physiographic sampling areas. Abbreviations: CP= Coastal Plain; PP= Piedmont Plateau; BP= Blackland Prairie; AP= Appalachian Plateau; HR= Highland Rim; RV= Ridge and Valley.

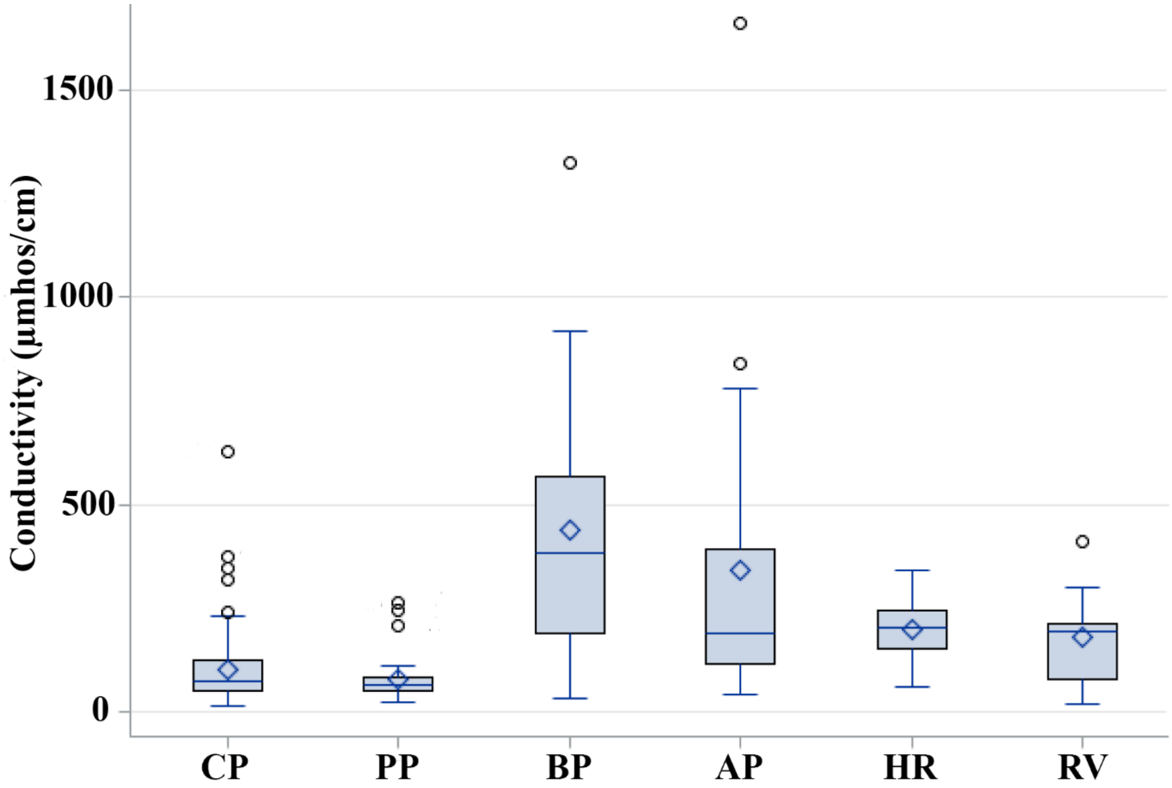

Figure 24. Boxplots for conductivity for Alabama streams in different physiographic sampling areas. Abbreviations: CP= Coastal Plain; PP= Piedmont Plateau; BP= Blackland Prairie; AP= Appalachian Plateau; HR= Highland Rim; RV= Ridge and Valley.

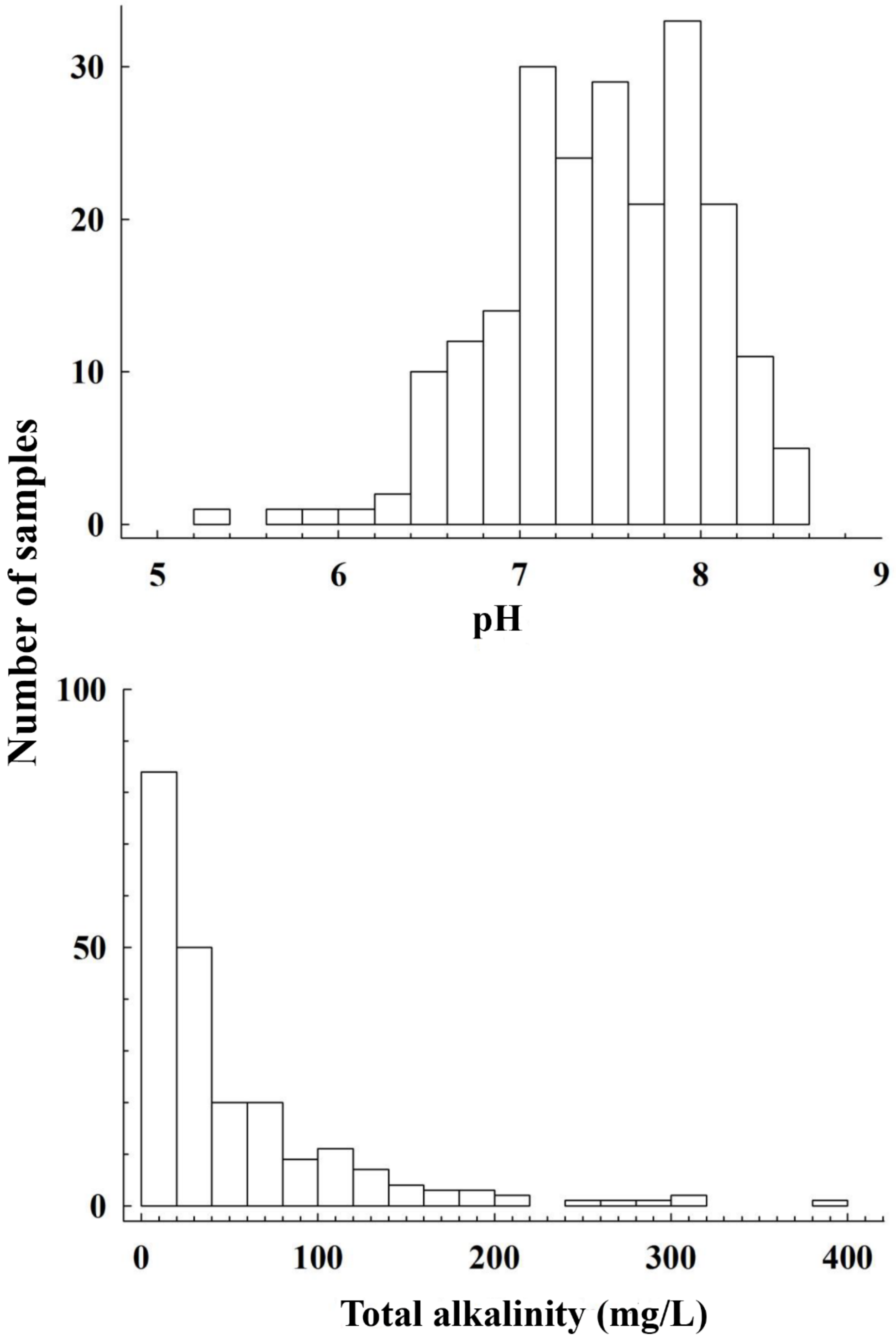

Figure 25. Frequency distribution histograms of pH and total alkalinity of samples taken in 2014 -2015 from 216 small stream representing major physiographic areas of Alabama.

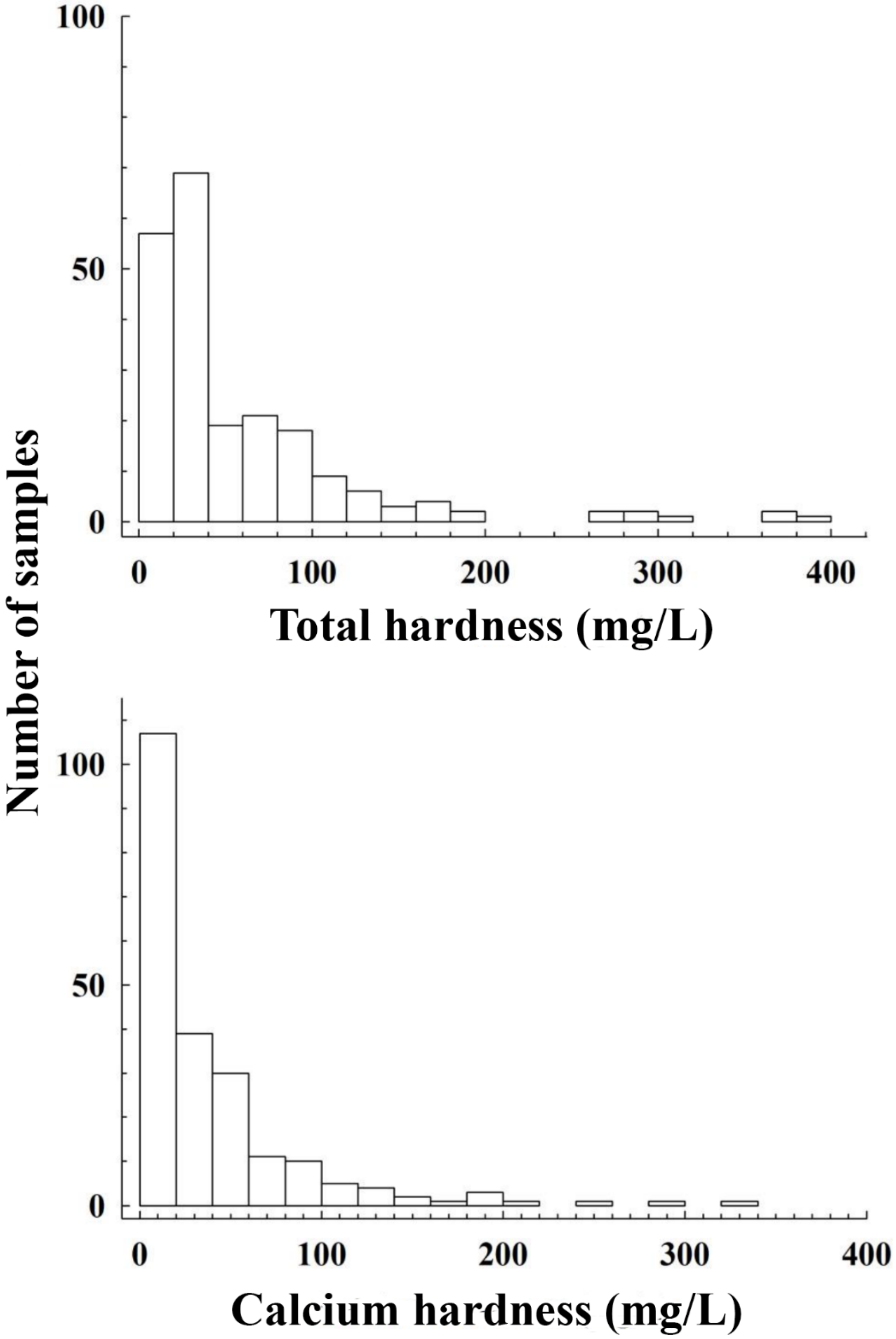

Figure 26. Frequency distribution histograms of total hardness and calcium hardness of samples taken in 2014 -2015 from 216 small stream representing major physiographic areas of Alabama.

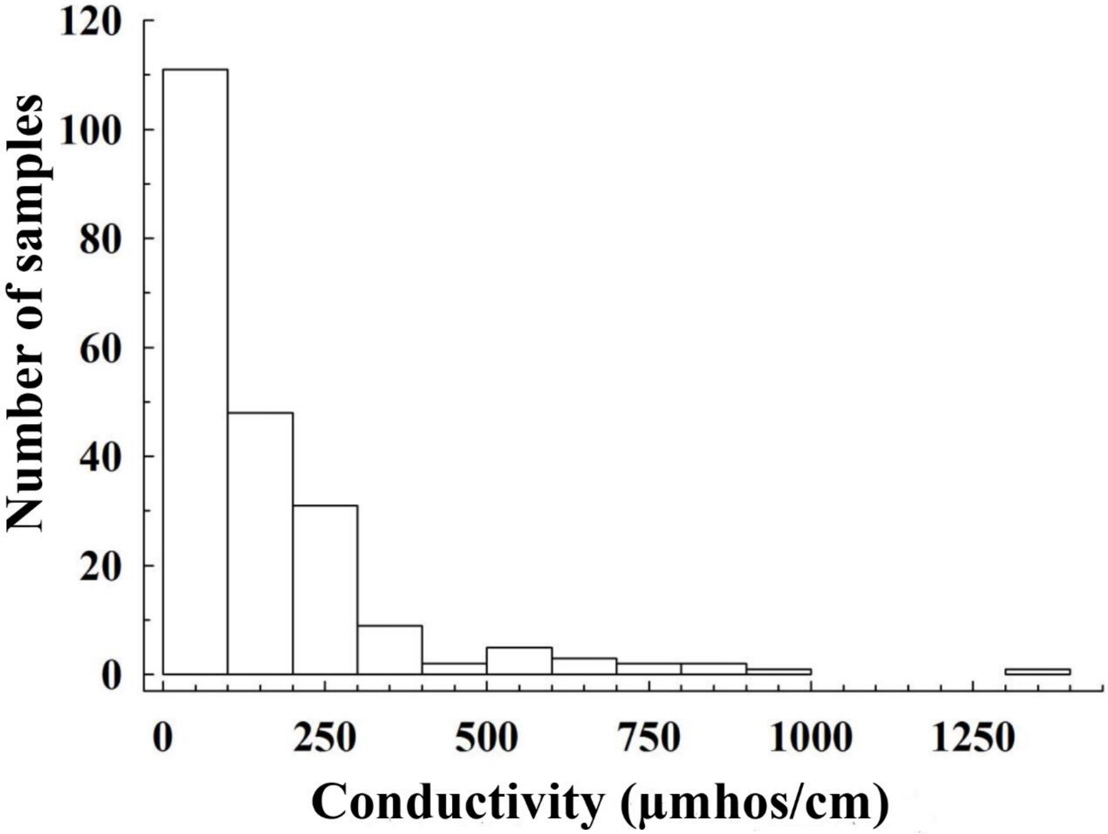

Figure 27. Frequency distribution histograms of conductivity of samples taken in 2014 -2015 from 216 small stream representing major physiographic areas of Alabama.

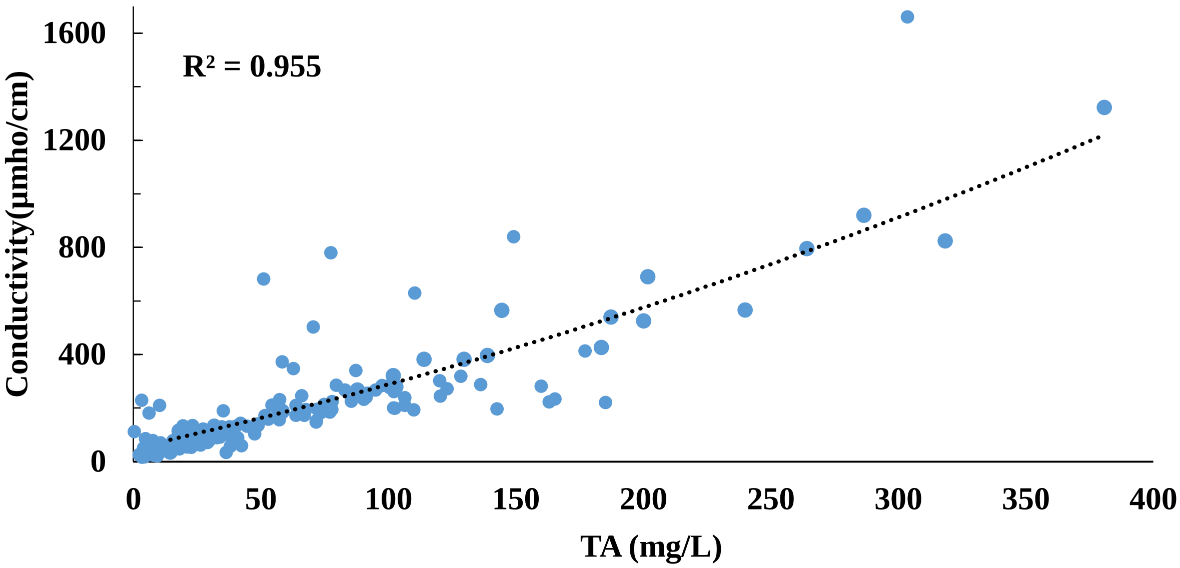

Figure 28. An example of relationship between total alkalinity (TA) and conductivity for all samples combined.