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COMMUNICATION

Interpretation of pH, Acidity, and Alkalinity in Aquaculture and Fisheries

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Abstract

Measurements of pH, acidity, and alkalinity are commonly used to describe water quality. The three variables are interrelated and can sometimes be confused. The pH of water is an intensity factor, while the acidity and alkalinity of water are capacity factors. More precisely, acidity and alkalinity are defined as a water's capacity to neutralize strong bases or acids, respectively. The term "acidic" for pH values below 7 does not imply that the water has no alkalinity; likewise, the term "alkaline" for pH values above 7 does not imply that the water has no acidity. Water with a pH value between 4.5 and 8.3 has both total acidity and total alkalinity. The definition of pH, which is based on logarithmic transformation of the hydrogen ion concentration ([H$^+$]), has caused considerable disagreement regarding the appropriate method of describing average pH. The opinion that pH values must be transformed to [H$^+$] values before averaging appears to be based on the concept of mixing solutions of different pH. In practice, however, the averaging of [H$^+$] values will not provide the correct average pH because buffers present in natural waters have a greater effect on final pH than does dilution alone. For nearly all uses of pH in fisheries and aquaculture, pH values may be averaged directly. When pH data sets are transformed to [H$^+$] to estimate average pH, extreme pH values will distort the average pH. Values of pH conform more closely to a normal distribution than do values of [H$^+$], making the pH values more acceptable for use in statistical analysis. Moreover, electrochemical measurements of pH and many biological responses to [H$^+$] are described by the Nernst equation, which states that the measured or observed response is linearly related to 10-fold changes in [H$^+$]. Based on these considerations, pH rather than [H$^+$] is usually the most appropriate variable for use in statistical analysis.

Temperature, salinity, hardness, pH, acidity, and alkalinity are fundamental variables that define the quality of water. Although all six variables have precise, unambiguous definitions, the last three variables are often misinterpreted in aquaculture and fisheries studies. In this paper, we explain the concepts of pH, acidity, and alkalinity, and we discuss practical relationships among those variables. We also discuss the concept of pH averaging as an expression of the central tendency of pH measurements. The concept of pH averaging is poorly understood, if not controversial, because many believe that pH values, which are log-transformed numbers, cannot be averaged directly. We argue that direct averaging of pH values is the simplest and most logical approach for most uses and that direct averaging is based on sound practical and statistical principles.

THE pH CONCEPT

The pH is an index of the hydrogen ion concentration ([H$^+$]) in water. The [H$^+$] affects most chemical and biological processes; thus, pH is an important variable in water quality endeavors. Water temperature probably is the only water quality variable that is measured more commonly than pH.

The pH concept has its basis in the ionization of water:

$$H_2O = H^+ + OH^-. \qquad (1)$$

The mass action expression for the ionization of water is

$$(H^+)(OH^-)/(H_2O) = K_w. \qquad (2)$$

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TABLE 1. Ionization constants (K_w), molar concentrations of hydrogen ion ($[H^+]$), and pH of pure water at different temperatures (data modified from Garrels and Christ 1965). The molar concentrations of hydroxyl ion ($[OH^-]$) are the same as $[H^+]$.

Water temperature (°C)	K_w	$[H^+]$	pH
0	$10^{-14.94}$	$10^{-7.47}$	7.47
5	$10^{-14.73}$	$10^{-7.36}$	7.36
10	$10^{-14.53}$	$10^{-7.26}$	7.26
15	$10^{-14.35}$	$10^{-7.18}$	7.18
20	$10^{-14.17}$	$10^{-7.08}$	7.08
25	$10^{-14.00}$	$10^{-7.00}$	7.00
30	$10^{-13.83}$	$10^{-6.92}$	6.92
35	$10^{-13.68}$	$10^{-6.84}$	6.84
40	$10^{-13.53}$	$10^{-6.76}$	6.76

Parentheses in equation (2) denote molar activities of reactants and products, and K_w is the ionization constant of water (Table 1). Ionic activities and measured molar concentrations of ions in freshwater can be considered equal for practical purposes (Boyd 2000). Moreover, pH is measured with a glass electrode that responds to H^+ activity. The degree of ionization of water is exceedingly small and does not change the concentration of water. Equation (2) may be simplified to

$$[H^+][OH^-] = K_w, \tag{3}$$

where brackets denote molar concentrations.

In pure water, one hydrogen ion and one hydroxyl ion (OH^-) result from ionization of one water molecule; $[H^+]$ is calculated by substituting $[H^+]$ for $[OH^-]$ in equation (3) and taking the square root of both sides of the following expression,

$$[H^+][H^+] = K_w,$$

which results in

$$[H^+] = \sqrt{K_w}.$$

Concentrations of H^+ and OH^- at different temperatures are provided in Table 1. The $[H^+]$ of water from different sources varies greatly, but the product of $[H^+]$ and $[OH^+]$ will always equal K_w, allowing $[OH^-]$ to be estimated from $[H^+]$:

$$[OH^-] = K_w/[H^+]. \tag{4}$$

In 1909, Danish chemist S. P. L. Sørensen recommended taking the negative logarithm of $[H^+]$ to avoid the use of small molar concentrations when referring to $[H^+]$ (Chang 1994). The negative logarithm of $[H^+]$ was eventually called pH:

$$\text{pH} = -\log_{10}[H^+]. \tag{5}$$

Pure water at 25°C has a pH of 7.00 because

$$\text{pH} = -\log_{10}(10^{-7.00}) = -(-7.00) = 7.00.$$

Pure water is neutral—neither acidic nor basic in reaction—because $[H^+]$ is equal to $[OH^-]$. It is common practice to use a pH scale of 0–14 with a pH value of 7 as the midpoint; pH values below 7 represent progressively more acidic reactions, and pH values above 7 represent progressively more basic or alkaline reactions. However, the pH of pure water is 7.00 only at a temperature of 25°C. At lower temperatures, the pH of pure water is above 7; at higher temperatures, the pH is below 7 (Table 1). It is possible to have negative pH values as well as values above 14. At 25°C, a solution that is 2 M (i.e., $10^{0.3}$ M) in H^+ has a pH of −0.3, while a solution that is 2 M in OH^- has a pH of 14.3.

RELATIONSHIPS AMONG pH, ACIDITY, AND ALKALINITY

Considerable confusion exists regarding the relationships among pH, alkalinity, and acidity. Confusion arises because solutions with pH values above 7 are sometimes described as "alkaline," and solutions with pH values below 7 are sometimes described as "acidic"; the same root words are present in the terms "acidity" and "alkalinity." In water chemistry, however, acidity and alkalinity have precisely defined meanings that are largely unrelated to whether water is "acidic" or "alkaline" with respect to pH.

The pH of water is an intensity factor, whereas the acidity and alkalinity of water are capacity factors. More precisely, acidity is a water's capacity to neutralize strong bases, and alkalinity is a water's capacity to neutralize strong acids. Base-neutralizing or acid-neutralizing capacity can be defined for any type of buffer system, but in water chemistry they are defined based on the carbonate system, which is the most common inorganic buffer system in natural waters (Stumm and Morgan 1996).

A common substance causing acidity in water is CO_2. For example, freshly distilled water has a pH of 7.00 at 25°C, but if this water is exposed to the air to equilibrate with atmospheric CO_2, it will be acidic: about pH 5.6 at the current atmospheric CO_2 concentration of 0.038%. Carbon dioxide will not cause pH to be below 4.5 in natural waters; a lower pH results from strong mineral acids, often sulfuric acid. Carbon dioxide does not occur in water at pH levels above 8.3. The total acidity of water typically is determined by titrating a sample with standard base (usually sodium hydroxide) to the phenolphthalein endpoint (pH = 8.3); the results are expressed in terms of equivalent calcium carbonate. However, acidity from hydrolysis of certain metals in acidic mine drainage may not be completely expressed by titration to pH 8.3 unless the metals are first oxidized by hot hydrogen peroxide (Eaton et al. 2005). Alkalinity is usually the result of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) from dissolution of limestone, calcium silicate, and feldspars (Boyd 2000; Ittekkot

2003). Bicarbonate does not exist in measurable concentration below a pH level of 4.5, so total alkalinity is measured by titrating a sample with standard acid (usually sulfuric acid) to the methyl orange endpoint ($pH = 4.5$), and the results are also expressed in terms of equivalent calcium carbonate. Waters with pH values less than 4.5 do not contain alkalinity, while waters with pH values greater than 8.3 do not contain acidity. However, natural waters in the pH range of 4.6–8.2 contain both acidity and alkalinity. Two waters of the same pH can differ greatly in acidity and alkalinity. Likewise, the pH in a water body may vary by 1–3 pH units during a 24-h period because of changes in CO_2 concentration in response to differences in rates of photosynthesis and respiration.

THE CONCEPT OF AVERAGE pH

Multiple pH measurements are made for various purposes in almost every field of biology. As is the case with most data, scientists often wish to summarize and describe pH data with a simple and meaningful measure of central tendency, such as the average. However, pH data are unlike most other water quality data in that pH values, as explained above, are negative $\log_{10}$ transformations of a concentration. As such, it has been extensively argued that pH values cannot be directly averaged but rather must be back-transformed to $[H^+]$ and then averaged. The average $[H^+]$ may then be re-transformed to average pH if desired.

Confusion regarding the proper method of describing pH data has engendered lively discussion in fields as wide-ranging as anesthesiology (Feinstein 1979; Giesecke 1979a, 1979b; Pace et al. 1979; Grogono 1980), water pollution (Barth 1975; Erlap 1978), and soil science (Shuie and Chin 1957). In aquaculture and fisheries, Kinney (1973) discussed the determination of average pH and concluded that averaging of $[H^+]$ is the correct approach but would be a tedious task for some data sets (this was before the advent of personal computers). Kinney (1973) suggested that it would be more meaningful and easier to report the range of pH data sets and explain any unusual deviations of pH; thus, he suggested avoiding the problem altogether by not calculating average pH. Current recommendations (FAO 2010) are to transform pH data to $[H^+]$ before averaging.

Below, we will discuss the two approaches to calculating and reporting average pH. First, however, we describe the pH of mixtures because arguments for $[H^+]$ averaging are sometimes based on this concept.

MIXTURES OF DIFFERING pH

Because pH is logarithmic, when estimating the pH of a simple mixture made by combining solutions of different pH values, it is necessary to (1) convert the pH of each solution to $[H^+]$, (2) determine the $[H^+]$ of the final mixture, and (3) convert the $[H^+]$ to pH. To illustrate, consider a mixture that is composed of 1 L of a pH 2 solution ($[H^+] = 10^{-2}$ M $= 1 \times 10^{-2}$ mol of H^+), 5 L of a pH 5 solution ($[H^+] = 10^{-5}$ M $= 0.005 \times 10^{-2}$ mol of H^+), and 4 L of a pH 7 solution ($[H^+] = 10^{-7}$ M $= 0.00004 \times 10^{-2}$ mol of H^+). Assume that the acidic solutions are made by using a completely dissociated acid, such as HCl (the need for this assumption will be described shortly). The resulting 10-L mixture contains 1.00504×10^{-2} mol of H^+; its $[H^+]$ is 1.00504×10^{-3} M, and the pH is

$$pH = -\log_{10}(1.005 \times 10^{-3}) = -[0.002 + (-3)] = 2.998.$$

The calculation is simpler when equal volumes of solutions are mixed. A mixture made from equal volumes of pH 5 ($[H^+] = 0.00001$ M), pH 6 ($[H^+] = 0.000001$ M), and pH 7 ($[H^+] = 0.0000001$ M) solutions would have a mean $[H^+]$ of 3.7×10^{-6} M, resulting in a calculated pH of 5.43:

$$pH = -\log_{10}(3.7 \times 10^{-6}) = -[0.57 + (-6)] = 5.43.$$

DIRECT AVERAGING OF pH OR AVERAGING OF HYDROGEN ION CONCENTRATION

The concept of calculating the pH of mixtures by transforming data to $[H^+]$ leads to the common conclusion that pH data cannot be averaged directly but rather must be transformed to $[H^+]$ before the mean is calculated and then retransformed to obtain the average pH. In fact, Giesecke (1979b) offered apparent proof that $[H^+]$ averaging (rather than pH averaging) was the best measure of central tendency by making solutions of various pH values with either HCl or NaOH, mixing the solutions, and comparing the calculated pH with the actual pH measurement. Under these conditions and as expected, the measured pH agreed exactly with the average pH that was calculated by $[H^+]$ averaging, as explained above.

The opinion that pH values must be averaged only by first transforming the pH data to $[H^+]$ seems logical in some of the cases encountered in the fields of aquaculture and fisheries. For example, if rainfall pH is measured as part of a study to assess the impact of acidic precipitation on a watershed, statistics (such as averaging) should be performed with $[H^+]$ because the situation is analogous to the examples above wherein waters of different pH are mixed. There is, however, an important difference between waters found in nature and aqueous solutions made from strongly dissociated acids and bases.

Most "nonlaboratory" waters (i.e., in nature or in an aquaculture setting) contain various amounts of weakly dissociated acids and bases. Good examples include HCO_3^- and phosphates. These substances are sometimes called buffers because they react with either acids or bases, thereby reducing the pH change resulting from the addition of acids or bases. When buffers are present in waters, the mixing of waters will result in a pH that differs from the average pH obtained by simple $[H^+]$ averaging or by calculating the amount of H^+ in each solution (if of different volumes) and dividing by the combined volume to obtain $[H^+]$ and pH.

We will illustrate the buffering capacity of natural waters by describing an example in which 10-mL aliquots of a hydrochloride acid solution of pH 2 are mixed with (1) 1,000 mL of unbuffered water with a pH of 8.3 ($[H^+] = 5.01 \times 10^{-9}$ M) and (2) 1,000 mL of natural water that also has a pH of 8.3 but has a total alkalinity of 50 mg/L. Bicarbonate is the main source of alkalinity in most natural waters of pH 8.3 or less. Thus, the natural water contains $[HCO_3^-]$ of 10^{-3} M, but it will not initially contain measurable free CO_2. Adding the acidic solution to the unbuffered water (no alkalinity) will cause a large drop in pH as calculated below:

$$(0.01\,\mathrm{M\,H^+})(0.01\,\mathrm{L}) = 0.0001\ \mathrm{mol\ of\ H^+}$$

and

$$(5.01 \times 10^{-9}\,\mathrm{M})(1.00\,\mathrm{L}) = 0.00000000501\ \mathrm{mol\ of\ H^+}.$$

Thus, 1.01 L contains about 0.0001 mol of H^+,

$$[H^+] = (0.0001\,\mathrm{mol\,H^+}/1.01\,\mathrm{L}) = 9.9 \times 10^{-5}\,\mathrm{M},$$

and the pH is 4.004.

In the natural water, H^+ from the acidic solution will react as follows:

$$HCO_3^- + H^+ = CO_2 + H_2O. \qquad (6)$$

The 10-mL aliquot of acidic solution contains 0.0001 mol of H^+ that will neutralize 0.0001 mol of HCO_3^- and produce 0.0001 mol of CO_2. The natural water contained 0.001 mol of HCO_3^- initially, and after addition of the acidic solution it will still contain 0.0009 mol of HCO_3^-. The $[HCO_3^-]$ and $[CO_2]$ of the final mixture of the two solutions will be

$$[HCO_3^-] = (0.0009\,\mathrm{mol}/1.01\,\mathrm{L}) = 10^{-3.05}\,\mathrm{M}$$

and

$$[CO_2] = (0.0001\,\mathrm{mol}/1.01\,\mathrm{L}) = 10^{-4.00}\,\mathrm{M}.$$

The $[H^+]$ immediately after the addition of the acidic solution can be calculated as follows:

$$([HCO_3^-][H^+])/[CO_2] = 10^{-6.35}\,\mathrm{M}$$

and

$$\begin{aligned}[H^+] &= ([CO_2] \times 10^{-6.35})/[HCO_3^-] \\ &= [(10^{-4.00} \times 10^{-6.35})/10^{-3.05}] = 10^{-7.30};\end{aligned}$$

therefore, the pH is equal to 7.30.

Of course, much of the CO_2 will diffuse from the water to the air, causing the pH to rise to near its original value. The total alkalinity will be about 45 mg/L, and the water will still be well buffered.

Note that when waters containing weak acids or weak bases are mixed, the resulting average pH cannot be calculated either by $[H^+]$ averaging or by direct pH averaging. Nearly all natural waters are buffered to some extent, and if the compositions of the waters are known, it may be possible to calculate the pH of mixtures based on appropriate dissociation constants, mass balance, charge balance, and proton condition, but this is only possible if the systems are very simple and if their compositions are known.

The upshot is that $[H^+]$ averaging provides the correct pH of mixtures only under specific, extremely simple conditions and has little meaning in the usual situation where natural waters contain substances that cause $[H^+]$ to act in a nonconservative manner (i.e., $[H^+]$ is not affected only by dilution or concentration). As such, arguments for using $[H^+]$ averaging to calculate average pH are inherently wrong when they are based on mixtures. We believe that in most instances, direct calculation of average pH is mathematically correct and more meaningful than $[H^+]$ averaging.

Many measurements of pH in aquatic systems are made to assess the potential response of plants or animals to $[H^+]$. A simple example is the effect of pH on fish survival, which may be used to assess a particular water's suitability for fish culture. In many such cases, $[H^+]$ averaging is misleading and may even be incorrect, especially when there is a wide range in pH.

A pH difference of 1 unit represents a 10-fold difference in $[H^+]$, and the lowest or highest pH value may distort the average pH obtained by $[H^+]$ averaging. For example, warmwater fish typically will die at pH values less than 4.0 (Boyd and Tucker 1998). Suppose that a survey of ponds in an area reveals pH values of 3.0, 7.0, 7.2, 7.5, 7.8, 7.9, 8.0, and 8.1. Direct averaging produces an average pH of 7.06, but $[H^+]$ averaging yields an average pH of 3.90. Based on direct pH averaging, the average pH of the sample of ponds is within the suitable range for warmwater fish, although one pond has an excessively low pH. If the $[H^+]$ averaging method is used, the average pH of these pond waters is outside the range for fish survival and suggests that the waters are unsuitable for fish culture. Clearly, this would be an erroneous conclusion because only one pond had excessively low pH. Of course, Kinney's (1973) suggestion to report the pH value and explain unusual deviations would lead to the same conclusion for this data set.

Data on water pH in 233 culture ponds for channel catfish *Ictalurus punctatus* in Alabama were presented by Silapajarn et al. (2004), and a second data set (C.E.B., unpublished data) described sediment pH from 131 ponds in Thailand (Table 2). For both data sets, but especially for the sediment data, the average pH based on $[H^+]$ averaging was considerably less than the average pH obtained by direct averaging of pH. In addition, coefficients of variation were much greater for $[H^+]$ than for pH. The two pH data sets also were used to evaluate the distribution of pH and $[H^+]$. Visual inspection of the frequency distribution histograms of the data suggests that pH conforms more closely to a normal distribution than does $[H^+]$ (Figures 1, 2); this

TABLE 2. Summarized results of statistical evaluation of pH and hydrogen ion concentration ([H$^+$]) in 233 water samples from channel catfish ponds in Alabama (Silapajarn et al. 2004) and 131 sediment samples from freshwater aquaculture ponds in Thailand (C.E.B., unpublished data).

	Water		Sediment	
Statistic	pH	[H$^+$]	pH	[H$^+$]
Mean[a]	7.96	6.73×10^{-8} M	6.31	1.4×10^{-5} M
SD	0.742	3.03×10^{-7} M	0.914	7.62×10^{-5} M
CV (%)[b]	9.33	450	14.4	552
Shapiro–Wilk *W*	0.981	0.193	0.946	0.172
P-value	< 0.0058	<0.001	< 0.0001	<0.001
Skewness	−0.108	7.839	−0.868	8.023
Kurtosis	1.043	66.299	1.671	70.269

[a] The pH obtained by [H$^+$] averaging is 7.13 for pond water and 4.85 for sediment.

[b] Coefficient of variation (CV) = (SD/mean) × 100.

result was confirmed by the Shapiro–Wilk *W*-test for normality (Table 2). Although the null hypothesis (i.e., that the sample represents a population with a normal distribution) could not be rejected at an α level of 0.01 for either pH or [H$^+$], the *W*-values were much greater for pH than for [H$^+$], and the closer the *W*-value is to 1.00, the more normally distributed is the data set. Tests for skewness and kurtosis resulted in much greater values for [H$^+$] than for pH, indicating greater skewness and greater

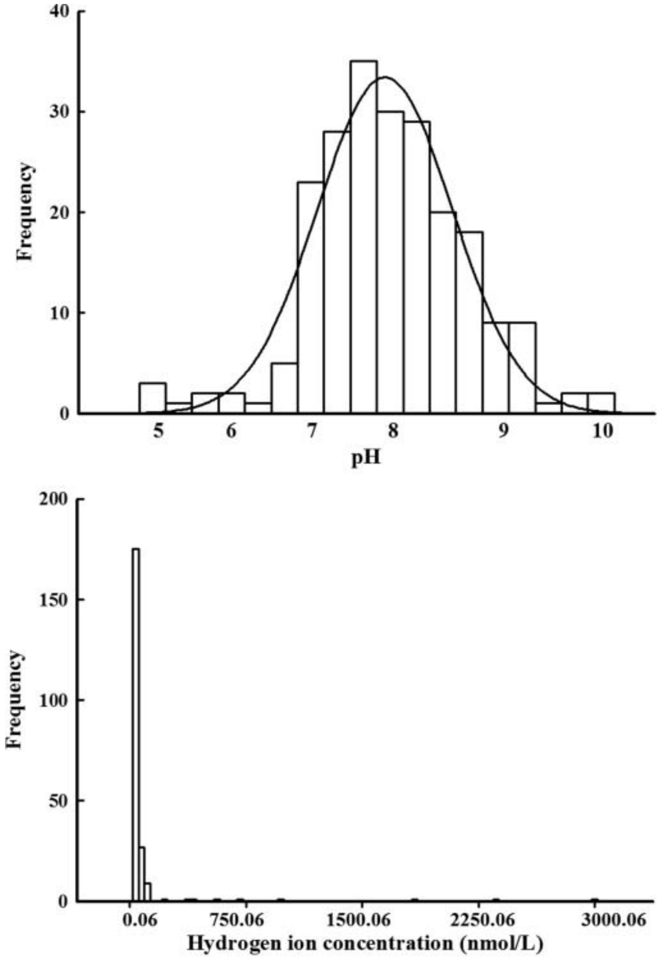

FIGURE 1. Frequency distribution histograms for the pH and hydrogen ion concentration of 233 water samples collected from channel catfish ponds in Alabama (sampled between 1100 and 1500 hours; data source: Silapajarn et al. 2004).

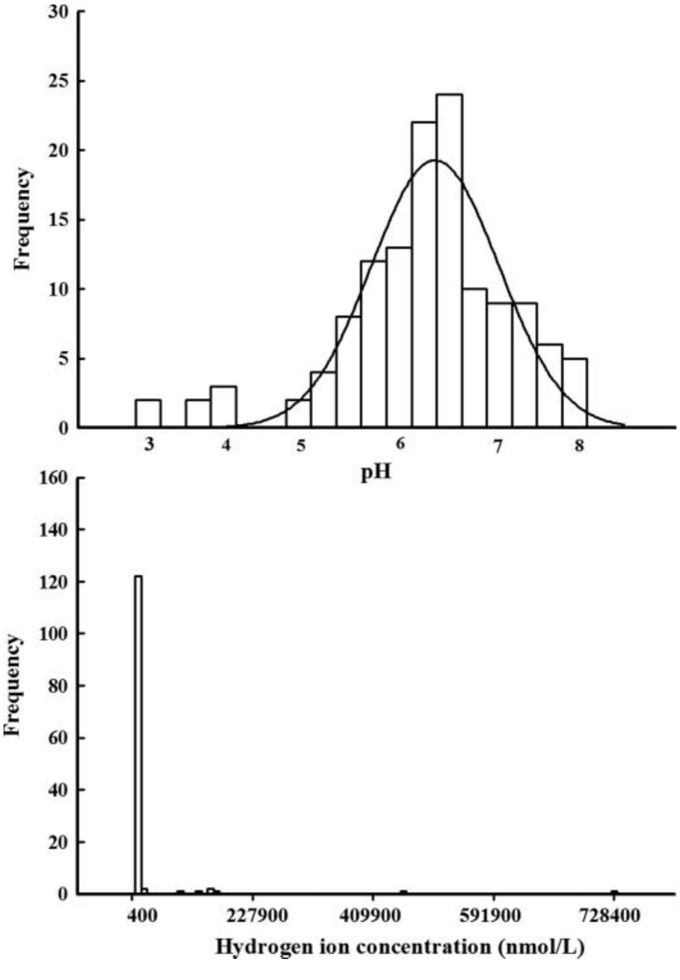

FIGURE 2. Frequency distribution histograms for the pH and hydrogen ion concentration of 131 sediment samples obtained from freshwater aquaculture ponds in Thailand (data source: C.E.B., unpublished data).

peakedness for $[H^+]$. The fact that pH is more normally distributed than $[H^+]$ for both data sets is worth noting; it suggests that in some situations, pH would be more appropriate for use in statistical analyses that require an assumption of normally distributed populations.

In addition to distorting the interpretation of statistics involving pH measurements, the most common method of measuring pH—potentiometry—is based on the Nernst equation, which in effect states that 10-fold changes in $[H^+]$ cause a linear change in electrode potential. This fact was recognized by J. W. Tukey (as reported by Feinstein 1979), who was a chemist prior to his illustrious career as a statistician. Tukey believed that arithmetic averages of pH are appropriate because based on the Nernst equation, pH is the measured value and $[H^+]$ is the derived value rather than the other way around.

Interestingly, a similar argument has been made for using statistics based on pH rather than $[H^+]$ when describing biological responses to pH. Many biological responses to stimuli are logarithmic and therefore may be normally distributed on a logarithmic (pH) scale but not on a linear ($[H^+]$) scale. This is not surprising because the membrane potential generated when a neuron is stimulated is also described by the Nernst equation. Boutilier and Shelton (1980) believed that this argument can be generalized and that the receptor system in animals probably responds to H^+ as a logarithmic function, which means that statistics based on pH are more appropriate than those based on $[H^+]$. The nature of the relationship between a biological response and either a linear or logarithmic measure of $[H^+]$ probably differs depending on the specific response of interest. However, the point here is that there is no a priori reason to reject the direct averaging of pH when assessing a biological response to $[H^+]$.

Although $[H^+]$ averaging may distort the interpretation of pH averages when the pH range is great, the difference in the two approaches becomes mathematically less meaningful when the pH range is small. In replicate pH analyses of a given sample or when pH is measured for replications of an experimental treatment, the pH seldom differs greatly and there usually is little difference between direct pH averaging and $[H^+]$ averaging for determining the average pH. For example, in an experiment on the use of copper sulfate to control phytoplankton in ponds at Auburn University, afternoon pH levels for five replications of a treatment were 8.3, 8.6, 8.2, 8.5, and 8.7. The average pH was 8.46 by direct averaging and was 8.42 by $[H^+]$ averaging.

It is rare to find a published paper in which the authors state that the $[H^+]$ averaging technique was used to calculate pH; this suggests that most authors use the direct averaging method. Therefore, when comparing the results of a current study with those of a previous one, it seems more appropriate to use the direct averaging method to estimate average pH in the current study unless it is known that $[H^+]$ averaging was used in the prior study. This would be especially true in cases where regression equations for estimating intensities of other chemical and biological variables from pH are based on direct measurements of pH.

Clearly, the appropriate method for pH averaging depends on the reason for making the measurements and the intended use of the statistic. When mixing certain waters of different pH values or in other circumstances where mixing is the conceptual context, $[H^+]$ averaging is the correct approach. In practice, however, there will be very few times when $[H^+]$ averaging provides a correct average pH because the buffers that are present in natural waters will affect the final pH above and beyond the effect of dilution. Direct averaging of pH is appropriate for most uses in fisheries and aquaculture, but the method used to average pH data—whether $[H^+]$ averaging or direct averaging—should always be indicated.

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