The Use of Carbon Dioxide to Construct pH Curves for the Measurement of Productivity

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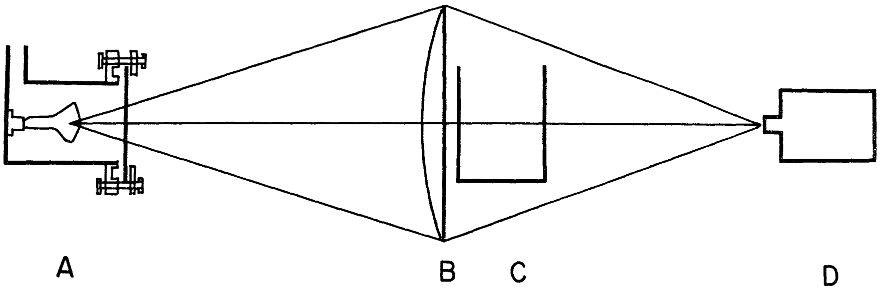

FIG. 1. Diagram of the illumination system: *A* is the light source; *B* is a 14 x 18 inch plastic Fresnel lens; *C* is the aquarium containing experimental animals; *D* is a movie camera.

To test for changes in linear velocity we invoked the null hypothesis that there is no significant difference in linear velocities in white light with and without infra red. Such a null hypothesis is amenable to the statistics of small samples.

The experiment was performed as follows. A population of *Daphnia magna* adapted to a given intensity of white light was continuously photographed with a movie camera for a period of thirty seconds. The animals were illuminated with white light for the first 15 seconds and with white light plus infra-red for the last 15 seconds. Analysis of the photographic record thus obtained shows an average linear velocity of 0.201 cm/sec. in white light and 0.196 cm/sec. in white plus infra-red. By the use of the Wilcoxon Sum for ranked differences of paired data, it was concluded that the mean differences of velocities are not significantly different from one another within the limits of the data.

Analysis of the same photographic record for angular velocities reveals an average angular velocity of 5 degrees/sec. in white light and 6 degrees/sec. in white plus infra-red. Again the use of the Wilcoxon Sum for the paired angular velocities indicates that they are not significantly different from each other within the limits of the data. The failure of *Daphnia magna* to respond to infra-red should not be extrapolated to other organisms without appropriate testing.

Finally, many behavior patterns are statistical in nature and are not readily observable over short periods of time. Such statistical behavior typically results in a net movement of a population in one direction to produce an aggregation at some given locus. The infra-red radiation employed in these studies whether with or without visible light failed to produce any aggregations of the population either toward or away from the source, or at the bottom of the container or at the surface of the water in a period of 2 hours.

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The Use of Carbon Dioxide to Construct pH Curves for the Measurement of Productivity[1]

INTRODUCTION

In recent years there has been increasing use of pH measurement for the determination of carbon metabolism in natural waters and in closed containers with living organisms (Verduin 1951, 1956a, 1956b; Jackson and McFadden 1954; Odum 1957a, 1957b; McQuate 1956; Park *et al.* 1958). A graph of pH against CO_2 is constructed for the particular water. The data for this graph are obtained either experimentally or by theoretical computation from alkalinity. Then the observed pH changes are converted into CO_2 changes by means of the graph. For some time, it has been apparent to us that one of the customary experimental procedures in this method leads to considerable error. The purpose of this note is to point out the error and suggest a better procedure applicable to both sea water and fresh water based on the use of CO_2 water.

In fresh water the theoretical equilibrium equations of Moore (1939) were used by Dye (1944) to compute nomograms so that with a known alkalinity, pH changes can be converted to CO_2 changes without actually determining the pH-CO_2 curve experimentally. In this work the alkalinity is determined by titration with a strong acid to approximately pH 4.5. Either a color indicator (such as methyl purple or methyl orange) or a pH electrode is used to locate the end point.

In sea water the constants in the equilibrium

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equations were determined by Buch and Wattenberg as summarized by Harvey (1957). With these constants for different salinities and temperatures, it is possible to compute a pH-CO_2 graph from alkalinity in sea water as well as in fresh water.

Because natural waters are not simple solutions containing CO_2 equilibria alone, it was considered advisable by Verduin (1956a) to refine the method, determining the pH-CO_2 curve by direct titration instead of by use of the theoretical equations and nomograms. This was an admirable advance since it was found that titration graphs were quite different and not readily predictable from alkalinity measurements alone. However, a conceptual error was involved which has affected many of the papers using this method, for example, Verduin (1956b, 1957), McQuate (1956), and Frey and Stahl (1958). As is well known, the titration curve of a strong acid and a weak base is quite different in shape from the titration curve of a weak acid and a weak base where there are common ions. Thus, when NaOH or HCl are used to obtain pH-CO_2 curves an erroneous assumption is introduced that an equivalent weight of strong acid or base is equal in effect on pH to an equivalent weight of CO_2.

METHODS

In order to provide a method for empirical determination of pH-CO_2 curves, and to show the error in using strong acids or bases, a titration was carried out with CO_2 water. Distilled water was allowed to come into equilibrium (both temperature and saturation) with a pure CO_2 atmosphere. The gas was bubbled through the water and, being heavier than air, was presumed to displace the air above the water in a two-liter Erlenmeyer flask. Thus, the water was assumed to be approximately in equilibrium with the CO_2 at one atmosphere pressure and at the measured temperature. For further refinement of the method, the CO_2 content of the water should be directly determined by an independent method. Saturation where bubbles are used is not likely to be exact.

From tables of CO_2 saturation in The Handbook of Chemistry (Lange 1952), the moles of total dissolved CO_2 per ml were computed. The CO_2 water was then introduced under the surface of a 100-ml sample of natural water by means of a volumetric pipette. pH was measured against added CO_2. Stable pH's were achieved approximately 5 seconds after the introduction. Bubbles of gas were rising through the flask of distilled water at all times. To avoid any unknown effects of suspended particulate matter, all samples of natural waters were first "Millipore" filtered. Initial high pH was obtained by use of N_2 gas to clear the sample water of free CO_2 before starting the titrations.

RESULTS AND DISCUSSION

Four graphs of pH against CO_2 were obtained with this method for a hard natural spring water and for a coastal sea water. Since these graphs were essentially identical, only one is given for each type of water. Figure 1 is a representative graph for the spring water and Figure 2 is one for the marine water. Also plotted in Figures 1 and 2 are the curves produced by titrations of the same waters with a strong acid (0.02 N H_2SO_4). The dashed curve in Figure 1 is the theoretical

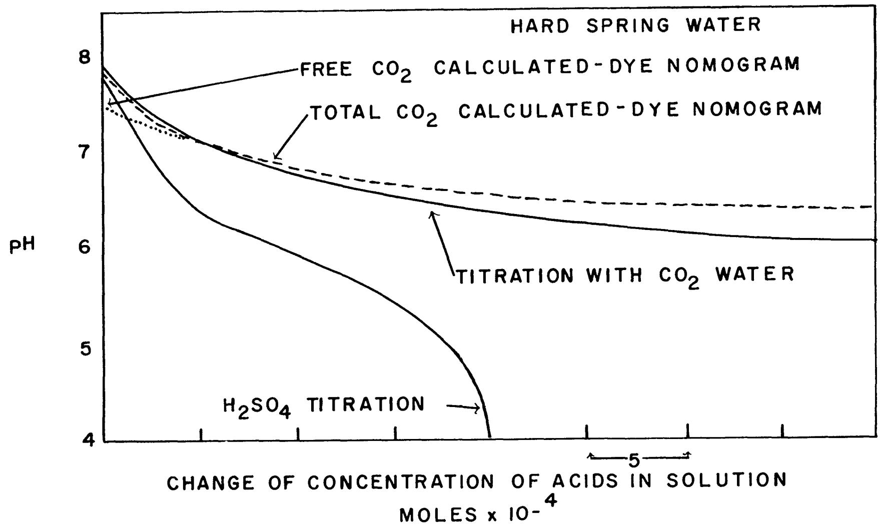

FIG. 1. Titration curves showing the pH as a function of acids added to hard spring water from San Marcos Spring, San Marcos, Texas. Temperature 28°C. Titration curves are given for sulfuric acid and water saturated with carbon-dioxide. The moles total carbon-dioxide calculated in solution are plotted as a function of pH as estimated with Dye nomograms. The dotted line is the free carbon-dioxide present at each pH.

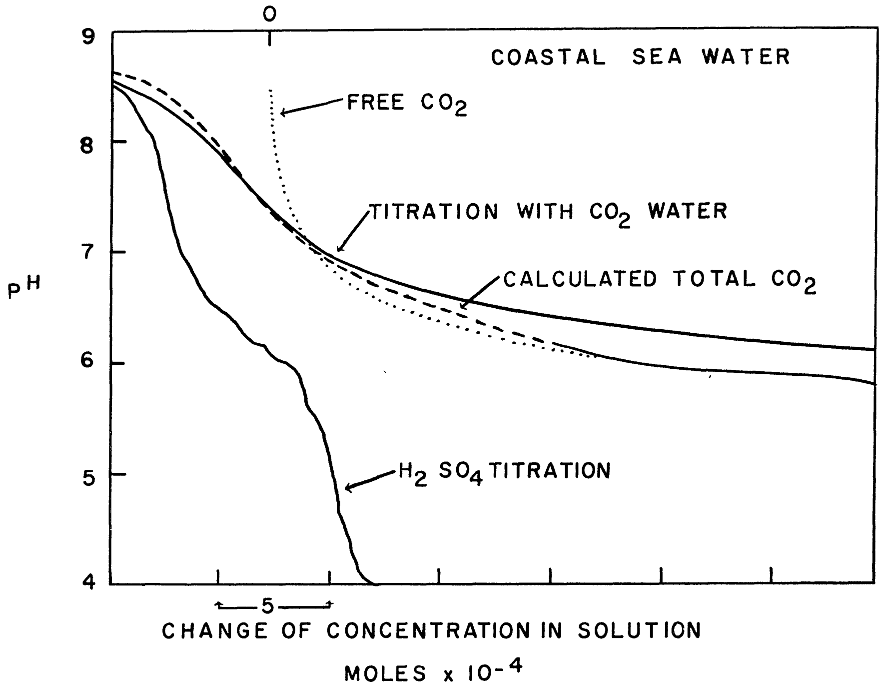

FIG. 2. Titration curves showing the pH as a function of acids added to sea water from a bay near Port Aransas, Texas. Temperature 28°C. Curves as in Figure 1.

curve determined with a Dye nomograph (Dye 1944). The dashed curve in Figure 2 is the theoretical curve computed from constants given by Harvey (1955).

The theoretical curve is similar to the curve determined with CO_2 water, but as expected on theoretical grounds, the titration curve of the strong acid is of entirely different shape. Metabolic rates computed by previous workers with strong acid curves may be in error in being too small.

At pH's below 7.5, increases in total CO_2 lead to increases in free CO_2 of the same amount. Thus the curves of pH and free CO_2 have the same general slope as curves of pH and total CO_2. As pH approaches 8, shifts in alkalinity equilibria occur so that curves of pH and free CO_2 have a different slope from curves of pH and total CO_2. The difference in total CO_2 and free CO_2 (dotted line) may be observed in Figure 2 for sea water. In studies of metabolism it is the total CO_2 that one is interested in measuring. Where data on diurnal free CO_2 changes have been used for computations of metabolism and respiratory quotient (Odum 1957a, 1957b), values are only approximate where the pH approaches 8.

Another example of a titration curve of CO_2 and pH is provided by McFarland and Norris (1958) in a paper that describes the pH changes taking place in enclosed vessels containing marine fish. In this case the fish provides a steady CO_2 supply which was computed from its metabolism. The pH-CO_2 curves thus provided are similar to those in Figures 1 and 2 obtained with artificial CO_2 water. Oppenheimer and Kornicker (1958) injected CO_2 gas into sediments and also obtained similar titration curves of marine sedimentary materials.

Thus it may be concluded that the pH-CO_2 curve method for measuring natural metabolism in free water is effective and empirically simple with the carbon-dioxide water procedure both in marine and fresh waters. Production and respiration values in a number of the previous papers involving titrations with strong acid or base are probably too small and should be recomputed.

Some have criticized the pH method for sea water on the grounds that the considerable alkalinity leads to an insensitive measurement (Ryther 1956). On the other hand Wells (1922) and Park *et al.* (1958) have found that coastal waters provide distinct diurnal curves for computation of carbon metabolism. These curves are even more easily obtained with the sensitive instruments presently available. Comparisons of metabolism in oxygen bottles and in free water (Odum and Hoskins 1958) indicate that bottle measurements are 2 to 100 times too small in shallow water. If bottle procedures such as the Winkler oxygen and radio-carbon light and dark bottle methods are not representative in shallow systems, then carbon metabolism is best measured with free water pH or oxygen methods. The diurnal measurement of metabolism with pH and

CO_2 has two great advantages over diurnal oxygen measurements: (1) No diffusion correction is ordinarily necessary with CO_2 curves; thus CO_2 curves reveal the course of night respiration (Park *et al.* 1958). (2) pH measurements are not as susceptible to pollution interference as are oxygen measurements by the Winkler process. However, both oxygen and pH curves are necessary for full interpretation of community metabolism.

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Memorial Resolution Respecting Dr. Bell M. Shimada and Townsend Cromwell, Esquire

Many members of the Society and other readers of Limnology and Oceanography will wish to endorse the memorial resolution of the Inter-American Tropical Tuna Commission which was made at their eleventh meeting in San Pedro, California on February 5, 1959.

Whereas, Dr. Bell M. Shimada and Townsend Cromwell, Esquire, were senior scientists on the staff on the Inter-American Tropical Tuna Commission since its early days

Whereas, their devotion to their researches and their outstanding abilities resulted in scientific contributions of great importance both to advancing basic knowledge in oceanography and marine biology and to the conservation of the living resources of the sea

Whereas, their scientific studies are of far reaching importance to the welfare of the fishing industries of the Americas

Whereas, these men lost their lives in the performance of their duties for the Commission. Now, therefore, be it

RESOLVED: That in the passing of Dr. Bell M. Shimada and Townsend Cromwell, the Inter-American Tropical Tuna Commission has lost two of the most important members of its scientific staff, marine science has lost two of its leaders, and the fishing industry of the American has lost two of its finest friends and supporters. Be it further

RESOLVED: That a copy of this resolution be incorporated in the Minutes of the Commission's Meeting, and copies thereof sent to their families and their associates.