

THE LIMNOLOGICAL AND
ECOLOGICAL SIGNIFICANCE OF
DISSOLVED YELLOW ORGANIC ACIDS
IN NATURAL WATERS

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ABSTRACT

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by Eugene Howard Buck

Yellow organic acids were extracted from natural waters and subsequently added to aquaria and experimental ponds. A quantitative procedure for the measurement of these compounds was developed using their characteristic fluorescence. This procedure is temperature sensitive and encounters interference when detergents are also present.

In natural water yellow organic acids may be allochthonous, mainly from runoff and leaf fall, or autochthonous, from the sediments and decay of aquatic vegetation, in origin. Loss to the environment was through a light-induced polymerization reaction and destruction by organisms as a source of energy or carbon. Diurnal and possibly annual cycles in acid concentration exist.

Chemically and physically changes in the concentration of these compounds produced changes in pH, conductivity, alkalinity and optical density. The first three may be explained by a hypothetical union between these acids and calcium carbonate while optical density changes are a product

of the characteristic light absorbance properties exhibited by the yellow organic acid molecule. Biologically these acids appeared to stimulate the growth of Navicula sp., Closterium sp., Arthrodesmus sp. and Surirella sp. while large copepods and cladocera were adversely affected.

This study indicates the significant role the yellow organic acids play in the chemical environment and the substantial effects these compounds may exert in the eutrophication process.

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By
Eugene Howard Buck

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
The problem	2
METHODS.	5
Acid recovery	5
Acid measurement and addition	19
Temperature	25
Alkalinity.	26
Conductivity.	26
Spectroscopy.	27
pH.	27
Light penetration	28
Oxygen.	31
Metal ions.	31
Fungi and bacteria aquaria.	32
Periphyton.	32
Centrifuged plankton.	33
Net plankton.	34
Other aquaria	35
Fate of acids in the ponds.	35
Environmental acid profiles	36
Aquaria contaminants.	36
RESULTS.	38
Acid concentration--aquaria	39
Acid concentration--ponds	42
pH--aquaria	43
pH--ponds	50
Conductivity--aquaria	51
Conductivity--ponds	52
Optical density--aquaria.	57
Optical density--ponds.	61
Alkalinity--aquaria	61
Alkalinity--ponds	66
Carbonate.	66
Bicarbonate.	66
Total.	67

TABLE OF CONTENTS - continued

Page

Temperature--aquaria.	67
Temperature--ponds.	67
Light penetration--ponds.	68
Oxygen--ponds	69
Metal ions--aquaria	70
Metal ions--ponds	70
Fungi and bacteria--aquaria	70
Fungi and bacteria--ponds	71
Centrifuged plankton--ponds	71
Centrifuged plankton--aquaria	75
Periphyton--ponds	75
Periphyton--aquaria	75
Net plankton--ponds	82
Net plankton--aquaria	82
Bottom organisms--ponds	87
Bottom organisms--aquaria	87
Fish--ponds	87
Fish--aquaria	87
Fate of the acids in the ponds.	87
Conditions in other natural bodies of water	89
Cause of viscosity in the aquaria	95
DISCUSSION	97
Method of acid measurement.	97
The origin and fate of the acids.	101
Effect of acids on chemical and physical features	109
Effect of acids on biological communities	121
Implications of this study.	123
SUMMARY.	127
LITERATURE CITED	129
APPENDIX	131

LIST OF TABLES

TABLE	Page
1. Summary of the acid addition to pond C.	23
2. Summary of the acid additions to the experimental aquaria	24
3. Significance of physical factors in the explanation of acid change	44
4. Observed changes in acid concentration in light and dark bottles suspended for three weeks in pond C (Data in ppm.)	88
5. Calculated effects of different sources of acid change as determined by light and dark bottle experiments. (Data in ppm.)	90
6. Acid concentration of various selected bodies of water	96
7. Dynamics of acid change in a pond on a summer day	106

LIST OF FIGURES

FIGURE	Page
1. Acid extraction efficiency from natural water as monitored by fluorescence during column flow on Duolite A-4 anion exchange resin.	8
2. Relationship between fluorescence of several random acid concentrations in distilled water and temperature.	14
3. Relationship between fluorometer units and weight of acids on the 3x scale.	18
4. Relationship between fluorometer units on the 10x scale and concentration of acids computed from fluorescence readings on the 3x scale . . .	21
5. Relationship between gram-calories per centimeter square per thirty minutes as measured by pyrhelimeter to units recorded by the transistORIZED light meters	30
6. Acid concentrations of the aquaria through the experiment	41
7. Experimental relationship between pH and acid concentration determined in the aquaria.	47
8. pH--acid concentration relationship in the individual aquaria with the theoretical trend of pH superimposed	49
9. Experimental relationship between conductivity and acid concentration determined in the aquaria	54
10. Conductivity--acid concentration relationship in the individual aquaria with the theoretical trend in conductivity superimposed	56
11. Experimental relationship between optical density and acid concentration determined in the aquaria.	60

LIST OF FIGURES - Continued

FIGURE	Page
12. Optical density--acid concentration relationship in the individual aquaria with the theoretical trend in optical density superimposed .	63
13. Bicarbonate alkalinity in the fish aquaria pair during the study period	65
14. Index values for centrifuged plankton from the ponds	74
15. Index values for centrifuged plankton from the aquaria	77
16. Index values for the dominant species found in the periphyton in the ponds	79
17. Index values for the dominant species found in the periphyton in the aquaria	81
18. Net plankton index values for the ponds through the summer.	84
19. Net plankton index values for the designated aquaria pair.	86
20. Acid concentration profiles of three lakes. . .	92
21. Acid concentration profile of the Clam River. .	94
22. Diurnal and annual acid cycles in a pond. . . .	108
23. Revised pH--acid concentration relationship . .	112
24. Revised conductivity--acid concentration relationship.	116

INTRODUCTION

Investigators have long known of the distinct communities which exist in highly colored bodies of water. A few have alluded to correlations of color with noticeable ecological changes in these systems (Transeau, 1905; Anthony and Hayes, 1964). It is only lately that interest has been directly focused on these colored compounds in an effort to explain their chemical and biological effects upon aquatic ecosystems. Most noteworthy in their attempts at chemical characterization of these compounds have been Shapiro (1957; 1958), Povoledo (1964), Povoledo and Gerletti (1964), Christman and Ghassemi (1966a;b) and Christman and Minear (1967). Shapiro (1964), Kent and Hooper (1965) and Christman (1967) have studied the interaction of these compounds with metallic ions in water while Anthony and Hayes (1964) have found color to be significant in quantitative expressions of bacterial standing crop.

Qualitatively these colored molecules are a diverse mixture of acidic phenolic residues and multipolymeric chains of such units. Being so heterogeneous it is not possible to set forth any definite chemical structure. Their derivation is probably from plant debris decomposition in the surrounding soil and within the aquatic system.

It is the universality of distribution, the prominence of occurrence and the possible ecological significance of these compounds which influenced the choice of this problem.

The problem

It was decided that a general investigation into the ecological and limnological significance of these compounds could best be conducted with a field study involving the application of these acids to a natural ecosystem supplemented by laboratory studies of aquaria containing the several distinct communities found in this system. The objectives were to develop methods for the study of and to study the possible changes in the physical and chemical environment upon acid addition and to attempt to relate these changes to any ecological modifications. Quantification of these changes would also be attempted.

The field work was conducted on two ponds, one experimental and one control, located at the Michigan State University Agriculture Experiment Station at Lake City, Michigan. These ponds will be further designated as C, the experimental with a surface area of 0.17 acres, and D, the control with a surface area of 0.18 acres. Both ponds had an average depth of 3 to 4 feet and were situated adjacent to each other with a 10 to 15 foot wide dike as separation. They both had inlets from a common holding pond and so were probably as similar in chemistry and biology as could be found for this purpose.

Each pond was provided with connections to a constant monitoring system in the laboratory where temperature, oxygen and pH could be continuously recorded. Either pond could be connected to this system and the changeover between ponds could be accomplished in 5 minutes. A Little Giant submersible pump, model 3E-12NR, was placed two feet beneath the surface near the center of each pond on a submerged screened platform and connected by $\frac{1}{2}$ " I.D. tygon tubing buried three inches underground leading to the laboratory. Water was forced into the laboratory at a rate of 670 gallons per hour where it flowed into a $5\frac{1}{2}$ inch diameter cylindrical plexiglas monitoring site and came in contact with the measuring electrodes inserted through specially constructed ports. Water left the laboratory and returned to the pond of origin along similar tubing.

The aquaria studies were conducted in adjacent laboratory facilities using water from pond D. Aquaria were set up with different community structure including bottom organisms, zooplankton, phytoplankton, periphyton, fungi and bacteria, and fish. Each aquaria was illuminated with a bank of two fluorescent lights about one foot above the water surface. Water lost by evaporation was replaced with distilled water each week.

Several independent experiments were planned and carried out in the effort to learn more of the mechanism of acid accumulation and disappearance from natural waters.

Several lakes and a stream were also sampled to yield information on the range and variability of acid occurrence in various environments.

METHODS

Acid recovery

In the initial search for a readily available and easily recoverable organic compound to work with, I used a modification of the separation procedure outlined by Aronoff et al. (1947). This involved passing filtered lake water through first a column of Duolite C-3 cation exchange resin followed by passage through Duolite A-4 anion exchanger. After finding the most abundant organic substances were the colored organic acids removed by the anion exchange column, the extraction process was considerably shortened.

The final procedure as was used during the field work consisted of a 55 gallon oil drum with a plastic liner elevated atop a three foot stand to serve as a reservoir for gravity flow to the resin columns. This apparatus was located ten feet from the holding pond which served ponds C and D and was filled twice daily by either a Homelite gasoline pump or a Little Giant submersible pump. Water was siphoned out of the barrel through tygon $\frac{1}{4}$ inch I.D. tubing to porcelain filters holding glass wool at the level of the base of the barrel. The rate of flow was regulated in the tubing by adjustable hose clamps and the greater portion of the suspended particulate matter was removed by

the filters. The glass wool was removed and replaced whenever the filtration capacity was lowered appreciably so as to obstruct maximum column flow due to the accumulated material in the filter.

The filtrate then flowed into the top of the resin column, passed through the column and discharged into the ground. A maximum of three columns with separate filters were maintained in operation during the summer. By adjustment for maximum flow, the columns were kept in operation around the clock. A total of 105 column-days (one column-day is equal to one column in operation for one day) was the actual operation time of the apparatus. By comparison of fluorescence of the water in the barrel reservoir to that of the column effluent, these columns averaged 82% efficiency in the removal of acids from the water during the summer. Columns were removed from the barrel when this efficiency dropped in the range of 75% recovery which averaged about 13 days flow time with variation dependent upon flow rate through the column. The general pattern of acid recovery on the column is shown in Figure 1. By comparison with the breakthrough patterns of this type of resin (Duolite Ion Exchange Manual) the resin is filled to approximately 85% of exchange capacity.

The columns were constructed of discarded water deionizer columns. The top was cut off just below the cap, the old resin removed and the plastic tube washed. As the

Figure 1. Acid extraction efficiency from natural water as monitored by fluorescence during column flow on Duolite A-4 anion exchange resin.

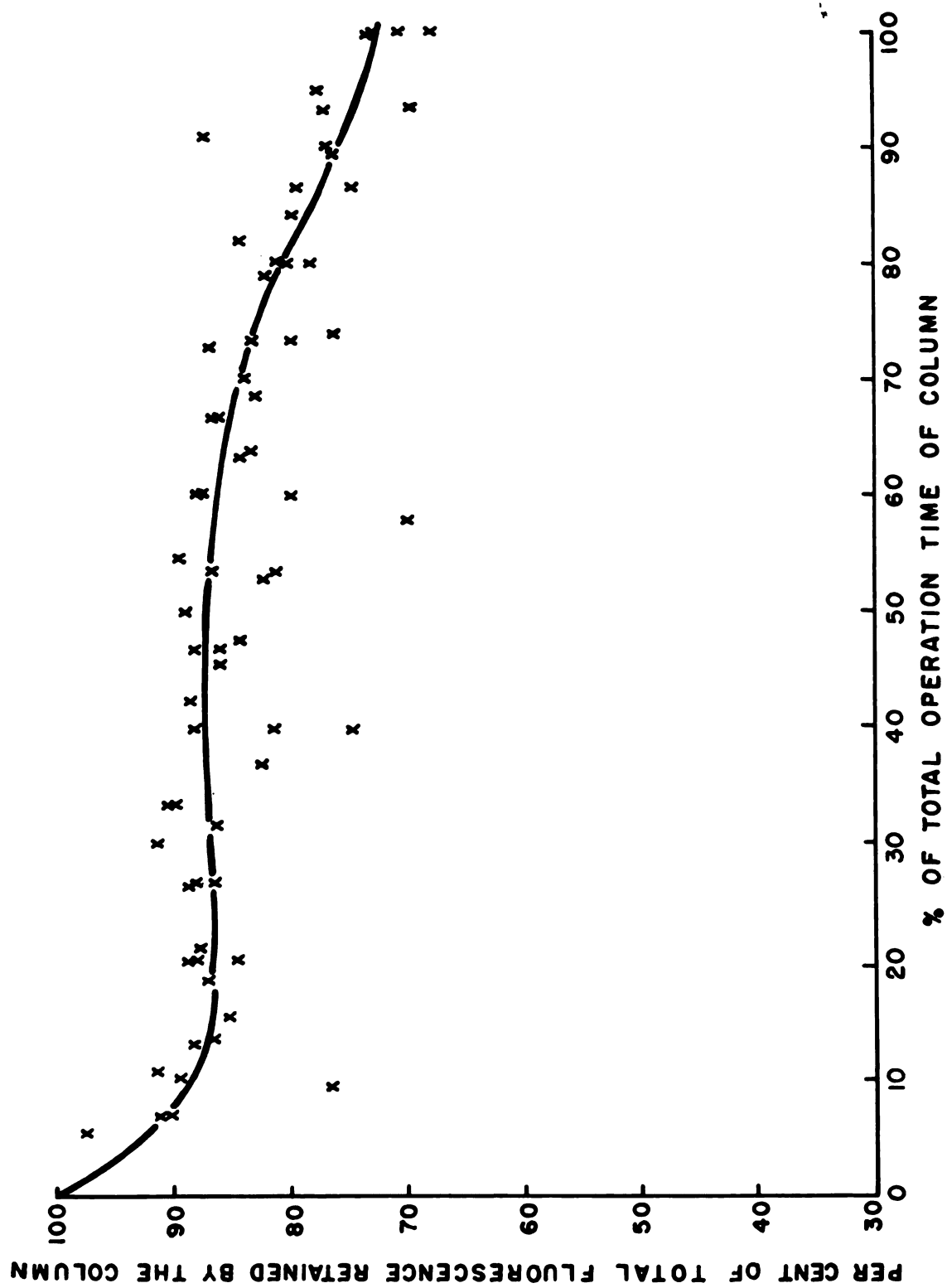


FIGURE 1

A-4 anion exchanger was received from the company as a dry resin, it was necessary that it be soaked overnight in water before packing the columns. During this soaking the resin expanded 12% by volume. In the morning the water-resin slurry was poured into the column until about 50 cubic inches or 800 cubic centimeters of resin had been added. A water flow was then introduced at the base of the column which forced any trapped air out and arranged the resin particles within the column by size. Upon draining the water to within one inch of the surface of the resin, the column was ready for conditioning (Duolite Data Leaflet No. 5).

First, two bed-volumes (about 1600 milliliters) of 1.5 N sodium hydroxide were introduced at the top of the column and the flow regulated for a passage time through the column of ten minutes. Next the column was rinsed with five bed-volumes of distilled water at the same flow rate. Then two bed-volumes of 2 N hydrochloric acid were passed through the column and it was rinsed again with five bed-volumes of distilled water. This entire cycle was repeated once more and was followed by a rinse with two bed-volumes of 95% ethanol to remove any non-polar impurities. After a final wash with five bed-volumes of distilled water, the column was ready for use. It was clamped into position beneath the filter of the collection apparatus and water was allowed to enter and flow through the column.

When the rate of acid extraction had decreased to the 75% efficiency range, the column was removed from the apparatus and taken into the laboratory for acid recovery and processing. The resin was removed from the column, placed in a large glass cylinder and one liter of 2 N sodium hydroxide was added. This mixture was stirred from time to time and allowed to stand for four to six hours.

At this time the supernatant was poured off into an enamel pan and concentrated hydrochloric acid was stirred in until a distinct lightening of color occurred. This happened when the solution became acidic and the free acids were released. The acidified supernatant was then evaporated to dryness in a drying oven held at a constant temperature of 36°C. At this temperature no decomposition of the acid molecule should have occurred (Shapiro, 1957). Another liter of 2 N sodium hydroxide was added to the resin in the glass cylinder and this process repeated until little observable color could be extracted from the resin.

When the combined extracts were completely dry, 100 ml of 95% ethanol were added to the pan. The mixture was stirred for maximum color extraction and this supernatant decanted into a small glass cylinder. This process was repeated several times until only the white sodium chloride residue remained in the pan. The combined ethanol extracts were evaporated to dryness and extraction repeated on this dry residue with 95% ethanol until all color was removed and

only a white sodium chloride residue remained. The final product was a dark brown waxy amorphous substance with a penetrating odor similar to vanillin. This was then dissolved in 95% ethanol and stored in a refrigerator at 40°F. in as saturated a solution as could be made, or a maximum of about 15 grams per 100 ml 95% ethanol.

The observation by Shapiro (1957) of the intense fluorescence produced by these compounds led to the attempt at quantitative measurement by fluorometric methods. Spectrofluorometric analysis by Dr. Robert E. Phillips of G. K. Turner Associates found an intense fluorescence peaking at 4700 Å with the maximum wavelength of activation at about 3350 Å. This compares favorably with the fluorescence measured for organic color in water by Christman and Ghassemi (1966a;b). The emission peak varied with excitation wavelength which characterizes a heterogeneous molecular mixture. From these data it was decided to use the Turner Filter Fluorometer Model 111 with the primary filter #7-60 and the secondary filter combination of #2A and #48. The excitation by the mercury line at 3660 Å with filter #7-60 was thought adequate for these compounds (R. E. Phillips, personal communication). All readings were of samples in the standard 12 x 75 mm, 8 cc Pyrex cuvettes with sample fluorescence being recorded immediately when the maximum dial reading was reached, usually within 15 seconds.

When this combination was tried it was found to give variable results. Several physical and chemical factors were investigated for possible interference. Temperature was known to effect fluorescence (Udenfriend, 1962) so this was studied first.

Erlenmeyer flasks were prepared with 100 ml of distilled water and several levels of acid concentration. Fluorescence was measured in arbitrary units as marked on the fluorometer while the temperature was adjusted through the range of 0° to 40° C by the application of ice or heat. A standard of distilled water was used and a zero level was set as five units so that fluctuations of the standard could be more easily noticed and adjusted. The resultant plots are seen in Figure 2. From these it can be calculated that fluorescence decreases 0.61% per degree Centigrade temperature increase. The expression for fluorescence corrected to 36° C., the temperature standard used in this study, is therefore:

$$\text{Fluorescence units corrected to } 36^{\circ} \text{ C.} = \left[\begin{array}{cc} \text{Fluorescence} & \text{Zero} \\ \text{units read} & \text{point} \end{array} \right] \left[\begin{array}{c} 1 - 0.0061 (36.0 \\ - \text{temperature}) \end{array} \right]$$

Christman and Ghassemi (1966a;b) report a direct relationship between pH and color intensity. This change in color was utilized in the preparation procedure to visually approximate acidification of the extracts, but closer investigation showed that the variation in pH seen to affect color would not affect fluorescence to anywhere as

Figure 2. Relationship between fluorescence of several random acid concentrations in distilled water and temperature.

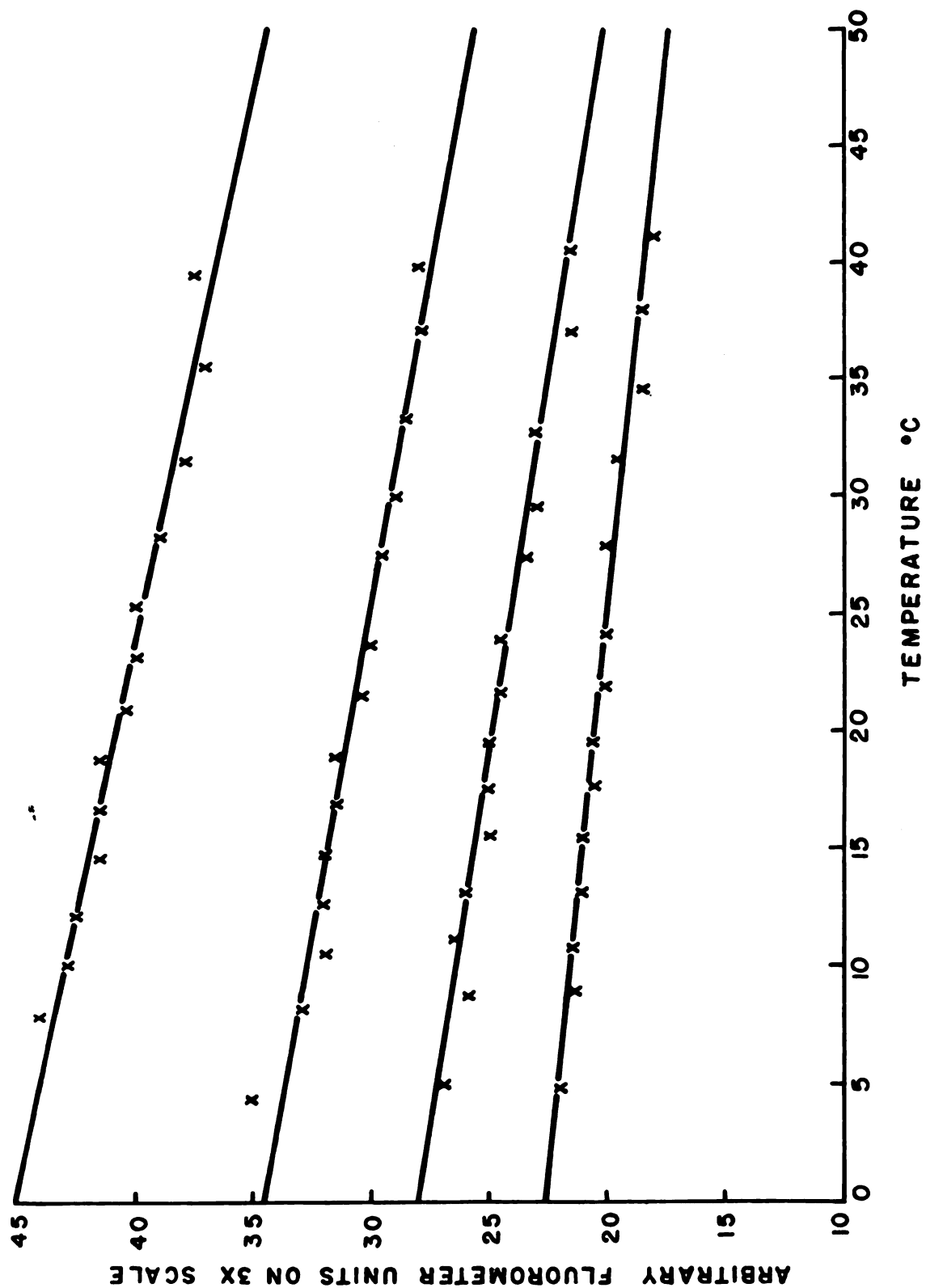


FIGURE 2

significant a degree under the conditions found in natural waters. In purified lignin solutions, Christman and Minear (1967) found that fluorescence was significantly decreased in both acid (pH less than 4.0) and alkaline solutions (pH above 7.0). When whole wastes were analyzed, little variation in fluorescence was found between pH 4.0 and pH 10.0 for low concentrations of the measured material. The pH effect does not appear significant enough in the range of natural waters to warrant correction of the measured values.

This last mentioned study also found chloride ion concentrations of up to 20,000 milligrams per liter had little effect upon fluorescence intensity.

Fluorometer units, corrected to a standard 36° C., were then used to establish a quantitative measurement for the dissolved yellow organic acids. One-tenth of a milliliter was chosen for the basic volume measure for convenience. From the supply of concentrated acids in solution, 0.1 ml was diluted to 15 ml with distilled water. This dilution was read on the fluorometer, corrected to 36° C. and the arbitrary units designated as the fluorometer units of acids in 0.1 ml of the dilute solution. The total number of units in the diluted 15 ml solution would then be equal to the number of units originally in 0.1 ml of the concentrated stock solution or a dilution factor of 150.

A powder paper was weighed on a Mettler analytical balance model H6T to 0.1 milligram accuracy and 10 ml of the

stock solution was placed in a depression formed by this paper in a plastic petrie dish. This was dried 24 hours at 36° C. in a drying oven. At that time the paper with the acid residue was weighed again. A blank of distilled water and three concentrations of acid in a wide range were measured on the fluorometer. The weight of the paper initially was subtracted from the dried weight to obtain the uncorrected acid weight. This weight was then corrected for the change in the blank during drying resulting in the weight of the dried acids. The relationship between the total number of fluorometer units in 10 ml of concentrated acid and the weight of this quantity was then known allowing a standard curve to be drawn (Figure 3).

The result was an average of 17020 fluorometer units per milligram of dried acids. The expression then for converting arbitrary fluorometer units previously corrected to 36° C. and measured on the 3x scale of the Turner model 111 fluorometer with the above stated filter combination into milligrams per liter (ppm) of yellow organic acids dissolved in water is:

$$\text{Milligrams per liter of acids} = \frac{(10000) (\text{fluorometer units corrected to } 36^{\circ} \text{ C. in } 0.1 \text{ ml})}{17020 \text{ fluorometer units per milligram acids}}$$

The factor of 10000 is necessary to convert the numerator to fluorometer units in one liter. Since the limit of sensitivity on the fluorometer is one-half an arbitrary fluorometer unit, sensitivity in this range is ± 0.3 ppm.

Figure 3. Relationship between fluorometer units and weight of acids on the 3x scale.

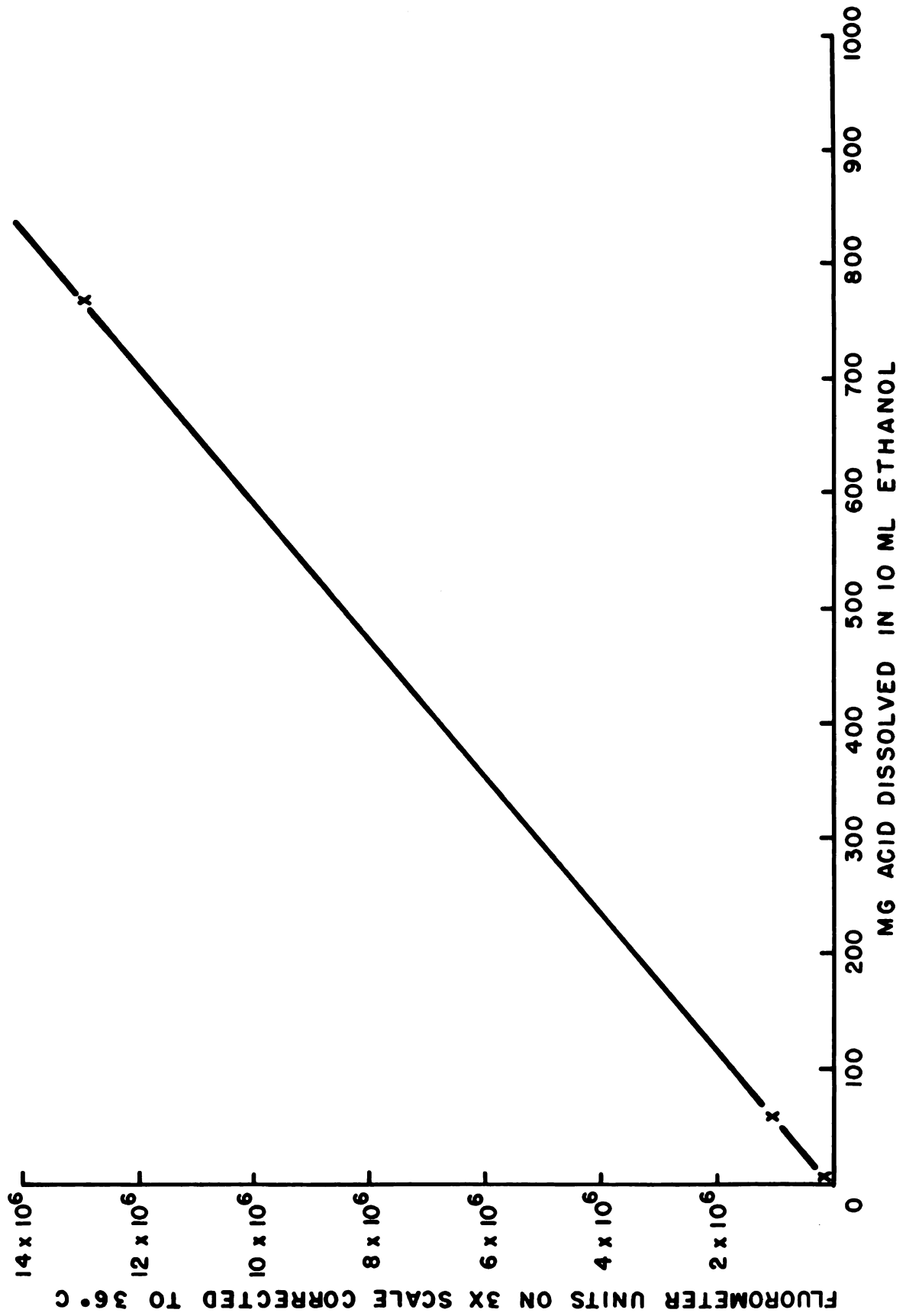


FIGURE 3

Figure 3. Relationship between fluorometer units and weight of acids on the 3x scale.

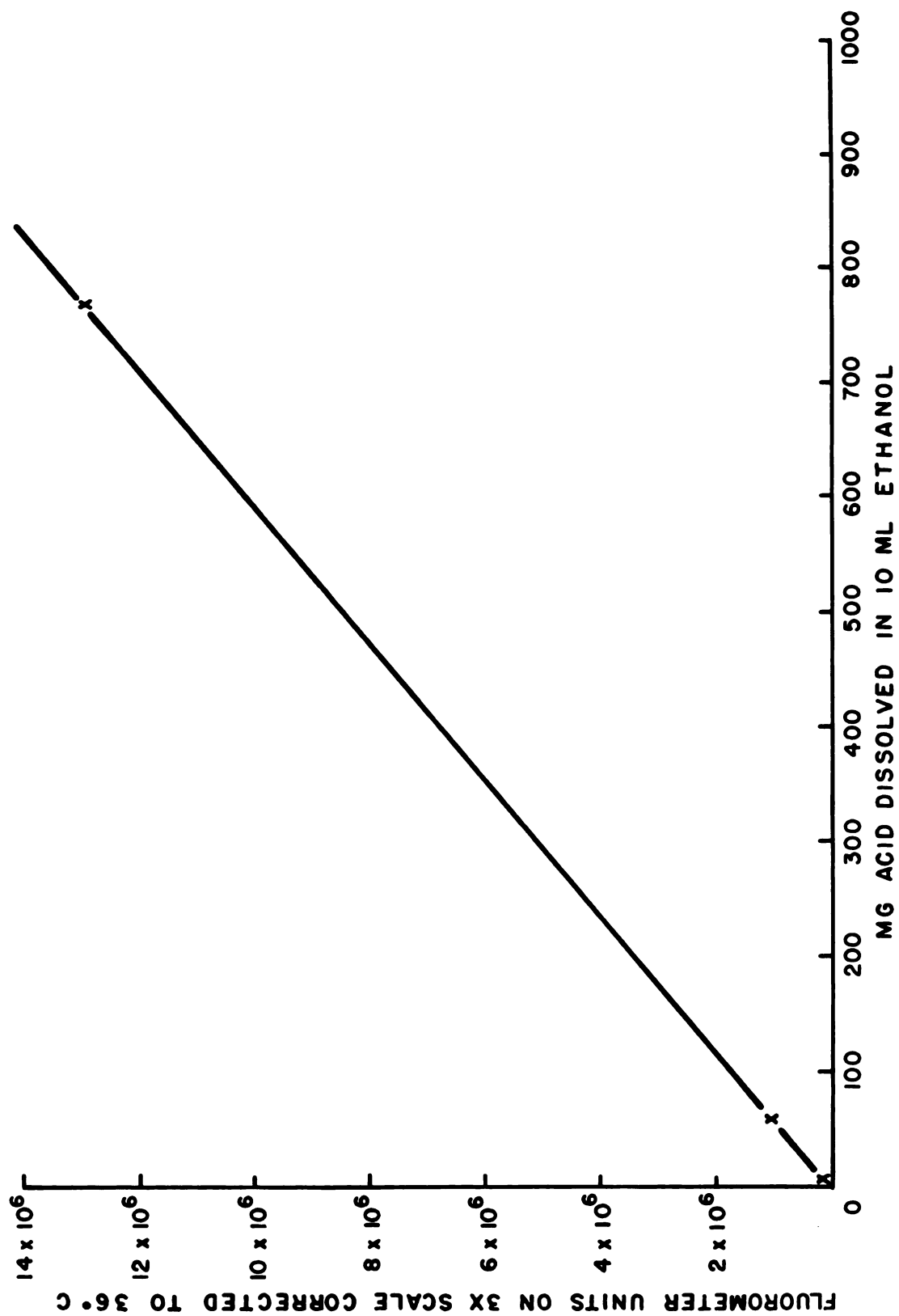


FIGURE 3

By relating the readings on the other scales, 10x and 30x, to the 3x scale we can develop a conversion factor for these ranges. Temperature must still be corrected for in the same manner before conversion from fluorometer units to ppm. On the 10x scale the regression between ppm figured from 3x scale readings and the 10x scale fluorometer units (Figure 4) yields the expression:

$$\text{Milligrams per liter acids} = \frac{(10000) (\text{fluorometer units corrected to } 36^{\circ} \text{ C.})}{64680 \text{ fluorometer units per milligram on 10x scale}}$$

$$N = 15 \quad r = 0.997 \quad \text{Sensitivity} = \pm 0.08 \text{ ppm}$$

On the 30x scale the conversion likewise becomes:

$$\text{Milligrams per liter acids} = \frac{(10000) (\text{fluorometer units corrected to } 36^{\circ} \text{ C.})}{183540 \text{ fluorometer units per milligram on 30x scale}}$$

$$N = 6 \quad r = 0.999 \quad \text{Sensitivity} = \pm 0.03 \text{ ppm or } 30 \text{ ppb}$$

Acid measurement and addition

Four water samples from each pond were taken twice weekly with a one liter Kemmerer water sampler at $1\frac{1}{2}$ feet depth at randomly selected sampling points. These samples were placed in 120 ml polyethylene bottles and stored in a drying oven set at 36° C. to minimize possible error from temperature changes at the time of reading. When the water samples had reached the vicinity of 36° C. , after about five hours in the oven, they were removed one at a time, their temperature recorded and a 5 ml aliquot transferred to the

Figure 4. Relationship between fluorometer units on the 10x scale and concentration of acids computed from fluorescence readings on the 3x scale.

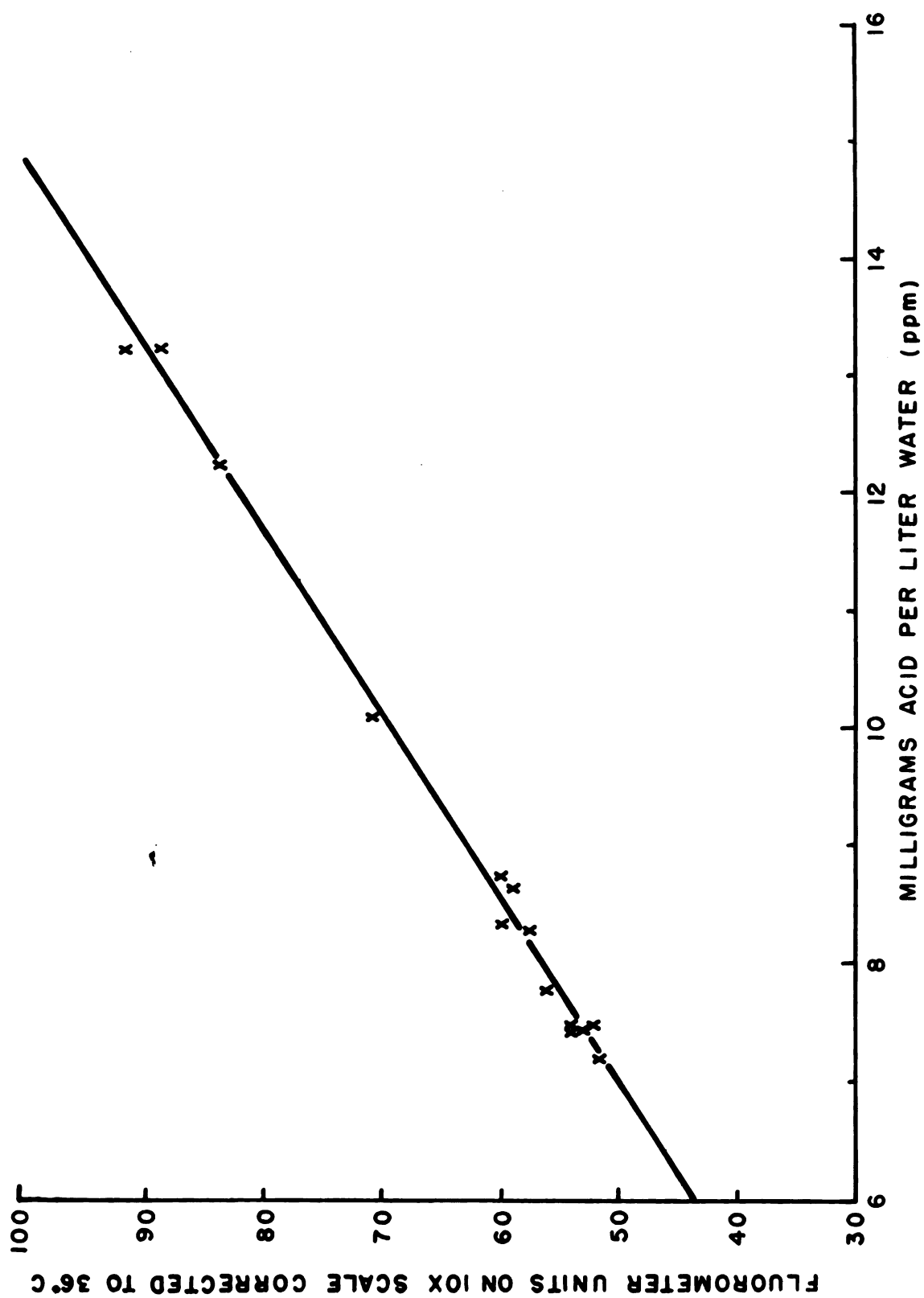


FIGURE 4

Figure 4. Relationship between fluorometer units on the 10x scale and concentration of acids computed from fluorescence readings on the 3x scale.

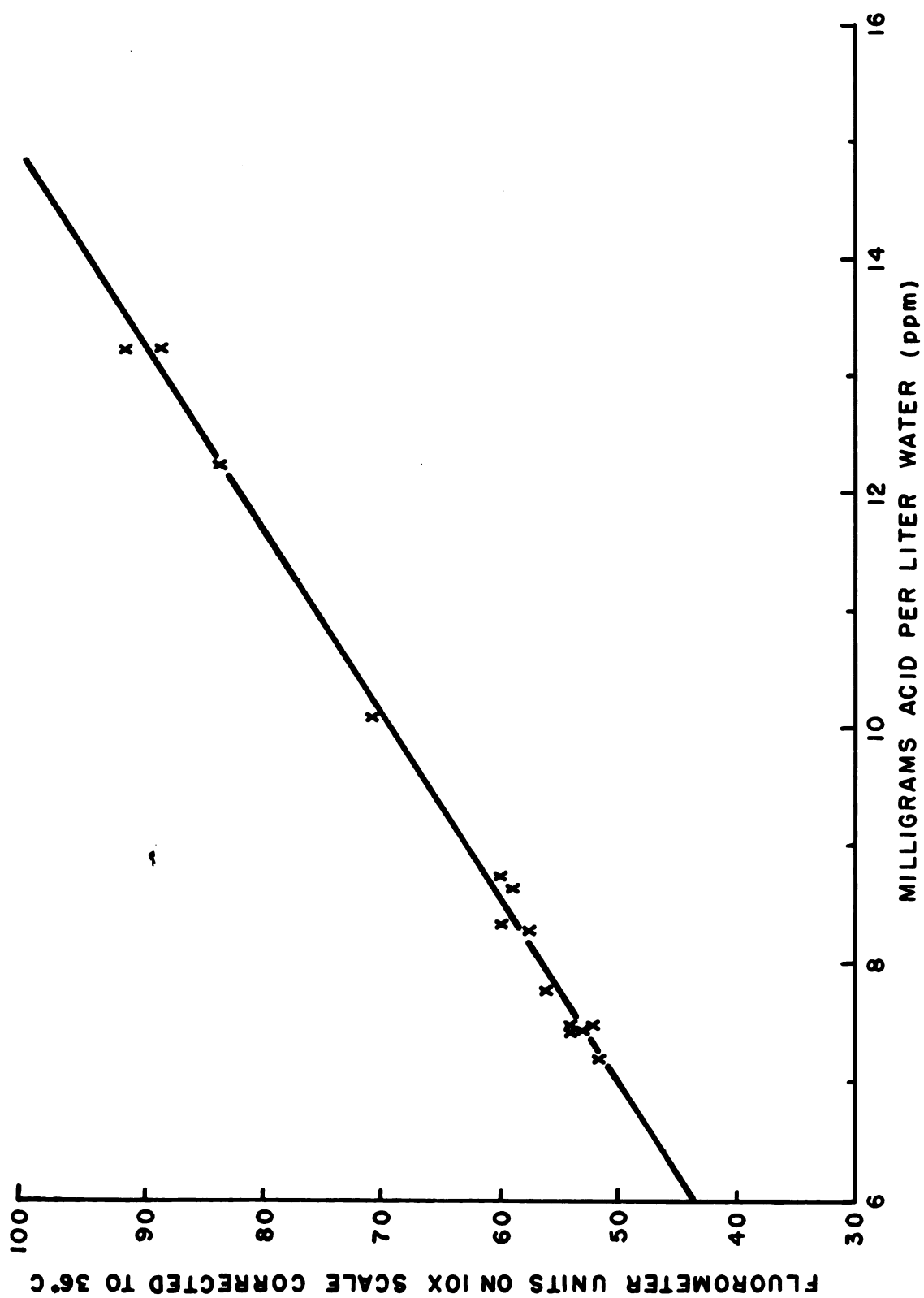


FIGURE 4

fluorometer cuvette. The Turner Filter Fluorometer model 111 with primary filter #7-60 and secondary filter combination #2A and #48 was used on the 3x sensitivity scale for all normal measurements. Other scales were used infrequently when the range of acid concentration made a different scale more applicable. A distilled water blank was used to set a zero point at five fluorometer units and this calibration was rechecked between each sample and the next.

A 120 ml polyethylene bottle was filled at mid-depth in each aquaria twice weekly and the identical procedure followed with these samples.

Purified acids extracted from the local water source were added to pond C three times during the summer (Table 1). These acids were dissolved in 95% ethanol and distributed over the surface of the pond from the stern of a moving rowboat. The acid solution was added dropwise and mixed by the action of the rowboat. Only on the third acid addition was an equal volume of ethanol added to the control pond.

Acids were added to the experimental aquaria four times during the summer (Table 2). One to three milliliters (depending on the aquaria volume) of the concentrated stock solution of acids were added at the water surface and mixed thoroughly. The aim was to raise the level of acid concentration several ppm each time although some of the aquaria were increased as much as 20 ppm after certain additions. An equal volume of ethanol was always added to the control aquaria at the same time.

Table 1. Summary of the acid additions to pond C

Date	Time	g acid	ml ethanol	ppm theoretical acid increase
July 21	11:15 AM	5.91	109	0.008
July 28	8:00 PM	11.23	393	0.016
August 30	10:00 AM	70.46	1000	0.10

Table 2. Summary of the acid additions to the experimental aquaria

Date	Time	g acid	ml ethanol	ppm acid concentration increase
July 26	10 AM - 2 PM	.045 - .100	1 - 3	3.0 - 3.3
August 4	2 PM - 6 PM	.110 - .115	1.9 - 2	3.7 - 6.9
August 10	11 AM - 2 PM	.144	2	4.8 - 9.0
August 17	11 AM - 3 PM	.317	2	10.0 -20.0

Analysis of variance was performed on the pond data to determine if the acid additions in pond C were detectable. One set of samples was collected every hour for 25 hours and was tested by one-way analysis of variance for significant changes during a diurnal cycle. An attempt was made to analyze changes in the ponds attributable to physical factors such as rain, temperature, sunlight, etc.

Aquaria measurements were analyzed by computer for significant relationships with other chemical factors.

Temperature

Pond D was continuously monitored with a Taylor recording water thermograph with the sensing probe at a two foot depth. This probe was shaded so solar heating would not affect the readings. Both ponds C and D were measured by the monitoring system in the laboratory when that pond was connected to the system. Here temperature was measured with a Yellow Springs Instrument Tele-thermometer using a thermistor probe and continuously recorded on the YSI model 80 laboratory recorder. Standardization of the instruments was checked every week. Temperatures of the aquaria were measured irregularly with a mercury thermometer. The collected data were analyzed to determine if the addition of these acids had any effect on the water temperature or the rate of heat exchange of the body of water.

Alkalinity

Carbonate and bicarbonate alkalinity were measured using the method described by Welch (1948). Commercially prepared and standardized N/50 sulfuric acid was used in all titrations. Duplicate samples were taken twice weekly from randomly selected sampling sites in both ponds with a one liter Kemmerer water sampler at a depth of $1\frac{1}{2}$ feet. The water was transferred to 120 ml polyethylene bottles and titrated immediately on return to the laboratory.

Analyses were performed on the differences in carbonate and bicarbonate between the ponds to determine if acid addition significantly affected this system.

One set of aquaria, that with fish present, was observed for alkalinity changes. A 100 ml volumetric pipette was used to withdraw a sample at mid-depth twice weekly which was then transferred to a beaker and titrated. The change in bicarbonate (the only form of alkalinity recorded in the aquaria) between experimental and control was followed through increasing acid concentrations.

Conductivity

The water samples collected for acid concentration measurement were also used for conductivity measurements. Eighty milliliters of the water sample were placed in a 100 ml graduated cylinder and the probe of an Industrial Instruments type RC conductivity bridge inserted. The temperature of the sample, scale reading in micromhos per centimeter,

scale multiplier and probe constant were recorded and all measurements were adjusted to micromhos per centimeter at 36° C. The temperature coefficient for the water from the ponds was experimentally determined to be 0.0157 per degree Centigrade or an increase in conductivity of 1.57% per degree Centigrade temperature increase.

Aquaria samples were processed in the same manner as those from the ponds and all data were analyzed in the same manner as the acid concentration data.

Spectroscopy

The water samples used for conductivity and acid concentration were also used for spectroscopic-optical density measurements. Ten ml of the sample were placed in the cuvette and optical density was read at 330 mμ on a Bausch and Lomb Spectronic 20. Aquaria samples were treated in a like manner. Analysis of the data was in the same manner as the acid concentration data.

pH

Measurement of hydrogen ion concentration of the ponds was conducted in the laboratory at the monitoring site with a Beckman Expanded Scale pH Meter equipped with automatic temperature compensation. The first half of the summer no recorder was available so irregular readings were made during the day. During the later summer a Sargent Recorder Model SR was used and a continuous pH record made during the day.

Recording was done at a chart speed of twelve inches per hour and a chart span from pH 5.0 to pH 10.0. pH was standardized once a week. Aquaria samples were taken and measured by the same instrument once a week.

Analysis of variance was performed on the pond data to determine if any significant changes in pH occurred which might have resulted from the acid additions. Aquaria measurements were used to define the relationship between chemical changes in the environment.

Light penetration

A Bristol pyrheliometer with chart recorder and digital read-out was in operation each day from 4 AM to 8 PM to measure the solar energy. The pyrheliometer bulb was located between the two ponds and about two feet above ground level. Also in operation were two transistorized light sensing meters. One was positioned adjacent to the pyrheliometer bulb while the other was mounted at one foot beneath the water surface in one of the ponds. This sensor was moved between ponds every three to four days.

A calibration run was made for both sensing meters against the standardized pyrheliometer so that the meter units could be converted into gram-calories per centimeter square per time period. A time period of thirty minutes was chosen for convenience (Figure 5). Using these conversions, the meter readings every day were expressed as gram-calories per centimeter square per thirty minutes and the percent of

Figure 5. Relationship between gram-calories per centimeter square per thirty minutes as measured by pyrheliometer to units recorded by the transistorized light meters.

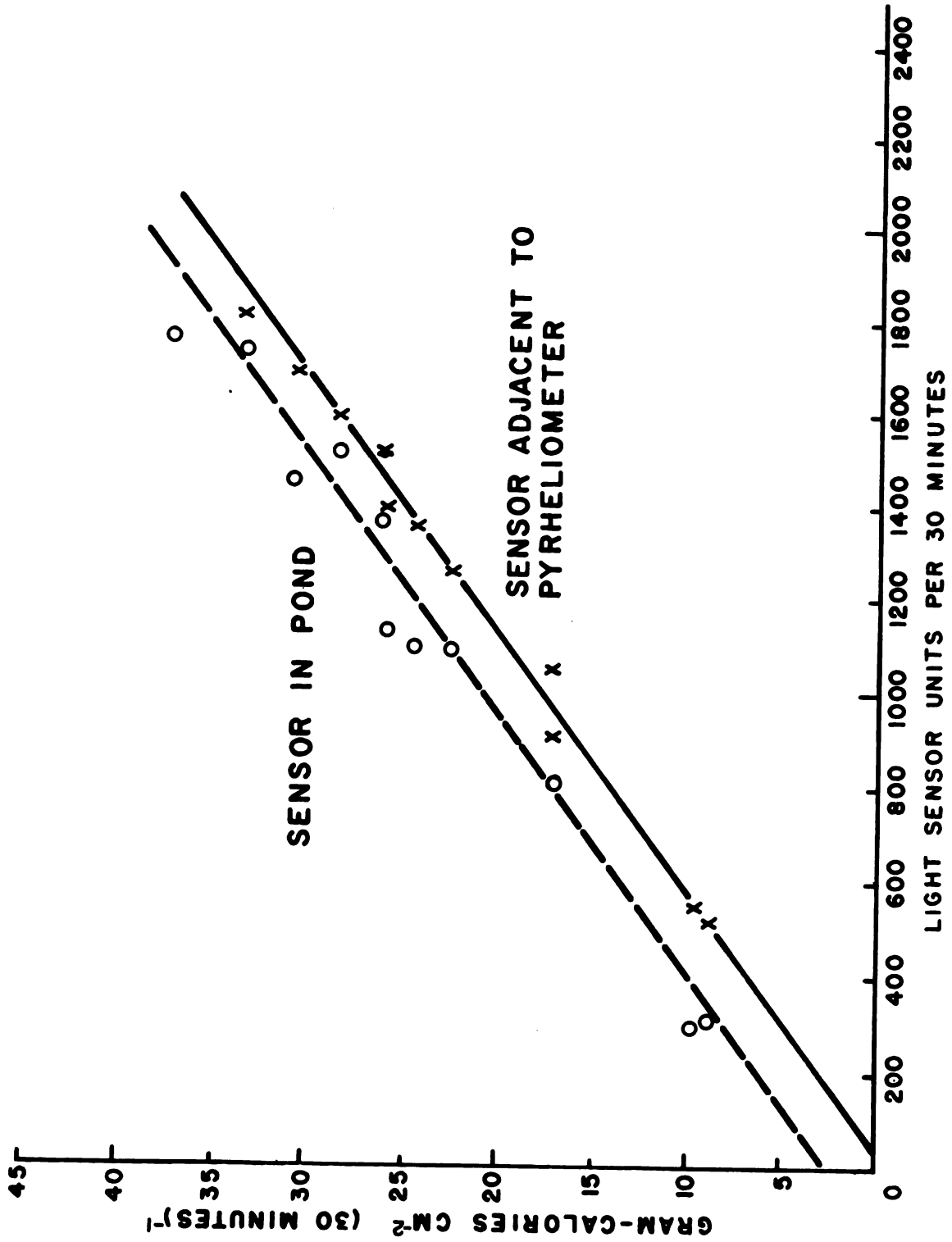


FIGURE 5

the ground level solar energy reaching a depth of one foot recorded.

Analysis of variance was performed to determine if light penetration were significantly influenced by the quantity of acid added to the pond. No measurements were made in the aquaria.

Oxygen

A Beckman Oxygen Analyzer was used for all oxygen measurements. The probe of this unit was placed in the monitoring site and the output recorded in percent saturation on a Sargent Recorder Model SR with a scale span of 0 to 100%. Using the temperature data these readings were transformed to milligrams oxygen per liter water. Analysis of variance was performed on the oxygen concentration data from the ponds both for the readings at 10 PM each evening and for the net rate of oxygen change between 10 PM at night and 4 AM the following morning.

No measurements were made on the aquaria as air was constantly bubbled through these units by a compressor.

Metal ions

A Beckman Model B Flame Spectrophotometer was used for flame photometry analysis of all water samples. A hydrogen and oxygen flame was used for measuring magnesium at 383 m μ , calcium at 554 m μ and sodium at 589 m μ . Standards were prepared with distilled water and the relationship between

percent transmission and concentration established from the measurement of these samples.

Each week five 500 ml water samples were taken from pond C and two from pond D. These samples were passed through a Foerst Plankton Centrifuge to remove the larger particulate material and then evaporated to near dryness in a 60° C. drying oven. These samples were then stored until the time of measurement on the flame photometer when they were diluted to their original volume with distilled water. The transmission data were then analyzed to determine if any significant changes had occurred.

The aquaria were sampled at the end of the summer and measured for the comparison of metal ion concentrations.

Fungi and bacteria aquaria

The water for two aquaria was passed through a Foerst Plankton Centrifuge to remove all large particulate material. Physical and chemical measurements were taken on these aquaria and both were observed for general conditions.

Periphyton

Four plexiglas shingles (0.014 meter square area each) were exposed in each pond at one foot depth for two weeks for periphyton accumulation. When the shingles were removed from the ponds they were placed in individual marked plastic bags and frozen. After thawing, the periphyton was scraped from the shingles into two ounce collecting bottles containing

95% ethanol. The samples were diluted to 50 ml with 95% ethanol and allowed to stand for 48 hours in a refrigerator for complete chlorophyll extraction before optical density was read at a wavelength of 663 m μ on a Bausch and Lomb Spectronic 20 Photometer. Analysis of the optical density data was conducted in an effort to determine if any significant changes had occurred in periphyton accumulation which might be traceable to acid addition.

Two shingles were removed from each pond early in the summer and placed in the corresponding experimental and control aquaria. An area of approximately one-fourth of one shingle was scraped into a vial from each aquaria each week and preserved with 95% ethanol. Each sample was then viewed under a microscope for a standard number of fields and counts taken of the different groups of organisms seen in each field. Since this procedure is highly variable with sample size, all data were transformed into ratios between organism types and comparisons were made between aquaria on this basis.

Sample shingles were also counted from the ponds each week in the same manner to see if any significant change could be detected in the periphyton community.

Centrifuged plankton

Each week five 4 liter water samples from randomly selected points in each pond were taken just beneath the surface and passed through a Foerst Plankton Centrifuge.

The particulate matter removed from the water was scraped into a 10 ml plastic vial, 5 ml of 95% ethanol added and the vial marked for identification. Later a standard number of fields were counted for each sample under the microscope and a tally kept of the organisms encountered. The total number for each organism group in the sample was computed and the data transformed into ratios between the different organisms groups. Analysis was then made to determine if any significant changes occurred which might be attributable to acid addition.

Also the same procedure was carried out each week with samples of 100 ml of water from each of the phytoplankton aquaria pair.

Net plankton

One night each week a small Wisconsin plankton net was towed behind a rowboat a standard distance in each pond. The sample collected was preserved in 95% ethanol until counting. Five fields were counted in each concentrated sample under a binocular microscope and the total number of organisms in each group in the sample computed. The data were handled in the same manner as that for the centrifuged plankton samples.

This same procedure was carried out with the zooplankton aquaria pair except that a sample of one liter of water was used. The zooplankton in the aquaria were initially stocked from one evening's catch from both ponds combined.

Other aquaria

Aquaria pairs containing bottom organisms (Odonata, Zygoptera, Hyalella sp. in each) and fish (four green sunfish, Lepomis cyanellus, in each) were observed for the general condition of these organisms at weekly intervals throughout the summer.

Bottom organisms were not studied in the ponds as this investigation was of short duration and at the time of emergence of insect forms. It was felt that under these conditions little of value could be learned with the limited sampling time available.

The ponds were watched for signs of abnormal activity of the fish but no directed effort was made toward following their reaction to the addition of the acids.

Fate of acids in the ponds

Several special experiments were conducted during the summer as interesting features of the acid's nature came to light. One study was designed to investigate the possible breakdown mechanisms for these molecules in the aquatic environment.

Several 250 ml BOD bottles were paired with matching bottles covered with black polyethylene sheeting to exclude all light. Five pair were set up as follows:

1. distilled water, no acids
2. distilled water, acids added
3. filtered (0.45 μ pore) pond water, acids added
4. centrifuged (Foerst) pond water, acids added
5. unmodified pond water, acids added

These bottles were measured for acid concentration and temperature before they were sealed and suspended just beneath the surface of the pond. After three weeks the bottles were removed, the acid concentration and temperature again measured and comparisons were made with the initial readings.

Environmental acid profiles

Measurements of acid concentration were taken from other bodies of water for a knowledge of vertical stratification in three lakes, comparison between different areas and the acid profile along the length of a stream. In lakes, a Kemmerer sampler was used to take samples at a series of depths while temperature was also recorded with a YSI Tele-thermometer with the thermistor probe taped to the sampler. In streams, samples were taken at the surface near shore at selected points along the watercourse. All samples were returned to the lab for fluorescence measurements. Records of acid concentration were taken from other areas whenever sampling apparatus was available. These readings also included in this section.

Aquaria contaminants

It was noticed during the summer that the water in several of the aquaria was becoming quite viscous and that these aquaria were giving off a slight odor which resembled watermelon rind. Several chemical tests were performed to determine the nature of the interfering substance.

A sample of aquaria water was filtered through a 0.45 μ pore size membrane filter and subjected to a seven minute 2 N hydrochloric acid hydrolysis. This hydrolyzed sample, along with an unhydrolyzed sample and a glucose standard, was chromatographed on Whatman No. 1 paper in isopropanol-acetic acid-water (3:1:1) and n-butanol - acetic acid - water (4:1:5) solvent systems. These chromatograms were tested with periodate-benzidine, aniline-acid-oxalate and ninhydrin sprays. The hydrolyzed sample was also co-chromatographed with arabinose, galactose, fucose, glucuronic acid, mannose, glucose, rhamnose, ribose and xylose. A Seliwanoff's test was conducted on the unknown. Samples were subjected to specific oxidation by periodate and the moles of periodate consumed and formic acid produced were assayed. All these tests were aimed at a qualitative and quantitative determination of the compound present causing the increased viscosity observed in some of the aquaria. All the methods used were from Clark (1964).

RESULTS

The factors leading to changes in the water chemistry of the aquaria were first investigated so that the ponds might be viewed in the light of the hypotheses formed after looking at the simpler system. Since water for the aquaria all originated from the same pond at the same time, the system was treated as a homogeneous group consisting of matched pairs of experimental and control. At the start of the experiment, readings of acid concentration, pH, conductivity, optical density and alkalinity were taken for all aquaria. On subsequent weekly readings, the change since the previous measurement was computed for both aquaria in a pair. The change in the control was then subtracted from the change in the experimental with the difference being added to the previous likewise corrected reading of the experimental aquaria to yield the corrected experimental reading.

$$\begin{array}{l} \text{Corrected} \\ \text{experimental} \\ \text{reading} \end{array} = \left[(R_e - P_e) - (R_c - P_c) \right] + \begin{array}{l} \text{Previous} \\ \text{corrected} \\ \text{experimental} \\ \text{reading} \end{array}$$

where R_e = Recent uncorrected experimental reading
 R_c = Recent uncorrected control reading
 P_e = Previous uncorrected experimental reading
 P_c = Previous uncorrected control reading

This was done to cancel out changes unrelated to acid concentration which may have occurred in both aquaria. The process was conducted for each of the five factors stated above.

Multiple regression was then performed on the Michigan State University CDC 3600 Computer System using the prepared MSU Agricultural Experiment Station STAT series routine for least squares with automatic stepwise deletion of variables from a least squares equation (LSDEL). This routine forms an equation of best fit by least squares including all parameters programmed and subsequently deletes, one by one, the factor which contributes least to the explanation of the desired parameter until a preset level of significance is reached. In this study all factors having less than a 95% probability of significance in the explanation of the desired variable were deleted. A series of 45 measurements were available for each factor from the paired aquaria.

Acid concentration--aquaria

The change in acid level in the individual aquaria during the experimental period is shown in Figure 6. The large increase in the control of the fish aquaria pair was most likely caused by the introduction of plant material along with zooplankton used in feeding. In mid-August a change was made to feeding emergent insects and acid concentration did not continue to rise in the control.

Figure 6. Acid concentrations of the aquaria through the experiment.

- Fig. 6a - Zooplankton aquaria
- Fig. 6b - Bacteria and Fungi aquaria
- Fig. 6c - Bottom organism aquaria
- Fig. 6d - Phytoplankton aquaria
- Fig. 6e - Periphyton aquaria
- Fig. 6f - Fish aquaria

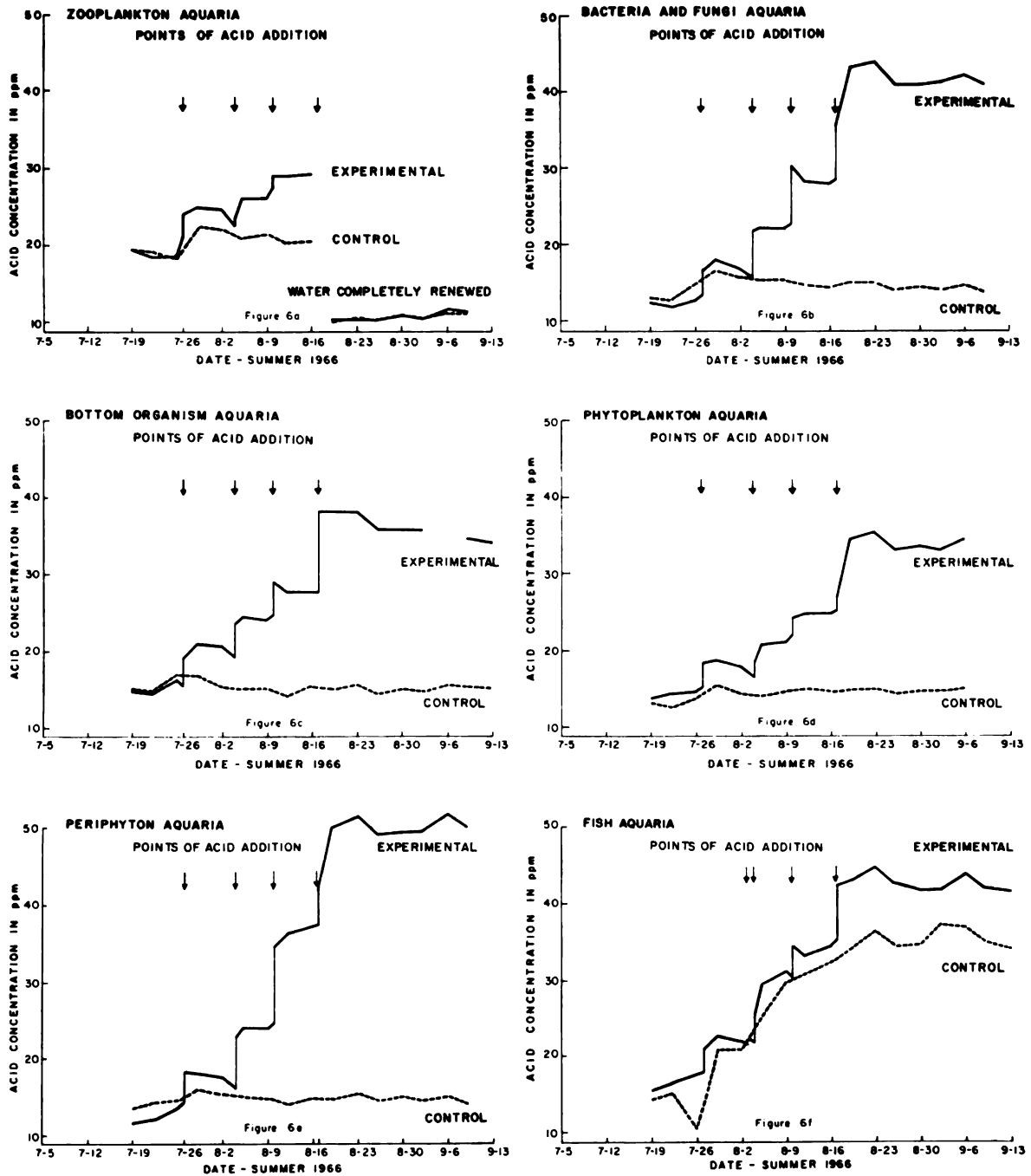


FIGURE 6

The result of acid addition to each of the experimental aquaria was an average total increase of 19.7 ppm acids over their initial acid concentration or a final concentration of about twice the control level. The periphyton experimental aquarium attained the highest acid concentration of 51.7 ppm or 36.1 ppm higher than its control. The zooplankton aquarium showed the lowest experimental concentration of 29.4 ppm with the fish aquaria exhibiting the smallest difference (7.3 ppm) between experimental and control. These results were used for computer analysis of the relationship among chemical parameters in the aquaria.

Acid concentration--ponds

Two-way analysis of variance was performed on the fluorescence data from the ponds divided by two ponds and 24 sampling times with a total of 216 readings (see Appendix, Tables 1 and 2). Pond D had a higher mean acid concentration (10.5 ppm) than did pond C (9.7 ppm) while both ponds showed a gradual decline through the summer. Significant interaction showed pond C higher than expected in concentration before the first addition, between the first and second additions and after the third addition. This same pond was below the expected acid levels between the second and third additions. Expected values were calculated on the assumption that the effect of pond and time on acid concentration was additive.

For the readings taken in pond D hourly over a 25 hour period of August 4 and 5, acid levels remained nearly constant around 11.4 ppm during the hours of darkness and exhibited a general falling trend during daylight to nearly 10.0 ppm in mid-afternoon (see Appendix, Tables 3 and 4).

None of the physical factors tested showed any significance in the explanation of the resultant changes in acid concentration through the summer (Table 3). The correlation value indicates the variation of acid concentration which can be accounted for by the designated variable such as rain after the variation attributable to all other independent variables had been removed. The probability factor indicates the amount of the variation which would be accounted for if the independent variable were a constant value.

pH--aquaria

The possible factors included for the explanation of changes in pH were acid concentration, conductivity, acid concentration squared, the cross product of acid concentration and conductivity, the cross product of acid concentration and pH, the pH at the start of the experiment, the acid concentration at the start of the experiment and the conductivity at the start of the experiment. The factor acid concentration squared was included to incorporate changes which might have occurred if acid concentration affected pH through some intermediate mechanism and was thus magnified

Table 3. Significance of physical factors in the explanation of acid change

Factor	Correlation with acid change	Probability factor not significantly different from constant
Rain	0.11456	0.820
Solar energy	0.43425	0.463
Days (time)	0.44365	0.627
Constant = -2.1	---	0.012

in effect. In the solution when all insignificant parameters had been deleted the remaining factors, which all had a probability of greater than 99% significance in the explanation of pH changes, were acid concentration, the pH at the start of the experiment, the cross product of acid concentration with pH and a constant (see Appendix, Table 5). This equation was transformed so that the pH at the start of the experiment became the theoretical pH when the acid concentration would be equal to 0.0 ppm and the entire expression simplified. This then became:

$$\text{pH} = \frac{-0.2415 (\text{acid concentration}) + \text{pH}_i}{1 - 0.0301 (\text{acid concentration})}$$

where pH_i indicates the theoretical pH when acid concentration is 0.0 ppm. This expression is based on measurements over a range of acid concentrations of 10.3 to 50.2 ppm and pH values from 7.20 to 8.45. In general this equation indicates a decrease in pH with increasing acid concentration below an initial pH (pH_i) of 8.02 and an increase in pH with increasing acid concentration above this pH. A discontinuity with pH values approaching 0 and 14 exists around 33 ppm acid concentration (Figure 7). Above this value the relationship between pH and acid concentration is reversed with pH once again approaching the initial pH values with increasing acid concentration.

The individual experimental aquaria with the theoretical trend in pH superimposed are presented in Figure 8. For the

Figure 7. Experimental relationship between pH and acid concentration determined in the aquaria.

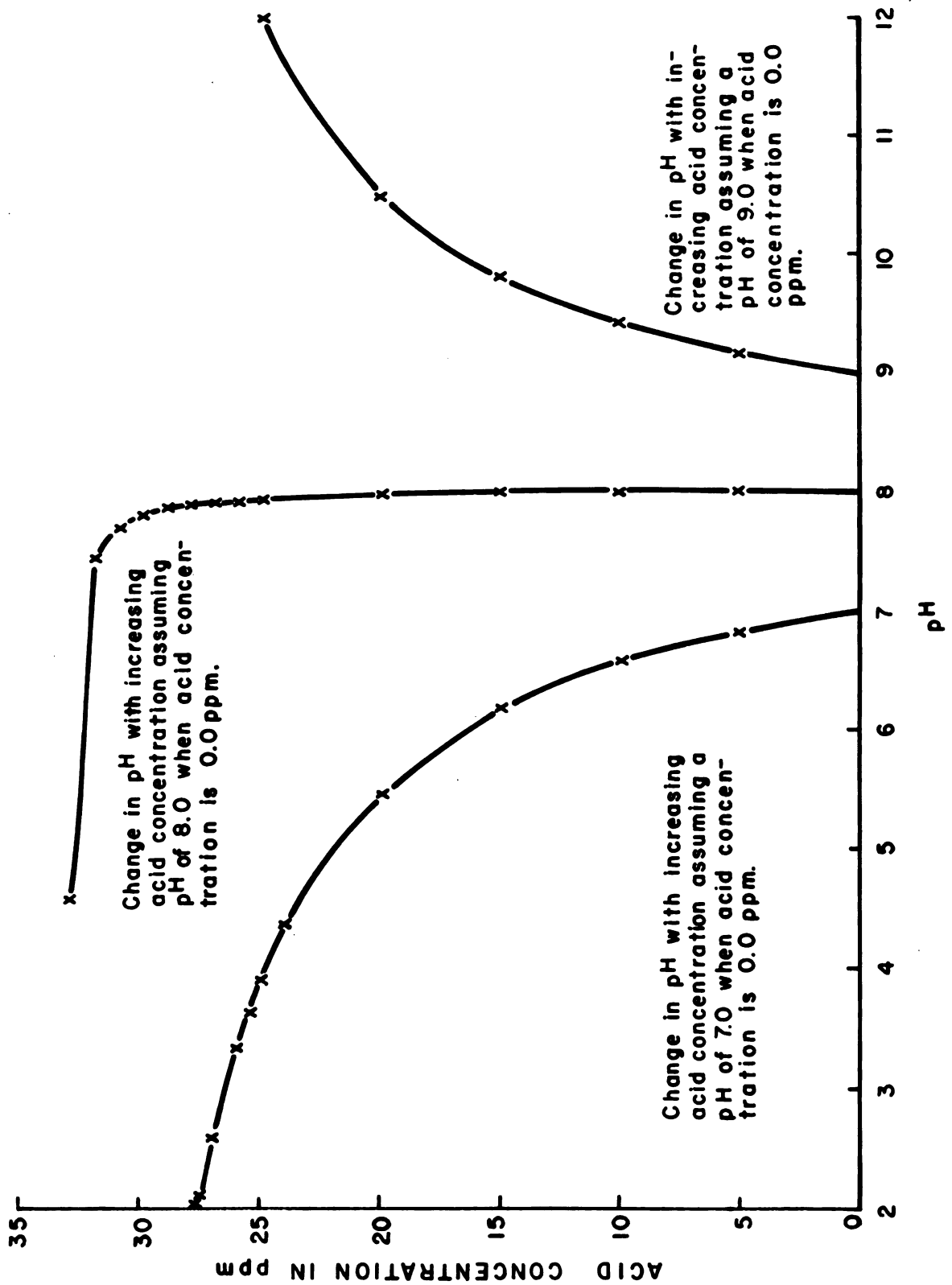


FIGURE 7

Figure 8. pH--acid concentration relationship in the individual aquaria with the theoretical trend of pH superimposed.

- Fig. 8a - Zooplankton aquaria
- Fig. 8b - Bacteria and fungi aquaria
- Fig. 8c - Bottom organism aquaria
- Fig. 8d - Phytoplankton aquaria
- Fig. 8e - Periphyton aquaria
- Fig. 8f - Fish aquaria

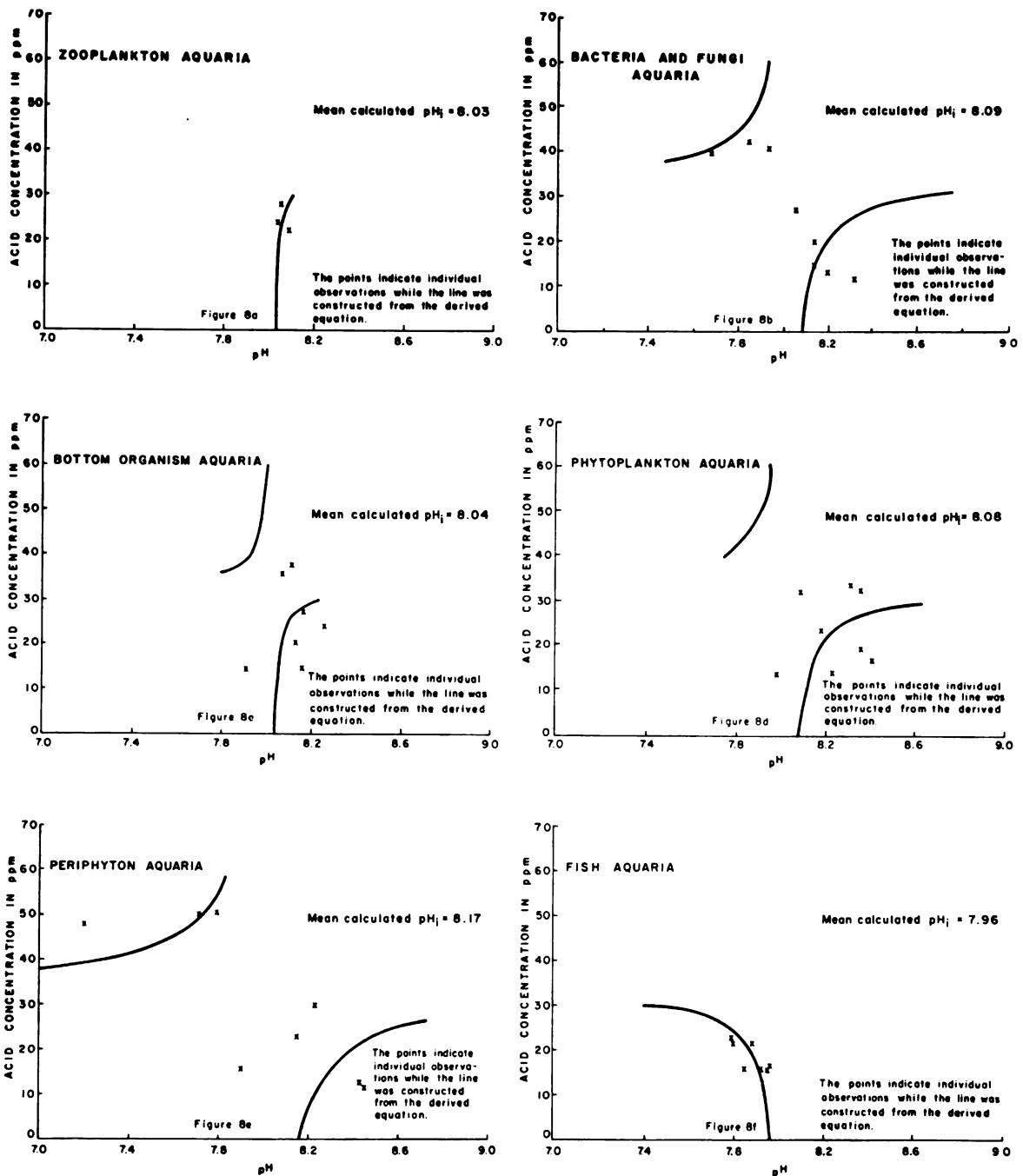


FIGURE 8

theoretical line, pH_i was taken as the average of individual pH_i 's computed from each acid concentration-pH data set for that aquaria.

pH--ponds

Three-way analysis of variance was performed on the pH data from the ponds divided by ponds (C and D), dates (before the first acid addition, between the first and second additions, between the second and third additions, after the third and last addition) and times (early morning, late morning, early afternoon, late afternoon) with a total of 204 pH readings (see Appendix, Tables 6 and 7).

A significant difference in times reflects the increase in pH through the day as carbon dioxide is removed from the water during photosynthesis. A significant interaction between dates and ponds is a sign that the pH change was not the same in both ponds during the summer. Upon inspection this interaction shows the pH in pond C increased through the summer as pond D decreased as compared to expected values generated for each sampling period under the assumption that the effects of time and of pond upon pH were additive. As the mean pH of pond C was 8.72, thus above the pH of 8.02, this pond may have reacted in the manner predicted by the equation for pH change derived from the aquaria experiment (see page 45 and Figure 7--pH increases with an increase in acid concentration in waters with a pH above 8.02).

Conductivity--aquaria

The possible factors included for the explanation of changes in conductivity were acid concentration, pH, acid concentration squared, the cross product of acid concentration and conductivity, the cross product of acid concentration and pH, the pH at the start of the experiment, the acid concentration at the start of the experiment and the conductivity at the start of the experiment. The parameter acid concentration squared was included to incorporate changes which might have occurred if acid concentration affected conductivity through some intermediate mechanism and was thus magnified in effect. After solving the regression equation, the remaining factors, all of which had a probability of greater than 99% significance in the explanation of conductivity changes, were acid concentration, conductivity at the start of the experiment, pH, the cross product of acid concentration with conductivity and a constant. This equation was transformed so that conductivity at the start of the experiment would be replaced by a factor equal to the theoretical conductivity when the acid concentration was 0.0 ppm and the entire expression simplified:

$$\begin{array}{l} \text{Conductivity} \\ \text{(micromhos/cm at } 36^{\circ} \text{ C.)} \end{array} = \frac{-3.2814 (\text{acid concentration}) + C_i}{1 - 0.02003 (\text{acid concentration})}$$

where C_i (initial conductivity) indicates the theoretical conductivity when acid concentration is 0.0 ppm. This

expression was derived from measurements over the range of acid concentration from 10.3 to 50.2 ppm and range of conductivity from 149 to 231 micromhos/cm at 36° C. (see Appendix, Table 8).

In general this expression indicates a decrease in conductivity with increasing acid concentration below an initial conductivity (C_i) of 164 micromhos/cm and an increase in conductivity with increasing acid concentration above this initial conductivity. A discontinuity exists around 50 ppm acids with the relationship of conductivity to acid concentration reversed at higher concentration levels (Figure 9). The transformed equation does not include pH since the regression coefficient of this parameter approached the limit of zero when C_i became an expression of the theoretical conductivity when acid concentration equals 0.0 ppm. The individual experimental aquaria with the theoretical trend in conductivity change superimposed are presented in Figure 10. For this theoretical line, C_i was taken as the mean of individual C_i 's computed from each acid concentration-conductivity data pair for that aquaria since each aquaria would be expected to have a characteristic C_i that could be found only by this method of back-calculation.

Conductivity--ponds

Two-way analysis of variance was performed on the conductivity data from the ponds with these data divided into 29 times (the roughly twice-weekly sampling times) and

Figure 9. Experimental relationship between conductivity and acid concentration determined in the aquaria.

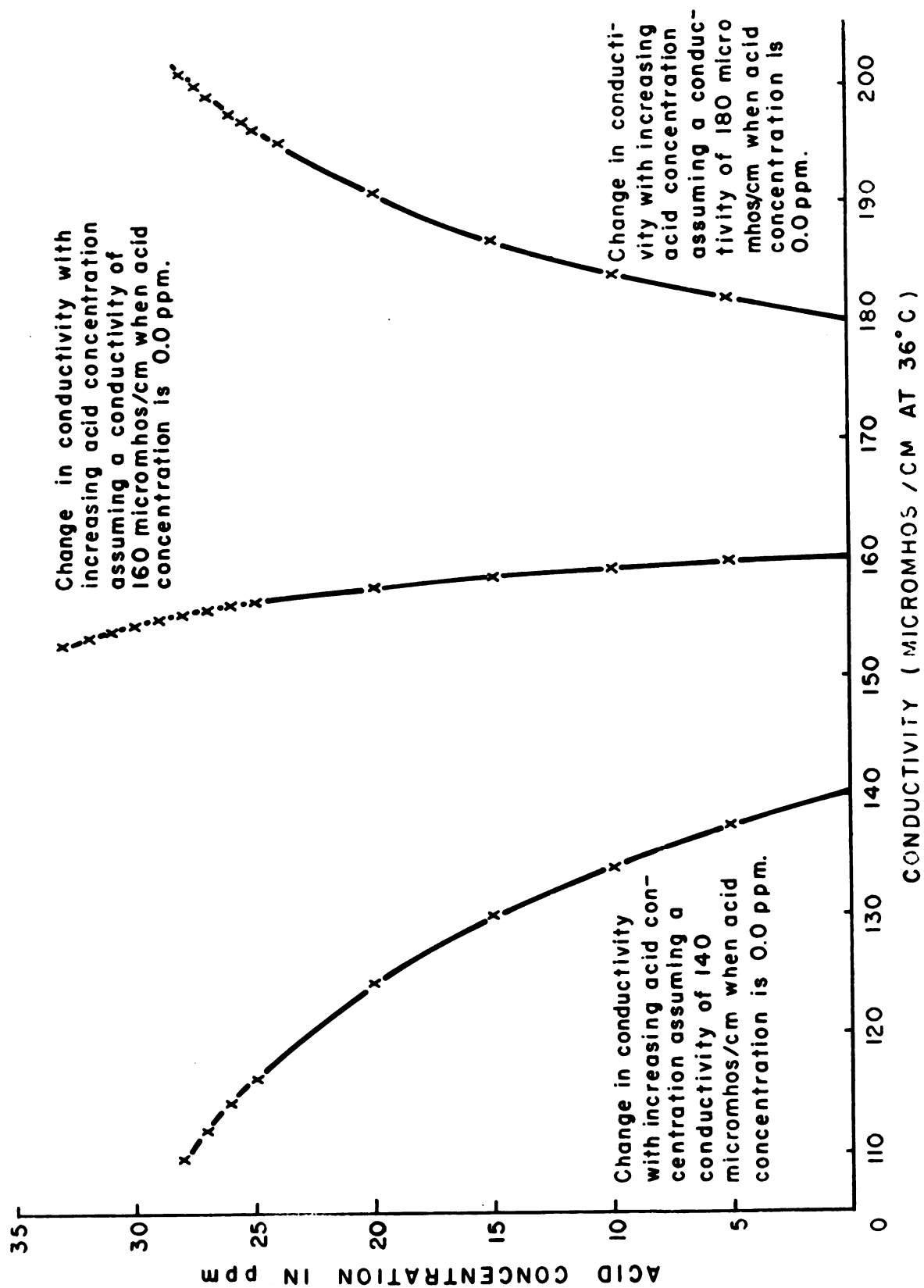


FIGURE 9

Figure 10. Conductivity-acid concentration relationship in the individual aquaria with the theoretical trend in conductivity superimposed.

- Fig. 10a - Zooplankton aquaria
- Fig. 10b - Bacteria and fungi aquaria
- Fig. 10c - Bottom organism aquaria
- Fig. 10d - Phytoplankton aquaria
- Fig. 10e - Periphyton aquaria
- Fig. 10f - Fish aquaria

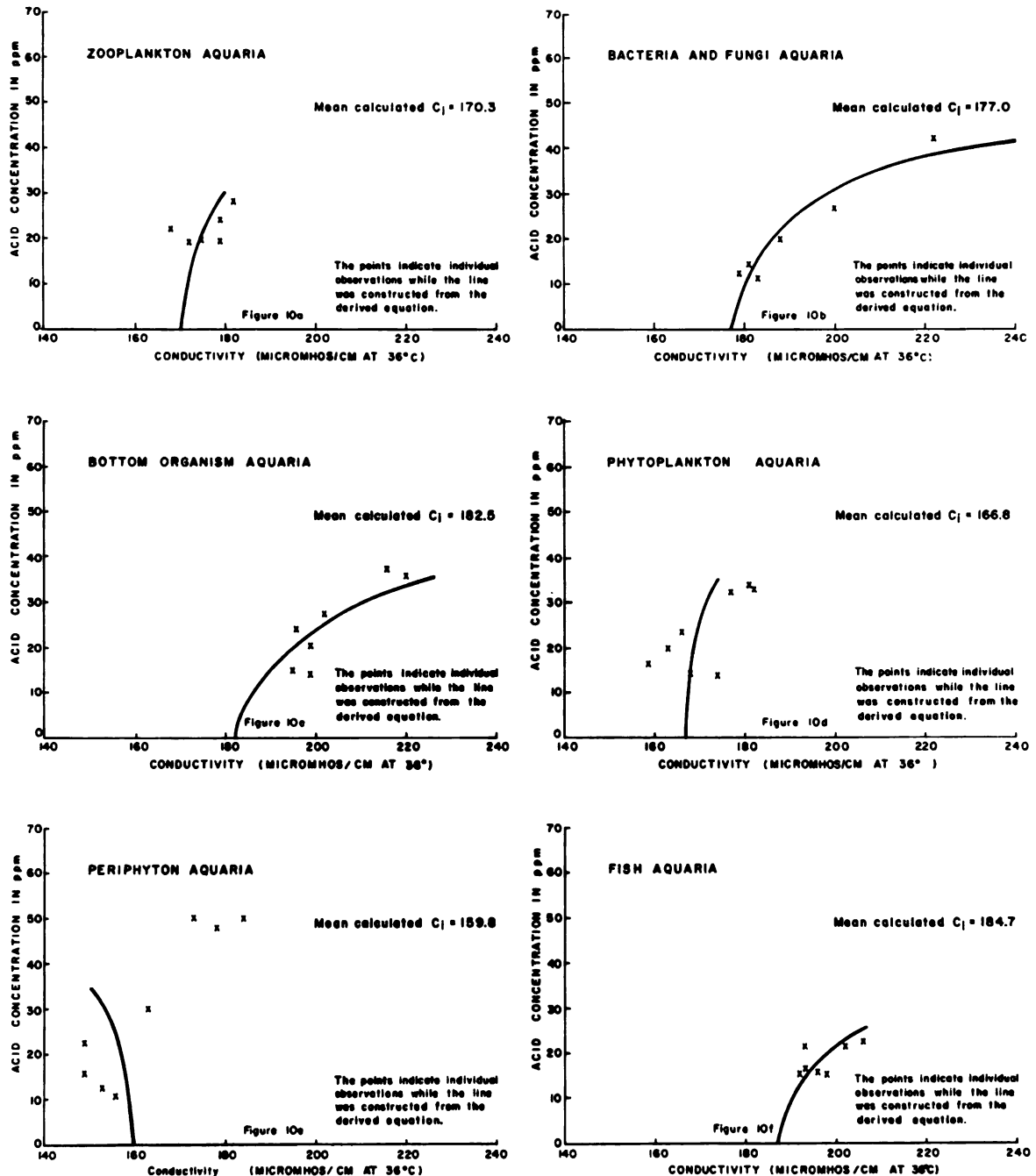


FIGURE 10

2 ponds (C and D) with a total of 304 samples taken (see Appendix, Tables 9 and 10).

A significant difference in times shows that conductivity did not remain constant but changed (generally increased) through the summer in both ponds. Pond D ($\bar{x} = 158.7$) was significantly higher in conductivity than pond C ($\bar{x} = 157.0$). The significant interaction between ponds and times shows the conductivity change to be different between the ponds during the sampling period. In this case, the conductivity in pond C decreased through the summer as pond D comparatively increased. The first acid addition was followed by a rise in conductivity in pond C with a gradual return to pre-addition levels. The second addition was also followed by an initial rise but soon conductivity decreased markedly. This conforms to the theoretical prediction from the aquaria results as the conductivity was below the 164 micromho/cm value in pond C. At the third acid addition the conductivity of pond C was slightly below 164 micromho/cm and, as would be predicted, conductivity decreased very little even though more acid was added at this time than at the second addition.

Optical density--aquaria

The possible factors included for the explanation of changes in optical density were acid concentration, pH, acid concentration squared, the cross product of acid concentration and conductivity, the cross product of acid concentration and pH, the cross product of acid concentration and optical

density, the optical density at the start of the experiment, the pH at the start of the experiment, the acid concentration at the start of the experiment and the conductivity at the start of the experiment. The parameter acid concentration squared was included to incorporate changes which might have occurred if acid concentration affected optical density through some intermediate mechanism and was thus magnified in effect. The regression solution was a complicated expression of many factors showing that optical density depends upon many different environmental conditions. Those parameters having a significance of less than 0.01 were acid concentration, acid concentration squared, the cross product of acid concentration with conductivity and the cross product of acid concentration with optical density. Initial pH and initial acid concentration had a significance between 0.01 and 0.05. The simplified expression became:

$$\text{Optical density at 330 m}\mu = \frac{0.0046 (\text{ppm acid}) - 0.00017 (\text{ppm acid})^2 + 0.0000066 (\text{ppm acid}) \times (\text{conductivity})}{1.0 - 0.0309 (\text{ppm acid})}$$

over a range of acid concentration of 10.3 to 50.2 ppm, conductivity of 149 to 231 micromhos/cm and optical density of 0.0311 to 0.2599 (see Appendix, Table 11).

In general this expression shows increasing optical density with an increase in acid concentration (Figure 11). Discontinuities exist in the regions of 32 and 50 ppm acids. The individual experimental aquaria data with the theoretical trend in optical density superimposed are presented in

Figure 11. Experimental relationship between optical density and acid concentration determined in the aquaria.

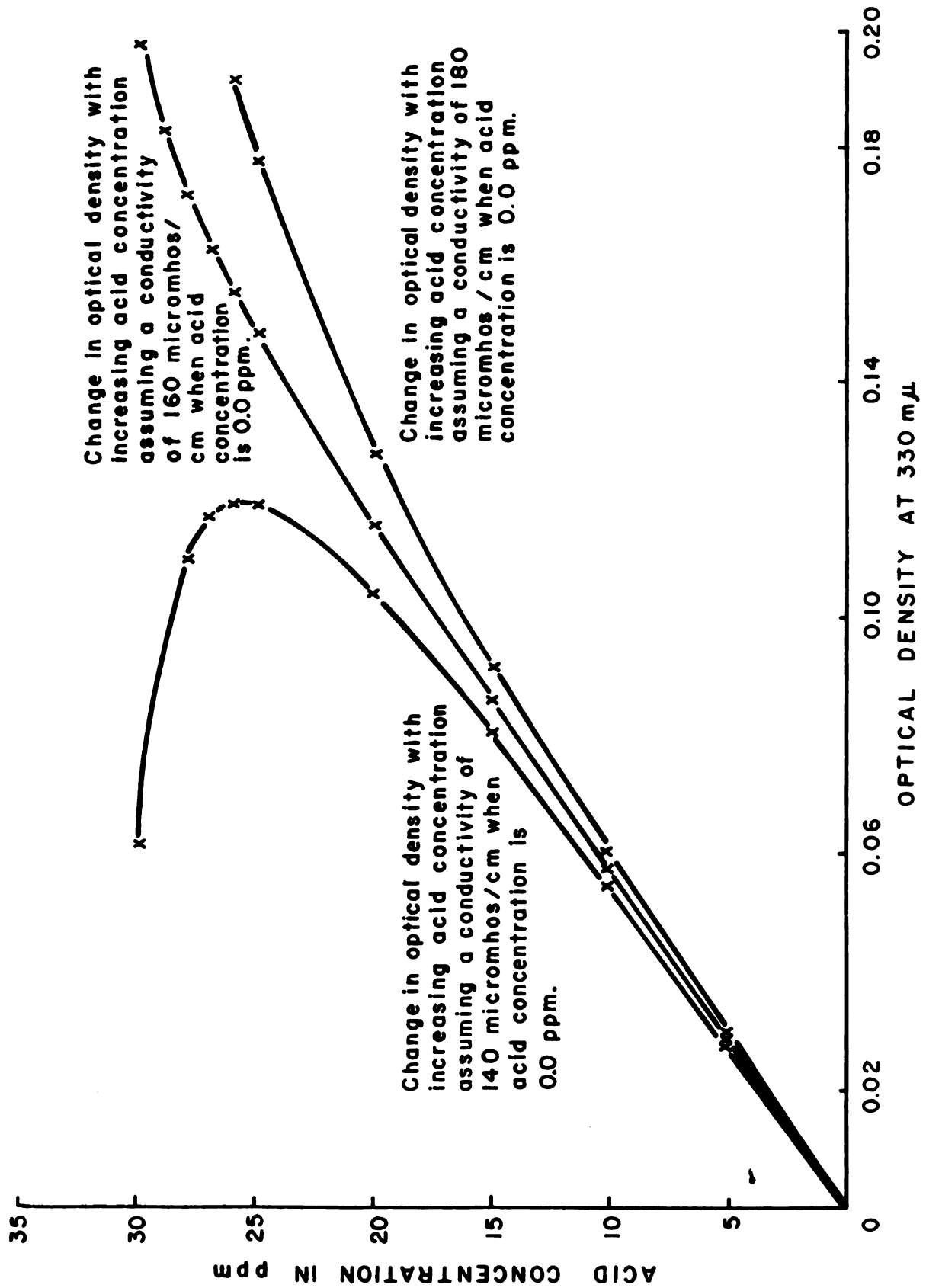


FIGURE 11

Figure 12. The same mean C_i used in the computation of conductivity for each aquaria was used here in determining the conductivity values.

Optical density--ponds

Two-way analysis of variance was performed on the optical density data from the ponds as they were originally recorded--percent transmission. These data were divided into 29 sampling times and 2 ponds with a total of 296 samples taken after the elimination of those samples containing visually large amounts of suspended plant material (see Appendix, Tables 12 and 13).

Pond D had a significantly lower percent transmission mean (88.87) than did pond C (89.73). Both ponds showed significant changes (decreasing to mid-summer, then increasing) during the sampling period. The significant interaction again showed a difference between the way both ponds changed through the summer. The percent transmission of pond C decreased during the summer as pond D comparatively increased. Such is as would be predicted from the theoretical model with increasing acid concentration.

Alkalinity--aquaria

Methyl orange (bicarbonate) alkalinity was the only form present in the aquaria. Not enough data was collected for regression analysis although measurements from the fish aquaria pair indicate a decrease in bicarbonate alkalinity as acid concentration increases (Figure 13).

Figure 12. Optical density-acid concentration relationship in the individual aquaria with the theoretical trend in optical density superimposed.

- Fig. 12a - Zooplankton aquaria
- Fig. 12b - Bacteria and fungi aquaria
- Fig. 12c - Bottom organism aquaria
- Fig. 12d - Phytoplankton aquaria
- Fig. 12e - Periphyton aquaria
- Fig. 12f - Fish aquaria

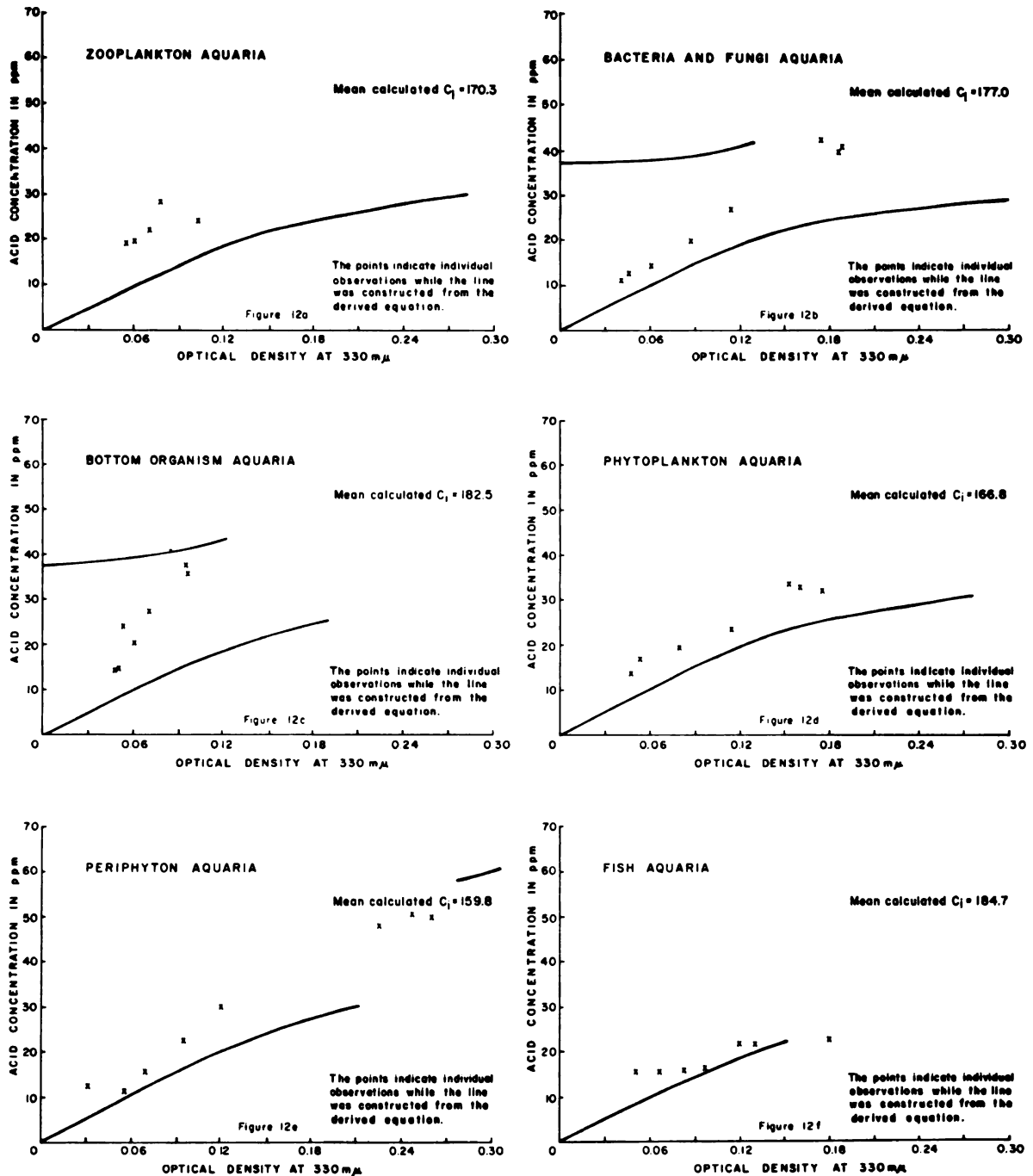


FIGURE 12

Figure 13. Bicarbonate alkalinity in the fish aquaria pair during the study period.

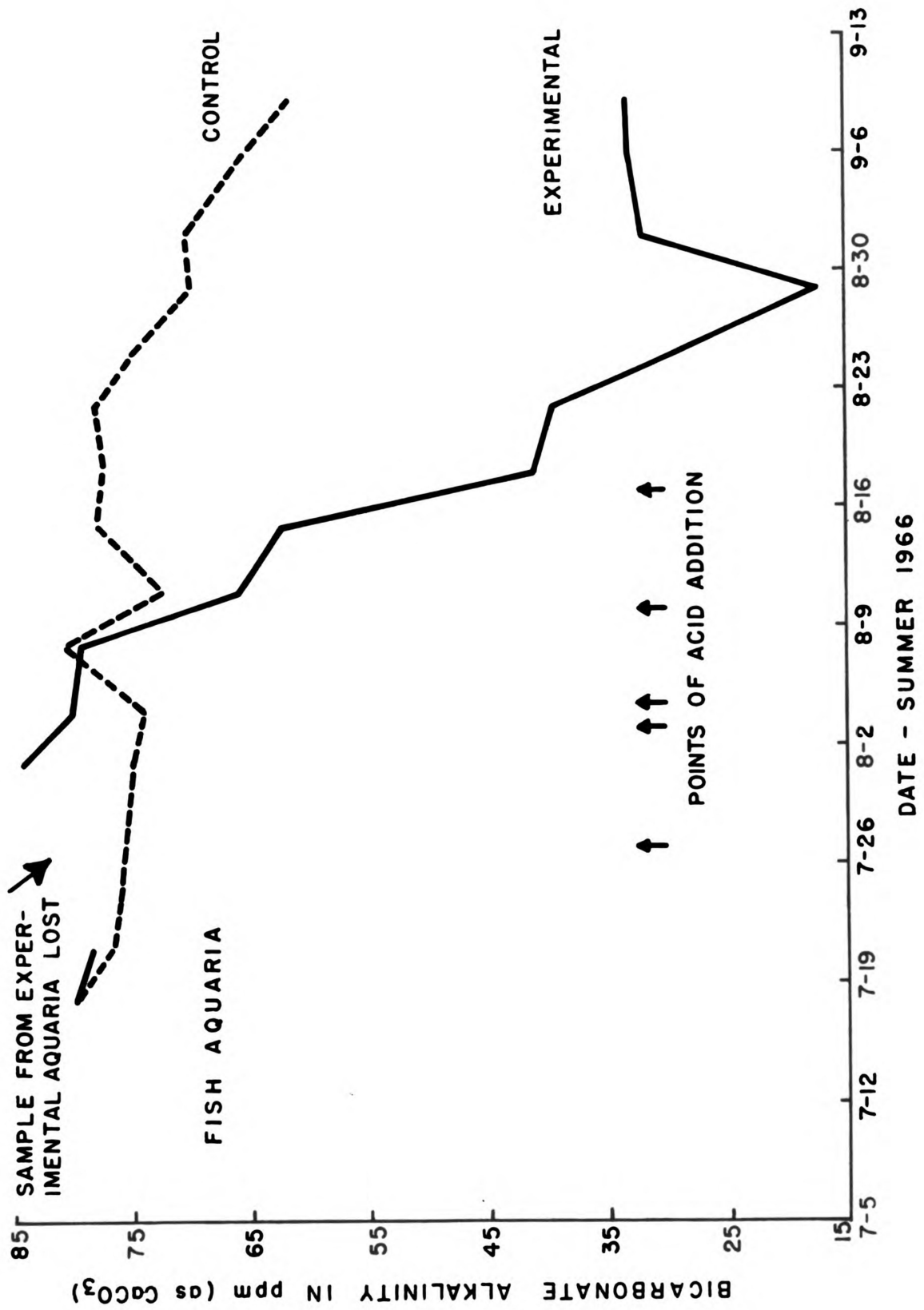


FIGURE 13

Alkalinity--ponds Carbonate

Two-way analysis of variance was performed on the data divided by two ponds and 19 sampling times with a total of 76 measurements taken (see Appendix, Tables 14 and 15).

Pond D had a higher mean carbonate alkalinity (14.20 ppm) than did pond C (11.14 ppm) while both ponds changed (increased to mid-summer, then decreased) over the summer. Interaction appeared as pond C was lower than expected the first half of the summer and higher than expected the second half compared to pond D. Expected values were generated for each sampling period under the assumption that the effects of time and of pond upon carbonate alkalinity were additive. Following acid addition, carbonate alkalinity in pond C showed consistently smaller increases comparative to pond D.

Alkalinity--ponds Bicarbonate

Two-way analysis of variance was performed on the data from the ponds divided in the same manner as for carbonate (see Appendix, Tables 16 and 17).

Pond D had a higher mean bicarbonate alkalinity (46.6 ppm) than did pond C (45.5 ppm) while both ponds changed (decreased to mid-summer, then increased) through the study. Interaction occurred as pond C decreased in bicarbonate relative to pond D after the second acid addition.

Alkalinity--ponds Total

Both ponds started in July at nearly the same total alkalinity (67.05 ppm for D and 69.65 for C). By September 12, pond C was 56.6 ppm--much less than the total alkalinity of pond D (72.9 ppm) which had remained nearly constant through the study period. The decrease in pond C began after the second acid addition.

Temperature--aquaria

The extreme temperature range in the aquaria during the summer was from 14.3 to 25.2°C. Usually all aquaria were within 3.5°C with differences coming from placement in the room. No significant differences could be detected between controls and experimentals.

Temperature--ponds

Two-way analysis of variance was performed on the temperature data from the ponds. For each sampling period the temperature reading obtained from the water thermograph in pond D was subtracted from the temperature reading for the pond connected at that time to the measuring site in the laboratory. This yielded a positive or negative value which was assigned to that pond connected at the indoor measuring site. This enabled the variation between temperature units to confirm the accuracy of calibration of both units. These data were divided by two ponds and four time periods in the same manner as the pH data and included a total of 512 measurements (see Appendix, Tables 18 and 19).

The ponds did not differ in temperature over the summer but both were indicated to have changed relative to the water thermograph in pond D. This change in time most likely indicated a change in instrument calibration during the study. Interaction shows pond C to be comparatively cooler than expected before the first addition and after the third addition. Between these times pond C was warmer than expected. Expected values were generated for each sampling period under the assumption that the effects of time and of pond upon temperature were additive. Both ponds exhibit the same directional changes except after the third addition when D becomes warmer as C cools. This interpretation of interaction may be invalid also if calibration varied erratically over short time periods as observation tended to indicate.

Light penetration--ponds

No measurement of light penetration was made with the aquaria.

Three-way analysis of variance was performed on the light data from the ponds divided by two ponds, four sampling time periods and two comparisons (ratios of pond underwater meter to pyrhelimeter and of pond underwater meter to air meter) with a total of 156 measurements (see Appendix, Tables 20 and 21).

Pond D had a greater ratio of light penetration (0.5200) than did pond C (0.4619) while both changed (increased to mid-summer, then decreased) over the summer. The lack of significant interaction fails to reveal any effect which can be

attributed to acid addition. No significant difference between pyrliometer and light meters indicates the calibration of the light meters with the pyrliometer was accurate. The absence of comparisons x times interaction indicates calibration did not change through the summer.

Oxygen--ponds

No measurement of oxygen was made in the aquaria since an air compressor was used at all times to maintain a high level of dissolved oxygen.

The mean change in ppm dissolved oxygen per hour from 10 PM to 4 AM the following morning was used as a rough estimate of total respiration. Two-way analysis of variance was performed on the pond data divided into two ponds and four sampling periods with a total of 64 measurements (see Appendix, Tables 22 and 23).

The lack of significant interaction fails to indicate any effect of acid addition on the total respiration in the ponds.

The ppm oxygen concentration at 10 PM was used for the comparison of oxygen production in the ponds since at this time dissolved oxygen was usually at its highest concentration of the day. Two-way analysis of variance was performed on these data divided in the same manner as that for respiration (see Appendix, Tables 24 and 25).

The lack of significant interaction fails to indicate any effect of acid addition on photosynthetic oxygen production in the ponds.

Metal ions--aquaria

A Wilcoxon matched-pairs signed-ranks test was performed on the aquaria flame photometry data for experimental-control pairs. Calcium ($T = 10\frac{1}{2}$, $N = 7$) and magnesium ($T = 12\frac{1}{2}$, $N = 7$) showed no difference between experimental and control while sodium ($T = 0$, $N = 7$, $P = .02$) was higher in the experimentals. Sodium was most likely added to the experimental aquaria as an impurity along with the acids. The sodium could not have originated from the glass walls or it would have been of equal concentration in both the experimental and control studies.

Metal ions--ponds

Two-way analysis of variance was performed on the flame photometry data for three metal ions (calcium, magnesium, and sodium) from a total of 89 samples from the two ponds for 13 sampling times (see Appendix, Tables 26 and 27).

There was no difference between ponds of any of the metals. Ion concentration changed with time (generally increased through the summer) but this change may have been related to the difficulty and variability in redissolving the concentrated salt mixtures. There was no interaction to indicate any effects attributable to acid addition.

Fungi and bacteria--aquaria

A colorless filamentous growth appeared in both aquaria of this pair though slightly more prominent in the experimental. Both aquaria subsequently gave off an odor resembling that of watermelon rind and the water became quite viscous. No identification of the growth was made.

Fungi and bacteria--ponds

Pond D was noticed to have a large growth of what appeared to be the same filamentous material as in the aquaria. During the first half of August, a dense layer formed among the Chara sp. near the bottom of the pond. None of this growth was noticed in pond C.

Centrifuged plankton--ponds

Knowing that the method for the collection of plankton I used on the ponds could not be relied upon for quantitative data, a measure of population dominance was derived to express the relationship of different organism types between the two ponds (or aquaria).

$$\text{Index value} = \left(\sum_{\text{pond C}} \frac{A}{n_{x-A}} - \sum_{\text{pond D}} \frac{A}{n_{x-A}} \right)$$

where A = estimated number of organisms of one type from either pond

n = estimated number of organisms of each type other than A of the same pond.

The ratio of one species to each other species is calculated in turn and all ratios summed for that species in one pond. The sum of ratios is calculated for the other pond and the difference between the summed ratios is the index value. When the index value is negative, the organism being compared is dominant in pond D. Conversely, a positive index value indicates dominance of the organism in pond C.

The index values of ten types of organism found in the centrifuged plankton from the ponds through the summer are shown in Figure 14.

The initial variability likely arises from variance in sampling and counting procedures before a constant method was adopted. Dominance appears random with the exception of July 27, after the first addition, and September 14, following the third addition. On July 27, seven types were dominant in pond C while three were dominant in pond D. With a probability of 0.117 for this distribution significance is questionable although those types showing dominance in pond C do so to a large degree (index values of 100 to 550). Possibly this initial small acid addition did stimulate the growth of planktonic organisms. On September 14, seven types were dominant in pond D while two were dominant in pond C (one type shows no dominance). With a probability of 0.07 for this distribution, it appears possible that the large third acid addition has depressed the growth of the planktonic organisms in pond C and allowed the majority to show greater dominance in pond D.

With the exception of Arthrodesmus sp. showing dominance in pond C after the last acid addition, no other type shows appreciable deviation from the general trends previously mentioned.

Figure 14. Index values for centrifuged plankton from the ponds.

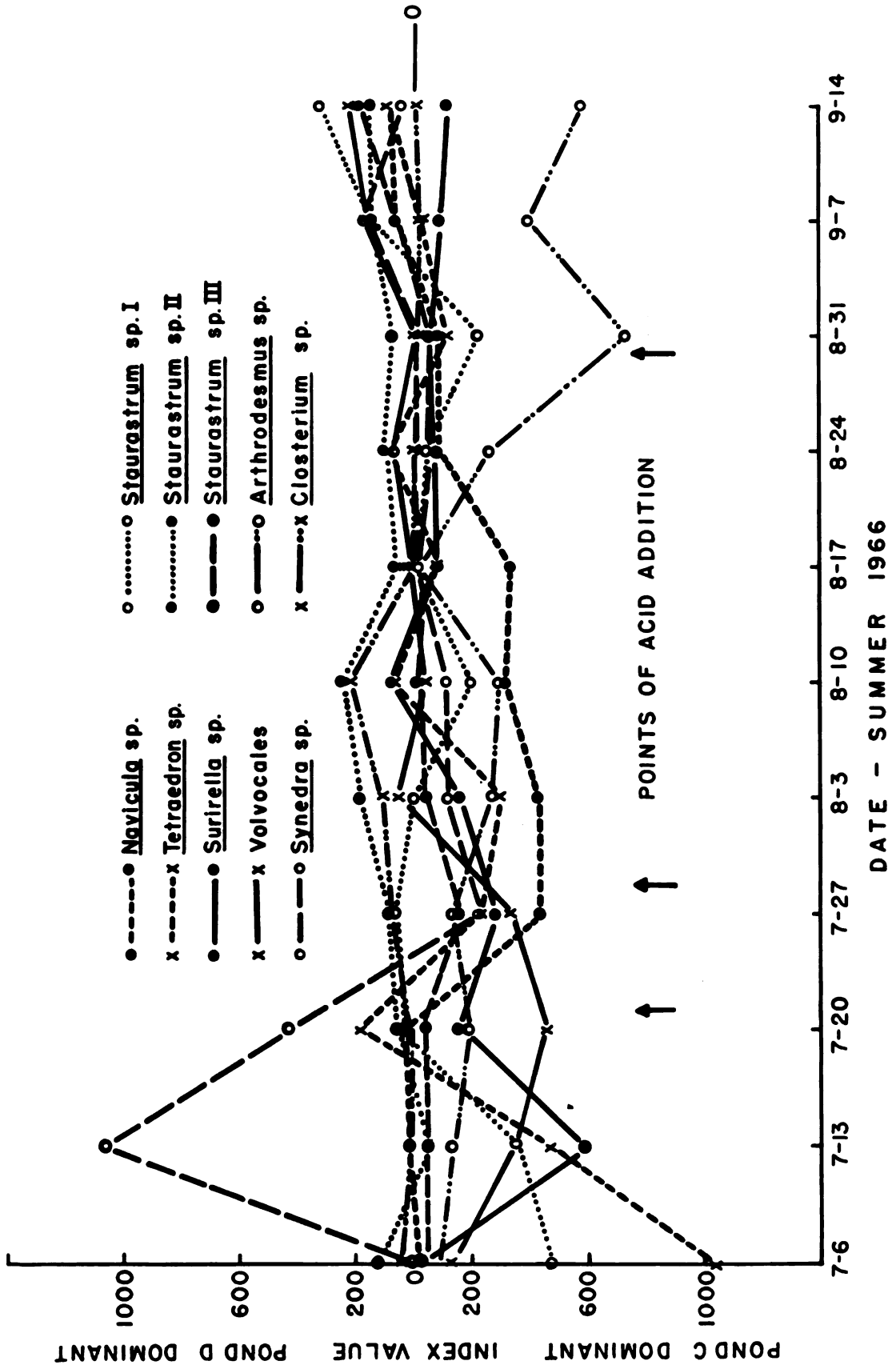


FIGURE 14

Centrifuged plankton--aquaria

The index values during the later portion of the summer are shown in Figure 15. The only possible significant points would be the dominance of Closterium sp. in the acid aquaria. All other prominent species do not differ between experimental and control.

Periphyton--ponds

Two-way analysis of variance was performed on the optical density data from the periphyton-extracted chlorophyll. A total of 81 samples were divided by two ponds and 9 two-week sample times (see Appendix, Tables 28 and 29).

Pond D had a higher growth of periphyton as shown by the chlorophyll values while the significant interaction showed pond C to have been lower than expected in chlorophyll values for the later part of the summer. Both ponds decreased to mid-summer then increased to higher than starting chlorophyll values.

The index values for the three dominant types of organisms found in the periphyton are shown in Figure 16. Growth of Navicula sp. may have been promoted while that of Synedra sp. might have been inhibited in pond C after the addition of acids.

Periphyton--aquaria

The index values for the three dominant types of organisms found in the periphyton in the aquaria are shown in Figure 17. No trends appear significantly related to the

Figure 15. Index values for centrifuged plankton from the aquaria.

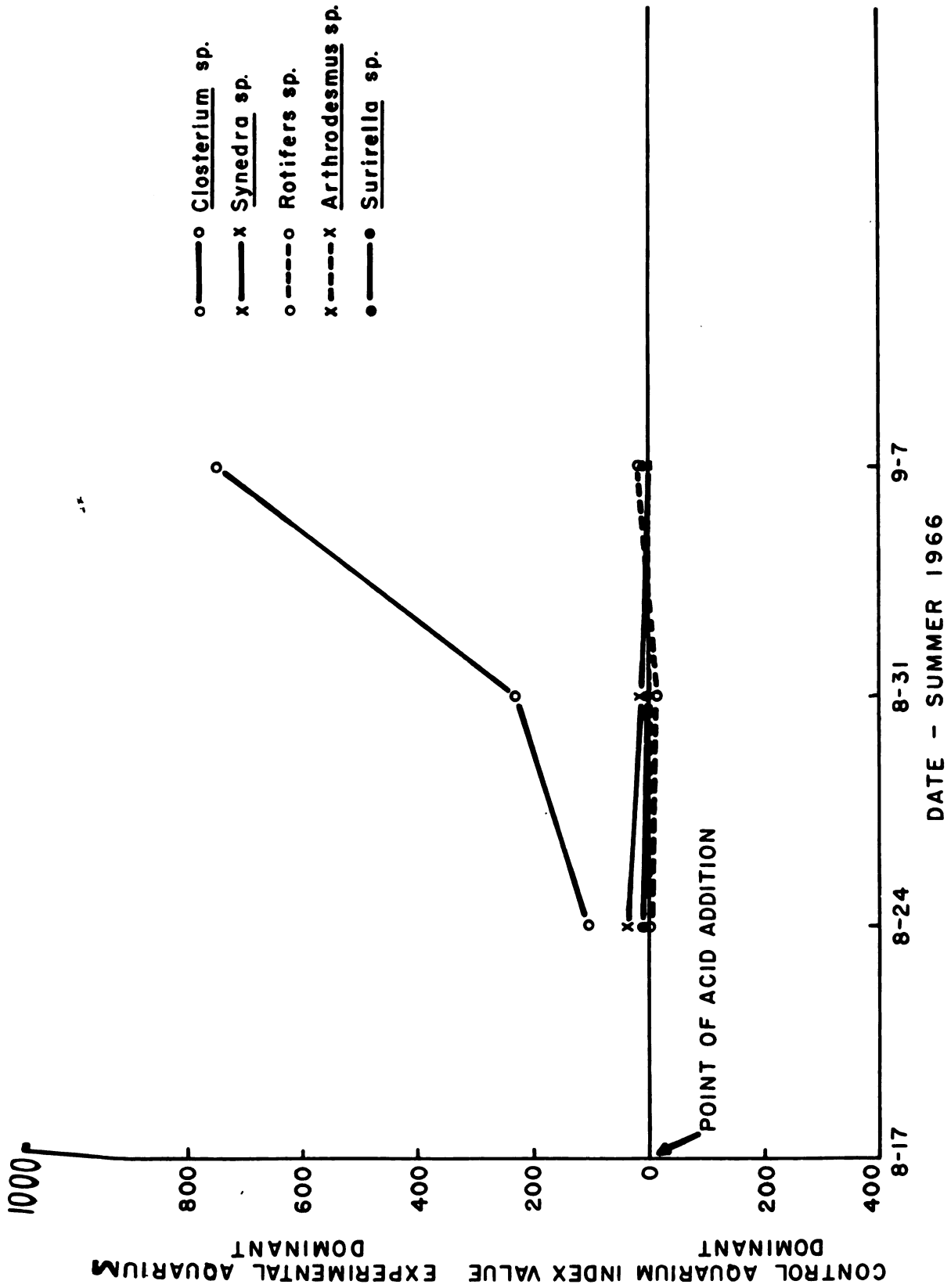


FIGURE 15

Figure 16. Index values for the dominant species found
in the periphyton in the ponds.

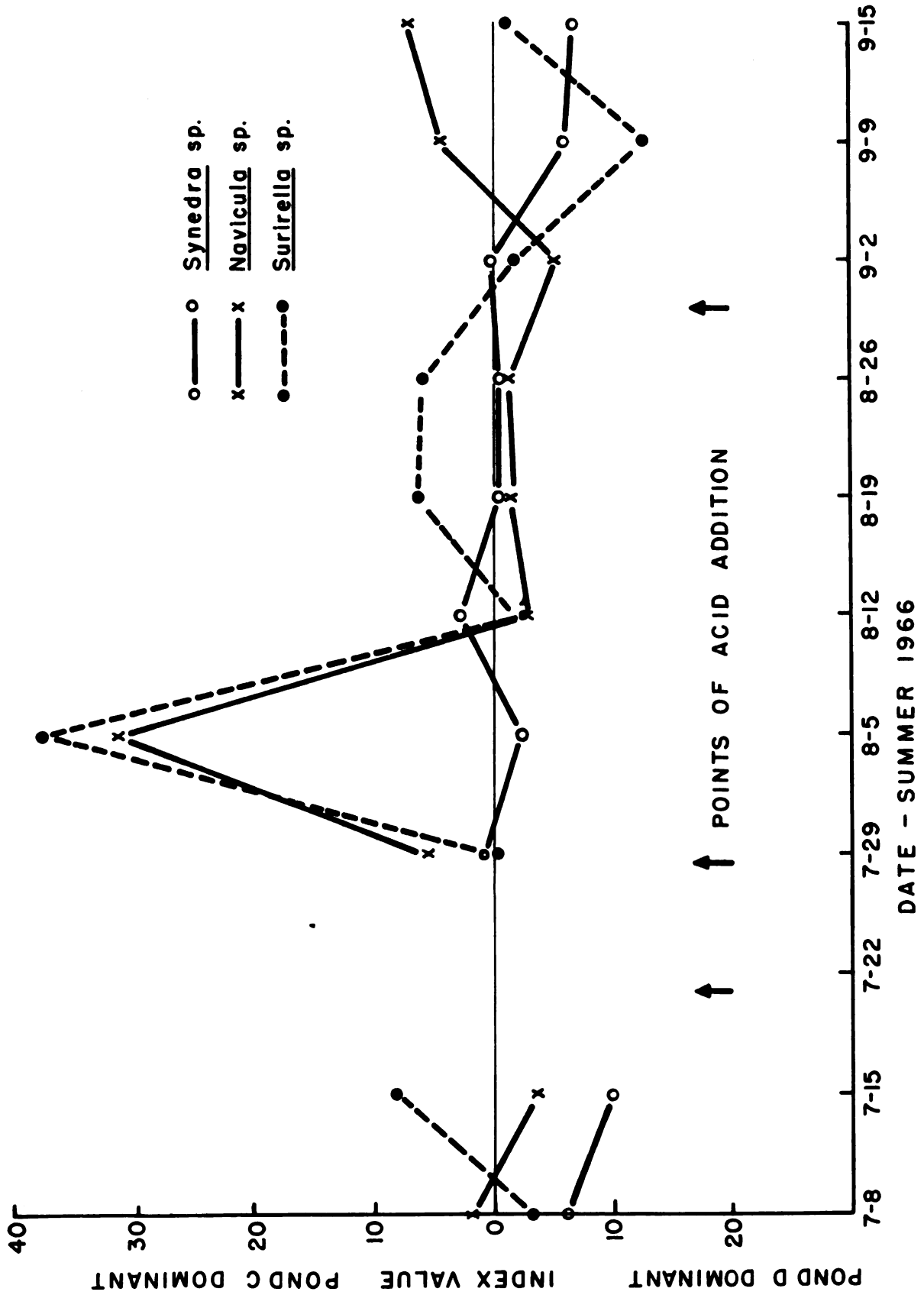


FIGURE 16

Figure 17. Index values for the dominant species found
in the periphyton in the aquaria.

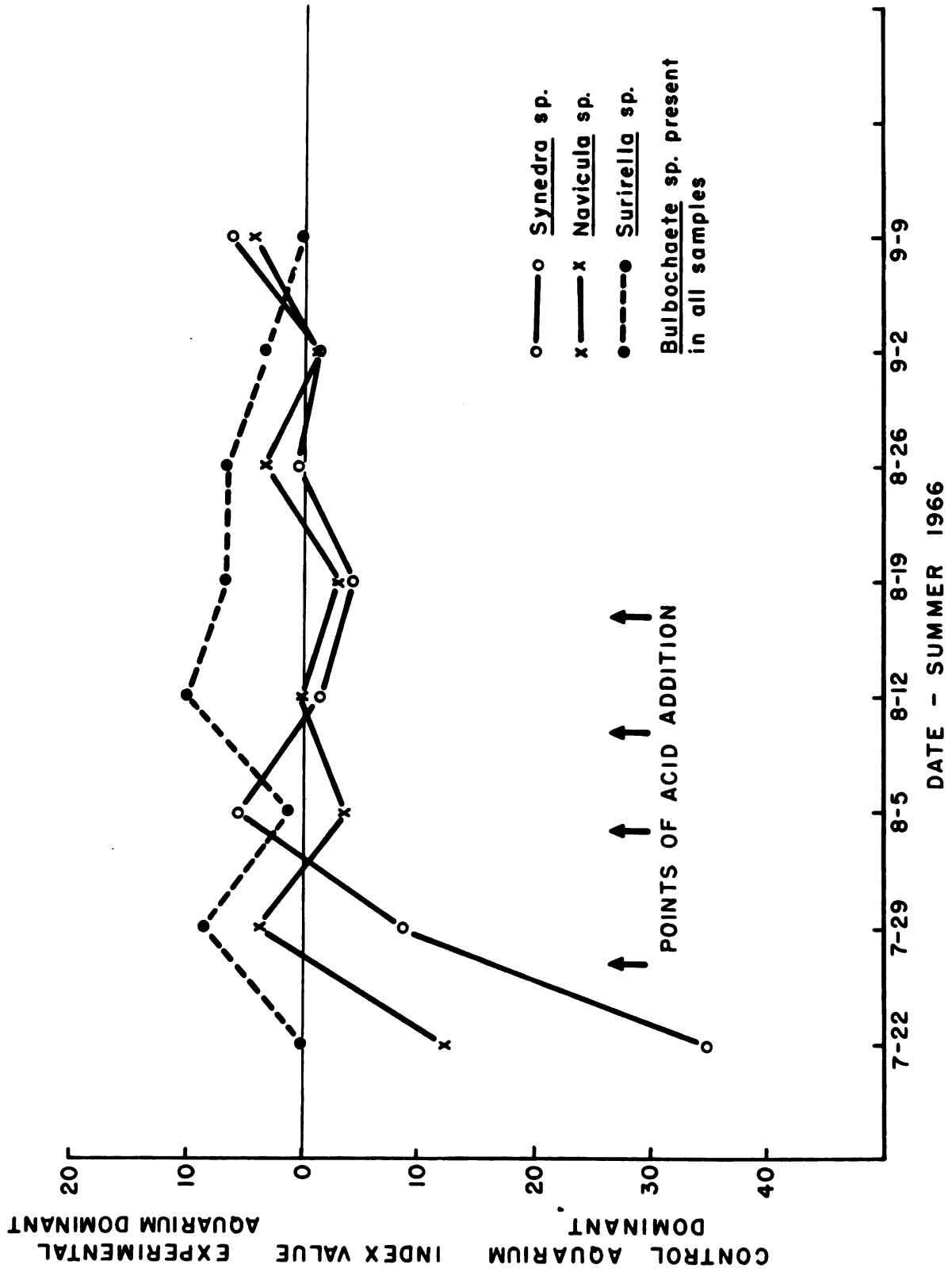


FIGURE 17

addition of acids.

Net plankton--ponds

The same type of index used for centrifuged plankton and periphyton was used again for net plankton. Figure 18 shows the change in this index for several organism types through the summer. Significance would be hard to define since variability could be introduced from many sources. The extremes would be the only possible significant feature. Here large copepods and cladocera seemed to be greatly reduced in dominance in pond C during the middle of August. If this was a result of addition of acid its effect was indirect since no immediate response was seen at the time of addition. This may also be why no response was seen immediately in the week after the third and largest acid addition.

Net plankton--aquaria

Net plankton were of small number and only survived for several weeks after the start of the experiment in both aquaria. As both populations were going to extinction the scant data may have little significance in explaining the effect of acid addition though the large copepods and cladocera were observed to decline in dominance in the experimental prior to their decline in the control (Figure 19).

Figure 18. Net plankton index values for the ponds
through the summer.

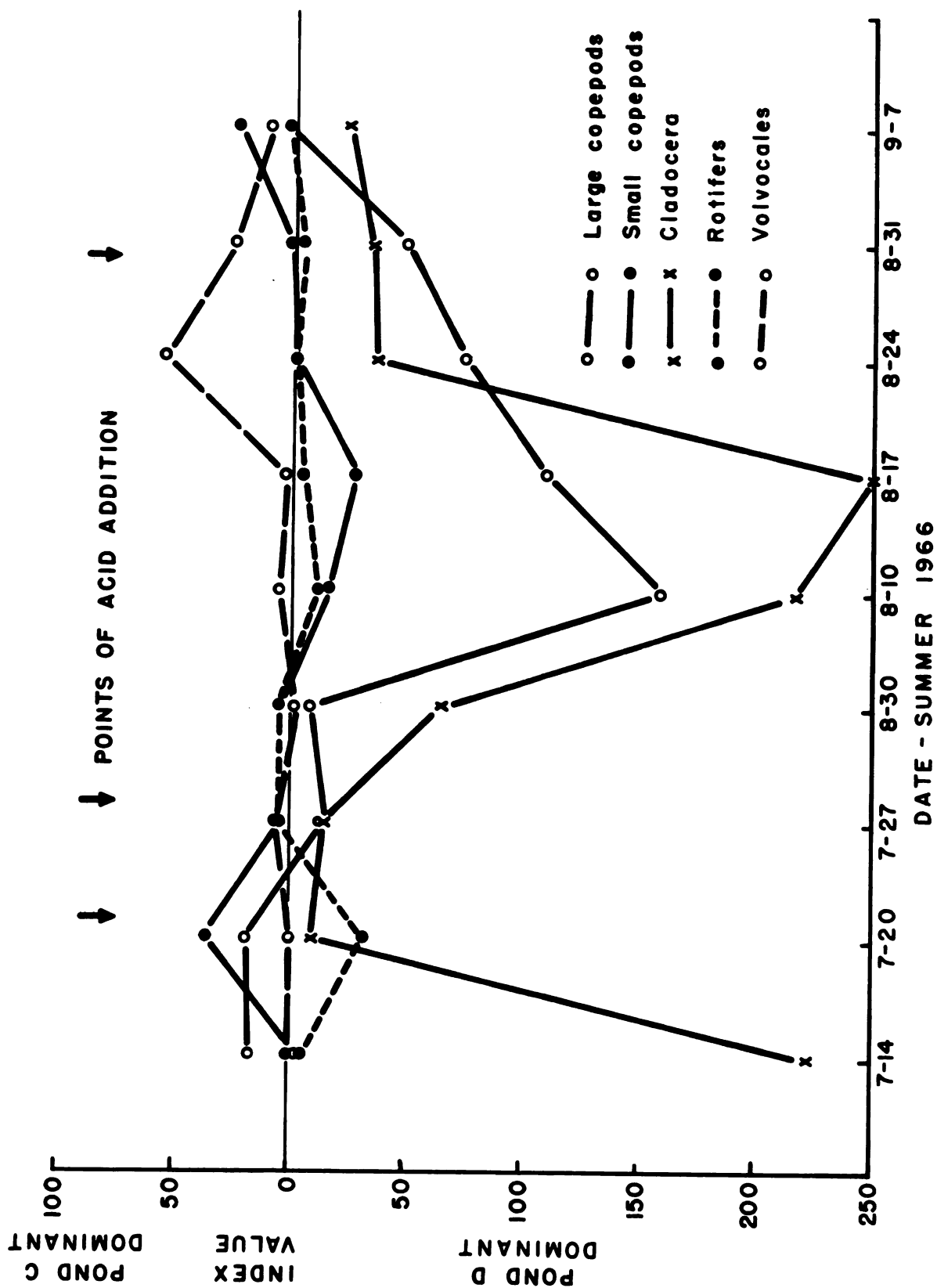


FIGURE 18

Figure 19. Net plankton index values for the designated aquaria pair.

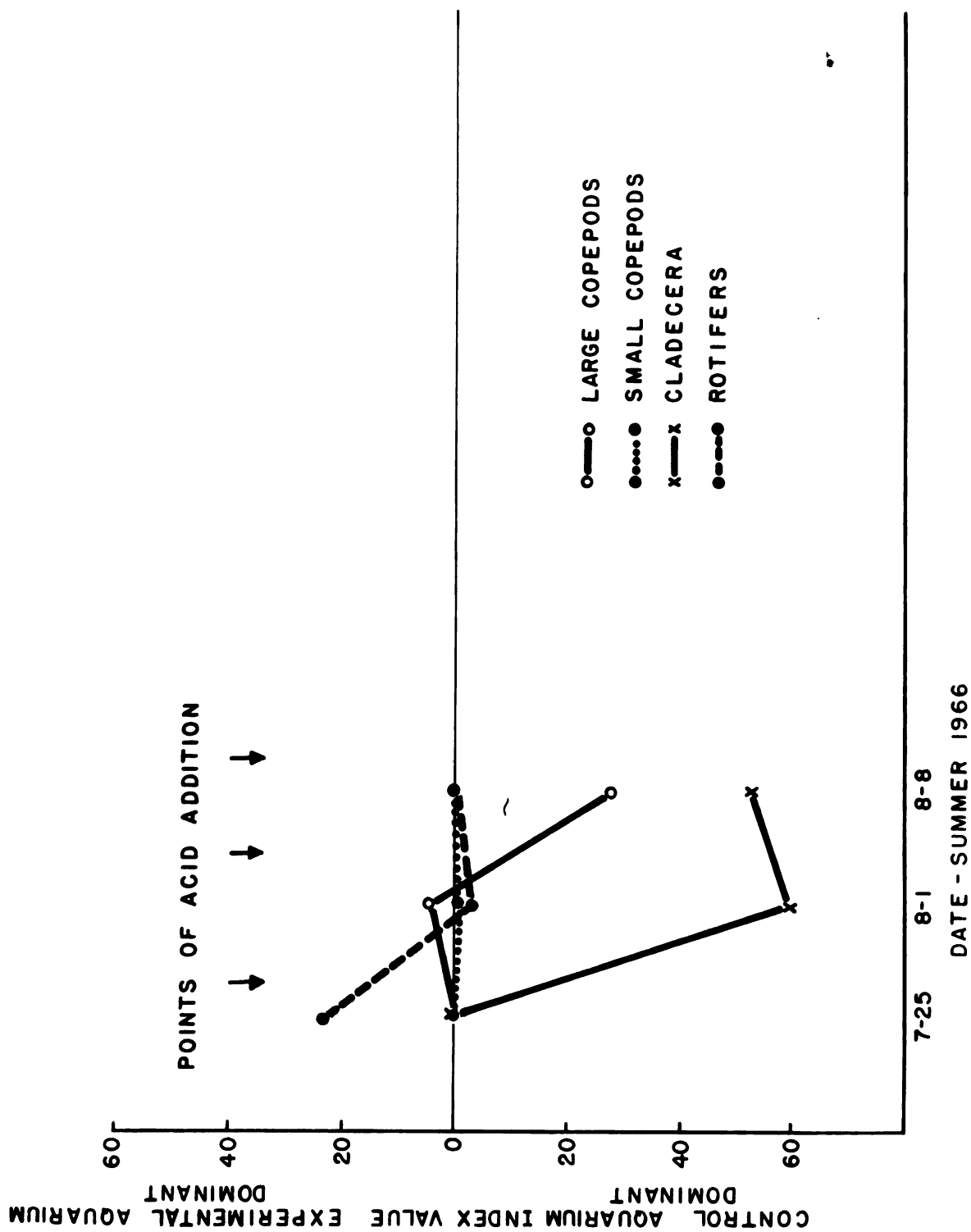


FIGURE 19

Bottom organisms--ponds

It was felt that a short term experiment such as this one, falling at the emergence time of many of the dominant bottom species would not provide any significant data with the limited sampling time available.

Bottom organisms--aquaria

There was no large difference noticed in the survival of the introduced organisms. Several Odonata survived in each aquaria while all other forms perished. Both aquaria developed the previously mentioned filamentous growth.

Fish--ponds

No dead fish were noticed in the ponds during the summer and all appeared to be in no distress.

Fish--aquaria

The four green sunfish in both aquaria survived the entire experiment with no ill effects noted. None of the colorless filamentous material was observed in either of these aquaria.

Fate of the acids in the ponds

The total changes in ppm acid concentration in the light and dark bottles during the three week exposure in the pond are presented in Table 4.

The change in the sample containing acids in distilled water showed that change which might be attributed to the effect of light on the acids. The difference between this

Table 4. Observed changes in acid concentration in light and dark bottles suspended for three weeks in pond C. (Data in ppm.)

Bottle type	Distilled water and acids	Filtered pond water and acids	Centrifuged pond water and acids	Untreated pond water and acids
Light	-1.3	-11.9	-11.4	-14.6
Dark	-4.6	+0.2	+2.5	-0.7

change and that observed for the 0.45 μ filtered pond water indicated the magnitude of the effect of dissolved substances in the water. The difference between the filtered and the centrifuged samples likewise indicated the result of bacteria and ultraplankton activity. Any effect on the acid concentration by phytoplankton and zooplankton was revealed when the difference between the untreated and the centrifuged samples was found. Table 5 shows the calculated values attributed to these separate sources.

A decrease in acid concentration measured by fluorescence indicates either destruction of the acid molecule or polymerization of two acid groups (Christman and Minear, 1967). Conversely an increase in fluorescence would indicate acid production or a breakdown of polymerized acid groups.

Conditions in other natural bodies of water

In Figure 20 the acid concentration was seen to increase only near the bottom on those lakes which lack an established thermocline, Goose and Long Lakes. In Titus Lake, an oligotrophic marl lake, acid concentration increased markedly as the thermocline was passed between 24 and 30 feet. Concentration remained relatively constant in the well-mixed upper water.

On a stream such as the Clam River, Figure 21, changes were seen in acid level along the flow. The substantial rise near mile 1 can be traced to the effect of a sewage plant operated by the city of Cadillac and emptying into the river.

Table 5. Calculated effects of different sources of acid change as determined by light and dark bottle experiments. (Data in ppm.)

Bottle type	Sunlight	Dissolved substances	Bacteria and ultra-plankton	Phytoplankton and zooplankton
Light	-1.3	-10.6	+0.5	-3.2
Dark	-4.6	+4.8	+2.3	-3.2

Figure 20. Acid concentration profiles of three lakes.

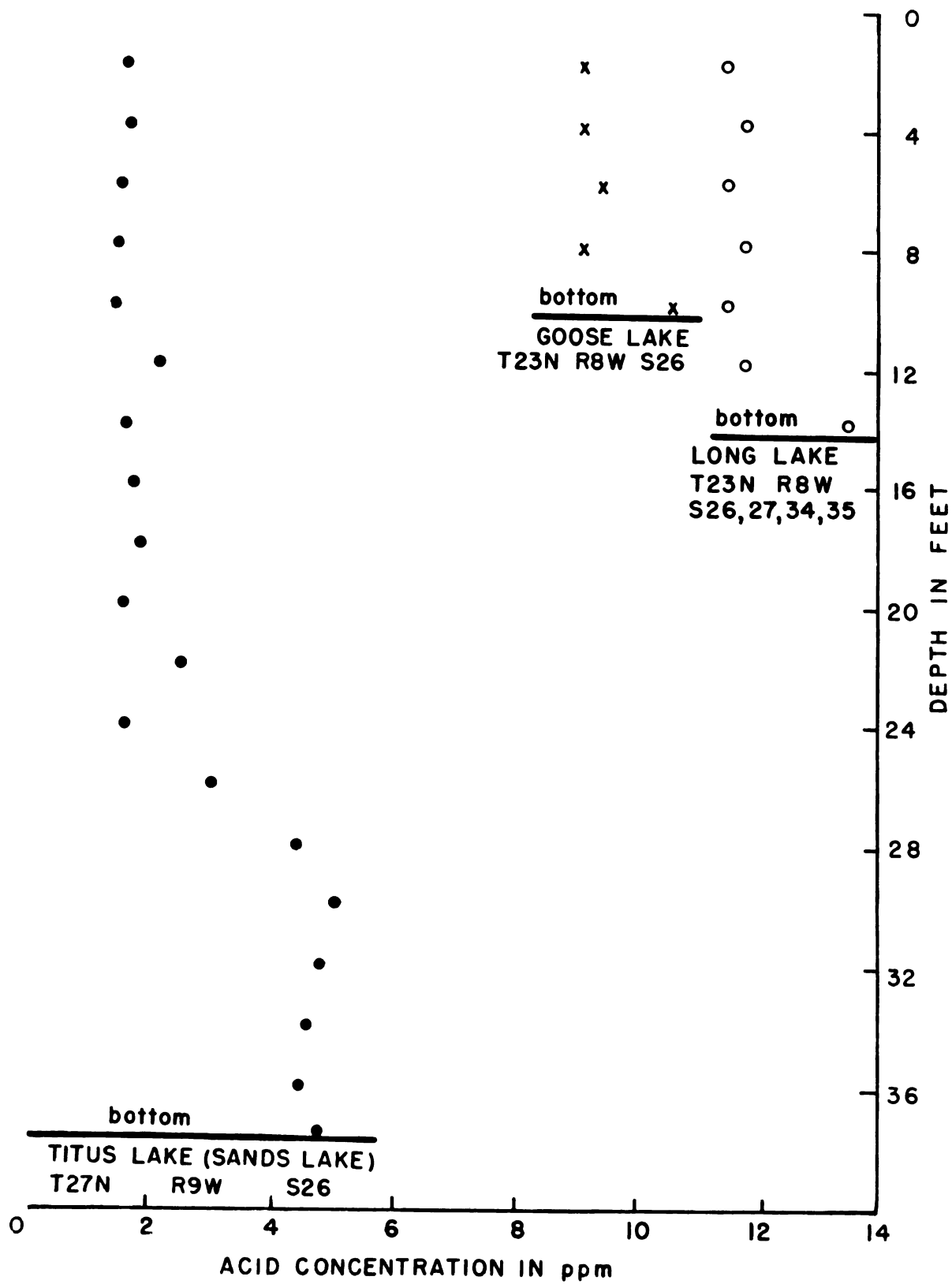


FIGURE 20

Figure 21. Acid concentration profile of the Clam River.

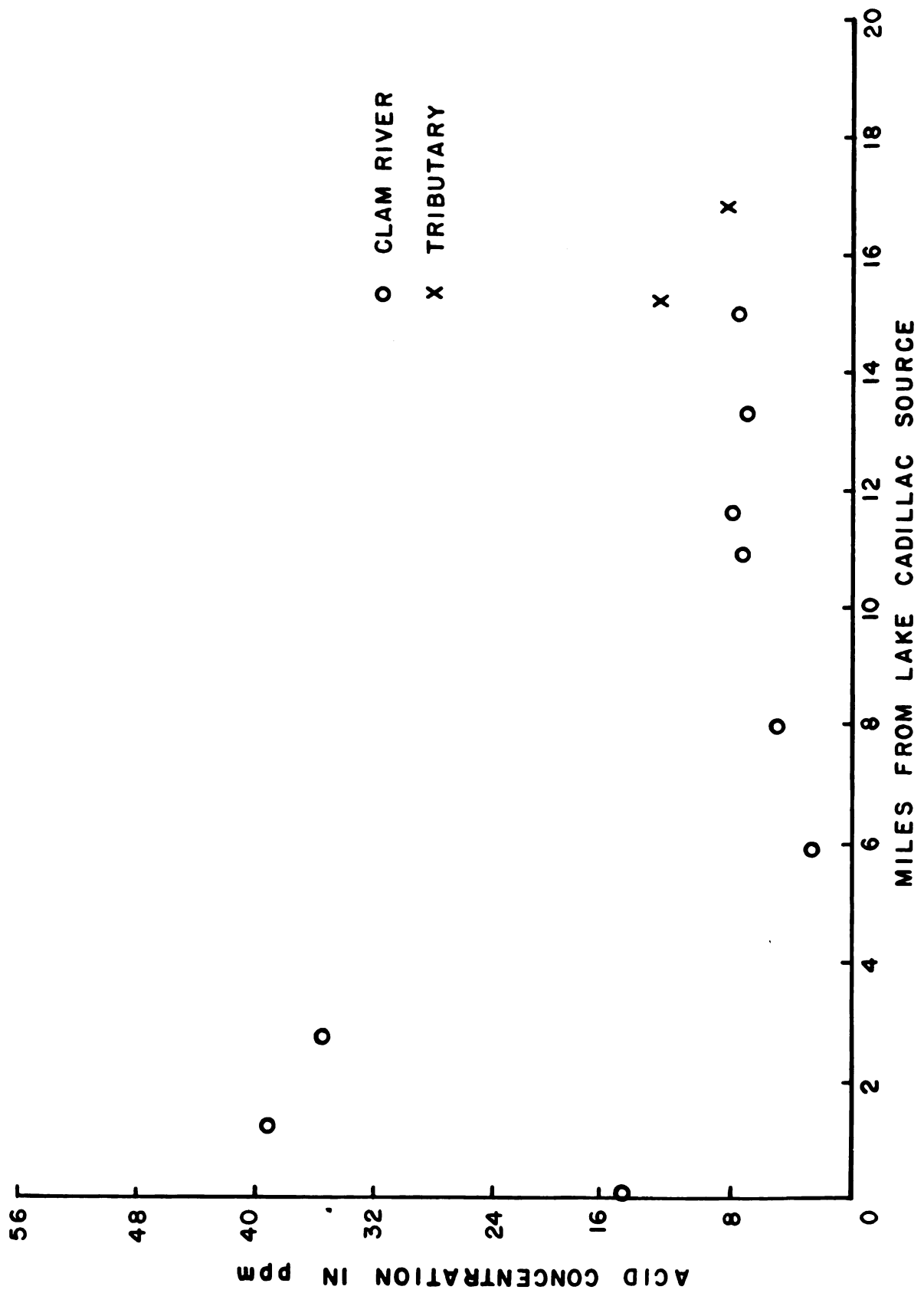


FIGURE 21

Detergents fluoresce at approximately the same wavelength as the colored acids and interfere with accurate determinations. In the next four miles essentially all the material added by this plant and most of the acids entering the river from the lake origin have been removed as the stream flows through the Cadillac moraine.

Table 6 presents measurements of acid concentration for various other bodies of water.

Cause of viscosity in the aquaria

Upon spraying the chromatogram run in the isopropanol:acetic acid:water solvent with sodium periodate reagent followed by benzidine, no spots were found in the unhydrolyzed sample while one spot, not corresponding to glucose, was found in the hydrolyzed sample. This spot had an r_f value of .73-.82. The same spot also had a reddish tint when sprayed with aniline-acid-oxalate reagent. This indicated the presence of aldopentose structure. No amines were present as no spots were seen following ninhydrin spray. In the *n*-butanol:acetic acid:water solvent this same spot had an r_f of .54. This compound consistently had a greater r_f value than any of the other sugars co-chromatographed. Seliwanoff's test proved negative for ketose. Determinations of periodate consumed upon oxidation and formic acid produced were inconclusive. The chemical causing the increased viscosity was most probably a polymonosaccharide of an uncommon sugar produced by bacterial or fungal growth.

Table 6. Acid concentration of various selected bodies of water.

Location	ppm acids	Remarks
Lake Michigan		
1. NE holland	0.68	at 77 meter depth
2. $\frac{1}{2}$ way across lake	0.62	at 157 meter depth
3. Racine	0.47	at 55 meter depth
Lake Mendota	7.65	surface near shore
Crawfish R., Columbus, Wis.	62.5	following heavy rain very turbid
Charlotte R., Davenport, N. Y.	6.03	clear cool stream

DISCUSSION

Method of acid measurement

Color, as measured by the comparison of samples of platinum-cobalt standards, was the only quantitative method widely used to measure the concentration of yellow organic acids in natural waters prior to the introduction of fluorescence. When compared to fluorescence methods, the measurement of color by association with platinum-cobalt standards shows several disadvantages in the measurement of concentrations of these compounds.

Color is more sensitive to pH change than is fluorescence in the pH range of natural waters (Christman and Ghassemi, 1966a,b; Christman and Minear, 1967). In fact, differences in pH may nullify the comparative value of color when several bodies of water are investigated. Recourse may be made to an expression of color at a standard pH as in Christman and Ghassemi (1966b) for streams in Western Washington, but this further complicates the methodology. The color to carbon ratios presented in this same previously mentioned study also indicate a high variability, most probably arising from differing chemical structure. A modification used by Anthony and Hayes (1964) where measures of color and turbidity are separated spectroscopically possesses these limitations also.

Shapiro (1957) arrived at a value of about 0.4 ppm acids per color unit at pH of 7.0 with acids from Linsley Pond. A comparison of both his and the values of Anthony and Hayes for Lake Mendota with my fluorescence measurements about ten years later shows a value of nearly 0.7 ppm acids per color unit. It is possible that Lake Mendota has changed in the intervening years or seasonally though from the similarity of both the previous measurements, I would believe this not to be the case to this great an extent. As I have already mentioned, detergents may increase fluorescence in the same wavelengths as the acids. This may have been the case in Lake Mendota. Otherwise chemical differences in the acid molecules between Lake Mendota, Linsley Pond and my experimental ponds may be indicated. Assuming these acids to be 53.5% carbon as found by Shapiro and that the entire dissolved carbon is derived from these colored compounds, we can compare Christman and Ghassemi's Western Washington streams with values from 0.2 to 0.55 ppm acids per color unit. This wide range of values can be attributed to the poor quantitative relationship between color and acid concentration probably arising from differences in the origin and chemistry of the acid molecules. Sensitivity of this method is about the same as that of fluorescence on the 3x scale or ± 0.3 ppm.

Christman and Ghassemi (1966a) used fluorescence for quantification of these colored compounds by almost the same

method as I have presented. The one difference is their use of only filter 2A for a secondary (emission) filter before measurement of fluorescence. This gives their method a wider response to all wavelengths above 415 m μ whereas the method used in this study measures only those wavelengths around 460 m μ . A product of these differences is a greater sensitivity (± 0.3 ppm on 1x scale) but less assurance of freedom from interfering substances for Christman and Ghassemi as opposed to lesser sensitivity (± 0.3 ppm on 3x scale) and lessened interference from detergents by my method.

Several problems do exist with the fluorescence method. A change in pH continues to cause a small change in fluorescence although Christman and Minear point out that fluorescence is insensitive to pH fluctuations over a broad range of pH values. pH was not found to be bothersome or necessary of adjustment for consistent reproducible results in this study.

Christman and Minear also report a polymerization reaction which is accompanied by a decrease in fluorescence. The extent of polymerization of the acid molecules in a body of water in addition to other differences in chemical structure or origin may also affect the accuracy of this method and its application to the comparison of different bodies of water.

Detergents have been mentioned previously as possible sources of interference in fluorescence measurements. This

is most probably the cause of the extremely high values recorded below the sewage plant for two miles or more on the Clam River. Interference of this type would not be expected to affect the measurements from the ponds or aquaria in this study. When the presence of detergents is suspected, it might be well to use the methylene blue method (Standard Methods) to measure their concentration while at the same time preparing a standard curve for detergent concentration and fluorescence at the interfering wavelengths. Hence in this way one might be able to separate the two factors and gain considerably more confidence in what has actually been measured.

Because of the high probability of unknown interference causing a loss of sensitivity in measurement, it would be best to measure fluorescence over as small a range of wavelengths as is possible. Correction should be made for detergents if they are present in quantity while pH may be ignored if changes are small and in the center of the range. The unknown effect of the extent of polymerization, different chemical structure or origin and the enhancement of fluorescence by certain salts must be merely acknowledged at this time as a possible source of error in the comparison of different waters until the magnitude of the differences arising from these sources is better understood. It may be that with fluorescence we can actually measure the chemically or biologically active part of the molecule such that differences

in overall structure are of little consequence. If this is the case, instead of parts per million, some activity unit should be proposed.

The origin and fate of the acids

It has long been assumed that these yellow acids originate from soil runoff and the decomposing vegetable material or humus. Shapiro (1957) supports this conclusion by the observation that soil extracts are very similar to the compounds isolated from lake water. Christman and Ghassemi (1966a) further clarify, based upon the chemical analysis of degradation products from these acids, that lignins, lignans and other wood phenolics are the most probable sources.

It therefore seems that two general sources may exist for the colored acids found in water. Origin may be allochthonous from decomposing terrestrial vegetation and/or soil organic matter and this matter then carried into a body of water with runoff. This was not revealed to be a large source of acids in the ponds studied since rainfall had no significance in the explanation of acid changes measured and no increase of fluorescence was noticed following even heavy rains. I do not feel that subsurface seepage into the ponds accounts for an appreciable influx of acids since these molecules are highly adsorbed in the soil and may travel no more than a few inches from their point of release into the environment.

Autochthonous acids released from aquatic vegetation decomposition and extraction from bottom sediments of the ponds would be a second source. From the higher concentration of acids near the bottom of the three lakes measured, this second source would appear to be important. In Titus Lake, where a thermocline is present, these acids can be seen to build up higher concentrations relative to the upper waters than in the other two lakes where mixing occurs between all strata.

In streams such as the Clam River, allochthonous sources would be the more important. Between mile six and mile twelve, where acid concentration more than doubles, the stream flows through wooded land as opposed to open farm land between mile twelve and mile fifteen where little change in acid concentration occurs. The slight increase below mile fifteen might be traced to input of acids from tributaries.

From the variations seen in the lakes, ponds, and streams, this acid system is certainly not stable. In addition to the previously mentioned sources, autumn leaf fall and spring runoff may also be of importance.

The loss of acids from a body of water is less understood than their source. Whipple (1927) has reported a lightening of colored waters which he attributed to "bleaching" by the sun. When Christman and Minear found a rapid decrease in fluorescence during measurement, they proposed the concept of polymerization occurring between acid molecules which

reduces the number of available sites for a fluorescent reaction. I believe these two observations were of a similar phenomenon.

The light and dark bottle experiment of this study was conceived to investigate this problem. A decrease of 1.3 ppm was measured in the light bottle having yellow organic acids dissolved in distilled water, but an even greater fall in concentration of 10.6 ppm was recorded in the light bottle containing acids dissolved in filtered pond water. Clearly some dissolved substance which occurs naturally in the pond water is necessary for this reaction. The decrease in the distilled water with acids was recorded for both light and dark bottles so this was probably not by the same mechanism.

The concept of a polymerization seems to fit the circumstances. Acid molecules may be joined together in a reaction catalyzed by sunlight and involving a dissolved substance (ion; molecule ?) naturally occurring in the water. It is not known whether this dissolved material may act as a catalyst also or be involved directly in the reaction. It is my belief that this dissolved substance may be (a) doubly positive charged metal ion(s), mainly calcium. I support this by pointing out the relatively abundant nature of this type of ion in natural waters, the low color of hard water lakes, the fact that Christman and Minear were working with a 0.01 M calcium chloride solution of these acids when they observed this phenomenon and that Shapiro (1958) was able to produce

and associate bands on paper chromatograms with distinct acid-metal associations. One of Shapiro's possible explanations was that these zones may represent different salts or complexes of one or more acids.

Through this polymerization reaction the molecular weight of the acid group would be increased. It would be theoretically possible for a point to be reached where the molecule would not be able to remain suspended or dissolved in the water column and would fall to the sediments. Mixing would tend to counteract this along with possible reactions breaking down the polymer bonding. The higher concentrations near the bottom of lakes could not be caused by this settling alone. This is especially true in stratified lakes where the bottom waters are exposed to little turbulence. In such undisturbed waters it would seem that settling would be more rapid in the absence of resuspension by mixing and that zones of increased concentration would be less likely to occur. Precipitation is thought to be of minor importance in the loss of acids to a body of water.

Other possible mechanisms causing changes in acid concentration within a body of water, as inferred from the light and dark bottle experiment, might be a breakdown of polymer structure, thus an increase in measured fluorescence, when bacterial enzymes attack the acid molecule for use as a carbon and/or energy source; and also the destruction of the total molecule when used for the same purposes by higher organisms.

A diagram of acid balance in these ponds can now be constructed to help visualize the dynamics of this system. Over the summer the ponds showed an average loss of about 0.05 ppm acids per day. A diurnal cycle was evident with a net loss for the daylight hours and a net gain during the hours of darkness. When the change for the light and dark bottle experiment was calculated, there was an average net loss of 0.71 ppm acids per day for the three weeks. Since the loss rate was so much greater in the bottles than the ponds, the source of acids that must have been replenishing the ponds was being excluded from the bottles. As this source was most likely the bottom sediments and vegetation decomposition in the ponds, we can assume an average net gain of 0.65 ppm acids per day from this area in order to achieve a balance between the two estimates of daily change. Table 7 shows the calculated movement of these acids in the experimental ponds on a typical summer day.

Figure 22 presents an explanation of the diurnal and annual cycles. The effect of light polymerization is a net loss during the day while at night, when this reaction does not occur, acids continue their constant release from the sediments and through decomposition to create a net gain. In this same manner the annual cycle is created. A net loss is incurred in summer under conditions of high solar radiation. In winter the acids show a net increase when this loss through light-induced polymerization is reduced.

Table 7. Dynamics of acid change in a pond on a summer day.

Loss of acids	
0.50 ppm per day	Light-induced polymerization
<u>0.21 ppm per day</u>	Destruction by higher organisms
0.71 ppm per day	Total loss per day
Increase of acids	
0.65 ppm per day	Release from sediments and through decomposition
<u>0.01 ppm per day</u>	All other sources (runoff, polymer dissociation)
0.66 ppm per day	Total increase per day
0.71 ppm per day	Total loss per day
<u>0.66 ppm per day</u>	Total increase per day
0.05 ppm per day	Net change (loss) observed per day

Figure 22. Diurnal and annual acid cycles in a pond.

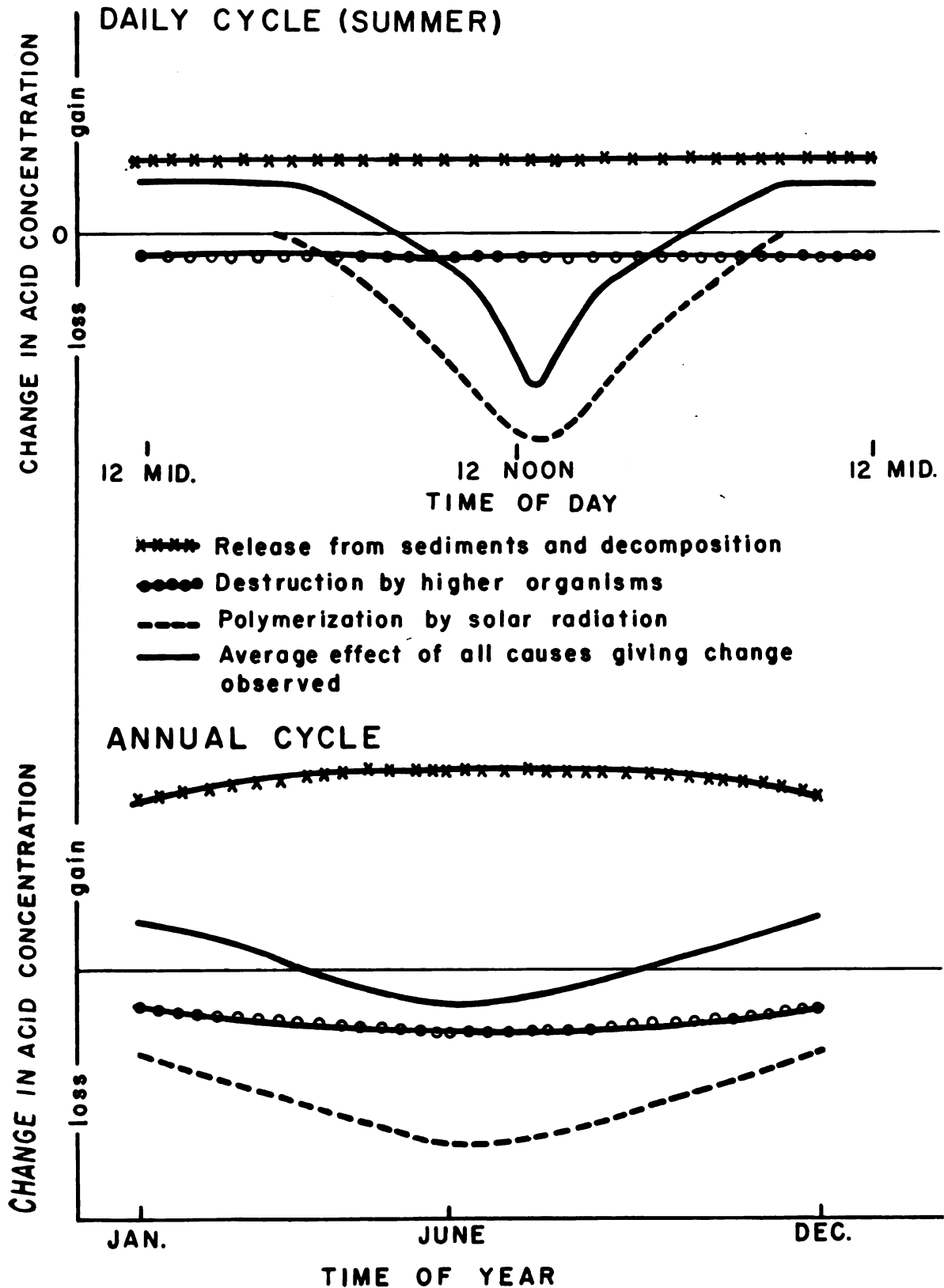


FIGURE 22

Effect of acids on chemical and physical features

A basic assumption upon which all the data of this section rests is that the acids extracted, purified and added once again to the aquaria and ponds were not changed in structure during the process. This assumption is probably not completely met as 2 N sodium hydroxide was used in the extraction process and would tend to alter the molecular structure. This section must therefore be considered with certain reservations as to whether the observed changes can be normally attributed to the native acids or are the product of structural fragments not naturally occurring. It is my belief that structural alteration was minimal as little change was noticed in the spectra and in the chemical behavior of the acids after the recovery process. Also any fragments that might be produced may resemble smaller polymer units which are normally present in the system.

The use of a computer to solve for the relationship between different chemical parameters in the environment was helpful in understanding the effect of acids on these features though some problems were encountered. The asymptotes seen in all three theoretical equations are artifacts produced by the method used in solving for the parameter to be described. If higher power functions were included for all factors the asymptotes would be smoothed out to a more representative curve at these points. The equations described are also completely symmetrical and certain portions of the

curves are not supported by the data. If these features can be taken into account, the meaning of these equations may be explained.

The action of acids on pH seems to show an interaction dependent upon the initial pH of the water. The partial correlation coefficient of acid concentration is negative showing generally decreasing pH with increasing acid concentration. I believe the general action of acid concentration on pH actually to be a decrease in pH with rising acid concentration for waters with initial pH values below 8.02 while, for those waters with an initial pH above 8.02, a rise in pH proportionate to the extent the initial pH exceeds 8.02 until, in the region of 25 to 30 ppm acids, pH decreases with increasing acid concentration (Figure 23). This representation seems the more true to nature as no return approach to pH values near the initial pH was recorded for the aquaria at high acid concentrations.

A clue to the possible cause for this strange pH behavior may be taken from the value of the pivotal initial pH--8.02. The presence of increased acid is normally expected to produce a decrease to a lower or more acidic pH as occurs at initial pH's below 8.02. The pH value of 8.02 is the approximate border pH where carbonate exists at higher pH's and bicarbonate only may be found at lower pH values. It could be that carbonate or calcium bonded as carbonate is joined in some way to the acid molecule preventing the

Figure 23. Revised pH--acid concentration relationship.

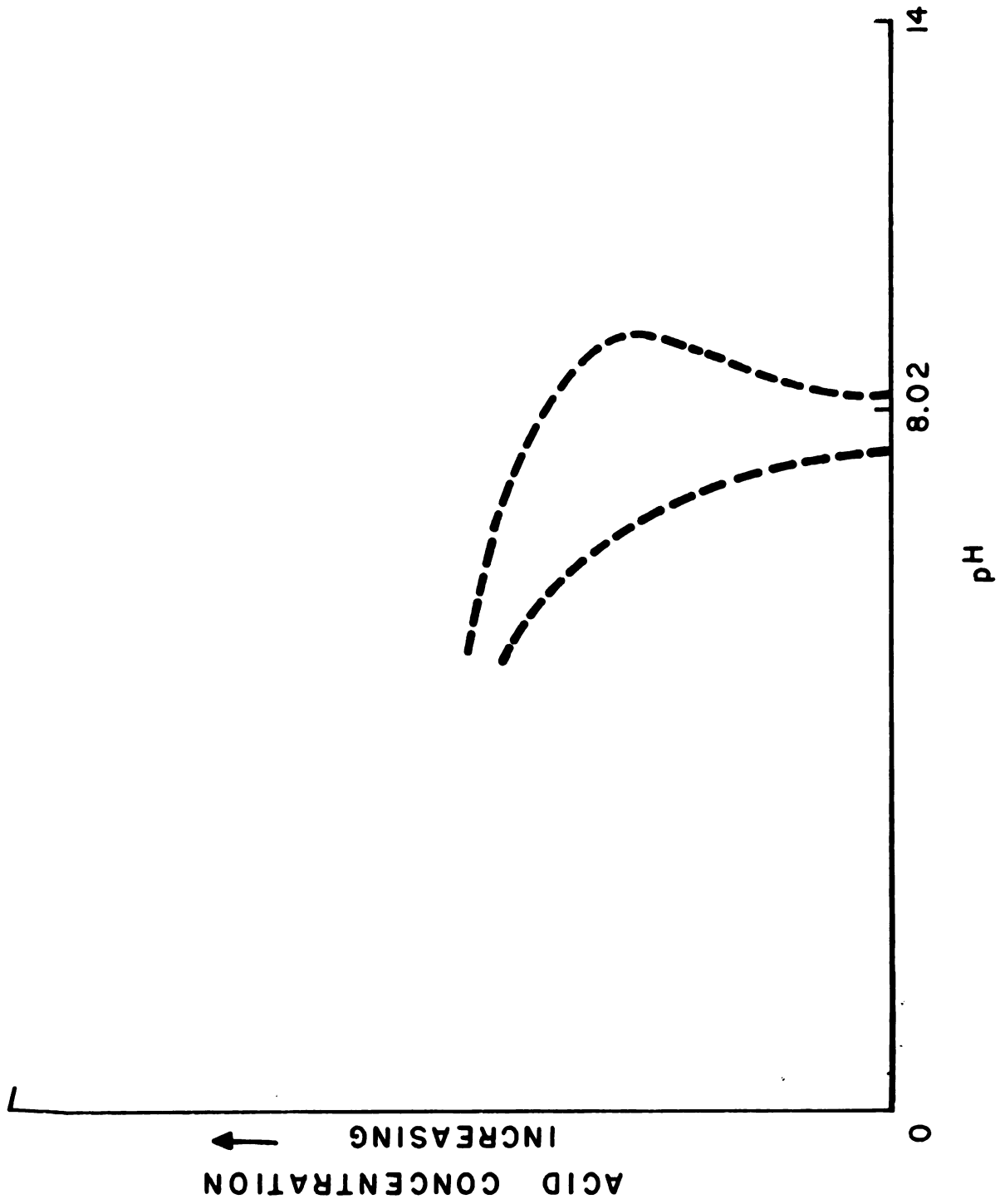


FIGURE 23

expression of acidity or releasing molecular fragments which are basic in pH. In turn, the carbonate so bonded may become untitratable so no expression of phenolphthalein alkalinity would be observed. This appears very possible when it is also recalled that alkalinity dropped markedly at high acid concentrations in the aquaria and that the polymerization reaction may involve calcium.

So it may be said that the expression of pH under different acid concentrations is dependent in some way upon the action of these acids on the carbonate-bicarbonate buffering system. As acid concentration becomes greater the capacity of the buffering system is eventually overcome (if the rate of acid increase is faster than the rate at which available insoluble calcium carbonate is dissolved and mobilized) and pH behaves in the generally accepted manner of decreasing as acids continue to rise. In the environment this relationship depends upon the rate of acid change in a body of water, the magnitude of the carbonate-bicarbonate system and the size and availability of the insoluble calcium carbonate reservoir. Through the rates of change of these three parameters it might be decided whether a lake might remain as hard water and relatively unproductive or become highly productive and more colored.

Conductivity appears to be affected in a manner somewhat like that of pH. The asymptotes can again be excluded as products of the method of solving the expression. The action

of acid concentration on conductivity then is seen to be a mirror image of its action on pH. When an initial pH was above 8.02 with a rise in pH accompanying an increase in acid concentration, the initial conductivity for the same aquaria was usually near to or less than the pivotal initial conductivity value of 164 micromhos per centimeter. As acid concentration was increased, conductivity decreased as might happen if two molecules were brought into union as was suggested in the explanation of the rise in pH. As acid concentration continues to rise the fall in conductivity slows and then reverses direction to show a direct relationship between these two parameters at high acid levels. For water above an initial conductivity of 164 micromhos per centimeter, a steady rise in conductivity occurs when acids are increased (Figure 24).

From the similar reaction of both pH and conductivity to a change in acid concentration it seems clearly evident that both these effects arise from the same source--an interaction between these acids and calcium carbonate to form a complex. In the absence of calcium carbonate, at a pH below 8.02 or after all the available carbonate has been complexed, conductivity rises as it normally would when molecules capable of dissociation are added to water.

Optical density, when the asymptotes are eliminated, shows an almost linear increase with increased acid concentration. This is what would be expected when a molecule

Figure 24. Revised conductivity--acid concentration relationship.

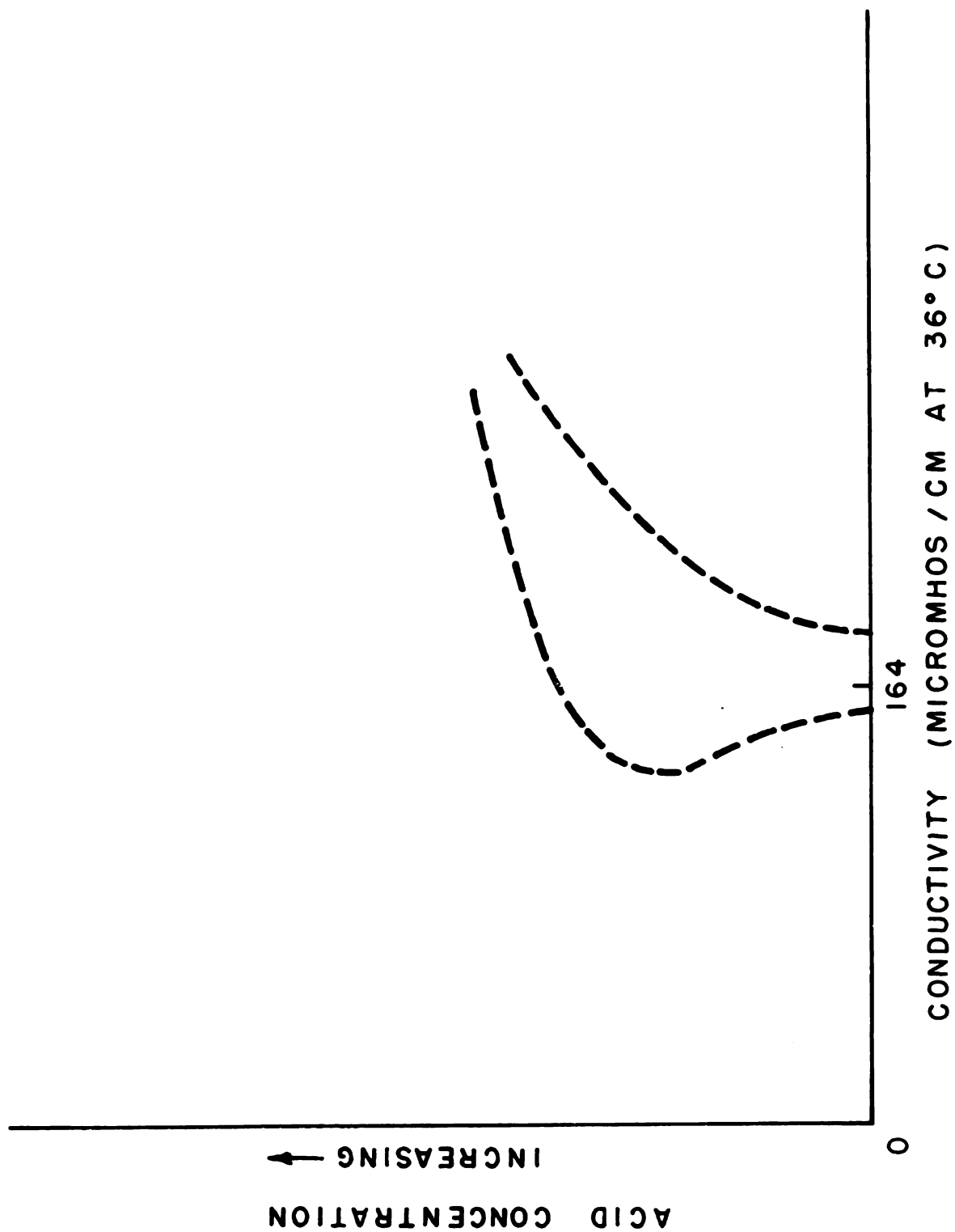


FIGURE 24

Figure 24. Revised conductivity--acid concentration relationship.

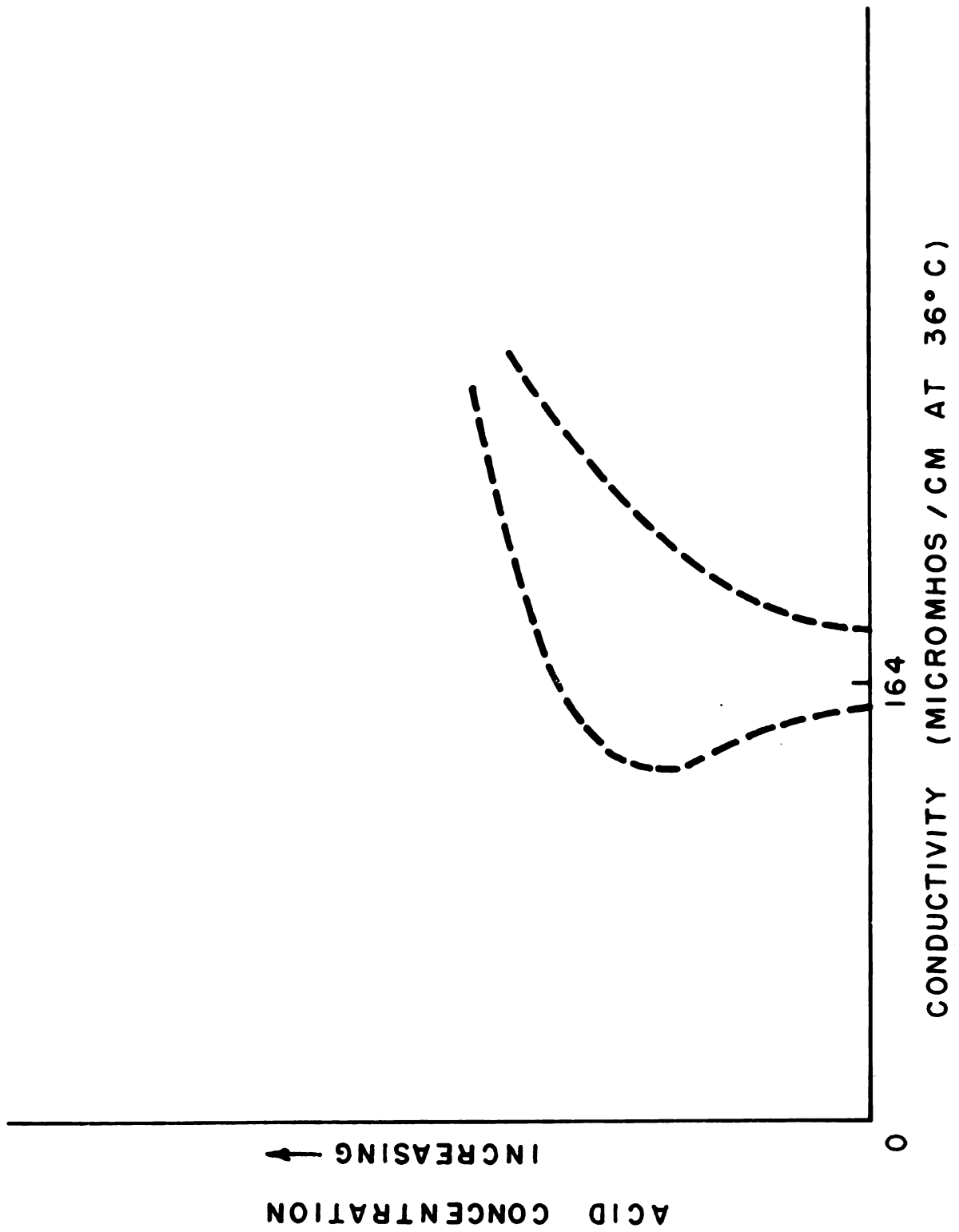


FIGURE 24

absorbing light at 330 m μ is measured at that wavelength and plotted against the concentration of that molecule.

Before the ponds may be viewed in the light of these aquaria predictions, any possible sources of interference in the ponds must be evaluated. Since there was a large filamentous growth only in pond D during the later portion of the summer, this would be a source which might yield interaction between the ponds in the same manner as acid addition. This source of interference will have to be evaluated when the ponds are considered.

The pH interaction in the ponds, pond C increased as compared to pond D, may have arisen from either acid addition in pond C, where the mean pH was 8.72, or from the decomposition and carbon dioxide production of the filamentous growth in pond D. Either or both of these may have caused the observed interaction and the effects cannot be separated.

Again with conductivity the causes of interaction cannot be separated. An increase in acid concentration in pond C, mean conductivity of 157 micromhos per centimeter, and the decomposition of vegetation in pond D would both produce interaction showing a decrease in conductivity in pond C relative to pond D.

With optical density it can be said with more confidence that the interaction recorded resulted from the addition of acids to pond C. The decomposition of a growth of filamentous material in pond D would be expected to decrease the percent transmission in this pond the same as would the addition of

acids to pond C. Interaction was shown to indicate a decrease in percent transmission in pond C relative to pond D and would thus be a more likely indication of an observable effect of acid addition. This might in turn be taken as proof that the effects of acid addition were observable in the ponds and that the interaction for pH and conductivity probably do indicate, at least in part, the effect of acid addition.

From the general decrease in bicarbonate alkalinity observed in the fish aquaria, some reaction must be taking place which involves the bicarbonate or calcium bicarbonate molecule. There are two possibilities for this reaction-- (1) bicarbonate combines with the acid molecule in the same manner as carbonate mentioned previously but at a slower and less preferential rate or (2) bicarbonate combines with the acid at an entirely different site causing different observable effects. Of these I believe the first to be the best explanation because it doesn't complicate the system through a set of assumptions for a new mechanism and the data can be fully explained by this same reaction. It may be that the action of acids with carbonate is so reactive that when carbon dioxide is removed from bicarbonate for photosynthesis, the transitory calcium carbonate is complexed with the acid molecule before combination with a free carbon dioxide molecule can transform it back into bicarbonate. This would be where the slower or less preferential combination would be observed depending upon the probability of

a chance encounter by a transitory calcium carbonate with an acid molecule before a free carbon dioxide molecule.

In the ponds changes in alkalinity may be entirely explained by the extreme decomposition of vegetation in D. Carbonate alkalinity decreased in D relative to C the later half of the summer as would be expected with a high rate of decomposition in pond D and the subsequent combination of carbon dioxide produced with calcium carbonate to form bicarbonate. This same process in pond D would produce the increase in bicarbonate relative to pond C. It is possible that the magnitude of these changes in pond D masked any noticeable effects of acid addition which may have occurred in pond D.

Total alkalinity also did not show any effects traceable to acid addition. The lower total alkalinity in pond C at the end of the summer most likely indicates the excess of fixed carbon dioxide removed through photosynthesis to respired carbon dioxide replaced. In pond D the carbon dioxide released through decomposition held the total alkalinity at a high value throughout the summer.

The temperature comparisons between ponds will not be evaluated as the calibration of the instruments was questionable. Light penetration, oxygen and metal ions failed to show any effects which might be traced to the addition of acids to the ponds.

In summary for this section, it might be said that very little chemical or physical effect was noted for the ponds that could be clearly called a response to the addition of yellow organic acids. It is only reasonable that this should be so since the amount of acids added was in the magnitude of one-one hundredth of the total concentration of these acids naturally found in the ponds. Even the fluorescent measurement of acid concentration showed no significant increase in pond C until after the third addition. Soon after this time the observations were terminated before any other effects might have become noticeable. The process of extracting acids from the water for re-use in addition was underestimated in planning and forced emphasis to be placed on the aquaria experiments.

These aquaria experiments did turn out to be very revealing. One reaction involving the complexing of carbonate salts, most likely calcium, to the acid molecules can be used to explain all the observed phenomena. This reaction may cause a rise in pH through the union with a part of the acid molecule blocking an acidic group. This union also shows a decrease in conductivity as two molecules capable of dissociation are complexed so that at least a portion of the resultant grouping does not dissociate. This reaction depletes the titratable carbonate alkalinity and may compete for bicarbonate when acid concentration is high. It may well be that this is the same mechanism that Christman and Minear described as light-induced polymerization.

Effect of acids on biological communities

No effect of increased acid concentration was evident for bacteria and fungi, bottom organisms or fish.

Centrifuged plankton from the ponds did appear to show some significant changes which might be associated with acid addition. The use of the index value method appeared most adequate when the counts made were an average of twenty or more individuals per organism group. At counts less than this any differences in dominance were not evident unless the dominance was extreme. After the third acid addition the majority of organism groups exhibited greater dominance in pond D. Though this may be an indication of the adverse effect of the acids on growth, I would rather believe that it indicates a large dominance of one group, Arthrodesmus sp., in pond C whose growth may have been stimulated by the acid introduction. This same feature may be recognized in the aquaria centrifuged plankton, only here it is Closterium sp. which exhibits the increased dominance in the acid aquarium.

The interaction seen with the periphyton chlorophyll extracts cannot be separated between the promotion of periphyton growth in pond D after the filamentous bloom die-off and lower chlorophyll production in pond C from the change in dominance of phytoplankton species. The growth of Navicula sp. appears to have been stimulated by the addition of acid while the slower growth apparent for Synedra sp. following the addition of acid may be only a lack of stimulation to the

extent of that expressed by Navicula sp. Little of significance can be seen in the aquaria studies of centrifuged plankters save a slight possibility of increased dominance of Surirella sp. in the acid aquarium.

The net plankton studies of the aquaria and ponds tend to show similar results. In these cases large copepods and cladocera appear much more dominant in the control aquarium and pond starting one to two weeks following the introduction of acid. These forms may be adversely affected by the increased acids in the experimental aquarium and pond C.

Since the variability of this index value method has not been defined, the significance of all these results is uncertain. Many other factors may have varied between the ponds and aquaria to cause the observed values.

If the observed effects were the result of acid addition they might be explained in several possible ways. Shapiro (1957) stimulated the growth of several algal cultures with the addition of purified yellow organic acids. Saunders (1957) has compiled a sizeable listing of algae capable of using organic compounds for energy or growth. The effect of growth stimulation may have been observed in this study with certain species of net plankters and in the periphyton.

Observed changes in species as a lake becomes an acidic bog may be the result of many environmental changes of which some are probably related to the increase of yellow organic acids. Patrick (1948;1954;1963) reports that certain species

of Navicula and Surirella tend to become the characteristic algal species under dystrophic conditions. Transeau (1905) reports for several Michigan bogs that "there have been marked variations within short periods of time in the color of the water and in the presence of such animals as Daphnia and Cyclops."

Though no more can be added to the understanding of why these changes have been observed in this study, it might be said that more intense study should be made of certain species which are known to show a response to acids or acid-related conditions. It appears that the causes cannot be entirely explained by large chemical and/or physical changes in the environment but more likely relate to (1) the increase in an essential nutrient or growth substance or (2) little understood or recognized physiological responses.

Implications of this study

From the scattered observations reported in this study, a relationship between acid concentration as measured by fluorescence and the character of a body of water can be seen. Oligotrophic lakes such as Titus and Lake Michigan are low in acids while the experimental ponds, Lake Mendota and other lakes measured are more eutrophic with higher acid levels. This seems a natural result as the more productive a body of water becomes, the more vegetation is produced which may in turn decompose with the release of acids. It therefore appears possible that there is a close relationship

between the process of eutrophication and the observed increase of dissolved yellow organic acids. If this is so the stage of eutrophication of a lake might be more easily determined through a simple observation of the acid concentration.

What are the possible effects of this acid buildup as eutrophication progresses? The extreme chemical and physical conditions in dystrophic lakes can be explained through the mechanisms elucidated in this study. Low metal ions and low alkalinity can be explained by the formation of the calcium-acid complex and light-induced polymerization. Low pH is explained by the high concentration of acids and little buffering action by the carbonate-bicarbonate system. Low conductivity most likely results from the fact that weak organic acids such as these being studied tend to dissociate most in dilute solution and remain largely undissociated in more concentrated solutions. Also large quantities of calcium and possibly other ions measured as conductivity may have been removed by these acids and precipitated. If these effects can be seen in dystrophic lakes, these acids must be able to exert an effect, although diminished, in waters where less acids are present.

Some algal species might be influenced in the development of blooms by these compounds since they are capable of growth stimulation. The mixing occurring after ice breaks up in the spring and after the fall overturn may bring to the

upper waters those acids contained in the concentrated layers near the bottom where they have been released. These acids may help to promote the spring and autumn growth pulses regularly observed in lakes in the temperate zones.

Stressful conditions in waters of high acid concentration may impose an added burden on the organism trying to survive in this environment. At these high acid levels, a small change in acid concentration as might happen in the diurnal cycle causes a much greater change in the chemical and physical parameters than a similar change at lower acid levels.

Although the acid concentration in a body of water is important, the rate of change in this concentration through time should be an indication of the rate of eutrophication for this water. A lake in which there is a balance between acids coming in and those lost should show more stable conditions than a lake which shows a deficit in one direction.

A method for measuring the direction and magnitude of acid change could be devised by measuring the acid concentration of a body of water several times during the year. Along with this a light and dark bottle experiment could be conducted to evaluate the rate of change in acids from different causes at different seasons. These rates would vary for different waters since the solar energy and the rate of availability of calcium carbonate would not necessarily be the same. A less involved but also less exact method would be the comparison of acid measurements taken at a standard

time of the year, such as after spring ice breakup and complete circulation, over a period of several years.

The better understanding of the dynamics of these acids in natural waters may lead to the development of better control of environmental quality. Acid increase in a lake might be halted or slowed by attacking one of two vital steps in the system--(1) interference with the release of acids from the sediments and vegetation decomposition or, more likely, (2) encouragement of light-induced polymerization by supplementing the available carbonate with fully dissolved calcium carbonate applied at the surface. It might be found that some other form of carbonate may work as well or better than calcium carbonate and would dissolve more readily.

Other interesting sidelights to this problem are the possibilities of using the calculated conductivity and pH values at a theoretical acid concentration of 0.0 ppm in the characterization of bodies of water. These values must indicate the exchange balance of ions, excluding organic acids, within a basin or drainage system and would thus be of certain limnological significance. Other conditions of the environment in the aquatic system might be found to be related to these acids once their dynamics become better understood.

SUMMARY

Fluorometric measurement of yellow organic acid concentration in natural waters can be a sensitive quantitative procedure if fluorescence is adjusted to a standard temperature and interference from such sources as detergents is minimized or taken into account. One must realize in the comparison between different bodies of water that the effect of differing chemical structure, the extent of polymerization and the action with salts on the fluorescence of the molecule has not been adequately defined.

The origin of yellow organic acids found in waters may be either allochthonous or autochthonous with the later source, from the sediments and the decay of aquatic vegetation, being the more important in the experimental ponds studied. These acids were primarily lost through a light-induced polymerization reaction with secondary loss attributed to their destruction by organisms as a source of energy or carbon. Diurnal and annual cycles were described which illustrate the integrated response of a pond to these causes of change.

The addition of yellow organic acids to natural waters produces changes in pH, conductivity, alkalinity and optical density. The first three may be explained by a hypothetical

union between these acids and calcium carbonate while optical density changes are a product of the characteristic light absorbance properties exhibited by the yellow organic acid molecule.

Following the addition of acids the growth of Navicula sp., Closterium sp., Arthrodesmus sp. and Surirella sp. appeared to be stimulated while large copepods and cladocera were adversely affected. These changes in population dominance do not appear to be caused by large chemical or physical changes of the environment but more likely by subtle physiological responses to the acids.

The concentration of these acids may indicate the stage of eutrophication. The direction and rate at which this process is moving may be easily calculated from a series of acid measurements through time. Many of the unique aspects of dystrophic lakes may be explained through the interaction of these acids with the environment. The effect of low concentrations of these acids in waters is not entirely understood at present though it is possible that many ecological relationships exist dependent upon the dynamics of yellow organic acids.

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APPENDIX

Table 1. Acid concentrations of the ponds through the experiment.

Date	Pond D		Pond C		Date average
	N	ppm	N	ppm	
7-19-1966	10	11.43	10	10.72	11.08
7-21 9 AM	4	11.46	4	10.65	11.05
7-21 3 PM	4	9.03	4	8.52	8.78
7-22	10	10.19	10	9.46	9.83
7-26 8:30 AM	4	10.80	4	10.06	10.43
7-26 9:30 AM	4	10.21	4	9.91	10.06
7-26 7:30 PM	4	10.21	4	9.62	9.91
7-28 7:30 PM	4	13.07	4	11.75	12.41
7-28 9 PM	4	12.93	4	11.75	12.34
7-29	4	11.60	4	10.87	11.24
8-2	4	11.53	4	10.65	11.09
8-5	4	10.06	4	9.25	9.66
8-9	4	10.80	4	9.69	10.25
8-12	4	10.36	4	9.33	9.84
8-16	4	10.65	4	9.55	10.10
8-19	4	10.06	4	9.40	9.73
8-23	4	10.72	4	9.62	10.17
8-26	4	9.25	4	8.45	8.85
8-30 8:30 AM	4	9.25	4	8.81	9.03
8-30 11 AM	4	9.55	4	9.25	9.40
8-30 4 PM	4	9.11	4	8.67	8.89
9-2	4	9.11	4	8.67	8.89
9-6	4	9.40	4	8.74	9.07
9-9	4	9.40	4	8.89	9.14
Pond averages		10.47		9.72	10.10
					Grand average

Table 2. Analysis of variance table for acid concentration of the ponds.

Source	Degrees of freedom	Sum of Squares	Mean sum of squares	F	Probability
Ponds	1	86.26042	86.26042	632.45	less than .01
Times	23	615.66251	26.76794	196.26	less than .01
Interaction	23	10.69040	.46480	3.408	less than .01
Error	168	22.9133	.13639		
Total	215	735.52663			

Note: Analysis was performed on the data in fluorometer units corrected to 36°C. before conversion to parts per million (3x scale).

Table 3. Acid concentration of pond D over a 25 hour period.

Time		N	ppm
8-4-1966	3 PM	4	9.62
	4 PM	4	9.62
	5 PM	4	9.55
	6 PM	4	9.47
	7 PM	4	9.47
	8 PM	4	11.09
	9 PM	4	11.46
	10 PM	4	11.60
	11 PM	4	11.53
	12 midnight	4	11.31
8-5-1966	1 AM	3	11.16
	2 AM	4	11.53
	3 AM	4	11.53
	4 AM	4	11.38
	5 AM	4	11.46
	6 AM	4	11.24
	7 AM	4	10.87
	8 AM	4	10.87
	9 AM	4	10.28
	10 AM	4	10.06
	11 AM	4	10.94
	12 noon	4	10.94
	1 PM	4	10.72
	2 PM	4	10.21
	3 PM	4	10.43
pond mean (8 PM through 3 PM only)			10.73

Note: From the start of the experiment through 7 PM a contaminated standard was in use yielding the lower values.

Table 4. Analysis of variance table for acid concentrations in pond D during 25 hour sampling, August 4 and 5.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Hours	24	152.7967	6.36652	28.77	less than .01
Error	74	16.375	.22128		
Total	98	169.1717			

Note: Analysis was performed on the data in fluorometer units corrected to 36° C. before conversion to parts per million (3x scale).

Table 5. Analysis of factors significant in the explanation of pH in the aquaria.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Prob.
Regression	3	2.58477103	0.86159034	132.7471	less than .01
Error	41	0.26610897	0.00649046		
Total	44	2.85088000			
multiple correlation coefficient $r = 0.9522$					
standard error of estimate 0.08056					
partial correlation coefficients					
			acid concentration		-0.94428
			initial pH		0.70489
			acid concentration x pH		0.93992

Table 7. Analysis of variance table for pH of the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	0.190776	0.190776	1.870	greater than .1
Times	3	12.457362	4.152454	40.705	less than .01
Dates	3	0.183281	0.061093	.599	greater than .1
Ponds x times	3	0.615719	0.205239	2.012	greater than .1
Dates x times	9	1.035038	0.115004	1.127	greater than .1
Dates x ponds	3	3.238623	1.079541	10.582	less than .01
All three factors	8	0.374275	0.046784	.459	greater than .1
Error	173	17.648414	0.102013		
Total	203	35.743488			

Table 8. Analysis of factors significant in the explanation of conductivity in the aquaria

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Regression	4	20282.5875	5070.6469	356.3411	less than .01
Error	40	569.1902	14.2298		
Total	44	20851.7777			
multiple correlation coefficient $r = 0.9863$					
standard error of estimate 3.7722					
partial correlation coefficients					
			acid concentration		-0.87009
			initial conductivity		0.87312
			pH		-0.44843
			acid concentration x conductivity		0.91680

Table 9. Conductivity of the ponds through the experiment.

Date	Pond D		Pond C		Date average
	N	$\mu\text{mhos/cm}$	N	$\mu\text{mhos/cm}$	
7-5-1966	10	121.2	10	136.6	128.9
7-8	10	172.3	10	188.4	180.35
7-12	10	161.2	10	167.4	164.3
7-15	10	154.9	10	162.2	158.55
7-19	10	151.1	10	155.1	153.1
7-21 9 AM	4	140.25	4	144.25	142.25
7-21 3 PM	4	159.0	4	167.5	163.25
7-22	10	153.3	10	160.2	156.75
7-26 8:30 AM	4	146.25	4	150.0	148.125
7-26 9:30 AM	4	152.25	4	152.5	152.375
7-26 7:30 PM	4	148.5	4	151.25	149.875
7-28 7:30 PM	4	145.0	4	139.75	142.375
7-28 9 PM	4	143.5	4	144.5	144.0
7-29	4	144.0	4	141.5	142.75
8-2	4	144.25	4	142.5	143.375
8-5	4	152.25	4	148.75	150.5
8-9	4	148.75	4	147.5	148.125
8-12	4	157.5	4	149.0	153.25
8-16	4	162.75	4	150.25	156.5
8-19	4	166.25	4	151.75	159.0
8-23	4	176.75	4	154.5	165.625
8-26	4	179.25	4	162.25	170.75
8-30 8:30 AM	4	179.75	4	163.0	171.375
8-30 11 AM	4	179.0	4	161.75	170.375
8-30 4 PM	4	174.75	4	158.75	166.75
9-2	4	188.75	4	167.75	178.25
9-6	4	184.25	4	162.75	173.50
9-9	4	184.5	4	161.0	172.75
9-13	4	187.0	4	169.0	178.0
Pond averages		158.67		157.01	157.84
					Grand average

Table 10. Analysis of variance for conductivity of the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	208.895	208.895	52.1	less than .01
Times	28	55703.496	1989.4106	496.2	less than .01
Interaction	28	11045.780	394.4921	98.4	less than .01
Error	246	986.25	4.0091		
Total	303	67944.421			

Table 11. Analysis of factors significant in the explanation of optical density in the aquaria.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Regression	7	0.15091815	0.02155974	554.9359	less than .01
Error	37	0.00143748	0.00003885		
Total	44	0.15235563			
multiple correlation coefficient $r = 0.9953$					
standard error of estimate 0.00623					
partial correlation coefficients					
			acid concentration		0.79043
			acid concentration squared		-0.91887
			acid concentration x conductivity		0.47159
			acid concentration x optical density		0.97524
			initial acid concentration		-0.38584
			initial pH		-0.35281

Table 12. Percent transmission of the pond water at 330 mμ through the study.

Date	Pond D		Pond C		Date average
	N	%	N	%	
7-5-1966	10	88.85	10	89.30	89.075
7-8	10	88.65	10	89.70	89.175
7-12	9	88.78	10	90.65	89.763
7-15	10	89.55	10	90.70	90.125
7-19	10	88.05	10	89.05	88.55
7-21 9 AM	4	88.125	4	89.50	88.812
7-21 3 PM	4	88.625	4	90.25	89.438
7-22	10	88.25	10	89.75	89.00
7-26 8:30 AM	4	87.875	4	89.00	88.438
7-26 9:30 AM	4	87.625	4	89.50	88.562
7-26 7:30 PM	4	89.125	4	90.375	89.75
7-28 7:30 PM	4	86.875	4	88.125	87.50
7-28 9 PM	4	87.50	4	88.125	87.812
7-29	4	87.375	4	88.875	88.125
8-2	4	87.875	4	88.50	88.188
8-5	4	89.625	4	90.625	90.125
8-9	3	88.667	3	88.833	88.75
8-12	3	88.667	4	89.50	89.143
8-16	4	88.25	3	89.333	88.714
8-19	4	89.875	4	90.0	89.938
8-23	4	88.625	4	90.125	89.375
8-26	4	90.0	4	90.75	90.375
8-30 8:30 AM	4	90.50	4	90.25	90.375
8-30 11 AM	4	90.375	3	89.833	90.143
8-30 4 PM	4	90.0	2	90.0	90.0
9-2	4	91.125	4	90.50	90.812
9-6	4	89.75	4	89.375	89.562
9-9	4	90.375	4	90.25	90.312
9-13	4	89.625	4	90.0	89.812
Pond averages		88.866		89.728	89.294
					Grand average

Table 13. Analysis of variance for percent transmission of the pond waters.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	54.9979	54.9979	127.93	less than .01
Times	28	179.2831	6.4030	14.89	less than .01
Interaction	28	34.8341	1.2441	2.894	less than .01
Error	238	102.3140	.4299		
Total	295	371.4291			

Table 14. Carbonate alkalinity of the ponds through the study.

Date	Pond D		Pond C		Date average
	N	ppm	N	ppm	
7-11-1966	2	18.3	2	6.6	12.45
7-14	2	18.5	2	7.9	13.2
7-18	2	19.7	2	8.6	14.15
7-21 AM	2	21.0	2	10.9	15.95
7-21 PM	2	24.1	2	13.0	18.55
7-25	2	24.3	2	11.3	17.8
7-28	2	17.7	2	10.6	14.15
8-1	2	21.3	2	12.8	17.05
8-4	2	20.9	2	14.4	17.65
8-8	2	16.3	2	13.9	15.1
8-11	2	15.5	2	13.7	14.60
8-15	2	12.5	2	13.4	12.95
8-18	2	11.2	2	13.0	12.1
8-22	2	5.6	2	8.6	7.1
8-25	2	4.2	2	10.7	7.45
8-29	2	9.8	2	17.1	13.45
9-1	2	4.0	2	9.6	6.8
9-9	2	2.0	2	7.4	4.7
9-12	2	3.0	2	8.2	5.6
Pond averages		14.20		11.14	12.32
					Grand average

Table 15. Analysis of variance of carbonate alkalinity in the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	178.27579	178.27579	221.4	less than .01
Times	18	1348.887504	74.93819	93.1	less than .01
Interaction	18	958.464075	53.248004	66.1	less than .01
Error	38	30.60	.80526		
Total	75	2516.227369			

Table 16. Bicarbonate alkalinity of the ponds through the study

Date	Pond D		Pond C		Date averages
	N	ppm	N	ppm	
7-11-1966	2	48.75	2	63.05	55.90
7-14	2	48.65	2	59.35	54.00
7-18	2	42.05	2	53.70	47.875
7-21 AM	2	39.65	2	50.35	45.00
7-21 PM	2	35.90	2	48.20	42.05
7-25	2	31.05	2	45.50	38.275
7-28	2	36.60	2	45.65	41.125
8-1	2	34.3	2	41.70	38.00
8-4	2	34.85	2	38.35	36.60
8-8	2	43.30	2	42.5	42.90
8-11	2	40.65	2	38.15	39.40
8-15	2	45.00	2	38.55	41.775
8-18	2	45.85	2	38.60	42.225
8-22	2	57.65	2	43.10	50.375
8-25	2	61.25	2	42.4	51.825
8-29	2	49.30	2	37.95	43.625
9-1	2	54.20	2	38.00	46.10
9-9	2	67.40	2	50.35	58.875
9-12	2	69.90	2	48.40	59.15
Pond averages		46.65		45.47	46.056
					Grand average

Table 17. Analysis of variance of bicarbonate alkalinity in the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	26.5265	26.5265	15.7	less than .01
Times	18	3602.1993	200.1277	118.7	less than .01
Interaction	18	2863.9660	159.1092	94.33	less than .01
Error	38	64.0950	1.6867		
Total	75	6556.7868			

Table 18. Temperature difference (pond temperature at measuring site minus temperature of pond D at water thermograph) of the ponds through the study.

Time period	N	Pond D	N	Pond C	Period average
Before first addition	78	+0.004	106	-.973	-.5587
Between first and second	9	-3.978	14	-1.779	-2.6391
Between second and third	129	-1.230	107	-.628	-.9572
After third addition	26	+0.042	43	-.977	-.5928
Pond averages		-.798		-.878	-.8404 Grand average

Table 19. Analysis of variance of temperature difference (pond temperature at measuring site minus temperature of pond D at water thermograph) of the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	.8287315	.8287315	0.49	greater than .10
Times	3	96.468104	32.156034	19.16	less than .01
Interaction	3	107.5261942	35.842065	21.36	less than .01
Error	504	845.7900763	1.678155		
Total	511	1050.613106			

Table 20. Light penetration ratios (underwater to surface) of the ponds through the study.

Time period	Pond	N	Underwater: Air meter	N	Underwater: Pyrehliometer	Period average
Before first addition	C	7	.4851	5	.5382	.5467
	D	4	.5640	4	.6475	
Between first and second	C	6	.5685	6	.5202	.5544
	D	4	.5795	4	.5598	
Between second and third	C	24	.4502	24	.4439	.4765
	D	18	.5007	16	.5377	
After third addition	C	13	.3842	13	.4767	.4215
	D	4	.3748	4	.4105	
Comparison averages			.4711		.4965	
Pond averages	C		.4619	D	.5200	
Grand average					.4835	

Table 21. Analysis of variance of light penetration ratios (underwater:surface) of the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	.12295883	.12295883	8.90	less than .01
Comparisons	1	.02512256	.02512256	1.818	greater than .10
Times	3	.31502653	.10500884	7.60	less than .01
Ponds x comparisons	1	.00293396	.00293396	0.213	greater than .10
Ponds x times	3	.03602462	.01200821	0.869	greater than .10
Times x comparisons	3	.06319568	.02106523	1.525	greater than .10
All three	3	.00895286	.00298429	0.216	greater than .10
Error	140	1.93440996	.01381721		
Total	155	2.508631			

Table 22. Mean change in ppm dissolved oxygen per hour between 10 PM and 4 AM the following morning as a measure of total respiration in the ponds during the study.

Time period	Pond C		Pond D		Period average
	N	ppm/hr	N	ppm/hr	
Before first addition	9	-.1187	7	-.1529	-.1337
Between first and second	4	-.1742	3	-.1994	-.1850
Between second and third	19	-.1341	14	-.1375	-.1356
After third addition	6	-.1484	2	-.1016	-.1367
Pond average		-.1369		-.1460	-.1406
					Grand average

Table 23. Analysis of variance of mean change in ppm dissolved oxygen per hour between 10 PM and 4 AM the following morning in the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	.001276332	.001276332	0.291	greater than .10
Times	3	.015524345	.005174781	1.180	greater than .10
Interaction	3	.007771220	.002590406	0.590	greater than .10
Error	56	.245497583	.00438389		
Total	63	.270069480			

Table 24. Ppm oxygen concentration at 10 PM as a measure of total photosynthetic oxygen production in the ponds.

Time period	Pond C		Pond D		Period mean
	N	ppm	N	ppm	
Before first addition	9	6.776	7	7.730	7.193
Between first and second	4	6.760	3	7.537	7.093
Between second and third	19	6.954	14	6.897	6.930
After third addition	6	6.738	2	6.365	6.645
Pond mean		6.857		7.154	6.978
					Grand mean

Table 25. Analysis of variance of ppm oxygen concentration at 10 PM in the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	1.362877	1.362877	1.818	greater than .10
Times	3	1.796910	.59897	.798	greater than .10
Interaction	3	3.492954	1.16432	1.553	greater than .10
Error	56	41.981553	.749670		
Total	63	48.634294			

Table 26. Metal ion concentrations in the ponds through the study.

Date	#	Pond C (ppm)			#	Pond D (ppm)			Date means		
		Ca	Na	Mg		Ca	Na	Mg	Ca	Na	Mg
7-6-1966	5	8.69	3.43	5.54	4	15.03	5.58	5.08	11.51	4.38	5.33
7-13	4	10.47	3.74	4.22	5	7.05	3.43	5.90	8.56	3.57	5.16
7-20	2	16.12	3.55	3.20	2	16.68	4.51	4.25	16.41	4.03	3.70
7-27	5	7.63	3.60	6.44	2	7.90	3.36	5.60	7.70	3.53	6.20
8-3	5	6.78	3.44	6.16	2	6.65	3.32	6.00	6.76	3.41	6.11
8-10	5	9.57	4.65	7.20	2	6.41	3.93	6.20	8.67	4.44	6.95
8-17	5	8.42	4.78	7.42	2	7.05	3.92	7.00	8.02	4.53	7.28
8-24	4	7.51	4.96	7.85	2	6.97	4.08	6.60	7.33	4.66	7.43
8-30 AM	4	12.81	4.74	7.45	4	12.09	4.45	7.02	12.45	4.60	7.24
8-30 PM	2	7.53	4.34	7.20	2	12.42	4.56	7.65	9.99	4.45	7.42
8-31	5	11.91	5.27	8.08	2	9.21	4.41	6.85	11.12	5.03	7.73
9-7	5	19.68	4.68	9.30	2	15.62	4.64	8.80	18.51	4.67	9.16
9-14	5	26.41	5.07	7.28	2	27.91	4.40	8.25	26.81	4.88	7.56
Pond means		11.9	4.36	6.89		11.4	4.23	6.43	11.7	4.31	6.72
										Grand means	

Table 27. Analysis of variance for metal ions in the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Calcium (data in % transmission from flame photometer)					
Ponds	1	39.17393	39.17393	0.208	greater than .10
Times	12	24657.40123	2054.78343	10.9	less than .01
Interaction	12	1755.36964	146.28080	0.776	greater than .10
Error	63	11879.59853	188.56505		
Total	88	38331.54333			
Sodium (data in ppm)					
Ponds	1	.3631406	.3631406	0.535	greater than .10
Times	12	24.0065407	2.000545	2.949	less than .01
Interaction	12	15.8189487	1.3182457	1.943	greater than .10
Error	63	42.733015	.6783018		
Total	88	82.9716450			
Magnesium (data in ppm)					
Ponds	1	4.282945	4.282945	4.673	greater than .05
Times	12	143.390244	11.949187	13.036	less than .01
Interaction	12	12.93734	1.078111	1.176	greater than .10
Error	63	57.747000	.916619		
Total	88	218.357529			

Table 28. Optical density data of extracted chlorophyll from periphyton from the ponds.

Date	Pond C		Pond D		Date mean
	N	O.D.	N	O.D.	
7-4 to 7-18	3	.020	4	.010	.0143
7-11 to 7-25	4	.0252	5	.0224	.0237
7-18 to 8-1	5	.0112	5	.0174	.0143
7-25 to 8-8	5	.0114	4	.0178	.0142
8-1 to 8-15	5	.0100	5	.0166	.0133
8-8 to 8-22	5	.0094	5	.0172	.0133
8-15 to 8-29	5	.0066	5	.0158	.0112
8-22 to 9-5	5	.0322	5	.0382	.0352
8-29 to 9-12	3	.0220	3	.0377	.0298

Table 29. Analysis of variance for optical density data for chlorophyll extracted from periphyton from the ponds.

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	Probability
Ponds	1	.000557926	.000557926	38.15	less than .01
Times	8	.005338558	.000667319	45.63	less than .01
Interaction	8	.000748026	.000093503	6.393	less than .01
Error	63	.000921367	.0000146248		
Total	80	.007565877			

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