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9. ABSTRACT

Lime improves conditions for plant growth, thereby providing a greater food base for fish production. Because there are few data on the effects of lime on aquatic plants, this investigation was undertaken to gather information on phytoplankton density and composition in fish ponds after liming. Fish ponds limed with dolomitic limestone increased primary production as determined by increased numbers of phytoplankters and greater phytoplankton volumes. The specific composition of phytoplankton communities was not affected obviously by liming. Favorable influences of lime resulted from one or more of the following observed changes in the pond environments: 1) increased carbon for photosynthesis following increases in bicarbonate, 2) greater availability of phosphorus added in fertilizers, and 3) increased concentrations of calcium and magnesium.

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## **Effects of Agricultural Limestone on Phytoplankton Communities of Fish Ponds\***

By CLAUDE E. BOYD and ELLEN SCARSBROOK

With 11 figures and 3 tables in the text

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### **Abstract**

Liming of fish ponds with dolomitic limestone increased primary production as determined by increased numbers of phytoplankters and greater phytoplankton volumes. Species composition of phytoplankton communities was not obviously affected by liming. Favorable influences of lime resulted from one or more of the following observed changes in the pond environments: 1) increased carbon for photosynthesis following increases in bicarbonate, 2) greater availability of phosphorus added in fertilizers, and 3) increased concentrations of calcium and magnesium.

### **Introduction**

Liming of ponds with soft waters and acid bottom muds increased fish production in central Europe (NEESS 1948), Asia (HICKLING 1962), and the United States (ZELLER & MONTGOMERY 1957, THOMASTON & ZELLER 1962). Lime will increase total hardness, total alkalinity, and pH of water (PAMATMAT 1960) and clear water of brown stain from humic substances (HASLER, BRYNILDSON & HELM 1951). Liming of acid waters increased zooplankton production (JOHNSON & HASLER 1954, STROSS, NEESS & HASLER 1961), presumably following increases in phytoplankton productivity. Neutralization of acid muds apparently liberates phosphorus for use by plants (WATERS 1958, ZELLER & MONTGOMERY 1957).

The primary action of lime is obviously to improve conditions for plant growth, thereby providing a greater food base for fish production. Since there are few data on the effects of lime on aquatic plants, this investigation was undertaken to gather information on phytoplankton density and composition in fish ponds after liming.

### **Materials and Methods**

Effect of lime on phytoplankton in ponds. — Six ponds on Piedmont soils of the Auburn University Fisheries Research Unit, Auburn, Alabama, were used in the study (Table 1). Limed ponds were in one watershed and located within a 200 m radius. One control pond, S-9, was in a second watershed but only

Table 1. Sizes of ponds, lime requirements of muds, and amounts of agricultural limestone applied.

Pond	Area (ha)	Lime requirement (kg/ha CaCO <sub>3</sub> )	Limestone applied (kg/pond)
S-11	1.12	3,696	4,140
S-12	0.89	2,688	2,932
S-13	0.85	4,704	3,998
S-19	0.69	6,496	4,482
S-9	1.44	5,040	0
S-22	0.79	2,676	0

200 m from the nearest limed ponds. The second control, S-22 was in a third watershed and about 1 km from other ponds. On 24 January 1973, mud samples were collected from ponds with an Ekman dredge and their lime requirements determined by the procedure described by ADAMS & EVANS (1962). Required amounts of agricultural limestone, finely ground dolomite, were broadcast over water surfaces of four ponds on 23 February 1973 (Table 1). Inorganic fertilizers were broadcast along shallow edges of all ponds at a rate of 45 kg/ha of 20-20-0 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) on 7 March, 21 March, 4 April, 25 April, 16 May, 20 June, 18 July, 15 August, and 12 September. Control ponds were stocked with *Micropterus salmoides* and *Lepomis macrochirus* while treated ponds contained *M. salmoides*, *L. macrochirus*, and *Pomoxis annularis*.

Water samples were collected at approximately weekly intervals between 0700 and 0800 hours with a 90-cm water column sampler (BOYD, 1973 a). Total carbonate, and bicarbonate alkalinities were determined by titration with 0.02 N sulfuric acid and soluble inorganic phosphorus was measured by the stannous chloride method (American Public Health Association, 1971). Water for total phosphorus analysis was treated with sulfuric acid and autoclaved at 2 atmospheres pressure for 30 minutes to convert organic phosphorus to soluble orthophosphate. Phytoplankton concentrated by centrifugation was counted in a Sedgwick-Rafter chamber under a microscope fitted with a Whipple disk micrometer (American Public Health Association, 1971). Beginning on 2 July, phytoplankton volume measurements were obtained (LUND & TALLING, 1957).

Laboratory studies of the influence of lime on algae. — Lime requirements were determined for muds from five ponds. Two 250 g portions of dry, pulverized mud (0.85 mm or less) were placed in plastic pans and required amounts of calcium hydroxide in 100 ml distilled water were mixed with one portion of each mud. An equal amount of distilled water was mixed with the second portion of each sample and pans covered with plastic sheets were incubated for 6 weeks at 25 C. After 6 weeks, muds were air dried and pulverized. Fifty gram portions of each limed and unlimed mud were treated at rates of 0, 10, 50, and 100 mg/kg of phosphorus by mixing with appropriate quantities of potassium dihydrogen phosphate in distilled water and storing damp for 1 week at 26 C. Covers were then removed and muds allowed to dry. Dried muds were pulverized and the wetting and incubation steps repeated twice using distilled water.

Two 0.5 g samples from each treatment of the five dried muds were weighed into individual 125-ml erlenmeyer flasks and 50 ml modified Zehnder

and Gorham No. 11 solution (Boyd, 1973 b) which contained no phosphorus were added to each flask. A 0.1-ml aliquot from a rapidly growing culture of *Scenedesmus dimorphus* was transferred to each flask. After incubation for 5 days at 25 C under a light intensity of 8,600 lux (16 hr light and 8 hr dark), the density of *S. dimorphus* in each flask was determined by enumeration of individuals per milliliter.

Responses of seven species of algae to different concentrations of calcium and magnesium were evaluated in laboratory cultures. These algae, *Coelastrum microporum*, *Chlorella pyrenoidosa*, *Selenastrum* sp., *Scenedesmus dimorphus*, *Staurastrum* sp., *Coccolithis penocystis* and *Navicula pelliculosa*, were obtained from Indiana University (STARR, 1964). Each alga was grown in modified Zehnder and Gorham No. 11 solution containing either 2.5 mg/l magnesium or 2.5 mg/l calcium. Aliquots of 0.1 ml from these cultures were inoculated into solutions (Zehnder and Gorham No. 11) containing 0 to 10 mg/l of calcium or magnesium. At each concentration, a species was inoculated into three 125-ml erlenmeyer flasks which contained 50 ml solution. Flasks were incubated at 8,600 lux (16 hr light and 8 hr dark) and 25 C for 10 days, at which time algal density was estimated by determination of optical density at 420 m $\mu$ .

### Results and Discussion

Initial concentrations of total alkalinity in the six ponds ranged from 6.3 to 16.0 mg/l (Fig. 1). Liming caused total alkalinity to increase three to four fold in S-11, S-12, and S-13 and eight to ten fold in S-19 (Fig. 1). Total alkalinity was 3 to 5 times greater in limed than in control ponds. Heavy phytoplankton growth in late spring and summer often depleted free carbon dioxide in control and limed ponds. In absence of free carbon dioxide, bicarbonate was the carbon source for photosynthesis and much higher concentrations of bicarbonate were present in limed ponds (Fig. 1).

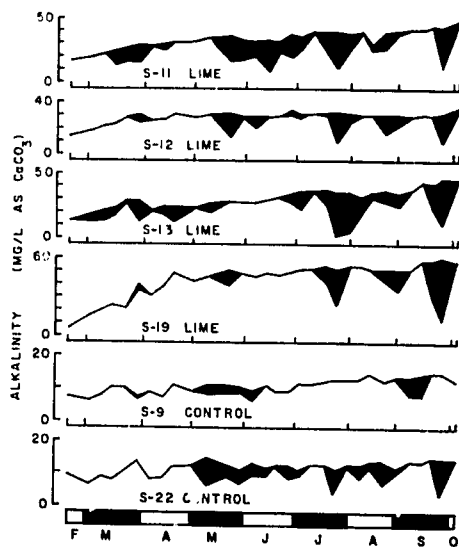


Fig. 1. Total, carbonate, and bicarbonate alkalinity in four ponds which were limed and in two control ponds. The highest points on curves represent total alkalinity, darkened areas depict carbonate alkalinity, and the difference in total and carbonate alkalinity is the bicarbonate alkalinity.

Total phosphorus concentrations were occasionally higher in limed than in control ponds during spring, but after early July concentrations of total phosphorus were usually greatest in limed ponds (Fig. 2). Concentrations of soluble inorganic phosphorus were almost always higher in limed than in control ponds (Fig. 2). Prior to liming, pH values of muds in S-11, S-12, S-13, and S-19, were 5.5, 5.7, 5.2, and 5.1, respectively. On 14 May 1973, pH values in these ponds were 6.4, 6.5, 6.6, and 7.2. HEPHER (1958, 1966) demonstrated that phosphorus added to ponds in fertilizers was ad-

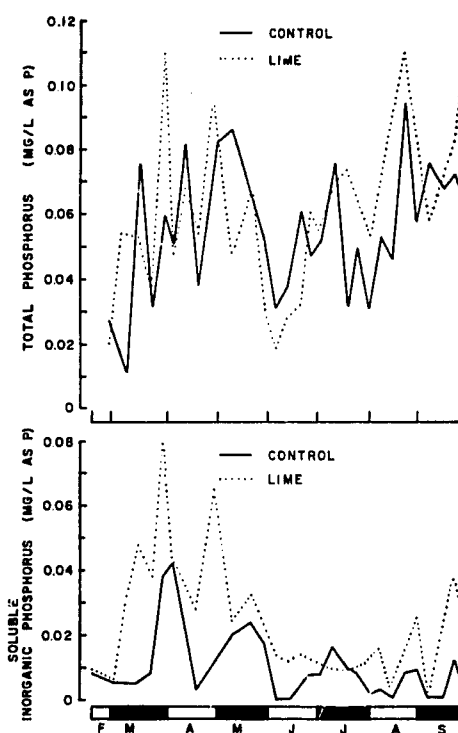


Fig. 2. Average concentrations of total and soluble inorganic phosphorus in four limed ponds and in two control ponds.

sorbed by muds and availability of phosphorus to phytoplankton was regulated by the equilibrium between phosphorus adsorbed on mud and phosphorus dissolved in water. Liming to increase mud pH apparently increased solubility of phosphorus in mud, as reflected in higher concentrations of soluble phosphorus (Fig. 2).

In bioassay experiments, additions of phosphorus to unlimed portions of muds A, D, and E resulted in little or no growth response (Fig. 3). Algal growth increased as a function of phosphorus rates for unlimed portions of muds B and C. Mud A, D, and E were more acid, pH 5.2, 4.6, and 5.3, respectively, than muds B and C, pH 5.5 and 5.6, respectively, suggesting increased pH favored phosphorus availability. The pH values of limed

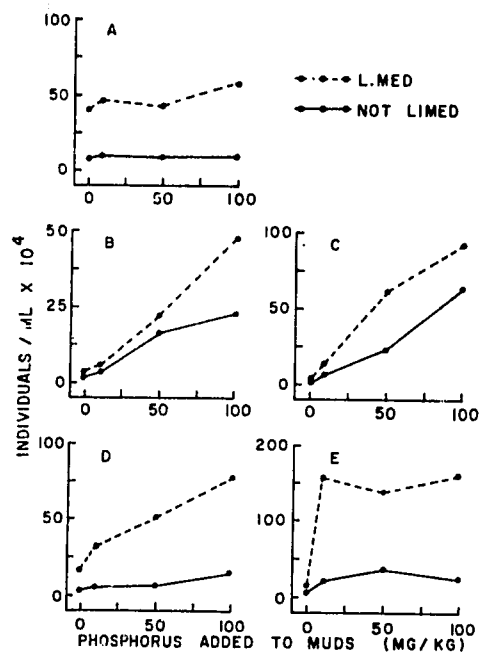


Fig. 3. Growth of *Scenedesmus dimorphus* in cultures where limed or unlimed muds served as the only sources of phosphorus. Phosphorus was added to muds at rates of 0, 10, 50, and 100 mg/kg and allowed to react prior to use of muds in cultures.

muds were in the range 6.3 to 6.6 and phosphorus availability increased as evidenced by increased growth of *Scenedesmus dimorphus*. Numbers of *S. dimorphus* increased linearly with respect to phosphorus additions to limed muds B, C, and D. Liming increased phosphorus availability in muds A and E, but enough phosphorus was released at low phosphorus addition rates to produce maximum growth of *S. dimorphus* under conditions of culture. This experiment corroborates pond studies (Fig. 2) which suggested liming increased phosphorus availability.

Data on phytoplankton density are summarized in Figure 4. A phytoplankton bloom developed earlier in S-9 than in other ponds. The second control pond, S-22, and two treated ponds, S-12 and S-19, developed blooms of phytoplankton in mid April while dense blooms were not observed until late June in S-11 and S-13. Prolific production of macrophytic algae, *Spirogyra* spp. and *Hydrodictyon reticulatum* in S-11 and S-13 from March until late June likely reduced spring growth of phytoplankton by competition. Three limed ponds S-11, S-12, and S-13, usually had greater numbers of phytoplankton than control ponds during summer and early fall, suggesting that liming increased phytoplankton growth. Smaller numbers of phytoplankton cells occurred in S-19 than in the other five ponds. Average volumes of algal cells per milliliter of water were generally two or more times greater in limed than in unlimed ponds (Table 2), also suggesting a positive influence of liming on phytoplankton production. Cell

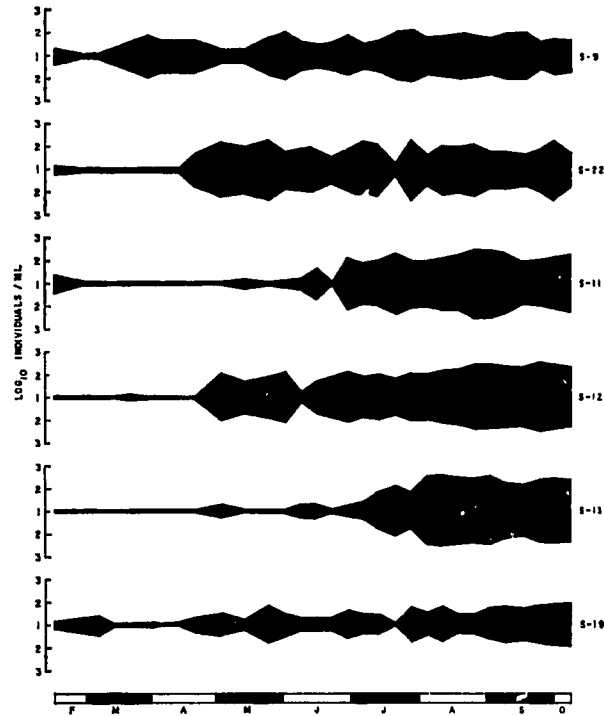


Fig. 4. Total phytoplankton counts in four limed ponds (S-11, S-12, S-13, and S-19) and in two unlimed ponds (S-9 and S-22).

Table 2. Phytoplankton volumes ( $\mu$ l/ml) in fertilized ponds which were either limed or not limed (Control).

Date	Control			Limed				Average
	S-9	S-22	Average	S-11	S-12	S-13	S-19	
7/2	0.3	12.6	6.4	3.1	2.1	0.1	0.1	1.4
7/9	0.8	1.0	0.9	0.2	3.7	0.4	0.2	1.1
7/16	3.3	1.4	2.4	2.4	3.0	2.4	0.1	2.0
7/23	5.2	10.0	7.6	32.2	4.4	0.6	1.6	9.7
7/30	1.1	1.5	1.3	0.9	3.2	13.7	0.4	4.6
8/6	2.6	2.5	2.6	1.8	3.9	17.3	0.9	6.0
8/13	3.1	1.3	2.2	3.2	4.1	10.9	0.4	4.6
8/20	2.5	3.3	2.9	4.8	8.4	9.5	0.2	5.7
8/27	0.4	0.6	0.5	4.2	7.0	11.9	0.4	5.9
9/4	3.2	0.4	1.8	1.6	4.8	2.6	0.5	2.4
9/11	1.1	0.5	0.8	0.6	4.5	2.3	0.3	1.9
9/17	0.1	1.2	0.6	1.4	7.6	7.6	0.7	4.3
9/26	0.6	1.0	0.8	3.5	3.6	9.4	1.4	4.5
10/8	0.2	0.5	0.4	3.0	1.8	7.1	0.4	3.1



volume values for S-19 were generally less than values for other ponds (Table 2). No explanation for relatively poor phytoplankton growth in S-19 is available.

The genera and species of phytoplankton observed in the ponds are listed below:

Chlorophyta. *Closteriopsis* spp., *Staurastrum* sp., *S. natator*, *Ankistrodesmus falcatus*, *Chlamydomonas* spp., *Sphaerocystis schroeteri*, *Dictyosphaerium pulchellum*, *Selenastrum* sp., *Scenedesmus quadricauda*, *S. dimorphus*, *Quadrigula chodatii*, *Volvox* sp., *Coelastrum proboscideum*, *C. microporum*, *Oocystis borgei*, *Kirchneriella* sp., *Hormidium* sp., *Actinastrum hantzschii*, *Nephroclytium agardhianum*, *Golenkinia* sp., *Pediastrum simplex*, *P. duplex*, *P. tetras*, *Pectodictyon cubicum*, *Tetraedron* sp., *Cosmarium* spp., *Treubaria* sp., *Arthrodesmus* sp., *Crucigenia* sp., *Euastrum* sp., *Gonium pectorale*, *Pandorina* sp., *Errerella bornhemiensis*, and *Bambusina* sp.

Euglenophyta. *Euglena* spp., *Trachelomonas* spp., and *Phacus* spp.

Chrysophyta. *Dinobryon* sp., *Bumilleria sicula*, *Tribonema* sp., *Mallomonas* sp., and several unidentified genera and species of diatoms.

Pyrrophyta. *Ceratium hirundinella* and *Gymnodinium* sp.

Cyanophyta. *Anabaena circinalis*, *A. flos-aquae*, *Oscillatoria* sp., *O. rubescens*, *Spirulina princeps*, *Microcystis aeruginosa*, *Aphanocapsa* sp.,

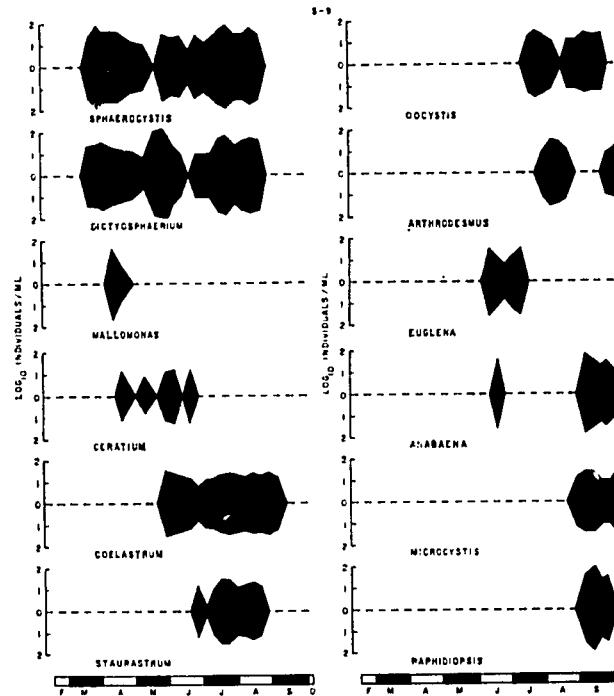


Fig. 5. Dominant phytoplankton populations in S-9, a control pond.

*Gomphosphaeria* sp., *Gloeocapsa* sp., *Raphidiopsis curvata*, and *Merismopedia tranquilla*.

Increases and declines of dominant genera in the six ponds are illustrated in Figures 5, 6, 7, 8, 9, and 10. Dominant genera or species were those comprising 10% of the total phytoplankters in a sample. There was no instance of a species maintaining dominance throughout the study. Some taxa were dominant for several months, eg., *Dictyosphaerium* and *Sphaerocystis* in S-9 (Fig. 4), while other taxa were dominant for brief periods, eg., *Anabaena* and *Oocystis* in S-11 (fig. 7). Some genera were

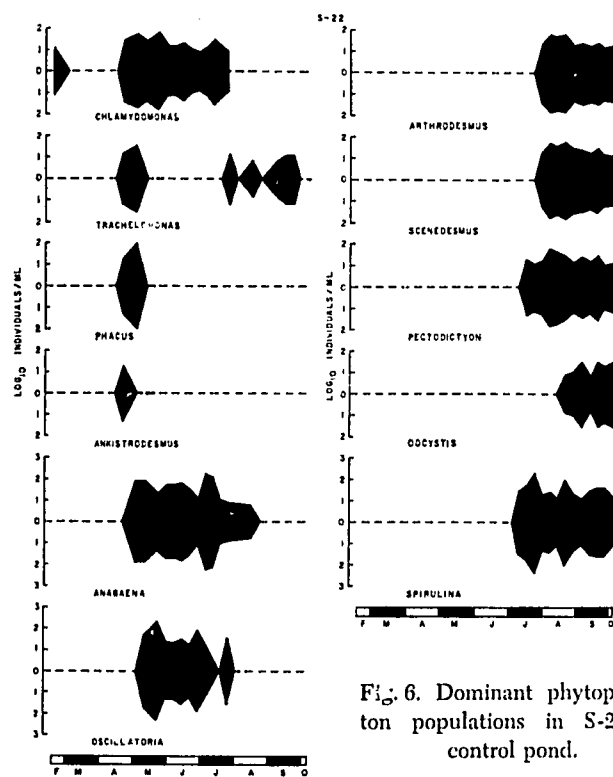


Fig. 6. Dominant phytoplankton populations in S-22, a control pond.

dominant early, disappeared from the community, and reappeared again in large numbers as with *Closteriopsis* and *Bumilleria* in S-12 (Fig. 8). Waxing and waning of populations of phytoplankton is apparently common in ponds (ROUND 1971).

Some genera were observed in three to six ponds and in both treatments: *Sphaerocystis*, *Oocystis*, *Scenedesmus*, *Oscillatoria*, *Arthrodesmus*, *Staurastrum*, *Anabaena*, *Raphidiopsis*, and *Coelastrum*. Other genera occurred in one pond of each treatment: *Trachelomonas*, *Ankistrodesmus*, *Chlamydomonas*, *Spirulina*, *Dictyosphaerium*, and *Ceratium*. The majority

of dominant algae were species of Chlorophyta, but species of Cyanophyta were important in each pond sometime during the study. The cyanophycean *Raphidiopsis curvata* was the most abundant alga in S-11 from early July until October (Fig. 7). Two cyanophyceans, *Anabaena* and *Oscillatoria*, dominated the phytoplankton of S-22 during May, June, and July (Fig. 6). Problems associated with dense populations of cyanophyceans are well known (SWINGLE 1968), but none of the cyanophyceans increased to nuisance proportions. Data from the present study reveal no effect of liming on species composition of phytoplankton communities.

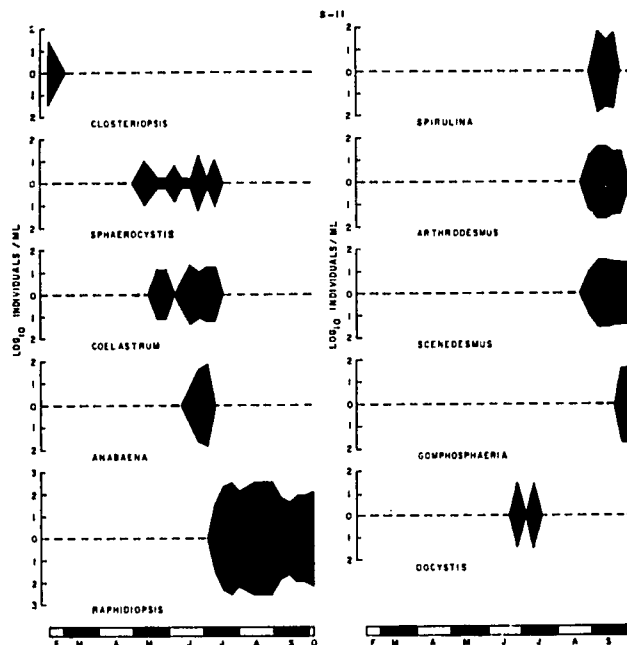


Fig. 7. Dominant phytoplankton populations in S-11, a pond which was limed.

Several influences of lime on the chemical environment conceivably resulted in increased phytoplankton growth. Agricultural limestone increased availability of carbon for photosynthesis by raising bicarbonate alkalinity. Neutralization of bottom muds increased availability of phosphorus, a key nutrient for growth. Finally, concentrations of two other essential nutrients, calcium and magnesium, increased following liming. Calcium and magnesium requirements of species of algae differ (GOLDMAN 1965), but many species require only small amounts of these two nutrients. Results from laboratory studies with seven species, some which were common in experimental ponds, indicated maximum growth at 5 mg/l or less of calcium and 2 mg/l or less of magnesium (Fig. 11). Many algae capable of

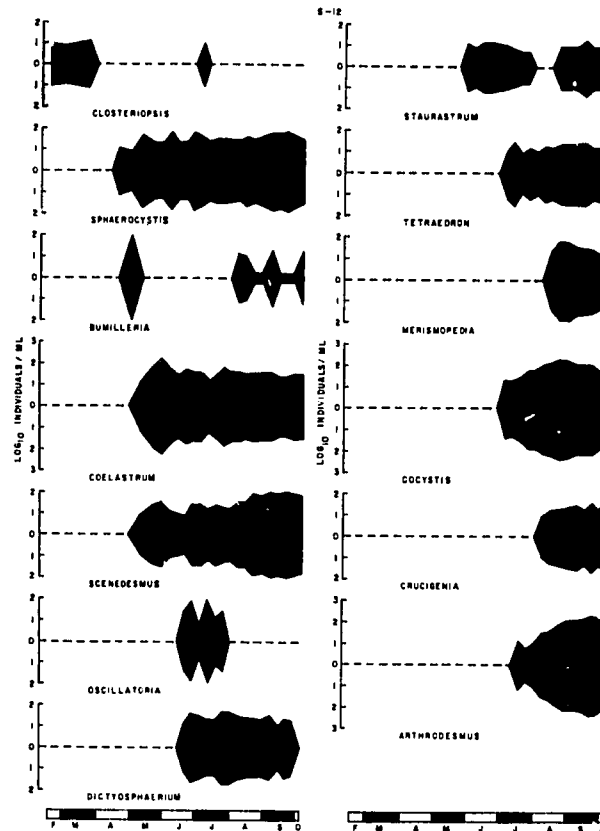


Fig. 8. Dominant phytoplankton populations in S-12, a pond which was limed.

rapid growth at these concentrations of calcium and magnesium are assumed present in most ponds. POLISINI, BOYD, & DIDGEON (1970) reported increased phytoplankton photosynthesis following additions of calcium and magnesium to a pond containing 1.09 mg/l calcium and 0.36 mg/l magnesium. Control ponds for the present study contained about 2.5 mg/l calcium and 1 mg/l magnesium (Table 3), so increases in concentrations of these ions following liming may have benefited primary productivity.

### Summary

The influence of liming on water chemistry and phytoplankton growth in fertilized fish ponds was evaluated by comparison of data from four limed ponds with data from two control ponds. Agricultural limestone caused increases in total and bicarbonate alkalinity and calcium and magnesium concentrations in water. Soluble phosphorus increased in water following neutralization of muds to about pH 6.5, reflecting greater solubility of phosphorus from muds at this pH as opposed to pH values of about 5.5 in control ponds. Bioassay experiments using limed and unlimed muds as sources of phosphorus for algae also showed

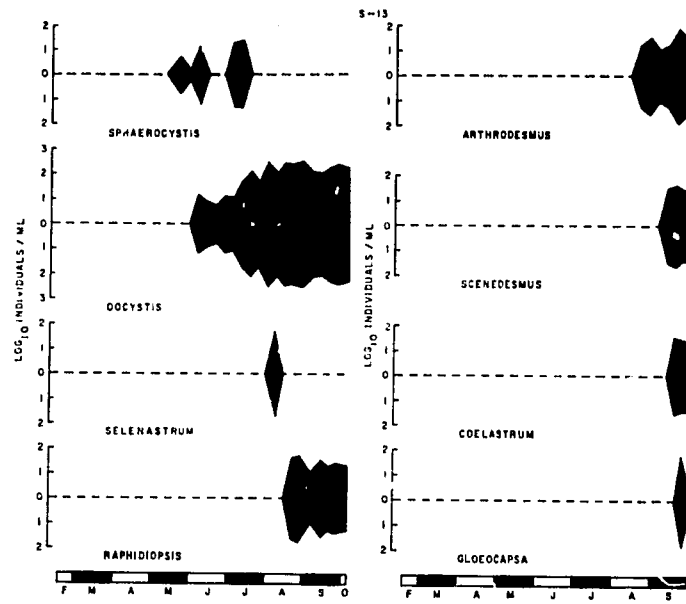


Fig. 9. Dominant phytoplankton populations in S-13, a pond which was limed.

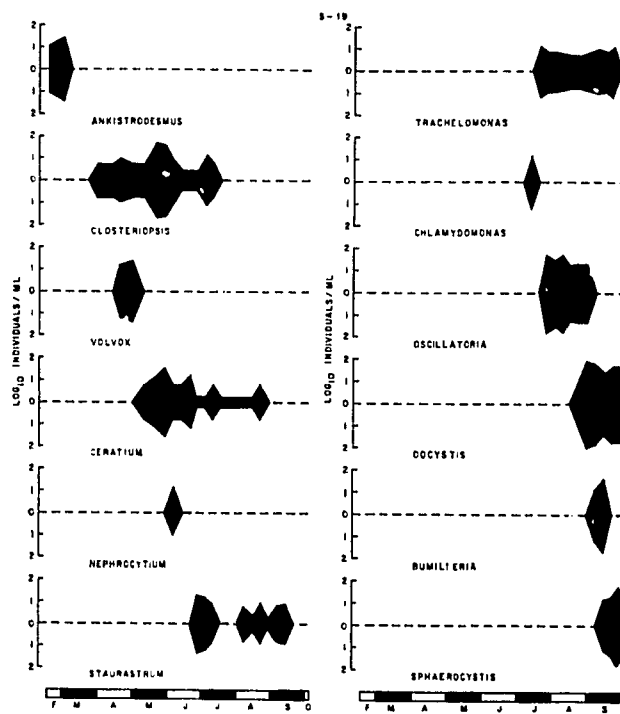


Fig. 10. Dominant phytoplankton populations in S-19, a pond which was limed.

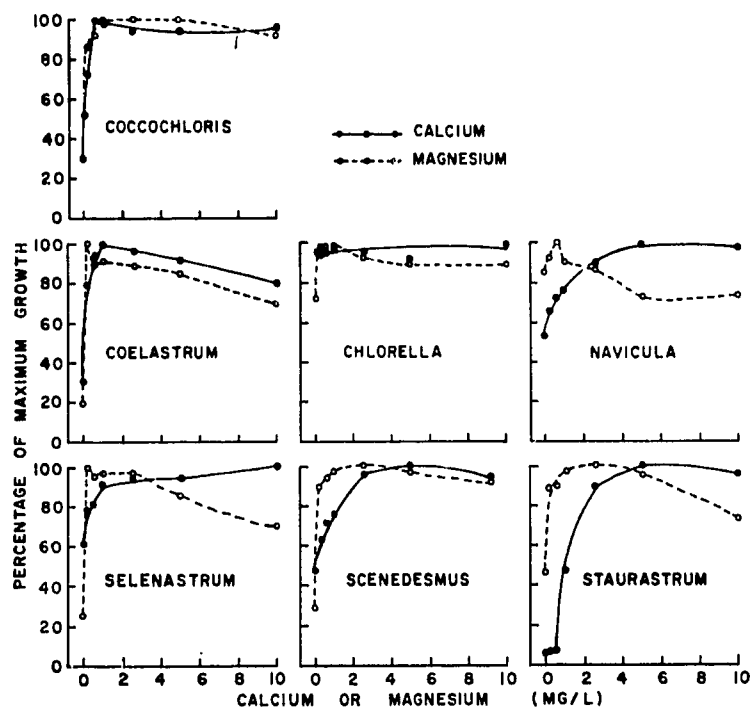


Fig. 11. Growth of different algae at various concentrations of calcium and magnesium. Maximum growth was the best growth obtained over the concentration ranges employed.

Table 3. Representative concentrations of calcium and magnesium in waters of control and limed ponds. The values are for 27 July.

	Control			Limed				
	S-9	S-22	Average	S-11	S-12	S-13	S-19	Average
Calcium (mg/l)	2.5	2.4	2.45	7.4	6.5	7.3	9.4	7.65
Magnesium (mg/l)	0.8	1.1	0.95	3.1	2.4	3.2	6.2	3.73

that phosphorus availability increased following liming. Phytoplankton density as estimated from counts of total individuals and phytoplankton volume values was greater in limed than in control ponds. Liming had no influence on species composition of phytoplankton.

Favorable influences of lime resulted from one or more of the following changes in the environment: 1) increased carbon for photosynthesis following increases in bicarbonate, 2) greater availability of phosphorus added in fertilizers, and 3) increased concentrations of calcium and magnesium.

### Zusammenfassung

Der Einfluß einer Kalkung auf die Wasserchemie und das Phytoplankton-Wachstum in gedüngten Fischteichen wurde durch vergleichende Untersuchung von vier gekalkten Teichen und zwei ungekalkt gebliebenen Kontrollteichen festgestellt. Landwirtschaftlicher Kalk verursachte im Wasser eine Zunahme der Hydrogencarbonat-Alkalinität und auch des Calcium- und Magnesium-Gehaltes. Nach Kalkbehandlung des Schlammes bis zu einem pH-Wert von 6,5 nimmt die Menge an gelöstem Phosphat zu, im Gegensatz zu der stärkeren Phosphatbindung in den Kontrollteichen mit pH-Werten um 5,5. Experimentaluntersuchungen mit gekalkten und ungekalkten Schlämmen als Phosphorlieferanten für Algen zeigten gleichfalls, daß nach erfolgter Kalkung mehr Phosphor verfügbar war. Die Phytoplankton-Dichte, aufgrund von Zählungen aller Individuen, und die Werte des Phytoplankton-Volumens waren in den gekalkten Teichen größer als in den Kontrollgewässern. Jedoch übte die Kalkung keinen Einfluß auf die Artenzusammensetzung des Phytoplanktons aus.

Günstige Einwirkungen des Kalkes basierten auf einer oder mehreren der folgenden Milieu-Änderungen: 1. Größere Kohlenstoffmengen für die Photosynthese aufgrund der Hydrogencarbonat-Zunahme. — 2. Größere Verfügbarkeit von Phosphat, das mit den Düngern zugefügt wurde. — 3. Größere Konzentrationen an Calcium und Magnesium.

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