THE REGULATION OF BLUE-GREEN ALGAE BY IRON AVAILABILITY AND CALCITE PRECIPITATION

by

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ABSTRACT

The primary objective of this research was to determine if changes in iron availability influence the periodicity of blue-green algal growth. A secondary goal was to resolve how iron availability was related to events such as calcite (calcium carbonate) precipitation and sediment nutrient release.

The biogeochemical regulation of blue-green algal succession was studied in three eutrophic hardwater lakes located upon the Thompson Plateau in south-central British Columbia. The experimental approaches included *in situ* bottle and limnocorral experiments, sediment core analysis, monitoring of seasonal changes in water chemistry, and whole-lake manipulation by hypolimnetic aeration, or calcium hydroxide addition. Growth and primary production bioassays were used to evaluate iron availability. Microbial chelators were isolated from algal cultures and lake water, quantified by a chelation assay, and used to determine their *in situ* effects on algal productivity and bacterial heterotrophy.

Microbes were able to regulate the bioavailability of iron. Algal siderophore isolates were rapidly assimilated in lake water and they were highly specific for iron chelation. Moreover, chelator concentrations in Black Lake usually exceeded the dissolved iron concentration. Algae excreted chelators that could suppress growth of some other species of algae by 90%, enhance the primary production of some other algal species by 30%, or suppress the heterotrophic activity of bacteria by 14-98%.

The degree of iron limitation varied greatly during the summer. In Black Lake, iron limitation was more than ten-fold

more intense in early summer than in late summer. Dense blooms of blue-green algae occurred in Black Lake only after the iron content of the lake increased from 20 to more than 100 ug/L. An increase in iron concentration in the water column of the three lakes was caused by a midsummer sediment release of iron.

Although sediment pyrite formation converted available iron into refractory iron in both Chain and Frisken lakes, the degree of iron limitation varied greatly among the lakes. Unlike in Black Lake, the algae in Chain Lake were not limited by iron availability. Phosphorus solubility was a good index of iron availability. Black and Frisken lakes had too little iron for iron phosphate to precipitate, but the higher iron concentration in Chain Lake regulated phosphorus solubility. The differences among lakes was primarily a function of external iron loading, not sediment iron release. Chain Lake received 10^3 more iron per m^2 than Frisken or Black lakes.

Carbonate equilibria integrated the microbial responses to iron enrichment. When iron availability was increased in the epilimnion of Black Lake, algal productivity was enhanced which resulted in an increase in pH and the coprecipitation of more calcite and phosphorus than in control treatments. The precipitation of calcite could sediment as much as 90% of the algae and 97% of the phosphorus from the epilimnion. The hypolimnia of the iron-enriched limnocorrals had the lowest pH and highest dissolution of precipitated phosphorus.

Three reactions, iron chelation, sediment iron release, and calcite precipitation, can regulate much of the periodicity of blue-green algal growth in hardwater lakes.

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Glossary

Andesite, a volcanic rock composed of andesine and a variety of magnesium silicates.

Allelopathy, the suppression of the growth or occurrence of another plant or microbe by excreted chemicals.

Apatite, a phosphorus containing mineral group containing fluorapatite, $Ca_5(PO4)_3F$, or hydroxylapatite $Ca_5(PO_4)_3(OH)$. The principal mineral is a carbonate containing variety of fluorapatite.

Axenic, without bacteria.

BOD, biochemical oxygen demand.

Calcite, a mineral, calcium carbonate, $CaCO_3$. Hexagonal-rhombohedral crystals.

Chl a, chlorophyll a, a photosynthetic pigment.

Chlorite, an iron containing clay mineral.

EDAX, energy dispersive analysis for x-rays.

EDTA, ethylenediamine tetraacetic acid, a chelating agent.

Epilimnion, the surface layer of a stratified lake.

FeBC, iron binding capacity, the ability of organic matter to maintain iron in solution.

Fibril, electron-opaque, colloidal size particles that often form a mucilaginous sheath around certain algae.

Heterotrophy, the microbial utilization of organic matter for energy and growth.

Hydroxamate, a functional group of a powerful group of siderophores, containing two reactive oxygen atoms that are bound to adjacent carbon and nitrogen atoms.

Hypolimnion, the bottom layer of a stratified lake.

Limnocorrals, large enclosures that isolate a portion of a lake.

PCS, a xylene based scintillation fluor produced by Amersham.

Picoplankton, microscopic unicellular algae that are less than 2.0 μ in size.

Pyrite, a mineral FeS_2, that forms in anoxic environments.

Sephadex, a modified dextran. The dextran macromolecules are crosslinked to give a three-dimensional network; the various size pores retard the elution of molecules as a function of size.

SRP, soluble reactive phosphorus.

Siderophore, a low molecular weight organic compound (500-daltons) that has a high specificity for strongly chelating ferric iron and that is produced by microbes subjected to iron deficiency to assist the solubilization and assimilation of iron.

1 INTRODUCTION

The primary goal of this research was to determine if changes in iron availability influence the periodicity of blue-green algal growth and regulate the blue-green algal dominance over other algae. A secondary objective was to resolve if events such as calcite precipitation, and sediment nutrient release, change iron availability.

The periodicity of algal growth in temperate lakes often follows very similar annual cycles (Lund et al. 1963). A typical species progression, in temperate lakes that stratify in summer, is from spring diatoms to summer blue-green algae. The seasonal periodicity in the growth of phytoplankton species is driven by a mixture of biological processes and physical perturbations (Trimbee and Harris 1984) that can lead to periodic changes in nutrient availability (Lund et al. 1975). However, the factors that regulate algal succession and the reactions that determine the timing of the periodicity are incompletely understood (Hutchinson 1967, Reynolds 1982).

1.1 Factors Regulating the Periodicity of Algal Growth

Water column stability plays a major role in the determination of phytoplankton periodicity. Reynolds et al. (1982) have shown that sedimentation of diatoms in stratified water is a major factor in the suppression of diatom growth in summer. Blue-green algae possess gas vacuoles that enable them to regulate their vertical distribution (Walsby 1977); thus, they may have a competitive advantage in a stratified lake.

Buoyancy control provides blue-green algae some regulation of exposure to light, temperature, and nutrients (Ganf and Oliver 1982). Although the response of species is variable, blue-green algae are generally enhanced by high illumination and warm water; whereas, diatoms are more tolerant of high turbidity and cool water (Hutchinson 1967). Apparently, in some lakes, the vertical movement mediated by gas vacuoles can allow blue-green algae to move into nutrient rich deep water and return to the euphotic zone (Ganf and Oliver 1982).

The suppression of diatom growth is also related to changing nutrient availability. Diatoms require much more silicon than other algae, and silicon availability often limits diatom growth (Lund et al. 1975, Hurley et al. 1985). After the vernal utilization of nitrate by diatoms, nitrogen fixing blue-green algae often replace diatoms which cannot utilize atmospheric nitrogen (Wetzel 1975). Declining concentrations of other nutrients such as phosphorus or carbon dioxide can also enhance blue-green algal dominance (Shapiro 1973). Blue-green algae usually start growing when the concentration of inorganic nutrients is minimal (Hutchinson 1967).

Blue-green algae are less likely to be eaten than are other algae. The large size of blue-green algal trichomes limits zooplankton grazing (Ferguson et al. 1982); however, more complex factors are involved. Trophic level structure such as the density of predatory fish can profoundly alter the amount of zooplankton algal grazing (Shapiro 1980). Lynch and Shapiro (1981) have shown that the relationship between Aphanizomenon and Daphnia is a complex symbiotic relationship. Furthermore, blue-green algae can

excrete organic compounds that suppress zooplankton grazing (Gentile and Maloney 1969; Lampert 1982; Porter 1973, 1977; Porter and Orcutt 1980; Snell 1980).

The ability of blue-green algae to suppress other algae by the excretion of toxic compounds is another method of reducing competition (Fogg et al. 1973, Hellebust 1974, Elbrachter 1976, Keating 1978, Kayser 1979, Wilson et al. 1979, Wolfe and Rice 1979, Chan et al. 1980). Natural waters are strikingly inhibitory when collected from areas rich in algae (Hutchinson 1967). The allelopathic reactions that occur in blue-green algal blooms contribute to the low-species diversity of the algal blooms (Hutchinson 1967).

Blue-green algae are also able to suppress bacterial heterotrophy (Chrost 1973, 1975; Delucca and McCracken 1977, Reichardt 1981). Chrost (1975) found that the inhibitory agent which blue-green algae excreted was more active in light. He proposed that once algae settle from the euphotic zone, bacteria would then rapidly decompose algae and enhance oxygen depletion in the dark hypolimnia. In contrast to the epilimnion, the hypolimnion does not have a renewable supply of oxygen.

At first, iron solubility is increased as the hypolimnion becomes anoxic. However, as the hypolimnion becomes more anoxic, sulphate reduction forms sulphide which precipitates ferrous sulphides (Banoub 1977). An enhanced hypolimnetic consumption of oxygen can result in anoxia, and the formation of pyrite (FeS_2) which is a stable mineral in anoxic environments (Berner 1971). In environments with little available iron and rapid pyrite formation, a reaction that displaces heterotrophy from the

epilimnion to the hypolimnion and enhances the seasonal development of anoxia would reduce the availability of iron in the hypolimnion.

The timing of anoxia development will directly influence blue-green algal succession. The periodicity of blue-green algal growth is often linked to the recruitment of algal cells from lake sediments (Trimbee and Harris 1984). In general, Aphanizomenon recruitment is favoured when the bottom water is oxygenated (Lynch 1980, Lynch and Shapiro 1981, Trimbee and Harris 1984). Other blue-green algae are usually stimulated when the bottom water approaches anoxia (Reynolds et al. 1981, Trimbee and Harris 1984). Lund and Reynolds (1982) suggest that Microcystis propagules are not stimulated by an increase in nutrients, but respond to changes in lake dynamics which permit the formation of an anoxic hypolimnion.

The acceleration of algal succession when a lake becomes stratified could be related to changes in the availability of iron. At first, iron becomes more soluble when hypolimnia become anoxic (Stumm and Morgan 1981). The following section discusses why blue-green algal response to a changing supply of iron should be considerable.

1.2 Iron-Requiring Reactions of Blue-Green Algae

The ecology of blue-green algal blooms is complex and much of the complexity is related to reactions involving iron (Fig. 1). As early as 1937, Guseva (1937, 1939) stated that iron was the controlling biogenic element for Anabaena and Aphanizomenon. More recently, Clasen and Berhardt (1974) observed that blue-

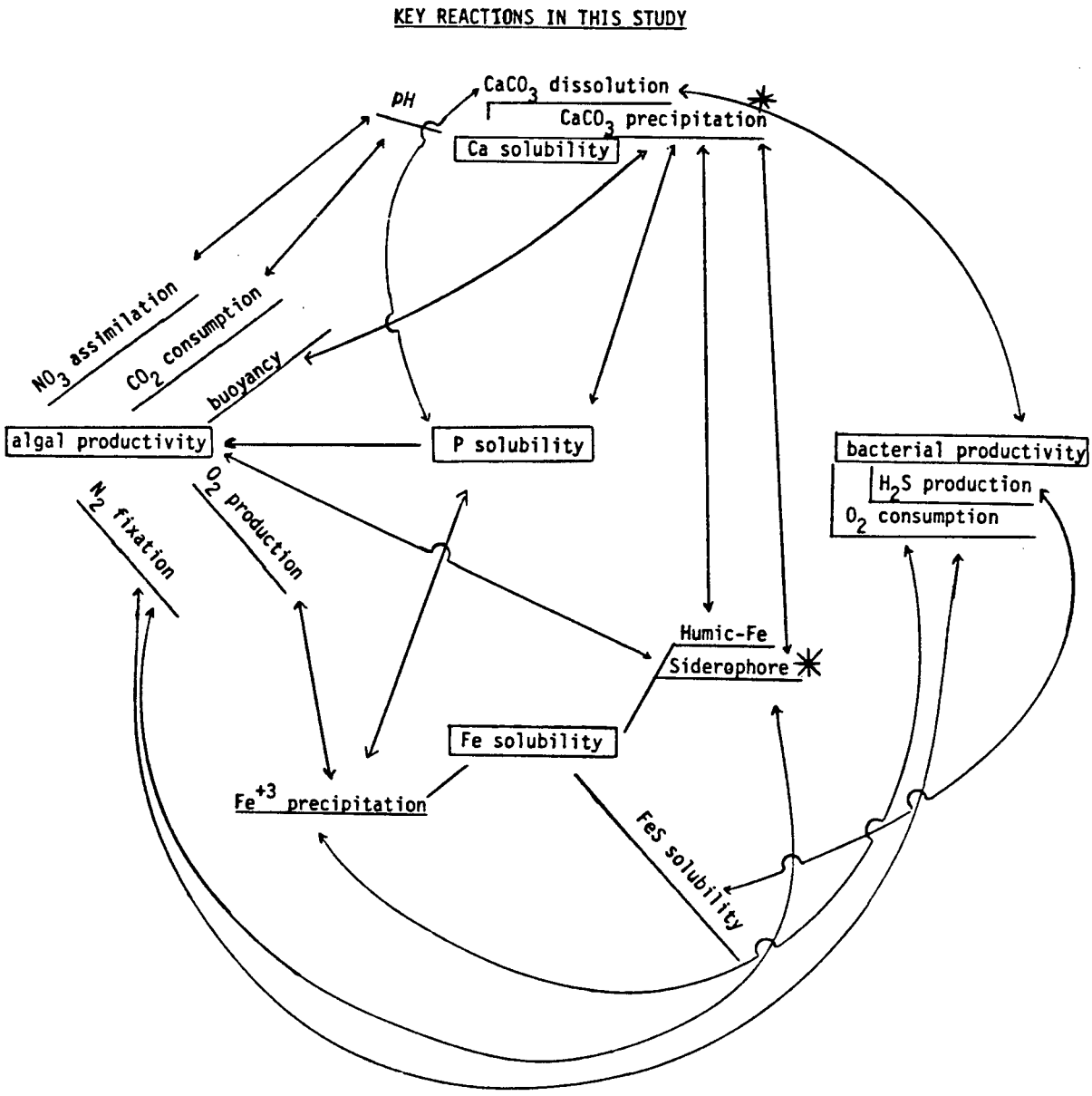


Figure 1 Key reactions in this study. Lines denote a reaction influencing another process. * denotes a key reaction.

green algae responded to iron enrichment with more growth than green algae. At least two laboratory studies have shown that blue-green algae require more iron than green algae (Soeder et al. 1971, Morton and Lee 1974). These observations are supported by the following studies demonstrating that iron-requiring reactions are important in blue-green algae.

The enzymes required for nitrogen fixation contain iron (White et al. 1968); therefore, when blue-green algae fix nitrogen they require additional iron (Carnaham and Castle 1958, Stewart 1969, Murphy 1976). Simpson and Neilands (1976) found that an iron-deficient Anabaena grew poorly when it had to fix nitrogen. Wurtsbaugh and Horne (1983) found that iron addition into Clear Lake stimulated nitrogen fixation by as much as 500%.

The ability of blue-green algae to recover from periods of nitrogen limitation is often dependent upon nitrogen fixation (Murphy and Brownlee 1981, Brownlee and Murphy 1983). Healey and Hendzel (1976) have reported that deficiency of an unknown nutrient, not phosphorus, was responsible for the periodic inability of Aphanizomenon to fix nitrogen, and the bloom collapse. Lean et al. (1978) suggested that a lack of iron may have been the factor that restricted nitrogen fixation of Anabaena blooms. Blue-green algae are the only algae which can fix nitrogen. Thus, a requirement for iron may make iron chelation more of a key reaction in blue-green algal blooms than in other types of algal blooms.

This hypothesis can be supported by marine studies. Stewart (1969) has proposed that the requirement for additional iron for nitrogen fixation and the general lack of iron in the oceans

(Ryther and Kramer 1962, Smayda 1969, Matsunaga et al. 1984, Rao and Yeats 1984) are responsible for the relative paucity of planktonic heterocyst containing blue-green algae in marine environments.

The requirement for iron in the repair of photooxidative damage also seems important. The iron-containing enzymes (catalase, peroxidase, and superoxidase dismutase) are required to repair photooxidative damage (White et al. 1968, Neilands 1981b). Catalases are known to become dramatically reduced in iron-deficient bacteria before growth is affected in a low light environment (Price 1968); these cells would be vulnerable to photooxidation. Lange (1974) observed that iron-deficient blue-green algae were killed by direct sunlight within three hours, but that iron-enriched algae were still healthy after 90 hours of exposure to direct sunlight. Photooxidative death of blue-green algae has often been proposed as a mechanism of bloom collapse (Abeliovich and Shilo 1972, Eloff et al. 1976, Coulombe and Robinson 1981, Eloff et al. 1976).

If blue-green algae are more susceptible to photooxidation than are other algae, the sensitivity could be a result of an impaired buoyancy control. Blue-green algae are the only algae with gas vacuoles. A reduced ability to regulate gas vacuoles would lead to the formation of surface scums and exposure to full sunlight. A lack of iron would slow respiratory reactions requiring iron (cytochromes, Lankford 1973) and respiration is required to regulate gas vacuoles (Walsby 1977). Thus, iron-requiring enzymes that prevent photooxidation (catalases, peroxidases, and superoxide dismutase) may be more important in

blue-green algal blooms than in other algal blooms.

Bacteriophages have also been observed in dying blue-green algal blooms (Coulombe and Robinson 1981). Although other factors influence viral attacks, they should occur more often during periods of iron limitation. Bacteriophages enter bacteria via the same proteins that siderophores use to enter the cell (Neilands 1981b). These proteins are produced in higher concentrations during iron limitation to enhance the assimilation of siderophores (Neilands 1982). This aspect of iron limitation is apparently unresolved in lakes.

Several observations that blue-green algae produce siderophores (Smayda 1974, Murphy 1976, Murphy et al. 1976, Simpson and Neilands 1976, Armstrong and Van Baalen 1979, Bailey and Taub 1980, McKnight and Morel 1980) and the rare production of siderophores by other algal divisions (Trick et al. 1983a, 1983b) indicate that blue-green algae have a large iron requirement. Siderophores are excreted by many prokaryotes when the supply of iron is limited (Neilands 1966, 1967, 1972, 1973; Lankford 1973; Emery 1982). Siderophores are low molecular weight organic compounds that selectively and strongly chelate iron; they either readily exchange the chelated iron with protein receptors sites on microbial cells or they enter the cell before releasing the iron.

Only a few studies have used rigorous chemical analysis to identify algal siderophores. Simpson and Neilands (1976) were fortunate in that the Anabaena species they studied produced a compound (Schizokinen) that earlier studies in their laboratory had isolated from bacteria. Trick and coworkers (1983b) found

hydroxamate type siderophores in isolates from marine algae. Other studies have relied upon bioassays, radiochemical assays, or chemical analysis of functional groups to detect siderophores.

In a laboratory, Murphy (1976) used the latter combination to study siderophores. Sephadex chromatography with 55Fe was able to detect, partially purify, and determine the approximate molecular weight of chelators. Compounds with no chelation such as glycine could not transmit iron through a Sephadex column, but a chelator such as citrate could. Chemical analysis indicated that the chelators excreted by Scenedesmus basiliensis and Anabaena cylindrica were cyclic peptides; these compounds had molecular weights around 300 daltons. Anabaena flos-aquae produced a chelator with a molecular weight of about 900 daltons that contained a hydroxamate group. The chelator from Anabaena flos-aquae formed a brick red complex when saturated with iron. The chelators from Anabaena flos-aquae, Anabaena cylindrica and Scenedesmus basiliensis were further studied in this thesis.

The hydroxamate production by Anabaena flos-aquae was radically different from the hydroxamate production in Trick's study (Trick et al. 1983a). Murphy (1976) observed that the concentration of excreted hydroxamate increased quickly up to about ten days, slowly increased for another ten days and then remained constant. Trick et al. observed that hydroxamate was excreted in a pulse and stayed in solution for only a few days.

The selective advantage that blue-green algae have over green algae in high-pH water (Shapiro 1984) may be related directly to iron assimilation. Iron is less soluble in high-pH water (Stumm and Morgan 1981); thus, more complex iron-

assimilation mechanisms may be required for growth in high pH water.

1.2.1 Iron Limitation

Iron limitation often has been observed in lakes without calcium carbonate precipitation (Goldman 1966, Bernhardt et al. 1971, Sakamoto 1971, Allen 1972, Clasen and Bernhardt 1974, Gerhold 1975, Lund et al. 1975, Thurlow et al. 1975, Happey-Wood and Pentecost 1981, Lin and Schelske 1981, Paerl 1982a). Complexation of iron with humic matter is the best documented reaction mediating iron limitation in softwater (Jackson and Hecky 1980). Although the reactivity and availability of humic iron varies greatly among lakes, in many lakes, most of the humic iron is unreactive (Shapiro 1969). Recent studies have shown that iron is rapidly coated by humic acids (Picard and Felbeck 1976, Tipping 1981, Baccini et al. 1982); presumably in some lakes, the coatings of humic acids reduce the reactivity and availability of iron.

The availability of humic iron is difficult to measure. Although a chemical assay, the ferrigram, can determine the reactivity and availability of humic iron in some lakes (Shapiro 1969), it is unlikely that any chemical fractionation can resolve the complexities of all humic-iron chemistry. For example, Powell et al. (1980) and Akers (1983) discovered a variety of siderophores in soil extracts. Previously, these siderophore isolations would have been called fulvic or humic acids.

The reactions of siderophores can be complex. The availability of siderophore iron can be restricted to only some species (O'Brien and Gibson 1970). Some siderophores have

antibiotic properties; they mimic iron-sequestering siderophores (Neilands 1981a). The iron biochemistry of lakes should be as complex as soil iron biochemistry.

Hardwater lakes that precipitate calcium carbonate have often been cited as being iron limited (Schelske 1961, 1962; Schelske et al. 1963; Wetzel 1965, 1966, 1968; Lange 1971; Horne 1974; Murphy and Lean 1975; Murphy et al. 1976; Elder 1977; Elder and Horne 1977; Wurtsbaugh and Horne 1983). The relationship between calcium carbonate precipitation and iron limitation is expected due to chemical and biochemical reasons.

Calcium carbonate precipitation can interfere with iron uptake. Iron is less soluble at the high pH found before and during calcium carbonate precipitation (Stumm and Morgan 1981). Also, the coprecipitation of dissolved organic matter with calcium carbonate will reduce the solubility of iron in the euphotic zone (Wetzel 1975).

Szaniszlo et al. (1981) found that the addition of calcium carbonate to culture medium enhanced the production of siderophores, but that calcium concentration alone had no effect upon siderophore production. Other studies have indicated that some siderophores have an appreciable affinity for chelation of calcium (Hider et al. 1982). Perhaps calcium or calcium carbonate can suppress some limnetic iron-siderophore reactions. Calcium is often concentrated on the surface of aquatic bacteria by excretion from microbial cells (Morita 1980). The surface of blue-green algae is often composed of microenvironments created by a covering of fibrils (Leppard et al. 1977; Leppard 1984a, 1984b). Thus, the concentration of calcium at the sites of iron

uptake could be much higher than bulk water chemistry would suggest. A suppression by calcium of iron assimilation would be much stronger in hardwater lakes than in softwater lakes.

Calcium carbonate precipitation is closely linked to iron biogeochemistry via algal productivity. Any enhancement of algal productivity would increase the pH and carbonate concentration of the epilimnion. Thus, increased iron availability in an iron-limited lake should enhance algal productivity and could result in precipitation of calcium carbonate. Small changes in carbonate equilibria could influence several limnetic processes that are not directly related to iron chemistry (Fig. 1). Calcium carbonate precipitation can precipitate algae (Rossknecht 1980) and phosphorus (Murphy et al. 1983) from the epilimnion. These two processes can suppress the concentration of algae in hardwater lakes in summer (Stauffer 1985). The induction of carbonate precipitation may be the major limnetic reaction that modifies the effects of a change in iron availability.

Stauffer (1985) has also proposed that a lack of mobile iron in calcareous areas results in too little iron to control phosphorus chemistry. In other areas that are rich in iron, geochemical phosphorus reactions are controlled by iron reactions (Bostrum 1984, Ryding 1985). As well as this spatial variation in the control of phosphorus solubility and of iron availability, there may be a temporal variation in iron availability. Stumm and Morgan (1981) proposed that the degree of iron limitation should vary among lakes and that iron availability should influence the timing of an algal bloom, not the biomass.

The temporal variation in iron limitation was demonstrated

in a study by Murphy (1976). In the Bay of Quinte, iron limitation that was detected by rapid iron uptake and the presence of siderophores, lasted for less than a week. However, the iron limitation that occurred during a blue-green algal bloom was followed by a four week period of reduced algal biomass.

Seasonal changes in iron geochemistry and the periodicity of blue-green algae are both well known, but the processes are usually linked to phosphorus availability. Iron is stable in oxidized sediments (Williams et al. 1971, Shukla et al. 1971). However, iron and phosphorus are often released from lake sediments when the oxygen concentration is depleted. Much more is known about the seasonal release of phosphorus from lake sediments than about iron release; however, in several studies iron and phosphorus were released in synchrony from the sediments (Mortimer 1941, 1942; Banoub 1977; Lijklema 1977). In some lakes, the solubility of phosphorus has been shown to be controlled by the precipitation of ferric phosphate (Birch 1976). In extreme anoxia, ferric sulphide is precipitated leaving phosphorus in solution (Banoub 1977).

Ryding (1985) has proposed that organic iron chelators may enhance the solubility of phosphorus by complexing iron and thus preventing the precipitation of ferric phosphate. Thus, the geochemistry of phosphorus may be an excellent signal of iron availability. Little is known about the coupling of iron release from lake sediments and the availability of iron as a micronutrient. To resolve the impact of sediment iron release from seasonal changes in phosphorus availability would require a lake with high phosphorus concentrations to saturate biological

requirements.

The goals of this study were to determine if the seasonal changes in blue-green algal growth were related to changes in iron availability and to resolve if the blue-green algal dominance over other microbes could be mediated by a siderophore regulation of iron availability. To achieve this objective, field studies were conducted in three hypertrophic lakes that had high phosphorus concentrations. The seasonal changes in water chemistry and phytoplankton periodicity were studied.

A series of enclosure experiments were conducted to assist the interpretation of limnetic observations. Short-term bottle incubations were used for primary production and heterotrophy assays. For long-term incubations, larger enclosures (limnocorrals) were used to avoid containment artifacts. Long-term incubations in small enclosures are prone to several artifacts. The high surface area may stimulate mineral precipitation and growth of species that are not common in the open water. Moreover, the isolation of the water prevents gas exchange which in turn can lead to unnatural changes in pH. Limnocorrals also allowed observation of algal succession.

Large enclosures can still have enhanced mineral precipitation on the walls. Thus, to confirm the long-term responses, whole-lake manipulations were also conducted. The interpretation of the long-term responses to iron enrichment required an evaluation of calcium carbonate precipitation. My studies were integrated with detailed laboratory bioassay and chemical studies where specific interactions could be investigated under more controlled conditions.

2 METHODS

2.1 Study Area

The Thompson Plateau is located in south-central British Columbia (Fig. 2). This area has a temperate continental climate. The plateau is in the rainshadow of the Cascade Mountains which border the plateau on the west. Rainfall varies from 75 cm on the highlands to less than 35 cm in the semiarid valleys (MacMillan-Bloedel 1972). Two stations at Hedley, 48 km west of Black Lake, receive a mean of 29 cm or 54 cm of rain a year at elevations of 425 m and 1,738 m, respectively (Environ. Can.). More rain and less evaporation at higher elevations results in runoff that supplies much of the water requirements of the valleys.

Black, Chain, and Frisken lakes were the main study sites (Fig. 2, Table 1). These lakes all had blue-green algal blooms, high phosphorus concentrations, and fish kills. The water flow in midsummer ceased at all sites; thus, the changes in water chemistry during summer were largely controlled by processes in the lakes. Some samples were also collected from Yellow Lake (next to Black Lake) and from Roche Lake (next to Frisken Lake).

2.1.1 Black Lake

Black and Yellow lakes are situated upon a highland 16 km S.W. of Penticton (Northcote and Halsey 1969, Northcote 1980). The neighboring mountains rise 750 m above a steep glacial valley (Mathews 1944, Little 1961, Nasmith 1961). The surface rock around Black Lake is predominately an apatite rich Eocene andesite (Bostock 1966, Church 1973, Parsons 1974).

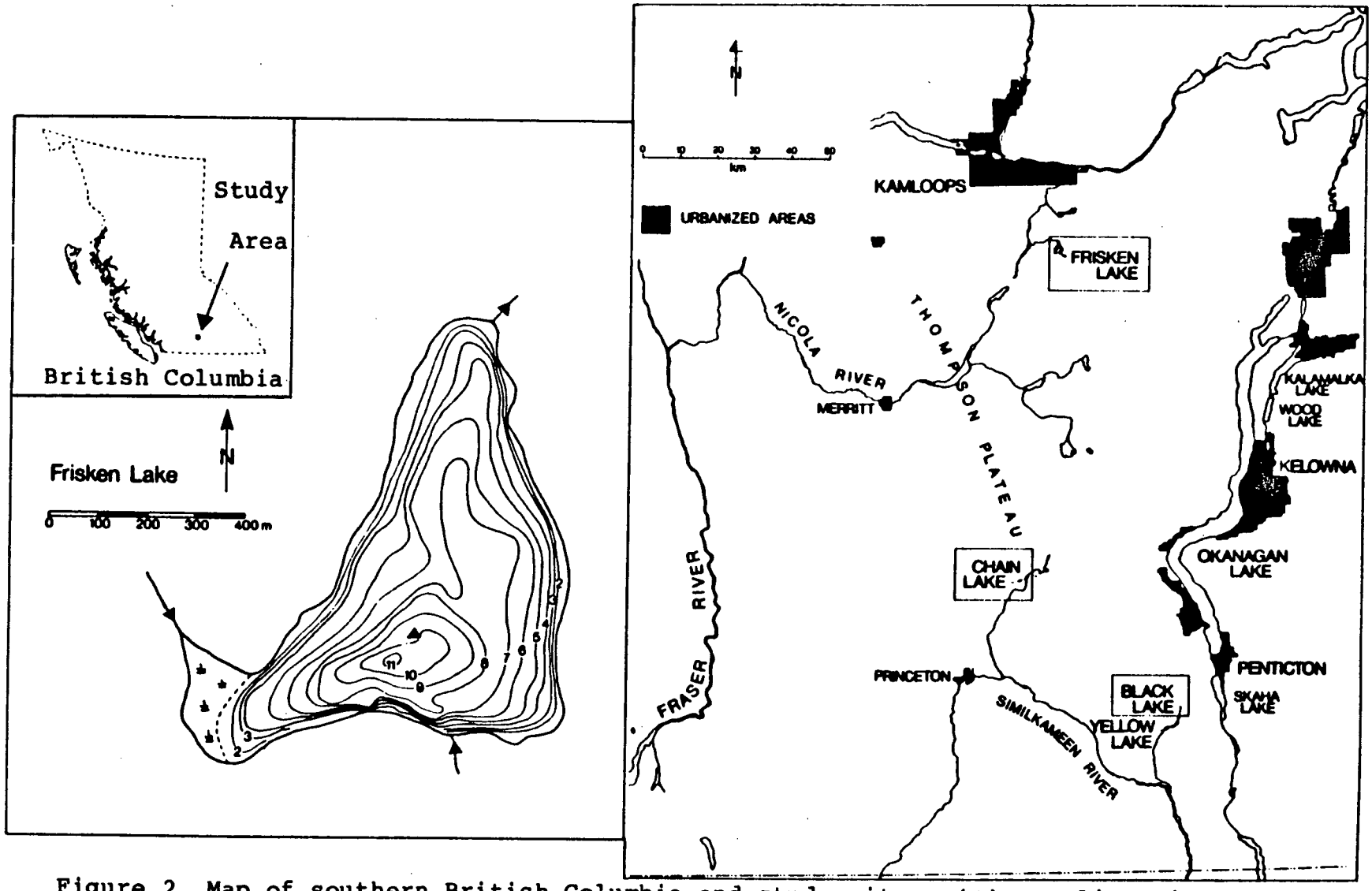
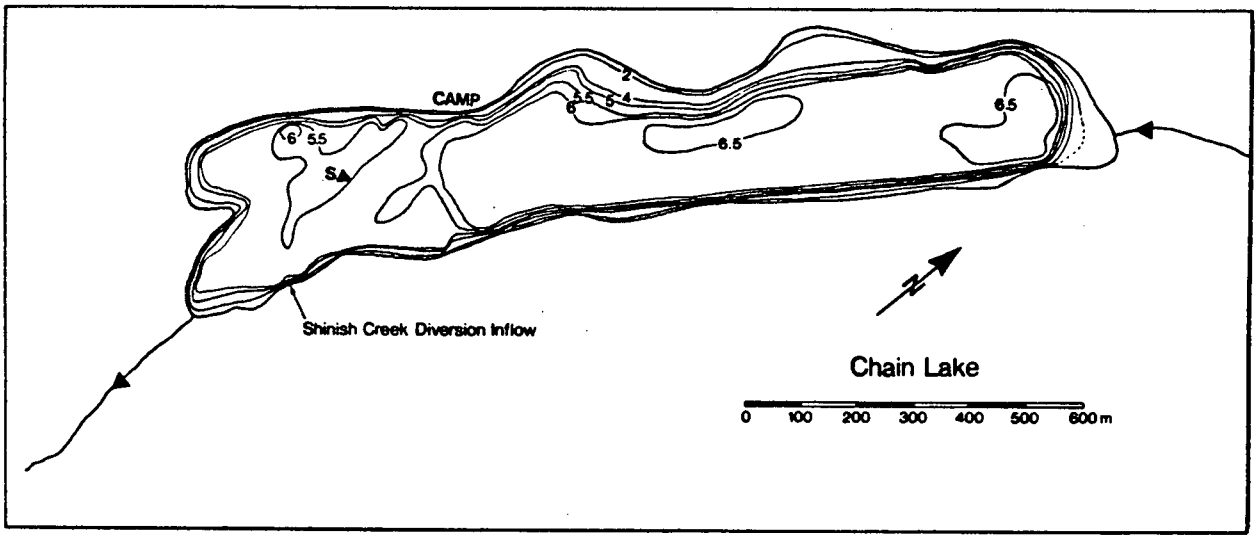


Figure 2 Map of southern British Columbia and study sites. (▲) sampling sites, contour intervals in meters



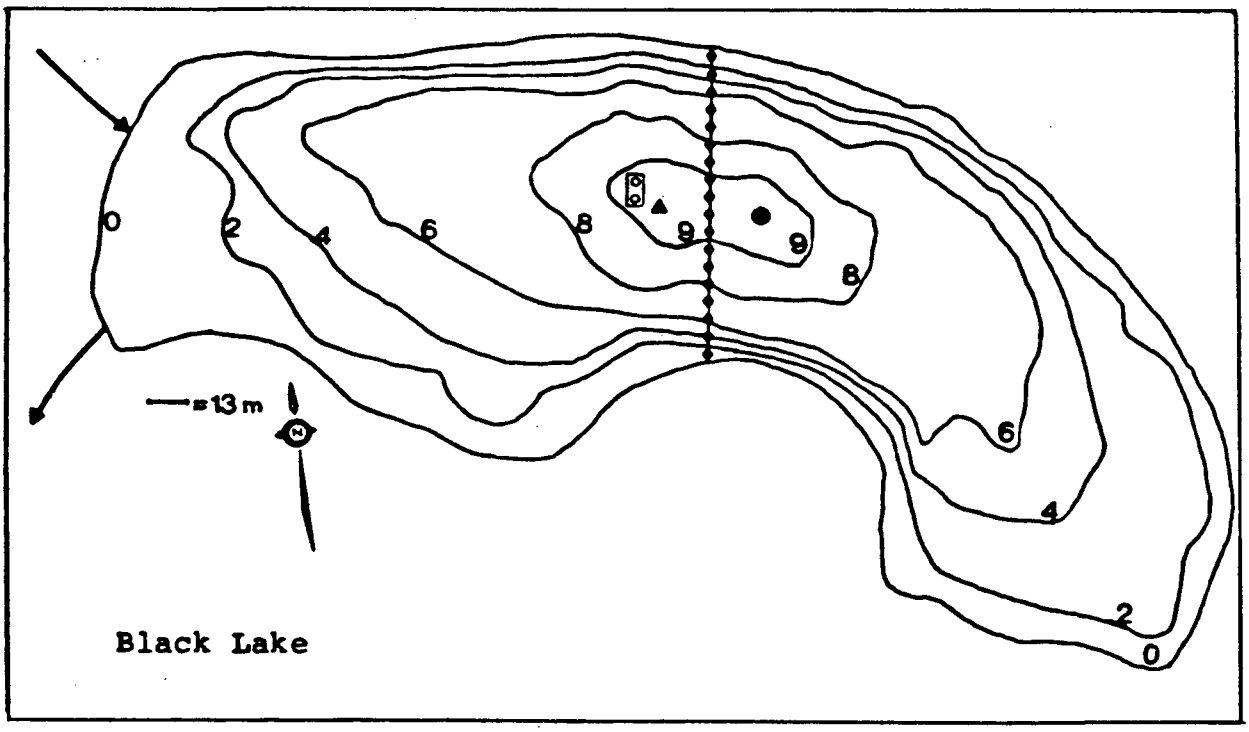


Figure 2 continued

Erosion of a surface layer of a porous volcanic rock is very pronounced. Black Lake had several aspects that made it appropriate for study: small size, good road access, power supply, and intense eutrophication without anthropogenic wastes.

Table 1 Some Physical and Chemical Features of the Study Lakes

Characteristic	Lake Black	Chain	Frisken
Latitude	490°20'	490°42'	500°27'
Longitude	1190°45'	1200°16'	1200° 8'
Elevation (m)	750	1006	1138
Drainage Area (ha)	1532	4060	267
Lake Area (ha)	4	43.7	33.8
Max. Depth (m)	9	7.1	11
Mean Depth (m)	4.5	6.1	5.5
Retention (yr)*	1-5	0.3	20
Conductivity #	506	220	374
Spring Fe (μg/L)	20	370	20
Spring TP** (μg/L)	300	20	300
Summer/Spring TP**	0.2-0.5	10	0.5
IAP/Ksp $CaCO_3$ &	7-23	2-10	5-20

All values are epilimnetic. * Retention time in Black and Frisken lakes is estimated from several measurements of stream flow. The hydrology of Chain Lake is continuously monitored by the British Columbia Ministry of Environment.

μS/cm at 25°C.

** TP = total phosphorus. & Range of calcium carbonate supersaturation in midsummer.

2.1.2 Frisken Lake

Frisken Lake is a hypertrophic lake 30 km S.S.E. from Kamloops. This lake is situated upon a poorly defined volcanic intrusion called the Wildhorse Mountain Batholith that formed during the Jurassic or post-lower Cretaceous period (Cockfield 1947, 1948). The rocks of this volcanic intrusion are rich in apatite. Frisken Lake was chosen for study because it has a longer residence time than Black Lake; thus, the long-term effects of a whole lake experiment could be monitored.

2.1.3 Chain Lake

Chain Lake is situated 45 km N.E. of Princeton. The lake lies upon a Jurassic volcanic intrusion that is similar in age and composition to the intrusives around Frisken Lake (Rice 1946). The plateau around Chain Lake is 300 m higher than the other sites; thus, more rainfall should fall in the Chain Lake basin. The only data available is for the valley which receives a mean rainfall of 52.7 cm a year (Envir. Can. 26 years of data). Two properties of Chain Lake support the hypothesis that Chain Lake receives more rainfall than Black and Frisken lakes; the conductivity is half, and the residence time of Chain Lake water is only a tenth that of the other lakes (Table 1).

Chain Lake is similar to the other sites in that it receives a high phosphorus loading from the natural weathering of apatite (Murphy 1985). In spite of having similar geology, Chain Lake has a much higher iron concentration than the other two lakes (Table 1). The water entering Chain Lake is rich in iron (Water

Investigations Branch of British Columbia, WIB, 1977). Chain Lake was chosen for study so that the biogeochemistry of lakes with low iron concentrations (Black and Frisken lakes) could be compared to an iron-rich lake with a similar habitat.

2.2 Field Experiments

Whole-lake perturbations, large enclosure, and bottle incubation experiments were conducted in Black, Frisken and Chain lakes (Table 2).

Table 2 Timing and Sites of Experiments

Year	Site			
	Black L.	Frisken L.	Chain L.	Laboratory
1979	monitoring aeration			chelator isolation
1980	monitoring aeration Fe-EDTA limnocorrals			
1981				initial FeBC* assay
1982	Fe-citrate limnocorrals			
1983		$Ca(OH)_2$ addition	lake monitoring	improved FeBC assay
1984		$Ca(OH)_2$ addition	lake monitoring	

*FeBC Iron-Binding Capacity, the ability of organic matter to maintain iron in solution

2.2.1 Sample Collection

Water samples were collected with a three-liter Van Dorn water sampler at stations located in the deepest part of the lakes. Samples were filtered within six hours. In 1983, samples

were collected from two stations in Frisken Lake which were 100 m apart. The two sites replicated very well; thus, in 1984, samples were collected from only one station. Chain Lake water samples were collected at one station in the middle of the lake between the provincial campground and the diversion. As WIB (1977) had found, this site appeared to represent all of Chain Lake well.

2.2.2 Aeration of Black Lake

Black Lake was divided into two equal parts by a Fabrene curtain (woven polyethylene) as part of a hypolimnetic aeration study of the western side of the lake (Ashley 1983). Ashley aerated the lake to allow trout to survive. The aeration experiment was continued so that samples could be collected to monitor the effect of a change in oxygen concentration on iron chemistry, and microbial responses to a change in iron availability.

2.2.3 Chemical Treatments of Frisken Lake

Calcium hydroxide was added to Frisken Lake to induce precipitation of calcium carbonate; the natural precipitation of calcium carbonate is too slow and unpredictable for study. The induction of calcium carbonate precipitation allowed more detailed study of the effects of carbonate precipitation on nutrient (iron and phosphorus) availability and algal productivity. A slurry maker on a barge was used to add the lime to the lake (Murphy et al. 1985). In 1983, lime was added to the lake during three relatively dry periods: June 16-17, 8.4 tonnes;

July 26-27, 7.2 tonnes; and August 16-17, 7.2 tonnes (total 22.8 tonnes). In 1984, lime was added in one trip: May 26-28, 16 tonnes. The lake received a total of 38.8 tonnes of $Ca(OH)_2$ over the two year period (114 g/m^2, 20.9 mg/L).

The degree of calcium carbonate saturation was determined with the computer program PHREEQE (Parkhurst et al. 1980). PHREEQE can perform complex titrations with data from each layer of a lake and then simulate destratification or calcite precipitation. The program used the Debye-Huckle method to correct for ionic strength (Berner 1971).

2.2.4 Limnocorral Experiments

Large containers (2.0 m in diameter, 5 or 7 m deep and 15,000 or 21,700 L) of transparent woven polyethylene were used to test the long-term effect of nitrate, EDTA, Fe-EDTA, citrate, or Fe-citrate on algal productivity, calcium carbonate precipitation, and phosphorus solubility. See Appendix 1 for details.

2.2.5 Small In-Situ Incubations

2.2.5.1 Calcium Chloride Experiment

To facilitate observations of calcium carbonate precipitation, water samples were incubated with calcium chloride. Water samples were collected 1.0 meter from the surface of Black Lake on Aug. 24 and Aug. 25, 1983, and enriched with 10 and 20 mg/L of $CaCl_2$ respectively. Samples with each treatment were incubated either in full sunlight in the lake (1000 $\mu Ei\ m^{-2}$

s^{-1}) or in the shade (10 μEi m^{-2} s^{-1}) for ten hours. All incubations were done in open ten-liter Plexiglas cylinders. After the incubation, the vessels were stirred and subsamples were immediately filtered through 0.45 μm Millipore cellulose acetate filters for analysis of soluble reactive phosphorus (SRP) and dissolved calcium. Subsamples for SRP were analyzed within three hours of collection. Both unfiltered and filtered samples were treated with 1.0 ml of 50% HCl per 100 ml of sample and later analyzed for calcium.

To facilitate collection of particles in the $CaCl_2$ experiment, the vessels were left undisturbed for 14 h. By decanting the surface water, the settled particles could be concentrated 20 fold. Fifty milliliters of the concentrate were filtered through Whatman GF/C filters. The filters were immediately placed in 50 ml syringes with 20.0 ml of distilled water and 10.0 ml of either CO_2 or air. The syringes were shaken for 20 s, and the gases were purged and replaced with fresh CO_2 or air. The syringes were incubated at 40°C for one hour. The syringe samples were filtered through Whatman GF/C filters. SRP analyses were immediately done, and samples for dissolved calcium were preserved for later analysis.

2.2.5.2 Primary Production

Primary productivity response to iron and chelators (EDTA, desferrioxamine-B, and three algal chelators) was measured by algal uptake of 1.48 x 10^6 Bq of ^{14}C-HCO_3 in 300-ml BOD bottles that were incubated in the lake. Relatively long incubations (48

h) were used to avoid misleading signals that short-term incubations can produce (Lean and Pick 1981). Dark bottles were used for controls. Samples were filtered (0.45 μm Millipore filters), treated with 1 ml of 0.5 N HCl for 24 h, dried (110°C), dissolved in PCS fluor (Amersham), and counted with a Beckman scintillation counter.

2.2.5.3 Heterotrophy

Microbial uptake of citrate was measured with ^{14}C-citrate. Two active and one control (killed with 0.2 ml formalin) samples were enriched with 7.11 μg C/L of 1,5 ^{14}C-citric acid (specific activity, 4.11 GBq/mM, Amersham). Ten-milliliter samples were incubated *in situ*, in 20.0 ml plastic syringes for 2.0 h.

After the incubation, a filter assembly containing a 0.2 μm, 25 mm membrane filter was attached to the syringe and the sample was filtered into the reservoir tubes of a CO_2 purging apparatus. The filters were washed with 10.0 ml of distilled water, transferred to scintillation vials and immediately inactivated with 10.0 ml of scintillation fluid. The filtrate in the purging apparatus was acidified by injecting 0.2 ml of 2 N H_2SO_4 through the stopper.

The samples were purged with CO_2-free air from an aquarium pump. The ^{14}C-CO_2 liberated from the solution was trapped in 5.0 ml of scintillation solution containing 0.3 ml of hyamine hydroxide in a scintillation vial connected to a Vigreaux column. The hyamine hydroxide is an effective absorber of CO_2; the Vigreaux column provides a large surface area for good absorption

of carbon dioxide. After purging for 20 min, the Vigreaux columns were washed with 5.0 ml of scintillation solution. The samples were counted on a Beckman Isocap scintillation counter and corrected for background and for quenching using an external standard. The control samples were subtracted from the active samples to determine the active uptake. The same procedure was used for ^{14}C-glucose and ^{14}C-acetate uptake studies. Recovery of ^{14}C from test samples of ^{14}C-bicarbonate in the purging apparatus was 100.5% (n=10).

2.3 Laboratory Studies and Analysis of Field Samples

2.3.1 Chemical Analysis

Oxygen and temperature were measured with YSI meters that were calibrated by Winkler titration (APHA 1976). The pH was measured with a Corning pH meter. Inorganic carbon was measured by gas chromatography at the lake (Stainton et al. 1977).

Samples for chlorophyll *a* analysis were filtered onto GF/C filters within two hours of collection, frozen, and analyzed by DMSO extraction (Burnison 1980). Although GF/C filters are unable to trap picoplankton, this limitation was not important for at least the summer period. Direct observation with a fluorescent microscope of water samples that were concentrated onto 0.2 µm Nuclepore filters and stained with acridine orange (Daley 1979) indicated that picoplankton were only a minor constituent of the algal biomass. For algal enumeration and identification, samples were preserved with Lugol's solution (Vollenweider et al. 1974) and settled with the Ultermohl (1931) technique.

Iron content in the samples was measured by a modified bathophenanthroline method (Strickland and Parsons 1972). Samples for iron analysis were filtered at the lake through Whatman GF/C filters. The filtrate was acidified with 2.0 ml of concentrated HNO_3 per 500 ml of sample and refrigerated until analyzed. Particulate iron samples were kept frozen until analyzed.

Samples for citrate analysis were preserved by freezing. Citrate was analyzed as the trimethylsilyl derivative by a gas chromatography method (Stumpf and Burris 1979). The method was changed in the following ways; the samples were eluted through an anion exchange resin to trap citrate (Dowex AG1-X8, formate form), citrate was eluted from the column with 1.0 M formic acid, the eluant was freeze-dried and resuspended in 0.1 M NH_4OH, dried in a derivatization vial at 50°C with argon, and derivatized with BSTFA [N,O-bis(trimethylsilyl)trifluoroacetamide] and pyridine.

Some algal siderophore isolates were hydrolyzed and analyzed for bound hydroxamate groups (Csaky 1948).

Arsenic and silica were measured by the silver diethyldithiocarbamate method and heteropoly blue method respectively (APHA 1976). Phosphorus was measured as total P and total dissolved P after perchloric acid digestion.

Samples for nitrate, ammonium, nitrite, dissolved organic nitrogen, particulate nitrogen, particulate carbon, dissolved inorganic carbon, total inorganic carbon, chloride, sulphate, calcium, magnesium, potassium and sodium were prepared in a field laboratory, and shipped on ice to a laboratory for analysis using Technicon autoanalyzer methods (Environment Canada 1979).

2.3.2 ^{32}P-SRP Analysis

Stream and extracts of surface rock from the Black Lake area were analyzed by gel chromatography to separate orthophosphorus from polymerized-P or organic-P of a different molecular size (Lean 1973). Rocks were extracted by first grinding them with a mortar and pestle and then extracting 3.0 g of powder with 100 ml of either distilled water or 1.0 N HCl in a wrist action shaker. $^{32}P\text{-}PO_4$ was used as a tracer of orthophosphate elution. Five ml samples were co-injected with $^{32}P\text{-}PO_4$ onto 2.5 by 30 cm columns packed with G-25 Sephadex beads (Pharmacia) and eluted with 0.3% sodium chloride and 0.02% sodium azide. Five-milliliter aliquots of the eluant were analyzed for ^{32}P activity by Cerenkov counting and then for SRP.

2.3.3 Iron-Binding Assay

The iron-binding capacity (FeBC) of lake water was determined with a radioisotope assay (Murphy et al. 1983a). In this assay, any unchelated iron was precipitated with magnesium carbonate. The chelators were calibrated with desferal (desferrioxamine-B, Ciba-Geigy) and the FeBC was expressed in equivalents of desferal (μM).

Some FeBC samples were further processed by gel-permeation chromatography (Murphy 1976). Five milliliters of the treated sample were injected onto 2.5 cm by 30 cm columns packed with G-25 Sephadex beads (Pharmacia) and eluted with 0.3% sodium chloride and 0.02% sodium azide. Five-milliliter aliquots of the eluant were analyzed for ^{55}Fe.

For a comparative binding assay of iron and other cations, the FeBC assay was improved by substituting 0.2 for 0.45 μm cellulose acetate filters, cacodylate buffer (pH 7.0) for TRIS buffer, and increasing the incubation to one hour. These assays used algal filtrates that had been frozen, and passed through a PM-30 ultrafiltration membrane (Amicon). ^{55}Fe was measured by scintillation counting. The efficiency of the PCS fluor (Amersham) was optimal when 1.0 ml of 500 μM desferal was added to the samples prior to addition of the fluor. Quench curves were needed for the coloured metal solutions. Metals were added in 0.001 N HCl.

A typical iron-binding experiment consisted of 2.0 ml of algal filtrate, 0.5 ml of ^{55}Fe-$FeCl_3$ (7.4×10^4 Bq in 0.01 N HCl), 0.5 ml of 0.01 N NaOH, 1.0 ml of buffer, and enough double distilled water to make a final volume of 10.0 ml. One-milliliter samples were then taken for determination of total ^{55}Fe. After the initial one hour incubation with the metals, $MgCO_3$ (60 mg) was added to each flask. After another one hour incubation, the solutions were filtered and 1.0 ml of filtrate was collected for determination of ^{55}Fe remaining in solution. Between experiments, the algal filtrates were frozen.

To determine the effect of pH changes during chelator isolation, a filtrate of Anabaena flos-aquae was pretreated with various amounts of HCl or NaOH for one hour prior to the FeBC assay.

Later in the study, the adsorption of siderophore isolates to the algal colloidal coatings was studied. The iron-binding

assay was used on filtrates from algal cultures that had been recently filtered; filtered, frozen, thawed, and refiltered; filtered, freeze-dried, redissolved, and refiltered; or passed through an ultrafiltration membrane (PM-30, Amicon). The former treatments dehydrated and precipitated colloids; whereas, the latter treatment physically removed the colloids.

2.3.4 Chelator Isolation

Chelators were isolated from Texas University algal cultures, Scenedesmus basiliensis Vischer 79, Anabaena flos-aquae [Lyngbye] 1444, and Anabaena cylindrica Lemm 1447. These cultures were grown with a 16 h photoperiod, in 100 $\mu E\ m^{-2}\ s^{-1}$ of light from fluorescent lamps. Tests for bacterial contamination of algal cultures were carried out before and after each experiment with yeast extract, beef extract, and thiogylcollate test media (Difco). Cultures were regarded as axenic if no bacterial or fungal growth appeared in the test media for one week at 25°C. Confirmation of the axenic status of Anabaena cylindrica, Anabaena flos-aquae, and Scenedesmus basiliensis was obtained several times by transmission electron microscopy.

Stock cultures were maintained in Chu-10 medium (Nichols 1973). To reduce the amount of iron in the experimental medium, algae were subcultured at least twice prior to the experiment in medium without added iron. Although iron-rich algae subcultured easily with a 1% inoculum, iron-deficient Anabaena flos-aquae needed a 10% inoculum to grow well. Some iron-deficient cultures of Anabaena flos-aquae would not grow. To minimize this problem

in establishing new cultures, several flasks were inoculated and the best growing cultures were used.

Chelators were isolated from axenic exponential phase batch cultures (ten days old). For the larger isolations, 100 ml cultures were used to inoculate bubbled 20 L cultures. Prior to chelator extraction, the air bubbler in the culture was turned off, the algae were allowed to settle for four hours, and the medium was decanted and filtered through cellulose acetate membranes with 0.45 µm pores.

Twenty liters of filtered medium were first acidified to pH 4.0, aerated for 30 min, adjusted to pH 10, and stirred with 100 g of AG1-X8 anion exchange resin (Bio-Rad, Cl^- form) for 20 min. The resin was packed into 1.0 cm diameter columns and the chelator was eluted off the resin with 0.01 N HCl. The first isolations used ^{14}C-HCO_3 to label the organic compounds (37 x 10^6 Bq per 100 ml culture), and heterotrophic bioassays with lake water samples were used to detect the most active peak.

This peak was then purified further by using ^{55}Fe-$FeCl_3$ and Sephadex chromatography (Murphy et al. 1976). The chelators were desalted by elution through an ion retardation resin (Bio-Rad AG11A8). ^{14}C and ^{55}Fe were not necessary once the isolation procedure was developed. The pH of the eluant from the ion exchange column was used to detect the important fraction. Gel-permeation columns were reproducible as shown by periodic calibration with blue-dextran and ^{14}C-glucose. All compounds that were used in the heterotrophy studies, but not the iron-binding assay, were passed through Sephadex columns.

2.3.5 Electron Microscopic Analysis

Samples from the $CaCl_2$ experiment in Black Lake and the lime treatment of Frisken Lake were analyzed at the Ontario Research Foundation or at McMaster University on a Semco Nanolab 7 scanning electron microscope equipped with an EDAX microprobe for elemental analysis. The beam was directed at the centre of the larger particles. The standardless analysis used the Magic V computer program to calculate the elemental composition (Yakowitz et al. 1973).

2.3.6 Sediment Analysis

Sediment cores were taken from Yellow, Roche, Frisken, and Chain lakes with a Williams lightweight corer (Williams and Pashley 1978). The cores were divided into 2.0 cm sections within an hour with the Williams extruder (Williams and Pashley, unpublished). The samples were then frozen and later freeze-dried at the Canada Centre for Inland Waters. Prior to analysis, the samples were ground in a sediment grinder, pelletized and analyzed for major elements (Si, Al, Ca, Mg, Fe, Na, K, P, and Mn) by X-ray fluorescence spectrometry (Mudroch and Duncan 1986). Determination of minerals present in subsamples was carried out qualitatively by a Philips X-ray diffraction spectrometer. The pyrite content of the unground, freeze-dried sediment was determined by Mossbauer spectrometry (Manning et al. 1979). The rates of sediment accumulation in Frisken and Chain lakes were determined by Pb-210 radiochemical dating (Robbins and Edgington 1975).

RESULTS

3.1 Biogenic Response to Iron Availability

3.1.1 Iron-Chlorophyll a Relationships

The yearly oscillations of algal biomass in Black Lake (chlorophyll a, Fig. 3) were closely correlated to the particulate iron concentration (Fig. 3) in both the aerated ($r=0.93$, $n=12$) and the control sides of the lake ($r=0.87$, $n=12$). The higher iron concentration on the aerated side of the lake was associated with a much higher algal biomass (chl a, μg/L aerated/control: 97/45, 1979; 160/75, 1980, Fig. 3: values are means of five replicates from 1.0 m). The higher algal biomass on the aerated side of the lake produced more oxygen in the surface of the lake, but the greater oxygen demand in the aerated hypolimnion resulted in a shallower zone of oxidized surface water (Fig. 4).

After the precipitation of the algal bloom by the second lime treatment of Frisken Lake, the Frisken Lake chl a-Fe data showed a trend similar to the Black Lake data (Fig. 5). The total iron concentrations in the epilimnion were strongly correlated to the epilimnetic chlorophyll a concentration ($r= 0.82$, $n=23$). Prior to the lime treatment, no correlation existed between iron concentration and chlorophyll a (Fig. 6). Although the iron concentration of Chain Lake also increased in midsummer of 1983, the correlation of total iron to chlorophyll a concentration was not significant (Fig. 6).

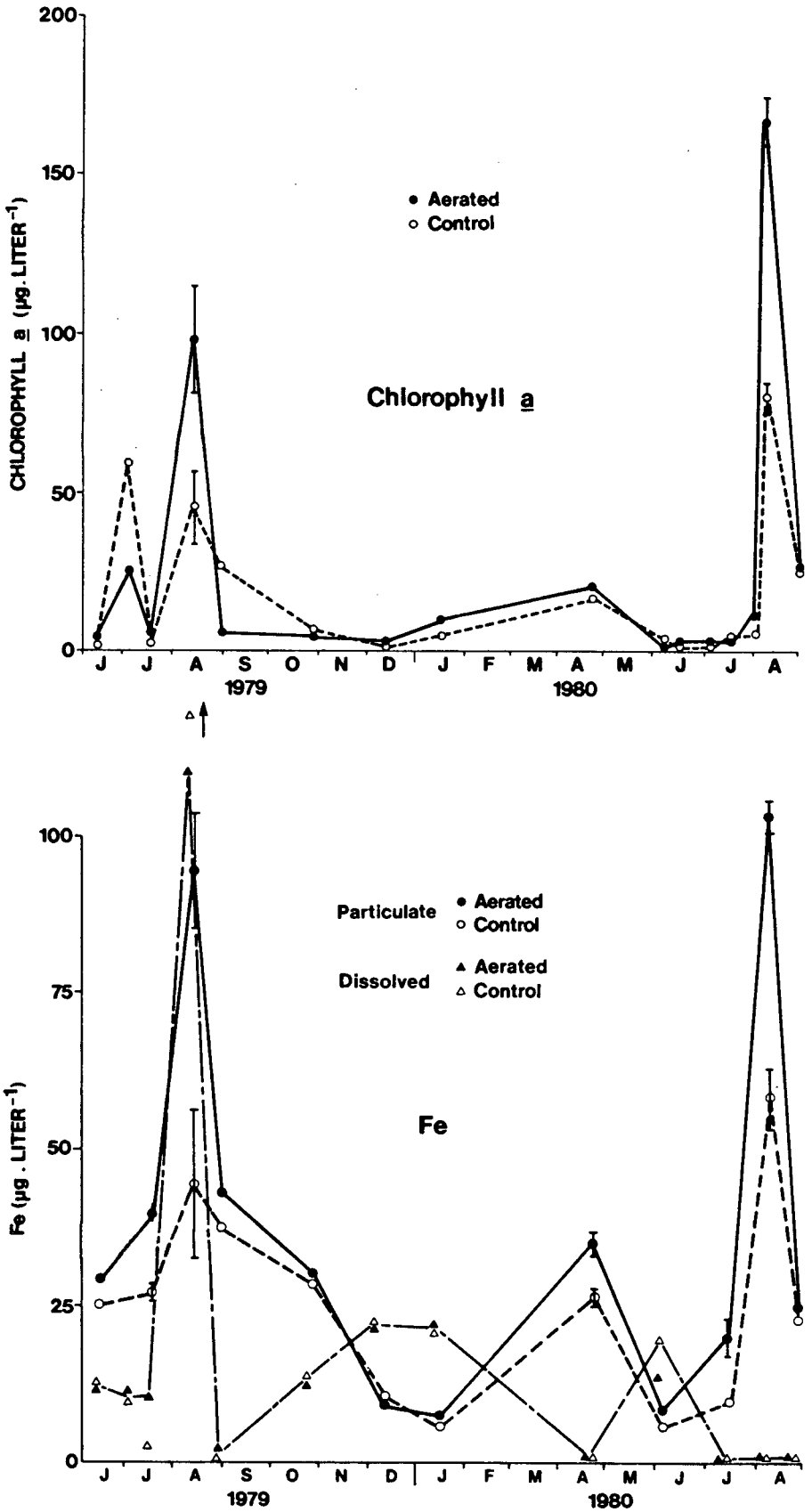


Figure 3 Seasonal changes in phytoplankton biomass (chlorophyll a) and particulate Fe in Black Lake. Values are means of two replicates, except August, 1979, 1980 where n=5. Error bars are one standard deviation.

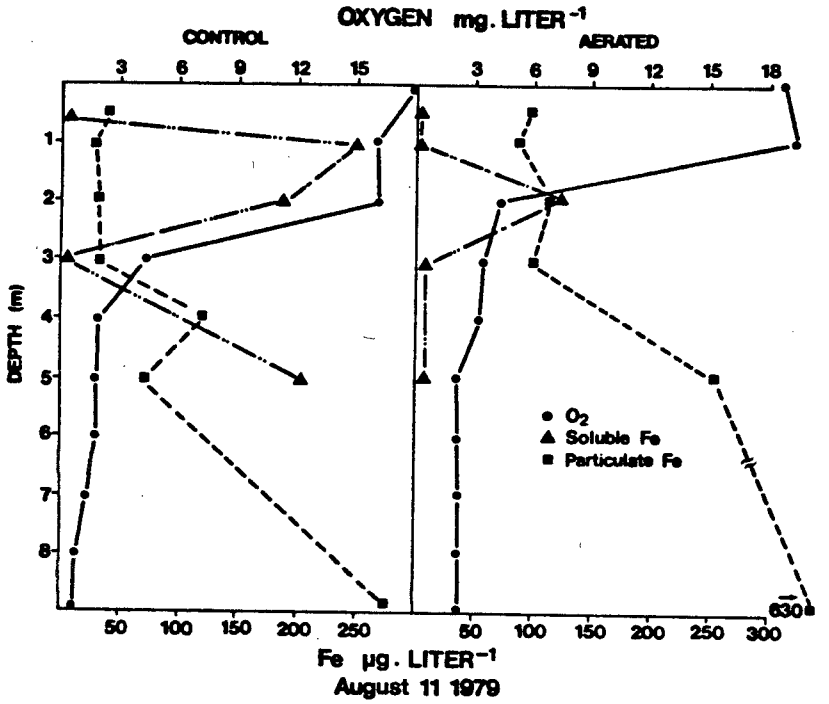


Figure 4 Black Lake iron concentrations in an algal bloom.

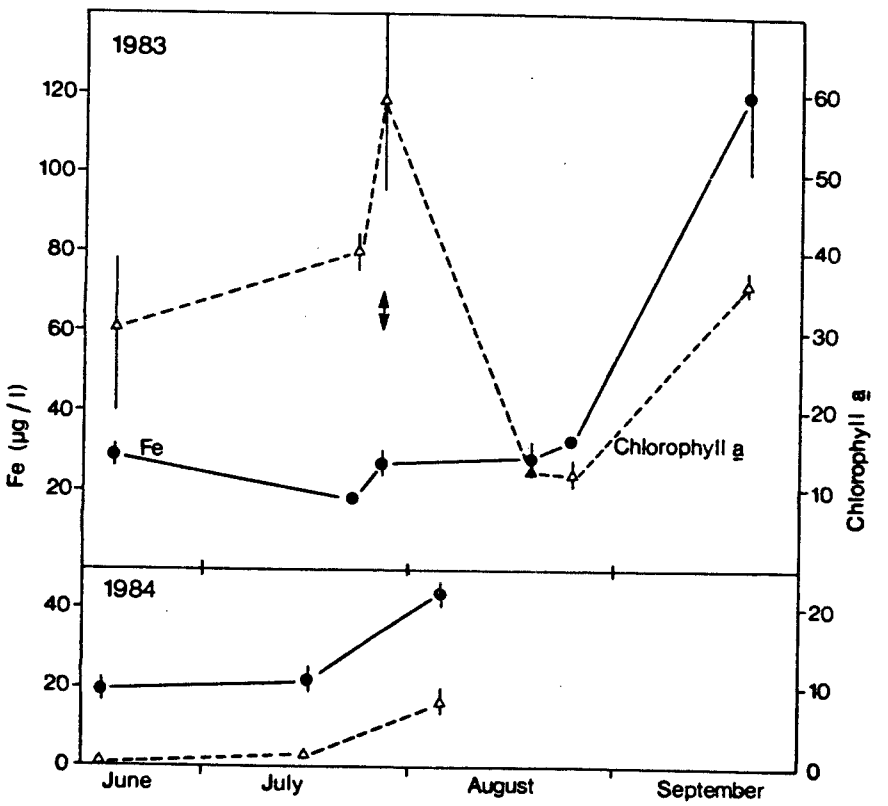


Figure 5 Iron and chlorophyll a in Frisken Lake at 1.0 m. Values are means of four samples. Error bars are one standard deviation. ↕ Denotes second lime addition.

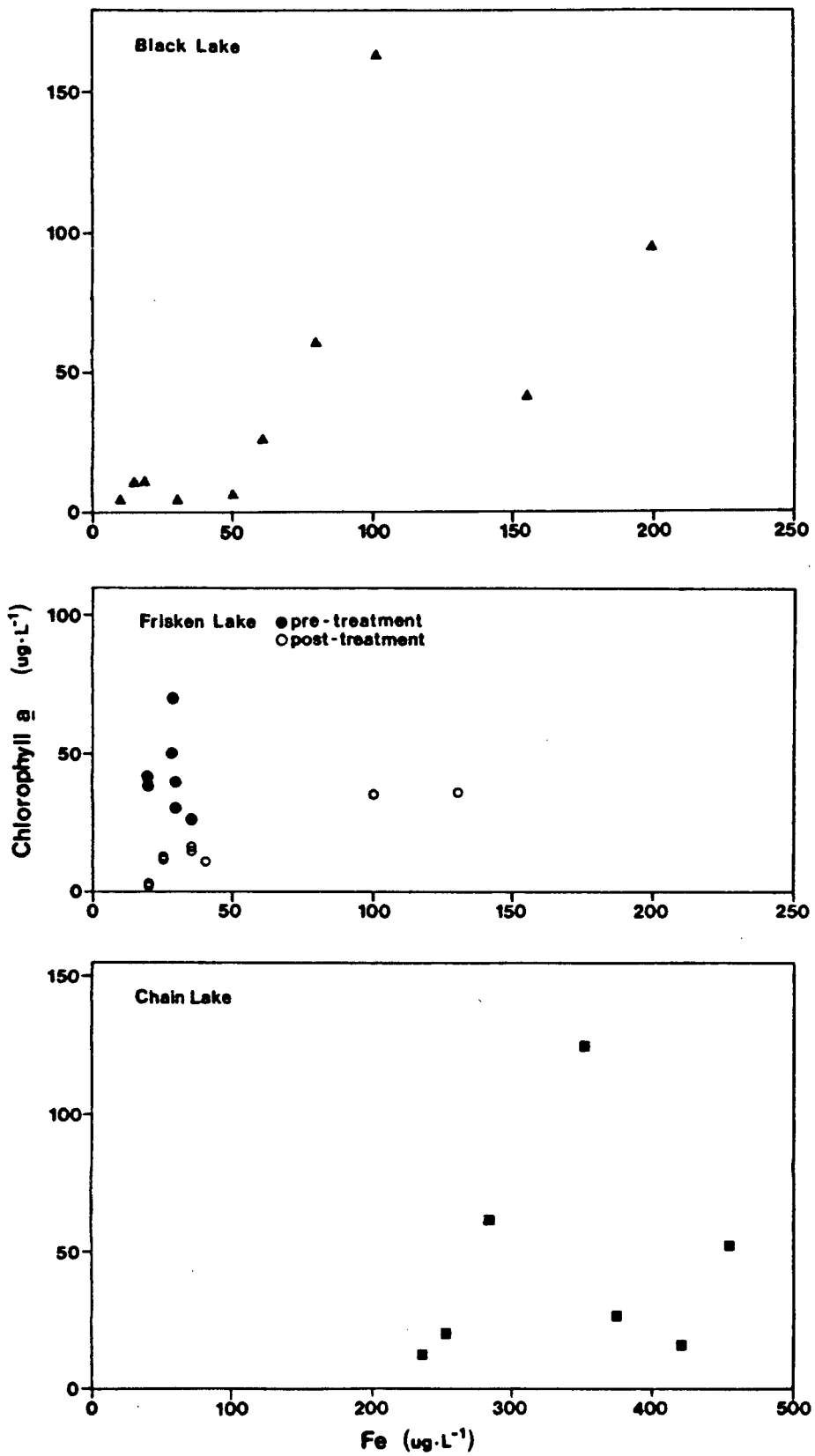


Figure 6 Chlorophyll and iron concentrations in Black, Chain, and Frisken lakes.

3.1.2 Seasonal Algal Succession

The seasonal changes in iron and chlorophyll a concentrations were associated with changes in the algal species. During the spring algal bloom in April 1980 in Black Lake (Mallomonas caudata, Cyclotella meneghiniana, Cocconeis scutellum, Diatoma elongatum, Cryptomonas erosa, Meridion circulare, and Gomphonema sp.), the dissolved iron decreased to less than 2 µg/L. During the same period, the total iron in the epilimnion remained constant (20-25 µg/L, Appendix 2). The low dissolved iron indicated the potential for iron limitation but rapid biological succession pre-empted a study of iron limitation.

The distribution of the algae and zooplankton indicated that the spring bloom in Black Lake was terminated by intense zooplankton grazing. On April 22, 1980, the chlorophyll a distribution in different areas of the lake was quite uniform (sample size 200 ml, n=10, $\bar{x}$=21.3±3.7 µg chl a/L, coefficient of variation 17.4%). On May 1, 1980, the chlorophyll a distribution indicated that the phytoplankton distribution was patchy (sample size 200 ml, n=18, $\bar{x}$=10.6±6.3 µg chl a/L, coefficient of variation 59%). On May 1, Daphnia pulex was distributed in dense swarms. At this time, the filtration of water samples produced filters that were either green without Daphnia or they were almost clear with 4-8 large Daphnia per sample. Similar effects of zooplankton on blue-green algae were not observed.

All lakes in this study developed Aphanizomenon blooms in mid to late summer, but the timing of the blooms varied greatly.

The lake with the highest iron concentrations, Chain Lake, developed Aphanizomenon blooms three weeks earlier than Black Lake.

The blue-green algal succession could occur very quickly. After the diatom bloom collapse in Black Lake in 1979 and 1980, Anabaena and Aphanizomenon coexisted at low concentrations in Black Lake for at least four weeks. Much of the biomass of these algae was in clumps; thus, they appeared to have separate niches. Before the August 1979 and August 1980 increases in iron, Anabaena died rapidly. On the morning of July 3, 1979, Anabaena was observed concentrated in the surface meter of water in green clumps with brown edges. Four hours later, Anabaena was found as brown clumps at 5.0 m (chl a 35 µg/L), well below the optimal light level and at a depth where chlorophyll a levels were usually less than 5 µg/L. Initially, the Aphanizomenon seemed unaffected by the death of the Anabaena; however, the death of the Anabaena coincided with enhanced hypolimnetic oxygen depletion, an increase in iron content of the water column, and the initiation of the Aphanizomenon bloom.

3.1.3 Seasonal Changes in Iron Concentration

The increase of iron in lake water, in Black, Chain, and Frisken lakes, occurred when stream flows were insignificant; thus, iron must have been released from the sediments. The influence of oxygen concentrations on iron release was different in the three lakes. In Frisken Lake and the control side of Black Lake, sediment iron release was not associated with a midsummer

reduction of oxygen content. The hypolimnia of Black and Frisken lakes were rarely oxidized (Fig. 7). The hypolimnion of Chain Lake was usually oxidized but it was anoxic when iron was released in August (Fig. 7).

In both Frisken and Chain lakes, about 20% and 50% respectively of the iron is converted into pyrite (FeS_2, a mineral containing reduced iron, Fig. 8). Once pyrite was buried in anoxic sediments, pyrite would be stable, and no refluxing of this iron into the lake would occur. Some ferric iron is metastable in these anoxic environments and a pulse of organic matter decay could enhance the rate of iron reduction. Some of the ferrous iron could enter the water column and some would form pyrite.

Temperature was an important variable in the sediment iron release at all sites. Iron release occurred earliest in Chain Lake, in mid-July (Table 3). This shallow lake mixes readily; thus, the sediments were warmer than the other sites (Fig. 9).

Table 3 Total Iron Concentrations in Chain Lake - 1984

	Iron Concentrations (μg/L)			
Depth (m)	July 6	July 16	Aug 10	Sept 14
1	426	377	236	252
2			322	633
3	264	379	812	1525
4			784	777
5	385	654	1967	741
Inlet	893	219		

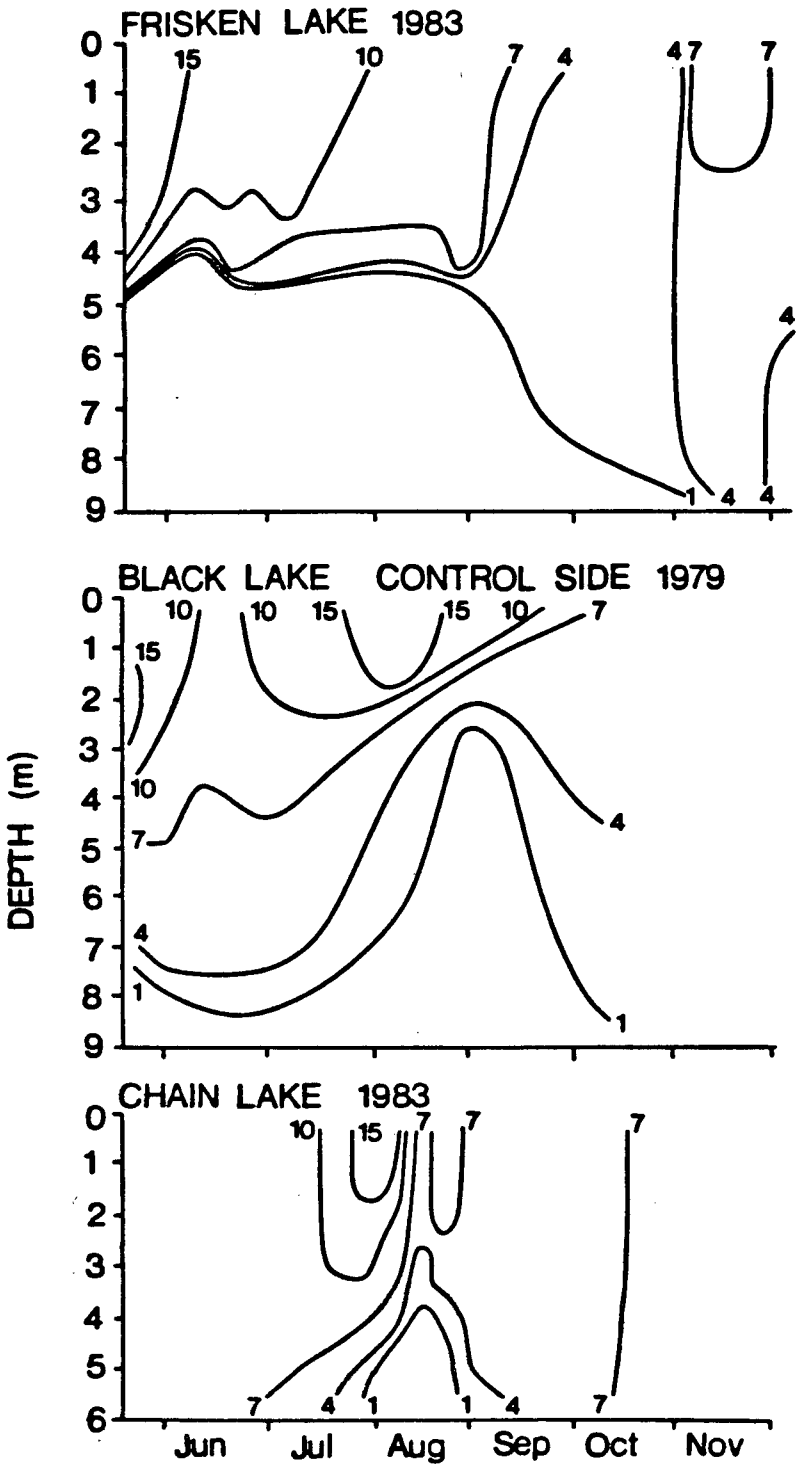
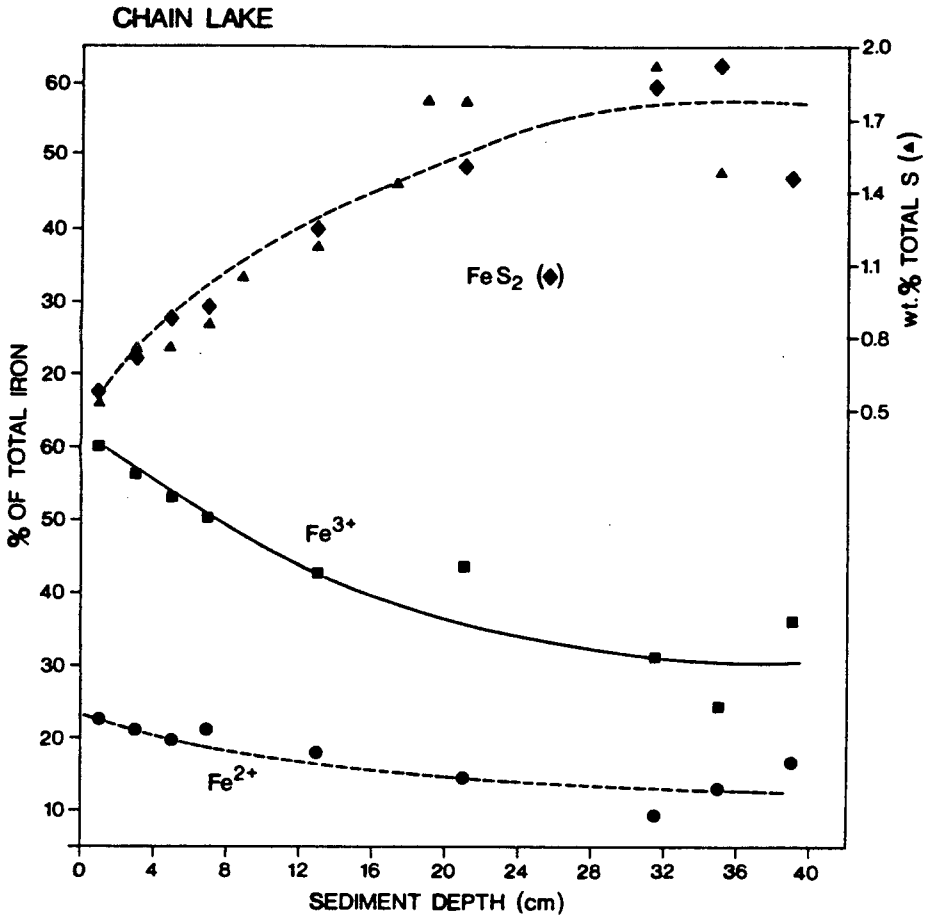


Figure 7 Seasonal changes in oxygen concentration in Chain, Black, and Frisken lakes. All values are mg/L.



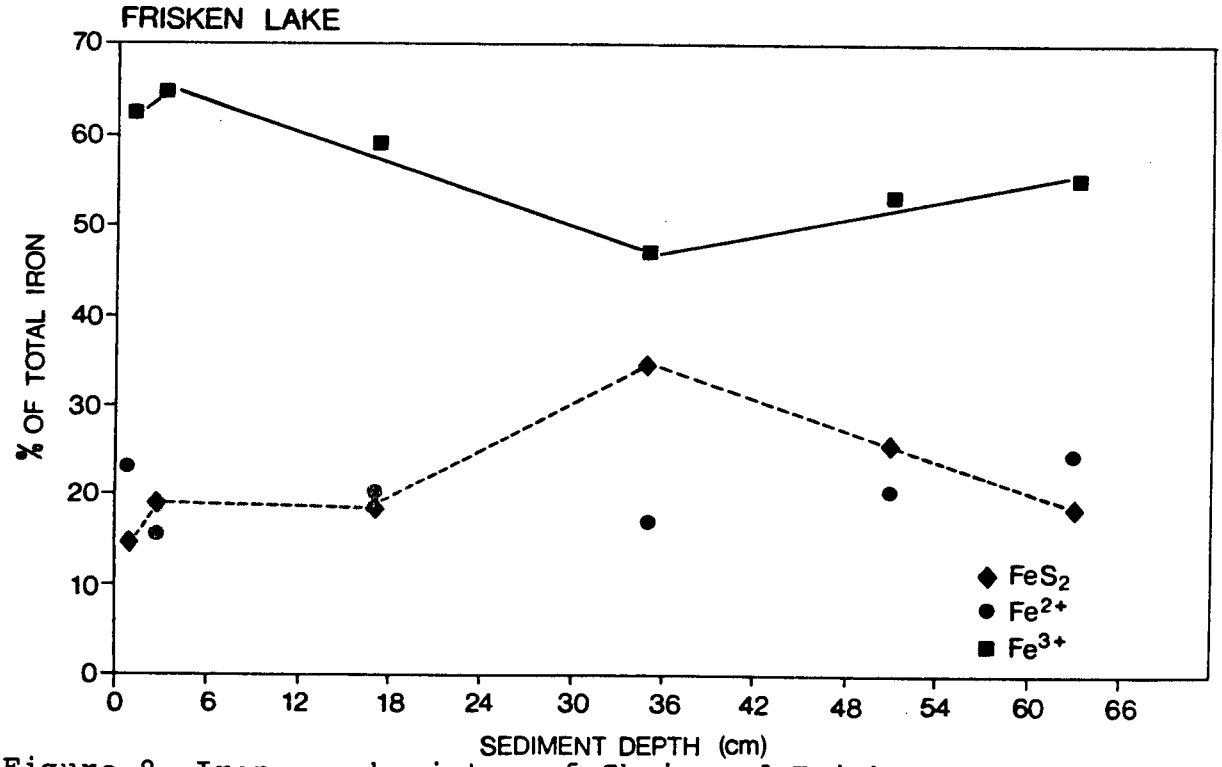


Figure 8 Iron geochemistry of Chain and Frisken lake sediments.

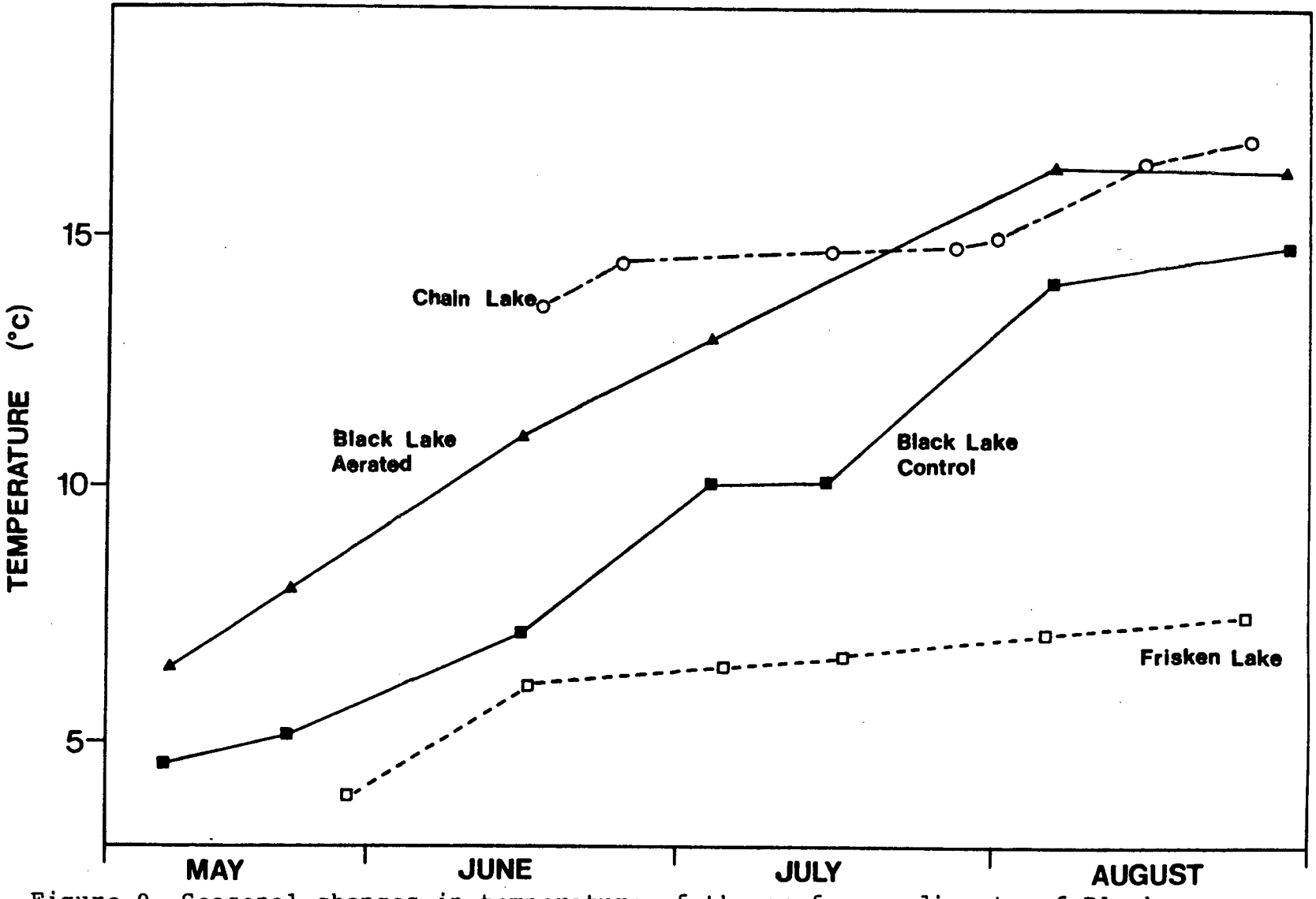


Figure 9 Seasonal changes in temperature of the surface sediments of Black, Chain, and Frisken lakes.

Iron release in Black Lake did not occur until the sediments were warmer than 10°C (Appendix A1, late July). A 2.6°C warming of the sediments by lake aeration appeared to enhance iron release on the aerated side of Black Lake (Fig. 9). Iron release occurred last in Frisken Lake perhaps because the profundal sediments did not warm above 5°C until August (Table 4, Fig. 9).

3.2 Confirmation of the Iron-Biomass Relationship

Although the iron concentration in Black and Frisken lakes was strongly correlated with the algal biomass (Figs. 3&5), these observations are not conclusive proof that iron availability regulated algal growth. The following microcosm studies were used to test the iron-limitation hypothesis.

3.2.1 Laboratory Bioassays with Black Lake Water

When water samples, collected on June 19, 1979, were enriched with 500 μg Fe/L, as $FeCl_3$, in an incubator, the algal biomass increased significantly (Fig. 10). Control samples had a lag of several days before growth was initiated and although the control samples then grew rapidly, the final biomass produced was less than the iron-enriched samples. In all samples that responded positively to iron enrichment, Anabaena continued to dominate the phytoplankton. Samples collected late in the summer (Aug. 11, 1979), when the iron content of the water had increased, did not show this iron-enrichment response (Fig. 10). The dominant algal species in late summer, Aphanizomenon, would not grow in the laboratory.

Table 4 Total Iron Concentrations in Frisken Lake[#]

1983 Depth[*]	June 16	July 23	July 27	Aug 18	Aug 25	Sept 20
1	27±4	18±1	26±5	24	36	83
2				22	31	137
3	18±2			34	30	134
4				32±2	35	129
5	68±3	39±10	45±12	131	125	43
6				559	536	80
7				791	923	39
8	94±11	226±102	219±245	1065±224	1253	444

1984 Depth	June 15	July 16	Aug 5
1	15	18	41
2	19	24	41
3	25	17	48
4	16	23	49
5	18	38	52
6	65	84	233
7	186	104	311
8	185	194	625

[#] Fe µg/L [*] depth in meters

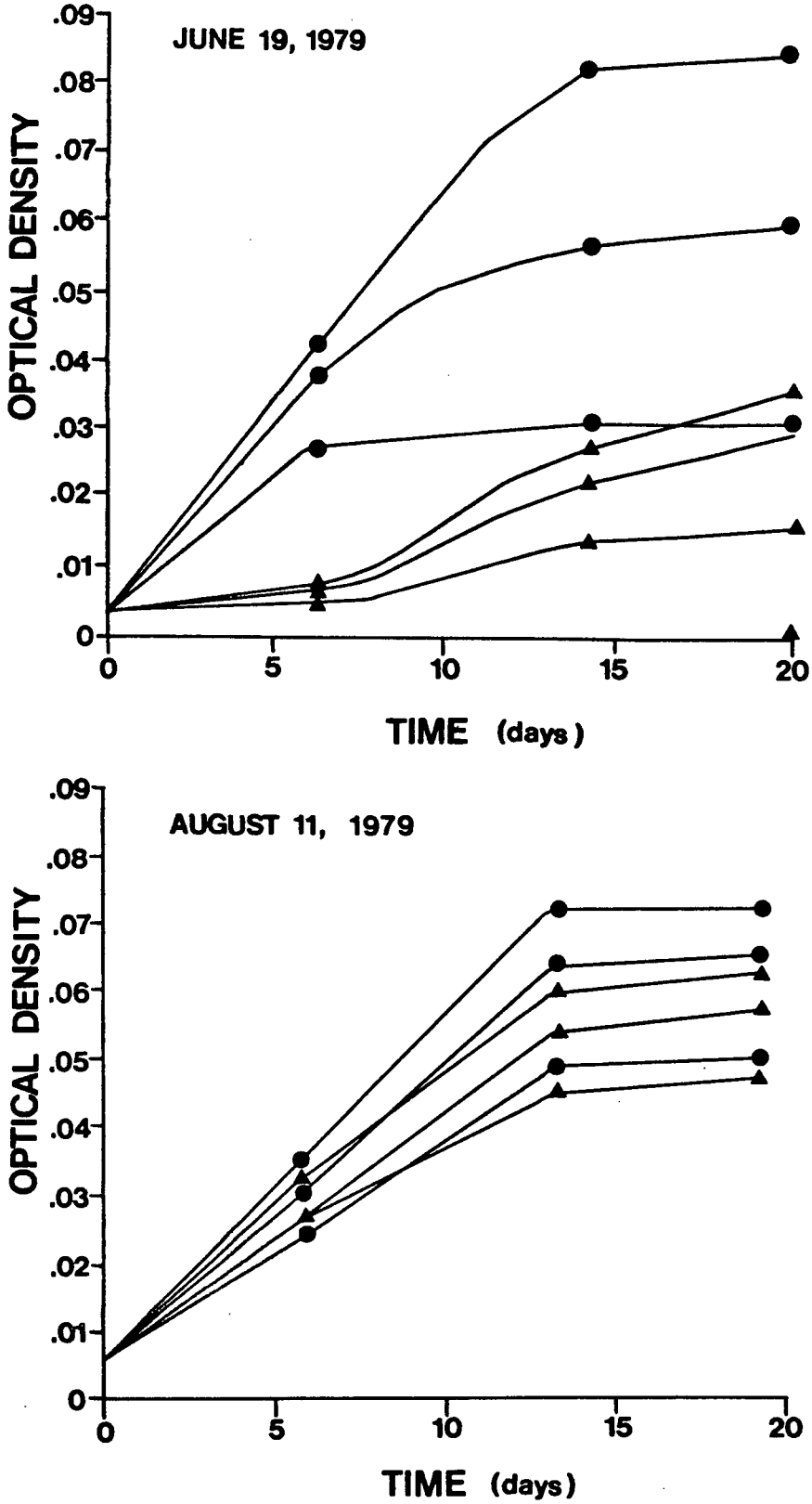


Figure 10 Effect of iron enrichment on growth of algae from Black Lake. Three samples were enriched with 500 µg Fe/L (●). (▲) were control samples. Optical density analysis has a coefficient of variation of 1.5%.

Although the prime controlling variable in the laboratory growth assays was probably iron concentration, the ability of the dominant alga to grow in the laboratory appeared to an important variable. In another assay on July 3, 1979, some of the flasks with Anabaena dominance showed a positive response to iron enrichment but others, in which diatoms replaced Anabaena, did not show any response to iron. Perhaps the high variance in the iron-enriched flasks of June 19, 1979 (Fig. 10) was caused by the varying proportion of diatoms present with Anabaena. Similar variable results occurred in 1980 when a large diatom, Rhopaladia gibba, which was never observed in the lake, quickly dominated Anabaena in laboratory cultures.

Primary production bioassays in the lake were brief (48 h); thus, the problem with algal succession encountered in laboratory incubations was avoided. The algal ^{14}C-assimilation indicated a significant stimulation from iron enrichment, as $FeCl_3$, only in early summer (compare C to Fe in Fig. 11). The similar stimulation of algal ^{14}C-assimilation by an algal siderophore isolate (5 μM FeBC) on June 17 but not in August (Fig. 11) confirmed that more iron was available in August than in June; iron chelated by this Anabaena chelate could be utilized by all tested species (three Anabaena species and two Scenedesmus species).

The moderate stimulation of primary production observed from $FeCl_3$ addition in the early summer of 1979, 1980 and 1981 was modest compared to the responses observed with algal chelators (section 3.3.5). These short in situ incubations were able to assess the immediate effect of iron addition. An

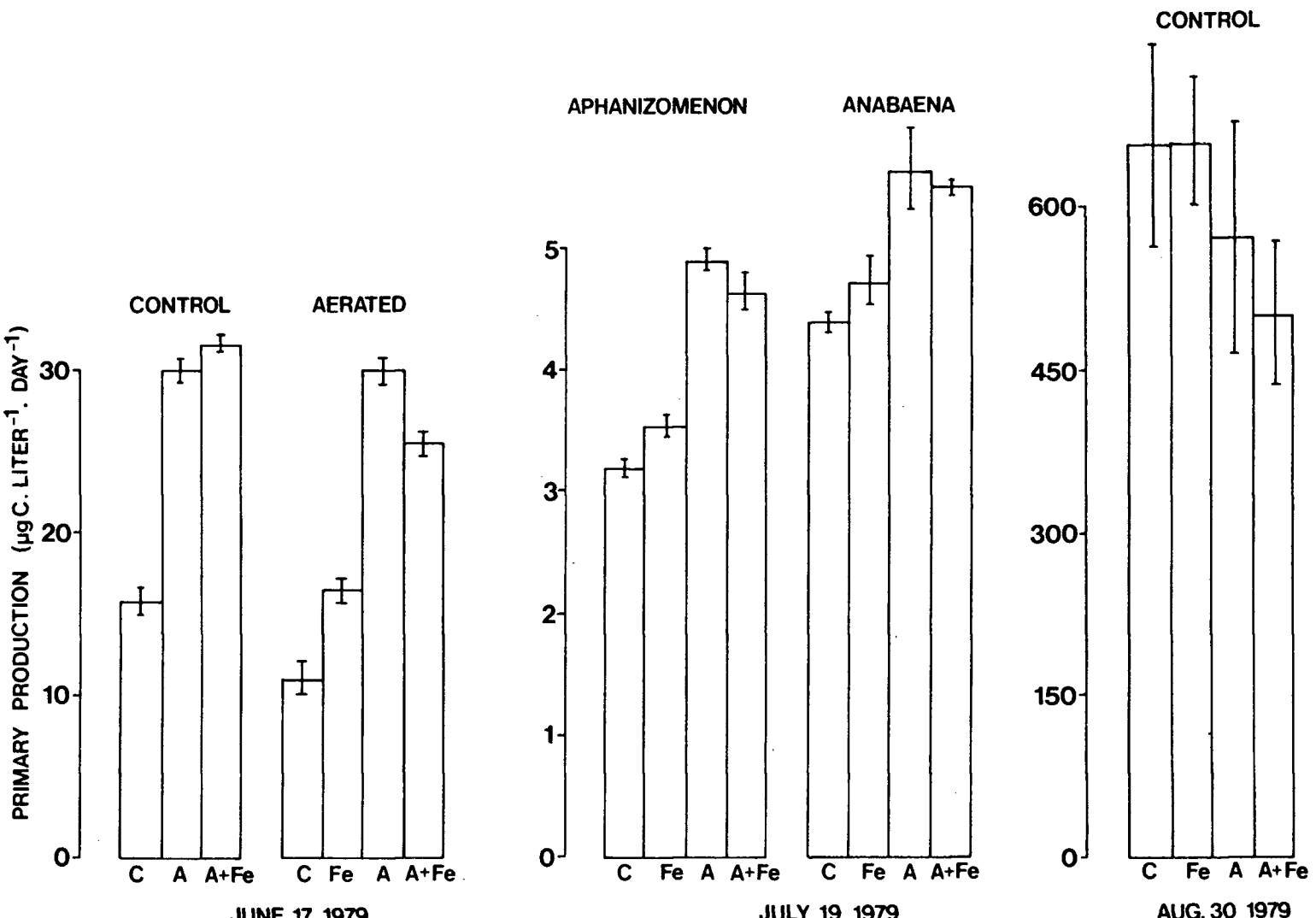


Figure 11 Effect of addition of Fe or the siderophore isolate from Anabaena cylindrica on primary production in Black Lake. C, A, Fe, and A+Fe are the control, siderophore, iron alone, and siderophore plus iron treatments. Control and aerated refer to the two sides of the lake. On July 19 the two types of algae were separated prior to incubation. Values are means of two replicates. Error bars are one standard deviation.

evaluation of long-term responses to iron enrichment required the use of limnocorral incubations.

3.2.2 Fe-EDTA Limnocorrals in Black Lake

In Black Lake in 1980, limnocorrals were enriched with Fe-EDTA or Na-EDTA. The iron concentration was maintained between 100 and 200 µg/L by monitoring the iron concentration and adding iron as needed (3 and 4 additions in first and second experiments respectively). Limnocorral bioassays are more restricted in the type of iron source than are small bottles. Unchelated iron would precipitate to the bottom of the limnocorral. A chelator must be used to maintain iron in solution; thus, an additional control must be added to determine if the chelator has an important effect.

The initial enrichment of limnocorrals on April 22, with Fe-EDTA and Na-EDTA resulted in higher oxygen concentrations (Fig. 12), presumably by increasing algal production. By May 25, calcium carbonate precipitation and perhaps zooplankton grazing had terminated the algal bloom; thus, no differences between chlorophyll a concentrations in the limnocorrals were apparent. The large reduction of phosphorus and calcium in the surface waters of the Fe-EDTA and Na-EDTA limnocorrals was primarily a result of coprecipitation of phosphorus with calcite (13 mg/L Ca and 140 µg/L P precipitated between May 25 and June 5; Ca/P molar ratio 77).

After this first set of limnocorrals had been emptied and refilled on July 30, 1980, there was no response to Fe-EDTA enrichment and only a slight response to the Na-EDTA enrichment

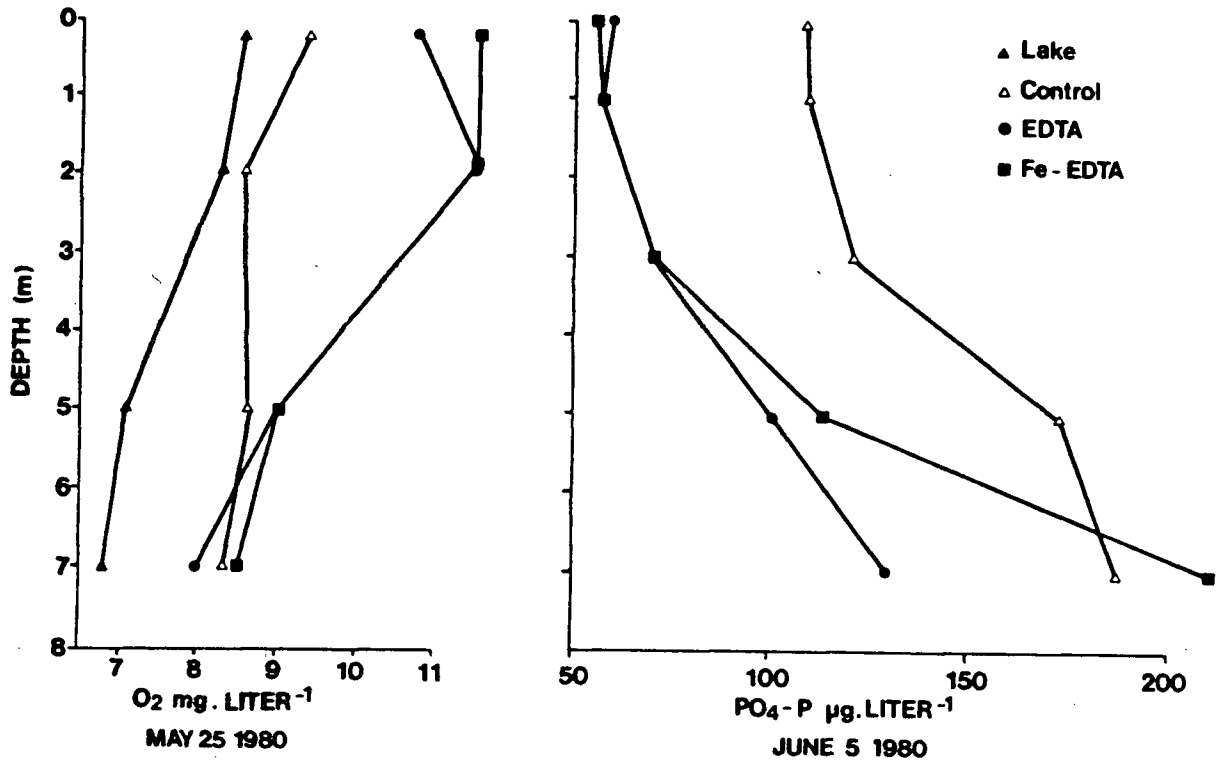


Figure 12 Oxygen and phosphorus in Na-EDTA and Fe-EDTA limnocorrals and Black Lake.

Table 5. Primary Production in Experimental Enclosures in Black Lake in 1980. [1]

	Limnocorral						
Date	Fe-1	ED-1	C-2	Fe-2	ED-2	NO_3	LAKE
May 22	28	40					
June 3	14	146					66
June 16	109	169	117	76	120	130	135
July 4	165	31	12	38	24	14	53
July 18	17	69	31	33	553	16	96
July 30			17	49	1030	28	175
Aug 12	77	251	192	115	976	56	730
Aug 28	46	166	38	156	589	13	124
Sept 10	50	350	52	33	310	20	83

[1] All samples collected at 1 m, all units as ($\mu g\ C\ L^{-1}\ d^{-1}$.).

(greater chl a, significant at the 90% confidence limits). This seasonal decrease in effect of iron enrichment was also observed in the growth and primary production bioassays and later limnocorral experiments. The seasonal increase of iron in the lake (Fig. 3) was probably responsible for the seasonal reduction in the response of iron enrichment in limnocorrals.

Primary production in the limnocorrals was highly variable; however, the Na-EDTA limnocorrals had the highest production measurements (Table 5). The heterotrophic activity was also variable in the surface water and the Na-EDTA limnocorrals had the highest heterotrophic rates. With only one exception, the heterotrophic activity was much higher in the Na-EDTA limnocorrals in the epilimnia and hypolimnia during late July and August (Fig. 13). The heterotrophic assay indicates the capacity for heterotrophic metabolism; the assay can not measure bacterial numbers or biomass.

Doubts about the suitability of EDTA as a model chelator led to the repetition of the iron enrichment experiments with citrate as the iron chelator. Citrate is a weak chelator that can be utilized by many microbes (Neilands 1981b) and plants (Tiffin 1966).

3.2.3 Fe-Citrate Limnocorral Experiment in Black Lake

Limnocorrals were enriched with sodium citrate or ferric citrate during the summer of 1982 in Black Lake. The citrate responses were quite different from the EDTA responses. Part of the difference was related to the microbial utilization of citrate. Within five days, citrate was undetectable in solution

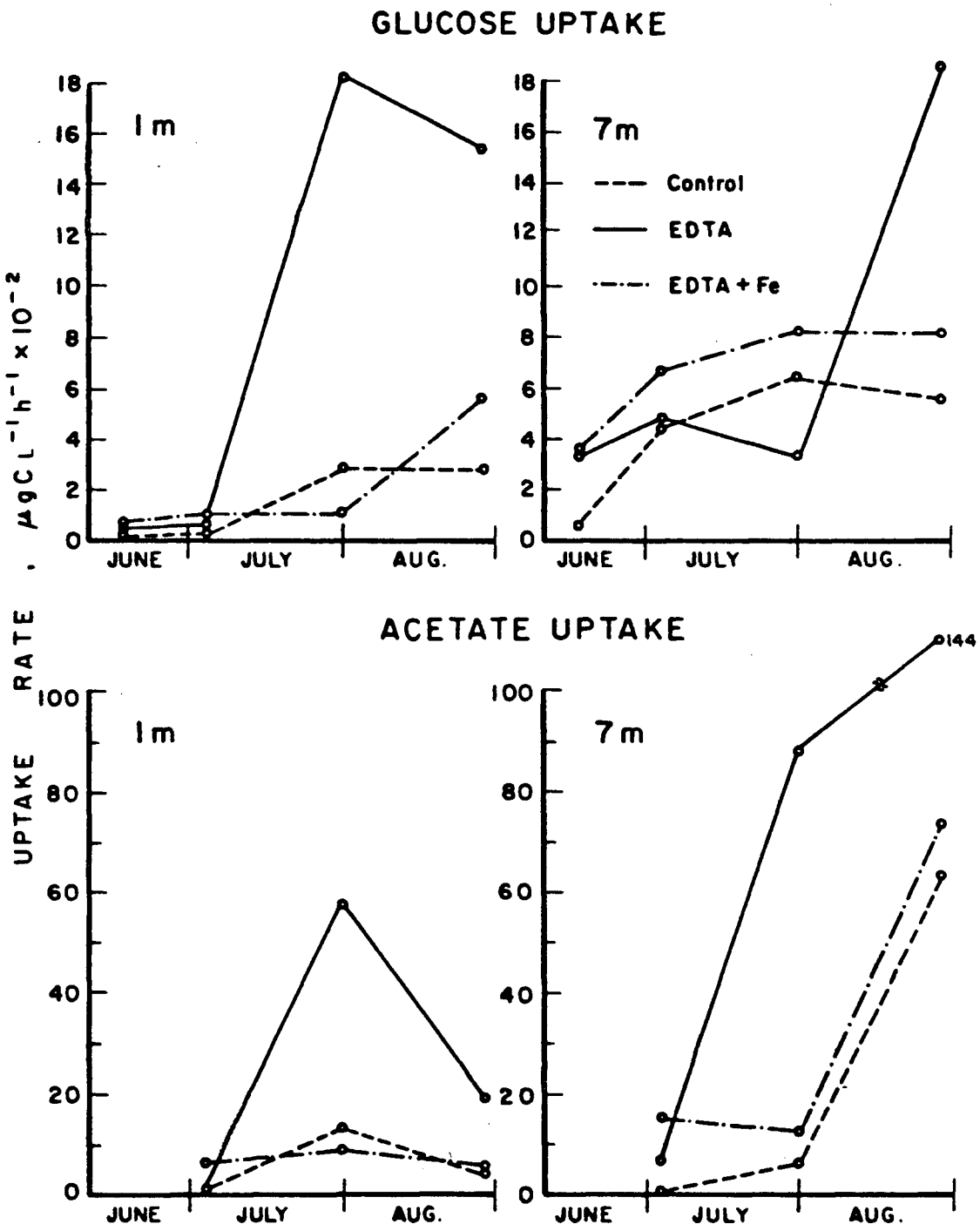


Figure 13 Temporal variation of heterotrophy in the 1980 limnocorrals.

(Fig. 14, Table 6). Heterotrophic uptake studies with ^{14}C-citrate indicated that 85±5.1% of the assimilated ^{14}C label was converted into carbon dioxide within two hours (Table 7).

Initially, the oxygen concentration in the sodium citrate-enriched limnocorrals decreased. Within three days, the ferric-citrate limnocorrals had significantly less oxygen than the control limnocorrals. On June 26 and June 28 (the third and fifth day after nutrient enrichment), the ferric-citrate limnocorrals had a mean of 0.90 mg O_2/L less O_2 than the control limnocorrals, and the sodium-citrate limnocorrals had a mean of 0.49 mg O_2/L less O_2 than the control limnocorrals. Each mean value was derived from 24 values; thus, the difference in treatments was highly significant (t test, less than 0.2% probability of the treatments not having an effect, Appendix 3).

After the citrate was utilized, oxygen concentrations increased in all limnocorrals (Appendix 3, Fig. 15). Within ten days, the oxygen content of the surface of the ferric-citrate limnocorrals increased more than the other limnocorrals (Fig. 15). The treatments replicated well, and by a t test, the ferric-citrate limnocorrals had significantly more oxygen than the sodium-citrate limnocorrals (t test, less than a 5% probability of the treatments not having an effect, Appendix 3).

The unusually cold and cloudy weather during this experiment appeared to suppress the intensity of the blue-green algal growth (Fig. 15). During this limnocorral experiment, the chlorophyll a content of the limnocorrals never exceeded 5 µg/L; the iron limnocorrals had the most chlorophyll. Flakes of the blue-green alga Aphanizomenon flos-aquae appeared only in the

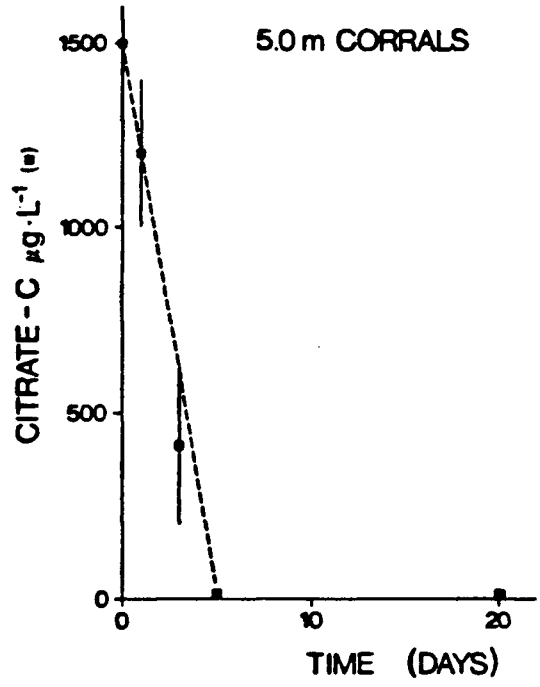


Figure 14 Citrate assimilation in limnocorrals. Analysis is on water 5.0 m from lake surface. The mean citrate concentrations (■) of the two sodium citrate and two ferric citrate limnocorrals is shown with error bars of one standard deviation. Day zero was June 23, 1982.

Table 6 Citrate Concentration in Limnocorrals

Limnocorral	June 24	June 26	June 28	June 30
Citrate 1				
1 m	55.0±2.7	2.8±1.5	ND	4.0
3 m	107.0	14.0±4.6	0.8±0.01	
Citrate 2				
1 m	89.8±0.7	2.3±0.2	4.6±1.8	ND
3 m	152.0±11.0	62.0±0.8	ND	
Iron 1				
1 m	144.0±20.0	2.2±1.44	1.0±0.6	
3 m	103.0±1.7	63.0±0.2	ND	
Iron 2				
1 m	122.0±3.2	1.2±0.4	1.2±1.2	
3 m	106.0	19.0±8.4	ND	

Samples analyzed twice are shown as the mean value ± one standard deviation. After the additions of citrate on June 23, June 30, and Aug. 12, the calculated concentration would be 140 µg C/100 mL. Samples of June 30 were collected 4 h after the citrate enrichment. All values are µg C/100 mL. ND is not detectable.

Table 7 Limnocorral ^{14}C-Citrate Assimilation

Sample	Depth	Cont-1	Cont-2	NO_3-1	NO_3-2	Cit-1	Cit-2	Fe-1	Fe-2
June 28									
Cit-Net	1.0 m	7.89	6.65	4.37	5.93	32.4	83.8	18.6	36.8
Cit-Net	3.0 m	4.92	3.99	3.28	4.41	203.5	103.1	165.3	105.3
June 30									
Cit-Net	1.0 m	.693	.042	*	*	89.8	79.4	84.2	68.8
Cit-Gr	1.0 m	6.68	.205	*	*	*	582.0	*	*
Cit-Net	3.0 m	1.64	.478	*	*	320.0	248.0	286.0	53.8
Cit-Gr	3.0 m	9.55	.674	*	*	1798.0	1702.0	1448.0	1270.0
Aug. 17									
Cit-Net	1.0 m	.73	.95	ND	ND	.22	ND	ND	.93
Cit-Net	3.0 m	.82	.69	ND	.439	.74	1.14	ND	3.72

ND=not detectable, *=no data, Cit=citrate, Gr=gross uptake (total carbon assimilated), Net=net uptake (gross C uptake-respired C).
Cont-1=control-1 limnocorral, Cont-2=control-2 limnocorral etc. Gross C uptake = net C uptake + respired C.
All rates expressed as $\mu g\ C\ L^{-1}\ h^{-1} x 10^{-2}$.

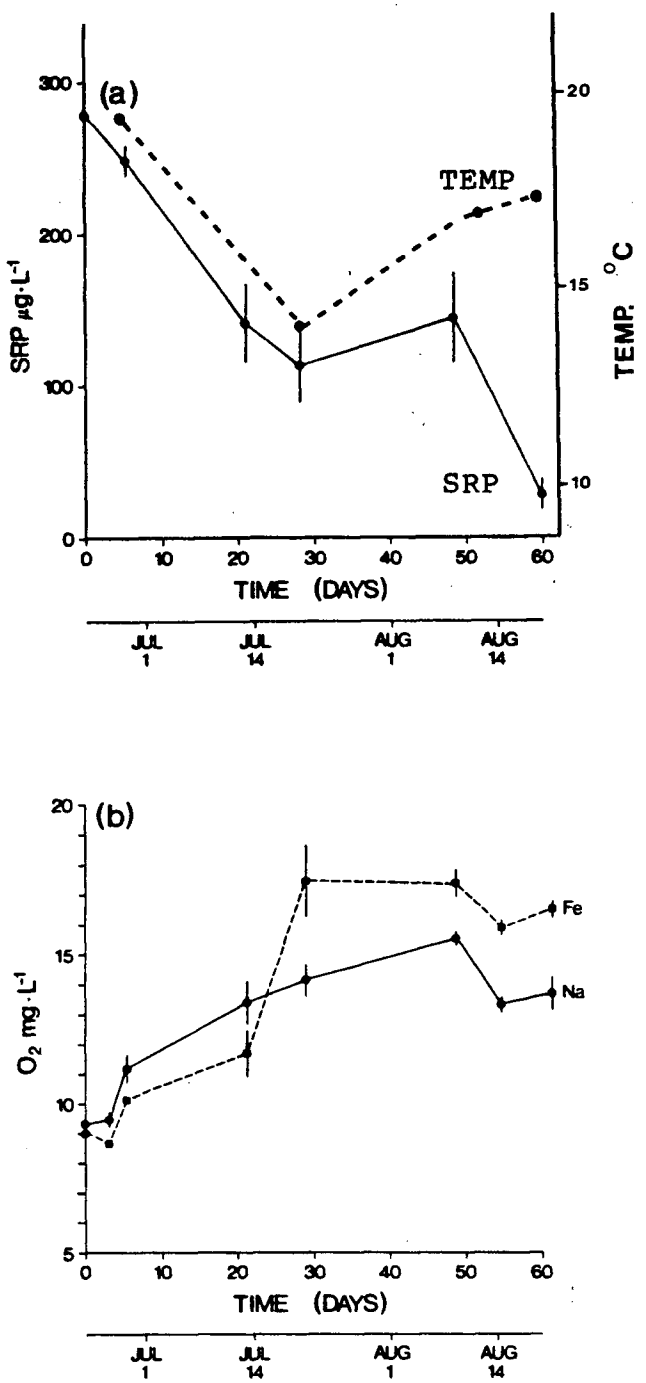


Figure 15 Phosphorus, oxygen, and temperature in the citrate limnocorrals in Black Lake.
(a) The mean SRP concentration (——) and temperature (----) of 1.0 m water from the 2 control, 2 nitrate, 2 Na-citrate, and 2 Fe-citrate limnocorrals.
(b) The mean oxygen content of the Na-citrate (●) and Fe-citrate (■) limnocorrals at 1.0 m . The error bars are 1 standard deviation. Day 0 was June 23, 1982.

ferric-citrate limnocorrals.

The final addition of sodium citrate and ferric citrate (Aug. 12) was not as closely studied as the earlier additions. Iron enrichment did not appear to stimulate oxygen consumption or production. Moreover, the oxygen content of all the limnocorrals decreased at this time. Any detailed interpretation of oxygen data was complicated by calcium carbonate precipitation which occurred in all the limnocorrals and in the lake.

3.2.4 Iron Availability in Chain Lake

Limnocorrals were filled in Chain Lake on July 15, 1983 to determine if algal oxygen production was suppressed by a lack of available iron. The iron response was much different from identical experiments conducted in Black Lake.

The limnocorral additions of ferric citrate did not result in a significant stimulation of algal oxygen production (Fig. 16). After the third day, the oxygen content of the sodium citrate limnocorrals was higher than the ferric citrate limnocorrals. In contrast to Black Lake, in Chain Lake the microbial utilization of citrate consumed more oxygen than the algae produced. This was true in both the sodium citrate and ferric citrate limnocorrals. Iron did not stimulate algal oxygen production; thus, it was concluded that the algae in Chain Lake were not limited by a lack of iron.

The Chain Lake limnocorral experiments indicate that iron in Chain Lake was available to algae, and was presumably reactive. The relationship between oxygen concentration and phosphorus solubility supports this hypothesis (section 3.4.1).

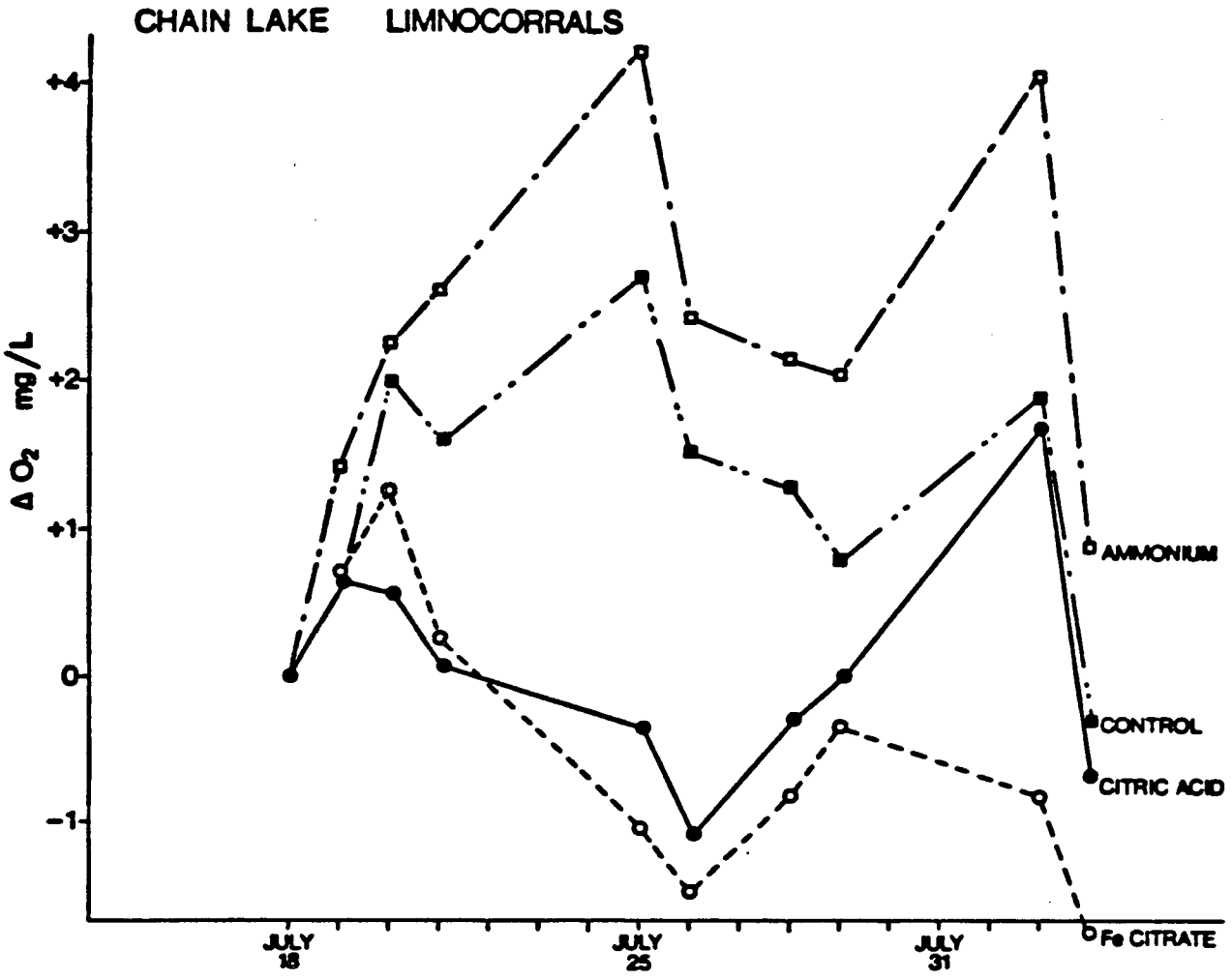


Figure 16 Oxygen in Chain Lake limnocorrals. ΔO_2 is the mean change in oxygen concentrations from mean initial oxygen concentration of 10.0 mg/L at 1.0 m. Two limnocorrals were used for each treatment. Coefficient of variation is <0.5%.

3.3 Siderophore Ecology

A series of experiments was conducted to determine how microbes regulated the availability of iron. An iron-binding assay was developed to quantify the amount of iron-complexing compounds present in cultures and in lake water. The selectivity of cation binding was evaluated to determine if these iron-complexing compounds were siderophores. The presence of siderophores indicates iron limitation; thus, a quantitative study of siderophores in the lake can provide insight into iron availability in the lake. The microdistribution of chelators in algal culture medium was also studied. The data on the microdistribution and chemistry of the chelators were used to design experiments on the effects of siderophore isolates on algal and bacterial productivity.

3.3.1 Chelator Quantification

An iron-binding assay was developed to estimate the concentration of iron-binding compounds. This assay can precisely and rapidly measure equivalents of a well characterized hydroxamate siderophore, namely, desferal (Appendix 4). The coefficient of variation of the iron binding was less than 3.1% (Table 8). The assay does not measure the molar concentration of the chelators. For example, a high concentration of weak iron-binding compounds could have the same iron-binding capacity as a dilute solution of strong iron-binding compounds.

Table 8 Effect of Freezing Anabaena Filtrates on Iron Chelation

Treatment	Filtrate		Totals		
	DPM[#]	C.V.[##]	DPM[#]	C.V.[##]	%Chelated
A.f.a.-1[*] Fresh Filtrate	89,727	.031	174,810	.015	51.2
Frozen Once	136,918	.011	180,160	.002	75.0
Frozen Twice	125,350	.013	122,956	.029	101.9
A.f.a.-2[*] Fresh Filtrate	10,151	.003	94,204	.007	10.7
Freeze-Dried	54,977	.007	69,802	.002	79.4
A. cyl.[**] Fresh Filtrate	35,744	.022	102,486	.055	34.9
Freeze-Dried	19,353	.018	47,171	.014	41.0

[*] A.f.a.-1 and A.f.a.-2 are two filtrates from cultures of Anabaena flos-aquae.

[**] A. cyl. is a filtrate from a culture of Anabaena cylindrica.

[#] DPM is the mean number of disintegrations per minute of the radioactive iron in two samples.

[##] C.V. is the coefficient of variation of DPM of radioactive iron.

3.3.2 Siderophore Association with Fibrils

Fortunately, I had supplied Dr. Leppard with samples of my cultures and their ultrastructure is well defined (Leppard et. al. 1977). Anabaena cylindrica produces 15 times more colloidal fibrillar material than Anabaena flos-aquae. The colloidal fibrils extend from the cell surface of Anabaena flos-aquae by 0.35 µm and Anabaena cylindrica by 2.6 µm. The establishment of a phycosphere, a microenvironment around a cell (Bell and Mitchell 1972), by siderophore adsorption to fibrils would greatly change the relationship between the producing species and competing microbes. Moreover, the microdistribution of siderophores influences the concentration of siderophore isolates used in bioassays.

Three different experiments indicated that the chelator excreted by Anabaena flos-aquae was loosely bound to a colloid.

1) When the filtrates from Anabaena flos-aquae were frozen and then refiltered, the chelation capacity increased. The percentage of 2.3 µM iron remaining in solution in the FeBC assay increased from 51% in unfrozen filtrate, to 76% in filtrate that was frozen once, and to 101% in filtrate that was frozen and thawed twice (Table 8). In a similar experiment, the chelation capacity of another filtrate of Anabaena flos-aquae increased 7.8 fold when the filtrate was freeze-dried, redissolved in an equivalent volume of distilled water, and refiltered (Table 8).

Note that the chelation capacity of an algal filtrate from Anabaena cylindrica increased only slightly after freeze-drying (Table 8). The freezing and refiltration of the thawed filtrate removes colloidal organic material. The colloidal "fibrils" that

coat many algae, including Anabaena flos-aquae (Leppard et al. 1977), do not redissolve from frozen algal filtrates.

2) The inability of fibrils to pass through an Amicon PM-30 ultrafiltration membrane seemed responsible for the apparent increase in iron-binding capacity of an algal filtrate from Anabaena flos-aquae from 2 to 80 µM (equivalents of desferal).

3) Another indication that chelators were not in true solution was found when the filtrate from Anabaena flos-aquae was titrated with iron. When the chelator was separated from the fibrils by freeze-drying and ultrafiltration and then used in the FeBC assay, a straight line was produced (Fig. 17). In two replicate titrations of the purified Anabaena flos-aquae chelators the coefficients of linear regression (r^2) were 0.961 and 0.968. The fresh algal filtrates had two inflection points in the titrations (Fig. 17). This step response of the fresh algal solutions may indicate that the chelator is more reactive when in solution than when it is adsorbed to the fibril. The purified algal chelator response was different from desferal or EDTA that each had one inflection point in the titration.

These experiments indicate that siderophores are associated weakly with the fibrillar surface of algae. This association supports the use in bioassays of solutions of siderophore isolates that are much more concentrated than that found in the bulk filtrate.

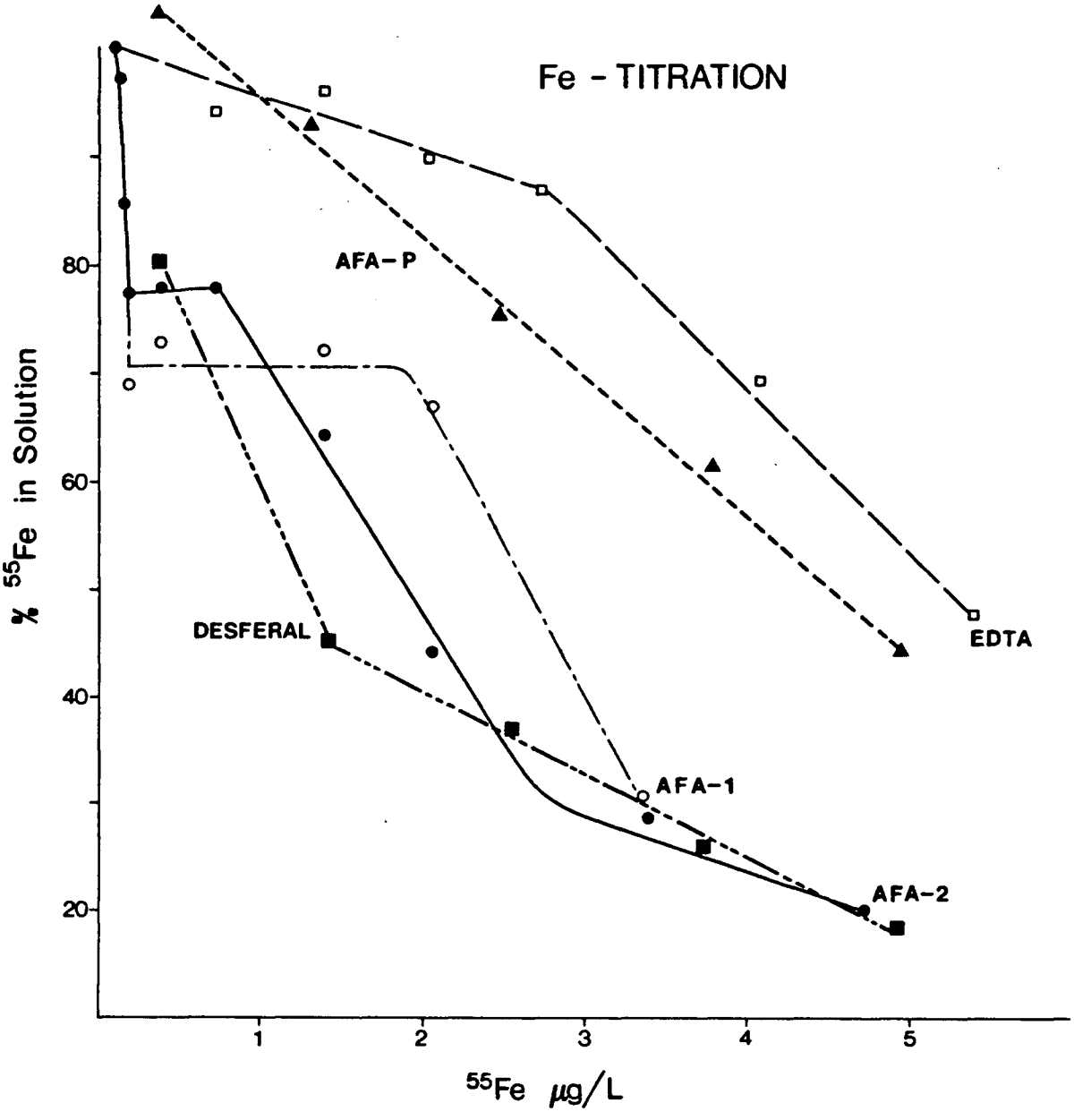


Figure 17 Iron titration of EDTA, desferal, and filtrates from Anabaena flos-aquae cultures.
AFA-1 and AFA-2 are fresh filtrates 20 and 14 days old respectively. AFA-P is a filtrate that was purifed by ultrafiltration and diluted five fold.

3.3.3 Siderophore Specificity for Iron

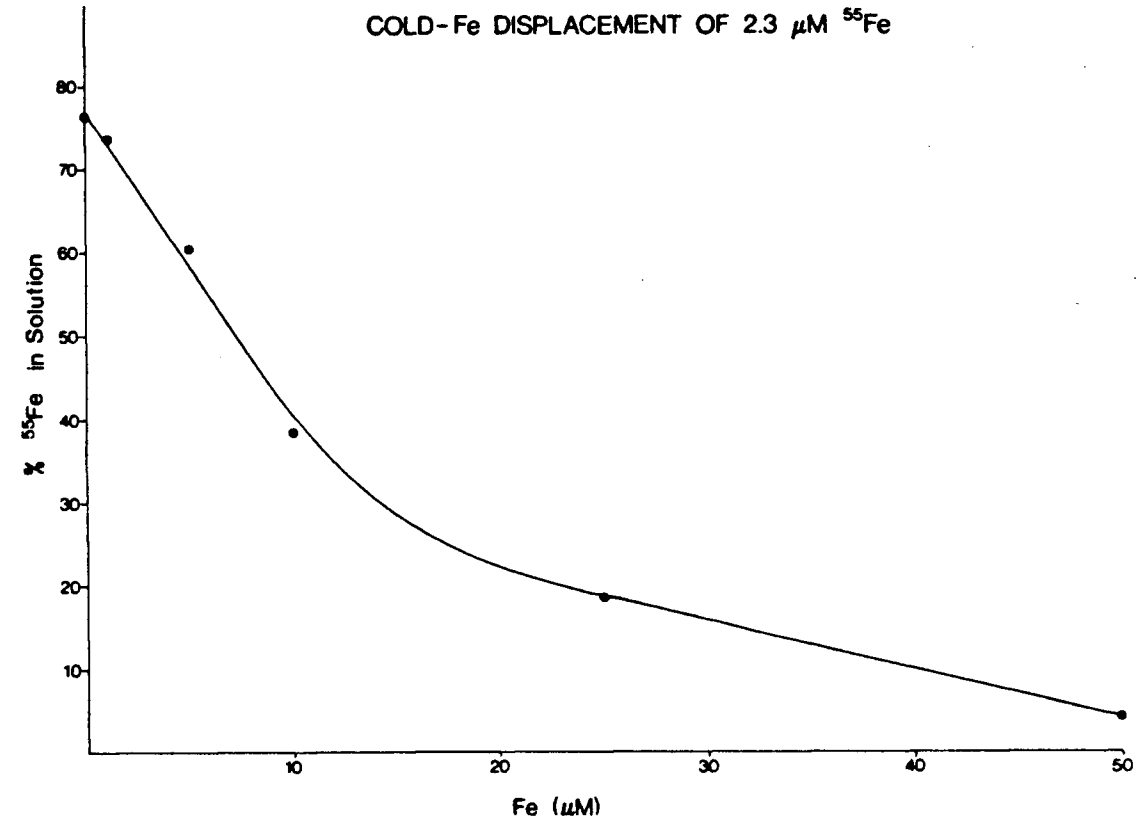
The iron-binding capacity assay also enabled an evaluation to be made of the specificity of the chelators for complexing iron. Relative to most organic compounds, siderophores have a high specificity for complexing iron. The siderophore isolate from Anabaena flos-aquae required two orders of magnitude more lanthanum or copper than iron to displace the radioactive iron (Fig. 18). Aluminum and chromium solutions had to be about three orders of magnitude more concentrated than unlabelled iron to displace the radioactive iron (Fig. 18). Solutions of calcium, cobalt, manganese, potassium, sodium, or zinc that were more than five orders of magnitude more concentrated than unlabelled iron, displaced little iron (Table 9).

Table 9 Effect of Metal Addition on Fe Chelation by the Siderophore Isolate from Anabaena flos-aquae.

Cation	Concentration[#] of Cation to displace Fe into solution	%Fe[*]
Ca	3.75 M	88, 87
Co	650 mM	87
K	1.63 M	94, 91
Mn	1.2 M	99, 99
Na	3.75 M	82, 88
Zn	6.5 M	88, 91

[#] highest attainable concentration in assay

[*] %Fe in solution, replicates



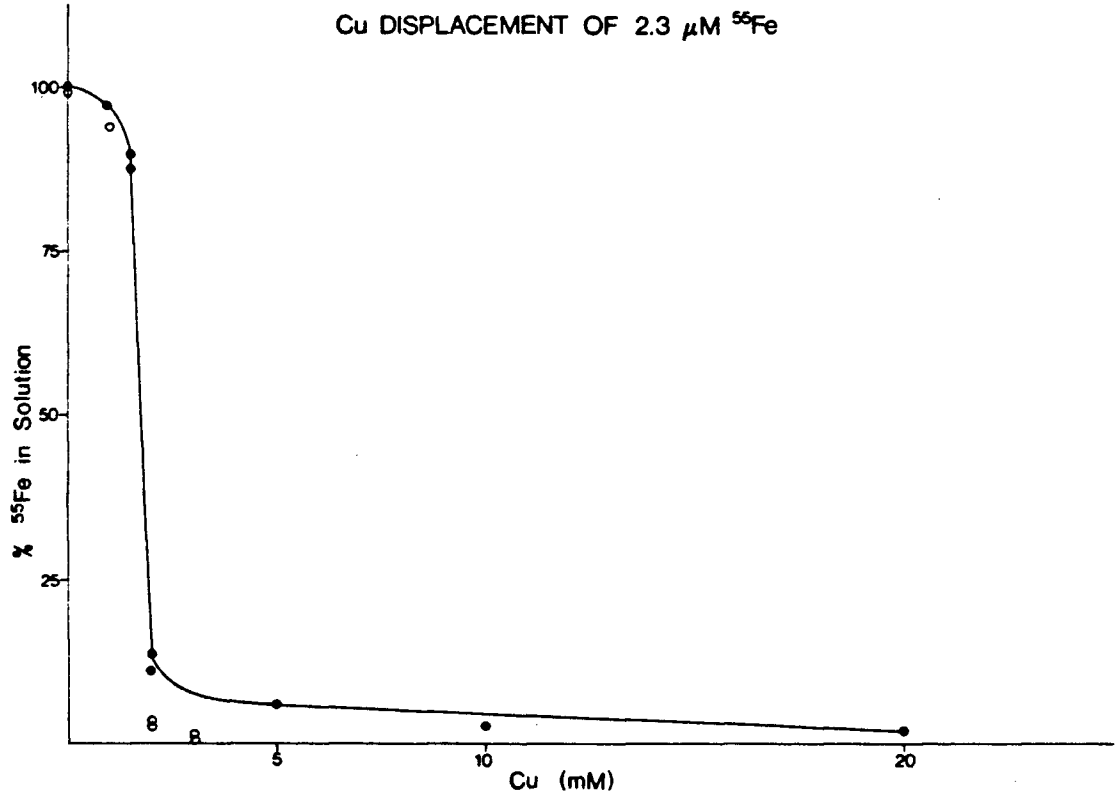
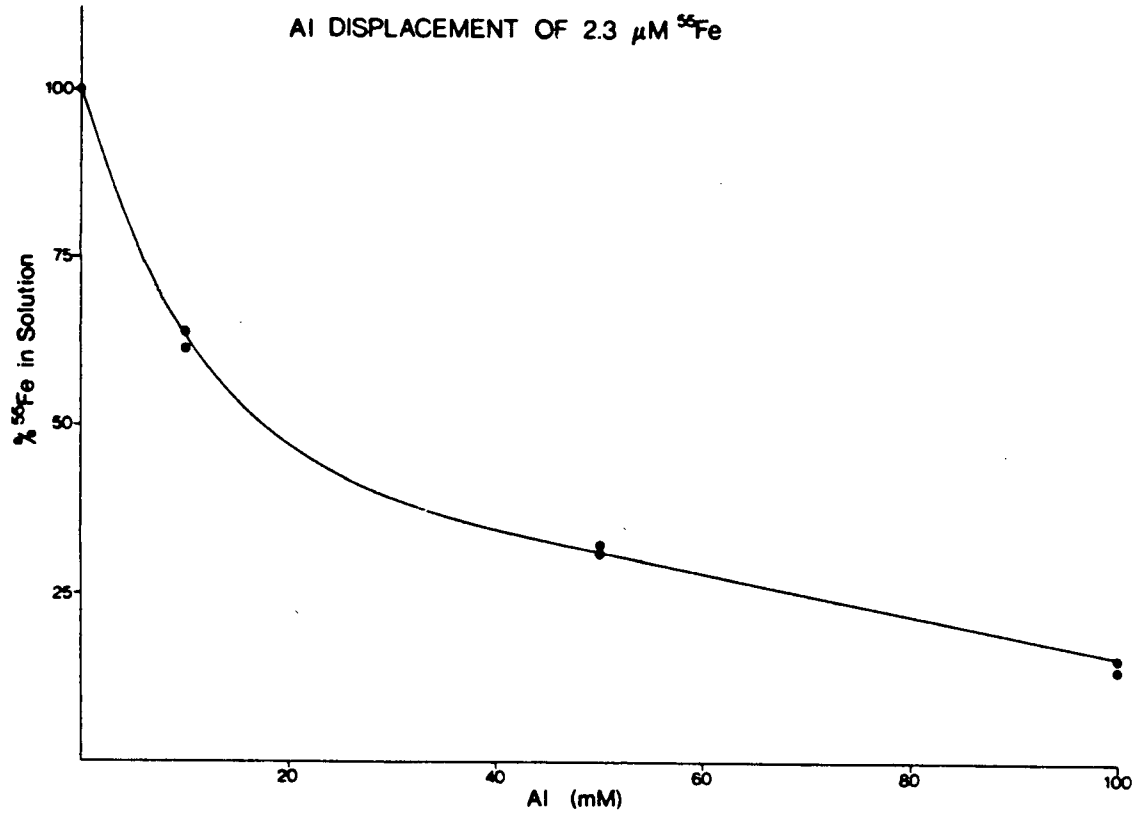
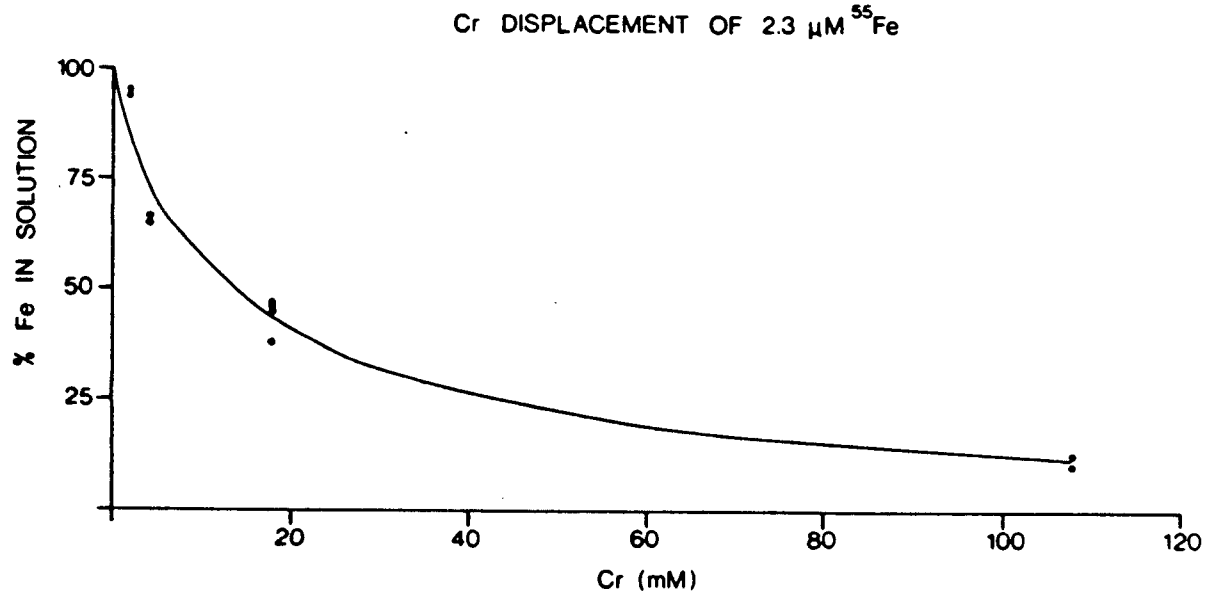


Figure 18 Metal displacement of iron from the Anabaena flos-aquae siderophore. Values are means of two replicates. Coefficient of variation is less than 3%.





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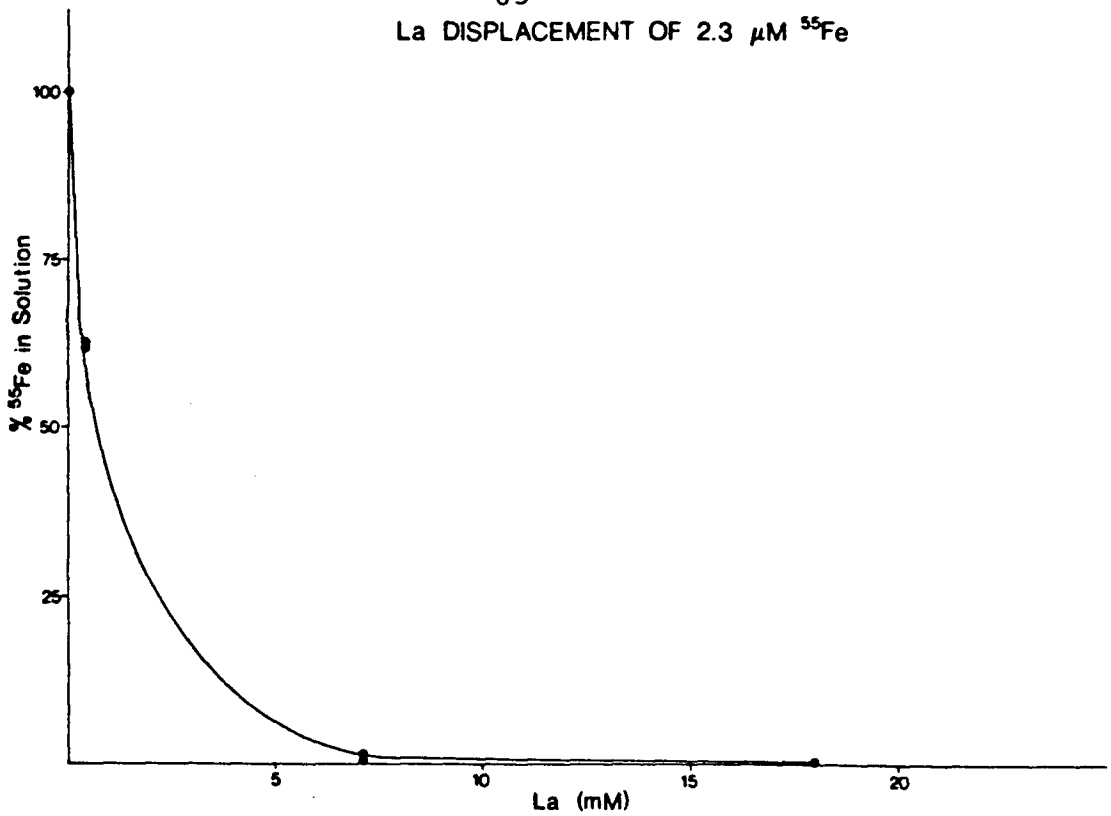

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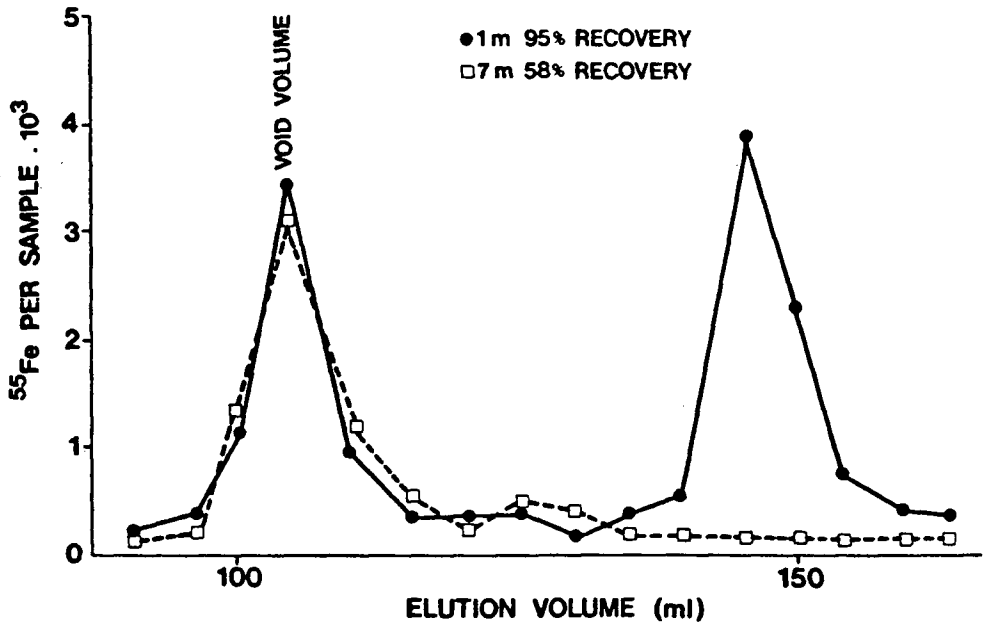

Figure 19 Elution of the ^{55}Fe-filtrate from an FeBC assay through a G-25 Sephadex column. The recovery refers to the proportion of ^{55}Fe that eluted through the column.

3.3.4 Lake Siderophores

The presence of low molecular weight chelators of iron in lake water could be observed either by the FeBC assay or a Sephadex-^{55}Fe assay. These assays indicated that chelators were present August 28, 1980 in the nitrate limnocorral, only in the surface water (Fig. 19). The low molecular weight peak (the compounds eluting later on a Sephadex column) contained a chelator. The high molecular weight peak could have been colloidal Fe-$MgCO_3$ that had not coagulated enough to be retained on a filter. The water from seven meters had comparatively little algal biomass and no apparent chelation capacity.

The chelator that was isolated from an Aphanizomenon bloom in Black Lake in 1982 was different from the Anabaena flos-aquae chelator in that the Aphanizomenon chelator did not have a hydroxamate group. The unconcentrated filtrate had a FeBC of 1.5 μM. Unlike the Anabaena flos-aquae chelator, concentrated solutions of sodium and cobalt suppressed iron chelation by the Aphanizomenon chelator (Table 10). In contrast to the Anabaena flos-aquae chelator, concentrated solutions of aluminum had little effect on chelation of iron by the Aphanizomenon chelator (Table 10). Although these chelators are very different, the chelation data indicate that both of these compounds are siderophores.

The iron binding capacity (FeBC) in the lake was never observed to be higher than 2 μM in 1980. The high levels of dissolved iron on Aug. 11, 1979 (Fig. 4) were observed in water supersaturated with oxygen, during a period of calcite precipitation. Since iron should be quite insoluble in this

Table 10 Effect of Metal Addition on Fe Chelation by the Aphanizomenon Siderophore

Cation	Concentration	%Fe in solution*
Al	0.25 M	69.9
Co	0.25 M	0.6, 0.55
Cu	1 mM	2.4, 2.4
Cu	0.1 mM	5.1, 4.1
Na	1.25 M	7.6, 7.8

* replicates

Table 11 Black Lake Iron Concentations and Chelation Capacity - July 17, 1980

Sample	FeBC μM Fe/L		Soluble Fe μg Fe/L		Particulate Fe μg Fe/L	
	1 m	7 m	1 m	7 m	1 m	7 m
C-1	0.88	0.35	<1	<1	40	80
Fe-1	350	230	150	200	80	100
EDTA-1	349	194	<10.*	<10.*	35	70
C-2	0.24	0.12	<1	<1	30	75
Fe-2	320	270	180	175	70	82
EDTA-2	204	331	<10*	<10*	70	80
NO_3	0.79	0.20	<1	<1	20	78
LC	1	0.24	<1	8.00	75	100
LA	1.40	0.25	<1	18.00	94	123

*EDTA interferes with bathophenanthroline reaction & digestion increased blank, samples with EDTA had to be diluted. LC - lake control side LA - lake aerated side. C-1, C-2 - control limnocorrals. Fe-1, Fe-2 - iron-EDTA limnocorrals.

water, the high iron concentration must have been maintained by either a high flux of iron from the hypolimnion or by about 4 μM of chelator (assumes 1:1 chelator to dissolved iron).

The chelator concentration in the epilimnion of Black Lake in 1980 was only weakly related to the algal biomass. However, hypolimnetic samples with minimal algal biomass had much less chelation capacity than the epilimnion (40% less chelation by FeBC assay, Table 11; 98% less chelation by gel-filtration assay, Fig. 19). The amount of chelation capacity in the lake was much less than in algal cultures. Even during the Aphanizomenon bloom of 1980, the FeBC of the lake (1.5 μM, chl a 150 μg/L) was much less than that observed in 20 day old blue-green algal cultures (Anabaena cylindrica 15 μM FeBC, 338 μg/L chl a; Anabaena flos-aquae 80 μM FeBC, 188 μg/L chl a). In spite of this large difference between lake water and culture filtrates, the chelation capacity of the lake water exceeded the dissolved iron concentration for much of the early summer. Thus, algal excretion could control iron bioavailability.

The differences in chelation capacity between lake water and culture filtrates may be a reflection of the utilization of siderophores in these two habitats. The algal cultures were unialgal and free of bacteria. In summer all lakes had one or two dominant algae, several rarer algae, and bacteria. If siderophores were important mediators of symbiotic associations or antibiotic competitions, the assimilation of siderophores should be quite different in culture medium and lake water. Bacteria may both consume and produce siderophores.

The rate of assimilation of ^{55}Fe-siderophore isolate, in an axenic culture of Anabaena flos-aquae, is relatively slow (<0.2 μM/d). However, rapid microbial assimilation of two ^{14}C-siderophore isolates was observed in Black Lake. The chelator from the Anabaena, that was isolated from Black Lake, was added back to lake water on July 4, 1979, at an equivalent concentration to the culture (FeBC not measured); 20± 1% of the chelator was utilized in one day.

In lake water samples from 1.0 m on June 17, 1979, 72 ±10% of the ^{14}C-labelled Anabaena cylindrica chelator (7 μM FeBC) was utilized in one day (5 μM/d). On July 4, 1979, 21 ±1% of this A. cylindrica chelator (7 μM FeBC) was utilized per day (1.5 μM/d). The reduction in chelator utilization from June 17 to July 4 was consistent with the seasonal increase in the iron content of the lake, the seasonal response of the in situ ^{14}C-primary production bioassays, the limnocorral bioassays, and the reduced effect of the Anabaena cylindrica chelator on primary production in late summer (Aug.30, A in Fig. 11).

3.3.5 Enhanced Iron Availability

The algal chelator isolated from a laboratory culture of Anabaena cylindrica greatly stimulated algal productivity in the lake in June but had less effect in July and little effect in August (A in Fig. 11). Unlike desferal and the chelators isolated from Anabaena flos-aquae and Scenedesmus basiliensis, the A. cylindrica chelator never caused algal cells to lyse. The former chelators deformed cells of two other Anabaena species and two Scenedesmus species at low concentrations (10-50 μM FeBC). At

higher concentrations these chelators could rupture cells of the above species.

The lack of antibiotic reactivity by the Anabaena cylindrica chelator makes it an excellent chelator to make iron available to algae. The stimulated algal productivity in June (Fig. 11) was probably a result of low levels of refractory iron being made more available to the algae. Some of the particulate iron may have been weakly adsorbed to humic matter.

The concentration of chelator used in the assay in Fig. 11 (5.0 µM FeBC) was two to five fold higher than any FeBC observed in the lake in summer of 1980, but the concentration would be much more dilute than in the phycosphere around the algal cells. As noted earlier, siderophores appear to be adsorbed weakly to the fibrils that are found on the surface of algal cells (3.3.2).

3.3.6 Allelopathic Properties of Siderophores

In Black Lake, Anabaena and Aphanizomenon had a significant portion of their biomass present as almost unialgal clumps. The macro appearance of the clumps was distinct enough to allow separation of two species of algae. Microscopic examination showed that the isolations were at least 95% pure. A chelator from the Anabaena clumps was isolated and added to fresh isolates of Anabaena or Aphanizomenon in the field. Five µM (equivalents of desferal) of the chelator isolated from the lake Anabaena stimulated the primary production of Aphanizomenon but not Anabaena (Fig. 20). Presumably, prior to my addition of chelator, the Anabaena chelator satisfied the Anabaena iron requirement, but Aphanizomenon was not receiving an adequate supply of iron.

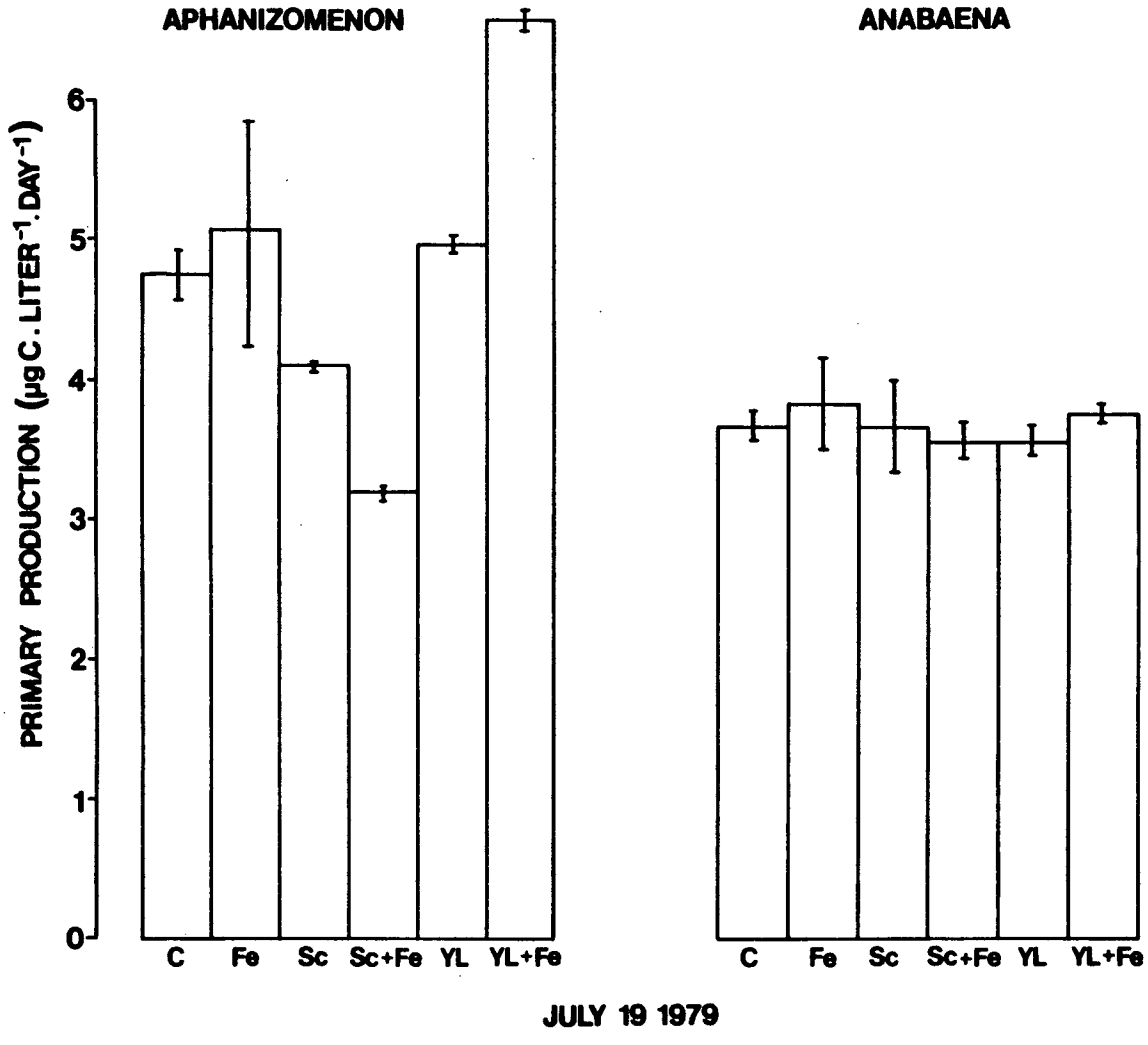

Figure 20 A demonstration of siderophore specificity. The effect of the control (C), 10 µM Fe/L (Fe), 5 µM Scenedesmus basiliensis chelate (Sc), 5 µM Scenedesmus chelate + 10 µM Fe/L (Sc+Fe), 5 µM chelate isolated from the Yellow Lake Anabaena (YL), and 5 µM YL chelate + 10 µM Fe/L (YL+Fe) on primary production of recent isolations of Aphanizomenon and Anabaena. Values are means of two samples. Error bars are one standard deviation.

The specificity of the chelator activity was also shown by the chelator isolated from a laboratory culture of Scenedesmus basiliensis (Sc., Fig. 20). Five μM of the Scenedesmus chelator suppressed primary production of Aphanizomenon, but not Anabaena. This suppression was stronger if the chelator was saturated with iron; thus, the chelator did not suppress Aphanizomenon by depriving it of iron. The enhanced toxicity may indicate that Aphanizomenon treated the Scenedesmus chelator as a source of iron; thus, the additional iron stimulated uptake of the toxic chelator. The results from Black Lake indicated that Aphanizomenon may require more iron than Anabaena.

The Aphanizomenon chelator was able to suppress the growth of competing species. The chelator was very toxic to Scenedesmus basiliensis when the siderophore isolate was not saturated with iron (Fig. 21). The toxicity could have been related to an enhancement of iron deprivation.

3.3.7 Siderophore Influence on Heterotrophy

Since siderophore isolates influence blue-green algal growth, siderophores probably influence the growth of many other bacteria. The assimilation of ^{14}C-labelled organic compounds was used to resolve the effect of siderophore isolates on bacteria. An additional control was used to test the possibility that other low molecular weight organic compounds were in the algal filtrate and were suppressing the uptake of the ^{14}C-labelled substrate.

In this control, the same techniques that were used for siderophore isolation were used to obtain a fraction from a culture of Anabaena flos-aquae that was grown with iron. This

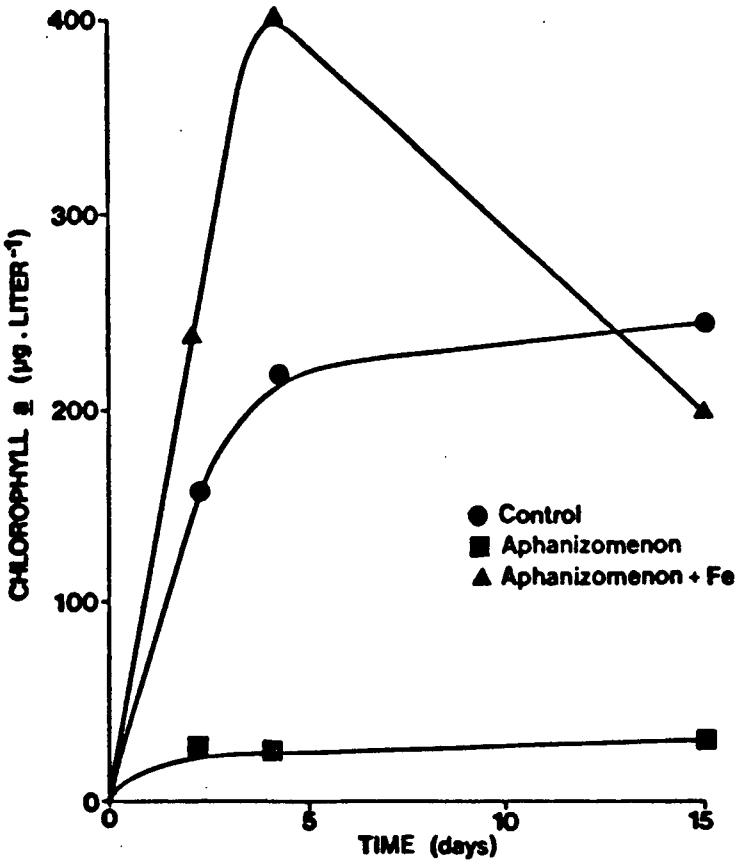

Figure 21 The effect of the Aphanizomenon siderophore on the growth of Scenedesmus basiliensis. Values are means of two cultures. Coefficient of variation is less than 10%.

Table 12 Effect of Iron Availability on the Toxicity of Anabaena Filtrates to Microbial Acetate Assimilation.

Algal Growth Conditions	Lake Water[#] Treatment	Gross Uptake Rate $\mu g\ C\ L^{-1}\ h^{-1} \times 10^{-2}$
Iron limitation	Algal extract contains chelator	2.39
Iron addition	Algal extract without chelator	10.85
----	Control no algal extract added	5.76

* acetate concentration=2.75 µg C/L. # Water collected at 1 m from control side of Black Lake on July 31, 1980.

culture should not have produced any siderophore (Murphy 1976). The isolate from the iron-saturated culture stimulated heterotrophy while the isolate from an iron-deficient culture depressed heterotrophy (Table 12). The stimulation of heterotrophy could indicate that iron was being made available to iron-limited bacteria or that a low molecular weight compound was stimulating heterotrophic assimilation of acetate. Fortunately, the stimulation did not prevent the use of heterotrophic bioassays in studies of siderophore-bacteria relationships; the algal siderophore isolates suppressed bacteria.

The ability of three algal siderophore isolates to suppress heterotrophic activity was strong (Fig. 22). Bacterial heterotrophy in samples from all water depths was suppressed, and the greatest suppression occurred in the hypolimnion. The suppression occurred equally well when these chelators were saturated with iron. Thus, the suppression was not related to an enhancement of iron deprivation.

The inhibition by the siderophore isolates appeared to be mediated by two reactions. The Anabaena siderophore isolates (400 µM FeBC) enhanced respiration of ^{14}C-labelled acetate much more than either the Scenedesmus siderophore isolate (10 µM FeBC) or control incubations (shaded area of Fig. 22). Another difference in the processing of the siderophore isolates was revealed in the effect of glucose on siderophore isolate toxicity (Fig. 23). The two siderophore isolates inhibited glucose uptake when the concentration of glucose was low. At higher glucose concentrations, the inhibitory effects of the Anabaena (400 µM FeBC), but not the Scenedesmus (10 µM FeBC) siderophore isolates,

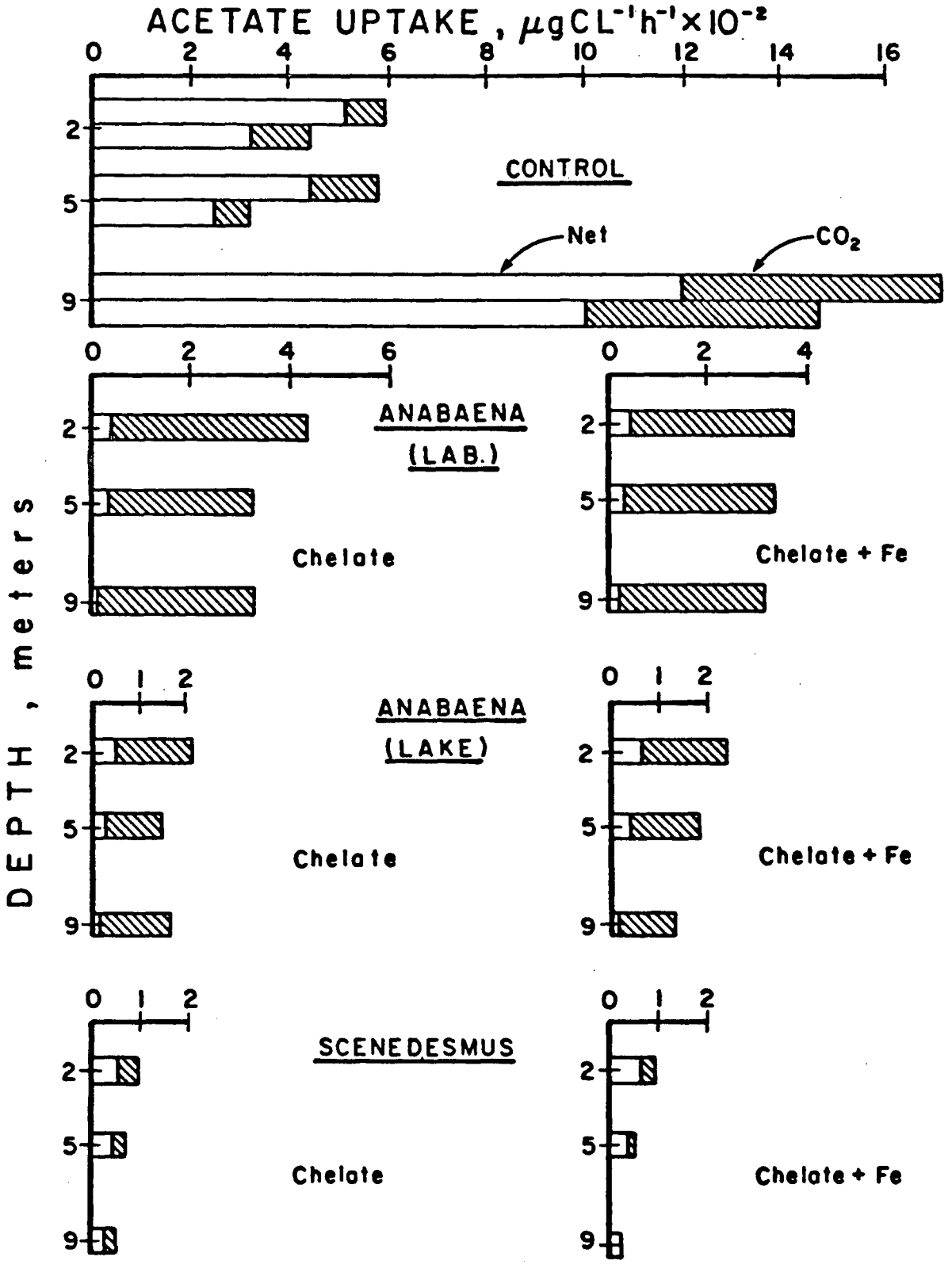

Figure 22 Suppression by siderophore isolates of bacterial assimilation of acetate. Samples from 2, 5, and 9 meters were incubated at those depths with 3 siderophore isolates and acetate. (\\\) represents acetate respiration.

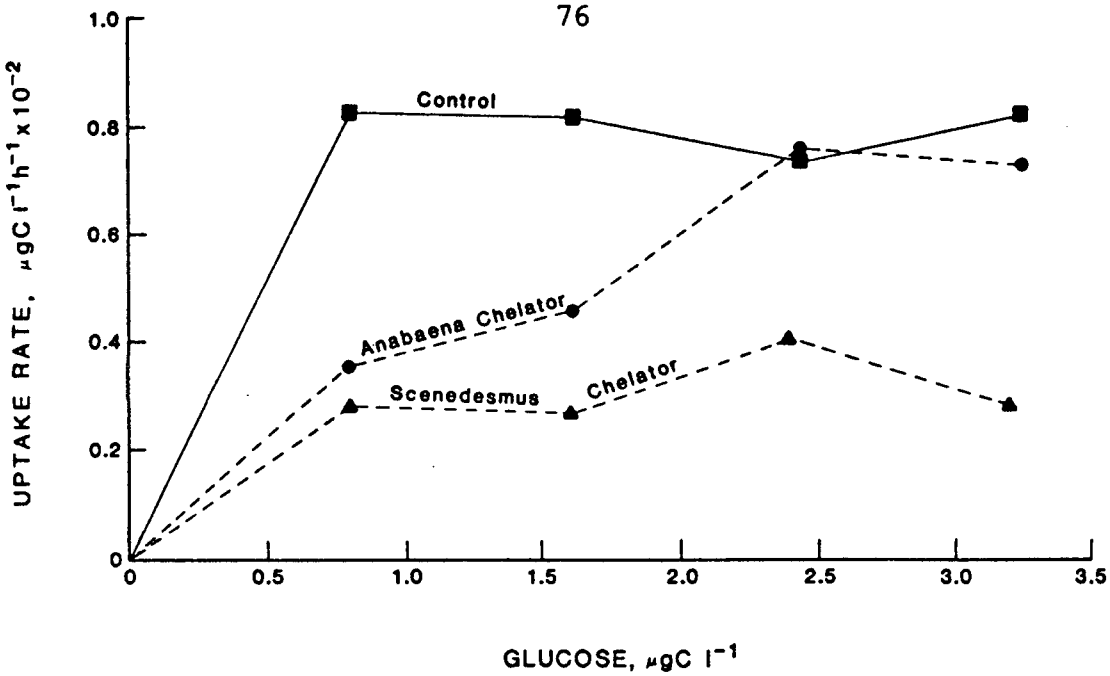

Figure 23 Effect of glucose on siderophore suppression of heterotrophy.

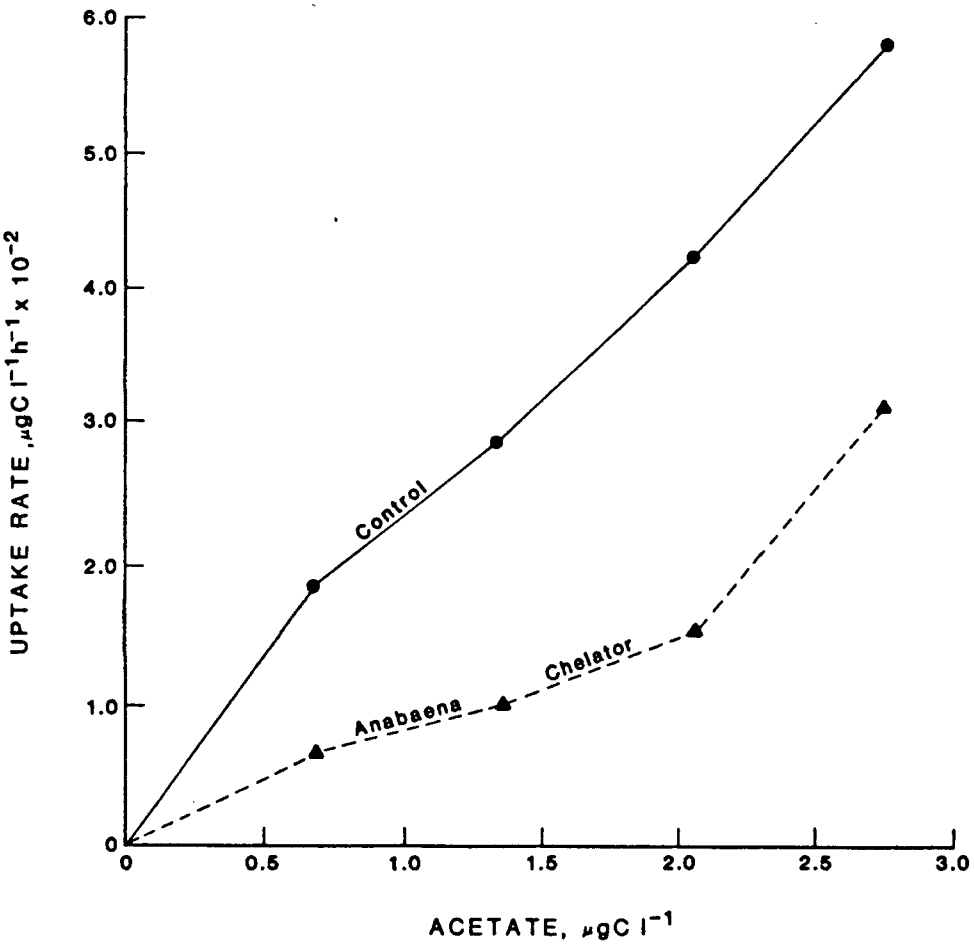

Figure 24 Effect of acetate on siderophore suppression of heterotrophy.

were overcome. The toxicity of the Anabaena siderophore isolate could not be overcome by high concentrations of acetate (Fig. 24).

The seasonal pattern of heterotrophic utilization of glucose in Black Lake formed a pattern that indicated suppression of bacterial activity (Fig. 25). A week after the collapse of the diatom bloom (May 11, 1979), the greatest uptake of glucose was observed. A week after the collapse of a denser Anabaena bloom (July 18, 1979), the lowest metabolic activity was observed (Fig. 25). The bacterial activity increased after the collapse of the Aphanizomenon bloom (Aug. 31, 1979), but the activity was not as high as the spring value. Two factors explain the difference between the two blue-green algal blooms. 1) The Anabaena siderophore isolate suppressed bacteria heterotrophy (Fig. 22) 2) The Aphanizomenon bloom was terminated by calcite precipitation which lysed algae and appeared to enhance bacterial activity.

Siderophore ecology was not studied in all lakes. However, the phosphorus geochemistry indicated that Black Lake was very similar to Frisken Lake and both of these lakes are quite different from Chain Lake. Siderophore reactions are probably more important in Black and Frisken lakes than in Chain Lake because the algae in Chain Lake would not need to produce siderophores.

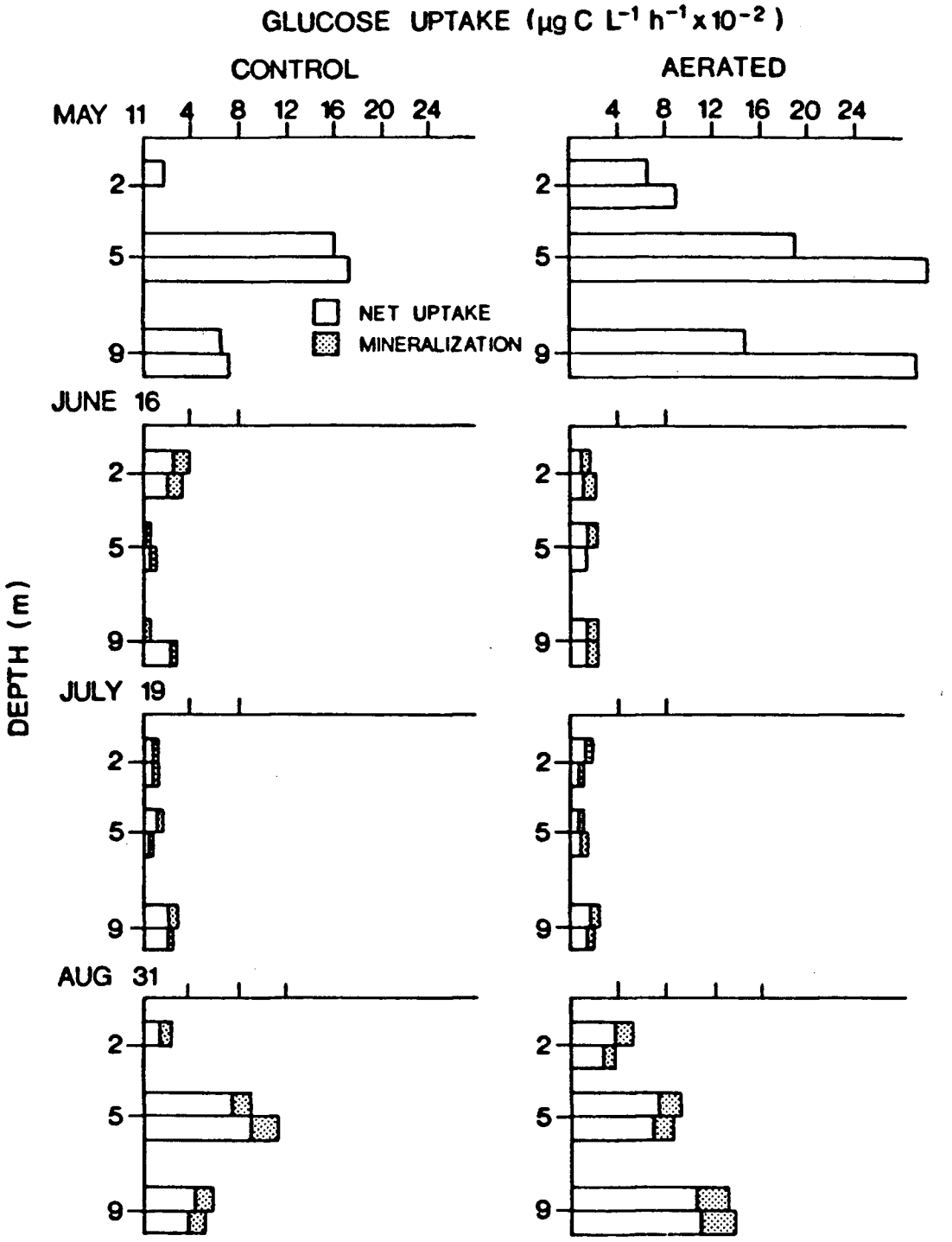

Figure 25 Temporal variation of heterotrophy in Black Lake.

3.4 Effect of Iron Availability on Phosphorus Chemistry

3.4.1 Geographic Variability in Fe-P Water Chemistry

The most striking difference between Chain Lake and Frisken and Black lakes was the seasonal change of phosphorus concentrations (Fig. 26). In Chain Lake, the concentration of soluble reactive phosphorus (Fig. 27) or total phosphorus (Fig. 28) was high only if the oxygen concentration was low (Fig. 7). In Black or Frisken lake, changes in oxygen concentration was not correlated with changes in phosphorus concentration. In contrast to Chain Lake, Black Lake (Murphy et al. 1983a,b) and Frisken Lake (Murphy et al. 1985) had very little reactive iron. Therefore, the best geochemical hypothesis for this difference was that higher reactive iron concentrations resulted in much more ferric phosphate precipitation in Chain Lake than in Black or Frisken lake.

In periods of high phosphorus concentrations in Chain Lake in 1973, 1976 (Fig. 3 and 7 in WIB 1977), and 1983 (Fig. 27) the lower water column was anoxic (Figure 7). The iron data support the hypothesis that in anoxic water, iron in the surface sediments is converted from insoluble ferric iron to the more soluble ferrous iron. The iron content of Chain Lake doubled from July 6, 1984 to September 14, 1984 (Table 3). The anoxic water contained a mean of 1.1 mg/L of iron.

The changes in water chemistry during November 1983 in Chain Lake were consistent with the precipitation of ferric phosphate or another iron-phosphorus complex. The oxygen concentration had increased from 4.4 to 8.0 mg/L. Similar changes occurred in 1974 and 1975 (Fig. 3 and p. 34 in WIB 1977).

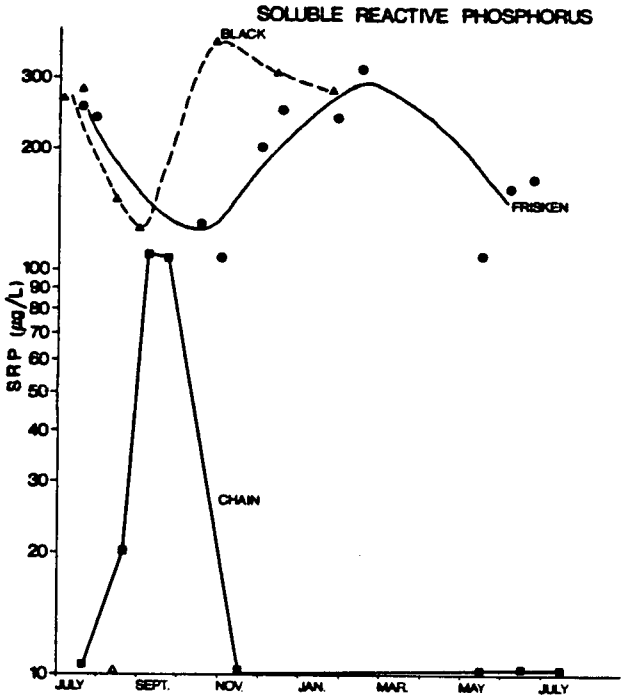

Figure 26 Seasonal comparison of soluble reactive phosphorus (SRP) concentrations in Black, Chain, and Frisken lakes. All Values are means of two samples. Black and Frisken lake values are means of two stations.

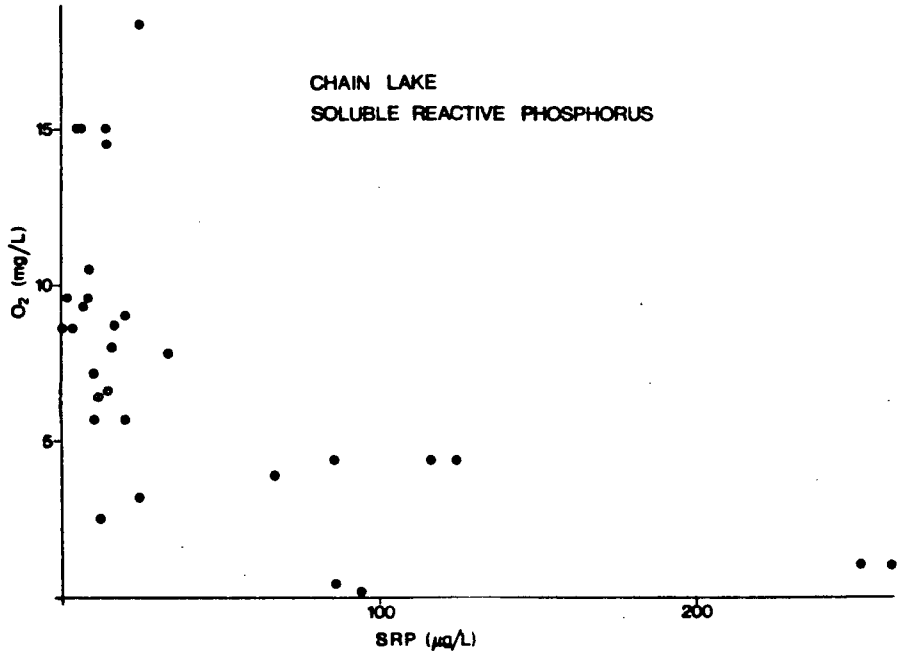

Figure 27 Chain Lake soluble reactive phosphorus (SRP). All values are means of two samples.

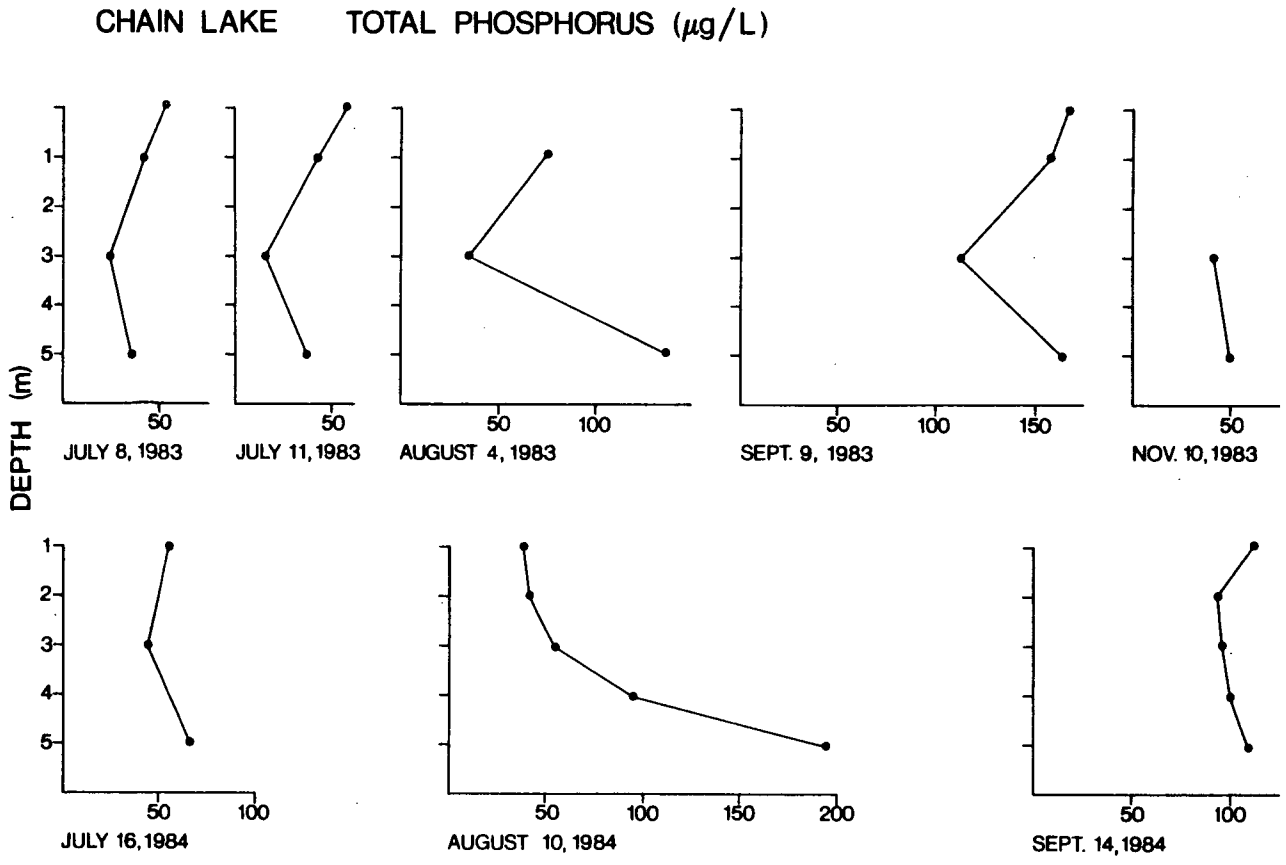

Figure 28 Chain Lake total phosphorus.

3.4.2 Geographic Variability in Sediment Iron Reactivity

The differences between the water chemistry of Chain Lake and Black and Frisken lakes are recorded in the lake sediments. The fluxes of iron into and out of the sediments provide insight into iron availability. Complete iron budgets can only be calculated for lakes that have pyrite data and lead-210 radiochemical dating (Chain and Frisken lakes, Table 13).

A comparison of the budgets has one large bias; the sediments of Chain Lake are relatively flat but the sediments of Frisken Lake have much more slope (Fig. 2). Resuspension from shallower sediments should result in enhanced sedimentation in the relatively small deep basin of Frisken Lake (sediment focussing). The discrepancy between the sedimentation of iron and hydraulic iron loading (Table 13) supports the hypothesis that the Frisken Lake sediment core exaggerates the net iron sedimentation of the whole lake. Thus, the higher flux of iron into Chain Lake relative to Frisken Lake (Table 13), is greater than the sediment core data indicate. The external iron loading is more than 200 fold higher and pyrite formation occurs more than 10 fold faster in Chain Lake.

Table 13 Fluxes of Iron in Chain and Frisken Lakes

Flux	Frisken Lake	Chain Lake	Chain/Frisken
Hydraulic Fe Loading[#]	3	730	240
Sedimentation Rate Pb-210[*]	1.25	7.8	6
Net Fe Sedimentation[#]	120	470	4
Retention of Fe in lake (%)	4000	47	-
Iron Reduction To Pyrite[#]	24	282	12
Sediment[**] Fe Efflux	35	238	7
Organic-C Sedimentation[##]	1400	4000	3

[#] $\mu g\ Fe\ cm^{-2}\ yr^{-1}$. [*] Sedimentation in mm per year. [**] Increase in water column iron from June to August, $\mu g/cm^2$. [##] $\mu g\ C\ cm^{-2}\ yr^{-1}$.

The proportion of iron found as pyrite, and the stability of ferric iron, are different in the sediments of Chain and Frisken lakes respectively. In the Chain Lake sediment core, the concentration of pyrite increases until about 60% of the iron is present as pyrite; iron in the sediment core from Frisken Lake of the same age as the bottom of the Chain Lake core is 20% pyrite.

Relative to Chain Lake, much more of the sediment iron in Frisken Lake appears to be stable as ferric iron. Iron in sediments that had precipitated fifty years ago (the bottom of the Chain Lake core) is 30% ferric iron. Iron in the Frisken Lake

sediments of the same age as the bottom of the Chain Lake core is 60% ferric iron. If differences in oxygen concentration produced this difference then Chain Lake would have less oxygen. However, the water overlying the sediments of Frisken Lake has less oxygen than does Chain Lake (Fig. 7). X-ray diffraction analysis indicated that in both lakes, some of the surface metastable iron is in chlorite. Most of this metastable iron could not be characterized; this technique can not measure iron bound to humic matter.

In both Chain and Frisken lakes, the amount of iron released from the lake sediments was similar to the rate of pyrite formation; thus, pyrite formation is an index of iron reactivity. Since most chemical variables are more favorable for pyrite formation in Frisken Lake than in Chain Lake, and Frisken Lake has proportionally less pyrite, these results indicate that the reactivity of iron is much lower in Frisken Lake than in Chain Lake.

The distribution of iron, calcium, and phosphorus in Chain Lake indicates that geochemical reactions do not restrict phosphorus mobility. The iron and calcium content is relatively constant, whereas the phosphorus content is enriched in the surface sediments. The phosphorus content was not correlated significantly to calcium ($r=0.53$) or iron content ($r= -0.11$) (Fig. 29). Carbonates are too dilute to control phosphorus solubility. The top two cm of sediment is composed of only 2.1% calcium carbonate and the deeper sediments have no detectable calcium carbonate. With the probable exception of the surface sediments, iron is also unable to influence phosphorus

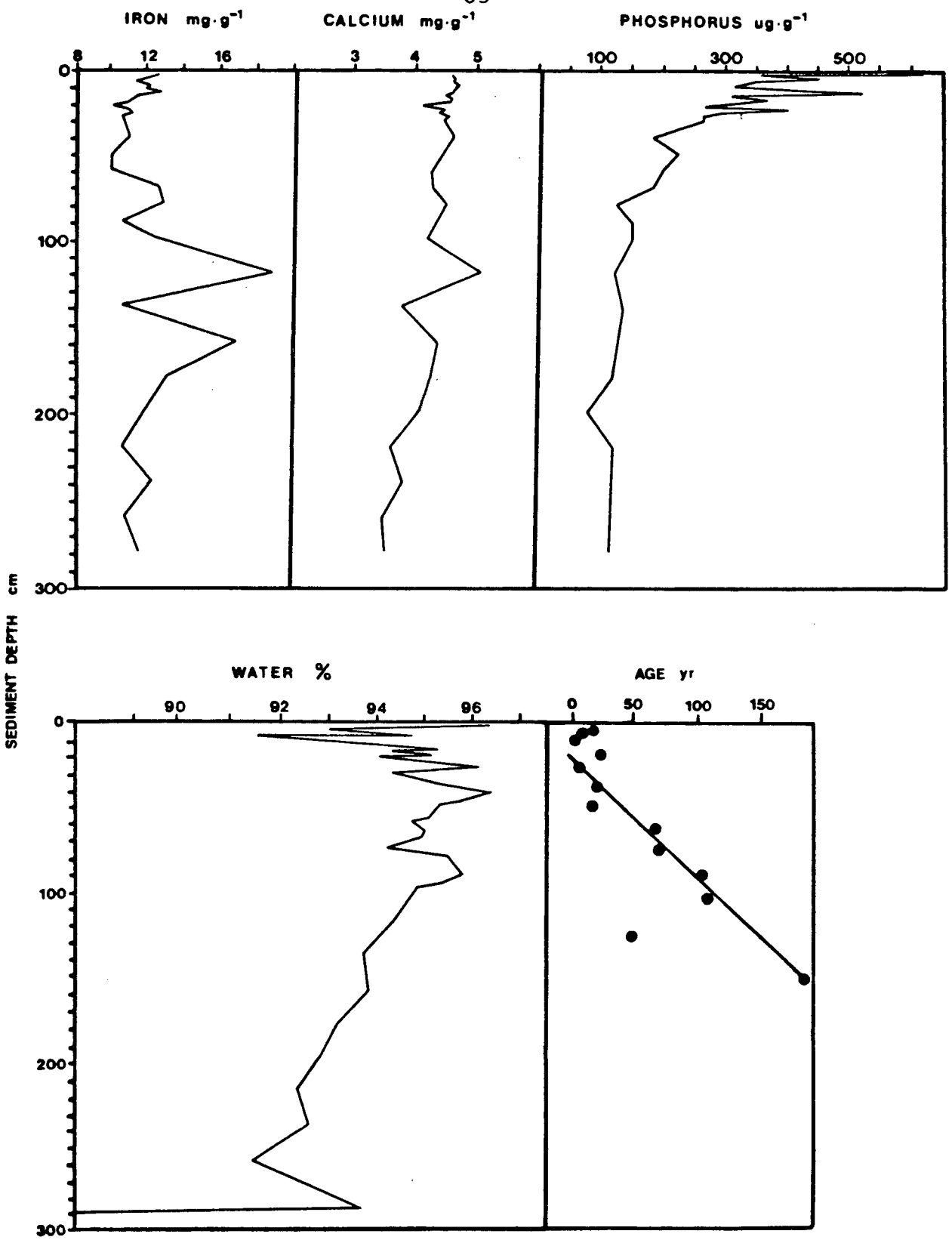

Figure 29 Chain Lake sediment chemistry; acid extractable iron and calcium, bioavailable phosphorus, water content, and age.

solubility.

In Frisken, Roche (downstream from Frisken Lake), and Yellow (downstream from Black Lake) lakes, the phosphorus content of the surface sediments was inversely correlated to the iron content (Frisken Lake r=-0.81, n=6; Roche Lake r=-0.86, n=7; Yellow Lake r=-0.86, n=7). The inverse correlation was a reflection of the strong control over phosphorus by calcium carbonate (Frisken Lake r=0.95, n=7; Roche Lake r=0.80, n=6; Yellow Lake r=0.95, n=7). The sediments contain over 30% calcium carbonate (Fig. 30, 31). X-ray diffraction analysis indicated that the only carbonate mineral was calcite.

3.5 Effect of Iron Availability on Calcite and Phosphorus Precipitation

The unusual biogeochemistry of the Thompson Plateau enabled the reactions between phosphorus and calcium to be used to assess iron availability.

3.5.1 Phosphorus Chemistry of Black Lake

The soluble reactive phosphorus (SRP) content of the Yellow Lake Creek that entered Black Lake ($\bar{x}$=257 µg/L) was very similar to that of the lake (mean integrated concentration varied from 228 to 379 µg/L). The SRP in the Yellow Lake Creek and the exposed volcanic rock extract appeared to be orthophosphate. The SRP coeluted through G-25 Sephadex beads with $^{32}PO_4$ (Fig. 32). Sephadex resin separates phosphorus from any organic-P of a different molecular size (Lean 1973). The arsenic content of the stream (25$\pm$ 2 µg/L, n=2) and volcanic rock extract (30 µg/L) were

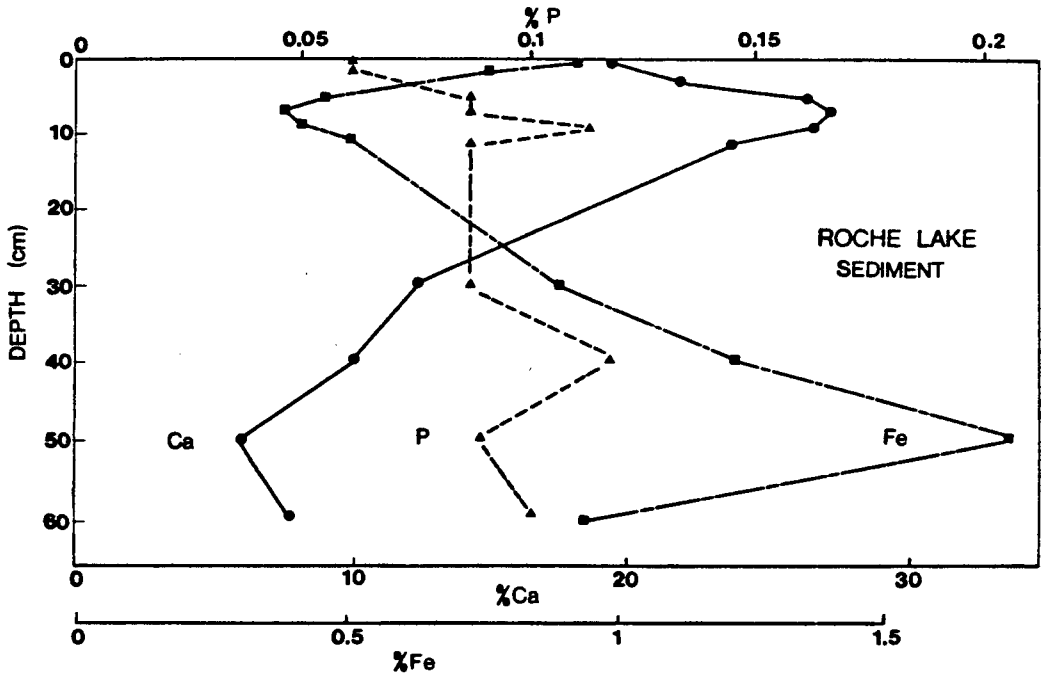

Figure 30 Roche Lake sediment chemistry; percent of total calcium, iron, and phosphorus in sample.

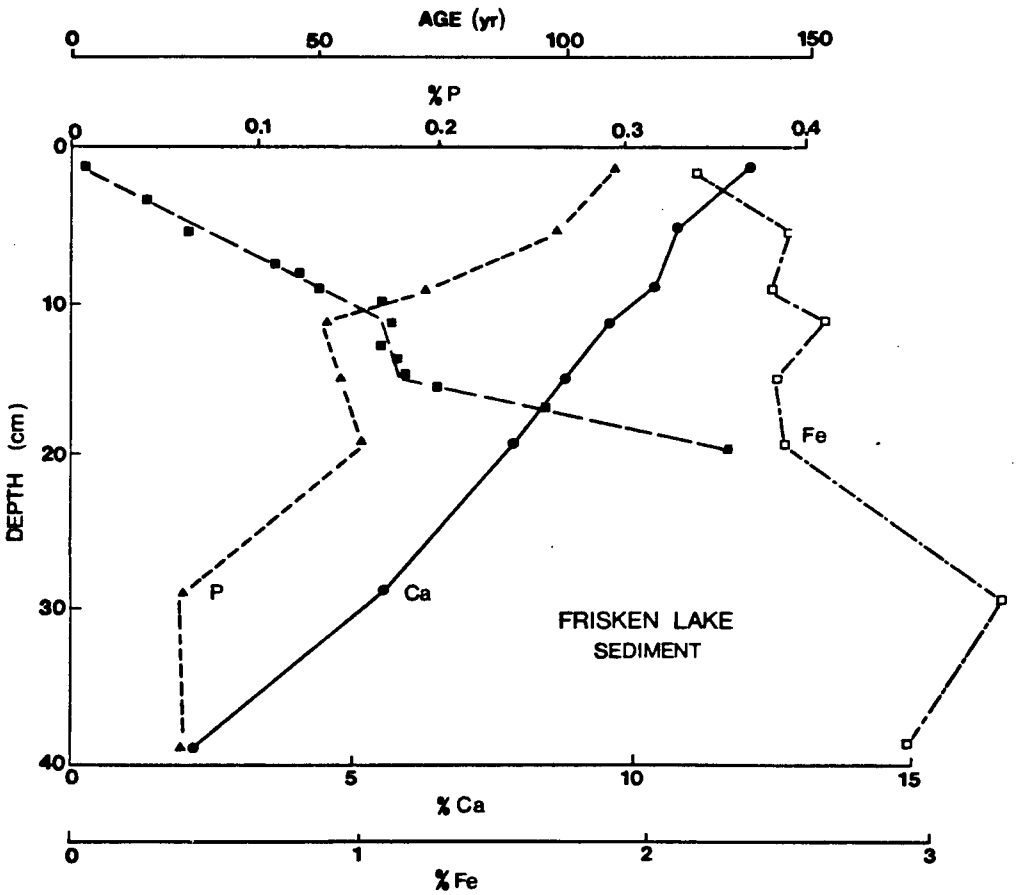

Figure 30 Frisken Lake sediment chemistry; percent of total calcium, iron, and phosphorus in sample.
(■) is the age of the sediment.

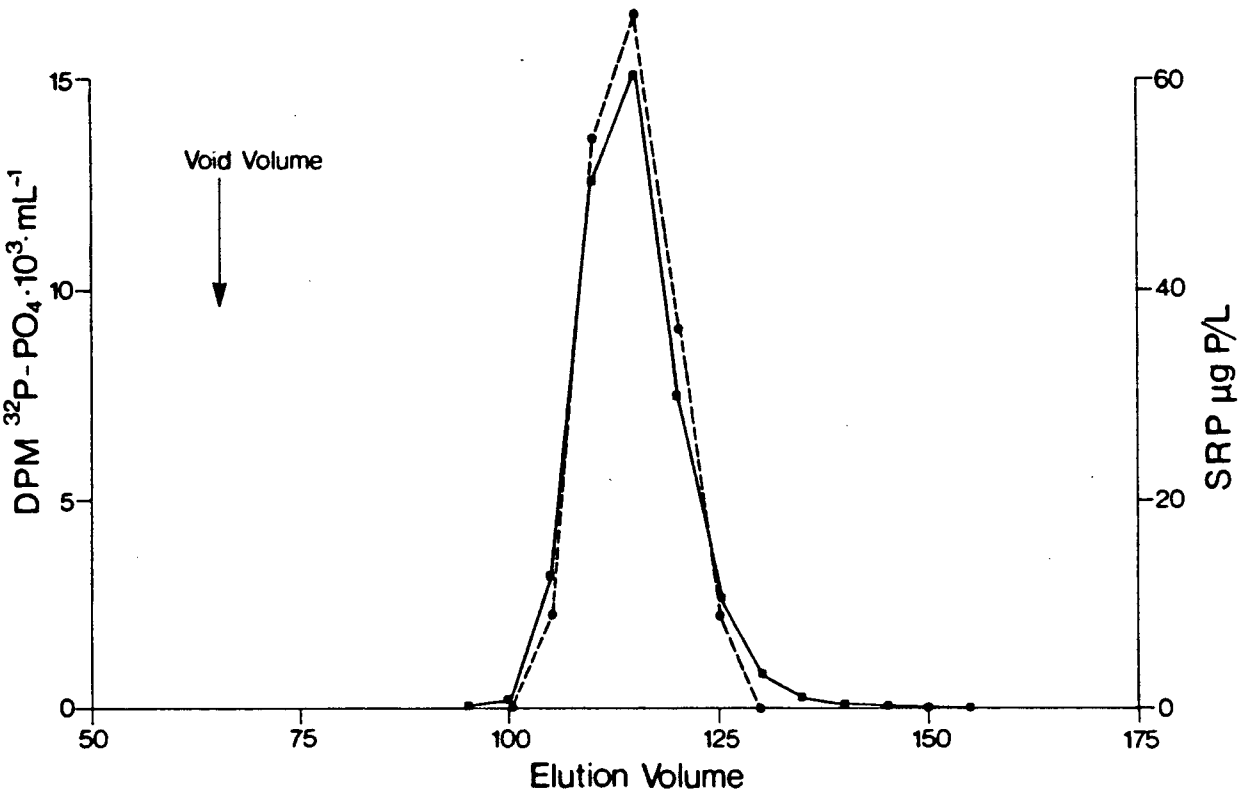

Figure 32 ^{32}P-phosphate analysis in Black Lake Creek. Soluble reactive phosphorus (SRP, ●) and ^{32}P content (■).

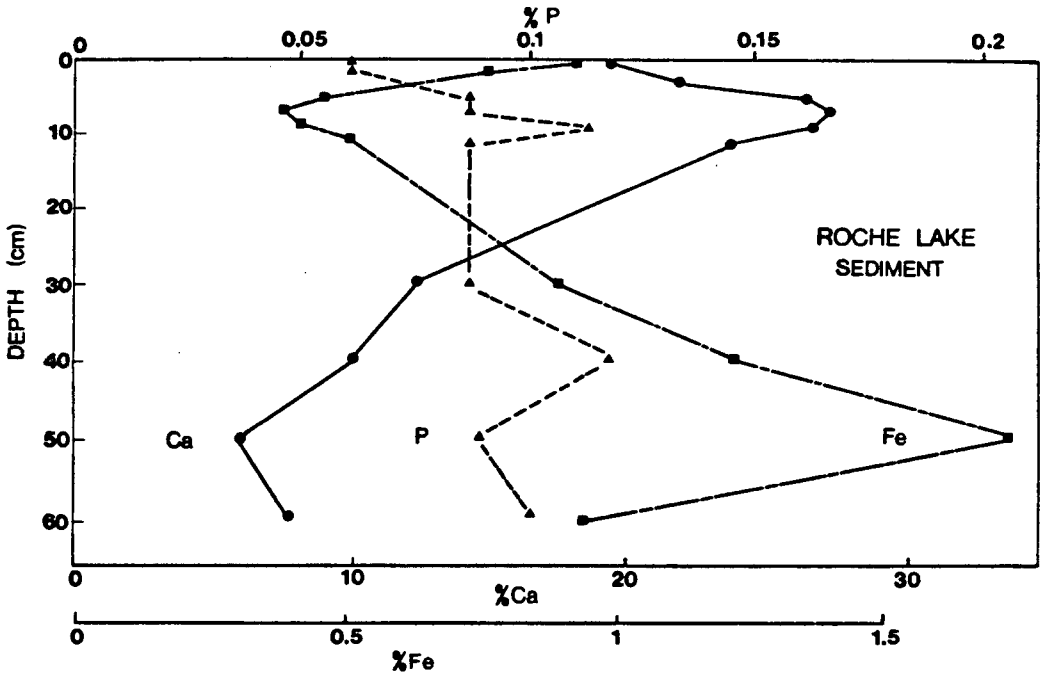

Figure 30 Roche Lake sediment chemistry; percent of total calcium, iron, and phosphorus in sample.

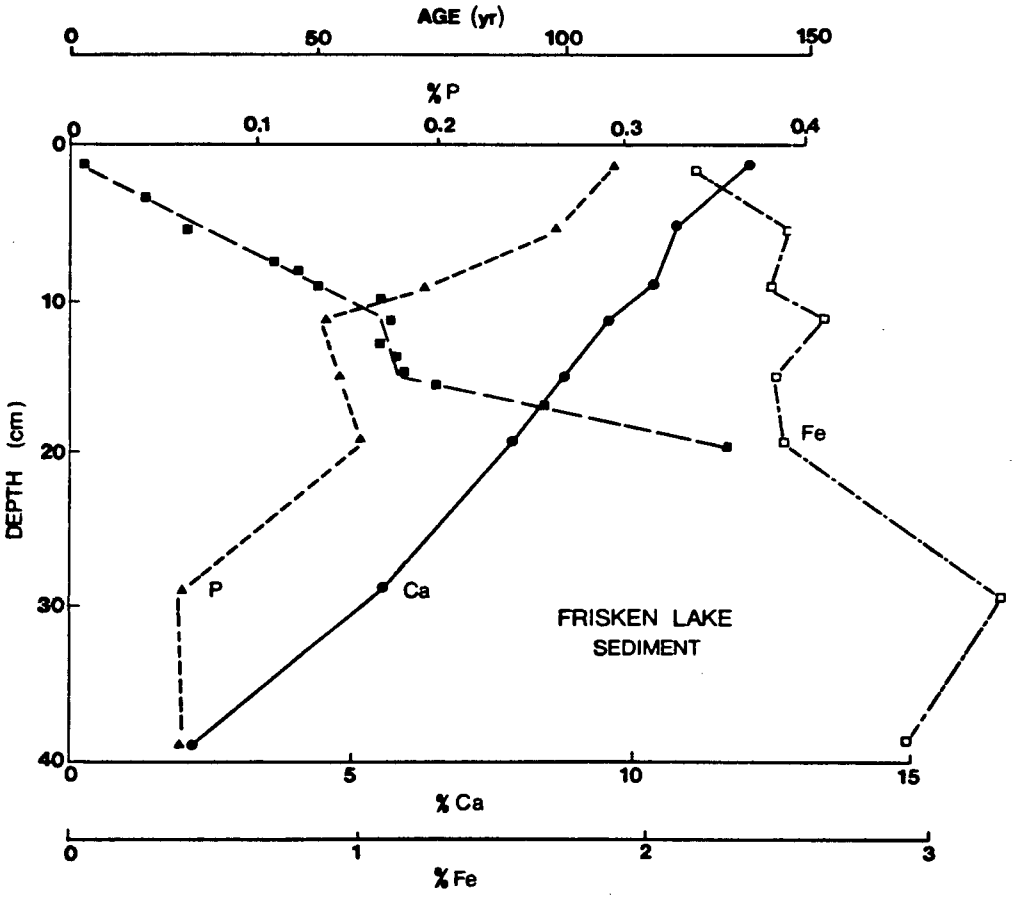

Figure 30 Frisken Lake sediment chemistry; percent of total calcium, iron, and phosphorus in sample.
(■) is the age of the sediment.

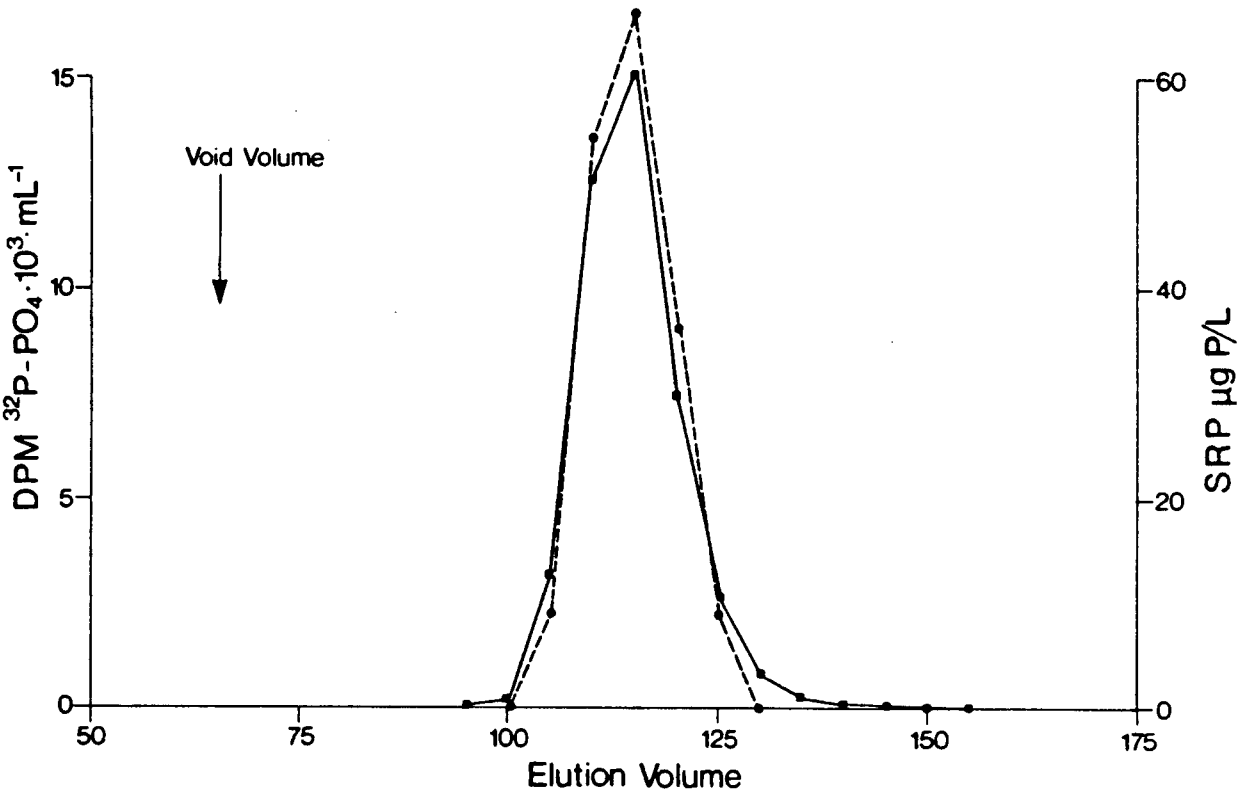

Figure 32 ^{32}P-phosphate analysis in Black Lake Creek. Soluble reactive phosphorus (SRP, ●) and ^{32}P content (■).

less than 10% of the phosphorus content of the samples; thus, arsenic could not have interfered with my analysis. Similarly, the organic phosphorus content of four samples was small (5-20 µg/L). The silica content of the volcanic rock extract (20 mg/L) was too dilute to interfere in the phosphorus analysis (APHA 1976).

3.5.2 Calcite Precipitation in Black Lake

During the Aphanizomenon bloom of 1979, large quantities of carbonate were observed precipitating on the incubation bottles on the iron-rich aerated side of the lake. The salts on the bottles effervesced vigorously when treated with 0.1 N HCl. These salts were not observed in the control side of the lakes. At this time, the pH of the lake surface increased while the alkalinity and phosphorus decreased with respect to samples collected two weeks earlier (Fig. 33). Phosphorus precipitation in the top 1.5 m of the Aphanizomenon bloom was at least 250 µg P/L in the two week period prior to August 11, 1979. The alkalinity and phosphorus content of the hypolimnion increased.

The 1980 observations contrast sharply with observations during the Aphanizomenon bloom of 1979 (Fig. 33). Calcite was not observed, alkalinity was constant, and the phosphorus concentration decreased slightly. In the two weeks before the 12 August 1980 sampling of the Aphanizomenon bloom, less than 10 µg P/L precipitated in the surface 1.5 m of water in both sides of the lake. The algal biomass and water stratification in 1980 was similar to that of 1979 (Fig. 33). These results were surprising, because the lake water in 1980 was 10-fold and 19-fold

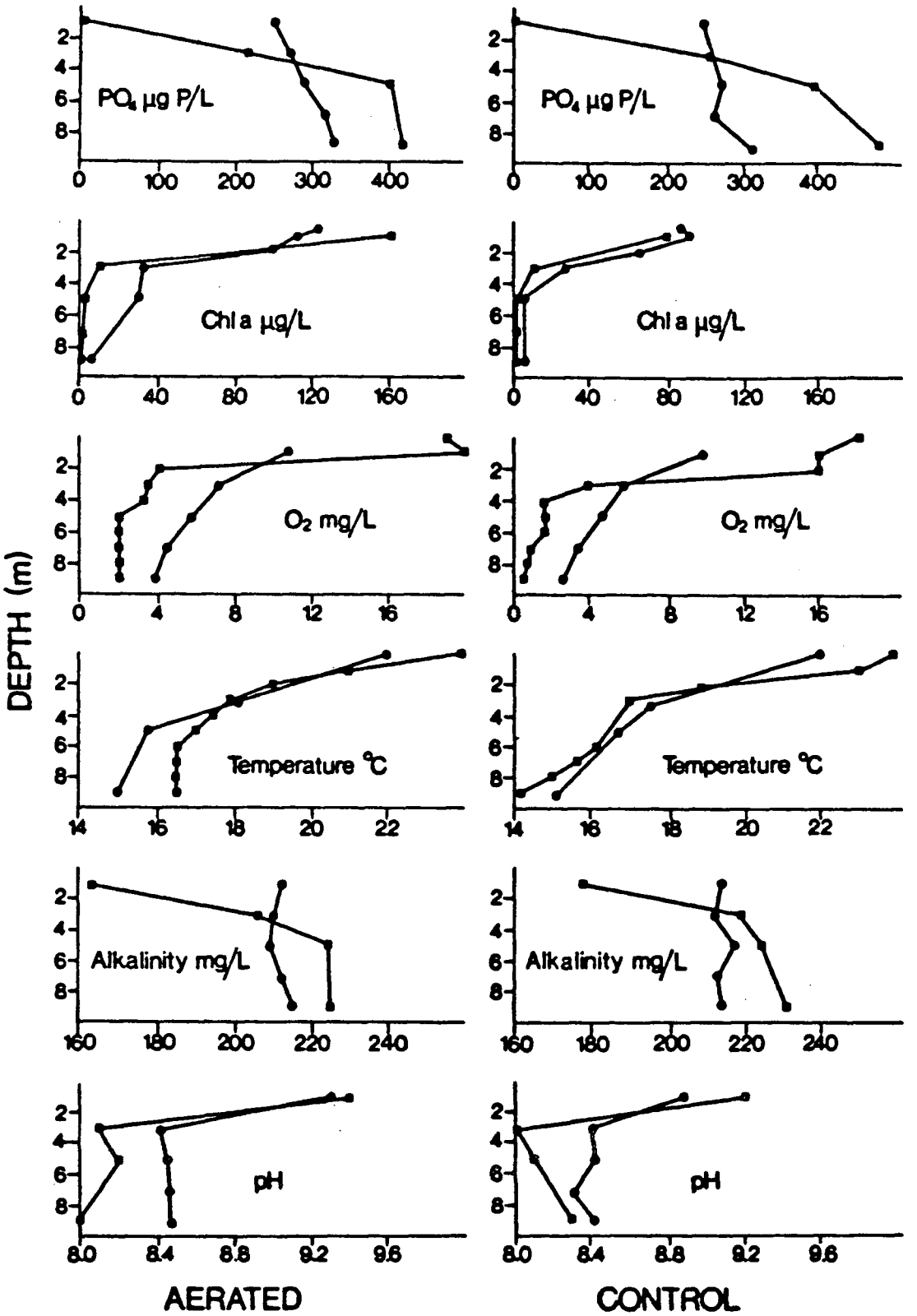

Figure 33 Lake chemistry during Aphanizomenon blooms; total phosphorus (PO_4), chlorophyll *a* (Chla), oxygen (O_2), temperature, alkalinity, and pH on the aerated and control sides of the lake, 12 August 1980 (●), 11 August 1979 (■).

supersaturated with respect to calcite on the control and aerated sides of the lake.

3.5.2.1 ^{32}P-Kinetics and P-Limitation

In July 1978, and June and July 1979, $^{32}P\text{-}PO_4$ assimilation was so slow that it could not be differentiated from phosphorus adsorption; the high SRP concentrations appeared to saturate phosphorus assimilation. During the 1979 bloom, precipitation of about 98% of the SRP resulted in rapid $^{32}P\text{-}PO_4$ uptake (Fig. 34). Phosphorus turnover time was 1.9 h in the aerated and 46.0 h in the control side of the lake. This large difference between the two sides of the lake appeared to be related directly to the amount of calcite formation and indirectly to the productivity. The bloom collapsed shortly after this period of calcium and phosphorus precipitation.

3.5.3 Calcite Precipitation in Limnocorrals

3.5.3.1 1980 Fe-EDTA Limnocorrals in Black Lake

Seven limnocorrals were used in a study of microbial regulation of iron availability. Early in the summer, Fe-EDTA and Na-EDTA treatments resulted in a pH of 9.5, a decrease of calcium and alkalinity, and complete precipitation of phosphorus in the top 1.5 m of water. After two weeks, the algal biomass was less than 15 µg chl *a*/L and the primary productivity was less than 170 µg C L^{-1} d^{-1}. Both values are an order of magnitude less than those from the algal blooms in other limnocorrals or the lake (n=8). The oxygen content of the water increased by only 3 mg/L in the Fe-EDTA and Na-EDTA treatments. Relative to the lake and

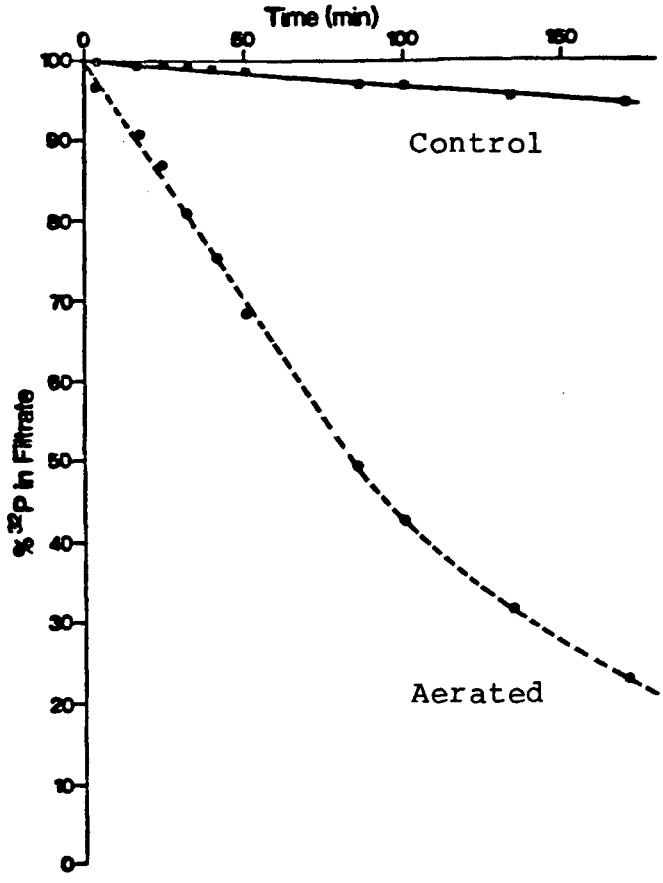

Figure 34 ^{32}P uptake during calcite precipitation.
Water from 1.0 m from the surface of aerated (- - -) and control (———) sides of lake.

some limnocorral experiments, these long-term responses indicate that either the algal bloom and the bloom effect on oxygen concentrations lasted less than a week, or that another process such as calcite precipitation, influenced phosphorus concentrations.

3.5.3.2 Nitrate Induction of Calcite Precipitation

The limnocorral treatment that had the greatest ability to induce calcite and phosphorus precipitation was nitrate enrichment. When nitrate was used as a source of N, the pH increased to 9.5 or 9.6 (Murphy et al. 1983). Within two weeks after a nitrate enrichment, the surface water became depleted of nitrate and phosphorus, the pH increased, and the alkalinity decreased much more than in other limnocorrals. Also, the minimum calcium content of the surface water (1.0 m) was much lower than any other limnocorral (nitrate limnocorral $\bar{x}$=18.5 mg Ca/L, other limnocorral with calcite formation $\bar{x}$=25.7 mg Ca/L, and limnocorrals with no calcite formation $\bar{x}$=35 mg Ca/L). Calcite formed a crust on the walls of the nitrate limnocorral. The biomass and productivity of the nitrate limnocorral were less than the control and the Fe-EDTA limnocorrals, and much less than in the Na-EDTA limnocorrals, or the lake (Murphy et al. 1983a,b).

In the hypolimnion, the phosphorus concentration was consistently much higher in the nitrate limnocorral than in the control limnocorral. Phosphorus increased in the hypolimnion by as much as 200 µg P/L with no increase in alkalinity. The precipitated phosphorus dissolved much faster than the precipitated calcite. This preferential phosphorus dissolution

indicates that phosphorus precipitation was a surface adsorption of phosphorus onto calcite rather than a precipitate of calcium phosphate.

3.5.3.3 1982 Fe-Citrate Limnocorrals

The 1982 iron-citrate limnocorral treatments provided additional information on the regulation of algal productivity by calcium carbonate precipitation.

Citrate was assimilated too quickly to have an effect on calcium precipitation via chelation of calcium (section 3.2.3). Thus, citrate could only influence calcium carbonate precipitation by microbial oxidation of citrate to carbon dioxide and by a subsequent lowering of pH. The effect of the citrate assimilation upon the pH of the epilimnia was too small to be detected (Fe-citrate 8.5, 8.6; Na-citrate 8.5, 8.5; NO_3 8.7; Control 8.6, 8.5). However, by Aug. 24, 1982, citrate conversion into CO_2 reduced the pH in the hypolimnia significantly (Fig. 35).

This vertical zonation of pH was a result of microbial productivity. After most of the citrate of the first enrichment had been assimilated, ^{14}C-citrate uptake (June 28, 1982) was much faster at 3.0 m than at 1.0 m (Fig. 36). Shortly after the second addition of citrate (June 30), citrate assimilation was again much faster at 3.0 m than at 1.0 m (Table 7). The assimilation of ^{14}C-citrate in the control limnocorrals was relatively consistent in samples collected at 1.0 and 3.0 m depths (Fig. 36, Table 7); thus, the enhanced microbial productivity in hypolimnia of the citrate treated limnocorrals must have been a result of the

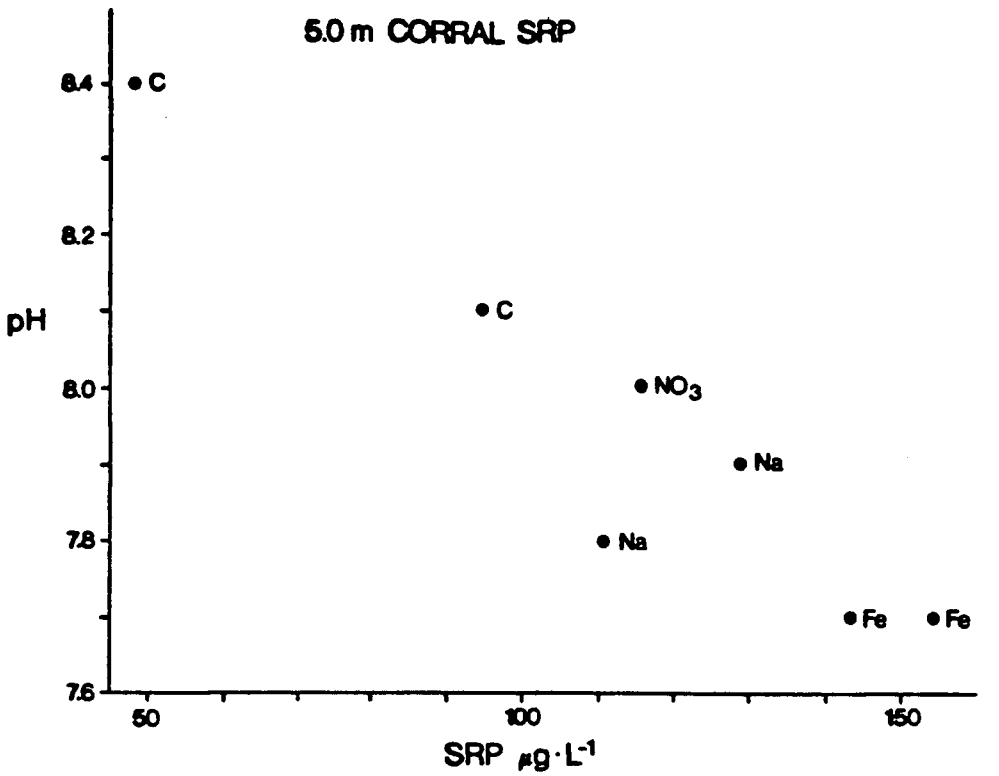

Figure 35 SRP and pH at 5.0 m in limnocorrals on Aug. 24, 1982. Limnocorrals were C, control; NO_3, nitrate; Na, sodium citrate; and Fe, iron citrate.

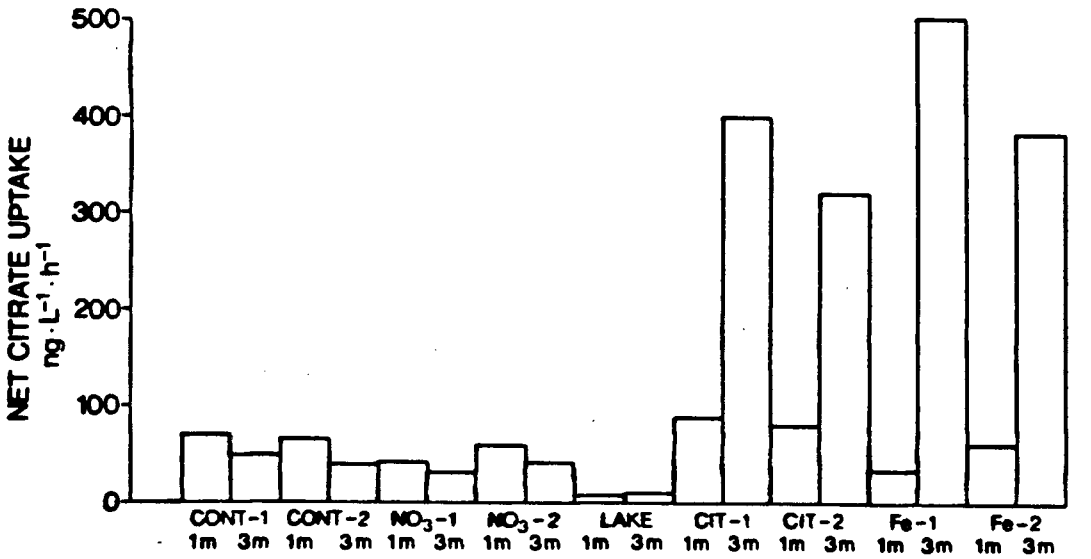

Figure 36 Depth profile of citrate assimilation. The net ^{14}C-citrate uptake on June 28, 1982 in the control (Cont-1, Cont-2), nitrate (NO_3-1, NO_3-2), sodium citrate (Cit-1, Cit-2), and ferric citrate (Fe-1, Fe-2) limnocorrals is shown.

citrate enrichment. The ^{14}C-assimilation data suggest that a change in water chemistry produced by citrate enrichment would only be observed in the hypolimnia.

3.5.3.4 Citrate Effect on P-$CaCO_3$ Indicates Iron Limitation

The phosphorus content of the limnocorral hypolimnia reflected the microbial response to iron enrichment. The phosphorus concentration was higher in the more acidic hypolimnia (Fig. 35). The hypolimnia of the ferric citrate limnocorrals had the highest phosphorus concentration and the lowest pH. The lower pH values in the hypolimnia of the ferric citrate limnocorrals were presumably a result of the stimulation of microbial productivity by iron enrichment. The higher phosphorus concentration in the more acidic hypolimnia could be a result of either greater microbial regeneration of organic-P, of inhibition of phosphorus coprecipitation with calcium carbonate, or of release of phosphorus from calcium carbonate as it settled into the lower pH hypolimnia.

3.5.3.5 Influence of Weather on P-$CaCO_3$ Precipitation

The enigma of why sediment iron release resulted in calcite precipitation in Black Lake 1979, but not in 1980, was resolved in the 1982 studies. The asynchrony in algal oxygen production and changes in lake chemistry indicate that calcium carbonate precipitation of phosphorus from the epilimnia of limnocorrals was partly controlled by weather.

The seasonal pattern of phosphorus depletion in the epilimnia was very similar in all limnocorrals (Fig. 15). The

rate of phosphorus depletion varied greatly among three periods: warm water of June, cool water of July, and warm water of August (Fig. 15). The cool water of July, 1982 was associated with unusually cloudy cold weather.

Phosphorus was rapidly precipitated in June from the epilimnion (>6.0 μg P L^{-1} d^{-1}). The water was warm, 20°C, and supersaturated several fold with calcium carbonate. The calcium concentration appeared to decrease within 15 days by an average of 2.0 mg Ca/L at a depth of 1.0 m (Table 14).

In the cooler 15°C water of July, phosphorus utilization was undetectable and the calcium concentration changed little. The cooling of the water reduced the degree of calcite supersaturation from 11.8 to 5.5 fold. The peak of algal biomass and oxygen production occurred in the cool water of July. Although the surface water in the limnocorrals in July was never less than five-fold supersaturated with calcium carbonate, the rapid precipitation of phosphorus and calcium did not occur until 2-3 weeks after the peak oxygen values.

When the water warmed back up to 17°C in early August, the mean phosphorus depletion at a depth of 1.0 m in the limnocorrals became very rapid (>9.4 μg P L^{-1} d^{-1}). At this depth, the calcium concentration decreased within a month by a mean of 9.5 mg Ca/L.

In the 1980 Aphanizomenon bloom, the colder weather, mixing of the lake by a storm, and the natural delay in crystal formation probably prevented calcite precipitation.

Table 14 Calcium Concentrations in the 1982 Limnocorrals

Treatment	June30		July13		July23	Aug24		
	1m	3m	1m	3m	1m	1m	3m	5m
Control 1	49	50	48	49	46	41	41	41
Control 2	48	49	48	47	49	40	39	38
Na-Citrate 1	49	49	46	45	48	38	40	40
Na-Citrate 2	49	48	48	48	48	37	39	40
Fe-Citrate 1	49	49	46	48	45	33	36	38
Fe-Citrate 2	49	49	46	48	44	34	36	38
Nitrate 1	48	49	47	48	48	38	42	41
Nitrate 2	48	50	47	49	46	47	42	42

All values are mg/L of Ca. The calcium content of the water used to fill the limnocorrals was 51 mg/L.

3.5.4 Calcium Chloride Induction of Calcite Precipitation

In this experiment, the precipitation of calcium carbonate was induced by increasing the calcium concentration with calcium chloride (Fig. 37). Chemical extractions and direct microscopic analysis were used to determine if precipitated phosphorus was associated with calcium. Calcium salts were added to lake water taken during an algal bloom (chl *a* 50 µg/L) that was seven-fold supersaturated with calcite (pH 8.6, Ca 40 mg/L, ion activity product/solubility product = IAP/K_{sp} = 7.63) on Aug 24, 1982. This sample was collected after a period of rapid phosphorus and calcium precipitation (decreases of 110±10 µg P/L; 10±1 mg Ca/L in five days). The phosphorus concentration in the lake was constant during the experiment and for the next two days. The oxygen increase in the lake during the experiment indicated that the algae were photosynthetically active.

The phosphorus concentration decreased least in the control vessels (184 to 134 µg/L). Calcium chloride enrichment resulted in a phosphorus decrease from 184 to 123 and 83 µg P/L in the dark and light incubations respectively. All flasks, even the dark control, attained the same final pH (8.8±0.05).

Another small experiment was done the next day to replicate the Aug. 24 experiment. The temperature was maintained to within 2°C of the lake surface. Within seven hours (1100-1800) the phosphorus concentration (SRP) decreased from 184 µg P/L to 90 , 75, and 77 µg P/L in the control, 10 mg $CaCl_2$/L, and 20 mg $CaCl_2$/L treatments respectively. The dissolved calcium concentrations decreased in all of the $CaCl_2$ incubations (4.0±3.0 mg Ca/L).

INDUCED CALCITE PRECIPITATION

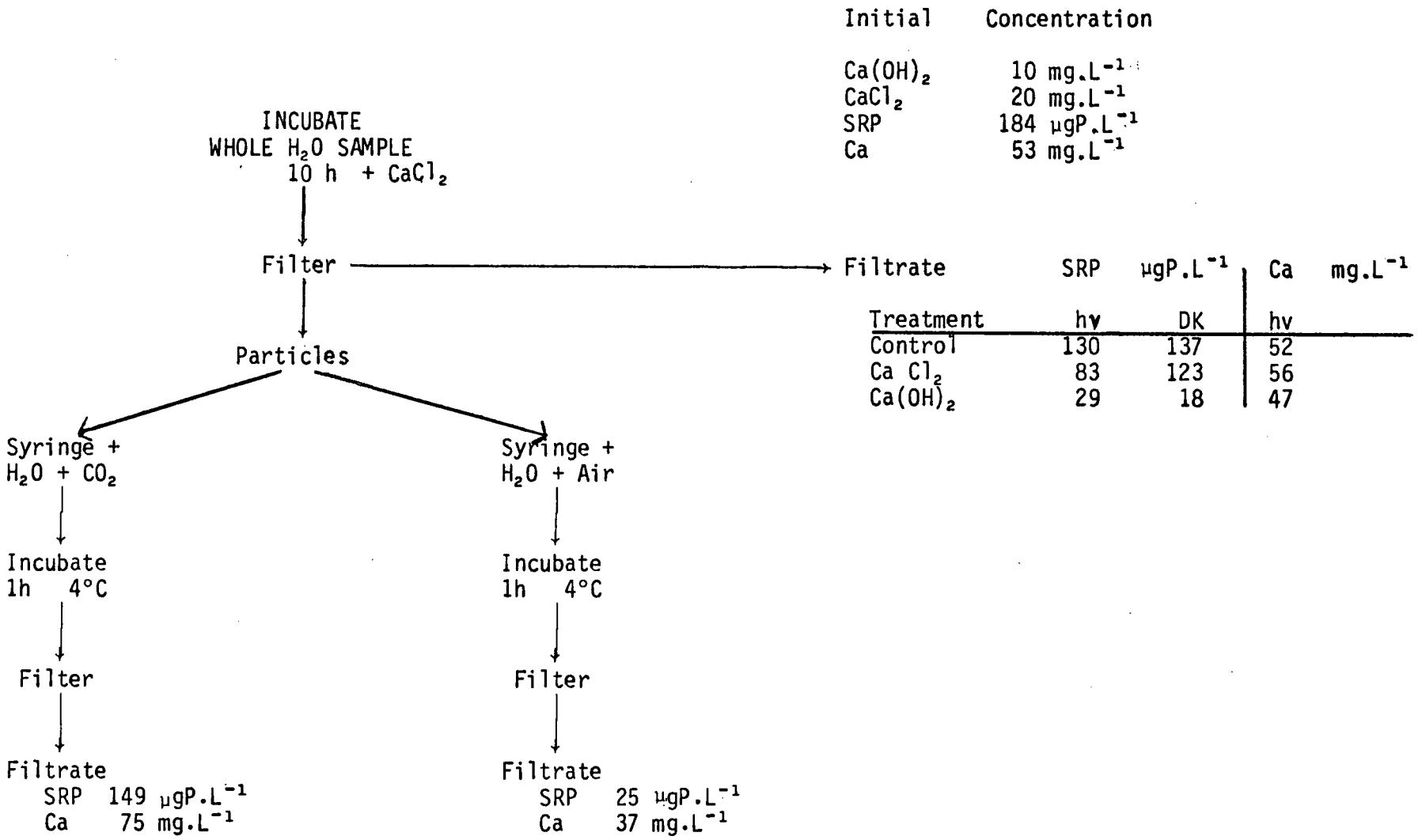

Figure 37 $CaCl_2$ induced calcite precipitation.
DK and hv represent dark and light incubations. Values are means of two analyses. Coefficient of variation was less than 5%.

3.5.4.1 Calcite Analysis

A weak acid extraction was used to characterize the phosphorus that precipitated in the calcium chloride incubations. The precipitated phosphorus could be quickly redissolved by cooling the particles and by equilibrating the sample with CO_2 (Fig. 37). The carbon dioxide reduced the pH to 5.7 which is low enough for rapid calcium carbonate dissolution (Berner and Morse 1974). Equilibration of the sample with air reduced the pH from 8.8 to 8.2 and had little effect on the phosphorus or calcium concentration. Carbon dioxide appeared to have little effect on the algae; algae that were collected from periods without calcite precipitation did not release phosphorus when equilibrated with CO_2.

Direct microscopic analysis of precipitates was used to assist the interpretation of the CO_2 extraction. Precipitates were collected from the Aug. 24 calcium chloride experiment at the end of the incubation period by filtration. Crystals had aggregated into large "balls" with a mean diameter of 20 μm in the control treatment but not in the carbon dioxide treatment. The elemental analysis indicated that the particles consisted mainly of calcium and that silicon was an important constituent. Potassium, phosphorus and zinc were minor constituents of the calcite particles (Table 15). The ratio of calcium to phosphorus in these particles (Ca/P molar ratio 72) corresponded to the changes in calcium and phosphorus observed earlier in periods of rapid calcium and phosphorus precipitation in the lake and limnocorrals (Table 16).

Table 15 Elemental Analysis of Calcite* Crystal

Oxide	Oxide%	Precision 2 sigma
CaO	51.78	0.59
SiO_2	17.11	0.24
K_2O	2.33	0.14
P_2O_5	0.92	0.07
ZnO	0.85	0.13
Al_2O_3	0.81	0.06
Na_2O	.14	0.02

* The computer program assumes all elements are present as simple oxides; the analysis is precise but not necessarily accurate. The technique could be accurate if the appropriate standards existed.

Table 16 Calcium/Phosphate Ratios in Precipitation Events, Precipitation Experiments, and Sediment

Sample	Ca mg/L	P µg/L	Ca/P*
L-1 May 25-June 5	13	140	77
L-2 July 17-July 31	7	105	52
L-2 July 31-Aug. 12	10	97	80
L-3 June 5- July 15	21.5	250	67
Black Lake Aug. 80-79	20	250	62
	Ca mg/g	P mg/g	
Sed-1 0-2 cm	11.2	.13	66
Sed-2 10-12 cm	22.7	.22	79
$CaCl_2$ Induced Precipitate	37.0	.40	72

* molar ratio. L-1 is the Fe-EDTA limnocorral from the first limnocorral experiment. L-2 and L-3 are the EDTA-2 and Nitrate limnocorrals from the second set of 1980 limnocorrals in Black Lake. The Black Lake Aug. 80-79 data is the difference observed in water chemistry from an algal bloom with no carbonate precipitation (Aug.12, 1980) to one containing rapid carbonate precipitation (Aug.11, 1979). All water samples were collected 1.0 meter from the surface. All water chemistry is the change in mg L^{-1} of Ca and µg L^{-1} of SRP between the two dates. Sed-1 and Sed-2 are sediment samples from the surface and 10-12 cm horizon of Yellow Lake.

These additional experiments with $CaCl_2$ confirm that calcium carbonate precipitation can control phosphorus solubility. The importance of crystal initiation and the susceptibility of the reaction to inhibitors results in highly variable precipitation. This key reaction could easily produce the variability observed in the iron experiments.

3.6 Calcite Precipitation - A Major Cause of Algal Periodicity

The processes that occur during calcite precipitation were confirmed by inducing calcite precipitation in Frisken Lake with lime, $Ca(OH)_2$.

3.6.1 Pretreatment Water Chemistry

In pretreatment samples from Frisken Lake, significant correlations of total inorganic carbon (TIC) and soluble reactive phosphorus (SRP) were observed in both the epilimnion/metalimnion ($r=.735$, $n=21$) and in the hypolimnion ($r=.973$, $n=11$). These two pretreatment data sets form two linear relationships (Fig. 38). The lowest TIC and SRP concentrations were in the epilimnion, and the highest were in the hypolimnion (Fig. 39). The same relationship existed between total inorganic carbon and total phosphorus (epilimnion and metalimnion $r=.753$, $n=21$; hypolimnion $r=.955$, $n=11$).

The similarity of the SRP-TIC and TP-TIC relationships was a reflection of the synchrony of carbonate and phosphorus biogeochemistry. The carbonate equilibria were closely related to phosphorus solubility and presumably algal production. Most of

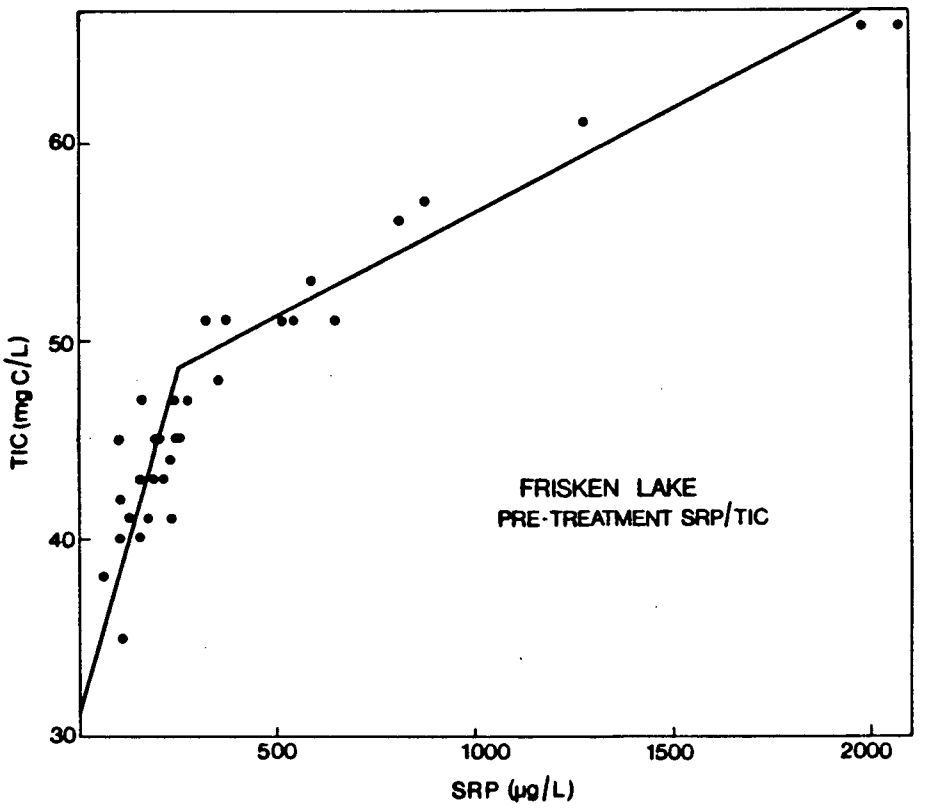

Figure 38 Frisken Lake pretreatment SRP/TIC.

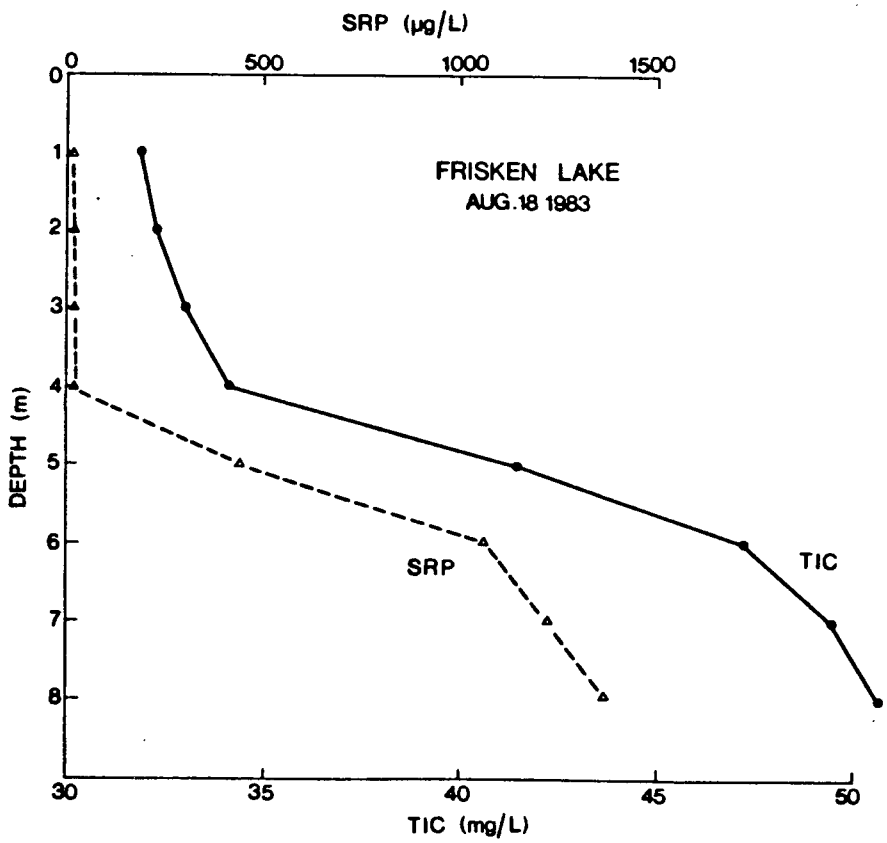

Figure 39 A depth distribution of SRP and TIC in Frisken Lake.

the total phosphorus was in solution (epilimnion and metalimnion 70.6%; hypolimnion 73.8%) and the soluble phosphorus was highly correlated to the total phosphorus (epilimnion and metalimnion r=.912, n=21; hypolimnion r=.959, n=11). This relationship was quite similar to that observed in Black Lake where 85% of the total phosphorus was in solution.

3.6.2 Lime-Induced Calcite Precipitation

The simple relationship between TIC and SRP (Fig. 38) predicted the results of the 1983 induction of calcite precipitation by lime application to Frisken Lake. The epilimnetic TIC value in August 1983 of 30.5 mg/L would have a SRP of zero if the relationship of Fig. 38 were extrapolated; the SRP was less than 20 µg/L (Fig. 40). Furthermore, examinations of particles with an EDAX microprobe of an electron microscope supported the hypothesis that calcite precipitation removed phosphorus from the epilimnion (Table 15). Samples collected in the epilimnion after the 1984 trial treatment contained particles rich in calcium and phosphorus. Over a hundred noncellular particles were analyzed. The ratio of P/Ca in ten particles varied between 0.64 to 0.04. Only two particles contained iron, and these particles contained no phosphorus; the high Fe/S ratio (0.35 to 0.56) indicates that these particles were pyrite or pyrite precursors. These particles were not well crystallized but they were not cellular.

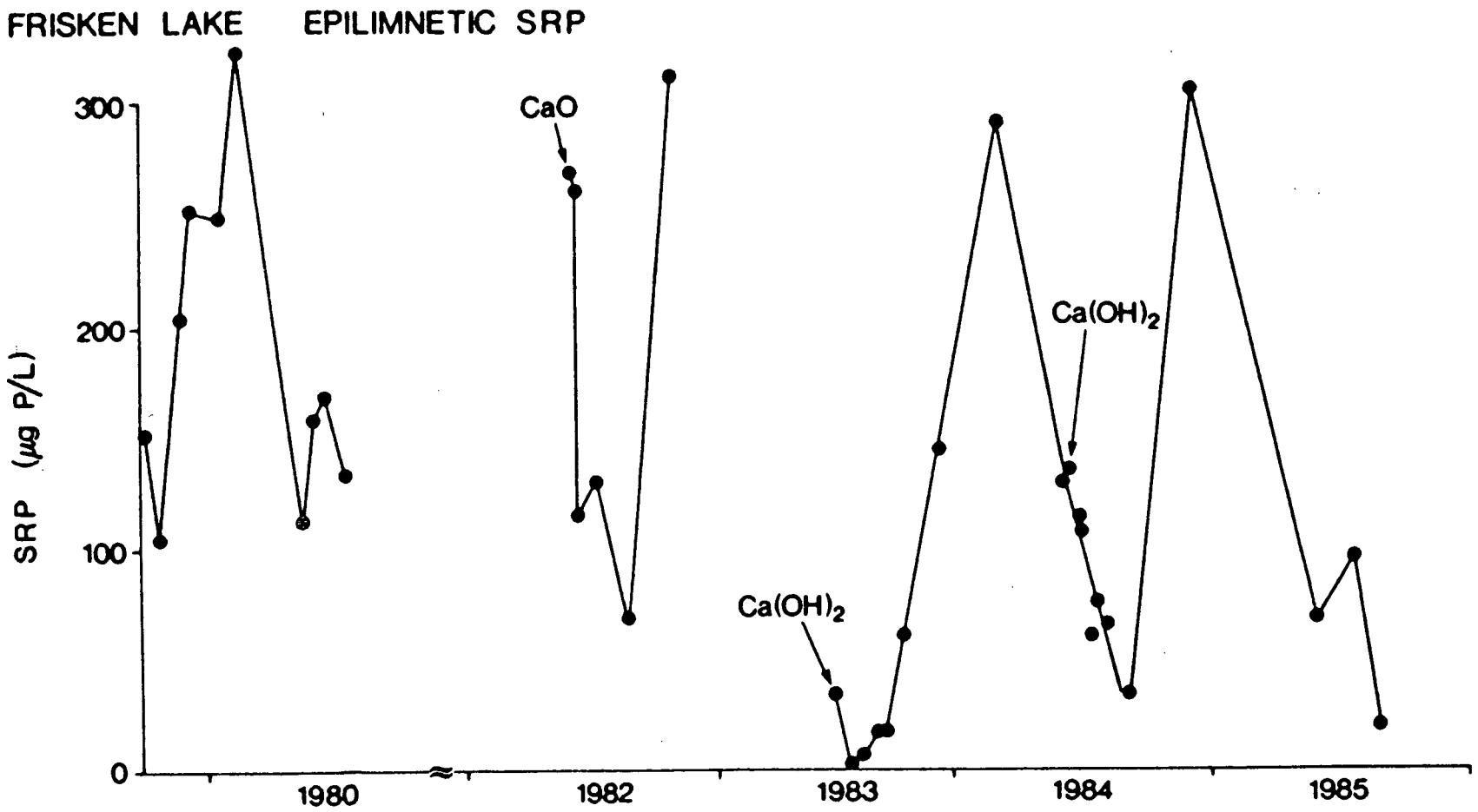

Figure 40 Frisken Lake epilimnetic SRP.

3.6.2.1 Suppression of Algal Growth

The induced calcite precipitation was able to suppress the blue-green algal blooms. The first addition of lime was too small to induce precipitation. The second addition did not have an effect after 48 h but after another two weeks, the chlorophyll a content had decreased (Fig. 41). The 1984 induction of calcite precipitation suppressed the chlorophyll a content of the lake throughout the summer (Fig. 41). The Secchi disk readings in 1984 exceeded four meters and were deeper than the pretreatment observations (Ashley, B.C. Environment).

The lime treatment produced a long-term suppression of algal growth and enhancement of phosphorus precipitation. Although no lime was added to the lake in 1985, the chlorophyll a content of the lake was lower than pretreatment values (Fig. 41; Ashley, B.C. Environment). The phosphorus concentrations in the epilimnion of 1985 were much lower than pretreatment values (Fig. 40). The Secchi disc readings in 1985 (5.5, 2.8 and 4.4 m) were much deeper than pretreatment ones which were less than 2.0 m.

3.6.3 Long-Term Enhancement of Calcite Precipitation

The reduction of algal biomass, the lower phosphorus concentrations, and improved clarity of 1985 appeared to be produced by the 1984 lime treatment. No lime was added to Frisken Lake in 1985. The improved water quality may have been produced by the dissolution of calcite in the hypolimnion in 1983 and 1984. The dissolution of calcite should enhance calcite precipitation in the epilimnion when the lake mixes.

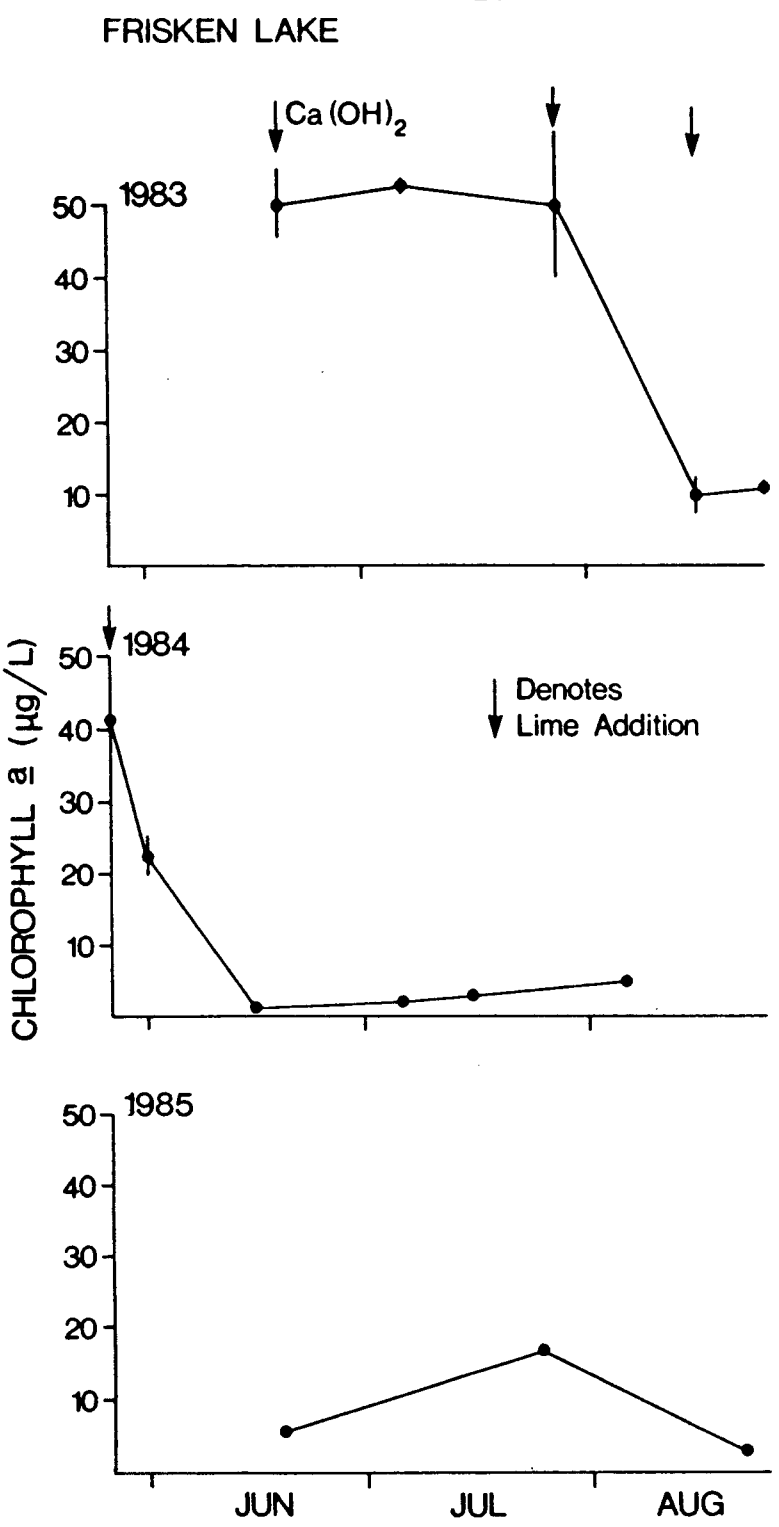

Figure 41 Suppression of algal biomass by lime application. Values are means of two samples. The error bars represent one standard deviation.

The dissolution of calcite in the hypolimnion was a composite of microbiological and geochemical reactions. The decomposition of sedimenting algae during either a natural or an induced calcite precipitation results in a rapid decrease in oxygen (Fig. 42) and pH in the hypolimnion (Fig. 43). The pH decreased by as much as 0.3 units within 48 h. The low pH and temperature of the hypolimnion both contribute to the dissolution of calcite; thus, the total inorganic carbon increased (Fig. 44).

The effect of calcium carbonate recycling is illustrated by a model mixing of Frisken Lake (Fig. 45). The model mixes sequentially the underlying one meter water layer with the overlying water column and calculates the carbonate equilibria for each mixing. Until the hypolimnetic water mixes, the mixing water is greatly supersaturated with calcite. The predicted sequence of changes in carbonate precipitation were observed when the water column destratified in the fall of 1983. The first reaction that occurred was an enhanced precipitation of calcite (Fig. 44). At this time, half of the phosphorus precipitated. However, as the lake continued to mix all of the phosphorus redissolved (Fig. 40).

An analogous enhancement of calcite precipitation should occur in the spring when the lake warms and becomes supersaturated with calcite. The 1985 water chemistry indicated that the enhanced precipitation of calcite did occur.

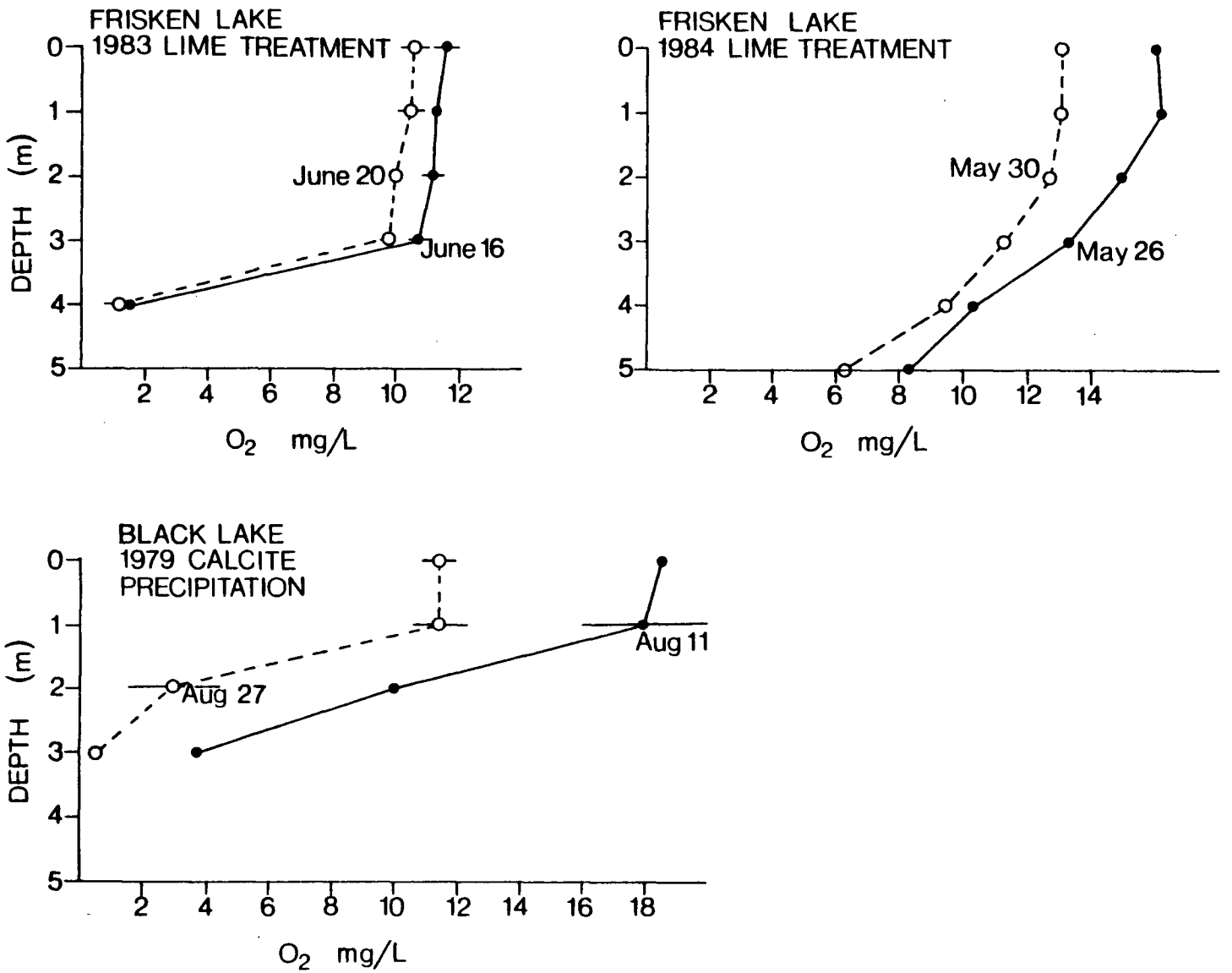

Figure 42 Effect of calcite precipitation on oxygen. Values from Black and Frisken lakes are means of data from two stations. Error bars represent one standard deviation.

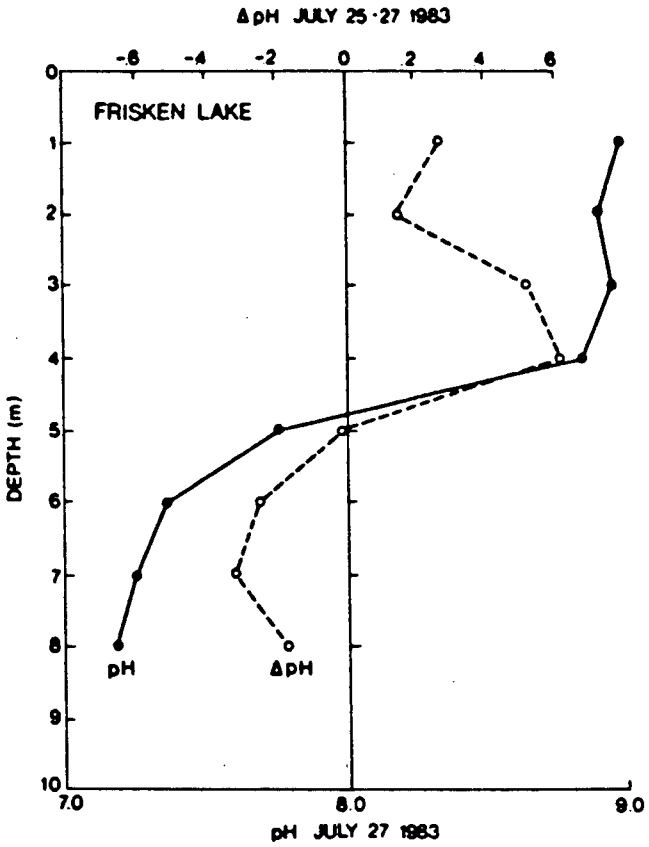

Figure 43 The pH (●) and change in pH (○) of Frisken Lake 48 hours after lime application.

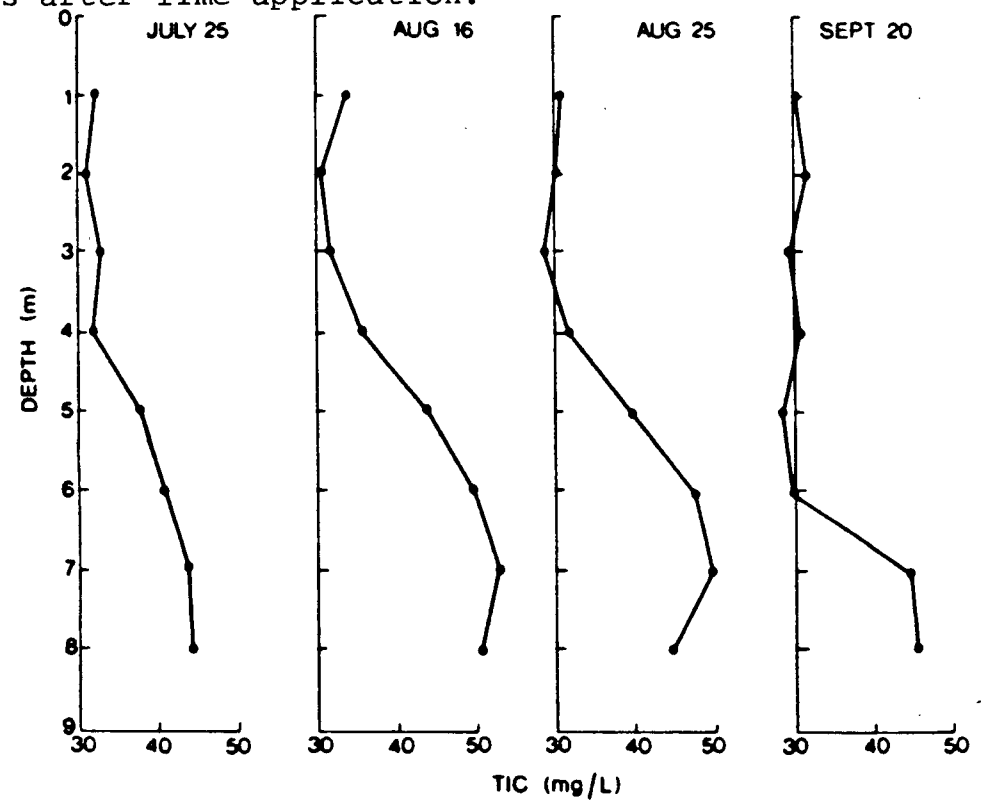

Figure 44 Total inorganic carbon (TIC) concentration of the water column of Frisken Lake in 1983.

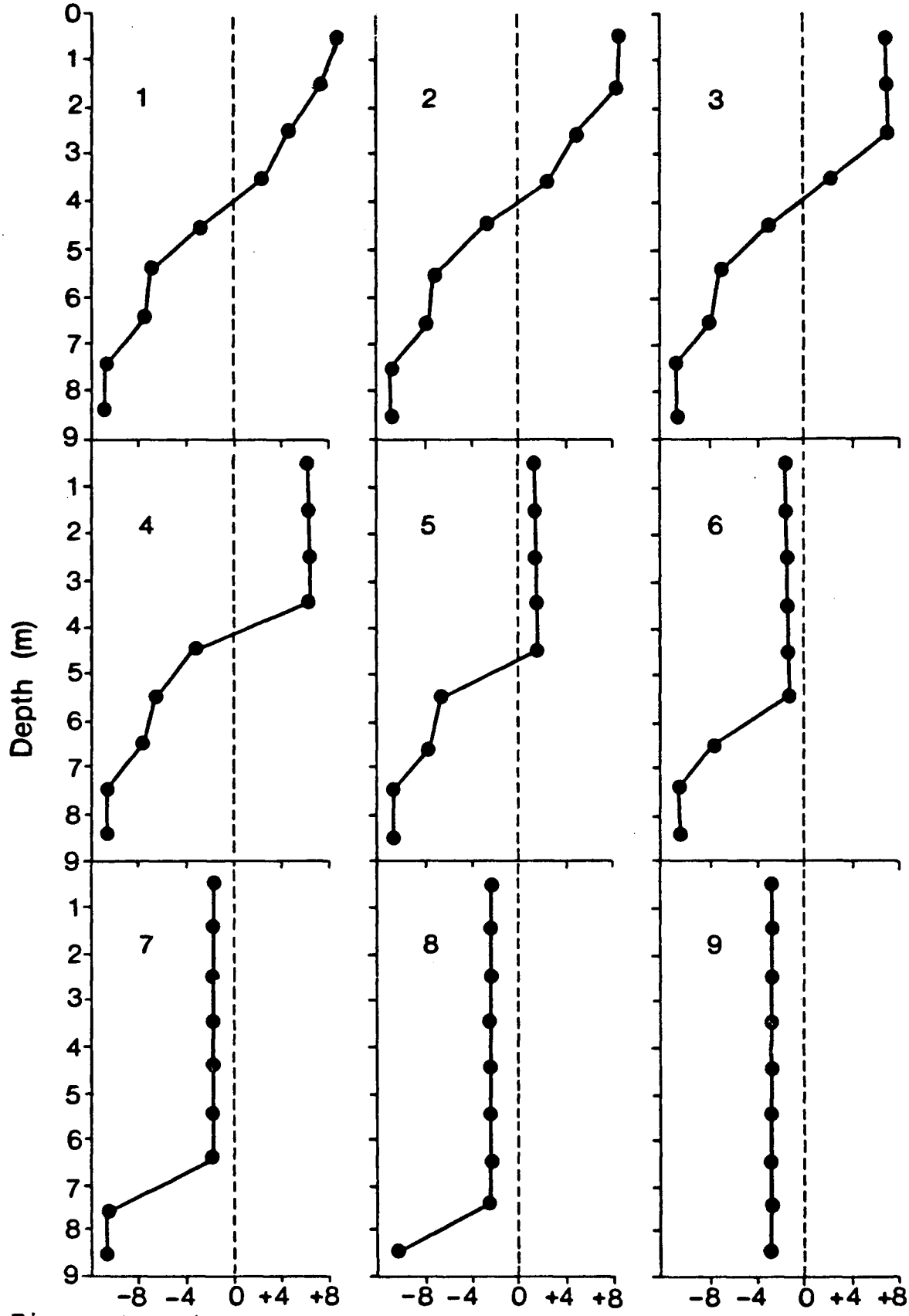

Figure 45 Simulated mixing of Frisken Lake. Numbers within graph refer to sequential mixing of one meter water layers. Numbers on x-axis are the degree of saturation of calcite (i.e. +4 is four fold supersaturated).

DISCUSSION

The primary objective of demonstrating that iron availability can affect the periodicity of blue-green algal blooms was achieved. New information on the spatial and temporal variation of iron limitation agrees with other recent studies (Stumm and Morgan 1981, Ryding 1985, Stauffer 1985). When and where iron limitation will occur is related to the epilimnetic concentration of iron; the phosphorus concentration in the lake water; the geochemistry of iron, calcium and phosphorus in the sediments; and the temperature of the lake sediments.

Microbes excreted chelators that influenced iron availability and microbial succession. The main limnetic event that regulated iron availability was iron release from the lake sediments. Pyrite formation regulated the amount of iron release. Another event, calcite precipitation, greatly altered the amount of algal biomass. In Black and Frisken lakes the sequential development of these four processes (iron chelation, iron release from the sediments, pyrite formation, and calcite precipitation) determined the types of species growing, the amount of algal biomass, and the duration of algal blooms. Both biochemical and geochemical reactions influenced or controlled the periodicity of blue-green algal growth.

4.1 Spatial and Temporal Variation in Iron Availability

The spatial variation in iron availability in my study confirms the recent hypotheses of Ryding (1985) and Stauffer (1985); the supply of iron varies greatly among lakes. The flux of iron into Frisken Lake was less than 1% of that entering Chain

Lake. The bedrock geology of the sites is similar (Cockfield 1947,1948; Rice 1947) but the water entering Chain Lake (WIB 1977) had 50% less alkalinity than the water entering Black Lake (Murphy et al. 1983b) and Frisken Lake (Murphy et al. 1985). On the Thompson Plateau, alkalinity may be a useful guide to where iron limitation could occur.

The concentration of iron in the water column was a rough guide to when iron limitation could occur. In Black Lake, addition of iron stimulated algal productivity when the ambient iron concentration was less than 50 µg/L and blue-green algal blooms only formed in lake water with high concentrations of iron. The Black Lake chlorophyll a (chl a) concentration was correlated to the iron concentration. Some of the variability of the chl a-Fe correlation was caused by calcite precipitation; the greatest chl a concentrations occurred during blooms with no calcite precipitation.

If my interpretation of the chl a-Fe correlation of Black Lake is correct then the absence of a chl a-Fe correlation in the Chain Lake data (Fig. 6) should reflect the high availability of iron in Chain Lake. The lowest iron concentration in Chain Lake (Table 3) was equal to the highest iron concentration in Black Lake (Appendix 2), and iron did not stimulate algal oxygen production in Chain Lake. Furthermore, the Black Lake studies indicated that Aphanizomenon needed more iron than other algae; therefore, the dominance of Aphanizomenon in Chain Lake confirmed that Chain Lake was not iron limited.

The iron data in Frisken Lake was more complex than in the other lakes. Initially, dense blue-green algal blooms (70 µg chl

a/L) formed in water with relatively little iron (28 µg Fe/L), and the chl a concentrations were not correlated to the iron concentrations. After the lime application, algal growth was restricted and chl a concentrations were correlated to iron concentrations.

The suppression of algal growth in 1984 in Frisken Lake was surprising in that the SRP concentration of the epilimnion was above 50 µg/L throughout the summer. The enhancement of carbonate precipitation may have been a factor regulating algal productivity; however, limited iron availability may also have been important. The total iron concentration in the epilimnion of Frisken Lake in the early summer of 1984 was less than 20 µg/L. The slight increase of chlorophyll a that was observed in August 1984 in Frisken Lake was associated with a doubling of the iron in the epilimnion (Fig. 5).

4.2 Calcite Induction of Iron Limitation

The establishment of the chl a-Fe relationship in Frisken Lake appeared to be a reflection of enhanced calcite precipitation. The enhancement of iron limitation by calcite precipitation is consistent with observations by Stauffer (1985). He proposed that hardwater lakes have little iron because iron mobility is suppressed in calcareous areas. Stauffer's hypothesis is consistent with soil studies that have shown that calcareous soils have less available iron (Brown 1979). Stauffer did not propose a mechanism for the suppression of iron mobility.

My observations in Frisken Lake were more specific than Stauffer's in that the carbonate suppression of iron mobility was

produced in the lake, not in the drainage basin. The simplest hypothesis to explain this observation, that iron adsorbs directly to calcite, is not supported by two observations. Iron was not associated with calcite crystals in the water column (Table 14) nor was iron positively correlated to calcite in the sediments (Table A3, Fig. 30, 31).

Without an assessment of iron availability, the lack of Fe-calcite precipitation would be surprising because during all observations of natural calcite precipitation in this study, iron concentrations in the lake water were at a yearly high. The most plausible explanation for these observations is that most of the epilimnetic iron in Frisken and Black lakes was unreactive. This hypothesis is consistent with the established hypothesis that in aerobic and alkaline lake water, the majority of iron occurs as physiologically unavailable forms (Wetzel 1968).

The reciprocal of Stauffer's hypothesis that lakes with little iron will have high phosphorus concentrations is consistent with the geochemistry of the lakes upon the Thompson Plateau; lakes with high phosphorus concentrations should be iron limited. This hypothesis is also consistent with observations by Ripl (1986). He found that iron reacts preferentially with sulphide; thus, for ferric phosphate reactions to occur, iron must first saturate sulphide precipitation reactions.

Ferric reactions were able to regulate the solubility of phosphorus in Chain Lake but not in Frisken or Black lakes (Fig. 26). The strong bonding of phosphorus to insoluble ferric iron resulted in low phosphorus concentrations (<10 µg/L) in Chain Lake's oxidized water in spring and early summer. Ferric

concentrations in Black and Frisken lakes were too low for precipitation or adsorption of phosphorus to ferric hydroxide (Birch 1976, Stumm and Morgan 1981). These observations indicate that sulphide precipitation of iron was not saturated in Frisken and Black lakes.

The iron geochemistry of the water column was reflected well in the lake sediments. Observations that the net sedimentation of organic-C in Frisken Lake is only a third that of Chain Lake may be a reflection of iron limitation (Table 13). In Frisken, Roche, and Yellow lake sediments, little of the sediment iron was able to react with phosphorus. The distribution of phosphorus was negatively correlated to the iron concentration and positively correlated to the calcite concentration.

Chain Lake sediments were different from sediments in other lakes in this study; iron and phosphorus concentrations were not correlated and calcium carbonate was undetectable below one centimeter. Moreover, the rate of pyrite formation in Chain Lake sediments was more than ten fold faster than in Frisken Lake sediments (Table 13, Fig. 8).

Without an evaluation of iron availability, the differences in the formation of pyrite between Chain and Frisken lakes would be a puzzle. The proportion of iron found as pyrite was three times higher in Chain Lake than in Frisken Lake. Chain Lake often had undetectable concentrations of sulphate (Murphy 1985); thus, sulphur availability should have limited sulphate reduction and pyrite formation (Berner 1971). Moreover, Chain Lake sediments were often oxidized (Fig. 7) and oxygen suppresses pyrite formation (Berner 1971). In spite of some adverse conditions, the

rate of pyrite formation in Chain Lake was more than ten times faster than in Frisken Lake; the availability of iron overcame the limiting factors that restrict pyrite formation.

Sulphate reduction in Frisken Lake was not strongly limited by the supply of sulphate; the lowest observed concentration of sulphate was 500 µg/L and the odour of hydrogen sulphide was always obvious in hypolimnetic samples (Murphy et al. 1985). Furthermore, the low oxygen concentrations (Fig. 7) in Frisken Lake should have enhanced pyrite formation. The presence of ferric iron in anoxic Frisken Lake sediments indicated an iron complex more stable than chlorite; pyrite formation proceeds readily by using the iron in chlorite (Berner 1971). The relatively slow rate of pyrite formation in Frisken Lake was largely a reflection of low availability of iron.

The mechanism of suppression of iron mobility in alkaline lakes is the formation of pyrite, a refractory iron mineral. Calcite precipitation of 90% of the algal bloom in Frisken Lake must have enhanced pyrite formation. The optimal site for pyrite formation in Frisken Lake would be the surface sediments; the constituents for pyrite formation, reactive iron, organic matter and bacteria to produce sulphide, would be highest here (Berner 1971). The formation of pyrite in an anoxic environment would minimize the recycling of iron.

A doubling of the rate of pyrite formation in Chain or Frisken lakes would block sediment iron release (Table 13). The long-term suppression of algal biomass by the lime treatment of Frisken Lake would be partially mediated by enhanced pyrite formation. The rapid decrease of pH by 0.1 to 0.2 units in 24 h

in the anoxic hypolimnion after lime treatment indicated rapid decay of algae. If most of the bacterial reductive activity were associated with sulphate reduction then the bacterial activity could have generated as much as 100 µM of H_2S. The concentration of sulphate was about 5 µM; thus, only about half of the iron could form pyrite.

The rate of pyrite formation is directly related to the concentration of sulphate (Berner 1971); thus, the degree of iron limitation would be much greater in lakes with more sulphate. The sulphate concentration in Black Lake was 5-10 times higher than in Frisken Lake. The typical epilimnetic and hypolimnetic sulphate concentrations in Black Lake in June were 5 and 4 mg/L. A faster rate of pyrite formation in Black Lake would result in less iron release from the sediments. As this hypothesis predicts, the hypolimnion of Black Lake had much less than half the iron concentration observed in the hypolimnion of Frisken Lake (Table 4, Appendix 2).

4.3 Sediment Iron Release

In this study, only the sediment iron release of Chain Lake was consistent with the model of Mortimer (1941, 1942), i.e. release with the onset of anoxia. Iron release in Black and Frisken lakes was not associated with a reduction in oxygen concentration. Pyrite would not dissolve in anoxic water and ferrous iron that was insoluble previously should remain insoluble. The gradual decrease in the concentration of ferric iron deeper in the sediments indicates that ferric iron is reduced to ferrous iron, apparently in mid summer, which then

either enters the water column or reacts with sulphide to eventually form pyrite.

Temperature was an important variable in the sediment iron release at all sites. Iron release occurred earliest in Chain Lake (mid-July). This shallow lake mixes readily; thus, the sediments were warmer than the other sites. Iron release in Black Lake did not occur until the sediments were warmer than 10°C (late July). A 2.6°C warming of the sediments by lake aeration appeared to enhance iron release on the aerated side of Black Lake. Iron release occurred last in Frisken Lake perhaps because the profundal sediments did not warm above 5°C until August. The effect of temperature on sediment iron release seemed to produce the strong seasonal change in iron concentration in the water columns.

The temperature of the sediment is largely controlled by physical mixing; these two variables act in close synchrony to influence the sediment iron release. Temperature was more closely related to the pattern of iron release than was physical mixing in my study; physical mixing can explain all observations except for the slower sediment iron release in Frisken Lake relative to the control side of Black Lake.

The coincidence of the sedimentation of the Anabaena bloom and the release of sediment iron indicates that microbial metabolic activity is an important aspect of sediment iron release. A microbial influence on sediment release of iron is analogous to the microbial mediation of phosphorus release from lake sediments (Ryding 1985); microbes enhance or mediate sediment phosphorus release and temperature enhances microbial

metabolic activity.

4.4 Interrelationship of Iron Limitation, Chelation and Calcite Precipitation

To assess completely the iron limitation of a lake requires the use of several assays. Moreover, the impact of associated reactions, such as calcite precipitation, must be resolved in synchrony or many iron bioassays cannot be resolved. The discovery that calcite precipitation could strongly regulate the response to iron enrichment provided insight into many observations. However, the experimental use of a chelator to maintain iron in solution made resolution of the effect of iron limitation and calcite precipitation difficult. EDTA appeared to stimulate algal and bacterial productivity more than Fe-EDTA; thus, a process other than iron availability may have been important.

A likely explanation for the apparent EDTA stimulation of primary production and bacterial heterotrophy is that EDTA may have suppressed calcite precipitation. Calcite precipitation is easily blocked by organic compounds, especially chelators (Reynolds 1978). The smaller degree of phosphorus and calcium precipitation in the EDTA limnocorrals indicated that less calcite precipitation occurred. Relative to limnocorrals with calcite precipitation, the algae in the Na-EDTA limnocorrals would have a much longer residence time in the euphotic zone which would result in greater productivity. The firm binding of iron would have prevented EDTA in the Fe-EDTA limnocorrals from suppressing calcite precipitation. Iron stimulated microbes in

the Fe-EDTA limnocorrals, but the use of EDTA as a control treatment weakened the detection of the iron enrichment response.

These doubts about EDTA led to the decision to repeat the iron enrichment experiments with citrate as the iron chelator. Citrate is a weak chelator and many microbes (Neilands 1981b) and plants (Tiffin 1966) can utilize iron chelated by citrate. The rate of microbial citrate assimilation in my study was fortunate in that iron was maintained in solution for days and the chelator was completely metabolized after a few days. This rate of citrate utilization provided an excellent opportunity to observe long-term responses to iron enrichment.

Initially, the ferric-citrate limnocorrals had lower oxygen concentrations than the control limnocorrals; iron seemed to enhance bacterial oxygen utilization. Once the citrate was assimilated, the ferric-citrate limnocorrals had significantly higher oxygen concentrations than the sodium-citrate limnocorrals (Appendix 3); iron stimulated algal oxygen production. The ferric-citrate limnocorrals in Black Lake provided highly significant evidence that an inadequate supply of iron was limiting the algal productivity of Black Lake (Fig. 15).

4.5 Microbial Control of Iron Availability

A study of algal siderophores was required to separate the effects of iron limitation from those produced by calcite precipitation. The siderophore isolates had many properties of siderophores (section 3.3); however, the purity of the siderophores was not rigorously established. Although Corbett and Chipko (1978) found that Sephadex chromatography of hydroxamic

acids resulted in compounds that were more than 99% pure, ion exchange and Sephadex chromatography may not have completely purified my siderophore isolates. With the possible exception of the bacterial heterotrophy, this uncertainty has no effect on the interpretation of my studies.

This study of the microbial aspects of iron availability required the use of limited supplies of naturally occurring chelators; thus, small bottles had to be used. Growth, primary production, and heterotrophy bioassays indicated that the microbial regulation of iron availability exerted a strong control over algal and bacterial growth. Analysis of lake water indicated that chelators could control the availability of all dissolved iron. The excretion of chelators was detected with ^{55}Fe by Sephadex chromatography and the FeBC assay. The ability of chelators to control microbial metabolic activity was demonstrated in primary production and heterotrophy assays.

The specificity of the siderophore isolates in chelating iron (Fig 18) supports the hypothesis that the activity of these isolates is related to siderophores. Moreover, this data disputes the alternative hypothesis that organic chelators enhance algal productivity by complexing toxic metals; in my assays, an iron-saturated siderophore would not detoxify another metal. In lakes, only a concentrated copper treatment could overcome a siderophore affinity for iron. This high degree of specificity is similar to that observed by Neilands (1957), Anderegg et al. (1963), Davis et al. (1971), and Emery (1971) with bacterial and fungal siderophores.

The specificity and the distribution of algal chelators indicate that a strong control of iron availability occurs near the cell. Blue-green algae appear to have two strategies of maintaining chelators in close proximity to an algal cell. Anabaena cylindrica produces a chelator that appears to be in true solution. This alga is covered with a very thick (2.6 μm) and dense layer of colloidal fibrillar material that should restrict water movement around the cell (Leppard et al. 1977). Anabaena flos-aquae produces much less colloidal material (0.35 μm thick layer) than A. cylindrica (Leppard et al. 1977). A. flos-aquae excretes a chelator that is loosely adsorbed to a colloidal fibril that is an extension of the cell surface.

Freeze-drying was a more effective method of isolating chelators from fibrils than was freeze-thawing. Chelation capacity was higher after freeze-drying than after one freeze-thaw. Frozen filtrates from Anabaena cultures that were thawed and refiltered twice still left a slimy deposit of fibrils on filters when frozen and thawed a third time. Isolates that were freeze-dried, thawed, and refiltered did not leave a slime layer when frozen, thawed, and refiltered a second time. Fibrils appear to be dehydrated more effectively by freeze-drying than by freeze-thawing. Dehydrated fibrils are relatively insoluble in water.

Resolution that the Anabaena flos-aquae chelator is loosely bonded to colloidal fibrils provides insight into the in situ concentration of chelators. In a culture filtrate that increased in FeBC from 2 μM to 80 μM after ultrafiltration, the equilibrium between dissolved and adsorbed chelators was 2/78 μM. The

following measurements were used to derive the *in situ* concentration of chelator; mean cell volume of 2 μm^3, cell length of 1.8 µm, cell width of 1.2 µm, total cell volume of 10^7 μm^3, and fibril length of 0.35 µm (Leppard 1977 et al.). The volume of the phycosphere was 1.8% of the culture medium; thus, the concentration of chelator in the phycosphere was 4.3 mM. The reactivity of the adsorbed chelator is unresolved; however, if the estimate that 2.5% of the chelator was in solution was accurate, then the concentration of dissolved chelator in the phycosphere was about 110 µM.

The weak association of chelators with fibrils explains why algae would not "lose" an excretion product in the bulk water. This discovery also justifies the use of solutions of chelators in bioassays that are more concentrated than those found in the freshly filtered medium. The criticism that concentrating lake water exaggerates the significance of organic-iron complexation (Plumb and Lee 1973) now seems less serious.

The study of chelator microdistribution provides useful insight into other chelator studies (Murphy 1976). Earlier observations with gel chromatography of ^{55}Fe-labelled algal filtrates could not be completely resolved. An unconcentrated filtrate could carry very little ^{55}Fe through the column and most of the ^{55}Fe that passed through the column was associated with a colloidal fraction. If the medium was freeze-dried, it could be diluted to the original concentration of the culture and virtually all of the iron could pass through a Sephadex column with a low molecular weight organic chelator. The results of my U.B.C. studies indicate that the enigma of my M.Sc. study was

produced by the adsorption of the chelator to fibrils in fresh medium and isolation of the chelator from fibrils by freeze-drying.

One problem related to the chelator fibril association found in the Black Lake study, is that some lake water samples were not concentrated, frozen, or passed through an ultrafiltration membrane. Chelation capacity in these samples may have been underestimated. Fortunately, interpretation of the data is still simple. The measured chelation capacity of the lake water was greater than the dissolved iron concentration. Thus, microbial chelators could complex all of the soluble iron and the access to iron could regulate which microbes could grow.

4.6 Siderophores as Mediators of Algal Succession

The observation that siderophore isolates could either inhibit or stimulate (Fig. 20) one algal species but not a coexisting species indicates that siderophores can influence algal succession. The decreasing rate of utilization of siderophore isolates in Black Lake as the iron concentration increased indicates that a siderophore mediation of microbial succession would decrease with a change in iron availability. Similar seasonal changes in allelopathy can be found in many culture and lake bioassays (Hutchinson 1967, Fogg et al. 1973, Hellebust 1974, Elbrachter 1976, Keating 1978, Kayser 1979, Wilson et al. 1979, Wolfe and Rice 1979, Chan et al. 1980). In all of these studies, the filtrate from either a culture or a lake water sample suppressed the growth of another alga. It is highly probable that some of these filtrates contained

siderophores.

Laboratory studies have demonstrated that excreted algal compounds, with many properties of siderophores, could suppress the growth of a competing algal species (Fig. 21; Murphy 1976). The antagonism was primarily a suppression of iron availability that iron addition could reverse. However, other inhibition reactions were present. The concentrated algal siderophore isolates (10x mature culture concentration) from Anabaena flos-aquae or Anabaena cylindrica could lyse Scenedesmus basiliensis but not the parent alga. Prior to lysis the cells became rounded and after several hours most of the cells had ruptured.

The specific toxicity of these siderophore isolates indicates that siderophores could regulate symbiotic associations in algal clumps. Symbiotic associations between bacteria and clumps of nitrogen fixing blue-green algae have been documented by Paerl (1982a). Iron complexed by the chelator produced by Anabaena oscillaroides was preferentially assimilated by both bacteria and algal cells near the heterocysts of A. oscillaroides (Paerl 1982b).

Other aspects of iron assimilation may also influence algal succession. The restricted growth of Aphanizomenon in iron-limited water and its rapid growth in iron-rich water indicates that during algal blooms this alga utilizes a low-affinity iron-uptake system. These assimilation systems are comparatively inefficient and non-specific (Neilands et al. 1980).

4.7 Siderophores as Regulators of Bacterial Heterotrophy

Three algal siderophore isolates were able to greatly suppress bacterial assimilation of simple organic compounds. The respiration of assimilated organic compounds was greatly enhanced by siderophore isolates from two Anabaena species. These disruptions indicated a suppression of bacteria by the algal siderophores. The seasonal pattern of heterotrophic activity in Black Lake also indicated that Anabaena inhibited bacteria. The suppression of bacteria by blue-green algae is well known (Chrost 1973, Delucca and McCracken 1977, Reichardt 1981); however, the idea that iron availability mediates algal suppression of bacteria is new.

The suppression of bacteria by siderophore isolates is similar to observations by Chrost (1973,1975) and Chrost and Siuda (1978). They hypothesized that algal excretory products suppressed bacteria more in the light than in the dark. Chrost's negative correlations between bacterial heterotrophy and chl a could be a reflection of greater iron limitation and higher siderophore or antibiotic production in the epilimnion. In Black Lake, the iron-binding data (Table 11, Figure 19) indicated that chelators are 4-10 fold more concentrated in the epilimnion than in the hypolimnion.

The production of siderophores should be minimal in iron-rich environments like the hypolimnion of a lake (Neilands 1967). A lack of siderophore production in hypolimnia may also reflect a lower iron requirement. Hypolimnia with little iron (i.e. iron-limited limnocorrals) had much less iron-binding capacity than the epilimnia. The lack of chelators in the

hypolimnia was not related to low microbial metabolic activity. Bacteria can produce siderophores (Neilands 1967) and the heterotrophy in the hypolimnia of these limnocorrals was rapid. The greater excretion of iron chelators by the microbes in epilimnia may indicate that autotrophic microbes require more iron than heterotrophic microbes. An enhancement by light of iron demand and subsequent siderophore excretion could reflect the need of iron-containing enzymes in reactions such as photooxidation (section 1.2).

In the lake, a suppression of microbial degradation of organic matter in the epilimnion by iron limitation could result in the organic matter being oxidized after it settles into the iron-rich hypolimnion. The hypolimnion does not have a renewable supply of oxygen; thus, the hypolimnion can become anoxic rapidly. Anoxic decay of organic compounds is inefficient (White et al. 1968); thus, a residual oxygen demand can accumulate in the hypolimnion. A suppression of oxygen consumption in the epilimnion during the Anabaena bloom in Black Lake as a result of inhibition of heterotrophy would influence the seasonal asynchrony in oxygen production/utilization which enhances pyrite formation.

The biochemical reactions in the heterotrophy inhibition experiments were incompletely resolved. In contrast to algal experiments, the addition of iron in heterotrophy experiments did not overcome the toxicity of the siderophore isolates. This unexpected response supports the alternative hypothesis that another compound with antibiotic properties was also in the siderophore isolates and was also induced by iron limitation.

Many microbes excrete antibiotics that are structural homologs of siderophores (Prelog 1968, Winkelmann 1974). These antibiotics can interact with siderophores to result in synergistic antagonism of other species (Musher et al. 1974). This uncertainty complicates an interpretation of the mechanisms of microbial competition; however, the significance of iron availability is unaltered by the potential presence of antibiotics that are induced by iron limitation.

The control that siderophore isolates had over aquatic microbes is analogous to siderophore regulation of microbes in soils (Neilands 1981b, Emery 1982), plant-pathogen relationships (Emery 1982), and enteric bacteria in humans (Weinberg 1974, 1975, 1978). The microbial control over iron availability is a general regulator of population structure and any force that changes iron availability can shift community structure.

4.8 Calcite Precipitation

Although this thesis began as a study of the effect of iron availability on algal periodicity, other factors modified the effect of iron availability on algal productivity. Interpretation of the long-term limnetic response to iron enrichment required a study of calcium carbonate geochemistry. The crash of blue-green algal blooms was sometimes related directly to calcite precipitation, and only indirectly to iron chemistry. Moreover, the precipitation of calcite seemed to overcome the microbial suppression of bacterial heterotrophy. Calcite precipitation is a key reaction that is related to iron chemistry in several ways.

Carbonate chemistry has been well documented; however, the importance of calcite precipitation as a regulator of algal growth has only recently been well established. Calcite precipitation suppresses algal growth by enhancing algal sedimentation and by precipitating phosphorus. Calcite precipitation of phosphorus has been demonstrated in laboratory studies (Otsuki and Wetzel 1972, Stumm and Morgan 1981) and recently in lakes (Rossknecht 1980, Avnimelach 1983, Murphy et al. 1983a).

4.8.1 Variability of Calcite Precipitation

Although the thermodynamics of carbonate reactions are well characterized (Stumm and Morgan 1981), the initiation of precipitation is difficult to predict. Calcite precipitation is restricted by kinetic limitations (Berner and Morse 1974), organic compounds (Chave and Suess 1970, Otsuki and Wetzel 1973, Reddy 1979) and phosphate (Berner and Morse 1974, Walter and Hanor 1979). In my studies, phosphate and dissolved organic matter were about 0.9% and 10% respectively of the precipitating calcite floc. Phosphate and dissolved organic carbon suppression of calcite precipitation must contribute to the high degree of supersaturation of Black and Frisken lakes with calcite.

In the July of 1982, cold weather restricted calcite precipitation. The cooling of the epilimnetic waters from 20°C to 14.6°C reduced the degree of calcite supersaturation from 11.8 to 5.5 fold. The cold weather was unusual; moreover, calcite precipitation was blocked when the water was still greatly supersaturated. Other factors must contribute to the variability

of calcite precipitation.

Geochemists believe that calcite crystal formation is prone to spontaneous nucleation (Reddy and Nancollas 1971, Reynolds 1978). Geochemists overcome the random length of time for nucleation to occur by saturating their laboratory solutions with calcium carbonate dust (Reddy et al. 1981). This technique would change too many variables (light intensity, dissolved organic carbon and phosphorus concentrations, and algal buoyancy) for most biological studies. A pH stat, such as Shapiro (1984) has used in limnocorrals, may be a very useful to prevent calcite precipitation in bioassay experiments.

Considerable variability in the initiation of calcite precipitation in lakes has been observed. Lehman (1980) found that "Lake water equilibria were so close to the saturation limit for $CaCO_3$ at the pH of ca. 8.2 that the increased productivity caused by the nutrients led quickly to calcite precipitation". Rossknecht (1977) proposed that a lack of "seed" crystals to initiate precipitation, resulted in the observed one week delay from the peak of algal productivity to precipitation of calcite. Similarly, Koschel et al. (1983) have observed delays of two to four weeks in the precipitation of calcite after the peak of oxygen production.

A stimulation of calcite precipitation could occur in any enrichment of hardwater with a limiting nutrient. The addition of the limiting nutrient causes an initial stimulation of primary production which raises the pH and causes calcite precipitation. This reaction was most pronounced in the Black Lake nitrate limnocorral. Nitrate assimilation results in hydroxide excretion

(Goldman and Brewer 1980). Moreover, nitrate has been used to stimulate the precipitation of calcium carbonate (Morita 1980, Novitsky 1981, Brownlee and Murphy 1983). Iron enrichment of the Black Lake limnocorrals also effectively stimulated calcite precipitation. The densest algal blooms in either the limnocorrals or in Black or Frisken lakes occurred when calcite precipitation was either not observed or was delayed by more than three weeks. At times, the periodicity of blue-green algal blooms was regulated by calcite precipitation.

4.9 Biological Induction of Calcite Precipitation

Another cause of the variable induction of calcite precipitation is biological regulation of calcium carbonate precipitation. Certainly, algae mediate crystal nucleation; the type of crystal precipitate formed is dependent upon the type of algae present (Darley 1974). However, whether microbes gain energy released in calcium carbonate precipitation is an old controversy in both geology and biology (Kuznetsov 1970). Calcium carbonate precipitation is so closely related to microbial metabolic activity that biochemical reactions cannot be readily discriminated from geochemical reactions (Megard 1968, Kelts and Hsu 1978).

However, some microbes are much better catalysts of calcium carbonate precipitation than others (Morita 1980). Stabel (1986) found that calcite precipitation in Lake Constance, Switzerland, was catalyzed by only some of the many species of algae observed growing in water supersaturated with calcium carbonate. Stabel (1986) also found that populations of algae able to initiate

calcite precipitation arise regularly at defined periods. Thus, the composition of the biological community can influence calcium carbonate precipitation; there is an important biological mediation of the algal periodicity imposed by calcite precipitation.

4.10 Microbial Responses to Calcite Precipitation

Other biological reactions during calcium carbonate precipitation are unresolved. The initiation of calcite precipitation by the additions of calcium chloride resulted in the lysis of about a third of the Aphanizomenon. None of the algae in the control flasks lysed. Lysis of algae also occurred during the calcite precipitation that was observed in Black Lake in 1979 and in the lime-mediated induction of calcite precipitation of Frisken Lake.

Rapid changes in microbial population structure would occur if calcite suppressed siderophore activity. Szaniszlo et al. (1981) found that calcium carbonate enhanced siderophore production. The microbial necessity to enhance siderophore production could indicate a suppression of siderophore reactivity. Three mechanisms could produce this suppression.

1) Calcite could react with the fibrillar material that siderophores are associated with. This reaction could physically prevent siderophore reactions. In the iron-binding study, failure to isolate the chelators from the fibrillar colloids resulted in carbonate coprecipitation of the chelators.

2) Calcium could compete directly with iron for siderophore complexation. The reactions of calcium (Hider et al. 1982) and

calcite (Reynolds 1978) with some chelators are well established; however, the specificity of the siderophore chelation suggests that other reactions exist.

3) Calcite could react with the sites of siderophore assimilation. This type of interference has been observed with polymyxin (Newton 1953). Newton found that divalent ions interfered with the antibacterial activity of polymyxin by blocking the sites of polymyxin assimilation.

The inhibition of siderophore activity by calcite would lead to a collapse of symbiotic associations mediated by siderophores. For microbes with weak iron assimilation systems, such as Aphanizomenon, iron limitation would be rapidly enhanced. The initiation of an iron-chlorophyll correlation after the two lime treatments of Frisken Lake (Fig. 5) may have been established by precipitation of iron chelators. Half of the dissolved organic carbon (30 --> 15 mg/L) was precipitated from Frisken Lake by the induction of calcium carbonate precipitation.

4.11 Phosphorus Coprecipitation with Calcite

Phosphorus limitation could be another mechanism producing the collapse of algal blooms during calcite precipitation. In Black Lake, the temporal separation of algal oxygen production and phosphorus precipitation (Fig. 15), and the coincidence of phosphorus and calcium precipitation showed that calcium carbonate precipitation could regulate phosphorus solubility. The rapid assimilation of $^{32}P\text{-}PO_4$ during calcite precipitation indicated that phosphorus limitation could be induced by calcite precipitation.

The induction of P-calcite precipitation could remove most of the phosphorus from the epilimnion of a hardwater lake. The maximum precipitation induced in the $CaCl_2$ incubation of Black Lake was 10 mg $CaCO_3$/L and 100 µg P/L. Linear extrapolation of this response to the largest amount of calcite precipitation observed in Black Lake (50 mg $CaCO_3$/L) indicates a potential precipitation of 500 µg P/L. Berner and Morse (1974) produced this degree of phosphorus precipitation in pseudo-seawater.

The relative importance of geochemical and biochemical phosphorus precipitation was not resolved. However, phosphorus precipitation during calcite precipitation events provided insight into the effect of iron availability on carbonate equilibrium and in turn heterotrophic production. The carbonate equilibria are a good integration of primary production and heterotrophy.

The pH in the hypolimnia of the citrate limnocorral experiments was lowest in the ferric citrate limnocorrals. A lower pH is the expected result of greater heterotrophic production of CO_2 in the hypolimnia which in turn, is the anticipated effect of greater primary production in the epilimnia; these Fe-limnocorrals had the greatest oxygen production in the epilimnia. The higher phosphorus concentration in the hypolimnia of the Fe-limnocorrals indicates that either more P-calcite dissolved as a result of greater CO_2 production or that more organic phosphorus was metabolized. Either reaction confirms that more heterotrophic activity occurred in the hypolimnia of the ferric citrate limnocorrals and that in turn, more primary production occurred in the epilimnia.

The strong correlation of phosphorus to calcium in the sediments of Yellow, Frisken, and Roche lakes indicates that not all of the precipitated P-calcite redissolved in the hypolimnion. Although the initial P-precipitate cannot be apatite (Koutsoukos et al. 1980), the stable component of P-calcite precipitation may be apatite. Initially, phosphorus adsorbs to kinks in the calcite crystal (Berner and Morse 1974) and this complex ultimately forms hydroxylapatite (Stumm and Leckie 1970, Griffin and Jurinak 1974). Brown (1980) suggested that hydroxylapatite could form in aqueous calcitic limestone suspensions. Ryding (1985) proposed that lime application to a lake would enhance apatite formation. In Frisken Lake, lime application did not appear to enhance apatite formation; about 95% of the precipitated phosphorus redissolved.

The inverse correlation between iron and phosphorus/calcite indicates that different variables control the solubility of these two geochemical subsets. Pyrite is very stable in these anoxic sediments; P-calcite can be dissolved by the heterotrophic production of CO_2. This difference in stability of these two minerals must contribute to the establishment of low iron and high phosphorus concentrations in many hardwater lakes.

4.11 CONCLUSIONS

Iron availability influences the periodicity of blue-green algal growth. Blue-green algal blooms occur in Black Lake only after iron is released from the lake sediments. Prior to the mid summer increase in iron, microbes in Black Lake produce low molecular weight chelators that selectively complex iron. The

bioavailability of chelated iron can be restricted to particular species. Furthermore, the chelator concentration in lake water can exceed the dissolved iron concentration; thus, microbial chelator production can control the availability of iron.

The competition for iron is associated with the suppression of competing algae and bacteria by either the direct toxicity of siderophores or an associated excretion of an antibiotic. These reactions enable some algae to produce their own microenvironment.

Iron limitation in lakes varies greatly within geological formations mainly because iron loading varies greatly between lakes in the same area. On the Thompson Plateau, the potential for iron availability is indicated by a high concentration of phosphorus in oxidized water in either the fall or spring. Iron-limited lakes can have too little iron for ferric phosphate reactions to regulate the solubility of phosphorus. The lack of iron to precipitate phosphorus and the high loading of phosphorus from the weathering of apatite results in very high concentrations of phosphorus (>200 µg/L) in Black and Frisken lakes. The high iron and phosphorus loading into Chain Lake results in high soluble phosphorus concentrations only for brief periods when oxygen concentrations are less than 4 mg/L.

Iron biogeochemsitry is closely coupled to calcite precipitation. Iron enrichment of iron-limited hardwater lakes can lead to rapid precipitation of calcite. However, calcite precipitation can be delayed for at least three weeks after the onset of calcite supersaturation. The delay period and the amount of precipitation varies greatly. In many bioassays in hardwater

lakes, the effect of iron enrichment can be interpreted more easily by an analysis of the shifts in the carbonate equilibria than by a measurement of algal biomass.

Phosphorus adsorbs directly to calcite. Over 90% of the precipitated phosphorus redissolves in the hypolimnion; thus, unlike in iron-rich lakes, the concentration of soluble phosphorus is very high in spring and fall when the hypolimnetic water mixes and becomes oxidized. However, the sedimented phosphorus that remains associated with calcite in the lake sediments is stable. In lake sediments with more than 10% calcium carbonate, the distribution of phosphorus is strongly correlated to the distribution of calcium.

The precipitation of calcite enhances the sedimentation of algae and many algae lyse during calcite precipitation. The decay of algae in the hypolimnion enhances the potential for pyrite formation which in turn minimizes the recycling of iron. Thus, calcite precipitation in eutrophic stratified lakes will enhance pyrite formation, and if the supply of iron to the lake is low, pyrite formation will result in iron limitation. In general, the enhancement of iron limitation by pyrite formation will be more important in hardwater lakes than in softwater lakes where sulphate reduction can be limited by an inadequate supply of sulphate.

Four reactions, iron chelation, sediment iron release, pyrite formation, and calcite precipitation, can regulate much of the algal succession and variability of microbial productivity in hardwater lakes.

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Appendix 1 Details of Limnocorral Experiments

1980 Black Lake Limnocorral Experiments

Seven limnocorrals of transparent woven polyethylene were used to test the effect of iron and nitrate enrichment on algal productivity. The limnocorrals were 2.0 meters in diameter and 7.0 meters deep with a sealed bottom. A float-collar of styrofoam and plywood prevented any water exchange with the lake.

Starting on 20 May 1980, one limnocorral was enriched every two weeks with 1.82 g of reagent grade KNO_3 (Fisher). A paddle was used to distribute the nitrate throughout the limnocorral. Ideally the top four meters would have been increased to 200 µg N/L. The surface nitrate concentration immediately after nitrate enrichment was 350 µg N/L.

Iron was added in the form of an EDTA-Fe complex (3:1 molar ratio EDTA:Fe). The concentration of iron was monitored biweekly by graphite furnace atomic absorption and the soluble iron levels were maintained between 100 and 200 µg Fe/L. EDTA-limnocorrals were enriched with equivalent concentrations of EDTA as were the Fe-EDTA treatments. Control limnocorrals were monitored along with the treatments.

1982 Black Lake Limnocorral Experiment

Limnocorrals of transparent woven polyethylene, 2.0 meters in diameter and 5.0 meters deep, were made by False Creek Plastics (Vancouver,B.C.). A collar that was resistant to ultraviolet light was sewn to the top and extruded styrofoam was inserted into the hollow collar for flotation. The bottom of the limnocorrals were sealed and anchored with ropes and concrete blocks via attached external loops. The improvements in design relative to 1980, facilitated filling of the limnocorrals and replication of the treatments.

Eight limnocorrals were filled June 13-14, 1982 with lakewater 2.0 meters from the lake surface using a large water pump. Two limnocorrals received three additions of 18.4 g of commercial grade $Ca(NO_3)_2$. Two limnocorrals received three additions of 18.4 g of $Ca(NO_3)_2$, and 78.76 g of sodium citrate. Two limnocorrals received 18.4 g of $Ca(NO_3)_2$, and an iron solution. The iron solution was made by first dissolving 4.55 g of $FeCl_3$ and 192.12 g of citric acid and then adjusting the pH to 7.0 with NaOH. Two limnocorrals were left untreated.

The nutrients were added June 23, June 30, and Aug. 12. In the August 12 treatment, nitrate was not added to the citrate limnocorrals. On June 23, the nutrients were dissolved in 50 liters of lakewater and pumped the solution to five depths. For the other two treatments the nutrients were dissolved in 1.0 liter of lakewater and added to the surface of the limnocorral with a paddle stirring the water. With ideal mixing, the initial

nutrient concentrations would have been: 200 µg N/L, 1.4 mg C-citrate/L, or 100 µg Fe/L.

Chain Lake Limnocorral Experiments

Limnocorrals with the 1982 design were used. Eight limnocorrals were filled July 15 and enriched on July 18. The nitrogen limnocorrals were enriched with 8.3 g of NH_4Cl which should have produced a concentration of 200 µg N/L. Iron (3.42 g $FeCl_3$ $6H_2O$ per limnocorral) was mixed with citric acid (96 g per limnocorral), the pH was adjusted to 7.0 with NaOH, and quickly the solutions were added to the limnocorrals. Two limnocorrals received 96 g of citric acid that was also neutralized with NaOH. All limnocorrals, including what is referred to in the text as "untreated limnocorrals" received 1.98 g of KH_2PO_4. All nutrient additions were made by mixing the nutrients in 50 L of water and then pumping the solution into the limnocorrals at five depths.

Appendix 2

Table A1. Iron analysis in Black Lake - May 9, 1978.[1]

Depth	Dissolved		Particulate	
	Aerated	Control	Aerated	Control
Surface	6	5	4	3
2M	8	4	19	12
4M	20	17	44	26
6M	22	26	182	57
8M	25	13	202	45
9M	28	88	119	69

[1] All values are in μg Fe/L.

Table A1 continued

1979 Data:

Date	Depth	Dissolved		Particulate	
		Aerated	Control	Aerated	Control
June 15	2M	13	-	29	25
	5M	22	-	30	11
	9M	19	-	30	11
July 4	Surf	17	11	31	28
	3M	10	10	29	20
	5M	20	20	84	27
	9M	22	24	97	57
July 17	2M	3	11	40	15
	5M	51	7	60	48
	9M	8	18	123	100
Aug 11	0.5M	<1	5	94	50
	1M	2	250	90	27
	2M	119	184	111	60
	3M	4	2	92	29
	5M	8	203	260	69
	9M	600	-	632	273
Aug 30	1M	<1	2	43	37
	5M	101	90	86	52
	9M	102	85	100	32
Oct 26	2M	10	12	30	28
	8M	14	18	44	62
Dec 13	2M	9	10	20	21
	8M	11	12	18	15

[1] All values are in µg Fe/L.

Table A1 continued

1980 Data:

Date	Depth	Dissolved		Particulate	
		Aerated	Control	Aerated	Control
Jan 22	2M	18	17	13	-
	8M	29	-	13	-
April 23	2M	2	2	35	27
	5M	3	4	39	45
	8M	10	13	42	41
May 12	2M	-	13	42	41
	8M	-	13	-	-
May 21	Surf	-	-	59	-
	2M	-	-	46	-
	5M	-	-	34	-
	7M	-	-	38	-
June 4	Surf	25	18	6	6
	1M	20	22	7	7
	2M	20	25	6	8
	3M	18	20	27	7
	5M	20	30	30	11
	8M	11	35	30	11
July 17	1M	1	1	10	12
	3M	4	2	20	17
Aug 11	1M	4	0	93	59
	3M	5	3	184	72
	5M	7	8	194	98
	7M	-	-	453	326
	9M	18	17	-	422

[1] All values are in μg Fe/L.

Appendix 3

Table A2 Oxygen Concentrations in Limnocorrals During Citrate Additions (mg/L)

Fe-Citrate Limnocorrals

Depth	June						July								August			
	24th		26th		28th		13th		22nd		10th		17th		24th			
0	8.5	8.8	8.4	8.7	9.2	11.2	13.3	12.2	18.0	20.0	17.9	18.2	18.2	16.6	17.4	16.8		
1	8.8	9.2	8.35	8.75	9.8	11.2	13.8	11.5	20.0	17.0	17.9	19.0	15.3	16.6	16.8	16.2		
2	9.4	10.2	9.4	10.4	9.5	10.3	11.3	8.6	20.0	16.6	10.2	17.0	12.8	13.1	14.0	14.4		
3	9.2	10.2	9.55	10.2	8.5	9.4	9.2	8.3	12.0	11.0	8.2	8.8	10.8	12.6	9.2	9.2		
4	9.1	9.9	9.25	10.0	8.0	8.5	7.9	7.0	3.0	6.0	8.1	7.8	10.1	10.6	6.7	2.0		
5	9.0	9.9	8.0	9.0	6.1	6.5	5.0	4.2	2.4	4.3	8.0	7.7	5.5	7.0	1.0	0.7		

Na-Citrate Limnocorrals

Depth	June						July								August			
	24th		26th		28th		13th		22nd		10th		17th		24th			
0	8.7	8.8	9.1	9.8	10.6	12.0	11.0	13.0	16.5	16.0	14.6	16.2	14.4	13.4	14.2	15.4		
1	9.0	9.5	9.1	9.8	10.5	12.0	12.2	15.0	13.5	14.6	15.2	16.2	13.6	12.9	14.3	13.1		
2	9.7	10.1	10.0	10.2	10.0	11.0	10.8	13.6	13.5	14.6	16.3	16.1	11.2	11.6	13.0	12.2		
3	9.6	9.9	9.9	10.2	9.2	9.0	9.1	9.0	12.6	11.8	9.4	8.8	11.2	10.6	10.0	9.1		
4	9.4	9.7	9.6	9.7	7.5	7.9	6.7	7.4	5.6	5.6	8.2	4.2	9.8	9.0	8.6	6.8		
5	9.0	9.4	8.1	8.4	6.7	7.0	6.5	4.1	4.0	1.8	7.6	3.6	8.0	7.0	3.6	1.3		

Control Limnocorrals

Depth	June						July								August			
	24th		26th		28th		13th		22nd		10th		17th		24th			
0	8.4	8.6	8.8	9.2	10.4	9.4	12.8	17.4	15.9	15.8	18.4	15.0	16.1	14.4	16.0	14.2		
1	8.5	8.8	9.0	9.2	10.5	9.4	13.0	20.0	15.9	16.1	18.4	14.4	16.0	12.0	16.4	14.4		
2	8.9	9.4	9.4	9.6	9.8	9.8	12.0	20.0	16.1	16.6	16.8	11.8	12.5	9.8	14.8	14.0		
3	9.0	9.5	9.4	9.6	9.3	9.4	9.5	12.4	9.8	14.4	10.4	4.2	12.4	9.6	11.4	8.0		
4	9.0	9.5	9.0	9.5	8.7	9.3	8.8	9.0	8.2	5.4	9.8	2.6	12.2	8.4	11.0	6.7		
5	8.7	8.4	8.3	7.8	7.8	7.3	6.4	6.0	7.7	3.8	9.4	2.5	3.0	6.4	4.2	3.2		

Appendix 4 Improvements to the Iron-Binding Assay

Unfortunately, the assay was initially used before the optimal incubation time was determined. The chelation is not complete within the half hour incubation that was initially used (Fig. A1). This procedure resulted in a smaller range of linear responses and a sampling error of about 10% (coefficient of variation). Half hour incubations were used for the following work: the iron binding capacity of the chelators used in the heterotrophy, primary production incubations, and measurements of lake siderophores.

In one hour incubations, the standard curve was linear throughout the concentration range that was encountered (Fig. A2). Blanks were very low and the sensitivity of the radioisotope method was good (Fig. A3). In one hour incubations, the coefficient of variation was less than 2%.

Another error may have arisen in the use of acids or bases with ion exchange resins. Some of the siderophore readily broke down in the presence of weak acid or base (Fig. A4). Presumably little of that break down product was in the bioassays. Freeze-drying is a better method of concentrating siderophores than the ion-exchange method.

The uncertainty associated with knowing the concentration of the siderophore in the microenvironment of the cells was a much bigger problem than the analytical problems. Strong qualitative statements are still possible.

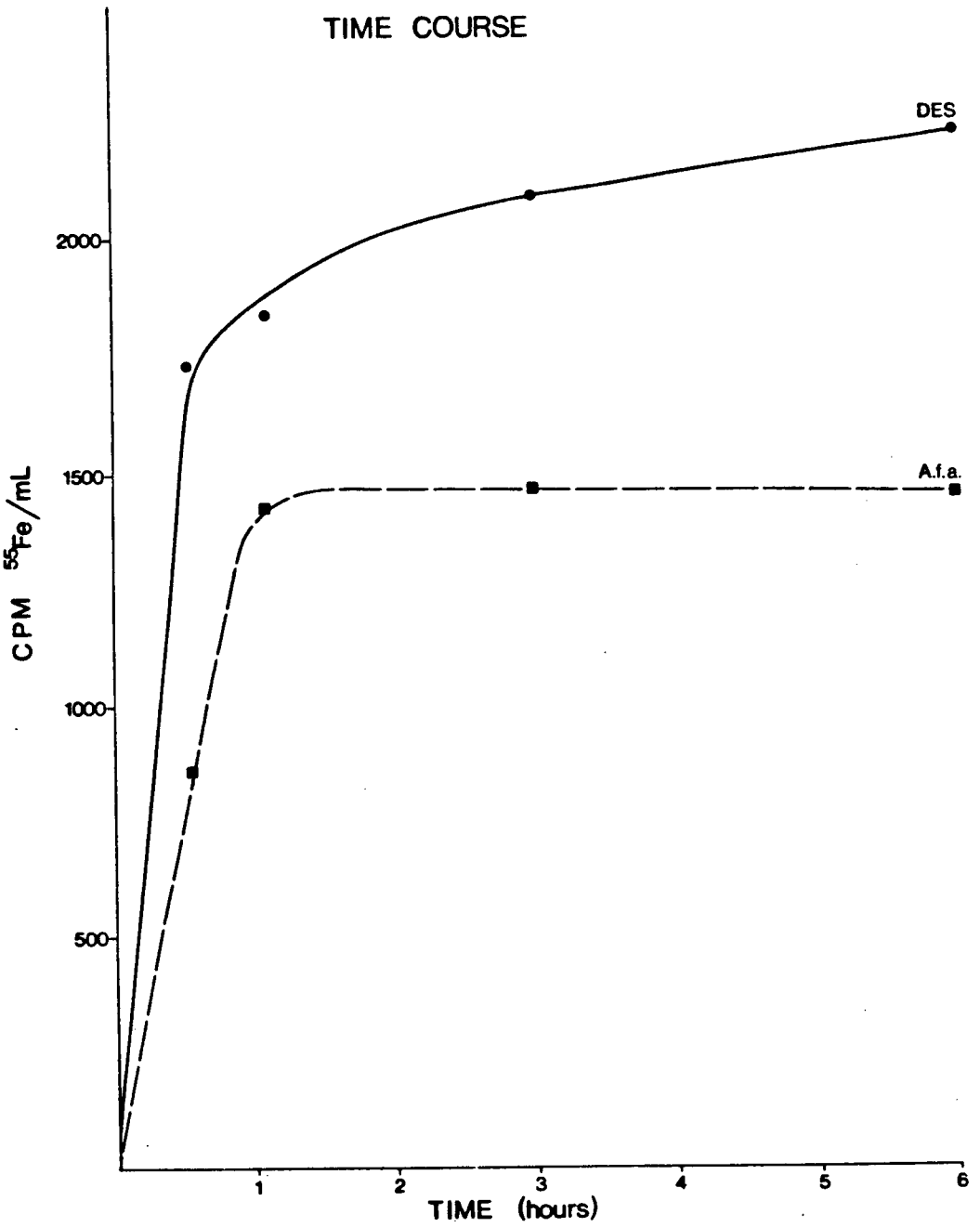

Figure A1 Effect of incubation time on chelation of iron by desferal (DES) and a filtrate from a Anabaena flos-aquae culture (A.f.a.).

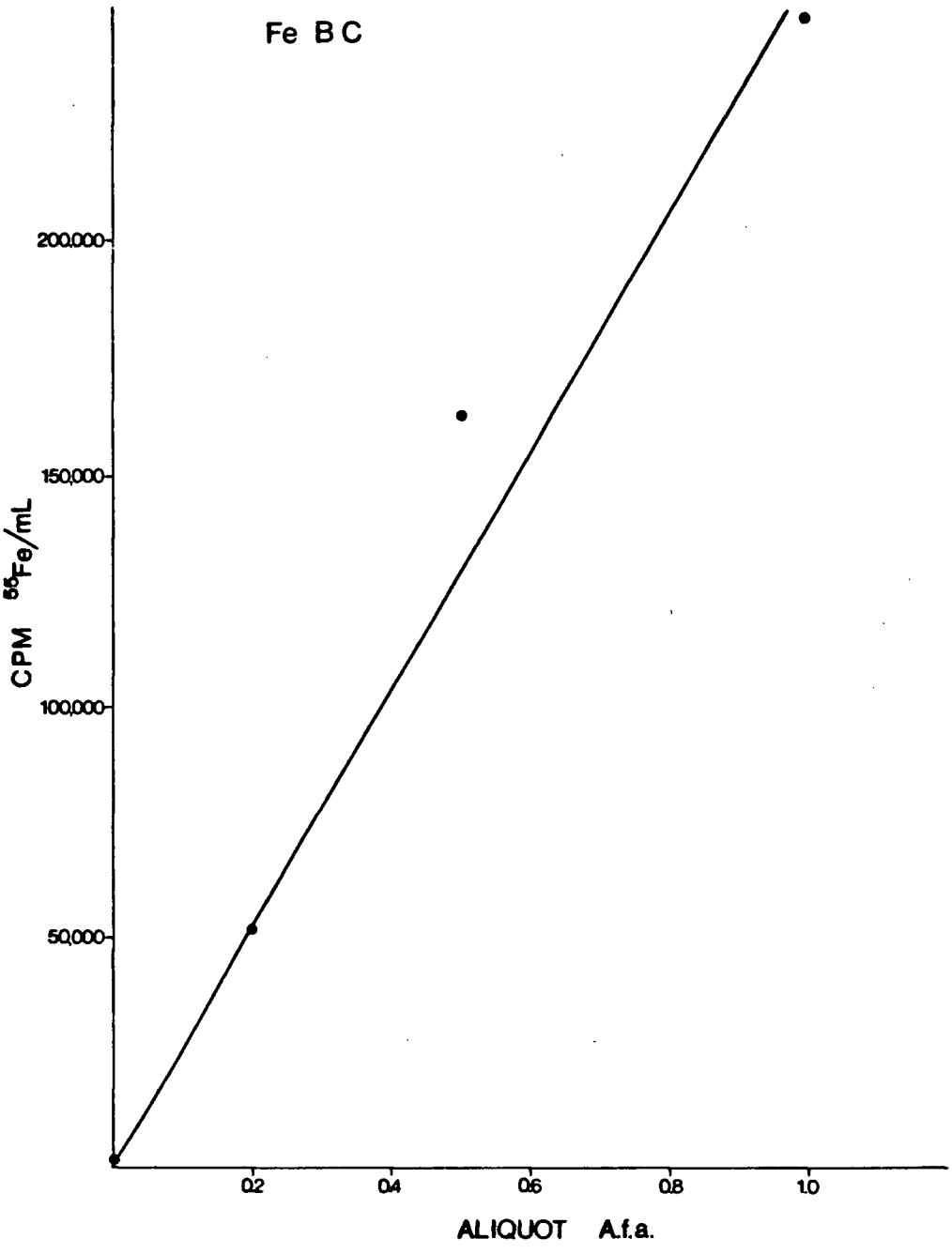

Figure A2 Iron binding capacity (FeBC) of a filtrate from a Anabaena flos-aquae culture. Aliquots of A.f.a. refers to the degree of dilution of the culture filtrate.

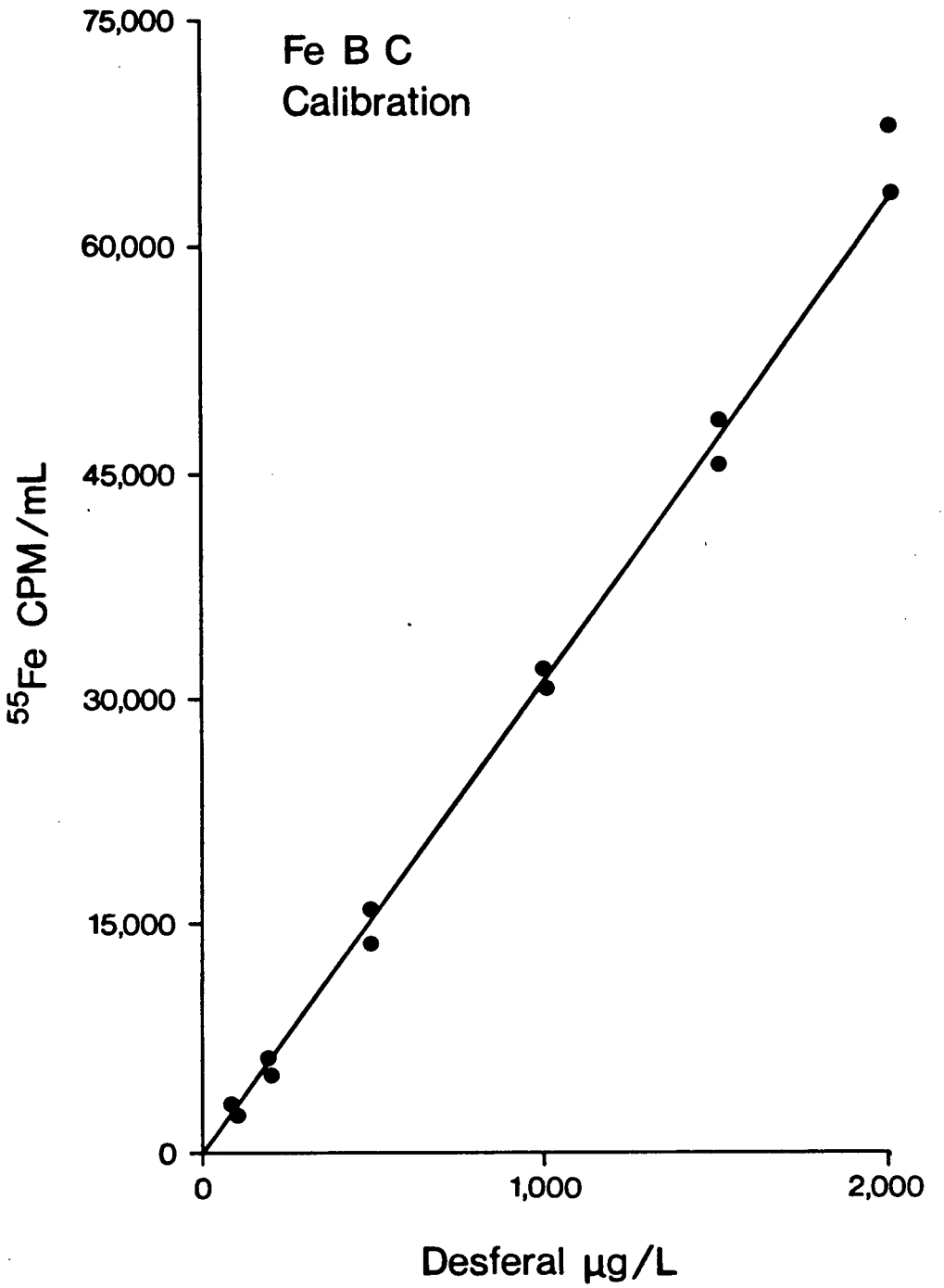

Figure A3 Iron binding capacity (FeBC) standardization with desferal.

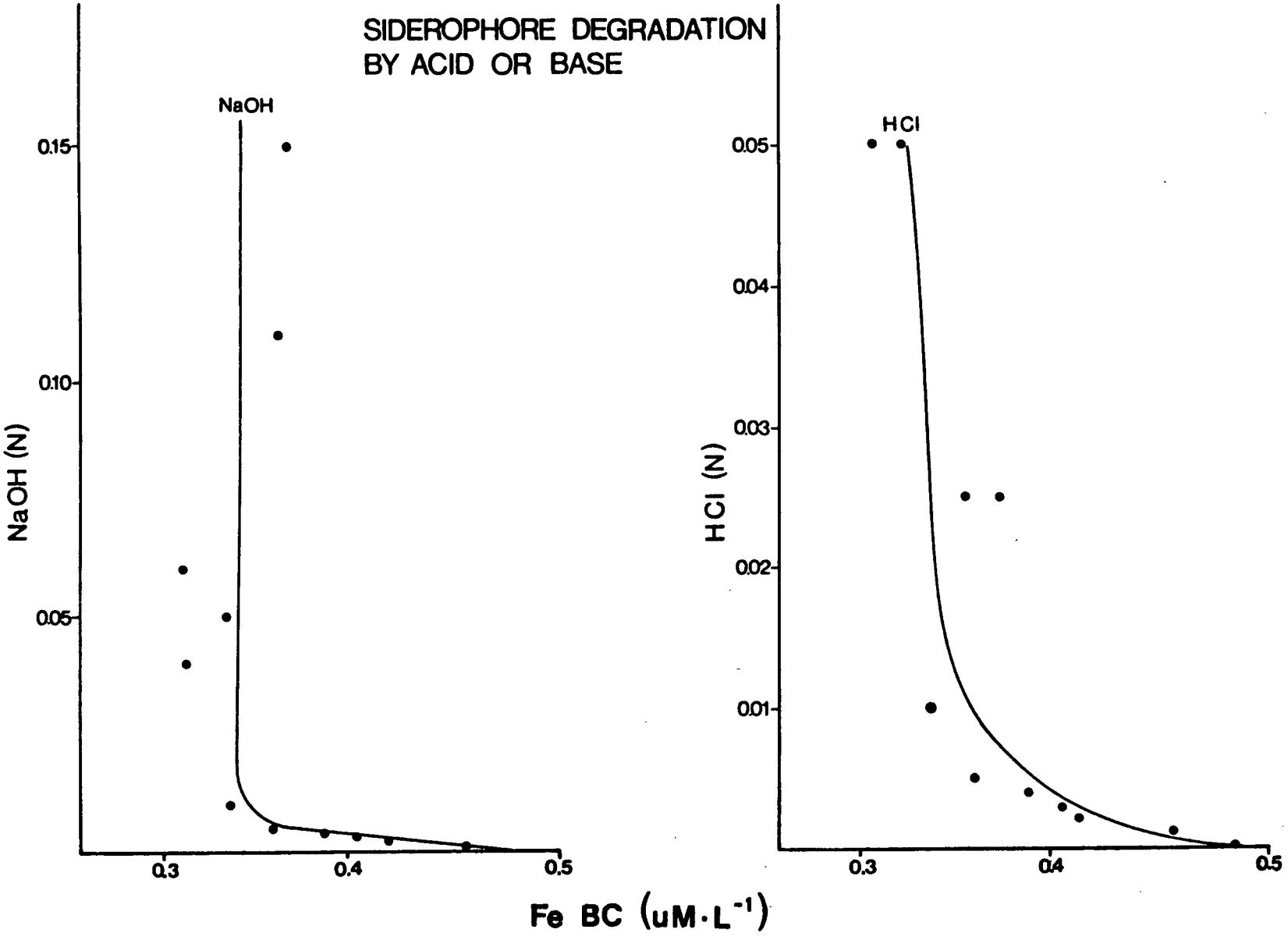

Figure A4 Degradation of the siderophore from Anabaena flos- aquae by acid or base.

Appendix 5
Table A3 Yellow Lake Sediment Chemistry

Depth	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	TiO_2	MnO	P_2O_5
cm										
0-2	54.86	6.11	3.55	2.16	15.72	0.82	1.85	0.41	0.14	0.30
4-6	43.75	8.70	3.99	2.22	19.36	0.87	2.25	0.42	0.06	0.32
6-8	38.07	6.17	2.39	1.36	26.89	0.81	1.64	0.28	0.10	0.38
8-10	33.96	4.06	1.65	1.02	31.65	0.76	1.11	0.19	0.05	0.44
10-12	33.70	3.94	1.73	1.04	31.83	0.54	1.04	0.19	0.05	0.51
12-14	36.77	5.45	2.58	1.50	28.52	0.64	1.42	0.24	0.05	0.43
14-16	31.96	4.83	2.23	1.56	31.82	0.80	1.10	0.20	0.04	0.48
18-20	42.66	4.27	2.65	0.96	20.35	0.35	0.88	0.29	0.06	0.48
28-30	47.33	1.22	1.50	0.52	21.82	0.20	0.10	0.09	0.05	0.62
38-40	44.85	2.55	1.70	0.60	23.36	0.36	0.64	0.18	0.04	0.58
48-50	49.09	1.63	1.57	0.53	20.42	0.34	0.28	0.13	0.05	0.52

All values are expressed as % dry weight.
The X-ray fluoresence analysis reports the total concentration as if the element was present as a simple oxide. The presence of calcite (calcium carbonate) was confirmed by X-ray diffraction analysis.