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SUPPRESSION OF MICROBIAL UPTAKE OR ORGANIC SOLUTES BY ALGAL SIDEROPHORES IN A HARDWATER LAKE

By

T.P. Murphy*, K.J. Hall+ and I. Yesaki+

* Aquatic Ecology Division, National Water Research Institute, 867 Lakeshore Road, Burlington, Ontario, L7R 4A6, CANADA.

+ Westwater Research Centre, 2075 Wesbrook Place, University of British Columbia, Vancouver, B.C., V6T 1W5, CANADA.

Sommaire :

On a isolé des agents de chélation du fer, soit des sidérophores, à partir d'algues cultivées en laboratoire dans des conditions d'inhibition de croissance par le fer. On a découvert que ces sidérophores pouvaient réduire la capacité des micro-organismes à absorber l'acétate et le glucose dans un lac à eau dure. Les sidérophores produits par l'algue Scenedesmus basiliensis pouvaient réduire de 81 à 97 p. 100 l'absorption d'acétate et de 92 à 98 p. 100 l'absorption de glucose. Les sidérophores isolés à partir d'algues de lac, Anabaena flos-aqua et Anabaena, pouvaient réduire de 14 à 92 p. 100 l'absorption d'acétate et augmenter de façon considérable la proportion de soluté respiré. Des additions de fer n'ont aucunement modifié les effets inhibiteurs des agents de chélation produits par les algues. Des changements temporels dans la quantité d'acétate, de glucose et de glycine absorbée par les micro-organismes du lac Black en Colombie-Britannique ont démontré qu'il y a eu une diminution des taux d'absorption en juin et au début de juillet. À ce moment, on a constaté que l'épanouissement d'Anabaena était prédominant dans la population de phytoplancton et que les quantités de fer dissous étaient faibles, situation qui devrait favoriser la production naturelle de sidérophores. Nous avons ajouté de l'EDTA-Fe et de l'EDTA dans des contenants d'essai, ce qui a contribué à stimuler la productivité du phytoplancton et l'activité des micro-organismes dans les conditions de faible disponibilité du fer qui prévalent durant l'été.

Perspective-gestion

On a constaté que les sidérophores sécrétés par des algues réduisaient la décomposition de composés organiques par les micro-organismes dans le lac Black en Colombie-Britannique. Les sidérophores sont des agents de chélation spécifiques du fer; ils sont produits lorsque les charges de fer limitent la croissance des micro-organismes. On a remarqué que les sidérophores produits par Scenedesmus réduisaient l'assimilation de l'acétate par les micro-organismes de 81 à 97 p. 100 et l'assimilation du glucose de 92 à 98 p. 100. Les sidérophores produits par les algues Anabaena flos-aqua et Anabaena du lac Black réduisaient l'absorption de l'acétate par les micro-organismes de 14 à 92 p. 100. Il faudrait tenir compte de ces importants résultats dans tout programme de restauration de lac qui modifie la disponibilité du fer et par conséquent, la production des sidérophores (aération, addition de chlorure ferrique, etc.).

Ce travail a été produit par T. Murphy pendant un congé pour fins d'éducation.

Management Perspective

The bacterial degradation of organic compounds was suppressed in Black Lake, British Columbia, by the algal excretion of siderophores. Siderophores are specific chelators of iron that are produced when the supply of iron limits microbial growth. The siderophore isolated from Scenedesmus suppressed the bacterial assimilation of acetate by 81 to 97% and of glucose by 92 to 98%. Siderophore isolates from Anabaena flos-aquae or an Anabaena isolated from Black Lake reduced the bacterial uptake of acetate by 14 to 92%. These results are important to any lake restoration procedure that alters the availability of iron and thus changes the production of siderophores (aeration, ferric chloride addition etc.).

This work was a product of an educational leave by T. Murphy.

SUPPRESSION OF MICROBIAL UPTAKE OF ORGANIC SOLUTES BY ALGAL SIDEROPHORES IN A HARDWATER LAKE[1]

Thomas P. Murphy[2], Ken J. Hall[3] and Itsuo Yesaki[3]

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[2] Aquatic Ecology Division, National Water Research Institute, 867 Lakeshore Road, Burlington, Ontario, L7R4A6.

[3]Westwater Research Centre, 2075 Wesbrook Place, University of British Columbia, Vancouver, B.C.,V6T1W5

Abstract:

Iron chelating compounds, siderophores, were isolated from algae grown in the laboratory under iron limiting conditions. These siderophores were capable of suppressing the microbial uptake of acetate and glucose in a hardwater lake. The siderophore from Scenedesmus basiliensis reduced the uptake of acetate 81 to 97 percent and the uptake of glucose 92 to 98 percent. Siderophores from Anabaena flos-aquae and an Anabaena lake isolate reduced the uptake of acetate 14 to 92 percent and greatly increased the proportion of the solute that was respired. Iron addition did not reduce the inhibitory effects of the algal chelates. Temporal changes in the microbial uptake of acetate, glucose, and glycine in Black Lake, British Columbia showed suppression of uptake rates in June and early July. At this time, an Anabaena bloom dominated the phytoplankton and dissolved iron levels were low, which should stimulate natural siderophore production. The artificial addition of Fe-EDTA or EDTA to experimental enclosures helped to stimulate phytoplankton productivity and microbial activity during summer conditions of low iron availability.

The interactions between algae and bacteria are complex. Bacteria can show a chemotatic response to algae or their excretion products (Bell and Mitchell 1972, Kagure et al. 1982) and they often utilize this excreted organic matter as an energy source (Chrost 1978, Larsson and Hogstrom 1979). Algae can also produce antibacterial substances which inhibit bacterial production and activity (Chrost and Wazyk 1978, Reichardt 1981). These bacterial inhibitors include a wide variety of chemicals such as acrylic acid, fatty acids, nucleosides, peptides, and polysaccharides (Jones 1982). Since heterotrophic bacteria degrade autochthonous organic matter in the aquatic environment, inhibition of aquatic bacteria could affect the seasonal pattern of decomposition, oxygen dynamics, and nutrient cycling in a lake.

The excretory compounds produced by algae can also have allelopathic interactions with other algae (Keating 1978, Wolfe and Rice 1979). Some of these compounds are chelating agents which could help algae compete for micronutrients. Murphy et al. (1976) found that blue-green algae, grown under iron-limiting conditions, excreted a chelator which inhibited the growth of other algae. These inhibitors can help to regulate the seasonal dynamics of a phytoplankon community especially in hardwater lakes where the solubility and availability of iron are low (Murphy et al. 1983a). These low molecular weight, water soluble chelating agents are highly specific for iron. Compounds that can regulate the supply of iron to the cell have been called siderophores (Neilands 1981).

Do these siderophores, induced by iron limitation, have any effect on bacterial processes? Here, we report some experiments to examine the effect of these algal siderophores on in situ microbial activity in a hardwater lake.

We thank the Environmental Engineering Laboratory, University of British Columbia for analytical assistance.

Black Lake is a hypereutrophic hardwater lake located on the Thompson Plateau, 16 km SW of Penticton, in south central British Columbia (49°20' N, 119°45' W). This small lake has an area of four ha, a maximum depth of 9.0 m, and a mean depth of 4.5 m. The lake lies in a steep valley at an elevation of 750 m with neighbouring mountains that rise to 1500 m. It is protected from the prevailing westerly winds by the Cascade Mountains ; the poor air circulation and infrequent mixing of the lakes in this area appear to be partly responsible for winter fish kills (Northcote and Halsey 1969). Experimental hypolimnetic aeration of half of this lake, which is split by a sea curtain, has been studied as a potential technique to improve conditions for fish survival in these lakes (Ashley 1983).

This lake is naturally enriched by the weathering of phosphorus from apatite rich volcanic rock, located at higher elevations in the watershed. A small stream which enters the west side of the lake contains over 200 ug SRP liter^{-1} (Murphy et al. 1983b). The lack of anthropomorphic nutrient sources to this lake provides an excellent opportunity to study the eutrophication process without the influence of high loadings of other contaminants.

Experimental enclosures constructed of transparent polyethylene were used to test the effects of chemical addition on phytoplankton, microbial activity, and water chemistry. These enclosures were 2.0 m in diameter and 7.0 m deep with a sealed bottom. They were supported by a flotation collar of styrofoam and plywood and were anchored in the lake with concrete blocks. Iron was added to the enclosures as an EDTA-Fe complex (3:1 molar ratio). The iron was monitored every two weeks by graphite furnace atomic absorption spectroscopy and levels were adjusted to keep the iron

concentration between 100-200 ug Fe liter^{-1}. Other enclosures received EDTA in equivalent concentrations or served as controls.

Natural siderophores were isolated from Texas University axenic cultures of algae, Scenedesmus basiliensis, Anabaena flos-aquae, and A. cylindrica or from cultures isolated from Black Lake. The cultures were grown in iron deficient Chu-10 media (Stein 1973) at 20°C, with a 16 h photoperiod, in 100 uE m^{-2} sec^{-1} of light from fluorescent lamps. The chelates were isolated from 10 day batch cultures in an exponential growth phase.

Media from settled cultures was decanted and filtered through 0.45 u cellulose acetate membranes. The media (approx. 20 liters) was acidifed to pH 4.0, aerated for 30 min, adjusted to pH 10.0, and stirred for 20 min with 100 g of anion exchange resin (AG1 X8, Bio-Rad, Cl^{-1} form) to adsorb the chelate. The resin was placed in a column (1 cm dia) and the chelator eluted from the resin with 0.01 N HCl. The chelator was further purified by gel filtration (Sephadex G-25, column dimensions 2.5 cm dia x 30 cm, eluted with 0.3% NaCl). The peak containing the low molecular weight chelator was desalted by elution through an ion retardation resin (AG11A8, Bio-Rad). The technique of chelator production and isolation was evaluated by the addition of ^{14}C-HCO_3^- to the cultures to label the organic compounds, and ^{55}Fe-$FeCl_3$ to extracts to determine the ability of the molecular size components to bind iron (Murphy 1976).

Water samples for microbial activity measurements were collected with a 3 liter Van Doran water sampler and processed immediately. Nine ml of lake water was placed in a 20 ml disposable plastic syringe. Chelator, radioisotope, and formaldehyde (added to blanks) were added separately to the incubating syringe, with 1.0 ml glass syringes, through the oriface of the

plastic syringe. Approximately one ml of air was left in the incubating syringe so that the contents could be mixed. The blanks received 0.2 ml of concentrated formaldehyde and were held for 5 min to kill the microorganisms, before the other chemicals were added. The syringes were preincubated with the chelator for one hour, followed by a two hour incubation with the ^{14}C-organic solute. Uniformly labelled solutes, namely ^{14}C-glucose, 333 mCi $mMol^{-1}$; ^{14}C-acetate, 57.6 mCi $mMol^{-1}$; and ^{14}C-glycine, 120 mCi $mMol^{-1}$ (Amersham);were ampulated in 2 uCi aliquots and diluted with distilled water prior to addition to the lake water in concentrations <5 ug C $liter^{-1}$. The syringes, two active samples and one blank, were incubated in the lake at the depth that the samples were collected. The samples were incubated under normal light conditions in the lake since a light and dark incubation experiment showed very little difference in the solute uptake rates.

After incubation, a Nuclepore filter assembly containing a 0.2 u, 25 mm cellulose nitrate membrane filter (Gelman) was attached to the syringe and the sample was filtered into a reservoir tube of a CO_2 purging assembly. The filters were washed with 10 ml of membrane filtered lake water to remove any soluble isotope on the filter and the filters were placed in 10 ml of PCS scintillation solution (Amersham) for counting.

The reservoir of the CO_2 purging apparatus was sealed and the filtrate acidified with 0.2 ml of 5N H_2SO_4, injected through a silastic septum in the stopper. A set of six filtrate samples set up in parallel was purged with CO_2 free air from an aquarium pump. The liberated ^{14}C-CO_2 from each sample was trapped in 5 ml of PCS, containing 0.3 ml of hyamine hydroxide or phenylethylamine, in a scintillation vial connected to a Vigreaux column. After purging for 20 min, the Vigreaux columns were rinsed with 5 ml of

PCS. Recovery of $^{14}CO_2$ from a series of $^{14}C\text{-}HCO_3^-$ samples in the purging apparatus was 100.5% (n=10).

Particulate and CO_2 samples were counted on a Beckman Isocap scintillation counter, corrected for background, and for quenching using an external standard. Control formalized samples were subtracted from the live samples to correct for physical adsorption. The gross uptake rate of the organic solute was determined by the addition of the particulate associated radioactivity (net uptake) and the respired component (CO_2).

The reproducibility of the in situ heterotrophic activity measurements was evaluated to determine experimental and sampling variability. Six replicate samples collected on 28 Aug. at 7.0 m from an experimental enclosure containing EDTA were incubated with glucose (1.2 ug C liter^{-1}) for two hours. The samples had an average respiration of 3.46 ± 0.52 ug C liter^{-1} h^{-1} (x 10^{-2}) and a net uptake of 19.34 ± 0.69 ug C liter^{-1} h^{-1} (x 10^{-2}). The percent respiration varied from 14.2 to 20.4 (av. 17.8%). This reproducibility was considered adequate to study variations that would occur in experimental manipulations.

Another experiment was conducted to determine if compounds, other than those induced by iron limitation in the algal culture, would have an effect on microbial activity. Algae were grown in media containing iron to suppress any siderphore production, and the same isolation procedure was used to prepare an extract that was used for siderophore isolation. The effects of this extract on heterotrophic activity was compared to an extract containing the siderophore and to a control (Table 1). The extract prepared from the Anabaena flos-aquae culture, containing iron, enhanced microbial uptake of acetate by a factor of two when compared to the control. The extract prepared from the culture grown without iron, to stimulate

siderophore production, reduced the microbial uptake of acetate to less than half of the control value. Although some compounds are present in the algal extract, which can stimulate microbial activity of the lake microorganisms, this stimulation is overshadowed by the inhibitory effects of the siderophores.

The effects of chelator concentration and preincubation time on inhibition of microbial activity were investigated. A chelator concentration range of 2-20 times the normal culture concentration did not result in further inhibition of microbial uptake of glucose (at 1.2 ug C liter^{-1}), indicating that chelators from A. flos aquae and S. basiliensis cultures were effective at low concentrations. Preincubation times of 5, 30, and 60 min with chelators, isolated from the above two cultures, did not result in further inhibition of glucose uptake. Therefore, the chelators act very rapidly. Since previous research with ^{14}C-labelled chelators added to lake water had demonstrated 20-70 percent degradation per day (Murphy et al. 1983a), preincubation times of one half to one hour were used in subsequent experiments, to provide adequate time to manipulate the samples.

Microbial uptake of acetate (conc. 2.75 ug C liter^{-1}) was suppressed in the presence of three different algal siderophores at three water depths in Black Lake during summer stratification (19 July) - see Fig. 1. The Scenedesmus chelator suppressed heterotrophy the most (81 to 97 percent reduction compared to the control). In addition to reducing the gross uptake from 19 to 92 percent, both Anabaena chelators increased the relative proportion of the adsorbed solute that was respired by the lake microorganisms. Iron addition with the chelator did not reduce the inhibitory effects of the algal chelators, which indicated that suppression of solute uptake was not directly related to iron availability.

A similar experiment was conducted a month later with glucose (1.2 ug C liter^{-1}) to make sure that the inhibition effects were not solute specific (Table 2). Both the Scenedesmus siderophore and the siderophore plus iron caused a large reduction (92 to 98 %) in the gross microbial uptake of glucose near the surface (1 m) and below the thermocline (9 m) in late August. The largest inhibition of solute uptake during both the July and August experiments occurred in the hypolimnion, where microbial activity was 2-3 times the level found in the epilimnion.

The inhibitory effects of the algal siderophores were investigated at different glucose (Fig. 2) and acetate (Fig. 3) concentrations. Glucose uptake was saturated over the concentration range studied (0.8-3.2 ug C liter^{-1}) and the uptake of acetate, in the same concentration range, was linear. Therefore, no attempt was made to calculate the kinetic values of K_m (transport constant) and V_m (maximum uptake rate). The Scenedesmus chelator reduced the uptake of glucose by 60 percent and there was very little variation in inhibition over the range of glucose concentrations studied. The Anabaena chelator reduced the glucose uptake at low glucose concentrations, but there was an apparent loss in this inhibition at the higher glucose levels. The Anabaena chelator reduced acetate uptake at all solute concentrations. There was not complete loss of inhibition at the higher acetate concentrations as occurred with glucose (Fig. 3). These different responses may indicate that the mechanism of inhibition of the two algal chelators is different.

The temporal pattern (May through August) of heterotrophic utilization of three solutes (glucose, glycine, and acetate) in Black Lake is presented in Figure 4. The data for May 11 only represent net uptake rates, since respiration was not being measured. The data for June, July, and

August include the respiration of the three radioactive solutes and are gross uptake rates. During June and July the uptake rate of glycine was often less than 1 ug C liter^{-1} h^{-1} x10^{-2} and could not be accurately represented on the histograms. Uptake rates usually followed the order acetate > glucose> glycine and they were usually higher on the aerated side of the lake.

There was a general pattern of suppression of bacterial activity from early to mid summer. This pattern was more evident on the side of the lake subjected to hypolimnetic aeration, which maintained more oxygen below the thermocline throughout the summer. A week after the collapse of a diatom bloom (May 11), uptake rates were relatively high. During a period when Anabaena dominated the phytoplankton community (June 16), and a week after the collapse of a dense Anabaena bloom (July 18), the lowest uptake rates for most solutes were recorded. The bacterial activity increased after an Aphanizomenon bloom (Aug. 31) when there was considerable autochthonous organic matter for microbial decomposition.

The following year (1980), glucose and acetate uptake were monitored at approximately monthly intervals during the summer (Table 3). Again there was suppression of glucose metabolism in early July, when Anabaena was the dominant algae, and acetate uptake rates were low compared to later in July and August.

The percent respiration, of the labelled solutes used by the microorganisms, varied from 18-22 percent for glucose and acetate in May and October when blue-green algae numbers were low. During the warm summer months, when blue-green algae were dominant in the lake, many respiration values of 30-40 percent were measured. This high rate of respiration could be attributable to the higher summer water temperatures and the higher energy requirements of the microorganisms. However, some

of the increased respiration could be caused by the apparent metabolic stress of the bacteria in the presence of natural algal siderophores, since the direct addition of Anabaena chelator to the lake water increased the proportion of the solute that was respired (Fig. 1).

The Anabaena chelator isolated from the lake culture showed strong inhibition of microbial activity and may help to explain microbial suppression in the lake from mid June to mid July. During this time, Anabaena was the dominant algae in the lake and iron concentrations were low. The low iron concentrations should stimulate natural siderophore production. A bloom of Aphanizomenon did not take place until there was more iron in the lake in late July and August (Murphy et al. 1983a) and at this time microbial activity was much higher.

Microbial activity was measured at monthly intervals in experimental enclosures to which Fe-EDTA and EDTA had been added. The experiment was designed to provide a consistent supply of soluble iron or to make the iron present in the lake more available and hopefully suppress any natural siderophore production (Fig. 5). Each value represents gross uptake of the solute and is an average for two samples corrected with an appropriate blank. In all enclosures, the uptake of acetate was higher than for glucose. With the exception of three samples collected the end of July, microbial activity at both 1 and 7 m was higher in the enclosures containing EDTA or Fe-EDTA, when compared to the controls.

The reduction of microbial activity by experimental addition of siderophores to lake water, the suppression of microbial activity in the lake during mid summer, and the stimulation of microbial activity by increasing the availability of iron provides evidence that algal siderophores affect the

ability of bacteria to assimilate and mineralize organic solutes in a hardwater lake.

Algal siderophores isolated from three species of algae were able to reduce the uptake of organic solutes, such as acetate and glucose, by the natural population of microorganisms during the summer in Black Lake. Chrost (1975) has also reported that bloom-forming algae can produce antibacterial substances, which can inhibit the growth and respiration of bacteria in a eutrophic lake in Poland. Gram positive bacteria appeared to be most susceptible to these inhibitors, while gram negative rods were resistant. Other research has substantiated the sensitivity of gram positive bacteria to these inhibitors (Sieburth 1959, Duff et al. 1966).

In further investigations, Chrost and Sinda (1978) found that there was a negative correlation between microbial activity (as measured by ^{3}H-thymidine uptake, and ^{14}C-acetate uptake and mineralization) and chlorophyll a concentrations in the photic zone of the lake. In contrast, there was a positive correlation between chlorophyll a and heterotrophic activity in the metalimnion and hypolimnion. Perhaps in lower zones of the Polish lake, iron was more available and the algae were in a senescent state, which would suppress siderophore production and not inhibit microbial degradation. It is also possible that the antibacterial inhibitors that Chrost and Sinda were studying were not chelating agents, regulated by micronutrients, since a variety of algal compounds can be produced by algae (Jones 1982).

Several studies in the marine environment (Sieburth 1959 &1971, Duff et al.1966, and Moebus 1972) and in a oxidation pond (Dor 1978) indicate that the production of bacterial inhibitors is widespread in the aquatic environment and suggests that antibiotic production in nature may

allow the producing organism to compete with other microorganisms more effectively. However, none of the above studies indicated that the formation of inhibitors can be related to the availability of iron, as is demonstrated by our experiments.

The mechanism of inhibition of these algal siderophores is unclear, since good saturation uptake kinetics were not obtained in our studies. Reichardt (1981) found that the uptake and respiration of ^{14}C-glucose by microbial isolates (Chromobacterium lividum and Arthrobacter sp.) was strongly inhibited by algal excretion products such as methylheptone. His kinetic studies suggested that inhibition was non-competitive since K_m remained fairly constant, while V_m decreased in the presence of the inhibitor. Kinetic studies over a much wider range of solute concentrations will be necessary to determine the mechanism of inhibition for the inhibitors produced by iron limitation in our studies.

Microbial uptake of organic solutes was inhibited during June and early July when compared to May and August. During this mid-summer period, Anabaena was the dominant phytoplankton bloom and it was capable of producing a siderophore which inhibits microbial activity. Moebus (1972) also found a larger inhibition of bacteria in the ocean in the summer months when phytoplankton numbers were high. In Black Lake, microbial activity increased in August after the death of the Anabaena bloom, which coincided with hypolimnetic oxygen depletion and an increase of iron in the water column. Dissolved iron concentrations were <25 ug Fe liter^{-1} during June and early July, but began to increase after mid-July (50 ug Fe liter^{-1}) to values of >100 ug Fe liter^{-1} in mid and late August as the Anabaena bloom decomposed (Murphy 1986). The Aphanizomenon bloom in August was not

iron limited, so no siderophore was produced and microbial activity increased.

There was an apparent stimulation in microbial activity in the area of the thermocline (5 m), on the control side of the lake, especially in May and August. This could be attributable to accumulation of settling algae, in this density stratified zone, which provides organic substrates and iron for microbial growth, as the algae decompose. Although no Fe data are available for the May period, dissolved iron concentrations were 203 and 90 ug Fe liter^{-1} at 5 m on Aug. 11 and 30 respectively (Murphy 1986).

In the enriched enclosures, the oxygen concentrations were higher than was found in the lake or control enclosure (Murphy et al. 1983a). This stimulation of phytoplankton production by iron or chelating agents, resulted in a stimulation of microbial activity.

Some suppression of microbial activity in the enriched enclosures relative to the control occurred in late July. This suppression could have been caused by a $CaCO_3$ precipitation event, which caused flocculation of microorganisms in the water column and coprecipitation of soluble reactive phosphorus (Murphy et al. 1983b). Calcium carbonate precipitation was favored by the high productivity in the enriched enclosures, which increased the water pH. Iron stimulation of primary productivity has been documented in marl lakes (Schelske et al. 1962), but the effects of iron on the bacteria in hardwater lakes has not been reported.

These observations provide evidence that siderphores, produced by algae grown under iron limiting conditions, can suppress microbial activity in a hardwater lake. Natural induction of siderophore production during the warm summer period, when available iron is low, appears to reduce microbial activity, even though high temperatures and organic matter from

an algal bloom should favor high rates of bacterial metabolism. The addition of available iron to the lake can stimulate primary productivity and help to overcome some of the inhibition of microbial activity. The suppression of heterotrophic activity could amplify the asynchrony of oxygen production and consumption in a lake. A delayed oxidation of organic matter could result in a more rapid decrease in oxygen concentration, when the siderophore inhibition has decreased. Suppression of microbial degradation of organic matter in the epilimnion could result in oxidation, after it has settled into the iron-rich hypolimnion, where oxygen renewal is low and the hypolimnion can become anoxic rapidly. Also, delays in decomposition until after an algal bloom collapses and siderophores are inactivated, could influence seasonal oxygen utilization and provide another factor in explaining the sensitivity of many hardwater lakes to winter fish kills.

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Table 1. Effect of extracts produced by Anabaena under different growth conditions on the microbial uptake of acetate[1]

Algal Growth Conditions	Lake Water[2] Treatment	Gross Uptake Rate (ug C $liter^{-1}$ h^{-1} x 10^{-2})
Iron limitation	Algal extract (contains chelator)	2.39
Iron addition	Algal extract (no chelator present)	10.85
-----	Control (no algal extract added)	5.76

[1] acetate concentration= 2.75 ug C $liter^{-1}$

[2] 1 meter water taken from the control side of Black Lake on July 31,1980.

Table 2. Suppression of glucose uptake by Scenedesmus siderophore[1]

Sample Treatment	Depth (m)	Gross Uptake (ug C liter^{-1}h^{-1}x 10^{-2}) Average	Range[2]
Control	1.0	4.40	---
	9.0	13.34	---
Siderophore	1.0	0.08	0.06-0.14
	9.0	1.05	0.91-1.32
Siderophore + Fe	1.0	0.35	0.26-0.44
	9.0	0.96	0.83-1.27

[1]Surface water from the control side of Black Lake on Aug.31,1979. Glucose concentration=1.2 ug C liter^{-1}.

[2] n=4

Table 3. Microbial activity in Black Lake in 1980[1]

Date	Depth (m)	Glucose Uptake[2] (ug C liter^{-1}h^{-1}x 10^{-2})	Acetate Uptake[2] (ug C liter^{-1}h^{-1}x 10^{-2})
May 1	1.0	3.22	0.72
	8.0	2.67	1.72
July 3	1.0	0.50	3.95
	7.0	0.19	5.56
July 30	1.0	2.89	13.13
	7.0	1.43	7.35
Aug. 28	1.0	5.70	23.46
	7.0	4.96	32.34

[1] Measurements made on the control side of the lake.

[2] Gross uptake rates. Glucose concentration= 1.44 ug C liter^{-1}, acetate concentration= 2.75 ug C liter^{-1}.

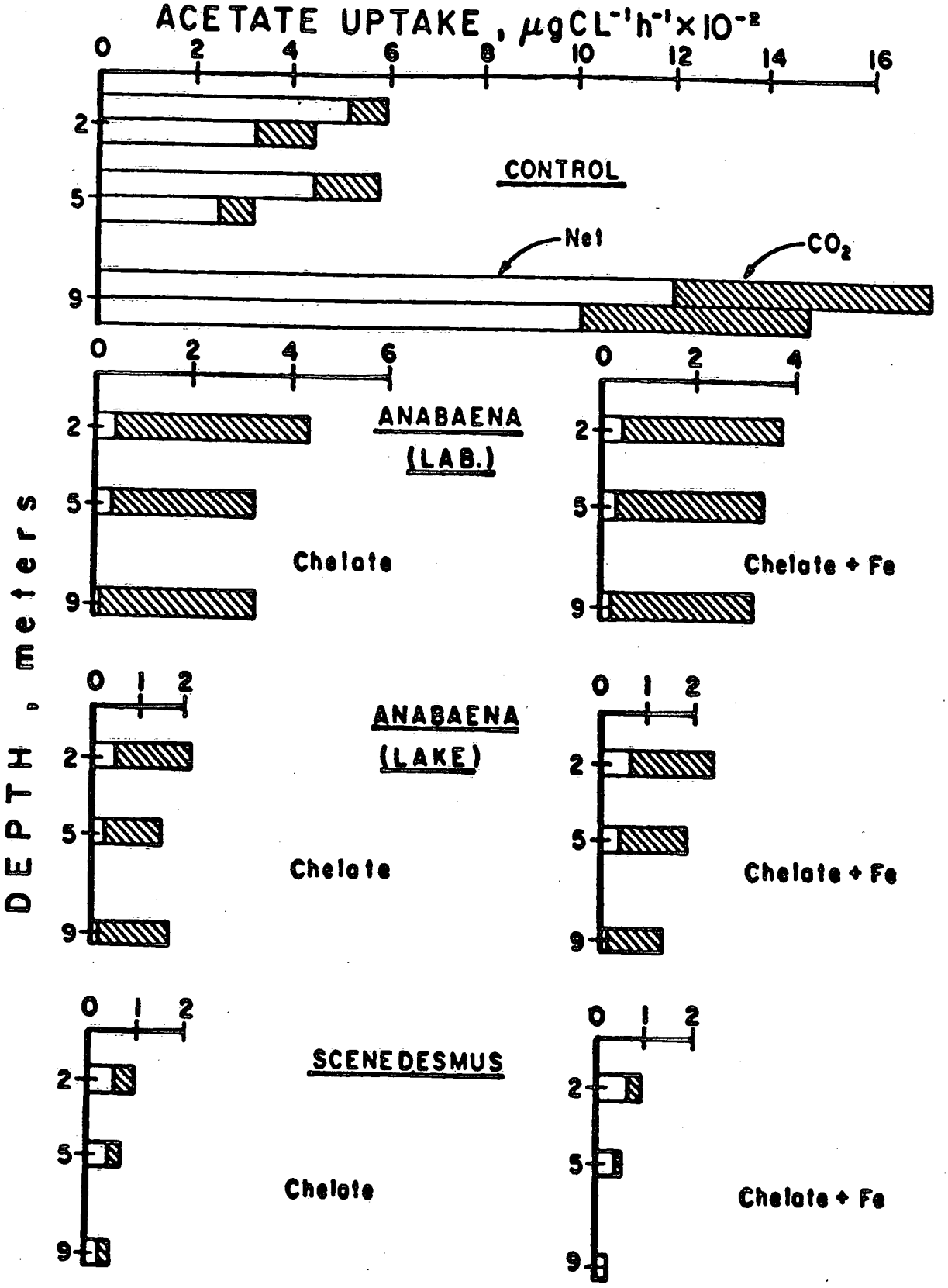

Fig. 1. Suppression of acetate uptake by algal siderophores.

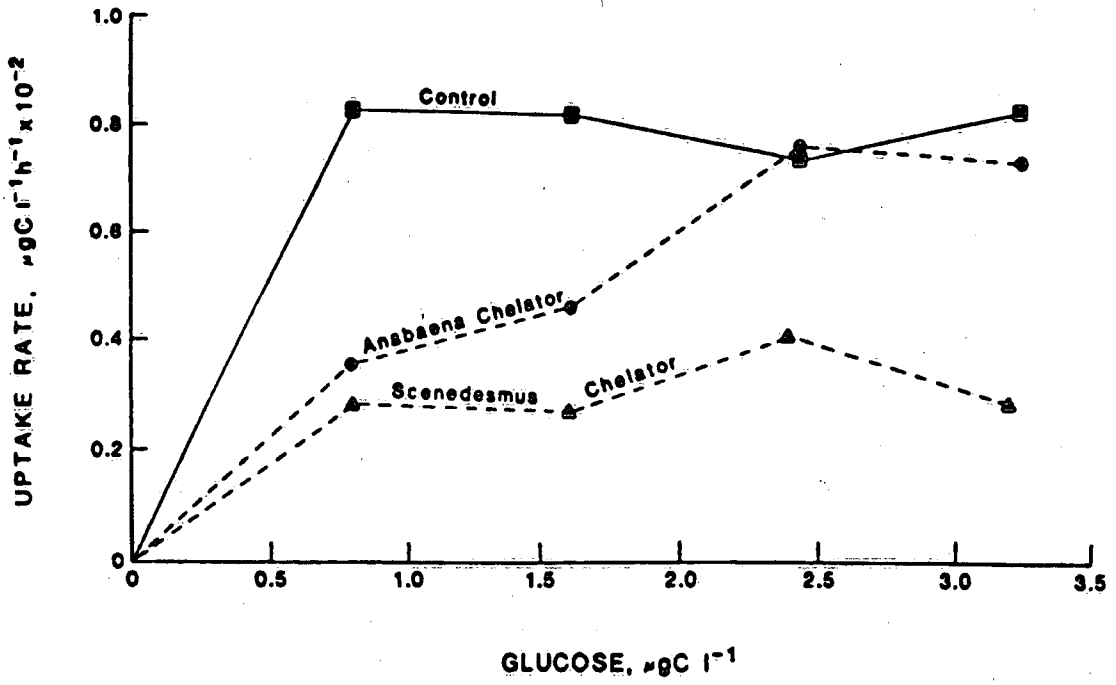

Fig. 2. Effect of glucose concentration on the suppression of microbial activity by algal siderophores.

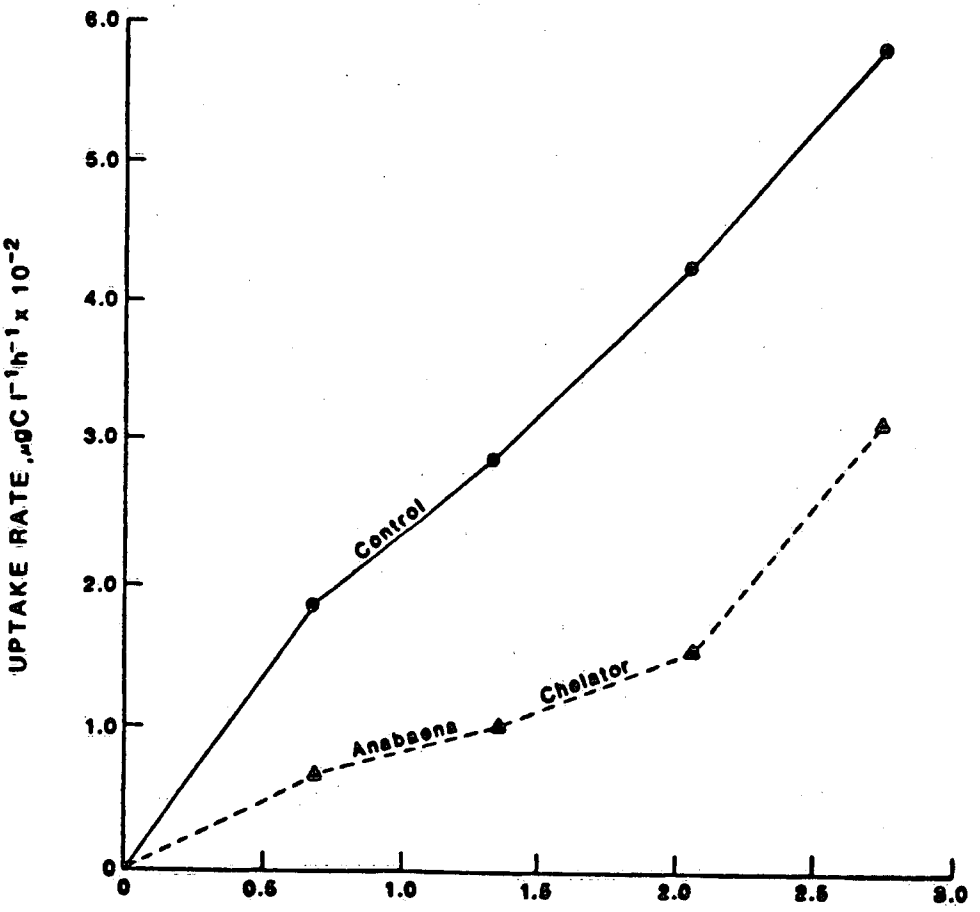

Fig. 3. Effect of acetate concentration on the suppression of microbial activity by an Anabaena siderophore.

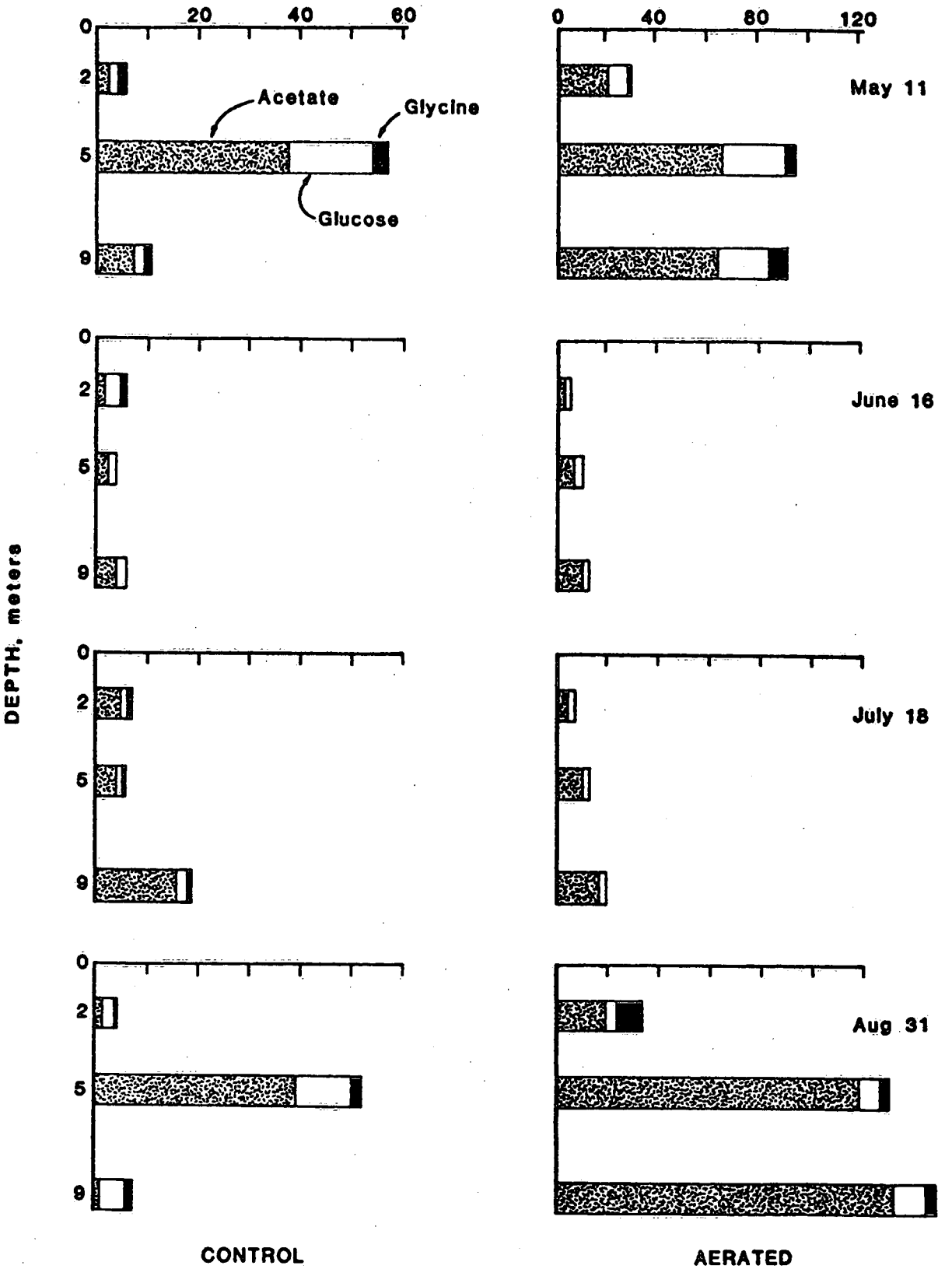

Fig. 4. Temporal variation in microbial activity, as measured by acetate, glucose, and glycine uptake, at three water depths in Black lake, B.C. Lake separated by sea curtain and one half of lake subjected to hypolimnetic aeration.

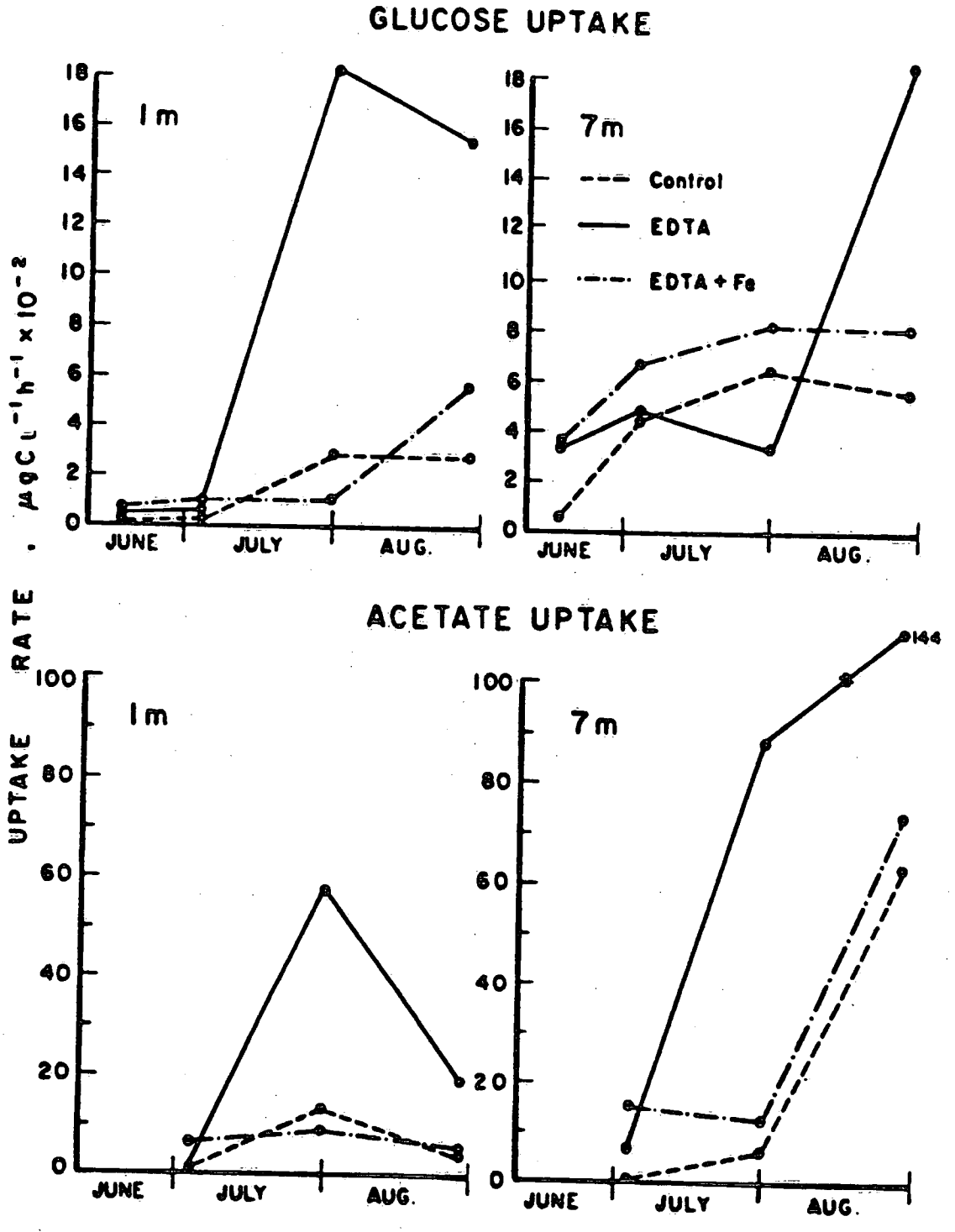

Fig. 5. Glucose and acetate uptake in experimental enclosures spiked with EDTA+Fe and EDTA.