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SOME INTERACTIONS OF LAKE PLANKTON WITH HEAVY METALS

by

RAMIRO TRUCCO

A Thesis

presented to the University of Ottawa

in partial fulfillment of the requirements

for the degree of Doctorate in Philosophy

in

Biology

Ottawa, Ontario, 1982

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ABSTRACT

Field experiments conducted at Heney Lake, Quebec, indicate that algal species composition is the major factor responsible for the binding capacity of the four metal ions tested, Cd^{2+}, Cu^{2+}, Hg^{2+} and Pb^{2+}. Only few species of the total pool, particularly green algae and diatoms, which constitute an important food source to planktonic consumers, did account for most of the binding capacity of the system.

Unialgal populations isolated in polyethylene enclosures at Heney Lake also show specificity for metal binding. When the green algae Ankistrodesmus falcatus and Gloeocystis gigas were tested for the ability to bind cadmium and lead, A. falcatus was found to be positively correlated with lead binding ($r=0.60$), whereas G. gigas showed a preference for cadmium ($r=0.66$).

Short term laboratory experiments indicate that the cladoceran Daphnia pulex is able to accumulate significant amounts of cadmium and lead from both the medium and the dietary route, in this case the alga Ankistrodesmus falcatus. Uptake of both metals occurs very rapidly and accumulation of lead by daphnids seems to be greater than that of cadmium. The rate of release of both metals is slower when they are accumulated through the diet than through the solution. Metal concentration from the food supply suggests that this mechanism may be an important pathway for bioaccumulation in the food web.

The potential implications of heavy metal binding and accumulation by planktonic organisms are then discussed in light of the ecology and management of freshwater systems.

RESUME

Des expériences de terrain conduites au Lac Heney, Québec, démontrent que la composition spécifique algale est le principal facteur responsable de la capacité de liaison des ions métalliques analysés, Cd^{2+}, Cu^{2+}, Hg^{2+} et Pb^{2+}. Seulement quelques espèces, en particulier des algues vertes et des diatomées, qui représentent une source alimentaire importante pour les herbivores planctoniques, sont responsables de la plus grande partie de la capacité de liaison du système.

Des populations unialgales isolées dans des enceintes de polyéthylène au Lac Heney demontrèrent plus avant le caractère spécifique de la liaison des ions métalliques. Quand les algues vertes Ankistrodesmus falcatus et Gloeocystis gigas furent analysées par rapport à leur capcité de liaison du cadmium et du plomb, on a trouvé que A. falcatus est relié positivement à la liaison du plomb ($r=0.60$) tandis que G. gigas montre une préférence pour le cadmium ($r=0.66$).

Des expériences en laboratoire à court terme démontrent que le cladocère Daphnia pulex est capable d'accumuler du cadmium et du plomb à partir du milieu ainsi que de sa nourriture, dans ce case l'algue Ankistrodesmus falcatus. La vélocité d'élimination des deux métaux est plus lente quand ils sont accumules via la nourriture plutôt qui via la solution. La concentration des métaux par l'intermédiaire de la nourriture suggère que ce mecanisme représente une route menant à la bioaccumulation dans la chaîne alimentaire.

Les implications possibles de l'accumulation et de la liaison des métaux lourds par les organismes planctoniques sont ensuite discutées par rapport à l'écologie et à la gestion des écosystemes lacustres.

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SECTION I

GENERAL INTRODUCTION

INTRODUCTION

Recent years have witnessed a sharp increase in public interest over the quality of the environment, and in particular over the extent of metal pollution. Reasons for concern appear numerous: heavy metals are now widely distributed throughout the environment; since they are not degraded they can persist in nature for extended periods of time; they are generally toxic to living organisms at fairly low concentrations and tend to be either biologically magnified or accumulated in plants and animals; finally, certain metals can be converted in the environment to more toxic forms.

Unlike most organic pollutants, metals and their salts occur naturally in the environment. In addition man discharges large quantities of metals into his surroundings in the course of his industrial and technological endeavors. This results not only from mining, smelting and processing operations, but also from the direct use and discharge of metals and their products, as well as from other human activities. For example, the burning of fossil fuels, including gasoline additives, appears to be currently the largest source of metal emission to the air. Consequently, metallic wastes have become distributed over the entire earth with excessive concentrations in localized areas, particularly in regions of high population density.

A major problem encountered with all metals is their persistence. Unlike organic pollutants, metals are not subjected to biological or chemical degradation in nature. Since metals are very stable, they may be transported over considerable distances via air or water, or they may be transferred along food chains, where they tend to accumulate (Friberg and Vostal 1972; Stopford and Goldwater 1975). Thus, concentrations several orders of magnitude greater than the original may be attained at the higher trophic level. Consequently, metallic wastes tend to remain indefinitely in the ecosystem, posing a continuous threat to the biota.

Since metals are naturally occurring substances, the geological characteristics of the rocks and soil also play a role in determining the metal content of water and sediments in a particular area. Thus, the concentration of metal in water varies naturally according to the region, season, and water characteristics. For example, Silker (1964) has shown that over a period of a year the copper concentrations, among other metals, of the Columbia River in the vicinity of Hanford, Washington varies 38-fold. Kopp and Kroner (1968) also report variations in metal concentrations of a large number of metals during a 4-year period in several water systems of the United States.

While contamination by toxic metals probably affects the entire biota, most attention has focused on the potential risk of metals to man. One of the factors determining toxicity concerns the chemical form to which a particular organism is exposed. Except for mercury vapor, the free metals generally present little or no toxicity. In contrast, the free ion activity of a metal (Sunda and Guillard 1976), its solubility, binding affinity and

biological activity will all determine its toxicity.

ROLE OF HEAVY METALS IN BIOLOGICAL SYSTEMS

Interest for this subject dates back at least one century. It related first to the effects of abnormal amounts of what are termed trace elements or oligoelements, i.e., that group of elements which occur biologically in exceedingly low concentrations. These were usually identified only qualitatively, as they could not be determined quantitatively; such was the case for cadmium, copper, lead and mercury, among others.

Heavy metals, it was found, can play both beneficial and detrimental roles in living systems. It is largely through catalytic participation, structure stabilization, or, alternatively, by modifying or disrupting biochemically poised structures such as proteins and membranes that metals express their normal role in biology. Deprivation studies have generally confirmed the biological necessity for these heavy metals (Lewin 1962). It is likely, however, that nutritional requirements for many elements have gone undetected, since it is difficult to create a deficiency state due to the very low concentrations required. Clearly the lack of success in establishing heavy metals deficiency states does not preclude the existence of a biologically essential function.

The known biological involvement of cadmium, lead, and mercury is expressed largely through their ability to form stable complexes with numerous biological ligands. These include links with -SH- and imidazole side chains of proteins, with nitrogenous bases (DNA, RNA), as well as

with phosphate groups occurring in phospholipids, DNA, RNA, coenzymes, vitamins and metabolites. Thus, the potential for heavy metals to influence viable poised structures is obvious.

While many heavy metals may induce or enhance enzymatic activity in a large number of enzymes (Vallee and Ulmer 1972), cadmium, lead and mercury have no known metabolic function. They are universally recognized as toxic elements, undesirable in any concentration in food (Shacklette et al. 1978; Russel 1978).

HEAVY METALS IN THE ENVIRONMENT

Cadmium

Cadmium is an element receiving increasing attention as a potential environmental hazard. It occurs in nature only in trace amounts, except when found in association with zinc and lead. According to Higgins and Burn (1975), the world-wide industrial use of cadmium increased markedly in mid-century as production rose from 6,000 tons in 1950 to 18,000 tons in 1970. World production appears to have declined since, and was recorded at 14,000 tons in 1980 (Wilson 1981). Because of its marked toxicity the presence of cadmium as a contaminant in the environment has been viewed with increasing concern in recent years. The dispersal of this element has been reviewed by Page and Bingham (1973).

Until recently most human toxic effects were identified from occupational exposure in workers involved in industrial manufacturing processes

using cadmium. Acute cadmium intoxication is not considered an environmental problem. The potential environmental problems are related to chronic exposures to low dosages.

The most striking effect of cadmium toxicity is the disorder known as Itai-Itai disease (Kobayashi 1978), which appeared in Japan following World War II. This very unusual form of renal disease leads to secondary softening of the bones from calcium/phosphate imbalances, resulting in multiple spontaneous fractures. The outbreak occurred as a result of ingestion of rice that had been grown in water containing high amounts of cadmium from industrial pollution. This illustrates how metal accumulation in plants may represent one potential human hazard. Schroeder (1965) drew further attention to this metal by linking hypertension to increased retention of cadmium in the kidney. Carroll (1969) later found cadmium levels in the air to be correlated with an increased incidence of mortality from hypertension and arteriosclerotic heart disease.

While numerous investigations have been devoted to the impact of heavy metals in aquatic systems (for a review, see Whitton and Say 1975) most of them have focused on fish. Only recently has the interaction between cadmium and algae been explored. Berland et al. (1977) found that sublethal doses of cadmium and other heavy metals reduce division rate of the diatom Skeletonema costatum. Klass et al. (1974) showed that cadmium inhibited population growth in the green alga Scenedesmus quadricauda. Conway (1978) found that low levels of cadmium which seems to be associated with the cell content reduced the growth, micronutrient utilization, and photosynthetic pigment composition in populations of the

freshwater diatom Asterionella formosa. Inhibitory effects of cadmium on cell division of the blue green alga Chlorella vulgaris have also been recorded by Rosko and Rachlin (1977). They suggest that cadmium was the most toxic element with respect to cell division, compared with copper, mercury or zinc. Overnell (1975) reported that low concentrations of cadmium inhibit oxygen evolution in the marine alga Dunaliella tertiolecta. Recently Adshead-Simonsen et al. (1981) found that cadmium not only inhibited growth of the freshwater alga Tabellaria flocculosa but they also found noticeable morphological changes induced at very low concentrations of this element. Work of this kind on a large number of species belonging to different divisions will be necessary to gain a comprehensive view of the toxic effect of cadmium on freshwater phytoplankton.

Laboratory studies on the rate of cadmium accumulation by phytoplankton species were conducted by Jennings and Rainbow (1979). They found that the algae studied did accumulate cadmium and that the accumulations appeared to be proportional to the concentration of the metal in solution up to a certain level (1.0 ppm). Above this concentration no further accumulation occurs, possibly due to limiting possiblities of binding sites. However, the nature of these binding sites on or within the cells remains unknown. Cossa (1976) also reported the uptake of cadmium by the marine diatom Phaeodactylum tricornutum. He found that the rate of uptake varies with the chemical form of cadmium in the culture medium as well as the growth stages of the algae. Therefore, the fact that algae do accumulate heavy metals may lead to biomagnification through the food web with potential consequences for the entire ecosystem.

Lead

Lead is an element used extensively, as indicated by world production figures, reaching 4 million tons in the early 1970's. The latest reports (see Stubbs 1981) indicate that lead production is maintained at similar levels. Measurements of lead content of both ancient and recent arctic snow and ice (Murozumi et al. 1969) showed that lead concentrations increased 4-fold between 1750 and 1940, and then nearly tripled again since 1940. The first increase reflects the great expansion of lead smelting following the Industrial Revolution, while the second increase reflects the addition of lead additives to gasoline. An excellent review of the history of lead use and atmospheric contamination is found in Shukla and Leland (1973). Nowadays, lead is used for a wide variety of purposes (Stubbs 1972): in batteries, which account for 40% of world consumption, in cable sheathing, in sheets and pipes, as pigment for paint, as glazing, in plastic, as a gasoline additive, etc.

Lead toxicity is a well documented issue, as can be expected for a metal utilized by man since ancient times because of its malleability, density and corrosion resistance. Gilfillan (1965) even suggested that widespread lead poisoning caused the downfall of the Roman Empire. According to his theory extensive oral lead exposure in Roman times produced a chronic intoxication that contributed to the Empire's decay.

The medical aspects of lead poisoning are discussed in Green et al. (1976). Aronson (1978) further gives detailed information on lead poisoning in domestic animals. Ecological effects of lead emitted from auto

exhaust are presented by Coello et al. (1974). Rosko and Rachlin (1977) show the depressive effect caused by lead and other heavy metal ions on cell division, growth, and particularly chlorophyll content in the freshwater alga Chlorella vulgaris. They found that lead exerts its toxicity by reducing chlorophyll a content but has very little effect on cell division, at least under the experimental conditions tested.

Bioaccumulation of lead has been documented in aquatic invertebrates (Spehar et al. 1978), algae (Trollope and Evans 1976) and aquatic macrophytes (Mayes et al. 1977). The uptake of lead by the aquatic biota and its subsequent release during decomposition of the organism represents one way for cycling lead in aquatic ecosystems.

Mercury

Mercury occurs in a natural state only in small amounts, estimated at 50-80 ppb of the earth's content (Vostal 1972). The production of mercury, largely centered in Spain, the U.S.A., Italy and China, is estimated at 10,000 tons/year (Mining Annual Review 1981). In ancient times, mercury was mainly used for cosmetics, for decorative purposes, and medically to treat syphilis and skin diseases. The current use of mercury, however, is mainly industrial.

Inorganic mercury is employed in the chlor-alkali industry (where chlorine is produced by electrolysis of sodium chloride using a mercury cathode), in the production of electric batteries, mercury vapor lamps and electrical relays with liquid contacts. It is also used in paint manufacture

and as a catalyst in the form of mercury chloride ($HgCl_2$) in the manufacture of vinyl chloride, urethane, plastic and acetaldehyde. Elemental mercury is universally used in chemical and physical laboratories and in thermometers and barometers.

Inorganic compounds of mercury such as calomel (Hg_2Cl_2) and mercury chloride are commonly employed as horticultural pesticides or used as fungicides in the paper-making industry. Although the use of mercury-containing pesticides is now probably in decline, it is still common in those countries where regulations limiting its application have not yet been promulgated.

The toxic effect of mercury and mercury compounds on man is well documented (Harada 1978; Cassidy and Furr 1978). The first details of a dangerous environmental mercury problem appeared from 1953 to 1960 where 111 fishermen and their families living along the shore of Minamata Bay, in southwestern Kyushu, Japan, developed a mysterious neurological disease and 44 people died. A similar case occurred in Niigata, Japan, 1964 with 26 cases and 5 deaths. The affected individuals suffered from neurological symptoms and brain damage and many of the survivors were paralyzed for life. The source of the disease was eventually determined to be from methyl-mercury that had been discharged into Minamata Bay by a plastic factory. A third case was reported from the same area in 1973, and the disease has now universally been referred to as Minamata disease.

In the late 1950's and early 1960's several ornithologists noted a marked decrease in the Swedish bird population. By 1960 evidence had pointed to mercury poisoning in these birds from the organic mercury compound used

extensively in Scandinavia for seed treatment (Vostal 1972). Recently, Charlebois (1978) reported mild symptoms of methyl-mercury intoxication in native communities in Canada. From the outbreak in Japan it was clear that methyl-mercury became concentrated in fish and-shellfish which were subsequently eaten by the victims. Similar mechanisms were involved in the case of Canadian natives.

In recent years the interaction of mercury with the freshwater biota and particularly phytoplankton has received more attention. Population growth in phytoplankton species under laboratory conditions was found greatly reduced by very small concentrations (0.03 ppm) of mercury (Bringmann and Kuhn 1959; Matida et al. 1971; Tompkins and Blinn 1976). Photosynthesis inhibition in freshwater populations of algae exposed to organo-mercurial fungicides was demonstrated by Harris et al. (1970). Similar effects were found also by Gächter (1976) in natural phytoplankton from two different lakes when adding $HgCl_2$, and by Blinn et al. (1977) using large volume plastic chambers in situ. Laboratory experiments (Boudou et al. 1977) have shown that accumulation of methyl-mercury by the green alga Chlorella vulgaris and the subsequent transfer to a third-rank consumer could occur. The effects of mercury on the primary producers and to consumers in the food chain is a cause of concern.

Copper

Copper is considered an essential element to life in animals (Underwood 1971) and in plants (Lewin 1962). It has varied and numerous biologic

effects, not only as an essential element, but also as a toxicant. Acute or chronic toxicity of copper to human beings from external exposure is rare (Cohen 1979). However, two specific genetic regulatory mechanisms for copper have been described (Scheinberg 1979). One appears to facilitate the transmembraneous transfer of copper; the absence of such a mechanism results in a hereditary form of copper deficiency which turns out to be fatal despite copper administration to the patient. The second mechanism, transmitted as an autosomal recessive, is essential to the excretion of copper via the glycoprotein ceruloplasmin. If this mechanism is absent chronic copper toxicity will develop, which would be fatal if not treated.

Many studies have been carried out on the effect of copper on algae. The early ones were concerned with copper as a mechanism of controlling algal blooms in freshwater lakes (Moore and Kellerman 1904). Most of the recent studies deal with the effects of copper in relation to physiological processes. For example, algal response to high concentrations of copper in water systems seems to change algal species richness as well as species composition (Butcher 1955; Stokes et al. 1973). Algal reponse to high concentrations of copper also includes reductions in growth rate (Stokes et al. 1973; Laube 1978), in photosynthesis (Steeman-Nielsen et al. 1969; Shioi et al. 1978), in respiration (McBrien and Hassall 1967; Overnell 1975), and in nitrogen fixation rates (Horne and Goldman 1974). Furthermore, laboratory studies have demonstrated algal accumulation of copper (Hassall 1963; Button and Hostetter 1977; Laube et al. 1979). This may lead to biomagnification of heavy metals, consequently affecting higher levels in the food web.

OBJECTIVES OF THIS STUDY

This thesis aims to increase our limited knowledge of the interactions between natural planktonic communities and four heavy metal ions, cadmium, copper, lead and mercury. In particular the question of whether phytoplankton play a major role in the bioavailability of these metals to aquatic systems is investigated.

To this effect three main sets of experiments were performed. First, field manipulations were conducted to determine whether algal biomass is related to heavy metal binding, and to test which species are actually responsible for the particular binding of the metal ions studied. Then, the rate of heavy metal uptake by unialgal populations maintained at different densities was determined under field conditions. Lastly, the transfer of heavy metal from algae to plantivorous species was investigated. Thus, there were four major questions tackled in this study:

A. Is total biomass related to heavy metal binding in natural lake systems?

B. Which species are responsible for heavy metal binding?

C. What is the rate of heavy metal binding in unialgal populations (edible or inedible) as a function of their densities?

D. Is there a transfer of specific heavy metal from phytoplankton species to higher trophic levels of the food web?

SECTION II

HEAVY METAL BINDING BY PHYTOPLANKTON COMMUNITIES

INTRODUCTION

Most heavy metal studies in aquatic systems have focused on the toxic effect on fish; only a limited amount of work has been carried out to determine the effects on organisms at lower trophic levels, particularly phytoplankton. Tompkins and Blinn (1976) showed the inhibitory effects of low concentrations of mercury on the growth rate of the freshwater diatoms Asterionella formosa and Fragillaria crotonensis. Their study also reported that A. formosa responds by an increase in size together with morphological variations in shape when exposed to sublethal mercury levels. This, the authors suggest, could remove the algae from a selected food chain. The mechanisms involved, however, and the ecological significance of such an effect remain unknown. Other studies illustrating the effects of metals to various populations of green algae in lotic systems are described by Whitton (1970). Mixtures of heavy metals have been found to have either synergistic or antagonistic toxic effects on different freshwater algae. Wong et al. (1978) showed that, when a mixture of metals were present in the culture medium of Scenedesmus quadricauda, inhibition of about 70% in primary productivity was observed. Hutchinson and Stokes (1975) also found synergistic toxic effects of copper and nickel, whereas cadmium and selenium acted antagonistically, on Chlorella vulgaris. Furthermore, the relative toxicity varied with different algal species. Wong et al. (1978)

reported that the most sensitive species in their studies was the diatom Navicula pelliculosa, followed by the blue-green Anabaena flos-aquae, and the green algae Scenedesmus quadricauda and Chlorella vulgaris. Similar patterns in terms of taxonomic groups were reported for copper by Maloney and Palmer (1956) on a variety of algal cultures.

All the three major components of aquatic systems, water, biota and sediments, interact with heavy metals. Sediments constitute an important compartment where heavy metals can be found at concentrations higher than those in the surrounding water (Ramamoorthy and Rust 1976). Mayes et al. (1977) showed that non-essential trace metals, such as cadmium and lead, are bound by sediments - to an extent depending on the organic matter content - and can be accumulated through the root system of aquatic macrophytes and then transported to stems and leaves. The subsequent release of the metal to ambient water gives one possible pathway for cycling of trace metals in aquatic ecosystems. The movement of metals from the sediments into water could result in an increase of metal availability to the organism in the system. Further, uptake of cadmium and copper from the sediment by the green alga Ankistrodesmus braunii and the blue-green alga Anabaena sp. (Laube et al. 1979), shows another possible route of heavy metal mobilization in planktonic communities.

Several laboratory studies have demonstrated that freshwater algae are able to concentrate heavy metal ions. Trollope and Evans (1976) reported the accumulation of copper, iron, lead, nickel and zinc in various freshwater algal blooms. Similar studies for a diverse variety of metals and other algal species have been carried out by Wihlidal et al. (1976); Filip

and Lynn (1972), Fujita and Hashizume (1975). Release of extracellular water soluble chelators under unfavorable conditions or during senescense (Lange 1973; Fogg 1971) seems to be another mechanism of heavy metal interaction with freshwater algae.

Although the toxicity of heavy metals to aquatic organisms is obvious, toxic effects, however, vary between species, suggesting that different mechanisms are involved in the process. Under similar conditions, some algae are affected severely by a particular element, while others are less so. Few alternatives have been proposed on how algae tolerate high concentrations of heavy metals. Silverberg (1975) suggests that the accumulation of lead inside the vacuoles of the alga Stigeoclonium tenue could act as a defence mechanism due to the impossibility of this element to interact there with other components in the cell. Copper exclusion by Chlorella vulgaris is presented as another way to explain heavy metal tolerance in this alga (Foster 1977). Recently Laube et al. (1980) found that Anabaena sp. and Ankistrodesmus braunii have different ways of dealing with copper and cadmium. Copper binding by Anabaena sp. appears to be related with cell content at an early stage of growth, whereas at a late stage the metal seems to be bound by external compounds originating from the algae. On the other hand, the cadmium mechanism appears to be different since the algae were able to grow even with substantial amounts of cadmium bound to the cell. The latter case is also applicable for A. braunii exposed to both cadmium and copper. There may be a mechanism for controlling the amounts of metals bound per cell during growth phases.

The toxic effects of heavy metals mentioned earlier bring drastic

changes to the flora and fauna in the environment by reducing biomass (Gächter and Mares 1979), species richness (Stokes et al. 1973), and altering community structure (Moore 1981). It is evident that heavy metals can interact with biotic components in water systems, but the question of whether or not algae affect the general availability and toxicity of heavy metal ions to aquatic food webs remains open. Since algae compose a major part of natural ecosystems as primary producers, the examination of their interaction with heavy metals under natural conditions will be considered as an initial step in this investigation. Thus, the first phase of the study consists of long term controlled field experiments to determine (1) whether there is a correlation between algal biomass and heavy metal binding in natural lake systems and (2) which indigenous species are actually responsible for this binding.

Several authors (McAllister et al. 1961; Anita et al. 1963; Takahashi et al. 1976; Lund 1972; Parson et al. 1978) have successfully employed relatively large enclosures to evaluate in situ the various effects of physico-chemical or biological parameters on aquatic environments. Similar types of enclosures were designed here so as to provide a broad data base from which to evaluate the various algal species and the four metal ions in the study.

To date, studies on algal-heavy metal interaction have been focused mainly on laboratory studies using few algal species. No attempt has been made to investigate the algal-heavy metal interaction with respect to the species pool within a particular community under natural conditions. The field approach together with various algal communities obtained as a result

of the field manipulations, the large number of samples taken and the aid of computer analysis permitted that all the species found could be analyzed with respect to the four metal ions. Thus, a better understanding of algal-heavy metal binding ability under natural conditions can be expected.

MATERIALS AND METHODS

Area of Study

The field experiments were performed in Heney Lake, Quebec (46°02'N - 75°55'W), where the Field Station of the Department of Biology, University of Ottawa, is located.

The lake is characterized by a low nutrient status, a maximum depth of 25 m, and a total area of approximately 12 km^2. The field experiments were carried out in a bay 10 m deep located at the northern end of the lake which offered protection against the prevalent northerly winds and was relatively free of human disturbance due to the lack of habitations on its shore.

Experimental Design

In order to carry out the field experiments with different planktonic communities, polyethylene enclosures were designed so as to contain an entire column of water with the associated biota. They were 10 m deep and 1 m in diameter (a volume of approximately 8,000 L), anchored at the bottom and suspended 20 cm above the water surface from a ring supported by floats. The launching procedure consisted of first sinking the collapsed containers to the bottom, then after the return to calm conditions drawing them up through the water column. To fill the enclosures to capacity, water from the lake was pumped into the bags using a 3 hp water pump. All enclosures were open at the top to permit gas exchange.

Several manipulations were carried out to generate appreciable variations in community structure. Two of the tubes were freely connected with the sediment, while the other four were sealed at the bottom. In two of the latter, zooplankton levels were reduced by passing a net of 135 µm aperture and 0.75 m in diameter through the enclosure. In addition, three enclosures, one of each set, were enriched weekly with nitrate and phosphate in concentrations of 100 µg N/L and 10 µg P/L respectively, while the others were left unfertilized.

Sampling Procedure

From June to November 1976, duplicate samples were taken weekly from each enclosure as well as from the lake using a Van Dorn bottle at 0, 3, 6 and 9 m, to determine changes in (1) chemical parameters, (2) phytoplankton biomass, and (3) species composition.

Plankton samples were preserved immediately after collection with Lugol's solution (see Appendix I), whereas samples destined for chemical analysis were carried to the laboratory for immediate assessment (see section on sample analysis below).

Enumeration, Identification and Volume Calculation Procedure

Phytoplankton samples were analyzed quantitatively and qualitatively using a Wild (M20) inverted microscope following the sedimentation method described by Utermöhl (1958). Using a 5 cc sedimentation chamber, usually

10 fields were enumerated and a minimum of 100 individuals were counted. In cases where cells occurred in aggregates or filaments, the cells of the aggregates or filaments were counted as individuals.

Organisms were identified to species when possible. For this purpose a wide taxonomic literature was used (see Appendix II). To facilitate the identification of diatoms, a sample aliquot was treated with hot hydrogen peroxide (50%) to remove the organic matter from the frustules. The cleaned diatoms were then mounted in slides with Hyrax Mounting Medium.

Phytoplankton volume was estimated using the geometric shapes devised by Kovala and Larrance (1966). Average cell size was measured and the mean value used for estimating the volume of a particular species.

Physico-Chemical Parameters

Along with the assessment of heavy metal binding, the following physico-chemical parameters were measured: pH, oxygen, temperature, redox potential, ionic background, conductivity, chloride and phosphate. In preliminary tests no significant difference in pH and redox potential was found between field and laboratory measurements. For this reason both parameters were assessed upon return to the laboratory.

pH: pH determinations were made using a Digital Orion pH meter (model 701A).

Oxygen and temperature: Both parameters were recorded weekly using a YSI Model 54 meter in both the lake and the enclosures.

Redox potential: The Orion redox electrode, model 96-78, connected to the Orion pH/mv meter model 801, was used to make direct measurements of redox potential in the samples. When the electrode is filled with 90-00-01 filling solution* its potential characteristics match those of a conventional calomel reference electrode (Chateau 1954).

Since it is customary to report redox potential readings relative to the normal hydrogen electrode (NHE), the potential developed by the reference electrode relative to the NHE is added to the potential developed by the platinum redox electrode. This is described in the following equation:

$$E_{NHE} = E_o + C$$

where E_{HNE} = oxidation reduction potential of the sample relative to the NHE following the international sign convention.

E_o = potential developed by the platinum redox electrode.

C = potential developed by the reference electrode portion relative to the NHE.

At 25°C the value of C is 241 mv, which was added to all the mv readings recorded by the platinum redox electrode so the redox potential values reported are relative to the NHE.

Conductivity: Conductivity of the samples was determined at 25°C by a Leeds and Northup conductivity bridge, using a glass conductivity cell electrode coated with platinum black, having a cell constant of 0.3358. The lower limit of conductance measurements using this cell was 3 umho ± 5%. At 50 umho, the reliability of the conductance is ± 0.001%.

* Orion Ionalyzer Instruction Manual for Platinum Redox electrode model 96-78, Orion Res. Inc., Mass., form IM 96-78/966, 1969.

Ionic background: Ionic background was calculated from the conductivity measurements using calibration values reported by Orion Research Inc.* relating the two values.

Chloride: Chloride ion concentration was determined by a Orion combination chloride electrode (Orion model 96-17) using an Orion Ionalizer (research model 801). Sodium chloride standards in the range 0.1 - 3500 mmg/L Cl^- were used for calibration. At 25°C the limit of detection of chloride ion concentration is 1.4×10^{-5} M.

Orthophosphate: Inorganic phosphorus content was measured by the Eibl and Lands method (1969).

Nitrate, nitrite: Nitrate and/or nitrite was determined by the Automated Cadmium Reduction method of Brewer and Riley (1965) as described by Traversy (1971).

Total Carbon: Total inorganic carbon (TIC) and total organic carbon (TOC) were determined in water samples using the method of the Federal Water Pollution Control Administration (1969) described by Traversy (1971).

* Orion Ionalyzer Instruction Manual for Divalent cation electrode model 92-93, Appendix III, Orion Res. Inc., Mass., form IM 92-93/7610, 1967.

Heavy Metal Binding Procedure

Ionic-specific electrode technique: The ability of the samples to bind the metal ions Hg, Cd, Pb and Cu, was measured by ion-specific electrode techniques using an Orion model 801 pH/mv meter in combination with model 851 digital print system and a 855 automatic electrode switch. The sensors used were Orion solid state specific ion electrode model 94-53 (Hg^{++}), 94-48A (Cd^{++}), 94-82A (Pb^{++}), 94-29A (Cu^{++}) and the reference electrodes suggested by the Orion Corp., which was a double junction model 90-02 (with 10% KNO_3 in the outer compartment) for Hg and Pb ions; and a single junction electrode model 90-01 for Cd and Cu ions (Ramamoorthy and Kushner 1975 a,b).

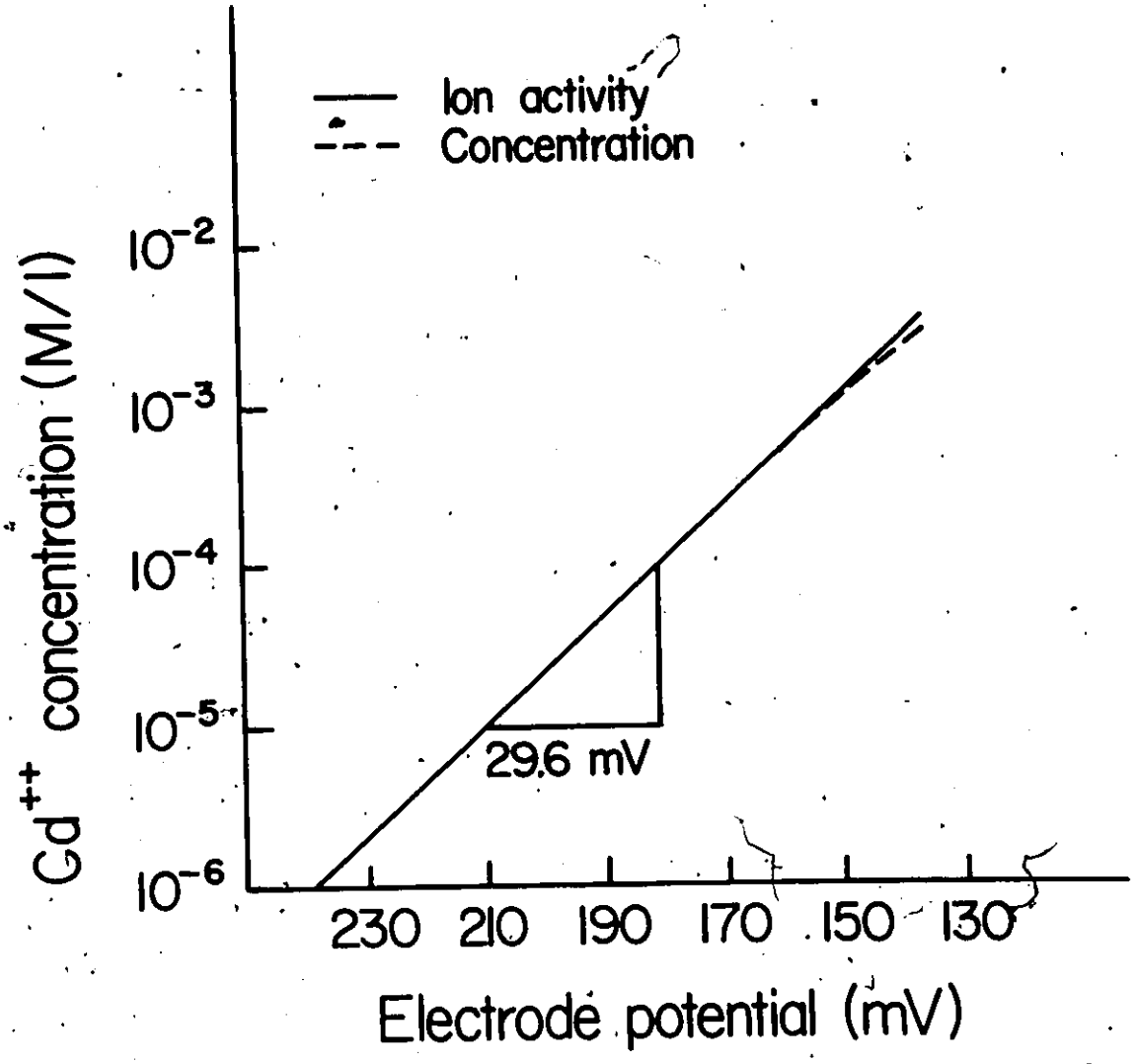
Analytical method: A typical electrode response for one of the metals studied (cadmium) is given in Figure 1.

For Cu, Pb and Cd ions, a change of 29.6 mv occurred for every tenfold change in metal concentration. For Hg ion the change was 59.2 for every tenfold.

Calibration curves of mv vs metal ion concentrations were prepared each time that a set of samples was measured. Metal nitrates were used throughout the experiment with a 10^{-2}M stock solution diluted to 10^{-3} -10^{-6}M. Standard solutions were prepared with deionized water. Calibration plots for each metal were made each time that a set of measurements was taken.

The electrodes develop a potential proportional to the logarithm of the

FIGURE 1 Typical electrode response to changes in Cd^{++} ion activity (solid line) and concentration (dotted line) in $Cd(NO_3)_2$ solutions at 25°C.


Ion activity
Concentration
Cd^{++} concentration (M/l)
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
29.6 mV
230 210 190 170 150 130
Electrode potential (mV)

activity of the metal ion in solution. This relationship is described by the Nernst equation.

$$E = E_0 + \frac{2.303\,RT}{nF} \times \log AM^{++}$$

where: E = measured total potential of the system

E_0 = potential due to the reference electrodes and internal solutions

R = gas constant

T = temperature in Kelvin degrees

n = valence of the metal ion

F = Faraday's constant

AM^{++} = metal ion activity in the sample.

The free divalent metal ion concentration (M^{++}) and the metal ion activity AM^{++} are related by a factor called the ion activity coefficient α in the following equation

$$AM^{++} = \alpha\,(M^{++})$$

At free metal ion concentrations below 10^{-3}M, α is approximately equal to unity, and the activity of the metal ion and free metal ion concentration become equal. Therefore, the potential developed by the electrodes is proportional to the logarithm of the concentration of the free metal ion in solution.

Calculation of the metal bound: Heavy metal binding in the water samples was expressed as α, the fraction bound, where

$$\alpha = Tm - (M^{++})/Tm = (M)\text{ bound}/Tm$$

Tm = total concentration of metal ions added to the sample.

(M^{++}) = free metal ion concentration detected by the ion specific electrode. Final results, however, are expressed in terms of concentration of metal ions bound on the system in $\mu M/l$.

Sample analysis: Measurements of the metal binding capacity at 20°C±1° were carried out in duplicate using two 50-ml of water samples to which the metal nitrate solution ($10^{-2}M$) was added in small increments with an adjustable calibrated sampler micropipette. Final concentration was never higher than $10^{-4}M$. The free metal ions in the solution were read as mv and converted to concentration according to standard curves established for known metal solutions.

Solutions were stirred magnetically during measurement, at a constant speed for all samples. To avoid external contamination, glassware was washed with a mixture of No-Chromix (Godax Laboratories, N.Y.) and sulfuric acid, then rinsed extensively with deionized water. This procedure was standard for all glassware when heavy metal measurements were involved. Previous studies (Bothner and Carpenter 1973; Ramamoorthy and Kushner 1975a) have shown that adsorption of heavy metal ions on the glass surface is negligible during the experimental period.

RESULTS

Community Structure

A total of 99 phytoplankton species (Table 1) was recorded during the period of this study, comprising 50 Chlorophyta (green algae), 20 Cyanophyta (blue-green algae), 19 Bacillariophyta (diatoms) and 10 species belonging to other groups.

The successions patterns in Heney Lake are characterized by a great abundance of blue-green algae in July and August. The chronological sequence of population peaks was Anabaena spiroides, Anabaena sp., Aphanizomenon flos-aquae, Phormidium sp. and Microcystis aeruginosa. Additional species, such as Anabaena flos-aquae, Dactylococcopsis acicularis, and Oscillatoria limnetica, appeared erratically throughout the entire sampling period.

Green algae are characterized for their sporadic blooms with maxima during summer months, with the exception of Sphaerocystis shroeteri and Scenedesmus obliquus which peak during September and October respectively.

In general diatoms are more abundant during the fall, when Tabellaria fenestrata, Fragilaria capucina, Asterionella formosa, Fragilaria crotonensis and Melosira granulata succeed each other. On the other hand, Rhizosolenia eriensis, Cyclotella sp. and Synedra delicatissima registered their greatest abundance earlier in the year.

The other groups, i.e., Cryptophyta, Xanthophyta, Pyrrophyta,

TABLE I
List of Species Recorded in the Lake

CHLOROPHYTA (Greens)

Ankistrodesmus braunii
A. falcatus
Chlamydomonas sp.
Closterium gracile
C. leiblenii
Coelastrum microporum
Cosmarium impressulum
C. regnelli
Crucigenia quadrata
C. rectangularis
Dictyosphaerium pulchellum
Elakatothrix gelatinosa
Euastrum insulare
Eudorina elegans
Franceia ovalis
Geminella interrupta
Gloeocystis ampla
G. gigas
Golenkinia paucispina
C. radiata
Gonatozygon brebisonii
Kirchneriella lunaris
Mougeotia sp.
Oocystis crassa
O. parva
O. pusilla
O. pyriforme
Oedogonium sp.
Pediastrum boryanum
P. duplex
P. tetras
Planktosphaeria sp.
Protoderma viridis
Quadrigula closteroides
Q. lacustris
Scenedesmus abundans
S. bijuga
S. dimorphus
S. obliquus
S. quadricauda
Schroederia setigera
Sphaerocystis shroeteri
Spirogyra sp.
Spondylosium planum
Staurastrum paradoxum
S. nannum
Tetraedron minimum
T. regulare
Tetrallantos lagerheimii
Ulothrix aequalis

CRYPTOPHYTA

Chroomonas nordstedti
Cryptomonas erosa
C. ovata

XANTHOPHYTA

Chlorochromonas minuta

CYANOPHYTA (Blue-greens)

Agmellenum thermale
Anabaena flos-aquae
A. spiroides
Anabaena sp.
Aphanizomenon flos-aquae
Chroococcus limneticus
Coelosphaerium naegelianum
Dactylococcopsis acicularis
D. fascicularis
D. smithii
Gloeòtrichia echinulata
Gomphosphaeria lacustris
Lyngbia birgei
Microcystis aeruginosa
Nostoc linckia
Oscillatoria limnetica
O. linosa
Phormidium sp.
Rhabdoderma sigmoidea
Spirulina sp.

BACILLARIOPHYTA (Diatoms)

Achnanthes deflexa
Asterionella formosa
Cocconeis placentula
Cyclotella meneghiniana
Cymatopleura solea
Cymbella sp.
Epithemia sp.
Eunotia arcus
Fragilaria capucina
F. crotonensis
Gomphonema acuminatum
G. olivaceum
Melosira granulata
Rhizosolenia eriensis
Rhopalodia gibba
Stephanodiscus niagarae
Synedra delicatissima
S. ulna
Tabellaria fenestrata

PYRRHOPHYTA

Ceratium hirundinella
Peridinium sp.

CHRYSOPHYTA

Dinobryon bavaricum
D. divergens
Mallomonas producta

EUGLENOPHYTA

Euglena proxima

Chrysophyta and Euglenophyta showed irregular fluctuations during the year.

As intended, the various manipulations resulted in marked changes in algal species richness (Figs. 2 to 8), standing stock (Figs. 9 to 15), and cell volume (Figs. 16 to 22) between the three sets of enclosures. Nutrient enrichment increased both the volume and the species number of the green algae significantly (Mann Whitney U test; $P < 0.05$) at the expense of the blue-green algae and diatoms. In terms of species richness, green algae tend to be more numerous in the enriched enclosure during late July to mid-September (see Figs. 3, 5, 7). On the basis of standing stock blue-green algae were dominant in the enclosures freely connnected with the sediment (Figs. 14 and 15). This dominance is not expressed in terms of cell volume (Figs. 21 and 22) due to the small size of the dominant blue-green algae. In the enclosures subjected to reduced zooplankton grazing, there was a shift from nannoplankton ($< 50\ \mu m$) to net-plankton ($> 50\ \mu m$) species. In terms of biomass (Fig. 19) and standing stock (Fig. 12) green algae dominated the enriched enclosures, whereas blue-green algae dominated the unenriched enclosures (Figs. 15 and 21).

Generally, blue-green algae and diatoms had higher frequencies in the lake than in the enclosures. Most noticeable were Aphanizomenon and Tabellaria, which were recorded in the lake throughout the entire sample period as opposed to the enclosures. On the basis of standing stock blue-green algae were dominant in the lake from June to late September, followed by diatoms (Fig. 9). A similar trend was observed in terms of cell volume, with a more restricted dominance of blue-green algae (Fig. 16). In terms of

FIGURE 2 Weekly fluctuations of species richness in the lake during the period studied.

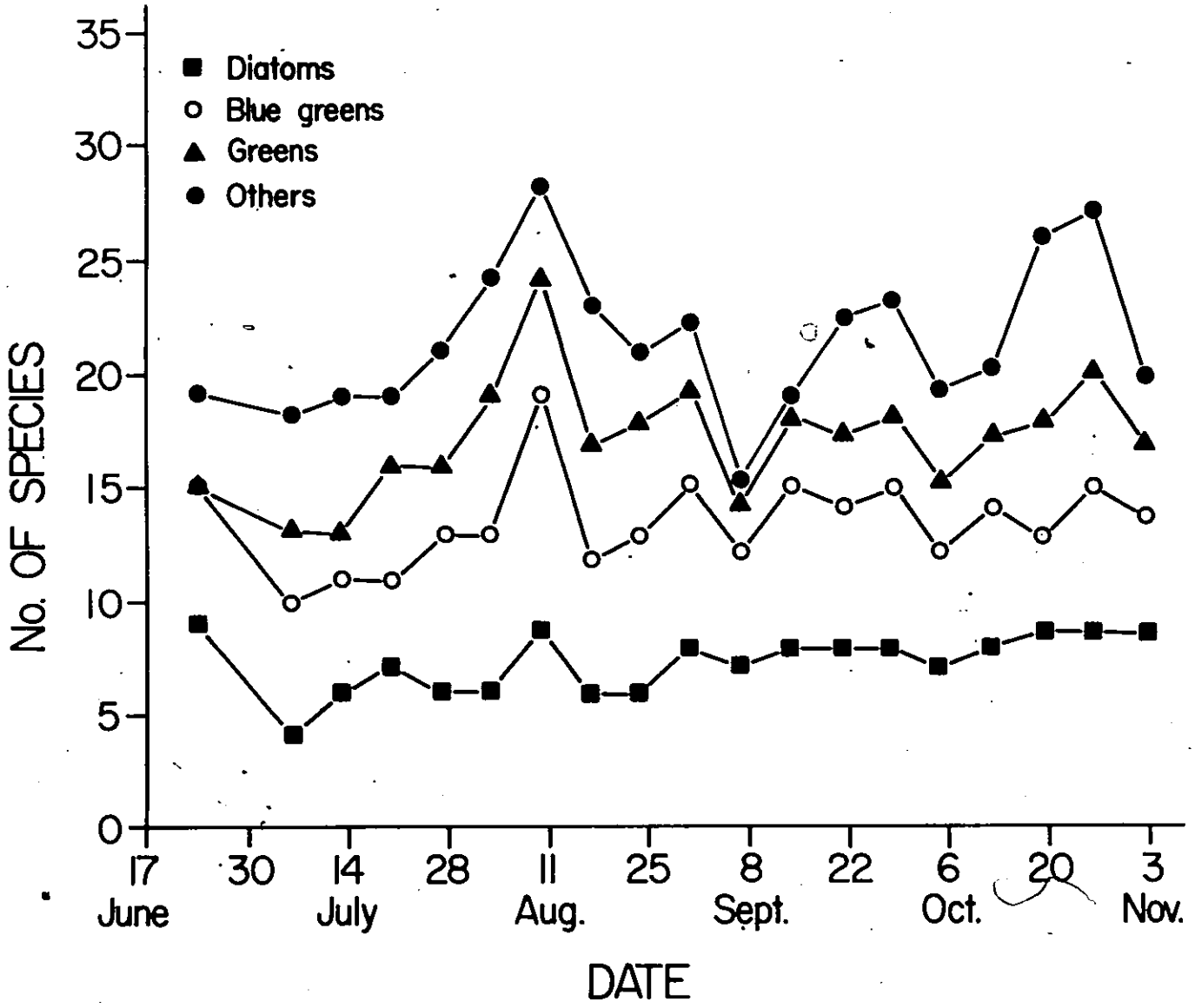

35
30
25
20
15
10
5
0
No. OF SPECIES
Diatoms
Blue greens
Greens
Others
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 3 Weekly fluctuations of species richness in an enriched enclosure during the period studied.

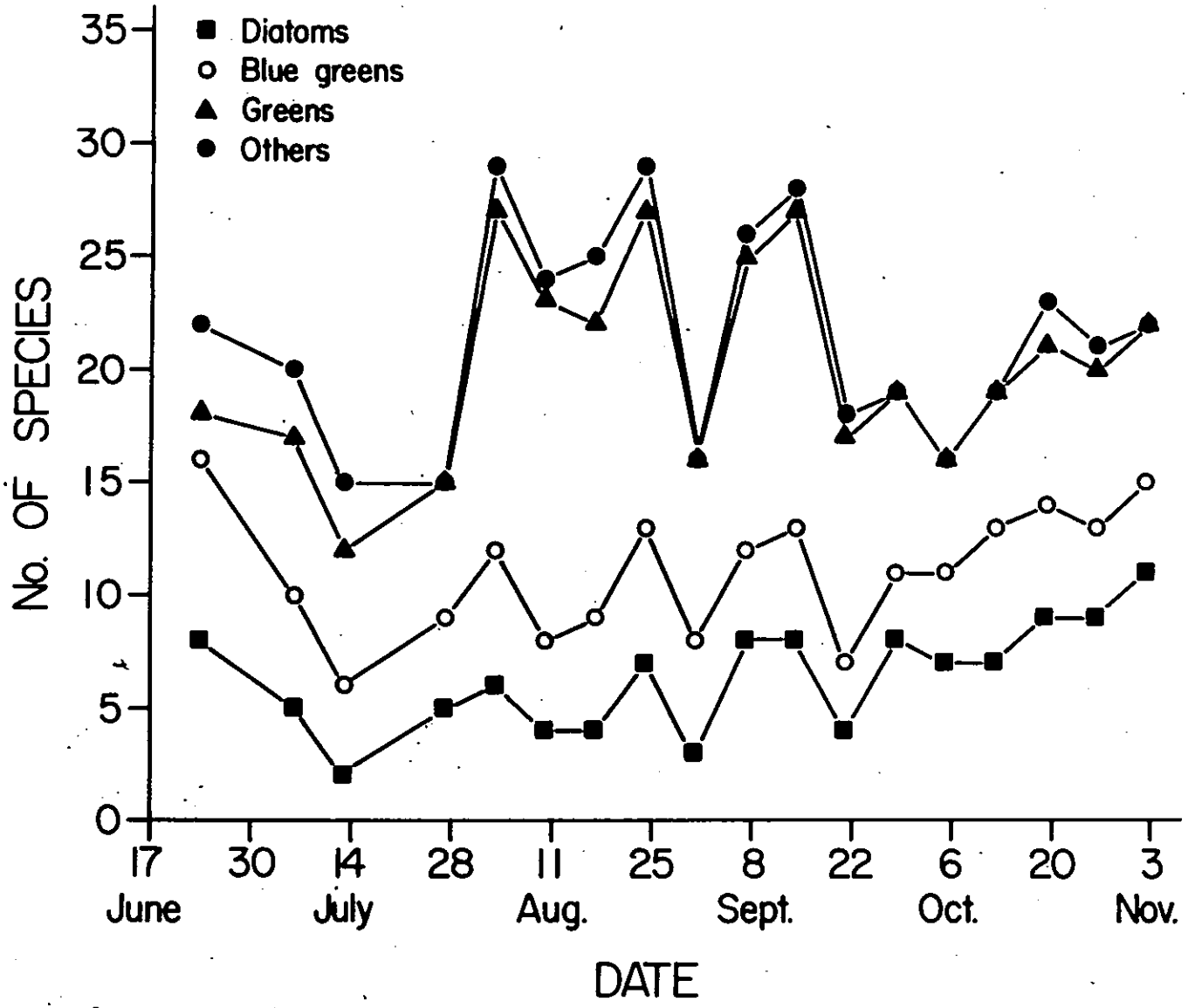

Diatoms
Blue greens
Greens
Others
No. OF SPECIES
35
30
25
20
15
10
5
0
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 4 Weekly fluctuations of species richness in an unenriched enclosure during the period studied.

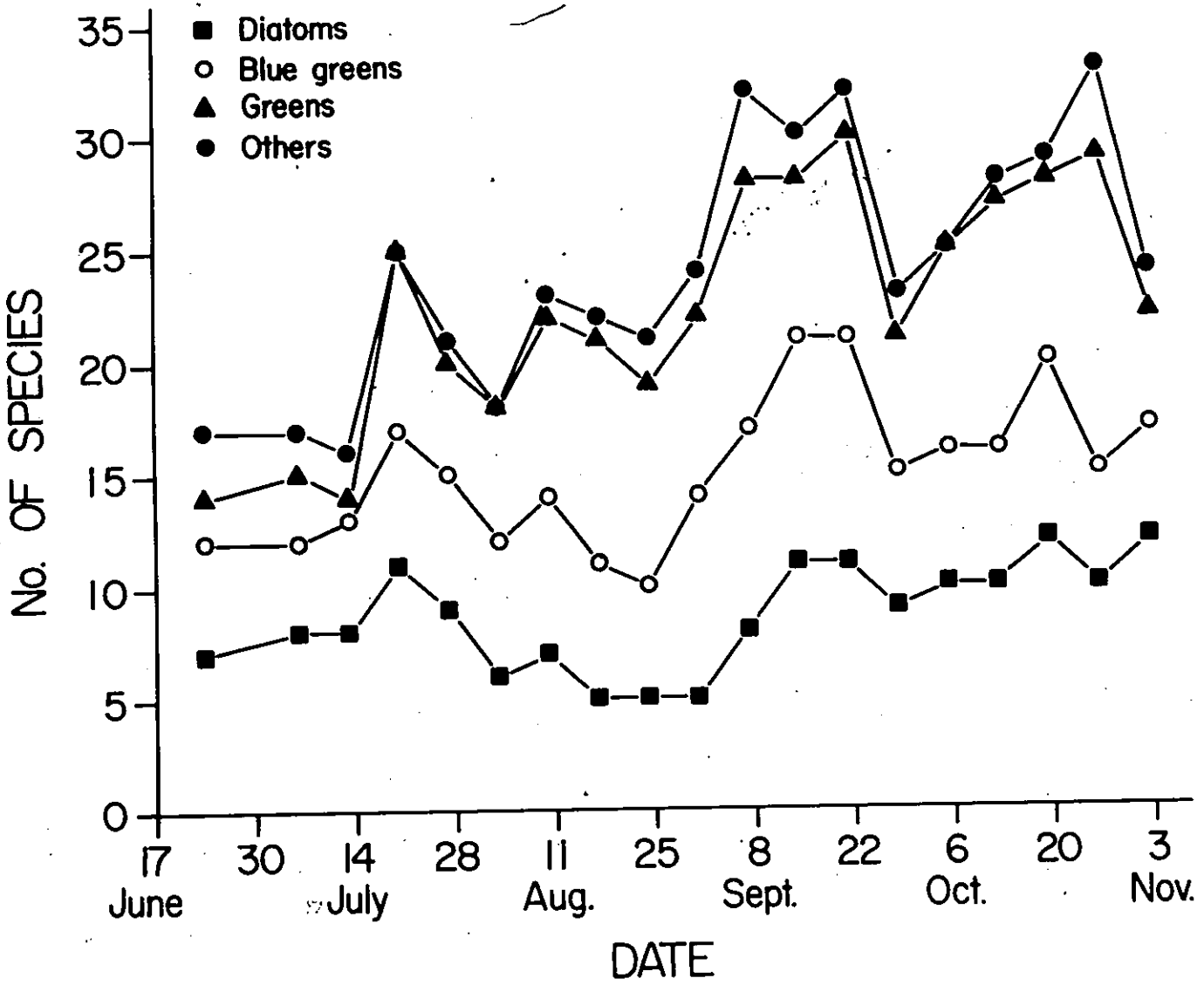

Diatoms
Blue greens
Greens
Others
No. OF SPECIES
35
30
25
20
15
10
5
0
17
June
30
14
July
28
11
Aug.
25
8
Sept.
22
6
Oct.
20
3
Nov.
DATE

FIGURE 5 Weekly fluctuations of species richness in an enriched enclosure with reduced zooplankton levels during the period studied.

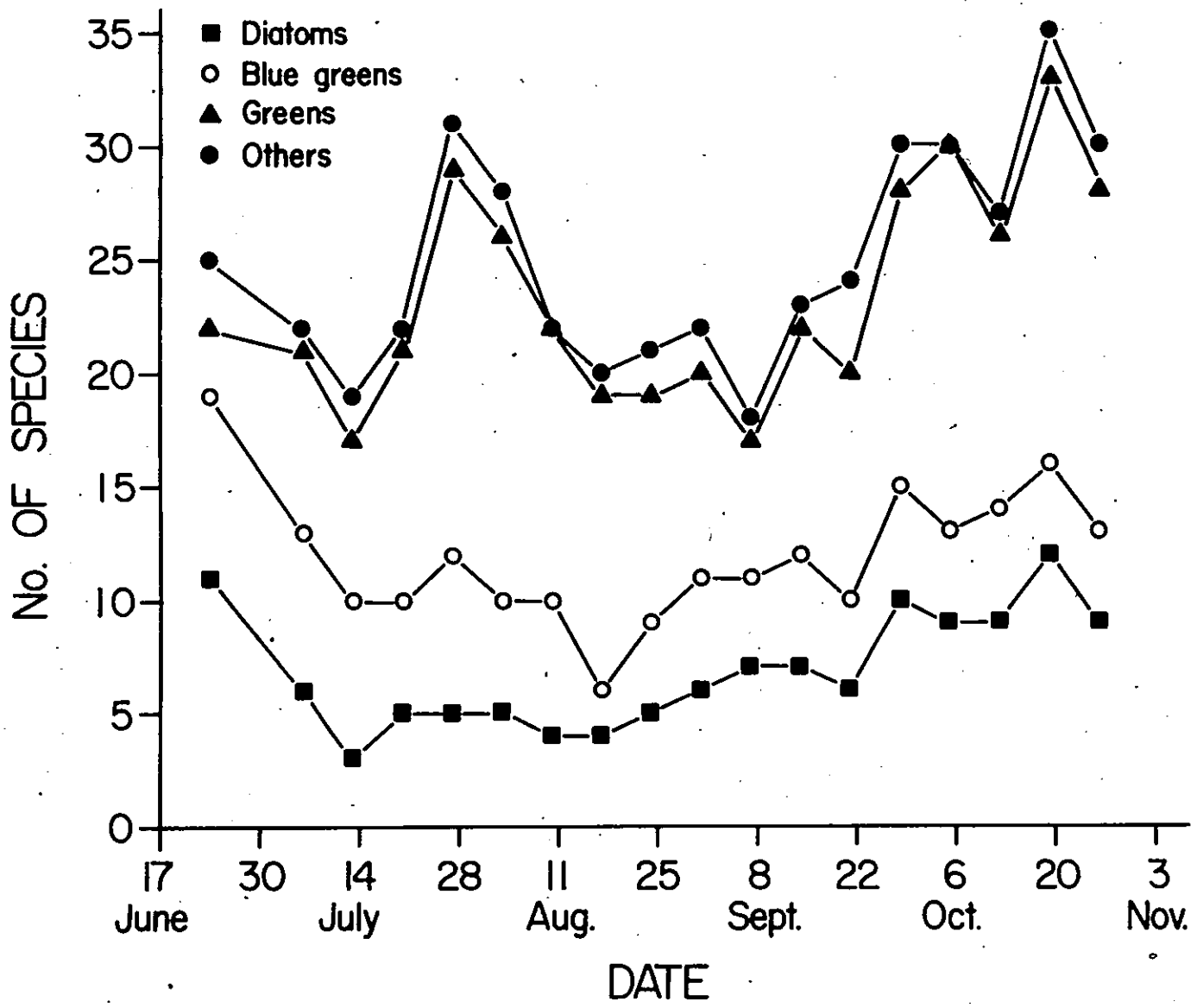

Diatoms
Blue greens
Greens
Others
No. OF SPECIES
35
30
25
20
15
10
5
0
17
June
30
14
July
28
11
Aug.
25
8
Sept.
22
6
Oct.
20
3
Nov.
DATE

FIGURE 6 Weekly fluctuations of species richness in an unenriched enclosure with reduced zooplankton levels during the period studied.

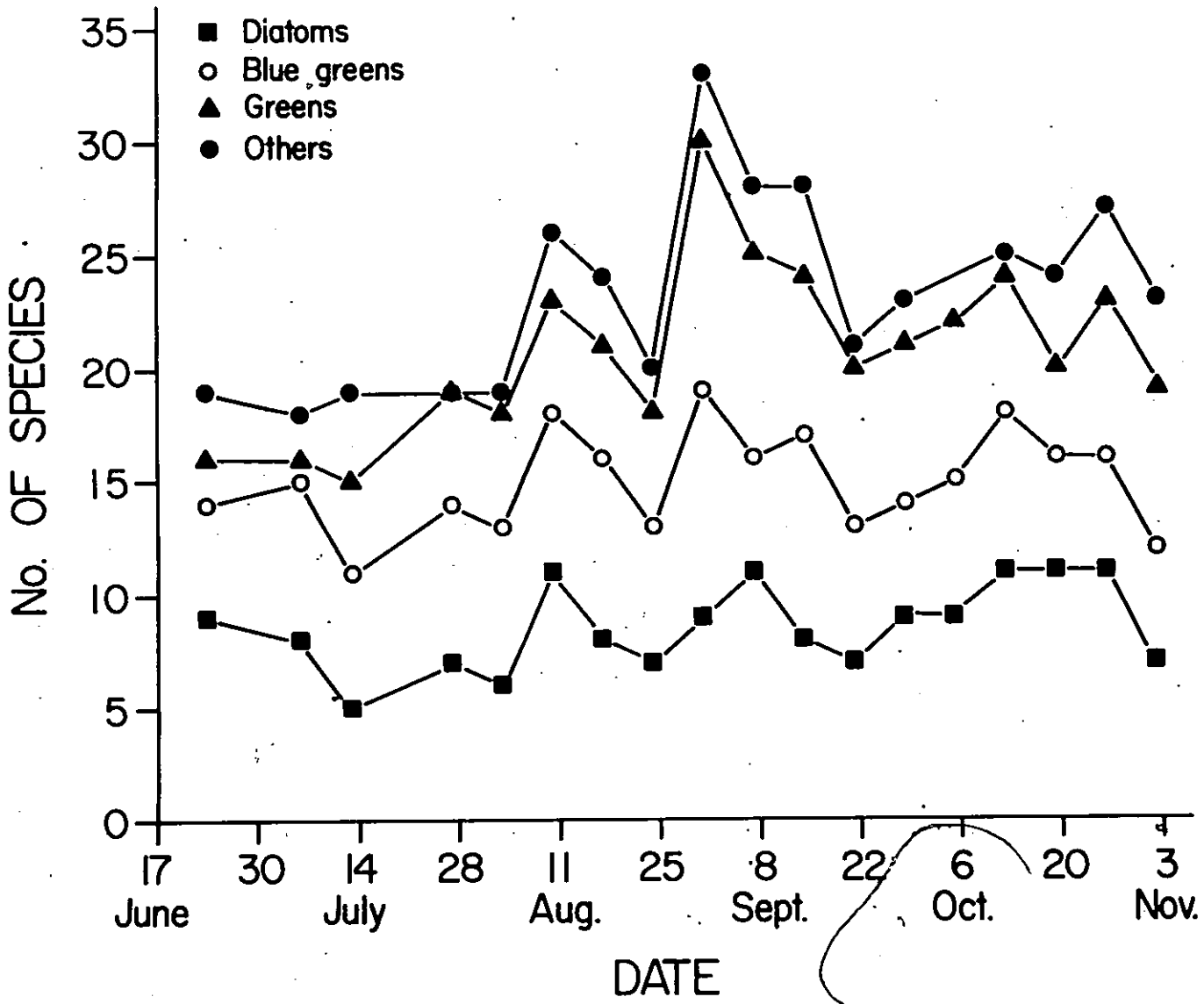

Diatoms
Blue greens
Greens
Others
No. OF SPECIES
35
30
25
20
15
10
5
0
17
June
30
14
July
28
11
Aug.
25
8
Sept.
22
6
Oct.
20
3
Nov.
DATE

FIGURE 7 Weekly fluctuations of species richness in an enriched enclosure in contact with the sediment during the period studied.

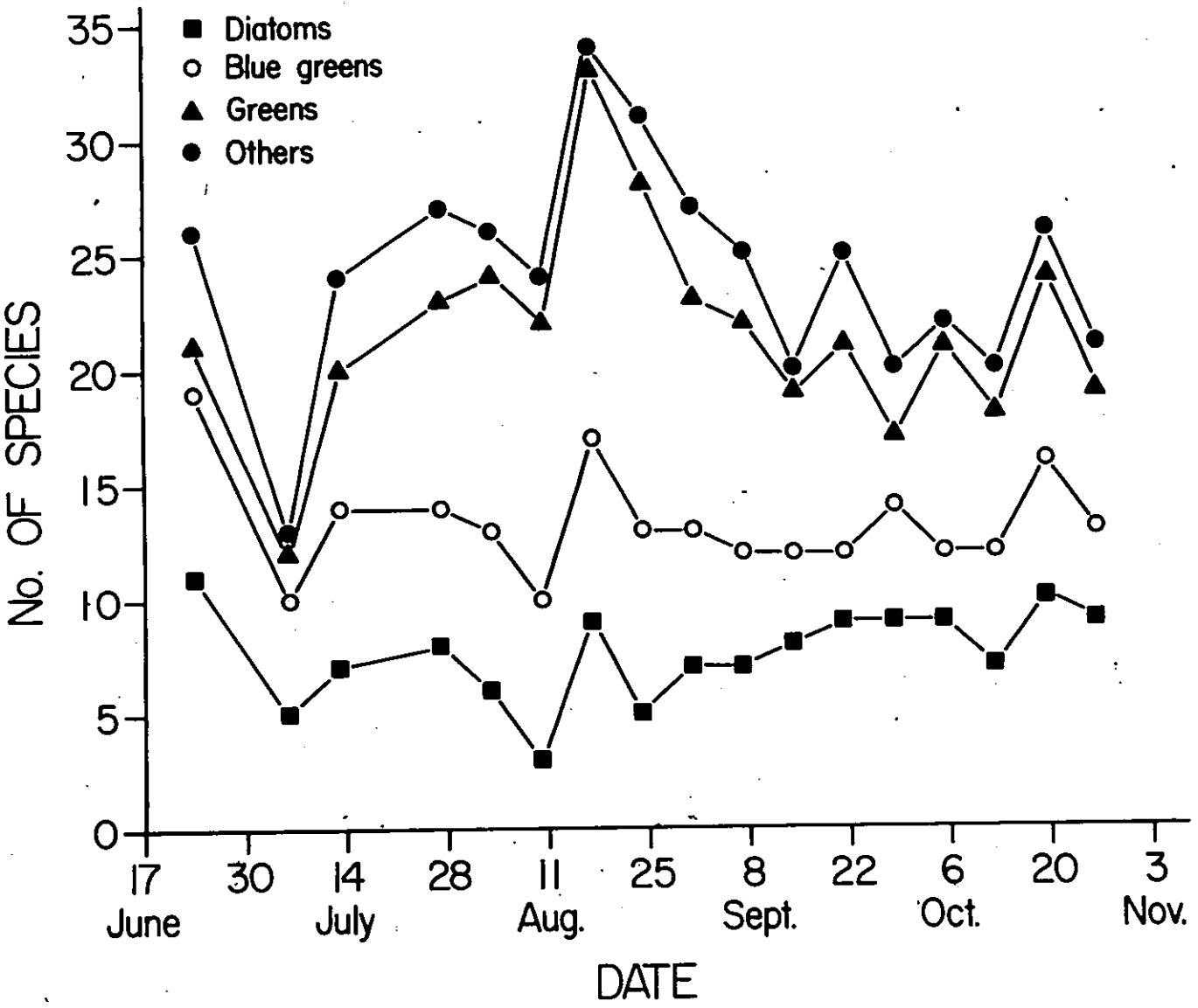

Diatoms
Blue greens
Greens
Others
No. OF SPECIES
35
30
25
20
15
10
5
0
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 8 Weekly fluctuations of species richness in an unenriched enclosure in contact with the sediment during the period studied.

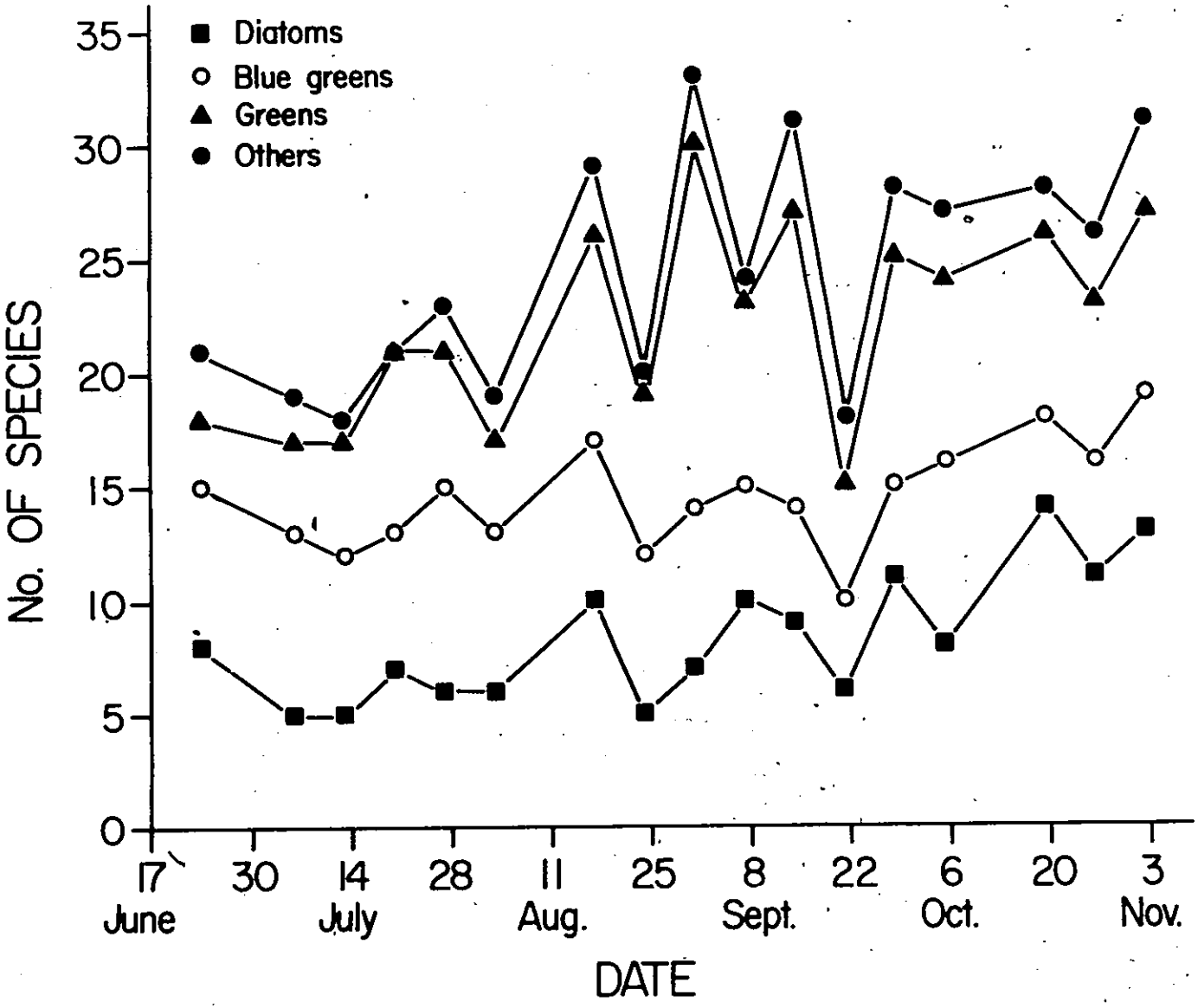

Diatoms
Blue greens
Greens
Others
No. OF SPECIES
35
30
25
20
15
10
5
0
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 9 Weekly fluctuations of phytoplankton standing stock in the lake.

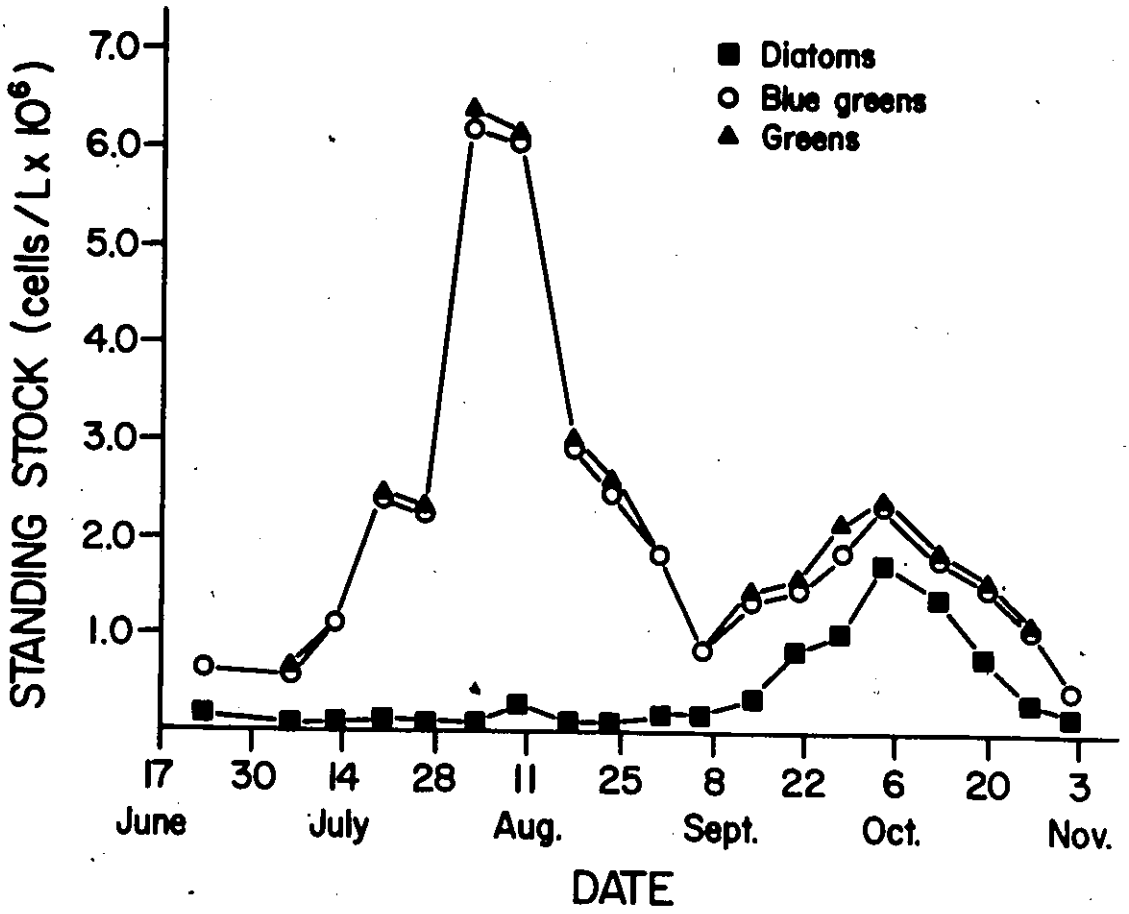

STANDING STOCK (cells / L x 10⁶)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Diatoms
Blue greens
Greens
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 10 Weekly fluctuations of phytoplankton standing stock in an enriched enclosure.

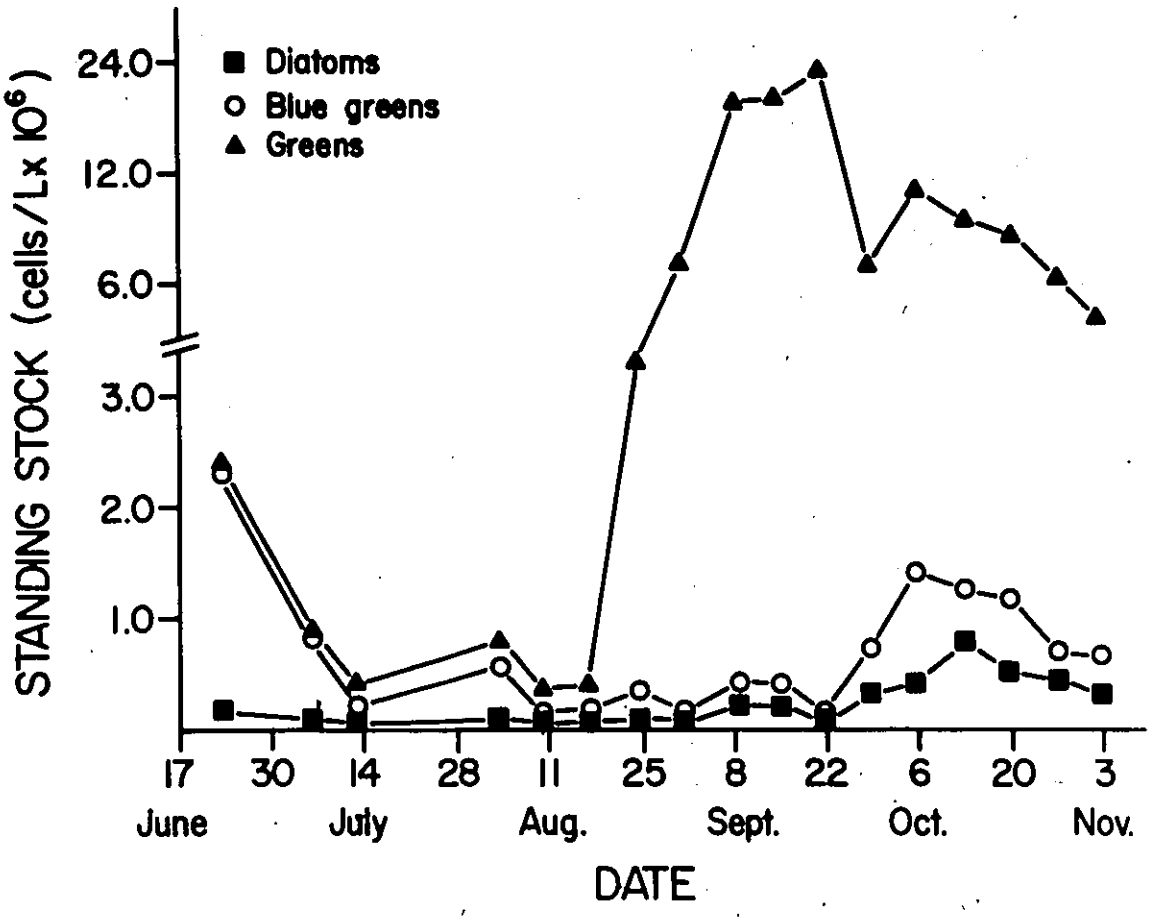

Diatoms
Blue greens
Greens
STANDING STOCK (cells/L x 10⁶)
24.0
12.0
6.0
3.0
2.0
1.0
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 11 Weekly fluctuations of phytoplankton standing stock in an unenriched enclosure.

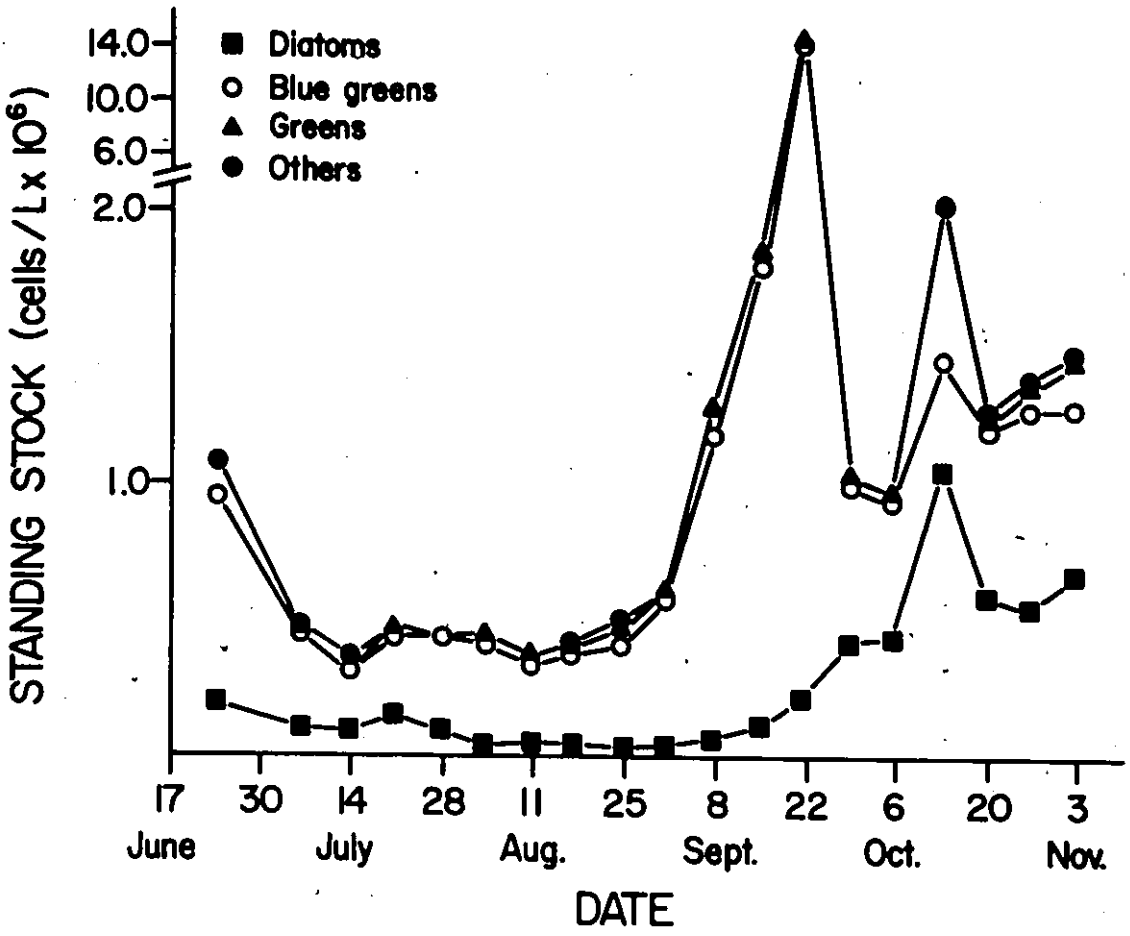

14.0
10.0
6.0
2.0
1.0
Diatoms
Blue greens
Greens
Others
STANDING STOCK (cells/L x 10⁶)
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 12 Weekly fluctuations of phytoplankton standing stock in an enriched enclosure with reduced zooplankton levels.

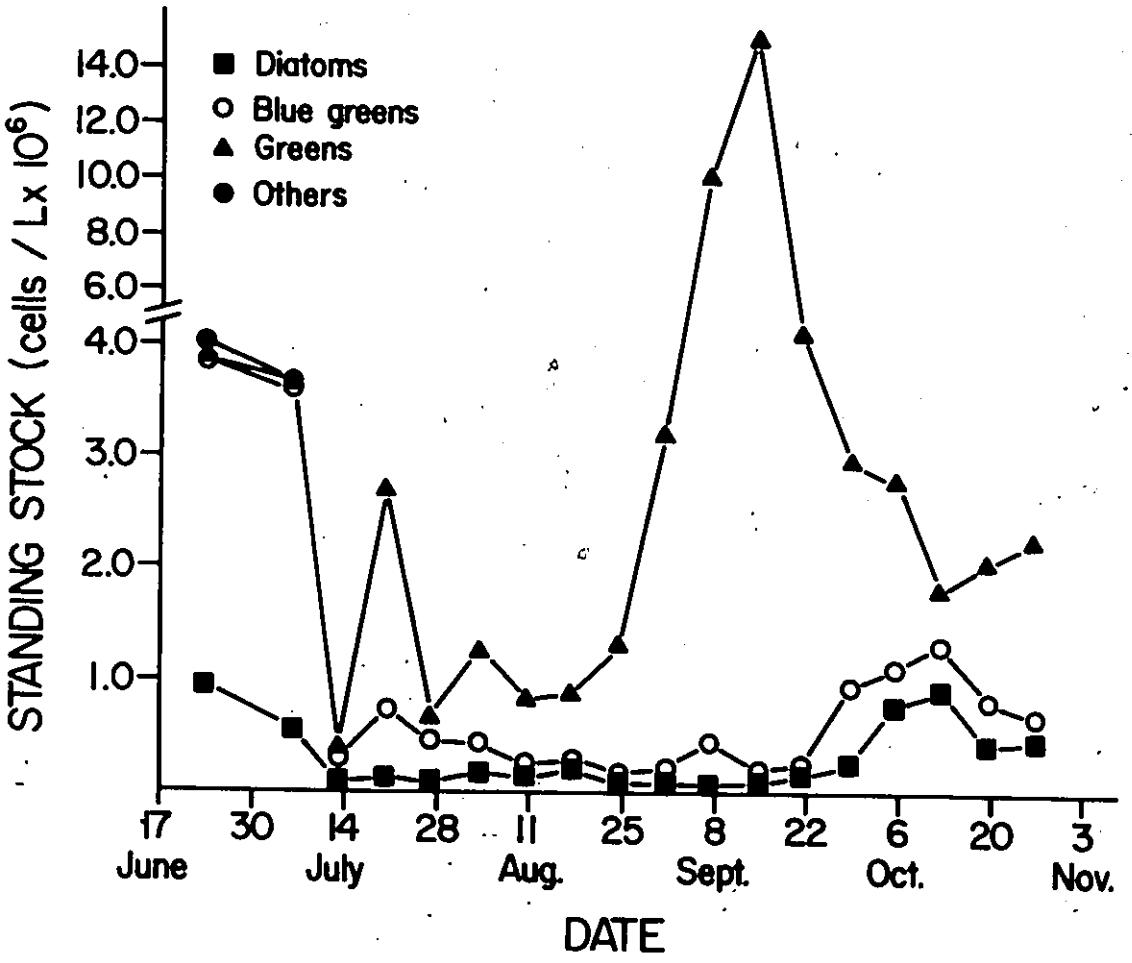

Diatoms
Blue greens
Greens
Others
STANDING STOCK (cells / L x 10⁶)
14.0
12.0
10.0
8.0
6.0
4.0
3.0
2.0
1.0
17 June
30
14 July
28
11 Aug.
25
8 Sept.
22
6 Oct.
20
3 Nov.
DATE

FIGURE 13 Weekly fluctuations of phytoplankton standing stock in an unenriched enclosure with reduced zooplankton levels.

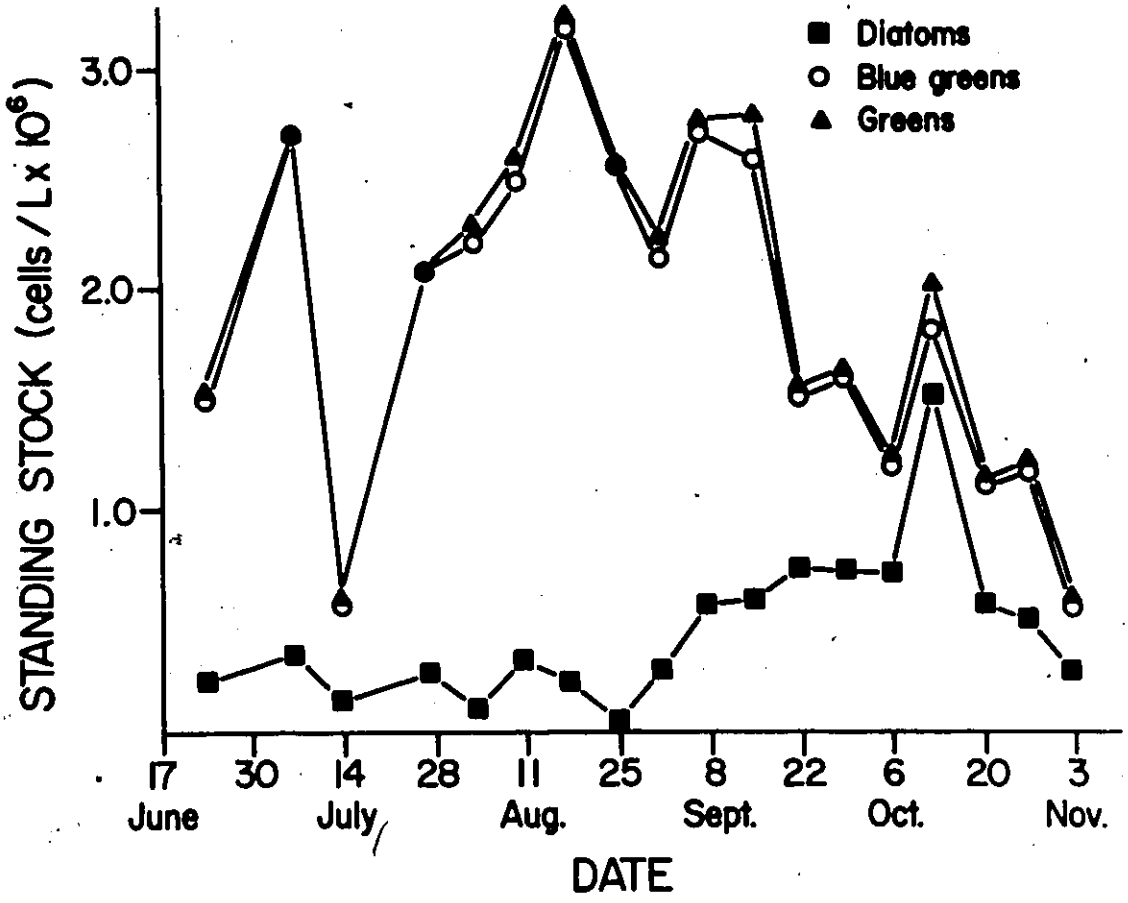

Diatoms
Blue greens
Greens
STANDING STOCK (cells / L x 10⁶)
3.0
2.0
1.0
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 14 Weekly fluctuations of phytoplankton standing stock in an enriched enclosure in contact with the sediment.

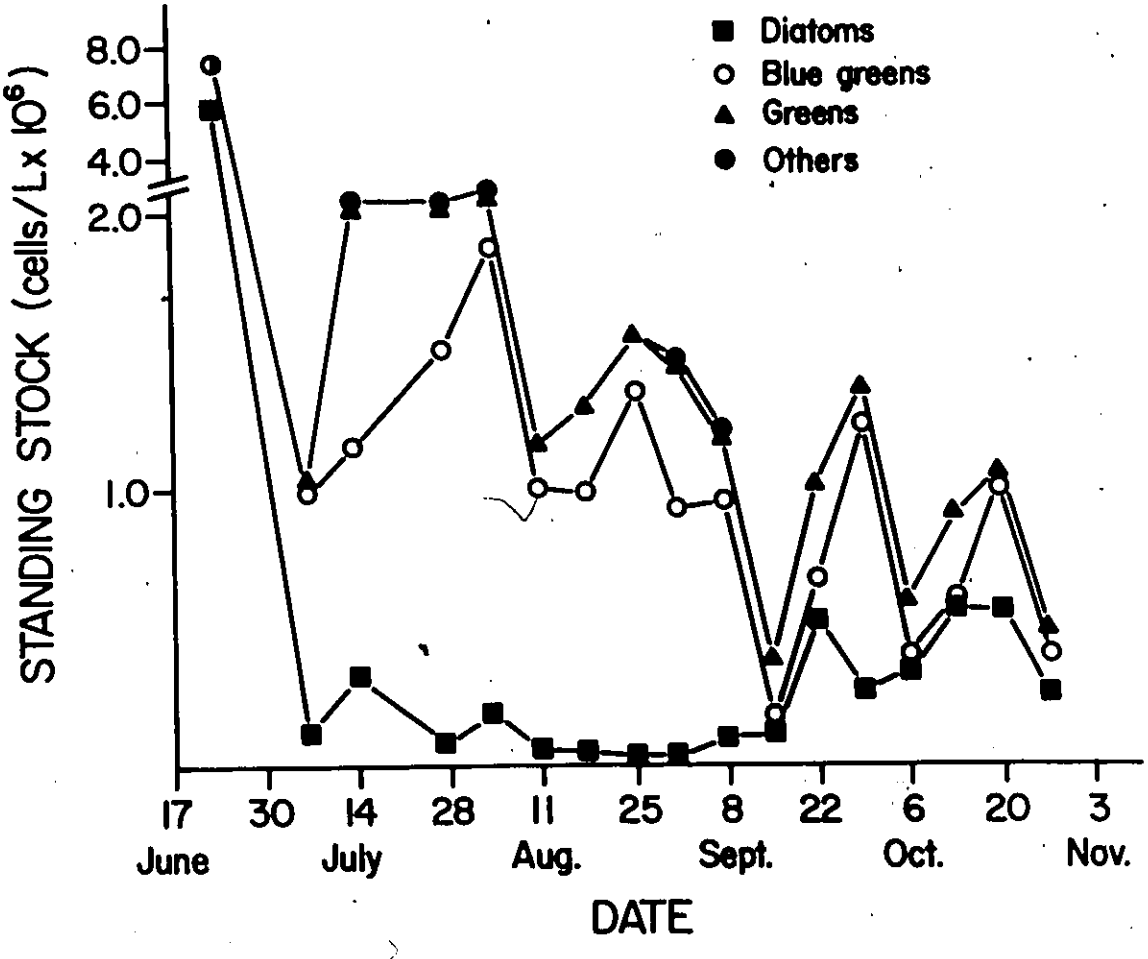

Diatoms
Blue greens
Greens
Others
STANDING STOCK (cells/L x 10⁶)
8.0
6.0
4.0
2.0
1.0
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 15 Weekly fluctuations of phytoplankton standing stock in an unenriched enclosure in contact with the sediment.

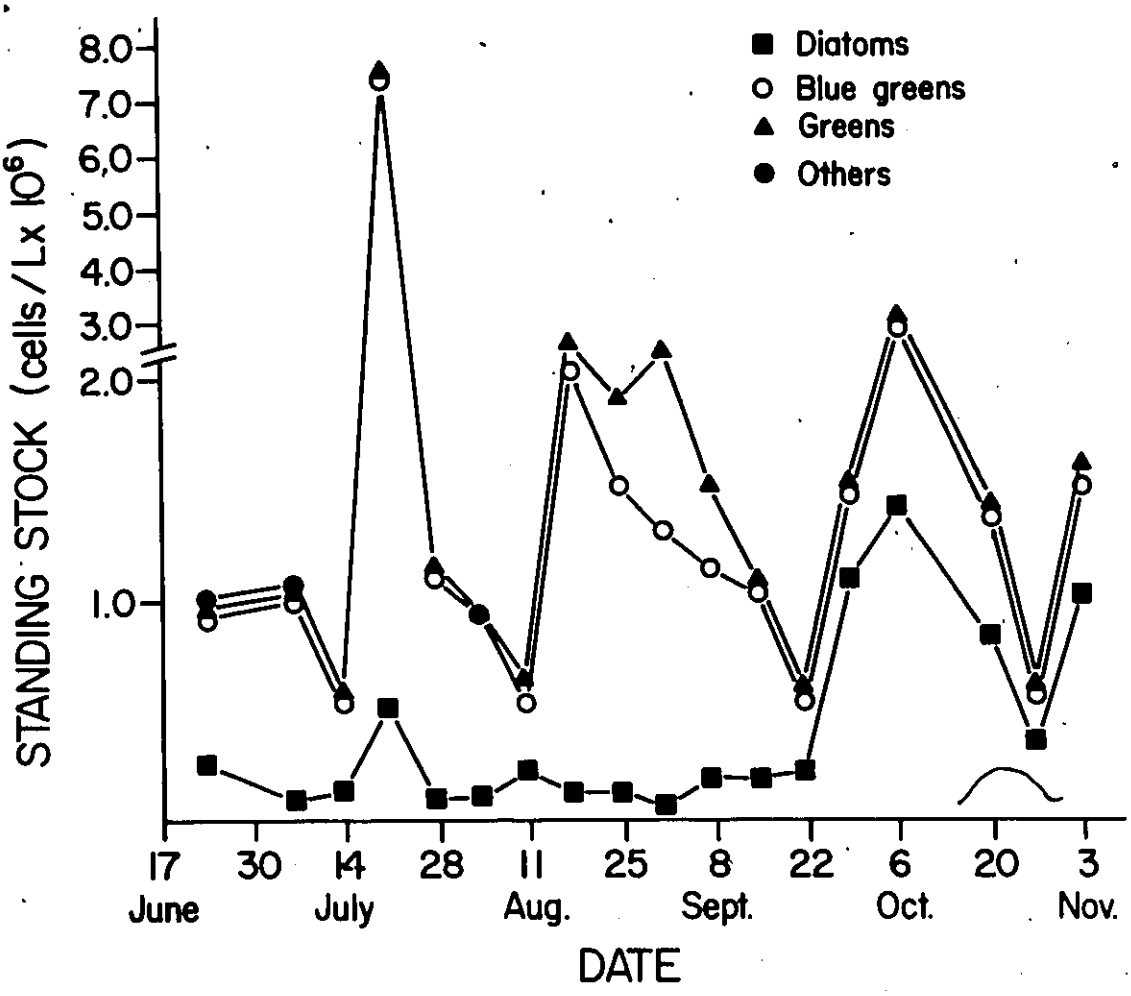

Diatoms
Blue greens
Greens
Others
STANDING STOCK (cells/L x 10⁶)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
17 30 14 28 11 25 8 22 6 20 3
June July Aug. Sept. Oct. Nov.
DATE

FIGURE 16 Weekly fluctuations of algal volume in the lake.

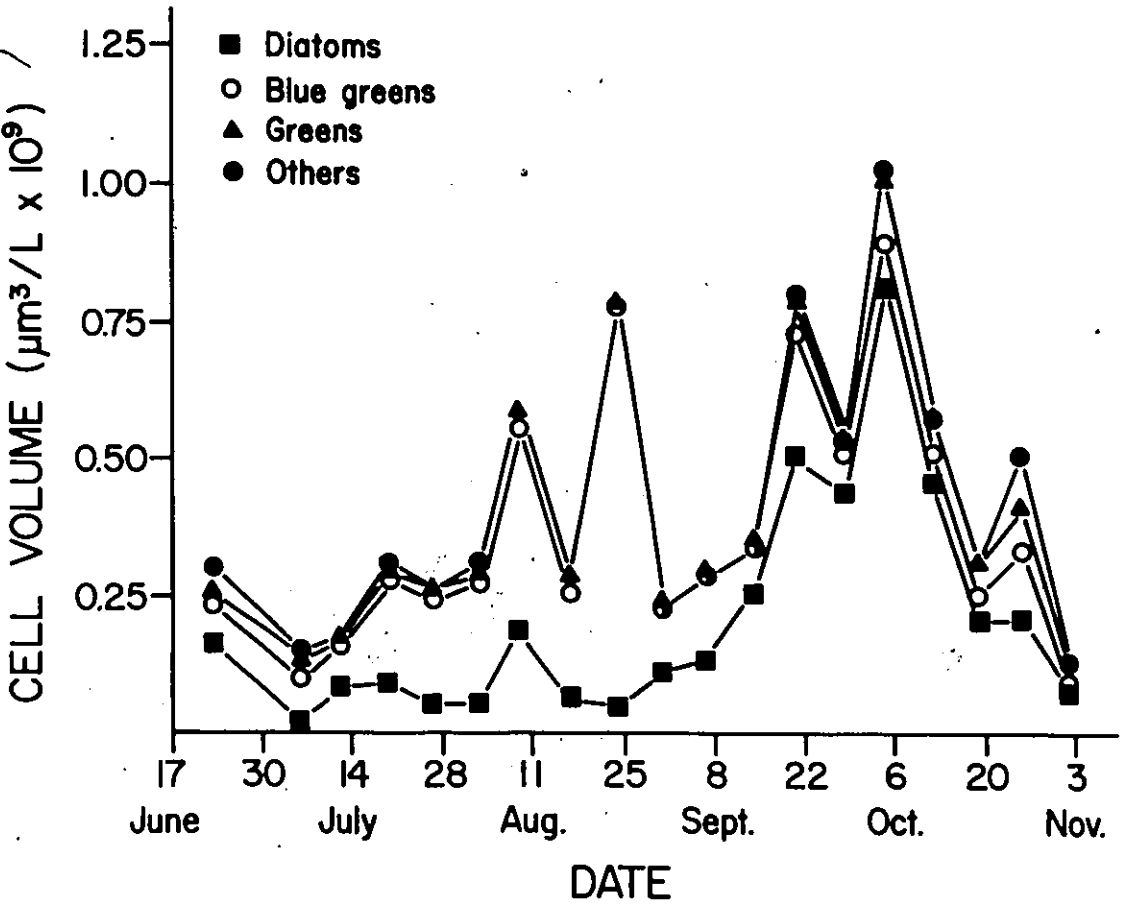

Diatoms
Blue greens
Greens
Others
CELL VOLUME (μm³/L x 10⁹)
1.25
1.00
0.75
0.50
0.25
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 17 Weekly fluctuations of algal volume in an enriched enclosure.

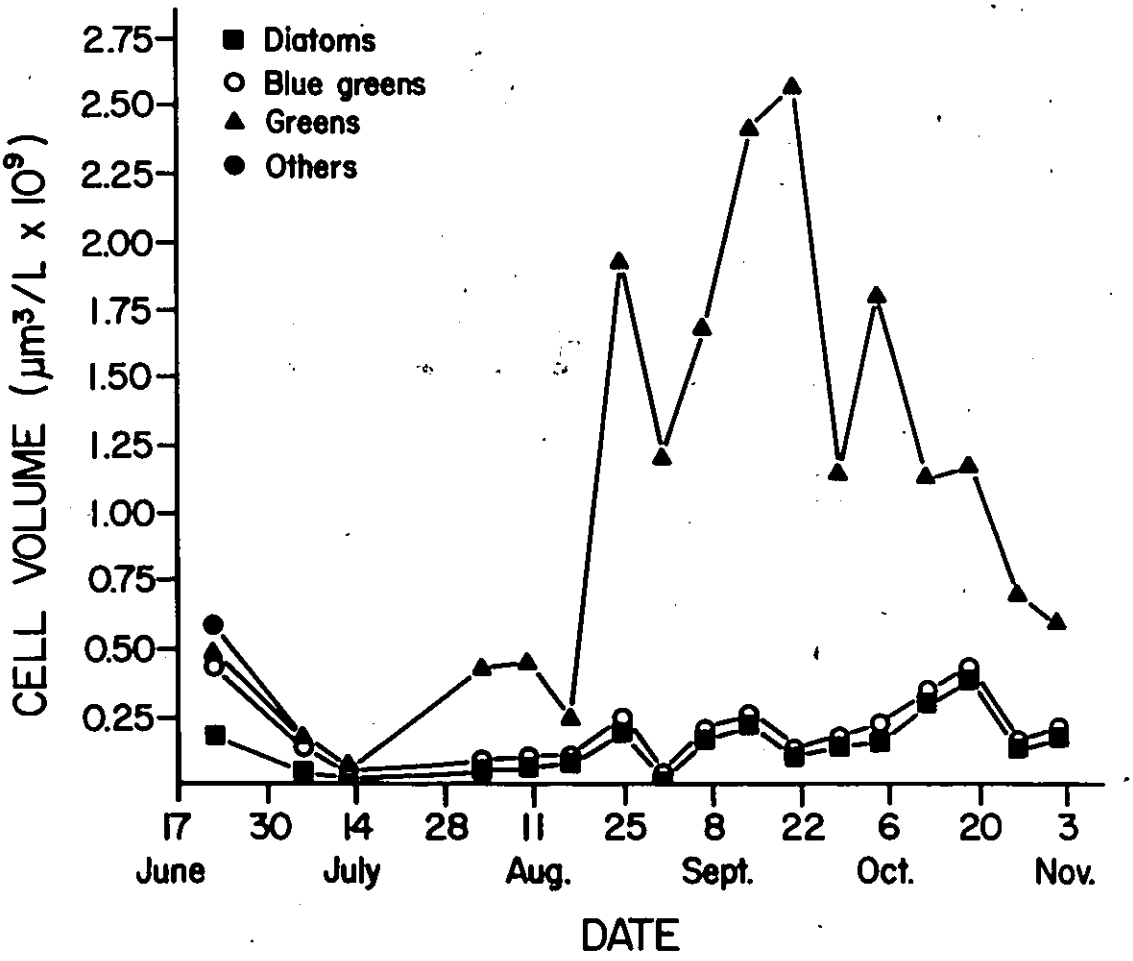

Diatoms
Blue greens
Greens
Others
CELL VOLUME (μm³/L x 10⁹)
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 18 Weekly fluctuations of algal volume in an unenriched enclosure.

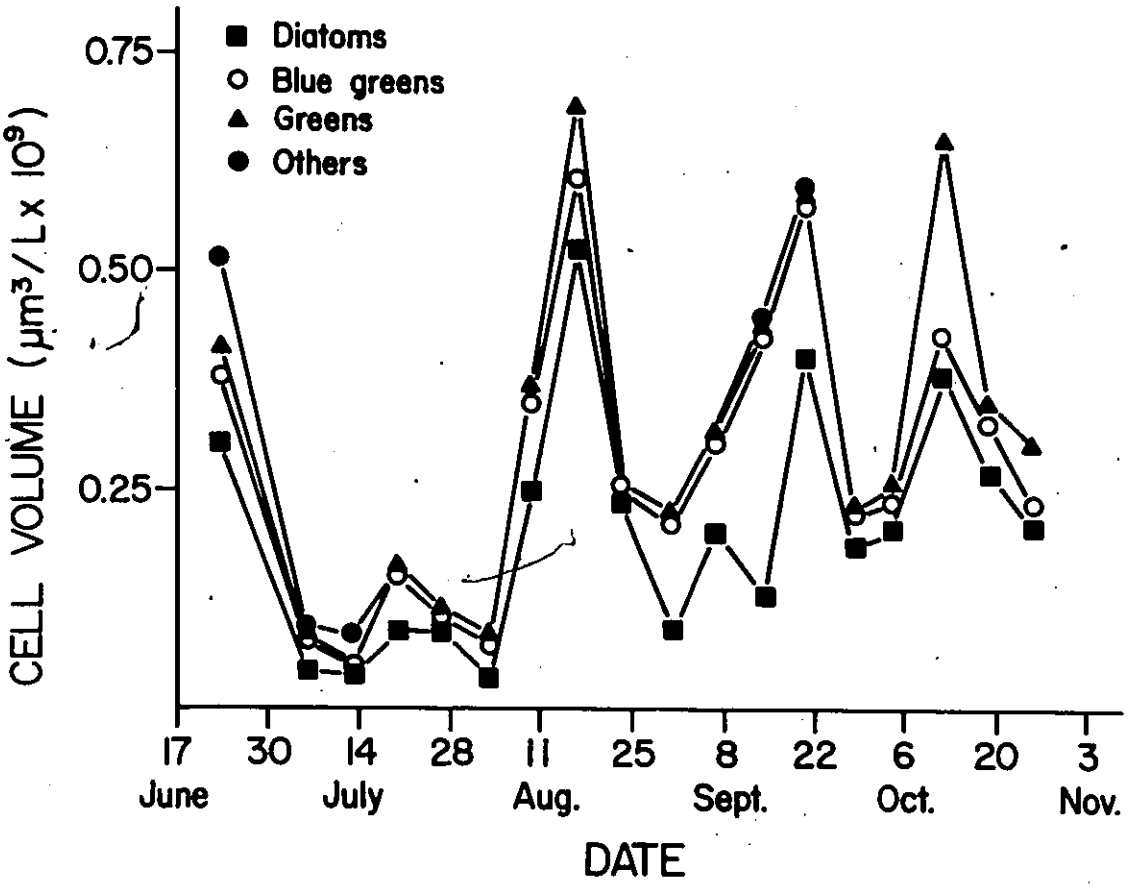

Diatoms
Blue greens
Greens
Others
0.75
0.50
0.25
CELL VOLUME (µm³/L x 10⁹)
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 19 Weekly fluctuations of algal volume in an enriched enclosure with reduced zooplankton levels.

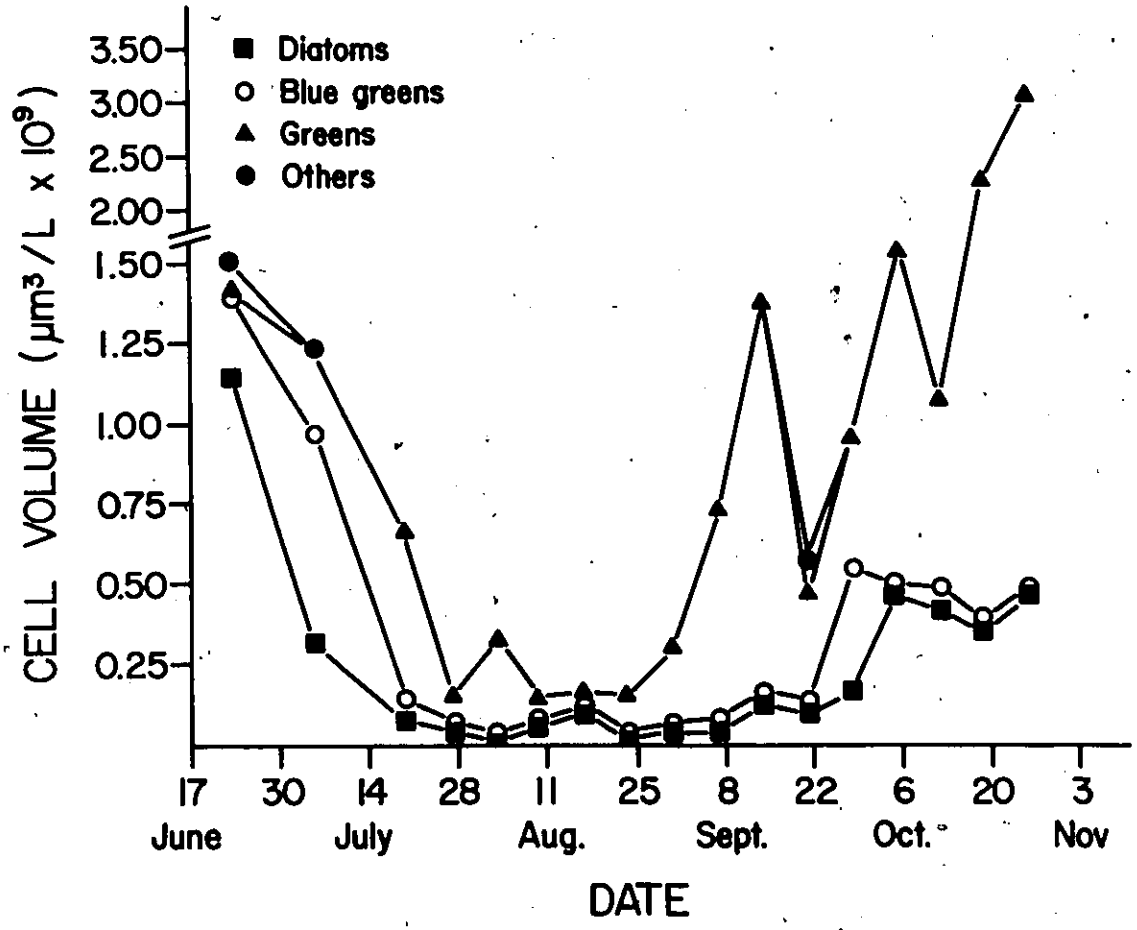

Diatoms
Blue greens
Greens
Others
CELL VOLUME (µm³/L x 10⁹)
3.50
3.00
2.50
2.00
1.50
1.25
1.00
0.75
0.50
0.25
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov
DATE

FIGURE 20 Weekly fluçtuations of algal volume in an unenriched enclosure with reduced zooplankton levels.

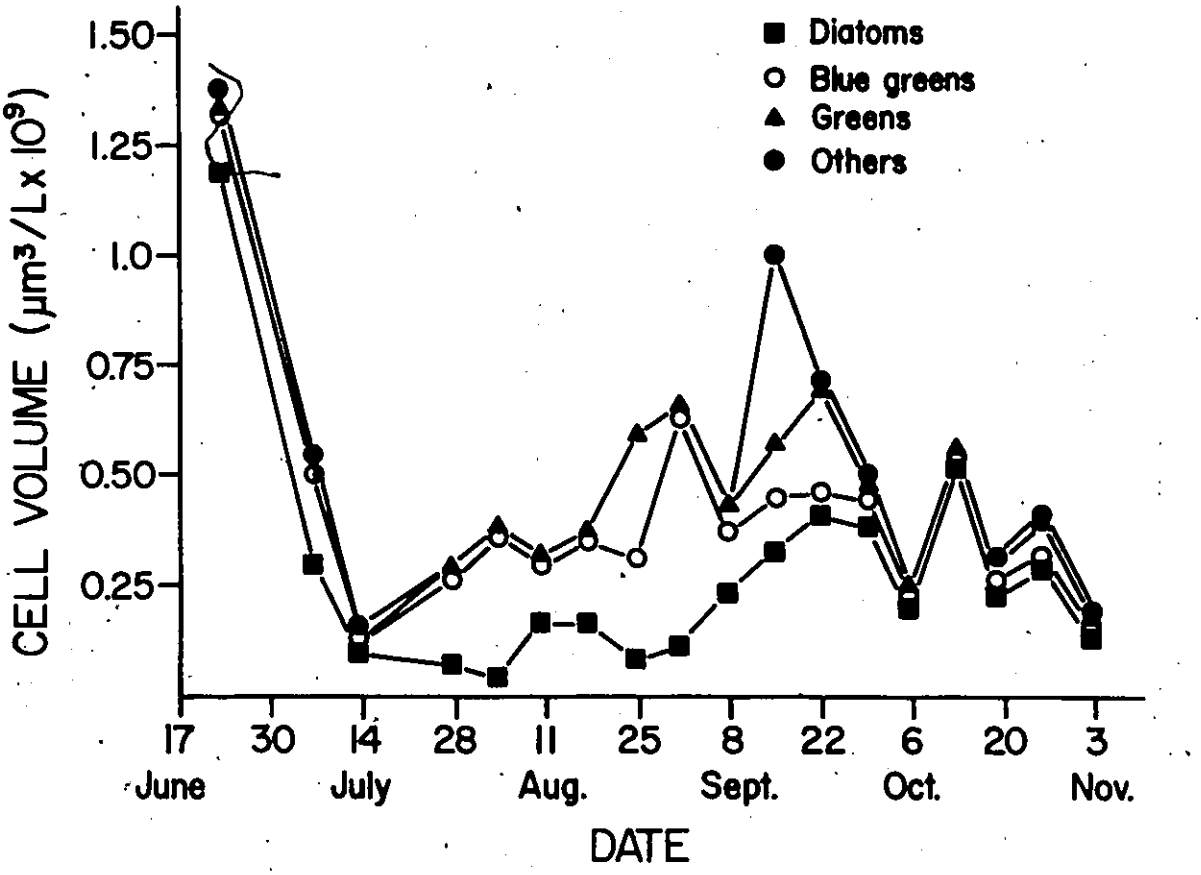

Diatoms
Blue greens
Greens
Others
CELL VOLUME (μm³/L x 10⁹)
1.50
1.25
1.0
0.75
0.50
0.25
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

FIGURE 21 Weekly fluctuations in algal volume in an enriched enclosure in contact with the sediment.

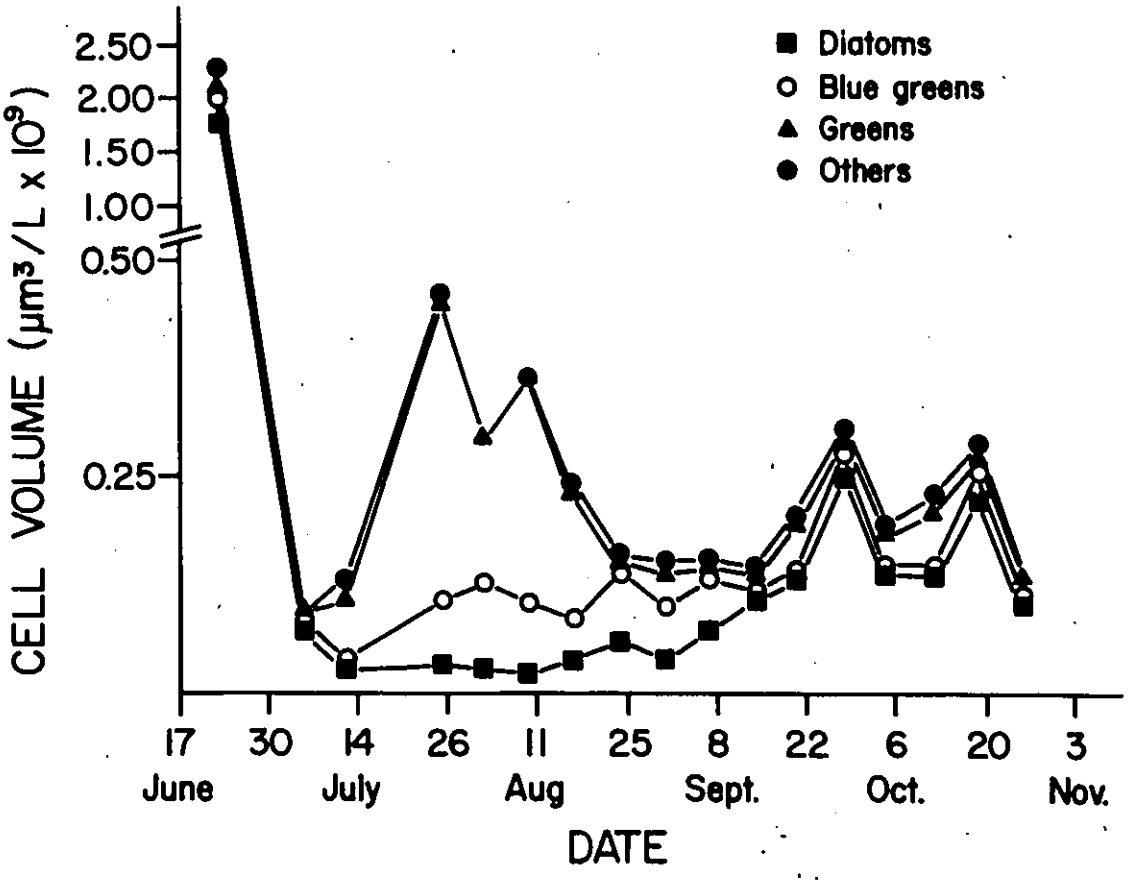
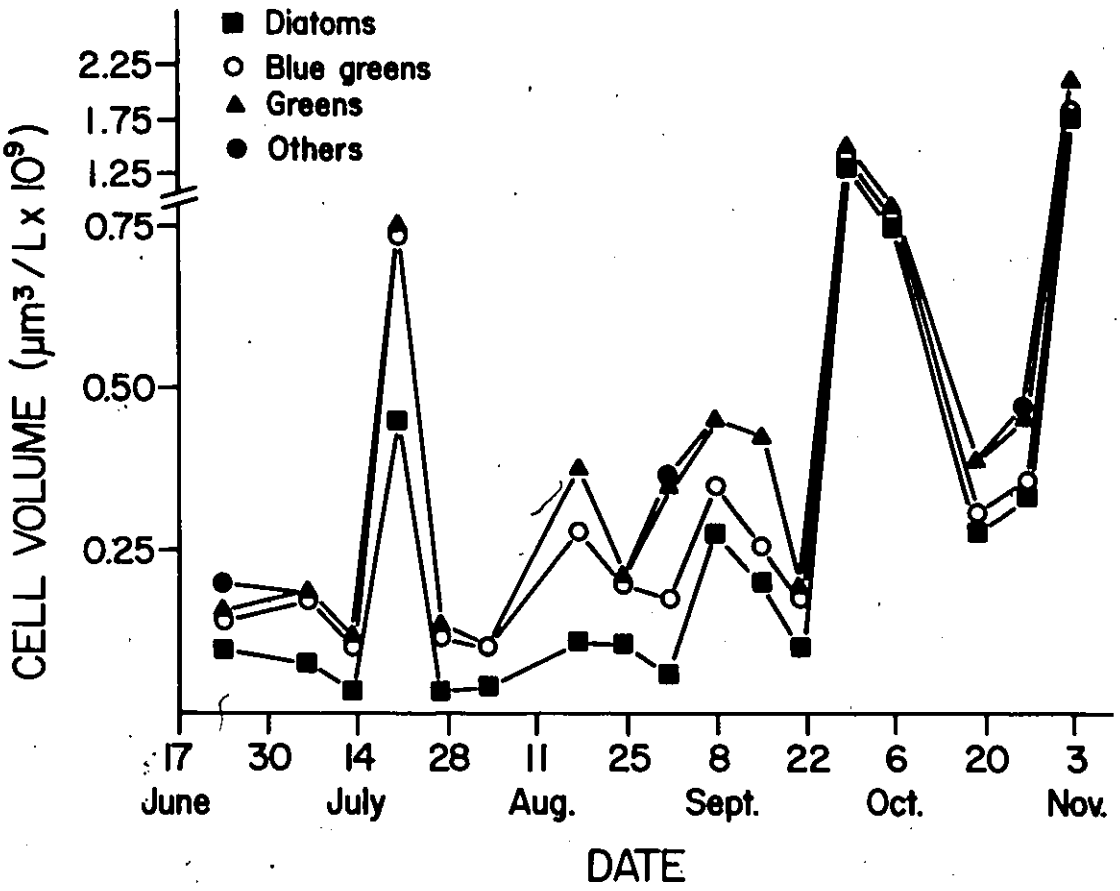

2.50
2.00
1.50
1.00
0.50
0.25
CELL VOLUME (μm³/L x 10⁹)
Diatoms
Blue greens
Greens
Others
17
June
30
14
July
26
11
Aug
25
8
Sept.
22
6
Oct.
20
3
Nov.
DATE

FIGURE 22 Weekly fluctuations in algal volume in an unenriched enclosure in contact with the sediment.


Diatoms
Blue greens
Greens
Others
2.25
1.75
1.25
0.75
0.50
0.25
CELL VOLUME (µm³/L x 10⁹)
17
30
14
28
11
25
8
22
6
20
3
June
July
Aug.
Sept.
Oct.
Nov.
DATE

species richness the enriched enclosures were markedly different (Figs. 5 and 7) when compared with either the lake or the unenriched enclosures. Clearly the green algae dominated under the enriched conditions.

Heavy Metal Binding

Fluctuations in the heavy metal binding capacity of the water samples (Table 2) were not related to the variations of total algal biomass (see, for example, Fig. 23) or of the physico-chemical parameters. For example, variations in pH (Table 3) could not account for the changes in heavy metal binding capacity observed. Since the amount of inorganic phosphate was never higher than 1 μM/L in any water sample (Table 4), a level too low to account for the heavy metal binding measured, phosphate can be eliminated as an important component of the metal binding capacity of the water samples. Despite changes in redox potential of the water samples, there was no correlation between these parameters and binding capacity (Table 5). Other parameters, such as chloride ion (Table 6), and ionic strength (Table 7) did not appear to be related with heavy metal binding capacity. Oxygen and temperature measurements indicate that the enclosures did not modify these parameters when compared to the lake (Table 8).

Multiple regression analysis was used to evaluate the interrelationship between heavy metal binding capacity and algal species composition. This technique analyzes the relationship between a dependent variable and a set of independent variables. Via a standard regression method, each variable is added in turn and its particular contribution weighted. Multiple

TABLE 2

Fluctuations of Metal Binding (uM/L) Capacity of the Water Samples in the Enclosures and the Lake

Date (1976)		6-7				13-7				20-7				27-7				3-8				10-8				17-8				24-8				31-8			
	Metal	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd
Sample Depth																																					
Lk	0	26	104		16	56	128	106	6	118	63	76	16	136	51	102	4	136	76	102		113	96		6	71	86	6	158	51	164	12	130	26	138	57
	3	120	161	16	16	86	179	146	6	110	81	66	36	106	71	66	4	129	71	71	4	128	66		4	71	86	6	120	51	66	16	96	31	132	86
	6	130	167	56	16	75	76	106	6	136	76	76	6	111	67			145	71	86	4	144	66			91	66	6	136	56	130	12	111	31	126	57
	9	126	170		6	104	184	76	6	118	76	26	6	118	81	66	26	131	66	61	4	128	66		6	76	76	22	132	46	116	35	118	26	122	35
UN	0	135	74	26	26	36	186	66	36	128	86	86	36	137	81	116	6	111	66	26	6	118	86		4	66	131	35	158	46	167	6	136	46	116	57
	3	46	56		6	129	128	106	16	110	86	96	6	126	71	131	4	130	66	45	6	118	86		4	66	86	35	150	51	161	6	96	36	120	
	6	104	136	46	26	126	128	146	36	128	61	86	6	130	67	116		124	66	57	6	116	26		2	66	96	45	141	76	151	6	116	46	116	57
	9	95	175	11	26	118	180	110	6	128	86	106		134	71	118	4	128	76		4	148	66			66	76	25	130	56	142	6	124	31	126	45
ENR	0	126	178	11	16	180	95	110		118	116	81		189	81	194	4	186	76	57	4	194	128		15	66		192	196	66		192	196	56		194	
	3	140	159		16	138	95	96	6					125	81	194	6	118	56	57	106	196	125		15	51		190	196	66		192	196	56			
	6	46	76	6	16	116	95	96	6									141	61	57	101	189	86		15	56		186	196	51		120	196	56		45	
	9	81	114	56	16	111	175	76	6									137	76		56	149	61		15	76		167	195	81	151	25	171	31	126	194	
GF	0	118	178	140	46	112	173	111	16					104	56	76		196	81	71	56	136	46		26	71	36	29	144	46	136	6	126	41	138	86	
	3	114	179	56	46	124	179	98	36					96	81	67		128	46	31	4	126	56		6	56	36	16	150	66	126		136	46	138	35	
	6	128	186	148	46	95	143							110	56	98	6	130	66	16	6	104	66		36	67		25	148	46	149		136	6	122	66	
	9	131	166	76		91	76	146	6					124	61	61	6	126	81			131	67		6	76	66		148	46	150	12	124	6	111	67	
GFR	0	104	100	56	6	111	177	26	16	126	86	81	6	182	76	61	26	106	66	16	46	186	46		6	76		190	196	41		163	129	66		171	
	3	81	182	86	46	116	100	66	36	135	81	16	36	194	81	61	96	86	66	16	75	182	46		6	66		190	196	46	144	194	126	16		182	
	6	116	175	36	46	112	180	56	6	136	96	61	6	188	81	61	96	128	86	16	75	196	51		6	67		186	196	56		161	169	36		176	
	9	112	91	26		122	128	66	36	128	86	86	6	184	76	61	96	144	81	16	4	185	41		6	76		180	196	36	130	61	151	36		171	
SD	0	124	124	26	6	61	100	66	6	144	96	90	116	193	76	86	6	114	86	61	4	96	76		6	76		125	180	26		127	149	16	86	141	
	3	116	110		6	156	86	106	6	193	96	35	107	154	76	45		165	96	61	4	164	86		6	71		145	196	76		143		46	96	153	
	6	86	124	36	6	114	180	146	6	110	104	35	107	152	81	116	6	144	86	61	4	138	81		96	56	76	115	196	76		76	111	46		135	
	9	148	105		46	130	169	106	6					122	66	57	6	116	86	61	6	131	66			70	76	16	144	61	136	12	134	31	110	135	
SDR	0	61	159	16	6	151	95			128	96	81	48	179	86	57	6	126	81	61	6	159	81		146				193	61		171	136	61	146	125	
	3	71	106	6	6	166	51	111		156	102	46	36	199	66	57	6	159	81	61	6	144	81		146	66		122	173	51		165	149	36	132	25	
	6	136	183		46	96	176	98	36	162	86	111	76	96	76	57	6	120	86	61	6	148	86		137	81		125	125	56		155	118	36	139	122	
	9	140	130	26	46	100	171	66	16	145	86	86	36	159	76	45	6	145	61	61	8	96	66		4	71	46	6	26	46	146	6	130	36	130	86	

LK Lake
UN Unenriched enclosure
ENR Enriched enclosure
GF Unenriched enclosure with reduced zooplankton levels
GFR Enriched enclosure with reduced zooplankton levels
SD Unenriched enclosure in contact with the sediment
SDR Enriched enclosure in contact with the sediment

TABLE 2 (cont.)

Fluctuations of Metal Binding (uM/L) Capacity of the Water Samples in the Enclosures and the Lake cont.

Date (1976)		7-9				14-9				21-9				28-9				5-10				12-10				19-10				26-10				2-11			
	Metal	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd	Cu	Hg	Pb	Cd
Sample Depth																																					
LK	0	96	116	136	46	36	142	149		191	61	142	66	66	100	106		138	106	151	54	121	102	36	63	175	158	114	27	156	56	135	45	146	185	116	27
	3		86	104	46	136	96	196	6	96	209	132	66	46	96	106		153	146	144	36	106	56	91	36	165	189	146	36	156	61	135	63	136	185	114	27
	6	106	91	122	46	140	140	98		96	100	136	76	26	100	86		106	86	142	45	96	56	76	36	162		160	45	162	177	135	63	152	185	114	27
	9	76	81	66	36	14	140	108	6	118	56	126	76	149	86	101		153	81	132	18	136	56	86	71	165	184	134	63	169	185	131	54	173	191	112	36
UN	0	146	96	111	66	104	112	106		96	100	36	6	144	76	108		96	81	138	66	136	61	112	80	167	186	138	63	167	187	102	54	155	185	140	27
	3	140	86	190	66	140	96	76	1	122	106	26		114	56	71		154	86	124	46	136	96	106	63	173	185	159	45	121	189	114	63	164	186	110	27
	6	128	76	111	56	138	194	66		138	131	26		120	76	96		136	121	120	56	136	86	96	45	171	185	156	54	165	184	108	54				
	9	136	76	106	76	124	96	122	1	106	100	26		114	66	96	6	134	91	128	36	146	51	106	45	165	56	152	45	166	183	108	63				
ENR	0	136	96		170	196	96		66	196	116			126	142	140	26	56	100	130	27	156	[illegible]	108		167	188	122	54	162	56	76	98	173	177	151	54
	3	180	96			196	112	106	16	196	102			106	66	86	56	162	98	124	36	149	180	93	27	181	188	116	45	169	56	91	98	169	188	114	54
	6	174	86			196	101		6	196	98			76	96	86	36	101	96	142	71	121	36	91	54	173	184	132	63	159	71	95	89	170	189	126	36
	9	159	96			176	82	140	16	196	112			108	56	86	35	41	86	132	63	152	186	121	54	150	183	108	63	159	66	104	89	169	184	120	36
GF	0	118	86	116	66	66	99	131	1	96	81	128	46	106	76	91		156	100	138	66	126	56	56	63	158	185	118	36	169	176	130	63	173	61	76	27
	3	124	7	111	66	140	96	98		111	86	96	25	96	76	101		100	114	120	45	142	66	106	36	158	185	128	36	158	169	106	63	171	185	86	45
	6	111	86	86	66	6	96	121		111	121	126	66	51	86	191		140	86	142	36	152	56	116	45	165	185	121	54	146	186	116	71	174	179	86	18
	9	138	86	96	86	118	91	98		106	100	116	46	66	76	91		126	112	140	54	140	56	112	27	153	178	118	63	159	182	116	54	169	184	120	36
GFR	0	173	86		142	96	112	98		114	86		86	126	76		56	154	96	91	63	146	56		36	176	188	136	89	156	186	36	148	173	61	76	27
	3	176	86		151					136	66	96	96	132	76	116	56	146	106	96	18	106	96	106	63	190	186	114	54	171	182	106	156	171	185	86	45
	6	166	96		166					129	66	116	76	146	66	106	36	184	120		36	126	46	120	36	180	[illegible]	124	54	171	188	76	134	174	179	86	18
	9	154	86		152					158	86	106	76	124	100	161	16	175	86	126	54	142	56	132	54	182	186	134	63	169	185	46	132	169	184	109	27
SD	0	96	86	146	66	186	132	86		171	168	153	86	134	71	98	46	144	96	148	63	149	184	94	63	146	96	106	36	167	182	144	27	167	185	96	27
	3	146	98		114	177	112	122	26	151	66	116	76	96	91	91	46	56	86	156	54									159	181	140	27	164	186	88	27
	6	132	96		121	161	106	96	6	130	106	136	66	136	86	106	6	56	106	156	63									171	186	134	63	179	184	96	27
	9	130	102	86	76	136	112	96		130	114	122	41	116	162	108	6	149	114	153	45									156	177	128	54	165	185	86	9
SDR	0	130	96	56	121	196	106	102	26	189	66		156	156	86		66	191	100	106	124	161	168		124	185	186	76	71	162	182	91	131				
	3	151	86		132	186	106	6	36	174	56		96	140	76	56	66	138	106	91	63	106	61		54	175	186	120	36	178	169	66	136				
	6	124	96		116	118	106	147		184	76		96	76	96	106	56	132	96	91	27	149	183	114	45	184	184	110	45	149	177	61	176				
	9	144	76	67	96	118	183	159	26	130	96		76	96	66	36	56	136	102		27	146	96	91	54	182	188	116	27	159	183	91	18				

FIGURE 23 Weekly fluctuations of total algal volume and binding capacity of Cu^{2+} and Hg^{2+} in surface samples from an enriched enclosure in contact with the sediment.

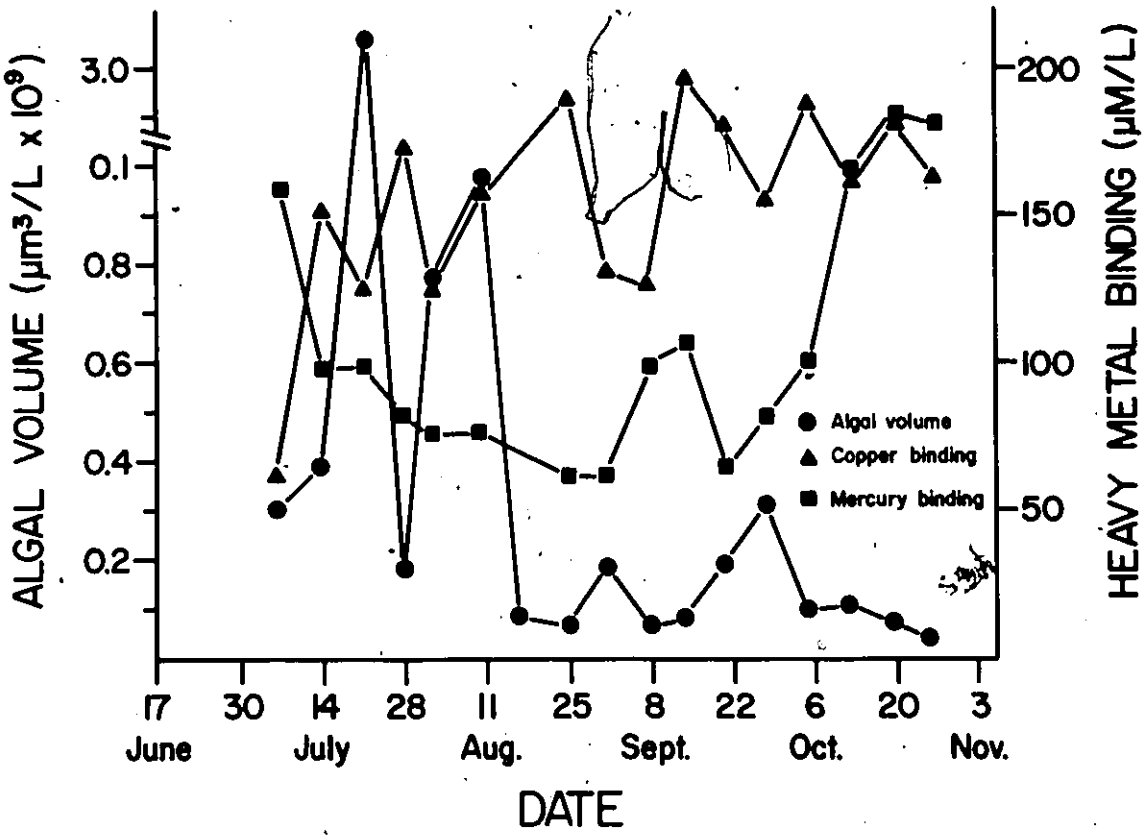

ALGAL VOLUME (μm³/L x 10⁹)
3.0
0.1
0.8
0.6
0.4
0.2
200
150
100
50
HEAVY METAL BINDING (μM/L)
Algal volume
Copper binding
Mercury binding
17 30 14 28 11 25 8 22 6 20 3
June July Aug. Sept. Oct. Nov.
DATE

TABLE 3
pH

DATE (1976)		28-6	6-7	13-7	20-7	27-7	3-8	10-8	17-8	24-8	31-8	7-9	14-9	21-9	28-9	5-10	12-10	19-10	26-10	2-11
Sample	Depth																			
LK	0	7.37		7.56	8.68	7.24	7.94	8.03	8.33	8.27	7.86	7.89	8.62	7.88	7.99	7.79	7.41	7.62	7.55	7.79
	3	7.36	7.63	7.36	8.57	7.59	8.04	8.35	8.19	8.33	7.51	7.77	8.55	7.98	7.89	7.77	7.60	7.61	76.7	7.63
	6	7.27	8.02	7.58	8.55	8.15	8.02	7.97	7.73	8.13	7.61	7.93	8.65	7.92	7.51	7.68	7.56	7.52	7.53	7.75
	9	7.08	7.33	7.60	8.51	7.79	7.99	7.67	7.46	8.08	7.95	8.09	8.51	7.94	7.93	7.46	7.32	7.51	7.63	7.73
UN	0	7.74	7.55	7.56	8.61	8.08	8.21	8.74	7.76	8.81	7.98	7.61	8.65	8.20	8.16	8.02	7.55	7.77	7.56	7.96
	3	7.74	7.70	7.51	8.58	7.73	8.30	8.76	7.99	8.84	7.71	7.88	8.62	8.26	7.95	7.89	7.78	7.24	7.64	7.78
	6	7.45	7.93	7.61	8.51	8.12	7.50	8.81	7.97	8.38	7.79	7.63	8.02		8.13	7.69	7.28	7.86	7.35	
	9	7.56	7.83	7.28	8.00	7.76	8.17	8.53	8.44	7.83	7.58	8.01	8.46	7.74	8.12	7.49	7.27	7.70	7.55	
ENR	0	7.65	7.43	7.95	7.67	10.02	10.04	10.20	10.11	10.51	10.38	9.70	10.39	10.63	9.24	9.46	9.14	9.25	8.78	9.15
	3	7.43	8.10	7.92		7.61	9.79	10.41	10.37	10.58	10.15	9.77	10.32	10.64	9.13	9.38	9.22	9.25	8.77	9.20
	6	7.53	8.49	7.94			9.80	10.34	9.72	9.93	9.92	9.38	10.39	10.54	9.44	8.09	8.75	9.08	8.88	9.15
	9	7.61	7.57	7.17			9.70	10.17	9.23	8.86	7.63	8.68	9.84	10.38	9.20	8.82	8.09	8.74	8.94	9.27
GF	0	7.66	7.53	7.34		7.50	7.43	8.54	8.03	8.40	7.80	8.19	8.66	7.61	7.73	7.83	7.35	7.46	7.80	7.99
	3	7.75	7.61	7.51		7.33	8.14	8.51	7.54	8.36	8.14	7.82	8.32	8.19	8.05	7.54	7.28	7.08	7.69	7.87
	6	7.58	7.95			7.58	8.05	8.34	7.94	8.36	7.54	7.46	8.61	7.75	8.05	7.68	7.51	7.61	7.75	7.68
	9	7.55	7.87	7.17		7.45	7.85	7.79	7.12	8.34	7.12	7.73	8.49	7.74	7.65	7.80	7.27	7.65	7.67	7.78
GFR	0	7.93	7.70	7.32	9.13	9.11	9.61	10.08	9.69	10.24	9.39	9.32	10.20	9.17	9.17	9.53	8.06	9.49	9.53	
	3	8.20	7.26	8.15	9.27	9.60	9.65	10.24	10.14	8.80	9.16	9.49		9.12	9.28	9.13	7.89	9.52	9.48	
	6	7.82	6.87		9.06	9.51	9.51	10.21	9.45	9.95	9.36	9.42		8.58	9.35	9.17	8.02	9.08	9.37	
	9	7.52	7.45	7.74	9.03	9.18	9.05	10.02	9.35	8.79	9.58	9.28		8.80	9.20	8.80	7.09	9.02	9.24	
SD	0	7.10	7.87	9.01	10.06	7.96	10.16	10.02	7.88	9.91	8.81	7.98	9.87	9.00	9.07	8.90	7.18	7.28	8.31	8.16
	3	7.87	7.64	7.57	10.09	7.68	9.85	9.94	7.94	9.57	9.24	8.00	9.96	8.89	9.11	8.34			8.27	7.71
	6	7.72	7.25	6.96	10.09	8.41	9.73	9.57	9.14	9.80	9.00	8.74	9.57	8.16	8.80	8.06			8.26	7.84
	9	7.55	7.65	7.23	9.70	7.26	8.19	7.80	7.35	8.18	9.46	8.28	8.43	8.33	8.44	8.15			7.84	8.00
SDR	0	7.45	7.73	8.86	9.16	9.59	9.72	9.88		9.77	8.91	8.60	10.28	10.07	9.44	10.00	9.49	9.45	9.68	
	3	7.70	8.34	9.03	9.37	9.55	8.57	9.89	9.01	10.05	8.01	9.13	10.32	9.69	9.43	9.58	9.15	7.44	9.54	
	6	7.59	8.71	7.44	9.57	8.85	9.37	9.60	8.02	9.93	7.82	8.66	9.68	9.52	9.12	9.57	9.28	9.38	9.54	
	9	7.48	7.97	7.28	9.32	7.89	7.99	7.67	8.03	8.17	7.64	8.36	9.31	9.04	9.40	9.80	9.40	7.38	9.21	

TABLE 4
Phosphate (ppb)

DATE (1976)		28-6	6-7	13-7	20-7	27-7	3-8	10-8	17-8	24-8	31-8	7-9	14-9	21-9	28-9	5-10	12-10	19-10	26-10	2-11
Sample	Depth																			
LK	0	.463						.7	.63	.15					.32		2.13	.315	.63	.63
	3	.463						1.56	.925						.32	.158		1.16		.63
	6	.463						.98	.63				.15	.32	.32		1.41	.472	1.26	.315
	9	.63						.32	1.26		.32		.32		.32		.315	.315	.63	
UN	0	.3					.17	.32		.63										
	3	.3					.93	.32										.63		
	6	.63					.56	.32							.63			.315		
	9	.3					.63	.32						.95		.315	.158	.315		
ENR	0							.32			1.56	.95	.63			.315		.945	.315	.315
	3	.13						.63	.63	.31	.95	1.26	1.26			.945	.315	.63	.945	.63
	6	.3	.3					1.26	.925	.63	.63	1.89	.93	.06	1.26	.63		1.11	.63	.63
	9	.37					.17	.63	1.3	.315	1.26	1.89	1.89			.945		1.11		
GF	0	.37					.32	.63	.32			.32					2.8	.315		
	3	.42	.32				.46	.17	.51			.32						.472	1.26	.315
	6	.37						1.85	.63		.63	.32	.63		.32		2.13	.788	.945	.315
	9	.63	.32				.17	1.24	.98		.32	.63	.63				2.13		.158	.63
GFR	0						.46	.925	.63		.32	.63	.32			.315	.315			
	3	.63	.32					.32	.63	.63	.32	.32				.63	.315			
	6							.63	.63	1.26	.63	.95		.32	.63	.315	.315		.315	
	9		.33				.63	.63	.98	1.26	.463	.32					.63			
SD	0	.3	.17				.32										.788			
	3						.17	.63		.26	.15								.472	
	6							.32		.26					.63					
	9							.63			.15					.63				
SDR	0						.17	.17									.63	.315		
	3						.17	.17	.32		.32								.63	
	6									.15	.32		.15				.965	.63		
	9		.63						.17	.15							.315			

TABLE 5
Redox (MV)

DATE (1976)		28-6	6-7	13-7	20-7	27-7	3-8	10-8	17-8	24-8	31-8	7-9	14-9	21-9	28-9	5-10	12-10	19-10	26-10	2-11
Sample	Depth																			
LK	0			523.3	468.5	459.3	500.4	317.9	305.2	393.1	381.2	383.7	336.3	400.4	390.1	455.9	487.3	351.8	501.9	488.5
	3		421.1	517.8	412.1	456.1	500.2	318.1	305.4	394.6	385.9	383.1	347.4	395.8	388.6	443.0	467.4	352.3	500.7	484.8
	6		419.2	511.1	419.2	453.6	498.2	319.6	305.9	394.7	476.3	383.2	350.4	392.5	392.3	439.0	468.4	354.7	503.7	493.0
	9		430.4	517.1	420.0	454.4	499.8	321.7	306.0	393.9	384.0	384.0	354.1	389.2	396.5	437.1	470.6	354.5	505.6	495.1
UN	0		515.0	500.3	435.9	459.3	466.1	387.9	304.0	381.5	379.3	389.2	357.0	355.4	395.0	392.8	483.0	347.0	503.6	539.6
	3		507.9	496.1	428.1	463.4	467.6	391.3	303.6	382.7	387.5	398.0	357.3	356.2	394.7	387.7	477.5	355.8	493.9	532.1
	6		488.5	499.6	427.2	464.0	469.4	393.1	303.9	386.7	384.0	394.3	362.0		395.6	392.3	478.0	353.3	498.3	
	9		498.6	494.1	433.1	465.5	468.9	394.0	301.0	388.5	383.0	391.0	362.3	360.7	392.1	397.3	477.1	352.9	504.8	
ENR	0		514.3	497.5	4475.0	433.9	449.3	382.6	288.8	365.6	349.9	374.9	303.5	336.1	399.1	401.0	471.6	350.9	486.1	489.4
	3		483.0	495.5		457.4	445.0	371.1	286.9	361.4	352.5	372.9	305.5	332.3	394.2	393.0	489.4	336.8	483.2	481.2
	6		460.0	494.3			453.3	370.1	289.2	367.6	352.5	373.7	305.4	329.4	386.7	402.3	481.6	332.7	477.6	484.9
	9		516.1	503.8			454.6	368.6	291.6	379.6	372.5	379.5	317.3	329.0	385.7	394.9	490.8	332.0	488.3	479.9
GF	0		433.4	505.0		456.2	464.1	312.0	359.0	393.6	475.5	378.7	360.9	384.4	395.6	328.1	479.1	369.3	508.6	523.1
	3		423.4	501.2		458.0	489.9	313.3	335.1	394.0	472.5	380.0	366.3	379.8	395.1	322.2	477.3	385.2	516.3	521.1
	6		424.1			457.1	493.2	315.8	328.7	394.2	471.2	388.5	365.3	383.7	396.3	321.2	475.0	387.9	512.2	519.3
	9		422.2	507.3		459.6	496.8	317.6	322.9	394.0	480.4	386.0	374.0	385.9	418.7	318.3	474.5	387.0	506.4	512.3
GFR	0		511.4	500.0	473.1	455.3	463.4	300.2	308.0	370.3	462.8	372.0	352.3	342.6	382.9	377.4	507.5	342.0	470.5	
	3		531.1	492.1	469.9	450.6	459.0	298.0	301.4	384.2	448.8	370.0		342.3	381.0	380.3	509.7	337.6	453.6	
	6		551:2		474.6	447.5	461.3	297.8	304.1	373.6	444.2	367.7		359.1	381.7	367.0	503.7	337.3	452.5	
	9		514.0	491.5	478.3	448.4	464.5	300.3	300.5	384.3	452.3	367.7		356.5	376.6	374.8	503.4	336.1	453.8	
SD	0		415.3	479.1	385.1	458.7	439.1	381.4	300.1	374.7	443.3	378.3	361.2	364.4	376.5	372.7	466.3	345.5	465.3	491.4
	3		414.8	490.1	384.6	459.3	434.1	376.4	302.0	380.3	433.2	384.2	358.0	360.8	374.3	380.5			464.1	494.8
	6		421.1	497.7	381.2	453.0	440.2	379.6	294.9	375.3	437.7	376.2	363.8	373.5	379.7	385.9			463.1	493.7
	9		419.1	493.5	397.2	460.4	451.5	391.0	303.5	389.1	431.4	379.1	375.2	364.6	381.3	388.2			468.2	494.4
SDR	0		506.6	491.1	475.7	445.9	285.2	306.1		385.4	435.4	385.9	341.0	341.0	399.8	377.8	439.6	335.6	488.8	
	3		462.2	482.1	460.1	448.7	298.0	307.2	388.4	379.0	377.0	381.5	341.6	342.1	392.2	374.5	449.0	351.5	486.8	
	6		433.4	505.0	459.6	452.6	284.6	308.5	396.2	377.0	375.5	383.1	354.7	341.6	389.9	374.8	448.2	338.5	481.3	
	9		440.7	506.2	464.6	459.9	297.0	318.3	392.5	390.0	378.5	381.3	356.6	348.0	374.0	374.6	444.0	352.1	482.9	

TABLE 6
Chloride (M) x 10^{-5}

DATE (1976)		28-6	6-7	13-7	20-7	27-7	3-8	10-8	17-8	24-8	31-8	7-9	14-9	21-9	28-9	5-10	12-10	19-10	26-10	2-11
Sample	Depth																			
LK	0	2.6		6.0	6.5	11.0	10.0	5.9	3.3	4.4	3.3	2.8	8.2	13.0	8.5	1.7	14.6	6.0	10.0	15.0
	3	2.4	5.5	7.0	7.5	10.0	9.5	4.7	2.9	5.0	6.4	2.5	5.6	10.5	9.0	1.9	12.0	7.0	10.0	17.0
	6	2.9	4.0	7.4	6.5	15.8	20.0	5.2	3.7	4.8	6.4	2.5	6.6	9.5	9.4	2.2	9.5	7.0	9.5	22.0
	9	4.0	5.0	6.5	7.0	5.0	10.0	5.4	3.7	4.5	8.8	3.0	7.2	10.5	10.5	3.0	9.0	7.5	10.5	22.0
UN	0	6.6	7.5	11.0	3.5	5.0	5.0	4.5	6.2	6.9	1.6	3.0	6.6	8.5	8.5	4.0	11.0	5.0	10.5	13.0
	3	4.2	7.4	7.5	4.0	9.0	10.0	3.7	3.9	6.2	1.7	2.7	6.2	9.0	8.5	4.8	8.9	5.0	11.0	11.0
	6	5.7	7.4	8.5	4.2	9.0	11.5	3.7	3.4	6.0	1.8	3.1	6.2		14.0	3.8	7.4	5.0	11.0	
	9	6.7	7.5	7.5	4.2	9.5	11.5	3.9	5.0	6.8	1.8	3.4	6.2	9.0	9.4	3.5	6.2	5.0	10.5	
ENR	0	17.0	9.0	11.5	10.5	5.0	17.0	5.0	6.2	6.9	2.8	4.0	14.0	13.5	6.0	6.6	10.0	7.0	11.0	17.0
	3	6.7	7.4	9.5		7.5	19.0	5.8	5.9	8.5	2.3	3.7	11.0	12.5	7.5	7.8	10.0	4.8	12.0	14.0
	6	6.5	9.0	9.5			13.0	5.2	5.4	7.6	2.3	3.7	9.5	12.5	8.0	6.6	12.0	5.0	12.0	18.0
	9	4.9	8.0	8.5			13.0	5.0	4.8	6.8	1.8	3.7	9.5	13.5	8.6	7.0	10.0	5.0	35.0	16.0
GF	0	25.0	13.0	8.0		5.0	17.0	5.0	3.5	7.6	10.0	2.8	1.3	9.0	8.5	3.5	7.4	3.3	10.5	12.0
	3	25.0	6.5	7.2		8.5	9.0	5.8	2.7	5.8	13.0	2.3	6.2	9.4	7.0	3.7	5.2	3.1	11.0	16.0
	6	23.0	12.0			10.0	9.5	5.2	2.5	6.8	9.6	2.3	5.6	9.2	5.2	4.2	5.8	3.8	95.0	14.5
	9	22.0	8.0	8.7		10.0	5.0	5.0	2.2	4.7	8.6	2.3	5.0	8.5	6.2	3.8	6.0	4.5	10.5	14.0
GFR	0	22.0	10.0	7.5	9.5	9.5	17.0	7.2	3.3	6.8	19.0	2.6	7.5	8.5	7.0	7.0	9.0	8.5	12.0	
	3	25.0	8.0	12.0	11.0	5.0	13.0	8.0	3.7	6.0	8.6	2.3		7.0	6.6	6.2	7.0	8.2	12.0	
	6	32.0	8.0		10.0	12.0	14.0	7.2	2.9	7.8	9.6	3.1		13.0	7.0	11.0	10.0	9.2	12.0	
	9	29.0	9.5	8.5	18.0	12.0	12.5	6.5	3.0	8.6	7.0	2.6		11.0	7.4	7.2	10.5	9.2	13.0	
SD	0	16.0	7.5	10.0	5.0	17.0	14.0	4.8	3.2	6.0	8.0	4.6	6.2	10.0	9.0	7.0	14.0	4.5	11.0	21.0
	3	20.0	9.0	9.0	5.5	9.0	30.0	6.5	4.0	6.2	8.0	3.0	6.0	10.5	10.0	4.5			10.0	20.0
	6	19.5	9.5	6.5	7.4	9.0	13.0	5.0	5.0	6.0	7.8	2.7	5.8	11.0	9.0	3.8			10.5	21.0
	9	18.0	10.5	8.8	1.4	9.5	10.0	4.4	4.0	5.4	10.0	3.2	5.2	9.4	9.0	4.5			11.0	20.0
SDR	0	18.0	13.0	9.5	20.0	14.0	9.5	5.8		7.5	2.5	3.0	7.8	12.0	13.0	8.0	8.9	8.5	15.0	
	3	16.5	180.0	8.5	14.0	14.0	11.5	5.0	4.8	8.5	1.4	2.8	9.0	13.0	8.5	8.0	9.0	6.5	12.0	
	6	16.5	9.5	7.5	11.0	12.5	10.0	5.0	5.0	7.4	1.5	3.0	9.0	11.0	11.0	8.0	9.5	8.2	12.0	
	9	16.5	15.5	8.5	95.0	11.5	5.0	4.8	3.7	5.8	4.3	2.8	5.2	9.5	10.5	6.8	8.5	7.5	10.5	

TABLE 7
Ionic Strength (M) x 10^{-3}

DATE (1976)		28-6	6-7	13-7	20-7	27-7	3-8	10-8	17-8	24-8	31-8	7-9	14-9	21-9	28-9	5-10	12-10	19-10	26-10	2-11
Sample	Depth																			
LK	0	1.43		1.37	1.32	1.32	1.41	1.41	1.32	1.44	1.32	1.32	1.32	1.32	1.32	1.32	1.38	1.32	1.35	1.35
	3	1.43	1.45	1.38	1.26	1.32	1.40	1.39	1.30	1.38	1.32	1.32	1.26	1.20	1.32	1.32	1.37	1.32	1.32	1.32
	6	1.43	1.45	1.39	1.32	1.32	1.50	1.39	1.32	1.41	1.32	1.32	1.32	1.32	1.32	1.32	1.35	1.32	1.32	1.32
	9	1.43	1.50	1.39	1.20	1.34	1.44	1.41	1.32	1.44	1.32	1.32	1.32	1.296	1.32	1.32	1.38	1.32	1.38	1.32
Un	0	1.38	1.44	1.38	1.20	1.32	1.39	1.37	1.32	1.46	1.26	1.31	1.35	1.32	1.32	1.35	1.32	1.32	1.32	1.32
	3	1.39	1.49	1.39	1.32	1.32	1.42	1.37	1.32	1.46	1.29	1.32	1.35	1.26	1.32	1.35	1.32	1.32	1.32	1.32
	6	1.39	1.44	1.38	1.32	1.32	1.47	1.38	1.32	1.39	1.29	1.32	1.38		1.32	1.35	1.35	1.32	1.35	
	9	1.38	1.44	1.41	1.20	1.32	1.44	1.39	1.32	1.46	1.31	1.31	1.35	1.32	1.32	1.35	1.32	1.32	1.35	
ENR	0	1.38	1.39	1.32	1.38	1.70	1.62	1.68	1.54	2.16	1.90	1.32	1.20	1.824	1.296	1.08	1.20	1.26	1.22	1.26
	3	1.39	1.38	1.38		1.80	1.68	1.79	1.39	2.18	1.90	1.392	1.20	1.764	1.296	1.08	1.20	1.22	1.26	1.26
	6	1.38	1.44	1.39			1.68	1.79	1.44	2.11	1.86	1.38	1.14	1.8	1.296	1.176	1.20	1.26	1.26	1.32
	9	1.44	1.50	1.38			1.56	1.72	1.32	1.62	1.68	1.296	1.20	1.68	1.26	1.20	1.30	1.22	1.30	1.20
GF	0	1.38	1.50	1.38		1.20	1.49	1.35	1.32	1.38	1.224	1.32	1.35	1.32	1.32	1.35	1.35	1.32	1.20	1.32
	3	1.38	1.45	1.39		1.32	1.41	1.32	1.32	1.39	1.27	1.35	1.35	1.296	1.32	1.32	1.38	1.32	1.35	1.35
	6	1.42	1.42	1.43		1.33	1.42	1.37	1.32	1.38	1.32	1.32	1.344	1.32	1.32	1.32	1.38	1.32	1.32	1.32
	9	1.39	1.45	1.37		1.32	1.45	1.37	1.38	1.38	1.30	2.35	1.35	1.20	1.35	1.32	1.35	1.32	1.35	1.32
GFR	0	1.38	1.44	1.38	1.416	1.38	1.42	1.41	1.44	1.78	1.26	1.32	1.14	1.20	1.248	1.26	1.33	1.26	1.20	
	3	1.38	1.42	1.38	1.428	1.38	1.50	1.43	1.38	2.04	1.26	1.26		1.20	1.224	1.26	1.39	1.20	1.22	
	6	1.39	1.47	1.39	1.44	1.38	1.47	1.43	1.38	2.04	1.26	1.26		1.20	1.296	1.26	1.37	1.20	1.22	
	9	1.38	1.47	1.39	1.42	1.38	1.44	1.38	1.32	1.74	1.212	1.296		1.20	1.26	1.296	1.38	1.30	1.32	
SD	0	1.35	1.50	1.38	1.26	1.30	1.50	1.38	1.20	1.58	1.212	1.44	1.23	1.224	1.26	1.32	1.38	1.32	1.32	1.32
	3	1.00	1.45	1.39	1.38	1.30	1.66	1.39	1.20	1.54	1.212	1.32	1.23	1.224	1.26	1.32			1.32	1.35
	6	1.44	1.49	1.39	1.32	1.26	1.50	1.32	1.20	1.44	1.212	1.32	1.26	1.224	1.32	1.38			1.35	1.32
	9	1.43	1.44	1.37	1.32	1.32	1.40	1.32	1.30	1.35	1.212	1.35	1.32	1.26	1.32	1.32			1.32	1.32
SDR	0	1.38	1.38	1.35	1.416	1.38	1.44	1.35		1.584	1.20	1.20	1.14	1.20	1.176	1.20	:96	1.10	1.08	
	3	1.38	1.47	1.33	1.428	1.32	1.49	1.31	1.20	1.50	1.176	1.20	1.08	1.14	1.176	.96	.96	1.35	1.08	
	6	1.39	1.47	1.32	1.416	1.32	1.44	1.26	1.20	1.584	1.188	1.20	1.08	1.10	1.20	.90	.96	1.08	1.08	
	9	1.38	1.44	1.39	1.416	1.36	1.38	1.32	1.32	1.38	1.212	1.32	1.10	1.14	1.20	.96	.99	1.38	1.20	

TABLE 8 Examples of Oxygen (ppm) and Temperature (°C) Profiles Recorded from Inside Enclosures and from the Lake during 1976.

	Oxygen (ppm) July 13		Temperature (°C) July 13		Temperature (°C) August 2	
Depth (m)	Enclosure	Lake	Enclosure	Lake	Enclosure	Lake
subsurface	8.4	8.2	20.8	20.8	20.2	20.2
1	8.4	8.2	20.5	20.5	20.2	20.2
2	8.4	8.3	20.2	20.2	20.2	20.2
3	8.5	8.2	20.0	20.0	20.2	20.2
4	8.6	8.2	19.0	19.0	20.2	20.2
5	8.0	7.9	17.1	17.0	20.2	20.2
6	7.6	7.4	16.0	16.0	20.2	20.2
7	7.2	7.2	15.2	15.5	20.2	20.0
8	7.1	6.8	13.8	14.3	20.1	20.0
9	6.7	6.5	13.0	12.5	20.1	20.0

regression analysis on the data, based on 504 samples collected weekly at four different depths from the six enclosures and from the lake, revealed that fluctuations in heavy metal could be largely accounted for by a few phytoplankton species (Table 9), which were usually minor contributors to the total algal volume. For each individual metal ion, only one or two selected species were sufficient to account for more than 50% of the variations in the binding capacity of the water. As an example, the relative contribution of each algal species to the total algal volume is given for the enriched enclosure in contact with the sediment (Table 10).

The species showing a capacity to bind a particular metal belong mostly to two major taxonomic divisions, Chlorophyta (green algae) and Bacillariophyta (diatoms). Among the green algae, certain species, such as Scenedesmus and Coelastrum showed a high affinity for mercury binding. Shroederia was highly correlated with lead binding, whereas the desmids Euastrum and Cosmarium preferentially bound copper and cadmium, respectively.

Diatoms were also well represented, in particular by Cymbella and Rhizosolenia with respect to mercury and lead affinity. Other groups that showed a capacity for metal binding and usually occurred together with the diatoms were the cryptophytes, represented by Cryptomonas, and the chrysophytes by Dinobryon.

Of the blue-green algae, which are found in high numbers in mid summer, only Anabaena indicated a correlation with heavy metal binding, in this case copper and mercury. Graphic examples of the relation between the four metals studied and those particular algae are given in Figures 24 to 27.

TABLE 9 Positive Correlation Coefficients ($r \geq 0.30$) Between Heavy Metal Binding Activity and Phytoplankton Species Volume in 504 Samples from Enclosures and Lake. Statistically Significant Correlations Indicated as Follows: * for $P < 0.05$; ** for $P < 0.01$; *** for $P < 0.001$.

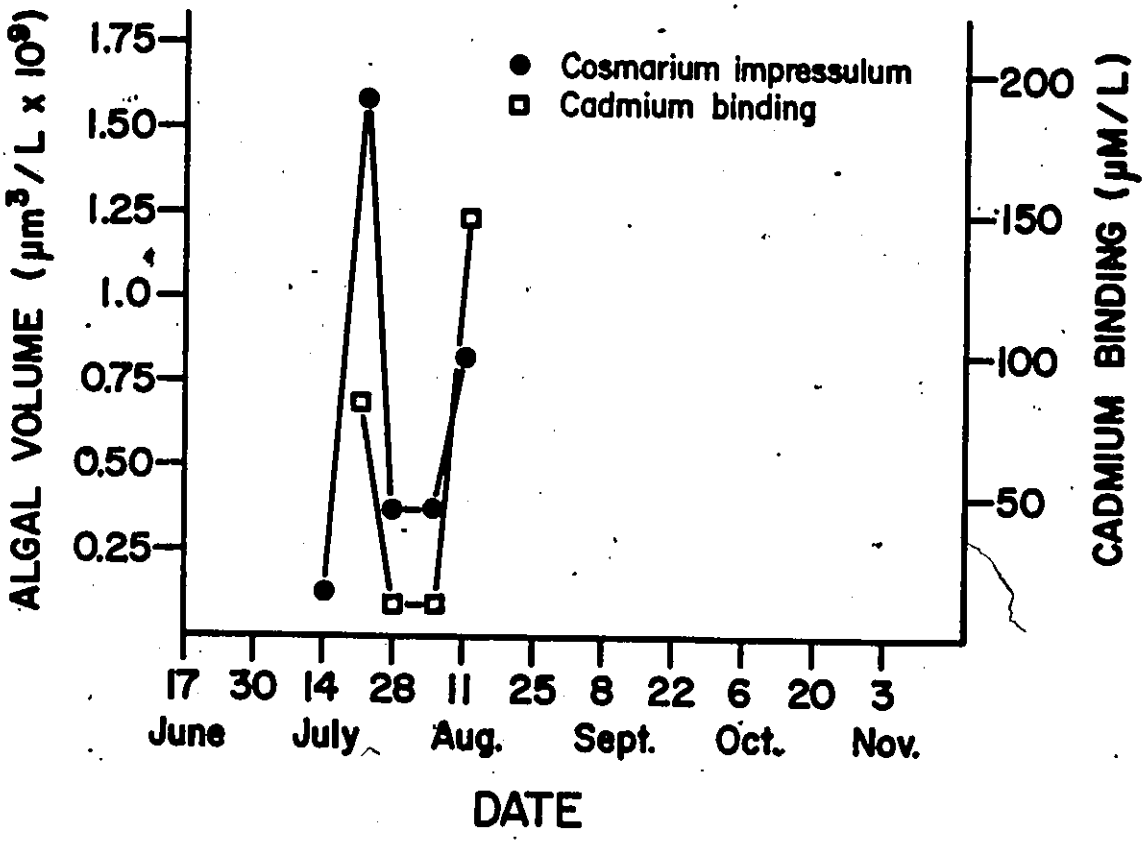
	Metal			
Algae	Cu	Hg	Pb	Cd
GREENS				
Ankistrodesmus falcatus			0.31	
Coelastrum microporum		0.44*		
Cosmarium impressulum				0.35*
Elakatothrix gelatinosa	0.54	0.51		
Euastrum insulare	0.47*			
Scenedesmus dimorphus			0.32	
S. obliquus		0.31***		
S. quadricauda		0.44**		
Schroederia setigera			0.91***	
Staurastrum paradoxum			0.30	
Tetraedron minimum		0.33		
BLUE-GREENS				
Anabaena spiroides	0.30*	0.52***		
DIATOMS				
Stephanodiscus niagarae		0.44		
Cyclotella meneghiniana		0.34		
Cymbella sp.		0.73***		
Melosira granulata			0.32	
Rhizosolenia eriensis			0.30**	
OTHERS				
Cryptomonas ovata		0.64*		
Dinobryon bavaricum	0.60	0.43		
D. divergens	0.53		0.46	0.52

TABLE 10 Percentage Contribution of Specific Algae to Total Algal Volume in Surface Sample from an Enriched Enclosure in Contact with the Sediment.

	July				August					September				October			
Algae	6	13	20	27	3	10	17	24	31	7	14	21	28	5	12	19	26
Ankistrodesmus falcatus	2.0	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coelastrum microporum	-	-	-	-	-	0.6	1.3	-	-	-	-	-	-	-	-	0.7	-
Cosmarium impressulum	-	40.8	62.7	-	37.4	70.4	-	-	-	-	-	-	-	-	-	-	-
Elakatothrix gelatinosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Euastrum insulare	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Scenedesmus dimorphus	-	0.2	-	-	-	-	-	-	0.2	-	11.8	-	-	-	-	-	-
S. obliquus	-	-	-	-	-	-	-	-	3.5	9.1	-	2.4	2.6	1.1	0.5	2.3	8.7
S. quadricauda	-	-	-	-	-	-	-	-	11.8	-	-	-	-	-	-	-	-
Schroederia setigera	-	-	-	-	-	0.3	20.0	-	15.6	28.9	27.1	-	-	-	-	-	-
Staurastrum paradoxum	-	-	-	-	-	-	-	-	-	-	-	53.2	-	-	-	-	-
Anabaena spiroides	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stephanodiscus niagarae	-	7.2	-	31.2	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyclotella meneghiniana	43.4	2.7	0.4	-	-	-	6.1	-	-	-	-	-	-	-	-	7.2	-
Cymbella sp.	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melosira granulata	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-	-
Rhizosolenia eriensis		-	-	-	-	-	-	-	-	-	-	7.3	-	-	-	-	-
Cryptomonas ovata	-	-	0.5	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-
Dinobryon bavaricum	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D. divergens	-	4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL	47.4	56.2	64.3	31.2	37.4	71.3	27.4	-	31.1	38.2	38.9	62.9	2.6	1.5	0.5	10.2	8.7

FIGURE 24 Weekly fluctuations of Cosmarium impressulum cell volume and cadmium binding capacity from surface samples in an enriched enclosure in contact with the sediment.

FIGURE 25 Weekly fluctuations of Euastrum insulare cell volume and copper binding capacity from 3 m samples in an enriched enclosure in contact with reduced zooplankton levels.


ALGAL VOLUME (μm³/L x 10⁹)
1.75
1.50
1.25
1.0
0.75
0.50
0.25
Cosmarium impressulum
Cadmium binding
200
150
100
50
CADMIUM BINDING (μM/L)
17 30 14 28 11 25 8 22 6 20 3
June July Aug. Sept. Oct. Nov.
DATE

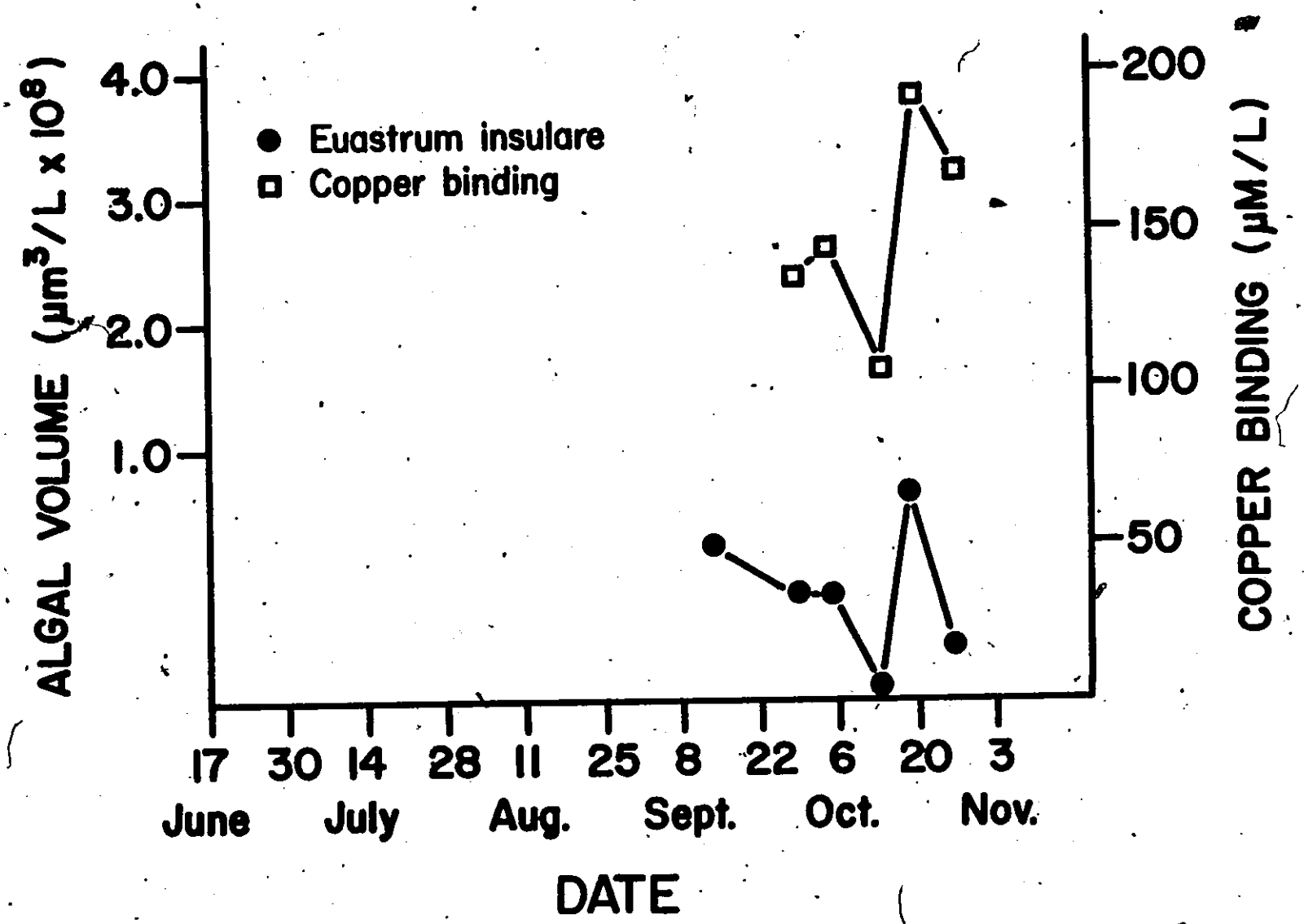

ALGAL VOLUME (μm³/L x 10⁸)
4.0
3.0
2.0
1.0
Euastrum insulare
Copper binding
200
150
100
50
COPPER BINDING (μM/L)
17 30 14 28 11 25 8 22 6 20 3
June July Aug. Sept. Oct. Nov.
DATE

FIGURE 26 Weekly fluctuations of Rhizosolenia eriensis cell volume and lead binding capacity from 3 m samples in the lake.

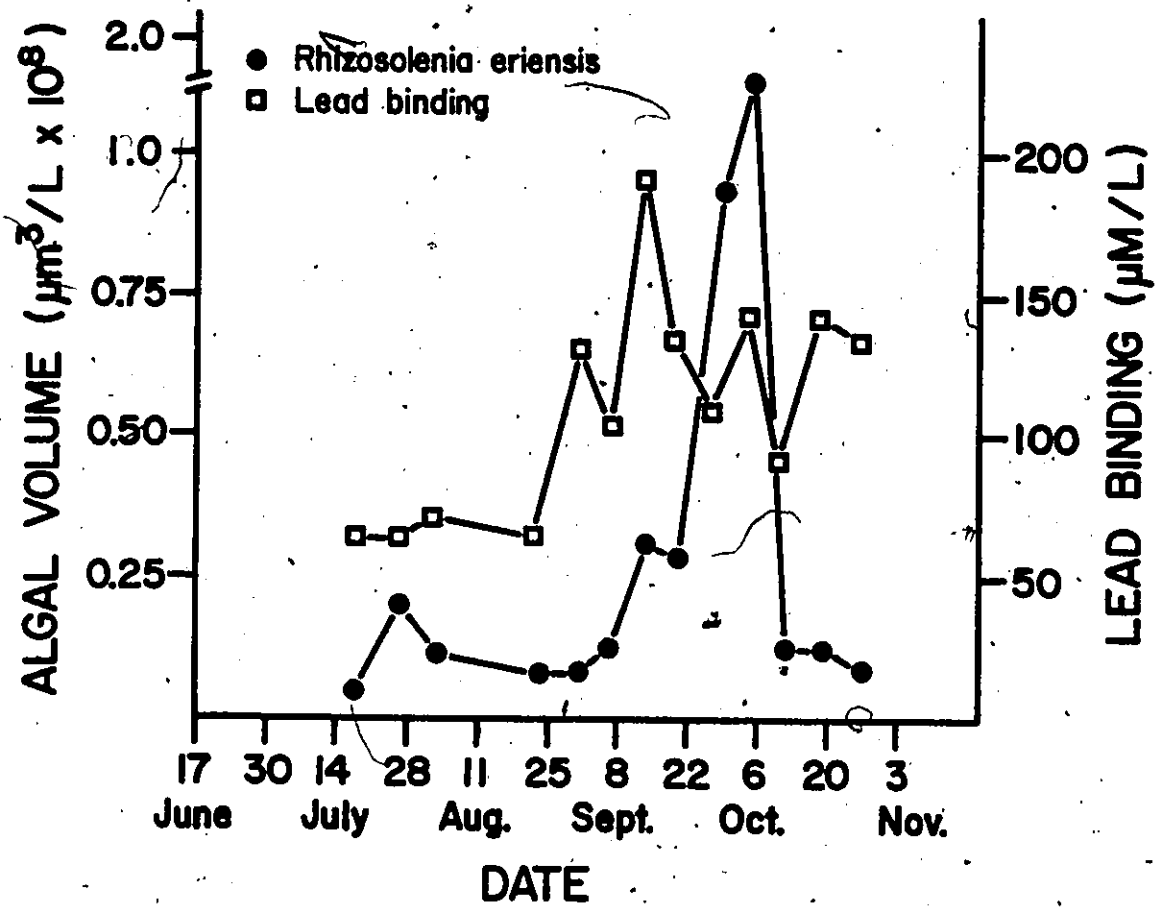
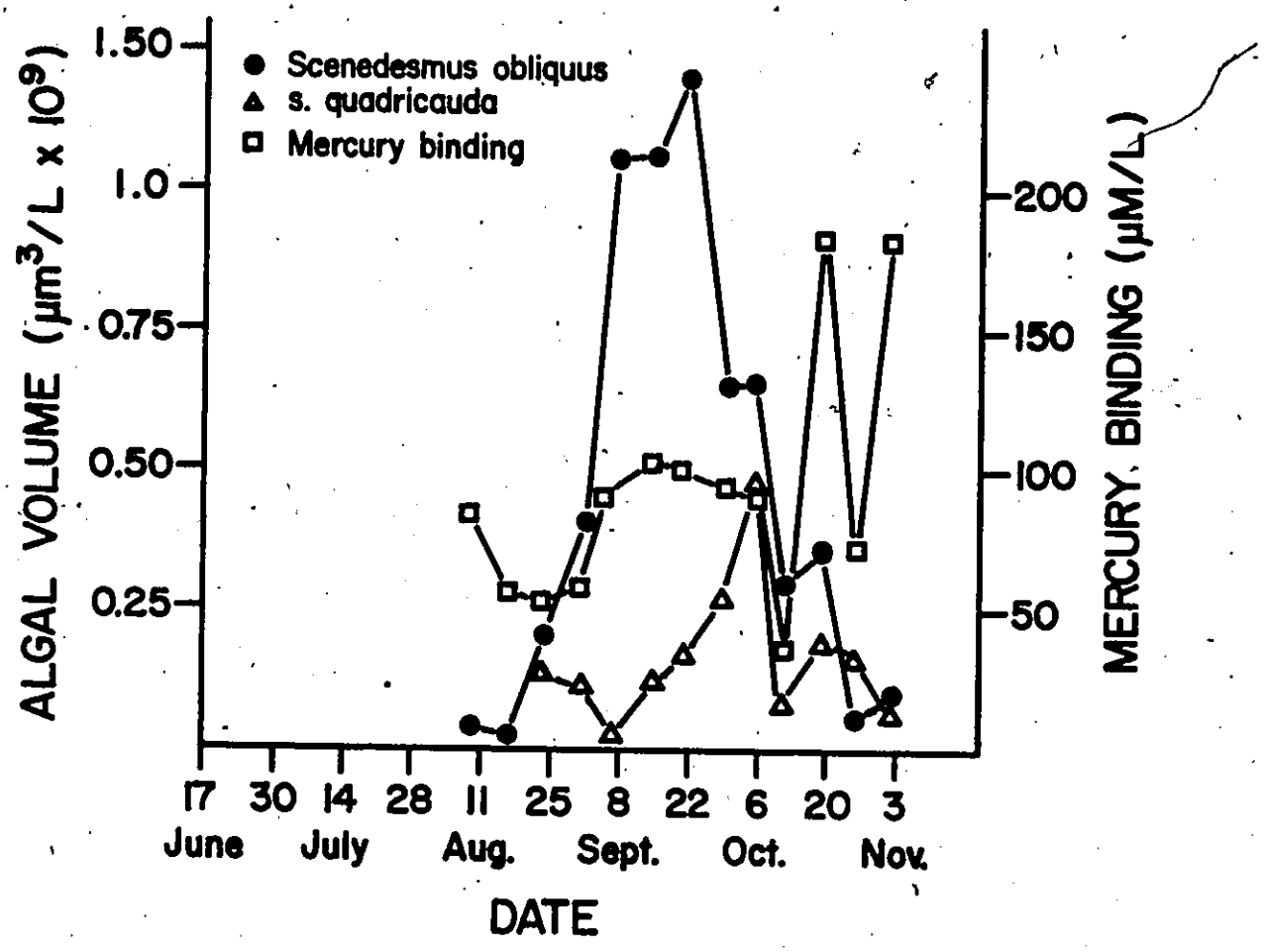

Rhizosolenia eriensis
Lead binding
ALGAL VOLUME (μm³/L x 10⁸)
LEAD BINDING (μM/L)
2.0
1.0
0.75
0.50
0.25
200
150
100
50
17 30 14 28 11 25 8 22 6 20 3
June July Aug. Sept. Oct. Nov.
DATE

FIGURE 27 Weekly fluctuations of Scenedesmus obliquus and S. quadricauda cell volume and mercury binding capacity from 6 m samples in an enriched enclosure.


ALGAL VOLUME ($\mu m^3/L \times 10^9$)
1.50
1.0
0.75
0.50
0.25
Scenedesmus obliquus
s. quadricauda
Mercury binding
200
150
100
50
MERCURY. BINDING ($\mu M/L$)
17 30 14 28 11 25 8 22 6 20 3
June July Aug. Sept. Oct. Nov.
DATE

DISCUSSION

The succession pattern and algal species composition in Heney Lake follow the general trend typical of a northern temperate lake (see Rodhe et al. 1958; Prescott 1962; Hammer 1964; Porter 1977). Phytoplankton pulses in the spring are characterized by a dominance of diatoms followed by blooms of blue-greens in summer. This shift in dominance seems to be caused by density independent factors such as temperature (Patrick 1969; Jones 1977), nutrient levels (Hutchinson 1967; Lund 1965), and thermal stratification of the water column (Reynolds 1976; Knoechel and Kalff 1975). Biological factors such as predation (Porter 1973), allelopathy (Keating 1978) among others could also play a role in determining community structure.

Diatoms in general are favored by higher nutrient levels (Lund 1954). The measured abundance of this group in the lake is in agreement with the general prediction, since diatoms were more abundant in the spring after the lake turnover brought about high nutrient levels. Diatoms also peak in late summer as a result of nutrient cycling.

Blue-green algae become more abundant when a decrease in nutrient levels is observed (Reynolds and Walsby 1975); this accounted for their emergence during the summer months, along with warmer temperatures (see Patrick 1969). Green algae made up the third major group in the lake, showing more erratic population fluctuations than the others. Finally chrysophytes and cryptophytes became more important before the onset of summer stratification.

The various manipulations performed on the enclosures had the desired effects in terms of altering community structure. During summer the enriched enclosures created conditions more favorable for the growth of green algae. This group is preferentially found in ponds and lakes with high nutrient levels during the summer period (Hutchinson 1967), conditions recreated here by the addition of nutrients. A detailed analysis of the succession patterns in the lake and the enclosures used in this study is given by Wall (1980).

Blue-green algae are often considered prime candidates for metal binding due to their ability to release low-molecular-weight compounds of high chelating capacity (Lange 1973). It comes as a surprise, therefore, to find only one representative of this group, the filamentous species Anabaena spiroides listed here. This suggests that the well documented release of metabolites by blue-greens may represent a shock response rather than an adaptation to bind metal ions. Sharp (1977) for instance states that such a release of organic compounds is mainly due to artifacts in the methodology, as well as to shock due to laboratory conditions. Although his conclusion was based on experimental studies using marine phytoplankton, there is no evidence to suggest that marine and freshwater blue-green algae behave differently with respect to organic excretion.

The species correlated with heavy metal binding (see Table 9) are all common inhabitants of temperate lakes and, with the exception of Anabaena spiroides, constitute a major food source of planktonic consumers. It is therefore critical to determine whether the respective metal ions are bound inside or directly to the cell surface, or externally by metabolite released

by the various algae. In the first case, heavy metal concentrations would increase by bioaccumulation and magnification through the food web. In the latter case, this would not occur due to external sequestration.

Laube et al. (1980) studied two species in this light and found that Anabaena sp. and Ankistrodesmus braunii mobilize sediment-bound copper and cadmium. In Anabaena, copper was bound in an extracellular form, whereas cadmium was present in both an extracellular and a cell-bound form. In Ankistrodesmus, both metals were present in cell-free and cell-bound form.

The variations in morphology found between the algae correlated with heavy metal binding suggest that heavy metal binding by algae in nature is not due to sorption. The algal species involved (Table 9) differ greatly in size and shape, ranging from small single cells (with large surface area) to long filaments (minimum surface area with reduced charge due to interrepulsion of the neighboring charge sites). The random variation in heavy metal specificity of these algae and the similarity of the charge and size of the four metal ions studied further rule out sorption mechanism, since sorption of heavy metal ions by surface is usually non-specific, and depends on the charge and ionic radii of the hydrated metal ions. In a previous study Button and Hostetter (1977) showed that Cyclotella meneghiniana accumulated as much as five times the amount of copper bound by Chlamydomonas reinhardtii. Yet the surface area and volume of both species are quite similar.

The amount of phosphate was very low in the water column. This indicates that the phosphate spiked weekly into the enclosure was

consumed by the algae and/or removed from the water column by sedimentation or hydrolysis following precipitation. The former could explain the dominance of green algae in the enriched enclosures, knowing the preference of this group for high nutrient levels. Considering that neither phosphate nor other physico-chemical parameter was correlated with metal binding, the possibility exists that natural ligands of non-algal origin such as fluvic acid, and/or persistent metabolites derived from previous algal blooms could account for the residual heavy metal binding capacity found in the samples. It is also quite possible that the bacterial fraction present in the water column and associated with the algal surface could account for some binding.

Since the purpose of this section was to investigate which species of algae are responsible for heavy metal binding no attempt was made to study the effect of heavy metals on the various communities. However, for epipelic algal communities in a metal-polluted northern lake in the Canadian subarctic Moore (1981) could not designate any particular species as indicator of heavy metal pollution despite the observed changes in species dominance, reductions in densities and diversity. Using controlled field experiments to monitor the effect of increased heavy metal loads on phytoplankton in Lake Baldegg, Switzerland, Gächter and Mares (1979) also showed that heavy metal concentrations caused shifts in phytoplankton species composition towards populations less susceptible to heavy metals. In general, the number of species observed in the contaminated limnocorrals was lower than in the controls. Over the long term however, higher phytoplankton densities were observed in the contaminated

enclosures compared to the controls, due to reduced grazing pressure from metal toxicity. Clearly, the possible mobilization of heavy metals in all compartments of the water system will depend to a large extent on the type of community developed.

A qualitative analysis of the data presented by Gächter and Mares (1979) reveals that of the sixteen most abundant algal species recorded in their artificially polluted limno-corrals, no less than six species are also listed in Table 9. Thus, although both communities differ in terms of algal species composition, a limited and very similar species pool appears to interact with the metals in both cases. The species involved comprise the green algae Ankistrodesmus falcatus, Elakatotrix sp., Shroederia setigera and Staurastrum sp., the diatom Cyclotella sp., and the cryptomonad Cryptomonas ovata. Clearly the relative and absolute abundance of these particular algae, combined with their specific palatability to grazers, will be of great significance regarding the introduction of heavy metals into the food web.

SECTION III

HEAVY METAL BINDING BY UNIALGAL POPULATIONS

INTRODUCTION

In the previous section, manipulation of seven algal communities and their interactions with four heavy metal ions were analyzed. In view of the specificity of heavy metal binding found in algal communities this section will focus on heavy metal binding ability of selected species commonly found in freshwater systems. Recent evidence indicates that phytoplankters accumulate non-essential trace elements such as cadmium (Cossa 1976; Conway 1978; Jennings and Rainbow 1979; Laube et al. 1980), lead (Schulz-Baldes and Lewin 1976; Trollope and Evans 1976) and mercury (Fujita and Hashizume 1975; Gerhards and Weller 1977). Such accumulation of non-essential metals varies qualitatively and quantitatively with different algal species. For example, Riley and Roth (1971) showed that under laboratory conditions accumulation of trace metals by phytoplankton varies among species. However, they found no differences in their distributions that could be correlated with a particular taxonomic group. In another study Gibson (1972) demonstrated that Anabaena flos-aquae accumulates more than twice the amount of copper accumulated by Scenedesmus quadricauda when subjected to a range of concentrations under laboratory conditions. Recently Gächter and Geiger (1979) found that temporal variation of metal uptake in their enclosures was linked to seasonal variations in phytoplankton species composition. De Hann et al. (1981) reported that copper

binding capacity is related with algal composition in Tjeukemeer Lake, Netherland. These studies agree well with our controlled field experiments (see section II) where most of the binding was accounted for by only a few species, all common inhabitants of temperate lakes and usually minor contributors of the total algal volume. This raises the important question of whether those phytoplankton species act selectively as a biological carrier of heavy metals to higher trophic levels.

It is now well documented (e.g., Gliwicz 1969, 1975; Porter 1973; Nadin-Hurley and Duncan 1976) that herbivorous plankton feed selectively on algae according to size, taste and morphology of their prey. The distinction between edible and inedible algae carries large implications for the biological transport of heavy metal pollutants in aquatic systems. Uptake of edible algae would tend to transfer heavy metals to higher trophic levels, while uptake by inedible algae would tend to remove heavy metals from the food web.

Together with the biological transfer of heavy metals into the food chain (Small 1969; Jennings and Rainbow 1979) and their direct accumulation from the ambient water (Hutchinson and Stokes 1975; Sick and Baptist 1979) or sediments (Laube et al. 1980), other factors such as chemical speciation (Anderson and Morel 1978) and free-ion activity in the metal solution (Sunda and Guillard 1976) should be considered in the dynamics and effects of metal pollutants in aquatic environments.

Two green algae, Ankistrodesmus falcatus and Gloeocystis gigas, and the diatom Tabellaria fenestrata were used in these experiments. The three species chosen were able to grow under laboratory conditions and are

commonly found and widely distributed in northern temperate lakes and ponds (Prescott 1962), sometimes reaching high numbers in response to local conditions. Both Ankistrodesmus and Tabellaria were considered as edible as opposed to Gloeocystis. In general A. falcatus is the most common species of the genus and occurs as solitary or loosely clustered cells with an acicular morphology. It is well established from field and laboratory studies on the feeding behavior of freshwater crustaceans (Berman and Richman 1974; Porter 1975) that A. falcatus is a readibly edible species. Tabellaria fenestrata is characterized by its elongate rectangular frustules which are attached at the corners, one to another, forming straight filaments. In spite of its size and morphology Tabellaria is commonly ingested by copedods, as it can be easily fragmented by grazers. The third species, G. gigas, is spherical in shape, arranged in groups of two or more and enclosed by a distinct concentric sheath of mucilage. In spite of a small individual cell size, G. gigas is considered inedible since the findings of Porter (1975) that gelatinous green algae ingested by zooplankton grazers are not digested. She showed that 90% of those algae had intact cell walls and cell contents after their ingestion by primary consumers, and further that the gut passage of the gelatinous algae stimulated cell numbers and productivity.

Cadmium, lead and mercury were considered in order to test the binding abilities of the three species mentioned above. Cadmium and lead binding capacity was evaluated in populations of A. falcatus and G. gigas whereas mercury binding capacity was assessed in populations of T. fenestrata. No information, with the exception of photosynthesis

inhibition in A. falcatus by copper (Shioi et al. 1978) and our previous studies (see section II), was known on the relationship between these three species and heavy metals.

More generally, no attempt had been made to our knowledge to study unialgal populations and their interaction with heavy metals in situ. This phase of the study will use the same experimental design as in section II, so as to monitor large populations in natural physico-chemical conditions and thus correlate fluctuations in population densities with metal binding affinity.

MATERIALS AND METHODS

Unialgal populations of A. falcatus, G. gigas and T. fenestrata were each innoculated at three different densities in polyethylene enclosures suspended in Heney Lake. The initial densities expressed in 1000 cells/L were: 100, 195, 350; 9, 16, 24; and 180, 270, 380 respectively. The enclosures, each 1 m in diameter and 2 m deep were open at the top to provide gas exchange with the atmosphere. Water from the lake was pumped into the enclosures after filtration through a 10 μm Unicomb filtering apparatus (Model WYTF 18) that removed most planktonic organisms. The filter tubes used were W19 R10 A.

Duplicate samples were taken periodically at a depth of 1 m to estimate changes in algal density and in heavy metal binding capacity. Sampling and enumeration of phytoplankton were carried out as described in the previous section, except that this time samples were counted with a Zeiss inverted microscope, using a Zeiss eyepiece with adjustable counting lines in combination with a stage micrometer. All cells were counted in an appropriate number of strips (each 100 μm wide, 10 mm high and 10 mm long). Algal volume was calculated as described previously (see section II).

Chemical analysis was conducted in the laboratory on the day of sampling. Metal binding was determined in the laboratory at 20± 1°C using two subsamples of 50 ml, one with, the other without, the algae. The metal solution was added with an adjustable sampler micropipette in small increments from a metal nitrate solution of 10^{-2}M. The ability of the sample

to bind cadmium, mercury and lead was measured by the ion-specific electrode technique. All procedures were fully explained in the previous section.

RESULTS

Population Densities

Variations in total cell volume following the initial inoculations are given in Figs. 28 to 32. Populations of Ankistrodesmus falcatus reach their maximum densities in late July (Fig. 28). This noticeable pulse was followed by a steep drop extended to the end of the experimental period. Gloeocystis gigas tends to decrease in density shortly after inoculation. The small increase in cell numbers recorded in late July was never higher than the initial inoculations numbers (see Fig. 30). Populations of Tabellaria fenestrata showed a steady decline in density following their inoculation in the enclosures (Fig. 32).

Heavy Metal Binding

The correlations found between metal binding capacity and cell volume for the three species studied are indicated in Table 11. Ankistrodesmus falcatus was positively correlated with lead ($r = 0.60$; $P < 0.01$). The relation was quite pronounced (Fig. 28) and confirms a similar trend observed (see Table 9) at the whole community level. When the same unialgal population was tested for the ability to bind cadmium, no significant correlation was found (Fig. 29 and Table 11). The other green alga, Gloeocystis gigas, was found to bind cadmium significantly ($r = 0.66$; $P < 0.01$) (see Table 11 and Fig. 30). However, no significant correlation

FIGURE 28 Fluctuation in total algal volume and lead-binding capacity for Ankistrodesmus falcatus in each enclosure (A_1, A_2, A_3).

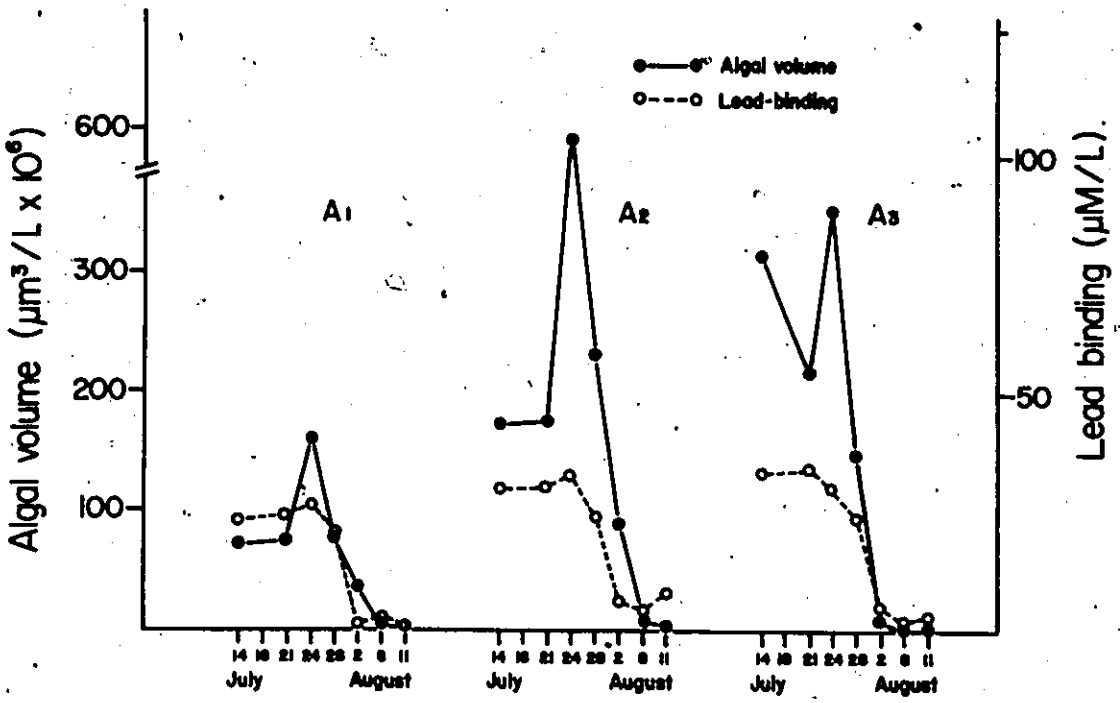

Algal volume
Lead-binding
Algal volume (µm³/L x 10⁶)
Lead binding (µM/L)
600
300
200
100
100
50
A1
A2
A3
14 18 21 24 28 2 6 11
July
August

FIGURE 29 Fluctuation in total algal volume and cadmium-binding capacity for Ankistrodesmus falcatus in each enclosure (A_1, A_2, A_3).

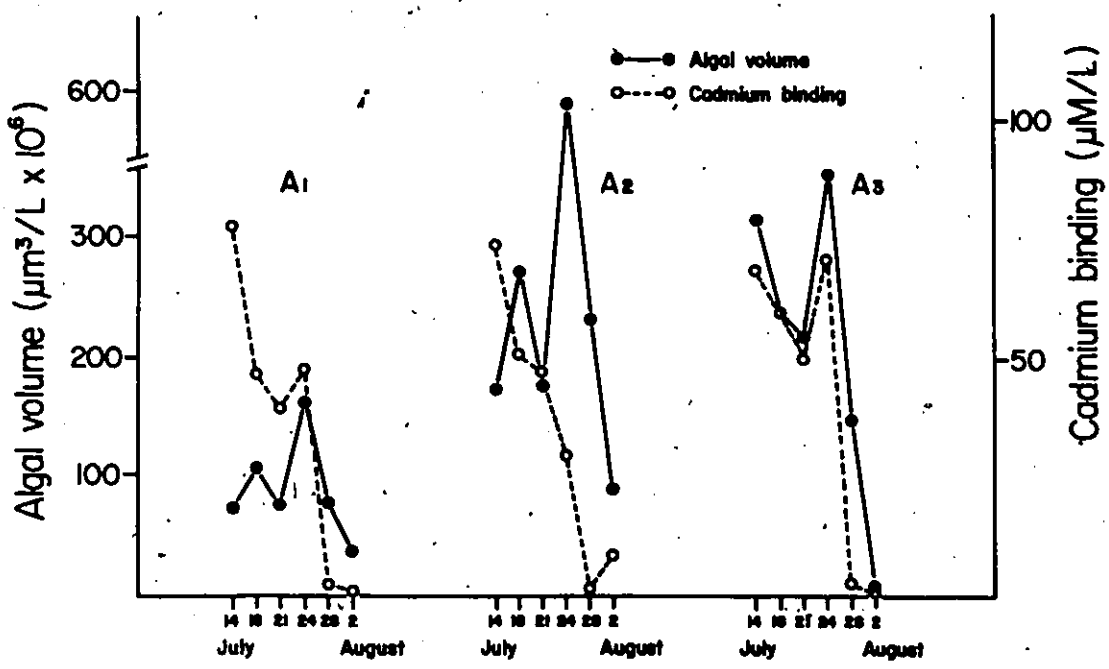

Algal volume
Cadmium binding
Algal volume (µm³/L x 10⁶)
Cadmium binding (µM/L)
600
300
200
100
100
50
A1
A2
A3
14 18 21 24 28 2
July
August

FIGURE 30 Fluctuation in total algal volume and cadmium-binding capacity for Gloeocystis gigas in each enclosure (B_1, B_2, B_3).

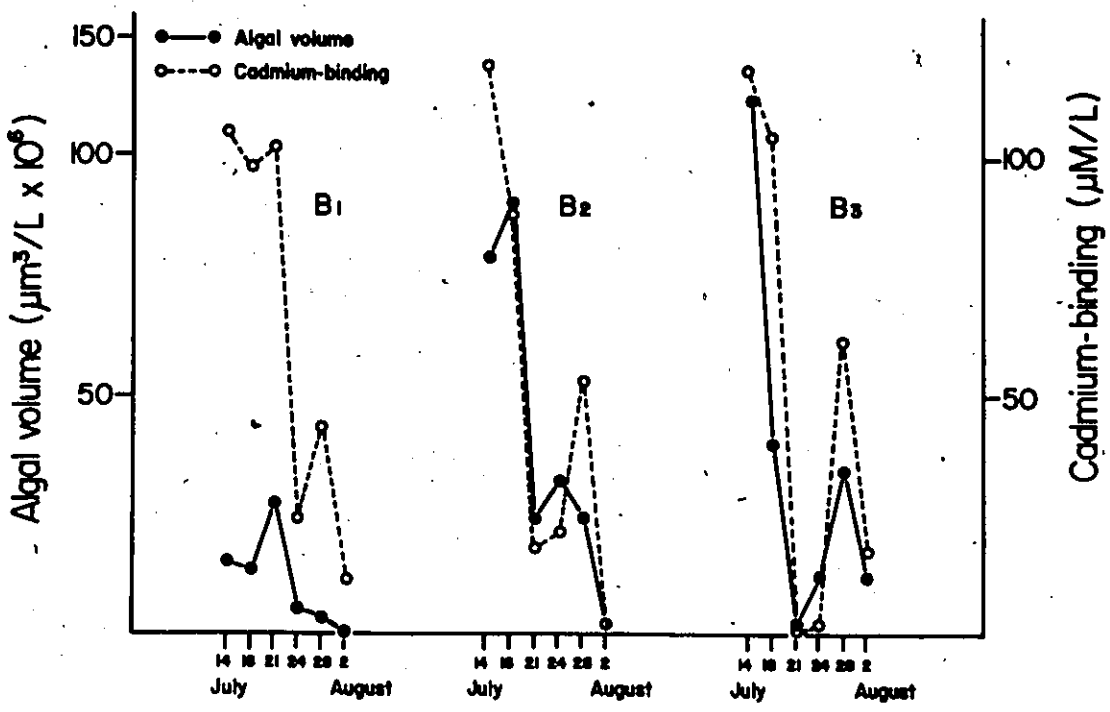

Algal volume
Cadmium-binding
150
100
50
Algal volume (µm³/L x 10⁶)
Cadmium-binding (µM/L)
B1
B2
B3
14 16 21 24 28 2
July
August

FIGURE 31 Fluctuation in total algal volume and lead-binding capacity for Gloeocystis gigas in each enclosure (B_1, B_2, B_3).

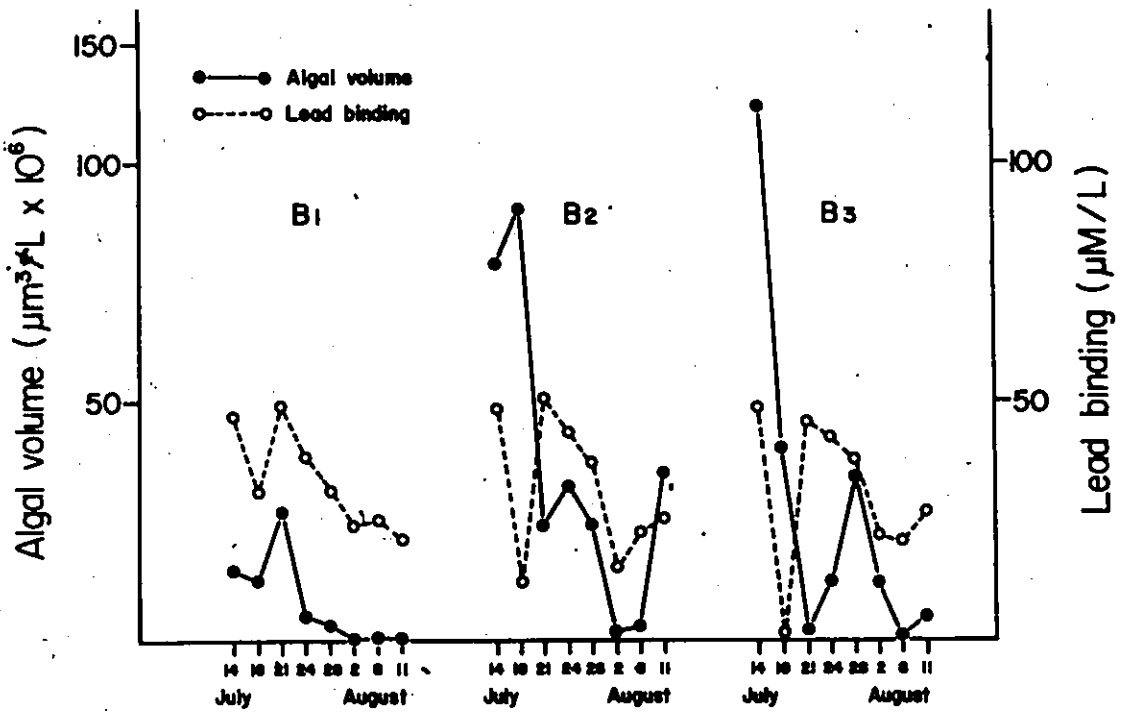

Algal volume
Lead binding
B1
B2
B3
150
100
50
Algal volume (µm³/L x 10⁶)
Lead binding (µM/L)
July
August

FIGURE 32 Fluctuation in total algal volume and mercury-binding capacity for Tabellaria fenestrata in each enclosure (C_1, C_2, C_3).

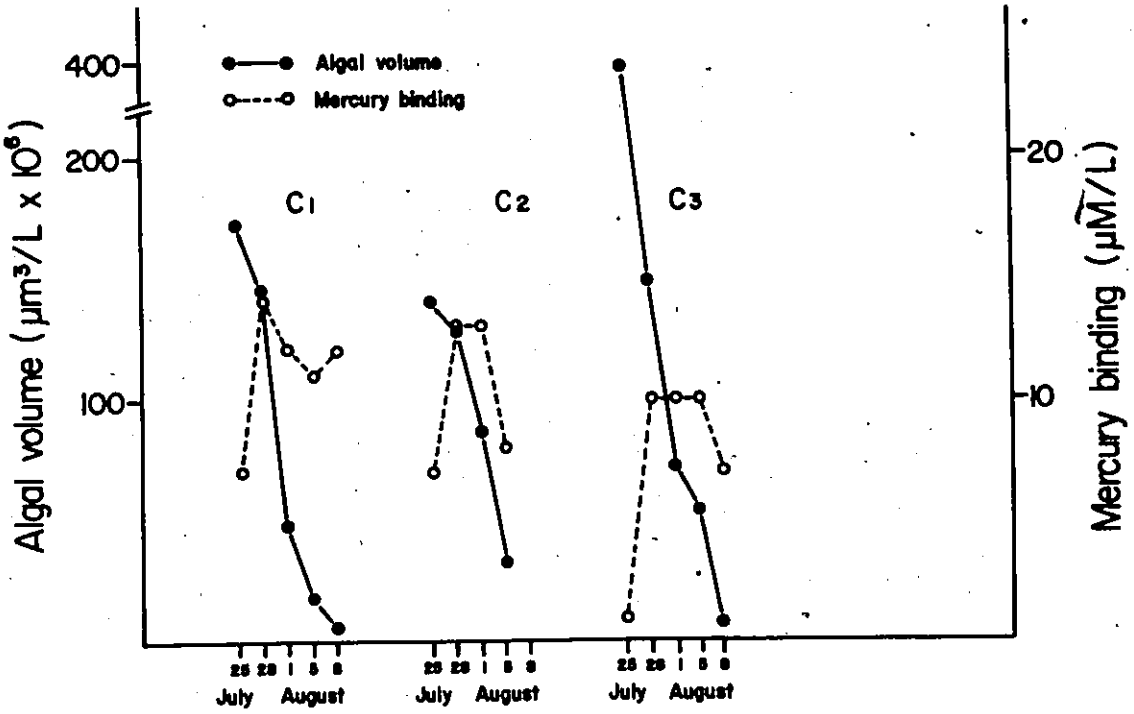

Algal volume
Mercury binding
400
200
100
20
10
C1
C2
C3
Algal volume (µm³/L x 10⁶)
Mercury binding (µM/L)
25 28 1 5 8
July August

TABLE 11 Correlation Between Cell Volume and Binding Capacity for the Species Studied. Statistically Significant Correlations are Indicated as Follows: * for $P < 0.05$; ** for $P < 0.01$

Algae	Time of Experiments	Metal	r
Ankistrodesmus falcatus			
	14 July - 11 Aug. 78	Pb^{++}	.60**
	14 July - 2 Aug. 78	Cd^{++}	.33
Gloeocystis gigas			
	14 July - 11 Aug. 78	Pb^{++}	.19
	14 July - 2 Aug. 78	Cd^{++}	.66**
Tabellaria fenestrata			
	25 July - 8 Aug. 77	Hg^{++}	-.58*

for lead binding capacity was observed. The third alga tested, Tabellaria fenestrata showed a significant negative correlation with mercury binding capacity ($r = -58$; $P < 0.05$).

DISCUSSION

As mentioned earlier, size, taste and morphology are the main criteria involved in the edibility status of a particular algal species. Since natural grazing rates in plankton communities may remove as much as 100% of the daily phytoplankton production (Haney 1971, 1973), the ability of A. falcatus to bind lead may provide a significant transfer mechanism of this non-essential metal to higher trophic levels. This active mechanism of bio-accumulation may become particularly significant compared to other mechanisms of accumulation such as direct incorporation from the ambient water, when lead levels are low in the aquatic environment, since non-selective accumulation of heavy metals by algae will depend in great measure on the concentration factors involved (Stokes 1979).

The ability of Gloeocystis gigas to bind cadmium together with its inedibility status in the trophic web suggests that in cadmium polluted waters this alga would tend to remove the metal and make it unavailable to planktivorous species.

Gloeocystis gigas was not among the species found to be highly correlated with the metals studied in section II. This apparently contradictory result, at least with respect to cadmium binding, may be explained by the fact that Gloeocystis occurred only at very low densities in the multi-algal enclosures.

The significant negative correlation in mercury binding detected for Tabellaria fenestrata may be explained by two mechanisms that are not mutually exclusive. Densities of Tabellaria in all enclosures decreased

continuously; since those enclosures were free of grazers an increase of organic matter due to algal decay is expected, resulting in a higher non-selective binding capacity. Recently, De Hann et al. (1981) suggested similar mechanisms based on the high metal chelating ability of organic matter following algal blooms. The second alternative relates to the observation made during the quantitative analysis of the samples that another alga, the blue-green Anabaena sp., was present as a contaminant in the enclosures (the density of Anabaena was not recorded). This may have caused an increased mercury-binding capacity and thus the observed negative correlation. This would agree well with our previous findings that showed a related species, Anabaena spiroides to be highly correlated to mercury binding (see Table 9).

The overall decline of algal densities observed in the enclosures was likely due to nutrient depletion as physico-chemical parameters were not significantly different throughout the experiment to account for such a reduction. Inhibition due to biological interactions can be discarded as an explanation since no other organisms (except for Anabaena in one set of enclosures) were present.

Since heavy metal uptake by algae appears clearly species-specific, one must take the algal species composition of a lake and its potential for change into account when assessing the possible impact of pollutants on freshwater systems. High phytoplankton densities followed by peaks in zooplankton numbers, together with low numbers of algal cells in the presence of high numbers of grazers, have long suggested the importance of herbivores in controlling algal numbers. In this respect the differential

impact of zooplankton grazing (Porter 1977; Briand and McCauley 1978; McCauley and Briand 1979) on the edible and inedible groups may be put to profitable use. For example, several algae are similar to Gloeocystis, in having a durable cell wall and/or gelatinous sheaths. The massive introduction of such inedbile species into a polluted lake would bind heavy metals away from the food web. On the other hand, a bloom of readily edible alga, such as Ankistrodesmus, known for its metal binding ability, would serve as an indicator of a potentially health-threatening situation when heavy metal concentrations are known to be present.

SECTION IV

ACCUMULATION OF CADMIUM AND LEAD BY *DAPHNIA PULEX*: SHORT TERM EXPERIMENTS

INTRODUCTION

In section III, the uptake of various metals by three phytoplankton species together with the importance and potential ecological consequences of heavy metal accumulation by algae was discussed. One of the edible species, *Ankistrodesmus falcatus*, was considered a good organism to continue our studies on the significance of phytoplankters as carriers of heavy metals to other levels of the food web. This section investigates the uptake and release of cadmium and lead by the primary consumer *Daphnia pulex*.

Accumulation of metals by aquatic organisms in general seems to be species dependent and varies according to the metal type (Riley and Roth 1971). The pathways of such accumulations may be by uptake from solution and/or via the food source. The data available from the few studies conducted previously on the uptake of heavy metals by aquatic organisms have raised a controversy regarding the relative importance of the pathways that allow the transfer of heavy metals to higher trophic levels.

The results of the present study draw attention to the findings of Jennings and Rainbow (1979) on the accumulation of cadmium by a marine herbivore, *Artemia salina*. They emphasized the importance of algae as

carriers of metals to other levels in the food web, since at lower concentrations of cadmium (1.0 ppm) uptake from the food was the major route of metal accumulation. However, at cadmium concentrations higher than 1.0 ppm, accumulation from the solution became the major route of uptake. An earlier study by Rice (1963) also provides evidence that the major route of zinc uptake in the same species, A. salina is due to the food source Carteria sp.

On the other hand, Gächter and Geiger (1979) working with a simple food chain (phytoplankton-zooplankton) in an unpolluted and artificially metal-polluted freshwater system suggested that heavy metals are not accumulated through the food chain, based on the fact that in their studies metal content was found to be highest for phytoplankton and lower in zooplankton.

When metals are found at very low levels in the environment their uptake by phytoplankton will be of great ecological importance since the algae are not only involved in the geochemical distribution, but also in the transfer of the metal to their consumers. For example, lead-enriched algae can increase the lead content of the marine bivalve, Mytilus edulis to the same extent as elevated lead concentrations in the surrounding medium (Schulz-Baldes 1974).

Despite current interest in the fate and effects of heavy metals in freshwater ecosystems and organisms, relatively little experimental work has been done using crustacean herbivores, a group especially important in the planktonic food web. The most abundant zooplankters in freshwater lakes are crustaceans, and particularly cladocerans such as Daphnia

pulex. Cladocerans are found throughout the year, and their populations can increase rapidly due to their short parthenogenic generation times. They have high filtering rates and could account for 80% of the community grazing rate (Porter 1977). Their effects on primary producers contribute, among other factors, to the character of a particular community (Brooks and Dodson 1965). Daphnia is also a favorite prey of several carnivorous zooplankters and fish, contributing in this way in biotransfer and bio-accumulation to high trophic levels.

Due to the fact that heavy metal accumulation by aquatic organisms has its origin in the water solution and/or the food supply it was necessary to design a set of experiments that allowed the independent evaluation of the relative importance of each source of heavy metal uptake by the cladoceran D. pulex. The difficulties of differentiating between the relevance of each pathway and the algal role in heavy metal accumulation by D. pulex was ultimately overcome by a combination of experimental procedures based on field and laboratory situations.

In this section, two separate experiments will be described, investigating the accumulation of cadmium and lead by Daphnia pulex. The first set evaluates the uptake of lead and cadmium by Daphnia using the food and the ambient water as exposure vectors. The second experiment determines the release of both metals by the daphnids. The green alga Ankistrodesmus falcatus was chosen as food supply. Both Daphnia and Ankistrodesmus were originally isolated from Heney Lake.

MATERIALS AND METHODS

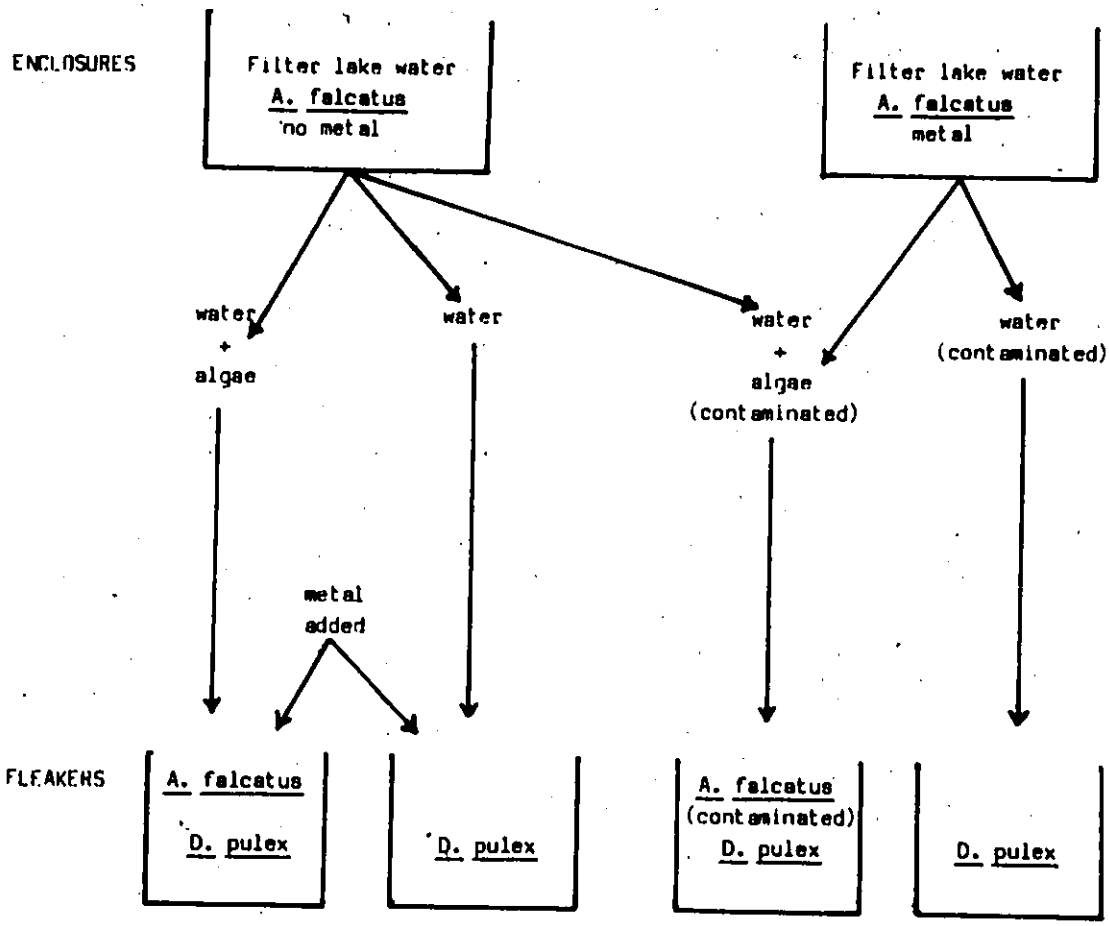
Field Procedure

Populations of Ankistrodesmus falcatus (Corda) Ralfs, from laboratory stock originally isolated from the lake, were inoculated into three separate polyethylene enclosures, each having a volume of 2000 L of filtered water. Twelve hours after the inoculation, cadmium, as $Cd(NO_3)_2$, and lead, as $Pb(NO_3)_2$, were added to two of the enclosures by spiking the metal solution in small pulses to reach a final concentration of 1000 ppb and 100 ppb respectively. Twenty-four hours later water from each enclosure was taken to the laboratory in order to proceed with the uptake phase of the experiment.

Uptake Experiments

For each experiment, four sets of 1.0 L fleakers (Pyrex), with three replicas each, were filled with water from the enclosures. The protocol is described in Fig. 33. Two sets were filled with water from the metal free enclosure. The algae were removed by filtration in one of these. Prior to adding the zooplankton a known amount of metal was added to each set of fleakers. The third set of fleakers was filled with water from the metal-free enclosure and a known amount of algae which had been previously in contact with the metal was added. The algae were rinsed with filtered lake water before the inoculation. The fourth set was filled with water from the

FIGURE 33 Diagram illustrating the procedure followed for the uptake experiments.


ENCLOSURES
Filter lake water
A. falcatus
no metal
Filter lake water
A. falcatus
metal
water
+
algae
water
water
+
algae
(contaminated)
water
(contaminated)
metal
added
FLEAKERS
A. falcatus
D. pulex
D. pulex
A. falcatus
(contaminated)
D. pulex
D. pulex

metal enclosure, although the algae were previously removed by filtration, using a .45 um millipore filter.

All fleakers from the four sets were then inoculated each with 40 Daphnia pulex. The daphnids had been kept in starving conditions for 24 hours prior to the beginning of the experiment. They were selected for a uniform size range, varying between 1.9 and 2.1 mm in length. The experiment was carried out at 16°C, and samples of water, algae and zooplankton were taken for analysis at 3, 6 and 12 hour intervals. The animals were washed and transferred to filtered lake water containing uncontaminated algae, on which they grazed for 30 minutes to ensure removal of metal from their gut. Immediately after, they were transferred to deionized water in which a known amount of 1:1 nitric-perchloric acid (Baker Reagent Grade) was added to reach a final concentration of 10% in order to kill the daphnids and fix the metal.

Release Experiments

Separate experiments were carried out in order to measure the loss of metal from contaminated daphnids during depuration. This was determined by treating 120 daphnids for 24 hours in filtered water containing the metal, then transferring them to uncontaminated filtered water containing algae as a food source. At time intervals of 0, 12, 24 and 48 hours, 30 animals were removed and the metal content was measured. A group of 120 daphnids was fed for 24 hours on algae containing the metal, and four groups of 30 daphnids each were measured for metal content at time

intervals of 0, 12, 24 and 48 hours. Prior to each measurement, daphnids were fed uncontaminated algae for 30 minutes in order to clear the gut content of contaminated algae.

Cultures

Stock cultures of A. falcatus were grown at 16°C under fluorescent illumination, using Westinghouse Cool White (F40CW) and Agro-Lite (F40/Agro) fluorescent tubes in a 16L/8D photo-period. The growth medium was Bold Basal (Nichols and Bold 1965): its detailed composition is given in Appendix III.

Daphnia pulex was isolated from Heney Lake and kept in filtered water at 16°C. A. falcatus was added periodically as a food supply.

Enumeration procedure

Algal densities were estimated as described in the previous sections, using the Utermöhl technique. Algal inoculation densities were 1.0×10^7 cells/L, the amount at which the filtering rate is independent of food concentration (Rigler 1961).

Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectroscopy is a process whereby a sample solution is vaporized and the metal present converted to neutral atoms. The

neutral atoms thus formed absorb radiation from an external light beam. The amount of absorption is then measured and correlated to the concentration of the element in the study via a calibration curve. Here the metal content of samples was analyzed by AAS using a Jarrel-Ash model 810.

Sample Preparation of AAS

After digestion with 1:1 nitric-perchloric acid (Stokes et al. 1973; Kobayashi 1972) the samples were heated in a 160°C oven for 3 hours. A light-colored residue indicated complete digestion of the specimens. Deionized water was added to a desired volume. At this stage, samples were ready to be measured by AAS. Fresh standard solutions were made on the same day of analysis from stock solutions of $Cd(NO_3)_2$ or $Pb(NO_3)_2$ (Baker Reagent Grade). Stock solutions were diluted using deionized water and an appropriate amount of nitric-perchloric acid was added to obtain the same concentrations as the samples. In order to calibrate the instrument, at least three known concentrations of each metal were prepared every time that a set of samples was analyzed. Blanks and samples were always subjected to the same treatment. Glassware was carefully washed with a solution of No-Chromic (Godax Lab., N.Y.) in sulfuric acid and afterwards rinsed several times with deionized water.

RESULTS

Cadmium Uptake by Daphnia Pulex

Most of the cadmium uptake by Daphnia pulex occurred very rapidly whether from the water solution and/or from the algae contaminated with the metal. The amount of cadmium accumulated (expressed as weight of cadmium) by D. pulex from each pathway during the experimental period is shown in Fig. 34.

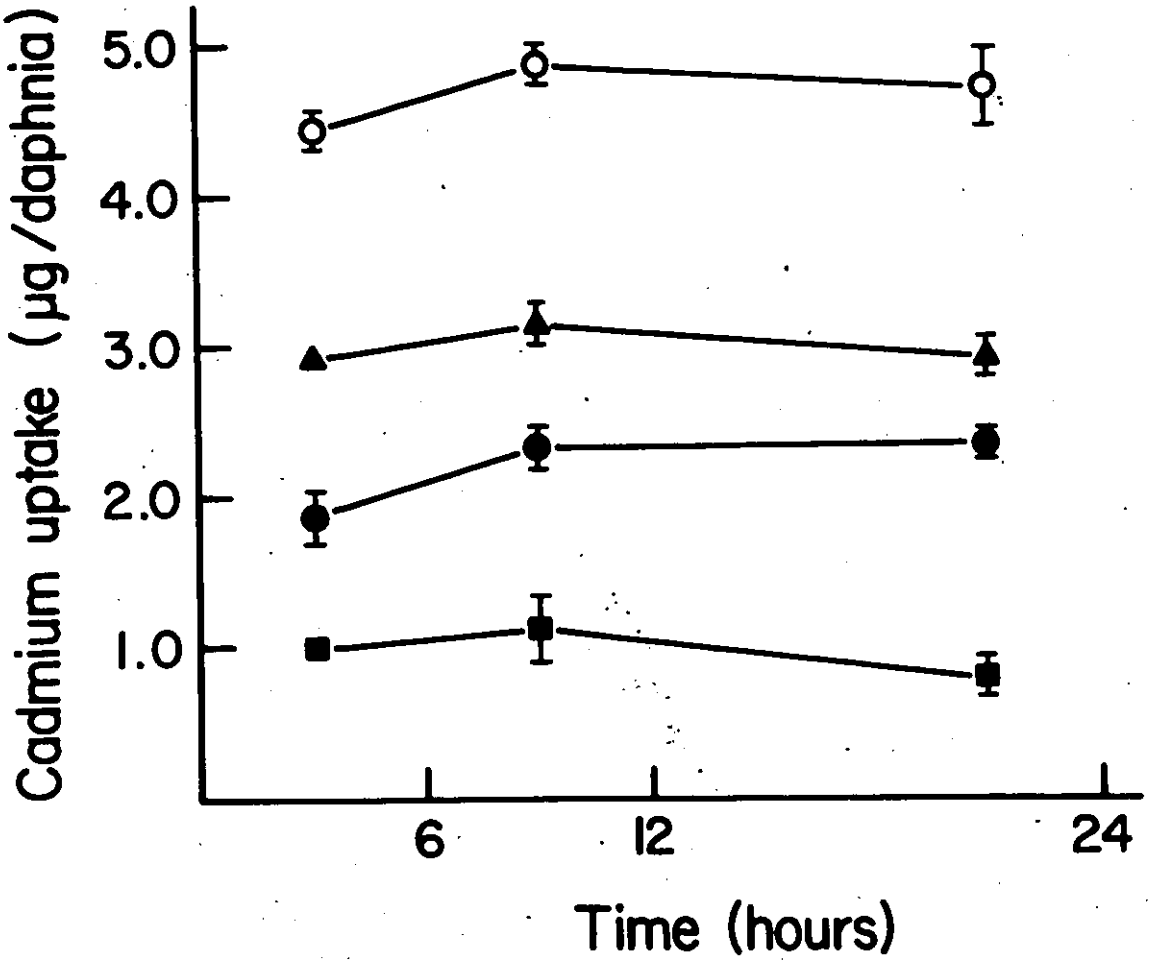
It is significant that D. pulex accumulated a detectable quantity of the metal (1 µg/daphnid) from the ingestion of previously contaminated A. falcatus, when the latter was the only source of metal available to the daphnids. The largest metal uptake occurred however when both ambient water and food source had been contaminated with the metal.

When cadmium was present in the water, the amount of metal accumulated by the daphnids was higher in the absence of food than when algae were grown in the solution but not given as a food source.

Absorption and/or adsorption of the metal by D. pulex seems to be 2 or 3 times greater than assimilation from the food source alone. Although it is emphasized that the total concentration of the metal was much higher when no algae were present than when algae were the sole source of cadmium.

The sum of the measured amount of cadmium in the water, and the metal accumulated by the algae and/or by Daphnia pulex was generally less than the total amount of metal added originally, indicating some adsorption

FIGURE 34 Cadmium uptake by Daphnia pulex. Level of exposure was 1.0 ppm Cd. Open circles: cadmium in solution and in Ankistrodesmus falcatus; triangles: cadmium in solution; solid circles: cadmium in solution from Cd-algae enclosure; squares: cadmium in A. falcatus only.


Cadmium uptake (µg/daphnia)
5.0
4.0
3.0
2.0
1.0
6
12
24
Time (hours)

by the surface of the fleakers. The amount was never higher than 5%.

At concentrations of 1 ppm of cadmium in the solution, A. falcatus was able to accumulate about 10.8 µg of Cd/10^6 cells/L. Average cadmium concentrations were approximately 6,000 to 12,000 times greater in D. pulex than in the water.

Release Experiments

Figure 35 shows the metal clearance in daphnids. The release of cadmium in daphnids in which the metal had been in solution occurred very rapidly and after 48 hours was slightly lower than when the metal present in daphnids was due to the food source.

Lead Uptake by Daphnia pulex

The amount of lead accumulation by D. pulex from each source during the experimental period is shown in Fig. 36.

When D. pulex was fed with contaminated algae, they accumulated as much as 1.12 µg. The amount of lead accumulation by D. pulex when exposed to the dissolved metal alone, was approximately half the amount accumulated by daphnids when exposed to the contaminated food only. However, as with cadmium, the highest accumulation by the daphnids occurred when both surrounding water and food were contaminated with the metal.

FIGURE 35 Daphnia pulex. Loss of cadmium upon return to cadmium-free conditions. Time 0 indicates time of removal to metal-free conditions. Triangles: cadmium in solution only; squares: cadmium in Ankistrodesmus falcatus only.

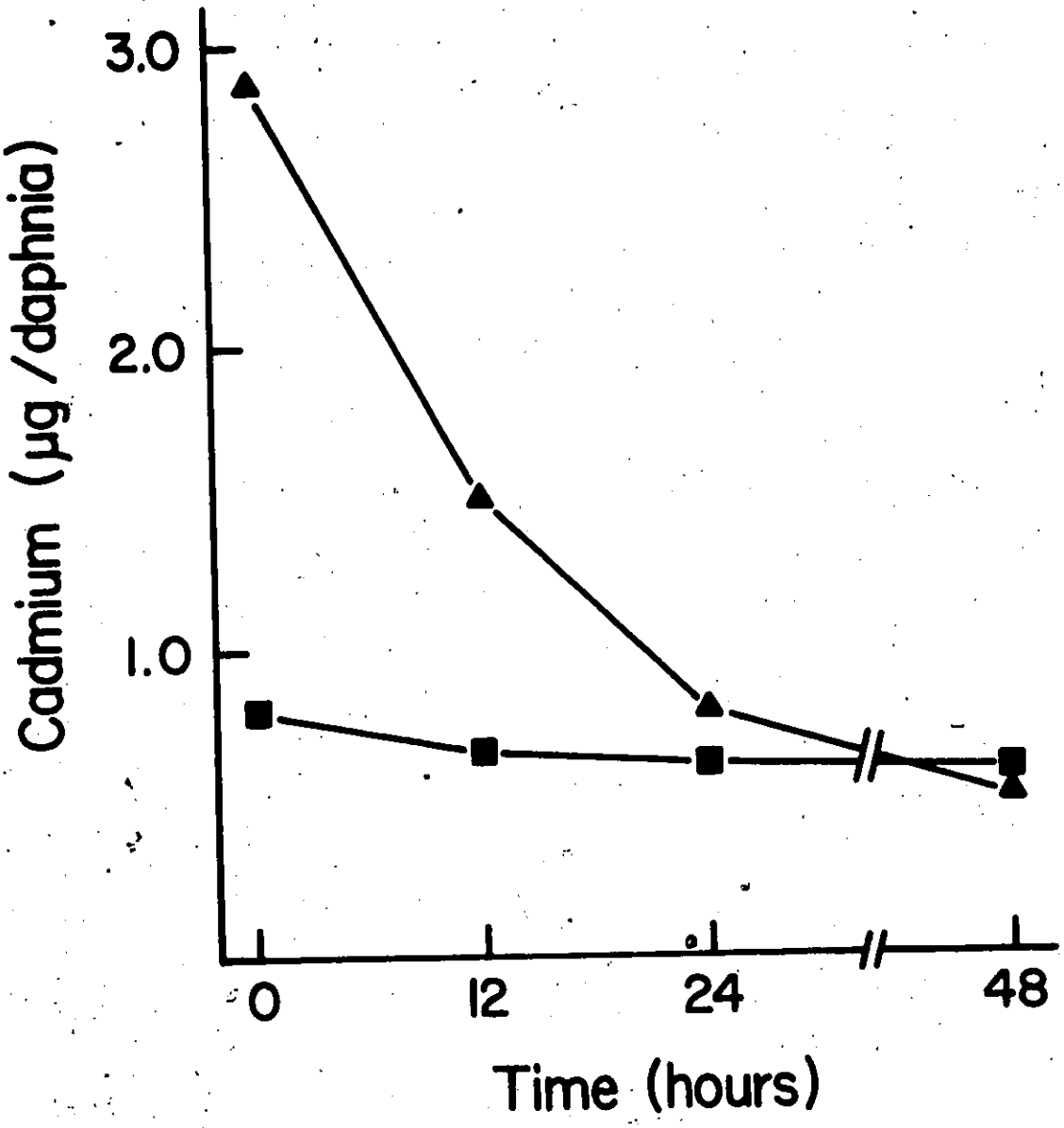
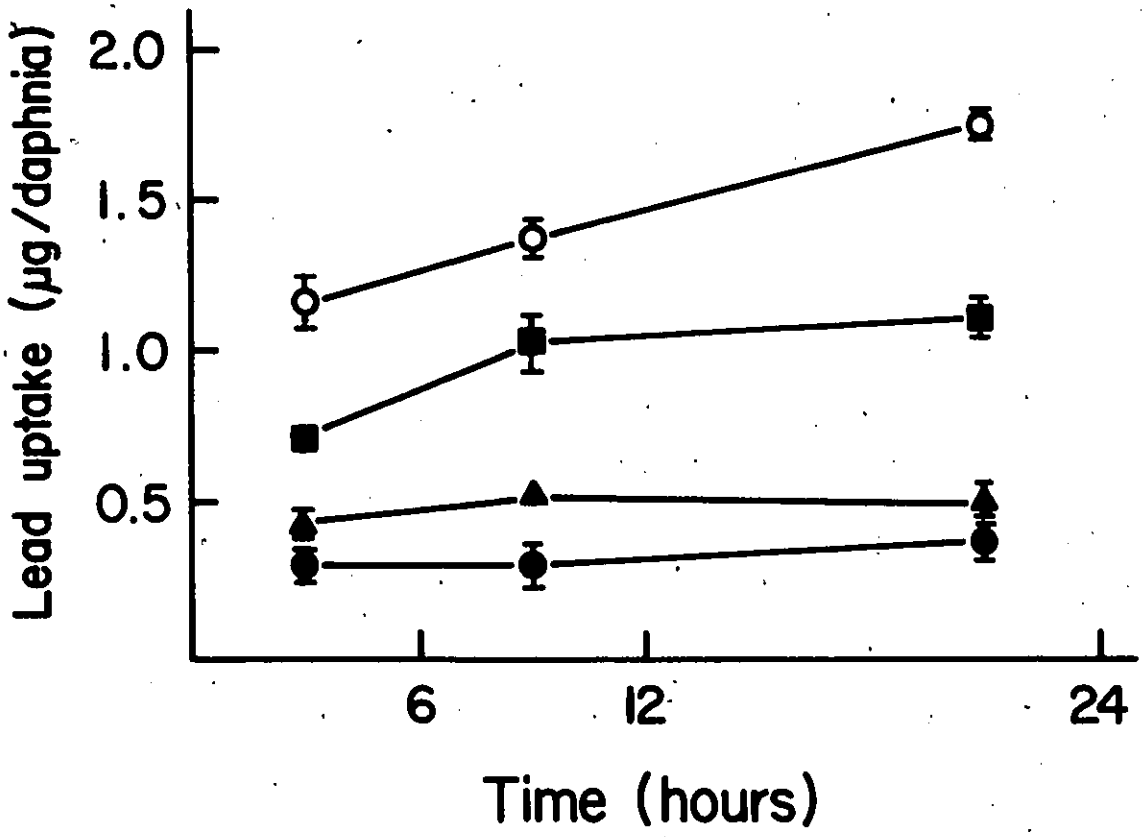

3.0
2.0
1.0
Cadmium (µg /daphnia)
0
12
24
48
Time (hours)

FIGURE 36 Lead uptake by Daphnia pulex. Level of exposure was 0.1 ppm Pb. Open circles: lead in solution and in Ankistrodesmus falcatus; triangles: lead in solution only; filled circles: lead in solution from Pb-algae enclosure; squares: lead in A. falcatus only.

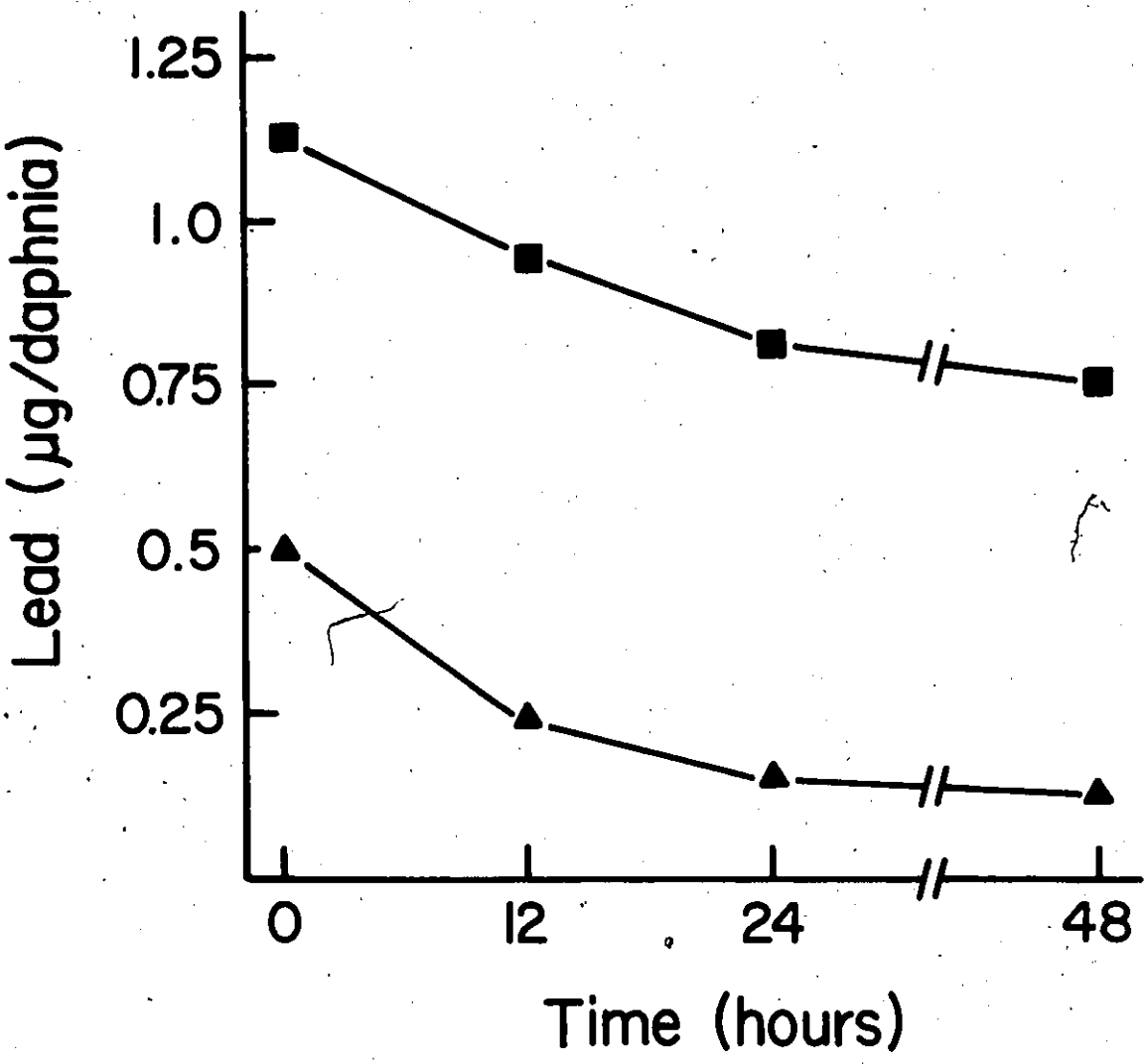

Lead uptake (µg/daphnia)
2.0
1.5
1.0
0.5
6
12
24
Time (hours)

With lead concentrations of 100 μg/L in the solution, A. falcatus accumulated about 9.5 $\mu g/10^6$ cells/L. Average lead residues in D. pulex were approximately 10,000 to 43,000 times greater than in the water. These values are up to four times greater than those found for cadmium uptake.

Release Experiments

Release of lead by the daphnids that had initially been fed on contaminated algae and transferred in a lead-free medium are given in Fig. 37. Also included in this figure are the data showing the release of metal that had been accumulated directly from solution. Both cadmium and lead curves show a slower rate of release from the daphnids having accumulated the metal through the diet. After 48 hours, the amount of lead remaining in daphnids fed with contaminated algae was about three times higher than the ones which were exposed to the metal from solution.

FIGURE 37 Daphnia pulex. Loss of lead upon return to lead-free conditions. Time 0 indicates time of removal to metal-free conditions. Triangles: lead in solution only; squares: lead in Ankistrodesmus falcatus only.


1.25
1.0
0.75
0.5
0.25
Lead (µg/daphnia)
0
12
24
48
Time (hours)

DISCUSSION

Heavy metal contaminants introduced into freshwater systems will not remain in solution in the water proper. As time progresses, their concentration in the water column will decrease by adsorption to the various compartments, sediments, detritus and plankton. In such a system, the transfer of heavy metals to the consumer level will presumably occur via different routes.

This study shows that the dietary route of metal uptake may be more important, especially at lower concentrations of the metal, than previously thought. Furthermore, the rate of depuration in daphnids is slower when the metal is provided through the food supply than when the metal is accumulated directly from the solution. This points to the importance of phytoplankton as a pathway of heavy metal accumulation by primary consumers.

The suggestion made by Gächter and Geiger (1979) that heavy metals seem not to be accumulated through the food chain, could be explained by the fact that they did not analyze quantitatively the specific differences between algal species; therefore, their results are based on the phytoplankton community as a whole. They did not take into account that zooplankton feed selectively on algae (Porter 1973; Gliwicz 1975; Nadin-Hurley and Duncan 1976), nor that phytoplankton may vary their capacity to sorb metals as shown in previous sections. One possible reason for their findings that zooplankton was found to have lower metal content than phytoplankton could be the possibility that inedible algae were mainly

responsible for metal binding in their communities.

The fact that cadmium accumulated by daphnids through the solution was lower in the sets where algae were present than in those without (Fig. 34) suggest that algal metabolites not retained by filtration did bind cadmium thus lowering its availability to the herbivores. In this respect studies by Murphy et al. (1976) provide evidence that blue-green algae are able to excrete selective chelators. This mechanism has not been reported for green algae. Recently De Hann et al. (1981) found a clear dependence between metal binding capacity and the growth and death of algae. Their results show that in some periods, algae were responsible for the binding capacity while in others they attribute the binding ability of the system to the excretion of metal chelators, particularly by Aphanizomenon sp. Therefore, metal binding by organic ligands represents another alternative to lower the metal availability for consumers. This mechanism is expected to be more significant after periods of algal blooms and when a high content of organic matter is present.

The release of metal observed upon transferring the daphnids to a metal-free system shows that depuration can be at least partially effective in lowering the metal concentration in contaminated cladocerans. However complete cleansing in the system, were it to occur, would require a long time. Depuration of the metal by Daphnia follows the same pattern in both metals, that is, a much slower decrease of the metal for the daphnids which accumulated the metal via the food supply. However, not all metals are necessarily released at the same rate. For lead, after 48 hours depuration (Fig. 37), the concentration in the zooplankters remains about three times

greater when the uptake was via the food vector than directly from the solution. Cadmium and lead accumulation by Ankistrodesmus at 1.0 ppm and 0.1 ppm respectively, do not differ greatly after 24 hours exposure, suggesting that accumulation of cadmium by the alga reaches a maximum value at concentrations lower than 1.0 ppm in the ambient water.

The dynamics of metal uptake and release by zooplankters during metal exposure is of particular interest because the concentration actually incorporated into the organism is likely to be critical in exerting sub-lethal effects in terms of biochemical, physiological and behavioral processes. Also it is the metal that is likely to be transferred to higher trophic levels. Concerning possible toxic effects, Urech (1979) found that the lower zooplankton densities observed in an artificially polluted field experiment were caused directly by the adverse effect of the metal and not indirectly by changes in food supply. With regard to the question of transfer of heavy metals to predators, it would be interesting to determine if the patterns observed in D. pulex accumulation are similar to other organisms of the trophic web. Since Daphnia species have been recognized as representative for toxicological work (Adema 1978) as well as ecological research (Porter 1977) at the herbivorous level, one would expect that accumulation of heavy metals will occur in a similar way and will depend in a good measure upon the dietary preference. However this aspect has not yet been well defined.

The question of whether the metal is bound to the cell wall, the plasma membrane or to the cytoplasma is not well understood. If the binding occurs in the cell wall one would expect a direct effect at the consumer

level, however, if the binding occurs mainly inside the algal cell the effect would probably interfer with the normal functioning of the cell and consequently changes in population densities would be expected. With respect to primary consumers the question of edibility would be of primary importance under low metal levels.

The present findings on accumulation and depuration of cadmium and lead by D. pulex are based on short-term exposure to the metal. Further research based on long-term experiments under similar conditions, and using other zooplankters such as rotifers and copepods, should allow one to investigate to what extent the amount of metals accumulated and retained after depuration represent an ecological hazard.

SECTION V

GENERAL CONCLUSIONS

The major conclusions to be drawn from this study are as follows:

A. Heavy metal binding is correlated with algal composition rather than algal biomass.

B. Neither morphology nor taxonomy of algae is related to heavy metal binding capacity.

C. The rate of heavy metal binding by unialgal populations confirms that algal specificity plays a major role in heavy metal binding in natural systems.

D. Dietary route is a significant pathway of metal transfer to primary consumers.

The field approach, using polyethylene enclosures under various treatments such as enriched conditions, reduction of grazing pressure and sediment interaction, appears to be adequate to monitor the various algal communities of a particular lake. The same type of approach is also valid for other experiments such as the maintenance of unialgal populations under field situations.

Despite the large number of species present throughout the experimental period, few algae were highly correlated with metal binding indicating that algal species compposition plays a major role in the binding capacity of freshwater systems. The algal specificity, however, is not related to a particular taxonomic group or to a particular morphology. This

suggests that the mechanism regulating heavy metal binding capacity by algae is more complex than a pure sorption process.

The bioavailability of heavy metals to aquatic systems is greatly influenced by particular species that tend to accumulate specific ions: depending on their edibility status they would either transfer or remove metals from the food chain. Herbivorous species and in particular Daphnia accumulate significant amounts of metals that will ultimately be transferred to their predators. The ultimate ecological implications of such mechanisms remain to be investigated.

Although it is clear that an integrated approach would be the ideal way to clarify the food chain relationship of metal bio-accumulation, useful information is being generated by individual scientists in various disciplines, studying different organisms at various trophic levels. Further research into the less understood areas of the mechanisms of heavy metal uptake and excretion, the relationship between toxicity and uptake, and the role of aquatic organisms in the cycling of heavy metals in aquatic ecosystems is essential to overcome the problems of metal pollution in lakes.

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APPENDIX I

PRESERVATION OF PHYTOPLANKTON SAMPLES

Lugol's Solution:

Potasium Iodide (KI)	10.0 g
Distilled Water	20.0 mL
Iodine (I_2)	5.0 g
Distilled Water	50.0 mL
Sodium Acetate	5.0 g (avoids destruction of calcareous structures).

APPENDIX II
MAJOR TAXONOMIC REFERENCES

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APPENDIX III

GROWTH MEDIUM

Bold Basal Medium	mg/L
$NaNO_3$	250
$CaCl_2.2H_2O$	25
$MgSO_4.7H_2O$	75
K_2HPO_4	75
KH_2PO_4	175
NaCl	25
EDTA	50
KOH	31
$FeSO_4.7H_2O$	4.98
H_2SO_4	.0001 ml/L
H_3BO_3	11.42
$ZnSO_4.7H_2O$	8.82
$MnCl_2.4H_2O$	1.44
MoO_3	.71
$CuSO_4.5H_2O$	1.57
$Co(NO_3)_2.6H_2O$	.49