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An in Situ Evaluation of Nutrient Effects in Lakes

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AN IN SITU EVALUATION OF NUTRIENT EFFECTS IN LAKES

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ABSTRACT

A method for performing *in situ* nutrient enrichment experiments on natural lake phytoplankton communities was developed and evaluated. One set of experiments in which it was employed was designed to detect limiting nutrients and to provide a basis for predicting future experiment results. Productivity increased in response to all three of the treatment variables used, N, P, and EDTA, but response patterns varied from experiment to experiment. Individual species responded differently to different treatments, and interactions among the treatment variables were important in shaping the community responses to mixtures of two or three variables. The most consistent features of the productivity results were incorporated into a "most probable response pattern," which was partially validated by a second series of experiments.

The second experiment series was also used to test the ability of NTA to stimulate phytoplankton productivity. Stimulation was continually obtained.

In a third series of experiments sewage effluents were tested in parallel with N and P. Varying degrees of overlap between the species complexes responding to the sewage and to the N and P treatments were found.

Recommendations for the use of *in situ* enrichment experiments in eutrophication studies are presented.

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CONCLUSIONS AND RECOMMENDATIONS

PROCEDURAL ASPECTS OF IN SITU NUTRIENT ENRICHMENT EXPERIMENTS

Statistical Design

Growth of phytoplankton in samples of natural waters was frequently stimulated by more than one nutrient at a given time, and interactions among different nutrient treatments frequently appeared. Therefore it was concluded that in experiments intended to determine what may be limiting phytoplankton growth in a natural system, treatments with several potentially important nutrients should be employed. The factorial statistical design is particularly useful in multivariate experiments of this type because it permits identification of interactions as well as independent effects.

Response Measurement

The ultimate response of interest in an enrichment experiment is algal growth. Since growth is difficult to measure directly in natural communities productivity is used as a rapidly obtainable index of growth. In this study the validity of productivity as a growth index was verified in numerous comparisons between productivity and cell count data. However, because of published reports of inconsistencies between productivity and cell count results the relationship should be tested routinely in any study employing in situ nutrient enrichment experiments. Experiments should be conducted for several days in order to allow detectable growth responses to occur and in order for changes in productivity response patterns to be followed.

Sensitivity

The sensitivity of an in situ enrichment experiment in detecting treatment effects depends strongly upon the degree of replicability attained between experimental units receiving identical treatments. In this study, within-treatment replicability was continually improved so that in some of the later experiments productivity elevations of as little as 10% over the control levels represented statistically significant treatment effects.

INTERPRETATION OF RESULTS

Variability in Response Patterns within Experiments

In this study, day to day changes in productivity response patterns within an experiment were found to reflect changes in the relative contributions of individual species to community productivity. Therefore, all productivity responses detected in an experiment, whether or not they persisted throughout the experiment, were assumed to contribute to the accumulation of algal biomass and were regarded as legitimate treatment effects.

Variability in Response Patterns between Experiments

Within series of similar experiments conducted in the ice free seasons of 1968, 1969, and 1970 certain features of the response patterns were relatively consistent, while other features varied from experiment to experiment. The consistent features appeared to relate to physio-chemical environmental conditions, while the variations could be attributed to changes in the species composition of the lake phytoplankton. The composition of the total community shifted gradually from experiment to experiment, while the composition of the fraction of the community that responded to the nutrient treatments shifted abruptly. Species that responded to treatments in one experiment did not necessarily respond when they were present in others. It was concluded that the best way to overcome the variability among experiments was to conduct enough experiments within any temporal series to determine tne consistent features, and use these features to characterize the average response.

TREATMENT EFFECTS

Selectivity of Treatment Effects

When a treatment effect is detected by productivity measurements it does not necessarily mean that the entire algal community responded to the treatment. Actually, nutrient treatments in in situ enrichment experiments are highly selective, and in many experiments in this study only one species was found that could account for the productivity responses. Productivity measurements detect the quantitative component

of community response; cell counts are necessary to determine the qualitative component.

Individual species did not necessarily respond to treatments in all experiments in which they were present. There was some suggestion, however, that a given species was more likely to respond during a period of ascendancy in the natural community than during periods of relatively stable population size. Thus it did not often happen that the species shifts in the experiment containers resulted in dominance by a normally rare form, a result reported by Thomas (1964).

The fact that treatment effects were highly selective implies that it cannot be concluded that a nutrient that stimulates productivity in an in situ enrichment experiment is limiting community productivity. It may be limiting the growth of the species that respond to it. This point is particularly evident when it is considered that species that did not respond to the nutrient treatments often were able to continue growing in the control communities, in the absence of enrichment.

In Third Sister Lake species of bluegreen algae responded more frequently than species of other groups, implying that enrichment of the lake could favor dominance by bluegreens.

Importance of Interactions

Interactions among the nutrient treatments frequently appeared in the productivity data. The most consistently important interaction was synergism between N and P. Species counts verified that the interactions occurred on the species level as well as on the community level. Interactions of the types observed undoubtedly contribute to the overall effects of nutrient mixtures entering natural waters.

Stimulation by Chelators

Both EDTA and NTA stimulated productivity in most of the experiments in which they were employed. Their effects were generally independent of the effects of accompanying N and P treatments. When both chelators were tested at equal molarities in the same experiment their effects usually differed in intensity. EDTA, whose complexes with metals have higher stability constants than those of NTA, usually stimulated more strongly. Occasionally one compound stimulated and the other did not, implying that their effects may have differed

in mechanism as well as in intensity.

APPLICATIONS OF IN SITU ENRICHMENT EXPERIMENTS

Identification of Limiting Nutrients and Prediction of Future Responses

One application of *in situ* enrichment experiments is to identify growth-limiting nutrients and to develop background data on the responses of the phytoplankton community to additions of those nutrients, that can be used to predict future responses. In this study the results of two years of experiments were used to develop a "most probable response pattern," and this pattern was compared to the results of a third year of experiments.

The experiments of the first two years indicated that all three of the nutrient variables employed could stimulate algal growth, and that their order of importance as growth stimulators was P>EDTA>N. The prediction that this order would be maintained in the following experiment series was successful, but more specific predictions regarding seasonal variations of the response patterns were not. Thus it was concluded that *in situ* enrichment experiments could be used to identify important controlling nutrients in natural systems, but their results could be used to make only very general predictions concerning when or how additions of these nutrients are likely to stimulate algal growth.

From analyses of the behavior of individual species in the experiments it was concluded that treatment effects on the species level could not be predicted as readily as treatment effects on the community level. One reason is that similar productivity response patterns were obtained in different experiments in which different species groups responded to the treatments. A second reason is that individual species did not necessarily respond similarly in different experiments in which they were present.

Testing of a Potential Environmental Contaminant

This application was attempted for NTA, and it was found that this substance could stimulate algal productivity fairly consistently at a treatment level of .252 mg/l. Side experiments indicated that NTA stimulation was due more probably to a chelation mechanism than to its

utilization as a nitrogen source.

The effects of the NTA treatments varied from experiment to experiment. Therefore in this as well as in the first application of in situ enrichment experiments, it is important that a series of experiments be performed if the "average" effect is to be determined. Several experiments are necessary, also, to avoid the possibility of concluding from one or two experiments with negative results that the treatment is biologically inert, when under other conditions or for other species it may be active.

Interpreting Stimulation by a Natural Nutrient Mixture

A third application of in situ enrichment experiments is to interpret the stimulatory effect of a natural nutrient mixture in terms of its components. This was attempted for samples of sewage effluents in several experiments. Effects of the sewage treatments tended to exceed the effects of parallel treatments with N and P. It was found that because of the high selectivity of treatment effects the extent to which N and P contributed to the sewage effects could not be reliably determined by comparing only productivity responses to the treatments. In one experiment responses to treatments with sewage and with a known nutrient mixture were similar in terms of productivity, but when individual species growth responses were determined it was found that entirely different groups of species responded to the two treatments. Two other experiments revealed different degrees of overlap between the species complexes responding to the sewage and to the nutrient mixtures. Thus when the effects of treatments are compared in this way, species counts must be done to avoid drawing erroneous conclusions from the productivity results.

Although the high selectivity of treatment effects complicates the comparison of productivity responses to two treatments, it is the main reason why in situ bioassays can be used very effectively in interpreting stimulatory effects of complex nutrient mixtures. It is conceivable that if enough of the components of the mixture were tested, separately and in combinations, most of the stimulatory effects of the mixture could be explained.

Examination of the species data for one of the experiments suggested that interactions between nitrogen and

phosphorus, on the species level, contributed to the overall sewage effect.

CONTAINMENT EFFECTS

Community Level versus Species Level

Containment effects appeared on the community level as declines in productivity with time in the experimental vessels or as changes in productivity in the vessels relative to productivity in the lake. On the species level the containment effects were highly selective, with different species exhibiting many different patterns of response. Responses often changed from experiment to experiment for the same species. The resultant of the individual species responses was a shift in the species composition of the experimental communities.

A species that did not decline but remained stable in a control community was not necessarily neutral to containment, since it may have increased in the lake. Thus the true measure of a containment effect is the difference in the fate of the species in the jug from its fate in the lake.

Responses to Treatments and Containment

Species that responded to the nutrient treatments were usually neutral to containment or responded positively to it, but occasional positive treatment effects in conjunction with negative containment effects appeared.

Consistent Sensitivity to Containment

Of the algae that exhibited negative containment effects, the taxonomic group that was the most sensitive was the Cryptophyta. One species, Chroomonas acuta, was present in the initial community of almost every experiment, but almost invariably disappeared from the experimental vessels by the end. Since this species was often very abundant in the initial communities it can be concluded that the jug environment, at least for Third Sister Lake phytoplankton, tended to make abundant species rare more often than it made rare species abundant.

Dependence on Type of Container

Containment effects depend to some degree on the type container employed. In this study species responded in different ways to a closed 19 liter jug suspended in

the lake and to an open 4 liter jar incubated in a water bath beside the lake.

Rate of Development

Containment effects develop rapidly, often within a few hours of the start of incubation.

Effects on Application of Results

Superficial analysis of results, ignoring containment effects, is sufficient for approaching general questions such as whether a treatment nutrient is limiting some member of the community or could stimulate productivity in the natural system. Because of the uncertainties involved, however, in situ enrichment experiments employed in eutrophication studies should be used in conjunction with other evaluation procedures, such as studies of nutrient dynamics in the natural system or physiological assays for nutrient limitation, and should not be used as the sole basis for conclusions.

SECTION II

INTRODUCTION

THE ENRICHMENT PROBLEM

The biological productivity of a body of water is influenced by many factors (Rawson 1939). Among these are its morphometry, the climate in which it is found, and its content of dissolved and suspended matter, loosely referred to as its "water quality." Morphometry and climate tend to change relatively slowly, but water quality can change rapidly in response to changes in the surrounding drainage basin. Man is an increasingly widespread and potent effector of changes in drainage basins, but he has failed to control his activities sufficiently to avoid unplanned consequences in neighboring waters. As a result numerous pollution problems have developed that reduce the suitability of natural waters for indigenous organisms and for use by man.

One consequence of uncontrolled human activities can be the enrichment of a water body with plant nutrients, a process referred to as "eutrophication" (Stewart and Rohlich 1967). The relationship between human activities and the release of nutrients into natural waters is most easily established where sewage effluents enter lakes and streams (Hasler 1947), less readily confirmed where fertilizers applied to agricultural soils are washed in (Stanford et al. 1970), and even more obscure where natural terrestrial nutrient cycles are destroyed, permitting soil nutrients to escape into drainage waters (Bormann and Likens 1970).

Eutrophication is not inherently bad, and in fact it has been done deliberately in many instances in attempts to increase fish production. However, there have been enough cases of inadvertent fertilization resulting in undesirable changes in natural waters that nutrient enrichment is now recognized as one of our most serious water quality problems.

Most of the unfavorable consequences of eutrophication are not direct effects of the nutrients themselves, but side effects of resultant excessive growths of aquatic plants (Lee 1970). Excessive algae in drinking water supplies can increase the clogging rate of sand filters, increase the chlorine demand of the water, and contribute color, tastes, and odors to the finished water. In recreational lakes algal accumulations can

impair aesthetic qualities as well as cause deterioration of fisheries. In the long run eutrophication accelerates the rate at which lakes fill up with sediment, thereby hastening their extinction.

NUTRIENT MANAGEMENT

The pressure for corrective action has become sufficiently intense for government agencies to become involved (e.g. U.S. Congress 1970, Bueltman et al. 1969), and it has been encouraged by recent reports of the rapid improvement of Lake Washington following elimination of the inflow of sewage treatment plant effluents (e.g. Edmondson 1970). In this case the complete effluents were diverted to a different drainage basin, solving the problem by exporting it rather than by instituting improved methods of nutrient management.

In most cases effluents cannot be exported but must be managed more effectively within a drainage basin. The ultimate approach will most probably have to be recycling of waste water into drinking water, without releasing wastes into natural waters, but this is in the future. The nutrient control method that is the most compatible with contemporary wastewater management practice, the foundation of which is the production and discharge of an effluent, is removal of nutrients prior to effluent release. This can be done by reducing the levels of nutrients entering wastewaters, the approach that is the basis of the current pressure for removal of phosphates from detergents, and by improving wastewater treatment to remove the nutrients that cannot be initially excluded.

It is generally believed that not all of the nutrients that enter natural waters as a result of human activities are responsible for the resultant problems. This is because not all nutrients are present in natural waters in the proportions required by plants. Natural supplies of some nutrients are in great excess relative to supplies of other nutrients, and their abundance is therefore unlikely to be limiting to plant growth. Wastewater inflows are more likely to enhance aquatic plant growth by supplying nutrients that are naturally scarce than nutrients that are naturally abundant, and it is logical to conclude that removal of the naturally scarce nutrients from wastewater should improve conditions in receiving waters.

A further point that must be considered, however, is

that there are many sources of nutrient enrichment other than wastewaters, and some of them, such as rainfall, are impossible to control. Consequently it is not sufficient to identify the scarcest nutrient in a given water body and remove it from wastewater inflows, but it is also necessary to compare the extent to which the total inputs of different nutrients can be reduced by removing them from wastewater.

An example of the integration of these two aspects of enrichment, nutrient limitation and nutrient controllability, is provided by a study of eutrophication in the Potomac estuary (Jaworski et al. 1971). Nitrogen was found to be more influential than phosphorus in controlling phytoplankton growth, but phosphorus input could be reduced more effectively than nitrogen input by improved waste treatment. Therefore in the proposal for future nutrient management phosphorus removal from effluents was emphasized more strongly than nitrogen removal.

As long as the approach to nutrient management is to be selective, emphasizing control of only the key substances, there is a need for methods that can be used to evaluate each body of water for which nutrient control is desired. In order to alleviate existing problems techniques are needed for (1) identifying limiting nutrients, (2) identifying nutrient sources, (3) identifying the substances in nutrient sources that are stimulating growth, and (4) evaluating the effectiveness of nutrient management practices. In order to protect aquatic communities from future problems methods are needed for (1) evaluating the sensitivity of an aquatic system to additional enrichment and (2) screening potential future environmental contaminants for possible effects.

RESEARCH METHODS

Limiting nutrients can be identified with some degree of confidence by investigating nutrient changes in the system of interest. For example phosphorus, rather than nitrogen, was identified as the key nutrient in the eutrophication of Lake Washington (Edmondson 1970) because its decline subsequent to nutrient diversion was more closely correlated with the decline in phytoplankton biomass, whereas nitrogen was found to be more influential than phosphorus in the coastal waters off New York City, where nitrogen levels decline more rapidly than phosphorus levels with distance from the harbor (Ryther and Dunstan 1971). In the former case, however,

the limiting nutrient was identified after its input had been curtailed.

Several techniques applicable to eutrophication studies employ algae as analytical tools. One group of these can be classed as biochemical assays, because they exploit various biochemical traits of algae to assess their nutritional state. For example limitation of growth by phosphate can be inferred from (1) high activity of alkaline phosphatase enzymes that are produced by phosphate deficient cells and function by releasing inorganic phosphate from organic compounds or (2) essential absence of the intracellular stores of surplus inorganic phosphates that are characteristic of cells growing in an environment rich in phosphate (Fitzgerald and Nelson 1966). In a similar way nitrogen limitation can be inferred from (1) high rates of ammonia utilization in the dark (Fitzgerald 1968) or (2) low activity of the nitrate reductase enzyme (Eppley et al. 1969). However, these techniques are difficult to apply to natural diverse algal communities because of the physiological variations that occur among species. Thus a "high" enzyme level for one species may be "low" for another species. Also, different species within a community may be limited by different nutrients at the same time (Fitzgerald 1969), precluding any conclusion about what is limiting the community. Nonetheless they have been used successfully to complement other types of measurements, such as in the Potomac study (Jaworski et al. 1971) where high rates of ammonia utilization in the dark in conjunction with low alkaline phosphatase activity and the presence of stored phosphate in algal samples supported the conclusion that nitrogen was limiting in the reaches where ambient nitrogen levels were low.

A second group of approaches employing algae, which can be applied to most of the questions that arise in eutrophication studies, consists of the productivity bioassays, or enrichment experiments. In these techniques algal growth in response to a treatment is measured, and the methods vary according to the algae used (single species cultures or samples of natural communities) and the growth conditions (batch or continuous culture, laboratory or in situ incubation). The Joint Industry-Government Task Force on Eutrophication (Bueltman et al. 1969) has been developing a series of standardized "algal assay" methods, concentrating so far on a bottle test employing separate cultures of four standard species grown under laboratory conditions (Environmental Protection Agency 1971). The procedural and statistical

aspects of the method have been intensively studied, but the problems of using the results to evaluate or predict events in natural waters have not yet been resolved.

The second method that is under study is similar to the first, only it employs continuous rather than batch culture conditions. The third method, however, differs fundamentally from the other two in employing samples of natural algal communities as the test organisms.

In enrichment experiments employing single species cultures the results are easy to interpret because the only difference between a treatment and a control culture is the nutrient treatment itself. However the results are difficult to apply to natural waters where environmental conditions are different, cell densities are generally lower, and many different species are present (Lund and Talling 1957). This last difference is particularly important in interpreting negative results of single species bioassays, and its importance relates to the fact that nutrient requirements vary greatly among different algal species (Chu 1942, 1943; Rodhe 1948). If a sample of lake water is filtered to remove the natural algae, inoculated with a test species, and spiked with a treatment substance, stimulation of growth can be safely interpreted as an indication that the test substance is biologically active and that it could stimulate algal growth in the natural system. If growth in the culture is not stimulated, can it be concluded that growth would not be stimulated in the natural system? Not necessarily, for some other species present in the natural community could respond.

When samples of the natural community are enclosed in containers, treated with nutrients, and incubated in situ; natural cell densities are maintained, numerous species are present, and environmental conditions such as light and temperature are fairly natural. Thus it seems that results of this type of experiment would be much easier to apply to the natural system than are the results of single species laboratory tests. It is true that the difference between a treatment and a control experimental unit in an in situ enrichment experiment is still just the nutrient treatment. However, there are differences between the environment in the control unit and the environment in the natural system that act as "treatments," and that are not evaluated by the normal statistical procedure of comparing control units with treatment units. An in situ enrichment experiment is a nested procedure in which the true control is the

natural water body, the first set of treatments, which is applied similarly to all experimental units, is a consequence of containment, and the second set of treatments consists of the nutrients added according to the experimental design. The magnitudes of the effects of containment influence the validity of using the responses to the nutrient treatments in evaluating or predicting events in the natural system.

Although the in situ technique is not as "direct" an approach to studying algal nutrition in natural systems as it seems on the surface, it still has appeal mainly because it employs the natural species assemblage in the system being studied. Thus, for reasons discussed above, it should be more sensitive in detecting treatment effects than the single species laboratory method, and more conservative in protecting natural waters.

Enrichment experiments employing samples of natural phytoplankton communities have been used by many investigators to study nutrient limitation in natural systems (e.g. Goldman 1960a, Biesinger 1967, Thomas 1964, Wetzel 1966, McLaren 1969, Kemmerer 1968, Hutchinson 1941, Gächter 1968, Schelske and Stoermer 1971, Powers et al. 1972, Moss 1969, Hamilton 1969, Tranter and Newell 1963, Menzel and Ryther 1961), but few thorough evaluations of the methods have been performed, and few guidelines for their application are available. If an in situ technique is to be incorporated into the standard Algal Assay Procedure, statistical properties and containment effects need particular attention so that "the results can be applied with judgment to field conditions" (Bueltman et al. 1969).

OBJECTIVES

The initial objective of this study was to develop a method for performing in situ enrichment experiments with samples of natural lake phytoplankton. Statistical properties were to be evaluated, and effects of containment were to be investigated.

The resulting method was to be used in a lake for three interrelated studies. In the first of these a series of similar experiments was to be performed and the results analyzed in an effort to evaluate (1) the ability of the method to detect limiting nutrients and (2) its predictive potential. In the second a potential environmental contaminant was to be evaluated for its

ability to stimulate algal growth. In the third the method was to be employed in an effort to interpret the stimulatory effects of some natural nutrient mixtures in terms of the effects of identifiable components. It was hoped that from the results of these studies guidelines for the utilization of *in situ* enrichment experiments in the field of eutrophication could be developed.

CHOICE OF VARIABLES

Identifying Limiting Nutrients and Evaluating Predictive Potential

In most of the enrichment experiments performed by previous investigators nitrogen or phosphorus or both have had stimulatory effects (e.g. Goldman and Carter 1965, Edmondson and Edmondson 1946-7, Menzel and Ryther 1961, McLaren 1969, Kalff 1971, Kemmerer 1968, Wetzel 1966, Hutchinson 1941, Gächter 1968, Lange 1971, Thomas 1964, Powers *et al.* 1972). As there is little doubt that these two elements have major roles in determining the production, periodicity, and species composition of primary producer populations (Lund 1965), they were natural choices for use as variables in the present study. Treatment additions were chosen to cause ecologically reasonable increases in N and P over the ambient lake levels.

In a number of enrichment experiments in which nitrogen and phosphorus were tested independently and in mixtures, their effects in the mixtures were found to be interdependent. For example in an experiment by Hutchinson and Riley (Hutchinson 1941) phosphorus or nitrogen treatments added to separate jugs of Linsley Pond water stimulated phytoplankton growth, but stimulation by a mixture of the two nutrients greatly exceeded the sum of the effects of the separate additions. This was one of the first published examples of a synergistic interaction between nitrogen and phosphorus, exhibited on the community level. In a more recent study by Goldman and Armstrong (1969) Lake Tahoe phytoplankton samples were treated with several combinations of nitrate and phosphate levels. Maximum growth of the dominant species, *Fragilaria crotonensis*, occurred in samples receiving 20 µg/l of nitrate-N and 2 µg/l of phosphate-P. The same phosphorus treatment added along with 10 µg/l of nitrogen produced little or no response. In this case the nitrogen-phosphorus interaction was detected on the species level.

While these and other studies demonstrated that nitrogen

and phosphorus treatments could interact synergistically in stimulating algal growth they did not reveal the mechanism of the interaction. Evidence of a direct physiological link had appeared in some work by Ketchum (1939) who found that the rate of phosphate absorption by cultures of Nitzschia closterium was dependent upon the concentration of nitrate in the medium. A reverse influence was suggested later by the discovery by Eppley et al. (1969) that the activity of nitrate reductase extracted from Ditylum brightwellii was dependent upon the concentration of phosphate present.

From these two lines of evidence, enrichment experiments and physiological studies, it was concluded that interactions between nitrogen and phosphorus may occur whenever nutrient mixtures containing available forms of both of these elements enter natural waters. Therefore it was decided to employ mixtures as well as independent additions of nitrogen and phosphorus as treatments in the present study, so that interactions could be detected.

Although nitrogen and phosphorus are undoubtedly important contributors to the effects of natural nutrient mixtures on algal growth, efforts to attribute the stimulatory abilities of such mixtures entirely to their nitrogen and phosphorus contents have generally been unsuccessful. Rodhe (1958) reported that in experiments involving additions of small amounts of phosphate, nitrate, and hypolimnetic water to samples of natural phytoplankton communities ^{14}C uptake after 24 hours was increased by as much as 80% by the hypolimnetic water while N and P never caused increases greater than 30%. Goldman and Armstrong (1969) reported similar results in experiments employing treatments with N, P, and water from enriched streams entering Lake Tahoe: the stream samples produced more stimulation than their N and P contents could explain. In both of these studies it was hypothesized that unknown organic compounds present in the nutrient mixtures could have been responsible for the growth stimulation above that produced by N and P.

One mechanism by which certain organic compounds can influence algal nutrition is chelation of micronutrients (Saunders 1957), and it has been demonstrated that chelating agents added with or without micronutrients to samples of natural waters can stimulate algal growth (Schelske 1960, Johnston 1964). In a study reported by Wetzel (1966) additions of EDTA (ethylene diamine tetraacetic acid) to water samples from marl lakes in

Indiana interacted indirectly with added phosphate, resulting in stimulation of algal productivity. The EDTA presumably chelated excess cations that otherwise would have rendered the phosphate unavailable by precipitation. Because of the possible role of such chelators in natural nutrient sources, and because of the demonstrated effects of EDTA in enrichment experiments it was decided to include EDTA as a third variable, in addition to N and P, in this phase of the present study.

Testing a Potential Environmental Contaminant

In recognition of the necessity for the complete or partial removal of phosphates from their products, the detergent industries have been evaluating potential alternative builders for a number of years (U.S. Congress 1969). One compound, NTA (nitrilotriacetic acid), has been under serious study since the early 1960's (Duthie 1972), and consequently has been under scrutiny by government agencies in the U.S. and Canada as a potential environmental contaminant.

Because of the potential importance of this compound, and in consideration of the results of the experiments with the related compound EDTA, it was decided in the spring of 1970 to initiate experimentation with NTA. A sample of trisodium nitrilotriacetate was obtained for this purpose from R.N. Sturm of the Proctor and Gamble Company, Research and Development Department, on May 4, 1970.

It has been estimated that under full utilization by the detergent industry NTA would be produced at the rate of over one billion pounds per year, and could enter sewage treatment plants at levels of 8 mg/l (Shumate et al. 1970) to 20 mg/l (Hamilton 1972). Average levels in surface waters have been anticipated to approximate .05 mg/l (Sturm and Payne 1971), but actual levels, of course, would vary widely depending on proximity to sources.

The potential for NTA to exert an environmental impact would depend on its biodegradability in sewage treatment plants and in natural waters, and on the nature of its degradation products. The compound was found by Thompson and Duthie (1968) to be readily broken down in sewage treatment plants, where it is claimed to degrade to CO_2, H_2O, and inorganic nitrogen compounds. Bunch and Ettinger (1967) suggested that the rapid

breakdown of NTA by sewage organisms was related to the presence of only one nitrogen atom in the molecule, since several other chelators, including EDTA, containing two or more nitrogens persisted much longer under their test conditions. Biodegradation of NTA in Ohio River water was studied by Thompson and Duthie(1968), who found that after acclimatization periods of 8-12 days organisms in river water samples could remove doses of NTA in 2-6 days. Similar results were obtained by Warren and Malec (1972) for Detroit and Meramec River waters.

Tests of the effects of NTA on algal growth by several investigators have yielded a variety of results. Studies employing single species cultures (Christie 1970, Sturm and Payne 1971) indicated little or no effect of NTA treatments. Studies employing natural species assemblages (Mitchell 1971, Sakamoto 1971, Goldman 1972), however, frequently detected stimulatory effects, demonstrating the greater sensitivity of multispecies communities used in testing potential pollutants. The effects in these studies were attributed to enhancement of the availability of iron chelated by the NTA. This hypothesis was supported in the study by Sakamoto (1971), in which NTA added to lake water increased the amount of iron passing through a filter.

Moreover, interest in studying the ability of NTA to stimulate algal growth was by no means unique to this investigation. Objective evaluation of its potential environmental significance should be based on a large amount of data, covering many different systems in many different geographical regions. In this sense there is no duplication of effort.

Interpreting Stimulation by a Natural Nutrient Mixture

It is well established that sewage treatment plant effluents characteristically contain biologically available forms of nitrogen and phosphorus (Mackenthun et al. 1964), organic compounds that have chelating properties (Vallentyne 1957), and numerous other biologically active substances. Therefore it was decided to conduct a series of side experiments in which effects of known nutrient mixtures and effects of sewage effluents could be compared, permitting assessment of the contributions of the components of the known mixtures to the stimulatory effects of the effluent samples.

Effluent samples for this purpose were obtained from

the Ann Arbor, Michigan sewage treatment plant, a secondary treatment plant employing the activated sludge process. Samples were taken from a point just preceding the chlorination unit.

STUDY SITES

Third Sister Lake

All but one of the enrichment experiments were performed in Third Sister Lake, Washtenaw County, Michigan. This lake has a surface area of 3850 sq m (Eggleton 1931) and a maximum depth of 16.5 m, and its drainage area is partly forested and partly agricultural land. It stratifies sharply in the summer, and the hypolimnetic oxygen becomes severely depleted during this period. The phytoplankton community is continually dominated by cryptophyte flagellates and a mixture of species of green and bluegreen algae. Further background data will appear in the discussion of results.

Crystal Lake

One experiment was performed in Crystal Lake, Benzie County, Michigan. With a surface area of almost 4×10^7 sq m and a maximum depth of almost 50 m (Michigan Conservation Department 1940), this lake provided a very different research environment from that of Third Sister Lake. This lake is regarded as a valuable recreational asset to the State of Michigan, based largely on the high clarity of its water resulting from low phytoplankton productivity. The dominant species at the time of the experiment were diatoms.

SECTION III

METHODS

REQUIREMENTS OF THE FIELD METHOD

Statistical Design

A review of the pertinent literature indicated that interactions among the three variables N, P, and EDTA could occur when they were applied in mixtures to experimental phytoplankton communities. Therefore a statistical design capable of detecting interactions as well as independent treatment effects was needed. The complete factorial design, illustrated in Table 1, satisfied this requirement.

TABLE 1

2 x 2 x 2 REPLICATED FACTORIAL DESIGN

Variable	Dose (µg/l)							
Nitrate-N	0				25			
Phosphate-P	0		5		0		5	
EDTA	0	500	0	500	0	500	0	500
No. of Replicates	2	2	2	2	2	2	2	2

The design presented in the table consists of three variables, each at two treatment levels. Eight combinations of the different treatments are possible, and all of these were included in the design. Each combination was applied to at least two experimental units, which for our purposes were identical lake water samples. After responses of the experimental communities to the treatment combinations were measured, the results were subjected to analysis of variance procedures which separated all possible individual or interactive effects and indicated their statistical significance.

In order for analysis of variance techniques to be applicable the data must satisfy three conditions: (1) They must be normally distributed. (2) The variance due to experimental error must be separable from that due to treatment effects. (3) The error variances of

the different treatment communities must be homogeneous. Raw data which do not possess these properties can be changed to other forms such as logarithms or square roots to make them suitable for analysis (Barnes 1952).

In the present study, the separability of error and treatment variances was assured by providing duplicate experimental units for each treatment combination (Table 1). The raw data from each experiment were tested for normality and variance homogeneity by plotting the standard deviations within the pairs of treatment replicates versus the treatment means. The corner test (Walker and Lev 1953) was performed on each plot to check for dependence of the standard deviation on the mean. Normally distributed data exhibit no relationship between the standard deviation and the mean, so when the plot showed this lack of relationship transformation was regarded as unnecessary. When a relationship was observed, log transformation was performed to eliminate it.

Computer facilities at the University of Michigan and at the Virginia Institute of Marine Science were utilized in the analysis of the data.

Incubation of Experimental Communities

Container. Once the statistical design had been selected the physical problems of applying it to natural phytoplankton communities were confronted. In situ enrichment experiments have been performed employing vessels ranging in size from 500 ml flasks (Goldman 1960 a) to 4000 liter plastic bags (Schelske and Stoermer 1971). Small containers are objectionable because they limit the size and number of subsamples that can be taken and because of their high surface area to volume ratios, which can exaggerate the effects of containment. Extremely large containers, on the other hand, are difficult to manipulate and, more important, are difficult to mix. Thorough mixing of the contents of a container is necessary if subsamples are to be representative.

A further point that was considered in selecting a container was the problem of distributing the experimental community uniformly among the treatment vessels at the beginning of an experiment. Uniform distribution was necessary in order to assure that treatment effects would not be obscured by initial discrepancies. It was concluded from a study by Jackson and Bender (1964) that this could be accomplished best by initially

isolating, in a single tank, all of the lake water to be used. The contents of the tank could then be mixed continuously while the treatment vessels were filled from it.

The experimental vessels selected were 19-liter (5 gallon) Pyrex® glass jugs. They were large enough to permit repeated subsampling and to, hopefully, avoid or retard the development of at least some containment effects. They were small enough to be conveniently handled and to permit a large number of them to be filled from a reasonable sized tank. Choice of the jugs was strengthened by a report by Abbott (1966) who concluded that his set of 18 jugs exhibited sufficient statistical replicability for use in aquatic research.

Depth. In previous studies experimental vessels have been incubated at the lake surface (Goldman 1960 b, Biesinger 1967) as well as at various depths in the water column. For the present study the lake surface was rejected for incubation because of reports in the literature (e.g. Edmondson 1956, Wetzel 1966) of depression of photosynthesis by surface light intensities. This phenomenon was subsequently demonstrated for Third Sister Lake phytoplankton on numerous occasions.

The alternative was subsurface incubation at some depth in the epilimnion. It was desirable to operate in the epilimnion because it is in this layer that most of the photosynthetic activity occurs in a natural lake. Furthermore there is a depth interval in this layer within which the rate of photosynthesis is maximal, or light saturated, during most of the day, and it is there that nutrient deficiencies are most likely to develop. Assuming that nutrient deficient algae would be the most responsive to experimental nutrient treatments, it was decided that the zone of light saturation would be optimal for incubation.

For the initial experiments the depth of the shoulders of the jugs was set at 1 m below the lake surface. This choice was based on a statement by Edmondson (1956) that the maximum rate of photosynthesis will lie between 0.3 and 1.7 m in a "least clear" lake and between 2.0 and 6.4 m in a "most clear" lake. It was estimated that Third Sister Lake was closer to "least" than to "most" clear.

Subsequent ^{14}C productivity profiles confirmed that 1 m was within the zone of light saturation in Third Sister Lake on most clear days. In 1969 profiles were

determined from measurements made at 1 m intervals, and the maxima usually appeared near 1 m depth, with depression of uptake at the surface (Figure 1). In 1970 four near-surface profiles were measured in order to more clearly define the regions of light inhibition and saturation (Figure 2). Samples were taken from jugs suspended at 1 m depth and were incubated at 20 cm intervals from the surface to 2 m, for 4 hours bracketing midday. Except for one day that was extremely overcast the depth interval occupied by the jugs was within the zone of light saturation. Surface depression occurred on all days.

In 1971 a series of 5 productivity profiles was measured on one day to delineate the depth zones of light inhibition, saturation, and limitation, and to indicate the variations of these phenomena with time. Figure 3 presents the temperature and percent light transmittance profiles for that day. Light penetration was measured with a submarine photometer (G.M. Manufacturing and Instrument Company, model 268 WA 310). Figure 4 shows the productivity profiles plotted separately for each time interval of incubation. In Figure 5 productivity at each depth is plotted as a function of time and, concurrently, of incident surface light intensity. Light intensity was measured with a Solar Radiation Recorder (Weather Measure Corporation, model R401), and the value plotted in each time interval is the maximum recorded during that interval. Light inhibition is evident at the surface, where the shape of the productivity distribution in Figure 5 approximates the mirror image of the incident light curve. Light saturation during the central 3 time intervals is evident at 1 m and 2 m, while light limitation, indicated by productivity distributions that closely follow the light intensity curve, shows up at 4 m and below.

Response Measurements

Method. The final major question to be resolved concerned the types and frequencies of response measurements to be made. The actual response of interest was algal growth, but this is difficult to measure directly at cell densities characteristic of natural waters (Lund and Talling 1957). Consequently, numerous indirect methods have been employed, including chlorophyll changes (Hutchinson 1941), oxygen production (Edmondson & Edmondson 1946), ^{14}C uptake rates (Goldman 1960a, 1960b, 1964; Goldman and Wetzel 1963; Goldman and Carter 1965; Schelske 1960; Biesinger 1967; Wetzel 1966), and combinations of these (McLaren 1969: chlorophyll and ^{14}C uptake, Kemmerer

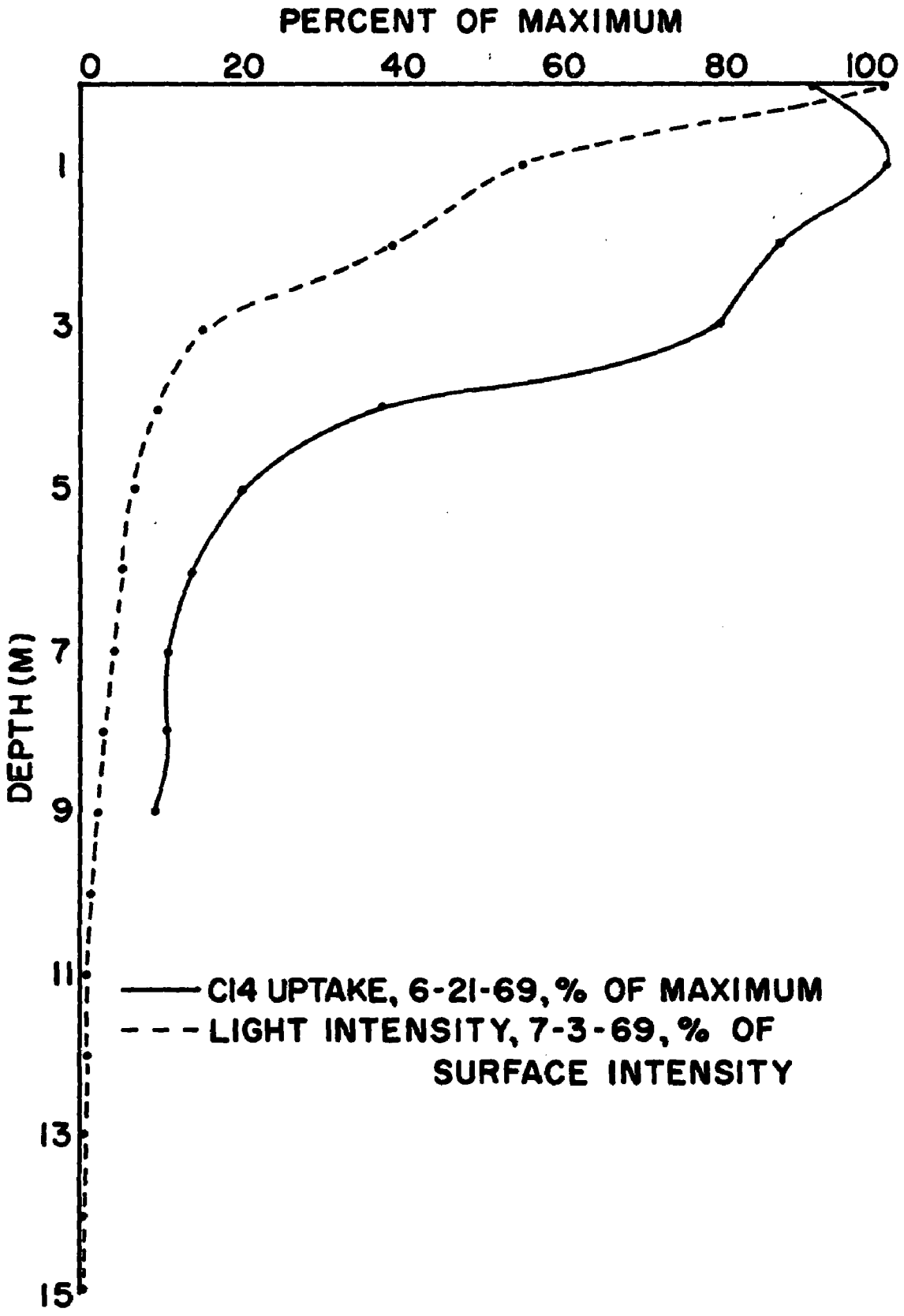


Figure 1. Representative Summer Photosynthesis and Light Intensity Profiles in Third Sister Lake, 1969

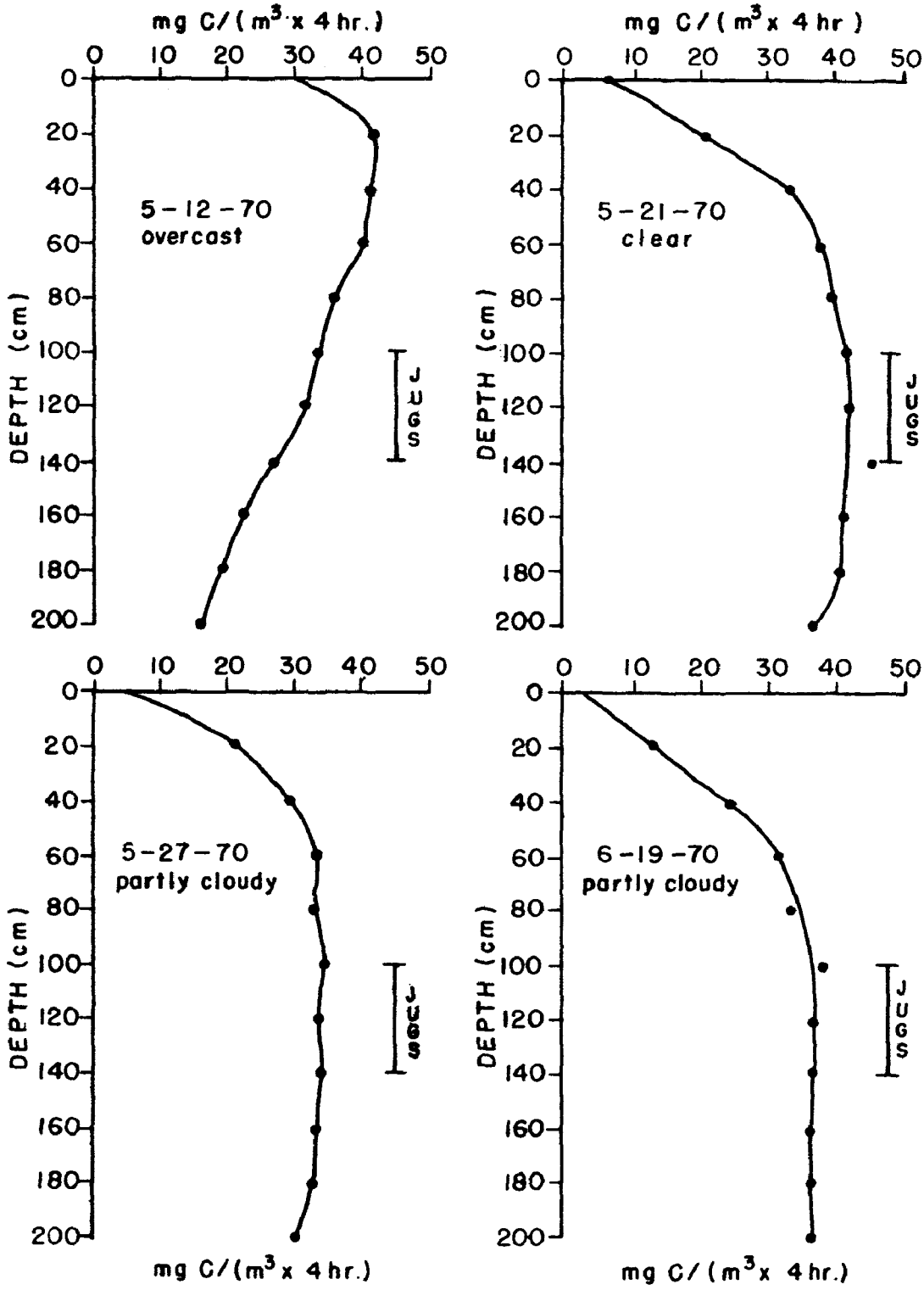


Figure 2. Productivity Profiles, Third Sister Lake, 1970

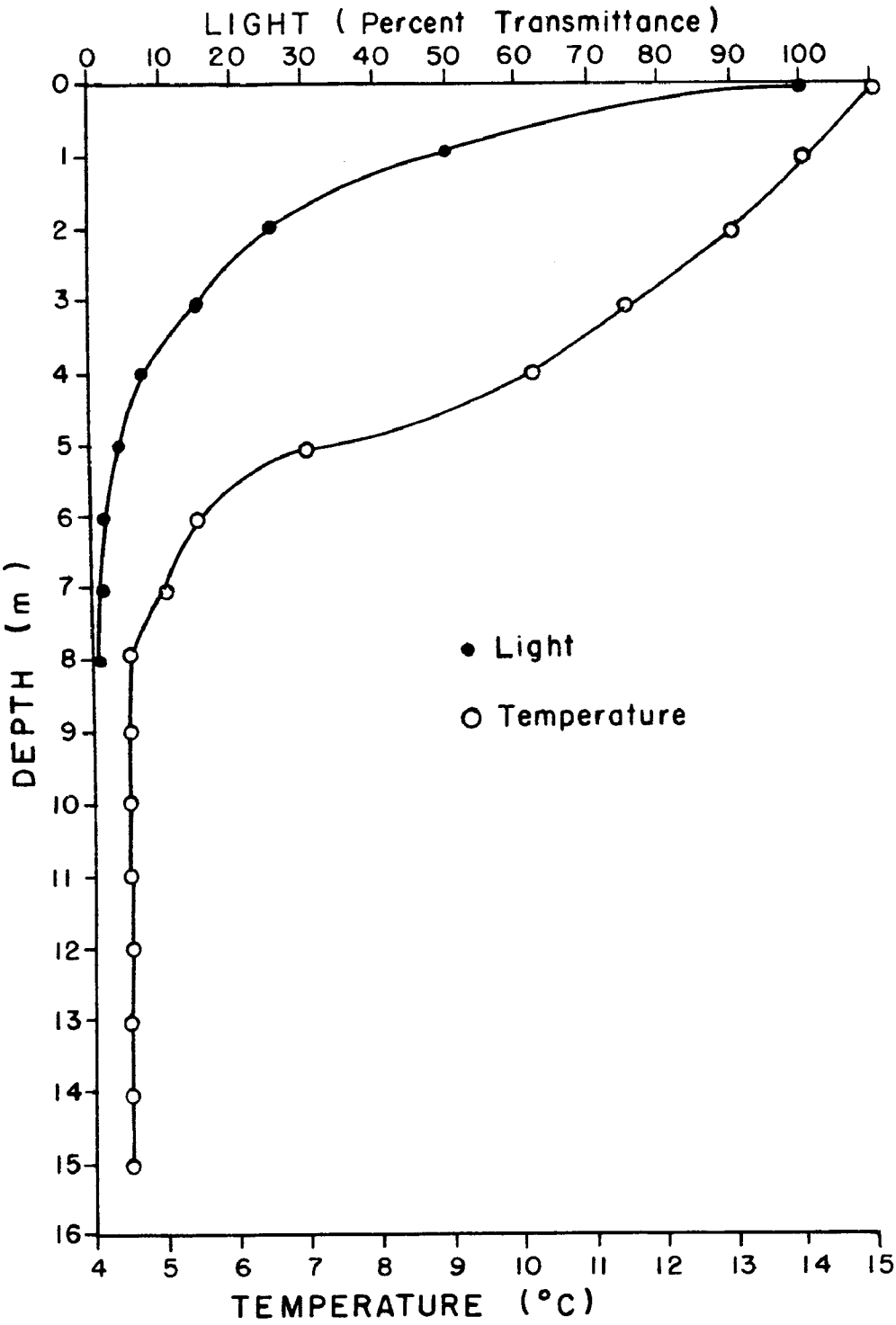


Figure 3. Light and Temperature Profiles, Third Sister Lake, 5-6-71

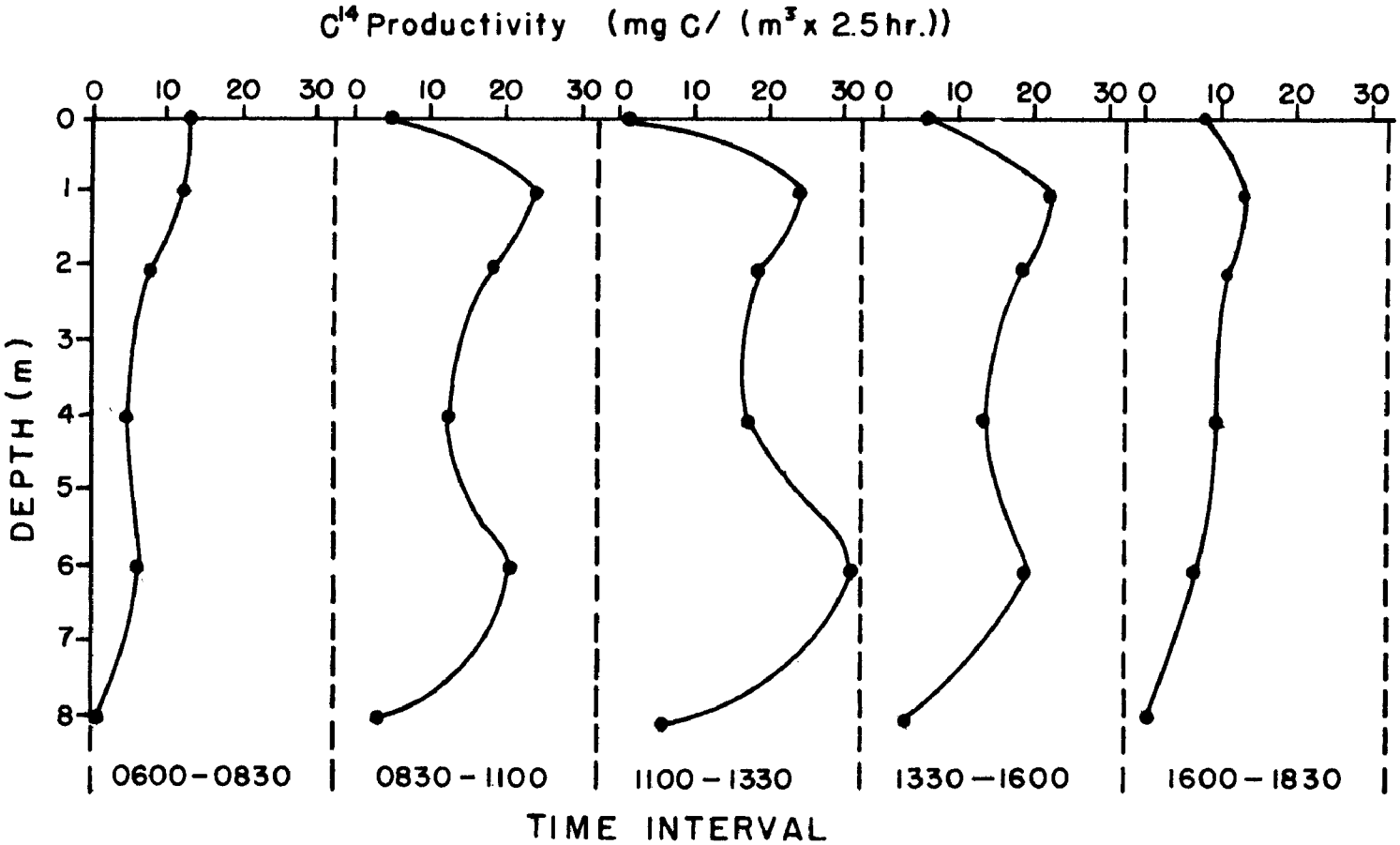


Figure 4. Productivity Profiles, Third Sister Lake, 5-6-71

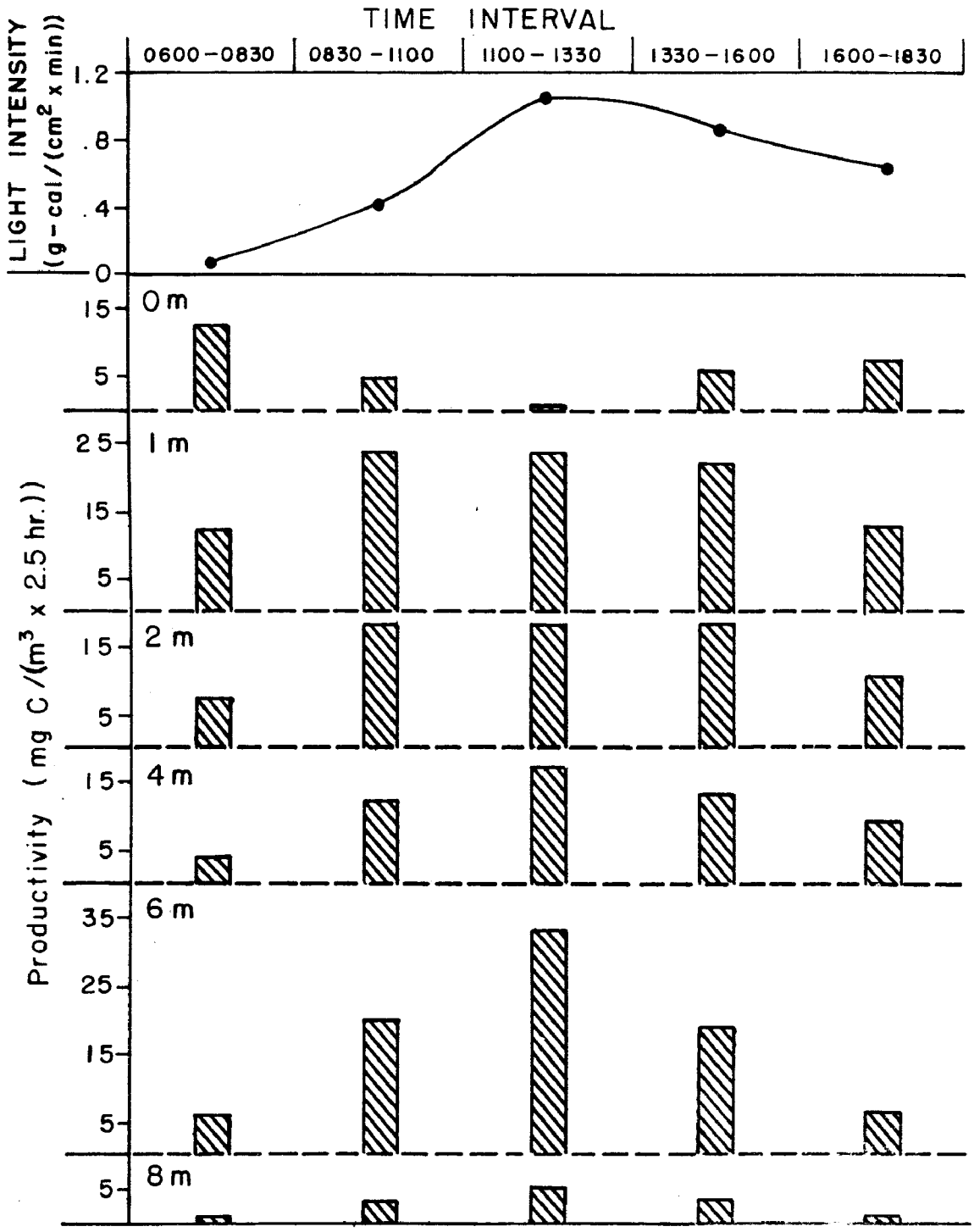


Figure 5. Productivity and Incident Light Levels, Third Sister Lake, 5-6-71

1968: chlorophyll and oxygen production, Gächter 1968 and Schelske and Storermer 1971: ^{14}C uptake and species changes). The obvious preference for the ^{14}C method is attributable to its rapidity and sensitivity to small changes in population size or photosynthetic activity. In some instances responses measured by ^{14}C uptake differences have been detected within minutes or hours after nutrient addition--much more rapidly than they could appear as significant increases in cell numbers (Goldman 1960b, Wetzel 1966). However, extension of these immediate responses to prediction of actual population increase is risky since the substances added may give only transitory stimulation to the photosynthetic process (Dugdale 1967). Also, measurement of only the immediate responses in terms of ^{14}C uptake precludes detection of responses that might appear after a lag period, such as those observed by Menzel et al. (1963).

Schedule. Because of the large designs to be used in this study and the desire to detect small responses to low level nutrient additions the ^{14}C method of response measurement was the most attractive. Considering that transitory effects or lag periods might arise in the responses it was decided to conduct each experiment for several days so that a series of ^{14}C measurements could be performed. To make certain that differences in these measurements actually meant differences in production initial and final plankton counts for representative experimental units were to be performed.

In skeleton form the three components of the method at this point can be summarized as: (1) a factorial statistical design, (2) in situ incubation of experimental communities in 19-liter jugs at 1 m depth and (3) measurement of responses by ^{14}C uptake and plankton examination techniques. The next section will examine in detail the development of specific procedures. This development process was inseparable from the evaluation of experimental results, and data will be drawn freely from specific experiments when needed.

DEVELOPMENT OF FIELD PROCEDURES

Experiment 1: June 1968

Objectives. From the many literature accounts of enrichment experiments the only generalization that could be drawn was that an added nutrient was likely to cause a detectable response. This response would occur if some segment of the phytoplankton community

TABLE 2

DESIGN OF EXPERIMENT 1

Variable	Dose (µg/l)								
Nitrate-N	0	100				250			
Phosphate-P	0	20		50		20		50	
EDTA	0	500	2500	500	2500	500	2500	500	2500
No. of Replicates	3	1	1	1	1	1	1	1	1

found the enriched environment more favorable than the control environment for growth, and would be detected if the measurements were made at the appropriate time. Thus the first experiment was approached with the expectation of answering the following basic questions: (1) Was the proposed method capable of detecting responses of Third Sister Lake phytoplankton communities to low level additions of nitrate, phosphate, and EDTA? (2) Did the response pattern vary from day to day? (3) Did the nutrients elicit responses independently or interactively? (4) Did replicate experimental units behave identically?

Design. Since the first question was the most critical it was decided that in this initial trial it was more important to cover a range of doses, extending upward to higher concentrations than were to be employed routinely, than to satisfy the statistical requirement of full replication. Thus if the high level treatments caused responses and the low level treatments did not, it would be concluded that the proposed low level doses were insufficient rather than that the nutrient variables themselves were insignificant in Third Sister Lake. Accordingly, the design presented in Table 2 was chosen for experiment 1.

Eight jugs were to receive different nutrient combinations, while three control jugs were to be included to provide base line measures of productivity and of replicability among jugs receiving identical treatments. Low and high level doses of nitrate and phosphate were intended to be approximately two and five times the ambient lake levels. The EDTA levels were intended to fall within the range of dissolved organic matter in lakes quoted by Hutchinson (1957, p. 883).

Procedures. The experiment was begun on June 12, 1968. Lake water was pumped from 1 m depth into a rectangular 75 gallon (284 liter) translucent polyethylene tank until about 250 liters were accumulated. The contents of the tank were then mixed continuously with a wooden paddle while the water was dispensed into the eleven jugs. The nutrient solutions were added in small volumes by pipetting into each jug before it was completely full, so that the turbulence accompanying the final lake water addition would provide thorough mixing. A float consisting of a 4 foot length of 2 x 2 with a one gallon plastic jug tied to each end was then attached to each jug to support it so that its shoulders were 1 m below the lake surface.

Carbon-14 uptake measurements were made on June 12, 13, 15, and 19. Sampling was accomplished by pulling each jug from the water, shaking it, and pouring about 1.2 liters into a dispensing funnel. While the contents of the funnel were stirred, four 300 ml BOD bottles (consisting of two light bottles, one dark bottle, and one chemical sample bottle) were filled from it. Each ^{14}C incubation bottle received the tracer in an injection of 2 ml of solution, prepared with an activity of approximately 1 microcurie per ml and stored in a sealed ampoule. After injection the bottles were placed on their sides in two racks, each having a capacity of 18 bottles, and the racks were suspended at 1 m depth for 4 hours, from 1000 to 1400. While the productivity samples were incubating the chemical samples were analyzed for pH and total alkalinity, so that ^{14}C counts could be converted to carbon fixed at a later date. At the end of incubation the productivity bottles were placed in the dark and duplicate 100 ml volumes from each bottle were filtered under suction through separate 47 mm, .45μ pore size membrane filters (Millipore Corp.). The filters were rinsed with small volumes of distilled water, dried, glued to planchets, and counted for duplicate 2 minute periods in a proportional, gas flow, ultrathin window counter (Beckman-Sharp Laboratories Low Beta II). After the last sampling run the jugs were emptied and acid cleaned to remove all organic matter and adsorbed nutrients so that no carryover would affect the next experiment.

To prepare the results for analysis the raw ^{14}C counts for each bottle were averaged, and the dark bottle average subtracted from each of the two corresponding light bottle averages. This yielded two independent observations (bottle means) for each jug for each sampling day. The means (jug means) and standard deviations of

TABLE 3

EXPERIMENT 1 (June 1968): RAW DATA
(JUG MEANS AND STANDARD DEVIATIONS
IN COUNTS PER MINUTE)

Treatment Jugs

Date	NO_3-N		PO_4-P: 20 µg/l		PO_4-P: 50 µg/l	
			EDTA: 0.5mg/l	EDTA: 2.5mg/l	EDTA: 0.5mg/l	EDTA: 2.5mg/l
6-12	100µg/l	$\bar{x}$	552.3	620.0	555.2	591.2
		s	88.10	21.85	59.54	57.06
	250µg/l	$\bar{x}$	415.2	559.6	517.3	370.0
		s	59.47	66.33	26.94	39.24
6-13	100µg/l	$\bar{x}$	637.1	548.8	654.8	625.3
		s	71.70	6.647	95.81	16.55
	250µg/l	$\bar{x}$	667.0	562.0	754.2	693.8
		s	7.283	145.0	40.16	11.67
6-15	100µg/l	$\bar{x}$	1120.	1276.	851.2	1013.
		s	129.0	22.13	157.4	60.88
	250µg/l	$\bar{x}$	1127.	1240.	1665.	2166.
		s	29.91	216.1	119.4	3.041
6-19	100µg/l	$\bar{x}$	837.6	1223.	948.8	1031.
		s	78.49	55.86	132.8	45.25
	250µg/l	$\bar{x}$	1885.	1480.	1716.	1858.
		s	156.2	138.6	52.33	269.5

Date		Control A	Control B	Control C	Grand Control Mean
6-12	$\bar{x}$	516.3	507.7	425.4	483.2
	s	4.525	31.11	78.70	50.15
6-13	$\bar{x}$	417.3	204.8	385.0	335.7
	s	25.03	107.1	88.18	114.5
6-15	$\bar{x}$	303.1	414.0	637.2	451.4
	s	39.60	120.9	30.41	170.2
6-19	$\bar{x}$	167.2	140.4	240.2	182.6
	s	17.96	170.9	53.25	51.66

these data appear in Table 3. The two bottle means were independent observations from a jug, but they were not independent observations from a treatment since they both came from a single treatment vessel. Fully independent observations, suitable for variance analysis in which all treatment effects could be resolved, would have to come from two different jugs receiving the same treatment. Nonetheless, for the purposes of this first trial it was decided that bottle means would be subjected to analysis of variance so that some separation of treatment effects could be achieved. A corner test of the jug means and standard deviations indicated that no transformation was necessary.

Results. The results of the data analysis will be included in a later section, and only the general features of the response pattern will be described here. It is obvious from Table 3 that the phytoplankton communities responded strongly to the nutrients added, even at the lower levels. The response was not immediate, however, but built gradually to a maximum 3 to 7 days from the start of incubation. In general replicate samples from the same jug exhibited similar uptake rates although some pairs differed from one another by several hundred counts per minute. The control means tended to differ widely. On the first three sampling days the standard deviation associated with the grand mean of the three control jugs exceeded most of the standard deviations between the bottle means, implying poor between-jug replicability relative to between-sample replicability.

The results were encouraging and enlightening. The response pattern showed that the nutrients employed were highly stimulatory, at the lower as well as the higher treatment levels. The ^{14}C method of response measurement was sufficiently sensitive to detect this stimulation. The pattern changed with time and confirmed the need for several ^{14}C runs during each experiment. Finally, the observations on replicability between samples and between jugs called for complete replication of future experiments, with at least two jugs for each treatment, and secondarily for refinement of sampling methods. The latter process extended into a continuing effort to identify and remove sources of extraneous variance from all parts of the method.

The importance of extraneous variance can be appreciated by briefly considering the significance test employed in analysis of variance. A series of calculations results in a set of mean squares, one for each treatment

and one representing the component of the total variance contributed by experimental error. Ratios are then calculated, each with a treatment mean square as the numerator and the error mean square as the denominator, and the significance of a treatment effect depends upon the size of its ratio. The ability of the method to detect subtle treatment effects, then, depends just as much upon the size of the denominator as it does upon the size of the numerator, so that any procedural refinements that can reduce the experimental error will enhance the sensitivity of the method. The many manipulations involved in carrying out an enrichment experiment provide many opportunities for the introduction of error. The following discussion will treat each source of error that was explored and will conclude with a description of the final form of the method employed in most of the experiments performed after 1968.

Sources of Statistical Error

^{14}C Technique. The basic response measurement employed was ^{14}C uptake. Before other sources of error could be identified a reliable method of preparing, storing, calibrating, and introducing the tracer had to be developed.

The first procedure attempted involved the preparation of ampoules containing 1 or 2 ml of a stock ^{14}C solution. The stock solution was prepared by diluting with distilled water a commercial preparation obtained from Nuclear-Chicago, so that the theoretical final activity of the solution was 1 microcurie per ml. Nonlabelled carrier was added (.35 g $NaHCO_3$/l) to reduce the probability of escape of $^{14}CO_2$ molecules, and the pH was adjusted to 9 so that virtually no free CO_2 would be present. Ampoules were filled by pipetting from the final solution, and after flame sealing were autoclaved to prevent bacterial growth (Vollenweider 1969, p. 54). Submersion of the ampoules in a solution of 1% methylene blue during autoclaving permitted detection of leaks (Steemann-Nielsen 1958). These ampoules were used in three experiments before a sample of them was tested for uniformity, by scintillation counting.

The testing procedure involved diluting the contents of an ampoule to 100 ml with pH 9 buffer, adding .5 ml of the diluted solution to a scintillation medium consisting of 7 ml of scint toluene and 2.5 ml of NCS,® and counting for several 2 minute periods in a scintillation counter (Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3375). Table 4 shows the results for

TABLE 4

STANDARDIZATION OF ^{14}C AMPOULES
BY SCINTILLATION COUNTING

Batch	Ampoule mean (cpm)	Batch mean (cpm)	Standard Deviation	95% Confidence Interval
1	(423.7) 14382.0 13139.3	13760.6	878.7	5866.4-21654.8
2	10899.2 11020.0 8074.7 9919.8	9978.4	1361.4	7812.4-12144.4
3	3359.2 3104.1 3647.0	3370.1	271.6	2695.4-4044.8
4	2168.8 2674.2 2134.6	2325.9	302.1	1575.5-3076.3

cpm = counts per minute

TABLE 5

COMPARISON OF REPLICABILITY OF AMPOULES AND SYRINGE SHOTS

	Batch	Ampoule Mean (cpm)	Batch Mean ($\bar{x}$)	Std. Dev. (s)	95% Confidence Interval
Ampoules	4	2976.2 2191.6 3790.4 1393.8	2588.0	1029.5	949.9-4226.1
	Group	**Shot Mean (cpm)**	**Group Mean ($\bar{x}$)**	**Std. Dev. (s)**	**95% Confidence Interval**
Syringe Shots	1	1850.0 1880.0 1833.2 1962.2	1881.4	57.3	1790.4-1971.4
	2	1935.5 1847.2 1969.8 1945.0	1924.4	53.4	1839.4-2009.4

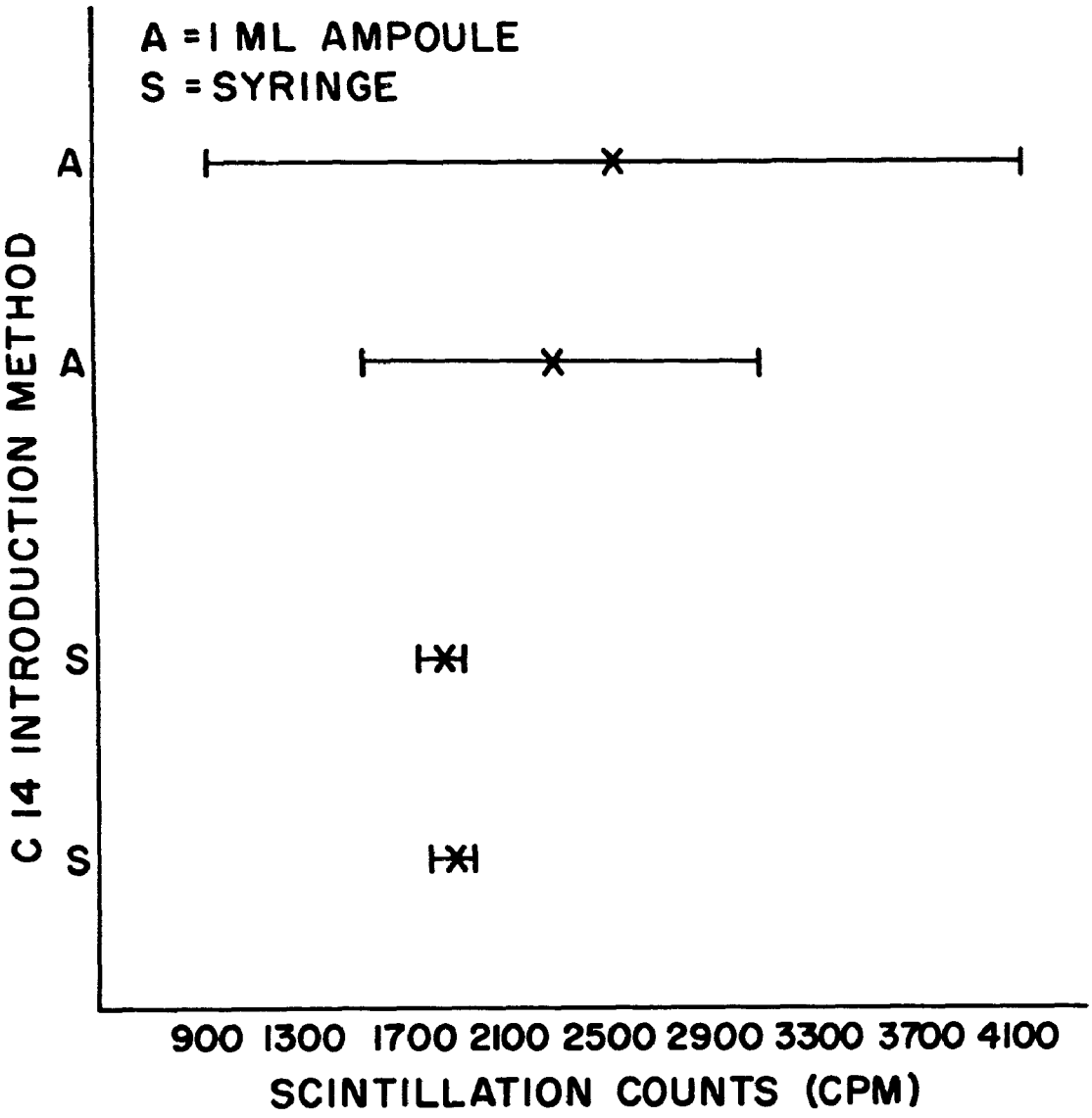


Figure 6. Comparison of Means and 95% Confidence Intervals for Batches of 1 ml Ampoules and Series of 1 ml Shots from an Automatic Syringe

the four ampoule batches prepared. The great variability within batches, summarized by the 95% confidence intervals, indicated that ^{14}C addition error was probably a major source of between-sample variance. Thus it was decided to test an alternative procedure: injecting into the sample bottles directly from the stock solution. Table 5 contrasts scintillation counts for a batch of 4 ampoules with counts for 2 series of 4-1 ml shots from an automatic syringe, diluted and counted in the same way. The second group of shots was taken four days after the first group, from the same stock, and the results indicated that stability of a bulk stock, at least for a short period, was excellent. Figure 6 compares the confidence intervals for the syringe shots with those for two batches of ampoules.

It was concluded that direct injection into samples from a stock solution involved less chance of introducing experimental error than did the use of ampoules. Other investigators report precisely the opposite finding (Doty and Oguri 1958). Fogg (1958), however, reported that he used a stock solution and maintained it by passing it through a membrane filter and determining its activity before each use. Goldman (1960b) reported using an automatic syringe.

The method that was chosen for routine operation involved injection of 1 ml volumes of tracer from a stock solution into the sample bottles, using an automatic syringe fitted with a Teflon® needle. Prior to injection a 1 ml volume of lake water was displaced from each sample bottle so that insertion of the stopper after injection would not splash out any ^{14}C. After the last sample was injected a final shot was added to a volumetric flask containing 99 ml of distilled water. Three 3 ml aliquots of the diluted tracer were then pipetted into scintillation vials containing 20 ml of a medium prepared by dissolving 100 g of napthalene and 6 g of PPO (2,5-diphenyloxazole) in 1 liter of dioxane. These samples were counted for duplicate 5 minute periods in the scintillation counter. It was determined from counting samples of this type prepared from a solution of known activity that 40290 cpm corresponded to 1 microcurie/ml of stock solution. Thus by scintillation monitoring of the tracer used in each experiment it was possible to determine its activity during each sampling run. Figure 7 shows that activity varied only slightly from run to run within a ^{14}C batch.

Two other modifications were made in the ^{14}C method to reduce error. First, 125 ml Pyrex® sample bottles were substituted for the 300 ml BOD bottles so that the

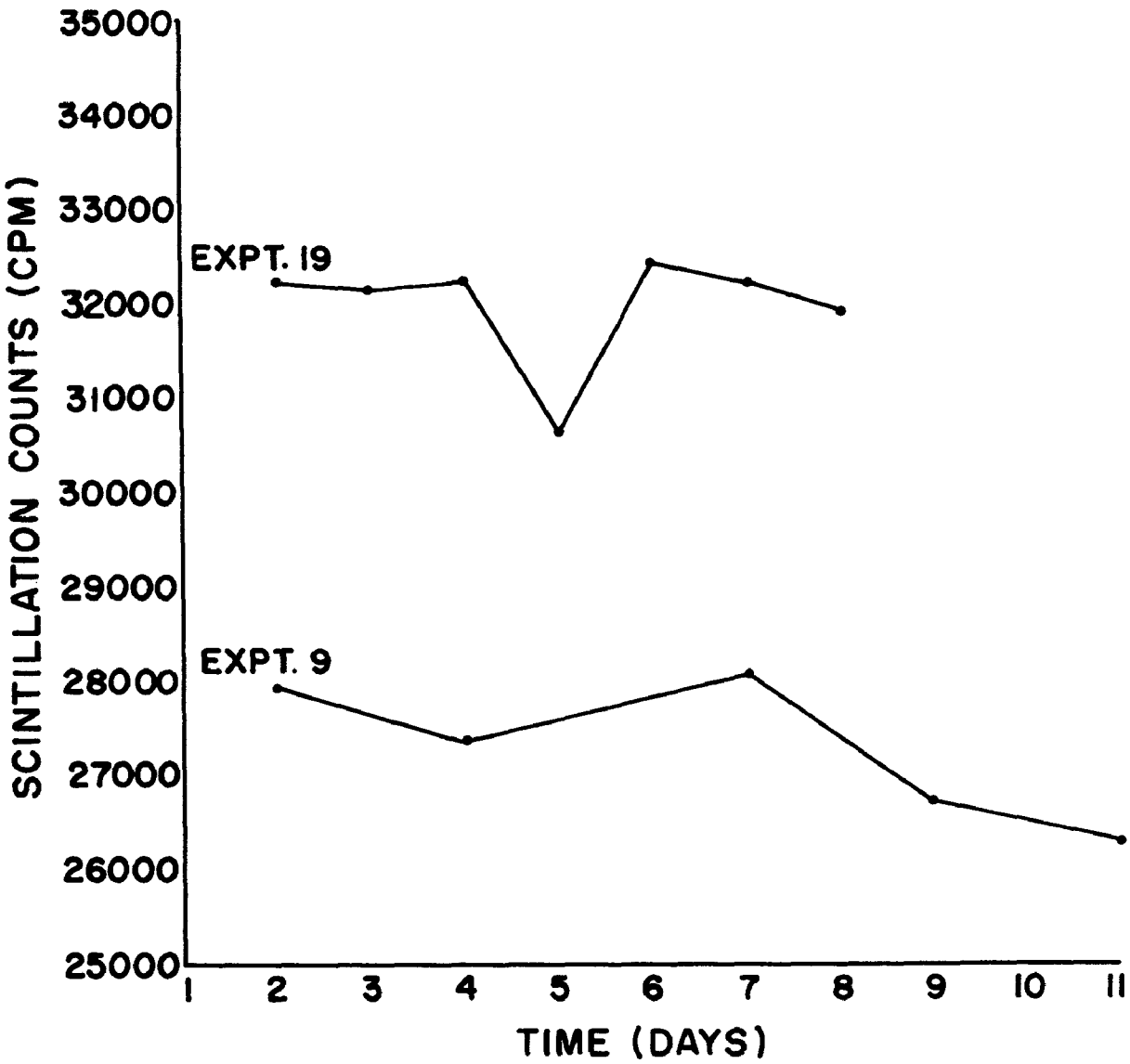


Figure 7. Activity of ^{14}C Stock Solutions as Monitored by Scintillation Counts on Days of Use

entire samples could be filtered. This was done to reduce filtration error as well as the time required for filtration. It should be noted that several attempts were made to detect effects of the length of time samples were stored before filtration (up to 3 hours between the first and the last sample in some cases), but no effects were ever found. The samples were held in closed boxes, without the addition of preservative.

The second modification involved extending the counting period to 10 minutes to improve counting accuracy. For example, in the case of a sample counting at 1000 cpm, the 0.95 error for a 10 minute count is 18 cpm compared to 45 cpm for a 2 minute count (U.S. Dept. of HEW 1960, p. 131).

Rinsing of the filters with dilute HCl to remove inorganic ^{14}C absorbed in the filter and adsorbed to detritus was considered, but was rejected on the basis of a report by McAllister (1961) that the decontamination process was more likely to increase the error of the measurement than to decrease it. Fogg (1958) had preceded this finding by expressing his opinion that rinsing filters with distilled water rather than HCl was "satisfactory in removing inorganic ^{14}C, from freshwater phytoplankton at least."

Loss of ^{14}C from phytoplankton stored dry on filters has been reported by Wallen and Geen (1968). Since most of the loss that they detected occurred within the first 24 hours of storage, and most of our samples were stored at least that long before counting, this source of error was not assessed.

Carbon-14 results were converted to carbon fixed by the equation of Saunders et al. (1962): $P = \frac{r}{R} \times C \times f$, in which P is photosynthesis in mg C per cubic meter, r is uptake of radioactive carbon in counts per minute, R is the total available radioactive carbon in counts per minute, C is the total available inorganic carbon in mg/m^3, and f is the isotope correction factor. R is further defined as microcuries of radioactivity used x efficiency of counter x correction for Millipore absorption effect x disintegrations per minute per microcurie. Table 6 contains the values for these factors and the final equation that was used in these studies.

During ^{14}C runs the samples were usually incubated in the lake for a period of 4 hours. Longer incubation

TABLE 6

CONVERSION OF CPM TO CARBON FIXED

Conversion Equation	$P = \frac{r}{R} \times C \times f$ (Saunders et al. 1962)
P	Photosynthesis in mg C/m^3
r	cpm counted, if entire sample is filtered
C	20.4×10^3 mg C/m^3 (Third Sister Lake)
f	1.06
R	Total available radioactive carbon, in cpm
Microcuries used	$\frac{\text{Scintillation cpm}}{40290 \text{ cpm/microcurie}}$
Counter efficiency	0.25
Millipore absorption factor	0.838
dpm/microcurie	2.22×10^6
Final equation for Third Sister Lake	$P = r \times \frac{1873}{\text{Scint. cpm}}$

periods were rejected on the basis of reports that ^{14}C uptake rates tend to decline due to bottle effects in samples incubated for more than a few hours (Barnett and Hirota 1967, Vollenweider and Nauwerck 1961). While bottle effects were undesirable, it was felt that the incubation period should be significantly longer than the period required for sampling the jugs and adding the ^{14}C. Thus differences among sample productivities due to differences in sampling time or injection time would, hopefully, be overcome.

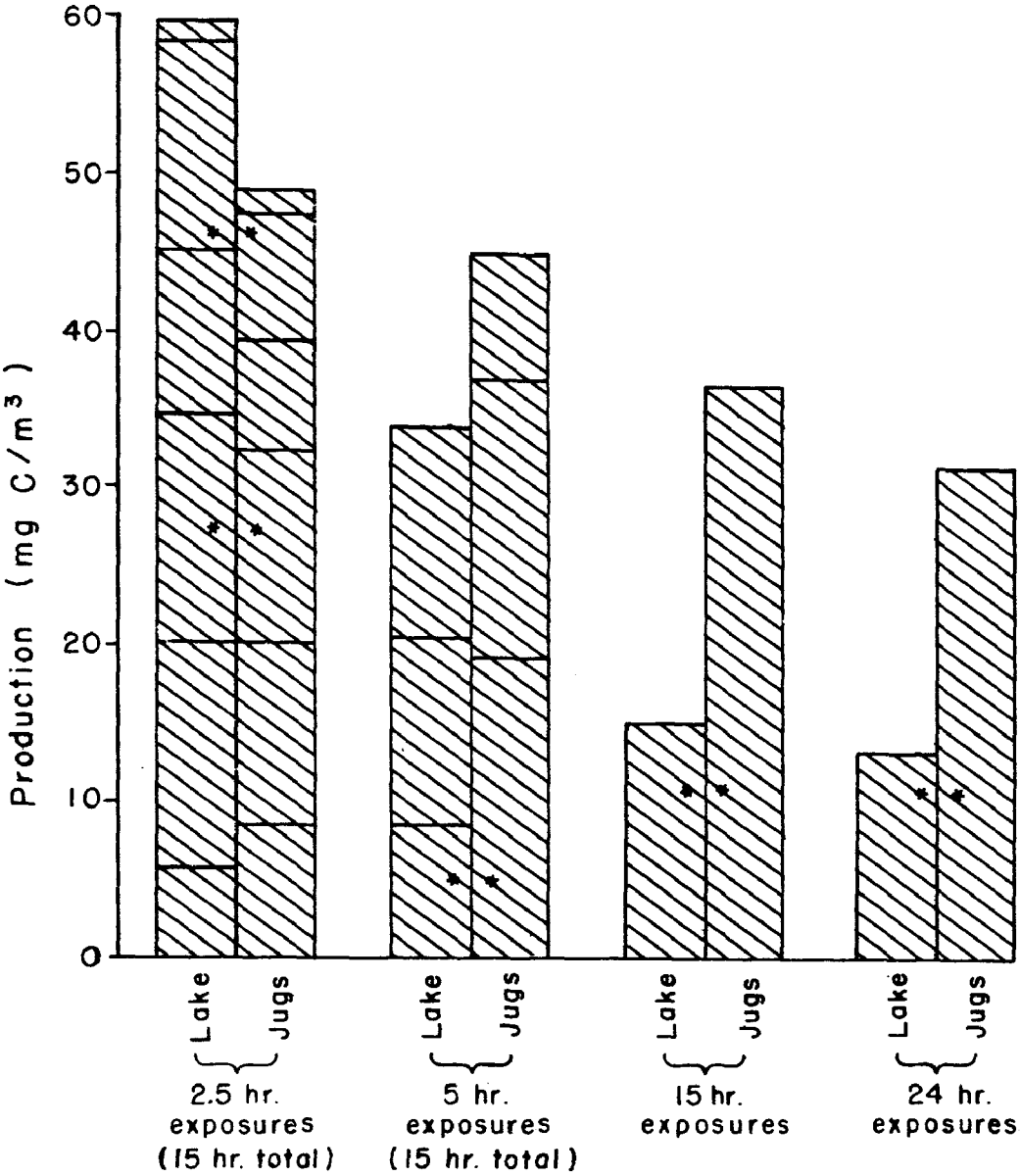
In order to test the influence of incubation time on ^{14}C uptake rate a special experiment, experiment 15, was performed in August 1969. The experiment was designed to compare four exposure durations: 2.5, 5, 15, and 24 hours. This was accomplished by exposing four stimultaneous series of samples taken from the same plankton

community periodically over the course of the day. In addition to samples taken directly from the lake, samples were also taken from jugs filled the previous day and incubated in the lake. The first batch of samples began incubation at 0600 on August 15, 1969. This batch consisted of eight light bottles and four dark bottles from each of two jugs and two lake stations, 48 bottles in all. All samples were taken from 1 m depth and were incubated at that depth. At the end of 2.5 hours one set of bottles from each of the four sampling stations was replaced by fresh samples, and immediately filtered. The fresh samples were taken from the same two lake stations, but from two previously unsampled jugs. After 2.5 hours more the second batch of 2.5 hour samples and the first batch of 5 hour samples were similarly replaced. This process continued until 2100 so that, altogether, six 2.5 hour sets, three 5 hour sets, and one 15 hour set of samples were processed. At 0600 the next day the 24 hour set was filtered. The results are plotted in Figure 8.

Each bar is a summation of means, with its total length representing the total amount of carbon fixed by a given series of samples over the course of the day. There were differences in uptake rates between series exposed for different lengths of time, but there were also large differences between samples taken directly from the lake and samples taken from jugs. Significant differences between jug and lake samples within exposure periods are indicated.

Comparison of the two 2.5 hour series shows that fresh lake samples were more productive than were jug samples for 2 of the time intervals. Comparison of the two 5 hour series shows greater productivity by the jug samples for one time interval, and in the 15 and 24 hour series lake and jug samples diverged even more widely. Comparison of the 2.5, 5, and 15 hour lake series shows a very great reduction in uptake rate with exposure time, and all differences among these three series are significant (.05 level, Tukey's Test). A similar comparison of the jug series shows a mild depression, and none of the differences are significant.

The reduced sensitivity to bottle effects of the jug samples relative to the open lake samples implies that the phytoplankton communities that had been imprisoned in the jugs since the previous day had become adapted to the enclosed environment. They had already responded to bottle effects, and had somewhat compensated for them. Therefore samples from the jugs could tolerate longer



* Adjacent bars or segments of bars significantly different at .05 level.

Figure 8. Productivity Series, Third Sister Lake, 8-15-69

exposure periods in productivity bottles before experiencing further bottle effects, than could fresh lake samples. A more accurate interpretation of the apparent adaptation to the jug environment will be discussed in a later section.

In a similar experiment conducted in 1971 (Figure 9) incubation periods of 1, 2, 3, 4, 5 and 6 hours were compared. Little difference in ^{14}C uptake rates showed up among the different periods, except that the rates for samples incubated only 1 hour were significantly lower than the maximum (4 hour) rates (.05 level, Tukey's Test). This difference implies that there was a lag period between the start of incubation and the onset of ^{14}C uptake. The only significant difference between jug and lake samples was for the 1 hour time interval (.05 level), implying that the lag period may have been related to adaptation to the bottle environment. As in experiment 15, jug samples were preadapted to containment.

In processing the productivity data all counts were converted to mg carbon fixed per m^3 per 4 hour incubation period, rather than per day, since as Vollenweider and Nauwerck (1961) pointed out the distribution of photosynthesis in relation to time during a day is assymetric, with a maximum in late morning. Thus multiplying the result of a 4 hour incubation by an arbitrary factor and calling the product a daily rate would merely introduce error.

With the use of a reliable ^{14}C technique other parts of the overall method could be tested and improved.

Sampling Technique. The best method for subsampling from the jugs into the ^{14}C incubation bottles would be the method which provided the best replicability between samples. The initial method of shaking and pouring was undesirable because of the danger of accident as much as because of its inherently sloppy nature. An improved method was developed which was based upon agitation of the jug contents with a plunger and removal of samples by suction. The major objection to this method was the insertion of foreign materials into the jugs since Doty and Oguri (1958) reported that a variety of materials including plywood, tygon, neoprene, plastic hose material, and metal pumps depressed photosynthesis in samples which contacted them, while bottles, buckets, and funnels made of plastic had no effect. Thus it was attempted to assure the inertness of the materials to be inserted, by constructing the plunger

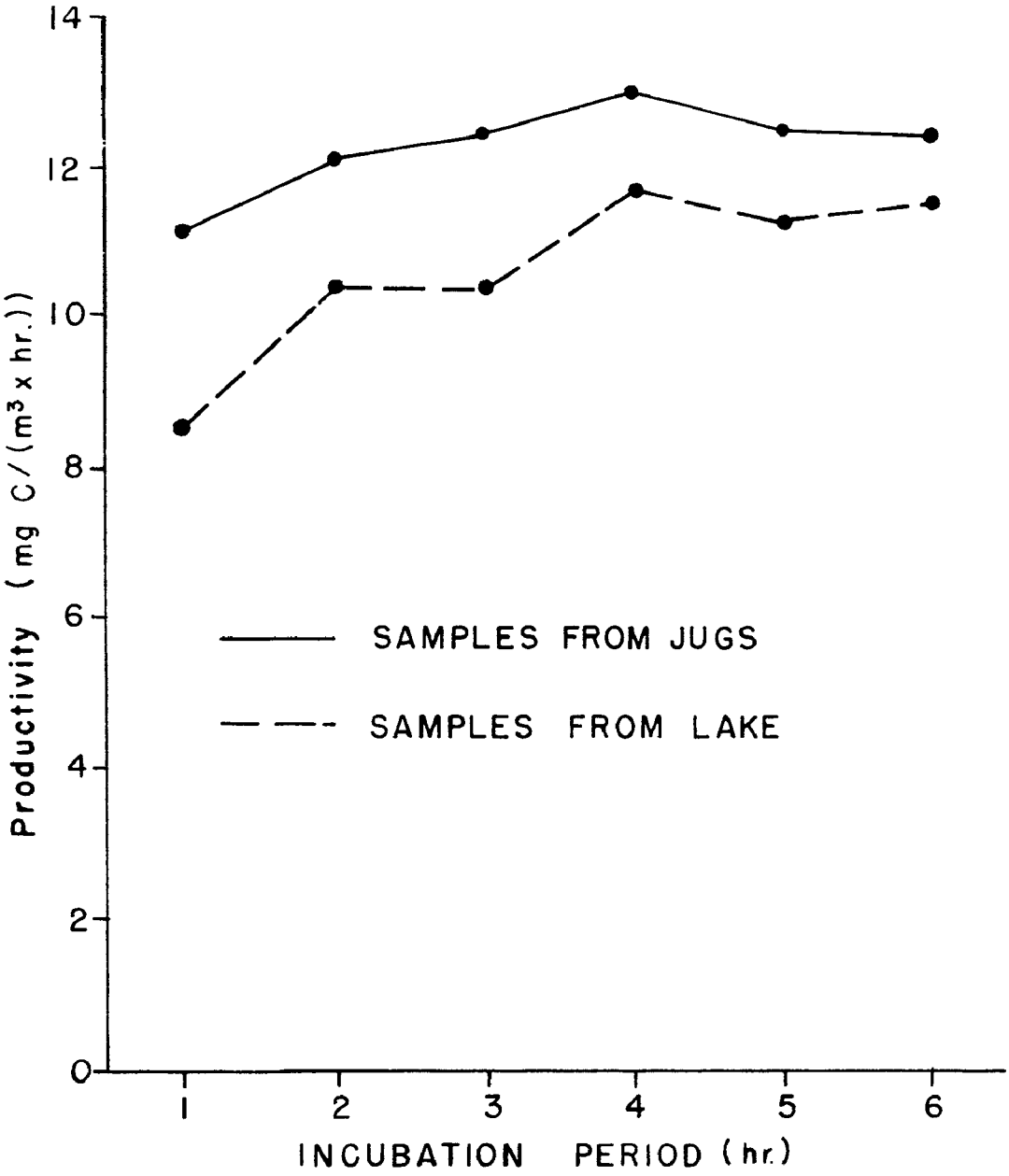


Figure 9. Productivity Series, Third Sister Lake, 6-3-71

from the top of a plastic bottle and a broom handle coated with epoxy paint, and by using a short length of rubber tubing for sample removal.

Several versions of the suction method were compared to determine which one provided the best replicability (see Figure 10). In the first trial two methods were compared. In the first method four bottles were filled simultaneously from a suction apparatus consisting of a suction pump and 4 liter trap on one end, the tube and plunger combination on the other end, and a manifold of tubes and stoppers in the middle. Water flowed from the jug through the tube and into the bottles, with overflow accumulating in the trap. Sufficient water was collected in the trap to fill a second series of four bottles. These bottles were filled one by one by dispensing from the trap in a manner similar to dispensing from the filling funnel employed in the shaking and pouring method. Two series of four bottles were filled by each method, and ^{14}C uptake was measured by the improved technique. Means and 95% confidence limits were calculated for each set of 4, and these are presented in the top graph of Figure 11, labelled Experiment 4A. Simultaneous refers to the first method, and indirect sequential refers to dispensing from the trap. Neither method performed very well. The lack of overlap of the confidence intervals of the two methods was interpreted to mean that overflowing of the simultaneous bottles to fill the trap caused concentration of plankton in those bottles, and consequent reduction in the population available for filling the indirect sequential bottles.

In the second trial (Experiment 4B) the simultaneous method was replaced by the direct sequential method, in which water flowed through the tube and into a single bottle which was linked in turn by a second tube to the trap. This time overflow was prevented. Two sets of four bottles were filled sequentially in this way. The indirect sequential method was again employed, but with water flowing directly to the trap, and a third method, the batch sequential method, was added. This method was similar to the indirect sequential method except that the ^{14}C was added to the contents of the trap before they were distributed among the bottles. As Figure 11 shows this method worked poorly compared to the indirect sequential method, which in turn was far excelled by the direct sequential method. Consequently in all subsequent experiments the direct sequential sampling method was employed, with agitation of the contents of the jug by plunging and prevention of overflow

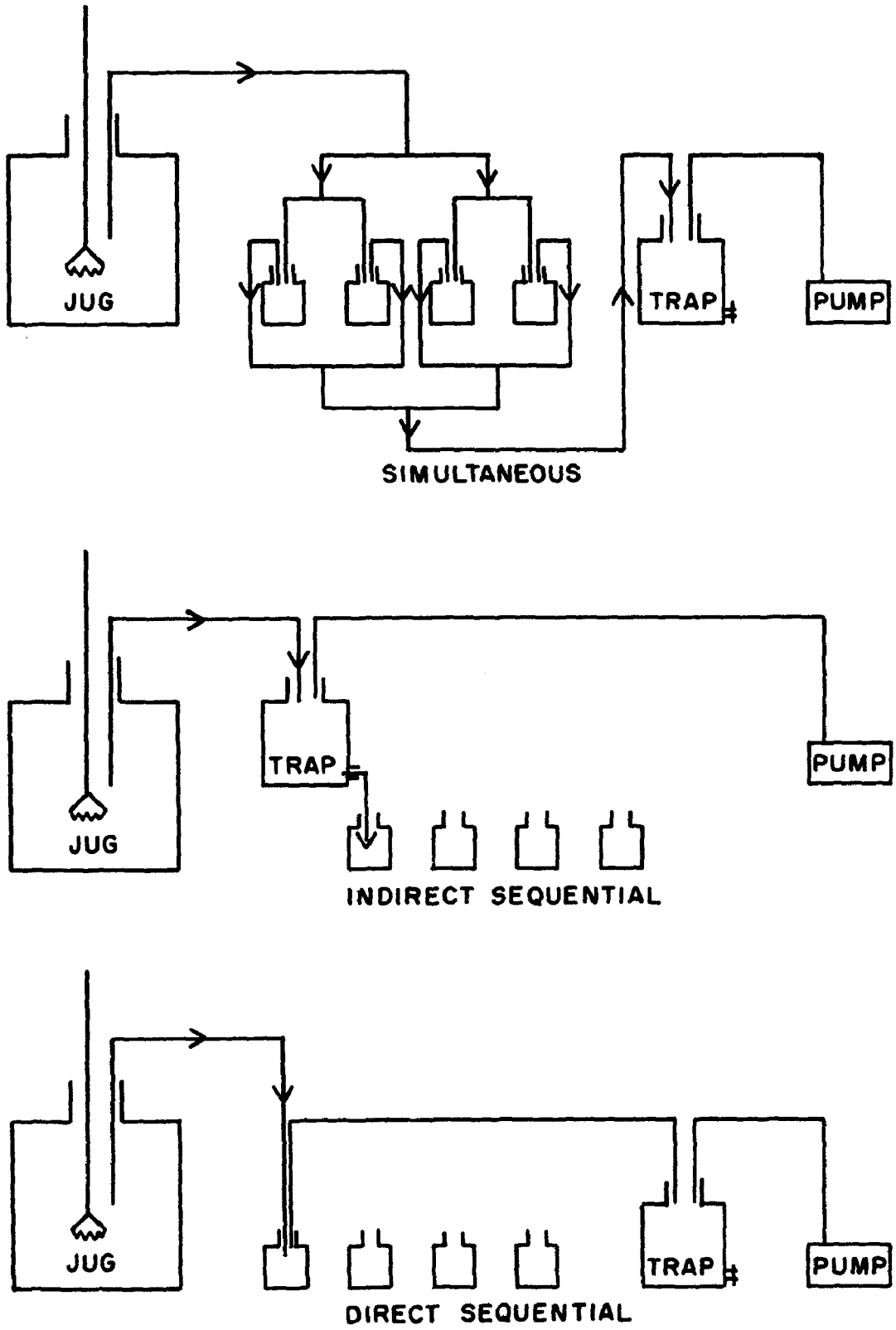


Figure 10. Sampling Methods Employed in Experiments 4A and 4B

TABLE 7

EXPERIMENT 3 (8-7/8-10-68): RAW DATA

EFFECT OF EXPOSURE RACK ON ^{14}C UPTAKE

(COUNTS PER MINUTE, JUG MEANS)

Date	Nutrient Treatment		Rack 1	Rack 2	Rack 3
8-8	1		337.3	707.3	723.4
	2		459.6	817.1	716.1
	3		713.2	837.2	746.0
		$\bar{x}$	503.4	787.2	728.5
8-10	1		132.4	324.4	366.7
	2		488.8	365.8	498.1
	3		188.2	567.5	541.0
		$\bar{x}$	269.8	419.2	468.6

from the sample bottles.

Sample Incubation Technique. In the first three experiments two or three separate racks, each with a capacity of 18 BOD bottles, were employed for incubating the ^{14}C samples at 1 m depth. In experiment 3 the distribution of the bottles among the three racks was controlled. It was found that consistent differences developed between samples on different racks (Table 7), presumably because their exposure periods and orientations toward the sun were slightly different. It was concluded that one large rack capable of holding all the sample bottles would remove a significant source of extraneous variance. This rack was constructed and employed in all subsequent experiments.

Jug Filling Technique. The 75 gallon (284 liter) rectangular tank used in the jug filling process in the first small experiments limited the size of the design that could be employed to a 2 x 3 replicated factorial. However, it was desired to perform larger experiments, based on 2 x 2 x 2 or 2 x 3 x 2 replicated designs, so this tank was replaced by a 150 gallon (568 liter) cylindrical polyethylene tank. The larger tank was first used in experiment 5, a 2 x 3 x 2 experiment, performed in October and November of 1968. For mixing

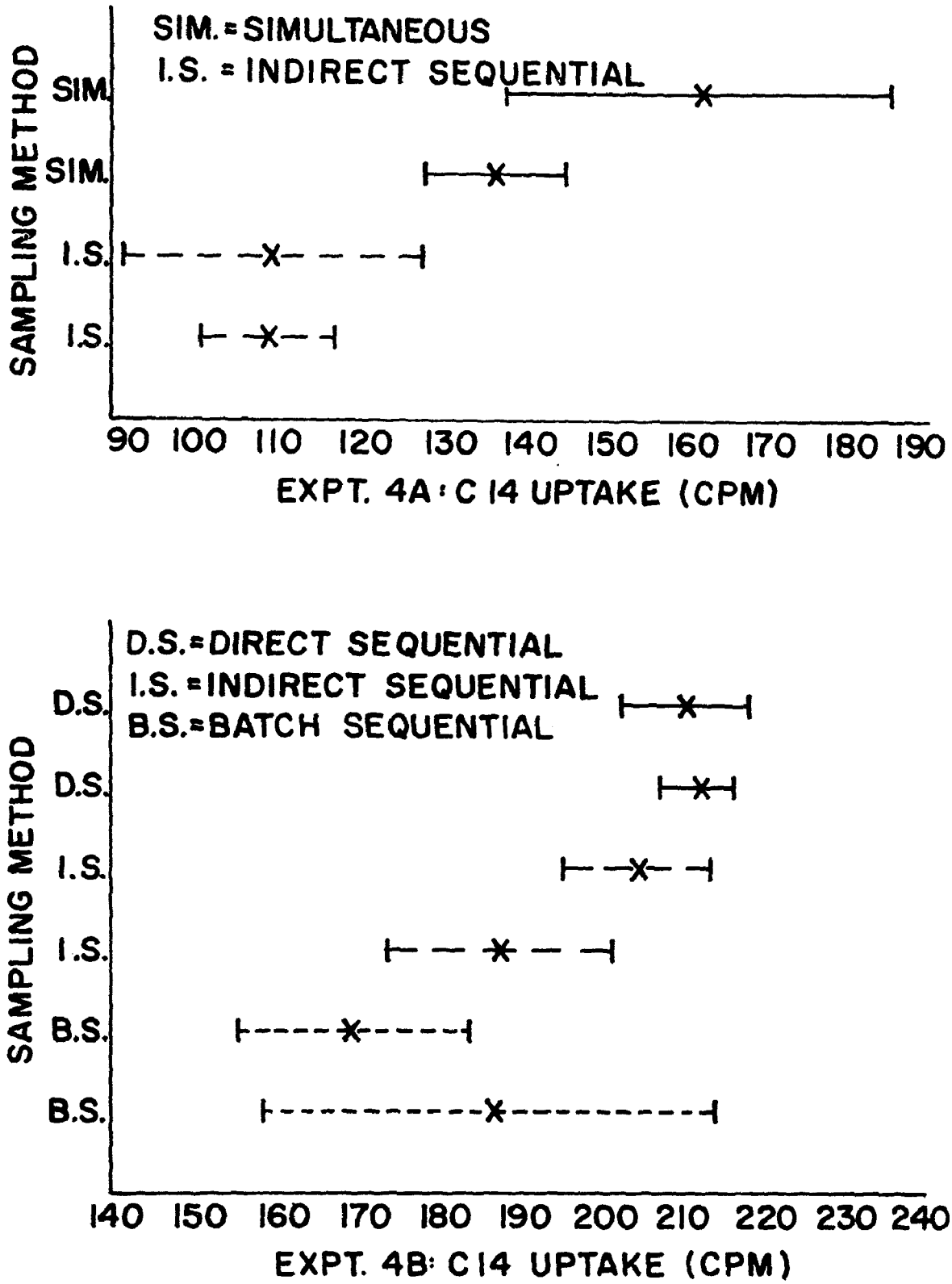


Figure 11. Comparison of Means and 95% Confidence Intervals for Groups of Lake Water Samples Taken from a Jug by Four Different Sampling Methods

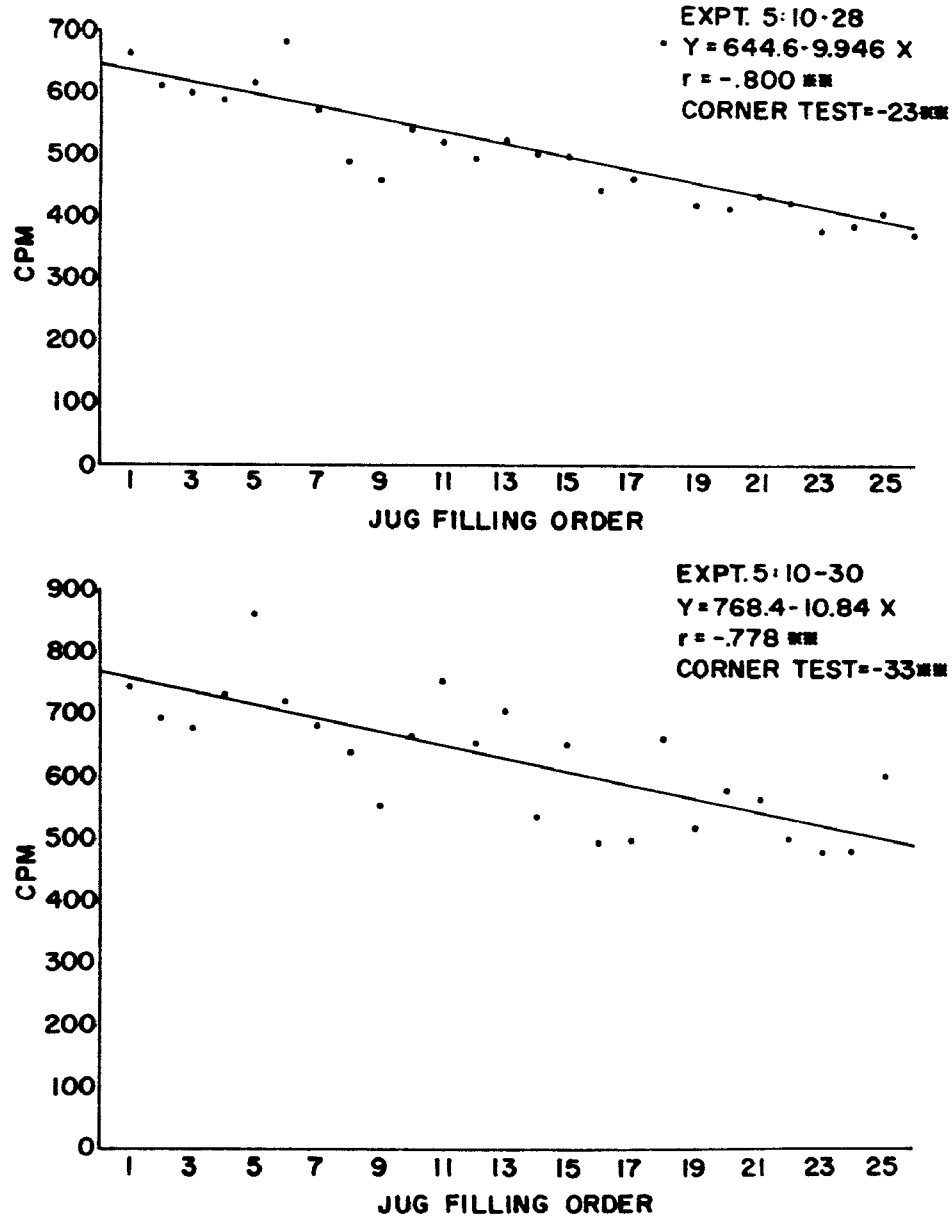


Figure 12. Effect of Nonuniform Jug Filling in Experiment 5

the tank during filling of the jugs a larger plunger was constructed from a broom handle and several large plastic bottles. It was thought that in a cylindrical tank vertical currents had to be induced to keep the phytoplankton in suspension. Unfortunately, the plunger broke and was replaced by the wooden paddle early in the filling process. The result of the consequent inadequate mixing is presented in Figure 12, in which ^{14}C uptake during the first two sampling runs is plotted versus jug filling order. The regression slopes and corner tests are highly significant indicating that the jugs filled early received more plankton than did the jugs filled later. Since the water was dispensed into the jugs via a tube passing through the wall of the tank just above the bottom, this nonuniform distribution of plankton was clearly due to settling of organisms in spite of the mixing effort.

Because of the closeness of the slopes of the two regression lines subtraction of the first day uptake values from the second day values removed the slope and resulted in data that could be analyzed for treatment effects (Figure 13). The second graph in this figure shows that after several more days of incubation the treatment effects had overcome the initial discrepancies, the slope had disappeared, and these final data could also be analyzed normally. Before the next experiment a more sturdy plunger was constructed, and as the plots in Figure 14 show jug filling problems were no longer encountered.

Summary of Statistical Performance.

Most of the major sources of statistical error discussed in the preceding sections were identified and reduced during the first 5 experiments, performed in 1968. In Table 8 the statistical performance of the method is summarized for each experiment in terms of the 5% least significant difference (Steel and Torrie 1960, p. 106) divided by the control mean. When more than one productivity run was performed in an experiment the smallest ratio obtained is tabulated.

The ratio for experiment 1, .238, is smaller than for the other 1968 experiments. This is because the experiment 1 least significant difference was computed from a data analysis performed on replicate sample values from single jugs, rather than on replicate jug means. Experiment 2 was the first experiment employing 2 separate jugs for each treatment combination, and its lsd

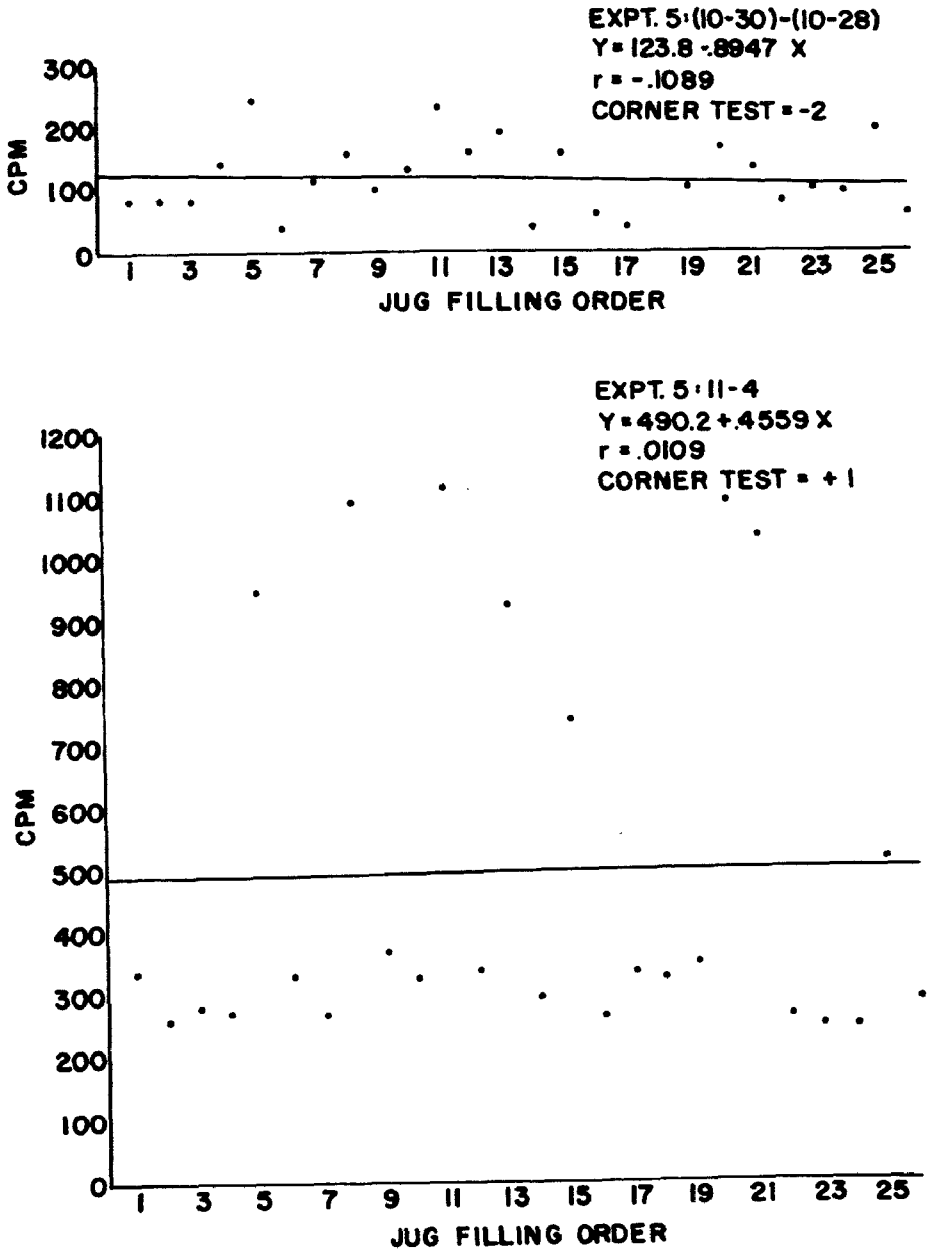


Figure 13. Removal of Jug Filling Effect

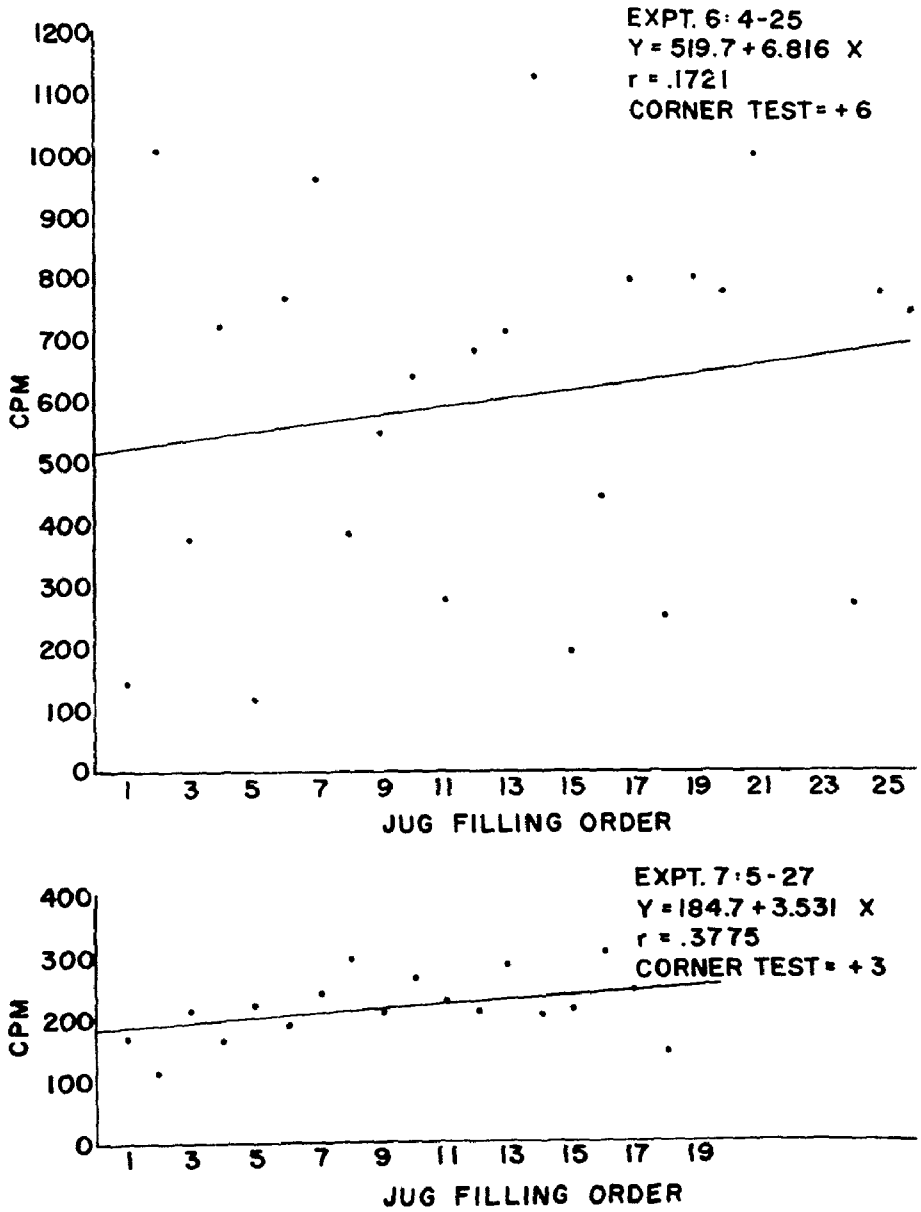


Figure 14. Subsequent Experiments: Results of Improved Jug Filling Technique

TABLE 8

EVALUATION OF SENSITIVITY OF METHOD

Experiment Number	Error df	5% lsd $\bar{x}$ Control
1	8	.238
2	8	2.20
3	9	1.01
4	6	.358
5	12	.535
6	8	.298
7	8	.193
8	8	.151
9	8	.280
16	8	.239
17	8	.149
19	8	.091
20	4	.106
22	8	.130
23	10	.085
24	8	.135
25	12	.151
29	8	.148
30	8	.110
31	9	.077
32	4	.126
34	4	.176

is consequently the highest obtained. The reduction in the ratio from experiment 2 to experiment 3 is attributable to the inclusion of ^{14}C sample exposure rack as a variable in the analysis, and consequent reduction in error variance.

The greatest proportional reduction in statistical error occurred between experiments 3 and 4. This was due to several modifications in the method, including the substitution of a single ^{14}C incubation rack for the three racks employed previously, the use of a common stock solution of ^{14}C rather than the ampoules, and use of the batch sequential sampling method. The direct sequential sampling method was used for the first time in experiment 5, but the jug filling problem more than negated the benefit of this improvement.

The improved jug filling method was introduced in experiment 6, and from that point on the statistical quality of the method remained essentially constant. The slight

improvement between experiments 6 and 7 was due partly to substitution of 125 ml reagent bottles for the 300 ml BOD bottles used for the productivity samples, and partly to the use of two aliquots of water to fill each jug. In experiment 6 the jugs were completely filled in succession, while in experiment 7 they were all filled to half capacity, and then to full capacity. Beginning with experiment 17 each jug was filled in thirds.

As a result of the various improvements introduced into the method, after the initial developmental phase productivity means exceeding the control means by about 15% routinely represented treatment effects significant at the .05 level. In some experiments the sensitivity limit was below 10% of the control productivity levels. Further improvement in sensitivity could probably be achieved by using more than 2 experimental units per treatment combination, but the degree of improvement possible would not justify the expense and effort involved.

SUMMARY OF METHOD

The final form of the method, incorporating all improvements, can be summarized as follows:

Setting up an Enrichment Experiment

The total volume of water to fill all the jugs required was pumped from 1 m depth into a 150 gallon cylindrical polyethylene tank. The contents of the tank were mixed continuously with a Plexiglass® plunger while the jugs were filled by gravity flow. Jugs were filled with three volumes of water, each volume approximating one third of the capacity of a jug. During the jug filling process four samples were taken, two of which were membrane filtered and frozen for later chemical analysis, and two of which were preserved with Lugol's solution (Saraceni and Ruggiu 1969, p. 7) for later plankton counting. After the jugs had been filled, the randomly assigned nutrient mixtures were added by pipetting, while the jug contents were continuously mixed with a plastic plunger. From this point until the jugs were suspended at the incubation depth, they were shielded with black polyethylene bags to prevent light shock.

^{14}C Uptake Measurement

On at least two separate days during the experiment the jugs were raised one by one and samples were removed by

the direct sequential sampling method into one dark and two light 125 ml glass stoppered bottles. Carbon-14 solution was added by injecting a 1 ml aliquot via an automatic syringe into each bottle, following removal of 1 ml of lake water to prevent splashout with reinsertion of the stopper. The bottles were incubated on their sides on a plywood rack suspended in the lake at 1 m depth, where they remained for 4 hours. After incubation the entire samples were membrane filtered; and the filters were rinsed with distilled water, dried, glued to planchets, and counted for 10 minute periods in a beta counter. The raw counts were converted to mg C fixed per m^3 per 4 hours, using a formula presented earlier and measurements of the activity of the tracer obtained at time of use.

Background Environmental Measurements

During the course of an experiment measurements of water temperature, using a thermistor unit, and Secchi disk transparency were made, to supplement the ^{14}C data. Chemical analyses performed on the stored samples included total dissolved phosphate, using persulfate oxidation (U.S. Dept. Int. 1969) to liberate bound phosphate and employing ascorbic acid as the reducing agent (Murphy and Riley 1962), and dissolved nitrate, employing the salicylate method (Schering 1931). Periodic analyses of fresh samples including ortho as well as total phosphate measurements, were performed to verify the results for the stored samples. The instrument used for reading the per cent transmittance of the developed samples was a Beckman DK-2 spectrophotometer, with a 10 cm path length. The limits of the sensitivity of this instrument were 1 µg PO_4-P/1 and 10 µg NO_3-N/1.

Plankton Counting

The plankton samples were concentrated by settling and were observed with an inverted microscope (Unitron, model BN-13). Counts of the important phytoplankton species (i.e., nannoplankters occurring in at least half of the 400 power fields observed and larger organisms that could be effectively enumerated in 200 or 100 power transects or in 100 power full scans) were made for selected samples. Individuals were measured, and cell numbers were converted to biomass based on calculated cell volumes and assumption of a density of 1 g/cc for all species except diatoms, which were assigned 1.1 g/cc based on data presented by Hutchinson (1967, p. 248). References used in species identification were

Desikachary (1959), Huber-Pestalozzi (1938, 1941, 1942, 1961, 1968), Patrick and Reimer (1966), Prescott (1962), and West and West (1908).

Statistical Analysis

Carbon-14 uptake data were tested for normality and transformed if necessary to base 10 logarithms before being subjected to analysis of variance. Correlation analyses were performed to detect relationships between differences in plankton counts and differences in ^{14}C uptake measurements obtained for control and high treatment populations. This data analysis was intended to detect treatment effects and to identify the algal species that had experienced these effects.

SECTION IV

EXPERIMENTS TO IDENTIFY LIMITING NUTRIENTS AND EVALUATE PREDICTIVE POTENTIAL

DESIGNS

This series of 13 experiments was conducted in the ice free seasons of 1968 and 1969, and ended with an experiment in the spring of 1970. The experimental designs are summarized in Table 9. In experiments 1-5 several designs were tested while the statistical properties of the method were being explored. Experiments 6-19 were all of similar designs, based on a 2 x 2 x 2 factorial setup with 2 replicate experimental units per treatment combination. Experiment 20 combined a 2 x 2 factorial with a one way design. Addition levels were varied somewhat from experiment to experiment.

PRODUCTIVITY RESULTS: APPENDIX A

The tables in Appendix A summarize the treatments, productivity results, and variance analyses for the 13 experiments. In experiments 1-5 extraneous sources of variance tended to obscure the treatment effects. Nonetheless, the major features of the response patterns are discernible. Experiments 6-20 employed the improved method and were more sensitive in detecting treatment effects. Experiments 10-15 and 18 were intended to investigate various features of the method, and their results are discussed elsewhere.

INTERPRETATION OF RESPONSE PATTERNS

Stable versus Variable Patterns

Examination of the variance analyses (Appendix A) reveals that in some of the experiments the response patterns developed immediately and remained relatively unchanged in all of the ^{14}C runs conducted, while in other experiments the response pattern changed significantly from the first to the last ^{14}C run. Experiment 8 provides an example of the first, "stable" type of response pattern, and its productivity results are plotted in Figure 15. The pattern consisted of a primary stimulatory effect of P that was enhanced by N, and it appeared in all 3 sets of productivity

TABLE 9

SUMMARY OF EXPERIMENTAL DESIGNS

EXPERIMENTS 1-20

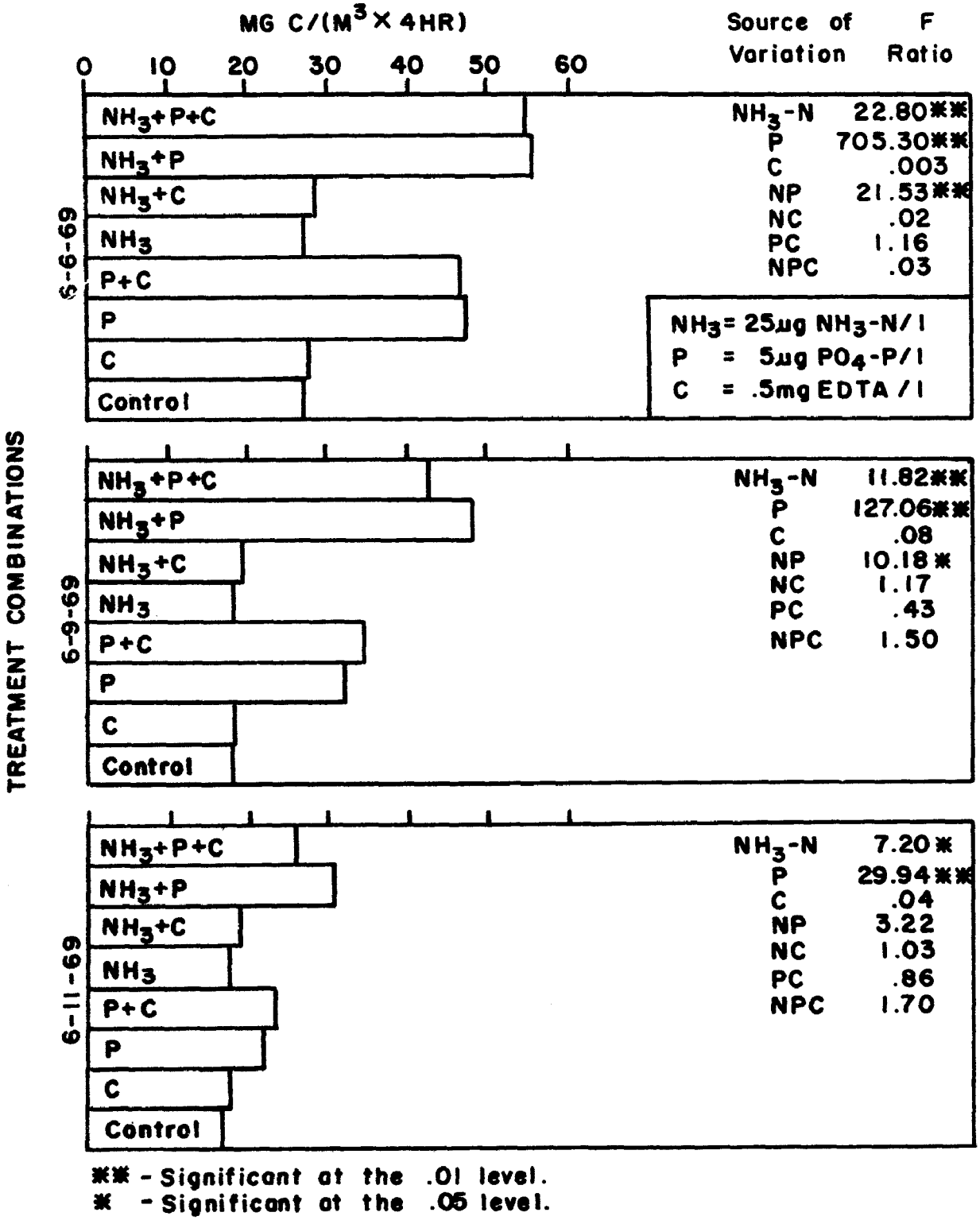
Experiment	Starting Date	Variables Treatment	Addition	Design
1	6-12-68	NO_3-N	.100 mg/l	2 x 2 x 2 Factorial,
			.250 mg/l	unreplicated,
		PO_4-P	.020 mg/l	+ 3 control jugs
			.050 mg/l	
		EDTA	.500 mg/l	
			2.50 mg/l	
2	7-18-68	NO_3-N	0 mg/l	2 x 2 x 2 Factorial,
			.100 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.020 mg/l	
		EDTA	0 mg/l	
			.500 mg/l	
3	8-7-68	EDTA	0 mg/l	1 way design,
			.500 mg/l	3 replicates
			2.5 mg/l	per cell
4	9-19-68	NO_3-N	0 mg/l	2 x 3 Factorial,
			.100 mg/l	2 replicates
		Poly-P	0 mg/l	per cell
			.050 mg/l	
			.100 mg/l	
5	10-27-68	NO_3-N	0 mg/l	2 x 3 x 2 Factorial,
			.100 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.010 mg/l	
			.050 mg/l	
		EDTA	0 mg/l	
			.500 mg/l	
6	4-23-69	NO_3-N	0 mg/l	2 x 2 x 2 Factorial,
			.025 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.005 mg/l	
		EDTA	0 mg/l	
			.500 mg/l	

TABLE 9 (Continued)

Experiment	Starting Date	Variables Treatment	Addition	Design
7	5-26-69	NO_3-N	0 mg/l	2 x 2 x 2 Factorial,
			.025 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.005 mg/l	
		EDTA	0 mg/l	
			.500 mg/l	
8	6-4-69	NH_3-N	0 mg/l	2 x 2 x 2 Factorial,
			.025 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.005 mg/l	
		EDTA	0 mg/l	
			.500 mg/l	
9	7-1-69	NO_3-N	0 mg/l	2 x 2 x 2 Factorial,
			.025 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.005 mg/l	
		EDTA	0 mg/l	
			.050 mg/l	
16	8-21-69	NO_3-N	0 mg/l	2 x 2 x 2 Factorial,
			.100 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.003 mg/l	
		EDTA	0 mg/l	
			.500 mg/l	
17	9-13-69	NO_3-N	0 mg/l	2 x 2 x 2 Factorial,
			.025 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.005 mg/l	
		EDTA	0 mg/l	
			.500 mg/l	
19	10-25-69	NO_3-N	0 mg/l	2 x 2 x 2 Factorial,
			.025 mg/l	2 replicates
		PO_4-P	0 mg/l	per cell
			.005 mg/l	
		EDTA	0 mg/l	
			.500 mg/l	
20	4-23-70	NO_3-N	0 mg/l	2 x 2 Factorial,
			.025 mg/l	2 replicates per
		PO_4-P	0 mg/l	cell for N and P;
			.005 mg/l	Independent
		EDTA	0 mg/l	treatment of EDTA
			.382 mg/l	

FIGURE 15

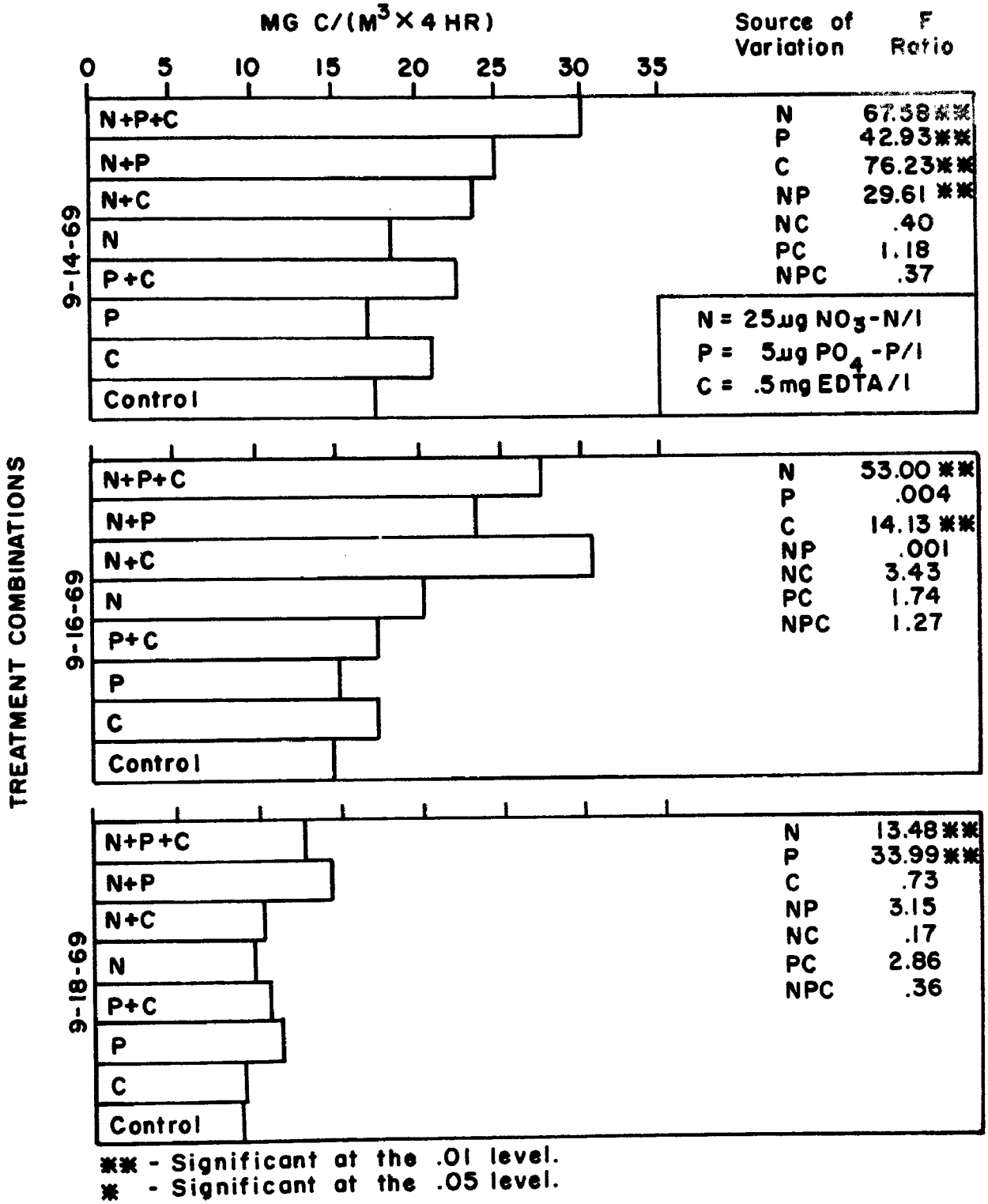
EXPERIMENT 8: TREATMENT EFFECTS



** - Significant at the .01 level.
* - Significant at the .05 level.

FIGURE 16

EXPERIMENT 17: TREATMENT EFFECTS



measurements. Experiment 17, in contrast, exemplifies the type that varied with time (Figure 16). On the first day of measurement a main effect of EDTA was clearly evident, as was a synergistic interaction between N and P. On the second day the EDTA effect still appeared, but an independent N effect had replaced the NP interaction. Finally, by the third day the EDTA effect had disappeared leaving independent but weakly expressed N and P effects.

In the case of a stable response pattern, interpretation of the results is clear--the one set of effects observed constitutes the response of the experimental community to the treatments. When the pattern varies with time, however, the nature of the true response is uncertain and interpretation of the results is more complicated. Brief discussions of two studies reported in the literature will illustrate this point.

In the first study Biesinger (1967) applied a series of micronutrient treatments to samples of Alaskan lake phytoplankton. Carbon-14 productivity measurements made after 12 hours of incubation detected stimulation by Li, Co, V, B, and Mn. After 24 hours of incubation only Co, V, and Fe effects were still present, while after 48 hours only the samples treated with Fe showed an effect. The author concluded that many substances added to natural waters can increase ^{14}C uptake in short term experiments, and that longer term experiments are necessary to reach adequate conclusions about limiting factors.

In a study of nutrient limitation of Sargasso Sea phytoplankton, Menzel and Ryther (1961) detected stimulation by Fe treatments, but the Fe effects lasted only 24 hours in the absence of added N and P. Still it was concluded that Fe was the limiting nutrient. In further experiments which lasted up to 9 days (Menzel et al. 1963) it was found that a mixed treatment of N, P, and Fe produced a rapid response, but that after a lag period a comparable response to N and P, added without Fe, developed. Addition of Al to the N+P treatment also accelerated the response, so it was concluded that the metals somehow exerted catalytic effects on the samples treated with N and P. In this study, as well as in the work by Biesinger, the interpretation of results was strongly influenced by the durations of the experiments.

^{14}C Uptake versus Growth

One other basic factor that contributes to the

uncertainty in interpreting response patterns that are expressed exclusively in terms of ^{14}C uptake is that ^{14}C uptake is not always directly related to growth. This applies to long term as well as to transitory responses. In a study reported by Goldman and Armstrong (1969), for instance, nitrate and phosphate treatments were applied to samples of pelagic Lake Tahoe phytoplankton. In one experiment cell counts increased in response to treatments with P alone, but ^{14}C uptake declined below control levels. In another experiment the reverse occurred: ^{14}C uptake was stimulated to levels 50% above controls, but cell counts did not exceed the control counts. If it is assumed that ^{14}C uptake is used as a response variable in enrichment experiments because it is a rapid and convenient way to measure population growth, then the relationship between the two processes should be confirmed frequently.

Experimental Evaluation: Experiment 12

Questions Asked. In the present study two important questions were posed that required answers before conclusions could be derived from the results. (1) Should the early effects that subsequently disappeared be regarded as transitory stimulations of ^{14}C uptake similar to those encountered by Biesinger (1967), and therefore be discounted in favor of the final patterns that developed? (2) Could productivity responses in general be interpreted to signify growth responses? Some insights into these questions have been provided by a detailed analysis of the species growth responses that occurred in a special experiment, experiment 12, conducted in 1969.

Design and Procedures. This experiment was conducted in Crystal Lake, Benzie County, Michigan beginning July 22, 1969. The design is shown in Table 10 which indicates the treatment additions, the number of replicate jugs per cell, and the ambient nutrient levels.

The field procedures were the same as those employed in the Third Sister Lake experiments. Responses were detected on three separate days following the start of the experiment by measuring carbon-14 productivity of subsamples from the jugs by the standard technique. Counts were made for the 15 most important species, and the numerical results were converted to biomass estimates by multiplying by volume and density estimates.

TABLE 10

DESIGN OF EXPERIMENT 12

Variable	Dose (µg/l)							
NO_3 - N	0				50			
PO_4 - P	0		5		0		5	
EDTA	0	500	0	500	0	500	0	500
Number of Replicates	2	2	2	2	2	2	2	2

Ambient concentrations NO_3^- - N : 30 µg/l
Total dissolved P : 6.5 µg/l

Productivity Results versus Growth Responses. The results of the productivity measurements appear in Figure 17. In order to maximize the clarity of this and of the other figures in this section, values that were insignificantly different (.05 level) were averaged, and the averages were plotted for all treatments to which they applied. On July 24, two days after the start of the experiment, the response pattern indicated stimulation by EDTA alone but not by either N or P alone. However, the mixture of N and P did cause stimulation, and the statistical analysis revealed a significant NP interaction. Three days later, on July 27, an independent P effect appeared, as did the NP interaction, but the EDTA effect did not. Instead, two types of interactions involving EDTA appeared. One of these was apparent blockage of the P effect, and the other was enhancement of the effect of the NP mixture. This last effect showed up more strongly on July 30, while both of the two way interactions had disappeared leaving the independent P effect. Thus this experiment belonged to the second type discussed above, with three different sets of productivity measurements yielding three different response patterns.

Various elements of the productivity response patterns can be elucidated by considering growth responses of individual species within the experimental communities, Figure 18. The response pattern of Synedra nana, one of the two dominant species, embodies most of the features shown in the productivity patterns: an independent P effect, blockage of this effect by EDTA, and enhancement of the effect by N + EDTA. The coefficient of correlation between the biomass estimates for this species and the productivity results on July 30 is .956.

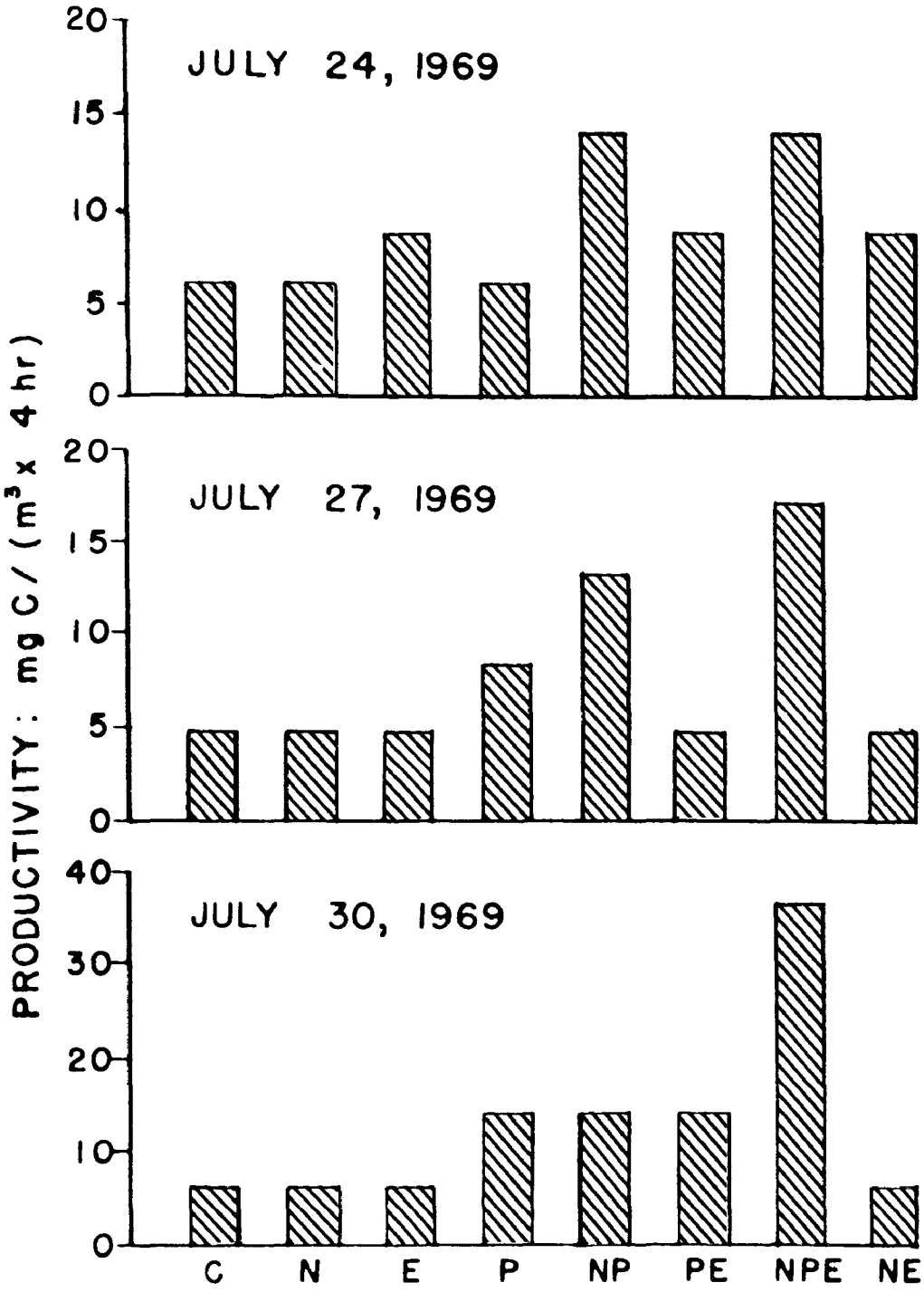


Figure 17. Experiment 12, Productivity Response Patterns

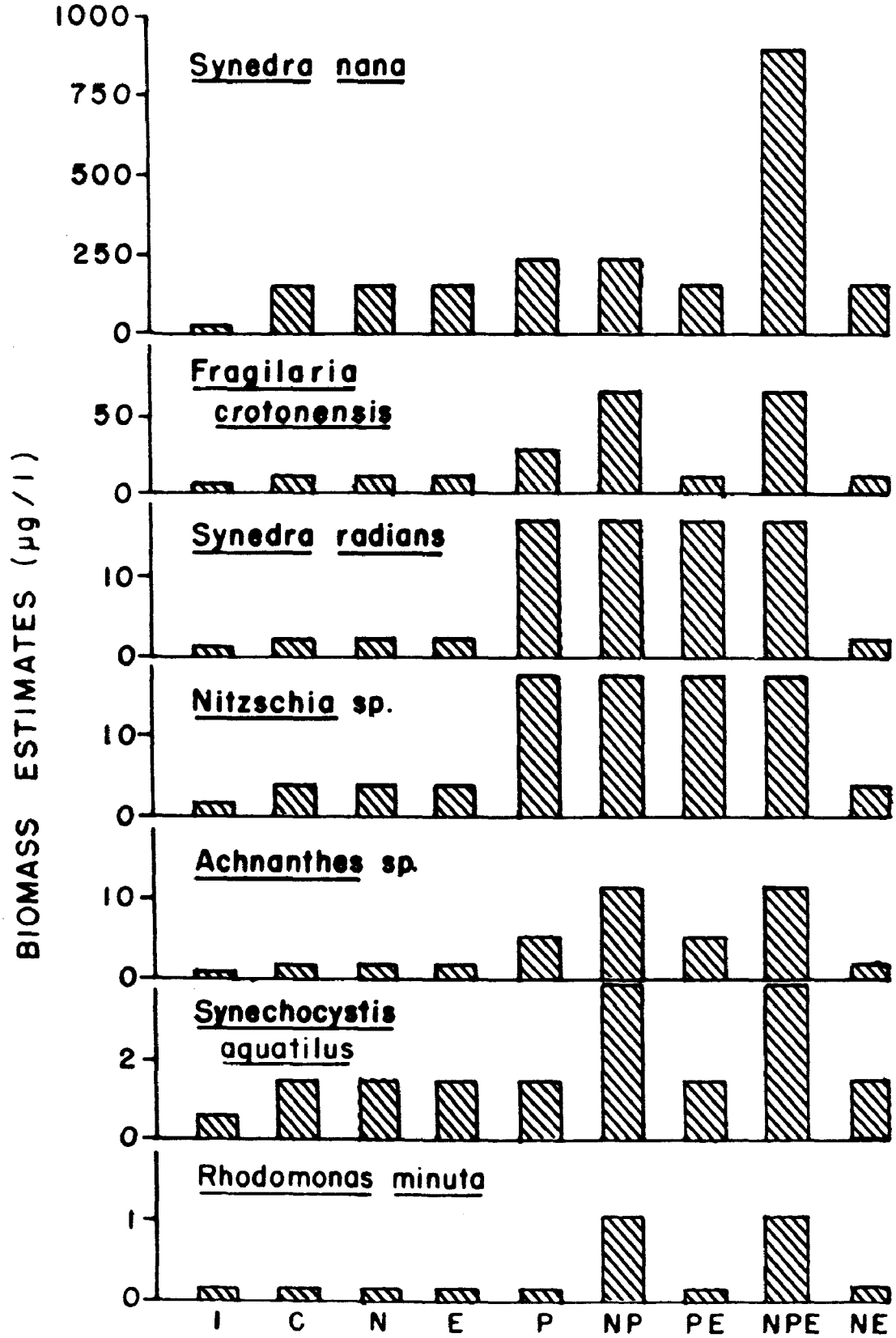


Figure 18. Experiment 12, Growth Response Patterns I

Fragilaria crotonensis, the second dominant species, shows stimulation by P alone, enhancement of the P effect by N, and blockage of the P effect by EDTA. These are three of the features of the productivity response pattern of July 27, and the correlation between the Fragilaria biomass estimates and the July 27 productivity results is .977.

If the growth patterns of Synedra nana and Fragilaria crotonensis are pooled the correlation between biomass and productivity on July 30 is increased to .961, and all but one of the features of the productivity pattern are accounted for. What remains is the response to the PE mixture, that appeared on July 24 as well as July 30. This can be covered by including the next three species, Synedra radians, Nitzschia sp., and Achnanthes sp., since for them the effect of P was not blocked by EDTA. Synedra radians and Nitzschia sp. responded to P alone, with no modification of the effect by either N or EDTA, while Achnanthes sp. responded more strongly to the NP mixture. Addition of the biomass estimates for these three species to the sums of the estimates for Synedra nana and Fragilaria crotonensis raises the correlation between biomass and productivity on July 30 to .973.

These correlation analyses indicate that the relative contributions of individual species to community productivity changed with time. Thus on July 27 most of the productivity responses could be attributed to Fragilaria crotonensis, although the influence of Synedra nana had begun to appear, at least in the response to the NPE mixture. By July 30 dominance of the productivity pattern had shifted to Synedra nana, but five species in all were required to account for all of the important productivity results. Thus each set of productivity measurements provided instantaneous community response estimates integrated over the active species, while the final biomass determinations provided individual species response estimates integrated over time. Since the day to day changes in the productivity response patterns reflected genuine changes in the growth activities of individual species, all of the productivity patterns observed were legitimate components of the community response to the nutrient treatments. Thus in this experiment and in each Third Sister Lake experiment, all of the productivity responses obtained, regardless of their persistence, were included in summarizing the results.

In experiment 12 there were two additional species, Synechocystis aquatilus and Rhodomonas minuta (Figure 18),

that responded positively to nutrient treatments. Both of these responded only to the NP mixture. Eight other species, shown in Figure 19, either responded negatively to the jug environment, or negatively to some of the nutrient treatments, or not at all. Most of these were minor species, but three of them Cyclotella ocellata, Cryptomonas ovata, and Cyclotella stelligera, accounted for significant fractions of community biomass.

The variety of response patterns observed on the species level in this experiment exemplifies the ability of nutrient enrichments to alter the species composition of phytoplankton communities. Species shifts in response to nutrient treatments have been observed in numerous other enrichment studies (Menzel et al. 1963, Thomas 1964, Barlow et al. 1971, Gächter 1968, Schelske and Stoermer 1972), and were encountered in the Third Sister Lake series of experiments, to be discussed.

The complex response patterns exhibited by some of the species in experiment 12, e.g. Synedra nana and Fragilaria crotonensis for which positive and negative interactions among treatments appeared, indicate that more than one nutrient treatment can influence a given species at a given time in a natural community. The growth responses of these two species, as well as all of the others, were totally dependent upon the treatment additions of P, while the other two treatments acted only to modify the P effects. In this sense P was the nutrient limiting species growth, and consequently community productivity, in this experiment.

PRODUCTIVITY RESPONSE PATTERNS

Summary of Treatment Effects

Table 11 is an attempt to summarize the productivity response patterns obtained in experiments 1-20. The treatments listed under independent effects caused significant stimulation of productivity in the presence or absence of the other treatment substances. Independent effect in this sense does not necessarily mean that the treatments did not interact with other substances in the lake water, since for EDTA chelation of ambient trace metals was probably the basis for its effect. When two or more substances had independent effects, these effects were essentially additive in mixtures of the substances. Interactions occurred when a treatment substance that did not have an independent effect

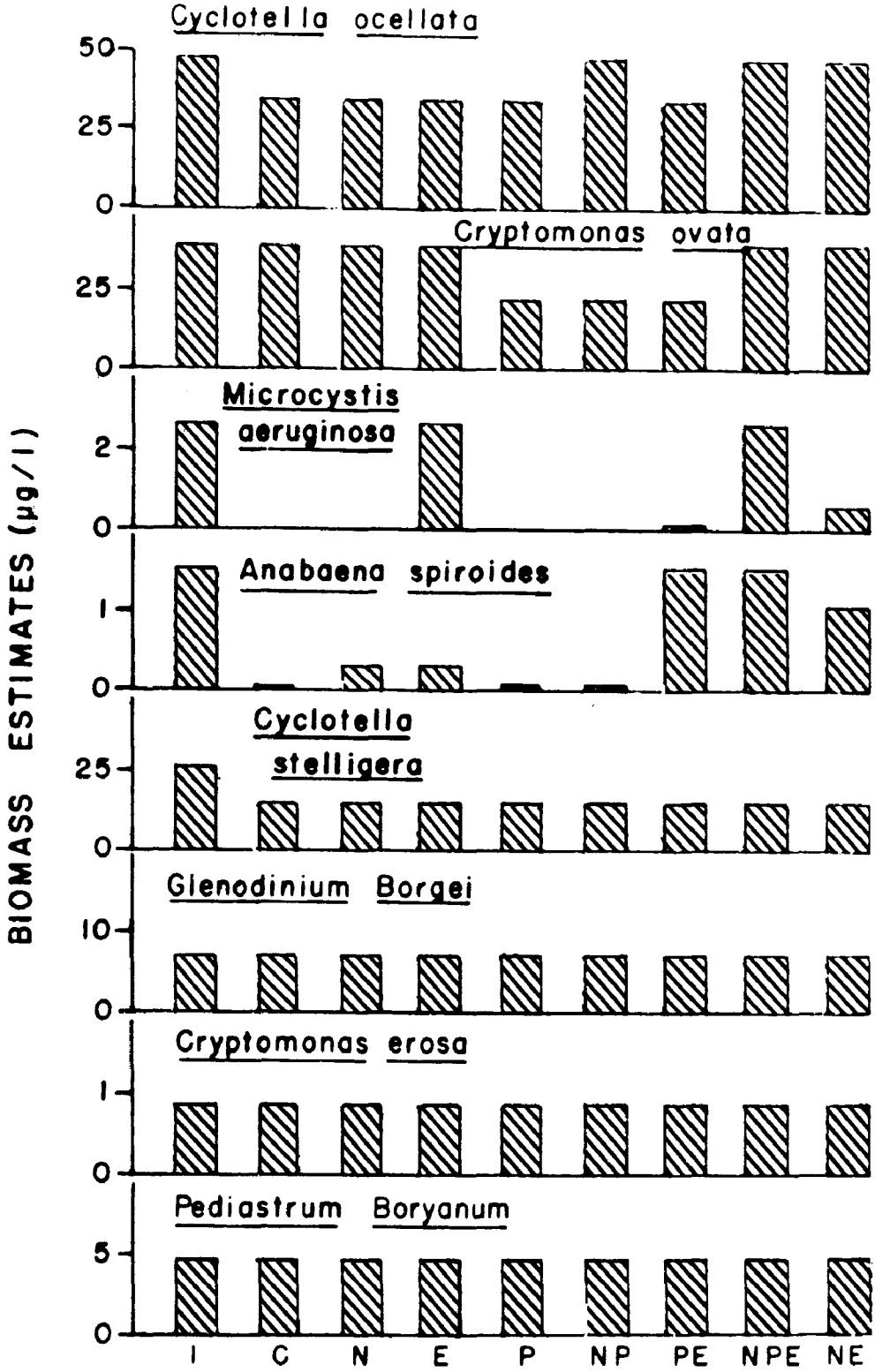


Figure 19. Experiment 12, Growth Response Patterns II

TABLE 11

SUMMARY OF PRODUCTIVITY
RESPONSE PATTERNS,
EXPERIMENTS 1-20

	Month:	Apr	May	Jun	Jul	Aug	Sep	Oct
1968	Expt:			1	2	3	4	5
	Independent effects			N EDTA		EDTA	P	
	Interactions			PN	NP			NP
	Notes					N, P Not Tested	EDTA Not Tested	
1969	Expt:	6	7	8	9	16	17	19
	Independent effects	EDTA	P	P	N P EDTA	P EDTA	P EDTA	P
	Interactions		NP N EDTA	NP			NP	NP EDTA
	Notes			NH_3-N employed				
1970	Expt:	20						
	Independent effects	P EDTA						

TABLE 12

FREQUENCY OF RESPONSES,
EXPERIMENTS 1-20

Treatment	No. of Expts. Tested	No. of Expts. with Independent Effects	No. of Expts. with Interactions Only	No. of Expts. with No Effect
N	12	2	6	4
P	12	8	3	1
EDTA	12	7	2	3

enhanced the otherwise independent effect of a second treatment (e.g. N in experiment 8, Figures 15 and 20), or when two treatments that had no effect when applied separately had an effect when applied in mixtures (e.g. N and P in experiment 17, Figures 16 and 20).

Table 12 summarizes Table 11 indicating the overall importance of each of the treatments. Of the three treatment substances P stimulated productivity in the largest number of experiments. In 8 experiments it acted independently, and in 3 its action depended upon the presence of added N. EDTA produced independent effects in 7 experiments, while N stimulated productivity only twice in the absence of added P. Thus if the three treatments are ranked according to their abilities to stimulate primary productivity in the experimental system the order is P, EDTA, and N.

The results of these 13 experiments could be pooled without further analysis in order to predict productivity responses in future experiments. The prediction, which might be called the "most probable response pattern," would include an independent effect of EDTA and an independent effect of P that would be enhanced in the presence of added N. Examination of Table 11, however, indicates that this theoretical pattern actually appeared in only one experiment, number 17, and that substantial variation in response patterns occurred from experiment to experiment.

Variations Among Experiments

Literature Examples. Variability seems to be a common feature in the results of studies in which series of experiments have been conducted on a single natural system. In the most general sense Rodhe (1958) attributed season to season changes in enrichment effects to changes in environmental conditions. He observed that nutrient treatments were most stimulatory during the period of thermal stratification, when nutrient availability to the photic zone was reduced, and least stimulatory during the fall circulation. However, experiment to experiment changes in enrichment effects during a season have been more difficult to explain in terms of environmental changes.

In one study Lange (1971) employed a procedure similar to the PAAP technique (Bueltman et al. 1969) to test for limiting nutrients in water samples from Lake Erie. Fifteen experiments were performed, most of them in a biweekly series from April-October 1969. In each

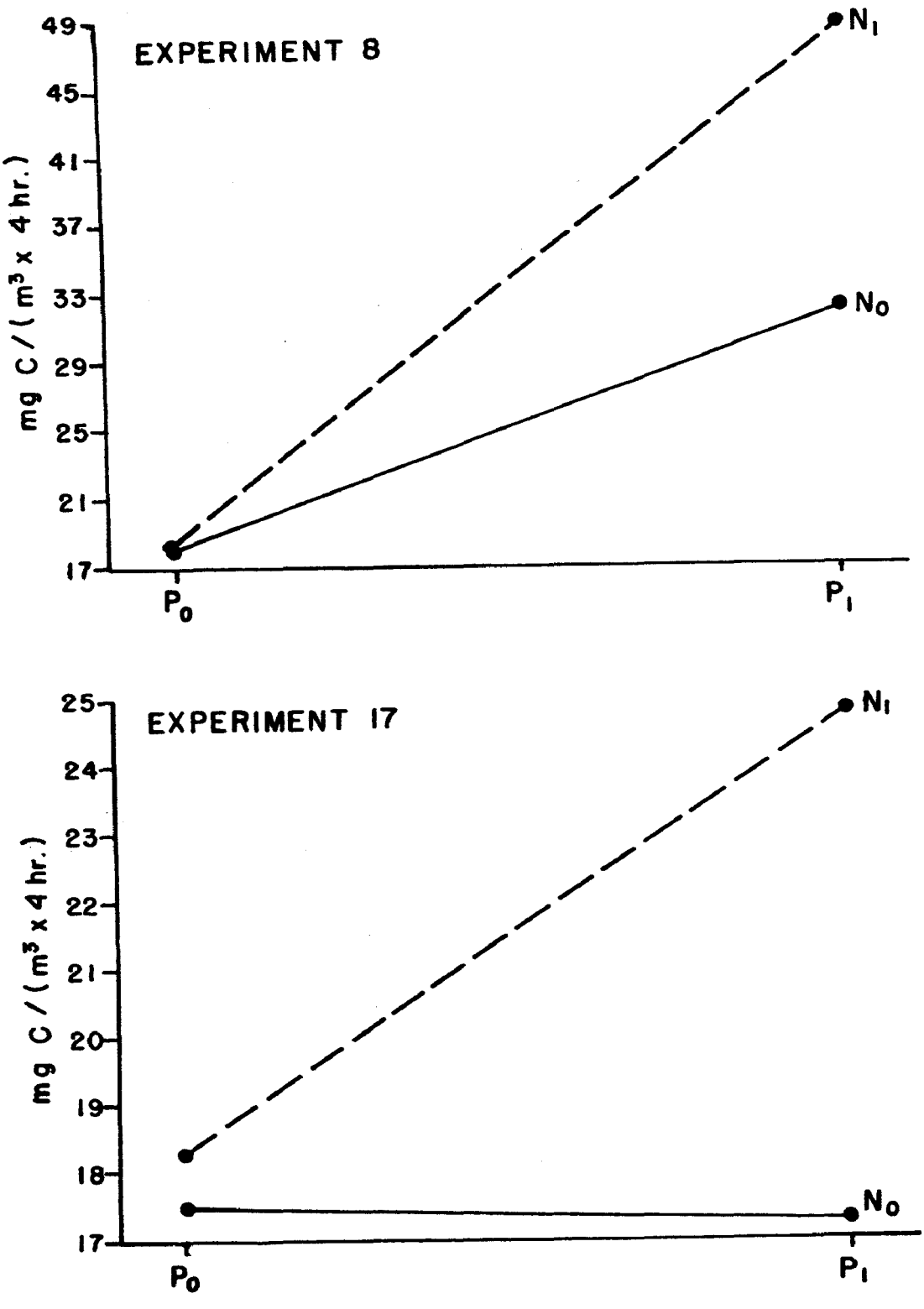


Figure 20. Examples of NP Interactions

experiment four cultured algal species were grown separately in filtered lakewater samples to which numerous pure and mixed nutrient treatments were added. Responses were determined by cell counts. There were response variations among experiments within species and among species within experiments, and no obvious correlations between the responses and ambient nutrient levels emerged. Conclusions about the relative roles of the different nutrients as limiting factors in the lake were based upon the number of experiments in which each one stimulated. The nutrient that stimulated the most frequently was N (39/60 cases), and it was concluded therefore that N was in adequate supply in the smallest number of samples tested. Based on these results the "most probable response" to a single nutrient in future experiments would be to N, but the probability would be only .65. One advantage claimed for the PAAP bottle test over *in situ* tests is reproducibility of results, since species composition can be eliminated as a variable by employing the same species in the same stage of growth in every experiment. If the results of Lange (1971) are representative, however, reproducibility seems to be an elusive quality when using the culture technique as well as when using the *in situ* technique.

Biological conditions have been invoked in some studies, in attempting to account for varying response patterns. Goldman and Wetzel (1963) reported that bioassay responses in Clear Lake, California related to the overall activity levels of the phytoplankton present. During periods of high primary productivity treatments were stimulatory, and when productivity was low no treatment effects occurred. In studies reported by Goldman (1960b) and Powers *et al.* (1972), direct examination of the phytoplankton communities present in different experiments revealed that the species composition changed from experiment to experiment. Changes in the experimental response patterns were thought to have related to the species shifts.

In attempting to interpret the results of the Third Sister Lake series of enrichment experiments, an effort will first be made to determine if the variability among experiments was random or if gradual changes occurred. If the changes were gradual, it seems more logical to expect to relate them to changes in environmental conditions or in the species present, than if the changes were random.

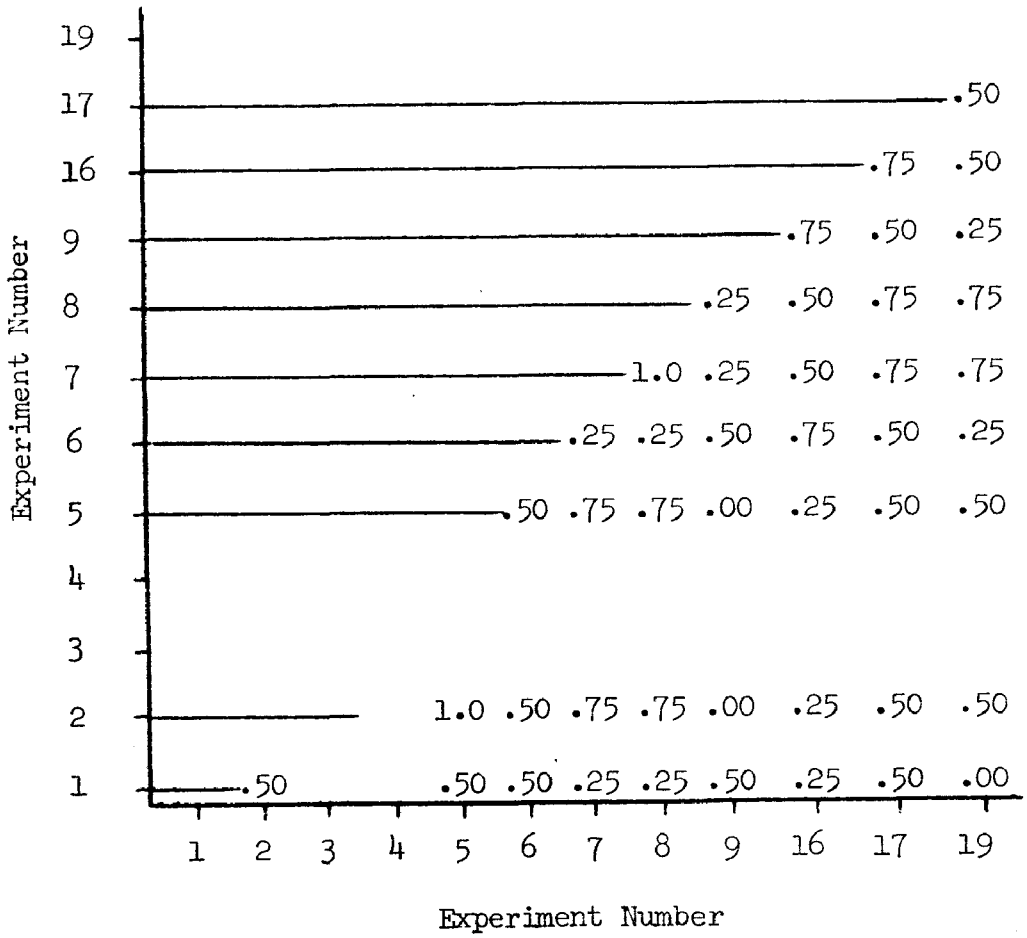


Figure 21. Comparison of Productivity Response Patterns, Experiments 1-20

TABLE 13

TESTS FOR SIMILARITIES AMONG "NEAR" VERSUS "DISTANT" EXPERIMENTS

Experiment	Sums of Comparison Values	
	Near	Distant
2	3.00	1.25
5	3.00	1.25
6	1.75	1.75
7	2.00	3.00
8	2.00	3.00
9	1.75	.75
16	2.50	1.75
17	2.50	2.25
19	2.00	2.00
Overall Sum:	20.50	17.00

Intercomparison of Experiments. The comparisons appear in Figure 21. For the purpose of this figure each productivity response pattern was summarized in terms of four segments: independent responses to N, to P, and to EDTA, and an interaction. Experiments 3, 4, and 20 were omitted because they did not include all treatment combinations. Pairs of experiments in which all 4 segments were similar, such as experiments 2 and 5 (See Table 11), in which an interaction between N and P showed up but in which no independent effects occurred, were awarded a 1. Pairs with dissimilar segments were awarded fractions from 0 to .75 based on the number of similar segments divided by 4. In experiments 8 and 16, for example, the responses to N and P were similar while the responses to EDTA and the interaction differed. The comparison value is .5.

These comparison values can be used to test for a tendency for experiments conducted close together in time to have more similar results than experiments that were more widely spaced. For this test two sums were obtained for each experiment (Table 13). The first is the sum of the values obtained by comparing the experiment in question with the four other experiments that were closest to it in time. The second is the sum of the values for the four experiments farthest from this experiment in time. For the purpose of this test experiment 1 was omitted so that each experiment could be compared to an even number of others. The sums of these sums are 20.5

for the "near" experiments versus 17.0 for the "distant" experiments, indicating that "near" experiments were more similar than "distant" experiments. This suggests that changes in response patterns from experiment to experiment were tied to gradual changes occurring in the lake system, rather than being totally random variations.

Environmental Conditions. Table 14 summarizes the ambient environmental conditions associated with the starting dates of experiments 1-20. The nutrient data indicate that nitrate was available at the working depth of 1 m for the entire summer of 1969, while orthophosphate declined gradually from a spring peak and reached the detection limit in late fall. These data seem to be consistent with the relative roles of N and P as treatments in the enrichment experiments (Table 11). Phosphorus additions consistently stimulated productivity in experiments 7 through 19, during the period of declining ambient P, while N additions were of secondary importance and usually stimulated only in conjunction with P.

Stimulation by N was entirely absent from the two spring experiments, numbers 6 and 20. This seems to relate to the ambient nitrate levels, which were at seasonal maxima in both of these experiments. In experiment 20 the ambient nitrate level was much higher than in experiment 6, and this may relate to the slower warming rate of the surface waters in 1970 compared to 1969. As shown in Figure 22 a more stable thermal structure had developed prior to experiment 6 than prior to experiment 20, with consequent reduction in the nutrient pool of the photic zone and reduction in nitrate levels. This line of reasoning, however, does not explain why the ambient orthophosphate level for experiment 20 was so much lower than for experiment 6. This difference, however, is consistent with the occurrence of stimulation by phosphate additions in experiment 20 and the absence of a P effect in experiment 6.

Reference to Table 11 indicates that stimulation by EDTA was essentially absent in the two fall experiments, 5 and 19. Figure 23 shows that prior to both of these experiments there had been periods of steadily declining surface water temperatures. The consequent erosion of the thermocline permitted reintroduction into the epilimnion of quantities of suspended and dissolved material that had been accumulating in the thermocline during the summer. This material may be assumed to have included trace metals and dissolved organic compounds with

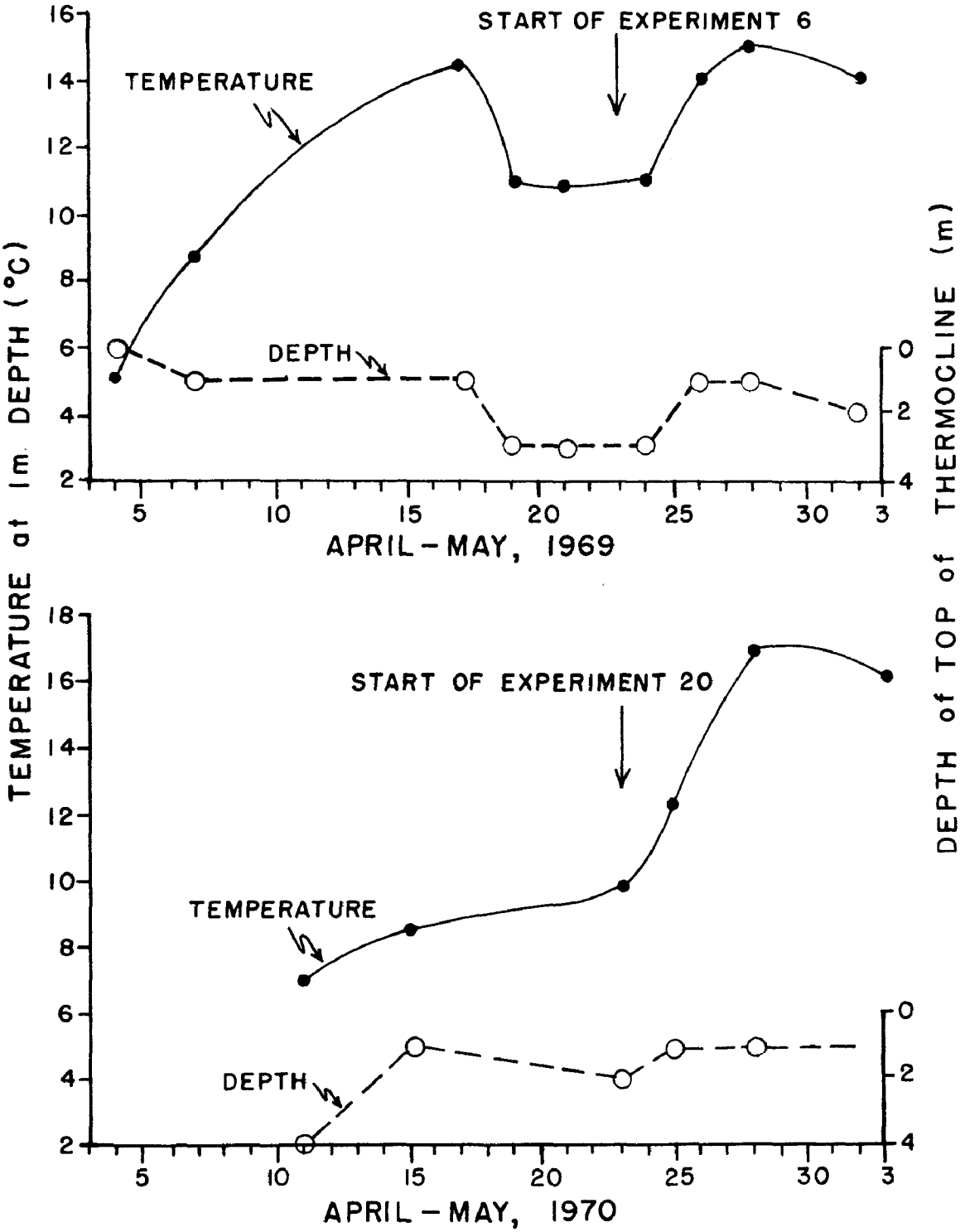


Figure 22. Spring Thermal Conditions in Third Sister Lake - 1969, 1970

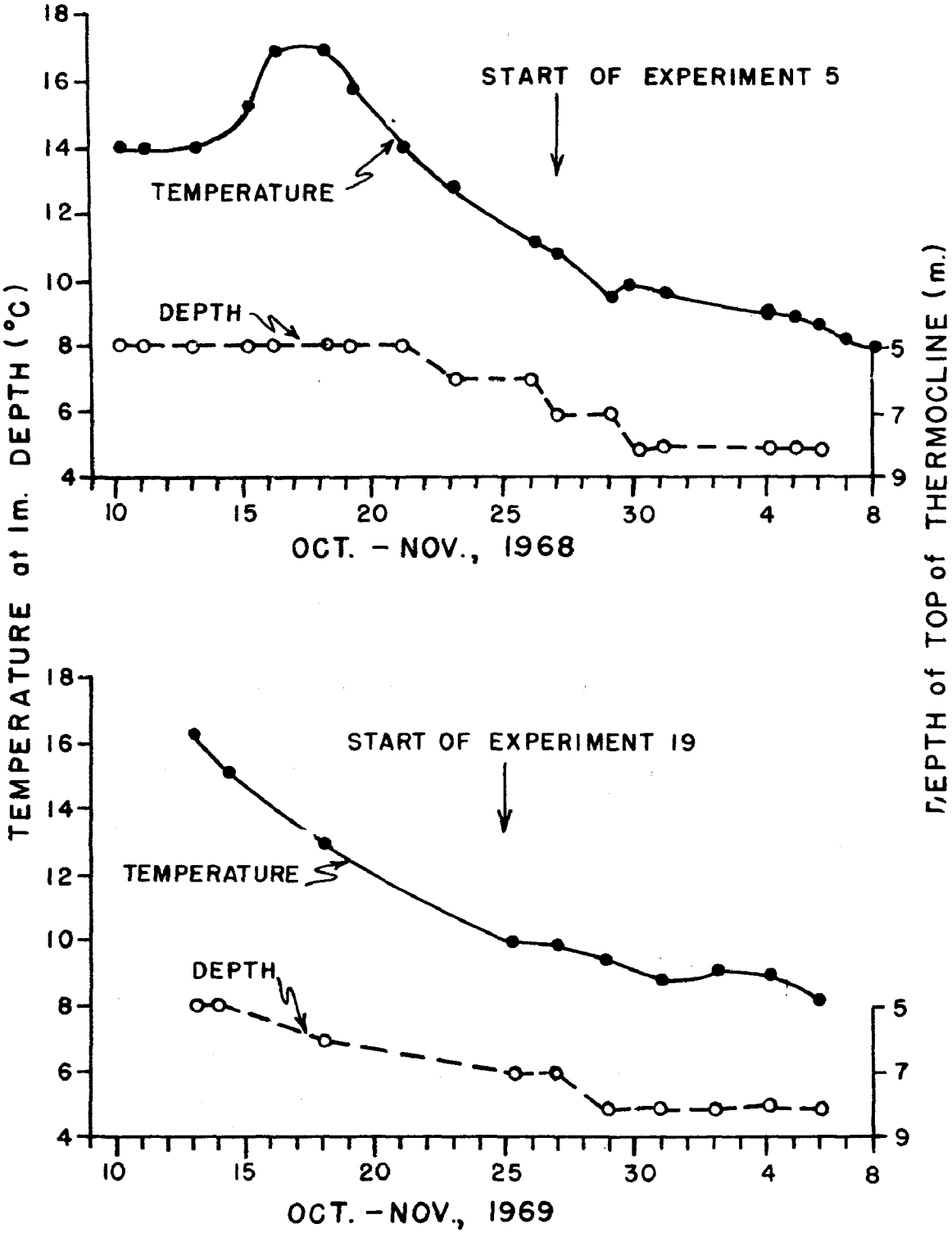


Figure 23. Fall Thermal Conditions in Third Sister Lake - 1968, 1969

TABLE 14

AMBIENT LAKE CONDITIONS: EXPERIMENTS 1-20

Expt. No.	Starting Date	Temp. at 1 m Depth (°C)	Light Extinction Coefficient	NO_3-N (µg/l)	PO_4-P, (µg/l) Ortho	Total
1	6-12-68			56	10	
2	7-18-68					
3	8-07-68	26				
4	9-19-68	21	.90			
5	10-27-68	10	1.20		10	
6	4-23-69	15	.98	50	10	10
7	5-26-69	18		40	6	7
8	6-04-69	19	.69	41	7.5	7.5
9	7-01-69	25	.46	42.5	5	5
16	8-21-69	25	.78	40	4	8
17	9-13-69	21	.78	38	<1	6.5
19	10-25-69	10	1.17	62	<1	2
20	4-23-70	10		230	5	

chelating abilities, thus reducing the sensitivity of the system to treatment with additional chelators.

In two other experiments, 2 and 8, EDTA effects were absent with no apparent relation to normal seasonal changes within the lake. Prior to experiment 2, however, on June 25, 1968 a massive inflow of surface runoff water occurred as a consequence of an unusually severe series of rainstorms. The runoff carried sufficient suspended particles to render the entire lake brown in color and to reduce light penetration to essentially nil. The particles gradually settled out, and the lake was visually normal by the start of experiment 2, on July 18. Nonetheless it is possible that allochthonous organic compounds carried in with the runoff had remained in sufficient concentrations to make the treatment additions of EDTA superfluous. It is also possible, of course, that stimulation by EDTA did occur but was obscured by the high error variance that showed up in experiment 2. This experiment, as mentioned in the methods section, was the least sensitive in detecting treatment effects of all the experiments performed (Table 8).

A further consistency that can be observed between experiments performed at similar times in two different

years is the absence of a nitrate effect, either independent or interactive, in experiments 4 and 16. Nothing in the ambient lake data suggests an explanation for this pattern.

Examination of ambient physico-chemical conditions has offered explanations for several features of the response patterns: (1) the greater stimulatory ability of phosphate treatments relative to nitrate treatments in the summer, (2) the absence of nitrate effects in the spring experiments, (3) the occurrence of a phosphate effect in experiment 20, but not in experiment 6, and (4) the essential absence of EDTA effects in the fall experiments, 5 and 19. The physical and chemical data provide little help in interpreting the variations in response patterns among the summer experiments. Changes in the species composition of the phytoplankton community may be partially responsible for the variations, and will be examined next.

Phytoplankton--Communities. In experiment 12 it was found that most of the features of the productivity response patterns, including the changes in these patterns that occurred from day to day, could be explained in terms of the growth response patterns of individual phytoplankton species. The productivity response patterns for experiments 1-19 can be assumed to be, likewise, integrals of the various growth responses of the species present. Shifts in the species composition of the phytoplankton community, as well as changes in the physiological condition of constant members of the community, can be expected to have caused changes in the productivity response patterns from experiment to experiment.

Table 15 summarizes the species composition of the phytoplankton communities present at the beginning of each of the 1968 and 1969 experiments. It will be noted that several species were present in nearly all of the experiments while the majority were present only occasionally, sometimes at characteristic times of the year. Many rare species were undoubtedly missed, so this table should be regarded as a compilation of the occurrence data for the major species.

In Figure 24 the experiments are intercompared on the basis of the number of major species that overlapped in each possible pair of experiments. Figure 25 presents these comparisons in terms of Sorensen's Index (Sorensen 1948) values, in which the number of overlapping species is doubled and divided by the total number of species present in both experiments. The range of possible

TABLE 15

OCCURRENCE OF PHYTOPLANKTON SPECIES IN INITIAL COMMUNITIES - 1968, 1969

Species	Experiment Number											
	1	2	3	4	5	6	7	8	9	16	17	19
Chroomonas acuta	x		x	x	x	x	x	x	x	x	x	x
Cryptomonas ovata	x		x		x	x	x		x	x		x
Cryptomonas erosa	x			x		x	x		x			
Ankistrodesmus falcatus	x	x	x	x	x	x	x	x	x	x	x	x
Crucigenia tetrapedia	x		x	x	x		x	x		x	x	x
Tetraedron minimum	x		x	x	x	x	x	x		x		
Tetraedron caudatum	x			x				x		x	x	x
Oocystis parva	x		x	x					x	x	x	
Pediastrum tetras	x				x			x		x		x
Rhabdoderma sigmoidea	x		x	x	x	x	x	x	x			
Lyngbya limnetica	x		x	x	x	x	x	x				
Chroococcus dispersus	x	x	x	x	x	x	x	x	x			
Gomphosphaeria lacustris	x		x						x	x		
Synedra rumpens	x	x	x			x	x	x				
Fragilaria crotonensis	x	x		x		x					x	x
Asterionella formosa	x					x	x					
Gonium pectorale	x		x	x				x		x		
Cosmarium truncatellum	x			x								
Scenedesmus bijuga	x									x		x
Synedra radians		x	x									x
Synedra acus		x	x				x	x				
Anabaena wisconsinense			x	x	x					x	x	x
Cylindrospermum stagnale			x	x						x	x	
Spirulina major			x	x						x	x	
Aulosira sp.			x	x								
Microcystis incerta				x	x			x	x	x	x	x
Elaktothrix gelatinosa				x			x	x				
Chrysidalis sp.						x	x					
Ochromonas sp.						x						
Microcystis aeruginosa						x				x	x	x
Sphaerocystis Schroeteri						x		x	x	x	x	x
Oscillatoria rubescens						x				x	x	x
Chroococcus minutus						x						
Fragilaria capucina						x						
Pediastrum Boryanum						x				x		
Aphanothece nidulans							x	x			x	
Aphanocapsa elachista										x	x	x
Aphanizomenon flos-aquae										x		
Chlamydomonas pseudopertyi										x		
Glenodinium pulvisculus										x	x	
Coelastrum microporum										x	x	
Oscillatoria tenuis										x	x	x
Asterococcus limneticus											x	
Anabaena Scheremetievi											x	x

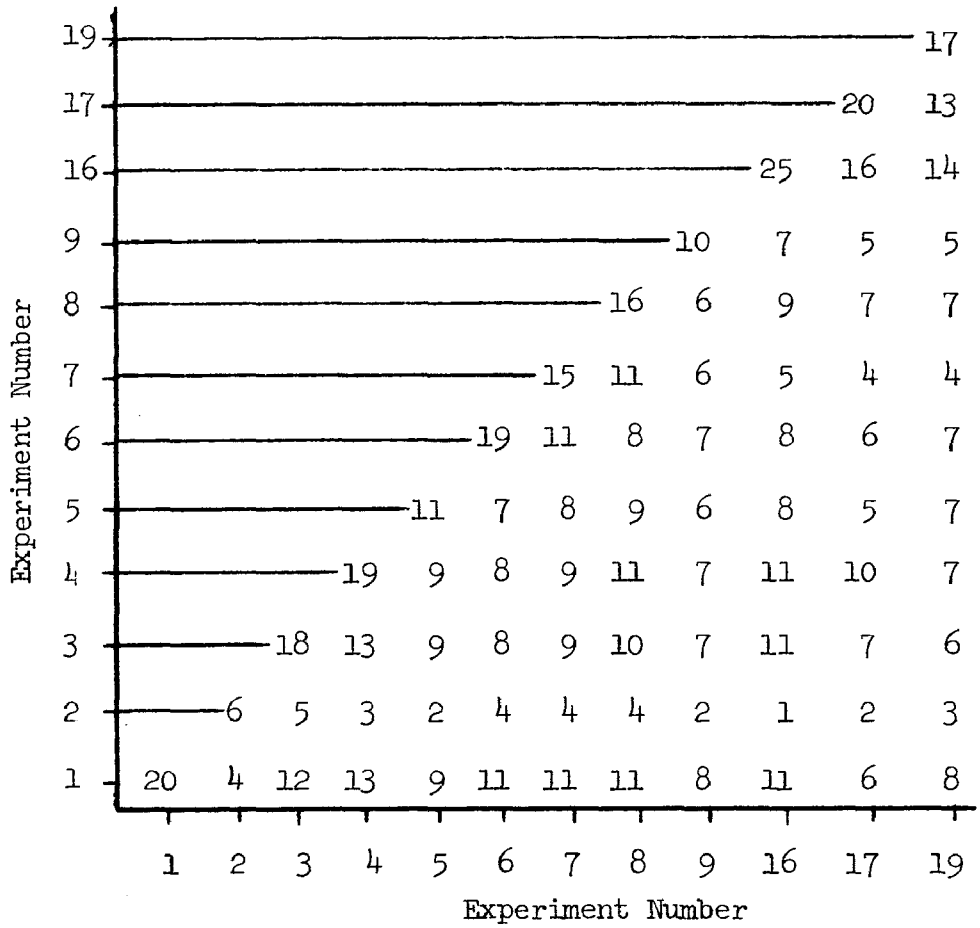


Figure 24. Numbers of Species in Common in Each Pair of Experiments-1968, 1969

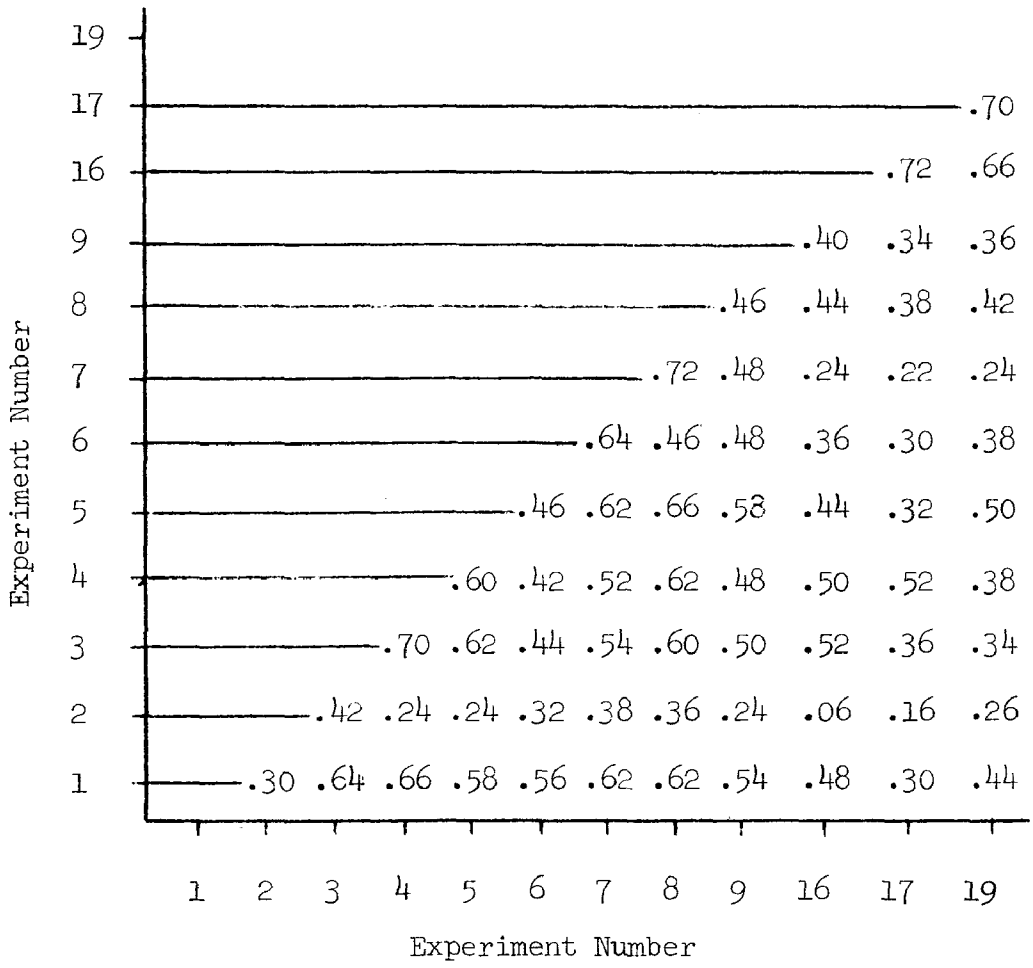


Figure 25. Sorensen's Index Values for Total Species Complexes - 1968, 1969

TABLE 16

SUMS OF SORENSEN'S INDEX VALUES - 1968, 1969

Year	Expt.	Near	Distant
1968	1	2.74	2.56
	2	1.52	1.20
	3	2.82	2.52
	4	2.62	2.64
	5	2.50	2.62
1969	6	2.40	2.04
	7	2.30	2.68
	8	2.46	2.86
	9	2.04	2.28
	16	2.46	1.88
	17	2.36	1.66
	19	2.38	1.86
Overall Sum:		28.60	26.80
1969	6	1.58	1.04
	7	1.84	.70
	8	1.64	1.24
	9	1.34	1.18
	16	1.78	1.04
	17	1.76	.90
	19	1.72	1.04
Overall Sum:		11.66	7.14

values is therefore from 0 to 1. The maximum value that shows up is .72, while the minimum is .06.

In Table 16 the "near" and "distant" values of Sorensen's Index are summed for each experiment in a way similar to the treatment of the productivity comparisons in Table 13. When all experiments are included, the grand "near" sum is only slightly higher than the grand "distant" sum, while when only the 1969 experiments are used the near sum is substantially greater. This discrepancy implies that continual shifts in species composition occurred within a year, but species complexes recurred in the separate years.

Not all of the species present in a given experiment responded to the treatments in the experiment, and often the number of responding species was a small minority. In Table 17 the responding species are listed, and the experiments in which they responded to nutrient

TABLE 17

RESPONDING SPECIES - 1968, 1969

Species	Experiment Number 1	2	3	4	5	6	7	8	9	16	17	19	Code Letter for Figure 38
Ankistrodesmus falcatus	x				x		x					x	E
Rhabdoderma sigmoidea	x						x						C
Chroococcus dispersus	x												
Synedra rumpens	x	x						x					G
10 μ Sphere	x												
Synedra radians		x										x	P
Anabaena wisconsinense			x							x			N
Elaktothrix gelatinosa				x									
Lyngbya limnetica					x		x						D
Cryptomonas ovata					x							x	Q
Chrysidalis sp.						x							A
Ochromonas sp.						x							B
Chroomonas acuta							x					x	F
Synedra acus								x					H
Microcystis incerta								x					I
Aphanothece nidulans								x					J
Gomphosphaeria lacustris									x	x			K
Aphanocapsa elachista										x	x		O
Aphanizomenon flos-aquae										x			L
Chlamydomonas pseudopertyi										x			M
Microcystis aeruginosa												x	R
Oscillatoria tenuis												x	S

treatments are indicated. The largest number of responding species was 6, in experiment 19, while in several experiments only one species responded. Comparison of this table with Table 15 indicates that changes in responding species were much more abrupt than were changes in the total species complex. This observation exemplifies how subtle changes in environmental conditions can induce shifts in the species composition of natural phytoplankton communities.

Figure 26 intercompares the experiments on the basis of the number of overlapping responding species, while Figure 27 expresses the comparisons in terms of Sorensen's Index. Most of the index values are zero and are omitted. This general lack of overlap of responding species supports the hypothesized role of changes in species occurrence or physiological state in causing changes in response patterns among experiments.

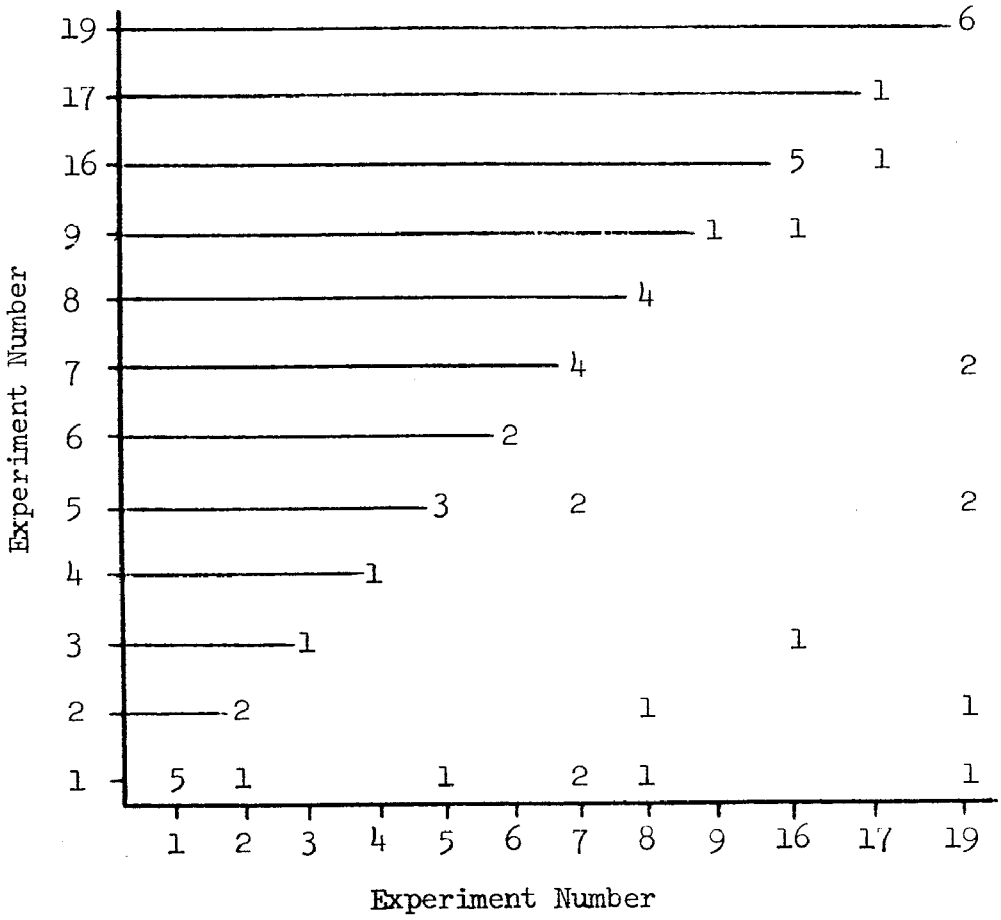


Figure 26. Numbers of Responding Species in Common in Each Pair of Experiments - 1968, 1969

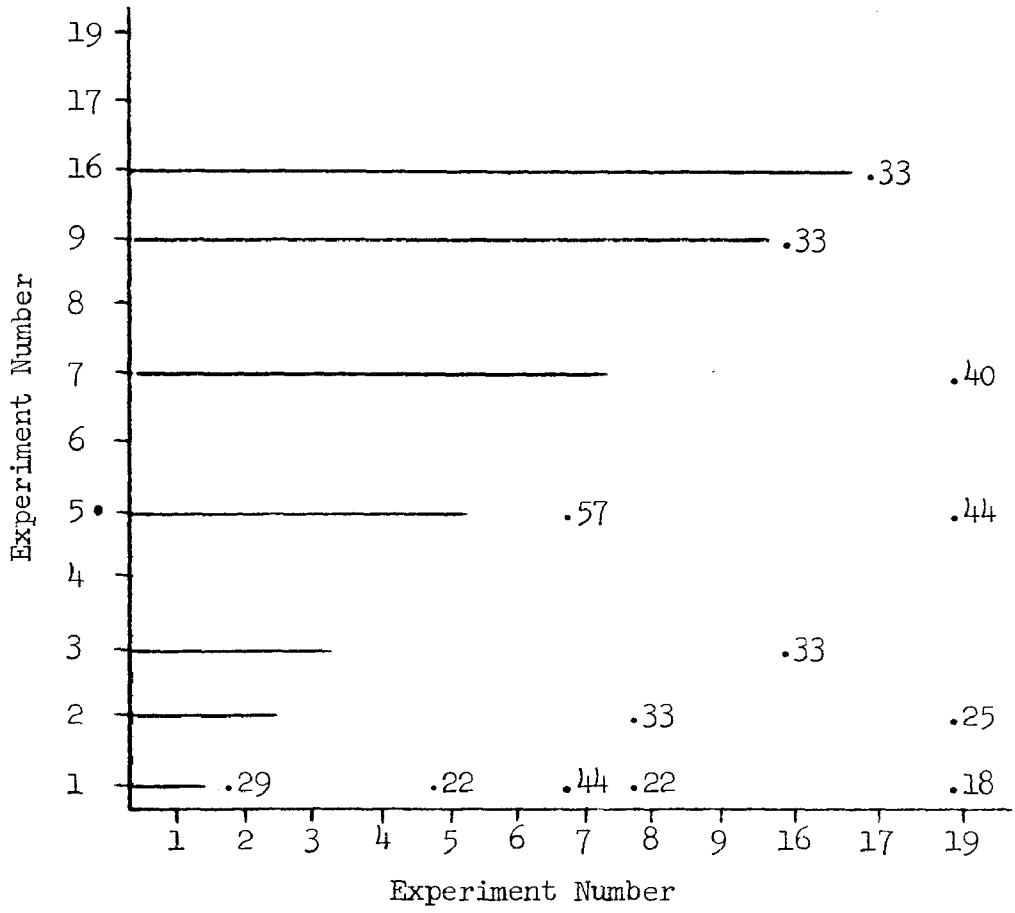


Figure 27. Sorensen's Index Values for Responding Species - 1968, 1969

In Appendix B quantitative data for the most important species in experiments 1-19 are tabulated. In most of the experiments samples from initial, final control, and final high treatment communities were counted, and the counts were converted to biomass estimates. Correlation analyses between the biomass estimates and the final productivity values were performed when possible, to indicate those species whose growth responses were most influential in shaping the productivity response patterns. Certain of these data are useful in further attempts to interpret consistencies and shifts in the productivity patterns among experiments.

Phytoplankton--Individual Species. It was noted that in experiments 4 and 16 no nitrate effects appeared, and that no environmental change that could explain this was apparent. It was determined from examining the species present that in both of these experiments heterocyst-forming bluegreen algae were important members of the phytoplankton community. Figure 28 shows that one of these organisms, Anabaena wisconsinense, had its peak in abundance in August and September of both 1968 and 1969, and that it responded to treatments in experiments 3 and 16. Examination of Table 15 reveals that other heterocyst-formers, Cylindrospermum stagnale, Aulosira sp., and Aphanizomenon flos-aquae were present in one or both of these experiments, and absent at other times of year. Thus it is reasonable to hypothesize that these organisms, heterocyst-formers and therefore potential nitrogen fixers, could have contributed fixed nitrogen to the lake system in the late summer of both 1968 and 1969, thereby eliminating the impact of the nitrate added as treatments at these times.

The biomass estimates for a number of other species have been plotted in Figures 29-37, and a variety of temporal distribution patterns appear. The first three species, Ankistrodesmus falcatus, Cryptomonas ovata, and Chroomonas acuta were present in all or nearly all of experiments 1-19. Ankistrodesmus maintained constant low population levels in the lake except for the spring and early summer of 1969 when a population peak occurred. Responses to treatments were confined to the early summer and late fall of both years. Cryptomonas population levels were low except in October of both years, when it peaked and responded to treatments. Chroomonas differed from the first two species in that its lake populations were generally high, at least for most of 1969, while its final population levels in the experimental jugs were low, if not nonexistent.

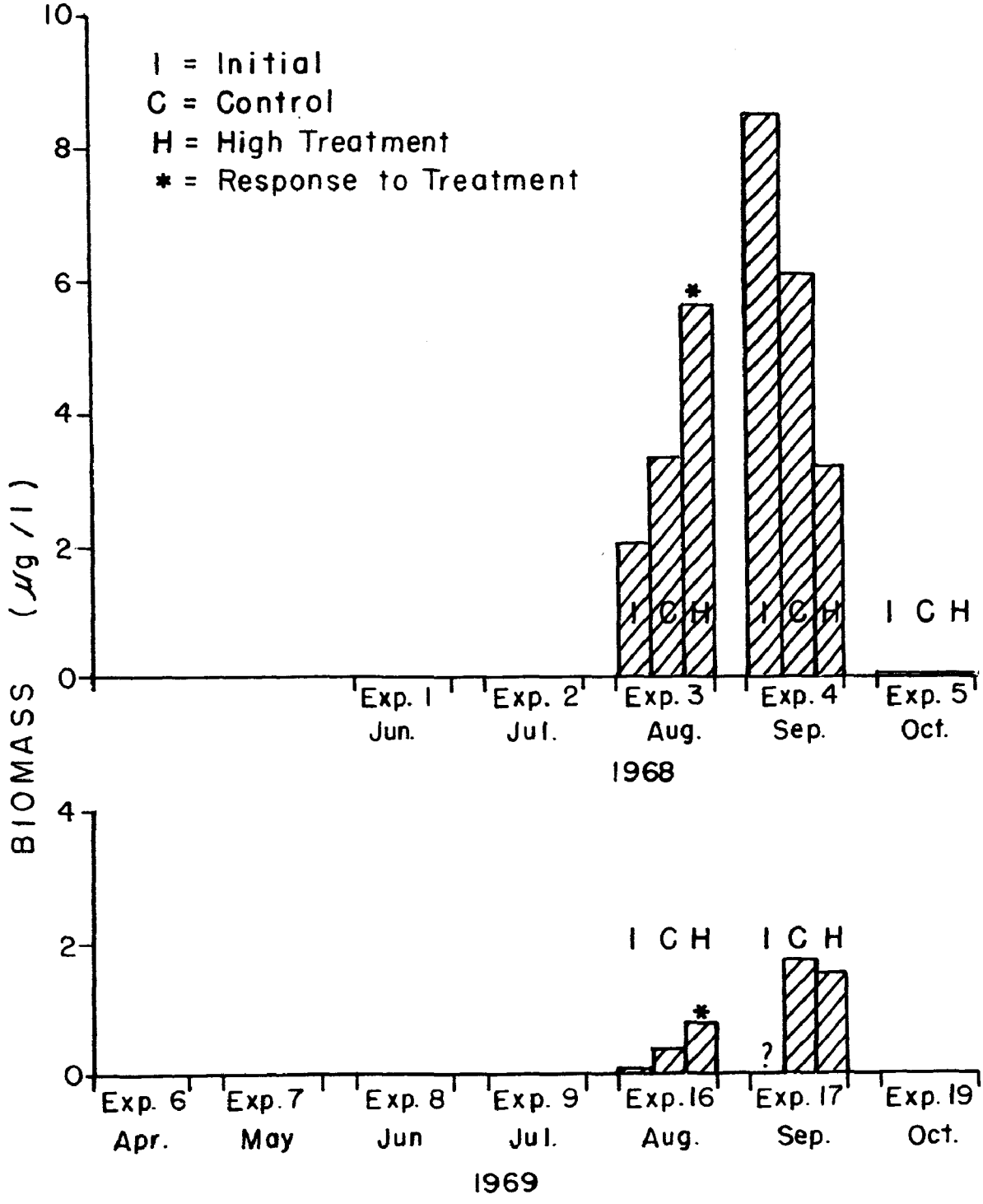


Figure 28. Anabaena wisconsinense Biomass Estimates-1968, 1969

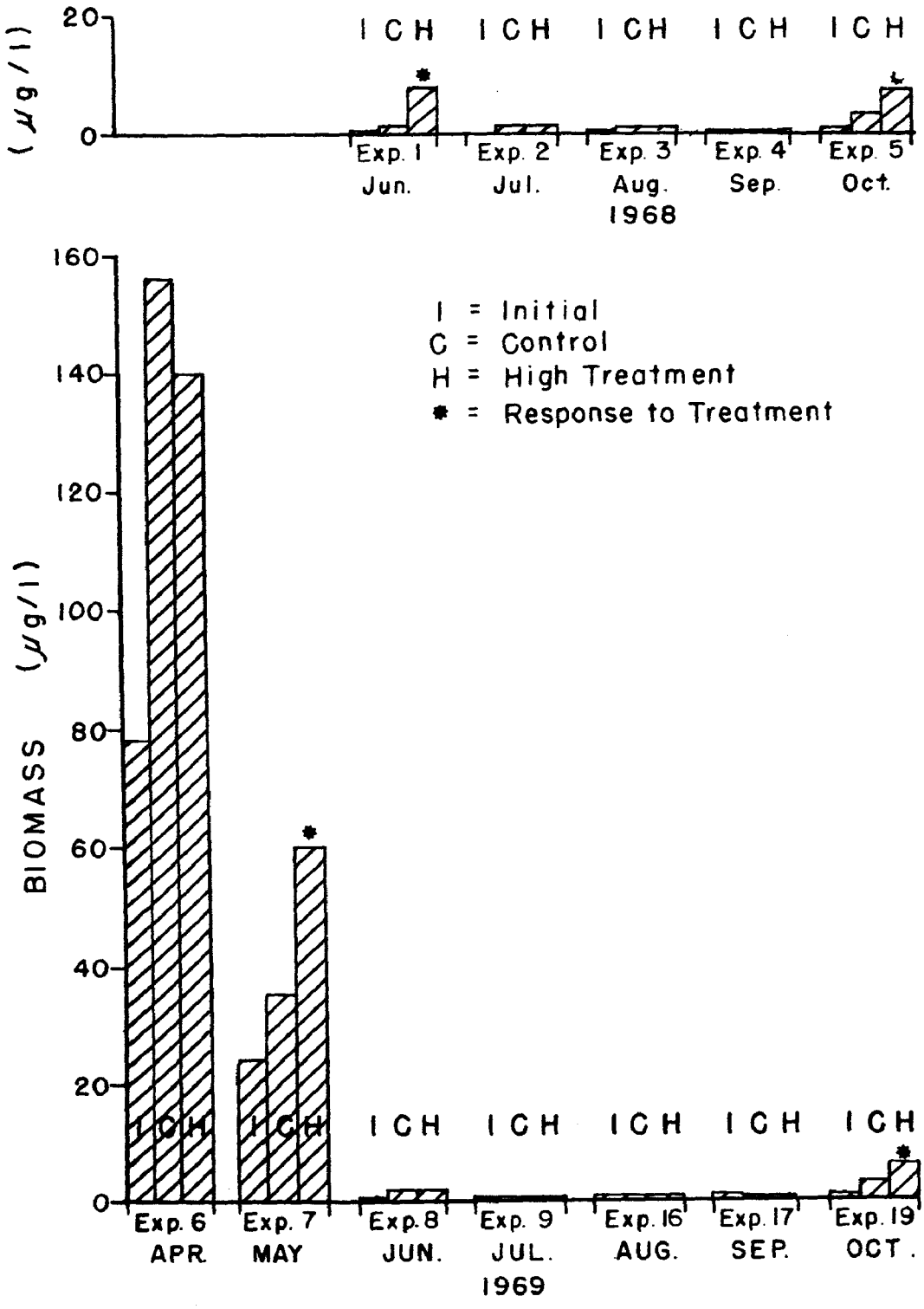


Figure 29. Ankistrodesmus falcatus Biomass Estimates - 1968, 1969

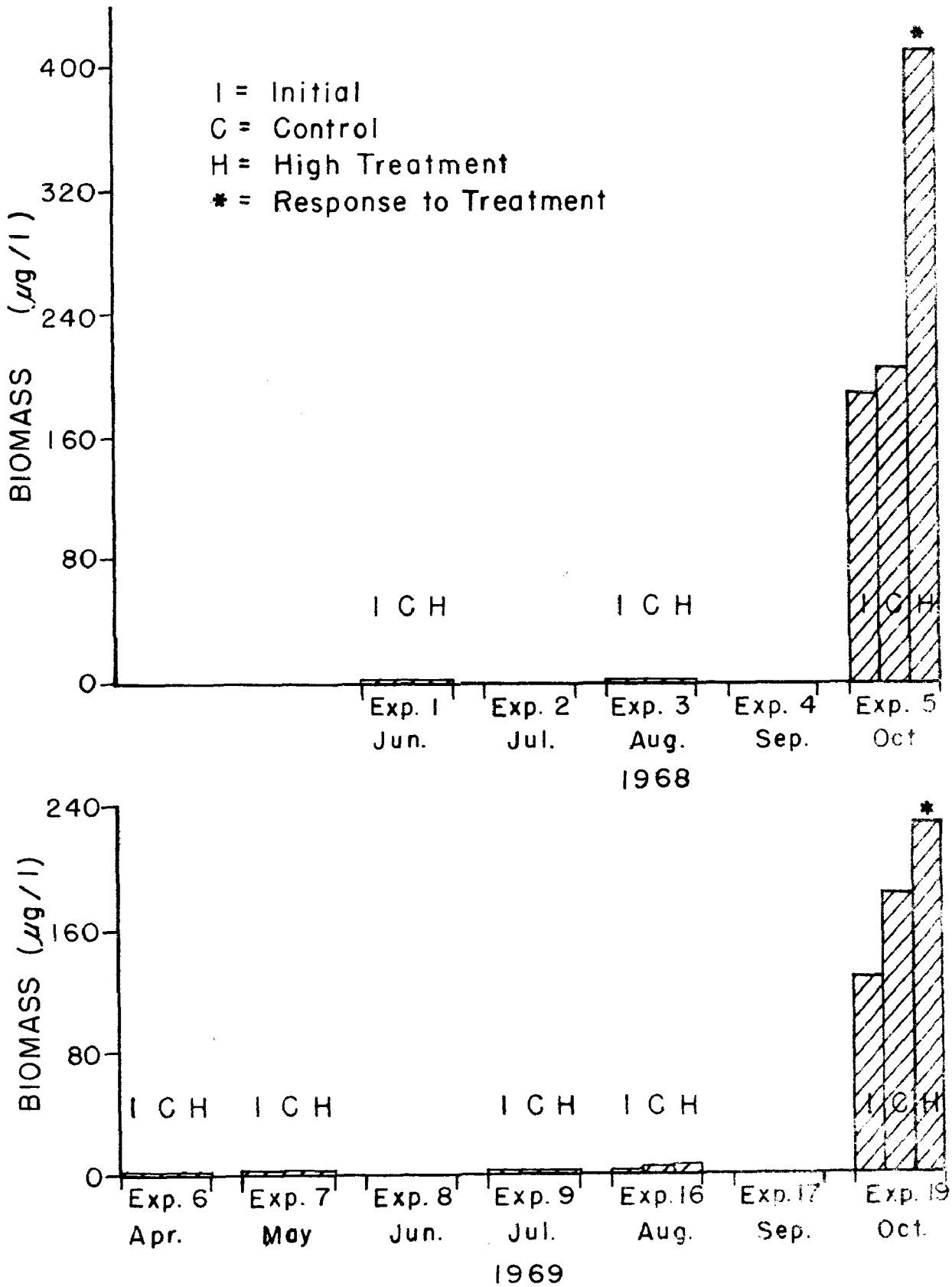


Figure 30. Cryptomonas ovata Biomass Estimates-1968, 1969

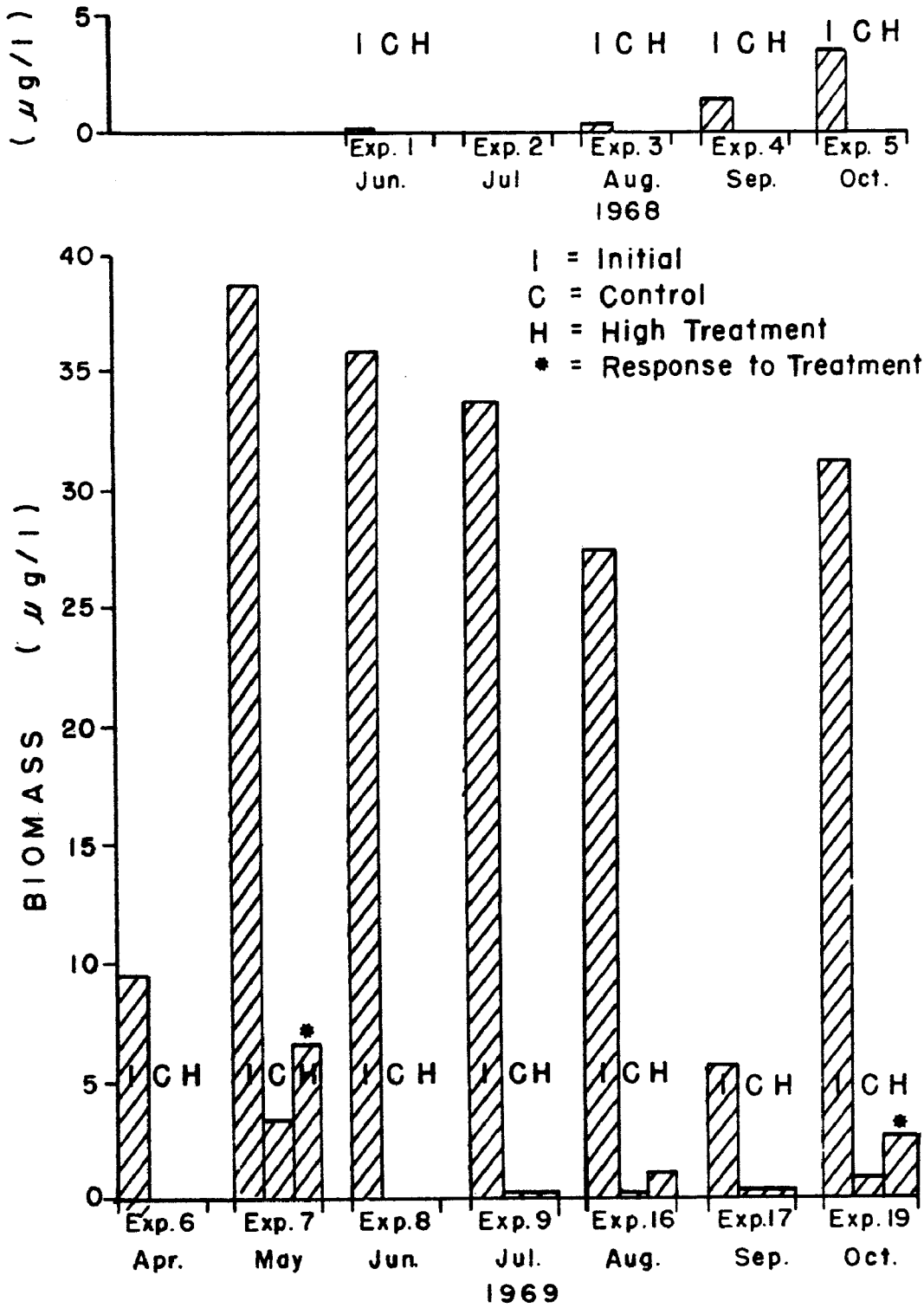


Figure 31. Chroomonas acuta Biomass Estimates-1968, 1969

Treatment effects that appeared for this species in experiments 7 and 19 did not represent actual growth of the populations in response to the nutrient treatments. Instead, they showed up because treatment populations declined less than the control populations. This organism was evidently sensitive to the jug environment, and will be discussed further in the section dealing with containment effects.

The next three species, Rhabdoderma sigmoidea, Synedra rumpens, and Lyngbya limnetica were significant responding species in both 1968 and 1969, but were not present at all times during both years. Rhabdoderma and Synedra responded in the early summer of both years, while Lyngbya responded in the fall of 1968 and early summer of 1969.

The last three figures present the biomass data for three species that were essentially limited in their occurrence in the experimental communities to short time periods in 1969. Chrysidalis sp. showed up in experiment 6 and was one of the two species that responded to the standard treatment mixture in that experiment. Its population in the lake had declined drastically by the start of experiment 7, and was not seen after that. Gomphosphaeria lacustris was seen in two experiments in 1968, but did not appear in countable quantities until experiment 9. It responded in that experiment and in experiment 16, then disappeared. Aphanocapsa elachista showed up in experiment 16, reached high levels in experiment 17, then declined, after responding in both of these experiments.

These examples illustrate the fact that the successional changes of responding species were almost as sudden as was implied in Table 17. Species were generally present in more experiments than those in which they responded to treatments, but usually preceded or followed their responses to treatments with bursts of growth in the lake. Figure 38 serves to compare the gradually changing productivity response pattern in 1969 with the abruptly changing complex of responding species. It seems surprising that the changes in response patterns were as gradual as they were in the face of the general lack of overlap of responding species.

It is implied in the preceding two discussions that changes in the physico-chemical environment and changes in the phytoplankton species composition both contributed to the variations that appeared in productivity response patterns. It seems reasonable to propose that the environmental conditions served to shape the major

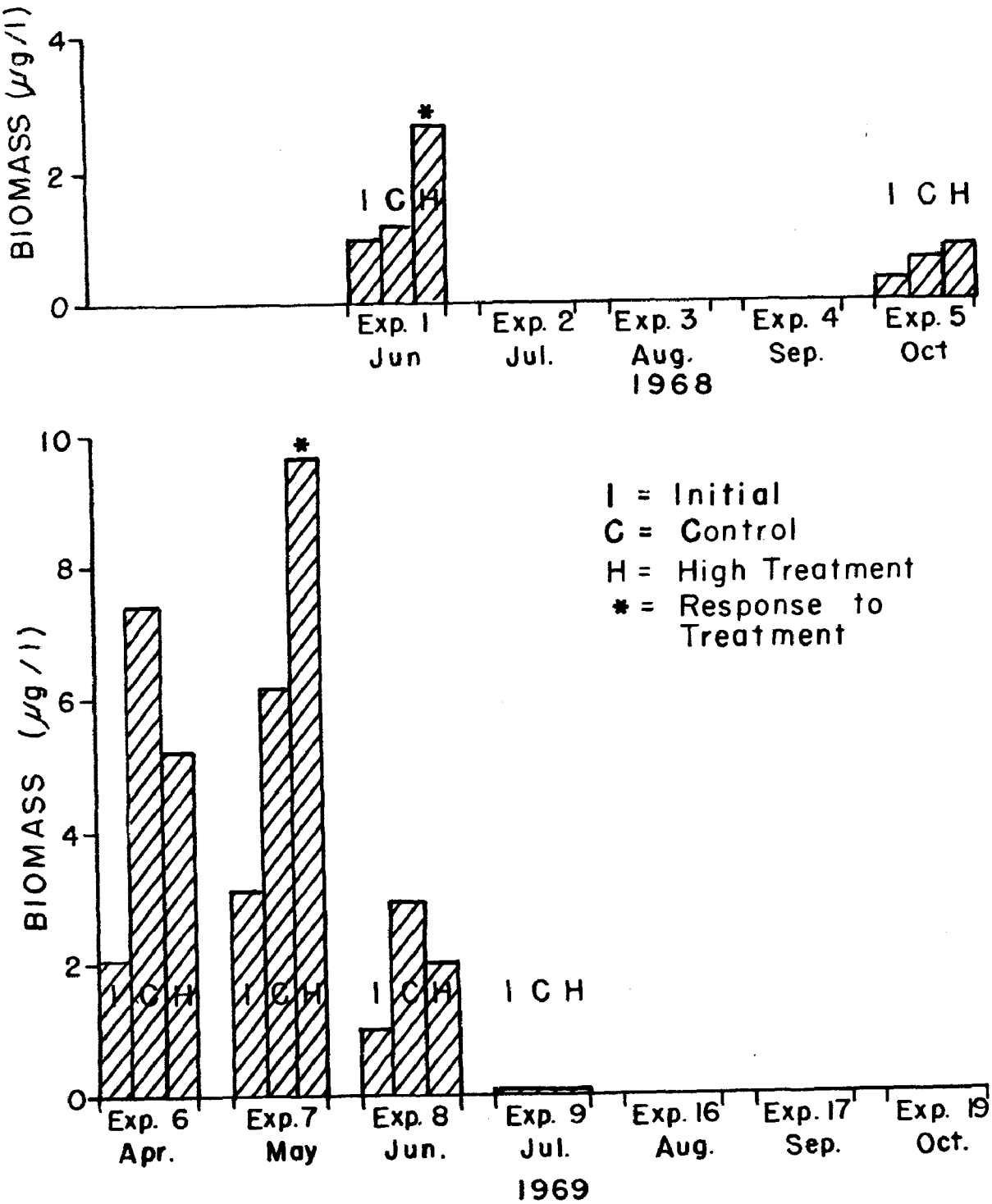


Figure 32. Rhabdoderma sigmoidea Biomass Estimates - 1968, 1969

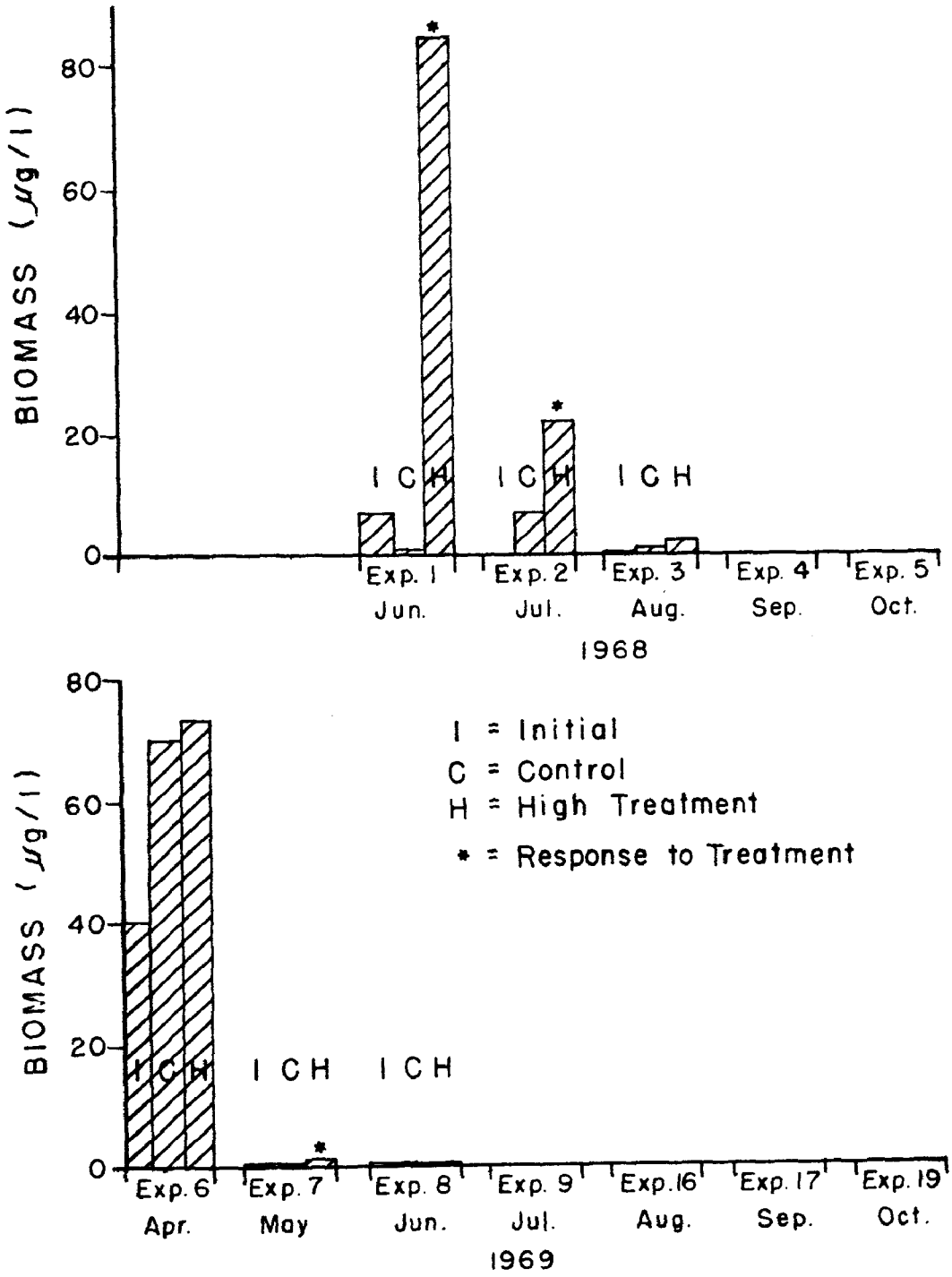


Figure 33. Synedra rumpens Biomass Estimates - 1968, 1969

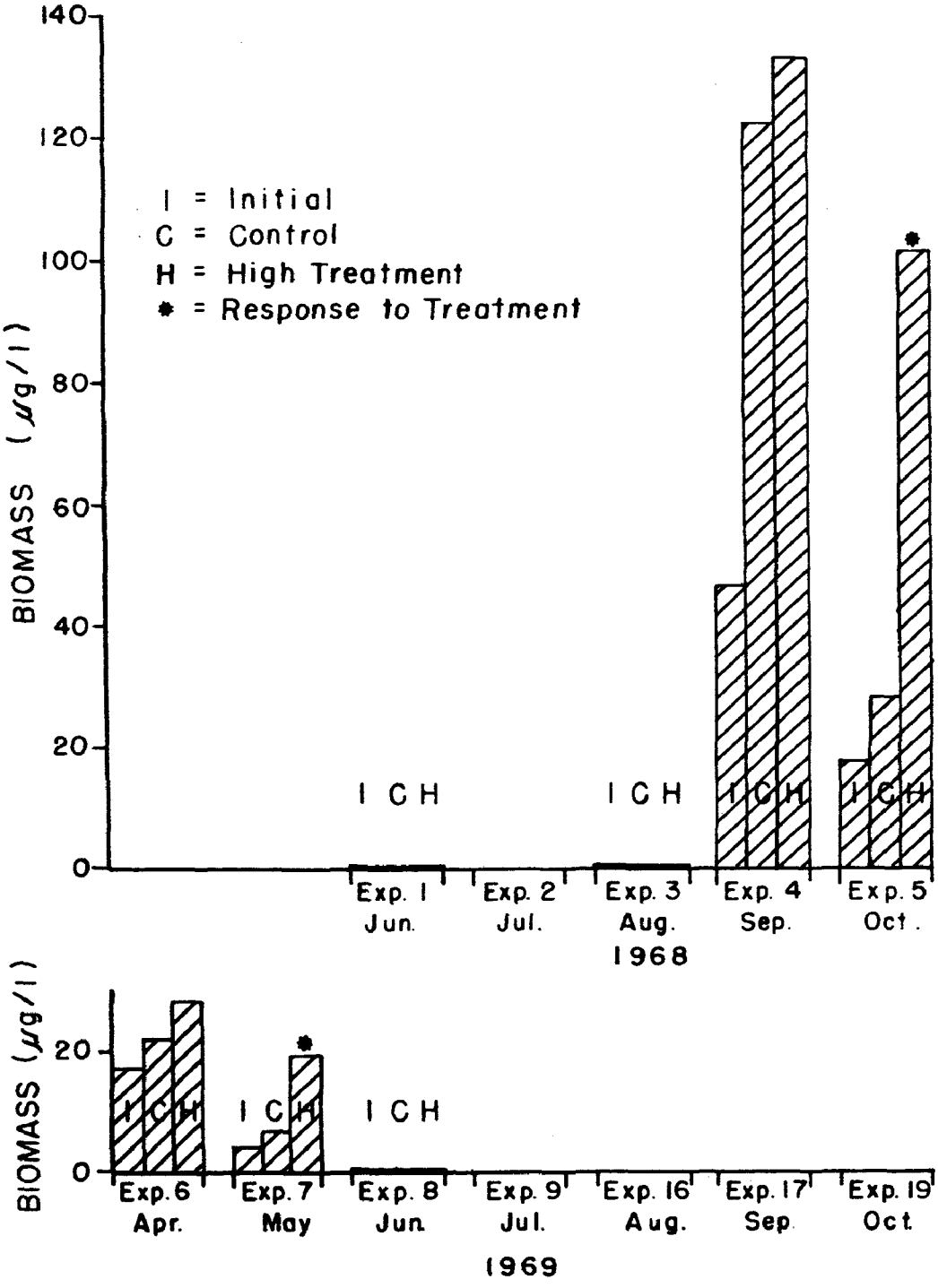


Figure 34. Lyngbya limnetica Biomass Estimates - 1968, 1969

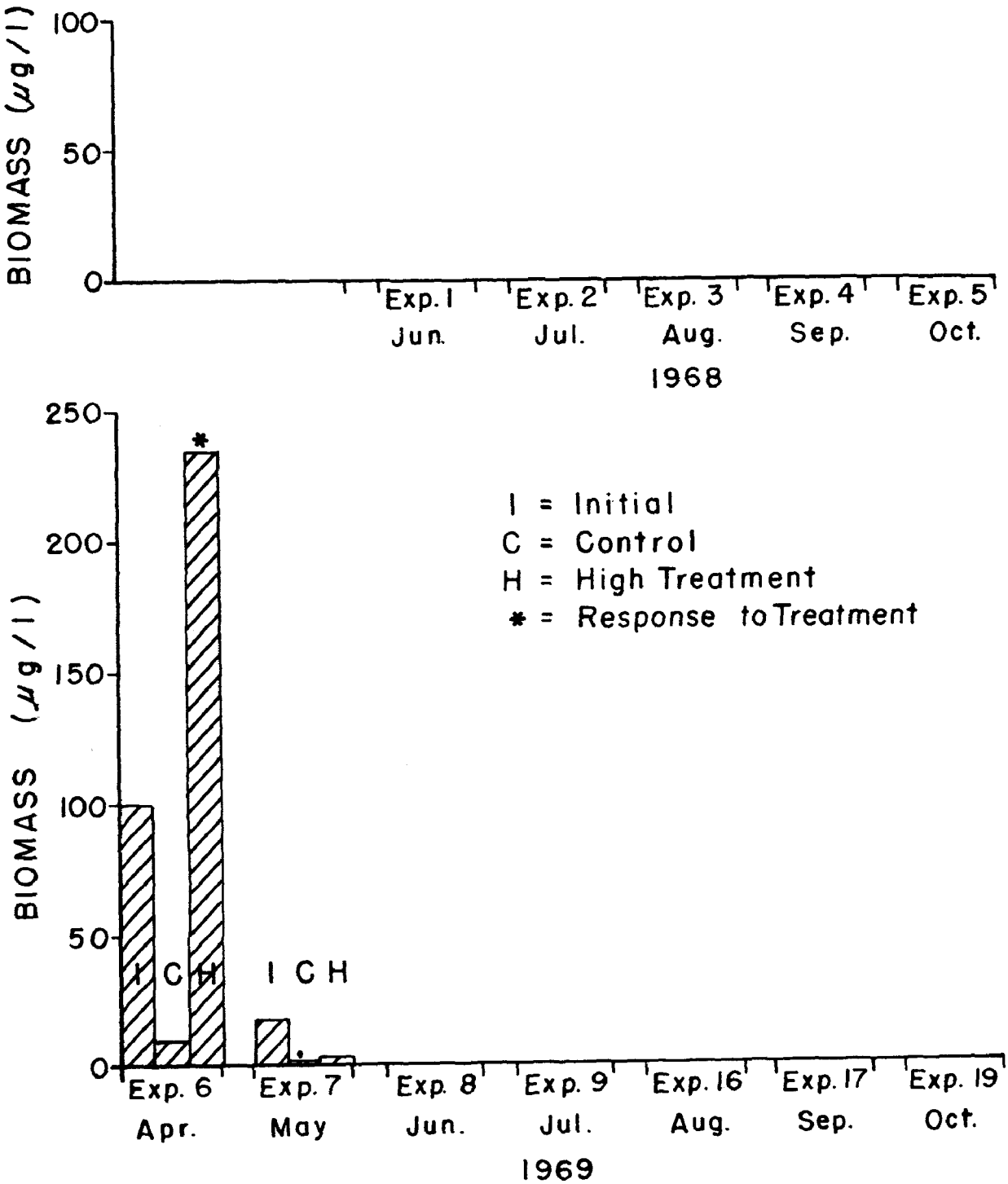


Figure 35. Chrysidalis sp. Biomass Estimates-1968, 1969

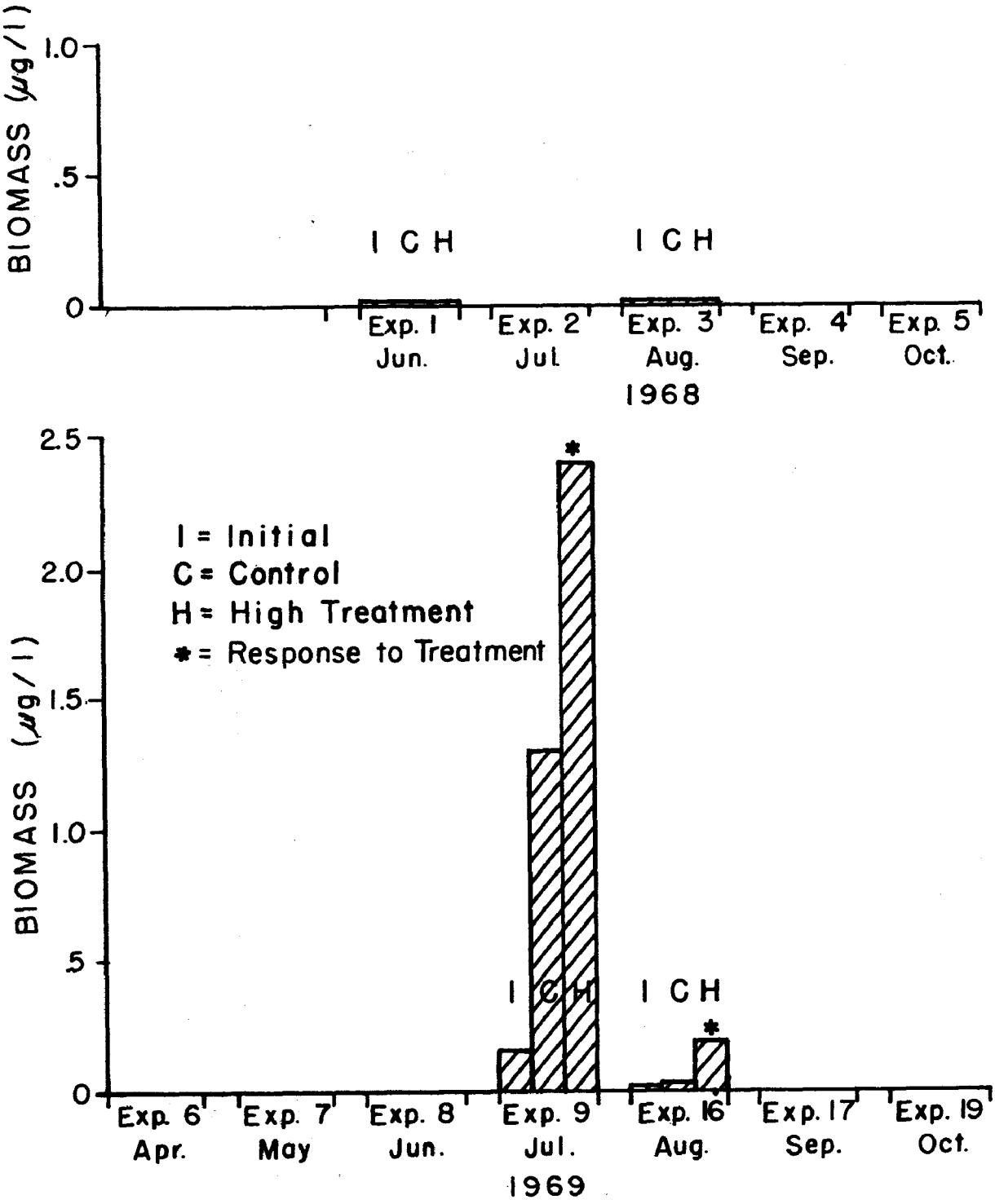


Figure 36. Gomphosphaeria lacustris Biomass Estimates - 1968, 1969

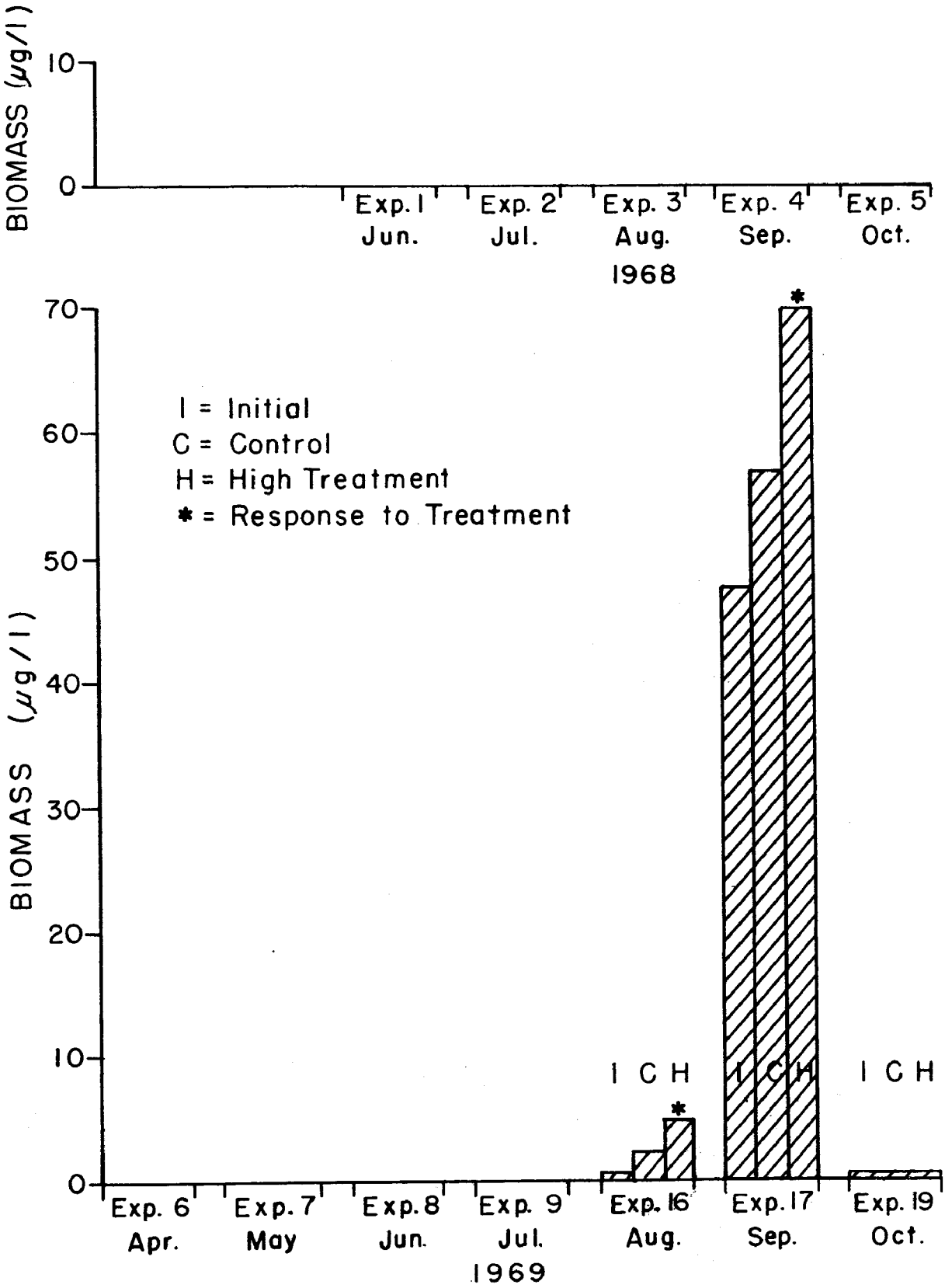


Figure 37. Aphanocapsa elachista Biomass Estimates-1968, 1969

features of the response patterns, such as the relative importance of P and N in the present series and certain seasonal aspects of the treatment effects, while species changes were reflected in the short term changes between successive experiments.

Use in Predicting Future Responses

After examining the factors that influenced the variations in the productivity response patterns, it can be concluded that for in situ enrichment bioassays to be used as predictive tools series of experiments in each season in the water body of interest should be conducted. One, or perhaps a seasonal series of "most probable response patterns" could then be proposed if clear-cut relationships appeared between ambient conditions and the experimental results.

From the set of experiments performed in Third Sister Lake in 1968 and 1969, "most probable response patterns" for the spring, summer, and fall can be proposed. In the spring, treatments with inorganic P or N are less likely to stimulate primary productivity than are organic chelators. In the summer, as inorganic P declines in the epilimnion, phosphate treatments become consistently stimulatory. Stimulation by inorganic nitrogen treatments generally is dependent upon the presence of added P, except in late summer when heterocysted bluegreens become active, and N treatments are no longer effective. Stimulation by chelators is fairly consistent throughout the summer, but is reduced in the fall as the thermocline is pushed downward, and decomposition products are released into the epilimnion.

Data concerning the species involved in enrichment experiments can considerably amplify predictions based strictly on productivity responses. This is because the composition of the phytoplankton as well as its quantity influences whether or not water quality problems develop from increased productivity. Of the species of algae that responded to treatments in the experiments discussed, 11 were bluegreens, 4 were greens, 3 were diatoms, 2 were cryptophytes, and 2 were chrysomonads. Of the 44 major species that were identified in initial phytoplankton communities, 17 were bluegreens, 16 were greens, 6 were diatoms, 3 were cryptophytes, 2 were chrysomonads, and 1 was a dinoflagellate. Thus the nutrient treatments tended to shift the community structure from approximate equality between green and bluegreen species to a dominance of bluegreen species. Moreover responses by bluegreens occurred at most times

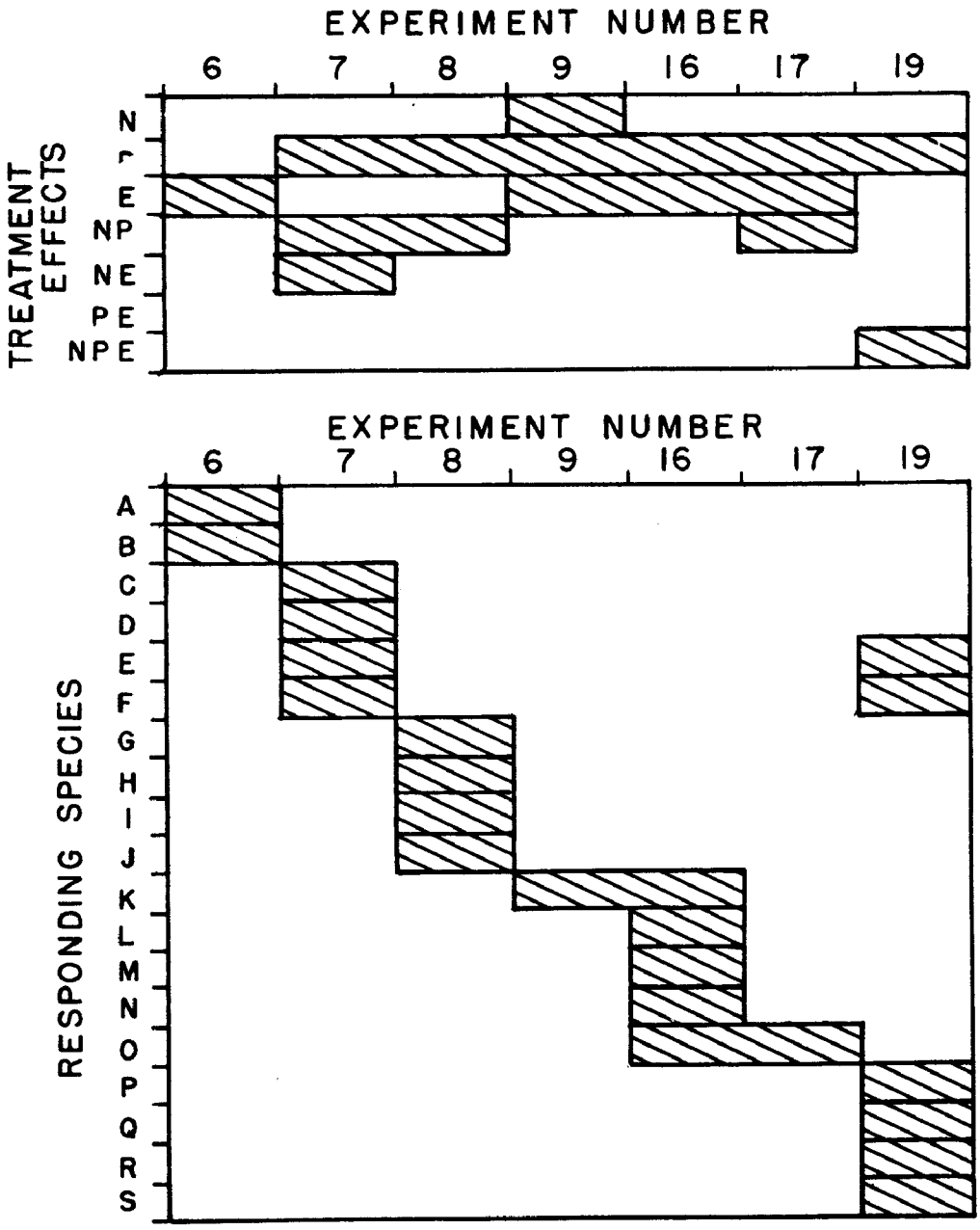


Figure 38. Productivity Response Patterns vs Responding Species - 1969

of the year, while responses by the other groups tended to be limited to the spring, early summer, or fall. In the late summer of 1969, July, August, and September, the responding species were almost exclusively bluegreens, one of which, Aphanizomenon flos-aquae, is a well known nuisance bloom former. Blooms of planktonic bluegreen algae have not been a problem in Third Sister Lake, but these results imply that increases in the nutrient input to the photic zone in the summer could favor bloom formers and lead to nuisance conditions. In the next section, subsequent experiments designed to test the stimulatory ability of NTA will be discussed. Nitrate and phosphate treatments were also employed in some of these experiments, permitting their comparison with the predictions derived from the initial series.

SECTION V

EXPERIMENTS TO TEST A POTENTIAL ENVIRONMENTAL CONTAMINANT

DESIGNS

In 1970 and 1971 nine in situ enrichment experiments were performed with NTA as a treatment. The designs, productivity results, and data analyses are tabulated in Appendix C. The NTA treatment level employed in most of the experiments was .252 mg/l (1.3×10^{-6}M). In addition to NTA, treatments with N, P, and EDTA were frequently included while treatments with glycine, vitamin B-12, Zn, and Mo were included in one experiment. Productivity in the open lake was monitored during most of the experiments, but discussion of these results will be deferred until the section on containment effects.

PRODUCTIVITY RESULTS

Comparison with 1968, 1969 Results

Table 18 summarizes the productivity response patterns, while Table 19 indicates the frequency of occurrence of each possible independent effect. Considering the complete response patterns, it will be noted that the three treatments N, P, and NTA each achieved independent stimulation of productivity in the majority of the experiments in which they were included. Responses to EDTA occurred in only half of its experiments. The consistent effects of P and chelator treatments in this series, therefore, repeated the main results of the 1968-69 series. The major change in the 1970-71 experiments was the greater significance of the nitrogen treatments, which achieved independent effects rather than the secondary effects in conjunction with phosphorus that occurred previously.

In both 1968 and 1969 EDTA failed to stimulate productivity in the fall experiments, and this was interpreted to relate to the onset of overturn. In 1970 thermal conditions at the time of experiment 30 were similar to conditions in the previous two falls, yet both NTA and EDTA effects appeared, casting doubt on the hypothesis that thermocline erosion reduced the sensitivity of the system to added chelators.

TABLE 18

SUMMARY OF PRODUCTIVITY RESPONSE PATTERNS, EXPERIMENTS 22-34

	Month:	May	May	May	Jun	Aug	Oct
1970	Expt:	22	23	24	25	29	30
	Independent effects	NTA EDTA	N	P NTA	N+P NTA EDTA Zn(-)	N NTA EDTA	N P NTA EDTA
	Interactions				NTA Zn		
	Notes	P not Tested	P, EDTA not Tested				
1971	Expt:		31	32	34		
	Independent effects		NTA		N P		
	Interactions						
	Notes		N, P not Tested	N, P not Tested			

TABLE 19

FREQUENCY OF RESPONSES EXPERIMENTS 22-34

Treatment	No. of Expts. Tested	No. of Expts. with Independent Effects	No. of Expts. with Interactions Only	No. of Expts. with No Effect
N	7	5	0	2
P	5	4	0	1
NTA	9	6	0	3
EDTA	8	4	0	4
Glycine	1	0	0	1
B 12	1	0	0	1
Zn	1	1	0	0
Mo	1	0	0	1

The differences between the results of the 1970-71 experiment series and the previous series indicate that year to year variations in response patterns could be greater than anticipated on the basis of the 1968-69 data. Nonetheless the major elements of the "most probable response pattern" formulated from the experiments of the first two years would be altered little by incorporation of the later results. They would simply be broadened to include a greater likelihood of independent stimulation by nitrogen and the probability of stimulation by chelators throughout the ice free season.

Combining all of the experiment results and ranking the treatments on the basis of frequency of effects, the order of importance in stimulating productivity in Third Sister Lake is still (1) P, (2) chelators, and (3) N. Thus a recommendation for nutrient management in the Third Sister Lake watershed would stress control of P inputs, but would advise that all inputs be prevented if possible since the phytoplankton of the lake is evidently highly sensitive to stimulation by allochthonous substances of many types.

With regard to formulating predictions on the basis of in situ enrichment experiments the results indicate that only the most general predictions can be made with a high degree of confidence. Predictions of the influences of particular environmental conditions or species assemblages on response patterns can be attempted, but can be expected to fail more often than predictions of average community responses over entire seasons. Thus nutrient management policies that relax restrictions on inputs during periods of supposedly reduced sensitivity to enrichment are less desirable than policies that assume that the most probable response pertains at all times.

NTA Effects

Turning to the effects of NTA, it is apparent from Tables 18 and 19 that this compound could stimulate productivity as consistently as had EDTA throughout the study. Table 20 summarizes the relative effects of these two compounds in the experiments in which both were employed. In most of the experiments stimulation by EDTA treatments was either similar to or stronger than that achieved by NTA treatments of equal molarity, while in one experiment NTA stimulated but EDTA did not. The occurrence of differences in the effects of the two substances is not surprising, since the stability constants

TABLE 20

COMPARISON OF NTA AND EDTA EFFECTS, EXPERIMENTS 22-34

Experiment	Treatments Stimulating Productivity	Relative Effects
22	EDTA, NTA	EDTA > NTA
24	NTA	
25	EDTA, NTA	EDTA > NTA
29	EDTA, NTA	EDTA > NTA
30	EDTA, NTA	EDTA = NTA
31	EDTA, NTA	EDTA = NTA
32	Neither	

for EDTA-metal complexes are stronger than for NTA-Metal complexes for all metals (Pollard 1966).

It seemed most probable that NTA treatments were stimulating productivity by means of a chelation mechanism, since the experiments were terminated before biodegradation was likely to occur. Also, results of studies with cultures of estuarine phytoplankton (Erickson et al. 1970) indicated lack of utilization of NTA as a nitrogen source by algae. Nonetheless the possibility of its utilization as a nitrogen source existed, and this was tested in experiments 22, 23 and 24. In these experiments side treatments with inorganic nitrogen, half as ammonia and half as nitrate, were included at levels comparable to the nitrogen levels added as NTA (Appendix C, Tables 1, 2, and 3). In no case did the nitrogen treatments have effects comparable to the NTA effects (Table 18). In experiments 22 and 24 NTA stimulated productivity and nitrogen did not, and in experiment 23 the reverse occurred.

While these results confirmed that the NTA was not utilized as a nitrogen source, they did not prove that chelation was the mechanism actually involved. A more direct test was employed in experiment 25, in which treatments with the metals Zn and Mo were included in conjunction with the NTA treatments. No effect of Mo occurred, either when added alone or in combination with NTA, but NTA stimulated productivity when added alone. The Zn treatments, however, strongly inhibited productivity, and this inhibition was partially canceled by the addition of NTA (Figure 39). A similar interaction

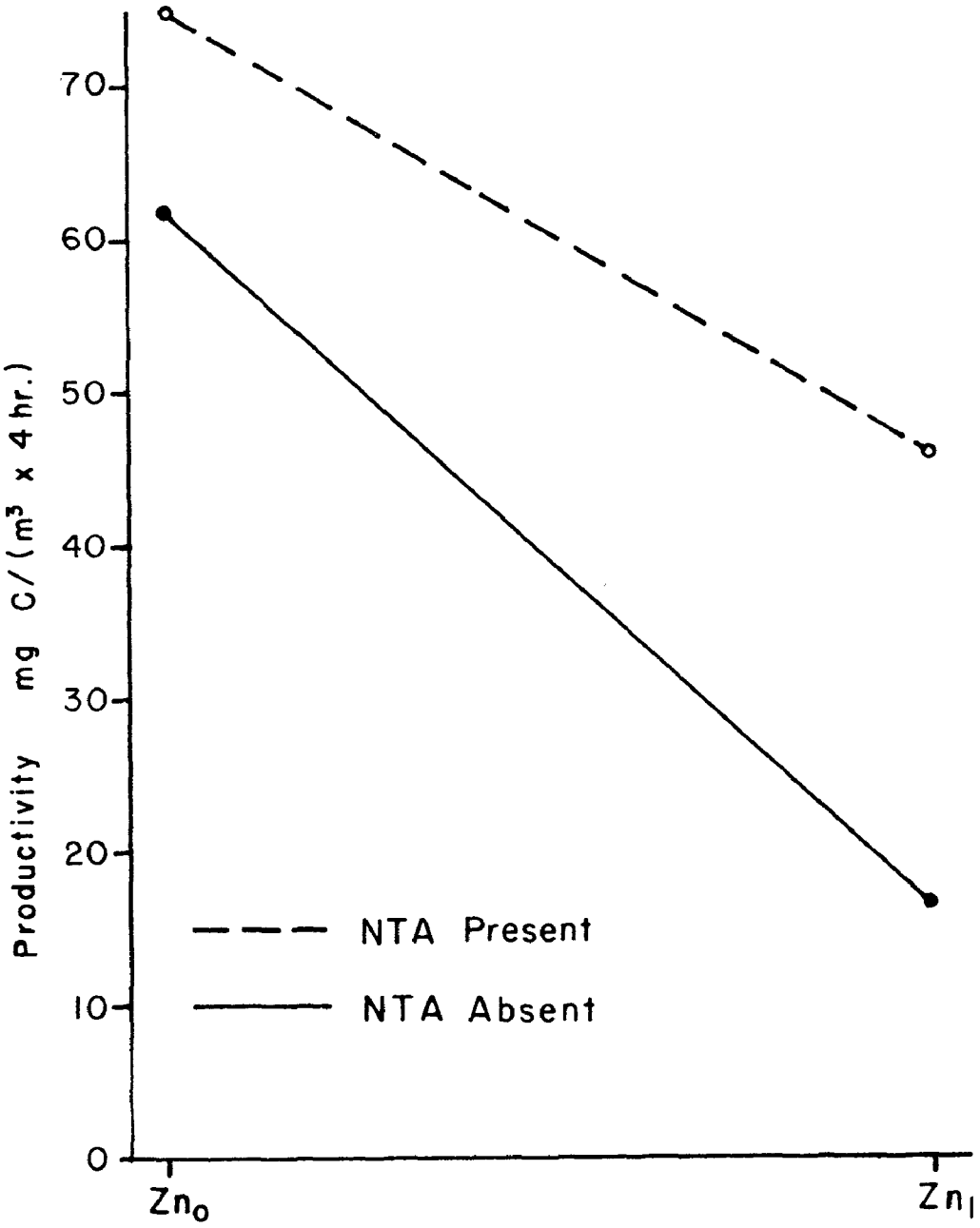


Figure 39. Interaction between NTA and Zn, Experiment 25

between Zn and EDTA was found by Glooschenko and Moore (1971) and was postulated by them as the mechanism by which EDTA and citrate stimulated productivity of phytoplankton in water samples from polluted Hamilton Harbor. Since there are no known inputs of toxic metals to Third Sister Lake, it is more logical to assume that the chelate effects observed in the present experiments were due to enhanced availability of trace metal nutrients, rather than sequestration of toxicants.

In additional parts of experiment 25 treatments with vitamin B-12 and with glycine, a possible breakdown product of NTA (Thompson and Duthie 1968), were tested for independent effects, but none were found (Appendix C, Table 4).

SPECIES COUNTS

NTA Effects

Limited plankton counts were performed for two of the NTA experiments in an attempt to confirm that productivity stimulation signified actual growth stimulation. In experiment 22 three major species were counted (Table 21). Both Chroomonas acuta and Cryptomonas ovata declined in the control jugs, but declined less in the jugs treated with NTA and EDTA. Thus the treatment effects for these species consisted of differential survivals rather than differential growth rates. Ankistrodesmus falcatus, however, did exhibit an actual growth response, but only to EDTA. Thus in this experiment it could be claimed that the NTA effect was an artifact of the method since it appeared for species whose major responses were to the jug environment.

In experiment 29 (Table 22) a similar problem of interpretation arises, although the response pattern is more complicated. Anabaena wisconsinense declined in the control jugs, less in the jugs treated with N, and not at all in those treated with P. In response to NTA, however, actual growth above the initial population level occurred. Thus NTA caused a growth response, but since the species involved was evidently strongly affected by the jug environment it could be argued that, once again, the NTA effect was an artifact. In the same experiment Ankistrodesmus falcatus grew to similar population levels in all experimental units.

TABLE 21

EXPERIMENT 22

SPECIES RESPONSE PATTERNS

TREATMENT MEANS (µg/l)

	Treatment			
Species	Initial	Control	NTA_3	$EDTA_3$
Chroomonas acuta	167.0	33.7	53.6	72.6
Cryptomonas ovata	22.7	6.71	7.85	10.02
Ankistrodesmus falcatus	31.2	28.6	29.3	41.8

Significant Simple Effects (Tukey's Test)

	Species		
	Chroomonas acuta	*Cryptomonas ovata*	*Ankistrodesmus falcatus*
Comparison			
$EDTA_3$ - Control	**	*	*
$EDTA_3$ - NTA_3		*	*
NTA_3 - Control			

TABLE 22

EXPERIMENT 29

SPECIES RESPONSE PATTERNS

TREATMENT MEANS (µg/l)

	Treatment				
Species	Initial	Control	N	P	NTA
Anabaena wisconsinense	2.06	.246	1.46	1.90	3.62
Ankistrodesmus falcatus	.322	.628	.730	.668	.680

Significant Simple Effects (Tukey's Test)

	Species	
	Anabaena wisconsinense	*Ankistrodesmus falcatus*
Comparison		
NTA - Control	**	
NTA - N	**	
NTA - P	*	
N - Control	*	
N - P		
P - Control	*	

Comparison with 1968, 1969 Results

The species counted in these two experiments had been significant members of the responding species complexes in experiments 1-19. Ankistrodesmus falcatus, it will be recalled, was present in all of the 1968 and 1969 experiments, peaked in the spring, and responded in the early summer and fall. The 1970 data are consistent with this pattern, with the occurrence of high population levels and of a response in a May experiment versus low population levels and lack of response in an August experiment. Chroomonas acuta and Cryptomonas ovata both declined in the jugs as had been the case, at least for Chroomonas acuta, in all of the 1968 and 1969 experiments. Finally, Anabaena wisconsinense, which had appeared in the late summer in both 1968 and 1969, appeared again in August 1970.

In this year, in contrast to the preceding two, nitrogen treatments stimulated productivity in the presence of this species. Thus one other specific prediction that nitrogen treatments would not stimulate in the presence of heterocyst forming bluegreen algae, that was included in the "most probable response pattern" must be eliminated or at least reduced from a probability to a possibility.

SECTION VI

EXPERIMENTS TO EVALUATE STIMULATION BY SEWAGE EFFLUENTS

DESIGNS

During the course of the complete study five side experiments were performed in which volumes of membrane filtered (.45μ pore size) secondary sewage treatment plant effluents were employed as treatments. The results of the effluent treatments were then compared to the results of treatments with mixtures of N and P, which in two cases were comparable to the levels added in the effluents. The productivity results and data analyses are tabulated in Appendix D.

The results of three of these experiments, 6-S, 25-S, and 34-S, have been analyzed in particular detail, including extensive species counts, and this discussion will be restricted to them. The other two experiments, 24-S and 29-S, evaluated only in terms of productivity, were similar in that the responses to the sewage additions exceeded the responses to the standard nutrient mixtures.

TABLE 23

SEWAGE EXPERIMENTS: BACKGROUND DATA

Experiment		Lake Levels		Treatments: Mixture			Treatments: Sewage	
		NO_3-N μg/l	PO_4-P μg/l	NO_3-N μg/l	PO_4-P μg/l	EDTA μg/l	NO_3-N μg/l	PO_4-P μg/l
6-S	April '69	50	10	25	5	500	(325 ml→19 l)	
							(200 ml→19 l)	
25-S	June '70	40	6	3	23	0	3	23
34-S	June '71	40	7	210	368	0	(200 ml→19 l) 210	368

Table 23 presents background data for the three experiments to be discussed. In experiment 6-S the NO_3 and PO_4 levels in the known nutrient mixture were chosen to approximate half the ambient lake levels. The levels of NO_3 and PO_4 added in the sewage were not determined. In experiments 25-S and 34-S the levels added in the NP mixtures were similar to the levels added in the sewage. Nitrogen forms other than nitrate in the sewage were not considered, and therefore the inorganic nitrogen levels in the NP mixtures were not strictly comparable

to the levels in the sewage. Thus in the comparisons to follow, conclusions about the relative contributions of inorganic nitrogen to the sewage effects will not be attempted.

PRODUCTIVITY RESULTS

Figure 40 shows the results of the productivity measurements, expressed in mg C/(m^3 x 4 hr), and plotted versus time since the start of each experiment. All differences that are shown on a given day are significant at the .05 level, and most at the .01 level according to Tukey's test (Steel and Torrie 1960). Values that were not significantly different have been averaged and appear as one point. In experiments 25-S and 34-S productivity in the open lake was monitored during the experiments, and these results appear as the dotted lines.

In experiments 6-S and 34-S the productivity levels of the experimental units treated with sewage and with the nutrient mixture followed similar trends with time, and ultimately converged. In experiment 25-S the response to the mixture declined rapidly, while the response to sewage intensified before it declined.

The only certain conclusion that can be drawn from the productivity data is that sewage was more stimulatory to community productivity than was the nutrient mixture in all three experiments. Since effects on community structure are not revealed it cannot be concluded whether the sewage and mixture treatments affected the same species to different degrees, or completely different species, or some of the same species and some different species. Such qualitative species information would be required if the bioassay were intended to determine the extent to which nitrate and phosphate in the sewage contributed to its overall effect.

The divergence of the trends of control productivity and lake productivity in experiments 25-S and 34-S implies that the algae in the jug communities responded to containment as well as to the nutrient treatments. This aspect will be discussed in the section dealing with containment effects.

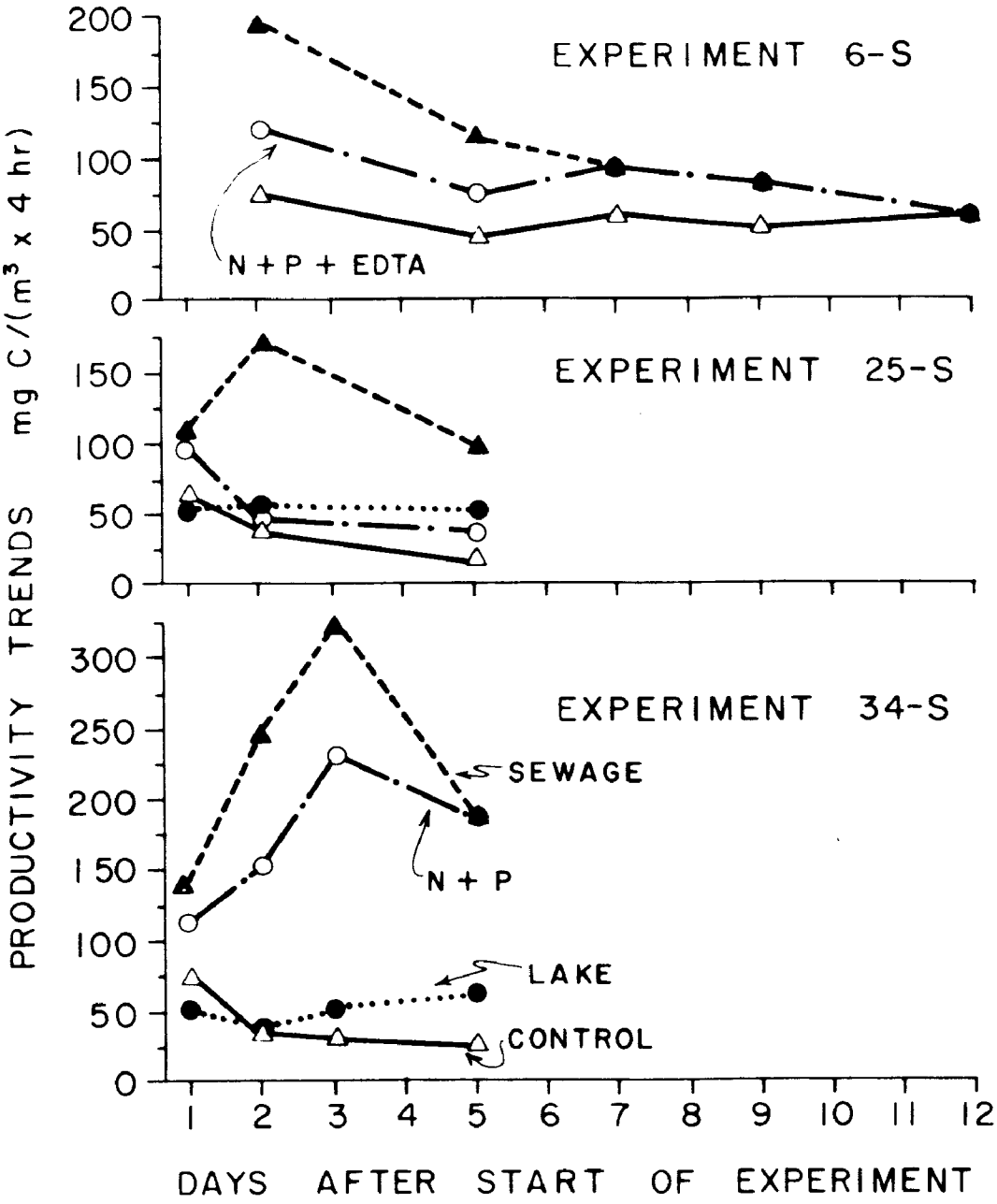


Figure 40. Sewage Experiments: Productivity Response Patterns

TABLE 24

SEWAGE EXPERIMENTS:
SUMMARY OF TREATMENT EFFECTS
(Number of Species Showing Each Effect)

Response Pattern	Experiment Number		
	6-S	25-S	34-S
No Treatment Effect	5	9	2
Mixture* Effect Only	2	0	0
Sewage Effect Only	6	7	2
Both Effects			
Same Magnitude	0	4	5
Sewage > Mixture	0	2	2

*Mixture = N + P + EDTA in Experiment 6-S

N + P in Experiments 25-S and 34-S

SPECIES COUNTS

To determine whether the treatment effects were general or selective, biomass data for individual species were analyzed. Mean biomass estimates and the results of variance analyses of the individual estimates are presented in Appendix E.

Table 24 summarizes the results for the mixture and sewage treatments in terms of the number of species that exhibited each possible treatment effect. In experiment 34-S 2 species responded to neither treatment, 2 others responded to sewage alone, and 7 others responded to both treatments, 5 of them to the same degree. Thus there was a strong qualitative overlap between the two treatment effects.

In experiment 25-S 9 species did not respond, 7 responded only to sewage, while only 6 responded to both. The overlap, therefore, was not as strong as in experiment 34-S. Reference to Figure 40 will reveal that the different degrees of species overlap relate closely to the different degrees of similarity between the sewage and mixture effects observed in terms of productivity in these two experiments. Nine species responded

differently in experiment 25-S while only 4 responded differently in experiment 34-S.

Returning to Table 24, we find that in experiment 6-S there was no species overlap at all--the species either did not respond, responded only to the mixture or only to the sewage. The productivity data in Figure 40, however, contain no evidence of this qualitative discrepancy.

In addition to data for the treatment units receiving N + P and sewage, the experiment 34-S table (Appendix E, Table 5) includes species data for units receiving P alone and N alone. Factorial analysis of variance techniques were employed to test for interactions between N and P on the species level and detected positive interactions for Chroomonas acuta, Elaktothrix gelatinosa, Sphaerocystis schroeteri, Synedra sp., Cryptomonas ovata, and, to some extent, Crucigenia rectangularis. Of the other species, Aphanothece nidulans responded only to P, while Ankistrodesmus falcatus responded only to N, but not in the presence of P. These data further exemplify the variety of patterns that different species may exhibit in response to components of nutrient mixtures, that was shown for experiment 12 in section IV.

SEWAGE VERSUS MIXTURE EFFECTS

The comparisons of the productivity and individual species responses to the nutrient mixture with the responses to the sewage treatments in these three experiments indicate that in all cases factors in addition to those included in the known mixture contributed to the effect of the sewage. Presumably additional substances suspected as potential contributors to the sewage effect could be added to the mixture until eventually most or all of the stimulation by the sewage could be accounted for. As indicated by the additional species data for experiment 34-S interactions at the species level are likely to be important in shaping the overall community response to any nutrient mixture.

The varying degrees of overlap between the species complexes responding to the sewage and those responding to the nutrient mixtures emphasize the necessity for basing comparisons of treatment effects on species data as well as productivity patterns, whenever it is to be concluded whether or to what degree the components of one treatment contributed to the effects of another treatment. This is due to the high selectivity of treatment effects in enrichment experiments employing mixed communities of algae.

SECTION VII

CONTAINMENT EFFECTS

BACKGROUND

Thus far the results of the in situ enrichment experiments conducted in this study have been discussed chiefly with regard to their internal consistencies. The results of individual experiments have been compared, as have the major response patterns that have shown up in series of experiments. The question that has been asked is: To what extent can the results of future experiments be predicted on the basis of the results of past experiments? This is one step removed from the ultimate question that must be considered in evaluating the utility of the method: To what extent can the responses of the community in the natural system to future enrichment be predicted on the basis of past experiments? To deal with this question data on the response of the natural plankton community to the experimental environment are required.

A plankton community enclosed in an in situ incubation vessel is subjected to several important environmental alterations. First and probably most important is a reduction in turbulence from that normally experienced in the open water. Turbulence affects the organisms by producing relative motion of the lake water past them, which in turn enhances nutrient availability by maintaining a steep concentration gradient between the environment and the cell (Hutchinson 1967, p. 293). The importance of turbulence to algal growth was demonstrated by Fogg and Than-Tun (1960), who found that growth of Anabaena cylindrica in culture was doubled when the flasks were shaken at 90 oscillations per minute instead of 65, and was prevented at 140 oscillations per minute.

A second set of environmental changes can result from the presence of the surface of the vessel (Lund and Talling 1957). Bacterial growth on and solute exchange with the walls of the vessel can alter the nutrient environment to an extent that should be related to the material composing the vessel and to the ratio of the surface area of the vessel to the volume it contains. Also the vessel surface presents a barrier to the exchange of solutes with the outside environment, thus permitting further modifications of the interior environment as nutrients are utilized and metabolic wastes accumulate.

Thirdly, the quality and quantity of light reaching the enclosed algae will be modified by passing through the walls of the container. Glass absorbs more light than does water, especially in the shorter wavelengths. This absorption can enhance productivity by protecting the plankton from excessive light intensity on bright days, or it can depress productivity on cloudy days. Plastic and quartz containers modify light conditions less than do glass containers (Soeder and Talling 1969).

OBSERVED EFFECTS

Productivity Declines

Some indications of the influence of containment on the behavior of the communities employed in the 1968 and 1969 series of enrichment experiments can be obtained by examining the productivity results for control communities in individual experiments. In Figure 41 the mean productivity of the control jugs involved in each experiment is plotted versus time since the beginning of the experiment. The apparent trend observed in 10 of the 12 experiments is decline of productivity with time. The two exceptions were experiments performed in the spring and late fall of 1969, while those that showed decline were performed in the summer and early fall, implying a relationship to season.

While the productivity trends for the control jugs in these experiments suggest that the jug environment depressed community productivity, this could not be concluded for certain because productivity in the open lake was not concurrently monitored. Beginning in the spring of 1970 comparisons between jug and lake productivity were made, and the results appear in Table 25.

Reference to this table reveals that in four experiments (22, 23, 31, and 32) productivity in the control jugs maintained levels that were comparable to the levels achieved by the lake samples throughout the experiments. In experiment 24 control productivity exceeded lake productivity by almost 10 mg C/(m^3 x 4 hr) on both days of measurement. This was probably due to a decline of the lake community subsequent to the start of the experiment. The experiment was set up in the morning of May 25, and by noon all of the jugs had been filled and suspended at the incubation depth. In the afternoon a severe rainstorm washed in large amounts of silt that raised the turbidity of the lake and apparently carried the phytoplankton downward as it settled.

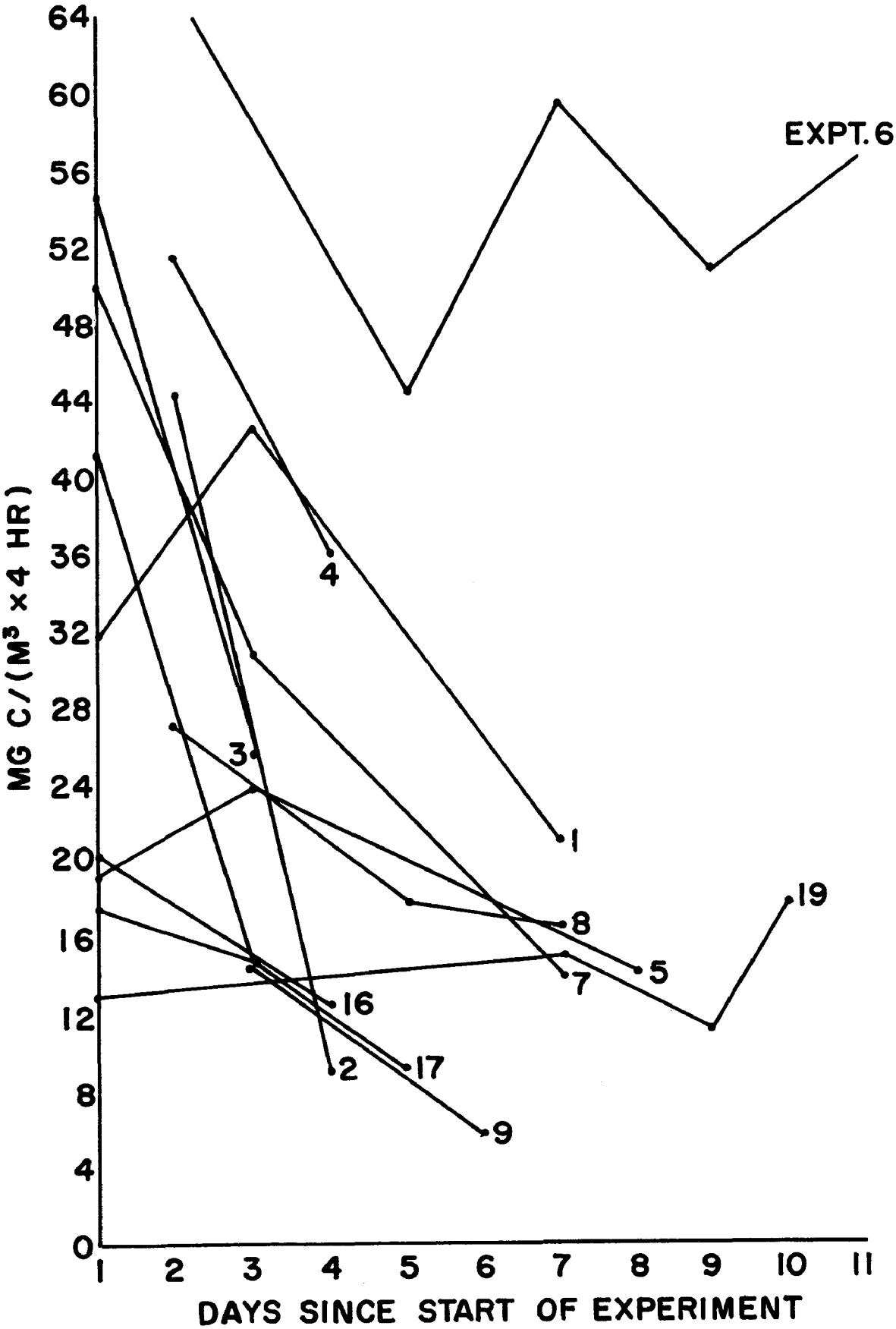


Figure 41. Productivity Trends in Control Jugs- 1968. 1969

TABLE 25

COMPARISON OF LAKE AND CONTROL PRODUCTIVITY MEASUREMENTS,

1970 AND 1971 EXPERIMENTS

Experiment	Date	Control	Lake	Lake / Control	Lake / High Treatment
		mgC/(m^3x4hr)			
20	4-24-70	110.8	114.6	1.03	.993
	4-25-70	124.4	130.4	1.05	.936
	4-26-70	122.4	139.4	1.13	1.02
	4-27-70	118.6	154.5	1.30	1.15
	4-28-70	120.4	118.9	.99	.800
	5-3-70	55.8	77.2	1.38	1.10
22	5-13-70	41.9	49.2	1.17	.924
	5-15-70	52.4	57.2	1.09	1.04
23	5-20-70	56.2	57.5	1.02	.908
	5-21-70	42.0	-	-	
24	5-26-70	42.2	33.3	.79	.643
	5-27-70	33.2	24.8	.75	.544
25	6-18-70	61.9	52.3	.84	.552
	6-19-70	35.4	52.9	1.49	1.21
	6-22-70	17.2	50.8	2.95	1.39
29	8-22-70	32.5	-	-	
	8-23-70	56.2	100.0	1.78	1.11
	8-24-70	46.0	87.8	1.91	1.08
	8-25-70	40.8	71.2	1.74	.984
	8-26-70	61.6	69.3	1.12	.748
30	10-25-70	69.9	85.3	1.22	.960
	10-27-70	51.4	88.2	1.72	.938
	10-29-70	18.1	36.1	1.99	1.21
31	5-19-71	22.7	21.3	.94	.873
	5-20-71	26.6	29.0	1.09	.976
	5-21-71	29.4	28.1	.96	.900
32	5-26-70	39.4	36.2	.92	.881
	5-27-70	37.8	33.3	.88	.826
	5-28-70	36.3	29.9	.82	.860
34	6-10-71	69.8	50.3	.72	.455
	6-11-71	31.9	37.2	1.17	.246
	6-12-71	30.0	53.7	1.79	.233
	6-14-71	25.0	61.5	2.46	.314

In the remaining experiments (20, 25, 29, 30, and 34) control productivity and lake productivity diverged. In three of these experiments (25, 30, and 34) the divergence increased with time as the control productivity declined and the lake productivity remained relatively stable.

Species Shifts

The species data for experiments 25-S and 34-S were discussed in the preceding section with regard to responses to the nutrient treatments. Responses to containment were also evident for a number of species (Appendix E, Tables 3 and 5) and these can be examined in an attempt to interpret the productivity changes that occurred in the 2 experiments. The numbers of species exhibiting each possible containment effect are summarized in Table 26. In both experiments there were species that responded positively as well as negatively to containment, yet the resultant effect at the community level was a decline in productivity in both cases.

Two of the species that exhibited negative containment effects in experiment 25-S were Chroomonas acuta and Cryptomonas ovata, species that had been found in previous experiments to be intolerant of the jug environment. The third species, Cryptomonas erosa, is closely related to the first two. All three of these species declined somewhat in the open lake, but essentially died out in all of the jugs.

The fourth species that responded negatively to containment was a species of Synedra (sp. 1) that increased in all jugs, but did not increase in the control jugs as much as it did in the open lake. Aphanothece nidulans in experiment 34-S exhibited this same type of pattern, which was also interpreted as a negative containment effect. Implicit in these latter evaluations is the assumption that the final lake populations were sampled from the same water mass as were the initial populations --an assumption that is open to criticism. However, considering the small size of Third Sister Lake, the protection from wind afforded by the surrounding forest, and the fact that many species did not change in abundance between the initial and final lake samples, such an assumption is not highly unreasonable in this case. In larger bodies of water containment effects would probably have to be evaluated exclusively by comparing initial lake and final control samples which would, however, confuse containment effects with increases or declines in populations that would have occurred naturally.

TABLE 26

SEWAGE EXPERIMENTS:
SUMMARY OF CONTAINMENT EFFECTS
(Number of Species Showing Each Effect)

Response Pattern	Experiment Number	
	25-S	34-S
No Containment Effect	13	7
Positive Containment Effect	5	3
Negative Containment Effect	4	1

TABLE 27

SEWAGE EXPERIMENTS: SUMMARY OF
TREATMENT AND CONTAINMENT EFFECTS

Experiment Number:	25-S				34-S			
Containment Effect:	0	+	-	Σ	0	+	-	Σ
Response Pattern								
N+P Effect Only				0				0
Sewage Effect Only	5	2		7	1	1		2
Both Effects								
Same Magnitude	3	1		4	3	1	1	5
Sewage > Mixture		1	1	2	1	1		2
Σ	8	4	1	13	5	3	1	9
No Treatment Effect	5	1	3	9	2			2

In experiment 34-S Chroomonas acuta maintained control populations little changed from the initial lake populations, indicating that even the historically most sensitive species did not always follow its normal decline.

Table 27 summarizes the complete species data for experiments 25-S and 34-S, showing for each species the combination of treatment and containment effects that it exhibited. Most of the species that responded to the nutrient treatments were either neutral to containment or were favored by it. However, in each experiment there was one species that responded to a treatment but responded negatively to containment.

The patterns presented in Table 27 exemplify the true

response of a natural phytoplankton community in an *in situ* enrichment experiment. The responses measured on the community level in terms of productivity or of any other gross measurement represent the summation of a set of individual species responses to containment and to the nutrient treatments. The declines in control productivity detected in experiments 1-19 were thus, for the most part, secondary effects of species shifts in response to the jug environment.

The results of experiment 15 (See Section III) indicated that the enclosed plankton communities adapted rapidly to the jug environment. It is now apparent that the "adaptation" did not involve some physiological adjustment shared by all species, but rather resulted from selective removal of the species most sensitive to containment. Since experiment 15 was performed at a time when *Chroomonas acuta* comprised about 50% of the phytoplankton biomass, it is logical to hypothesize that the adaptation observed was due to rapid selective removal of this species from the jug communities.

Since the containment effects in experiments 25-S and 34-S were highly species specific, it is possible to subtract out the sensitive species and determine if the interpretations of the experiment results are affected. This is done in figure 42, in which biomass estimates for the neutral species were summed and plotted on the left, and sums of all the species biomass estimates were plotted on the right. The superficial conclusion, that sewage was more stimulatory than the nutrient mixtures, is not changed in either experiment. Thus if the questions asked of an enrichment experiment are simply whether a given treatment might affect phytoplankton growth, or which of two treatments might be more potent, the answers appear to be similar whether or not containment effects are evaluated. Response measurement in terms of productivity is adequate as long as it is confirmed that productivity signifies growth. More detailed questions, such as whether two treatments have similar effects, require more detailed analyses of individual species responses.

Experiments with Alternative Containers

Up to this point, containment effects have been discussed as if they were one set of effects common to all containers. Evidence has accumulated in this study as well as in others to indicate that containment effects depend to some extent on the type of container employed.

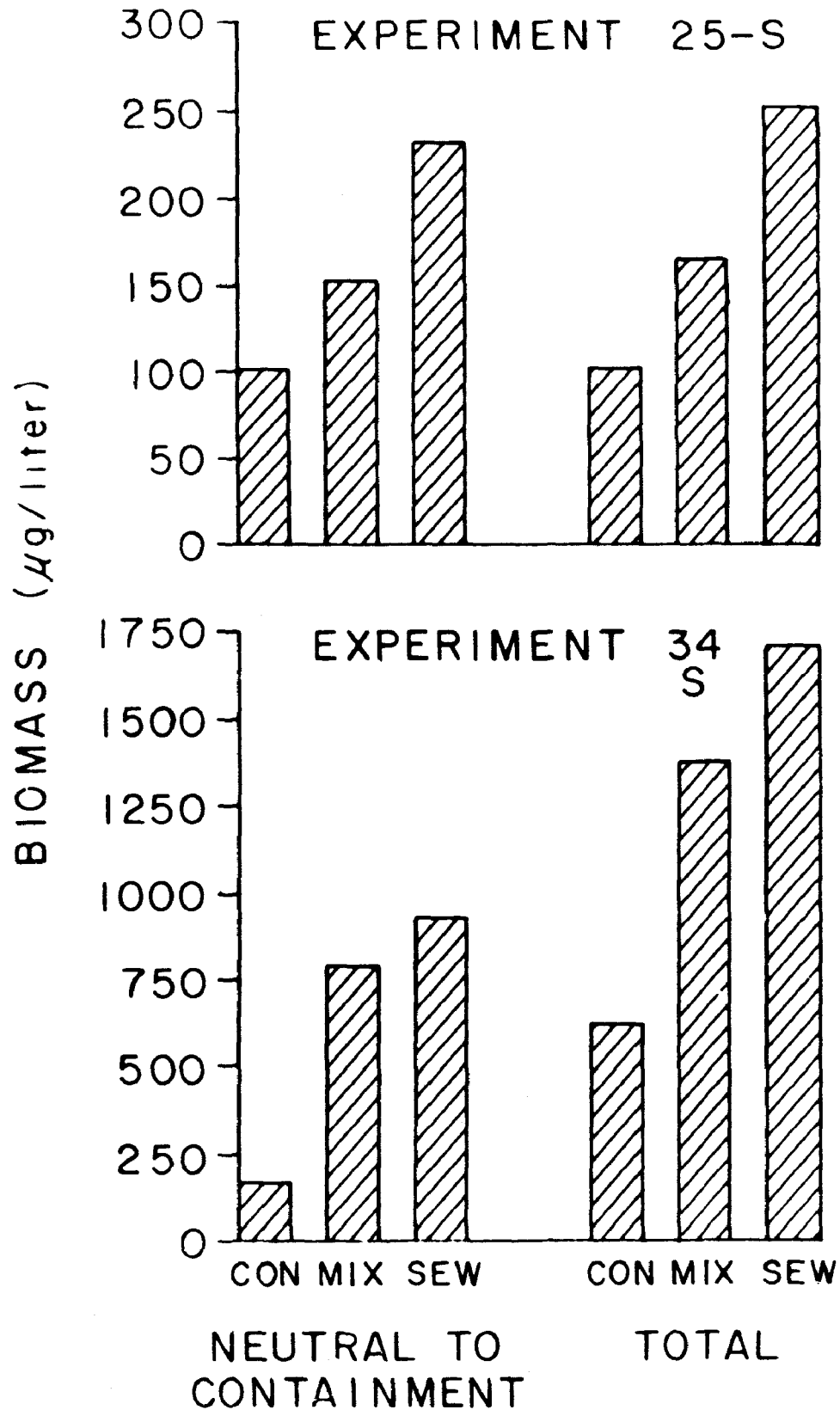


Figure 42. Biomass Estimates for Neutral Species vs Complete Communities

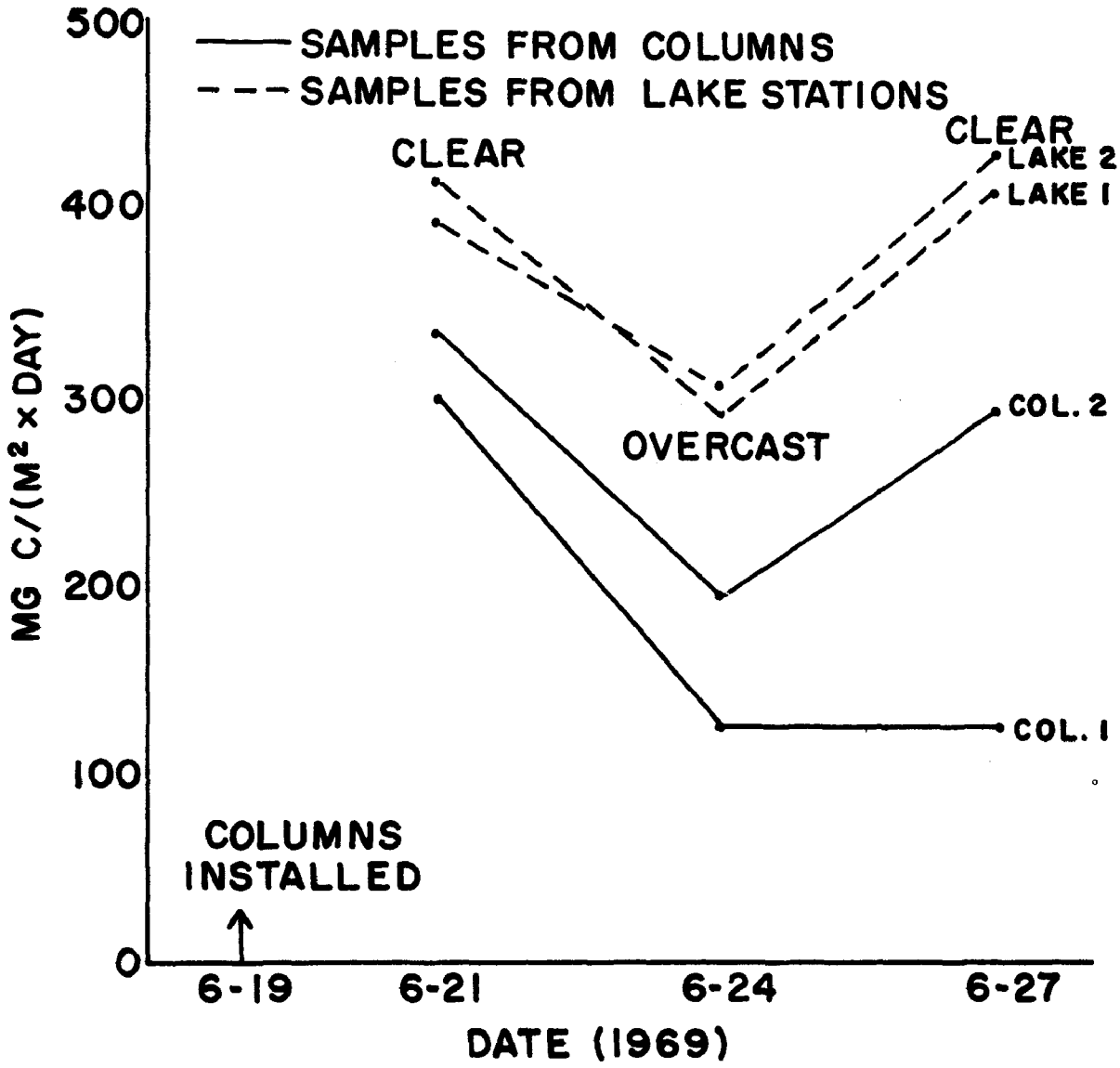


Figure 43. Comparison of Productivity Profiles: Two Columns vs Two Lake Stations

Polyethylene Columns. The long term trend of productivity decline in the jugs was noticed long before plankton counts were made that related this trend to selective elimination of particular species. It was felt that if surface effects were responsible for this property, perhaps larger containers with smaller surface area to volume ratios would perform better. Accordingly some experiments were performed with large polyethylene columns (Goldman 1962). Substitution of these vessels for the jugs would also have added the dimension of depth to the experimental design which with the jugs was restricted to a single depth.

Data on the long term productivity of populations enclosed in large bags are rare in the literature. The most complete record appears in Gächter's paper (1968), in which he reported a gradual increase followed by a gradual decline in productivity in a control vessel over an 18 day period. The productivity never declined below the initial level. McLaren (1969) found that productivity in a fertilized column greatly exceeded that in the open lake over a three week period. However, no data could be found directly comparing productivity in a control column with that in open water.

The first column experiment in the present study was performed early in June, 1969. Carbon-14 productivity profiles were measured using samples taken from within a single column and from a single lake station. The column was constructed from 6 mil polyethylene film, 0.5 m in diameter by 10 m long, open at both ends, and was suspended in Third Sister Lake with its upper end about 10 cm above the water surface. Profiles were measured three times during a 12 day period, and showed a gradual decline in productivity in the column relative to the lake. A second experiment was performed employing duplicate columns and duplicate lake stations. The results, which appear in Figure 43, showed not only that productivity declined in the columns relative to the lake, but also that the columns diverged from one another. Thus the columns proved to be no better than the jugs at maintaining the photosynthetic activity of phytoplankton communities, and they showed that they would perform much worse as experimental units in a statistical design. A more detailed discussion of these results is found in Bender and Jordan (1970).

Glass Tubes and Jars. Information about the behavior of two other types of containers emerged indirectly from efforts to develop a screening method for testing a large variety of nutrients for potential use in jug experiments. One container was a glass tube sealed to a glass

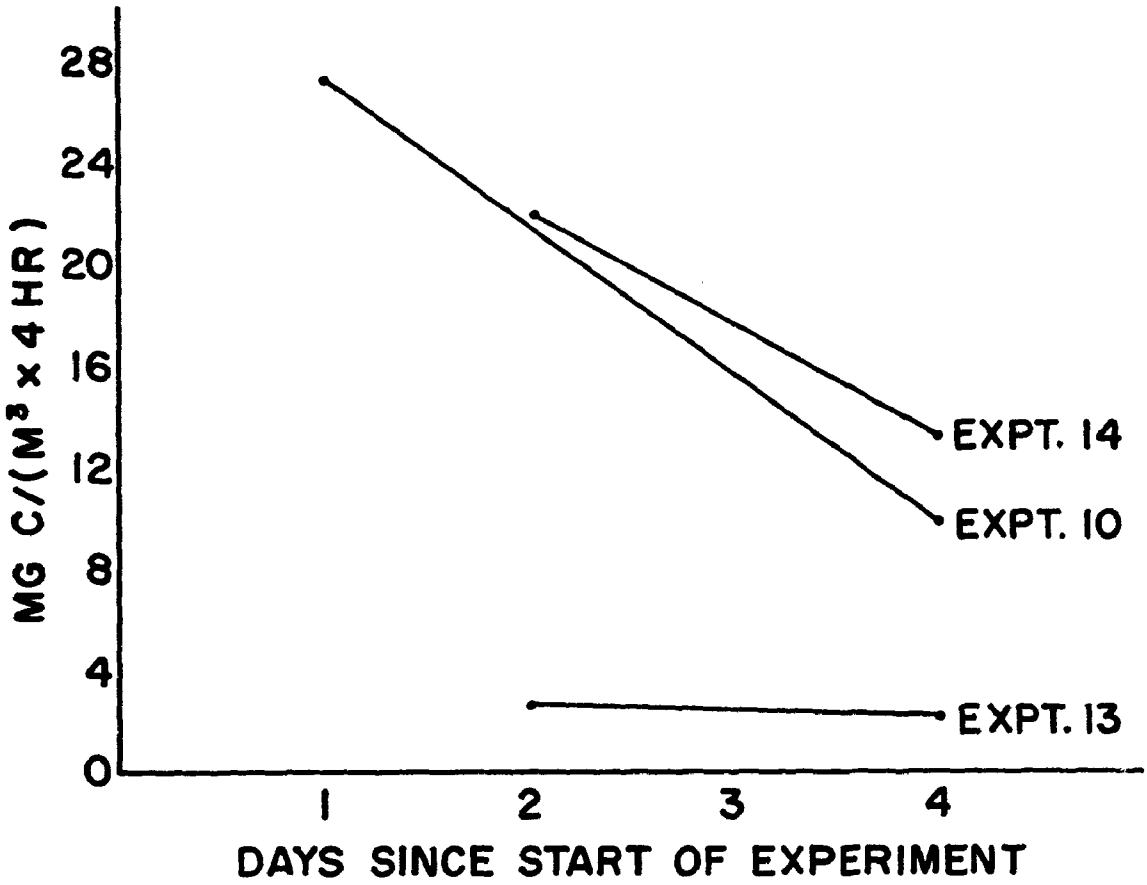


Figure 44. Productivity Trends in Control Tubes

plate at one end and open at the other, with dimensions 10 cm diameter by 40 cm high. The experimental lakewater volume added to each tube was two liters. The second container was a glass jar with dimensions 16 cm diameter by 24 cm high, and with a mouth of 8 cm diameter. Two or three liters of lake water were added. During an experiment these vessels were incubated alongside the lake in a water bath through which lake water was pumped for cooling. The mouths of the containers were left open.

Figure 44 shows productivity trends in control tubes for the three experiments in which they were employed. Productivity declined with time, and replicability between duplicate tubes receiving identical treatments was poor. The jars were then tested in hopes that they would at least provide better replicability. These vessels were employed in two experiments, 16 and 18.

Direct comparison of the behavior of control jars with that of control jugs was possible in experiment 16 since both sets of vessels were filled from the same tank of lake water, and ^{14}C measurements were run simultaneously. The surprising results appear in Figure 45. Productivity of samples from the control jars more than doubled between the first and second measurements, while productivity in jug samples dropped almost 50%.

In experiment 18, a 2 x 3 design employing N and P (Table 28), jars alone were employed, and productivity again increased with time. Examination of plankton samples revealed that Chroomonas acuta, which had never increased in numbers in a control jug but almost always declined or disappeared, had actually increased in the control jars (Table 29). The productivity results of experiment 18 differed from the results of the jug experiments of the same series in that the former indicated no stimulation by the P treatments but stimulation only by added N, in contrast to the P effects consistently obtained in the jug experiments. This could be due in part to the use of lower treatment levels of P and higher levels of N than were normally used in the jug experiments.

The difference in the fates of Chroomonas acuta in the jar environment and in the jug environment indicates that this species was not intolerant of containers in general, but rather of the jug container in particular. Differential responses of phytoplankton species to different types of incubation containers were reported by Thomas (1961), who traced species changes in flasks and in 7 m plexiglass columns, all suspended in a lake.

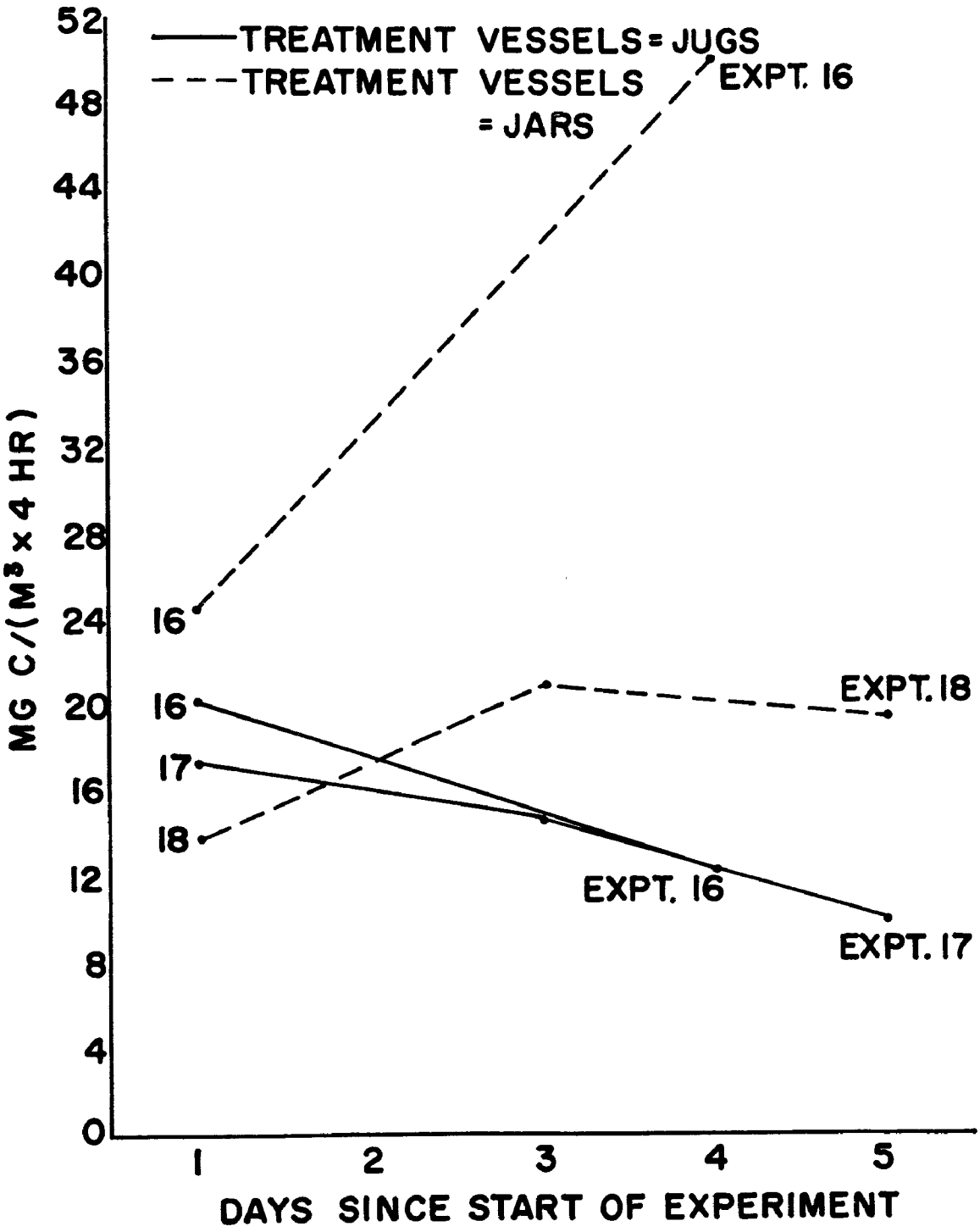


Figure 45. Productivity Trends: Control Jars vs Control Jugs

TABLE 28

EXPERIMENT 18
10-13-69 - 10-18-69

Design

Enrichment	Concentration
NO_3 - N	333 µg/l
+	+
NH_3 - N	150 µg/l
PO_4 - P_1	1.0 µg/l
PO_4 - P_2	1.7 µg/l

Productivity Results - $mgC/(m^3x4hr)$

	10-14-69		10-16-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
$N+P_2$	19.5	1.44	41.4	2.03
$N+P_1$	18.6	1.38	31.5	1.54
N	16.8	1.24	30.3	1.49
P_2	15.4	1.14	20.2	.99
P_1	15.6	1.16	19.9	.98
Control	13.5	1.00	20.4	1.00

ANOVA

	10-14-69	10-16-69
Source	F	F
N	17.79**	47.59**
P	3.09	2.91
NP	.14	2.88

Significant Simple Effects

	10-14-69	10-16-69
Comparison	F	F
N - Control		7.62*
$N+P_1$ - P_1		10.6*
$N+P_2$ - P_2	8.2*	35.4**
P_1 - Control		
P_1+N - N		
P_2 - Control		
P_2+N - N		

TABLE 29

EXPERIMENT 18: BIOMASS ESTIMATES (µg/l)

Species	Treatments		
	Initial	Control	N+P
Chroococcus dispersus	15.7	34.7	38.4
Microcystis aeruginosa	6.08	24.3	22.1
Aphanocapsa elachista	36.4	29.1	49.1
Microcystis incerta	16.7	18.3	19.6
Synedra rumpens	.022	.154	.194
Synedra radians	.379	2.70	3.90
Chroomonas acuta	38.6	54.3	67.5
Cryptomonas ovata	50.8	75.2	117.9
Cryptomonas erosa	48.8	37.4	82.1
Total	213.5	276.2	400.8
MG C/(m^3x4hr)		23.8	35.1

Oscillatoria rubescens disappeared from the flasks but not from the columns, while Fragilaria crotonensis and Mougeotia sp. grew better in the flasks than in the columns.

The jar environment differed from the jug environment in four major ways: (1) greater surface area to volume ratio, (2) access to sunlight unaltered by passage through water or glass, (3) opportunity for gas exchange with the atmosphere, and (4) opportunity for wind-induced turbulence. Which one or which combination of these factors favored Chroomonas is uncertain, especially since an abundant population of this organism (10.9 µg/l) was found under 30 cm of snow-covered ice on February 3, 1970. From this observation it appears that Chroomonas acuta does not require high light intensities, gas exchange, or high turbulence, and its abundant occurrence in the open lake water in the summer implies no need for close association with a surface.

If pure speculation is employed briefly, interspecific interactions such as predation or competition can be proposed to explain the different responses of this species to the two environments. Both of these hypothetical mechanisms depend upon the reduced turbulence in the jug environment, which could allow planktonic

organisms to sink toward the bottom. Flagellates such as Chroomonas acuta could be expected to settle less rapidly than nonmotile species, and could, after a sufficient period of time, be the major remaining components of the phytoplankton in the open water in the jugs. Swimming grazers would then feed selectively upon these flagellates because they were the most available prey.

If the Chroomonas cells settled with the other species, competition for nutrients could occur in the concentrated mass of organisms accumulated at the bottom of the jug. If Chroomonas were a poor competitor, it could decline as a result. Calculations by Hurlburt (1970) indicated that competition for nutrients in plankton communities could not occur unless cell densities exceeded 3×10^8 per liter, at which point nutrient depleted zones surrounding cells could overlap. In Third Sister Lake cell densities rarely exceeded 1×10^6 per liter. Therefore to achieve densities conducive to competition all of the organisms present in a 19 liter jug at a density of 1×10^6 per liter would have to settle into a bottom layer of approximately 60 ml volume. This seems unlikely, particularly since the jug contents were thoroughly mixed during sampling for each ^{14}C run.

Studies of the Jug Environment

During the course of the study various attempts were made to identify factors that contributed to the containment effects exhibited by the jugs. While none of these efforts produced an explanation for the response of Chroomonas acuta to the jug environment, they will be discussed to indicate the approaches that were tried.

It was mentioned at the beginning of this section that the chemical environment within a jug could change with time, due to the lack of interchange of water with the outside. In experiments 1-6 alkalinity and pH were frequently measured in the jugs (Table 30). Neither parameter appeared to change with time within a jug or to differ significantly between treatment and control jugs.

It was mentioned that radiation passing through the jug walls could be modified and consequently modify the jug environment. One result could have been a greenhouse effect, but when temperatures inside the jugs were compared to the ambient lake temperatures no differences were found (Table 30).

TABLE 30

PHYSICO-CHEMICAL CONDITIONS IN
CONTROL AND HIGH TREATMENT JUGS

Parameter	Jug	Expt.	Productivity Run				
			1	2	3	4	5
ALK*	Control	1	87.4	87.7	86.0	86.0	
	HHH	1	86.0	84.8	86.0	87.0	
pH	Control	1			8.2		
	HHH	1			8.2		
ALK	Control	2	81.0	79.0	80.0		
	HHH	2	81.0	80.0	80.0		
ALK	Control	3	78.0	77.0			
	H	3	78.5	78.0			
pH	Control	3	8.16	8.26			
	H	3	8.13	8.24			
Temp.	Control	3	26.1	25.6	(identical to lake)		
	H	3	26.1	25.5			
ALK	Control	4	78.0	78.0			
	HH	4	76.0	76.0			
pH	Control	4	8.20	8.10			
	HH	4	8.13	8.10			
Temp.	Control	4	20.5	21.6	(identical to lake)		
	HH	4	20.5	21.3			
ALK	Control	5	82.0	83.2			
	HHH	5	81.0	82.0			
pH	Control	5		7.81			
	HHH	5		7.85			
ALK	Control	6	86.0	89.0	86.0	81.0	84.0
	HHH	6	86.0	83.0	84.0	82.0	86.0
pH	Control	6			8.4	8.65	8.26
	HHH	6		8.6	8.7	8.7	8.50

* mg $CaCO_3$/l

There were variations in wall thickness among the jugs, which ranged in weight from 10 lb to 16 lb 12 oz. It was at one time suspected that light could have been modified more by passing through the thicker walls than through the thin walls, and that this could have contributed to variance among the jugs. In experiment 4 wall thickness (inferred from jug weight) was included as a testable variable, and no effects on the productivity results were found.

Since reduction in turbulence was suspected as a factor contributing to the containment effect, various mixing regimes were tested to see if they altered productivity results. In experiment 30 an extra pair of control jugs was included that was not sampled until the day of the last ^{14}C run. These jugs remained undisturbed in the lake for 5 days while the other jugs in the experiment were sampled on days 2, 3, and 5. The productivity results for the unmixed controls were insignificantly different from the results for the normal controls on day 5. In experiment 32 half of the jugs were mixed three times per day while the other half were mixed once a day. Again no productivity differences appeared.

The possibility that the sulfuric acid-chromate solution used to clean the jugs was leaving toxic residues was tested in experiments 31 and 32, in which jugs cleaned with this solution were compared with jugs cleaned with HCl and with water only. No productivity differences appeared.

Finally, in experiments 30 and 32 attempts were made to remove the zooplankton from certain jugs so that predation effects could be studied. Results were erratic, indicating that the technique used for zooplankton removal (straining the lake water through a # 20 net) was disrupting the phytoplankton community as well.

SIGNIFICANCE OF CONTAINMENT EFFECTS

Although the series of enrichment experiments conducted in this study produced many examples of containment effects and demonstrated that they can be highly and consistently selective for particular species, notably cryptomonads, the mechanisms of the effects were not explained. Attempts to evaluate the general significance of containment effects were more successful, as shown in the analysis of the data for experiments 25-S and 34-S. These results indicated that containment effects do not totally negate all value claimed for *in situ*

enrichment experiments in interpreting or predicting responses to enrichment in the natural system, but rather they limit the precision of the predictions that can be made. Substances may be tested to determine if they might affect algal growth in a given system, but conclusions about how much growth could result from a given amount introduced into the natural system cannot be drawn.

SECTION VIII

ACKNOWLEDGEMENTS

The field portions of this study were conducted at the University of Michigan. Numerous faculty members and students in The Department of Environmental Health, School of Public Health assisted with the project, and are acknowledged with sincere thanks.

The cooperation of the Department of Forestry, School of Natural Resources, who permitted the use of Third Sister Lake was greatly appreciated. Deserving of particular gratitude is Professor Frank F. Hooper, Department of Wildlife and Fisheries, who initially suggested the use of this ideal research site.

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SECTION IX

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SECTION X

PUBLICATIONS

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SECTION XI

APPENDICES

APPENDIX A

Productivity Results

Experiments 1-20

TABLE 1

EXPERIMENT 1
6-12-68 - 6-19-68

Design

Enrichment	Concentration
NO_3 - N_1	100 µg/l
NO_3 - N_2	250 µg/l
PO_4 - P_1	20 µg/l
PO_4 - P_2	50 µg/l
$EDTA_1$	.5 mg/l
$EDTA_2$	2.5 mg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	6-13-68		6-15-68		6-19-68	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
$N_2+P_2+EDTA_2$	65.6	2.06	204.9	4.80	175.8	10.16
$N_2+P_2+EDTA_1$	71.4	2.24	157.5	3.69	162.3	9.38
$N_2+P_1+EDTA_2$	53.2	1.67	117.2	2.74	140.0	8.09
$N_2+P_1+EDTA_1$	63.1	1.98	106.6	2.50	178.2	10.30
$N_1+P_2+EDTA_2$	59.2	1.86	95.8	2.24	97.6	5.64
$N_1+P_2+EDTA_1$	63.2	1.99	80.5	1.88	93.2	5.39
$N_1+P_1+EDTA_2$	52.0	1.63	120.8	2.83	115.8	6.69
$N_1+P_1+EDTA_1$	60.3	1.90	106.0	2.48	79.2	4.58
Control	31.8	1.00	42.7	1.00	17.3	1.00

ANOVA

	6-13-68	6-15-68	6-19-68
Source	F	F	F
N	2.37	69.86**	110.96**
P	5.19	16.19**	.36
EDTA	4.26	16.19**	.40
NP	.84	74.28**	.88
N EDTA	.12	1.62	6.52
P EDTA	.57	2.92	.58
NP EDTA	.01	2.73	10.63*

Significant Simple Effects

Comparison	6-13-68 F	6-15-68 F	6-19-68 F
$N_2+P_1+EDTA_1$ - $N_1+P_1+EDTA_1$			59.4**
$N_2+P_2+EDTA_1$ - $N_1+P_2+EDTA_1$		49.1**	29.0**
$N_2+P_1+EDTA_2$ - $N_1+P_1+EDTA_2$			
$N_2+P_2+EDTA_2$ - $N_1+P_2+EDTA_2$		98.7**	37.1**
$N_1+P_2+EDTA_1$ - $N_1+P_1+EDTA_1$			
$N_2+P_2+EDTA_1$ - $N_2+P_1+EDTA_1$		21.4**	
$N_1+P_2+EDTA_2$ - $N_1+P_1+EDTA_2$			
$N_2+P_2+EDTA_2$ - $N_2+P_1+EDTA_2$		64.0**	
$N_1+P_1+EDTA_2$ - $N_1+P_1+EDTA_1$			
$N_1+P_2+EDTA_2$ - $N_1+P_2+EDTA_1$			
$N_2+P_1+EDTA_2$ - $N_2+P_1+EDTA_1$			
$N_2+P_2+EDTA_2$ - $N_2+P_2+EDTA_1$		18.6**	

* .05 level
** .01 level

TABLE 2

EXPERIMENT 2
7-18-68 - 7-22-68

Design

Enrichment	Concentration
NO_3 - N	100 µg/l
PO_4 - P	20 µg/i
EDTA	.5 mg/l

Productivity Results - mgC/(m^3x4hr)

	7-20-68		7-21-68		7-22-68	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	290.2	6.54	134.6		131.3	
N+P	95.4	2.15	73.5		132.4	
N+EDTA	94.4	2.13	174.0		148.0	
N	59.0	1.33	65.0		56.6	
P+EDTA	14.3	.32	8.0		82.2	
P	49.4	1.11	61.8		70.8	
EDTA	76.2	1.72	64.6		86.0	
Control	44.4	1.00			8.9	

ANOVA

	7-20-68	7-22-68
Source	F	F
N	7.42*	7.02*
P	.28	3.57
EDTA	1.26	6.50*
NP	8.71*	1.45
N EDTA	4.88	2.51
P EDTA	1.78	5.88*
NP EDTA	1.97	.70

EXPERIMENT 2 (Continued)

Significant Simple Effects

	7-20-68	7-22-68
Comparison	F	F
N - Control		9.85*
N+P - P		
N+EDTA - EDTA		
N+P+EDTA - P+EDTA	21.6**	
P - Control		10.0*
P+N - N		
P+EDTA - EDTA		
P+N+EDTA - N+EDTA		
EDTA - Control		13.6**
EDTA+P - P		
EDTA+N - N		
EDTA+N+P - N+P		

* .05 level
** .01 level

TABLE 3

EXPERIMENT 3
8-7-68 - 8-10-68

Design

Enrichment	Concentration
$EDTA_1$	.5 mg/l
$EDTA_2$	2.5 mg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	8-8-68		8-10-68	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
$EDTA_2$	72.4	1.30	40.9	1.57
$EDTA_1$	62.8	1.13	42.6	1.64
Control	55.7		26.0	

ANOVA

	8-8-68	8-10-68
Source	F	F
Treatments	.820	1.28

Productivity Results - $mgC/(m^3 x 4hr)$

		8-8-68		8-10-68	
Treatment		$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
Rack 1	$EDTA_2$	67.5	2.12	17.8	1.42
Rack 1	$EDTA_1$	43.5	1.36	46.2	3.70
Rack 1	Control	31.9		12.5	
Rack 2	$EDTA_2$	79.2	1.18	53.7	1.55
Rack 2	$EDTA_1$	77.3	1.16	34.6	1.13
Rack 2	Control	66.9		30.7	
Rack 3	$EDTA_2$	70.6	1.03	51.2	1.48
Rack 3	$EDTA_1$	67.7	.99	47.1	1.36
Rack 3	Control	68.4		34.7	

ANOVA

	8-8-68	8-10-68
Treatment	F	F
Rack	5.98*	4.94*
EDTA	2.08	4.33*

Significant Simple Effects

	8-8-68	8-10-68
Comparison	F	F
R_2 Control - R_1 Control	6.08*	
R_2 $EDTA_1$ - R_1 $EDTA_1$	5.66*	
R_2 $EDTA_2$ - R_1 $EDTA_2$		10.9**
R_3 Control - R_1 Control	6.62*	4.26*
R_3 $EDTA_1$ - R_1 $EDTA_1$		
R_3 $EDTA_2$ - R_1 $EDTA_2$		9.6*
R_1 $EDTA_1$ - R_1 Control		10.5**
R_1 $EDTA_2$ - R_1 Control		
R_2 $EDTA_1$ - R_2 Control		
R_2 $EDTA_2$ - R_2 Control		4.53*
R_3 $EDTA_1$ - R_3 Control		
R_3 $EDTA_2$ - R_3 Control		

* .05 level
** .01 level

TABLE 4

EXPERIMENT 4
9-19-68 - 9-23-68

Design

Enrichment	Concentration
NO_3 - N	100 µg/l
Poly - P_1	50 µg/l
Poly - P_2	100 µg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	9-21-68		9-23-68	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
$N+P_2$	93.8	1.82	86.8	2.40
$N+P_1$	81.4	1.58	73.8	2.04
N	50.0	.97	29.2	.81
P_2	86.4	1.67	72.6	2.01
P_1	81.4	1.58	77.4	2.14
Control	51.6	1.00	36.2	1.00

ANOVA

	9-21-68	9-23-68
Source	F	F
N	.09	.29
P	35.96**	128.43**
NP	.03	4.96

Significant Simple Effects

	9-21-68	9-23-68
Comparison	F	F
N - Control		
$N+P_1$ - P_1		
$N+P_2$ - P_2		
P_1 - Control	19.5**	72.5**
P_1+N - N	22.25**	108.2**
P_2 - Control	25.5**	61.4**
P_2+N - N	37.5**	150.0**

* .05 level
** .01 level

TABLE 5

EXPERIMENT 5
10-27-68 - 11-4-68

Design

Enrichment	Concentration
NO_3 - N	100 µg/l
PO_4 - P_2	50 µg/l
PO_4 - P_1	10 µg/l
EDTA	.5 mg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	(10-30-68)-(10-28-68)		11-4-68	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P_2+EDTA	6.5	1.44	48.9	3.42
N+P_2	9.4	2.09	47.0	3.29
N+P_1+EDTA	9.4	2.09	47.0	3.29
N+P_1	8.0	1.78	28.9	2.02
N+EDTA	3.1	.69	15.7	1.10
N	2.6	.58	12.8	.90
P_2+EDTA	3.6	.80	12.6	.88
P_2	2.7	.60	12.7	.89
P_1+EDTA	3.8	.84	15.2	1.06
P_1	6.2	1.38	13.9	.97
EDTA	4.1	.91	13.5	.94
Control	4.5	1.00	14.3	1.00
Cation Control	2.7	.60	13.7	.96

ANOVA

	(10-30-68)-(10-28-68)	11-4-68
Source	F	
N	13.58**	188.79**
P	9.05**	46.33**
EDTA	.54	7.25*
NP	9.05**	51.29**
N EDTA	.08	6.90*
P EDTA	.23	4.08
NP EDTA	3.06	2.68

Significant Simple Effects

Comparison	(10-30-68)-(10-28-68) F	11-4-68 F
N - Control		
$N+P_1$ - P_1		18.25**
N+EDTA - EDTA		
$N+P_1$+EDTA - P_1+EDTA	10.9*	82.3**
$N+P_2$ - P_2		95.5**
$N+P_2$+EDTA - P_2+EDTA	18.2**	107.0**
P_1 - Control		
P_1+N - N	12.1**	
P_1+N+EDTA - N+EDTA	16.5**	79.7**
P_2 - Control		
P_2+N - N	19.0**	94.7**
P_2+N+EDTA - N+EDTA	4.65*	90.0**
EDTA+N+P_1 - N+P_1		26.5**

* .05 level
** .01 level

TABLE 6

EXPERIMENT 6
4-23-69 - 5-5-69

Design

Enrichment	Concentration
NO_3 - N	25 µg/l
PO_4 - P	5 µg/l
EDTA	.5 mg/l

Productivity Results - mgC/(m^3x4hr)

	4-25-69		4-28-69		4-30-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	120.0	1.68	73.6	1.65	88.2	1.48
N+P	87.8	1.23	57.2	1.28	76.4	1.28
N+EDTA	96.8	1.35	61.1	1.37	84.2	1.41
N	54.7	.76	42.2	.95	57.7	.97
P+EDTA	91.8	1.28	58.8	1.32	80.4	1.35
P	70.5	.98	43.0	.96	70.2	1.18
EDTA	93.5	1.31	53.5	1.20	74.8	1.25
Control	71.6	1.00	44.6	1.00	59.7	1.00
Cation Control	75.2	1.05	50.0	1.12	53.3	.89

	5-2-69		5-5-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	81.6	1.60	75.2	1.25
N+P	70.1	1.37	82.0	1.36
N+EDTA	69.0	1.35	65.8	1.09
N	44.8	.88	53.2	.88
P+EDTA	61.4	1.20	62.6	1.04
P	63.6	1.24	64.3	1.07
EDTA	68.0	1.33	66.6	1.11
Control	51.1	1.00	60.2	1.00
Cation Control	42.0	.82	45.7	.76

ANOVA

Source	4-25-69 F	4-28-69 F	4-30-69 F	5-2-69 F	5-5-69 F
N	.99	1.55	1.60	2.72	1.46
P	2.78	1.31	5.18	11.08*	4.27
EDTA	13.39**	4.82	13.92**	14.71**	.32
NP	3.38	.75	1.15	5.89*	4.21
N EDTA	.94	.15	.59	2.59	.002
P EDTA	.11	.25	1.31	5.78*	2.20
NP EDTA	.09	.12	.34	.24	.38

Significant Simple Effects

Comparison	4-25-69 F	4-28-69 F	4-30-69 F	5-2-69 F	5-5-69 F
N - Control					
N+P - P					
N+EDTA - EDTA					
N+P+EDTA - P+EDTA					
P - Control					
P+N - N				14.6**	
P+EDTA - EDTA					
P+N+EDTA - N+EDTA					
EDTA - Control				6.45*	
EDTA+P - P					
EDTA+N - N	6.94*		9.65*	13.5**	
EDTA+N+P - N+P					

* .05 level
** .01 level

TABLE 7

EXPERIMENT 7
5-26-69 - 6-2-69

Design

Enrichment	Concentration
NO_3 - N	25 µg/l
PO_4 - P	5 µg/l
EDTA	.5 mg/l

Productivity Results - mgC/(m^3x4hr)

	5-27-69		5-29-69		6-2-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	61.9	1.23	63.8	2.13	24.1	1.68
N+P	55.5	1.10	50.2	1.68	22.6	1.58
N+EDTA	57.9	1.15	52.0	1.74	23.9	1.67
N	45.7	.90	30.9	1.03	16.3	1.14
P+EDTA	55.8	1.10	36.3	1.21	24.0	1.68
P	52.1	1.03	29.3	.98	22.8	1.59
EDTA	48.4	.96	30.8	1.03	17.9	1.25
Control	50.5	1.00	29.9	1.00	14.3	1.00
Cation Control	45.0	.89	26.4	.88	18.8	1.31

ANOVA

	5-27-69	5-29-69	6-2-69
Source	F	F	F
N	2.85	46.92**	5.02
P	7.45*	12.13**	37.26**
EDTA	5.78*	17.19**	16.23**
NP	.32	6.50*	5.57*
N EDTA	4.12	6.72*	1.59
P EDTA	.00	.02	6.26*
NP EDTA	1.89	1.75	1.20

EXPERIMENT 7 (Continued)

Significant Simple Effects

Comparison	5-27-69 F	5-29-69 F	6-2-69 F
N - Control			
N+P - P		16.6**	
N+EDTA - EDTA		16.7**	17.8**
N+P+EDTA - P+EDTA		28.4**	
P - Control			24.5**
P+N - N	5.5*	14.1**	13.3**
P+EDTA - EDTA			12.2**
P+N+EDTA - N+EDTA			
EDTA - Control			
EDTA+P - P			
EDTA+N - N	8.6*	16.7**	19.7**
EDTA+N+P - N+P		6.9*	

* .05 level
** .01 level

TABLE 8

EXPERIMENT 8
6-4-69 - 6-11-69

Design

Enrichment	Concentration
NH_3 - N	25 µg/l
PO_4 - P	5 µg/l
EDTA	.5 mg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	6-6-69		6-9-69		6-11-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	54.8	2.01	42.8	2.38	26.0	1.55
N+P	55.8	2.05	48.9	2.72	30.7	1.83
N+EDTA	28.2	1.04	19.2	1.07	19.0	1.13
N	27.0	.99	18.2	1.01	17.6	1.05
P+EDTA	46.5	1.71	34.7	1.93	23.0	1.37
P	47.5	1.75	32.1	1.78	21.3	1.27
EDTA	27.8	1.02	18.5	1.03	17.4	1.04
Control	27.2	1.00	18.0	1.00	16.8	1.00
Cation Control	25.0	.92	17.8	.99	16.9	1.01

ANOVA

	6-6-69	6-9-69	6-11-69
Source	F	F	F
N	22.80**	11.82**	7.20*
P	705.30**	127.06**	29.94**
EDTA	.003	.08	.04
NP	21.53**	10.18*	3.22
N EDTA	.02	1.17	1.03
P EDTA	1.16	.43	.86
NP EDTA	.03	1.50	1.70

EXPERIMENT 8 (Continued)

Significant Simple Effects

Comparison	6-6-69 F	6-9-69 F	6-11-69 F
N - Control			
N+P - P	21.8**	20.2**	11.4**
N+EDTA - EDTA			
N+P+EDTA - P+EDTA	21.8**		
P - Control	129**	14.2**	
P+N - N	261**	67.1**	22.4**
P+EDTA - EDTA	110**	18.9**	
P+N+EDTA - N+EDTA	246**	39.4**	6.16*
EDTA - Control			
EDTA+P - P			
EDTA+N - N			
EDTA+N+P - N+P			

* .05 level
** .01 level

TABLE 9

EXPERIMENT 9
7-1-69 - 7-7-69

Design

Enrichment	Concentration
NO_3 - N	25 µg/l
PO_4 - P	5 µg/l
EDTA	50 µg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	7-2-69		7-4-69		7-7-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	70.7	1.69	26.1	1.80	12.2	2.09
N+P	70.3	1.68	26.5	1.83	11.5	1.97
N+EDTA	56.3	1.34	24.6	1.70	11.5	1.97
N	45.1	1.08	20.6	1.42	8.93	1.53
P+EDTA	65.0	1.55	17.3	1.19	13.2	2.26
P	48.6	1.16	14.7	1.01	10.2	1.75
EDTA	49.3	1.18	14.1	.97	9.10	1.56
Control	41.9	1.00	14.5	1.00	5.84	1.00
Cation Control	36.9	.88	12.5	.86	5.17	.88

ANOVA

	7-2-69	7-4-69	7-7-69
Source	F	F	F
N	194.87**	173.02**	9.67*
P	503.60**	14.43**	39.42**
EDTA	175.18**	4.43	26.07**
NP	48.20**	1.98	7.51*
N EDTA	11.08*	.23	2.59
P EDTA	.12	.20	1.22
NP EDTA	35.11**	6.77*	.80

Significant Simple Effects

Comparison	7-2-69 F	7-4-69 F	7-7-69 F
N - Control		18.76**	10.78**
N+P - P	227.35**	69.55**	
N+EDTA - EDTA	23.10**	54.96**	6.60*
N+P+EDTA - P+EDTA	33.92**	38.69**	
P - Control	21.31**		21.31**
P+N - N	305.67**	17.05**	7.66*
P+EDTA - EDTA	118.44**		19.40**
P+N+EDTA - N+EDTA	141.62**		
EDTA - Control	26.51**		11.99**
EDTA+P - P	130.33**		10.57*
EDTA+N - N	59.98**	7.94*	7.55*
EDTA+N+P - N+P			

* .05 level
** .01 level

TABLE 10

EXPERIMENT 16
8-21-69 - 8-25-69

Design

Enrichment	Concentration
NO_3 - N	100 µg/l
PO_4 - P	3 µg/l
EDTA	.5 mg/l

Productivity Results - $mgC/(m^3x4hr)$

	8-22-69		8-25-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	42.2	2.08	23.7	1.93
N+P	31.7	1.56	15.8	1.28
N+EDTA	25.7	1.27	15.0	1.22
N	17.7	.87	11.2	.91
P+EDTA	39.5	1.95	26.7	2.17
P	28.3	1.39	18.5	1.50
EDTA	27.1	1.33	15.1	1.23
Control	20.3	1.00	12.3	1.00
Cation Control	20.3	1.00	10.1	.82

ANOVA

	8-22-69	8-25-69
Source	F	F
N	.25	4.71
P	147.28**	99.39**
EDTA	74.95**	52.97**
NP	5.76*	2.11
N EDTA	.02	.04
P EDTA	2.66	9.38*
NP EDTA	.21	.19

EXPERIMENT 16 (Continued)

Significant Simple Effects

Comparison	8-22-69 F	8-25-69 F
N - Control		
N+P - P		
N+EDTA - EDTA		
N+P+EDTA - P+EDTA		
P - Control	14.6**	15.9**
P+N - N	45.0**	8.93*
P+EDTA - EDTA	34.7**	55.8**
P+N+EDTA - N+EDTA	61.0**	30.8**
EDTA - Control	10.5**	
EDTA+P - P	28.4**	27.6**
EDTA+N - N	14.6**	5.87*
EDTA+N+P - N+P	24.4**	25.4**

* .05 level
** .01 level

TABLE 11

EXPERIMENT 17
9-13-69 - 9-18-69

Design

Enrichment	Concentration
NO_3 - N	25 µg/l
PO_4 - P	5 µg/l
EDTA	.5 mg/l

Productivity Results - mgC/(m^3x4hr)

	9-14-69		9-16-69		9-18-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	30.3	1.73	27.7	1.87	12.9	1.44
N+P	24.8	1.42	23.6	1.59	14.6	1.63
N+EDTA	23.3	1.33	30.8	2.08	10.3	1.15
N	18.3	1.05	20.4	1.38	9.82	1.10
P+EDTA	22.6	1.29	17.3	1.17	10.74	1.20
P	17.2	.98	15.1	1.02	11.5	1.28
EDTA	21.1	1.21	17.5	1.18	9.28	1.04
Control	17.5	1.00	14.8	1.00	8.96	1.00
Cation Control	13.4	.77	13.9	.94	8.11	.91

ANOVA

	9-14-69	9-16-69	9-18-69
Source	F	F	F
N	67.58**	53.00**	13.48**
P	42.93**	.004	33.99**
EDTA	76.23**	14.13**	.73
NP	29.61**	.001	3.15
N EDTA	.41	3.43	.17
P EDTA	1.18	1.74	2.86
NP EDTA	.37	1.27	.36

EXPERIMENT 17 (Continued)

Significant Simple Effects

Comparison	9-14-69 F	9-16-69 F	9-18-69 F
N - Control			
N+P - P	45.5**	10.5*	10.6*
N+EDTA - EDTA		25.2**	
N+P+EDTA - P+EDTA	45.5**	15.7**	
P - Control			7.15*
P+N - N	32.4**		24.5**
P+EDTA - EDTA			
P+N+EDTA - N+EDTA	38.4**		7.15*
EDTA - Control	10.2*		
EDTA+P - P	23.4**		
EDTA+N - N	19.4**	16.0**	
EDTA+N+P - N+P	24.2**		

* .05 level
** .01 level

TABLE 12

EXPERIMENT 19
10-25-69 - 11-4-69

Design

Enrichment	Concentration
NO_3 - N	25 µg/l
PO_4 - P	5 µg/l
EDTA	.5 mg/l

Productivity Results - mgC/(m^3x4hr)

	10-26-69		10-28-69		10-30-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	13.7	1.06	5.22	1.31	6.51	1.50
N+P	14.2	1.10	5.46	1.37	6.48	1.49
N+EDTA	12.4	.96	4.21	1.05	4.82	1.11
N	12.4	.96	3.94	.99	4.46	1.03
P+EDTA	14.4	1.12	5.55	1.39	6.58	1.52
P	14.7	1.14	5.30	1.32	6.54	1.51
EDTA	13.2	1.02	4.21	1.05	4.61	1.06
Control	12.9	1.00	4.00	1.00	4.34	1.00
Cation Control	12.9	1.00	3.51	.88	4.06	.94

	11-2-69		11-4-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+EDTA	4.58	1.36	30.4	1.70
N+P	4.38	1.30	23.5	1.31
N+EDTA	2.71	.80	18.0	1.01
N	3.01	.89	17.1	.96
P+EDTA	3.99	1.18	22.5	1.26
P	5.04	1.50	23.1	1.29
EDTA	2.94	.87	18.4	1.03
Control	3.37	1.00	17.9	1.00
Cation Control	2.70	.80	15.4	.86

EXPERIMENT 19 (Continued)

ANOVA

Source	10-26-26 F	10-28-69 F	10-30-69 F	11-2-69 F	11-4-69 F
N	5.91*	.05	.03	.64	6.55*
P	37.57**	23.77**	51.34**	51.26**	99.33**
EDTA	.28	.20	.41	3.58	7.42*
NP	.02	.01	.18	.40	11.19**
N EDTA	.26	.17	.01	2.72	7.57*
P EDTA	1.37	.19	.26	.02	3.04
NP EDTA	.00	.27	.01	1.85	6.21*

Significant Simple Effects

Comparison	10-26-69 F	10-28-69 F	10-30-69 F	11-2-69 F	11-4-69 F
N - Control					
N+P - P					
N+EDTA - EDTA					
N+P+EDTA - P+EDTA					30.9**
P-Control	12.7**	6.02*	16.0**	16.1**	13.5**
P+N - N	5.71*	8.36*	13.4**	10.8*	23.0**
P+EDTA - EDTA	13.5**	6.40*	13.1**	5.9*	8.64*
P+N+EDTA - N+EDTA	6.5*		9.44*	20.1**	75.6**
EDTA - Control					
EDTA+P - P					
EDTA+N - N					
EDTA+N+P - N+P					24.1**

* .05 level
** .01 level

TABLE 13

EXPERIMENT 20
4-23-70 - 5-3-70

Design

Enrichment	Concentration
NO_3 - N	25 µg/l
PO_4 - P	5 µg/l
EDTA	.382 mg/l

Productivity Results - mgC/(m^3x4hr)

	4-24-70		4-25-70		4-26-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
EDTA	115.1	1.04	139.2	1.12	139.0	1.13
N+P	113.2	1.02	133.6	1.07	135.0	1.09
N	96.9	.87	119.8	.96	115.4	.93
P	111.4	1.01	134.1	1.08	140.3	1.14
Control	110.8	1.00	124.4	1.00	122.4	1.00
Lake	114.6	1.03	130.4	1.05	139.4	1.13

	4-27-70		4-28-70		5-3-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
EDTA	134.2	1.13	148.9	1.24	70.4	1.26
N+P	131.4	1.11	136.2	1.13	69.0	1.24
N	114.7	.97	113.4	.94	56.2	1.01
P	135.0	1.14	121.8	1.01	65.4	1.17
Control	118.6	1.00	120.4	1.00	55.8	1.00
Lake	154.5	1.30	118.9	.99	77.2	1.38

ANOVA

	4-24-70	4-25-70	4-26-70	4-27-70	4-28-70	5-3-70
Source	F	F	F	F	F	F
N	3.55	.387	3.41	.342	.722	.784
P	6.93	7.90*	32.18**	6.78	7.72*	24.81**
NP	6.06	.240	.066	.0004	5.98	.502

EXPERIMENT 20 (Continued)

Significant Simple Effects

Comparison	4-24-70 F	4-25-70 F	4-26-70 F	4-27-70 F	4-28-70 F	5-3-70 F
N - Control						
N+P - P						
P - Control			14.8*			7.94*
P+N - N			18.25*		14.0*	16.6*

ANOVA

Source	4-24-70 F	4-25-70 F	4-26-70 F	4-27-70 F	4-28-70 F	5-3-70 F
EDTA	2.18	8.22	9.05	4.84	51.97*	14.51

Lake: Significantly higher than control on 5-3-70 (Tukey's Test)

* .05 level
** .01 level

APPENDIX B

Phytoplankton Species Biomass Estimates

Experiments 1-19

TABLE 1

EXPERIMENT 1: BIOMASS ESTIMATES (µg/l)

Species	Treatments							Corr Coef[1]
	Initial	Control	n+p+c	N+p+c	n+P+c	n+p+C	N+P+C	
Chroococcus dispersus	10.4	3.12	7.14	6.10	2.97	6.05	11.8	.677
Ankistrodesmus falcatus	1.06	1.48	7.91	9.89	9.32	11.7	18.6	.842*
Rhabdoderma sigmoidea	.944	1.15	2.63	2.16	1.28	1.68	23.2	.548
Synedra rumpens	7.06	1.41	85.4	68.5	43.8	29.7	17.7	.265
Scenedesmus bijuga	T	T	.578	1.60	.578	1.60	8.25	
Chroomonas acuta	T		T					
10µ sphere	2.01	4.01	74.2	145.	71.2	60.2	105.3	.925**
Total	21.5	11.2	177.8	233.2	129.1	110.9	184.8	
MG C/(m^3 x 4hr)		15.8	79.2	178.2	93.2	115.8	175.8	

[1] - Correlation coefficient computed with ^{14}C uptake on last day of experiment

** - Significant at the .01 level

* - Significant at the .05 level

T - Not numerous enough for reliable estimate

C,c - Chelator (EDTA); high and low levels, respectively

TABLE 2

EXPERIMENT 2: BIOMASS ESTIMATES (µg/l)

Species	Treatments				Correlation Coefficients
	Control	N+C	N+P	N+P+C	
Chroococcus dispersus	x	15.2	16.9	13.3	
Ankistrodesmus falcatus	2.07	3.35	2.23	2.19	-.279
	7.32	11.0	23.6	22.3	.629
Synedra rumpens	3.26	4.35	6.74	11.3	.963*
Synedra radians	4.54	2.45	4.95	4.21	.126
Synedra acus					
Total	17.2+	36.4	54.4	53.3	
MG C/(m^3 x 4hr)	44.4	94.4	95.4	290.2	

x - Obscured by high detritus content

TABLE 3

EXPERIMENT 3: BIOMASS ESTIMATES (μg/l)

Species	Treatments								Corr. Coef.
	Initial		Control		c		C		
	(a) [1]	(b)	(a)	(b)	(a)	(b)	(a)	(b)	
Chroococcus dispersus	6.07	10.9	7.00	7.06	7.00	7.70	9.66	8.09	.195
Ankistrodesmus falcatus	.211	.633	1.64	1.08	1.42	.994	1.44	.832	-.486
Anabaena wisconsinense	1.13	2.79	3.25	3.34	4.51	7.18	6.33	4.93	.509
Cylindrospermum stagnale	.130	.318	.155	.214	.160	.263	.174	.141	-.201
Spirulina major	.031	.005	.045	.048	.087	.092	.049	.038	.287
Aulosira sp.	.001	.014		.010		.010			
Synedra rumpens	.372	.124	1.49	.248	2.11	1.62	2.61	1.99	.610
Synedra radians	T	T	T	T	T	T	T	T	
Total	7.91	14.8	13.6	12.0	15.3	17.9	20.3	16.0	
MG C/(m^3 x 4hr)			68.4	66.9	77.3	67.7	67.5	79.2	

(a) and (b) are labels for two separate plankton samples

TABLE 4

EXPERIMENT 4: BIOMASS ESTIMATES (µg/l)

Species	Treatments						Correlation Coefficients
	Initial		Control		N+P		
	(a)	(b)	(a)	(b)	(a)	(b)	
Chroococcus dispersus	6.46	8.02	2.98	7.54	3.64	3.61	-.388
Elaktothrix gelatinosa	1.15	.650	.146	.190	.312	.281	.949*
Microcystis incerta	2.59	3.37	3.26	4.63	3.86	3.95	.049
Anabaena wisconsinense	10.4	7.01	5.40	6.69	2.40	4.07	-.815
Cylindrospermum stagnale	.182	.409	.162	.554	.361	.442	.239
Lyngbya limnetica	54.5	37.2	111.8	133.9	126.7	138.0	.570
Aulosira sp.	8.90	11.6	6.14	12.5	4.35	5.59	-.615
Spirulina major	.125	.282	.075	.209	.315	.151	.479
Oocystis parva	5.99	8.27	3.92	6.28	4.64	5.92	.189
Cosmarium truncatellum	12.7	14.0	54.7	58.0	29.8	50.4	-.653
Chroomonas acuta	1.47	1.20					
Total	104.5	92.0	188.6	230.5	176.4	212.4	
MG C /(m^3 x 4hr)			33.7	38.7	82.8	90.8	

TABLE 5

EXPERIMENT 5: BIOMASS ESTIMATES (µg/l)

Species	Initial	Treatments				Correlation Coefficients
		Control		N+P+C		
		(a)	(b)	(a)	(b)	
Chroococcus dispersus	16.5	20.0	14.6	21.1	86.1	.680
Ankistrodesmus falcatus	1.30	2.71	4.59	8.24	7.42	.894
Rhabdoderma sigmoidea	.337	.899	.337	.337	1.01	.217
Lyngbya limnetica	17.5	27.8	29.4	82.8	120.0	.956*
Crucigenia tetrapedia	1.54	2.89	1.73	2.50	5.78	.679
Chroomonas acuta	3.34					
Cryptomonas ovata	187.2	230.0	181.9	519.0	315.6	.784
Total	227.7	284.3	232.6	634.0	535.9	
MG C/(m^3 x 4hr)		17.1	11.6	47.7	50.4	

TABLE 6

EXPERIMENT 6: BIOMASS ESTIMATES (µg/l)

Species	Treatments						Corr. Coeff.
	Initial		Control		N+P+C		
	(a)	(b)	(a)	(b)	(a)	(b)	
Chroococcus minutus	57.4	42.0	112.0	96.6	92.4	81.2	-.766
Chroococcus dispersus	22.8	18.7	30.1	13.9	17.0	20.1	.080
Ankistrodesmus falcatus	77.0	79.2	143.0	169.4	151.8	129.8	-.748
Rhabdoderma sigmoidea	2.10	2.10	10.5	4.20	4.20	6.30	-.181
Lyngbya limnetica	15.0	20.4	19.8	24.0	30.6	26.4	.654
Oscillatoria rubescens	.290	.278	.054	.090	.391	.202	.678
Synedra rumpens	34.2	43.5	95.2	43.5	73.0	73.5	.269
Fragilaria crotonensis	.097	.105	.692	.321	.553	.637	.501
Fragilaria capucina var. mes.	.085	.338	1.20	1.91	1.41	1.06	-.699
Fragilaria capucina (35 µ)	.135	.593					
Fragilaria capucina (70 µ)	.023	.093	.131	.523	.150	.126	
Tetraedron minimum	5.74	8.05	12.4	11.8	10.4	13.4	.176
Chrysidalis sp.	74.2	126.0	7.00	12.6	254.8	212.8	.913*
Ochromonas sp.	8.8	17.6	26.4	44.0	308.0	325.6	.958*
Chroomonas acuta	8.50	10.3					
Cryptomonas erosa	10.3	19.6					
Total	309.0	388.9	458.5	422.8	944.7	891.1	
MG C/(m^3 x 4hr)			62.0	58.5	72.5	77.8	

TABLE 7

EXPERIMENT 7: BIOMASS ESTIMATES (µg/l)

Species	Treatments						Correlation Coefficients
	Initial		Control		N+P+C	N+P	
	(a)	(b)	(a)	(b)			
Chroococcus dispersus	.753	.621	.770	.687	.642	.764	-.254
Ankistrodesmus falcatus	16.8	30.6	45.8	25.0	55.9	65.0	.866
Rhabdoderma sigmoidea	2.70	3.48	8.26	4.21	9.38	9.89	.818
Lyngbya limnetica	4.27	4.65	7.27	6.52	19.9	18.5	.998**
Synedra rumpens	.063	.063	.069	.056	.075	.213	.564
Synedra acus	.313	.313	1.57	1.88	.940	2.51	-.119
Chrysidalis sp.	15.9	20.1	.467	.467	.467	1.63	.490
Chroomonas acuta	37.2	40.1	2.14	4.41	6.69	6.55	.845
Cryptomonas ovata	T	T					
Cryptomonas erosa	T	T					
Total	78.0	100.0	66.3	43.2	94.0	105.1	
MG C/(m^3 x 4hr)			15.1	13.7	23.8	22.6	

TABLE 8

EXPERIMENT 8: BIOMASS ESTIMATES (µg/l)

Species	Initial	Treatments				Correlation Coefficients
		Control		N+P+C		
		(a)	(b)	(a)	(b)	
Chroococcus dispersus	9.02					
Ankistrodesmus falcatus	.120	1.52	2.12	.636	.742	-.959
Rhabdoderma sigmoidea	1.01	2.60	3.27	2.73	1.05	-.784
Lyngbya limnetica	.257	.144	.174	.038	T	-.999
Microcystis incerta	.009	T	T	.193	.916	
Aphanothece nidulans	.089	7.32	3.24	20.2	22.2	.997**
Synedra rumpens	.048	.051	.110	.286	.176	.719
Synedra acus	.198	.257	.257	1.21	1.14	.958**
Crucigenia tetrapedia	.915	1.46	1.69	1.32	1.56	-.488
Sphaerocystis Schroeteri	T	2.76	1.85	1.38	2.52	-.080
Chroomonas acuta	35.7					
Total	47.4	16.1	12.7	28.0	30.3	
MG C/(m^3 x 4hr)		18.0	15.6	24.9	27.1	

TABLE 9

EXPERIMENT 9: BIOMASS ESTIMATES (µg/l)

Species	Treatments						Correlation Coefficient
	Initial		Control		N+P+C	N+P	
	(a)	(b)	(a)	(b)			
Chroococcus dispersus	5.49	5.15	4.32	3.67	4.83	4.39	.840
Ankistrodesmus falcatus	T	T	T	T	T	T	
Rhabdoderma sigmoidea	T	T	T	T	T	T	
Microcystis incerta	.740	.304	.203	.249	.539	.399	.917*
Gomphosphaeria lacustris	.168	.151	1.55	1.03	1.93	2.81	.826
Oocystis parva	1.60	2.10	2.58	1.71	2.73	2.18	.533
Chroomonas acuta	37.2	30.1	T	T	T	T	
Cryptomonas ovata	T	T	T	T	T	T	
Total	45.2	37.8	8.65	6.66	10.0	9.78	
MG C/(m^3 x 4hr)			6.52	5.17	12.6	11.9	

TABLE 10

EXPERIMENT 16: BIOMASS ESTIMATES (µg/l)

Species	Treatments					Correlation Coefficients
	Initial	Control		N+P+C		
		(a)	(b)	(a)	(b)	
Microcystis incerta	.017	.192	.055	.336	.184	.613
Aphanocapsa elachista	.380	2.77	1.98	4.82	4.20	.922*
Gomphosphaeria lacustris	T	.032	.008	.256	.120	.772
Anabaena wisconsinense	.039	.408	.328	.712	.896	.988**
Aphanizomenon flos-aquae	.131	1.77	2.19	3.44	3.88	.974**
Cylindrospermum stagnale	.273	.304	.144	.312	12.5	.695
Chlamydomonas pseudopertyi	.120	6.06	6.58	20.6	32.9	.964**
Tetraedron caudatum	2.29	9.49	10.4	5.90	14.2	.160
Oocystis parva	14.2	8.54	12.5	4.05	9.31	-.564
Sphaerocystis Schroeteri	10.2	10.0	11.2	12.0	10.5	.323
Chroomonas acuta	27.1	.160	T	T	.968	
Cryptomonas ovata	.603	.720	1.08	1.08	3.62	
Total	55.4	40.4	46.5	53.5	93.3	
MG C/(m^3 x 4hr)		12.7	11.9	22.6	25.0	

TABLE 11

EXPERIMENT 17: BIOMASS ESTIMATES (µg/l)

Species	Treatments						Correlation Coefficients
	Initial		Control		N+P+C		
	(a)	(b)	(a)	(b)	(a)	(b)	
Aphanocapsa elachista	44.3	49.2	62.7	52.3	62.1	80.7	.752
Anabaena wisconsinense	x	x	1.79	1.68	1.53	1.56	
Ankistrodesmus falcatus	1.41	1.14	.377	.474	.406	.293	
Microcystis incerta	T	T	4.77	4.82	7.11	4.64	
Tetraedron caudatum	.748	.416	.276	1.41	.744	1.33	.432
Oocystis parva	T	T	5.26	6.29	4.11	6.29	-.052
Sphaerocystis Schroeteri	4.00	5.44	7.80	7.40	3.28	6.80	-.539
Asterococcus limneticus	6.72	4.48	161.3	156.8	67.2	156.8	-.394
Glenodinium pulvisculus	T	T	6.22	7.44	3.25	4.11	-.832
Chroomonas acuta	4.32	6.72	T	T	T	T	
Total	61.5	67.4	250.5	238.6	149.7	262.5	
MG C/(m^3 x 4hr)			8.54	9.39	12.2	13.6	

x = not counted

TABLE 12

EXPERIMENT 19: BIOMASS ESTIMATES (µg/l)

Species	Treatments						Corr. Coeff.
	Initial		Control		N+P+C		
	(a)	(b)	(a)	(b)	(a)	(b)	
Microcystis aeruginosa	32.3	32.8	30.6	29.0	45.5	49.8	.994**
Microcystis incerta	7.80	6.96	10.2	7.68	11.3	8.64	.332
Oscillatoria tenuis	.080	.280	.600	.400	1.04	1.28	.971**
Oscillatoria rubescens	.440	.024	.420	.120	.760	.080	.140
Anabaena Scheremetievi	.680	.680	.808	.200	.320	.280	.845
Ankistrodesmus falcatus	.190	.220	3.52	2.78	5.80	5.91	.984**
Synedra radians	4.32	4.88	8.64	9.60	22.9	21.4	.986**
Chroomonas acuta	31.0	31.0	.600	.800	1.40	3.60	
Cryptomonas ovata	110.6	146.0	172.6	192.2	220.8	235.8	.941*
Total	187.4	222.8	227.3	242.8	309.8	326.8	
MG C/(m^3 x 4hr)			18.1	17.7	29.8	31.0	

APPENDIX C

Productivity Results

Experiments 22-34

TABLE 1

EXPERIMENT 22
5-12-70 - 5-15-70

Productivity Results - $mgC/(m^3 x 4hr)$

Treatment	5-13-70 $\bar{X}$	5-13-70 $\bar{X}$/Control	5-15-70 $\bar{X}$	5-15-70 $\bar{X}$/Control
N: 92 µg/l NO_3-N+ 92 µg/l NH_3-N	45.8	1.09	56.2	1.07
NTA_3: 2.52 mg/l	53.3	1.27	54.8	1.05
NTA_2: 252 µg/l	46.6	1.11	55.4	1.06
NTA_1: 25.2 µg/l	44.0	.89	56.0	1.07
$EDTA_3$: 3.82 mg/l	59.2	1.41	56.6	1.08
$EDTA_2$: 382 µg/l	53.7	1.28	52.4	1,00
$EDTA_1$: 38.2 µg/l	45.6	1.09	55.4	1.06
Control	41.9	1.00	52.4	1.00
Lake	49.2	1.17	57.2	1.09

ANOVA

Source	5-13-70 F	5-15-70 F
Treatments	12.69**	.52

Significant Simple Effects (Tukey's Test)

Comparison	5-13-70	5-15-70
NTA_3 - Control	*	
$EDTA_3$ - Control	**	
$EDTA_2$ - Control	*	

* .05 level
** .01 level

TABLE 2

EXPERIMENT 23
5-19-70 - 5-21-70

Productivity Results - $mgC/(m^3 x 4hr)$

Treatment	5-20-70 $\bar{X}$	5-20-70 $\bar{X}$/Control	5-21-70 $\bar{X}$	5-21-70 $\bar{X}$/Control
N_3: 92 µg/l NO_3-N+ 92 µg/l NH_3-N	63.4	1.13	60.6	1.44
N_2: 18.5 µg/l NO_3-N+ 18.5 µg/l NH_3-N	61.2	1.09	53.4	1.27
N_1: 4.5 µg/l NO_3-N+ 4.5 µg/l NH_3-N	65.0	1.16	49.0	1.17
NTA_6: 2.5 mg/l	58.7	1.04	41.6	.99
NTA_5: 1.25 mg/l	58.2	1.04	40.6	.97
NTA_4: 500 µg/l	61.0	1.09	40.2	.96
NTA_3: 250 µg/l	57.0	1.01	42.3	1.01
NTA_2: 125 µg/l	60.0	1.07	41.8	1.00
NTA_1: 25 µg/l	57.3	1.02	39.0	.93
Control	56.2	1.00	42.0	1.00
Lake	57.5	1.02		

ANOVA

Source	5-20-70 F	5-21-70 F
Treatments	3.57*	15.11**

Significant Simple Effects (Tukey's Test)

Comparison	5-20-70	5-21-70
N_3 - Control		**
N_2 - Control		*

* .05 level
** .01 level

TABLE 3

EXPERIMENT 24
5-25-70 - 5-29-70

Design

Enrichment	Concentration
NO_3-N	25 µg/l
PO_4-P	5 µg/l
NTA	.252 mg/l
NO_3-N+NH_3-N	18 µg/l
EDTA	.382 mg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	5-26-70		5-27-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P+NTA	51.8	1.23	45.6	1.37
N+P	51.5	1.22	41.8	1.26
N+NTA	44.8	1.06	39.8	1.20
N	37.4	.89	31.6	.95
P+NTA	54.0	1.28	42.7	1.29
P	51.2	1.21	41.2	1.24
NTA	45.2	1.07	38.2	1.15
Control	42.2	1.00	33.2	1.00
EDTA	42.3	1.00	35.6	1.07
NO_3-N+NH_3-N	41.3	.98	32.2	.97
Lake	33.3	.79	24.8	.75

ANOVA

	5-26-70	5-27-70
Source	F	F
N	2.09	.388
P	62.94**	23.96**
NTA	7.50*	10.35*
NP	.432	.326
N NTA	.155	.872
P NTA	2.15	1.87
NP NTA	1.92	.024

Significant Simple Effects

Comparison	5-26-70 F	5-27-70 F
N-Control		
N+P - P		
N+NTA - NTA		
N+P+NTA - P+NTA		
P - Control	13.6**	7.65*
P+N - N	31.9**	12.2**
P+NTA - NTA	12.9**	
P+N+NTA - N+NTA	8.0*	
NTA - Control		
NTA+P - P		
NTA+N - N	9.19*	8.02*
NTA+N+P - N+P		

* .05 level
** .01 level

TABLE 4

EXPERIMENT 25
6-17-70 - 6-22-70

Design

Enrichment	Concentration
NO_3-N	2.57 µg/l
PO_4-P	23 µg/l
NTA	.252 mg/l
EDTA	.382 mg/l
Glycine	.098 mg/l
Zn	42.8 µg/l
Mo	9.6 µg/l
B 12	1.0 mg/l

Productivity Results - mgC/(m^3x4hr)

	6-18-70		6-19-70		6-22-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
B 12	70.6	1.14	40.6	1.15		
N+P	94.7	1.53	43.5	1.23	36.4	2.12
Glycine	64.0	1.03	41.2	1.16		
NTA + EDTA	72.7	1.17	47.0	1.33		
EDTA	78.2	1.26	46.8	1.32	22.0	1.28
NTA	74.8	1.21	40.4	1.14	17.9	1.04
Zn	16.6	.27	11.3	.32		
NTA + Zn	46.4	.75	28.0	.79		
Mo	59.6	.96	36.3	1.03		
NTA + Mo	72.6	1.17	43.2	1.22		
Control	61.9	1.00	35.4	1.00	17.2	1.00
Lake	52.3	.84	52.9	1.49	50.8	2.95

ANOVA

	6-18-70	6-19-70
Source	F	F
NTA	87.86**	378.22**
Zn	262.97**	1064.22**
NTA Zn	13.83**	111.84**
Source	F	F
NTA	18.23**	15.69**
Mo	.53	1.54
NTA Mo	.0003	.407
Source	F	F
NTA	.744	.925
EDTA	2.74	11.52*
NTA EDTA	4.55	.819
Source	F	F
Treatments	39.69**	239.37**

Significant Simple Effects (Tukey's Test)

	6-18-70	6-19-70
Comparison		
N+P - Control	**	

* .05 level
** .01 level

TABLE 5

EXPERIMENT 29
8-21-70 - 8-26-70

Design

Enrichment	Concentration
NO_3 - N	25 µg/l
PO_4 - P	5 µg/l
NTA	.252 mg/l
EDTA	.382 mg/l

Productivity Results - $mgC/(m^3 \times 4hr)$

	8-22-70		8-23-70		8-24-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
EDTA	51.3	1.58	99.1	1.17	80.0	1.74
N+P+NTA	48.2	1.49	90.4	1.61	81.8	1.78
N+P	35.3	1.09	65.8	1.17	58.0	1.26
N+NTA	42.7	1.31	85.1	1.51	77.0	1.67
N	37.0	1.14	62.8	1.12	56.2	1.22
P+NTA	43.4	1.33	81.4	1.45	69.8	1.52
P	34.6	1.06	61.8	1.10	49.1	1.07
NTA	39.7	1.22	74.4	1.32	65.4	1.42
Control	32.5	1.00	56.2	1.00	46.0	1.00
Lake			100.0	1.78	87.8	1.91

	8-25-70		8-26-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
EDTA	67.2	1.65		
N+P+NTA	72.4	1.77	92.8	1.51
N+P	57.0	1.40		
N+NTA	69.0	1.69		
N	58.2	1.43		
P+NTA	61.2	1.50		
P	48.9	1.20		
NTA	59.2	1.45		
Control	40.8	1.00	61.6	1.00
Lake	71.2	1.74	69.3	1.12

ANOVA

	8-22-70	8-23-70	8-24-70	8-25-70
Source	F	F	F	F
N	5.13	17.52**	50.41**	61.41**
P	2.86	8.31*	5.45*	4.39
NTA	36.49**	138.19**	199.06**	92.67**
NP	.095	.376	.025	1.69
N NTA	.209	1.63	.567	.580
P NTA	2.42	.282	.496	.061
NP NTA	.940	.008	.092	3.20

Significant Simple Effects

	8-22-70	8-23-70	8-24-70	8-25-70
Comparison	F	F	F	F
N - Control			11.5**	34.4**
N+P - P			8.8*	7.44*
N+NTA - NTA		8.95*	14.9**	10.9*
N+P+NTA - P+NTA		6.22*	17.3**	14.2**
P - Control				
P+N - N				
P+NTA - NTA				
P+N+NTA - N+NTA				
NTA - Control	6.32*	25.4**	41.7**	8.0*
NTA+P - P	9.47*	29.4**	47.5**	17.2**
NTA+N - N		38.4**	51.7**	13.2**
NTA+N+P - N+P	20.2**	46.5**	62.8**	27.0**
Tukey's Test				
EDTA - Control	**	**	**	*
Lake - Control		**	**	**

* .05 level
** .01 level

TABLE 6

EXPERIMENT 30
10-24-70 - 10-29-70

Design

Enrichment	Concentration
NO_3 - N	25 µg/l
PO_4 - P	5 µg/l
NTA	.252 mg/l
EDTA	.382 mg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	10-25-70		10-27-70		10-29-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
EDTA	75.3	1.08	63.3	1.23	21.1	1.16
N+P+NTA	88.8	1.27	94.1	1.83	29.8	1.65
N+P	81.1	1.16	74.7	1.45	24.4	1.35
N+NTA	75.2	1.08	72.1	1.40	28.6	1.58
N	75.1	1.07	64.0	1.24	22.8	1.26
P + NTA	85.8	1.23	62.6	1.22	22.2	1.23
P	85.3	1.22	57.7	1.12	18.4	1.02
NTA	76.1	1.09	57.1	1.11	21.8	1.20
Control	69.9	1.00	51.4	1.00	18.1	1.00
Lake	85.3	1.22	88.2	1.72	36.1	1.99

ANOVA

	10-25-70	10-27-70	10-29-70
Source	F	F	F
N	.236	79.42**	31.24**
P	45.66**	27.12**	.618
NTA	4.85	19.87**	17.30**
NP	.672	5.98*	.206
N NTA	.034	3.96	.624
P NTA	.085	1.55	.005
NP NTA	4.05	2.01	.012

Significant Simple Effects

Comparison	10-25-70 F	10-27-70 F	10-29-70 F
N - Control		8.72*	
N+P - P		15.9**	7.07*
N+NTA - NTA		12.4**	9.05*
N+P+NTA - P+NTA		54.4**	11.4**
P - Control	21.6**		
P+N - N		6.28*	
P+NTA - NTA	8.54*		
P+N+NTA - N+NTA	14.4**	26.6**	
NTA - Control			
NTA+P - P			
NTA+N - N			6.6*
NTA+N+P - N+P		20.6**	5.74*
Lake - Control	**	**	**
Control - C	**	*	

* .05 level
** .01 level

TABLE 7

EXPERIMENT 31
5-17-71 - 5-21-71

Design

Enrichment	Concentration
NTA	.252 mg/l
EDTA	.382 mg/l

Productivity Results - mgC/(m^3x4hr)

	5-19-71		5-20-71		5-21-71	
Treatment	$\bar{X}$	$\bar{X}$/ Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
NTA	24.4	1.07	29.7	1.12	31.2	1.06
EDTA	25.5	1.12	29.1	1.09	29.5	1.00
Control	22.7	1.00	26.6	1.00	29.4	1.00
Lake	21.3	.94	29.0	1.09	28.1	.96

Significant Simple Effects (Tukey's Test)

	5-19-71	5-20-71	5-21-71
Comparison			
NTA - Control		*	
EDTA - Control			
Control - Lake			

* .05 level

TABLE 8

EXPERIMENT 32
5-24-71 - 5-28-71

Design

Enrichment	Concentration
NTA	.252 mg/l
EDTA	.382 mg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	5-26-70		5-27-70		5-28-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
NTA	41.1	1.04	40.3	1.07	34.8	.96
EDTA	41.4	1.05	41.2	1.09	34.0	.94
Control	39.4	1.00	37.8	1.00	36.3	1.00
Lake	36.2	.92	33.3	.88	29.9	.82

Significant Simple Effects (Tukey's Test)

	5-26-70	5-27-70	5-28-70
Comparison			
NTA - Control			
EDTA - Control			
Control - Lake		*	*

* .05 level

TABLE 9

EXPERIMENT 34
6-9-71 - 6-14-71

Design

Enrichment	Concentration
NO_3 - N	.198 mg/l
PO_4 - P	.350 mg/l
NTA	.252 mg/l
EDTA	.382 mg/l

Productivity Results - $mgC/(m^3 x 4hr)$

	6-10-71		6-11-71		6-12-71	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
N+P	110.5	1.58	151.2	4.74	230.6	7.69
N	69.2	.99	57.6	1.81	47.0	1.57
P	79.3	1.14	36.6	1.15	40.8	1.36
NTA	65.8	.94	30.0	.94	26.3	.88
EDTA	65.0	.93	28.6	.90	26.6	.89
Control	69.8	1.00	31.9	1.00	30.0	1.00
Lake	50.3	.72	37.2	1.17	53.7	1.79

	6-14-71	
Treatment	$\bar{X}$	$\bar{X}$/Control
N+P	195.8	7.83
N	28.0	1.12
P	38.2	1.53
NTA	24.5	.98
EDTA	22.9	.92
Control	25.0	1.00
Lake	61.5	2.46

ANOVA

	6-10-71	6-11-71	6-12-71	6-14-71
Source	F	F	F	F
N	23.97**	224.44**	1799.02**	64.84**
P	66.15**	110.37**	1591.23**	82.26**
NP	26.06**	90.07**	1258.65**	60.08**

Significant Simple Effects

	6-10-71	6-11-71	6-12-71	6-14-71
Comparison	F	F	F	F
N - Control		15.0*	24.0**	
N+P - P	50.1**	299.0**	3030.0**	124.7**
P - Control			9.68**	
P+N - N	87.7**	199.0**	2822.0**	141.4**
Tukey's Test				
Lake - Control	*		*	**

* .05 level
** .01 level

APPENDIX D

Productivity Results

Sewage Experiments

TABLE 1

EXPERIMENT 6-S
4-23-69 - 5-5-69

Sewage Addition 325 ml to 19L

Productivity Results - $mgC/(m^3 x 4hr)$

	4-25-69		4-28-69		4-30-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
Sewage	196.8	2.75	114.4	2.56	97.0	1.62
N+P+EDTA	120.0	1.68	73.6	1.65	88.2	1.48
Control	71.6	1.00	44.6	1.00	59.7	1.00

	5-2-69		5-5-69	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
Sewage	81.6	1.60	48.9	.81
N+P+EDTA	81.6	1.60	75.2	1.25
Control	51.1	1.00	60.2	1.00

ANOVA

Significant Simple Effects (Tukey's Test)

	4-25-69	4-28-69	4-30-69	5-2-69	5-5-69
Comparison					
Sewage - Control	*	*	*	*	
N+P+EDTA - Control	*	*	*	*	
Sewage - N+P+EDTA	*	*			

* Significant at .05 level.

TABLE 2

EXPERIMENT 24-S
5-25-70 - 5-29-70

Sewage Addition 200 ml to 19L

Productivity Results - $mgC/(m^3 x 4hr)$

	5-26-70		5-27-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
Sewage	77.6	1.84	105.6	3.18
N+P	51.5	1.22	41.8	1.26
Control	42.2	1.00	33.2	1.00

ANOVA

Significant Simple Effects (Tukey's Test)

	5-26-70	5-27-70
Comparison		
Sewage - Control	**	**
N+P - Control	**	
Sewage - N+P	**	**

** Significant at .01 level.

TABLE 3

EXPERIMENT 25-S
6-17-70 - 6-22-70

Sewage Addition 200 ml to 19L

Productivity Results - $mgC/(m^3 x 4hr)$

	6-18-70		6-19-70		6-22-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
Sewage	109.2	1.78	169.2	4.78	98.2	5.71
N+P	94.7	1.53	43.5	1.23	36.4	2.12
Control	61.9	1.00	35.4	1.00	17.2	1.00

ANOVA

Significant Simple Effects (Tukey's Test)

Comparison	6-18-70	6-19-70	6-22-70
Sewage - Control	**	**	**
N+P - Control	**	*	*
Sewage - N+P	**	**	**

* Significant at .05 level.
** Significant at .01 level.

TABLE 4

EXPERIMENT 29-S
8-21-70 - 8-26-70

Sewage Addition 400 ml to 19L

Productivity Results - mgC/(m^3x4hr)

	8-22-70		8-23-70		8-24-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
Sewage	38.2	1.18	75.3	1.34	88.1	1.92
N+P+NTA	48.2	1.49	90.4	1.61	81.8	1.78
Control	32.5	1.00	56.2	1.00	46.0	1.00

	8-25-70		8-26-70	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
Sewage	102.4	2.51	168.9	2.74
N+P+NTA	72.4	1.77	92.8	1.51
Control	40.8	1.00	61.6	1.00

ANOVA

Significant Simple Effects (Tukey's Test)

	8-22-70	8-23-70	8-24-70	8-25-70	8-26-70
Comparison					
Sewage - Control			*		
N+P+NTA - Control	**	*	*		
Sewage - N+P+NTA					

* Significant at .05 level.
** Significant at .01 level.

TABLE 5

EXPERIMENT 34-S
6-9-71 - 6-14-71

Sewage Addition 200 ml to 19L

Productivity Results - mgC/(m^3x4hr)

	6-10-71		6-11-71		6-12-71	
Treatment	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control	$\bar{X}$	$\bar{X}$/Control
Sewage	139.2	1.99	245.0	7.68	323.8	10.79
N+P	110.5	1.58	151.2	4.74	230.6	7.69
Control	69.8	1.00	31.9	1.00	30.0	1.00

	6-14-71	
Treatment	$\bar{X}$	$\bar{X}$/Control
Sewage	174.4	6.98
N+P	195.8	7.83
Control	25.0	1.00

ANOVA

Significant Simple Effects (Tukey's Test)

	6-10-71	6-11-71	6-12-71	6-14-71
Comparison				
Sewage - Control	**	**	**	**
N+P - Control	**	**	**	**
Sewage - N+P	*	**	**	

* Significant at .05 level.
** Significant at .01 level.

APPENDIX E

Phytoplankton Species Biomass Estimates

Sewage Experiments

TABLE 1

EXPERIMENT 6-S

Species Biomass Estimates
Treatment Means - µg/l

Species	Initial Lake	Control	N+P+EDTA	Sewage
Ochromonas sp.	13.2	35.2	316.8	32.4
Chrysidalis sp.	100.1	9.80	233.8	10.8
Synedra rumpens	38.8	69.4	73.2	200.3
Fragilaria capucina	.211	1.56	1.24	11.55
Fragilaria crotonensis	.101	.506	.595	8.23
Ankistrodesmus falcatus	78.1	156.2	140.8	314.0
Tetraedron minimum	6.90	12.1	11.9	33.8
Chroococcus dispersus	20.3	22.0	18.6	246.4
Chroococcus minutus	49.7	104.3	86.8	123.2
Lyngbya limnetica	17.7	21.9	28.5	27.7
Rhabdoderma sigmoidea	2.10	7.35	5.25	2.10
Cryptomonas erosa	15.0	0	0	0
Chroomonas acuta	9.9	0	0	0

TABLE 2

EXPERIMENT 6-S

Species (Continued)
Treatment Effects (Tukey's Test)

Species	Comparison		
	Sewage - Control	Sewage - N+P+EDTA	N+P -Control
Ochromonas sp.		**	**
Chrysidalis sp.		**	**
Synedra rumpens	*	*	
Fragilaria carucina	*	*	
Fragilaria crotonensis	*	*	
Ankistrodesmus falcatus	*	*	
Tetraedron minimum	*	*	
Chroococcus dispersus	**	**	
Chroococcus minutus			
Lyngbya limnetica			
Rhabdoderma sigmoidea			
Cryptomonas erosa			
Chroomonas acuta			

* Significant at .05 level
** Significant at .01 level

TABLE 3

EXPERIMENT 25-S

Species Biomass Estimates

Treatment Means - µg/l

Species	Initial Lake	Final Lake	Control	N+P	Sewage
Coelosphaerium Kuetzingianum	20.70	36.49	30.90	59.20	73.05
Oscillatoria sp.	5.32	3.90	8.56	27.35	25.50
Mallomonas sp.	.01	.01	1.15	5.85	7.10
Crucigenia tetrapedia	.100	.081	.108	.174	.218
Synedra sp. 1	.001	.153	.034	1.06	4.43
Synedra sp. 2	.108	.108	.237	1.77	3.88
Crucigenia rectangularis	7.50	5.44	5.44	8.94	57.0
Sphaerocystis Schroeteri	5.64	4.44	2.69	8.36	23.3
Microcystis incerta	.280	.112	.168	.352	7.26
Scenedesmus bijuga	.245	.228	.216	.542	3.01
Ankistrodesmus falcatus	.01	.01	.008	.021	.081
Coelastrum microporum	.072	.145	.362	.434	3.18
Nephrocytium sp.	.211	.036	.355	.598	1.109
Chroomonas acuta	43.75	22.87	.228	.019	0
Cryptomonas ovata	58.85	27.66	.456	.342	.233
Cryptomonas erosa	23.50	6.58	.518	.032	.027
Oocystis submarina	56.35	37.17	31.45	27.00	28.85
Ankistrodesmus falcatus var. acicularis	.017	.024	.018	.035	.047
Ankistrodesmus falcatus var. 2	.142	.104	.068	.090	.085
Synechocystis aquatilus	1.42	2.53	2.76	2.74	4.78
Aphanocapsa elachista	7.67	15.45	7.33	19.05	13.50
Tetraedron caudatum	.025	.036	.058	.114	.147

TABLE 4

EXPERIMENT 25-S

Species (Continued)

Significant Treatment Effects (Tukey's Test)

Species	Comparison		
	Sewage - Control	Sewage - N+P	N+P - Control
Coelosphaerium Kuetzingianum	**		*
Oscillatoria sp.	*		*
Mallomonas sp.	**		*
Crucigenia tetrapedia	*		*
Synedra sp. 1	**	**	*
Synedra sp. 2	**	*	*
Crucigenia rectangularis	**	**	
Sphaerocystis Schroeteri	**	**	
Microcystis incerta	*	*	
Scenedesmus bijuga	*	*	
Ankistrodesmus falcatus	**	**	
Coelastrum microporum	**	**	
Nephrocytium sp.	*	*	

* .05 level
** .01 level

TABLE 5

EXPERIMENT 34-S

Species Biomass Estimates
Treatment Means - µg/l

Species	Initial Lake	Final Lake	Control	P	N	N+P	Sewage
Chroomonas acuta	130.0	144.5	98.6	136.2	185.6	636.2	676.0
Elaktothrix gelatinosa	.381	.551	.342	.372	.382	.937	1.16
Sphaerocystis Schroeteri	2.75	2.40	4.29	2.78	3.78	8.15	9.02
Aphanothece nidulans	7.45	48.7	24.9	65.9	27.4	53.0	57.0
Synedra sp.	.218	0	.436	.709	.463	2.18	2.94
Cryptomonas ovata	34.2	24.7	24.2	31.5	50.2	119.4	181.9
Crucigenia rectangularis	43.3	1.90	106.2	157.4	130.8	220.4	304.8
Oocystis sp.	136.8	144.8	320.8	319.6	290.6	288.5	426.4
Chlamydomonas sp.	5.29	3.78	3.58	2.88	4.06	4.40	73.3
Ankistrodesmus falcatus	3.91	3.99	5.36	3.96	7.85	5.23	6.11
Aphanizomenon flos-aquae	.580	.832	1.01	.706	1.26	.895	.738

TABLE 6

EXPERIMENT 34-S

Species ANOVA

Species	ANOVA		Simple Effects	
Chroomonas acuta	Source	F	Comparison	F
	N	655.7 **	N - Control	28.6 **
	P	453.6 **	N+P - P	951 **
	NP	324.9 **	P - Control	
			P+N - N	771 **
			Sewage - Control	**
			Sewage - N+P	
			N+P - Control	**
Elaktothrix gelatinosa	Source	F	Comparison	F
	N	11.27 *	N - Control	
	P	10.54 *	N+P - P	18.9 *
	NP	8.52 *	P - Control	
			P+N - N	19.1 *
			Sewage - Control	*
			Sewage - N+P	
			N+P - Control	*
Sphaerocystis Schroeteri	Source	F	Comparison	F
	N	6.87	N - Control	
	P	2.39	N+P - P	16.8 *
	NP	10.06 *	P - Control	
			P+N - N	11.1 *
			Sewage - Control	*
			Sewage - N+P	
			N+P - Control	*
Aphanothece nidulans	Source	F	Comparison	F
	N	.67	N - Control	
	P	27.4 **	N+P - P	
	NP	1.49	P - Control	20.8 *
			P+N - N	8.05 *
			Sewage - Control	*
			Sewage - N+P	
			N+P - Control	*

EXPERIMENT 34-S

Species ANOVA (Continued)

Species	ANOVA		Simple Effects	
Synedra sp.	Source	F	Comparison	F
	N	22.34 **	N - Control	
	P	39.44 **	N+P - P	43.0 **
	NP	20.79 *	P - Control	
			P+N - N	58.8 **
			Sewage - Control	**
			Sewage - N+P	
			N+P - Control	*
Cryptomonas ovata	Source	F	Comparison	F
	N	34.58 **	N - Control	
	P	15.58 *	N+P - P	40.8 **
	NP	10.17 *	P - Control	
			P+N - N	25.4 **
			Sewage - Control	**
			Sewage - N+P	*
			N+P - Control	**
Chlamydomonas sp.	Source	F	Comparison	F
	N	2.25	N - Control	
	P	.075	N+P - P	
	NP	.612	P - Control	
			P+N - N	
			Sewage - Control	**
			Sewage - N+P	**
			N+P - Control	
Ankistrodesmus falcatus	Source	F	Comparison	F
	N	21.17 **	N - Control	18.5 *
	P	24.20 **(-)	N+P - P	
	NP	2.23	P - Control	
			P+N - N	20.5 *
			Sewage - Control	
			Sewage - N+P	
			N+P - Control	

EXPERIMENT 34-S

Species ANOVA (Continued)

Species	ANOVA		Simple Effects	
Aphanizomenon flos-aquae	Source	F	Comparison	F
	N	1.26	N - Control	
	P	3.01	N+P - P	
	NP	.021	P - Control	
			P+N - N	
			Sewage - Control	
			Sewage - N+P	
			N+P - Control	
Crucigenia rectangularis	Source	F	Comparison	F
	N	18.07 *	N - Control	
	P	46.68 **	N+P - P	18.6 *
	NP	3.51	P - Control	12.2 *
			P+N - N	37.7 **
			Sewage - Control	**
			Sewage - N+P	*
			N+P - Control	*
Oocystis sp.	Source	F	Comparison	F
	N	2.34	N - Control	
	P	.007	N+P - P	
	NP	.001	P - Control	
			P+N - N	
			Sewage - Control	*
			Sewage - N+P	*
			N+P - Control	

* Significant at .05 level
** Significant at .01 level

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16. Abstract

A method for performing in situ nutrient enrichment experiments on natural lake phytoplankton communities was developed and evaluated. One set of experiments in which it was employed was designed to detect limiting nutrients and to provide a basis for predicting future experiment results. Productivity increased in response to all three of the treatment variables used, N, P, and EDTA, but response patterns varied from experiment to experiment. Individual species responded differently to different treatments, and interactions among the treatment variables were important in shaping the community responses to mixtures of two or three variables. The most consistent features of the productivity results were incorporated into a "most probable response pattern," which was partially validated by a second series of experiments.

The second experiment series was also used to test the ability of NTA to stimulate phytoplankton productivity. Stimulation was continually obtained.

In a third series of experiments sewage effluents were tested in parallel with N and P. Varying degrees of overlap between the species complexes responding to the sewage and to the N and P treatments were found.

Recommendations for the use of in situ enrichment experiments in eutrophication studies are presented.

17a. Descriptors

*Eutrophication, *Nutrients, *Phytoplankton, Primary Productivity, Nitrogen, Phosphorus, Chelation, Lakes

17b. Identifiers

*Enrichment Experiments, *Factorial Designs, Individual Species Responses

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