THE EFFECTS OF NUTRIENT ENRICHMENT ON THE PLANKTON COMMUNITY IN EIGHT EXPERIMENTAL PONDS

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ABSTRACT

THE EFFECTS OF NUTRIENT ENRICHMENT ON THE PLANKTON COMMUNITY IN EIGHT EXPERIMENTAL PONDS

By

William John O'Brien

The concentrations of nitrogen and phosphorus present in a body of water may limit the amount of energy fixed by the primary producers of that water body. Little, however, is known of the relationship between varying amounts of these nutrients and the density of the phytoplankton in a water body. The relationship between varying amounts of phytoplankton and the density of the zooplankton is also poorly understood. To investigate the effect of different levels of nutrients on the plankton community, Frank deNoyelles and I organized a controlled fertilization study in eight experimental ponds. Two controls were maintained, and an inorganic nitrogen and phosphorus fertilizer was added at three increasing levels, each replicated, to create conditons ranging from the oligotrophic control ponds to the highly eutrophic level in the highest fertilized ponds. The fertilization regime was conducted throughout two summers, 1968 and 1969.

Chemical and biological observations were made primarily during the two summers of the fertilization treatment with less frequent observations during the rest of the year. The factors of major interest were the species composition and abundance of the phytoplankton and zooplankton, with Mr. deNoyelles studying the phytoplankton and I concentrating on the zooplankton. Many factors of common interest were also measured: concentrations of nitrogen and phosphorus in the pond water, chlorophyll a concentration and primary production of the phytoplankton, various chemical conditions, and other limnological parameters thought to be important.

The concentrations of nitrogen and phosphorus in the ponds ranged over three orders of magnitude. Classification of the nutrient concentration, especially that of nitrogen, and the level of primary production showed the treatments ranged from oligotrophic to highly eutrophic. A bioassay and other evidence suggested that nitrogen was the "governing" nutrient.

The concentrations of nitrogen and phosphorus largely followed treatment level and each treatment was distinct from the others, especially in 1969. In 1969 this was also true of the phytoplankton density as measured by chlorophyll a concentration and primary productivity. In 1968 the phytoplankton density according to treatment level was erratic, with the medium treatment level ponds having the highest phytoplankton density and the high

treatment level ponds generally having phytoplankton densities little greater than the low treatment ponds.

The relationship between treatment level and crustacean zooplankton density was variable in both years. In 1968 the high nutrient ponds had the highest average cladoceran density with the other treatments differing only slightly from one another. In 1969 even this relationship was no longer apparent. However, there was a clear, positive correlation, with four exceptions, between the average chlorophyll a concentration and the average cladoceran density in a pond. The four exceptional ponds had the highest chlorophyll a concentrations of all the ponds, and the relationship between the amount of phytoplankton and the cladoceran density may have been consideraly altered by the high phytoplankton densities in these ponds.

The species composition of the phytoplankton varied markedly with treatment level. Phytoplankton algae occurring in the control ponds were those common to oligotrophic conditions, while those occurring in the high and medium treatment level ponds were those typical of eutrophic conditions. The most interesting distribution of a species by treatment level was that of Microcystis aeruginosa, an organism often associated with eutrophic conditions, which occurred in the low and medium treatment level ponds but did not occur at all in the high treatment level ponds. The species diversity of the phytoplankton,

as measured by the average number of summer species, decreased with increasing nutrient input in both years.

While the same species of zooplankton occurred in all the ponds, the relative species composition changed dramatically, with Ceriodaphnia reticulata completely dominating the species composition in the higher treatment ponds. Considering species diversity to be the evenness with which the total biomass of the zooplankton is distributed among species rather than the overall number of species present, the species diversity of the zooplankton decreased with increasing nutrient input.

In the four ponds in which average cladoceran density did not increase with average chlorophyll a concentration, the relationships between the phytoplankton and the zooplankton were analyzed in detail. Three of these cases occurred in 1969 when the phytoplankton density was very high in late July and August yet no crustacean zooplankton at all were present at this time. Laboratory life tables investigating the survivorship of Ceriodaphnia reticulata in this water and other laboratory experiments along with field correlation suggested a pH mortality threshold at pH values over 10.8. The high pH values which occurred in these ponds were produced by high primary production. The other exceptional pond was pond 8 in 1968, a medium treatment pond. In this pond it was demonstrated that a large part of the chlorophyll a

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measured was from large algal species such as colonial Volvocales and Microcystis aeruginosa which would be unavailable to the zooplankton as food. The high zooplankton densities which preceded blooms of both these large phytoplankton forms may have played a role in the development of these blooms.

Large field experiments such as this one are valuable in determining what factors are important in the functional coupling between trophic levels, and how these factors are altered as basic ecosystem parameters change. Necessary to such experiments are constant monitoring by the investigator and the performance of critical experiments at appropriate times to confirm causal relationships.

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appreciation for detail and accuracy that was outstanding. Gary Hitchcock worked as the most full time part time employee anyone ever had the good fortune to work with.

Finally, I wish to fully acknowledge the efforts and understanding of my wife, Marion. She helped in every phase of this work with understanding and often with unnerving critical questions.

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INTRODUCTION

Brandt (1899) was perhaps the first to suggest that the amount of nitrogen and phosphorus present in a body of water limits the amount of energy fixed by the primary producers in that water body. Since then it has been well documented that the amount of energy fixed through photosynthesis determines the amount of energy available for consumer trophic levels. The general steps in the flow of energy are known for plankton communities: the phytoplankton fix carbon through photosynthesis and are fed upon by filter-feeding zooplankton which in turn are consumed by both invertebrate predators and small fish.

A number of investigators have manipulated nutrient input to small bodies of water, primarily in an attempt to understand factors affecting fish production (Swingle and Smith, 1939; Ball and Tanner, 1951; Nelson and Edmondson, 1955; and McIntire and Bond, 1960). Since the fish production in most of the waters studied did increase with fertilization, it is apparent that the effect of nutrient addition is "transmitted" to higher trophic levels. Most workers on experimental nutrient enrichment have left unstudied the details of the coupling between trophic

levels. Hall, Cooper, and Werner (1970), in a cross-classified study of nutrient enrichment and predation level on experimental ponds, demonstrated that not only did fish production increase with increasing nutrient input, but that this increased fish production was clearly related to the abundance of food particles greater than a certain critical size.

Some of the relationships between nutrients, phytoplankton, and zooplankton have been suggested through various correlations. Sakamoto (1966) shows a strikingly positive relationship between nitrogen and phosphorus concentrations and chlorophyll a concentrations in various Japanese lakes of widely varying nutrient content. This strongly suggests a corresponding increase of phytoplankton with increasing nutrient concentration. Hrbacek (1966) has shown a direct relationship between the fixed nitrogen in water, determined by Kjeldahl analysis, and the amount of nitrogen in zooplankton, which suggests that the zooplankton biomass is directly related to the amount of particulate matter (mostly phytoplankton) in the water. Other workers have shown that the abundance of zooplankton in a particular water body is inversely related to the abundance of phytoplankton (Anderson, Comita, and Engstrom-Heg, 1955). Nauwerck (1963) has shown that in the lake he studied the phytoplankton, if considered the only source of zooplankton food, cannot account for the observed abundance of the zooplankton. While these studies have

produced some insight into the mechanisms by which changes in one trophic level affect the others, the functional couplings between trophic levels are not well understood.

To investigate the effect of different levels of nutrients on the phytoplankton and zooplankton community, while minimizing the variability among the bodies of water under observation, Frank deNoyelles and I organized a controlled fertilization study of eight experimental ponds. We established three levels of fertilization, with one replicate pond at each level, and two control ponds. The fertilizer was added to create conditions ranging from slightly higher than the unproductive oligotrophic control ponds, up through intermediate ranges to a very productive, eutrophic level.

A fixed fertilization schedule was followed throughout two summers, early June to late August of 1968 and 1969. The pond nutrient concentrations and plankton communities were observed weekly during the time of fertilization and at less frequent intervals during the rest of the year. A concentrated observation period during the time of maximum possible biological change was felt to be a better analytical procedure than a less intense observation schedule throughout the entire two years. The factors of prime interest in this study were the numerical abundance and species composition of the phytoplankton and zooplankton, with Mr. deNoyelles studying the phytoplankton

and I concentrating on the zooplankton. Many factors of common interest were also measured: concentrations of nitrogen and phosphorus in the pond water, chlorophyll *a* concentration and primary production of the phytoplankton, various chemical conditions, and other limnological parameters thought to be important.

As an initial working hypothesis, we assumed that the relationship between nutrients and phytoplankton and the relationship between phytoplankton and zooplankton were simple, proportional functions. That is, the more nutrients added, the more phytoplankton production and biomass would result; the more phytoplankton biomass, the more zooplankton biomass would result. As specific contradictions to this hypothesis arose, experiments were performed to determine what relationships were involved in these responses. A series of bioassay experiments was performed to determine what factors were limiting the production of the phytoplankton in each of the four treatment levels. The filtering rate of the dominant zooplankter, *Ceriodaphnia reticulata* Jurine, was measured at different food densities in order to assess the capacity of this species to influence phytoplankton abundance through grazing. Several life table experiments were performed using *C. reticulata* to investigate the decrease and disappearance of crustacean zooplankton in certain of the higher nutrient ponds which had high phytoplankton densities.

Precedence was given to analyzing averaged zooplankton samples and individual samples from particular ponds where preliminary observations showed dynamic interactions between phytoplankton and zooplankton were affecting the pond community. Approximately 50 per cent of the phytoplankton samples were analyzed, with emphasis placed on characterizing seasonal trends in the phytoplankton species composition of all the ponds and the relationship between phytoplankton species composition and treatment level (deNoyelles, 1970). Although there is need for further analysis, this thesis presents the major response of the phytoplankton to increasing nutrients, along with the analysis of the average cladoceran response to treatment level. Each pond with high average phytoplankton density which showed no marked increase of average cladoceran biomass is analyzed in depth, and mechanisms causing these anomalies are suggested.

The concentration of nutrients in a body of water also affects the relative distribution of the plankton biomass among particular species or size categories. A preliminary analysis of the species diversity, as measured by average number of species in the case of the phytoplankton and by number of dominant species in the case of the zooplankton, is reported. Margalef (1964) and others have predicted that as the primary production of a body of water is increased through nutrient addition, the ratio of primary production to biomass will increase and this will

decrease the species diversity of the community through differential increase of certain species. Although much of our work could and will be used in the future to test this and other hypotheses put forth on the subject of species diversity, this consideration is much too complex and lengthy an undertaking for this dissertation.

MATERIALS AND METHODS

The Ponds

The ponds used in this experiment were constructed by the Department of Agronomy at Cornell University in the summer of 1964 at Cornell Pond Site #2. The general area is in the Erie-Langford Soil Association developed on glacial till with poor drainage. Site #2 was constructed on an old field and marsh. There are 50 ponds at the site, 40 built in the summer of 1963 and the 8 used in this study built the next year. The entire area was scraped down and the ponds constructed by building up dikes to form a square depression. The bottom area was 1/10 of an acre (0.04 hectare), the top area 1/3 of an acre (0.14 hectare), and the dikes were 3 meters high. Following construction the ponds were allowed to fill with rain water and snow; however, a canal system and reservoir were constructed at the site to fill the ponds if necessary. No effort was made to seed the 8 ponds used in this study with any organisms, and they remained undisturbed except for the maintenance of a 1.22 m water level from 1964 until the summer of 1968.

Because the bottom of the ponds is clay and relatively impermeable and the general locale has a high water table, the ponds lose very little water. During both years of the experiment, water had to be pumped out of the ponds each spring to reduce the pond depth from 1.83 m to 1.37 m. At this depth the pond volume is approximately 8.1×10^5 l. Prior to fertilization, all the pond bottoms were covered with Chara sp., and the water was very clear.

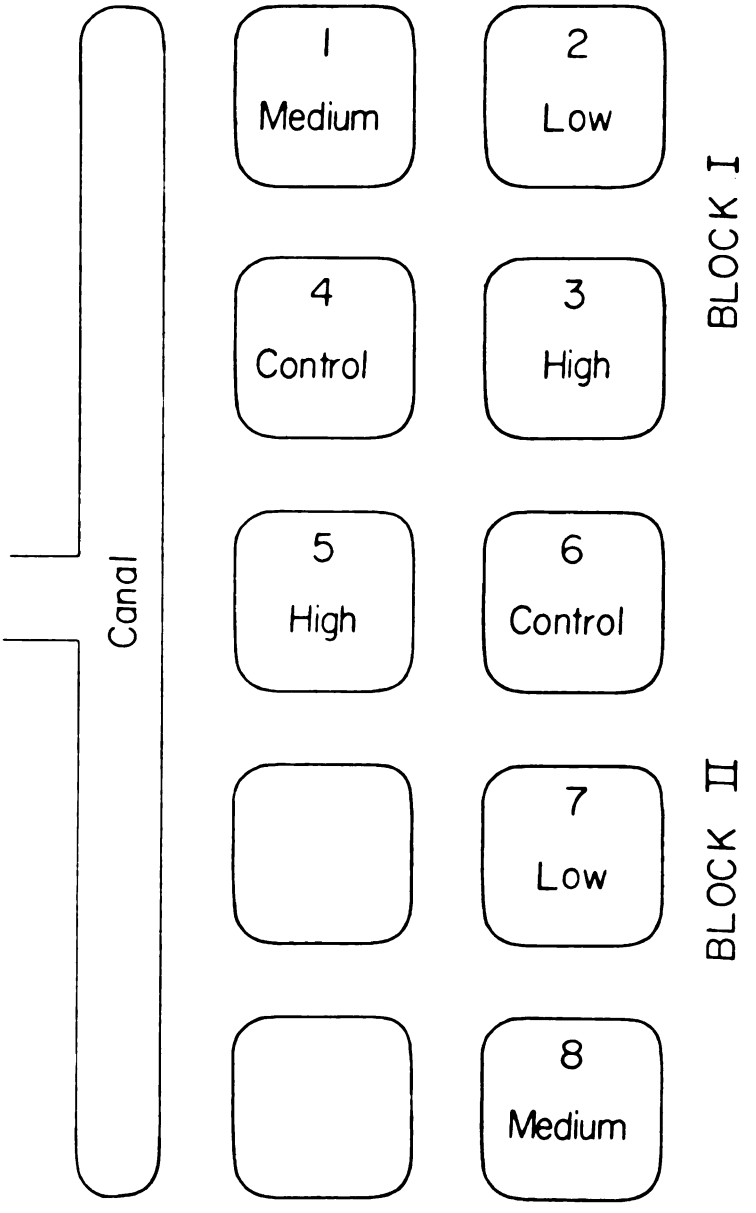
Experimental Design and Numerical Analysis

The pond nutrient treatments were organized in a randomized complete block design (Federer, 1955) with three levels of nutrient addition and a control, each replicated. Thus there were two blocks, an upper one and a lower one, and the ponds were numbered as shown in Figure 1. Ponds 4 and 6 were control ponds (here called 4 C and 6 C), ponds 2 and 7 were low nutrient level ponds (2 L and 7 L), ponds 1 and 8 were medium nutrient level ponds (1 M and 8 M), and ponds 3 and 5 were high nutrient level ponds (3 H and 5 H).

Usually an experimental design utilizing a block procedure is aimed at minimizing the effects of a known environmental gradient, and the blocks run along this gradient of assumed importance. In this case there was no a priori knowledge of an important gradient, and a determination of the concentrations of various elements made on 15 November 1967 showed no major differences among

Figure 1. Randomized complete block experimental design. Block I ponds 1-4; Block II ponds 5-8.

Figure 1.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3
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5
High
6
Control
7
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8
Medium
BLOCK I
BLOCK II

ponds (Table 1). However, a block design was used because of the arrangement of the ponds (Figure 1).

Most parametric statistical techniques have only limited use in analyzing sequential environmental or population data because the observed values on different sampling dates are not independent. Although summer averages are reported in several figures and tables, no confidence limits may be associated with these means because the autocorrelation between sampling dates violates the assumptions of parametric statistics. Block effects and asynchronous biological events within replicate ponds severely affected the magnitude of treatment mean variance for sequential data. This data is analyzed primarily for major, consistent trends. Ranges and means of chemical and biological parameters are reported to indicate the extent of within-pond and within-treatment variability throughout the summer.

Nutrient Treatment

Nutrients were added as high quality agricultural fertilizer purchased from the Agway farm supply store in Ithaca, New York. The treatment was a mixture of nitrogen, phosphorus, and potassium. The nitrogen source was ammonium nitrate fertilizer which is rated as 32.5 per cent nitrogen by weight. The phosphorus source was a fertilizer termed triple superphosphate, rated 20 per cent phosphorus by weight. The potassium source was potash

TABLE 1.--Photoelectric spectrometer analysis of important elements. Three dates: before fertilization, during the summer of 1968; and during the summer of 1969.

	15 November 1967				15 August 1968				10 August 1969			
	K	Na	Ca	Mg	K	Na	Ca	Mg	K	Na	Ca	Mg
Control Ponds												
#4	5.0	1.83	30.0	11.0	4.5	2.20	22.0	12.5	3.0	2.14	20.5	10.5
#6	5.0	1.13	26.0	8.0	5.0	1.58	23.5	11.0	1.3	1.44	21.5	10.0
Low Ponds												
#2	3.5	1.81	26.0	8.5	2.4	2.32	13.5	10.5	0.5	2.42	19.0	9.0
#7	4.5	1.59	26.0	9.5	ND	1.91	14.0	11.0	2.0	2.24	21.5	11.0
Medium Ponds												
#1	6.0	2.07	29.0	10.0	3.5	4.91	28.0	10.5	3.5	2.02	21.5	9.5
#8	4.5	1.19	26.0	7.0	4.5	1.55	28.5	8.5	4.5	1.66	25.5	6.0
High Ponds												
#3	5.5	1.53	33.0	9.0	8.0	1.84	28.5	8.0	6.5	2.02	30.0	6.0
#5	6.5	1.88	30.5	9.5	7.5	2.04	27.0	9.0	7.5	2.31	37.0	7.0

fertilizer rated 20 per cent potassium by weight. These three fertilizers were mixed in a ratio of 13 parts ammonium nitrate to 5.4 parts triple superphosphate to 1 part potash, a nutrient ratio of 8:2:1 (N:P:K) by weight which gives an atomic ratio of roughly 24 to 3 to 1. The fertilizer was mixed in 13.62 kg lots, and from this mixture appropriate amounts were taken and applied to the ponds.

The actual amounts added to the ponds per week were 1.36 kg, 3.63 kg, and 7.26 kg of the fertilizer mixture to create low, medium, and high nutrient treatment levels. See Table 2 for the amount of nutrients which would have resulted from these treatments if all the fertilizer went into solution and none of the nutrients was acted upon biologically or physically. The rates of addition were chosen such that a complete span of trophic conditions from the oligotrophic control ponds to a very eutrophic high level of nutrients would be created.

While the figures in Table 2 are expressed on a weekly basis, the fertilizer was actually applied three times per week, on Monday, Wednesday, and Saturday afternoons. In all cases, the fertilizer was added after water samples were taken for chemical analysis. The three times a week treatment schedule was begun on 7 June 1968 and continued until 28 August 1968; fertilizer was added 1 September, 6 September, 15 September, and 22 September 1968. The three times a week schedule was resumed on 4 June 1969 and terminated on 27 August 1969. To apply

TABLE 2.--Amount of fertilizer added at each treatment level and the concentration of each nutrient theoretically added each day.

	Amount of Fertilizer Added				Concentration of Each Nutrient Theoretically Added Each Day		
	lbs/pond/wk	kg/pond/wk	lbs/acre/yr	kg/ht/yr	N	P	K
Treatment Level:							
Low	3	1.36	144	161	53	13	6
Medium	8	3.63	384	430	140	35	16
High	16	7.26	768	861	281	70	32

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the fertilizer, 1/3 of each pond's weekly allotment was divided and mixed into four pails of water from that pond, then broadcast from each side of the pond.

Water Samples

All the water samples for water chemistry, chlorophyll *a* concentration, primary productivity measurements, and enumeration of phytoplankton species were taken using a column sampler designed and built of inert plastic tubing by Mr. deNoyelles. This sampler was usually lowered to within 8 cm of the bottom of the pond (1.37 m), enclosing about 700 ml of water. When water samples were taken for the chlorophyll determinations, the sampler was lowered to a depth of only 1.22 m.

The number of samples taken at a particular time and the size of the container used varied depending upon the analysis to be performed. However, the samples were always placed in a Nalgene screw cap bottle 0.94 l, 1.89 l, or 3.79 l in size and taken to the laboratory soon after collection.

A column sample was used to eliminate from consideration any vertical differences in distribution. Because of the almost constant breeze at the pond site, the ponds never thermally stratify for more than a few days, and then only during particularly calm, hot periods. For this reason, vertical heterogeneity was not thought to be important.

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Chemical Analysis

The reactive phosphorus content of all the ponds was determined weekly during the summer of 1968; five determinations were made throughout the fall, winter, and spring of 1968-1969; and determinations were made every two weeks during the summer of 1969. The method used to determine the amount of reactive phosphorus was that of Strickland and Parsons (1965) with a detection limit of 1.0 µg P/l. This technique utilizes the complexing of molybdic acid, ascorbic acid, and trivalent antimony with phosphorus which results in a blue solution the light extinction of which was measured with a Klett-Summerson photoelectric colorimeter using a red filter and a 2 cm cell. Solorzano and Strickland (1968) and Rigler (1968) have pointed out that this technique may include forms of phosphorus unavailable for plant nutrition. However, the measurement demonstrates major nutrient differences among treatments.

The reactive phosphorus content of the ponds varied from below detection in the control ponds up to 893 µg/l in one of the high nutrient ponds.

The inorganic nitrogen content of the pond water was determined for all three forms of inorganic nitrogen (nitrite, ammonia, and nitrate) each week during the summer of 1968, five times between September 1968 and May 1969, and every two weeks in the summer of 1969, on alternating weeks with the phosphorus measurements.

The nitrite procedure used was that of Strickland and Parsons (op. cit.) with a detection limit of 0.15 µg N/l, in which sulphanilamide complexes with nitrite to form an azo dye, the light extinction of which was measured with a Klett photometer using a green filter and a 2 cm cell. The nitrite concentrations varied from a low in the control ponds of 0.7 µg/l to a high in the high nutrient ponds of 100 µg/l.

The other forms of nitrogen were measured as nitrite. The ammonia content of the pond water was determined using the method given in Strickland and Parsons (op. cit.) with a detection limit of 1.5 µg N/l, in which ammonia is oxidized to nitrite by alkaline hypochlorite and determined as nitrite using the technique described above. The concentrations of ammonia ranged from a low of 5.5 µg/l in the control ponds to 280 µg/l in the high nutrient ponds.

The determination of the nitrate content of the pond water was made using the technique of Wood, Armstrong, and Richards (1967) and Strickland and Parsons (op. cit.) with a detection limit of 0.7 µg N/l. In this method, the sample is exposed to tetrasodium ethylenediamine-tetraacetate solution and passed through a column of copperized cadmium filings. During this procedure the nitrate is reduced to nitrite and is determined in this form using the method described above. To keep the readings within the range of detection of the instrument used, it was often necessary to dilute the high nutrient

pond samples with 0.0015 N HCl and adjust the values arithmetically. The amount of nitrate nitrogen in the water extended from less than 1.5 µg/l in the control and low treatment ponds to almost 1400 µg/l in the high treatment ponds.

At infrequent intervals throughout both summers and occasionally in the winter of 1968-1969, water samples were taken to the Cornell University Department of Pomology for analysis of the concentrations of various important elements (P, Ca, K, Mg, Na, Zn, Mn, Fe, Cu, and B) in the pond water; the measurements were made with a photoelectric spectrometer manufactured by Applied Research Laboratories, Inc., Glendale, California. The water samples were Millipore filtered and put in Nalgene containers before being sent to the Pomology laboratory for analysis. During the summer of 1968 and throughout the winter, the Millipore filters used were not pre-rinsed to remove possible contaminants. The pre-rinsing procedure was instituted for all the sampling dates during the summer of 1969 but seems to have been an unnecessary precaution. See Table 1 for a summary of some of these data.

The hydrogen ion concentration of the water was measured in conjunction with a determination of alkalinity. During the summer of 1968 both these factors were determined infrequently. During the summer of 1969 they were determined weekly between 10:30 a.m. and 11:30 a.m.

in conjunction with carbon-14 primary production measurement. pH was measured using a Corning Glass electrode pH meter (Model 7). Because of the general basic nature of the ponds, the meter was consistently calibrated using a buffer of pH 10. The control ponds generally maintained a pH from 8.8 to 9.3 while the high and medium ponds occasionally reached a pH of 10.6 to 11.0.

The alkalinity of the ponds was always measured at the same time as the hydrogen ion concentration was determined. The method used was that described in Standard Methods for the Examination of Water and Wastewater (1965): titration with 0.02 N H_2SO_4 to the color change of phenolphthalein and methyl orange. The phenolphthalein alkalinity was at times non-existent in the control and low ponds, and ranged up to 1 meq/l weak acid salts (largely HCO_3^- and CO_3^{--}), often expressed as 50 mg $CaCO_3$/l, in the high nutrient ponds. The methyl orange alkalinity varied from 1.2 meq/l weak acid salts (60 mg $CaCO_3$/l) in the lower treatment level ponds to an occasional maximum of 2 meq/l weak acid salts (100 mg $CaCO_3$/l) in the high nutrient ponds.

The oxygen concentration of the ponds was occasionally determined during the morning using the Azide modification of the basic Winkler technique as described in Standard Methods for the Examination of Water and Wastewater (op. cit.). Because the ponds are shallow and mix almost

continuously, the oxygen concentration was generally very close to saturation, or greater than 8 mg O_2/l.

Water temperature was measured at about 11:00 a.m. once a week during the summer of 1968, and varied from 22° to 30°C with no difference among ponds nor among depths within ponds. In 1969 a maximum-minimum thermometer was placed in pond 4 C and read daily in the morning. The summer range was 20° to 30°C with the daily range never exceeding 5°C.

Plant Pigments

The chlorophyll *a* concentration of the pond water was determined weekly from 11 July to the end of August 1968, and weekly from the beginning of June to the end of August 1969. The basic procedure used was very similar to that described in Strickland and Parsons (*op. cit.*), but several important changes were made at various intervals throughout the study. The procedure was to filter the phytoplankton from the water, extract photosynthetic pigments using 90 per cent aqueous acetone, and measure the light extinction at various wavelengths of light as specified in Richard's equation (Strickland and Parsons, *op. cit.*) using a Coleman Hitachi 101 spectrophotometer. At the same time, the light extinction at 480 nm was measured, and using the equation given by Strickland and Parsons, the carotenoid concentration was determined.

For the first four weeks of chlorophyll determinations in 1968, Millipore filters and an 18-hour extraction period were used. Because the Millipore filters tended to become cloudy in acetone thus greatly reducing the sensitivity of the technique, Gellman glass fiber filters were used in later determinations. These filters must be ground up in a tissue homogenizer and the suspended glass fibers removed by centrufugation. Because the grinding of the glass filters completely ruptures the phytoplankton cells, the extraction time was shortened to approximately 1/2 hour. This greatly enhanced the precision of the technique, but on occasion the variance of replicates within a pond was large and appeared to be due to occasional contamination of the sample with periphyton algae from the bottom of a pond. Therefore, on and after 16 August 1968, the column water sampler was lowered to within 15 cm of the bottom of the ponds instead of to within 8 cm of the bottom. The number of samples taken per pond was also increased from two to three. On 30 August 1968 the light path length in the spectrophotometer was changed from 1 cm to 2 cm by using larger cuvettes. The method then remained unaltered throughout the summer of 1969.

The amount of pond water filtered varied greatly depending upon the amount of suspended material in the water. It was never more than 1 liter nor less than 50 ml.

The chlorophyll a concentrations averaged about 10 to 20 mg/m^3 in the control ponds and generally increased with increasing treatment level to a high value of almost 1000 mg/m^3 in ponds 3 H and 5 H at the end of August 1969.

Primary Productivity

During the summer of 1969, the carbon-14 primary production was measured weekly using the technique of Steemann Nielsen (1952) with modifications suggested by Vollenweider and Nauwerck (1961) and Wetzel (1964). The procedure used to fill the incubation bottles was designed to assure an even distribution of pond water and tracer among the bottles used for a particular pond. First, several column samples were taken from a pond and mixed together. This water was then put in an opaque 2 l separatory funnel to which was added an appropriate amount of carbon-14 in the form of sodium bicarbonate (New England Nuclear Corp., Boston, Mass.). The formula for adding the water and tracer was as follows: 250 ml of pond water and 1 ampoule of 1 ml of 50 µg sodium bicarbonate in sterile distilled water at pH 9.5 at 5 µcurie activity for every two bottles to be incubated. The maximum volume of the BOD bottles used was about 130 ml, but they were filled only to the 100 ml mark. In a typical pond, six bottles would be filled, two to be incubated at a depth of 0.31 m, two incubated at a depth of 0.92 m, and two dark bottles incubated at a depth of 0.31 m.

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At times more than six bottles were used per pond. In the late summer in ponds 3 H, 5 H, and 8 M the phytoplankton was so dense that an extra set of bottles was incubated at a depth of 5 cm in order to obtain an estimate of the effect of extreme light extinction on production. Additional bottles were also used for a series of experiments in which a nutrient or nutrients thought likely to be limiting were added to pond water and the uptake of the tracer compared with a control of unaltered pond water. The only change in procedure for these bottles was mixing a 1 ml nutrient solution with the pond water at the time of filling.

As soon as a bottle was filled, it was placed in a dark box. When all the bottles for a particular pond were filled, they were immediately taken to that pond and incubated by suspending them to the appropriate depth from a permanent crosspiece in the pond.

The experiments were begun at about 8:30 a.m., bottles were incubated in the first of the ponds by 8:45 and in the final pond usually by 10:30 and never later than 11:00 a.m. The bottles were removed beginning at 2:00 or 2:30 p.m. and 1 ml of 10 per cent formalin was added to stop carbon fixation. This procedure took about 1/2 hour. Thus the incubation period of the bottles in different ponds varied from 3 1/2 to 5 1/2 hours, as suggested by Barnett and Hirota (1967).

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Due to the need for rapid placement of the bottles in all the ponds so as to assure similar incubation conditions, bottles were incubated in ponds in the most efficient possible order, one of two linear series running from pond 1 M to pond 8 M, or pond 8 M to pond 1 M, decided randomly each week. The bottles were always removed in the same order in which they were suspended in order to reduce any effect due to time of day of incubation. Water samples for pH and alkalinity determinations were taken during the incubation period.

The volume of carbon-14 labeled sample filtered (47 mm HA Millipore filter) varied with the amount of phytoplankton in the pond, but was consistent for all the samples from that pond on that day. The volume varied from 50 to 100 ml. Occasionally only 4 ml from a particular bottle were filtered and compared with a larger volume filtered to check for decreasing measurable radioactivity as a function of increasing volume filtered (Arthur and Rigler, 1967). This same procedure should demonstrate increasing color or chemical quench with increasing amount of cellular material on the filter, as predicted by Pugh (1970). As there was no significant difference in the radioactivity measured per unit volume between large and small volumes filtered, neither of these potential errors seems to have been large in the present technique. The funnels were rinsed with 10 ml of distilled water, and 10 ml of 0.003 N HCl was passed through the filters at the

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very end of filtration. The filters were then treated in a fashion similar to that of Lind and Campbell (1969). They were placed in 20 ml liquid scintillation vials which were desiccated over Drierite ($CaSO_4$) for two days. Wallen and Geen (1968) report loss of radioactivity during the drying process, but offer no easily adaptable alternatives. After the filters were dry, the vials were filled with a toluene-based scintillation fluid: POPOP [1, 4-bis-2-(5-phenyloxazolyl)-benzene] and PPO (2, 5-diphenyloxazole). They were placed in a Model 6850 Unilux Liquid Scintillation System (Nuclear Chicago, Chicago, Ill.), and each vial counted twice for a period of 10 minutes each time. Using the channels ratio technique (Wang and Willis, 1965) to determine the counting efficiency, the amount of radioactivity on each filter can be expressed as the number of disintegrations per minute. With this information and knowledge of the amount of total carbon in each pond, the formula proposed by Saunders, Trama, and Bachmann (1962) can be used to determine the productivity.

$$P = r/R \times C \times f$$

where

P = photosynthesis in mg C^{12} per cubic meter

r = uptake of radioactivity in disintegrations per minute

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R = total available radioactive carbon in disintegrations per minute

C = total available stable inorganic carbon in mg per cubic meter

f = isotope correction factor (1.06)

The radioactivity of the two dark bottles was averaged and subtracted from the r value in the above formula. The method of determining C is as described in Saunders, Trama, and Bachmann (op. cit.). This formula yields results in terms of carbon fixed per cubic meter, which can be expressed as carbon fixed per square meter of pond surface by extrapolating the values determined at 0.31 m and 0.92 m from the surface to the bottom of the pond on the basis of a logarithmic extinction curve. When extra bottles were incubated at 5 cm the values obtained agreed well with an extrapolation of such a curve.

The primary production varied immensely over the difference treatment levels. Carbon fixation in the control ponds averaged about 20 mg $C^{12}/m^3/hr$ and generally increased with increasing treatment level to a high value of over 2000 mg $C^{12}/m^3/hr$ in pond 5 H on 20 August 1969.

Phytoplankton Enumeration

Column water samples for the enumeration of phytoplankton species were taken from the ponds and thoroughly mixed in a container by a gentle whirling motion. Two 50 ml samples were then taken and preserved, one with

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5 per cent formalin and glacial acetic acid and one with 1 per cent Lugol's iodine solution. Two preservatives were used due to their different disruptive properties: formalin and glacial acetic acid may destroy small flagellates while Lugol's may break the colonial organisms (colonical Volvocales, Syncryta, and Uroglenopsis) into single cells. The sampling and enumeration were performed by Frank deNoyelles (1970). The technique (deNoyelles, 1968) consisted of filtering previously stained organisms onto an MF Millipore AA filter (0.8 μ pore size) using a Millipore Swinny filter holder. This filter with the retained organisms was then mounted on a glass slide and the algae counted at 788 magnifications. The volume filtered varied from 0.5 ml to 6 ml depending on the density of the phytoplankton in any given sample; to keep constant the "effective pond volume observed" (0.2 ml), the area of the filter observed was a function of the amount of water filtered.

The mean dimensions of most of the phytoplankton species were measured from samples preserved in Lugol's solution which may cause some slight cell shrinkage, especially of some of the Cryptomonadales. By applying some assumptions about the geometric shape of an organism, these measurements can be converted to average cell volume (Table 3).

TABLE 3.-

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Cosmariu Probably
Cryptomo Ehrenber
Cryptomo Skuja
Cryptomo Bachmann
Erkenia Skuja
Eudorina Ehrenber Colony eaten
Euglena Quite l food
Kirchner (Kirchne Colony 250 µ i inedible

TABLE 3.--Calculated cell volumes of phytoplankton species present in pond 8 M 1968.

Species	Dimensions (μ)	Volume (μ^3)
Ankistrodesmus falcatus (Corda) Ralfs	cell actually 3 dia. 72 long but pointed; assume 3 dia. and 60 long	420
Chlamydomonas pertusa Chodat	cells 8.8 dia. 12.3 long	570
Chlamydomonas Reinhardtii Dangeard	cells 4 dia. 6 long	58.6
Chlamydomonas sp.	cells 4.5 dia. sphere	47.7
Chlorella sp.	cells 4.0 dia. sphere	33.5
Chroomonas caudata Geitler	cells 4.2 dia. 11.5 long, flattened ovate (1/2 ovate)	70
Cosmarium sp. Probably not edible	cell a flattened plate assume 26 dia. and 4 thick	2,123
Cryptomonas erosa Ehrenberg	cells 14.5 dia. 32.8 long, ovate	4,618
Cryptomonas marssonni Skuja	cells 8 dia. 18 long	770
Cryptomonas pulsilla Bachmann	cells 4 dia. 9.3 long	100
Erkenia subequiciliata Skuja	cells 4 dia.	33.5
Eudorina elegans Ehrenberg	cells 15 dia.	1,767
Colony too large to be eaten	colony (assuming 32 cells/colony)	56,551
Euglena sp. Quite large; marginal food	cells 20 dia., 68 long	19,000
Kirchneriella lunaris (Kirchner) Moebius	cells 2 dia. 16	48.2
Colony may be 100 to 250 μ in diameter; inedible	(straightened) colony (assuming 16 cells/ colony)	770

TABLE 3.--(cont'd.).

Species	Dimensions (μ)	Volume (μ^3)
Microcystis aeruginosa (Kuetz.) Elenkin Colonies too large to be eaten	cells 4 dia. sphere	33.5
Oocystis parva West and West Colony may be 44 μ in diameter; may just barely be edible	cells 8 dia. 12 long colony (assuming 4 cells/colony)	469 1,876
Pandorina morum Bory Probably too large to be edible	cells 15 dia. colony (assuming 16 cells/colony)	1,766 28,266
Pediastrum boryanum (Turp.) Meneghini Too large to be eaten	assume a plate of cells 80 dia. and 15 in depth/2	37,700
Peridinium gatunense (Nygaard) Not edible	cells (assume a sphere 46 dia.)	50,900
Peridinium palatinum Lautenborn Not edible	cells (assume a sphere 44 dia.)	44,600
Pleodorina californica Shaw Colony much too large to be edible	vegetative cells 10 dia. reproductive cells 34 dia. colony (assuming 64 veg. cells/ colony and 64 repr. cells/colony)	523 20,574 1,350,000
Scenedesmus abundans (Kirch.) Chodat	cells 5 dia. 12 long	202.9
Scenedesmus bijuga (Turp.) Lagerheim	cells 6 dia. 12 long	282.7
Scenedesmus dimorphus (Turp.) Kuetzing	cells 5 dia. 18 long	320.7
Scenedesmus quadricauda (Turp.) deBrebisson	cells 8 dia. 15 long	619.9
Schroederia Judayi (G. M. Smith) Spines so long it may be inedible	cell of peculiar shape; assuming an oval 4 dia. 30 long	360.2

TABLE 3.--(cont'd.).

Species	Dimensions (μ)	Volume (μ^3)
Tetraspora lacustris Lemmermann	colony 23 dia. sphere	6,371
Uroglenopsis americana (Calkins) Lemmermann Colony diameter may reach 500; inedible	cells 5 dia. colonies with perhaps 200 cells	65.4 13,000
Volvox aureus Ehrenberg Not edible	cells 5 dia. colony (assuming 1000 cells/ colony)	65.4 65,400
Volvox globator Linnaeus Not edible	cells 3 dia. colony (assuming 10,000 cells/colony)	14.1 141,000
Misc. ciliates	cells roundish 20 dia.	4,200
Misc. flagellates	cells 2 dia. 4 long	10.5

The abundances of phytoplankton cells varied from a low value of less than 1000 cells/ml to over 900,000 cells/ml in the high nutrient ponds.

Particle Size Analysis

During the summer of 1969, samples were taken twice weekly for particle size analysis using a Coulter Counter. The water samples were taken with a sampler which could be swung out over the pond and which takes a 0.7 m deep column sample. Samples were taken from each of the four sides of the ponds; then two were mixed together to give two samples per pond. These were than taken directly to the laboratory. The means of analysis was a Model B Coulter Counter using a 100 μ orifice which can effectively size and count particles within a 2 to 50 μ size range. Various size ranges were selected and the total number of particles within each was recorded. The size ranges, selected to encompass the most common phytoplankton species, were 3 to 6 μ, 4 to 6, 6 to 12, 12 to 24, and 24 to 40.

Zooplankton Enumeration

Zooplankton were sampled using a 0.92 m long #20 plankton net (100 μ mesh opening) mounted on a brass ring 0.31 m in diameter which was attached to a sturdy pole. A #20 plankton net will retain all the crustacean zooplankton and many of the common rotifers.

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The general sampling design was fairly simple. The pond is considered to consist of three corridors running north and south and three corridors running east and west. Each corridor is assigned a number, 1 through 3, from left to right as one faces south and east. On a given sampling date, two random numbers from 1 to 3 are chosen per pond. These numbers specify which corridor in each direction is sampled. Thus on each sampling date, two discrete samples were taken from each pond with the direction of the tows at right angles.

At the time of sampling a small boat was rowed backwards across the pond. The net was held at the back and starting at one edge of the pond was raised and lowered in a sine wave-like pattern to the opposite shore. The ponds are square, 27.45 m on a side, and one may therefore assume a column of water 0.31 m in diameter and 27.45 m in length has been filtered.

As the cladoceran zooplankton have a strong negative phototaxis and clump just above the bottom throughout the day, all sampling was carried out at night, commencing at about 1/2 hour after sunset. The ponds were sampled weekly from June through August and at less regular intervals throughout the year. When there was ice on the ponds, a vertical column was taken during the day by lowering the net to the bottom, leaving it there for about 5 minutes, and rapidly hauling it to the surface. The only other deviation from the procedure described is that

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on occasion large phytoplankton became so abundant in a particular pond during the summer that an across-pond tow would completely clog the net. It was then necessary to take shorter, vertical tows, here called hauls. In this situation, the boundaries of the corridors were considered as describing a grid of 9 small squares numbered 1 through 9 from left to right starting from the southeast corner. To determine sampling points, two random numbers from 1 to 9 were selected; these represented squares in which a haul was taken. The contents of both hauls were placed in one jar and were considered one of the two replicate samples from that pond. All the samples were preserved in a mixture of 95 per cent alcohol and 2 per cent formalin.

To enumerate the zooplankton, a 1 ml subsample was taken from the original sample jar by means of a calibrated glass tube. A subsampling sequence proceeded as follows: the sample jar was vigorously shaken and a 1 ml volume withdrawn. This was then placed on glass slides having three depressions, and the tube was rinsed with a soap solution. More soap solution was added to each depression to reduce the turbulence created by the rapid evaporation of the alcohol. The slides were allowed to stand for about 1/2 hour in a humid chamber to allow the organisms to settle for easier counting. The crustaceans and large rotifers were enumerated with an M-5 Wild dissecting microscope at either 12 X or 25 X magnification.

From each sample the number of individuals and eggs, carried or loose, present for each species of crustacean zooplankton, and the number of eggs carried by each Ceriodaphnia reticulata individual were counted. Other than Asplanchna sp., rotifers were not routinely counted, since they generally made up only a small percentage of the zooplankton biomass and rarely numbered more than 200 per liter. A similar finding is reported by Hall et al. (1970) in ponds lacking fish.

Two different methods of crustacean zooplankton sample enumeration were followed. The more common was to count a subsample taken from one of the two samples from a particular pond on a specific date. The other type of sample enumeration was an averaging procedure in which 3 ml from each sampling date of the summer, taken randomly from one of the two jars for each date, were mixed and the zooplankton counted. A 3 ml sample from each of the remaining jars was also taken and these were mixed. Then a 1 ml subsample was taken from the pooled samples and counted. Any sampling dates involving vertical hauls were pooled and counted separately, then combined with the full tow data through weighted averages. In this way an estimate of average standing crop of zooplankton throughout the summer was obtained.

The pooled estimates of average zooplankton biomass were compared with arithmetic averages of zooplankton biomass in the five cases in which a complete summer's

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sequence of weekly samples was counted. A Wilcoxon's Signed Rank Test showed no significant difference between the two methods at the 5 per cent confidence level.

Zooplankton Dry Weight Measurements

The dry weight biomass of different size categories of both Ceriodaphnia reticulata and Daphnia pulex (Leydig) Richard were determined using a Cahn Electrobalance. Lengths of the animals were measured from the front of the head to the posterior of the carapace, excluding the posterior spine in the case of D. pulex. Such data normally follow an allometric growth curve of the form,

$$\text{Weight (W)} = \text{constant} \times \text{length (L)}^{\text{exponent}}$$

which is a straight line using a logarithmic transformation. The regression equation fitted to the data by the least squares method, for C. reticulata is

$$\log_{10} W = \log_{10} 21.6 + 3.29 \log_{10} L$$

with a coefficient of determination (r^2) of 0.96, and for D. pulex is

$$\log_{10} W = \log_{10} 6.99 + 2.72 \log_{10} L$$

with a coefficient of determination (r^2) of 0.98. Knowing these relationships and the size frequency distribution of each species in a particular pond at a specific time, it is possible to estimate the average weight of each species.

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This value may then be multiplied by the number of each species present to determine the biomass per liter of each species. In the case of the pooled samples, an average weight of C. reticulata (1.64 µg ± s.e. 0.088) was determined from all the pond samples (a total of 73) counted up to that particular time, and this value was multiplied by the number of C. reticulata counted in each of the pooled samples. For D. pulex the average weight (10.51 µg ± s.e. 1.849) was determined from 11 samples.

Laboratory cultures were used to find the dry weights of both species. This was done not only for convenience but also with the assumption that the weight per unit length within a species is independent of pond or population history. This would seem to be justified since the above equation for D. pulex agrees quite well with the data given by Burns (1969). No such comparison is available for C. reticulata.

For dry weight measurements, animals of a particular size class were selected using an optical micrometer in an M-5 Wild binocular dissecting scope and transferred to a tared aluminum pan containing a drop of triple distilled water. Once a sufficient number of animals of a particular size were on the pan, most of the water was removed using a hypodermic needle and syringe, and the pans were placed in a 51°C oven. After two days the pans were removed from the oven and placed in a desiccator over Drierite ($CaSO_4$) for a day. Finally the pans were weighed using a

Cahn Electrobalance set at the 1 mg level. Dry weight values for other species were taken from Hall et al. (1970) and multiplied by the number of individuals of that species occurring at a particular time.

Filtering Rate Measurements

In order to estimate the grazing impact of the zooplankton on the algae, an estimate is needed not only of the zooplankter's abundance but also of its feeding rate. A measure of filtering rate of C. reticulata, the dominant zooplankter, was undertaken using a modification of a technique developed by Rigler (1961), and specifically Burns and Rigler (1967). The main difference between the method used in the present experiment and that of Burns and Rigler was the use of carbon-14 and a liquid scintillation counting procedure rather than phosphorus-32 and a planchet counting method.

A group of animals was taken from a laboratory culture and placed in a plastic tube 3 cm in diameter and 8 cm long which had #20 netting on one end. The tube was then suspended in a plastic beaker 7 cm high containing about 100 ml of medium. This arrangement facilitated rapid transfer of the animals to various labeled and non-labeled foods. For a 1 hour pre-feeding period, the animals in the tube were placed in a beaker containing the same type and concentration of food that was to be tested. The animals were then transferred to a similar beaker in which

labeled food was suspended. They were left in this container for 2 minutes, a necessarily short period of time to prevent defecation of labeled food (Bourne, 1959). The time was determined by actual observation to be short enough that the animals feeding on different foods and concentrations of food did not completely fill their guts in 2 minutes. It was also observed that the amount of measurable radioactivity per unit time increased at exposure times of 1/2 hour, 5 minutes, and 2 minutes.

The test algae used were always either a pure culture of carbon-14 labeled Ankistrodesmus falcatus (Corda) Ralfs or 2 ml of carbon-14 labeled A. falcatus suspended in 98 ml of water from a specific pond. The pure cultures of A. falcatus varied in density from 4,200 to 220,000 cells/ml and were labeled by exposing 500 ml of this culture to 1 ml of 20 μcurie sodium bicarbonate for 12 hours under strong fluorescent illumination. The A. falcatus to be suspended in the pond water was prepared at a concentration of 100,000 cells/ml and labeled in the manner described above. Two ml of this labeled culture solution was always added to the pond water just before the animals were placed in the test algae solution.

After 2 minutes exposure to labeled algae, the animals were returned to the unlabeled food for 1 minute so they could ingest or reject radioactive food present in their food grooves. Then they were plunged into a carbonated water solution (club soda) used as a narcotic,

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transferred to a very dilute acid rinse and back to the club soda. The tube was then removed from the water and the plankton netting, now containing all the animals, was removed from the bottom of the tube and placed under an M-5 Wild microscope. A number of animals of a specific length were picked from the plankton netting and placed in a small container of distilled water. When a sufficient number of animals of a particular size had been collected, the animals were transferred to a liquid scintillation counting vial using a pipette. As water severely quenches toluene based liquid scintillations, it was important to remove as much of the water as possible. This was done by placing the open vials in a drying oven set at 60°C and frequently observing the amount of water driven off. Not all the water was removed, as the Nuclear Chicago NCS animal tissue solubilizer requires that just a trace of water be present in the tissue and on the tissue surface. When most of the water was removed, 1 ml of NCS was added to the vials, the caps were put on, and the vials were placed in the drying oven. After a 24-hour digestion period, the vials were removed from the oven and allowed to cool to room temperature before the toluene based counting fluid was added. The vials were then placed in complete darkness for 6 to 7 days as experience showed that the NCS and counting fluid were severely affected by fluorescent lighting. It took 6 or 7 days for the extraneously induced fluorescence to disappear. The vials

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were then placed in a liquid scintillation counter with the room lights completely off. The samples were counted using the channels ratio method. Both channels were counted twice for 10 minutes and an average was taken.

At the time the animals were removed from the labeled food, a 50 ml sample of the food was Millipore filtered after having been preserved with formalin. These filters were treated in an identical fashion to those of the primary production study.

Using the channels ratio method to correct the measured counts per minute to disintegrations per minute (dpm), the absolute amount of radioactivity in the algae ranged from 1,000 to 15,000 dpm and averaged 10,000 dpm per 50 ml. Knowing the amount of radioactivity in 1 ml of the original algal test solution, and the amount of radioactivity picked up by the animals in a 2 minute exposure to the algae, the amount of algae eaten by the animals can be calculated, and expressed as volume of water filtered per unit time.

In the case of the algae, the level of labeling was such that any background radiation was negligible in comparison to the amount of radiation in the algae themselves, and no correction was made. In the case of the animals, this was far from true. A good percentage of the radioactivity recorded from a counting vial containing perhaps 25 animals which had been exposed to labeled algae for

2 minutes was extraneous and not due to the consumption of the labeled algae. This background activity may come from small quantities of radioactive isotopes already in the bodies of the animals, the counting fluid, or the counting vial itself. It can also come from room, terrestrial, and cosmic radiation striking the recording sensors.

A background radiation value for the animal vials was determined by treating a series of animals exactly as has been described above except that rather than being exposed to labeled algae the animals were exposed to the labeled culture of algae from which all particulate material had been removed by Millipore filtration. The animals were exposed to this water for 2 minutes and in every way treated as described above. Presumably this method would expose the animals to all the various organic by-products which the algae might have formed and excreted into the culture water during the 12 to 18 hour labeling time. However, in another measurement of background radiation, in which the animals were exposed to 20 μcuries of bicarbonate carbon-14 diluted in 500 ml of Millipore filtered tap water, an almost identical background activity value was determined. This value was 70.8 dpm. Because the tissue solubilizer and the toluene counting fluid alone accounted for 90 per cent of the background radiation, the number of animals, which varied only slightly from experiment to experiment, was not considered in these calculations, and the background was

considered constant. No correction was made for self-absorption, which is consistent with the data of Ward, Wong, and Robinson (1970).

There are three major factors that can be important in applying lab-derived filtering rates to field populations: water temperature, concentration of particles in the water, and the size of the animal considered (Schindler, 1968; Burns, 1969). In the present study, laboratory experiments were performed at or near pond temperatures, and Burns (op. cit.) has shown that slight variation at this temperature range has little effect on filtering rate.

Four times during the summer of 1969, the filtering rate of Ceriodaphnia reticulata was measured in water from four ponds which differed greatly in the abundance of particles within the size range 3-24 μ. Using a logarithmic transformation, the Ceriodaphnia filtering rates with increasing particle concentration fit a straight line (Figure 2) with an equation

$$\log_{10} \text{F.R. (0.8 mm animals)} = 2.4405 - 0.734 \times \#\ \text{particles (3-24 } \mu\text{)} \quad (1)$$

The data fit the line with a coefficient of determination (r^2) of 0.96. This equation predicts the filtering rate of animals 0.8 mm in length in water containing from 14,000 to 500,000 particles/ml between the sizes of 3 and 24 μ. As no data were gathered at concentrations lower

Figure 2. Relationship between the log of the filtering rate of Ceriodaphnia reticulata and the log of the number of particles in the water. The straight line is fitted by least squares for only the pond water estimates of filtering rate.

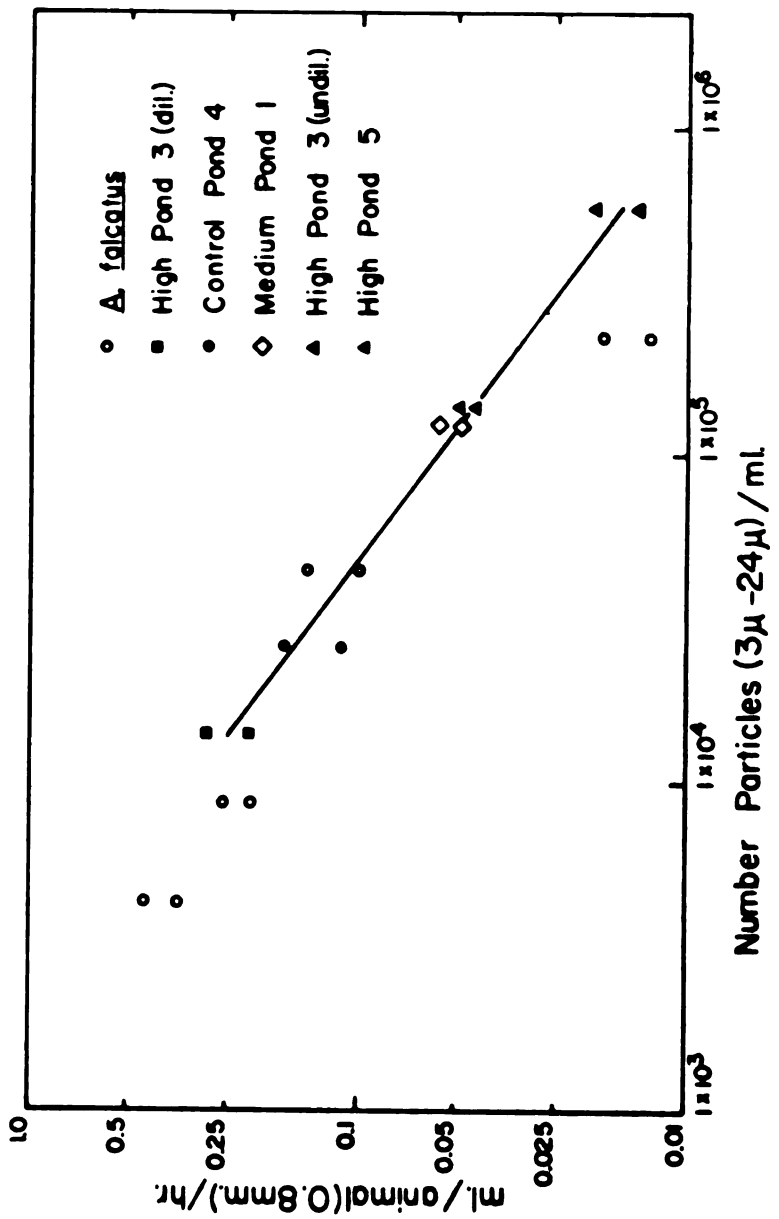
A. falcatus
High Pond 3 (dil.)
Control Pond 4
Medium Pond 1
High Pond 3 (undil.)
High Pond 5
ml./animal(0.8mm.)/hr.
1.0
0.5
0.25
0.1
0.05
0.025
0.01
1x10³
1x10⁴
1x10⁵
1x10⁶
Number Particles (3μ-24μ)/ml

Figure 2.

than 14,000 particles/ml in the present study, it was assumed that the filtering rates remained constant below this point. This assumption was conservative in that it presumed a filtering rate lower than that which would be obtained by a strict extrapolation of the equation.

The size structure of the pond population must also be taken into account. Burns (op. cit.) has shown that the filtering rates of several species of Daphnia increase with increasing body length as a power function. However, she determined this relationship at very low food concentrations, when the filtering rate is at its maximum. In the present study the relationship between body length and filtering rate was determined over a wide range of food concentrations. Such data generate a family of curves with y intercepts which vary as a function of the number of particles in the water. Logarithmically transformed, the slopes of these lines varied from 0.88 to 5.2 with a mean of 2.9. This mean value is quite similar to the range reported by Burns (op. cit.). A generalized filtering rate curve was generated using this average slope

$$\log_{10} \text{F.R.} = 0.28103 + 2.9 \times \log_{10} \underset{\text{body length (mm)}}{\text{Ceriodaphnia}} \qquad (2)$$

In this equation the filtering rate for animals 0.8 mm long equals 1.0 ml/hr. Filtering rates for each size category of animal were obtained using the above equation,

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young

Nalge

volum

then multiplied by the percentage frequency distribution to derive the average generalized filtering rate for a particular pond population. This value was then multiplied by the value obtained from equation (1) for the appropriate number of particles/ml present in the pond. To obtain the total Ceriodaphnia filtering rate per liter per hour, the above value was multiplied by the number of Ceriodaphnia/l. Assuming that the filtering rate remains constant throughout the day (Schindler, op. cit.), the hourly value was multiplied by 24 to obtain a total daily filtering rate.

Ceriodaphnia Life Tables

A series of life table experiments was performed during the late summer of 1969 to test pond 8 M water for possible toxic factors.

In order to get animals of almost the same age, embryo-carrying animals were isolated in the medium in which the young animals were to be raised. The embryo-carrying animals were observed at short intervals throughout the day, and any newborn animals removed and discarded, until a time interval occurred in which enough newly born animals were present for the experiment. In such a way animals of almost identical age could be obtained. These young were immediately placed in rearing containers, Nalgene #10 hollow stoppers of approximately 20 ml in volume, which contained the medium to be tested.

There were several media for growing the young animals: pond 8 M water, Millipore filtered pond 8 M water with Ankistrodesmus falcatus added, pond 8 M algae removed from the pond water through centrifugation and resuspended in Millipore filtered dechlorinated tap water, and Millipore filtered dechlorinated tap water with A. falcatus added. The pond water was collected in the morning using the column sampler. The amount of A. falcatus to be used was determined by measuring the light absorbance of a stock culture and diluting the culture to the desired concentration (10 Klett units or about 100,000 cells/ml). The pond algae were separated from the pond water using a Foerst Continuous Centrifuge, and the algae were resuspended in Millipore filtered dechlorinated tap water. The water to be tested was made up and placed in rearing containers and the animals transferred daily to fresh media. At the time of transfer any mortality or reproduction was noted. Every other day the animals were removed with a pipette, placed in a depression slide from which the water was withdrawn so as to immobilize the animals, and the length of the animals was measured using an ocular micrometer with an M-5 Wild dissecting microscope. The animals were then placed in the fresh medium with one animal to a container; all the containers were arbitrarily positioned in an enamel tray and placed in a dimly lit chamber at a constant 21°C.

EFFECTS OF FERTILIZATION ON NUTRIENT LEVELS

The addition of varying levels of fertilizer to ponds does not necessarily result in the creation of distinct nutrient conditions. It is possible that fertilizer added to ponds does not become available for plant growth. This would invalidate any investigation as to differential nutrient impact on the phytoplankton and zooplankton. There are many documented cases in which fertilizers have been added to small lakes and little or no change has resulted (M. W. Smith, 1969). Often when fertilizer is added, the concentration dissolved in the water very quickly decreases to pre-treatment levels (Einsele, 1941). This decrease is often thought to be due to a combination of factors: physical adsorption (Holden, 1961), chemical precipitation, and biological absorption (Hepher, 1966). To determine if a significant fraction of the nutrients added in the present study were in solution and therefore presumably available for plant growth, the dissolved inorganic nitrogen and reactive phosphorus contents of the pond water were measured frequently.

Figure 3, a plot of the inorganic nitrogen treatment means, shows that the high and medium treatments were decidedly different during the summer of 1968, but the low and control treatments were indistinguishable from each other. In the summer of 1969 there was a separation of all four treatments. The same figure also shows that even the high treatment level ponds returned to near control levels of inorganic nitrogen concentration by the spring of 1969, and then quickly responded to the addition of fertilizer that summer.

Figure 4 shows the reactive phosphorus treatment means as measured during both summers and a few times during the winter. The response of the phosphorus was much like that of inorganic nitrogen; the low and control treatments were indistinguishable, but the medium and high treatments showed definite separation during the first summer. In the second summer there were four very distinct levels of phosphorus concentration. Unlike inorganic nitrogen, not all treatment level ponds returned to control levels by the spring of 1969. The high ponds remained at a constant level throughout the winter while the phosphorus concentrations in the medium and low ponds fell to control levels.

To summarize the reactive phosphorus and inorganic nitrogen responses to the fertilization regime: there were three measurable and distinct chemical conditions in 1968,

Figure 3. Inorganic nitrogen treatment means throughout the study. The arrows on the abscissa mark the periods of fertilization. The arrows on the ordinate mark the 200 and 500 µg/l level. B.D. indicates below detection.

Figure 3.

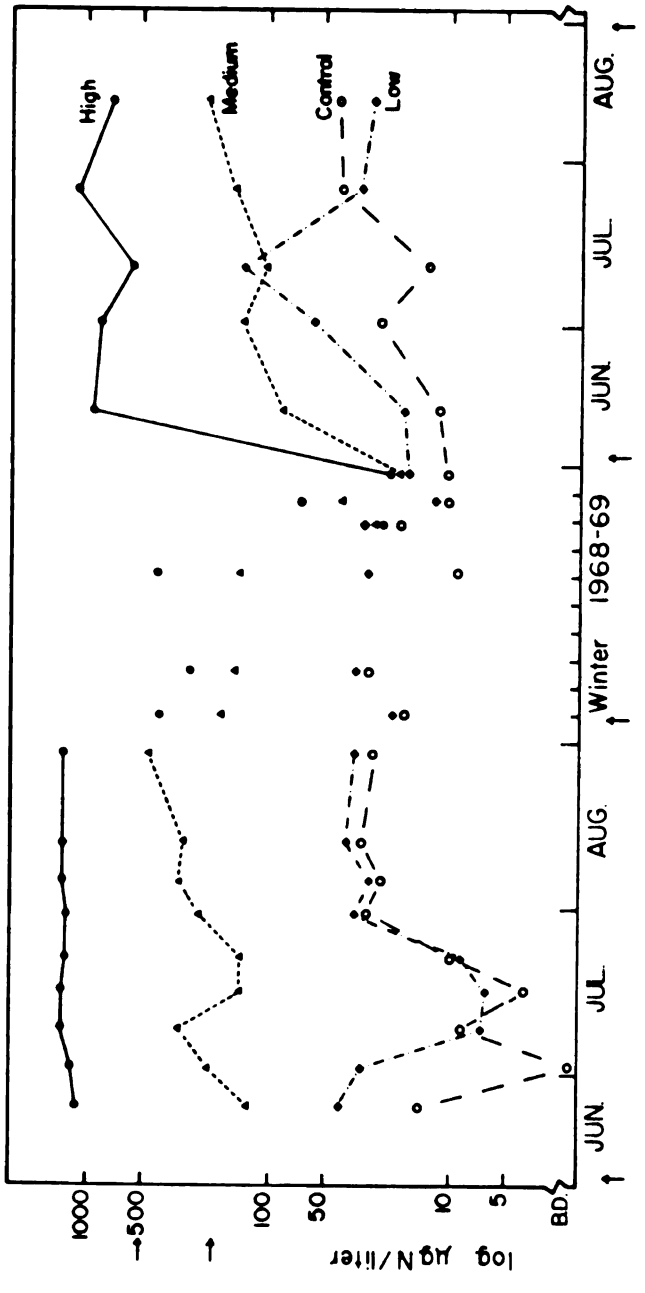

High
Medium
Control
Low
log µg N/liter
1000
500
100
50
10
5
BD
JUN.
JUL.
AUG.
Winter 1968-69
JUN.
JUL.
AUG.

Figure 3.

Figure 4. Reactive phosphorus treatment means throughout the study. The arrows on the abscissa mark the periods of fertilization. The arrows on the ordinate mark the 10 and 30 µg/l level.

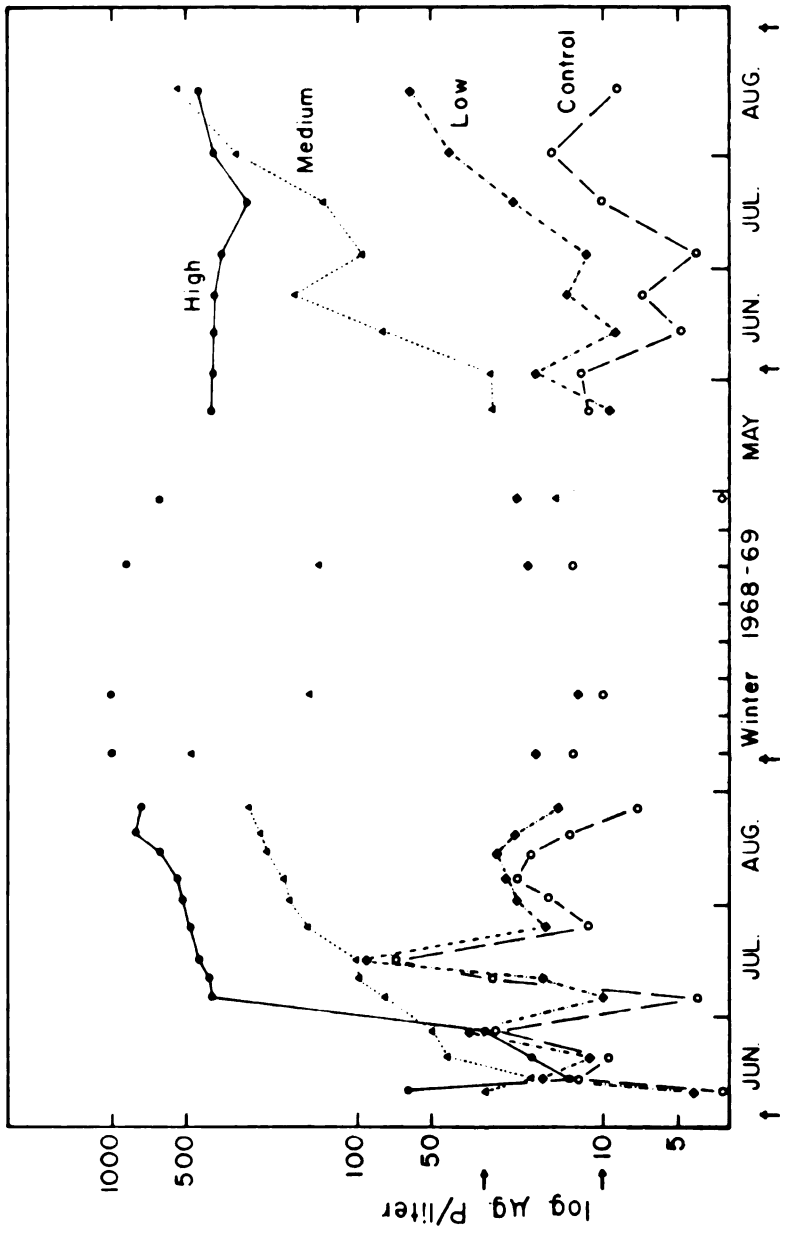

log Ag P/liter
1000
500
100
50
10
5
JUN.
JUL.
AUG.
Winter 1968-69
MAY
JUN.
JUL.
AUG.
High
Medium
Low
Control

Figure 4.

and four conditions in 1969. The increase in nitrogen and phosphorus levels in the low nutrient ponds in 1969, which thereby distinguished them from the control ponds, may have been due in part to a saturation of the binding properties of the clay sediments of the ponds (Mortimer, 1941). Hall et al. (1970), in a fertilization experiment at the Cornell site, report no difference in nitrogen and phosphorus levels between the low and medium treatment ponds for the first two years.

To put the nutrient conditions of the ponds in perspective, it is necessary to compare the nutrient levels with those of other ponds and lakes, and with the growth requirements of plankton algae.

In extensive studies of the growth rates and final yields of several planktonic algae as a function of initial concentrations of nutrients, Chu (1942, 1943) determined upper and lower tolerances for phosphorus and nitrogen. He found that 9-18 mg $P(PO_4)$/l was lethal or over the optimal limit for algal growth, while concentrations below 18-90 µg $P(PO_4)$/l resulted in poor growth.

This upper limit cited by Chu is much higher than any concentration encountered in the ponds, regardless of treatment level. The lower limit of 18 µg $P(PO_4)$/l is less than that normally found in the control ponds in 1968 (Table 4). Typically, low nutrient pond phosphorus concentrations were just above the sub-optimal level. On this admittedly broad basis, the control pond phosphorus

TABLE 4.--Summer range, pond mean, and treatment mean of inorganic nitrogen concentration, reactive phosphorus concentration, chlorophyll *a* concentration, and primary production level.

Trt. Level	Control		Low		Medium		High	
Pond #	4	6	2	7	1	8	3	5
Inorganic Nitrogen 1968 (µg/liter)								
Pond Range	BD--31.9	BD--35.8	2.10--45.6	9.52--60.2	25.2--622	51.7--344	1200--1870	1320--1750
Pond Mean	16.4	22.5	20.4	30.1	287	220	1530	1520
Trt. Mean	19.5		25.3		253		1520	
Inorganic Nitrogen 1969 (µg/liter)								
Pond Range	9.80--32.9	10.5--58.8	21.4--104	12.3--179	74.3--333	37.2--106	360--1260	629--1370
Pond Mean	19.6	28.3	46.1	52.2	209	74.6	786	1010
Trt. Mean	23.9		49.1		142		898	
Reactive Phosphorus 1968 (µg/liter)								
Pond Range	BD--69.4	BD--127	3.72--59.2	4.65--80.9	27.3--179	11.8--304	4.03--893	23.3--741
Pond Mean	22.5	25.7	23.9	22.7	102	140	363	444
Trt. Mean	24.1		23.3		121		404	
Reactive Phosphorus 1969 (µg/liter)								
Pond Range	BD--21.1	BD--11.5	8.40--93.3	9.42--34.0	44.1--732	69.1--326	208--574	188--615
Pond Mean	11.0	6.11	33.1	20.6	293	172	333	414
Trt. Mean	8.56		26.9		232		373	
Chlorophyll *a* 1968 (mg chlorophyll *a*/m^3)								
Pond Range	4.09--24.61	3.32--21.12	11.95--51.40	2.24--15.75	3.92--47.46	36.42--341.1	BD--70.06	2.75--148.9
Pond Mean	14.75	10.38	34.75	11.00	25.13	140.3	25.63	47.31
Trt. Mean	12.57		22.88		82.72		36.47	
Chlorophyll *a* 1969 (mg chlorophyll *a*/m^3)								
Pond Range	6.39--24.19	5.98--22.33	4.50--52.64	6.67--46.70	4.61--273.2	54.26--329.2	14.56--972.9	58.16--928.1
Pond Mean	12.38	12.03	24.11	17.08	61.74	144.2	181.3	221.9
Trt. Mean	12.21		20.60		102.97		201.6	
Primary Production 1969 (mg C/m^2/hr)								
Pond Range	14.09--32.48	16.19--40.01	72.11--218.3	5.77--159.5	45.08--1177.3	190.7--828.6	62.56--911.4	42.86--2454.3
Pond Mean	21.22	26.59	128.79	56.28	212.57	549.21	309.8	630.44
Trt. Mean	23.91		92.54		380.89		470.62	

concentrations may have been below optimum, at least for some organisms, especially in 1969, while all the fertilized ponds should have been within the optimal range for algal growth.

According to Chu, the maximum growth of planktonic algae is attained in a culture medium containing between 300 and 1,300 µg N/l, whereas growth is sub-optimal below concentrations of 100 µg N/l. This lower threshold would fall within the normal summer range of the medium nutrient ponds, and be above the levels of the control and low treatment ponds. The upper range for maximum growth (300 µg N/l) was attained only in the higher nutrient ponds (Table 4, Figure 3).

In comparison to other fertilized and non-fertilized fish ponds (Stangenberg-Oporowska, 1966), the present experiment produced quite a wide range of phosphorus concentrations. Forney (1957), in a survey of farm ponds of central New York State, summarizes the phosphorus content of both fertilized and unfertilized ponds. The unfertilized ponds averaged 30 µg P/l, which is similar to the low treatment ponds or occasionally the medium treatment ponds of the present experiment. The fertilized ponds in his survey averaged 160 µg P/l, which is well within the range of the medium treatment ponds.

The mass of data on the chemistry and biology of experimental fish ponds in Poland shows generally higher phosphorus concentrations. Ferenska and Lewkowicz (1966)

state phosphorus values for a series of experimental ponds both fertilized and unfertilized. The fertilized ponds range from 200 to 1600 µg P/l, which corresponds to medium or high nutrient ponds in the present study. The unfertilized ponds, which had received fertilizer in the past, had from 20 to 400 µg P/l, which corresponds to a little higher than control levels up to medium treatment levels.

Hall et al. (1970) added nitrogen and phosphorus fertilizer to a set of ponds at the Cornell site. The reactive phosphorus concentrations in their highest fertilized ponds rarely reached more than 25 µg P/l, and averaged 12 µg P/l, which is similar to the low and control treatment ponds in the present experiment. Hall et al. report very high concentrations of ammonia nitrogen up to 1000 µg NH_3-N/l in the high ponds, about the same as the total nitrogen concentration in the high ponds of the present experiment.

Using primarily the data of Thomas (1953), Vollenweider (1968) prepared a table relating the trophic characteristics of water bodies to their concentrations of total phosphorus and inorganic nitrogen (Table 5). Sakamoto (1966) has a classification derived from Japanese lakes that is very similar. The control ponds averaged 24.1 µg P/l in 1968, which would characterize them as meso-eutrophic according to the Vollenweider classification. In 1969 the control ponds averaged 8.6 µg P/l

TABLE 5.--Classification of lake types by nutrient concentration (after Vollenweider, 1968).

Trophic Characteristics	Total P (µg/l)	Inorganic N (µg/l)
1. Ultra-oligotrophic	less than 5	less than 200
2. Oligo-mesotrophic	5-10	200- 400
3. Meso-eutrophic	10-30	300- 650
4. Eu-polytrophic	30-100	500-1,500
5. Polytrophic	greater than 100	greater than 1,500

characterizing them as oligo-mesotrophic. The rest of the treatments were generally in the range of eu-polytrophic.

According to these classifications, then, the control ponds were high in phosphorus, and on this basis alone would have been judged mesotrophic. All the treated ponds would have been classified as high mesotrophic or eutrophic.

On the basis of nitrogen, however, this classification would be radically changed. Vollenweider classes as ultra-oligotrophic a water body with less than 200 µg inorganic N/l. The control and low nutrient ponds always had less than this amount, and the medium nutrient ponds often did. The high treatment ponds ranged from 360 to 1750 µg inorganic N/l, which spans the rest of the range of the classification system but would surely have placed them as eutrophic. Only the medium treatment ponds would at times have been classed mesotrophic with respect to

nitrogen. In Sakamoto's classification, both the control and low ponds would have been oligotrophic with respect to nitrogen.

The apparent imbalance between phosphorus and nitrogen levels is even more obvious when the pond nitrogen to phosphorus ratios (Table 6) are compared with those reported by other workers (Sakamoto, 1966). According to Liebig's "law of the minimum" (Odum and Odum, 1959), nitrogen may well have been the "governing" nutrient in determining productivity in the experimental ponds.

TABLE 6.--Average nitrogen to phosphorus ratios.

Trt. Level	Control		Low		Medium		High	
Pond #	4	6	2	7	1	8	3	5
			1968					
Pond Average	2.56	3.06	2.09	2.94	5.26	3.86	9.00	6.76
Trt. Average	2.81		2.52		4.61		7.88	
			1969					
Pond Average	3.07	6.20	5.21	5.84	2.02	1.34	5.67	6.34
Trt. Average	4.64		5.53		1.68		6.01	

A bioassay experiment was performed to indicate which nutrients might be most important as limiting factors (Hitchcock, 1970). Water from each of the four treatments (ponds 6 C, 7 L, 1 M, and 3 H) was Millipore filtered and Pandorina morum Bory used as the test organism. To water

from each treatment level were added various major and minor nutrients creating four different combinations for each of the four treatment levels: 1.33 mg/l N as NH_4NO_3; 0.84 mg/l P as $Ca(H_2PO_4)_2 \cdot H_2O$; a combination of these two; and a minor nutrient mixture containing 40 µg/l each of some important minor nutrients (Zn, Mn, Mo, B, Cu, Fe, and Co) and 0.475 mg/l of EDTA. The unaltered pond water from each treatment level and the above-mentioned treatments were incubated in an illuminated temperature chamber at 20°C. The flasks were sampled every two days and the number of P. morum was determined using a Coulter Counter. The final yields of the P. morum after two weeks are presented in Table 7. There was a consistent increase in number of colonies/ml with increasing fertilizer level; the low and control pond waters showed the slightest difference. At no time did phosphorus or the minor nutrient mixture stimulate the final yield to concentrations greater than those stimulated by the pond water itself. In every case except the high treatment pond water, nitrogen stimulated a final yield greater than the pond water alone. However, in the control and low treatment ponds there was an interaction between nitrogen and phosphorus such that together they stimulated a greater final yield than nitrogen alone, yet phosphorus alone was not stimulatory at all. In the medium and high treatment waters, this interaction was no longer present. This experiment would seem to indicate that the "governing"

TABLE 7.--Laboratory bioassay of pond water from each treatment level to determine important growth factors.

	Maximum Growth Concentrations (# *Pandorina morum* colonies/ml)			
	Pond Water	Pond Water +0.84 mg/l P	Pond Water +1.33 mg/l N	Pond Water +0.84 mg/l P +1.33 mg/l N
Control Pond 6	350	350	3,600	9,000
Low Pond 7	450	400	6,800	11,500
Medium Pond 1	2,300	2,400	14,000	14,000
High Pond 3	19,000	18,000	23,000	20,000
	Per Cent Growth Increase Over Unaltered Pond Water			
Control Pond 6	. .	. .	1029%	2571%
Low Pond 7	. .	. .	1478%	2500%
Medium Pond 1	. .	. .	609%	609%
High Pond 3	. .	. .	121%	105%

nutrient in these ponds was nitrogen. The possibility that the phosphorus did not stimulate the P. morum due to luxury uptake of phosphorus by the P. morum prior to its being added to the pond water may effectively be ruled out. The inoculum was kept very small compared to the final yields in even the unaltered control pond water, and the organisms used for the inoculum had been grown on a soil water extract culture medium which was relatively poor in phosphorus.

EFFECTS OF NUTRIENTS ON PLANKTON DENSITIES

Phytoplankton Densities

As a measure of the total phytoplankton response to fertilization, the chlorophyll *a* concentrations of the pond waters were measured every week during the summers of 1968 (Figure 5) and 1969 (Figure 6). In 1968 the only obvious trend was that the control level ponds generally showed the lowest concentration and the medium nutrient ponds the highest concentration of chlorophyll *a* (Figure 7). During 1969, there was a more definite treatment trend: the control and low treatments were always distinct after June, and all four treatments were separate and in ascending order by treatment level during all of August (Figure 8).

The difference in degree of phytoplankton biomass response from 1968 to 1969 may partly be a function of an absence of algal species adapted to high nutrient conditions in the first part of the summer of 1968. *Scenedesmus quadricauda* (Turp.) de Brebisson and *S. abundans* (Kirch.) Chodat, which became very dense in both high treatment ponds in 1969, were either unrecorded or recorded only late in the summer of 1968. Filamentous

Figure 5. Chlorophyll a treatment means throughout summer 1968.

Figure 5.

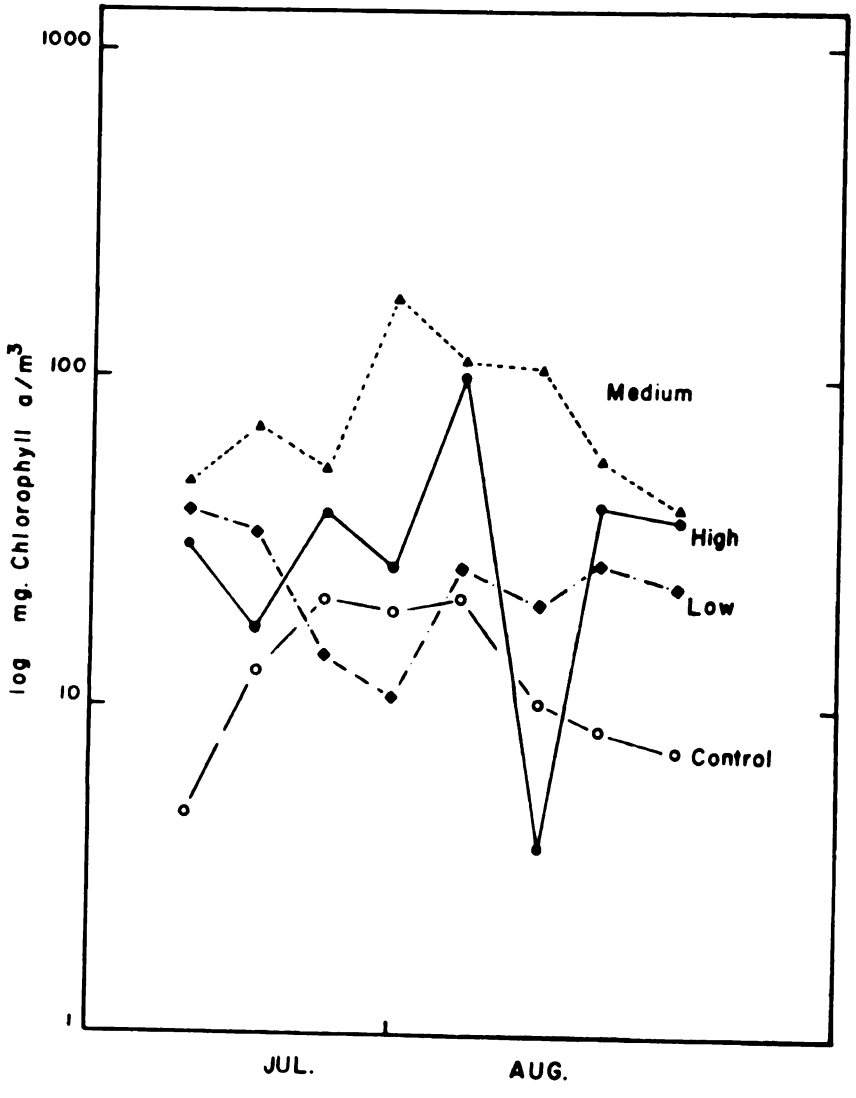

1000
100
10
1
log mg. Chlorophyll a/m3
Medium
High
Low
Control
JUL.
AUG.

Figure 6. Chlorophyll a treatment means throughout summer 1969.

Figure 6.

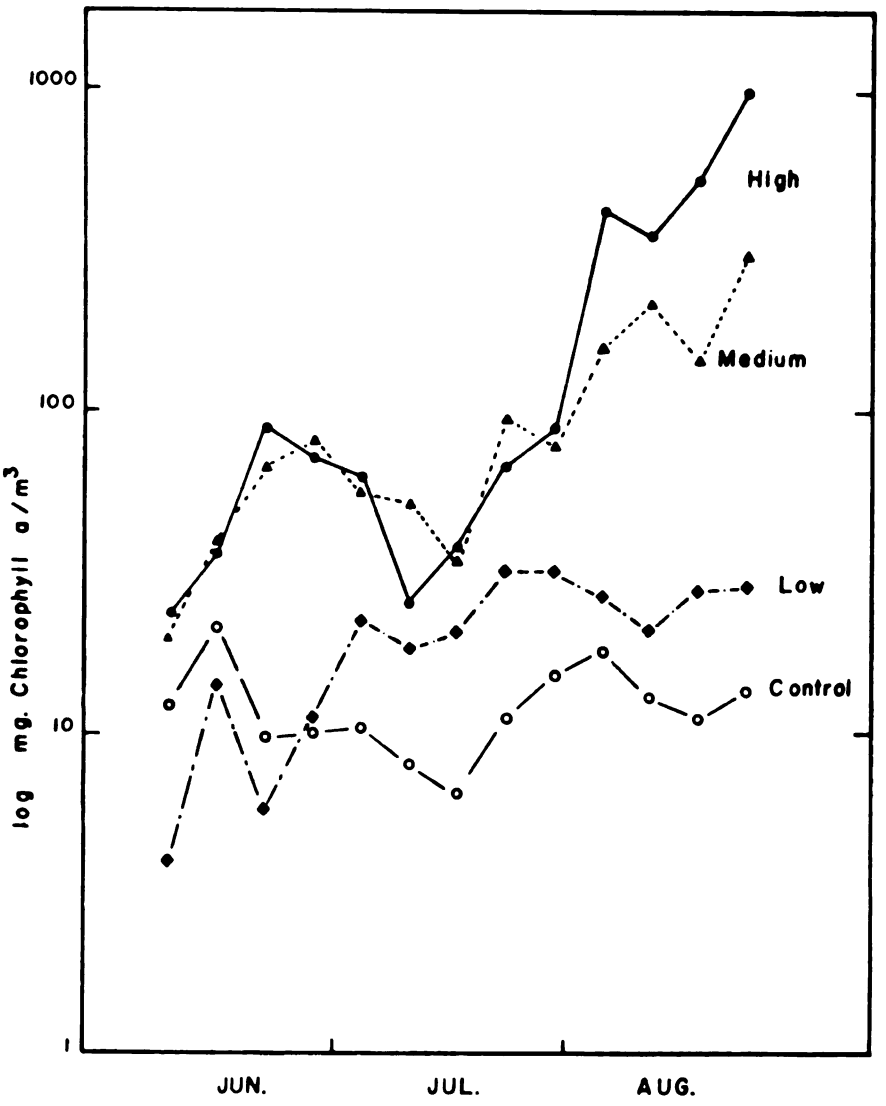

1000
100
10
1
log mg. Chlorophyll a/m3
High
Medium
Low
Control
JUN.
JUL.
AUG.

Figure 7. Chlorophyll a pond and treatment means. Summer 1968.

Figure 7.

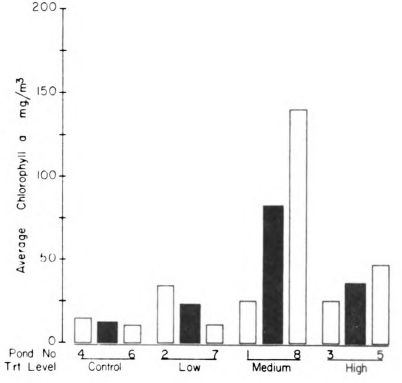
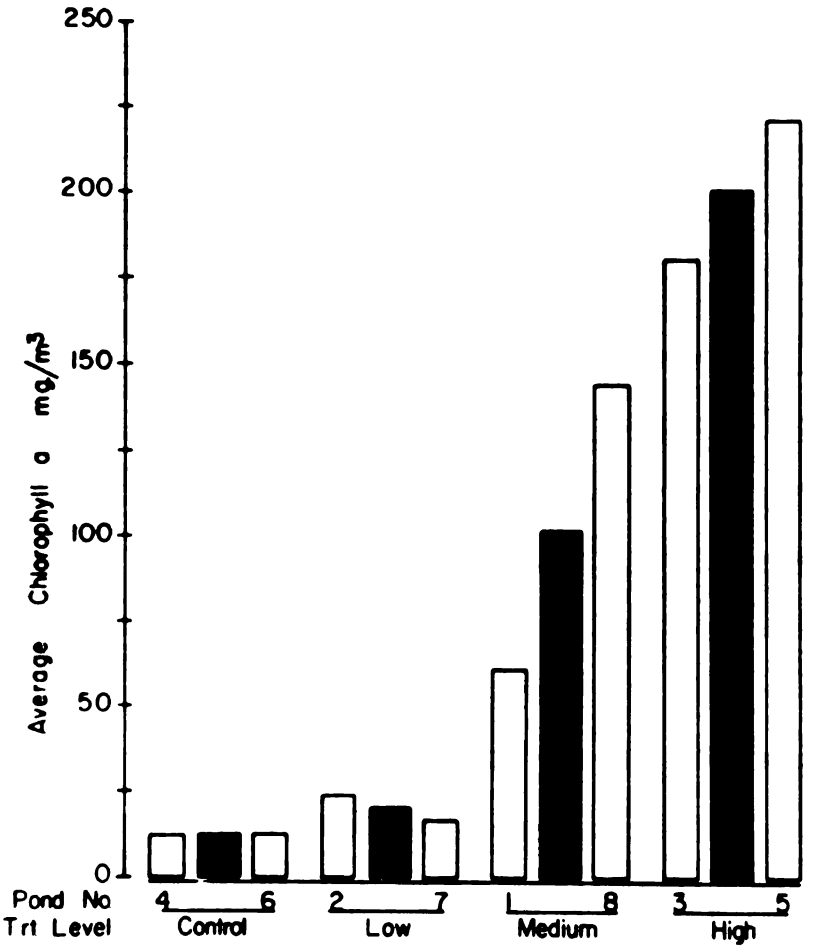

200
150
100
50
0
Average Chlorophyll a mg/m³
Pond No
Trt Level
4 6
Control
2 7
Low
1 8
Medium
3 5
High

Figure 8. Chlorophyll a pond and treatment means. Summer 1969.

Figure 8.


250
200
150
100
50
0
Average Chlorophyll a mg/m³
Pond No
Trt Level
4 6
Control
2 7
Low
1 8
Medium
3 5
High

algae were more abundant in the higher treatment ponds during the first summer, and may have suppressed phytoplankton growth. Another important change from 1968 to 1969 in ponds 8 M, 3 H, and 5 H was the decrease and disappearance of grazing zooplankton in these ponds late in the summer of 1969.

Sakamoto (1966) presents data on the chlorophyll a content of Japanese lakes in different trophic states. His values for lakes during summer stagnation were: 5-120 mg chlorophyll a/m^3 in eutrophic lakes, 1-5 mg chlorophyll a/m^3 in mesotrophic lakes, and less than 1 mg chlorophyll a/m^3 in oligotrophic lakes. Given this scale of comparison, the control ponds in the present experiment were mesotrophic and sometimes reached eutrophic levels of 10 mg chlorophyll a/m^3 during the summer. Only the medium and high ponds reached or, in the case of the high nutrient ponds, vastly surpassed the upper levels indicated by Sakamoto.

Copeland, Minter, and Dorris (1964) reported chlorophyll a concentrations approaching 100 mg/m^3 in oil refinery effluent ponds. They state that these values, which were exceeded by both the high and medium ponds in the present experiment, were as high as those reported from sewage treatment ponds. Wrobel (1965), in work with Polish experimental fish ponds, cited values of 25 mg chlorophyll a/m^3 for ponds without fertilizer, and peaks up to 200-300 mg chlorophyll a/m^3. These data agree well

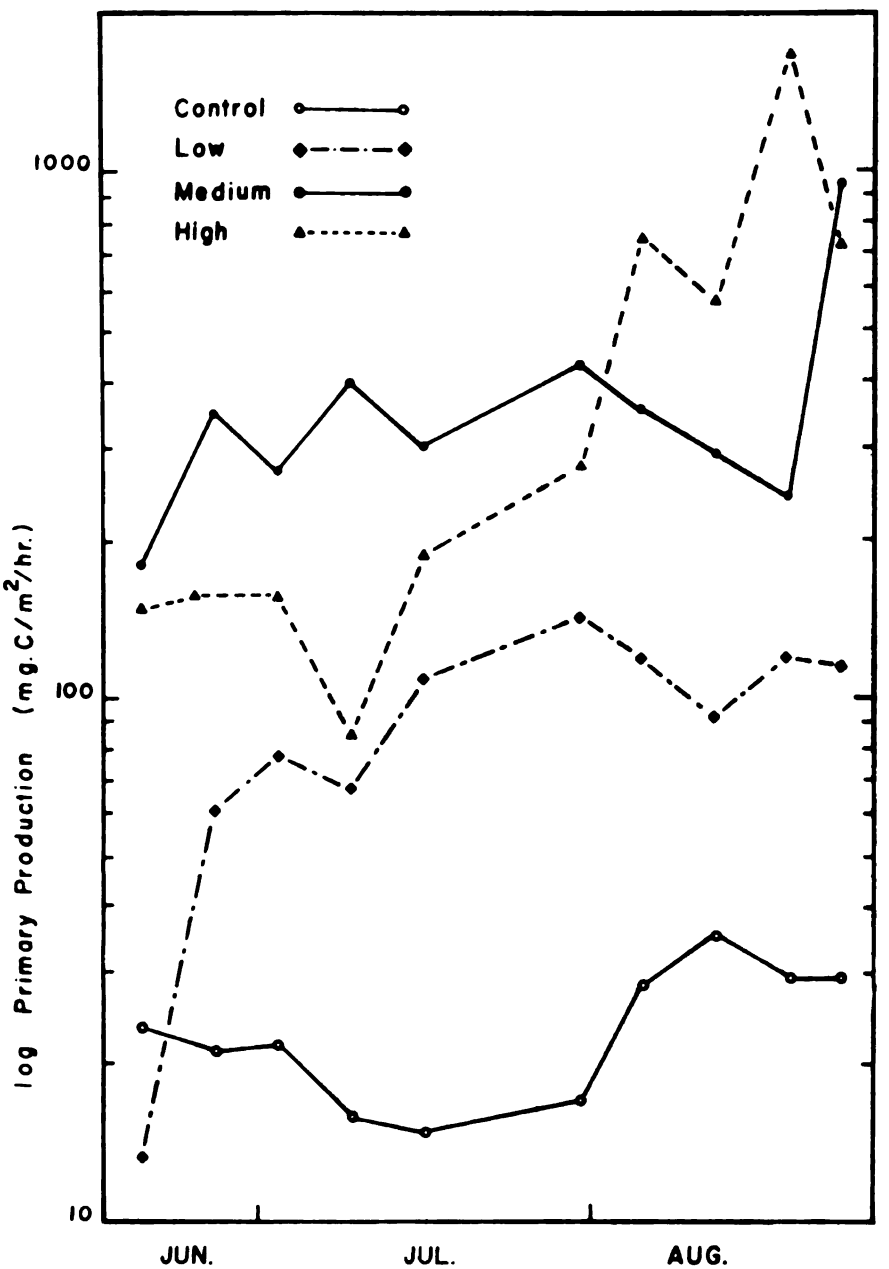
with chlorophyll a concentrations in the present study, except that the chlorophyll levels in the control ponds were somewhat lower than those reported from Poland, and those in the medium and high nutrient ponds went above 300 mg chlorophyll a/m^3 only when grazing organisms were absent. Abeliovich (1969), working on Israeli fish ponds receiving high levels of fertilizer at high temperature, reports a maximum of 1,400 mg chlorophyll a/m^3 in May, although most of the ponds studied had under 400 mg chlorophyll a/m^3.

Hall et al. (1970) report chlorophyll a average values of 2.9, 6.0, and 55.5 mg chlorophyll a/m^3 in the low, medium, and high treatment ponds of their experiment during the latter part of the summer of 1965. In the summer of 1967, their average values were 5.7, 13.9, and 37.9 in the low, medium, and high treatment ponds, respectively.

The primary production as measured by carbon-14 uptake was determined weekly in each pond throughout the summer of 1969, but not at all in 1968. As might be expected from numerous correlations of chlorophyll a and primary production (Ketchum et al., 1958; Sakamoto, 1966), the mean primary production increased directly with increasing treatment level (Figure 9). In fact, the production rate responded more markedly to the fertilizer regime than did the standing crop of algae as measured by chlorophyll a concentration.

Figure 9. Primary production treatment means throughout summer 1969.

Figure 9.


Control
Low
Medium
High
1000
100
10
log Primary Production (mg.C/m²/hr.)
JUN.
JUL.
AUG.

The primary production of the ponds had a large range: from 10 to 2,500 mg $C/m^2/hr$. So as to compare primary production rates with other work, the hourly rates were multiplied by 6 (Vollenweider, 1965). These values then become 60 mg C/m^2day to 15,000 mg $C/m^2/day$.

By comparison with production values reported elsewhere, the minimum daily rate is one of the lowest summer production rates noted. Wetzel (1966), working on marl lakes in Indiana, reports lower values. These, however, were winter samples; the annual mean daily primary production on all his lakes was higher.

Knight, Ball, and Hooper (1962), working on four experimental ponds in Michigan, report a summer average of 5.5 mg $C/m^3/hr$ for the control pond and a range of 9.1 to 14.8 mg $C/m^3/hr$ for ponds to which various combinations of phosphorus fertilizer and a chelating agent had been added. Even this level for the fertilized ponds is below the average production of the control ponds in the present study. Hepher (1962), working on fertilized fish ponds in Israel, reported from 140-190 mg $C/m^2/hr$ in unfertilized ponds and up to 4 or 5 times more in fertilized ponds. The unfertilized ponds would correspond roughly to the medium and high ponds, and the fertilized ponds approach or exceed the high ponds of the present experiment.

The upper value occurring in the present ponds, 15,000 mg $C/m^2/day$, is the highest primary production value ever reported, and most probably is in error in that a

multiplication of an hourly rate implies that the same level of production is maintained all day. Even so, it is certain that the high treatment ponds late in the summer of 1969 were as productive as the most eutrophic bodies of water. Other high primary production values have been reported by Elster and Vollenweider (1961), who cite values of 8,000-10,000 mg C/m^2/day, and Sreenivasan (1964), who used the oxygen method of measuring primary production in a tropical pond and got a value of 11,000 mg C/m^2/day. Aleem and Samaan (1969), working on a sub-tropical shallow eutrophic lake, report high values varying between 6,000 and 8,000 mg C/m^2/day. They state their value to be one of the highest reported.

Vollenweider (1968), summarizing a great deal of work on primary productivity from Europe, gives the maximum daily production along with an assessment of the trophic state of the lake. According to his classification, the production in a eutrophic lake falls between 600 and 8,000 mg C/m^2/day, in a mesotrophic lake between 250 and 1,000 mg C/m^2/day, and in an oligotrophic lake between 65 and 300 mg C/m^2/day. The treatment levels of the present study fall nicely within Vollenweider's classification. The control ponds varied over the summer from 80 to 240 mg C/m^2/day, which agrees with Vollenweider's oligotrophic value. The low treatment ponds ranged from 60 to 800 mg C/m^2/day, which is at times within Vollenweider's oligotrophic range but more often falls within the

mesotrophic limits. While both the medium and high ponds had periods of low production, they most often ranged well over 600 mg C/m^2/day with the highest value being 15,000 mg C/m^2/day.

Hall et al. (1970) report average primary production values on a per unit volume basis (mg C/m^3/hr) for the summers of 1965 and 1967. In 1965 the average primary production in the low (control) ponds was 28, in the medium treatment ponds 34, and in the high treatment ponds 282 mg C/m^3/hr. In 1967 these values were 66, 207, and 994 mg C/m^3/hr. The present data are quite similar (Figure 9).

The phytoplankton biomass and productivity data show that not only were four separate conditions created in the present experiment by the nutrient enrichment regime, but that these conditions ranged from oligotrophic to very eutrophic. Using the concentrations of nitrogen and phosphorus as criteria for classification, conflicting results are obtained. The phosphorus concentrations in the control ponds were high and would indicate they were close to being eutrophic. The other treatment level ponds, of course, ranged upward from this. The nitrogen concentrations in the control ponds were very low, and would indicate the ponds were very oligotrophic. That the nitrogen concentration in the ponds was the "governing" factor is supported by the bioassay experiments performed on pond water from each treatment level (Hitchcock, 1970).

Also supporting this is the close correspondence between the trophic classification of the ponds on the basis of nitrogen and classification on the basis of primary production.

Zooplankton Densities

According to chlorophyll a and primary production measurements, a wide range of phytoplankton concentrations resulted in the ponds from the nutrient treatment regime. Assuming that the zooplankton respond directly to increases in phytoplankton, large differences in zooplankton densities according to treatment level would also be expected.

To determine the response of the crustacean zooplankton to the fertilizer treatments, pooled samples were prepared and treated as average values for each pond for each summer. The total filter feeding crustacean plankton biomass in 1968 showed an inconsistent trend (Figure 10) with increasing fertilizer level. The control ponds were lowest in biomass and the high treatment ponds were highest; however, the low and medium treatments were reversed in their response. This was caused by two ponds: pond 2 L had a very high average biomass, and pond 1 M had the lowest average biomass of all the ponds.

However, if the calanoid copepod Diaptomus pallidus Herrick, which occurred in large numbers in pond 2 L, is eliminated from the calculation and just the cladoceran

Figure 10. Crustacean zooplankton biomass pond and treatment means. Summer 1968. Central bar represents the treatment mean. Solid and cross hatched areas represent total cladoceran biomass. Clear area represents biomass of Diaptomus pallidus.

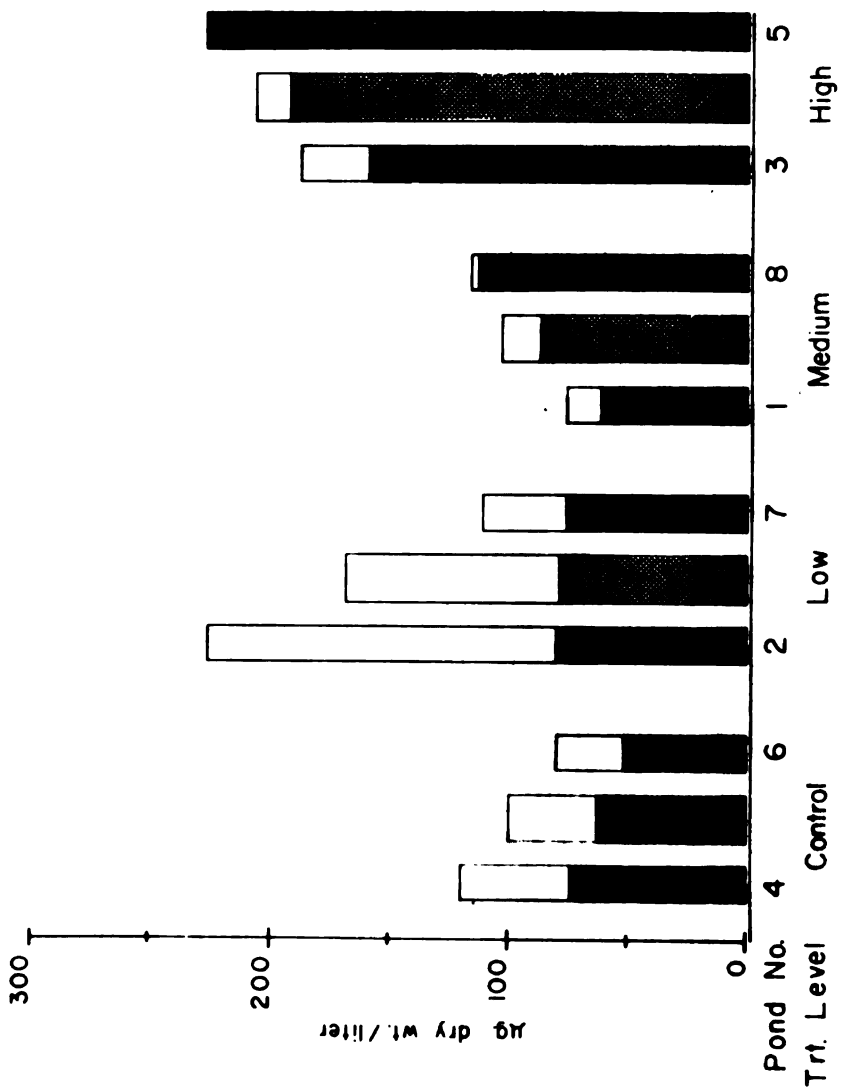

300
200
100
0
mg dry wt./liter
Pond No.
Trt. Level
4
6
2
7
1
8
3
5
Control
Low
Medium
High

Figure 10.

zooplankton are considered, there is a definite trend of increasing biomass with increasing treatment level. Since D. pallidus reproduces sexually and has a life cycle extending over at least several months, it might be expected that it could not have responded as quickly to erratic fluctuations in edible phytoplankton algae as could the parthenogenic cladocerans. In the case of pond 2 L in 1968, there was a large population of D. pallidus at the beginning of the fertilization regime and this population decreased only slowly. In the high ponds D. pallidus populations were never large and decreased quickly after fertilization, never after the first few weeks of 1968 representing more than 5 per cent of the total biomass in these ponds. Thus this species may be excluded from the analysis both on the grounds that it is an inappropriate measure of the effect of increased phytoplankton on filter feeding crustaceans and due to the fact that its initial abundance and distribution in the ponds may have altered its response to treatment levels.

In 1969 (Figure 11), there was again no simple trend of increased total crustacean zooplankton biomass with increasing fertilizer input. The high treatment ponds were higher than the controls, but pond 2 L once again had exceptionally high total zooplankton biomass. At the same time, levels in pond 5 H and especially pond 8 M were extremely low. When just the cladocerans are considered,

Figure 11. Crustacean zooplankton biomass pond and treatment means. Summer 1969. Central bar represents the treatment mean. Solid and cross hatched areas represent total cladoceran biomass. Clear area represents biomass of Diaptomus pallidus.

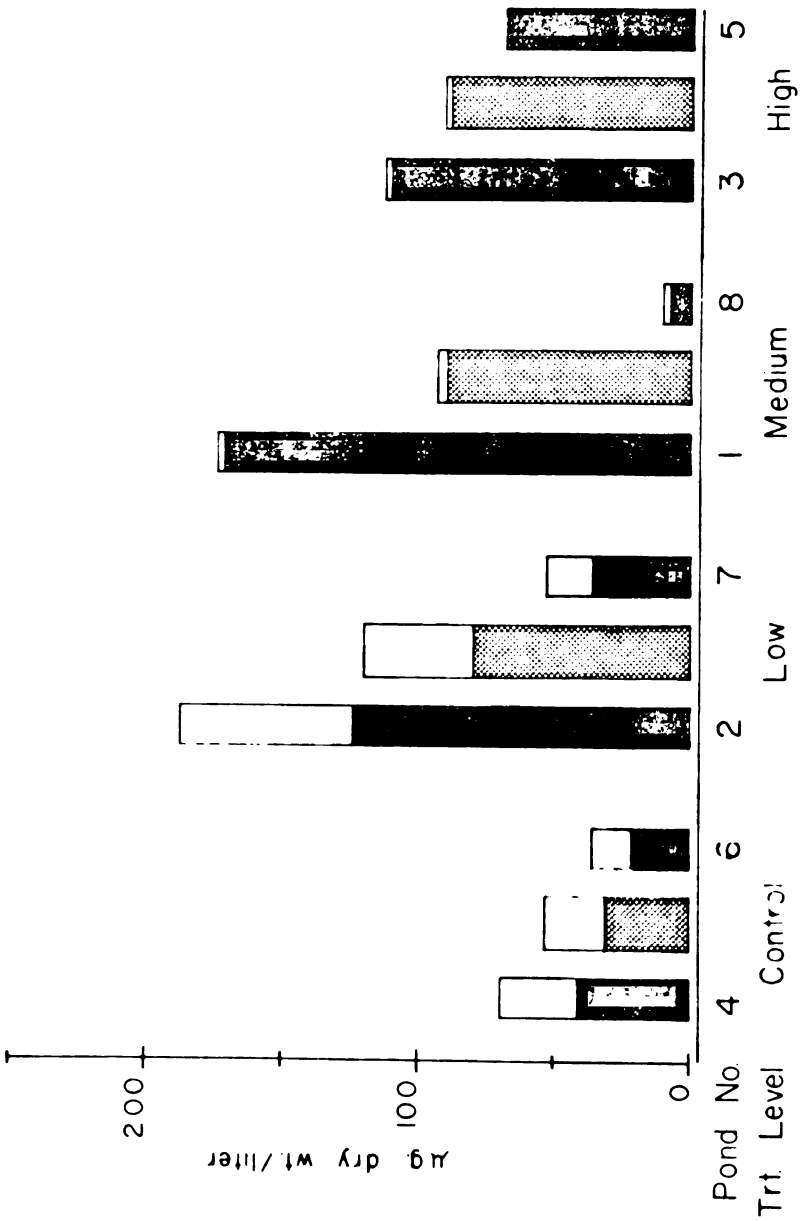

200
100
0
µg dry wt/liter
Pond No. 4 6 2 7 1 8 3 5
Trt. Level Control Low Medium High

Figure 11.

the relationship is somewhat more clear, but at best all the treatment means are the same except for the controls.

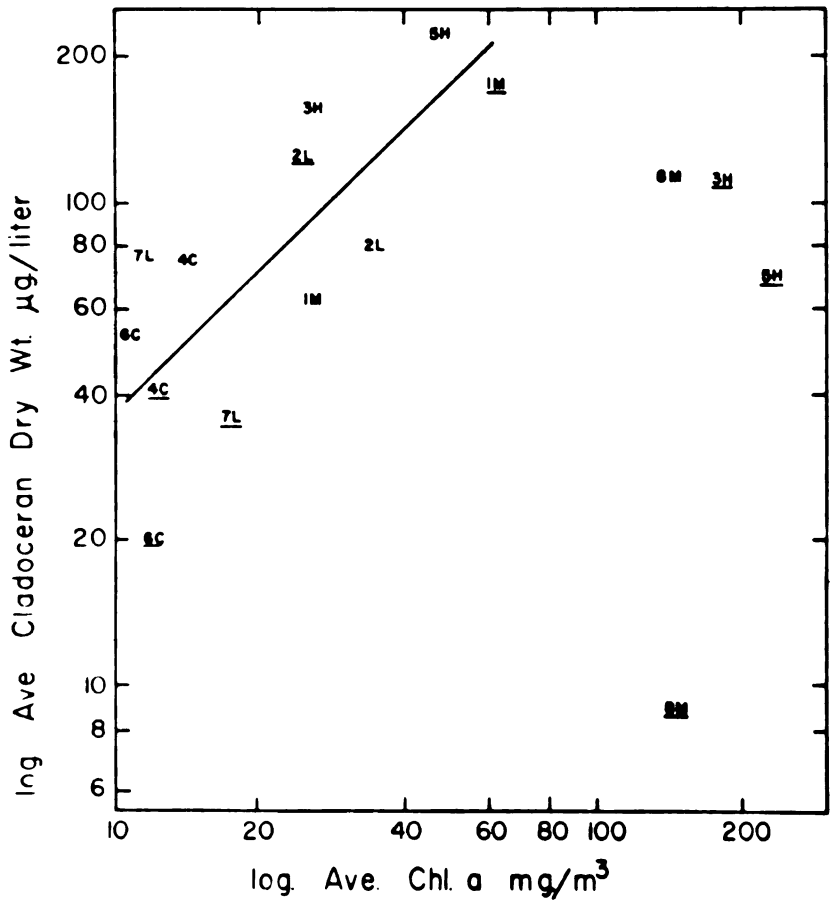
However, there is a good correlation between the average summer chlorophyll a concentration in a pond and the average cladoceran biomass of that pond. Excluding the values from pond 8 M for both years and ponds 3 H and 5 H for 1969, a straight line with a correlation coefficient of 0.75 was obtained using a logarithmic transformation and the least squares method (Figure 12). All four of the excluded ponds had average chlorophyll a concentrations notably higher than any of the other ponds. These exceptional cases are discussed in a later section.

Although cladoceran zooplankton production was not calculated, it is unlikely that the relationship between production and treatment level differed greatly from the relationship between cladoceran biomass and treatment level. Preliminary but incomplete results indicate there was no change in one index of production, the ratio of number of eggs to number of animals, as a function of treatment level. Hall et al. (1970) found that the biomass and production of zooplankton responded in a similar fashion to increased nutrient input.

The zooplankton densities recorded for the fertilized ponds and even the controls were somewhat higher than those of deeper lakes and other large bodies of fresh water (Tressler, Wagner, and Bere, 1940; Applegate and Mullin, 1967). Even fairly eutrophic lakes have lower zooplankton

Figure 12. Relationship between the log of the average summer chlorophyll a concentration and the log of the average summer cladoceran biomass. The straight line was fitted using the least squares method with a correlation coefficient of 0.75.

Figure 12.


log Ave Cladoceran Dry Wt. µg/liter
200
100
80
60
40
20
10
8
6
log. Ave. Chl. a mg/m³
10
20
40
60
80
100
200
5H
1M
3H
2L
6M
3H
7L
4C
2L
5H
1M
6C
4C
7L
8C
8M

densities (Davis, 1969). Zooplankton densities reported in ponds and other shallow water bodies, however, are usually as great as or greater than the average recorded in the high ponds during the present experiment (Straskraba, 1965; Ferenska and Lewkowicz, 1966; Cummins et al., 1969).

The problem of comparing zooplankton densities and production in various bodies of water is complicated by the lack of standard sampling and weighing methods. In the present study, samples were taken at night, whereas many workers sample during the day. Unlike many shallow bodies of water, the experimental ponds had negligible weed growth. Attempts to compare weights are confused by variation in procedures and by the use of wet weights by many European workers. Because of such methodological differences, it is often best simply to compare the reported densities of particular species rather than total zooplankton biomass.

The control ponds in the present study averaged 25 to 30 Ceriodaphnia reticulata per liter during both summers (an exception is pond 4 C in 1968 when Diaphanosoma brachyurum Liéven was present at about this abundance while C. reticulata densities were low). The main zooplankton production in the control ponds occurred in June and early July, during which time there were up to 100 to 150 animals per liter. Late July and August abundances were lower, around 10 animals per liter. Similar seasonal

trends have been reported by Borecky (1956), Straskraba (1965), Ferenska and Lewkowicz (1966), and Hall et al. (1970).

The control ponds in the present study appear to have been quite low in cladoceran density. In only a few pond studies (McIntire and Bond, 1960; George, 1966) are lower cladoceran densities recorded. The highest average zooplankton density of the fertilized ponds was recorded in pond 5 H in 1968: 138 C. reticulata per liter. The peak population density of the ponds--750 C. reticulata per liter--was recorded in pond 8 M in mid-July of 1968. Several other workers have reported similar or higher densities (Ferenska and Lewkowicz, op. cit.; Straskraba, 1967; Armitage and Smith, 1968). Hall et al. (op. cit.) report peaks of almost 1,000 C. reticulata per liter in some of their fertilized ponds.

In 1969, both high nutrient ponds in the present experiment averaged about 10 Daphnia pulex per liter, but each had pulses in which D. pulex reached 50 per liter. Armitage and Smith (op. cit.) report almost 1,000 per liter, and Ferenska and Lewkowicz (op. cit.) report up to 140 per liter.

As mentioned previously, it is difficult to compare total zooplankton biomasses. However, some approximate comparisons may be made if the wet weight figures given by several European workers are reduced by a factor of 10, a conversion factor which is supported by the data of

Lovegrove (1962). Ferenska and Lewkowicz (op. cit.) report almost 800 µg/l total zooplankton biomass in unfertilized ponds, and from 1,000 to 1,400 µg/l in fertilized ponds.

Hall et al. (op. cit.), working on similar ponds at the Cornell site and using the same sampling procedure as in the present study, report higher total zooplankton dry weights in their highly fertilized ponds, using a slightly higher dry weight conversion factor. They observed roughly 460 µg/l total zooplankton dry weight in the high fertilized ponds in 1965 as compared to 206 µg/l in the high nutrient ponds of the present experiment in 1968. There is much better agreement between the medium treatment ponds of Hall et al. and the low ponds of the present experiment, in which the total zooplankton dry weight was about 100 to 170 µg/l in both studies. In 1965, the zooplankton dry weight in the low treatment ponds (controls) of Hall et al. averaged 70 µg/l as compared with 100 µg/l in 1968 and 50 µg/l in 1969 in the control ponds of the present study.

Hall et al. did demonstrate a general increase in zooplankton biomass with increasing fertilization. McIntire and Bond (1960) also show this response of the zooplankton, with total crustacean numbers increasing dramatically with the increase of phosphorus and nitrogen fertilizer. Gliwicz (1969b) reports greater zooplankton

densities in eutrophic lakes than in meso-oligotrophic ones. However, Bakhtina (1968), working on four fish ponds in Russia in which two different levels of phosphorus and nitrogen fertilizer were applied, shows just the opposite effect. Cladoceran zooplankton were most abundant in his lesser fertilized ponds and almost non-existent in the higher fertilized ponds. He did not believe this was due to fish predation. This result is similar to that observed in some of the ponds in the present study in 1969.

The first part of the initial hypothesis, that increasing nutrient input would increase the standing crop of algae, appears to have been largely correct in 1969. During both years of the study, the amount of nutrients was distinct and increased with treatment level. In 1969, the planktonic algae, as measured by chlorophyll a content, increased with increasing treatment level. In 1968, however, the medium nutrient ponds, especially pond 8 M, showed the greatest phytoplankton biomass, and the phytoplankton levels in the high nutrient ponds were quite a bit lower (Figures 7 and 8).

A simple and consistent relationship between treatment level and average zooplankton biomass is not apparent (Figures 10 and 11). In 1968 the high nutrient ponds had the highest average cladoceran biomass, but all the other treatments were alike. In 1969 there was even less of a consistent relationship between cladoceran biomass

and treatment level with all the treatments about the same except the control ponds. However, there is a clear, positive correlation, with four exceptions, between the average chlorophyll a concentration and the average cladoceran biomass in a pond. From this correlation it can be seen that had the chlorophyll a content increased directly with increasing treatment level, the average cladoceran biomass would have done the same. Thus the inconsistent relationship between average cladoceran biomass and treatment level was due in part to the erratic response of the phytoplankton to treatment level. This was especially true in 1968 (Figure 7). However, in the four ponds in which the average chlorophyll a concentration was greater than 60 or 70 mg/m^3, the relationship between the amount of phytoplankton and the cladoceran biomass may have been considerably altered. The relationship between phytoplankton and zooplankton in these four ponds is discussed in a later section.

EFFECTS OF NUTRIENTS ON PLANKTON SPECIES COMPOSITION AND DIVERSITY

Phytoplankton Species Composition and Diversity

All the ponds were notably similar in phytoplankton species composition prior to fertilization. The species composition in the control ponds stayed largely the same through both summers of the two-year experiment, and some of the fertilized ponds, especially the low treatment level ponds, returned to their previous composition by the spring of 1969, before the nutrient regime was resumed in the summer.

Cryptomonas Marssonii Skuja and C. pulsilla Buchmann were generally present and often abundant in the control ponds. Chlorella sp. was also an important species in both control ponds during both summers. Perhaps the most characteristic abundant species was Erkenia subsequiciliata Skuja, which was initially present in all the ponds but persisted only in the control ponds once the other ponds were fertilized. Oocystis parva West and West and Kirchneriella lunaris (Kirchner) Moebius often dominated the phytoplankton of pond 4 C for long stretches during both summers.

In 1968 the low treatment ponds remained dominated by many of these same species until the development of Microcystis aeruginosa (Kuetz.) Elenkin blooms in both ponds late in the summer. M. aeruginosa blooms reoccurred in both ponds in late summer of 1969. This species never occurred in the control ponds. In pond 2 L the most important species were Tetraspora lacustris Lemmermann and Oocystis parva, while in pond 7 L Chlorella sp. dominated during 1968 and Chromulina sp. through much of 1969. In both ponds there was a greater number of species of Scenedesmus than appeared in the control ponds.

Microcystis aeruginosa also occurred in both medium treatment ponds in great abundance, in pond 8 M in 1968 and in pond 1 M in 1969. M. aeruginosa was absent from pond 1 M in 1968 and was infrequent in pond 8 M in 1969. Chroomonas caudata Geitler was often very abundant in both ponds in 1968 but appeared only early in the summer of 1969 in pond 1 M and not at all in pond 8 M. Chlamydomonas sp. was important in pond 1 M throughout both summers. Pandorina morum and Pleodorina californica Shaw were very abundant in pond 8 M in the early summer of 1968, and P. morum was very abundant in pond 8 M again in 1969. Chlorella sp. and several species of Scenedesmus were abundant in pond 8 M during both summers. Pond 8 M had far more species represented during both summers than did pond 1 M (Figures 13 and 14).

Figure 13. Average number of summer phytoplankton species. Summer 1968. Clear area represents individual pond. Cross hatched area represents treatment mean. Based on 10 sampling dates.

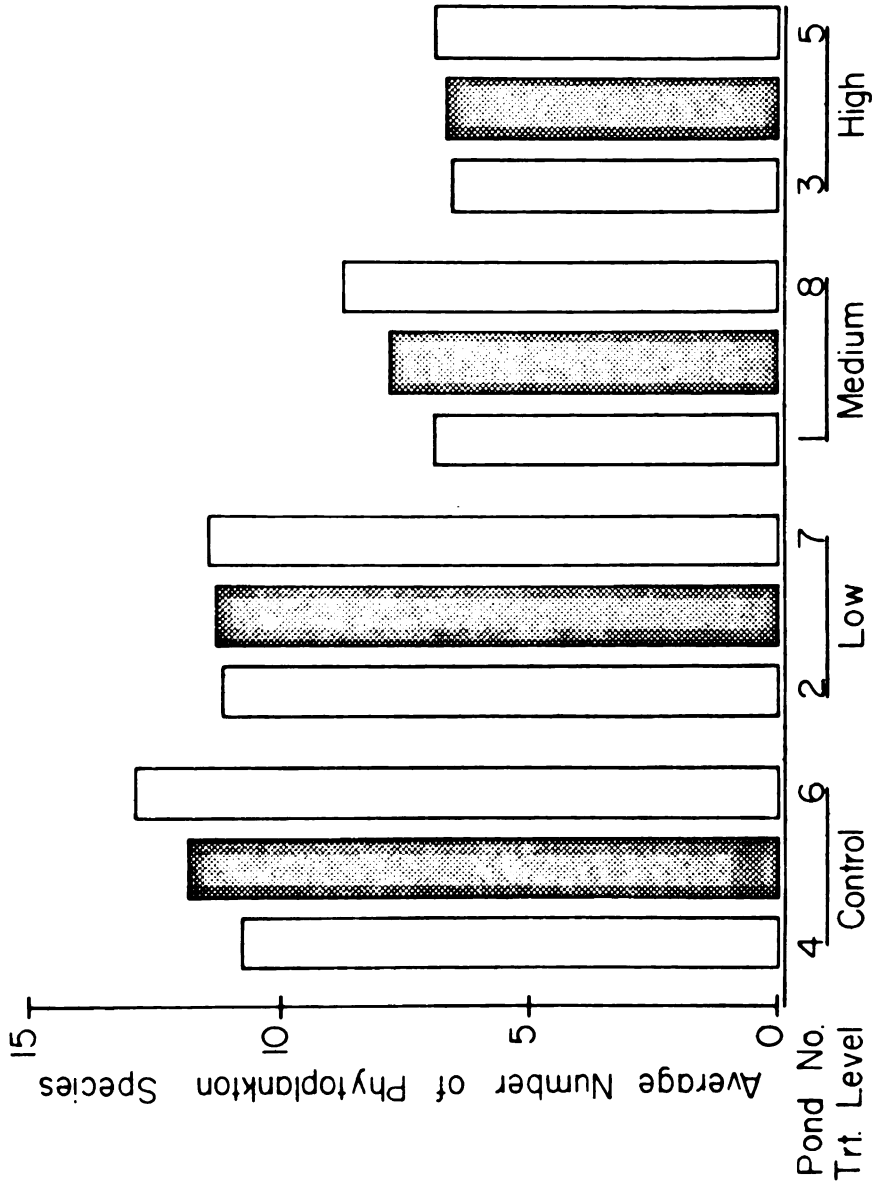

Average Number of Phytoplankton Species
15
10
5
0
Pond No.
Trt. Level
4
6
Control
2
7
Low
1
8
Medium
3
5
High

Figure 13.

Figure 14. Average number of summer phytoplankton species. Summer 1969. Clear area represents individual pond. Cross hatched area represents treatment mean. Based on 9 sampling dates.

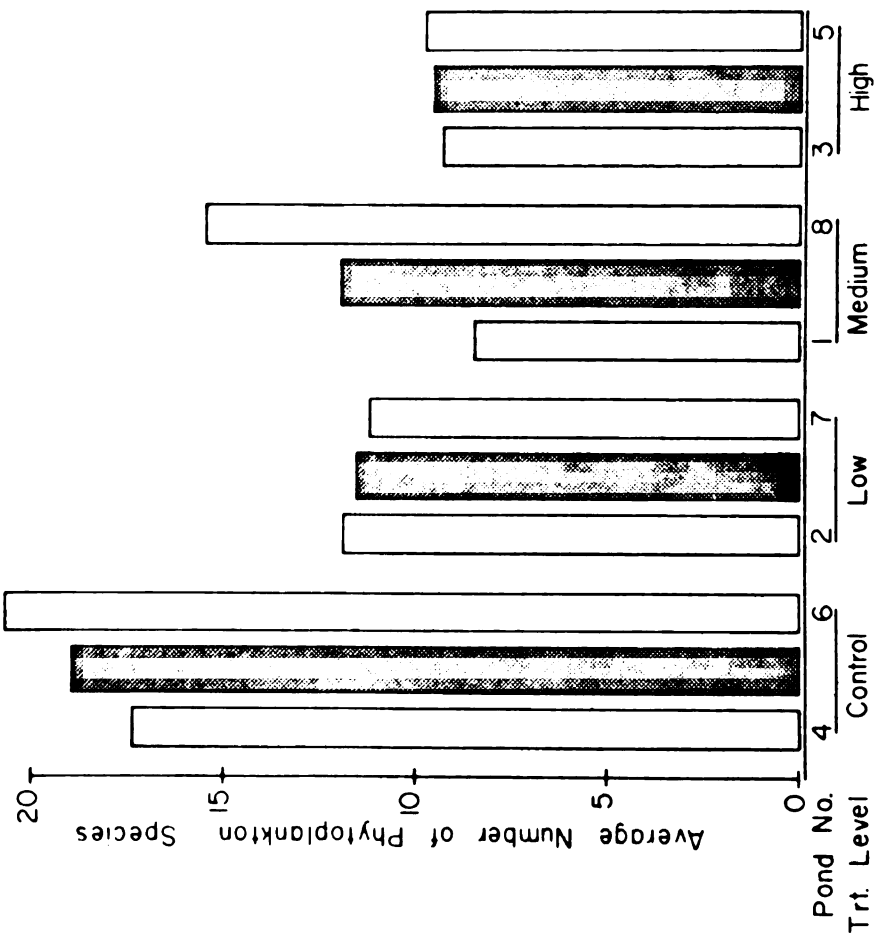

Average Number of Phytoplankton Species
20
15
10
5
0
Pond No.
Trt. Level
4
6
Control
2
7
Low
1
8
Medium
3
5
High

Figure 14.

The high treatment level ponds were quite alike in their species composition. Several species of Cryptomonas were dominant in both ponds at various times during both summers. Pleodorina californica and several representatives of the genus Chlamydomonas were abundant in both ponds. The greatest difference in species composition occurred in 1969 when Chlorella sp. and several species of Scenedesmus became extremely abundant in pond 5 H, sometimes reaching densities of 500,000 cells/ml. At the same time, pond 3 H had a bloom of Tetraspora lacustris, a species which occurred in pond 5 H only in the early summer of 1969. Perhaps the most striking aspect of the species composition of the high ponds was the absence of Microcystis aeruginosa. This species occurred in both the low nutrient ponds and both the medium nutrient ponds and is often found in eutrophic waters (Hammer, 1964), yet never occurred in the high treatment ponds.

There were some very marked species distributions with treatment level: Erkenia subsequiciliata occurred only in the control ponds; Microcystis aeruginosa occurred only in the low and medium nutrient ponds; Chlamydomonas pertusa Chodat, while abundant only in pond 5 H, occurred in the medium and high ponds but not in the control or low treatment ponds.

However, the phytoplankton species composition of the ponds also showed some remarkable consistencies

considering the great differences in chemical environments created in the experiment. Cryptomonas erosa Ehrenberg occurred in all the ponds during both summers with the exception that it was lacking entirely from pond 2 L during the summer of 1968. It was often abundant in all the ponds where it occurred, and shared relative dominance even in highly fertilized ponds with high populations of other species. Chlorella sp. and Tetraspora lacustris also occurred in all the ponds throughout the study. They often became very abundant in the medium and high nutrient ponds, and occurred with regularity in the low and control ponds. The genus Scenedesmus was well represented in all the ponds with the species S. bijuga (Turp.) Lagerheim occurring in every pond and other species being abundant in the higher treatment level ponds. The last group with general occurrence was the colonial Volvocales, which occurred in all the ponds.

The most simple measure of species diversity--the average number of species occurring in each pond--shows a consistent trend in both years (Figures 13 and 14). In 1968, the control ponds had the greatest average number of species, 12.8, with the other treatments ranging downward in order of increasing nutrient input to a high treatment average of 6.7 species/pond/summer. The same general trend existed in 1969: the control ponds had the greatest average number of species and the high treatment ponds the

lowest number. Hall et al. (1970), utilizing the data of Mulligan (1966), show the same trend in 1965 with the low ponds having the highest average number of species and the high treatment pond the lowest. In 1967, however, the highest average number of phytoplankton species occurred in their medium nutrient level ponds, based on only a few sampling dates. The control ponds in the present experiment markedly increased in the number of species generally present from a 1968 average of 12.8 to a 1969 average of 19.0. With the exception of both the low nutrient ponds and pond 1 M, all the ponds increased in average number of species present in 1969. Pond 8 M showed the highest increase.

The planktonic algal composition and species diversity of any body of water is controlled by a number of interacting factors. Margalef (1964) states that the increased production caused by eutrophication increases the ratio of algal production to biomass which lowers diversity through uneven partitioning of biomass among the different species. Using primarily the data of Järnefelt (1958), Margalef demonstrates a consistent trend of decreasing phytoplankton diversity with increasing eutrophy in lakes. Yount (1956), working on the attached diatoms of Silver Springs, Florida, says that areas with the highest primary production had the lowest number of diatom species, whereas areas with the greatest number of diatom species had considerably

lower production. Hohn (1961), however, questions some of these data, but also demonstrates a decrease in average number of diatom species at the higher production site. Ewing and Dorris (1970) found no correlation between phytoplankton diversity and nutrient concentration in nine experimental fish ponds in Kansas.

One explanation for the decrease in species diversity or change in the species composition with increasing nutrient input is that the nitrogen and/or phosphorus concentrations may create intolerable physiological conditions for certain species. High concentrations of these nutrients, especially phosphorus, may be lethal to many species (Chu, 1943; Rodhe, 1948; Lund, 1964). However, Järnefelt (1952), studying more than 300 Finnish lakes, found only six species of plankton algae restricted to oligotrophic waters, but he found more than 30 restricted to eutrophic waters. In the present study, some species seem to have been inhibited by the high nutrient conditions in the fertilized ponds. Uroglenopsis sp. occurred only in the control ponds after the fertilization schedule was begun, but had occurred in all the ponds just prior to fertilization. Lund (op. cit.) suggests that this species may be particularly sensitive to high phosphorus concentrations.

In the control ponds of the present experiment, and perhaps in the low nutrient ponds, which differed only slightly in nitrogen and phosphorus content from the

controls, some phytoplankton organisms may have been excluded due to the low nutrient conditions. In the fertilized ponds, except for perhaps the low treatment ponds, there was apparently more than enough nitrogen and phosphorus to permit the occurrence of most fresh-water algae (Chu, 1942, 1943).

In the present experiment, algae may have been in competition for chemical factors other than nitrogen and phosphorus, such as vitamins (Provasoli, 1963), minor nutrients (Goldman, 1961), or carbon dioxide (Steemann Nielsen, 1955). One factor which occurred only in the higher treatment ponds in 1969 was light limitation or self-shading (Talling, 1960). Any organism subject to light shock would have been at a distinct disadvantage in ponds 8 M, 3 H, and 5 H in August 1969 as it was circulated out of the virtual darkness below one foot from the surface into the light (Javornicky, 1970).

The opportunities for other types of interactions within various treatments are great. Either autoinhibition through the secretion of some organic molecules or allelopathic effects among various algal species could well determine certain species compositions and diversities. See Hartman (1960) for a summary of work in this area.

Chemical changes other than the increase of those nutrients added to the ponds accompanied the treatment regime. The pH increased quite markedly with increasing

nutrient input from a general range of 8.8 to 9.3 in the control ponds to a maximum of 11.0 in pond 8 M in late August of 1969. Many algal species must certainly be sensitive to the direct effects of high pH (Sparling and Nalewajko, 1970). In the high treatment ponds, when the pH reached 10.5 and higher, those species best able to utilize bicarbonate as a carbon source for photosynthesis would have been favored. Felfoldy (1960) shows particularly clearly that many Scenedesmus spp., which were often dominant during blooms in the high ponds during the summer of 1969, are able to utilize this carbon source. However, Chlorella sp. was also abundant at some of these times, and Steemann Nielsen and Jensen (1958) show that Chlorella pyrenoidosa Chick can utilize bicarbonate only to a slight extent.

The effect of extremely high pH on the phytoplankton species diversity was variable. In pond 8 M, where conditions were most severe and lasted over the longest period of time, the average number of species in June and early July 1969, before the extreme primary productivity and high pH, was 20.5. In late July and August the number of species in the pond fell to an average of 10.8. In ponds 3 H and 5 H, which experienced high pH levels for only a month (August 1969), the average number of phytoplankton species did not change. However, the conditions were less severe than in pond 8 M.

While the concentrations of nitrogen and phosphorus certainly changed with increasing fertilizer level in both years, so did the nitrogen to phosphorus ratio (Table 6). The control ponds averaged an N/P ratio of 3.5 for both summers, and the ratio in the high treatment ponds increased to 7.0. The N/P ratio of 3.5 in the control ponds is quite low (Sakamoto, 1966). While the relative uptake of each element by plants may determine the N/P ratio, it would seem more likely that the addition of fertilizer with an N/P ratio of 8:1 was responsible.

Pearsall (1932) states that desmids tend to occur in waters with low nitrate to phosphate ratios. This agrees well with the desmid distribution according to treatment level in the present study. Of the three species of desmids which occurred in the ponds, none was present in the medium and high nutrient ponds, and Staurastrum sp. occurred only in the control ponds. Chu (1943), however, found that the N/P ratio had no effect on the planktonic algae he studied, so long as the concentrations of these nutrients remained within the optimal range.

The ratio of divalent to monovalent ions in the pond water also changed along with treatment level. Miller and Fogg (1957) state that algal growth may improve as this ratio decreases. This ratio dropped from an average of all the ponds of 8.2, measured before the addition of fertilizer, to a low in the high ponds of 4.4 measured in mid-August of 1969. This change was due mainly to the

differential addition through the fertilizer of relatively large amounts of calcium and especially potassium (Table 8).

Selective zooplankton grazing could also play a key role in the determination of the composition and diversity of the phytoplankton. Edmondson (1965) shows that certain rotifers seem to have rather particular feeding preferences as to algal species and sizes. Gliwicz (1969a) demonstrates that rotifers and small cladocerans, which tend to be dominant in eutrophic lakes, feed on minute food particles, whereas the filtering copepods present in more oligotrophic lakes feed on larger food items. Hrbacek et al. (1961) and Brooks and Dodson (1965) propose that the larger cladocerans can consume larger food items; the presence or absence of these large bodied cladocerans, which is partially a function of the fish predation in the water body, could alter the phytoplankton species assemblage.

F. E. Smith (1969) argues on theoretical grounds that enrichment of a three trophic level aquatic ecosystem stimulates primarily the plants and carnivores. As increased enrichment places greater predatory pressure on the herbivores, the most susceptible species may be eliminated, thereby narrowing the range of grazing on the algae. The algae relieved of grazing pressure could then increase differentially. Such differential shifts would tend to reduce the diversity of plants.

This hypothesis does not appear to be supported by the data reported by Hall et al. (1970) from ponds of three

TABLE 8.--Photoelectric spectrometer analysis of the fertilizer used in the nutrient enrichment and concentrations of elements which would result in the ponds. B.D. indicates below detection.

Weight of Fertilizer/Unit Volume	Results of photoelectric spectrometer analysis of fertilizer (Elements mg/l)									
	P	Ca	K	Na	Mg	Zn	Mn	Fe	Cu	B
10 g/l	615	352	419	17.9	4.5	0.9	0.5	11.5	B.D.	0.4
	Concentrations which would result in the ponds (Elements µg/l)									
	P	Ca	K	Na	Mg	Zn	Mn	Fe	Cu	B
3 lbs (1361 g)/Pond (8×10^5 l)	103	59	71	3	0.8	0.15	0.09	1.9	B.D.	0.07
8 lbs (3629 g)/Pond (8×10^5 l)	275	158	188	8	2.0	0.40	0.24	5.2	B.D.	0.18
16 lbs (7257 g)/Pond (8×10^5 l)	550	315	376	16	4.0	0.80	0.48	10.3	B.D.	0.37

nutrient levels having a constant fish predation pressure. The fish increased the zooplankton species diversity at all nutrient levels, thereby presumably widening the range of grazing on the algae. If we assume a direct relationship between zooplankton species diversity and phytoplankton species diversity, then the ponds with fish should have shown a marked increase in phytoplankton diversity. From the data available in Hall et al. there appears to have been no notable increase in the species diversity of the phytoplankton. It would seem that the presence or absence of fish in these ponds had little impact on phytoplankton diversity, and that the nutrient level was of much greater importance. Losos and Hetesa (1969), in a pond experiment in which planktivorous fish were introduced to some of the ponds, report that the number of large cladocerans was severely reduced in the ponds in which fish were present. In these same ponds, when the amount of zooplankton grazing was reduced, it appears that the number of phytoplankton species increased. This would support the concept that heavy zooplankton grazing may decrease phytoplankton species diversity.

The intensity of grazing in a pond, rather than the diversity of the grazing community, may be related to the phytoplankton species diversity. In the present study, zooplankton biomass and therefore presumably grazing pressure generally increased with treatment level, and phytoplankton species diversity decreased with treatment

level. Increased grazing pressure would give greater advantage to those phytoplankton species able to grow rapidly, and would tend to selectively remove slowly growing species. Because slowly growing algal species may have a higher ratio of carotenoids to chlorophyll a than do actively growing species (Margalef, 1964), this selective removal could alter the carotenoid to chlorophyll a ratio, which preliminary analyses show decreased with treatment level. Further analysis of these data is needed and intended. Margalef also suggests that increased nutrient input may decrease the carotenoid to chlorophyll a ratio.

The only time the ponds in the present experiment were completely free of zooplankton grazing was at the time of the cladoceran zooplankton disappearance from ponds 3 H, 5 H, and 8 M in late summer of 1969. The only one of these ponds to show a marked change in species diversity after the zooplankton disappeared was pond 8 M. In that case, the species diversity decreased. However, as the chemical conditions were extreme in these ponds, the phytoplankton may have responded atypically. It is still most likely that overall the nutrient treatment level was the primary factor in determining phytoplankton species diversity.

One of the most important and intriguing species distributions occurring in this study was that of Microcystis aeruginosa, which was present in the low and

medium nutrient ponds but never, even in small amounts, in the control or high ponds. The work of Gerloff and Skoog (1957) and many others demonstrating the prevalence of this species in eutrophic waters, would preclude its presence in the control ponds, especially considering their low nitrogen levels. The real question is why no Microcystis occurred in the high ponds. Zehnder and Gorham (1960) have shown through laboratory studies that M. aeruginosa is not sensitive to elevated nitrogen and phosphorus levels such as those that occurred in the high ponds. However, Hammer (1964) states that of those lakes containing M. aeruginosa the ones with the highest phosphate concentrations usually had less M. aeruginosa than those lakes with less phosphate. Pearsall (1932) and many others have pointed out that blue-green algae often tend to appear in a body of water in midsummer when macronutrients are in low concentrations. While blue-green algae are thought of as indicative of eutrophic conditions, this may not include such high nutrient conditions as those created in the high ponds. M. aeruginosa rarely occurs in sewage ponds (deNoyelles, 1967). Fitzgerald (1969) demonstrates a bacterial sized organism present in sewage plant effluent which inhibits the growth of M. aeruginosa but does not inhibit the growth of Chlorella pyrenoidosa. Perhaps the high nutrient ponds allowed the growth of such an organism. Vance (1965) suggests that certain species of algae may secrete substances inhibitory to Microcystis.

McLachlan and Gorham (1961) suggest the possibility of a potassium inhibition of M. aeruginosa. Potassium averaged 5 mg/l in the high nutrient ponds, and only 3 mg/l in the medium nutrient ponds, but there certainly were times when the potassium levels were lower in the high nutrient ponds. Some data suggest that the zooplankton may influence M. aeruginosa growth by grazing on nannoplankton algae and bacteria which may be competing directly with Microcystis or secreting inhibitory substances. There were times of heavy grazing in both ponds 3 H and 5 H in 1968, but never to the extent that immediately preceded the bloom of M. aeruginosa in pond 8 M in 1968 and 1 M in 1969.

Zooplankton Species Composition and Diversity

Crustacean zooplankton species common to all the ponds during the summer months included primarily Ceriodaphnia reticulata and Diaptomus pallidus, occasional representatives of the family Chydoridae (Chydorus sphaeicus O. F. Müller, Alona costata Sars, and Pleuroxus denticulatus Birge), and sometimes Bosmina longirostris (O. F. Müller). Several species of cyclopoid copepods were often present, none of which contributed more than 5 per cent of the total zooplankton biomass in any pond.

Common rotifers were Asplanchna sp., Brachionus sp., Filinia pejleri Hutchinson, Keratella cochlearis (Gosse),

and Polyarthra sp. The rotifers generally represented only a small fraction of the total zooplankton biomass.

The nutrient treatments had little effect on the species occurrence in the ponds, but had drastic effects on the relative abundances of particular species. All the above-mentioned species occurred in all the ponds at some time during both summers, but in the higher treatment level ponds one or two species clearly dominated the zooplankton community.

As shown in Figure 15, C. reticulata totally dominated the species composition of the high and medium treatment ponds in 1968. It was only in the two low treatment ponds that Bosmina longirostris was abundant enough to be of importance.

If species diversity is considered to be the evenness with which biomass is distributed among species rather than the overall number of species present (Hall et al., 1970), there is a marked trend of decreasing diversity with increasing nutrient level in 1968. The control ponds had a relatively even distribution of biomass between dominant species, while the low treatment ponds had a biomass distribution among three or four dominant species. In the medium and high level ponds, C. reticulata dominated to the extent of making up 80 to 90 per cent of the zooplankton biomass.

In 1969, several species were greatly decreased in importance in certain ponds (Figure 16). Diaphanosoma

Figure 15. Average crustacean zooplankton species composition by per cent of total biomass. Summer 1968. Individual species as indicated in the key. Clear area represents several rarely abundant cladoceran species.

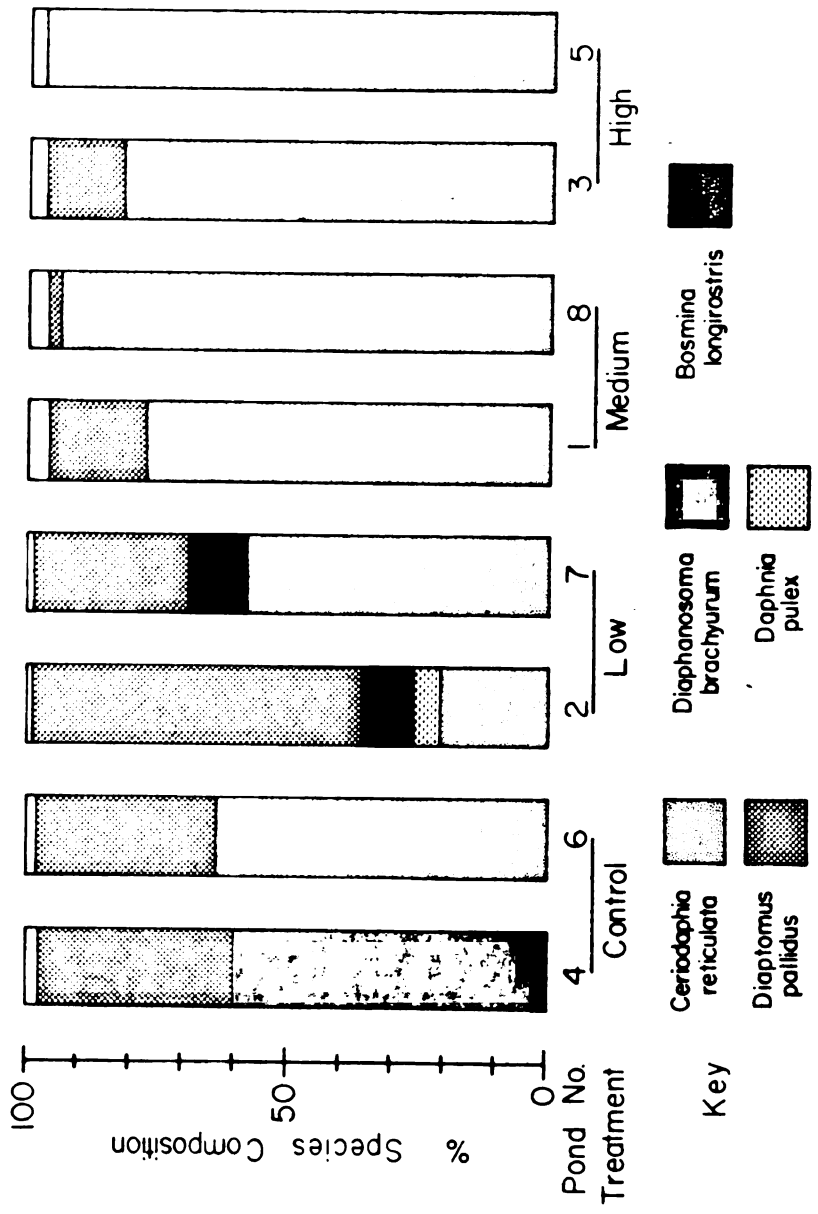

100
50
0
% Species Composition
Pond No.
Treatment
4
6
Control
2
7
Low
1
8
Medium
3
5
High
Key
Ceriodaphnia reticulata
Diaptomus pallidus
Diaphanosoma brachyurum
Daphnia pulex
Bosmina longirostris

Figure 15.

Figure 16. Average crustacean zooplankton species composition by per cent of total biomass. Summer 1969. Individual species as indicated in the key. Clear area represents several rarely abundant cladoceran species.

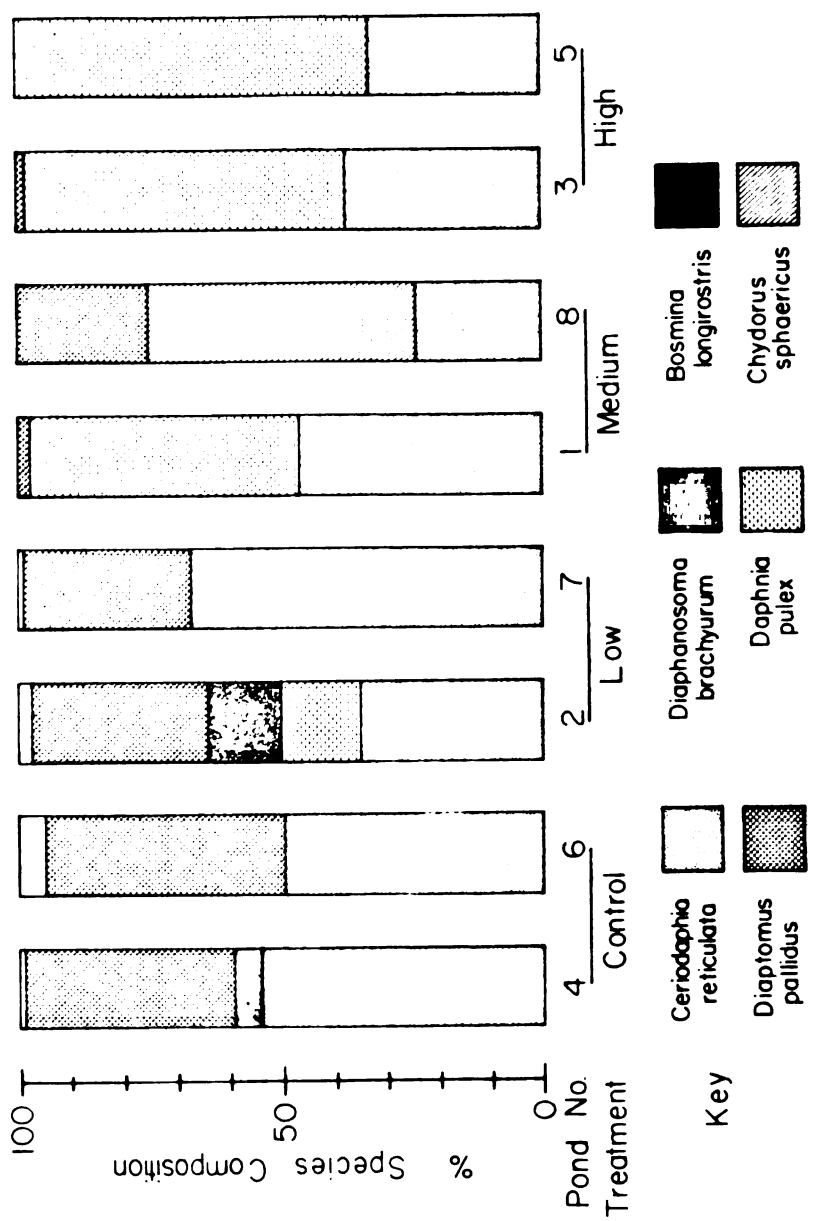

100
50
0
% Species Composition
Pond No.
Treatment
4
6
Control
2
7
Low
1
8
Medium
3
5
High
Key
Ceriodaphnia reticulata
Diaptomus pallidus
Diaphanosoma brachyurum
Daphnia pulex
Bosmina longirostris
Chydorus sphaericus

Figure 16.

brachyurum was almost completely replaced by C. reticulata in pond 4 C. Bosmina longirostris greatly decreased in importance in pond 7 L. Daphnia pulex was much more abundant in the high nutrient ponds in 1969. There was also an increase and almost complete dominance of pond 8 M zooplankton by Chydorus sphaericus. This was due primarily to the extremely low zooplankton biomass of any type; during the latter half of the summer, there was virtually no crustacean zooplankton in pond 8 M at all.

Weekly counts of ponds 1 M, 3 H, and 5 H in 1969 showed that these ponds were dominated by two different species at two different times. D. pulex was virtually the only zooplankter in these ponds early in the summer; later it disappeared and C. reticulata became dominant. At any one time, then, there was almost complete domination by just one species.

In comparing the species composition of the ponds each year, a definite order in zooplankton succession as influenced by nutrients becomes evident. With a slight increase in nutrient concentration, the number of important species increased, as seen in pond 2 L and 7 L in 1968, and in pond 2 L in 1969. With a greater increase in nutrients, C. reticulata became dominant, as seen in all the medium and high nutrient ponds in 1968. As the nutrients increased even more, D. pulex became relatively abundant in the early summer, as seen in ponds 3 H and 5 H in 1969.

All the zooplankton species occurring in these ponds are characteristic of ponds throughout the Northern Hemisphere. Both D. pulex and the entire genus of Ceriodaphnia are considered by Hutchinson (1967) to be pond forms. Elgmork (1966) determined which zooplankton species were pond forms by comparing the length of survival of many zooplankton species washed into ponds during flooding. Those which survived are considered pond forms. He determined that Ceriodaphnia spp., Diaphanosoma spp., and Bosmina longirostris are all definitely pond organisms. Workers in both Europe and the United States report similar zooplankton species composition of ponds (Bucka and Kyselowa, 1967; Armitage and Smith, 1968). Most reports indicate a greater number of species than reported in the Cornell ponds. Bucka and Kyselowa (op. cit.), working on experimental ponds in Poland, report almost all the species recorded from the Cornell ponds as well as three additional species of Ceriodaphnia and two additional species of Daphnia. As in the present study, however, only four species were abundant in the Polish ponds, and these were the same or members of the same genus as those which were abundant in the Cornell ponds. Patalas and Patalas (1966) state that the number of species present and dominant in a body of water is strongly dependent upon the size of the water body. Elgmork (1964) suggests the amplitude of change within the zooplankton community decreases with

increasing size of the water body. Since the Polish ponds studied by Bucka and Kyselowa are 60 times larger than the Cornell ponds, it may not be surprising that there was a difference in the number of species present. The stage of succession of a pond may also play a major role in determining the number of species which occur. In later seral stages macrophytes become abundant, increasing the physical diversity of the pond. In the ponds in this study, only one macrophyte, Chara sp., was prominent, and this plant alters the physical relief and heterogeneity only slightly.

There are few published data comparing the zooplankton species composition and diversity in water of different trophic types. Patalas and Patalas (1966), studying zooplankton from 650 Polish lakes of different trophic types, report a definite decrease in the number of dominant species in the more eutrophic lakes. The average number of dominant zooplankton species in mesotrophic lakes is 3.6; in ponds the average is 2.8. Patalas and Patalas show no increase in number of dominant species in slightly eutrophic lakes, whereas in the present study the low fertilizer level ponds had a greater number of dominant species than the control ponds. Four species were abundant in pond 2 L compared with two abundant species in the control ponds and only one in the higher fertilized ponds. Borecky (1956) and Cummins et al. (1969), both working

on the same eutrophic reservoir, report four or five species abundant during the summer months.

Species compositional changes similar to those observed in the present study have been reported in Gliwicz (1969a, 1969b). He notes an increase in the dominance of the members of the genus Bosmina in more eutrophic lakes, while the abundance of calanoid copepods decreases with increasing eutrophy. Members of the genus Daphnia also decrease slightly in importance in more eutrophic lakes and reservoirs. Bakhtina (1968) reports that all the crustaceans disappeared from the highly fertilized ponds in his study, in a manner similar to the 1969 cladoceran decline in the highly fertilized ponds of the present experiment.

Hall et al. (1970), working at the Cornell pond site with a similar nutrient treatment regime, show little or no change in species dominance with increasing treatment level. They do report that Bosmina longirostris was essentially restricted to the lower treatment levels containing fish, but apparently its abundance was not enough to influence the species dominance. They also showed a similar response of D. pulex dominating the high nutrient ponds in the early summer as occurred in the present study in 1969. Throughout most of the summer months, Hall et al. show C. reticulata to be the dominant species regardless of treatment level.

In general, many chemical factors were altered with increasing fertilizer level. One explanation for the decrease in number of dominant species in the higher fertilized ponds may be that the chemical conditions existing there were outside the physiological tolerance limits for particular zooplankton species.

It is probable, however, that competitive interactions played a part in reducing the number of abundant species. Gliwicz (1969a, 1969b), in studying several Polish lakes and reservoirs, states that the zooplankton food base shifts from nannoplankton algae in oligotrophic lakes to bacteria in eutrophic lakes. If true, then species best adapted to feed on the small bacteria would be at an advantage in eutrophic lakes (Saunders, 1969) over those species able to use only nannoplankton algae or some fraction of the nannoplankton algae. Because the size diversity of food items decreases from oligotrophic lakes to eutrophic ones, the number of species feeding on this food may also decrease. There are no data on the presence and abundance of bacteria in the Cornell ponds, but there was in fact, an increase in the abundance of nannoplankton present in the higher treatment ponds.

One possible explanation for the decrease in crustacean species diversity with increasing nutrients is that interspecific competition may increase in importance with increased nutrient input. If a body of water is quite low in an important plant nutrient and thus has a very low

density of phytoplankton algae (the control ponds in the present study), the only zooplankton species present will be those which can grow and reproduce at low density food levels. The density of these species, in the present case C. reticulata and D. pallidus, may be so low that their grazing can have only slight impact on the density of the phytoplankton and thus little effect on the amount of food available. For this to be true, the rate of nutrient regeneration must be such that the phytoplankton can grow enough to replace small grazing losses but not enough to markedly increase in density. If the amount of nutrients available to the plants is increased and an increase in phytoplankton density follows (the low nutrient ponds in the present study), more zooplankton species might be able to exist but still there would be little grazing impact on the amount of food available. In ponds 2 L and 7 L, D. pulex and B. longirostris were present along with C. reticulata and D. pallidus. With further increases in available nutrients and increased density of the phytoplankton (the medium and high nutrient ponds of the present study), one particular species may be able, at least on occasion, to reach high numerical densities. This species could then severely graze the phytoplankton, reducing it to a low density and thereby affecting populations densities of other zooplankton species. On at least one occasion, pond 8 M in 1968, the abundance of C. reticulata

was high enough to have an apparent effect on the phytoplankton densities. This effect is discussed in a later section.

The data of Hall et al. (1970) do not wholly support the idea that interspecific competition is minimal in low nutrient level ponds. In their experiment in 1967, fish were introduced into ponds of each nutrient level, and the fish almost completely removed C. reticulata. If the presence and abundance of zooplankton in their low (control) ponds are solely dependent upon the density of phytoplankton and not influenced by the presence and abundance of other zooplankton species, one would expect no change in the species composition and density with the removal of C. reticulata. However, small bodied rotifers and Bosmina longirostris increased. This is suggested in Hall et al. as a competitive response of the rotifers to the decrease of C. reticulata. In the present study, in ponds 8 M, 3 H, and 5 H in 1969, when C. reticulata disappeared there was only one case in which another species became abundant (Brachionus sp.), and that lasted only a short time. However, chemical conditions in these ponds were exceptional, and may have excluded other species.

FACTORS AFFECTING ZOOPLANKTON RESPONSE TO NUTRIENTS

High pH

As seen in Figure 12, the average summer zooplankton response to average chlorophyll *a* concentration was remarkably consistent with four notable exceptions. These exceptions are a decided contradiction of the initial hypothesis, that nutrient induced plant production would stimulate an increase in zooplankton biomass. The plant biomass was increased by high nutrient addition, as can be seen by the high chlorophyll *a* values recorded in both high nutrient ponds and pond 8 M. Not only do the average zooplankton values in these ponds fail to correspond with the chlorophyll *a* values, they are as low as some average values for the control and low nutrient ponds. At least in part, the low average zooplankton biomass in the higher treatment ponds was due to the complete disappearance of cladoceran zooplankton from ponds 8 M, 3 H, and 5 H during the midsummer after which the crustacean zooplankton never re-appeared.

This phenomenon was first noticed in pond 8 M in late July, by which time there were no rotifers or

crustacean plankton present in the pond. At that same time the pond phytoplankton were quite productive and the water had a definite green color. An initial explanation for the zooplankton disappearance was that the algae, while abundant, were an insufficient food source, and the zooplankton had not been able to maintain an adequate reproductive rate.

To test this idea, a life table experiment was initiated on 27 July 1969. There were two treatments: pond 8 M water, and Millipore filtered pond 8 M water to which was added 10 Klett units of Ankistrodesmus falcatus (about 100,000 cells/ml). The water for both these treatments was collected each day at mid-morning. Ten newly born Ceriodaphnia reticulata from laboratory cultures were placed in small cups containing the two types of water, then the growth, reproduction, and survivorship of the animals were noted. Unexpectedly, there was a very high death rate of animals in the unaltered pond water, rather than decreased reproduction of these animals (Figure 17). Since the animals in the Millipore filtered pond water did not die, it was thought that the pond algae must in some way be toxic. To test this hypothesis, another life table experiment was begun on 2 August 1969. This experiment had four treatments: pond 8 M water, A. falcatus in Millipore filtered pond 8 M water, Foerst centrifuged pond 8 M algae resuspended in Millipore filtered dechlorinated tap water, and A. falcatus in

Figure 17. Survivorship of Ceriodaphnia reticulata in two types of water: Solid line--Pond 8 M water; Dotted line--Pond 8 M water Millipore filtered with 100,000 Ankistrodesmus falcatus cells/ml added. Time zero equal to 27 July 1969.

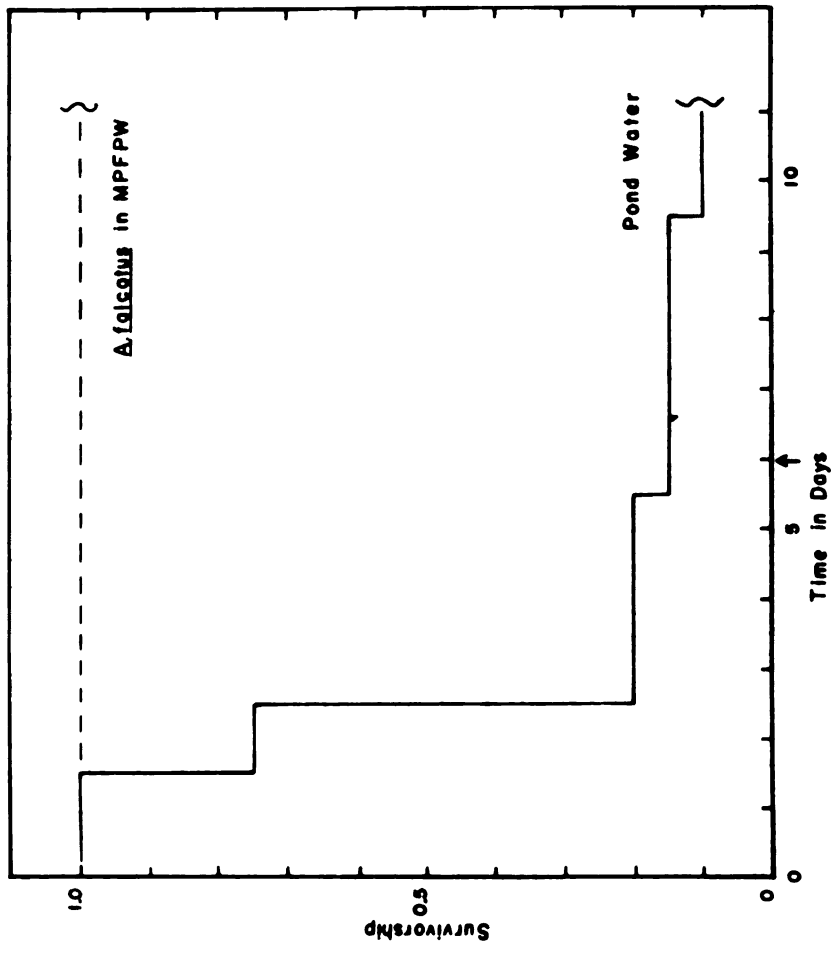

A. falcatus in MPFPW
Pond Water
Survivorship
1.0
0.5
0
0
5
10
Time in Days

Figure 17.

Millipore filtered dechlorinated tap water. Again, growth, reproduction, and survivorship were measured, and again, at least for the first three days, the unaltered pond 8 M water caused rapid death to the animals (Figure 18). Completely contrary to the hypothesis was the excellent growth and survivorship of animals in Millipore filtered dechlorinated tap water with pond 8 M algae added.

It was obvious that the algae themselves were not toxic or unnutritious. The only conclusion to draw was that the water itself was toxic, but that the toxic factor had somehow been altered in the experimental situation. The only important manipulation of the pond 8 M water from which the pond algae were removed was the Millipore filtration itself. Therefore, the pond 8 M water was tested before and after Millipore filtration for changes in chemical factors: oxygen content, ammonia content, and pH were all tested (Table 9). The oxygen content of the water was high at this time; filtration lowered the oxygen content but did not reduce it below an adequate level. The ammonia content was not particularly high to begin with and changed only slightly upon filtration. The pH was quite high at this time, and decreased slightly upon filtration. This suggested that the high pH of pond 8 M water was causing the animal mortality, and the slight change upon filtration indicated that there might exist a narrow threshold of pH from non-toxic to toxic.

Figure 18. Survivorship of Ceriodaphnia reticulata in three types of water: Solid line--Pond 8M water; Dotted line--Pond 8 M water Millipore filtered with 100,000 Ankistrodesmus falcatus cells/ml added; Dot-dash line--Pond 8 M algae in Millipore filtered dechlorinated tap water. Time zero equal to 2 August 1969.

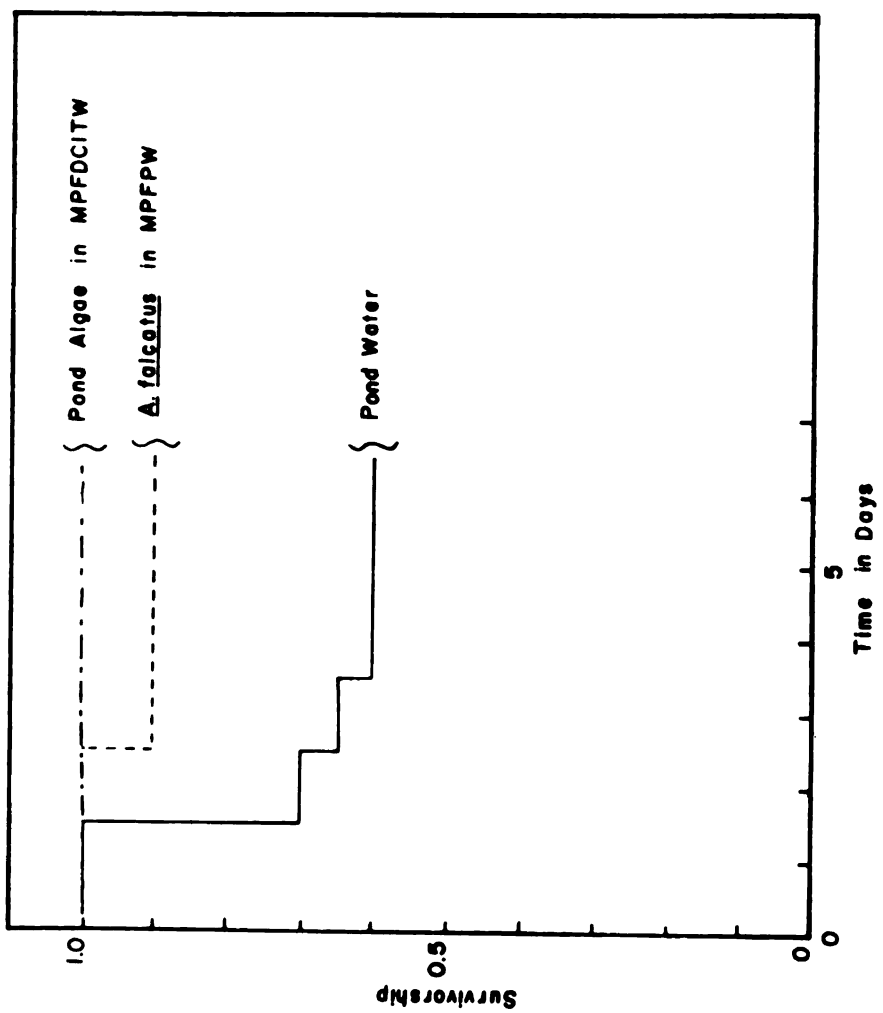

Pond Algae in MPFDCITW
A. falcatus in MPFPW
Pond Water
1.0
0.5
0
Survivorship
0
5
Time in Days

Figure 18.

TABLE 9.--Chemical factors in pond 8 M on 4 August 1968 and changes produced by Millipore filtration of the pond water.

	Oxygen Content (in mg O_2/l)	Ammonia Content (in µg N/l)	pH
Column of pond water	14.7 ± 0.09	46.2	10.8
Millipore filtered column of water	6.7 ± 0.12	42.0	10.6
Pond bottom water	5.5 ± 0.08		
Column of water left in dark 4 hours	10.9 ± 0.06		

A very simple experiment was performed to determine if such a narrow threshold exists. Three pH conditions were created experimentally, starting with Millipore filtered dechlorinated tap water and 10 Klett units A. falcatus to which a strong base (sodium hydroxide) was added. The pH was accurately adjusted by back titration using a dilute solution of hydrochloric acid. The three pH conditions created were 11.2, 10.8, and 10.4. The last was a "salt control"; that is, it had as much base added as the others, and more acid so as to neutralize the base. It therefore had more of the resulting salts than the other two treatments. Water of each pH level was placed in five Nalgene hollow stoppers to which were added two Ceriodaphnia reticulata less than 12 hours old, born of mothers that had survived in untreated pond 8 M water during the life table experiment. The stoppers were then put in a constant

temperature chamber at 21°C. All the animals placed in the 11.2 pH water died in less than 18 hours, while only one animal in the 10.8 pH water died and two animals in the salt control died. This experiment demonstrates a clear threshold of mortality, where a change of 0.4 pH units changes the water from almost totally harmless to completely toxic. Unfortunately, just where in terms of the pH scale such a threshold is cannot be determined from these data; the pH of the treatments decreased rapidly due to the respiration of animals and algae and exposure to atmosphere. Further incidental evidence of a pH mortality threshold is provided by the life table experiments (Figures 17 and 18). Throughout all the first life table experiment and for the first 3 days of the second life table experiment, the mid-morning pH of the pond 8 M water was 10.9 to 11.0. After 5 August 1969 (day 3 of the second life table experiment), the pH dropped below 10.8 (Figure 19) and the water was no longer lethal. This would seem to indicate a threshold of C. reticulata mortality when the pH is greater than 10.8.

If this hypothesis is correct and accounts for the decrease and loss of crustacean zooplankton in pond 8 M, there should be close agreement between the pond pH levels and the density of crustacean zooplankton once the pH reaches a high level. This is what was found (Figures 19, 20, 21, and 22). Both the high nutrient ponds show a marked decrease and disappearance of C. reticulata and all

Figure 19. Ceriodaphnia reticulata biomass per liter and pond pH. Pond 8 M 1969.

Figure 19.

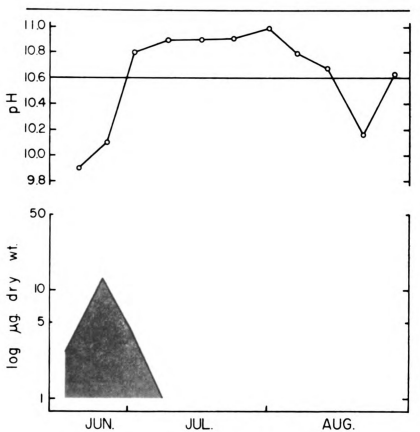


Figure 20. Ceriodaphnia reticulata biomass per liter and pond pH. Pond 3 H 1969.

Figure 20.

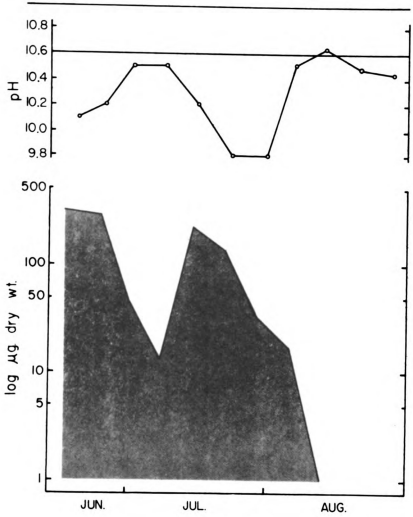

10.8
10.6
10.4
10.2
10.0
9.8
pH
500
100
50
10
5
1
log µg dry wt.
JUN.
JUL.
AUG.

Figure 21. Ceriodaphnia reticulata biomass per liter and pond pH. Pond 5 H 1969.

Figure 21.

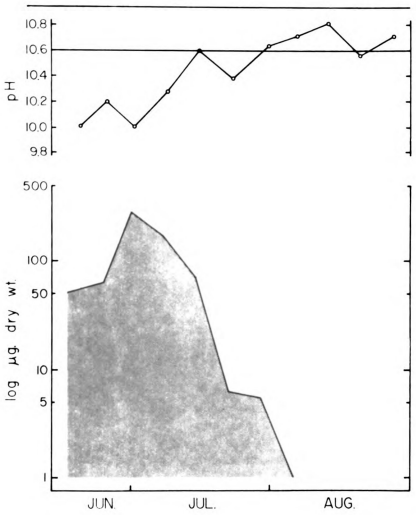
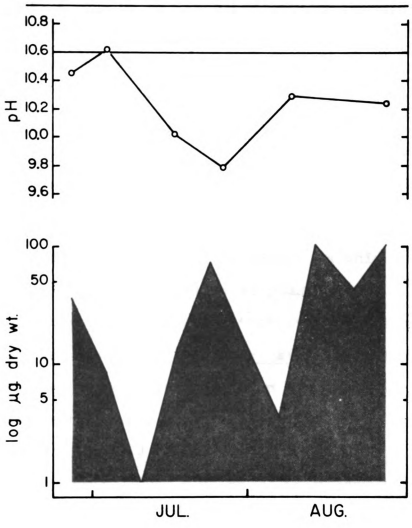

10.8
10.6
10.4
10.2
10.0
9.8
pH
500
100
50
10
5
1
log µg dry wt
JUN.
JUL.
AUG.

Figure 22. Ceriodaphnia reticulata biomass per liter and pond pH. Pond 1 M 1968.

Figure 22.



other crustacean zooplankton in mid-August. This disappearance correlated well with a mid-morning pH of 10.6. This is not to suggest that a pH of 10.6 is lethal (the pH threshold experiment would suggest it is not), but the pH undoubtedly increased above 10.6 by afternoon. The correlation with 10.6 is simply an index that the pond pH probably reached a lethal point that day.

Pond 8 M also had a die-off of crustacean zooplankton earlier in the summer of 1969, and pond 1 M had a similar occurrence in early July 1968, showing that the mortality factor in all four ponds was not due to a simultaneous meterological occurrence such as unusually high temperatures.

The only other time the mid-morning pH was recorded at 10.6 was in pond 2 L on 9 July 1969. In this case there was no die-off of the crustacean zooplankton.

In no other ponds during either summer were there complete disappearances of C. reticulata except for an extreme decrease in abundance in pond 8 M in 1968, which will be discussed in the following section.

On 8 July 1969, the time of the cladoceran die-off in pond 8 M, there were three species of Scenedesmus present (S. abundans, S. quadricauda, and S. sp.), totalling 200,000 colonies/ml. At this same time there were a few other species in the pond: Oocystis parva and 50 colonies/ml of Pandorina morum. During the time of the life table

experiments (27 July to 5 August 1969) P. morum became much more abundant and the Scenedesmus spp. became less so, while other phytoplankton species densities remained about the same. At the time when pond 8 M water was no longer lethal, the only changes in the pond water were that the Scenedesmus spp. had decreased to 100,000 colonies/ml while P. morum had reached 2,300 colonies/ml and the pH had dropped from 11.0 to 10.8.

In pond 3 H on 5 August 1969, the time of the cladoceran disappearance, the species composition of the phytoplankton was quite different. The phytoplankton was dominated by Tetraspora lacustris with 400,000 colonies/ml, and small amounts of O. parva and S. quadricauda were present. The other important phytoplankter was P. morum with 1,800 colonies/ml.

At this same time in pond 5 H there was a dense bloom of Chlorella sp. at a density of 900,000 cells/ml. Also present were three species of Scenedesmus totaling 50,000 colonies/ml and 103 colonies/ml of P. morum.

Pond 1 M, the only other pond to show these peculiar die-offs of cladocerans, had a phytoplankton species composition made up almost entirely of 440 colonies/ml of Pleodorina californica. In this case it would be hard to state whether the C. reticulata died of starvation or of the proposed pH mortality factor.

Pond 2 L, the only pond to have such a high pH and show no die-off of crustacean zooplankton, completely

lacked any colonial Volvocales. The phytoplankton composition included a trace of Microcystis aeruginosa, some Oocystis parva, and Tetraspora lacustris (50,000 cells/ml). A phytoplankton composition similar to this had occurred several times in several ponds with much greater numbers and without the accompanying high pH. The simultaneous occurrance of high phytoplankton abundance and a filamentous algae, mostly Rhizoclonium sp., present around the edges and on the bottom of pond 2 L, may explain why the pH was so high at this time.

The concept of aquatic plants acting to eliminate or inhibit other organisms by some chemical means is not new. Hardy (1936) was one of the first to propose an "exclusion" theory which Lucas (1947) later expanded to explain the often noted phenomenon in the ocean of high phytoplankton densities associated with low zooplankton densities in one locale and just the opposite in another area. Lucas proposed that the phytoplankton excreted chemical "ectocrines" into the environment which were either noxious to the animals and drove them away or killed them outright.

Many investigators have demonstrated the allelopathic effects of certain algal species on other algae and on microorganisms (Hartman, 1960; Fogg, 1962; Lefèvre, 1964; Fitzgerald, 1969) and showing the toxicity of algae, especially blue-green algae, to animals (Gorham, 1965; Gentile and Maloney, 1969; Shilo, 1964). Most of this work, however, has been concerned with the public health aspects

of these toxins on vertebrates. Little attention has been paid to their effects on aquatic invertebrates, although Arnold (1969) and Gentile and Maloney (op. cit.) do demonstrate the adverse effects of blue-green algae on zooplankton.

Similarly, little attention has been paid to the side effects of phytoplankton blooms such as increased pH due to high primary production during these algae blooms. Walter (1969), in a laboratory study, demonstrated the deleterious effects of high pH on Daphnia magna Straus and also demonstrated that these effects were independent of accompanying high oxygen concentrations of high primary production. Bogatova (1962) demonstrated lethal limits of pH between 10.6 and 11.0 for certain members of the Chydorid family. Ivanova (1969) showed that cladocerans have definite maximal filtering rates at certain optimal pH levels, and that the filtering rates are depressed as the pH gets higher or lower. Mortimer (1954) gives a pH value of 10.8 as the upper toxic limit for carp (Cyprinus carpio Linnaeus), and Swingle (1961) states that practically all pond fish die when the pH reaches 11.0. Walter (1969) demonstrates a toxic level of pH at 11.0 with British specimens of Daphnia pulex. To date, there has been no conclusive report of pH being lethal to zooplankton in a field situation.

There are several alternative explanations for the complete disappearance of crustacean zooplankton in ponds

3 H, 5 H and 8 M in late summer of 1969 and in pond 1 M in mid-July of 1968. Assuming that no important predator somehow entered those ponds unobserved, there would seem to be only three other possible explanations: starvation, excess or deficit of oxygen, or an allelopathic chemical.

In all but the case of pond 1 M in 1968, the idea of starvation through the lack of sufficient appropriate food can be eliminated. In ponds 3 H, 5 H, and 8 M, the algae which are known to be good food for cladocerans (Lefèvre, 1942) were present in great abundance throughout the late summer of 1969. This was clearly demonstrated in the case of pond 8 M in the life table experiments. It is not so easy to dismiss the possibility of oxygen depletion at night being the cause of the rapid zooplankton mortality. The night oxygen concentration was not measured, but pond 8 M was supersaturated when oxygen level was measured during the day in mid-August. Hall et al. (1970) report the night oxygen rarely reached dangerously low levels in their highly fertilized ponds at the same pond site, as the ponds usually mix thoroughly each night. Pacaud (1939) states that pond cladocerans are much more resistant to low oxygen concentrations than other cladocerans. Circumstantial evidence that the oxygen levels at least in pond 8 M of the present experiment never reached critically low levels during the late summer of 1969 is the fact that Fundulus diaphanus LeSueur, the banded killifish

accidentally introduced to pond 8 M some time before the experiment began, lived through the die-off of all cladocerans.

A reverse of the idea that low night oxygen levels caused the cladoceran disappearance is invoked by Bakhtina (1968). He stated the supersaturated water in the highly fertilized fish ponds he was studying was probably responsible for the death of all crustacean zooplankton. Walter (1969) demonstrated that this is unlikely, but because Bakhtina does not report pH values, it is impossible to show that his results were caused by high pH. One similarity between Bakhtina's studies and the present experiment is suggestive of elevated pH. He reports a peak Brachionus calyciflorus Pallas population soon after the disappearance of the crustacean zooplankton. In pond 8 M in mid-August there also occurred a remarkable population increase of Brachionus sp. This whole genus is known for its tolerance to high pH (Ahlstrom, 1940).

No blue-green algae occurred in the affected ponds at the time of the cladoceran disappearance. There is only one reported case of an allelopathic effect of a green algae inhibiting zooplankton growth and reproduction. This is the well known case in which Ryther (1954) claimed chlorellin or a like substance was responsible for the depression of the filtering rates of Daphnia magna. That a similar toxic chemical may have been responsible for the observed die-offs in the present experiment cannot be

dismissed easily, especially when the colonial Volvocales distribution is considered. During the die-offs, there were 50 colonies/ml of Pandorina morum in pond 8 M, 1,800 colonies/ml of P. morum in pond 3 H, 103 colonies/ml of P. morum in pond 5 H, and 440 colonies/ml of Pleodorina californica in pond 1 M. But in pond 2 L on 9 July 1969, when the pH reached 10.6, there were only 2 P. californica colonies/ml, and no die-off followed the high pH. However, it should be noted that while the pH values were quite constant in all four cases of cladoceran disappearance, the number of Volvocales colonies/ml varied from 50 to 1,800. During the two summers of the study, there were 12 times when the concentration of either Pleodorina or Pandorina was greater than 50 colonies/ml, and no marked decrease in cladoceran densities resulted. Regression analyses of the number of colonial Volvocales against the birth rate, the death rate, and the density of C. reticulata show no significant correlations. Further, in the life table experiment with pond 8 M water (5 August 1969), at the time the water was no longer lethal, the pH was decreasing yet the concentration of P. morum was 2,300 colonies/ml, the highest ever observed in the ponds.

It therefore seems unlikely that a direct allelopathic chemical factor is responsible for the cladoceran disappearance; however, the possibility of an interaction between the colonial Volvocales and the pH of the pond water cannot be denied. Perhaps it is the colonial

Volvocales which can photosynthesize well at high pH levels and thus drive the pH even higher. If Tetraspora lacustris and Rhizoclonium sp., which were abundant when the pH reached high levels in pond 2 L, are unable to photosynthesize well at high pH levels, they could not drive the pH to a lethal threshold. This would account for the absence of a die-off in pond 2 L.

A toxic factor such as that described by Ryther (op. cit.) does not seem to be supported by the present data. In fact, at least part of what Ryther described may well have been a pH mortality effect.

Ryther based his conclusions of the inhibitory effects of phytoplankton on zooplankton on three separate series of observations. First, he noted that the filtering rate of Daphnia magna decreased as the concentration of food particles increased. He interpreted this to be an increase of some inhibitory factor as the food concentration increased. Rigler (1961) and McMahon and Rigler (1965) and others have since demonstrated that the reduction in feeding rate is accommodation of the animals and occurs at what Rigler called the "incipient limiting concentration" above which the feeding rate is no longer proportional to the concentration of food but remains constant.

The second series of observations Ryther made was based on the statement of Pratt, Oneto, and Pratt (1945) that the amount of antibiotic substance produced by

Chlorella vulgaris Beijerinck increased with the age of the culture. Ryther fed senescent cultures of C. vulgaris to D. magna and found that the animals' filtering rate was depressed far below that of cultures used while still in log-phase growth, at the same concentration of cells. In a series of experiments using a radioactive tracer technique to measure the filtering rate, McMahon and Rigler (1965) showed that when senescent cultures are used, the filtering is indeed depressed far below what one would expect simply on the basis of cell concentration. They checked several possible explanations but advanced no conclusive mechanism to account for this phenomenon, other than showing it was not simply an external "taste" response but an internal action once the senescent cells were ingested.

During both of these series of experiments performed by Ryther there was little chance that the pH of the C. vulgaris cultures used could have become very high. The cultures were constantly agitated with 5 per cent carbon dioxide enriched air and then the algal cells were centrifuged out of the culture medium and resuspended in pond water. At this time the filtering rate of the D. magna was determined over a period of an hour. This experimental procedure is changed significantly in Ryther's third series of tests for an allelopathic effect. In this third series, the D. magna were exposed to cultures of from 0.05 to 0.5 million C. vulgaris cells/ml under 64 footcandles of light

for 12 hours. Presumably these cultures were not agitated; Ryther states that agitation altered or even stopped the D. magna feeding altogether. The animals were moved to fresh cultures every 3 hours in order to assure a relatively slight change in cell concentration over the period of the experiment. At the end of 12 hours, the filtering rate was determined over an hour. The experiment showed significant depression in the filtering rate of the animals that had been exposed to the higher concentrations of C. vulgaris.

Ryther does not report the chemical conditions of these cultrures. However, even though the animals were introduced to fresh cultures every 3 hours, it is quite possible that at the upper cell concentrations the pH could have increased to high levels under such lighting conditions and lack of agitation. It was only at the upper concentrations, greater than 0.15 million cells/ml, that the filtering rates were depressed below the rate of animals which had not been exposed to growing C. vulgaris for 12 hours. Ryther concluded that only in the upper concentrations of food would the animals have consumed enough cells for the toxin to become effective and thus depress the filtering rate. However, it is more likely that C. vulgaris is not toxic, and rather that at the upper concentrations of cells the pH reached toxic or inhibitory levels in the C. vulgaris culture media. In the cultures where the cell concentration was less

than 0.15 million cells/ml, the pH may not have increased to toxic levels. In the present experiment, ponds 8 M, 3 H, and 5 H at the time of the die-offs all had phytoplankton cell concentrations greater than 150,000 cells/ml.

That the above observation by Ryther may not be explained by an allelopathic effect and may best be explained by a toxic pH factor is also demonstrated by a control experiment performed by Ryther. An actively growing culture of C. vulgaris at a concentration of 0.478 million cells/ml was placed under constant illumination and agitated with 5 per cent carbon dioxide enriched air; after 48 hours the cells were filtered from the water. Various concentrations of C. vulgaris were resuspended in the "conditioned" water and used to test Daphnia magna filtering rates. The filtering rates were found to be identical to those of D. magna in unconditioned water and much higher than those in which the animals had been exposed to growing Chlorella. During the 48 hours the C. vulgaris were growing, the pH of the water would certainly have increased to a high level except for the agitation and the filtration of the water (see Table 9). Neither agitation nor filtration would have any effect on a toxic chemical; both surely would have lowered the pH.

Thus the one piece of work which was interpreted to suggest an allelopathic effect of a green alga on a cladoceran may instead support the concept of a pH inhibitory effect.

It is difficult to state the mode of action of the proposed pH mortality factor. Very little work has been done on the effects of high levels of pH on cladocerans. Ivanova (1969) investigated the change of filtering rate of various cladocerans including Ceriodaphnia reticulata and found that high pH had a definite effect. In C. reticulata she found that the filtering rate decreased by a factor of 2 as the pH increased from 7 to 9. Walter (1969) also confirms this decrease in filtering as the pH increased, but reports only a 25 per cent decrease from pH 7 to pH 10.5. In the present study, a set of experiments measuring the filtering rate of C. reticulata using water from ponds 3 H and 5 H during the time when the pH of both these ponds was well above 10 showed no change in the filtering rate which could not be accounted for as a function of increasing cell concentration (see Figure 2). However, these were short-term experiments since a carbon-14 technique was used. Ivanova used a particle decrease method with a longer exposure to measure the filtering rate. She does not state how long the measurements took, but it is possible that exposure time is a factor in C. reticulata filtering rate decrease because of high pH. Walter (1969), working on Daphnia magna, also showed decreased growth and reproduction with high pH levels. Her data would suggest a sub-lethal mechanism operating to lower growth and reproduction. However, the life table data from the present experiment (Figures 17 and 18)

support a rather narrow threshold from relatively normal physiological function to rapid death. Regression analyses of increasing pond pH values plotted against the birth rate, death rate, and density of C. reticulata show no decreasing trend of these parameters with increasing pH. Bogatova (1962), using four species of Chydorids, shows a rather narrow threshold of mortality with increasing pH. Eurycercus lamellatus (O. F. Müller), Acroperus harpae Baird, Chydorus sphaericus, and Peracantha truncata O. F. Müller all survived pH levels of 10.6, yet only E. lamellatus survived pH of 11.0, the point at which the other species died rapidly. These data not only confirm a narrow threshold but indicate a threshold mortality value approximately the same as that indicated in the present life table experiments for C. reticulata. Walter (1969) found 100 per cent mortality of British specimens of Daphnia pulex after 5 hours in water of pH 11.0, but only 20 per cent mortality of Greenland specimens of D. pulex after 24 hours at this same pH. She suggests this might be an adaptation to the long summer day length in Greenland during which time the pH could build up to very high levels.

Inedible Algae

The average crustacean biomass of each pond correlates well with the average chlorophyll a concentration of that pond, with four notable exceptions (Figure 12).

Three of these unusual results are accounted for by the pH mortality factor. The other case in which average pond zooplankton falls below what would be expected on the basis of chlorophyll a content may be explained by the fact that much of the phytoplankton in pond 8 M in 1968 was unavailable for zooplankton consumption.

There are two main problems in determining the importance of a particular phytoplankton species for growth of the zooplankton. The first is whether the zooplankter under consideration can ingest the algal particle; the second is the question of the nutritive value of the algal species once ingested. Setting aside the latter problem, which very quickly becomes a detailed physiological question, there are several factors which may make a particular species of alga unconsumable by a particular zooplankter (see Edmondson, 1957, and Jørgensen, 1962 for full review of these problems). The most obvious factor, and the only one considered in the present study, is the size of the phytoplankter. Burns (1968) shows a very definite relationship between the length of the cladoceran and the maximum sized particle which it can ingest. The largest particle ingested by cladocerans the size of Ceriodaphnia reticulata was 25-30 μ in diameter. Using this measurement as a rough criterion for consumable algae, a listing was made of algal species within this size range occurring in the ponds. Species or colonies with a mean dimension greater than 30 μ (assumed inedible by

C. reticulata) were grouped into three categories: colonial Volvocales, Microcystis, and other large algae. The volume of each spherical was calculated using mean dimensions estimated during the phytoplankton counting and assuming a geometric shape approximately either cylindrical with hemispheric ends. The total volume of the three significant groups--the edible algae, the colonial Volvocales, and the Microcystis--was calculated. These three algal groups were plotted with the dry weight of C. reticulata for pond 8 M in 1968 (see Figure 23). These data show clearly that C. reticulata biomass in pond 8 M is correlated not with total phytoplankton biomass but only with that calculated as consumable phytoplankton cell volume.

Several workers have reported either no correspondence between phytoplankton density and zooplankton abundance or an inverse relationship (Anderson et al., 1955; Nauwerck, 1963). However, Edmondson (1965) shows a good correlation between "micro-algae" and the reproductive rate of Keratella cochlearis in four English lakes. If the primary production of Cyanophyceae are excluded from the calculations, Straskraba (1966) shows a fairly direct relationship between zooplankton standing crop and primary production of a water body.

Phytoplankton competition and succession are most often cited as the reasons for the differential growth of edible and inedible phytoplankton. However, it is

Figure 23. The relationship of phytoplankton cell volume and biomass of Ceriodaphnia reticulata in pond 8 M 1968. Solid line connecting solid circles: dry wt. C. reticulata. Dashed line connecting open circles: cell volume of "edible" phytoplankton. Dotted line connecting open squares: cell volume of colonial Volvocales. Dot-dash line connecting solid triangles: cell volume of Microcystis aeruginosa. B.D. indicates below detection.

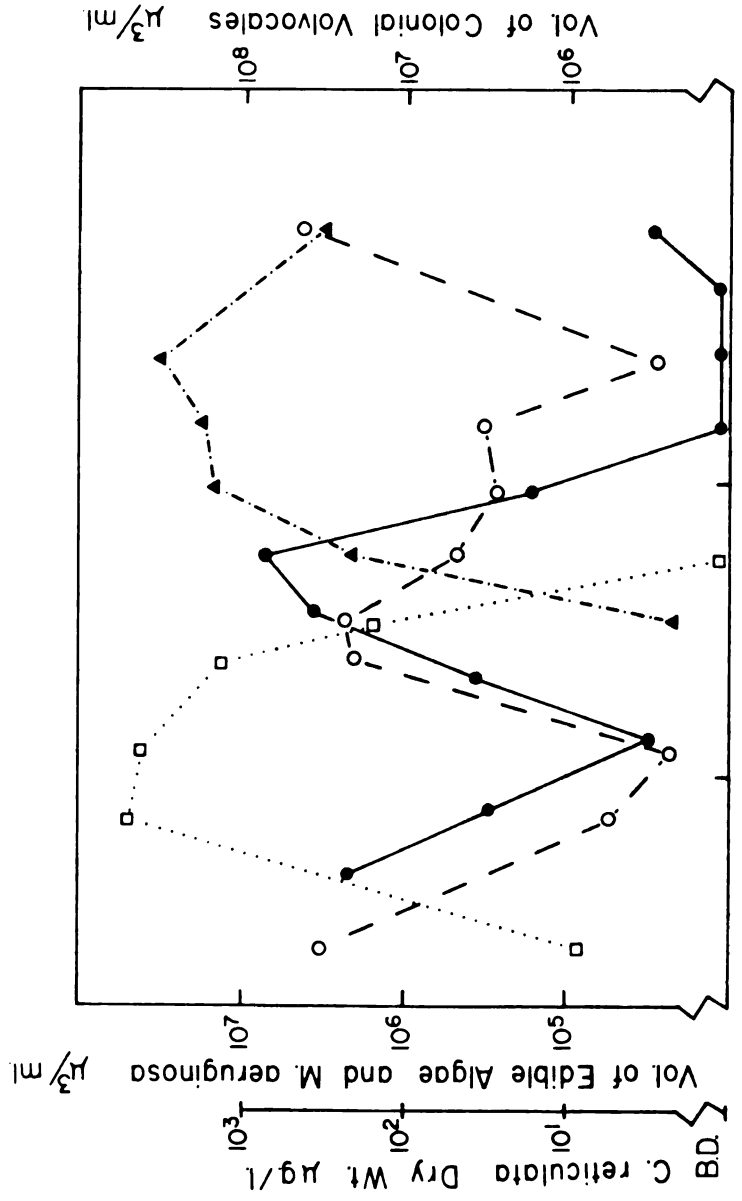

Vol of Colonial Volvocales μ³/ml
10⁸
10⁷
10⁶
JUN
JUL.
AUG.
Vol of Edible Algae and M. aeruginosa μ³/ml
10⁷
10⁶
10⁵
B.D.
C. reticulata Dry Wt. μg/l
10³
10²
10¹

Figure 23.

interesting to speculate as to the possible role of the zooplankton. The exceptionally high zooplankton densities just preceding the colonial Volvocales bloom and especially the Microcystis aeruginosa bloom in pond 8 M in 1968 suggest that such high zooplankton densities and accompanying high grazing may initiate these blooms by altering the competitive relationships among phytoplankton species. The amount of grazing by C. reticulata in pond 8 M at the peak of its population density (23 July 1968) was estimated by the method previously described to be 1.23 liters per liter, or 123 per cent of the pond volume per day. However, Haney (1970) shows that laboratory measurements of this type may ignore environmental factors of unknown importance. A further problem in accurately estimating the decrease of phytoplankton due to grazing is the difficulty in estimating the growth rates of phytoplankton species. Still, it is certain that in late July of 1968, many organisms which could be eaten by C. reticulata must have been experiencing a severe mortality due to Ceriodaphnia grazing.

Bucka and Kyselowa (1967) recorded up to 1,700 Ceriodaphnia sp. per liter just preceding an extremely dense Microcystis bloom. Hrbacek (1964), noting the common association of members of the genus Daphnia with blue-green blooms, suggests that the Daphnia may reduce the numbers of nannoplankton algae which may be competing with the blue-green algae for nutrients. Conversely,

Losos and Hetesa (1969) have reported that the large blue-green alga, Aphanizomena flos-aquae Ralfs, which dominated all the experimental ponds they studied, disappeared about 2 to 3 weeks after the introduction of planktivorous fish into some of the ponds. Since A. flos-aquae did not disappear in the ponds without fish and the fish virtually removed all the large cladocerans, they suggest that the large cladocerans play a role in maintaining the large blue-green algae. Most of the factors of possible competition which can affect the species composition could be altered through the reduction of numbers of one particular group of algae. Any type of inhibiting substance, such as those suggested by Vance (1965) or Fitzgerald (1969), would also be much reduced with the reduction of the cells producing the substance.

Once larger inedible forms have firmly established themselves in a body of water, they may well have an advantage because they are inedible. Smaller algae are often thought to be able to compete with larger forms because of their greater relative surface area to absorb nutrients. In enriched situations such as those existing in the experimental ponds, efficient nutrient uptake may not be a major factor of competition. Thus, due to their lack of grazing losses, the larger algal forms may remain in competition with the smaller algae. There is of course the possibility that the colonial Volvocales and especially

Microcystis secrete substances which inhibit smaller algae (Vance, 1965).

The size of phytoplankton species seems to be a major factor in availability as food. In the case of pond 8 M in 1968, the zooplankton increased directly with amount of edible algal biomass. On only two occasions in pond 8 M did C. reticulata increase to such numbers as to have a serious grazing impact. Immediately following such heavy grazing, blooms of large algae appeared, suggesting that heavy grazing by zooplankton may influence the succession of the size of phytoplankton species. A high population of Ceriodaphnia also preceded a heavy bloom of Microcystis aeruginosa in pond 1 M in August 1969. Heavy zooplankton grazing would give an advantage to the larger, inedible forms which, once abundant in a pond, may exclude smaller algae for a time even after the grazing pressure is decreased. This type of mechanism certainly has analogies in terrestrial grasslands where large vertebrate herbivores, through over-grazing, can shift the vegetation of the range to short grasses much less susceptible to grazing (Ellison, 1960).

Shifts in the size of phytoplankton associated with high zooplankton densities also occurred to a lesser extent in ponds 3 H and 5 H in 1968. The large species which developed in these ponds were Pleodorina californica and Volvox spp. However, the predominance of large phytoplankton was apparently not great enough to alter the

positive relationship of average cladoceran biomass to average chlorophyll a for these ponds (Figure 12).

Cycles of alternating low chlorophyll a concentrations accompanied by high C. reticulata densities and high chlorophyll a concentrations accompanied by low C. reticulata densities also occurred in the high nutrient ponds in 1968. A simple mathematical simulation and analysis of those cycles using estimated filtering rates of C. reticulata were originally planned. However, such analysis requires an estimate of the phytoplankton growth rate. Two different methods were used in an attempt to measure the growth rate of the phytoplankton. The first was an in situ enclosure technique in which large amounts of pond water were enclosed in two large cylinders (1.5 m high, 0.6 m in diamter) of clear polyethylene plastic supported by a metal screen frame. These were constructed such that the pond water entered through an opening in the bottom in which a number 20 plankton net could be placed to remove all the crustacean zooplankton entering the container. The enclosures were also fitted with plexiglass tops through which projected a wind-driven stirring mechanism which it was hoped would keep the phytoplankton in suspension in a manner similar to wind action on the pond. The change in the density of the phytoplankton from day to day in the enclosure without zooplankton was expected to provide an estimate of the absolute growth rate of the phytoplankton with no death rate due to grazing. However,

for the estimate to be accurate, the enclosure must have little or no effect on the rate of growth of the phytoplankton. In this case, even after all metal surfaces of the enclosure had been painted with epoxy paint, the enclosure somehow caused extreme mortality of the phytoplankton.

The second method of measuring the growth rate was based on the premise that an observable cytological event indicating cell division or reproduction may be used as an index of the rate of cell division or reproduction. This is similar to the egg ratio method for estimating the birth rate of egg-carrying zooplankton. The duration of the stage indicating cell division must be known, and so must the ratio of the number of dividing cells to the total number of cells. While theoretically possible, the practical application of the technique is quite difficult. Unlike the egg-bearing zooplankton, the reproductive events of phytoplankton do not occur randomly during a 24 hour period. This means that a series of samples must be taken throughout a 24 hour cycle. A second major problem is that not all phytoplankton species have a suitable cytological event indicating cell division or reproduction, and even when present such an event may be difficult to observe and of short duration. This necessitates the microscopic examination with high resolution of large numbers of cells. Both the 24 hour sampling and the arduous microscopical analysis needed for this

technique limited its usefulness in a study involving eight ponds, and little actual data on the growth rates of phytoplankton were ever obtained.

Until there is a solution to the problem of estimating such an important factor as the rate of growth of phytoplankton, zooplankton grazing rates cannot be applied to field situations to predict the population dynamics of the phytoplankton.

SUMMARY AND CONCLUSIONS

The initial goal of this experiment was to observe the response of the plankton community over widely varying nutrient conditions. The nutrient levels created in the ponds by the addition of fertilizer ranged over three orders of magnitude, and especially the nitrogen concentrations corresponded well with a general trophic classification of lake types from oligotrophic to extremely eutrophic on the basis of both inorganic nitrogen concentration and primary production levels. A bioassay experiment and other evidence suggest that nitrogen was the "governing" nutrient in the ponds.

In 1969, the phytoplankton biomass, as estimated by chlorophyll *a* concentration, and the primary productivity largely followed the increasing nutrient levels and generally responded proportionately to the nutrient input in also ranging over three orders of magnitude. In 1968, the phytoplankton response to treatment level was considerably more variable. Pond 8 M had the highest phytoplankton biomass, due mainly to heavy *Microcystis* and Volvocales blooms. The phytoplankton biomass in the high

treatment ponds was extremely variable throughout the summer, although the summer averages were relatively high. The overall response of the crustacean zooplankton to treatment level was erratic. In 1968 the high treatment ponds showed a three-fold increase of average cladoceran biomass over the control ponds, but the low and medium treatment levels hardly differed in average cladoceran biomass from the control ponds. In 1969 even the difference in the high treatment ponds was absent; the treatment averages were all about the same and great variability existed within treatments at the low and medium levels. Much of the response of the zooplankton in 1968 was attributable to variable phytoplankton response. The average cladoceran zooplankton biomass correlated well with the average chlorophyll a concentration at the lower treatment levels. With increased production and average chlorophyll a concentrations greater than 60-70 mg/m^3, this positive correlation between average chlorophyll a and average zooplankton ceased.

Dramatic shifts in the size and abundance of the phytoplankton in pond 8 M in 1968 affected the relationship between average cladoceran biomass and average phytoplankton biomass. When only the "edible" phytoplankton in pond 8 M are considered, there was again a very direct relationship between phytoplankton abundance and zooplankton abundance. While many factors may be responsible

for the development of blooms of large phytoplankton, it is possible that heavy zooplankton grazing may be active in the determination of the phytoplankton assemblage size structure and abundance.

The lack of a clear relationship between treatment level and average cladoceran biomass in 1969 was due in part to the complete disappearance of crustaceans from both high nutrient ponds and pond 8 M in midsummer. Analysis of these unusual disappearances showed a possible narrow threshold of zooplankton mortality at high pH levels. The rapid zooplankton mortality appears to have been not a direct allelopathic effect of algae on zooplankton, as might be suggested by the data of Ryther (1954), but simply the end result of an extremely high rate of primary production stimulated by nutrient addition.

The plankton community diversity, especially that of the phytoplankton, decreased with increased nutrient input. This may have been due simply to the increasingly physiologically intolerable conditions created by the experimental manipulations which fewer and fewer organisms could withstand. Nutrient enriched conditions would give maximum selective advantage to algae which could respond rapidly to change and which would at times be resistant to grazing through large size or resilient to grazing through rapid growth. Only a limited number of species could meet these requirements; those which could not would quickly

be eliminated through grazing or through rapid differential growth of a few phytoplankton species.

Much work remains to be done to obtain a clear understanding of the species diversity changes during the experiment. All the available samples must be enumerated and the phytoplankton species composition must be expressed on a volume basis rather than a numerical one before a clear picture can be obtained of the response of the phytoplankton species diversity to nutrient enrichment. An effort is planned to relate measured primary production, phytoplankton biomass, and zooplankton biomass to phytoplankton species diversity and various pigment ratios. Also planned is a more detailed investigation of particular pond events.

As with all studies having a broad scope, one can see in hindsight certain factors and events which deserved more investigation. An in situ estimation of zooplankton filtering rates would have greatly strengthened understanding of the importance of zooplankton grazing on the phytoplankton. Even more important in this regard would have been a measure of the growth rate of the phytoplankton, which would have allowed a detailed investigation of the predator-prey type relationship between these two trophic levels. Another project which would have been valuable was a laboratory or field bioassay as to the factors limiting the distribution of Microcystis aeruginosa to the low and medium treatment ponds.

One of the greatest strengths of this study may lie not in any of the specific results but in an increased awareness of the type of approach needed to study relationships as complex as those existing between trophic levels. Large field experiments certainly are essential in determining what factors are important in the functional coupling between trophic levels, and how these factors are altered as basic ecosystem parameters change. Necessary to such experiments are constant monitoring by the investigator and the performance of critical experiments at appropriate times to confirm causal relationships.

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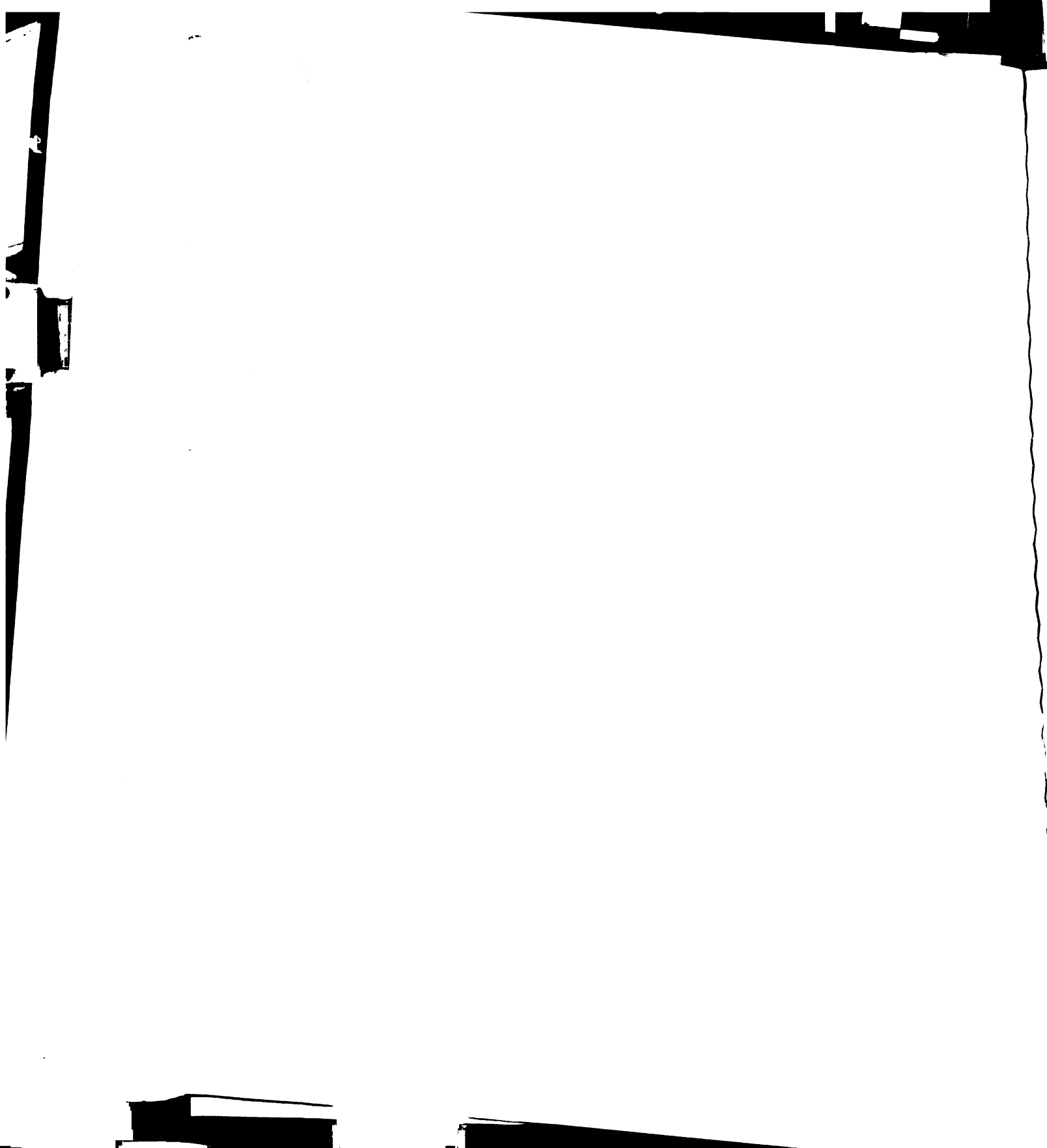

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