EFFECTS OF ARTIFICIAL FERTILIZATION ON A DAPHNIA PULEX POPULATION

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY
DAVID LEE EGGER

1969

ABSTRACT

EFFECTS OF ARTIFICIAL FERTILIZATION
ON A DAPHNIA PULEX POPULATION

By

David Lee Egger

A study area on the north side of a pond on the Michigan State University campus was partitioned into four sections with plastic sheeting. Two of these sections were fertilized once during the summer of 1969 with di-basic sodium phosphate.

The addition of fertilizer resulted in an increase in the concentration of soluble phosphorus. The two sections fertilized remained high in concentration of soluble phosphorus for the rest of the study period.

Algal blooms primarily of filamentous types were evident in the fertilized sections.

Daphnia pulex populations were sampled in the four sections throughout the summer. Numbers of adults, and young, of *Daphnia pulex* increased significantly after fertilization. Egg ratios were increased as a result of fertilization indicating that the *Daphnia pulex* population and reproduction were food limited.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Biological Science

1969

ACKNOWLEDGMENTS

I wish to express my sincere appreciation and indebtness to: Dr. T. Wayne Porter who directed the graduate work; Dr. Niles Kevern for guidance and help with water analysis problems; Dr. Richard Sauer for guidance and help with statistical analysis; Dr. John Cantelon and Dr. William Cooper for suggestions on the manuscript and method of analysis; my wife without whose assistance and encouragement this thesis would not be a reality.

Equipment, laboratory space, and financial assistance for this work were provided by Dr. Julian Brandou through the Science and Math Teaching Center of Michigan State University.

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INTRODUCTION

Water is the most important resource available to man. It is a biological necessity for him and all other living things in our biosphere. Man has been able to utilize water for a myriad of tasks including food production, heating and cooling, waste removal, and industrial uses. Water also provides recreation in the form of swimming, boating, and fishing for millions of people every year.

Only recently has the public become aware of a need to develop and protect this great resource. Interest and concern with developing sound management procedures and techniques is reflected in the recent anti-pollution laws as well as increased federal, state, and private funds for research and study of natural waters. Much of the research and funding has been directed towards developing methods which will control aquatic animal and plant populations to the advantage of man.

Inorganic nutrients are necessary for growth and reproduction of the living cell. Chemicals added to water bodies as artificial fertilizer are thought to be a means of increasing productivity of some bacteria, protozoa, algae, and higher aquatic plants. These primary producers of aquatic ecosystems use the added nutrients along with sunlight energy and carbon dioxide to produce food and growth by photosynthetic activity. The aquatic food web begins with this primary production. Available protoplasm is eaten by primary consumers which are in turn eaten by secondary consumers and so on through the food web. The ultimate consumer may be the largest fish of the system or may be man if he eats the fish.

Artificial fertilization of water is a relatively new practice in this country. The Chinese, however, have been fertilizing ponds to increase fish growth and production since before the birth of Christ. A summary presented by Smith (1934) indicates that the first experiments were conducted to try to increase the production of algae and plankton in relation to carp rearing. Embody (1921) started American experimentation along these same lines. This was followed by works of Wiebe (1930), Embody and Sadler (1934), Howell (1942), Langford (1948), Ball and Tanner (1951), Alexander (1956), and others which have shown that increases in growth of higher aquatic plants, plankton, bottom fauna and flora, and fish result from addition of fertilizer. Pertinent European and American literature has been summarized by Maciolek (1954).

The purpose of this investigation was to determine the affect of artificial fertilizer on a cladoceran population. Daphnia pulex was the species chosen as it is common to lakes, ponds, and streams of Michigan. A pond on the Michigan State University campus containing a population of Daphnia pulex was used.

A new technique was used for the study. A portion of the pond was divided into four sections by the use of plastic sheets. Two of the sections were artificially fertilized with phosphorus and two were left undisturbed as controls.

Results were evaluated in terms of chemical and physical changes of the water and biological changes in the Daphnia pulex populations both before and after fertilization. This thesis reports the work completed during the summer of 1969 from mid-April until mid-August when the study area dried up.

Description of Pond and Study Area

The pond utilized for this study is located on the Michigan State University campus in Meridian Township, Ingham County (T.4N., R.1W., Sec. 19). It lies about one mile south of East Lansing, Michigan where Farm Lane and the Grand Trunk railroad tracks cross. It is directly north of the tracks and west of Farm Lane. It has a surface area of about one acre and a maximum depth of five feet in the spring before drying. More than 70 percent of the pond is less than two feet deep. Pond temperatures were nearly homothermous for the duration of the study.

Most waters of this area are alkaline with the pH varying from 8.0 to 9.5 during the summer months. Alkalinity of the pond averaged 160 ppm. Bulldozing done by Campus Parks and Planning left the far eastern shore with sandy patches. The remainder of the pond bottom has a dark brown color and contains large amounts of organic matter.

The pond water has the brown, muddy appearance characteristic of ponds in this area. Water is supplied to the pond by melting snow and ice which inundate it in the spring. During the study the water level of the pond fluctuated with rainfall and dried to one-half its spring size and depth by early fall.

Steep banks are found on the east shore as a result of bulldozing and on the south shore where the Grand Trunk roadbed extends to the pond edge.

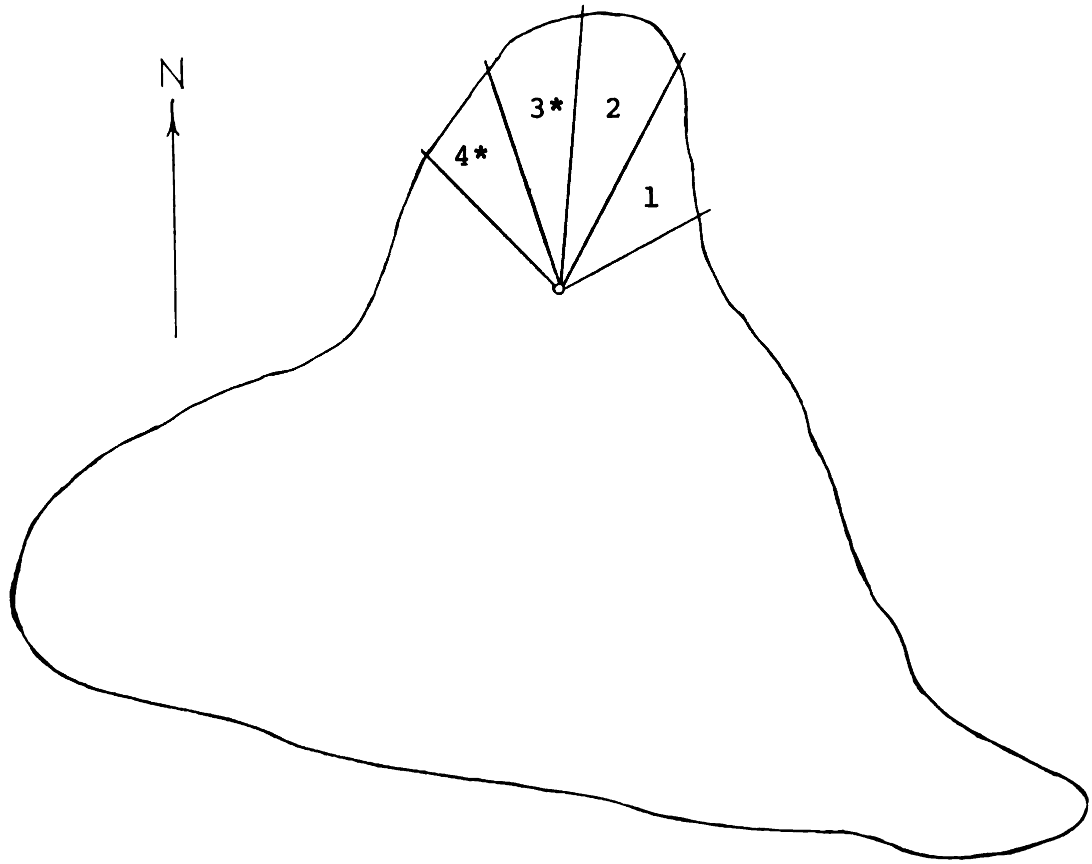
Pondweed (*Potamogeton* sp.) was the predominant aquatic plant and grew profusely throughout summer, eventually choking the pond by early fall. Sedge (*Carex* sp.) was found in abundance along the shoreline and small patches of cattail (*Typha latifolia*) were present along the north and east banks.

Aquatic insects, including borrowing mayflies (Ephemeridae), dragonfly nymphs (Odonata), diving beetles (Coleoptera), and Diptera, were present in abundance.

Although previously thought not to contain fish life the author found the bluntnosed minnow (*Pimephales notatus*) and the yellow bullhead (*Ictalurus natalis*) to be present.

The area selected for study was on the north shore where an indentation created a small cove (see Figure 1). At the center of this cove a six foot long 4 by 4 was driven into the pond bottom. Depth of the pond at this point was 28 inches. Heavy gauge wire was strung from the post to the shore and six inches above the water surface. Five wires were staked down at intervals of about 14 feet along the shoreline. Plastic sheeting (10 mil) was cut into five 50 by 5 foot pieces. These pieces were folded over lengthwise, and laid in the cove with the crease on the bottom directly under the five wires. This crease was filled with coarse gravel and dirt which effectively sealed the plastic to the pond bottom. The top of the plastic was attached to the wires with spring type clothes pins. The wires were supported at regular intervals with wooden stakes.

This construction divided the area into four triangular-shaped sections of approximately equal size



*Fertilized

FIGURE 1

MAP OF POND AREA SHOWING LOCATION
OF STUDY AREA AND SECTIONS

(see Figure 2). Each section was 28 inches deep at the center post and gradually decreased in depth to the shore. These sections each held approximately 3800 liters of water.

FIGURE 2

STUDY AREA LOOKING FROM THE EAST BANK TO THE WEST

METHODS AND EQUIPMENT

Fertilization

Di-basic sodium phosphate was added once to test sections 3 and 4 during the summer of 1969 (Figure 1). The sodium phosphate (1.9 grams per section) was dissolved in eight liters of distilled water and distributed evenly over sections 3 and 4 with a sprinkling can. Theoretical and actual concentrations in the four sections as a result of fertilization are given in Table 1. The section volume used in these calculations was 3800 liters. Sections 1 and 2 were maintained as control areas.

TABLE 1

THEORETICAL AND ACTUAL CONCENTRATION OF SOLUBLE PHOSPHORUS AFTER FERTILIZATION OF SECTIONS 3 AND 4

	Concentration (ppb)	
	Theoretical	Actual (7-8)
Section 3	20.0	20.0
Section 4	20.0	21.6

Sampling

Two liter water samples for physical and chemical analysis were taken from the center of each section. These samples were taken between 10:00 A.M. and 12:00 noon on a weekly basis throughout the study. All chemical and physical determinations were completed the same day as sampling.

Physical

Temperature. Water and air temperatures were taken on a weekly basis throughout the study. A Science Associates thermometer was used. These results are shown in Table 2.

Chemical

Hydrogen ion concentration. The pH was determined using a Beckman electric pH meter.

Alkalinity. The titration method outlined in Standard Methods (1965) was used to determine alkalinity. Water samples were tested for both methyl orange and phenolphthalein alkalinity.

Dissolved oxygen. The Winkler Iodometric method as described by Standard Methods (1965) was used to determine the dissolved oxygen.

TABLE 2

AIR AND WATER TEMPERATURE
(°F)

Date		Water Temperature	Air Temperature
April	16	67	68
	23	58	48
	30	62	62
May	6	72	75
	13	66	65
	20	64	60
	27	69	72
June	3	63	55
	10	68	70
	17	71	75
	24	69	66
July	1	66	67
	8	69	75
	15	77	85
	22	76	83
	29	77	82
August	5	70	74
	12	73	80

<u>Phosphorus</u>. Soluble phosphorus determination was made by the molybdate method as outlined in Standard Methods (1965). The Klett-Summerson colorimeter with no. 66 filter was used to take readings and these were converted into parts per billion by comparison with standard solutions. Particulate matter was filtered from samples using a Millipore filter and 0.45 micron membrane paper before phosphorus determinations were made.

<u>Biological</u>

<u>Daphnia pulex populations</u>. Samples of the <u>Daphnia pulex</u> populations were taken between 9:30 P.M. and 11:30 P.M. These population samples were taken on the same day as samples for water analysis. The populations were divided into young and adults on the basis of total length. Those larger than 1.4 mm were considered adults and found to reproduce while those smaller were considered juveniles and did not reproduce. This corresponds well with the literature (Anderson, Lumer, and Zupancic, 1937). Adults and young were separated in all samples by viewing them with a binocular microscope against a centimeter grid glued to the bottom of a petri dish.

Two different methods were used in sampling the <u>Daphnia pulex</u> populations. For the first four weeks of sampling a six inch Wisconsin plankton net attached to the end of a broom handle was employed. The net was swept through the water for a distance of ten feet. This sweep started on the bottom of the section and ended at the surface. The sweep started two feet from the centerpost and ended 12 feet from the centerpost. It was directed from the centerpost down the center of the section toward the shore. By knowing the diameter of the plankton net and the length of the sweep the volume of water sampled was computed in the following manner:

$$\text{Volume} = \pi r^2 h$$

$$\text{Volume} = 3.14\ (3)^2\ 120$$

$$\text{Volume} = 3391.20\ \text{in.}^3$$

or

$$\text{Volume} = 55.62\ \text{liters}$$

Daphnia pulex from these sweeps were preserved in ten percent formaldehyde for laboratory examinations.

Samples for the first four weeks of the study period were counted and numbers per liter calculated in the following manner: Daphnia were spread randomly in a petri dish which had a centimeter grid glued to the bottom. Counts were taken over 10 one-centimeter square areas. A mean was calculated for these ten areas and then multiplied by the area of the petri dish. This gave an estimate of the total number of Daphnia pulex in the sweep. The above estimate was then divided by the volume in liters which gave a final estimate of the numbers of daphnia per liter.

It became apparent that rapidly growing aquatic plants and algae would soon make this type of sampling unreliable. Therefore a second method of sampling the Daphnia pulex populations was initiated and used for the remainder of the sampling period. A 500 ml wide-mouthed jar was attached to the end of a four foot long 2 by 4 so that jar and 2 by 4 were parallel. A cork which fit snugly into the jar mouth was attached to one end of a three foot section of 1/4 inch bar stock. The bar stock was secured to the 2 by 4 with screw eyes so that the cork was directly above and in line with the wide-mouthed jar. The sampler was lowered to the desired depth with cork firmly in place. The top of the bar stock was pulled disengaging the cork and allowing the jar to fill with a sample. When completely full the bar was pushed down reengaging the cork and trapping the sample. Samples were poured through the Wisconsin plankton net and condensed in vials. They were preserved in ten percent formaldehyde and counted in the laboratory.

Six samples were taken in each section on a weekly basis at the stations indicated in Figure 3. A mean was calculated for the six samples and then doubled to give an estimate of Daphnia pulex per liter. All samples were taken at one-half the depth of the sampling station. Therefore as pond level fluctuated so did sample depth.

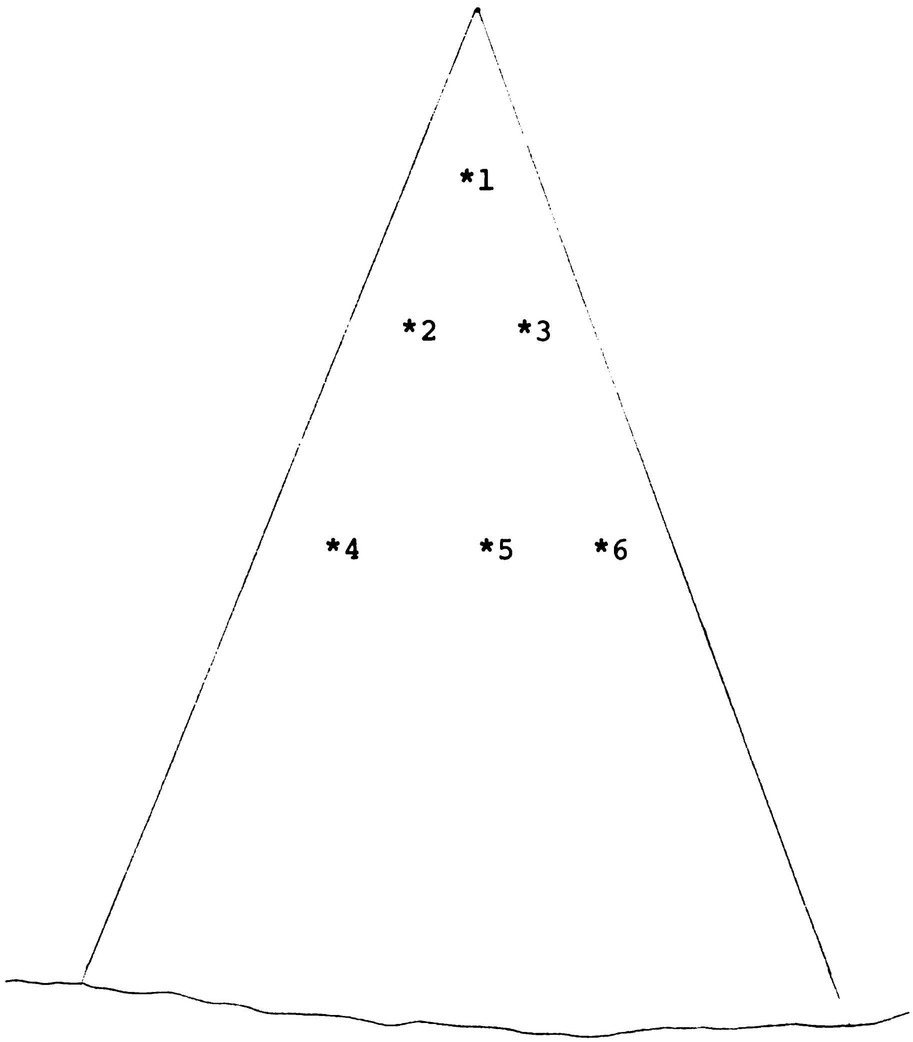


FIGURE 3

SECTION SHOWING APPROXIMATE LOCATION OF SAMPLING STATIONS

Eggs. Egg counts were taken in section 2 and 3 both before and after fertilization. Ten adults from each section were dissected and their eggs counted. A mean was then calculated for these ten adults. All adults used for egg counts were selected at random.

RESULTS

Sampling

The addition of artificial fertilizer to sections 3 and 4 brought about an immediate physical change. The surface of these sections took on a green color characteristic of an algal bloom. Filamentous algae grew rapidly and covered the water surface along with increased growth of duckweed (*Lemna* sp.). Chemical parameters other than phosphorus showed little response to fertilization (see Appendix A). Alkalinity continued to average 160 ppm. The pH of the sections remained between 8.0 and 9.0. Oxygen levels showed no change continuing to vary between 7.0 and 11.0 ppm. This lack of response corresponds well with the works of Wiebe (1930), Ball and Tanner (1951), and Alexander (1956).

Biological response in the *Daphnia pulex* populations were slow to occur; however, significant changes did appear during the summer of 1969.

Physical

Temperature. Water temperature varied between 57 degrees and 77 degrees for the duration of the study.

Temperature records are entered in Table 2. The highest temperatures occurred in July and early August. This hot, dry weather caused a general drying of the pond and study area. June was cool with heavy rainfall which kept temperatures down.

Chemical

Chemical parameters for all four sections may be found in Appendix A.

Hydrogen ion concentration. The pH of the four sections varied as follows: section 1, 7.4 - 10.2; section 2, 8.2 - 10.0; section 3, 7.9 - 9.6; and section 4, 8.3 - 9.9. After fertilization on July 1 the pH of sections 3 and 4 did not show a response.

Alkalinity. Total alkalinity varied around 160 ppm in the study area over the summer. Methyl orange alkalinity varied in the four sections as follows: section 1, 90 - 170 ppm; section 2, 64 - 164 ppm; section 3, 120 - 174 ppm; and section 4, 140 - 180 ppm. Phenalphthalien alkalinity was present in all sections throughout the study except in section 1 during the last two weeks of July. Fertilization seemed to have no effect on alkalinity.

Dissolved oxygen. Dissolved oxygen increased in all four sections during the study period. On August 5 the oxygen in sections 1 and 2 showed a sharp increase. The weather on this date was clear and bright and increased photosynthetic activity of submerged vegetation may have caused these high concentrations.

Phosphorus. Soluble phosphorus in the four sections just before fertilization on July 1 averaged 4.0 ppb. Tests were run on all four sections on the three days following fertilization (Table 3). Section 3 increased from 3.6 ppb just before fertilization to 29.4 ppb one day after. On the second and third days after fertilization phosphorus levels in section 3 dropped to 26.0 ppb and 25.2 ppb, respectively. Section 4 increased to 32.0 ppb one day after fertilization and then declined on the second and third days to 29.3 ppb and 26.0 ppb, respectively.

TABLE 3

CONCENTRATION OF SOLUBLE PHOSPHORUS IN SECTIONS 1 THROUGH 4 AS A RESULT OF FERTILIZATION (ppb)

Date	Section 1	Section 2	Section 3	Section 4
July 1	3.1	4.1	3.6	5.4
July 2	3.3	4.0	29.4	32.0
July 3	3.3	3.7	26.0	29.3
July 4	2.7	3.6	25.2	26.0

Phosphorus levels in sections 1 and 2 remained below 4.0 ppb for the rest of the summer. Levels of phosphorus in sections 3 and 4, however, did not drop below 11.0 ppb (Table 3 and Figure 6).

Biological

Daphnia pulex populations. The populations of Daphnia pulex showed a peak in early June (Figures 4 and 5). The peak for adults in sections 1 and 3 occurred on June 10 while the peak for section 2 and 4 occurred on June 3. According to Pennak (1953) these population peaks should have occurred earlier than they did.

In the spring of 1968 I did a population study on Daphnia pulex in a pond in Baker Woodlot. Baker Woodlot is immediately adjacent to the pond used for the 1969 study. The 1968 study showed population peaks to occur in early May.

Populations in control sections 1 and 2 decreased steadily from their June high until the end of the study in August. Effects of the artificial fertilizer were seen in experimental sections 3 and 4. Adults in these sections did not drop below ten individual per liter as did those in sections 1 and 2.

The population of young in the experimental sections showed an even sharper reaction to the

FIGURE 4

MEAN OF ADULT AND YOUNG DAPHNIA PULEX IN SECTIONS 1 AND 2 THROUGHOUT THE SAMPLING PERIOD

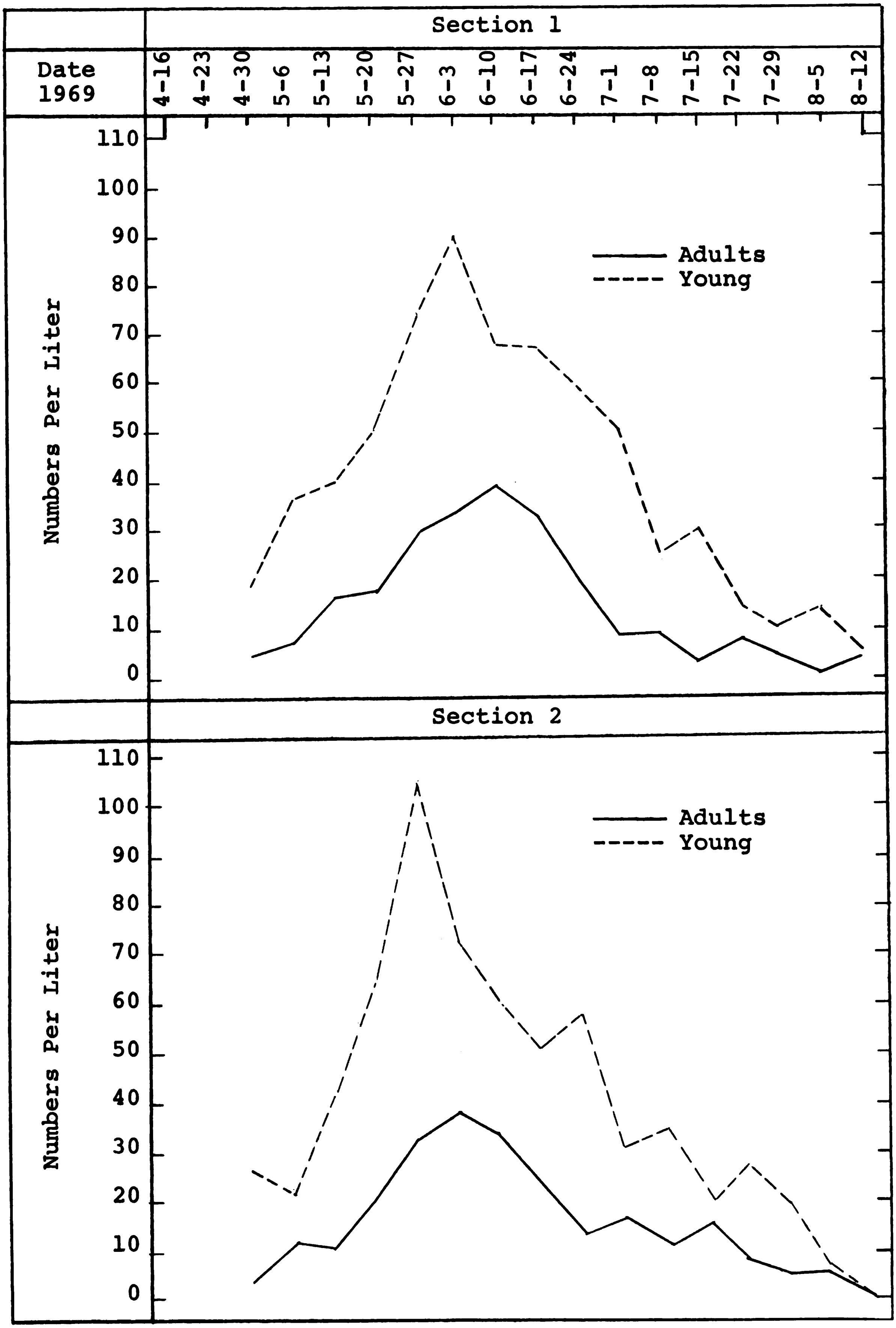
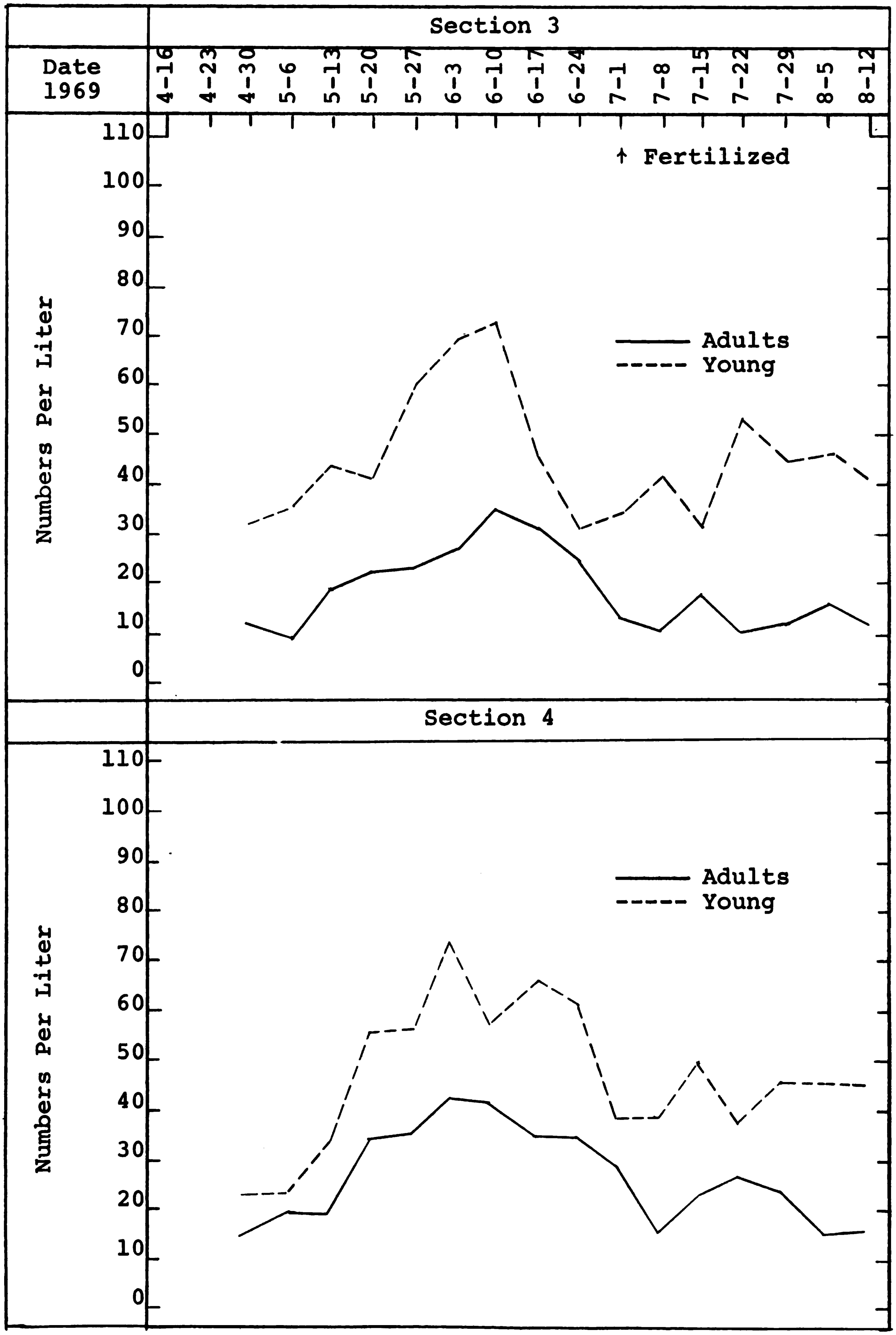

Section 1
Date 1969
4-16
4-23
4-30
5-6
5-13
5-20
5-27
6-3
6-10
6-17
6-24
7-1
7-8
7-15
7-22
7-29
8-5
8-12
Numbers Per Liter
110
100
90
80
70
60
50
40
30
20
10
0
Adults
Young
Section 2
Numbers Per Liter
110
100
90
80
70
60
50
40
30
20
10
0
Adults
Young

FIGURE 5

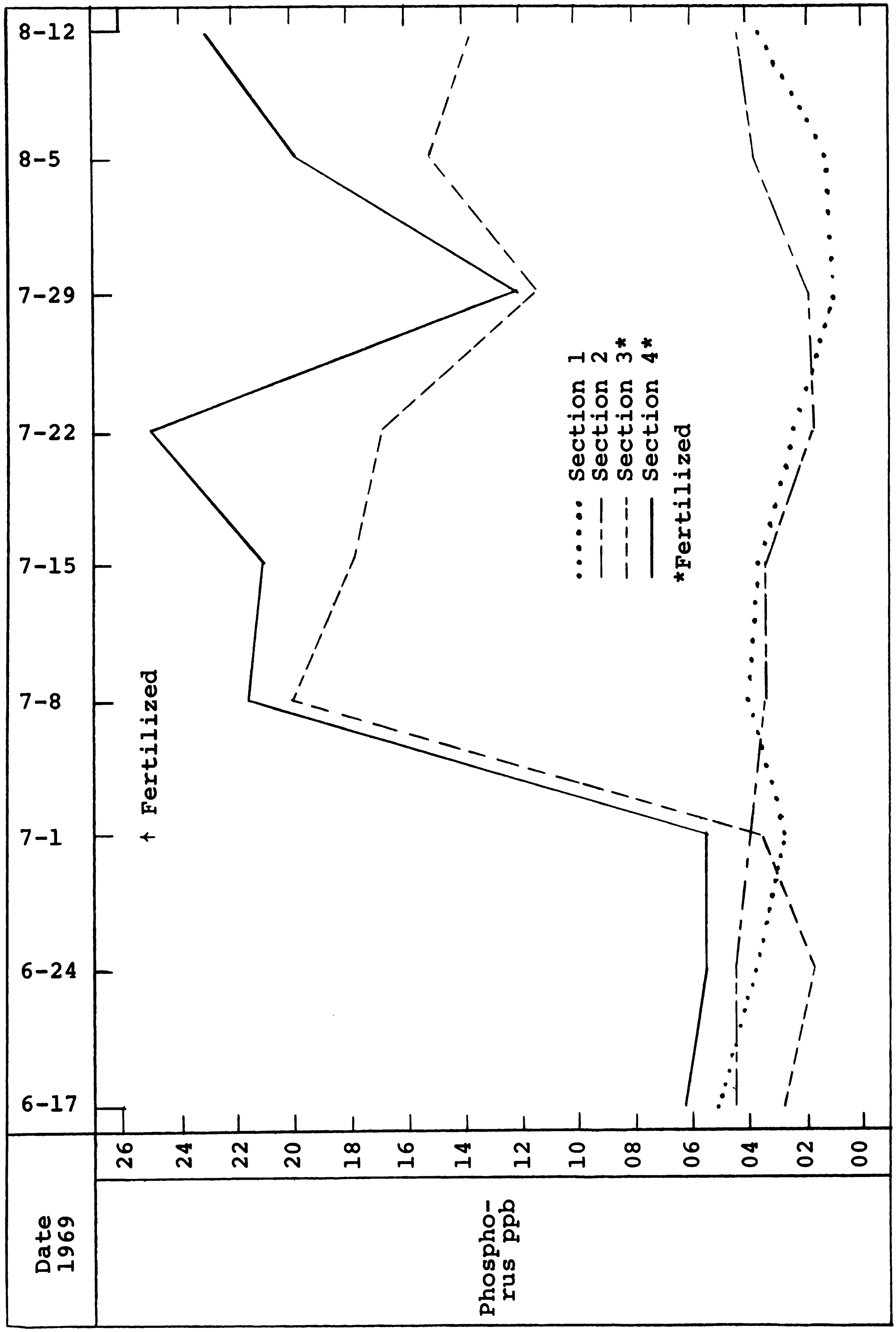
MEAN OF ADULT AND YOUNG *DAPHNIA PULEX* IN SECTIONS 3 AND 4 THROUGHOUT SAMPLING PERIOD



Section 3
Date
1969
4-16
4-23
4-30
5-6
5-13
5-20
5-27
6-3
6-10
6-17
6-24
7-1
7-8
7-15
7-22
7-29
8-5
8-12
↑ Fertilized
Adults
Young
Numbers Per Liter
110
100
90
80
70
60
50
40
30
20
10
0
Section 4
Adults
Young
Numbers Per Liter
110
100
90
80
70
60
50
40
30
20
10
0

FIGURE 6

CONCENTRATIONS OF SOLUBLE PHOSPHORUS IN SECTIONS 1 - 4 THROUGHOUT THE STUDY PERIOD


Date 1969
6-17
6-24
7-1
7-8
7-15
7-22
7-29
8-5
8-12
Phospho-rus ppb
26
24
22
20
18
16
14
12
10
08
06
04
02
00
↑ Fertilized
Section 1
Section 2
Section 3*
Section 4*
*Fertilized

fertilization. Their numbers did not drop below 30 individual per liter while those in sections 1 and 2 dropped well below ten individuals per liter.

Adult and young populations were subjected to statistical analysis in the following way. The means of the weekly samples from May 27 to June 24 before fertilization and from July 15 to August 12 after fertilization were collapsed to give a mean of means (Table 4). This mean of

TABLE 4

MEAN OF MEANS FOR ADULT AND YOUNG POPULATIONS FROM MAY 27 TO JUNE 24 BEFORE FERTILIZATION AND FROM JULY 15 TO AUGUST 12 AFTER FERTILIZATION

Section	May 27 to June 24	July 15 to August 12
	<u>Adults</u>	
1	28.8	3.8
2	28.0	6.8
3	27.6	13.4
4	38.4	17.3
	<u>Young</u>	
1	66.6	14.8
2	69.4	14.8
3	55.4	44.4
4	62.6	44.8

means was regarded as a sample and a two way analysis of variance was run on these samples. As seen in Table 4 this produced four cells with two replicates in each.

Specifics of analysis are entered in Table 5 for adults and Table 6 for young. The interaction F values indicate whether or not there was a significant difference in the population as a result of fertilization. Analysis run on adults reveals an F value of 8.76 which is significant at the five percent level. Analysis run on young reveals an F value of 100.77 which is also significant at the five percent level.

TABLE 5

ANALYSIS OF VARIANCE OF ADULT POPULATION MEAN OF MEANS BEFORE AND AFTER FERTILIZATION

Source of Variability	Degrees of Freedom	Sum of Squares	Mean Square	F Ratio
Sections	1	139.45	139.45	5.86
Dates	1	748.85	748.85	33.00
Interaction	1	28.12	28.12	8.76*
Error	4	12.84	3.21	
Total	7	929.26		

*Significant at the five percent level.

TABLE 6

ANALYSIS OF VARIANCE OF YOUNG POPULATION MEAN OF MEANS BEFORE AND AFTER FERTILIZATION

Source of Variability	Degrees of Freedom	Sum of Squares	Mean Square	F Ratio
Sections	1	216.30	216.30	28.93
Dates	1	2284.80	2284.80	305.86
Interaction	1	752.80	752.80	100.77*
Error	4	29.90	7.47	
Total	7	3263.80		

*Significant at the five percent level.

Eggs. Egg counts per ten adults for sections 2 and 3 averaged 23.8 and 27.0, respectively. These counts were made on June 17 before fertilization. On July 22 (after fertilization) egg counts were again made in sections 2 and 3. The average egg count per ten adults in section 2 was 9.4. Section 3 showed an average egg count per ten individuals of 19.6. All adults dissected for egg counts were selected at random.

DISCUSSION

Addition of artificial fertilizer in the form of sodium phosphate on July 1 produced an algal bloom in experimental sections 3 and 4. This bloom was not evaluated quantitatively. Mats of *Spirogyra* sp. and *Lemna* sp. were present in all four sections prior to fertilization; however, these growths increased in fertilized sections 3 and 4. Amounts of cellular material in the biological samples from sections 3 and 4 indicate that unicellular algae on which *Daphnia pulex* feeds also increased as a result of fertilization. Unicellular algae in the study area was primarily made up of *Trachelomonas* sp., *Scenedesmus* sp., *Chlorococcum* sp., and diatoms.

Table 3 shows that phosphorus increased to 29.4 ppb and 32.0 ppb in sections 3 and 4, respectively, on the day following fertilization. These levels decreased over the week following fertilization to 20.0 ppb in section 3 and 21.6 ppb in section 4. Phosphorus levels varied over the rest of the sampling period but did not fall below 11.0 ppb. Control sections 1 and 2 were never above 4.2 ppb after fertilization. Most investigators show phosphorus levels after fertilization quickly returning to pre-fertilization levels (Alexander (1956), Tanner (1956) and others).

Soluble phosphorus levels are reduced in two ways: it can be biologically assimilated by phytoplankton, periphyton, bacteria, and higher aquatic plants or it may be tied up chemically by calcium, carbonate, iron and other ions (Neese, 1949). The chemically tied up phosphorus may precipitate out or be held in suspension in an insoluble form.

The bloom which followed fertilization of sections 3 and 4 indicates that phosphorus was a limiting factor in the study area. Phosphorus levels did not drop back to prefertilization levels indicating that there were one or more limiting factors other than phosphorus. Nitrogen, potassium, or carbon dioxide may have been present in small enough quantities to be limiting factors thereby not allowing complete biological uptake of phosphorus.

Maciolek (1954) states that lakes which are high in organic matter and low in marl may retain phosphorus that is absorbed by sediments and is in an exchangeable form. High phosphorus content after fertilization in experimental sections may have been due to this phenomenon.

The works of Hutchinson (1967), Hall (1962), Ingle, Wood, and Banta (1937), and others indicate that reproduction in cladocerans is directly related to both temperature and food availability. Increased algal production in fertilized sections 3 and 4 led to significantly greater numbers of *Daphnia pulex* adults, young, and eggs per female as compared to adults, young, and eggs per female in control sections. Since temperatures were homothermal in all four sections over the study period *Daphnia pulex* populations were declining because of food limitations.

Temperature is very important in control of zooplankton populations. An even greater response might have occurred in experimental sections 3 and 4 if water temperatures had not increased in July and August. Embody and Sadler (1934) indicate that temperatures above 82 degrees were deleterious to Daphnia and other organisms in small ponds. Temperatures in the pond and study area never reached this maximum during the summer of 1969.

Weather during the summer of 1969 was atypical of that normally found in Michigan. Early spring was quite cold and wet and may have had an adverse affect on the *Daphnia pulex* population. This may partially explain why peaks in the populations of the four sections were so late in occurring.

Neese (1949) states that duplication of results from one research station to another (or one pond to another) is possible only in rare cases. Chemical and physical properties are much more similar between sections as outlined in this study than between two separate ponds. Duplication of results and chemical parameters for future aquatic research would more easily be achieved by this sectioning method.

The plastic sheeting used to partition the study area into four sections undoubtedly changed the immediate aquatic environment of those sections. Light entering the area was decreased and mixing or currents as a result of wind action was practically eliminated. The plastic created an unnatural substrate upon which grew copious amounts of algae and numbers of snails. Chemical changes in the water may have occurred as a result of contact with the plastic sheeting especially in the case of phosphorus which is readily absorbed by plastic. The author believes, however, that the advantages of using this sectioning technique and, therefore, using the pond as its own control, outweigh the disadvantages.

SUMMARY

1. A study area on the north side of a one acre pond located on the Michigan State University campus was partitioned into four sections with ten mil plastic sheeting.
2. Inorganic fertilizer was applied to two of these sections. A study was made of the physical, chemical, and biological characteristics in the four sections before and after fertilization.
3. An algal bloom formed in the two sections which received fertilizer.
4. No change in pH, alkalinity, or dissolved oxygen was observed following fertilization.
5. Concentrations of soluble phosphorus in the fertilized sections increased after the application of fertilizer. Concentrations of soluble phosphorus remained at a high level in these sections throughout the study.
6. Numbers of *Daphnia pulex* adults, young, and eggs per adult increased significantly in the fertilized sections as compared to those in the unfertilized sections.

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APPENDICES

APPENDIX A

CHEMICAL PARAMETERS OF POND SECTIONS

FIGURE 7

CHEMICAL PARAMETERS OF SECTION 1

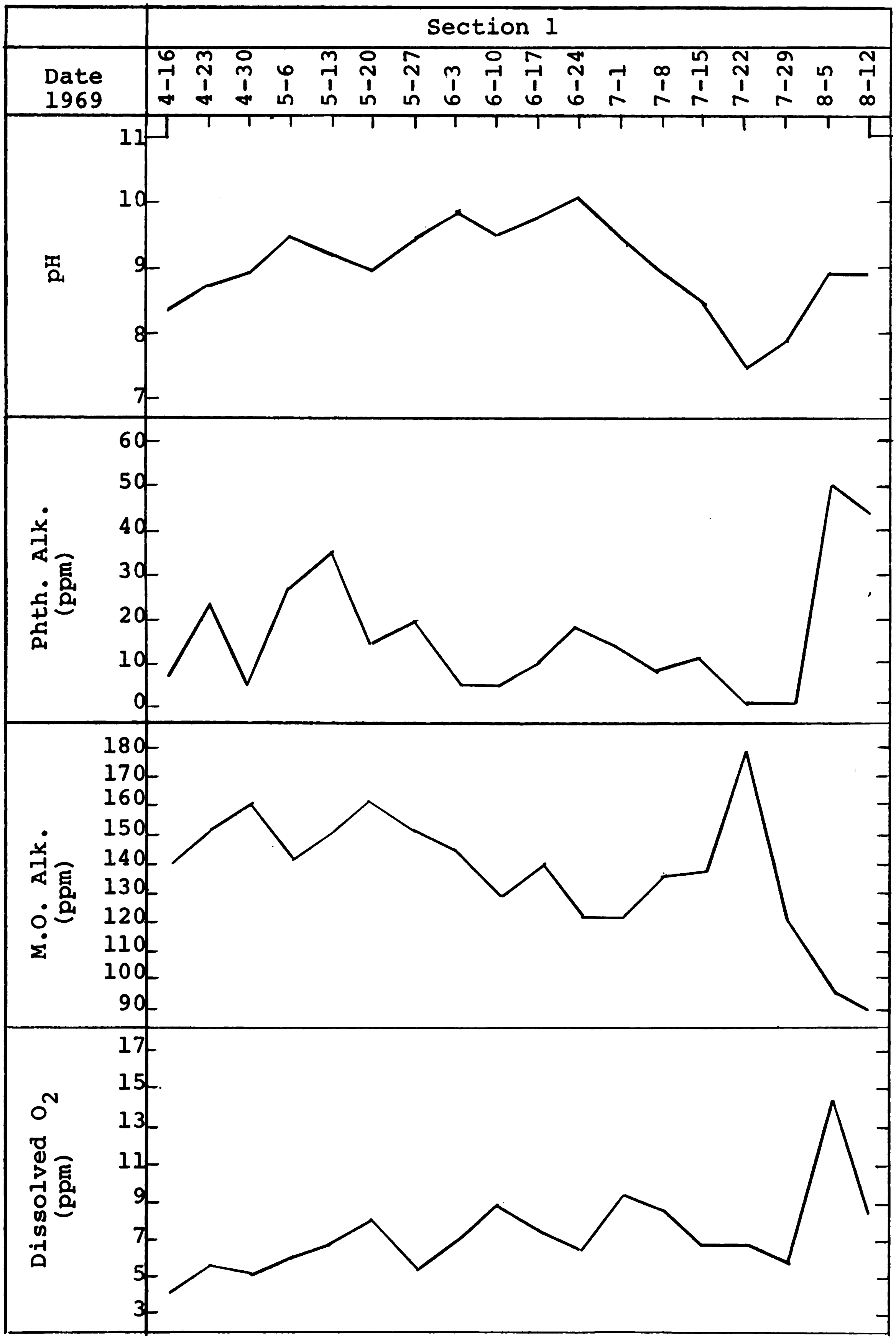
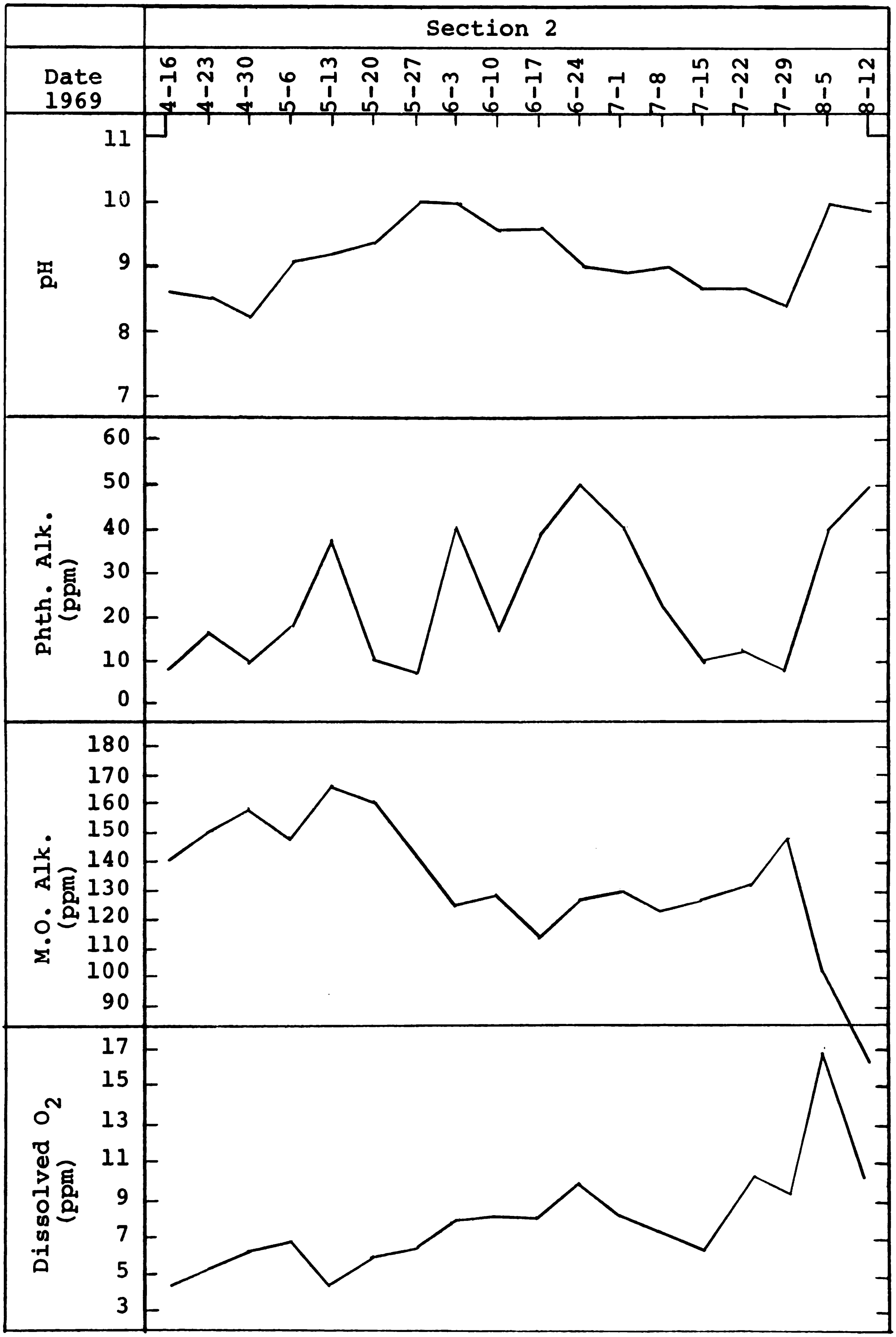

Section 1
Date 1969
4-16
4-23
4-30
5-6
5-13
5-20
5-27
6-3
6-10
6-17
6-24
7-1
7-8
7-15
7-22
7-29
8-5
8-12
pH
Phth. Alk. (ppm)
M.O. Alk. (ppm)
Dissolved O_2 (ppm)

FIGURE 8

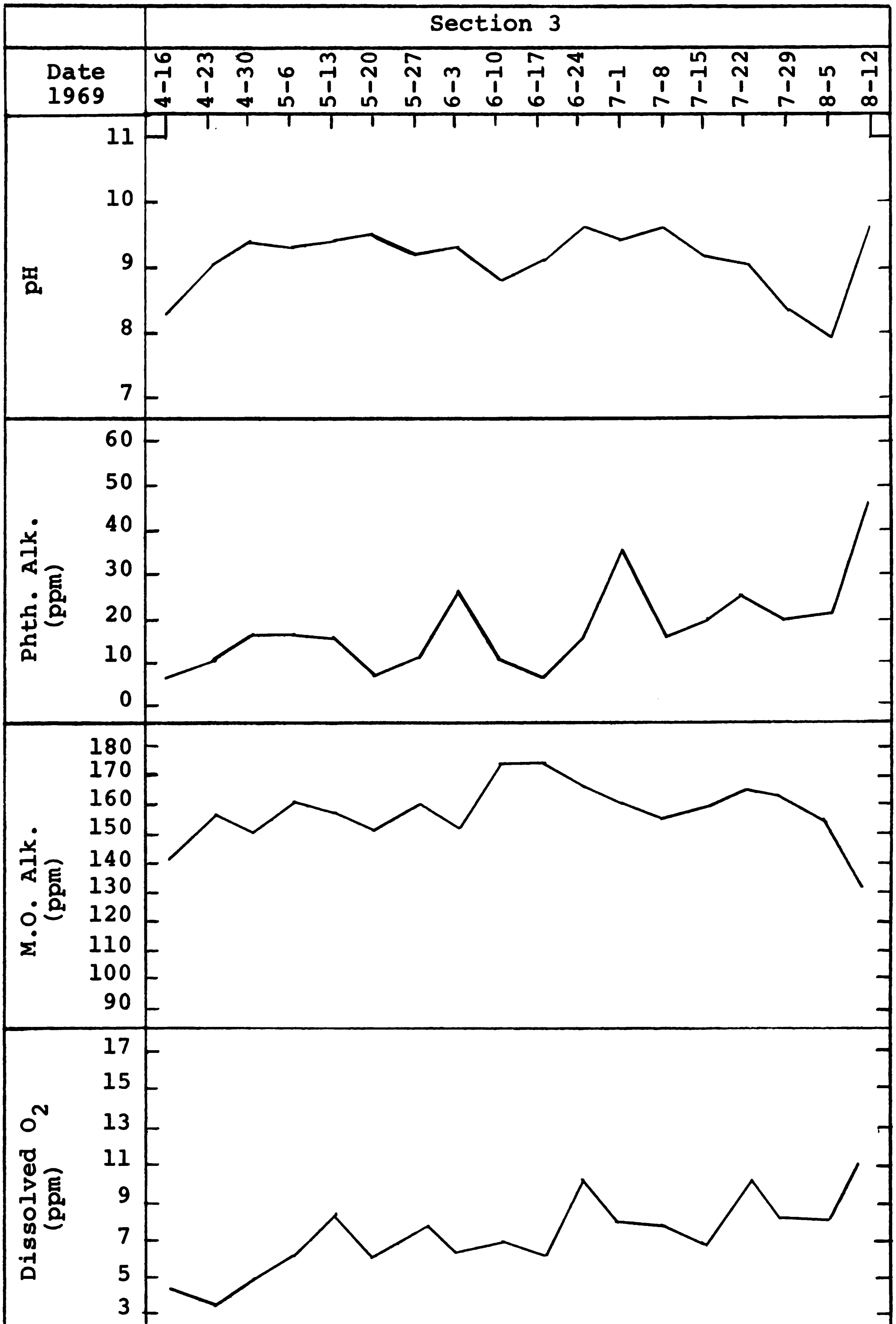
CHEMICAL PARAMETERS IN SECTION 2



Section 2
Date 1969
4-16
4-23
4-30
5-6
5-13
5-20
5-27
6-3
6-10
6-17
6-24
7-1
7-8
7-15
7-22
7-29
8-5
8-12
pH
11
10
9
8
7
Phth. Alk. (ppm)
60
50
40
30
20
10
0
M.O. Alk. (ppm)
180
170
160
150
140
130
120
110
100
90
Dissolved O_2 (ppm)
17
15
13
11
9
7
5
3

FIGURE 9

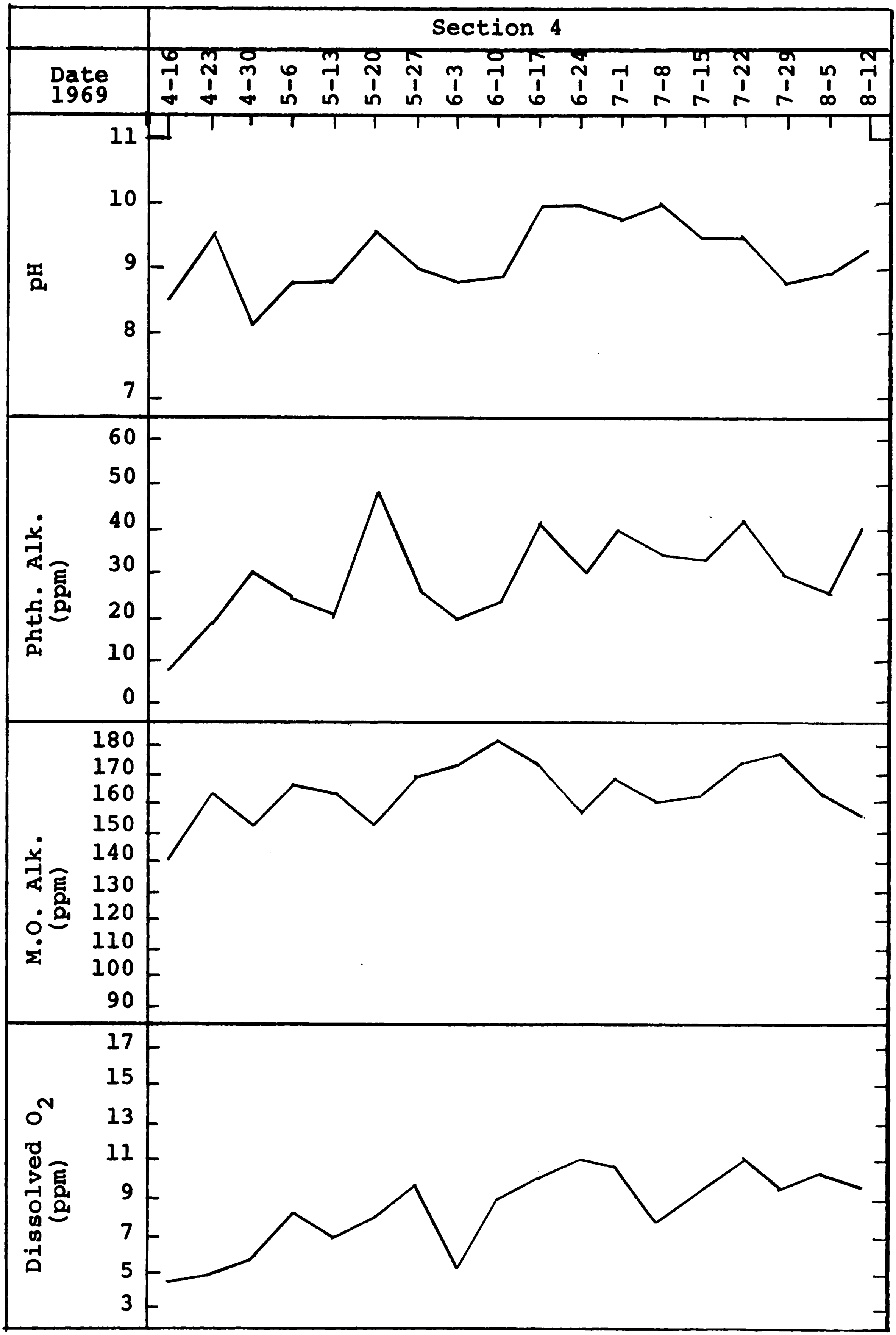
CHEMICAL PARAMETERS IN SECTION 3



Section 3
Date 1969
4-16
4-23
4-30
5-6
5-13
5-20
5-27
6-3
6-10
6-17
6-24
7-1
7-8
7-15
7-22
7-29
8-5
8-12
pH
11
10
9
8
7
Phth. Alk. (ppm)
60
50
40
30
20
10
0
M.O. Alk. (ppm)
180
170
160
150
140
130
120
110
100
90
Dissolved O_2 (ppm)
17
15
13
11
9
7
5
3

FIGURE 10

CHEMICAL PARAMETERS IN SECTION 4


Section 4
Date 1969
4-16
4-23
4-30
5-6
5-13
5-20
5-27
6-3
6-10
6-17
6-24
7-1
7-8
7-15
7-22
7-29
8-5
8-12
pH
11
10
9
8
7
Phth. Alk. (ppm)
60
50
40
30
20
10
0
M.O. Alk. (ppm)
180
170
160
150
140
130
120
110
100
90
Dissolved O_2 (ppm)
17
15
13
11
9
7
5
3

APPENDIX B

SAMPLER

FIGURE 11

HALF-LITER SAMPLER USED FROM MAY 27 TO AUGUST 12
FOR SAMPLING DAPHNIA

FIGURE 12

HALF-LITER SAMPLER USED FROM MAY 27 TO AUGUST 12
FOR SAMPLING DAPHNIA

APPENDIX C

DATA SHEET

WEEKLY WATER SAMPLE

CHEMICAL PARAMETERS:

ALKALINITY

METHYL ORANGE __________ PPM

PHENOLPHTHALEIN __________ PPM

TOTAL------------ __________ PPM

PH-------------------- __________

FREE CARBON DIOXIDE __________ PPM

WEATHER:

TEMPERATURE

AIR------------- __________

WATER

SURFACE------ __________

BOTTOM------- __________

GENERAL CONDITIONS:

WIND DIRECTION________ SPEED______

MISCELLANEOUS:

INVESTIGATOR____________________

APPENDIX D

TABLES

TABLE 7

ACTUAL NUMBERS OF ADULT DAPHNIA PULEX PER SAMPLE

Section	5-27		6-3		6-10		6-17		6-24		7-1		7-8		7-15		7-22		7-29		8-5		8-12	
	12	20	14	17	16	23	17	10	11	10	9	7	11	4	1	0	2	7	0	3	0	0	2	1
1	13	15	12	23	22	24	13	17	5	8	0	4	2	3	3	2	3	3	2	4	1	2	0	0
	16	8	14	16	14	12	15	16	6	13	2	5	7	1	7	0	4	1	0	0	1	0	3	7
	17	20	27	13	14	15	14	13	8	10	8	10	6	8	4	10	4	0	0	3	0	5	--	--
2	13	12	21	18	19	20	7	17	6	4	8	5	2	5	11	7	1	7	4	3	5	4	--	--
	22	16	17	13	15	15	9	10	7	4	7	11	10	3	5	9	2	6	1	5	2	4	--	--
	11	14	10	11	16	20	15	15	12	13	3	5	8	5	13	7	5	8	4	9	11	4	4	5
3	7	14	18	15	13	23	21	14	12	15	6	10	7	3	10	5	3	6	6	5	6	8	3	6
	13	8	14	11	14	19	18	9	13	6	6	9	3	4	6	10	4	4	6	8	3	10	8	11
	12	17	30	18	25	22	22	17	21	9	20	9	4	7	17	9	7	17	11	11	19	8	1	8
4	16	24	25	20	29	14	18	12	24	19	3	18	3	12	9	11	18	10	7	20	9	5	5	9
	17	19	27	19	17	17	21	19	14	15	12	12	11	8	8	14	11	16	9	--	8	10	15	7

TABLE 8

ACTUAL NUMBERS OF YOUNG DAPHNIA PULEX PER SAMPLE

Section	5-27	6-3	6-10	6-17	6-24	7-1	7-8	7-15	7-22	7-29	8-5	8-12
	22 36	33 50	27 22	25 31	32 38	31 29	5 16	22 18	10 1	3 4	4 7	0 2
1	31 20	39 48	39 35	42 28	19 24	19 24	10 15	16 9	9 6	0 8	3 8	3 7
	29 18	55 46	34 40	44 30	29 31	16 30	9 18	13 12	11 8	7 8	11 8	1 1
	62 44	35 44	22 34	20 28	24 17	18 13	20 13	6 14	11 12	9 14	2 12	-- --
2	70 58	34 39	30 38	34 32	31 32	12 20	12 24	0 7	19 19	3 8	0 3	-- --
	36 50	32 29	19 37	16 25	31 29	6 24	18 17	20 12	13 7	6 17	6 8	-- --
	24 38	37 48	39 42	22 18	12 17	24 21	24 21	12 22	24 36	18 16	20 28	22 25
3	28 21	28 32	24 45	32 30	21 16	23 8	19 12	11 18	20 31	25 30	30 19	23 20
	27 40	32 31	30 38	17 25	8 16	14 11	30 19	17 14	27 20	25 18	27 19	14 18
	28 33	45 33	30 26	22 38	24 38	19 21	18 15	31 21	26 13	28 21	32 22	28 12
4	28 34	44 28	38 24	37 31	24 30	17 28	12 29	34 20	18 15	31 22	20 16	36 27
	24 21	31 38	24 31	29 40	27 37	16 12	13 31	17 24	22 16	14 20	29 25	10 23

TABLE 9

MEAN NUMBER OF DAPHNIA PULEX PER LITER

Section	4-30	5-6	5-13	5-20	5-27	6-3	6-10	6-17	6-24	7-1	7-8	7-15	7-22	7-29	8-5	8-12
Adults																
					Before Fertilizer							After Fertilizer				
1	5	8	15	16	28	32	37	29	18	9	9	4	7	3	1	4
2	3	12	10	20	33	38	33	23	13	16	11	15	7	5	7	0
3	11	8	18	22	22	26	35	31	24	13	10	17	10	13	15	12
4	15	20	18	22	35	46	41	36	34	28	15	23	26	23	20	15
Young																
					Before Fertilizer							After Fertilizer				
1	18	36	40	48	52	90	66	67	58	50	24	30	15	10	14	5
2	26	21	43	64	107	71	60	52	57	31	35	20	27	19	8	0
3	31	34	45	40	59	69	73	46	30	34	42	31	53	45	46	41
4	23	24	33	55	56	73	58	66	60	38	39	49	37	45	48	45