WITH COPPER SULFATE AND
SODIUM ARSENITE

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ABSTRACT

ECOLOGICAL ALTERATIONS PRODUCED BY THE TREATMENT OF POND ECOSYSTEMS WITH COPPER SULFATE AND SODIUM ARSENITE

by Leonard Paul Sohacki

The ecological alterations produced by the addition of herbicides to pond ecosystems were investigated. Three ponds were used in the study and each received treatment as follows: pond A, 8 ppm sodium arsenite; pond B, 8 ppm sodium arsenite and 2 ppm copper sulfate; pond C, 2 ppm copper sulfate.

Changes in water chemistry, which included dissolved oxygen, alkalinity, pH, and carbon dioxide, were exhibited by all of the treated ponds. In general, the severity of changes produced by the treatments were related to the concentration of plant biomass killed. Dissolved oxygen and pH values of the pond waters decreased shortly after the herbicide treatments but returned to the normal range of values within two weeks. The alkalinity values of the ponds increased to as high as 20 ppm above the normal range of values and took as long as one and one-half months to attain normal conditions again. Carbon dioxide concentrations increased simultaneously with plant decomposition and returned to normal within two weeks after the herbicide applications.

Herbicide induced changes in the productivity of the macrophytes, phytoplankton and periphyton were observed. Copper sulfate effectively eliminated the macroscopic alga, *Chara*. Sodium arsenite selectively killed the higher aquatic plants but showed no toxicity towards *Chara*. Once the higher aquatic plants were killed, they showed no signs of recovery for the remainder of the summer. Contrarily, *Chara* exhibited new growth within one month after herbicide treatment.

The periphyton and phytoplankton productivity were altered by sodium arsenite as well as by copper sulfate. Phytoplankton productivity as measured by the carbon-14 method showed that the phytoplankton were inhibited immediately after the herbicides were added and for five to ten days thereafter. No evidence of a phytoplankton bloom accompanied the release of nutrients from the decomposing vegetation. Unlike the phytoplankton, the periphyton showed little inhibition from the herbicide treatments, but responded to the release of nutrients as shown by increased standing crop levels.

A comparison of the pretreatment and post-treatment benthic invertebrate populations showed significant changes which were attributed to the toxicity of the herbicides. Both copper sulfate and sodium arsenite produced serious decreases in the invertebrate populations. However, the greatest decrease was shown in pond B where the bottom fauna was subjected to the combined toxicities of copper sulfate and sodium arsenite. Although all invertebrates were affected, the mayfly naiads showed extreme susceptability particularly to sodium arsenite.

ECOLOGICAL ALTERATIONS PRODUCED BY THE TREATMENT OF POND ECOSYSTEMS WITH COPPER SULFATE AND SODIUM ARSENITE

By

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INTRODUCTION

Aquatic weed control has become an indispensable practice for successful pond management because of the detrimental effects nuisance weeds exert upon fish populations. Fish management is hampered by aquatic weeds in the following ways: (1) weeds protect small fish from predators and thereby enhance the development of overcrowded, stunted fish populations, (2) weeds compete for nutrients with the desirable primary producers which comprise the base of the food chain, (3) heavy weed infestations lead to winter and summer fish kills, and (4) weeds interfere with fish removal from the ponds.

Weeds are removed from the ponds by mechanical or chemical means, however, chemical control is usually preferred. Sodium arsenite and copper sulfate are the most commonly used chemical controlling agents; sodium arsenite is utilized primarily for higher aquatic weed control, whereas copper sulfate is used for algae. These two herbicides have remained popular for over fifty years despite the discoveries of newer organic herbicides. The lasting popularity of sodium arsenite and copper sulfate can be attributed to their lower cost, and of more importance, their effectiveness in removing common aquatic nuisance weeds.

In spite of their extended use as aquatic herbicides, very little is known of the ecological alterations induced by the addition of copper sulfate or sodium arsenite. The more apparent changes such as reduction in the amounts of vegetation and depletion of dissolved oxygen concentrations are well-known. Investigators have devoted considerable study to the alterations which produce fish mortality, but the insidious changes which accompany herbicide treatment have been neglected. However, the absence of mortality doesn't necessarily mean the fishes escape all detrimental effects. Lawrence (1958) showed that bluegill production was reduced by sodium arsenite treatments even though the concentrations used were considered "safe" by herbicide specialists. Although Lawrence)op. cit.) did not attribute the reduced production to any specific factor, it is presumed that a combination of herbicide toxicity and the accompanying ecological changes were responsible for the inhibition of fish growth, with the latter enhancing the toxic effects of the former. The objective of this study was to discover the magnitude and duration of the latent ecological changes which are caused by treatment of sodium arsenite, copper sulfate or combination thereof.

Of the various latent changes initiated by the herbicide addition, the following are thought to most seriously impair the welfare of the aquatic community: (1) alteration of the water chemistry, and (2) inhibition of the primary producers.

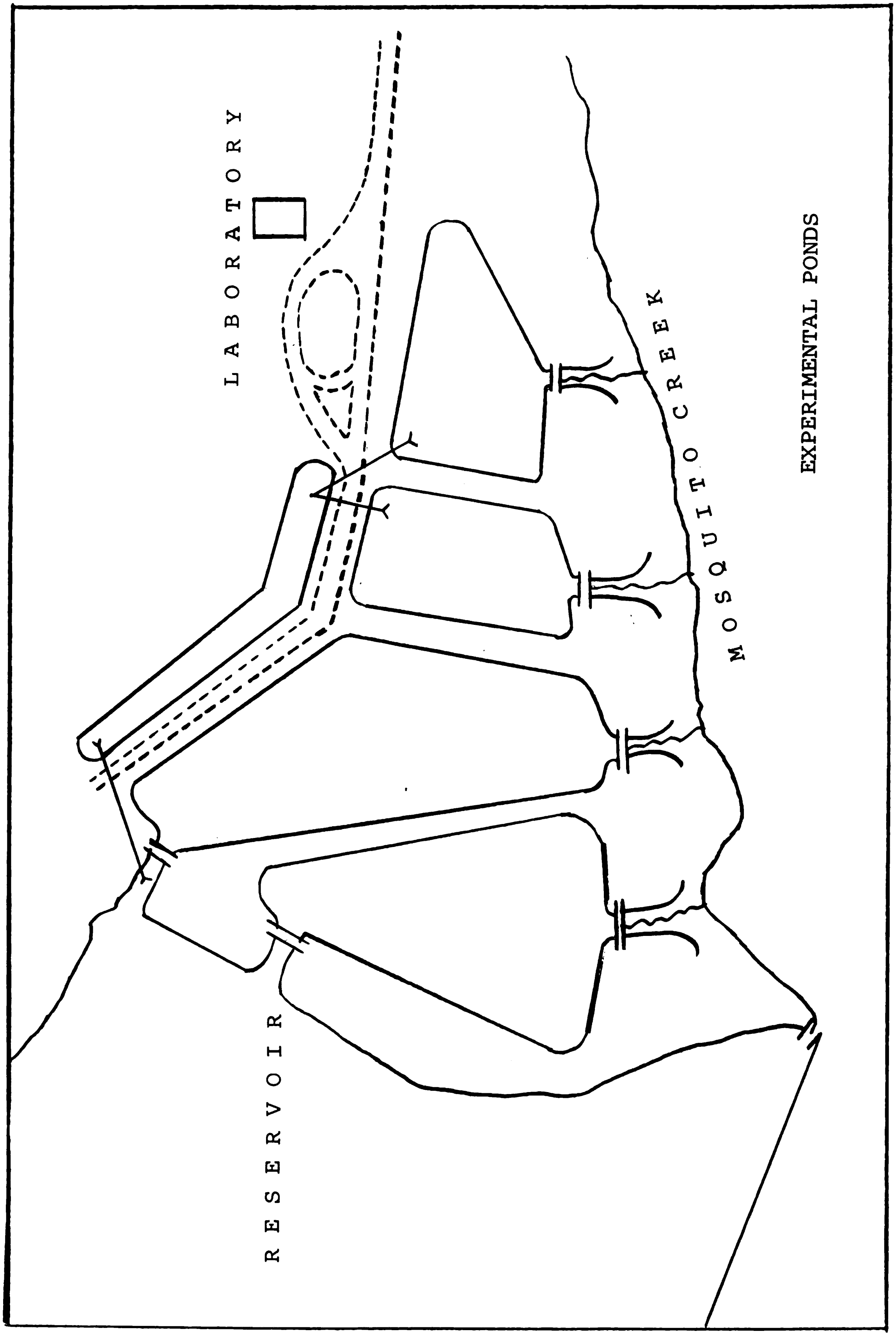
The chemical alteration of pond waters which follows herbicide treatment results primarily from the death and decomposition of the aquatic vegetation. The most obvious change is the reduction of dissolved oxygen in the water. Since severe oxygen depletion results in fish mortalities, this phase of water chemistry has received considerable attention from the investigators. However, other important chemical changes are known to accompany plant decomposition. Of particular significance is the increase in carbon dioxide concentrations. Because carbon dioxide, pH, and alkalinity are interrelated, a change in one is usually accompanied by changes in the other two factors. This is especially true in aquatic systems which are not well buffered. Although radical alterations in the pH can deleteriously influence the aquatic biota, its effects are not thought to be as toxic as changes in carbon dioxide and oxygen. Low oxygen concentrations are considered more toxic in the presence of high carbon dioxide levels (Welch, 1952). Such conditions often follow herbicide applications.

Due to the non-specificity of sodium arsenite and copper sulfate, the desirable as well as the undesirable primary producers are killed or inhibited in growth. In pond ecosystems the phytoplankton and periphyton are principal contributors to the base of the food chain, and hence all aquatic heterotrophs are directly or indirectly dependent upon these algae for existence. Consequently, the absence or inhibition of these primary producers for an extended period of time would adversely influence all trophic levels. Yet little information can be found pertaining to the magnitude and duration of algal inhibition following herbicide applications. Smith (1940) reported that algal production was impaired for over a year in soft water lakes which received 3 parts per million (ppm) copper sulfate. On the other hand, Moyle (1949) stated that a decrease in undesirable forms is followed shortly by an increase in desirable forms. The information presented in these two articles dealt only with planktonic forms. No data pertaining to the influence of copper sulfate upon periphyton could be found.

Although sodium arsenite is considered a higher aquatics-specific herbicide it also exhibits algacidal properties, and hence could also inhibit the beneficial primary producers. Surber (1943) and Lawrence (1958) demonstrated the effectiveness of sodium arsenite upon filamentous algae such as *Hydrodictyon*, *Spirogyra*, and *Pithophora*. Since these nuisance algal forms are inhibited, it seems reasonable to assume that the desirable forms are inhibited also. However, this has never been proven.

A meager amount of information is available concerning the toxicity of copper sulfate and sodium arsenite treatment upon benthic fauna. Lawrence (1958) is the only investigator who demonstrated that sodium arsenite reduced bottom insect populations under natural conditions. Surber (1931) showed that low concentrations (2.5 ppm) killed susceptable insects under laboratory conditions. Even less is known about the influence of copper sulfate. Although Mackenthun and Cooley (1952) demonstrated that copper sulfate accrual in lake sediments from prior treatments had no effect upon the benthic fauna, no data are available on its direct influence. Because of the important role played by the bottom fauna in the aquatic community, further investigation was warranted.

Figure 1. Map of the Lake City ponds.


LABORATORY
EXPERIMENTAL PONDS
MOSQUITO CREEK
RESERVOIR

DESCRIPTION OF STUDY AREA

This study was conducted at the Lake City Experimental Farm which is located approximately two miles south of Lake City, Michigan. The farm, owned and operated by Michigan State University, is used for agricultural research projects conducted by the Agricultural College. The Department of Fisheries and Wildlife maintains four experimental ponds and a field laboratory on the premises where limnological research work is conducted during the summer seasons.

The experimental ponds were constructed during the period 1943-1945 on a marshy area near Mosquito Creek, a small stream which rises on the farm property. A dam, located on the stream, forms a backwater area which is used as a reservoir for filling the ponds. Each pond has its own inlet and outlet and hence can be filled or drained independently of the others (Fig. 1). Downstream from the dam the stream bed passes behind the pond outlets and serves as a drainage channel when the ponds are emptied.

From west to east the ponds are designated by the letters A, B, C, and D; only ponds A, B, and C were used in this project. The areas and average depths of the test ponds are as follows:

Pond A - .36 acres, 3.2 ft. average depth

Pond B - .46 acres, 3.3 ft. average depth

Pond C - .17 acres, 3.4 ft. average depth.

All ponds have a maximal depth of 6 ft. at the spillway.

The ponds were originally built on a sandy base, but presently the sand is partially covered by a mucky deposit interspersed with small woody particles.

METHODOLOGY

Sodium Arsenite Application

Sodium arsenite ($NaAsO_2$) is a herbicide well-known for its toxicity to terrestrial as well as aquatic plants. Liquid sodium arsenite is recommended for treating aquatic habitats in preference to the dry forms (Hooper and Cook, 1957). The liquid form is manufactured by a number of companies under different brand names, but in each case the product contains four pounds of arsenic trioxide (As_2O_3) per gallon.

On July 20, 1962, pond A was treated with a form of sodium arsenite known as Atlas-A, which is manufactured by the Chipman Chemical Company. Enough sodium arsenite was added to the pond to bring the arsenic concentration up to 8 ppm As_2O_3.

Before being added to the pond, the concentrated sodium arsenite was thoroughly mixed with 150 gallons of pond water in a large plastic lined tank located at the pond's edge. A portable engine-driven pump was used to pump the diluted herbicide from the tank through a length of 2 inch diameter hose into the pond. An operator at the nozzle directed the stream of dilute herbicide across the surface of the pond. Immediately after dispensing the herbicide, a small boat equipped with a 5 horsepower outboard engine was used to agitate the pond and mix the herbicide to all depths.

Copper Sulfate Application

Copper sulfate ($CuSO_4 \cdot 5H_2O$) is an algae specific herbicide which has been used for controlling algal blooms since 1904. With the exception of a few species, most algae readily succumb to low concentrations of copper sulfate. As little as .3 ppm effectively control obnoxious phytoplankton blooms, but as much as 1.5 ppm are required to kill *Chara*. Since *Chara* was the nuisance alga in the test ponds, higher concentrations of copper sulfate were necessary. However, the susceptibility of the algae is not the only factor which determines the concentrations of copper sulfate to be used; the water chemistry of the system must also be considered. Bicarbonates and suspended organic matter react with copper sulfate to inactivate its algacidal properties. Consequently, adjustments must be made to compensate for these reactions and are described by Smith (1935).

Powdered copper sulfate, which is the most soluble form, was used to treat pond C on July 12, 1962. The amounts necessary to bring the copper sulfate concentration of the pond water to 2 ppm was measured out to the nearest gram, placed in a fine meshed sack and towed through the pond behind an outboard powered boat.

Copper Sulfate and Sodium Arsenite

Pond B received a treatment of 2 ppm copper sulfate and 8 ppm sodium arsenite on August 3, 1962. These herbicides were applied within one-half hour using the methods of application described in the preceding sections.

Physical Measurements

Solar Radiation

The incident solar radiation in the vicinity of the Lake City ponds was measured by a pyrheliometer-recorder assembly. The pyrheliometer is a radiation detector of the thermopile type which consists of a number of series connected thermocouples that generate an electromotive force (emf) proportional to the intensity of the incident radiation. An Eppley pyrheliometer was mounted on a platform located between ponds C and D, and was connected by an underground cable to a Bristol strip-chart recorder located in the laboratory. The recorder converted the emf produced by the pyrheliometer into units of gram calories per square centimeter per minute. The gram calorie is a unit of pyrheliometry defined as the quantity of heat necessary to change the temperature of one gram of water from 3.5 to 4.5 degrees centigrade.

Chemical Measurements

With few exceptions, chemical analyses were conducted daily throughout the summer. Surface water samples were collected between 1400 and 1600 hours, and transferred to the laboratory for immediate processing.

Dissolved Oxygen

The unmodified Winkler method (Standard Methods, 1961) was used to determine the dissolved oxygen content of the pond waters.

Alkalinity

Alkalinity, or the acid destroying capacity imparted to the water by carbonates, bicarbonates, and hydroxides, was determined by titrating the water samples with N/50 sulfuric acid using phenolphthalein and methyl orange as end point indicators.

Hydrogen Ion Concentration

The hydrogen ion concentration, or pH, was determined with a Beckman Model H2 pH meter.

Carbon Dioxide Concentration

The alkalinity and pH values were used in conjunction with Moore's nomograph to determine the carbon dioxide concentrations.

Biological Measurements

Macrophytes

The macroscopic bottom plants (aquatic macrophytes) of each pond were sampled twice during the summer, once before and once after the herbicide applications. Each sampling consisted of thirty randomly selected Petersen dredge samples (.802 M^2). The individual samples were placed in a 30 mesh sieve and washed to remove the silt and sand particles. Then the plants were carefully separated from the material remaining in the sieve and washed again to remove any adhering particles of sand, detritus, etc.; the residue in the sieve was saved for invertebrate analysis. Subsequently, the plants were dried in a drying oven maintained at a temperature of 55°C., and finally weighed to the nearest .001 gram.

Phytoplankton

The carbon-fourteen method, introduced by Steemann Nielsen (1952) with modifications by Rodhe (1958), was used throughout the summer of 1962 to study phytoplankton production. Rodhe (op. cit.) exposed his light and dark bottles from sunset of one day to sunset of the next. He maintained that quarter or half-day production values extrapolated for the whole day are subject to error because of unequal insolation.

Glass reagent bottles (250 ml.) were employed as light and dark exposure containers. Light was excluded from the dark bottles with a coat of black paint and a layer of black plastic tape, as recommended by Strickland (1960). Shortly after sunset water samples for the C^{14} method were taken from the 32 inch depth with a modified Meyer bottle and poured into three containers, a light and a dark bottle plus a sample bottle for alkalinity determination. Two microcuries of C^{14} as sodium carbonate ($NaC^{14}O_3$) were injected into the contents of the light and dark bottles with a 2 ml. insulin syringe. (The $NaC^{14}O_3$, contained in 2 ml ampoules, was purchased from the Volk Chemical Company.) Then the exposure bottles were stoppered, clamped to a removable pipe support and exposed at the 32 inch depth. The sample taken for alkalinity determination was immediately transferred to the laboratory and titrated.

The exposed bottles were removed from the ponds on the sunset of the following date and processed immediately. A 100 ml aliquot was measured from each bottle and filtered separately through .45 micron Millipore Filters. Then 10 ml .003N HCl was passed through the filter to remove inorganic C^{14}, and followed by 10 ml 10% formalin to kill and preserve the plankton. The filters were removed from the funnels, glued to stainless steel planchets and stored in a dessicator which contained silica gel.

All samples were counted in a Tracerlab low background Omni/Guard counter for ten minutes. Since a zero thickness was assumed, no corrections for self absorption were made.

The basic principle underlying the C^{14} method is the photosynthetic conversion of carbon dioxide to organic carbon by phytoplankton. Unlike the terrestrial environment, free carbon dioxide in many aquatic ecosystems is practically nonexistent. Instead, carbon dioxide exists in the chemically combined forms of bound and half-bound carbonates such as $CaCO_3$ and $Ca(HCO_3)_2$. Aquatic plants possess physiological mechanisms which enable them to extract carbon dioxide from these carbonates. When a small tracer dose of radioactive carbon (C^{14}) in a sodium carbonate ($NaC^{14}O_3$) carrier is added to a sample of pond or lake water and exposed to favorable conditions, the phytoplankton convert the $C^{14}O_2$ from the artificial carbonate into organic matter. Upon completion of the exposure period, the phytoplankters are filtered from the water sample and assayed for radioactivity. The ratio of $\frac{C^{14}\text{ assimilated}}{C^{14}\text{ available}}$ represents the proportion of C^{14} uptake from the original concentration. Since stable carbon (C^{12}) from the natural occurring carbonates is assimilated simultaneously with C^{14} in about the same proportions, the total original C^{12} content of the water sample multiplied by the preceding ratio yields the C^{12} assimilated during the exposure period.

Calculations:

The presumed constant relationship between the assimilation of stable and radioactive carbon is expressed in the following equation:

$$\frac{C^{12}\text{ Assimilated}}{C^{12}\text{ Available}} = \frac{C^{14}\text{ Assimilated}}{C^{14}\text{ Available}} \quad (K)$$

For every fraction of C^{14} assimilated from the total available supply, a proportional amount of C^{12} is assimilated from the stable carbon supply.

1. C^{14} assimilated:

Non-photosynthetic C^{14} assimilation by phytoplankton and bacterial physiological processes obscures the true photosynthetic values. Fortunately, the non-photosynthetic assimilation progresses at the same rate in the darkness as in the light, and hence this assimilation is equal in both, the light and dark bottles. The activity of the dark bottle in counts per second (cps) subtracted from the cps of the light bottles yields net photosynthetic activity.

The counts registered by radiation detectors are not enumerations of particle emissions, but manifestations of particle initiated ionization events in the counting chamber. Because not all beta emissions initiate an ionization, not all particles are counted. The fraction of particles counted of the total emitted constitutes the counter efficiency, a factor which must be multiplied times the cps of each sample to convert relative counts into absolute disintegrations per second (dps). For example, if the counter efficiency is 25% all counts must be multiplied by a factor of 4.

Correction must also be made for subsample size if the entire sample is not filtered. For example if a 100 ml aliquot of a 300 ml sample is filtered, the dps must be multiplied by a factor of 3. With all factors included, the C^{14} assimilated value of the preceding equation may be calculated as follows:

C^{14} assimilated in dps = (cps light bottle-cps dark bottle) (counter efficiency) $\left(\frac{\text{sample size}}{\text{subsample size}}\right)$.

2. C^{14} available:

This value represents the total activity in dps added to the sample bottle. In this study, 2 microcuries or 7.4×10^4 dps were injected into the sample.

3. C^{12} available:

The C^{12} available is calculated from the alkalinity determination made at the time of sampling. Alkalinity titrations with .02N H_2SO_4 results in alkalinity values expressed as milligrams per liter (mg/l) $CaCO_3$; hence C^{12} = (.12) (mg/l $CaCO_3$). The factor .12, which represents the fraction of $CaCO_3$ weight contributed by carbon, was calculated as follows: Molecular Wt. (M.W.) Carbon = 12; M.W. $CaCO_3$ = 100; $\frac{12}{100} = .12$.

4. K:

K equals the discrimination constant. Nielsen (1952) and others demonstrated that C^{14} is assimilated slightly slower than C^{12}, hence a correction of 6% is added to the C^{12} value.

Periphyton

The autotrophs which are found growing on the surfaces of organic and inorganic objects in aquatic environments, and forming a slimy layer upon these objects are known as periphyton. These organisms, comprised mainly of algae, differ from other primary producers by their habit of attaching to but not penetrating the surface upon which they live.

Various methods have been used for measuring the production of periphyton, however, the artificial substrate method was used in this study. Twenty-four plexiglass substrates (140 cm^2 exposure area) were attached to an exposure rack and exposed horizontally 15 inches below the pond's surface. Periodically, four of the substrates were removed from each pond; three were processed for productivity estimations and the organisms from the fourth were preserved in a formal-alcohol solution for future microscopic analysis.

The substrates used for production estimations were processed in a manner similar to Grzenda's (1960). Each substrate was scraped of periphyton and rinsed with 95% ethanol. This mixture was then increased in volume to 50 ml with more ethanol, transferred to a 2 oz. bottle and placed in the dark for at least 24 hours for phytopigment extraction. After extraction, 25 ml of the phytopigment solution was pipeted into a colorimeter cell and absorbency readings were made using a Klett Colorimeter equipped with a red filter (643-700 mμ range). Special care was taken during the pipeting process to prevent the settled algal cells from being disturbed, since the turbidity caused by the suspended cellular material would interfere with the colorimeter readings. Any samples which were accidentally agitated were allowed to settle before the absorbency reading was made.

Grzenda (1960) showed that ethanol-chlorophyll solution absorbency conformed to the Lambert-Beer Law up to a reading of 0.20 (Klett Colorimeter with 600-700 mμ filter). Beyond this range the deviation from the Lambert-Beer Law increased proportionally with higher phytopigment concentrations, thereby requiring the use of a correction graph. In this study the absorbency readings never exceeded 0.20 and hence no correction of figures was necessary. Consequently, the absorbency figures presented here are directly equivalent to Grzenda's (op. cit.) phytopigment units. For convenience, the phytopigment units were multiplied by a factor of 10^3 for all graphical and tabular presentations.

Ultimately, each periphyton sample was transferred to a preweighed evaporating dish and completely dried at 55°C. After the dry weight was determined, the samples were placed in a muffle furnace and ashed at 550°C; the dry weight minus the ash free dry weight constituted the organic weight of the periphyton.

Bottom Invertebrates

The material remaining in the sieve after the plants were removed consisted of coarse bottom sediments, organic debris, and benthic invertebrates. This agglomeration was transferred to pint jars, preserved in formalin, and eventually inspected for invertebrates. A sugar flotation method was used to separate the insects from the bottom refuse. Sugar water, concentrated enough to float the invertebrates, was added to the samples and the mixture agitated. The agitating action caused most of the invertebrates to float to the surface where they were easily removed with forceps; the residue was then thoroughly inspected for non-floating invertebrates.

After the invertebrates were separated into their respective categories, the wet weights were determined by the following method. First, these organisms were soaked in distilled water for 30 minutes. Then they were transferred to special centrifuge screens, and centrifuged for

30 seconds to 1800 rpm. After centrifuging, the insects were immediately weighed on an analytical balance to the nearest .0001 gram.

MACROPHYTES

Pretreatment standing crop estimates showed _Chara_ to be the sole macrophyte in pond C, and the dominating plant in the other ponds. In addition to _Chara_, ponds A and B also contained higher aquatics plants. The macrophyte composition of the test ponds along with the herbicide treatment data are included in Table 1.

A comparison of the pretreatment and post-treatment standing crop estimates revealed the effectiveness of the herbicide treatments. This is shown in Table 2. All macrophytes except the _Chara_ in pond A were successfully eliminated or retarded by the herbicides.

Copper Sulfate

The _Chara_ in pond C showed first signs of reacting to the copper sulfate treatment three days after the herbicide was added. At this time, these plants exhibited a pale yellow color. Within a few days after the abnormal coloration was exhibited, all of the _Chara_ slumped to the bottom and decomposed. Very little growth activity was shown by the _Chara_ for the remainder of the season. In fact, thirty post-treatment bottom samples yielded only a fraction of a gram of this macrophyte.

Table 1. Herbicide concentrations, treatment dates, and pretreatment macrophyte composition of the Lake City ponds.

Pond	Herbicide	Ppm.	Treatment Date	Macrophytes
C	$CuSO_4$	2	7-12	Chara sp.
A	$NaAsO_2$	8	7-20	Chara sp. Elodea canadensis Najas flexilis Potamogeton sp.
B	$CuSO_4$ $NaAsO_2$	2 8	8-3	Chara sp. Elodea canadensis Najas flexilis Potamogeton sp.

Table 2. Standing crop estimates of the macrophyte populations before and after herbicide treatment.

	Higher Aquatics		Chara sp.	
	Mean*	Standard Error	Mean*	Standard Error
Pond C				
Pretreatment (7-10-62)	0.00	----	96.4	12.7
Post-treatment	0.00	----	trace	
Pond A				
Pretreatment (7-18-62)	32.0	11.9	49.6	9.1
Post-treatment (9-5-62	0.0	----	53.8	9.3
Pond B				
Pretreatment (8-1-62)	57.5	9.8	61.7	14.7
Post-treatment (9-12-62)	0.0	----	6.9	1.7

*Grams per square meter dry weight.

Sodium Arsenite

The plants in pond A exhibited a delayed response to the toxicity of sodium arsenite. Six days elapsed before the higher aquatic plants showed any signs of unhealthiness. Shortly thereafter, these plants slumped to the bottom. Mackenthun (1955), Hooper and Cook (1957) and others state that the plants usually slump to the bottom in approximately five days or less after herbicide treatments. Pond A was the only pond which required a longer time to respond to the herbicide action. It is believed that the cloudy conditions which prevailed on the date of herbicide treatment and the ensuing days were responsible for the delay (see Appendix for solar energy values). Low light intensities accompanied by reduced metabolic activities in the plants would result in a slower uptake of the herbicide and hence a delayed kill.

Although the higher aquatic plants were completely eliminated by sodium arsenite (Table 2), the *Chara* exhibited resistance. A slight yellowing of the *Chara* "stems" was the only visible sign of reaction to the herbicide. Inhibition of growth, if any, was slight, for a matched observations test revealed the post-treatment population to be significantly greater in grams per square meter than the pretreatment population (Table 3). This indicated that the *Chara* was actively growing during the period between

Table 3. Matched pairs test (mean of the differences) of the pretreatment and post-treatment standing crop (mg/M^2 dry weight) of _Chara_ in pond A.

$\Sigma d = 9.22$

$\Sigma d^2 = 21.83$

$\frac{(\Sigma d)^2}{n} = 2.83$

$\bar{d} = .307$

$s_{\bar{d}} = 0.145$

$t = 2.115$ *

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*Significantly different at the .05 level (d.f. = 30).

samplings. The resistance of this alga to the toxicity of sodium arsenite was demonstrated by Riggs (1955) and is mentioned in most herbicide manuals.

Copper Sulfate and Sodium Arsenite

The aquatic plants in pond B responded to the combined toxicities of copper sulfate and sodium arsenite within two days after application. And although the plants in this pond succumbed more rapidly than those in the other ponds, they were the first to recover. A new growth of *Chara* was evident at the post-treatment sampling, only forty days after the herbicide application (Table 2). However, the higher aquatic plants showed no signs of recovery during the remainder of 1962.

DISSOLVED OXYGEN

The dissolved oxygen concentrations of any aquatic ecosystem are subject to diurnal fluctuations normally brought about by changes in the biological processes and physical components of the environment. According to Welch (1952) the two most important sources of oxygen in an aquatic system are diffusion from the atmosphere and photosynthetic production by plants. Deoxygenation is produced by the following: (1) animal respiration, (2) plant respiration, (3) bacterial decomposition, and (4) chemical oxidation of organic matter in solution (Hutchinson, 1957). Usually the oxygen depletion is "balanced" by replacement so the oxygen content of the water is seldom reduced to levels deleterious to the aquatic biota. Addition of herbicides to an aquatic environment upsets this "balance" and tends to reduce the oxygen concentrations, sometimes to critical levels.

The most important factors responsible for a herbicide induced oxygen reduction are the cessation of photosynthetic mechanisms of the primary producers and increase in bacterial respiration. Undoubtedly the first reaction of the primary producers to the toxicity of the herbicide is the halting of photosynthetic oxygen production.

When this happens, the major portion of the oxygen entering the waters is by direct diffusion from the atmosphere. However, the diffusion rates are not sufficient to satisfy the increased metabolic demands of the decomposer organisms, particularly the bacteria. As the primary producers succumb to the toxic action of the herbicides, their tissues are reduced to simpler compounds by oxidative decomposition of the aquatic bacteria. The greater the bulk of decaying plant tissues, the more numerous are the bacteria and the greater their oxygen demand. For this reason herbicide experts recommend repeated partial treatment of ponds heavily choked with weeds. Under such circumstances, an entire treatment would result in a kill of the desirable fauna due to the oxygen deficiency caused by the decay of the large bulk of weeds.

All of the test ponds exhibited a decrease in dissolved oxygen concentrations shortly after the herbicide treatments. The herbicide induced alterations of dissolved oxygen are illustrated in Figure 2. As expected, the magnitude and duration of oxygen reduction was directly related to the amounts of vegetation killed in the pond. And hence it is not surprising to note that pond A exhibited the least reduction in oxygen concentrations. Since the <u>Chara</u> in pond A was apparently unaffected by the sodium arsenite, its production of photosynthetic oxygen alleviated the reduced oxygen condition and promoted rapid recovery of the pond.

Figure 2. Dissolved oxygen concentrations in the test ponds during the summer of 1962.

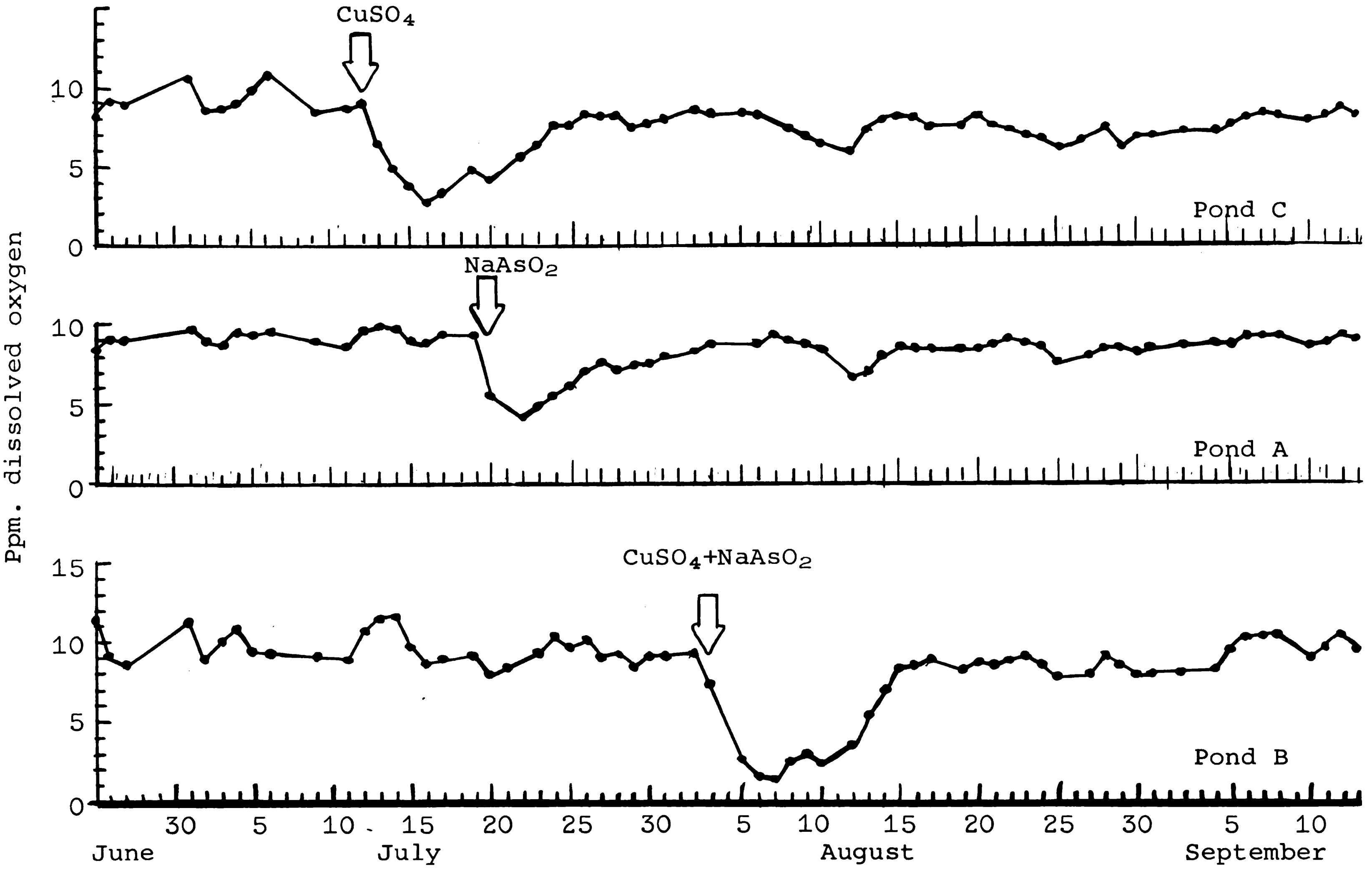
CuSO$_4$
NaAsO$_2$
CuSO$_4$+NaAsO$_2$
Pond C
Pond A
Pond B
Ppm. dissolved oxygen
0
5
10
15
30
5
10
15
20
25
30
June
July
August
September

Figure 2

More plant material was killed in ponds C and B than in A, and therefore the deflections in the oxygen curve are greater and longer in duration. Both, higher aquatic plants and Chara were killed in pond B by the copper sulfate-sodium arsenite combination. The bulk of decomposable plant tissues exceeded the amounts in either of the other test ponds; this is reflected in the post-treatment oxygen concentrations. Dissolved oxygen concentrations in pond B decreased to 1.52 ppm four days after the herbicide treatment, and remained lower than the pretreatment levels for approximately two weeks.

The oxygen concentrations in ponds B and C were reduced to concentrations of less than 3 ppm. In general, 3 ppm oxygen at 25°C. is critical to most fresh water fishes (Ellis, M. M., Westfall, B. A., and Ellis, M. D., 1948). Although no visible fish kill was noted in pond C, a considerable number of bullheads, green sunfish, and minnows were found dead or dying in pond B four days after the herbicide treatment. In addition, many invertebrates, mainly crayfish and dragonfly naiads were also found.

Although the oxygen reduction was undoubtedly important in killing the aquatic organisms, it most certainly was not the sole factor responsible. The direct toxicity of the sodium arsenite-copper sulfate undeniably had some effect. In addition, other factors such as changes in the pH and carbon dioxide are known to enhance the toxicity of poisons. The alterations in the latter two factors will be discussed in the next section.

CARBON DIOXIDE, pH AND ALKALINITY

Carbon dioxide, pH and alkalinity are interdependent chemical properties of natural waters which are predominant factors in determining the composition of the aquatic community. A change in one of these chemical properties is usually reflected in an alteration of the others. Any rapid increase or decrease, particularly in the pH and carbon dioxide can deleteriously effect the organisms of the aquatic environment. Ordinarily such drastic changes seldom occur, however, a disturbance such as the addition of an herbicide can produce such undesirable consequences.

The addition of a herbicide to an aquatic ecosystem initiates a series of biological responses which alter the chemical composition of the water. Of the various chemical changes that occur, the increase in free carbon dioxide is of prime importance. Besides altering the pH and alkalinity of the water, excessive free carbon dioxide influences the respiration of fishes and other aquatic animals directly by reducing the affinity of blood for oxygen (Welch, 1952). In fact, high carbon dioxide tensions may result in the suffocation of fishes even though ample oxygen is available (Prosser and Brown, 1961). Although carbon dioxide increases may not always be severe enough to induce mortality, lesser concentrations could possibly produce sufficient physiological stress to retard growth and/or impair the fertility of the aquatic fauna.

Free carbon dioxide is produced as a waste product during the utilization of dead organic matter by decomposer organisms. All of the aquatic organisms which succumb to the toxicity of the herbicide immediately become available to the decomposers. During decomposition under aerobic conditions, 61 to 67 per cent of the carbon in algae and net plankton were found to be released as carbon dioxide within 31 days (Allgeir, R. J., Peterson, W. H., and Juday C., 1934). Once the free carbon dioxide is released into the water, some or all of it combines immediately with monocarbonates to form bicarbonates (Welch, 1952). In the absence of carbonates, carbon dioxide reacts with water to form carbonic acid (op. cit.). Consequently, the fate of carbon dioxide depends upon the chemistry of the aquatic environment.

Chemical determinations made on the Lake City ponds in the undisturbed condition (summer, 1961) showed the waters to be moderately hard and alkaline. Total alkalinity values ranged between 55 and 75 ppm, and the pH varied between 8.0 to 9.0. Under these alkaline conditions little free carbon dioxide (less than 1 ppm) is found, instead it exists in the combined form of half-bound bicarbonates (HCO_3^-) and bound carbonates ($CO_3^=$). Similar chemical conditions existed in the ponds during the summer of 1962, prior to the herbicide treatment.

Within 24 hours after the herbicide treatments, the water chemistry of the ponds began to exhibit changes. Carbon dioxide and alkalinity concentrations increased and the pH decreased. These changes are readily recognizable from Figures 3 and 4. Changes in pH and alkalinity were due to the increase in free carbon dioxide released from the decomposing organic matter. As mentioned previously, free carbon dioxide reacts immediately with carbonates to form bicarbonates. As long as the carbonates are available, the formation of carbonic acid is prevented and the pH of the water remains unchanged. Hence, the carbonates serve as a buffer substance; one which resists changes in pH. However, the buffer action is limited by the concentration of carbonates. Once the carbonates are depleted and free carbon dioxide continues to be released, a change is inevitable. The pH changes exhibited by the test ponds indicated that the carbonate concentrations were insufficient to buffer the water during the duration of carbon dioxide release from the decaying plants. In fact, the water chemistry data show that the carbonate alkalinity was completely absent from the water in pond C within 24 hours after the herbicide was added. Ponds A and B are assumed to have reacted similarly although alkalinity determinations were not made on the day following herbicide addition. Nevertheless, the carbonate alkalinity disappeared completely from these ponds within three days after the herbicide additions.

Figure 3. Carbonate and bicarbonate alkalinity values of the Lake City ponds during the summer of 1962.

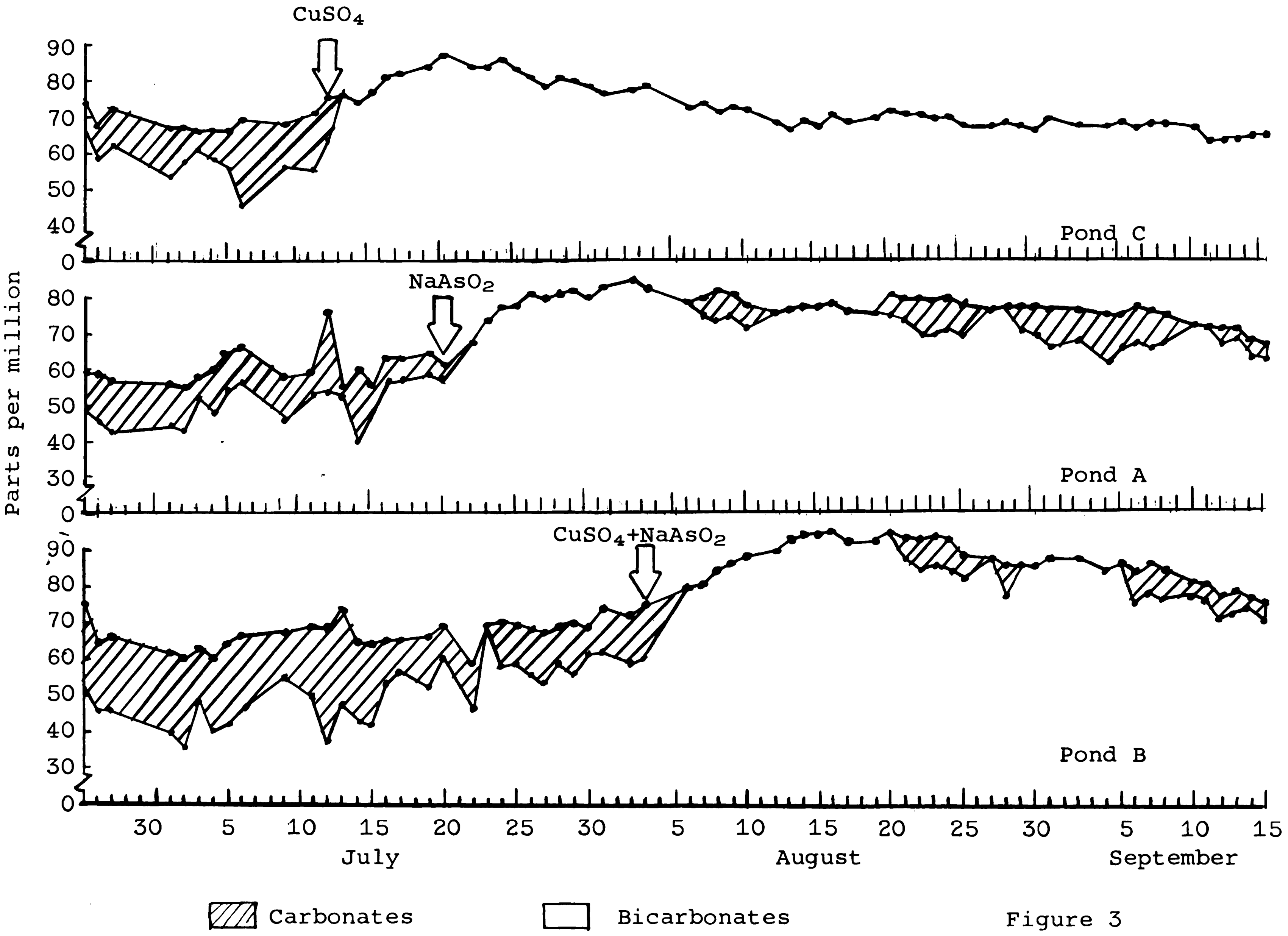
$CuSO_4$
$NaAsO_2$
$CuSO_4+NaAsO_2$
Pond C
Pond A
Pond B
Parts per million
90
80
70
60
50
40
30
0
30
5
10
15
20
25
30
5
10
15
20
25
30
5
10
15
July
August
September
▨ Carbonates
☐ Bicarbonates

Figure 3

Figure 4. Hydrogen ion concentration (pH) and free carbon dioxide concentrations greater than 1 ppm. of the test ponds during the summer of 1962.

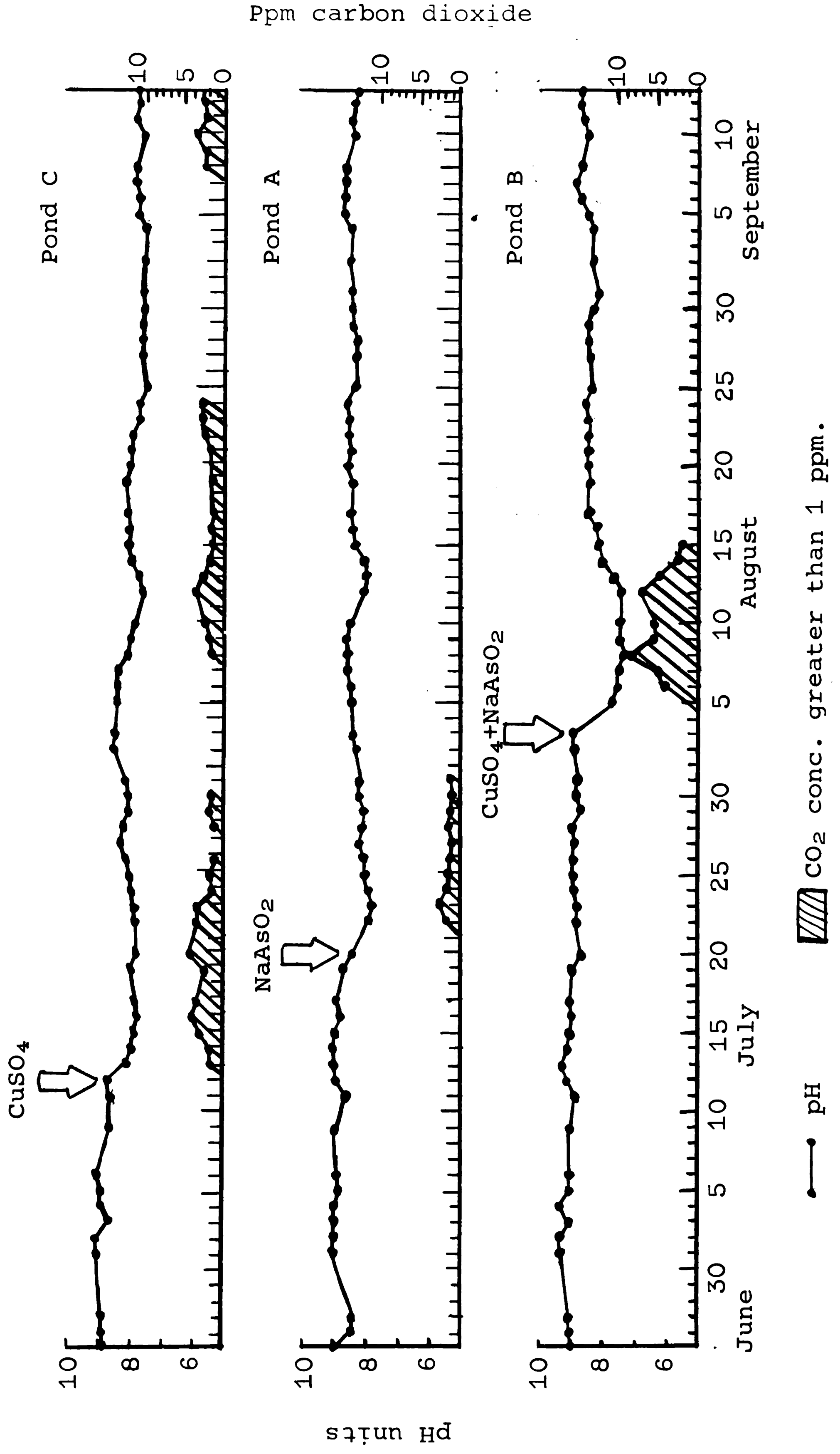
Ppm carbon dioxide
10
5
0
Pond C
Pond A
Pond B
CuSO$_4$
NaAsO$_2$
CuSO$_4$+NaAsO$_2$
10
8
6
pH units
June
30
5
10
15
July
20
25
30
5
10
15
August
20
25
30
5
September
10
pH
CO$_2$ conc. greater than 1 ppm.

Figure 4

The reduction in pH which followed the depletion of carbonates was also accompanied by an increase in carbon dioxide. Free carbon dioxide concentrations (calculated from Moore's nomograph) in all ponds exceeded 1 ppm within three days after the herbicide treatments. A minimum pH of 7.3 and a maximum carbon dioxide concentration of 7.0 ppm were recorded in pond B. The pH and carbon dioxide changes of the other ponds are easily recognizable, but not as pronounced as in pond B.

Of all chemical factors measured, the bicarbonate alkalinity exhibited changes which were greatest in magnitude and longest in duration. Post-treatment maximum values exceeded the pretreatment values by 21 ppm in pond B, 14 ppm in pond A, and 12 ppm in pond C. The initial post-treatment increase was attributed to the conversion of carbonates to bicarbonates. However, it has been mentioned that the carbonate alkalinity was completely depleted from the pond waters within three days after the herbicide additions. And yet the bicarbonate alkalinities continued to rise for as long as eight days thereafter. Evidently a source of carbonates other than that contained by the water was being utilized. Since the only other available carbonates were contained in the bottom muds and plant encrustations, the reactions of the carbon dioxide and carbonic acid must have included these sources.

The most severe changes in the water chemistry of the ponds took place within two weeks after the herbicide applications. The time required to return to normal varied between ponds and between the various chemical factors. By the end of the summer, the carbon dioxide and pH levels of ponds A and B had attained the pretreatment range of values. Contrarily, the pH in pond C remained lower than the pretreatment values for the remainder of the season. In addition, the carbon dioxide levels of this pond continued to exhibit sporadic increases above 1 ppm.

PHYTOPLANKTON

Because of the non-specific action of copper sulfate and sodium arsenite, treatment to eliminate macrophytes may result in the elimination of phytoplankton and periphyton as well. Most heterotrophic forms of life in lentic systems depend directly or indirectly upon the phytoplankton and periphyton for their existence. Since these producers comprise an important segment of the food chain, any prolonged decrease in their production would be reflected in the production at all trophic levels.

Although copper sulfate and sodium arsenite exhibit algacidal properties, their long-term effects upon the phytoplankton populations are not fully understood. During this study, the phytoplankton productivity was followed from the time of herbicide application until complete recovery was attained. Post-recovery productivity estimates were continued to ascertain whether the productivity was increased by the release of nutrients from the decaying macrophytes.

Immediate Response of Phytoplankton Productivity to Herbicides

In order to detect the immediate influence of herbicide toxicity upon phytoplankton productivity, the routine C^{14} method was modified on the dates of herbicide application.

Instead of exposing the samples for an entire day, three series of exposures were placed in the pond to be treated for the following time periods: 0600-1000, 1000-1400 and 1400-1800. In each case, the herbicides were applied immediately preceding the 1400-1800 series. The phytoplankton productivity values for the days of herbicide application appear in Table 4 along with the incident light energy values of the exposure periods.

All of the ponds exhibited a greatly reduced productivity immediately after the introduction of the herbicides. These reductions were too drastic to be caused by natural limiting factors. Light is normally considered as the most important limiting factor to phytoplankton production. However, the sunlight energy values on the herbicide application dates show that no severe decrease in sunlight energy followed any of the herbicide applications. On the contrary, all of the sunlight energy values for the afternoon exposures were greater than those for the mornings. Yet, a tremendous difference in productivity is shown between the two periods. These data seem to indicate that the reduction in phytoplankton productivity could hardly have been attributed to low sunlight energy.

Other limiting factors could have contributed to the reduction in phytoplankton productivity, but this is unlikely. Depletion of an essential nutrient or combination thereof would produce such a reduction, but certainly not within a

Table 4. Phytoplankton productivity and solar energy determinations of the Lake City ponds on the dates of herbicide application.

Time	Gram calories cm^{-2}	Assimilated Carbon (mg. m^{-3})
	<u>Pond C -- July 12, 1962</u>	
0600-1000	187.68	18.22
1000-1400	257.55	30.67
1400-1800	225.08	2.12
	<u>Pond A -- July 20, 1962</u>	
0600-1000	22.7	7.67
1000-1400	33.3	15.44
1400-1800	30.6	3.96
	<u>Pond B -- August 3, 1962</u>	
0600-1000	125.70	10.06
1000-1400	232.95	18.21
1400-1800	130.65	2.10

few hours. Furthermore, the phytoplankters probably maintain a reserve supply of nutrients which would sustain them for at least several hours after such a mishap. Although changes in water chemistry, particularly pH, could induce such an effect, no such change was detected. Moreover, the change which reduced the phytoplankton production gives all indications of being immediate rather than gradual. Since the only radical change that occurred on these days was the addition of herbicides, any anomalous disturbance of the productivity must have been due to the herbicide toxicities. Additional evidence of phytoplankton inhibition is provided by the post-treatment productivity measurements.

Copper Sulfate

Copper sulfate is a very potent algacide. As little as .3 to .5 ppm will control undesirable plankton blooms, but concentrations as high as 1.5 ppm are required to control the macroscopic alga, *Chara*. If low concentrations are inhibitive to phytoplankton growth, how much more extensive is the inhibition when high concentrations are used? Smith (1940) reported that 3 ppm of copper sulfate added to soft water lakes inhibited the phytoplankton population for over a year. Moyle (1949) implied that the phytoplankton populations of lakes are not seriously inhibited by standard concentrations of copper sulfate. However, most of the reports dealing with the influence of copper sulfate upon phytoplankton have been casual observations; no specific production studies were conducted.

The decline of phytoplankton productivity is quite evident in Figure 5. The pretreatment productivity values averaged 28.2 mg assimilated carbon/M^3/day, but decreased to 2 mg on the day following the copper sulfate treatment. This depressed condition continued for nine days before the pretreatment range of productivity values was once again attained.

After recovering from the copper sulfate toxicity, the phytoplankton population maintained normal productivity except on two occasions, August 6th and 13th (Figure 5). On these dates the productivity decreased tremendously. In fact, the magnitude of decline in productivity was similar to that which took place during herbicide inhibition. At least one of these erratic values may be attributed to a physical change in the pond waters. After the dead Chara had decomposed, the waters of pond C became highly colored and turbid. This condition is assumed to have arisen from the decomposition products released from the plants. But regardless of the source, these factors were responsible for attenuating the sunlight in deeper areas of the pond. On one of the days of low production (August 13th), the incident sunlight energy was only 108.6 gram calories. It is assumed that the attenuation of the already low

Figure 5. Phytoplankton productivity of the test ponds during the summer of 1962.

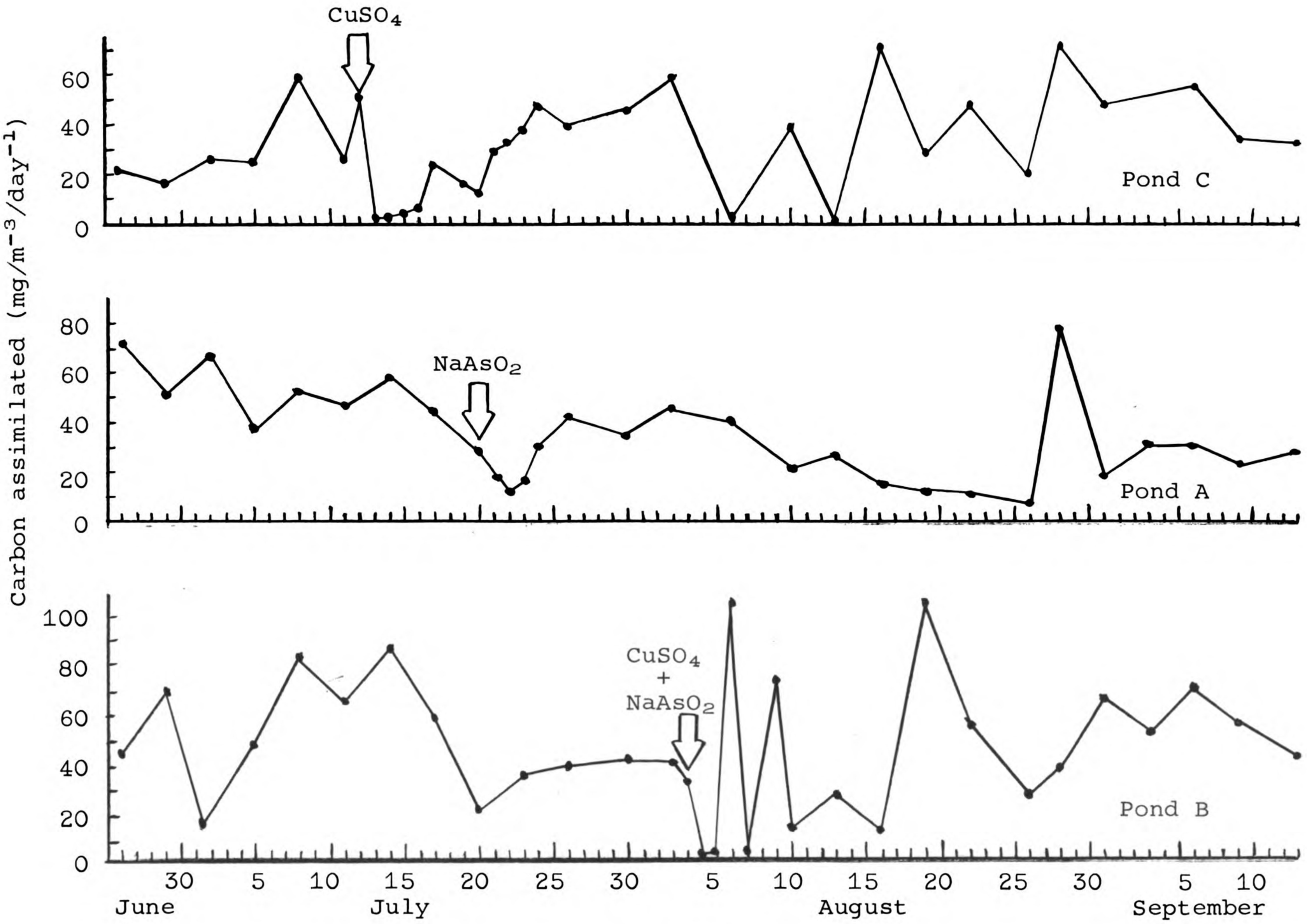
Carbon assimilated (mg/m^{-3}/day^{-1})
CuSO$_4$
Pond C
60
40
20
0
NaAsO$_2$
Pond A
80
60
40
20
0
CuSO$_4$
+
NaAsO$_2$
Pond B
100
80
60
40
20
0
30
5
10
15
20
25
30
5
10
15
20
25
30
5
10
June
July
August
September

Figure 5

incident light resulted in sub-optimal light conditions at the level of C^{14} exposure. Hence, the extreme reduction in assimilated carbon. Since the other ponds did not develop the turbid conditions, they did not react similarly. The other low productivity value shown in pond C (August 6th) cannot be explained as above for the light on this date was normal.

Sodium Arsenite

Little is known about the influence of sodium arsenite upon the phytoplankton even though its algacidal properties were demonstrated over thirty years ago. Surber (1931) warned against exceeding the recommended concentration of 2 ppm because of its toxicity towards microscopic plants and animals. Dupree (1960) discovered that plankton concentrated tremendous amounts of arsenic from waters treated with 4 ppm sodium arsenite. Further studies on the algacidal properties of this herbicide dealt mainly with filamentous pond scum algae, although a few general comments on the reactions of the phytoplankton were usually included. But that is about the extent of information available.

Phytoplankton productivity decreased following the sodium arsenite application (Figure 5), but the decrease was not as great as that produced by copper sulfate. In addition the phytoplankton recovered sooner from the toxic effects of sodium arsenite. Only six days elapsed before the pretreatment range of productivity values were attained. The slight reduction in productivity and short recovery time demonstrates the resistance of algae toward sodium arsenite toxicity.

After recovering from the toxicity of sodium arsenite, the phytoplankton productivity in pond A gradually decreased until a seasonal low of 8.2 mg carbon/m^3/day was recorded on August 6th. From this date until the end of the summer the productivity showed a marked increase. The gradual decline in productivity was assumed to be part of a seasonal cycle and not associated with herbicide inhibition.

Copper Sulfate and Sodium Arsenite

The phytoplankton population of pond B reacted to the combined toxicities of sodium arsenite and copper sulfate by exhibiting post-treatment productivity reductions. But unlike the responses shown in the other ponds, those in pond B were highly erratic. Pond B exhibited an initial decrease like the other ponds, but only three days after herbicide application productivity increased abnormally (Figure 5). Productivity values again decreased, but repeated the unusual increase three days later, whereupon the anticipated levels were finally attained permanently. Judging from the post-treatment responses of the other ponds, the erratic values observed could hardly have been so great, especially since there were two algae inhibitors present. The only possible explanation for the extreme increases is human error. This error could have occurred during the processing of the C^{14} samples, and the following factors are suspected: (1) failure to rinse the Millipore Filters of inorganic C^{14} during processing, or (2) contamination of the filter with inorganic C^{14} during or after processing. If the data suspected of being in error are deleted, then the period of reduced phytoplankton productivity caused by the addition of the herbicide combination lasted ten days.

Post-treatment Response to Increase in Nutrients

Treatment of a pond or lake with herbicides usually results in a post-treatment production decrease followed by a tremendous increase (Mackenthun, 1955; Moyle, 1949). These increases are initiated by the release of nutrients from the decaying macrophytes and other primary producers which succumb to herbicide toxicity. In order to detect a possible increase in productivity, the pretreatment productivity values were compared to the post-recovery values (exclusive of the inhibited period). The comparisons for the three ponds are shown in Table 5. No significant differences were shown between the pretreatment and post-recovery values of pond B or pond C. A significant difference did exist between these values in pond A, but was exactly opposite the results expected; the pretreatment

Table 5. Comparison of phytoplankton productivity of the pretreatment and post-recovery periods using the Student's t-test.

		Pretreatment*			Post-recovery		
Pond	Dates included	Mean	Standard error	Dates included	Mean	Standard error	
C	6-23-62 to 7-11-62	28.18	5.33	7-20-62 to 9-9-62	40.76	4.42	0
A	6-23-62 to 7-17-62	56.55	5.20	7-26-62 to 9-9-62	29.71	4.60	X
B	6-26-62 to 7-17-62	48.70	6.00	8-13-62 to 9-9-62	50.38	8.31	0

*Productivity measurements in milligrams carbon assimilated per cubic meter per day.

X = significantly different at the .05 level.

0 = no significant difference at the .05 level.

productivity was greater than that of the post-treatment period. From these data, no evidence of a post-treatment bloom is shown.

The lack of response to the nutrient increase merits further discussion. Evidence that the nutrients, in fact, were released was shown by the increases in periphyton productivity in all test ponds. These results will be discussed in the next section. However, the periphyton data offer proof that nutrients were not concentrated near the bottom sediments and unavailable to the phytoplankton, for the periphyton substrates were exposed only 15 inches below the surface. Any nutrients available at this depth would certainly be available for phytoplankton also. But for some reason the phytoplankters were unable to utilize these nutrients. A possible explanation of this phenomenon may reside in the reaction of these unicellular algae to intense sunlight conditions.

Knight, Ball and Hooper (1962) observed low phytoplankton productivity values in the Lake City ponds during the summer of 1960, and attributed the reductions to photoinhibition effects produced by extended exposure to critical light intensities. Supra-optimal light intensities can lead to decreasing photosynthetic activity and loss of chlorophyll. The phenomenon of photoinhibition has been observed in marine phytoplankton and is discussed in detail by Steemen Nielsen (1952).

Both, Steemen Nielsen's (loc. cit.) and Rhode's (1958) data, show that the greatest amount of inhibition occurs in the first meter below the ocean's surface. However, the phytoplankton in the ocean are not constantly subjected to the deleterious intensities for they are carried by the currents to more optimal light conditions at lower depths. In the test ponds, which average 1 meter in depth, the phytoplankters are constantly exposed to intense sunlight without relief. If this inhibitive effect persisted during the post-treatment period, the phytoplankton could not use the nutrients even though they were available.

PERIPHYTON

Although more emphasis is usually placed upon the production of phytoplankton in lentic environments, the role of the periphyton producers cannot be ignored. This is particularly true in shallow lakes and ponds where the littoral zone encompasses all depths (Wetzel, 1964). In such systems, a major portion of the total pond production is due to the microscopic sessile algae. The greatest significance attached to these algal forms is the amount of organic matter contributed to the higher trophic levels as food. In fact, Lauff (1959) stated that a direct correlation exists between the periphyton and fish production in many lakes. If this is true, then a long term inhibition of the periphyton producers would ultimately be accompanied by a reduced production at all trophic levels.

Despite the fact that periphyton, like all other primary producers, are susceptible to the toxicity of herbicides, no information is available concerning the extent and duration of inhibition initiated by these toxic substances.

Periphyton productivity is normally expressed as milligrams of carbon produced per unit area per day, and I originally intended to express my data as such. But the extreme variability which existed between the subsample weights precluded such treatment. Unequal settling of disturbed bottom detritus upon the horizontally exposed substrates was suspected as the variability inducing agent. Fortunately, phytopigment readings were made on the pigment extracts within a few days after the samples were collected. And furthermore, the presence of organic detritus gave no indication of interfering with the phytopigment determinations. Since phytopigment concentration has been proven to be closely correlated with the organic weights of periphyton (Grzenda and Brehmer, 1960), these determinations were used to detect the herbicide induced responses of the periphyton communities.

Copper Sulfate

Because algae are extremely sensitive to the toxicity of copper sulfate, a serious reduction in periphyton standing crop was expected after treatment of pond C. However, this did not occur. Instead, a very slight decrease in standing crop was elicited in response to the treatment (Figure 6). But the magnitude of this reduction could have been deceptive due to the method of measurement. Dead algal cells are known to retain detrital chlorophyll which when measured during pigment analysis contribute to the total chlorophyll concentration and limit the accuracy of the method (Strickland, 1960). The periphyton algae which

Figure 6. Periphyton standing crop of the test ponds expressed as phytopigment units per square decimeter.

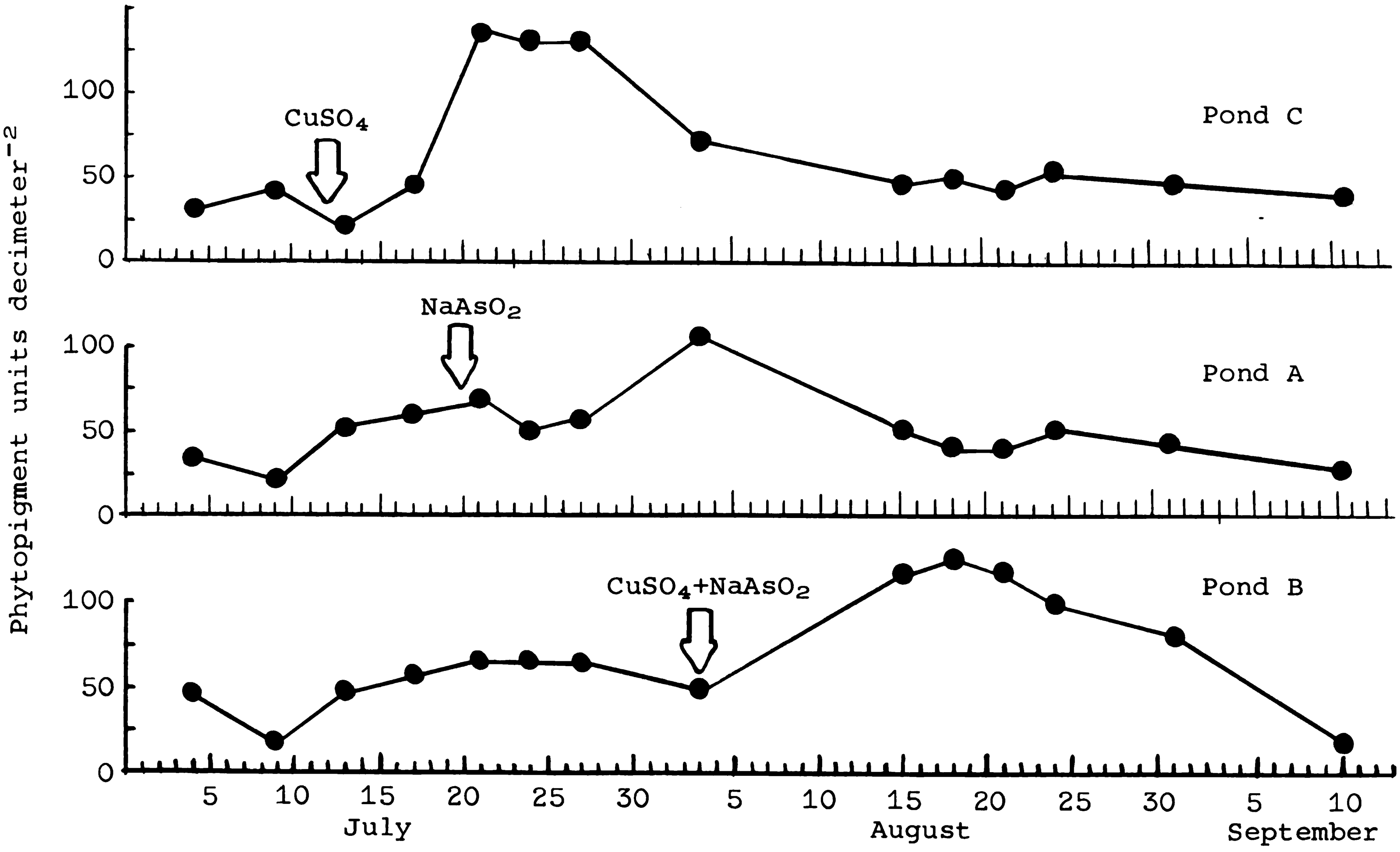
Phytopigment units decimeter^{-2}
100
50
0
$CuSO_4$
Pond C
$NaAsO_2$
Pond A
$CuSO_4+NaAsO_2$
Pond B
5
10
15
20
25
30
5
10
15
20
25
30
5
10
July
August
September

Figure 6

succumbed to the herbicide toxicity probably contributed some of the phytopigment during the post-treatment decline, and hence these particular values do not represent the true standing crop. Such a phenomenon would lead to an underestimation of the actual inhibitory response exhibited by the periphyton. Nevertheless, any inhibition which might have occurred was short-lived for five days after treatment the standing crop was once again normal.

Upon recovering from the copper sulfate toxicity, the periphyton increased rapidly until the pretreatment standing crop was exceeded by over 200 per cent. This tremendous increase took place in less than ten days from the time of treatment and endured for two weeks. Subsequently, the standing crop gradually decreased to the normal pretreatment values which were maintained for the remainder of the season.

The period of high production undoubtedly was initiated by the release of nutrients from the decomposing Chara. If extrinsic factors, chemical or physical, were responsible for this accelerated growth, then the other ponds, proximal to pond C, should have reacted similarly. As one may see from Figure 6, the undisturbed ponds showed no similar responses. Post-treatment data from the other ponds showed the same effect; while the treated pond(s) responded to the herbicide treatment, the non-treated pond(s) maintained normal standing crop values. The fact that this was repeated three times during the summer provided undisputable proof that the accelerated production of periphyton was associated with the after effects of herbicide treatment; furthermore, the increase in nutrient concentration was the most probable causative factor.

Sodium Arsenite

Sodium arsenite is usually not considered as being an algacide. However, Suber (1943) and Lawrence (1958) proved that sodium arsenite effectively controlled filamentous algae such as *Hydrodictyon*, *Pithophora*, *Oedogonium* and *Cladophora*. Lawrence (loc. cit.) obtained effective control of *Pithophora* over the entire summer by using one application of 4 ppm sodium arsenite. Despite the high concentrations of this herbicide used in this study, the periphyton standing crop which consisted primarily of *Oedogonium* was not greatly reduced (Figure 6). In fact, the magnitude of decrease in standing crop was not any greater than that produced in pond C by copper sulfate. But the limitations of the pigment analysis (discussed previously) may have resulted in the underestimation of inhibition.

After a five day period of inhibition, the standing crop increased in response to the release of nutrients from the decomposing macrophytes. The duration of increased production was not nearly the magnitude or the duration of the other ponds. A possible explanation resides in the fact that less vegetation was killed in this pond, and hence the nutrients were not as abundant.

Copper Sulfate and Sodium Arsenite

Although the data from pond B show no post-treatment standing crop decrease, the combined toxicities of these two herbicides are assumed to have initiated such a response. Nonetheless, within twelve days after the herbicide treatment, the standing crop increased 100 per cent (Figure 6). These high values were maintained for approximately three weeks before normal values were once again attained. The extended production increase was attributed to the large mass of vegetation killed in this pond. More macrophytes were killed in pond B than in any other pond.

BOTTOM INVERTEBRATES

Immature aquatic insects comprised the greater portion of the invertebrates in the test ponds with the midge family (Tendipedidae) being the most numerically abundant group. Mayfly naiads (Ephemeroptera), comprised mainly of the genus <u>Caenis</u>, were the second most numerically abundant group. Dragonfly and damselfly naiads of the order Odonata (families Libellulidae, Gomphidae, and Coenagrionidae) contributed the most insect biomass to ponds A and B, while midge larvae contributed most in pond C. The most commonly encountered members of the group classified as Other Insects included the caddis flies (Trichoptera), biting midges (Diptera) and water boatmen (Hemiptera). These insects were not as prevalent as the other groups and did not contribute much in numbers or in biomass.

The non-insect segment of the invertebrate population was categorized into two groups, the Molluscs and Miscellaneous Invertebrates. The latter group was comprised mainly of the orders Amphipoda (scuds), Hydracarina (water mites), and Oligochaeta (aquatic annelids). Small immature clams made up most of the Mollusc group, although an occasional snail was encountered.

Since the bottom invertebrate populations were quite similar in all ponds, the same general classification was used for each.

In order to determine whether the herbicide treatments deleteriously influenced the bottom organisms of the test ponds, a comparison was made of the pretreatment and post-treatment populations. Two parameters, numbers per square meter (no/m^2) and milligrams per square meter (mg/m^2) were compared. In addition, the post-treatment per cent reduction in numbers was calculated for each group.

Copper Sulfate

A meager amount of information is available concerning the effects of copper sulfate upon the aquatic invertebrates. Mackenthun (1952) investigated the long-range effects of copper accumulations in bottom sediments upon midge larvae populations. He discovered no significant difference between the midge populations of a lake which received extensive copper sulfate treatments and a non-treated control lake. No information seems to be available on the direct influence of copper sulfate upon aquatic invertebrate populations or the insect types which are most susceptible to its toxicity.

Pond C, the copper sulfate treated pond, showed significant reductions in all invertebrate groups following treatment. This is shown in Table 6. The data in Table 9 illustrate the magnitude of reduction which occurred in each

Table 6. Comparison of the mean pretreatment and post-treatment invertebrate populations of pond C ($CuSO_4$) via the Student's t-test.

Group	Pretreatment (7-10-62) Mean	Pretreatment (7-10-62) Standard Error	Post-treatment (8-29-62) Mean	Post-treatment (8-29-62) Standard Error	
<u>Tendipedidae</u>					
no/m^2	7626	1623	2269	264	*
mg/m^2	2235.7	265.7	937.2	101.6	*
<u>Ephemeroptera</u>					
no/m^2	2498	446	406	142	*
mg/m^2	1454.3	229.5	122.1	38.6	*
<u>Odonata</u>					
no/m^2	254	47	22	5	*
mg/m^2	1428.4	101.6	588.9	203.7	**
<u>Other Insects</u>					
no/m^2	107	16	8	2	*
mg/m^2	52.9	11.1	2.6	1.1	*
<u>Molluscs</u>					
no/m^2	145	48	14	7	*
mg/m^2	140.6	39.6	10.5	5.8	*
<u>Misc. Invert.</u>					
no/m^2	696	126	78	13	*
mg/m^2	480.6	69.8	26.3	7.9	*

*Significantly different at the .01 level.

**Significantly different at the .05 level.

invertebrate group. The odonata and Other Insects groups appeared to be most severely reduced, whereas the Tendipedids were least effected.

Sodium Arsenite

The toxicity of sodium arsenite to bottom organisms has been demonstrated by several investigators under both laboratory and natural conditions. Under laboratory conditions, Surber (1931) discovered that 2.5 ppm As_2O_3 equivalents were necessary to kill such organisms as midge larvae; mayfly, damselfly and dragonfly naiads. He also learned that certain resistant species of the order Odonata could tolerate 14 ppm concentrations for a period of five days with low resultant mortalities. Under natural conditions, Lawrence (1958) demonstrated the reduction of organisms in ponds treated with 4 to 8 ppm As_2O_3 equivalents. But he failed to mention which organisms were most susceptible.

After the sodium arsenite treatment, most of the invertebrate groups of pond A exhibited reductions, but with varying degrees of significance (Table 7). Furthermore, the two parameters of each group differed in the degrees of reduction; when no/m^2 were greatly reduced, mg/m^2 showed a lower degree of reduction or vice versa. No significant decrease in numbers was exhibited by the Other Insects, and similar results were shown by the weights of the Molluscs.

Table 7. Comparison of the mean pretreatment and post-treatment invertebrate populations of pond A ($NaAsO_2$) via the Student's t-test.

	Pretreatment (7-18-62)		Post-treatment (9-5-62)		
Group	Mean	Standard Error	Mean	Standard Error	
Tendipedidae					
no/m^2	1347	214	694	127	*
mg/m^2	1283.3	580.5	268.1	11.7	***
Ephemeroptera					
no/m^2	927	133	0	0	
mg/m^2	746.0	100.7	0	0	
Odonata					
no/m^2	137	28	55	11	*
mg/m^2	2322.6	700.6	1092.2	231.5	***
Other Insects					
no/m^2	70	18	44	8	0
mg/m^2	142.2	54.7	23.1	4.7	**
Molluscs					
no/m^2	36	8	13	6	***
mg/m^2	282.2	64.3	23.7	11.6	0

[*] Significantly different at the .01 level.

[**] Significantly different at the .05 level.

[***] Significantly different at the .10 level.

[0] No significant difference.

The inconsistency of reduction between the numerical counts and weight determinations of the invertebrate groups was possibly caused by one of the following factors or combination thereof: (1) A selective kill of either the larger or smaller invertebrates, (2) increase in the numbers of small invertebrates between the samplings, or (3) emergence of mature larval insects.

The Ephemeroptera was the only group completely eliminated from the pond. Not a single mayfly was found in any of the thirty bottom samples examined.

With the exception of the Ephemeroptera, the invertebrate groups of pond A showed less reductions in numbers than identical groups in the other test ponds (Table 9).

Copper Sulfate and Sodium Arsenite

The combined toxicities of copper sulfate and sodium arsenite in pond B produced the most severe invertebrate reduction (Table 8). This was the only pond which exhibited visible signs of an invertebrate kill. Dragonfly naiads were found floating along the pond margin two days after the herbicide treatment. At this time some vertebrates (bullheads and minnows) were also found. All invertebrate groups except the Tendipedids were reduced in numbers 90 per cent or more (Table 9). The Tendipedids showed an 88 per cent reduction.

Table 8. Comparison of the mean pretreatment and post-treatment invertebrate populations of pond B ($CuSO_4$+$NaAsO_2$) via the Student's t-test.

Group	Pretreatment (8-1-62)		Post-treatment (9-12-62)		
	Mean	Standard Error	Mean	Standard Error	
Tendipedidae					
no/m^2	1363	211	160	23	*
mg/m^2	1082.2	278.6	99.6	18.8	
Ephemeroptera					
no/m^2	609	115	Trace		
mg/m^2	597.4	115.2			
Odonata					
no/m^2	348	76	11	3	
mg/m^2	4036.8	733.1	1253.9	329.6	
Other Insects					
no/m^2	64	20	Trace		
mg/m^2	301.3	70.6			
Molluscs					
no/m^2	328	95	19	5	
mg/m^2	5749.2	1922.8	313.7	204.5	
Misc. Invert.					
no/m^2	379	52	29	6	
mg/m^2	390.8	130.1	14.8	2.8	

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* All pretreatment and post-treatment population means significantly different at the .01 level.

Table 9. Per cent reduction in bottom invertebrates of the test ponds following herbicide treatment.

Invertebrate Group	Per cent reduction in no./m^2		
	Pond C	Pond A	Pond B
Tendipedidae	70.3	48.5	88.3
Ephemeroptera	83.8	100.0	99.0+
Odonata	91.3	59.9	96.8
Other Insects	92.5	37.2	99.0+
Molluscs	90.4	63.9	94.2
Miscellaneous Invertebrates	88.8	----	92.4

The death of insects in the test ponds was attributed to the combined effects of direct toxicity plus alterations in the chemistry of the aquatic environment. Chemical alterations, such as reduction in dissolved oxygen, are known to enhance the toxicity of poisons (Hynes, 1963). And so, it is not surprising that pond B displayed the most serious invertebrate reductions. In the first place, the invertebrates were subjected to fairly high concentrations of two toxic substances. Secondly, the alteration in the water chemistry of this pond was most severe because of the large quantities of aquatic plants killed.

Comparisons of Herbicide Toxicities

The invertebrate data obtained from ponds A and C revealed some information concerning the susceptibility of different invertebrates to the toxicities of sodium arsenite and copper sulfate. The most conspicuous alteration in these ponds was the drastically reduced mayfly populations. And it is interesting to note that the complete or near complete elimination of mayflies occurred only in the ponds which received sodium arsenite applications. Although the mayfly population was significantly depleted in the copper sulfate treated pond, the kill was not nearly as complete as that produced by sodium arsenite. But the mayflies were the only invertebrates highly susceptible to arsenic toxicity; the magnitude of reduction shown by the other invertebrates of pond A was far less than that exhibited by identical groups in other ponds.

Copper sulfate was not as specific to a single invertebrate group as was sodium arsenite. Results from pond C indicate that all insects plus the non-insect groups were reduced substantially in quantity.

In general, 2 ppm copper sulfate displayed a higher degree of toxicity toward the bottom invertebrates than did 8 ppm sodium arsenite. Sodium arsenite exhibited a higher toxicity than copper sulfate to only one invertebrate group; the mayflies. Furthermore, treatment of a pond with simultaneous applications of sodium arsenite and copper sulfate produced an additive effect which was more destructive to bottom fauna than a single individual application of either herbicide.

Because no control pond was available for comparison with the test ponds, the validity of the preceding conclusions may be questioned. Admittedly, the reductions could be attributed to factors other than herbicide toxicity alone. For example, seasonal fluctuations of aquatic insects occurs under undisturbed conditions because of emergence. In addition, total or partial denudation of the pond substrate by the elimination of weeds could have influenced the invertebrate populations. Under such conditions the bottom organisms are more susceptible to predation by fishes. However, available evidence indicated that the above factors could not account for the total reductions observed.

Although emergence during the summer months tends to reduce the aquatic insect populations, the depletion would never be expected to be as complete as shown by some groups in the test ponds. The mayflies, for example, would normally not be expected to be reduced to the extent shown in ponds A and B. Data from the preceding year (1961) showed that the mayfly population of pond B consisted of 1278 per square meter. In the same pond one year later, only three mayflies were found in a total of 30 bottom samples.

Additional evidence which supports the contention that emergence was only a minor factor in the invertebrate abatement was provided by the Mollusc and Miscellaneous Invertebrate groups. Since these invertebrates are non-emerging types, their disappearance had to be due to some other factor.

Increased predation by fishes could have contributed to the reduction in invertebrates, but this factor was not thought to be influential for two reasons. First, special care was taken to prevent the entry of fishes into pond C when the pond was filled. And although a few fishes managed to gain entry, they were insufficient to produce substantial predation losses. Regardless of the small quantity of fishes in pond C, nonetheless, the invertebrate population was reduced immensely. Secondly, only a partial denudation of the pond substrate occurred in pond A because only the higher aquatic plants were killed by the sodium arsenite. Even though an abundant growth of <u>Chara</u> remained as a protective cover for invertebrates, yet the mayfly population was completely eliminated.

SUMMARY

Herbicide treatments were conducted to study the insidious alterations of the aquatic ecosystem induced by the addition of copper sulfate and sodium arsenite, two popular aquatic herbicides whose overall influence upon the aquatic environment is not fully understood. Three ponds, designated A, B, and C, were treated during the summer of 1962 with 2 ppm sodium arsenite, 8 ppm sodium arsenite and 2 ppm copper sulfate, and 2 ppm copper sulfate, respectively.

These herbicide treatments were successful since all susceptible macrophytes were temporarily eliminated. The resistance of *Chara* to sodium arsenite was demonstrated in pond A. This macroscopic alga exhibited active growth while the higher aquatic plants died from the sodium arsenite toxicity.

The water chemistry of the treated ponds exhibited alterations following herbicide treatment. As anticipated, the magnitude and duration of water chemistry change was directly related to the mass of vegetation killed in the ponds.

Dissolved oxygen concentrations of all ponds were reduced following herbicide additions. The oxygen concentrations in ponds B and C were reduced to less than 3 ppm;

levels which are critical to warm water fishes. However, pond B was the only pond to exhibit visible signs of a fish kill.

Notable changes were also evident in the carbon dioxide, pH and alkalinity concentrations of the ponds. All ponds exhibited abnormal increases in carbon dioxide concentrations during the post-treatment recovery period. As a result of the increase in carbon dioxide, the pH was reduced and the normal carbonate disappeared. A simultaneous increase in bicarbonate alkalinity accompanied the carbonate disappearance. The bicarbonate alkalinity continued to increase until the pretreatment range of values were exceeded by as high as 20 ppm.

All water chemistry values returned to the normal pretreatment range within two weeks after the herbicide addition, with the exception of the bicarbonate alkalinity. The alkalinity maintained its high post-treatment values for as long as one and a half months before the normal pretreatment values were once again attained.

In addition to changes in water chemistry, the ponds also showed alterations in the primary productivity. Phytoplankton productivity, as measured by the C^{14} method, decreased immediately after the addition of the herbicides and remained depressed for as long as ten days before attaining normality. Sodium arsenite, erroneously thought by many to be higher aquatics-specific in its toxicity, reduced the phytoplankton productivity, however, this reduction was not as notable as that produced by the copper sulfate. The inhibition of phytoplankton productivity produced by the sodium arsenite-copper sulfate combination in pond B inhibited the phytoplankton to a greater extent and for a longer time than the single applications of the individual herbicides.

Although the availability of nutrients was assumed to increase because of the decomposing macrophytes, no post-treatment phytoplankton bloom was detected in any of the ponds.

Contrarily, the periphyton responded to the release of nutrients provided by the decomposing macrophytes, but showed only slight inhibitive reactions to the herbicides. Inhibition of the periphyton organisms was definitely suspected, but the method used for determining productivity was not sensitive enough to detect the actual decreases which undoubtedly occurred. However, any inhibition which may have taken place was short-lived, for within seven days following the herbicide treatment periphyton productivity increased and remained significantly higher than that of the control pond(s) for as long as two weeks. Once the bloom was completed, the productivity returned to the normal range of values.

The bottom invertebrate populations of the test ponds exhibited decreases which were attributed to the herbicide toxicities. Pond B, which received the sodium arsenite-copper sulfate combination, showed the greatest decrease of invertebrates. The invertebrate loss resulting from the copper sulfate application was not as serious as that caused by the copper sulfate-sodium arsenite combination, but nevertheless was highly significant. The single sodium arsenite application in pond A produced the slightest invertebrate alteration. In general, the copper sulfate was more toxic than sodium arsenite to all invertebrates except the mayflies. The mayfly populations of the ponds which received sodium arsenite treatment exhibited complete or near complete elimination.

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APPENDIX

Incident solar energy measured in the vicinity of the Lake City ponds and expressed as gram calories per square centimeter per day.

Date	Gram cal. cm^{-2} day^{-1}	Date	Gram cal. cm^{-2} day^{-1}
6-25	618.4	8-2	675.8
6-26	671.3	8-3	496.1
6-27	731.2	8-4	190.0
6-28	658.6	8-5	640.6
6-29	503.7	8-6	479.5
6-30	683.6	8-7	614.2
7-1	638.1	8-8	452.0
7-2	285.3	8-9	642.6
7-3	569.3	8-10	659.7
7-4	700.8	8-11	612.2
7-5	539.8	8-12	99.1
7-6	593.9	8-13	108.6
7-7	738.2	8-14	651.3
7-8	630.9	8-15	579.4
7-9	615.1	8-16	591.4
7-10	533.9	8-17	545.2
7-11	550.0	8-18	588.6
7-12	699.0	8-19	537.9
7-13	528.6	8-20	398.8
7-14	599.7	8-21	602.7
7-15	326.0	8-22	541.4
7-16	419.5	8-23	504.3
7-17	324.2	8-24	478.1
7-18	671.8	8-25	108.5
7-19	443.6	8-26	209.5
7-20	98.4	8-27	486.9
7-21	427.2	8-28	548.6
7-22	380.7	8-29	529.8
7-23	477.4	8-30	393.8
7-24	484.8	8-31	325.6
7-25	518.4	9-1	559.3
7-26	648.2	9-2	468.5
7-27	658.1	9-3	440.1
7-28	182.5	9-4	235.3
7-29	198.7	9-5	504.4
7-30	439.6	9-6	535.3
7-31	676.2	9-7	425.8
8-1	674.5	9-8	396.6
		9-9	247.5

Parts per million phenolphthalein and methyl orange alkalinity for the Lake City ponds--summer, 1962.

Date	Pond A Phth.	Pond A M.O.	Pond B Phth.	Pond B M.O.	Pond C Phth.	Pond C M.O.
6-25	5.0	54.0	11.0	65.0	3.0	71.0
6-26	6.0	52.0	9.0	55.0	4.0	63.0
6-27	7.0	49.0	10.0	56.0	5.0	67.0
7-1	6.0	50.0	11.0	52.0	7.0	60.0
7-2	6.0	49.0	12.0	48.0	5.0	62.0
7-3	3.0	55.0	7.0	56.0	3.0	63.0
7-4	6.0	54.0	10.0	50.0	4.0	62.0
7-5	5.0	59.0	11.0	53.0	5.0	61.0
7-6	5.0	61.0	10.0	56.0	12.0	57.0
7-9	6.0	52.0	6.0	61.0	6.0	62.0
7-11	3.0	56.0	10.0	59.0	8.0	63.0
7-12	11.0	65.0	16.0	52.0	6.0	69.0
7-13	1.0	54.0	13.0	61.0	0.0	76.0
7-14	10.0	50.0	11.0	54.0	0.0	74.0
7-15	4.0	51.0	11.0	53.0	0.0	77.0
7-16	3.0	60.0	6.0	59.0	0.0	81.0
7-17	3.0	60.0	4.0	61.0	0.0	82.0
7-19	3.0	61.0	7.0	59.0	0.0	84.0
7-20	2.0	69.0	4.0	65.0	0.0	87.0
7-22	0.0	67.0	6.0	52.0	0.0	84.0
7-23	0.0	74.0	0.0	69.0	0.0	84.0
7-24	0.0	77.0	6.0	64.0	0.0	86.0
7-25	0.0	78.0	5.0	64.0	0.0	83.0
7-26	0.0	81.0	6.0	62.0	0.0	81.0
7-27	0.0	80.0	7.0	60.0	0.0	79.0
7-28	0.0	81.0	5.0	64.0	0.0	81.0
7-29	0.0	82.0	2.0	68.0	0.0	80.0
7-30	0.0	80.0	4.0	65.0	0.0	79.0
7-31	0.0	83.0	6.0	68.0	0.0	77.0
8-2	0.0	85.0	6.0	65.0	0.0	78.0
8-3	0.0	83.0	7.0	68.0	0.0	79.0
8-6	0.0	79.0	0.0	80.0	0.0	73.0
8-7	2.0	78.0	0.0	81.0	0.0	74.0
8-8	4.0	78.0	0.0	85.0	0.0	72.0
8-9	3.0	78.0	0.0	87.0	0.0	73.0
8-10	3.0	75.0	0.0	89.0	0.0	72.0
8-12	0.0	76.0	0.0	90.0	0.0	69.0
8-13	0.0	77.0	0.0	94.0	0.0	67.0
8-14	0.0	78.0	0.0	95.0	0.0	69.0
8-15	0.0	78.0	0.0	95.0	0.0	68.0
8-16	0.0	79.0	0.0	96.0	0.0	71.0
8-17	0.0	77.0	0.0	93.0	0.0	69.0

Continued

Alkalinity data -- continued

	Pond A		Pond B		Pond C	
Date	Phth.	M.O.	Phth.	M.O.	Phth.	M.O.
8-19	0.0	76.0	0.0	93.0	0.0	70.0
8-20	3.0	78.0	0.0	95.0	0.0	72.0
8-21	3.0	77.0	3.0	91.0	0.0	71.0
8-22	5.0	75.0	4.0	89.0	0.0	71.0
8-23	5.0	75.0	4.0	90.0	0.0	70.0
8-24	4.0	76.0	4.0	89.0	0.0	70.0
8-25	4.0	74.0	3.0	86.0	0.0	68.0
8-27	0.0	77.0	0.0	88.0	0.0	68.0
8-28	0.0	78.0	4.0	86.0	0.0	69.0
8-29	3.0	75.0	0.0	86.0	0.0	68.0
8-30	4.0	74.0	0.0	86.0	0.0	67.0
8-31	5.0	72.0	0.0	88.0	0.0	70.0
9-2	4.0	73.0	0.0	88.0	0.0	68.0
9-4	7.0	69.0	0.0	85.0	0.0	68.0
9-5	5.0	71.0	0.0	87.0	0.0	69.0
9-6	5.0	73.0	4.0	80.0	0.0	68.0
9-7	5.0	72.0	4.0	83.0	0.0	69.0
9-8	4.0	72.0	4.0	81.0	0.0	69.0
9-10	0.0	73.0	2.0	80.0	0.0	68.0
9-11	0.0	73.0	2.0	79.0	0.0	65.0
9-12	2.0	70.0	3.0	78.0	0.0	65.0
9-13	1.0	71.0	3.0	76.0	0.0	65.0
9-14	2.0	67.0	1.0	76.0	0.0	66.0
9-15	2.0	68.0	2.0	74.0	0.0	66.0
9-16	2.0	67.0	4.0	70.0	0.0	65.0

Phth. = phenophthalein alkalinity
M.O. = methyl orange alkalinity

pH Values for the Lake City Ponds--Summer, 1962.

Date	Pond A	Pond B	Pond C	Date	Pond A	Pond B	Pond C
6-25	9.0	9.1	8.7	8-17	8.5	8.5	8.1
6-26	8.7	9.1	8.8	8-19	8.4	8.4	8.2
6-27	8.7	9.1	8.9	8-20	8.6	8.5	8.0
7-1	9.1	9.3	9.0	8-21	8.4	8.5	7.9
7-2	9.1	9.4	9.1	8-22	8.6	8.4	7.8
7-3	9.0	9.1	8.7	8-23	8.5	8.5	7.7
7-4	9.1	9.4	8.9	8-24	8.6	8.6	7.7
7-5	8.8	9.1	8.9	8-25	8.3	8.3	7.4
7-6	8.9	9.1	9.1	8-27	8.3	8.4	7.6
7-9	8.9	9.0	8.7	8-28	8.3	8.4	7.6
7-11	8.7	8.9	8.6	8-29	8.4	8.4	7.6
7-12	9.0	9.2	8.7	8-30	8.4	8.3	7.5
7-13	9.1	9.3	8.1	8-31	8.4	8.2	7.6
7-14	9.1	9.2	7.9	9-2	8.5	8.3	7.5
7-15	9.0	9.1	7.7	9-4	8.5	8.3	7.5
7-16	8.8	9.0	7.6	9-5	8.7	8.5	7.7
7-17	8.9	9.1	7.7	9-6	8.7	8.6	7.7
7-19	8.7	9.0	7.8	9-7	8.6	8.8	7.8
7-20	8.5	8.7	7.6	9-8	8.6	8.7	7.8
7-22	7.9	8.8	7.7	9-10	8.4	8.5	7.6
7-23	7.8	8.8	7.8	9-11	8.4	8.6	7.8
7-24	7.9	8.9	8.0	9-12	8.4	8.7	7.7
7-25	8.0	8.9	8.0	9-13	8.3	8.7	7.7
7-26	8.1	8.9	8.2	9-14	8.3	8.6	7.7
7-27	8.2	8.9	8.3	9-15	8.6	8.6	7.7
7-28	8.1	8.9	8.2	9-16	8.5	8.8	7.7
7-29	8.1	8.7	8.1				
7-30	8.2	8.8	8.1				
7-31	8.2	8.8	8.2				
8-2	8.3	8.9	8.5				
8-3	8.4	8.9	8.5				
8-5	8.5	7.7	8.4				
8-6	8.5	7.6	8.4				
8-7	8.6	7.5	8.3				
8-8	8.6	7.3	8.1				
8-9	8.6	7.5	7.9				
8-10	8.5	7.5	7.8				
8-12	8.1	7.4	7.6				
8-13	8.0	7.6	7.7				
8-14	8.1	7.9	7.9				
8-15	8.3	8.2	8.0				
8-16	8.4	8.3	8.0				

Parts per million dissolved oxygen concentrations of the Lake City ponds--summer, 1962.

Date	Pond A	Pond B	Pond C	Date	Pond A	Pond B	Pond C
6-25	8.7	11.3	8.1	8-8	9.1	2.6	7.6
6-26	9.2	9.1	9.1	8-9	8.9	3.0	7.1
6-27	9.2	8.7	9.0	8-10	8.6	2.4	6.8
7-1	9.8	11.4	10.6	8-12	6.8	3.6	6.2
7-2	9.0	9.0	8.6	8-13	7.1	5.5	7.5
7-3	8.8	10.0	8.8	8-14	8.1	7.0	8.2
7-4	9.5	10.8	9.0	8-15	8.7	8.4	8.4
7-5	9.0	9.5	9.8	8-16	8.6	8.6	8.3
7-6	9.7	9.4	10.9	8-17	8.6	8.9	7.7
7-9	9.0	9.1	8.5	8-19	8.6	8.4	7.8
7-11	8.6	8.9	8.7	8-20	8.6	8.8	8.3
7-12	9.8	10.7	9.0	8-21	8.9	8.7	7.8
7-13	9.9	11.6	6.5	8-22	9.1	8.9	7.4
7-14	9.9	10.7	4.8	8-23	9.0	9.2	7.2
7-15	9.3	9.6	3.9	8-24	8.7	8.8	7.0
7-16	8.9	8.8	2.8	8-25	7.7	7.9	6.4
7-17	9.4	9.0	3.4	8-27	8.1	8.0	6.9
7-19	8.8	9.2	4.9	8-28	8.6	9.1	7.6
7-20	5.6	7.9	4.2	8-29	8.6	8.6	6.4
7-22	4.3	8.5	5.8	8-30	8.4	8.0	7.0
7-23	4.9	9.4	6.4	8-31	8.6	8.1	7.1
7-24	5.7	10.4	7.6	9-2	8.9	8.2	7.4
7-25	6.2	9.8	7.8	9-4	9.0	8.5	7.0
7-26	7.2	10.0	8.4	9-5	9.2	9.6	8.0
7-27	7.7	9.1	8.4	9-6	9.6	10.2	8.4
7-28	7.2	9.3	8.4	9-7	9.4	10.5	8.6
7-29	7.5	8.6	7.7	9-8	9.5	10.6	8.5
7-30	7.7	9.0	7.9	9-10	8.9	9.2	8.2
7-31	8.0	9.0	8.1	9-11	9.2	9.9	8.7
8-2	8.4	9.2	8.8	9-12	9.6	10.5	8.9
8-3	8.9	7.4	8.6	9-13	9.4	9.6	8.5
8-5	---	2.5	---	9-14	9.7	9.8	8.4
8-6	9.0	1.6	8.6	9-15	10.5	9.9	8.3
8-7	9.4	1.5	8.4	9-16	10.0	10.5	8.6

Periphyton standing crop samples of the test ponds expressed as phytopigment units per square decimeter.

Exposure Period	Pond A	Pond B	Pond C
June 26	34.27	44.27	38.56
to	35.70	45.69	32.84
July 4	42.84	49.98	25.70
June 26	28.56	17.14	42.84
to	21.42	21.42	52.84
July 9	24.28	12.85	34.27
June 26	58.55	49.98	25.70
to	49.98	45.70	22.85
July 13	54.26	48.55	21.42
June 26	71.40	64.26	44.29
to	55.69	58.55	47.12
July 17	55.69	48.55	48.55
June 26	63.54	72.83	145.66
to	65.69	65.69	124.24
July 21	82.83	61.40	138.52
July 4	52.84	78.54	134.23
to	51.41	75.68	137.09
July 24	52.84	44.27	119.95
July 9	49.98	62.83	139.94
to	71.40	82.82	138.52
July 27	55.69	51.41	112.81
July 9	99.96	48.55	79.97
to	104.24	35.70	67.12
August 3	122.81	67.12	74.26
August 9	52.84	118.52	48.55
to	57.12	114.24	51.41
August 15	52.84	118.52	41.41
August 9	38.56	121.38	47.12
to	44.27	129.95	54.26
August 18	44.27	131.38	54.26
August 9	28.56	121.38	44.27
to	52.84	111.38	37.13
August 21	52.84	124.24	51.41
August 9	54.26	99.96	57.12
to	51.41	101.39	55.69
August 24	51.41	104.24	54.26
August 9	39.98	79.97	45.69
to	47.12	84.25	42.84
August 31	48.55	85.68	57.12
Sept. 1	31.42	24.28	45.69
to	35.70	19.99	29.99
Sept. 10	22.85	21.42	45.69

Carbon dioxide concentrations greater than one part per million in the Lake City ponds during the summer of 1962.

Date	Pond A ppm CO_2	Pond B ppm CO_2	Pond C ppm CO_2
7-13			1.5
7-14			1.8
7-15			3.0
7-16			4.0
7-17			3.3
7-19			2.6
7-20			4.3
7-22	1.9		3.4
7-23	2.4		3.6
7-24	1.9		1.7
7-25	1.6		1.7
7-26	1.3		1.0
7-27	1.0		
7-28	1.3		1.0
7-29	1.3		1.6
7-30	1.0		1.6
7-31	1.1		
8-6		4.0	
8-7		5.0	
8-8		8.4	1.2
8-9		5.3	1.8
8-10		5.5	2.3
8-12		7.0	3.5
8-13		4.7	2.7
8-14		2.4	1.8
8-15		2.0	1.4
8-16			1.4
8-17			1.2
8-20			1.4
8-21			1.8
8-22			2.3
8-23			2.8
8-24			2.8
9-8			2.3
9-10			3.5
9-11			2.1
9-12			2.5
9-13			2.5
9-14			2.5

Summary of the phytoplankton productivity as measured by the C^{14} method in the Lake City ponds during the summer of 1962.

Date	Pond A Mg.C/M^3/day	Pond B Mg.C/M^3/day	Pond C Mg.C/M^3/day
6-23	49.19		19.21
6-26	72.23	42.29	22.74
6-29	51.90	67.11	17.64
7-2	67.70	14.71	26.64
7-5	37.16	46.75	25.47
7-8	52.91	81.89	59.13
7-11	47.15	63.02	26.42
7-12			51.01
7-13			1.96
7-14	58.05	86.24	3.59
7-15			4.43
7-16			6.61
7-17	44.50	57.88	24.12
7-19			16.84
7-20	27.07	20.30	13.22
7-21			29.05
7-22	12.28		33.55
7-23	17.82	34.68	38.76
7-24	30.46		48.81
7-26	42.01	38.33	40.94
7-30	35.74	40.51	45.50
8-2	45.60	39.35	59.37
8-3		30.36	
8-4		1.81	
8-5		2.21	
8-6	41.12	102.88	2.21
8-7		4.19	
	8-9	71.58	
8-10	22.76	12.60	40.68
8-13	27.35	26.74	0.26
8-16	15.29	11.89	72.40
8-19	13.80	103.17	28.54
8-22	11.09	54.13	48.86
8-26	8.23	26.90	20.83
8-28	78.76	37.30	72.28
8-31	19.58	65.29	48.95
9-3	30.44	52.72	
9-6	30.79	70.13	56.15
9-9	23.06	55.54	35.54
9-13	27.66	41.62	33.48