RESPONSE OF A POND METABOLISM TO SODIUM ARSENITE

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ABSTRACT

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by Jack Deverick Bails

The metabolic responses of a small pond to a sodium arsenite treatment were investigated. Two ponds were used in the study and one received an application of 8 mg/l sodium arsenite in two successive years. The other pond was utilized as a control.

Dissolved oxygen, alkalinity, conductivity and pH were measured in both ponds. The dissolved oxygen was recorded automatically on a twenty-four hour basis and gross oxygen production values were calculated using a computer program. Periphyton and phytoplankton production was also measured during the second year of this study.

The dissolved oxygen, pH, and alkalinity in the treated pond all reacted immediately to the sodium arsenite application. The pH dropped from 8.65 to 7.1 within one week after the herbicide was added in the first year (1963). The total alkalinity increased over 30 mg/l after the herbicide was added in 1963. Dissolved oxygen was

reduced from 8.5 mg/l before treatment to a summer low of 4.8 mg/l within 72 hours after the arsenite was added in 1963. Oxygen production, respiration, efficiency and production-to-respiration ratios were all depressed following the 1963 treatment.

Similar results were obtained in the 1964 application and the control pond served to confirm the observation of the effects of the sodium arsenite. The degree of changes in 1964 were less than those observed in 1963 indicating that the metabolic reaction was related to the quantity of plant biomass killed. The periphyton and phytoplankton measurements in 1964 indicated the sodium arsenite temporarily interfered with their growth.

The immediate effects of the herbicide treatment were short lived (i.e. two to three weeks). However, the removal of the macrophytes did effect production-to-respiration ratios, and the primary efficiencies throughout the study period.

RESPONSE OF A POND METABOLISM TO SODIUM ARSENITE

Ву

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TABLE OF CONTENTS

																							Page
ACKNO	WLE	DGN	ÆN	TS		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	ii
LIST (OF	TAI	3LE	S		•	•	•	•	•	•	•	•	•		•	•	•	•	•	•	•	v
LIST	OF	FIC	SUR	ES			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	vi
INTRO	DUC	TIC	NC		•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•		1
DESCR:	IPI	101	1 O	F	SI	UĽ	Y	ΑF	RE <i>P</i>	1	•	•	•	•		•	•		•	•	•	•	6
метно	DOI	.OG3	Č	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•	•	•	•	10
Sod: Phys													•				•	•					10 11
So	ola	era r H Pre	Ene	rg	У	•	•		•	•	•	•		•	•	•			•	•		•	11 12 12
Cher	mic	al	Me	as	ur	en	ner	nts	}	•	•	•	•	•	•	•	•	•	•	•	•	•	13
H ₂ Co	ydr ond	lir oge luct	en ∶iv	Io it	n Y	Cc·	nc •	en	tr •	at	:i	on •	•	•	•	•	•	•	•	•	•	•	13 13 13
Auto Bio								_			Che	em:			M∈					nts •	5	•	14 18
Pe	eri	.phy .opl	/to	n	•	•	•	•		•	•	•	•										18 22
Оху	gen	. M ∈	eta	bo	li	.sn	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	24
D: Re Co P, E:	iff esp omp /R ffi	ual usi oira outa Rat	ion ati er cio enc	on An s	al s	· .ys	sis	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	24 25 26 27 30 31
Co	onv	ers	sio	n	of	C	ху	ge	n	Va	1 1	ıe:	s 1	to	Er	er	gy	, [Jni	Lts	3		34

																								Page
RESUI	LTS	ANI	ם כ)IS	C	JSS	SIC	N	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	37
196	53 E	rel	Lin	nin	aı	Э	St	u	ly	•	•	•	•	•	•	•	•	•	•	•	•	•	•	37
	Iydı																							37
P	٩Īka	alir	nit	ΞУ	(1	1et	h	71	Oı	cai	ng	e ·	-]	Pho	end	olj	oht	ta:	lei	in)				40
F	act	ors	s j	n	A]	Lka	ali	ni	Lty	7 8	an	d j	РΗ	Re	esi	001	nse	25		•				41
	Diss																							42
C	ХУ	ren	Me	eta	ιbo	οĺ:	sn	α																45
ī	emr	era	atu	ıre	<u> </u>													•		•		•		49
7	Cond	luct	- i v	, <u> </u>	·v	•	•	•	•	•	•	•	•		·	•	•	•	•		•	•	•	50
	,0		1		-1	•	•	•	•	•	٠	•	•	•	•	•	•	•	•	•	•	•	•	30
196	54 5	Stud	ly	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	50
P	Peri	.phv	/tc	n																				51
	hyt																							61
	iss																							65
7	xyc	on.	M _C	.+=	h	11	en		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	68
	lydi																							71
1.	ıyaı	.oge	211	10	,11	aı	ıu	M	LNC	1 Д.	T 11.	_ L	Y	•	•	•	•	•	•	•	•	•	•	, 1
Con	npar	isc	on	Ве	tv	ve	en	19	963	3 a	an	d :	19	64	T	rea	atr	neı	nts	3	•	•	•	72
Con	npar	isc	n	of	E	Res	sul	Lts	5 V	vi†	th	0.	the	er	St	tu	die	es		•		•	•	74
c	iol a	r I	a d	ii a	+ +		1																	75
	Sola Oxyg	ian I	Mc	1+3	h	101	en	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	75 75
	Effi	i	1.16	;) <u></u> .	LOI		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	79 79
F	Peri	.pny	/tc	n	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	86
SUMMA	ARY	•	•	•	•		•			•	•	•	•	•	•	•	•	•	•	•		•	•	90
LITER	JTAS	JRE	CI	TE	D		•			•	•	•			•	•	•	•	•	•	•	•		93
APPEN	IDI	ζ.	•	•	•	•				•	•	•	•		•	•	•	•	•	•	•	•		99

LIST OF TABLES

Table		Page
I.	Analysis of Covariance of Periphyton Production in Pond C (Treated) Versus Pond D (Control)	56
II.	Analysis of Covariance of Periphyton Production in Pond C (Treated) Versus Pond D (Control)	57
III.	Range of Efficiency and Gross Oxygen Pro- duction Values from Various Aquatic Communities Compared to Lake City Ponds .	81

LIST OF FIGURES

Figure		Page
1.	Map of Lake City ponds showing location of sample devices	9
2.	Diagramatic representation of automatic monitoring equipment and photograph of laboratory apparatus	16
3.	Correction for respiration in the deter- mination of gross oxygen production	29
4.	Energy flow diagram adapted from E. P. Odum (op. cit.) where:	33
5.	Daily maximum and minimum hydrogen Ion concentrations in Pond C after 1963 treatment	39
6.	Maximum-minimum dissolved oxygen (D.O.) and production/respiration (P/R) ratios in Pond C following 1963 treatment	44
7.	Available solar energy (La), efficiency (Pg/La), respiration (Rt) and production (Pg) in Pond C following 1963 treatment .	47
8.	Periphyton production on vertical substrate in Pond C and Pond D in 1964	53
9.	Periphyton production on horizontal substrate in Pond C and Pond D in 1964 .	55
10.	Index to standing crop of phytoplankton in 1964 from 16 liter centrifuged samples	63
11.	Maximum-minimum dissolved oxygen and production/respiration (P/R) ratios in Pond C during 1964 study	67

Figure		Page
12.	Available solar energy (La), efficiency (Pg/La), respiration (Rt) and production (Pg) in Pond C (treated) and Pond D (control) during 1964 study	70
13.	Range of oxygen metabolism values from several ponds:	77
14.	The inverse, curvilinear relationship between solar energy and efficiency in the Lake City ponds	85
15.	The linear relationship between optical density of chlorophyll content and dry weight of periphyton in Pond D and Pond C in 1964	88

INTRODUCTION

The over-abundance of aquatic plants often limits the full use of a water resource. Extensive research has been carried out to develop controls for aquatic weeds and as a result, many chemical herbicides have been developed. Most of the studies involving herbicides have dealt with their effectiveness in removing aquatic weeds; very little work has been done to determine what effects these chemicals have on the metabolism of an aquatic community.

Many methods have been presented which attempt to measure a part, or the total metabolism of an aquatic community. With the refinements presented by Odum (1956), the diurnal oxygen curve method is one of the most efficient means of obtaining a total picture of the metabolic rates of an aquatic community. The purpose of this study was to utilize diurnal oxygen curves and other measures of primary production to determine the effects of sodium arsenite on the metabolism of a small pond.

Sodium arsenite is the herbicide most often used to kill submergent aquatic weeds. It has remained popular for over fifty years, primarily because of its effectiveness in removing weeds and its relatively low cost (Mackenthum, K. M., 1964).

It has been well established that sodium arsenite, when applied to an aquatic environment, reduces the dissolved oxygen. However, little research has been done to:

(1) quantify this loss of dissolved oxygen in terms of primary production; (2) determine the duration of this oxygen depletion; or (3) evaluate possible changes in the community production to respiration ratios. Without this information, the long term effects of a herbicide treatment on the biota of an aquatic ecosystem are difficult to evaluate.

Sohacki (1965) made estimates of the duration of the dissolved oxygen depletion following the application of sodium arsenite on a small pond. Although the dissolved oxygen determinations were only made once a day during his study, Sohacki reported that the treatment pond exhibited below normal oxygen readings for thirty days after the herbicide was added. In this same study, he also attempted to make estimates of the loss of primary production due to the sodium arsenite by determining the post and pre-treatment production of macrophytes, plankton and periphyton. His results indicated that all types of primary producers were at least temporarily inhibited by the application of the herbicide.

Copeland and Whitworth (1963) attempted to measure the effects of a new chemical herbicide on the oxygen

metabolism of a small Oklahoma farm pond. Their analysis of the diurnal oxygen curves, taken before and after the pond was treated, indicated that the decaying vegetation, resulting from the treatment, increased the community respiration and significantly reduced the community production-to-respiration ratio. With the exception of this single study, the free oxygen (diurnal oxygen curve) method has not been utilized to measure the effects of a herbicide treatment. However, since 1956 diurnal oxygen curves have been used to measure the metabolic rates of lenthic aquatic communities under a variety of other conditions.

Minter and Copeland (1962) studied the wintertime oxygen production and respiration relationships of a small lake and found that diffusion of oxygen from the atmosphere played a major role in maintaining the oxygen balance when photosynthetic oxygen production was limited.

Copeland and Dorris (1964) investigated the photosynthetic oxygen productivity in ponds polluted with oil refinery effluent. They found exceptionally high oxygen production values and correspondingly high community oxygen respiration values. They reported that the oxygen production-to-respiration ratio was often less than unity in the oil polluted ponds. Copeland (1963) reported similar results on several other oil effluent holding ponds.

The oxygen metabolisms of four distinctly different unpolluted farm ponds were studied by Copeland and Whitworth (op. cit.). The results of their study indicated that the sources of water for these ponds and the terrain that it passed over, determined to a great extent the rate of oxygen metabolism.

Each of these studies demonstrated that diurnal oxygen curves can be utilized effectively to measure the metabolic rates of aquatic communities. They also indicated that the effects of a sodium arsenite application could be measured quantitatively by this same method. There is one major disadvantage in determining primary production values with the diurnal oxygen curve method. Periodic dissolved oxygen readings must be taken throughout a twenty-four hour period to determine oxygen production. Obviously, to maintain daily oxygen production records through chemical analysis would be an arduous task, even for a short period of time. The oxygen metabolism studies previously mentioned relied upon two or three diurnal curves for results. The nature of this study made it imperative that a complete daily oxygen metabolism record be kept.

Preliminary studies by the author indicated that it was feasible to obtain continuous dissolved oxygen readings through the utilization of automatic sensing and

recording equipment. The unique manner in which the data for this study was obtained and compiled is perhaps as significant as the results themselves and consequently these methods will be discussed in detail.

DESCRIPTION OF STUDY AREA

The research for this study was conducted at the Michigan State University Agricultural Experiment Station, located two miles south of Lake City, Michigan. The ponds used in this study are maintained by the Department of Fisheries and Wildlife for limnological and fisheries research during the summer.

There are six ponds located on the farm, four of which are connected to a five acre impoundment. The impoundment was built in the early 1940's on a small stream, Mosquito Creek. It serves both as a reservoir for maintaining the experimental ponds and as a source of irrigation water. The two ponds used in this study (Pond C and Pond D) have an inlet from the reservoir and an outlet to the Mosquito Creek (Fig. 1).

Pond C and Pond D were chosen for this study primarily because of their close proximity to the field laboratory buildings. In previous studies (Sohacki, op. cit.), Pond C had been treated with both sodium arsenite and copper sulfate. Pond D, on the other hand, had a history of having very few rooted aquatic plants and had not been used in previous algalcide or herbicide experiments.

The substrate of all the ponds was originally sand. However, the process of eutrophication has deposited a layer of mucky organic debris over most of the bottom of the two ponds used in this study.

Physically, Pond D and Pond C are quite similar.

The maximum depth of both ponds during the study, was

4.5 feet. Their areas and average depths were as follows:

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

Pond D - .19 acres, 3.2 ft. average depth

The research for this study was conducted throughout the summer months of 1963 and 1964.

Figure 1. Map of Lake City ponds showing location of sample devices

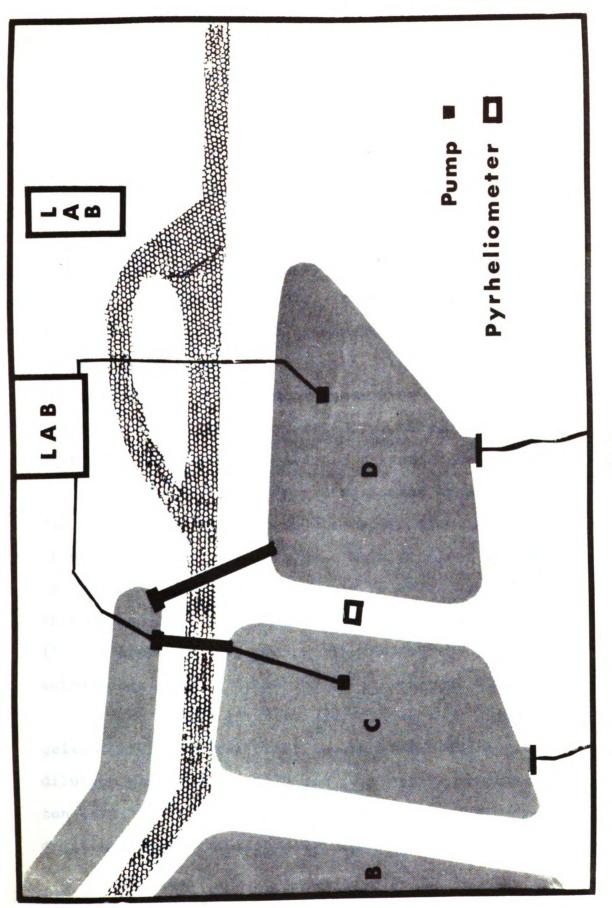


Fig. 1

METHODOLOGY

Sodium Arsenite Application

In the preliminary study in 1963, and in the final study in 1964, a liquid form of sodium arsenite (NaAsO₂) was added to Pond C. Enough sodium arsenite was added to the pond in each treatment to bring the total concentration of arsenic trioxide (As₂O₃) up to 8 mg/l. The liquid form of sodium arsenite used in this study ("Atlas - A", Chipman Chemical Co., Chicago, Illinois) contained four pounds of arsenic trioxide per gallon.

In 1963, the concentrated sodium arsenite was diluted with water prior to the application in a 50-gallon drum, lined with polyethylene. This drum was placed on the shore and the diluted arsenic solution was pumped through one-half inch tubing to an applicator on a boat. It took approximately twenty minutes for the 110-volt submersible pump to drain the 50-gallon drum.

The sodium arsenite was diluted in a sixteen gallon plastic barrel prior to the 1964 treatment. The dilution was approximately one part sodium arsenite to ten parts water. The barrel was placed on a boat and the diluted arsenic solution was pumped through the applicator.

A wet-cell storage battery located on the boat, provided the power to operate the 12-volt submersible pump used for this treatment.

For both treatments, a boom-type copper applicator was used to distribute the sodium arsenite evenly over the entire surface of the pond. The boat, with applicator attached, was pulled over the pond's surface with ropes in 1963. And in 1964, the boat was simply rowed while the arsenite was being applied. No additional effort was made to mix the herbicide into the pond water, after either application.

Physical Measurements

The physical measurements of solar energy, atmospheric pressure and temperature were obtained in the same manner in 1963 and 1964.

Temperature

A continuous record of air temperature was maintained using a Taylor air thermograph. Water temperatures in Pond C were continuously recorded on a Taylor water thermograph. Numerous determinations of the water temperatures in Pond D indicated that there was no significant difference between the water temperatures of Pond D and Pond C. Thus, one measurement of water temperature was assumed to be indicative of both ponds.

The thermographs, used to obtain air and water temperatures, were located near the spillway of Pond C (Fig. 1).

Solar Energy

Solar energy was measured with an Epply pyrheliometer which was mounted on a permanent concrete cylinder
between ponds C and D (Fig. 1). The pyrheliometer is a
thermopile type radiation detector which generates an electromotive force (emf), through a series of thermocouples,
proportional to the incident radiation.

A Bristol strip-chart integrated recorder was used to record, and convert the signal from the pyrheliometer to gram-cal/cm²/day. The recorder assembly was located in the laboratory and the signal from the pyrheliometer was carried by an underground cable to the recorder. The recorder was automatically timed to go on at sunrise and turn off at sunset. The timer was preset manually and adjusted periodically to approximate, as closely as possible, the actual hours of sunrise and sunset.

Air Pressure

Barometric pressure was measured with a continuously recording Taylor barograph. Air pressure readings were maintained primarily to record sudden storms which might

account for unusually low pyrheliometer and/or dissolved oxygen readings.

Chemical Measurements

Alkalinity

Total alkalinity was measured by titrating samples with 0.02 sulfuric acid, using methyl-orange and phenol-phthalein as indicators (American Public Health Association et al., 1960).

Hydrogen Ion Concentration

Hydrogen ion concentration was determined using a Beckman expanded scale pH meter (Model 76) and continuously recorded on a Bausch and Lomb recorder.

Conductivity

Conductivity was obtained with an Industrial Instruments continuously recording conductivity meter (Model RQ).

Dissolved Oxygen

Dissolved oxygen was determined with a Beckman polarographic oxygen analyzer (Model 777) and a Beckman polarographic oxygen analyzer adapter in conjunction with a Model 76 pH meter. Dissolved oxygen values were continuously recorded on Sargent recorders (Models MR and SR).

The polarographic oxygen analyzers were calibrated daily using the Alsterberg (Azide) modification of the Winkler method (American Public Health Association, et al., op. cit.).

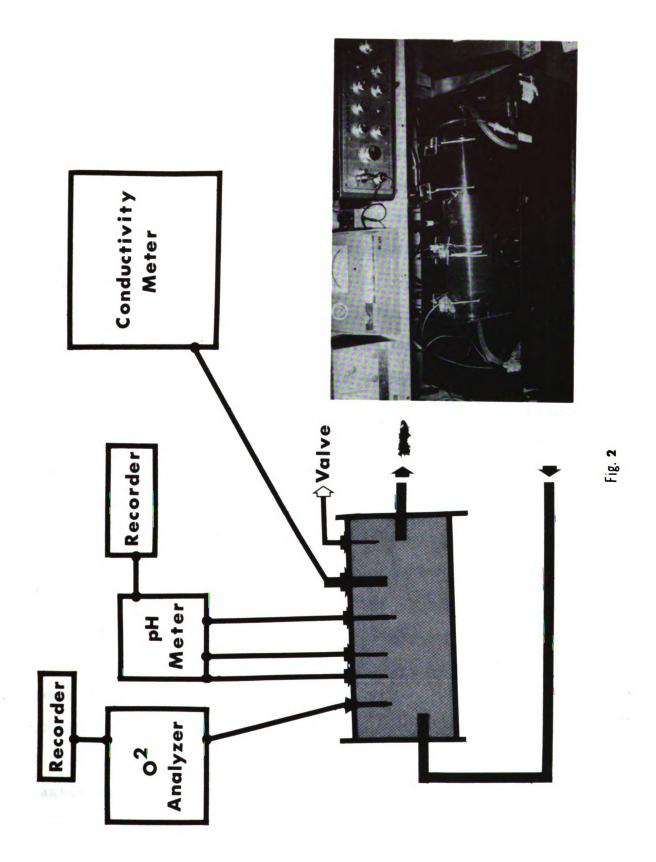
Automatic Monitoring of Chemical Measurements

During the two year study period, changes in dissolved oxygen, pH, and conductivity were simultaneously analyzed and recorded inside the laboratory on a closed extension of the pond water. A 110-volt submersible pump was located in each pond. The water was pumped from the pond through 1/2 inch Tygon tubing to a plexiglass cylinder (18" long, and 6" in diameter), and eventually returned to the pond. The sensing probes for the numerous analyzers were inserted in the top of the cylinder (Figure 2). The system was entirely closed and continuously running such that the chemical measurements made of the water passing through the cylinder were at any given moment, representative of the water in the pond. A diagramatic representation of the system is illustrated in Figure 2.

Although instruments which measure conductivity and pH have been in general use for some time, dependable dissolved oxygen analyzers have only recently been developed and utilized in biological research.

Odum and Hoskin (1957) attempted to adapt the gold-zinc electrode developed by Ohle (1953) to make

Diagramatic representation of automatic monitoring equipment and photograph of laboratory apparatus Figure 2.



polarographic oxygen measurements in a laboratory stream microcosm. However, their efforts were unsuccessful and their results indicated that the presence of zinc in the system inhibited the growth of algae.

Odum and Hoskin (op. cit.), reported that Ambuhl (1955), and Lynn and Okum (1955) also had failed to obtain reliable dissolved oxygen readings in a flowing system with either gold-zinc or solid platinum polarographic analyzers.

In England, Gameson and Griffin (1959) were able to obtain a six-month record of dissolved oxygen in a polluted stream using photographic equipment to record the dial readings on a dropping mercury electrode. Electrical recorders have also been used with mercury electrodes to record dissolved oxygen (Briggs, Dyke and Knowles, 1959).

Newly developed polarographic dissolved oxygen analyzers were evaluated by Macklin, Baumgartner, and Ettinger (1959), and used successfully by Bartsch (1959) to measure diurnal oxygen in Ohio streams.

Sneed and Dupree (1962) developed an oxygentemperature meter designed particularly for fishery biologists. Their polarographic oxygen analyzer consisted of a platinum, silver oxide electrode pair covered with a plastic membrane. Sneed and Dupree credit Clark, Wolf, Granger and Taylor (1953) with the development of plastic films to cover the electrodes which prevents "poisoning"
(i.e., plating of electrodes with other metalic ions in
the water). This development allowed continuous recording
of oxygen with polarographic analyzers over long periods
of time.

Very recently, Copeland and Duffer (1964) used polarographic oxygen analyzers and automatic multi-channel recorders to simultaneously measure both diurnal dissolved oxygen and oxygen diffusion.

As indicated previously, in this study a Beckman polarographic dissolved oxygen analyzer was used. The sensing probe consisted of gold and silver electrodes covered with a conductive salt gel which was held in place by a semi-permeable Teflon membrane. This same type of analyzer was used by Pamatmat (1965) to measure the metabolic activity of benthic communities.

Biological Measurements

Periphyton

In many recent publications, periphyton has been used interchangeably with the term aufwuchs (Odum, E. P., 1959). Aufwuchs, as proposed by Ruttner (1953), includes all organisms (both plant and animal) attached or clinging to stems and leaves of rooted plants or other surfaces projecting above the bottom (Odum, E. P., loc. cit.). In this study, periphyton is used to describe only the

autotrophic material growing on, but not penetrating the surface of organic or inorganic objects below the surface of the water (Sohacki, op. cit.). Thus, in this study periphyton includes all primary producers not defined as either phytoplankton (free-floating autotrophs) or as macrophytes (rooted aquatic plants).

Recent advances in the quantitative measurement of periphyton have generally been attributed to original work done by Newcombe (1950). In his research work on Sodon Lake, Newcomb (loc. cit.) reported that studies of the growth of attached materials had been made as early as 1915 by Hentschel. Until the early 1950's, most studies of aufwuchs had been conducted in Europe.

The method used to measure periphyton in this study was a combination of techniques developed by several authors (Welch, 1959; Grzenda and Brehmer, 1960; Kevern, 1962; and Sohacki, op. cit.). The sampling period and choice of substrates closely paralleled those used by King (1964).

Periphyton production was measured as the accumulation of chlorophyll on plexiglass substrates (140 cm² exposure area) suspended 18 inches below the pond's surface. The substrates were removed at periodic intervals, placed in individual plastic bags, and frozen to facilitate removal of the attached algae. After freezing, the plexiglass

substrates were scraped clean with a rubber spatula and washed with 95% ethanol. The ethanol wash and scraped materials were placed in a 2 oz. bottle, and ethanol was added to bring the total volume of the sample to 50 ml. Each sample was then shaken vigorously and placed in a dark box for at least twenty-four hours to complete the chlorophyll extraction.

A number of trials revealed that if kept in the dark, extracted chlorophyll samples would retain a constant absorbency reading for at least thirty days from the time they were processed. Similar results were reported by Grzenda and Brehmer (op. cit.).

Absorbency readings of the chlorophyll extract were made on a Klett-Summerson Colorimeter using a filter in the 643-700 m μ range. To prevent cellular particulate matter from interfering with the absorbency readings, a 25 ml aliquat of the chlorophyll extract was carefully pipetted from the top of each sample for the colorimetric readings.

An ethanol-chlorophyll solution absorbency curve conforms to the Lambert-Beer Law up to a reading of 200 (Klett Units with a 600-700 mµ filter) (Grzenda and Brehmer, op. cit.). All readings taken during this study were under 200 and no correction factor was applied. Klett units were multiplied by .004 to obtain optical density, which is equivalent to phytopigment units used

by other authors cited (i.e., Grzenda and Brehmer; Kevern; Sohacki; and King). For all graphical and tabular presentations, all periphyton phytopigments units were multiplied by a factor of 10³.

Possible sources of error in measuring the absorbency of the extracted chlorophyll were carefully avoided. Samples which were accidentally shaken during the pipetting process, were allowed to settle before the Klett readings were made. The colorimetric cell was carefully rinsed and cleansed between samples and zero absorbency instrument readings were made with pure ethanol in the colorimetric cell.

All periphyton samples were dried in pre-weighed crucibles at 55°C., after chlorophyll absorbency readings had been made. A representative number of the dried samples were placed in a muffle furnace at 550°C. and the loss of weight due to muffling was used as an estimate of the organic weight of the periphyton samples.

King (op. cit.) and others have shown that periphyton growth on plexiglass plates placed vertical with respect to the surface of the water is significantly different than periphyton growth on plexiglass plates placed horizontally. Both horizontal and vertical substrates were used in this study and the results from each type of substrate were evaluated separately.

Periphyton growth was measured during three periods. At the beginning of each period twenty-four substrates were placed in the pond and every four days four substrates were sampled. To obtain significant colorimetric readings, the four substrates were pooled into two paired samples each consisting of two substrates (a total of 280 cm² exposure area per sample).

This sampling procedure was followed in measuring periphyton growth on both horizontal and vertical substrates in the two study ponds during each of three periods.

Phytoplankton

Several methods have been developed to quantitively measure the production of phytoplankton. The light and dark bottle methods (Odum, 1956) and physical removal of phytoplankton by filtration (Copeland and Dorris, op. cit.) have been used by numerous authors to measure phytoplankton productivity. However, both of these methods depend upon a relatively large quantity of plankton for accuracy. The ponds used in this study have comparatively small amounts of phytoplankton and attempts by the author to utilize either one of these methods failed to produce reliable results.

The carbon-14 technique was used with some success in two previous studies to measure the phytoplankton

production in the Lake City ponds (Knight, et al., 1962; and Sohacki, op. cit.). Both of these studies indicated that phytoplankton contributed very little of the total energy fixed by the primary producers. Thus, during the course of this study, only the standing crop of phytoplankton was estimated.

Periodically throughout the summer of 1965, 16

liter samples of water were collected and passed through
a Foerst centrifuge (at a rate of 1 liter per 4 minutes).

The precipitant was then scraped into a 2 oz. bottle and
mixed with 50 ml of 95 per cent ethanol. The resultant
chlorophyll-ethanol mixture was shaken vigorously and
allowed to settle for 24 hours in the dark. The phytopigment concentration was read on a Klett colorimeter in
the manner described previously for periphyton.

Chlorophyll extracts of concentrated phytoplankton have been used by other authors to measure the standing crop of phytoplankton (Kosminski, 1938 from Prescott, 1951; and Welch, 1948). Recent studies lead E. P. Odum (op. cit.) to state, chlorophyll may be, in some cases, a good measure of primary productivity when it is expressed on a square meter basis. Several authors have used extracted phytoplankton chlorophyll as an index to potential productivity (Odum, 1957; Odum, Burkholder and Rivero, 1959; Minter, Copeland and Dorris, 1964; and Knudson and Dorris, 1963).

Oxygen Metabolism

Diurnal changes in dissolved oxygen were used to estimate gross production by methods similar to those presented by Odum (1956) and Odum and Hoskin (1958). The free-water gas curves (diurnal curves) are particularly well suited to measure productivity in shallow homogeneous aquatic ecosystems, such as the ponds used in this study.

Many factors may contribute to the observed dissolved oxygen readings which are not a direct result of photosynthetic production. Diffusion from the atmosphere and oxygenated water entering the system from another source (accrual oxygen) may increase the dissolved oxygen in the water. While other factors, such as oxygen diffusing out of the water and respiration, may decrease the dissolved oxygen. If these factors, which add to or subtract from the photosynthetic oxygen, are accounted for, the rate-of-change between two dissolved oxygen readings during the daylight hours will be a measure of gross photosynthetic production.

Accrual Oxygen

Accrual oxygen was discounted as a possible source of oxygen to the ponds as they were essentially closed systems isolated from any source of outside water, including run-off.

Diffusion

Diffusion was determined indirectly by methods presented by Odum (1956). Initially several oxygen curves in the control and treated ponds were corrected for diffusion by the indirect method. The rate of diffusion depends on the degree of saturation of the water. Odum (loc. cit.) presented the following formula for determining the rate of diffusion:

D = KS

where D is the diffusion rate of an area basis, S is the saturation deficit between water and air and K is the gas transfer coefficient (i.e., g/m²/hr for 0% saturation). To make practical use of this formula, K must be determined or at least reasonably estimated. Although several K values were determined by a method presented by Odum (loc. cit.), this author questioned the reliability of these values because they were based on the assumption that respiration remained a constant at night.

Gross oxygen production values were calculated from both diffusion corrected and uncorrected diurnal curves for several days and the greatest difference between any two of the values was less than 5 per cent. Because of the questionable reliability of this indirect measurement of diffusion and the small differences realized when diffusion corrections were made, oxygen

production values were calculated from diurnal oxygen curves not corrected for diffusion. Diffusion into or out of the ponds was assumed not to be a major source of error in determining either oxygen production or respiration values from the observed diurnal curves.

This assumption was reinforced by two recent reports by other authors. Further studies of reaeration by Odum and Wilson (1962) lead them to state that because of changes in nighttime respiration the diffusion correction had been previously miscalculated and when the diurnal curves were corrected as outlined by Odum (1956) the total respiration was often overestimated. Copeland and Duffer (1964) used a plexiglass dome to make diffusion estimates. They found that indirect measurements of diffusion in lentic communities were gross overestimates of the actual diffusion. They also illustrated that observed diurnal oxygen rate-of-change curves from shallow ponds changed very little when they were corrected for diffusion by the direct dome method.

Respiration

The rate of oxygen consumption (respiration) in an aquatic community may vary considerably from one day to the next. However, changes in the respiration rate during any twenty-four hour period are generally gradual and the average respiration rate can be closely

approximated from the nighttime dissolved oxygen rate-ofchange curve.

Figure 3 illustrates the correction made for respiration. Respiration was estimated by averaging the nighttime dissolved oxygen readings from the uncorrected rate-of-change curve. This average respiration was then added to each of the daytime rate-of-change values and a respiration-corrected rate-of-change curve was calculated. This area shaded in the corrected rate-of-change curve (Fig. 3) represents the gross photosynthetic production for one day (gm O₂/m³/day).

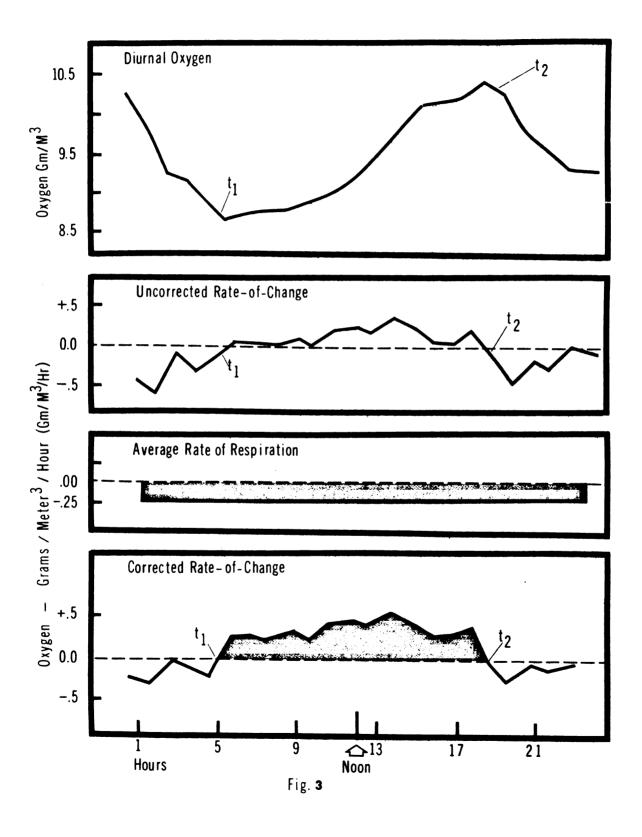
The average respiration rate $(gm O_2/m^3/day)$ times twenty-four was used as an estimate of the total community respiration $(gm O_2/m^3/day)$.

For comparative purposes the volume production and respiration values were multiplied by the average depth to obtain values on a square meter or area basis. Since both ponds were maintained with an average depth of close to one meter, the correction factor for conversion to an area basis was 1.0.

Computer Analysis

Diurnal oxygen curves were analyzed through the use of the Control Data Corporation 3600 Digital Computer at the Michigan State University Computer Center. The computer program was designed by the author with assistance

Figure 3. Correction for respiration in the determination of gross oxygen production (T1 equals sunrise and T2 equals sundown). Shaded area in corrected rate-of-change curve is gross oxygen production for one day.



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from Computer Center personnel. Details of this program can be found in the appendix.

The computer program calculated average respiration, gross production, and total respiration from observed hourly diurnal oxygen readings in generally the same manner that was previously described. Although this program was designed for a specific use, it could be utilized to analyze diurnal oxygen curves from any aquatic community in which diffusion was not a significant contributor to the observed dissolved oxygen values.

In some respects, the computer program used in this study is similar to the program designed by Armstrong (1963) to calculate production using carbon dioxide curves. Armstrong's program has been used successfully by Butler (1964) and others.

P/R Ratios

Ratios of gross oxygen production to community respiration (P/R ratios) were proposed by Odum (1956) to logically classify aquatic communities into autotrophic and heterotrophic types. When oxygen production and respiration values are used as coordinates of a graph, quantitative comparisons of a wide variety of aquatic communities can be made (Fig. 13).

In this study, P/R ratios were used to illustrate changes in community metabolism which were a result of the

sodium arsenite application. They were also used to compare the Lake City Ponds with similar aquatic ecosystems analyzed in other studies.

Efficiencies

Ecological efficiencies are ratios of energy at different points in the food chain, expressed as percentages. Since there are many types of efficiencies, an abbreviated energy flow diagram has been used to define exactly what ratios are being considered (Fig. 4).

In Figure 4, the abbreviations are defined as follows:

L = Total light

La = Absorbed light (visible range) reaching
 surface of plant

Pg = Total photosynthesis (gross production)

Pn = Production of biomass (net production)

R = Respiration

Rt = Total Respiration (community respiration)

S & E = Storage and export

Consumer-decomposer organisms as used in Figure 4 include herbivores, carnivores, bacteria and any other non-producers.

These abbreviations and definitions are presented by H. T. Odum (op. cit.), and they have been widely used by a number of authors to express ecological efficiencies. Energy flow diagram adapted from E. P. Odum (op. cit.)
where: Figure 4.

L = Total Light

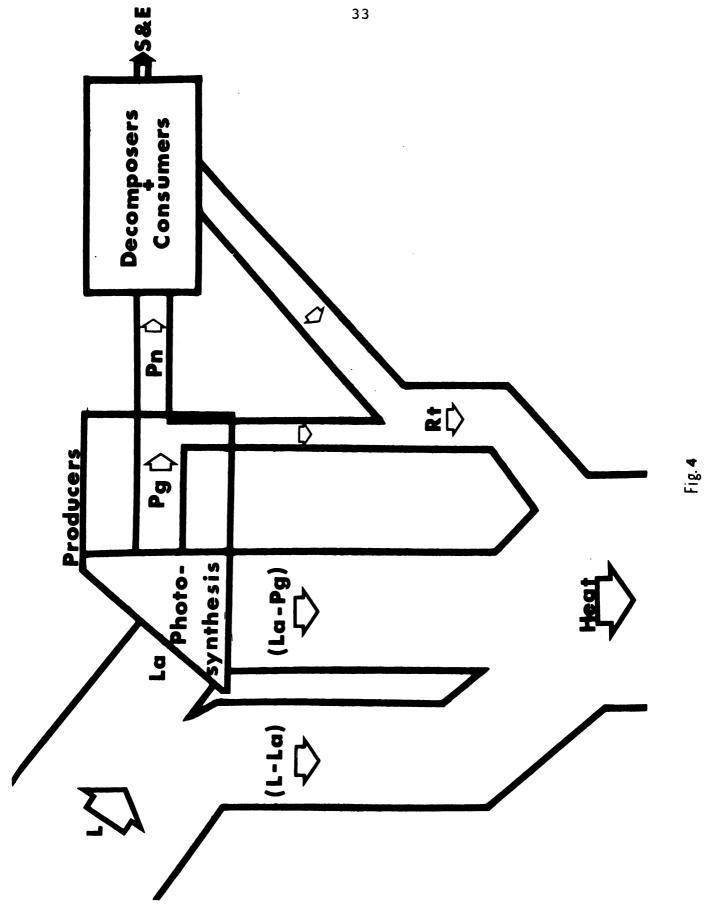
La = Absorbed light (visible range), reaching surface
 of plants

Pg = Total photosynthesis (gross production)

Pn = Production of biomass (net production)

Rt = Total respiration (community respiration)

S & E = Storage and export



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Trophic level energy intake efficiency (Lindeman's Efficiency) for the primary level is then

$$\frac{\text{Pg}}{\text{L}}$$
 or $\frac{\text{Pg}}{\text{La}}$

where L is total sunlight energy at the surface of the water and La is the total visible light energy available at the surface of the plants.

To calculate efficiencies, oxygen production values (gm $O_2/m^2/day$) were converted to energy units (kg-cal/m²/day) by an approximate conversion factor of 4 kg-cal/gm O_2 metabolized. Total light energy (L) as determined with the pyrheliometer (g-cal/cm²/day), was converted to kg-cal/m²/day and absorbed light energy (La) was estimated to be approximately 20 per cent L (Odum, Burkholder, and Rivero 1959; Reid 1961).

Conversion of Oxygen Values to Energy Units

Several factors are involved in the conversion of oxygen production values to energy units. These factors vary depending on the composition of -(CH₂O)-(the photosynthetic product) in the following reaction:

$$CO_2 + H_2O = -(CH_2O) - +H_2O + O_2$$

The caloric content of the photosynthetic product can be determined using bomb calorimetry. Kevern (op. cit.) demonstrated that the caloric content of the photosynthetic product is greater than that of carbohydrate

alone, thus indicating the presence of either protein and/or fats which have higher caloric values.

By direct analysis Kevern (op. cit.) found that the photosynthetic product of certain algae was 76% carbohydrate, 21% protein, and 3% lipid with an average caloric value of 4522. cal/gm dry weight.

The synthesis of protein indicates a PQ (photosynthetic quotient, moles of O₂ produced/ moles CO₂ consumed) greater than one (Strickland, 1960). The higher the caloric content of the organic product the greater the PQ. PQ values ranging from 1.0 to 1.6 have been reported by various authors (Kevern, op. cit.; Strickland op. cit.; Ryther, 1956; and Odum, 1957). Thus, if the caloric content of the photosynthetic product is determined, the PQ value can be reasonably estimated.

An estimate of the PQ is essential to establish the relationship between photosynthetic oxygen production and the organic product. If the PQ is 1.0, 6 moles of CO_2 will produce 6 moles of O_2 and 1.0 mole of $-(CH_2O)-$.

To convert oxygen gas production to energy units, you must: (1) establish the number of moles of O_2 released for every mole of $-(CH_2O)$ - produced using the estimated PQ value; (2) divide the molecular weight of the organic product by the weight of the moles of O_2 released to obtain the organic weight equivalent of the gas; and (3) multiply the organic weight equivalent by the average caloric

content of the organic product. The result of these steps is a conversion factor by which gm $O_2/m^2/day$ can be converted into kg-cal/m²/day.

Time did not allow the actual determination of caloric values of the photosynthetic products of the Lake City ponds so the factor used to convert oxygen production values to energy units was estimated from those found in the literature.

Duffer and Dorris (1966) used a factor of 3.75 kg-cal/gmO₂; Copeland (op. cit.) and Butler (op. cit.) used a factor of 3.5 k-cal/gmO₂; and Odum and Wilson (op. cit.) used 4.0 k-cal/gmO₂. Kevern (op. cit.) used several different factors depending on the caloric content of the organic product and the estimated PQ.

Since the comparisons to be made later are primarily with results obtained by other authors using 4.0 k-cal/gmO₂, this same conversion factor was used in this study. Actually, Kevern's (op. cit.) work indicates most conversion factors should fall in the range between 3.5 and 3.9, unless caloric values or PQ's are actually determined, however, the factor chosen is somewhat arbitrary.

RESULTS AND DISCUSSION

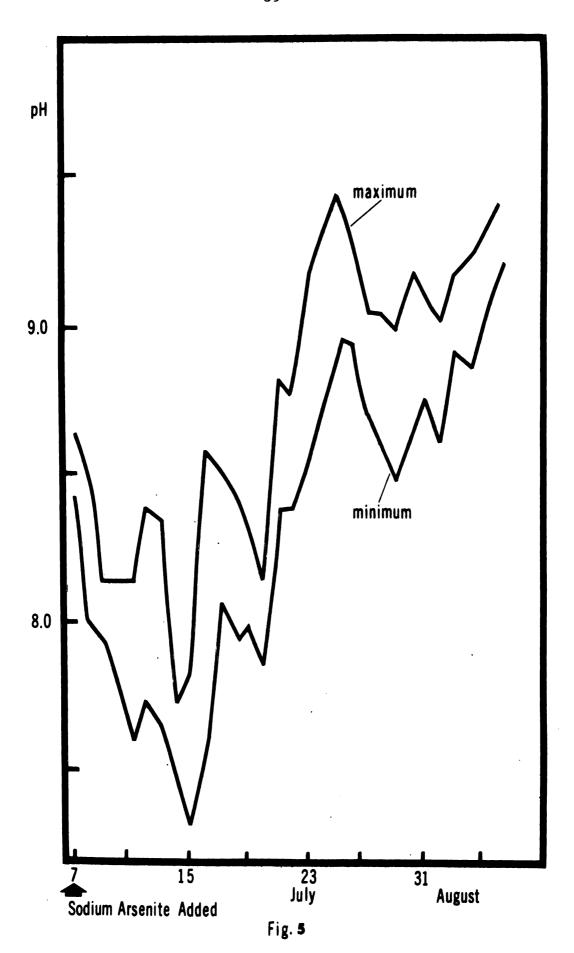
1963 Preliminary Study

The results of the 1963 study reported here were one phase of a larger more comprehensive study involving the ecological effects and translocation of radioactively tagged (arsenic 74) sodium arsenite in the Lake City ponds (Ball and Hooper, 1966). Automatic monitoring of the pH and dissolved oxygen was begun on the day of treatment as were the periodic measurements of alkalinity.

Hydrogen Ion Concentration

The continuous pH measurements in Pond C were initiated some four hours after the arsenite was added in 1963. Subsequently, little is known about the immediate response of the pH to the treatment. However, grab samples indicated afternoon pH values in Pond C were 8.8 or greater before the pond was treated. The maximum pH recorded on the day of treatment was 8.65. Within twenty-four hours after the application, the pH had been reduced to 8.0. The minimum pH in Pond C (7.1) occurred a week after the treatment (Figure 5).

Figure 5. Daily maximum and minimum hydrogen Ion concentrations in Pond C after 1963 treatment



It was two weeks after the herbicide application before pH values greater than 9.0 were recorded in Pond C (Figure 5). Periodic samples from adjacent ponds during the same period ranged in pH from 8.4 to 9.8.

Within four weeks the pH values in the treated pond returned to normal when compared to adjacent ponds.

Alkalinity (Methyl Orange - Phenolphthalein)

Periodic sampling of the treated pond, indicated the total alkalinity increased a minimum of 30 mg/l within two days after the arsenite was added. Pretreatment total alkalinity in Pond C ranged below 70 mg/l and a maximum of 106 mg/l was recorded two days after the addition of the herbicide.

Adjacent ponds ranged below 70 mg/l total alkalinity throughout the study period. The total alkalinity in Pond C appeared to return to normal within three weeks after the treatment.

Phenolphthalein alkalinity in Pond C declined with corresponding decreases in pH following the application. Four days after the treatment, phenolphthalein alkalinity was completely absent. For two weeks following the application of the sodium arsenite, phenolphthalein alkalinity remained low (below 6 mg/l) and periodically was absent when the pH dropped below 8.0.

In adjacent ponds phenolphthalein alkalinity averaged above 6.0 mg/l and ranged in values from 1.0 mg/l to 19 mg/l during the three weeks following the applications.

Factors in Alkalinity and pH Responses

As indicated by the results, pH and alkalinity are interdependent. In Pond C the rapid decay of aquatic vegetation produced large quantities of carbon dioxide (CO₂) which initiated changes in both pH and alkalinity.

Free carbon dioxide released into an aquatic environment as a product of respiration or bacterial decomposition of organic matter, immediately combines with available monocarbonate to form bicarbonate. No change in pH or total alkalinity occurs if sufficient monocarbonate is available to combine with all the carbon dioxide (Welch, 1952; Welch 1948).

In the absence of monocarbonates, free carbon dioxide combines with water to form carbonic acid and the pH of the aquatic environment is lowered (Ibid.).

Under normal conditions the Lake City ponds are slightly alkaline (pH 8.0 to 9.0) and have sufficient monocarbonates in solution to resist large fluctuations in pH.

When the sodium arsenite was added to Pond C rapid decomposition of organic material followed, producing

large quantities of carbon dioxide. Available carbonate (CO₃), measured as a function of phenolphthalein alkalinity, was quickly converted to bicarbonate (HCO₃). However, the production of carbon dioxide continued and eventually exceeded the buffering capacity of the available monocarbonates. The excess carbon dioxide formed carbonic acid, thus lowering the pH. Total alkalinity of the treated pond was increased when bound carbonates in the bottom muds and plant encrustations were brought back into solution as bicarbonates.

The reverse process was initiated when production of carbon dioxide decreased and photosynthesis of the remaining plants increased. The pH returned to the normal range and some of the bicarbonates were precipitated as insoluble carbonates lowering the total alkalinity to the pretreatment levels.

Dissolved Oxygen

The dissolved oxygen (D.O.) in Pond C showed an immediate response to the addition of the sodium arsenite. The pond was treated early in the morning when D.O. normally begins to increase with increasing photosynthetic activity. Just prior to treatment the D.O. was 8.5 mg/l. Just after the arsenite was added, it began to decrease and in less than twenty-four hours, it had dropped to 5.8 mg/l (Figure 6).

Figure 6. Maximum-minimum dissolved oxygen (D.O.) and production/respiration (P/R) ratios in Pond C following 1963 treatment

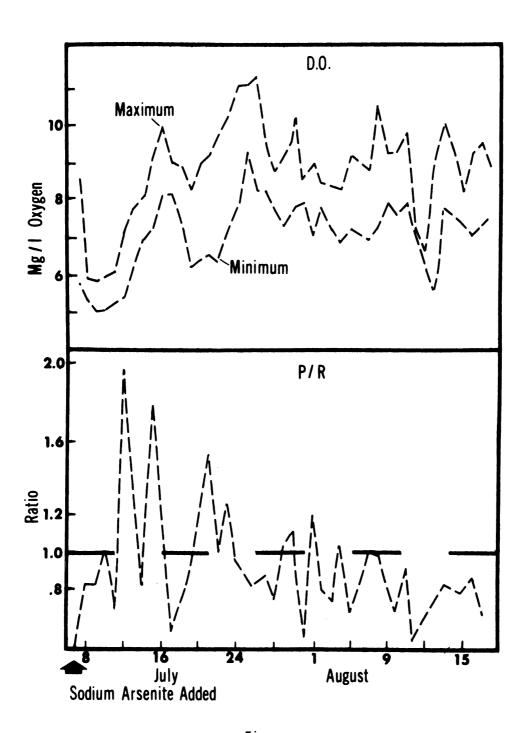


Fig. 6

Although the D.O. increased slightly during the daylight hours of the next day, the values continued to decline further during the night. The lowest D.O. (4.8 mg/l) was recorded three days after the application of the herbicide. A D.O. greater than the pretreatment value of 8.5 mg/l was not recorded for more than a week after the treatment (Figure 6).

During the week following the treatment, grab samples of the three adjacent ponds (ponds A, B, and D) showed no significant decrease in D.O. and they ranged in values daily from 7.0 mg/l to 12.0 mg/l.

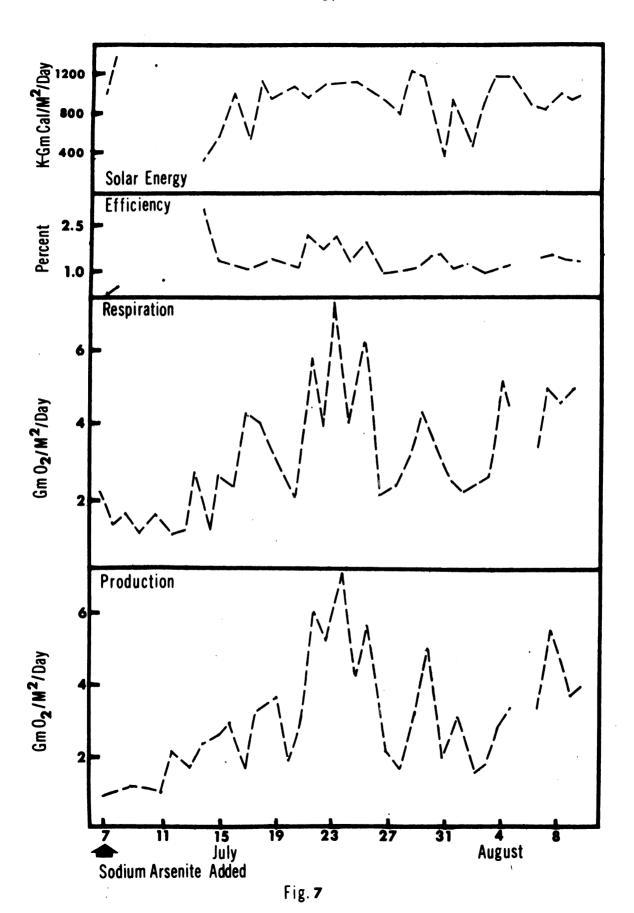
Throughout July and August of 1963, D.O. values from Pond C averaged much lower than those of the adjacent ponds.

Oxygen Metabolism

The dissolved oxygen readings suggest that the addition of sodium arsenite and the subsequent removal of higher, rooted aquatic plants severly limited photosynthetic activity in the treated pond. Although there were no pretreatment or control oxygen metabolism determinations made in 1963, Figure 7 clearly suggests a major reduction of gross photosynthetic production (Pg) occurred immediately after the herbicide was added.

Gross oxygen production increased gradually from a summer low of 1.0 gm-O $_2/m^2/day$ on the day of treatment

Figure 7. Available solar energy (La), efficiency (Pg/La), respiration (Rt) and production (Pg) in Pond C following 1963 treatment



to a high of 6.9 gm- $O_2/m^2/day$, seventeen days after the application (Figure 7).

Community respiration in Pond C showed similar trends. A summer low of just over $1.0~\mathrm{gm}$ - $\mathrm{O_2/m^2/day}$ occurred three days after the arsenite application. The highest respiration value (7.2) occurred the same day as the highest production value, seventeen days after the treatment (Figure 7).

While oxygen production and respiration values closely paralleled each other throughout the summer, they demonstrated very little correlation with solar energy until three weeks after the arsenite was added (Figure 7).

The lowest observed gross production-community respiration ratio (P/R ratio) of 0.4 was recorded on the day of treatment. Although the P/R ratios in Pond C increased significantly during a short recovery period following the treatment, less than one third of the observed readings were greater than 1.0, indicating the pond was essentially a heterotrophic community during most of the growing season (Figure 6).

The efficiency (Pg/La) in Pond C was less than 1.0 per cent for four days following the treatment. A high of 3.0 per cent occurred a week after treatment and corresponded to lowest observed solar energy reading. During the remainder of the summer the efficiency ranged between 1.0 and 2.5 per cent (Figure 7).

Gross production, community respiration, efficiency and P/R ratios all indicate the metabolism of Pond C was at least temporarily inhibited by the addition of the herbicide. The P/R ratios and the efficiency calculations suggest the productive potential of the pond may have been significantly reduced by the removal of the higher aquatics. A more thorough discussion of these results will be made later.

Temperature

The water temperature in Pond C varied between 70° and 90°F. throughout the summer study period in 1963 and corresponded very closely with changes in air temperature. Although increases in solar energy were often reflected in temperature, the oxygen metabolism of the study pond showed no direct correlation with changes in temperature.

The role of temperature in aquatic community metabolism regulation was discussed by Odum and Wilson (1962). Their analysis of one hundred and twenty-three diurnal oxygen curves from Texas bays indicated community production and respiration values corresponded very closely with incident radiation and that even rather large fluctuations in temperature had little effect. They concluded that while changes in temperature have a pronounced effect on the rate of physical gas exchange, an aquatic system and its organisms are sufficiently organized in their activity

to keep total metabolism in phase with food conditions as developed seasonally from light energy, generally independent of temperature.

Temperature fluctuations in the near optimum range (70-90°F.) in the study ponds probably played a very minor role in controlling community metabolism.

Conductivity

When compensations for changes in water temperature were made, fluctuations in conductivity of the study pond water were below the sensitivity of the automatic analyzing equipment. There is little doubt that some changes in conductivity did occur.

Immediately after the herbicide was added, the total alkalinity increased, indicating a rise in the total dissolved solids. Although not measured on the conductivity meter, this increase in dissolved solids probably affected the conductivity.

1964 Study

An analysis of the 1963 study results uncovered many areas where further information and adjustments in sampling techniques were needed. Thus in the 1964 study, a control pond (Pond D) was selected to provide a comparison for changes occurring in the treated pond (Pond C).

Measurements of phytoplankton and periphyton were made in

both ponds in 1964, and simultaneous dissolved oxygen readings were maintained for both ponds during critical periods.

Automatic monitoring of pH and conductivity was discontinued in 1964. However, periodic measurements of pH and alkalinity were made in both ponds as a check of the 1963 results.

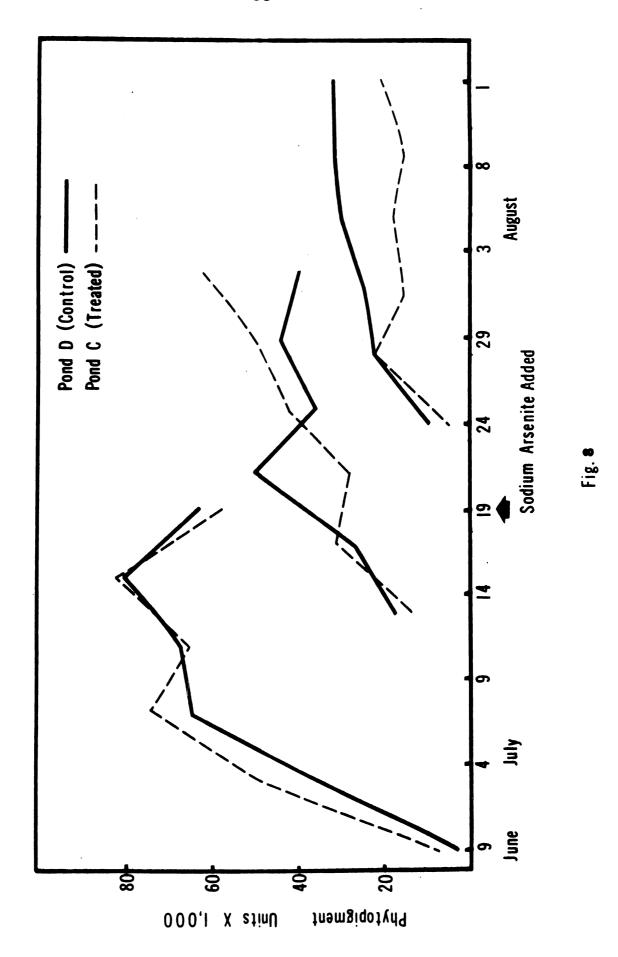
The date of the sodium arsenite treatment in 1964, was moved to July 19 to provide pretreatment information in Pond C.

Periphyton

The production of periphyton was designated as a rate of accumulation of chlorophyll on artificial substrates expressed as phytopigment units (optical density of extracted chlorophyll). In the 1964 study, both horizontal and vertical substrates were used and the results were analyzed separately. The slope of each periphyton growth curve (Fig. 8 and Fig. 9) was considered the rate of production. The periphyton production rates of Pond C and Pond D were compared using methods presented by Li (1957) for analysis of covariance with replicate samples (Tab. I and Tab. II).

Three intervals were used to compare periphyton production on vertical substrates in Pond C and Pond D. Interval I (June 25 - July 18) was chosen to compare the

Periphyton production on vertical substrate in Pond C and Pond D in 1964 Figure 8.



Periphyton production on horizontal substrate in Pond C and Pond D in 1964 Figure 9.

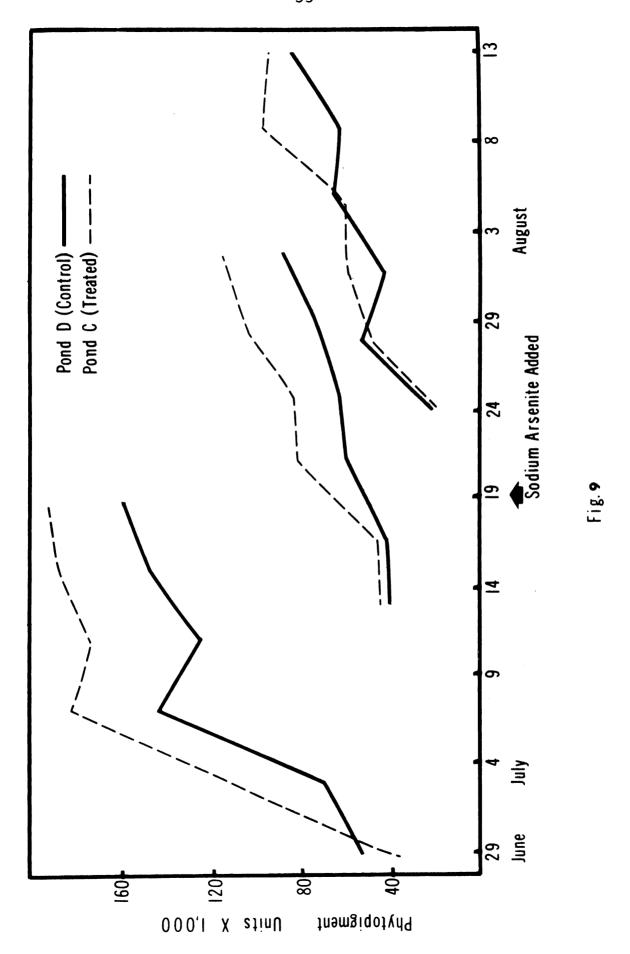


TABLE I

Analysis of Covariance of Periphyton Production in Pond C (Treated) Versus Pond D (Control)

(Comparisons made in terms of accumulation of phytopigment on vertical substrates in 1964)

F Ratio	0.41		7.03*		6.41*	
Mean Square	141.72	340.94	589.90	83.89	107.73	16.82
Degrees of Freedom	ı	ω	ı	œ	п	80
Sum of Squares	141.72	2,727.56	589.90	671.12	107.73	134.55
Source of Variation	Variation among slopes**	Pooled residual	Variation among slopes**	Pooled residual	Variation among slopes**	Pooled residual
Growth Interval	6-25 to 7-19	Interval I	7-7 to 8-2	Interval II	7-20 to 8-13	Interval III

*Significant difference at the 95% level.

^{**} Variation among slopes equals variation between production rates in Pond C and Pond D.

TABLE II

Analysis of Covariance of Periphyton Production in Pond C (Treated) Versus Pond D (Control)

(Comparisons made in terms of accumulation of phytopigment on horizontal substrates in 1964)

	287.93	7	2,015.51	Pooled residual	Interval III
2.95	849.61	1	849.61	Variation among slopes**	7-20 to 8-2
	37.93	ω	303.51	Pooled residual	Interval II
7.76*	288.70	1	288.70	Variation among slopes**	7-7 to 8-2
	1,694.50	8	13,552.40	Pooled residual	Interval I
0.31	519.59	1	519.59	Variation among slopes**	6-25 to 7-19
F Ratio	Mean Square	Degrees of Freedom	Sum of Squares	Source of Variation	Growth Interval

*Significant difference at the 95% level.

^{**} Variation among slopes equals variation between production rates in Pond C and Pond D.

production rates in ponds C and D under natural conditions prior to any treatment. Interval II (July 7 - Aug. 2) was chosen to illustrate the effects of sodium arsenite on periphyton which had been allowed to establish some growth on the substrates before the treatment. Interval III (July 20 - Aug. 13) began one day after Pond C was treated with sodium arsenite and it was used to determine the effects of the herbicide on the establishment of new periphyton communities (Fig. 8).

Periphyton production on vertical substrates in the two ponds showed no significant difference during Inverval I. During Interval II the treated pond exhibited significantly greater periphyton production, and in Interval III the periphyton production on vertical substrates was significantly greater in the control pond (Fig. 8 and Tab. I).

Although at first the results appear to be contradictory, a closer analysis reveals a correlation between the arsenite treatment and the observed periphyton production. Under natural conditions the periphyton production in the two ponds is essentially equal. The periphyton which had been allowed to establish itself on the substrates in Pond C before the herbicide treatment, was apparently not affected by the sodium arsenite and eventually utilized the nutrients made available by the decaying macrophytes, to increase production. Colonization

of clean substrates placed in Pond C after the treatment was probably inhibited by the presence of the sodium arsenite in the water, and hence, observed Pond C periphyton production during Interval III was less than that in the control pond--even though an increased quantity of nutrients was available.

Horizontal substrates were also sampled in the same three intervals indicated for vertical substrates. Periphyton production on horizontal substrates during Interval I and Interval II showed the same results as the vertical substrates. The production of periphyton on horizontal substrates was not significantly different in the two ponds during Interval I, and the periphyton production was significantly greater in the treated pond during Interval II (Fig. 9 and Tab. II).

Periphyton production on horizontal substrates during Interval III was not significantly different in the two ponds. Although this measurement of periphyton production in Interval III does not correspond to the results obtained from vertical substrates, it does support the contention that colonization of the new substrates was inhibited by the sodium arsenite. If the herbicide had not interfered with colonization, the periphyton production on horizontal substrates during Interval III should have been significantly greater in the treated pond when compared to the control pond because of the increase in

nutrients--it was not. The difference between the results on the vertical and horizontal substrates can be explained by the fact that horizontal substrates always "seed on" or colonize quicker than vertical substrates.

A certain minimum quantity of plant material on a substrate is required before the logarithmic or arithmetic growth phase of the periphyton community begins (Kevern, op. cit.; King, op. cit.). Normally, this requires less than four days in the Lake City ponds. The accumulation of organic material begins more rapidly on horizontal substrates. Thus, if colonization is inhibited, the horizontal substrates could be expected to demonstrate greater periphyton production than the vertical substrates in a short term comparison.

In summary, the periphyton production in Pond C, as measured by the artificial substrate method, increased significantly after treatment. Even though the arsenite inhibited the colonization of clean substrates, the natural periphyton growing in the pond was probably capable of utilizing the new source of nutrients to temporarily increase production. This increase in production in Pond C was estimated to have lasted for thirty days after the treatment.

Large floating mats of <u>Spirogyra</u> <u>sp.</u> appeared in Pond C ten days after the application of the herbicide and persisted for about one week, which gave further evidence

that the decaying macrophytes had released nutrients.

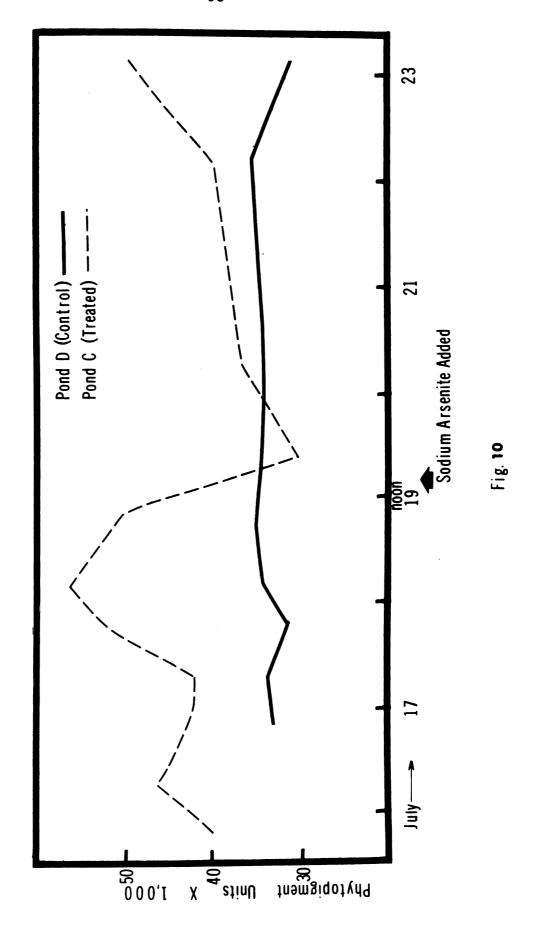
Spirogyra blooms of this type were characteristic of fertilization treatments applied to these same ponds in previous studies.

Phytoplankton

Because of the time involved in sampling, only a limited number of plankton samples were taken. However, samples obtained did provide an adequate index to the standing crop of phytoplankton expressed as phytopigment units. Figure 10 illustrates the effect of the sodium arsenite on the phytoplankton in Pond C compared to the untreated control, Pond D. The most dramatic evidence, showing the immediate effect of sodium arsenite on phytoplankton, was the difference between the two samples taken on the day of application. One sample was taken in the morning before treatment and yielded .051 phytopigment The afternoon sample, after treatment, exhibited only .031 phytopigment units (Fig. 10). The control pond showed no difference between morning and afternoon samples on the same day. The sodium arsenite apparently destroyed a portion of the phytoplankton on contact; however, as the concentration of the herbicide diminished, the phytoplankton recovered.

Sohacki (op. cit.) reported similar results when he measured the effects of sodium arsenite on phytoplankton

Index to standing crop of phytoplankton in 1964 from 16 liter centrifuged samples Figure 10.



productivity using the C¹⁴ method. He concluded that the slight reduction in photoplankton productivity that occurred following the treatment and the short recovery time demonstrated the resistance of algae toward sodium arsenite toxicity.

Sohacki (op. cit.) reported that the phytoplankton in the treated pond recovered to pretreatment production levels within six days after the application. As can be seen in Figure 10, the standing crop of phytoplankton in Pond C returned to pretreatment levels within four days after the arsenite was added in 1964.

Unfortunately, the <u>Spirogyra sp.</u> blooms which occurred in Pond C after the treatment did not lend themselves to quantitative measurement by either of the methods being employed to measure changes in periphyton and phytoplankton production. These blooms were of sufficient magnitude to have significantly increased the total oxygen production in Pond C. With the exception of these blooms, no increase in phytoplankton standing crop was detected in Pond C after the treatment, although periodic samples were taken for a month after the application of the herbicide.

Properly considering these <u>Spirogyra</u> <u>sp.</u> floating mats as part of the phytoplankton population, the phytoplankton production in Pond C certainly increased significantly a short time after the herbicide was added.

Although no quantitative measurements of this increase in production were made, visual observations indicated it lasted for about seven days.

Dissolved Oxygen

The D.O. in Pond C in 1964 again demonstrated an immediate response to the sodium arsenite treatment. The herbicide was applied in the middle of the afternoon and within two hours the D.O. dropped from 9.5 mg/l to 8.0 mg/l. Within two days the D.O. reached a summer low of 4.2 mg/l (Fig. 11).

It was almost two weeks after the application before the D.O. in Pond C exceeded pretreatment values. The control pond during this same interval had no D.O. values below 7.5 mg/l, and some values ranged as high as 11.5 mg/l. Pretreatment D.O. values in Pond D and Pond C were nearly equal.

It was misreported in an early paper on this same study that the D.O. values in the treated pond reached a minimum of only 6.0 mg/l (Bails and Ball, 1966). A rechecking of twenty-four hour dissolved oxygen recording charts revealed this previous error. The possible reasons for a lower minimum D.O. following the 1964 treatment, rather than the 1963 treatment as previously reported, will be discussed later.

Figure 11. Maximum-minimum dissolved oxygen and production/respiration (P/R) ratios in Pond C during 1964 study

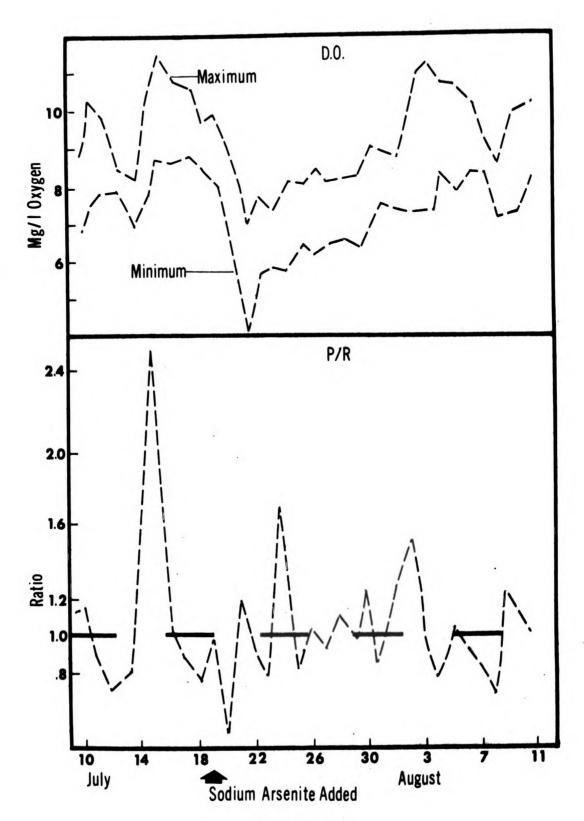


Fig. 11

Oxygen Metabolism

The pretreatment oxygen production values in Pond C ranged from 2.3 to 9.5 $\text{gm-O}_2/\text{m}^2/\text{day}$. Within one day after treatment, the gross oxygen production in Pond C was reduced to 1.6 $\text{gm-O}_2/\text{m}^2/\text{day}$ from a 4.2 value the day before.

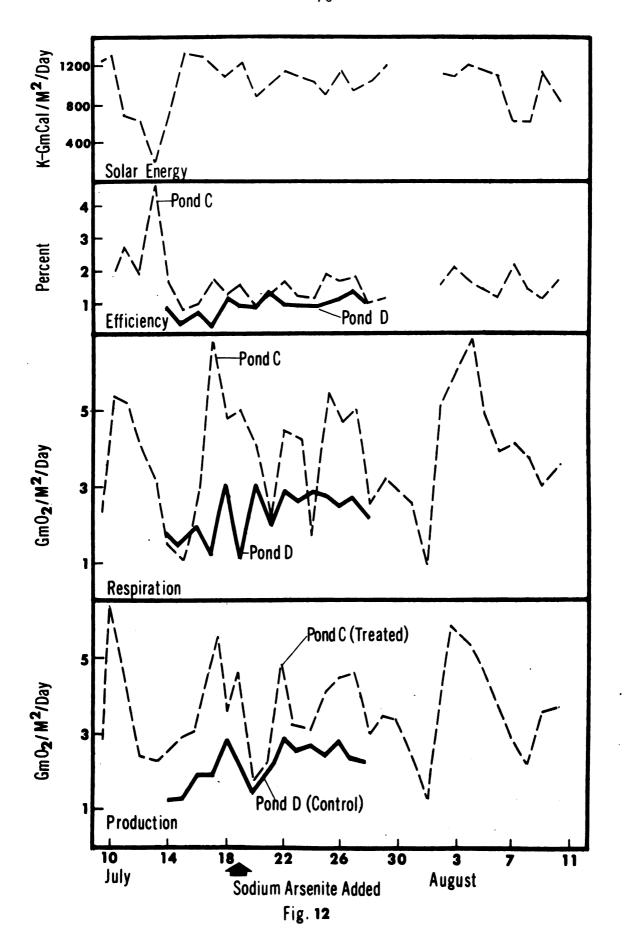
The control pond, Pond D, also exhibited a reduction in oxygen production (i.e., from 2.1 to 1.4 $gm-O_2/m^2/day$) the day after the arsenite was added to Pond C. It was very small in comparison, however, and it was attributed to a decrease in solar energy (Fig. 12).

Efficiency in Pond C was reduced to a summer low of 0.7 the day following the arsenite application. The pretreatment efficiency percentages in Pond C showed an inverse relationship with solar radiation (i.e., high radiation, low efficiency) and they ranged between .72 and 5.0. Post-treatment efficiency values in Pond C never exceeded 2.0.

The efficiency values in Pond D were considerably less than Pond C. They remained relatively constant and inversely related to solar energy throughout the study period. The inverse relationship of solar energy and oxygen metabolism efficiency will be discussed later.

The P/R ratios in Pond C ranged from .8 to 2.4 before treatment and dropped to a summer low of .45 the day following the treatment. Post-treatment P/R ratios

Figure 12. Available solar energy (La), efficiency (Pg/La), respiration (Rt) and production (Pg) in Pond C (treated) and Pond D (control) during 1964 study



in Pond C did not exceed 1.65. The R/R ratios in Pond D were quite low all summer and their comparison to Pond C is nearly meaningless except to say that P/R ratios in Pond D showed no significant change during the period immediately following the treatment of Pond C.

Summarizing the reaction of oxygen metabolism in Pond C to the arsenite treatment, it appears that while the oxygen production recovered to pretreatment levels quite rapidly, the efficiency and P/R ratios were significantly reduced for the entire study period.

Hydrogen Ion and Alkalinity

The hydrogen ion content of the treated pond in 1964 increased after the application of the sodium arsenite as was predicted from the preliminary study. The pH of Pond C varied between 7.5 and 8.7 before treatment and dropped as low as 6.00 after treatment. The control pond maintained a pH above 8.0 throughout the study period. Again, as predicted by the preliminary study, the total alkalinity increased in Pond C after treatment, while the total alkalinity of the control pond remained relatively constant throughout the study. The total alkalinity in Pond C ranged between 60 and 70 mg/l before treatment and climbed as high as 97 mg/l after treatment. Pond D maintained a total alkalinity between 60 and 70 mg/l all through the summer.

Comparison Between 1963 and 1964 Treatments

In both 1963 and 1964, Pond C was treated with sodium arsenite, and although the reaction of the pond metabolism to each treatment was similar, the magnitude and duration of the effects were different. Before discussing these differences, the condition of the pond ecosystem before each treatment and timing of the arsenite application in each year will be analyzed.

In 1962, Pond C was treated with copper sulfate for a previous study (Sohacki, op. cit.). This treatment removed much of the algae, Chara sp. which, under normal conditions, is common in these hardwater ponds.

By the time the sodium arsenite was applied in 1963, the higher aquatic macrophytes, predominately Potamageton natans and P. praelongus, were well established. The abundance of the macrophytes in Pond C in 1963, would not have, under normal circumstances, required removal to obtain full utilization of the pond. The Chara sp. in Pond C, although present in early 1963, had not recovered fully from the previous year's copper sulfate treatment. Periphyton and planktonic algae in Pond C were probably exhibiting normal production before the 1963 sodium arsenite application.

The 1963 treatment removed all submergent aquatic macrophytes with the exception of <u>Chara sp.</u> Although these higher aquatics showed some signs of recovery by September

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In 1963, the sodium arsenite was applied early in the morning. In 1964, the arsenite was added at the peak of photosynthetic activity in the late afternoon. The treatment came two weeks later in the growing season in 1964.

In general, the herbicide produced more pronounced effects in 1963. The pH dropped much lower, the total alkalinity increased more, and the gross oxygen production was suppressed much longer in 1963 than in 1964. These differences can be accounted for by the fact that there was a larger quantity of macrophytes susceptible to removal by sodium arsenite before the 1963 treatment. Thus, the entire metabolism of the pond was more dependent on these plants and the ecosystem responded more dramatically to their removal.

The minimum D.O. in Pond C was lower after the treatment in 1964 compared to 1963, and this observation is difficult to explain in view of the relative abundance of macrophytes in the two years. Perhaps the answer lies in the unusual weather conditions that persisted in 1964.

The days immediately preceding the 1964 treatment were very cloudy and solar radiation was less than 1000 $kgm-cal/m^2/day$. The maximum D.O. in Pond C the day before the arsenite was added in 1964 was only 10.0 mg/l. In the

two days following the 1964 treatment, the D.O. was reduced to 4.2 mg/l--a gross D.O. reduction of 5.8 mg/l from the pretreatment high.

The overcast weather following the 1964 treatment restricted even the remaining algae from producing sufficient oxygen to replace that being consumed by the decaying macrophytes.

The gross reduction in D.O. following the 1963 treatment amounted to 6.5 mg/liter in a period of three days from a maximum the day before treatment of 11.5 mg/l, to a low of 5.0 mg/l two days following the application.

Thus, even though the minimum D.O. recorded after sodium arsenite treatment was less in 1964 than in 1963, the gross D.O. reduction from a pretreatment high was greater in 1963. The overcast conditions in 1964 perhaps obscured the more pronounced effects of the arsenite treatment in 1963 when the minimum D.O. values of Pond C after the two treatments, are compared.

Comparison of Results with Other Studies

One of the best means of testing the validity of the methods used in this study is through comparing the results with those obtained by numerous other authors in similar attempts to measure aquatic community metabolisms.

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Solar Radiation

An accurate measure of total solar radiation is essential to daily calculations of metabolism efficiency. Solar radiation figures used in this study were recorded at the study site with an Epply pyroheliometer and they were very similar to those recorded by Crabb (1950) in his studies at East Lansing, Michigan. While the maximum daily total solar radiation values recorded at Lake City were higher than those recorded by Crabb (op. cit.) the mean weekly values were not significantly different.

Oxygen Metabolism

Oxygen production and respiration, and P/R ratios have been calculated for numerous lentic communities (Copeland, Whitworth 1963; Odum, 1956; Knudson and Dorris, 1963; and Odum, 1957). The range of values reported by these authors are graphically presented in Figure 13.

This oxygen production, oxygen respiration graph was first proposed by Odum (1956) to classify biological communities into heterotrophic or autotrophic types. The diagonal line from the lower left hand corner to the upper right hand corner of the graph represents P/R ratios of one or unity. Points to the right of this line have P/R ratios less than one and points to the left have P/R ratios greater than one.

Figure 13. Range of oxygen metabolism values from several ponds: (1) Lake City Ponds; (2) Four Oklahoma Farm Ponds (Copeland and Whitworth, 1963); (3) Several Northern Ponds (Odum, 1956); (4) Oklahoma Ponds (Knudson, and T. C. Dorris, 1963); and (5) Marine Turtle Grass Communities (Odum, 1957). Shaded areas for (1) and (2) encompass the wide range of values reported in those studies

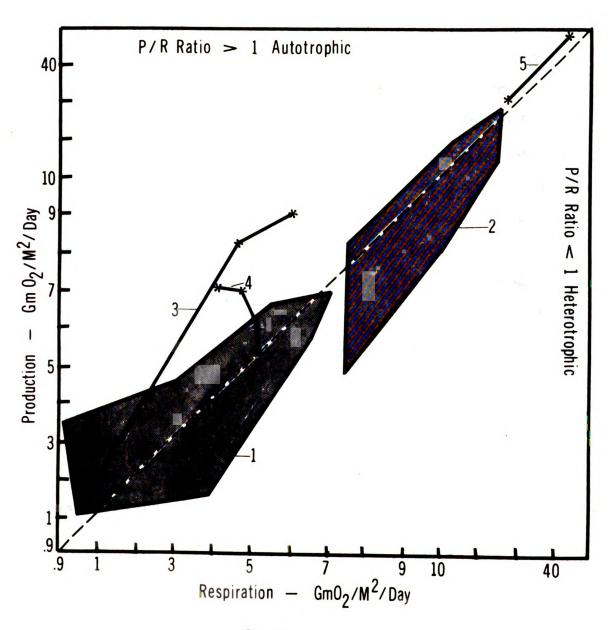


Fig. 13

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This type of graphic presentation not only allows for a visual comparison of production and respiration values from several different communities, it can also be used to classify community types and predict the ecological succession in terms of oxygen production and respiration values.

The values recorded in Figure 13 for the Lake City ponds represents 95% of the total range of values. Extremely low production and respiration values in Pond C, occurring after the herbicide was added, were deleted so that the scale of this graphic comparison would be meaningful. The values recorded for this study are in the right magnitude when compared to the results of these other authors (Ibid.).

The range of values recorded in Figure 13 represent only the productive summer periods for all the communities. The annual herbicide treatments in the Lake City ponds are at least partially responsible for the fact that the Lake City pond values are somewhat lower and to the left of the values recorded for other ponds in Figure 13.

The range of oxygen production and respiration values in Figure 13, most similar in magnitude to the Lake City ponds, were reported by Odum (1956). These values calculated and presented by Odum, were extracted from information collected by Juday, Blair, and Wilda (1943), on Little John Pond, Wisconsin; and by Riley (1940) on

Linsley Pond, Connecticut. It is interesting to note that both these ponds are in nearly the same latitude as the Lake City ponds and all three sites would, therefore, be exposed to nearly equal solar radiation.

The oxygen metabolism values reported by Copeland and Whitworth (1963) exhibit wide variation both to the right and to the left of the P/R unity line in Figure 13. One of the four ponds studied by Copeland and Whitworth was also treated with a herbicide, which perhaps accounts for the wide range of values found in both the Oklahoma ponds and the Lake City ponds. The four Oklahoma ponds were all regularly subjected to fertilization through runoff from land under varied forms of intensive agriculture, making them considerably more productive than the Lake City ponds. This is clearly illustrated in Figure 13.

The oxygen metabolism values of a Marine Turtle

Grass Community (Odum, 1957), were included in Figure 13

to compare the magnitude of one of the most productive,

naturally occurring lentic aquatic communities with various

small ponds.

Efficiencies

Figure 4 indicates the various types of ecological efficiencies that can be calculated for the transfer of energy from solar radiation through the first trophic level--the producers. Generally, these efficiencies are

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one of four types: (1) Pg/Li; (2) Pg/La; (3) Pn/Li; or (4) Pn/La. Each of these ratios represent a different type of energy transfer efficiency.

Table III compares the calculated efficiencies in these four categories from various aquatic communities with the ratios obtained from the Lake City ponds.

With the exception of Lindeman's figures on the Cedar Bog Lake, all the Pg (gross production) efficiencies presented in Table III were calculated on the basis of gross oxygen production values obtained from diurnal oxygen curves. For these studies the range of gross oxygen production values reported by each author were also included in Table III.

The upper range of Pg/Li, Pg/La and gross oxygen production values recorded for the Lake City ponds are generally below those reported by the several other authors in Table III. However, for each of these same three parameters the Lake City ponds exhibited the lowest values recorded in these various aquatic communities. There are probably two primary reasons why the lowest efficiency and oxygen production values would be observed in the Lake City ponds. First, the sodium arsenite application significantly reduced oxygen production and efficiency levels in the treated pond far below normal summer levels. Secondly, since both efficiency and oxygen production were calculated on a daily basis the lowest values occurring would be

TABLE III

Range of Efficiency and Gross Oxygen Production Values from Various Aquatic Communities Compared to Lake City Ponds

Aquatic Community		Efficie	Efficiencies*		Gross O, Production
	Pg/Li	Pg/La	Pn/Li	Pn/La	$(Gm-O_2/M^2/Day)$
Minnesota Pond ¹			0.02		
Lake Mendota ²			0.27	0.35	
Cedar Bog Lake	0.1		90.0		
Artificial Stream	8.8	12.5	2.90	4.10	
Blue River ⁵	2.7				2.4 - 12.0
Stream Microcosm		3.0			25.0
Silver Springs ⁷	1.6	5.3	0.52		8.0 - 35.0
Range of Above	8.8	12.5	2.90 0.02	4.10 0.35	2.4 - 35.0
Lake City Ponds	1.02	5.1 0.4			6.9 -60.0

*Efficiencies expressed as percentages--when two values occur they indicate range.

⁽¹⁾ Dineen, 1952; (2) Juday, 1940; (3) Lindeman, 1942; (4) Kevern, 1962; (5) Duffer and Dorris, 1966; (6) Odum and Hoskin, 1957; and (7) Odum, 1957a.

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recorded in the Lake City ponds while the actual lowest values may have been missed in the sampling procedure used by these authors.

None of the other authors cited in Table III utilized automatic dissolved oxygen recording equipment and, thus, they were restricted to a few twenty-four hour sampling periods in their studies. Kevern (1962) made the most frequent diurnal oxygen determinations and he sampled on a weekly basis.

No net production values were calculated for the Lake City ponds. Attempts to accurately measure the standing crop of Chara sp. in the Lake City study were unsuccessful, and periphyton production was not converted by bomb calorimetry to obtain net periphyton on a gramcalorie basis. However, using Table III as a guide, the net production efficiencies of the Lake City ponds can be estimated.

In three studies recorded in Table III (Lindeman, 1942; Kevern, 1962; and Odum, 1957a) both gross and net production efficiencies were calculated. In each case, the net production efficiencies were about one third the corresponding gross production efficiencies. E. P. Odum (op. cit.) cites several studies that indicated that fifty percent or more of the gross photosynthetic production may be used up by the plants themselves.

It is perhaps reasonable to assume, therefore, that net production efficiencies for the Lake City ponds, during the summer period, were from thirty to fifty per cent less than the gross production efficiencies recorded in Table III. The maximum Pn/Li for the Lake City ponds would then fall between .3 and .51 and the maximum Pn/La between 1.7 and 2.5.

The inverse curvilinear relationship between efficiency and solar energy has been observed by many authors. Recently, Duffer and Dorris (op. cit.) were able to demonstrate this relationship by calculating efficiencies and recording solar energy on an hourly basis. They concluded that, "In general, efficiency calculated from total solar radiation indicates the same relationship as that calculated from total radiation each hour (higher efficiency with low light intensity)." E. P. Odum (op. cit.) theorized that low efficiency is necessary for maximum power output of a biological system, and that more rapid growth per unit time has a greater survival value than maximum efficiency in the use of available energy.

Figure 14 illustrates the relationship between solar energy (La) and efficiencies (Pg/La) during the two summer study periods at Lake City. The period immediately following the sodium arsenite application in each year was excluded from this curve so that it would more closely represent the relationship between solar energy and

Figure 14. The inverse, curvilinear relationship between solar energy and efficiency in the Lake City ponds

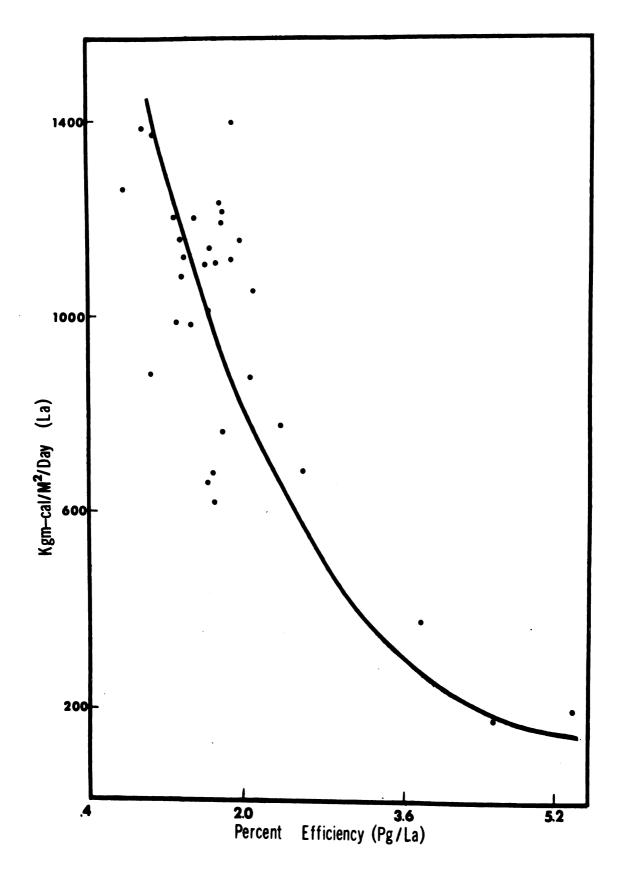


Fig. 14

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efficiencies under normal conditions. The variance of the points plotted in Figure 14 is fairly high; however, the inverse curvilinear relationship between solar energy and efficiency is, nevertheless, clearly illustrated.

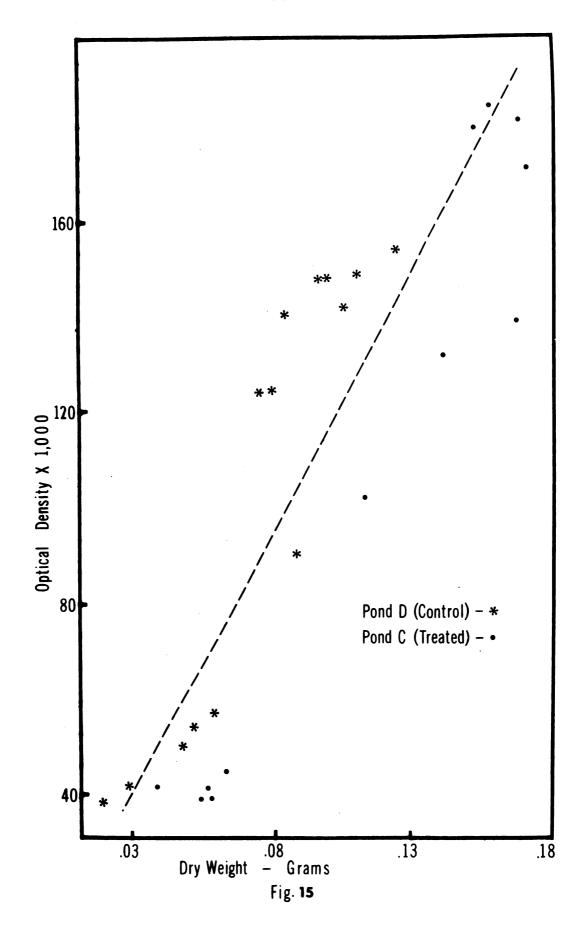
Periphyton

As was mentioned earlier, a number of periphyton samples were dried and weighed to obtain an estimate of the relationship between dry weight and optical density of the chlorophyll content. A portion of the dried samples were then placed in a muffle furnace to obtain an estimate of the ash-free dry weight (organic weight).

Only the vertical substrates were used to obtain the relationship between dry weight and optical density. King (op. cit.) and others have found that substrates placed horizontally with respect to the surface usually have significant quantities of heterotrophic and inert material which tends to distort the true relationship between the chlorophyll content and dry weight of the autotrophic material. Figure 15 illustrates the dry weighoptical density relationship on vertical substrates from both study ponds.

The linear relationship between these two measures of organic material is clear, however, if all optical density measurements were to be converted to dry weight measures of periphyton two separate curves would have to

Figure 15. The linear relationship between optical density of chlorophyll content and dry weight of periphyton in Pond D and Pond C in 1964



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be calculated. Figure 15 indicates that in general more chlorophyll was observed for the same dry weight in Pond D than in Pond C. The reason for this difference is not entirely clear, however, it may be a result of the numerous herbicide treatments applied to Pond C. The species composition of the periphyton community in Pond C may have been selectively altered by sodium arsenite.

King (op. cit.) found that different zones of the Red Cedar River had different periphyton communities and that dry weight-optical density conversion factors had to be calculated independently for each zone.

The ash-free dry or organic weights obtained from the Lake City periphyton samples were extremely variable. Organic weights ranged between 80 and 47 per cent of the dry weights. The organic weights showed little or no relationship to either the dry weights or the optical densities. The only plausible explanation seems to be an inherent error in the procedure used to obtain organic weight.

The dry weight of each individual sample was always less than .1 grams and usually less than .05 grams. Since each sample had to be handled several times to obtain organic weight, even small errors in either handling or weighing were magnified and became significant because of the small weight of each individual sample.

SUMMARY

Automatic analyzing equipment was utilized to measure the oxygen metabolism of two small ponds, one of which was treated with sodium arsenite. Other measures of primary productivity were also made to determine the effects of the herbicide treatment.

A system was developed to automatically monitor and record dissolved oxygen and pH in the two study ponds inside the laboratory. A computer program was designed to calculate the gross oxygen production, community respiration, and the production to respiration ratios from the hourly dissolved oxygen readings.

The pH in the treated pond (Pond C) in the preliminary study (1963) dropped from a maximum on the day of treatment of 8.65 to a low of 7.1 one week after the herbicide was added. Adjacent control ponds had a range of pH 8.4 to 9.8 during the same period. It was approximately one month after treatment before the pH returned to normal levels in Pond C.

Pretreatment total alkalinity in Pond C in 1963, ranged below 70 mg/l and a maximum of 106 mg/l was recorded two days after the addition of the herbicide. Adjacent

ponds ranged below 70 mg/l total alkalinity throughout the study period. The total alkalinity appeared to return to normal levels in the treated pond in three weeks.

Oxygen production, community respiration and production to respiration ratios were all significantly reduced in Pond C following the 1963 sodium arsenite treatment. Both oxygen production and respiration reached a summer low of 1.0 gm-O₂/m²/day just after the treatment. The lowest P/R ratio (0.4) was recorded in Pond C on the day of treatment. Dissolved oxygen in Pond C dropped from 8.5 mg/l to a summer low of 4.8 mg/l within 72 hours after the arsenite was added. The dissolved oxygen remained relatively constant in the adjacent control ponds during the study period.

Pond C was again treated with sodium arsenite in 1964, and the changes in both pH and alkalinity were similar to those observed in the 1963 preliminary study.

The effects of the sodium arsenite on the production of periphyton was also measured in 1964. The periphyton production in Pond C, as measured by the artificial substrate method, increased significantly after treatment when compared to the control pond (Pond D). However, the periphyton colonization of new substrates appeared to be inhibited by the arsenite application.

Samples of the standing crop of phytoplankton in Pond C and Pond D in 1964, indicated that phytoplankton

production was temporarily restricted by the herbicide.

Spirogyra sp. blooms occurred in the treated pond about ten days after the sodium arsenite was added and lasted about a week.

The pretreatment oxygen production values in Pond C ranged from 2.3 to 9.5 gm-O₂/m²/day. Within one day after treatment, the gross oxygen production in Pond C was reduced to 1.6 gm-O₂/m²/day. Efficiency in Pond C was reduced to a summer low of 0.7 the day following the treatment. While oxygen production and respiration recovered to pretreatment levels quite rapidly, the efficiency and P/R ratios were significantly reduced for the entire study period.

Results of the Lake City study compare very favorably with the results of various other authors using similar methods, particularly with respect to oxygen metabolism and efficiency calculations. The inverse curvilinear relationship between light energy and primary efficiency could be illustrated with the Lake City data. And, the straight line relationship between chlorophyll content and dry weight of periphyton samples was confirmed by the Lake City data.

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APPENDIX

Record of solar energy, oxygen production, respiration and efficiency in Pond C and Pond D in 1963 and 1964. The following abbreviations were used in the tables:

L = Total light energy in gm-cal/cm²/day

La = Absorbed light in $kgm-cal/m^2/day$

Max. D.O. = Maximum observed dissolved oxygen in mg/l

Min. D.O. = Minimum observed dissolved oxygen in mg/l

Pg = Gross production in $gm-O_2/m^2/day$

Rt = Community respiration in $gm-O_2/m^2/day$

Pg/Rt = Production-respiration ratio

Pg/La = Efficiency in per cent

Pond C

Date	L	La	Max. D.O.	Min. D.O.	Pg	Rt	Pg/Rt	Pg/La
7- 7-63	507	1015	8.4	5.7	.1	2.0	.05	.04
8	727	1454	5.8	5.3	1.1	1.3	.81	.30
9			5.7	5.0	1.4	1.7	.81	
10			5.9	5.0	1.2	1.1	1.11	
11	649	1299	6.0	5.2	1.1	1.7	. 62	.33
12			7.0	5.4	2.1	1.0	2.05	
13			7.6	6.1	1.7	1.3	1.29	
14	156	313	7.9	6.7	2.4	2.8	.85	3.01
15	346	691	9.0	7.0	2.5	1.3	1.87	1.44
16	573	1145	9.8	8.2	3.0	2.7	1.11	1.04
17	322	643	8.8	8.2	1.3	2.2	.58	.81

Date	L	La	Max. D.O.	Min. D.O.	Pg	Rt	Pg/Rt	Pg/La
7-18-63	606	1211	8.7	7.3	3.1	4.3	.71	1.01
19	474	948	7.9	6.2	3.7	4.0	.90	1.54
20	550	1101	8.8	6.4	1.9	1.7	1.25	.64
21	545	1191	9.1	6.6	3.2	2.1	1.50	1.09
22	504	1008	9.6	6.2	6.1	5.8	1.05	2.42
23	621	1241	10.1	7.0	5.3	4.1	1.28	1.70
24	600	1200	10.8	7.6	7.0	7.2	.97	2.33
25	626	1252	10.8	9.2	4.1	4.3	.94	1.31
26	567	1134	11.0	8.0	5.7	6.8	.83	2.01
27	474	948	9.2	8.2	2.0	2.2	.91	.85
28	381	762	8.5	7.5	1.7	2.3	.72	.88
29	636	1272	9.2	7.1	3.6	3.3	1.08	1.13
30	608	1216	10.5	7.7	5.3	4.8	1.11	1.76
31	173	346	8.2	7.8	1.7	3.6	.46	1.92
8- 1-63	558	1116	8.9	6.7	3.3	2.6	1.24	1.17
2	235	470	8.4	7.5	1.6	2.3	.71	1.39
3	402	804	8.2	7.0	1.7	2.5	.69	.86
4	643	1286	8.3	6.6	2.7	2.7	1.01	.85
5	634	1268	9.3	7.0	3.4	5.4	.64	1.09
6	450	900						
7	415	830	8.5	6.7	3.1	3.0	1.06	1.07
8	560	1120	10.5	7.1	5.3	5.3	1.02	1.02
9	517	1034	9.3	7.7	3.7	4.4	.83	.83

Date	L	La	Max. D.O.	Min. D.O.	Pg	Rt	Pg/Rt	Pg/La
8-10-63	577	1154	9.4	7.3	3.9	5.3	.74	.74
7- 9-64	634	1268	8.6	6.9	2.9	2.5	1.16	.90
10	711	1423	10.2	6.9	6.4	5.5	1.16	1.80
11	341	681	9.8	7.8	4.8	5.2	.91	2.81
12	317	633	8.5	8.8	2.6	4.0	.65	1.64
13	97	194	8.2	7.1	2.6	3.1	.84	5.34
14	338	677	10.1	6.9	2.6	1.6	1.65	1.56
15	698	1396	11.4	8.6	3.0	1.2	2.48	.85
16	692	1384	10.6	8.6	3.0	2.9	1.04	.88
17	616	1233	10.4	8.6	5.4	6.6	.88	1.77
18	509	1018	9.6	8.4	3.7	4.7	.76	1.40
19	622	1244	9.8	7.8	4.7	5.1	.93	1.52
20	440	881	8.4	6.0	1.6	4.0	.41	.74
21	508	1016	7.0	4.0	2.8	2.3	1.23	1.10
22	594	1188	7.5	5.4	5.0	5.1	.97	1.67
23	554	1107	7.2	5.7	3.2	4.3	.75	1.17
24	555	1109	7.9	5.5	3.1	1.7	1.79	1.12
25	452	904	7.9	6.4	4.1	5.5	.85	2.05
26	566	1132	8.3	6.1	4.6	4.5	1.02	1.64
27	479	959	7.9	6.4	4.6	5.1	.91	1.93
28	508	1015	8.1	6.4	2.9	2.5	1.14	1.14
29	632	1264	8.1	6.3	3.5	3.7	.93	1.10
30			8.9	6.8	3.6	2.9	1.22	
31			8.7	7.4	2.2	2.5	.88	

Da	ate	L	La	Max. D.O.	Min. D.O.	Рg	Rt	Pg/Rt	Pg/La
8-]	1-64			8.5	7.3	1.1	. 9	1.23	
	2	564	1128	10.4	7.2	4.5	3.1	1.44	1.60
	3	559	1117	11.1	7.3	5.8	5.9	.99	2.09
4	4	638	1277	10.4	8.3	5.3	6.8	.77	1.66
	5	635	1270	10.6	7.7	4.8	4.6	1.05	1.43
(6	563	1127	10.1	8.1	3.7	3.8	.95	1.30
•	7	588	1176	9.1	8.0		4.1		.58
8	8	325	650	8.3	6.9	2.5	3.7	.67	1.53
9	9	599	1199	9.7	7.1	3.6	3.0	1.21	1.21
10	0	395	791	10.0	7.9	3.6	3.7	.97	1.82

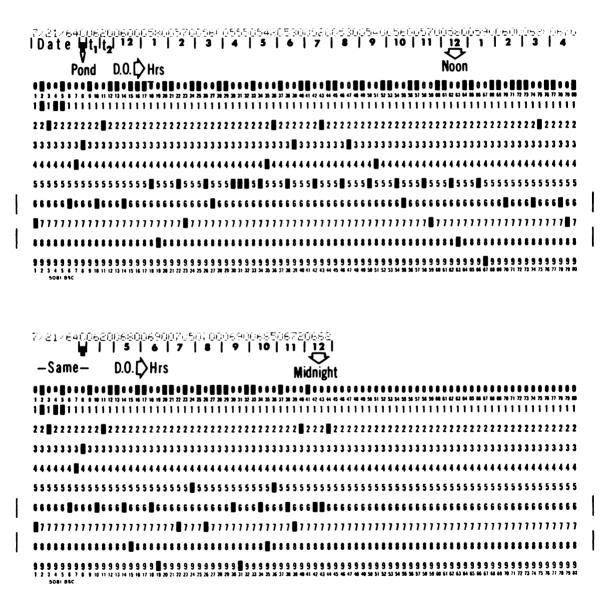
Pond D

Date	L	La	Pg	Rt	Pg/La
7-14-64	338	677	1.2	1.7	.73
15	698	1396	1.3	1.5	.39
16	692	1384	2.0	2.0	. 57
17	616	1233	1.9	1.1	.31
18	509	1018	2.9	3.0	1.10
19	622	1244	2.2	1.0	.75
20	440	881	1.5	3.0	.72
21	508	1016	2.3	2.0	1.02
22	594	1188	2.9	2.8	.98
23	554	1107	2.6	2.7	.94
24	555	1109	2.7	2.8	.97
25	452	904	2.5	2.7	1.10
26	566	1132	2.8	2.5	.98
27	479	959	2.5	2.6	1.04
28	508	1015	2.4	2.0	.94

Fortran computer program developed to calculate average respiration, gross oxygen production, and total community respiration for each day from observed hourly dissolved oxygen readings.

```
PROGRAM BAILS
    DIMENSION DAY(25). ADJDAY(25)
    PRINT 11
 12 READ 1, DATE, N1, N2, (DAY(1), 1=1,25)
    IF(EOF,60)999,998
998 L1=N1-1
    SUM1=0.0
    DO 2 1=2,L1
    POINT1=DAY(I)-DAY(I-1)
    IF(POINT1)3,3,2
  3 SUM1=SUM1+POINT1
  2 CONTINUE
   L2=N2+1
    SUM2=0.0
    DO 4 1=L2,25
    POINT2=DAY(I)-DAY(I-1)
    IF (POINT2)5.5.4
  5 SUM2=SUM2+POINT2
  4 CONTINUE
    AREA=SUM1+SUM2
    POINTS=(L1-1)+(25-N2)
    AVENITE1=AREA/POINTS
    AVENITE=ABSF(AVENITE1)
    DO 6 1=N1.N2
    POINT3=DAY(I)-DAY(I=1)
    ADJDAY(I)=POINT3+AVENITE
  6 CONTINUE
    L3=N2-1
    SUM3=0.0
    DO 7 I=N1,L3
    IF(ADJDAY(I))7,8,8
  8 [F(ADJDAY([+1))7,9,9
  9 TRAPE=0.5+(ADJDAY(I)+ADJDAY(I+1))
    SUM3=SUM3+TRAPE
  7 CONTINUE
    TOTAL = AVENITE + 24.
    PRINT 10, DATE, AVENITE, SUM3, TOTAL
    GO TO 12
 1 FORMAT (A8,212,17F4,2/12X,8F4,2)
 10 FORMAT (1X, A8, 18X, F10, 5, 21X, F10, 5, 21X, F10, 5)
 11 FORMAT (1H1,2X, *DATE*,12X, *AVERAGE OXYGEN USED AT NIGHT*,10X, *PPM
   10xygen/Day+,10x, +TOTAL Oxygen used in 24 hours+/2x)
999 CONTINUE
   END
```

Sample Data Cards



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