THE EFFECTS OF LIMITING CONCENTRATIONS OF NITROGEN ON PRIMARY PRODUCTION IN AN ARTIFICIAL STREAM

Thesis for the Degree of M. S.

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Robert Mitchell Stokes

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THESIS

THE EFFECTS OF LIMITING CONCENTRATIONS OF NITROGEN ON PRIMARY PRODUCTION IN AN ARTIFICIAL STREAM

by

Robert Mitchell Stokes

AN ABSTRACT

Submitted to the College of Agriculture of Michigan
State University of Agriculture and Applied
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requirements for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1960

Approved: ______________________________

Robert Mitchell Stokes

ABSTRACT

The effects of growth-limiting nitrogen concentrations upon lotic primary production were studied under the controlled environment of an artificial stream. The stream water contained an excess of all major nutrients except nitrogen. Effects of variable water velocity (riffle and pool areas) and light intensity were also examined.

Three successive additions of this element (1 mg l^{-1} each) brought about the establishment of an attached algal community followed by increased levels of phytopigment, organic nitrogen, and total dry weight. The concentration of all major nutrients which were measured decreased with growth of periphyton. The reduction of each new supply of nitrogen was inversely proportional to a rise in cellular chlorophyll. After nitrate reduction the chlorophyll content decreased.

Correlation coefficients indicated that the relationship of pigment concentration to total dry weight and pigment to organic N. were high. The cellular nitrogen content approximated two percent on a dry weight basis.

The production of organic matter (measured as total dry weight), phytopigment, and organic nitrogen was significantly different between riffle and pool areas. Early in the project pool values were higher than riffle values. Later the converse of this situation occurred. Similar

fluctuations occurred in the percent of cellular nitrogen. This difference between areas could have been produced by three factors: variable incident radiation, variable water velocity, and competition.

The incident radiation on pool and riffle surfaces approximated daylight. This radiation along with limiting nitrogen concentration accounted for a primary production of 250.2 and 201.4 mg dry wt $m^{-2} day^{-1}$ in the riffle and pool respectively. In diminishing light intensities phytopigment production decreased, but even in this area production increased with nitrogen additions.

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This study was conducted under a graduate research assistantship from the National Institute of Health.

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INTRODUCTION

In recent years the rapid expansion of the human population has created many problems which affect our streams. One of the major problems results from a need for increased sport and food fish production while paradoxically an increase of pollution is unfavorably altering many of the present environments.

Many limnologists have looked to primary production, which has direct bearing on basic stream ecology, for the answer to this perplexity. Primary production is the rate at which organic matter is formed by photosynthetic and chemosynthetic activity of producers from basic raw materials (Odum 1953, Ruttner 1953). If the efficiency of this process can be increased and be applied in a practical and successful manner, a broader base would be provided for the production of consumers such as fish. Variation in primary production rates and the composition of the periphyton community may also provide a useful method of detecting sub-lethal and chronic pollution.

Keeping those problems in mind, an artificial stream was constructed in which communities of periphyton could be grown under controlled environmental conditions. The present study included investigations of community growth patterns under limiting concentrations of nitrogen with

variations of light intensity and current. All other physical and chemical conditions were held constant.

Although there have been numerous studies on the subjects of primary production, photosynthesis, and nutrient metabolism in both natural waters and laboratory vessel cultures, few have incorporated the use of an artificial stream. Odum and Hoskin (1957) studied the metabolism of algal communities under artificial stream conditions in a recirculating apparatus which consisted primarily of clear plastic tubing. Several other projects employing artificial streams are now being conducted throughout the country which indicates this approach is now receiving consideration.

This study was undertaken to determine the effects of limiting concentrations of nitrogen upon the growth patterns and composition of an artificial stream algal community under variable light intensity and current.

METHODS

Equipment

The recirculating mechanism employed for this project was designed with the following requirements in mind: simple, sturdy, light-weight construction; ample stream bottom for extensive sampling procedures; elimination of contamination; high degree of flexibility; an area for efficient bacterial decomposition; maintainence of desired fluid temperature; source of controlled light; and economy. This apparatus is illustrated in figures 1 and 2.

An aluminum trough 24 feet X 14 inches X 10 inches formed the stream bed. This length was a product of joining six sections each 4 feet in length. These were held together by small c-clamps. Flexibility of section construction allowed an 8 inch variation in depth at each junction. Pools or variations in gradient might then be formed as desired. Contamination from the aluminum was prevented by lining the stream with 4 mil, clear polyethylene sheets.

The trough was supported by a ¼ inch steel frame of channeling and bent pieces under each joint. Adjustments for the desired depth were located upon the steel structure. Coarse adjustment of 1 inch or more was made by

Figure 1. Photograph of Artificial Stream

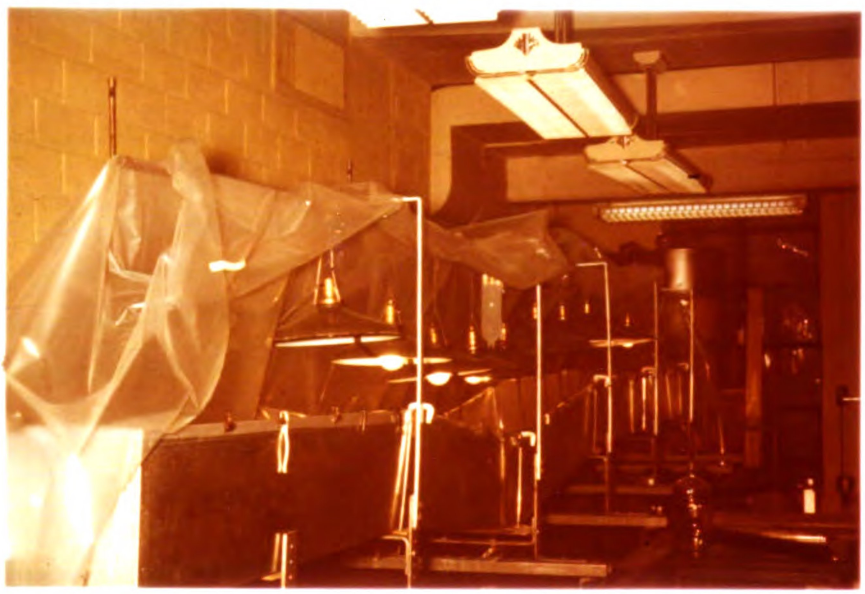
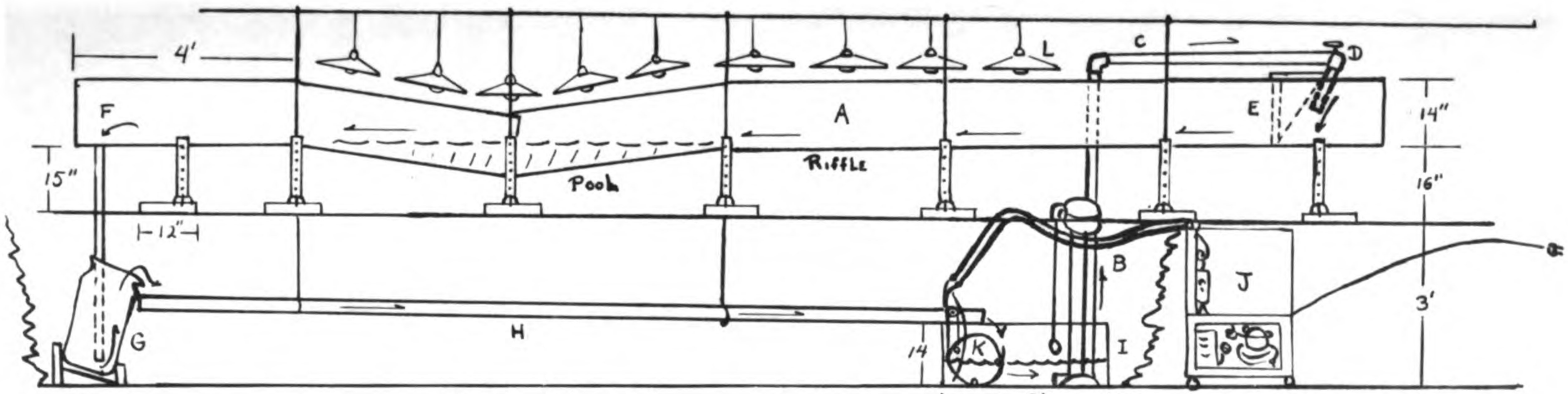
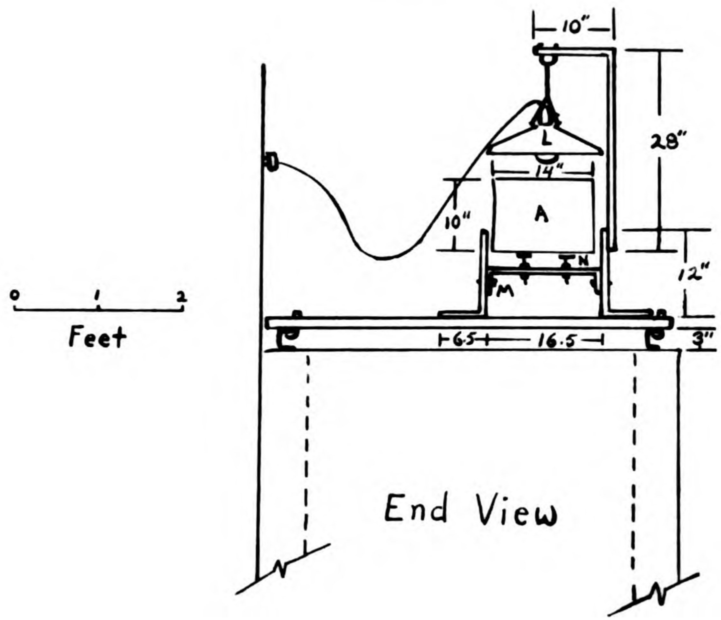


Figure 2. Diagram of Artificial Stream



Side View



End View

— LEGEND —

A. Stream Trough
B. Pump
C. Inlet Pipe
D. Inlet Valve
E. Baffle
F. Stream Outlet
G. Filter
H. Return
I. Reservoir
J. Cooler
K. Cooling Unit
L. Lights
M. Coarse Adjustment
N. Fine Adjustment

moving the horizontal cross beams supporting the stream joint. Fine adjustments, consisting of two adjacent bolts vertically movable within a 1.5 inch range, were located on each cross beam. Metal plates were welded to each bolt head for stream support.

Water was pumped into the stream from a reservoir located beneath the trough by a Model 259.481 Homart sump pump. The plumbing into the stream consisted of 1.5 inch polyethylene pipe containing a valve which regulated the flow.

A centrifugal pump best suited the requirements for recirculating a relatively high volume of 50 gallons per minute under low pressure and a 5 foot head. Any pump, classified as "noncontaminating" and meeting the requirements above, would have been expensive and difficult to obtain. The inexpensive sump pump used met all requirements except contamination, and this was reduced by using a bronze intake. A constant pump discharge was possible by adjustment of the inlet valve and maintainence of a relatively even water depth in the reservoir.

Originally a wooden baffle had been constructed to decrease turbulence of inflowing water. Since there might have been a possible release of harmful resins from the wood, this baffle was replaced by one of plexi-glass construction.

The stream outlet included a 1.5 inch sink fitting and

drain connected to 1.75 inch polyethylene tubing. Maximum filtering surface was obtained by entering this stream outlet into the bottom of a 10 gallon milk can filter. Upon entrance the solution upwelled through rocks, coarse gravel, and aquarium sand separated by layers of fiberglass. The liquid then spilled over the can lip into the reservoir return. This return consisted of galvanized eaves trough lined and covered with polyethylene. This filter was so designed because maximum drop from outlet to the reservoir was only 9 inches, and a trickle type might provide insufficient filtering depth.

The first operation of the filter proved ineffective in that the force of upwelling water was so great that amounts of sand from the filter were carried into the reservoir. This problem was finally solved by placing two layers of fiber glass screen over the filtering material surface. Stiff wire was used to hold the screen edges solidly against the can sides.

The inner surface of the filter was painted with Krylon plastic paint to prevent rust and iron contamination. Black paint was used to insure that all parts had received paint. This inert paint was highly insoluble in water and remained intact during the entire experiment. All filtering materials were soaked overnight in one percent hydrochloric acid solution and rinsed before use.

The reservoir was a 3 feet X 3 feet X 14 inches wooden

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box lined and covered with clear polyethylene sheeting. It had a capacity of 298 liters which was in excess of the circulating fluid.

Before each operation all polyethylene pipes were cleaned of all mineral and algal deposits by circulating a dilute formalin - HCl solution for a period of 24 hours. To control the corrosive action of HCl upon the brass pump intake the pH of the cleaning solution was kept within a range of 6 to 7.

The polyethylene sheeting seems to be the most versatile component of the stream. It was used to line the stream bed, reservoir, and return. Only the brass pump intake and valve could contribute heavy metal contamination. Also the lined parts need not be watertight. When an experiment has terminated, the polyethylene can be easily removed, discarded, and the system relined.

During preliminary operations of the stream it was noted that large amounts of water were being lost via evaporation. Since plans had been made to circulate distilled water, this loss, amounting to 38 liters per day, was important because the demand exceeded the water supply. The polyethylene covering of exposed surfaces reduced the loss to 4 liters per day. Excessive amounts of air contamination also were eliminated by this covering.

A cooler, containing a movable cooling unit of stainless steel coils, was designed and constructed by the

college for wide range temperature control. The cooling unit was immersed into the reservoir and only used in this project to hold temperatures constant at 70° ± 2°F. The 4° variability was due to the fact that the temperature control of the cooler operated within a 2.5°F range. Reservoir temperatures were recorded upon a Taylor Recording Thermometer.

Illumination for the stream was provided by a rack of nine 100 watt incandescent bulbs, each with a 14 inch shade reflector suspended 14 inches above the stream bottom. Although a great deal of heat was created by the incandescent bulbs, it was considered unimportant in this system provided with circulation and a cooler. To reduce the number of variables constant illumination was maintained.

The artificial stream was set up for experimentation in the following manner. It was adjusted to contain an 8 feet X 14 inches pool with a maximum depth of 6 inches. This pool was preceded by a riffle area 12 feet X 14 inches X one inch. The pump was valved to deliver a flow of 25 gallons per minute. This produced a velocity of approximately 1 foot per second in the riffle. The velocity in the pool was not measured with acceptable accuracy. Velocity was measured by a Micro Gurly Current Meter. The total stream fall from origin to outfall was set at 1 inch per 24 feet. This is the equivalent of a stream with a gradient of 18 feet per mile. This represents a fast-flowing

stream.

Nutrient Medium

A nutrient medium B with A_5 microelements was selected from several media employed by Kratz and Meyer (1955) in the culturing of blue-green algae (Table 1). Such a medium (plus initial elimination of nitrogen) with various minor modifications would insure the presence of excessive amounts of all major elements necessary for culture of algae except nitrogen. This view must be taken although excessive quantities might themselves be limiting to algal production.

The following modifications of medium B were made. Nitrogen sources, both KNO_3 and $Ca(NO_3)_2 \cdot 4H_2O$, were removed. Calcium nitrate was then added to the stream at regular two-week intervals. A total of three additions were made with each addition introducing one milligram per liter of nitrogen.

Silica, not included in the orginal medium, was added in the form of silica gel. Since the entrance and growth of any organism was of interest in this project, the silica inclusion provided a possible basis for establishment of diatom communities.

Ethylene diamine tetra-acetic acid (EDTA) was substituted for sodium citrate. Either of these chelating agents can be used to form soluble complexes with various insoluble,

TABLE 1

CONCENTRATIONS OF MAJOR SALTS (g l^{-1}), MAJOR ELEMENTS (mg l^{-1}), AND MICROELEMENTS IN NUTRIENT MEDIUM B CONTAINING A_5 MICROELEMENTS STOCK SOLUTION INCLUDING MODIFICATIONS*.

Salt	Concentration[1]	Ion	Concentration
$MgSO_4 \cdot 7H_2O$	0.250	Mg	24.3
KH_2PO_4	1.000	K	287.0
Na_2CO_3	0.700	Na	303.8+[3]
$Fe(SO_4)_3 \cdot 6H_2O$	0.004	Si	63.6
$Na_2O_3 \cdot SiO_2$*	0.350	Fe	0.4
EDTA*	0.050	P	228.0
Microelements A_5[2]	1.0 ml	CO_3	395.2
- - - - - - - -	- - -	SO_4	98.2

[1] Concentration in 1 liter distilled water

[2] Stock solution micronutrients (g l^{-1}):

H_3BO_3 - - - 2.86	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ - 0.18*
$MnCl_2 \cdot 4H_2O$ - 1.81	$CuSO_4$ - - - - - - - - 0.05*
$ZnSO_4 \cdot 7H_2O$ - 0.22	$Co(NO_3)_2 \cdot 6H_2O$ - - - 0.49*

[3] Sodium from sodium silicate not accounted for

thus unavailable, nutrients and maintain a precipitate-free alkaline medium (Kratz and Meyer 1955).

The micronutrient modifications included the substitutions of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ for 85% MoO_3 as a source of molybdenum, and anhydrous $CuSO_4$ for $CuSO_4\cdot 5H_2O$. The A_5 micronutrients were also supplemented with cobalt nitrate.

Calcium initially was absent from the medium except as an impurity by the exclusion of calcium nitrate; but each addition of the nitrate salt introduced elemental calcium in a molecular concentration higher than that of the nitrogen, i.e. 25 mgl^{-1} $Ca(NO_3)_2.4H_2O$ contained 4.2 and 3.0 mgl^{-1} of calcium and nitrogen respectively. In general, calcium is required in minor amounts by organisms. Most nutrient media contain only traces except when the nitrogen source is calcium nitrate; a few such as that used by Warburg and Burk (1950) contain none. Bold (1942) reports that calcium is unnecessary for certain algae such as Chlorella. Allison, Hoover, and Morris (1937) found calcium to be essential for nitrogen-fixation by Nostoc musorum but unimportant for growth. Media with higher magnesium content often reduces any requirement (Chu 1942). Still others found any limiting concentration to be far below that of nitrogen (Gerloff, Fitzgerald, Skoog 1950, Kratz and Meyer 1955).

All nutrients were placed into the stream channel source as a solution or suspension. This prevented settling out in the reservior and facilitated solution of undissolved.

salts.

Water Chemistry

A sampling program set up for this project included analysis of alkalinity, pH, and conductivity at weekly intervals and analysis of total available nitrogen, ammonia nitrogen, and total phosphorus at two-day intervals. Total available nitrogen was also determined immediately before and one hour after each addition of calcium nitrate. All samples were collected from the pool zone of the stream.

alkalinity

Phenolphthalein and methyl orange alkalinity were determined by titration methods described in Welch (1948). Results were expressed in milligrams per liter of calcium carbonate.

pH

Hydrogen ion concentration was determined on a Beckman Model H pH meter.

conductivity

Electrical resistance was measured with an Industrial Instrument Company Model RC-7 portable conductivity meter. All readings were corrected to 18°C and expressed in units of specific conductance as micromohs per centimeter (Industrial Instruments Operating Manual).

hardness

Values of hardness in milligrams per liter were

obtained by the versonate method (Catalog No. 4, Hach Chemical Company). Determinations were made only before EDTA had been added to the stream as EDTA was the titrant used in the versonate method.

silica

Three determinations of silica were made: at the beginning, middle, and end of the experiment. A gravimetric method taken from the Chemical Laboratory Manual of the American Cast Iron Pipe Company, Birmingham, Alabama, was employed. Results were expressed in milligrams per liter.

sodium and potassium

Concentrations of these cations in milligrams per liter were determined from samples removed for silica determinations. Values were obtained from a Perkin-Elmer Flame Photometer.

total phosphorus

Values of total phosphorus were resolved by a colorimetric method described by King (1932). A Beckman Model B Spectrophotometer at wavelength 860 mu was used in the procedure. Results were obtained in milligrams per liter.

total available nitrogen

Total available nitrogen included all inorganic forms except atmospheric nitrogen. Determinations were made by using the reduction method described in Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (APHA, AWWA, FSIWA, 1955). These determinations were made

immediately after collecting the sample to prevent loss of ammonia. All results are expressed in milligrams per liter.

ammonia nitrogen

Ammonia nitrogen in milligrams per liter was measured by the distillation method described in "Standard Methods" (APHA, AWWA, FSIWA, 1955). Determinations were begun on April 25.

Periphyton Analysis

sampling procedure

Artificial plexi-glass substrates 7 mm thick with an exposed area of 150 cm^2 were employed to sample the community of periphyton. These plates were held stationary in the stream by plastic coated wire racks.

A total of 54 shingles received use in this project (Fig. 3a). The riffle and pool areas each contained 24, and the remaining 6 were placed into an unlighted zone preceding the riffle. The 24 shingles per area were subdivided into 8 sets of 3 shingles each, 7 of which were incorporated into a 12-day overlap sampling system with one set remaining to be used as an extra.

The 12-day sampling system was devised to obtain an approach to the measurement of instantaneous primary production. To help visualize this procedure figure 3b is provided with the 5 two-week nitrogen periods labled. Each of the 7 shingle sets was exposed to two weeks of stream

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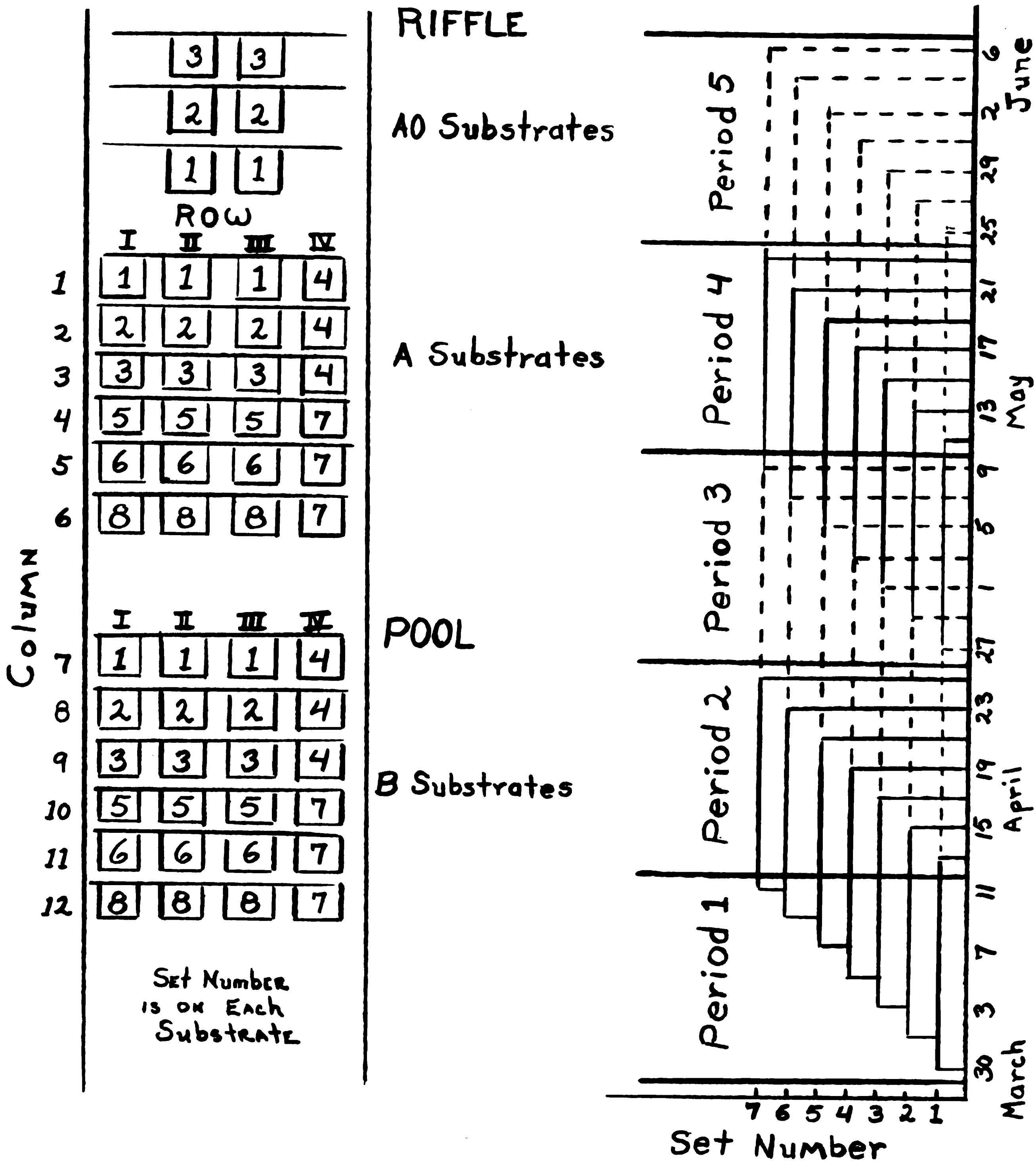
Figure 3. Diagrams of Substrate Arrangement in Artificial Stream (a) and Dates Sampled by Each Shingle Set (b)


RIFFLE
A0 Substrates
ROW
I II III IV
A Substrates
POOL
B Substrates
Column
1 2 3 4 5 6 7 8 9 10 11 12
Set Number is on Each Substrate
Period 1
Period 2
Period 3
Period 4
Period 5
March 30 3 7 11 15 19 23 27
April
May
1 5 9 13 17 21 25 29
June
2 6
7 6 5 4 3 2 1
Set Number

a. b.

conditions, but overlapped in a manner which allowed each set to sample two days longer than the preceding set. Before the first addition of nitrogen on April 12, two weeks were required to set up the operation; set one being added 13 days before adding nitrogen; set two 11 days before; ... set seven one day before. Therefore, set one was removed after one day of exposure to nitrogen, set two after three days, ... and set seven after 13 days. This process was kept in motion throughout the project by removal and replacement of the designated set of shingles every two days from both riffle and pool zones. All light shingles were collected after exposure to periods 2 through 5.

After the substrate set had been removed from the stream, shingles I and II were analyzed for phytopigment concentration and organic nitrogen content. Each shingle was divided laterally with organic nitrogen and chlorophyll being determined from the upstream and downstream halves respectively. Shingle III was reserved as an extra.

<u>phytopigment</u>

The growth on the entire surface including bottom and sides was removed to obtain maximum material in periods of low production. This material was scraped and washed with 95 percent ethanol into 250 ml beakers. Remaining shingle halves were stored in the freezer. Complete chlorophyll extraction was insured by soaking the material for a period exceeding 24 hours in complete darkness. Tests indicate

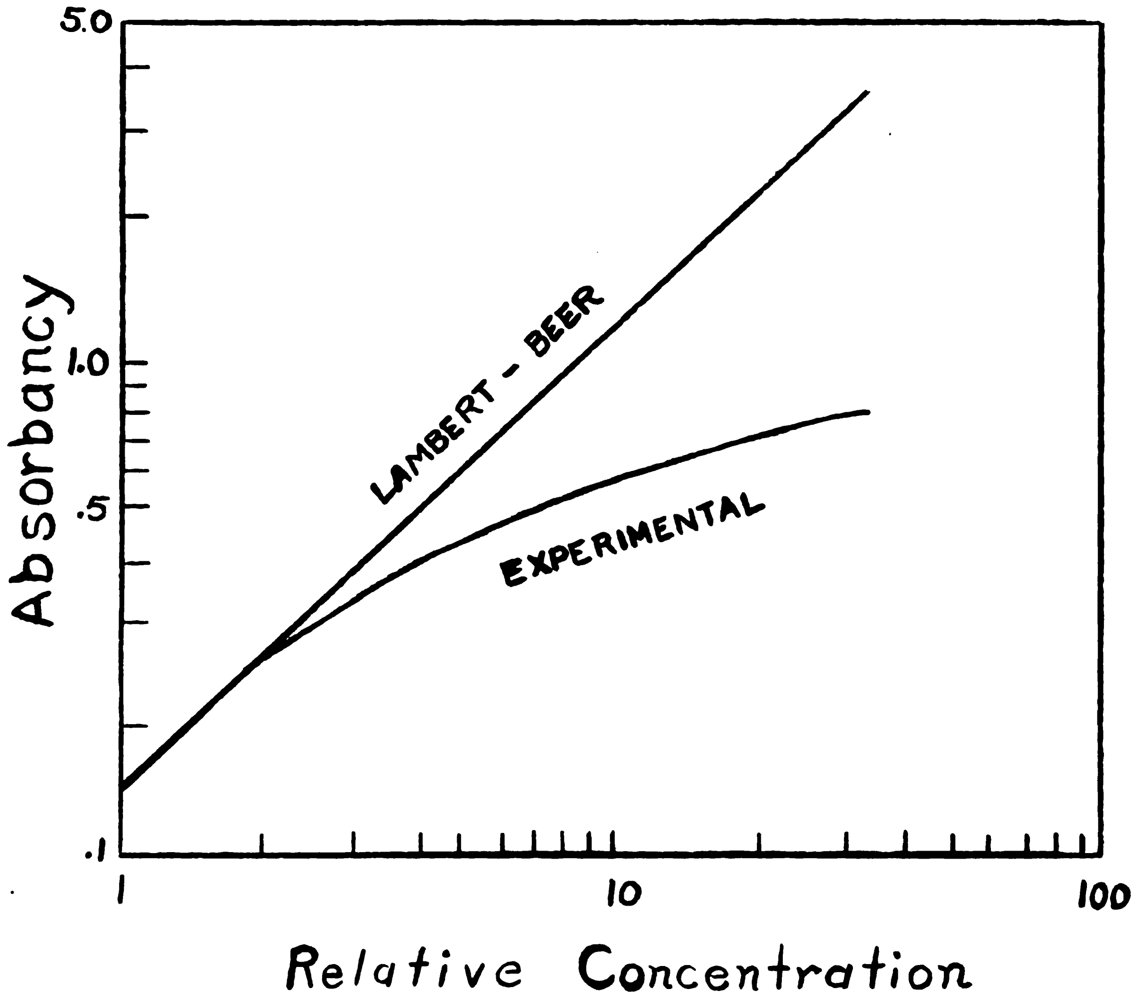
that samples can be stored in this manner for as long as 30 days without a loss of phytopigment due to decomposition (Brehmer, PhD Thesis). Alchol was used as a solvent in preference to acetone since the latter dissolves plexiglass.

After soaking, the samples were filtered through glass wool, and the extract volume was adjusted to 50 ml by dilution or evaporation. Phytopigment concentration was determined in a Klett-Summerson colorimeter using a 640-700 mu red filter.

Brehmer and Grzenda (in press) have shown that the absorbancy (640-700 mu) of 95 percent ethanol pigment extracts is not linerally related to the concentration except at low values. Hence, they have provided a graph (Fig. 4) for converting the measured absorbancy into the theoretical absorbancy which follows the Lambert-Beer Law. The necessary corrections were made by locating measured absorbancy on the ordinate and following this value to a point of interception on the experimental line. A vertical extension of this point will intercept the Lambert-Beer line, and the absorbancy unit directly opposite this intercept represents the corrected absorbancy reading. The unit of adjusted absorbancy is termed (AA) and is multiplied by 10^3 to avoid use of the decimal.

Shingles in the area of diminished light were measured only for their chlorophyll content. For comparison purposes

Figure 4. Correction Graph for Adjusting Measured Phytopigment Absorbancy Values to Units Related to Concentration



5.0
1.0
.5
.1
1
10
100
Absorbancy
Relative Concentration
LAMBERT - BEER
EXPERIMENTAL

half of each shingle was analyzed.

organic nitrogen

The remaining shingle halves, which had been frozen after chlorophyll determinations, were removed, thawed, scraped, and the aufwuchs was washed into 250 ml beakers with distilled water. Each was analyzed by the semi-micro Kjeldahl procedure described in "Standard Methods" (APHA, AWWA, FSIWA 1955). Results are expressed in milligrams organic nitrogen per 75 cm^2 (area of half shingle).

total dry weight

An estimate of the total dry weight of periphyton which accumulated within two weeks was obtained by removing the growth from a 37.5 cm^2 section of shingle III. These determinations were made after completion of the project and only certain shingles were available at that time.

The chlorophyll was extracted upon removal as described in the section on Phytopigment, except that gooch crucibles were used to collect the algal residue. This was necessary for weight analysis. The residue was dried overnight in an oven at 55°C then placed into a dessicator to cool. Successive weight measurements were conducted upon an analytical balance until a constant weight of ± .5 mg was reached.

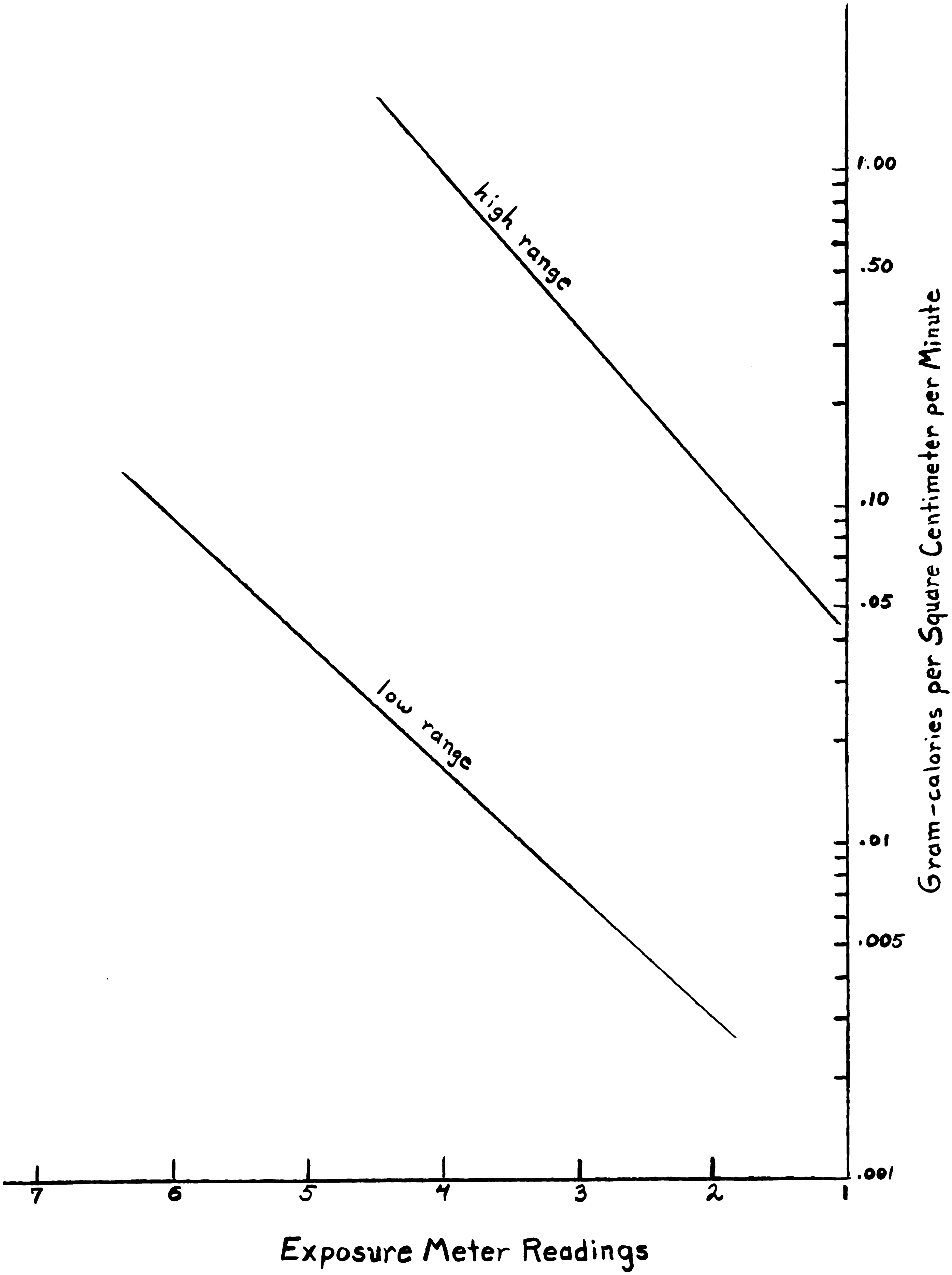
The total dry weight values will tend to underestimate the actual values since chlorophyll and lipids were removed with the filtrate.

Light

Measurements of light intensity were obtained by using a PR-1 General Electric Exposure Meter. This instrument was calibrated by direct comparison with an Eppley pyrheliometer maintained at the Michigan Hydrologic Research Station on the Michigan State University campus. The calibration was conducted on a clear summer afternoon from 1:00 PM until sundown. Light meter readings were taken at 15 minute intervals.

The intensities recorded by the pyrheliometer were converted to gram-calories per square centimeter per minute by dividing the direct measurement which was recorded in millivolts by a constant, 1.71. A straight line relationship was obtained by plotting the exposure meter readings against gm-cal $cm^{-2} min^{-1}$ on semi-log scale. The line of best fit was adjusted by eye and extrapolated to give an estimate of the energy received at lower light intensities. Two lines are indicated which represent the adjustment of the exposure to read at high and low light intensities.

Figure 5. Correction Graph for Converting Exposure Meter Readings to Gram-calories per Square Centimeter per Minute



high range
low range
1.00
.50
.10
.05
.01
.005
.001
Gram-calories per Square Centimeter per Minute
7
6
5
4
3
2
1
Exposure Meter Readings

RESULTS

Species Composition

The first attempt to establish an algal community in the artificial stream was successful using tap water enriched with small amounts of water from both the Red Cedar River and the fish tanks in the laboratory. A few stones from the Red Cedar, well encrusted with aufwuchs, were introduced to seed the system. Diatoms were the dominant organism in this material with Navicula and Gomphonema as the major species.

The stream was in continuous operation two weeks before new growth appeared on the seed rocks and stream bottom. This growth, primarily Chroococcus, first appeared in the pool.

During the third week a commercial fertilizer rated 17-17-17 (NH_3-P_2O_5-K) was added to the reservoir. Within three days a dense algal bloom of Chlorella and Navicula had begun. Navicula seemed to dominate the riffle area while Chlorella was more abundant in the pool. This phase of the project indicated that a reproducing algal community could be established under atypical lotic conditions.

On March 28 the quantitative experimental program described in "Methods" was begun. Seed material scraped from Red Cedar River stones was added on March 29.

The first green cells appeared in the pool on April 15, three days after the first addition of nitrogen. No growth was noted in period 1. The pioneer community was essentially composed of diatoms although representatives of green and blue-green algae were present.

From April 15 to 25 unidentified unicellular blue-greens and diatoms dominated the pool area while a lesser population of diatoms existed in the riffle. The diatom Navicula was most abundant. Filaments of Stigeoclonium and Ulothrix also entered both zones about April 19 and remained until early May.

After April 25 a major community change took place as colonies of Anabaena oscillarioides (identified by Dr. Francis Drouet) appeared. Thereafter this species of blue-green algae completely dominated the habitat, and eventually it formed a spongy mat of cells about one-fourth of an inch thick. By June 6 the mat had begun to break loose from the stream bottom. This was most likely due to death of the cells adjacent to the bottom and formation of gas bubbles under the material. Moreover, the community appeared to be senile; but production, described in later sections, was still great and was visually evident by the repopulation of areas left bare after sloughing.

Initially it seemed that Anabaena succession had eliminated other organisms, but careful examination found a large community of Navicula living within the blue-green mat.

Other species of algae such as Stichococcus bacillaris and Schizochalamys delicatula were minor constituients of the community for a short time after April 25. About June 12 filaments of Stigeoclonium again entered the area at the pool head.

Throughout most of the experiment there was little evidence of an invertebrate community. However, toward the end a population of midges was beginning to develop.

Water Chemistry

alkalinity

Weekly determinations indicated that high levels of both phenolphthalein and methyl orange alkalinities were maintained throughout the experiment (Fig. 6). Initial high values were expected since the proportions of monobasic potassium phosphate and sodium carbonated used buffered medium B in the alkaline range (Kratz and Meyer 1955).

It was also calculated that approximately 400 mgl^{-1} of carbonate ion would exist in the system upon complete solution of sodium carbonate (Table 1). Conversion of this ion to bicarbonate would be enhanced by exposure of carbonate ion to carbon dioxide in the air resulting from the turbulent conditions and shallow depths of this stream.

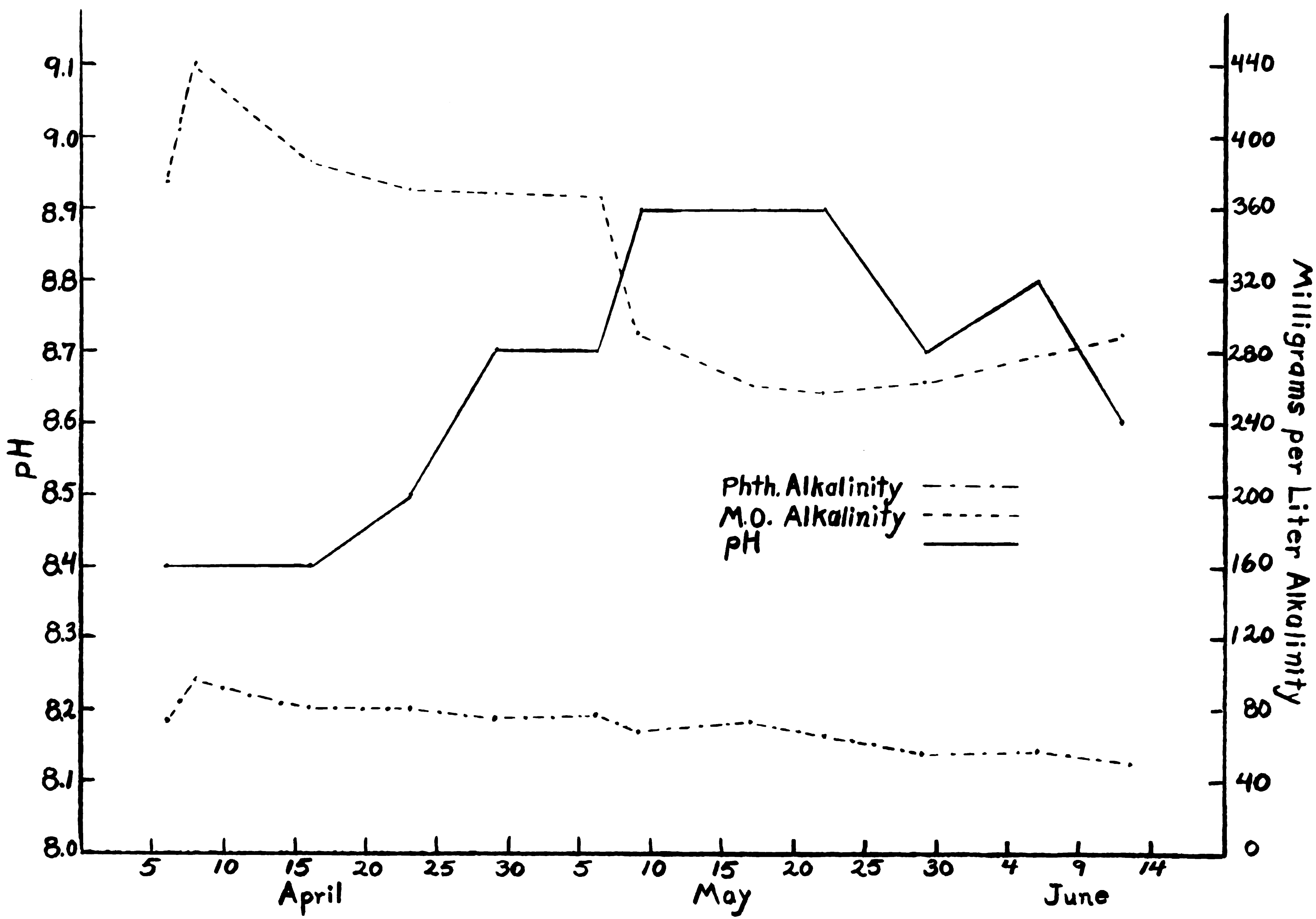
With the accrual of algal cells the concentrations of 440 mgl^{-1} bicarbonate ion and 96 mgl^{-1} monocarbonate ion were reduced to lows of 257 and 50 mgl^{-1}, respectively. A gradual

TABLE 2

WATER CHEMISTRY

Date	pH	Alkalinity (mg 1) Phth.	M.O.	Resistance (ohms)	Conductivity (micromhos)
4-6	8.4	75	375	1150	1610
4-8	8.4	96	440	- - -	- - -
4-16	8.4	80	386	1300	1425
4-23	8.5	80	370	1320	1403
4-29	8.7	75	368	1380	1342
5-6	8.7	77	366	1280	1447
5-9	8.9	68	290	1630	1136
5-17	8.9	72	261	1670	1109
5-22	8.9	66	257	1950	950
5-29	8.7	56	264	2000	926
6-6	8.8	58	278	1950	950
6-12	8.6	50	290	- - -	- - -

Figure 6. Alkalinity and pH in Artificial Stream Water


pH
9.1
9.0
8.9
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8.0
Milligrams per Liter Alkalinity
440
400
360
320
280
240
200
160
120
80
40
0
5 10 15 20 25 30 5 10 15 20 25 30 4 9 14
April
May
June
Phth. Alkalinity
M.O. Alkalinity
pH

rise in bicarbonate which occurred after May 23 plus continued monocarbonate reduction might indicate that the latter was being converted to the former by presence of increased carbon dioxide from organic decomposition.

pH

The hydrogen ion concentration increased from a value of 8.4 at the beginning of the project to 8.9, which remained for some time after the third addition of nitrogen. A decrease again occurred toward the project termination (Fig. 6).

The slight fluctuation in pH seemed to follow production levels and possible nitrate assimilation. On May 29 production dropped in both riffle and pool areas after a general increase prior to this date (Fig. 11). A production rise again occurred after this date to correspond with pH rise. In certain vessel cultures the assimilation of nitrate by growing cells was accompanied by an increase in pH (Rodhe 1948, Kratz and Meyer 1955).

It is evident from figure 6 that pH was inversely related to the bicarbonate alkalinity. This effect could be a product of many factors since alkalinity results from the solution of Na_2CO_3, and pH results from solution of both buffer salts plus the presence of sodium silicate which was not included in the medium used by Kratz and Meyer. Sodium silicate and sodium carbonate are often combined to maintain an alkaline buffering capacity (Gerloff, Fitzgerald, Skoog 1950).

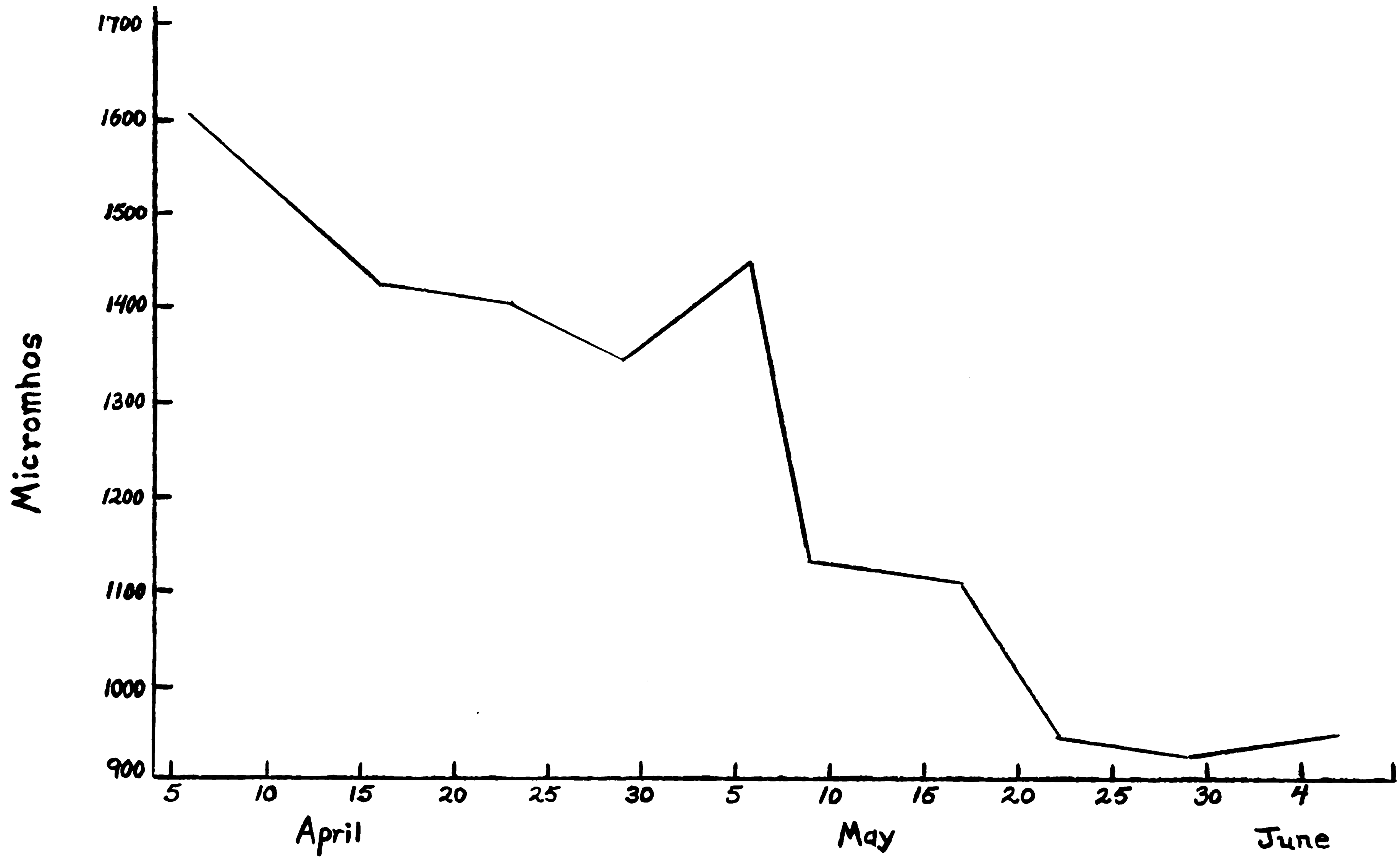
The pH and alkalinity, no doubt, have played an important role in determining the presence of Anabaena and Navicula. Using a modified Chu 10 medium Gerloff, Fitzgerald, and Skoog (1952) noted optimum growth of Microsystis aeruginosa was at pH 10. Cells of Anabaena variabilis experienced maximum growth at pH 6.9 to 9.0 (Kratz and Meyer 1955). Bold (1942) cites Gietler as finding it necessary to grow Navicula on agar of pH 9 in order to secure formation of auxospores.

conductivity

Values of specific conductance, plotted in figure 7, implied that a constant decrease in total ionized constituents in the water took place. As expected, diminution followed aufwuchs increase. It is interesting to note that conductivity variations followed closely to those of bicarbonate alkalinity.

The alterations in alkalinity, pH, conductivity, and other specific ion concentrations were in part due to an irreparable leak which developed at the filter lip on or about May 4. The exact date it began was unknown but was estimated from observations of standing water under the filter. Corrections for this loss were made by collecting and measuring the liquid from the leak for a period of three weeks. The determined average leakage rate indicated that approximately 38 liters of solution would have been

Figure 7. Specific Conductance of Artificial Stream Water



Micromhos
1700
1600
1500
1400
1300
1200
1100
1000
900
5
10
15
20
25
30
5
10
15
20
25
30
4
April
May
June

lost in 20 days--a length of time known to exceed the actual days of leakage.

hardness

Calcium hardness (EDTA) was determined on April 8 to confirm the fact that alkalinity was not due to calcium carbonate. Results indicated that calcium was present in amounts too small to be detected. On April 13 total hardness (EDTA) was measured twice and found to be 68 mgl^{-1}. Since the total hardness is lower than the alkalinity, it appeared that most hardness was due to magnesium carbonate. The calcium hardness was rechecked on April 13, but results remained negative.

total phosphorus

The phosphorus levels were extremely high throughout the experiment, ranging from 100 to 170 mgl^{-1}, with only one exception which occurred on April 15 (Fig. 8). An even greater concentration of phosphorus would have occurred if complete solution of KH_2PO_4 had taken place (Table 1). Therefore, the gradual rise from the minimal to maximal concentration from April 11 to 21 probably was due to increased disintegration and mixing of the undissolved salt.

After April 21 it appeared that plant production began gradually to reduce the phosphate level to a low on May 25. Throughout this reduction several pulses of increase were observed. These pulses might stem from liquification of undissolved phosphate salt or regeneration and recycling of

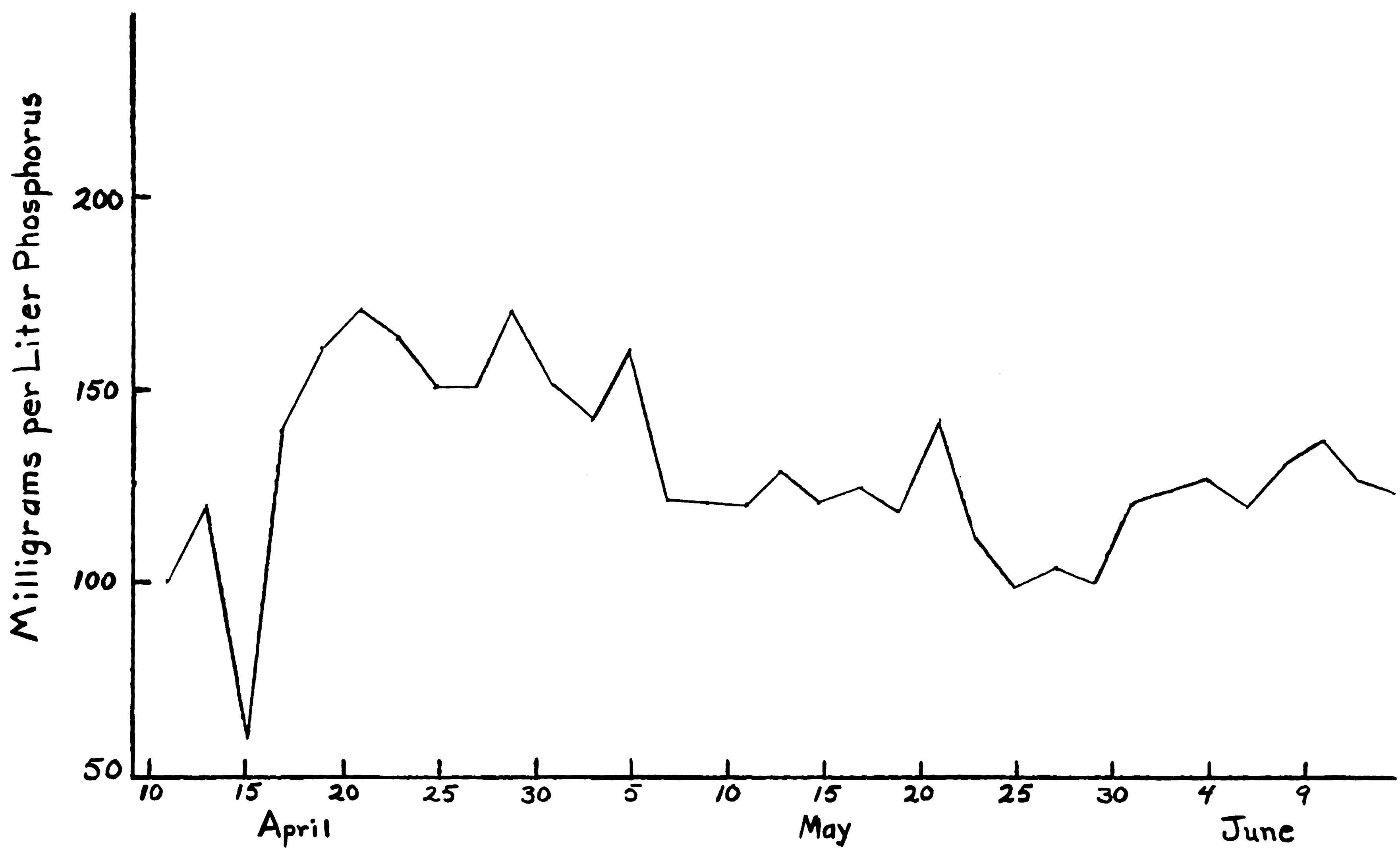
TABLE 3

NITROGEN AND PHOSPHORUS DETERMINATIONS
IN MILLIGRAMS PER LITER

Date	Total Available Nitrogen	Ammonia	Total Phosphorus
4-10	.043	- - -	- - -
4-12	.130*	- - -	100
	.340°	- - -	- - -
4-13	.310	- - -	120
4-15	.370	- - -	60
4-17	.570	- - -	140
4-19	.260	- - -	160
4-21	.015	- - -	170
4-23	.090	- - -	164
4-25	.040	.000	150
4-26	.080*	- - -	- - -
	.250°	- - -	- - -
4-27	.420	.000	150
4-29	.085	.000	170
5-1	.090	.000	150
5-3	.140	.015	140
5-5	.330	.060	160
5-6	.090	- - -	- - -
5-7	.105	.025	120
5-9	.150	.005	120
5-10	.210*	- - -	- - -
	.970°	- - -	- - -
5-11	.180	.025	120
5-13	.120	.010	130
5-15	.120	.120	120
5-17	.150	.000	125
5-19	.160	.020	118
5-21	.110	.015	142
5-23	.105	.000	110
5-25	.150	.000	100
5-27	.100	.050	104
5-29	.140	.000	100
5-31	.175	.000	120
6-2	.110	.000	122
6-4	.105	.085	126
6-6	.070	.000	120
6-8	.300	.090	130
6-10	.185	.030	136
6-12	.110	.080	126
6-14	.110	.060	124

* Sample taken immediately before nitrogen addition
° Sample taken one hour after nitrogen addition

Figure 8. Total Phosphorus in Artificial Stream Water


Milligrams per Liter Phosphorus
200
150
100
50
10
15
20
25
30
5
10
15
20
25
30
4
9
April
May
June

this nutrient in the plant material.

Total phosphorus levels began to rise again after May 25. This seemed to coincide with the sloughing of large algal fragments from the aged mat. If bacterial decomposition took place in the filter, phosphorus in organic form would be recirculated through the system.

Losses of phosphorus due to leakage were estimated to be approximately 16 mgl^{-1}.

As was mentioned earlier, certain elements in the artificial stream might be detrimental to growth as a result of their enormous concentrations. Phosphorus is one such element; and since it plays a vital role in plant nutrition, the large stream supply needs consideration. Chu (1942) noted that strong phosphorus concentrations inhibited growth of certain diatoms and green algae, but it varied with the species. Later he found less inhibition when nitrate-nitrogen was used as a nitrogen supply (Chu 1943). Osterlind (1947) mentions that use of phosphate buffers in high concentrations are often injurious to algae. In direct conflict with these findings Kratz and Meyer (1955) varied K_2HPO_4 from .25 to 1.5 gl^{-1} without effect on growth of Anabaena, Anacystis, and Nostoc. Other nutrient media used by Ketchum, Lillick, and Redfield (1949); Warburg and Burk (1950); and Harris (1941) to grow many algal species also contained phosphate in similar or even higher amounts than those occurring in the artificial stream. Views on this subject conflict, but it seems much depends on tolerance ranges of the particular algae species and the phosphate concentration range with which one works.

silica

The depletion of silica was of interest since the ubiquitous diatoms remained throughout this study. Data from three determinations (Table 4) indicated that only about one half of the added silica gel went into solution (Table 1). This provided an initial concentration of 34 mg l^{-1}, which according to Krauss (1958) is about optimum for dense cultures of Navicula. Certain greens and diatoms exhibit an optimum growth in nutrient solutions containing 30 mg l^{-1} of silicon dioxide (Chu 1942).

The concentration of silica was reduced 6 mg l^{-1} during the experimental period, although leakage accounted for about two-thirds of this loss. Moreover, the probable solution of salts, yet undissolved, complicated the picture.

potassium and sodium

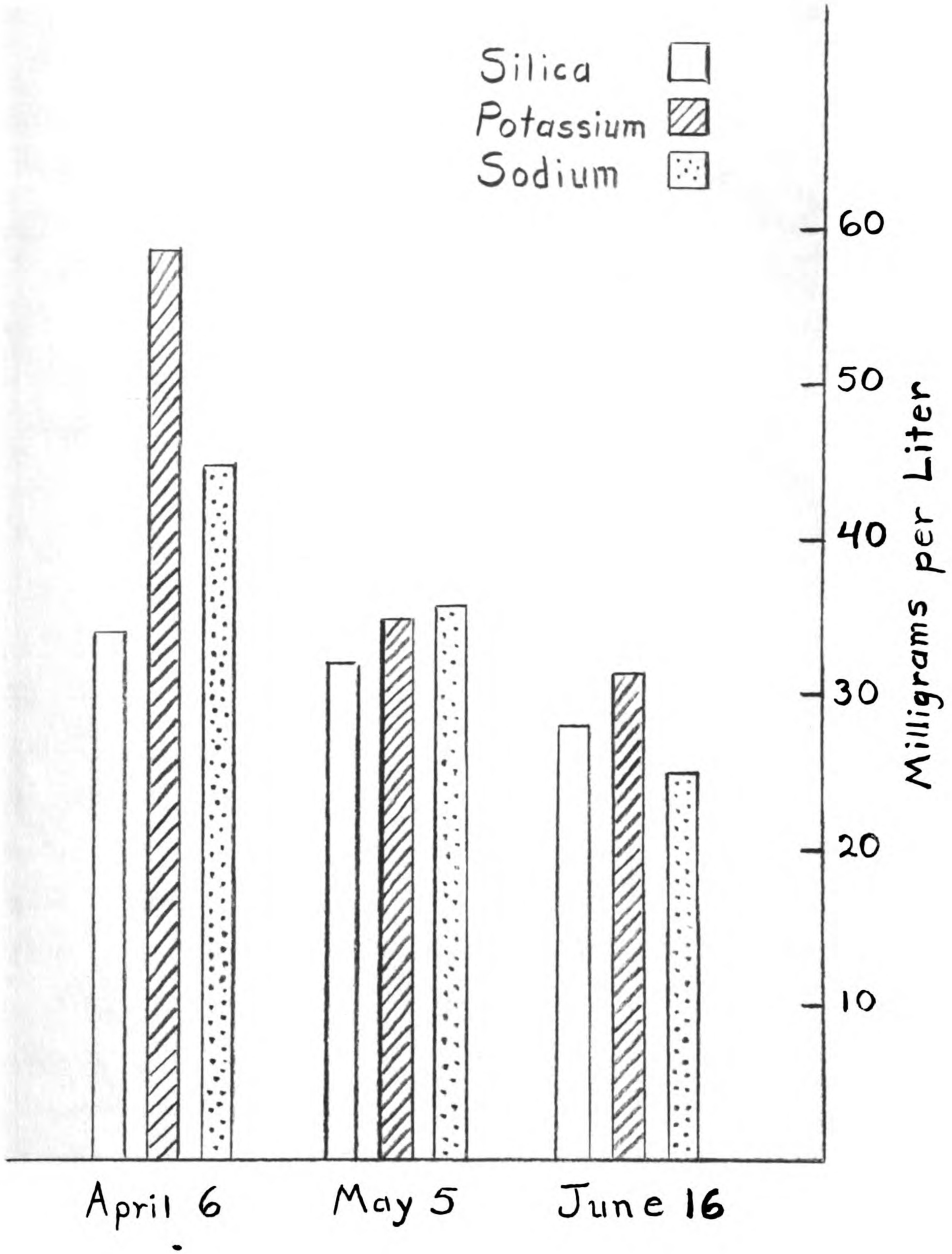
The relative concentrations of 58.7 mg K l^{-1} and 44.8 mg Na l^{-1} gave evidence that monobasic potassium phosphate was more soluble than sodium carbonate at the beginning of the experiment (Table 4). In table 1 it should be noted that complete solution of the two salts would insure a high pH. In view of this, pH should have been much lower than it actually was. The actual high pH value was probably a product of sodium silicate.

TABLE 4

POTASSIUM, SODIUM, AND SILICA ION CONCENTRATIONS AND CORRECTED TOTAL REDUCTION OF EACH IN MILLIGRAMS PER LITER

Date	Potassium	Sodium	Silica
4-6	58.3	45.0	- - -
4-6	58.8	44.5	34.0
5-5	35.0	35.8	32.0
6-16	31.3	25.0	28.0
Concentration Reduction	27.2	19.8	6.0
Leakage Loss	4.75	4.9	4.3
Corrected Reduction	22.45	14.9	1.7

Figure 9. Potassium, Sodium, and Silica Ion Concentrations of the Artificial Stream

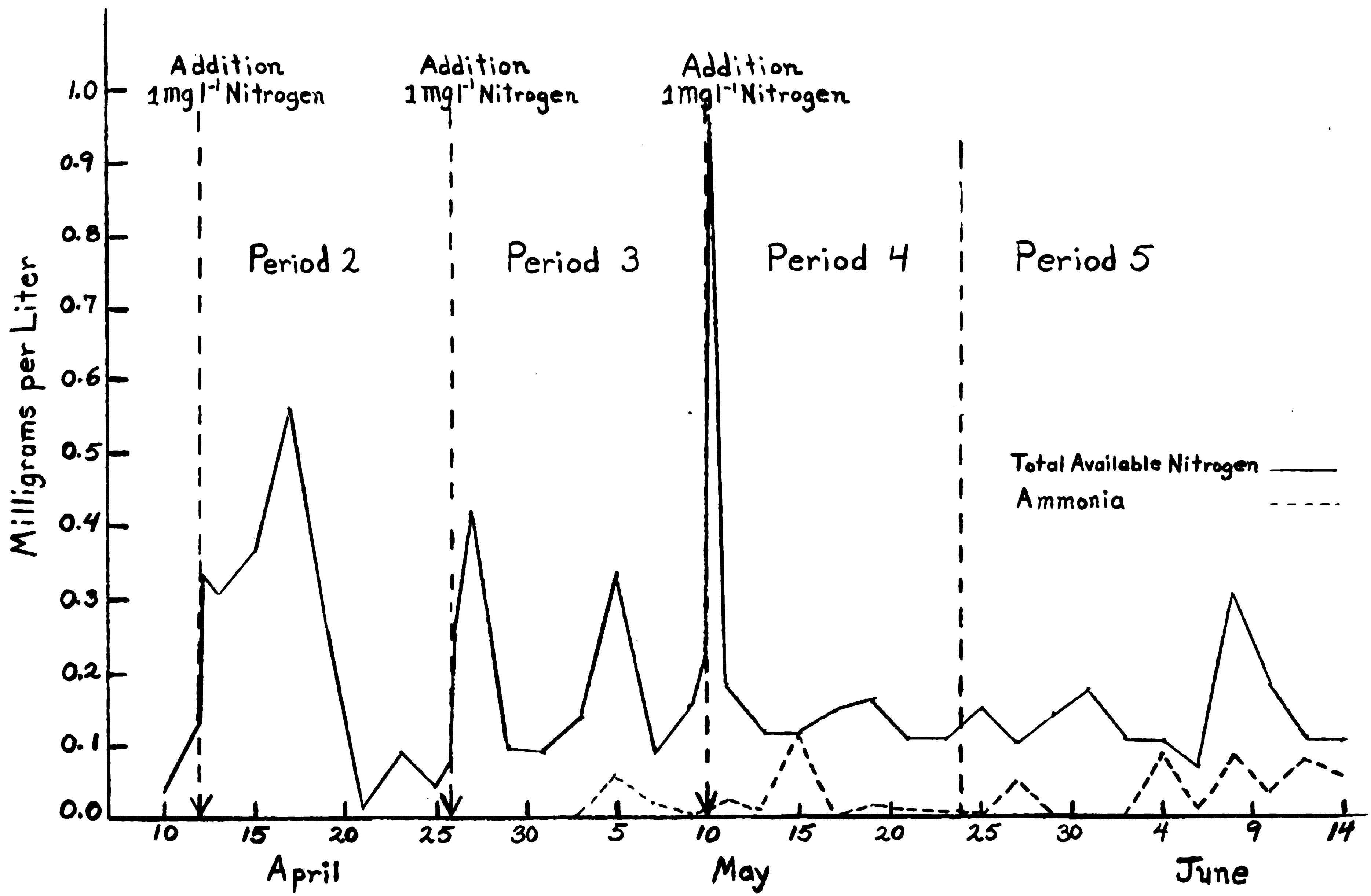

Silica
Potassium
Sodium
60
50
40
30
20
10
Milligrams per Liter
April 6
May 5
June 16

As plant growth increased both potassium and sodium were significantly reduced (Fig. 9). When compared to silica their respective losses due to leakage were minor. The total corrected reduction of potassium was the largest, but analysis indicated that sodium depletion was relatively greater when the community was dominated by Anabaena oscillarioides. Potassium ion is found almost universally as the principle inorganic cation of cells, whereas the sodium ion is known to be dispensable for most plants with the exception of blue-green algae (Fruton and Simmonds 1959). It is necessary to point out that few conclusions can be drawn about these ionic reductions when quantities of undissolved salts in the system provided a continuous source for nutrient replenishment.

total available nitrogen

The concentrations of total available nitrogen in the artificial stream are illustrated in figure 10. Successive calcium nitrate additions, each introducing 1 mg l^{-1} of nitrogen, caused an immediate rise in the total available nitrogen. These high initial levels were significantly reduced by the growing algal community. The algal production peaks in figure 11 followed each initial peak of inorganic nitrogen. It also should be noted that the fall of initial levels was more rapid as the standing crop developed. A total of nine days passed before the April 12 nitrogen supply fell to trace amounts (April 21), whereas the

Figure 10. Total Available Nitrogen and Ammonia Nitrogen in the Artificial Stream


Addition
1 mg l⁻¹ Nitrogen
Addition
1 mg l⁻¹ Nitrogen
Addition
1 mg l⁻¹ Nitrogen
Period 2
Period 3
Period 4
Period 5
Milligrams per Liter
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10
15
20
25
30
5
10
15
20
25
30
4
9
14
April
May
June
Total Available Nitrogen
Ammonia

period 4 supplement on May 10 dropped from .97 mg l^{-1} to .18 mg l^{-1} in one day. A strict two day sampling would have missed this rise and fall.

After the new supplies of nitrogen had been depleted the "normal" stream concentration remained in the vicinity of .1 mg l^{-1}. In periods 4 and 5 this is particularly noticeable. Many authors working on the subject of nitrogen as a limiting factor and using many varieties of algae found that the lower limit of this element for optimum growth occurs well above the value of .1 mg l^{-1} (Gerloff, Fitzgerald, and Skoog 1950; Rodhe 1948; Chu 1943). Moreover, Brehmer (PhD thesis) has interpreted nitrogen to be the limiting factor of the Red Cedar River where mean values of inorganic nitrogen are .7 mg l^{-1} above the sewage outfall. Riley (1940) also indicated that the plankton of Linsley Pond becomes dominated by diatoms and blue-green algae in the summer months when the nitrate ranges between .01 and .04 mg l^{-1}. These populations and concentrations compare somewhat to those of the artificial stream. In conjunction with low levels of nitrogen the presence of Anabaena and diatoms in the artificial stream gives further evidence that nitrogen was the limiting factor.

It was noted earlier that the salts of the nutrient medium did not dissolve completely. Calcium nitrate was no exception to the rule. Less than 35 percent of the period 2 addition went into solution; and when algal growth began to affect a reduction on April 17, only 55 percent had been dissolved. Since these undissolved salts might always be present, the nutrient could be immediately assimilated upon release into solution. The solution of undissolved salts plus organic breakdown, nitrogen fixation, and release from living plants might also account for the fact that some nitrogen was present at all times in the stream.

<u>free ammonia</u>

Free ammonia determinations were begun after some growth had accumulated in hopes that pulses of this product would be indicative of organic decomposition by heterotrophic bacteria. The values ranged between .12 and 0 milligrams per liter for the entire period (Fig. 10), and it appeared there might be a tendency for the concentration to rise toward the end of the experiment. Plant material was breaking loose and being washed into the filter at this time.

Periphyton Analysis

phytopigment

Relative rates of primary production were obtained by comparison of the phytopigment concentration per half shingle. The mean phytopigment units are plotted versus time in figure 11.

The first detectable shingle growth occurred six days after the first nitrogen had been added. No growth was noted in period 1. In view of these facts the introduction of nitrogen became a mechanism for triggering the reproduction of cells which had lain dormant for almost three weeks.

Figures 11 and 12 illustrate that successive additions of calcium nitrate brought about an increase in the relative production rates in all lighted areas of the artificial stream. Within periods 2, 3, and 4 pigment concentration dropped after the assimilation of new nitrate (Fig. 10). This drop might be an actual decrease of pigment. Yentsch and Vaccaro (1958) report that nitrogen deficiency produces a decrease in chlorophyll which may be attributed to the decomposition of the pigment protein complex.

Another possible reason for reduction of chlorophyll might merely be a result of the physical condition of the shingle. For example, the surface of a two-week substrate removed on April 19 could have been altered by the environment to accept adhering cells. Cells throughout the stream at this time would be entering a period of rapid division,

TABLE 5

RIFFLE PHYTOPIGMENT ABSORBANCY PER UNIT AREA (75 CM^2)
AFTER TWO WEEKS EXPOSURE

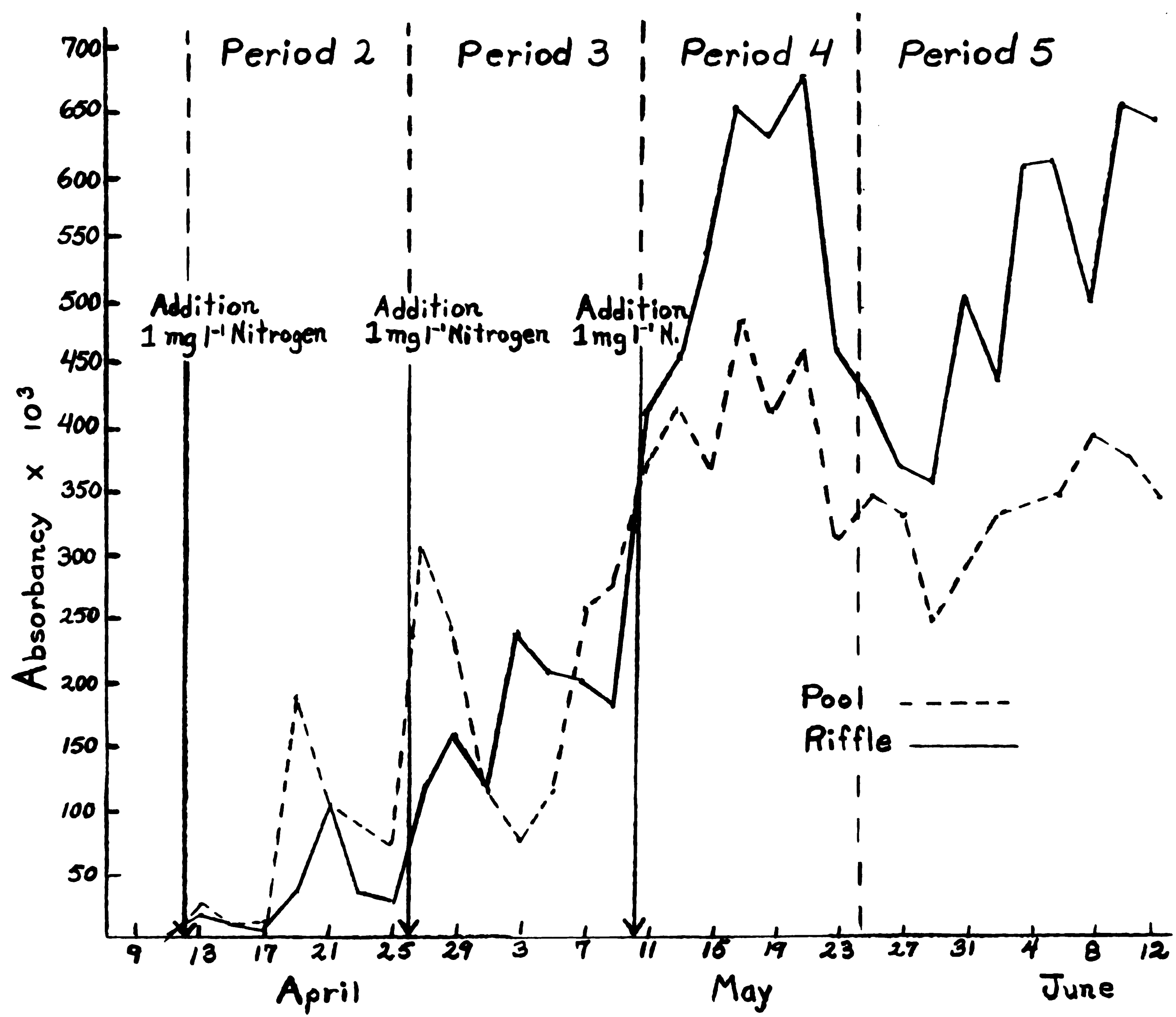
Date	AAX10^3	Mean	Var.	Sta. Dev.	Date	AAX10^3	Mean	Var.	Sta. Dev.	Date	AAX10^3	Mean	Var.	Sta. Dev.
4-10	0				5-1	115				5-23	415			
	0	0	0	0		118	117	4	2.0		500	458	3612	60.1
4-12	0				5-3	360				5-25	484			
	0	0	0	0		120	240	28800	169.7		356	420	2592	50.9
4-13	12				5-5	226				5-27	368			
	25	19	84	9.2		188	207	722	26.9		364	366	8	2.8
4-15	12				5-7	184				5-29	372			
	8	10	8	2.8		220	202	648	25.5		331	352	840	29.0
4-17	4				5-9	204				5-31	409			
	6	5	2	1.4		158	181	1058	32.6		585	497	15488	124.5
4-19	44				5-11	403				6-2	435			
	23	34	220	14.8		412	408	40	6.3		435	435	0	0
4-21	98				5-13	403				6-4	670			
	96	97	2	1.4		504	454	5600	74.8		532	601	9522	97.6
4-23	36				5-15	516				6-6	610			
	38	37	2	1.4		556	536	800	28.3		600	605	50	7.1
4-25	16				5-17	630				6-8	475			
	40	28	292	17.1		670	650	800	28.3		508	492	544	23.3
4-27	120				5-19	610				6-10	680			
	108	114	72	8.5		640	625	450	21.2		620	650	1800	42.4
4-29	160				5-21	696				6-12	580			
	160	160	0	0		640	668	1568	39.6		696	638	6728	82.0

TABLE 6

POOL PHYTOPIGMENT ABSORBANCY PER UNIT AREA
AFTER TWO WEEKS EXPOSURE

Date	AAX10^3	Mean	Var.	Sta. Dev.	Date	AAX10^3	Mean	Var.	Sta. Dev.	Date	AAX10^3	Mean	Var.	Sta. Dev.
4-10	0				5-1	91				5-23	251			
	4	2	8	2.8		132	112	1040	32.2		368	310	6844	82.7
4-12	0				5-3	65				5-25	418			
	0	0	0	0		85	75	200	14.1		274	364	10368	101.8
4-13	28				5-5	124				5-27	328			
	32	30	8	2.8		102	113	242	15.6		334	331	18	4.2
4-15	12				5-7	261				5-29	247			
	12	12	0	0		245	253	128	11.3		245	246	2	1.4
4-17	22				5-9	289				5-31	286			
	2	12	200	14.1		261	275	392	19.8		283	285	4	2.0
4-19	202				5-11	364				6-2	274			
	176	189	338	18.4		364	364	0	0		376	325	5202	72.1
4-21	96				5-13	427				6-4	277			
	116	106	200	14.1		406	417	220	14.8		392	355	6572	81.1
4-23	82				5-15	372				6-6	344			
	100	91	162	12.7		360	366	72	8.5		348	346	8	2.8
4-25	70				5-17	496				6-8	372			
	74	72	8	2.8		475	486	220	14.8		412	442	800	28.3
4-27	245				5-19	412				6-10	396			
	367	306	7442	86.3		403	408	40	6.3		356	376	800	28.3
4-29	226				5-21	465				6-12	249			
	253	239	364	19.1		455	460	50	7.1		237	243	72	8.5

Figure 11. Mean Phytopigment Absorbancy Units per Unit Area After Two Weeks Exposure. Arrows Indicate Bi-weekly Nitrogen Additions



Period 2
Period 3
Period 4
Period 5
Addition
1 mg l⁻¹ Nitrogen
Addition
1 mg l⁻¹ Nitrogen
Addition
1 mg l⁻¹ N.
Absorbancy x 10³
700
650
600
550
500
450
400
350
300
250
200
150
100
50
9
13
17
21
25
29
3
7
11
15
19
23
27
31
4
8
12
April
May
June
Pool
Riffle

thus reducing the available nitrogen content. At this time the shingles which were to be removed on April 25 were not conditioned for growth and only became conditioned during the reduced nitrogen concentrations, therefore lowering the cumulative cell production to that date.

A two-way analysis of the variance was used to analyze the variability of phytopigment concentrations between riffle and pool zones (location) and between periods 2, 3, 4, and 5. The "F" values obtained from this test show that there was a significant difference between location, between periods, and the interaction between location and periods at the one percent level (Table 7). The relative production differences between the riffle and pool zones can be narrowed to one of three factors or the interactions between them since other variables are assumed constant. These three factors were variations in water velocity, variable light intensity striking the stream bottom, and competition effects. Although all lights were placed equidistant from the bottom, the variations in light intensity are mentioned because light waves must pass through different depths of water.

The effects of competition seem very plausible when considering the differences between locations and between nitrogen periods. Briefly, seed material settles out in the pool and begins to grow. Riffle areas lag in cell establishment due to the physical effects of the current.

TABLE 7

ANALYSIS OF VARIANCE OF RIFFLE AND POOL AREAS
FOR FOUR PERIODS OF SHINGLE EXPOSURE

Phytopigment

Variation	Sum of Squares	Degrees of Freedom	Mean Square	" F "
Within	257,135	48	5,357	- - -
Cells	1,696,478	- -	- - -	- - -
Location	46,633	1	46,633	8.705
Period	1,538,863	3	51,288	9.574
Interaction	110,982	3	36,994	6.906
Total	1,953,613	- -	- - -	- - -

Organic Nitrogen

Variation	Sum of Squares	Degrees of Freedom	Mean Square	" F "
Within	5.5140	48	.1149	- - -
Cells	11.5851	- -	- - -	- - -
Location	0.6343	1	.6343	5.521
Period	9.7683	3	3.2561	28.339
Interaction	1.1825	3	.3942	3.431
Total	17.0991	- -	- - -	- - -

TABLE 8

MEAN PHYTOPIGMENT ABSORBANCY UNITS
AND MILLIGRAMS ORGANIC
NITROGEN PER PERIOD

Period	Mean Phytopigment		Mean Organic Nitrogen	
	Riffle	Pool	Riffle	Pool
1	33	73	0.02	0.11
2	174	196	0.27	0.31
3	543	402	1.17	0.77
4	468	316	1.32	0.73

Figure 12. Mean Phytopigment Absorbancy Units per Period

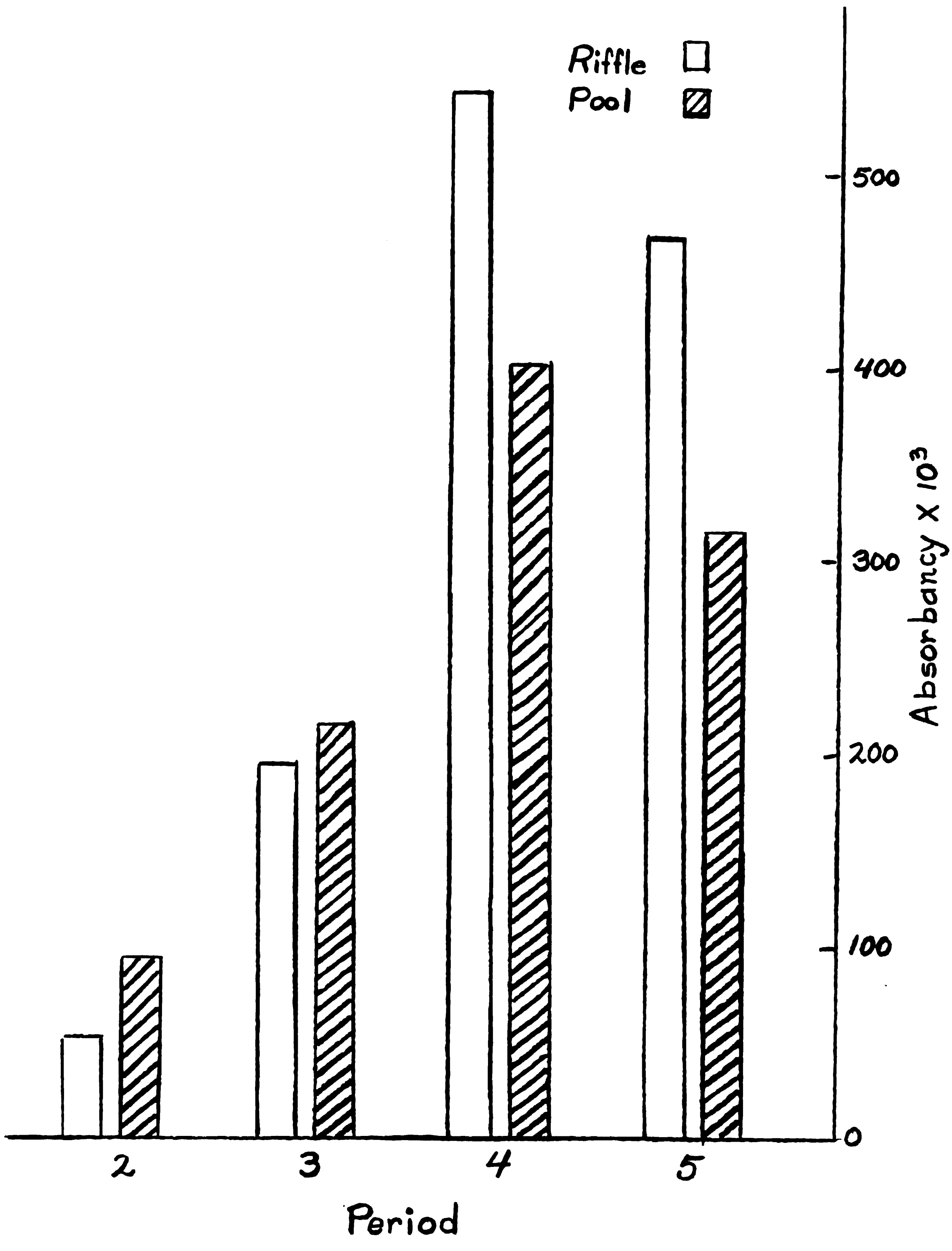

Riffle
Pool
500
400
300
200
100
0
Absorbancy X 10^3
2
3
4
5
Period

Figure 11 shows that growth began earlier in the pool; and the histogram in figure 12, which contains period averages, illustrates that growth was greater in the pool during initial colonization.

Eventually the riffle growth became equal to and exceeded that of the pool because this area had the first opportunity to extract nutrients coming from the reservoir. It is also interesting to note that pool pigment concentration peaks preceded peaks in the riffle until the middle of period 3 (Fig. 11).

The standard deviations of two phytopigment samples removed on the same date are listed in tables 5 and 6 along with means and variances. Pigment variability between these adjacent shingles might be a result of sloughing algal cells at certain times, highly variable growth on the shingle bottoms, and initial colonial growth of Anabaena. Single large Anabaena colonies were noted on one of the two April 27 pool and May 3 riffle samples. It should be mentioned that variable growth on the shingle bottoms might have been a product of upper surface shading and/or adherence of cells which were torn loose from the stream mat.

organic nitrogen

The mean values of organic nitrogen in milligrams per half shingle (each exposed two weeks) are plotted versus the time exposed in figure 13. In conjunction with pigment production an increase in organic nitrogen followed each

TABLE 9

MILLIGRAMS OF ORGANIC NITROGEN PER UNIT AREA AFTER A TWO WEEK EXPOSURE PERIOD IN THE POOL AREA

Date	Mg N	Mean	Var.	Sta. Dev.	Date	Mg N	Mean	Var.	Sta. Dev.	Date	Mg N	Mean	Var.	Sta. Dev.
4-10	.016				5-1	.105				5-23	.543			
	.016	- -	- -	- -		.158	.14	.0014	.0374		.289	.42	.0300	.1732
4-12	.016				5-3	.210				5-25	.945			
	.016	- -	- -	- -		.280	.25	.0018	.0424		.777	.87	.0141	.1187
4-13	.016				5-5	.245				5-27	.495			
	.016	- -	- -	- -		.228	.24	.0001	.0118		.560	.53	.0021	.0458
4-15	.016				5-7	.455				5-29	.508			
	.016	- -	- -	- -		.263	.36	.0184	.1356		.560	.54	.0014	.0374
4-17	.016				5-9	.560				5-31	1.155			
	- -	.02	0	0		.623	.59	.0020	.1407		1.138	1.27	.0001	.0118
4-19	.240				5-11	.655				6-2	.263			
	- -	.24	0	0		.545	.60	.0061	.0781		1.698	.98	1.030	1.063
4-21	.035				5-13	.753				6-4	.262			
	- -	.04	0	0		.438	.60	.0496	.2227		.385	.33	.0086	.0927
4-23	.252				5-15	1.803				6-6	.315			
	.350	.30	.0048	.0693		.805	1.13	.4980	.7057		.840	.58	.1380	.3715
4-25	.193				5-17	.998				6-8	.980			
	- -	.19	0	0		.700	.85	.0444	.2107		2.503	1.79	1.160	1.077
4-27	.263				5-19	.749				6-10	.595			
	.298	.28	.0006	.0247		.593	.67	.0122	.1105		.525	.56	.0018	.0424
4-29	.333				5-21	.686								
	.280	.31	.0014	.0374		1.155	.93	.1100	.3317					

TABLE 10

MILLIGRAMS OF ORGANIC NITROGEN PER UNIT AREA AFTER
A TWO WEEK EXPOSURE PERIOD IN THE RIFFLE AREA

Date	Mg N	Mean	Var.	Sta. Dev.	Date	Mg N	Mean	Var.	Sta. Dev.	Date	Mg N	Mean	Var.	Sta. Dev.
4-10	.016				5-1	.114				5-23	2.783			
	.016	- -	- -	- -		.114	.11	0	0		.717	1.75	2.1320	1.4600
4-12	.016				5-3	.182				5-25	.508			
	.016	- -	- -	- -		- -	.18	- -	- -		.427	.47	.0033	.0574
4-13	.016				5-5	.368				5-27	.455			
	.016	- -	- -	- -		.263	.32	.0055	.0742		.315	.39	.0098	.0990
4-15	.016				5-7	.158				5-29	.455			
	.016	- -	- -	- -		.350	.26	.0184	.1356		1.715	1.09	.8438	.9186
4-17	.016				5-9	.998				5-31	1.372			
	.016	- -	- -	- -		.575	.76	.1166	.3415		1.925	1.65	.1529	.3910
4-19	.016				5-11	.508				6-2	.735			
	- -	.02	- -	- -		1.383	.95	.3828	.6189		1.967	1.86	.0269	.1640
4-21	.025				5-13	.578				6-4	2.200			
	- -	.03	- -	- -		.392	.49	.0173	.1315		1.100	1.65	.6050	.7778
4-23	.053				5-15	2.188				6-6	2.741			
	.053	.05	0	0		.777	1.49	.9950	.9975		1.488	2.12	.7863	.8867
4-25	.016				5-17	.665				6-8	.612			
	- -	.02	- -	- -		1.260	.97	.1770	.4207		2.258	1.44	1.3514	1.1620
4-27	.105				5-19	1.925				6-10	2.415			
	.105	.11	0	0		.851	1.39	.5778	.7601		1.768	2.10	.2093	.4574
4-29	.123				5-21	.875								
	.123	.12	0	0		1.488	1.14	.1885	.4342					

Figure 13. Mean Organic Nitrogen per Unit Area After Two Weeks Exposure. Arrows Indicate Bi-weekly Nitrogen Additions

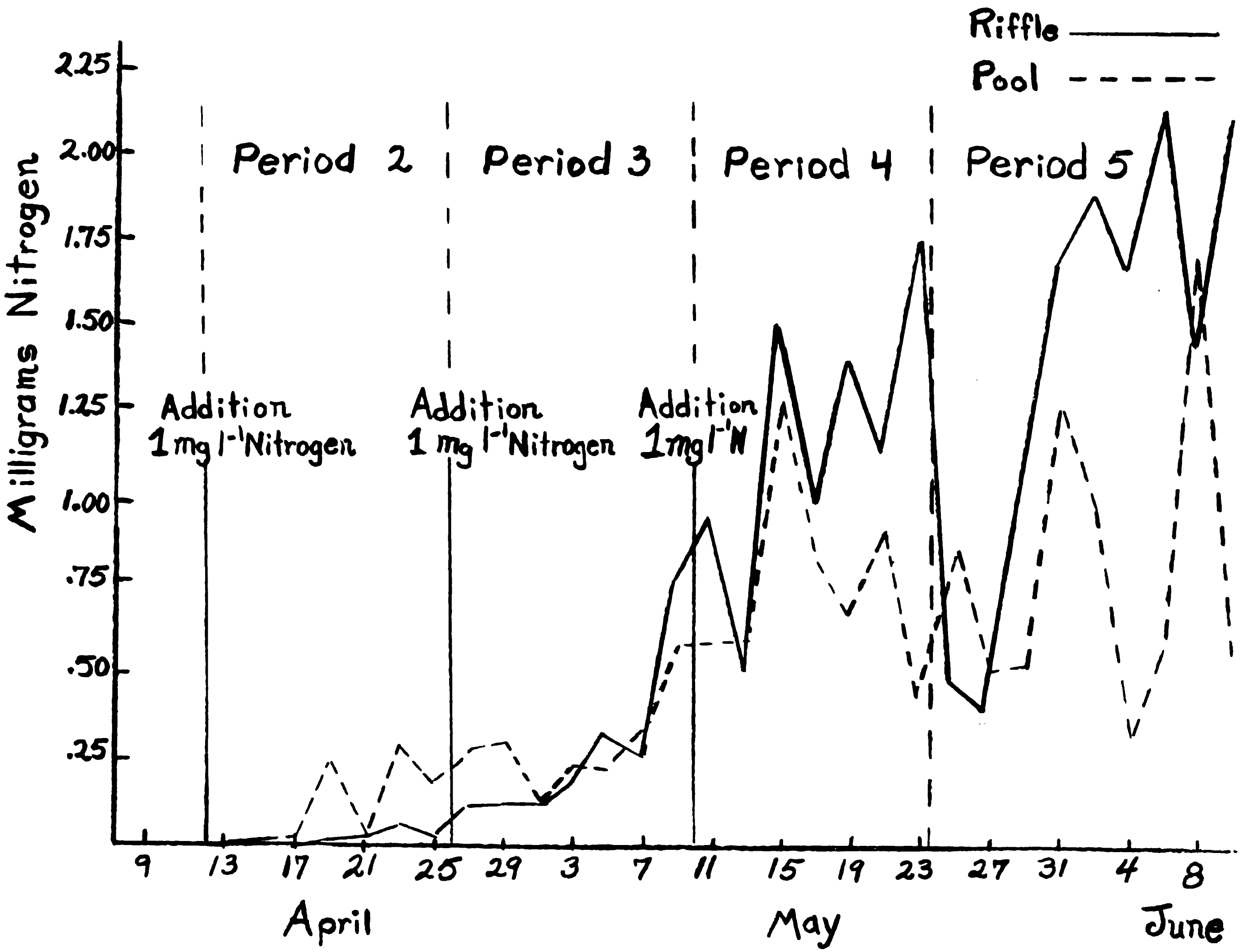
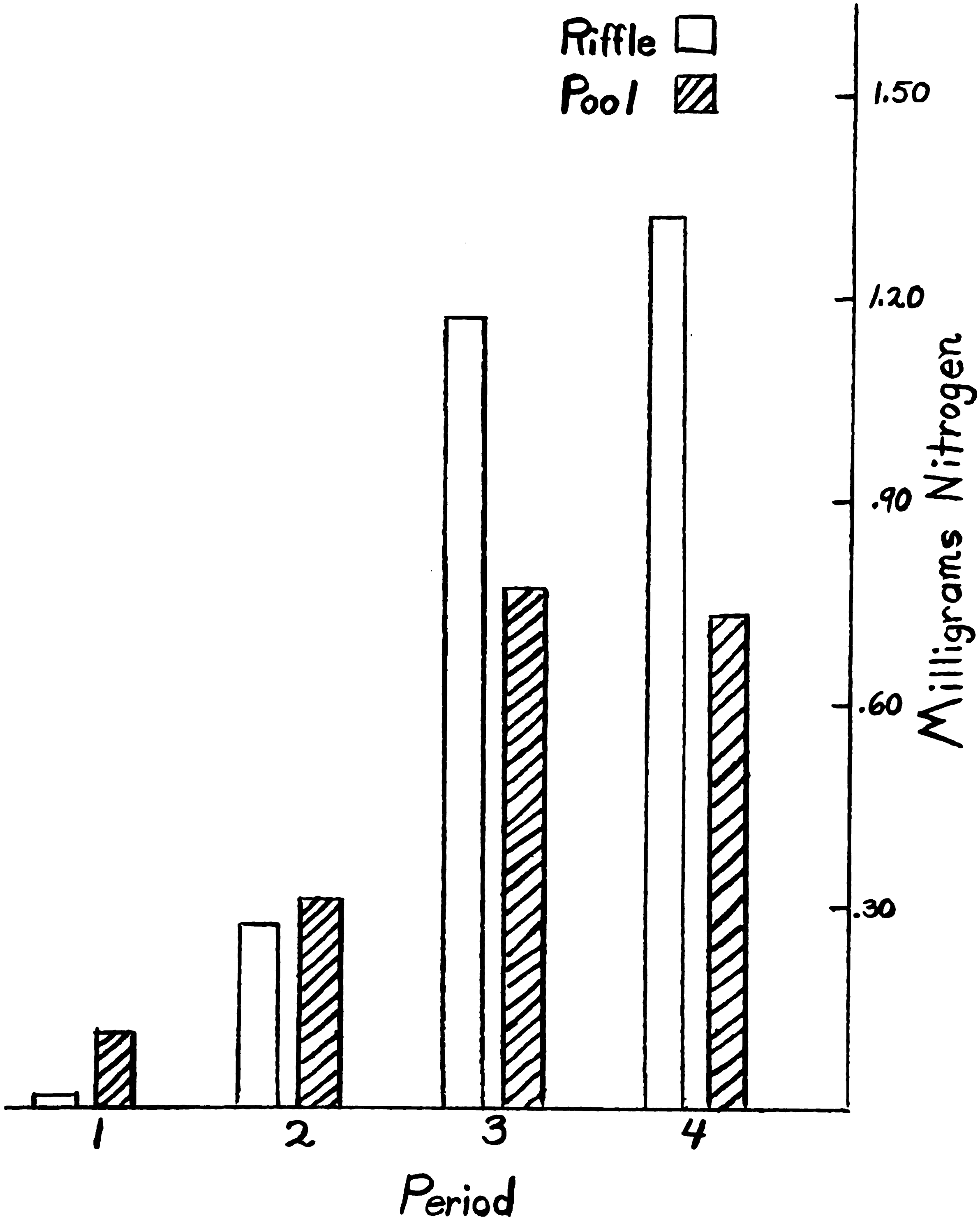

Riffle
Pool
Period 2
Period 3
Period 4
Period 5
Addition
1 mg l-1 Nitrogen
Addition
1 mg l-1 Nitrogen
Addition
1 mg l-1 N
Milligrams Nitrogen
2.25
2.00
1.75
1.50
1.25
1.00
.75
.50
.25
9
13
17
21
25
29
3
7
11
15
19
23
27
31
4
8
April
May
June

Figure 14. Mean Milligrams of Organic Nitrogen per Period


Riffle
Pool
1.50
1.20
.90
.60
.30
Milligrams Nitrogen
1
2
3
4
Period

calcium nitrate addition. Enormous fluctuations were also noted in periods 4 and 5.

Figure 14, which consists of values averaged for periods 1, 2, 3, and 4, illustrates the most profound increment of organic nitrogen, which occured after period 2. The averages of periods 4 and 5 indicate that cellular nitrogen content may have reached a leveling off point, but this is only an assumption as enormous fluctuations occurred in these latter periods (Fig. 13). The point of change, just discussed, roughly corresponds to the entrance of Anabaena which later dominated the awfwuchs community.

A two-way analysis of the variance indicated that there were significant differences between the location means and between the interaction of location and periods at the 5 percent level (Table 7). Means between nitrogen periods are significant at the one percent level. The levels of significance show that the differences between periods were greater than differences between locations. The five percent level for location also indicates that differences between mean values of organic nitrogen were not as great as those between mean phytopigment units in the riffle and pool areas. As with pigment the difference in riffle and pool areas was probably a product of current, light, and competition. Note that the trend again follows that of phytopigment in that the early concentrations of organic nitrogen, highest in the pool, were eventually overtaken and succeeded by a higher concentration in the riffle.

The difference in nitrogen periods, which resulted from an increase of mean organic nitrogen concentrations per period, appears to be a product of nitrate additions, although some increase could have occurred from nitrogen fixation by Anabaena.

Within a particular two-shingle sample the variability was often quite large (Tables 9 and 10). The same factors that were responsible for variability within chlorophyll samples are believed to be largely responsible for this. However, the variability does not seem large enough to destroy the value of this procedure since the increases and decreases in averaged period values of organic nitrogen and phytopigment are closely related.

Phytopigment--organic nitrogen relationship

A linear regression was used to determine the phytopigment concentration--organic nitrogen relationship (Fig. 15). This regression is given by the formula:

$$\hat{Y} = a + bX$$

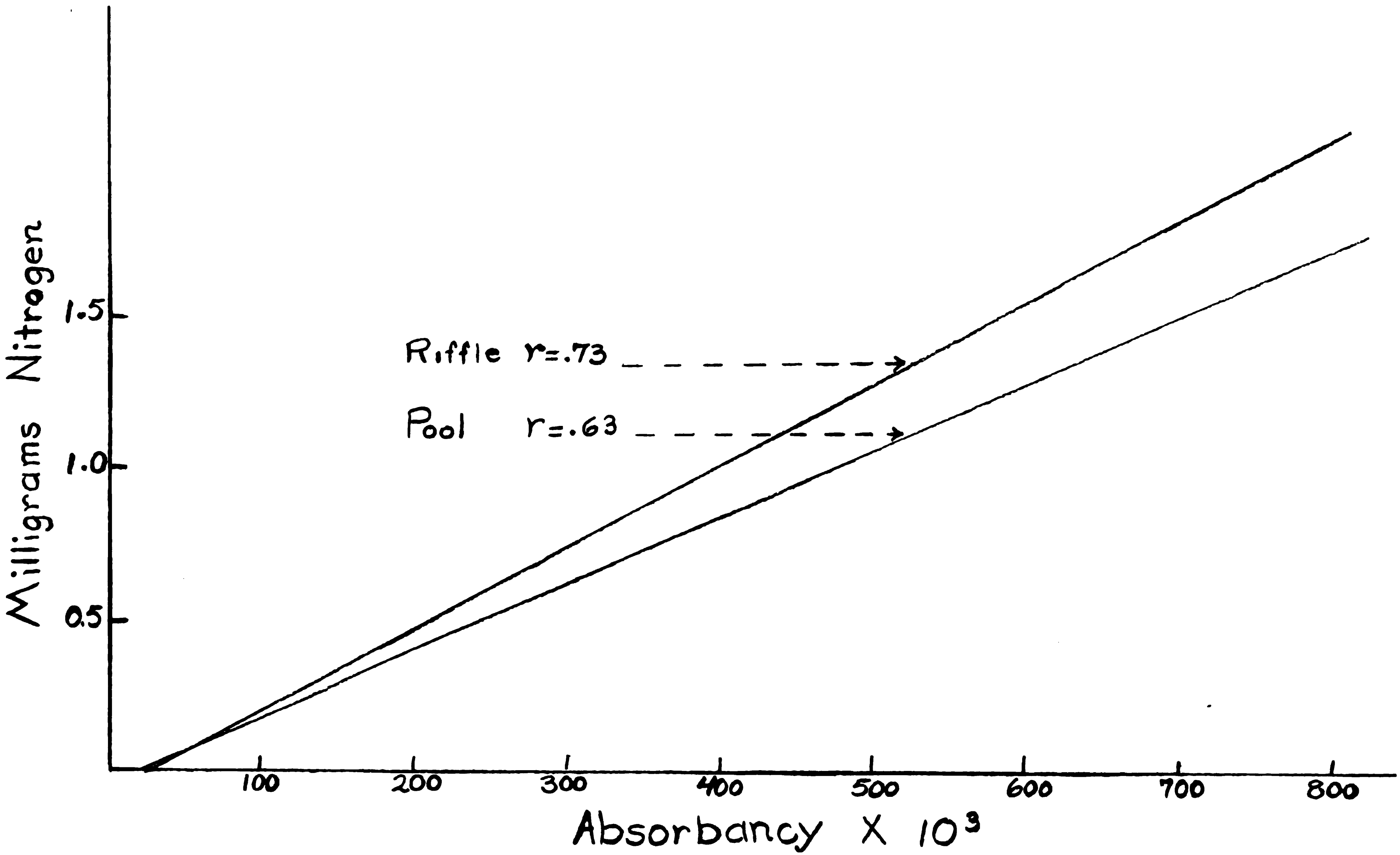
where Y is the predicted value of organic nitrogen; X is the known phytopigment concentration; b is the slope of the regression line; and a is the Y intercept.

The slope is found by the equation:

$$b = \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{n}}{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}$$

and the Y intercept is calculated by the formula:

Figure 15. Regression Lines Expressing the Relationship Between Phytopigment Absorbancy Units and Milligrams of Organic Nitrogen for Riffle and Pool Zones



Milligrams Nitrogen
1.5
1.0
0.5
Riffle r=.73
Pool r=.63
100
200
300
400
500
600
700
800
Absbrbancy X 10³

$$a = \frac{\Sigma Y - b\Sigma X}{n}$$

In the riffle and pool zones $Y = -.089 + .0027X$ and $Y = -.023 + .00215X$, respectively. Although not determined, the variance of points about the regression appeared to be large.

The coefficient of correlation between phytopigment and organic nitrogen is given by the formula:

$$r = \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{n}}{\left[\left(\Sigma X^2 - \frac{(\Sigma X)^2}{n}\right)\left(\Sigma Y^2 - \frac{(\Sigma Y)^2}{n}\right)\right]^{\frac{1}{2}}}$$

The calculated correlation coefficients for riffle and pool regions are .73 and .63 respectively.

It can be seen that a fairly good correlation was obtained, which probably was due to each variable's relationship to cellular weight. The higher correlation in the riffle indicates that for a given unit of chlorophyll the riffle community contains slightly more organic nitrogen. This might mean that nitrogen pumped from the reservoir first becomes available to the riffle area.

total dry weight--pigment relationship

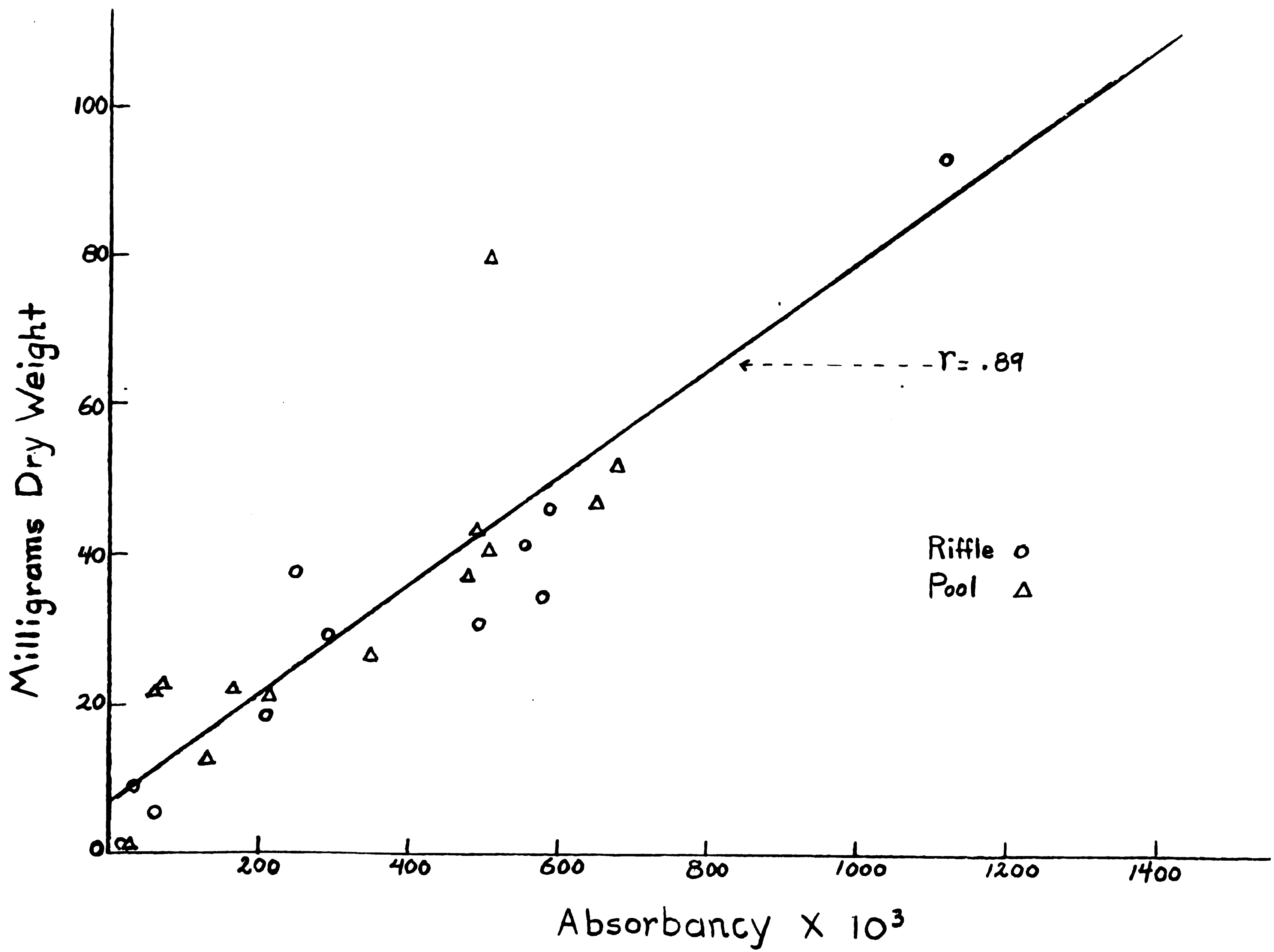
The results of total dry weight--pigment analysis are listed in table 11. The relationship of these data is expressed by a linear regression of $Y = 3.47 - .0719X$ (Fig. 16). Most of these data came from the *Anabaena* community. Only the lowest points on the slope were represen-

TABLE 11

PHYTOPIGMENT PER UNIT DRY WEIGHT PER UNIT AREA AFTER TWO WEEKS OF EXPOSURE

Date	Riffle AAX10^3	Pool AAX10^3	Riffle mg. dry wt.	Pool mg. dry wt.
4-13	12	12	0.8	0.8
4-21	56	68	5.6	22.8
4-23	32	64	9.2	21.6
4-29	208	132	18.4	12.4
5-3	288	164	29.0	21.8
5-9	252	352	37.8	27.6
5-11	- -	510	- - -	79.6
5-17	554	510	41.2	40.4
5-23	584	208	35.6	21.6
5-25	584	486	46.6	43.6
5-31	- -	674	- - -	52.4
6-6	1112	478	92.8	37.4
6-8	494	646	30.6	47.0

Figure 16. Regression Expressing the Relationship of Phytopigment Absorbancy and Milligrams of Total Dry Weight for All Artificial Stream Communities



Milligrams Dry Weight
0
20
40
60
80
100
200
400
600
800
1000
1200
1400
Absorbancy X 10³
r= .89
Riffle o
Pool Δ

tative of the diatom-green algae pioneers since growth was slight during their presence. The calculation of separate slopes did not seem justifiable.

The coefficient of correlation was calculated to be .89. A lower correlation of .616 between organic weight and chlorophyll was given by Riley (1940). However, in certain Red Cedar River diatom communities Peters (M. S.) found that a common correlation of .93 occurred using organic weight versus ethanol pigment extracts. If it is assumed that total dry weight minus ethanol soluble compounds approaches Peter's organic weights which were corrected for the loss of soluble compounds, then the correlation coefficient for blue-green algae is slightly less than that for diatoms. Riley (1940) indicates that partial correlations are slightly lower for the blue-greens.

In view of the statements above, it is still interesting to note that two such diverse groups of algae with different pigment characteristics are so closely comparable. This might in part be why deviations of all points from the common calculated slope gave no trend to justify computation of separate slopes.

organic nitrogen--total dry weight relationship

The relationship between organic nitrogen and total dry weight is expressed as percent organic nitrogen (Table 12). Only two shingles were analyzed per period in question. Of the two shingles one was removed near the beginning

TABLE 12

PERCENT CELLULAR NITROGEN PER UNIT AREA AFTER TWO WEEKS EXPOSURE AT THE BEGINNINGS AND ENDS OF PERIODS 2, 3, AND 4

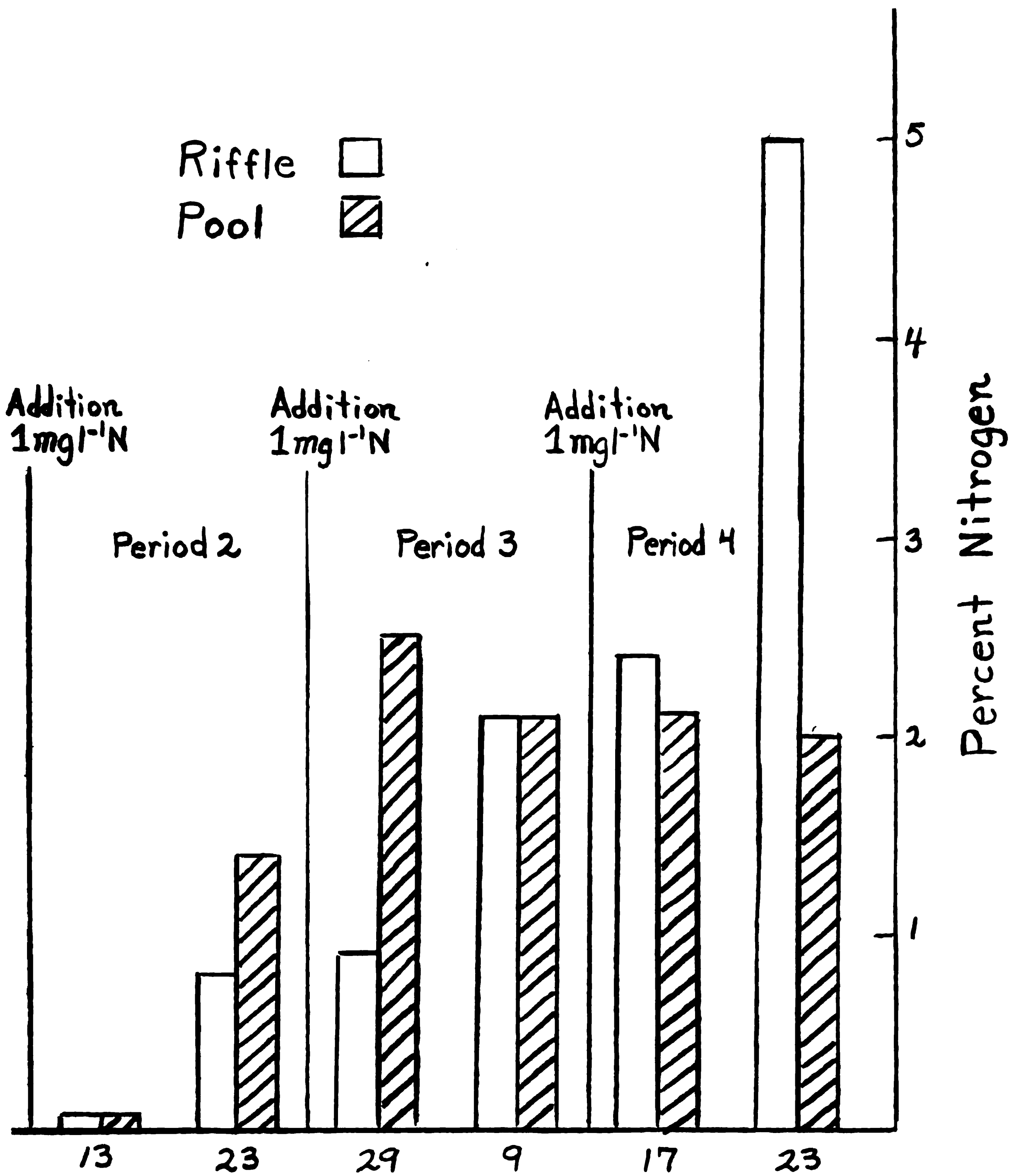
Date	Pool			Riffle		
	Mg N	Mg Dry Wt	% N	Mg N	Mg Dry Wt	% N
4-13	trace	0.8	trace	trace	0.8	trace
4-23	.30	21.6	1.4	.05	9.2	0.6
4-29	.31	12.4	2.5	.12	16.4	0.8
5-9	.59	27.6	2.1	.76	37.8	2.1
5-17	.85	40.4	2.1	.97	41.2	2.4
5-23	.42	21.6	2.0	1.75	35.6	4.9

and one near the end of the period. This analysis was only conducted to obtain further insight into the nitrogen metabolism within periods of nitrogen addition. Data for periods 2, 3, and 4 are illustrated in figure 17.

It is evident that the cellular nitrogen content remained below 2.5 percent most of the time. Only on May 23 in the riffle area was this value exceeded. Fogg (1944) states that nitrogen fixing blue-green algae contain a rather high organic nitrogen content near 7 or 8 percent. Gerloff and Skoog (1954) have pointed out that an internal concentration of nitrogen above four percent in cells of Microcystis is luxury consumption, whereas growth below this amount is proportional to the supply of the element.

The organic nitrogen in the pool zone increased until the beginning of period 3. Thereafter a slight drop in the level occurred. In the riffle area the percent of nitrogen rose consistently throughout the experiment. This rise might be accounted for by the proximity of the riffle to the incoming nitrogen from the reservoir influent, with the riffle community depleting most of the nitrogen in solution before it reaches the pool. This explanation would account for the stabilization or drop of percent organic nitrogen in the pool after period 2. However, since Anabaena dominated the stream community from the middle of period 3 until the end of the project, nitrogen fixation may in part be responsible for increases within periods 3 and 4. It is

Figure 17. Percent of Cellular Nitrogen at the Beginnings and Ends of Periods 2, 3, and 4



Riffle
Pool
Addition 1mgl⁻¹N
Addition 1mgl⁻¹N
Addition 1mgl⁻¹N
Period 2
Period 3
Period 4
5
4
3
2
1
Percent Nitrogen
13
23
29
9
17
23
April
May

interesting to note that the percent nitrogen relationship between pool and riffle compares closely to mean period organic nitrogen and phytopigment values (Figures 12 and 14).

stream primary production

Up to this time only relative rates of primary production have been mentioned. The calculation of absolute rates treating data from all samples would be difficult if not impossible since there was considerable overlap of shingle exposure. It was necessary to use only one set which was removed and replaced at two week intervals for the absolute estimate. This group chosen, set 1 of both riffle and pool, sampled each nitrogen period in entirety and thus represents the accumulation of algae during that period. These values should give a close approximation to actual primary production since consumers were not noted to exist in the algal mat until shortly before the experiment terminated. All chlorophyll values were converted to total dry weight by employing the regression in figure 16. Results of calculations are listed in table 13.

An increase in absolute production per period followed the cumulative additions of nitrate with a mean daily production based on 8 weeks of 250.2 and 201.4 milligrams dry weight per square meter per day for riffle and pool respectively. The higher riffle production was due to a greatly accelerated rate during periods 4 and 5 (Figures 18 and 19). This more than made up for higher pool rates in the

TABLE 13

ARTIFICIAL STREAM PRIMARY PRODUCTION

TABLE 13

ARTIFICIAL STREAM PRIMARY PRODUCTION

Riffle Production

Period	Date	Cumulative mg l Nitrogen	AA X 10^3 75 cm^{-2} 2 wks^{-1}	mg dry wt 75 cm^{-2} 2 wks^{-1}	mg dry wt m^{-2} 2 wks^{-1}	mg dry wt m^{-2} 2 wks^{-1}
2	4-11 to 4-25	1	28	5.3	706.7	50.5
3	4-25 to 5-9	2	181	16.4	2,186.4	156.2
4	5-9 to 5-23	3	458	36.4	4,853.3	346.7
5	5-23 to 6-6	3	605	47.0	6,266.7	447.6

Total milligrams 75 cm^{-2}8 $weeks^{-1}$- - - - 105.1
Total milligrams m^{-2}8 $weeks^{-1}$- - - - - - - - - - - - - -14,013.4
Mean milligrams $m^{-2}day^{-1}$- - - - - - - - - - - - - - - - - 250.2

Pool Production

Period	Date	Cumulative mg l Nitrogen	AA X 10^3 75 cm^{-2}2 wks^{-2}	mg dry wt 75 cm^{-2}2 wks^{-1}	mg dry wt m^{-2} 2 wks^{-1}	mg dry wt m^{-2} 2 wks^{-1}
2	4-11 to 4-25	1	72	7.8	1,040.0	74.3
3	4-25 to 5-9	2	275	23.0	3,066.7	219.1
4	5-9 to 5-23	3	310	25.5	3,400.0	242.9
5	5-23 to 6-6	3	346	28.3	3,773.3	269.5

Total milligrams 75 cm^{-2}8 $weeks^{-1}$- - - 84.6
Total milligrams m^{-2}8 $weeks^{-1}$- - - - - - - - - - - - 11,280.0
Mean milligrams $m^{-2}day^{-1}$- - - - - - - - - - - - - - - - 201.4

preceding periods 2 and 3. These calculations seem to substantiate earlier assumptions based upon relative rates.

When compared to other works, the artificial stream is relatively unproductive with rates approximating those of sterile springs and oceanic waters. Odum (1956) states that gross production of ten Florida springs during July and August ranged between 600 and 59,000 mg m^{-2} day^{-1}. He also quotes Riley in saying oceanic waters range between 170 and 1,600 mg m^{-2} day^{-1}. Although production was low, the standing crop became quite high, forming a thick spongy mat of cells which eventually began to break loose from the stream bottom. This atypical situation was probably due to lack of grazing consumers. In summary it seems that lack of nitrogen has significantly decreased the overall production of the artificial stream with successive nitrate additions determining the attainment of higher production rates.

It is interesting to note that pool production rates for periods 2, 3, and 4 were closely grouped, whereas those of the riffle were more widely separated. This might indicate that a steady-state community was nearly reached in the pool.

Light

A continuous influx of energy was supplied to the artificial stream community by incandescent lamps. To review, all lighted substrates were 36 centimeters from

Figure 18. Primary Production of Riffle Area in Grams per Square Meter After Two Weeks Exposure to Periods 2, 3, 4, and 5

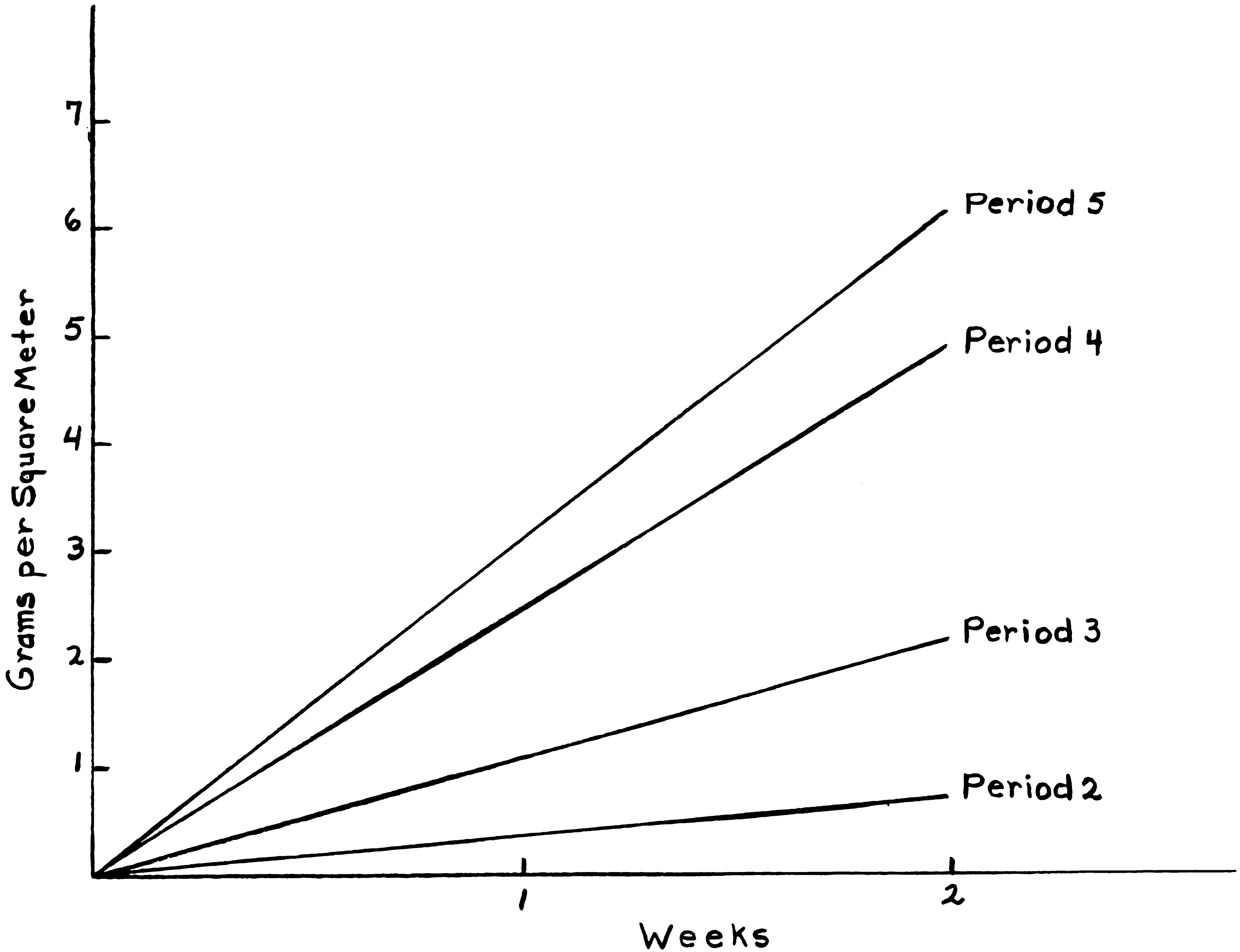
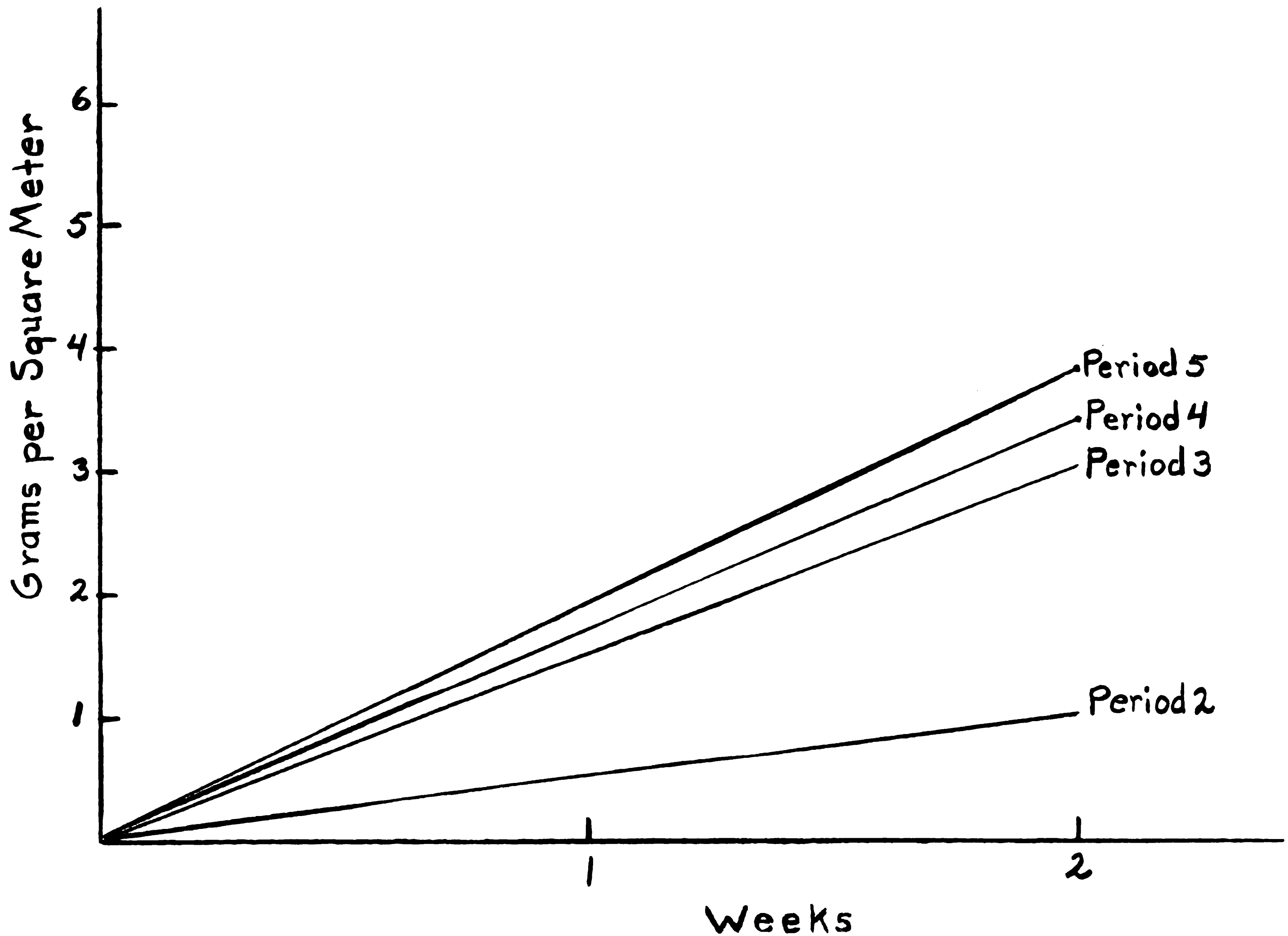

Grams per Square Meter
7
6
5
4
3
2
1
Period 5
Period 4
Period 3
Period 2
1
2
Weeks

Figure 19. Primary Production of Pool Area in Grams per Square Meter After Two Weeks Exposure to Periods 2, 3, 4, and 5


Grams per Square Meter
6
5
4
3
2
1
Period 5
Period 4
Period 3
Period 2
1
2
Weeks

and directly beneath the lamps in order to obtain equal and maximal perpendicular radiation. However, the equal conditions were only approximated since light had to pass through a greater depth of water in the pool.

The average maximum radiant energy striking the pool surface was measured at 1.0 $\pm$ .05 gm-cal $cm^{-2} min^{-1}$. In the riffle region the energy striking was approximated at .63 gm-cal $cm^{-2} min^{-1}$. The higher pool value stems from the fact that the pool surface was closest to the light source since lights followed the contour of the stream bottom. An actual value of energy reaching the bottom could not be obtained due to the position of the exposure meter window.

In nature the total radiation at the water surface (temperate zones) seldom exceeds 1.5 gm-cal $cm^{-2} min^{-1}$, and only about half is used in photosynthesis (Edmondson 1956). Except for quality of light the energy entering the artificial stream compared closely to natural sunlight. This assumption can be made since the exposure meter was calibrated directly from a pyrheliometer which is equally sensitive to all wavelengths (Strickland 1958). Pringsheim (1950) indicates that in comparison to luminscent tubes, incandescent illumination has a spectral emanation that is more in conformity to the absorption of algal pigments.

In view of the quantity of constant energy supplied to the artificial system, Photosynthetic inhibition could have affected the community composition as a whole. Ryther (1956) working with marine phytoplankton groups found that growth of the group Chlorophyta was inhibited before that of diatoms by increasing light. Meyer and Bun (1940) report that at light intensities of 12,000 foot-candles (roughly .2 gm-cal $cm^{-2} min^{-1}$) complete inhibition of Chlorella occurred. Further studies state that mixed populations of marine phytoplankton became inhibited at about .5 gm-cal $cm^{-2} min^{-1}$ (Strickland 1958). Moreover, floating forms of blue-green algae appear to have their maximum photosynthetic rate near the water surface (Davis 1955 reported by Verduin). Therefore, it appears that light intensities in the artificial stream could be excluding such algae as Chlorophyta, but permitting diatoms and blue-green forms to flourish.

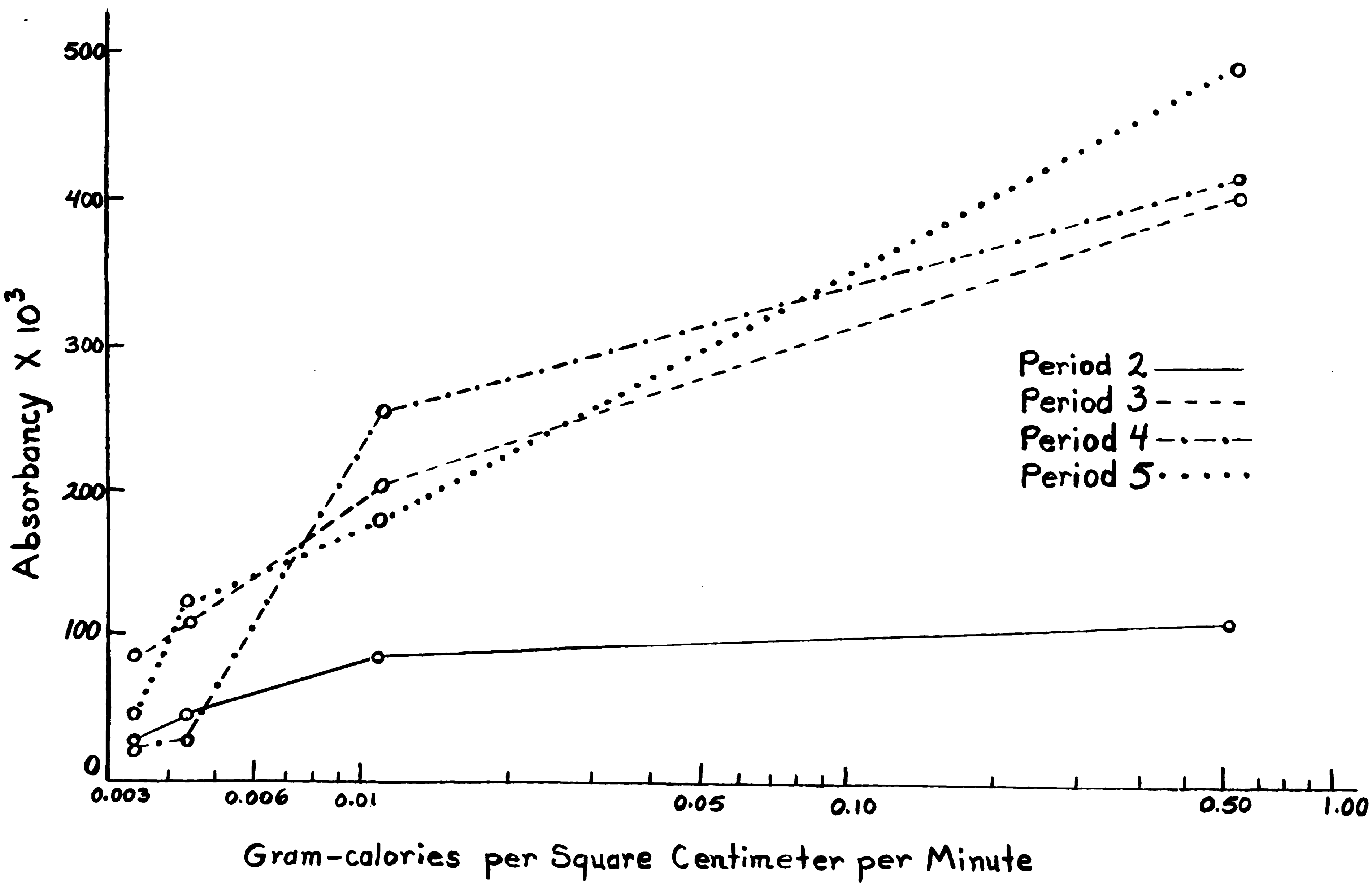
The effects of reduced light intensities were studied by comparing the chlorophyll content of illuminated shingles with those in the unlighted area preceding the riffle. As expected, a decrease in light intensity significantly reduced the primary production (Fig. 20). Edmondson (1956) found little or no correlation between light income and rate of photosynthesis in surface waters due to surface inhibition of growth and range of saturation intensity for photosynthesis, but at six meters or more a correlation of the two variables became evident. The lowest light intensity to which shingles were exposed was 3.4×10^{-3} gm-cal $cm^{-2} min^{-1}$. This value corresponds to the compensation intensity of many algal forms which centers around 3×10^{-3} gm-cal $cm^{-2} min^{-1}$ (Strickland 1958).

TABLE 14

MEAN ABSORBANCY UNITS (AAX10^3) OF PHYTOPIGMENT PER UNIT AREA AFTER EXPOSURE TO FOUR PERIODS OF NITROGEN AT DECREASING INTENSITIES OF LIGHT ENERGY (g-cal cm^{-2} min^{-1})

Shingle Number	Energy	Period 2	Period 3	Period 4	Period 5
A1	.6300	114	408	420	492
A01	.0110	85	202	253	180
A02	.0040	45	110	27	122
A03	.0034	27	88	22	46

Figure 20. Mean Phytopigment Absorbancy Units per Unit Area at Decreasing Intensities of Light Energy for Periods 2, 3, 4, and 5



Absorbancy X 10³
500
400
300
200
100
0
0.003
0.006
0.01
0.05
0.10
0.50
1.00
Gram-calories per Square Centimeter per Minute
Period 2
Period 3
Period 4
Period 5

It can also be seen that factors other than light affected production in the unlighted zone. The successive additions of nitrogen in general appeared to increase phytopigment production here as in fully lighted areas. The change is particularly noticeable from period 2 to the other periods. However, at energies below .011 gm-cal cm^{-2} min^{-1} this effect is not well defined.

Nitrogen Fixation

Throughout this paper references have been made to the possibilities of nitrogen fixation being partially responsible for increased period production. Therefore, it seems useful to summarize both pros and cons of this occurence.

First of all *Anabaena oscillarioides* dominated the artificial stream during the latter two-thirds of the project. Seven species of *Anabaena* have been known to fix nitrogen (Fogg 1947); however, *A. oscillarioides* was not listed.

The artificial stream media was conducive to fixation since the nitrogen concentration never exceeded 1 mg l^{-1}. Fogg (1942) reports that fixation occurs in *A. cylindrica* if the concentration falls below 4 mg l^{-1}. It was noted that *Anabaena* appeared in the artificial stream at the end of period 2 when the concentration was very low. This genus often exists in waters of low inorganic nitrogen content (Riley 1940; Edmondson, Anderson, and Patterson 1956; Hutchinson 1944; and Pearsall 1932), but few authors have concluded that fixation was occurring. This genus may be highly tolerant of certain environmental conditions regardless of fixation tendencies. It is almost universally accepted that blue-green algae prefer alkaline media (Bold 1942) and strong light intensities (Davis 1955). Due to a variety of pigments in the cell a high photosynthetic efficiency is produced (Strickland 1958).

In direct opposition to the occurrence of biological fixation is the fact that the artificial stream *Anabaena* cells contained a low percent of nitrogen. Nitrogen fixers have a high cellular nitrogen content (Gerloff and Skoog 1954). Only in the riffle during periods 3 and 4 did an increase in organic nitrogen take place while inorganic levels were low, but this increase could be explained by the riffle proximity to nitrogen in the influent. The low rate of primary production throughout this experiment indicates that substantial amounts of nitrogen fixation could not have occurred.

SUMMARY

1. A community of algal cells established itself and grew in an artificial stream with controlled water temperatures of 70° ± 2°F, continuous illumination, constant flow, excess of all major nutrients except nitrogen, and presence of trace elements. Conditions that were varied included nitrogen content, water velocity, and incident stream bottom radiation.

2. The first addition of nitrogen to the stream, which contained only traces of this element, triggered growth of a pioneer community which was predominately diatoms. During the second addition period this community was replaced by one of blue-green algal dominance, which remained until the experiment terminated.

3. Three successive supplements of calcium nitrate (1 mg l^{-1} each) elevated period production of phytopigment, organic nitrogen, and total dry weight. All increases were due to nitrate additions and possibly nitrogen fixation, but there is little evidence to support the latter theory.

4. The assimilation of nitrogen after each addition resulted in a rise of cellular chlorophyll and a fall of inorganic nitrogen, which usually leveled off at a concentration near .1 mg l^{-1}. Following this reduction chlorophyll content decreased.

5. A significant difference in production, phytopigment content, and organic nitrogen occurred between riffle and pool areas. Pool and riffle values were respectively higher at the beginning and end of the project. This difference can be attributed to one or more of these factors: variable incident radiation, variations in current, or competition.

6. A good relationship was expressed between total dry weight and pigment for a community of mixed algal groups. Also the correlation between phytopigment and organic nitrogen was fairly good for both pool and riffle areas. Correlations in the pool were slightly lower.

7. The "normal" organic nitrogen of cellular material approximated two percent with one exception. This exception might be a product of nitrogen fixation.

8. Total radiation striking the pool and riffle surfaces was measured at 1.0 ±.05 to .63 gm-cal $cm^{-2} min^{-1}$ respectively. This accounted for a primary production of 250.2 mg dry weight $m^{-2} day^{-1}$ in the riffle and 201.4 in the pool.

9. Shingles placed in diminishing light intensities showed a definite decrease in phytopigment production. Even under these conditions production increased with nitrogen additions.

10. During the experiment initially high concentrations of

alkalinity, conductivity, total phosphorus, silica, sodium, and potassium dropped as time progressed. The variation in pH was slight due to a complex buffering system. Reductions appear to be primarily from cellular growth although other factors are partially responsible. An instantaneous concentration of any ion in the stream is equal to the solution of the initial addition, organic decomposition, and release from living cells minus plant assimilation, leakage, and undissolved salts.

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