ALTERATION OF THE PRODUCTIVITY OF A TROUT STREAM BY THE ADDITION OF PHOSPHATE

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ALTERATION OF THE PRODUCTIVITY OF A TROUT STREAM

BY THE ADDITION OF PHOSPHATE

By

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ABSTRACT

Diammonium phosphate was added to the west branch of the Sturgeon River at a point approximately four stream miles above where it crosses U. S. Highway 27 near Wolverine. At times the presence of excessive phosphorus was detected as far downstream as Highway 27.

In the period in which phosphate was added, increased periphyton growth at a point about one and a half miles downstream was shown. The ratio of phosphorus to organic nitrogen in the periphyton population at all times, both upstream and downstream, was found to be one to ten by weight.

No change in volume of benthos one and a half miles downstream from the point of phosphate addition could be correlated with this addition.

A study of the composition of the pigment complex in ninety-five percent ethanol extracts of periphyton from the west branch of the Sturgeon River and a ninety percent acetone extract of fresh periphyton from the Red Cedar River was carried out.

Robert C Ball

ACKNOWLEDGMENTS

The author wishes to express his appreciation and gratitude to Dr. Robert C. Ball and Dr. Frank F. Hooper for their guidance and advice during this project. The author is also indebted to Mr. Lowell Keup, who helped carry out the field work; and Dr. Philip J. Clark, for his assistance in the statistical analysis of the data.

This study was carried out under a graduate research fellowship from the Institute for Fisheries Research, Ann Arbor, Michigan.

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INTRODUCTION

Civilization with its expanding populations and rapidly developing industrialization has created an ever increasing problem of pollution. One important phase of this problem is the municipal pollution of streams. In order to handle this problem in such a way as to serve the best interests of man we must gain a much more thorough understanding of the normal biology of relatively unpolluted streams. We must also study the effects of the addition of known amounts of extraneous materials.

The present study is the fourth in a series of experiments on the effects, both biological and chemical, of the addition of inorganic nitrogen and phosphorus to the west branch of the Sturgeon River. In all three of the previous studies (1954-56), these elements were applied to Hoffman Lake, the source of the stream concerned (Grzenda, 1956; Colby, 1957; Carr, M.S.). During the present study diammonium phosphate was added directly to the stream in a continuous flow for a short period in August, 1957. The effects of this addition were studied in cooperation with Keup (M.S.).

Although there have been many publications on the subject of the fertilization of ponds and lakes, very few studies of this type have been made on streams. Huntsman (1948) observed an increase in production as a result of inorganic fertilization of a stream in Nova Scotia.

GENERAL DESCRIPTION OF STUDY AREA

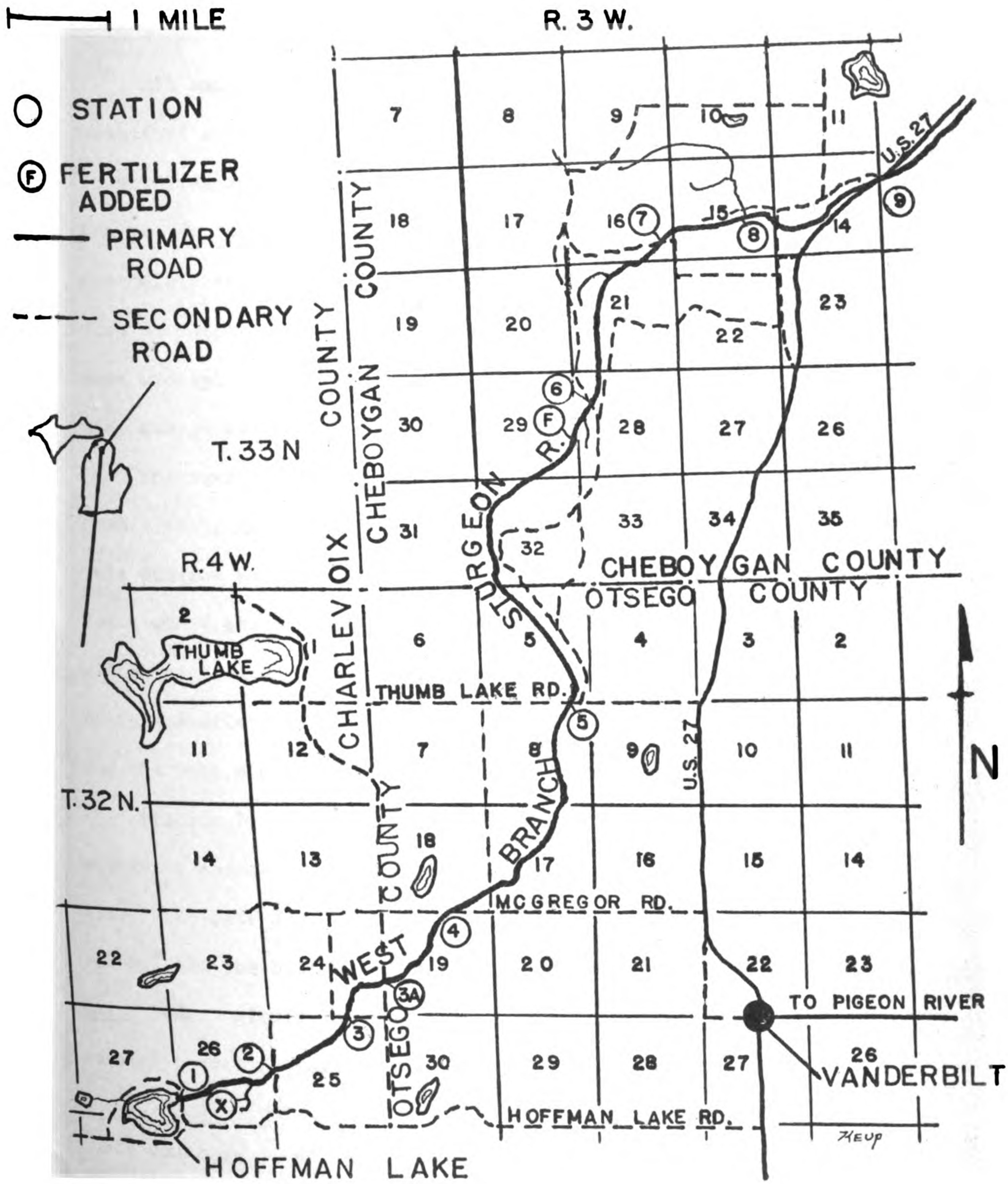
The west branch of the Sturgeon River is a cold, clear trout stream which originates in Hoffman Lake, Charlevoix County, Michigan; and joins the main branch of the Sturgeon River at Wolverine in Cheboygan County. Hoffman Lake is a marl lake of about 120 acres and the outflow from it is about one cubic foot per second. The stream flows through the northwest corner of Otsego County and on into Cheboygan County.

Due to the large number of moraines in the area, the watershed is restricted to a small area and the surface runoff is only rarely a major contribution to the volume of flow. The stream picks up the bulk of its water from springs and the outflow of several small lakes and beaver ponds. A large part of the watershed is within the Pigeon River State Forest, however there are a number of summer cottages on both Hoffman Lake and the stream. There are also some scattered farms on the watershed. Figure 1 shows the study area and adjacent roads in detail.

Figure 1

Map of Study Area
Showing Sampling
Stations

WEST BRANCH STURGEON RIVER AREA



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DESCRIPTION OF SAMPLING STATIONS

All sampling stations are marked on the map (fig. 1). The principal control station was located on the Charlevoix-Otsego County line and was designated as 3A. The stream is small (approx. ten cu. ft. per sec.)* at this point and is often broken up into many channels through a heavy arbor vitae swamp. The flow is relatively slow as compared to downstream and the water temperature fluctuates more widely. About half a mile upstream there is a beaver dam, which also has an effect on the stream.

The next station used extensively was located in Cheboygan County about two miles north of the Otsego-Cheboygan County line. This station was designated as 6 and is not far downstream from a point where the stream leaves an arbor vitae swamp which extends almost continuously from the source of the stream to this point. At this station the base flow is about 30 cubic feet per second* and the bottom of the stream is gravel in most places.

Station 7 is about one and a half stream miles downstream from station 6 and about three miles north of the Otsego-Cheboygan County line. The base flow at this station is about 45 cubic feet per second* and the bottom is marked by the presence of numerous large *Chara* beds. Within this stretch a brook, which originates in several inactive beaver ponds, joins the stream.

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*Data courtesy of Mr. Arlington D. Ash, U.S.G.S., Lansing, Michigan.

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Station 8 is located about one stream mile downstream from station 7 and is at the point where Fulmer Creek joins the stream. Fulmer Creek is a small brook which drains Fulmer Lake to the north of station 8. There is also a small spring-fed brook which joins the stream from the south and sometimes brings in nutrients from a cow pasture.

Station 27 is the place at which the stream crosses U. S. Highway 27. This station was only used on a temporary basis.

METHODS

Fertilization

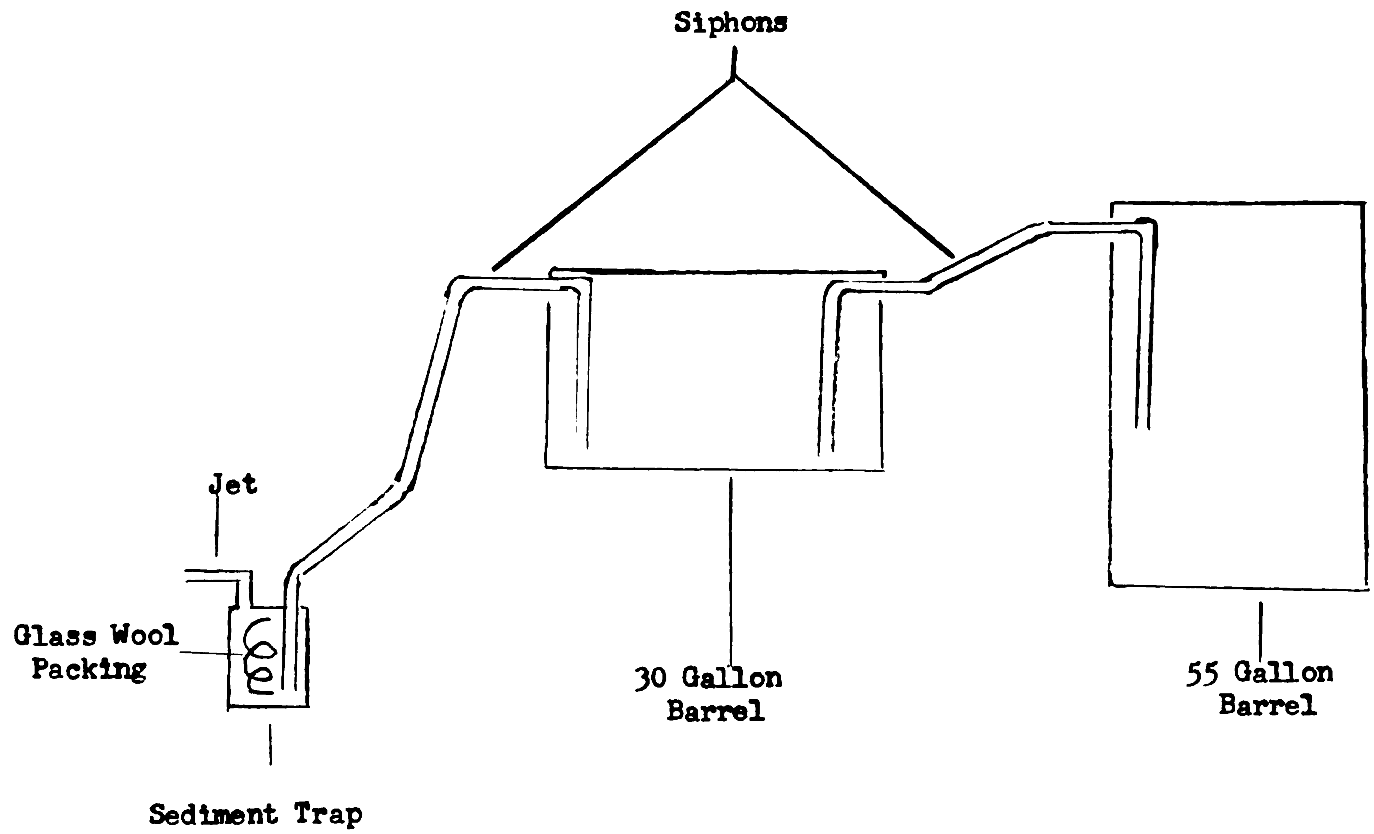
Four hundred and ten pounds of diammonium phosphate, $(NH_4)_2HPO_4$, which was rated 21-53-0 (NH_3-P_2O_5-K) was divided into 35 eleven and a half pound portions and each portion was put in a plastic bag. These bags were then transported as needed to the fertilization site and each was mixed with ten gallons of stream water in a galvanized tub and poured into the fertilization apparatus (fig. 2).

The fertilization apparatus was installed about one hundred yards upstream from the bottom sampling area at station 6. The barrels were placed on the bank and the sediment trap was located on a log in such a manner as to direct the jet of diammonium phosphate into the main core of the current. Fertilization proceeded from 2:45 p.m. August 8, 1957 until sometime in the early morning of August 17 for a total of about eight and a half days or about 204 hours.

The calculation of flow at the point of fertilization based on the figure of 30 cubic feet per second was 6.7×10^6 pounds of water per hour. The total amount of fertilizer added contained about 95 pounds of phosphorus and 71 pounds of nitrogen. The rate of addition was as even as conditions permitted but fluctuated somewhat due to variations in height of the head of solution in the barrels, which amounted to about 18 inches. During the ninth

Figure 2

Schematic Diagram of Apparatus Used
in Fertilization of the West Branch
of the Sturgeon River.


Siphons
Jet
Glass Wool
Packing
Sediment Trap
30 Gallon
Barrel
55 Gallon
Barrel

and tenth of August there were also some periods when the rate was greatly reduced due to particles clogging the jet tip. However, the assumption is made here that the rate was uniform. This would give additions of 0.466 pound of phosphorus per hour and 0.348 pound of nitrogen per hour. In terms of parts per billion this would be 70 parts per billion (p.p.b.) phosphorus and 52 p.p.b. nitrogen. These figures give an approximation of the average rate of addition of nutrients at station 6 and may be multiplied by two-thirds for corresponding values to be expected at station 7 if dilution were the only factor to be considered. This would give values of 47 p.p.b. phosphorus and 35 p.p.b. nitrogen.

Physical

Temperature

Air and water temperatures were taken with a Taylor pocket thermometer at each station on all sampling trips along with the time of day. Air temperatures were taken in the shade, but over the stream. Notes were also taken on weather conditions.

Gauge Height

A depth gauge calibrated in hundredths of feet was fastened in a permanent position at station 7 where the stream is confined between two vertical concrete bridge abutments. The gauge was installed on July 12 and readings were recorded for the remainder of the study.

Water-mass Movement Data

The time required for a patch of water dyed green with fluorescein dye to move from station 6 to various points downstream was measured.

Bottom Fauna

Six sets of bottom samples were taken at both station 3A and station 7 at two week intervals from July 5 to September 13. The samples were taken with a Surber sampler in gravel riffles. Each set consisted of a ten sample transect immediately upstream from the previous sample transect. The samples were transferred to pint bottles containing enough formalin to make a final concentration of about five percent formaldehyde.

The benthic fauna in the samples were separated by floatation using a saturated sugar solution. This work was done by prisoners at the state prison camp at Waterloo, Michigan. Total volumes were determined with ten ml. calibrated centrifuge tubes and ten ml. burettes. Counts of total numbers of organisms were also made. These data were then analyzed statistically by the use of an F test for homogeneity.

Chemical

Water Chemistry

hardness

Hardness was determined in parts per million (p.p.m.) using the versonate method.* Titra Ver and Mono Ver were used in the determination.

alkalinity

Total alkalinity was determined in p.p.m. using the titration method described in Ellis, Westfall, and Ellis (1948). Methyl orange and phenolphthalein were used as indicators.

hydrogen-ion concentration

A Beckman Model H pH Meter was used to determine pH on fresh water samples.

total phosphorus

Total phosphorus was determined by a modification of the method in Ellis, Westfall, and Ellis (1948). A Klett-Summerson Photoelectric Colorimeter was used with a red (660 millimicron) filter. Results are expressed as parts per billion (p.p.b.).

* Catalog No. 4, page 5. Hach Chemical Co. Ames, Iowa.

soluble, ortho phosphate

A series of phosphorus tests were run during fertilization in which digestion of the sample was omitted. Otherwise the samples were treated as in total phosphorus determinations. Since this method will detect all soluble ortho phosphates and the diammonium phosphate added comes under this heading, this test proved to be a valuable aid in tracing the progress of the fertilization.

Periphyton

Samples of periphyton were collected on cedar shingles which were sawed to a uniform twelve by four inch size. These shingles were attached at the butt end to logs and extended downstream parallel to the current. Each shingle had two slots cut in it in such a way that it could easily be split into three pieces, each twelve by one and one-fourth inches. Average calculated surface area of actual subshingles was 38.76 square inches, of which 16.125 square inches was the area of the upper surface. These shingles were fastened along the stream in sets of ten at both station 3A and station 7 and left for two week intervals. The sets were replaced five times, each individual shingle being replaced in the same spot by the new one. The six consecutive sets were labeled Periods A through F and are referred to by this convention for the remainder of the text. As each shingle was removed it was split into three subshingles, each of which was sealed in a plastic bag. Two parts were frozen until the periphyton could be analyzed for organic nitrogen and total phosphorus. The remaining one was scraped into a white enameled pan and washed with distilled water. The scrapings and wash water were filtered through Whatman no. 1 filter paper in Büchner funnels. The filtered material was extracted with 95 percent ethanol in one ounce glass bottles. The bottles were stored in complete darkness until they could be analyzed for pigments. In no case was any effort made to remove invertebrates from the periphyton complex before analysis.

pigments

The 95 percent alcohol extracts were filtered through Whatman no. 1 filter paper and the residue washed with enough 95 percent alcohol to bring the volume of extract up to 50 ml. The color of this solution was then read in a Klett-Summerson Photoelectric Colorimeter using a 660 millimicron filter and the reading obtained was converted to Harvey units (Harvey, 1934) by comparison with a standardization curve. The Harvey units can be converted to absorbency units by multiplying by the factor 12×10^{-3}.

organic nitrogen

One frozen subshingle from each shingle was thawed and then scraped and washed as in the pigment determination, then transferred to a 300 ml. Erlenmeyer flask. It was then acidified with sulfuric acid, concentrated by boiling, and analyzed by a semi-micro Kjeldahl procedure as described by Belcher (1945). Results are expressed in milligrams organic nitrogen per unit area (subshingle).

total phosphorus

One frozen subshingle from each shingle was thawed and then scraped and washed as in the pigment determination, then transferred to a 300 ml. Erlenmeyer flask and analyzed in the same manner as the water samples for total phosphorus. Results are expressed in micrograms phosphorus per unit area (subshingle).

<u>periphyton ratios</u>

Three ratios were calculated for each shingle; phosphorus to pigment, phosphorus to nitrogen, and pigment to nitrogen.

<u>statistical analysis</u>

All six types of periphyton chemical data were analyzed by the application of F tests and multiple range tests (Duncan, 1957).

Bottom Organisms

On July 22 and again on August 18 samples of *Chara*, mayfly naiads (*Hexagenia*), stonefly naiads (Plecoptera), and dragonfly naiads (Odonata) were collected and frozen until they could be analyzed. Rough volumes were run on the samples before freezing using 25 ml. centrifuge tubes and a 25 ml. burette. These samples were later digested and analyzed for total phosphorus in the same manner as the water samples. More acid was used in digesting the samples, however.

Isolations and Identifications of Pigments

A large volume of the ethanolic extracts of pigments from the west branch of the Sturgeon River and a large volume of an acetone extract of pigments from periphyton growing in the Red Cedar River upstream from the Michigan State University campus were separately evaporated to dryness in a vacuum desiccator under reduced lighting. These samples were then repeatedly fractionated by column chromatography using powdered sucrose and anhydrous alumina as adsorbents and a solvent system of petroleum ether-benzene (9:1). Various developers were used which incorporated petroleum ether, benzene, and isopropyl alcohol.

A Beckman Model B Spectrophotometer was used to follow the progress of the fractionations and curves were plotted for various separated components. The results of this work are reported in the appendix.

RESULTS

Physical

Temperature

A rather large diurnal fluctuation in water temperature occurs in the stream, especially on clear, warm days. This fluctuation is well illustrated by figure 3, which was recorded during a period of this type of weather. Figures 4 and 5 show the seasonal temperature fluctuations for stations 3A, 6, 7, and 8. It is easily seen that the highest water temperatures occurred during the period from the middle of July into early August at stations 7 and 8. Furthermore, the lowest overall temperatures and the least fluctuation in temperatures were recorded at station 6.

Apparently station 3A has higher temperatures and larger fluctuations due to the fact that the stream is sluggish and divided into many small channels at this point. By the time it reaches station 6 it has gained a large volume of cold spring water and this tends to stabilize the temperature at a fairly low value. The greatest fluctuation observed during the course of the study at station 6 was eight degrees Fahrenheit.

Stations 7 and 8 are subjected to more intense solar radiation and therefore have a greater range of fluctuation. It is interesting to note the close parallel in temperatures at these two stations. All available temperature and weather data are recorded in table 1.

Figure 3

Temperature Curve, Station 7, July 17, 1957, Degrees F.

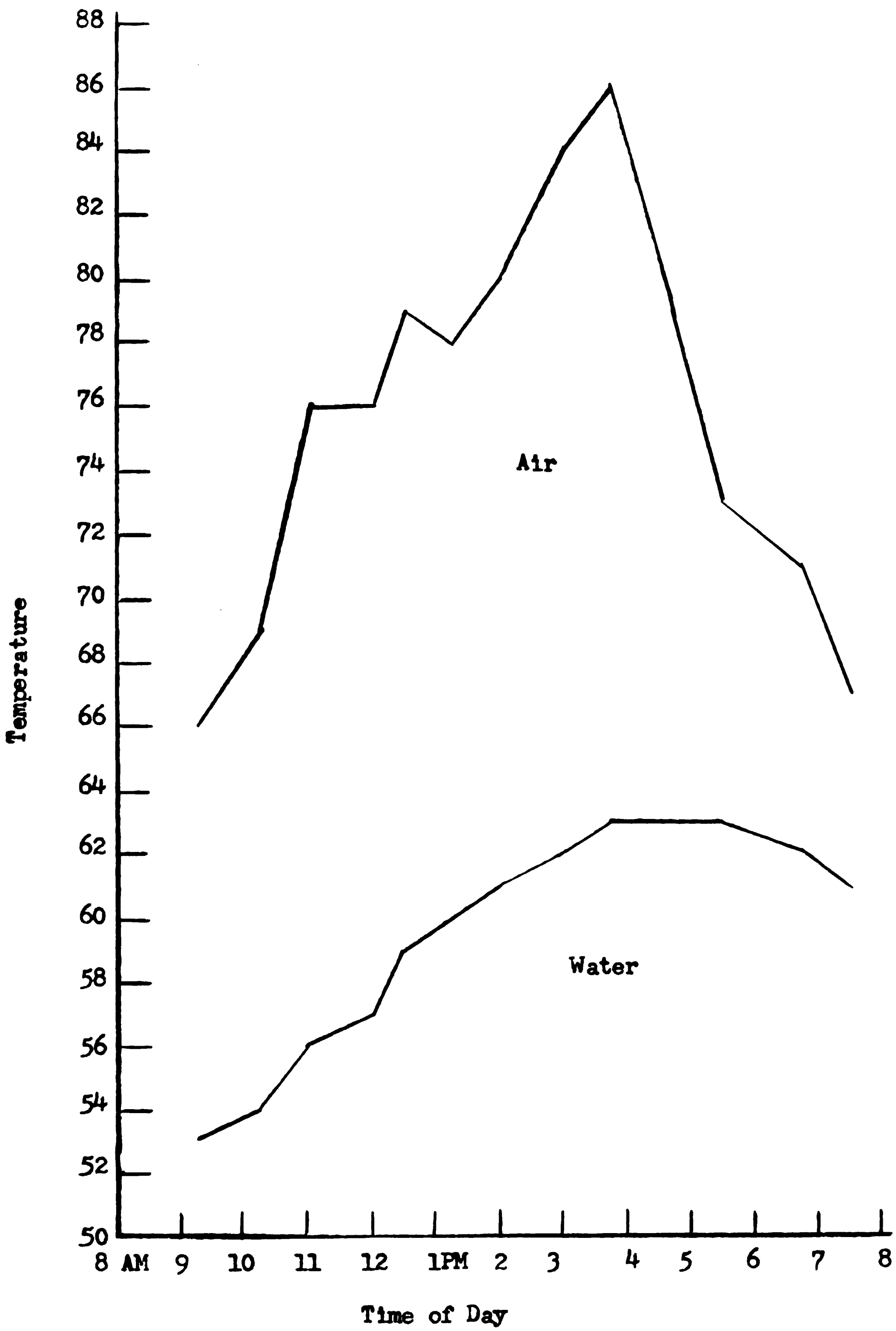

Air
Water
Temperature
88
86
84
82
80
78
76
74
72
70
68
66
64
62
60
58
56
54
52
50
8 AM
9
10
11
12
1PM
2
3
4
5
6
7
8
Time of Day

Figure 4

Water Temperatures, Stations 3A and 6, in Degrees F. (All Readings Taken Between 8 A.M. and 12 Noon)

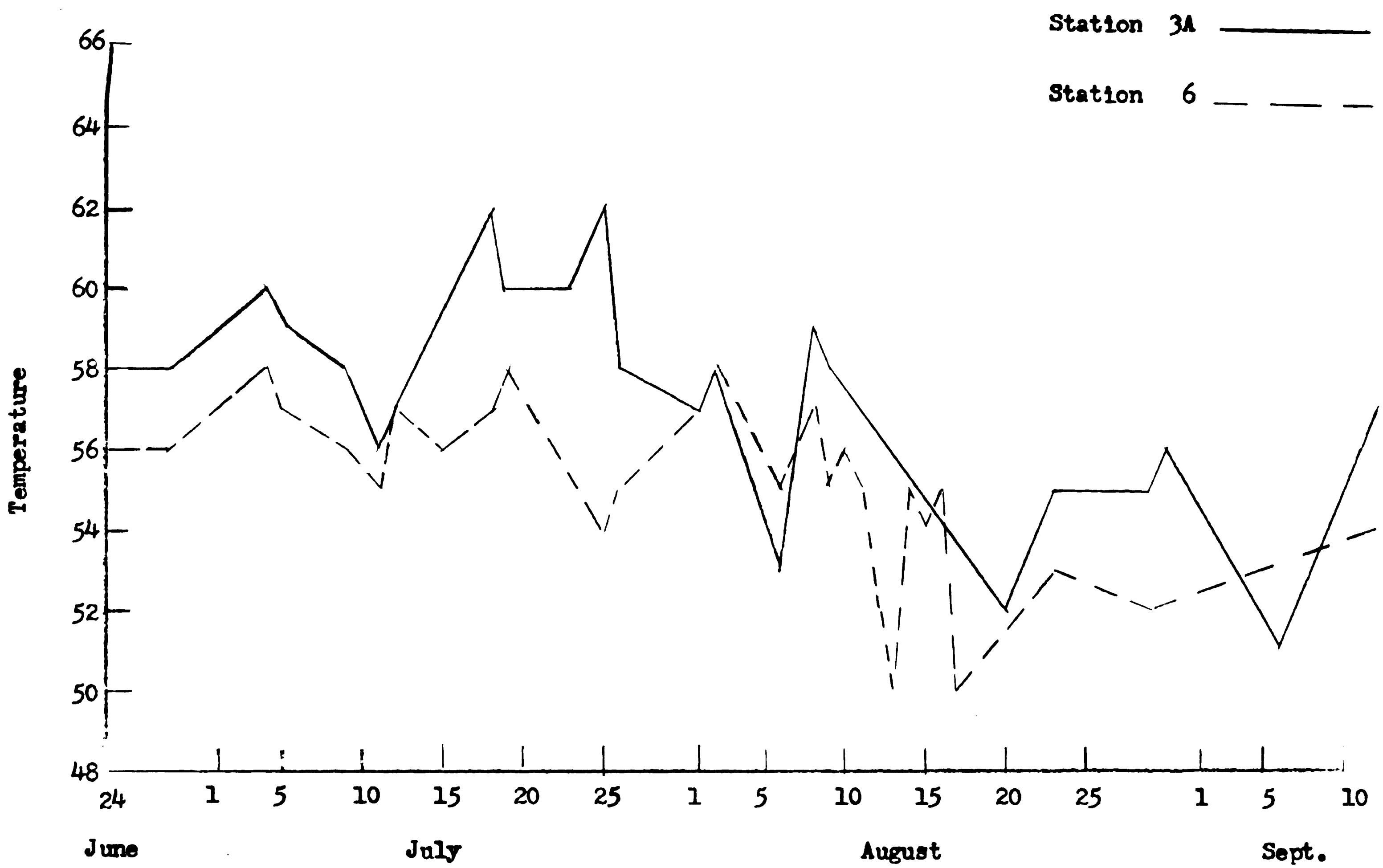

Station 3A
Station 6
Temperature
66
64
62
60
58
56
54
52
50
48
24
1
5
10
15
20
25
1
5
10
15
20
25
1
5
10
June
July
August
Sept.

Figure 5

Water Temperatures, Stations 7 and 8, in Degrees F. (All Readings Taken Between 1 P.M. and 5 P.M.)

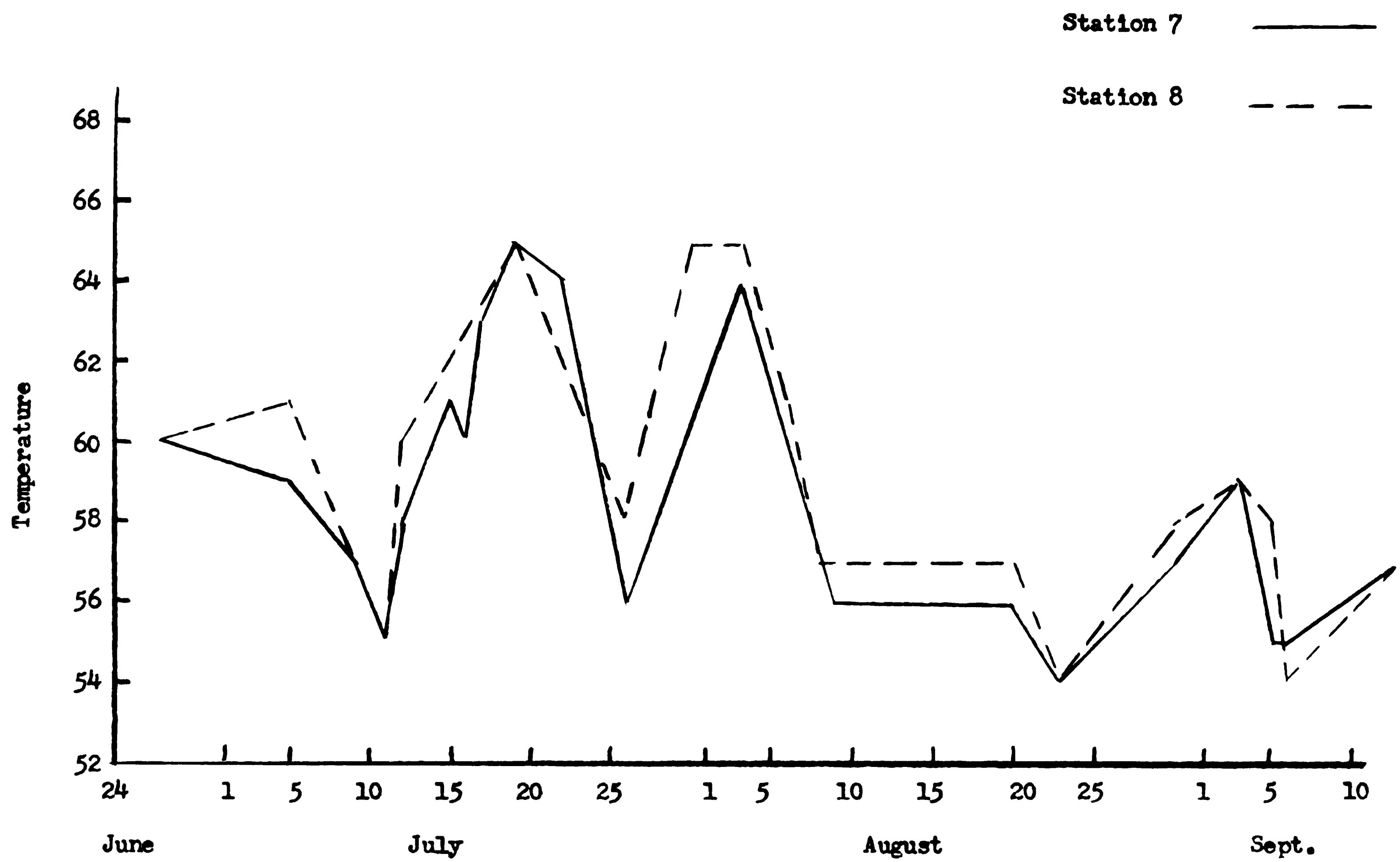

Station 7
Station 8
Temperature
68
66
64
62
60
58
56
54
52
24
1
5
10
15
20
25
1
5
10
15
20
25
1
5
10
June
July
August
Sept.

TABLE 1

Temperature Data

date	sta.	water temp.	air temp.	time	weather *	date	sta.	water temp.	air temp.	time	weather *
6-25	3A	58	60	morn.	cl,lt	7-11	8	55	66	9:30am	p
6-25	6	56	63	morn.	cl	7-12	3A	57	68	9:30am	c
6-25	7	57	62	morn.	cl	7-12	6	57	71	11:10am	cl
6-25	8	57	65	morn.	cl	7-12	7	58	64	1:40pm	cl
6-27	3A	67	73	aft.	cl	7-12	8	59	66	2:50pm	cl,lt
6-27	6	60	76	aft.	cl	7-15	6	56	64	9:45am	c
6-27	7	60	68	aft.	cl	7-15	7	61	68	1:45pm	c
6-27	8	60	65	aft.	cl	7-16	7	53	68	9:15am	p
6-28	3A	58	64	morn.	cl,r	7-16	7	57	70	12:01pm	p
6-28	6	56	63	morn.	cl,r	7-16	7	60	78	3:50pm	p
6-29	7	57	74	morn.	p	7-16	7	61	73	6:15pm	p
6-29	8	61	68	morn.	p	7-17	7	63	86	3:45pm	c
7-3	7	58	74	9:30am	c	7-18	3A	62	71	10:30am	c
7-4	3A	60	68	9:15am	c	7-18	6	57	71	10:00am	c
7-4	6	58	71	9:55am	p	7-18	7	55	71	9:40am	c
7-4	7	58	72	10:15am	p	7-18	8	55	72	9:30am	c
7-4	8	58	71	10:30am	p	7-19	3A	60	68	9:15am	c
7-5	3A	59	58	9:15am	c	7-19	6	58	74	10:50am	c
7-5	6	57	59	10:50am	p	7-19	7	65	84	2:50pm	c
7-5	7	59	73	2:10pm	p	7-19	8	65	82	3:45pm	c
7-5	8	61	64	3:30pm	cl	7-22	7	64	77	12:30pm	c
7-9	3A	58	57	10:30am	c	7-23	3A	60	67	11:30am	c
7-9	6	56	57	11:45am	p	7-23	6	58	68	12:45pm	c
7-9	7	57	66	2:15pm	cl	7-23	7	62	70	3:00pm	c
7-9	8	57	60	2:45pm	cl	7-23	8	61	62	3:45pm	c
7-11	3A	56	64	8:15am	cl	7-25	3A	62	56	10:00am	cl
7-11	6	55	61	8:45am	p	7-25	6	54	65	10:40am	cl
7-11	7	55	64	9:15am	p	7-25	7	54	69	10:55am	cl

date	sta.	water temp.	air temp.	time	weather *
7-25	8	54	69	11:05am	cl
7-25	27	54	71	11:15am	cl
7-26	3A	58	65	8:45am	cl
7-26	6	55	66	10:45am	cl
7-26	7	56	71	12:15pm	cl
7-26	8	58	73	2:50pm	cl
7-30	6	60	71	12:30pm	p
7-30	8	65	76	4:15pm	p
8-1	3A	57	70	9:10am	c
8-1	6	57	76	10:15am	c
8-1	7	57	76	10:40am	c
8-1	8	57	75	11:00am	c
8-1	27	59	77	11:10am	c
8-2	3A	58	77	9:40am	p
8-2	6	58	83	11:40am	p
8-2	7	64	83	3:35pm	c
8-2	8	65	86	4:50pm	c
8-6	3A	53	68	9:25am	c
8-6	6	55	70	11:40am	c
8-6	7	60	75	2:10pm	c
8-6	8	61	74	3:35pm	c
8-8	3A	59	71	10:00am	p
8-8	6	57	68	10:45am	cl
8-8	7	58	66	3:20pm	cl
8-8	8	57	70	4:00pm	cl
8-8	27	57	72	4:25pm	cl
8-9	3A	57	66	10:30am	cl,r
8-9	6	55	66	9:45am	cl,lr
8-9	7	55	67	9:20am	cl
8-9	8	55	67	9:00am	cl,lr
8-9	27	55	65	8:45am	cl,lr
8-9	7	56	69	1:10pm	cl,lr

date	sta.	water temp.	air temp.	time	weather *
8-9	8	57	70	2:55pm	cl,lr
8-10	6	54	68	9:25am	c
8-10	7	54	68	9:10am	c
8-10	8	54	68	9:00am	c
8-10	27	54	67	8:50am	c
8-11	6	55	69	9:45am	p
8-11	7	54	70	9:35am	p
8-11	8	54	70	9:25am	p
8-11	27	54	69	9:10am	p
8-12	6	52	60	9:50am	cl
8-12	7	51	60	9:40am	cl
8-12	8	52	60	9:30am	cl
8-12	27	52	60	9:15am	cl
8-13	6	50	59	9:45am	cl
8-13	7	50	60	9:30am	cl
8-13	8	49	60	9:20am	cl
8-13	27	49	60	9:10am	cl
8-14	6	55	70	10:40am	cl
8-14	7	54	72	10:20am	cl
8-14	8	54	70	10:10am	cl
8-14	27	54	68	9:50am	cl
8-15	6	54	58	9:05am	cl,lr
8-15	7	54	59	8:50am	cl
8-15	8	54	59	8:40am	cl
8-15	27	54	60	8:25am	cl
8-15	3A	57	62	10:40am	cl
8-16	3A	54	60	10:30am	p
8-16	6	55	64	11:50am	p
8-16	7	54	64	11:30am	p
8-16	8	53	67	11:20am	p
8-16	27	53	67	11:10am	p
8-17	6	50	62	10:20am	c

date	sta.	water temp.	air temp.	time	weather *	date	sta.	water temp.	air temp.	time	weather *
8-17	7	49	64	10:00am	c	9-3	7	59	64	4:15pm	cl,lt
8-17	8	49	62	9:20am	c	9-3	8	59	64	5:00pm	cl,r
8-17	27	49	62	9:10am	c	9-5	3A	59	63	1:10pm	c
8-18	7	51	69	10:45am	-	9-5	6	55	57	1:45pm	c
8-18	8	51	68	10:35am	-	9-5	7	55	62	2:00pm	c
8-18	27	52	67	10:25am	-	9-5	8	58	63	4:30pm	c
8-20	3A	52	70	10:15am	c	9-6	3A	51	57	10:30am	cl
8-20	6	56	71	1:45pm	p	9-6	6	53	61	1:00pm	p
8-20	7	56	69	3:10pm	cl	9-6	7	55	62	2:15pm	p
8-20	8	57	64	3:50pm	cl,lr	9-6	8	54	64	2:45pm	p
8-23	3A	55	69	10:05am	cl	9-10	7	50	56	10:45am	cl
8-23	6	53	68	11:35am	cl	9-11	7	50	61	9:50am	cl
8-23	7	54	63	2:15pm	cl,lr	9-12	3A	57	63	11:15am	cl
8-23	8	54	63	3:10pm	cl,lr	9-12	6	54	61	11:50am	cl
8-27	7	50	58	morn.	p	9-12	7	54	62	12:05pm	cl
8-29	3A	55	60	11:20am	cl	9-12	8	54	63	12:15pm	cl
8-29	6	52	59	11:50am	cl	9-13	3A	56	65	10:15am	cl
8-29	7	52	60	12:01pm	cl	9-13	6	55	63	2:00pm	cl
8-29	8	52	60	12:10pm	cl	9-13	7	57	64	3:20pm	p
8-30	3A	56	66	10:15am	cl	9-13	8	57	63	4:00pm	p
8-30	6	56	65	1:10pm	p	9-17	3A	49	54	10:00am	c
8-30	7	57	69	2:30pm	p	9-17	6	53	62	2:20pm	c
8-30	8	58	72	3:30pm	c	9-17	7	54	66	2:40pm	c
9-3	3A	61	76	12:30pm	cl	9-17	8	55	65	4:00pm	c
9-3	6	58	64	2:30pm	cl						

*weather code: c, clear; cl, cloudy; p, partly cloudy; r, rain; lr, light rain

Gauge Height

In the period from July 12 to September 17 the stream level underwent only minor fluctuations even though this period included several rains in early September. The data is plotted in figure 6. The maximum fluctuation during this period was less than three inches. Early in the study, however, the stream was considerably higher and roily. This was particularly true on June 29, at which time the stream was at least twelve inches above base flow.

Water-mass Movement Data

On August 1, at which time the stream was near base flow, a patch of water was dyed green at station 6. It required two hours and twenty minutes for the dye to reach station 7 and an additional hour and fifteen minutes to reach station 8.

Bottom Fauna

Total Volumes of Bottom Fauna

The means of the total volumes of bottom fauna for stations 3A and 7 are plotted in figure 7 along with twice the standard deviation of the mean. It can be seen by this rough method that no two consecutive periods show a difference that is valid at a 95 percent confidence limit at either station. There are, however, significant differences between some pairs of values at both stations.

The application of an F test to station 3A data showed that the means were not significantly different at the five percent level. Thus it may be said with considerable assurance that the volumes

Figure 6

Relative Guage Height at
Station 7

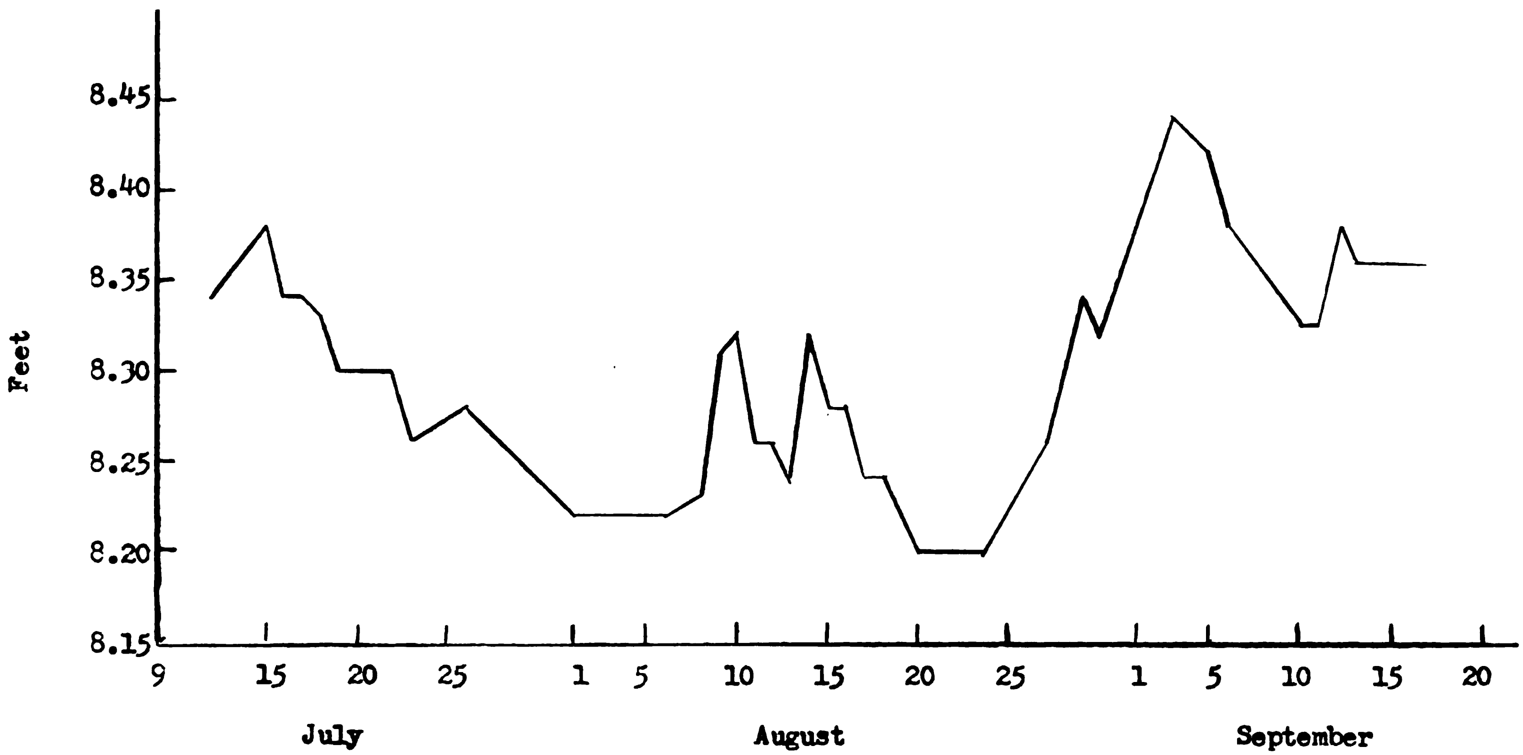
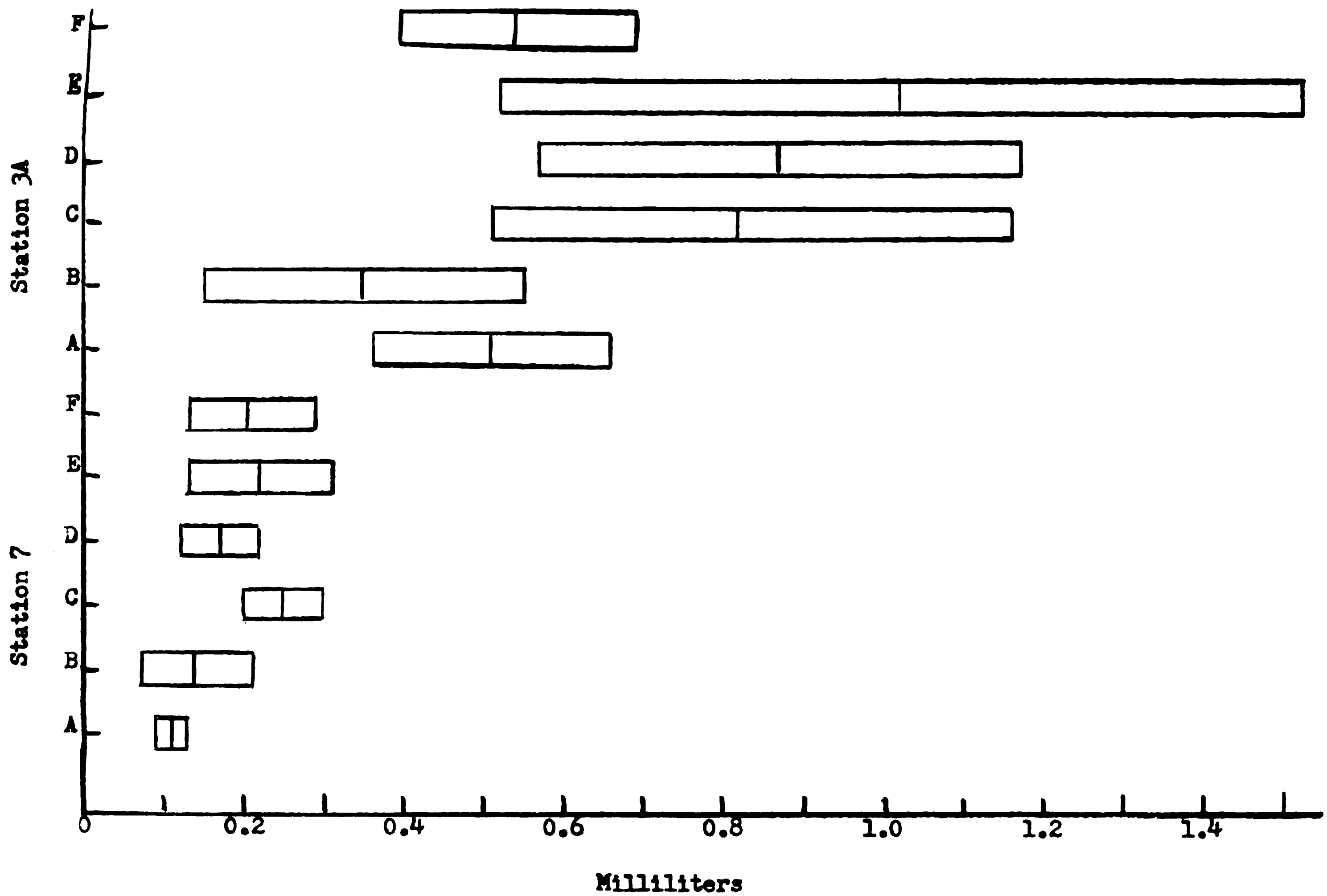

Feet
8.45
8.40
8.35
8.30
8.25
8.20
8.15
9
15
20
25
1
5
10
15
20
25
1
5
10
15
20
July
August
September

Figure 7

Total Volumes of Bottom Fauna in Milliliters per Surber Sample at Six Times During the Summer. (Mean ± 2 Standard Deviations of the Mean).


Station 3A
Station 7
F
E
D
C
B
A
F
E
D
C
B
A
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Milliliters

of bottom fauna at station 3A, the control for this study, had little if any seasonal fluctuations.

An F test on the data from station 7 also showed that these means were not significantly different at the five percent level. The data and statistical analysis are summarized in tables 2 and 3.

Total Numbers

The data and a limited amount of statistics concerning them are summarized in tables 4 and 5. A rough plot of the means and twice the standard deviations of the means is shown in figure 8 for both station 3A and station 7. Nothing further was done with total numbers statistically since it is easily seen that it would be difficult and certainly meaningless to attempt to correlate the erratic fluctuations shown without breaking the samples down into taxonomic units.

Chemical

Water Chemistry

hardness

In general hardness was a function of surface runoff. Heavy rains resulted in lower hardness and when the stream was at base flow the hardness was highest, since the spring water was saturated with calcium bicarbonate. Table 6 records the data obtained for stations 3A, 6, 7, 8, and 27. On August 22 a sample was taken at the point where the stream originates in the lake. At this point the hardness was 150 p.p.m., while on the same day the water at

TABLE 2

Total Volumes of Bottom Fauna-Station 3A in Cubic Centimeters per Square Foot During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	0.25	0.32	0.17	0.66	0.89	0.74
2	1.10	0.31	1.28	0.70	0.40	0.85
3	0.18	0.26	1.01	1.33	0.58	0.82
4	0.36	0.40	0.76	0.58	0.88	0.43
5	0.28	0.23	1.41	0.48	1.68	0.37
6	1.53	0.22	0.37	0.42	0.67	0.15
7	0.15	0.12	0.23	1.37	2.97	0.64
8	0.14	0.14	1.45	0.33	0.55	0.36
9	0.21	0.33	0.57	1.28	0.62	0.27
10	0.94	1.18	0.99	1.59	0.96	0.78
sum	5.14	3.51	8.24	8.74	10.20	5.41
$\bar{X}$	0.51	0.35	0.82	0.87	1.02	0.54
ΣX^2	4.82	2.06	8.85	9.59	15.76	3.51
$(\Sigma X)^2/n$	2.64	1.23	6.79	7.64	10.40	2.93
Σx^2	2.18	0.83	2.07	1.95	5.36	0.58
var.	0.24	0.09	0.23	0.22	0.60	0.06
sta. dev.	0.49	0.30	0.48	0.46	0.77	0.25
$(\Sigma X)^2$	26.42	12.32	67.90	76.39	104.04	29.27

$SS_T = 15.78$, df $= 59$

$SS_B = 2.81$, df $= 5$

$SS_W = 12.97$, df $= 54$

$s^2_B = 0.562$

$s^2_W = 0.240$

$F = 2.34$

CODE

$\bar{X}$ = mean

ΣX^2 = sum of squares

$\Sigma x^2 = \Sigma X^2 - (\Sigma X)^2/n$

var. = variance

sta. dev. = standard deviation

$(\Sigma X)^2$ = sum squared

SS_T = sum of squares total

SS_B = sum of squares between groups

SS_W = sum of squares within groups

s^2_B = mean square between, s^2_W = mean square within

TABLE 3

Total Volumes of Bottom Fauna-Station 7 in Cubic Centimeters per Square Foot During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	0.11	0.08	0.33	0.28	0.25	0.47
2	0.15	0.05	0.22	0.18	0.14	0.21
3	0.08	0.04	0.39	0.17	0.17	0.15
4	0.05	0.06	0.24	0.07	0.20	0.04
5	0.11	0.02	0.36	0.08	0.26	0.14
6	0.13	0.20	0.13	0.10	0.11	0.20
7	0.10	0.19	0.19	0.15	0.22	0.12
8	0.11	0.14	0.17	0.15	0.07	0.22
9	0.15	0.37	0.22	0.23	0.60	0.21
10	0.14	0.22	0.23	0.29	0.17	0.35
sum	1.13	1.37	2.48	1.70	2.19	2.11
$\bar{X}$	0.11	0.14	0.25	0.17	0.22	0.21
ΣX^2	0.14	0.30	0.68	0.34	0.67	0.58
$(\Sigma X)^2/n$	0.13	0.19	0.62	0.29	0.48	0.45
Σx^2	0.01	0.11	0.06	0.05	0.20	0.13
var.	0.00	0.01	0.01	0.01	0.02	0.01
sta. dev.	0.03	0.11	0.08	0.07	0.15	0.12
$(\Sigma X)^2$	1.28	1.88	6.15	2.89	4.80	4.45

$SS_T = 0.67$, df = 59

$SS_B = 0.10$, df = 5

$SS_W = 0.57$, df = 54

$s_B^2 = 0.020$

$s_W^2 = 0.011$

$F = 1.89$

TABLE 4

Total Numbers of Bottom Fauna-Station 3A per Square Foot During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	72	72	187	314	351	129
2	77	104	228	431	258	172
3	119	72	226	398	202	110
4	197	109	256	313	253	101
5	141	119	257	350	201	212
6	175	147	199	203	316	65
7	42	85	217	238	444	65
8	49	69	219	250	253	93
9	112	163	255	338	166	214
10	111	261	271	285	192	127
sum	1,095	1,201	2,315	3,120	2,636	1,288
$\bar{X}$	109.5	120.1	231.5	312.0	263.6	128.8
ΣX^2	143,619	175,511	542,731	1,019,052	760,400	192,494
$(\Sigma X)^2/n$	119,902	144,240	535,922	973,440	694,850	165,894
Σx^2	23,717	31,271	6,809	35,612	65,550	26,600
var.	2,635	3,475	757	3,957	7,283	2,956
sta. dev.	51.3	59.0	27.5	62.9	85.3	54.4
$(\Sigma X)^2$	1,199,025	1,442,401	5,359,225	9,734,400	6,948,496	1,658,944

TABLE 5

Total Numbers of Bottom Fauna-Station 7 per Square Foot During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	15	53	50	82	102	288
2	17	45	58	89	107	248
3	13	31	37	85	107	220
4	8	45	45	30	299	8
5	12	23	58	32	115	173
6	15	64	44	65	267	153
7	6	44	55	36	91	94
8	18	27	78	95	198	114
9	27	66	60	84	350	276
10	23	48	65	88	239	196
sum	154	446	550	686	1,875	1,770
$\bar{X}$	15.4	44.6	55.0	68.6	187.5	177.0
ΣX^2	2,734	21,770	31,492	53,140	434,323	382,674
$(\Sigma X)^2/n$	2,372	19,892	20,250	47,060	351,562	313,290
Σx^2	362	1,878	11,242	6,080	82,761	69,384
var.	40.2	208.7	1,249.1	675.6	9,195.7	7,709.3
sta. dev.	6.3	14.4	35.3	26.0	95.9	87.8
$(\Sigma X)^2$	23,716	198,916	302,500	470,596	3,515,625	3,132,900

Figure 8

Total Numbers of Bottom Fauna per Surber Sample at Six Times During the Summer. (Mean $\pm$ 2 Standard Deviations of the Mean).

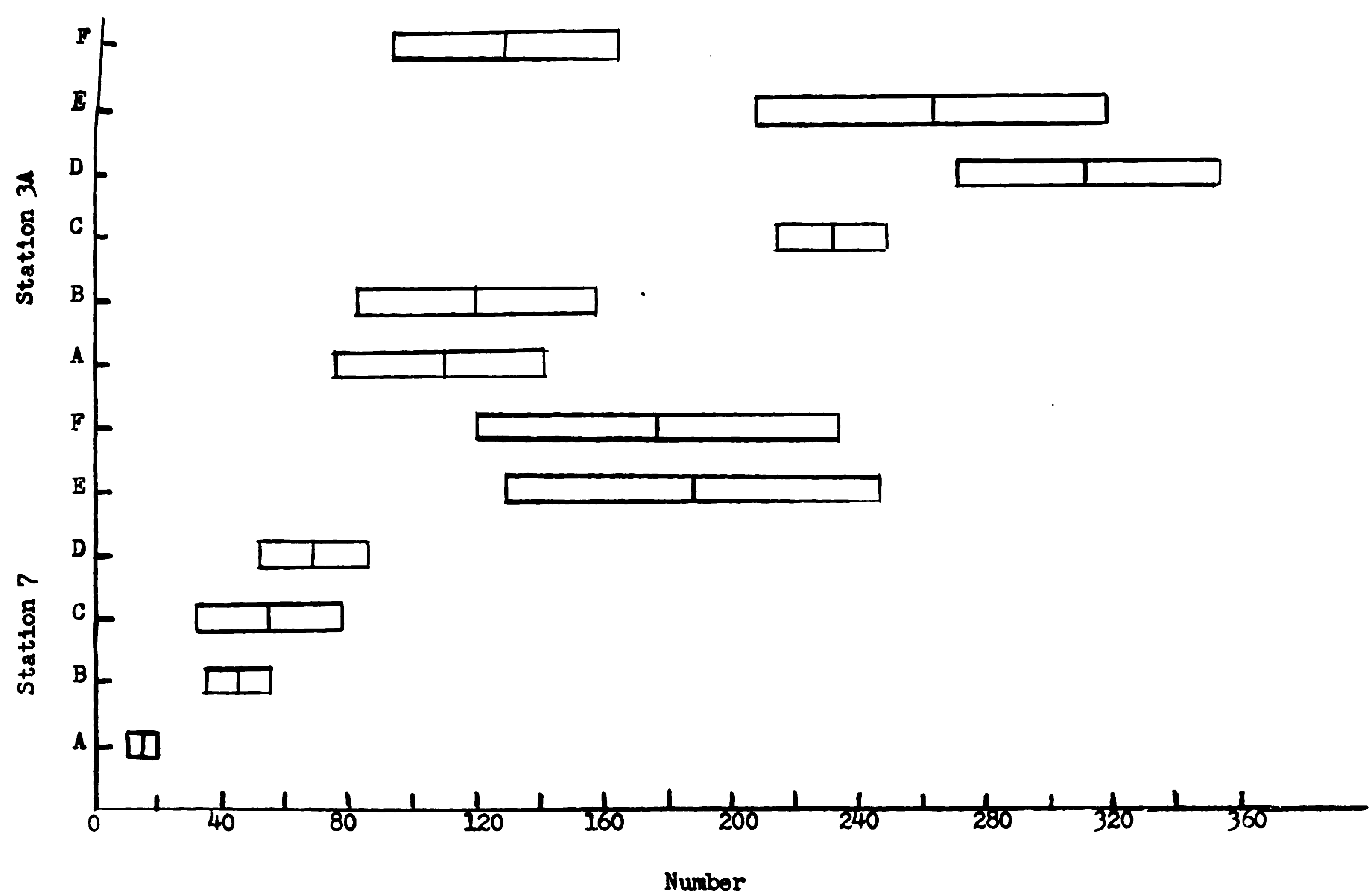

Station 3A
Station 7
F
E
D
C
B
A
F
E
D
C
B
A
0
40
80
120
160
200
240
280
320
360
Number

TABLE 6

Water Hardness in Parts per Million

date	sta. 3A	sta. 6	sta. 7	sta. 8	sta. 27
6-27	192	188	190	188	...
7-4	175	168	170	173	...
7-11	194	196	194	193	...
7-18	187	187	186	196	...
7-25	194	195	193	197	197
8-1	194	195	193	196	197
8-8	196	191	190	193	192
8-9	193	188	193	...	...
8-10	...	196	196	201	200
8-11	...	196	196	202	196
8-12	...	197	196	199	200
8-13	...	194	196	196	196
8-14	...	193	192	194	195
8-15	194	195	194	197	197
8-16	...	195	193	195	196
8-17	...	193	193	193	195
8-18	...	196	192	195	197
8-22	194	191	193	195	...
8-29	199	196	196	197	...
9-5	192	194	193	195	...
9-12	195	196	195	196	...

station 3A had a hardness of 194 p.p.m. The highest value recorded in this study is 202 p.p.m. at station 8 on August 11. The lowest value, 168 p.p.m., was found at station 6 on July 4 during the period in which the water level was very high due to heavy rains over a prolonged period. The data from stations 3A and 7 are plotted in figure 9.

<u>alkalinity</u>

On June 27 alkalinity was run on stations 3A, 6, 7, and 8. All detectable alkalinity was methyl orange alkalinity and checked within two parts per million with total hardness run on the same samples. It appears from this data that all or almost all of the alkalinity and hardness was due to calcium bicarbonate in solution.

<u>hydrogen-ion concentration</u>

The pH of the water samples was restricted to the range between 7.8 and 8.4 with only one exception and the majority were between 8.0 and 8.3. These values are recorded in table 7. It is interesting to note that the lowest pH values were recorded from August 8 to August 12, but it is not very likely that this fact had anything to do with the addition of fertilizer. This series of low values was also found at station 3A and may have been due to considerable amounts of organic acids being produced in the upper swampy section of the stream and in the beaver ponds since it was warm weather and the water was low.

<u>total phosphorus</u>

The data compiled for the results of total phosphorus determinations are shown in table 8. Normal levels are about ten parts per

Figure 9

Total Harness of water,
Stations 3 and 7

TABLE 7

Hydrogen-ion Concentration of Water
in pH Units

date	sta. 3A	sta. 6	sta. 7	sta. 8	sta. 27
6-27	8.2	8.3	8.3	8.3	...
7-4	8.1	8.2	8.2	8.2	...
7-11	8.1	8.3	8.3	8.3	...
7-18	8.0	8.3	8.3	8.2	...
7-25	8.1	8.3	8.3	8.3	8.3
8-1	8.2	8.3	8.3	8.3	8.3
8-8	8.1	8.0	8.1	8.1	7.9
8-9	7.6	7.8	8.0	...	...
8-10	...	7.9	7.9	7.9	8.0
8-11	...	7.9	7.9	7.9	7.9
8-12	...	8.2	8.1	8.2	8.2
8-13	...	8.3	8.2	8.2	8.3
8-14	...	8.3	8.2	8.2	8.2
8-15	8.1	8.2	8.1	8.1	8.2
8-16	...	8.3	8.2	8.2	8.2
8-17	...	8.2	8.2	8.2	8.2
8-18	...	8.1	8.2	8.2	8.2
8-22	8.4	8.4	8.3	8.3	...
8-29	8.3	8.3	8.2	8.2	...
9-5	8.4	8.3	8.2	8.3	...
9-12	8.2	8.2	8.2	8.2	...

TABLE 8

Total Water Borne Phosphorus
in Parts per Billion

date*	sta. 3A**	sta. 6	sta. 7	sta. 8	sta. 27
6-27	11	11	20	9	..
7-4	11	17	13	17	..
7-11	1	6	5	5	..
7-18	6	4	4	4	..
7-25	8	7	5	5	7
8-1	4	3	2	5	0
8-8 (am)	14	13	17	17	8
8-8 (8pm)	..	38	22	12	13
8-9 (1am)	..	16	25	10	9
8-9 (6am)	..	21	18	15	28
8-9 (9am)	..	23	19	13	7
8-10	..	19	19	14	..
8-11	..	31	24	51	27
8-12	..	71	51	32	37
8-13	..	71	55	47	32
8-14	..	69	39	51	41
8-15	10	49	..	29	25
8-16	..	65	38	33	28
8-17	..	8	18	25	26
8-18	..	5	4	4	10
8-22	11	14	11	13	..
8-29	20	12	14	29	..
9-5	9	10	11	9	..
9-12	11	13	10	13	..

* Fertilizer applied August 8-17, 1957.
** Control Station.

billion or less at all stations. There are a number of factors which can raise this level, however.

Heavy rain tends to bring organic debris and nutrients into the stream in the surface runoff. During long warm periods the stream has a higher phosphorus level due to more rapid decay of organic materials in such places as beaver ponds. Life cycles of various aquatic plants may also play a role in this phenomenon. Higher values than normal were found on June 27, July 4, and the morning of August 8. The results of the entire study period for stations 3A and 6 are plotted in figure 10 and for stations 7 and 8 in figure 11.

During the period in which the diammonium phosphate was added there can be no doubt that the addition was somewhat erratic and the water samples taken are only an attempt to obtain a rough idea of the amount of phosphorus moving downstream. This period for stations 6, 7, 8, and 27 is shown in figures 12 and 13. It is interesting to note that when fertilization was stopped early in the morning on August 17, there was a sharp drop in phosphorus values back to the normal range. The rise in values for August 29 at both stations 3A and 8 could be due to rain which is recorded in the gauge height data as a rainy period. It could also be due to regeneration of phosphorus from upstream in the case of station 8.

Although it is quite possible that there was a time lapse in the build-up of phosphorus in stations which were progressively farther downstream, this cannot be shown in a clear-cut manner due to a period from the early morning of August 9 to 10:00 a.m. on

Figure 10

Total Phosphorus in Water,
Stations 3A and 6

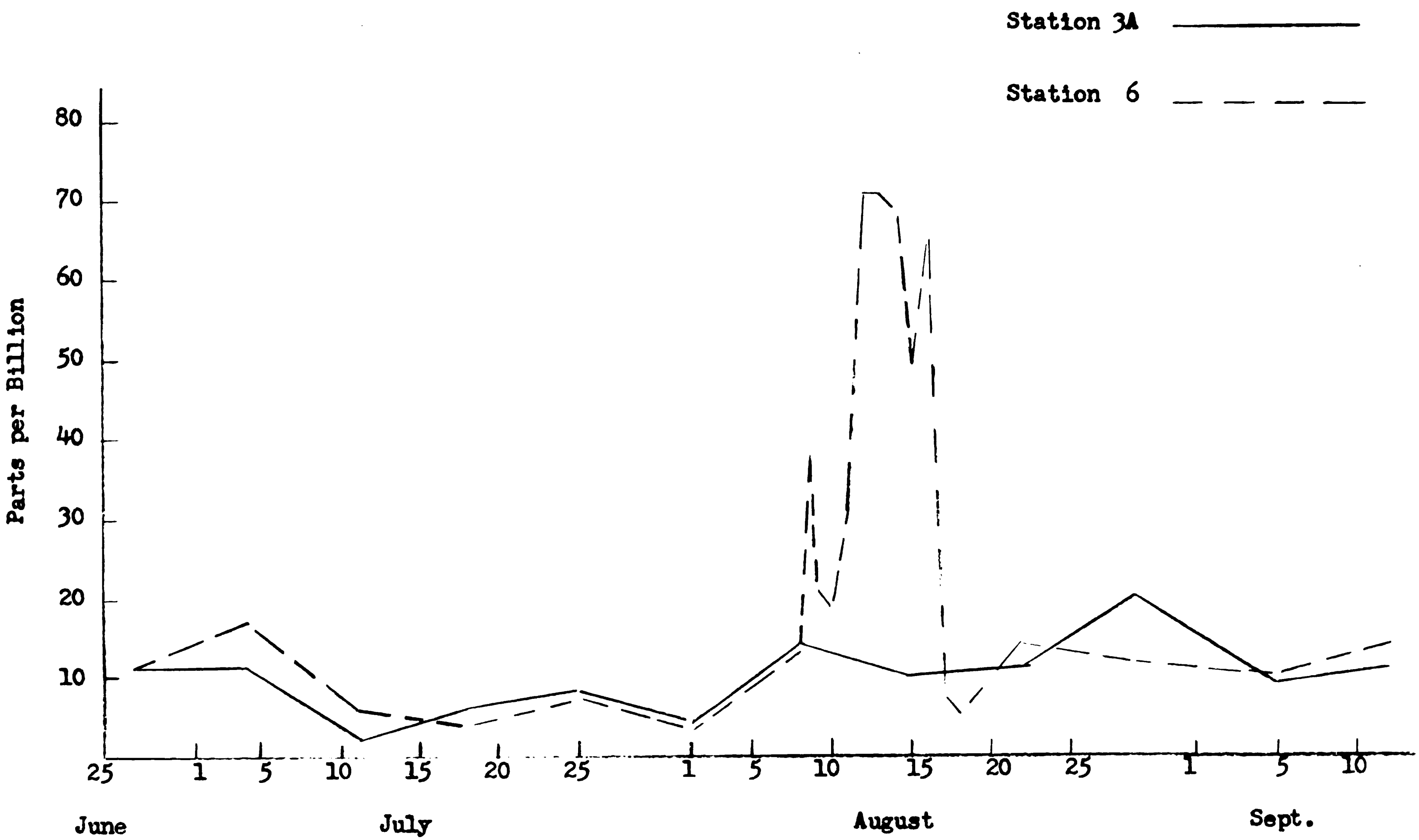

Station 3A
Station 6
Parts per Billion
80
70
60
50
40
30
20
10
25
1
5
10
15
20
25
1
5
10
15
20
25
1
5
10
June
July
August
Sept.

Figure 11

Total Phosphorus in Water,
Stations 7 and 8.

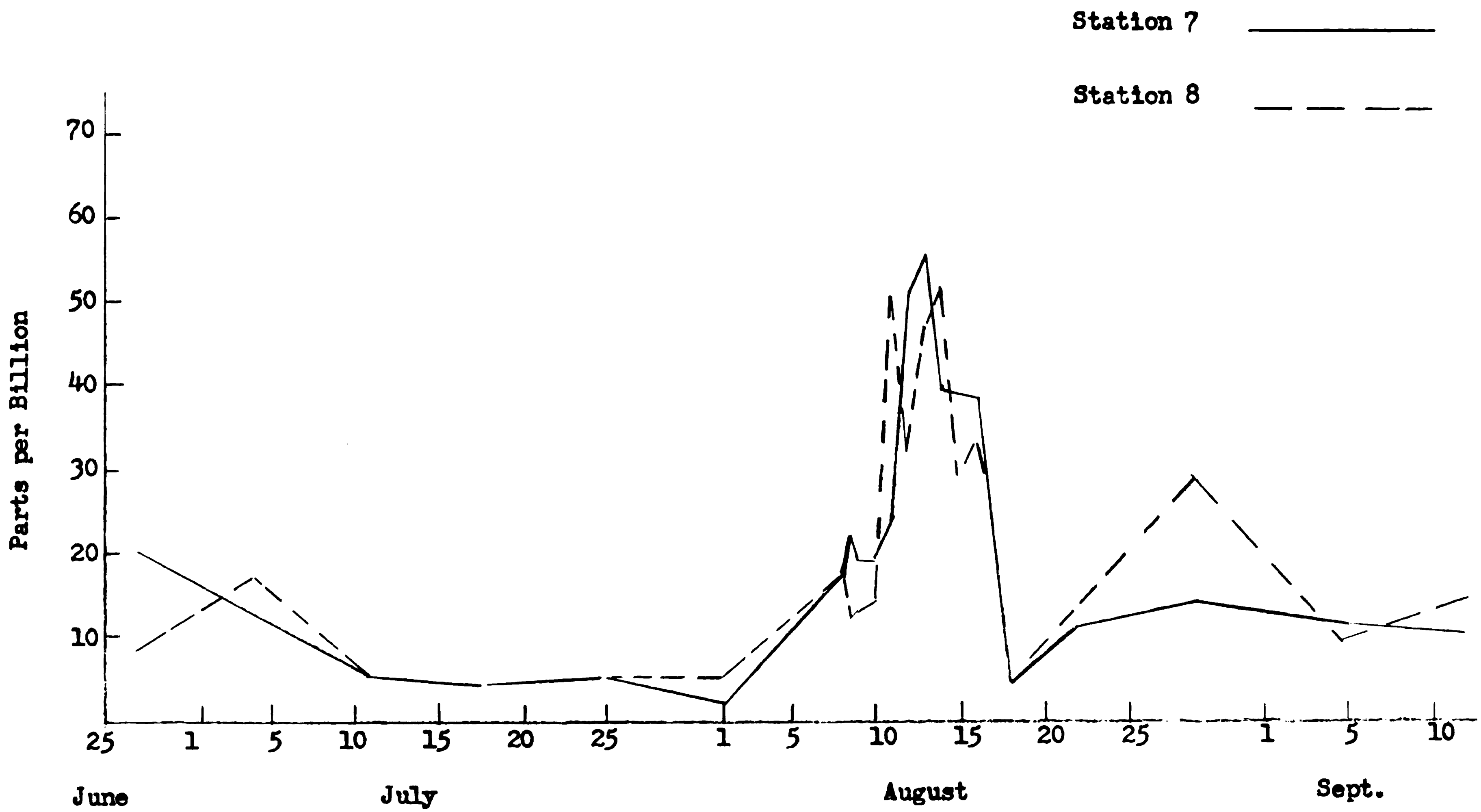

Station 7
Station 8
Parts per Billion
70
60
50
40
30
20
10
25
1
5
10
15
20
25
1
5
10
15
20
25
1
5
10
June
July
August
Sept.

Figure 12

Total Phosphorus in Water During Period of July 24 to August 29.

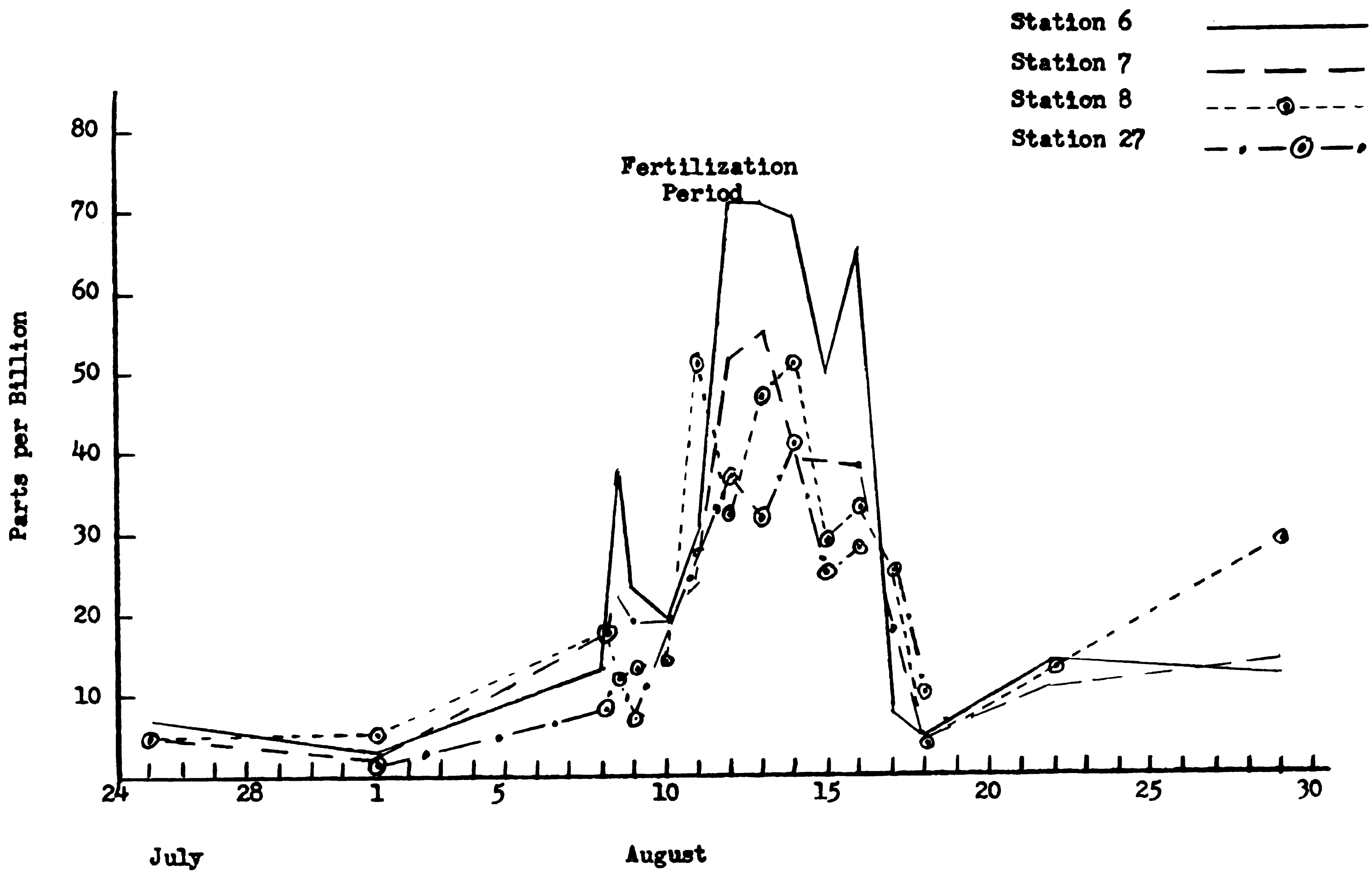
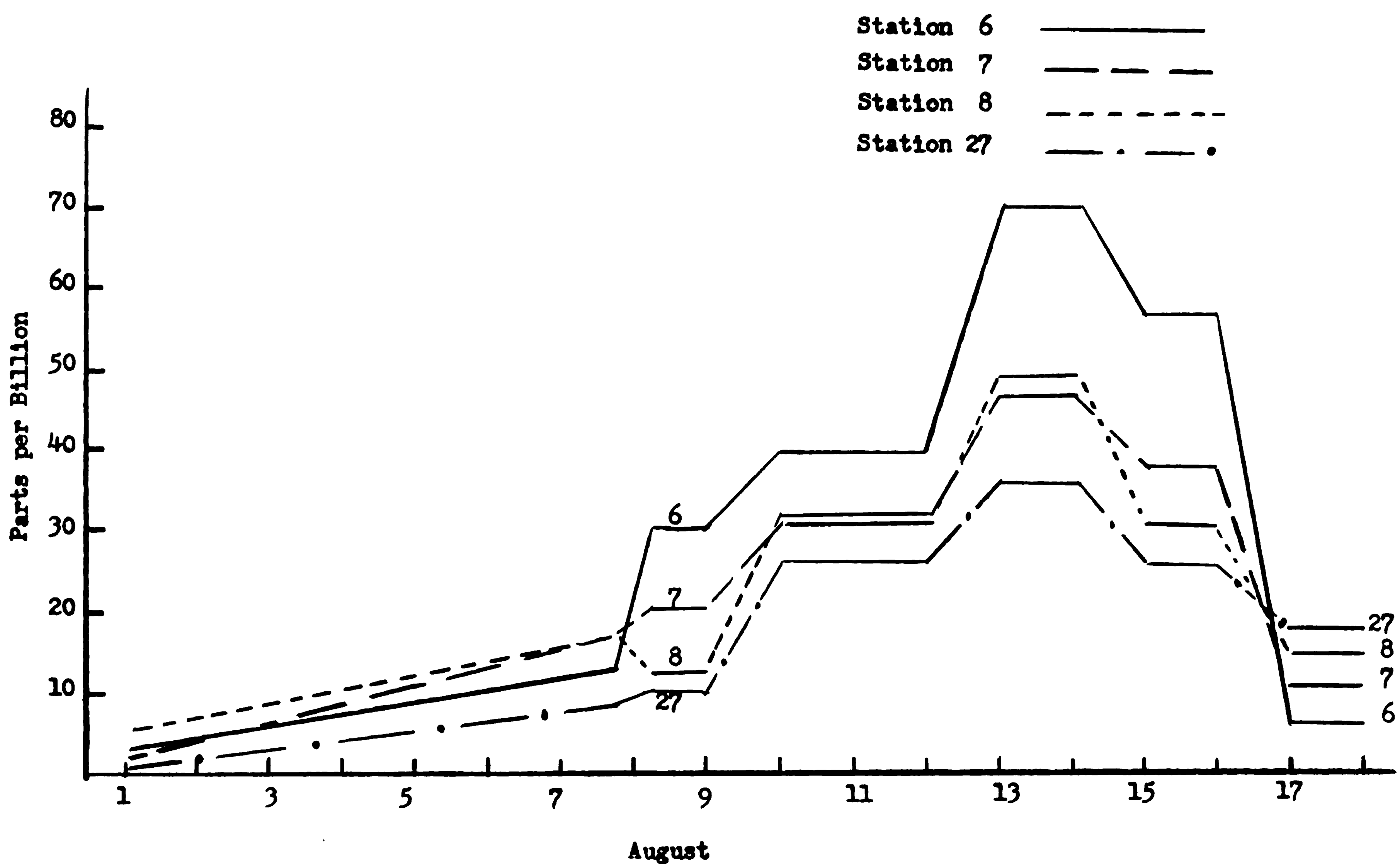

Station 6
Station 7
Station 8
Station 27
Fertilization Period
Parts per Billion
80
70
60
50
40
30
20
10
24
28
1
5
10
15
20
25
30
July
August

Figure 13

Total Phosphorus in Water During
Period August 1 to August 18
(Idealized by Grouping and
Averaging)


Station 6
Station 7
Station 8
Station 27
Parts per Billion
80
70
60
50
40
30
20
10
1
3
5
7
9
11
13
15
17
August
6
7
8
27

August 10 during which the fertilizer was added at an erratic rate due to the filter becoming plugged several times.

About August 18 it was noted that there was a fairly heavy growth of filamentous green algae in the part of the stream above the point of fertilizer input (station 6). On August 22 water samples were taken along the upper section of the stream and all had total phosphorus values around 22 to 24 p.p.b. Samples of the filamentous algae were found to be made up of Spirogyra and Mougeotia in a proportion of roughly two to one (Keup, M.S.). The effects of these slightly higher nutrient levels seems, however, not to have been very great in terms of increased periphyton growth on shingles. The proportion of total phosphate available to the algae may have been much lower. There is no way of knowing from this data, how much soluble phosphorus was present at this time.

soluble ortho-phosphate

Determinations of soluble ortho-phosphate were only made during the period from the evening of August 8 to the morning of August 18. The data is recorded in table 9. It is interesting to note that the highest values recorded correspond very closely with the values theoretically calculated as average values assuming no biological uptake. The calculated values were 70 p.p.b. and 47 p.p.b. for stations 6 and 7 respectively and the highest values are 70 p.p.b. and 46 p.p.b. The data for stations 6 and 7 are plotted in figure 14.

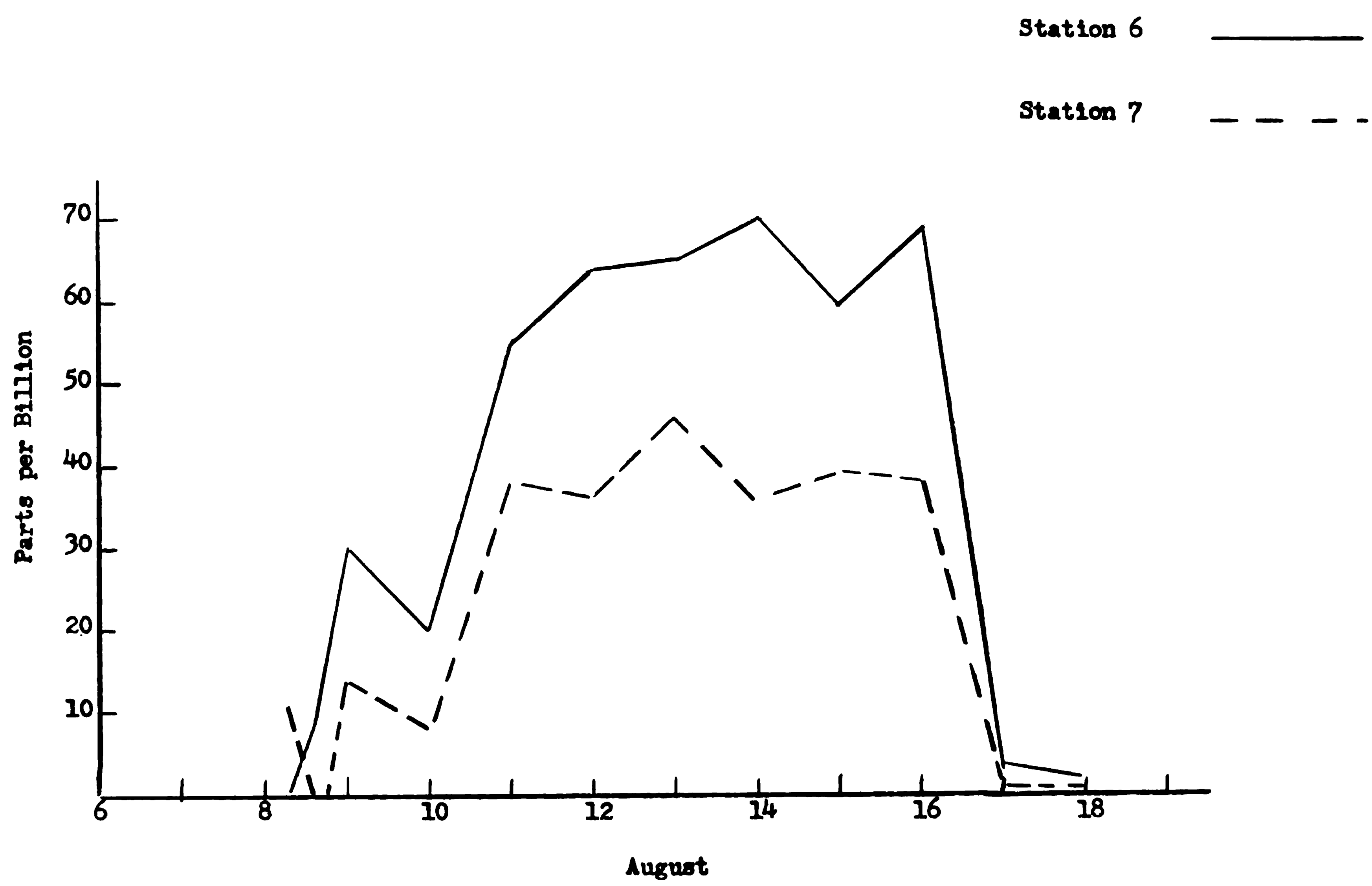
TABLE 9

Soluble Ortho Phosphate in Water
Expressed in Parts per Billion

date	sta. 6	sta. 7	sta. 8	sta. 27
8-8 (8pm)	0	11	1	6
8-9 (1am)	8	0	1	0
8-9 (6am)	12	0	6	0
8-9 (9am)	30	13	8	6
8-10	20	8	11	3
8-11	55	38	30	17
8-12	64	36	30	20
8-13	65	46	39	30
8-14	70	36	28	21
8-15	59	39	33	23
8-16	69	38	33	23
8-17	1	3	19	19
8-18	0	2	0	2

Figure 14

Soluble "Ortho" Phosphate in Water During Period August 8 to August 18.



Station 6
Station 7
70
60
50
40
30
20
10
Parts per Billion
6
8
10
12
14
16
18
August

Periphyton

pigments

The mean pigment values in Harvey units per unit area (sub-shingle) for each period at stations 3A and 7 are shown as a histogram in figure 15. The three fold change in absorbency as represented here would be even larger if the fact that the pigment complex doesn't follow the Lambert-Beer Law at values above 16 Harvey units were taken into account. This fact has become evident as a result of work being carried out by Brehmer (M.S.). The corrected value would be much higher for station 7, period D.

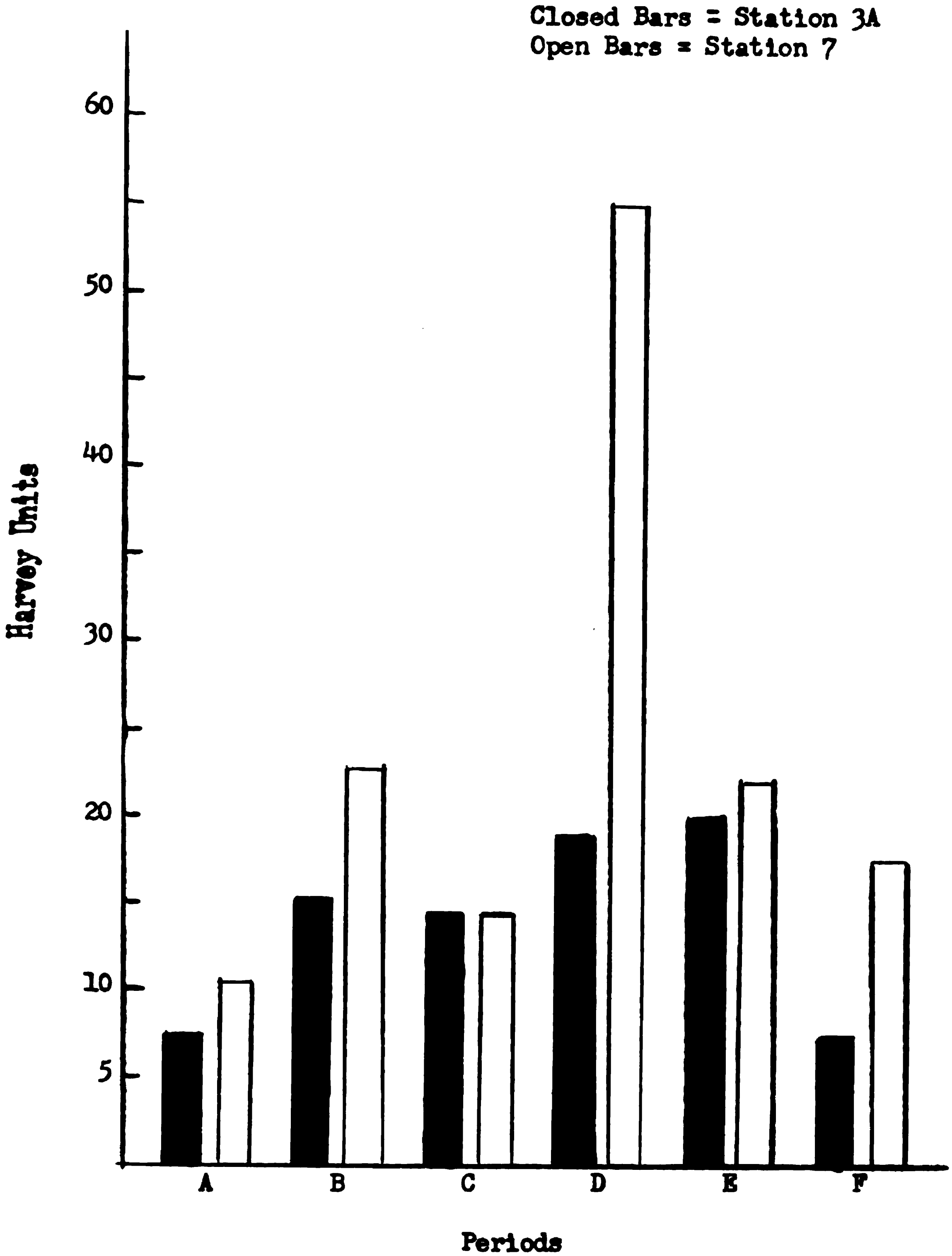
In order to show how significant the change is and to compare with station 3A, the means and twice the standard deviation of the mean are plotted in figure 16. It is easily seen that all the means for both stations are in a fairly compact group with the exception of station 7, period D.

F tests showed both sets of means to be significantly different even at the one percent level. Using the method of Duncan (1957) it was found that for station 3A, periods B, C, D, and E could not be shown to be significantly different at the five percent level and similarly periods A and F are not significantly different at this level. However, A and F are significantly different from B, C, D, and E at the five percent level.

For station 7 it was found that periods A and D are each significantly different from all other periods at the one percent level. It is obvious that this would also be true at the five

Figure 15

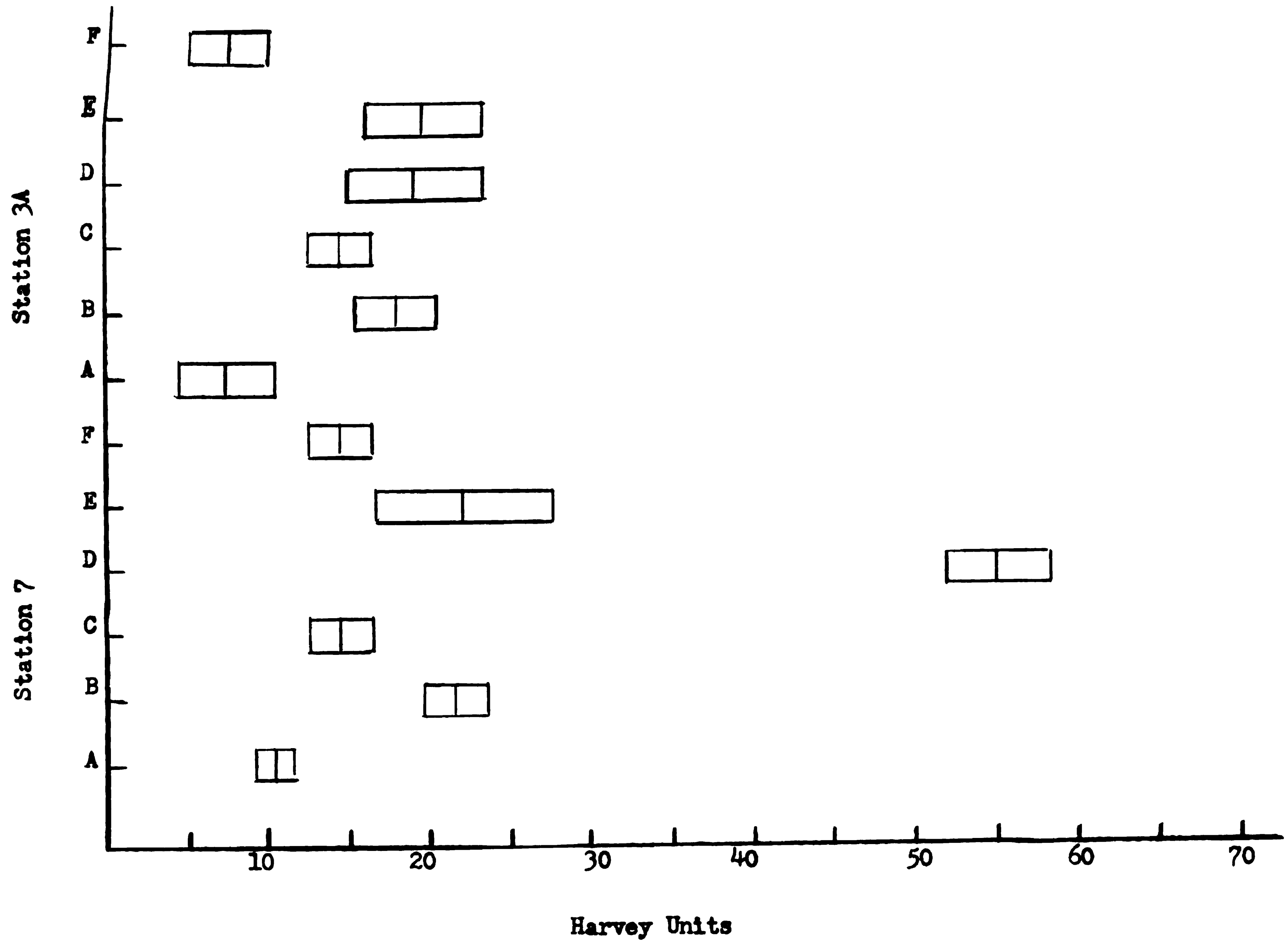
Means of Pigment per Unit
Area in Harvey Units During
Six Periods of the Summer



Closed Bars = Station 3A
Open Bars = Station 7
Harvey Units
60
50
40
30
20
10
5
A
B
C
D
E
F
Periods

Figure 16

Harvey Units Per Unit Area During Six Periods of the Summer (Mean Per Sub-shingle $\pm$ 2 Standard Deviations of the Mean)



Station 3A
F
E
D
C
B
A
Station 7
F
E
D
C
B
A
10
20
30
40
50
60
70
Harvey Units

percent level. Periods B, E, and F can not be shown to be significantly different at the one percent level. The data and statistical analysis for stations 3A and 7 are shown in tables 10 and 11 respectively.

It is easily seen from this data that the addition of diammonium phosphate did increase the amount of pigment per shingle to a large extent, but the amount of pigment was also influenced by other factors.

<u>organic nitrogen</u>

The mean values of organic nitrogen per unit area (subshingle) in milligrams nitrogen are shown as a histogram in figure 17. The change at station 7 from period C to D is almost three fold while the values for station 3A decrease throughout the study period. Figure 18, which shows the means and twice the standard deviations of the means, points out the large variance found in organic nitrogen values. The only mean which is different from those adjacent to it at the 95 percent confidence level is station 7, period D.

F tests showed both sets of means to be heterogenous at the one percent level and further tests showed that for station 3A periods C, D, E, and F are not significantly different at the five percent level and similarly periods A and B are not significantly different at this level. Periods A and B are significantly different from periods C, D, E, and F at this level.

It was found for station 7 that in terms of organic nitrogen development periods B, C, E, and F are not significantly different from each other at the five percent level and similarly periods A, B, and E are not significantly different from each other.

TABLE 10

Harvey Units of Pigment per Subshingle* Station 3A
During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	19.4	20.2	12.1	28.0	28.7	10.3
2	4.4	14.0	12.6	8.7	13.1	3.8
3	10.4	22.8	20.9	32.3	27.8	8.4
4	5.1	20.8	18.2	16.6	19.2	14.9
5	6.4	10.4	13.0	21.3	17.3	5.3
6	3.0	21.6	11.6	19.2	27.4	7.9
7	6.6	14.9	13.9	16.4	20.8	10.7
8	7.0	20.1	18.5	14.2	15.2	5.1
9	5.3	18.0	11.9	20.4	15.0	5.6
10	...	16.6	13.2	14.7	13.3	3.0
sum	67.6	179.4	145.9	191.8	197.8	75.0
$\bar{X}$	7.5	17.9	14.6	19.2	19.8	7.5
ΣX^2	700.5	3,356.8	2,228.1	4,103.7	4,252.4	684.5
$(\Sigma X)^2/n$	507.7	3,218.4	2,128.7	3,678.7	3,912.5	562.5
Σx^2	192.7	138.4	99.4	425.0	339.9	122.0
var.	24.1	15.4	11.1	47.2	37.8	13.6
sta. dev.	4.9	3.9	3.3	6.9	6.1	3.7
$(\Sigma X)^2$	4,569.8	32,184.4	21,286.8	36,787.2	39,124.8	5,625.0

$SS_T = 2,864$, df = 58

$SS_B = 1,547$, df = 5

$SS_W = 1,317$, df = 53

$s_B^2 = 309$

$s_W^2 = 24.8$

$F = 12.4$

*surface area equals 38.76 square inches

TABLE 10 (CONT.)

Multiple Range Test*-Station 3A

(a)

Source	df	m.s.	s
Between treatments	5		
Error	53	24.84	5.0

(b) $R'p = s\ z_p$

p:	2	3	4	5	6
5% z_p:	2.84	2.99	3.09	3.15	3.21
5% R'_p:	14.20	14.95	15.45	15.65	16.05

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	7.50	7.51	14.59	17.94	19.18	19.78
n:	10	9	10	10	10	10

(d) Test Sequences: at 5% level

(f-b)' greater than R'_5; (f-c)' not greater than R'_4

(e-b)' greater than R'_4; (e-c)' not greater than R'_3

(d-b)' greater than R'_3; (d-c)' not greater than R'_2

(c-b)' greater than R'_2

(b-a)' not greater than R'_2

(e) Conclusions: at 5% level

(c, d, e, f) can not be shown to be different
(a, b) can not be shown to be different

*Duncan (1957)

TABLE 11

Harvey Units of Pigment per Subshingle-Station 7
During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	11.0	18.3	10.9	60.4	11.9	9.1
2	11.2	23.7	11.9	53.1	...	11.2
3	12.8	21.6	11.9	53.1	12.1	14.2
4	8.7	18.5	13.0	60.2	17.4	12.8
5	7.7	17.4	12.1	58.8	34.2	22.4
6	14.2	23.2	16.6	57.8	18.2	30.7
7	10.4	20.6	15.0	58.0	33.4	18.2
8	9.1	20.6	15.7	52.6	23.0	14.2
9	9.5	27.6	17.8	53.6	22.9	18.7
10	10.5	25.8	20.6	43.1	27.8	19.7
sum	105.1	217.3	145.5	550.7	200.9	171.2
$\bar{X}$	10.5	21.7	14.6	55.1	22.3	17.1
ΣX^2	1,138.4	4,823.0	2,206.0	30,571.0	5,033.5	3,288.6
$(\Sigma X)^2/n$	1,104.6	4,722.0	2,117.0	30,327.1	4,484.5	2,930.9
Σx^2	33.8	101.0	89.0	244.0	549.0	357.7
var.	3.7	11.2	9.9	27.1	0.1	39.7
sta. dev.	1.9	3.3	3.1	5.2	0.4	6.3
$(\Sigma X)^2$	11,046.0	47,219.0	21,170.0	303,270.5	40,360.8	29,309.4

$SS_T = 14,281$, df = 58

$SS_B = 12,906$, df = 5

$SS_W = 1,375$, df = 53

$s_B^2 = 2,581.0$

$s_W^2 = 25.94$

$F = 99$

(

(

(c

(d)

(e)

TABLE 11 (CONT.)

Multiple Range Test-Station 7

(a)

Source	df	m.s.	s
Between Treatments	5		
Error	53	25.94	5.1

(b) $R'_p = s\ z_p$

p:	2	3	4	5	6
1% z_p:	3.79	3.95	4.06	4.14	4.20
1% R'_p:	19.33	20.14	20.71	21.14	21.42

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	10.51	14.6	17.1	21.7	22.3	55.1
n:	10	10	10	10	9	10

(d) Test Sequences: at 1% level

(f-e)' greater than R'_2
(e-b)' greater than R'_4; (e-c)' not greater than R'_3
(d-b)' greater than R'_3
(c-a)' greater than R'_3: (c-b)' not greater than R'_2
(b-a)' not greater than R'_2

(e) Conclusions: at 1% level

(c, d, e) can not be shown to be different
(b, c) can not be shown to be different
(a, b) can not be shown to be different

Figure 17

Mean Milligrams of Organic
Nitrogen per Unit Area During
Six Periods of the Summer

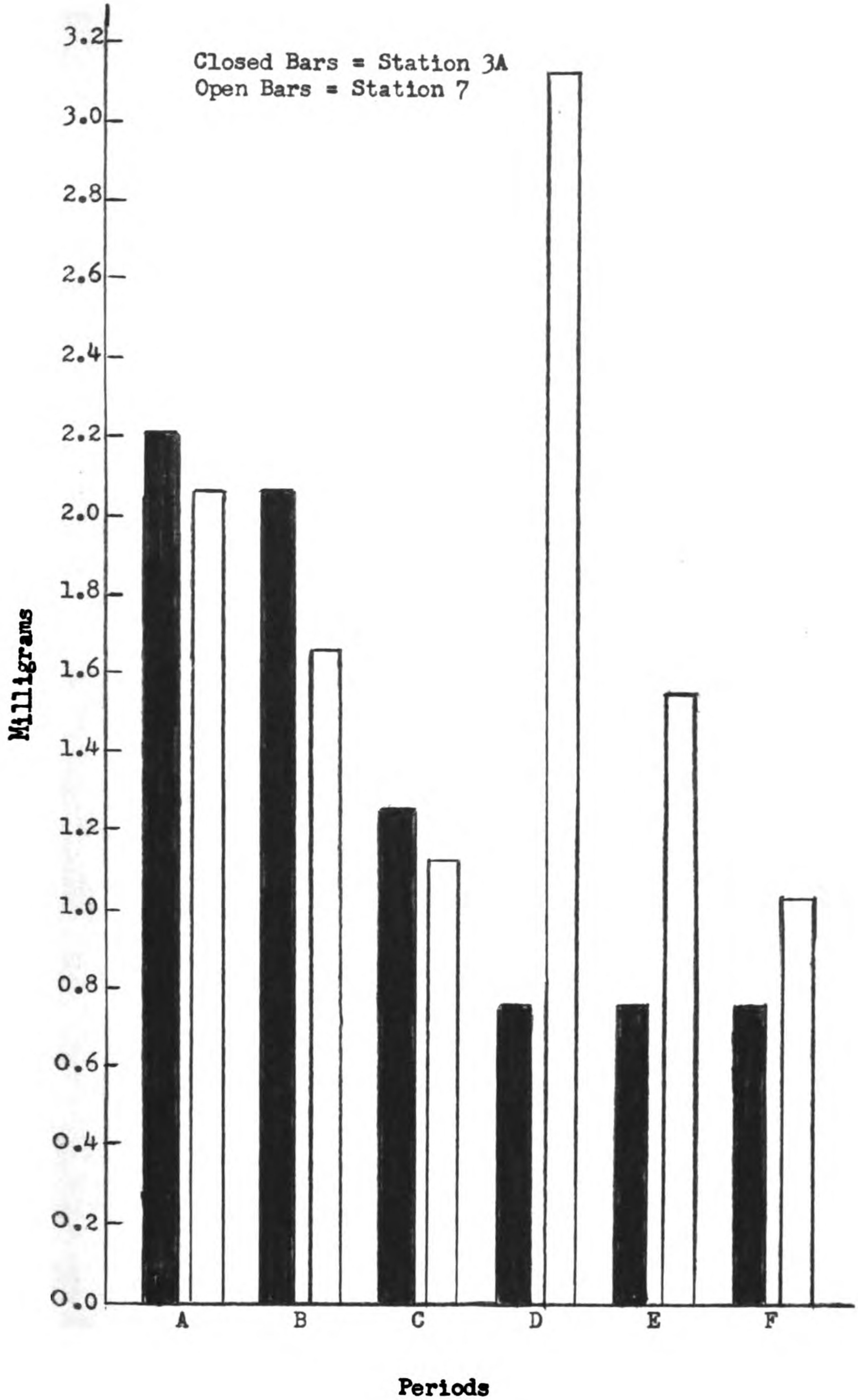
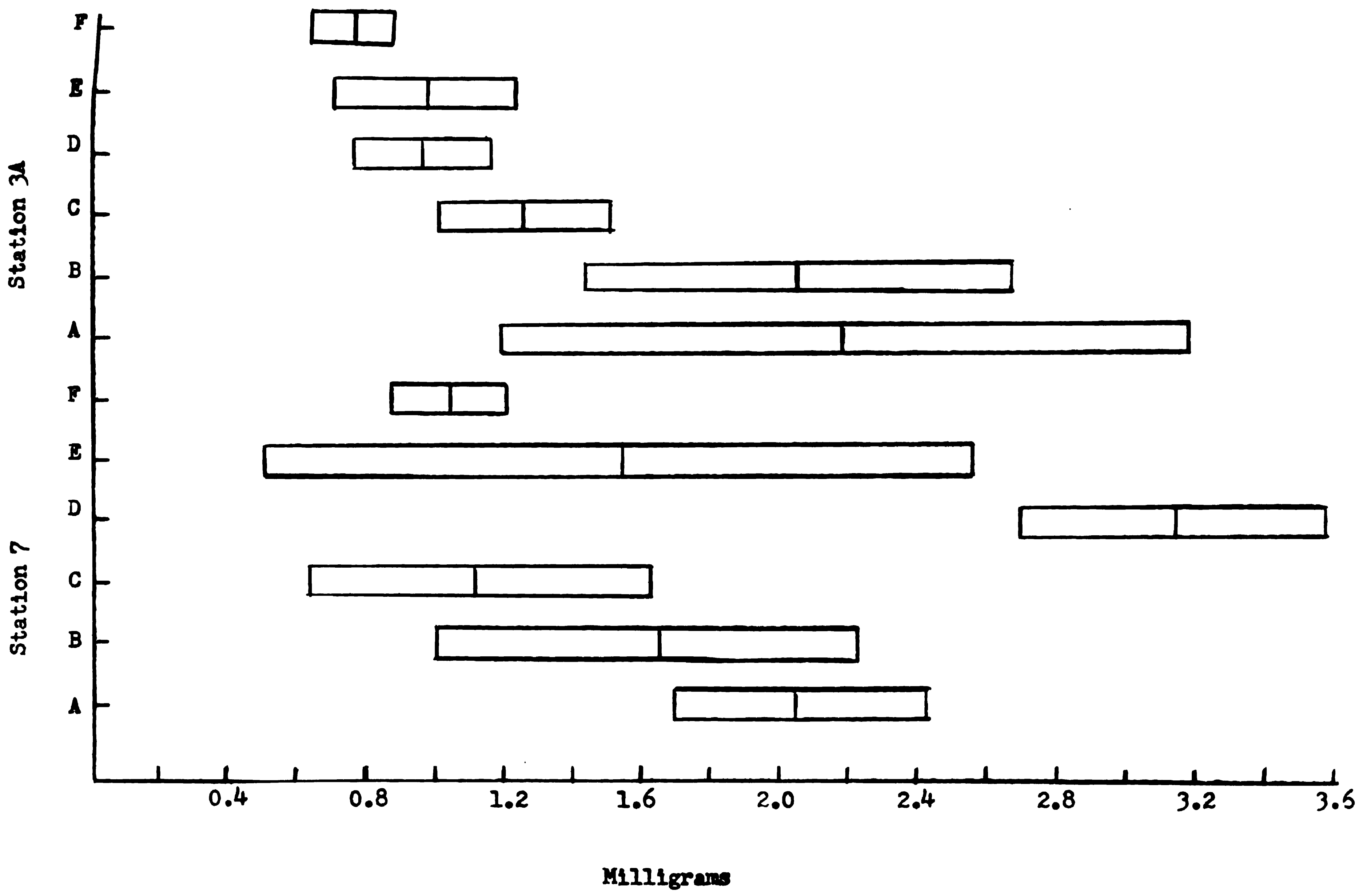

Closed Bars = Station 3A
Open Bars = Station 7
Milligrams
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A
B
C
D
E
F
Periods

Figure 18

Organic Nitrogen Per Unit Area in Milligrams Nitrogen During Six Periods of the Summer. (Means $\pm$ 2 Standard Deviations of the Mean).


Station 3A
F
E
D
C
B
A
Station 7
F
E
D
C
B
A
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
Milligrams

Period D is significantly different from all others at this level.

Although the organic nitrogen data was not as distinctly indicative as the pigment data, it does show a statistically valid increase in organic nitrogen during the period in which fertilizer was added.

The data and statistical analysis for stations 3A and 7 are shown in tables 12 and 13 respectively.

total phosphorus

The mean values of phosphorus in micrograms of phosphorus per unit area (subshingle) are plotted as a histogram in figure 19. There is almost a four fold increase in phosphorus at station 7 between periods C and D.

Figure 20 shows the mean phosphorus values and twice the standard deviation of the means. The only set which is not within the grouping is station 7, period D.

F tests showed the periods of both stations to be heterogenous at the one percent level. Multiple range tests on station 3A indicate that periods C, D, E, and F can not be shown to be significantly different from each other at the five percent level and similarly periods A and B are not significantly different at this level. However, these two groups of means are significantly different from each other at this level.

Multiple range tests for station 7 show that all periods except period D are not significantly different at the five percent level. Period D is significantly different from all others at this level.

TABLE 12

Milligrams of Organic Nitrogen per Subshingle-Station 3A During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	5.59	2.19	1.91	0.60	0.95	0.75
2	0.98	1.61	0.96	1.64	0.78	0.52
3	2.03	4.43	0.75	0.71	0.33	0.80
4	1.03	2.02	0.89	1.10	1.82	1.13
5	1.60	1.38	1.61	0.80	1.23	0.60
6	3.32	1.61	1.40	0.95	0.79	1.00
7	1.32	1.79	1.52	0.88	1.28	0.88
8	0.88	1.44	0.77	0.55	1.25	0.63
9	2.84	2.92	1.03	1.27	0.63	0.66
10	...	1.06	1.78	1.13	0.63	0.58
sum	19.59	20.45	12.62	9.63	9.69	7.55
$\bar{X}$	2.18	2.04	1.26	0.96	0.97	0.76
ΣX^2	61.56	50.52	17.61	10.27	11.06	6.05
$(\Sigma X)^2/n$	42.64	41.82	15.93	9.27	9.39	5.70
Σx^2	18.92	8.70	1.68	1.00	1.67	0.35
var.	2.36	0.97	0.19	0.11	0.19	0.04
sta. dev.	1.5	0.99	0.43	0.33	0.43	0.20
$(\Sigma X)^2$	383.78	428.20	159.26	92.74	93.90	57.00

$SS_T = 48.02$, df = 58

$SS_B = 15.70$, df = 5

$SS_W = 32.32$, df = 53

$s_B^2 = 3.1$

$s_W^2 = 0.61$

$F = 5.0$

TABLE 12 (CONT.)

Multiple Range Test-Station 3A

(a)

Source	df	m.s.	s
Between Treatments	5		
Error	53	0.6098	0.78

(b) $R'_p = s\ z_p$

p:	2	3	4	5	6
5% z_p:	2.84	2.99	3.09	3.15	3.21
5% R'_p:	2.22	2.33	2.41	2.46	2.50

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	0.76	0.96	0.97	1.26	2.04	2.18
n:	10	10	10	10	10	9

(d) Test Sequences: at 5% level

(f-d)' greater than R'_3; (f-e)' not greater than R'_2

(e-d)' greater than R'_2

(d-a)' not greater than R'_4

(e) Conclusions: at 5% level

(a, b, c, d) can not be shown to be different
(e, f) can not be shown to be different

TABLE 13

Milligrams of Organic Nitrogen per Subshingle-Station 7
During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	1.16	0.99	0.80	3.37	1.81	0.75
2	2.35	1.28	0.88	3.29	...	1.08
3	1.86	2.70	...	2.20	1.45	0.92
4	1.98	0.99	1.15	4.41	1.53	1.01
5	2.29	1.26	0.79	3.44	1.56	0.73
6	1.14	1.47	0.91	1.97	0.74	1.31
7	2.58	1.05	3.08	2.56	2.14	1.40
8	2.57	1.73	0.58	3.53	1.70	0.92
9	1.68	3.73	1.04	3.49	1.59	0.86
10	2.89	1.40	0.96	3.07	1.28	1.37
sum	20.50	16.60	10.19	31.33	13.80	10.35
$\bar{X}$	2.05	1.66	1.13	3.13	1.53	1.04
ΣX^2	45.23	34.61	16.02	102.80	22.34	11.27
$(\Sigma X)^2/n$	42.02	27.56	11.54	98.16	21.16	10.71
Σx^2	3.21	7.05	4.48	4.64	1.18	0.56
var.	0.36	0.78	0.56	0.52	0.15	0.06
sta. dev.	0.60	0.89	0.75	0.72	0.38	0.25
$(\Sigma X)^2$	420.25	275.56	103.84	981.57	190.44	107.12

$SS_T = 46.98$, df = 57

$SS_B = 25.86$, df = 5

$SS_W = 21.12$, df = 52

$s_B^2 = 5.17$

$s_W^2 = 0.4062$

$F = 12.73$

TABLE 13 (CONT.)

Multiple Range Test-Station 7

(a)

Source	df	m.s.	s
Between Treatments	5		
Error	52	0.4062	0.64

(b) $R'_p = s\ z_p$

p:	2	3	4	5	6
5% z_p:	2.84	2.99	3.09	3.15	3.21
5% R'_p:	1.82	1.91	1.98	2.02	2.05

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	1.04	1.13	1.53	1.66	2.05	3.13
n:	10	9	9	10	10	10

(d) Test Sequences: at 5% level

(f-e)' greater than R'_2

(e-b)' greater than R'_4; (e-c)' not greater than R'_3

(d-a)' not greater than R'_4

(e) Conclusions: at 5% level

(a, b, c, d) can not be shown to be different
(c, d, e) can not be shown to be different

Figure 19

Mean Total Phosphorus per Unit Area During Six Periods of the Summer

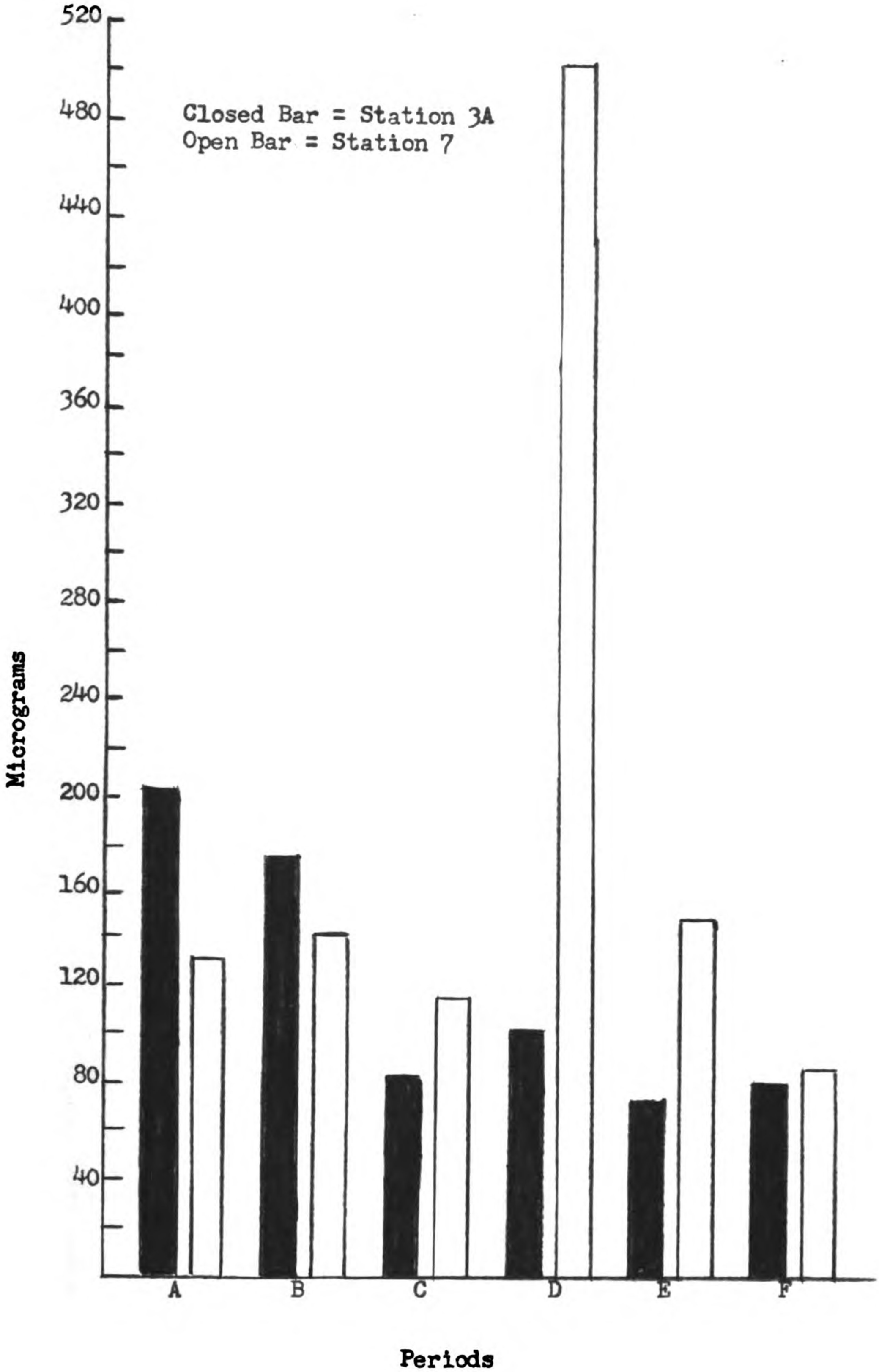
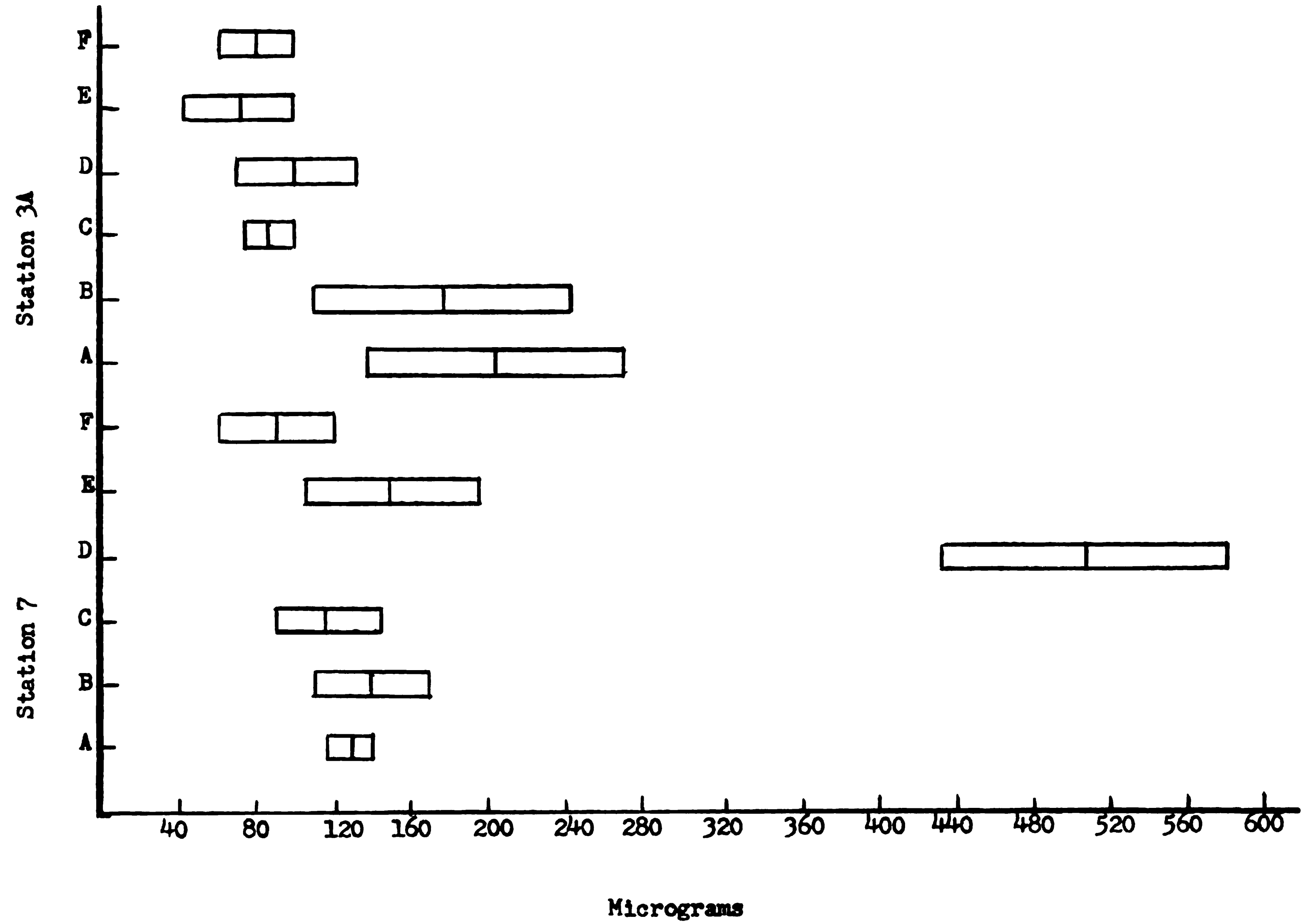

Closed Bar = Station 3A
Open Bar = Station 7
Micrograms
520
480
440
400
360
320
280
240
200
160
120
80
40
A
B
C
D
E
F
Periods

Figure 20

Total Phosphorus Per Unit Area During Six Periods of the Summer (Means $\pm$ 2 Standard Deviations of the Means).


Station 3A
F
E
D
C
B
A
Station 7
F
E
D
C
B
A
40
80
120
160
200
240
280
320
360
400
440
480
520
560
600
Micrograms

The data on phosphorus show the most striking effects of fertilization of the three analytical methods partly due to the accuracy of the analytical method itself. The data and statistical analyses are shown for stations 3A and 7 in tables 14 and 15 respectively.

<u>periphyton ratios</u>

<u>phosphorus to organic nitrogen</u>: The ratios and statistical data for stations 3A and 7 are shown in tables 16 and 17 respectively. A histogram of the means of the ratios for stations 3A and 7 is shown in figure 21. Figure 22 shows a plot of the means and two standard deviations of the means. This method shows, roughly, that no period at either station is significantly different from the adjacent periods at the 95 percent confidence level.

An F test on station 3A shows that the means are not significantly different even at the 25 percent level. An F test on all periods at both stations gives a value that shows that the means are not significantly different at the one percent level, but are significantly different at the five percent level.

A multiple range test of this set of twelve means indicates that all but station 7, period D can not be shown to be significantly different at the five percent level. Station 7, period D can not be shown to be significantly different from station 7, period C or station 3A, periods A and D.

These data show that with the exception of the period of fertilization at the downstream station, the phosphorus to nitrogen ratio is statistically valid and could be representative of a fairly

TABLE 14

Micrograms of Phosphorus per Subshingle for Station 3A
During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	182	218	87	230	59	137
2	148	295	118	70	48	54
3	435	185	98	109	50	93
4	132	112	94	86	48	92
5	265	98	69	68	70	78
6	213	133	54	103	67	85
7	162	418	78	112	50	80
8	138	90	92	88	91	80
9	151	132	88	95	191	90
10	...	81	95	73	47	29
sum	1,826	1,762	873	1,034	721	818
$\bar{X}$	203	176	87	103	72	82
ΣX^2	445,360	415,420	78,927	126,952	69,449	73,848
$(\Sigma X)^2/n$	370,475	310,464	76,927	106,916	51,984	66,912
Σx^2	74,885	104,956	2,714	20,036	17,465	6,936
var.	9,361	11,361	302	2,226	1,941	771
sta. dev.	97	108	17	47	44	28
$(\Sigma X)^2$	3,334,276	3,104,644	762,129	1,069,156	519,841	669,124

SS_T = 371,360; df = 58

SS_B = 144,368; df = 5

SS_W = 226,992; df = 53

s_B^2 = 28,874

s_W^2 = 4,283

F = 6.74

TABLE 14 (CONT.)

Multiple Range Test-Station 3A

(a)

Source	df	m.s.	s
Between Treatments	5		
Error	53	4,283	65.4

(b) $R'_p = s\ z_p$

p:	2	3	4	5	6
5% z_p:	2.84	2.99	3.09	3.15	3.21
5% R'_p	185.74	195.55	202.09	206.01	209.93

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	72.1	81.8	87.3	103.4	176.2	203.0
n:	10	10	10	10	10	9

(d) Test Sequences: at 5% level

(f-d)' greater than R'_3; (f-e)' not greater than R'_2
(e-d)' greater than R'_2
(d-a)' not greater than R'_4

(e) Conclusions: at 5% level

(a, b, c, d) can not be shown to be different
(e, f) can not be shown to be different

TABLE 15

Micrograms of Phosphorus per Subshingle for Station 7 During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	124	58	205	340	127	41
2	132	115	62	375	...	78
3	151	202	68	592	104	56
4	151	133	97	542	90	66
5	119	149	112	370	113	64
6	140	116	87	502	120	137
7	149	214	160	635	187	184
8	81	79	144	552	348	98
9	125	198	130	462	129	108
10	131	131	106	692	142	75
sum	1,303	1,395	1,171	5,062	1,360	907
$\bar{X}$	130	140	117	506	151	91
ΣX^2	173,711	219,000	154,487	2,689,594	242,323	98,871
$(\Sigma X)^2/n$	169,781	194,600	137,124	2,562,384	205,511	82,265
Σx^2	3,930	24,500	17,363	127,210	36,812	14,606
var.	437	2,722	1,929	14,134	4,602	1,845
sta. dev.	21	52	44	119	68	43
$(\Sigma X)^2$	1,697,809	1,946,000	1,371,241	25,623,844	1,849,600	822,649

SS_T = 1,452,743; df = 58

SS_B = 1,226,322; df = 5

SS_W = 226,421; df = 53

s_B^2 = 245,264

s_W^2 = 4,272

F = 57.41

TABLE 15 (CONT.)

Multiple Range Test-Station 7

(a)

Source	df	m.s.	s
Between Treatments	5		
Error	53	4,272	65.4

(b) $R'_p = s\ z_p$

p:	2	3	4	5	6
5% z_p:	2.84	2.99	3.09	3.15	3.21
5% R'_p:	185.74	195.55	202.09	206.01	209.93

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	90.7	117.0	130.3	140.0	151.1	506.2
n:	10	10	10	10	9	10

(d) Test Sequences: at 5% level

(f-e)' greater than R'_2

(e-a)' not greater than R'_5

(e) Conclusions: at 5% level

(a, b, c, d, e) can not be shown to be different

TABLE 16

Ratio of Phosphorus to Organic Nitrogen in Periphyton-Station 3A (μg. P/mg. N) During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	32.56	99.54	45.55	383.33	62.11	182.67
2	151.02	183.23	122.92	42.68	61.54	103.85
3	214.29	41.76	130.67	153.52	151.52	116.25
4	128.16	55.45	105.62	78.18	26.37	81.42
5	165.62	71.01	42.86	85.00	56.91	130.00
6	64.16	82.61	38.57	108.42	84.81	85.00
7	122.73	233.52	51.32	127.27	39.06	90.91
8	156.82	62.50	119.48	160.00	72.80	126.98
9	53.17	45.21	85.44	74.80	303.17	136.36
10	...	76.42	53.37	64.60	74.60	50.00
sum	1,088.53	951.25	795.80	1,277.80	932.89	1,103.44
$\bar{X}$	120.95	95.12	79.58	127.78	93.29	110.34
ΣX^2	160,241	126,489	75,797	248,990	146,033	133,904
$(\Sigma X)^2/n$	131,655	90,488	63,330	163,277	87,028	121,758
Σx^2	28,586	36,001	12,467	85,712	59,004	12,146
$(\Sigma X)^2$	1,184,898	904,877	633,298	1,632,773	870,284	1,217,580
var.	3,573	4,000	1,385	9,524	6,556	1,350
sta. dev.	60	63	37	98	81	37

$SS_T = 240,455$; df = 58

$SS_B = 16,538$; df = 5

$SS_W = 233,917$; df = 53

$s_B^2 = 3,308$

$s_W^2 = 4,414$

$F = 0.749$

TABLE 17

Ratio of Phosphorus to Organic Nitrogen in Periphyton-Station 7 (µg. P/mg. N) During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	106.90	58.59	256.25	100.89	70.17	54.67
2	56.17	89.84	70.45	113.98	...	72.22
3	81.18	74.81	...	269.09	71.72	60.87
4	76.26	134.34	84.35	122.90	58.82	65.35
5	51.96	118.25	141.77	107.56	72.44	87.67
6	122.81	78.91	95.60	254.82	162.16	104.58
7	57.75	203.81	51.95	248.05	87.38	131.43
8	31.52	45.66	248.28	156.37	204.71	106.52
9	74.40	53.08	125.00	132.38	81.13	125.58
10	45.33	93.57	110.42	225.41	110.94	54.74
sum	704.28	950.86	1,184.07	1,731.45	919.47	863.63
$\bar{X}$	70.43	95.09	131.56	173.14	102.16	86.36
ΣX^2	56,689	110,554	199,140	341,501	113,502	82,190
$(\Sigma X)^2/n$	49,601	90,413	155,780	299,792	93,936	74,586
Σx^2	7,088	20,140	43,359	41,709	19,566	7,605
$(\Sigma X)^2$	496,010	904,135	1,402,022	2,997,919	845,425	745,857
var.	788	2,238	5,420	4,634	2,446	845
sta. dev.	28	47	54	68	49	29

F Test on Data of Station 3A + Station 7

SS_T = 458,918; df = 116

SS_B = 85,533; df = 11

SS_W = 373,385; df = 105

s_B^2 = 7,776

s_W^2 = 3,556

F = 2.187

TABLE 17 (CONT.)

Multiple Range Test-Station 3A + Station 7

(a)

Source	df	m.s.	s
Between Treatments	11		
Error	105	3,556	59.7

(b) $R'_p = s\ z_p$

p:	2	3	4	5	6	7
5% z_p:	2.80	2.95	3.05	3.12	3.18	3.22
5% R'_p:	167.2	176.1	182.1	186.3	189.8	192.2

p:	8	9	10	11	12
5% z_p:	3.26	3.29	3.32	3.34	3.36
5% R'_p:	194.6	196.4	198.2	199.4	200.6

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	70.43	79.58	86.36	93.29	95.09	95.12
n:	10	10	10	10	10	10

Code:	g	h	i	j	k	l
$\bar{X}$:	102.16	110.34	120.95	127.78	131.56	173.14
n:	9	10	9	10	9	10

(d) Test Sequences: at 5% level

(l-h)' greater than R'_5; (l-i)' not greater than R'_4

(k-a)' not greater than R'_{11}

(e) Conclusions: at 5% level

(a, b, c, d, e, f, g, h, i, j, k) can not be shown to be different

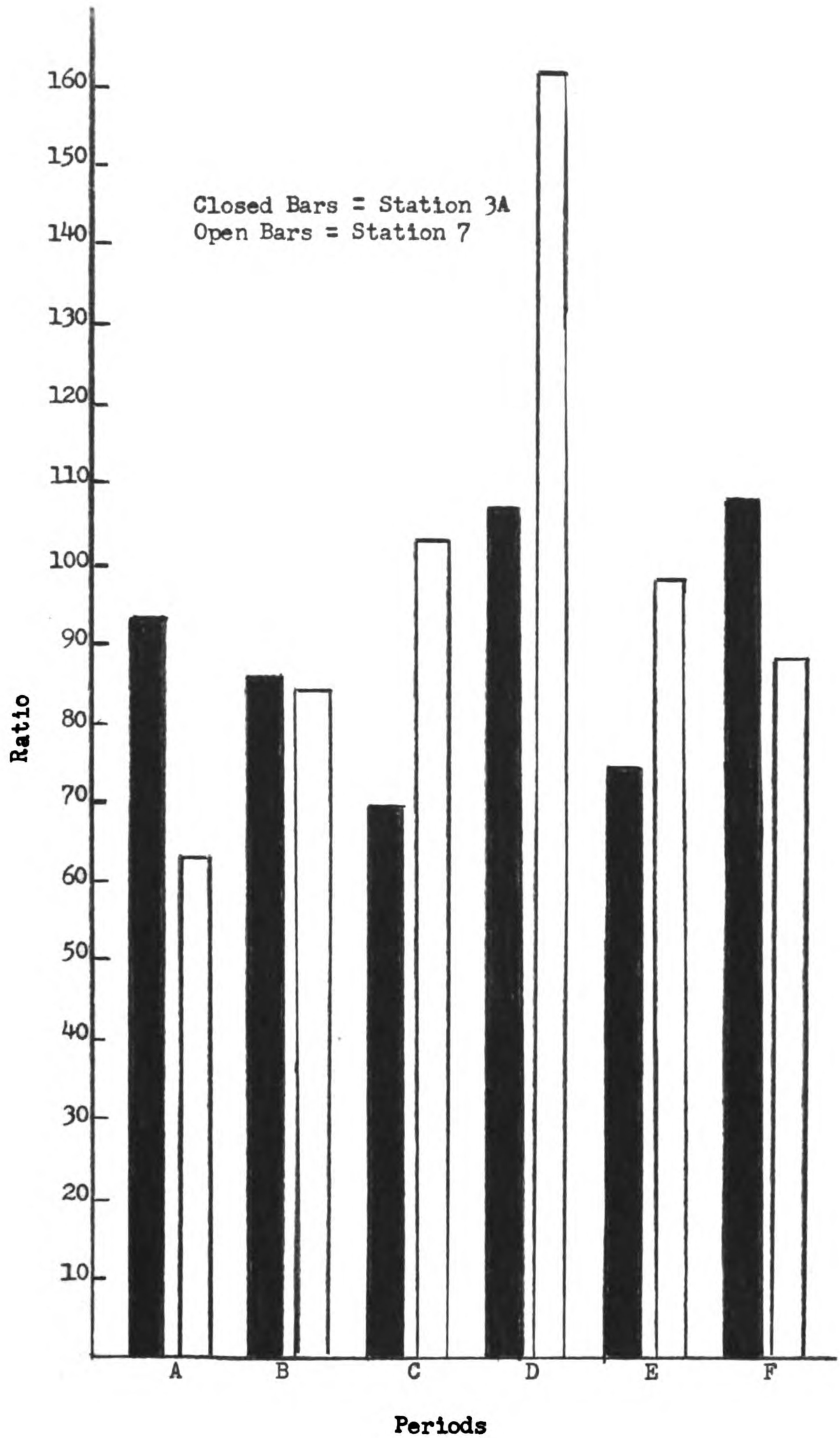
(i, j, k, l) can not be shown to be different

(f) Micrograms of Phosphorus per Milligram of Organic Nitrogen:

$$\frac{\Sigma \bar{X}}{12} = 101.15$$

Figure 21

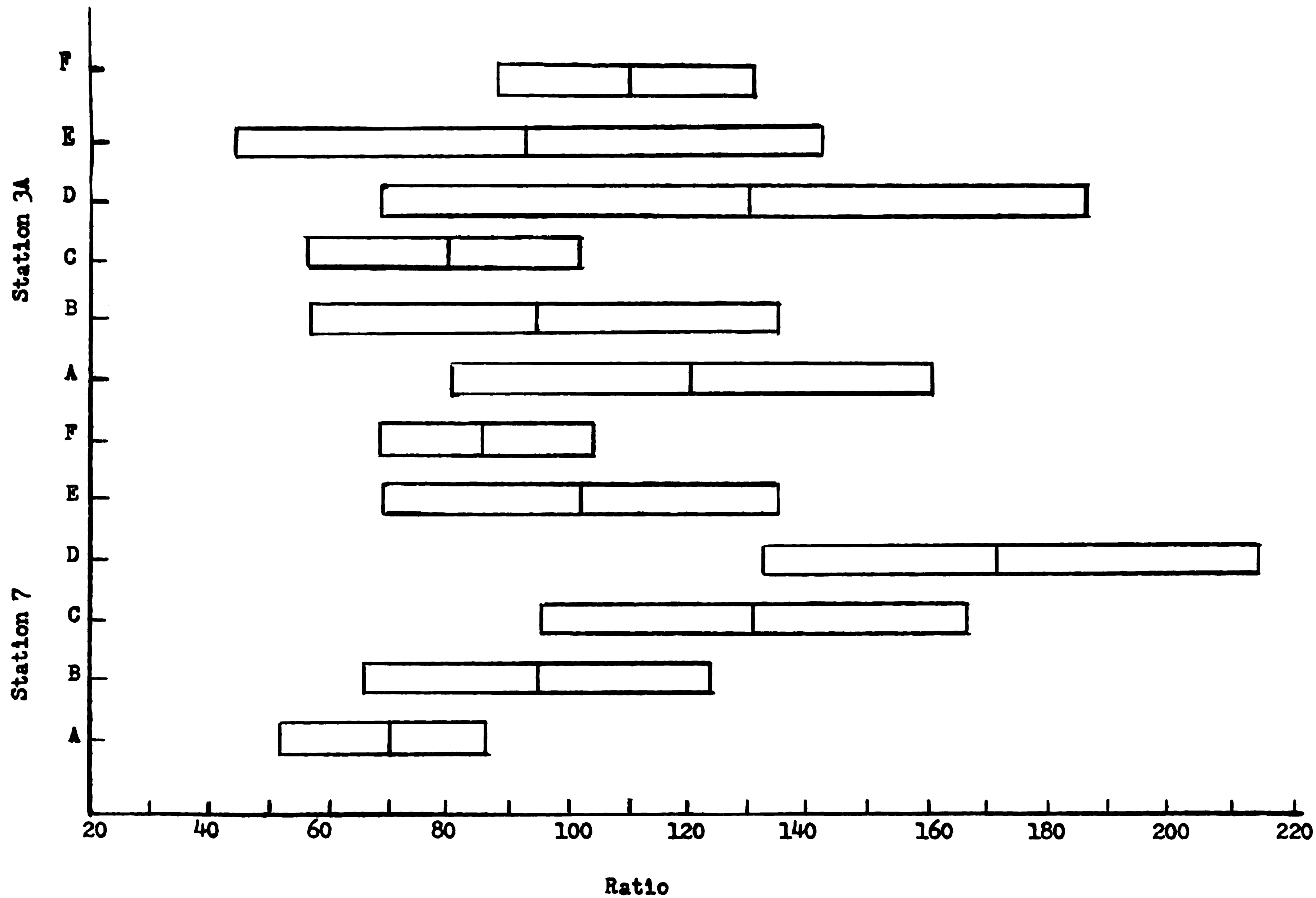
Mean Ratio of Total Phosphorus (in μg.) to Organic Nitrogen (in mg.) During Six Periods of the Summer.



Closed Bars = Station 3A
Open Bars = Station 7
Ratio
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
A
B
C
D
E
F
Periods

Figure 22

Mean Ratio of Total Phosphorus (in μg.) to Organic Nitrogen (in mg.) During Six Periods of the Summer. (Means ± 2 Standard Deviations of the Means).



Station 3A
F
E
D
C
B
A
Station 7
F
E
D
C
B
A
20
40
60
80
100
120
140
160
180
200
220
Ratio

homogeneous population. If all the data are combined and the units are equalized this ratio becomes 1.01 g. phosphorus per 10.0 grams organic nitrogen. Ketchum (1949), and Ryther (1956) obtained ratios within the general range of one g. phosphorus to five g. organic nitrogen in cultures of freshwater algae and marine algae, respectively.

The effects of phosphorus storage by algae undoubtedly have an influence upon the phosphorus to nitrogen ratio. It is possible that the periphyton quickly became established on the shingles and stored phosphorus in the cells until fertilization ceased, after which there was a four day period in which the periphyton could have grown enough to reduce the ratio of phosphorus to nitrogen almost back to normal.

Einsele (1941) found that the planktonic algae of Schleinsee were capable of storing up to ten times the necessary amount of phosphorus per cell. The fact that planktonic algae are capable of storing phosphorus is also shown by Lund (1950). This work was done on Asterionella formosa in various English lakes. Lund also illustrated that algae can take up phosphorus from lake water when the concentration is as low as one part per billion. Work done on the Red Cedar River by Grzenda (M.S.) tends to show the same type of phenomenon in periphyton. In his studies he has found a periphyton phosphorus to nitrogen ratio of about one to one during some periods. However, it may be true, in some situations, that species composition of the periphyton community is what changes rather than the phosphorus to nitrogen ratio in a given species.

The data on the phosphorus to organic nitrogen ratio in the west branch of the Sturgeon River are especially interesting since it effectively proves that phosphorus is the limiting nutrient for periphyton growth in the stream rather than nitrogen. This is true since the amount of nitrogen added was considerably lower than phosphorus added, but the amount of nitrogen per subshingle increased several fold. It is possible that nitrogen would have become the limiting factor if the addition was continued over a period of some length.

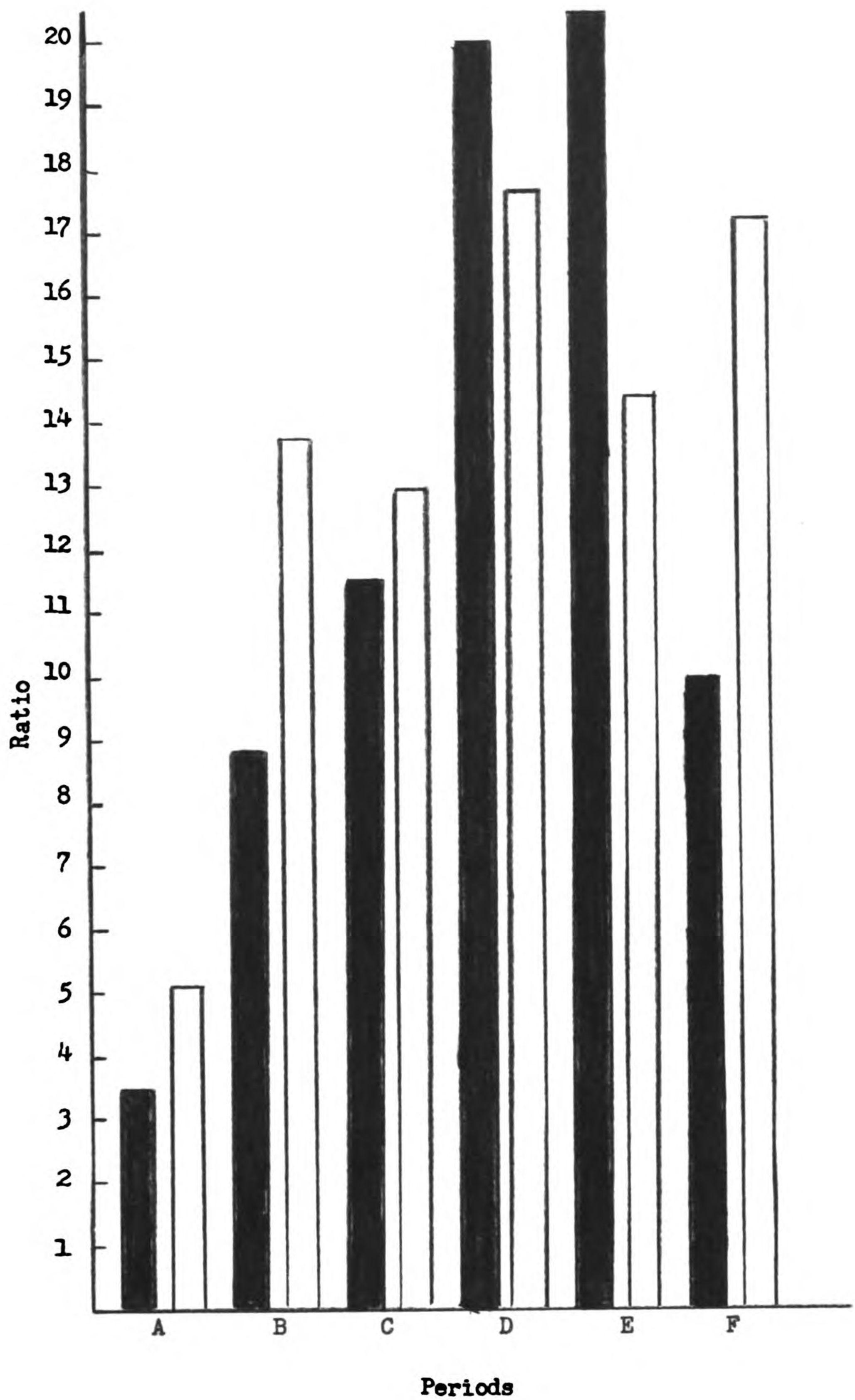
<u>pigment</u> <u>to</u> <u>organic</u> <u>nitrogen</u>: Figure 23 is a histogram of the means of the ratios for stations 3A and 7 at various times during the summer. A combination of progressively decreasing organic nitrogen values for station 3A and depressed pigment values early in the study at both stations tends to give a skewed graph. The reasons for the low pigment values are probably related to higher water turbidity and lower temperatures. Figure 24 shows these means and two standard deviations of the means.

Further analysis shows that periods B, C, D, E, and F from station 7 and periods B, C, and F from station 3A cannot be shown to be significantly different at the five percent level.

Although there is a certain amount of stability in the ratio of pigment to organic nitrogen, it is by no means as constant as the phosphorus to nitrogen ratio. The data and statistical analyses are recorded for stations 3A and 7 in tables 18 and 19 respectively.

Figure 23

Mean Ratio of Pigment (in Harveys) to Organic Nitrogen (in mgs.) During Six Periods of the Summer.



Ratio
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
A
B
C
D
E
F
Periods

Figure 24

Ratio of Pigment (in Harveys) to Organic Nitrogen (in mg.) During Six Periods of the Summer (Mean $\pm$ 2 standard Deviations of the Mean).

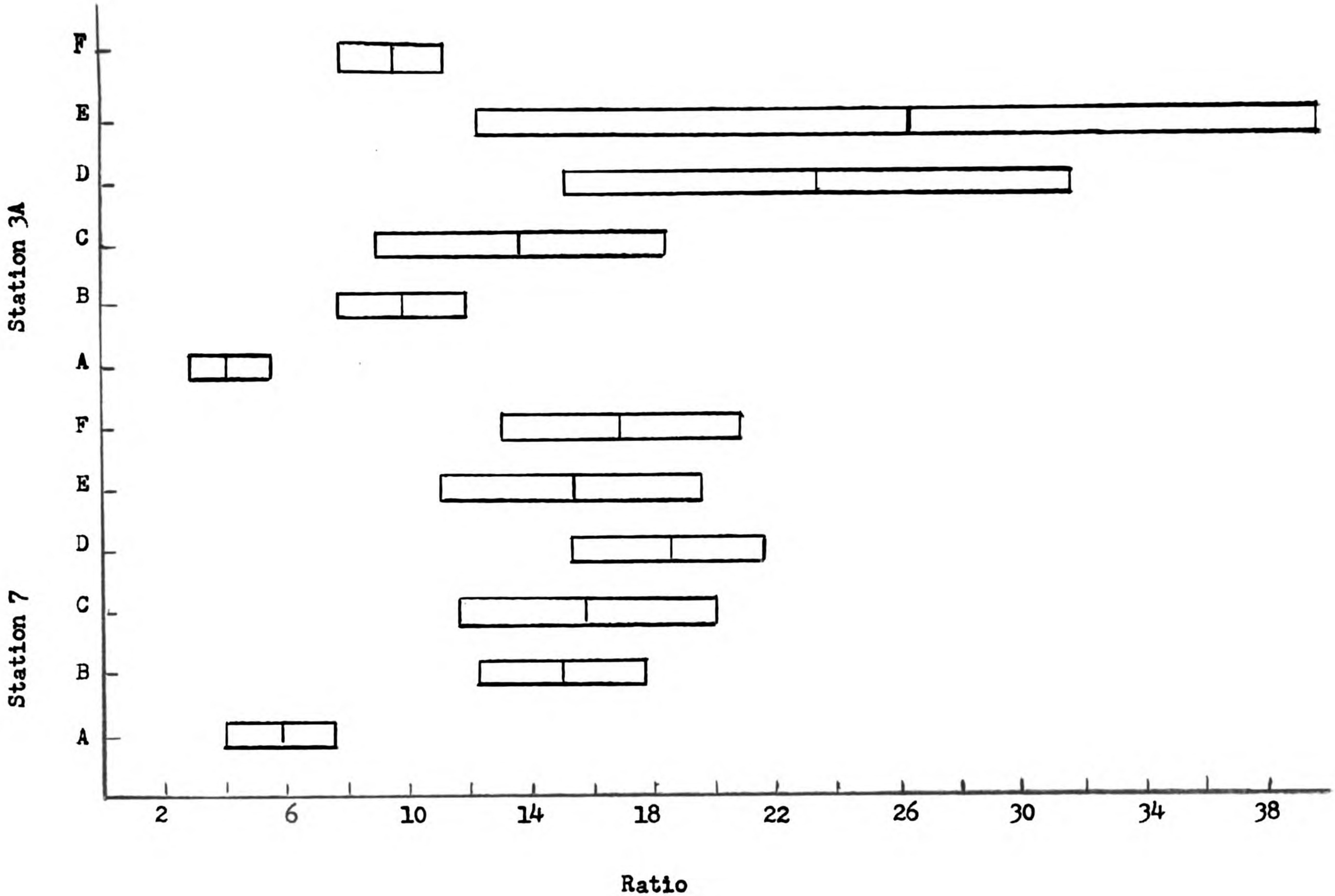

Station 3A
Station 7
F
E
D
C
B
A
F
E
D
C
B
A
2
6
10
14
18
22
26
30
34
38
Ratio

TABLE 18

Ratio of Pigment to Organic Nitrogen in Periphyton-Station 3A (Harvey units pigment/mg. N) During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	3.47	9.22	6.34	46.67	30.21	13.73
2	4.49	8.70	13.12	5.30	16.79	7.31
3	5.12	5.15	27.87	45.49	84.24	10.50
4	4.95	10.30	20.45	15.09	10.55	13.19
5	4.00	7.54	8.07	26.62	14.06	8.83
6	0.90	13.42	8.29	20.21	34.68	7.90
7	5.00	8.32	9.14	18.64	16.25	12.16
8	7.95	13.96	24.03	25.82	12.16	8.10
9	1.87	6.16	11.55	16.06	23.81	8.48
10	...	15.66	7.42	13.01	21.11	5.17
sum	37.75	98.43	136.28	232.91	263.86	95.37
$\bar{X}$	4.19	9.84	13.63	23.29	26.39	9.54
EX^2	191.43	1,077.54	2,390.56	7,061.60	11,227.1	978.67
$(EX)^2/n$	158.34	968.85	1,857.22	5,424.71	6,962.2	909.54
Ex^2	33.09	108.69	533.34	1,636.89	4,264.9	69.13
$(EX)^2$	1,425.06	9.688.46	18,572.2	54,247.1	69,622.1	9,095.44
var.	4.14	12.08	59.26	181.88	473.88	7.68
sta. dev.	2.0	3.5	7.7	13.5	21.8	2.8

TABLE 19

Ratio of Pigment to Organic Nitrogen in Periphyton-Station 7 (Harvey units pigment/mg. N) During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	9.48	18.48	13.62	17.92	6.57	12.13
2	4.77	18.52	13.52	16.14	...	10.37
3	6.88	8.00	...	24.14	8.34	15.43
4	4.39	18.69	11.30	13.65	11.37	12.67
5	3.36	13.81	15.32	17.09	21.92	30.68
6	12.46	15.78	18.24	29.34	24.59	23.44
7	4.03	19.62	4.87	22.66	15.61	13.00
8	3.54	11.91	27.07	14.90	13.53	15.43
9	5.65	7.40	17.12	15.36	14.40	21.74
10	3.63	18.43	21.46	14.04	21.72	14.38
sum	58.19	150.64	142.52	185.24	138.05	169.27
$\bar{X}$	5.82	15.06	15.84	18.52	15.34	16.93
ΣX^2	419.64	2,458.76	2,573.51	3,672.13	2,433.00	3,230.48
$(\Sigma X)^2/n$	338.61	2,269.24	2,256.88	3,431.39	2,117.53	2,865.23
Σx^2	81.03	189.52	316.63	240.74	315.47	365.25
$(\Sigma X)^2$	3,386.08	22,692.4	20,311.9	34,313.9	19,057.8	28,652.3
var.	9.00	21.06	39.58	26.75	39.43	40.58
sta. dev.	3.0	4.6	6.3	5.2	6.3	6.4

TABLE 19 (CONT.)

Multiple Range Test-Station 3A + Station 7

(a)

Source	df	m.s.	s
Between Treatments	11		
Error	105	77.66	8.8

(b) $R'_p = s\ z_p$

p:	2	3	4	5	6	7
5% z_p:	2.80	2.95	3.05	3.12	3.18	3.22
5% R'_p:	24.64	25.96	26.84	27.46	27.98	28.34

p:	8	9	10	11	12
5% z_p:	3.26	3.29	3.32	3.34	3.36
5% R'_p:	28.69	28.95	29.22	29.39	29.57

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	4.19	5.82	9.54	9.84	13.63	15.06
n:	9	10	10	10	10	10

Code:	g	h	i	j	k	l
$\bar{X}$:	15.34	15.84	16.93	18.52	23.29	26.39
n:	9	9	10	10	10	10

(d) Test Sequences: at 5% level

(l-i)' greater than R'_4; (l-j)' not greater than R'_3
(k-e)' greater than R'_7; (k-f)' not greater than R'_6
(j-b)' greater than R'_9; (j-c)' not greater than R'_8
(i-b)' greater than R'_8
(h-b)' greater than R'_7
(e-a)' greater than R'_5; (e-b)' not greater than R'_4
(d-a)' not greater than R'_4

(e) Conclusions: at 5% level

(j, k, l) can not be shown to be different
(f, g, h, i, j, k) can not be shown to be different
(c, d, e, f, g, h, i, j) can not be shown to be different
(b, c, d, e) can not be shown to be different
(a, b, c, d) can not be shown to be different

<u>phosphorus</u> <u>to</u> <u>pigments</u>: Figure 25 shows in histogram form the means of the ratios for stations 3A and 7. The reason for the excessively high value during period A is low pigment values which seem to be associated with high water, as explained previously. Figure 26 is a plot of the means and two standard deviations of the means.

An F test on station 7 showed the means to be heterogeneous even at the one percent level. Further analysis showed that periods B, C, D, E, and F for station 7 and periods C, D, and E for station 3A can not be shown to be significantly different at the five percent level. The data and statistical analysis for stations 3A and 7 are recorded in tables 20 and 21 respectively.

The phosphorus to pigment ratio is similar to the pigment to nitrogen ratio in that it is usually fairly close to a mean value, but there are many exceptions and this ratio is by no means as useful as the phosphorus to nitrogen ratio.

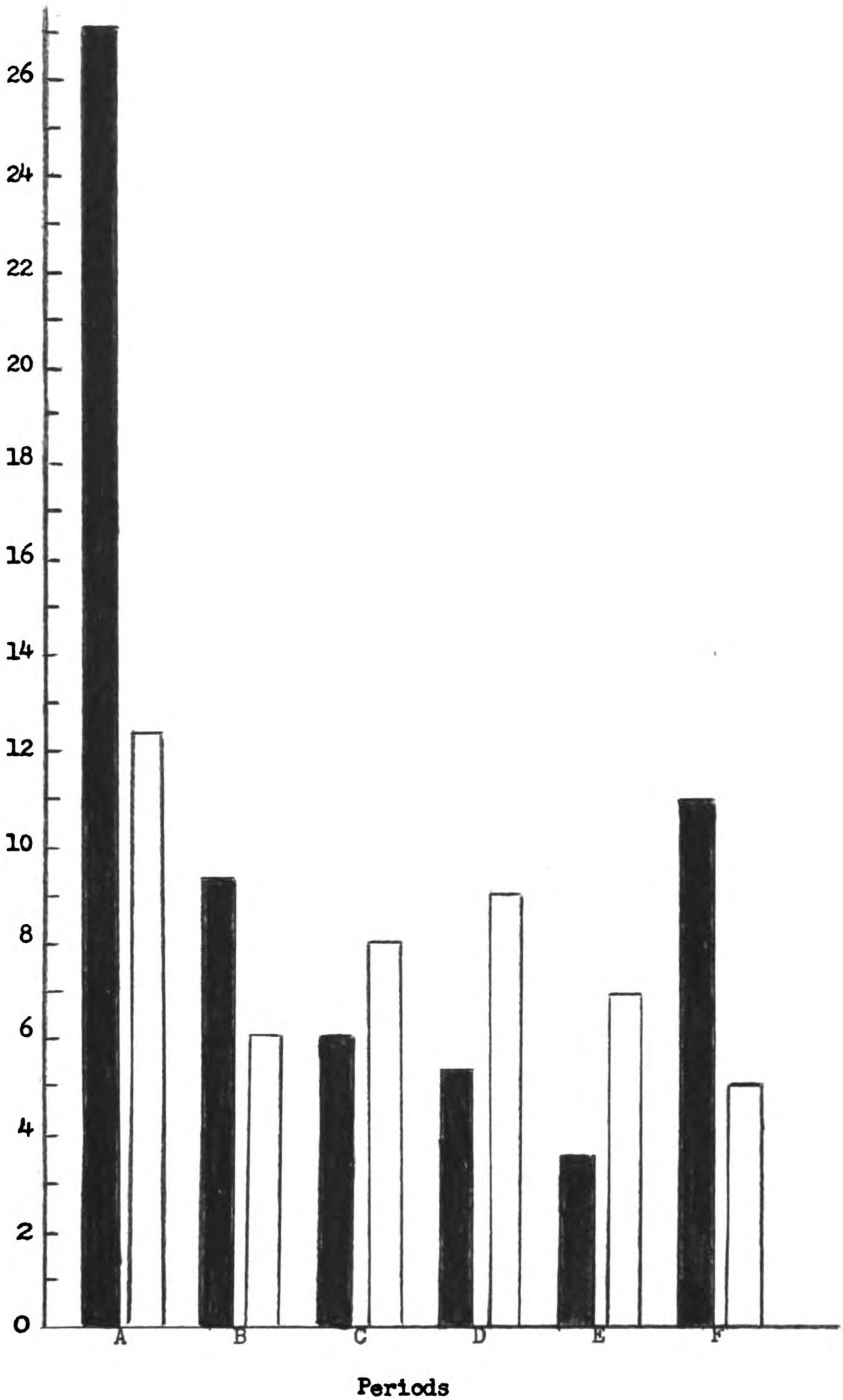
bottom organisms

The results of total phosphorus analysis of various benthic organisms are reported in terms of micrograms phosphorus per milliliter of organisms in table 22. It was felt by the author that there would be a value in knowing at least the approximate amount of phosphorus occurring in these organisms. However, the values are not very accurate, since volumes were measured crudely and not enough samples were taken to carry out a statistical analysis.

Whether a small increase in the percentage of phosphorus in these organisms took place after fertilization is not known, but

Figure 25

Mean Ratio of Phosphorus (in $\mu g.$) to Pigment (in Harveys) During Six Periods of the Summer.



26
24
22
20
18
16
14
12
10
8
6
4
2
0
A
B
C
D
E
F
Periods

Figure 26

Ratio of Total Phosphorus (in μg.) to Pigment (in Harveys) During Six Periods of the Summer (Means $\pm$ 2 Standard Deviations of the Means).

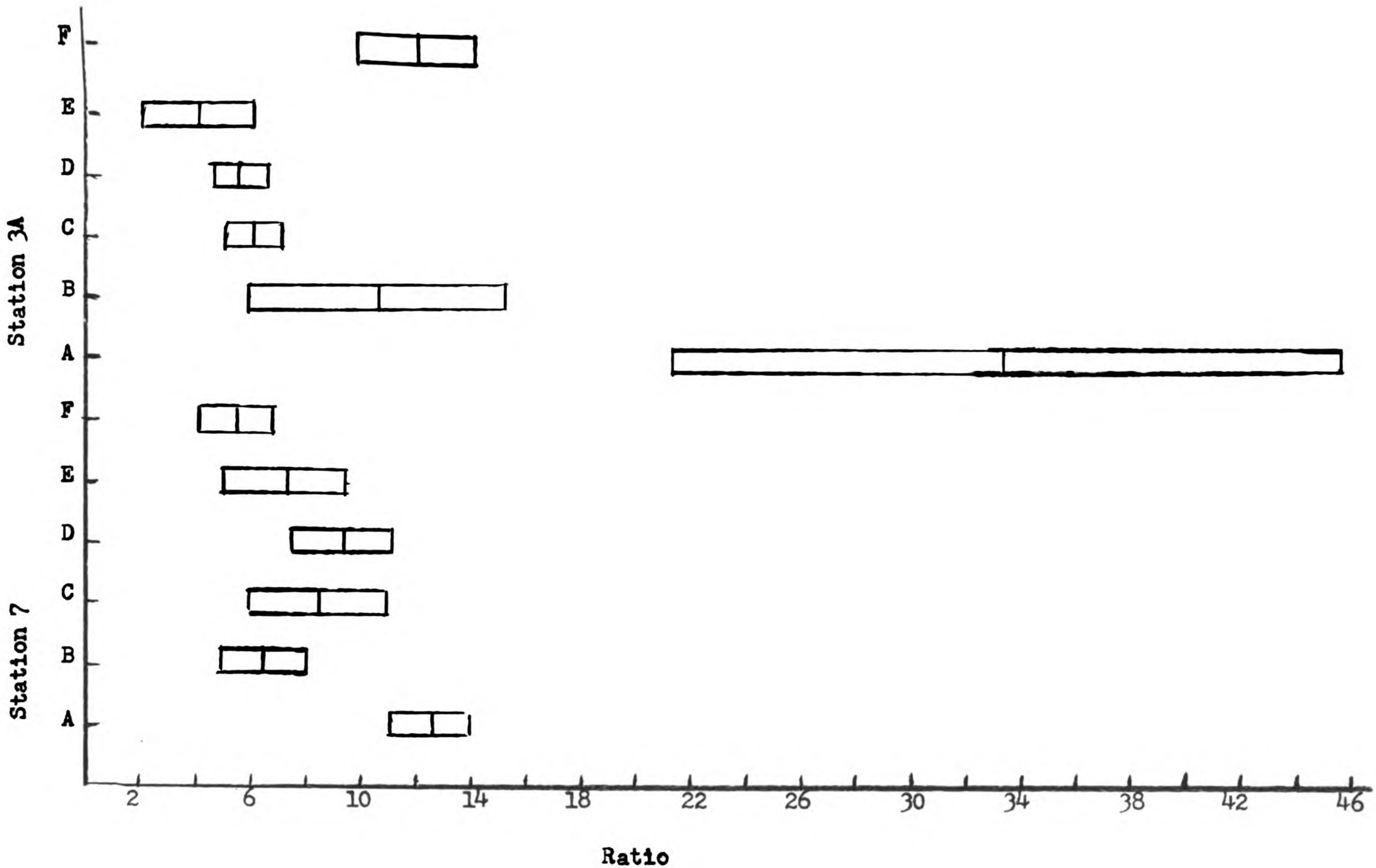

Station 3A
Station 7
F
E
D
C
B
A
F
E
D
C
B
A
2
6
10
14
18
22
26
30
34
38
42
46
Ratio

TABLE 20

Ratio of Phosphorus to Pigment in Periphyton-Station 3A (μg. P/Harvey units pigment) During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	9.38	10.79	7.19	8.21	2.06	13.30
2	33.64	21.07	9.36	8.04	3.66	14.21
3	41.83	8.11	4.69	3.37	1.80	11.07
4	25.88	5.38	5.16	5.18	2.50	6.17
5	41.41	9.42	5.31	3.19	4.05	14.72
6	71.00	6.16	4.66	5.36	2.44	10.76
7	24.54	28.05	5.61	6.83	2.40	7.48
8	19.71	4.48	4.97	6.20	5.99	15.69
9	28.49	7.33	7.39	4.66	12.73	16.07
10	...	4.88	7.20	4.96	3.53	9.67
sum	295.88	105.67	61.54	56.00	41.16	119.14
$\bar{X}$	32.88	10.57	6.15	5.60	4.12	11.91
ΣX^2	12,197.32	1,666.18	400.46	340.55	265.64	1,525.76
$(\Sigma X)^2/n$	9,727.22	1,116.62	378.72	313.60	169.42	1,419.43
Σx^2	2,470.10	549.56	21.74	26.95	96.22	106.33
$(\Sigma X)^2$	87,544.97	11,166.2	3,787.17	3,136.00	1,694.15	14,194.3
var.	308.76	61.06	2.44	2.99	10.67	11.81
sta. dev.	17.6	7.8	1.6	1.7	3.3	3.4

TABLE 21

Ratio of Phosphorus to Pigment in Periphyton-Station 7 (μg. P/Harvey units pigment) During Six Periods of the Summer

sample no.	A	B	C	D	E	F
1	11.27	3.17	18.81	5.63	10.67	4.50
2	11.78	4.85	5.21	7.06	...	6.96
3	11.80	9.35	5.71	11.15	8.60	3.94
4	17.36	7.19	7.46	9.00	5.17	5.16
5	15.45	8.56	9.26	6.29	3.30	2.86
6	9.86	5.00	5.24	8.68	6.59	4.46
7	14.33	10.39	10.67	10.95	5.60	10.11
8	8.90	3.83	9.17	10.49	15.13	6.90
9	13.16	7.17	7.30	8.62	5.63	5.78
10	12.48	5.08	5.14	16.06	5.11	3.81
sum	126.39	64.59	83.97	93.93	65.80	54.48
$\bar{X}$	12.64	6.46	8.40	9.39	7.31	5.45
ΣX^2	1,655.81	470.80	860.07	963.94	586.94	336.66
$(\Sigma X)^2/n$	1,597.44	417.19	705.10	882.28	481.07	296.81
Σx^2	58.37	53.61	154.97	81.66	105.87	39.85
$(\Sigma X)^2$	15,974.43	4,171.87	7,050.96	8,822.84	4,329.64	2,968.07
var.	6.49	5.96	17.22	9.07	13.23	4.43
sta. dev.	2.5	2.4	4.1	3.0	3.6	2.1

$SS_T = 818.67$, df = 58

$SS_B = 324.34$, df = 5

$SS_W = 494.33$, df = 53

$s_B^2 = 64.87$

$s_W^2 = 9.33$

$F = 6.95$

TABLE 21 (CONT.)

Multiple Range Test-Station 3A + Station 7

(a)

Source	df	m.s.	s
Between Treatments	11		
Error	106	35.52	6.0

(b) $R'_p = s\ z_p$

p:	2	3	4	5	6	7
5% z_p:	2.80	2.95	3.05	3.12	3.18	3.22
5% R'_p:	16.80	17.70	18.30	18.72	19.08	19.32

p:	8	9	10	11	12
5% z_p:	3.26	3.29	3.32	3.34	3.36
5% R'_p:	19.56	19.74	19.92	20.04	20.16

(c)

Code:	a	b	c	d	e	f
$\bar{X}$:	4.12	5.45	5.60	6.15	6.46	7.31
n:	10	10	10	10	10	9

Code:	g	h	i	j	k	l
$\bar{X}$:	8.40	9.39	10.57	11.91	12.64	32.88
n:	10	10	10	10	10	9

(d) Test Sequences: at 5% level

(l-k)' greater than R'_2
(k-e)' greater than R'_7; (k-f)' not greater than R'_6
(j-c)' greater than R'_8; (j-d)' not greater than R'_7
(i-a)' greater than R'_9; (i-b)' not greater than R'_8
(h-a)' not greater than R'_8

(e) Conclusions: at 5% level

(f, g, h, i, j, k) can not be shown to be different
(d, e, f, g, h, i, j) can not be shown to be different
(b, c, d, e, f, g, h, i) can not be shown to be different
(a, b, c, d, e, f, g, h) can not be shown to be different

TABLE 22

Total Phosphorus in Bottom Organisms

date	organism	sample volume	μg. P/ml.	mean
7-22	*Chara*	3.0 ml.	200	
7-22	"	5.6 ml.	172	
7-22	"	5.0 ml.	245	
				206
8-18	*Chara*	6.6 ml.	250	
8-18	"	9.5 ml.	266	
				258
7-22	Stoneflies	1.6 ml.	1,875	
8-18	Stoneflies	3.7 ml.	1,311	
7-22	Mayflies	4.0 ml.	812	
7-22	"	1.8 ml.	472	
				642
8-18	Mayflies	7.3 ml.	911	
8-18	"	4.9 ml.	1,163	
8-18	"	5.2 ml.	1,082	
				1,052
7-22	Dragonflies	3.4 ml.	1,235	
7-22	"	0.5 ml.	1,999	
7-22	"	3.3 ml.	985	
				1,406
8-18	Dragonflies	1.0 ml.	1,425	

there was no large increase. Even if a small change could be shown, other factors such as life cycles could also influence the percentage.

It is evident that *Chara* has only about one-fourth as high a percentage of phosphorus as the insects studied. It is also interesting to note that none of the analyses indicated more than 0.2 percent phosphorus (wet weight).

CONCLUSION

It was concluded from the results of this study that the addition of inorganic phosphate to the west branch of the Sturgeon River resulted in a large increase in the primary production in a section extending at least several miles down the stream. Colonization and growth upon new substrates was no more rapid after the cessation of fertilization than before. The phosphorus to nitrogen ratio in the periphyton complex was one to ten by weight during the entire study with the possible exception of the period of fertilization. There is good reason to believe that phosphorus is the limiting nutrient in primary production.

No significant increase in total volumes of bottom fauna at station 7, the downstream station, could be correlated with fertilization or its after effects.

APPENDIX

Introduction

There has been a concerted effort for many years to develop a method of measuring primary production. The greatest problem has been the difficulty of getting an exact, quantitative method. This is especially difficult in lotic situations since current is a factor. Any method which is developed must be of a type which can be applied in an efficient manner to have any practical value.

One channel of effort has been based upon the fact that certain types of pigments are essential to the photosynthetic process. These methods are based upon the extraction and measurement of pigments found in the plants which are carrying out primary production in a given case.

Harvey (1934) formulated a method for the estimation of the quantity of chlorophyll present in an extract based upon a visual comparison with a set of inorganic standards. This method was the basis for much work in the fields of Limnology and Oceanography. In a modification of this method in which absorbency of the extract is measured in the region from 640 to 700 millimicrons with a photoelectic colorimeter and a correction is made at higher absorbencies for the deviation from the Lambert-Beer Law, Grzenda (M.S.) has correlated the amount of pigment with dry organic weight of periphyton in the Red Cedar River.

One of the more important recent attempts to improve on this type of measurement is based upon the light absorbed by a 90 percent acetone extract using a spectrophotometer at certain specific wavelengths (Richards, 1952) and the use of nomographs to simplify calculations of the components causing this absorbency (Duxbury, 1956). In this method certain assumptions as to the composition of the pigment complex must be made, unless supplementary studies are made. If all the pigments which absorb light in this area are known and their specific absorbency can be determined, this method should give accurate results.

It is the belief of the author that before any method of this type can completely succeed, a more thorough understanding of the pigment complex in algae is necessary. The physiological importance of the various pigments needs further study and the relative stability of the quantities of these pigments within the cell should be known.

Spectroscopy and Chromatography

(historical)

The pigment complex found in algae is complicated and has been studied a great deal. A good review of the subject is found in The Manual of Phycology (Smith, 1951) in the section on pigments, which is written by H. H. Strain. The xanthophylls are discussed more thoroughly in Leaf Xanthophylls (Strain, 1938).

Pigments may be separated efficiently by chromatography. Lind (1953) made some rough separations by means of two-dimensional ascending paper chromatography. However, column chromatography

has proven more useful in most applications. The subject of chromatography is discussed on an introductory level by Brimley (1953) and many applications and references to specific methods are given by Zechmeister (1950). A thorough review of the literature on the physical properties of pigments in general is given by Zacheile (1941).

The structures of chlorophyll a and b have been known for a number of years. Chlorophyll c has been found to be a magnesium complex lacking phytol, and it is probably a modified magnesium pheoporphyrin (Granick, 1949). Chlorophylls a and b are found in the Chlorophyta, a and c in the Bacillariaceae, and only a in the Cyanophyta, (Smith, 1951). Although the chlorophylls are the only pigments which are known to take a direct part in photosynthesis, the Cyanophyta also possess phycobilin type pigments which are protein containing pigments that absorb in the green range of the spectrum and fluoresce in green light. These pigments are believed to act to aid in energy transfer to the red-absorbing chlorophylls by absorbing green light and fluorescing in a lower frequency (French, 1952).

It is interesting to note that although the red peak of chlorophyll a is generally quoted as about 665 millimicrons, upon direct measurement in leaves the peaks were found to be shifted about twelve millimicrons toward the red end of the spectrum (Shpolskii, 1947). This may be due to the association of chlorophyll with proteins in the chloroplast.

Chlorophyll, when acted upon by the enzyme chlorophyllase in the presence of ethanol, forms ethyl chlorophyllide in which the phytol is replaced by ethanol. Weak acids remove the magnesium from chlorophyll to form pheophytins and the action of a strong acid upon either the chlorophyll, the ethyl chlorophyllide, or the pheophytin yields a pheophorbide in which both the phytol group and the magnesium are missing (Bonner, 1950).

In order to detect which pigment is being studied after separations and to follow the progress of the separations, spectrograms of the visible range are usually used. The absorbency peaks for the algal pigments are given by Smith (1951). Bacteriochlorophyll a solutions have maxima at 360, 390, 570, and 770 millimicrons (Holt, 1954). Protochlorophyll has peaks at 435, 530, 575, and 625 millimicrons (Smith, 1948). Holt (1952) also reported the absorption spectra of ethyl chlorophyllides a and b as being the same as the original chlorophylls. He reports a shift of the peaks to 408, 500, 532, 605, and 645 millimicrons for the pheophorbide of chlorophyll a and peaks of 432, 525, 600, and 645 millimicrons for the pheophorbide of chlorophyll b. He reports the same spectra for the pheophytins as for the pheophorbides. This would mean that the removal of the alcohol or a change of the alcohol would have no effect on the spectra.

Using ethanol Evstigneev (1954) reports a reversible shift of chlorophyll a to a semiquinonoid form with peaks at 415, 518, 585, and 665-670 millimicrons. In aerobic conditions this form shifts to the pheophytin.

Experimental

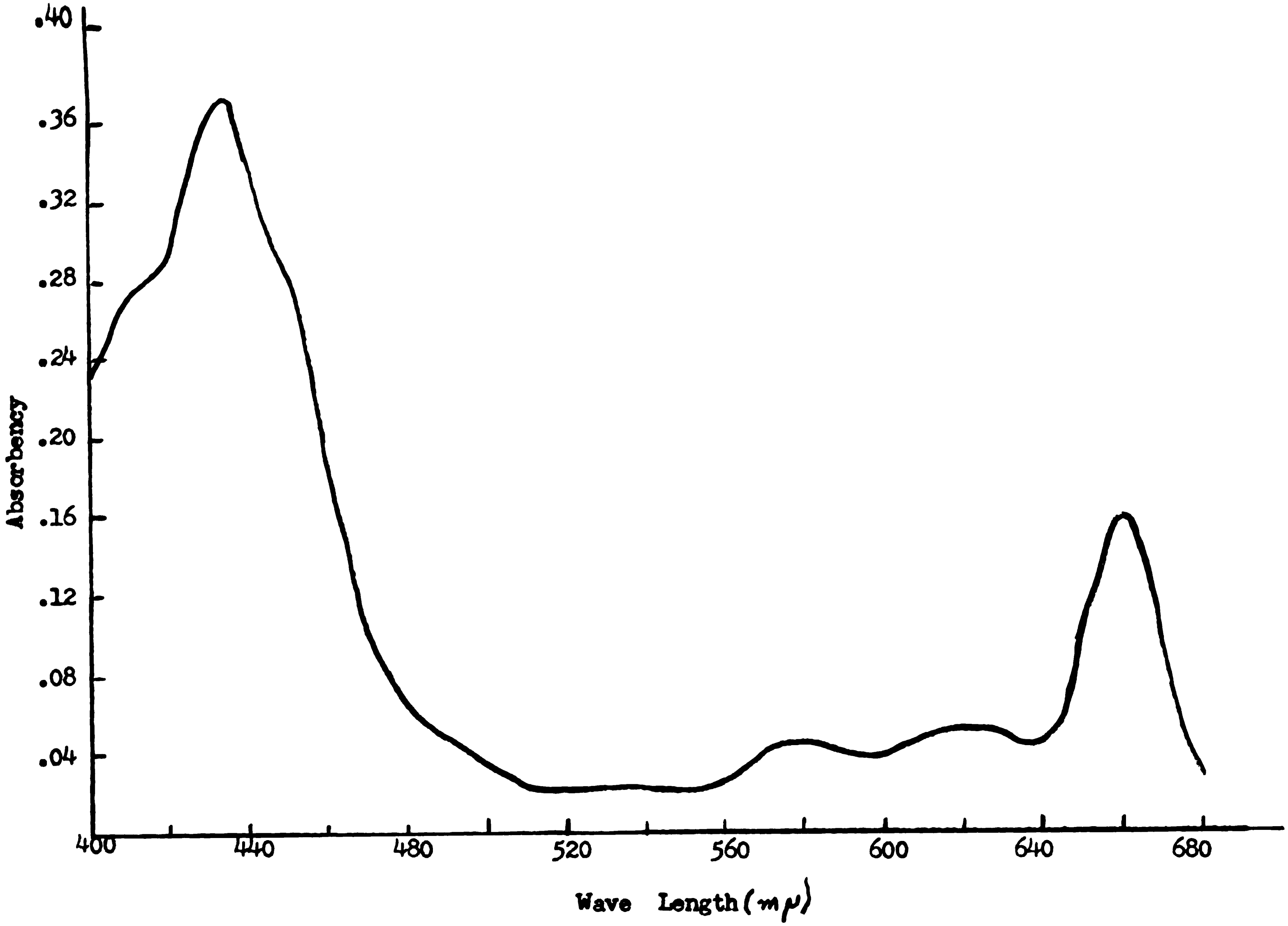
Red Cedar River

Fresh periphyton material was obtained from the Red Cedar River in Ingham County in the water upstream from the sewage treatment plant at Williamston. In a preliminary experiment a small amount of 90 percent acetone extract was fractionated in a column packed with powdered sucrose. The columns were always packed with a slurry of adsorbent in the solvent. Petroleum ether and benzene (9:1) was used as a solvent. Four fractions were obtained. Fractions A and C are shown in figures 27 and 28. Fraction A contains a large amount of chlorophyll a and probably a relatively small amount of chlorophyll c. Similarly fraction C probably contains a considerable amount of chlorophyll b, which is obscured by some persistent chlorophyll a. It will be noticed in all of the experimental results that there tends to be a shift of absorbency peaks several millimicrons toward the blue end, probably due to instrumental error.

In order to get a better idea of the pigments present in this complex another sample was obtained and a large initial volume of 90 percent acetone extract was transferred to a petroleum ether, benzene solvent. This was run through a powdered sucrose column 16 millimeters in diameter and about 30 centimeters in length. By visually controlled fractionation 19 fractions of eluent were obtained and the pigment remaining in the sugar was extracted to form fraction 20.

Figure 27

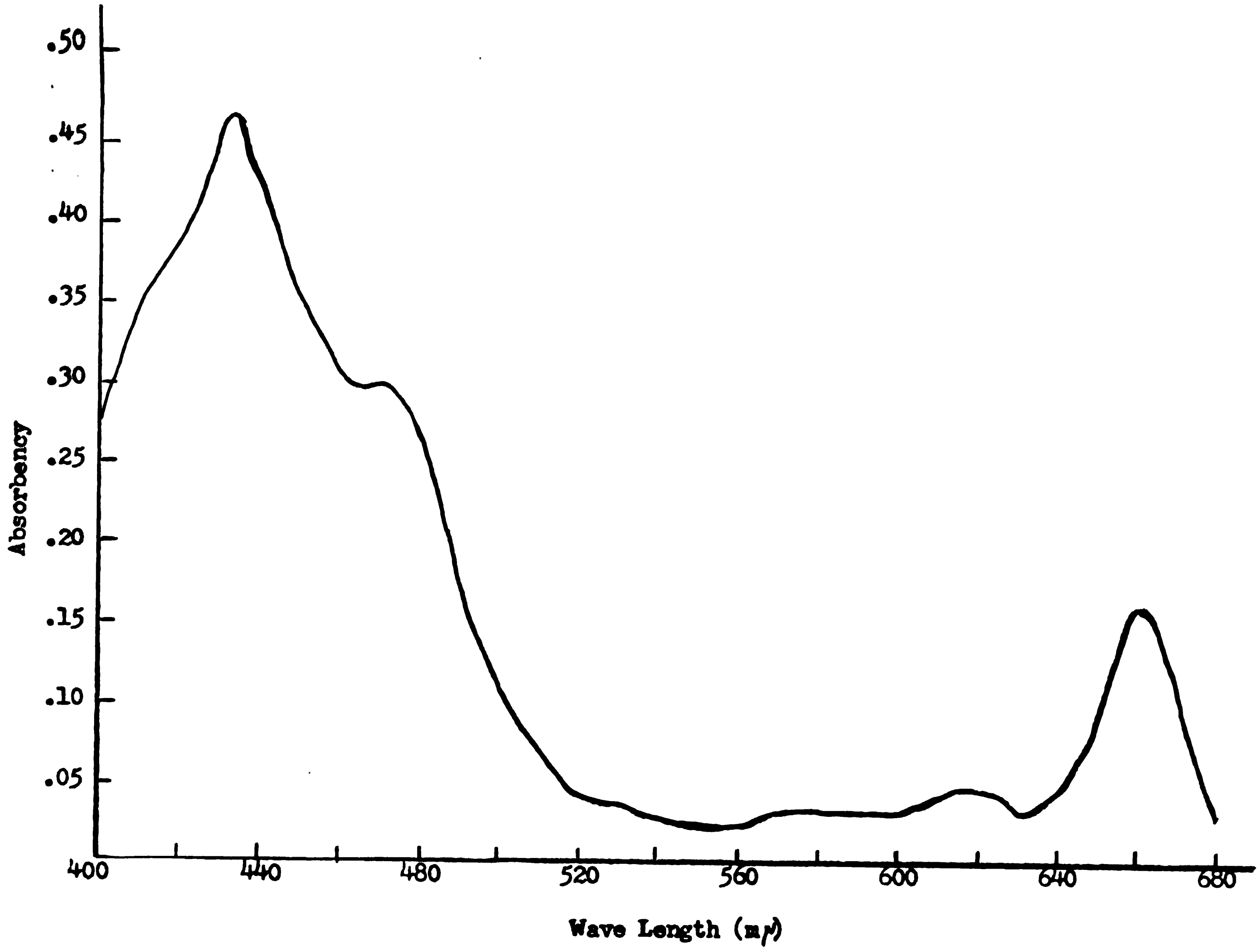
Absorption Spectra of Red
Cedar Periphyton Pigment
(Fract. A).



.40
.36
.32
.28
.24
.20
.16
.12
.08
.04
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 28

Absorbency Spectra of Red
Cedar River Periphyton Pigment,
Fraction C.



.50
.45
.40
.35
.30
.25
.20
.15
.10
.05
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

A graph of the spectra of the total mixture is shown in figure 29. Fraction 1 was run on a ten millimeter column of alumina dried at 60° C. and broken into three more fractions. Fraction 1' is shown in figure 30 and has a single peak at about 448 millimicrons. In all probability this reddish pigment was fucoxanthin, which is the most abundant xanthophyll found in diatoms. Fraction 3' is shown in figure 31 and is identical with fraction 9' and will be discussed later. It is shown here only to point out that two different fractionation methods arrived at precisely the same peaks for this important constituent of the pigment complex.

Fractions 7 through 11 were combined and rechromatographed in precisely the same manner as the original mixture, except that the pigments were separated on the basis of color regions within the column rather than elution. The column was cut into six color regions to form fractions 4' through 9' and the color that came through made up fraction 10'. Fraction 6' is shown in figure 32. It was an olive green color. This fraction was shown to be composed of at least two pigments by combining it with fraction 5', a similar fraction, and then rechromatographing on a ten millimeter column of sucrose and separating into fractions 1" through 4" on the basis of colored bands.

Fraction 2" is shown in figure 33. It is believed to represent almost entirely chlorophyll _a_ in an unmodified form. This fraction was dried under reduced light in a vacuum desiccator and weighed. The weight of the sample was 2.3 milligrams. This was redissolved in ten milliliters of ethanol and the spectra determined immediately.

Figure 29

Absorbency Spectra of Red Cedar River Periphyton Pigment, Initial Mixture.

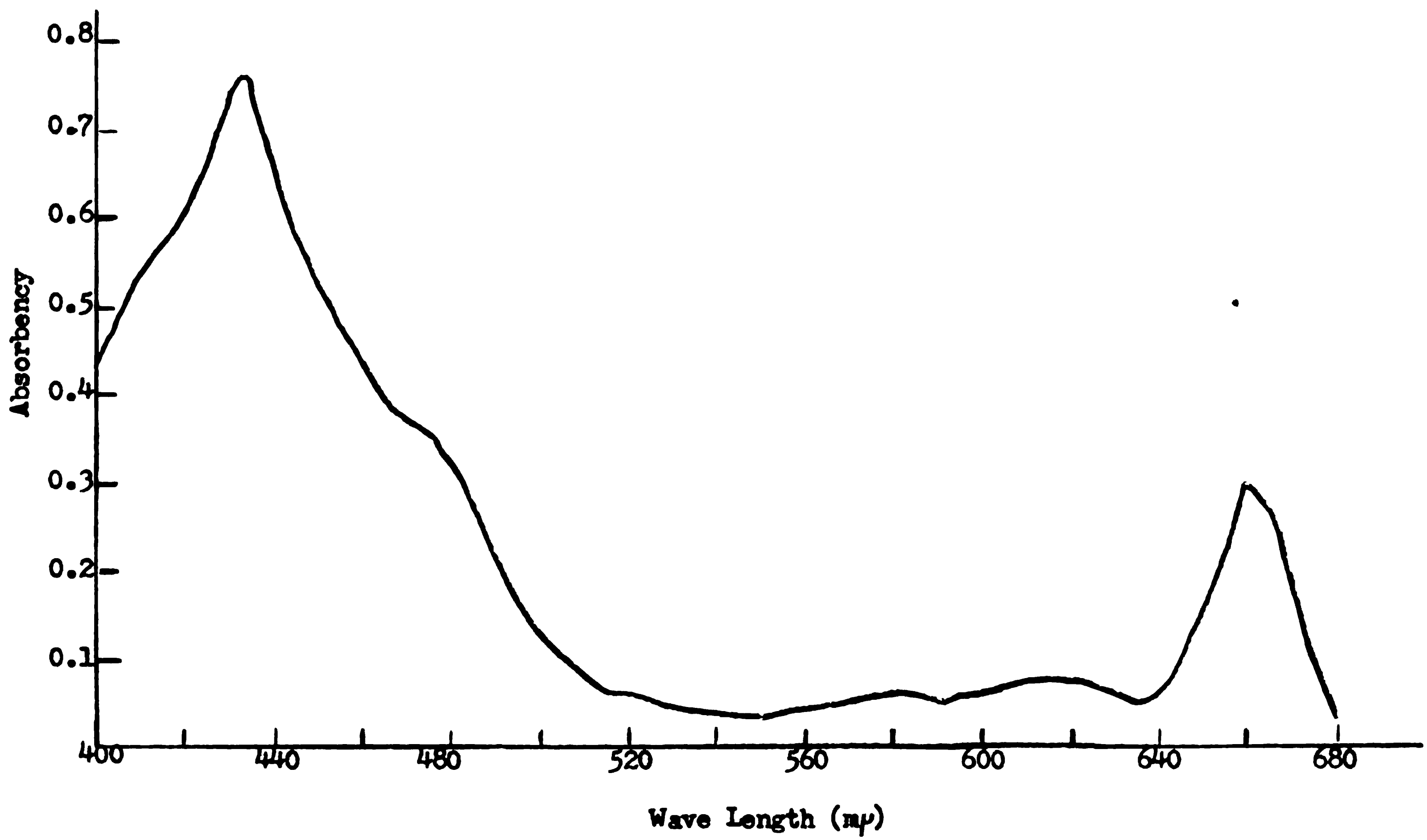

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 30

Absorbency Spectra of Red
Cedar River Periphyton Pigment,
Fraction 1'.

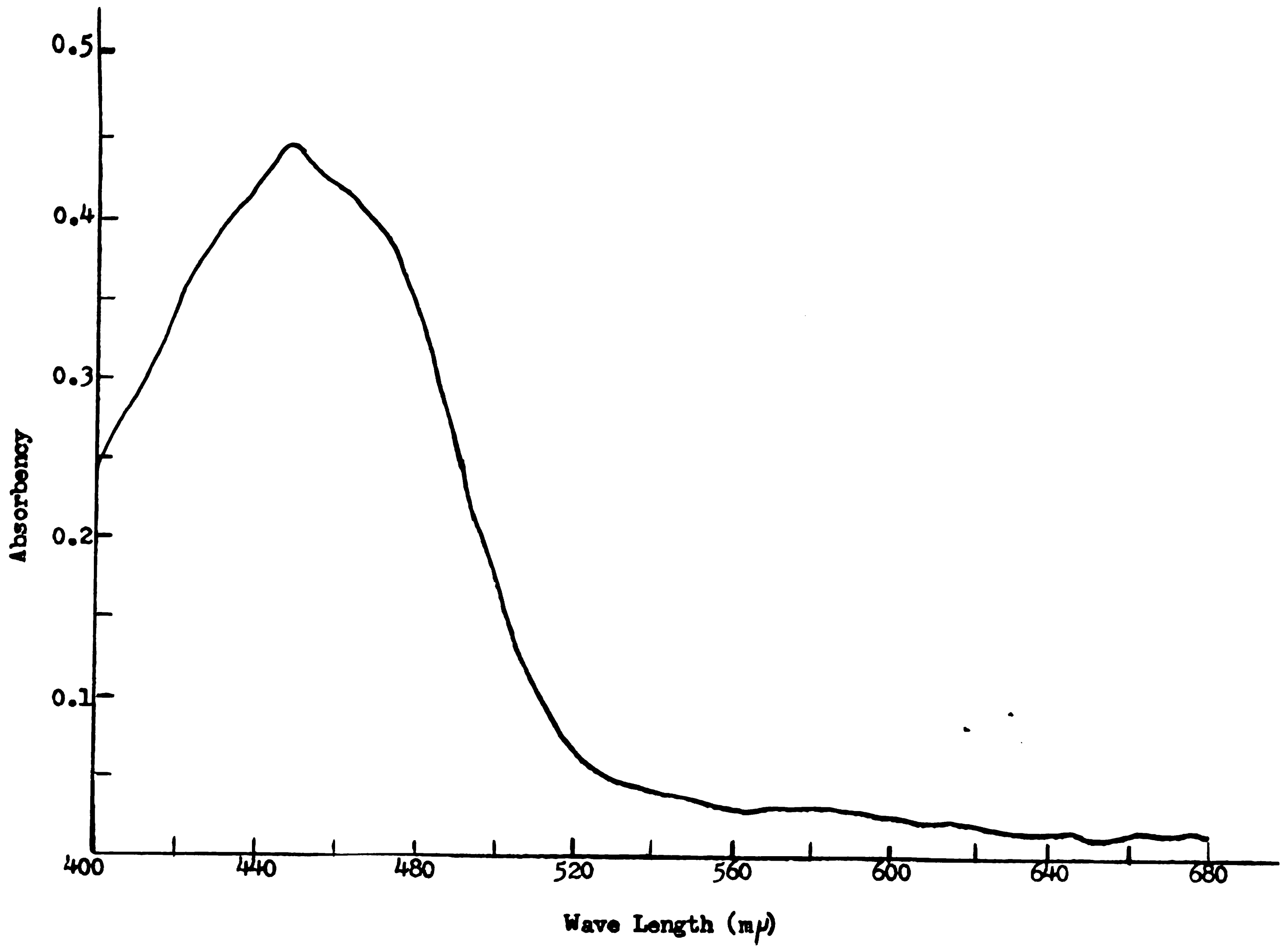

0.5
0.4
0.3
0.2
0.1
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 31

Absorbency Spectra of Red
Cedar River Periphyton
Pigment, Fraction 3'.

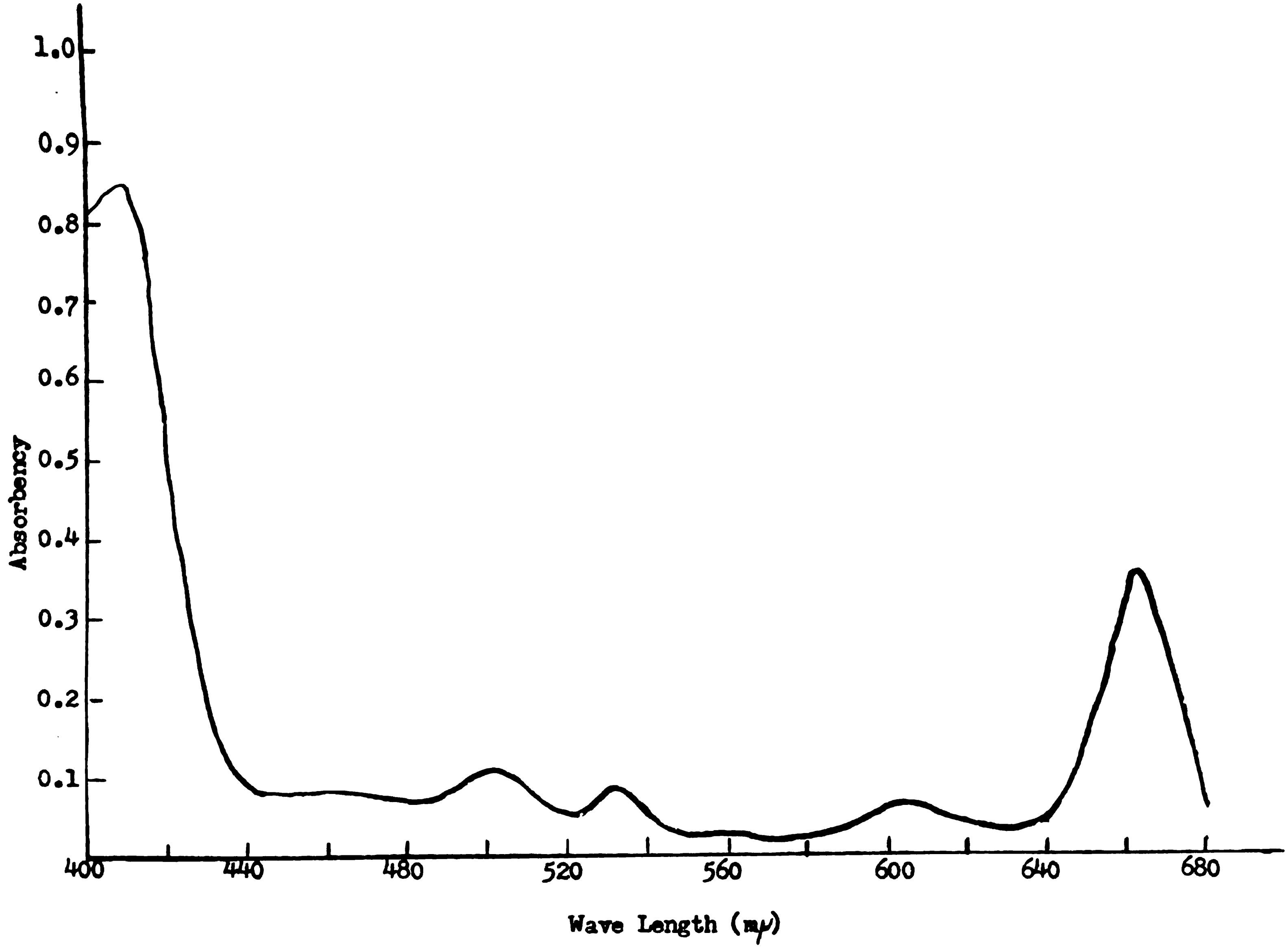

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 32

Absorbency Spectra of Red Cedar River Periphyton Pigment, Fraction 6'.

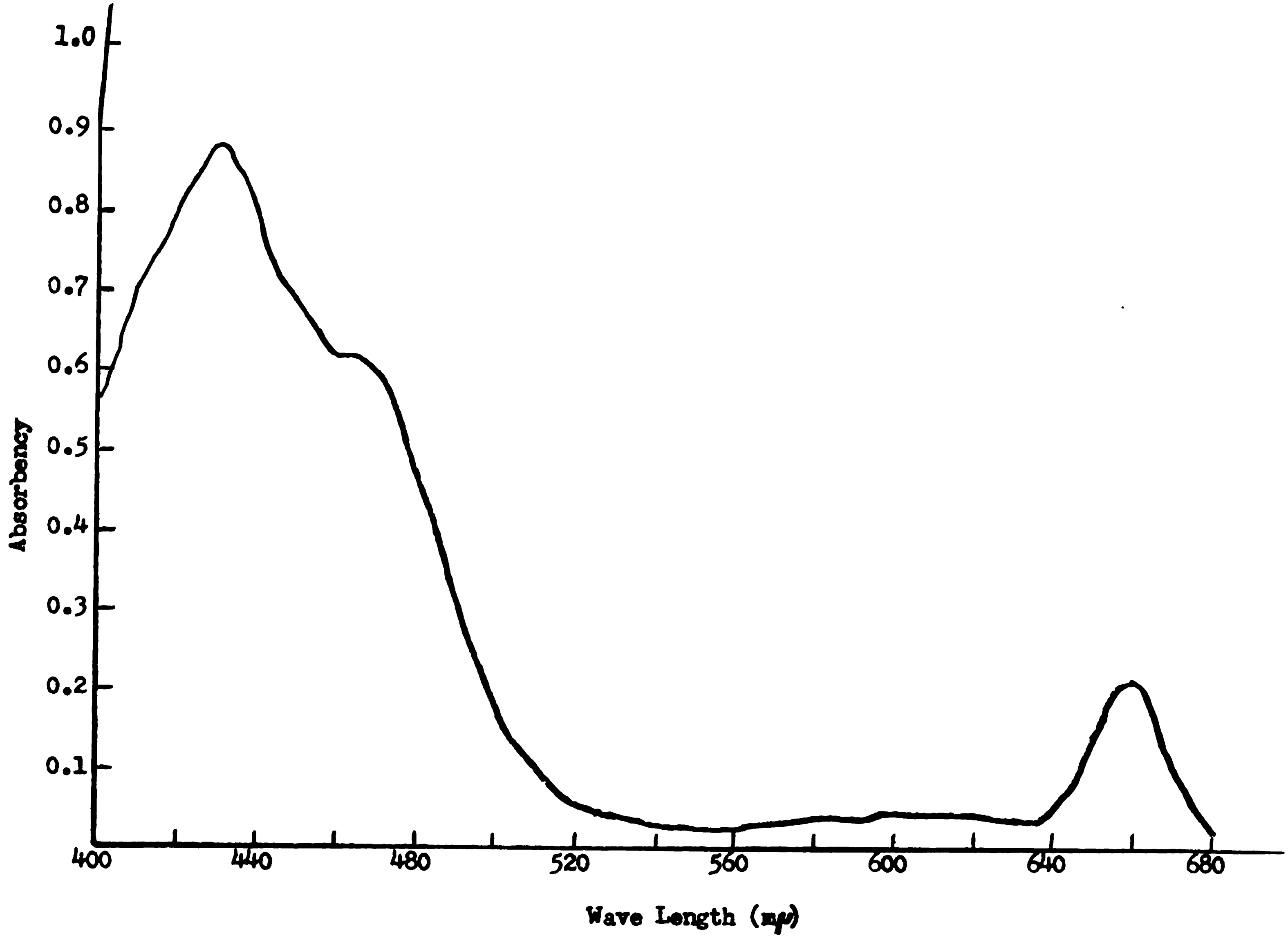
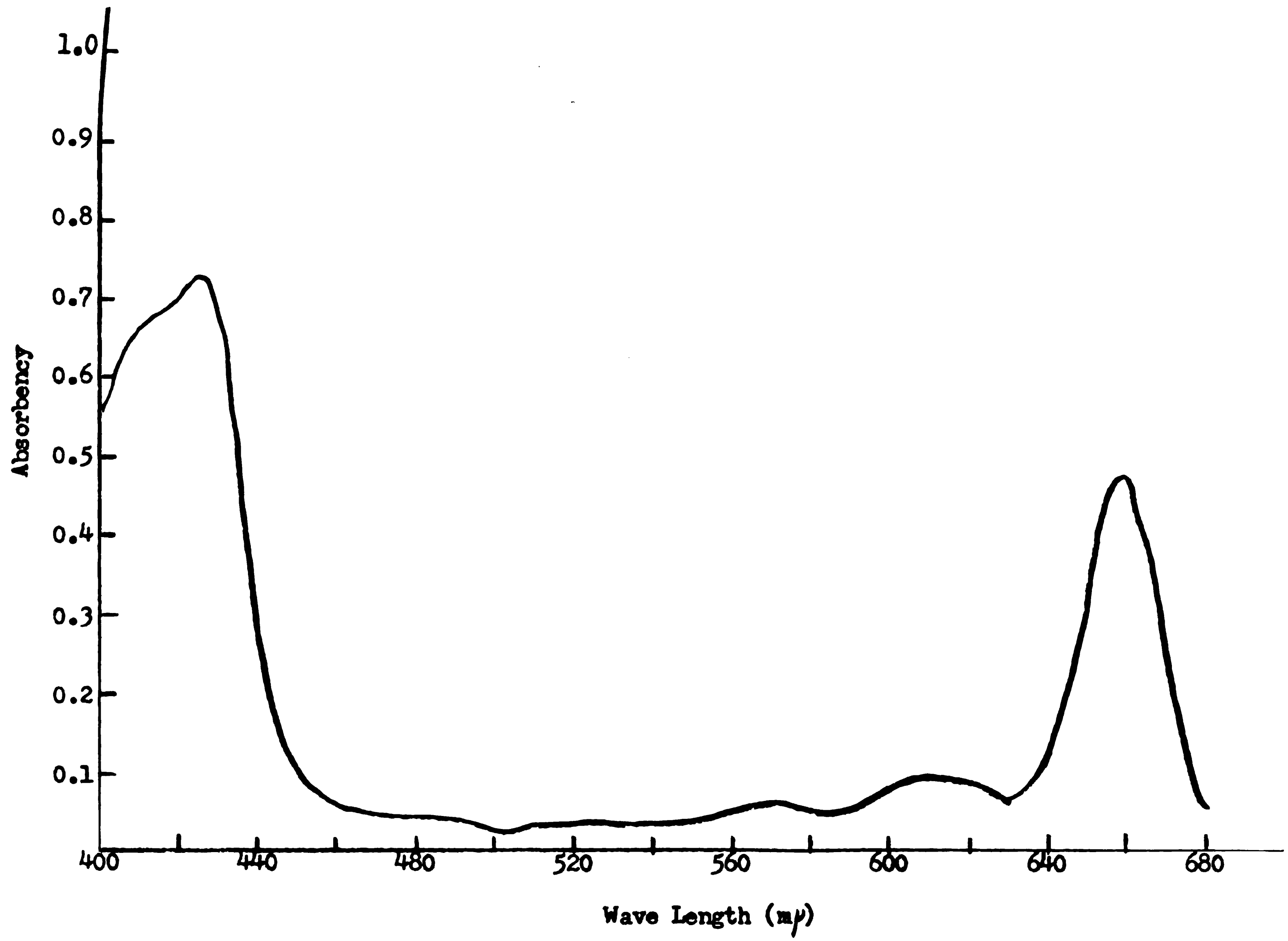

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 33

Absorbency Spectra of Red
Cedar River Periphyton
Pigment, Fraction 2".



Absorbency
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
400
440
480
520
560
600
640
680
Wave Length (mμ)

The spectrogram is shown in figure 34. The peak which was originally at about 428 millimicrons shifted to about 415 millimicrons while the red peak remained unchanged. This corresponds to the results of Evstigneev (1954) as described earlier.

Fraction 4" is shown in figure 35. This was an orange pigment and the peaks are at about 442 and 465 millimicrons. It was probably violaxanthin, which is found in the green algae.

Fraction 8' is shown in figure 36 and is quite apparently a combination of several pigments. Further fractionations were of no value due to lack of sufficient yields. The author would propose that the peaks are a result of a combination of the chlorophyll intermediate described by Evstigneev (1954) and a pigment with peaks at about 442 and 473 millimicrons. This might be lutein which absorbs at 446 and 476 millimicrons and is a major pigment in the green algae, or it might be diadinoxanthin which is found in diatoms and absorbs at 448 and 478 millimicrons.

Fraction 9' was a definitely blue-green pigment with a very high specific absorbency. It is shown in figure 37 and has definite peaks at 408, 502, 532, 605, and 662 millimicrons. This pigment occurs in fresh periphyton and since it has a strong peak at 662 millimicrons and occurs in large enough quantities to be easily separable, the method used by Richards (1952) would be very misleading if applied to this material. The peaks found for this pigment correspond to the peaks reported by Holt (1954) for pheophorbide _a_ except for the red peak at 662 millimicrons. This pigment is only adsorbed weakly by sucrose. Fraction 9' was dried

Figure 34

Absorbency Spectra of Red Cedar River Periphyton Pigment, Fraction 2" (after transfer to Ethyl Alcohol).

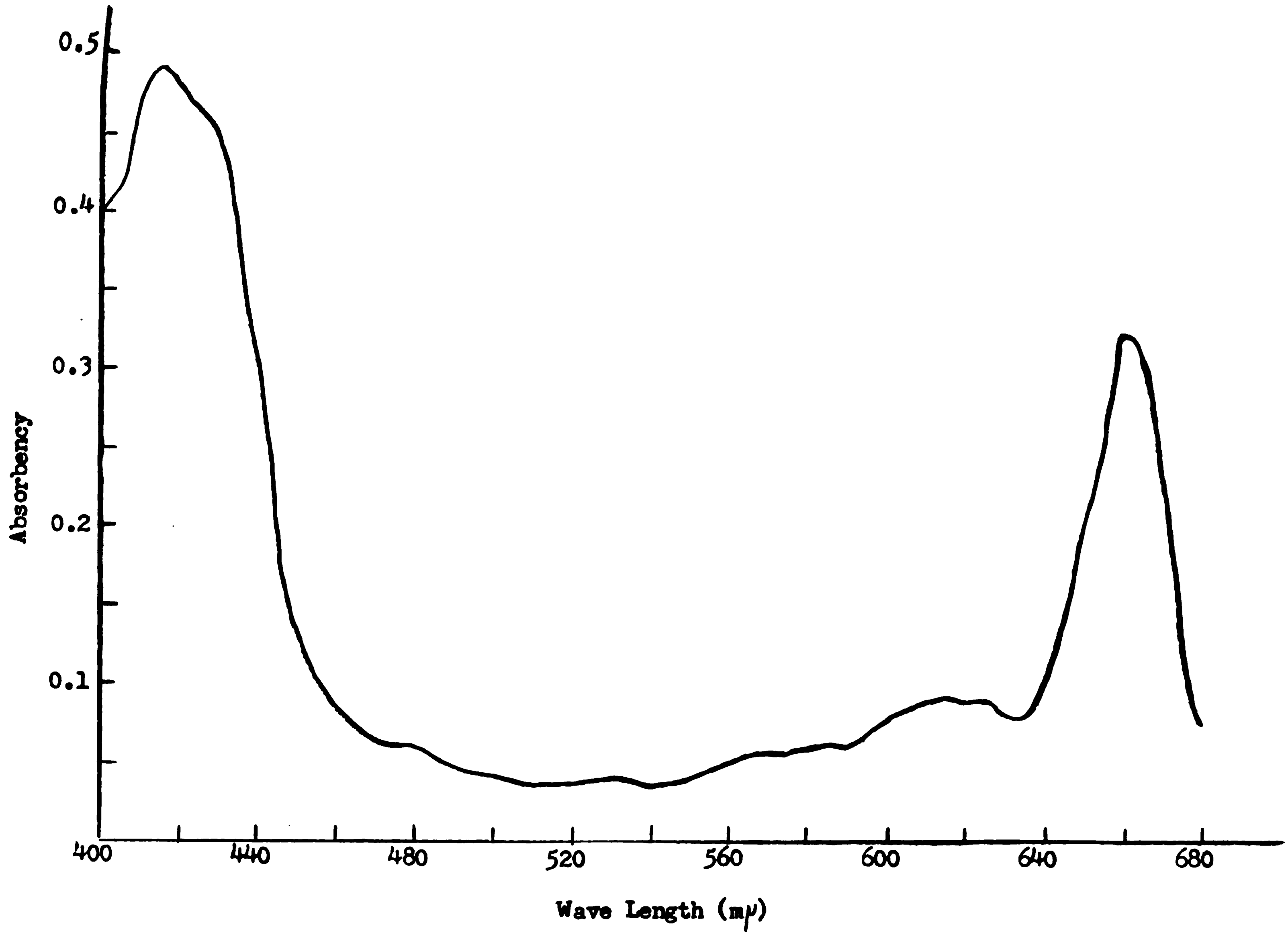
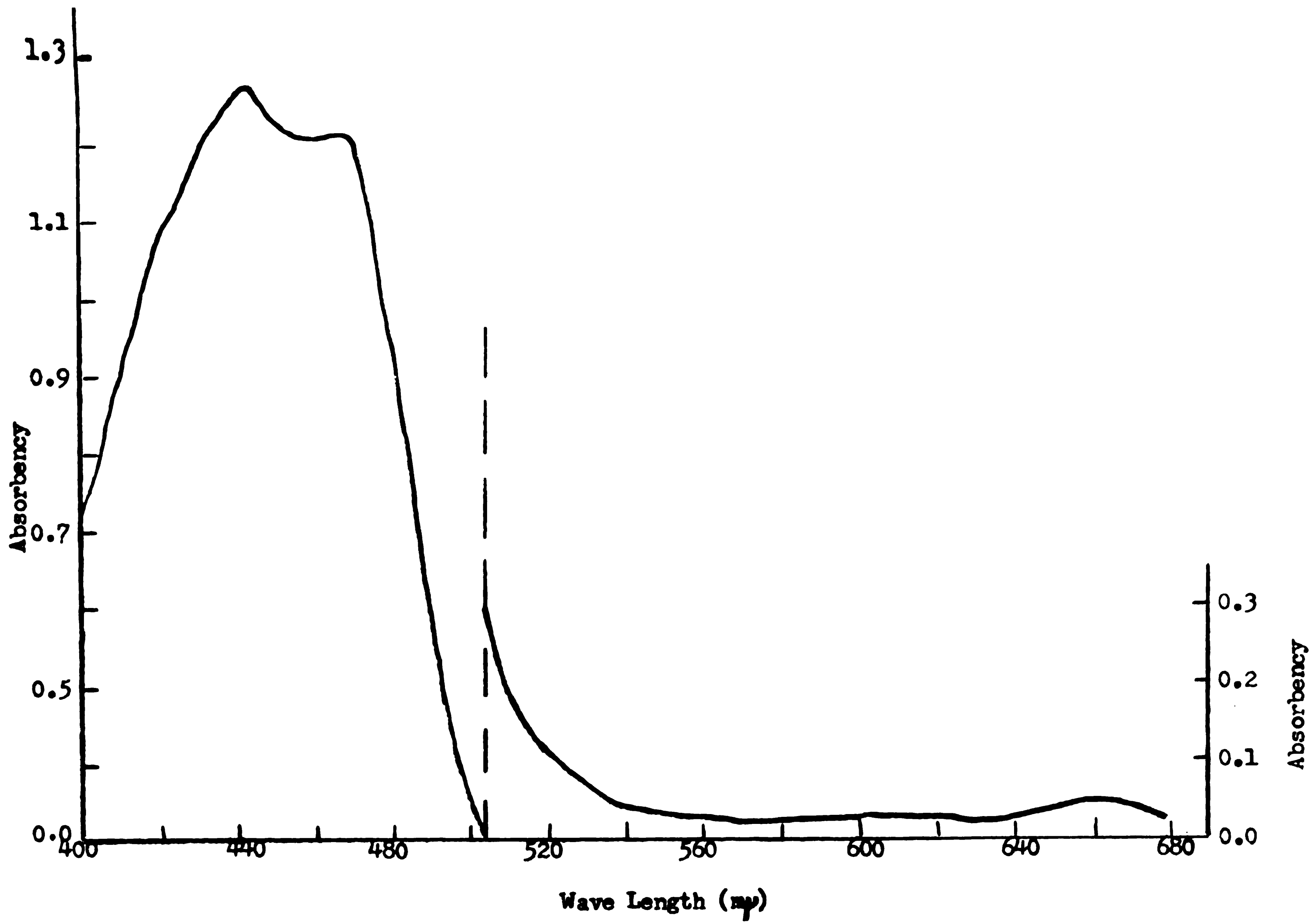

0.5
0.4
0.3
0.2
0.1
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 35

Absorbency Spectra of Red Cedar River Periphyton Pigment, Fraction 4".



1.3
1.1
0.9
0.7
0.5
0.0
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)
0.3
0.2
0.1
0.0
Absorbency

Figure 36

Absorbency Spectra of Red
Cedar River Periphyton
Pigment, Fraction 8'.

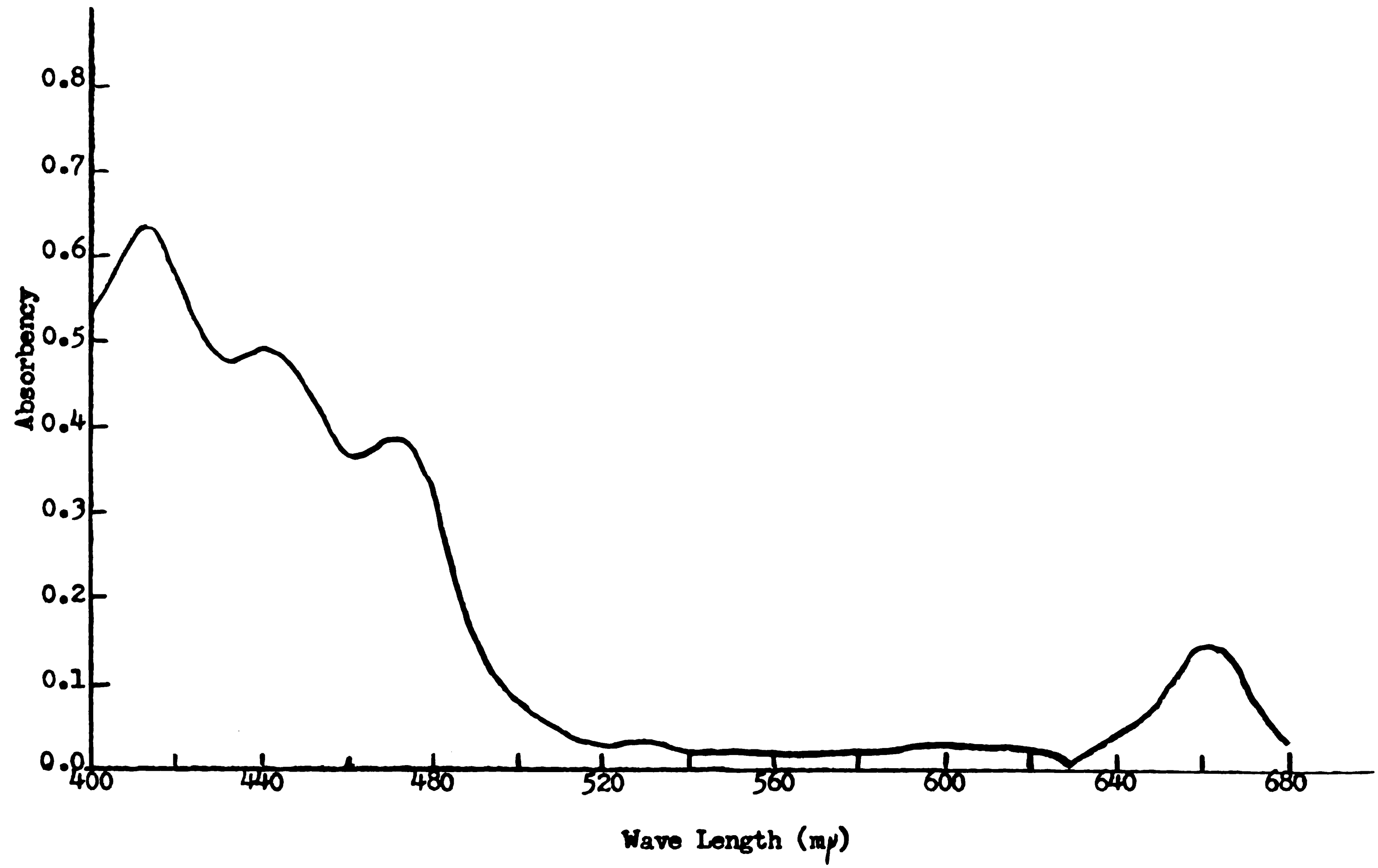
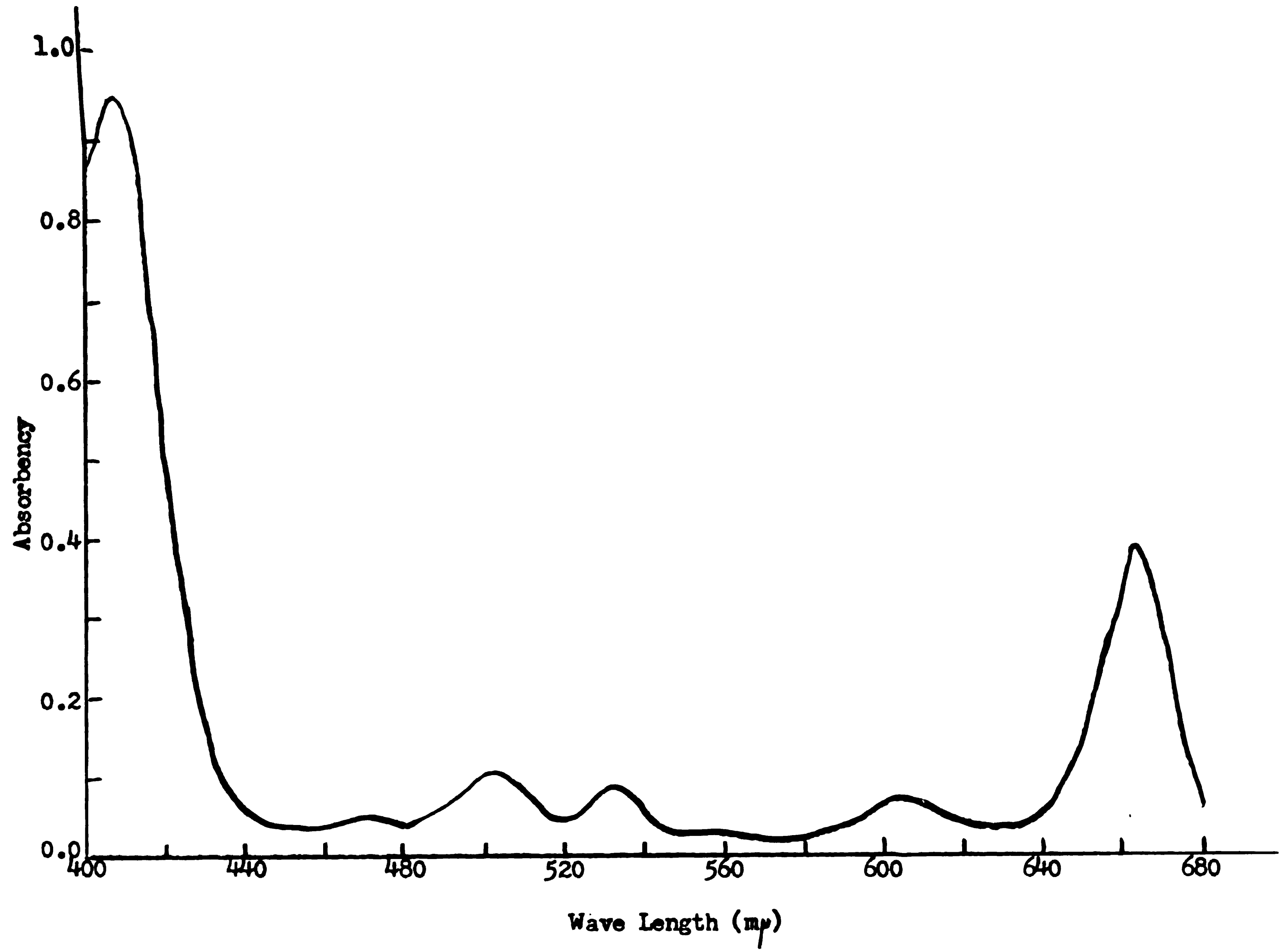

Absorbency
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 37

Absorbency Spectra of Red
Cedar River Periphyton
Pigment, Fraction 9'.


1.0
0.8
0.6
0.4
0.2
0.0
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

in a vacuum desiccator under reduced lighting and found to weigh 0.7 milligrams. This amount was dissolved in ten milliliters of 95 percent ethanol and the spectrogram determined immediately. The result is shown in figure 38. It is interesting to note that the use of alcohol as a solvent doesn't shift these peaks.

Fractions 14 through 17 were combined and rechromatographed using a ten millimeter column of alumina. The column was separated into four fractions on the basis of color bands and the fractions were labeled 11' to 14'. Fraction 11' is shown in figure 39. It is an orange pigment with a single peak at about 442 millimicrons. It is probably neofucoxanthin A (447 mμ), neofucoxanthin B (446 mμ), or a combination of the two. Both pigments are found in fair amounts in diatoms.

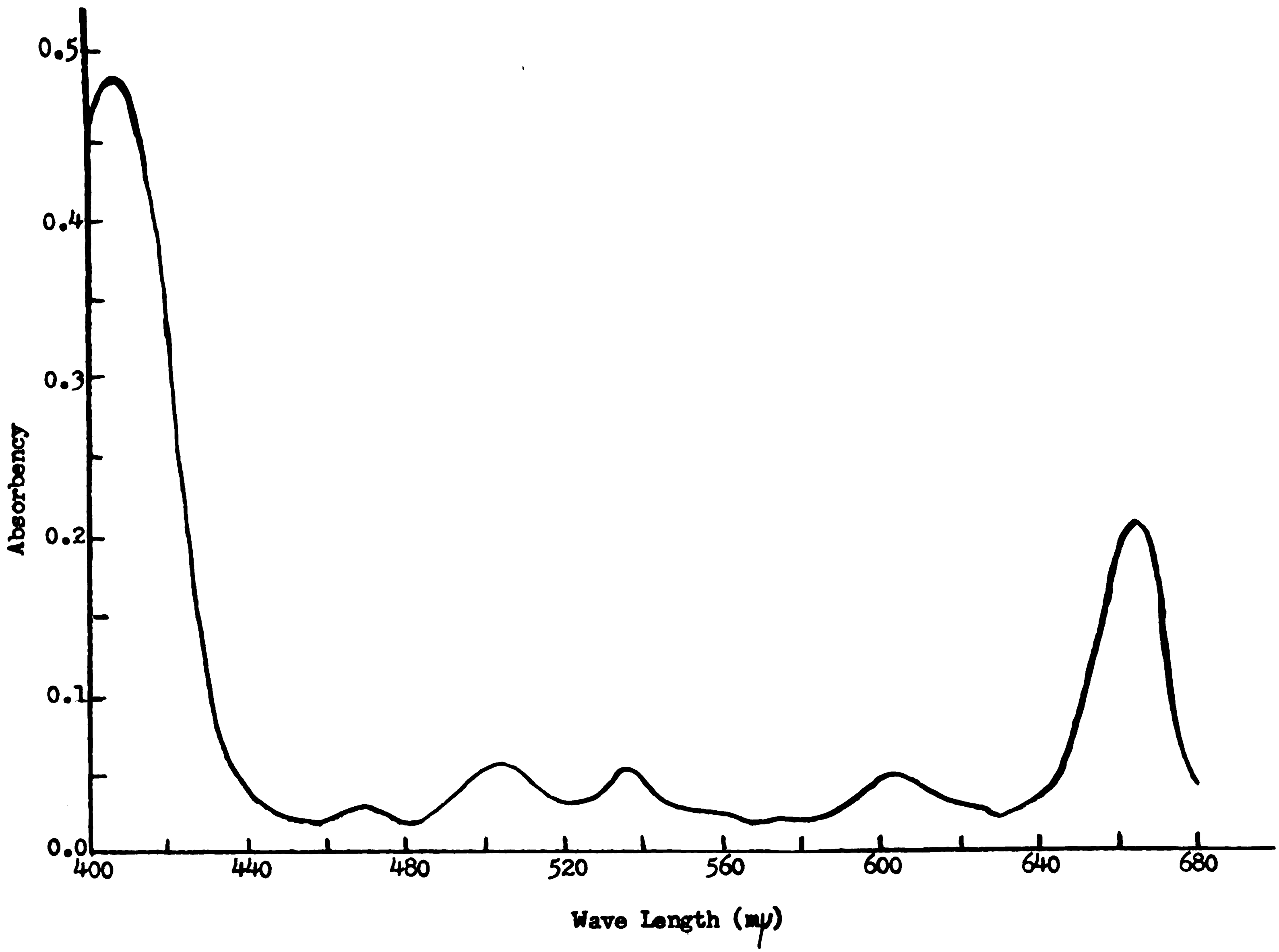
An outline of these fractionations and the colors of the fractions is shown in table 23. The spectrophotometer data, which was obtained using a Beckman Model B Spectrophotometer, is shown in table 24.

West Branch of the Sturgeon River

In order to determine what was actually measured when the absorbencies of 95 percent ethanol extracts of periphyton were determined in the west branch of the Sturgeon River study, a chromatographic separation was carried out on a large volume of the extract. The extract was dried in a vacuum desiccator under reduced lighting and chromatographed in a 16 millimeter column packed with sucrose. The eluent was separated into seven fractions

Figure 38

Absorbency Spectra of Red Cedar River Periphyton Pigment Fraction 9[1] (transferred to ethyl alcohol and at a known concentration of 70 mg/l.).



0.5
0.4
0.3
0.2
0.1
0.0
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 39

Absorbency Spectra of Red
Cedar River Periphyton
Pigment, Fraction 11'.

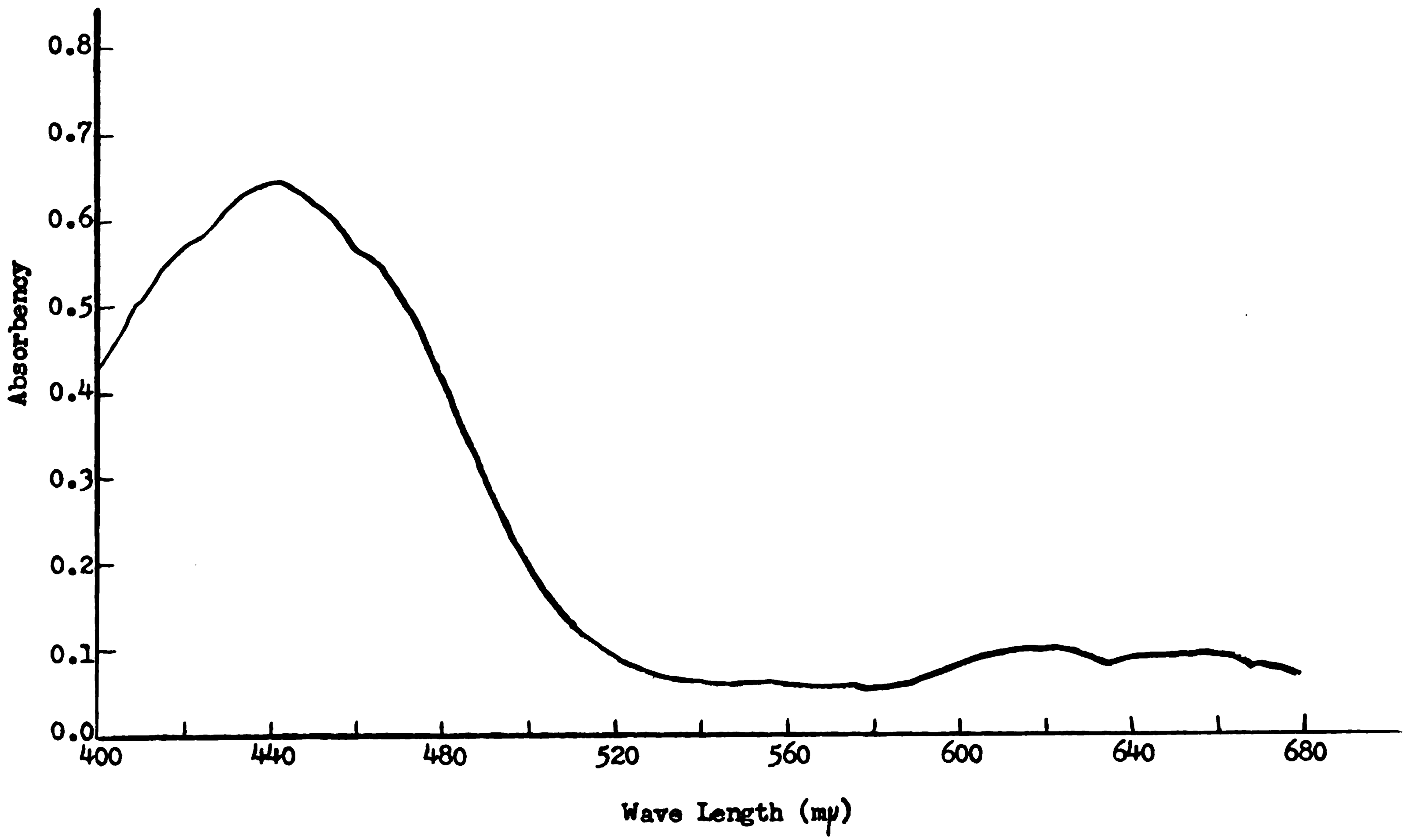

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

TABLE 23

Red Cedar River Periphyton Separations

MIXTURE

sucrose

Fract. 1 - yellow-green
" 2 - green
" 3 - green
" 4 - green
" 5 - green
" 6 - dark green

alumina (Fract. 1):
Fraction 1' - red
" 2' - red-green
" 3' - green

Fract. 7 -
" 8 -
" 9 - dark
" 10 - green
" 11 -

sucrose (Fract. 7–11):
Fract. 4' - light green
" 5' - green *
" 6' - olive green*
" 7' - green
" 8' - green-yellow
" 9' - blue-green
" 10' - yellow

Fract. 12 - dark green
" 13 - green-yellow

Fract. 14 -
" 15 -
" 16 - yellow
" 17 -

alumina (Fract. 14–17):
Fract. 11' - orange
" 12' - green-yellow
" 13' - green-yellow
" 14' - green

Fract. 18 - dark green
" 19 - light green
" 20 - light green

* Fract. 5' - green
" 6' - olive green

sucrose

Fract. 1" - light green
" 2" - dark green
" 3" - 1st orange and green
" 4" - 2nd orange

TABLE 24

Absorbency Data Obtained with a Beckman Model B Spectrophotometer on Pigments from Red Cedar River Periphyton

Wave-length in mμ	Absorbency					
	fract. A	fract. C	mixture	fract. 1'	fract. 3'	fract. 6'
400	0.229	0.272	0.432	0.241	0.810	0.560
405	0.250	0.320	0.490	0.264	0.835	0.625
410	0.271	0.348	0.543	0.285	0.845	0.698
415	0.279	0.372	0.572	0.313	0.750	0.740
420	0.292	0.391	0.610	0.348	0.510	0.800
425	0.329	0.420	0.672	0.370	0.322	0.845
430	0.360	0.460	0.750	0.390	0.187	0.880
435	0.368	0.460	0.738	0.404	0.118	0.855
440	0.336	0.418	0.658	0.422	0.094	0.795
445	0.302	0.375	0.578	0.440	0.083	0.720
450	0.279	0.350	0.523	0.444	0.081	0.695
455	0.241	0.324	0.481	0.431	0.080	0.655
460	0.191	0.302	0.430	0.420	0.080	0.622
465	0.150	0.295	0.392	0.411	0.079	0.620
470	0.103	0.298	0.369	0.400	0.077	0.602
475	0.083	0.284	0.352	0.378	0.075	0.555
480	0.065	0.260	0.318	0.342	0.070	0.469
485	0.056	0.221	0.273	0.302	0.069	0.390
490	0.048	0.170	0.211	0.257	0.081	0.292
495	0.045	0.134	0.164	0.212	0.097	0.228
500	0.036	0.104	0.126	0.173	0.108	0.170
505	0.032	0.085	0.105	0.136	0.104	0.136
510	0.029	0.070	0.080	0.110	0.084	0.101
520	0.023	0.042	0.056	0.064	0.049	0.060
530	0.024	0.034	0.043	0.048	0.083	0.042
540	0.025	0.025	0.035	0.040	0.049	0.030
550	0.024	0.023	0.034	0.035	0.028	0.026
560	0.028	0.023	0.039	0.032	0.029	0.028
570	0.040	0.028	0.051	0.030	0.022	0.031
580	0.044	0.030	0.059	0.029	0.023	0.033
590	0.040	0.027	0.052	0.028	0.035	0.032
600	0.040	0.033	0.060	0.024	0.060	0.040
610	0.047	0.041	0.071	0.020	0.057	0.046
620	0.052	0.043	0.075	0.019	0.036	0.042
625	0.053	0.038	0.071	0.018	0.035	0.039
630	0.053	0.033	0.061	0.017	0.031	0.034
635	0.045	0.034	0.049	0.016	0.037	0.036
640	0.048	0.040	0.063	0.016	0.044	0.054
645	0.058	0.059	0.092	0.017	0.078	0.083
650	0.094	0.093	0.155	0.011	0.137	0.140
655	0.137	0.133	0.233	0.010	0.223	0.197
660	0.161	0.159	0.298	0.015	0.318	0.210
665	0.143	0.138	0.272	0.016	0.338	0.167
670	0.095	0.101	0.185	0.013	0.252	0.106
680	0.027	0.027	0.038	0.013	0.058	0.023

TABLE 24 (CONT.)

Wave-length in mμ	Absorbency						
	fract. 8'	fract. 9'-AC	fract. 9'-ET	fract. 11'	fract. 2"-AC	fract. 2"-ET	fract. 4"
400	0.530	0.870	0.462	0.425	0.558	0.400	0.710
405	0.574	0.930	0.482	0.463	0.615	0.412	0.785
410	0.620	0.940	0.483	0.502	0.658	0.462	0.900
415	0.628	0.770	0.433	0.540	0.680	0.490	0.985
420	0.578	0.522	0.328	0.563	0.700	0.482	1.08
425	0.515	0.307	0.210	0.582	0.725	0.468	1.12
430	0.485	0.150	0.114	0.612	0.685	0.450	1.19
435	0.482	0.080	0.058	0.628	0.490	0.383	1.23
440	0.490	0.058	0.039	0.642	0.282	0.308	1.26
445	0.485	0.043	0.024	0.641	0.158	0.206	1.26
450	0.455	0.040	0.022	0.620	0.100	0.138	1.23
455	0.401	0.039	0.018	0.600	0.078	0.105	1.21
460	0.373	0.039	0.018	0.565	0.061	0.085	1.21
465	0.373	0.048	0.025	0.552	0.055	0.072	1.21
470	0.384	0.049	0.024	0.520	0.048	0.064	1.20
475	0.382	0.048	0.025	0.480	0.046	0.057	1.08
480	0.330	0.041	0.018	0.420	0.044	0.057	0.930
485	0.238	0.049	0.022	0.366	0.042	.....	0.760
490	0.161	0.062	0.032	0.298	0.037	0.044	0.590
495	0.110	0.085	0.043	0.242	0.034	.....	0.444
500	0.080	0.105	0.050	0.191	0.030	0.039	0.350
505	0.063	0.107	0.055	0.161	0.027	.....	0.258
510	0.046	0.084	0.045	0.131	0.028	0.036	0.189
520	0.031	0.046	0.031	0.092	0.033	0.037	0.112
530	0.035	0.084	0.038	0.070	0.035	0.040	0.067
540	0.022	0.050	0.043	0.060	0.033	0.036	0.043
550	0.020	0.031	0.025	0.057	0.032	0.040	0.031
560	0.021	0.029	0.022	0.052	0.049	0.051	0.027
570	0.020	0.021	0.017	0.051	0.059	0.056	0.023
580	0.020	0.022	0.017	0.056	0.051	0.061	0.023
590	0.024	0.036	0.025	0.063	0.055	0.059	0.024
600	0.030	0.065	0.043	0.080	0.080	0.077	0.019
610	0.031	0.064	0.038	0.094	0.095	0.087	0.023
620	0.024	0.042	0.028	0.105	0.083	0.086	0.017
625	0.017	0.037	0.028	0.101	0.077	0.089	0.022
630	0.009	0.035	0.020	0.095	0.068	0.078	0.020
635	0.027	0.038	0.026	0.078	0.087	0.077	0.020
640	0.040	0.054	0.032	0.088	0.124	0.106	0.025
645	0.055	0.084	0.048	0.090	0.208	0.146	0.027
650	0.082	0.154	0.081	0.093	0.343	0.207	0.034
655	0.120	0.267	0.134	0.097	0.450	0.266	0.042
660	0.147	0.358	0.188	0.096	0.470	0.322	0.042
665	0.142	0.373	0.205	0.095	0.357	0.301	0.042
670	0.108	0.277	0.167	0.080	0.238	0.222	0.036
680	0.031	0.062	0.041	0.072	0.049	0.072	0.021

by visual control. The upper, middle, and lower parts of the column were extracted to give fractions 8, 9, and 10 respectively. A spectrogram of the orginal mixture is shown in figure 40.

Fractions 1 and 2 were combined and rechromatographed on a ten millimeter column of alumina. A red layer (fract. 1') was separated and is shown in figure 41. It is the belief of the author that this material represented the chromatophore grouping of a phycobilin type pigment which was no longer connected with the protein portion of the molecule. The phycobilins are known to be unstable in solvents at room temperature.

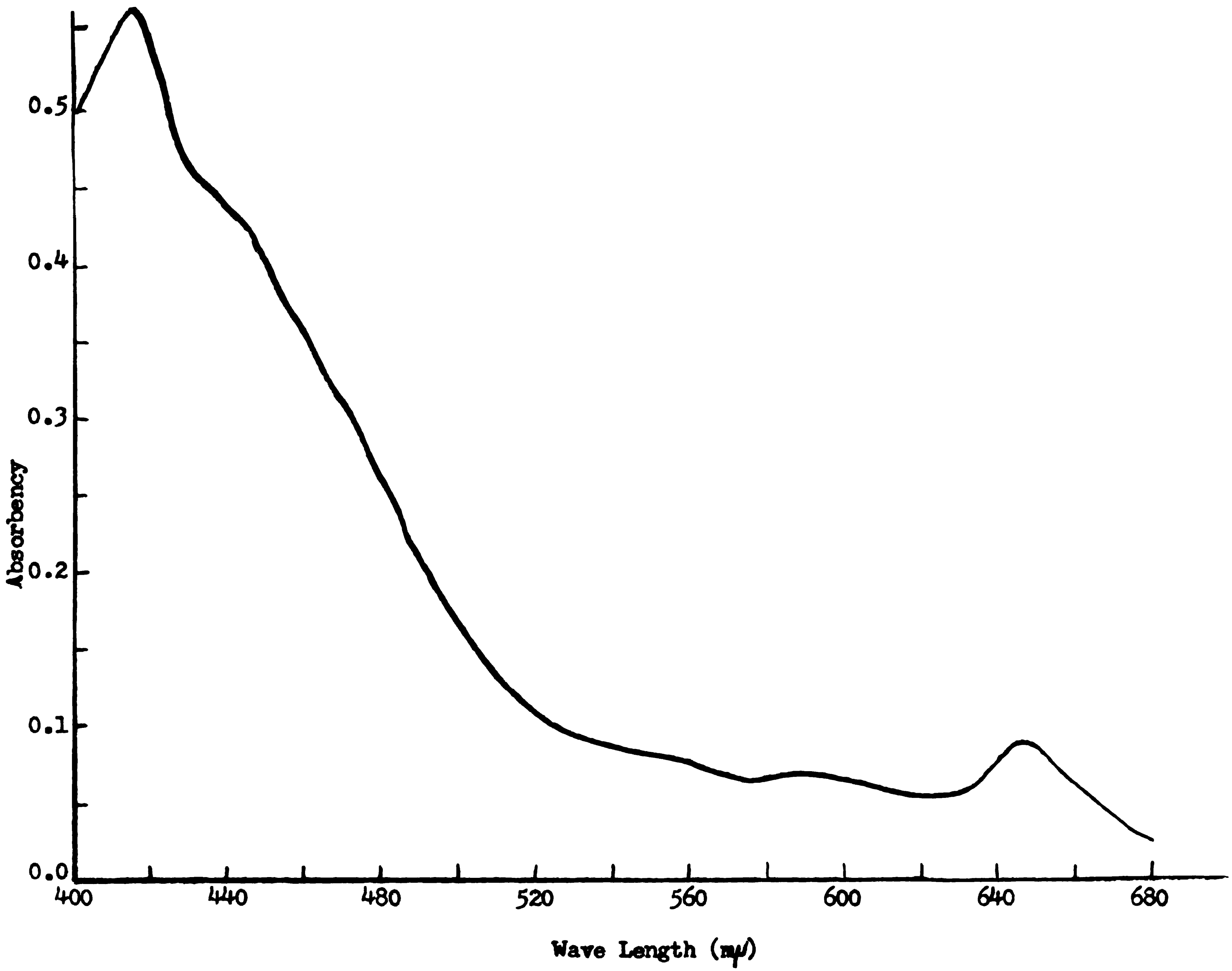
Fraction 7 is shown in figure 42. It is a golden-colored pigment with a single peak at about 445 millimicrons. It may be one of the neofucoxanthins.

Fraction 8 is the component of greatest interest, since it represents the bulk of the pigment measured in this study. It is shown in figure 43. The major peaks are at 415 and 645 millimicrons. The red peak corresponds to the value reported by Holt (1954) for pheophorbide a, and the blue peak corresponds to the value reported by Evstigneev for the semiquinonoid form of chlorophyll a.

Since the periphyton was obtained by scraping periphyton from wood shingles, some of the cedar wood was soaked for several months in 95 percent ethanol and the resulting solution's spectra determined. The spectrogram is shown in figure 44. The amounts of wood fibers in the samples would have been very small and the peak is at 452 millimicrons which doesn't correspond with any of the separated components. The absorbency in the 660 millimicron region is also negligible.

Figure 40

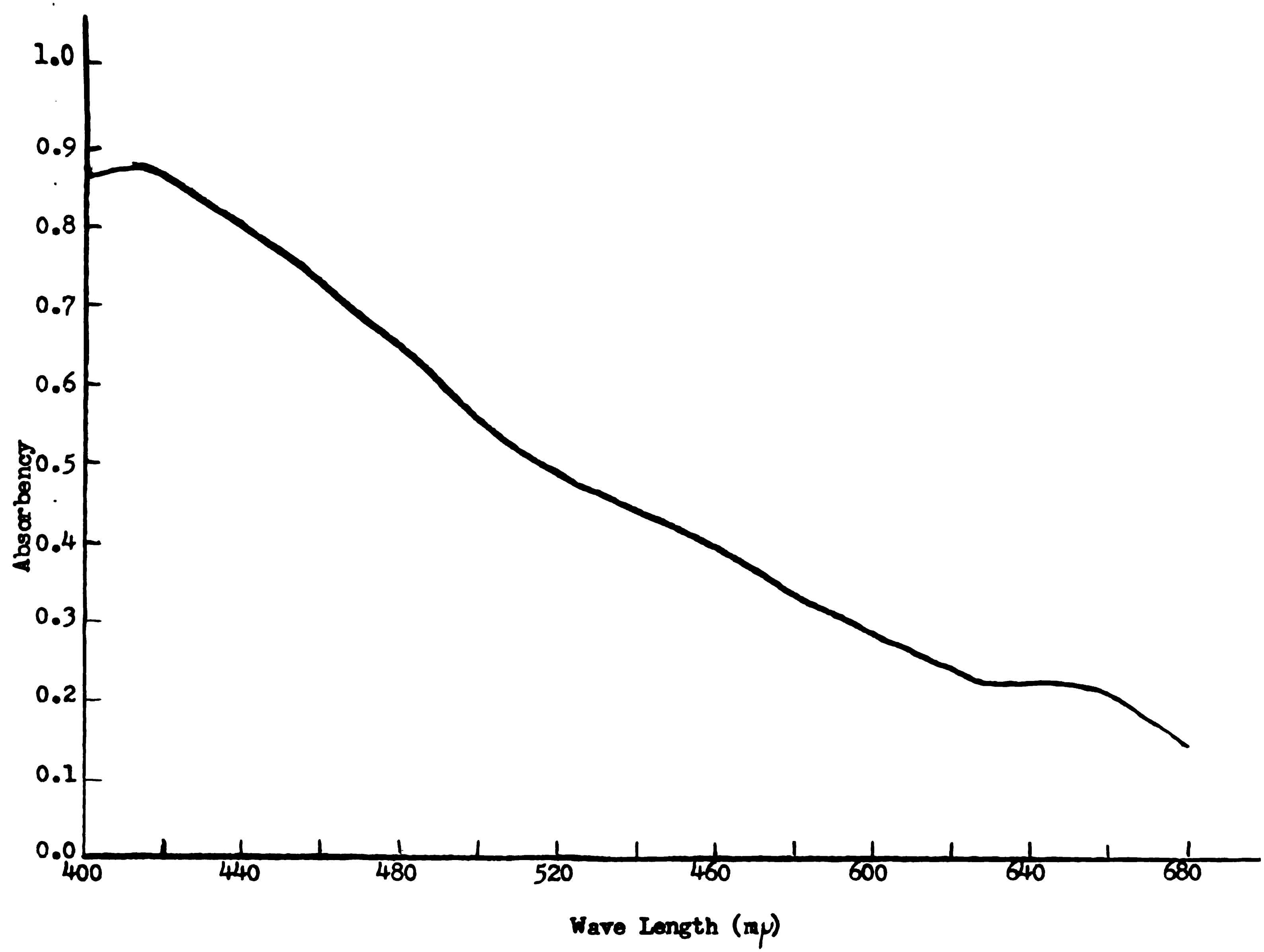
Absorbency Spectra of West
Branch of Sturgeon River
Periphyton Pigment, Initial
Mixture.



Absorbency
0.5
0.4
0.3
0.2
0.1
0.0
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 41

Absorbency Spectra of West
Branch of the Sturgeon River
Periphyton Pigment, Fraction 1.



Absorbency
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
400
440
480
520
460
600
640
680
Wave Length (mμ)

Figure 42

Absorbency Spectra of West
Branch of Sturgeon River
Periphyton Pigment, Fraction 7.

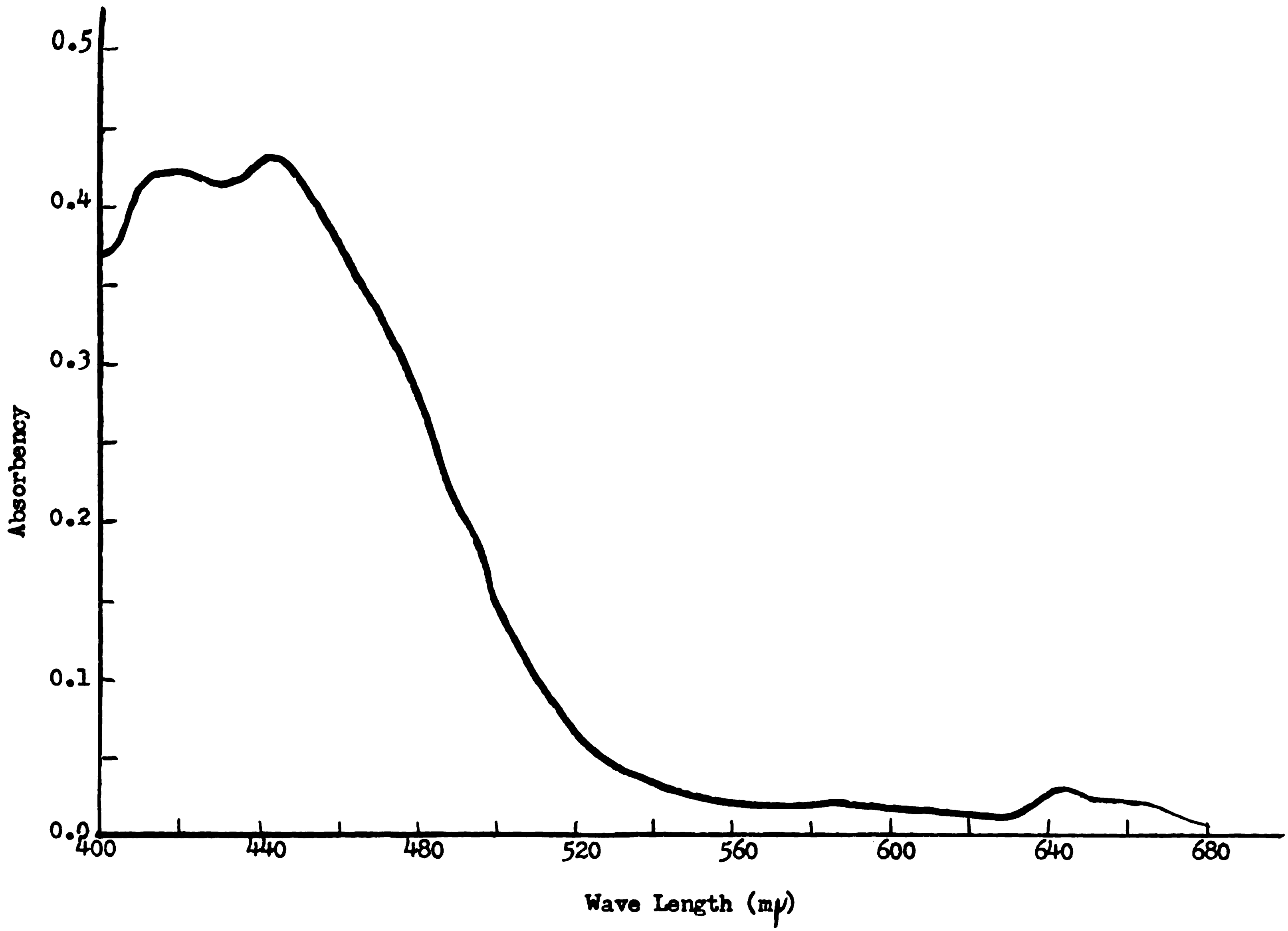
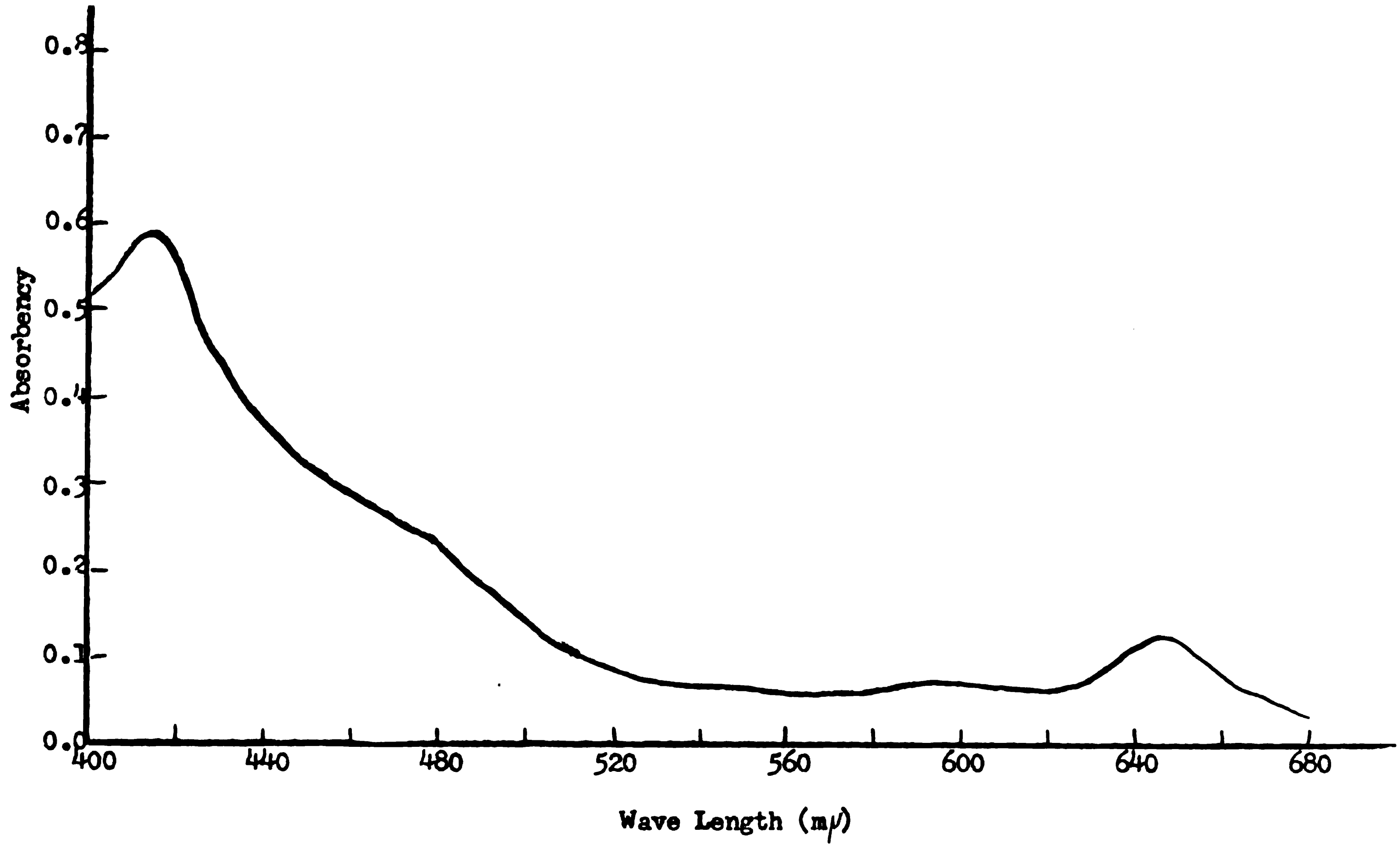

0.5
0.4
0.3
0.2
0.1
0.0
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 43

**Absorbency Spectra of West
Branch of Sturgeon River
Periphyton Pigment, Fraction 8.**


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

To illustrate the amounts of the red pigment (fract. 1') found in some samples, the spectra of an unusually reddish sample was determined and is shown in figure 45. The sample was station 3A, period E, sample 10. Its graph is essentially a line with a negative slope. There is only a slight peak at 415 millimicrons due to the presence of a little pheophorbide _a_, or whatever the pigment in fraction 8 is.

An unusually green sample from station 3A, period D, sample 7 is shown in figure 46. It has peaks at 417 and about 650 millimicrons. The spectrophotometric data for the figures are shown in table 25. It is evident from this analysis that the pigments measured in the study were not chlorophylls, but rather the decomposition products of chlorophyll. If the method is understood to yield only a relative index to periphyton abundance, this in no way invalidates the method. If measurements of actual quantities of various pigments are to be made, it is evident that ethanol should not be used as a solvent.

Figure 44

Absorbency Spectra of Ethyl
Alcohol Extract of Cedar Wood
(from shingles).

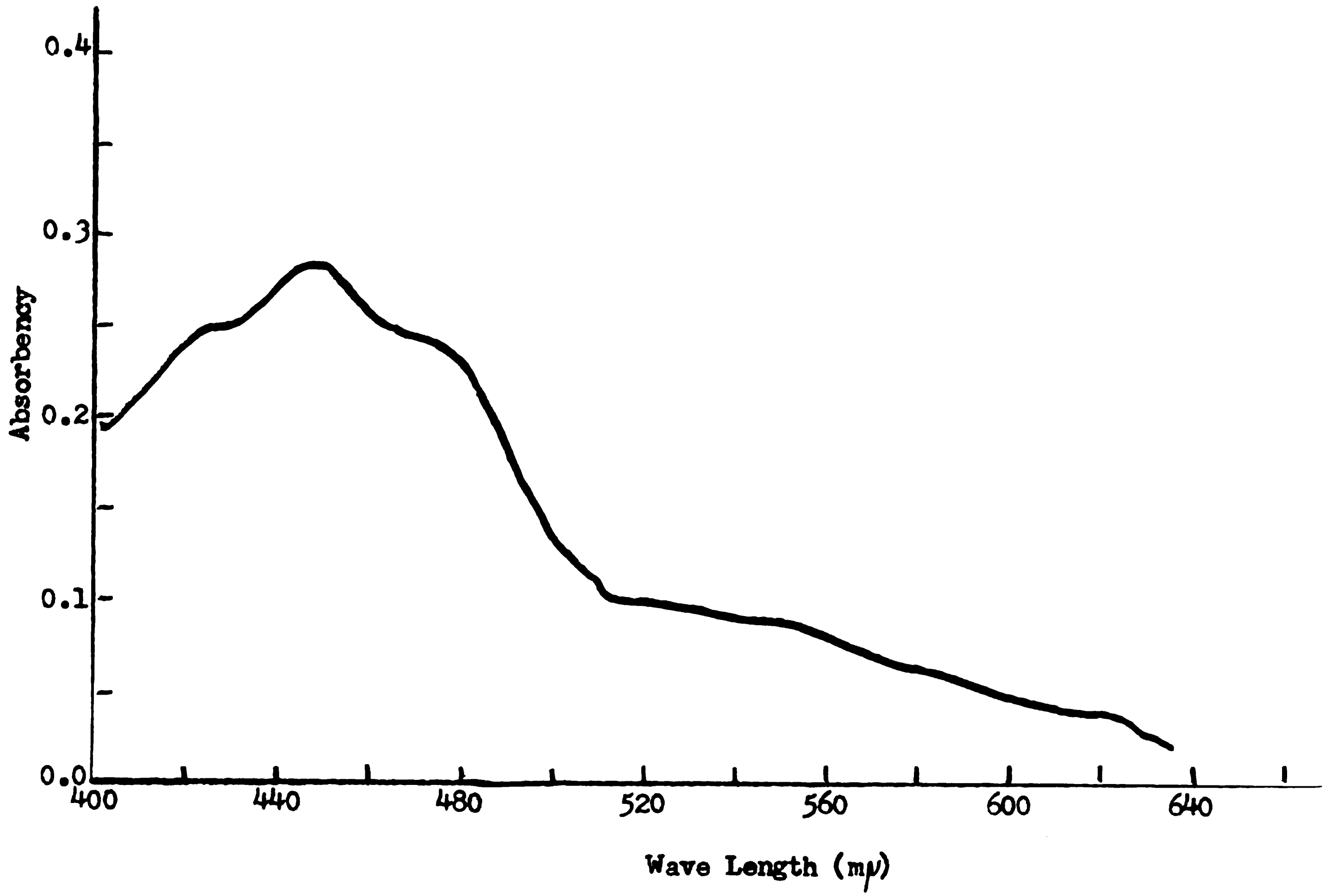

0.4
0.3
0.2
0.1
0.0
Absorbency
400
440
480
520
560
600
640
Wave Length (mμ)

Figure 45

Absorbency Spectra of West
Branch of Sturgeon River
Periphyton Pigment (sample 10,
period E, station 3A).

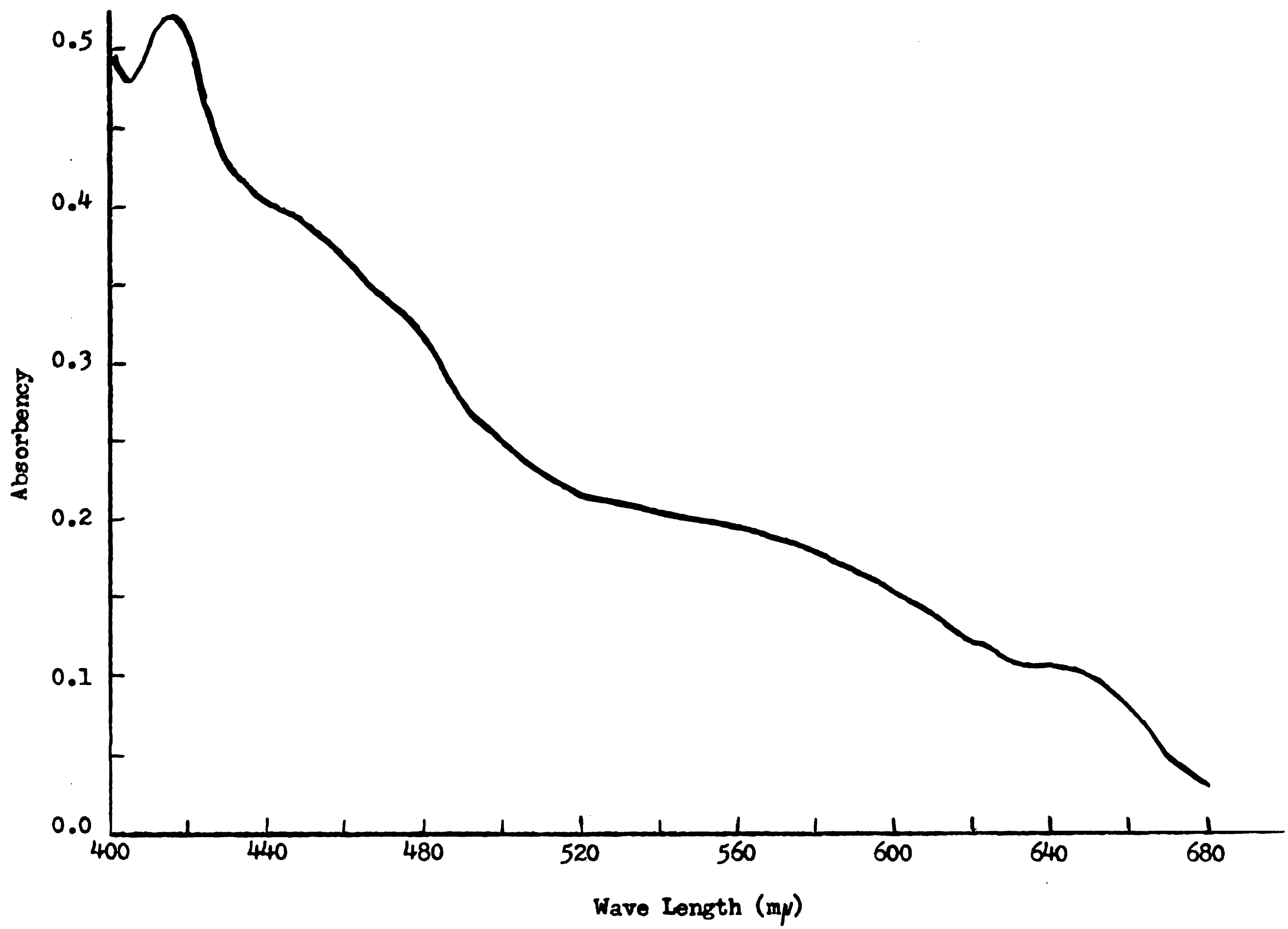
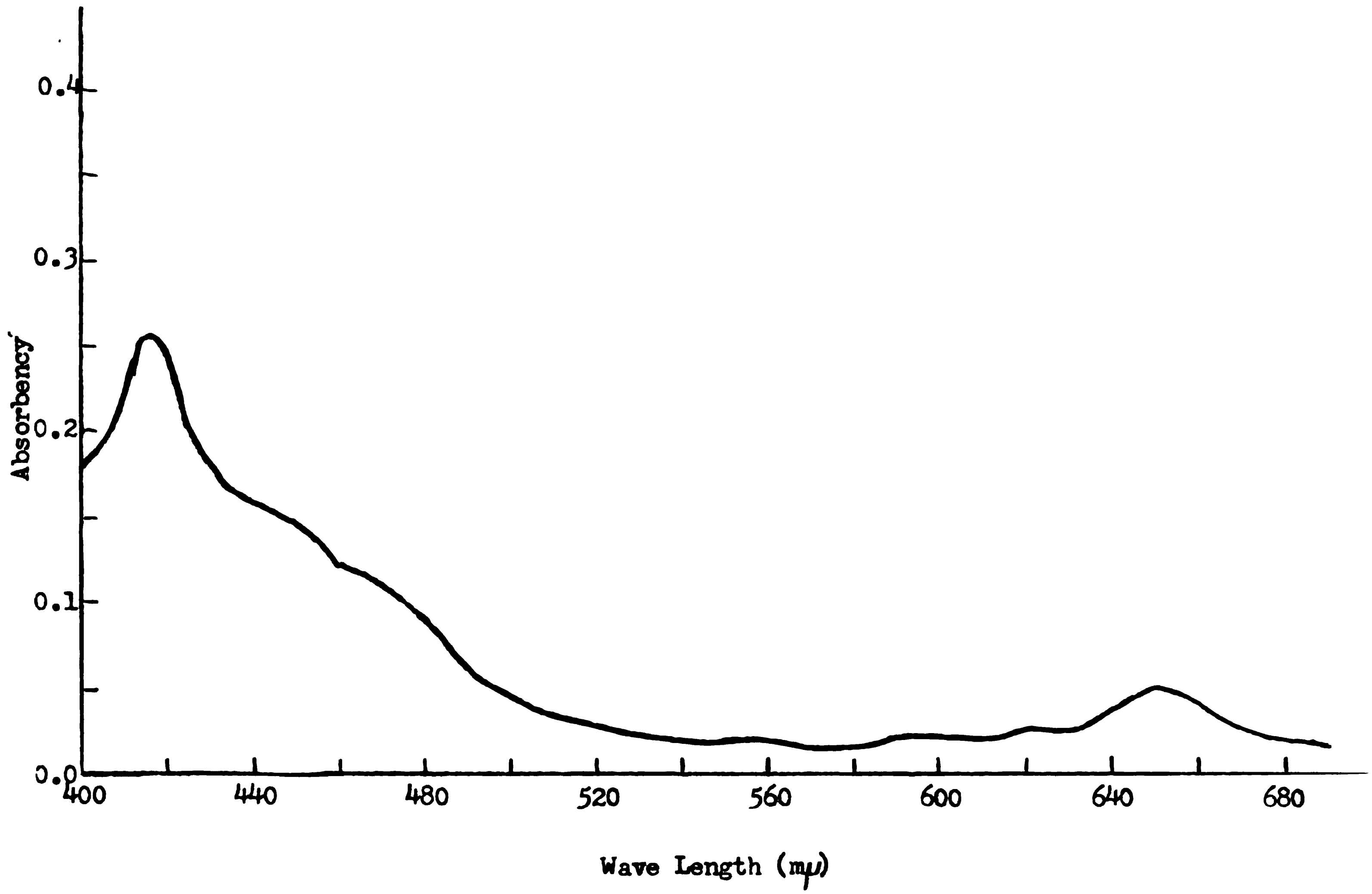

0.5
0.4
0.3
0.2
0.1
0.0
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

Figure 46

Absorbency Spectra of West Branch of the Sturgeon River Periphyton Pigment (sample 7, period D, station 3A) in Ethyl Alcohol



0.4
0.3
0.2
0.1
0.0
Absorbency
400
440
480
520
560
600
640
680
Wave Length (mμ)

TABLE 25

Absorbency Data Obtained with a Beckman Model B Spectrophotometer on Pigments of Periphyton from the west branch of the Sturgeon River

Wave-length in mμ	mixture	fract. 1'	fract. 7	fract. 8	cedar extract	3A-10-E	3A-7-D in ETOH
400	0.501	0.865	0.369	0.204	0.196	0.496	0.183
405	0.521	0.870	0.382	0.210	0.200	0.480	0.193
410	0.543	0.875	0.410	0.226	0.214	0.500	0.225
415	0.559	0.875	0.420	0.232	0.224	0.521	0.255
420	0.545	0.865	0.422	0.233	0.238	0.508	0.244
425	0.504	0.850	0.419	0.227	0.248	0.463	0.200
430	0.468	0.825	0.414	0.222	0.252	0.433	0.180
435	0.451	0.815	0.421	0.220	0.259	0.412	0.164
440	0.438	0.800	0.427	0.221	0.272	0.402	0.159
445	0.420	0.780	0.429	0.220	0.282	0.395	0.151
450	0.405	0.767	0.418	0.214	0.281	0.389	0.143
455	0.380	0.750	0.398	0.210	0.273	0.377	0.136
460	0.359	0.730	0.374	0.199	0.259	0.367	0.122
465	0.333	0.720	0.352	0.196	0.251	0.353	0.115
470	0.310	0.685	0.330	0.187	0.243	0.339	0.108
475	0.290	0.665	0.310	0.177	0.241	0.331	0.099
480	0.260	0.645	0.279	0.164	0.230	0.317	0.088
485	0.237	.....	0.244	.....	0.209	0.300	0.074
490	0.210	0.600	0.210	0.127	0.179	0.276	0.061
495	0.189	.....	0.184	.....	0.154	0.260	0.052
500	0.165	0.560	0.150	0.093	0.132	0.248	0.045
505	0.149	.....	0.125	.....	0.119	0.239	0.039
510	0.136	0.520	0.100	0.064	0.108	0.230	0.034
520	0.110	0.488	0.064	0.047	0.099	0.213	0.027
530	0.094	0.462	0.045	0.037	0.093	0.207	0.021
540	0.085	0.440	0.032	0.029	0.091	0.202	0.019
550	0.080	0.418	0.027	0.029	0.088	0.200	0.018
560	0.074	0.390	0.020	0.027	0.079	0.195	0.019
570	0.068	0.357	0.019	0.024	0.071	0.186	0.017
580	0.066	0.327	0.018	0.025	0.062	0.174	0.017
590	0.068	0.304	0.019	0.026	0.056	0.166	0.021
600	0.064	0.281	0.013	0.022	0.046	0.154	0.022
610	0.057	0.260	0.014	0.020	0.039	0.139	0.021
620	0.053	0.237	0.013	0.017	0.033	0.122	0.023
625	0.056	.....	.....	.....	0.031	0.118	.....
630	0.056	0.220	0.013	0.018	0.024	0.108	0.026
635	0.059	0.218	.....	0.020	0.021	0.106	.....
640	0.076	0.218	0.025	0.023	0.017	0.104	0.038
645	0.085	0.218	0.028	0.023	.....	.....	0.044
650	0.082	0.212	0.023	0.022	0.012	0.101	0.049
655	0.069	0.209	0.020	0.018	.....	.....	0.045
660	0.060	0.197	0.020	0.017	0.008	0.078	0.039
665	0.051	.....	0.017	0.016	.....	.....	0.032
670	0.042	0.169	0.012	0.015	0.002	0.044	0.028
680	0.024	0.143	0.005	0.010	0.003	0.032	0.021

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