RESPONSE OF PERIPHYTON TO PHOSPHORUS INTRODUCED INTO A MICHIGAN TROUT STREAM

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RESPONSE OF PERIPHYTON TO PHOSPHORUS INTRODUCED INTO A MICHIGAN TROUT STREAM

By

HUGH F. CLIFFORD

AN ABSTRACT

Submitted to the College of Agriculture of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1959

Approved _____

ABSTRACT

The summer program of 1958 for the West Branch of the Sturgeon River was divided into two closely related phases.

For the second consecutive summer, phosphate in the form of an inorganic fertilizer was added directly to the river. No immediate positive response by the standing crop of the periphyton complex could be detected, although the periphyton mass increased in total phosphorus. When the amount of fertilizer was increased and the substrates were allowed to remain in the water a longer period of time, the standing crop of periphyton gave indications of increasing. Fast river currents appeared to affect the standing crop of periphyton adversely.

Twenty three millicuries of P^{32} was applied to the West Branch on August 5, 1958. The periphyton complex was initially responsible for the great amount of P^{32} retained in the experimental area. Downstream from the point of addition, the initial uptake of radiophosphorus decreased. Seven days after the addition of the isotope, the P^{32} of the periphyton was uniformly distributed in the experimental area. The P^{32} was believed rapidly lost from the periphyton; biologically to other organisms, and physically to the inanimate bottom complex and the current which transports it out of the system.

H. F. C.

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INTRODUCTION

In a relatively short span of years, the theories and practices of fish production in our inland waters have passed from the uncomplicated ideas limited to legislation and stocking, to complex theories involving the entire environment of the fish. This environment encompasses a vast array of biotic and abiotic factors. One of the primary abiotic factors is nutrients. Their translocation through the food chain makes them inseparably interrelated to the living organisms of a particular environment. The nutrient, phosphorus, and algae of the primary producers are two of these specific inseparable components that contribute to the food chain in a lotic environment. A better understanding of the relationship and interaction of these two components would contribute knowledge that could help formulate future theories and practices of fish production in our inland waters.

In 1954 an experimental program was initiated on the West Branch of the Sturgeon River that was designed to evaluate the chemical, physical, and biological responses to the addition of nutrients in the form of inorganic fertilizer. The nutrients studied included both phosphorus and nitrogen; the biota of the food chain encompassed all trophic levels with the exception of converter organisms. This

thesis contains part of the research performed on the West Branch during the fifth summer of the program, 1958, and is concerned with the response of the periphyton[1] to the nutrient, phosphorus.

The experimental program of 1958, pertaining to periphyton, was divided into two closely related phases. The initial phase, from June 15 to July 28, was concerned with evaluation of periphyton responses to inorganic fertilizer, detection of total phosphorus in the periphyton mass, and measurement of certain physical factors that may be related to periphyton growth. These studies are treated in the initial section of the thesis. The remaining section of the thesis is concerned with the second phase of the summer's program, namely that of uptake, accumulation, and translocation of radioactive phosphorus in periphyton.

Description of the Study Area

The West Branch of the Sturgeon River is a hard-water stream which originates in Hoffman Lake, a marl lake located in Charlevoix county (T. 32N., R. 4W, Sec. 26, 27, 34, and 35) Michigan. The river flows approximately thirteen miles through a narrow watershed before its confluence with the Sturgeon River near Wolverine,

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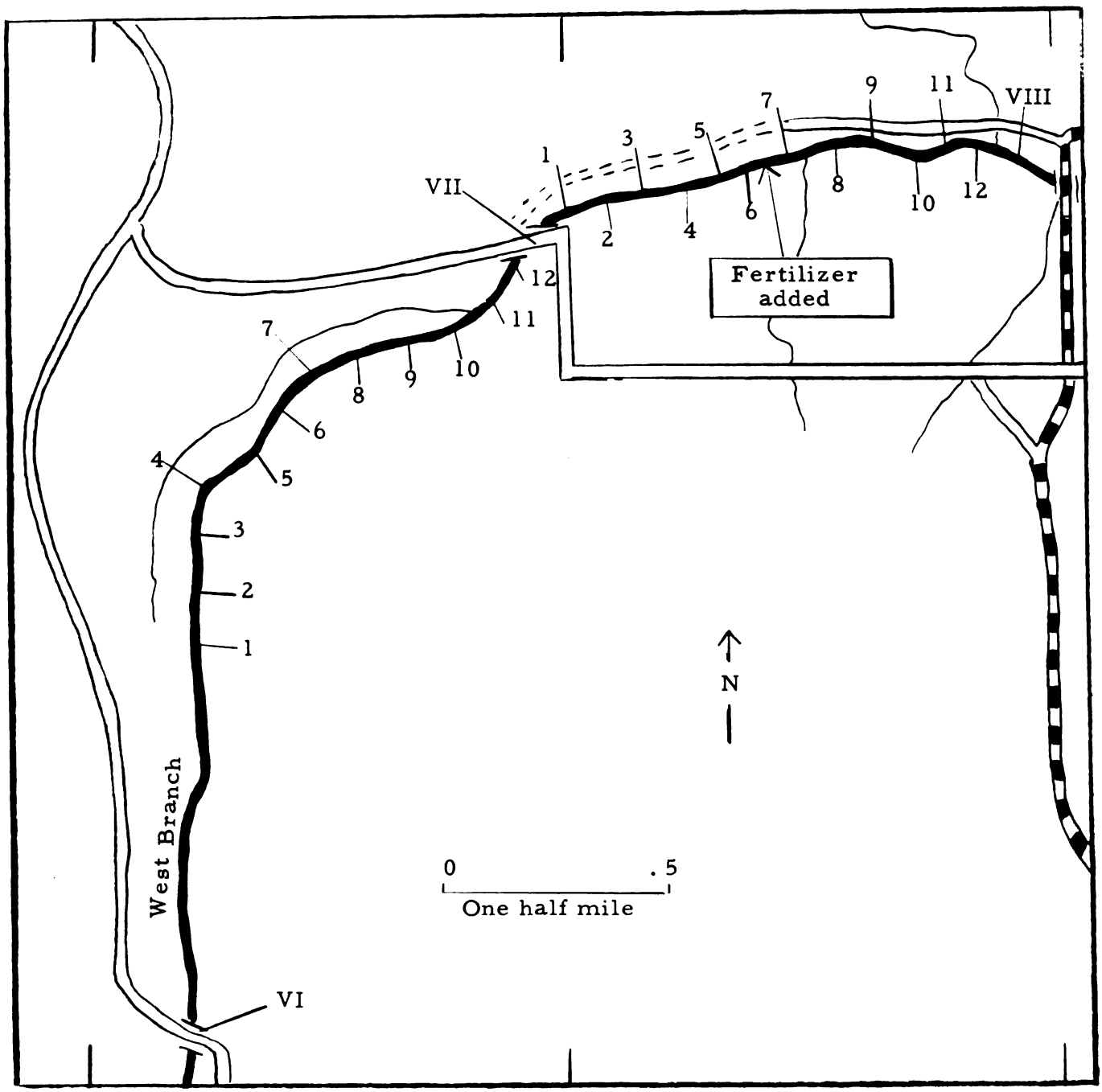
[1] Periphyton in this study follows closely that definition by Young (1945), pertaining to the assemblage of benthic or encrusting algae growing on free surfaces. It does not include animal materials as defined by Newcombe (1949).

FIGURE I. --Map of the West Branch of the Sturgeon River area, showing the stations used in the preliminary study.


VIII
VII
1
2
3
4
5
6
7
8
9
10
11
12
Fertilizer added
N
0
.5
One half mile
West Branch
VI

Cheyboygan county. The topography and general features of the river have been described by Grzenda (1955), Colby (1957), Keup (1958), and Carr (1959) and need not be elaborated on here.

The experimental area for both the preliminary and isotope study of 1958 is a 2, 700 yard section of the river situated between permanent stations VI and VIII (Figure I). In the first 250 yards of the area, the stream is well sunlit and supports an abundant growth of Chara. Below this for the next 300 yards the gradient increases and the river is heavily shaded by cedars, hemlock, nine bark, and tag alder. Within this section is found the most impoverished growth of Chara for the entire experimental area (Knight, unpublished). From here to the terminus of the study area, 100 yards above Fulmer Creek (Station VII, 12), the gradient of the stream decreases and the stream in most places is well sunlit. Luxuriant growths of Chara are to be found throughout this section, where they have developed on bars of sand and organic detritus. In this latter part of the experimental area, stream deflectors have created a habitat of pools alternating with swift-running areas.

The entire experimental area is devoid of domiciliary and agricultural habitation as are all feeder streams, with the exception of a small run located approximately 600 yards above the terminus of the area which may deliver small amounts of extraneous nutrients to the river.

Flow data indicates a uniform increase in water for the entire experimental area. Flow in cubic feet per second on July 7, 1959 for the beginning of the area, middle section (Station VII), and terminus of the area were 38.17, 43.48, and 49.72 c.f.s. respectively.[2]

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[2]Courtesy of Carr and Vannote, 1959.

PRELIMINARY STUDY

Sampling Stations

Figure I shows the location of the sampling stations for the preliminary study of periphyton. The twelve stations in the upper area (stations VI, 1-12) are located at approximately 150 yard intervals. These stations served to evaluate data relating to the amount of periphyton that could be measured at the end of seven and fourteen day intervals at these specific localities.

The twelve stations below station VII (VII, 1-12) were also located at approximately 150 yard intervals. Stations VII, 1-6 were above the point of fertilizer application while stations VII, 7-12 were subjected to the fertilizer effects. An attempt was made to establish all twelve stations in an environment that would be uniform to such physical factors as current velocity, light, and depth. Stations VII, 1-12 were used for the evaluation of fertilizer effect on periphyton growth and the amount of total phosphorus in the periphyton.

Methods and Procedures

Composition of Periphyton Communities on Artificial Substrates

A study of the periphyton complex was started in the summer of 1958 and completed in the summer of 1959. Along with

the study of the periphyton composition on plexiglass substrates, an attempt was made to identify the major filamentous and other algae of the West Branch.

In 1958 the substrates taken from the stream were frozen immediately upon arrival at the field laboratory. Analysis was made the following winter. Since this method would preclude identification of algae other than the diatoms, during the summer of 1959 periphyton on artificial substrates was stored in a 6-3-1 algal preservative.

The organisms were scraped from the substrates and filtered through a Millipore filter having a pore diameter of 0.45 microns. The density of organisms on a single substrate was such as to allow the entire periphyton complex of that shingle to be concentrated in this way. Tabulation of frequency of occurrence of algae, and in some cases identifications, were made directly from the pad. The number of times an organism was observed in 30 fields was used as a measure of frequency of occurrence. Organisms that could not be identified with certainty on the filter pad were studied in water mounts.

Fertilization

To determine the response of periphyton to artificial enrichment, fertilizer in the form of di-ammonium phosphate was added to the river between stations VII, 6 and VII, 7. The growth

of periphyton below this location (stations VII, 7-12) was compared with the growth in a section where the periphyton was not exposed to added nutrients (stations VII, 1-6). If an appreciably greater growth of periphyton occurred in the section of the river exposed to fertilizer (VII, 7-12) than in the unfertilized water (VII, 1$\bar{\emptyset}$6) then fertilizer would be added at the point of isotope release in order to assure a rapid uptake of the radioactive phosphorus and to insure a measurable amount of periphyton. Eighty pounds of inorganic fertilizer was first added to the river between stations VII, 6 and VII, 7 on July 3, 1958. The apparatus used to distribute the fertilizer was essentially the same as that described by Correll (1958). Two 55 gallon drums were placed on the stream bank and the fertilizer mixed with river water in the drums. The siphoning apparatus was designed so that both drums would empty at a predetermined constant rate. Fertilization proceeded for a total of 48 hours. The rate of addition was not uniform. Mechanical failures in the sediment trap and particles clogging the jet were considered to be major factors in this erratic behavior of the fertilizer apparatus. Because of this non-uniformity in distribution of the fertilizer, no attempt was made to calculate the actual rate that the nutrients (phosphorus and nitrogen) entered the river. The 80 lbs. of fertilizer was distributed sometime within this 48 hour period.

The second application of fertilizer between stations VII, 6 and 7 was started on July 20, 1958 and continued intermittently until July 26, 1958, a period of six days. Between these dates, approximately 150 lbs. of fertilizer was added to the river.

Periphyton

The method used for collecting periphyton on artificial substrates in 1958, the fifth year of the program, was modified from those used the four previous years. Grzenda (op. cit.), used both cedar shingles and bricks. The accrued periphyton was measured at thirty day intervals. Colby, in 1955, also used bricks and cedar shingles, measuring the changes in the periphyton mass at seven and thirty day intervals. Carr, in 1956, modified the schedule of removal of the cedar shingles so that measurements could be made at intervals of one, two, three, and four weeks. In that year, bricks and cedar shingles were evaluated at thirty day intervals. In 1957, Keup and Correll dispensed with the bricks and collected all samples pertaining to periphyton on cedar shingles. Substrates were collected at fourteen day intervals and a pigment analysis was made from periphyton on one third of the shingle.

In 1958, the number of sampling stations was increased and the stations were concentrated in a much shorter area of the river (Figure I). The type of artificial substrate also differed from

previous years. Periphyton data were collected from "2 x "5 plexiglass substrates. The substrates were placed six on a cross-bow; the cross-bow was supported by a steel stake and the whole assemblage was placed approximately 8 inches below the surface of the water.

This program was coordinated with the isotope study that was to take place in August of 1958. It was decided to allow the plexiglass substrates, to remain in the river a period of seven days, instead of fourteen, for the first two collecting periods. During the first seven day period (July 1-8) the substrates at stations VII, 7-12 were subjected to fertilizer, while stations VII, 1-6 were above the point of fertilizer application. During the second seven day collection period none of the stations were exposed to fertilizer. If it was found that measurable (by weight) amounts of periphyton accrued in seven days, this time interval could be used for the isotope study and would make it possible to increase the number of sampling dates for radioactive periphyton. After the first two evaluations it was decided that weighable amounts of periphyton could not be accrued in seven days. The periphyton on the artificial substrates was then collected at the end of a fourteen day period.

The procedure for all three evaluations was to pick up two substrates for pigment analysis at each station. The substrates were processed immediately upon return to the laboratory. All

macro-invertebrates, chiefly Trichoptera and Simuliidae larvae, were first picked off the substrates. The remaining plant material was washed with 95 percent ethyl alcohol and scraped off the substrates. This mixture was poured into a glass funnel and filtered through glass wool. The filtrate which contained the pigment was made up to a volume of fifty millili ters with additional alcohol. Final laboratory work consisted of measuring the density of pigment in each sample with a Klett-Summerson photoelectric colorimeter. A number 66 red filter was used in the colorimeter. The Klett unit was used for comparison of results.

Phosphorus Content of the Periphyton

The total phosphorus in the periphyton was determined by analyzing two substrates from each station. All macro-invertebrates were picked from the substrates to be analyzed. Total phosphorus was determined by digesting an unfiltered sample with sulfuric, nitric, and hydrochloric acids and determining the phosphate content by the molybdate method as described by Ellis, Westfall, and Ellis (1948). Maximum color development was determined with a Klett-Summerson photoelectric colorimeter. Total phosphorus in p. p. b. was obtained from a graph based on known phosphorus standards.

Stream Fluctuation and Water Temperature

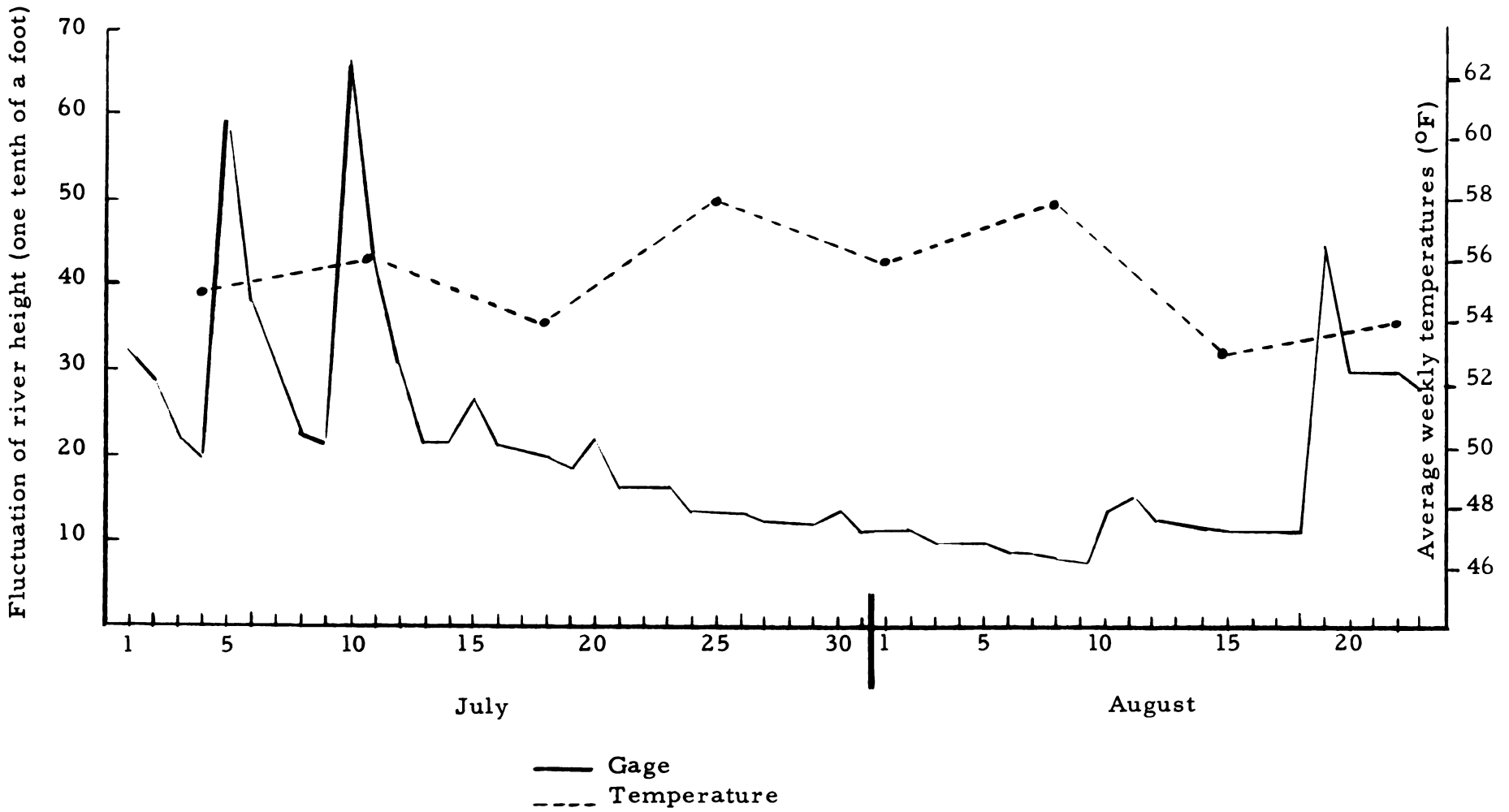
Water fluctuation in the West Branch of the Sturgeon River for July and August, 1958 was determined from a river staff gage located 10 yards above permanent station VII. Temperatures were also taken at station VII with a pocket thermometer. A composite of both weekly temperatures and daily gage readings are shown in Figure II. Extensive water fluctuations in the West Branch during July and August, 1958 were confined to three periods, July 4-8, July 9-13, and August 18-23. The greatest of these was only five tenths of a foot indicating the stable condition of the water level in the West Branch of the Sturgeon River. Figure II also indicates stable conditions in the river for the period preceeding the isotope release on August 5, 1958 and for the fourteen days immediately following the isotope addition.

Since there was considerable variation in the time of day that temperatures were measured, only a general picture of fluctuations of the temperatures in the river water was obtained.

Velocity of the River Water

An attempt was made to measure surface velocity of the river water at the odd numbered stations below permanent station VII. A wooden float timed over a short distance of the stream was the method used and it was consistent enough to allow for comparative results.

FIGURE II. --Mean weekly water temperatures and daily staff gage readings from station VII on the West Branch of the Sturgeon River--July and August, 1958


Fluctuation of river height (one tenth of a foot)
70
60
50
40
30
20
10
Average weekly temperatures (°F)
62
60
58
56
54
52
50
48
46
1
5
10
15
20
25
30
1
5
10
15
20
July
August
Gage
Temperature

Results and Discussion

Composition of Periphyton Communities on Artificial Substrates

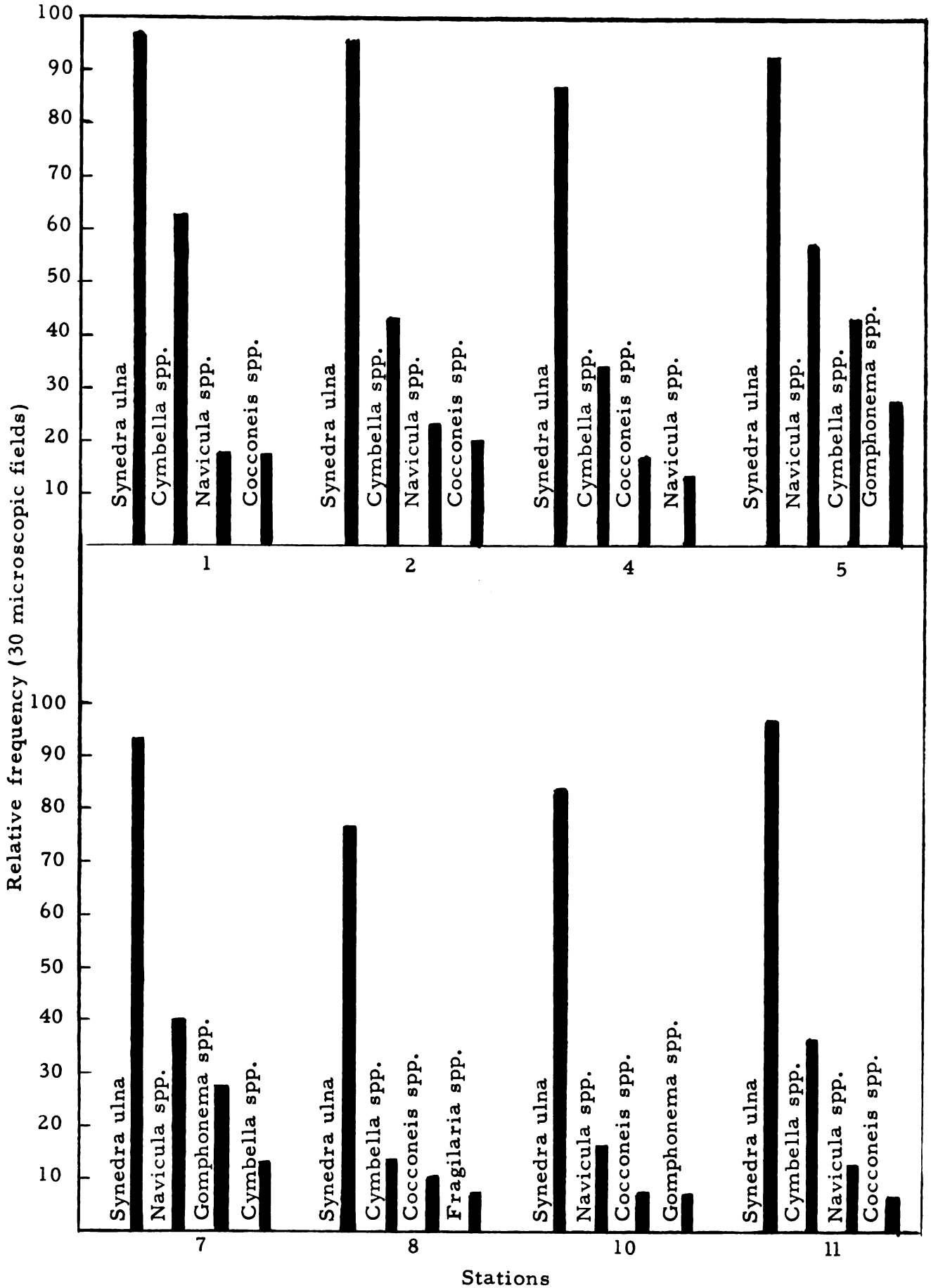
The results of the study of periphyton on the artificial substrates in the West Branch of the Sturgeon River indicate a community made up almost entirely of diatoms. Table 1 indicates that Synedra ulna was the dominant species on the substrates at all stations investigated during the period of July 11 to July 18, 1958. Although no attempt was made to establish patterns of community periodicity, substrates removed from the river in August of 1959 also showed a predominance of this species. Figure III shows the four major organisms at the various stations for the period of July 11 to July 18, 1958. It is evident that at this time Synedra ulna accounts for the greatest portion of the periphyton complex on the artificial substrates. All stations were quite uniform as to community composition. Cymbella spp., Navicula spp., Cocconeis spp., and Gomphonema spp. were the other principal diatoms making up the periphyton at this time. None of these, however, achieved a dominant position at any of the stations.

When relating the periphyton community on the artificial substrates to the periphyton complex of the West Branch itself many factors must be considered. Colonization of the bare artificial areas

TABLE 1. --Relative frequency of diatoms counted in thirty microscopic fields selected from artificial substrates placed in the West Branch of the Sturgeon River July 11, 1958, and removed July 18, 1958.

	Relative frequency		Relative frequency
Station 1#		Station 7#	
Synedra ulna	97	Synedra ulna	93
Cymbella spp.	63	Navicula spp.	30
Navicula spp.	17	Gomphonema spp.	27
Cocconeis spp.	17	Cymbella spp.	13
Gomphonema spp.	10	Cyclotella spp.	13
Cyclotella spp.	3	Cocconeis spp.	7
Station 2#		Station 8#	
Synedra ulna	97	Synedra ulna	77
Cymbella spp.	43	Cymbella spp.	13
Navicula spp.	23	Cocconeis spp.	10
Cocconeis spp.	20	Fragilaria spp.	7
Gomphonema spp.	10	Navicula spp.	3
Cyclotella spp.	3	Gomphonema spp.	3
		Stephanodiscus spp.	3
Station 4#			
Synedra ulna	87	Station 10#	
Cymbella spp.	34	Synedra ulna	83
Cocconeis spp.	17	Navicula spp.	17
Navicula spp.	13	Cocconeis spp.	7
Gomphonema spp.	7	Gomphonema spp.	7
Fragilaria spp.	3	Fragilaria spp.	3
Cyclotella spp.	3	Synedra spp.	3
Station 5#		Station 11#	
Synedra ulna	93	Synedra ulna	97
Navicula spp.	57	Cymbella spp.	37
Cymbella spp.	43	Navicula spp.	13
Gomphonema spp.	27	Cocconeis spp.	7
Cyclotella spp.	3	Gomphonema spp.	7
		Stephanodiscus spp.	3

FIGURE III. --Relative frequency of the four major diatoms from artificial substrates placed in the West Branch of the Sturgeon River July 11, 1958 and removed July 18, 1958 -- Calculated in thirty fields from a millipore filter pad.


Relative frequency (30 microscopic fields)
100
90
80
70
60
50
40
30
20
10
Synedra ulna
Cymbella spp.
Navicula spp.
Cocconeis spp.
1
Synedra ulna
Cymbella spp.
Navicula spp.
Cocconeis spp.
2
Synedra ulna
Cymbella spp.
Cocconeis spp.
Navicula spp.
4
Synedra ulna
Navicula spp.
Cymbella spp.
Gomphonema spp.
5
Synedra ulna
Navicula spp.
Gomphonema spp.
Cymbella spp.
7
Synedra ulna
Cymbella spp.
Cocconeis spp.
Fragilaria spp.
8
Synedra ulna
Navicula spp.
Cocconeis spp.
Gomphonema spp.
10
Synedra ulna
Cymbella spp.
Navicula spp.
Cocconeis spp.
11
Stations

in relation to the time substrates are removed is one of the most important factors. The difference between periphyton on non-living and that on living substrates is another. Butcher (1932) could observe no differences in sessile algae on artificial substrates (glass slides) from that found on natural substrates in the river. Young (1945), however, failed to find any Gloeotrichia on rope or on glass slides placed within a clump of bullrushes bearing the blue-green algae. Periodicity of the various algae also must be known to establish the true picture of the periphyton complex. Blum (1957) reports that algae sampled at weekly intervals will often reveal the complete disappearance within a period of only 6 to 10 days of an erstwhile conspicuous algae. Peters (1959) reports a marked seasonal periodicity of algal organisms which become attached to artificial substrates in the Red Cedar River, Michigan. Periodicity and living substrates were not investigated in the West Branch. To what degree the periphyton sampled on the substrates between July 11 and July 18, 1958 is related to the over-all periphyton complex in the West Branch can only be surmised. It is evident, however, that the community on the substrates is sufficiently uniform at all stations sampled to justify comparisons between stations as to chlorophyll content and uptake of phorphorus.

Table 2 thows a list of the algae identified in the West Branch of the Sturgeon River during the summers of 1958 and 1959.

TABLE 2. --A qualitative list of the algae identified in the West Branch of the Sturgeon River in the summers of 1958 and 1959.

Chlorophyta

Class: Charophyceae
- Chara sp.

Class: Chlorophyceae
- Oedogonium sp.
- Mougeotia sp.
- Dichotomosiphon sp.

Rhodophyta

Class: Rhodophyceae
- Batrachospermum moniliforme

Chrysophyta

Class: Xanthophyceae
- Vaucheria sp.

Class: Bacillariophyceae[1]*
- Synedra ulna
- Synedra sp.
- Cocconeis placentula
- Cocconeis sp.
- Cymbella turgida
- Navicula spp.
- Cyclotella sp.
- Gomphonema sphaerophorum
- Fragilaria harrisonii
- Meridion circulare
- Amphora ovalis
- Surirella biseriata
- Stephanodiscus sp.

*The list of Bacillariophyceae is based on the taxonomic keys presented in Tiffany and Britton (1952). It represents only the species occuring on artificial substrates.

All the major fresh water phyla are represented with the exception of Cyanophyta. Plosila (1958) identified a number of blue-greens in the plankton samples of Hoffman Lake, the origin of the West Branch. Prescott (1951) reports that a cyanophycean flora is most frequently encountered in water that is eutrophic in type. The most conspicuous filamentous aglaes were Batrachospermum moniliforme and Oedogonium sp. B. moniliforme was especially abundant during June and July. By the middle of August this alga had almost completely disappeared from the river. Oedogonium sp., on the other hand, reached its greatest numbers in August and early September. It was particularly abundant after heavy rains in August. Between August 27 and August 30, 1959, a period immediately following a considerable rise in the water level of the West Branch, this alga covered practically every extending substrate in a 300 yard section immediately upstream from permanent station VII. Vaucheria sp., although inconspicuous because its thallus is frequently covered by sand on the river bottom, was also found in considerable quantities. Dichotomosiphon sp. and Mougeotia sp. were collected only rarely.

Periphyton

Investigations pertaining to primary producers in aquatic situations have an early root in limnological history. Early investigations ot the standing crop of primary producers included counting,

TABLE 3. --Mean density of chlorophyll extracted from periphyton on 7-day substrates from stations VII, 1-12. Expressed as Klett units.

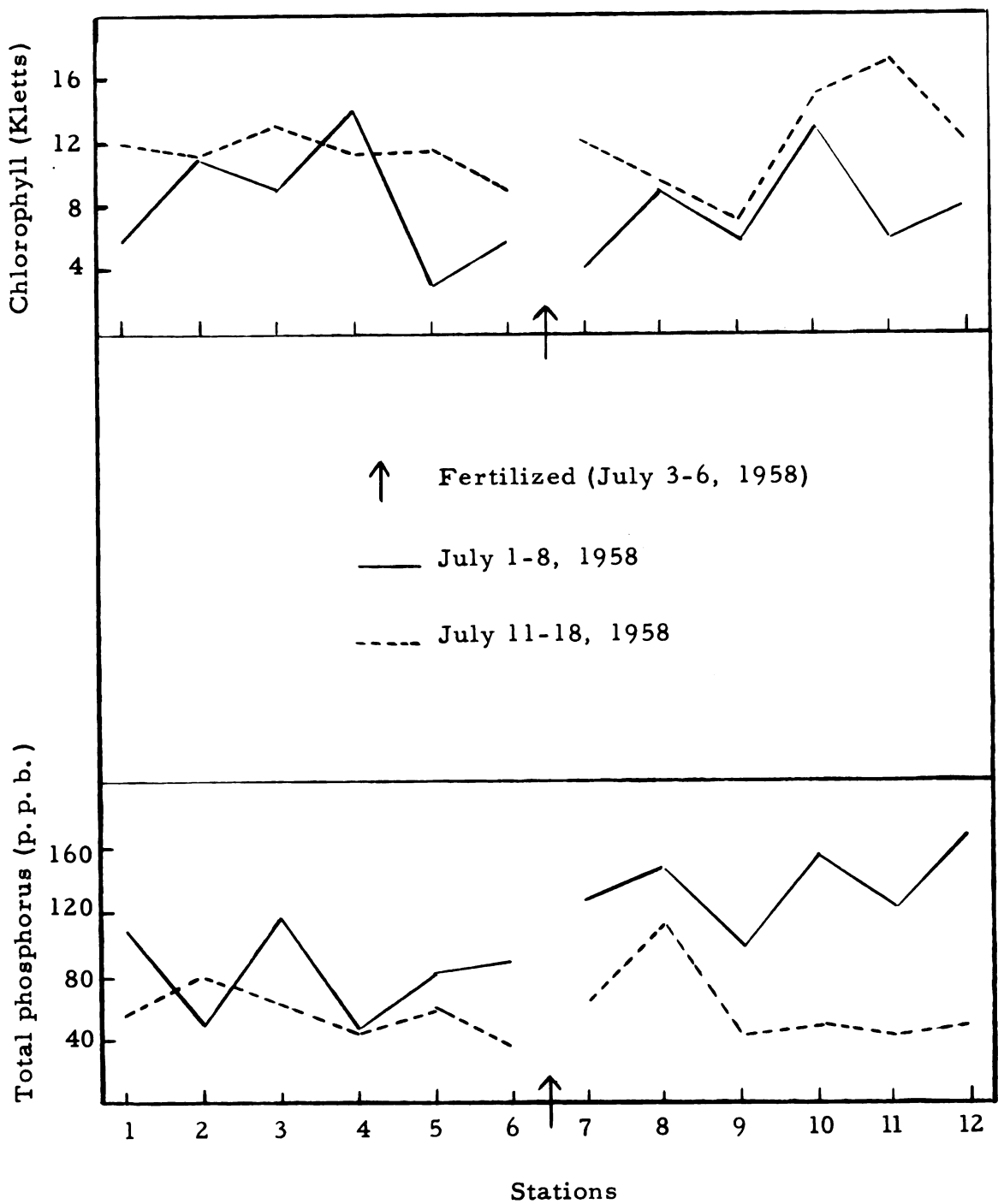
Station	Date	
	July 1-8	July 11-18
1	6	12
2	11	11
3	9	13
4	14	11
5	3	11
6	6	9
7	4*	12
8	9*	10
9	6*	7
10	13*	15
11	6*	17
12	8*	12

*Stations exposed to 80 lbs. of fertilizer, July 3-5, 1958.

weighing, or determining the cell constituents. Because of the lack of universal acceptability and sometimes applicability, the diversity of such techniques offered only limited usefulness when comparisons of production rates were made. In 1934, Harvey introduced a method which allowed quantitative estimates of chlorophyll to be made from extracted pigments. The chlorophyll method is still undergoing refinements, but has become a useful indicator of the standing crop. The swiftness of such a technique has made it a valuable tool for the limnologist. In this study the standing crop of periphyton is measured by the density of extracted pigments.

Previous studies of the periphyton in the West Branch of the Sturgeon River indicate an increase in the standing crop following fertilization. In the first three years of the program (1954, 1955, and 1956) the fertilizer was applied to Hoffman Lake. Grzenda (op. cit.) and Colby (op. cit.) both found a positive response to the fertilizer by periphyton downstream from Hoffman Lake. Carr, in 1956, observed an increase in the standing crop after fertilization but also observed a natural increase in production at the control station. This natural increase may be a seasonal phenomenon. In 1957, when the fertilizer was applied directly to the river, Keup (op. cit.) found large increases of periphyton which he could not attribute to natural flucuations.

FIGURE IV. --Mean density of chlorophyll and total phosphorus from periphyton on weekly shingles of stations below permanent station VII--Expressed as Klett units and p. p. b., 1958.


Chlorophyll (Kletts)
16
12
8
4
Fertilized (July 3-6, 1958)
July 1-8, 1958
July 11-18, 1958
Total phosphorus (p. p. b.)
160
120
80
40
1
2
3
4
5
6
7
8
9
10
11
12
Stations

In 1958, the substrates remained in the water for seven days (July 1-8) and 80 lbs. of fertilizer was added between stations VII, 6 and VII, 7 for a period of forty eight hours between July 3 and July 5, 1958. No statistically significant difference in periphyton could be detected between the six stations (VII, 7-12) that were exposed to the fertilizer and the six stations (VII, 1-6) above the fertilizing point (Figure IV and Table 4, test 1). The substrates were then again placed at all stations in the river (VII, 1-12) for another seven day period (July 11-18). To ascertain if there might be a delayed response to the 80 lbs. of fertilizer already added, no additional fertilizer was added during this period. Again no statistically significant differences could be detected in the periphyton of stations VII, 7-12 when compared to stations VII, 1-6 (see test 2, Table 4).

To determine if there might be a natural flucuation between stations VII, 7-12 and VII, 1-6 for the two sampling periods, July 1-8, and July 11-18, 1958, statistical comparisons were made between these two sections for the two different sampling periods. Tests 3, 4, 5, and 6, Table 4 indicate that no such flucuations occurred. Statistical analysis was not made for the five stations (VII, 1, 3, 5, and 9) used to evaluate the shingles exposed to fertilizer and removed after fourteen days (Figure V). Examination of these data (Table 5) indicates erratic results for stations 7 and 9, the two stations exposed to the fertilizer. It is believed that complete mixing of the added

TABLE 4. --Results of "t" tests calculated from mean chlorophyll content of stations VII, 1-6, and stations VII, 7-12; for the period of July 1-8, 1958, and July 11-18, 1958.

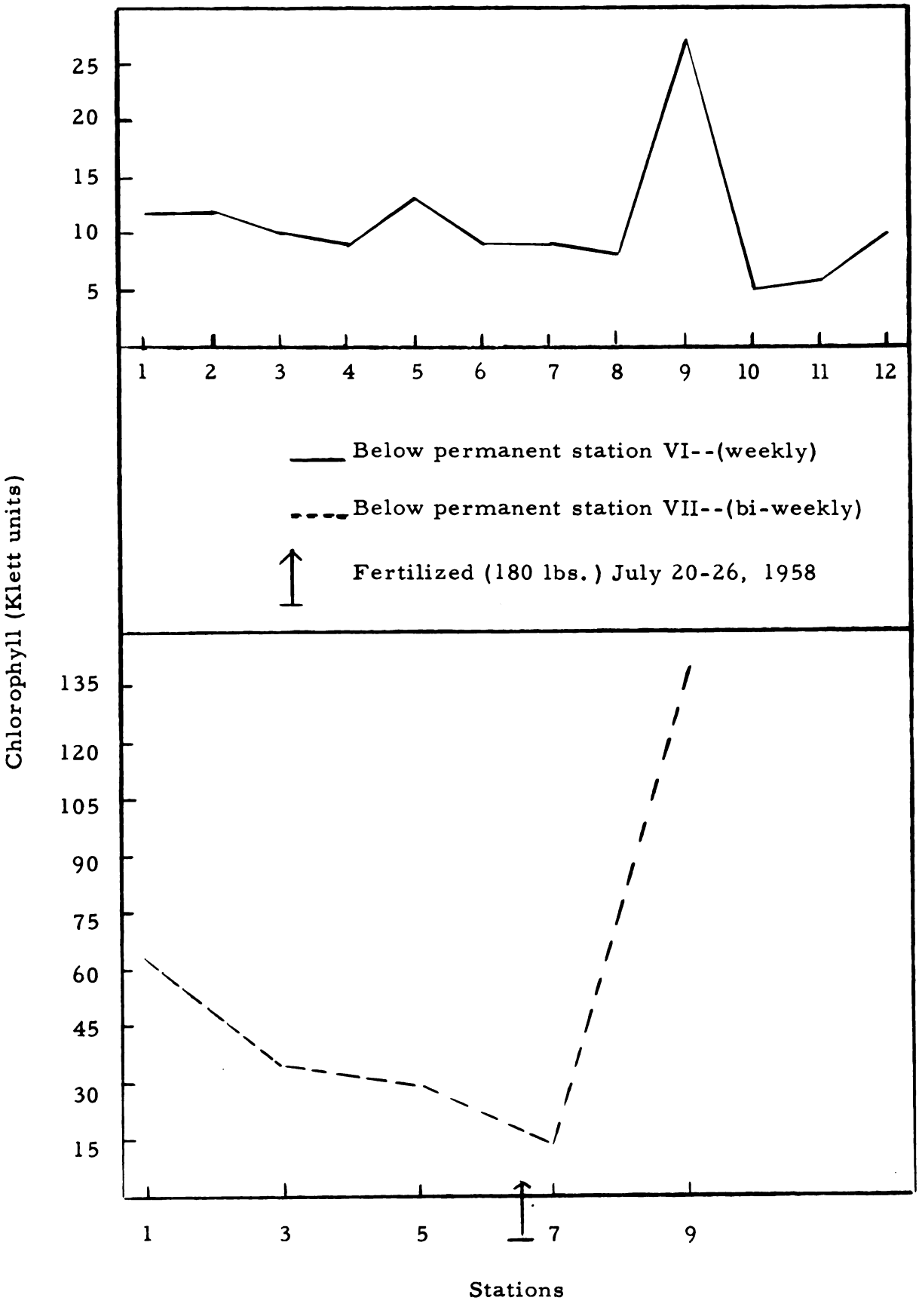
Test Number	Test	Result
1	Means of stations 7-12 (July 1-8) is greater than means of stations 1-6 (July 1-8).	t = 0.139
2	Means of stations 7-12 (July 11-18) is greater than means of stations 1-6 (July 11-18).	t = 0.373
3	Means of stations 1-6 (July 1-8) is significantly different from means of stations 1-6 (July 11-18).	t = 1.010
4.	Means of stations 1-6 (July 1-8) is significantly different from means of stations 7-12 (July 11-18).	t = 1.062
5	Means of stations 7-12 (July 1-8) is greater than means of stations 1-6 (July 11-18).	t = 1.240
6	Means of stations 7-12 (July 1-8) is greater than means of stations 7-12 (July 11-18).	t = 1.043

Degrees of freedom = 10

Critical value of "t" for tests 1, 2, 5, 6 = 1.812
Critical value of "t" for tests 3 and 4 = 2.228

All tests made at the five percent level. Hence no significant increase in chlorophyll content could be detected between the stations concerned for test 1, 2, 5, and 6; no significant differences in chlorophyll content could be detected between the stations concerned for test 3 and 4.

FIGURE V. --Mean density of chlorophyll from weekly substrates of stations above permanent station VII, and bi-weekly substrates from stations below permanent station VII--Klett units, 1958.


Chlorophyll (Klett units)
25
20
15
10
5
1
2
3
4
5
6
7
8
9
10
11
12
Below permanent station VI--(weekly)
Below permanent station VII--(bi-weekly)
Fertilized (180 lbs.) July 20-26, 1958
135
120
105
90
75
60
45
30
15
1
3
5
7
9
Stations

fertilizer with the river water may not have occurred by the time the nutrients would be passing station 7, a distance of 75 yards from the point of application.

An explanation of why the standing crop of periphyton did not increase following the first application of fertilizer involved many factors. The artificial substrates were only in the water seven days, a period believed to be insufficient for maximum growth of periphyton in the West Branch. The amount of fertilizer added (80 lbs.) was not as great as that added in 1957 (410 lbs.). The microhabitat of the plexiglass shingles may have varied from those of the cedar shingles used in 1957. Colby (op. cit.) found a difference in the community complex between the wooden and brick substrates in 1955. The first two possibilities appear to be closer to the problem. When the substrates were exposed for fourteen days and a greater amount of fertilizer (150 lbs.) was added, the few data available indicate a positive response of the periphyton to the fertilizer.

The information obtained from stations VI, 1-12, where the plexiglass substrates were exposed for seven days without benefit of fertilizer (see Table 5 and Figure V), indicated that this section of the river should receive added nutrients in the form of fertilizer in order to insure that there would be a weighable amount of periphyton present when the isotope was to be released into the river. It was also decided to allow the plexiglass substrates to be in the

TABLE 5. --Mean density of chlorophyll extracted from periphyton on weekly substrates from stations VII, 1-12, and bi-weekly substrates from the odd numbered stations below station VII--Klett units, 1958.

Station	Date	
	July 23-30 (Below VI)	July 18-31 (Below VII)
1	12	63
2	12	-
3	10	35
4	9	-
5	13	29
6	9	-
7	9	13*
8	8	-
9	27	141*
10	5	-
11	6	-
12	10	-

*Stations exposed to 150 lbs. of fertilizer, July 20-26, 1958.

water a period of fourteen days instead of seven, a procedure that would entail lengthening the sampling periods for radioactive periphyton.

Phosphorus Content of the Periphyton

After the first application of fertilizer to the West Branch (July 1-8), a significant increase in total phosphorus in the periphyton was detected in those stations (VII, 7-12) accessible to its effects (Tables 6 and 7). This increase of total phosphorus in periphyton did not carry over for the July 11 to 18 period, a period when no fertilizer was added to the river.

An analysis of the first period (July 1-8) does not indicate progressively smaller amounts of phosphorus in the periphyton mass proceeding downstream from station 7 to station 12 (see Table 6 and Figure IV). This phenomenon, which did not occur, could be expected if enough phosphorus was lost, either biologically or physically, so as to dilute the amount of available phosphate as it passed each station. A possible reason why this did not occur in this study may lie in the tremendous amount of extraneous phosphate that would be available to the periphyton complex when fertilizer is added to the river. From the point of fertilization to station VII-12, a distance of 800 yards, it is possible that phosphate may have been available in such quantities as to allow the periphyton at these distances to all reach maximum accumulation values. It is also possible that the periphyton at these

TABLE 6. --Mean value of total phosphorus in the periphyton mass on weekly substrates from stations below permanent station VII-- Expressed as p. p. b., 1958.

Station	Date	
	July 1-8	July 11-18
1	107	56
2	50	79
3	117	60
4	48	44
5	76	60
6	89	35
7	125*	64
8	144*	111
9	94*	42
10	153*	47
11	119*	42
12	166*	47

*Stations exposed to 80 lbs. of fertilizer, July 3-5, 1958.

TABLE 7.--Result of "t" tests calculated from mean p.p.b. of total phosphorus in the periphyton of stations VII, 1-6, and 7-12, July 1-8, and July 11-18, 1958.

Test Number	Test	Results
1	Means of stations 7-12 (July 1-8) is greater than means of stations 1-6 (July 1-8).	t = 1.911*
2	Means of stations 7-12 (July 11-18) is greater than means of stations 1-6 (July 11-18).	t = 0.146
3	Means of stations 1-6 (July 1-8) is significantly different from means of stations 1-6 (July 11-18).	t = 1.113
4	Means of stations 1-6 (July 1-8) is significantly different from means of stations 7-12 (July 11-18).	t = 0.805
5	Means of stations 7-12 (July 1-8) is greater than means of stations 1-6 (July 11-18).	t = 3.656*
6	Means of stations 7-12 (July 1-8) is greater than means of stations 7-12 (July 11-18).	t = 2,825*

Degree of freedom = 10.

Critical value of "t" for tests 1, 2, 5, and 6 = 1.812.
Critical value of "t" for tests 3 and 4 = 2.228.

*Significant at the 5 percent level.

distances have simply satisfied a phosphorus debt that the complex may be subjected to in this river. That it may be just a case of satisfying a phosphorus debt is somewhat substantiated by the work of Rodhe (1948). Working with Scenedesmus spp. (a small green algae), he found that it could assimilate phosphorus very rapidly (one day) to satisfy a phosphate debt, but the accumulation of extra phosphorus required a fairly long time (seven days). In any case, a progressive decrease in the uptake of phosphorus by periphyton, proceeding downstream from where it entered, would not be expected to endure long. Harvey et al. (1935), working with phytoplankton, found that only a fraction of the phosphate utilized by the plankton could be found as phosphorus compounds in the planktonic population. Goldberg et al. (1951) also found that as much as 50 percent of the radioactive phosphorus in cells containing more than the minimum requirements could be removed by washing with sea water free of radiophosphorus.

This rapid loss of phosphorus from marine algae, noted by the above authors, appears to have occurred also for the periphyton in this river experiment (see test 6, Table 7). Since the actual amount of periphyton that was analyzed for total phosphorus could not be known with certainty, it may at first appear to be unwarranted to make such a conclusion from this test. A comparison of the chlorophyll content for each of these periods, however, indicates that stations VII, 7-12 for the second period (July 11-18) had an amount of periphyton

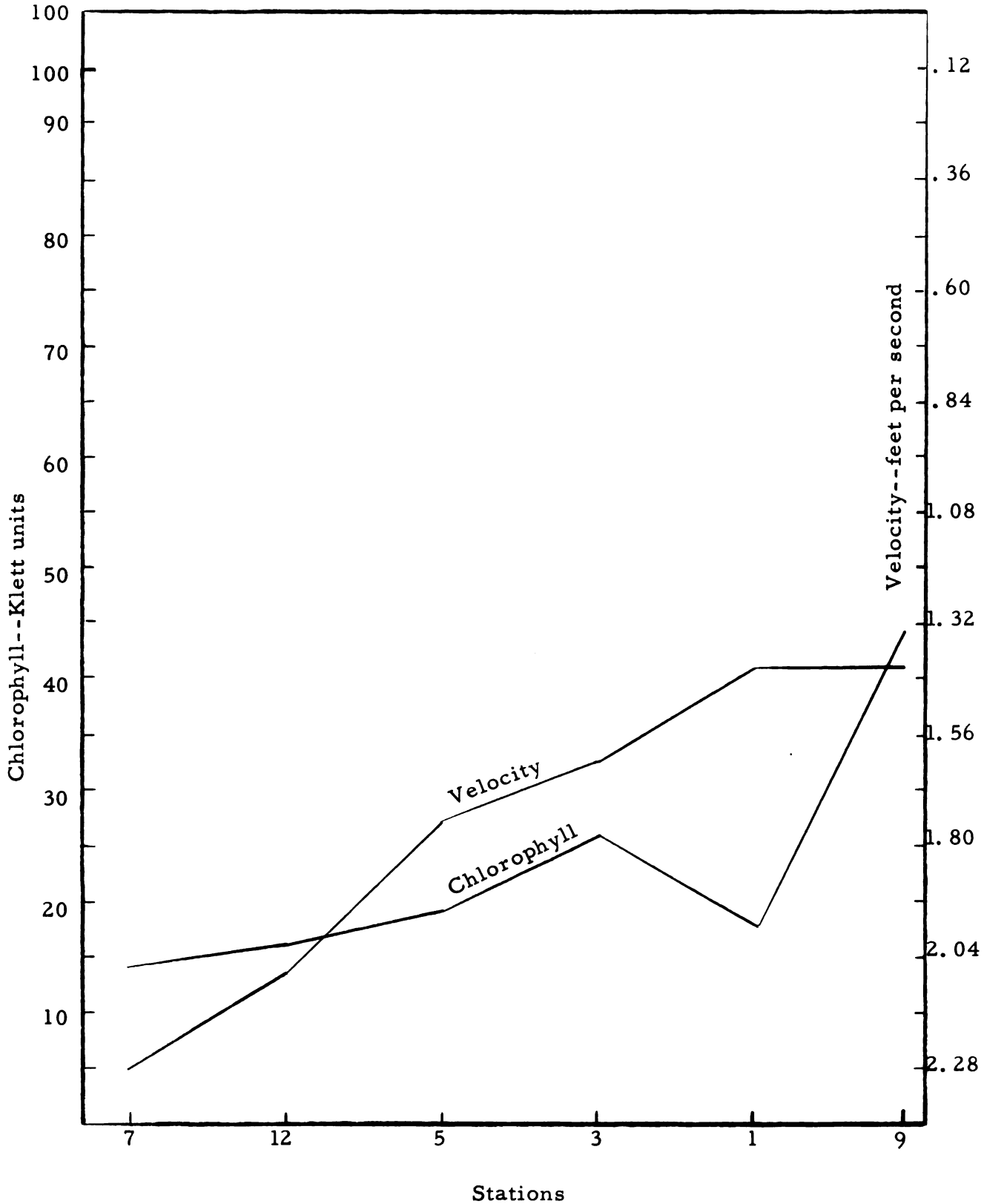
that was not significantly different from stations VII, 7-12 for the first period (July 1-8). (See test 6, Table 4.) If this is the actual case, the above inference from test 6 appears to be justified. That is, excessive amounts of phosphorus in the periphyton appear to be lost rapidly.

With the methods employed in the preliminary study, it would be impossible to differentiate between naturally occurring phosphorus and that introduced extraneously. The actual amount loss and rate of loss could not be determined. It was decided to add artificial substrates to the river following the release of P32 in order to detect any re-accrual of the added P32, either from biological "feed back," or from delayed eddy diffusion.

Velocity of the River Water

Many workers have observed that certain algae will grow more luxuriously in rapid water. Neel (1951) believes greater consumption of nutrients occurs in rapids than in pools. Whitford (1956) emphasizes the beneficial effect of current on algal communities. Ruttner (1953) is one of the chief proponents of rapid currents manifesting beneficial effects on the quantity of organic production per unit area. He states: "In the rushing water of rapids the stones are thickly overgrown with mosses and algae and in addition there is richly developed animal life, such as one would not expect in an

FIGURE VI. --Comparison of the velocity of the surface water over the odd numbered stations below permanent station VII with the chlorophyll concentration on substrates for these stations, 1958


100
100
90
80
70
60
50
40
30
20
10
Chlorophyll--Klett units
.12
.36
.60
.84
1.08
1.32
1.56
1.80
2.04
2.28
Velocity--feet per second
Velocity
Chlorophyll
7
12
5
3
1
9
Stations

oligotrophic mountain water. The stones of the lentic regions, on the other hand, exhibit a much smaller aufwuchs and usually fewer animals as well." Other investigators are inclined to believe the opposite is true; that there is more production in slower waters. Blum (1956) does not believe that there is enough evidence to indicate better growth in riffles. He cites an investigation in Brazil where it has been concluded that rapids are less productive than the quiet portions of rivers. In a recent paper by Douglas (1958), working with a diatom population, it was noted that an increase in the flow of water caused a marked decrease in the diatom population. Butcher (1932), using glass slides as artificial substrates, found the rate of increase of diatoms inversely proportional to the velocity of the current.

Comparison was made in the West Branch between the crude surface velocity of the water over the odd numbered stations and the mean amount of periphyton found at these stations (Figure VI). There are indications that rapid current of the water may have a negative influence on the standing crop of periphyton on the plexiglass substrates. To see if there was a correlation between the velocity of the river water and the standing crop of periphyton, a regression line was computed from chlorophyll and water velocity at four stations (3, 5, 7, 9) where measurements were made. A test was then performed to see if the regression line was significantly different from zero. The results (Table 8) indicate that the regression

TABLE 8.--Test of regression line computed from velocity of the river water and chlorophyll at four stations below permanent station VII on the West Branch of the Sturgeon River, 1958.

Station	X (velocity, c.f.s.)	Y (Klett units)
3	1.60	119
5	1.73	90
7	2.31	71
9	1.38	209

$b = -126.062$ $\quad Sy.x^2 = 1840.640$

$a = 343.489$ $\quad S_b = 62.361$

$t = -2.021$ with 2 degrees of freedom.

Critical value of $t = -2.920$ at 10% level.

Critical value of $t = -1.886$ at 20% level.

b is only significantly different from 0 at the 20% level.

line was significantly different from zero only at the 80 percent level, not the 95 percent or 90 percent level. This further indicates that faster velocities may influence adversely the standing crop of periphyton. Observations made later, of periphyton collected for radioactive analysis, also indicates a greater biomass at stations almost lentic in nature. Since the periphyton complex consists almost entirely of diatoms (see Table 1), it is possible that this greater standing crop at lower velocities was due to a settling out of the potamoplankton on the artificial substrates. The smaller crop of periphyton in fast water does not mean that the production rate was lower here. Diatoms continually grow and break off in the fast water. The rate of production would be governed by the turnover rate of diatoms, a phenomenon that was not investigated in this study.

RADIOPHOSPHORUS STUDY

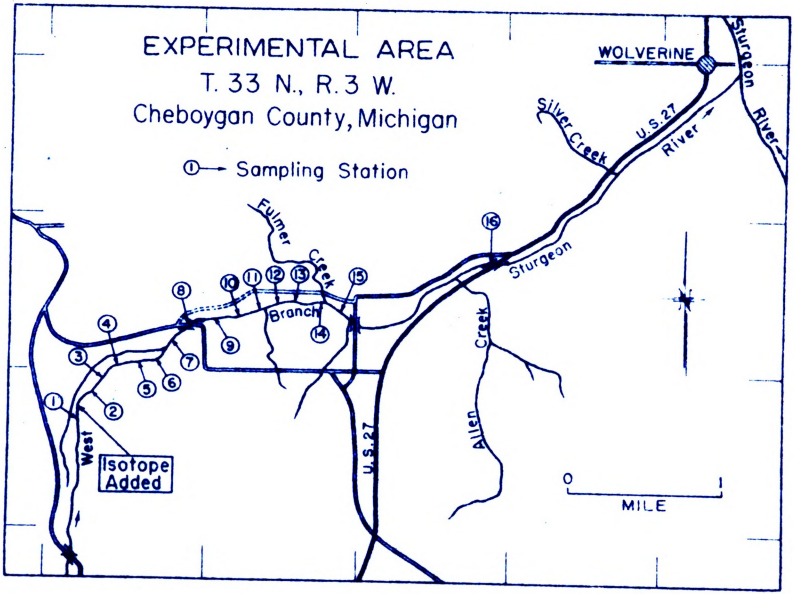
Methods and Procedures

Description and Preparation of the Study Area

The study area for measuring the response of periphyton to radioactive phosphorus encompassed stations both above and below permanent station VII (Figure VII). The stations for this phase of the program differed from those used during the preliminary study even though the same stretch of the river was used for both. The entire length of the area was 2,700 yards long. Fourteen sampling stations were used for measurements of radioactive periphyton. Station 1 (control) was approximately 100 yards above the point of isotope addition. Stations 2 through 9 were at 150 yard intervals below the point of tracer entrance. Stations 10 through 14 were at 300 yard intervals.

Preliminary investigation of this area revealed a low production of periphyton (see Table 5). An inorganic fertilizer, diammonium phosphate, was used to "prime" this area in order to insure a rapid uptake of the P32 and to provide a measurable amount of periphyton. The fertilizer was applied continuously for three days at the point where the isotope was to be released on August 1, 1958.

FIGURE VII. --Map of the West Branch of the Sturgeon River area, showing the stations used in the radiophosphorus study.


EXPERIMENTAL AREA
T. 33 N., R. 3 W.
Cheboygan County, Michigan
① Sampling Station
WOLVERINE
Sturgeon River
Silver Creek
U.S. 27
Fulmer Creek
Branch
Sturgeon
Allen Creek
West
Isotope Added
U.S. 27
0
1
MILE

Description and Distribution of the Isotope

Radiophosphorus (P32) was used as the tracer to determine the uptake and movement of naturally occurring phosphorus. The P32, which was carrier-free, was obtained from the Atomic Energy Commission, Oak Ridge, Tennessee as phosphate (PO_4) dissolved in a weak hydrochloric acid. The P32 was assayed at 8 A. M., August 4, 1958 and found to have an activity of 23. 1 millicuries.

The method of distributing the P32 into the West Branch of the Sturgeon River was similar to that used earlier in the summer to distribute di-ammonium phosphate fertilizer to the river. The 23 millicuries of P32 were diluted with 50 gallons of river water in a 55 gallon drum. The diluted tracer was siphoned into the river by means of polyethylene tubing. The flow was calibrated to distribute the entire amount of P32 in 37 minutes. This was accomplished by constructing a board with 38 nails at 0. 8 inches. The nails allowed the discharge end of the siphoning tube, extended by a long bamboo pole, to rest at each interval for one minute. The siphoning of the P32 commenced at 2:01 P. M., August 5 and terminated at 2:38 P. M. A green florescent dye was released at the point where the isotope was to be released and its movement timed over the stations downstream. It was determined that complete mixing of the isotope with the river water would occur by the time the isotope had reached a

distance of 300 yards below the point of distribution. This was further substantiated by radioactivity found in samples of periphyton.

Field Sampling Methods

Periphyton was collected both from rocks and plexiglass substrates. The substrates were of two sizes, 2" x 5" and 4" x 10". The smaller ones (2" x 5") were arranged eight on a stand. The remainder of the assemblage is similar to that described for the preliminary study. The large plates (4" x 10") were fastened separately to convenient objects (logs, roots, etc.) below the surface of the water.

Collecting methods consisted of picking up four of the small or one of the large substrates from each station, 4 hours after the addition of the isotope and again three and seven days after the isotope had been released. Thereafter, periphyton was sampled only at stations 1, 3, 5, 8, 11, and 14. This procedure was followed thirteen, nineteen, and twenty six days after the addition of the isotope. Two rocks, about the size of the small plexiglass substrates were also picked up at the latter mentioned stations. The periphyton on the rocks was sampled in order to detect differences in the response of periphyton to P32 in its natural condition when compared to that analyzed from the artificial substrates. Rocks were collected five, twelve, and nineteen days after tracer entrance.

Laboratory Procedures

The periphyton samples, in which radioactivity was to be determined, were immediately prepared in the laboratory after they were removed from the stream. All obvious forms of animal life were picked off the substrates. The periphyton was scraped from the substrates, placed in weighed deep walled planchets and weighed. It was found necessary to use a small amount of distilled water (approximately 3 c. c.) to facilitate transfer of all the periphyton. The planchets containing the samples were then placed in an oven at 100° C. and evaporated to a point at which no water movement could be detected when the planchet was tipped at an angle. Even though water did not flow over the planchet, the samples at this stage were still wet. The planchets were reweighed and the difference taken as wet weight of periphyton. Ten milliliters of 2N nitric acid was then added directly to the planchets containing the periphyton and this was boiled under a heat lamp until yellow-brown. Five milliliters of concentrated nitric acid was then added to the digestate and the sample was again heated under a heat lamp until it was completely dry. The planchet containing the digestate was then transferred to a furnace and muffled until red hot. After the periphyton samples in the planchets were allowed to cool, they were ready for counting.

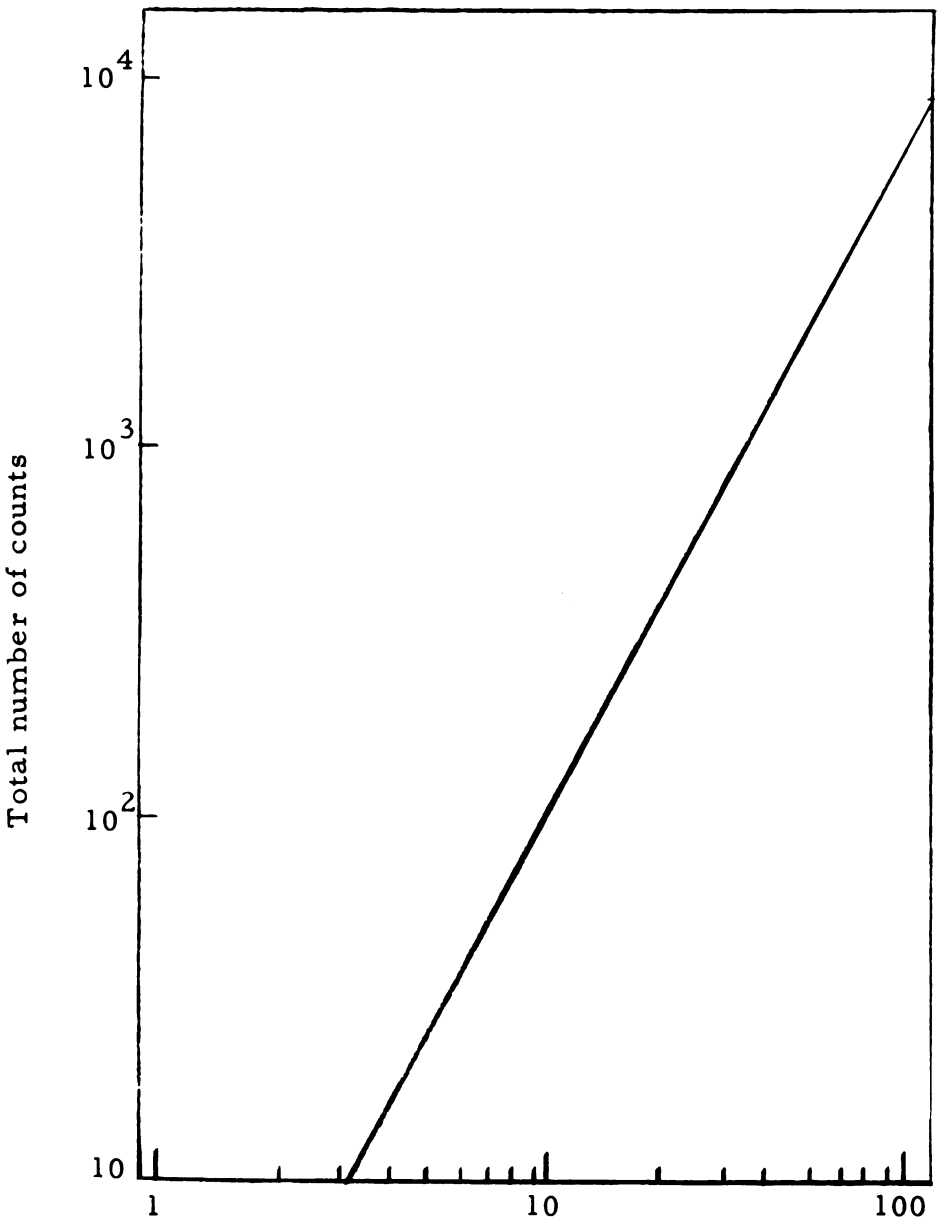
Because of the large amount of marl closely associated with the encrusting periphyton on rocks, it was necessary to slightly

alter the procedure for preparing rock samples. The procedure is similar to that of above but the periphyton was brushed into a beaker using approximately 15-30 milliliters of distilled water, depending on the size of the rock. The sample was then decanted to a 150 ml. beaker and this decanted to a 50 ml. graduated cylinder. A 5 ml. aliquot was taken and this was put in a weighed deep walled planchet. The remainder of the procedure (evaporation, reweighing, etc.) is identical with that used for the artificial substrates.

Measurement of Activity

Radioactivity was measured with a Nuclear Measurement Corp. type PC-3A gas flow proportional counter. This particular counter had a background of 47-54 c.p.m. (counts per minute). All measurements were corrected for sample size, background, and radioactive decay (P32 loses half its activity in 14.3 days). Counting efficiency of the machine included factors of self absorption and backscatter. The counting efficiency of the PC-3A counter was approximately 46 percent for radioactive phosphorus. Since comparisons and interpretations will be meaningful without multiplication by this constant, counting efficiency was not included in final tabulation of data. Samples were counted for two minutes. Kinman (1954) shows the error involved for counts of such a period of time (Figure VIII). It is evident that such a counting time is satisfactory when dealing with

FIGURE VIII.--Error in counts per minute for two minute counts, 95 percent confidence level. (After Kinsman, from Krumholz, 1954).


Total number of counts
10^4
10^3
10^2
10
1
10
100
Error in counts per minute, 95 percent confidence interval

samples relatively high in activity level. After the third collecting trip (seven days after the release of the isotope) the activity of periphyton fell to a point where a two minute count gave a considerable amount of variability in the final tabulation. Nevertheless, the large number of samples to be counted at all levels (water, periphyton, aquatic plants, aquatic insects, and fish) coupled with the short summer season indicated that a two minute count would be the most satisfactory.

Results and Discussion

Initial Uptake of P32 by Periphyton

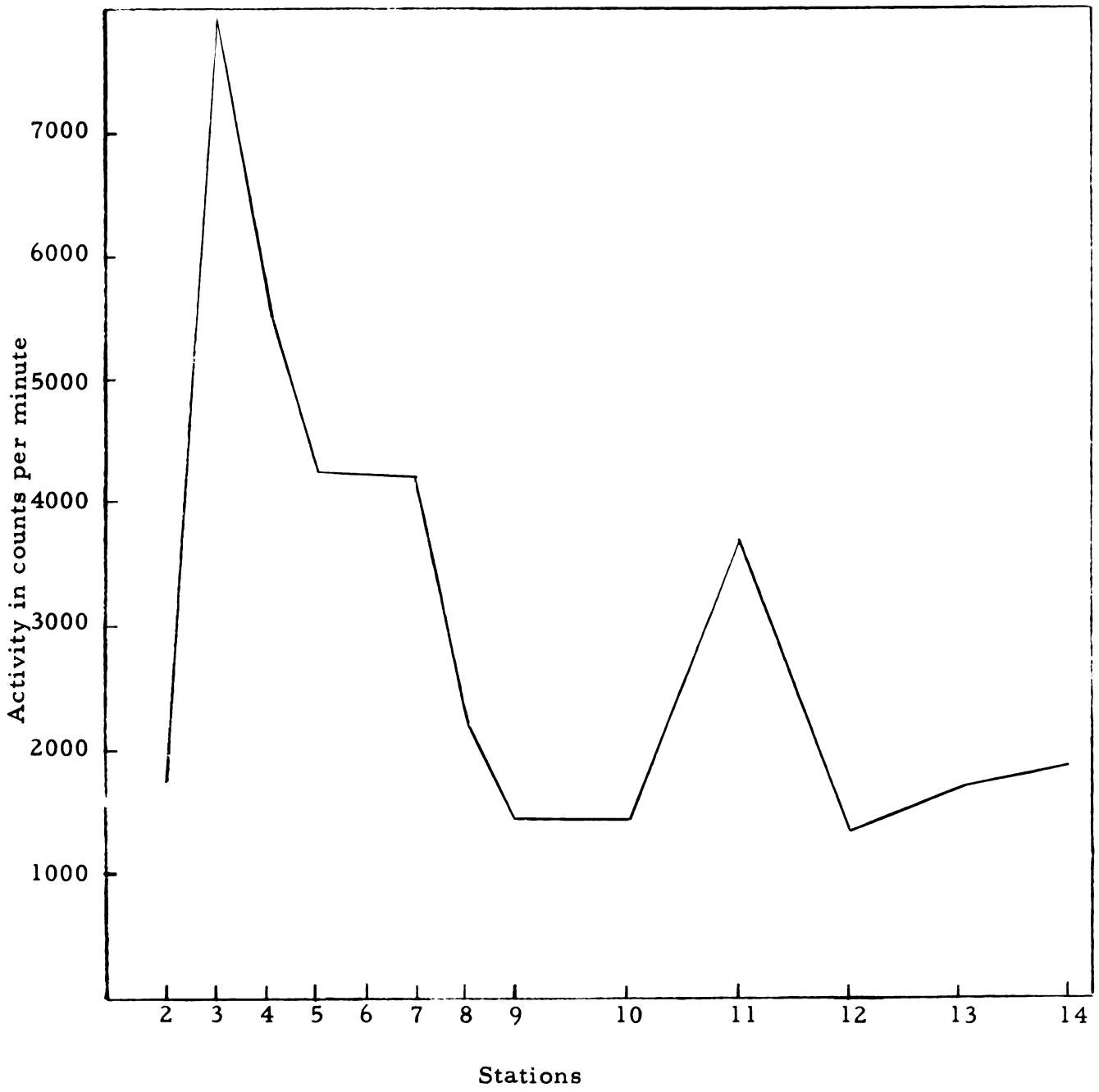
Table 9 indicates that the initial uptake of P32 by periphyton was, in general, progressively smaller in amounts proceeding downstream from station 3 to station 14. The magnitude of the initial uptake of P32 may best be viewed graphically (Figure IX). It is evident that at station 2, the isotope was not completely mixed with the river water. The high activity level of periphyton at station 11 dictates closer investigation. This station was also high in activity three days after the release of the isotope. Because substrates at this station were particularly impoverished in periphyton, a weighing error for both dates could have occurred. It is also possible that the periphyton community in this section of the river was different from the other

TABLE 9. --Initial uptake of P32 by periphyton four hours after the addition of the isotope to the West Branch of the Sturgeon River, August 5, 1958.

Station	Counts per minute* per gram
1	0
2	1733
3	7972
4	5611
5	4266
6	4222
7	4236
8	2222
9	1482
10	1481
11	3707
12	1390
13	1728
14	1934

*Counts are corrected for background and decay.

FIGURE IX. --Initial uptake of P32 by periphyton on artificial substrates four hours after the addition of the isotope for the entire experimental area of the West Branch of the Sturgeon River, August 5, 1958.


7000
6000
5000
4000
3000
2000
1000
Activity in counts per minute
2
3
4
5
6
7
8
9
10
11
12
13
14
Stations

areas. In light of the taxonomic investigation (see Table 1), this possibility would not appear to be the case.

A large amount of P32 was taken up initially by periphyton a distance of 2, 750 yards (station 14) downstream from the point of release. This indicates to some degree the amount of available P32 that must have remained in the water after passing through this distance of the river. Borgeson (1959) calculated that three percent of the isotope dosage passed through the study area of the West Branch of the Sturgeon River. Hutchinson (1950) observed the entire amount of radioactive phosphorus (35 millicuries) to be utilized by plankton in the epilimnion of a small eutrophic lake within minutes. Rigler (1956) found that over 95 percent of the radiophosphorus added to a lake was taken up by plankton within twenty minutes. Data indicate that in the West Branch, a lotic situation, a considerable amount of P32 is still available at the end of 108 minutes, the calculated time of movement of the initial pulse of P32 from the point of release to station 14 (Table 9). Whittaker (1953), working with an aquarium community, noted that P32 during the first few hours after tracer introduction, was rapidly absorbed by planktonic algae but after 15 hours plankton activity densities decreased while the P32 was more gradually taken up by bottom and side-wall algae. In the West Branch the primary producers are limited to periphyton and higher aquatic plants,

there being no plankton in the strictest sense. It is therefore reasonable to suppose that the ability of the P32 to remain free in the water for longer periods of time than would be found in standing water series, is due to the lack of plankton in the community composition of the stream.

Accumulation of P32 by Periphyton

Since the amount of P32 that enters the periphyton after the initial pulse (uptake) could not be ascertained with certainty, it appears best to view the remainder of the data as values of accumulation (P32 that is present in the periphyton at a given time). Table 10 shows the amount of radiophosphorus accumulated in the periphyton for the third and seventh day after the isotope release, August 7 and August 11 respectively. The amount of P32 accumulated by the third day still shows indications of decreasing as one proceeds downstream from the point of release, although the gradiation is not as striking as on the first day. By the seventh day, there is no evident decrease of the P32 in the periphyton proceeding downstream from station 3 to station 14. This is better illustrated if the stream is partitioned into sections and the mean value of P32 for each section is calculated. It can be seen (Table 11) that by the seventh day the P32 in the periphyton is quite evenly distributed in the entire experimental area. Davis and Foster (1958) showed that a radioactive

TABLE 10. --Accumulation of P32 in periphyton of the West Branch of the Sturgeon River for the third and seventh day after the release of the isotope, August 7, and August 11, 1958.

Third day		Seventh day	
Station	CPM/ per gram*	Station	CPM/per gram*
1	0	1	0
2	2139	2	2178
3	2910	3	1167
4	5298	4	991
5	1427	5	440
6	1368	6	-
7	1202	7	350
8	3559	8	2191
10	1504	10	1943
11	3834	11	1491
12	1393	12	-
13	1107	13	910
14	182	14	1174

*Counts are corrected for background and decay.

element, such as P32, will eventually become uniform throughout the biota. This is because the isotope will be initially diluted with its stable form, first in solution, and eventually by exchange with its stable form which has not been in solution. Borgeson (op. cit.) found that by August 15, this phenomenon also held for Chara sp. and Potamogeton pectinatus. Because the diatoms of the periphyton complex have a shorter retention time of P32 than the aquatic plants, it would be expected that the periphyton would first show a uniform distribution of the isotope.

The retention time of an element, which is a function of the biochemistry of the particular element and components involved, is an especially important biological consideration when tracing the element through the various trophic levels and when considering values of accumulation. Davis and Foster (op. cit.) have shown that retention is likely to be inversely related to the size of the "pool" or "reservoir" for the element in that trophic level. The element will remain for a longer period of time in the larger consumer organisms than in the smaller plants, although a major fraction of the element will at first be accumulated by the periphyton because of its relatively large total biomass. Odum et al. (1958) demonstrated that the ability of the biomass to accumulate large amounts of the element initially to be a function of the high surface to volume ratio of the small plants.

TABLE 11. --Mean value of P32 accumulated in periphyton of the West Branch of the Sturgeon River per section for the first seven days after the addition of the isotope.

Section	Counts per minute, per gram, corrected for background and decay		
	August 5	August 7	August 11
0-----700 yards	4761	2628	1194
700--1400 yards	2355	2021	1494
1400-2600 yards	2190	1629	1191

TABLE 12. --Accumulation of P32 (corrected counts per minute) in periphyton of the West Branch of the Sturgeon River for the thirteenth, nineteenth, and twenty-seventh day after the addition of the isotope, August 17, August 23, and August 30, 1958.

August 17		August 23		August 30	
Station	CPM per gram	Station	CPM per gram	Station	CPM per gram
1	0	1	0	1	0
3	1500	3*	284	3*	268
5	2718	5*	263	5*	364
8*	310	8*	447	8*	368
11*	616	11*	321	11*	357
14	1860	14	274	14*	621

*Denotes substrates introduced to the water after the release of the isotope.

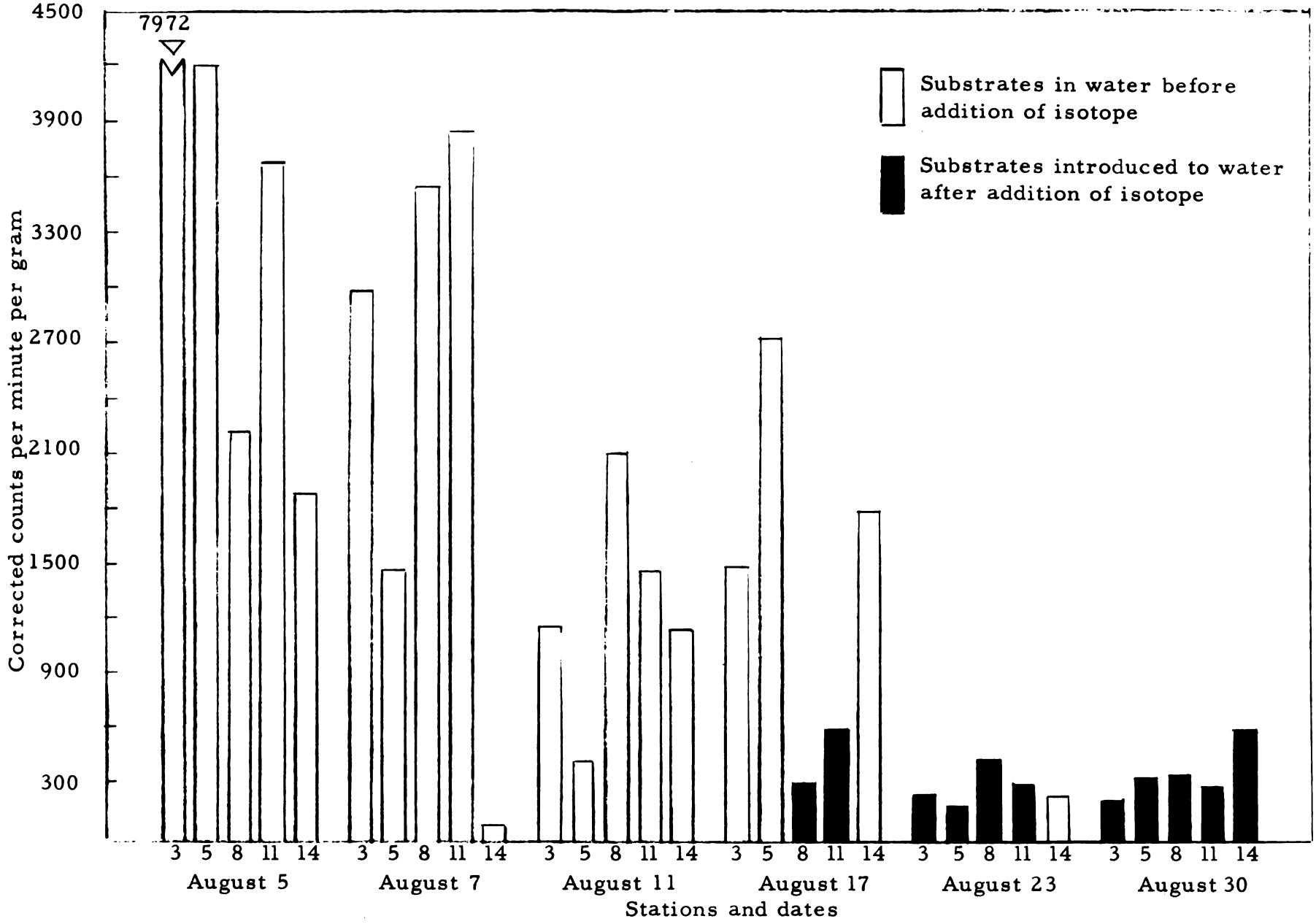
With the exception of station 14 on August 30, 1958, the uniformity in distribution of P32 in the periphyton continued for the remainder of the experiment. These remaining values (Table 12) are chiefly from substrates introduced after the application of P32 and will be treated separately from the above in a later section. The relatively large amount of radioactivity found in the periphyton at station 14 for August 30, 1958 may indicate that the accumulated "drift" of radioactivity is reaching a downstream station.

Exchanged and Regenerated P32

Data from substrates placed in the water after the release of the isotope indicate that exchanged and/or regenerated P32 entered the system rapidly and in considerable amounts (Table 12). Since the substrates were not placed in the river until well after the initial pulse of P32 (the first post treatment shingles were not put in the river until two days after the release of the isotope), it is apparent that this re-accrual of P32 by periphyton could not have taken place from P32 held in situ, either by eddies or other entrapments.

Figure X shows that for the thirteenth day of the experiment, August 17, approximately 33 percent of the total measured radiophosphorus on the substrates was accumulated in the exchangeable and regenerated form. Substrates placed in the river August 23 (19 days after the release of the isotope) and collected August 30 still

FIGURE X. --Summary of P32 activity in periphyton of the West Branch of the Sturgeon River, 1958.


4500
3900
3300
2700
2100
1500
900
300
7972
Corrected counts per minute per gram
Substrates in water before addition of isotope
Substrates introduced to water after addition of isotope
3 5 8 11 14 3 5 8 11 14 3 5 8 11 14 3 5 8 11 14 3 5 8 11 14 3 5 8 11 14
August 5
August 7
August 11
August 17
August 23
August 30
Stations and dates

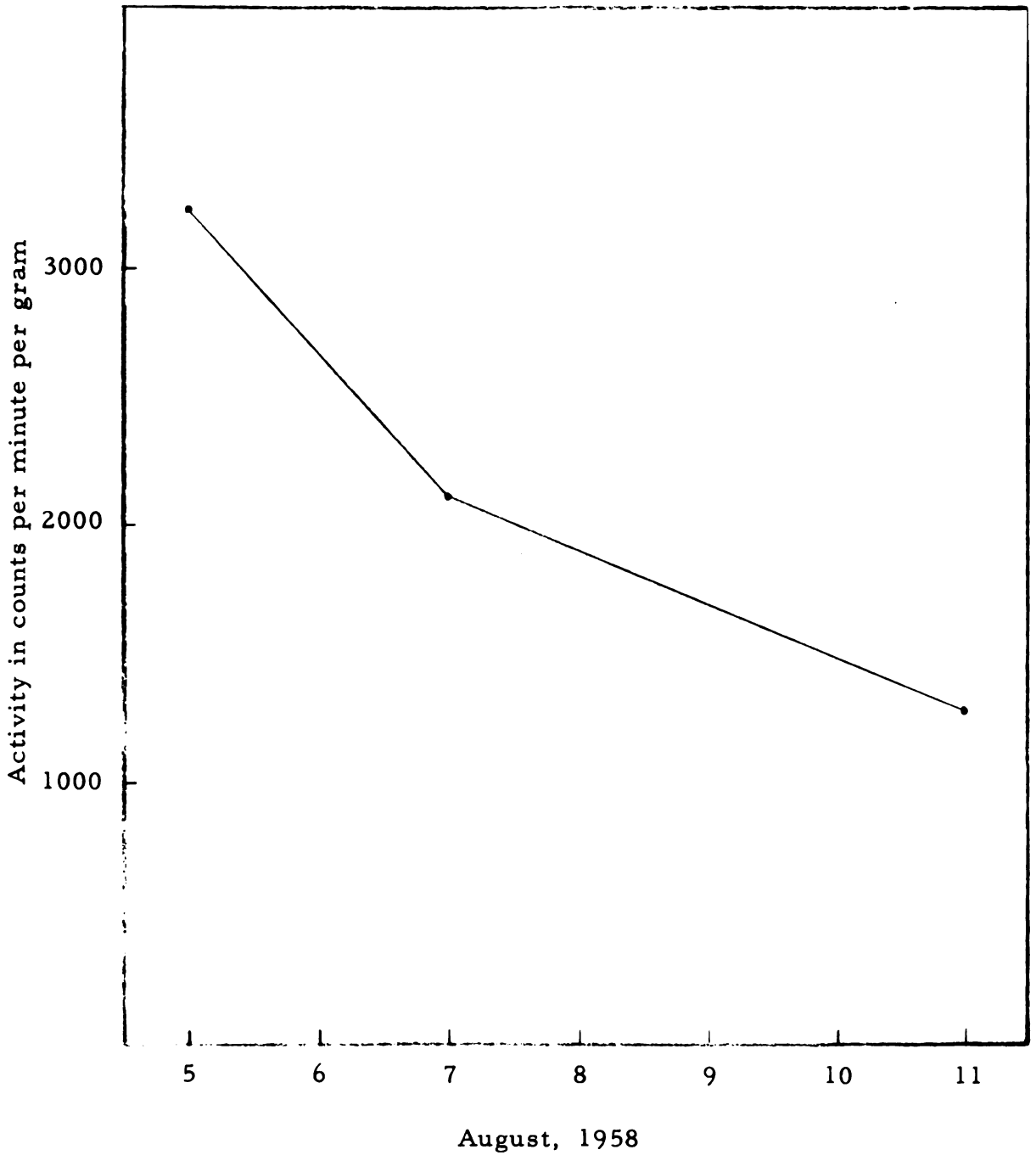
showed an appreciable amount of P32, most of which must have been previously adsorbed on, or incorporated into the periphyton (Table 12). In all cases a slight allowance must be made for the "seeding on" of diatoms and other algae. These forms could be radioactive when they were seeded on, thus contributing activity which would not necessarily be exchanged or regenerated in form.

In systems such as the West Branch, where the periphyton complex may be surviving perilously close to phosphorus deficient conditions, the re-utilization rate of phosphorus will depend to a great extent upon the retention time of phosphorus in the previous organism. When measurements are made from artificial substrates, the re-utilization of phosphorus may depend also on the age of the community on the substrate. It is possible that new substrates may pick up phosphorus faster than older ones. The substrates with the older community may be picking up only a replacement ration of phosphorus while those substrates that are new will be picking up phosphorus at a faster rate because of the "seeding on" and rapid initial growth of the community.

Loss of P32 from Periphyton

The loss of P32 from periphyton of the experimental area is determined on a relative basis. The assumption is made that the amount of P32 accumulated on each substrate when considered on a

FIGURE XI. --Mean loss of P32 activity in periphyton on artificial substrates for the entire experimental area during the first seven days after isotope addition, 1958.


Activity in counts per minute per gram
3000
2000
1000
5
6
7
8
9
10
11
August, 1958

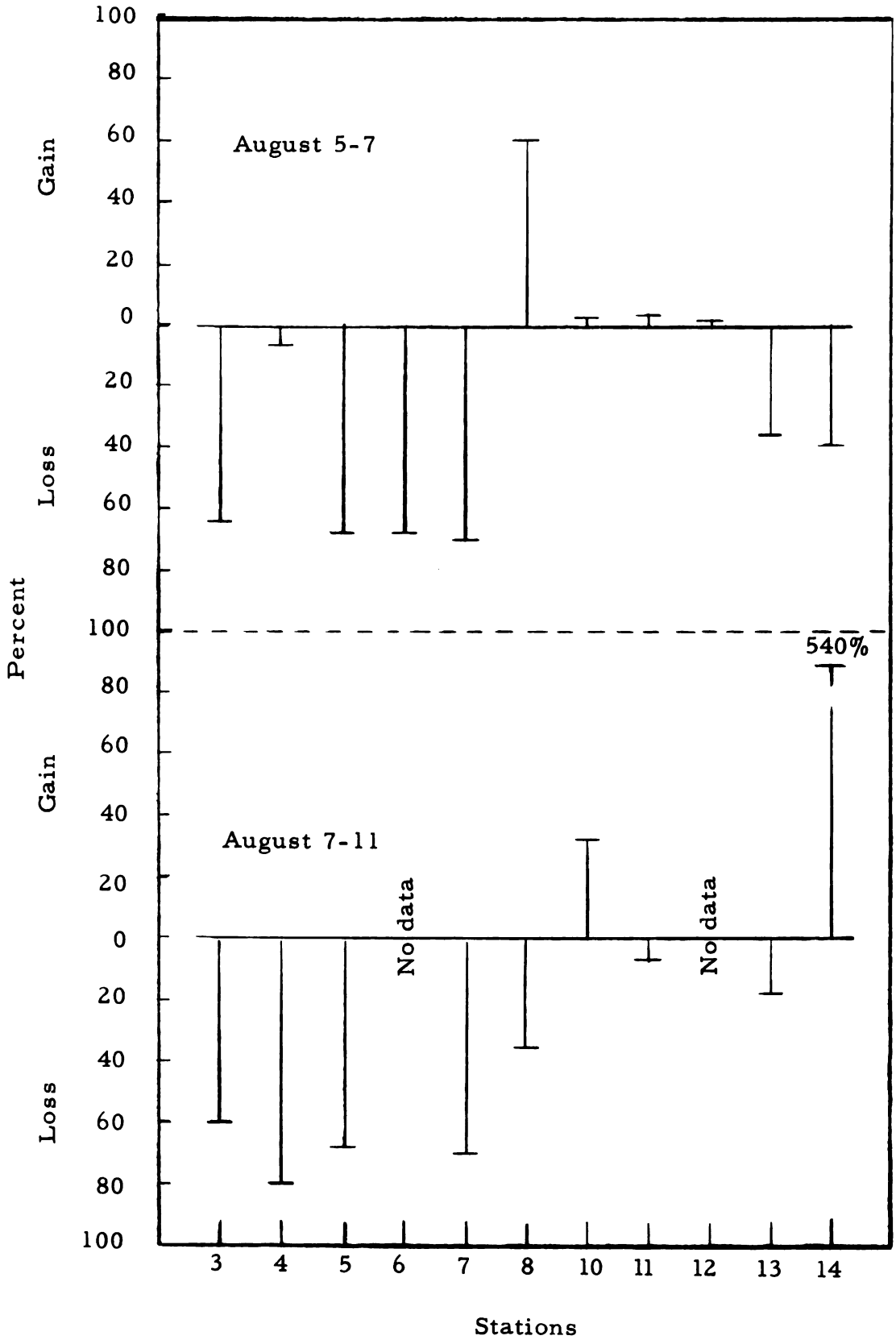
weight basis, is equal to the amount accumulated for each of the other shingles for that particular station and date. Knight (op. cit.) determined that the variation of activity within stations is not extensive for periphyton.

Whittaker (op. cit.) found that in his aquarium experiment periphyton did not show a loss of P32 until after the second week. His data were drawn from a periphyton complex consisting mainly of filamentous algae and a system offering continual exposure of the algae to the free P32. The mean value of P32 in periphyton of the entire experimental area of the West Branch declined very rapidly for the first seven days after the isotope release (Figure XI). This would be expected since this is a uni-directional system and even with a rapid re-utilization of the regenerated and exchanged P32 the free marked atoms and Potamoplankton particles are continually and permanently moving out of the system. Because of this early and rapid loss of P32 from periphyton, it is unlikely that at any time a degree of equilibrium existed, where the uptake and loss of P32 would be constant and the resultant accumulation would be maximum and also constant. Davis and his associates (op. cit.) found such a state existing in the Columbia River below the Hanford Works where the organisms are subject to continual or chronic exposure from radiomaterials. In systems such as these, concentration values can be calculated with a great deal of certainty. Krumholz (1954)

in White Oak Lake, Tennessee, found that the concentration factor for phosphorus in Spirogyra sp. to be approximately 850,000 times the normal amount.

Although there is a rapid loss of P32 from the periphyton of the experimental area as a whole, there is a somewhat different picture when individual stations are compared. Figure X shows the activity of periphyton for the stations sampled throughout the entire program. Indications are that stations in the lower half of the area gain considerable P32 from the upper area. When this is figured on a percentage basis, Figure XII indicates a large loss by the third day from the upper stations, with the exception of station 4, a slight gain for the middle stations and a loss for the last two stations. Between the third and seventh day of the experiment, station 10 continued to gain P32 while all other stations, with the exception of 14, lost P32. From this graph it can ge seen that station 14 showed the greatest loss (third day) and also the greatest gain (seventh day). It should be expected that much of the flucuation in the gain or loss of P32 for the various stations is due to variability inherent in the techniques of measurements and the method used for comparisons. Still, enough of a pattern persists to warrant further investigation as to the movement of P32 through the system. A prime possibility is that the isotope, once released into the system and taken up by the periphyton, is continually and constantly being lost to periphyton

FIGURE XII. --Percent gain or loss of P32 activity in periphyton from August 5, 1958 to August 7, 1958; and from August 7, 1958 to August 11, 1958.


Percent
Gain
Loss
100
80
60
40
20
0
20
40
60
80
100
August 5-7
Gain
Loss
100
80
60
40
20
0
20
40
60
80
100
540%
August 7-11
No data
No data
3
4
5
6
7
8
10
11
12
13
14
Stations

further downstream, with of course some being removed to a higher tropic level. If this is true, it would be unlikely that any station would show an appreciable gain of radiophosphorus after the initial pulse of P32. The quantity of P32 that is continually and constantly being lost from the periphyton in the upper reaches of the experimental area would never be available to the periphyton at the stations in the lower part of the area in amounts great enough for these stations to show a gain of P32. The periphyton in the lower area would also be continually and constantly losing the P32 initially incorporated into it. Another possibility is that the P32 moves through the system in more or less secondary pulse-like manners. The possibilities of secondary pulses are complicated not only by biological factors that contribute to the disappearance of P32 from the organisms involved, but also by physical factors of the system itself. Precipitation of P32 and sorption by silt particles will account for some loss of P32 (Foster, 1956). Hutchinson (1957) found that different algae varied in efficiency to utilize phosphorus. In a uniform periphyton complex, such as is found on the substrates in the West Branch, such a factor would have minimal affect on complicating a pulse like movement.

Table 13 gives some indication that the P32 does move through the area in a manner that is not indicative of a constant and continued loss of the isotope from the system. During the study in the West Branch, the upper seven stations, which encompass the first

TABLE 13. --Percent gain or loss of P32 activity in periphyton of the West Branch of the Sturgeon River from August 5, 1958 to August 7, 1958; and from August 7, 1958 to August 11, 1958.

Station	August 5 to August 7		August 7 to August 11	
	Percent		Percent	
	gain	loss	gain	loss
3		63		60
4		6		81
5		67		69
6		68	-	-
7		72		71
8	60			38
10	2		29	
11	3			9
12	1		-	-
13		35		18
14		39	540	

850 yards of the experimental area, did not show a gain of P32 after the initial pulse of the radioactive phosphorus (Figure XII). That is to say that stations 1-7 did not show an increase of P32 in relation to the time the substrates were removed from the river, it possibly being that the time of sampling was spaced at intervals too far apart to indicate if a gain of P32 occurred at any of these stations. The failure to capture an increase in P32 at these upper stations, in relation to the time samples were taken, would be expected if the turnover time for the periphyton is rapid. A rapid turnover of phosphorus by plankton, including diatoms, was substantiated by Rigler (op. cit.) Goldberg et al. (op. cit.), and Rice (1953).

Because some of the downstream stations did show a gain of P32 in the periphyton after the initial pulse, it does not necessarily indicate that secondary pulses of P32 occur in the system. Three sets of factors may be operating to determine the level of P32 in the periphyton.

1. Pure exchange in a kinetic sense. This could bring about a rapid loss of marked atoms when a considerable amount of marked atoms are present in the cells of the periphyton. Hence the loss would be greatest at the upstream stations which have the initial largest uptake. The downstream stations would not lose P32 as fast because the P32 level in water is higher due to drift etc. and because these

stations have a smaller initial proportion of marked to unmarked atoms.

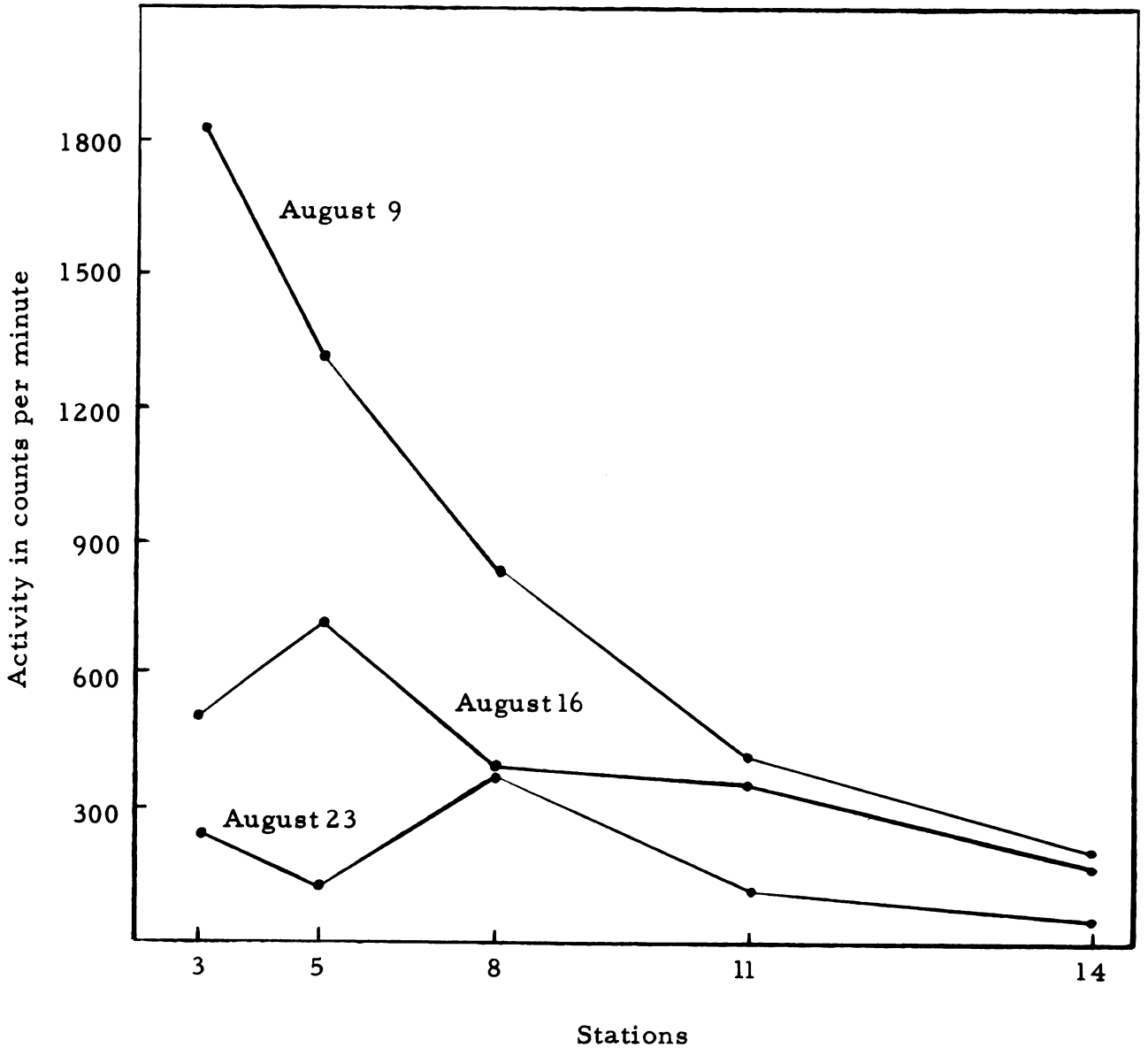
2. Growth. Uptake rate of cells will vary with the physiological state of the community. Some of the colonies will be more active in accumulating phosphorus than others. In this respect the age of the community on the substrate must be considered.

3. Quantity of phosphorus in the cells. Starved cells will have a high P32 to total phosphorus ratio while cells subjected to fertilizer will have a low P32 to total phosphorus ratio. If the ratio of P32 to total phosphorus is low, it will take longer to get rid of the marked atoms. Since fertilizer was added to the experimental area prior to the isotope release, this factor will be important in this system.

Accumulation of P32 in Rock Periphyton

P32 accumulation in periphyton collected on rocks followed a pattern similar to that of the artificial substrates. On the fifth day of the experiment, August 9, 1958, P32 values were greatest at station 3 and declined progressively at the downstream stations (Figure XIII). By the twelfth day of the experiment, August 16, the P32 in rock periphyton was quite evenly distributed throughout the

FIGURE XIII. --Summary of activity of P32 in rock periphyton in the experimental area of the West Branch of the Sturgeon River for the summer of 1958.


1800
1500
1200
900
600
300
Activity in counts per minute
August 9
August 16
August 23
3
5
8
11
14
Stations

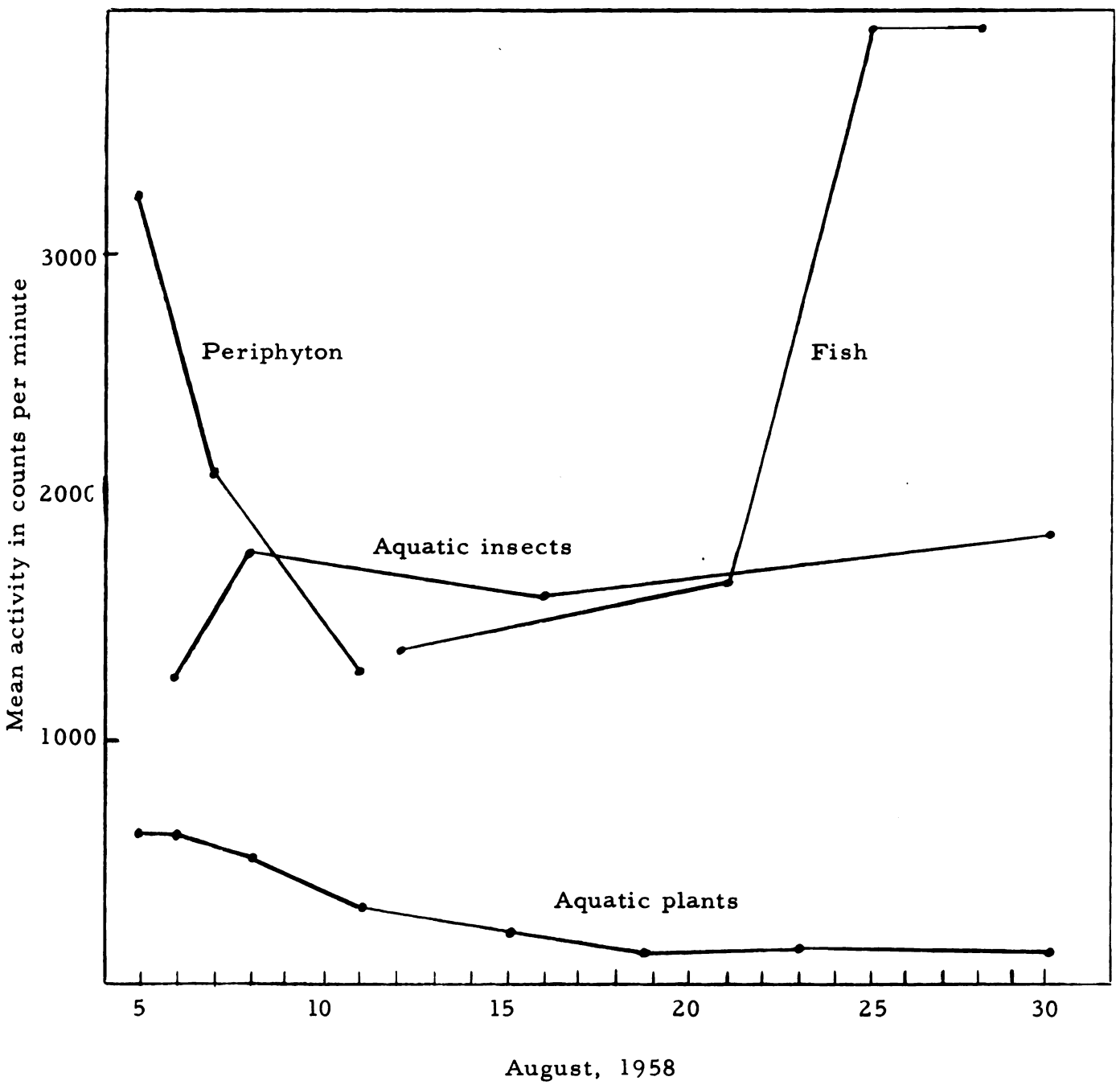
entire experimental area. Because of the minute amount of marl and rock particles that could not be separated from the periphyton at the time of preparation, the rock periphyton never obtained as high specific activity as found on the artificial substrates. It is also believed that some of the P32 measured as activity of rock periphyton may in reality be P32 that has been encorporated into the marl and consequently lost permantly from the system.

Position of Periphyton in Relation to Phosphorus

Since P32 is biologically the same as its stable counterpart P31, its movement through the system will also indicate the movement of naturally occurring phosphorus.

Figure XIV indicates to some degree the position the periphyton complex holds in relation to phosphorus entering the system. Because of the relatively large total biomass and the high surface to volume ratio possessed by the periphyton, it appears to be initially responsible for holding the majority of the phosphorus entering the system. The other producer organisms (aquatic plants including Chara sp.) do not appear to be initially responsible in retaining much of the phosphorus entering the system. Hutchinson (op. cit.) also found this phenomenon in the epilimnion of a small eutropic lake. He hypothesized that it is apparently the diatoms and other algae of

FIGURE XIV. --Mean activity of radiophosphorus for the various biological levels sampled in the experimental area of the West Branch of the Sturgeon River during August, 1958.


Mean activity in counts per minute
3000
2000
1000
Periphyton
Fish
Aquatic insects
Aquatic plants
5
10
15
20
25
30
August, 1958

short life spans that make up the great reservoir of littoral phosphorus. Knight (op. cit.) reports filamentous algae to be intermediate between the aquatic plants and the periphyton in regards to initial uptake of P32 in the West Branch. Although the initial uptake of phosphorus by periphyton is high, it also loses phosphorus much faster than the other biological levels sampled (see Figure XIV). The majority of the phosphorus concentrated by the primary consumers must come directly from that initially retained by the periphyton. The rapid loss of phosphorus from periphyton indicates that the permanent pool of phosphorus is small in periphyton in relation to aquatic insects and fish.

SUMMARY

1. The periphyton sampled on artificial substrates in the West Branch of the Sturgeon River was quite uniform in community composition. Diatoms made up the great majority of the algae on the substrates. A single species, Synedra ulna, accounted for the greater part of the diatom population on all artificial substrates.

2. No statistically significant increase in the standing crop of periphyton could be detected after the first application of eighty pounds of fertilizer to the West Branch of the Sturgeon River. The substrates were in the river a period of seven days.

3. Data available gave an indication of increase of periphyton after the second application of fertilizer. During this fertilization period, twice as much fertilizer was applied and the substrates were collected at the end of a fourteen day period.

4. A significant increase of total phosphorus in the periphyton mass was detected after the first application of fertilizer.

5. There are indications that rapid currents had a negative influence on the standing crop of periphyton on the plexiglass substrates.

6. The initial uptake of P32 by periphyton declined progressively downstream from the point of application.

7. The amount of P32 accumulated by periphyton three days after the isotope release into the area gave indications of decreasing progressively downstream from the point of addition. Seven days after the isotope was released into the system, the P32 was quite evenly distributed in the periphyton throughout the experimental area.

8. Data from substrates placed in the river after the addition of the isotope indicate that exchanged and/or regenerated P32 occurred in considerable amounts.

9. The re-utilization of phosphorus in systems such as the West Branch depend on the retention time of phosphorus in the previous organism and the age of the community on the substrates sampled.

10. Loss of P32 from periphyton was rapid for the first seven days after the release of the isotope.

11. During the study, the first seven stations below the point of isotope addition did not show a gain of radiophosphorus after the initial pulse of P32. Seven days after the isotope addition, all of the seven lower stations, with the exception of station 13, had gained P32 from that measured four hours after the addition of the tracer.

12. Accumulation of radiophosphorus in rock periphyton followed a pattern similar to that accumulated on artificial substrates. Activity measurements of rock periphyton did not attain those of the artificial substrates.

13. The periphyton complex in the West Branch of the Sturgeon River appears to be more responsible initially for removing the P32 from the water mass than the aquatic plants and larger filamentous algae.

14. The permanent reservoir of phosphorus in the periphyton is smaller in relation to large consumer organisms.

APPENDIX

Summary of the computations performed in determining corrected counts per minute for the periphyton at each station during the summer of 1958.

TABLE 14. --Summary of weights, counts, and computation data per station (artificial substrates) of P32 in periphyton, August 5, and August 7, 1958.

Date (1958)	Decay factor	Back-ground	Sta-tion	Raw count	Wet weight (grams)	Corrected CPM/gm.	Corrected CPM/gm. plus decay
August 5	.9000	46	1	44	.025	-	-
			2	241	.125	1560	1733
			3	2342	.320	7175	7972
			4	1056	.200	5050	5611
			5	545	.130	3829	4266
			6	122	.020	3800	4222
			7	1285	.325	3812	4236
			8	766	.360	2000	2222
			9	453	.305	1334	1482
			10	326	.210	1333	1481
			11	463	.125	3336	3707
			12	365	.255	1251	1390
			13	1049	.645	1555	1728
			14	664	.355	1741	1934
August 7	.8200	46	1	50	.184	-	-
			2	502	.260	1754	2139
			3	702	.275	2386	2910
			4	589	.125	4344	5298
			5	549	.430	1170	1427
			6	377	.295	1122	1368
			7	697	.660	986	1202
			8	367	.110	2918	3559
			10	379	.270	1233	1504
			11	439	.125	3144	3834
			12	280	.205	1142	1393
			13	184	.152	908	1107
			14	104	.390	149	182

TABLE 15. --Summary of weights, counts and computation data per station (artificial substrates) of P32 in periphyton, August 11, to August 30, 1958

Date (1958)	Decay factor	Back-ground	Sta-tion	Raw count	Wet weight (grams)	Corrected CPM/gm.	Corrected CPM/gm. plus decay
August 11	.5800	85	1	79	.063	-	-
			2	234	.118	1263	2178
			3	263	.263	677	1167
			4	268	.318	575	991
			5	179	.368	255	440
			6	-	-	-	-
			7	299	1.050	203	350
			8	174	.070	1271	2191
			10	209	.110	1127	1943
			11	232	.170	865	1491
			12	-	-	-	-
			13	180	.180	528	910
			14	166	.119	681	1174
August 17	.5000	52	1	61	.010	-	-
			2	112	.170	353	706
			3	217	.220	750	1500
			5	211	.117	1359	2718
			8*	135	.535	155	310
			11*	129	.250	308	616
			14	159	.115	930	1860
August 23	.3800	54	1	59	.095	-	-
			3*	80	.240	108	284
			5*	119	.653	100	263
			8*	82	.165	170	447
			11*	89	.286	122	321
			14	80	.250	104	274
August 30	.2800	52	1*	55	.076	-	-
			3*	58	.080	75	268
			5*	69	.167	102	364
			8*	83	.300	103	368
			11*	73	.210	100	357
			14*	68	.092	174	621

*Denotes shingles introduced to experimental area after release of radioactive phosphorus.

TABLE 16. --Summary of weights, counts, and computation data per station (rocks) of P32 in periphyton, August 9, 1958 to August 23, 1958.

Date (1958)	Decay factor	Back-ground	Sta-tion	Raw count	Wet weight (grams)	Corrected CPM/gm.	Corrected CPM/ gm. plus decay
August 9	.7800	47	1	51	.195	-	-
			3	161	.079	1443	1850
			5	221	.167	1042	1336
			8	205	.245	645	827
			11	140	.290	320	412
			14	76	.228	127	163
August 16	.5000	52	1	45	.150	-	-
			3	154	.395	258	516
			5	156	.300	347	694
			8	154	.370	276	552
			11	114	.315	197	394
			14	99	.440	107	214
August 23	.3700	54	1	55	.290	-	-
			3	72	.200	90	243
			5	72	.360	50	135
			8	105	.360	142	383
			11	67	.290	45	122
			14	65	.600	18	49

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