THE TRANSFER OF RADIOPHOSPHORUS WITHIN A STREAM AND ITS RELATIONSHIP TO LIGHT INTENSITY AND INSECT MIGRATION

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ABSTRACT

THE TRANSFER OF RADIOPHOSPHORUS WITHIN A STREAM AND ITS RELATIONSHIP TO LIGHT INTENSITY AND INSECT MIGRATION

by Thomas A. Wojtalik

The present study was conducted to determine the agencies of transfer of phosphorus in a stream ecosystem. On July 10, 1962 twenty-three millicuries of P^{32} were added to the West Branch of the Sturgeon River. Samples of the water mass were fractionated and eight fractions were analyzed for amounts of radioactivity present in the water passing given points in the study area. By comparison of the amounts of tracer present in particular fractions at the different points, loss from the water mass, uptake by the different fractions, change in mode of transfer and possible agents of that change were calculated. In earlier work on this stream, stream organisms had been investigated with reference to their specific concentration of stable and radioactive phosphorus, but not with reference to the possible modes of transfer of the element other than whole organism ingestion by other organisms. Transfer modes were investigated indirectly by analyzing the water fractions for soluble organic materials and insoluble organic materials.

A study of the movement of aquatic insects showed that an appreciable amount of radiophosphorus moved upstream through the migration of both immature forms and the flights of adult insects.

Thomas A. Wojtalik

Light measuring and integrating instruments were developed and the relationships of light energy reaching the stream to production of periphyton and uptake of P^{32} in a light area, a dark area, and an intermittently lighted area was studied.

Robert C Ball

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By

Thomas A. Wojtalik

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INTRODUCTION

In many aquatic ecosystems phosphorus has been found to be a consistently limiting factor to the productivity. Its limited availability was evident at all trophic levels in the West Branch of the Sturgeon River and it appeared to be especially limiting at the primary level since all phosphorus fertilization experiments indicated a rapid uptake and subsequent bloom of periphyton near the source of fertilizer introduction which then gradually decreased with time.

In the fertilization studies conducted on the West Branch of the Sturgeon River a series of phosphorus additions were conducted over a period of more than five years. These additions were at its source, Hoffman Lake; at the lake outlet, and at several points approximately five to eight miles downstream. Each study indicated phosphorus uptake was rapid in the immediate area of introduction, but little uptake in any of the trophic levels could be found in downstream areas. Therefore in 1958, additions of tracer phosphorus were made using the radioactive material P^{32}. It was intended that pathways of phosphorus transfer be followed from the inorganic form introduced, through the primary producers to the primary consumers, secondary consumers and all other levels up to and including fish which were considered the terminus for the ecosystem. A secondary consideration of the fish terminus was from a public health view of contamination due to fallout over watersheds which furnish both food and water to humans.

The West Branch of the Sturgeon River is a representative of the small, cold-water trout streams which formerly were typical of Michigan. Its source is a marl lake, but most of its summer flow of approximately 49.7 cubic feet per second at nine miles from its source, is supplied by ground water fed out of underground springs present in the surrounding forest covered moraines. Its morphology is one of nearly equal proportions of riffles and pools or runs which are underlain by a marly gravel interspersed with larger marl concretions. The average width is 28.3 feet and the average depth is 1.4 feet for the section used during the present study. From its source to the lower end of the study area, a section approximately nine miles long, it winds through heavy cedar swamps and densely forested valleys.

Its summer steady state of phosphorus concentration was approximately 7 p.p.b. with spring peaks of up to 20 p.p.b. (Correll, 1958) during the high runoff period. Oxygen content was always high due to the large amount of oxygenation by the large number of riffles which keep the stream at least in equilibrium with the atmosphere. Alkalinity was exclusively of the methyl orange variety and maintained itself at 180 p.p.m. Turbidity and color were low during most of the three months of the study, only immediately after heavy rains did the turbidity increase appreciably. Large amounts of organic detritus were moved downstream by continuous saltation and sliding of the fragments over the bottom substrates.

In several of the years of the fertilization study the pulse of periphyton close to the source of introduction indicated a rapid uptake of phosphorus which created a large exchange pool in that area alone. Therefore in the radioactivity studies of 1958, 1959, and 1960

pre-tracer additions of two to three times the normal phosphorus load were made to increase the exchange pools. During the 1961 and present study no pre-tracer additions of any fertilizer were made. Each of the additions of tracer was used to further our knowledge of phosphorus pathways in a lotic ecosystem, trophic levels were indicated (of which some had long been established by other means of study and which were only confirmed with the tracer study), new ones were established and food webs were constructed. Recycling was indicated as was a reserve phosphorus pool in the primary producers (periphyton in particular) which maintained ratios of nitrogen to phosphorus as high as 10:1 when it was commonly believed this ratio was 5:1 (Correll, 1958). Diatoms were believed to be the major primary producers during the early studies because filter feeders like Simulium sp. were always found to be filled with them. The bacterial contribution to a food chain was hard to evaluate and misleading when only stomach analyses were run. Therefore to definitely isolate the bacterial contribution of phosphorus to filter feeders and other parts of the food chain or web the 1961 study (Bender, 1962) was conducted using Escherichia coli 0111 as the organic bacterial carrier of P^{32}. The results were negative and helped confirm a definite diatom base for the food web of the stream and the pyramid of numbers built thereupon. The 1961 study also raised a question concerning the food of the fishfly larvae, Chauloides sp., since they accumulated from 10 to 100 times the activity recorded in earlier studies when the tracer introductions were of an inorganic nature. Their head capsules and mouthparts are definitely not suited to filter, scrape, or catch bacteria.

Each of the several inorganic introductions of P^{32} and the single

incorporated organic tracer addition had told much about the food chains and stream food web, but none defined exact proportions contributed by different agents that were probably present in the water. Nor did they indicate modes or amounts of change between sampling areas or with increased distance for organic fractions. Therefore, the present study was initiated to do much of this type of isolation of fractions. Water was fractionated into diatom; bacteria; adsorbed; soluble organic; non-water soluble, non-ether soluble; non-water, non-ether, non-acid soluble organic; and small "particulate" fractions. Amounts of P^{32} were determined in terms of the radioactivity passing a given point while in a given fraction. Changes were followed as well as sources of possible contributions to the total organic fractions, such as plankton contributions and periphyton, plants, insects and fish. Modes of uptake among the sediments were investigated as were areas of deposition of the organic materials and carriers. Movement of the isotope due to insect activity was investigated using drift organisms and actively migrating adult and immature insects in areas above the point of isotope introduction. Movements due to fish activity as well as their possible physiological activity were investigated from the same areas above the point of tracer addition.

A secondary consideration concerned the pools of exchangeable phosphorus present and the amount by which they could be altered by fertilization methods especially designed for a given stream. Dry weight and phosphorus conversion factors were determined to milligrams per individual in previous years, especially by Zettelmaier (1961).

Productivity figures were evaluated for a lighted, open area of stream and a densely shaded tunnel using submersed light meter units

in both areas of similar depth and nearly similar current. The light-dark study was conducted with respect to possible use as a management tool along non-productive, shaded streams.

Introduction to the Stream and Study Area

The West Branch of the Sturgeon River originates from Hoffman Lake, a 128 acre marl lake located in Charlevoix County, Michigan. From its origin, the general pattern of flow is in and out among wooded moraines that create a northeasterly directed valley. Its entire length from source to confluence with the Sturgeon River south of Wolverine, Michigan is 14 miles. This study was conducted upon a 3230 yard section approximately five miles above its terminus.

During the course of its flow the West Branch of the Sturgeon River passes through Cheboygan, Otsego, and Charlevoix Counties, each of which is heavily wooded, glacial country where the river flows. The vegetation about the stream is chiefly of birch, aspen, alder, and balsam fir on the higher moraines. At streamside it is chiefly cedar, tamarack, aspen, alder, and ninebark.

The river is one of the few cold-water trout streams left within the state, its temperature varies between 52° F. and 58° F. during the summer low water months (Clifford, 1959). It remains open throughout the winter and has a flow little increased from that of the summer lows. The majority of summer flow is from underground water supplied from surrounding pockets and springs part way up the side of gravelly moraines.

The actual study area was located in T. 33 N, R. 3 W. Its average width was 28.3 feet and its average depth was 1.4 feet. The gradient

was sufficient for a morphology of half riffles and half pools or runs. The bottom varies in composition from sand and a marly gravel to silt and detritus. The vast majority was of marly gravel and large marl concretions.

General stream conditions were as follows: the stream flow had a mean flow of 43.75 cubic feet per second (Knight, 1961), it entered the area at 38.75 cubic feet per second and left Station 14 at 49.37 cubic feet per second. The gain in flow was from two small tributary creeks and 14 small springs along with underwater contributions of ground water. Rains rarely affect the summer level, its color or turbidity. The alkalinity was normally 180 p.p.m. of methyl orange, and carbonates precipitate in concretion rings of about 3/8 of an inch per year. The total phosphorus concentration during the summer months was approximately 7 parts per billion. A pH of approximately 7.9-8.3 was present during the summer. A high dissolved oxygen content existed due to the riffles and excellent diffusion. Aquatic organisms were abundant, especially aerophilic groups.

The chief aquatic vegetation present was in dense beds, of such species as Chara sp.; the water moss, Fontinalis antipyretica; and water cress, Nasturium officinale; with scattered clumps of Ranunculus sp.; and a Potamogeton sp. The aquatic plants were abundant in most of the stream, an exception was a stretch from 300 yards to 600 yards below the point of isotope introduction. It was an area of nearly complete canopy which passed less than 500 foot-candles of sunlight during the day except for scattered light flecks.

The trophic structure was topped by three salmonids: the brown trout, Salmo trutta fario; the rainbow, Salmo gairdnerii; and the brook

trout, Salvelinus fontinalis in their respective order of abundance. An end in itself as well as a part of the structure, was the sculpin group composed of two members: the eastern slimy sculpin, Cottus cognathus; and the northern mottled sculpin, Cottus bairdii.

Most of the typical trout stream insects were present, i.e. members of the Diptera, Ephemeroptera, Megaloptera, Neuroptera, Plecoptera, and Tricoptera. Species represented were ones common to clean, cold water that was swift and well oxygenated. Coleoptera, Hemiptera, and Odonata were not represented in large numbers. Oligochaetes and Hydracarina were also not abundant. Gammarus was represented only at the lowermost station (14), and from there downstream. No crayfish have ever been found by the author or his associates.

A table of area per station, flow, and depth follows:

Station	Total Area	Average Depth	Flow
3	17430 ft.^{2}	12.8 ins.	38.74 cfs.
5	21000 ft.^{2}	13.5 ins.	40.15 cfs.
8	37924 ft.^{2}	17.2 ins.	43.50 cfs.
12	134245 ft.^{2}	14.3 ins.	45.56 cfs.
14	52992 ft.^{2}	13.3 ins.	49.75 cfs.

Sampling Stations

Typical trout streams provide a variety of habitats that must be sampled in their entirety to give a valid representation to an ecosystem nutrient study. In other years, 16 stations had been selected with reference to flow, bottom type, vegetation, and shade. Of these, five were used in 1962 along with six new ones established for particular

phases of study. The old stations used were 3, 5, 8, 12, and 14. The new stations were five for migration studies, at 100 yard intervals beginning at the point of isotope introduction and proceeding upstream, and a new shade area 130 yards below Station 3 and its opposite light area 200 yards above Station 8 (Figure 1).

Samples of water, periphyton, plants, insects and fish were taken at Stations 3, 5, 8, 12, and 14. Collections of insects and fish were made at 100, 200, 300, 400, and 500 yards above the transect of isotope introduction. Light readings and periphyton substrates were collected in both the shaded area and the light area. Area descriptions of the sampling stations are as follows:

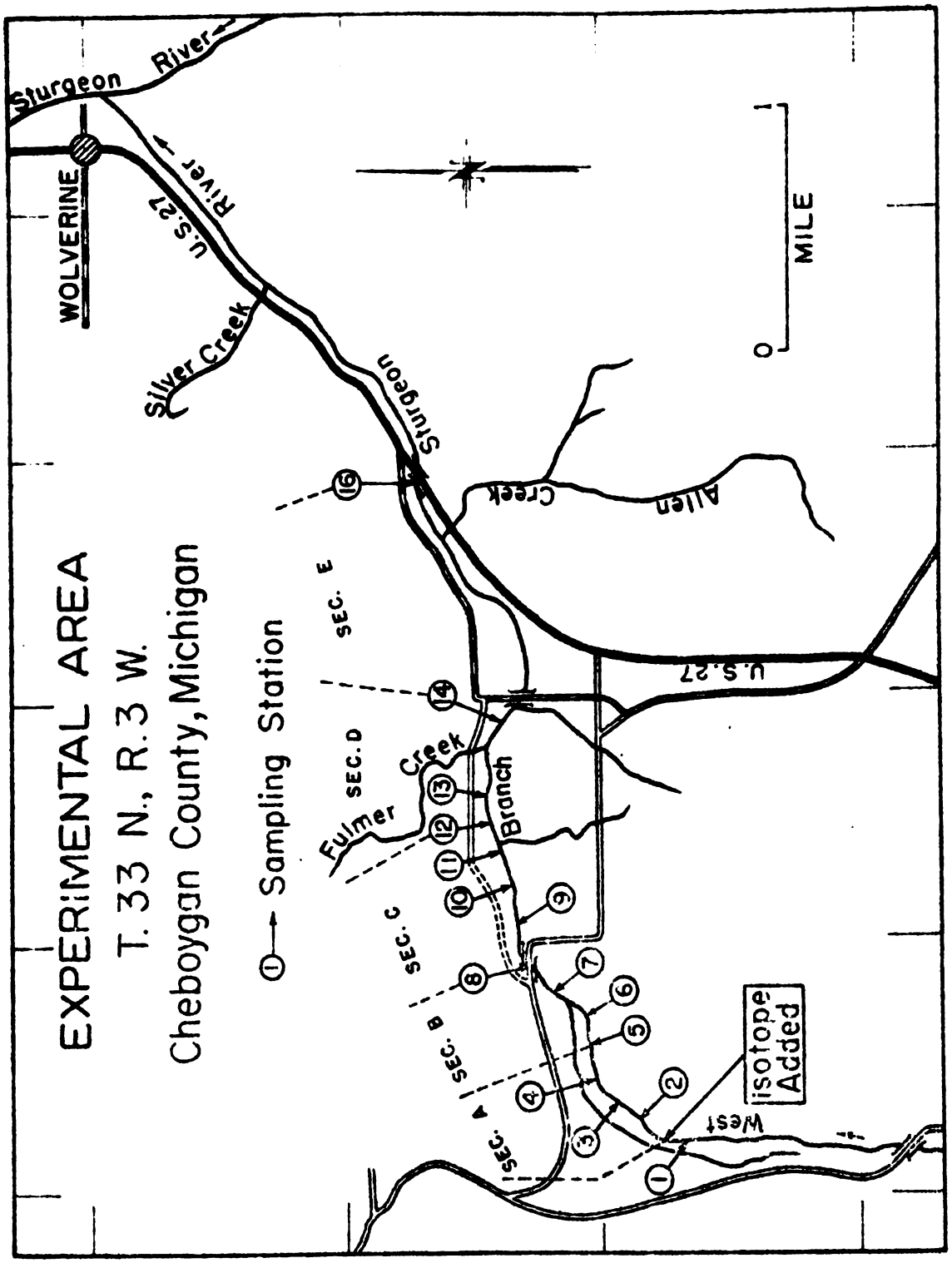
Station 3

A site located 240 yards downstream from the point of isotope introduction. The immediate sides were well shaded, while the upstream section down to the sampling point was open to the sun. All the section was log strewn. The logs were covered with mats of Fontinalis which grew out of the crevices and marl concretions on the logs. Scattered areas of plants consisted mostly of Chara sp. were present, as were small amounts of Ranunculus sp. and a little greater amount of Nasturium officinale and Potamogeton sp. Flow was about 38.74 cfs and the average depth was 12.8 inches (Zettelmaier, 1961). The shallow, rapid flow helped concentrate the invertebrates in backwaters and on projecting log surfaces depending upon their requirements.

Station A--Shaded Area

A 200 foot section of complete or nearly complete canopy over a bare marl gravel with no aquatic vegetation other than periphyton. Its

Fig. 1.--Map of the West Branch of the Sturgeon River with sampling stations and stream study areas designated.


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

maximum level of sunlight was only 500 foot-candles with a few sun-dapple flecks of higher intensity which shifted with every breeze. The average depth was 13.5 inches and the flow was approximately 39.25 cubic feet per second. Lack of aquatic vegetation and logs left only the bare gravel substrate for invertebrate habitation. Only the tricopteran, Glossoma sp. with its heavy stone case was abundant (500 per square foot) on the surface.

Station 5

Was situated at a point 540 yards downstream from the addition point. It was the termination point of nearly complete canopy in the upper reaches of the study area. Flow was approximately 40 cubic feet per second. Average depth was 13.5 inches.

Light Area--Station B

Was situated at 840 yards, it was 200 feet long and had an average depth of 14.8 inches. Logs and vegetation studded one side of the strip. The middle and opposite side were bare gravel. There was no brush closer than 10 feet from the water edge and no trees for approximately 125 feet on either side.

Station 8

Was located in an open portion of the stream 1,040 yards below the point of addition. Flow was through a nearly straight run. The near laminar flow was approximately 43.5 cfs. Vegetation consisted mainly of Chara sp.; Fontinalis antipyretica; Potamogeton sp.; and Ranunculus sp. Mean water depth was 17.2 inches (Zettelmaier, 1961).

Station 12

Was a site located on an open sweeping "S" bend that was much deeper on the outside of the curves than on the inside. Logs were abundant in the area as were large, dense beds of all the plant species studied. Flow was about 45.56 cfs. and the average depth was 14.3 inches. The combination of factors made it the most productive station in terms of numbers and kinds of invertebrates present.

Station 14

Was located at a point where the stream valley and bed both opened or spread out and leveled off. The resultant area was a continuous series of fast, shallow riffles with numerous interspersed rocks and little backwater or edge pools. Plants were abundant only along the sides except for the water moss, Fontinalis antipyretica which grew on the rocks situated in the fast current. Invertebrates were numerous and very dispersed. It was the only station at which amphipods were found. The only major backwater areas were immediately behind stream improvement sturctures and diverters. Flow was 49.75 cfs. and the average depth was 13.3 inches.

Migration stations above the point of isotope introduction follow:

100 Yards Above

The site was located at the upstream tip of an island which divided the stream unequally and created a deep, fast, log-studded run on one side and a slow, log-studded trickle on the opposite side. Several large logs laid parallel to the current along the deeper side. Plants were abundant along both sides and invertebrates were easily

found among them. Flow was approximately the same as that entering the area below the point of isotope introduction, i.e. 38 cfs. with an average depth of 12.0 inches.

200 Yards Above

It was a deep run located above two deep pools with little vegetation and few logs to offer sites of substrate or avenues of movement. The average depth was 19.5 inches.

300 Yards Above

It was a slanting pool bottom with bare sides and a log jam caused by the shelving bottom. The jam caused the major part of the flow to become a torrent. Average depth was 15.0 inches, but varied from 1 to 30 inches.

400 Yards Above

It was a twisting rapids jammed with logs which projected from the sides. Little vegetation and a 2/3 canopy were characteristic of the area. The average depth was 13.0 inches, over a bottom of uniform pea gravel. Several backwater areas were present behind logs lying parallel to the current.

500 Yards Above

The area was an open stretch of rapid current on one side only, the opposite was log studded. Little vegetation was present on the firm bottom. A number of insects were present that were not common in other areas. The average depth was 12.2 inches with a flow of about 33.78 cfs.

METHODS AND PROCEDURES

General Methods

The general methods used in following the nutrient through the stream ecosystem were as follows:

1. A study section of 3230 yards was selected which included representative areas of all the major types of water, bottom, organisms, and physical conditions with special areas set aside for study of unusual microhabitats, physical factors, and chemical additions or exclusions.
2. A chemical survey was made in 1958 (Correll) to determine if phosphorus and nitrogen were limiting factors. Zettelmaier (1961) added an iron chelating agent to investigate the possibility of iron being a limiting factor. Every year alkalinity, pH, hardness, carbon dioxide and phosphorus were checked. Samples from several points within the study area were taken. The quantitative results were used as the basis of comparison in the present study.
3. A physical survey of stream length, depth, width, flow, current, morphology and topography was conducted again in 1962. Most of the results compare with the results determined by similar methods of measurement done in other years. Of particular interest was the near comparable flow between the present year and the 1958 measurements of Vannote.
4. Organisms resident in the stream were identified to the most specific

group available keys would allow, under field magnifications. Some were only to family, others were to species. In former studies their specific concentration of the stable nutrient had been determined (Zettelmaier, 1961). The values of stable phosphorus per gram of dry weight of the organisms were used as the basis for expansion to biomass estimated for similar organisms. In this way the pools of phosphorus that were present in the stream were determined and the exchangeable amounts determined by tracer accumulations were to be used to estimate the contribution a specific dose of stable phosphorus fertilizer would make to the stream exchange pools. Along with estimations of the pool of stable phosphorus an attempt was made to determine the rates of uptake and turnover of the isotope, assuming a similar uptake rate and turnover exists for both stable P^{31} and radioactive P^{32}.

5. An attempt was made to measure specific area losses, by soluble forms, of the isotope and apply the values to each area's contribution of the stable form.

Special Consideration with the Isotope

1. An isotope of known activity, form, concentration, and specific activity had to be used. Being biologically active the phosphorus isotope P^{32} was ideal. It also had the advantage of direct application for measurement of a limiting factor, had a short half life, and sufficient energy to count with a reasonable counter efficiency.
2. Being a low energy beta emitter it did not furnish a health hazard to humans or aquatic organisms in the concentrations used.
3. A secondary consideration was the low purchase price for sufficient

amounts to use in a field study.

4. No attempt was made to determine different rates of uptake between the stable and radioactive forms of nutrient. The literature does not indicate any difference.
5. Suggested recycling patterns were indicated by previous studies on this stream and an attempt was made to specifically isolate them during the present study. This involved a series of fractionations and investigation of the physical factors when a graphic pattern indicated a recycling trend.
6. Uptake patterns followed in other years were further investigated and reaffirmed among the major forms of fauna and flora of the ecosystem.
7. The terminal aquatic organisms were investigated to demonstrate that they did not concentrate amounts of radioactivity that could be dangerous to humans.

Combined Study Methods

1. An addition of 23.54 millicuries of the phosphorus isotope P^{32} was made without pre-tracer additions of fertilizers.
2. A dye front was used to alert sampling crews when the tracer was introduced and sampling was to be performed. It was also used to indicate the extent of physical mixing.
3. The form introduced was inorganic $P^{32}O_4$ in dilute HCl.
4. Sampling sites were selected to cover the complete range of possible losses from the area and yet insure a good cross-section of the changes undergone by the isotope.
5. The study was long enough to indicate where the tracer was incorporated

and to what extent it occurred in each trophic level up to and including the terminal trophic level of aquatic organisms present.

The Isotope

The phosphorus isotope had been used in several studies connected with the West Branch of the Sturgeon River.

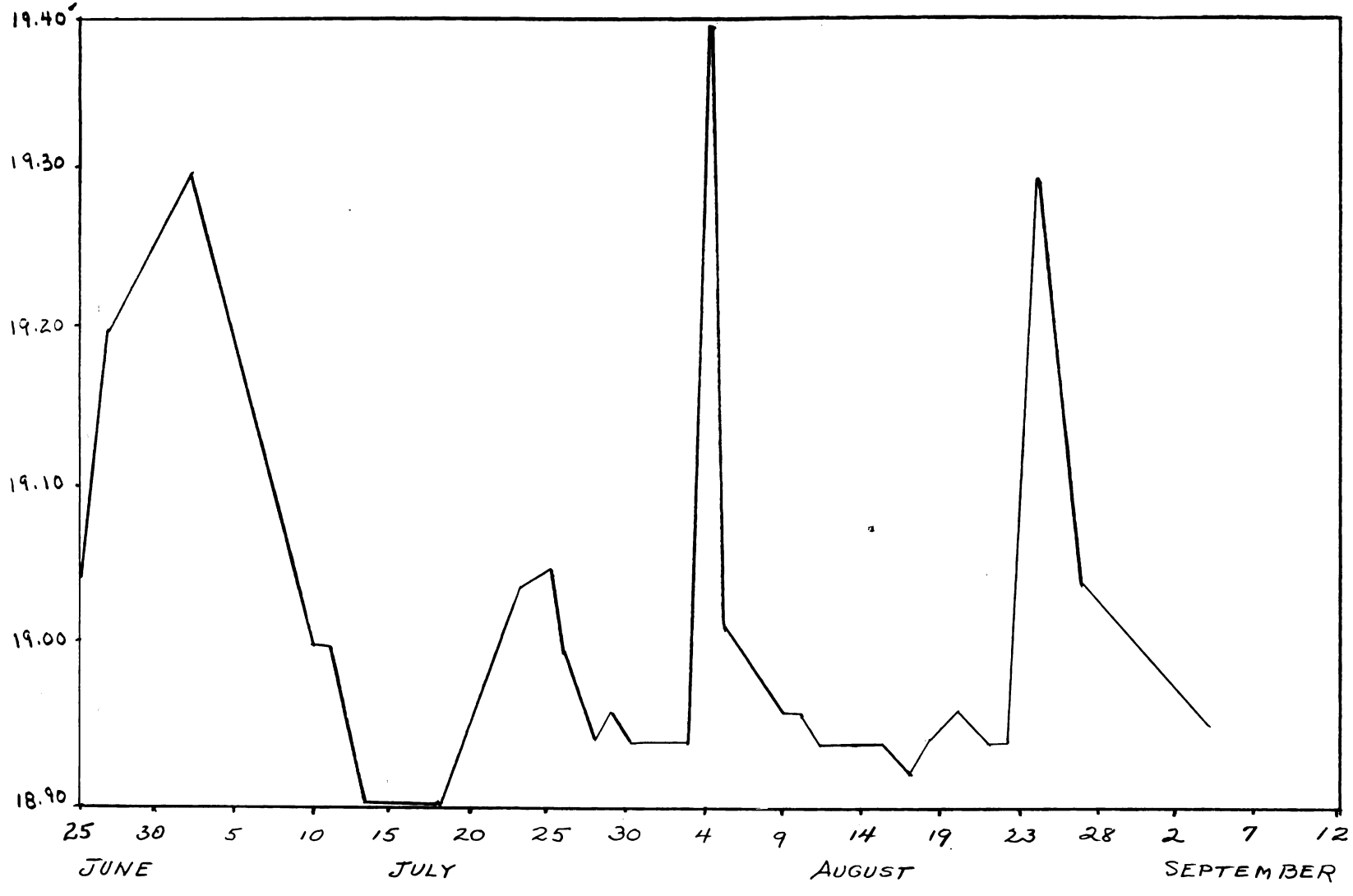
Specifically the phosphorus isotope (P^{32}) was a beta-ray emitter with a maximum energy of radiation of exactly 1.712 Mev (Chase, 1960) and has a half-life of between 14.2 and 14.3 days. The isotope was supplied with a specific analysis by the Oak Ridge National Laboratory, Oak Ridge, Tennessee as phosphate (PO_4) in 1.2 milliliters of weak HCl.

It was diluted in a fifty-five gallon drum and the dilute material was then fed into the stream where the flow was approximately 38 cubic feet per second. A boom was used to distribute the isotope across a transect of the stream (Figure 2).

Isotope Introduction

A new procedure of band introduction of the isotope was used in 1962. A submersible electric pump and copper pipe fitted with capped "T's", which was drilled to calibrate the overall system to deliver a specific amount of the dilute tracer per unit of time was used. The head was furnished by a 55 gallon oil drum suspended over the stream on a fallen tamarack tree. Polyethylene tubing connected the pump in the drum to the boom. The pump furnished a constant pressure to all areas of the copper pipe during the delivery. Previous to assembling the system on the stream it was calibrated at the laboratory and set to deliver the 55 gallons over a period of 31 minutes elapsed time.

Fig. 2.--Stage readings of the stream gauge by date. Taken as relative values from a stream gauge graduated in hundredths of a foot at Station 8.


19.40
19.30
19.20
19.10
19.00
18.90
25
30
5
10
15
20
25
30
4
9
14
19
23
28
2
7
12
JUNE
JULY
AUGUST
SEPTEMBER

The boom spread the isotope through 10 nozzles spaced at regular intervals along the 22 feet of pipe to effect a band spread of the dilute tracer. The band spread into a sheet of radioactivity between 25 and 30 feet wide within the first 25 to 50 feet of stream, by mixing of the adjacent bands. The flow pattern for 1962 is shown in Figure 2.

Experiments with fluorescein dye showed good physical distribution and showed no effective backwash or eddying in the immediate area of introduction. In previous years the introduction had been essentially from a point source, a single siphon in midstream. Our curtain type was to insure better mixing in the immediate downstream areas and to insure a more strict delineation of radioactivity free water for our migration studies.

Measurement of Radioactivity

An Omni/Guard Low Background Counting system was used that was an improved beta counting machine with ultra low background (less than 1 count per minute) and high counting efficiency (48 per cent for P^{32}). The machine was operated automatically in conjunction with a SC-100 Multi/Matic sample changer and an SC-87A Auto/Printer and SC-88 Auto/Computer all built by Tracerlab of Waltham, Massachusetts.

The detector used in this system employs a guard counter which envelops the sample counter except for the window area. The cavities of the two counters use the same flow of geiger gas. An ultra thin window, approximately 150 micrograms/cm^2, permits the counting of low energy emissions. The efficiency of the thin window counter approaches that obtained with a windowless counter.

An anti-coincidence counting circuit and complete shielding with

four inches of alloyed lead further insure low backgrounds and high counter efficiency.

Calculation of Radioactivity

Raw counts from the counter were registered in terms of counts per minute. These values must be corrected for background levels of cosmic radiation, differences in sample size, backscatter, self-adsorption and decay. A description of each of these factors follows:

Correction Factors

Self-Adsorption

Self-adsorption is an interference which causes a lower observed count due to the residue adsorption of some of the particles, especially low energy levels.

Back Scatter

Back scatter is the interference which causes an increase in observed counts due to the deflection of radiation by the sample support or shielding.

Decay Factor

Decay factor is due to the decrease with time of the number of radioactive atoms in a sample. Counts must be corrected to time zero for decay. The table given by Kinsman (1957) was utilized to arrive at the correction factor.

Background

Background radiation is caused by natural cosmic radiation,

and/or fallout of radioactive substances in or near the counter. The determination of background was carried out daily by operating the counter for 30 minutes with muffled planchets in the planchet holders. The Background observed on each day never was out of the 95 per cent confidence range, therefore each could be subtracted from the readings made that day or even any made that week.

Volume Factor

Due to the difference in sample size of the various materials collected, all counts were corrected to counts per minute per gram or milliliter. During the study period, counts were corrected as indicated below:

$$\text{corrected value} = (\text{cpm} - \text{bkg}) \times (\text{Vf}) \times (\text{Df})$$

where:

bkg = background

Vf = volume factor

Df = decay factor

cpm = counts/minute

Correction of counts per minute to millicuries and microcuries can be derived from the following factors given by Robeck, et al. (1954).

1 curie (c)	$= 3.7 \times 10^{10}$ disintergrations per second (dps)
1 microcurie (uc)	$= 3.7 \times 10^{4}$ dps
	$= 2.22 \times 10^{6}$ dpm
1 dpm - disintergration per second	$= 1/2.22 \times 10^{6} = 4.5 \times 10^{-7}$ uc

If the results are desired in terms of microcuries, then microcuries = $(\text{cpm} - \text{bkg}) \times (\text{Vf}) \times (\text{Df}) \times (1.001 \times 10^{-6})$ with a counter efficiency

of 48 per cent. If micromicrocuries are desired then micromicrocuries = (cpm - bkg) x (Vf) x (Df) x (1.001×10^{-12}).

BIOLOGICAL SAMPLING PROCEDURES

Water Samples

All water sampling was conducted within 48 hours before and 48 hours after the radioactive spike was introduced. The samples taken prior to the isotope introduction were used to indicate any background levels of radioactivity concentrated from fallout. Samples taken during and shortly after the radioactive spike passage were used to indicate the form the tracer took upon entering the ecosystem, its rate of uptake, and changes undergone during the short interval of passage past an area and which was sampled at a given point along the stream. The majority of the samples were taken to determine the time of maximum activity present in an area, relative time it remained free in the area and the amounts transferred or by-passed through the area. Fractionation was conducted to isolate the actual carrier forms at different periods during the time of maximum radioactivity within a given area. A breakdown of the procedures used to collect and process each sample series follows with statements of their intended purpose.

Water Sampling Procedures

Duplicate liter samples were taken at given points in the stream. Each was given a station designation, i.e. 3, 5, 8, 12 or 14 to locate them longitudinally and an A or B designation to denote left and right sides of the fixed reference point upon a stake respectively. Each

stake was placed in the maximum current determined at that station by using a Price Pattern Gurley Current Meter. All were also positioned at four-tenths the total depth at that point. A rack was attached to each stake and the polyethylene bottles were placed within the rack at each sampling interval.

From the duplicate samples taken at selected Stations 3, 8, and 14 fractions were made by the following methods. Station 3 and 8 were sampled after the dye front reached the station, but before the isotope was due. Thereafter all samples were taken at ten minute intervals for a period of 80 minutes at which time the spike had passed the station entirely. At Stations 12 and 14 samples were taken in a similar manner for the first 30 minutes, then at 15 minute intervals thereafter until eight samples were taken.

Water Processing Procedure for Radioactive Counting

Each liter sample was divided into two 500 milliliter aliquots, of which one was processed for colony counts of bacteria from culture plates and diatoms from filters, and the other for fractions.

The colony counts from culture plates were made from plates in which the filter from a millipore apparatus had been imbedded and the bacteria grown in the mesothermophilic range. A universal Tripticase agar medium was used for culturing the bacteria endemic to the stream. It was realized that the medium may have been lacking in some essential nutrients that some of the bacteria needed to grow. Also, the temperature range may have been limiting to some of the flora. Within these limits reproducibility was excellent.

Using millipore filtration flasks and different size filters,

the following fractions were separated from the water. One-hundred milliliters were drawn through a 5 micron filter by suction, this removed 100 per cent of the diatoms and eight to nine per cent of the larger bacterial forms.

The filter was washed with 25 milliliters of distilled water and then dried after being glued to a flat planchet. The combined filter and planchet were then placed in an Omni/Guard counting system to measure the beta emissions.

From a second portion a filtration was made and 4 squares of each grid filter were examined by direct examination under the high dry power of a compound microscope. By expansion to the whole filter the total number of diatoms was determined and the number per milliliter calculated. Immersion oil was used to clear the filter and diatoms in the enumeration procedure.

The 125 milliliters from the diatom filtrate were then drawn through a 1.2 micron millipore filter to collect the larger bacteria. This filter was also washed with 25 milliliters of distilled water, dried and counted for radioactivity.

The filtrate, consisting of 100 milliliters of stream water and 50 milliliters of distilled water was drawn through a 0.45 micron millilpore filter, washed with distilled water, dried, and counted for radioactivity assuming it would contain small bacterial forms, colloids, and inorganic particulate materials, it was specifically designated "particulate".

A fourth fraction was determined before each filter was dried by washing the filter with 0.01 N hydrochloric acid (25 ml.), which was pooled from the three filters. This was the adsorbed phosphorus

fraction.

One-hundred milliliters of the water sample were placed in a separatory funnel. Ten milliliters of ether (benzene, petroleum ether) were added to the funnel. The mixture was shaken to insure mixing, using care during the shaking to release pressure through the stop cock.

Time was allowed for the water and ether to separate within the funnel and then the water was taken off through the stop cock with the ether layer on top left within the funnel. The water drawn off at the bottom of the funnel was then poured through a charcoal column. The water collected in the receiving flask was counted for radioactivity after air drying as the non-water soluble, non-ether soluble fraction.

The time interval involved for the air drying of the above fraction allows time for the little remaining water to drain out of the ether. The water was poured off after the separatory funnel was drained of the solution.

After the ether was removed from the separatory funnel and the water was decanted, the ether was transferred to a charcoal column. Time was allowed for drainage of the column and an extra five minutes were allowed for the column to quit draining. The column was washed with ten milliliters of ether. The ether wash was added while some ether was still adhering to the sides of the column. The wash ether and sample ether were combined in a clean beaker after any water present was decanted. If the column clogged it was shaken into a beaker and washed in that container. The quantity of ether uses in both washings remained a fixed percentage of the total volume of the water sample throughout the test.

The combined ether volumes were partially evaporated and then

transferred quantitatively to a ten milliliter test tube for complete drying.

When the ether in the test tube was evaporated to complete dryness 0.1 milliliter of ether-ethyl acetate (1:1, v:v) was added. The test tube was turned while washing the sides as the ether-ethyl acetate was added. Mixing was conducted by a hand rod.

A 20 lambda pipette was used to spot chromatograph paper with 50 lambdas of the mixture. A quick drying spot represented a fraction containing little or no water. A spot that did not dry indicated a ruined fraction, and a second spot was made upon a different paper using the same source mixture for a check.

The chromatograph paper was placed in a chromatography jar which contained a half inch of a solution composed of the following materials: 100 milliliters of ten per cent 2-phenoxyethanol in diethyl ether, plus 100 milliliters of 2,2,4-trimethylpentane mixed thoroughly together. Enough time was allowed to pass so that the paper was 2/3 saturated by the solution traveling from bottom to top. Then the paper was removed and air dried for 2 or 3 minutes.

Each paper was sprayed with an aerosol of toxaphene. Just enough was used to wet the paper, not saturate it. The toxaphene spray used was composed of 6.25 grams of $AgNO_3$, 1.25 milliliters of HOH, 25 milliliters of 2-phenoxyethanol, 2 drops of 30 per cent H_2O_2, and acetone made up to 500 milliliters.

Handling the paper by its edges, it was transferred to a development plate under an ultra-violet light for a period of approximately 45 minutes or until developed. The spot could then be quantified chemically or cut out and counted for radioactivity as an ether soluble

organic fraction.

Any filtrate left was placed within a Whatman No. 1 filter and filtered, washed with 0.1 N hydrochloric acid and placed in a separatory funnel where specific gravity differences separate the acid and a fraction containing a non-water soluble, non-ether soluble, and non-acid soluble constituent.

No attempt was made to analyze the ether soluble organic fraction, nor the non-water, non-ether soluble fraction, or the non-acid soluble fraction. Each of these composite organic fractions was counted for radioactivity separately.

Samples from Stations 5, 12, and 16 were run in duplicate by a separate process which was as follows: 500 milliliter aliquots were taken of which 100 milliliters were filtered through a 0.45 micron millipore filter. The filter was washed with 25 milliliters of distilled water and then dried before counting for radioactivity. The second sample of 100 milliliters was filtered through a 0.45 micron filter also. The filter was washed with 25 milliliters of 0.01 N hydrochloric acid into a separate beaker, dried, and the three fractions: particulate, adsorbed, and soluble filtrate were counted separately for radioactivity.

Transect Collection Procedures

A series of samples of water were replicated longitudinally and latitudinally across the stream to investigate the possibility of cross-sectional differences in tracer passage. Station 9 was the site chosen because of near laminar flow, accessibility, and favorable mixing time. The apparatus used was a pole which was operated by one individual.

Each pole was located in a rack and held suspended parallel to the flow, between fixed stakes. Along the length of the pole, heavy rubber bands were used to hold four 140 milliliter polyethylene bottles. Three across-the-stream replicates were collected in the same manner. Radioactivity in the longitudinal replicates indicated some variance, but was not significantly different at the 95 per cent level. Across the stream replicates indicated a ten per cent level of significant difference which was presumed due to the channel shape and current. A check with a micro-Price Pattern Gurley Current Meter indicated a microstructure of current within the area similar to the activity passage, i.e. more rapid current from right to left and more rapid passage which was erratic and highly variable on the right due to hangbacks and possibly from particulate additions in the slightly slower water.

Processing Procedure for Activity Assay in Transects

Fifty milliliter aliquots were taken from the 140 milliliter samples after acidification with three milliliters of concentrated nitric acid and a thorough shaking. Each aliquot was evaporated to a two or three milliliter volume and transferred by quantitative washing with 0.1 N HCl and a rubber policeman to a numbered planchet. A second washing and scraping was conducted. A final wash with distilled water and a rubber policeman completed the transfer. The planchet and contents were dried in a muffle furnace maintained at 600° C. after initial drying on a stove hot plate. After cooling the planchet was counted for radioactivity in the Omni/Guard system.

Mud Samples

Little work has been conducted on radioactive materials collecting upon aquatic substrates within a stream. Major contributions have been for dangerous, long-lived isotopes such as zinc or strontium and calcium. Most have been conducted as studies on the Clinch River by the Oak Ridge National Laboratories or the Columbia River studies connected with the Hanford reactor site. Because of the nature of the long-lived isotopes used, no large biological incorporation of activity in the sediments was apparent. With phosphorus there was assumed to a possibility of measuring loss of the nutrient to the sediments. Possible modes of loss were numerous; precipitation as tricalcium phosphate, as dead or entrapped biological organisms or ionically bound colloids were a few of the many ways that might be expected. Sampling was conducted to determine if radioactive phosphorus precipiated and if so in what form and to what extent.

Procedure for Mud Sampling

Backwater areas at Stations 8 and 12 included two types of substrates; silt (grading in color from dark gray to black) and sand (grading from reddish to light gray) were mapped on graph paper. Then sample positions were determined from a table of random numbers which was applied to the map. Ten sampling locations were designated for each substrate at each station for a total of 40 samples among the four sites at the two stations. Planchets were pushed into each substrate at the random locations and then filled with sediments by stirring the substrate immediately upstream. Enough sediment was allowed to deposit on each planchet to cover all except the outer edge, (e.g. the outer

rim was flush with the top of the substrate). The planchets were imbedded about 30 minutes before the spike reached the area and removed about 30 minutes after the spike had passed. To remove a planchet, a flat planchet was placed over the exposed rim and the buried planchet was carefully transferred to a plastic petri dish.

Processing Procedure for Radioactive Counting

At the laboratory the contents of each planchet was transferred to a 150 milliliter beaker and acidified with 25 milliliters of 0.1 N HCl. The slurry was mixed and filtered through a No. 1 Whatman filter. Each filtrate was evaporated on a hot plate, muffled, and counted for radioactivity. Of the slurry, a small true volume aliquot was taken by suspending the bottom materials in 25 milliliters of distilled water and taking a five milliliter subsample. Each subsample was placed in its own planchet, dried, weighed, and counted for radioactivity.

Periphyton Samples

Aufwuchs or periphyton was the major unit of primary production present within the stream. It was due almost exclusively to diatoms. The diatoms present were of nine major genera: Navicula, Fragilaria Synedra, Diatoma, Coccinodiscus, Coconeius, Tabellaria, Cymbella, and Surrirella. All were uniformly distributed within the study section with the exception of heavy concentrations in areas of low light levels, where no aquatic macrophytes were competing. A sheet of periphyton covered every projecting surface in the stream bed as well as the entire stream bottom. To measure the radioactivity initially taken up by periphyton from the P^{32} in the dissolved inorganic state it was

necessary to determine the amount incorporated by the drifting and attached diatoms. The attached producers and the available light energy determined the amount of tracer incorporated into the ecosystem and thus set the maximum limit for the amounts available for transfer to other trophic levels.

Sampling Procedure

A series of metal stakes with cross-bows were positioned in areas of nearly uniform current at each station. To the cross members, at 14 and .6 tenths the total depth, a bank of 8 plexiglass substrates of known area were attached. These were allowed to remain in place for a minimum of two weeks which had been determined by experience to be the optimum period for periphyton accrual in the stream. Beginning upon July 5, 1962, one from each level was selected at random, removed, and replaced by a similar clean substrate. Thereafter a weekly sample was collected. All invertebrates were removed immediately after removal of the substrate from a cross member and then each was placed in a plastic bag or jar bearing the date and time of removal from the stream. Five stations were used as collecting sites in the main stream, they were Stations 3, 5, 8, 12, and 14. Two stakes with substrates were placed in a tributary stream, Allan Creek, which were used to determine the pattern of biological loss by attrition. These units were sampled in a manner similar to those present in the main stream. One was located at Station 3 during the spike passage and the other was in place at Station 5, immediately after the passage of the spike they were transferred to Allan Creek and maintained there in pre-selected sites.

Substrates collected once a week were frozen upon returning to the laboratory and processed the next day. Processing for radioactive counting was as follows:

Radioactive Counting Preparations

Processing involved scraping the accumulated periphyton from each substrate into a common beaker. After all the periphyton was removed the substrates and scraper were thoroughly washed with distilled water. The composite in the beaker was transferred quantitatively to a millipore apparatus which contained a previously weighed, wet, 0.45 micron millipore filter. A wet weight for the filter was determined by soaking the filter in place within the apparatus with 20 milliliters of distilled water and then applying suction for 15 seconds. Each wet filter was immediately removed and placed on a flat planchet of known weight and combined weight was taken for them from a Mettler balance. The mixture was washed with 3 milliliters of 0.01 N hydrochloric acid when the filter appeared dry, under suction. Immediately after the acid wash five milliliters of distilled HOH were drawn through the filter. Ten seconds after the filter appeared water free, suction was removed and the filter extracted, to be placed upon a weighed flat planchet. Filter and planchet were weighed and the periphyton weight taken by difference. Each weighed filter was then placed under a heat lamp or on a stove hot plate and left there until all traces of the filter and material were gone. Care was exercised to allow no boiling or spattering. The mineralized materials present in the planchet were burned to a final mineralization in a muffle furnace maintained at 600° C. After removal and cooling, the planchet

was counted in an Omni/Guard counting system to determine the radioactivity present.

Stream Macrophytes

Macrophytes contribute less to the overall productivity of the ecosystem than does the periphyton group. It would appear that the plants serve as nutrient pools that resupply the primary producers of lower organization upon their breakup and decay. They do not contribute much to the stream bottom stabilization in the study area. By estimates of the area of the bottom covered, per ten yard interval along the length of the stream, the overall average of plant cover was only about seven to eight per cent of the total area. Macrophytes appear to be the major habitats for invertebrates. It was common to find four to six times as many specimens per unit area of plants as on the bottom substrates.

It was apparent that macrophytes in the West Branch of the Sturgeon River serve more importantly as areas of attachment and avenues of movement than as producers. They were hiding and resting places for upstream migrating forms as shown by concentrations in the beds, along the edges and lower end in particular. Visual observation along the edges indicated a directed movement upstream of many forms. This has been confirmed by tracer study in the present project. More sedentary forms and injured or disoriented organisms washed loose in upstream areas were collected among the leaves at the upper end of the beds. Overall the macrophytes seem to help stabilize invertebrate populations by slowing their sweep downstream and furnishing a means of returning them by affording resting and traveling sites.

The concentration of invertebrates, particularly insects, was not passive though, many microhabitats were observed among the macrophyte framework. As an example: Isoperlid stoneflies actively patrolled a limited section of each stalk or stem and fed upon net-spinning midges and caddisflies. Also, from ten foot sections of recumbant aquatic macrophytes selected at random, as many as 1000 midges were collected, each held on the stem in its own net case. Interspersed among them were caddisflies that built their nets on the leaves and in the leaf axils. They were observed to be feeding by grazing upon the clumps of attached periphyton, drift materials trapped among the leaf axils and materials present in the quiet water held among the dense clumps of stems and leaves of the macrophytes.

With these factors in mind a sampling program was conducted to collect information about the incorporated radioactivity and the exchangeable pools of phosphorus. No emphasis was placed on tracing a pathway to higher trophic levels since no macrophyte grazers were believed present in our study area, except for the omnivorous pouch snail, Physa sp.

As previously stated five major genera comprise the macrophyte flora, they include Chara, Fontinalis, Nasturium, Potamogeton, and Ranunculus. Within the study area, community composition was dominated by the hard water alga, Chara sp. with lesser amounts of four others, specifically Fontinalis antipyretica, Nasturium officinale, a Potamogeton sp., and a Ranunculus sp. At the time of isotope introduction Chara was four times as abundant as any of the other species on a dry weight basis. Later in the study Fontinalis was the dominant at the lowermost station, 14. Nasturium officinale made up less than one per cent of the

wet weight and less than one-hundredth of one per cent of the total dry weight of macrophytes. Ranunculus sp. makes up less than two per cent of the wet weight of the total plant weight and less than one per cent of the dry weight of the total plant biomass. If these two species are disregarded in calculations of percentage composition the ranking was Chara 86.65 per cent, Fontinalis 12.38 per cent, and Potamogeton 0.97 per cent at the time of isotope introduction. Later the shift was to Fontinalis 79.01 per cent, Chara 19.35 per cent, and Potamogeton 1.64 per cent. The change was due to a slight shift downstream of sampling sites to compensate for sites which previously had their macrophytes removed. The flora about Station 14 affects the whole area distribution adversely because of the lack of suitable substrates for bed making plants and the preponderance of rocks and logs suitable for Fontinalis. The single station influence was very large and not true for the other station areas of differing habitat. If the contribution of macrophytes from Station 14 was disregarded momentarily the same relative abundance was present except for a slightly higher frequency of Chara. Zettelmaier (1961) has also pointed this out and has reached the same conclusion of area influence at Station 14.

At Station 3 Chara, Fontinalis, and Potamogeton were the only relatively abundant macrophytes. Ranunculus was found as sprigs all summer, but was never represented by a large group of plants. Nasturium was never represented by more than one or two complete plants from which only tips could be collected on each sampling date. Station 5 was well represented by abundant, well-developed plants in the area below the sampling point, but no plants were present in most of the area between 3 and 5 because of the low light levels, approximately 500

foot-candles maximum. Station 8 had numerous representative beds of all the genera except Nasturium which was represented by only two complete plants during the entire sampling period. Station 12 was a veritable jungle of aquatics with complete species representation as was Station 14.

Plant Collections

In the field the grouped or clumped nature of the plants nullified any systematic random sampling, therefore all specimens were sampled at the stations by seeking the specific genus of plant and clipping only the leaf tips or stem ends where possible. Immediately after clipping they were washed in the stream to remove silt, debris, and organisms. The washed plants were placed in bags designated with station, date and time, or else placed in two ounce collecting bottles with the same information. Approximately two grams was collected from each plant genus, and where possible specimen tips were taken from several plants.

Laboratory Processing For Radioactivity

The next day each leaf tip or fragment was washed with 0.01 N hydrochloric acid to remove the adsorbed phosphorus. That material collected was processed separately by drying on a hot plate and muffling in a furnace set at 600° C. The mineralized fraction was then counted in the Omni/Guard system after cooling. A distilled water rinse was then used on the plants after which they were allowed to dry upon paper toweling for a period of five minutes. The semi-dry plants were then broken into small pieces and placed in a numbered, previously weighed, deep wall planchet and the combined material and planchet

weighed on a Mettler balance. The wet weight was obtained by difference. The weighed planchet and contents were transferred to a stove hot plate set at low heat and covered with concentrated nitric acid. Acid and heat were added until the material was a light brown or white ash and no crust remained. No boiling or spattering was allowed to take place. The dry planchet and contents were transferred to a muffle furnace and heated to a cherry red color, 600° C. On removal and cooling the planchet and its mineralized contents were counted in the Omni/Guard system.

Plant Standing Crop and Productivity

Random sites for plant collection were obtained by application of the numbers from a table of random numbers to the stream transects. Locations were found exactly by chain tape measurements. All samples were taken by hand, removing all the vegetative materials within the limits of a Surber square foot sampler and collecting the material in the attached bag. Each sample was sorted to species, weighed on a triple beam balance after removal of rocks, marl concretions, and washing to remove clinging invertebrates. The weighed material was returned to the stream as debris. The first standing crop estimate was made on July 7, 1962. Prior to sampling it was decided that a second standing crop estimate would be made from the same sites in late August. Comparison of the two would be the area productivity and rate. All procedures were the same for both collecting periods. The second estimate was made on August 31, 1962.

Invertebrate Samples

In the stream invertebrates act as a two way shuttle system, they convert the primary producer energy into consumer flesh which was transferred directly and indirectly into carnivore flesh. They also convert carnivore flesh and wastes to elemental or near elemental forms. Their pathways of transfer were not as important to the study as the function performed by them and the level at which they attacked the compounds or materials to which the tracer phosphorus was bound.

The large numbers of organisms present and their relative metabolic rates were important to the turnover and cycling of the phosphorus. At any given time large amounts of phosphorus may have been present in the organisms which may or may not have been exchangeable with additional amounts of radiophosphorus. They also served as active concentrating agents for the radioactive material which was important to all the trophic levels present. The bound phosphorus compounds were very important to all the trophic transfer considerations related to migration and drift movement of the invertebrates which had concentrated some of the radioactive phosphorus. The study was intended to answer the question about insect migration upstream and possibly indicate a potential human health hazard created by invertebrates concentrating wastes from reactors or fallout and then moving into unrestricted public areas.

Members of the Diptera, Ephemeroptera, and Tricoptera were by far the most abundant aquatic species. Specifically mayflies of the Baetic family were the most abundant insects with Ephemerella needhami the most abundant and Ephemerella cornuta a close second. They were

followed in abundance by several caddis flies, primary of which was the net-spinning caddis Hydropsyche sp. Later members of the stonecase group, especially Glossoma sp. took the Hydropsychids place in the rankings. Blackflies, Simulium sp. were very abundant in riffles and the associated sands as larvae and pupae respectively. Omnivorus Plecoptera, Pteronarcys sp.; Ephemeroptera, Hexagenia sp.; and the pouch snail, Physa sp. were quite abundant in limited areas. In some cases the Physa were very scarce. Megaloptera, Chauloides sp. in particular were not abundant as was true of the Dipteran, Atherix variegata.

Invertebrates Collection Procedures

In reiteration, the organisms were only identified from available keys to a convenient point which would separate insects according to field identifiable characteristics. Because of the non-random distribution of each insect no systematic statistical sampling procedure was followed. Since large numbers of small insects were needed the sampling was conducted by going to specific known habitats and hand picking members of the particular insect needed. It was made a prerequisite that no more than two large insects or 25 small insects be taken from a single area of the substrate. A minimum sample size for radioactivity assay was arbitrarily set at five for larger genera, i.e. Chauloides, Pteronarcys, and Physa. While 50 mayflies of the Baetid group were set as the lower limit and 100 blackflies were minimal to obtain a weight minimum of about 0.1 gram. In the majority of sampling areas, enough specimens for a weighable sample were collected within 50 yards up or down stream from the station. Physa sp. and Hydropsyche sp. were the exception, the former because of habitat considerations

and the latter because of life history stages. Therefore several samples consisting of two or three specimens were used. Sometime during the summer all habitats at each station were sampled.

Migration Collections

Collections of insects above the point of isotope introduction were made to attempt to answer the question of how the upstream areas were repopulated when it was known that currents constantly denuded them of organisms. It has been suggested that adult forms undergo periodic nuptial flights upstream or that egg laiden females fly upstream in small groups or as individuals. Little work has been done on immature forms with respect to a directed movement in streams. The few instances mentioned in the literature only suggest a rare occurrence of streaming movements among some of the larger insects, scuds, and a few species of snails. It was thought that the incorporated tracer would serve as a conclusive indicator of upstream movements in both larval and adult forms. Quantities of isotope present in the area above the point of isotope introduction that were higher than prelimnary background sample radioactivity would definitely indicate a carriage of activity upstream by some active agent probably the organism itself. Directed adult insect flights were checked with a directional trap and light traps.

Adult Insect Collections

Adult insects were collected qualitatively by hand nets, sweeping, light traps, and directional traps. Only the light traps and directional trap were quantitative. A Malaise directional trap (Figure 3) with a barrier in the center was used to collect adults at a point 60 yards

Fig. 3.--A Malaise directional trap.

upstream from the point of isotope introduction. Due to its inaccessibility, maintenance was restricted to daily checks and samples collected during the 24 hour period thus contained both diurnal terrestrial insects and crepuscular and nocturnal aquatic insects. Terrestrial insects were removed from all samples, leaving only aquatic insects or semi-aquatic, associated groups, i.e. some diptera. A second quantitative method was based on the use of a series of battery powered black or ultra-violet light traps. The data was necessarily based upon the weight and radioactivity contained within an entire night's collection. To make the nightly basis uniform, no samples were included for which the time of collection may have been reduced by faulty electrical equipment. A minimum of 8 hours of operation was acceptable. The power package for each night of operation for each light was a 12 volt wet storage battery, an inverter which changed the 12 volt D.C. to 110 volt A.C. and a fluorescent ultra-violet light. The battery and inverter were housed in a box at the side of the stream, while the light was suspended over the stream in a metal housing.

The lights were biased with respect to sampling, those forms attracted to light were caught exclusively. They consisted of only aquatics and a few moths. Each sample of the ten weighable samples obtained was collected immediately after a major weather change from either cool, cloudy weather or cool, clear weather to a warm humid period. A total of 30 nights were sampled by each of several light traps and a total of 70 days and nights were sampled by the Malaise trap.

Larval Migrant Collections

The larval forms were collected and processed in the same manner as those from the down stream areas which were used for radioactivity assay. The sites of sampling were as previously described in the section on stations. Specific genera were collected by hand picking when located by turning over logs and stones and examining weeds.

Drift Insect Collections

Drift samples were collected in duplicate using No. 14 mesh Nitex mounted on a 12 inch by 18 inch metal frame and positioned with stakes midway across the river at Station 5 and at the confluence of Allan Creek and the West Branch of the Sturgeon River. Samples were taken for one hour periods just before and after dusk on several nights during the summer and two 24 hour series were conducted, sampling at two hour intervals before each net was emptied into a preservative jar. Sorting was conducted by hand picking the insects and insects parts with a pair of BB forceps while the sample was under 100 X magnification. Only weights were determined and the rates of accumulation per hour made. The expansion to rate per unit area were made using the formula developed by Water's (1961) based on his standard foot.

Processing Procedures for Insect Radioactivity

Larval Insects

Larval insects were processed one day after collection by spinning them in a clinical centrifuge which was run at full speed for ten seconds then turned off and allowed to stop by its own friction. The insects were then transferred to a weighed planchet and the planchet and contents

were weighed again. The wet insect weight was determined by difference. Each planchet of insects or individual insect, for the larger ones, was then placed on a stove hot plate and concentrated nitric acid was added until the heat and acid reduced the insects to a film of mineralized material on the bottom of the planchet. The planchet was moved back on low heat and ten drops of acid were added again. After complete evaporation of the acid by low heat, the planchet and contents were muffled in a furnace maintained at 600° C. until cherry red. After removal from the furnace and cooling the planchet and contents were counted in an Omni/Guard system.

Adult Insect Procedures for Radioactivity

Adult insects were sorted to order when weighable samples were obtained and then run separately by order. They were weighed as composite groups when not separable into weighable order units. Each group was digested with concentrated nitric acid and heat. After digestion the insects were muffled in a furnace to a cherry red color, 600° C., cooled, and counted for P^{32}.

Biomass Collection Procedures

An invertebrate biomass was estimated after collecting the insects from 90 square foot quadrants selected by applying random numbers from a table of random numbers to the stream transects. A Surber square foot sampler was used to collect the material. Exact locations were determined by use of a 100 foot chain tape. The sampling was to a depth of about four inches in all substrates except the logs and rocks.

Processing for Enumeration and Weights

Field preserved samples were transported to East Lansing and sorted by hand picking after screening each sample several times through a 30 mesh per inch screen. The number per family was determined for insects, and oligochaetes were enumerated as a group. Weights were determined as total insect weight per sample on a Mettler balance accurate to milligrams, after reconstituting the insects, which had been preserved in 70 per cent alcohol. They were reconstituted by suspending them in distilled water for 30 minutes, centrifuging for ten seconds and weighing.

Fish Samples

Three specimens of each of the following vertebrates were collected for radioactivity assay at two week intervals at each of the five main stream stations: the brown trout, Salmo trutta fario; the rainbow, Salmo gairdnerii; the brook trout, Salvelinus fontinalis; the slimy sculpin, Cottus cognathus; the northern mottled sculpin, Cottus bairdii; and the northern brook lamprey, Ichthyomyzon fossor. They were considered to be the terminal trophic level present in the ecosystem. By radioactivity assay the amount of isotopes reaching the final organisms was determined along with the rate of exchange with the stable phosphorus. The maximum accumulation per gram of fish was analyzed to determine if the isotope was concentrated to a level that could be dangerous to humans.

By ranked order of abundance the brown trout was the dominant large fish, with the rainbow trout a close second even though no rainbow trout over 10 inches were encountered during the sampling period.

Large rainbow trout were encountered only during spring spawning runs and shortly thereafter, after which they return to deeper water downstream. If all fish were considered, the two sculpins were by far the most numerous fish, but on a weight basis they were the least abundant. No significant difference in distribution was observed in the whole study area and they were equally available at all five stations. All specimens were collected by means of a 230 volt D.C. shocker.

Procedures for Radioassay of Fish

Upon collection the fish were placed in jars, transported to the laboratory and frozen until processed the following day. Each fish was measured and weighed before processing. The initial spike was to open the salmonids and assay the contents of the digestive tract with respect to plant, invertebrate, or other fish material. Then the fish and its stomach contents were placed in a Waring blender with a volume of water equal to its weight and homogenized by the blades at full speed for 30 seconds or until complete homogenization occurred. An aliquot of the homogenate was removed with a pipette and placed in a pre-weighed planchet, weighed, and covered with concentrated nitric acid on a hot plate. After complete mineralization, the planchet and contents were muffled in a furnace at 600° C. and removed from the furnace, cooled, and counted for radioactivity.

Fish Biomass

Estimations of trout and muddler populations in the West Branch of the Sturgeon River during 1962 were conducted at Stations 3, 5, 8, 12, and 14. A segment of stream 100 yards long was selected at each station for estimation of the trout population and a 50 foot section

was selected for estimation of the muddler population.

The muddler population was estimated one week after the trout shocking was finished. Muddlers were captured in the section by shocking, placed in M.S. 222, measured, the right pectoral fin clipped and after species determination weighed as a group. Following this the fish were placed in fresh water until they revived, then redistributed in the 50 foot section. A 30 minute waiting period was allowed to elapse. Then a single recapture was conducted as in the Petersen method. The Petersen and Snabel methods were compared over the same areas later and no significant difference was found in the estimates. A previous comparison of the two methods on this stream also showed no significant difference in the two methods of estimation.

All species of trout of all age groups were collected in a 100 yard section by the same methods as used for muddlers. Lengths, weights, and species were recorded. The right pectoral fin was clipped and after reviving they were redistributed within the 100 yard section. After a 30 minute waiting period, the section was reshocked.

RESULTS AND DISCUSSION

Water Samples

On July 10, 1962 a mock run was made on the stream using a fluorescein dye suspended in alcohol as the spike. The dye was used to prepare a sampling schedule for each station and to determine the total time of passage by the given points. On the day of isotope introduction a similar dye front was introduced five minutes ahead of the isotope to alert the sampling crew.

During a thirty-one minute period beginning at 9:30 a.m. on July 11, 1962, 23.54 millicuries of radioactive phosphorus (P^{32}) were added to the water of the West Branch of the Sturgeon River. The addition was made by means of a boom with ten evenly spaced nozzles, the tracer was sent to the boom by a submersible electric pump immersed in a 55 gallon drum. Through the ten nozzles the tracer was introduced at a concentration of 11.9447 micro-microcuries per milliliter during the full 31 minute period. No pre-tracer fertilizer application to increase the exchange pools was made.

Sampling was begun first at a point 240 yards downstream from the point of isotope introduction. Duplicate liter samples were taken at ten minute intervals for 80 minutes according to the sampling schedule determined in the mock run, it was assumed that physically the behavior of the radioactive material would be the same as that of

the dye. At the laboratory fractionation was conducted using the previously described methods. Eight duplicate fractions were run at five stations with each station sampled at a fixed point during each interval. According to the sampling schedule time for the attenuation of the spike was allowed for and sampling was conducted accordingly. The assumption was that there was no significant difference in the passage of the tracer spike as compared to a dye spike, both applied in the same manner. It was known that no change in discharge took place during the addition or between the day of timing and day of application.

By fractionation methods the form of the tracer passage past a point was determined as was the amount in each form and the change in mode of transport with increased distance from the point of tracer introduction. The amount present in the different fractions at given times was calculated as well as totals for the water mass passing a given point. By difference between points the amount of uptake, loss, or change was computed. By adding all fractions, the total amount of tracer leaving the area above 240 yards was only 17.01 per cent of the total introduced activity. Thus over 80 per cent of the tracer was taken up by some means in the first 240 yards of stream. This was not comparable to other years, but the difference was presumed to be due largely to the method of introduction. In the four years previous to 1962, the isotope introduction had been from a single point source which produced an unmixed cone of tracer which was still not fully dispersed across the stream at 300 yards downstream, if it can be assumed that tracer spikes are directly comparable to dye spikes. The large number of point sources from the boom changed the single cone to ten small intermixing cones.

Table 1 gives the percentages of the total available tracer activity in each fraction based upon the mean values of the duplicate samples taken at a given time at a given station. From the values in the table it was evident that adsorption was the major form of tracer uptake by particulate materials in the first 240 yards, with actual known biological incorporation second and dissolved forms making up the remainder. The relative percentages were about one third in the dissolved state, more than one third in the adsorbed forms, and less than one third in incorporated biological forms as the tracer leaves the area above 240 yards. Downstream the proportion of biologically incorporated fractions rapidly increased. At Station 14, the furthest downstream station at which fractions were determined, over 70 per cent of the available activity was in an organic form.

From the same table it can be seen that an important part of the isotope was carried by a fraction designated as "particulate" material. The actual composition of which was not investigated, but from the large amount of tracer incorporated in the first 240 yards, the small particle size (0.45 microns) and the consistency, a semi-solid slime, it was assumed that it was of an organic nature. It was much more probable that it was a nannoplankton form of bacteria or fungi, or even a psammolittoral type of organism, than an inorganic material since the water was not entirely mixed with the bottom sediments, but only with their surfaces and the stream side interfaces.

Diatoms and large endemic bacterial forms incorporated tracer phosphorus by the same relative order of magnitude when filtered from the same volume of water. Both progressively incorporated more tracer with distance and time. On a per unit cell basis diatoms accumulated

TABLE 1.--Radioactivity levels of the water fractions at Station 3, 8, and 14 in corrected micro-microcuries per milliliter. Corrected for background and decay.

Fraction	Station 3		Station 8		Station 14	
	$\bar{X}$ uuc/ml	% total	$\bar{X}$ uuc/ml	% total	$\bar{X}$ uuc/ml	% total
Dissolved	0.2233	29.29	0.1363	24.13	0.0258	15.43
Adsorbed	0.2883	37.82	0.1060	18.77	0.0239	14.29
Diatom	0.0469	6.15	0.0476	8.42	0.0258	15.46
Bacteria	0.0385	5.05	0.0435	7.79	0.0216	12.90
Particulate	0.1250	16.40	0.1213	21.48	0.0494	29.54
Ether soluble	0.0326	4.28	0.0976	17.28	0.0132	7.90
Non-acid soluble	0.0074	0.97	0.0114	2.02	0.0072	4.31
Acid soluble	0.0003	0.05	0.0011	0.20	0.0003	0.16
Total	0.7624	100.00	0.5648	100.00	0.1671	100.00

a phosphorus 32 concentration 16 times greater than bacteria. Of all fractions only the dissolved and adsorbed decreased with time and distance. The variation within the ether soluble organic fraction and the non-water soluble, non-ether soluble, and non-acid soluble organic fractions would appear to be a function of the particulate fraction composition and the changes undergone in it. The basis of this conclusion was the probable increase in amount of inorganic sediments with distance. Little tracer was present when the water was known to be not thoroughly mixed, then it increased in both fractions after passing through an area of rapid current, low sunlight, little or no silt and high periphyton composition. The radioactivity levels decreased again after passing through an area of more moderate current, high sunlight, much sand and silt and intermediate-to-high periphyton composition. It was recognized that tracer concentrations in the "particulate" forms increased with time of contact with a more dilute medium such as Krumholz and Foster (1957) found when they reviewed the effect of concentration on uptake. Their conclusion was that, for substrates, when the concentration was low the uptake was higher.

Table 2 was an evaluation of the radioactivity entering each station, the uptake and the amount leaving each area. When sampling was terminated on July 11th some tracer was still leaving the study area at the lowermost station at which fractions were run (Station 14). Therefore based upon the measured total radioactivity passing that point, in water borne forms, up to the time of termination of sampling on July 11th, 93.72 per cent of the tracer was taken up. In terms of radioactivity units this would have been 98.6 per cent of the initial concentration. Figure 4 illustrates the trend of total activity (from

TABLE 2a.--Concentration of isotope, amount retained, amount leaving each station. Corrected micromicrocuries per milliliter.

TABLE 2b.--Total millicuries retained and leaving each station.

	Station	Conc. uuc/ml	% Leaving	% Total Retained	Cumulative % Retention	Conc. Retained uuc/ml	Conc. Leaving uuc/ml	Cumulative % Leaving
	0	11.9436	100.0	-----	-----	11.1818	0.7624	-----
	3	0.7624	6.38	93.62	93.62	0.1975	0.5645	6.383
2a.	8	0.5648	74.08	25.92	95.27	0.3968	0.1677	4.729
	14	0.1671	29.71	70.29	98.60	------	------	1.398
	Total					11.7760	0.1676	
	0	23.54 mc.	100.0	-----	-----	19.5800	4.01 mc.	-----
	3	4.01	17.01	82.99	82.99	0.6749	3.33	17.01
2b.	8	3.34	83.17	16.83	85.84	1.8570	1.48	14.16
	14	1.48	44.40	55.60	93.72	------	----	6.28
	Total					22.1119	1.48	

Fig. 4.--Total activity passage past Stations 3, 8, and 14. Corrected micromicrocuries per milliliter from duplicate samples taken during spike passage.

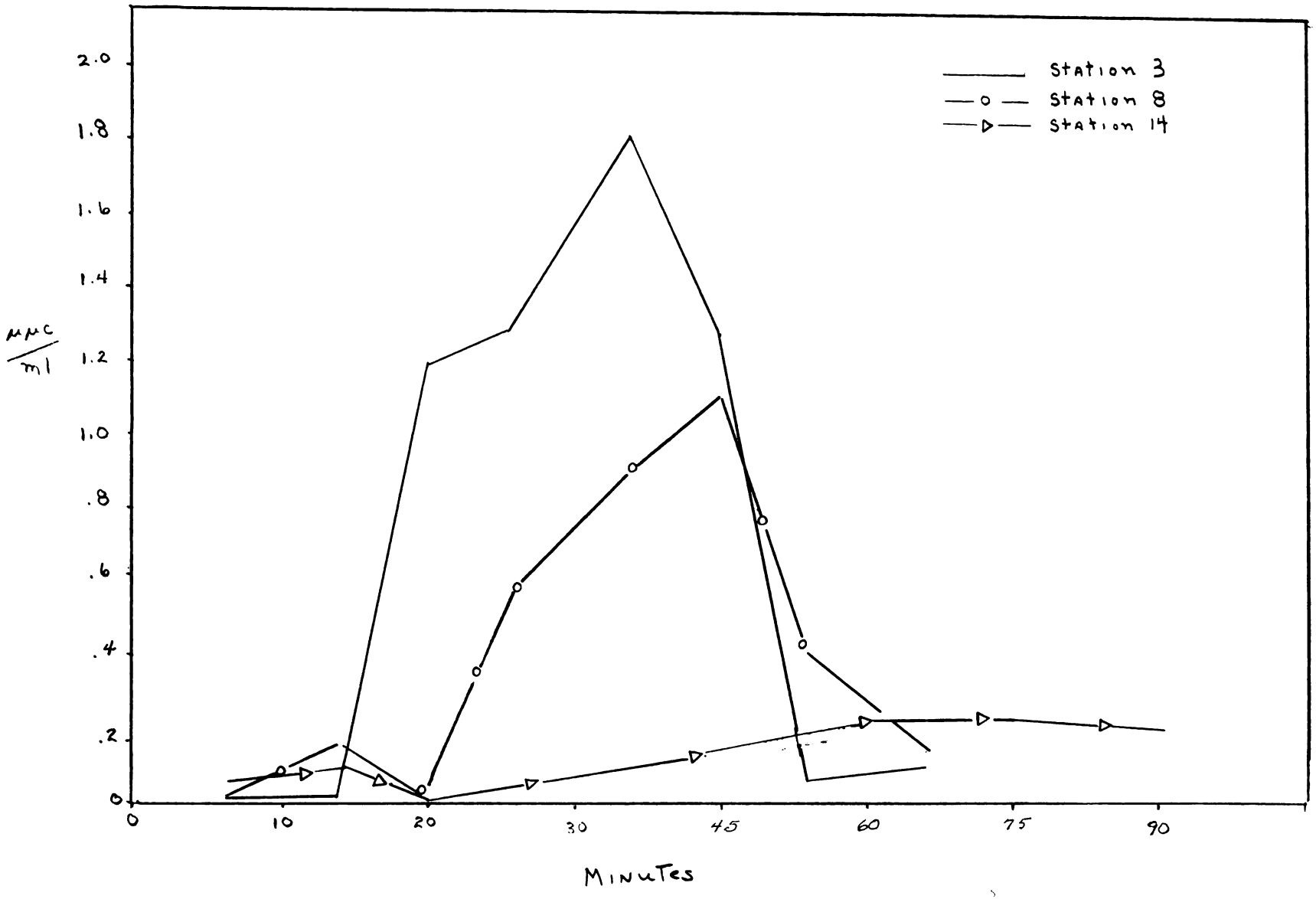

Station 3
Station 8
Station 14
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
μμc/ml
0
10
20
30
45
60
75
90
MINUTES

TABLE 3.--Radioactivity levels in eight fractions of water taken as P^{32} spike passed Stations 3, 8, and 14. Corrected micromicrocuries per milliliter.

Fraction	A	B	$\bar{X}$	A	B	$\bar{X}$	A	B	$\bar{X}$
	Series 3-1			Series 3-2			Series 3-3		
1	0.00138	0.00000	0.00069	0.00035	0.00000	0.00018	0.00000	0.00107	0.00054
2	0.00511	0.00408	0.00460	0.00459	0.00100	0.00280	0.35829	0.34708	0.35269
3	0.00529	0.00328	0.00428	0.00507	0.00485	0.00496	0.52364	0.59870	0.56008
4	0.00050	0.00000	0.00025	0.00000	0.00181	0.00091	0.14777	0.18758	0.16768
5	0.00000	0.00033	0.00017	0.00000	0.00000	0.00000	0.00286	0.00000	0.00143
6	0.00000	0.00173	0.00087	0.00000	0.00000	0.00000	0.00142	0.00000	0.00071
7	0.00022	0.00218	0.00120	0.00023	0.00025	0.00024	0.03931	0.05250	0.04439
8	0.00000	0.00022	0.00011	0.00000	0.00066	0.00033	0.06901	0.09762	0.07952
Total			0.01217			0.00942			1.20704
	Series 3-4			Series 3-5			Series 3-6		
1	0.00000	0.00074	0.00037	0.00000	0.02171	0.01086	0.00072	0.00000	0.00036
2	0.47083	0.31535	0.39309	0.47771	0.39956	0.43864	0.30646	0.79675	0.55160
3	0.89950	0.25920	0.57935	0.59867	0.80159	0.70013	0.33983	0.48321	0.41152
4	0.16002	0.26557	0.21280	0.39865	0.38766	0.39316	0.16395	0.25203	0.20800
5	0.00035	0.00000	0.00017	0.00146	0.00109	0.00128	0.00000	0.00000	0.00000
6	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00035	0.00000	0.00017
7	0.04281	0.05526	0.05327	0.10352	0.14371	0.13431	0.06465	0.05242	0.06359
8	0.14676	0.09129	0.11479	0.13999	0.13890	0.12876	0.07076	0.03778	0.04921
Total			1.35384			1.80714			1.28445

	Series 3-7			Series 3-8		
1	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
2	0.01372	0.00000	0.00686	0.04945	0.02333	0.03639
3	0.01498	0.01295	0.01397	0.02802	0.03623	0.03213
4	0.00780	0.00806	0.00793	0.01040	0.00806	0.00923
5	0.00000	0.00247	0.00124	0.00000	0.00000	0.00000
6	0.00035	0.00105	0.00070	0.00105	0.00000	0.00053
7	0.00502	0.00306	0.00425	0.00633	0.00153	0.00667
8	0.00197	0.00284	0.00240	0.00218	0.00415	0.00043
Total			0.06155			0.08538

	Series 8-1			Series 8-2			Series 8-3		
1	0.00000	0.00660	0.00330	0.00181	0.00070	0.00126	0.00249	0.00000	0.0012
2	0.00382	0.00518	0.00450	0.00456	0.23480	0.11969	0.02171	0.00581	0.0138
3	0.00122	0.00081	0.00102	0.01225	0.00391	0.00808	0.00443	0.00384	0.0041
4	0.00129	0.00000	0.00065	0.00159	0.00286	0.00223	0.00181	0.00181	0.0018
5	0.00000	0.00138	0.00069	0.00000	0.00000	0.00000	0.00070	0.00000	0.0004
6	0.00033	0.00000	0.00018	0.00140	0.00140	0.00140	0.00142	0.00000	0.0007
7	0.00284	0.00000	0.00154	0.02031	0.00066	0.01011	0.00022	0.00066	0.0005
8	0.00000	0.00044	0.00022	0.00087	0.00000	0.00006	0.00415	0.00000	0.0019
Total			0.01198			0.14283			0.0244

TABLE 3.--Continued

Fraction	A	B	$\bar{X}$	A	B	$\bar{X}$	A	B	$\bar{X}$
	Series 8-4			Series 8-5			Series 8-6		
1	0.00000	0.01212	0.00606	0.00000	0.00000	0.00000	0.00186	0.00000	0.0008
2	0.33164	0.25037	0.29101	0.35671	0.06316	0.21005	0.21571	0.20093	0.208
3	0.20556	0.15803	0.18180	0.18118	0.23624	0.20872	0.17389	0.21440	0.194
4	0.20193	0.00000	0.10097	0.07513	0.42870	0.25192	0.52219	0.41234	0.467
5	0.00000	0.00072	0.00036	0.00000	0.01212	0.00006	0.00072	0.00144	0.0011
6	0.00035	0.00074	0.00055	0.00192	0.00000	0.00096	0.00000	0.00109	0.0006
7	0.03058	0.04652	0.04188	0.10025	0.12143	0.12043	0.10964	0.10199	0.114
8	0.06399	0.05635	0.05684	0.12776	0.14087	0.12473	0.17188	0.10920	0.1314
Total			0.67947			0.92287			0.1186
	Series 8-7			Series 8-8					
1	0.00000	0.00000	0.00000	0.00035	0.05220	0.02628			
2	0.13737	0.00655	0.07196	0.04508	0.02426	0.03467			
3	0.19125	0.00686	0.09906	0.03558	0.05493	0.04525			
4	0.15793	0.09990	0.12891	0.03352	0.00000	0.01676			
5	0.00000	0.00035	0.00018	0.00000	0.00035	0.00018			
6	0.00000	0.00035	0.00018	0.00000	0.00000	0.00000			
7	0.04259	0.03342	0.04129	0.01747	0.01158	0.01579			
8	0.68141	0.06399	0.06278	0.01835	0.01835	0.00256			
Total			0.40436			0.14149			

	Series 14-1			Series 14-2			Series 14-3		
1	0.00275	0.00000	0.00138	0.00070	0.00000	0.00035	0.00000	0.00000	0.00000
2	0.01818	0.00000	0.03973	0.00633	0.00253	0.00444	0.00625	0.00422	0.00523
3	0.02970	0.00895	0.01933	0.00895	0.00513	0.00705	0.00290	0.00192	0.00242
4	0.00103	0.00076	0.00090	0.00076	0.00135	0.00106	0.00232	0.00203	0.00220
5	0.00000	0.00000	0.00000	0.00070	0.00000	0.00035	0.00000	0.00074	0.00037
6	0.00044	0.00000	0.00022	0.00000	0.00000	0.00000	0.00107	0.00000	0.00054
7	0.00066	0.00000	0.00033	0.00197	0.00022	0.00913	0.00000	0.00000	0.00000
8	0.00000	0.00000	0.00000	0.00066	0.18520	0.08489	0.00066	0.00087	0.00029
Total			0.06189			0.10727			0.01153

	Series 14-4			Series 14-5			Series 14-6		
1	0.00000	0.00179	0.00090	0.00000	0.00000	0.00000	0.00072	0.00035	0.00054
2	0.03313	0.01437	0.02375	0.00000	0.04038	0.02019	0.02691	0.01540	0.02115
3	0.02638	0.01428	0.02034	0.03503	0.02948	0.03226	0.03403	0.03400	0.03402
4	0.01979	0.00753	0.01368	0.05780	0.06041	0.05611	0.12479	0.11315	0.11898
5	0.00035	0.00000	0.00018	0.00000	0.00072	0.00036	0.00111	0.00000	0.00056
6	0.00000	0.00000	0.00000	0.00072	0.00035	0.00054	0.00074	0.00072	0.00073
7	0.00022	0.00502	0.00502	0.00699	0.02381	0.02885	0.05831	0.04390	0.06846
8	0.02752	0.00699	0.01486	0.03123	0.02381	0.01407	0.03975	0.06770	0.03638
Total			0.07873			0.15238			0.28009

TABLE 3.--Continued

Fraction	A	B	$\bar{X}$	A	B	$\bar{X}$
	Series 14-7			Series 14-8		
1	0.00070	0.00282	0.00176	0.00000	0.00072	0.00036
2	0.00330	0.10278	0.05304	0.04043	0.03704	0.03874
3	0.07574	0.02774	0.05174	0.00000	0.00000	0.00000
4	0.06482	0.13643	0.10063	0.08904	0.01136	0.10131
5	0.00035	0.00000	0.00017	0.00000	0.00070	0.00035
6	0.00000	0.00105	0.00053	0.00000	0.00070	0.00035
7	0.05067	0.01005	0.03304	0.01966	0.02927	0.02710
8	0.01704	0.04499	0.02834	0.02948	0.03145	0.02783
Total			0.26926			0.19587

duplicate samples) passing the three stations at which fractions were run. Full fractionation procedures were not conducted on the samples from Stations 5 and 12, but partial fractions agree in their intermediate tracer level with the intermediate station level relative to the other three stations. In all 22.1119 millicuries were removed from the water mass. (Table 3)

Preliminary analyses of the diatom and endemic bacterial flora conducted in May and again in July prior to the tracer addition revealed a normal composition of 8.89 ± 1.65 diatoms per milliliter of water and about $1.65 \times 10^2 \pm 42$ bacteria per milliliter of the same volume of water. The values obtained were based on direct counts of diatoms on millipore grid filters and bacterial plate counts, both counted under high dry power of a compound microscope. The bacterial counts may well be less than actual stream numbers because of bias of the medium nutrients, a universal Tripticase agar was used, and the culture temperature which was in the mesothermophilic range. Millipore filter pads were imbedded on the agar surface and cultured for about a day, then counted while colonies were still distinguishable. During the entire study period bacteria and diatoms maintained their same relative numbers. In connection with a similar tracer study conducted in 1961 the normal bacterial flora was of the order of 1.27×10^3 bacteria per milliliter (Bender, 1962). The difference was presumed to be due to high bacterial populations washed into the stream from surrounding wooded areas by a series of rains during the period of pre-tracer sampling immediately preceding the 1961 tracer study.

Using these values a rate of uptake was computed and found to be 0.0058 micromicrocuries per diatom and 0.00026 micromicrocuries per

bacterium, maxima respectively. Table 4 was a listing of the computed activity incorporated per individual organism per station from that available at the time of isotope passage. The decrease from Station 8 to Station 14 was a function of the distance, availability, and time of contact. Distance decreases the availability due to dilution from the water entering the area behind the spike and contributions from springs and underground water. The difference was evident even though the time of contact with the attenuated water mass was much greater. Incorporation itself would appear to be a function of the available form of the tracer. From the results of this study and previous ones conducted on the same river as well as other studies on other streams, it was known that dissolved, inorganic ions were readily adsorbed, and incorporated after absorption, but organically bound or released products were utilized at a very slow rate, apparently after some type of breakdown or change.

Labaw, et al. (1950) found that bound phosphorus was not released into the media as cell death proceeded. In 1961, using Escherichia coli 0111 as the carrier, (a bacterium non-endemic to the stream) only about ten per cent of the tracer was released from the cells within the study area. The rest was carried out of the study area still in the incorporated form (Bender, 1962). This strongly suggests that organic products were not released immediately or if so, they were not soon utilized by incorporation. Rather they were utilized only as adsorbed phosphorus complexes which were slowly broken down to usable forms. It has been shown that turnover times of five minutes for bacteria, in relation to radioactive phosphorus in water, were possible (Hayes and Phillips, 1958). The ionic or atomic turnover must involve some loss

TABLE 4.--Concentrations of radiophosphorus in diatoms and bacteria. Corrected micromicrocuries per organism.

	Station	Counted Radioactivity uuc/ml.	Concentration uuc/diatom	Total ml. per sec.	Total Micro-microcuries per Second
Diatoms per ml. 8.89 ± 1.65	3	0.0469	0.0053	1097272	61296
	8	0.0476	0.0053	1230125	68717
	14	0.0258	0.0029	1408020	43038
Bacteria per ml. 165 ± 42	3	0.0385	0.00023	1097272	52241
	8	0.0435	0.00026	1230125	69435
	14	0.0216	0.00013	1408020	37890

of organically bound phosphorus since there was energy loss during the exchange. It may have been possible that some organic phosphorus was lost when the cells were duplicated during reproduction, also. The cells separated as rapidly as once every half hour in some experiments.

It does not seem reasonable to assume that the phosphorus released would be only displaced inorganic phosphorus since this would be utilized by adsorption and/or incorporation by other organisms by the time it reached the last station. It would even have been incorporated by the time it reached the second station. It has been noted that of the inorganic P^{32} 83 per cent was utilized by organisms and the substrate in the first 240 yards of stream below the point of introduction. It was possible that it was a displaced metabolic product in the dissolved state, with small amounts being utilized by organisms present within the stream.

The increased percentage incorporation of available tracer may be a function of the increased dilution and time of contact with the tagged water mass. Boroughs, et al. (1957) have shown by using zinc in the habitat of the alga, Nitzchia sp. that as the concentration of tracer zinc in the habitat decreased there was a greater per cent of uptake by individual cells. With more contact time the adsorbed tracer may also have been incorporated. If the diatom and bacterial differences in radioactivity were compared between stations with the decreased activity of adsorbed tracer between the same stations, the close agreement may furnish an answer to the changes undergone. For the differences in the dissolved state radioactivities, the solution may be in the amounts of increase found within the "particulate" fraction. Carryover to increase the organic fractions may have been from the excess

adsorbed tracer.

Whittaker (1961) has reported that losses from algae back to the water may be in the form of some organic compound. When a suspension of the alga Chlorella or the alga Scenedesmus was allowed to photosynthesize for the very short time of five seconds, in the presence of carbon dioxide made with radioactive carbon isotopes, a very large proportion (about 70 per cent) of the labelled carbon was found by subsequent analyses of the cells to be present in the compound, phosphoglyceric acid, (Calvin and Benson, 1949; Benson and Calvin, 1950). It was also known as an intermediate compound in the process of respiration. The above authors have stated that a plausible explanation may be that the carbon dioxide reduction phase of photosynthesis may proceed at least in part by a reversal of the sequence of reactions occurring in one phase of respiration. It suggests that a phosphorus bearing compound rapidly constructed by cells may also be rapidly returned to the water as a waste product.

Diatoms and bacteria may incorporate an inorganic form of the tracer, rapidly convert it to an organic form, and then rapidly return parts of the organic products to the ecosystem. This was the first time that they had been analyzed in total, much less as specifics. The actual mechanisms and agents contributing the organic fractions of incorporated phosphorus were not determined by this study. Trends of possible contributors to the organic fractions were indicated by computing the losses in activity between stations in the adsorbed and dissolved fractions and then comparing them with the gains in the organic fractions. The increases in incorporated tracer within organisms was nearly directly proportional to the increases in the organic

fractions.

By multiplying plant, periphyton, insect, and fish biomass by their mean summer uptake values, adding them and the water borne activity of the adsorbed fraction, a total uptake was found that was very nearly the same as computed for the loss from the water mass the initial day of isotope introduction. The two values agree to within ten per cent, which was well within the error of the sampling methods employed and possible estimation errors. Therefore the amount of isotope not incorporated immediately, but lost to the area above a station may well have been incorporated at some later date during the summer. It would suggest that the tracer was held in some manner that caused it to eventually be available after undergoing a change. The largest possible reservoir for such retention as that suggested would be the substrate interfaces of the bottom. The vast majority of downstream tracer activity would then have to have been recycled phosphorus to give the total cumulative summer activity the lower stations reached. The mass estimates cannot be calculated by mean uptake values and biomass estimates because of recycling and drift organisms which greatly increase the station variability for a given organism. As an example of the drift values: plankton diatoms amounted to 16 per milliliter later in the summer with a range of from nine to 45 when measured by collections from a plankton net and computed by the Welch formula (1948) and 8.89 ± 1.65 per milliliter by the millipore filtration methods using five micron filters. A value for drifting insects was not available, but it was known to be very large, indications being about 2.5 million insects per square foot of stream area per week as computed by the Water's standard foot formula (1961). Fragments of plants were

periodically washed downstream, especially after heavy rains or winds. Insects were periodically abundant in the drift with diurnal and seasonal patterns as well as irregular abundance after some disturbance had washed them loose. Table 5 gives the per unit area uptake as well as the total area uptake in the various years.

Sediment Samples

Sediment samples were taken to determine the amount of tracer precipitated by chemical means or settling of organisms from suspension in the water mass. It was assumed that settling out would be a function of the organisms that had sufficient specific gravity to settle from moderate-to-slow flowing water. With mixing known to be incomplete between the bottom materials and the water in the upstream areas, only Stations 8 and 12 were studied. Random samples of the sediments settling upon planchets were resuspended in the laboratory by making a slurry with dilute acid, after which it was filtered, and counted for radioactive phosphorus. Aliquots of the solids and the total filtrates were counted separately. Filtrates contained the adsorbed quantities of tracer while aliquots of the bottom materials contained the incorporated tracer.

Mud consisting of a dark brown, organic material or materials was sampled and processed independently of light brown or tan sand materials. The mud fraction was common in areas of little or no current in the downstream sections, especially among plant beds, on the inside of river bends, and behind stream improvement structures. Sand was a transient medium which was constantly being altered from bars in midstream to the slack current near the banks and below diverters.

TABLE 5.--Comparison of uptake by water fractions in different years at the different stations. Corrected microcuries per milliliter.

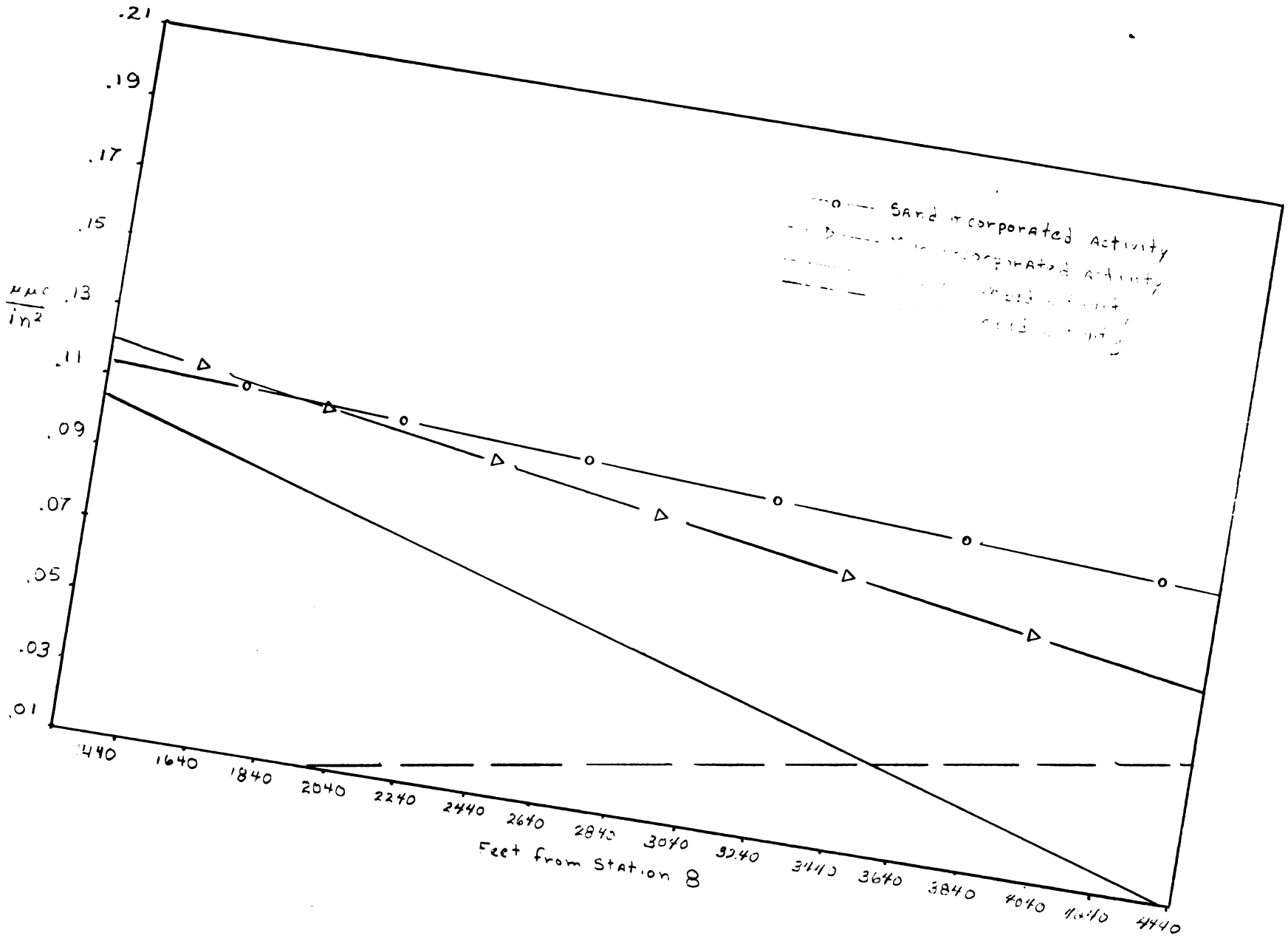
Section	Area in sq. yds.	Total Uptake uc/ml.	Uptake per sq. yd. uc/ml.	Proportion of Total Available Activity	Year
2-5	4,378	6,382	1.457	0.311	
5-8	4,036	4,084	1.011	0.289	
8-12	14,926	9,080	0.608	0.904	1959
12-14	5,322	962	0.180	0.999	
2-5	4,378	10,996	2.511	0.478	
5-8	4,036	476	0.118	0.036	
8-12	14,926	6,021	0.403	0.480	1960
12-14	5,322	982	0.184	0.150	
2-5	4,378	6,219	1.420	0.242	
5-8	4,036	857	0.212	0.044	
8-12	14,926	2,478	0.205	0.164	1961
12-14	5,322	-----	-----	-----	

By sight estimation at ten yard intervals along the longitudinal length of the study section, three investigators estimated the area covered by sand, silt, and plants; the remainder being a marly gravel and actual marl concretions. Average values of the independent estimates were used to calculate the area between the two sampling stations overlain by the two kinds of bottom materials and the area stabilized by plants. The values obtained by these methods were: 11,746 square feet of sand, 4604 square feet of mud, and 4457 square feet of bottom stabilized by plants. Figure 5 indicates the radioactivity of the two fractions for each type of sediment at each of the two stations. Assuming a strictly linear relationship between the sediments accumulated and the tracer settling out with increasing distance, it can be postulated that incorporation follows and may be estimated for the other stations.

The amounts of tracer incorporated in both sand and mud tend to decrease with distance downstream, but they differ in their trends of adsorption. Sand decreases in the amount of tracer adsorbed at the downstream station, while radioactivity on the mud increases at the same station. The indications were that adsorption was a function of the time of contact and possibly the available form of the tracer. Both stations had all samples in situ for the same relative amount of time even though the concentration of tracer in the water mass may have been much more attentuated at the lower station. This appears to follow the findings of Krumholz and Foster (1957) of a more avid uptake of a more dilute isotope.

The procedure in the field was to place the planchets in the stream approximately one half hour before the tracer was due to arrive

Fig. 5.--Illustration of the tracer accumulation among the sediments at Stations 8 and 12. Corrected micromicrocuries per square inch. Mean values of ten random samples at each station.


.21
.19
.17
.15
.13
.11
.09
.07
.05
.03
.01
μμc / in²
Sand incorporated Activity
1440
1640
1840
2040
2240
2440
2640
2840
3040
3240
3440
3640
3840
4040
4240
4440
Feet from Station 8

at each of the two stations, then collect them one half hour after the water sampling was terminated on the day of isotope introduction at that particular station. For the upstream station this amounted to 110 minutes exposure from time of arrival of the tracer, downstream it was 135 minutes because of allowance for the water mass attenuation in the water sampling program. Essentially the relative time of contact was the same. The individual sand and mud fractions at the two stations were approximately the same. They were presumed to have settled out from the same relative speed and type of current. Therefore no physical concentration of the tracer should have occurred at either of the stations. Other possible factors affecting the amount of tracer incorporated would be: the amount of tracer available and the form available. Amounts of free inorganic P^{32} in the watermass were definitely less at the lower station, while combined organic fractions would have been increased and the form changed. The assumption was made that the sediments settling out during the interval of passage of the radioactively spiked water mass were made up largely of dead organisms and their products. The radioactivity they carried was adsorbed previous to their death or ionically bound to their products. At the lower station time had been sufficient for some of the drift from upstream stations to incorporated the adsorbed tracer phosphorus, die, and settle out. Some of these were probably trapped in the sand and mud settling out on to the planchets. If the cells were not broken the incorporated tracer would increase downstream with more and more organisms settling out following the passage of the tagged water mass. It apparently did not. Downstream stations must receive more dead organisms from upstream areas that have high concentrations of incorporated radioactivity and

are subject to faster currents. The sampling interval was not sufficient to indicate a build up of numbers nor accumulated activity so that a rate could be determined.

The radioactivity incorporated by the substrates decreases between the two stations, while the adsorbed radioactivity on the mud substrate increases. This suggests that possibly an accumulation of organically bound tracer may be built up among the finer sediments. The build up may be of phsophorus from broken cells that have been ruptured by the molar action of shifting sands and larger particles in upstream areas. If the released phosphorus products were organic there would be a high probability that they would be ionic and attracted by finer sediments present in the water. A floc may build up and settle out in the quiet areas as a semi-suspended material.

The basis of this hypothesis was from the previously quoted work of Labaw, et al. (1950) in which he stated that bound phosphorus was not released from intact cells that had died. I am suggesting that the adsorbed difference was real and was due to a change of state or form of the phosphorus to an organically bound phosphate which was ejected from the living cell, had an ionic charge, and was not readily incorporated by living organisms present in the sediments or incorporated by organic semi-colloids. This change of form can be seen from the water fractions. The sediments were not examined directly or cultured to determine whether they were composed of living or dead materials nor were they examined for difference in floral or faunal forms present.

Table 6 gives the values of tracer incorporated, adsorbed, the losses between stations, and a per unit area uptake between the two stations sampled.

TABLE 6.--Stations 8 and 12 sediment activity in sand and mud fractions, their losses, and gains. Corrected micromicrocuries per square inch.

Station	Fraction	Incorporated uuc/in^2	Adsorbed uuc/in^2	Between Station Loss uuc/in^2		Station Area in sq. ft.	Station Uptake uuc/in^2	
				Inc.	Adsorbed		Inc.	Adsorbed
8	Sand	0.0986	0.0479				14.198	6.8976
8	Mud	0.0935	0.0269				13.464	6.3072
12	Sand	0.0912	0.0146				13.133	2.1020
12	Mud	0.0497	0.0389				7.157	5.6020
8 - 12	Sand			0.0074	0.0333	134,245		
8 - 12	Mud			0.0438	0.0120			

Holden, (1961) found an exponential decrease of tracer phosphorus in lake water, which he attributed to sediment incorporation. He has suggested that the loss or rate of removal was proportional to the concentration present. From his data he developed the formula:

$$\frac{dc}{dt} = Kc$$

where:

K = the rate constant

c = concentration

t = time

d = differential interval

where the rate constant K can be calculated as the percentage loss in concentration per day. This only applies to the case where the phosphate was removed by a small unit area of the surface, the surface being the surface of the bottom deposits. If the lentic time factor were changed to distance in a lotic environment the values obtained by the formula may also serve to describe the uptake of available radioactivity. The few measurements available from this study only suggest this possibility (Table 7).

Holden further discovered that when 700 micrograms of phosphorus per square centimeter were applied, 85 per cent of the phosphate was taken up by the aerobic zone of the sediments, and only six per cent could be extracted with deci-normal hydrochloric acid. His suggestion was that most of the added phosphorus had been converted to a stable organic form. He further suggests that much of the removed phosphorus may be converted to forms that were unavailable for later release. Although he did not separate his sediments into graded fractions the

TABLE 7.--Sediment fractions uptake by area and rate. Total area of substrate and its loss between stations.

Station	Fraction	Total Area[a] sq. ft.	% Total Area[b] (estimate)	Area Inc.[c] uuc.	Uptake Ads.[d] uuc.	Rate uuc/in^2/min. Inc.	Rate uuc/in^2/min. Ads.	Difference Between Stations in uuc/in^2 Inc.	Difference Between Stations in uuc/in^2 Ads.
8	Sand	37,925	9.93	14,205	25,170	.00089	.00067	0.0074	0.0333
8	Mud		3.91	84,078	9,070	.00081	.00037	0.0438	0.0120
12	Sand	134,245	8.75			.00043	.00011		
12	Mud		3.43			.00024	.00028		

[a]Total Area = Total area between the immediately preceding one and this one.

[b]% Total Area = Estimated area covered by the particular substrate.

[c]Inc. = Incorporated.

[d]Ads. = Adsorbed.

results of the lake studies were nearly comparable to the results of this study. Both suggest that once the material was incorporated from the dissolved inorganic state it was not readily released in an inorganic form. It was also indicated that in both experiments the released adsorbed material was bound to an organic compound. In disagreement, the two experiments do not indicate the fact that aerobic sediments take up activity only at a slow rate. Our study indicates an uptake rate of available radioactivity greater than twice that of either the plankton bacteria or diatoms, and more than their combined radioactivity.

Periphyton or Aufwuchs

Within the West Branch of the Sturgeon River periphyton, composed primarily of diatoms, was the major primary producing unit. Every part of the surface exposed to the water and to light was covered. Because of its nearly universal coverage, and the indications from stomach analyses that it was the base of the food chains in the ecosystem, periodic samples were taken to determine the amount of periphyton and its incorporated P^{32} accruing on plexiglass substrates. The composition had been examined in previous years, particularly by Clifford (1959) who found that Synedra ulna was the overwhelmingly dominant form in two separate studies one year apart. Cymbella sp.; Navicula sp.; Cocconeis sp.; and Gomphonema sp.; were other principal diatoms of the plexiglass substrates (Clifford, 1959). The time of sampling and the sampling interval were important to the colonization of the artificial substrates as indicated by Butcher (1932), Young (1954) and Blum (1957). Peters (1959) reported a marked seasonal

periodicity of algal organisms which attached to artificial substrates in the Red Cedar River, Lansing, Michigan. Of particular interest was the report of Butcher (1932) who could not observe any differences in sessile algae on artificial substrates (glass slides) from that found on natural substrates in the river. Periodicity of species on substrates was not observed during the present study. Diatom chains broke off in the current and dead diatoms were suspended for some distance before settling out. These were sampled quantitatively at all stations at weekly intervals.

Table 8 indicates the types collected, relative abundance, and any possible differences in composition at each sampling site.

Using a plankton net of No. 25 silk bolting cloth and sampling for two minutes at each station an average of 16.43 diatoms per milliliter were collected in the drift. These diatoms were of nine major genera. A two-way analysis of variance indicated no significant difference between stations at the 95 per cent level of confidence. By comparison of the types present in 1958 and 1959 with those of the present study a change in composition was evident. Navicula sp. had replaced Synedra ulna as the overwhelming dominant form and two other genera; Fragilaria sp. and Cocconeis sp., respectively were more abundant than Synedra in number of occurrences by frequency analysis. Table 9 represents the weekly frequency distributions and composite summer frequency distribution. From Table 9 it can be seen that certain genera may have been changed in numbers present, but with the exception of Cymbella sp., and Diatoma sp. the other seven genera show no change, in either percentage composition or total numbers, which would be indicated by a higher frequency. This would signify cyclic changes of

TABLE 8.--Drift diatom composition by station by week, between July 9, 1962 and August 20, 1962. Based on per cent frequency of occurrence of the total composition.

Week of:	Per Cent of Total Composition					
	July 9-16	July 16-23	July 23-30	July 30 Aug. 6	Aug. 6-13	Aug. 13-20
Station 3						
Synedra	12.5	2.63	5.71	17.65	7.69	3.85
Navicula	12.5	36.84	45.71	32.35	30.77	23.08
Fragellaria	62.5	68.18	11.43	0	15.38	69.23
Cymbella	6.25	2.63	2.86	0	7.69	0
Tabellaria	0	0	5.71	0	0	0
Diatoma	0	0	2.86	11.76	23.08	3.85
Cocconeis	6.25	2.63	14.29	23.53	15.38	0
Cocinodiscus	0	0	8.57	5.88	0	0
Surrirella	0	0	2.86	5.88	0	0
Station 8						
Synedra	17.86	22.22	7.32	4.76	16.67	0
Navicula	64.29	66.67	48.78	52.38	83.33	68.75
Fragellaria	3.57	0	12.20	4.76	0	0
Cymbella	3.57	11.11	0	0	0	0
Tabellaria	0	0	14.63	9.52	0	0
Diatoma	0	0	2.44	14.29	0	0
Cocconeis	7.14	0	12.20	0	0	12.50
Cocinodiscus	0	0	0	4.76	0	12.50
Surrirella	0	0	2.44	4.76	0	6.25
Station 14						
Synedra	0	0	8.33	4.26	0	10.34
Navicula	44.44	16.67	38.33	42.55	0	24.14
Fragellaria	33.33	33.33	30.00	36.17	0	0
Cymbella	11.11	16.67	6.67	6.38	0	0
Tabellaria	0	0	0	0	0	0
Diatoma	0	0	0	0	0	31.03
Cocconeis	0	8.33	6.67	4.26	0	24.14
Cocinodiscus	0	0	1.67	0	0	0
Surrirella	0	8.33	1.67	2.13	0	6.90

TABLE 9.--Summer composition of drift diatoms and mean weekly composition during the first six weeks of the study.

Genera	% Summer Composition	% of Total Weekly Composition Among All Stations					
		July 16th	July 23rd	July 30th	Aug. 6th	Aug. 13th	Aug. 20th
Navicula	42.18	42.80	46.30	46.54	47.65	60.37	38.66
Fragellaria	25.17	27.73	29.13	14.24	11.90	5.13	23.08
Cocconeis	9.01	4.31	2.74	9.96	11.12	5.13	9.16
Synedra	8.67	7.66	7.46	11.17	8.34	19.24	4.73
Cymbella	4.93	9.08	10.10	5.72	1.60	2.56	0
Diatoma	4.42	0	0	1.33	7.35	7.69	11.63
Surrirella	2.21	0	2.08	1.74	3.19	0.71	4.38
Tabellaria	1.70	0	0	5.09	2.38	0	0
Cocinodiscus	1.70	0.96	0	2.56	2.66	0	4.16

the composition on the total substrate of the stream.

The only other trends indicated were that generally the same genera were present along the entire length of the study area with minor shifts in total numbers and mean number of individuals, but not genera changes. Two possible seasonal changes were hinted at by Cymbella declining and Diatoma increasing during the interval sampled. These were suggested trends only.

Figure 6 indicates a trend in mean number of diatoms per milliliter which builds up to a peak which lasts for about ten days to two weeks and then rapidly falls off to a low. This period was followed by a slow building up of numbers again.

Welch formula for drift plankters

$$n = \frac{(a\ 1000)\ c}{1}$$

where:

n = number of plankters per liter of the original water

a = average number of plankters in all counts in counting unit of 1 milliliter capacity

c = volume of original concentration

1 = volume of original water mass sampled expressed in liters

$$n = \frac{(24.17 \times 1000)\ 150}{220{,}000} = \frac{3625500}{220} = 16{,}480 \text{ plankters/liter}$$

= 16.48 diatoms/ml.

A two-way analysis of variance revealed no significant difference between stations in the wet weights of the periphyton mass accruing upon the artificial substrates. A similar analysis of radioactivity present on the substrates also revealed no significant difference in uptake rate among the five stations. The conditions for

Fig. 6.--Mean number of diatoms of nine major genera by weeks. Mean number of diatoms by station by week.

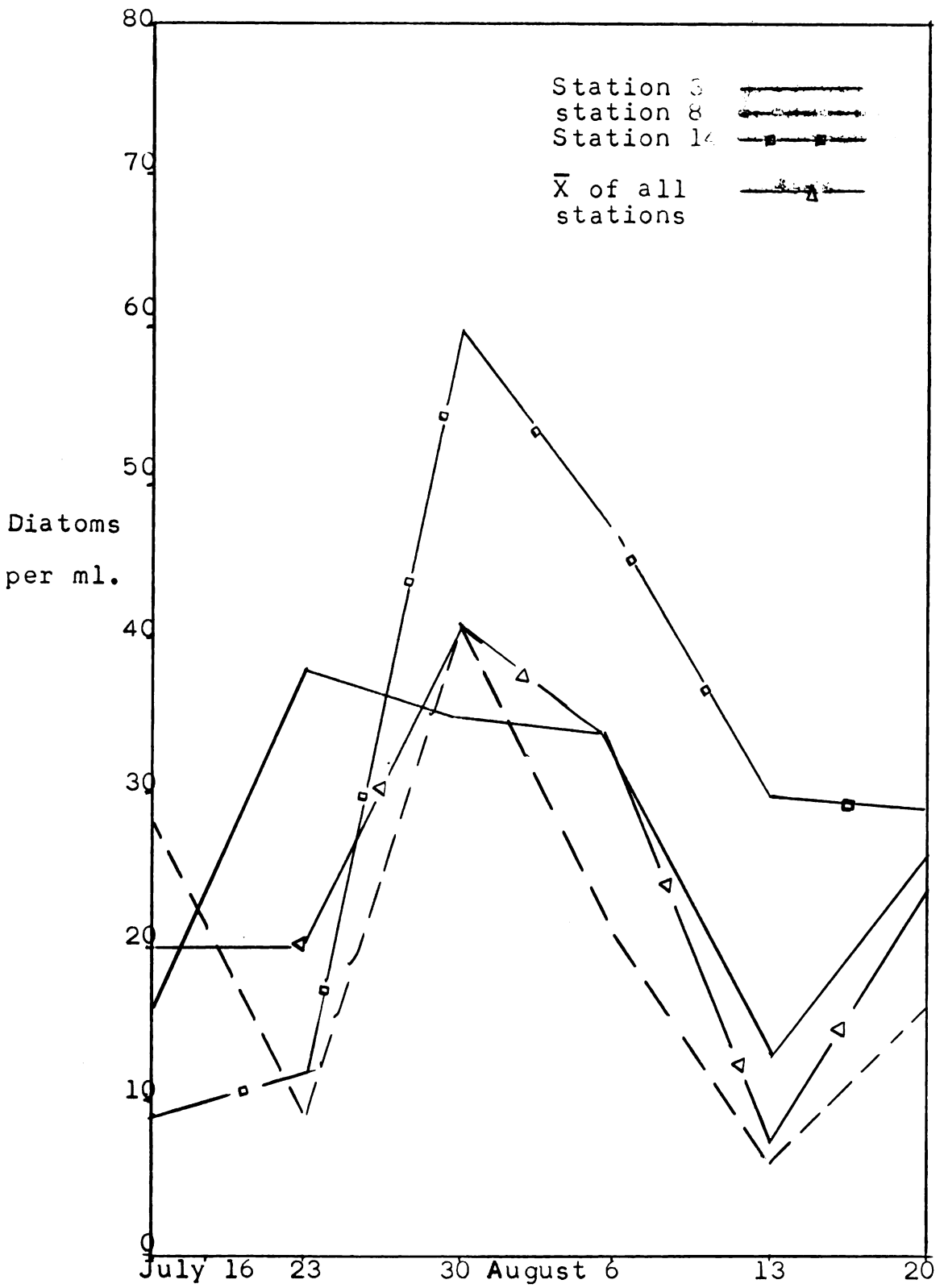
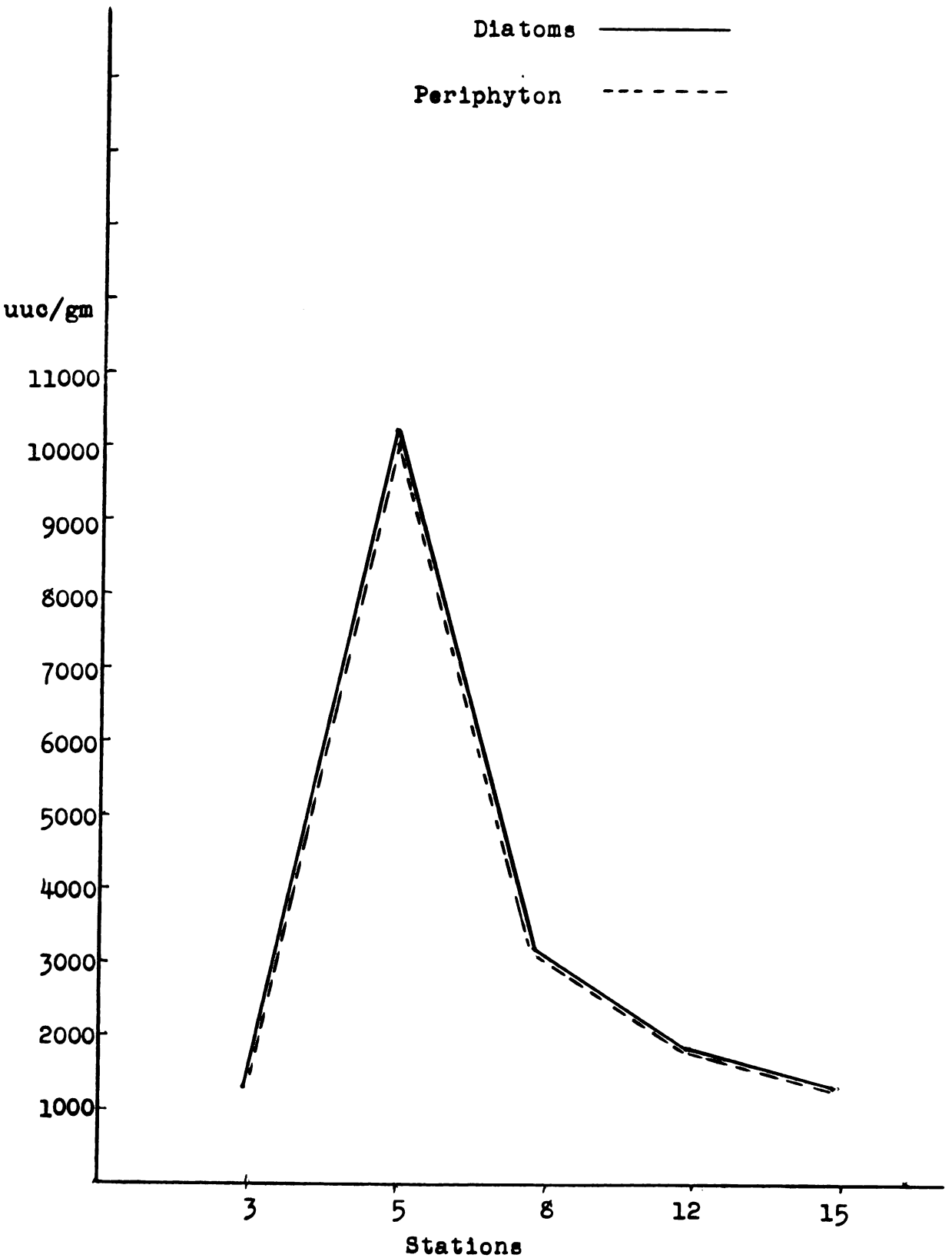

80
70
60
50
40
30
20
10
0
Diatoms per ml.
Station 3
station 8
Station 14
X̄ of all stations
July 16
23
30
August 6
13
20

Fig. 7.--Mean number of drift diatoms by station for total summer and mean radioactivity of periphyton by station.


Diatoms
Periphyton
uuc/gm
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
3
5
8
12
15
Stations

accrual and growth after invasion were as nearly comparable as experimentally possible under field conditions. All stakes were located in nearly identical currents and at uniform depth from the surface. Each stake at a station was placed in an area which received a noon light intensity of 8950 foot-candles the day of placement. Under these conditions two weeks were necessary to obtain a weighable amount of periphyton from two of the substrates at each station (Table 10).

Figures 8 and 9 represent the patterns of uptake of P^{32} at the five major sampling sites. Individually they indicate the following trends:

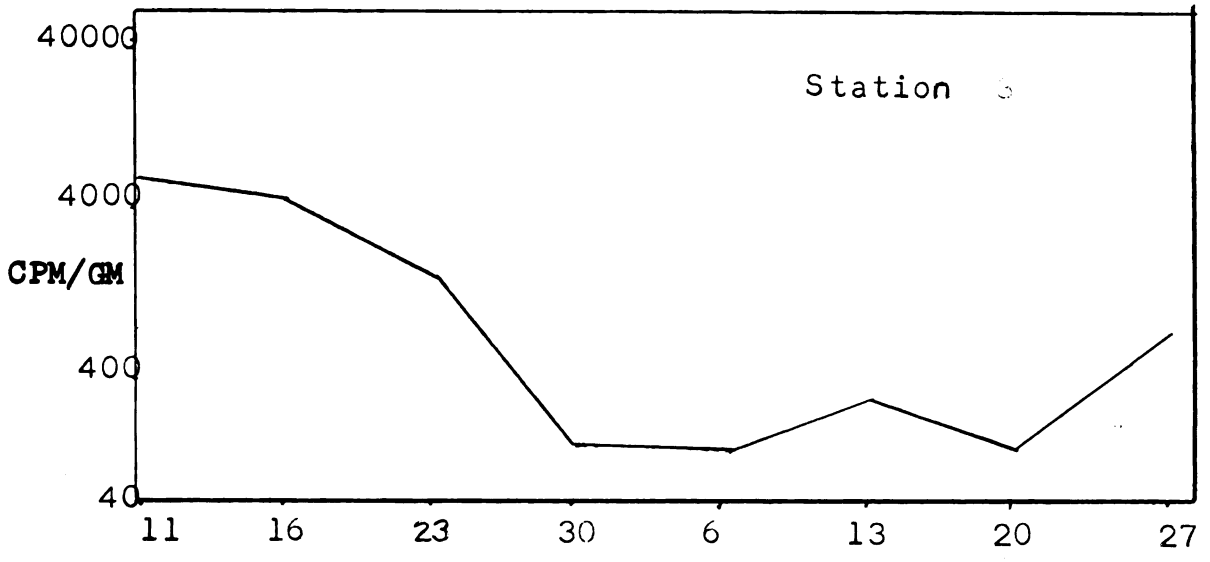
Station 3 had a peak activity of 5,944 cpm/gm on the day of isotope introduction, thereafter it lost activity gradually for four weeks. Sometime between August 6-13 either a recycling from biological forms or from sediments took place and built the activity to a secondary peak on the 27th of August.

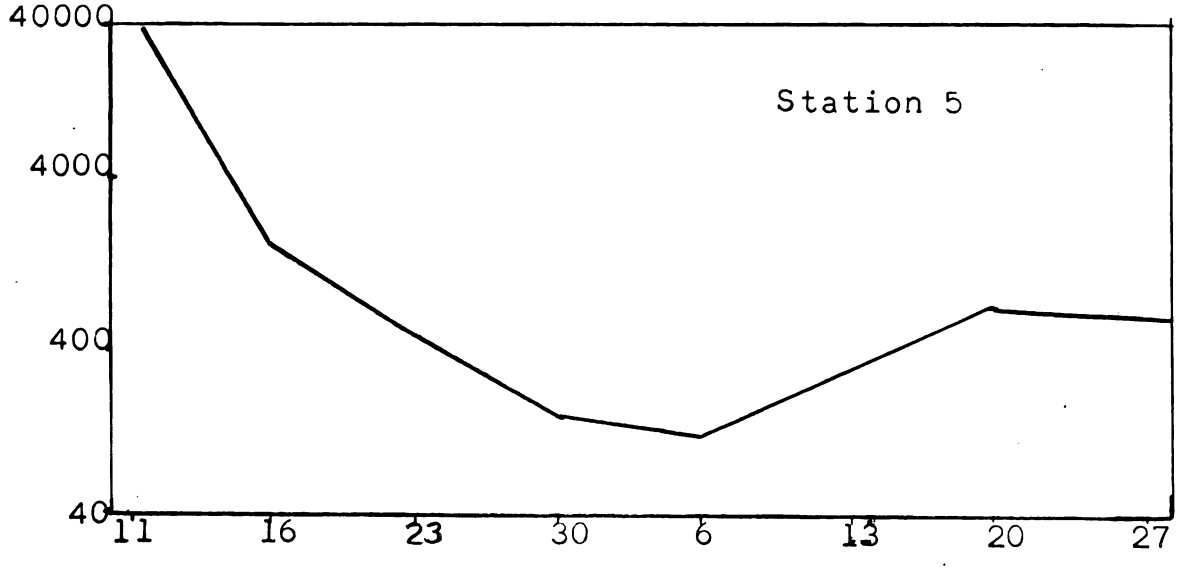
Station 5 had a peak activity of 41,531 cpm/gm on the day of isotope introduction, but a summer mean activity of only 5,716 cpm/gm with the activity reduced to only 1,856 cpm/gm by the second week. It then lost activity at a gradual rate until the 6th-13th period when a recycling occurred at this station also. It built the activity to a secondary peak on the 20th of August. The peak activity on the day of isotope introduction and the summer mean activity at this station were the highest of all five stations studied. The peak activity was presumed to be due to its being the first station sampled at which complete mixing had occurred and at which no backwater areas caused settling out of the suspended activity present in the water mass. The area immediately above this station for 300 yards was composed of

TABLE 10.--Periphyton radioactivity in corrected counts per minute per gram of wet material. Corrected for background and decay.

	Station 3 240 yds.	Station 5 540 yds.	Station 8 1040 yds.	Station 12 2580 yds.	Station 14 3230 yds.	Station 3 tributary	Station 5 tributary
				Activity (cpm/gm.)			
July 11	5,944	41,531	8,315	3,477	945		
16	4,821	1,856	601	1,394	938	3,146	2,428
23	1,296	530	196	207	113	550	151
30	173	197	307	479	238	1,033	296
August 6	133	118	76	113	453	93	26
13	383	301	430	165	123	239	538
20	187	523	782	82	1,227	493	572
27	702	688	650	481	827	2,255	897
				Mean Activity (cpm/gm.)			
	1,704	5,716	1,419	799	608	1,112	701

Fig. 8.--Activity levels of periphyton at Stations 3, 5, and 8. Corrected counts per minute per gram. Corrected for background and decay. Wet weighs only.


40000
4000
CPM/GM
400
40
Station
11
16
23
30
6
13
20
27


40000
4000
400
40
Station 5
11
16
23
30
6
13
20
27

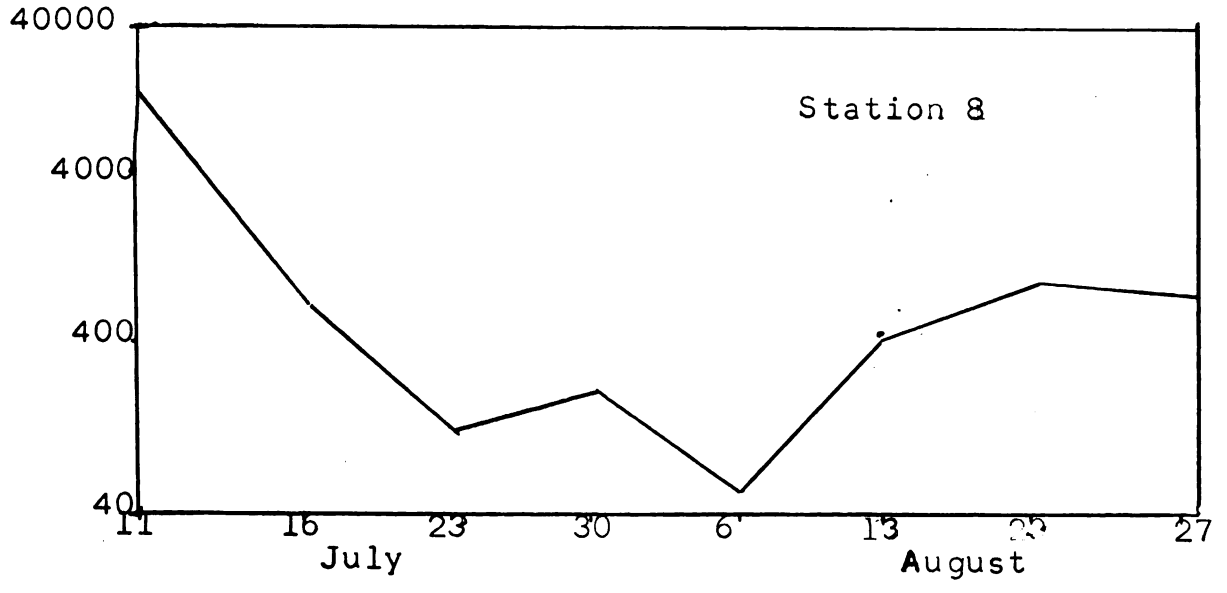
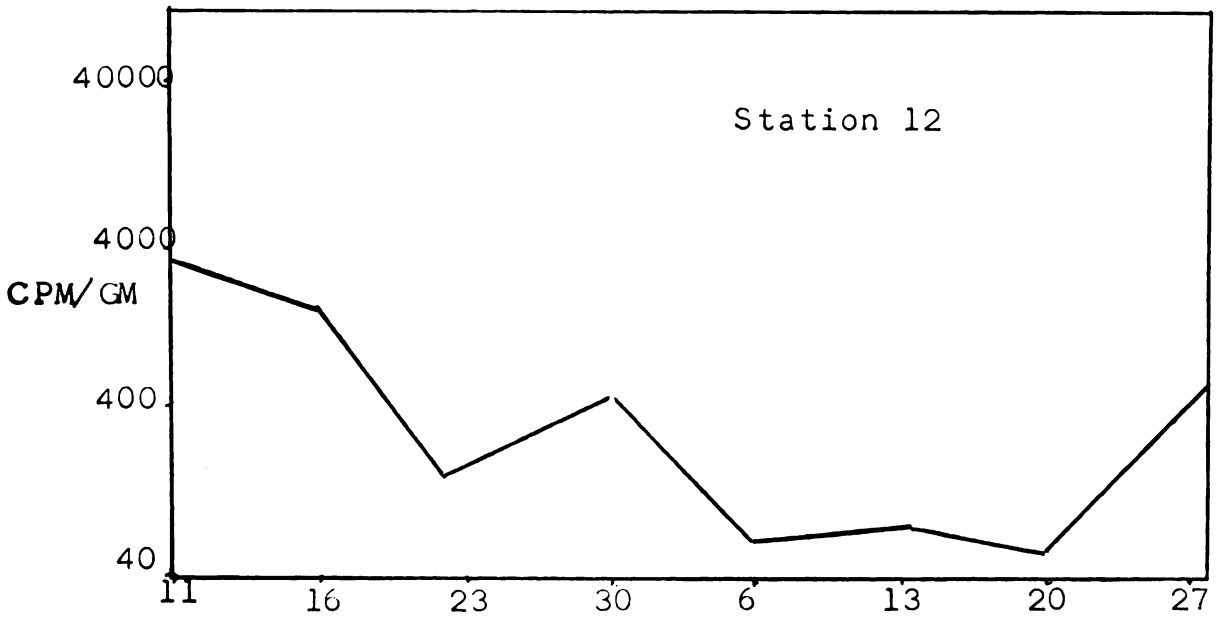
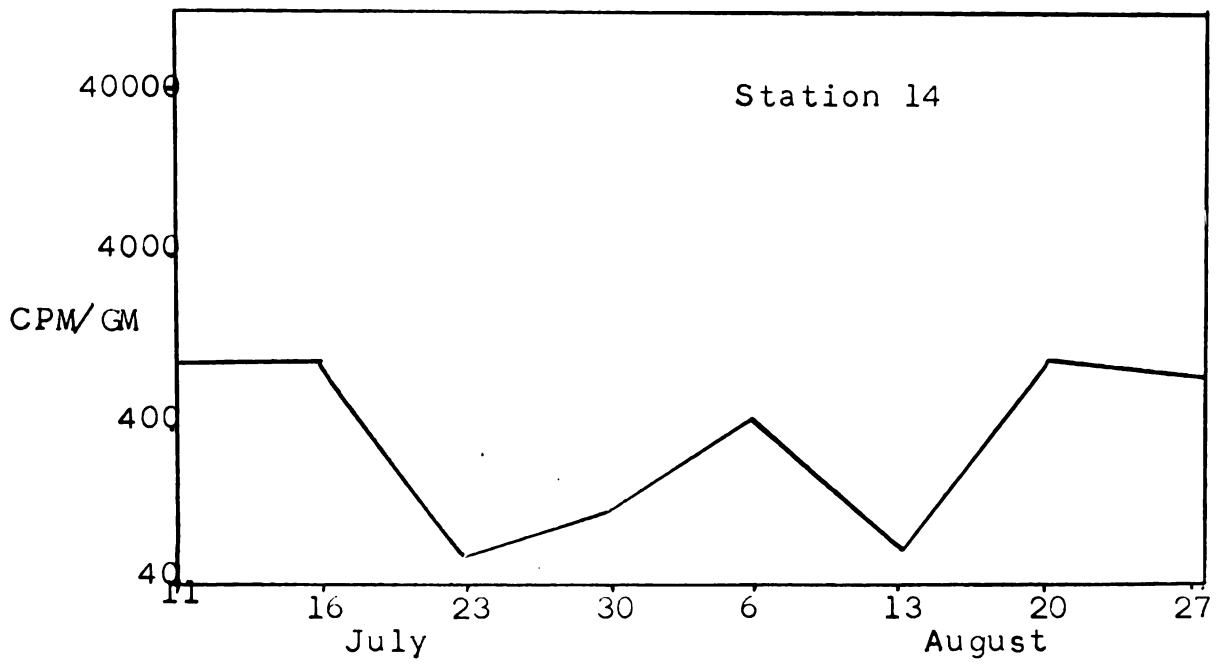

40000
4000
400
40
Station 8
11
16
23
30
6
13
27
July
August

Fig. 9.--Activity levels of periphyton at Stations 12 and 14. Corrected counts per minute per gram of wet material. Corrected for background and decay.


Station 12
40000
4000
400
40
CPM/GM
11
16
23
30
6
13
20
27


Station 14
40000
4000
400
40
CPM/GM
11
16
23
30
6
13
20
27
July
August

a shallow run covered overhead by a nearly complete canopy. No substrates other than the gravel and a few stream improvement structures were present. The current was rapid and no macrophytes were present due to the low level of light (less than 600 foot-candles at noon). Therefore with no competition from macrophytes and near ideal physical conditions it was to be expected that the primary producers would accumulate the most activity at this station, lower stations would have less activity available to them by reason of dilution alone.

Station 8 had a peak activity of 8,315 cpm/gm on the day of isotope introduction and a summer mean activity of 1,419 cpm/gm. The gradual loss of activity was exhibited, but erratic. A low at the station was also exhibited on the 6th of August following the decline, then it built up activity gradually beginning the period after the 6th of August. The secondary peak occurred on August 20th and was followed by a gradual loss. The area peak of activity had been at Station 8 in all other years. The difference this year was presumed to be due to the change in method of isotope introduction which allowed a more complete mixing of the water and isotope earlier in its passage through the study sections.

Station 12 had a peak activity of 3,477 cpm/gm and a summer mean activity of 799 cpm/gm. Distance had decreased the amount of isotope available to the primary producers as had the fact that some of the isotope had changed to organic forms.

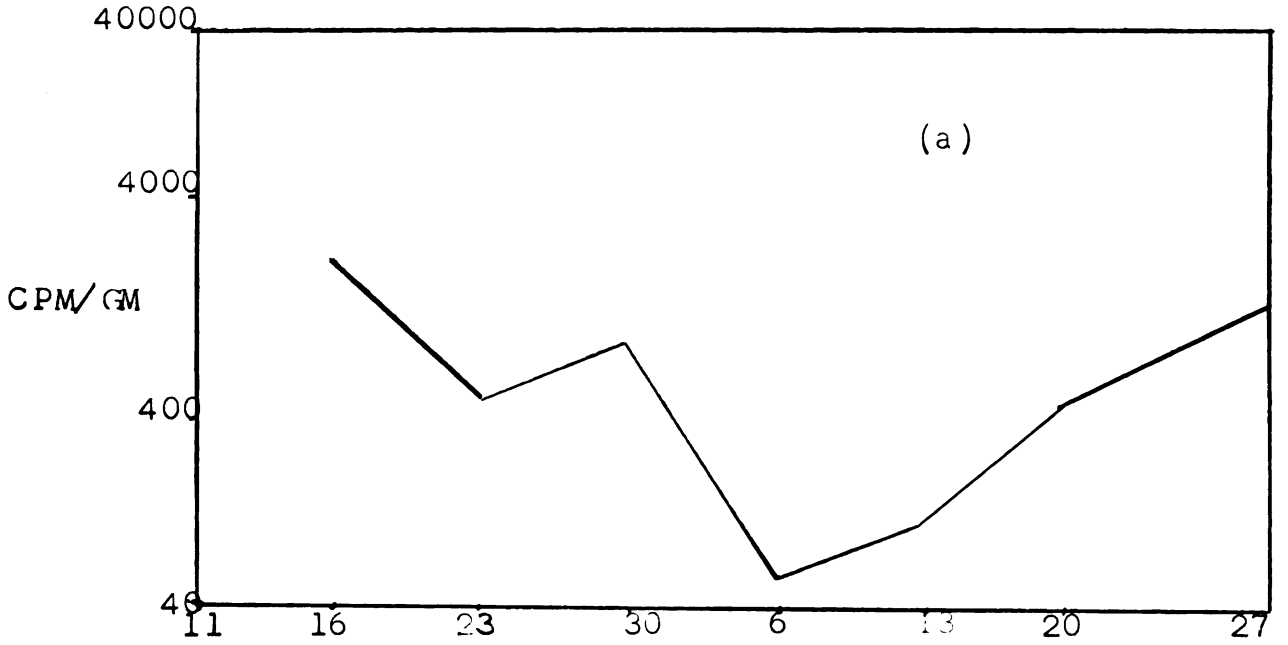
Station 14 had a peak of 945 cpm/gm and a summer mean activity of 608 cpm/gm. The mean activity level indicated does not indicate the high degree of variability at the station. The trend was similar to that exhibited by the other stations up to the end of the fourth week,

but differed on the 6th of August since it was not the date of lowest activity. The following week was the period of lowest activity. Thereafter the activity increased to a secondary peak on August 20th and gradually tapered off the following week. The graphic representation of Station 14 activity indicates the high degree of variability, which was different than that exhibited by any other station. It was presumed that the difference was due to drift organisms, from upstream areas, which may have settled out on the substrates and reattached if living or were wedged into the slime by the force of the current on impact with the substrates. The jammed material may have been either living or dead diatoms.

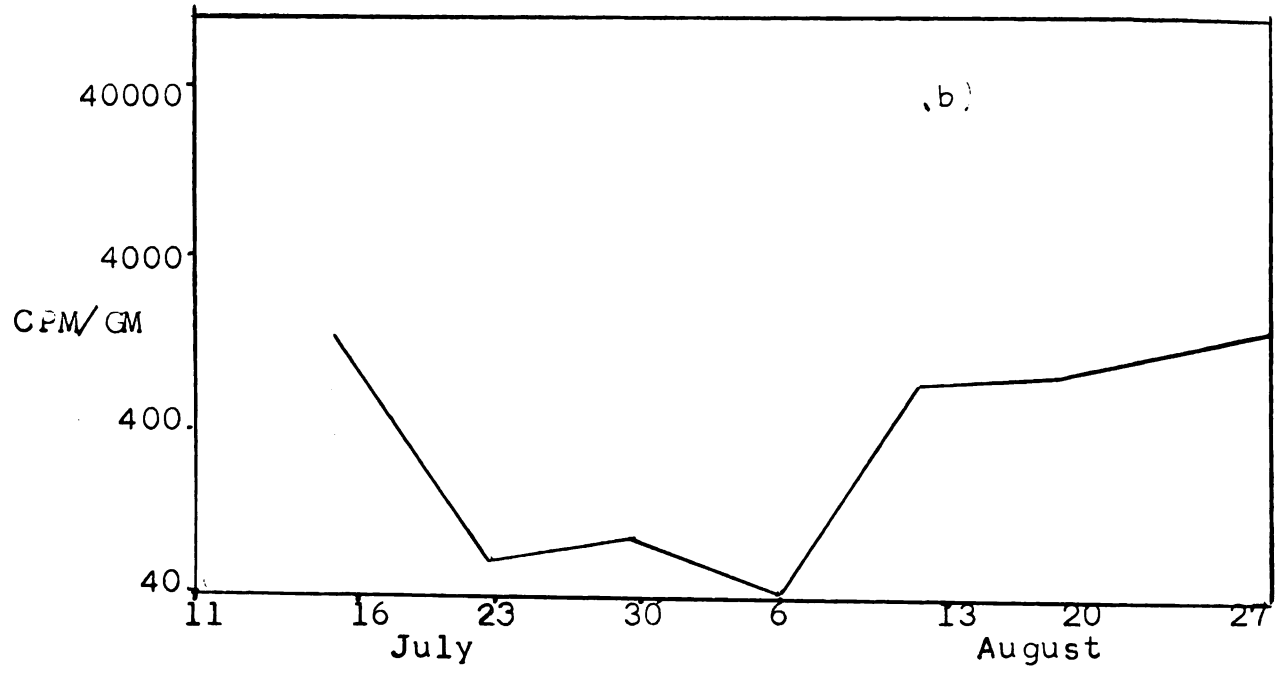
In general, all the stations exhibited a similar pattern of tracer uptake with initial differences due to reduced levels of available isotope and the changed form of the isotope. Peak activity was reached on the day of isotope addition, after which loss was gradual due to attrition of organisms and radioactive decay. The recycling indicated must, in part, be due to renewed periods of log phase growth of the organisms after the first major summer rain. The rain, after a long dry spell, was two days before the sampling date of lowest activity after which the samples accumulated activity for at least three weeks. Similar trends were exhibited during 1960 and 1962 and can be seen in Figure 10.

The slight delay in renewed accumulation of activity evident at Station 14 was presumed to be due to loss of the freed nutrient to upstream organisms. Indications of the renewed growth were evident in samples taken from stakes placed in the tributary, Allan Creek. The stakes present in the tributary were some that had been in position at

Fig. 10.--Activity curves of substrates spiked with radioactive phosphorus at Stations 3 and 5 respectively, and then moved to Allan Creek for the remainder of the study. Counts are corrected for background and decay.


40000
4000
CPM/GM
400
11
16
23
30
6
13
20
27

(a)

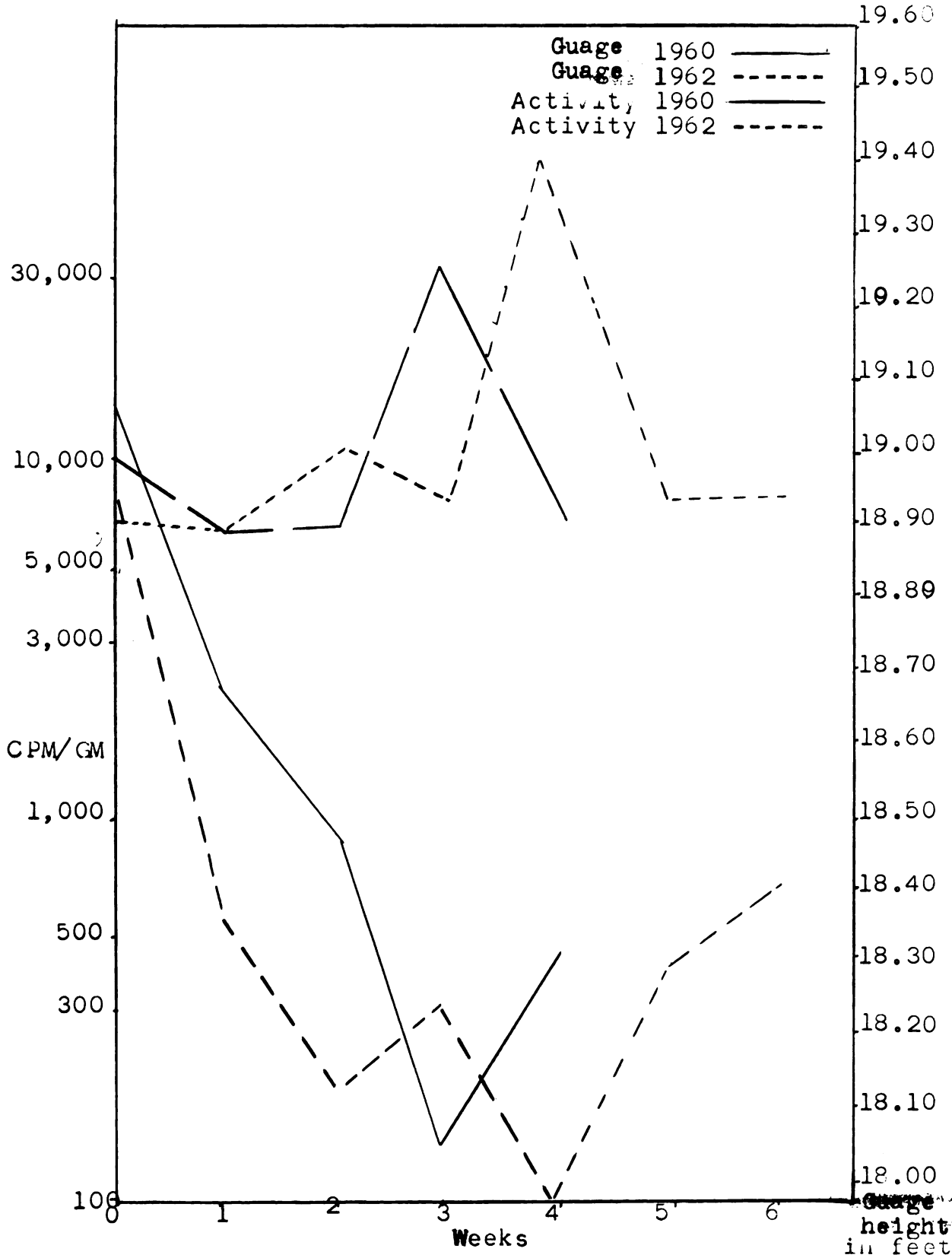

40000
4000
CPM/GM
400
40
11
16
23
30
6
13
20
27
July
August

(b)

Stations 3 and 5 during the interval of P^{32} spike passage the day of isotope introduction. They had been removed from their positions at Stations 3 and 5 immediately after the spike passed and then were placed in Allan Creek, between Stations 5 and 8, to study the effects of attrition and decay without any contributions from recycled P^{32} coming from some upstream area (Figure 11). It was evident that the pattern of loss and accumulation after four weeks was the same as that in the mainstream, but at a reduced level of activity. These results suggest that either the isotope was adsorbed differently by the artificial substrates or that the activity present was coming from fallout washed into the stream after the heavy rain. Foliage from the area upstream in the tributary was collected and counted for radioactivity, none was found that counted above cosmic radiation levels. Therefore, the activity on the substrates was different or some organisms that carried activity had moved on to the substrates and when picked off, left deposited activity.

The similar patterns of all stakes in the main stream and those in the tributary would not suggest a difference in substrate uptake, especially after thorough mixing of the water. The conclusion offered was that several organisms that carried radioactivity may have moved into the tributary out of the main stream and while grazing on the periphyton present on our artificial substrates, they released some wastes that adhered to the slime and the similarity of patterns of its build up and loss was due to coincidence. The pattern exhibited by the activity in the main stream would appear to be a true representation of tracer released by molar action of the materials carried by a swollen stream working against the substrates. The large increase in

Fig. 11.--Periphyton radioactivity levels and corresponding water levels. Counts per minute per gram of wet material, corrected for background and decay.


Guage 1960
Guage 1962
Activity 1960
Activity 1962
30,000
10,000
5,000
3,000
CPM/GM
1,000
500
300
100
0
1
2
3
4
5
6
Weeks
19.60
19.50
19.40
19.30
19.20
19.10
19.00
18.90
18.80
18.70
18.60
18.50
18.40
18.30
18.20
18.10
18.00
Guage height in feet

radioactivity was presumed to have come from the activity adsorbed and incorporated among the stream bottom substrates which were altered by the storm waters.

Compared with other years the uptake patterns were similar with the exception that the peak uptake was at Station 5 five-hundred years upstream from the point of maximum uptake in the four other years of tracer study on this stream. This difference may be due to differences in the modes of isotope introduction. A point source introduction of isotope had been made in previous years, this year a whole transect introduction had been made. The difference in mixing was evident in the dye front placed ahead of the isotope to alert the sampling crew.

The organisms making up the periphyton complex and their physiological state at a given moment greatly influence the pattern of uptake. It has been found by numerous authors that phosphorus is rapidly taken up by algae when it was normally deficient in the system. As an example, Rodhe (1948) found that Scenedesmus sp. could assimilate sufficient phosphorus in one day to fulfill a phosphate debt, but accumulation beyond this level was very slow, requiring as much as seven days to accumulate. In opposition were the findings of Einsele (1941) and Lund (1950) who agreed that planktonic algae were capable of storing up to ten times the necessary amount of phosphorus per cell. Lund further illustrated that algae can take up phosphorus from lake water when the concentration was as low as one part per billion. His work was conducted on the alga, Asterionella formosa in various English lakes. Grzenda (1960) found the same tendency in periphyton of the Red Cedar River in Michigan. These findings indicate the reason for a peak accumulation of radioactivity the day of introduction followed by

a gradual loss of activity for four weeks. At Station 14 it could be explained by the slight change in activity the first two weeks and the trend at all the lower stations, below Station 5, to be slightly erratic while slowly accumulating small amounts of radioactivity. The secondary peak of activity came one week after the peak number of diatoms occurred in the drift and during the summer low of drift diatoms at all stations. The diatoms and activity increased to a secondary peak together at a rapid rate for two or three weeks then leveled off (Figure 11).

Renewed growth of the diatoms may have occurred when nitrogen and phosphorus were washed into the stream after a heavy summer shower. During the dry period preceding the rain both may have been limiting, but phosphorus was more so, since the nitrogen to phosphorus ratio had been found to be 10:1 by Correll (1958) when it was commonly thought to be 5:1 in other aquatic studies, and this ratio probably was at least at this level during the entire study.

A change of form of the phosphate may have occurred if it followed the respiration or anabolism cycle previously discussed under the fractionation study. Expulsion of wastes that were in an organic form that was not readily taken up by other organisms may have built up a reserve of radioactive phosphorus that was of a form available to some decomposers after some time had elapsed, but the nitrogen may have been too low for their requirements. The rain increased both of the nutrients and triggered a recycling of the phosphorus.

The pool of total phosphorus in the water mass during the summer was only 7 p.p.b. (Correll, 1958) of which only about 20 to 25 per cent was indicated as being incorporated in diatoms. The total phosphorus pools for the diatom periphyton were dependent on a valid

biomass estimate and knowledge of the turnover rates. This year's figures were only rough indications on a wet weight basis. The average wet weight accruing upon artificial substrates in the dark area was 0.00113 gms/dm^2 and for the light area, 0.0006 gms/dm^2 per week.

Losses from the periphyton were usually not rapid, Whittaker (1953) found in aquaria studies that periphyton did not show a loss of P^{32} until the second week. During the initial uptake from the spike some P^{32} may exchange rapidly with either stable or tracer phosphorus already accumulated or it may be rapidly cycled as Calvin and Benson (1949 and 1950) have indicated. The uptake curves would indicate a rapid uptake of 1 day maximum duration and the author has found a five second minimum possible for diatoms, after which loss was constant and began immediately.

Macrophytes

Plants have several possible modes of tracer accumulation through any or all of three different structures, i.e. leaves, stems, and roots. The methods of tracer accumulation encompass external adsorption, ionic exchange, and active internal ion accumulation which requires the expenditureof energy. In this study it was assumed that the higher aquatics received their tracer phosphorus by either of the last two methods since washing the samples with 0.01 N hydrochloric acid released little or no activity during the first two weeks after isotope introduction. Thereafter, the acid washings were discontinued. Chara sp. may have obtained some tracer through its root-like structures, but this was assumed not the case.

During four years of study (1959 through 1962) there was wide

variability among the macrophyte biomass data. In the first two years introductions of inorganic superphosphate fertilizers were made before the tracer introductions, in the latter two years none was made. Between the two series of two years (1959-1960 and 1961-1962) there was wide variation in both biomass and activity.

With 300 pounds of low analysis fertilizer (12-12-12), introduced as inorganic phosphate before the isotope, the highest levels of accumulated radioactivity were recorded for aquatic plants. With 240 pounds of high analysis fertilizer (21-53-0) added in 1960 (Zettelmaier) the tracer accumulation in all plants studied was very erratic. The variability was high both within and among species. The peak radioactivity at Station 8, one day after introduction, in the water moss, Fontinalis antipyretica was approximately 1/8 the 1959 peak at the same station. A second peak of activity accumulated late in the summer of 1960 which was higher than that exhibited on the day of tracer addition. Several species of the plants studied indicated a similar secondary peak in 1960, but not in any of the other three years.

With no fertilizer added before the isotope introduction in 1961 and 1962, and a tracer addition in 1961 as an organically bound form (incorporated in Escherichia coli 0111), the peak of activity in Fontinalis was approximately one-tenth that found during 1959, the low analysis year, and approximately one-third that of the following year 1960 when high analysis fertilizer was added. The peak activity in 1962 followed the pattern exhibited during 1959 and 1961 when P^{32} was introduced as an incorporated organic form and about one-tenth that present in 1959 when added as inorganic P^{32}.

Figures 12, 13, and 14 represent the peak activity levels in

Fig. 12.--Comparative Station 8 uptake and loss for Chara sp. **Corrected micromicrocuries** per gram of wet plant material.

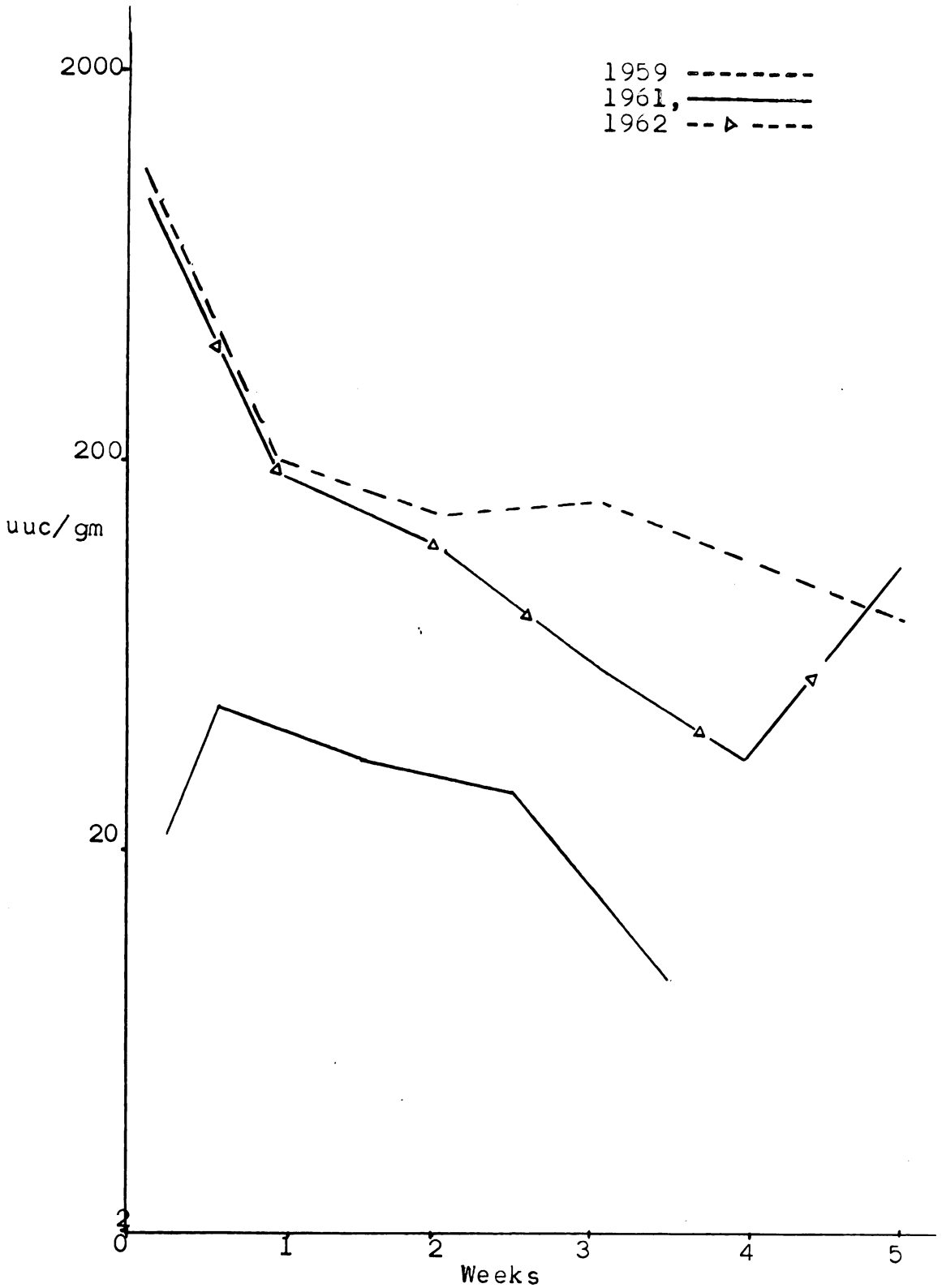

2000
200
20
2
uuc/gm
1959
1961,
1962
0
1
2
3
4
5
Weeks

Fig. 13.--Comparative Station 8 uptake and loss for Fontinalis antipyretica. Corrected micromicrocuries per gram of wet plant material.

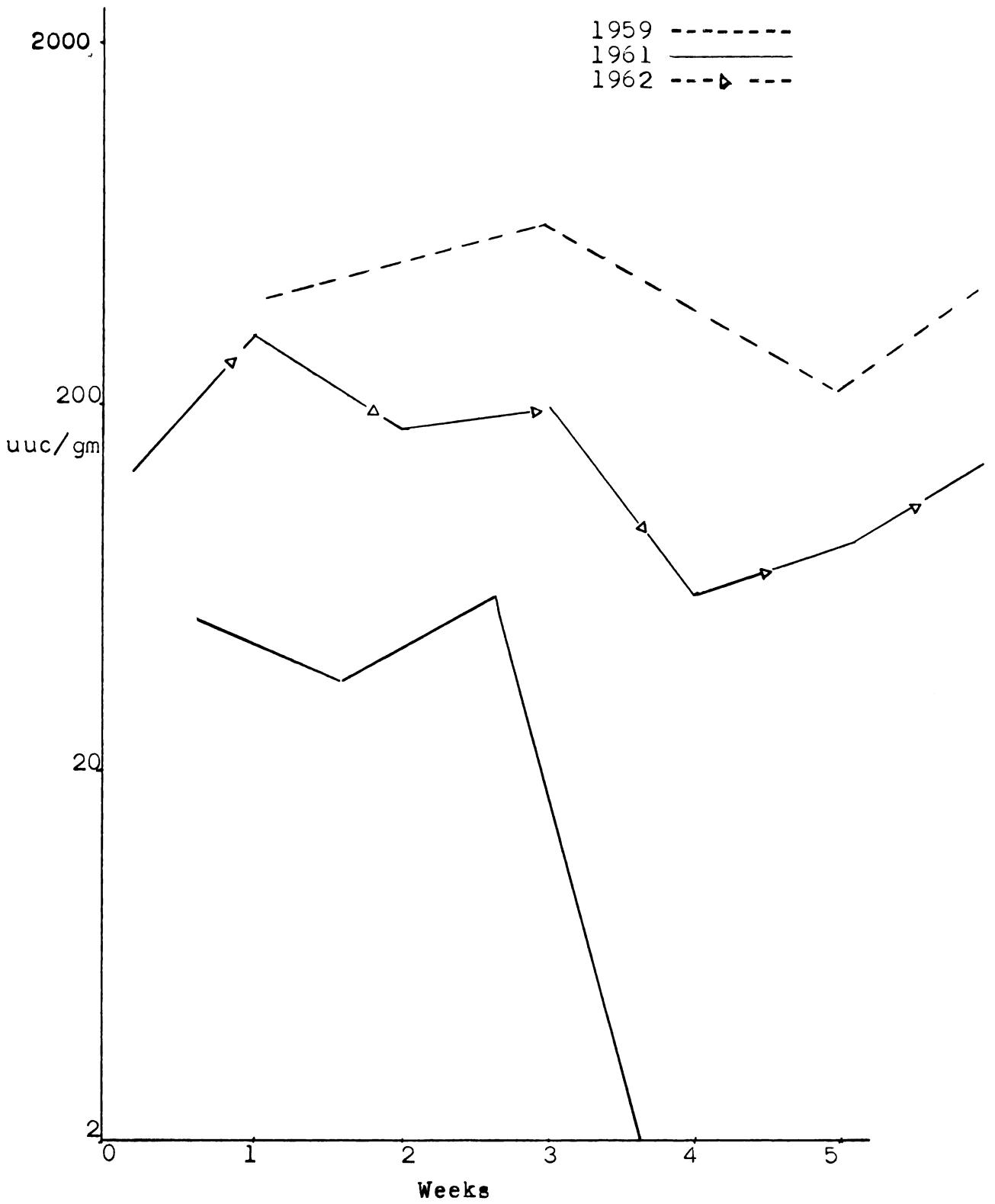
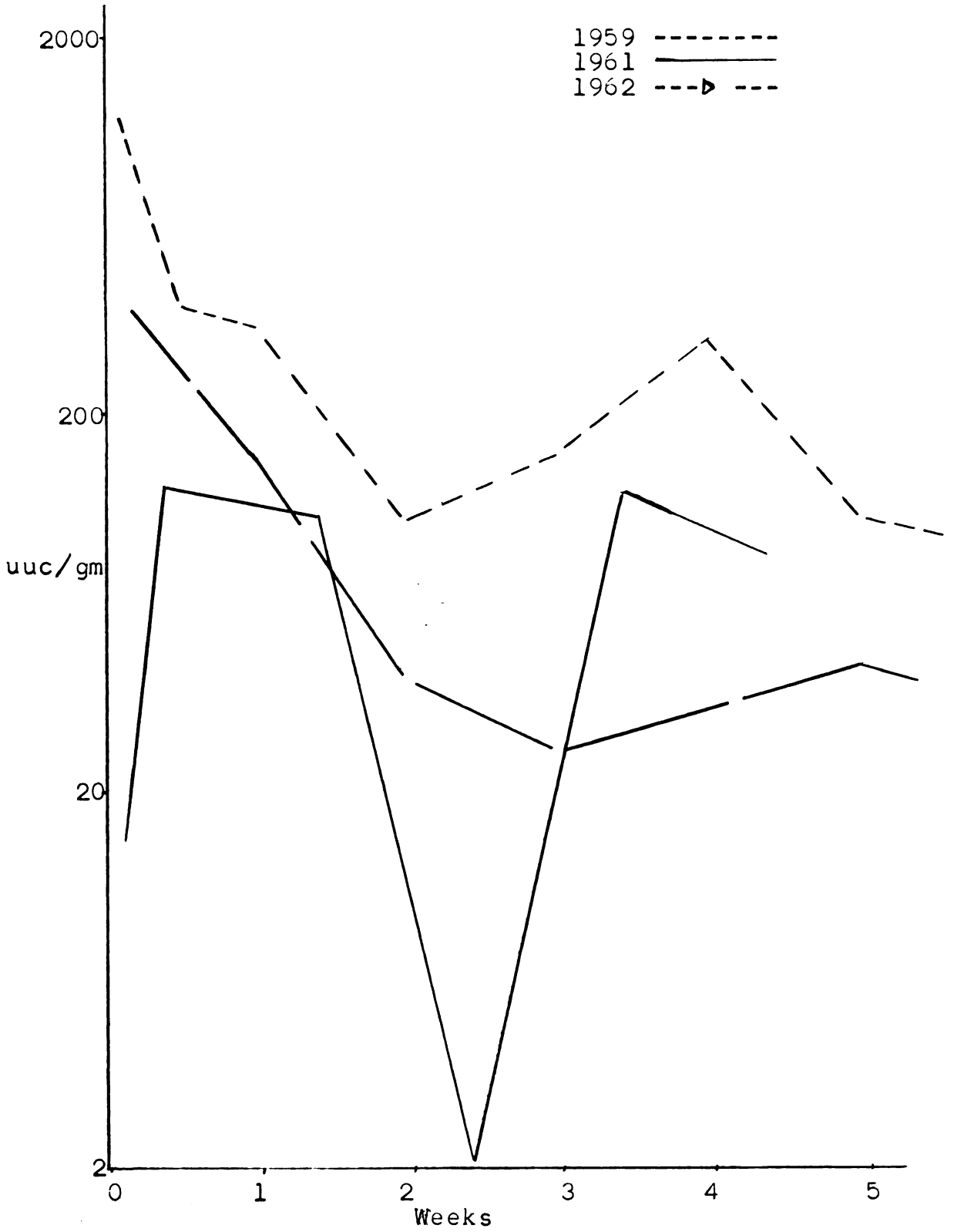

1959
1961
1962
2000
200
uuc/gm
20
2
0
1
2
3
4
5
Weeks

Fig. 14.--Comparative Station 8 uptake and loss for Potamogeton **sp. Corrected micromicrocuries per gram of wet plant material.**


1959
1961
1962
2000
200
20
2
uuc/gm
0
1
2
3
4
5
Weeks

plants collected during 1962. They are comparisons of the trends of activity during 1959 (Knight), 1961 (Bender) and the present study. Generally, the patterns of uptake agree in all three years with the agreement between the present year and 1959 especially good. Differences between the two years, 1959 and 1962, are only in order of magnitude as are the differences between the 1961 data of Bender and the present study. All species except Nasturium officinale exhibit their largest concentration of activity at all stations on July 11, 1962 then progressively decline to a low value between August 6th and 13th. This seemed to be a period of initiation of another increasing cycle of activity accumulation which reached its peak on August 27th.

Only Station 8, is illustrated in Figures 12, 13, and 14. Trends for all five plant species during the study were indicative of the initially lower concentrations available at the progressive downstream stations. The figures also indicate a slow decline in accumulated activity for five or six weeks, then a secondary accumulation of activity.

Nasturium officinale, the exception mentioned above, had a one week lag in activity accumulation. Other exceptions were on individual dates, i.e. July 30, for Ranunculus sp. and Chara sp. which were evaluated and found to have a level of tracer accumulation much higher than either the preceding or following weeks. No measurement or machine error was indicated or found by rechecking. The two samples were processed as usual which includes a washing to remove silt and periphyton, therefore it was presumed that entrapped detritus or invertebrates did not account for the difference, nor did adsorbed materials since the plants were washed with dilute acid before mineralization. I can

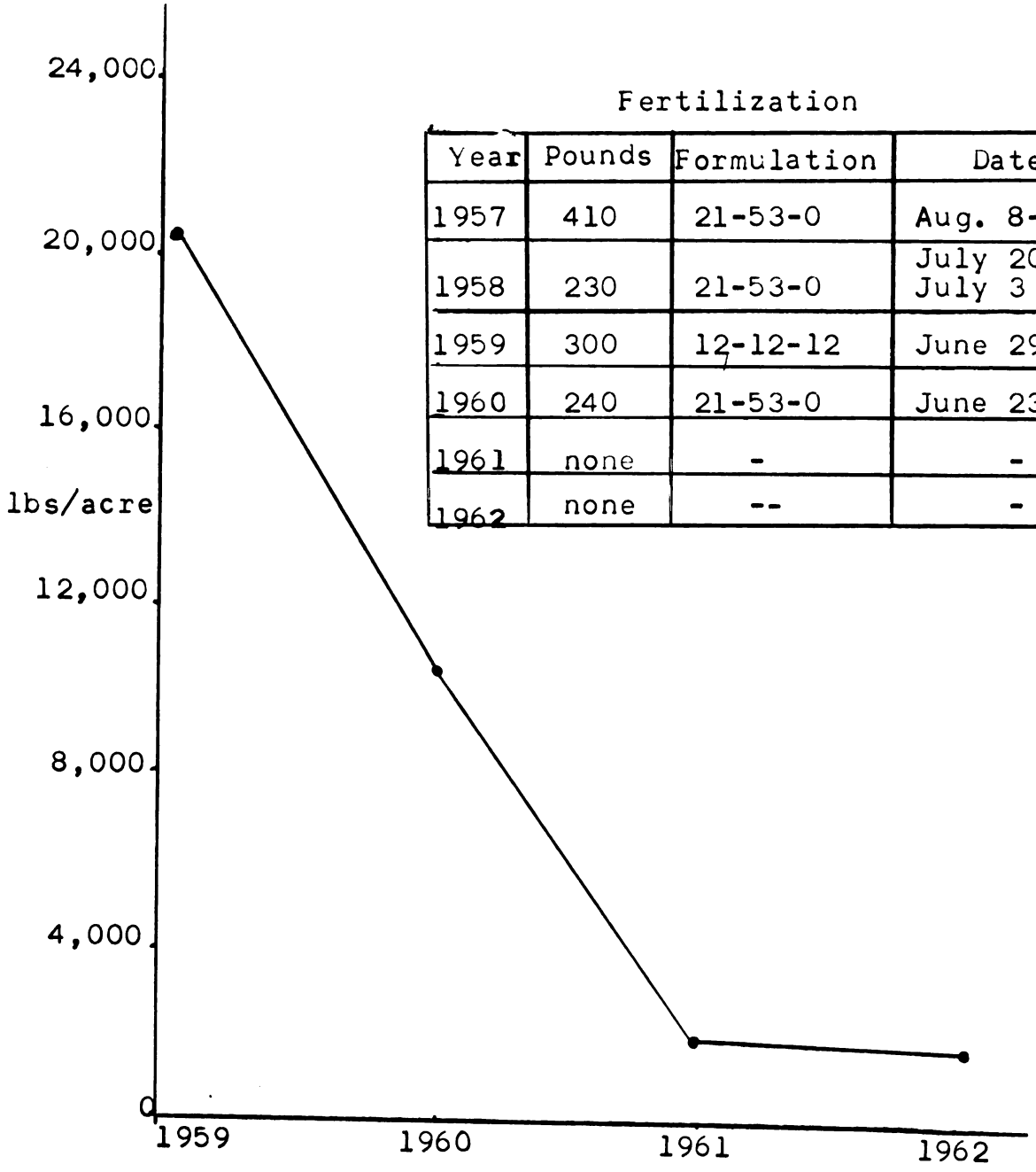
offer no explanation for the erratic values exhibited.

There was a strong indication that fertilization with low concentrations or low analysis fertilizer can cause an increase of tagged phosphorus utilization in macrophytes for as much as a mile or more downstream within a three month period. The increase was only an indication from the data (Figure 12, 13, and 14) as was the possible increases in biomass with similar fertilization (Figure 15). With large amounts of fertilizer added or a high analysis fertilizer it would appear that a disruptive pulse of stable phosphorus was produced which affected P^{32} uptake in macrophytes adversely.

The pulse of stable phosphorus may have been stored in a dense periphyton mat immediately below the point of introduction or it may have been stored as a precipitated floc of tricalcium phosphate immediately below the point of introduction (Keup, M.S., 1958). The excess P^{31} may have been cast into new pools or reservoirs which formed and then periodically released some of the stable and some of the tagged phosphorus by unknown methods, with no particular pattern other than a possible correlation with the first major summer rain after the isotope introduction. The high concentration of phosphorus (P^{31}) provided by the fertilizer may have made either nitrogen or some minor element a limiting factor which was renewed by runoff from rains.

Figures 16 through 25 exhibit the pattern of tracer uptake by macrophytes during eight weeks of the summer of 1962 and Table 11 gives the specific uptake and Table 12 gives the mean uptake and standard deviations. They all indicate a high initial incorporation of radiophosphorus followed by a gradual loss for a period of five to six weeks and then a minor secondary accumulation of P^{32}. Fontinalis antipyretica

Fig. 15.--Levels of standing crop of macrophytes as related to fertilization.



Fertilization

Year	Pounds	Formulation	Dates
1957	410	21-53-0	Aug. 8-17
1958	230	21-53-0	July 20-26 July 3
1959	300	12-12-12	June 29-July 6
1960	240	21-53-0	June 23-29
1961	none	-	-
1962	none	--	-

Fig. 16.--Stations 3, 5, and 8 Fontinalis antipyretica activity levels. Corrected micromicrocuries per gram of wet plant material.

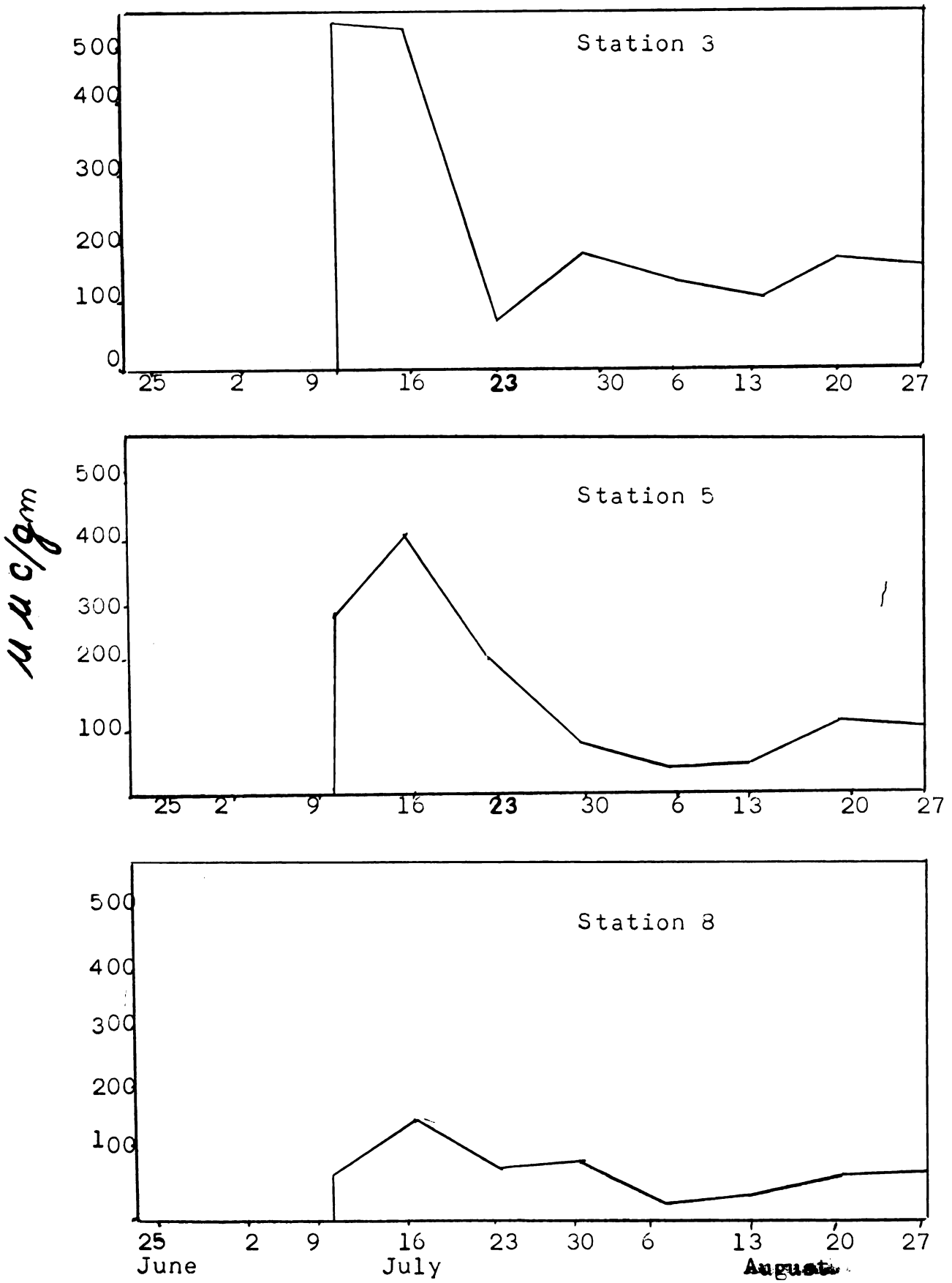
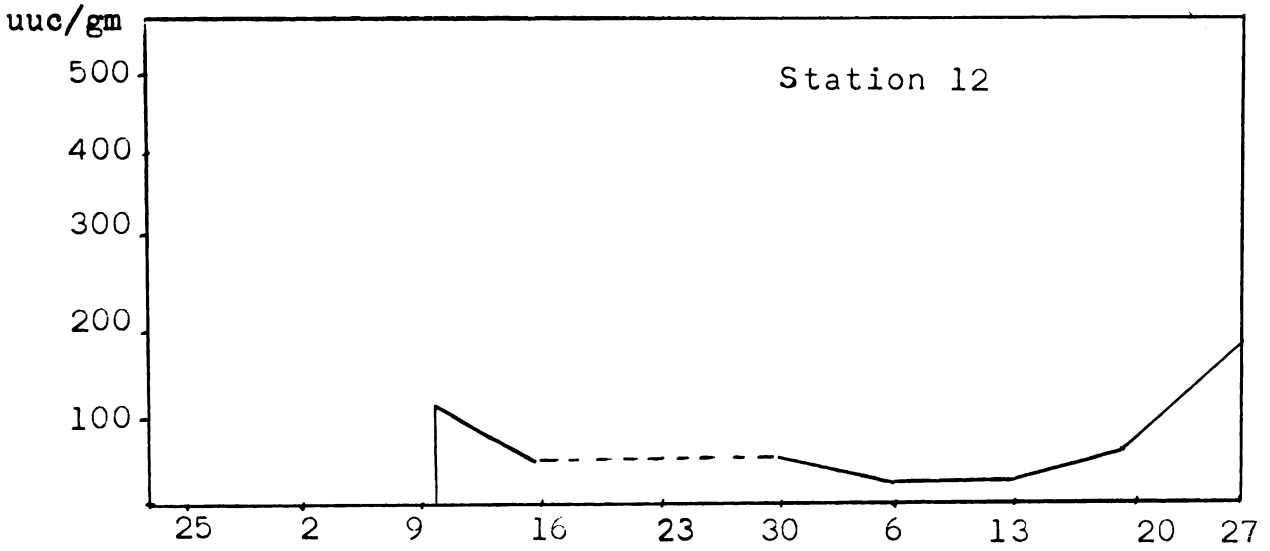

Station 3
Station 5
Station 8
μμc/gm
500
400
300
200
100
0
25
2
9
16
23
30
6
13
20
27
June
July
August

Fig. 17.--Stations 12 and 14 Fontinalis antipyretica activity levels. Corrected micromicrocuries per gram of wet plant material.


uuc/gm
500
400
300
200
100
Station 12
25
2
9
16
23
30
6
13
20
27

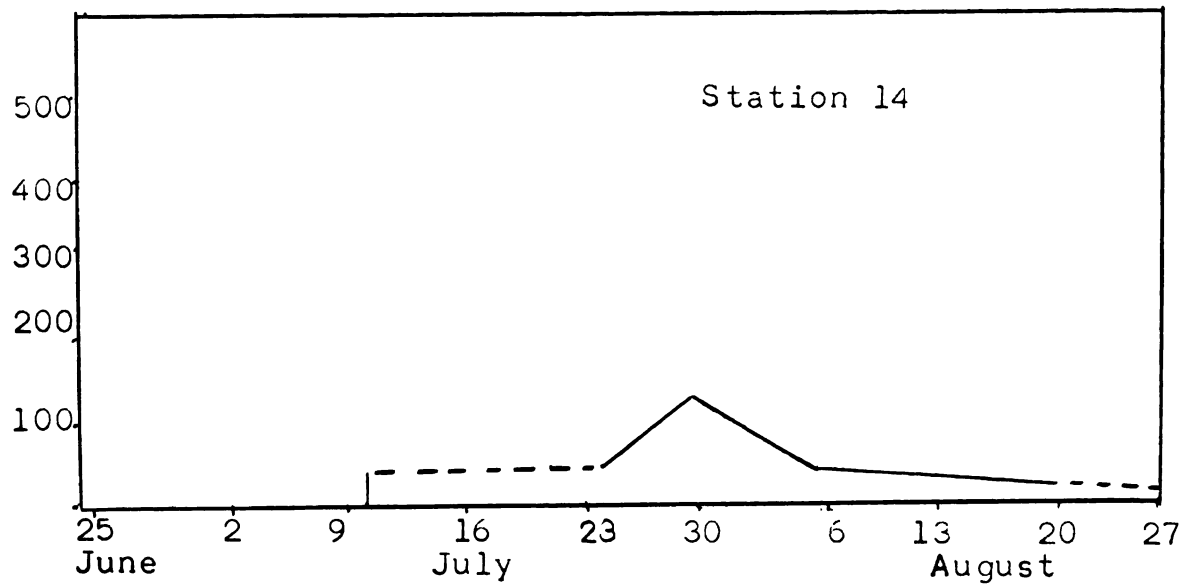

500
400
300
200
100
Station 14
25
June
2
9
16
July
23
30
6
13
August
20
27

Fig. 18.--Stations 3, 5, and 8 Potamogeton sp. activity levels. Corrected micromicrocuries per gram of wet plant material.

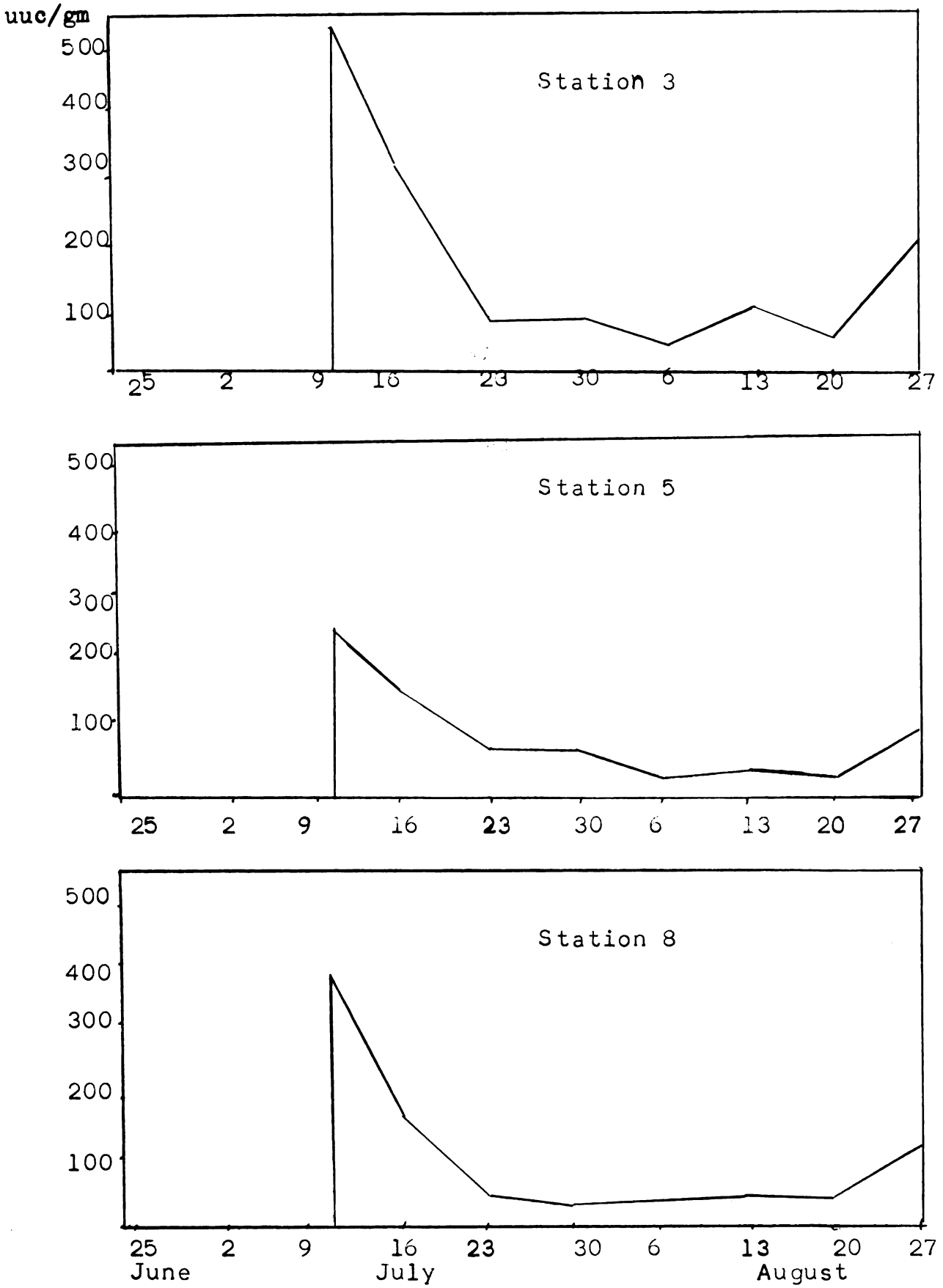
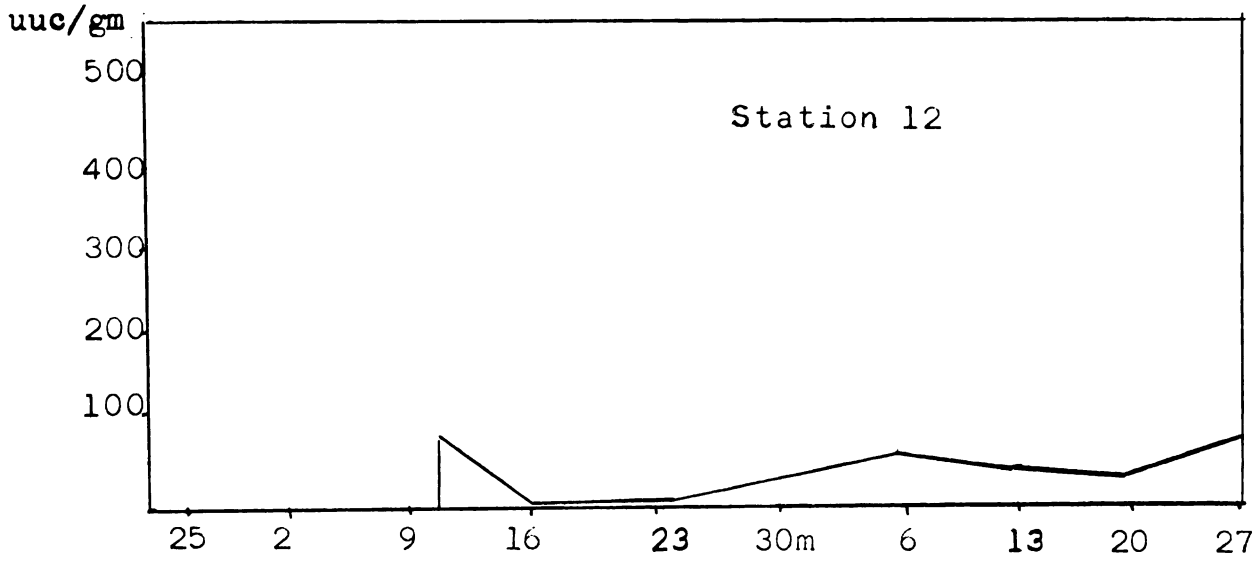

uuc/gm
500
400
300
200
100
Station 3
25
2
9
16
23
30
6
13
20
27
500
400
300
200
100
Station 5
25
2
9
16
23
30
6
13
20
27
500
400
300
200
100
Station 8
25
June
2
9
16
July
23
30
6
13
August
20
27

Fig. 19.--Stations 12 and 14 Potamogeton sp. activity levels. Corrected micromicrocuries per gram of wet plant material.


uuc/gm
500
400
300
200
100
Station 12
25
2
9
16
23
30m
6
13
20
27

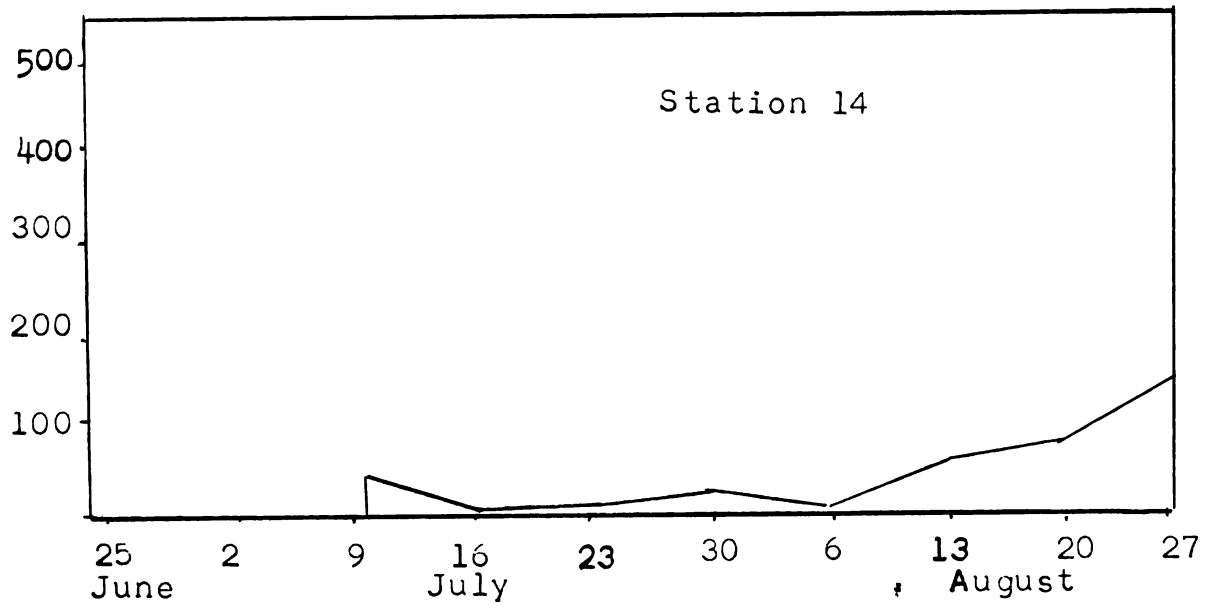

500
400
300
200
100
Station 14
25
June
2
9
16
July
23
30
6
13
August
20
27

Fig. 20.--Stations 3, 5, and 8 Chara sp. activity levels. Corrected micromicrocuries per gram of wet plant material.

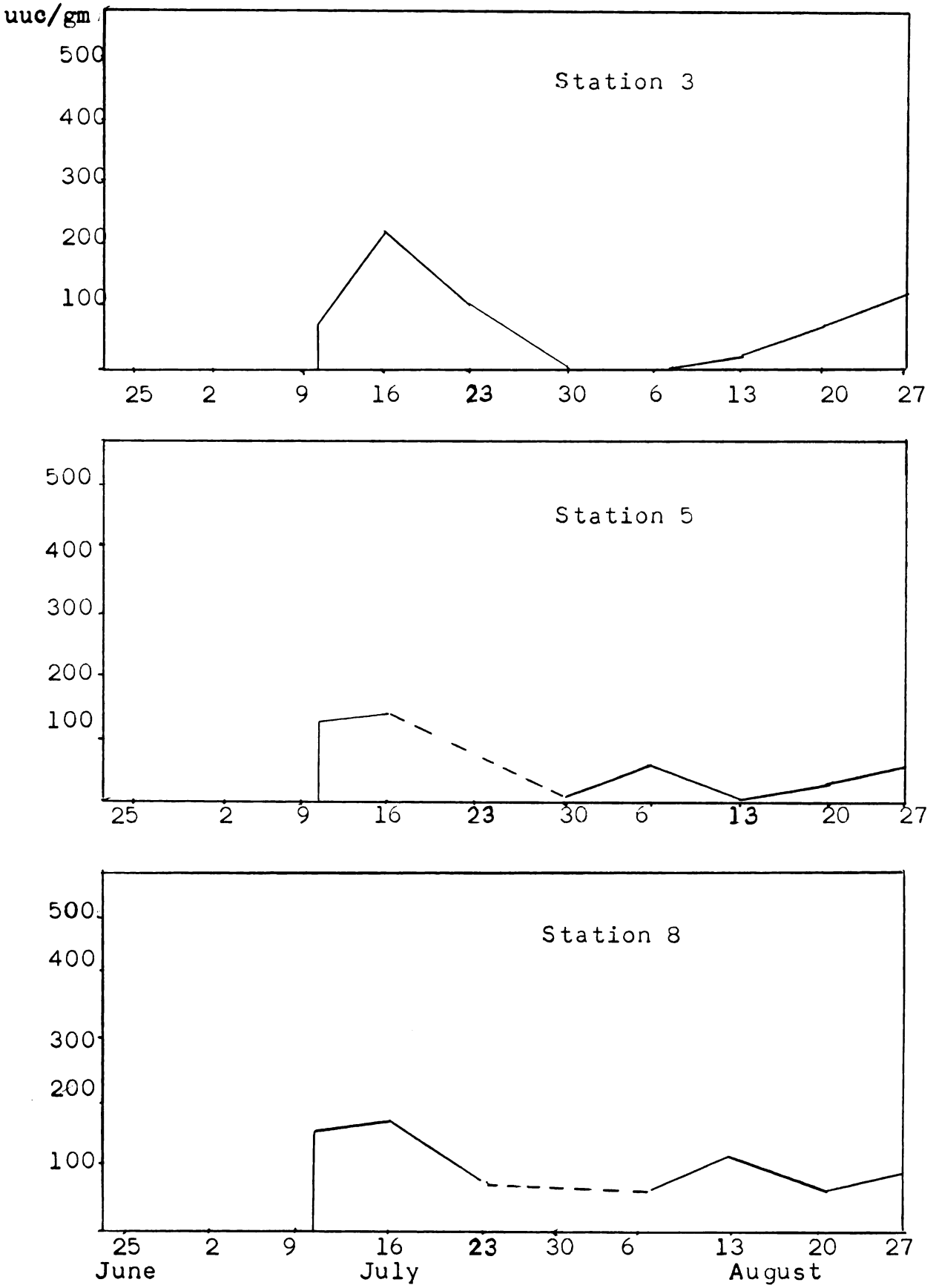
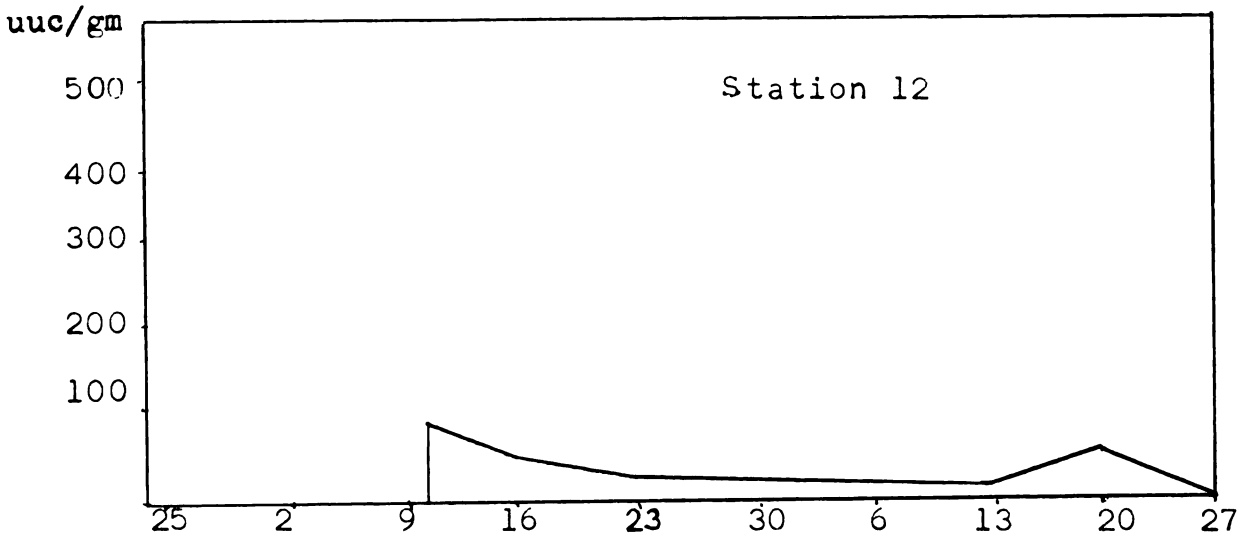

uuc/gm
500
400
300
200
100
Station 3
25
2
9
16
23
30
6
13
20
27
500
400
300
200
100
Station 5
25
2
9
16
23
30
6
13
20
27
500
400
300
200
100
Station 8
25
2
9
16
23
30
6
13
20
27
June
July
August

Fig. 21.--Stations 12 and 14 Chara sp. activity levels. Corrected micromicrocuries per gram of wet plant material.


uuc/gm
500
400
300
200
100
Station 12
25
2
9
16
23
30
6
13
20
27

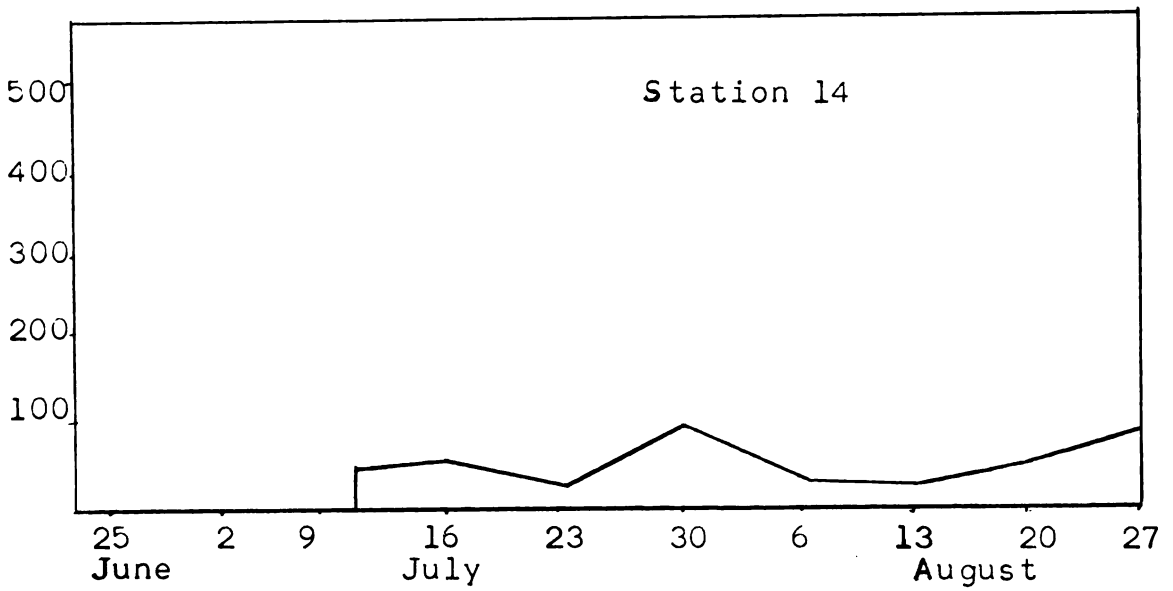

500
400
300
200
100
Station 14
25
2
9
16
23
30
6
13
20
27
June
July
August

Fig. 22.--Stations 3, 5, and 8 Ranunculus sp. activity levels. Corrected micromicrocuries per gram of wet plant material.

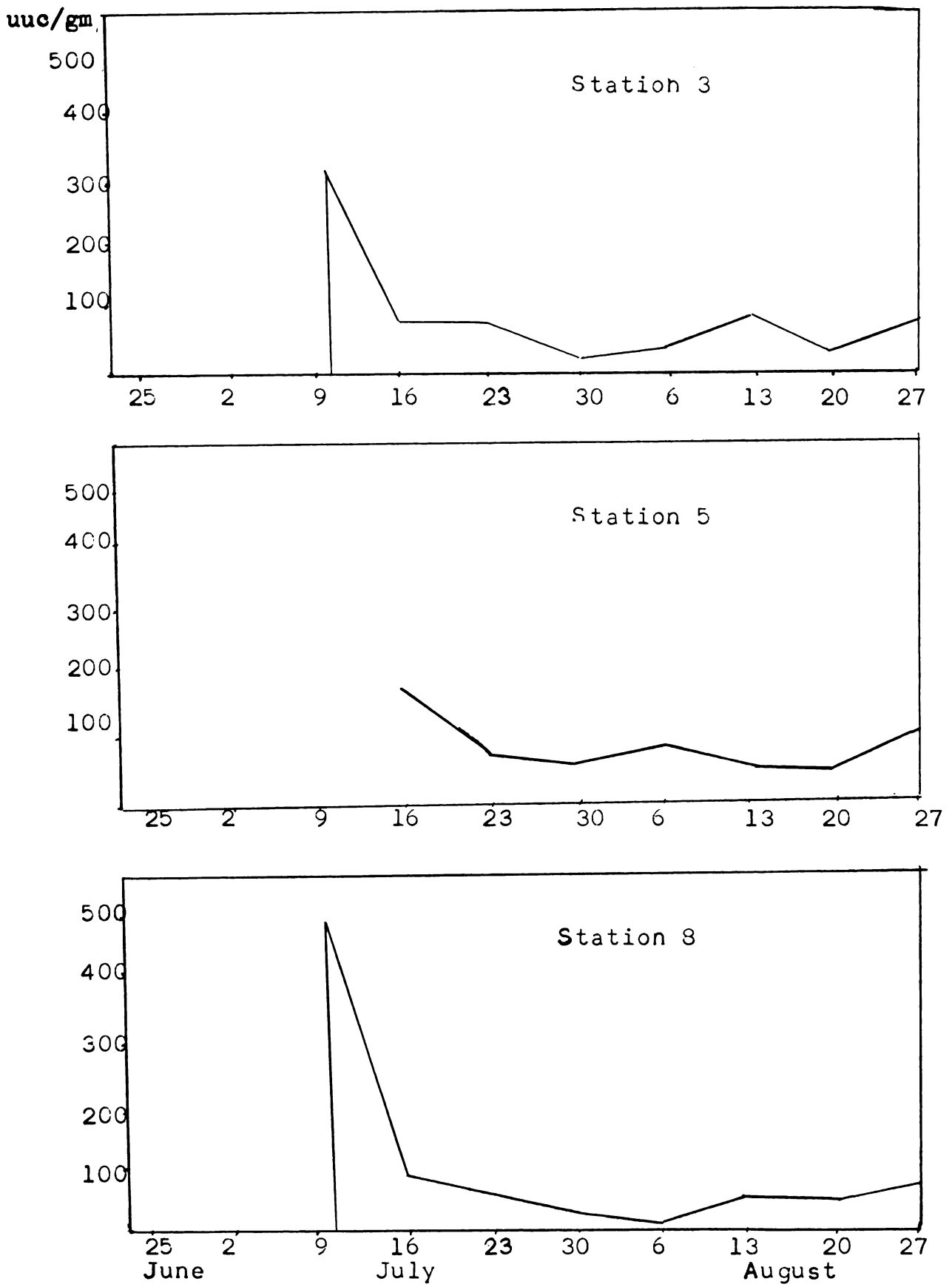

uuc/gm
500
400
300
200
100
Station 3
25
2
9
16
23
30
6
13
20
27
500
400
300
200
100
Station 5
25
2
9
16
23
30
6
13
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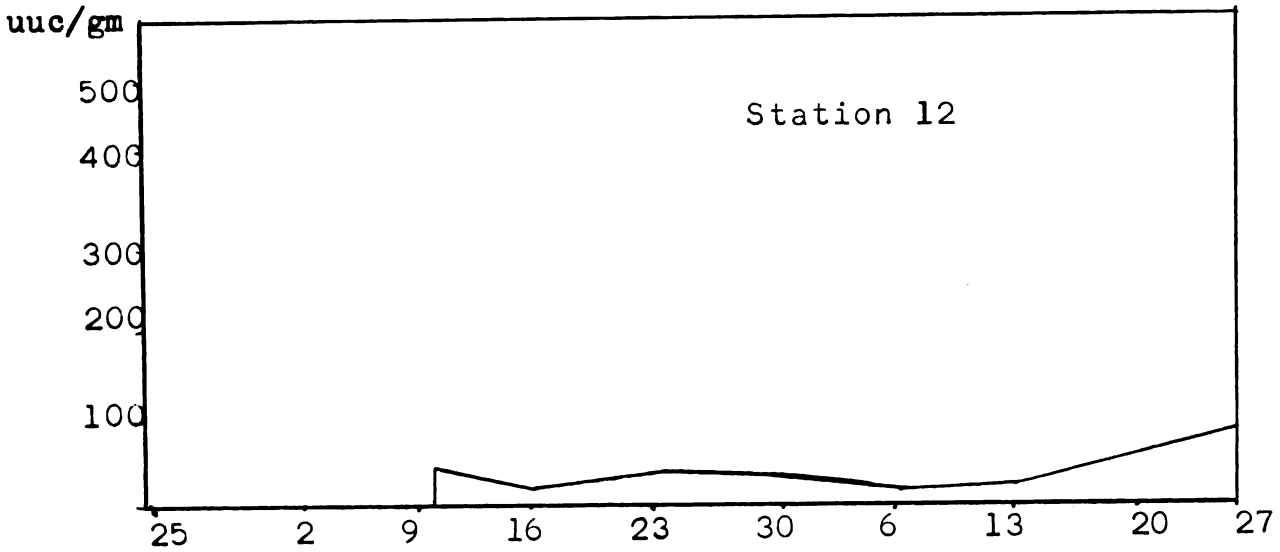
Fig. 23.--Stations 12 and 14 Ranunculus sp. activity levels. Corrected micromicrocuries per gram of wet plant material.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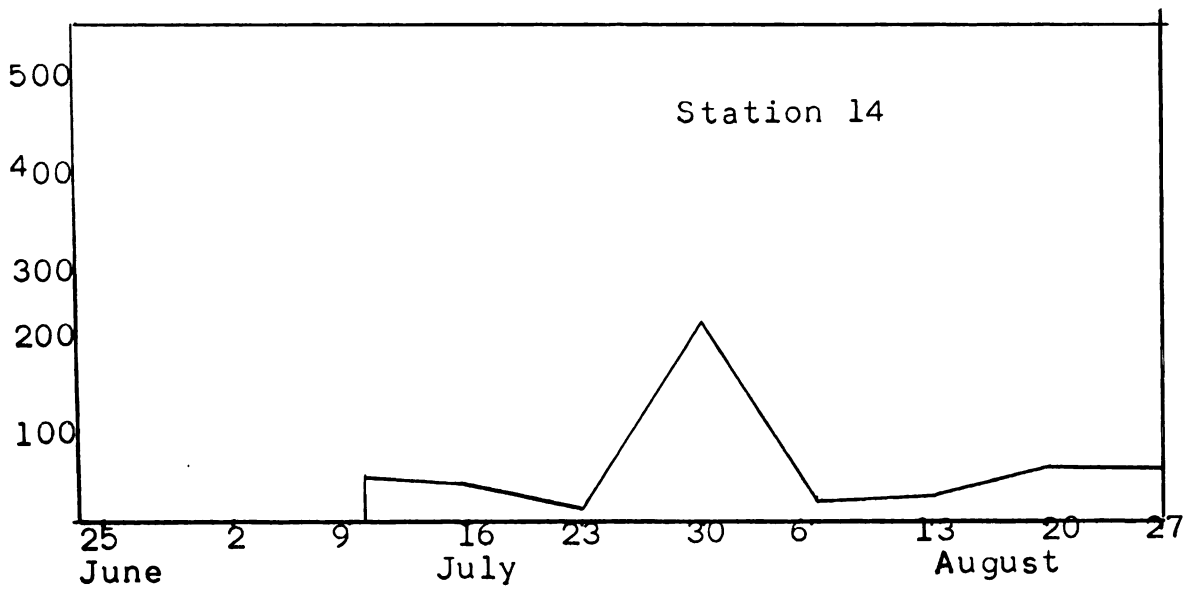
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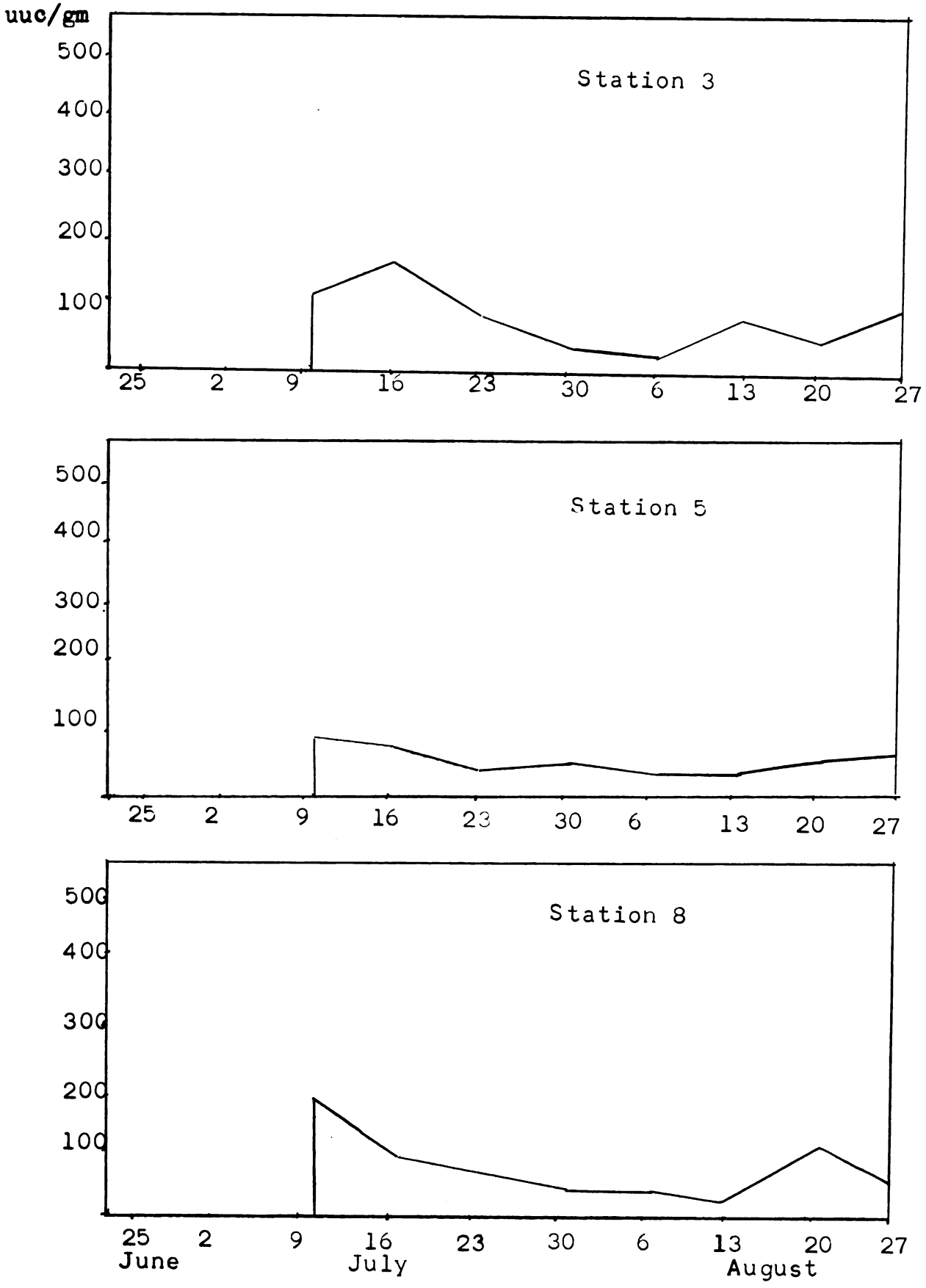
Fig. 24.--Stations 3, 5, and 8 Nasturium officinale activity levels. Corrected micromicrocuries per gram of wet plant material.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Station 8
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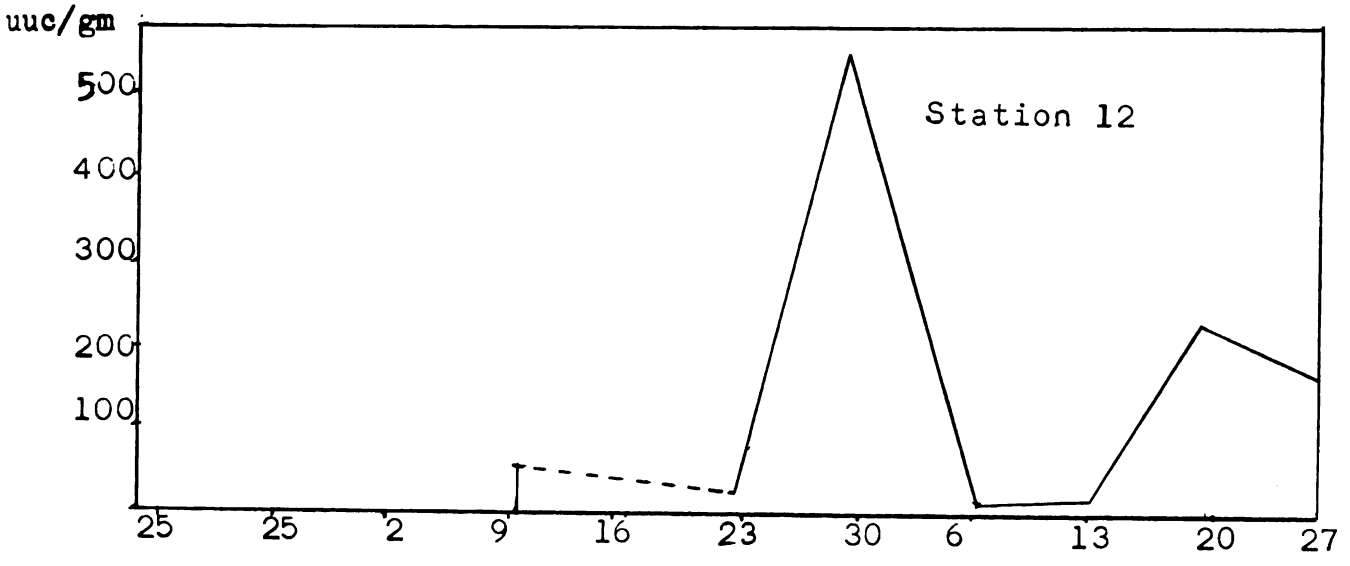
Fig. 25.--Stations 12 and 14 Nasturium officinale activity levels. Corrected micromicrocuries per gram of wet plant material.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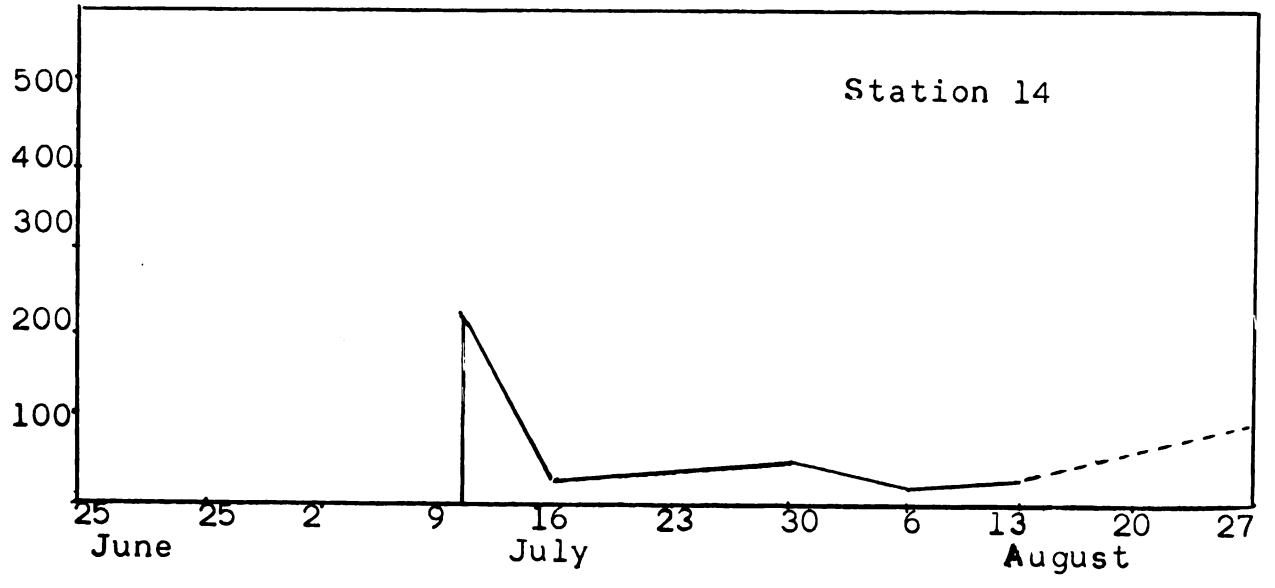
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TABLE 11.--Radioactivity levels of plant material from the West Branch of the Sturgeon River collected in 1962. Corrected micromicrocuries per gram of wet plant material.

	Micromicrocuries per Gram of Wet Plant							
	July 11	July 16	July 23	July 30	August 6	August 13	August 20	August 27
				Chara sp.				
# 3	297.7	76.0	74.5	24.9	38.7	79.7	32.1	72.6
8	418.3	86.6	56.2	27.5	15.2	49.3	46.2	59.7
14	47.0	41.9	16.2	255.8	19.8	23.7	57.3	48.5
				Fontinalis antipyretica				
# 3	416.4	401.0	68.5	179.5	137.7	102.6	165.9	144.7
8	62.7	142.5	82.4	91.7	28.5	37.9	68.6	68.8
14	38.7	---	43.5	123.2	42.4	38.0	33.3	---
				Nasturium officinale				
# 3	36.1	104.4	46.6	6.6	3.4	14.8	36.2	61.4
8	72.2	82.4	41.7	711.3	11.2	57.3	29.2	37.7
14	21.9	7.9	11.0	52.1	17.7	12.1	24.2	45.2
				Potamogeton sp.				
# 3	235.2	15.0	35.9	40.4	18.9	46.6	23.6	99.5
8	181.7	73.6	18.6	12.1	15.9	19.6	15.2	55.0
14	19.0	8.7	2.8	14.3	6.1	27.4	32.8	69.9
				Ranunculus sp.				
# 3	82.8	107.6	40.4	14.2	9.3	41.1	22.4	46.2
8	91.9	48.5	34.5	25.3	24.0	12.9	56.8	25.1
14	32.4	1.6	10.4	231.1	8.2	11.0	104.3	69.9

TABLE 12.--Variance and standard deviation of plant activity levels per species.

	Station 3 (uuc/gm.)	Station 8 (uuc/gm.)	Station 14 (uuc/gm.)
		Chara sp.	
S^2	64827.1	15204.9	5918.5
S	84.6	123.3	76.9
		Fontinalis antipyretica	
S^2	20398.3	1600.0	7703.3
S	142.8	40.0	87.8
		Nasturium officinale	
S^2	1092.3	45605.8	250.3
S	33.1	213.6	15.8
		Potamogeton sp.	
S^2	5670.2	2975.1	470.1
S	75.3	54.5	21.7
		Ranunculus sp.	
S^2	2330.6	1850.4	9726.9
S	48.3	43.0	98.6

S^2 = variance

S = std. deviation

and Potamogeton sp. were the two species which accumulated the most radioactivity at the upstream stations. The day of highest activity in both was the day of isotope introduction. The P^{32} was incorporated within the plants since washing them with dilute acid did not release any radioactive materials which counted above the cosmic background radiation levels, nor did samples of plants collected before the tracer introduction count above background. Lack of data in the sample values was indicated by broken lines, they resulted from the lack of specimens at several stations during the summer.

Macrophyte Biomass and Production

Biomass samples were taken before and after isotope introduction by use of a Surber square foot sampler on sites selected by application of the numbers from a table of random numbers. Analysis of the data, using a Chi-square test for randomness, revealed the plants to be highly clumped between stations. For the whole stream the plants found in random quadrats would have represented their relative abundance. Therefore, when standing crop figures of the pre-tracer period were expanded to the whole study area they indicated the size of the stable phosphorus pool present in plants. This was converted to dry weights from the field wet weights and the per cent phosphorus was computed from the values derived by Zettelmaier (1961), the conversions are given in Table 13. The second sample series from the same quadrants was used to compute the production figures and turnover rate of the macrophytes in the study section (Table 14). Square foot sites that had plants removed from them the first time were excluded the second time and new sites were added to replace them.

TABLE 13.--Macrophyte composition and production of three major species in 1962.

	Fontinalis	Potamogeton	Chara	Total
		July 6, 1962		
Species dry wt.	290.84 lbs.	5.29 lbs.	494.14 lbs.	765.27 lbs.
% of total wt.	38.00	0.69	61.30	
Area				26,366,490 sq. ft.
		August 31, 1962		
Species dry wt.	1589.85 lbs.	32.96 lbs.	389.45 lbs.	2012.26 lbs.
% total wt.	79.01	1.64	19.35	
Increase	1229.01 lbs.	27.67 lbs.		1246.99 lbs.
Loss			104.69	

TABLE 14.--Macrophyte production figures per unit area and phosphorus pool contained in each species.

Station	Wet Wt. in lbs.	Dry Wt. in lbs.	Dry Wt. Rate in lbs. per day	Dry Wt. per unit area lbs/acre/day	Grams/m^2/day of plants	Grams/m^2/day of stable phos.
3	253.4	40.60	0.73	1.03	0.203	0.034
5	169.8	6.12	0.11	0.23	0.026	0.004
8	1057.6	251.73	4.50	5.17	0.580	0.097
12	8093.1	545.91	9.75	3.17	0.355	0.059
14	4303.6	1168.00	20.86	17.10	1.918	0.320

Total P^{31} by species by dry wt.	Fontinalis	Potamogeton	Chara
July 7, 1962	0.1272 lbs.	0.0103 lbs.	0.3730 lbs.
August 31, 1962	0.1954 lbs.	0.0439 lbs.	0.1908 lbs.

Turnover time equals 79.66 days at rate of 0.107 gms/day/ft^2 wet weight of plants.

The area production and rate of production between Stations 3 and 5 was extremely low due to the reduced light, while the area between Station 12 and 14 was one of high light intensity, favorable substrates and currents for the macrophytes. Comparison between the area production rates of this stream and the Red Cedar River near Lansing, Michigan, a warm-water, enriched stream with the same volume of summer flow an average production of 2.6 gm-cal/m^2/day shows only the lower most section studied by us, was of the same order of magnitude (personal communication with Robin Vannote, research fellow at Michigan State University).

Invertebrates

Invertebrates, especially insects, were the major organisms which transferred energy and nutrients from the primary producers to the consumers in the West Branch of the Sturgeon River. Generally, the classic trophic system of primary producer, consumer, and decomposer or omnivore was present in the stream. Knight (1961) and Zettelmaier (1961) divided the invertebrates into particular niches for the stream. Using their classification nine invertebrates were studied during the summer of 1962. They included a filter feeder, Simulium sp.; a netspinning caddis, Hydropsyche sp.; two periphyton scrapers or grazers, Ephemerella needhami and E. cornuta; two secondary consumers, Atherix variegata and the fishfly larvae, Chauloides sp.; two omnivores, the pouch snail Physa sp.; and the riffle stonefly, Pteronarcys sp.; and the detritus feeder, Hexagenia sp. Each has been found to occupy a similar niche in studies conducted elsewhere.

A single exception to the above classification, Chauloides sp., was found in 1961 when it was found to be an extremely efficient

accumulator of radioactive phosphorus during the single year when the P^{32} was introduced as an organically bound complex (bacteria). The question was not resolved by the present study because with an introduction of inorganic tracer again, the fishfly larvae again had only a low level of incorporated P^{32}. The difference in magnitude was striking in view of the head construction and structure neither of which suits the organism to adapt it to feeding on bacteria. Observed feeding habits also contradict the accumulated activity levels present during the 1961 study. Numerous separate observations of the feeding habits had shown the organism to be a secondary carnivore on such organisms as Hydropsyche which they stalked at dusk among riffle substrates. From specimens collected at random, stomach analyses data reveal only insect head capsules were present. It was recognized that normal cursory investigation of the intestinal tract does not reveal even large clumps of slime that might contain bacteria. No insect sampled by investigators during the past four years (1959-1962) possessed enough activity in 1961 to contribute the amount present in the 1961 fishfly larvae, yet the same organisms were present in the intestines of preserved specimens from each year before and during 1961 and in those collected during the present year when the activity level was again low.

Either the fishfly was eating some detrital bacteria with its normal food or it greatly concentrated small amounts of the organically bound phosphorus from its food organisms which it had never done before nor did it during the present study. It was a changed form of introduction in 1961, but the high degree of loss to the organisms and substrate of P^{32} in the first 240 yards of stream in 1962 would seem to

nullify the advantage of changed form. During the present study much more activity was available in the upstream areas and much of its incorporation was into bacterial forms that were endemic to the stream while in 1961 the tagged bacterial form was foreign to the stream. The activity remained in the upstream areas for a long time with only gradual losses therefore, only a changed form of introduction (bacteria) cannot be the whole answer to this question.

In general, the principal mode of tracer accumulation in invertebrates is the same as that of fish namely, through the ingestion of food organisms. Radioactive materials are incorporated physiologically into the invertebrate tissue directly. In studies conducted by Robeck, et al. (1954) in the Columbia River it was indicated that radioactivity levels in most aquatic invertebrates were dependent upon the particular organism's metabolic rate and the radioactive material ingested. In this study it was assumed that no radioactivity was contributed by physiological transfer across membranes since no adsorbed activity was washed from the external parts of the invertebrates by 0.01 N hydrochloric acid.

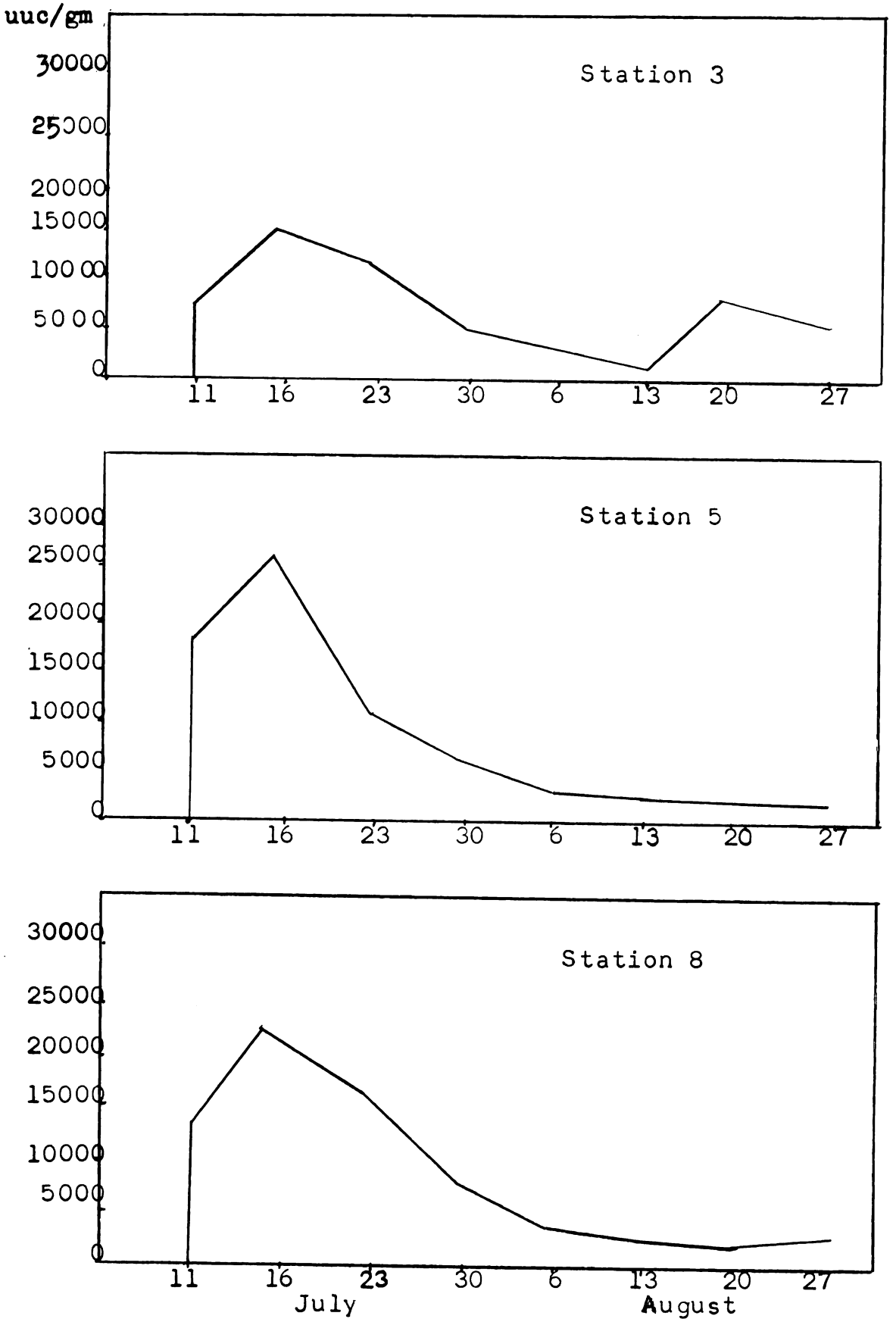
Figures 26 through 33 exhibit the pattern of tracer uptake in the order of trophic levels. Hydropsyche sp. was not included because weekly samples could not be systematically collected because of emergences that left too few for a weighable sample immediately after the emergence. Also, the replacement organisms had a very different metabolic rate and subsequent tracer accumulation. The caddisfly, Brachycentra sp. not previously mentioned had a similar emergence and replacement, but some of the stations were sampled for them after the first three weeks. Their activity levels were initially high, nearly

TABLE 15.--Insect radioactivity levels in micromicrocuries per gram of wet insect material at weekly intervals. Maximum values of activity by species by week.

	Micromicrocuries per Gram of Wet Insect Material							
	July 11	July 16	July 23	July 30	August 6	August 13	August 20	August 27
				Simulium sp.				
# 3	3309	6580	4115	2306	478	417	376	352
8	7356	9839	4634	3274	1444	1000	1000	832
14	5429	9072	6408	3435	1651	1188	898	1195
				Ephemerella needhami				
# 3	8183	25600	20372	13113	1909	3477	3695	2422
8	7083	5567	16354	11497	6050	3724	2782	2459
14	743	4121	6428	5030	2581	1959	5209	1018
				Ephemerella cornuta				
# 3	5228	23059	16906	17018	9706	5827	4451	3359
8	6080	6023	10954	10221	3599	5982	3562	4038
14	891	3313	4973	7157	2278	2542	1931	----
				Chauloides sp.				
# 3	332	376	1105	2374	319	1607	2511	1825
8	234	609	815	6666	686	2066	2278	537
14	74	46	229	1243	380	673	1002	898

				Atherix variegata				
# 3	1118	1880	5888	9743	4368	2413	3936	4075
8	245	1717	406	963	2739	1437	6030	5189
14	92	1035	1780	1520	2478	2548	1483	2418
				Pteronarcys sp.				
# 3	2416	5298	3960	2158	3508	6613	6832	6085
8	1496	780	5606	4995	2175	1734	3219	4464
14	382	1546	2007	673	1212	1358	1492	2256
				Physa sp.				
# 3								
8								
14								
				Hexagenia sp.				
# 3	48	153	87	199	103	221	384	---
8	28	232	144	245	129	400	620	480
14	37	164	306	---	336	459	284	1116

Fig. 26.--Stations 3, 8, and 14 activity levels of the blackfly, Simulium sp. Corrected micromicrocuries per gram of wet insect material. Corrected for background and decay.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August

Fig. 27.--Stations 3, 8, and 14 activity levels of the mayfly, Ephemerella needhami. Corrected micromicrocuries per gram of wet insect material.

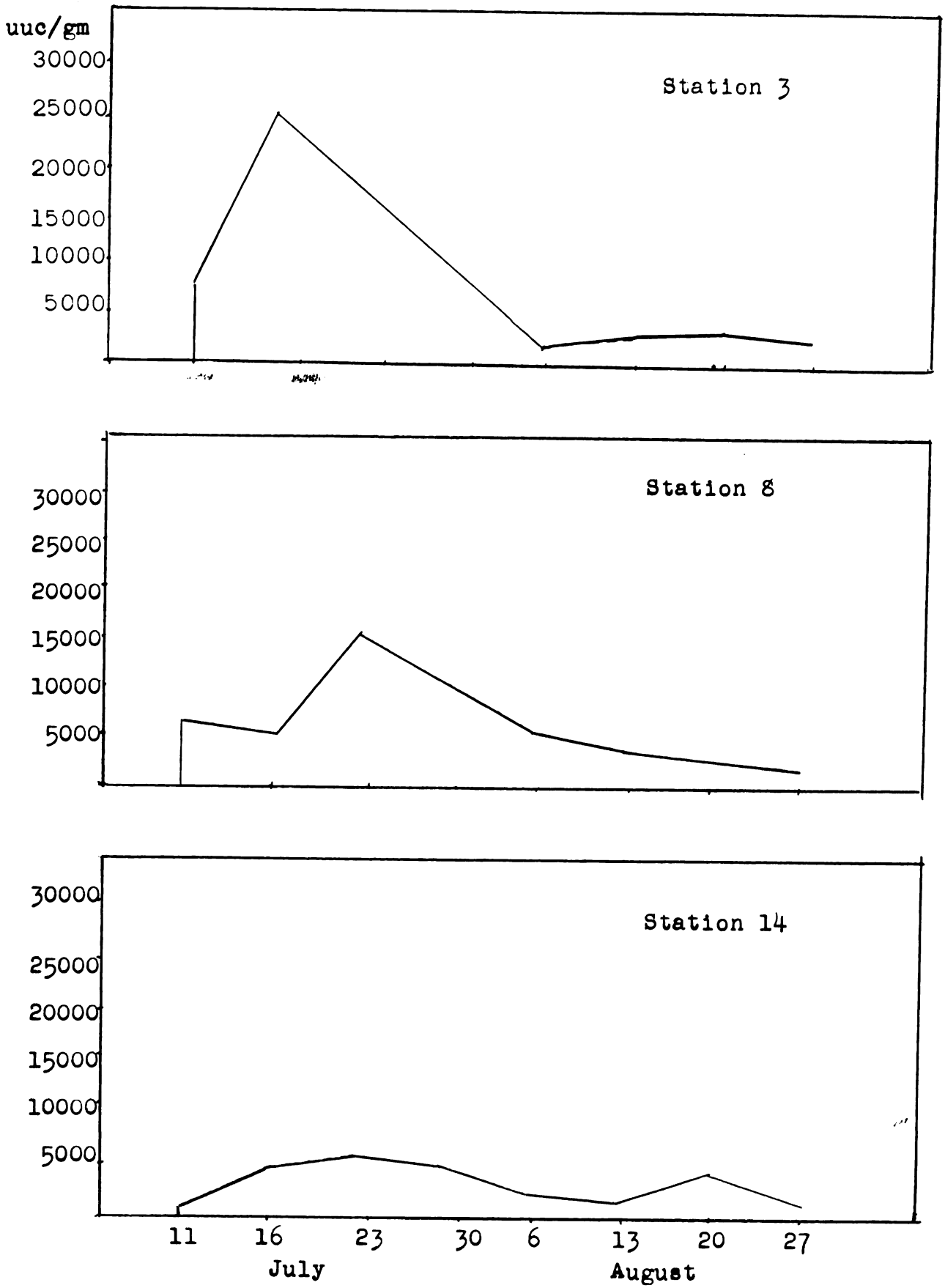
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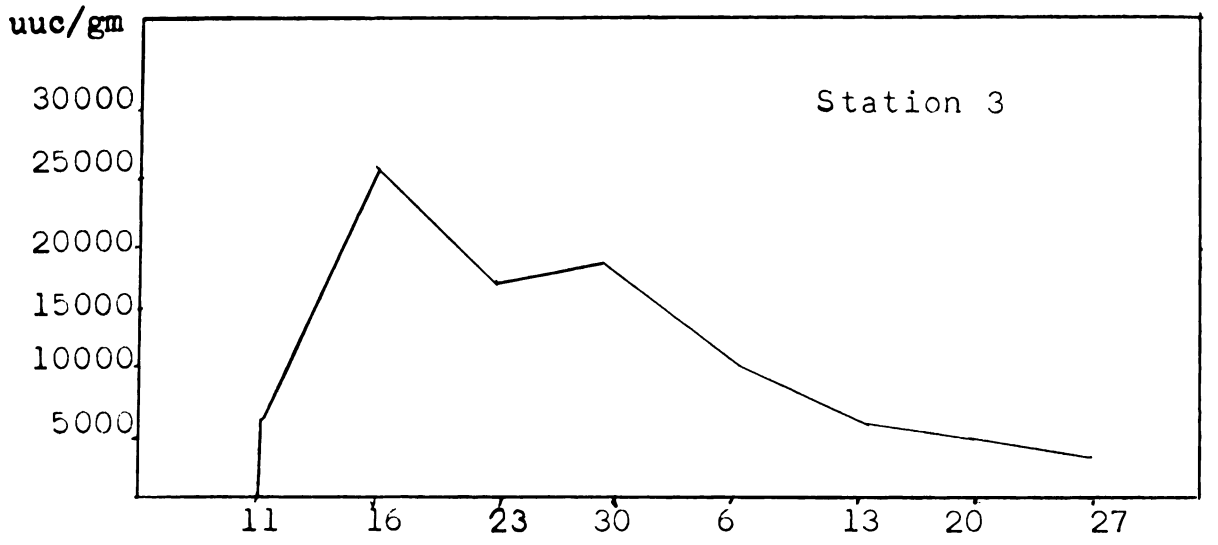
Fig. 28.--Stations 3, 8, and 14 activity levels of the mayfly, Ephemerella cornuta. Corrected micromicrocuries per gram of wet insect material.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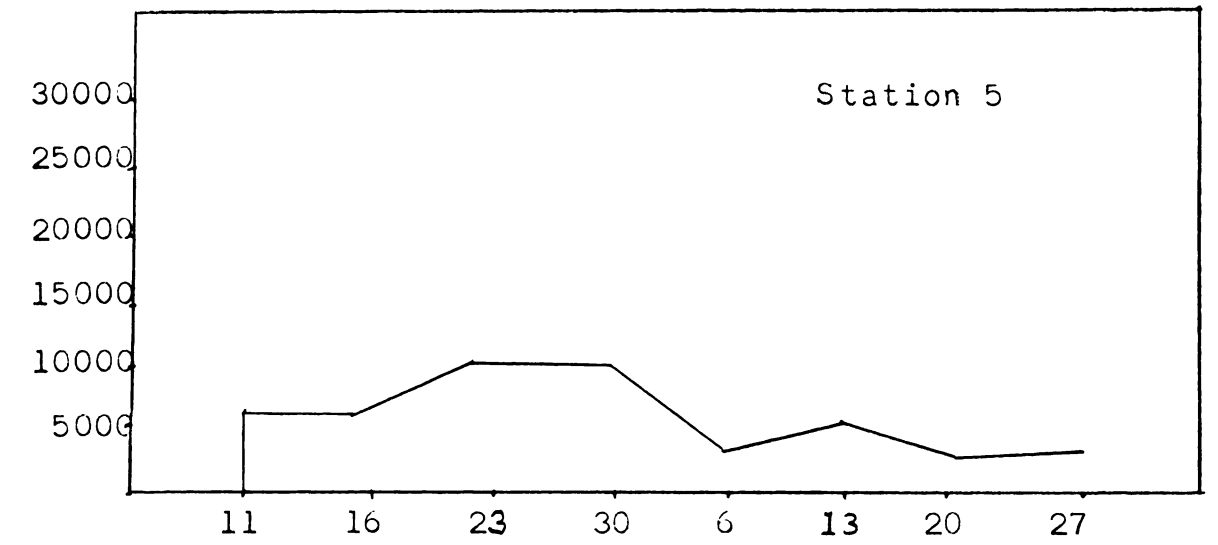
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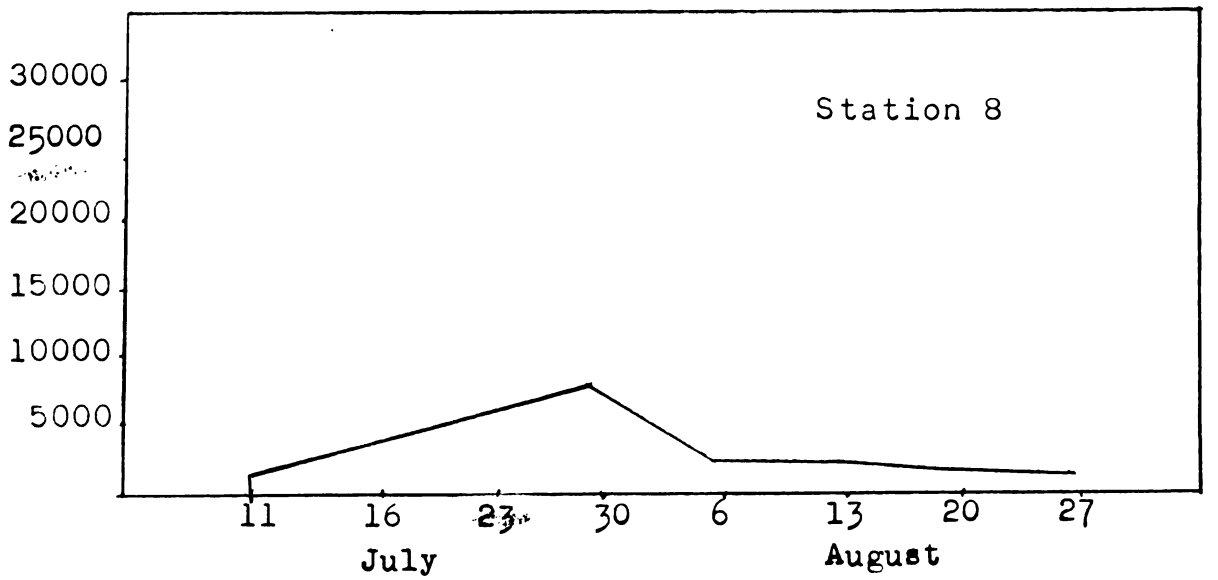
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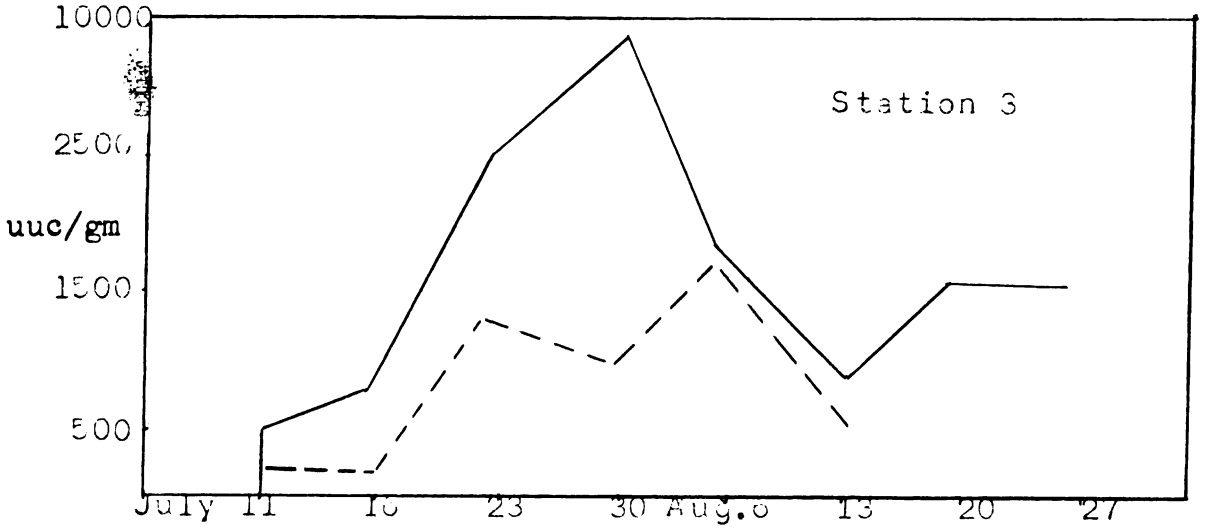
Fig. 29.--Stations 3, 8, and 14 activity levels of the snipefly, Atherix variegata. Corrected micromicrocuries per gram of wet insect material.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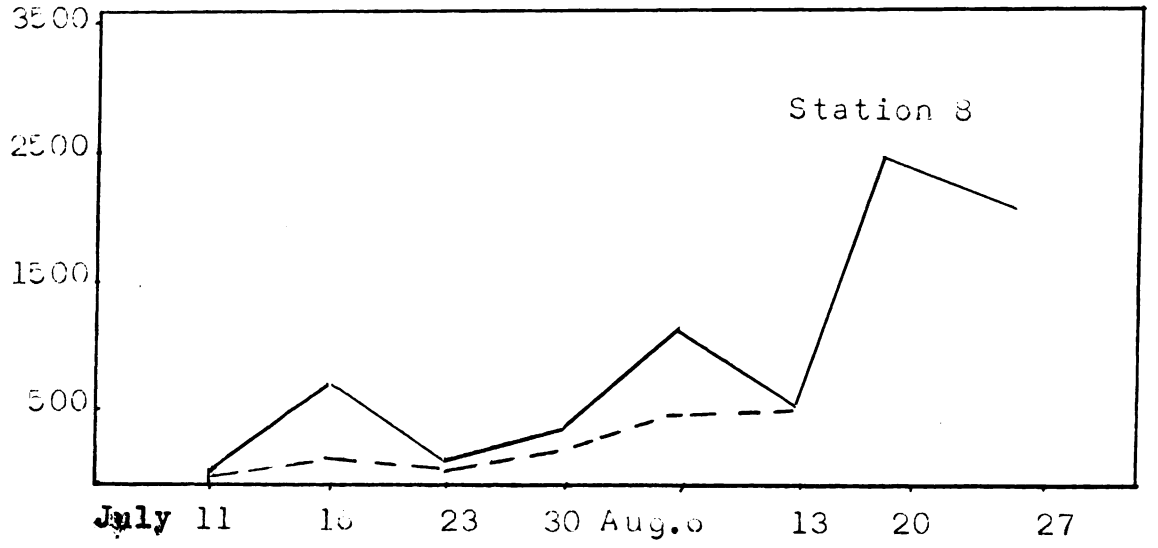
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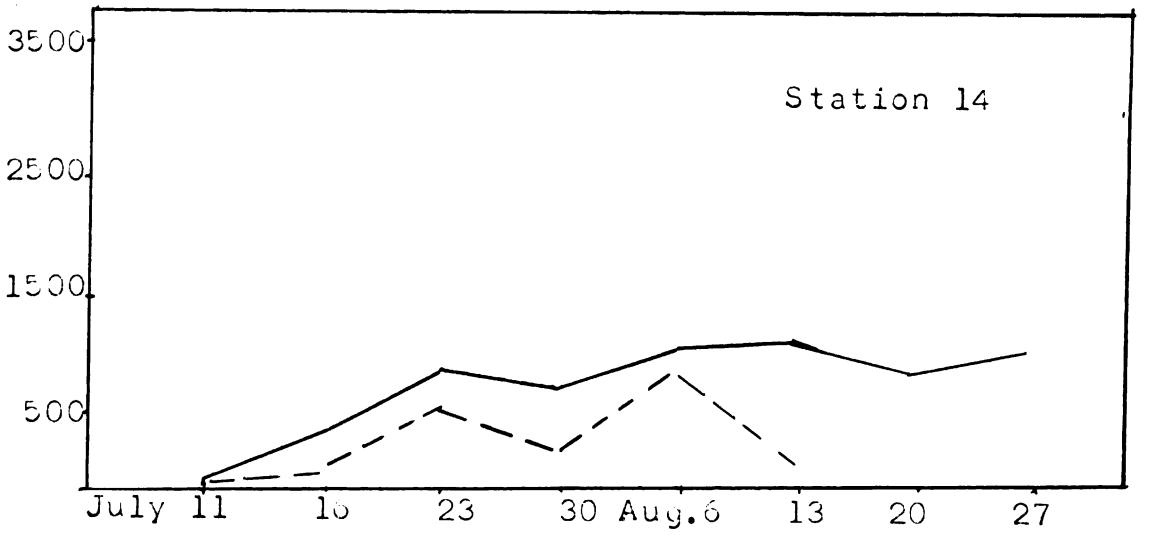
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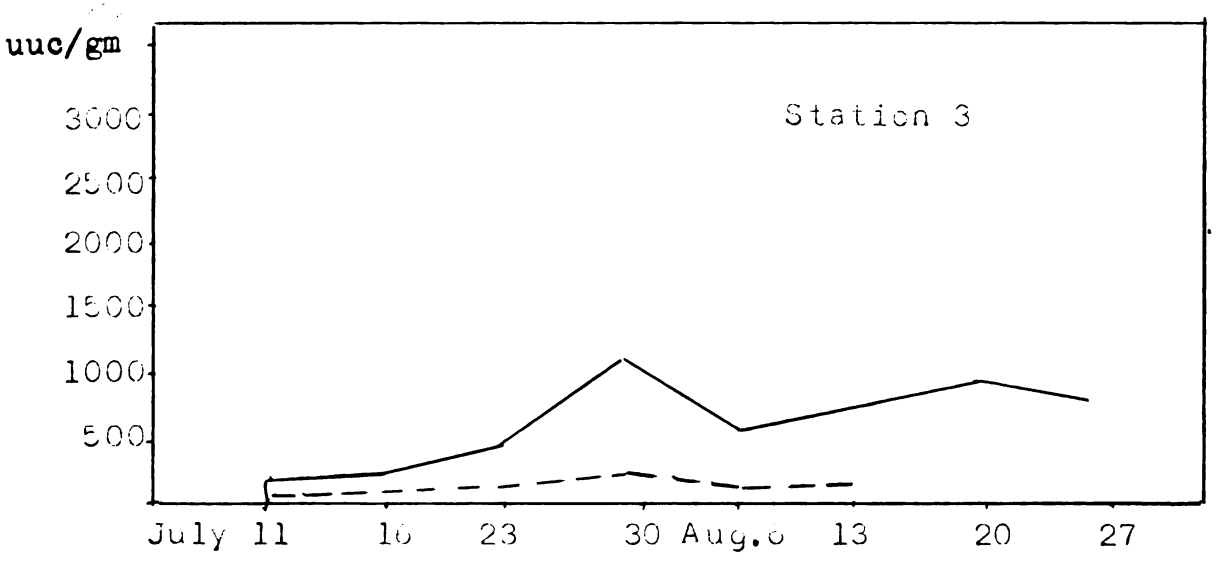
Fig. 30.--Stations 3, 8, and 14 activity levels of P^{32} in the fishfly larvae, Chauloides sp. Corrected micromicrocuries per gram of wet insect material.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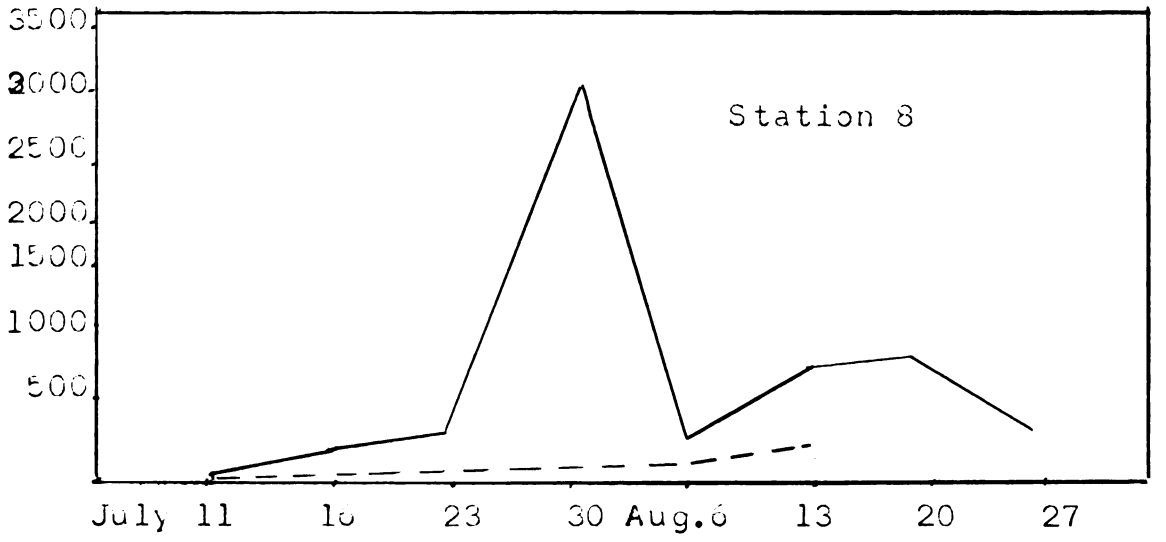
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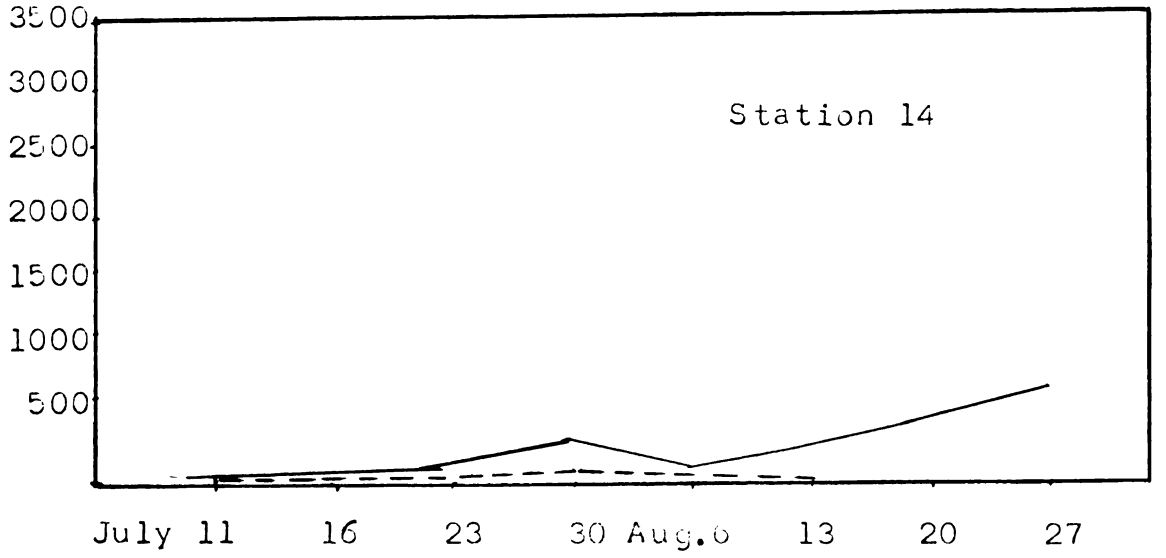
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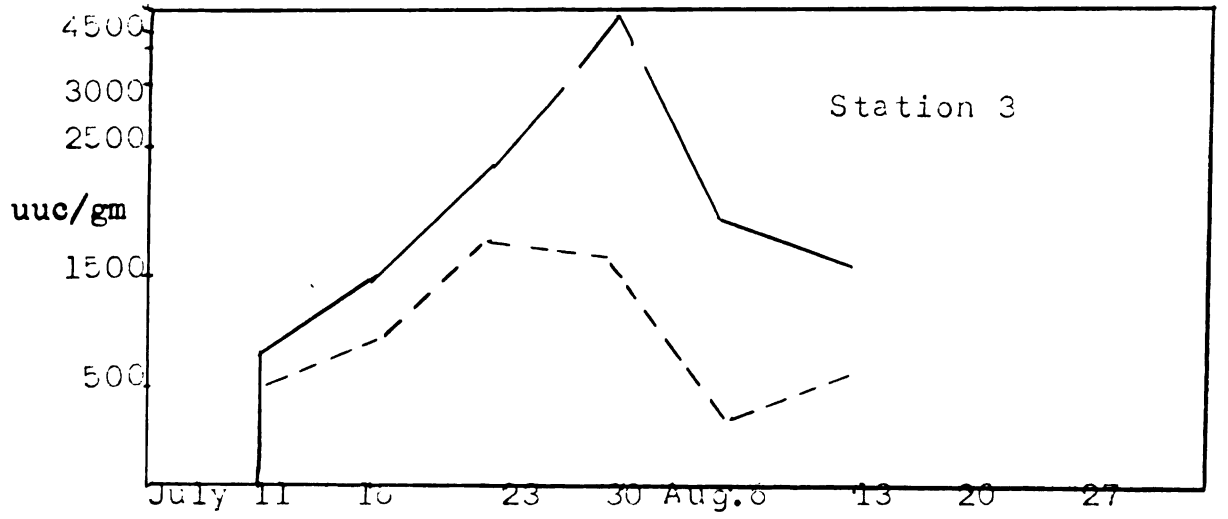
Fig. 31.--Stations 3, 8, and 14 activity levels of the pouch snail, Physa sp. Corrected micromicrocuries per gram of wet insect material.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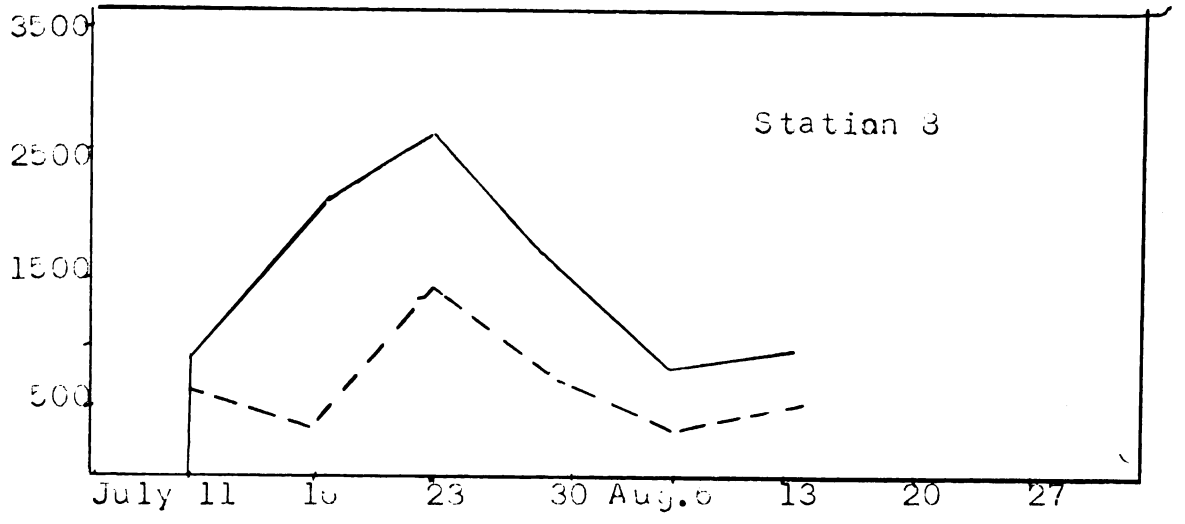
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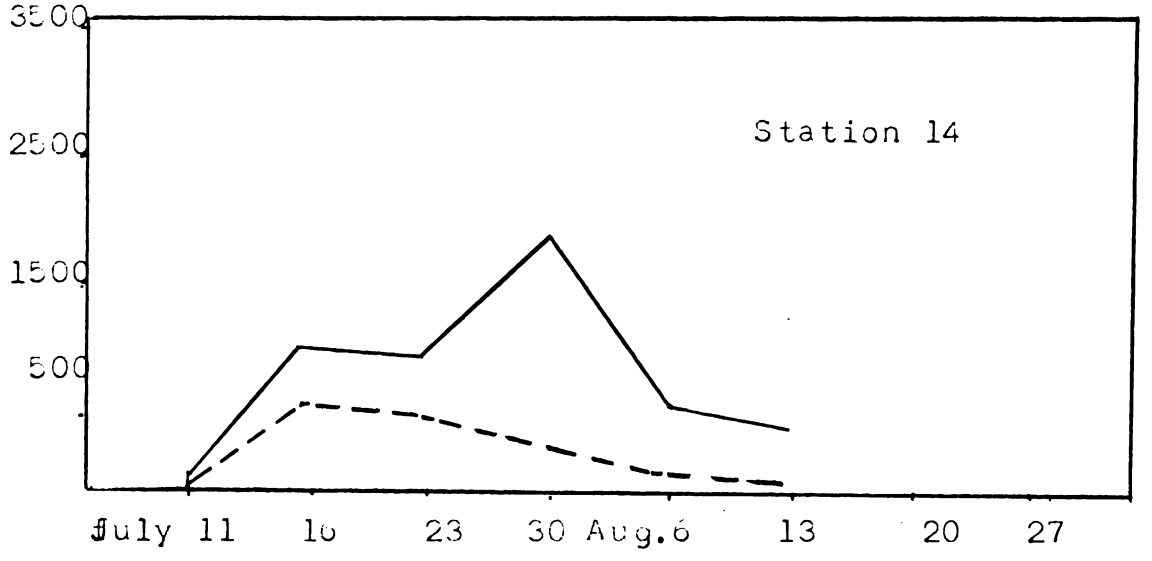
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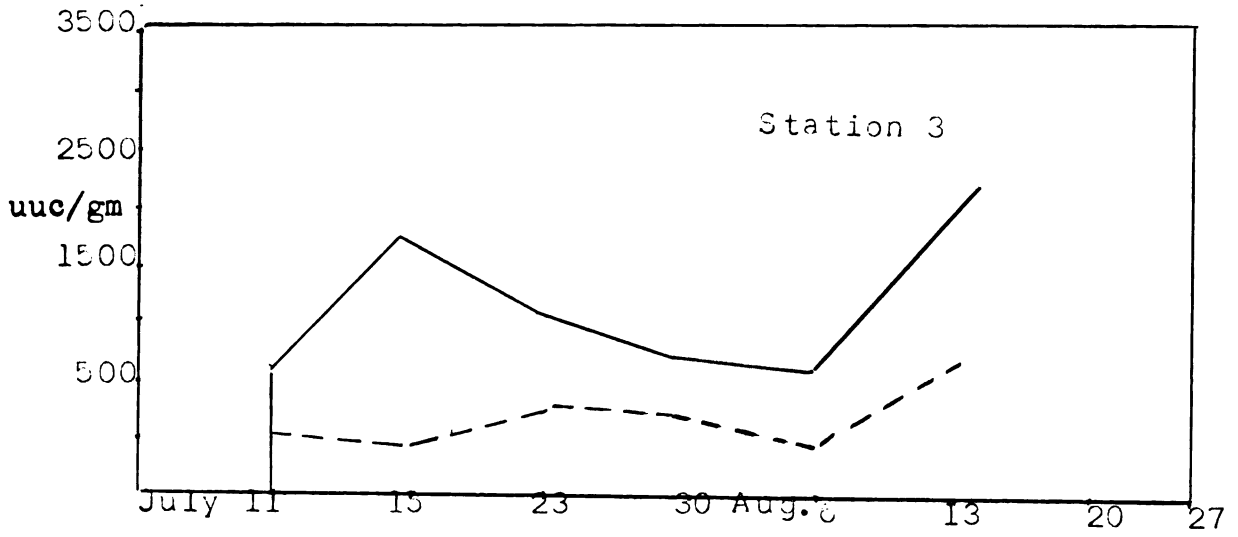
Fig. 32.--Stations 3, 8, and 14 activity levels of the riffle stonefly, Pteronarcys sp. Corrected micromicrocuries per gram of wet insect material.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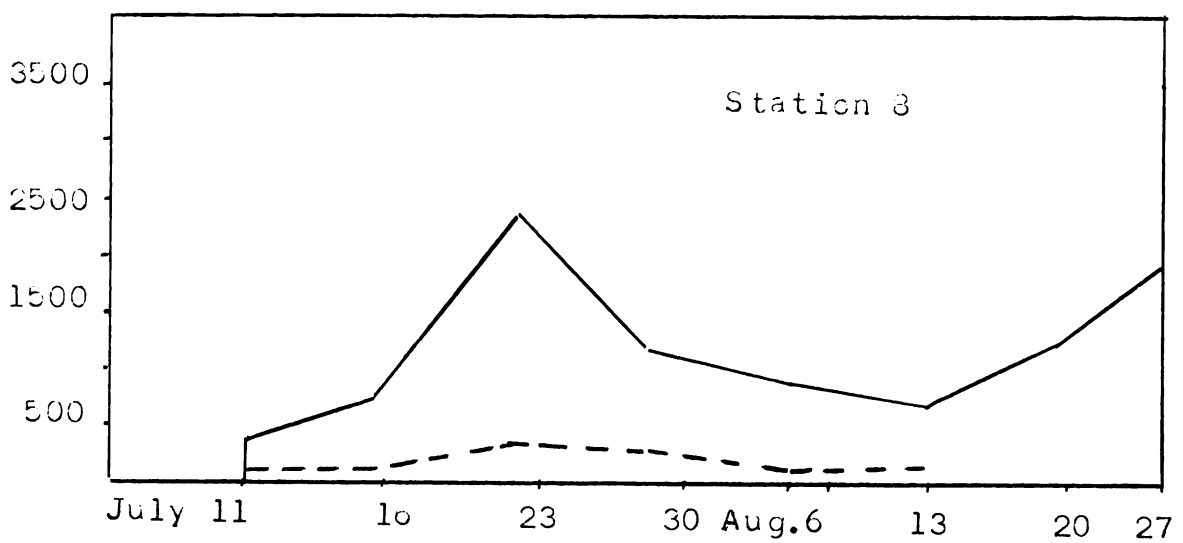
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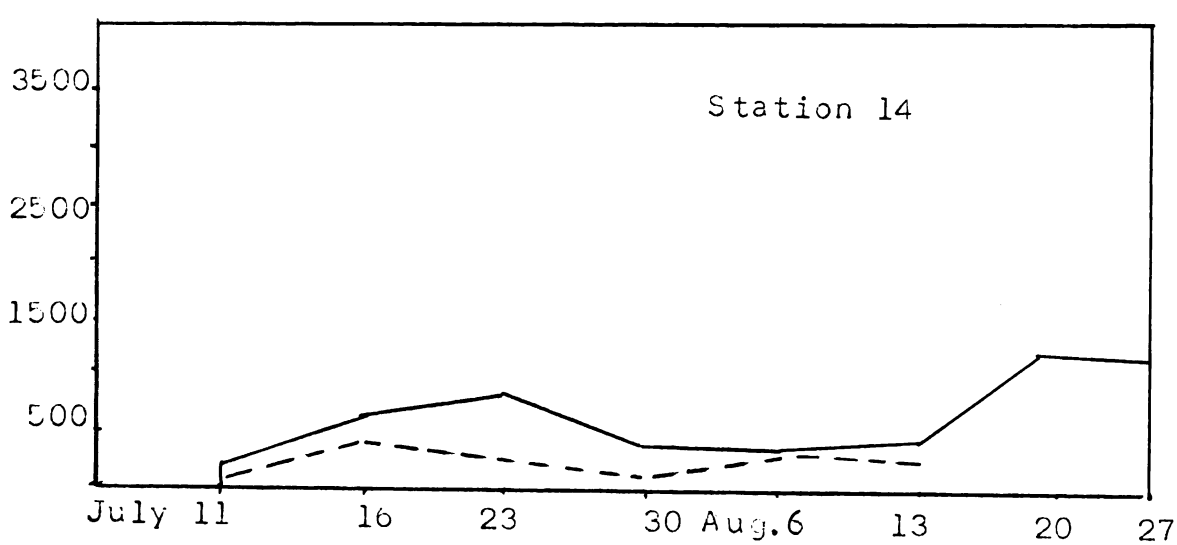
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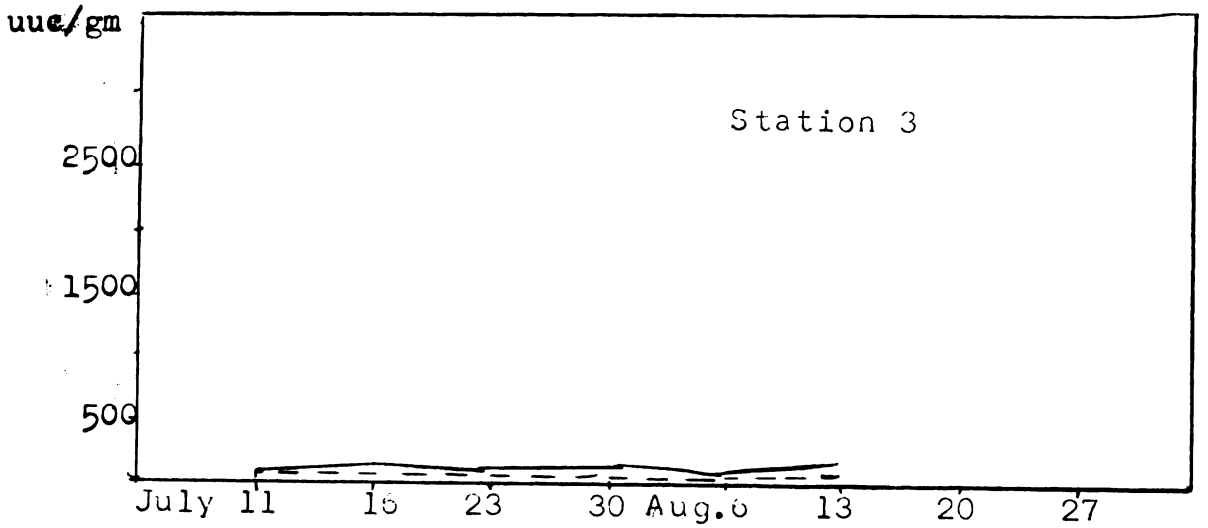
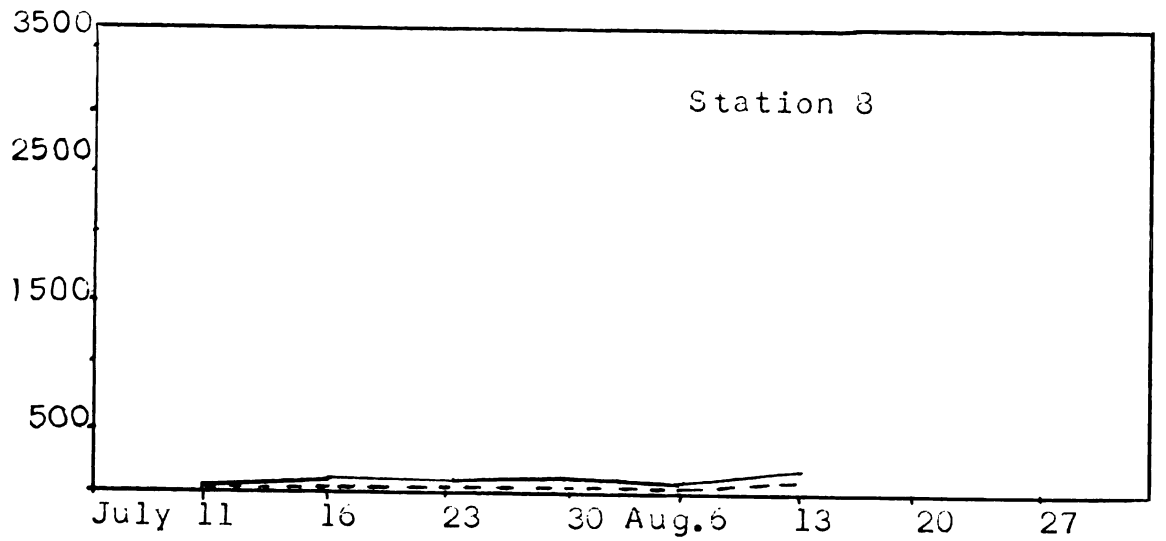
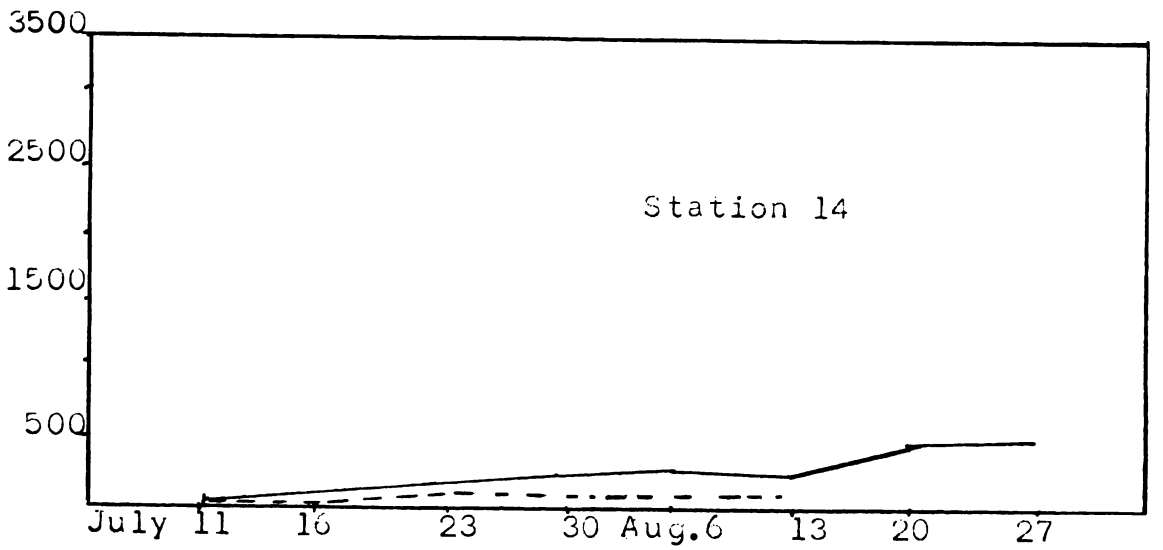
Station 14
July 11
16
23
30 Aug.6
13
20
27

Fig. 33.--Stations 3, 8, and 14 activity levels of the mayfly, Hexagenia sp. Corrected micromicrocuries per gram of wet insect material.


uuc/gm
Station 3
2500
1500
500
July 11
16
23
30
Aug.6
13
20
27


3500
Station 8
2500
1500
500
July 11
16
23
30
Aug.6
13
20
27


3500
Station 14
2500
1500
500
July 11
16
23
30
Aug.6
13
20
27

that of the blackflies, followed by a rapid tapering off of the activity levels. Samples were taken before the isotope introduction and indicated no significant activity above background levels.

The two grazing mayflies, Ephemerella needhami and E. cornuta, from Station 3 exhibited the highest levels of accumulated tracer one week after the isotope introduction. Both then indicated a gradual loss of activity during the following six weeks. The blackflies at Station 8 had the second highest levels of P^{32}. The difference between the highest peak of activity at Station 3 for the grazers and Station 8 for the filter feeders was presumed to be due to the difference of feeding habits and the activity incorporation by drift forms at the two stations. Each of the secondary consumers, Atherix and Chauloides, exhibit a lag of accumulation to a high peak level of P^{32} on the third or fourth week after the introduction. A secondary accumulation follows after a gradual loss of P^{32}. The two omnivores, Physa and Pteronarcys, indicate their feeding habits, reaching a high peak of activity on the first week after introduction when it may be presumed that they have been feeding on the periphyton and grazing mayflies while later they reach an equal secondary peak activity when it may be presumed they were feeding on excretory products and more invertebrates than periphyton or its grazing fauna. The lowest levels of activity were as expected in the detritus feeders, Hexagenia sp.

A progressively lower level of activity downstream was exhibited as was expected due to the lower level of available activity. No secondary peak of activity accumulation was exhibited in any of the diatom feeders, it was only exhibited by the trophic levels above the primary consumers. This was anomalous since the periphyton was grown

on substrates, collected, counted, and found to exhibit a secondary peak of tracer accumulation. This would seem to offer strong evidence of the secondary accumulation coming from organic sources that were precipitated upon the substrates or were ionically bound colloids and not utilized by the primary consumers. Above the primary consumer level the secondary activity increase may only be due to the recycling generated by detritus and omnivorus feeders which were eaten to a greater degree when the other food organisms were less available due to emergence and drift losses.

Migration

For years it has been known that aquatic stream organisms were normal members of the drift complex moving with the current and along the bottom by saltation due to eddy currents. Several studies have been made relating this drift to the feeding habits of fish (Needham, 1929; Ide, 1942; and Muller, 1954). Dendy (1944) appears to have been the first to formulate the concept that the drifting of stream organisms was a normal process in all streams, even in the absence of floods or adult stream insects and found females with mature eggs flying in a predominantly upstream direction. Muller (1954) proposed the term "colonization cycle" to describe this series of phenomena. In the case of insects and some crustaceans without a flying phase he recognized that the question of upstream repopulation was unanswered, he considered this question particularly significant with the crustacean, Gammarus, which has been observed in the drift in very large quantities.

With this in mind it was decided to use our tracer phosphorus as an aid to answering Muller's question. The advantages were obvious

and the only drawback was that a large series of samples must be counted to determine the background radiation before the tracer was introduced.

The methods of isotope introduction in 1962 was such as to effectively create a definite curtain barrier of activity free water from that bearing radioactivity. The curtain was such that anything upstream of the point of introduction that contained radioactive phosphorus must have flown, swum, or crawled to the point where it was collected.

The logical explanation seemed to be a colonization flight, therefore a light trap series using ultra-violet light was setup for collecting adults. The first was 100 yards upstream from the point of tracer addition, and was masked with aluminum foil so it effectively was uni-directional in the upstream direction. Anything attracted to it would have to have flown upstream past it and then circled back or flown downstream. Any P^{32} carrying specimens would have to have flown past and turned back into it. The second light trap was located at a roadside overhanging cedar about 1/2 mile upstream and was not directional, the third and fourth were located, at irregular times, at successive roadside bridges upstream. Since we had no way of knowing the possible length of flights, nor their intervals, the lights were operated on about every third night. Of thirty sample nights for at least two traps operated for a minimum of eight hours only ten weighable samples were collected after sorting to order. The points of activity levels on given nights are given with distance upstream in Table 16.

Samples of adults collected in a Malaise trap which was directional were of little use, presumably because the net was too large

TABLE 16.--Radioactivity levels of adult insects collected by ultraviolet light traps. Corrected counts per minute per gram of insects.

Date	Type	Distance	Activity
		1961	
7-27	mixed	100 yds	88.8 cpm/gm
7-27	mixed	200 yds	85.8 cpm/gm
7-27	mixed	300 yds	19.5 cpm/gm
7-28	mixed	100 yds	3.1 cpm/gm
7-28	mixed	200 yds	42.3 cpm/gm
8-9	mixed	100 yds	0.0 cpm/gm
8-9	mixed	200 yds	39.2 cpm/gm
8-9	mixed	400 yds	14.2 cpm/gm
8-15	mixed	1800 yds	8.7 cpm/gm
8-15	mayflies	1800 yds	105.3 cpm/gm
		1962	
7-31	mayflies	1800 yds	0.0 cpm/gm
7-31	mixed	100 yds	99.8 cpm/gm
8-3	mixed	1800 yds	23.8 cpm/gm
8-3	mixed	100 yds	138.0 cpm/gm
8-6	mixed	100 yds	151.2 cpm/gm
8-6	mixed	1800 yds	930.6 cpm/gm
8-10	mixed	100 yds	45.8 cpm/gm
8-12	mixed	100 yds	92.1 cpm/gm
8-16	mixed	100 yds	51.8 cpm/gm
8-17	mixed	100 yds	58.5 cpm/gm
8-20	mixed	100 yds	68.4 cpm/gm

mesh to collect small mayflies and caddis. The activity accumulated by composite samples of semi-aquatic and aquatic specimens indicated no directed flight pattern nor did weights of the insects collected. Examination of specimens with respect to egg carrying females on one side of the net or the other were also inconclusive of a directed flight.

The question of a species like Gammarus, led us to set up sampling sites at 100 yard intervals upstream from the isotope introduction point. They were only sampled as a precautionary measure in 1961, since we had no crustaceans in any of the upper stream study sections and none could be found in the area above the point of tracer addition. A few aquatic beetles and Hydracarina, both of which did not fly, but might move upstream, being large, active, and predatory. Two genera of snails present in the stream were also considered possible migrants since they might also be strong enough to overcome the current, especially along the edges and on logs.

In 1961 the isotope was introduced in the form of organically incorporated phosphorus within Escherichia coli 0111. The results of which were reported by Bacon (M.S., 1962) and Bender (1962) who both concluded that the majority of activity was not retained within our study area, but remained tied up within the bacteria and was washed out of our study area which was over two miles long. It was to our advantage that this happened because any activity found in adults or immature specimens that were above the point of isotope introduction, had to have accumulated their activity from the low level of radioactivity that was not incorporated by the bacteria, but was available for incorporation in the stream (about 10 per cent was so available,

as reported by Bender, 1962), and was then moved upstream. Among the immature forms sampling was conducted on the same species upstream of the tracer addition point as those collected downstream of the point.

It was found that the immature forms accumulated radioactivity and then progressively moved it upstream for more than 500 yards above the addition point over a period of seven or eight weeks. The data was tested for statistical significance and found to be a significantly greater than background radiation at the 90 per cent level. Furthermore it could be shown that the progressive increase in activity was along trophic lines to a culmination in the stronger, large bodied organisms that could be presumed to move more rapidly upstream. The most radioactive species was a stonefly of the family Isoperlidae, an active, large, predatory group. Second in radioactivity levels was the fishfly larvae, Chauloides, also large-bodied, active and considered a predator.

Tables 17 and 18 indicate the radioactivity levels present in the respective studies, 1961 and 1962. The difference in timing of the movement was presumed to be due to the difference in mode of isotope introduction.

Logically there were two methods of moving the radioactivity up into the area above the point of isotope introduction other than insect flights or crawling immature forms; mechanically by the investigators carrying debris on their equipment and possible fish movements.

During the 1962 study we again conducted the study, but with the changed method of isotope introduction and form of the isotope. The change from a point source in 1961 to a boom with several point

TABLE 17.--Activity density (corrected counts[1] per minute per gram) of stream invertebrates collected upstream from the site of P^{32} release. Isotope released July 11, 1961. Values presented are those in which the difference in count between sample and background was significant at the 90 per cent level. All other counts are recorded as zero.

	100 Yards Above							200 Yards Above							300 Yards Above						
	Days After Release							Days After Release							Days After Release						
	7	14	21	28	36	42	48	7	14	21	28	36	42	48	7	14	21	28	36	42	48
Atherix variegata[2]	0	0	0	40	0	78	45	0	12	0	0	0	0	0	0	0	0	0	0	4	40
Simulium sp.	41	--	--	--	--	--	--	-	--	--	--	--	--	--	-	--	--	--	--	--	--
Chauloides sp.[3]	0	275	0	2	2	31	47	0	78	1679	5	0	0	0	0	0	0	0	0	6	35
Brachycentia sp.[2]	0	0	163	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29
Hydropsyche sp.[2]	0	34	0	0	0	0	0	0	0	0	55	0	0	0	0	0	0	0	0	0	278
Pteronarcys sp.[2]	0	0	0	3	2	5	11	0	5	14	0	3	0	0	0	0	0	0	0	2	62
Isoperlinae[4]	69	--	--	--	--	0	--	-	--	--	--	--	--	--	-	--	--	--	--	--	540
Baetidae[2]	0	82	0	76	0	0	0	0	381	0	195	0	0	0	0	0	0	0	0	0	4885
Hexagenia sp.	0	0	--	--	--	0	0	-	--	--	--	--	--	--	-	--	--	--	--	--	--
Physa sp.[2]	19	29	26	4	0	0	0	0	4	0	0	8	0	0	0	0	0	0	0	0	0

[1]Counts corrected for background and decay.

[2]Samples of this species from Stations at 400 and 500 yards were analyzed on all dates, but a significant amount of activity was not found.

[3]Samples from Stations at 400 and 500 yards were analyzed on all dates. Samples from both of these stations had a significant amount of activity 36 days after P^{32} release.

[4]Samples from Station at 400 yards had a significant amount of activity 36 days after the release.

TABLE 18.--Activity density (corrected counts per minute per gram) of stream invertebrates collected upstream from the site of P^{32} release. Isotope released July 10, 1962. Values presented are those in which the difference in counts between sample and background was significant at the 95 per cent level. All other counts are represented by zeros.

Species	100 Yards Above							200 Yards Above							300 Yards Above						
	Days After Release							Days After Release							Days After Release						
	7	14	21	28	35	42	48	7	14	21	28	35	42	48	7	14	21	28	35	42	48
Atherix variegata	9	19	38	42	41	59	17	45	32	36	38	26	0	278	27	42	34	19	23	99	26
Simulium sp.	44	24	61	45	49	122	--	30	18	94	38	46	97	80	11	41	22	36	23	41	57
Chauloides sp.	7	4	20	6	16	48	49	19	6	0	59	21	84	121	21	--	12	20	12	46	67
Brachycentra sp.	18	22	27	51	68	32	163	39	0	14	55	0	89	91	--	16	4	139	111	37	151
Hydropsyche sp.	4	53	61	--	--	35	145	46	41	0	--	69	128	146	14	23	62	79	39	40	139
Pteronarcys sp.	7	41	31	22	35	63	154	33	29	14	17	76	19	142	20	33	13	9	38	50	213
Isoplerinae	0	--	--	--	--	--	--	--	11	--	--	7	--	111	--	--	16	0	--	--	--
Ephemerella needhami	0	15	42	116	6	49	--	21	7	15	77	37	0	142	10	0	11	225	12	66	58
Ephemerella cornuta	28	26	10	25	71	624	0	14	12	--	16	40	0	174	10	17	--	75	28	0	56
Hexagenia	8	13	14	26	20	119	131	6	14	14	5	18	39	124	12	17	12	380	12	60	63
Physa sp.	--	13	15	20	0	95	263	6	17	34	28	25	171	116	15	13	5	30	0	10	0

	400 Yards Above							500 Yards Above						
	Days After Release							Days After Release						
	7	14	21	28	35	42	48	7	14	21	28	35	42	48
Atherix variegata	35	19	4	11	0	31	73	48	31	20	26	56	55	21
Simulium sp.	18	19	44	23	58	50	273	20	35	33	23	48	34	80
Chauloides sp.	6	32	14	24	18	69	301	16	23	28	12	48	55	339
Brachycentra sp.	7	46	--	0	69	201	109	34	31	31	12	58	182	2252
Hydropsyche sp.	8	--	44	100	69	49	732	16	27	49	--	101	94	1013
Pteronarcys sp.	13	18	29	19	25	84	91	9	42	34	7	49	84	130
Isoperlinae	--	--	--	49	27	30	57	7	0	18	30	--	0	30
Ephemerella needhami	27	7	0	0	--	0	89	60	17	13	10	127	74	326
Ephemerella cornuta	15	0	4	0	29	112	0	18	122	0	10	30	45	81
Hexagenia	10	18	14	27	72	57	1240	20	18	21	18	26	52	291
Physa sp.	15	7	29	5	37	97	21	14	10	--	65	53	0	67

All values in corrected counts, corrected for background and decay.

sources was initiated to effectively change the activity pulse from a cone with 3° slopes to a curtain barrier with little or no slope. It was done to create a transect beyond which any insect carrying activity would have to have moved a definite minimum known distance rather than travelled a possible distance governed by the slope of the intermixing cone of activity. A table or graphic representation of the radioactivity levels is erratic because with the large amounts of drift and normal sedentary forms present the probability of always getting radioactive specimens was quite low.

As previously stated the Malaise trap was ineffective for aquatic adults and only the data from light traps were used to draw conclusions about the adult movements.

A precaution exercised in 1962 was that before entering the area upstream of the point of introduction, the personnel entering the area allowed the current in riffles to wash the debris off their boots and equipment. Mechanical hand washing was effected if needed, when they moved directly into the upstream area from downstream. Otherwise, they moved up to the area only by land and entered with no contact with the downstream waters at all.

The problem of fish passage of activity was met by shocking specimens in the areas of insect collection and counting them for radioactivity after processing. No activity above background levels was found in any of 125 specimens of fish of the three major salmonids five weeks after isotope introduction. The longer interval before sampling was allowed for the fish to build up activity after feeding on smaller fish and insects. The fish downstream which have been measured for radioactivity in the last four years have always shown a

slow accumulation of activity which was delayed one to two weeks after isotope introduction, presumably due to feeding habits and trophic level changes or transfers of the radioactivity. Robeck, et al. (1954) have shown this to be due to the fact that fish take up activity apparently only through the gut, and ingestion was the only means they have of getting it to the gut. If they went upstream after this the feces may have been released and been radioactive. Other workers have shown little or no movement from home areas to be a common salmonid behaviourial pattern, barring spawning runs in late fall or the early spring. Miller (1954) concluded for cutthroat trout little or no movement took place into or out of several 600 foot sections of stream. Logan (1963) working with cutthroat and rainbow trout had fewer than 40 per cent of his tagged fish move more than 400 feet. In fact, the average per cent of recaptures in excess of 400 feet for all fish within the study section was 6.8 per cent. It therefore was assumed that fish passage of activity could be ruled out.

Light traps in 1962 definitely proved that the adults do move upstream in huge flights at different times throughout the summer, dependent upon emergence periods and weather conditions. Again on 30 separate occasions the traps were used, but only captured twelve weighable samples. These weighable samples were always predominantly composed of egg laiden females and were collected as pulses that would fill a pint jar in short periods of time before dusk. Other samples of unweighable numbers collected by hand nets at bridges upstream showed high activity as much as five miles upstream close to the Hoffman Lake source of the stream. Thus quite possibly Muller's colonization cycle was an accurate hypothesis.

Immature forms collected in 1962 indicated more activity in more forms and more rapid movement into the upstream areas. Chauloides activity level was practically nil for the first few weeks and some of the omnivores had activity as rapidly as did the herbivores and predators. A possible explanation was that on the night of isotope introduction there was a large emergence of adult stoneflies and they may have carried some activity upstream in nuptial flights or incorporated in their egg masses. The lack of a time lag at the upper stations above the point of tracer addition may have been the result of this flight or the result of more radioactivity available in all forms immediately below the point of introduction. Thus the organisms didn't have to move as far upstream in 1962, from lower areas where the cone of radioactivity had reached them in the year before. Particular reference to the cone of activity was made because we cannot envision the immature forms moving upstream in areas of full exposure to the currents, instead they appear to move along the sides, along, over, in, and around the numerous logs which studded the area and the weed beds inbetween.

A study by Neave (1930) on the mayfly, Blasturus cupidus Say in an intermittent stream revealed similar progressive movements of the immature forms upstream to the extent of as much as one mile, but his conclusion was that they were moving up the intermittent stream in response to crowding and competition for food. He recorded movements as great as 600 feet per day by swimming activity of the nymphs in a current amounting to more than 85 to 110 inches per minute. Or they moved by crawling along the sides and bottom, and when the current was uncomfortable for them they even partially or wholly quit the stream

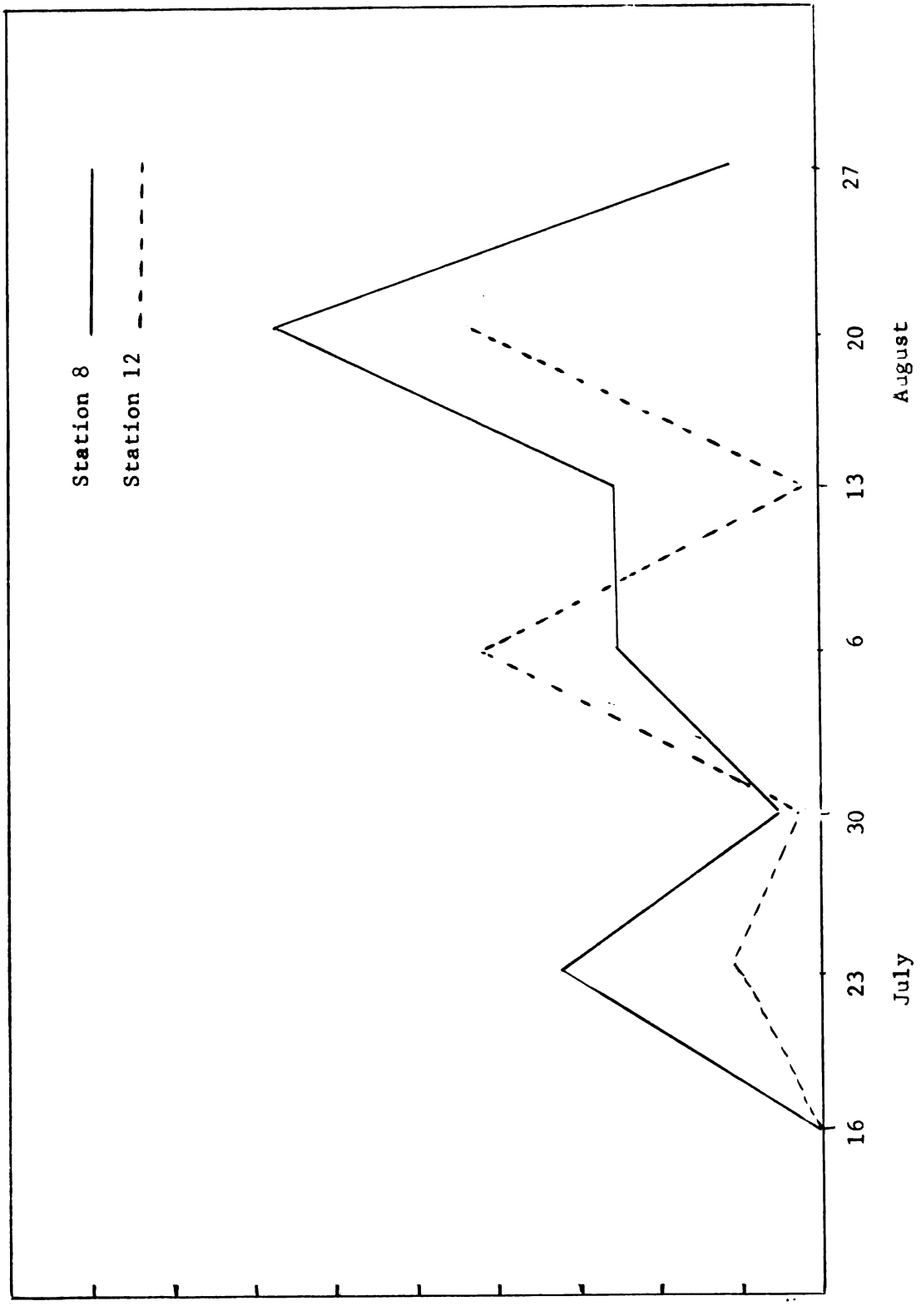
margin, crawling and wriggling forward along the wet border.

Fish Samples

Systematic sampling of the four major fish species: Salmo trutta fario, Salmo gairdnerii, Salvelinus fontinalis, and Cottus sp., was not a primary consideration during this study, therefore only four collections for tracer levels were made of the fish, beginning on July 16th five days after the isotope introduction. It was found that there was much variation within individual species on a given date as well as between species of a single collection. Figure 34 is a representative plot of tracer concentrations of the Cottus. Both species of sculpin, Cottus bairdii and Cottus cognathus were present and will be referred to here as "muddlers". The individual sample variation was typical of all the fish samples, without regard to species. It appears to be a function of the immediate past feeding history, age, condition, and habitat.

Knight (1961) regards the variability as a function primarily of the opportunistic nature of feeding within the four species and the drift composition of the mass passing each fish, Zettelmaier (1961) and Bender (1962) concur in this assumption as does this author. From stomach analyses of the fish used for radioactivity determinations this author would have to agree on both the opportunistic nature of feeding and the drift feeding, but would not place as much emphasis on drift feeding as a general food source at all times. The majority of trout examined had fed upon many bottom organisms that do not drift readily. These organisms would have to be assumed to have been selected and actively picked from the bottom or logs. Two out of every three

Fig. 34.--Curve of individual variability within a particular species at a given station demonstrated with muddlers at Stations 8 and 12. Corrected counts per minute per gram of wet fish.


cpm/gm
800
700
600
500
400
300
200
100
0
Station 8
Station 12
16
23
30
6
13
20
27
July
August

specimens that had food in their stomachs contained a species of stone-case caddis which when displaced mechanically, only drifted a few inches before reattaching to the substrate. A few specimens contained several snails of the genus, Physa, which the investigators could not find readily when sampling specifically for the snails for radioactivity processing.

According to Koster (1937) in a New York stream the diet of sculpin consisted largely of larval insects in all stages of development. Bailey (1952) and Dineen (1951) agree with this finding for different types of streams. Knight's work (1961) reveals this to be true of the West Branch of the Sturgeon River specimens.

Pentelow (1932) found brown trout to be largely piscivorous which was not shown by either Knight's work (1961) or the present study which revealed the contents of the stomachs to be mostly insect material with the remainder arachnids. Neil (1938) agrees with the insectivorus diet of brown trout. The discrepancy may be with the size of the specimens sampled in the three streams. Because of the high accumulations of activity in Salmo trutta fario it would seem more probable that the tracer concentrations resulted from an insectivorus diet.

Generally, the brook and rainbow trout can be classed as insectivorus fish.

A regression line for the tracer accumulation by species at the different stations was computed and reveals the difference in concentration of phosphorus 32 between trout species at a given station on a given date. Figures 35, 36, and 37 are the plotted regressions of tracer activity with time at Stations 3, 8, and 14 where comparisons of available water-borne tracer fractions have been made. All the fish

Fig. 35.--Regressions of activity with time in days. Salvelinus fontinalis was a negative regression at Station 3. Corrected micro-microcuries per gram of wet weight.

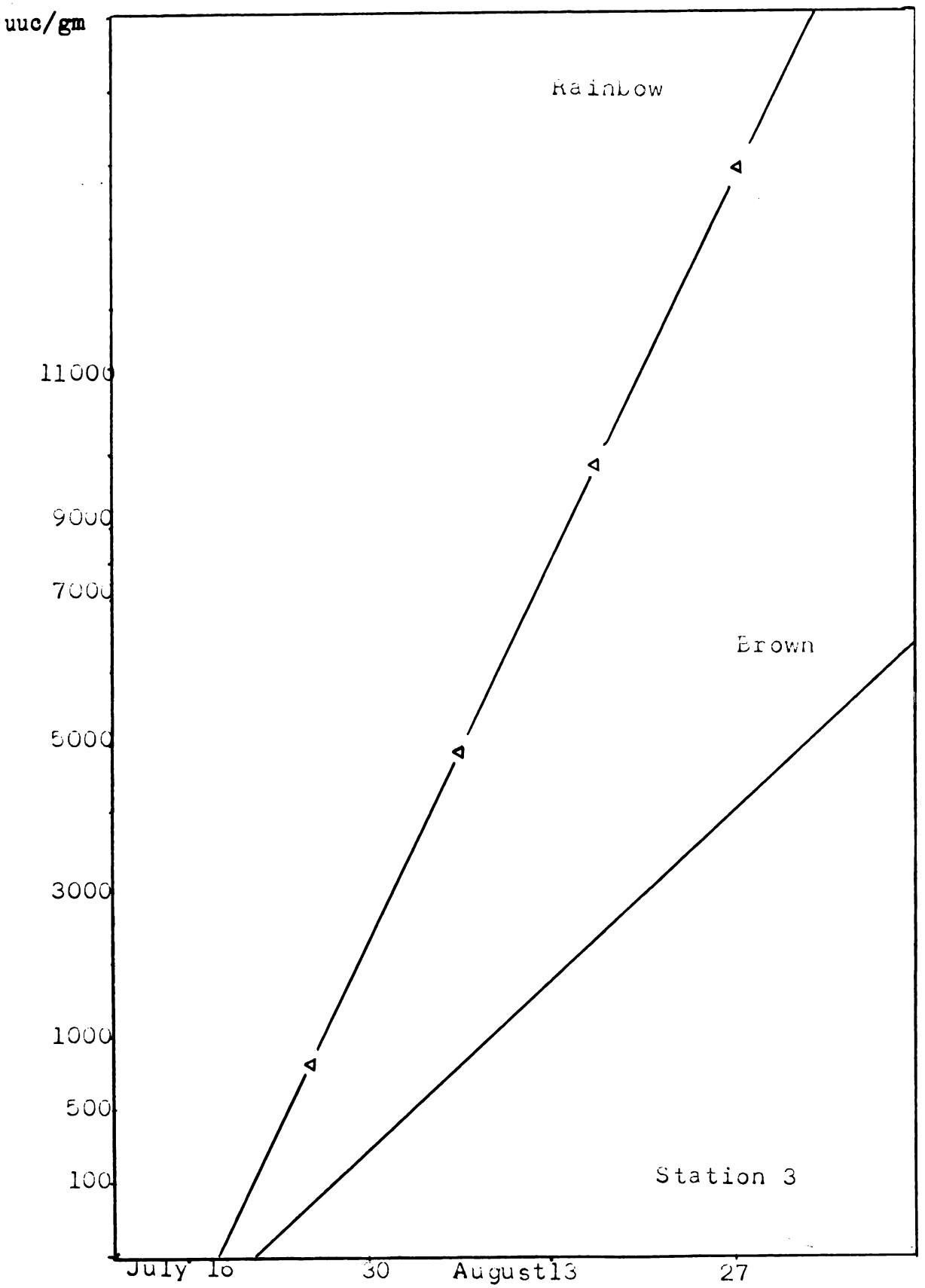

uuc/gm
Rainbow
11000
9000
7000
5000
3000
1000
500
100
Brown
Station 3
July 16
30
August 13
27

Fig. 36.--Regression of activity with time in days. Corrected micromicrocuries per gram of wet weight. Section 8 plots are given.

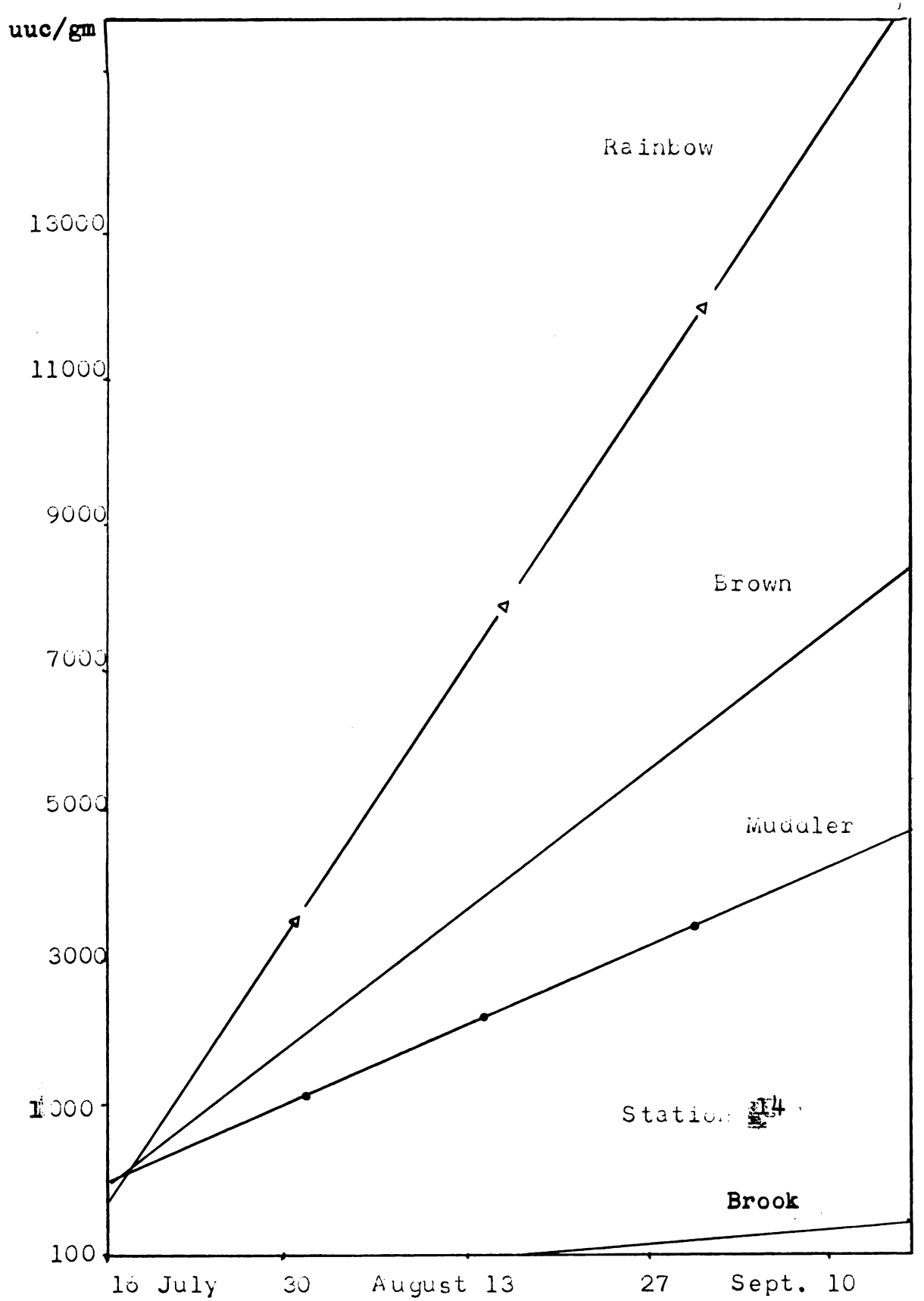
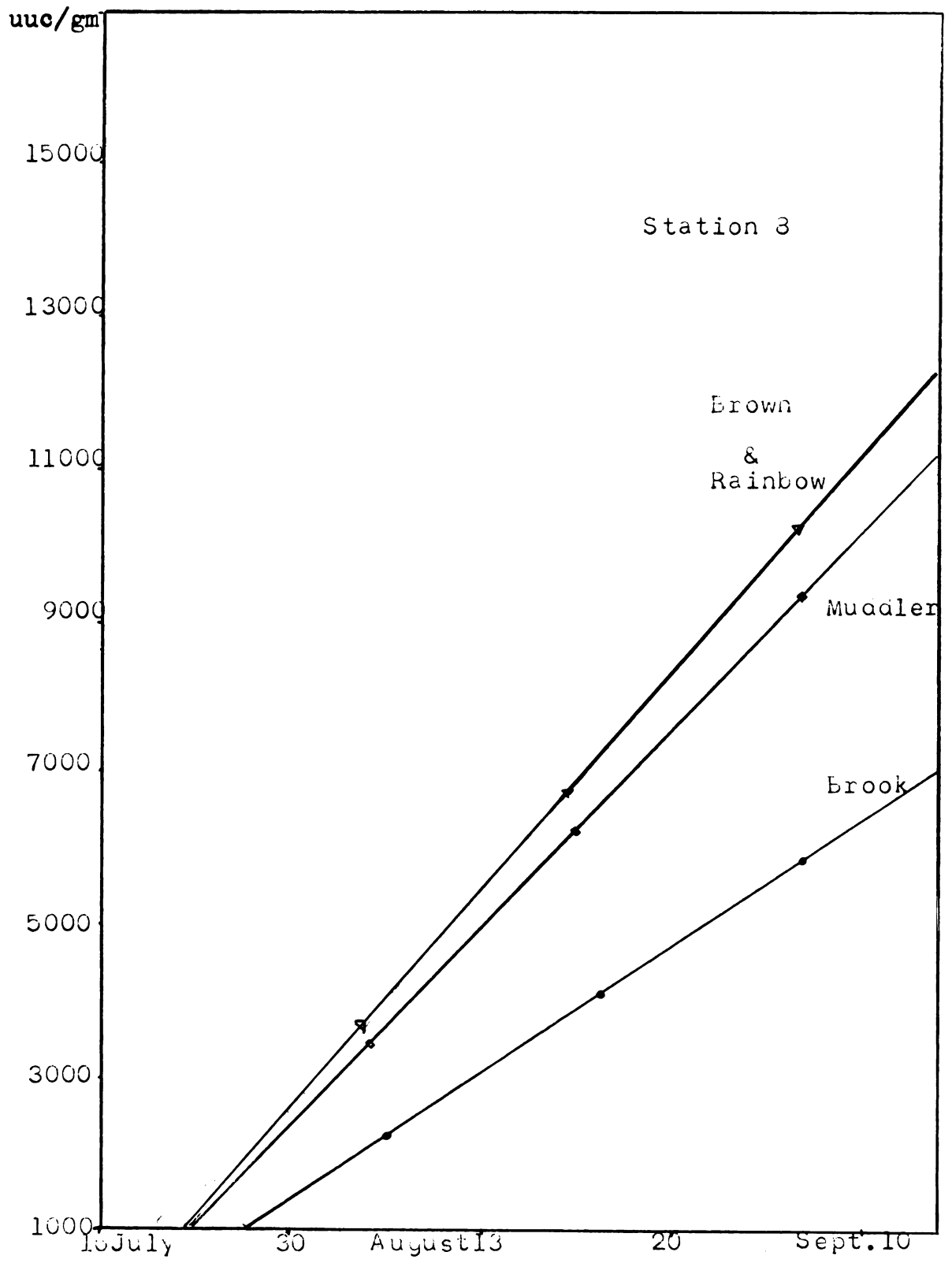

uuc/gm
13000
11000
9000
7000
5000
3000
1000
100
Rainbow
Brown
Muddler
Station 14
Brook
16 July
30
August 13
27
Sept. 10

Fig. 37.--Regression of activity of fish by time in days. Values from Station 14 samples. Corrected micromicrocuries per gram of wet weight.


uuc/gm
15000
13000
11000
9000
7000
5000
3000
1000
Station 8
Brown
&
Rainbow
Muddler
Brook
16July
30
August13
20
Sept.10

were washed with hundredth normal hydrochloric acid and the wash counted for radioactivity. The results of the counted washed showed that no activity was present as adsorbed phosphorus on the fish at any time during the study.

These data reveal no between station difference in rate of accumulation as tested by a two-way analysis of variance. They also revealed a trend of upstream concentration of tracer in the rainbow trout, which was followed closely in activity levels by the brown trout, then muddlers, and the least was found in brook trout. This trend was continuous downstream as concentrations built up with time, the only change being more activity in brown trout than in rainbow trout at the lower stations.'

A regression based upon parr would probably be very much different, but none was calculated because of the lack of enough samples at each station. When specimens were collected occasionally they had high concentrations of P^{32} in all species. The much higher concentrations in parr and fry has been established by Olsen and Foster (1952) who state that younger, more rapidly growing individuals accumulated relatively greater amounts of radioactivity than the older, more slowly growing fish. They attributed this to a reflection of the more rapid metabolism of the younger fish. In this study two to three times as much was accumulated.

Stress has been placed on food chain concentrations of tracer an ingestion by fish because in several types of studies conducted by Davis and Foster (1958), Phillips (1959), and Podoliak (1961) all revealed little incorporation of dissolved inorganic or organic phosphorus by sorption through either the gills or the skin. If one

considers loss of tracer through the feces and soluble wastes, a large amount must be so concentrated and returned to the stream in variously combined forms. Podoliak (1961) found indications that in water of 51° F. food which was dyed remained in a trout's stomach up to four days; accumulation in the tissues was slow, but efficient. At higher temperatures the time of contact was less as was the efficiency of removal of phosphorus from the food. The indication was that in 51° F. water and up to 66° F., two milligrams of phosphorus exceeded the digestive and absorptive capacity of the fish and was released only little changed.

Fish Biomass and Concentration of P^{32}

Fish populations were determined by using the Petersen formula, from collections made with a 220 volt D.C. shocker. The results indicate that brown trout are the numerically dominant salmonid and brook trout are the least abundant (Table 19). By use of the Bailey formula for variance (Bailey, 1951) the population variance and standard deviation were calculated.

From the regression slopes the rate of accumulation was inferred for any given time, as well as the total concentration of tracer at that time. Total stable phosphorus was determined by applying the conversion factor obtained by Zettelmaier (1961) for phosphorus per species per unit dry weight. A mean rate of accumulation was calculated, applied to the estimated population per unit area and the total accumulation derived. A minimum population was estimated since the samples for estimation were taken late in the summer after some losses to juvenile mortality, cannibalism, fishing pressure, and sampling.

TABLE 19.-- Trout population estimates of West Branch and their concentration of isotope.

	7/16	7/30	8/13	7/16
Station 3	Rainbow			Brown
Area population	412			563
Area weight in gms.	6226.6			8514.9
$\bar{X}$ uuc/gm	37.995	114.570	121.295	17.515
Area species accumulation by date	2.36×10^5	7.13×10^5	7.50×10^5	1.5×10^5
Station 8				
Area population	1623			1082
Area weight in gms.	19138.6			12759.1
$\bar{X}$ uuc/gm	58.367	98.383	488.600	4.679
Area species accumulation by date	1.1×10^6	1.9×10^6	9.4×10^6	5.9×10^4
Station 14				
Area population	1228			12213
Area weight	11247.5			111903.5
$\bar{X}$ uuc/gm	9.562	16.455	308.026	7.004
Area species accumulation by date	1.1×10^5	1.9×10^5	3.4×10^6	7.8×10^5
Total accumulation by date in uuc	3.9×10^5	1.34×10^6	1.22×10^6	1.2×10^6

TABLE 19.--(Cont.)

7/30	8/13	7/16	7/30	8/13
		Brook		
		68		
		1022.7		
74.456	54.399	7.224	29.794	5.845
6.34×10^5	4.6×10^5	7.4×10^3	3.0×10^4	5×10^4
		358		
		4227.4		
388.741	461.453	5.849	48.329	270.027
4.9×10^6	5.9×10^6	2.5×10^4	2.6×10^5	1.1×10^6
		437		
		4003.4		
25.890	304.300	3.064	6.066	11.412
2.9×10^6	3.4×10^7	1.2×10^4	2.4×10^4	4.5×10^4
7.05×10^6	1.64×10^7	9.0×10^5	3.1×10^6	3.8×10^7

From the calculated values in the Table 19 it is apparent that brook trout are not abundant, and because of their low tracer activity level they do not account for much of the phosphorus transfer within this ecoxystem. Brown trout by gross numerical abundance, weight, and a very high tracer level dominate the production terminus in the stream. Rainbow trout are a close second, but fall off because of reduced numbers of large fish, low stable phosphorus pools, and the period of feeding (diurnal) when insect activity or movement is slight. Rainbow trout seem to have a definite point of growth, beyond which they migrate downstream to larger lentic water areas. For this stream it is approximately nine inches and above. The larger fish are only present during spring spawning runs and shortly thereafter.

Accumulation of activity by muddlers is high and probably a large part of it never goes directly beyond their trophic level. It is assumed that they transfer their incorporated tracer by being eaten by larger trout. They are known food fish and are not large in size so that they don't outgrow their predators. By number alone they account for a tremendous quasi-terminal level of phosphorus accumulation.

Closer examination of fish biomass data (Table 19) and tracer levels reveals a series of trends that indicate the fish at the station located one-third of the longitudinal distance down the stream study section reached a peak of concentration in five days. Downstream levels were a function of the low tracer concentrations upstream. Station 14 accumulations were less, as were those of the upstream stations, 3 and 5, which had a large part of the available activity removed by various means. At the time of the second sampling, 19 days,

tracer was still accumulating at all stations within the fish. These tracer concentrations were building up the fastest at Station 8. However, by the third sampling period at 33 days, the tracer was being lost from Station 3, the uppermost station samples. It is assumed that adsorbed and incorporated tracer were being recycled and passed downstream, with continued accumulation evident at the lowermost station.

No attempt was made to isolate areas, within fish, that might accumulate more tracer than other areas might. Knight (1961) has a complete description of the level of tracer concentration within a series of fish of each species. He found that by ranked order of magnitude, bones are highest in activity, followed respectively by the head and gills, viscera, and muscle tissue with the least activity. From a public health standpoint, the amount concentrated in edible fish flesh, i.e. the muscle, was well within the amount given by Donaldson and Foster's (1957) suggested figure of 7×10^{-4} microcuries per gram, being slightly more than half, i.e. 3.7×10^{-4} microcuries per gram of phosphorus 32. I believe that similar values of concentration were maintained during this study period.

Light-Dark Study

An area of stream was selected for light-dark study of periphyton production on artificial substrates. The first segment was a 200 foot section between Stations 3 and 5 which was characterized by a nearly complete canopy of cedar and tamarack, no higher aquatic vegetation, a riffle over gravel and few logs or large stones for substrates. The mean noon level of sunlight was between 500 and 700 foot-candles depending upon the amount of sunflecks striking the light-meter sensing

unit directly and the amount of reflected light that was scattered. The second segment was between 400 and 500 yards further downstream between stations 5 and 8. It also was 200 feet long, but it was completely open to direct sunlight and no shore vegetation was present that was higher than one foot high for a radius of 125 feet in all directions. The flow was only half riffle in the sense that the bottom tilted from the left bank to the right facing upstream, and the right side was a deeper run. Numerous logs were present in this section.

An open field off to one side of the light area served as a control area and a comparison for the light readings obtained under the water's surface. The clearing was an oval area of approximately 200 feet in a north-south direction and about 280 feet in an east-west direction.

The areas were of slightly different flow characteristics, the "shade" zone being quite swift and the "light" zone being swift only on one side. The difference was not presumed significant since the supports for the light measuring units and periphyton substrates in both areas were placed randomly with the aid of a table of random numbers. An equal number of quadrats represented swift and slow habitats in each larger area during the course of the study.

The periphyton complex grew upon artificial plexiglass substrates attached to a cross-bow on a steel stake. Eight substrates of 1.2 dm^2 each, were used to accumulate periphyton for one-week intervals, at which time they were removed and replaced by 8 more substrates and the unit moved to a new randomly selected site. Each stake with its 8 substrates had a light meter attached to it during the week interval and all the incident light penetrating to a point one inch above the

substrates was recorded continuously by the solenoid recorder. The daily accumulated total readings were recorded each morning between nine and ten o'clock.

Maximum and minimum temperatures were taken at the downstream point of each area. At no time did they differ by more than 3° F. and no consistent pattern of difference was observed. The differences were presumed to be due to available light during some periods heating slow water areas, and to cold ground water contributions to the flow.

The substrates were collected in each area and transported to the laboratory in quart jars after the invertebrates were removed. They were frozen overnight and processed for radioactivity the following day. Processing for radioactivity was only slightly different from that of the processing previously described. The 8 substrates were scraped into a common large beaker and the periphyton was suspended in enough 95 per cent alcohol to make the solution up to 50 millimeters. The periphyton and alcohol were allowed to stand for 1 hour, while other substrates were scraped, in a dark container. Then the solution was drawn through a 0.45 micron millipore filter in a millipore apparatus. The filter was wet and pre-weighed. Ten seconds after the filter appeared water free, 3 milliliters of 0.01 N hydrochloric acid were added, followed immediately by a 5 milliliter distilled water wash. The suction was withdrawn after the filter appeared water free again for 15 seconds. The filter was removed and weighed, after which it was placed in a numbered deep-wall planchet. The filtrate was transferred to a Klett cuvette and read using a 660 millimicron filter. Then the filtrate was evaporated to a volume of 2-3 milliliters, added to a deep-wall planchet containing the filter and both were digested with

concentrated nitric acid and heat on a hot plate. After complete mineralization the planchet and contents were heated to a cherry red in a muffle furnace set at 600° C. After cooling, the planchet was counted in the Omni-guard system for radioactivity.

Light Measurement

A simple, portable, and inexpensive field instrument was built for us by Mr. Clinton Harris, an electronic engineer of Ann Arbor, Michigan. These instruments were built for long continuous operation in the field with a probable error of ± 20 per cent.

The main requirement was for a system which would measure the total amount of energy falling upon the sensing device over a period of time rather than measuring instanteous intensities. Therefore, the light striking a selenium photovoltaic cell was fed through a transistor, which served as a matching device and permitted the photovoltaic cell to work through a low resistance. The result of which was a linear response to a wide range of light values in the spectral range which covers the wavelengths involved in biological activity. The selenium cell also allows comparison with the work of other investigators who have used this type of cell.

The photovoltaic cell and transistors were packaged as a unit. Temperature considerations were important as related to the transistor since high ambient temperatures could damage it, therefore, the sensing unit and transistor were packed in a water-tight unit and placed directly in the stream which remains at a near constant temperature.

Current from the photovoltaic cell fed through the transistor and resistance was fed into a timing capacitor. The pulsed output from

the timing capacitor was directly proportional to the intensity and was sent through a second transistor to energize the solenoid in a five place mechanical counter. The second transistor was silicon to guard against high ambient temperatures since the timing capacitor, transistor, and counter were maintained in a box on shore, as were the batteries which powered the unit. Four 6 volt sport lantern batteries (NEDA 918) were used to power the 24 volt DC General Controls 5 digit, non-reset counter.

Calibration

The determination of the lower limit of usefulness of the light and dark units was found to be "dark current" which with proper selection of the component parts could be made such that the device was reliable down to a light level of well under 1 foot-candles incident directly on the photovoltaic cell if a reasonable counting rate was employed (2 counts per second). By inherent limitations the device stopped counting with a little under 0.1 foot-candles directly incident upon the photovoltaic cell.

Because selenium cells are non-linear at high light intensity and because light intensities in excess of 1000 foot-candles damage the cells it was decided that a maximum of 500 foot-candles should be incident upon the photovoltaic cells at any given moment. A range of 1 to 200 foot-candles was considered ideal and by filtering the cell should be representative of the stream.

To reduce the intensity of the incident light upon the cells neutral density filters were employed. Interchangeable filters were provided for the "light" and "shade" units.

Filters with a factor of 50 (density 1.7) were used in the "light" units. Thus the incident light range of 50 to 10,000 foot-candles resulted in a unit incident light on the photovoltaic cell of from 1 to 200 foot-candles. In "shade" areas where lower light levels were important in terms of their aggregate contribution, advantage was taken of the fact that very high light intensities were not expected. Therefore, filters with a factor of 5 (density 0.7) were used in the shade units.

In order to reduce the error resulting from angle or incidence the photovoltaic cell and the filter were mounted and sealed against moisture behind a glass hemisphere with a finely ground surface.

The counting interval was established as about 1 second for a light intensity of 200 foot-candles incident upon the cell. The devices were calibrated with the sensing unit immersed in water of the same temperature as that of the stream in which they were to be used in order to eliminate errors arising from the temperature characteristics of the photovoltaic cells and their matching transistors.

Figure 38 is a photo of a complete unit without the battery and counter housing (a wooden box).

Table 20 is a representative series of instantaneous light readings taken at 20 foot intervals downstream from the upstream edge of the dark area. The levels of incident light were measured with a Weston light cell which measured the light on an upper and lower scale with a range on the scale from zero to seventy foot-candles. The readings tables were on the lowest scale setting and were read directly. They are noon readings on a slightly hazy day when maximum open field light was 7000 foot-candles from the same meter.

Fig. 38.--A light sensing unit in its immersible covering, with glass hemisphere and cord to shore counter and power source.

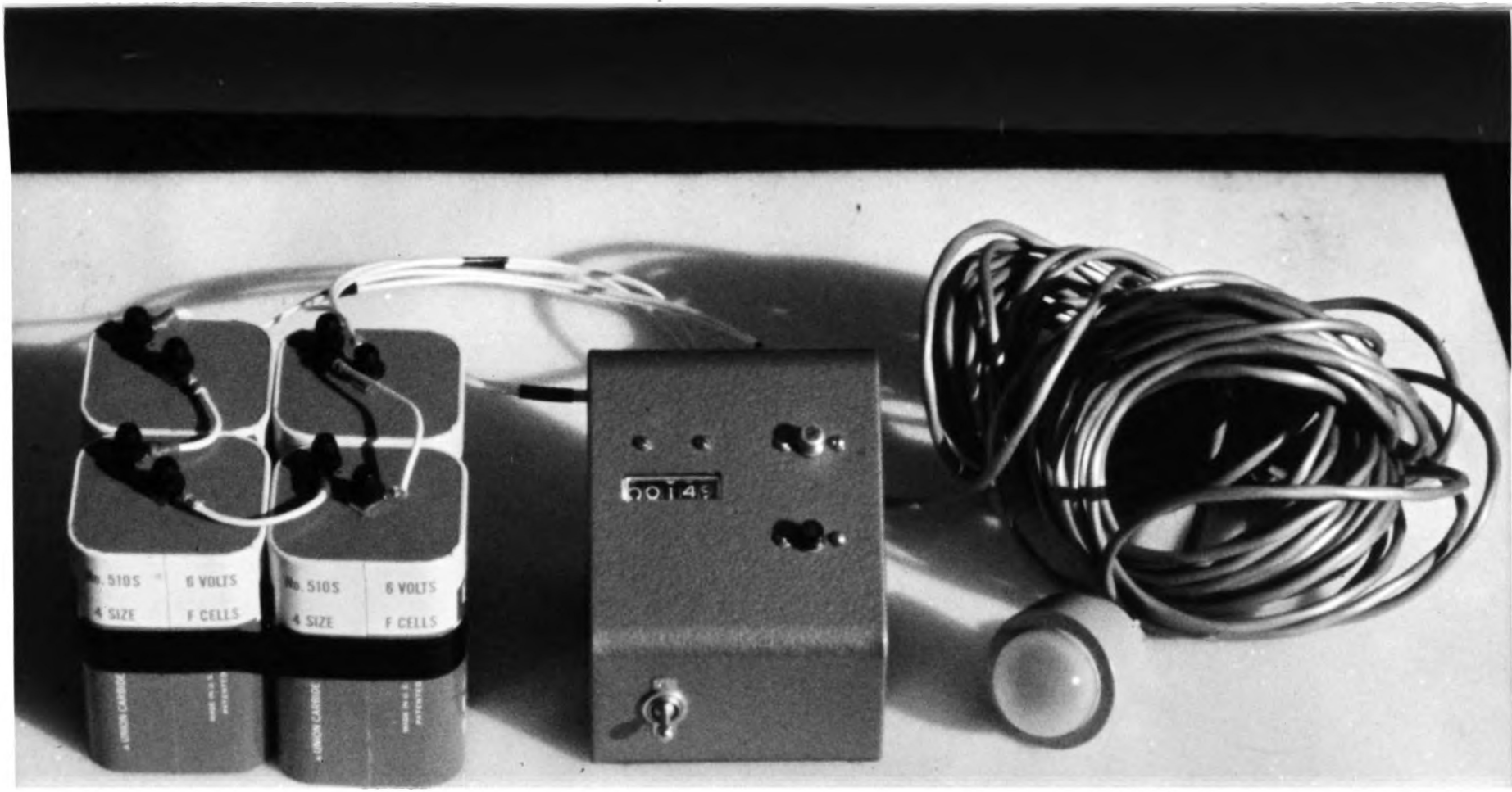

No. 510S
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TABLE 20.--Noon light readings in foot-candles, as measured by Weston 756 light cell. Taken at 20 foot intervals (longitudinally) on a slightly hazy day.

Transect	Distance From North Stream Bank, in Feet (Right Bank)						
	3	6	9	12	15	18	21
1	20	30	20	5	5	10	-
2	36	42	42	42	40	35	30
3	25	25	30	30	30	26	-
4	10	35	45	45	36	15	10
5	35	30	28	28	15	10	5
6	10	30	25	20	12	10	-
7	25	30	5-	1	3	5	-
8	40	40	25	10	6	4	-
9	70-	70-	70-	70-	60	56	55
10	60	70	70	60	55	50	45

Light reflecting off water--5 foot-candles.

Open sunlight 7000 foot-candles.

10 A.M. readings averaged 5-10 foot candles less.

3 P.M. readings averaged 10-15 foot candles higher.

Area Comparisons

The total light intensity values of replicated data from randomly located stakes from the two sites (light and dark) were computed by a matched observation method, the results of which are given in the appendices. These tests indicate that the two areas were significantly different at the .99 per cent level in the total light received. From the same stakes the substrate weights of periphyton and radioactivity accumulated during given intervals were measured and the weight of periphyton versus radioactivity tested for the light and dark area by a covariance analysis modeled after that given by Walker and Lev (1953). The results are given in the appendices and indicate that the two slopes are not significantly different, i.e. the rates of accumulation in both areas were the same. A test of covariance of the intercepts given in the appendices indicated that the intercepts were significantly different at the .99 level, i.e. the rate of accumulation in both areas was the same, but the amount of activity present from measured time intervals were different between areas. The light to dark area relationship was one where the higher weights present in the dark areas had less radioactivity associated with them at any given time than did the light areas.

It would appear then that the ratio of bound phosphorus to wet weight of periphyton of similar species in the two areas differ, with the dark area generally having a lower value than the light area. With the rate of weight accumulation and radioactivity accumulation the same in the two areas (light and dark) the lower amount of phosphorus compounds present at the time of measurement must be due to some type of organic phosphate degeneration. This may be due to the significantly

different amounts of light energy reaching the two areas. The light area receives nearly full light (95 per cent) while the dark area receives on 13 per cent of full light. The lesser intensity in the dark area may be compensated for by more of the light being of long wavelengths due to the canopy filtration favoring passage of the red end of the spectrum, but it apparently does not compensate by increased chlorophyll production, with associated phosphorus uptake. Increased efficiency of production may account for the difference in production on the substates since it is known that the rate of photosynthesis will increase with the light intensity at lower light levels up to a certain level of light intensity after which it rapidly falls off. Maximum efficiency is often reached at only one-third to one-fourth the peak intensity (Meyer and Anderson, 1952).

These factors must enter into the area production just as the fact that phosphorus deficient land plants tend to store excessive sugars and inorganic nitrates rather than proteins with their associated phospholipids and nucleoproteins. If it can be assumed the same physiological and biochemical mechanisms and processes are present in aquatic plants as in land plants then the above factors may account for the differences in organic weight accumulated in the two areas, but not the difference in radioactivity. The production of more material per se would have the same incorporation rate normally, as it does, and a higher total incorporated amount of phosphorus should result. The difference must lie in the total energy received in the two areas, they are known to be significantly different with the dark area receiving much less external light energy. Therefore, the energy required must come from stored products through the energy cycle utilizing

ATP and ADP and all the intermediate stored phosphorus compounds. The energy demand for the high rate of production must be met by a high respiratory rate. Calvin and Benson (1950) have shown that phosphoglyceric acid is both an anabolism and catabolism constituent and may well be a respiratory waste. The intermediate phosphates which were stored are transported to new building sites and used to build more phospholipids and nucleic proteins with organically bound phosphate constituents. During the building the stored phosphates are changed from the old forms to new forms for reincorporation by the high energy system utilizing ATP and ADP and the total result is a recycling of stored phosphates for the energy they can supply when the external environment does not supply enough energy for maintaining the photosynthetic rate.

With respect to the possible difference in efficiency of production between the two areas the work of Emerson and Arnold (1932) may well explain the observed differences in the West Branch of the Sturgeon River data. They exposed cultures of Chlorella to intermittent illumination at the rate of 50 flashes per second, the periods of illumination being much shorter (0.0034 second) than the intervening dark periods (0.0166 second), and obtained a photosynthetic yield per unit of light which was increased by about 400 per cent as compared with the rate in continuous light. Assuming the photochemical reaction comes first, the results were concluded as follows: when illumination is continuous the products of a light reaction are formed faster than they can be utilized in a relatively slower dark reaction. When the light is intermittent, all or most of the products are removed from the photochemical reaction, and the photosynthetic output per unit of

light is considerably greater.

They measured the dark reaction and found it to proceed in less than 0.04 second at 25° C., and to be greatly influenced by temperature. The light reaction takes place at a speed of about 0.00001 second and is unaffected by temperature. With low light intensities and adequate carbon dioxide, a photochemical reaction is limiting and temperature will have little effect on the rate of the process. With high light intensities and adequate carbon dioxide, but low temperatures, the rate of photosynthesis is limited by the dark reaction, and will increase considerably with a rise in temperature.

Considering the stream as a whole the water is well aerated and rich in carbon dioxide as well as being uniformly cold. The shaded areas were well interspersed with light flecks which changed instantaneously and continuously. This would well fit the conditions described by the above authors and in the light areas the dark reaction may well be limiting, whereas in the dark areas the photochemical reaction may be the limiting factor due to reduced energy, but still greater in terms of the amounts of products produced.

SUMMARY

The form of tracer available to the organisms of the ecosystem was shown to be an important factor in the time and amount of phosphorus take up by living and dead materials. Uptake by the sediments was high within the experimental zone, with larger amounts being found in the biologically incorporated state than in the adsorbed state. These differences increased with distance downstream from the point of application.

The particulate fraction and the organic fractions of the water were shown to be very important to the exchangeable phosphorus that may be recycled as it moved downstream. The amount of tracer incorporated in organic fractions increased in direct proportion to the increase of the producer organisms.

Bacteria were shown to incorporate as much tracer as the diatoms when both were endemic in the stream and in the same volume of water, but the magnitude and rate of diatom incorporation appear to be more important to the transfer of inorganic phosphorus to the trophic structure of the stream than that of the bacteria.

Movement of radio-activity was demonstrated in connection with the terrestrial and aquatic phase of the large, mobile aquatic insects, and to movements of snails which moved readily upstream even against rapid currents. Distances of 500 yards for the crawling forms and five miles for the flying forms were recorded. Fish movement was very

limited and accounted for very little dispersal of phosphorus. The volume and number of invertebrates drifting downstream was high and contributed considerably to the downstream movement of the isotope.

Light controlled the productive structure of the stream where it was on the border line of the duration and intensity required for periphyton growth. The levels of light were so low in the areas having a complete vegetative canopy that the food reserves built up during certain intervals of peak illumination were called on to supply the energy for continued maintenance at the low efficiency periods which occurred during much of the daylight hours.

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APPENDIX

Expansion of data presented in thesis.

Y- radioactivity (P^{32})

X_1 - light

X_2 - weight

Y	X_1	X_2	Y	X_1	X_2	Y	X_1	X_2
384.9	399640	.0255	400.2	980592	.0335	733.7	880000	.0570
142.0	114615	.0925	327.5	78006	.0290	176.1	127734	.0520
603.2	985500	.0285	42.0	1554056	.0415	403.8	1106180	.0600
0.0	109918	.0240	414.7	247032	.1030	324.4	259596	.2060
0.0	1075932	.0175	313.9	2088576	.0375	71.9	1089296	.0355
451.4	112735	.0125	152.2	149624	.0285	146.6	122176	.0465
309.0	496060	.0270	300.0	1285472	.0320	307.7	458936	.0325
322.2	109987	.0385	310.4	136030	.0695	300.0	95577	.0426
247.3	760256	.0620	197.5	1446384	.0880	309.1	955144	.0145
401.8	108464	.3650	75.3	105506	.0932	198.2	1041555	.0620
161.8	929244	.0241	300.0	1056936	.0475	170.0	633920	.0341
300.1	112464	.0425	201.9	105363	.0811	350.8	120141	.0480
3216.8	5314815	.7596	3035.6	10233577	.6843	3497.3	5952851	.5637

AREA

	X	Y	X	Y	X	Y	X	Y
light	.0255	384.9	.0336	400.2	.0570	733.7	.1160	1518.8
dark	.0925	142.0	.0290	327.5	.0520	176.1	.1175	645.6
light	.0285	603.2	.0415	42.0	.0600	408.8	.1300	1054.0
dark	.0340	0.0	.1030	414.7	.0790	324.4	.2060	739.1
light	.0175	0.0	.0375	313.9	.0355	71.9	.0905	385.3
dark	.0125	451.4	.0285	152.2	.0465	146.6	.0875	750.2
light	.0270	309.9	.0320	300.0	.0325	307.7	.0915	917.6
dark	.0385	342.2	.0695	310.4	.0426	300.0	.1506	952.6
light	.0620	247.3	.0330	197.5	.0145	309.1	.1045	753.9
dark	.3650	401.8	.0932	75.3	.0620	198.2	.5202	675.3
light	.0241	131.3	.0475	300.0	.0341	170.0	.1057	631.8
dark	.0425	300.1	.0311	201.9	.0430	350.8	.1716	852.8
sum X	.7596	3216.8	.6843	3035.6	.5637	3497.3		
Sum X^2	.1527	1269755.6	.0476	924642.6	.0296	1389306.9		
$(X)^2$	.5770	11687609.1	.4683	9214387.4	.3178	12231107.3		
$\frac{(X)^2}{n}$	.0481	973967.4	.0390	767905.6	.0265	1019258.9		
x^2	.1046	295788.2	.0086	156736.9	.0031	320548.0		

Treatments		
"	1	246.0
"	2	169.7
"	3	172.7
		583.4

Grand totals

	X	Y
X	1.9516	9877.5
X^2	.4718	8992650.2
$(X)^2$	3.8087	97565006.3
$(X)^2/n$	.3174	8130417.2

Matched observations test of significant difference between observations.

	1	2	3	sum		
light	399640	980592	880000	2260232		
	985500	1554056	1106180	3645736		
	1075932	2083570	1039296	4253524		
	496080	1285472	458936	2240488		
	750256	1446384	955144	3161784		
	929244	1050936	838920	2020100		
					difference	% light area light
dark	114615	78000	127734	320355	1939877	14.17
	109918	247032	259596	616546	3029190	16.91
	112735	149624	122176	384535	3868989	9.04
	109987	136030	95577	341594	1898874	15.25
	108464	105506	104151	318121	2843663	10.06
	112464	105363	120.41	337968	2282132	12.90
	X 15862725		X^2	448084589042l9	$\bar{X}$ =	13.06%
			$\frac{(X)^2}{n}$	$\frac{251626044425625}{6}$		

$\bar{d}$ = 2543787.5

$t = \frac{\bar{d} - 0}{s_d} = 3.49$ with 11 df.

Therefore: significant at .99 level

SS	41937670000000
$\frac{SS}{n-1}$	$\frac{2870788900000}{5}$
S^2	574157700000
S	757731.9

Covariance analysis of slopes of weight vs. radioactivity in the light vs. dark areas.

b	x^2	xy	y^2	N
12600.0	.0005	6.3	77705.9	3
366.7	.0075	2.5	19498.4	3
-3666.7	.0006	- 2.2	162426.4	3
5303.0	.0033	17.5	95121.9	3
9000.0	.0003	2.7	54088.9	3
-10200.0	.0005	- 5.1	60818.4	3
0.0	.0000	0.0	54.0	3
600.0	.0005	- 0.3	966.7	3
-1464.3	.0028	- 4.1	6551.4	3
879.5	.0556	48.9	54386.0	3
5333.3	.0003	1.6	12022.1	3
-3111.1	.0009	--2.8	11461.7	3

Test of whether 12 b's are significantly different from one another.

Find sum of d^2 about each regression by: $d^2 = y^2 - b\ xy$

df. = (n--2)

77705.9 - 79380.0 = - 1674.1

19498.4 - 5833.6 = 13364.8

162423.4 -- 3666.7 = 154359.7

95121.9 - 92802.5 = 2319.4

54088.9 - 24300.0 = 29788.9

60818.4 - 52020.0 = 8798.4

Covariance analysis of slopes of weight vs. radioactivity con't.-

54.0 -	0.0 =	54.0
966.7 -	180.0 =	786.7
6551.4 -	6003.6 =	247.8
54636.0 -	43007.6 =	11378.4
12022.1 -	8463.3 =	3468.8
11461.7 -	8711.1 =	2750.6

$(d^2)_{ind} = 223914.0$

df. ind = 12

$$b_{average} = \frac{\Sigma xy_1 + \ldots\ldots + \Sigma xy_{12}}{\Sigma x_1^2 + \ldots\ldots + \Sigma x_{12}^2} = \frac{62.0}{.0728} = 947.8$$

df. = (n-k-1) = (36-12-1) = 23 df.

$$\Sigma(\Sigma d^2)_{average} = \Sigma y_1 + \ldots\ldots + \Sigma y_{12}^2 - b_{average}(\Sigma xy_1 + \ldots + \Sigma xy_{12})$$

$$= 554301.8 - 947.8\ (69.0)$$

$$= 554301.8 - 65398.2 = 489403.6$$

unbiased estimate of $sigma_2^2 = S_2^2 = \frac{\Sigma(\Sigma d)_{ind}}{df\ ind} =$

unbiased estimate of $sigma_1^2 = S_1^2 = \frac{489403.6 - 19975.2}{11} = \frac{470327.4}{11}$

$$= 42757.0$$

$$F = \frac{42757.0}{489403.6} = .087 \qquad \frac{11\ df.}{12\ df.}$$

5% level F = 2.72 with 11/12 degrees of freedom

Therefore the slopes are not significantly different.

Analysis of covariance:

Test of intercepts that are assumed to be equal, adapted from Walker and Lev (1953).

Peripnyton weight vs. radioactivity

	sum X	sumXY	sumY	sum X^2	sum Y^2	$\bar{X}$	N
light	.1150	65.04	1513.8	.0050	840623.7	.0387	3
dark	.1175	31.79	845.8	.0121	1158431.5	.0392	3
light	.1300	43.46	1054.0	.0061	532721.7	.0433	3
dark	.2050	68.34	733.1	.0174	277211.5	.0683	3
light	.0905	14.32	385.8	.0060	103702.8	.0302	3
dark	.0875	16.80	750.2	.0031	248418.4	.0292	3
light	.0915	27.97	917.8	.0023	280717.3	.0305	3
dark	.1506	47.53	952.6	.0081	311576.3	.0502	3
light	.1645	37.19	753.9	.0118	195706.4	.1734	3
dark	.5202	165.96	875.3	.1458	206396.6	.0352	3
light	.1057	23.95	631.8	.0040	145079.2	.0352	3
dark	.1715	45.97	852.8	.0117	253884.3	.0142	3

Sum (sum X) = 1.9516 Sum(sumXY) = 588.23 Sum(sumY) = 9877.5

Sum(sum X^2) = .8299 Sum(sum Y^2) = 3560479.7

$\bar{X}_T = \frac{(X)}{N} = \frac{1.9516}{36} = .0542$

$\bar{Y}_T = \frac{(Y)}{N} = \frac{9877.5}{36} = 274.4$

$b_{average} = 947.8$

Determine adjusted means by

$Y = Y_T - b_T (X_T - X_1)$

Covariance of intercepts con't.--

274.4 - 947.8 (-.0618)

274.4 --947.8 (-.0633)

274.4 - 947.8 (-.1391)

274.4 - 947.8 (-.2909)

274.4 - 947.8 (-.0603)

274.4 - 947.8 (-.0335)

274.4 - 947.8 (-.0373)

274.4 - 947.8 (-.1357)

274.4 - 947.8 (-.1103)

274.4 - 947.8 (-.4000)

274.4 - 947.8 (-.0518)

274.4 - 947.8 (-.1174)

Calculate one grand regression equation pooling all data.

$$Y = \bar{Y}_T - b_T(\bar{X}_T - X_1)$$

where:

$$b_T = \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}}{\Sigma X^2 - \frac{(\Sigma X)^2}{N}} = \frac{588.23 - 535.47}{.2299 - .1058} = \frac{52.76}{.1241} = 425.1$$

$$a = \frac{1.9516 - 425.1\ (9877.5)}{12}$$

$$\Sigma YT^2 = (\Sigma Y^2) - \frac{(\Sigma Y)^2}{N} = 3560879.2 - 2710139.1 = 6849945.5$$

Covariance of intercepts con't.---

$$d_T^2 = YT^2 - b_T XY_T = 6849945.5 - 425.1\ (52.76) = 6827517.2$$

$$F = \frac{\frac{6827517.2 - 432403.6}{11}}{\frac{432403.6}{23}} = \frac{575192.1}{21278.4} = 27.08 \text{ with } \frac{11 \text{ df.}}{23 \text{ df.}}$$

Intercepts are significantly different at the .999 level.

Three-way analysis of variance for dates, groups, and distance in insects during 1961 immature movement study.

A = dates 7, 14, 21, 28, 36, 42, and 48 days

B = groups - *Athericix*, *Ptilomicrys*, etc.

C = distance - 100, 200, 300, 400, and 500 yards

Source	Sum of squares	df	mean square	F
A	4226.40	6	704.40	0.843
B	7440.26	5	1488.05	1.780
C	4801.10	4	1200.28	1.436
AB	23839.31	30	794.64	0.951
BC	21429.81	20	1071.49	1.282
AC	25671.17	24	1069.63	1.280
ABC	100309.12	120	835.91	

no significance from any source at alpha = quotient .05

Light - dark matched observations

399640	980592	880000	2260232	
985500	1554056	1106180	3645736	
1075932	2088576	1089296	4253524	
496060	1285472	458936	2240468	
760256	1446384	955144	3161784	
929244	1056936	633920	2620100	
114615	78006	127734	320355	1939877
109918	247032	259596	616546	3029190
112735	149624	122176	384535	3868930
109987	136030	95577	341594	1693874
108464	105506	104151	318121	2343663
112464	105363	120141	337968	2282132

sum X = 15862725

sum X^2 = 443034589042l9

$\frac{(\text{sum } X)^2}{n} = \frac{251620044425625}{6}$

SS = 41937670000000

$\frac{SS}{n-1} = \frac{23707883900000}{5}$

S^2 = 574157700000

S = 757731.9

$\bar{d} = 2643787.5$

$t = \frac{\bar{d} - 0}{S_{\bar{d}}} = 3.49$

with 11 df.

Significant at .99 level

Test of heterogenity of regression between areas.

Area	DF	x^2	xy	y^2	b_{yx}	$b_{yx}xy$	y_d^2	DF
1	11	.1046	37.2	295738.2	355.6	13228.3	282559.9	10
2	11	.0086	-3.4	156736.9	-395.3	13440.2	143296.7	10
3	11	.0031	8.4	320548.0	2709.7	22761.5	297786.5	10
Total	33	.1163	42.2	773073.1	2670.0	49430.0	723643.1	32

Total reduced sum of squares accounts for 32 degrees of freedom the reduced sums of squares for each area for only 3 x 10 = 30 degrees of freedom. Remaining 2 degrees of freedom represen t differences between the 3 regression coefficients.

Therefore:

	DF	SS	MS	F
Total	32	723643.1		
Areas	30	723643.1	24121.4	
Difference	2	0.0		0

Replicates

$$\frac{(.0255 \times 384.9) - \ldots - (.0425 \times 300.1)}{12} - \frac{1.9516 \times 2877.5}{36}$$

$$= \frac{1557.7}{12} - \frac{19276.9}{36} = 129.8 - 535.5 = -405.7$$

Treatments

$$\frac{(.7596 \times 3216.8) - \ldots - (.5637 \times 3497.3)}{12} - \frac{1.9516 \times 9877.5}{36}$$

$$= \frac{6492.2}{12} - \frac{19276.9}{36} = 541.0 - 535.5 = 6.0$$

Error

$$52.9 - (-405.7) - 6.0 = 452.6$$

	df.	x^2	xy	y^2	b_{yx}	$b_{yx}xy$	y_d^2
Replicates	12	-.0665	-405.7	-1960751.3			
Treatments	12	.0078	6.0	- 60657.7	769.2	4615.2	65272.9
Error	33	.1328	452.6	2845474.6	2475.9	1120592.3	1724881.8
Treatment plus error	45	.1906	458.6	2784816.9	3245.1	1125207.5	1790154.7

Test significance of regression error

	SS	DF	MS	F
Regression	1120592.3	11	101872.0	1.89
Error	1724881.8	32	53902.6	

Test significance of thedifferences between area means (treatments).

Treatment plus error	1790154.7	45		
Error	1724881.8	32	53902.6	0.15
Difference	65272.9	8	8919.1	
Adj. Treatments	4615.2	11		
$B_e - B_t$	60657.7			

Simultaneous across stream samples from 4 repeated transects. Samples were taken at 4 feet from the left bank, the center, and 4 feet from the right bank.

Mean values of the four replicates are given in terms of micro-microcuries per milliliter of water.

Left		Center		Right	
$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
0.0193	0.0084	0.1015	0.1644	0.1320	0.0580
0.0193	0.0059	0.0273	0.0065	0.1535	0.1032
0.0183	0.0123	0.0245	0.0093	0.0958	0.0266
0.0615	0.0184	0.1048	0.1208	0.1725	0.0693
0.1635	0.0343	0.1383	0.0483	0.3018	0.1481
0.8555	0.2676	0.4678	2.0981	0.6658	0.1097
2.8223	0.2827	1.2840	0.8586	1.6110	0.2914
2.6790	0.5036	1.6305	0.2831	1.6545	0.1713
1.5290	0.1225	1.5250	0.1637	1.0418	0.1992
0.2500	0.1807	0.4473	0-4473	0.0475	0.0366
0.0275	0.0429	0.1035	0.0820	0.1240	0.0023
0.0083	0.0080	0.1223	0.0104	0.1903	0.1895

$\bar{X}$ = mean activity

S = standard deviation