TRANSLOCATION OF RADIOPHOSPHORUS IN A STREAM BY BACTERIA AND INORGANIC IONS

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James P. Bacon Jr.

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By

JAMES P. BACON JR.

AN ABSTRACT

Submitted to
Michigan State University
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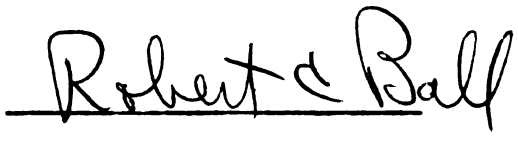
Approved 

ABSTRACT

Twenty-one millicuries of radiophosphorus (P^{32}) were incorporated into the cells of Escherichia coli Olll bacteria. On July 13, 1961, these bacteria were added to the West Branch of the Sturgeon River. In 1958, 1959, and 1960 P^{32} was added to this stream in an inorganic form. The purpose of the 1961 study was to tag only the consumer portion of the community with P^{32} atoms and to note any differences in metabolic pathways or activity density which occurred when phosphorus entered the food chain at a different point.

The data reveal that there was some soluble phosphorus in the spike, but that there was no significant uptake of this activity as the spike passed downstream. There was a logarithmic decrease in the amount of particulate activity in the water. This may have been the "fallout" of bacterial cells. These cells which were trapped in the study area seem to have been the major source of activity for the community. At least fifty-five per cent of the bacteria were carried through and beyond the study area and these bacteria showed no significant loss of P^{32} through the study area.

The periphyton and macrophytes showed activity density curves which were similar in pattern to those of previous years with two exceptions. First, they were all reduced by approximately ten fold, and second, the plants showed a period of uptake during the first four days which indicated they were receiving activity which was being released by the decomposition of bacteria which had remained in the study area. We did not succeed in isolating the producer level of the stream.

The invertebrate organisms of the stream showed little increase

in activity density over the plants when both are compared to the 1959 results. This suggests that very few bacteria were utilized as food organisms. If any of the stream consumers utilized bacteria to any extent they should have shown relatively higher activity density curves in 1961 since our bacteria spike more than doubled the number of bacteria in the water at some of the sampling stations. The bacteria in this study were also exposed to a greater concentration of P^{32} than were the stream bacteria in previous studies.

The movement of the P^{32} through the consumer portion of the food chain was quite comparable to that of past years except that the magnitude of the activity density curves were reduced by ninety per cent in most cases. Three organisms showed activity densities which differed from the other species sampled when compared with 1959. Simulium and Hexagenia apparently obtain and utilize bacteria in their diet. Nigronia showed a higher activity density in 1961 than it did in 1959. These differences were presumably brought about by the different form in which the isotope was added.

Activity density curves showed that the activity moved from the primary consumers to the secondary consumers to the tertiary consumers and finally to the decomposers.

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INTRODUCTION

One of the most useful tools with which the present day ecologist may examine the complexities of food webs is the radioactive nuclide. It may be used in the laboratory or, in special cases, in the field.

During the summers of 1958, 1959, and 1960 radiophosphorus (P^{32}) was added to the West Branch of the Sturgeon River, in an inorganic form. The movement of the P^{32} through the community was analyzed each year, and a firm foundation was gained concerning the translocation of inorganic phosphorus through a cold water stream community.

There was some doubt, however, as to what role the stream bacteria played in the movement of the phosphorus. They may have been intermediate in position between the inorganic P^{32} and the first producers to take it in, the diatoms. The present study also afforded the investigators a chance to find out of what importance the bacteria are as food organisms in a stream ecosystem. The role of bacteria in the phosphorus metabolism of lakes has been investigated by Hayes and Phillips (1958) and Rigler (1956), but just what role the bacteria play in stream ecology is unknown. It was for these reasons that in 1961 the radiophosphorus was added to the stream in the form of radioactive bacteria (Escherichia coli).

This study then, is comparative in nature. It represents a comparison of the differences in magnitude of concentration, metabolic pathways, and point of entry into the food web, which ensue when radiophosphorus is added to a stream community in an organic

form, as compared with an inorganic form.

With this as a major theme, it must also be remembered that while we are now only working with a relatively harmless level of radioactivity, such might not always be the case. The growth and development of our society, in the future, will depend more and more on radioactivity as a source of energy. Our present day energy sources are already dangerously low, and they will be inadequate to meet the demands of society in a relatively short time. The use of huge nuclear fission reactors as a major power source is already becoming quite common. These reactors use huge quantities of water as a coolant, and the water is usually pumped back into a stream or lake after it is used. This would not be dangerous, except that the fission process releases high energy particles and rays which alter the atoms of the elements found in the water and makes them unstable. It is now a well established fact that even very dilute, low level activity released in the environment may be dangerous because the plants and animals possess remarkable powers to concentrate many of these elements within their tissues (Odum, 1959). Those elements, like phosphorus, which are crucial to the existence of the organism are particularly susceptible to this concentration. Thus, with each investigation of this type we gain more insight into the problems of translocation and concentration of radioactive elements in natural ecosystems.

DESCRIPTION OF STUDY AREA

The study area selected was a 3,200 yard section of the West Branch of the Sturgeon River. This section has been used in past years for studies in which an inorganic source of P^{32} has been used. The river is approximately 14 miles long and has its source in Hoffman Lake in Charlevoix County, Michigan. The stream originates at the northeast corner of the lake and flows in a northeasterly direction through Charlevoix, Cheboygan and Otsego counties, Michigan (T.33N., R.3W.). The West Branch joins the Sturgeon River near the town of Wolverine. The Sturgeon River empties into Burt Lake.

The topography of the area shows the effects of glaciation. The terrain is very hilly, due to the presence of many glacial moraines. The course of the West Branch, located in the vicinity of several large moraines, receives a large amount of ground water from them.

The study area was located approximately three miles from the mouth of the river. The temperature of the stream has been found to remain quite constant throughout the summer. Clifford (1959) gives the range as between 11.1°C. and 14.4°C. These extremes are accounted for by warm runoff from rainstorms, and dry periods when the flow of the stream is composed mainly of cold ground water. The stream remains constantly cool for two reasons. First, the ground water flows to the surface at a temperature of approximately 8.3°C. Second, the stream is shaded for a large part of its length by the shrubs and trees which line its banks.

The trees and shrubs which occur near the West Branch were

found to be : Cedar, Juniperus virginiana; Ninebark, Physocarpus opulifolius; Tag Alder, Alnus glutinosa; and Aspen, Populus sp. The Tag Alder and the Ninebark were the predominant shrubs bordering the stream.

The flow of the stream had a mean value of 43.75 cubic feet per second (Knight, 1961). The total phosphorus concentration was approximately 7 p.p.b. (Borgeson, 1959). The total alkalinity was about 181 p.p.m.

The stream bed was composed of glacial gravels and sands. There were also frequent silt and marl deposits. Many fallen trees littered the stream bottom, and there were several artificial stream diversions in the study area. Most of the logs and rocks were encrusted with marl.

The vegetation of the stream bed varied both longitudinally and latitudinally, with stream and light conditions. The dominant forms were: the stonewort, Chara sp.; the water moss, Fontinalis antipyretica; the pond weed, Potomogeton pectinatus; Ranunculus sp.; and the red algae, Batrachospermum moniliforme. Also present, but in much smaller numbers, were: Water Cress, Nasturtium officinale; Vallisneria sp.; and Anacharis sp. The periphyton, or aufwuchs, of the stream was analyzed by Clifford (1959) and found to be made up almost entirely of diatoms. The dominant form was Synedra ulna. Also found in somewhat smaller numbers were: Cymbella spp., Navicula spp., Cocconeis spp., and Gomphonema spp.

The insect orders which were present in the stream were all typical representatives of a cold, lotic community. Those observed during the course of this study were: Odonata, Hemiptera, Coleoptera,

Ephemeroptera, Plecoptera, Trichoptera, Diptera, and Megaloptera.

Other invertebrates encountered were the freshwater oligochaetes, gastropods, pelecypods, and amphipods.

A complete list of all invertebrate species observed, will be found in Appendix I. The identifications have been carried as far as possible within the limits of time and the needs of the project.

There were also numerous vertebrate organisms in the stream. Among the fishes, the predominant forms were: brown trout, Salmo trutta; brook trout, Salvalinus fontinalis; rainbow trout, Salmo gairdnerii; northern mottled sculpin, Cottus bairdii; eastern slimy sculpin, Cottus cognatus; and the brook lamprey, Entosphenus sp. The central mudminnow, Umbra limi; brook stickleback, Eucalia inconstans; and various unidentified Cyprinidae were also present in small numbers. Two species of amphibians were found in or near the stream. The green frog, Rana clamitans, and the American toad, Bufo americanus, were both quite common. One reptile, the common water snake, Natrix sipedon, was also collected in the stream.

SAMPLING STATIONS

Collecting sites were established at various intervals along the length of the stream. The stations were approximately 50 yards long and were used to take samples of water, bacteria, periphyton, plants, insects, and fishes. These stations, numbered 1 through 10, corresponded to those used in earlier studies on this stream. The same stations are used each year to facilitate the comparison of data. The approximate location of the stations is shown on the map in Fig. 1. Water samples were taken at stations

3, 5, 7, 8, 12, 14, and 16. Bacterial samples were taken at all of the aforementioned stations except Station 16. Throughout the summer, insect, plant, and periphyton samples were taken at stations 3, 8, 12, and 14. Fish samples were taken at Stations 8 and 12. In addition, periphyton was sampled at Station 7.

When the stations were originally selected several factors were considered. Knight (1961) says, shade, stream velocity, bottom type, and vegetation composition were all taken into consideration when the stations were established.

It was thought that each station represented one, or more, of the various major environmental conditions present in the stream.

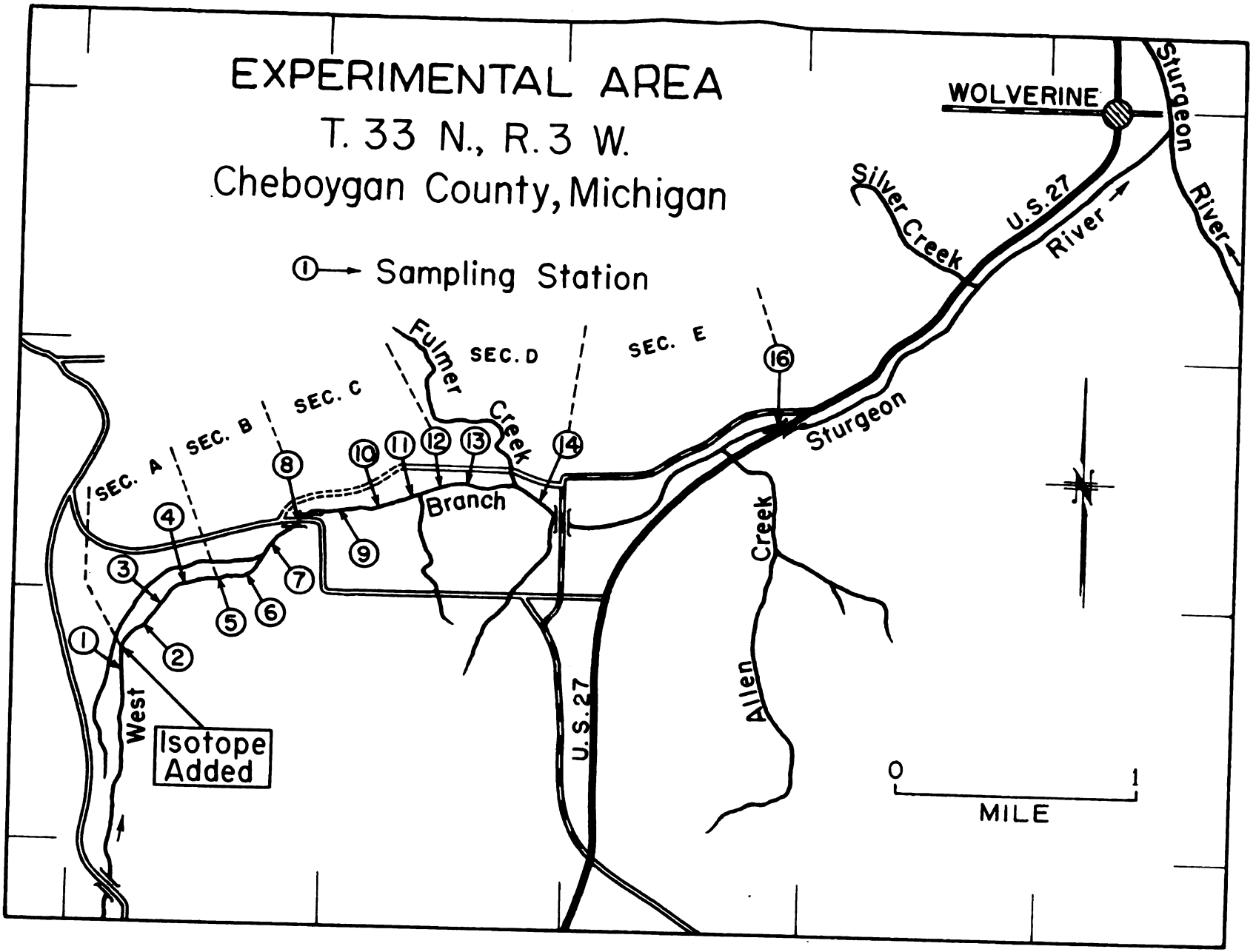
Station 3

Station 3 was the furthest upstream. It was approximately 300 yards downstream from the site of isotope entry. The bottom type was mainly glacial sand and gravel. The station was bounded by a long, moderately deep stretch of water on the upstream side, and a very deep pool (5-6 feet) downstream. It was well shaded, and the stream vegetation was limited to a few Chara sp. and Ranunculus sp. beds. Other species were hard to locate. All the insect forms used in the study were found at this station in ample numbers, throughout the summer.

Station 8

This station was located 1030 yards below the point of isotope addition. It was a relatively straight stretch of stream, partly shaded, of moderate depth (17.2 in., Zettelmaier, 1961) and velocity. The bottom consisted of sand and gravel, with a large number of submerged logs along the southeastern shoreline.

Figure 1. Map of the West Branch of the Sturgeon River area, showing the sampling stations and site of isotope entry.


EXPERIMENTAL AREA
T. 33 N., R. 3 W.
Cheboygan County, Michigan
① Sampling Station
WOLVERINE
Sturgeon River
Silver Creek
U.S.27
River
Fulmer Creek
SEC. D
SEC. E
SEC. C
SEC. B
SEC. A
16
8
10
11
12
13
14
Sturgeon
Branch
4
3
9
7
5
6
1
2
West
Isotope Added
U.S.27
Allen Creek
0
1
MILE

A riffle area was immediately upstream from this site and a wide bend downstream. All the necessary species of plants were found here in abundance, except Water Cress, Nasturtium officinale. Invertebrate organisms were also abundant here, with the exception of the caddis fly larva, Hydropsyche sp.

Station 12

Station 12 was situated directly on a sharp bend in the stream, 2580 yards downstream from the site of isotope entry. A long, straight, shallow riffle immediately preceded the bend, and a relatively deep (3-4 feet), slow moving stretch followed it. The bank was deeply undercut at this point and the water velocity was high. There was little overhanging cover to shade the stream, and there were luxuriant plant beds of several species here. Insect life was also abundant, but hellgrammites, Nigronia sp. and the caddis fly larvae, Hydropsyche sp. were scarce.

Station 14

This station was located on a straight, shallow, riffle area. Several large stumps and rocks were exposed here and two artificial stream diverters had been installed. This station was bounded upstream by more riffle, and a deep hole on the downstream side. There was no shade and the plant and animal populations were adequate for collecting purposes throughout the summer. This station was 3,280 yards below the point of isotope addition.

RADIOLOGICAL PROCEDURES

Isotope

The type of isotope used in tracer experiments is dictated by the experiment itself. Isotopes with extremely short half-lives cannot be used if the experiment takes place a long way from the isotope supplier, or if it is to be a long term experiment. If the isotope is to be released in the environment, however, and different studies are to be made every year, an isotope with a short half-life is desirable.

An isotope with a half-life which was suitable for our needs was radiophosphorus (P^{32}). It is a beta ray emitter with a maximum energy of 1.712 Mev. Its half-life is 14.3 days.

The isotope was supplied by the Oak Ridge National Laboratory, Oak Ridge, Tennessee, as phosphate (PO_4) dissolved in weak hydrochloric acid. Upon assay, it was determined that the sample contained twenty-five millicuries. The radiophosphorus was added to phosphorus-poor cultures of Escherisnia coli O111 (for bacteriological methods and results see Bender, M.S. thesis unpublished). The bacteria took up 97 per cent of the P^{32}. The cultures were then taken to the experimental area, diluted appropriately, and siphoned into the stream.

The addition of the isotope was carried out following the methods presented by Borgeson (1959) and Clifford (1959). A brief summary is presented. The bacteriawere placed in a fifty-five gallon drum and diluted with fifty gallons of water. Then, using a constant head, the bacteria were siphoned into the stream, at a constant rate, over a period of thirty-six minutes. The siphoning

was begun at 9:31 AM and finished at 10:07 AM on July 13, 1961. At the computed flow of 38 cubic feet per second it was found that the final dilution of the isotope in the stream was well below the limits set by the National Committee on Radiation Protection, sponsored by the National Bureau of Standards. Radiation safety officers from the Michigan State University Department of Public Safety supervised all isotope handling and made regular checks on activity levels in the laboratory and in the stream.

Activity measurement

Counting equipment: Activity was measured with two Nuclear Measurements Corporation, internal-flow proportional counters in connection with two decade scalers. Background activity was determined at regular intervals whenever counting was in progress. In past years fifteen-minute background counts were made each day, with the counter chamber empty. We found evidence that the presence of the stainless steel planchets in the counter chamber may depress the actual number of counts. Therefore, background counts were always made with freshly muffled (heated) planchets in the counter chambers. The time required for these counts varied from 15 to 30 minutes.

Over the entire summer the background counts ranged from 40 to 60 counts per minute.

The periphyton, plant, and insect samples were counted for varying lengths of time. Since we were dealing with a very low level of activity, long counts were used in all cases. Periphyton was counted for thirty minutes, plants for fifteen minutes, and the insect for five minutes. The increased length of time for

these counts was necessary in order to increase the statistical probability that the counts were significant and not due to chance variations in the natural background.

Laboratory conditions: The laboratory was kept at a temperature of from 70^{o} - 75^{o}F during counting. The relative humidity was kept as low as possible with a dehumidifier, and dust was removed from the air electrically. The outsides of the planchets were wiped clean with cheese cloth before counting, and the planchets were handled with forceps. The counter chamber was wiped clean with cheese cloth after each sample was counted.

Calculation of results

The raw counts per minute were computed from the counter data. Several correction factors must be applied to the counts before the activity levels of the various samples may be compared and contrasted. The correction factors used were as follows (Robeck, et al., 1954):

Background: The normal cosmic radiation and the presence of various radioactive substances in the vicinity of the counters cause what are called background counts. The method used in determining the magnitude of this factor has already been discussed. The background count is subtracted from the gross counts per minute.

Mass: As the samples were all of different masses, the counts per minute were adjusted by dividing the counts of each individual sample by its mass. The counts per minute were then on a per gram basis.

Radioactive decay: Because the half life of P^{32} is 14.3 days there is an appreciable decrease in the amount of P^{32} left

in the ecosystem, each week. Therefore, the counts per minute per gram are divided by a decay factor which converts the actual counts at time = t (the time of sample collection), to time = 0 (the time of isotope addition). Decay factors may be found in Kinsman (1957).

There are other correction factors which may be used, but as they are all constants and do not affect the relationships between the various samples, they are not used in this study. The formula used for computing corrected counts per minute is:

Activity Density = (CPM — BKG) x (mass factor) x (decay factor).

Whenever the term "corrected counts per minute" is used in this study, it refers to the gross counts per minute corrected for the aforementioned factors.

If it is necessary to convert the activity density in counts per minute to microcuries the following conversion is applicable. From Robeck (1954):

1 curie (c) = 3.7×10^{10} disintegration per second (dps)

1 microcurie (μc) = 3.7×10^{4} dps

= 2.22×10^{6} dpm

= $1/2.22 \times 10^{6} = 4.5 \times 10^{-7}$

Correction for counter efficiency = (45%) x (4.5×10^{-7})

Conversion factor is: (cf) = 2.025×10^{-6}

The final formula for correction to microcuries is:

microcuries = (CPM - BKG) x (MF) x (DF) x (2.025×10^{-6})

DISCUSSION OF BACTERIAL RESULTS

As it is of prime importance to the entire food chain to know in what chemical state the phosphorus was, and where it was in the ecosystem, a brief discussion of the bacterial results is presented here. For a complete account of all bacteriological procedure, data, and results see Bender (M.S. Thesis).

Upon examining the literature concerning the role of bacteria in fresh-water phosphorus metabolism several points become evident. First, because this field is relatively new it is often difficult to relate the results of the biochemist and laboratory biologist to those of the field biologist. Second, all the experiments have been carried out either in laboratories or in lakes. No work on the role of stream bacteria has yet been reported. I shall attempt to show some interrelations between various works from the literature and between these works and our results from the West Branch of the Sturgeon River.

Our data has revealed that by incorporating the P^{32} into bacterial cells before adding it to the stream, we reduced the total activity found at all trophic levels to 5% - 15% of the levels achieved in past experiments when inorganic P^{32} was used. The water samples from the station furthest downstream revealed that between 50% and 55% of the bacteria were carried past this station. Radiological assay of these samples from Station 14 revealed that the bacteria had nearly the same amount of activity they possessed when they were added to the stream. Thus, at least 15 mc of P^{32} was lost from the experimental section of the stream

in the first hour of the experiment. The effects of a severe storm and ensuing 8.7 in. rise in the stream on the night of July 13, 1961, are impossible to assess because of the sampling difficulties encountered.

The results from past years (Clifford, 1959; Knight, 1961; and Zettelmaier, 1961) seem to indicate that the periphyton of the stream is chiefly responsible for the fixation of inorganic phosphorus. The role of the bacteria, however, in the phosphorus metabolism of a lotic community was unknown. It was thought that perhaps the bacteria served as an intermediate level between the inorganic phosphorus and the plant life of the stream. In lakes, several workers (Hayes and Phillips, 1958; Rigler, 1956; and Reid, 1961) agree that it is very likely that there is a competition between the aquatic bacteria and phytoplankton for available inorganic phosphorus and that the bacteria are successful in this competition and change the phosphorus to organic compounds. Rigler (ibid.) added P^{32} to an enclosed portion of a lake and after 1.5 hours, more than 60% of the total amount of P^{32} was found to be in the bacteria of the system.

There is disagreement, however, concerning the fate of the phosphorus once taken in by the bacteria. Labaw, et al. (1950) found in his work with E. coli B that bacteria do not release any phosphorus, in the inorganic state, even at death. Harris (1957) noted that approximately 60% of the phosphorus he had used in his investigation had been converted to organic phosphorus compounds by microorganisms (mainly bacteria). Hayes and Phillips (1958) also found that bacteria change nearly all of the phosphorus they take

in to organic compounds. Williams, et al. (1940) substantiated this in their work with E. coli. Hayes and Phillips (ibid.) also discovered, however, that breakdown of the organic compounds to inorganic phosphorus and the subsequent recycling of this inorganic phosphorus resulted in a state of equilibrium between the bacteria, the organic compounds, and the inorganic breakdown products of the system. It was found that 10% - 20% of the phosphorus of this system was in the inorganic state.

Gest and Kamen (1948) found that there is a portion of the total phosphorus held by bacteria which is labile and easily lost and there is also a portion which is bound and is not cycled. Bacteria cultured under optimal phosphorus conditions possessed both labile and bound phosphorus, but bacteria cultured under low phosphorus conditions did not have any labile phosphorus. They also mentioned that other workers have found that under different chemical conditions, in the laboratory, various species of bacteria exhibited a phenomenon known as phosphorus leakage. This phosphorus was lost in different chemical states by different species. Some bacteria lost inorganic compounds, others lost organic, and still others lost a combination of organic and inorganic. The relation between these results and those of Labaw (ibid.) is not clear.

The same labile--bound relation was found in diatoms by Goldberg, et al. (1951). He also noted that the labile phosphorus was not the result of "active transport", or storage, but that the amount within the cells was a reflection of the concentration of phosphorus in the environment.

These results leave a very interesting question unanswered. Why did not the bacteria of the stream ecosystem take up most of the inorganic phosphorus used in previous years and change it to organic compounds like they seem to do in lake ecosystems? These compounds would be unavailable to the plants and periphyton, and yet there was more than ten times as much activity at the producer level in 1959 as there was in 1961.

It is impossible to tell, however, whether or not the P^{32} was cycled through the pool of labile phosphorus which the cells would possess in a medium with a phosphorus concentration similar to that of the West Branch. If it were cycled by the bacteria it would still be available to the plants as inorganic phosphorus.

One answer which seems plausible in light of the data and literature is that the temperature of the stream was such that the periphyton was successful in competing with the bacteria for the phosphorus, even though the bacteria outnumber the diatoms of the stream (Wojtalik, unpublished), and possess a greater affinity for inorganic phosphorus at room temperature. The temperature of the stream was approximately 12.8°C at Station 8 on the day the isotope was added. This would inhibit the metabolism of many bacteria. Photosyntheses, however, is not temperature dependent at temperatures of this magnitude. It may very well be that there are bacteria in the stream which can function quite normally at this temperature, but as no taxonomic survey was made of the bacterial fauna of the stream it is impossible to say whether or not any of these species were present. It is known that E. coli metabolism is inhibited by these low temperatures.

The data of Bender (M.S. thesis) show that the amount of filterable P^{32} and the amount of P^{32} per cell remained nearly constant in the stream, as the bacteria passed downstream. There was, however, a logarithmic decrease in the amount of particulate P^{32} as the spike passed through the study area, and the number of bacteria were increased twofold at many of the stations, by the addition of our E. coli O111.

In summary: the conditions which we encountered seem to be as follows. It appears as though the majority of the P^{32} may have been incorporated into the bacterial cells, possibly in the form ribose and desoxyribose nucleic acids and phospholipids (Labaw, 1950; Hayes and Phillips, 1958). This hypothesis is substantiated by the failure to show a change in the level of activity as the cells passed downstream.

Since the level of filterable P^{32} did not change significantly as the spike passed downstream, it does not appear as though this was in a form which was available to the stream community. The level would have decreased if there had been uptake from this source.

It is impossible to determine whether the P^{32} was cycled through the pool of labile phosphorus in the bacteria in 1959 and 1960. Because of the low stream temperatures, bacterial metabolism and reproduction are inhibited. Thus, even though the bacteria greatly outnumber the diatoms, the diatoms can successfully compete for the available phosphorus. If the stream bacteria were metabolizing and reproducing normally in 1958, 1959, and 1960, they should have fixed a large per cent of the P^{32} within their

cell walls, and made it chemically unavailable to the producers of the stream.

The phosphorus level of the stream was not increased by the addition of P^{32} in 1958, 1959, 1960, or 1961. However, in 1961, the concentration of bacteria in the stream was doubled at many of the sampling stations. It is obvious that the stream bacteria in 1958, 1959, and 1960, were exposed to much lower concentrations of activity than were our cultured E. coli cells.

It appears then that the only remaining source of radiophosphorus lay within the pool of cellular P^{32} trapped in the study area by cellular "fallout". This is substantiated by the decrease in activity in particulate form. The means by which this organic radiophosphorus was broken down to a form which was available to the producers of the stream is unknown. Labaw (ibid.) says that E. coli do not release inorganic phosphorus even upon cell death. It is possible, however, that decomposition of these organic compounds might have occurred in the pools and eddies where the greatest number of bacteria would have been trapped.

PERIPHYTON

Field Procedures

The periphyton, or aufwuchs, is an extremely important link in the complex food chain of stream communities. To obtain relatively pure and weighable samples of the diatoms and algae which make up periphyton populations is not always easy. To facilitate collection, plexiglass substrates were placed in the stream and periphyton was allowed to accrue thereto. These substrates were two by five inches and their exposed area was 1.4 square decimeters. The periphyton was easily scraped from the substrates with a clean glass slide in the laboratory.

The substrates were placed in the stream two weeks before the isotope was added to insure sufficient growth of the periphyton. Growth was slow this year, probably due to the fact that the stream was not fertilized.

The substrates were held in place on horizontal wooden racks by metal spring clamps. The horizontal racks were fastened to metal fence posts driven into the stream bed. They were placed in areas of water which received maximum sunlight and where the velocity of the stream was moderate.

Racks were located at Stations 3, 8, 12, 14 and at Station 7. Station 7 was situated about 150 yards upstream from Station 8, where a small stream joins the West Branch.

At Stations 8 and 12 there were four racks, each with seven substrates. Four substrates were removed each week, and none were replaced. It was possible to sample the same periphyton pop-

ulation throughout the summer, allowing for normal biological turnover.

At Stations 3, 8, 12, and 14 there was one post which had two racks on it. One was set at a distance of 1/3 the depth of the stream from the surface, at that point, and the other was set a predetermined distance below the upper rack. One substrate was taken from each (upper and lower) rack every week, and a replacement substrate was added. Their purpose was to determine whether or not depth affected the growth or activity uptake of the periphyton.

At Station 7, one rack with four shingles was placed in the small creek. This rack was moved to the West Branch four days after the isotope had been added and four new substrates were placed in the creek. Each week this procedure was repeated and the four samples taken from the West Branch were processed to determine the amount of recycled activity, while the four substrates from the creek were moved into the West Branch. A polyethylene stream diverter was constructed at the mouth of this small stream and extended downstream some 25 yards. The polyethylene was stapled to wooden posts which had been jetted with a high speed pump into the marl and gravel stream bed. In this way it was hoped that the flow of the two streams might be kept separate for at least this distance, and thus the biota of the small stream would not be exposed to the isotope as it flowed by. This would provide not only a nursery for activity-free periphyton, but also serve to check the horizontal movements of insects in the stream. It was discovered, however, that the phosphorus found its way inside

the diverter and as a result only the periphyton was free from activity as it had been located 15 - 20 yards upstream from the confluence of the two streams. It is thought that the circulation of ground waters was the source of this activity leak, and not an opening in the diverter itself.

Periphyton collections were made on July 14, July 17, and then once every seven days for the remainder of the summer.

The substrates were removed from the rack and immediately all larger invertebrate organisms were removed with forceps. The substrates were then placed in labelled plastic refrigerator bags for transport back to the laboratory.

Periphyton Laboratory Procedures

The periphyton laboratory procedures were relatively unmodified from those used in past years on this same project (Knight, 1961; Clifford, 1959; Zettelmaier, 1962) and will, thusly, only be briefly reviewed here.

The periphyton growth was scraped from the substrates with the edge of a polished glass slide, and then the substrate was thoroughly rinsed with distilled water into a 500 ml beaker.

The wet weight of a number .047 white grid filter was obtained by setting up a Millipore filter apparatus with the clean filter pad in it. Twenty ml of distilled water were then run through the filter. The pump was left running for 15 seconds. The pad was then placed on a clean, flat planchet and weighed on an analytical balance. This weight was then recorded.

Next, the mixture of periphyton and water was run through the filter apparatus. The beaker was rinsed with distilled water and

the rinse was also filtered. This was immediately followed by three cc of 0.01 N hydrochloric acid, and five cc of distilled water. The pump was allowed to run for ten seconds after the last traces of water had disappeared from the filter pad. The pad was immediately removed and placed on the same flat planchet and weighed on an analytical balance. The difference in the two weights was the weight of the periphyton sample.

Now the pad was placed in a deep-walled planchet and the planchet number was recorded with the corresponding sample data. The planchet was then filled with concentrated nitric acid and the sample was digested on a griddle on an electric stove and under a heat lamp. They were so arranged in relation to the heat sources that all boiling or spattering was avoided. When the sample was completely digested and the planchet relatively clear and dry, it was removed from the stove and heated to red heat in a muffle furnace at 600°C. The samples were then removed from the furnace, allowed to cool, and taken to the counting laboratory where the radiological investigations were made.

Periphyton Results and Discussion

As this paper is mainly comparative in nature no discussion of the physiology of phosphorus uptake and its use by plants will be presented. For comprehensive studies of this nature any of the following references involving inorganic phosphorus translocation in a stream community is recommended: Clifford (1959), Knight (1961), or Zettelmaier (1961).

Any comparisons in the following discussion are made with data from Station 8 alone. Station 8 was chosen because it was

very accessible and the conditions there were typical of much of the rest of the river.

The two most striking differences between the results of the radiological analysis of the periphyton in 1959 and 1961 are the low level of initial uptake found in 1961, and the apparently rapid rate at which it was lost in 1961.

This uptake pattern seems to substantiate the interpretation of the bacterial results. If, as was hypothesized, only 10% - 20% of the P^{32} was in the inorganic state and available to the plant life of the stream, then this low level of initial uptake is to be expected. Several authors agree that plants can only use inorganic phosphorus, (Green, 1907; and Bonner and Galston, 1952).

As no collections were made during the first 48 hours after the isotope was added, it is impossible to say when the activity peak was reached in the periphyton. In past years this peak has been found to vary a great deal in magnitude and in relation to the time the isotope passed. Knight (1961) infers that the further removed downstream from the site of isotope entry, the smaller the magnitude of the peak and the greater the time between the isotope passage and when the peak occurs. In 1959 activity peaks were reached at all stations within 24 hours of the addition of the isotope. This was probably also the case in 1961. The earliest periphyton samples taken in 1961 showed about 750 cpm, while at a comparable time in 1959 there were 1,300 cpm.

When total activity is compared it is found that in 1961 there was 6.4% of the activity present at Station 8, that was present in 1959. This result was arrived at by comparing the area beneath

the activity curves for the periphyton for both years. The obvious conclusion then is that the bacteria did not release much of the activity or did not release it in a form which was available to the flora of the stream. The bacterial results indicate that a combination of these factors was responsible. Those bacteria which were carried through the study area did not release the P^{32} they had taken up, and those that were trapped in the study area probably released it in organic compounds when they died.

Figure 2 shows the activity curves from Stations 3, 8, 12, and 14 for 1961 as compared with the results from Station 8 in 1959. The curves from 1961 are terminated after the fourth day because when samples were taken one week later, no significant activity was present at any of the stations. It was, thus, impossible to determine exactly when the activity dropped below the observable level, and an extension of the curves to this base level, on the date at which it was first recorded, would distort them.

It appears then that the only difference between the periphyton uptake in 1961 and previous years was in magnitude. In comparison, there was approximately one-tenth as much P^{32} available to the periphyton of the stream. The other nine-tenths was tied up by the bacteria and was never chemically available to the plant life of the stream.

Figure 3 shows the periphyton activity curves plotted on a semi-logarithmic scale. The dotted portion of the curves represent projections of the curves obtained from the first two col-

Figure 2. A comparison of the activity density curves of the periphyton at Stations 3, 8, 12, and 14, for 1961 with the periphyton curve from Station 8, for 1959. Counts are corrected for decay and background.

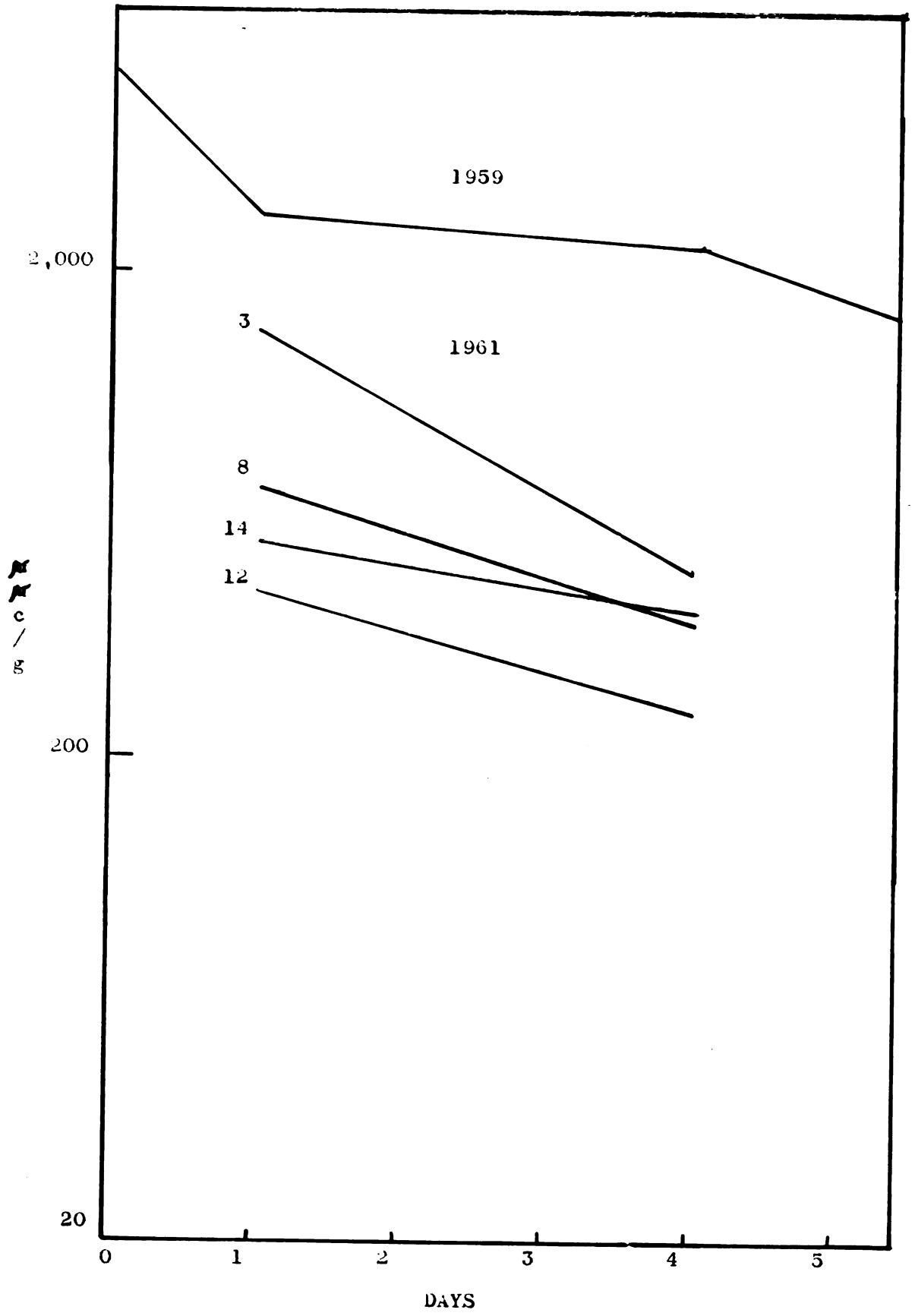
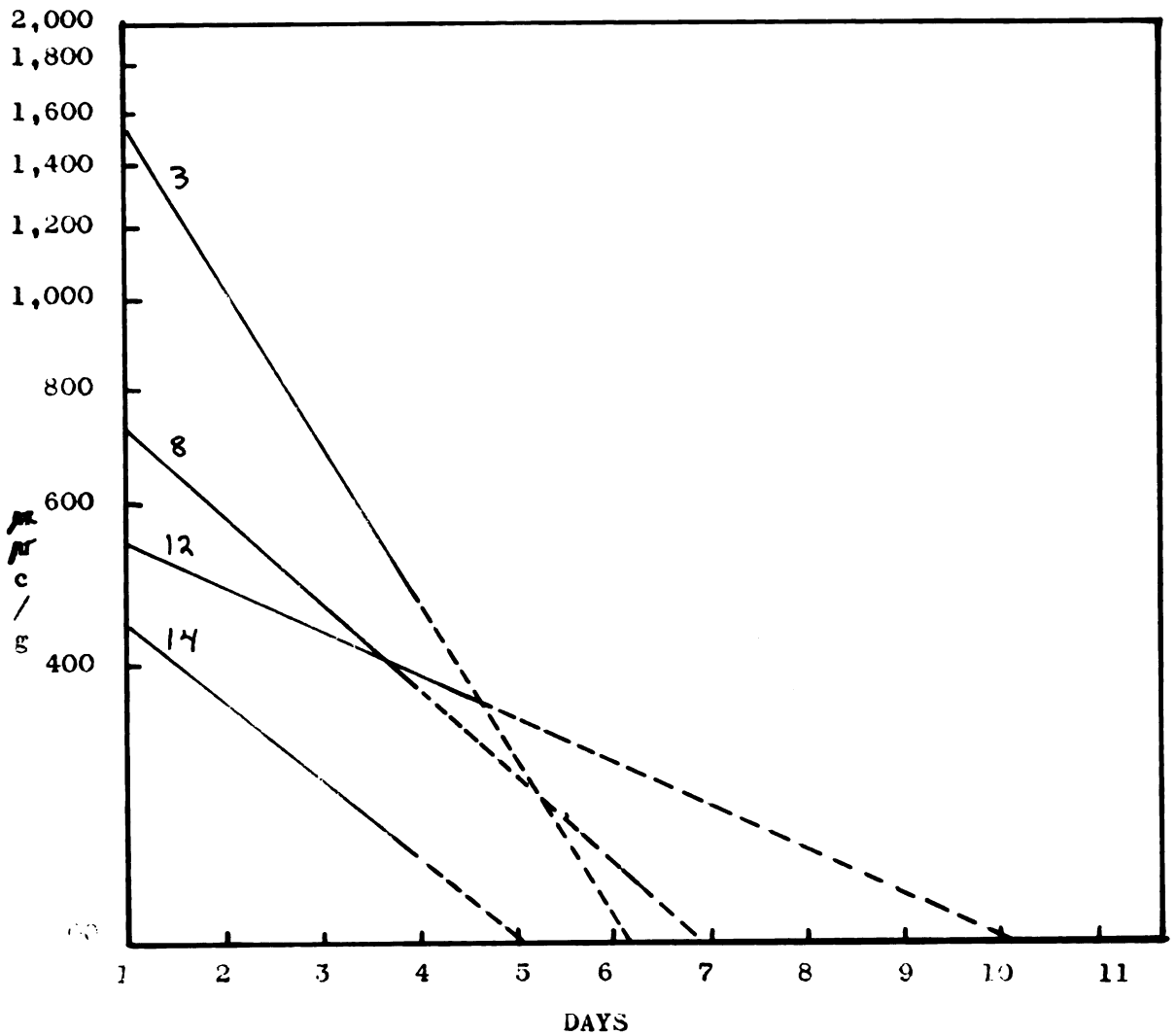

1959
2,000
3
1961
8
14
12
μμc/g
200
20
0
1
2
3
4
5
DAYS

Figure 3. Activity density curves of periphyton from Stations 3, 8, 12, and 14, plotted on semi-log paper. The curves are extended to activity = 0. Counts are corrected for background and decay.


2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
μμc/g
3
8
12
14
1
2
3
4
5
6
7
8
9
10
11
DAYS

lecting dates to a base level. This implies a phosphorus return to the stream at an exponential rate. This exponential phosphorus exchange has been found to be real (Hayes, et al., 1952). In past years, however, it has only occurred for a brief period immediately following the addition of the isotope. The dotted lines show that if this rate of return were continued, all activity would be gone at all of the four stations before the third collection was made.

One of the most interesting aspects of the periphyton analysis is concerned with the recycling of the P^{32}. It is generally agreed that the exchange of phosphorus into and out of plant cells takes place at an exponential rate (Hayes, 1952). In previous experiments (Knight, 1961) it has been shown that this recycling of the P^{32} results in a leveling or "plateau phenomenon" in plant activity. This means that whereas the rate of loss is still taking place at an exponential rate the subsequent uptake of recycled activity results in a net rate of return to the stream which is less that exponential. This activity comes from the biota upstream from substrates which have been exposed to the isotope.

In 1959, certain periphyton substrates were removed from the stream before the passage of the spike and were not returned until it had passed. It was assumed then that the only source of activity for these periphyton populations was that which had been recycled from the ecosystem upstream. The substrates showed quite conclusively that there is a recycling and that this "plateau phenomenon" is due to the recycling.

In 1961, it has been mentioned that activity-free periphyton populations were cultured in a small stream which empties into the

West Branch. Beginning four days after the isotope had passed and every week thereafter, these substrates were moved to the West Branch and then processed to determine the level of the recycled activity. The results from these substrates were quite uniform, and possibly misleading. It was found that there was no activity present on any of these substrates. This does not, however, necessarily mean there was no recycling of activity . It seems quite feasible that because of the low level of initial uptake, the level of recycled activity will also be greatly reduced. In other words, the activity was being recycled, but because of the small quantities of phosphorus involved and the dilution of this activity as it was washed downstream, the recycled phosphorus was not of a significant magnitude to be recognized on our counting equipment.

There may be greater ecological significance here than the mere retention of the P^{32} by the bacteria. It is realized that the bacteria which we used in our experiment were not normally found among the bacterial constituents of this stream community. However, they did succeed in keeping a great majority of the phosphorus which they possessed unavailable to the producer level of the stream. Rigler (1956) presents very convincing evidence that bacteria may successfully compete with the rest of the planktonic communities of warm-water lakes for available phosphorus. If this were true in aquatic communities in general, then under the proper conditions the bacteria could prove to be a limiting factor to the plant life, and thus, the entire food chain, of the community.

This hypothesis is in accord with Reid (1961), and he also notes that although the aforementioned "phosphorus competition"

is highly probable, it has proven very difficult to actually assess the "phytoplankton versus bacteria" problem.

MACROPHYTES

Field Procedures

Samples of five species of higher aquatic plants were collected at Stations 3, 8, 12, and 14. The species used were the stonewort, Chara sp.; the bryophyte, Fontinalis antipyretica; and the Tracheophytes, Ranunculus sp., Potomogeton pectinatus, and Nasturtium sp. The first two collections were made 24 and 96 hours after the isotope was added. Subsequent collections were made once a week until August 21, 1961. All samples were collected by hand. Where possible, the samples were made up of fragments of plants from more than one location at each station. This was not possible with Nasturtium sp. for it was rare at Stations 3, 8, and 12. At these stations it was necessary to remove samples from the same individual plant for two or three weeks to keep the species from being depleted before the experiment was concluded.

Each sample was thoroughly rinsed in stream water and was then placed in an appropriately labelled jar for transport back to the laboratory. Since all radiological processing was done on the day following collection the plants were stored overnight in their jars in a refrigerator.

Laboratory Procedures

The procedure followed in processing the aquatic plants were those given by Knight (1961). As at least one minor change was made, however, a brief review of the methods is presented.

A sample of from 1 - 2 g mass was taken from each individual collection bottle. This sample was thoroughly rinsed in distilled water and then blotted in paper toweling for five minutes.

Samples were not rinsed in dilute acid before processing. This is a procedure which was followed in previous years. It removes absorbed phosphorus from the plant cells and gives a measure of activity actually incorporated in the plant.

After drying, the samples were placed in individual evaporating dishes which had been previously cleaned, weighed, and numbered. The weight of the sample was the difference obtained by subtracting the weight of the evaporating dish from the weight of the sample and dish. All weighing was done on an analytical balance. After the weights had been recorded, the samples were covered with concentrated nitric acid and heated on a griddle on an electric stove. The samples were located far enough from the heat source to eliminate any spattering or boiling. When samples had been evaporated to a volume of 2 - 3 ml, they were transferred to a numbered stainless steel planchet. Evaporating dishes were rinsed with acid and the rinse was added to the planchet. Digestion and drying were then completed on the stove and under a heat lamp. When dry, the samples were placed in a muffle furnace at 600^{o}C to be ashed. This took from 3 - 5 minutes, or until the planchet was cherry red.

Care was taken to make sure the samples were thoroughly digested and completely dry. Partially digested samples tended to smoke and to ignite in the furnace. This was thought to be deleterious as activity may be lost in this manner.

The samples were removed from the muffle furnace and allowed to cool in covered trays. The trays were then taken to another room for counting.

Macrophyte Results and Discussion

Figures 4, 5, 6, 7, and 8 show the great amount of intraspecific and interspecific variability in the activity found in the higher aquatic plants. These curves were terminated after four weeks because the activity fell to a level which was not significantly above background.

In comparison with the results of 1959 there was a much lower level of initial uptake. The peak activity in 1959 was found in Potomogeton (1700 cpm) and in 1961 in Fontinalis (125 cpm). The 1961 peak was, however, more than twice as great as the next highest sample analyzed during that year. Activities in 1959 and 1961 were compared by determining the area beneath the activity curves. In 1961 Potomogeton had only 5% of the 1959 activity, Fontinalis had 4% and Chara had 7%. These values are in the range which would support the hypothesis presented in the discussion of the bacterial results that approximately 10% of the total P^{32} added to the stream was available to the producer level.

The data do not substantiate Knight's ranking of the plants according to their ability to absorb P^{32} on a surface per volume ratio. Our data may not invalidate Knight's ranking because the extremely low level of uptake and the less uniform distribution of the available P^{32} throughout the stream could cause these effects.

Comparing the higher plants with the periphyton there is a much lower level of initial uptake in the plants, and a much slower loss of activity, on the per gram basis. These differences are due to the differing metabolic and cell division rates in the two plant types. The periphyton has a much more rapid metabolic

Figure 4. Activity density of Fontinalis sp. in μμc/g at Stations 3, 8, 12, and 14, for the first four weeks of the study period. Counts are corrected for background, decay and mass.

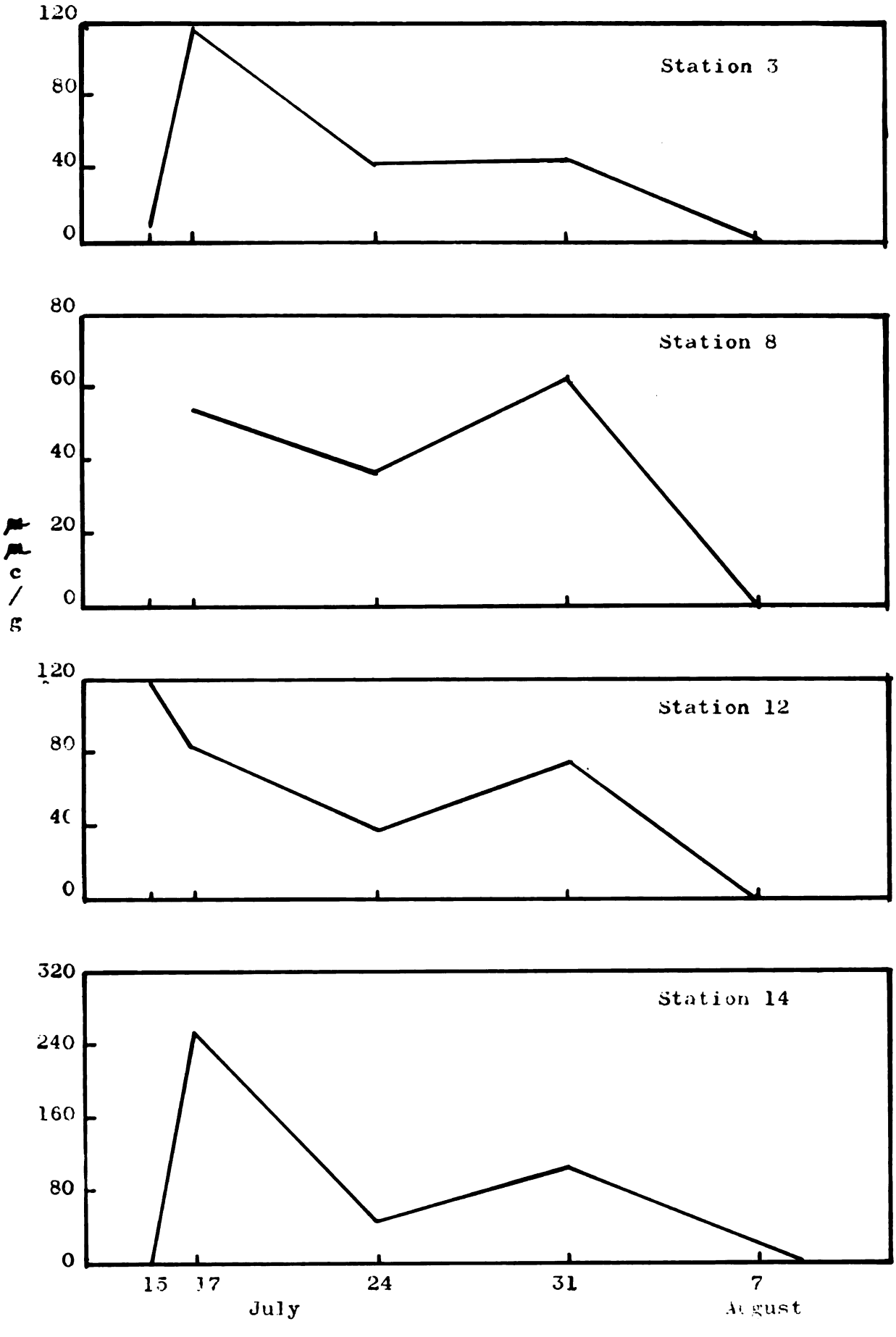

Station 3
Station 8
Station 12
Station 14
120
80
40
0
60
20
μμc/g
320
240
160
15
17
24
31
7
July
August

Figure 5. Activity density of Chara sp. in μμ c/g at Stations 3, 8, 12, and 14, for the first four weeks of the study period. Counts are corrected for background, decay and mass.

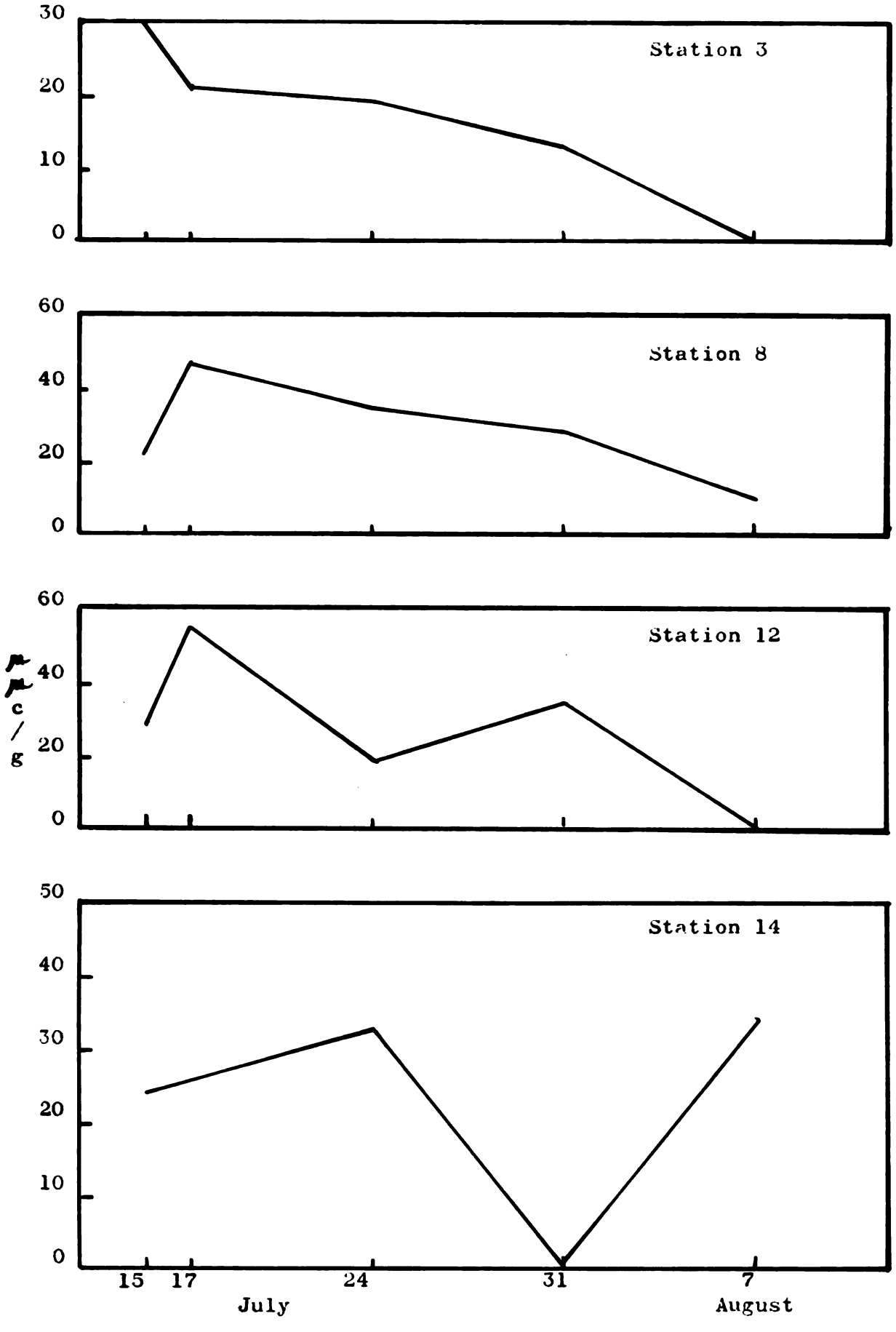

Station 3
Station 8
Station 12
Station 14
μμc/g
30
20
10
0
60
40
20
0
60
40
20
0
50
40
30
20
10
0
15
17
July
24
31
7
August

Figure 6. Activity density of Nasturtium sp. in μμc/g at Stations 3, 8, 12, and 14, for the first four weeks of the study period. Counts are corrected for background, decay and mass.

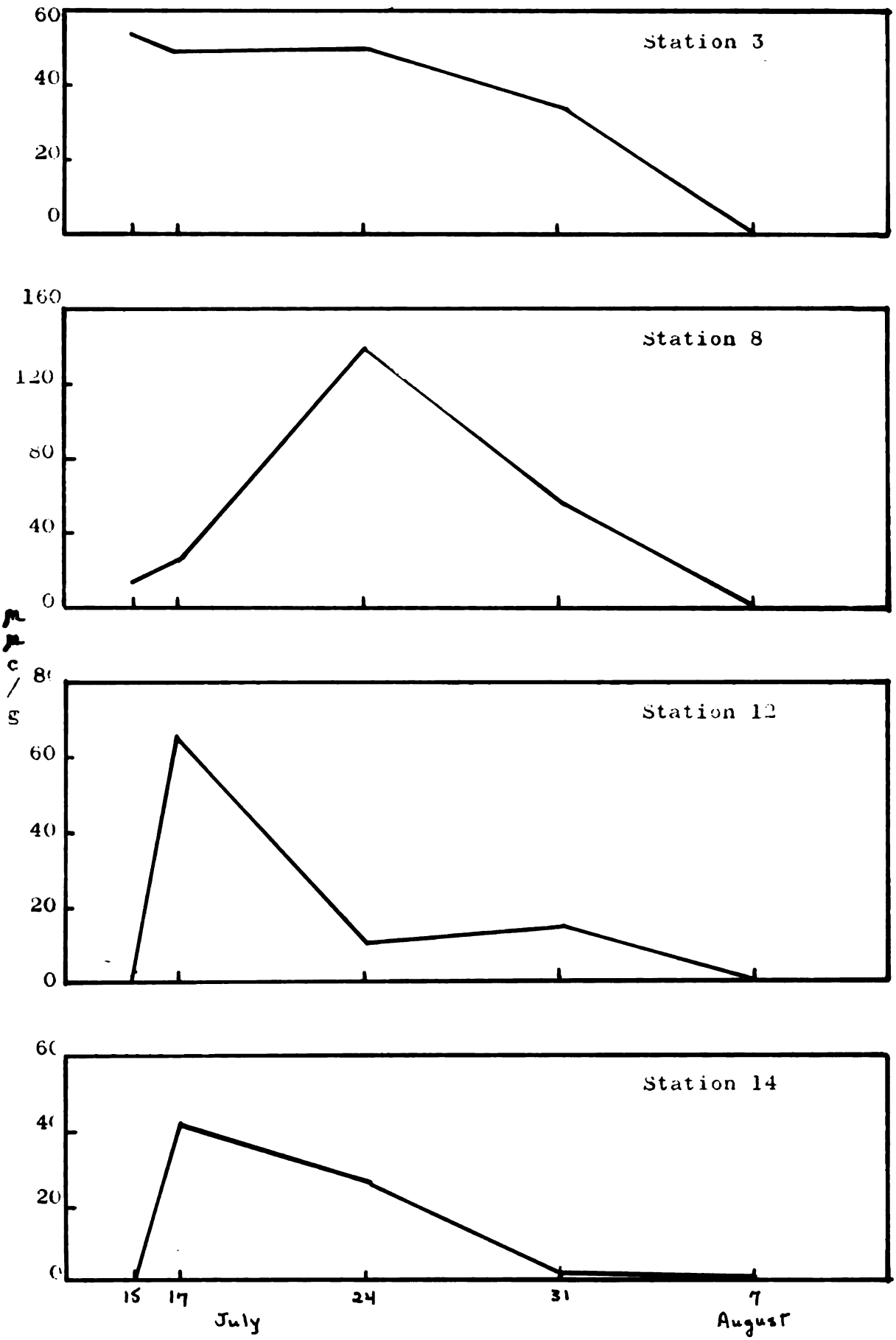

Station 3
60
40
20
0
Station 8
160
120
80
40
0
Station 12
60
40
20
0
Station 14
40
20
0
15
17
24
31
7
July
August

Figure 7. Activity density of Ranunculus sp. in μμ c/g at Stations 3, 8, 12, and 14, for the first four weeks of the study period. Counts are corrected for background, decay and mass.

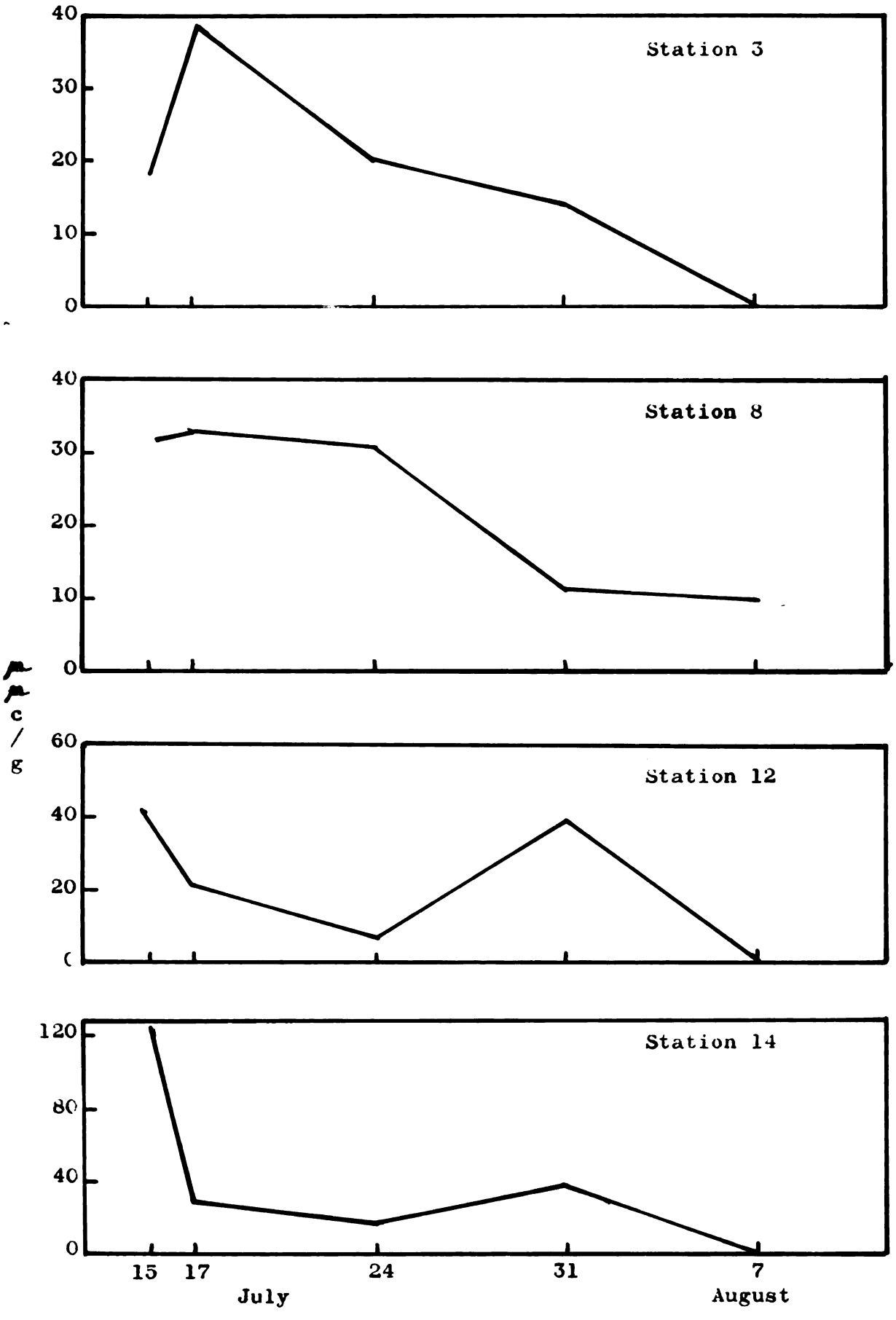
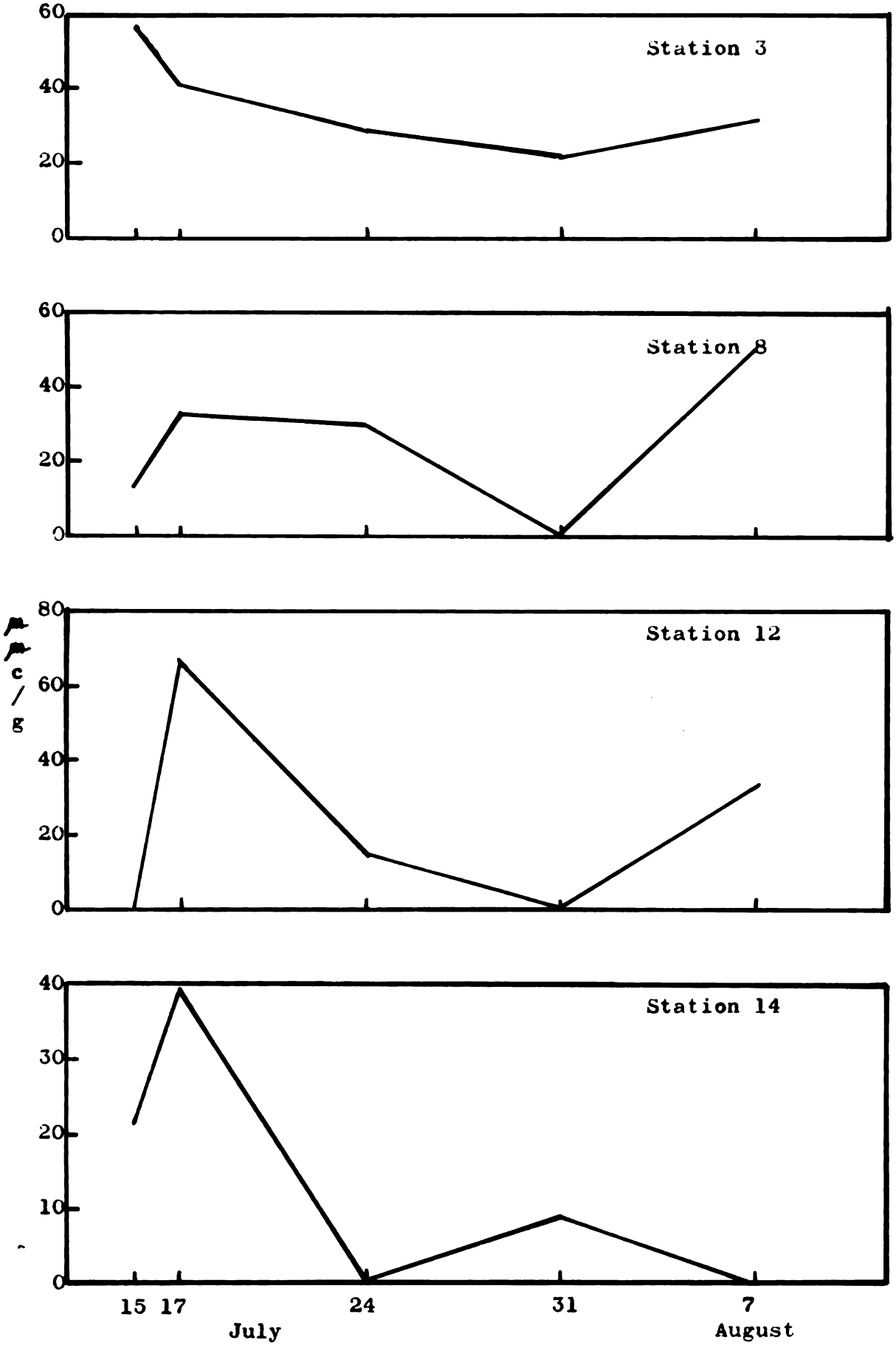

Station 3
Station 8
Station 12
Station 14
μμc/g
40
30
20
10
0
60
120
80
15
17
24
31
7
July
August

Figure 8. Activity density of Potamogeton sp. in $\mu\mu$c/g at Stations 3, 8, 12, and 14, for the first four weeks of the study period. Counts are corrected for background, decay and mass.


Station 3
Station 8
Station 12
Station 14
60
40
20
0
80
30
10
µµc/g
15 17
July
24
31
7
August

rate and rate of cell division. Therefore, when the higher plants and periphyton are both exposed to the same amount of radioactivity over the same amount of time the periphyton will take up more initially, and it will lose it to the stream and biological dilution more rapidly than the higher plants.

When the activity curves of Potomogeton, Fontinalis and Chara in 1959 and 1961 are examined (Figures 9, 10, and 11), it is apparent that after the first four days the activity patterns for 1959 and 1961 are similar. The differences in the curves during the ninety-six hours following the isotope treatment may be crucial, however. All the curves from 1959 indicate that during this period the plants are losing activity at nearly a logarithmic rate. This is activity which was taken in during the passing of the isotope spike. The curves for 1961 show that the plants were taking in activity during the four day period following the spike. This was true of four of the five species. Ranunculus was the only species which did not show this period of uptake.

These differences may have been due to the different form in which the activity was added. In past years the plants absorbed the P^{32} from the spike as it passed. In 1961 the plants may have gotten their activity from the breakdown of bacterial cells trapped in the study area. This would account for the period of uptake, since the P^{32} trapped in the cells would be released slowly. The similarity of the later portions of the curves would be expected since the chemical source of the P^{32} was probably the same in both years, inorganic ions.

Figure 9. Comparison of the activity density of Potomogeton sp. in micromicrocuries per gram for 1959 and 1961, at Station 8. The counts are corrected for background, decay and mass.

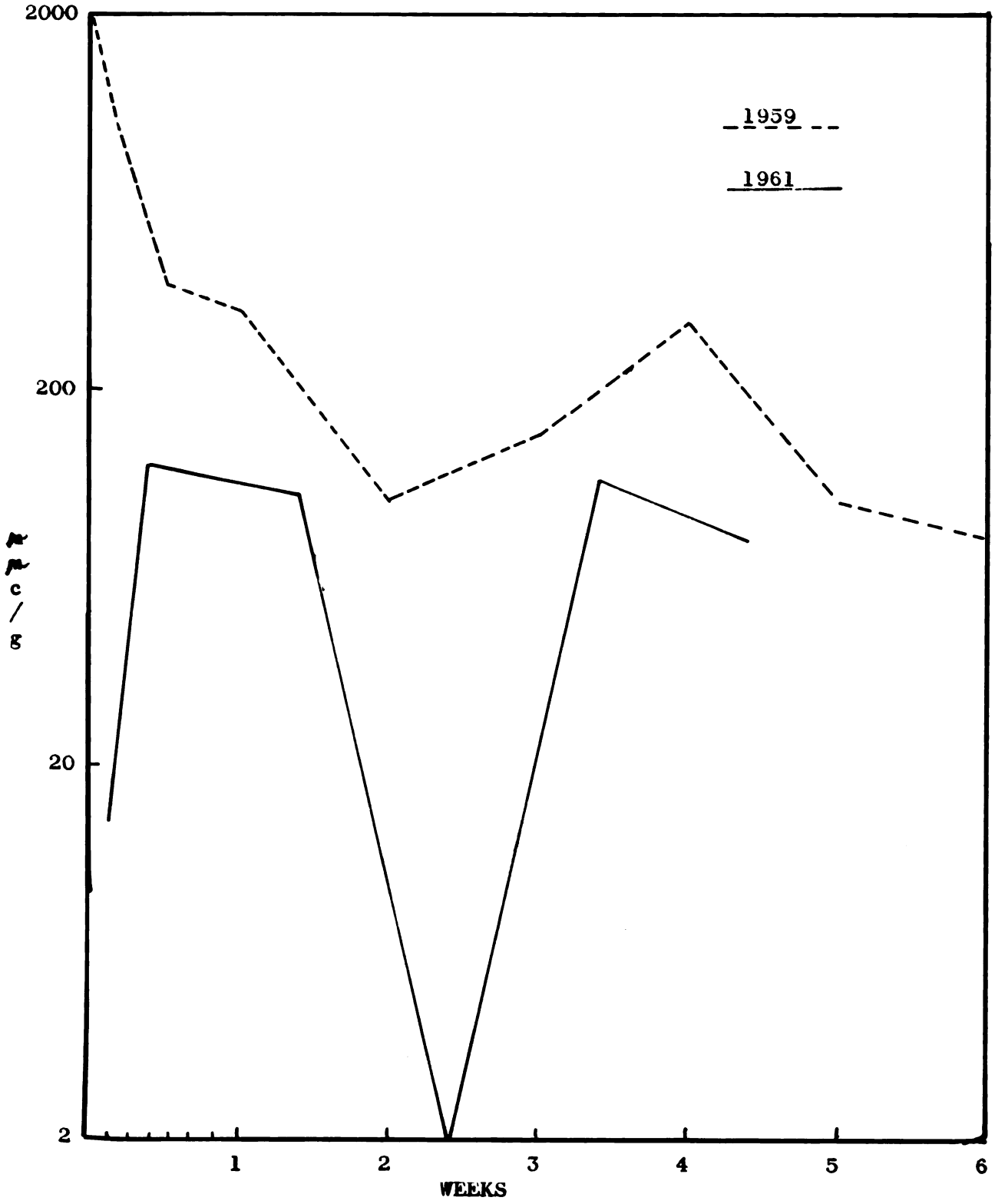

2000
200
20
2
1959
1961
μμc/g
1
2
3
4
5
6
WEEKS

Figure 10. Comparison of the activity density of Chara sp. in micromicrocuries per gram, for 1959 and 1961, at Station 8. The counts are corrected for background, decay and mass.

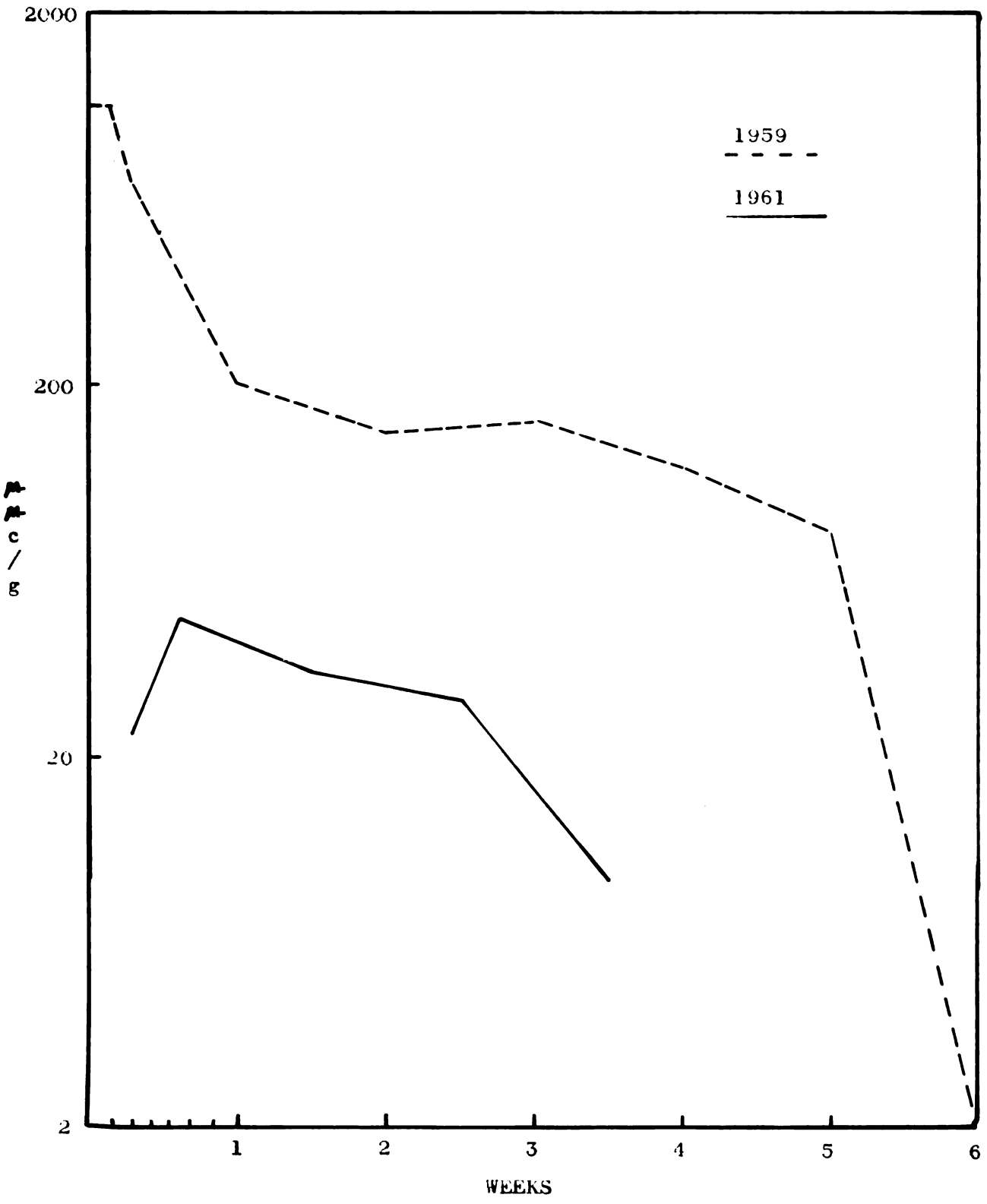
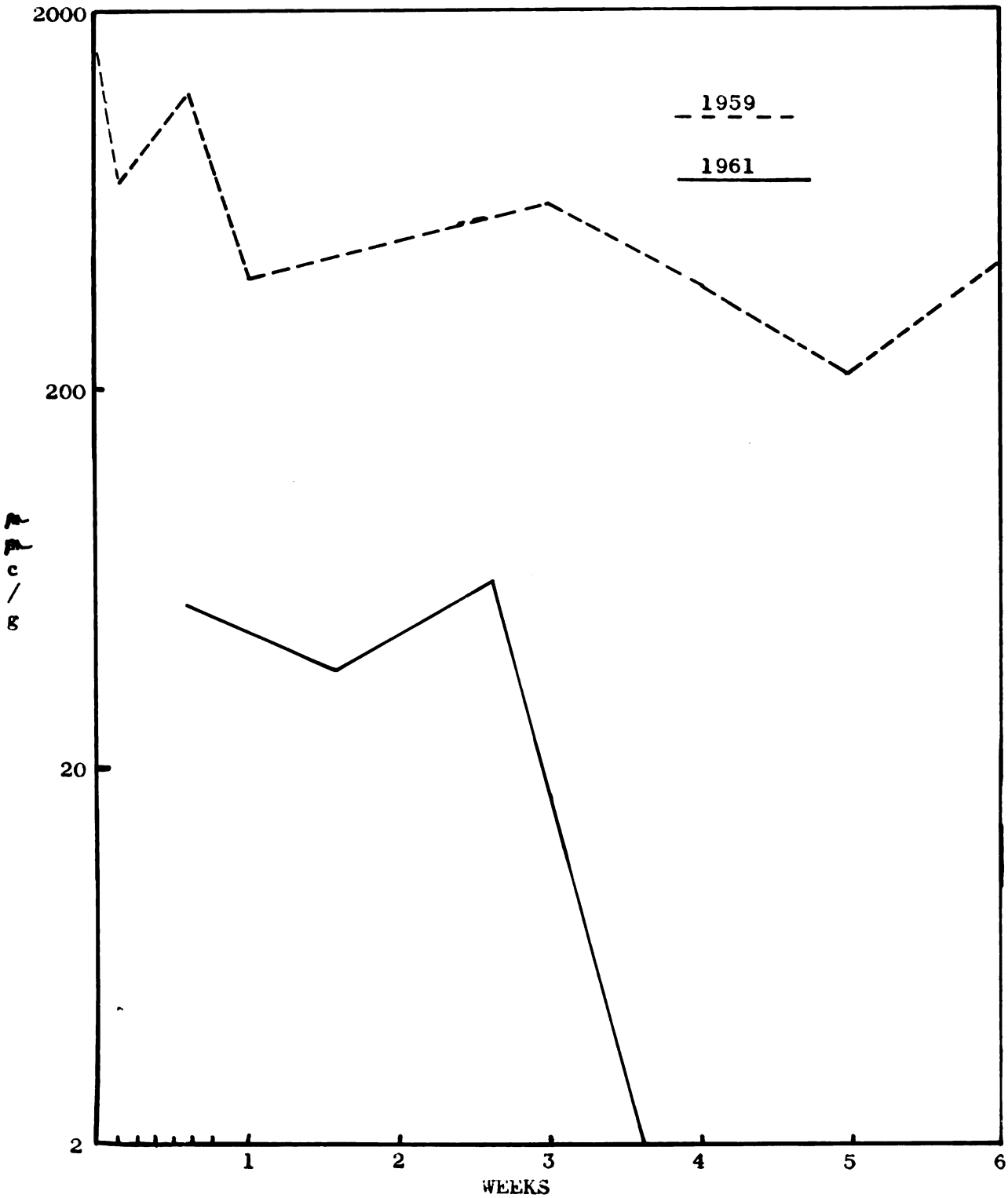

2000
200
20
2
μμc/g
1959
1961
1
2
3
4
5
6
WEEKS

Figure 11. Comparison of the activity density of Fontinalis sp. in micromicrocuries per gram, for 1959 and 1961, at Station 8. The counts are corrected for background, decay and mass.


2000
200
20
2
1959
1961
μμc/g
1
2
3
4
5
6
WEEKS

Plant Biomass

On September 4, 1961, an estimate of the biomass of the higher aquatic plants was made. The stream, from Station 2 (site of isotope entry) to Station 12, was divided into transects. Two samples, each one square foot in area, were taken at each transect. The sampling locations along each transect were determined with a table of random numbers.

Each sample, which included any living plants, was wet-weighed to the nearest gram in the field. No division of the samples, by species, was attempted.

The area sampled was 184,059 sq ft, or 5.5084 acres. The average biomass, at the time of sampling, was .0385 g/ft^2. The biomass for the entire section was 1681 lbs/acre. For a comparison with previous plant biomass estimates see Appendix II.

Bender (M.S. thesis, 1961) presents some insight into the interrelations of total P^{32} uptake by the aquatic plants and their biomass.

INVERTEBRATES

Insect: Field Techniques

Several criteria were used in selecting representative species of aquatic invertebrates for use in this study. One of the main objectives of the study was to see what differences occurred in phosphorus concentration, accumulation, and translocation when the phosphorus was added in an organic form, in comparison to previous studies which involved the addition of inorganic phosphorus to the same stream ecosystem. Thus the same species were used in this study as were used by Zettelmaier (1961), Knight (1961), and Bryant (1960). These were: the burrowing mayfly nymph, Hexagenia; the stonefly nymph, Pteronarcys; the fishfly larva, Nigronia; the snipe fly larva, Atherix; two species of caddisfly larvae, Brachycentrus and Hydropsyche; and the black-fly larva, Simulium. These species were chosen by earlier workers on the basis of availability and position in the food chain. It is necessary to select organisms from all trophic levels of a community when the movement of a nutrient through a community is being investigated. If this is not done it cannot be ascertained how long the nutrient was present at any level which had been omitted, and it could only be hypothesized that it was present at that level at all.

Collections were made starting July 15, 1961, two days after the isotope was added, then again two days later on July 17, 1961, and once each week thereafter until August 28, 1961.

Those species which were procured throughout the summer and the habitats in which they were most often found are listed below.

Fresh-water snails: Snails of the genus Physa were found on

logs and vegetation away from the main current of the stream. It is believed that they feed on dead animal and plant matter and periphyton.

Caddisfly larvae: Two species, representing two families, of caddisfly larvae were used. They were Brachycentrus and Hydropsyche. Brachycentrus was very abundant. It was usually found in moderate to swift water. Their rectangular or cylindrical cases were always found attached to a vegetable substrate. They were collected from twigs and roots which protruded from the bank of the stream down into the water, or from beds of aquatic plants found in swifter water, particularly Potomogeton and Fontinalis. Hydropsyche was never found in large numbers, but since it is so common elsewhere within its range, it was also collected. It is a net builder and most frequently was encountered in swift water on logs, roots, and clumps of detritus. The openings of the nets always faced upstream. Brachycentrus is considered to be a periphyton browser, and Hydrophsyche , a filter feeder.

Snipe fly larvae: These small, green, predaceous fly larvae, of the genus Atherix were collected often in Fontinalis beds on rocks and logs, and in cracks of submerged logs. They were most often encountered in and under the marl concretions which accumulate on the rocks and logs in the stream. Judging from the habitats favored by this species it seems logical that its prey would include mayfly nymphs and possibly caddisfly larvae. They were not found in association with the black-fly larvae during the day.

Fishfly larvae: Larvae, of the genus Nigronia, were col-

lected most frequently from beneath strips of wood peeled back from the surface of decomposing logs. They were seldom found anywhere in the stream except in close association with logs and stumps. The velocity of the water did not seem to affect their distribution. The only species which seemed to be safe from them, during the day, were the black-fly larvae.

Stonefly nymphs: Although several genera of stonefly nymphs were present, the only one collected was Pteronarcys. It was found on logs, stumps, clumps of aquatic vegetation, among leaves of terrestrial vegetation which dangled in the stream, and often in clumps of detritus which had become entangled in the roots and branches in the water. This species of insect was the most easily collected. Soon after a log, stump, root, etc. was lifted from the stream any Pteronarcys not in deep, water-filled crevices, would scurry from their hiding places due to their quick response upon exposure to the atmosphere.

Black-fly larvae: The black-fly, Simulium sp., was collected in substantial numbers from nearly any substrate in fast, relatively shallow, turbulent water. At Stations 3 and 8, they were abundant on periphyton substrates. This was not the case at Stations 12 and 14. Since a large number of specimens was needed to constitute a sample of mass 0.1 g, a special method was divised to speed up the collection of this species. The grass which grew along the shore and hung down into fast water proved to be a particularly fruitful substrate for them. It was relatively simple to remove all other macroscopic life from the individual blades of grass with sharp-tipped forceps and then strip the blades of grass between

the tips of the forceps and thus remove all the black-fly larvae.

The black-fly larva is one of the primary consumers of the stream. It is adapted to filter out diatoms and possibly bacteria and other small particulate matter from the passing water.

Mayfly nymphs: The only mayfly nymph used in this study was that of the large burrowing mayfly, Hexagenia limbata. It seemed selective concerning its habitat since it was very rarely found in any substrate other than nearly pure silt. It was never collected in pure sand and very rarely in marl beds.

These organisms are detritus feeders and are found in association with the lamprey, fresh-water oligochaetes and burrowing Odonata nymphs.

Oligochaetes: a few oligochaetes were collected from the silt beds along the shoreline of the stream.

Scuds: There was a substantial population of these organisms at Station 14 and they were collected and processed for a period of three to four weeks. They were members of the genus Gammarus (Zettelmaier, 1961).

Dragonfly nymphs: Several types of immature dragonflies were collected from the Chara beds of Station 12. They were all of the family cordulegastridae.

The number of specimens collected from each station varied with the species involved. When the gross counts per minute were corrected for mass it was thought best to have samples which were at least 0.1 g in mass. In the past, sample weights of 0.01 g and less were often used. Such small samples create weighing errors which are magnified when weights are expressed on a per

gram basis.

The average weights per sample and the average number of organisms per sample of all species used for more than four weeks are given in the following table.

TABLE 1. Insect sample size.

Genus	total indivs.	total weight	mean weight indivs.	number of samples	mean sample weight	mean number per sample
Nigronia	132	24.674g	.1870g	45	.5483g	2.93
Simulium	9,308*	8.049g	.0009g	48	.1677g	194*
Brachycentrus	558	8.492g	.0150g	42	.2022g	13
Hexagenia	263	22.484g	.0850g	47	.4784g	5.6
[illegible]	318	8.503g	.027g	47	.1809g	6.8
Pteronarcys	166	31.043g	.187g	45	.6898g	3.7

*approximate

Collecting Techniques

The several methods of collecting mentioned in the preceding section will be reviewed separately.

Hand picking: This term aptly describes our most successful collecting method. The only equipment used was a pair of sharp forceps, a Surber sampler, a shovel, and small collection bottles in which the specimens could be transported. Substrates such as logs, rocks, plant matter, bottom silt, or detritus clumps were examined and the desired organisms captured with fingers or forceps. Submerged logs were abundant in the stream and proved to be the most fruitful habitat. Nearly every species used could be found under the bark or marl covering, or in cracks or crevices. Stone-

fly nymphs, hellgrammites, snipe fly larvae, and snails were particularly numberous. If possible, logs were hauled out onto the bank and exposed to the air. This brought to the surface many of the hidden forms.

The aquatic moss, Fontinalis sp., also proved an excellent habitat for many species. Many snipe fly larvae were collected by carefully picking apart the Fontinalis mat and marl concretions which covered most of the rocks and logs. Other aquatic plants, which occurred in large beds, were handled somewhat differently. Large clumps of plant matter and substrate were shoveled out onto the bank of the stream and then carefully picked apart and washed with stream water. Dragonfly nymphs, scud, burrowing mayfly nymphs, and oligochaetes were collected in this way. Beds of the pond weed, Potomogeton sp., were found in relatively swift water and provided a very favorable habitat for clinging forms, such as caddisfly larva, Brachycentrus, and the black-fly larva, Simulium.

The species which lived in silt beds were collected in two ways. First, it was often possible to get a sufficient number of specimens by merely scooping silt from the bottom with a shovel, placing it on the shore, and then carefully examining it as it was washed with stream water. If this method was not productive it was possible to concentrate the silt by rinsing it in a Surber sampler. The concentrated detritus was then emptied from the sampler and washed as in the previous method. This technique was particularly sucessful in gathering the burrowing mayfly nymph.

Branches and roots of trees, both living and dead, in the stream hold detritus and it was in these clumps of detritus or

"trash" that many species were collected. These clumps were removed from the water and carefully dissected with fingers and forceps. Black-fly larvae, snipe fly larvae, stonefly nymphs, and caddisfly larvae were gathered in this fashion.

Clinging forms were also collected from living vegetation which grew on the banks and dipped down into the stream. Stonefly nymphs were abundant in clumps of live alder leaves which hung in the water.

Shocking: Although the D.C. shocker was used mainly in the collection of fish and lamprey specimens, it was useful in the gathering of burrowing forms such as the mayfly nymph, Hexagenia. When the positive electrode was placed in the silt, any types of burrowing organisms were driven out and were easily collected.

Transporation and Storage

As soon as all the necessary specimens were collected at a given station they were sorted according to species and bottled in jars of stream water which had been appropriately labeled. After the day's collection was completed all specimen jars were placed in labeled boxes and stored overnight in a refrigerator. All radiological processing was done on the day following collection.

Aquatic Insect Laboratory Procedures

Due to significant changes in laboratory procedure from those of preceding years for the processing of the insect samples, an analysis of the method is given. It was necessary to obtain an estimate of the variability in activity between samples of the same species, collected on the same day, and at the same location in the stream. The best answer to this problem would have been

to process all the insects individually, but time and manpower conditions did not allow this. Also, weighing individual specimens of most of the species would have been inaccurate because of their small weight. Samples weighing at least 0.1 g were required to obtain the necessary accuracy in weighing.

Each collection of a species from each collecting station was divided into two samples for processing, thus making possible an estimate of the variability between samples.

The dividing of each species into two aliquots made it necessary to develop the following procedures to conserve time. They were thought to be the best for our conditions.

Preparation: On the day preceding processing the stainless steel planchets, in which the processing was done, were made ready. New planchets were used for all samples. They were numbered by etching a number on the side of the planchet with a Vibra-tool. These marks held up well under the severe conditions encountered in processing samples. Planchets were individually weighed on an analytical balance and the weights and planchet numbers were recorded.

Processing: Each sample from the stream was divided into two aliquots which were of approximately equal mass. In previous years the samples were rinsed in 0.01 N hydrochloric acid. During this study, this step was omitted; the insects were rinsed with distilled water just prior to being dried.

The following procedure was used to obtain the wet weight. Each sample was placed in a screen basket which had been made to fit snugly in the open end of a centrifuge tube. The screen was

then rinsed with distilled water and placed in the centrifuge. The power was turned all the way on for ten seconds and then was turned off. The head was allowed to stop without interference. The insects were then immediately transferred to a numbered and weighed planchet. The number of organisms and the species in each sample and the planchet number were recorded. The planchet was then weighed on an analytical balance and the combined weight of the sample and planchet was recorded. The wet weight of the insects is the difference between the weight of the planchet with the insects and without the insects. This method permitted the removal of any foreign materials (marl, sand, vegetation, etc.) which might have been mixed in with the insects and not rinsed out in the centrifuge. Previously, when the insects were weighed in the screens, any foreign matter which escaped the rinse was weighed along with the sample, in the centrifuge basket. If it was discovered in the planchet it was either processed along with the sample, or the weighing had to be repeated. Also the previous method necessitated the reweighing of the screens after each use. The present procedure eliminated this weighing.

Up to this point the samples were processed in groups of four (the number of centrifuge tubes). This was necessary, because the insects dessicated rapidly if allowed to remain in open planchets for more than a few moments before they were weighed. Now the samples were digested with acid and heat. While they were being digested they were watched carefully, but since the process was a slow one, a large series of planchets could be attended while other samples were being dried and weighed. Digestion was carried

out on a hot plate and under an infrared lamp. The insects were then covered with concentrated nitric acid. A hood and exhaust fan above the stove removed the acid fumes from the laboratory. The planchets were placed on the hot plate at a distance remote enough from the source of heat to prevent any boiling or spattering. The amount of acid used and the time required to digest the samples to a point where the planchet was relatively clean and dry varied a great deal with the nature of the sample. Larger forms, such as hellgrammites and stonefly nymphs, took three hours or longer. Several acid treatments were necessary in order to break down all the organic matter present. This is a very crucial step, and care must be taken to see that as much organic matter as possible is broken down, and that the planchets are dry. When digestion was complete and the planchets dry, they were removed from the hot plate and stored in a covered tray until they were counted.

Before a sample could be counted, it had to be ashed in a muffle furnace. This removed any trace of organic matter and moisture which might have been left. If the planchet was not dry, or if there was a residue of undigested organic matter, the sample smoked excessively and often ignited. This was avoided wherever possible because there might be a loss of radioactivity in the smoke. A temperature of 600°C in the muffle furnace was necessary to thoroughly ash the sample. Planchets were removed from the furnace, allowed to cool in covered trays and then they were taken to another laboratory where the radiological counting was carried out.

So that this paper may serve as a reference for other investi-

gators on this same project it may be of some help if the procedures which have just been described are written in a step by step form.

Aquatic Insect Laboratory Procedures

1. All planchets are numbered and weighed individually. Weights and numbers are recorded.
2. Each species from each station is divided into two samples of approximately equal mass.
3. The sample is rinsed with 0.01 N hydrochloric acid before drying.
4. Each sample is placed on a screen and centrifuged. The centrifuge is turned on full speed for ten seconds and then turned off. The head is allowed to come to a stop without interference.
5. Each sample is placed in a planchet and all foreign matter is removed.
6. Samples are now weighed on an analytical balance and the combined weight of the sample and planchet is recorded.
7. The number of individuals and the species name is recorded.
8. The planchets are now placed on a hot plate, under an infrared lamp and concentrated nitric acid is added. The heat is regulated to avoid spattering and boiling.
9. The larger species may take several treatments with acid to digest them to the point where the planchet is relatively free of residue and dry.
10. Planchets are heated in a muffle furnace at 600°C until they are cherry red. The samples must be completely digested and dry before muffling.

11. Samples are removed from the furnace, allowed to cool in a covered tray, and are then taken to the counting laboratory.
12. The planchets are always handled with forceps in the counting laboratory.
13. The counter chamber should be cleaned out with a piece of cheese-cloth after each sample is counted.

Introduction to Insect Results

Relatively little work has been done concerning the importance of bacteria as food organisms in stream ecosystems. Several stream dwelling insects seem to possess mechanisms which might allow them to filter, or capture, bacteria. Whether bacteria play a significant role as an energy source for these organisms, however, is unknown.

In previous studies on the West Branch of the Sturgeon River, high activity peaks in the filter feeding organisms led the investigators (Knight, 1961; Zettelmaier, 1961; and Clifford, 1959) to the following hypotheses. (1) The filter feeders were consuming diatoms. Because the filter feeders' activity peaks were often higher than the periphyton activity peaks, (2) the filter feeders were feeding on diatoms and concentrating the P^{32}, or (3) the filter feeders were feeding on some organism which was not being sampled, possibly bacteria.

Aquatic invertebrates are incapable of taking in salts through any surface but the lining of the digestive tract (Harris, 1957). Krogh (1931) believed that no animal can utilize dissolved organic material to a significant extent. Thus, the only source of P^{32} for the consumer organisms should be the organisms upon which they

feed (Davis and Foster, 1958).

If bacteria made up a major portion of the diet of the filter feeders and detritus browsers, and were responsible for a large part of the activity found in these organisms in 1959, then there should have been an increase in the activity peaks of these organisms when compared with the data of 1959. This increase should have been the result of the increased number of bacteria available during the passage of the isotope spike (Bender, ibid.) and the increased amount of P^{32} per bacterial cell (Bender, ibid.). In this way we hoped to isolate that part of the food chain which could utilize bacteria and particulate organic detritus as food. Thus, in short, the purpose of the experiment was to separate the catabolic portion of the food chain from the anabolic portion by tagging the catabolic organism with P^{32} atoms.

We expected to find significant activity in the filter feeders and any organism which preyed upon them, the ooze browsers, and other detritus feeders.

If we had succeeded in changing the point of entry of the P^{32} into the food chain, the organism through which the influx occurred should have shown a very high level of activity in comparison with results of 1959. A species by species analysis will show that such was not the case.

Results and Discussion

Simulium: As the time of appearance of the P^{32} in any species is dependent upon its position in the food web, it seems logical to examine the various species by trophic groups. The classifications are, by necessity very general, for in a rigorous environ-

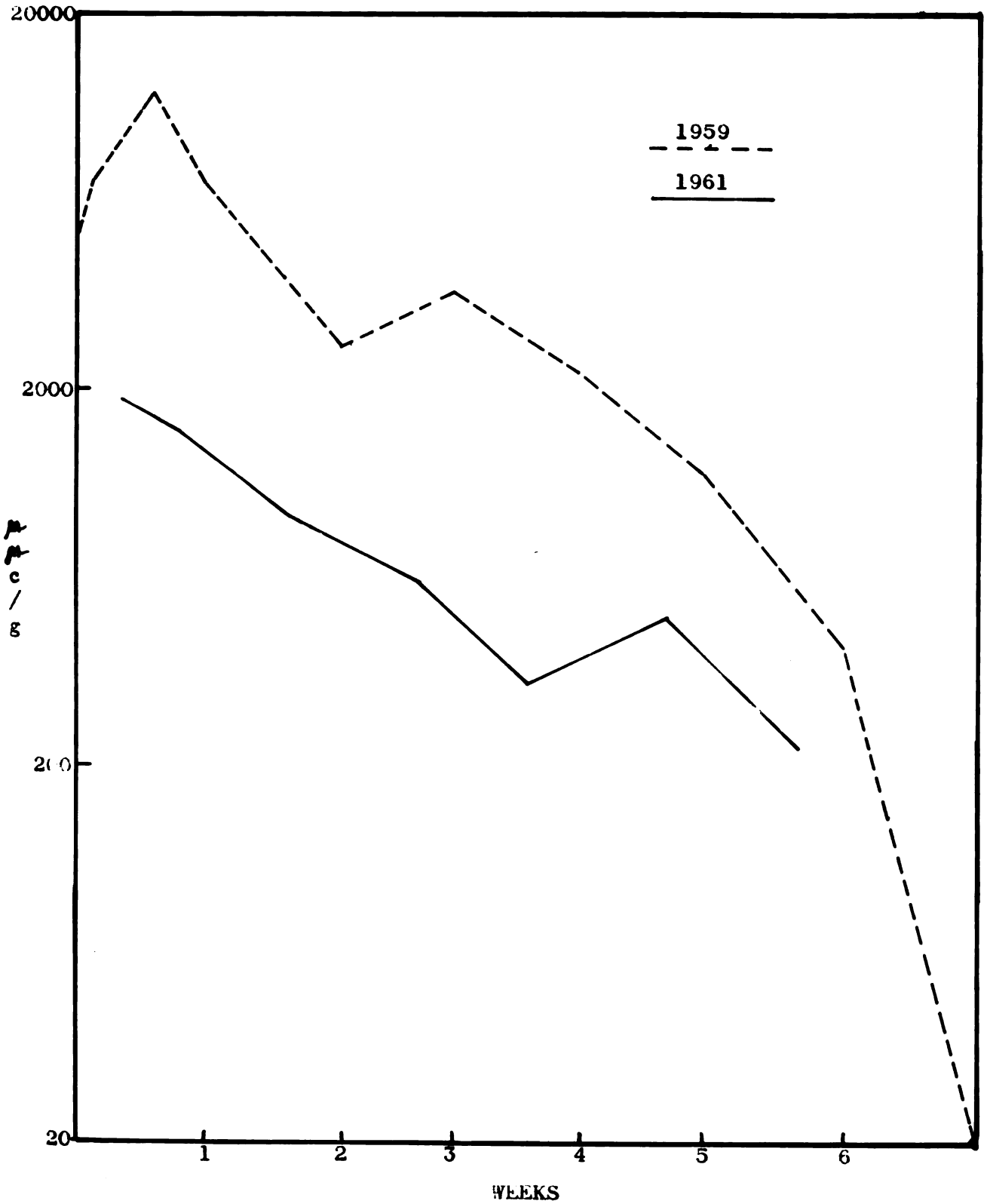
ment, like a stream, selective feeding is exceptional. The trophic groupings examined were: filter feeders, periphyton feeders, omnivores, carnivores, and detritus feeders.

The black-fly, Simulium sp., was thought to be the most likely macroscopic form which might feed on bacteria. It possesses two filtering fan mechanisms at the anterior end with which it strains food from the passing water (Pennak, 1953).

The results show that the black-fly larvae did not take in a great deal of P^{32} in relation to previous studies. Figure 12 shows a comparison between the results of 1959 and 1961, at Station 8. By comparison of the areas beneath the two curves it was ascertained that there was approximately eighty-six per cent less activity present in the black-fly larvae in 1961. This quantity of P^{32} could have come entirely from the inorganic P^{32} which was taken in by the periphyton. The activity of black-flies in 1961 represented a larger fraction of the 1959 activity than that of any species collected. This can be interpreted as indicating that the black-fly larvae take in bacteria, but that bacteria do not represent a major source of energy for these organisms. This would substantiate the hypothesis that the diatoms or other types of particulate detritus were a much more important energy source than the bacteria for the black-fly larvae.

Our data, and those of Zettelmaier (1961), seem to indicate that Simulium may not be a major item in the diet of stream carnivores. If the black-fly larvae were an important food item of the major carnivores of the stream it follows that the amounts of P^{32} taken in by these organisms would have been similar to the uptake

Figure 12. Comparison of the activity density curves in micro-microcuries of Simulium sp. for 1959 and 1961. Data used are from Station 8. Counts are corrected for background and decay.


20000
2000
200
20
1959
1961
μμc/g
1
2
3
4
5
6
WEEKS

by the black-fly larvae, in relation to the 1959 results (Roebeck, et al., 1954), but they were not. The black-fly larvae showed a higher percentage uptake than did any of the carnivorous organisms which were investigated. The answer to this problem may be found in the data of Zettelmaier (ibid.). He sampled one organism which was not investigated in either 1959 or 1961, the periphyton browsing mayfly nymphs of the family baetidae. These organisms are among the most abundant in the stream and they also reached the highest activity peak of any organism analyzed in 1960. Because of the habitat in which the black-fly larvae are found and their subsequent, relative inaccessibility to the carnivores, and the close association of the browsing mayflies and the stream carnivores, it is possible that the mayfly nymphs make up a larger portion of the diet of the carnivores than do the black-fly larvae.

Knight (ibid.) citing Harris (1957), hypothesizes that a latent period, or lag, before the peak activity in the black-fly larvae was due to the fact that it took forty hours for an equilibrium condition to be reached in the bacteria. Our results do not substantiate such a hypothesis. Collections following the isotope spike were begun two days later in 1961 than in 1959, however, a lag was still noticed at Stations 12 and 14. If the hypothesis were true no equilibration period would be expected for the activity was already inside the bacterial cells, and was never available to the stream community. Since Harris' (ibid.) work was done in glass containers with bacteria films it may not be comparable to a cold, rapidly-flowing stream situation.

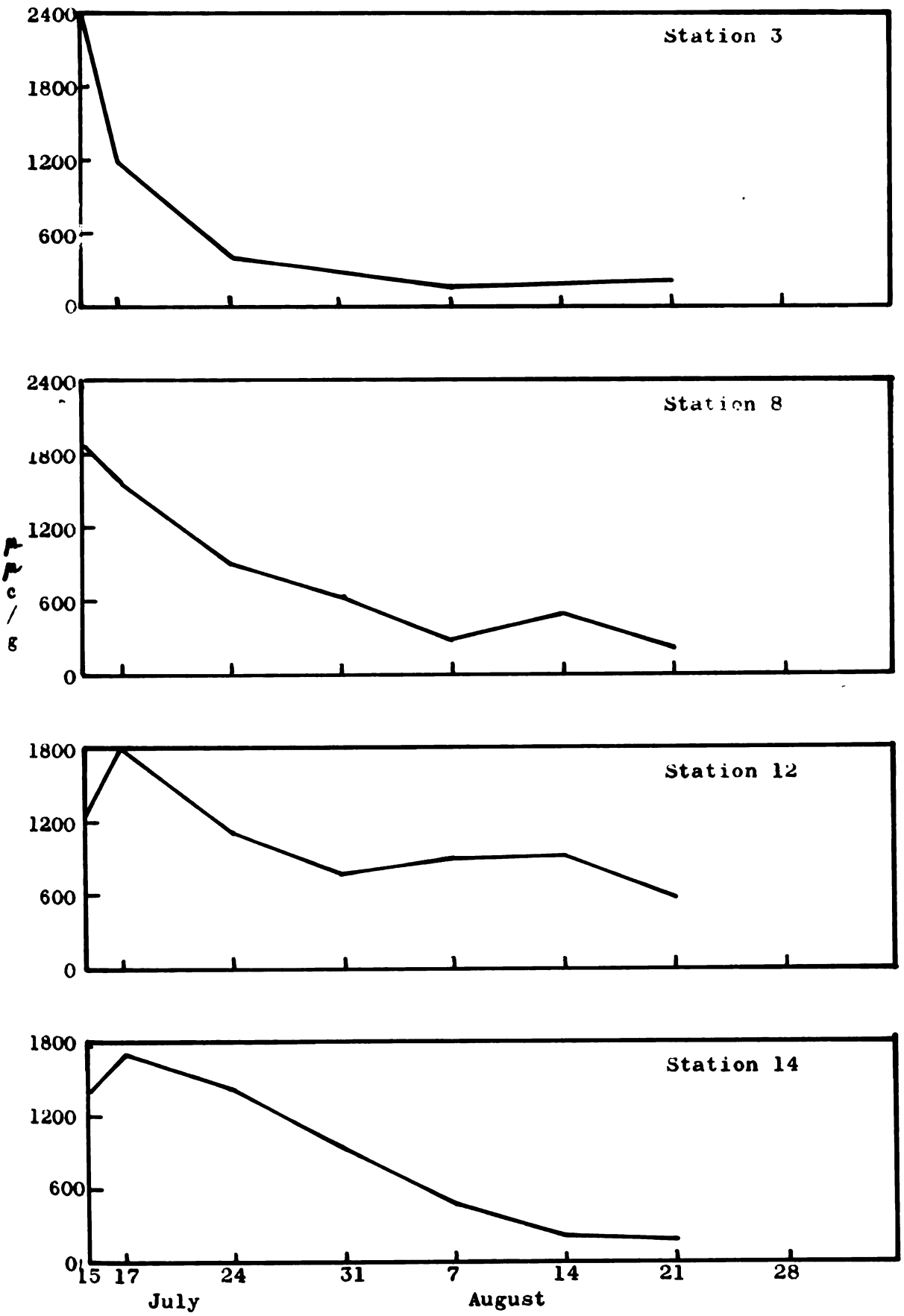
The days immediately following isotope addition are periods

when recycled quantities, and activity which had been trapped in eddies, will be maximal in the water. If the equilibration time of the black-fly larvae (at present unknown) were of the order of from four to seven days, it would mean that the larvae were merely taking in activity and holding it during this period. Then as the amount of recycled P^{32} dropped off and the larvae began to loøse P^{32} through equilibration with the P^{31} of the water, their specific activity would drop off.

This uptake of recycled activity is most evident at Stations 12 and 14, and possibly at Station 8. This follows since the further downstream the station is located, the greater the area above it releasing activity. It is also possible that because of the low level of uptake during the passing of the spike at the downstream stations, that the regenerated and recycled activity which was taken in would show up here as an increase. It might not appear at the upstream stations where the initial uptake was greater.

The work of Stuart et al. (1931) provides an interesting contrast. Their work on the cladoceran, Moina macrocopa, revealed that this species, which feeds almost exclusively on bacteria it filters from the water with its mouth parts, may feed with some degree of "selectivity". They placed bacterially sterile cladocerans in different containers and then fed each group one or more of eleven species of bacteria. By comparing reproduction rates and survival rates they found that, at one extreme, two species of bacteria promoted maximal cladoceran growth, and at the other extreme two species were actually toxic. They did not, however, differentiate between selective feeding and selective utilization.

Figure 13. Activity density in micromicrocuries of Simulium sp. at all stations, corrected for background and decay.


Station 3
Station 8
Station 12
Station 14
2400
1800
1200
600
0
μμc/g
15 17 24 31 7 14 21 28
July
August

Thus it may be that although the cladocerans, or our black-fly larvae, were taking bacteria into their digestive tracts, they lack the necessary enzymes to break down the bacterial cells. The possibility exists that because we used a bacterial form which was not found naturally in the stream we may have added a form which was not an available food organism to the black-fly larvae. Our results cannot resolve this problem. A possible clue may come from Stuart et al. (ibid.), since one of the species which yielded optimal results in their experiment was a coliform.

The activity curves of the black-fly larvae at all stations and over the entire study period are presented in Figure 13. There is an initial lag in uptake at Stations 12 and 14. After the activity peaks of this species have been reached, which is within four days, the activity is lost at a nearly exponential rate.

The black-fly larvae, as primary consumers, show activity peaks sooner than any species at a higher trophic level. This is in accord with the food-source hypothesis of phosphorus translocation presented earlier.

Brachycentrus: This trichopteran or caddisfly is classified as a periphyton scraper. The larva builds its case with the open end facing upstream in moderate to swift water. The food of these organisms is believed to be mainly algae and diatoms (Pennak, ibid.) although there is evidence that they may also eat other organisms and detritus which are carried within their reach (Mutkowski, 1929).

The comparison between activity curves for 1959 and 1961 for Brachycentrus is shown in Figure 14. The similarity in the pattern of activity uptake and loss is also evident in this species. When

the areas beneath the 1959 and 1961 curves are compared it is found that there was approximately ninety-two per cent less activity present in 1961. Again, this is in keeping with the hypothesis that eighty-five to ninety per cent of the P^{32} was never available to the metabolism of the community. The peak activity of Brachycentrus sp. in 1961 was approximately 870 counts per minute.

In comparison with the black-fly results we find that there was nearly fifty per cent less activity in Brachycentrus, in relation to the 1959 results. This is probably due to the different feeding mechanisms of the two organisms. The black-fly larvae could have filtered out some of the radioactive bacteria, but it is doubtful whether Brachycentrus is capable of such microscopic filter feeding. Thus, Brachycentrus could only ingest phosphorus which had been previously fixed by the producers of the community.

The activity curves for Brachycentrus at each of the stations are shown in Figure 15. The uptake-loss pattern is very similar to that observed in 1959. There was a relatively gradual uptake which reached its maximum after three to four weeks. This was followed either by a gradual decline, or a period of relative stability and then a decline. Knight (ibid.) believed that the relative stability of the Brachycentrus curves was due to the physiological behavior of the organism, which apparently tightly fixes the P^{32} and does not release it. Since there was no recognizable activity left in the periphyton after two weeks, it is apparent that Brachycentrus either retains the P^{32} taken in initially, or it is omnivorous enough to maintain a relatively constant activity level after the P^{32} is gone from the periphyton.

Figure 14. A comparison of the activity density curves, in micro-microcuries, of Brachycentrus for 1959 and 1961. The data are from Station 8. Counts are corrected for background and decay.

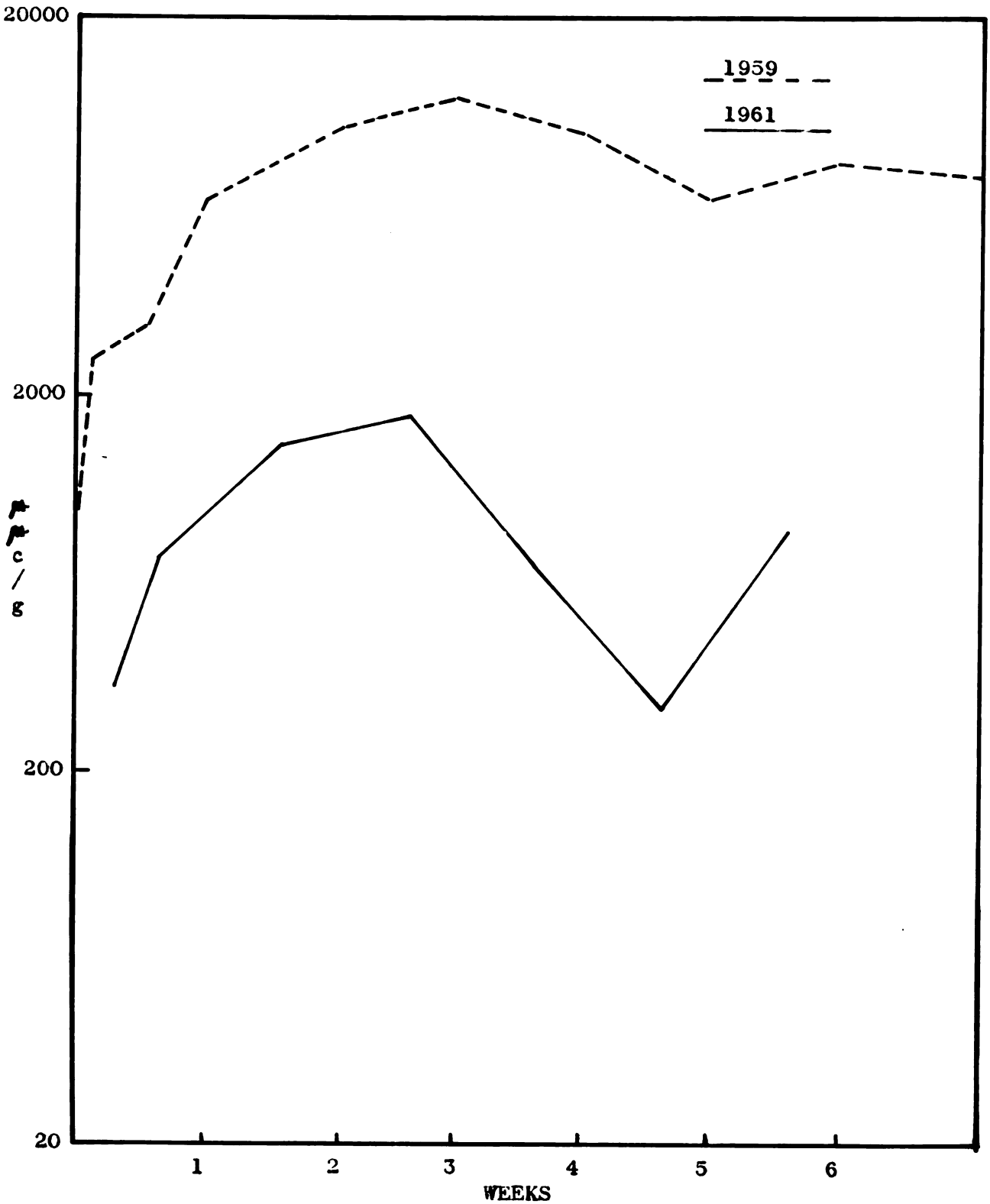
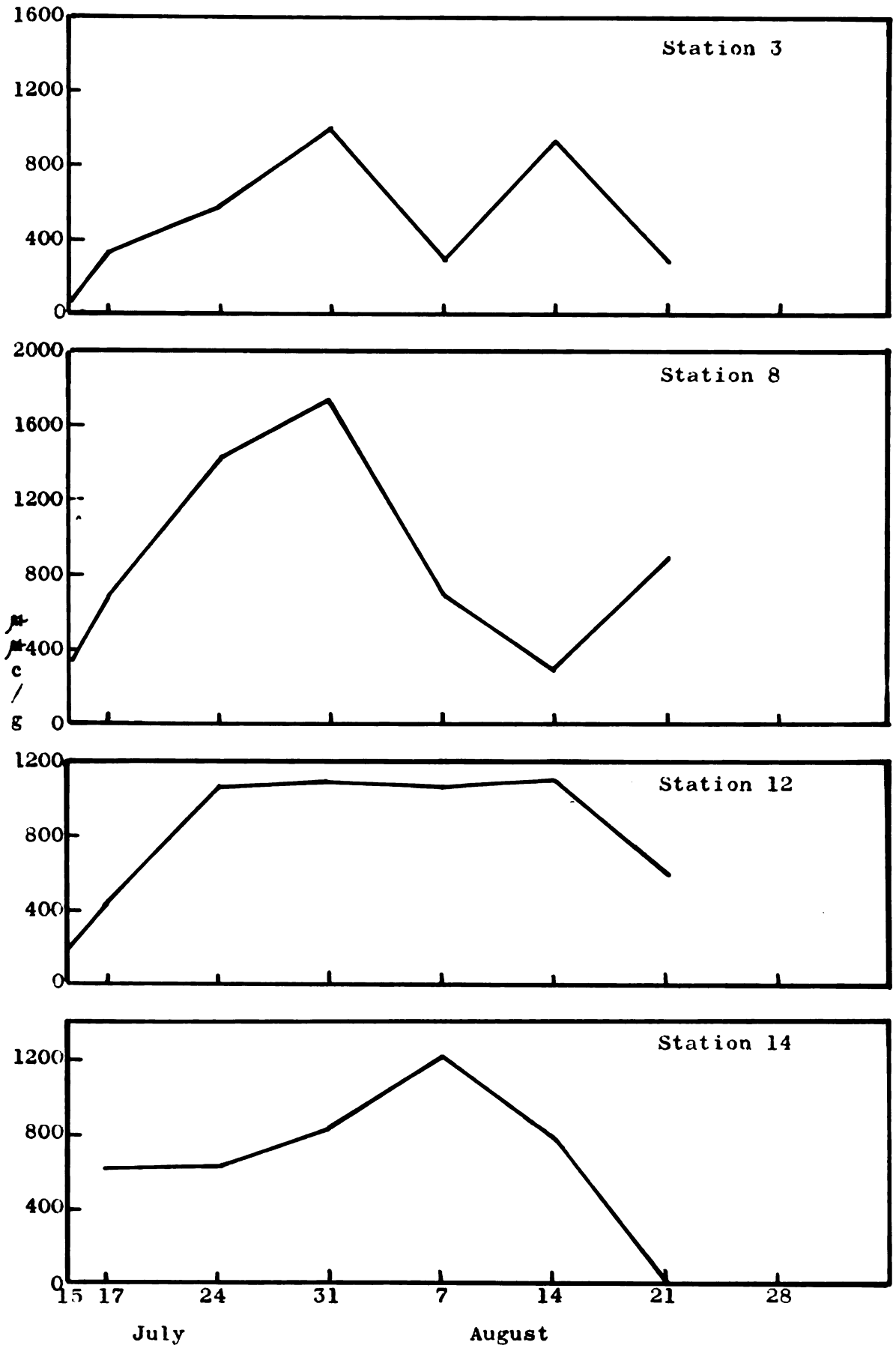

20000
2000
200
20
1959
1961
μμc/g
1
2
3
4
5
6
WEEKS

Figure 15. The activity density curves, in micromicrocuries, for Brachycentrus at Stations 3, 8, 12, and 14. Counts are corrected for background and decay.


Station 3
Station 8
Station 12
Station 14
1600
1200
800
400
0
2000
µg C/g
15 17 24 31 7 14 21 28
July
August

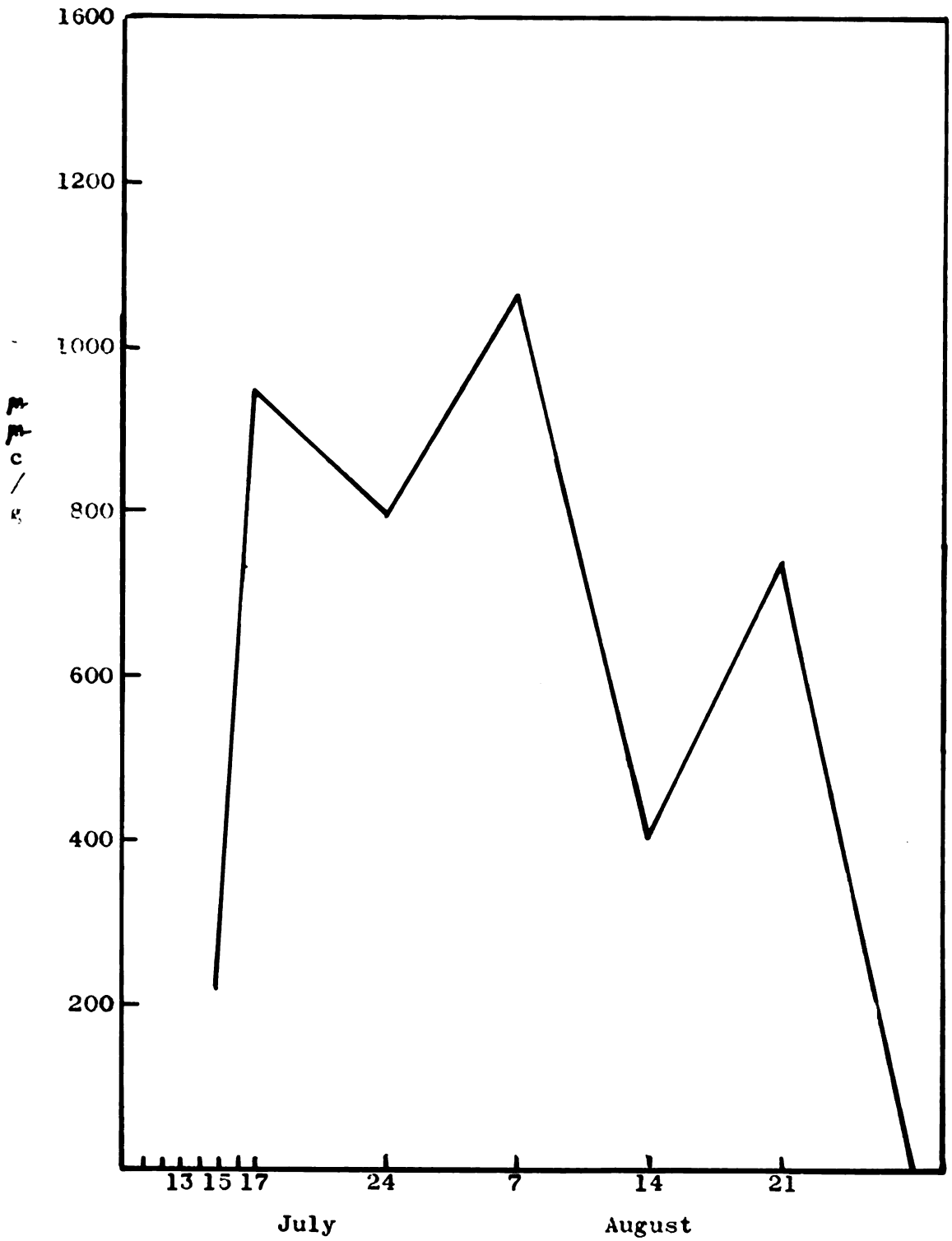
Hydropsyche: This net-building caddis proved to be a very unsatisfactory study organism. Its population was at an extremely low level during the summer of 1961. The species was studied in 1960, but during the 1961 investigation, it was found in sufficient numbers only at Station 3. Three weeks after the isotope was added it was very difficult to locate any full-sized larvae in the stream. After four weeks it was apparent that a new generation of larvae was present in the streams.

Figure 16 shows the activity curve for Hydropsyche at Station 3. This was the only station at which enough specimens could be procured to constitute weighable samples. This species was found to have an activity peak of approximately 11,000 counts per minute in 1959. In 1961 the peak was only 530 counts per minute.

The uptake-loss pattern of the species is representative of those organisms which feed on algae, diatoms, and detritus. These are trapped in, or grow upon, the organism's net dwelling. There is a rapid uptake followed by a gradual loss of activity. The sharp decrease after the third week may have been due to the maturation of the larvae and their subsequent emergence although it may have been due to chance variation alone. It is not known whether the new generation of larvae hatched from their eggs in time to be exposed to the activity spike.

Two species of predaceous insect larvae were studied as representative organisms of the carnivore trophic level. One, the Rhagionidae fly larvae, Atherix, is probably a secondary consumer. They are found in close association with the herbivorous mayfly nymphs. The second is the hellgrammite, Nigronia. It must be class-

Figure 16. Activity density of Hydropsyche sp., in micromicrocuries, at Station 3. Counts are corrected for background and decay.


1600
1200
1000
800
600
400
200
ppc/g
13 15 17
24
7
14
21
July
August

ified as at least a tertiary consumer, for one was observed devouring an Atherix larvae.

Carnivores are located, trophically, midway between the herbivores and the decomposers and detritus feeders. Thus they receive the P^{32} after the herbivores and before the decomposers. In general, their uptake-loss pattern shows a brief latent period, while the herbivores uptake is maximal, followed by a rapid increase. A relatively high level is then maintained for several weeks. There is, however, a great deal of variability in activity throughout the study period.

Atherix: The snipe fly larvae reached a peak activity density of approximately 1100 counts per minute. In comparison with the results of 1959 (see Figure 17) there was approximately ninety-two per cent less activity in 1961. The decrease at the predator level is believed to be a reflection of the form in which the activity was added.

Figure 18 shows the activity curves for Atherix at all stations. The aforementioned carnivore activity pattern is evident, as is the great deal of variability in the activity level.

Nigronia: The fishfly larva, or hellgrammite, presents one of the most interesting and perplexing problems encountered during the study. This is the only organism which showed as much activity in 1961 as it did in 1959. In some cases the peaks were even higher than they were in 1959. A sample collected on July 31 showed 7,918 counts per minute. There were seven samples which exceeded 2,000 counts per minute. The relative variability of the species was great. On July 17, at Station 8, one sample was found to

Figure 17. A comparison of the activity density curves, in micromicrocuries, of Atherix sp. for 1959 and 1961. The data are from Station 8. Counts are corrected for decay and background.

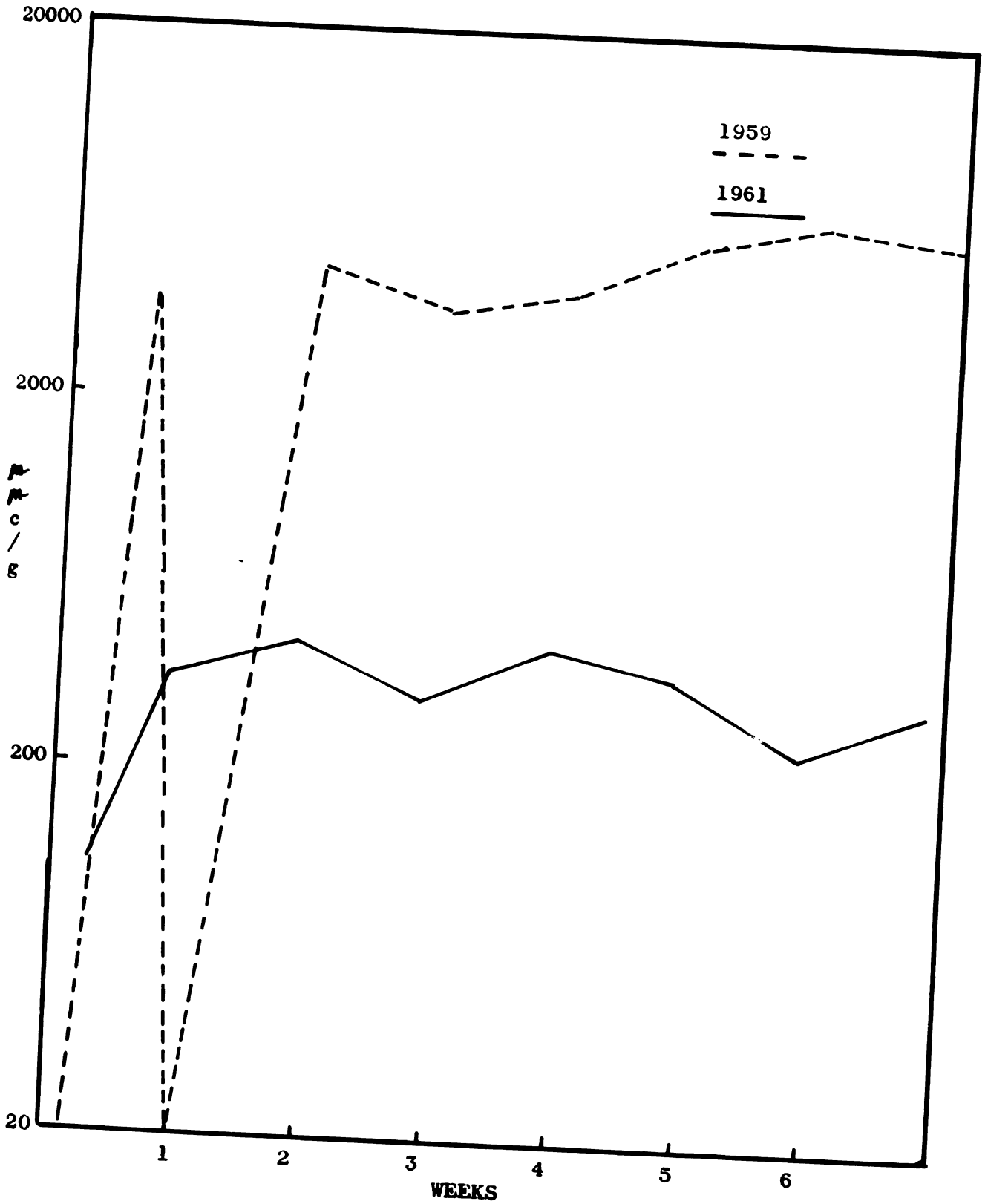
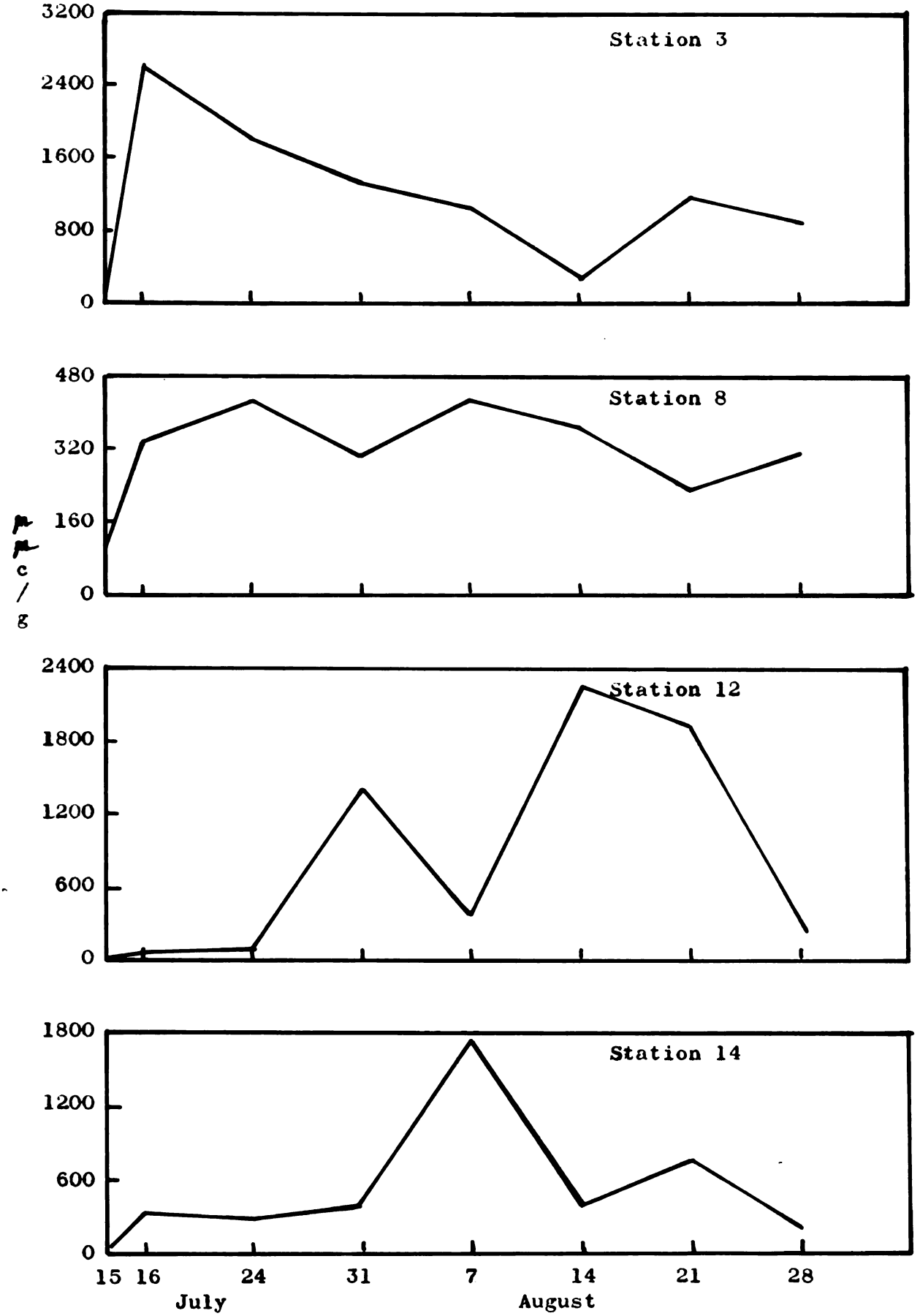

20000
2000
200
20
μμc/g
1959
1961
1
2
3
4
5
6
WEEKS

Figure 18. Activity density of *Atherix* sp., in micromicrocuries, at Stations 3, 8, 12, and 14. Counts are corrected for background and decay.


Station 3
3200
2400
1600
800
0
Station 8
480
320
160
0
μμc/g
Station 12
2400
1800
1200
600
0
Station 14
1800
1200
600
0
15 16 24 31 7 14 21 28
July
August

possess nine counts per minute, while the other sample showed 6,851 counts per minute. (For overall account of insect activity variability, see page 102.)

With this great amount of variability a graphical representation of the uptake-loss pattern for the hellgrammite is difficult. An attempt is made in Figure 19 to make such a presentation. First, all the points on each graph were used to compute a linear regression for the results at each station. This was done by the following method (from Dixon and Massy, 1957).

where: b = slope of regression line

X = time in days

Y = activity in counts per minute (natural logs must be used)

A = intercept value

$M_{y.x}$ = regression line formula for mean Y when X given

N = number of observations

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

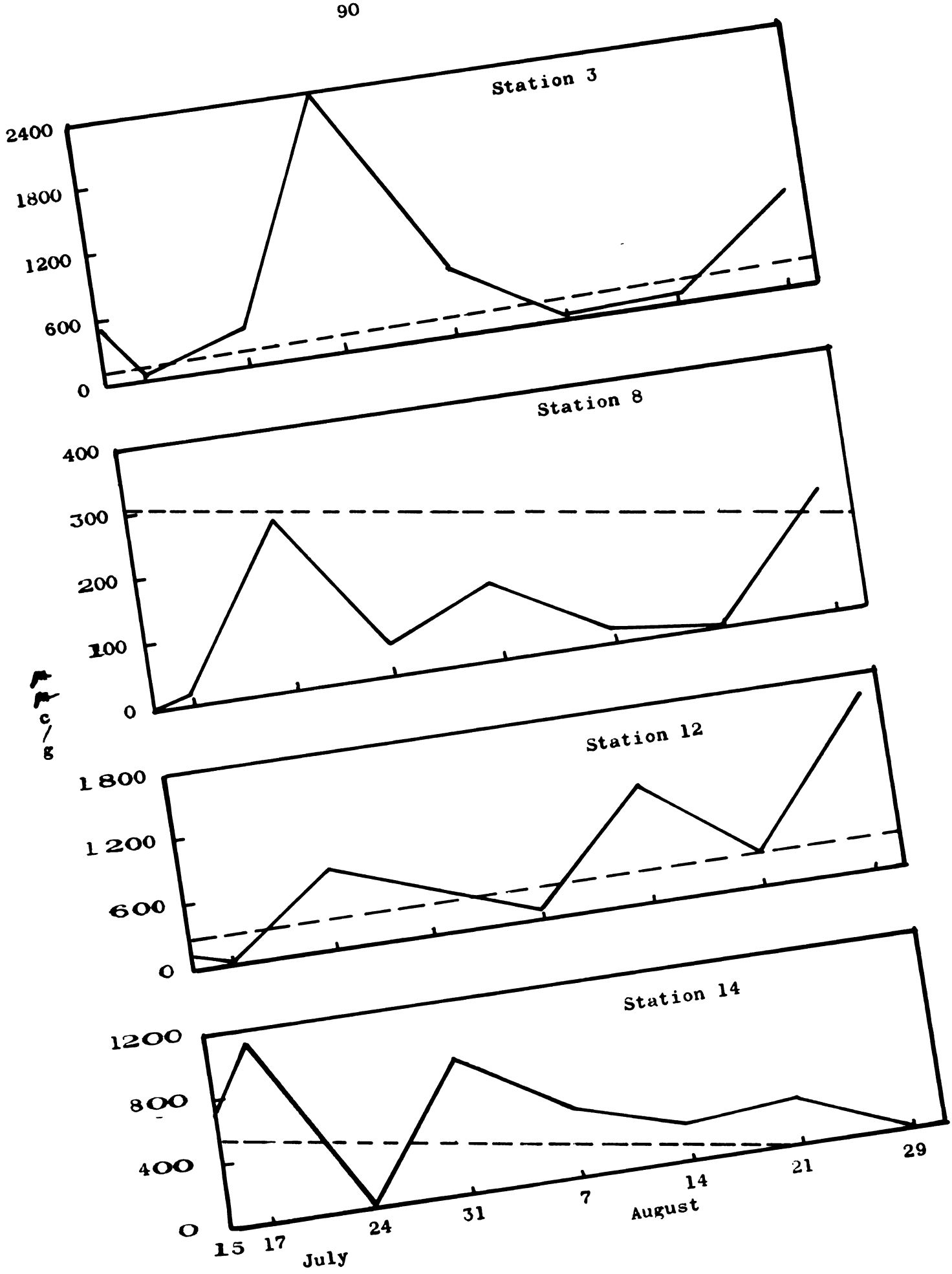
$$A = \bar{Y} - b\bar{X}$$

$$M_{y.x} = A + b(X)$$

The variation between these lines could be real, or it could be due to chance. No test was run to see if all the regression lines could have come from the same population.

Next, the eight highest points were all discarded and curves were drawn using the remaining points. The high points were excluded for the following reason. It is believed that the increased

Figure 19. Adjusted activity density curves, in micromicrocuries, for Nigronia sp. A regression line and adjusted activity density curve are shown for each station. Counts are corrected for background and decay.


Station 3
2400
1800
1200
600
0
Station 8
400
300
200
100
0
μg C/g
Station 12
1800
1200
600
0
Station 14
1200
800
400
0
15
17
July
24
31
7
14
21
29
August

variability in the activity of all predator species is due to their irregular feeding habits. Thus an organism which has just eaten a large amount will have more activity in its digestive tract, while an organism which has not eaten in several days will not possess this extra activity. Also, because of the euryphagous habits of many stream carnivores, one specimen may be feeding on one type of organism which is particularly abundant in its vicinity, while another specimen may be feeding on an entirely different species of prey animal. The activity levels of the prey species may be entirely different, and this will be reflected in the carnivores which feed upon them. These differences cannot be resolved here. Thus, the high points have been excluded from the graphs merely for the purpose of presenting a more intelligible graph. The possibility exists that these samples consisted of one or more specimens which had just eaten and possessed a great deal of activity in their digestive tracts which they might have lost in their droppings. The same argument might be offered for disregarding the very low points on the graphs.

Another very likely source of activity was the random settleing out of bacteria possessing P^{32}. The activity level seemed to be independent of time and distance downstream in relation to the site of isotope entry.

Whether the graphs are valid or not, the activity pattern is similar to that found in 1959 (Knight, 1961). Not only is the pattern similar, but also the magnitude does not seem to differ greatly.

At present, it seems that the only way in which this species

could possess the same, or a greater activity peak in 1961 than it did in 1959, is by eating some unknown organism which had a very high level of activity. This is at present hypothetical. It was not sampled, nor was it eaten by any of the other stream carnivores which were sampled. Nigronia sp. probably had a more direct source of activity than any of the other stream organisms which we sampled.

Two organisms were studied which represented the detritus feeding trophic group. They were the stonefly nymph, Pteronarcys, and the burrowing mayfly nymph, Hexagenia. These organisms are the last to receive the activity.

Pteronarcys: Pennak (ibid.) and Usinger (1956) indicate that this species belongs to a group whose nymphs are largely herbivorous. They are believed to feed on algae and plant detritus.

A comparison of the uptake and loss of activity by this species in 1959 and 1961 is presented in Figure 20. The similarity is again evident. There was approximately ninety-three per cent less activity in 1961. The activity peak in 1961 was 300 counts per minute. This, again, indicates a similar source of activity, but a greatly reduced magnitude.

The activity curves of Pteronarcys, at all stations, are shown in Figure 21. In general, there was a gradual increase in activity until a peak was reached sometime later than July 31. This period of uptake occurs while the higher plants are losing their activity. Pteronarcys continued to take up activity after all significant activity was gone from the plants. This indicates that either the feeding habits of the nymphs have been incorrectly

Figure 20. A comparison of the activity density curves, in micro-microcuries, of Pteronarcys sp. for 1959 and 1961. The data are from Station 8. The counts are corrected for background and decay.

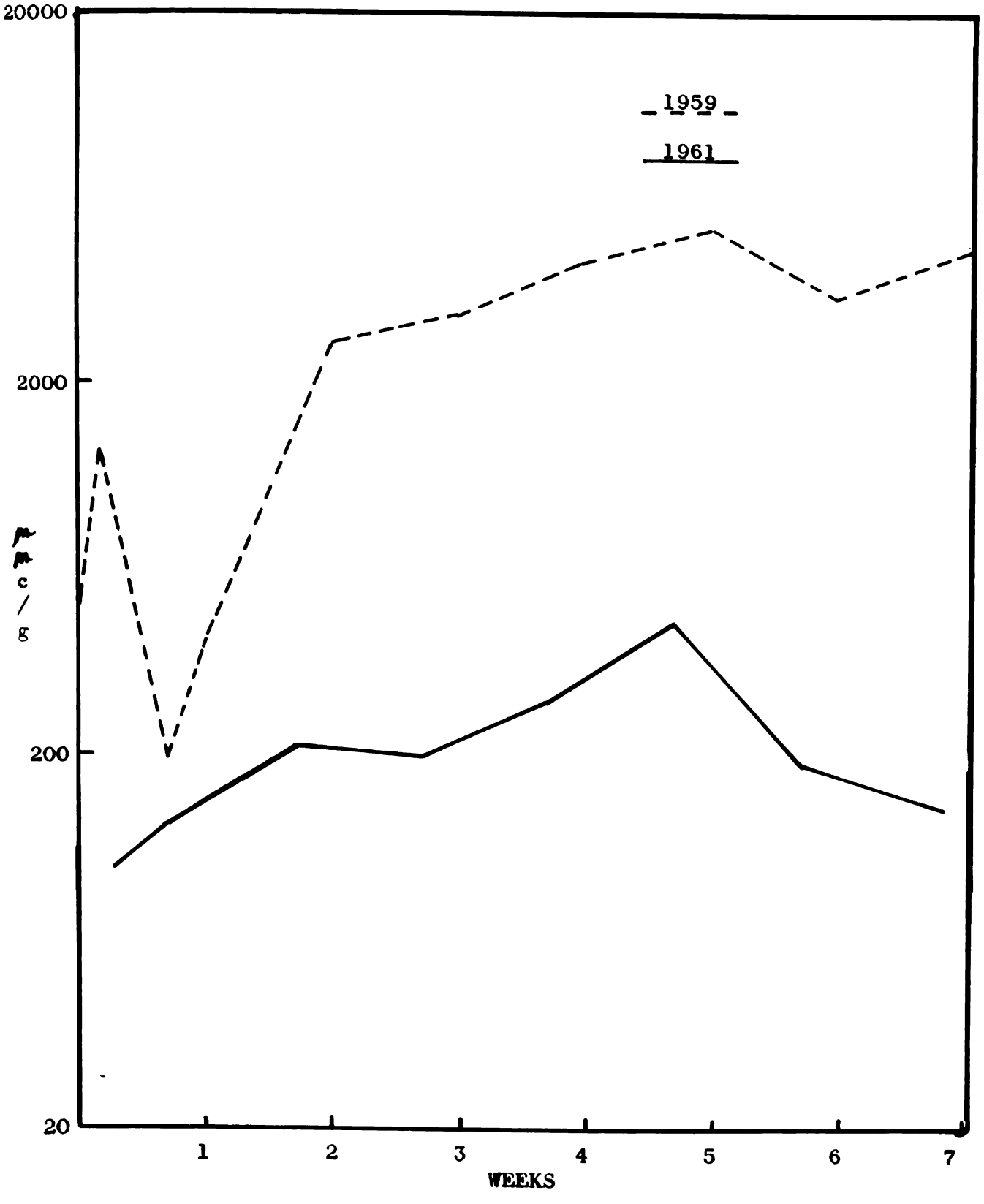
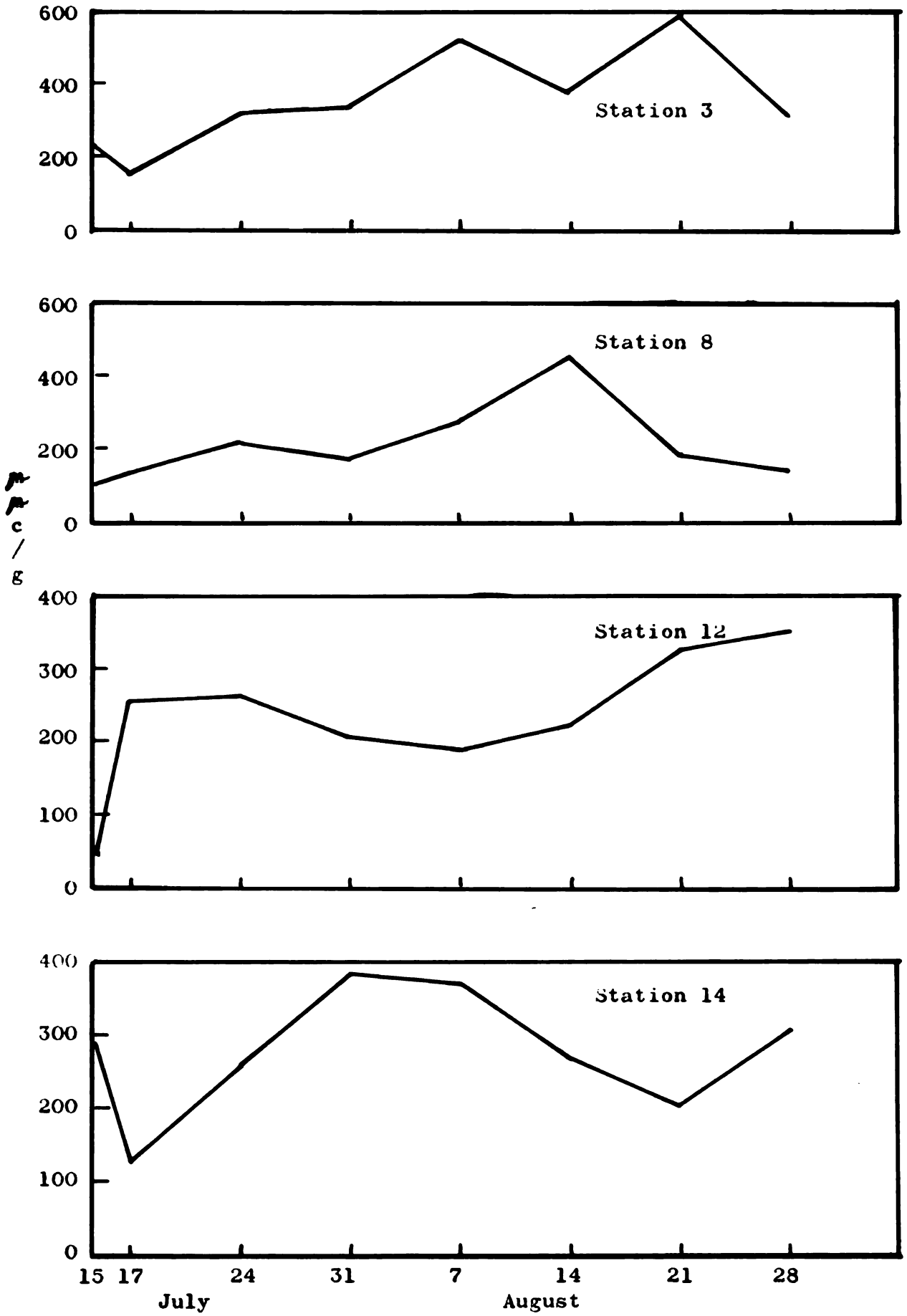

20000
2000
200
20
μμc/g
1959
1961
1
2
3
4
5
6
7
WEEKS

Figure 21. Activity density curves, in micromicrocuries, for Pteronarcys sp. at Stations 3, 8, 12, and 14. Counts are corrected for background and decay.


600
400
200
0
Station 3
600
400
200
0
Station 8
µµc/g
400
300
200
100
0
Station 12
400
300
200
100
0
Station 14
15 17 24 31 7 14 21 28
July
August

analyzed, or that they are capable of concentrating the activity which is left in their tissues.

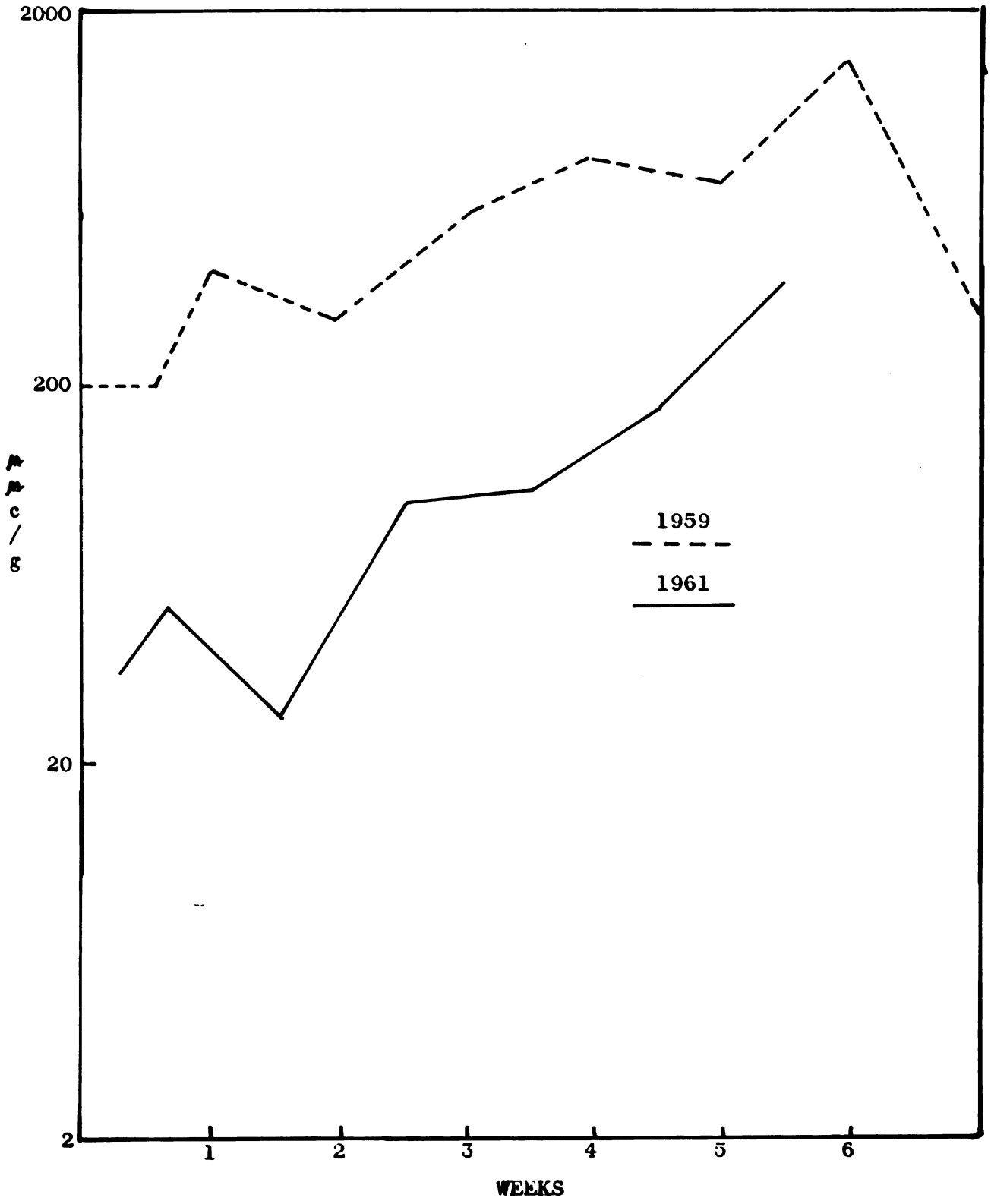
Hexagenia: The burrowing mayfly nymph gains its nutrition from bits of detritus and microscopic forms it encounters while burrowing through the many silt beds found along the stream course.

Upon examination of the activity curves of this species at all stations (Figure 22), it is evident that they are correctly classified as detritus feeders. They do not reach their peaks until very late in the study period. The one exception was Station 14. The counts were so close to background at this station that considerable variability was encountered when the counts were corrected for mass.

It is obvious that the peaks of activity are not very great. The maximum was less than 250 counts per minute. When these results are compared with those of 1959 (Figure 23), it is found that there was approximately 86 per cent less activity in 1961. This is relatively more uptake than any other species shows with the single exception of the black-fly larvae. This is reasonable, however, for these two organisms are the only macroscopic forms which seem capable of obtaining bacteria as food organisms. The habitat of the mayfly nymph also lends itself to their possible utilization of the bacteria. The silt beds in which the Hexagenia were invariably found, were located in areas of reduced water velocity, such as pools, eddies, and behind stream diverters. These would also be areas where bacteria would tend to be caught and settle out.

It is evident that Hexagenia at downstream stations shows

Figure 22. A comparison of the activity density curves, in micro-microcuries, of Hexagenia limbata for 1959 and 1961. The data are from Station 8. The counts are corrected for decay and background.


2000
200
20
2
μμc/g
1959
1961
1
2
3
4
5
6
WEEKS

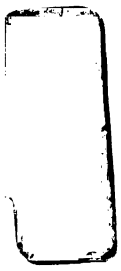
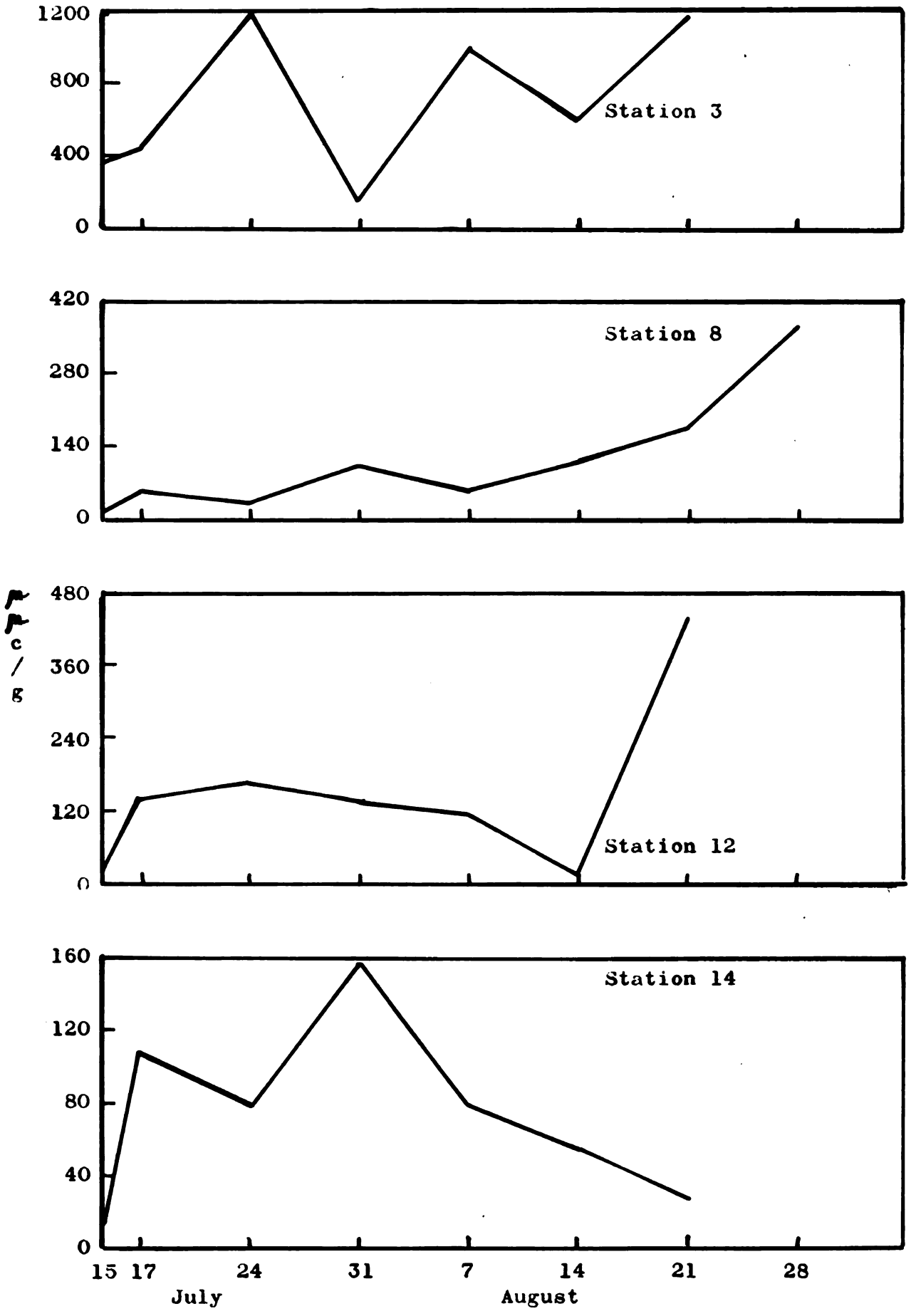


Figure 23. Activity density curves, in micromicrocuries, for Hexagenia limbata at Stations 3, 8, 12, and 14. The counts are corrected for decay and background.


1200
800
400
0
Station 3
420
280
140
0
Station 8
µµc/g
480
360
240
120
0
Station 12
160
120
80
40
0
Station 14
15 17 24 31 7 14 21 28
July
August

higher activity peaks than those at Station 3. This could be caused by nymphs drifting into Station 3 from areas upstream from the site of isotope entry. It could also have been due to the fact that the downstream stations were exposed to greater amounts of greater radioactive detritus. The results from Station 14 do not concur with this latter theory. This may be due to the fact that there are very few silt beds at Station 14. The beds are small and are located along the edges of the stream. This station is a riffle area.

Physa: The snail Physa was sampled as an omnivore. Pennak (ibid.) says that the organism feeds upon dead and living plant matter and dead animal matter. This feeding pattern may be reflected in the activity curve of the species.

Figure 24 shows the comparison between the activity levels of 1959 and 1961. Again, there is a similarity in the uptake-loss pattern. There was 94 per cent less activity in 1961.

The curves for all stations are shown in Figure 25. As was the case in 1959, the curves tend to be biomodal. I believe that Knight's (ibid.) interpretation of the situation is quite adequate. This hypothesis is that the snails are feeding on both plant and animal matter throughout the summer, and that the two peaks represent the maximum activity in the plant matter (first peak) and then after a lag, the animal detritus (second peak). The lag between the two peaks is supposedly due to the time it takes the P^{32} to be transferred from the plant material to the animal waste products.

Variation in P^{32} Uptake by Aquatic Insects

Figure 24. A comparison of the activity density curves, in micromicrocuries, for Physa sp. for 1959 and 1961. The data are taken from Station 8. Counts are corrected for background and decay.

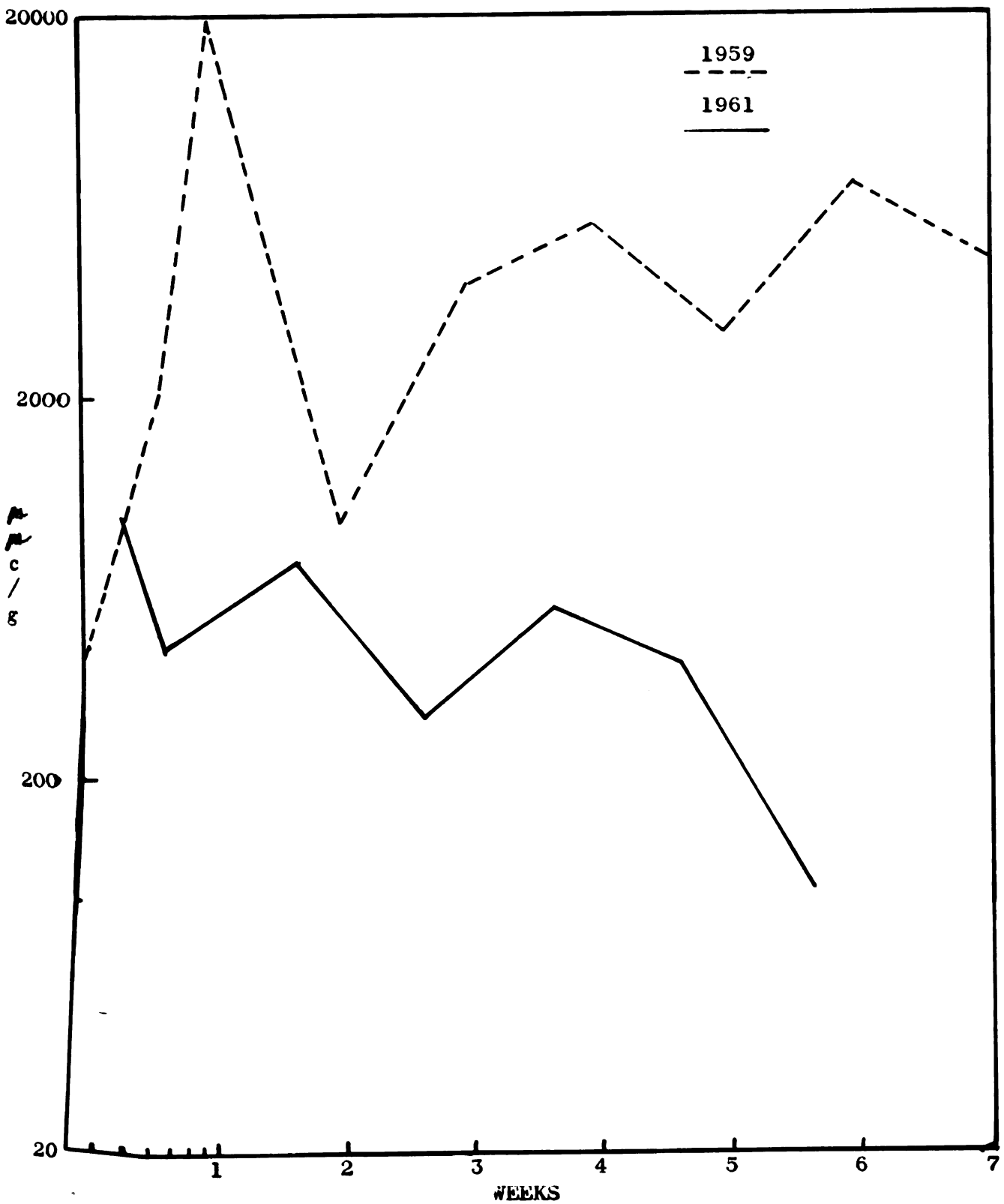
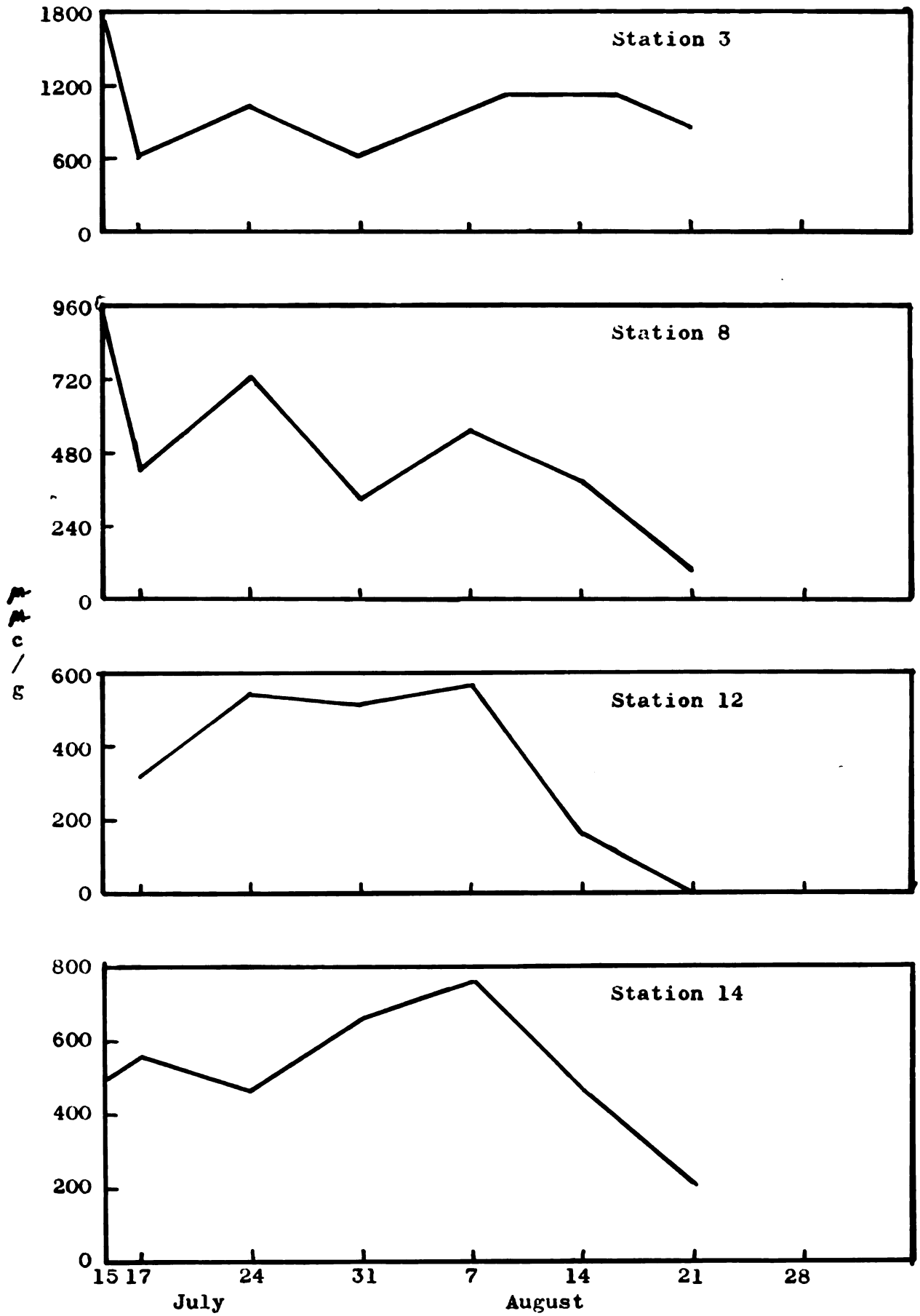

20000
2000
200
20
1959
1961
μμc/g
1
2
3
4
5
6
7
WEEKS

Figure 25. Activity density curves, in micromicrocuries, for Physa sp. at Stations 3, 8, 12, and 14. Counts are corrected for background and decay.


Station 3
1800
1200
600
0
Station 8
960
720
480
240
0
µµc/g
Station 12
600
400
200
0
Station 14
800
600
400
200
0
15 17
July
24
31
7
August
14
21
28

The problem of variation in sample activity was brought to light in 1959 by Knight. He processed two samples of each insect species over a period of several weeks. Marked differences were often noticed in the activity levels of the two samples. To discover whether or not these differences represented normal intraspecific variation, two samples of each species were collected from each station at each collection date during our investigation.

It was discovered that, in many cases, there is a great deal of variation in activity in samples of the same organism taken from the same station at the same time. There seems to be a logical pattern to this variation which may be related to the feeding habits of the organism. The age of the specimens used in the samples may have some effect too.

It was pointed out in the section of this paper dealing with the carnivorous insects that it is probably the irregular feeding times and euryphagous habits of these organisms which leads to the variation found in these species. In contrast with the carnivores, we find that the detritus feeders, the browsers, and the filter feeders feed more or less continuously, and they tend to be more stenophagous than are the carnivores. The variance in these species was found to be markedly less than that of the carnivores.

The age of the specimens used in the samples may also provide a source of variability. It is well established that younger organisms take up more radioactivity than do older, metabolically more stable individuals.

Knight (ibid.) also believes that the drift of organisms from outside the study area into the area may be a major source of

variability. A preliminary examination of this phenomenon in the West Branch revealed completely negative results. No drift organisms were captured by pumping and filtering large quantities of water at different times of the day and night. Further examination of the problem is needed, however. Undoubtedly, there is some downstream movement of individuals in the West Branch, but whether these movements result in long distance displacements or displacements of a few feet or yards is unknown.

The variance (s^2) for each species was calculated at each station by the sum of squares method. This procedure tells how much variation in the activity occurred within that portion of the total population which was sampled.

Paired samples = X_{11} and X_{12}, ... X_{k1} and X_{k2}

Weeks = 1, 2, 3, ... k.

	weeks		
	1	2	k
observation	X_{11}	X_{21}	X_{k1}
	X_{12}	X_{22}	X_{k2}

$$SS_1 = \text{Sum of Squares}_1 = (X_{11})^2 + (X_{12})^2 - \frac{(X_{11} + X_{12})^2}{2}$$

d.f. = degrees of freedom = 1 per SS

SS_t = Sum of Squares (total) = $SS_1 + SS_2 + \ldots SS_k$.

$$s^2 = \text{variance} = \frac{SS_t}{\Sigma \text{ d.f.}} = \frac{SS_t}{k}$$

This is the accepted method of computing variance in such cases. It should be obvious that this method does not take into consideration the actual height of the curve. Thus an absolute variance has been computed and not a relative variance. A vari-

ance which is relative to the height of the curve might be preferable, biologically, but I do not believe it is essential in this case and the statistical procedure is not widely accepted.

The results are presented in Appendix IV. The differences between the carnivores and all the other organisms sampled is marked. There also appears to be a difference between the detritus feeders (Pteronarcys, Hexagenia, and Physa), and those organisms which feed more on living plant matter (Brachycentrus and Simulium). No tests were run to see if the differences are significant, statistically.

Invertebrate Biomass

On August 30, 1961, an estimate of the invertebrate biomass was made on the West Branch of the Sturgeon River between Stations 11 and 12. This section of the stream is approximately 220 yards long. Horizontal transects were established every ten yards. Sampling sites were selected at random along the transects with a table of random numbers. One sample of one square foot was taken at each transect with a Surber sampler. The procedure is outlined in detail by Knight (ibid.).

The specimens were then preserved in alcohol for transportation and storage. In the laboratory the samples were separated by sugar floatation, counted, hydrated, and weighed. No identifications were made of these specimens. It was found that there were 0.2011 g of invertebrates per square foot, or 16.2 pounds per acre. The invertebrate biomass data for 1958, 1959, 1960, and 1961 are presented in Appendix V.

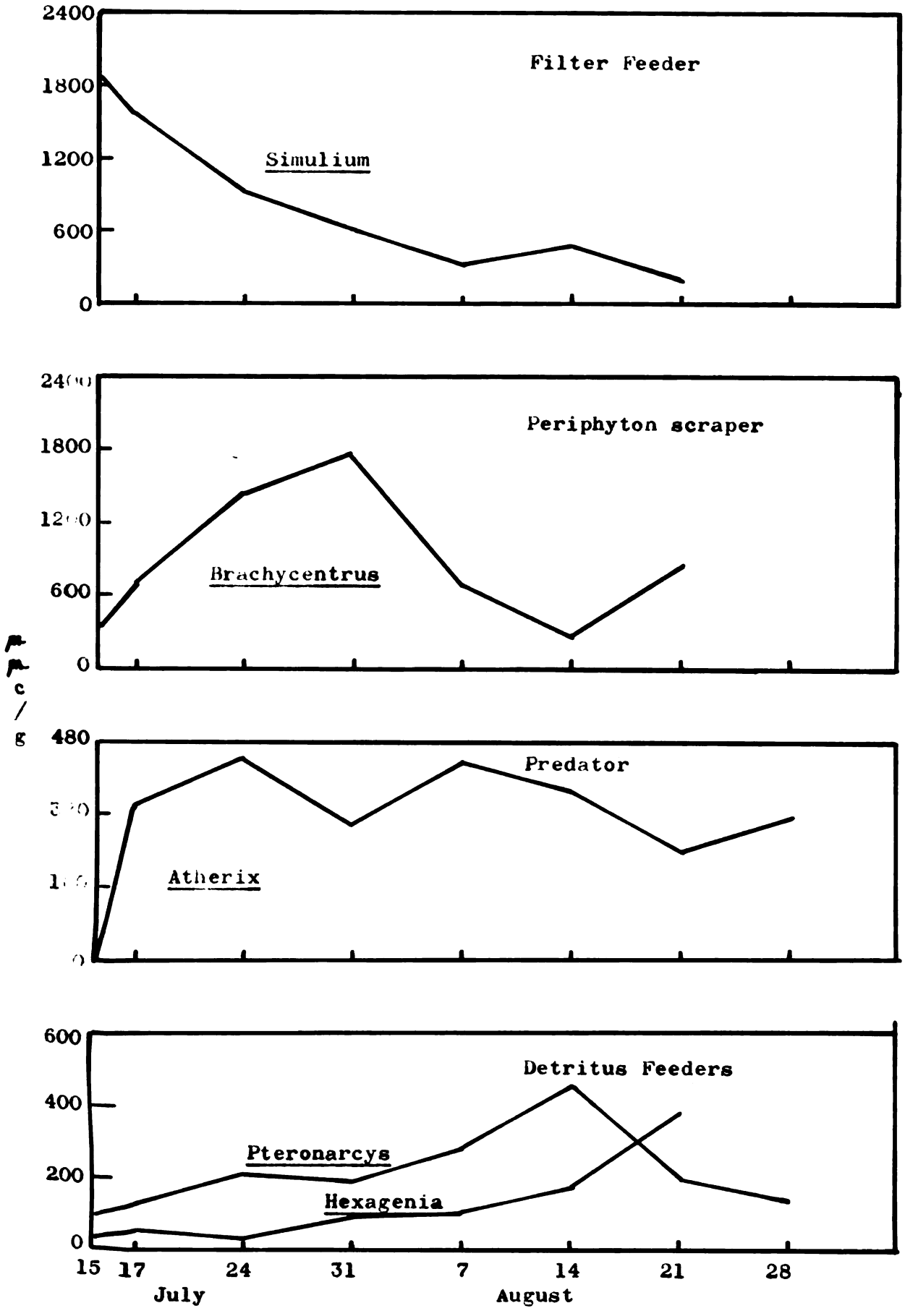
Summary of Invertebrate Results

To summarize the results of the invertebrates it may be said that the addition of radiophosphorus in the form of phosphorus inoculated bacteria instead of inorganic ions reduced the magnitude of the uptake of all forms examined. The single exception was Nigronia sp. The uptake was reduced at all levels by at least 85 per cent as compared to when inorganic P^{32} was used. Simulium sp. and Hexagenia limbata showed a slightly higher level of activity than the other organisms in 1961. Both of these forms seemed capable of taking in bacteria. The increased per cent activity was probably not passed on to predators because of the habitats of the two species. Neither were found in close association with any of the major stream predators.

Most of the P^{32} found in the aquatic invertebrates seems to have come from the inorganic P^{32} which was trapped by the plants, and not directly from the bacteria.

Figure 26 shows the movement of P^{32} through the consumer portion of the food chain of this ecosystem. By following peaks of activity density it is possible to trace the translocation of the radiophosphorus from one trophic level to another.

Figure 26. The movement of radiophosphorus through the invertebrate community of the West Branch of the Sturgeon River. All data are from Station 8. Activity density, in micromicrocuries, is corrected for background and decay.


2400
1800
1200
600
0
Filter Feeder
Simulium
Periphyton scraper
Brachycentrus
µgC/g
480
Predator
Atherix
600
400
200
0
Detritus Feeders
Pteronarcys
Hexagenia
15
17
24
31
7
14
21
28
July
August

SUMMARY

Twenty-one millicuries of radiophosphorus were incorporated into the cells of Escherichia coli O111. bacteria. The bacteria were then added to the West Branch of the Sturgeon River, Cheyboygan County, Michigan. Fifty-five per cent of the bacteria were carried through the study area in the first two hours with no significant loss of activity. Most of the P^{32} was apparently tied up in phospholipids and nucleic acids, and a small portion was exchanged in equilibration with the P^{31} of the stream. It appears as though this soluble portion was not the source of P^{32} for the community since there was no significant decrease in the amount of soluble P^{32} present in the water during the time the isotope passed. The source of activity for the stream community appears to have been the bacteria which were dropped in the study area. Their breakdown may have resulted in approximately ten to fifteen per cent of the P^{32} becoming available to the plants in an inorganic form.

The periphyton showed ninety-four per cent less activity in 1961 than in 1959, a year in which inorganic P^{32} was used. Among the higher aquatic plants, Chara sp. showed ninety-three per cent less activity in 1961; Potomogeton sp., ninety-five per cent less; and Fontinalis sp., ninety-six per cent less. There was a great deal of intraspecific similarity in the activity density curves of 1959 and 1961. A period of phosphorus uptake is noted during the first four days of the experiment. This P^{32} is probably from the bacteria trapped in the study area and the release of absorbed

phosphorus. The recycled activity was not recognizable. This was probably due to the extremely low level of uptake. The activity curves of Ranunculus sp. and Nasturtium sp. were of the same magnitude as the three previously mentioned species.

A polyethelene sheet was used as a stream diverter in an unsuccessful attempt to separate the waters of the West Branch from those of a small stream which joins the West Branch at Station 7. Activity-free periphyton was cultured in this small tributary of the West Branch.

The activity density of the invertebrates was comparable to that of the plants. Only three species showed a decline of less than ninety per cent of the 1959 levels. Two of these species, Simulium sp. and Hexagenia sp. possess feeding mechanisms or behavioral adaptations which might allow them to use bacteria as food. The third species, Nigronia sp., showed activity density curves which were comparable in pattern and magnitude to those of 1959. In many instances the peaks were higher than they were in 1959. No answer for this phenomenon was offered. The insect species also show a great deal of intraspecific similarity in their uptake-loss patterns for 1959 and 1961. All the activity in the insect species could have come from the inorganic P^{32} trapped by the plants. It is hypothesized that the bacteria of the stream do not play a significant role as food organisms in the community. It is also believed that because of the low temperatures encountered, the bacteria of the stream may not be successful in competition with the diatoms for the majority of the available phosphorus. The hypothesis presented by Knight (1961) and Zettelmaier

(1961) that the bacteria probably play an intermediate role between the inorganic phosphorus and the diatoms may be incorrect, at least they do not fix the phosphorus within their tissues and make it unavailable to the plants of the stream.

The variability of activity in the insect species was found to correspond closely with trophic level, feeding habits, and behavior. The greatest amount of variability was found in the carnivorous forms, Atherix and Nigronia. The least was found in the detritus feeders, Pteronarcys and Hexagenia. The species which feed on living plant material were found to occupy an intermediate position. Simulium and Brachycentrus are representatives of this group.

No insect species was found which could be used as an indicator organism for the level of activity present in the stream throughout the summer.

APPENDICES

APPENDIX I

A list of the insect species used in the experiment and frequently encountered in the West Branch of the Sturgeon River. The identifications have been carried as far as possible, within limits of time. This list is based on Usinger (1956), Pennak (1953), and Knight (1961).

Coleoptera

Haliplidae

Hemiptera

Gerridae

Gerris sp.

Belostomatidae

Neuroptera

Corydalidae

Nigronia sp.

Diptera

Rhagionidae

Atherix variegata

Simuliidae

Simulium sp.

Odonata

Cordulegasteridae

Cordulegaster sp.

Libellulidae

Plecoptera

Pteronarcidae

Pteronarcys sp.

Perlidae

Ephemeroptera

Heptageniidae

Heptagenia sp.

Ephemeridae

Ephemera sp.

Hexagenia limbata

Baetidae

Ephemerella sp.

Iron sp.

Trichoptera

Philopotamidae

Hydropsychidae

Hydropsyche sp.

Molannidae

Molanna sp.

Brachycentridae

Brachycentrus sp.

APPENDIX II

Estimates of the plant biomass of the West Branch of the Sturgeon River for 1959, 1960 and 1961.

1959 (Knight, 1961)	21,780.0 lb/acre
1960 (Zettelmaier, 1961)	10,260.0 lb/acre
1961	1679 lb/acre

APPENDIX III

Highest activity density recorded for organisms from the West Branch of the Sturgeon River in 1959 and 1961.

Organism	Date	Station	Activity (μμc/g)
Flora			
Periphyton	7/8/59	8	1.17×10^{-3}
	7/14/61	3	3.55×10^{-4}
Chara sp.	7/8/59	8	2.5×10^{-4}
	7/17/61	12	1.22×10^{-5}
Fontinalis sp.	7/9/59	3	7.2×10^{-4}
	7/17/61	14	5.63×10^{-5}
Potomogeton sp.	7/8/59	3	7.2×10^{-4}
	7/17/61	12	1.49×10^{-5}
Fauna (invertebrate)			
Simulium sp.	7/12/59	8	2.7×10^{-3}
	7/15/61	3	5.3×10^{-4}
Brachycentrus sp.	7/22/59	8	2.8×10^{-3}
	7/31/61	8	3.68×10^{-4}
Pteronarcys sp.	8/5/59	3	2.2×10^{-3}
	8/21/61	3	1.7×10^{-4}
Hexagenia limbata	8/19/59	8	3.3×10^{-4}
	8/21/61	12	1.3×10^{-4}
Atherix variegata	8/19/59	12	2.9×10^{-3}
	7/24/61	3	1.1×10^{-3}
Nigronia sp.	8/5/59	8	5.6×10^{-4}
	7/31/61	3	3.6×10^{-3}
Physa sp.	7/15/59	8	4.3×10^{-3}
	7/15/61	8	4.0×10^{-4}

APPENDIX IV

Variance of insect activity (S^2)

Simulium sp.

Station 3 - 2,181.667
Station 8 - 7,340.375
Station 12 - 49,512.800
Station 14 - 5,825.500

Atherix variegata

Station 3 - 190,295.750
Station 8 - 36,724.167
Station 12 - 274,096.875
Station 14 - 811,492.500

Hexagenia limbata

Station 3 - 250.640
Station 8 - 9,530.210
Station 12 - 1639.833
Station 14 - 679.100

Nigronia sp.

Station 3 - 3,354,908.500
Station 8 - 33,425,119.800
Station 12 - 44,454,155.000
Station 14 - 601,024.710

Brachycentrus sp.

Station 3 - 25,544.500
Station 8 - 25,451.500
Station 12 - 20,804.600
Station 14 - 37,212.750

Pteronarcys sp.

Station 3 - 11,169.250
Station 8 - 2,994.870
Station 12 - 11,166.214
Station 14 - 4,055.562

Physa sp.

Station 3 - 1,537.66
Station 8 - 11,434.000
Station 12 - 3,805.750
Station 14 - 7,973.000

APPENDIX V

The invertebrate biomass of the West Branch of the Sturgeon River for 1958, 1959, 1960, and 1961.

1958 (Bryant, 1960)	53.0 lb/acre
1959 (Knight, 1961)	36.6 lb/acre
1960 (Zettelmaier, 1961)	66.0 lb/acre
1961	16.2 lb/acre

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