THE TRANSLOCATION OF RADIOPHOSPHORUS THROUGH AN AQUATIC ECOSYSTEM

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Allen Warner Knight

1961

SS044

THE TRANSLOCATION OF RADIOPHOSPHORUS THROUGH AN AQUATIC ECOSYSTEM

By

ALLEN WARNER KNIGHT

AN ABSTRACT

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1961

Approved Robert A Ball

ABSTRACT

On July 8, 1959, twenty-one (21) millicuries of radioactive phosphorus were added to the West Branch of the Sturgeon River (Section 21, T. 33 N., R. 3 W.), Cheboygan County, Michigan. The main objective was to determine the fate of radioactive phosphorus released into the stream ecosystem and the manner by which the communities and populations participated in the distribution of the radioactivity. The uptake of such a tracer will differ according to anatomical characteristics, physiological properties, life histories and the relationships of the organisms to each other and their environment.

The radiophosphorus was first removed by suspended materials including the phytoplankton and bacteria. These forms, in turn, distributed the isotope to other members of the system through P^{32} "feed-back". The P^{32} uptake by the periphyton portion of the primary producers reached a peak in approximately 4 hours after isotope treatment, whereas the larger aquatic plants reached peak activity within 4 to 24 hours after isotope treatment. The uptake of radiophosphorus by primary consumer organisms was the result of these herbivorous forms ingesting plant material. The primary consumers reached a maximum activity level within 4 weeks after isotope treatment. Uptake of P^{32} by the secondary consumer organisms was a result of these organisms

ingesting the herbivores as food. The carnivores reached peak activity within 6 to 8 weeks from the date of isotope treatment.

The uptake of radiophosphorus by aquatic organisms may occur by absorption, as in aquatic plants; through membranes exposed to the surrounding water; and through ingestion of food or inert particles, as in the case of aquatic animals, which contain the radiophosphorus.

The accumulation of radiophosphorus, it is concluded, follows--for the most part--a definite and orderly pattern. The activity in the water is removed by primary producer organisms which are then fed upon by primary consumers; these in turn are fed upon by secondary consumers.

From a population estimate of the standing crop of fish and invertebrates in a 1,000-yard section of the study area, it was found that the trout and invertebrate production in the West Branch of the Sturgeon River is considered unproductive.

A. W. K.

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6/19/61

ACKNOWLEDGMENTS

In the study of the West Branch of the Sturgeon River, the writer was variously aided by different persons to whom he wishes to express his sincere gratitude: Mr. Frank Beekman and Mr. Kenneth Osborn for their assistance in the field; Mr. Hugh Clifford, a fellow graduate student, for his assistance in the field and laboratory; Dr. W. Carl Latta and his Pigeon River Staff, the writer is indebted to for the many courtesies, conveniences, facilities and direct aid; above all the writer owes his greatest indebtedness to Dr. Robert C. Ball, under whose guidance and direction this study was carried out; Dr. Frank Hooper, who is co-investigator in the overall project, which was sponsored by the Atomic Energy Commission; to his wife, Barbara, for her assistance in the exacting task of proof-reading, typing and unending support.

This study was made possible by a Graduate Research Fellowship from the Institute for Fisheries Research of the Michigan Department of Conservation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure | Page

Figure Page

Dedicated

to

MY FATHER

INTRODUCTION

When radioisotopes are released into the environment, they quite often become dispersed and diluted, but may also undergo unexpected movements and concentrations. At the present time very little is known about the mechanisms of uptake, concentration, retention, and excretion of radiophosphorus by fresh-water organisms. Even less is known about the fate of radiophosphorus released into lotic ecosystems and the manner by which the ecological communities and populations of such a system control the distribution of radioactivity. The necessity of obtaining such information is becoming increasingly urgent as more and more power-producing reactors are put into operation. In most cases the only ready means of disposal of large quantities of liquid effluent, by these reactors, is into fresh water. The nearby rivers or lakes will receive the discharge of radioactive wastes and, even though an isotope might be diluted to a relatively harmless level on release into the environment, it might become concentrated by aquatic organisms or a series of organisms to a point where it would be critical. In addition to valuable knowledge of a potential human health hazard, radiophosphorus can also be used to trace the metabolism of phosphorus through the entire ecosystem and thus provide information available by no other means.

It is the purpose of this paper to obtain information on: (1) the modes of transfer, accumulation and translocation of radiophosphorus between various components of a rapid stream biological system; (2) the interrelationships between particular species; (3) the metabolism and fate of nutrient material added to such an aquatic environment.

Description of the Study Area

The West Branch of the Sturgeon River is a cold water river, flowing through Cheboygan, Otsego and Charlevoix Counties, Michigan (T. 33 N., R. 3 W.). The West Branch of the Sturgeon River has its origin in Hoffman Lake, a hard water lake with an approximate area of 128 acres. The river flows from the northeast end of Hoffman Lake and continues in a northeasterly direction, through a narrow valley with steep and rolling glacial morainic hills. The river flows for about 14 miles before confluence with the Sturgeon River, at the town of Wolverine, Michigan.

The vegetation in the valley is chiefly birch, aspen, cedar, tamarack and balsam fir. Along the stream margin the vegetation is primarily cedar, tamarack, aspen, tag alder and ninebark.

The water temperature of the stream remains cool throughout the summer, due to water entry by way of springs and tributaries, as well as, shading by overhanging trees and mixing of the water, due to general turbulence. The summer water temperature remains between 52°F. and 58°F. throughout the summer (Clifford 1959).

The stream flow through the study area has a mean of 43.75 cubic feet per second.[1] The water level of the

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[1]Courtesy of Vannote and Carr, 1959.

stream remained relatively stable throughout the summer. Temporary turbidity is quite frequent and is caused by rains. Unless the rains have been very severe, the turbidity lasts only a few hours. The stream water in the study area has a total alkalinity of approximately 180 p.p.m., and a total phosphorus concentration of approximately 7 p.p.b. (Borgeson 1959). The thorough churning and mixing of water with air insures a high dissolved oxygen content in the stream water. In addition to the churning, the low water temperature permits the absorption of greater quantities of oxygen.

The area of the stream covered by this study is about 2,650 yards long (Figure 1). The stream bottom in this area varies from sand and gravel to silt and detritus.

For the first 250 yards, the vegetation is abundant with beds of Chara sp.; the water moss, Fontinalis antipyretica; mare's-tail, Hippuris vulgaris, with occasional growths of water cress, Nasturtium officinale. The stream bottom is gravel and sand down the middle with an occasional silt bed along the shore. The next 300 yards are almost devoid of any vegetation. The stream in this section becomes almost entirely riffle area with only a sparse growth of pondweed, Potamogeton pectinatus; water moss, Fontinalis antipyretica; mare's-tail, Hippuris vulgaris, and occasional beds of Chara sp. The stream bottom is predominately sand and gravel in this area.

The stream for the remainder of the study area supports a luxuriant growth of plants. The predominant plants are: Chara sp., which grows in beds of silt and detritus; Potamogeton pectinatus, which grows in riffle areas and in swift water near the shore; Batrachospermum moniliforme, which grows attached to rocks and logs; Oedogonium sp., which grows in filamentous strands attached to twigs, logs and rocks in the stream; mare's-tail, Hippuris vulgaris, which grows in silt and detritus beds near the stream bank; and tape-grass, Vallisneria sp., which grows occasionally in silt beds along the bank, as does occasional solid tangled masses of water cress, Nasturtium officinale. The stream bottom in this area varies from sand and gravel to silt and detritus.

The fish present are typical cold water forms: eastern slimy sculpin, Cottus cognatus; northern mottled sculpin, Cottus bairdii; brown trout, Salmo trutta; rainbow trout, Salmo gairdnerii; and brook trout, Salvelinus fontinalis.

The aquatic insects present are represented by the orders: Odonata, Ephemeroptera, Plecoptera, Trichoptera, Diptera, Megaloptera, Coleoptera, and Hemiptera--in general, only such insect types as require a high degree of oxygenation. Because of the currents, the biota is further limited to species that are either strong swimmers or strong clingers, the latter directly by means of structural adaptations (claws, suckers) or indirectly by means of

clinging devices constructed by the various species (webs, jellies, attached cases). The insects will be discussed more fully elsewhere. Other invertebrates present are the annelids, gastropods and pelecypods.

Sampling Stations

Sampling stations were established within the principal study area and were so located as to most nearly represent the various situations that were found to exist within the experimental area. Such factors as shade, stream velocity, bottom type and vegetation composition were the variables taken into account when each station was established. The sampling stations that were designated as permanent stations were numbers 3, 8, 12 and 14 (Figure 1). These stations were the permanent sites for collections of aquatic plants, periphyton, fish, lamprey and aquatic invertebrates throughout the study period.

Station 3. A well shaded area 300 yards below the isotope entry was selected for this station. The water depth was quite shallow with an average depth of 12.8 inches (Zettelmaier, unpublished). The stream bottom, at this station, was mainly of sand with isolated areas of gravel. The water flow at this site was somewhat retarded, due to a log obstruction immediately below this sampling range. Vegetation in this area was sparse and limited mainly to moss and Chara sp.

Station 8. This site was situated in a straightaway portion of the stream, 900 yards below the point of isotope entry, where there was a great deal of streamside vegetation which did not shade the stream to any extent. The mean depth of the water at this station was found to be 17.2 inches (Zettelmaier, ibid.) and flowing rather rapidly over a predominately gravel midstream channel with luxuriant beds of Chara bordering this main channel.

Station 12. The site chosen for this sampling range was a river bend, 2,200 yards below the point of isotope entry, with a gravel bottom, except for silt beds located near the shore. This site was devoid of any shade and was obstructed in part by a stream deflector. The mean water depth at this range was 13.3 inches (Zettelmaier, ibid.) with a rather rapid flow, due to the stream deflector. The vegetation consisted of huge beds of Chara sp. and a few patches of Potamogeton pectinatus.

Station 14. The site chosen for this station was a very rapid riffle area, some 2,650 yards below the point of isotope entry. There was little shading of the stream by streamside vegetation and only sparse aquatic plant growth. The bottom of this area ranged from gravel to huge boulders. The mean water depth was 12.2 inches (Zettelmaier, ibid.).

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

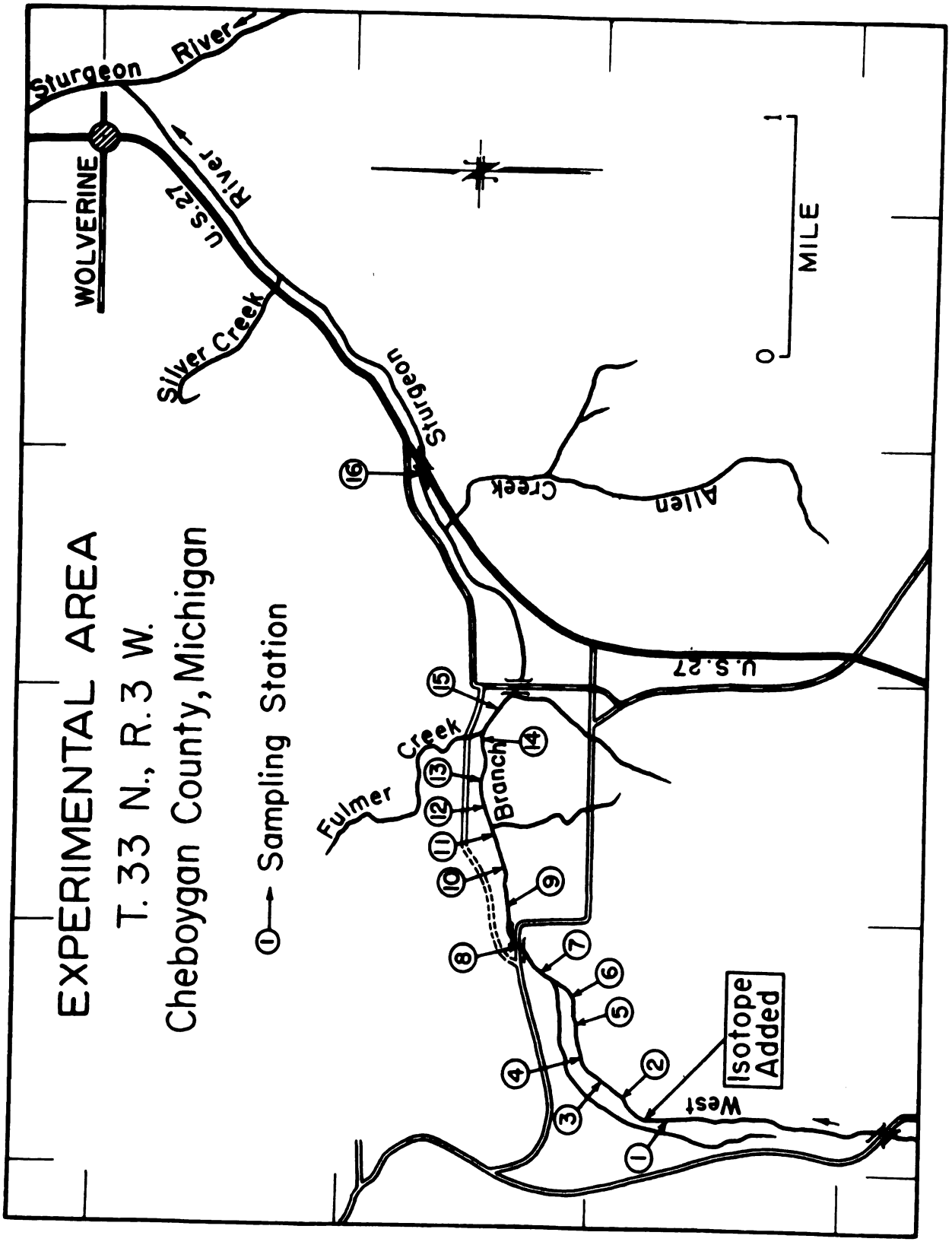
EXPERIMENTAL AREA
T. 33 N., R. 3 W.
Cheboygan County, Michigan
Sampling Station
Sturgeon River
WOLVERINE
U.S. 27
Silver Creek
Sturgeon River
Allen Creek
U.S. 27
Fulmer Creek
Branch
Isotope Added
West
MILE
0
1

Figure 1

METHODS AND PROCEDURES

Radiological Techniques

The Introduction of Isotope

The choice of a tracer to be used for a particular experiment is dependent on the specific nature of the experiment. A tracer with a very short half-life precludes the possibilities of shipment for appreciable distances, of synthesis of compounds, and of lengthy experiments. The tracer that was chosen for the present study was radiophosphorus (P^{32}) which is a beta ray emitter with a maximum energy of radiation of 1.712 mev. Radiophosphorus is further desirable because of ease of counting and possession of a satisfactory half-life (14.3 days).

The radioisotope for this study was supplied by the Oak Ridge National Laboratory, Oak Ridge, Tennessee, as phosphate (PO_4) dissolved in weak hydrochloric acid. The 1.1 ml. solution of radiophosphorus was assayed at 8:00 AM, July 6, 1959, and was found to have an activity of 23.29 ± 3% millicuries. The addition of isotope was made in Section 21, T. 33 N., R. 3 W. (Figure 1), on July 8, 1959. The activity of the radioisotope at the time of introduction into the river was calculated to be 21.17 ± 3% millicuries.

The method whereby the isotope was released into the West Branch of the Sturgeon River has been described in

detail by Borgeson (ibid.) and Clifford (ibid.), but it is felt that a brief description at this time would be desirable. The 1.1 ml. solution of P^{32} was thoroughly mixed with 50 gallons of stream water in a 55-gallon drum. The diluted isotope was siphoned from the drum by way of a polyethylene tube. The rate of siphoning was controlled in such a way that the isotope entered the river at a constant rate over a 33-minute period, commencing at 9:33 AM and terminating at 10:06 AM. The flow of the stream at the point of isotope introduction was approximately 38 cubic feet per second. Using this stream flow and the flow rate of the diluted isotope given above, the theoretical concentration of isotope in the water was calculated to be approximately 1.22×10^{-5} microcuries per milliliter.

Measurement of Activity

Counting Equipment. Radioactivity was measured with a Nuclear measurements, internal flow proportional counter converter, PCC-10A, and coupled to a decade scaler (model PC-1A). Each day a 15-minute count was made using an empty counting chamber. This value was plotted on a background control chart. The background varied very little from day to day, generally from 50 to 54 cpm with a mean value of 52 cpm. Each sample was counted for a minimum of three minutes.

Calculation of Results. To convert the relative value of counts to an absolute value of corrected cpm,

μc/gm., or μc/ml., it is necessary to apply various correction factors to the raw counts (the correction factors are from Robeck, et al., 1954). The factors are:

Background: the natural or instrument background count is caused by cosmic radiation and radioactive substances in or near the counter. The determination of background was carried out each day by operating the counter without a sample for 15 minutes. The mean background count was 52 cpm for the entire experimental period. This background count was subtracted from all observed counts to correct for any radioactivity arising from sources other than the one directly under consideration.

Volume factor: due to the various organisms and materials samples, tremendous variation in sample size resulted. To correct for this difference the observed cpm minus background was divided by the weight of the sample in grams, or, as in the case of water, it was divided by the volume in milliliters.

Decay factor: due to the decrease, with time, of the number of radioactive atoms in a sample as a result of their spontaneous transformation, counts must be corrected for this decay. The influence of this decay is very important when using a short-lived isotope, such as radiophosphorus, with a half-life of 14.3 days when sample transfer and preparation time is large. Kinsman (1957) presents a radiological decay table giving the fraction of radioactivity remaining at a given time. The table for

radiophosphorus can be used conveniently for the recorded time unit and calculations were made for times which fell beyond the time span given in the table. The value obtained from the table was divided into the observed counts corrected for background and volume factors. The reciprocal of the decay value obtained from the table can be used and, instead of dividing, one multiplies the corrected observed counts by this reciprocal value.

Various other correction factors are given by Robeck, et al., (ibid.), but if all observed counts were corrected for background, volume and decay, the resulting values would be meaningful without multiplication by correction factors remaining constant throughout the study period.

In this study the correction factors used were:

Background (BG) = variable from day to day, generally 50 to 54 cpm with a mean value of 52 cpm.

Volume factor (VF) = variable depending upon the size of the sample.

Decay factor (DF) = variable with time between time isotope was introduced into the river and counting time.

Activity density = (cpm - BG) x (VF) x (DF) = corrected cpm.

In order to facilitate comparisons with other investigators and determination of maximum permissible concentrations set down by the National Committee on Radiation

Protection, sponsored by the National Bureau of Standards (1953), it becomes necessary to convert corrected cpm into microcuries.

It is known that (Robeck, et al., ibid.):

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

1 microcurie (uc) = 3.7×10^{4} dps

= 2.22×10^{6} dpm

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

Therefore Conversion Factor (CF) = 4.5×10^{-7}

If results are desired in terms of microcuries, then:

$$\text{microcuries} = (\text{cpm} - \text{BG}) \times (\text{VF}) \times (\text{DF}) \times (4.5 \times 10^{-7})$$

Flow Data

Fluctuation of the water level in the West Branch of the Sturgeon River for the entire study period is shown in Figure 2. The measurements were made from a river staff gage located ten yards above Station 8 (Figure 1). The greatest fluctuation was recorded on August 17 and was due to a severe storm. The river, except for one or two dates, maintained a stable water level.

Flow data obtained on July 7, 1959, indicated a uniform progressive increase in water velocity as one proceeds progressively downstream through the experimental area.[1] The flow recorded at various locations in the experimental

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[1]Courtesy of Vannote and Carr, 1959.

area in cubic feet per second is as follows: Station 1, 38.17; Station 5, 38.73; Station 8, 43.48; Station 11, 44.85; 100 yards below Station 12, 47.53; and bridge below Station 15, 49.72 (Figure 1).

Fertilization

In the preliminary investigation of the stream in 1958 (Clifford, ibid.), a low level of periphyton production was revealed. It was assumed that a similar situation was operative in June of 1959. It was thus deemed necessary to "prime" the experimental area with inorganic fertilizer in order to insure a rapid uptake of isotope and provide a sufficient amount of periphyton for radiological examination on the isotope entry date. The inorganic fertilizer used was a commercial 12-12-12 fertilizer. At the point of isotope release, 200 pounds of the fertilizer were applied continuously during the period June 29 to July 6, 1959. Station 8 (Figure 1) received 100 pounds of the fertilizer continuously during the period July 3 through July 6, 1959.

Water

Field Procedures

In order to obtain water samples that would be representative of the detectable activity present during the entire period of isotope passage through the study area, samples were taken at intervals varying from 5 to 30 minutes.

Figure 2. Daily staff gage readings at Station 8 on the West Branch of the Sturgeon River, during the period June 29 to August 19, 1959.

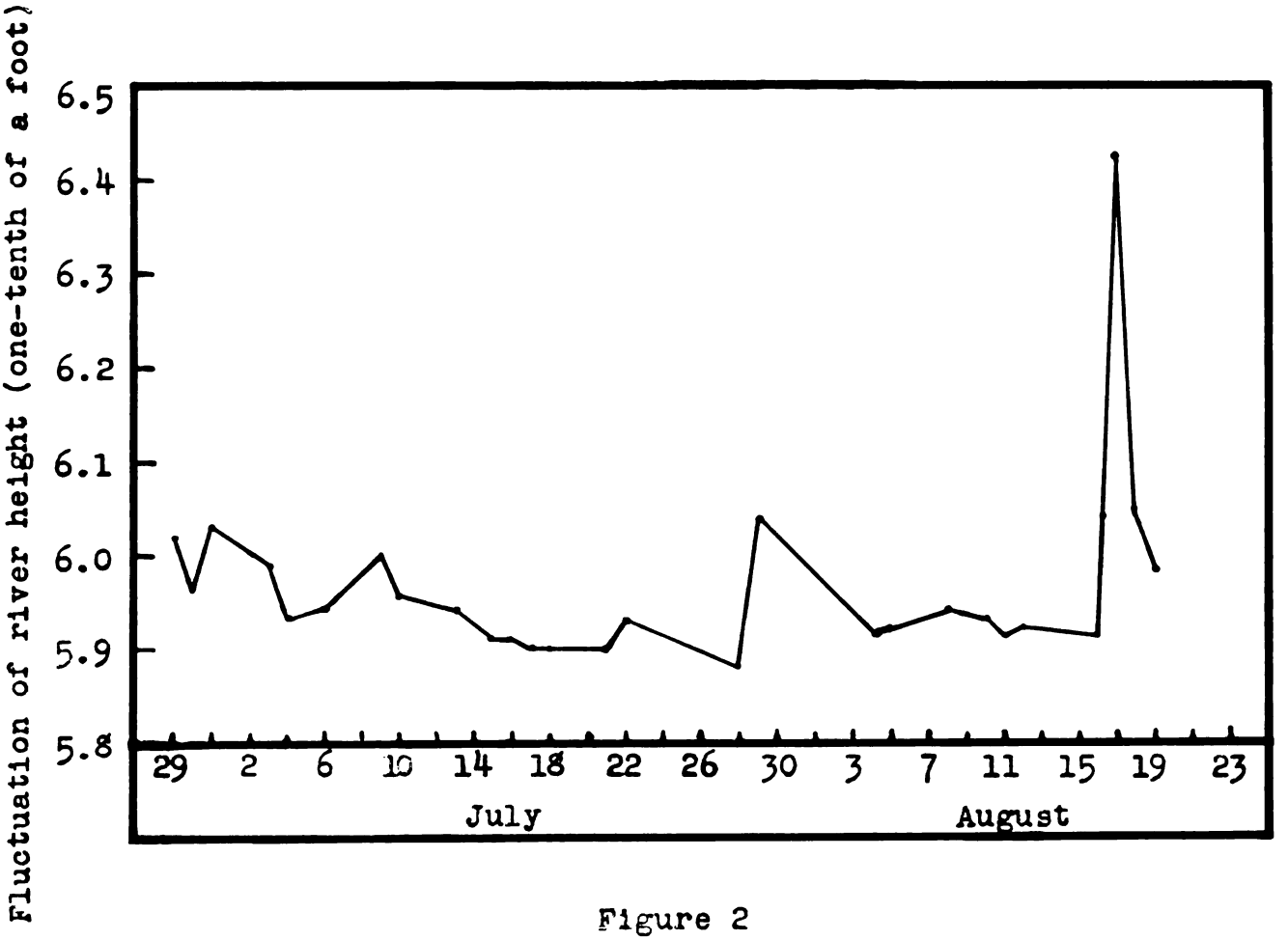
Fluctuation of river height (one-tenth of a foot)
6.5
6.4
6.3
6.2
6.1
6.0
5.9
5.8
29
2
6
10
14
18
22
26
30
3
7
11
15
19
23
July
August

Figure 2

The 5-minute interval samples were obtained--at some of the stations near the isotope release--when it was hypothesized that the isotope was flowing past that particular station. Stations maintaining a 10-minute sampling schedule were those stations located in the lower portion of the study area. At the expiration of the initial hour of sampling, the frequency of sampling was maintained at 15-minute intervals. At some of the more remote downstream stations, the sampling interval eventually lapsed into 30-minute periods.

The water sampling stations maintained in this study were Stations 3, 5, 8, 11, 12, 14 and 16. The seven sampling stations were located between the point of isotope entry and the State Highway Park adjacent to US-27 (Station 16, Section 14 of T. 33 N., R. 3 W.), a distance of approximately 3.3 miles (Figure 1).

Experiments were conducted (Borgeson, ibid.) in 1958 with fluorescein dye to determine flow time between stations and the feasibility of using such a dye as a visual indicator of the actual movement of isotope mass through the experimental area. This method proved satisfactory as such an indicator and the assumption was made that the isotope would perform physically in the same manner as the dye. The fluorescein dye was released into the water at the isotope entry point 10 minutes previous to the actual isotope entry and again at the termination of isotope release. The arrival of the dye at each station instigated

the water sampling. It was necessary to add additional dye at Stations 8 and 12, in order to supplement the fading color, due to dilution as it was transported through the study period.

The water samples were procured using 140 ml. polyethylene bottles, except for sixteen 500 ml. water samples taken while the isotope was moving downstream, which were rinsed for 30 seconds prior to obtaining each water sample. Samples were taken in the main current at each sampling station. The samples thus obtained were capped and transported to the laboratory for radiological analysis.

Laboratory Procedures

The preparation of water samples for radiological examination was carried out as suggested by Robeck, et al. (ibid.), except for minor modifications.

Three milliliters of concentrated nitric acid were introduced into the sample bottle containing 140 ml. of water for analysis, whereupon the bottle was thoroughly shaken. A 50 ml. subsample was placed in a 150 ml. beaker and allowed to evaporate to a small volume. The contents of the 150 ml. beaker were transferred to a stainless steel counting planchet. The 150 ml. beaker was then washed with 2N nitric acid and the washings thus obtained were placed in the planchet. This material was evaporated to dryness and placed in a muffle furnace for about one minute at 600°C. The planchet was removed from the furnace and

allowed to cool in an aluminum transfer pan, whereupon the samples were placed in the counting equipment for activity determination.

The 500 ml. water samples were filtered through a type HA, 47 mm. "millipore" filter. Solids collected on the filter were washed with one-tenth normal hydrochloric acid in some instances and washed with only water in other cases, then placed in planchets. These were dried in a drying oven, set at 90°-100°C. for 10 minutes. The planchets were cooled and placed in the counting equipment for activity determination.

Periphyton

Field Procedures

Plexiglass plates with a total exposed area of 1.4 decimeters (2" x 5") and a thickness of 7 millimeters were used to collect the periphyton growth. The plates were attached to a horizontal crossbar with 3/4" metal screws. The crossbar was attached to a steel post, by means of bolts and wing nuts, which was driven into the stream bed (Grzenda and Brehmer, 1960). The crossbar was lowered to approximately 8 inches below the stream surface when in the exposure position. A total of 35 plexiglass plates (2" x 5") were distributed on stands at each sampling station. In addition to the above plates, one large (4" x 10") plexiglass plate was also placed at each station. These large plates were attached to logs, roots and branches

below the water surface.

The sampling stations maintained in the periphyton study were Stations 3, 8, 12 and 14 (Figure 1). The schedule for the plexiglass plate removal was carried out as follows: ten minutes after the second dye marker passed each station (signifying the completion of isotope addition), 4 hours, 24 hours and 96 hours after the dye marker passed each station, samples were collected. Thereafter, samples were obtained each week on Wednesday for the remainder of the study period. The total number of plexiglass plates removed each sample date was 5 at each station. Four of the small (2" x 5") plates and one large (4" x 10") comprised a periphyton sample at each station. In addition to the above sampling procedure, a series of substrates that had been maintained at Stations 3 and 8 were removed from the stream temporarily during the period when the isotope pulse was passing through the experimental area and replaced immediately after the isotope dosage had passed the station.

The total period during which periphyton analysis was conducted was 35 days after the isotope was introduced into the stream. The plexiglass plates were transported to the laboratory as soon as possible for immediate radiological analysis. All animal forms, such as blackfly larvae and other invertebrates, were picked from the plexiglass plates immediately upon removal from the stream.

Laboratory Procedures

The plexiglass substrates, upon arrival at the laboratory, were scraped into a large beaker, using a polished glass slide, thus removing the periphyton community which had accrued thereon. The substrates were then rinsed to remove any periphyton that might be present after the scraping procedure. The resulting mixture of periphyton and water was filtered through a type HA, 47 mm. Millipore filter. While the filter pad (previously weighed) remained in place, .3 cc. of 0.01 N hydrochloric acid was introduced into the filter apparatus. The acid rinse was followed with a rinse of approximately 5 cc. of distilled water. The filter apparatus was allowed to remain in operation ten seconds after the filter pad was observed to be free of all visible moisture. The filter pad was then removed from the filter apparatus and placed in a previously weighed planchet. The wet weight of the periphyton was obtained by subtracting the weight of the planchet plus the filter pad from the total weight of the combined planchet, filter pad and periphyton. The material in the planchet was digested, using 5 ml. of concentrated nitric acid and then placing this under a heat lamp until completely digested. The digestate was placed in a muffle furnace set at 600°C. and remained therein until the planchet was heated to red heat. The samples were then cooled and removed to the counting room for activity determination. The digestion procedure given above was adapted from the method outlined by Robeck, et al., for the preparation of filamentous algae samples.

Aquatic Plants

Field Procedures

The collection of aquatic plants was done manually and routinely at Stations 3, 8, 12 and 14. The collection schedule was as follows: 4, 24 and 96 hours after the isotope arrived at each station; thereafter a weekly collection on Wednesday was maintained for a total of 7 weeks after the introduction of the radiophosphorus. The aquatic plants collected at each station were: Chara sp., Potamogeton pectinatus and Fontinalis antipyretica. The plant samples consisted of the entire plant, less roots, where such were present. The samples were rinsed in the stream water to remove adhering material or organisms and placed in 500 cc. polyethylene bottles for transport to the laboratory.

Laboratory Procedures

A 1-to-2 gram subsample of each plant type to be processed was washed with distilled water and dried with blotting paper. This blotted sample was placed in a previously weighed evaporating dish. The total weight was then determined, using a beam balance. The weight of the evaporating dish was subtracted from the total weight and the resulting weight recorded as the wet weight of the plant sample.

Concentrated nitric acid--enough to cover the plant sample--was added and the sample was placed on a hot plate

until completely digested. The digestate was placed in a muffle furnace, set at 600°C. The sample was removed from the muffle furnace and allowed to cool. After the sample had been allowed to cool, approximately 20 drops of 2N nitric acid were introduced into the evaporating dish containing the ashed sample. The introduction of the acid solution and the scraping facilitated the removal of residue adhering to the evaporating dish. This scraping and washing with acid solution was continued until complete removal of the residue was completed. The material obtained in the rinsing and scraping procedure was transferred into a stainless steel planchet.

The planchet and contents were placed on a hot plate and the sample evaporated to dryness. The planchet and dry sample were placed in a muffle furnace, set at 600°C.; the planchet was heated to red heat, removed from the furnace and allowed to cool. The planchet containing the sample was transferred to the counting room for activity determination.

Biomass Estimate

On August 15, 1959, a quantitative estimate of the aquatic vegetation biomass was conducted on 1,000 yards of the study area. The area was delimited by Station 1 and Station 8 (Figure 1). Numbers were taken from a Table of Random Numbers and numbers thus selected were designated as the cross-stream transects. The procedure for the aquatic

plants was exactly as for the aquatic insects, except for the collecting equipment, discussed elsewhere under aquatic insect biomass estimate, to which the reader is referred for a detailed discussion of the random sampling technique.

In order to isolate a square foot of aquatic vegetation, a metal frame measuring exactly one foot square was attached to a wooden handle. The sample was placed firmly against the stream bottom at the random collection site. Thereupon, a complete removal of all vegetation enclosed by the sampler was completed at each site. Each square foot sample of vegetation was blotted to remove excess moisture and weighed. No attempt was made to ascertain the individual weights of the several different plant types found in the samples. The results of the vegetation were recorded as pounds per square foot.

Aquatic Invertebrates

Field Procedures

The collection of aquatic insects for radiological analysis was made at Stations 3, 8, 12 and 14 (Figure 1). The sampling schedule for aquatic invertebrates was as follows: 4, 24 and 96 hours after the introduction of the radiophosphorus with weekly sampling every Wednesday thereafter, for a total of 7 weeks.

In a tracer study such as the present one, it is desirable to select those aquatic invertebrates for radiological purposes which are abundant, consistently and easily

collected. The animals were also selected as representatives of various trophic levels, in order to obtain data that were representative of both primary and secondary consumer invertebrates. With this in mind, the following invertebrates were obtained and processed throughout the study period: the stonefly nymph, Pteronarcys sp., blackfly larvae, Simulium sp.; mayfly nymph, Hexagenia sp.; snipe fly larvae, Atherix variegata Walker; caddisfly larvae, Brachycentrus sp.; fishfly larvae, Chauliodes sp.; and the pouch snail, Physa sp. Inasmuch as specific organisms were used in the radiological study, it was necessary that collection procedures obtain predetermined organisms consecutively at each collection site. In order to accomplish this, many specific methods were used. The following is a discussion of aquatic invertebrate collecting procedures used in the present study.

Hand Picking

Logs: Logs in the water are very rich sources of insect larvae. The crevices and cracks of logs usually harbor many insect larvae. The exterior surface of the log serves as a highway on which many organisms scurry back and forth in the acquisition of food. The pupal form of some insects can also be found in logs. Logs were either lifted from the water and examined, or were placed upon the shore for examination. As soon as the log was exposed to the atmosphere, many immatures crawled and ran from their

hiding places. The loose strips and rotten slivers were stripped from the log; thus, many insect larvae of the crawling type were located. If the log was in water that just covered it, one could, by using forceps, pick many larvae from it while it remained under water.

Moss and deposits: The water moss, Fontinalis antipyretica, growing on logs is an excellent habitat for many insect larvae. As one removes the moss from the log or stone, care should be exercised in obtaining those forms hiding under the tuft of moss. Once the moss was separated from its substrate and exposed to the atmosphere, insects crawled from it, making procurement easy. The moss tuft was then carefully dismantled, removing larvae in the process. Marly deposits on logs and rocks are also the hiding place for many species.

Stones: Stones were found to have many organisms adhering to, crawling on and clinging to them, as well as, many forms hiding under them. Some stones are of such a nature that hook and claw bearing larvae can cling to them in very fast water. Pupal cases and net and case bearing larvae also make use of rocks as a substrate for clinging and attachment. Some organisms use rocks as an attachment from which food is either filtered from the water, or obtained from the surface of the rock.

Aquatic vegetation: The aquatic vegetation offers many insects a substrate on which to cling in rapid water. For others, the vegetation is a protected place to

hide and prey on other insects and small organisms. Vegetation is also a place for other forms to feed on algae and diatoms. Some forms even feed on the aquatic plant itself. The aquatic plant of greatest abundance was the stonewort, Chara sp. Chara grows in submerged gardens or in large mats upon silt and debris. In order to obtain insect larvae from such a growth, it was necessary to isolate a portion of it from the bottom and from the rest of the Chara mat. The method found to produce the best results was to remove a large portion of Chara, roots, silt, debris, and all, and throw it out on the stream bank or on a log. As the water drained from the pile of Chara, insects began to crawl from it. In order to separate some of the forms from the silt and debris, the splashing of stream water over the Chara that had been placed on the shore was necessary. The above method was slightly modified later in using a shovel instead of hands to remove the Chara from the stream. The Chara produced great quantities of immature insect forms, along with some burrowing forms from the silt and debris, disengaged with the Chara.

The pondweed, Potamogeton pectinatus, grows rooted to the bottom, submerged in swift water. This pondweed afforded the collector with many clinging forms, especially those with cases.

Terrestrial vegetation: The stream edge, for the most part, was populated with overhanging trees and shrubs. The shrubs, tag alder and ninebark, occasionally dipped

their branches into the water. Clinging forms adhered to the branches and leaves of these shrubs dipping into the swift water.

Trash: In a swift stream such as the one in the present study, one finds trash in the form of leaves, algae, twigs and numerous other materials caught in bundles on projections or deflectors in the stream. These collections of trash offer an ideal habitat for many forms of aquatic insects. The forms range from the clinging forms to those that crawl and climb. This niche offered one of the richest habitats (with the exception of Chara) found in the stream as far as different types of organisms were concerned. The trash was pulled from its snag point and spread upon the shore or on a log. The organisms readily crawled from the trash. By picking over each piece of trash, many additional forms were isolated. The cover of the trash offers protection and a continually collecting food supply for carnivorous, herbivorous and omnivorous forms.

Surber Square-foot Sampler

The Surber sampler was used as a nonquantitative sampler. The mid-channel river bottom was, for the most part, of a gravel and rock character. The Surber sampler was used as a water net by holding it close to the bottom of the stream while gravel, stones and trash were disturbed as the collector moved upstream, scuffing the bottom with his boots. Numerous clinging and case forming insects were collected in this manner.

Direct Current Fish Shocker

The collection of certain immature insects with a fish shocker was found effective. The positive electrodes were thrust into Chara mats or silt and debris beds. This method was used primarily to obtain the burrowing mayfly, Hexagenia sp. Thrusting the positive electrode into a silt or debris bank produced the burrowing mayfly in great quantities, floating up from the substrate. The mayflies were easily scooped up in a mesh net.

The invertebrate organisms thus obtained were taken immediately to the laboratory for radiological processing.

Laboratory Procedures

The procedure for the preparation of aquatic invertebrates was modified from the method presented by Robeck, et al. (ibid). The organisms that were to comprise a sample were rinsed with 0.01 N hydrochloric acid and placed in wire centrifuge baskets. The basket, plus sample, was placed in a centrifuge and spun at 1,840 rpm for 15 seconds. At the termination of the 15-second period, the propelling power was shut off and the samples were allowed to run to a complete stop. It was felt that this procedure would produce the most consistent moisture removal from all types of organisms. The organisms thus prepared were weighed on an electric Mettler balance. Upon weighing the sample, it was transferred into a stainless steel planchet for the digestion procedure. Concentrated

nitric acid (0.1 to 1 ml.) was placed over the organisms and the planchet and sample were placed under a heat lamp until complete digestion had taken place. The digestate thus obtained was transferred to a muffle furnace set at 600°C. for 5 to 10 minutes. The planchet and residue were removed from the furnace and allowed to cool. The planchet was then removed to the counting room for activity determination.

Biomass Estimate

On August 31 and September 1, 1959, the author made a quantitative survey of stream bottom organisms in the West Branch of the Sturgeon River. The survey was made with a Surber square-foot sampler. Inasmuch as the survey area is predominantly riffle area, it was felt that the Surber sampler could be used in a random sample of the area.

The area of the stream used for this survey was delimited to 1,000 yards (Station 1 to Station 8). In order to eliminate bias or prejudice and to insure that the components of sample were completely independent from one another, random sampling techniques were brought into play. To facilitate the drawing of random samples, the Table of Random Numbers was used.

Since the survey area of the stream was 1,000 yards long, a maximum of four digits, groups of four numbers, were used. The random numbers were selected by starting at the beginning of the table and proceeding downward until

30 numbers had been selected. The 30 numbers thus selected were designated as the cross-stream transects, starting at the upper end of the survey area and proceeding downstream.

Each of these cross-stream transects was randomly subdivided. Each cross-stream transect was designated as 100 percent of stream width. Random numbers were selected in two digits. The number selected was converted into a percent. The percent selected at random was converted into a number in feet, corresponding to the percentage of the total width of each transect. This number (in feet), measured from the stream shore on the transect, was the sampling point. In selecting the sample point in this manner, the author believes the possibility of bias and prejudice has been held as low as possible, within the limitations of human error and equipment thus used. In using this method the sample points were selected on a random basis: i.e., any number of possible transects may have been selected for sampling and any number of points may have been selected on each transect.

As each cross-stream transect was located, the width of the stream at this point was measured and recorded. The random predetermined percentage of this width was computed and, when the resulting measurement was located on the cross-stream transect, it became the collection site. The frame of the Surber square-foot sampler was placed in position on the bottom, and the enclosed pebbles, gravel, rubble, sand and silt or other material were carefully gone

over with the fingers to dislodge the specimen. The material thus obtained was emptied into a collecting jar and labeled for sorting later in the day.

In the laboratory the samples were sorted, using a floatation method adapted from the one described by Anderson (1959). The organisms removed by the floatation process were transferred to preservation jars in which 70 percent alcohol was added and the sample was properly labeled.

In the laboratory, also, the organisms were sorted into groups and identified to order and--in some cases--to family. Each group was individually counted, then placed in a wire basket and spun for a consistent period of 30 seconds to remove surface moisture. Each group was then weighed on a Mettler multipurpose balance. The organisms were reported as number per square foot and the weights given in grams per square foot.

Fish and Lamprey

Field Procedures

The collection of fish and lampreys for radiological examination was made at Stations 3, 8, 12 and 14 (Figure 1). The fish and lampreys were collected, using a direct current fish shocker. The direct current power was furnished by a 230-volt, 2,500-watt, gasoline driven generator. The total weight of the generator is about 135 pounds. A sheet of copper, approximately 14 inches by 8 feet, mounted on

the bottom of the flat bottom boat used to float the shocker, served as the negative electrode. The two positive, hand held electrodes consisted of wooden handles, at the extreme end of which was affixed a piece of copper cable. The electrodes were thrust under the overhanging stream banks, under logs and other obstructions which furnish cover for fish. Lampreys were obtained in great numbers by thrusting the electrode into a Chara or silt bed.

The collection schedule for fish and lampreys was as follows: the first sample was obtained 48 hours after isotope treatment; thereafter, sampling was at weekly intervals with collections on each Wednesday. The fish and lampreys collected at each station were transported to the laboratory for radiological examination.

The brown trout, Salmo trutta fario Linnaeus, and the Eastern slimy sculpin, Cottus cognatus gracilis Heckel, were the fish collected and examined throughout the study period. The American brook lamprey, Entosphenus lamottenii lamottenii (LeSueur), was the lamprey collected and examined throughout the study period. The brown trout and the sculpin were the fish of choice, primarily because of their availability and because neither of these fish are stocked regularly in the West Branch of the Sturgeon River.

The weight of the brown trout collected varied from approximately 0.37 to 122 grams, while the sculpins collected ranged from 1.13 to 5.06 grams. The weight of the lamprey samples ranged from 0.69 to 4.42 grams. In addition

to the above collections, several brown trout were obtained for the purpose of determining the radioactivity levels in various organs and other parts of fish.

Laboratory Procedures

The lamprey samples were prepared and processed in the exact manner as were the fish, so the procedure is given as one method, as modified from Robeck, et al. (ibid).

The sample to be examined was washed with distilled water, blotted with blotting paper and weighed, using a beam balance. If the sample weight was in excess of 2 to 3 grams (exclusive of lamprey), the sample was placed in a Waring blender and ground up, thus facilitating the removal of an aliquot of approximately 2 to 3 grams.

The aliquot or entire organism, as the case may be, was placed in an evaporating dish, flooded with concentrated nitric acid and placed under an infra-red heater until dry. When the sample became dry, it was transferred to a muffle furnace, heated to 600°C. and allowed to ash for 5 to 10 minutes. This step was repeated until the sample was completely ashed (white ash) and then removed and allowed to cool. When sufficiently cool, about 20 drops of 2N nitric acid was introduced into the evaporating dish the the residue was scraped into a planchet, using a glass stirring rod. The addition of acid and the scraping procedure were continued until complete removal of all residue was realized.

The planchet and material contained therein were transferred to a hot plate and complete evaporation to dryness was completed. The planchet bearing the dry sample was placed in a muffle furnace heated to 600°C. and allowed to remain until the planchet was heated to red heat. The sample so treated was allowed to cool, whereupon it was transferred to the counting room for activity determination.

Biomass Estimate

Population estimates for fish in the West Branch of the Sturgeon River were made using an electric fish shocker. The shocker was operated by a field party throughout 1,000 yards (point of isotope release to Station 8) of stream section on August 25 and 28, 1959. On the first "run" through the stream section, fish were captured, marked by fin-clipping and released, giving a known number of marked fish (m) present. On the second "run" through the same stream section, the number of recaptures (r) of previously marked fish and the number of unmarked fish (u) were recorded. The numerical computation of the population was estimated, using the Petersen formula for population estimate, and is as follows:

$$P = m\,(u \neq r)/r$$

where: P = total fish population estimate,

m = number of fish captured and marked and released on the first run,

u = total number of unmarked fish captured,

r = number of marked fish recaptured on the second run.

In order to establish a weight-length relationship and obtain a mean weight value with which to calculate the biomass, a third "run" through the stream section was necessary on September 1, 1959. Each captured trout in the section was weighed and measured. Inasmuch as the sculpin population was extremely large, only the sculpins obtained from 100 yards of stream were used in the calculation of mean weight and length. The section of stream for which the biomass was estimated was 1,000 yards long and the average stream width in this section as determined on the basis of 30 random measurements was found to be 25.5 feet. The area of stream used in the fish biomass estimate was computed to be 1.76 acres. The fish biomass was calculated by multiplying the mean weight of the fish by the total estimated fish population and then computing the result in terms of pounds of fish per acre.

RESULTS AND DISCUSSION

When radioisotopes are released into an aquatic environment, they quite often become dispersed and diluted, but they may also undergo unexpected movements and concentrations. The accumulation of radioactive materials in an aquatic system follws a definite pattern. It is this pattern that will be discussed in the balance of this report.

It should be emphasized at this time that a tracer study such as the one under consideration is designed in such a way that the amount of radiophosphorus introduced is extremely small in comparison to the amount of non-radioactive phosphorus already present in the system. Thus there is no increase in the phosphorus present in the system, one is simply following the behavior of a few marked atoms. Therefore, neither the radioactivity nor the extra ions of phosphorus disturb the system; what happens to the tracer simply reflects what is normally happening to naturally occuring phosphorus as a continuous exchange is going on between the water, plants, animals and bottom complex of the stream.

The mode of uptake by which radiomaterials may become associated with fresh-water organisms occurs in one of three ways: through adsorption to surface area; through absorption from the surrounding medium; or through ingestion as food (Krumholz and Foster, 1957).

The fate of radiophosphorus released into the stream system and the manner by which the ecological communities and populations control the distribution of radioactivity will be discussed in detail in the following sections.

Water

The addition of a single dose of radiophosphorus into the stream ecosystem resulted in immediate uptake of the isotope (Figure 4). Inasmuch as the isotope introduced into the stream was in the inorganic form, adsorption and absorption of P^{32} by plants occurs almost instantaneously. All of the nutrient materials and thus the biologically important phosphorus, as well as, radiophosphorus, that are metabolized by plants are absorbed directly from the environment. Adsorption of radiophosphorus directly from water, however, cannot be neglected. The absorption and adsorption are the primary mechanisms by which inorganic materials are acquired by aquatic plants, which are the food sources of the animals.

It is hypothesized that immediately upon addition, the radiophosphorus was adsorbed or absorbed by particulate solids made up of--possibly--nanoplankton, bacteria and diatoms. It is seen that upon addition of P^{32} to water the immediate reaction within minutes is a transfer of the isotope through the bodies of unicellular floating forms of life. In view of this reaction, these forms must occupy a very important position in the distribution of phosphorus

(P^{32}) to other pools of the system. Bacteria and algae, according to Rigler (1956), are the two groups of planktonic organisms known to take up phosphate (P^{32}) from solution in sufficient amounts. The bacteria (Krumholz and Foster, ibid.) may have the greatest powers for concentrating radiomaterials of any fresh-water organism; their concentration factor for certain isotopes may exceed 1,000,000. Hayes and Phillips (1958) indicate that bacteria are continuously putting inorganic phosphate rapidly through their bodies, changing most of it enroute to the organic form. There is at the same time a regeneration of the inorganic phosphate by breakdown of the organic fraction. Hayes and Phillips (ibid.) further indicate that in their study, water bacteria rapidly incorporated 50% of the P^{32}, which was introduced as inorganic PO4, as part of their body protoplasm. As a result of the analysis of the filtered solids rinsed with dilute acid in the present study, it was found that only a small fraction of the activity was removed (Figure 3), lending supporting evidence that most of the filtered solids incorporated the tracer as part of their body protoplasm.

The results obtained at Station 3 indicated that approximately two-thirds of the water activity was in the form of solids, and at Station 8--sampled after the peak activity had passed--the data indicated that the solids made up 50% of the total activity. The data obtained at Station 12 appeared impossible, since the activity of the

solids was greater than the total water activity. This difference was probably due to sampling error. The water sample used to determine the total water activity was not from the sample used for solids determination. It is hypothesized that although the samples were taken from the same station at the same time, a different collection site was selected for the actual sampling procedure and a slight time difference in collection of these two samples was involved.

In conjunction with the radiophosphorus adsorption and absorption by the particulate solids, great quantities of radiophosphorus were also taken up by the periphyton and aquatic plants which possessed a mechanism of initial phosphorus uptake that might be due to physical processes unconnected with active cell metabolism (Coffin, et al., 1949). This uptake by periphyton and aquatic plants is discussed more fully elsewhere in this paper.

Water activity curves (Figure 4) show the water activity for the various stations throughout the period when the isotope was moving through the experimental area. The maximum activity value reached was approximately 12.4 cpm/ml. and was recorded at Station 5. Station 3 reached nearly the same value and may have surpassed this value between sample periods. A similar trend was common to the water curves at all stations. As the isotope arrived at the sampling station, there was an increase in activity until peak activity was recorded, in all cases, somewhat past the

Figure 3. Total water activity, and the activity of water-washed and acid-washed solids filtered from stream water by millipore filter. Counts were corrected for background and decay.

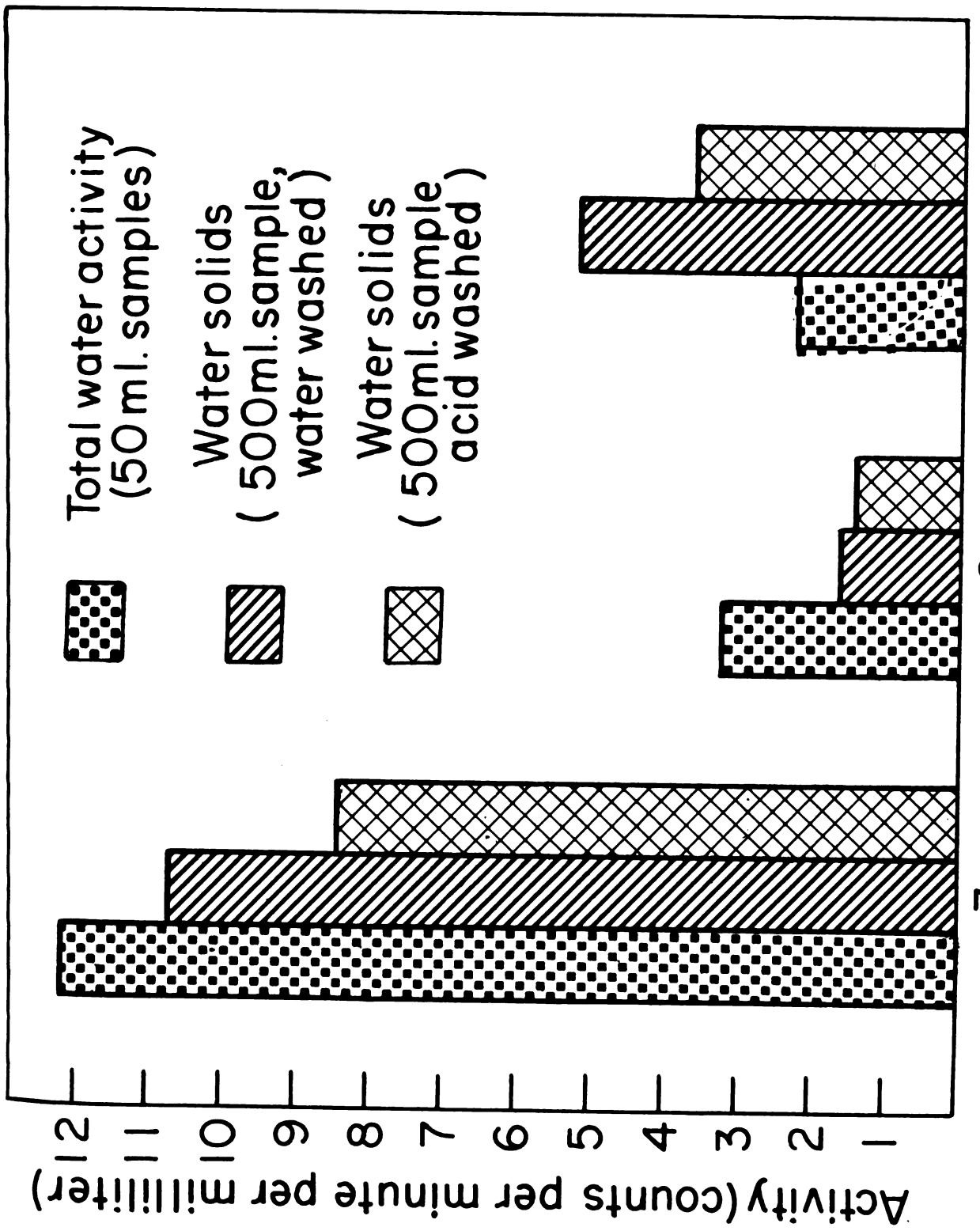
Activity (counts per minute per milliliter)
1
2
3
4
5
6
7
8
9
10
11
12
3
8
12
Stations
Total water activity (50 ml. samples)
Water solids (500ml. sample, water washed)
Water solids (500ml. sample acid washed)

Figure 3

midpoint of the time span. This was followed by a reduction in activity to almost background level at the expiration of 70 minutes after the isotope arrived at each station. Station 3 reached a peak water activity of 12.3 cpm/ml. approximately 40 minutes after the isotope arrived at this station. At Station 5, as previously mentioned, the maximum water activity value of 12.4 cpm/ml. was recorded approximately 35 minutes after the arrival of the isotope. Station 8 showed a somewhat reduced water activity when compared with the previous stations. The peak water activity of 7.1 cpm/ml. was recorded approximately 40 minutes after the radiophosphorus was detected. Station 11 initiates a series of four stations in the lower portion of the study area. Station 11 reached a peak activity of nearly 3 cpm/ml. 30 minutes after the arrival of P^{32} at this station. Station 12 reached a peak of 2.7 cpm/ml. 30 minutes after arrival of the isotope. A second peak of nearly equal magnitude as the first was recorded nearly 20 minutes after the first. Station 14 reached a peak of 1.6 cpm/ml. approximately 40 minutes after the arrival of the isotope.

The theory advanced in an attempt to explain the nearly symmetrical curve of activity resulting during the passage of isotope past each station proposes a purely physical process. The initial entry of radiophosphorus into the stream resulted in low concentration, due to tremendous dilution as it proceeded downstream. Water receiving a dosage of radiophosphorus at progressively later times

Figure 4. Total water activity at various collecting stations during passage of isotope. All counts were corrected for background and decay.

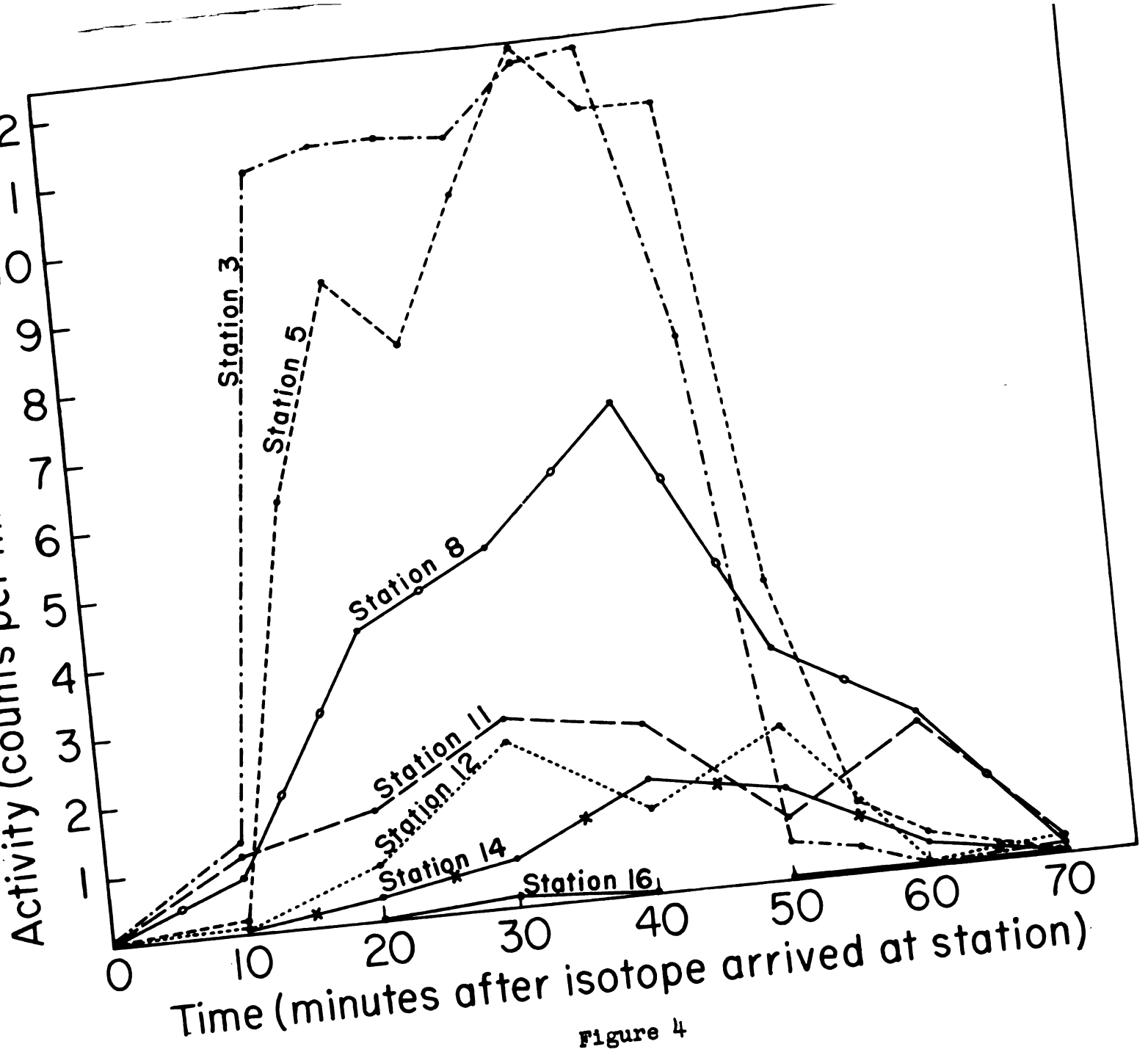
Activity (counts per minute per milliliter)
1
2
3
4
5
6
7
8
9
10
11
12
Station 3
Station 5
Station 8
Station 11
Station 12
Station 14
Station 16
0
10
20
30
40
50
60
70
Time (minutes after isotope arrived at station)

Figure 4

was also diluted by the stream water but was subjected to additional activity entering the main course of the stream by way of backwater, eddies and areas near stream bottom and surface. This additional activity built up higher and higher concentrations of activity passing a given point until the conclusion of the isotope entry, at which time a steady decline in activity was noted. The decrease in activity was due to the fact that activity was no longer being introduced into the stream. The tail of activity appeared as the isotope continued to re-enter the stream from backwater and embayment.

In order to demonstrate horizontal variation in water activity, a series of water samples were obtained on a transect across the stream. These samples were obtained at Station 8, during the passage of the isotope, just after the peak activity had passed this station. The data obtained indicated that the greatest activity occurred along the stream margin and that lower activity levels were recorded in the main current near midstream (Table 1).

Table 1. Water activity (corrected counts per minute per milliliter) at different points in the stream channel during passage of isotope dose.

Left Bank	Left Center	Right Center	Right Bank
3.0	2.1	2.3	3.3

Inasmuch as the peak activity had passed this station prior to sampling, the higher activity along the stream margin would be reentering the main channel of the stream, forming the tail of the activity curve.

Current velocity is not uniform in all parts of the transverse section of a stream (Welch 1952), but is reduced at and near the surface because of surface tension, and diminished as the bottom and sides of the channel are approached, owing to frictional effects. Welch (ibid.) further states --- "the distribution of velocities in natural streams is determined by several different factors operating simultaneously, such as, shape of channel, roughness of channel, size of channel and slope of channel". It can readily be seen from the above that it would be possible for many situations to develope which might sidetrack and detain portions of water containing a considerable amount of isotope, allowing it to reenter the main channel of the stream sometime later.

Figure 5 shows the mean activity value for each station during the period of isotope passage. From this curve, with some error expected, when using the mean value, it is possible to obtain the mean uptake of activity at each station during the passage of isotope. This activity curve indicates a nearly logarithmic decrease between Stations 5 and 11; thereafter, the downstream stations decreased at a reduced rate. The uptake pattern was rather uniform as the isotope proceeded downstream. The removal of isotope from

Figure 5. Mean water activity at various collecting stations during the passage of isotope. All counts were corrected for background and decay.

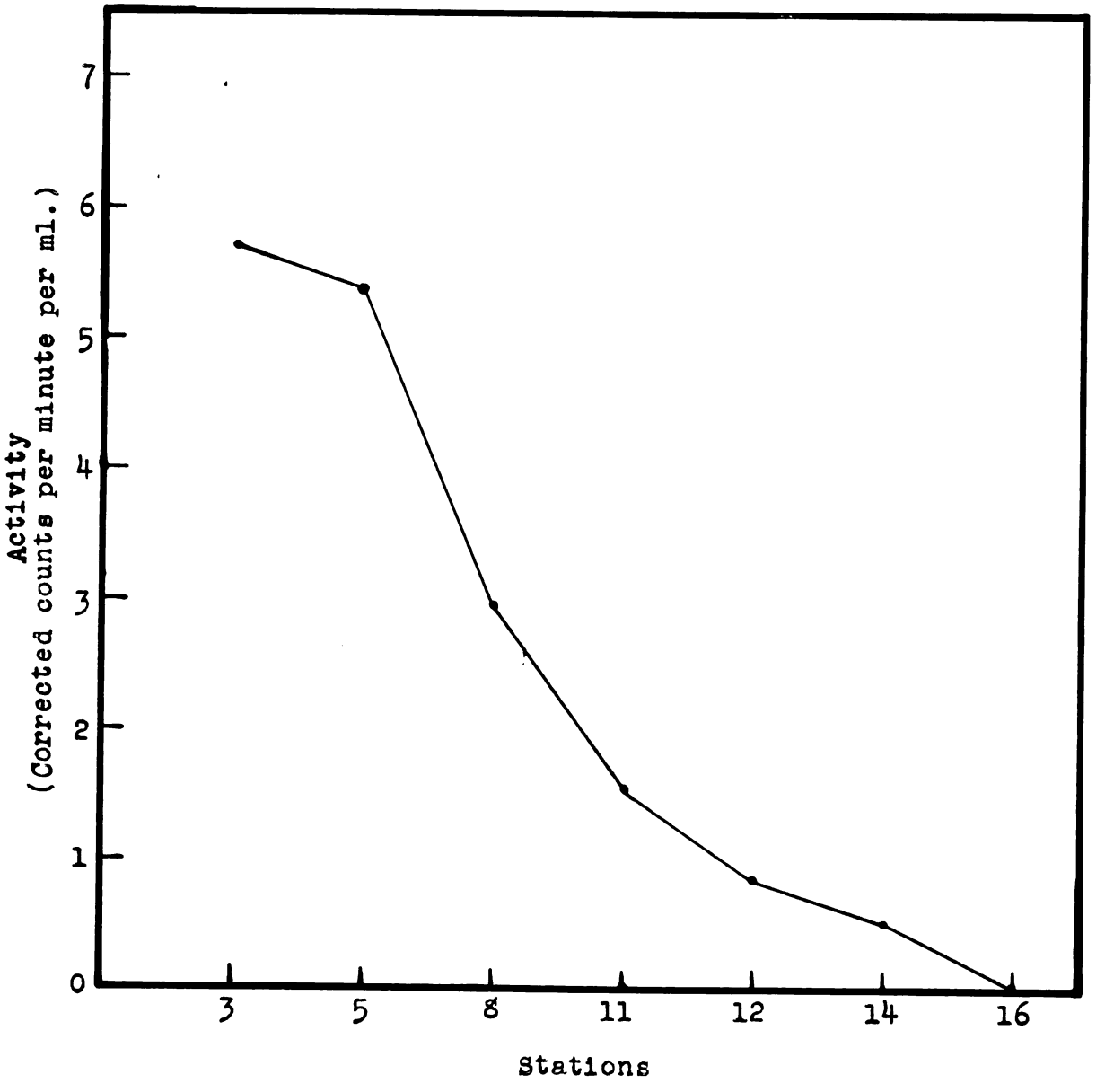
Activity
(Corrected counts per minute per ml.)
0
1
2
3
4
5
6
7
3
5
8
11
12
14
16
Stations

Figure 5

the water was nearly complete in passing through the experimental area. This depletion of activity from the water is not an actual loss of the isotope, but rather the tracer has become distributed into the various phosphorus pools of the ecosystem. The movement of the isotope from one compartment (water) into other compartments (vegetation, bottom silt, fish, etc.) illustrates the dynamic state of phosphorus in the ecosystem (Foster 1959). The only true loss of isotope will be through the tracer being swept out of the study area, through the harvest of fish or other crops which have concentrated the isotope, through the emergence of aquatic insects and through radiological decay.

Periphyton

Initial Uptake of Radiophosphorus

The periphyton biocenosis accruing on the artificial substrates in the West Branch of the Sturgeon River is made up almost entirely of diatoms (Clifford, ibid.). According to Clifford, Synedra ulna accounts for the greatest portion of the periphyton complex. In lesser numbers, Cymbella spp., Navicula spp., Cocconeis spp. and Gomphonema spp. were the other principal diatoms comprising the periphyton complex.

Figure 6 indicates that the uptake of radiophosphorus, ten minutes after the isotope passage, was greatest at Station 3 and progressively less at the downstream stations. This is undoubtedly due to the fact that Station 3 was

Figure 6. Activity of periphyton taken from artificial substrates at Stations 3, 8, 12 and 14. Counts were corrected for background and decay.

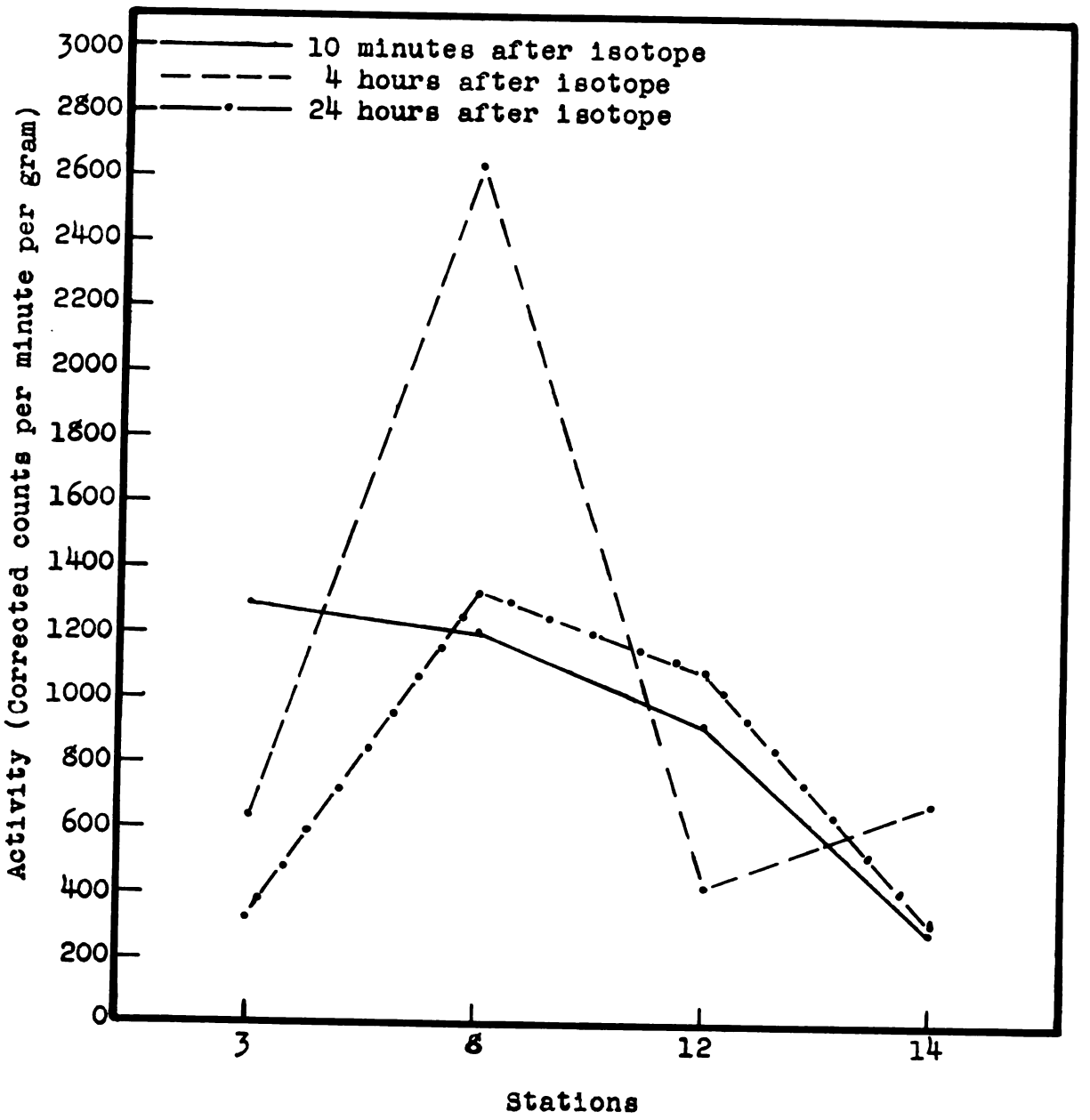
10 minutes after isotope
4 hours after isotope
24 hours after isotope
Activity (Corrected counts per minute per gram)
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
3
6
12
14
Stations

Figure 6

exposed to a greater water activity and, as the isotope proceeded downstream, the activity remaining in the water became progressively less due to uptake by the stream vegetation and dilution, The uptake of radiophosphorus by the periphyton showed a somewhat different trend when sampled four hours after the passage of the isotope dose. The maximum uptake at this time appeared at Station 8 and uptake was greatly reduced at Station 3, as well as, the stations downstream from Station 8. The uptake curve obtained 24 hours after the passage of the isotope dosage indicates that the maximum activity was recorded at Station 8 with decreased activity values recorded for stations upstream and downstream from this point.

The initial radiophosphorus uptake in periphyton was extremely rapid, reaching a peak at Station 8 some 4 hours after the initial dose. The isotope during this initial period apparently was entering what plant physiologists call the "outer space" of the plant, that is adsorbing onto the surface of the cell and is not incorporated into protoplasm (Odum, et al., 1958). Apparently this initial uptake might be due to physical processes unconnected with active cell metabolism. Some of the P^{32} taken up at Station 3 was apparently returned to the water and taken up by the vegetation progressively downstream. Inasmuch as Station 8 was the first station sampled below Station 3, it showed the greatest activity level of all other stations. Station 8 apparently utilized the radiophosphorus released from the

upstream stations, accumulating the isotope until a peak 4 hours after the isotope arrived at this station. Station 3 is located in a well shaded area, while Station 8 is fully exposed to direct sunlight. This difference in light intensity reaching the plants may have a pronounced effect on the periphyton community metabolism, and thus may be a factor in the greater P^{32} uptake at Station 8, when compared with Station 3. This phenomenon is shown in Figure 7. As was observed in Figure 7, Station 3 reached its peak activity 10 minutes after the isotope dosage, while Station 8 reached its peak 4 hours after isotope passage. Station 12 (Figure 7) did not reach peak activity until 24 hours after the isotope arrived at this station. The activity curve at Station 14 indicates a double activity peak: the first, 4 hours, and the second, 96 hours, after the isotope passage. This delayed peak activity developing progressively downstream indicates that release of the isotope at an upstream station was followed by a downstream uptake. This proceeded progressively downstream until it ultimately passed beyond the final periphyton collecting station. The magnitude of peak activity recorded at each station decreased progressively downstream in all cases except Station 3. The explanation for this might lie in the fact that the isotope entry point is only 300 yards upstream from Station 3 and the initial isotope uptake was greatest at this station, in comparison to the other stations, 10 minutes after the passage of the isotope dose because of exposure

Figure 7. Activity of periphyton taken from artificial substrates at Stations 3, 8, 12 and 14, for the entire study period. Counts were corrected for background and decay.

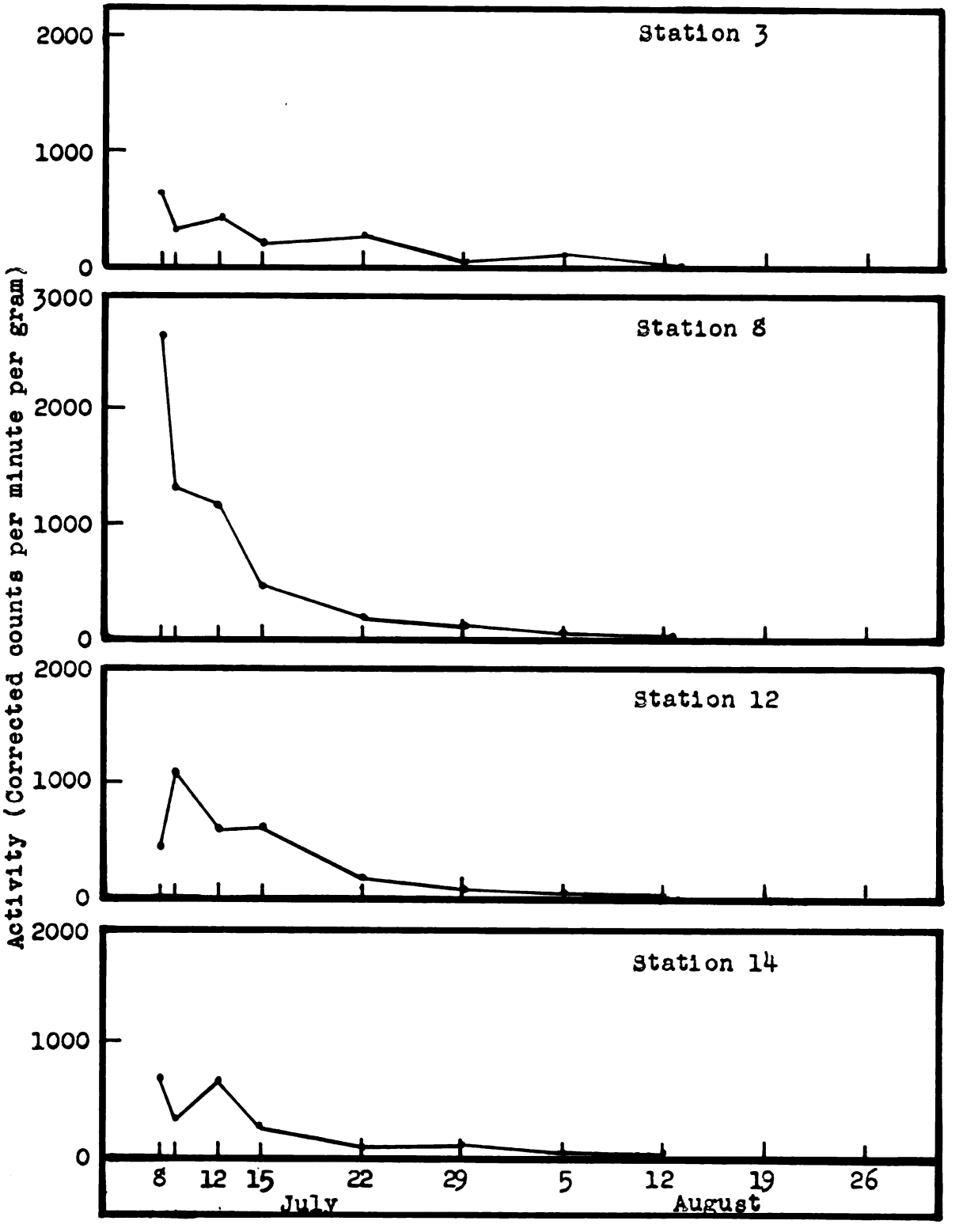
Station 3
Station 8
Station 12
Station 14
2000
1000
0
3000
Activity (Corrected counts per minute per gram)
8
12
15
22
29
5
12
19
26
July
August

Figure 7

to the greatest concentration of activity. The reason for Station 8 exhibiting a peak of greater activity than Station 3 was probably due to the fact that Station 8 received additional activity released from upstream vegetation, while Station 3 apparently received little activity from the area preceding it. This may also be due to incomplete mixing of the isotope. Complete mixing of the isotope probably was not completed until very close to Station 3.

The transport of the isotope prior to complete mixing would--for the most part--follow the main midchannel of the stream where very little vegetation grows and thus little opportunity was afforded the area above Station 3 for the uptake of the radiophosphorus. Conversely, little P^{32} would be available for exchange.

Exchange and Regeneration of Radiophosphorus

Figure 7 shows that periphyton activity at Station 3 decreased only slightly during the first week, and at a greatly reduced rate thereafter. The periphyton activity trend at Station 8 shows a sharp decrease at a logarithmic rate during the first week; thereafter, at a reduced rate. Station 12 periphyton activity shows a rapid decline in activity, after the initial peak, until the end of the second week; thereafter, at a reduced rate. Station 14 shows a trend similar to Station 12, inasmuch as the activity decrease was rather noticeable until the end of

the second week when a greatly reduced rate of activity decrease was observed. This much reduced rate of periphyton activity after the initial period of rapid uptake and release of radiophosphorus occurred at each of the sampling stations and indicates that periphyton apparently reached a plateau of activity. This plateau phenomenon, according to Hayes, et al. (1952), can be explained as a function of radiophosphorus exchange between water and plants. They further state that when a number of marked atoms are placed in the water, they will tend to leave the water exponentially and enter the plants. At the same time there is a return of material from the plants, which also proceeds exponentially. During the initial uptake period after the addition of radiophosphorus, little isotope can return from the plants because little has yet entered them. As soon as a significant fraction of the isotope has entered the solids, there will be a "feed-back" into the water (Foster, ibid.). This equilibration or plateau phenomenon may be the result of isotope uptake when radiophosphorus reentered the water as a result of phosphorus exchange, coupled with the continual release of radiophosphorus, due to regeneration of the isotope as a result of decomposing organisms.

Supporting evidence of this equilibration level of regenerated or exchanged radiophosphorus comes from measurements made of the activity of substrates removed from the stream during the isotope passage, but replaced immediately after the isotope dosage had passed the station (Figure 8).

Substrates thus exposed tend to show isotope uptake, but in a greatly reduced rate in comparison to those substrates remaining in the stream during isotope treatment. The radiophosphorus picked up by the substrates placed in the water after treatment was the result of periphyton uptake of regenerated or exchanged isotope reentering the water with subsequent uptake by the periphyton introduced after isotope treatment. This plateau or equilibration level exhibited by the periphyton may be the so-called minimum phosphate content of the periphyton components and may be designated as "bound phosphate" (Goldberg, et al., 1951), or the phosphate which is not readily exchangeable with that of the surroundings. Such a condition as this must exist whenever the phosphate (radiophosphorus) is incorporated irreversibly into the cell protoplasm. The immense initial isotope uptake shown by the stations remaining in the water during treatment (Figure 8) probably represents--in part--the labile or readily exchangeable phosphate in the organisms and only the activity of the substrates remaining out of the water during treatment are representative of the "bound phosphate", or that which is stored or taken up and incorporated into protoplasm. Once the periphyton cells have equilibrated (lost the labile P^{32}), further decline in activity can be attributed to three causes. First, a loss of P^{32} would occur as a result of isotope "feed-back" between the periphyton and the water. Secondly, as might be expected, the initial mass of cells

Figure 8. Comparison of activity of periphyton substrates at Stations 3 and 8. Counts were corrected for background and decay.

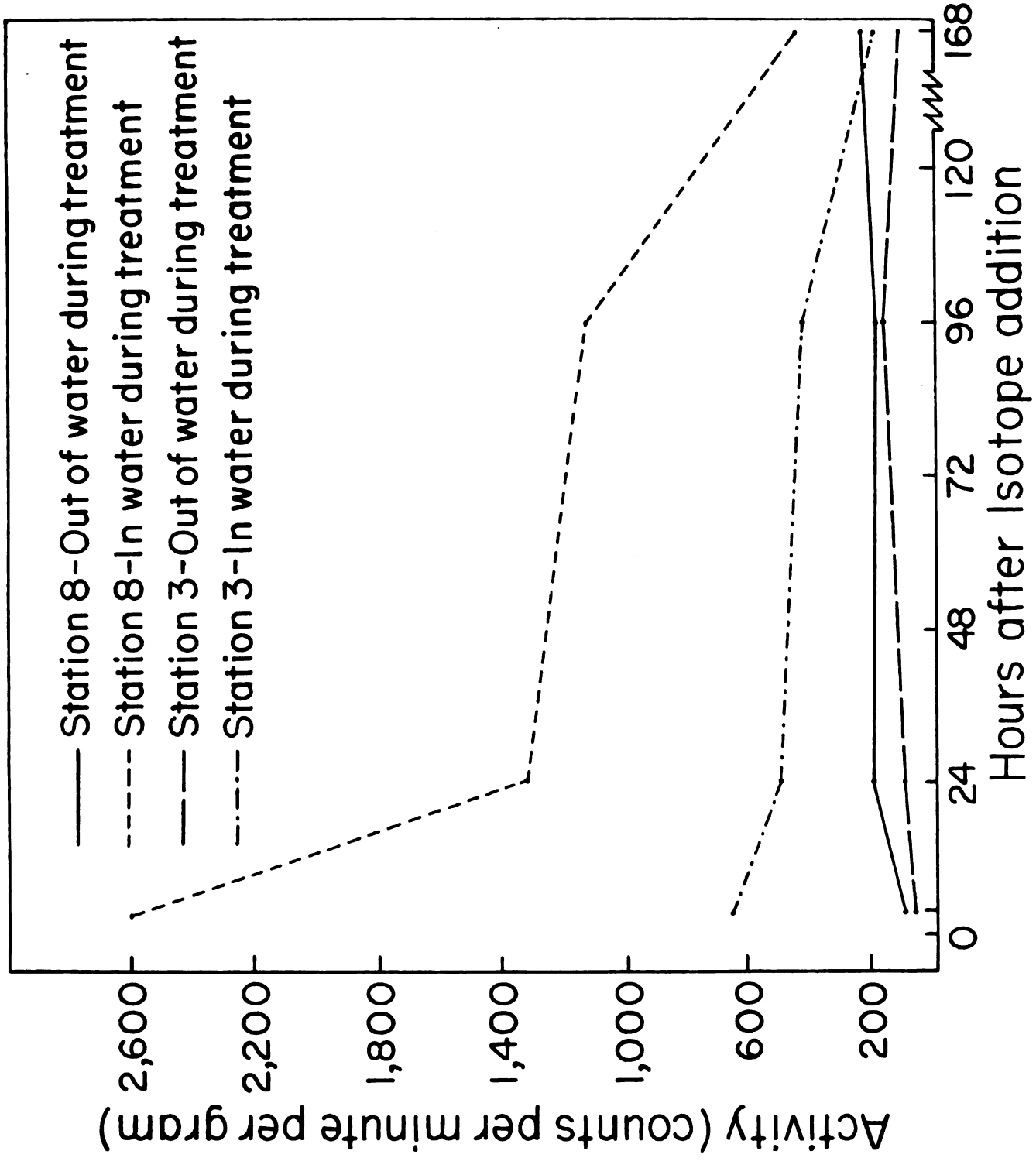
Station 8-Out of water during treatment
Station 8-In water during treatment
Station 3-Out of water during treatment
Station 3-In water during treatment
Activity (counts per minute per gram)
2,600
2,200
1,800
1,400
1,000
600
200
0
24
48
72
96
120
168
Hours after Isotope addition

Figure 8

containing P^{32} would be increased by biological dilution as a result of the addition of new cells, growth of cells and loss of old cells. This would have the effect of diluting the sample taken for analysis. Thirdly, the concentration of isotope would diminish as a result of radioactive decay.

Larger Aquatic Plants

Isotope Uptake and Accumulation

The variations in activity for the larger aquatic plants, by sampling stations, are shown in Figures 10, 11 and 12. The larger plants readily concentrate radiophosphorus, but, in most cases, to a lesser degree than periphyton. Maximum values found were approximately 1,700 counts per minute per gram, as against approximately 2,600 counts per minute per gram for periphyton. The curves showing the decrease in activity with time for Chara, Potamogeton and Fontinalis, are strikingly similar to the activity curves for periphyton. All of these aquatic plants show a rapid initial uptake of isotope (Figure 9). A very interesting situation developed when the ability of a gram of the different plant species to "absorb" the tracer was compared. The initial uptake of isotope for Station 3 in Potamogeton was twice that recorded for Fontinalis, and over four times greater than that recorded for Chara Figure 9). The downstream stations show a similar uptake pattern, except for Stations 12 and 14. The uptake recorded for these stations show activity values that do not

vary to any degree from each other. The uptake values at the downstream stations may reflect physical factors, such as exposure of certain areas of vegetation to greater or lesser amounts of water activity levels. Plants growing in the main water course (Chara, for example) might have been exposed to a greater water activity in some areas than other plant species, and, therefore, exhibited an uptake rate similar to the other plants even though isotope uptake was slower than for other plants. The upstream stations (3 and 8) may have shown the plant isotope uptake when there was a greater concentration of tracer in the stream water. This difference in the ability of different species to "absorb" the tracer may be due to surface-per-volume ratios discussed by Odum, et al. (ibid.). They suggest that tracer amounts of P^{32} and, therefore, phosphorus, in general, are absorbed at a rate determined by the inherent structural surface area features of the plants. High surface-per-volume ratio increases the uptake rate. On the basis of this surface-per-volume ratio, the values recorded for the present study indicate that the plants would rank as follow: periphyton, Potamogeton, Fontinalis and Chara, in descending order. In addition to the surface-per-volume ratio, the differential plant species uptake may be limited by the fact that each organism in each environment has specific requirements for the different chemical elements. In order to understand and interpret the role played by phosphorus in the metabolic processes,

Figure 9. Activity of stream vegetation on July 8, 1959. Counts were corrected for background and decay.

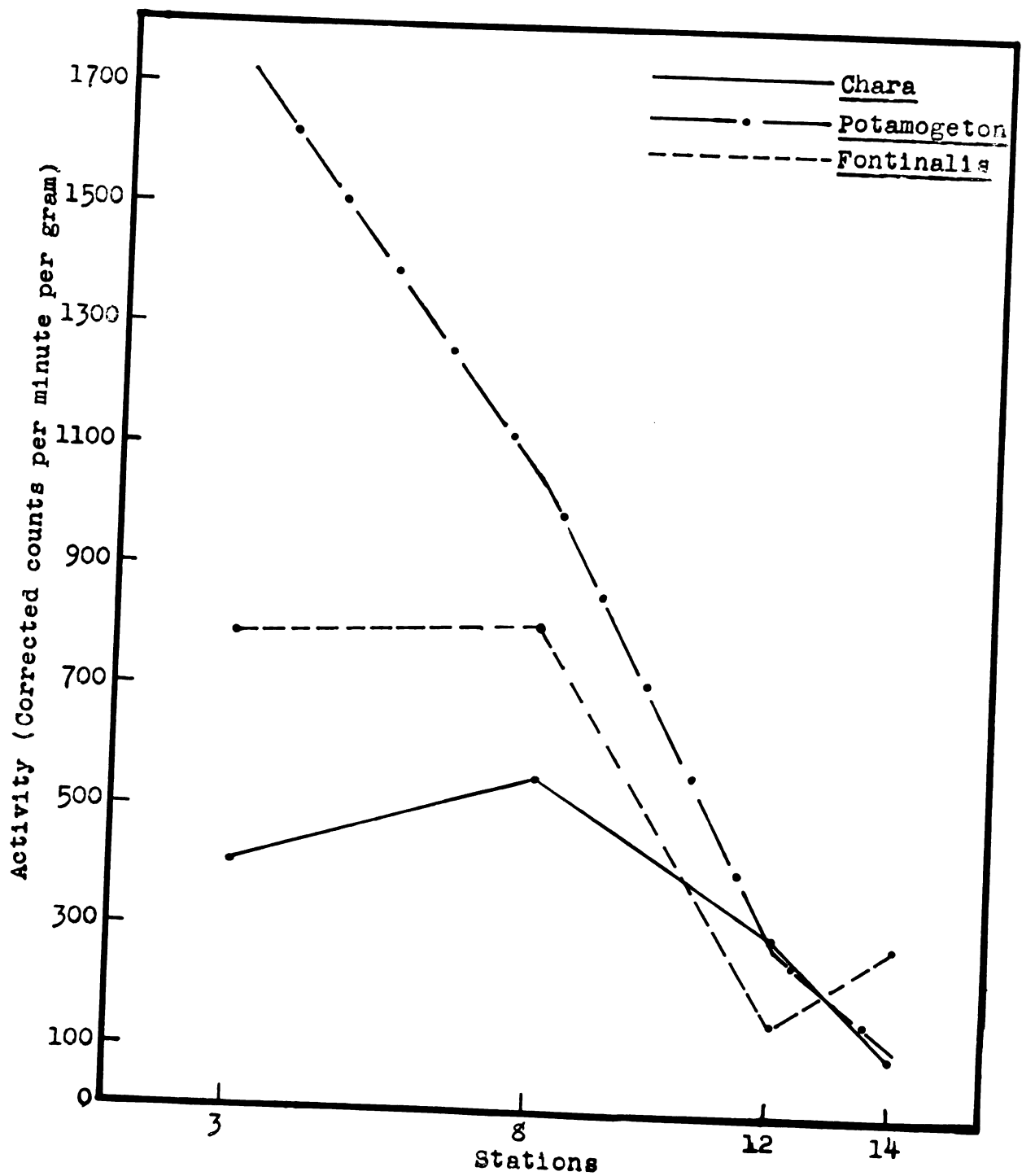
Chara
Potamogeton
Fontinalis
Activity (Corrected counts per minute per gram)
1700
1500
1300
1100
900
700
500
300
100
0
3
8
12
14
Stations

Figure 9

the specific phosphorus requirements and chemical composition of each plant species must be obtained. This information is not available for the organisms utilized in the present study.

The activity curves for Potamogeton are shown in Figure 10. The maximum value found for Potamogeton was approximately 1,700 counts per minute per gram at Station 3, on July 8. The peak activity for the stations below Station 3 decreased progressively downstream, apparently because there was less P^{32} in the water to be picked up by the plants, to a peak value of slightly over 100 counts per minute per gram at Station 14. Stations 3 and 8 showed an initial logarithmic decrease in activity until July 12, followed thereafter by an equilibration phase in which there was little or no rise in activity until a slight increase on July 5. Stations 12 and 14 showed a brief logarithmic decrease in activity until July 12, followed thereafter by a sharp rise in activity on July 15 at both stations. This increase in activity was apparently due to "back diffusion", or exchange of P^{32} between the organisms (periphyton and aquatic plants) and the water with immediate uptake at the downstream stations. The increased activity at Stations 12 and 14 took place at precisely the base of the logarithmic phase and the onset of the equilibration phase of Potamogeton at the upstream stations. The slightly increased activity levels at Stations 8, 12 and 14 on August 5 and at weekly intervals progressively downstream also

Figure 10. Activity of Potamogeton at Stations 3, 8, 12 and 14, for the entire study period. Counts were corrected for background and decay.

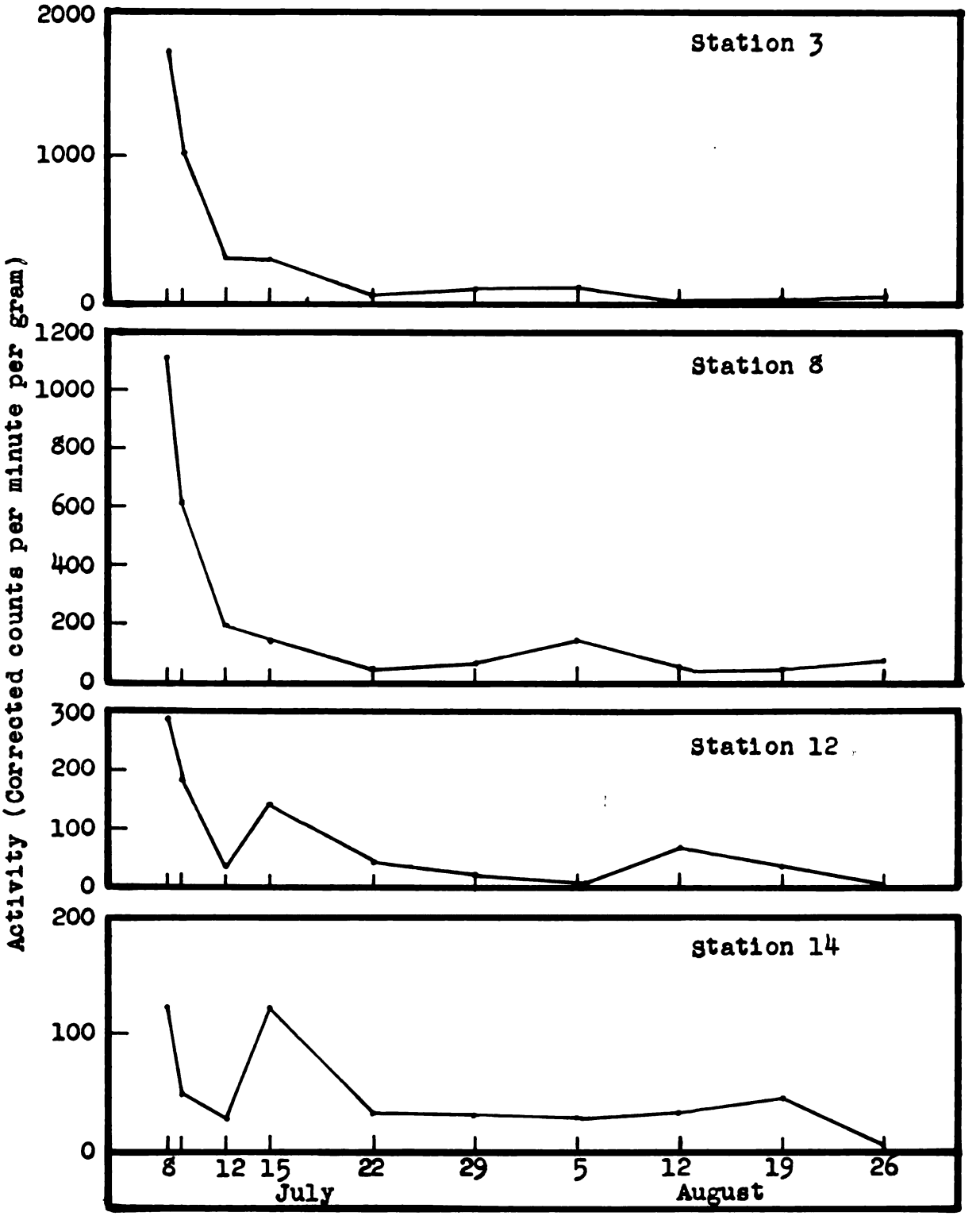
Station 3
Station 8
Station 12
Station 14
Activity (Corrected counts per minute per gram)
2000
1000
0
1200
800
600
400
200
300
100
8
12
15
22
29
5
12
19
26
July
August

Figure 10

Figure 11. Activity of Fontinalis sp. at Stations 3, 8, 12 and 14, for the entire study period. Counts were corrected for background and decay.

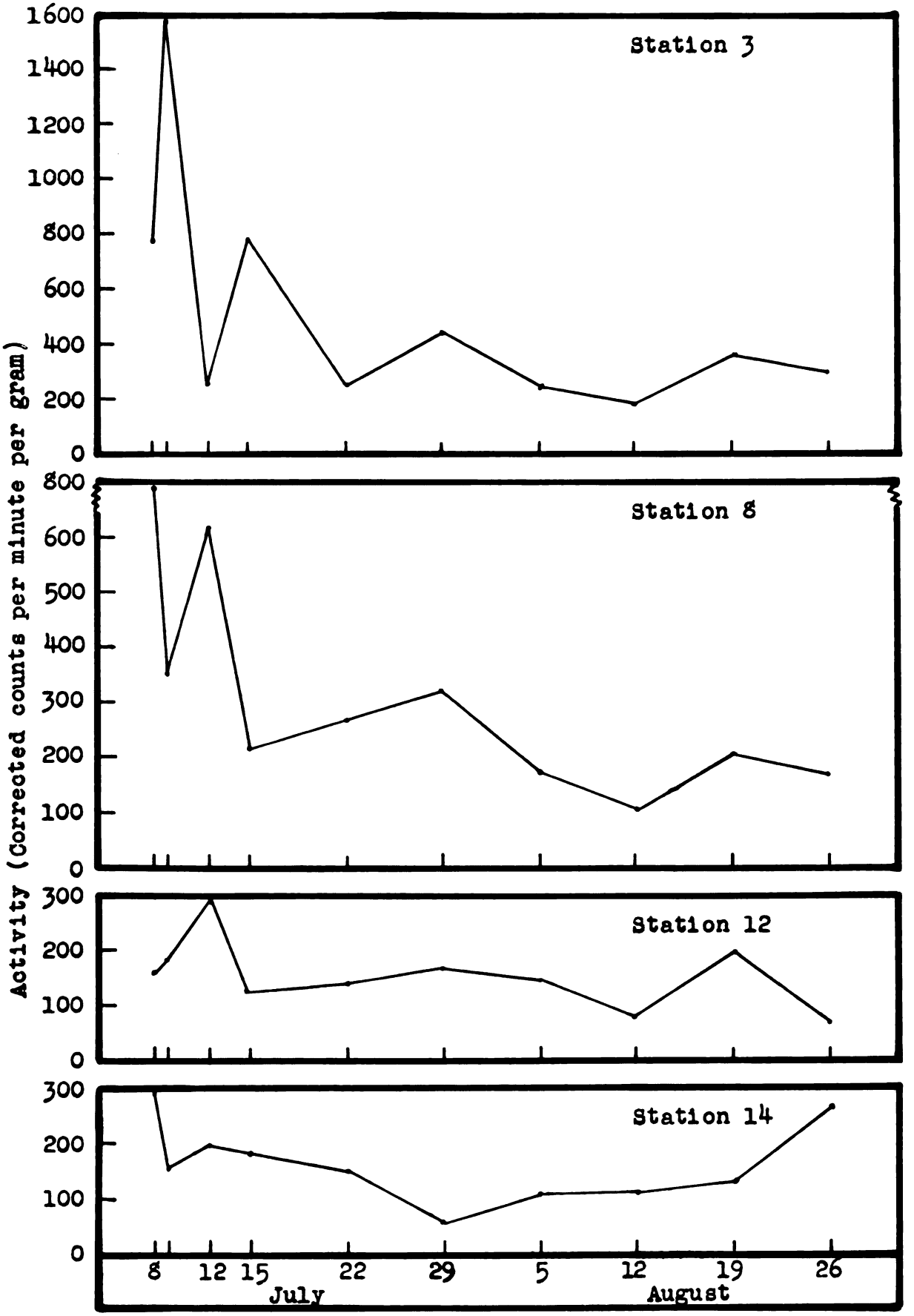
Station 3
Station 8
Station 12
Station 14
Activity (Corrected counts per minute per gram)
1600
1400
1200
1000
800
600
400
200
0
800
600
500
400
300
200
100
0
300
200
100
0
300
200
100
0
8
12
15
22
29
5
12
19
26
July
August

Figure 11

Figure 12. Activity of Chara at Stations 3, 8, 12 and 14, for the entire study period. Counts were corrected for background and decay.

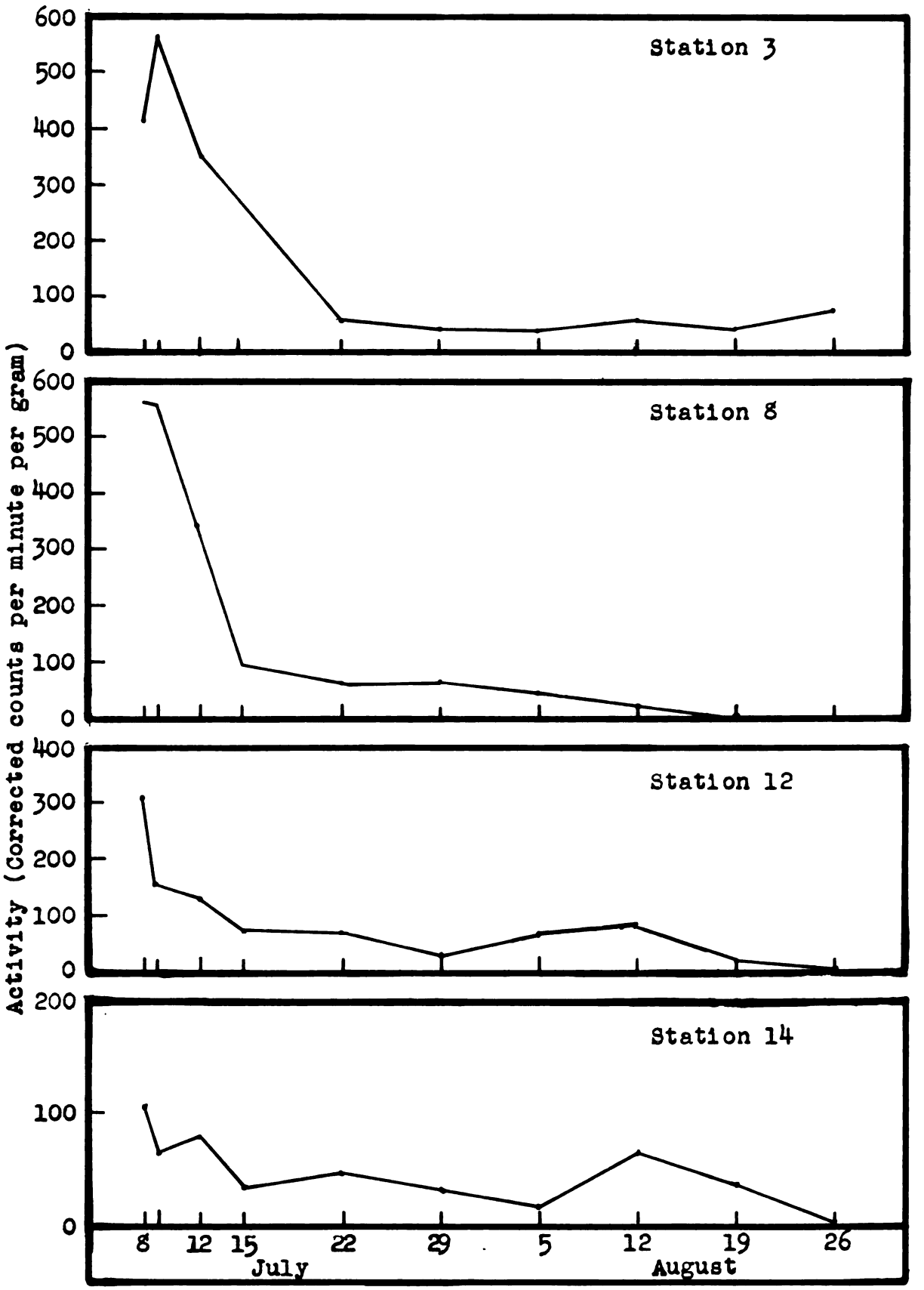
Station 3
Station 8
Station 12
Station 14
Activity (Corrected counts per minute per gram)
600
500
400
300
200
100
0
8
12
15
22
29
5
12
19
26
July
August

Figure 12

indicate "feed-back" with downstream uptake.

The activity curves for Fontinalis are shown in Figure 11. Maximum activity value recorded was approximately 1,600 counts per minute per gram at Station 3 on July 9. The peak activity value decreased progressively downstream (probably due to the fact that there was less P^{32} in the water to be picked up by the plants) to a peak value of approximately 300 counts per minute per gram, recorded at Stations 12 and 14. The peak activity at Station 3 did not, however, occur until 24 hours after the introduction of the tracer. This may indicate, as did Figure 9, that Fontinalis did not take up radiophosphorus as rapidly as Potamogeton. The activity loss observed at all of the stations did not follow that of Potamogeton. The activity curves at Stations 8 and 14 showed an initial loss of activity followed by a slight rise on July 12, followed by an equilibration phase. The reason for the belated activity peak exhibited by the Fontinalis at Station 3 is not known. The answer may lie in the fact that the sample collected on July 9 was obtained in an area that received a greater concentration of P^{32} during the isotope treatment than did the plant sample taken on July 8. Inasmuch as Station 3 is located rather close to the isotope entrance point, the major portion of tracer may have passed this station in the main channel of the stream, leaving areas exposed to lesser amounts of tracer. Station 12 showed a rather scanty initial P^{32} uptake with an increased uptake,

reaching a peak on July 12. This peak was apparently due to "feed-back" of radiophosphorus from upstream plants.

The activity curves for Chara are shown in Figure 12. The maximum activity value for Chara was approximately 560 cpm/gm., recorded on July 8 and 9 at Stations 8 and 3, respectively. The activity trends for Stations 3 and 8 are very similar except for the activity recorded for Station 3 on July 8. The activity value decreased progressively downstream (probably due to the fact that there was less P^{32} in the water to be picked up by the plants) to a peak of about 100 cpm/gm., recorded for Station 14. Stations 12 and 14 showed an initial isotope activity value relatively close to the equilibration level.

The activity curve of the red alga, Batrachospermum, is shown in Figure 13. This red alga was not sampled as extensively as were the other larger aquatic plants. This plant was discovered only while obtaining the routine plant samples reported in this paper. This alga was first processed for radiological analysis on July 12, four days after the isotope treatment date. It is suspected that the activity values for Batrachospermum greatly exceeded any value reported for the routine larger aquatic plants and periphyton. If this red alga followed a similar pattern of isotope uptake as the other algae and larger plants--as it apparently did--the theoretical activity value may have been as high as 4,000 cpm/gm. or more during the initial uptake period, just after isotope treatment. The activity

Figure 13. Activity of Batrachospermum at Stations 3, 8, 12 and 14, during July 1959. Counts were corrected for background and decay.

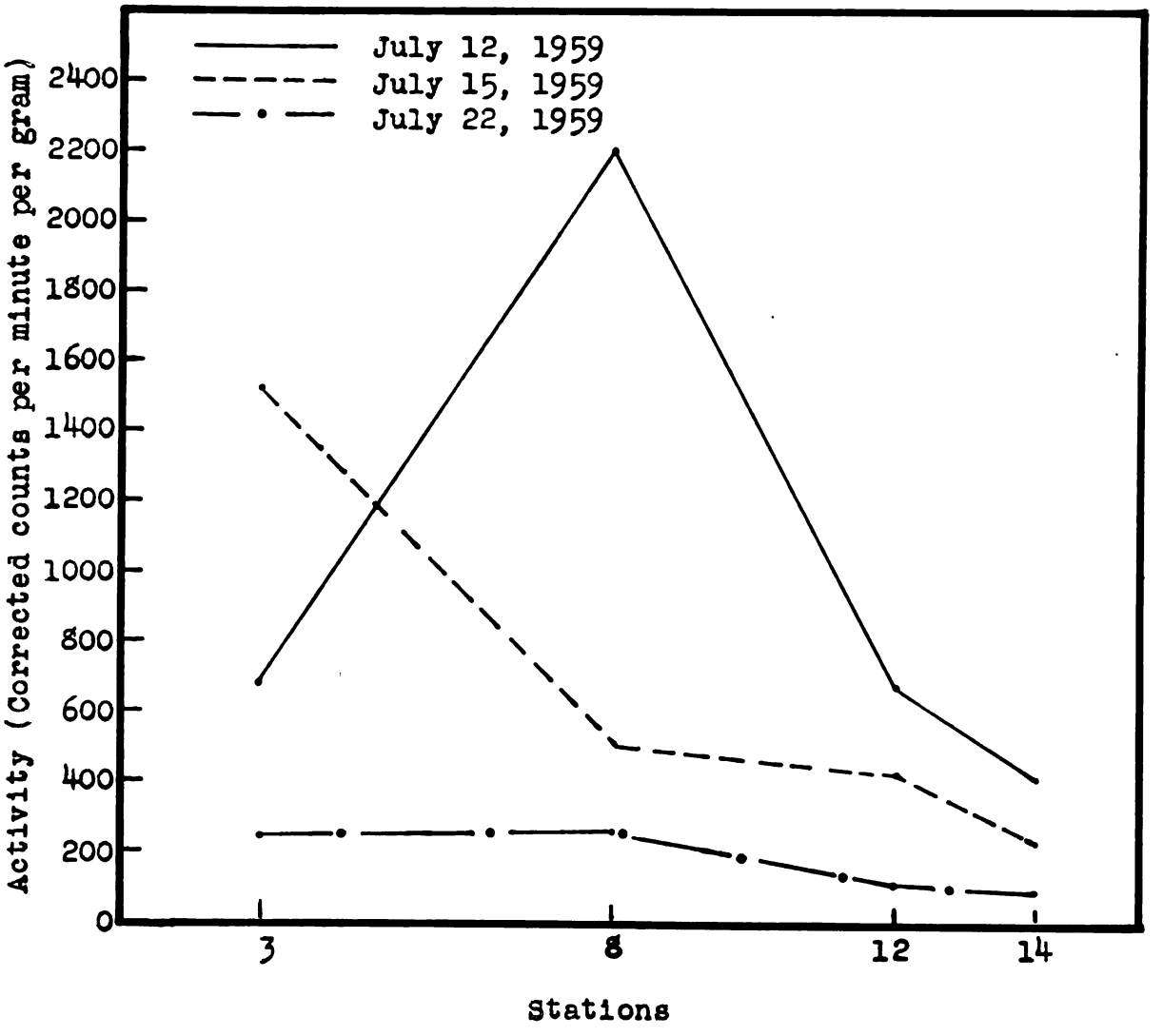
July 12, 1959
July 15, 1959
July 22, 1959
Activity (Corrected counts per minute per gram)
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
3
8
12
14
Stations

Figure 13

curve indicates that Batrachospermum released the isotope rather rapidly as an equilibration phase apparently was present on July 22 (Figure 13). In the study of phosphorus exchange in Bluff Lake, Hayes, et al. (ibid.) indicates that the alga, Batrachospermum, displayed a rapid P^{32} uptake from the first hours.

The uptake and accumulation of radiophosphorus by plant cells are characterized by two features. First, the living cells can accumulate ionic material, e.g., radiophosphorus. That is, they can continue to take up the isotope even if the concentration level of this tracer inside the cells is far above the concentration of the same ionic species (P^{32}) in the external medium. From the point of view of diffusion, the accumulation of phosphorus (and presumably P^{32}) by a cell is, not only unorthodox, but impossible. According to Bonner and Galston (1952), when substances penetrate into cells in response to a diffusion gradient, they may--at most--attain an internal concentration equal to the external concentration. It may be concluded, therefore, that the entrance into, and the increased internal concentration of, ionic materials (and presumably P^{32}) by cells is not a simple diffusion process. On the contrary, phosphorus accumulation is a process which requires the expenditure of energy by the plant--energy to do the osmotic work involved in moving the phosphorus (and presumably P^{32}) against a concentration gradient (Bonner and Galston, ibid.). Rice (1953) discusses the uptake and

accumulation of P^{32} in algae. He discusses the entry of radiophosphorus into the cell, indicating that P^{32} may enter algae by diffusion through the cell membrane or by entry of P^{32} into the cell through esterification at the cellular interface, or a combination of these two methods. The process of phosphorus (and presumably P^{32}) accumulation, which makes possible the uptake and retention by the plant of large quantities of phosphorus (and presumably P^{32}), is--at least in part--an energy-requiring process driven by, and wholly dependent on, the energy liberated in respiratory metabolism (Bonner and Galston, ibid.).

The second feature of radiophosphorus accumulation is that of equilibration between water and the aquatic plants which occurs within approximately one week. As soon as a significant fraction of isotope has entered the aquatic plants, there will be a "feed-back" of P^{32} into the water (Foster, ibid.). According to Foster, as more and more isotope enters the aquatic plants, the "feed-back" will increase until an equilibrium is established such that the amount of isotope leaving the water and entering the plants is balanced by the amount returned to the water from the solids. The data for Station 8 is shown in Figure 14, demonstrating exchange and equilibrium. In Figure 14 the logarithmic rate of decrease during the first week has been extrapolated to the X axis and a smooth curve has been fitted to the activity values by inspection. Had there been no "feed-back" of radiophosphorus between the solids

Figure 14. Activity of plants collected at Station 8. The dotted lines represent the extrapolation of the logarithmic rate of decrease of activity during early stages of the experiment. Counts were corrected for background and decay.

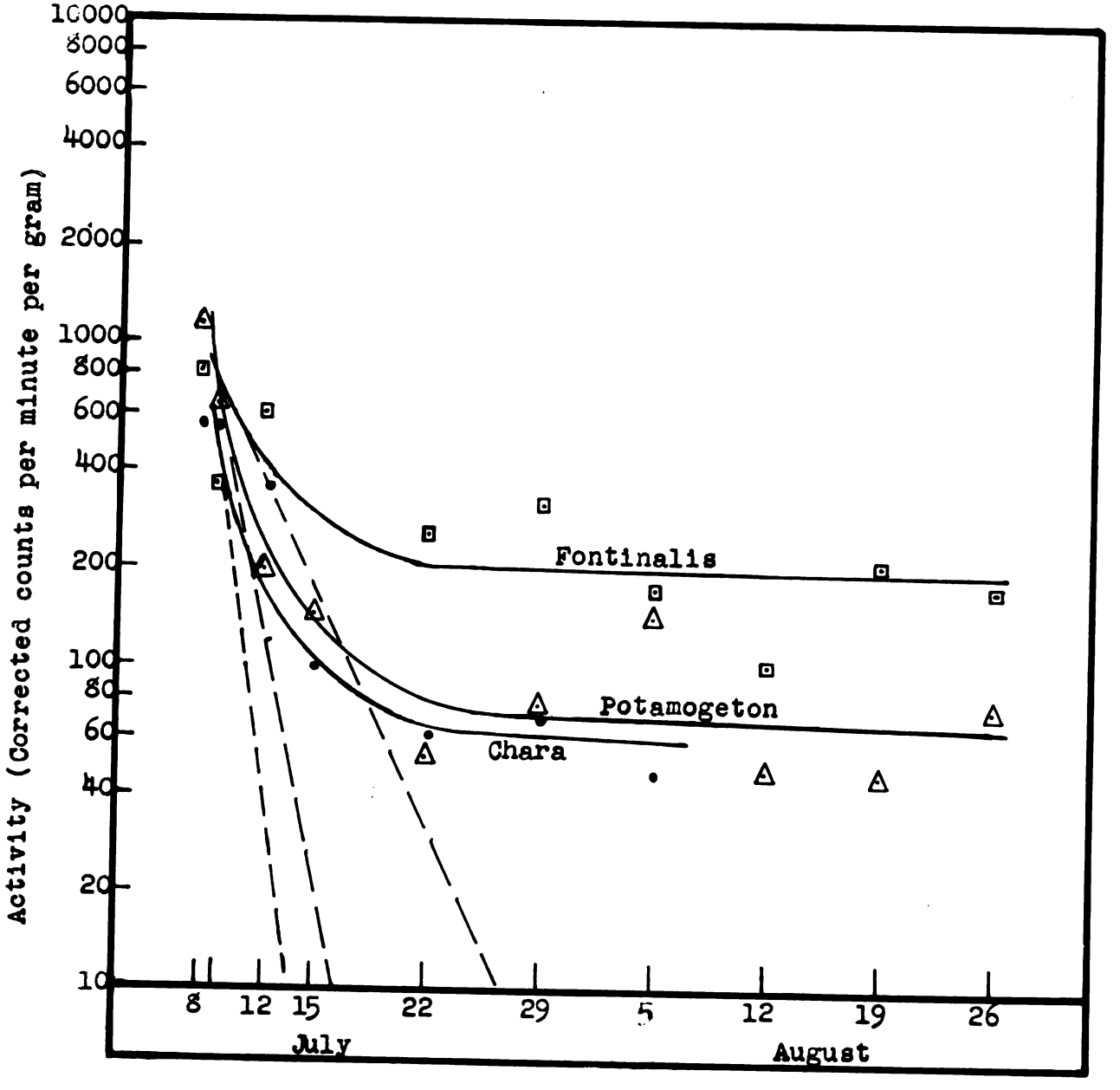
Activity (Corrected counts per minute per gram)
10000
8000
6000
4000
2000
1000
800
600
400
200
100
80
60
40
20
10
Fontinalis
Potamogeton
Chara
8
12
15
22
29
5
12
19
26
July
August

Figure 14

and the water, one would expect the decline in the concentration of isotope in the plants to follow the broken line. The increase with time in the amount of regenerated P^{32} returned from the water to the solids is evident from the space between the two curves (Foster, ibid.). On the basis of these data, the amount of regenerated P^{32} taken up by Fontinalis during the equilibration phase of the activity curve is greater than twice that taken up by either Potamogeton or Chara (Figure 14). Once the aquatic plants have equilibrated, further decrease in activity can be attributed to: (1) a decrease of P^{32} as a result of isotope loss from the plants back into the water; (2) increase in initial cell mass due to cell growth, new cell addition and loss of old cells, causing biological dilution; and (3) the concentration of isotope would diminish as a result of radioactive decay.

Larger Aquatic Plant Biomass Estimate

An estimate was made of the standing crop of larger aquatic plants for the first 1,000 yards of the stream below the point of isotope entry. The area encompassed by this area was from the point of isotope release to Station 8 (Figure 1), and represented an area of approximately 1.76 acres. Riffle area occupied a major portion of this section of the experimental area with a vast area of scanty vegetational growth observed in the middle portion of the sampling area. The mean plant biomass computed for this

portion of the experimental area was 21,780.0 pounds per acre. The dominant aquatic plant observed in the plant samples was Chara.

Aquatic Invertebrates

Isotope Uptake and Accumulation

Radioactive materials are taken into the body of an organism through physiological processes and incorporated directly into the tissues of the organism. The principal mode of accumulation of radiomaterials by invertebrate aquatic organisms is by ingestion of food. In their studies of the Columbia River, Robeck, et al. (ibid.), indicate that activity levels in most aquatic invertebrates are dependent upon their metabolic rates and the radioactivity values of the materials upon which they feed. In his work with Gammarus, Harris (1957) indicates that Gammarus does not take up appreciable quantities of phosphorus by direct absorption through the body wall, intestine or gills. It is assumed in the present study that no appreciable radiophosphorus entered the aquatic invertebrates by direct absorption. On the basis that the isotope enters the invertebrates through ingestion, it appears quite logical that the invertebrate organisms, in the present study, could be discussed most effectively if they were subdivided on the basis of their food habits.

The seven organisms collected and analyzed throughout the study period were subdivided into five food habit

categories, as follow: filter feeders, periphyton scrapers, detritus feeders, predators and an example of an organism with an omnivorous feeding habit. Even though the following section will subdivide the organisms into food habits, it is well recognized that precisely such a partitioning is impossible, due to various factors in nature. Environmental conditions in fast streams are strenuous; the strong current in particular makes life somewhat precarious and selective feeding difficult (Muttkowski 1929). It is recognized also that aquatic insects in rapid streams may become opportunists in regards to food and, out of necessity, may eat whatever is available.

Invertebrates Studied

The blackfly, Simulium sp., was selected to represent the filter feeder group. The activity curve for the blackfly larvae is shown in Figures 15, 16, 17 and 18. The maximum blackfly radiophosphorus concentration of approximately 6,000 cpm/gm. was recorded at Station 8 on July 12, four days after the isotope treatment. The peak activity at Station 3 was the lowest peak reported at any of the sampling stations. The maximum activity value of 6,000 cpm/gm. recorded at Station 8 was double the peak activity recorded for periphyton, indicating that the blackfly was either feeding on a material with a much higher activity concentration than anything analyzed in the present study or the blackfly is capable of concentrating great amounts

of the radiomaterial. The blackfly larvae possess two prominent fanlike structures located at the extreme anterior end of the organism. These anterior fans strain plankton and organic debris from the water for food (Pennak 1953). The material strained from the water by the blackfly larvae possibly might include bacteria and diatoms, containing a great concentration of radiophosphorus. According to Robeck, et al. (ibid.), radioactivity density of organisms is dependent upon the rate of metabolism and the activity of the organisms upon which they feed. If blackfly larvae follow the pattern of other organisms in that metabolism per gram decreases with increasing size of the individual, the small blackfly larvae would utilize relatively large quantities of food material which might be high in radiophosphorus and possibly concentrate P^{32} at a rate many times greater than the larger invertebrate organisms.

The activity curves for the blackfly larvae at all of the sample stations show strikingly similar trends. An initial period of increasing P^{32} concentration built up to a peak on July 12 at Stations 3 and 8, and on July 15 at Stations 12 and 14. Following the maximum activity level, the blackfly activity at all stations decreased at a logarithmic rate, indicating there was little uptake from regenerated materials and its source of activity had nearly disappeared. Studies on radiophosphorus metabolism by Harris (ibid.) indicate that bacteria in that particular

Figure 15. Activity recorded for Station 3 invertebrates representing four food niches. Counts were corrected for background and decay.

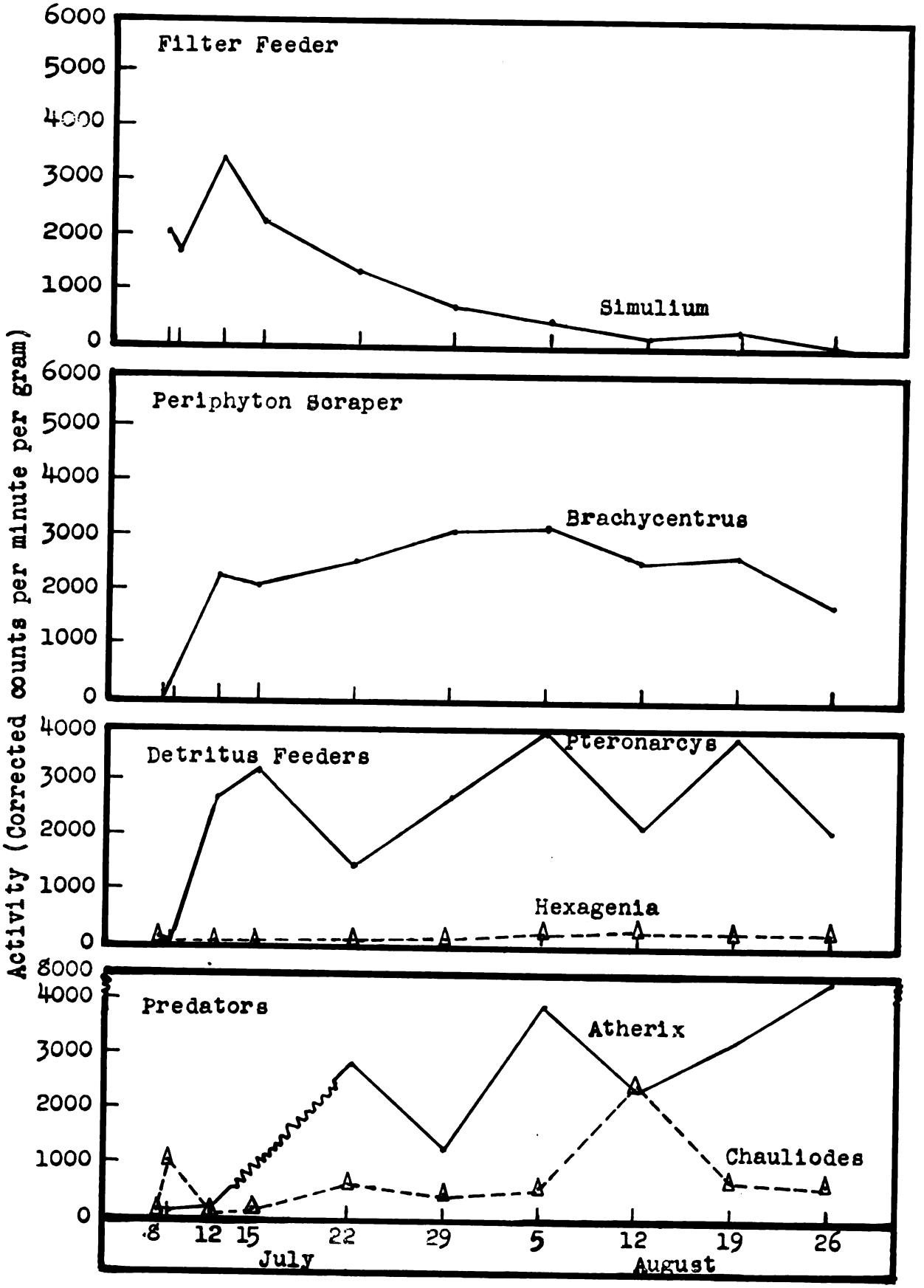
Filter Feeder
Simulium
Periphyton Scraper
Brachycentrus
Detritus Feeders
Pteronarcys
Hexagenia
Predators
Atherix
Chauliodes
Activity (Corrected counts per minute per gram)
6000
5000
4000
3000
2000
1000
0
8000
8
12
15
22
29
5
12
19
26
July
August

Figure 15

Figure 16. Activity recorded for Station 8 invertebrates representing four food niches. Counts were corrected for background and decay.

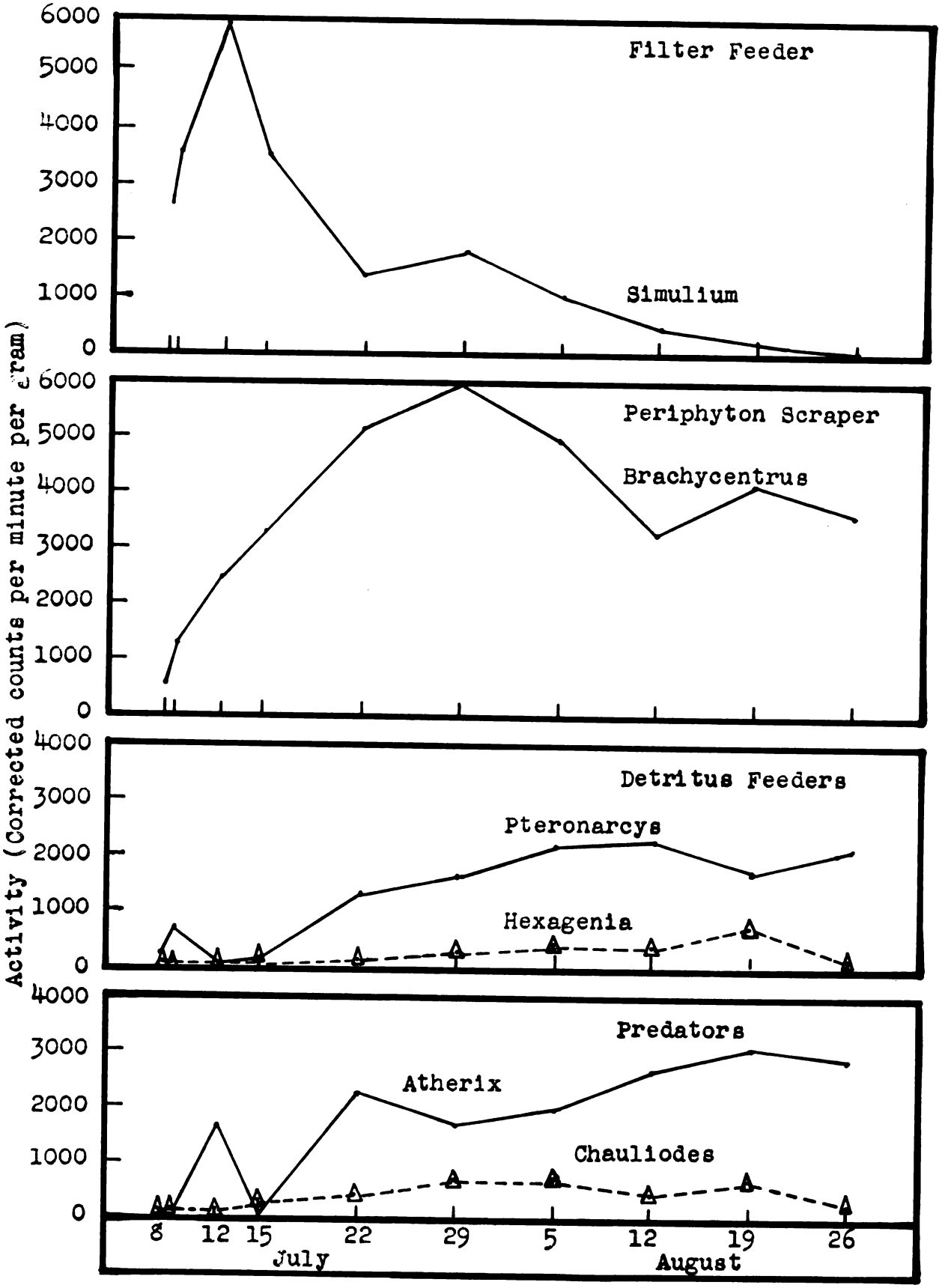
Filter Feeder
Simulium
Periphyton Scraper
Brachycentrus
Detritus Feeders
Pteronarcys
Hexagenia
Predators
Atherix
Chauliodes
Activity (Corrected counts per minute per gram)
6000
5000
4000
3000
2000
1000
0
8
12
15
22
29
5
12
19
26
July
August

Figure 16

Figure 17. Activity recorded for Station 12 invertebrates representing four food niches. Counts were corrected for background and decay.

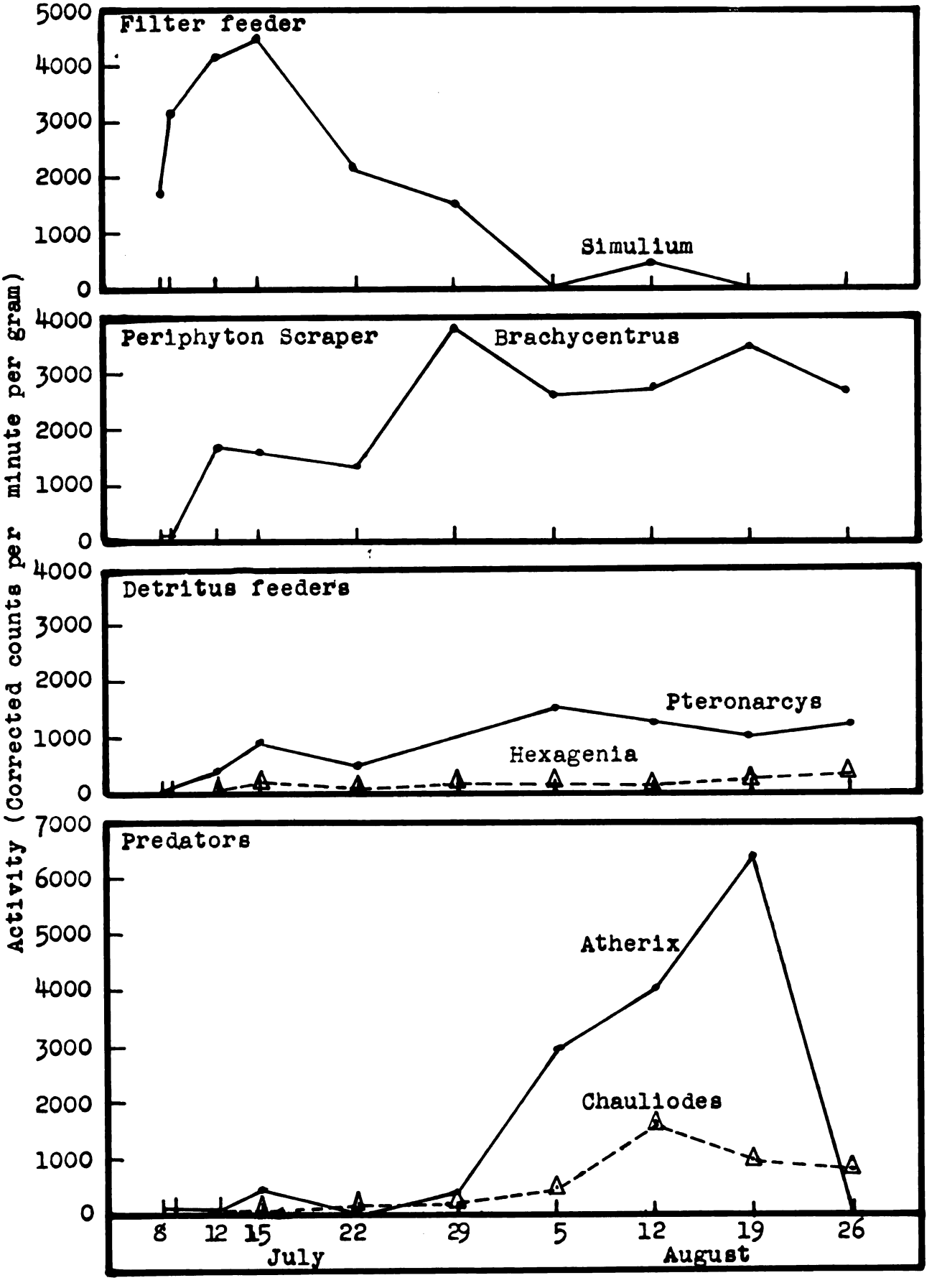
Filter feeder
Simulium
Periphyton Scraper
Brachycentrus
Detritus feeders
Pteronarcys
Hexagenia
Predators
Atherix
Chauliodes
Activity (Corrected counts per minute per gram)
5000
4000
3000
2000
1000
0
7000
6000
8
12
15
22
29
5
12
19
26
July
August

Figure 17

Figure 18. Activity recorded for Station 14 invertebrates representing four food niches. Counts were corrected for background and decay.

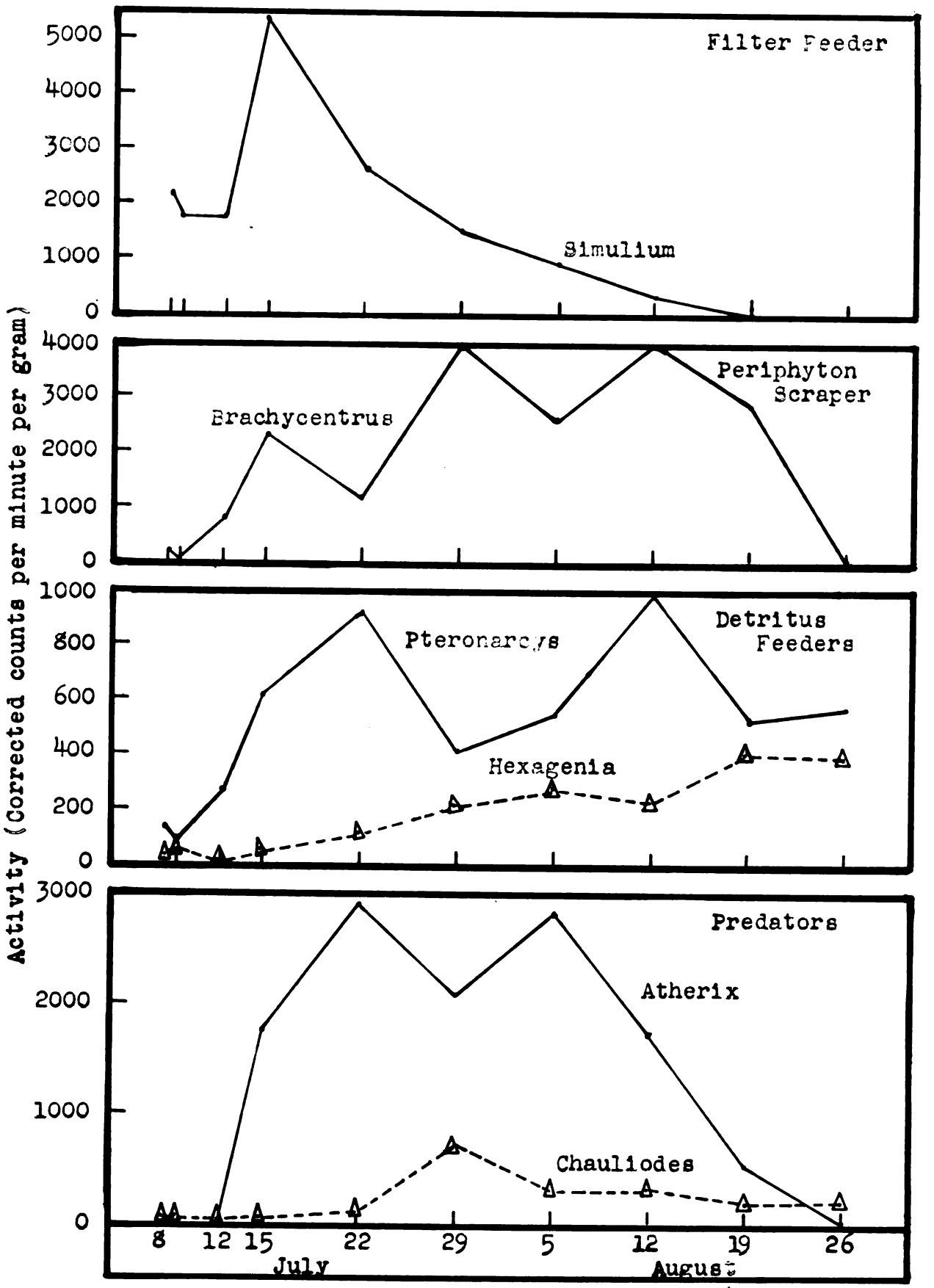
Activity (Corrected counts per minute per gram)
Filter Feeder
Simulium
Periphyton Scraper
Brachycentrus
Detritus Feeders
Pteronarcys
Hexagenia
Predators
Atherix
Chauliodes
July
August
8
12
15
22
29
5
12
19
26

Figure 18

study required nearly 40 hours before they began to equilibrate with the inorganic radiophosphorus in the solution. Harris found that about the same length of time was required before Gammarus began to take up the radiophosphorus more quickly. Harris (ibid.) attributed this to the fact that there are more bacteria containing radiophosphorus after about 40 hours, so that animals feeding on bacteria at a constant rate will take up more radiophosphorus in the bodies of the bacteria after 40 hours. Such a situation may have been operating in the present study, causing the initial relatively slow uptake of radiophosphorus by the blackflies. This initial slow uptake may be a result of the time interval required to accumulate P^{32} by way of food organisms.

The stations downstream from Station 3 show somewhat higher peak activity values. This situation probably developed as a result of downstream "feed-back" of P^{32}. The blackfly at the downstream stations would be in a position to filter out activity released from the upstream solids. Further evidence of this is indicated in the fact that peak activity values for the downstream Stations 12 and 14 were recorded three days after peak levels were recorded at the upstream Station 3 and 8.

The caddisfly, Brachycentrus sp., was selected as a representative of a periphyton scraper. The Brachycentrus sp. attaches its case to rocks or higher aquatic plants in exposed places and faces up-current so that the water

sweeps directly into the case. According to Morgan (1930), the larvae first live upon diatoms and then upon other algae; but when about six weeks old, it adds to this a diet of mayflies, water mites, midge larvae and small crustaceans. Muttkowski (ibid.) found that the diet of Brachycentrus consisted of 18.3% animal food, 72.7% plant food and 9% detritus.

The activity curves for the Brachycentrus are shown in Figures 15, 16, 17 and 18. The maximum activity of the caddisfly larvae was approximately 6,000 cpm/gm. and was recorded on July 29, three weeks after isotope treatment.

The activity for Brachycentrus at the four sampling stations all show similar activity trends. There is an initial period of gradual isotope concentration increase until a peak activity is reached on July 29, three weeks after isotope treatment. Apparently, by feeding on the periphyton on rocks and higher plants, the Brachycentrus was able to maintain a rather high activity plateau or equilibration shortly after peak activity was recorded. Radioisotopes will be deposited and retained in the organism according to the physiological behavior of the particular element involved and, in this case, phosphorus may be so tightly fixed that little loss occurs, except by radioactive decay and biological dilution (Krumholz and Foster, ibid.).

The stonefly nymph, Pteronarcys, and the mayfly nymph, Hexagenia, were selected to represent detritus feeders.

The Pteronarcys nymph is herbivorous feeding on algae and vegetable debris (Pennak, ibid.). The activity curves for Pteronarcys is shown in Figures 15, 16, 17 and 18. The maximum activity recorded for the stonefly was nearly 4,000 cpm/gm. collected at Station 3 on August 5, four weeks after isotope treatment. The activity curves of the stonefly show a trend in complete reverse of the aquatic invertebrates heretofore discussed. The peak activity recorded for the stonefly was collected at Station 3 and the activity peaks decreased progressively downstream. This downstream decrease in activity peaks was demonstrated previously when discussing the larger aquatic plants. The stonefly isotope activity curves show a lag period in which uptake was slow at precisely the period when larger aquatic plants exhibited their greatest uptake (Figures 10, 11 and 12). At the time when the larger aquatic plants showed the greatest decrease in activity, the stoneflies exhibited a sharp activity uptake trend. Inasmuch as the stonefly inhabits and feeds on collections of plant debris, this trend is not too surprising. In his study of the ecology of trout streams in Yellowstone National Park, Muttkowski (ibid.) observed some small Pteronarcys nymphs feeding on blackfly larvae. Whether or not the stoneflies in this study fed on blackfly larvae is not known. It is interesting to note that once the peak activity was reached, the stonefly activity never decreased below 1,000 cpm/gm. in any of the collections (except for Station 14) for the

remainder of the study period. This indicates that the stonefly nymphs continued to pick up activity for a considerable period.

The mayfly, Hexagenia sp., feeds on vegetable detritus and microscopic aquatic organisms, principally diatoms (Burks 1953), which it obtains from the rich ooze through which they plow their way (Morgan, ibid.). The activity curves of Hexagenia are shown in Figures 15, 16, 17 and 18. The maximum activity uptake by Hexagenia was approximately 700 cpm/gm. recorded on August 19, six weeks after the isotope treatment, in the sample obtained at Station 5. The activity trend recorded for all of the stations followed a continuous uptake pattern throughout the study period. This perhaps was due to a greater amount of radioactivity reaching the detritus and sediments as the study period progressed. The Hexagenia obtained radioactive phosphorus from diatoms and other plant materials as a result of fall out to the sediment surface in back water areas. The activity actually available to the mayfly was very much reduced due to various causes. In the first place, much of the plant material reaching the bottom sediments may be dead material sloughed off upstream and deposited in back waters downstream. For the most part, any plant material reaching the ooze would have lost the majority of its activity (corrected counts) due to radiological decay. Radiophosphorus would be introduced into the surface layers of the detritus beds by the fall out of

plankton which have died and fecal pellets of animals which have eaten materials containing radiophosphorus. A possible explanation for the reduced radiophosphorus uptake by the mayfly may lie in the fact that very little activity actually entered the ooze and silt beds. According to Hayes and Phillips (ibid.), a fall out of organisms to the sediment surface is followed by a bacterial breakdown to inorganic P^{32}, so that the P^{32} is restored to the water.

The snipe fly larvae, Atherix variegata Walker, and the fishfly larvae, Chauliodes sp., were selected as representatives of the predator feeding habit.

The snipe fly larvae activity curves are shown in Figures 15, 16, 17 and 18. The maximum activity recorded for the snipe fly was approximately 6,400 cpm/gm. for the sample collected at Station 12 on August 19, six weeks after isotope treatment. The dates on which the other stations were observed to reach peak activity varied from July 22 for Station 14 to August 19 for Stations 8 and 12. The activity curves show a great deal of variability in trends as might be expected for predacious organisms. The food eaten would depend upon what became available. Thus the activity curves would be expected to indicate an erratic pattern from station to station, as well as, at the same station, indicating the organism was feeding on food material of various activity levels. It appears possible that the snipe fly larvae at Station 12 may have found a certain type of food so much to their liking that they may

have preyed upon only certain individuals. It may be that a certain organism or several organisms high in activity became available at Station 12 shortly after July 29, thus bringing about a peak in the activity curve of the snipe fly. Similar situations may have been operative at Stations 3, 8 and 12, which show trends in their activity curves that may indicate variations in feeding habits.

The activity curves for the fishfly larvae, Chauliodes sp., are shown in Figures 15, 16, 17 and 18. The maximum activity recorded for fishfly larvae was approximately 2,500 cpm/gm., recorded for the sample collected at Station 12 on August 12, five weeks after isotope treatment. Even though the fishfly larvae are predacious, as are the snipe fly larvae, the isotope uptake did not show similar activity patterns. The peak activity of the fishfly larvae was less than one-half the peak value recorded for the snipe fly larvae, indicating that the two larvae either did not feed upon the same organisms or did not concentrate P^{32} in a like manner, due to variation in physiological make-up or phosphorus metabolism rate. The P^{32} uptake by the fishfly larvae showed a slow initial uptake period. Within 2 to 4 weeks--depending upon the station--there was an increase in P^{32} uptake, followed by an equilibration period with little activity variation from one sample date to the next.

The pouch snail, Physa sp., was selected as a representative of an organism with an omnivorous feeding

habit. Physa is a scavenger and is essentially omnivorous, eating materials that range from living and dead plant material to dead animal material (Pennak, ibid.).

The activity curves for Physa are shown in Figure 19. The maximum activity of approximately 8,500 cpm/gm. was recorded for the sample taken at Station 8 on July 15, one week after isotope treatment. The omnivorous feeding habit of this organism would lead one to suspect that the activity recorded on the various sample dates would actually represent the various activity levels of the food previously eaten by the snail. The activity curves show a bimodal type trend at each sample station. The explanation for this may lie in terms of trophic level shifting of P^{32} activity with time from an initial plant uptake to a later animal uptake of radiophosphorus.

It appears probable that the first mode was a result of the snail feeding on periphyton and other living and dead plant material. As the plant material decreased in activity, the snail activity decreased in response to the decreased activity of its food. The dead animal material that the snail fed upon during the first peak was low in activity but, with the passage of time, the animal material available to the snail contained more and more activity, thus accounting for the second peak activity in mid to late August. The snail, because of its omnivorous food habit, concentrates radiophosphorus from two trophic levels. The initial uptake of P^{32}, presumably, was a result of

feeding upon primary producers and the second uptake as a result of feeding upon dead consumer organisms (dead animal material).

Variation in Radiophosphorus Uptake by Invertebrates

In order to determine to what extent the concentration of P^{32} varied among organisms of the same species collected at the same sampling station at precisely the same time, a duplicate sample was obtained. The duplicate sample was processed for radiological determination exactly as was the routine sample. The data thus obtained indicated that in some organisms a rather large variation occurred between duplicate samples. The activity curves shown in Figure 20 illustrate this variation of duplicate samples. No attempt is made to show such variability for all samples or all organisms for the entire study period. A thorough treatment of such a subject would encompass a paper in itself. In lieu of such extensive treatment, it is believed that Figure 20 will suffice as an indication of variability in isotope concentration, operating in the present study.

Several factors may be responsible for the variation of activity in some of the invertebrate forms. Foremost of these factors is the fact that some aquatic invertebrates in rapid streams may become opportunists in regard to food and eat whatever becomes available under certain circumstances. Environmental conditions in streams with strong current makes life somewhat precarious and selective

Figure 19. Activity of Physa at all Stations
Counts were corrected for background and decay.

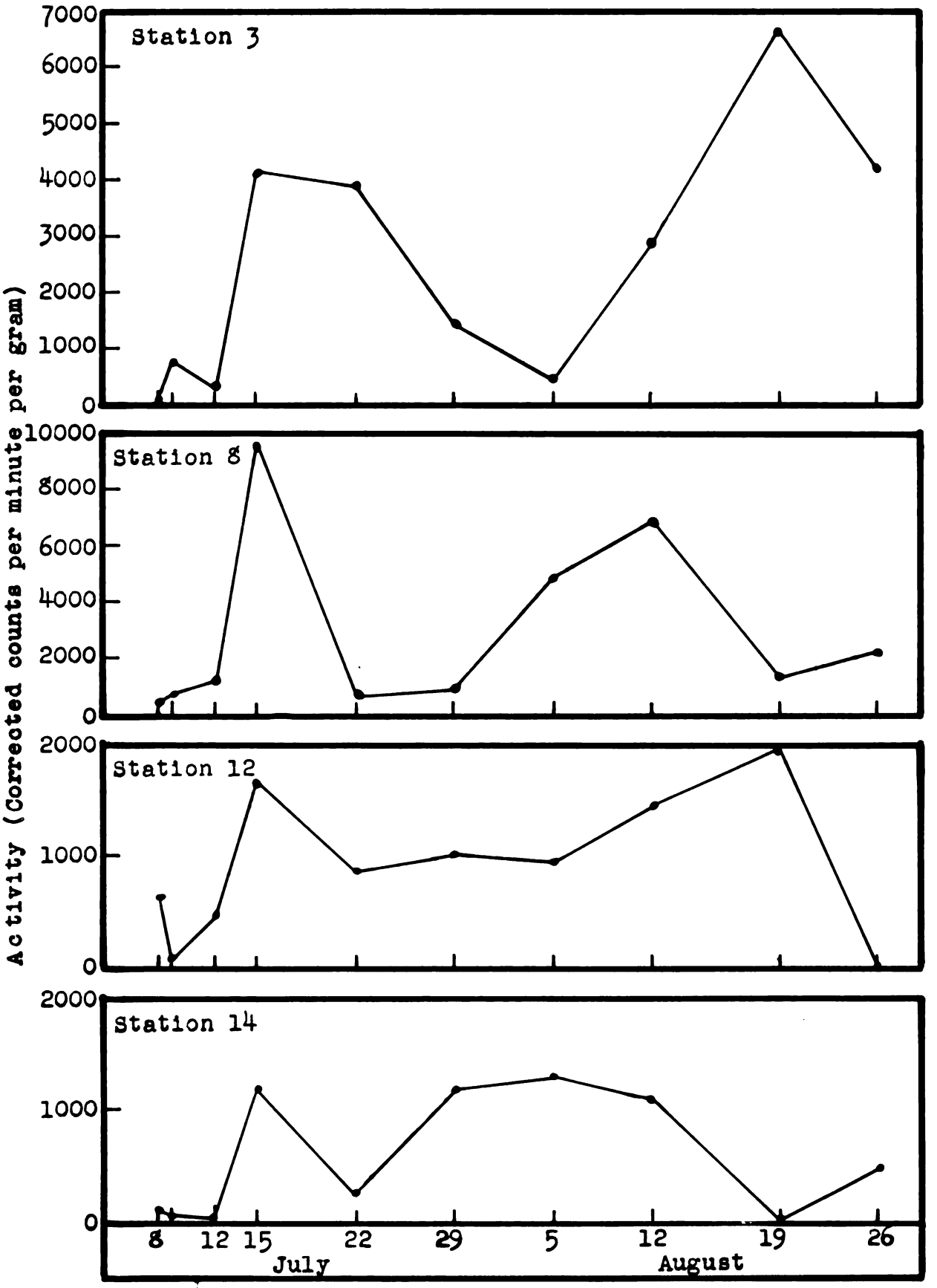
Station 3
Station 8
Station 12
Station 14
Activity (Corrected counts per minute per gram)
7000
6000
5000
4000
3000
2000
1000
0
10000
8000
6000
4000
2000
0
2000
1000
0
2000
1000
0
8
12
15
22
29
5
12
19
26
July
August

Figure 19

feeding at times may be difficult. The activity of food eaten just prior to collection, as well as the condition of the digestive tract at the time of sampling, would have a great bearing on the activity of the organism. It is apparent that an organism with a digestive tract gorged with material of great isotope concentration would tend to indicate a greater tracer concentration over a similar organism that had not eaten for several hours prior to collection. The factor of age may play an important part in activity uptake. It is generally agreed that younger, more rapidly growing individuals accumulate relatively greater amounts of radioactivity than the older, more slowly growing ones. This phenomenon is apparently a reflection of the more rapid anabolism that accompanies the growth of younger individuals. If a sample contained young individuals, a rather great variance in isotope accumulation might result over samples containing older individuals. Still another factor operating to cause variation may arise as a result of drift materials. Dendy (1944) presents results of his investigation on drift in three Northern Michigan streams. He found that 71 different kinds of macroscopic animals, representing 7 phyla, occurred in the drift during the summer months, as well as the fact that the presence of macroscopic animals, although highly variable in kind and quantity, was a constant feature. Dendy further indicates that in one of the streams, all species represented in bottom fauna samples were sooner or later found in the drift

Figure 20. Activity of invertebrate organisms at Station 14, following treatment of the stream with P^{32}. The variation of subsamples within routine samples is shown. Counts were corrected for background and decay.

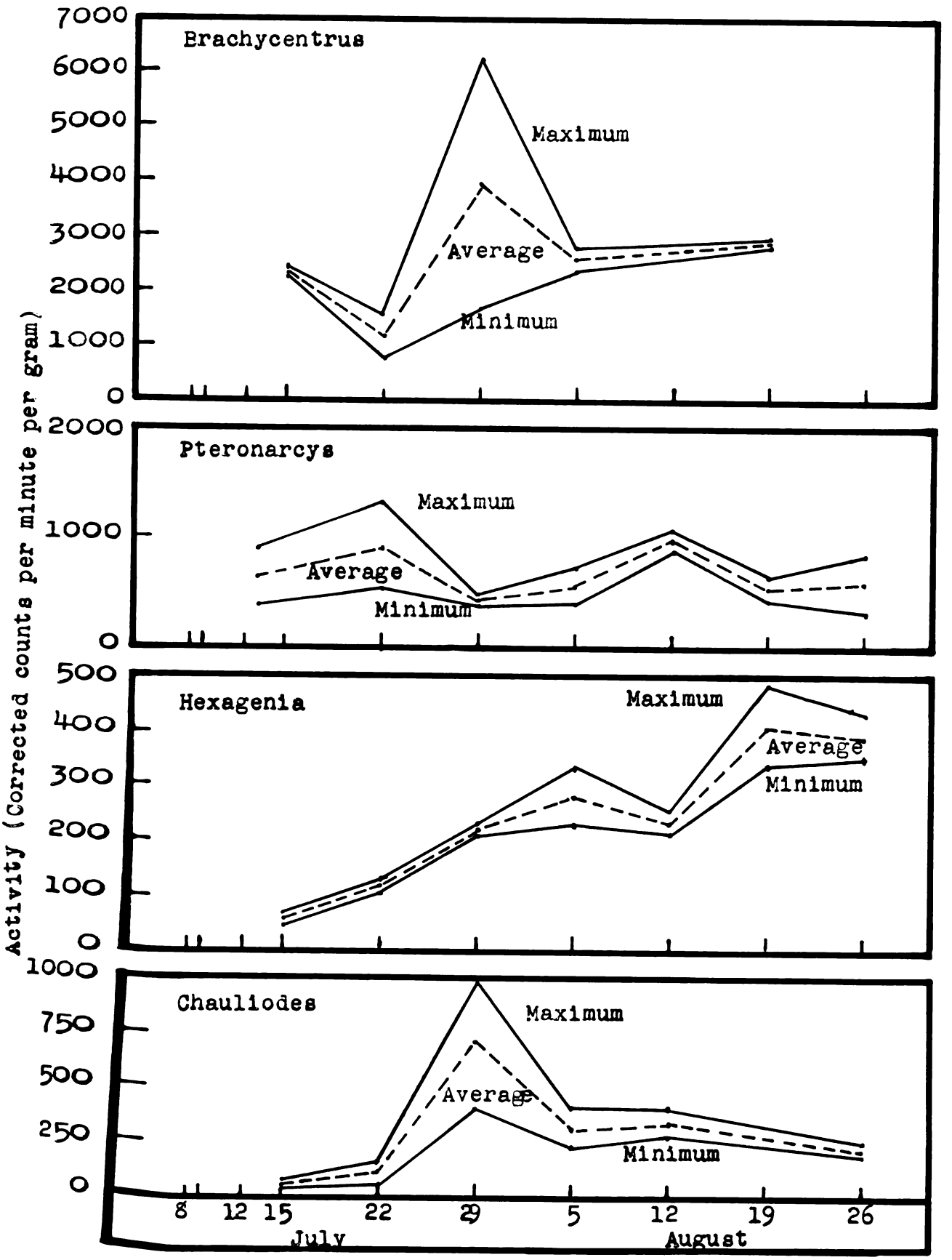
Brachycentrus
Maximum
Average
Minimum
7000
6000
5000
4000
3000
2000
1000
0
Pteronarcys
Maximum
Average
Minimum
2000
1000
0
Hexagenia
Maximum
Average
Minimum
500
400
300
200
100
0
Chauliodes
Maximum
Average
Minimum
1000
750
500
250
0
Activity (Corrected counts per minute per gram)
8
12
15
22
29
5
12
19
26
July
August

Figure 20

of that stream. On the basis of this information, it appears safe to assume that organisms collected at a station may have originated further upstream. In some cases a certain organism swept downstream and relocating would contain a greater or lesser accumulation of activity than a similar organism native to the area. In such a situation a great variation in isotope accumulation could occur and thus distort the actual isotope accumulation value representative of that area. It is not known just how much this stream drift phenomenon distorted recorded activity values.

Invertebrate Biomass Estimate

A random sample estimate of invertebrate standing crop was obtained for 1,000 yards of the experimental area (Station 1 to Station 8) on August 31 and September 1, 1959. The mean live weight of invertebrates was calculated to be 0.377 grams per square foot, or 36 pounds per acre. The mean number of organisms was found to be 109 per square foot. The organisms dominating the samples on a weight basis were mayfly nymphs (40%), caddisfly larvae (21%), stonefly nymphs (12%), fishfly larvae (10%) and Oligochaetes (8%). The dominant organisms on a numerical basis were the caddisfly (39%), Oligochaetes (28%) and mayfly (11%).

Fish and Lampreys

Assimilation of ingested materials is the chief means by which many radioactive materials accumulate in animals,

since the bulk of their essential elements is obtained from their food (Davis and Foster, 1958). The principal mode of accumulation of radiomaterials by fish is through ingestion (Krumholz and Foster, ibid.). Direct absorption of P^{32} by fish apparently is inconsequental. According to Krumholz and Foster (ibid.), in fish that live downstream from the Hanford reactors, sorption of radioactive materials directly from the effluents account for only about 1.5% of the total radioactivity.

The activity curves for the American brook lamprey, Entosphenus lamottenii lamottenii (LeSueur), are shown in Figure 21. The maximum activity for the lamprey was approximately 305 cpm/gm. recorded in the sample collected at Station 12 on August 19, 1959. The peak activity for Stations 3 and 8 were quite similar (about 150 cpm/gm.) but occurred on August 5 and 26, respectively. The peak activity recorded for Station 14 was about 215 cpm/gm., about two-thirds the peak activity recorded at Station 12. The activity of the brook lamprey was somewhat higher for the samples obtained at the downstream stations (12 and 14). This may be due to the fact that the detritus at the downstream stations possessed a greater activity due to "fall out" material from upstream areas. This increased activity at the lower stations, the low level of activity at its maximum and somewhat slow activity increase are suggestive of detritus feeders. The material in the digestive tract of numerous brook lampreys was found to contain large

quantities of sand, decaying plant material and other debris. The general activity trend--although somewhat variable--of the brook lamprey was a more-or-less steady increase after isotope treatment.

The activity curves of the Eastern slimy sculpin, Cottus cognatus gracilis Heckel, are shown in Figure 22. A maximum activity of about 7,400 cpm/gm. was recorded for the sculpin sample collected at Station 3 on August 23, 1959. The activity curves for the sculpin indicate a great deal of variation and thus mask, for the most part, any uptake pattern that might have been present. The activity pattern, even though somewhat variable, does show a general pattern of uptake throughout the study period. The variability of activity from one sample date to the next apparently was due to the activity of food consumed. As has been shown earlier in this paper, the various fish food organisms present in the stream showed considerable difference in radiophosphorus accumulation. On this basis the variation in sculpin activity probably reflects nothing more than difference in the past feeding experience of the individual fish. This becomes even more certain when one examines the food ingested by sculpins. The sculpin is an opportunist as far as food habits are concerned. According to Koster (1937), the bulk of the sculpin diet is made up of insect larval stages. The larval forms of Diptera, Tricoptera, Ephemeroptera and Plecoptera are the chief insect forms. In addition to the insects, the sculpin may

Figure 21. Activity of American brook lamprey at all stations. Counts were corrected for background and decay.

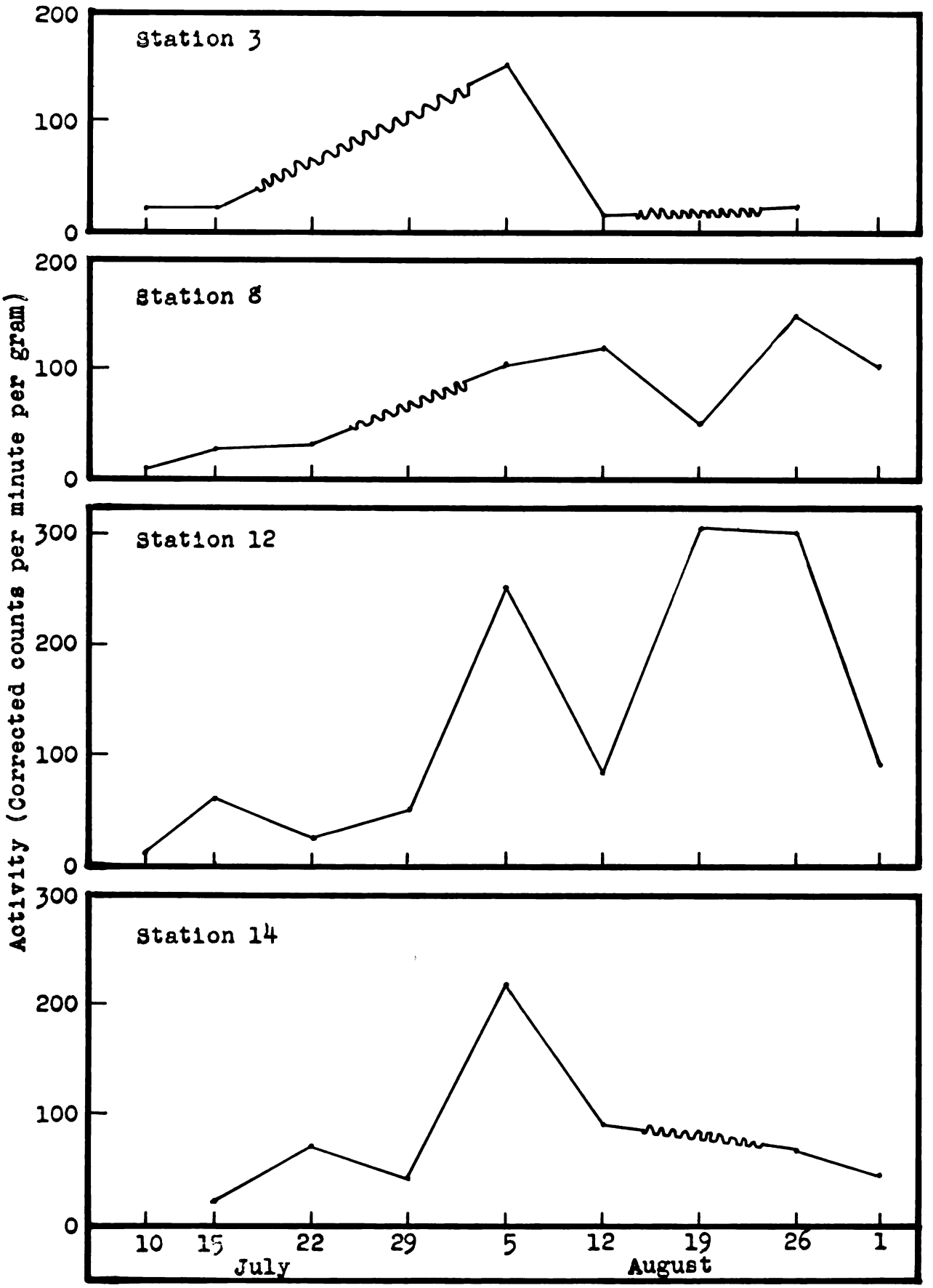
Station 3
Station 8
Station 12
Station 14
Activity (Corrected counts per minute per gram)
200
100
0
300
10
15
22
29
5
12
19
26
1
July
August

Figure 21

Figure 22. Activity of the sculpin at all stations. Counts were corrected for background and decay.

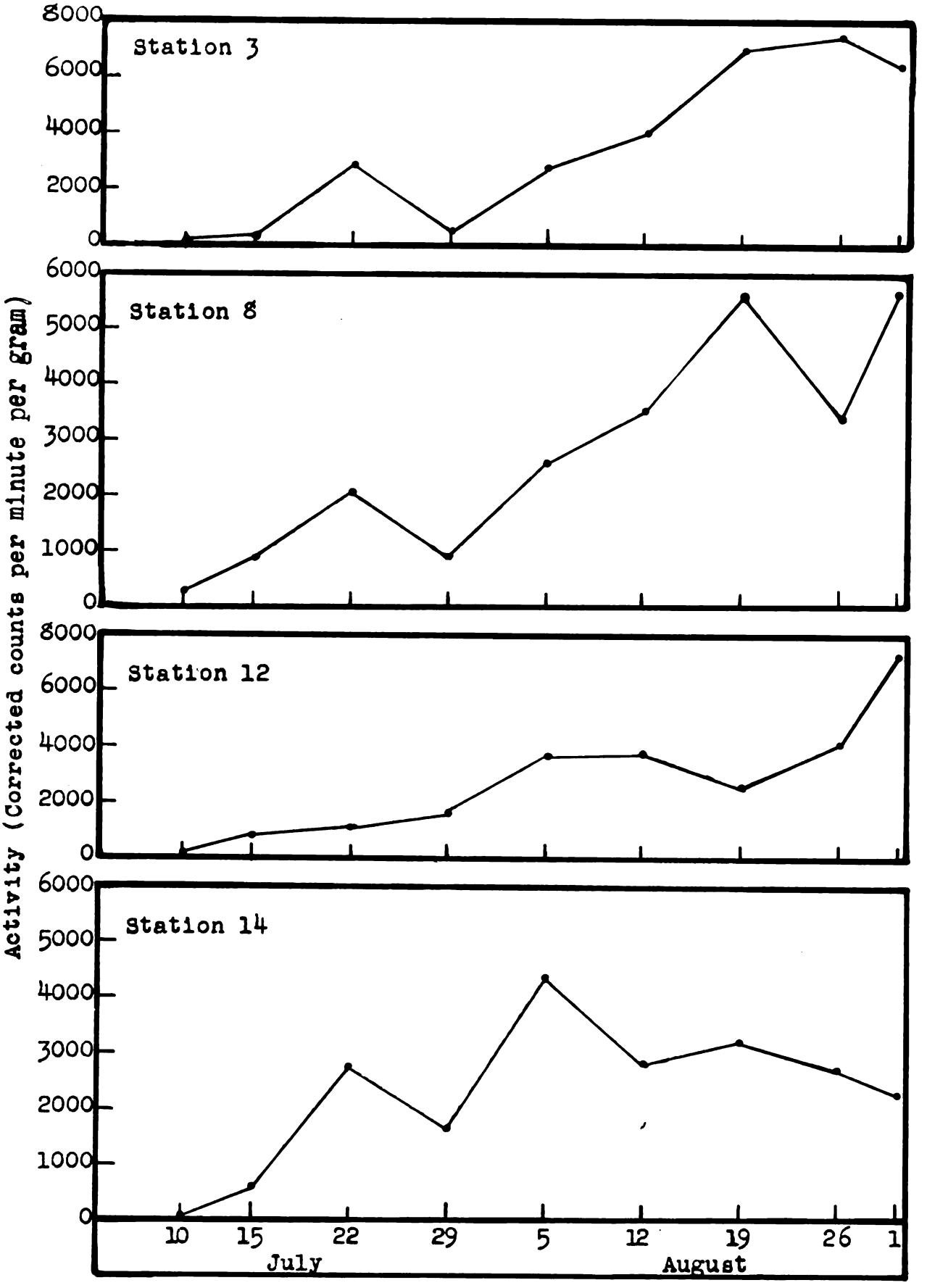
Station 3
Station 8
Station 12
Station 14
Activity (Corrected counts per minute per gram)
8000
6000
4000
2000
0
5000
3000
1000
10
15
22
29
5
12
19
26
1
July
August

Figure 22

eat a considerable amount of Chara and Oligochaetes. The stomach contents of approximately 200 sculpins taken from the West Branch of the Sturgeon River were examined in an attempt to determine the food habit of the sculpin in the experimental area. The food items found in the digestive tracts of the sculpin were represented chiefly by aquatic insect larvae. The immature insect forms occurring most frequently were: caddisfly larvae, mayfly nymphs, biting midge larvae, blackfly larvae, stonefly nymphs and dragonfly nymphs. Organisms occurring less frequently were Oligochaetes, pouch snails and fingernail clams. In addition to the items listed above the sculpin intestinal tracts were observed to contain great quantities of detritus and other debris. The writer realizes that studies based on contents of digestive tracts merely show what an animal will eat and has eaten shortly before capture. However, such facts do illustrate the fact that the sculpins, in the West Branch of the Sturgeon River, are apparently opportunists in regard to food and eat whatever becomes available. The accumulation of P^{32}, in the case of sculpins, would be as variable as the type of food ingested. The sculpins may, however, prefer to feed in a particular region and stay there until satiated. Thus, when feeding from a Chara bed, for example, they may eat whatever they can find there; and once they begin to feed from a Chara bed, they continue feeding there until their hunger is satisfied. Thus it was common to find stomachs filled with dozens of

Figure 23. Activity of brown trout at all stations. Counts were corrected for background and decay.

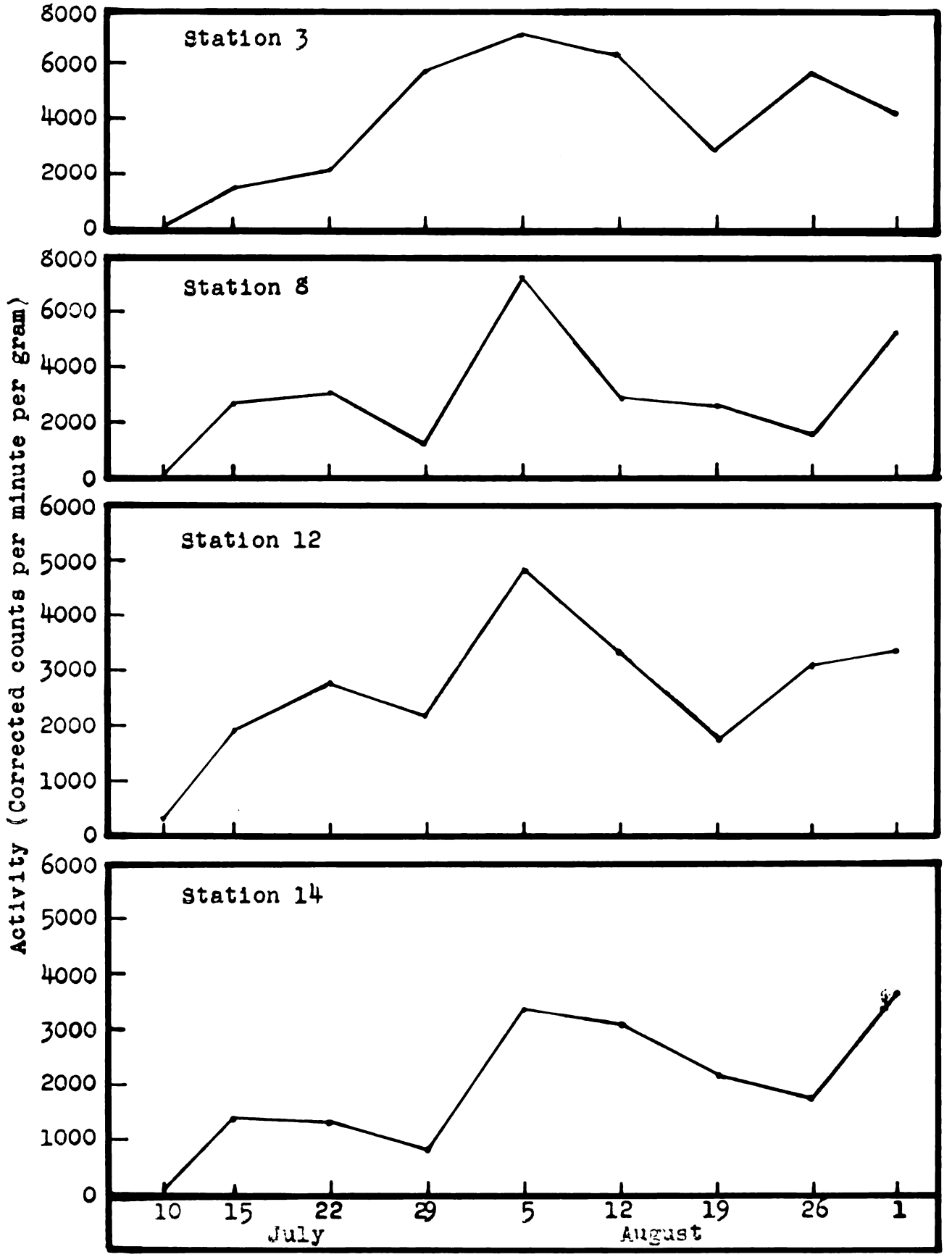
Station 3
Station 8
Station 12
Station 14
Activity (Corrected counts per minute per gram)
8000
6000
4000
2000
0
5000
3000
1000
10
15
22
29
5
12
19
26
1
July
August

Figure 23

individuals of one type of food. This behavioristic type of feeding would inject a great deal of variability into the activity pattern of the sculpin. The variability between sculpins collected at the same station at the same time showed great variability between each individual sculpin (Figure 24). For example, a sculpin that had fed on blackfly larvae or stonefly nymphs just previous to collection might show an activity of nearly 7,000 cpm/gm.; whereas a sculpin that had been feeding upon Hexagenia nymphs might exhibit an activity of less than 100 cpm/gm.

Still another factor in the variability of activity between individuals may be attributable to age. Among the fishes, it has been established by Olsen and Foster (1952) that the younger, more rapidly growing individuals accumulate relatively greater amounts of radioactivity than the older, more slowly growing ones. This phenomenon is probably a reflection of the more rapid anabolism that accompanies the growth of younger fish.

The activity curves for the brown trout, Salmo trutta fario Linnaeus, are shown in Figure 23. The maximum accumulation of P^{32} was 7,100 cpm/gm. reported for the collection at Station 8 on August 5, 1959, four weeks after isotope treatment. The initial uptake of P^{32} in the brown trout is somewhat slow at all stations until July 29, followed by a rapid uptake (except for Station 3) leading to a peak on August 5. The lag in initial P^{32} uptake may possibly be due to the fact that the brown trout is almost entirely

Figure 24. Activity of brown trout and sculpins following treatment of the stream with radiophosphorus. Counts were corrected for background and decay.

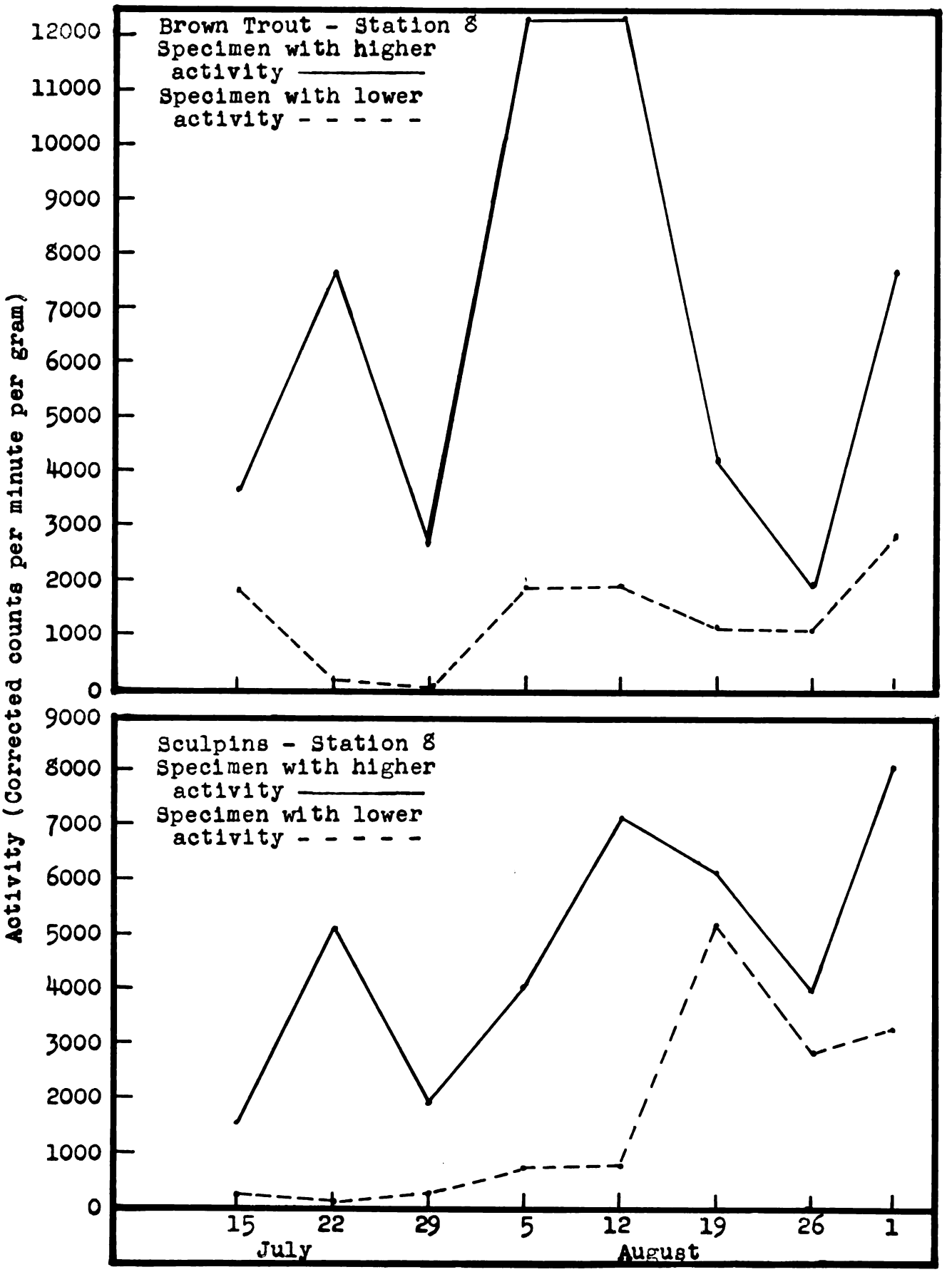
Brown Trout - Station 8
Specimen with higher activity
Specimen with lower activity
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Sculpins - Station 8
Specimen with higher activity
Specimen with lower activity
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Activity (Corrected counts per minute per gram)
15
22
29
5
12
19
26
1
July
August

Figure 24

carnivorous (Pentelow 1932). Radiophosphorus did not accumulate in the animals comprising the trout diet until the P^{32} was taken up by the primary producers and then accumulated in the trout only as a result of ingestion of plant material or other organisms. The appearance of a peak in activity, at all stations on August 5, further indicates the brown trout was feeding primarily on animal organisms which were probably reaching peak activity during this same period. The activity curves for the brown trout indicate a pattern. The higher activity values occurred at Stations 3 and 8 and decreased progressively downstream, a pattern similar to that found in many of the stream invertebrates.

The P^{32} activity values recorded for individual brown brout showed considerable variation. The variation between individual brown trout is shown in Figure 24. This variation is undoubtedly due to feeding habit and to the age of the individual. The influence age has on radiophosphorus uptake and accumulation in fish was discussed at length in the section on sculpin uptake and accumulation of P^{32}, and need not be repeated here. According to Muttkowski (ibid.), fish will take food that is easily captured and which is accessible. In general, trout are opportunists as far as their food is concerned. They eat what animal food is available, regardless of the origin. Neil (1938) found that the major items in the brown trout diet were the blackfly larvae, mayfly nymphs, caddisfly

nymphs, stonefly nymphs, leeches and other fish. Pentelow (ibid.) indicates somewhat similar food items for brown trout in his work on the Tees and Itchen Rivers. He found immature insects to be the item most frequently found in brown trout digestive tracts. The immature forms found by Pentelow (ibid.) were chiefly representatives of the stonefly, caddisfly and mayfly groups. He found water plants to be in complete absence in the brown trout diet.

In view of the tremendous variability in food items, it is little wonder that the activity of the individual brown trout varied considerably. It is further felt that variability between individuals may be due to feeding prior to capture. Because the samples were prepared from whole specimens of trout, the amount and kind of food material contained in the stomach would have some effect on the results in individual cases. For example, if a brown trout that had been feeding on the mayfly, Hexagenia, was captured and processed, the activity level would probably be greatly reduced when compared to an individual processed with its stomach gorged with stoneflies.

Radiophosphorus Accumulation in Various Tissues of the Brown Trout

Highest activity values in the brown trout were found in the bone, head and gills, and viscera, with much lower results in the muscle. The maximum value of 6,375 cpm/gm. (2.87×10^{-3} μc/gm.) was found in the bone of the brown trout. The value found in the head and gills was 2,111 cpm/gm.

(9.5×10^{-4} µc/gm.), while the value recorded for the brown trout viscera was 1,986 cpm/gm. (8.9×10^{-4} µc/gm.). The maximum value of 826 cpm/gm. (3.7×10^{-4} µc/gm.) was found in the muscle of the brown trout.

As trout may concentrate radioactive phosphorus many times above the level found in water, the use of trout for human consumption presents a potential public health problem (Robeck, et al., ibid.). Permissible levels of P^{32} of fish used for human consumption have been discussed by Donaldson and Foster (1957). They calculated the amount of radiophosphorus that would be expected in edible parts of fish, assuming an intake of 3 µc per week. This figure was based upon uptake from water containing the maximum permissible concentration for drinking water (International Committee on Radiation Protection). Donaldson and Foster's suggested maximum level is 7×10^{-4} µc P^{32} per gram for fish flesh, a figure which apparently includes a safety factor of 10. This value is substantially greater than the maximum recorded in trout muscle (3.7×10^{-4} µc/gm.) in the present study. Bone, viscera and head and gills of some of the fish in the present study exceeded this activity level slightly. It is pointed out, however, that the portion of the fish with the greatest activity value is not considered the edible part of the trout. Further, the fish are not eaten immediately after being caught, some time elapsing for cleaning, cooking and other preparation. Thus the actual permissible intake would be greater than that computed (Robeck, et al., ibid.).

Biomass Estimate

An estimate was made of the standing crop of fish in 1,000 yards of the West Branch of the Sturgeon River (isotope entry to Station 8) on August 25 and 28, 1959. The area encompassed by this section was calculated to be 1.76 acres.

The population estimate yielded the following data:

Table 2. Population and biomass estimates of fish in 1,000 yards of the West Branch of the Sturgeon River.

Fish Type	Pounds Per Acre	Population (Number of Fish)
Brown Trout	9.79	5,103
Brook Trout	1.12	91
Rainbow Trout	9.13	1,010
Sculpin	106.6	17,484

Standing crop estimates of fish, invertebrates and vegetation in various stream systems are given in Table 3.

On the basis of the data presented in Table 3, it becomes apparent that the West Branch of the Sturgeon River supported a very low standing crop of trout in 1959. The reason for this is not known but, according to professional sources acquainted with trout in this section of the river, the population of trout in 1959 was extremely low. The answer for this low standing crop may lie in factors

Table 3. A comparison of standing crop estimates of fish, invertebrates and aquatic plants for various streams.

Stream	Pounds of Invertebrates	Pounds of Trout Per Acre	Pounds of All Fish Per Acre	Pounds of Aquatic Plants Per Acre
West Branch, Sturgeon River (1958) (Bryant 1960)	53	86	101.8	
West Branch, Sturgeon River (1959)	36.58	20	126.6	21,780
Hunt Creek, Michigan (Shetter and Leonard, 1942)	72	94	104	
Houghton Creek Mich. (Ellis and Gowing, 1957)	138-411	120-127		
Big Spring Creek, Va. (Surber 1937)	483.9			
Prickly Pear Creek, Mont. (Sept. 1949) (Stefanich 1951)			92.3	
Prickly Pear Creek, Mont. (Sept. 1950) (Stefanich 1951)			58.9	

associated with the severe winter present in 1959. Whatever the cause, it affected the trout to a greater degree than invertebrates. The invertebrate estimate in 1959 is

about 68% of that reported for 1958, while the trout standing crop estimate is about 25% of that reported for 1958 (Bryant, ibid.).

The estimate for all fish in 1959 shows a greater standing crop than in 1958 or in any of the other streams in Table 3. This elevated value is due to a large estimated sculpin standing crop in 1959. Due to a scanty return of marked sculpin, a great deal of weight cannot be given the sculpin estimate in 1959.

Translocation of radiophosphorus Through the Ecosystem

The accumulation of radiophosphorus by aquatic organisms follows a definite pattern. The mean activity level of aquatic organisms and water, computed for all of the sampling stations, is shown in Figure 25. This figure provides a good picture of the fate of P^{32} as it passes along the food chain. In order to demonstrate the translocation of P^{32} along the food chain, the organisms are arranged according to broad trophic levels, represented by producers (plants), primary consumers (plant eaters) and secondary consumers (carnivores which eat the herbivores).

The P^{32} in the water reached a peak activity in 40 minutes after its arrival at the sampling stations. The radiophosphorus was first removed from the water by the periphyton and aquatic plants (producers), which reached a peak in approximately 240 minutes (4 hours) after isotope

Figure 25. Movement of radioactive phosphorus into the different components of a lotic ecosystem, following a single addition of P^{32} to the water. Counts were corrected for background and decay.

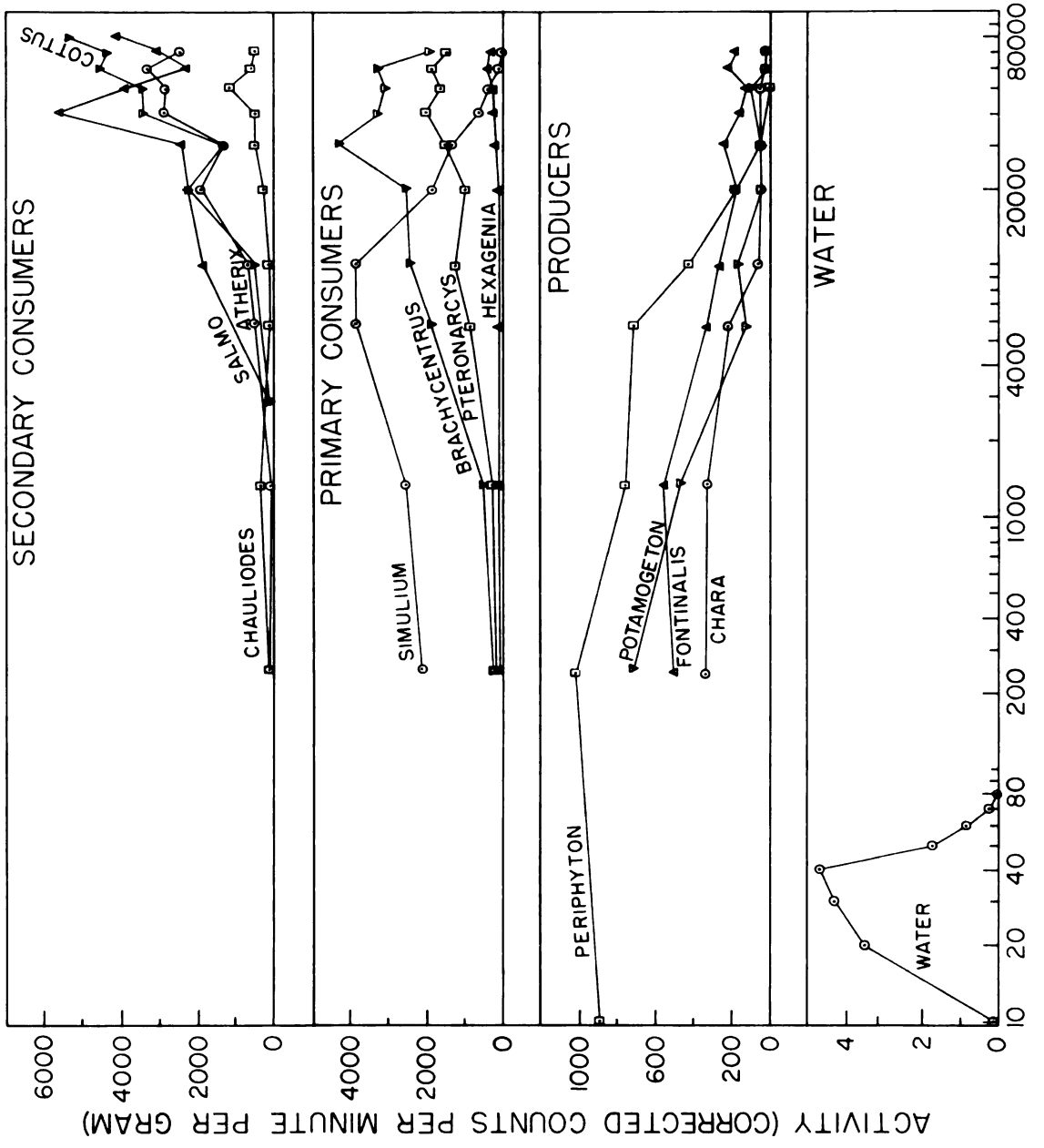
SECONDARY CONSUMERS
COTTUS
SALMO
ATHERIX
CHAULIODES
PRIMARY CONSUMERS
SIMULIUM
BRACHYCENTRUS
PTERONARCYS
HEXAGENIA
PRODUCERS
PERIPHYTON
POTAMOGETON
FONTINALIS
CHARA
WATER
WATER
ACTIVITY (CORRECTED COUNTS PER MINUTE PER GRAM)
6000
4000
2000
0
4000
2000
0
1000
600
200
0
4
2
0
10
20
40
80
200
400
1000
4000
20000
80000
TIME IN MINUTES

Figure 25

treatment. Uptake by the herbivores (primary consumers) was slower.

The blackfly larvae, Simulium, which filters bacteria and phytoplankton directly from the water, reached a peak in about 5,760 minutes (4 days) after isotope treatment; while maximum levels for the caddisfly larvae, Brachycentrus, and stonefly nymphs, Pteronarcys, were not reached for 30,240 minutes (21 days) and 40,320 minutes (28 days) respectively. The mayfly nymph, Hexagenia, did not reach a peak for 60,480 minutes (42 days) after isotope treatment. This slower uptake was due to the fact that it is a detritus feeder, living within detritus and silt beds and obtaining plant material only after its death and deposit in the back water silt and detritus deposits.

Uptake by the carnivores was even slower--as would be expected--than the primary consumers. Peak activity levels for animals feeding on herbivores were reached 40,320 minutes (28 days) and 80,640 minutes (56 days) for the brown trout, Salmo trutta fario Linnaeus, and sculpin, Cottus, respectively. The maximum activity levels for the fishfly larvae, Chauliodes, and snipe fly, Atherix, were not reached for 50,400 minutes (35 days) and 60,480 minutes (42 days), respectively.

On the basis of the data obtained in the present study, it becomes apparent that the radiophosphorus moves through an aquatic ecosystem in an orderly fashion, beginning with the primary producers and finally ending in the

secondary consumers. It is well recognized that the translocation does not stop at this point, but is further accumulated by secondary carnivores, which--in the present study--were not analyzed.

Water

The mean maximum activity level reported for the water was 4.74 cpm/ml.

Primary Producers

Periphyton: Periphyton organisms appear to accumulate P^{32} to a marked degree, the mean of the maximum levels observed at all of the stations being close to 230 times greater than that in the river water.

Larger aquatic plants: The larger aquatic plants rapidly accumulate P^{32} but not to the degree observed for periphyton. The mean maximum levels observed at all of the stations were about 72, 173 and 122 times greater for Chara, Potamogeton and Fontinalis, respectively, than that in the river water.

Primary Consumers

Aquatic insects: Radiological data on the herbivorous insects showed mean maximum activity accumulation levels of 818 for the blackfly larvae, Simulium; 884 for the caddisfly, Brachycentrus; 264 for the stonefly, Pteronarcys; and 87 for the mayfly, Hexagenia, times greater than that in the river water.

Secondary Consumers

Aquatic insects: Uptake by the predacious aquatic insects was slow with a mean maximum activity accumulation of 698 for the snipe fly larvae, Atherix, and 251 for the fishfly larvae, Chauliodes, times greater than that in the river water.

Fish: The fish accumulated the greatest mean maximum levels of P^{32} of all the organisms analyzed. The mean maximum activity accumulation for the sculpin, Cottus, is 1,139, while the brown trout, Salmo trutta fario L., is 1,186 times greater than that in the river water.

SUMMARY

Twenty-one (21) millicuries of radiophosphorus were added to the West Branch of the Sturgeon River (Section 21, T. 33 N., R. 3 W.) on July 8, 1959. The theoretical concentration of isotope in the water (at the point of entry) was calculated to be approximately 1.22×10^{-5} µc/ml. The translocation of radiophosphorus was followed throughout the entire ecosystem, thus obtaining information on the phosphorus dynamics of a natural system.

Radiophosphorus rapidly disappeared from solution, when introduced into the system, and entered the various components of the biomass. A large fraction of the isotope in open water moved immediately into the suspended material, including the phytoplankton and bacteria. These forms in turn distributed the isotope to other members of the system. The accumulation of radiophosphorus by aquatic organisms followed a definite pattern. The initial P^{32} uptake by the aquatic plant portion of the stream ecosystem collectively constituted a pool or retention reservoir for the isotope in that particular trophic level.

The components of the primary producer trophic level, which absorb nutrients directly from the water, showed variation in isotope uptake. The maximum activity level observed for the periphyton was approximately 2,600 cpm/gm., whereas the maximum values observed in the larger aquatic

plants were: Potamogeton, 1,700 cpm/gm.; Fontinalis, 1,600 cpm/gm.; and Chara, 560 cpm/gm. The explanation advanced for this variation in isotope uptake by plants is due to their differences in metabolic rate, as well as differences in the surface-to-volume ratio between the various components of the producer trophic level.

The producers in the present study exhibited an equilibration phenomenon. This was due to an initial physical uptake of P^{32} with rapid exchange with the water until an equilibrium was established, such that the amount of isotope leaving the water and entering the plants was balanced by the amount returned to the water from the solids.

The radiophosphorus accumulated in the primary producers became accumulated in the primary consumers as a result of ingestion as food. The maximum activity levels observed for the herbivorous invertebrates were: blackfly, Simulium, 6,000 cpm/gm.; caddisfly, Brachycentrus, 6,000 cpm/gm.; stonefly, Pteronarcys, 4,000 cpm/gm.; mayfly, Hexagenia, 700 cpm/gm.; and the American brook lamprey, 305 cpm/gm. The activity levels in the primary consumers were dependent upon their metabolic rates and the activity of the material on which they feed. The blackfly larvae, by way of its filter feeding habit, filter planktonic and organic material from the water and obtain--along with the caddisfly larvae--the greatest activity level of the herbivores. The caddisfly larvae which feed on periphyton accumulated P^{32} in nearly the same magnitude as the blackfly

larvae. The stonefly nymph which feeds on algae and vegetable detritus accumulated only about two-thirds the amount of maximum P^{32} observed in the blackfly larvae and caddisfly larvae.

The mayfly and the brook lamprey showed extremely low maximum activity levels. This may be due to the fact that they feed predominantly on detritus and may possess a low metabolic rate. The activity level of material available in the detritus was extremely low. The mayfly nymph showed a maximum P^{32} accumulation of only one-sixth of that observed in the stonefly and about one-ninth of the maximum level observed for the blackfly larvae and caddisfly nymph. The brook lamprey showed a maximum P^{32} accumulation of only one-half that of the mayfly nymph, about one-thirteenth that of the stonefly nymph, and about one-twentieth of that shown for the blackfly larvae and caddisfly larvae.

Radiophosphorus became accumulated in the secondary consumers as a result of ingesting primary consumers as food. The maximum activity values recorded for the secondary consumers were: slimy sculpin, Cottus cognatus gracilis H., 7,400 cpm/gm.; brown trout, Salmo trutta fario L., 7,100 cpm/gm.; fishfly larvae, Chauliodes, 2,500 cpm/gm.; and the snipe fly larvae, Atherix, 6,400 cpm/gm.

The maximum activity levels reported for the sculpin and brown trout were not surprising when one considers their food habits. Both of the fish feed predominantly upon aquatic insects, but eat insects or whatever else presents

itself to them. It is because of the opportunist feeding habit that a great deal of variation between the individuals arose.

The fishfly larvae is predacious but, apparently, feeds upon organisms accumulating only a rather low level of maximum activity because it accumulated only about one-third that observed for the fish and approximately a little less than one-half that reported for the predacious feeding snipe fly larvae. A variation in physiological make-up, phosphorus metabolism or preference of food items between the snipe fly larvae and the fishfly larvae may have been operating to bring about this difference in maximum P^{32} accumulation. It is interesting to note that the maximum P^{32} accumulation in the snipe fly larvae nearly approximated that reported for the sculpin and brown trout. Of course, the fact that the snipe fly is much smaller than the fish may make such a comparison somewhat invalid. It is generally accepted that the smaller the organism, the greater its metabolism per gram of biomass.

Uptake of P^{32} by the pouch snail, Physa, which is omnivorous (feeding on both plant and animal material) showed two nearly equal activity peaks. The first maximum reached nearly 9,600 cpm/gm. one week after isotope treatment and the second peak of 7,200 cpm/gm. occurred six weeks after the isotope treatment. The bimodal curve is a result of the snail's feeding habit. The first peak is a result of ingestion of plant material, while the second

peak is due to ingesting animal material. Thus the snail received P^{32} from both the producer and consumer trophic levels.

The movement of P^{32} through the various components of the stream ecosystem was found to follow a definite pattern. The mean activity of the stream water reached a peak 40 minutes after isotope treatment. The mean activity of the primary producers reached a peak approximately 4 hours after isotope treatment, whereas the primary consumers mean peak activity was reported to range from 4 days for the blackfly larvae to 28 days for the stonefly. The mean maximum activity levels observed for the secondary consumers reached peak activity during the period of 28 days for the brown trout to 56 days for the sculpin.

A population estimate of all the fish in 1,000 yards of stream was obtained, as was a random quantitative standing crop biomass estimate for bottom invertebrates and larger aquatic plants. The biomass estimate for the trout was computed to be 20 pounds per acre, while the biomass estimate for all of the fish was 127 pounds per acre. The biomass estimate for invertebrates was calculated to be 37 pounds per acre, while the estimate for larger aquatic plants was found to be 21,780 pounds per acre. The results showed that the West Branch of the Sturgeon River was very unproductive on the basis of trout and invertebrate production estimates.

APPENDIX

Table 4. Highest activity values recorded in aquatic organisms from the West Branch of the Sturgeon River after the addition of radiophosphorus.

Organism	Activity (Microcuries Per Gram at Time of Collection)	Location (Station)	Date 1959
Water	5.4×10^{-6}	5	7-8
Plant Material			
Periphyton	1.17×10^{-3}	8	7-8
Chara	2.5×10^{-4}	8	7-8
Fontinalis	7.2×10^{-4}	3	7-9
Potamogeton	7.7×10^{-4}	3	7-8
Batrachospermum	9.9×10^{-4}	8	7-12
Invertebrates			
Simulium	2.7×10^{-3}	8	7-12
Brachycentrus	2.8×10^{-3}	8	7-22
Pteronarcys	2.2×10^{-3}	3	8-5
Hexagenia	3.3×10^{-4}	8	8-19
Atherix	2.9×10^{-3}	12	8-19
Chauliodes	5.6×10^{-4}	8	8-5
Physa	4.3×10^{-3}	8	7-15
Vertebrates			
Entosphenus	1.4×10^{-4}	12	8-19
Cottus	5.2×10^{-3}	3	8-26
Salmo	5.99×10^{-3}	8	8-12

Table 5. A list of organisms found in the West Branch of the Sturgeon River, 1959. The organisms presented have been identified as far as possible or practical in the limited time available. This list is based on the taxonomic keys presented by Pennak (1953), Burks (1953), Usinger (1956) and Hubbs and Lagler (1958).

Vertebrata

Cottus bairdii bairdii Girard

Cottus cognatus gracilis Heckel

Salmo trutta fario Linnaeus

Salvelinus fontinalis (Mitchill)

Salmo gairdnerii irideus Gibbons

Entosphenus lamottenii lamottenii (LeSueur)

Diptera

Tipulidae

Tipula sp.

Simuliidae

Simulium sp.

Chironomidae (= Tendipedidae)

Pelopiinae

Diamesinae

Ceratopogonidae (= Heleidae)

Palpomyia sp.

Stratiomyiidae

Odontomyia sp.

Anthomyiidae

Table 5, continued

Rhagionidae

Atherix variegata Walker

Empididae

Tabanidae

Tabanus sp.

Chrysops sp.

Coleoptera

Elmidae

Dytiscidae

Haliplidae

Hemiptera

Corixidae

Gerridae

Gerris sp.

Odonata

Gomphidae

Cordulegasteridae

Cordulegaster sp.

Libellulidae

Megaloptera

Corydalidae

Chauliodes sp.

Table 5, continued

Plecoptera

Pteronarcidae

Pteronarcys sp.

Perlidae

Paragnetina sp.

Chloroperlidae

Ephemeroptera

Ephemeridae

Ephemera sp.

Hexagenia sp.

Heptageniidae

Heptagenia sp.

Baetidae

Ephemerella sp.

Caenis sp.

Iron sp.

Trichoptera

Rhyacophilidae

Glossosoma sp.

Rhyacophila sp.

Philopotamidae

Dolophilodes sp.

Psychomyiidae

Hydropsychidae

Hydropsyche sp.

Table 5, concluded

Limnephilidae

Astenophylax sp.

Limnephilus sp.

Brachycentridae

Brachycentrus sp.

Molannidae

Molanna sp.

Goeridae

Goera sp.

Leptoceridae

Triaenodes sp.

Leptocerus sp.

Miscellaneous Invertebrates

Annelidae

Oligochaeta

Hirudinea

Amphipoda

Gammarus sp.

Gastropoda

Physa sp.

Pelecypoda

Sphaerium sp.

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