THE TRANSLOCATION OF RADIOPHOSPHORUS THROUGH A LOTIC ECOSYSTEM

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John L. Zettelmaier

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THE TRANSLOCATION OF RADIOPHOSPHORUS THROUGH A LOTIC ECOSYSTEM

By
JOHN L. ETTEIMAIER

AN ABSTRACT

Submitted to
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ABSTRACT

On July 5, 1960, twenty-three millicuries of radioactive phosphorus (P^{32}) were added to the West Branch of the Sturgeon River, Cheyboygan County, Michigan. The main objective was to determine the physical and biological translocation pathways of nutrients in the stream.

The physical pathways include the removal of P^{32} from the current by adsorption, and the capturing of P^{32} from the main current by diversions and pools.

Most of the P^{32} was removed from the current before traveling the 3,200 yard experimental stream section. Much of the P^{32} was immediately incorporated in the organisms of the stream. The radiophosphorus was also adsorbed onto the stream bottom, particulate matter, and organisms.

The biological pathways included the removal of P^{32} from the water by biological incorporation, and the translocation of biologically incorporated P^{32} through the food chains of the stream ecosystem.

Uptake of P^{32} followed the trophic level scheme for the stream biota. The radiophosphorus was first incorporated by the autotrophic organisms (periphyton and other aquatic plants). The primary consumers which obtain immediate high activity values were those which possess a filtering-feeding mechanism. These animals are able to ingest the radiophosphorus which is adsorbed onto the particulate matter or in diatoms being carried along with the major isotope flow.

Most of the primary consumers obtained measurable amounts of P^{32} after the producers (plants) reached their activity peaks. Then, by ingestion, the activity was shifted to the consumer trophic level. Between the primary and secondary trophic level transition, the activity which accumulated in the bottom due to adsorption and death of radioactive organisms appeared in the organisms which inhabit the stream bottom.

The culmination of translocation by ingestion occurred in the secondary consumers. The P^{32} accumulation of these organisms occurred toward the end of the collecting period.

The radiophosphorus was biologically translocated through the trophic levels of the system. The producers accumulated the activity first, the secondary consumers accumulated the activity last, and the accumulation by the primary consumers occurred between these extremes.

J. L. T.

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INTRODUCTION

The study of physical and biological radioisotope translocation supplies information concerning several problems of civilized societies. The knowledge of nutrient cycles of streams and rivers supplies the information needed to ascertain the feasibility of increasing fish populations by applications of fertilizers. The pathways of radioisotope translocation gives information concerning the effects of environmental manipulation. As nuclear power plants are being installed, greater knowledge about radioactive effluent translocation will be necessary for the survival of man. Through the study of introduced isotopes, man may obtain the knowledge necessary to alter or retard the movement of radioactive contaminants by the manipulation of ecological factors.

Therefore, information on the ability of biological populations to concentrate radioactive materials from the water, and the role of these populations in redistribution of radioactive materials, may enable man to adjust his environment to preserve his species.

The information obtained from radioisotope translocation is also important in ascertaining feeding habits, food habits and food chains. The organisms can be related to their position in the trophic levels by noting the position and time of their activity curves, i.e., the time interval between initial isotope entry and their peak activity.

The purpose of this paper is to contribute information on 1) physical and biological modes of transfer, removal,

accumulation and translocation of radiophosphorus; (2) the position of organisms in the trophic levels within a stream; (3) the methods of translocation of nutrient materials and (4) the effect of chelates on the nutrient cycles of an aquatic environment.

Description of the Study Area

The West Branch of the Sturgeon River is a moderately rapid, cold water river, having its origin in Hoffman Lake, a marl lake of approximately 128 acres. The West Branch of the Sturgeon River flows through sections of Cheboygan, Otsego and Charlevoix Counties, Michigan (T. 33 N., R. 3 W), joins the main Sturgeon River at the town of Wolverine, Michigan, and empties into Burt Lake in Cheboygan County.

The West Branch of the Sturgeon River flows in a northeasterly direction, through a narrow valley with steep glacial morainic hills. The vegetation of the valley is primarily birch, aspen, cedar, balsam, fir and tamarack. The vegetation along the stream margin proper is cedar, aspen, tag alder, tamarack and ninebark.

The water temperature throughout the summer remains between 50°F and 59°F. The river temperature remains low due to heavy shading and the inflow of tributaries and numerous springs.

The rate of stream flow in the study area has a mean value of 43.75 cubic feet per second.[1] The water level, as measured by two gauges, remains approximately constant throughout the summer. Due to heavy rains, sharp changes in the water level occur, but the level descends to the average value in just a few hours.

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[1]Courtesy of Carr & Vannote, 1959.

The stream water in the study area has a total alkalinity and total hardness of 181 p.p.m. (Bryant, 1960) and a total phosphorus concentration of approximately 7 p.p.b. (Borgeson, 1959). High oxygen concentrations are insured through turbulence and low temperatures.

The length of the study area from Station 1 through Station 14 is 320[illegible]. The stream bottom is generally composed of sand, gravel and marl, with many silt beds along the periphery.

The biota is typical of cool, fast streams. The flora includes beds of Chara sp.; the water moss, Fontinalis antipyretica; mare's-tail, Hippuris vulgaris; water cress, Nasturtium officinale; pondweed, Potamogeton pectinatus; tape-grass, Vallisneria sp., and some sparse mats of Batrachospermum moniliforme and Oedogonium sp. The fauna includes the typical cold water forms. The fish present are the eastern slimy sculpin, Cottus cognatus; northern mottled sculpin, Cottus bairdii; brown trout, Salmo trutta; rainbow trout, Salmo gairdneri; brook trout, Salvelinus fontinalis and the American brook lamprey, Entosphenus lamottenii. The aquatic insects present are represented by the following orders; Odonata, Plecoptera, Ephemeroptera, Trichoptera, Coleoptera, Diptera, Megaloptera and Hemiptera. Other invertebrates present are annelids, gastropods and pelecypods.

Sampling Stations

Sixteen stations within the test section of the stream

were established. Stations 3, 8, 12 and 14 were the sites of weekly collections of aquatic plants, periphyton and aquatic invertebrates. The other stations were used for water samples during the flow of the isotope. The location of the stations is shown in Figure 1 and the descriptions of the stations where routine collections were made are as follows:

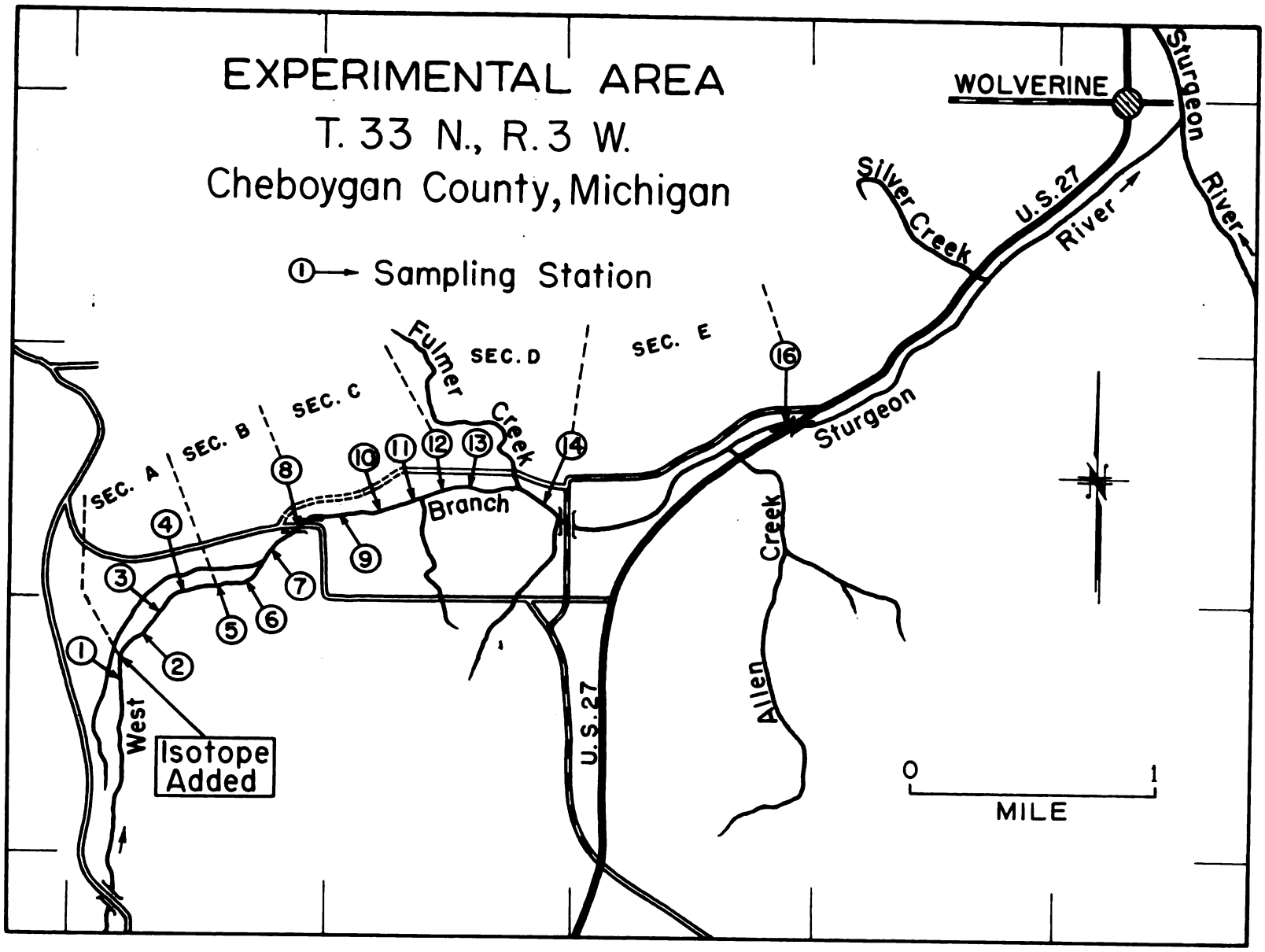
Station 3

This station is 200 yards below the isotope entry site which is designated Station 1. Station 3, thus, is the nearest station to the isotope entry at which collections were made. The station is 100 yards long, as are all the collections stations. The average water depth at this station is 12.8 inches. The average width is 25 feet. The stream bottom is mainly sand with smaller areas of gravel and silt. The vegetation is sparse, with a total mean wet-weight of 72 grams per 100 yards. The floral composition is as follows: Potamogeton pectinatus, 50%; Fontinalis antipyretica, 35%; and Chara sp., 14%. The area is heavily shaded by cedars and tamaracks. The rate of water flow is retarded at this site due to several log obstructions.

Station 8

Station 8 is 900 yards downstream from Station 1, the isotope entry site. The downstream portion of this area is crossed by a bridge which is 1030 yards below Station 1. This bridge serves as a dividing line, subdividing the complete study area into an upstream and downstream portion.

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


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

Thus, Stations 1 through 8, above the bridge, are considered to be the upstream half of the study area. Station 8 has the greatest average depth of the sampling stations. Its average depth is 17.2 inches. The average width is 26 feet. This relatively narrow and deep run supports a luxurious aquatic plant crop. The vegetation represents a total mean wet weight of 546 grams per 100 yards. The floral composition includes filamentous algae, 51%; Fontinalis antipyretica, 27%; Elodea sp., 5%; Potamogeton pectinatus, 4%; and Chara sp., 0.1%.

Station 12

Station 12 is 2540 yards below Station 1. The sampling area includes a long, straight run at the upstream portion and a sharp bend at the downstream portion. The average water depth at this station is 13.3 inches. The average width is 23 feet. The principle bottom type is gravel with silt beds along the periphery.

Chara sp. is the most abundant aquatic plant in this area. The total mean wet weight of the vegetation is 526 grams per 100 yards. Chara sp. represents 92% of this mass while Fontinalis antipyretica accounts for 4% and filamentous algae for 2%. Potamogeton pectinatus patches are very sparse and contribute only 0.1% of the total wet weight.

Station 14

The site of this station is 3110 yards below Station 1. The sampling area is a rapid riffle area having the least depth of the collecting stations. This shallow area has an

average water depth of 12.2 inches. The width of the sampling area, however, is the greatest of the collecting stations; the average width being 29 feet. This wide and shallow run of water supports the heaviest aquatic plant growth. The total mean wet weight for the aquatic plants is 689.6 grams per 100 yards. The principal constituents are: Fontinalis antipyretica, 35.7%; Chara sp., 25.1%; Elodea sp., 16.1%; Nasturtium officinale, 13.1%, filamentous algae, 7.7%; Hippuris vulgaris, 1.7%; and Potamogeton pectinatus, 0.7%. This station has the largest stands of water cress, Nasturtium officinale, along the banks. Water cress is quite sparse at the upstream stations and seems to prefer the shallow, silted sites.

METHODS AND PROCEDURES

Biomass Estimate Techniques

Aquatic Plants

On August 23 through August 30, 1960, a quantitative study was made of the aquatic plants in the West Branch of the Sturgeon River.

The entire stream was divided into transects which were 10 yards apart. The transects were, in turn, subdivided horizontally into 3-foot sampling spots for the entire width of the transect. In order to sample each station, each 100 yards apart, it was decided that three transects of plants would be collected. Thus, for Station 1, transect numbers 10, 20, and 90 were collected. The transect numbers were selected randomly to eliminate bias.

The samples were collected starting at the upper end of the survey area and proceeding downstream. The collection was made with the Surber square-foot sampler, placed at three-foot intervals along the transect. The entire plant population encompassed by the Surber sampler was removed. The samples were washed in the stream to remove debris. The samples were then segregated into taxonomic types and the types independently weighed for each transect. The weight recorded from the beam balance was designated as the wet weight of the plant sample. The weights and composition percentages are presented in Appendix I.

Aquatic Insects

An insect biomass estimate was obtained for 100 yards of

the experimental area. In order to preserve specimens at the radiological collecting stations, the estimate was taken at Station 11. The area was divided into five transects which were, in turn, subdivided horizontally. The transects and the sampling sites on the transects were picked randomly. The entire insect population encompassed by the Surber square-foot sampler was removed. There were four random sampling sites on each transect and a total of five transects collected.

The samples were weighed and the pounds of insects per acre were computed. The area of this section was .20 acres. The numerical composition was calculated by Knight (1961) and was not repeated in this study. The biomass estimate was obtained for comparison with that of previous years. A table comparing the invertebrate biomass of the West Branch of the Sturgeon River for the years 1958, 1959 and 1960 is presented in Appendix II.

Fish

A fish biomass estimate was taken for Stations 3, 8, 12 and 14. At each station, a 100 yard section was sampled. A fine-mesh screen was installed both above and below the sampling section. The screen was periodically brushed to prevent collapse from debris accumulation.

Electrical shocking was used to collect the samples. The unit was a 220 volt, DC generator mounted in a small, flat-bottomed skiff.

The fish samples were captured, clipped, and returned to the stream. The number of clipped fish recaptured gave an

estimate of the percent recovery. The collecting procedure was continued until no further fish were captured. The lowest percent recovery was at Station 12 where only 25% of the clipped fish were recaptured. The fish biomass estimate is presented in Appendix III.

Radiological Techniques

The isotope employed in the present study was radiophosphorus (P^{32}) which is a beta ray emitter with a maximum energy of radiation of 1.70 mev. The P^{32} is produced by the S(n,p)P reaction in the uranium pile at Oak Ridge, Tennessee at the Oak Ridge National Laboratory (Kamen, 1957). No appreciable gamma radiation is observable and the beta emission intensity can be cut to half-value by 0.5 mm aluminum foil. Radiophosphorus is a desirable tracer because it possesses a satisfactory half-life of 14.3 days, it is not dangerous in minute quantities to the aquatic organisms, and because of its low volatility and loss under heat. The low volatility is desirable because there is no appreciable loss of P^{32} in the digestion procedures.

The phosphate anion was dissolved in weak hydrochloric acid. The assayed value of the isotope on July 5, 1960 was 23.29 $\pm$ 3 percent millicuries. The isotope entered the West Branch of the Sturgeon River on July 5, 1960 at 9:30 a.m.

Borgeson (ibid.) and Clifford (1959) have described the method of isotope entry into the West Branch of the Sturgeon River. The method consists of diluting the 1.1 ml. solution

of P^{32} with 50 gallons of stream water in a 55-gallon drum. This solution is siphoned from the drum by a polyethylene tube. The isotope enters the stream at a constant rate for a 33-minute period. The flow of the stream at Station 1 is approximately 38 cubic feet per second and this figure combined with the rate of isotope entry gives the calculated concentration of the isotope in the water. The calculated value is approximately 1.22×10^{-5} microcuries per milliliter. The amount of isotope used is in keeping with the maximum permissible amount which may be used in water according to the National Bureau of Standards (1953). The National Bureau also states that permissible concentrations of radioactive materials beyond the control area in water should be 10^{-7} microcuries per milliliter.

Measurement of Activity

The beta emissions were measured with a Nuclear Measurements Corporation Internal Flow Proportional Counter, PCC-1CA, which was coupled to a decade scaler (Model PC-1A). Each morning a 30-minute background count was made using an empty counting chamber. The background varied from approximately 48 to 51 counts per minute with a mean value of 50 counts per minute. Samples were counted for a minimum of three minutes or until a count of 1,000 was reached.

Correction Factors

The actual counts obtained are meaningless unless they

can be compared with activities of other organisms. To convert the actual counts to an absolute value of corrected counts per minute, several correction factors are applied to the raw counts. The correction factors are given by Robeck et al. (1954).

Background: The raw counts are affected by a certain level of activity due to cosmic radiations or radioactive substances in the counting room. The mean background value of 50 counts per minute was, therefore, subtracted from all raw counts to correct for the radioactivity recorded from sources other than the sample.

Volume factor: Due to the variability in sample size, comparable counts must have some constant value. To arrive at this constant, the recorded counts per minute, minus background were divided by the weight of the sample in grams, or, as in the case of water, by the volume in milliliters.

Decay factor: Due to the decrease of one-half of the radioactive atoms in a sample in 14.3 days, counts must be corrected for radioactive decay. Tables for calculation of radioactive decay are available from isotope suppliers.

The comparable corrected counts were arrived at by the following method; where BG = background, VF = volume factor and DF = decay factor:

Corrected counts per minute = (cpm - BG) x (VF) x (DF)

It may be desirable to present the results in terms of microcuries. The calculation outline from Robeck, et al. (ibid.) is as follows:

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

1 microcurie (μc) = 3.7×10^{4} dps

= 2.22×10^{6} dpm

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

The conversion factor (CF) = 4.5×10^{-7}

Microcuries = (cpm - BG) x (VF) x (DF) x (4.5×10^{-7}).

Water

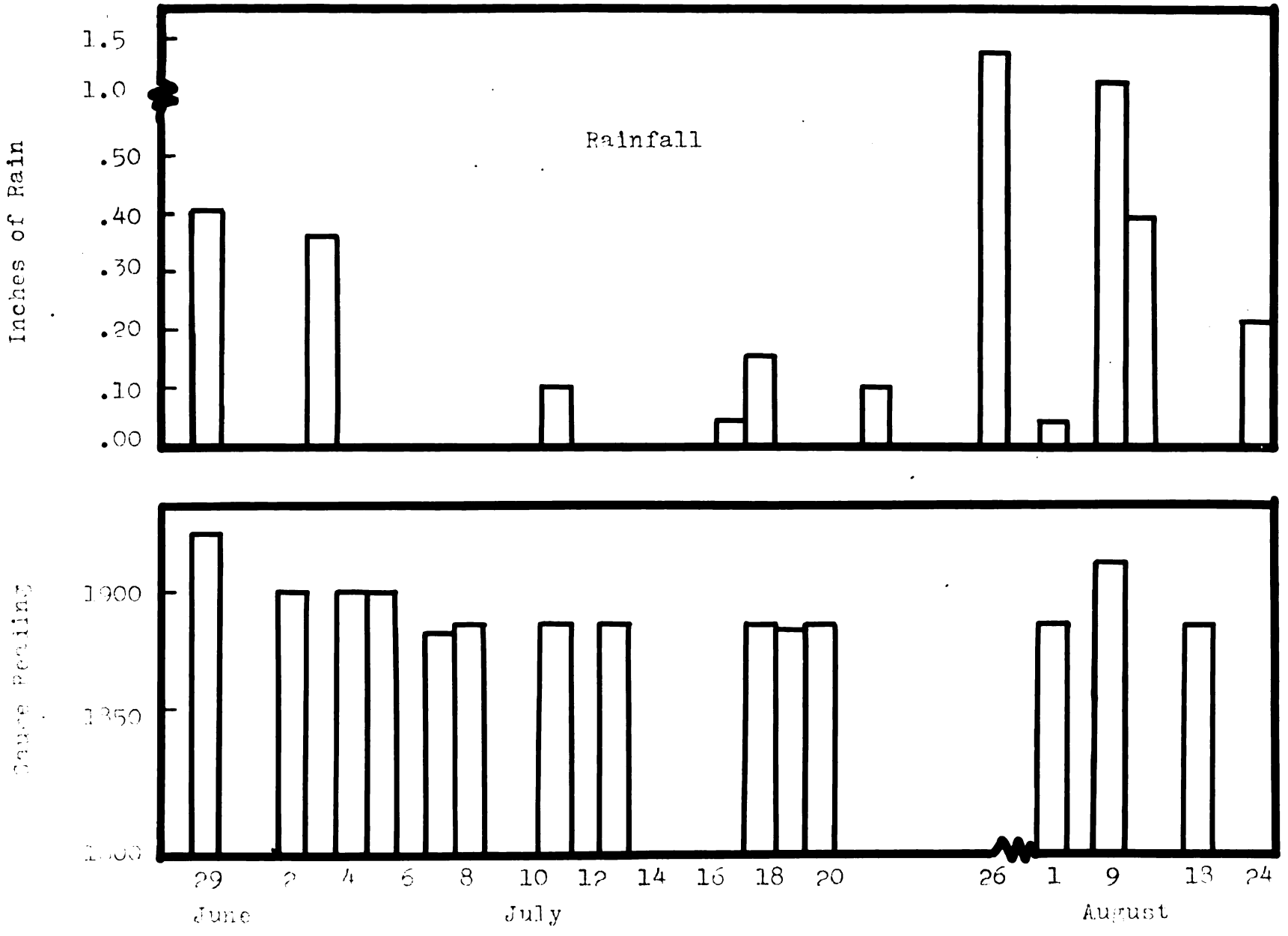
Stage and Rainfall

The fluctuation of the water level of the West Branch of the Sturgeon River was recorded for the duration of the summer project. The depth variations were read from a water level gauge located two yards below Station 8 bridge. The water level remained constant with the exception of periodic rises of short duration caused by heavy rainfall. Figure 2 presents the data for rainfall and water level fluctuation. Excess water entering the system is carried through in just a few hours, and in cases of night rainfall, the excess water was not detected as water level change on the gauge reading the following morning.

Field Procedures in Collection of Water Samples

Primary small samples. On July 3, 1960 a fluorescein dye was released at the isotope entry point to determine the approximate arrival time of the isotope at the individual stations. It was assumed that the dye would react to the water currents in a similar manner to the isotope. The stations maintained for water-activity sampling were Stations

Figure 2. Comparison of the rainfall and water level for the summer of 1960.


Rainfall
Inches of Rain
1.5
1.0
.50
.40
.30
.20
.10
.00
1900
1350
29
June
2
4
6
8
10
12
14
16
18
20
July
26
1
9
18
24
August

3, 5, 8, 12, 14 and 16. Three stations, 8, 11 and 12, were the sites of automatic water sampling devices constructed by Dr. R. C. Ball.

Five minutes before the isotope release at Station 1, the fluorescein dye was released to indicate to the collectors that the isotope flow was approaching. At each collecting station additional dye was added to insure that the green color would be visible at the lower collecting stations.

At the upstream stations, i.e., Stations 3, 5 and 8, the water samples were collected at five-minute intervals after the five-minute delay following the fluorescein dye flow. The duration of the sampling time at the upstream collecting stations was eighty minutes. The samples were collected in clean, labeled 140 ml. polyethylene bottles. Each bottle was immediately capped and the collection time was recorded.

At downstream Stations 12, 14 and 16, the primary samples were collected at ten-minute intervals. The duration of the collecting time at Stations 12 and 14 was eighty minutes. The duration of the collecting time at Station 16 was 160 minutes. Station 16 was the check station to measure the radioactivity that left the experimental area. The sampling of the downstream stations followed the same method as that of the upstream stations, with the exception of the collection time intervals.

Single large samples. When the peak of the isotope flow, as calculated from previous years, reached the station, a liter bottle was submerged, filled, capped, and labeled.

Plankton samples. At Stations 8 and 12, a plankton sample was collected. The sample permitted determination of radiological transportation in particulate matter. A fine mesh plankton net, No. 20 bolting silk, was washed in the stream before the dye reached the station. Then during the isotope flow, ten two-quart jars of stream water were poured through the net. Five of these were taken before the calculated isotope peak flow and five were taken after the calculated peak.

Automatic pump samples. Three automatic water-sampling devices were installed at Stations 8, 11, and 12. The units were basically six four-liter collecting bottles in a wooden frame, a 12 volt battery, a 120-110 volt inverter, a timer, and a small submersible pump. The apparatus was so designed that when the first bottle was filled, the water would be routed into the second bottle, and so on until all six were filled. In this way, the water was pumped into the bottles at prescribed times. During the isotope flow, the pumps were synchronized and collected water samples at fifteen-minute intervals. The four-liter polyethylene bottles were capped and taken to the laboratory for radiological analysis.

Laboratory Processing of Water Samples

The preparation of the samples for radiological analysis follows a modified version of the methods suggested by Robeck, et al. (ibid.).

Primary small samples. Three milliliters of concentrated nitric acid was added to the 140 milliliter sample bottle.

The bottle was then shaken thoroughly, a 50 ml. subsample measured into a 150 ml. beaker. The water in the beaker was evaporated on a hot plate until the sample just covered the bottom of the beaker and was then poured into a stainless steel counting planchet. The 150 ml. beaker was washed with 2N nitric acid. The sides of the beaker were scraped with a rubber policeman and the remaining drops removed with a medicine dropper. The contents of the planchet were evaporated to dryness on a hot plate, then placed in a covered aluminum pan and transferred to the counting room where the activity of the sample was determined.

Single large samples. A 500 ml. subsample was taken from each of the liter bottles collected at the calculated peak activity flow. The 500 ml. subsample was filtered through an HA, 47 mm. Millipore filter. The filtrate was then placed in a beaker and evaporated until only a thin layer was left on the bottom of the beaker. The contents were then emptied into a planchet and the beaker was washed with 0.1N nitric acid. The washings were also emptied into the planchet, evaporated to dryness, then placed in a muffle furnace at 725°C for two minutes.

The original subsample (minus the filtrate), with the filter intact, was then washed with 0.1N nitric acid. This was done to remove the adsorbed activity. The adsorbed activity differs from biologically incorporated activity in that it adheres to surfaces and is not incorporated into the organism. The second filtrate was then placed in a separate

planchet which was treated in the same manner as the first filtrate.

The filter pad containing only the solid particles from the water was removed and placed in a planchet. Due to the removal of adsorbed activity, these solid particles possessed only biologically incorporated activity. Concentrated nitric acid was added to the filter pad in the planchet which was placed under a heat lamp and additional acid added until the filter pad was completely digested. The planchet was then placed in the muffle furnace, heated, and then counted.

Plankton samples. The plankton samples were evaporated until little residue remained in the bottom of the beaker. The material was then transferred to a planchet, muffled and counted.

Automatic pump samples. Twenty milliliters of concentrated hydrochloric acid were added to the four-liter polyethylene bottles and the bottles thoroughly shaken. A 500 ml. subsample was removed and filtered in the Millipore filter. The filtrate was placed in a beaker and evaporated until only a small amount of sample remained. The contents were then transferred to a planchet, the beaker washed and scraped and the washing added to the planchet. The contents of the planchet were evaporated and muffled.

Periphyton

Fertilization

In preliminary investigations of the stream in 1958 (Clifford, ibid.) the periphyton growth was slow to become

established on the artificial substrates. In an effort to increase periphyton growth, an inorganic fertilizer was added to the stream to provide a sufficient growth for radiological examination during the 1959 experiment (Knight, ibid.). During the 1960 investigation, fertilizer again was added. Between June 23 and June 29, 240 pounds of diammonium phosphate had been added to the experimental area. One-half of the fertilizer was added at Station 1 and the remaining 120 pounds was added just above Station 7.

Field Procedures in Collection of Periphyton

The periphyton was collected by means of plexiglass plates which served as substrates upon which periphyton could grow. This gave a base of known area which could be removed easily from the stream and from which the attached material could be removed, weighed and examined for the presence of the isotope. Six plates 7 millimeters thick, with an area of 1.4 decimeters (2" x 5") were attached to a horizontal crossbar which was in turn attached to a steel post and lowered into the water.

The periphyton growths were started two weeks prior to the isotope entry. The stations used as collecting sites were 3, 8, 12 and 14.

Stations 3, 8, 12 and 14 each had a rack of 16 plates in the water when the isotope passed through the system. The substrates which experienced the direct isotope flow were called "in" units. The "in" units were collected four hours after isotope entry and weekly thereafter on Mondays.

A control procedure was utilized by placing one rack of 16 plates at Station 8 and at Station 12 approximately nine hours after the water mass containing the isotope had passed through the system. These substrates which were not in the isotope flow were designated "out" units. The "out" units were collected 24 hours after the isotope entry and weekly thereafter on Wednesdays. Stations 3, 8, 12 and 14 thus had a rack of 16 plates designated as "in" units. Stations 8 and 12, in addition to the "in" units, each had a rack of 16 plates designated as "out" units.

Two plates were removed from the cross-bars at each station and were immediately replaced by new ones for later collections. Insects and other aquatic invertebrates were removed from the plates, then the substrates were wrapped in polyethylene sheeting and taken to the laboratory.

Laboratory Processing of Periphyton

The periphyton was scraped off the plates and into a beaker with a polished glass slide. The wet weight of the periphyton was determined as follows: the wet weight of a 47 mm membrane filter was first obtained by pouring 20 ml. of distilled water into the filter apparatus with the vacuum pump running; the pump was allowed to run for 15 seconds. The filter was placed in a clean planchet and weighed. The periphyton was poured into the filter apparatus which contained the previously weighed filter paper. The filter apparatus was rinsed with 3 ml. of 0.01N hydrochloric acid. This was followed by a second rinse of 5 ml. of distilled water. Fifteen seconds after the filter was observed to be free of water,

the pump was turned off. The filter was removed and placed in the same planchet and this unit was weighed. The original planchet and filter weight was subtracted, giving the wet weight of the periphyton. This was assumed to be the "live" weight of the periphyton.

The sample was digested by adding 15 ml. of concentrated nitric acid, and heating the beaker on a hot plate. The evaporated contents were then placed in a planchet. The planchet was heated on the hot plate until dry and then placed in the muffle furnace.

Aquatic Plants

Field Procedures in Collection of Aquatic Plants

The collecting sites for aquatic plants were Stations 3, 5, 8, 12 and 14. Station 5 was used as the collecting site for Nasturtium officinale as it was not present at Station 3. The plants used in the study were Chara sp.; the pondweed, Potamogeton pectinatus; water cress, Nasturtium officinale; and the water moss, Fontinalis antipyretica. The four aquatic plants were collected at each of the stations with the exception of water cress.

The plants were collected by hand. Insects and debris were removed from the samples by washing them in the stream. The roots were discarded and only the leaves and stems were used for radiological analysis. Samples were placed in collecting bottles with dilute formalin and taken to the laboratory.

Laboratory Processing of Aquatic Plants

The plants were taken from the sample bottles and allowed to dry on paper towelling for five minutes, then placed in

evaporating dishes and weighed. The known weight of the evaporating dishes was subtracted from the total weight giving the plant weight. The plants in the evaporating dishes were covered with concentrated nitric acid and placed on a hot plate. When the material had been heated to a point where evaporation began, acid was added until the solution became clear. The clear sample was transferred to a planchet and evaporated to dryness. The planchet was then examined to determine if it possessed a grey or white cast. If not, the heating and acid procedure was repeated. When the planchet samples were properly processed, they were muffled and later counted.

Invertebrates and Larval Insects

Field Procedures in Collection of Invertebrates and Larval Insects

The invertebrates were collected at Stations 7, 8, 12 and 14. The scuds, Gammarus sp., were found only at Station 14. The invertebrates were collected 24 and 72 hours after the isotope entry and weekly thereafter on Mondays.

The invertebrates collected included the blackfly, Simulium sp.; the net caddis, Hydropsyche sp.; the mayflies Ephemerella cornuta sp.; Ephemerella needhami: scuds, Gammarus sp. and oligochaetes.

There were several methods employed in collecting the invertebrates. Hand picking was the principal method. The invertebrates were found on logs, aquatic vegetation, stones and terrestrial vegetation which dirped into the stream. Forceps were used to pick invertebrates from niches in stones, logs, and so on.

The sugar flotation method was found to be very effective in collecting oligochaetes. This process consists of placing a handful of detritus in a white enamel pan. Water and sugar are added. It was found that a few drops of dilute formalin facilitated the process. The organisms wiggle to the surface and can be easily collected. They were placed in collecting bottles containing dilute formalin and transported to the laboratory.

Laboratory Processing of Invertebrates and Larval Insects

The samples were first processed in a centrifuge. Screens were washed with distilled water and set in place in the centrifuge. With the centrifuge turned to full speed, they were spun for 10 seconds, then allowed to come to a stop. The centrifuge screens were then weighed. The insect samples were then added to the centrifuge screens and subjected to the same treatment as the empty screens. The weight of the insects was then computed by subtracting the known weight of the screen from the combined weight.

After weighing, the insects were transferred to a planchet, covered with nitric acid and placed on the hot plate. When the sample was completely digested, 10 drops of concentrated nitric acid were added to the planchet, evaporated to dryness and muffled to red heat.

Adult Insects

Field Procedures in Collection of Adult Insects

On July 12, July 25, and August 2, 1960, between the hours of 9 p.m. and 11 p.m., collections of mature insects

were made at Station 8 by means of a light trap with a "black light" tube. Since no electricity was available at the collecting point, the power was furnished by a 12 volt storage battery connected to an inverter. The collecting bottle contained pieces of chloroform-soaked paper. After the two-hour collecting period, the bottle was removed, capped, taken to the laboratory and refrigerated.

Laboratory Processing of Adult Insects

The mature insects were first segregated taxonomically. A known number of insects was added to a planchet of known weight and weighed on a Mettler balance and the weight of the insects determined by subtracting the planchet weight from the combined weight.

The insects, still in the planchet, were completely covered with concentrated nitric acid and placed on a hot plate. When the contents turned black, an additional 10 drops of the acid were added and evaporated to dryness. The planchet was then muffled to red heat.

Fish

Field Procedures in Collection of Fish

The eastern slimy sculpin, Cottus cognatus; the northern mottled sculpin, Cottus bairdii, and the American brook lamprey, Entosphenus lamottinii were collected periodically, primarily as a check on the data of previous years. The fish were collected by means of electric shocking. The power was generated by a 230 volt D.C. generator mounted in a small, flat-bottomed skiff. The fish were placed in pumple bottles

containing dilute formalin and transported to the laboratory.

Laboratory Processing of Fish

The samples were weighed, and placed in beakers, then covered with concentrated acid and allowed to digest. When the contents of the beaker had evaporated to a small volume, all material was transferred to a planchet. The beaker was washed with dilute nitric acid and the washings added to the planchet. When the planchet was completely dry, 10 drops of concentrated nitric acid were added and allowed to evaporate. The planchet was then muffled.

Chelation

Field Procedures in Collection of Chelate Samples

An iron chelate was introduced into the experimental area on July 7, 1960, 48 hours after the isotope treatment. The iron chelate was allowed to flow into the stream for a 4-hour period. The chelate was added at the Station 8 bridge. The iron chelate used was sodium N-hydroxyethylethylenediaminetriacetate (NaFeHEDTA). The concentration of the chelated iron in the stream was approximately one part per million.

Water samples were collected by hand and by automatic pumps. The hand-collected samples were taken in 140 ml. polyethylene bottles at five-minute intervals. The collection sites for these samples were Stations 12 and 14.

The automatic sampling devices were located at Stations 11 and 12. The automatic pumps took samples through July 8, 1960.

Laboratory Processing of Chelate Samples

The 140 ml. hand-collected samples were thoroughly mixed and a subsample was removed and sent to the Institute for Fisheries Research, Ann Arbor, Michigan for iron analysis. The remainder of the sample was processed for counting, the methods for which were described earlier.

The four-liter samples obtained from the automatic sampling devices were shaken thoroughly and 25 ml. of concentrated hydrochloric acid added to each. A subsample was removed and sent to the Institute for Fisheries Research, Ann Arbor, Michigan for iron analysis. The remainder of the sample was processed for activity as described earlier.

Total Phosphorus

Water

Field Procedures in Collection of Total Phosphorus Water Samples

Water samples were collected for total phosphorus analysis, by hand and by the automatic sampling devices. The hand-collected samples were taken on July 7, 1960 at five-minute intervals for a period of two hours. These samples were taken simultaneously with the chelate samples. The collecting stations for these samples were Stations 12 and 14.

The automatic sampling devices collected water samples from July 5 to July 8, 1960. The devices were located at Stations 8, 11 and 12.

Laboratory Processing of Total Phosphorus Water Samples

The 140 ml. hand-collected samples were mixed thoroughly. A subsample was removed and sent to the Institute for Fisheries

Research in Ann Arbor, Michigan for phosphorus analysis. The remainder of the sample was analyzed for trace P^{32} phosphorus by the same methods used for water hand samples.

The four-liter samples obtained from the automatic sampling devices were shaken thoroughly and 25 ml. of concentrated hydrochloric acid was added. Approximately two liters of this sample was removed and sent to the Institute for Fisheries Research in Ann Arbor, Michigan for phosphorus analysis. The remainder of the sample was analyzed for radiophosphorus by the same methods used for water hand samples.

Biota

Field and Laboratory Procedures for Total Phosphorus Biota Samples

Representative specimens of the biotic community were collected from Station 14. The specimens were placed in dilute formalin and sent to the Institute for Fisheries Research for phosphorus analysis. Appendix IV presents the analysis.

The fauna specimens included: Ephemerella cornuta, Ephemerella needhami, Hydropsyche sp., Gammarus sp., Hexagenia sp., Atherix variegata, Simulium sp., Brachycentrus sp., Pteronarcys sp., Physa sp., Cottus cognatus, Salvelinus fontinalis, Chauliodes sp., and Entosphenus lamottenii. The flora samples included: Chara sp., Fontinalis antipyretica, Potamogeton pectinatus, and Nasturtium officinale.

RESULTS AND DISCUSSION

Radiological

When a radioisotope is released into a stream, its transportation follows the physical and biological pathways of that stream. The current, pools, and riffles direct the translocation of the isotope at a fairly rapid rate through the stream. However, detectable amounts of the isotope remain in the lotic system for an appreciable length of time. Much of the isotope is immediately accumulated by the stream biota.

Fresh-water organisms may accumulate isotopes in three ways: through adsorption to the surface area, through absorption from the surrounding medium, or through ingestion as food (Krumholz and Foster, 1957).

The physical and biological factors of the stream will be described in the following section. The physical influence will be presented in the section dealing with water. The biological effects of radioisotope translocation will be presented in the sections concerned with population ecology.

Water

Current is one of the most important factors concerning life in a stream. Thus, many of the nutrient elements are derived from the headwaters and entering tributaries. Some of the nutrient elements, however, are derived from autolytic or bacterial decomposition of organic debris. Barnes (1957) found that bacterial decomposition of organic debris was a factor in returning inorganic phosphorus to the upper waters

of the sea. In this connection, one could picture some of the isotope flowing directly through the system and after a time lapse, small amounts of the isotope would reappear after having been biologically incorporated and then decomposed.

Figures 3 and 4 present the flow of the isotope through the system. There can be seen in the curves for the upstream stations, i.e., Stations 3, 5 and 8, that there is a direct time relationship between the arrival of the peak activity and the station's distance from the isotope entry point. The downstream stations, i.e., Stations 12, 14 and 16, show that much of the radioisotope has been removed from the main stream flow. Station 12, which is 2540 yards from the isotope entry point, shows that at least 64% of the original amount of the isotope has been removed from the water flow. Evidence will be offered in the following sections indicating that much of this removed isotope is biologically incorporated.

Figure 5 presents the data gathered from the continuous automatic sampling devices. The counts are recorded as corrected counts per 500 ml. to avoid the confusion of using fractional counts.

If the isotope had remained in pools or other diversions as free activity in the water rather than in some other form, the curves in Figure 5 would be expected to show greater fluctuations. The variability of the curves in Figure 5 are placed in perspective by noting that the activity is expressed in counts per 500 ml. in opposition to the water activities of the main isotope flow of Figures 3 and 4 which are expressed in counts per milliliter. It is apparent from Figure 5

Figure 3. Total water activity at upstream collecting Stations 3, 5 and 8 during the passage of isotope. All counts were corrected for background and decay.

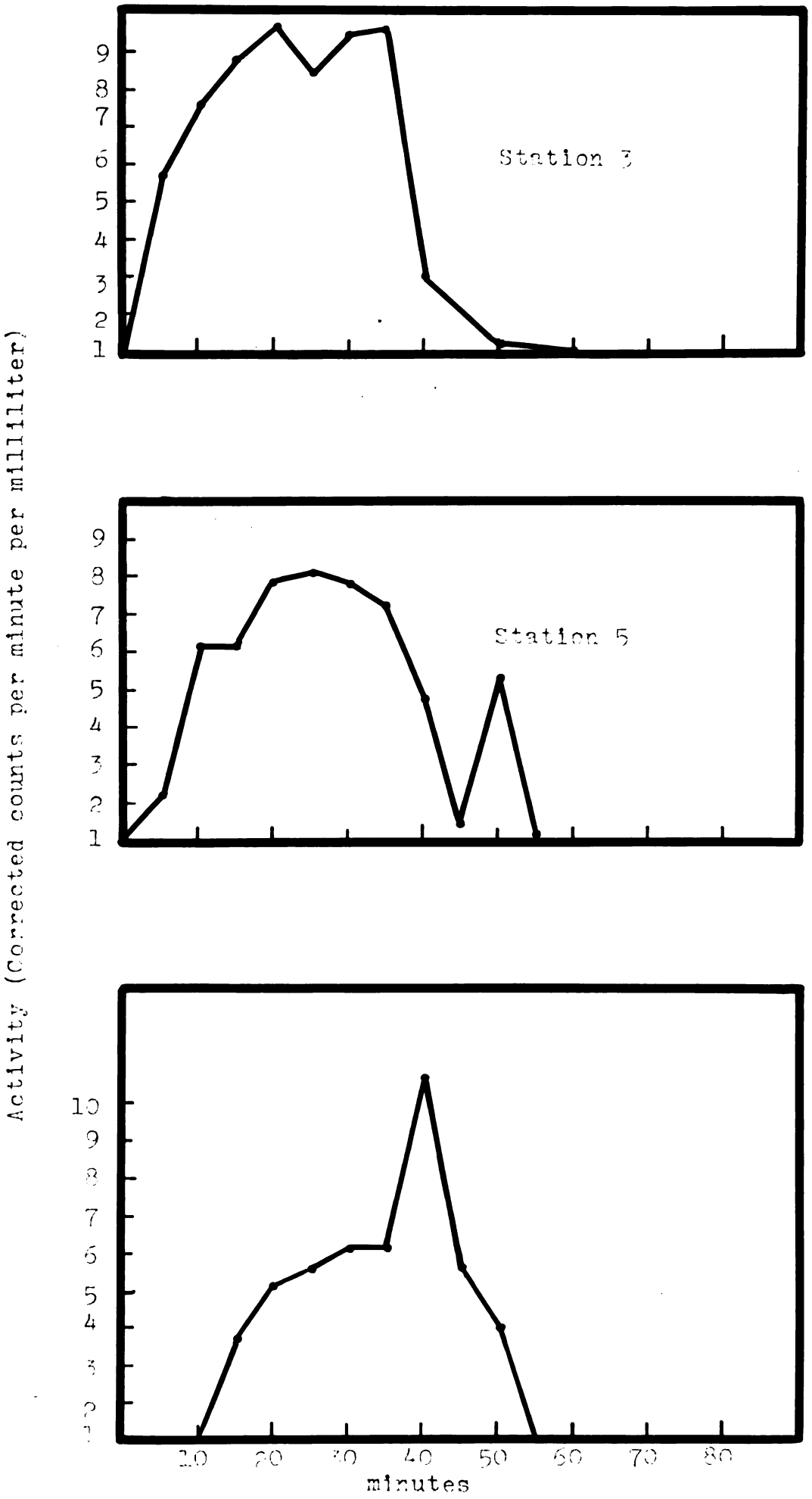

Station 3
Station 5
Activity (Corrected counts per minute per milliliter)
minutes
10
20
30
40
50
60
70
80

Figure 4. Total water activity at downstream collecting Stations 12, 14 and 15 during the passage of isotope. Counts were corrected for background and decay.

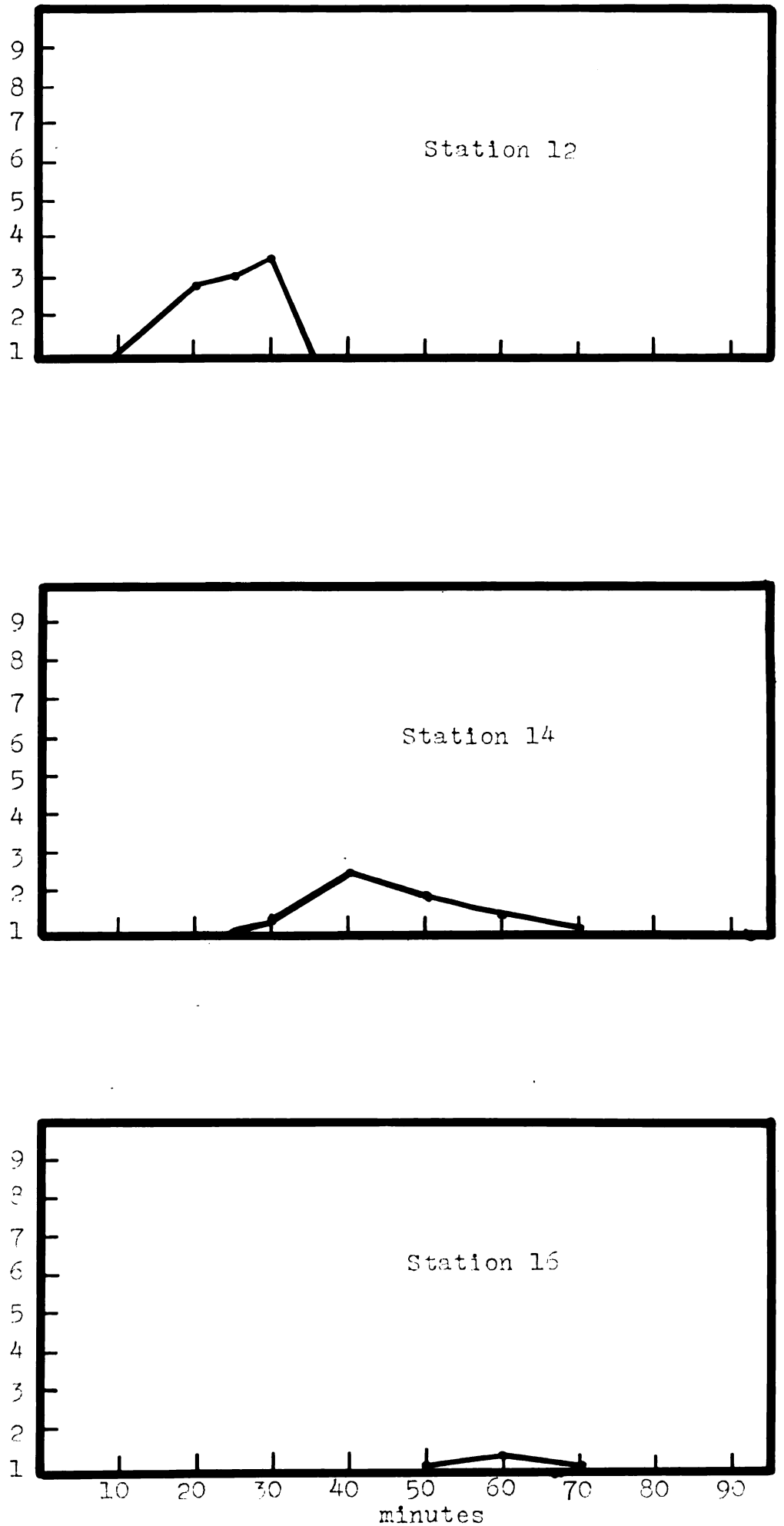

Activity (Corrected counts per minute per milliliter)
Station 12
Station 14
Station 16
1
2
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
minutes

Figure 5. Continuous total water activity at collecting Stations 8, 11 and 12 for July 6 and July 7, 1960. Counts were corrected for background and decay.

Station 8
Station 11
Station 12
Activity (corrected counts per minute per 500 milliliters)
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
11 noon 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7
July 6
July 7

that in the first 18 hours of sampling an approximate 90% activity decrease occurred in the samples obtained from the sampling devices. It is believed that the drastic loss of isotope from the main stream of isotope flow is due to biological incorporation or adsorption.

The adsorption factor can account for much of the lost isotope. Figure 6 shows the soluble isotope, the adsorbed isotope, and the isotope that is incorporated within particulate matter, such as diatoms or bacteria. These small organisms may be ingested by other animals or may settle to the bottom, thereby removing the activity they carry from the water flow. The adsorbed value for Station 12 was greater than the total water activity for Station 12 in Figure 7. This difference was probably due to sampling error as the peak total water activity sample was not, in this case, from the same sample that was used for determination of solids.

The plankton samples had extremely low activities. The activity was in the thousandth count per ml. range. The activities may have had different ranges if the samples were processed for solids determination rather than evaporating the samples and computing the activities on milliliter basis. The actual counts per minute for Stations 7 and 12 were 346 and 292 respectively. This low activity may have also been due to the dislodging of the adsorbed activity on the particulate matter when new samples are poured into the plankton net. The samples were taken 12 inches below the surface and this also may have a limiting effect on the capturing of the radio-

Figure 6. Soluble activity, acid soluble or adsorbed activity, and particulate activity filtered from the stream water by Millipore filter. Counts were corrected for background and decay.

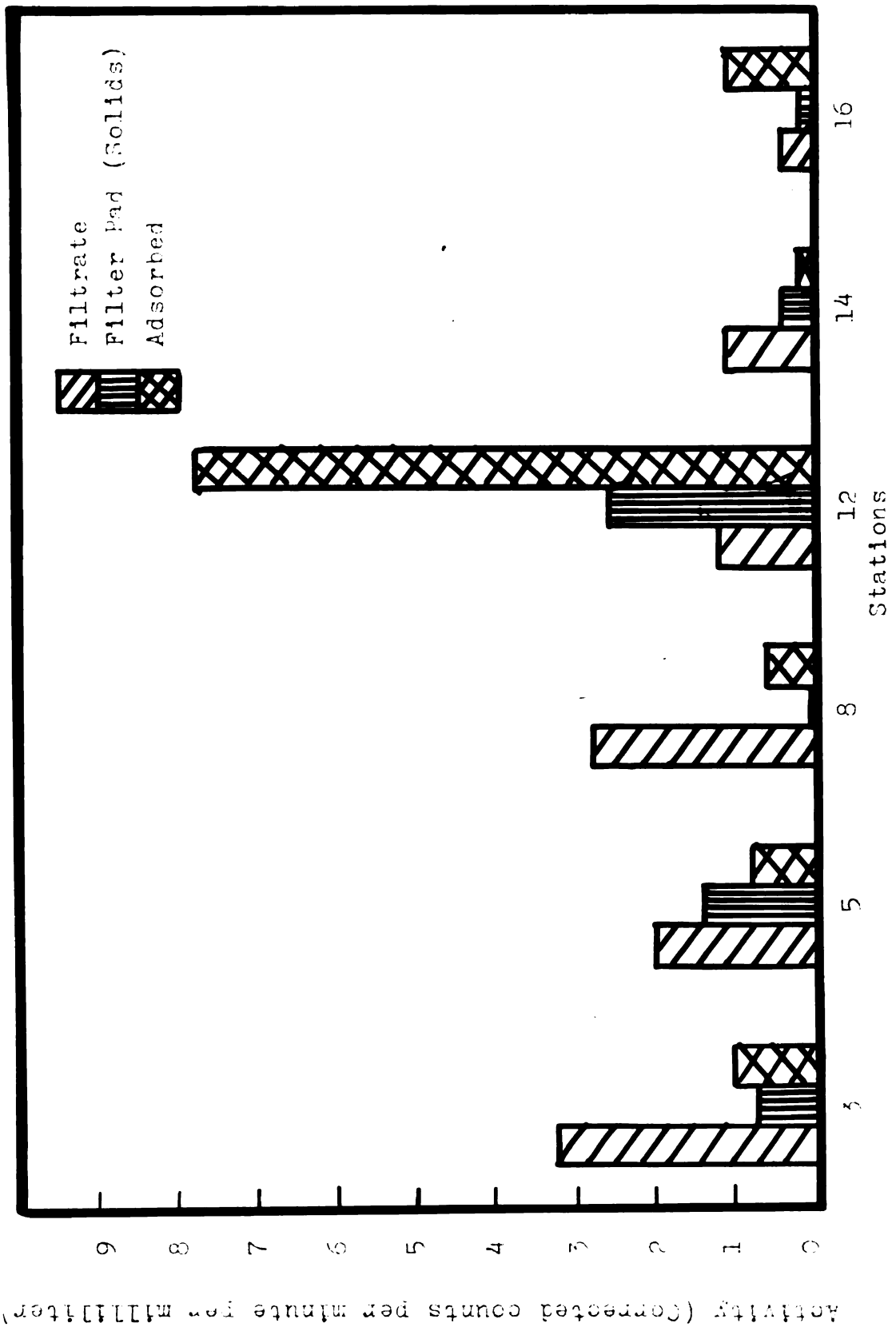
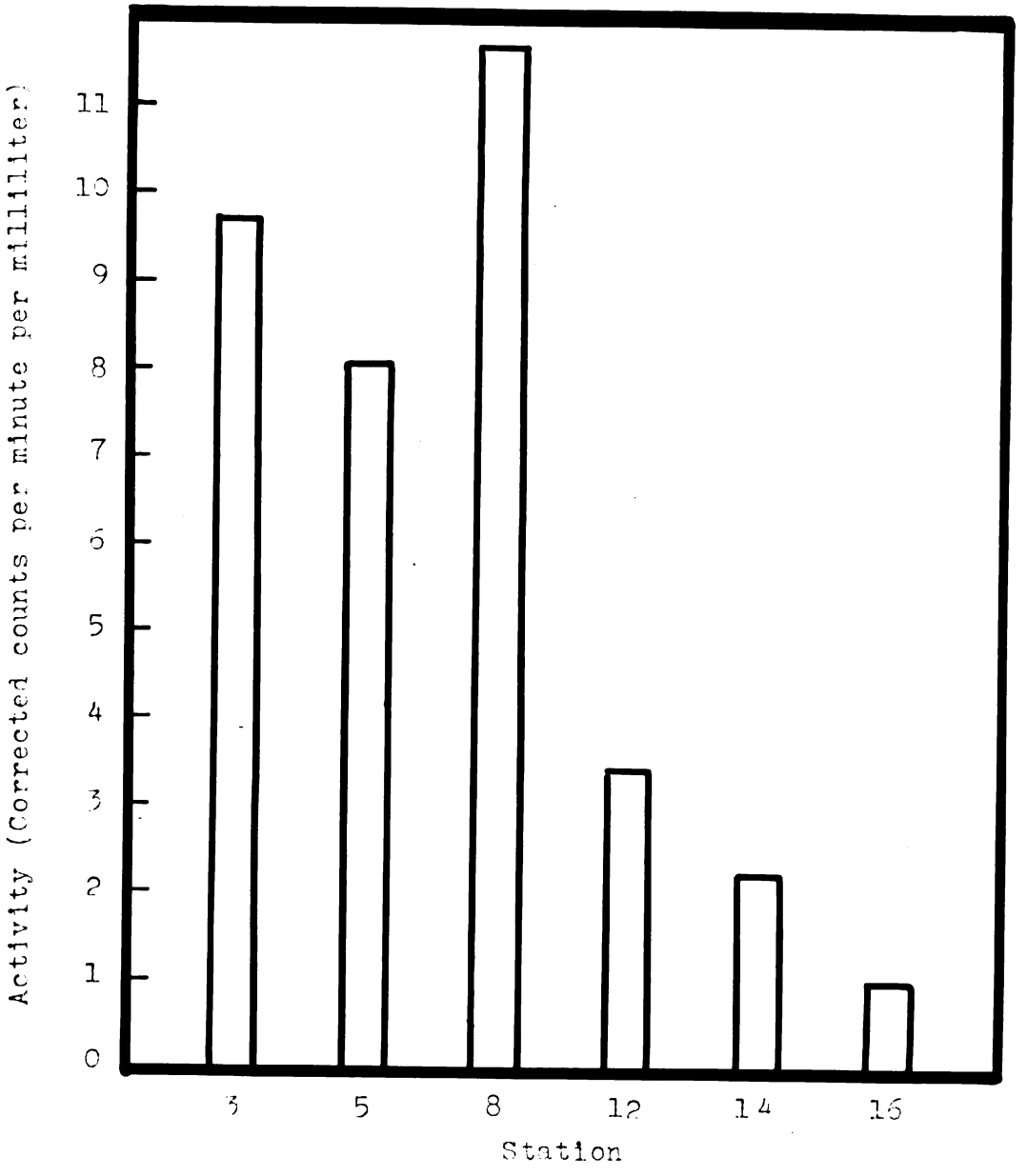

Filtrate
Filter Pad (Solids)
Adsorbed
0
1
2
3
4
5
6
3
5
8
12
14
16
Stations
Activity (corrected counts per minute per milliliter)

Figure 7. Total peak water activities at the various stations. Counts were corrected for background and decay.


Activity (Corrected counts per minute per milliliter)
0
1
2
3
4
5
6
7
8
9
10
11
3
5
8
12
14
16
Station

active particulate matter.

The peak activities were expected to diminish as the isotope-bearing water mass traveled downstream. The peak activity at Station 8 (Figure 7), however, was greater than that recorded at Station 3. This aberrancy may be due, in part, to sampling variability or error. For example, it was found that the water mass in the center of the stream contained more activity than the eddy areas of the side of the stream. This fact was brought out in the taking of simultaneous samples across the stream but the results of these findings could not be applied to these collections.

Periphyton

Periphyton, or aufwuchs, are attached organisms which cling to stems, leaves or other surfaces (Odum, 1959). Considerable interest revolves around periphyton activities, as they are key organisms in the food chain. The periphyton population on the artificial substrates in the West Branch of the Sturgeon River is composed principally of diatoms (Clifford, ibid.). According to Clifford, Synedra ulna is the most abundant form.

Periphyton is important not only for its niche in the food web, but also for its isotope accumulation abilities. According to Donaldson (1959) radioactive materials are quickly taken up by algae and these algae are capable of concentrating the radioactive materials more than a thousand times the amount of the radioactive substances found in the surrounding water.

Figures 8 and 9 show the rapid and large accumulation by periphyton of the radiophosphorus. The periphyton data for Figures 8 and 9, come from the periphyton growths which were started two weeks prior to the isotope entry. The substrates remained in the water during the isotope addition.

Station 14, the furthest downstream station from the point of isotope entry, shows greater isotope accumulation than the intermediary Stations 8 and 12 (Figures 8 and 9) on the first collection day, i.e., the day of isotope entrance. The radioisotope was present when it passed Stations 8 and 12 but it may have been adsorbed on particulate matter. This adsorbed activity may then have equilibrated or exchanged P^{32} for the stable phosphorus and thus become available for periphyton uptake at Station 14. A further explanation for the uptake variation may be provided by the locations of the periphyton collection apparatus. The periphyton stakes at Station 14 were in riffle areas while the stakes at both Station 8 and 12 were in pool areas. The current of the riffle area may have induced particulate matter equilibration by constant agitation.

The general picture, however, demonstrates the radiophosphorus accumulation abilities of periphyton. The initial counts ranged from 5,600 to 37,000 counts per minute per gram. The counts after a 72-hour period ranged from 1,400 to 12,600 counts per minute per gram.

Exchange and Regeneration

It was shown (Figure 4) that 6 % of the original amount

Figure 8. Activity of periphyton at Stations 3 and 8. The substrates remained in the water during isotope treatment. Counts were corrected for background and decay.

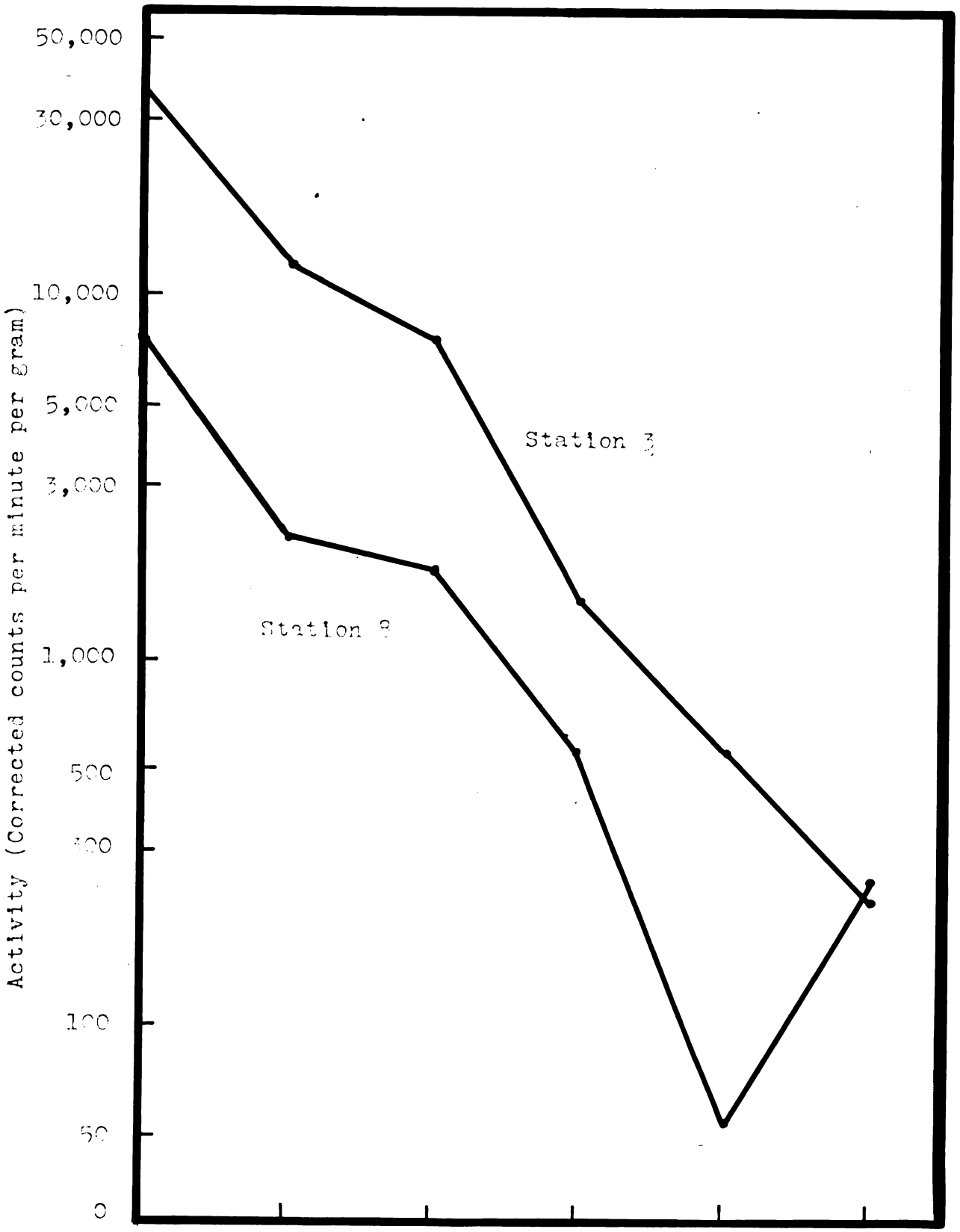
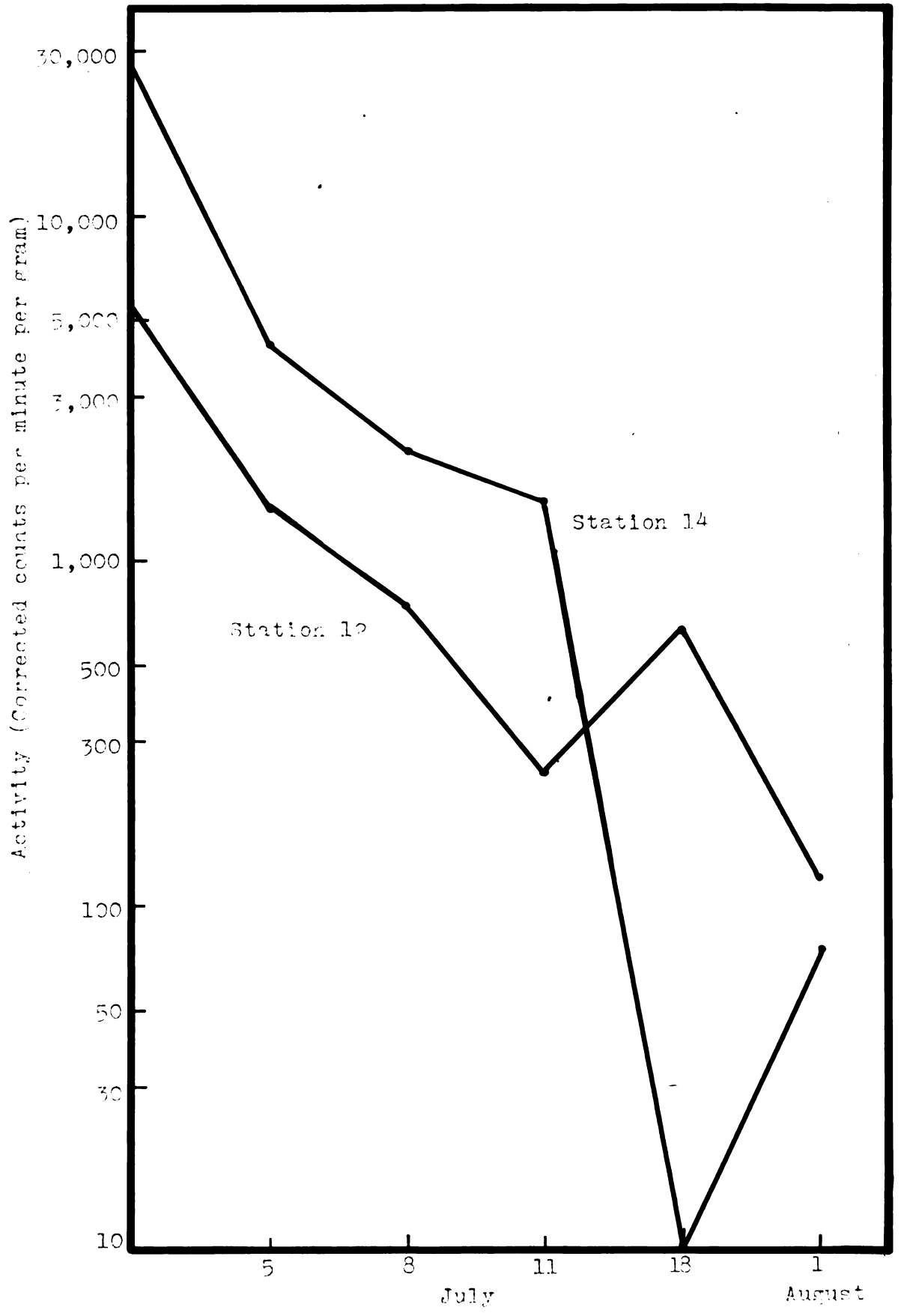

50,000
30,000
10,000
5,000
3,000
1,000
500
100
50
0
Activity (Corrected counts per minute per gram)
Station 3
Station 8

Figure 9. Activity of periphyton at Stations 12 and 14. The substrates remained in the water during isotope treatment. Counts were corrected for background and decay.


30,000
10,000
5,000
3,000
1,000
500
300
100
50
30
10
Activity (Corrected counts per minute per gram)
Station 14
Station 12
5
8
11
18
1
July
August

of isotope was removed from the main water flow by the time the water mass reached Station 12. Comparing the curves for Station 3 (Figure 3) and Station 14 (Figure 4) shows that approximately 80% of the original amount of the isotope was retained in the sampling area. Approximately 90% of the original amount of isotope was retained in the area between Stations 3 and 16. Figure 6 presented a picture of the adsorption of radiophosphorus from the water. The traceable phosphorus, however, reappears throughout the summer. The recurring radiophosphorus is believed to be regenerated (re-cycled) phosphorus that leaves the biotic element and re-enters the stream water after the original dosage. The re-cycling of phosphorus was apparent in the activity curves of several of the organisms collected throughout the summer.

Periphyton is an excellent experimental plant for showing the regeneration of radiophosphorus in the stream. In the section on periphyton methods, there was presented collecting procedures for "in" units and "out" units. These periphyton units represent those substrates that were in contact with the initial isotope-bearing water mass ("in" units) and those that were placed in the stream after the isotope-bearing water mass had passed through the stream ("out" units). An analysis of the two different treatments of periphyton substrates indicates the effects of radiophosphorus regeneration.

Figures 10 and 11 present a comparison of the percentages of activity, in counts per minute per gram, to the initial collection activity. The activity of the first collecting

Figure 10. Comparison of the magnitude of retained and regenerated radiophosphorus to the initial activity of the first collecting day at Station 8. The activity of the first collecting day was considered to be 100%.

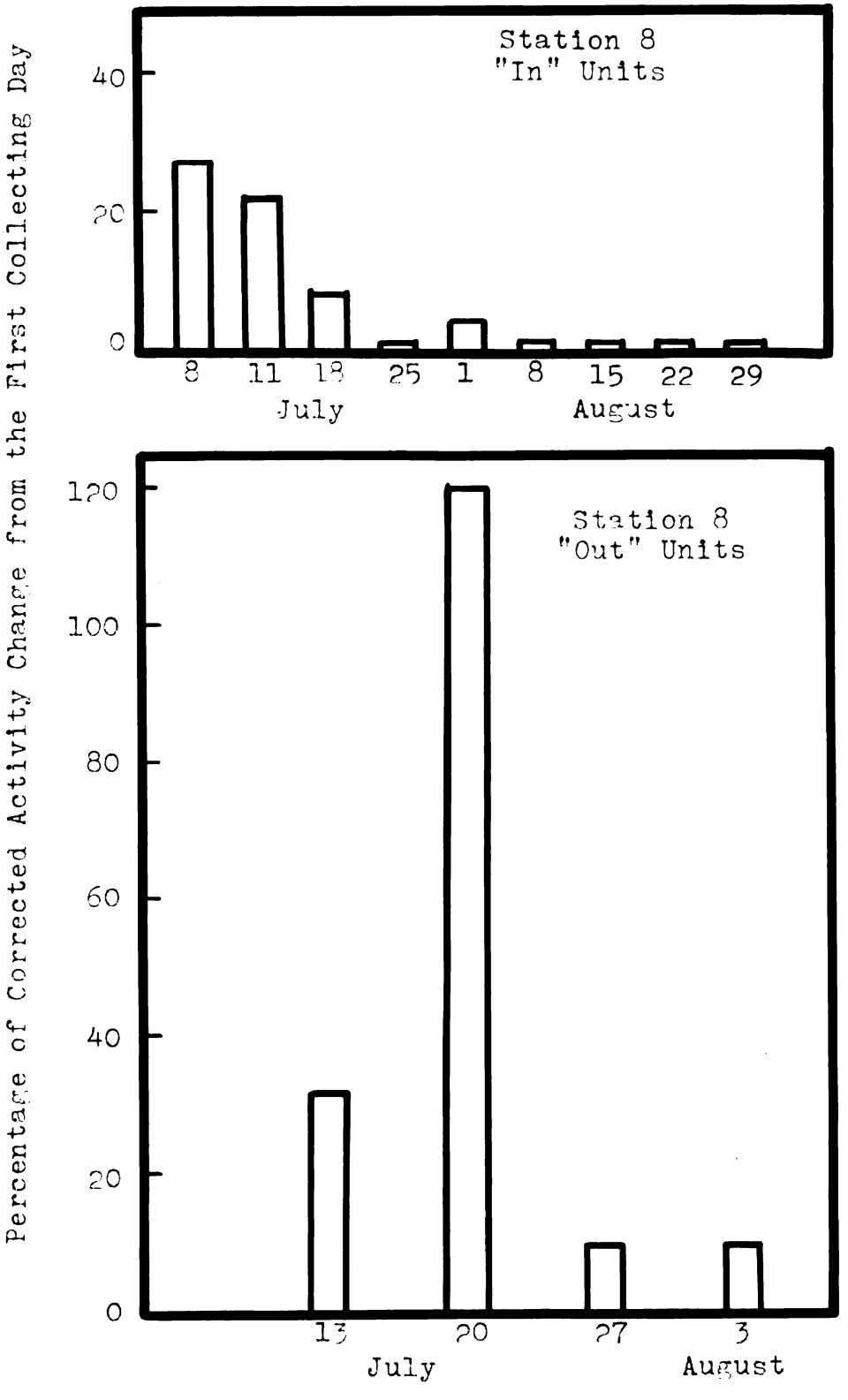
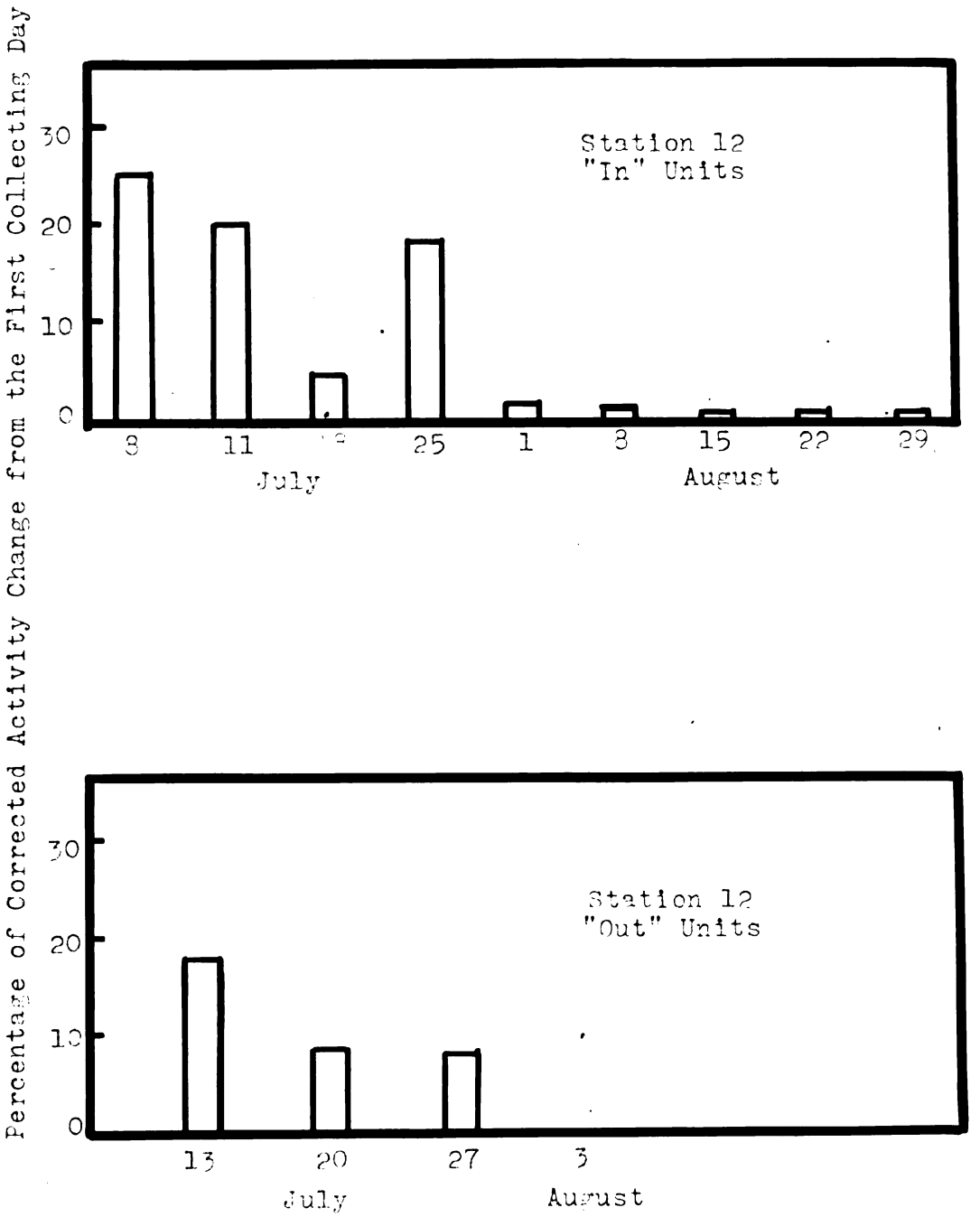

Percentage of Corrected Activity Change from the First Collecting Day
Station 8
"In" Units
40
20
0
8
11
18
25
1
8
15
22
29
July
August
Station 8
"Out" Units
120
100
80
60
40
20
0
13
20
27
3
July
August

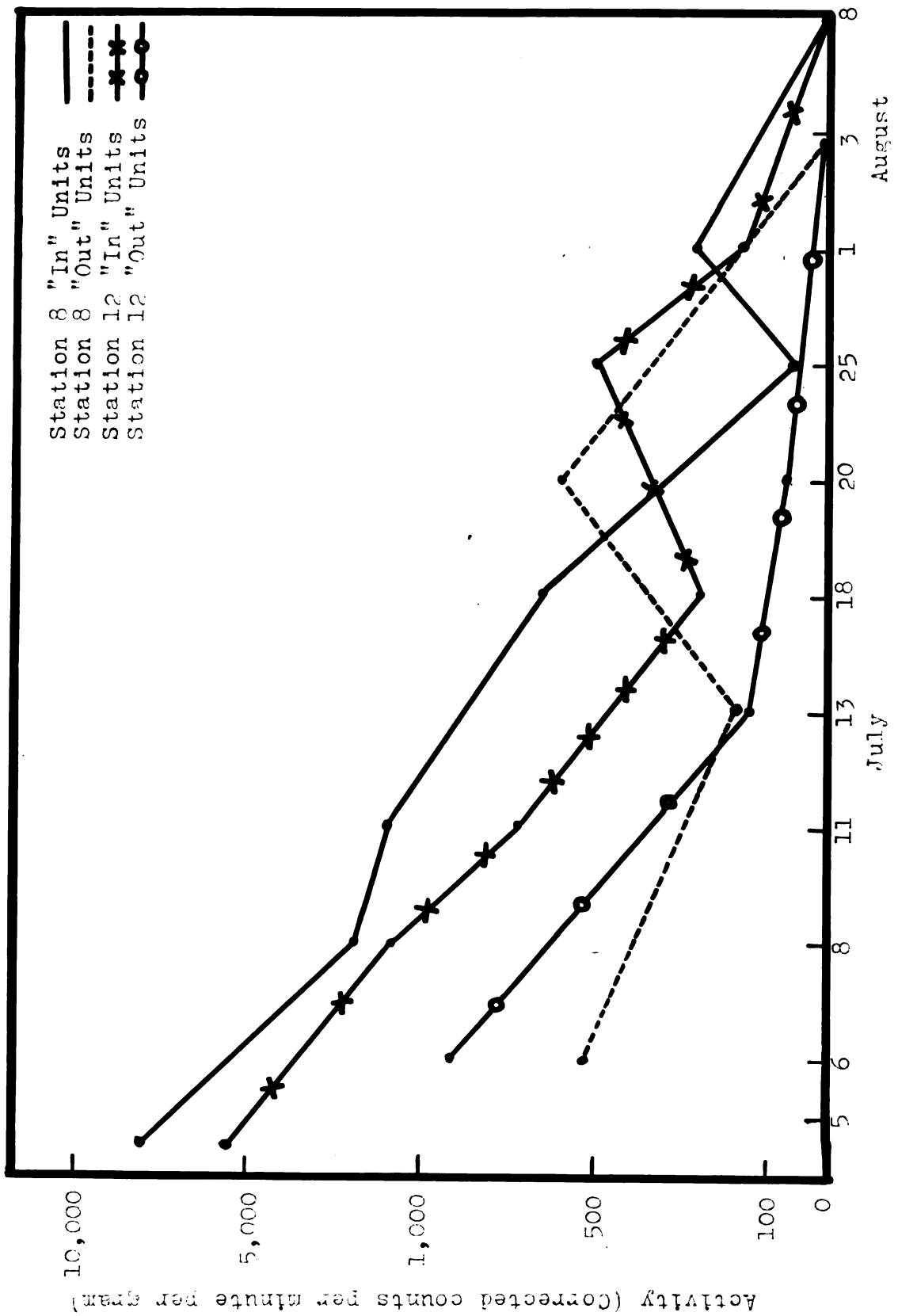
Figure 11. Comparison of the magnitude of retained and regenerated radiophosphorus to the initial activity of the first collecting day at Station 12. The activity of the first collecting day was considered to be 100%.


Percentage of Corrected Activity Change from the First Collecting Day
30
20
10
0
Station 12
"In" Units
8
11
25
1
8
15
22
29
July
August
30
20
10
0
Station 12
"Out" Units
13
20
27
3
July
August

day was considered to be 100%. The first collecting day for the "in" units was July 5. The first collecting day for the "out" units was July 6. The activities of these units on July 5 and July 6 was considered to be 100%, and the activities of the units collected later during the study were compared to the activities of the first collecting day. From these figures, there appears to be some radiophosphorus regenerated throughout the summer. The percentage of activity is expected to decrease (with no increase), if there were no regeneration. There is a general decrease due to biological dilution, e.g., growth of cells, physical dilution and the loss of old cells. However, Figures 10 and 11 show that there are some very marked increases in activity throughout the summer. The "in" units for Station 8 show an increase in activity of 1% on August 1, 1960. The "out" units which were exposed only to regenerated activity, show that considerable amounts of regenerated phosphorus is present. The "out" units at Station 8 show a 12% activity increase on July 20, 1960.

Figure 12 represents a composite picture of the averaged activities for the "in" units and "out" units of Stations 8 and 12. The data for the individual substrates of Figure 12 and "in" units of Stations 3 and 14 is presented in Appendix IV. The "in" units of Station 8 had an increase as late as August 1. From these data there seems to be no upstream-downstream differentiation other than the expected decrease in magnitude of the activity due to decay and biological dilution. As can be seen in Figure 12, the initial activity

Figure 12. Comparison of periphyton activity at Stations 8 and 12. Counts were corrected for background and decay.


Station 8 "In" Units
Station 8 "Out" Units
Station 12 "In" Units
Station 12 "Out" Units
Activity (Corrected counts per minute per gram)
10,000
5,000
1,000
500
100
0
5
6
8
11
13
18
20
25
1
3
8
July
August

decreases rapidly and then appears to level cut. Aside from the small increases, the data average out to a plateau level at approximately 200 corrected counts per minute, beyond July 18, 1960.

There are, as yet, few concisive explanations for the initial high activities of the first collecting day. Several physical and biological factors have been explored by various workers. The mechanism for the initial uptake and accumulation may be due to physical processes unconnected with active cell metabolism (Coffin, et al., 1949). Thus, the initial activity may be adsorbed on the surface of the cell and not incorporated into the protoplasm (Odum, et al., 1958). Correll (1961), working with synchronized cultures of Anabaena found that small amounts of radioactivity were found in sugar-phosphates, AMP, ADP and orthophosphate, while the polyphosphate was very radioactive. Correll (ibid.) also concluded that the total phosphorus of the cells was principally half polyphosphates and half orthophosphate. The percent of the orthophosphate declined sharply with age.

The initial high activities may be due to physical and biological factors. The radioactive phosphate ion may be adsorbed onto the periphyton cells. The traceable orthophosphate may be immediately incorporated biologically by phosphorylation or may be present as the orthophosphate ion.

The cell accumulation may give some insight into the regeneration effect. The adsorbed phosphate can be released throughout the study area by death of the cell or constant

washing in the currents. The biologically incorporated radiophosphates may be distributed via death, growth, and reproduction. The active orthophosphate within the cell may be equilibrated or exchanged with the water for the stable orthophosphate. The phosphate within the cells, in any form other than orthophosphate, is probably unavailable for direct release into the system. This is due to the energy required to form the sugar-phosphate bonds (Bonner et al., 1952) and these compounds are thus retained within the cell.

Variation in P^{32} Uptake by Periphyton

In order to determine to what extent the concentration of radiophosphorus varied among periphyton samples collected at the same station and at the same time, a duplicate sample was taken. The activity curves shown in Figures 13 and 14 illustrate the variation of the duplicate samples. The data obtained indicated that some samples had large variations.

The activity for the two samples at Station 8 on July 25 varied by more than 100 counts. The activity counts for the two samples at Station 14 on July 25 were both zero. This date, July 25, which is 20 days after isotope entry, may be the point at which the original isotope retained has been lost by the periphyton. The activities for other specimens collected during this time indicate that the counting equipment was functioning correctly. The increases in activity after July 25 may be due to regenerated activity.

Several factors may be responsible for the variation of the periphyton samples: 1) The position of the substrates in the stream. It was noted that the plates in the riffle areas

Figure 17. Activity of periphyton at Station 8. The variation of the subsamples within routine samples is shown. The routine sample consists of a set of four substrates. Counts were corrected for background and decay.

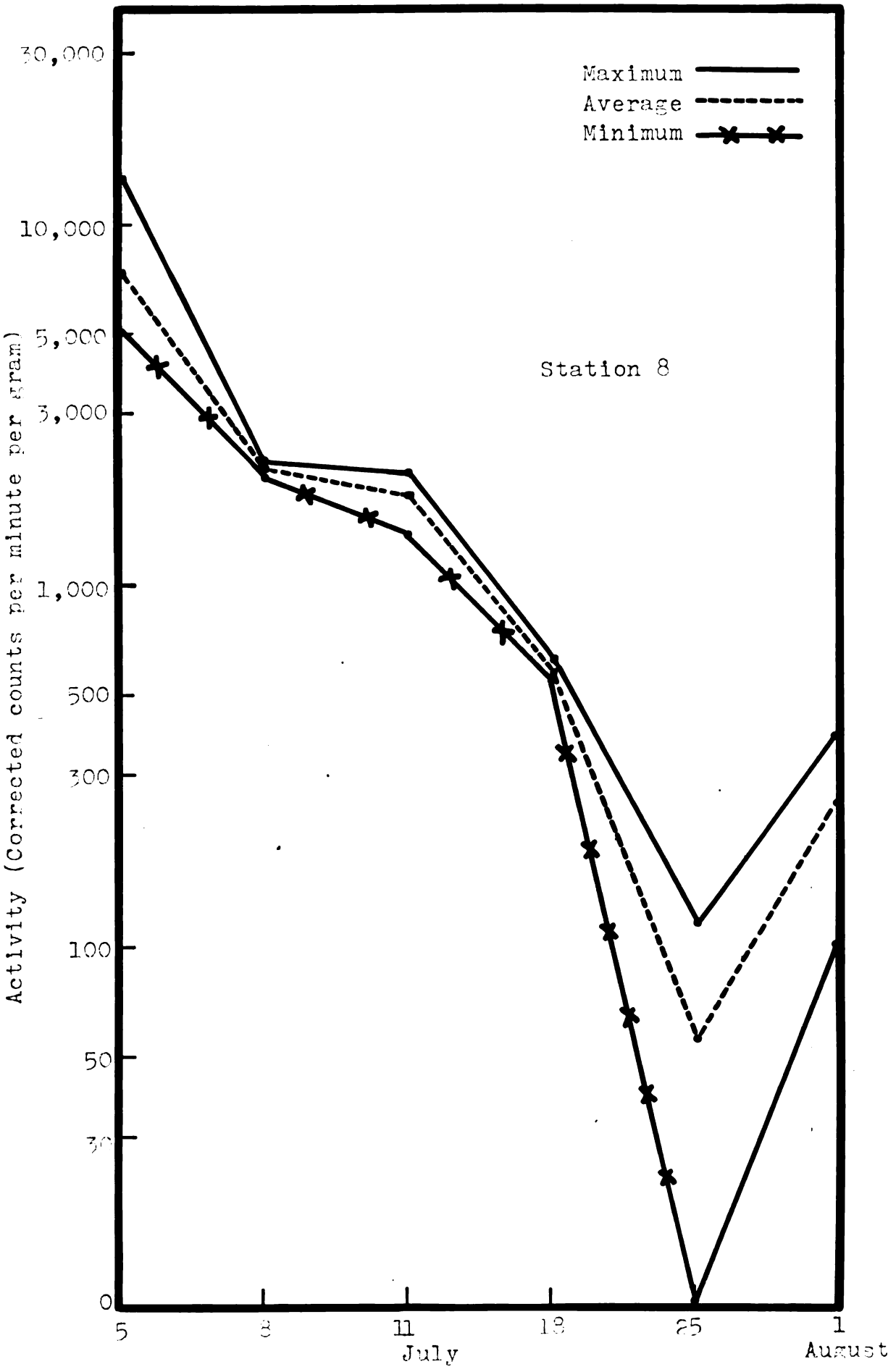
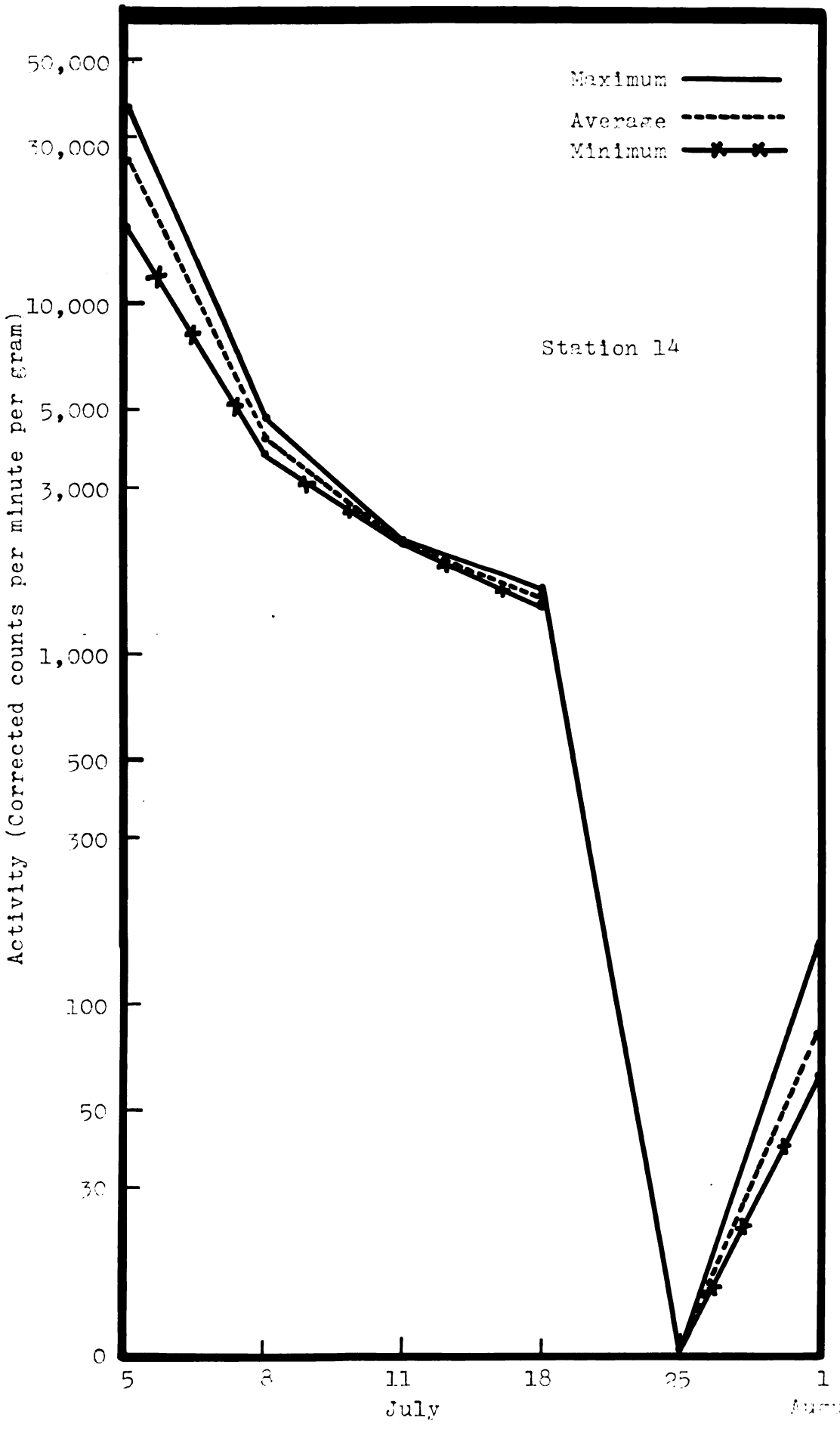

Maximum
Average
Minimum
Station 8
Activity (Corrected counts per minute per gram)
30,000
10,000
5,000
3,000
1,000
500
300
100
50
30
0
5
8
11
18
25
1
July
August

Figure 14. Activity of periphyton at Station 14. The variation of the subsamples within routine samples is shown. The routine sample consists of a set of four substrates. Counts were corrected for background and decay.


Maximum
Average
Minimum
Station 14
Activity (Corrected counts per minute per gram)
50,000
30,000
10,000
5,000
3,000
1,000
500
300
100
50
30
0
5
8
11
18
25
1
July
August

had larger periphyton growths. 2) Age and metabolism of the sample. The substrates may also have differed in their ratio of young cells to old cells. It is known that younger, more rapidly growing individuals accumulate relatively larger amounts of P^{32} than the older, more slowly metabolizing cells. Although duplicate substrates were in the stream the same length of time, their populations may have been metabolically different. 3) Species composition. The metabolism of the populations also may have varied due to different taxonomic types which composed the individual samples.

Aquatic Plants

The aquatic plants differ from the periphyton basically by the possession of roots or root-like structures. Physiologically, the aquatic plants differ from the periphyton by the acquisition of nutrients through the root structure as well as the foliage. The participation of a root-nutrient method plays an important role in the radioisotope uptake. Activity curves differing from those of periphyton can be expected due to the relationship of the roots and the stream bottom. Jitts (1959) found that silts are capable of adsorbing very large quantities of phosphates. At Congressional hearings in 1959 of the special subcommittee on radiation of the Joint Committee on Atomic Energy, it was found that some bottom types can absorb as much as 64% of the added radionuclides. Carritt and Goodgan (1954) have demonstrated that phosphate is adsorbed by silt in two stages: the first stage being temporary while the second stage is more permanent in

duration. Jitts (ibid.) suggests that the second, more permanent adsorption is due to a gradual physical penetration of the phosphate ions into the lattices of the mineral constituents of the silt.

Because of the important central role of phosphorus in the metabolic activities of the cell, it is important to state that the uptake of the phosphate ions may not necessarily be completely independent from other factors. Metabolic phosphorus is involved in the transfer of energy for the process of ion accumulation (Hagen, 1956). Some plant roots, however, have what is called "apparent free space" in the roots (Boyer, 1956). This free space is a possible site for simple ionic exchange. However, the radiophosphorus exchange may not pass into and out of the cell as proportionately as does water (Dainty and Hope, 1959). Dainty, Hope and Denby (1960) found that some ionic exchanges show differences in the permeabilities based on the concentrations of other ions. In particular, the investigators found that the amount of sodium incorporated within the cell wall of Chara australis was a function not only of the concentration of sodium but also of calcium concentration in the external solution.

Thus the radiophosphorus uptake by aquatic plants is not as simple as the ingestion method of the insects. There are several loci for uptake; roots, stems and leaves. Uptake may be due to external adsorption, simple ionic exchange, or energy requiring ion accumulation--or any combination of these methods.

Fontinalis antipyretica. Fontinalis is the water moss found growing on the stream bottom, logs or calcareous clumps. It was frequently collected in dense mats which were inhabited by various larval insects. The upstream collecting Stations 3 and 8, show a dramatic increase in activity on August 22, 1960 (Figure 15). The other stations show a steady decline in activity except for several slight increases. The increases in activity are probably due to the accumulation of radiophosphorus in the stream bottom or regenerated radiophosphorus. There was no evidence of inaccuracy due to the counting equipment as the activity of the stations varied for all samples and there was no definite increase in activity for all samples on August 22.

Between August 8 and August 29, there were five recorded rainfalls. This additional water may have disturbed the bottom and the agitation may account for the additional activity increases; however, if this were the case the downstream stations would be expected to also show an increase in activity. The rather large increase in activity for the upstream stations may have been due to the chelation effect on the downstream stations, making the upstream activity appear higher, or to the greater amount of isotope held in the upper portion of the sampling area.

Chara sp. Chara is the predominant plant, on a biomass basis, in the West Branch of the Sturgeon River. It was found growing in dense mats which were widely scattered. The activity curves (Figure 16) are similar to those of

Figure 15. Activity of Fontinalis at Stations 3, 8, 12 and 14, for the entire study period. Counts were corrected for background and decay.

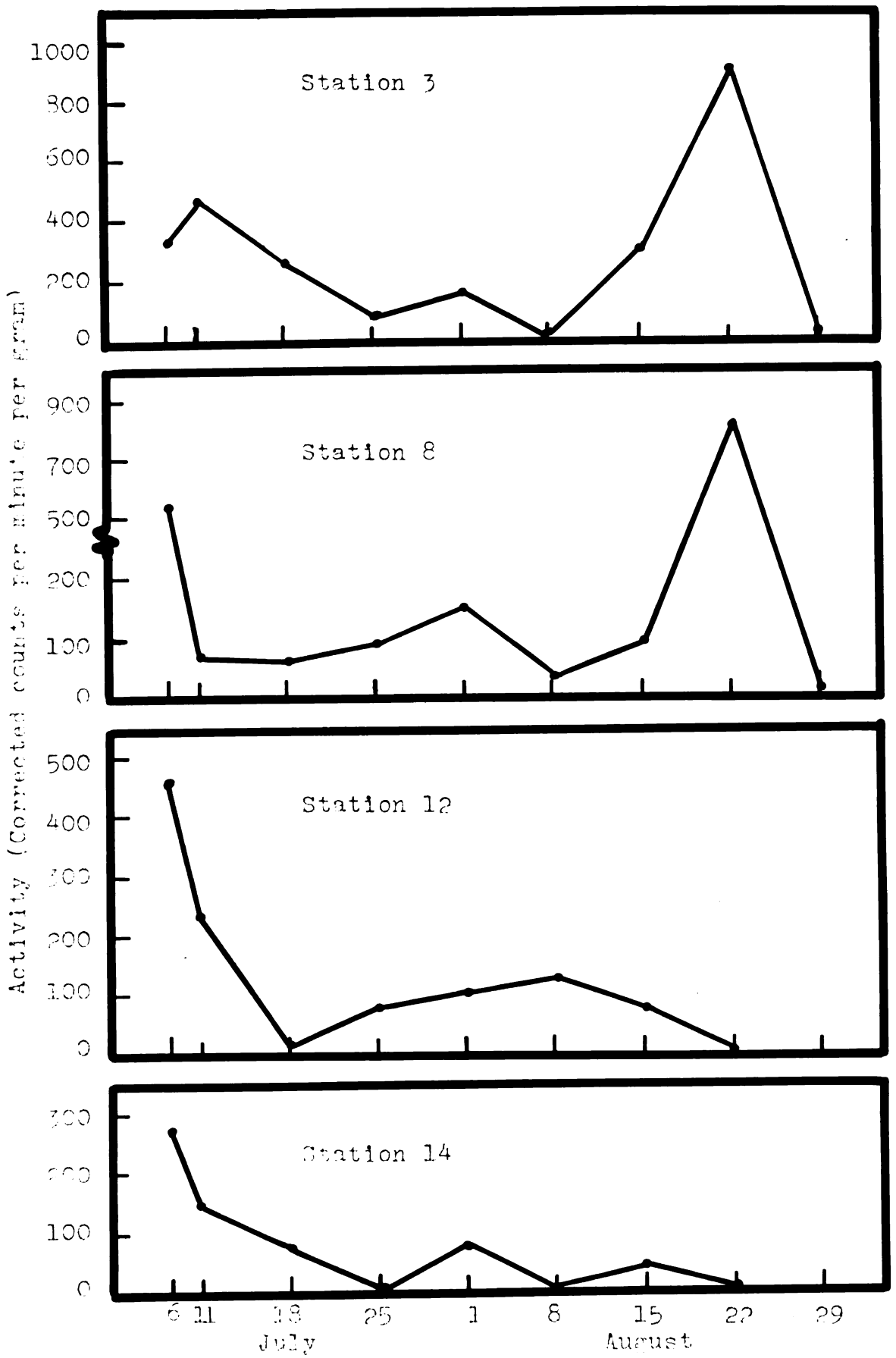
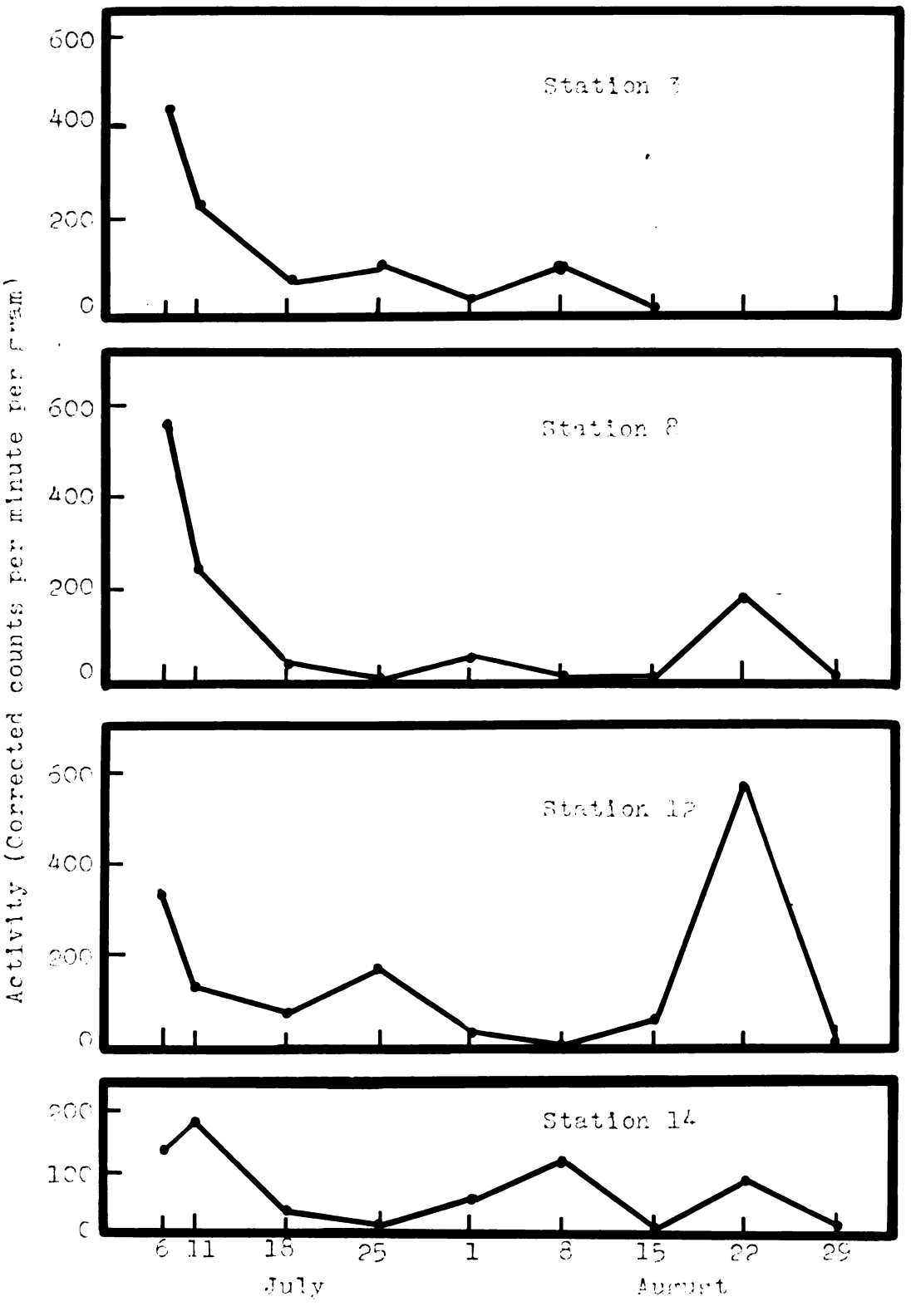

Station 3
1000
800
600
400
200
0
Station 8
900
700
500
200
100
0
Station 12
500
400
300
200
100
0
Station 14
300
200
100
0
6
11
18
25
1
8
15
22
29
July
August
Activity (Corrected counts per minute per gram)

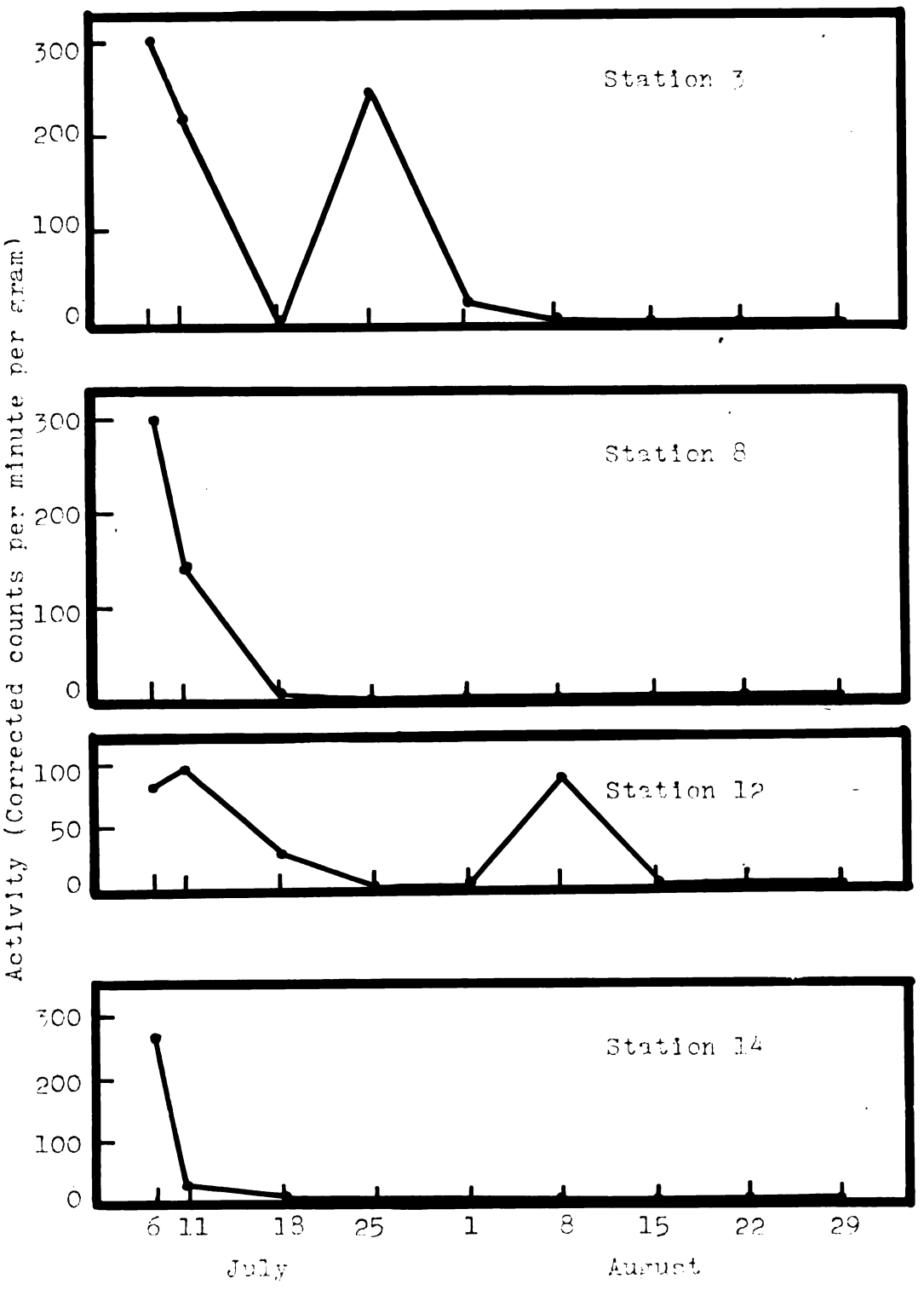
Figure 16. Activity of Chara at Stations 7, 8, 12 and 14 for the entire study period. Counts were corrected for background and decay.


Station 7
Station 8
Station 12
Station 14
600
400
200
0
100
6
11
18
25
1
8
15
22
29
July
August
Activity (Corrected counts per minute per gram)

Fontinalis with a few large peaks arriving late in the study period. The Chara peaks, however, show no upstream-downstream variation. The location of the Chara mats may be responsible for the activity curves at the respective stations. The Chara beds f[illegible] tations 3 and 14 were in shallow riffle sidewater areas, while at Stations 8 and 12, the beds were in deeper water near the middle of the stream. The Chara at Stations 8 and 12 had activity curves which include at least one point at which the corrected count was over 500 c.p.m. Station 8 had a maximum count of 434 c.p.m. and Station 14 had a maximum of 174 counts per minute per gram.

Potamogeton pectinatus. The pondweed, Potamogeton, was found growing on the bottom of the stream, usually in direct current and principally in the middle of the stream. Growing primarily in gravel bottom areas, it might be expected that the radiophosphorus bound by the silt and debris would not be available to the Potamogeton. Little P^{32} is held by the sand (Chadin, Kuznetsov and Timofeev-Resovsky, 1958). This may be a possible explanation for the activity curves shown in Figure 17 for Potamogeton which do not show the continuous variability shown by Chara (Figure 16) which inhabits silt beds. The corrected counts for Potamogeton samples collected after August 15, 1960 were zero. Two slight peaks which do not exceed that of the initial activity points were, however, found at Stations 3 and 12. Since the plants at these stations were found in gravel bottoms, a possible explanation for the peaks is "feed back." However, "feed back" or the

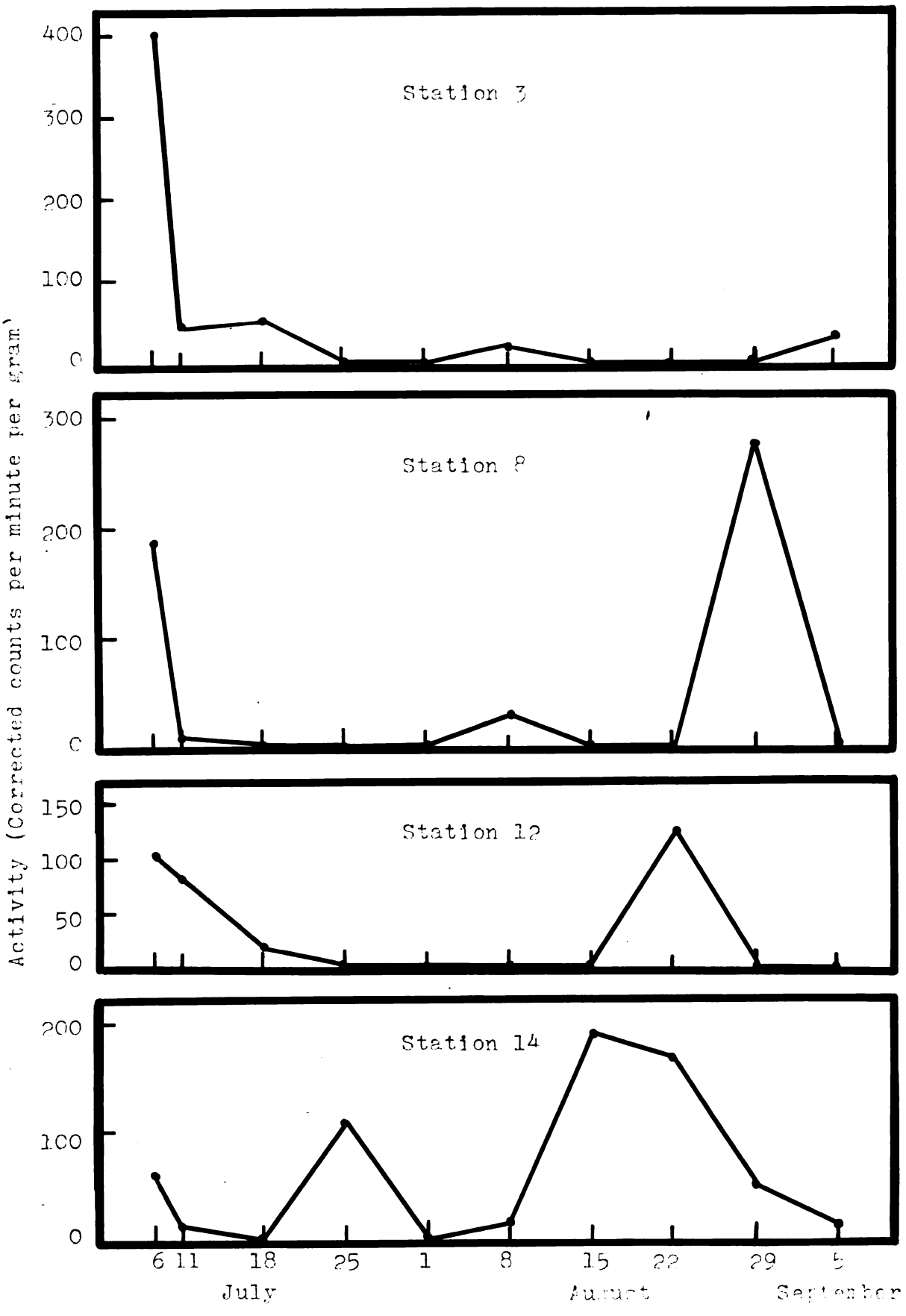
Figure 17. Activity for Potamogeton at Stations 3, 9, 12 and 14 for the entire study period. Counts were corrected for background and decay.


Station 3
Station 8
Station 12
Station 14
300
200
100
0
100
50
0
6 11 18 25 1 8 15 22 29
July
August
Activity (Corrected counts per minute per gram)

exchange of P^{32} for P^{31}, would appear not to be a satisfactory explanation for Station 3. Station 3 is 200 yards below the site of isotope entry and, presumably, with the rapid current, much of the activity of this upper area should have been removed by July 25. However, the curve for Fontinalis at Station 3 (Figure 15) shows that there is a large amount of activity still present in the upstream portion of the stream as late as August 22.

Nasturtium officinale. Water cress, Nasturtium, inhabited the silt and debris deposits. As water cress was not found at Station 3, most collections were made at Station 5, the next closest station at which Nasturtium was found. The activity curves for Nasturtium show large fluctuations in the latter part of the collecting season (Figure 18). The periods between August 15 and August 29, which are removed from the date of isotope entry by more than a month, show large activity increases. With the exception of Station 5, the corrected counts for Stations 8, 12 and 14 during the latter weeks of sampling exceed the initial activities of these respective stations on July 6. This latter increase in activities is due probably to the radiophosphorus collection in the silt and debris in which they are rooted. Further information collected on the last day of sampling, September 5, 1950, demonstrates that the accumulation of radiophosphorus by the stream bottom is an actual phenomenon. Some of the activity for detritus and stream bottom ran as high as 25 c.p.m. As the bottom materials do not concentrate this activity by

Figure 18. Activity of Nasturtium at Stations 5, 8, 12 and 14, for the entire study period. Counts were corrected for background and decay.


Station 3
400
300
200
100
0
Station 8
300
200
100
0
Station 12
150
100
50
0
Station 14
200
100
0
Activity (Corrected counts per minute per gram)
6 11 18 25 1 8 15 22 29 5
July
August
September

biological assimilation as do the aquatic plants, the counts are considered to be relatively large. An error in background counting during this latter part of the study would show large activities when the counts were corrected for decay. Appendix V presents the data of Nasturtium in counts corrected for weight but not for decay and background. This data shows that some of the large peaks of activity in the latter stages of the study were due to plant activity, while others appear to be due to counting equipment error. The activity for Station 8 on August 29 appears to be due to the plant activity while the activity for Station 14 on August 29 appears to be due to counting equipment error.

It should be stated again that the radiophosphorus of the bottom deposits is also available later in the season for uptake by the aquatic plants.

Several other plants which could not be sampled on a routine schedule were sampled intermittently and tested for activity. The filamentous algae which first appeared in the West Branch of the Sturgeon River on August 15, had counts of 40 c.p.m. on September 5, 1950. The water weed Anacharis sp. also had significantly high counts on September 5, 1950. Their counts were in the 20-30 counts per minute per gram range.

Invertebrates and Larval Insects

Oligochaetes. The oligochaetes, or segmented worms, occupy a peculiar niche in the stream's ecosystem. They are found inhabiting silt, debris or other materials of the

stream bottom. The activity curves of these invertebrates should give a more accurate picture of the bound radiophosphorus of the bottom. Some of the radiophosphorus is loosely bound to the silt and bottom materials and is quickly exchanged with the phosphorus of the water (Hutchinson and Vaughan, 1950; and Jitts, ibid.). The exchangeable radiophosphorus and the bound radiophosphorus are incorporated in the oligochaetes through ingestion of the detritus and silt. The bound radiophosphorus is available for longer periods of time for oligochaete ingestion than is the rapidly exchanged P^{32}.

The activity curves for the oligochaetes (Figure 19) present data which probably reflect the accumulation of radiophosphorus in the detritus in which they live. The activity curves show that 14 days following isotope entry, the worms had accumulated considerable amounts of activity.

Gammarus sp. The only station which supported a scud population, Gammarus, was Station 14. The scuds are voracious feeders, feeding on all kinds of plant and animal matter. They rarely feed upon living animals (Pennak, 1953). The activity curve for these omnivores shows that a considerable amount of radioactive food materials was available (Figure 20). A maximum count of 1,312 c.p.m. per gram was recorded for August 1, 1960. The collections on the dates of high activity were composed of small individuals. Hevesy (1948) noted that P^{32} accumulates in rapidly developing and growing organs. These large peaks on July 18 and August 1 may have been due

Figure 19. Activity for oligochaetes at Stations 5, 8, 12 and 14. Counts were corrected for background and decay.

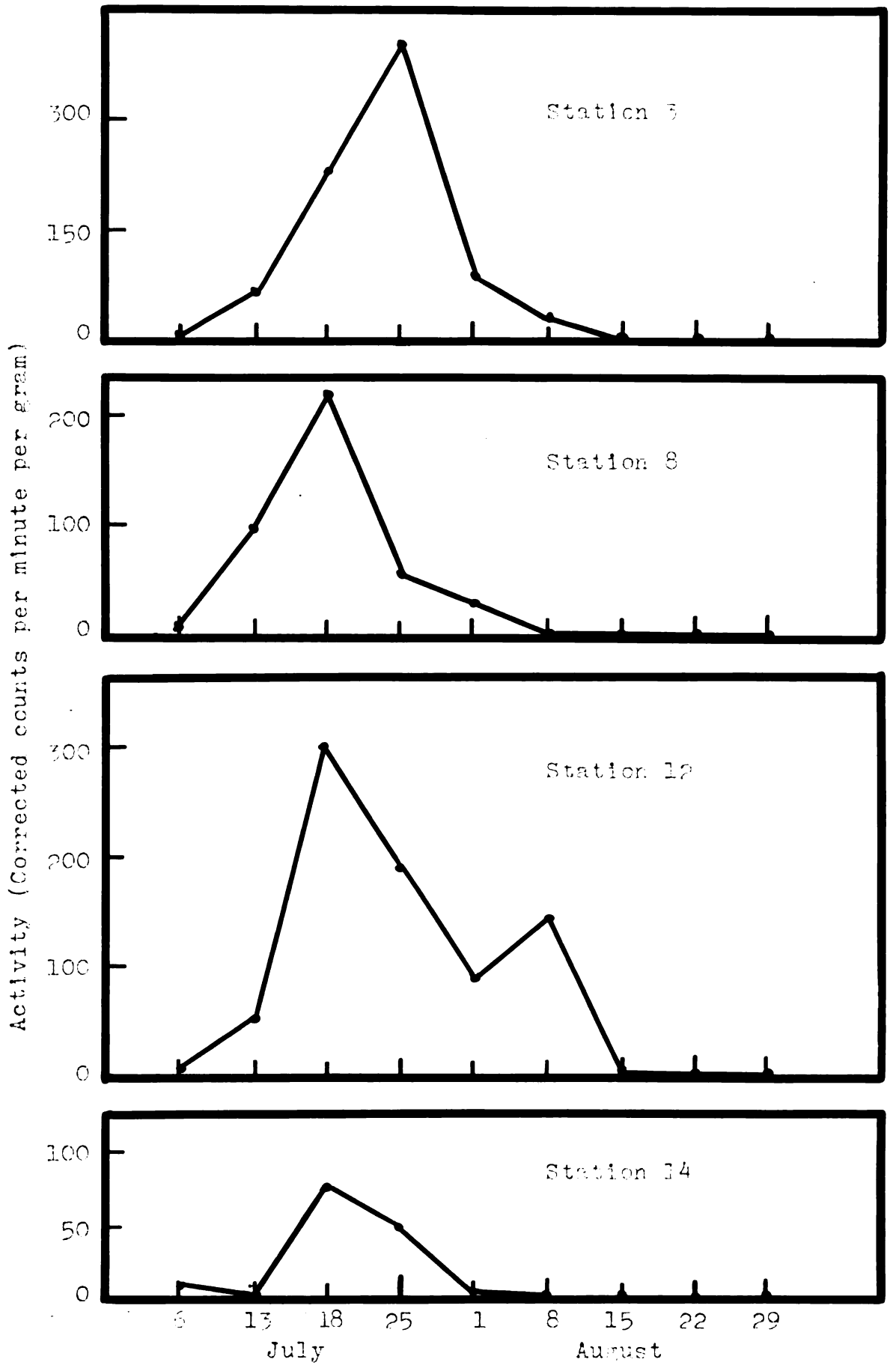
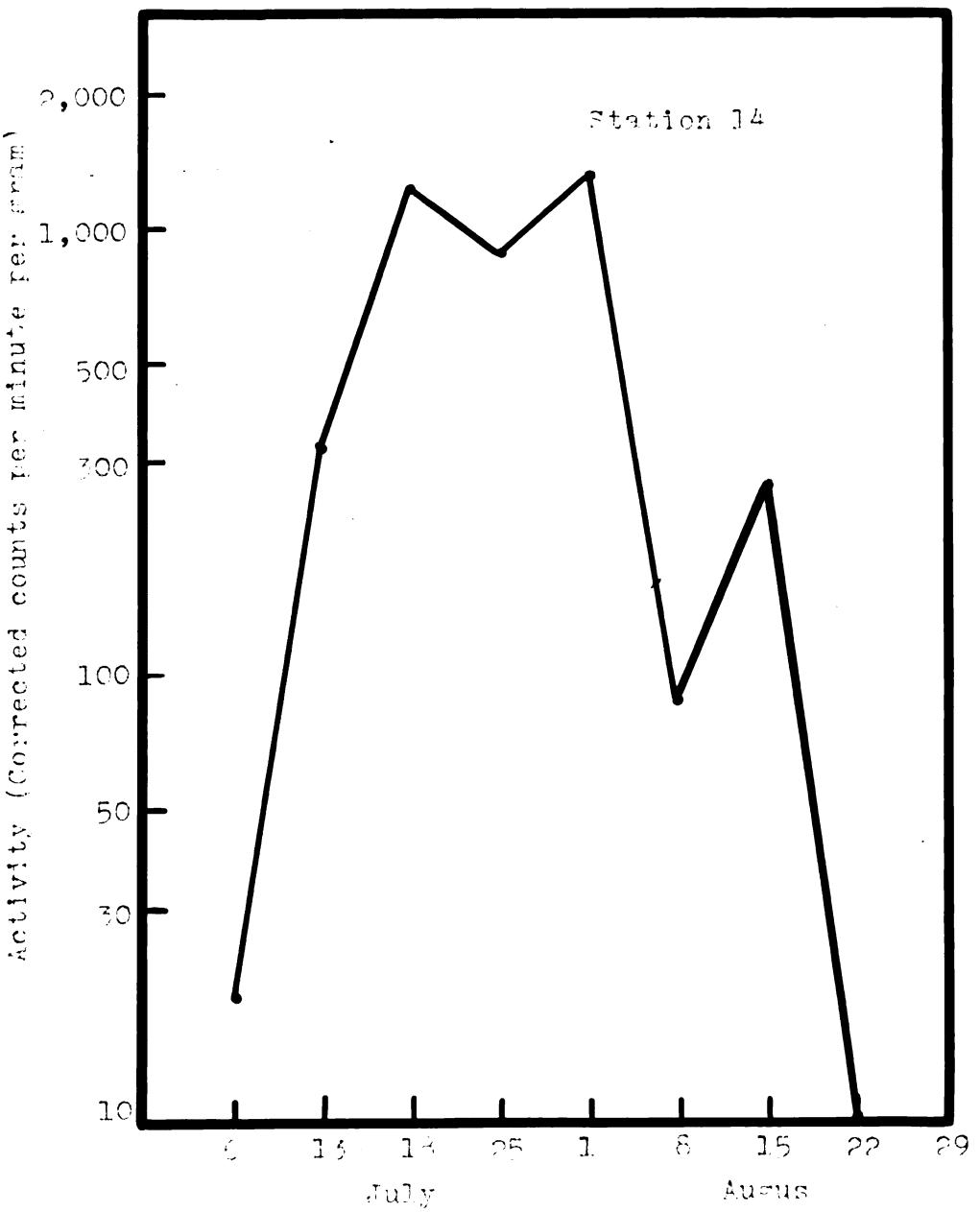

Station 3
Station 8
Station 12
Station 14
300
150
0
200
100
0
300
200
100
0
100
50
0
Activity (Corrected counts per minute per gram)
6
13
18
25
1
8
15
22
29
July
August

Figure 29. Activity of Gammarus at Station 14. Counts were corrected for background and decay.


Station 14
2,000
1,000
500
300
100
50
30
10
Activity (Corrected counts per minute per gram)
July
August

to the age of the animals collected. The peaks may also represent individual broods as Pennak (ibid.) states that some souls average 15 broods in 152 days.

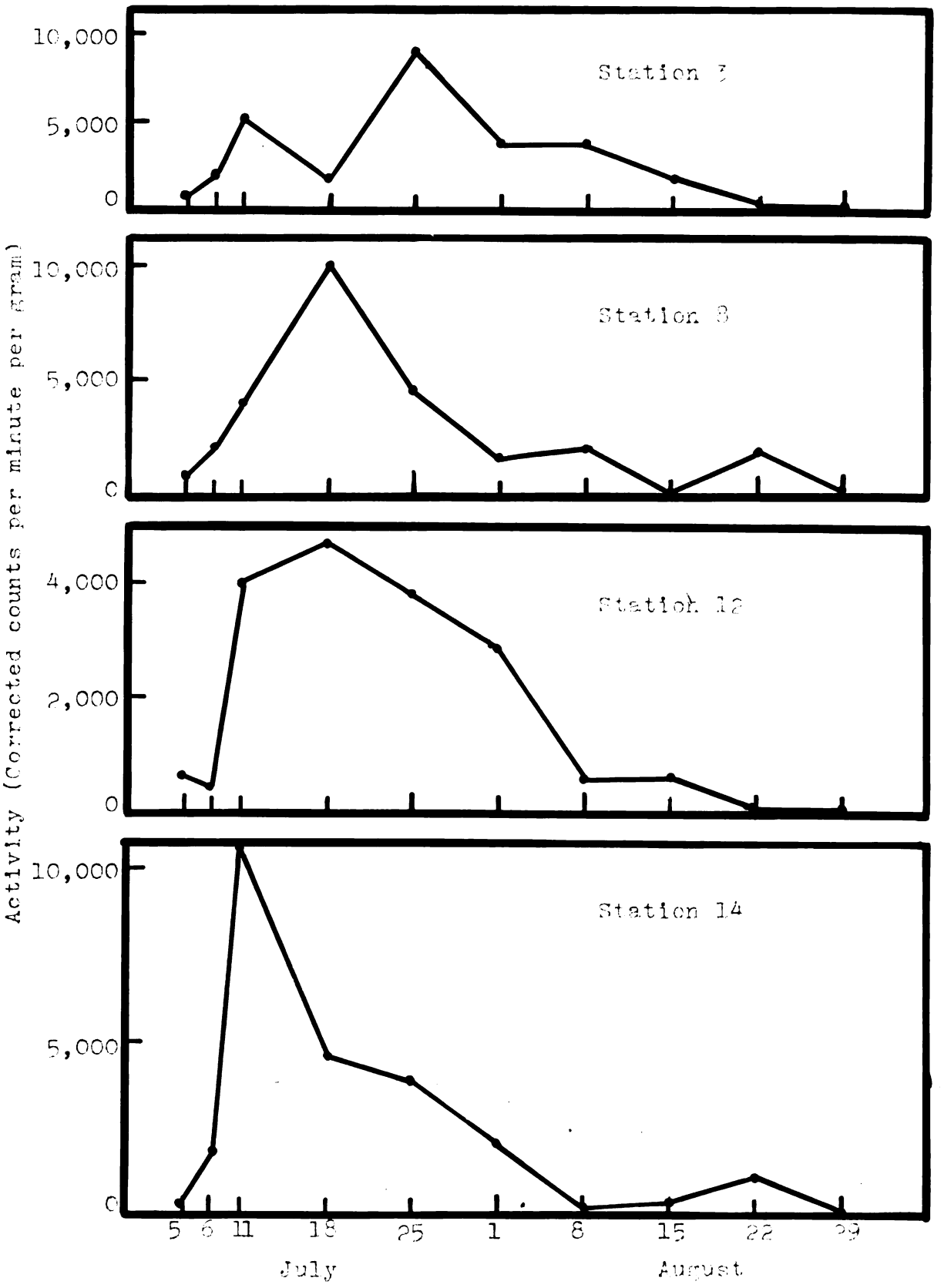
Hydropsyche sp. The hydropsychids attach their nets to rocks, logs, or large gravel and are seldom found in mud (Murray, 1938). The nets were found facing directly into the current. Hydropsyche sp. eats a preponderance of plankton, sessile diatom growths and other small organisms (Ross, 1944).

From the known feeding habits of Hydropsyche, the activity curves (Figure 21) may present a picture of the radiophosphorus of periphyton and plankton movement throughout the study period. The activity curves show that most of the radioactive food materials passed through the system in the first few weeks of the experiment. Small increases in activity occurred as late as August 22, which presumably shows that regenerated radiophosphorus was available for incorporation by the periphyton and plankton. There appears to be some counting equipment error during the latter part of the sampling period. This was demonstrated in the comparison of Figure 16 with Appendix V for Nasturtium activities.

Simulium sp. The blackflies, Simulium, are similar to the Hydropsyche in that both feed upon the moving particulate matter of the stream. The Simulium do not use the net as a collecting device as does the Hydropsyche, but instead use anterior plankton straining fans (Pennak, ibid.).

Simulium was one of the most numerous insects in the

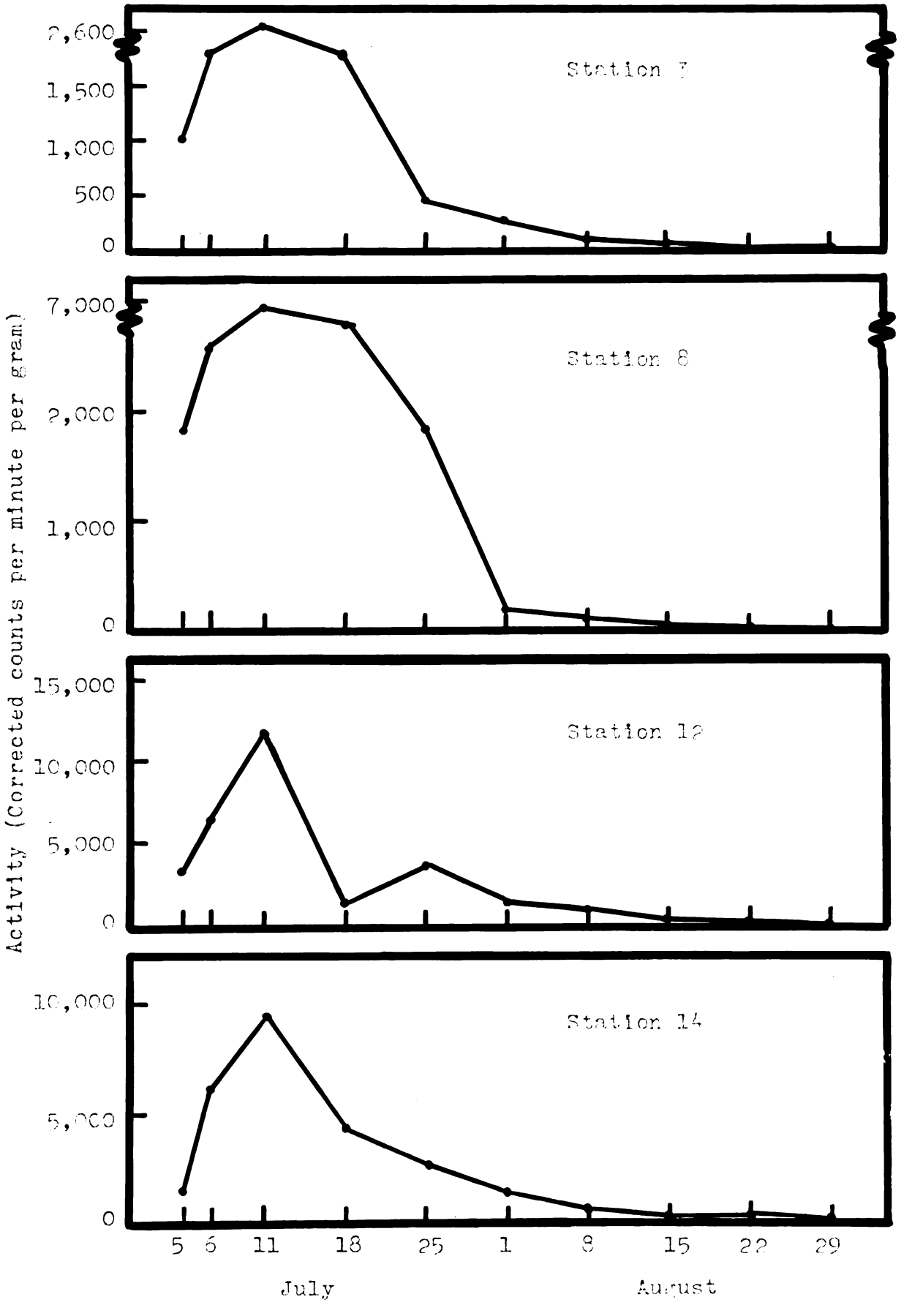
Figure 21. Activity of the net-caddis, Hydropsyche, at Stations 3, 8, 10 and 14. Counts were corrected for background and decay.


Activity (Corrected counts per minute per gram)
10,000
5,000
0
Station 3
10,000
5,000
0
Station 8
4,000
2,000
0
Station 12
10,000
5,000
0
Station 14
5
6
11
18
25
1
8
15
22
29
July
August

stream. Their adaptations for the lotic life include the filter fans and a sucker at the posterior end for attachment. It is also supplied with a safety device; a silk thread which it attaches to an anchorage which keeps it from floating away should the sucker become detached (Odum, ibid.). The blackflies were found on logs, twigs and aquatic plants, and were most abundant in swift current.

The activity curves for Simulium, like Hydropsyche, should present a picture of the moving particulate matter. The radioactive particulate matter also is an indication of the radiophosphorus regeneration. Unlike the net-caddis activity curves, the Simulium curves (Figure 22) show no increases in the latter part of the study period. Thus, from the Simulium activity curves, it might be hypothesized that little regenerated activity incorporated in the particulate matter (diatoms, etc.) passed through the system. However, there is a major habitat difference between Simulium and Hydropsyche. Simulium populations are most abundant along the stream margins. Hydropsyche populations are most abundant in deeper water and located generally near the center of the stream. Knight (ibid.) has indicated that there is horizontal variation in water activity along a stream transect. If so, it is likely that the locations of the populations, as well as the differing metabolism, accounts for the differences in the activities of Simulium and Hydropsyche. The metabolic differences may vary with the age of the sampled populations. The mayfly nymphs may mature within a minimum

Figure 22. Activity for blackflies, Simulium, at Stations 7, 8, 12 and 14. Counts were corrected for background and decay.


Activity (Corrected counts per minute per gram)
2,600
1,500
1,000
500
0
Station 7
7,000
2,000
1,000
0
Station 8
15,000
10,000
5,000
0
Station 12
10,000
5,000
0
Station 14
5 6 11 18 25 1 8 15 22 29
July
August

of a few months (Usinger, 1956) while dipteran larval stages may last for several weeks (Pennak, ibid.). The Simulium and Hydropsyche populations may have differed in their respective population ages. The increases in activity in the latter part of the sampling period of Hydropsyche may have been due to the population age and metabolism.

Ephemerella. The two mayfly nymphs studied were Ephemerella cornuta and Ephemerella needhami. The baetid naiad, Ephemerella needhami, was found primarily in the moss, Fontinalis, and thus fits into Usinger's classification of a rapid water moss-inhabiting form. The Ephemerella cornuta naiads were frequently found on sticks and submerged logs with the heptageneid naiad Iron sp. The naiads were in direct current, but they sheltered themselves from the current by staying behind the water moss or climbing in coveys in the sticks and logs.

Usinger (ibid.) compares the mayfly niche in the aquatic communities to that of cattle or rabbits in the terrestrial communities. Burks (1953) also stresses the herbivorous feeding habits of the mayflies. The nymphs are herbivores or scavengers, living on vegetable detritus and microscopic aquatic organisms, principally diatoms (Burks, ibid.).

The activity curves for the mayfly nymphs (Figures 23 and 24) are not as horizontally extended as are those for Hydropsyche. Although the food consumed is similar in both cases, the procurement of the food is quite different. The curves for Hydropsyche have slight peaks in the latter part of the sampling period. These peaks probably represent the

Figure 21. Activity of the baetid mayfly naiad, Ephemerella serrata, at Stations 1, 2, 13 and 14. Counts were corrected for background and decay.

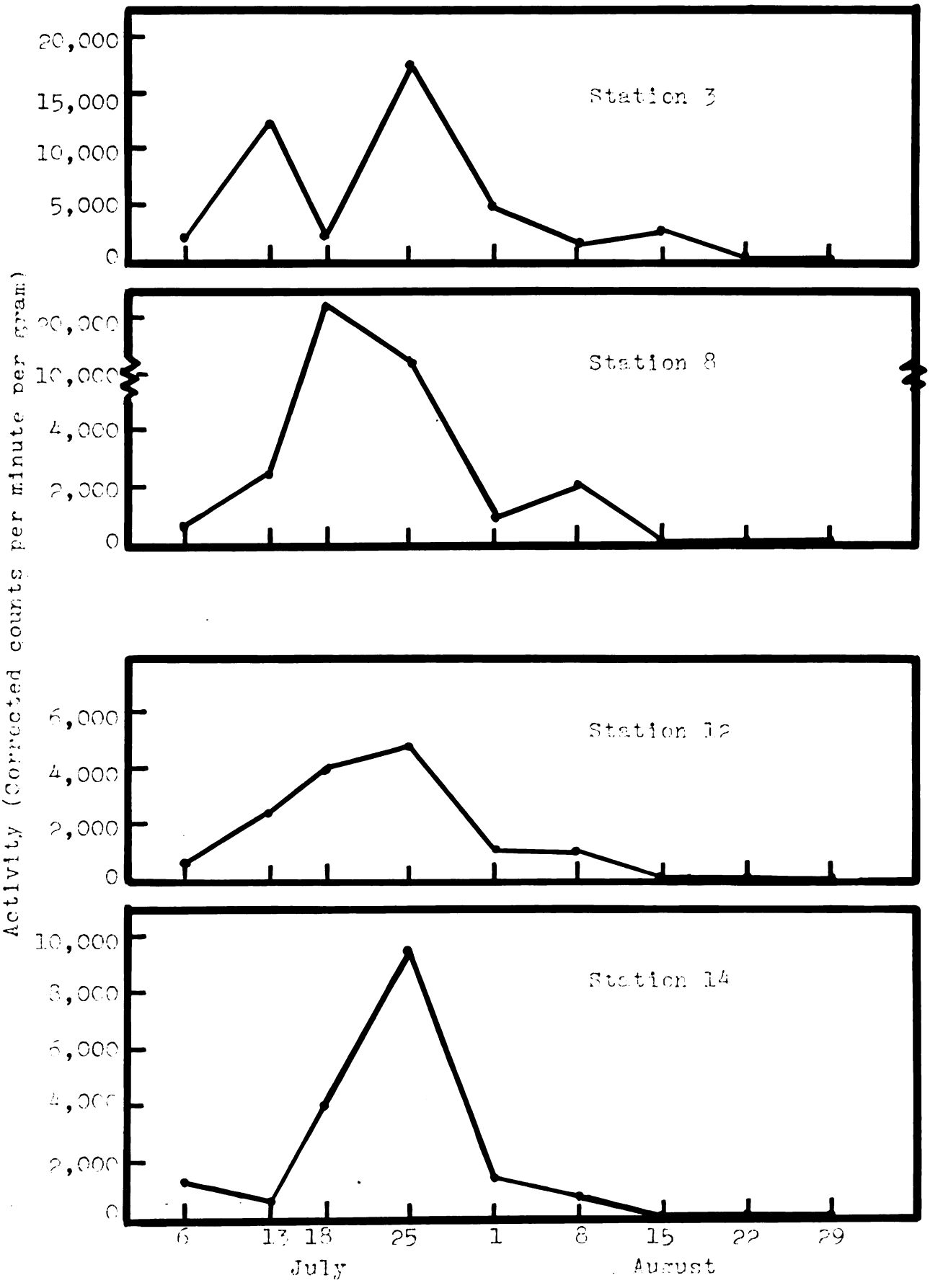
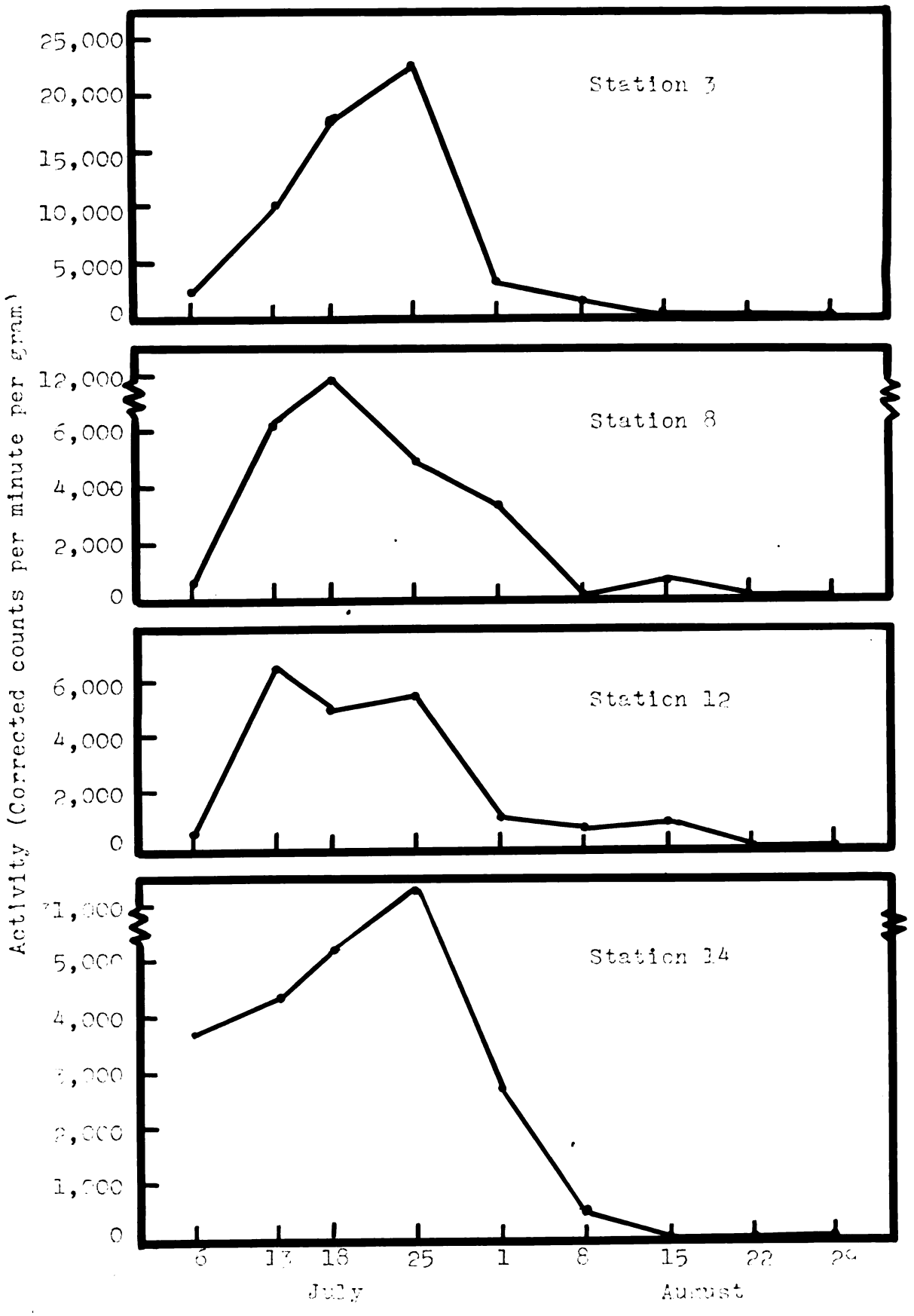

Station 3
20,000
15,000
10,000
5,000
0
Station 8
20,000
10,000
4,000
2,000
0
Station 12
6,000
4,000
2,000
0
Station 14
10,000
8,000
6,000
4,000
2,000
0
Activity (Corrected counts per minute per gram)
6
13
18
25
1
8
15
22
29
July
August

Figure 24. Activity of the baetid mayfly naiad, Ephemerella needhami, at Stations 5, 8, 12 and 14. Counts were corrected for background and decay.


Activity (Corrected counts per minute per gram)
Station 3
25,000
20,000
15,000
10,000
5,000
0
Station 8
12,000
6,000
4,000
2,000
0
Station 12
6,000
4,000
2,000
0
Station 14
5,000
4,000
1,000
0
6
18
25
1
8
15
July
August

incorporated radiophosphorus of the moving plankton and dislodged periphyton. Ephemerella, however, does not capture dislodged periphyton or plankton as does Hydropsyche. The mayfly nymphs browse over the substrates upon which they live and take up radiophosphorus primarily from sessile food forms.

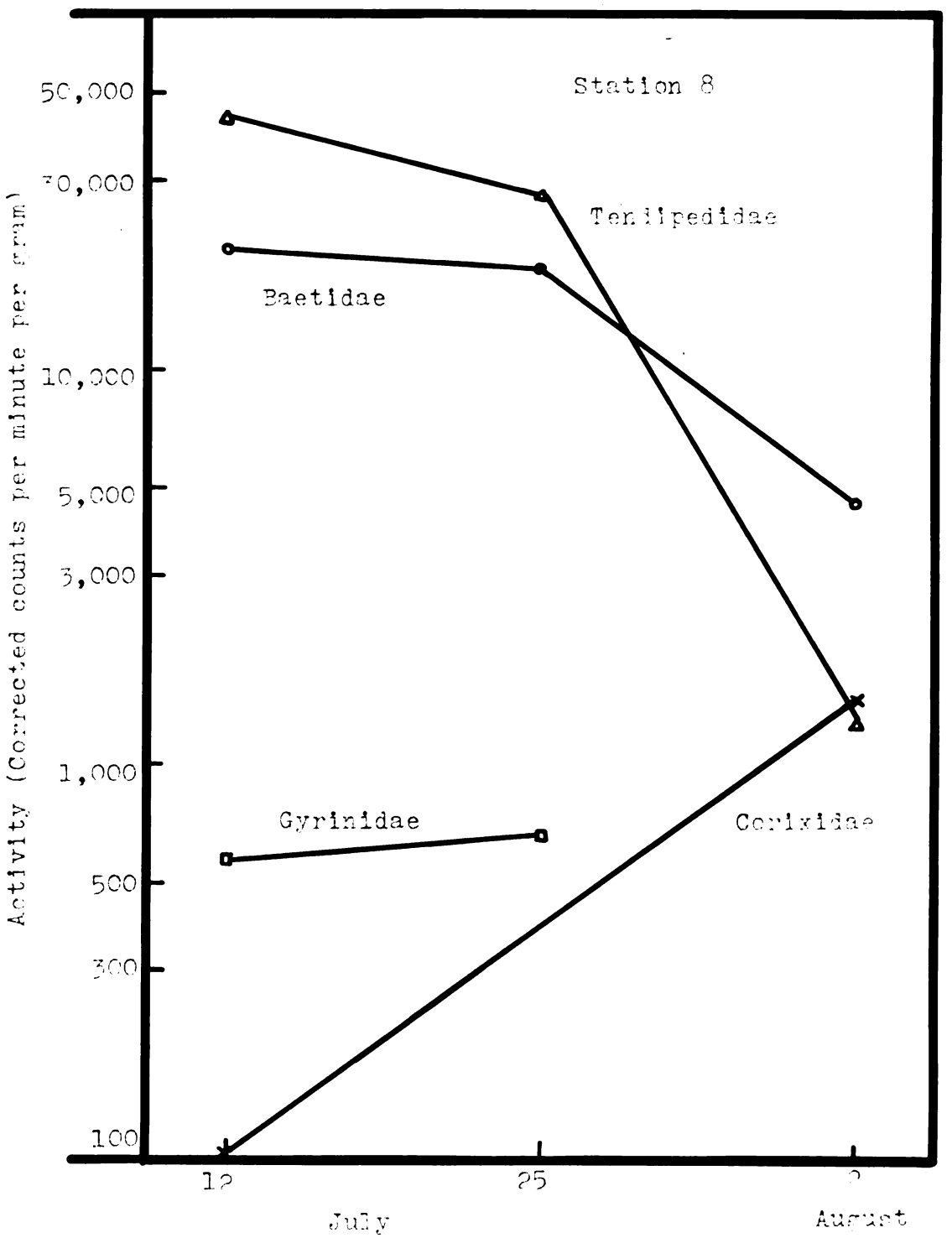
The mayfly nymphs are indeed important herbivores as Usinger (ibid.) has stated. The highest recorded activity for Ephemerella corruta was slightly over 21,000 counts per minute per gram. The maximum for Ephemerella needhami was over 78,000 counts per minute per gram.

Mature Insects

The adult insects were collected at Station 8 by means of a light trap. Although numerous specimens were obtained, only four families were present in sufficient numbers to be used in the study.

The corixid or water boatman was generally present in the pools and backwaters. The corixids are uniquely adapted to their environment by the possession of scoop-like front tarsi which enable them to browse through detritus to procure their food. The activity curve (Figure 25) shows that the corixids gradually increased in activity from the first to the last collection. In fact, at the first collection, their activity was zero, but by August 2, it was almost 2,000 counts per minute per gram. This may be accounted for by noting that although the activity in the stream was decreasing, a greater amount of activity was piling up in dead cells,

Figure 25. Activities of four families of adult insects collected by light trap. Counts were corrected for background and decay.


Station 8
50,000
10,000
10,000
5,000
3,000
1,000
500
300
100
Activity (Corrected counts per minute per gram)
Tenuipedidae
Baetidae
Gyrinidae
Corixidae
12
25
July
August

detritus and mud, and this is the browsing pasture for the corixids.

The Gyrinidae or whirligig beetles are found most frequently in small colonies inhabiting the backwaters. Their food consists of smaller insects and possibly detritus materials (Pennak, ibid.). Their morphological adaptations for the environment include; divided compound eyes, flattened fringed hindlegs, and long, hooked forelegs. They also carry an oxygen supply under the elytra. These insects were collected only on two of the three collecting trips, but from the data obtained, they appear to be detritus feeders, as a slight increase in activity was recorded.

The baetids or mayflies are herbivorous substrate browsers. The adults have activity curves (Figure 25) which are different from the nymphs (Figures 23 and 24). The activities for the adults on July 12 were 20,000 counts per minute per gram, while the nymphs on July 13 were in the range of 2,000 to 10,000 counts per minute per gram. The differences in activity may be explained as a function of weight determination.

The nymphs' wet weight is determined by the outline in the section on methods. The weight of the nymphs, however, contains much more water than the respective adult. Thus, the weight of the adult is less, giving a corresponding increase in the counts per minute per gram.

The highest activity recorded was that of the tendipedids. The midges (Tendipedidae) had recorded counts per minute per gram of slightly over 44,500. This great accumulation of

activity is again probably an effect of weight determination. The mides are herbivorous and feed chiefly on algae, higher aquatic plants, and detritus (Pennak, ibid.). The high activity may also be due to rapid metabolism.

The adult insects are a factor in the removal of radiophosphorus from the stream ecosystem, since great numbers of these insects are leaving the system throughout the summer.

Sculpins

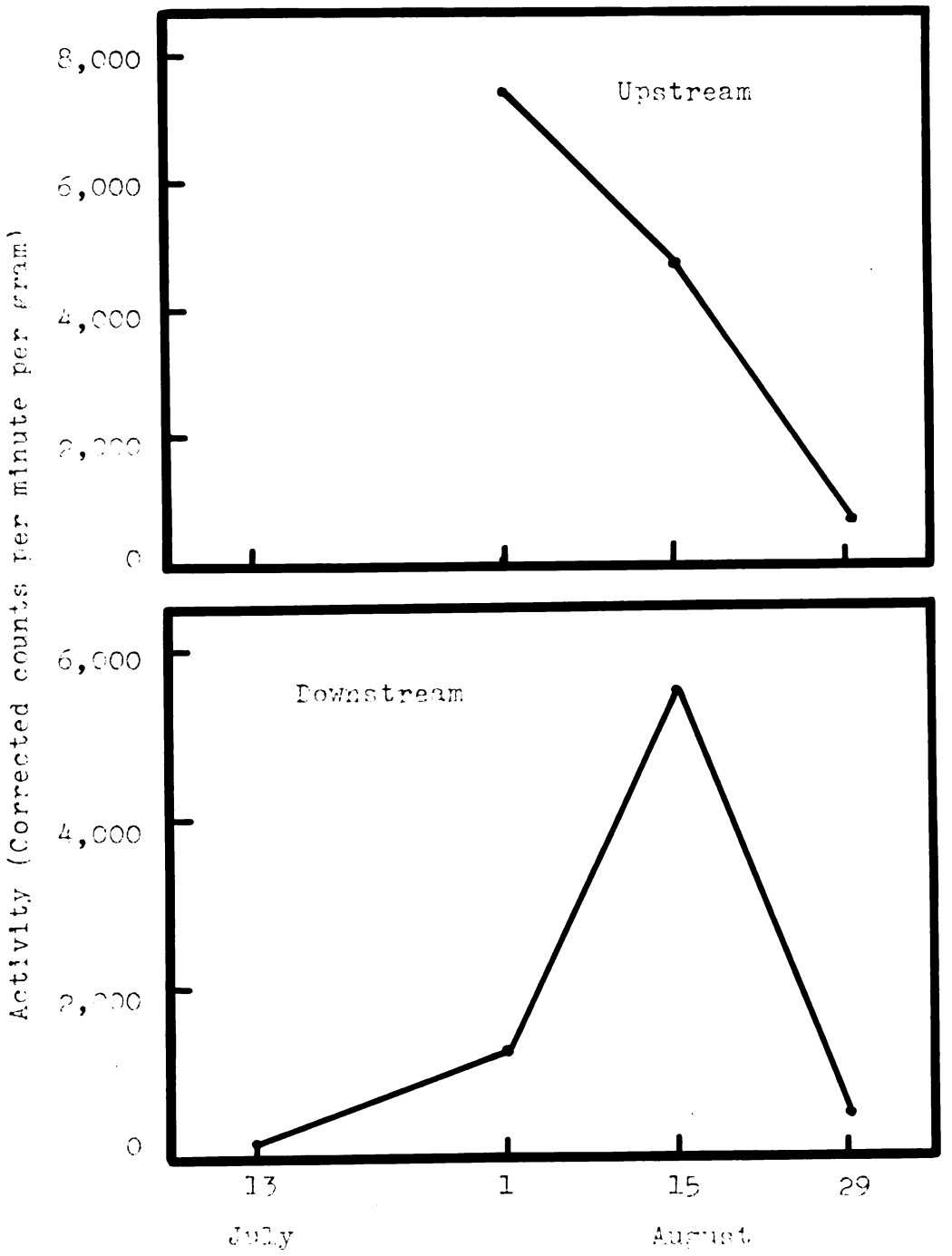
Although a continuous sampling of the fish was not part of the present study, some conclusions can be drawn from the intermittent collections made during the summer. The muddlers, or sculpins, Cottus bairdii and Cottus cognatus, were collected seven times throughout the summer. The muddlers lie on the bed of the stream on logs or under rocks. Their food is composed of entomastraca and small crustacea, with occasional insects, stonefly larvae, mayfly nymphs, Chironomus and Simulium larvae (Cahn, 1927).

The variability of the activity curves (Figure 26) is similar to Knight's (ibid.). These curves reflect the opportunistic nature of the sculpin's feeding habits. The upstream activity is the averaged data for Stations 3 and 8 and the downstream activity is the averaged data for Stations 12 and 14.

Translocation and Removal of P^{32} through Food Chains and the Ecosystem

As stated before, the translocation of the radiophosphorus follows the physical and biological pathways of the stream. The biological translocation can be seen from a food chain

Figure 26. Activity of sculpins at the upstream Stations 3 and 8 and at the downstream Stations 12 and 14. Counts were corrected for background and decay.


Upstream
Downstream
8,000
6,000
4,000
2,000
0
6,000
4,000
2,000
0
Activity (Corrected counts per minute per gram)
13
1
15
29
July
August

constructed from the food preferences and activity curves of the respective representatives. When reading the food chain (Figure 27) from bottom to top, it is apparent that the activity curves are steadily shifting to the right. The gradual shift also represents a picture of the trophic levels of the stream. As the activity of the periphyton diminishes, the activity of the periphyton feeders gradually increases. The curves for Ephemerella and Gammarus show a gradual shift from those of the periphyton feeders. The secondary consumer, Cottus, reaches its maximum activity late in the study period. The activity had been previously incorporated in the lower trophic levels. Food chain studies involving radioactive materials have compound importance in revealing patterns, cycles, rates, and in alerting us to possible dangerous concentrations of radioactivity (Odum, 1956).

The radiophosphorus translocation follows very complex patterns. Some of the P^{32} is removed directly with the current; some is immediately incorporated in the biological networks. Meanwhile, some of the P^{32} is removed from immediate biological incorporation by adsorbing to the stream bottom. Other radioactivity is removed from the system by the roots of the neighboring terrestrial flora. And finally, additional radioactivity is lost due to removal or emergence of organisms which have accumulated radiophosphorus. Figure 28 presents a graphic view of some of the mechanisms by which radiophosphorus is translocated or removed from the stream.

Figure 37. Translocation of radiophosphorus via food chains. The mean counts of the specimen were corrected for background and decay.

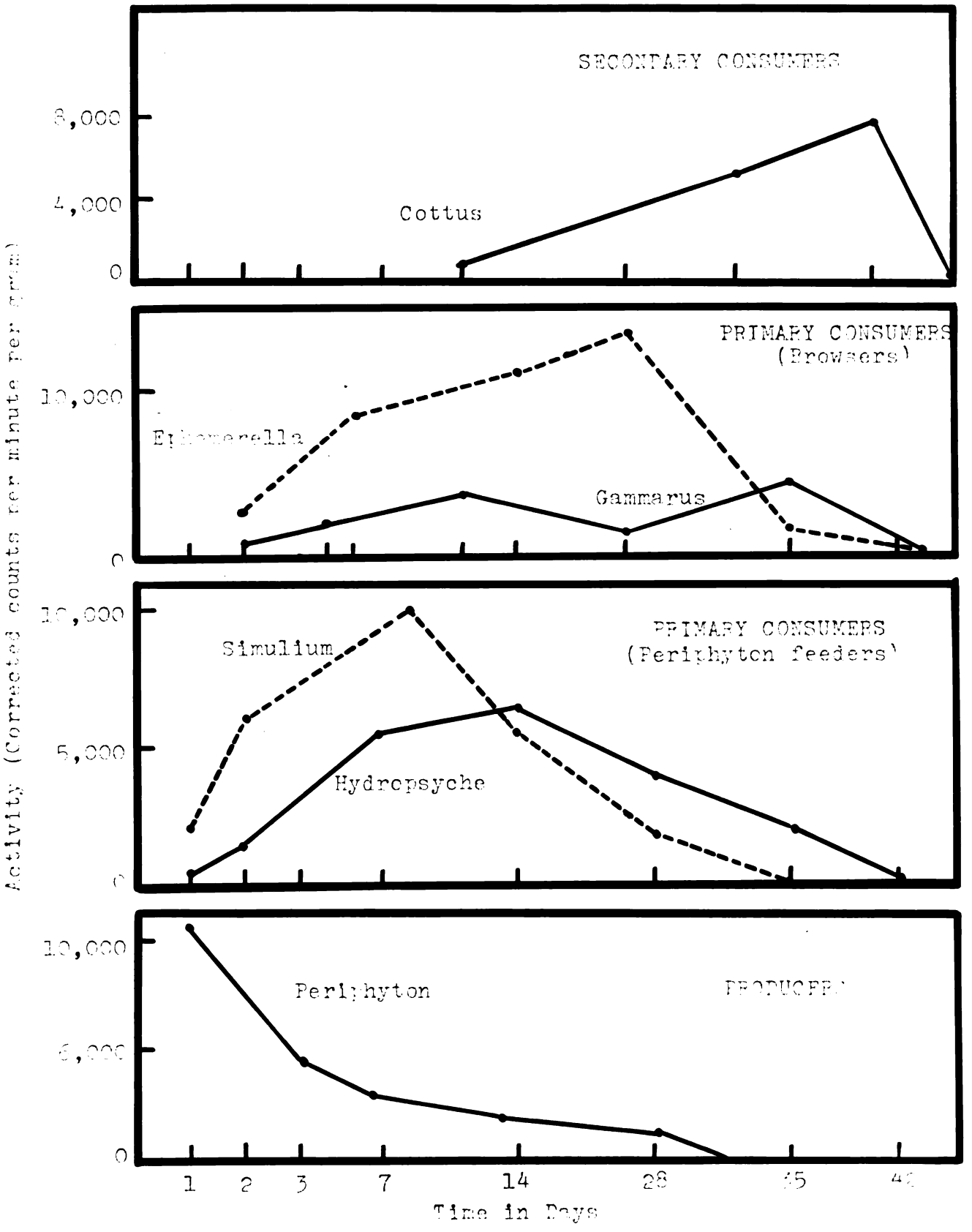
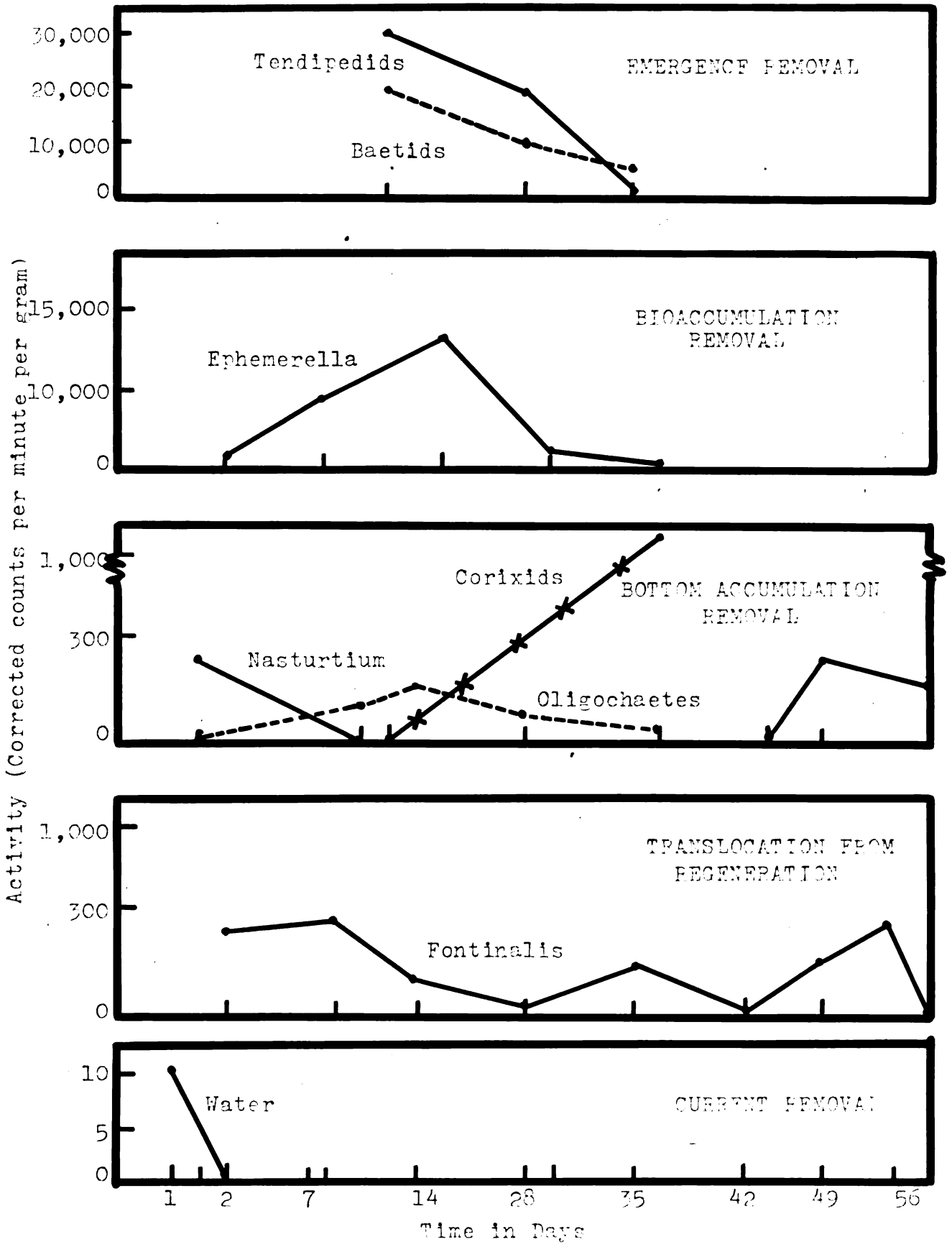

SECONDARY CONSUMERS
8,000
4,000
0
Cottus
PRIMARY CONSUMERS
(Browsers)
10,000
Ephemerella
Gammarus
0
10,000
5,000
Simulium
PRIMARY CONSUMERS
(Periphyton feeders)
Hydropsyche
10,000
Periphyton
0
1
2
3
7
14
28
Time in Days
Activity (Corrected counts per minute per gram)

Figure 28. Translocation and removal of radiophosphorus from the lotic ecosystem. The mean counts were corrected for background and decay.


30,000
20,000
10,000
0
Tendipedids
Baetids
EMERGENCE REMOVAL
15,000
10,000
0
Ephemerella
BIOACCUMULATION REMOVAL
1,000
300
0
Corixids
Nasturtium
Oligochaetes
BOTTOM ACCUMULATION REMOVAL
1,000
300
0
Fontinalis
TRANSLOCATION FROM REGENERATION
10
5
0
Water
CURRENT REMOVAL
1
2
7
14
28
35
42
49
56
Time in Days
Activity (Corrected counts per minute per gram)

Chelation

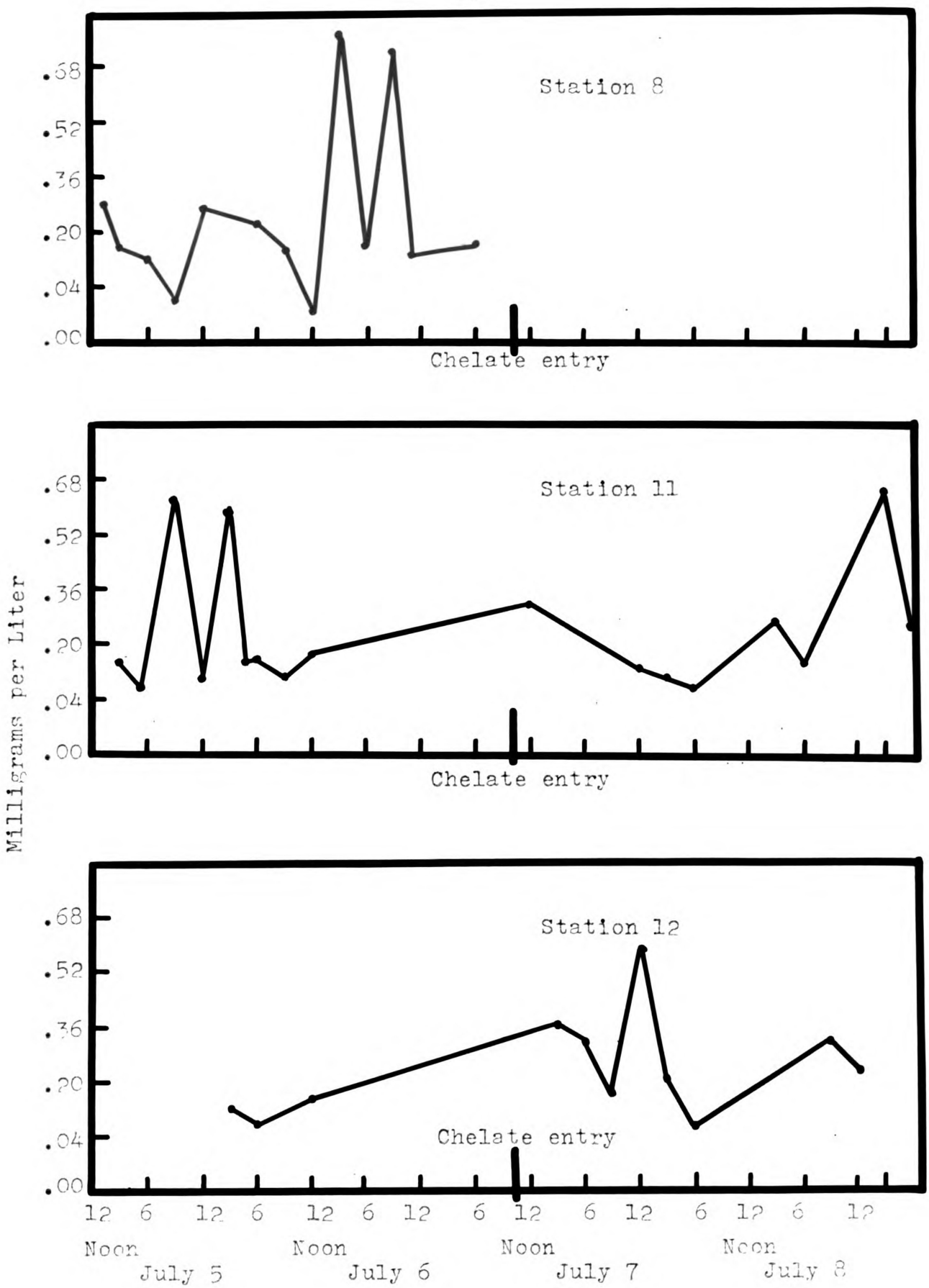
Ringed structures formed by coordination of a given species at two points are called chelate rings. Chelating agents are ringed complexes capable of stoichiometric reactions with metals, resulting in the formation of a ring in which the metal is held by coordinate valences (Stewart and Leonard, 1956).

Chelating of metals has been used to overcome soil fixation of plant nutrients (Stewart and Leonard, ibid.). Chelating agents are used to supply the micronutrients iron, zinc and manganese to plants (Wallace, 1960), and some of the chelating agents have produced auxin-like effects (Wallace, ibid.).

In an effort to determine the effects of chelating agents on the nutritional cycle, or the effects on activity displacement, an iron chelate was added to the stream at Station 8 on July 7, 1960. The concentration of the iron chelate NaFeEDTA (iron, sodium N-hydroxyethylethylenediamine) was approximately one part per million.

There seems to have been only a slight effect of the chelate on the iron in the system. The automatic pumps located at Stations 8, 11 and 12 sampled the area. The chelate entered the stream at 10:00 a.m. on July 7. The automatic sampling device at Station 8 was stopped before the entrance of the chelate. This was done to obtain data on the natural fluctuation of the stream before chelate entry. The duration and fluctuation of the iron curve (Figure 29) will give an indication of natural variability taken at Station 8.

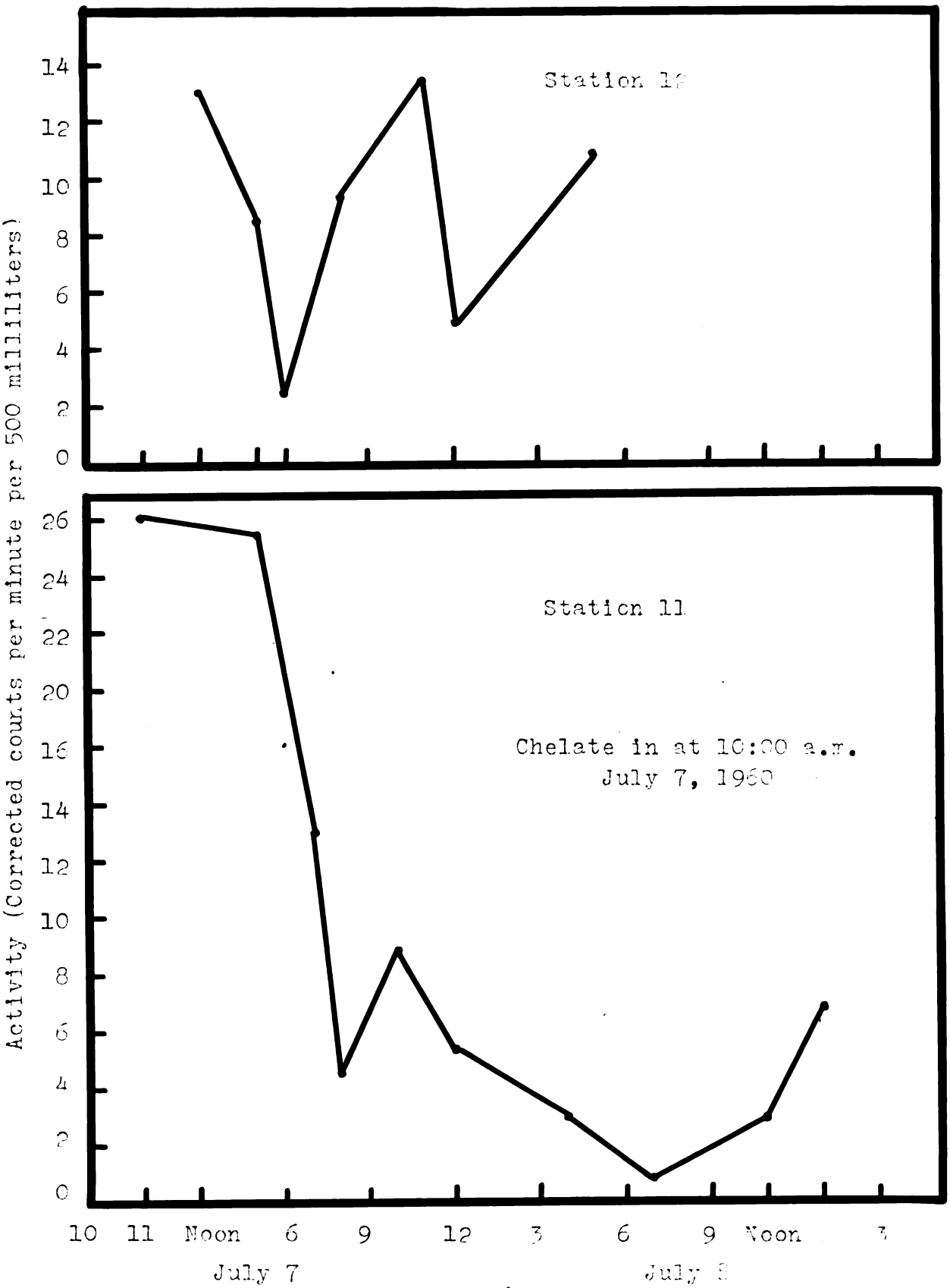
Figure 29. Iron content of chelated water for Stations 8, 11 and 12. The chelate entered the stream at 10:00 p.m. on July 7, 1960.


Station 8
.68
.52
.36
.20
.04
.00
Chelate entry
Station 11
Milligrams per Liter
Chelate entry
Station 12
Chelate entry
12 6 12 6 12 6 12 6 12 6 12 6 12 6 12
Noon
July 5
Noon
July 6
Noon
July 7
Noon
July 8

The concentrations of the iron expressed in milligrams per liter show a slight and possibly insignificant increase after the chelate entry. The activities of Figure 5 show the radiophosphorus before the chelate was added, while the activities of Figure 30 show the radiophosphorus after the chelate was added. From Figure 5, it is noted that Station 11 had a count of 15 counts per minute per 500 milliliters at 6:00 a.m. on July 7 while from Figure 30 at 11:00 a.m. of the same day and just one hour after the chelate was introduced, the count was 26 c.p.m. However, the effects at Station 11 are somewhat negated by the comparison of Station 12 in Figures 5 and 30. The counts were over 200 at 7:00 a.m. on July 7, while at 12:00 noon, just two hours after the chelate was introduced, the count was 13 c.p.m. It was noted that the chelate-containing water samples appeared to have an unusual and sometimes violent effect upon the stainless steel planchets when treated with concentrated acid and heated: sometimes turning the planchet black, and sometimes creating a black foam which bubbled over the sides of the planchet. This may have affected the counts.

From an analysis of the phosphorus content of the water at Stations 8, 11 and 12, little conclusion about the chelate effect can be drawn. There were slight increases in the concentrations of phosphorus immediately after the chelate was introduced, but in light of the natural variation as presented in the data of Station 8, the curves for Stations 11 and 12 may only show natural fluctuation.

Figure 40. Activity of chelated water for Stations 12 and 11. Counts were corrected for decay and background. Chelate entered at 10:00 p.m. on July 7.


Station 12
Station 11
Chelate in at 10:00 a.m.
July 7, 1960
Activity (Corrected counts per minute per 500 milliliters)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
10
11
Noon
6
9
12
3
6
9
Noon
3
July 7
July 8

Hand-collected samples were taken immediately after the addition of the chelate. The samples were taken on July 7, 1960 at Station 12 at five-minute intervals starting at 10:50 a.m. for the first hour and then at ten-minute intervals. The same procedure was followed at Station 14 with the starting time at this station of 11:07 a.m. of the same day.

The 140 ml. samples were analyzed for total phosphorus, iron content, and activity. There seems to be little consistency in the respective curves (Figures 32 and 33). During the first hour, however, the greatest peaks in activity appear. This activity may have been due to a release from flora and fauna upon which the chelate had an effect.

It seems that there is little that can be said with assurance about the chelate and its relationship to P^{32} or even iron release. There seems to have been only a slight increase in activity due to the chelating agents. It is important to stress the considerable distance (2080 yards from Station 8 bridge to Station 14) between the chelate entry site and the sampling stations. A station nearer the chelate entry point may have shown a more marked chelate effect.

Total Phosphorus and Specific Activity

In order to compare the translocation of radiophosphorus in the different biotic representatives, a specific activity calculation was made. The specific activity of an organism is the ratio of the quantity of radiophosphorus to the quan-

Figure 71. Phosphorus (P^{32}) content of chelated water for Stations 8, 11 and 12.

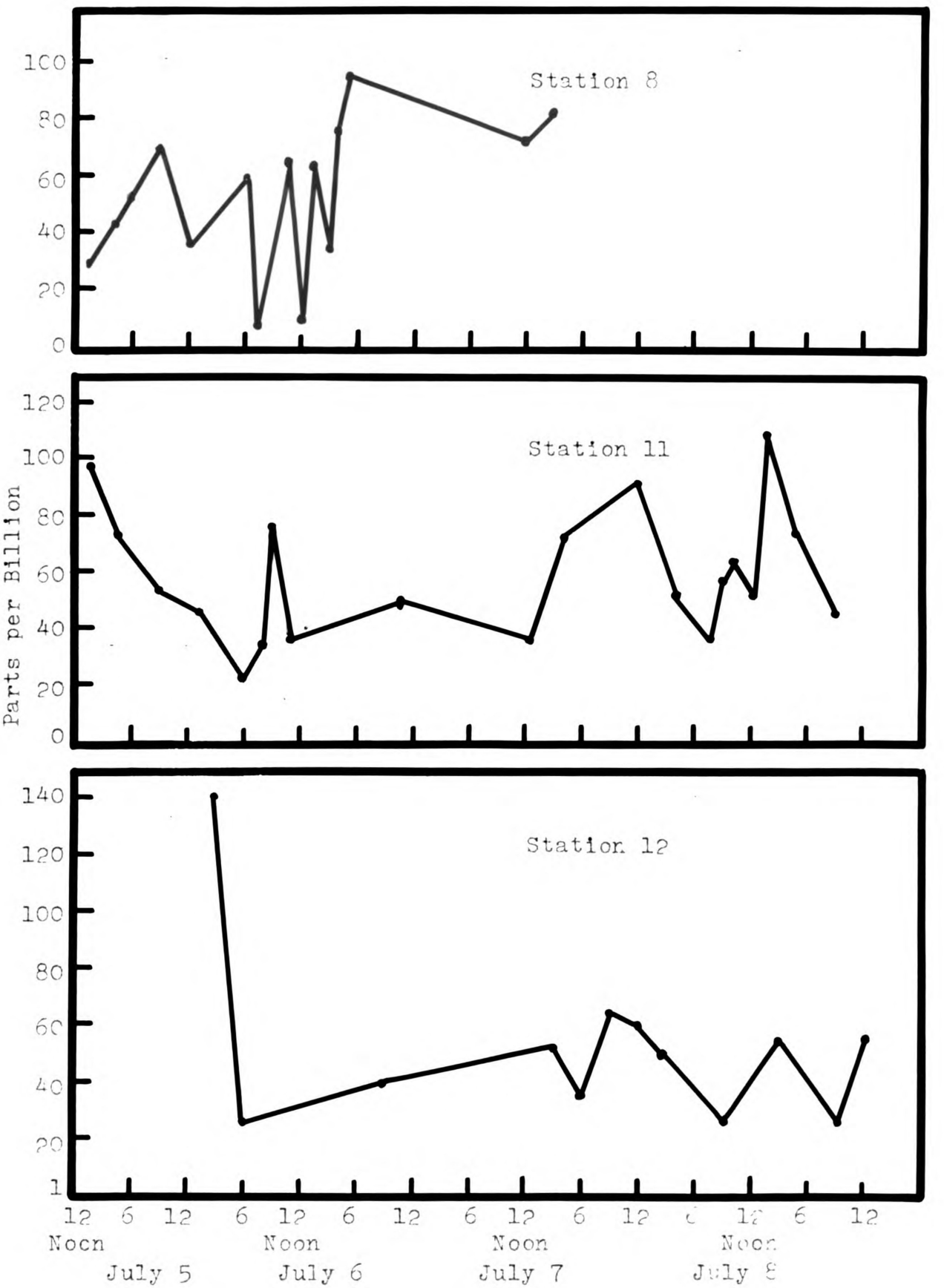

Station 8
Station 11
Station 12
Parts per Billion
100
80
60
40
20
0
120
140
1
12 6 12 6 12 6 12 6 12 6 12 6 12 6 12
Noon
July 5
July 6
July 7
July 8

Figure 32. A comparison of total phosphorus, iron, and activity for Station 12 during the chelate flow. Counts were corrected for background and decay. The chelate entered the stream at 10:00 a.m.

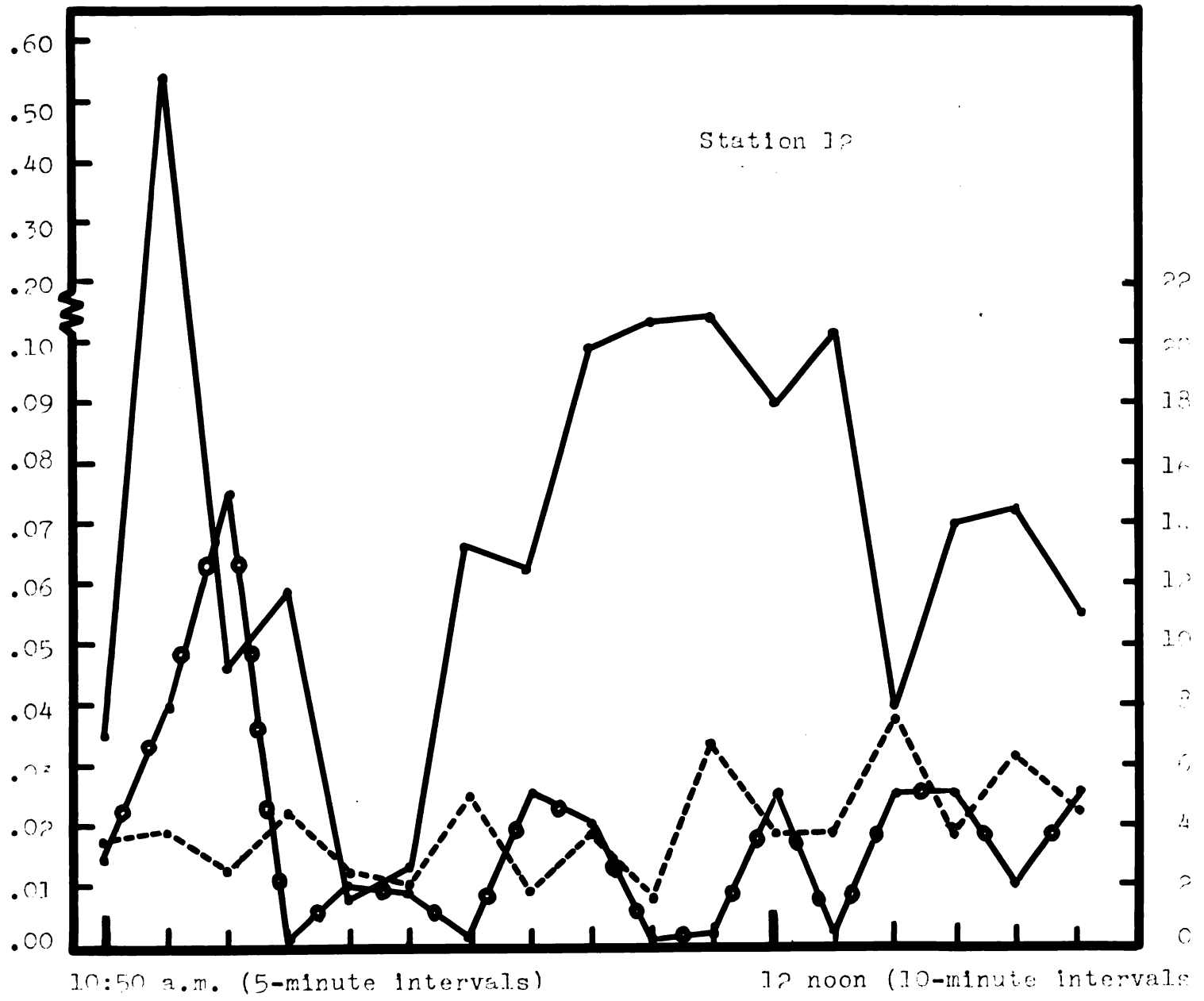

.60
.50
.40
.30
.20
.10
.09
.08
.07
.06
.05
.04
.02
.01
.00
Station 12
22
18
16
14
12
10
8
6
4
2
0
10:50 a.m. (5-minute intervals)
12 noon (10-minute intervals

Figure 33. A comparison of total phosphorus, iron, and activity for Station 14 during the chelate flow. Counts were corrected for background and decay. The chelate entered the stream at 10:00 a.m.

Activity (Corrected counts per minute per 140 milliliters)
Milligrams per Liter
Station 14
CPM
Iron
Phosphorus
10:30 a.m. (5-minute intervals)
12 noon (10-minute intervals)

tity of stable P^{31}. The specific activity calculations for Figure 34 represent the ratio of microcuries of P^{32} to the grams of P^{31}. In Figure 34, the values for the activities (microcuries of P^{32}) were selected from the maximum activities of the first collecting week, July 5 to July 12, for the various organisms.

The specific activities show much the same picture as the food chain curves in Figure 27. During this early stage, the producers and organisms which feed directly on the producers have the greatest specific activity. The range of specific activities ran from 3,625 for periphyton, to less than one for Cottus and the trout. It appears that Hydropsyche and the two mayfly nymphs utilize periphyton as a major portion of their food.

Figure 35 compares the specific activities of the same organisms on August 15, which is 41 days after the isotope entry. The most apparent change is one of magnitude. The specific activities of Figure 35 range up to the hundreds rather than in the thousands. The fish have specific activities which are greater than one. The bottom dwellers have also increased in specific activities. Although the curve for Fontinalis seems large, it should be placed in perspective by noting that the actual specific activity has been reduced by two thirds that shown in Figure 34. The tabular data for Figures 34 and 35 is presented in Appendix V.

The specific activities for the producers and primary consumers were high during the first part of the isotope

Figure 34. The specific activity of various organisms during the first week of the study. The isotope entered on 7-5-60 and the values are for 7-5-60 to 7-12-60.

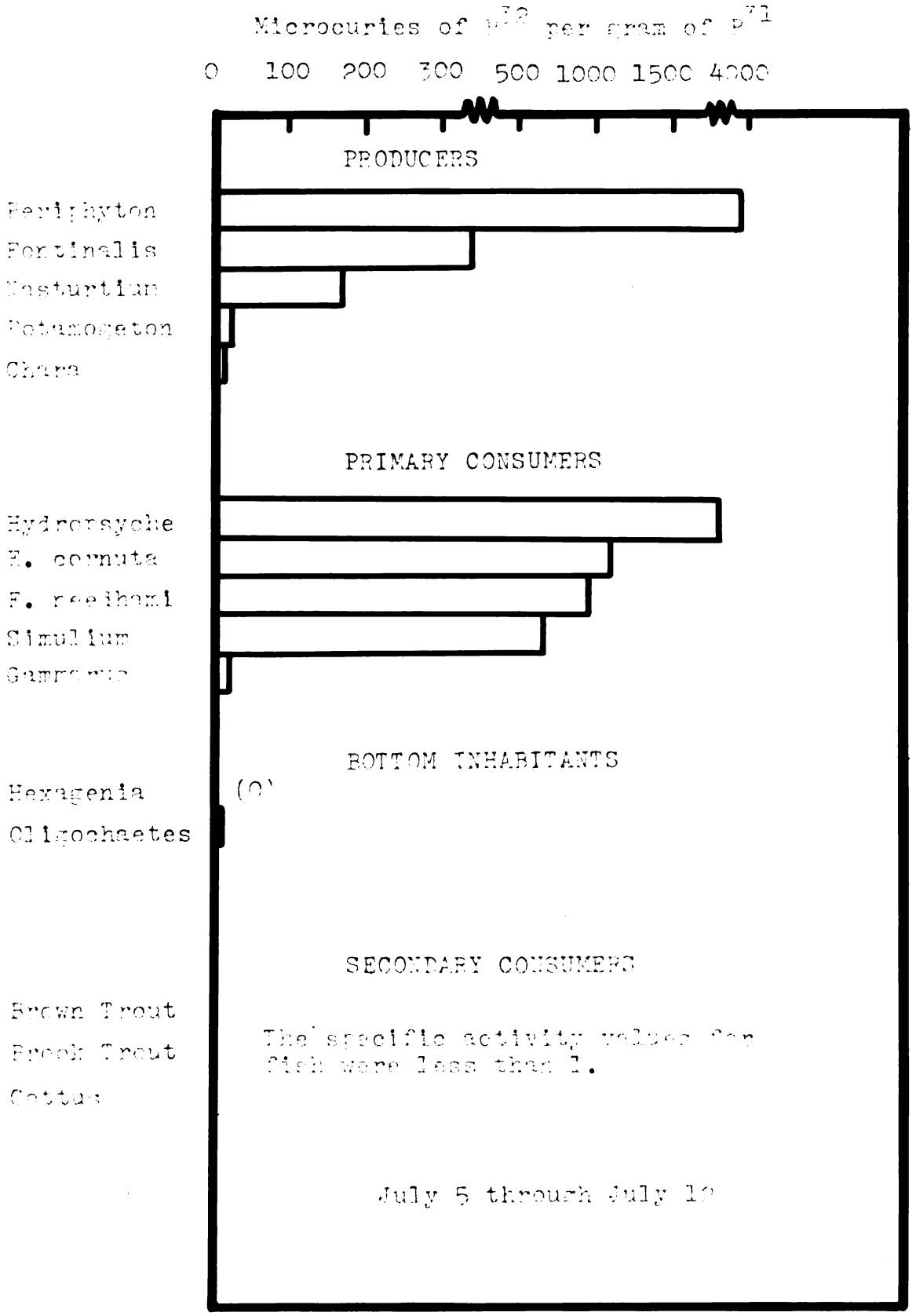
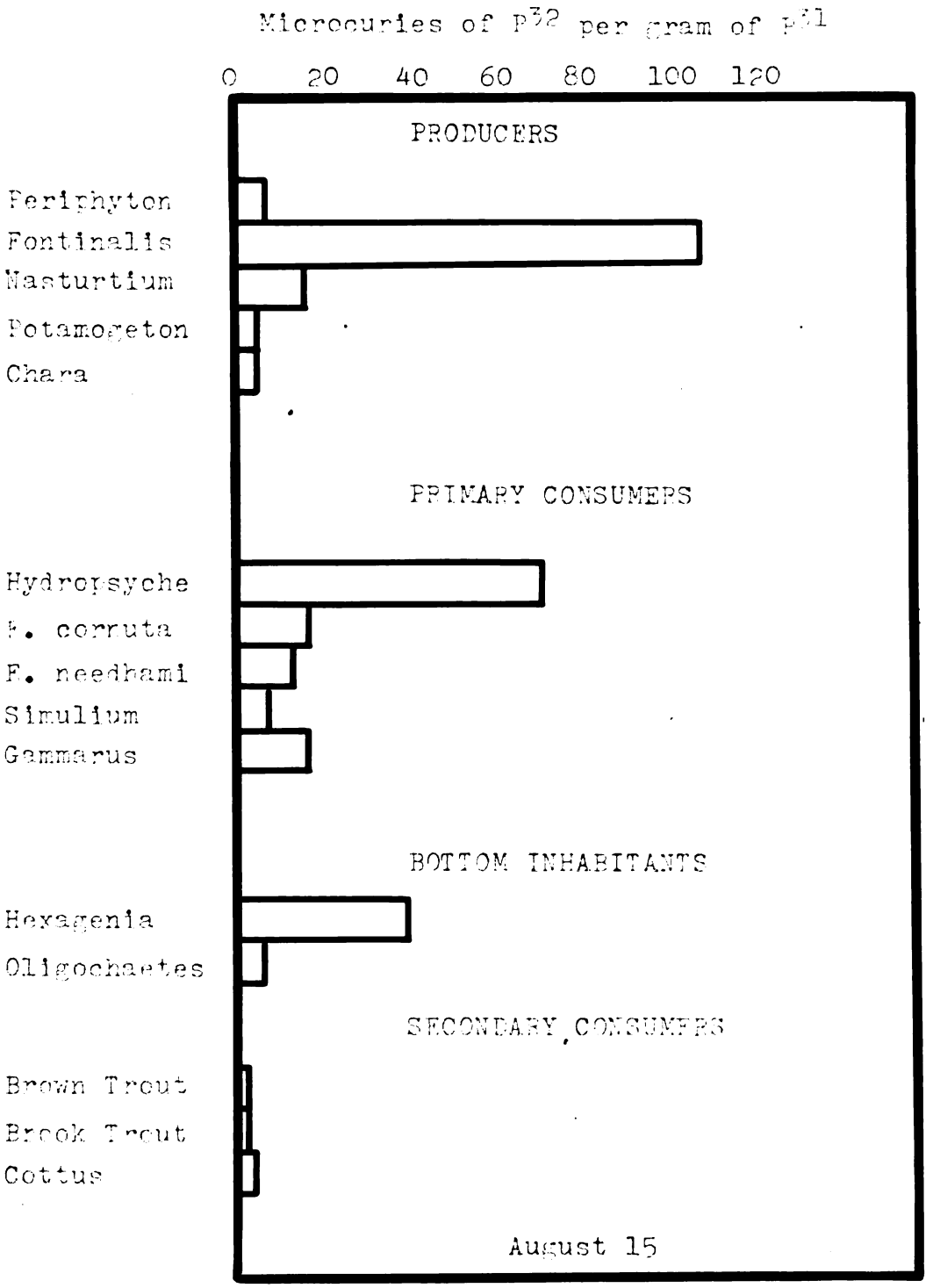

Microcuries of P32 per gram of P31
0 100 200 300 500 1000 1500 4000
PRODUCERS
Periphyton
Fontinalis
Nasturtium
Potamogeton
Chara
PRIMARY CONSUMERS
Hydropsyche
H. cornuta
H. reedhami
Simulium
Gammarus
BOTTOM INHABITANTS
Hexagenia
Oligochaetes
SECONDARY CONSUMERS
Brown Trout
Brook Trout
Cottus
The specific activity values for fish were less than 1.
July 5 through July 12

Figure 15. The specific activity of various organisms during the sixth week of the study. The isotope entered on 7-5-60 and the values are for 8-15-60.


Microcuries of P32 per gram of P31
0
20
40
60
80
100
120
PRODUCERS
Periphyton
Fontinalis
Nasturtium
Potamogeton
Chara
PRIMARY CONSUMERS
Hydropsyche
F. corruta
F. needhami
Simulium
Gammarus
BOTTOM INHABITANTS
Hexagenia
Oligochaetes
SECONDARY CONSUMERS
Brown Trout
Brook Trout
Cottus
August 15

study period. But 41 days later (Figure 35), the specific activities of the producers and primary consumers dropped from the original value by one third to one nine-hundredth of the specific activities of Figure 34. Thus, the specific activity curve changed with time, and the bottom dweller and secondary consumers received greater roles in the specific activity picture.

SUMMARY

On July 5, 1960, twenty-three millicuries of radioactive phosphorus (P^{32}) were added to the West Branch of the Sturgeon River, Cheboygan County, Michigan. The physical and biological pathways of the stream ecosystem were studied to determine the fate of the radiophosphorus.

The physical pathways include current and adsorption. The current is the principal source of radiophosphorus distribution. The water activity curves showed that the current carried the isotope to distances greater than 3,200 yards below the entry point. By comparing the upstream activity of Station 7 to that of the downstream Station 14, an 80% activity loss was recorded. It is believed that the activity removed from the current is held within the experimental area.

The physical phenomenon of adsorption accounts for the initial loss of activity from the current. The radiophosphorus can probably be adsorbed onto anything which it contacts. The adsorbed phosphorus was detected on moving particulate matter, biotic organisms and the stream bottom. Little of the adsorbed radiophosphorus appears in the current as the water activities obtained from the automatic sampling devices remained at a low level. The adsorbed radiophosphorus may be an incipient factor of biological accumulation.

The biological pathways of the stream include the organism's place in food chains and trophic levels, the habitat niche occupied by the organism, and the emergence of mature organisms.

The trophic level translocation is observed from the respective curves of producers, primary consumers and secondary consumers. For example, as the producer, periphyton, loses activity, the primary consumer, Hydropsyche, gradually increases in activity. The appearance of peaks in the activity curves of the secondary consumers, fish, occur when the activities of the producers and primary consumers are reaching low levels.

Variation in the trophic level activity shifts are due to the position of the organism in the food chain, the habitat niche occupied by the organism, and regeneration.

Gammarus has a variable position in the food chain. The scuds are voracious feeders, feeding on all kinds of plant and animal matter. Since they consumer food from two trophic levels, i.e., producers and consumers, the activity curves of Gammarus cannot concisely show trophic level activity shifts.

The habitat niche occupied by an organism also causes variation in the trophic level activity shifts. The oligochaetes inhabit the mud, silt and debris of the stream bottom. Because the materials of the stream bottom can themselves retain radiophosphorus, the activity curves of the oligochaetes are not exclusively a function of their position in the trophic level.

Water cress (Nasturtium) with its highly ramified root system is another case in which the activity curve is dependent upon the habitat niche. Water cress populations are

found primarily along the stream periphery and it is believed that there are definite physical factors which influence horizontal P^{32} distribution. The activities due to the habitat niche of Nasturtium, then, include two physical factors; activity of the mud, and horizontal variation, and the biological incorporation like other producers.

The final factors which cause variation in trophic level shifts are re-cycling and regeneration. Biological populations are able to participate in radiophosphorus accumulation by adsorption; simple ionic exchange, e.g., as the orthophosphates; and accumulation via energy transfer, e.g., as polyphosphates, sugar phosphates, etc. The adsorbed radiophosphorus is accumulated externally. This external radiophosphorus may be washed into the current: the current may also receive radiophosphorus due to the simple ionic exchange of the P^{32} orthophosphate with the surrounding P^{31} orthophosphate. This phenomenon is called regeneration.

The activity curves for the producers and the net spinning or filtering primary consumers, show the regenerated radiophosphorus of the stream. Generally, these curves show plateaus which are intermittently interrupted by slight or sometimes large peaks. It is believed that the intermittent peaks are largely due to the regenerated radiophosphorus. What brings about the abrupt release of phosphorus is unknown.

Re-cycled radiophosphorus is that which has been biologically incorporated in a trophic level, and is available for translocation due to the death of the organism or its ingestion

by another organism. The ingestion of the organism is the basic method of trophic level activity shifts. The death of the organism causes slight variations in the trophic shifts, and this radiophosphorus can be incorporated in the stream bottom or in omniverous organisms.

The final state in biological translocation occurs when insects emerge from the stream and the biologically incorporated P^{32} of these emerging insects is removed from the system. Some of their activity, however, may re-enter the system if the adult insects are consumed by secondary consumers. The adult insects may also die over the experimental area and thus liberate their activity to the system.

On July 7, 1960 an iron chelate, NaFeEDTA, was added to the West Branch of the Sturgeon River. The effect of chelation is not conclusive. There was an increase in iron, total phosphorus, and activity; however, in light of the natural fluctuations, the increases seem to be insignificant. Sampling stations closer to the chelate entry point may have shown a more marked chelation effect.

The specific activities, i.e., the ratio of P^{32} to P^{31}, were calculated from the activities and total phosphorus determinations. The specific activities gave the same general trophic level shift as the activity curves.

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APPENDIX I

Chart Number

Station	Water Depth in feet	Area in acres	Species	Grams per 100 yards	Percent Composition	Total mass in grams
3	10.4	.20	Chara	478	91	542 grams per 100 yards or .43 lbs. per acre
			Potamogeton	2	0.4	
			Nasturtium	1	0.1	
			Fontinalis	6	1.0	
			Hippurius	17	3.0	
			Elodea	12	2.0	
4	19.0	.14	Chara	36	14	72 grams per 100 yards or 0.06 lbs. per acre
			Potamogeton	76	50	
			Fontinalis	85	45	
5	24.4	.14	Chara	1	0.1	546 grams per 100 yards or 0.17 lbs. per acre
			Potamogeton	22	4	
			Elodea	40	5	
			Fontinalis	149	27	
			Filamentous algae	340	64	

APPENDIX I (Cont.)

Plant Biomass

Station	Water Plants in Cent	Area in acres	Species	Grams per 100 yards	Percent Composition	Total mass in grams
11	46.2	.20	Chara	48	21	222 grams per 100 yards or .09 lbs. per acre
			Potamogeton	12	5	
			Nasturtium	1	0.4	
			Elodea	5	0.2	
			Fontinalis	139	63	
			Filamentous algae	1	0.4	
13	27.7	.19	Chara	485	92	526 grams per 100 yards or 0.22 lbs. per acre
			Potamogeton	1	0.1	
			Fontinalis	21	4	
			Filamentous algae	12	2	
14	25.7	.24	Chara	153	25	629 grams per 100 yards or 0.55 lbs. per acre
			Potamogeton	4	0.7	
			Nasturtium	82	14	
			Elodea	100	16	
			Fontinalis	224	36	
			Filamentous algae	24	4	

APPENDIX II

The Invertebrate Biomass of the West Branch of the Sturgeon River for 1958, 1959 and 1960.

Year	Pounds of invertebrates per acre
1958 (Bryant, 1960)	53
1959 (Knight, 1961)	36.5
1960	66

APPENDIX III

Fish Biomass

Station	Species	Composition No. of fish per 100 yd. section	Total Weight in Grams	Pounds per acre
7	Trout		2,461	34
	Brook	25		
	Brown	16		
	Rainbow	1?		
	Muddlers	144	987	?
8	Trout		1,372	19
	Brown	91		
	Rainbow	52		
	Brook	20		
	Muddlers	319	819	9
12	Trout		3,952	41
	Brown	77		
	Rainbow	10		
	Brook	?		
	Muddlers	207	689	?
14	Trout		4,445	?
	Brown	148		
	Rainbow	28		
	Brook	15		
	Muddlers	114	841	7

Biomass of the West Branch of the Sturgeon River
for the Years 1958, 1959 and 1960

Year	Pounds of Trout/acre	Pounds of Muddlers/acre	Total Pounds of Fish/acre
1958 (Empire, Type C)	86	16.0	102.?
1959 (Empire, Type C)	20	176.5	196.
1960	55	6.5	61.5

APPENDIX IV

Activity of the Individual Periphyton Substrates in Figure 12 and the "In" Units of Stations 7 and 14.

Date	Station		Activity (Corrected Counts per Minute per Gram)
7-5-60	3		24,640
	3		43,825
	8		5,202
	8	"In" Units	10,473
	12		3,7[illegible]
	12		7,545
	14		[illegible],436
	14		17,697
7-[illegible]-60	3		790
	3		734
	8		714
	8	"Out" Units	[illegible]81
	12		810
	12		[illegible]99
	12		937
	12		905
7-[illegible]-60	3		18,53[illegible]
	3		10,[illegible]40
	8		2,363
	8	"In" Units	[illegible],[illegible]71
	12		[illegible],[illegible]30
	12		[illegible],[illegible]60
	14		4,371
	14		[illegible],728
7-11-[illegible]	[illegible]		7,455
	[illegible]		[illegible],[illegible]61
	3		[illegible],[illegible]68
	8	"In" Units	1,4[illegible]6
	12		[illegible]764
	12		758
	14		[illegible],[illegible]13
	14		[illegible],[illegible]37
7-[illegible]-[illegible]	[illegible]		[illegible]
	[illegible]		[illegible]
	[illegible]	"Out" Units	[illegible]
	[illegible]		[illegible]
	[illegible]		[illegible]
	[illegible]		[illegible]

Activity Data.

Date	Hour	Activity (Corrected Counts per Minute per Gram)
7-18-60	7	1,641
	8	1,154
	9	869
	10	744
	"In" Units	609
	12	554
	13	554
	14	1,459
	14	1,364
7-20-60	8	145
	9	1,504
	9	679
	10	459
	"Out" Units	295
	12	174
	12	0
	12	0
7-25-60	7	1,165
	8	0
	8	11
	9	0
	"In" Units	1,372
	10	0
	11	0
	11	0
8-1-60	7	1,[illegible]
	8	[illegible]
	8	[illegible]
	9	[illegible]
	"In" Units	[illegible]
	10	[illegible]
	11	[illegible]
	11	[illegible]
8-4-60	8	1,[illegible]
	9	[illegible]
	9	[illegible]
	10	[illegible]
	"Out" Units	0
	11	0
	12	0
	12	0
	12	[illegible]

APPENDIX IV (Cont'd.)

Date	Station	Activity (Corrected Counts per Minute per Gram)
8-8-60	1	0
	2	0
	4	0
	5	0
	6	0
	12	0
	13	0
	14	0
	14	0

"In" Delta

APPENDIX V

Activity of Nasturtium Corrected for Weight but not Corrected for Decay or Background

Date	Activity (Counts per Minute per Gram) Station			
	5	9	13	14
7-6-60	518	313	199	113
7-11-60	58	246	201	49
7-13-60	39	35	71	61
7-25-60	64	105	31	130
8-1-60	59	102	147	98
8-8-60	65	90	114	96
8-15-60	48	94	73	72
8-22-60	56	133	60	96
8-29-60	45	513		147
9-5-60	33	100	49	63

APPENDIX VI

Biota Phosphorus Analysis

Sample	Average Micrograms of Phosphorus Per Dry Weight Sample
Flora	
Periphyton	6.34
Fontinalis	0.72
Nasturtium	1.24
Potamogeton	4.09
Chara	3.54
Fauna	
Hydropsyche	3.35
Ephemerella cornuta	4.36
Ephemerella needhami	4.72
Simulium	3.54
Gammarus	7.49
Hexagenia	3.17
Oligochaetes	7.70
Brown Trout	7.71
Brook Trout	1.10
Cottus	0.74

APPENDIX VII

Phosphorus Content and Activity of Samples Used for Specific Activity Determination

Sample	Grams P^{31}	7-5 & 7-10 Activity us P^{32}	8-15 Activity us P^{32}
Fertilella	$.70 \times 10^{-6}$	2.38×10^{-4}	7.91×10^{-5}
Tetraedron	4.00×10^{-6}	1.38×10^{-4}	9.00×10^{-6}
Nasturtium	1.24×10^{-6}	2.05×10^{-4}	2.25×10^{-5}
Chara	8.54×10^{-6}	2.49×10^{-4}	2.11×10^{-5}
Fontinalis	6.04×10^{-6}	2.19×10^{-4}	4.50×10^{-5}
Hydrocharis	2.85×10^{-6}	4.70×10^{-4}	2.03×10^{-4}
F. communis	4.96×10^{-6}	6.10×10^{-3}	2.30×10^{-5}
F. needhami	4.72×10^{-6}	4.50×10^{-3}	6.75×10^{-5}
Simulium	8.54×10^{-6}	4.80×10^{-3}	6.75×10^{-5}
Gammarus	7.30×10^{-6}	1.59×10^{-4}	1.24×10^{-4}
Oligochaetes	3.70×10^{-6}	4.41×10^{-5}	1.24×10^{-5}
Potamogeton	8.17×10^{-6}	1.45×10^{-3}	1.40×10^{-4}
Cottus	2.74×10^{-3}	1.12×10^{-5}	2.66×10^{-3}
Brown Trout	3.74×10^{-3}	6.74×10^{-5}	5.99×10^{-3}
Brook Trout	1.70×10^{-2}	6.74×10^{-5}	5.99×10^{-3}