THE BACTERIAL TRANSLOCATION OF RADIOACTIVE PHOSPHORUS THROUGH A LOTIC ECOSYSTEM

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Michael E. Bender

1962

R. C. Ball
Institute of Water Research.

THE BACTERIAL TRANSLOCATION OF RADIOACTIVE PHOSPHORUS THROUGH A LOTIC ECOSYSTEM

By

MICHAEL E. BENDER

AN ABSTRACT

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

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1962

Approved _____

ABSTRACT

The recent availability of radioisotopes has enabled ecologists to follow nutrient cycles in many habitats with great precision. Radioactive phosphorus (P^{32}) has been widely employed in the study of the phosphorus cycle in lentic environments. The investigators of these lake systems have found that phytoplankton and bacteria are the primary fixers of phosphorus in the community.

In 1958, 1959, and 1960, inorganic P^{32} was applied to a stream system, the West Branch of the Sturgeon River. The transfer of P^{32} was followed throughout the biota of the stream and was found to follow the trophic levels rather closely.

In 1961, an experiment was designed to evaluate the importance of bacteria in the distribution of phosphorus within the stream system. E. coli O111 was selected as the carrier for the P^{32}, and experiments on its growth and uptake of P^{32} were performed. The organism was found to incorporate nearly 100% of the P^{32} available when the normal phosphorus concentration of the media was reduced to .3 mg/100 ml.

A bacterial culture containing 25 mc of P^{32} was added to the stream system on July 13, 1961. It was discovered that upon addition to the stream only a small proportion of the P^{32} was released into the soluble state; approximately 90% of the activity was held by the bacterial cells.

In the three previous years of the experiment, nearly all of the 23 mc of P^{32} added to the stream was fixed within the study area, but in this experiment only half of the activity added was retained within the area.

During the remainder of the study period, the various consumer organisms within the system showed lower activity densities than would have been expected if the bacteria remaining within the system distributed the P^{32} as was encountered in previous years.

An analysis of P^{32} uptake in various stream sections revealed heterogeneity of activity fixation. One section showed low uptake during all four years of the experiment. The most plausable hypothesis for this finding seems to be that lower populations of energy fixers are found in this section.

M. E. B.

THE BACTERIAL TRANSLOCATION OF RADIOACTIVE PHOSPHORUS THROUGH A LOTIC ECOSYSTEM

By

MICHAEL E. BENDER

A THESIS

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

MASTER OF SCIENCE

Department of Fisheries and Wildlife

1962

ACKNOWLEDGMENTS

During the preparation of this thesis, the author was aided and encouraged by many people. The author is particularly indebted to Dr. Robert C. Ball whose enthusiasm for the project made its completion possible, and to Dr. F. F. Hooper whose many ideas proved valuable throughout the study period. In the field, the author was assisted by Richard Bennett, Thomas Wojtalik, and fellow graduate student, James Bacon. For the assistance of these people, the author is deeply grateful. Dr. W. Carl Latta and his staff at the Pigeon River Trout Research Station also contributed a great deal to the project. The author also wishes to thank Dr. Philip J. Clark for his assistance with the statistical analyses. The author was assisted by his wife, Pat, throughout the study in typing, preparing of the figures, and proofreading.

The study was made possible through a grant held by Dr. R. C. Ball and Dr. F. F. Hooper from the Atomic Energy Commission. The author was supported by a Graduate Research Fellowship from the Institute for Fisheries Research of the Michigan Department of Conservation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

INTRODUCTION

Phosphorus and nitrogen have long been recognized as the major limiting elements in many habitats. The nitrogen cycle has been called a near perfect cycle since the element is circulated through and returns to various trophic levels by fairly definite routes. The phosphorus cycle, however, is not a perfect one because the means of returning phosphorus to the cycle may be inadequate to compensate for the loss (Odum, 1959). At the present time through the efforts of Hutchinson and Bowen (1950), Rigler (1956), Hayes, et.al. (1952) and others who have used P^{32} to study the cycle in lakes, a fairly definite pattern of phosphorus exchange has been established. Stream systems, however, have not been investigated as well.

In 1954, Alexander and Grzenda studied the effects of phosphorus fertilization on Hoffman Lake and its effluent stream, the West Branch of the Sturgeon River. These authors found an increase in periphyton growth downstream from any detectable phosphorus increase in the water. They attributed these results to minute increases in phosphorus concentrations below the sensitivity of normal chemical procedures.

In order to follow the pathways of phosphorus transfer within the stream system, P^{32} was employed as a tracer in four separate experiments during 1958, 1959, 1960, and 1961.

The trophic levels sampled during the study were the

consumers, various species of aquatic insects, other invertebrates and fish, and the primary producers, the periphyton.

The 1958 experiment was conducted by Borgeson, Clifford, and Bryant. These authors found that upon addition of the isotope, the periphyton populations showed immediate uptake of P^{32} and rapidly lost this activity which was then biologically incorporated into other organisms. The organisms which showed initially high activity levels were the plant feeding forms. Predators and omnivores had the next highest activity and the scavengers the least. Brown trout, Salmo trutta, and muddlers, Cottus cognatus, showed similar activity levels.

In 1959, Knight followed the transfer of P^{32} through the system and found much the same general pattern of phosphorus uptake. He noted, however, that much of the actitity during the initial isotope passage was in the form of particulate matter. He then postulated that either diatoms and/or bacteria were responsible for the initial fixation of phosphorus. Using activity-free periphyton substrates cultured outside of the experimental area he was able to detect, by placing these substrates in the stream immediately after the isotope passage, a recyling of activity within an hour after the isotope passed.

In the 1960 experiment, Zettelmaier found much the same uptake patterns throughout the stream biota.

Ball and Hooper (1962) have reviewed the results of the above authors and have calculated uptake values for the

several stream sections.

In the three previous years, the uptake pattern of P^{32} has been shown to follow the various trophic levels rather closely. The organisms responsible for the immediate removal of inorganic P^{32} are unknown. The results of P^{32} studies on lakes have shown that phytoplankton and bacteria are responsible for the initial removal. Knight (1961) has hypothesized that bacteria may be the organisms responsible for the initial removal in the West Branch of the Sturgeon River.

In the 1961 experiment, the P^{32} was incorporated into bacterial cells and then added to the stream system. The bacterium, Escherichia coli, was selected as a carrier organism because the absence of coliform organisms from the stream system would permit its separation from the normal stream flora. Before addition of the bacteria to the stream, the organisms' growth and uptake of P^{32} were followed in laboratory experiments. With the trophic relationships in the stream already well established, it was believed that initiating the phosphorus cycle in the particulate form would define the relationships and importance of bacteria in the distribution of phosphorus in a stream ecosystem.

Description of the Study Area

The West Branch of the Sturgeon River originates at Hoffman Lake, a 128 acre hard-water lake located in Charlevoix County, Michigan. From its origin, the river flows in a northeasterly direction for 14 miles before entering the Sturgeon River near Wolverine, Michigan.

As the West Branch of the Sturgeon River continues its northeasterly flow through Cheboygan, Otsego, and Charlevoix Counties, the vegetation encountered along its course consists chiefly of birch, aspen, cedar, tamarack, and balsam fir. Cedar, tamarack, aspen, alder, and ninebark are located along the margin of the river.

The West Branch of the Sturgeon River is a cold-water stream with the temperature of the water varying between 52^0 F. and 58^0 F. during the summer months (Clifford, 1959). The cool temperature of the water can be attributed to the numerous springs and tributaries which enter the stream and the shade produced by overhanging cover.

The study area of the river, located in (T.33 N, R. 3 W), consisted of a section approximately 5,280 yards in length with the stream bottom varying from sand and gravel to silt and detritus. The general conditions of the stream within this area are as follows.

The stream flow through the study area has a mean of 43.75 cubic feet per second (Knight, 1961). During the summer, rains rarely affected the stable water level or

brought turbidity to the normally clear water. The total phosphorus concentration in the stream water of the study area is approximately 6 parts per billion. A high dissolved oxygen content exists in the stream due to churning and mixing of the water with air. The aquatic life supported by the stream is rich in abundance and variety.

Among the main vegetational growths found in the stream were beds of Chara sp.; water moss, Fontinalis antipyretica; and water cress, Nasturtium officinale. Vegetation was fairly abundant for the first 550 yards of the stream, scarce for the next 480 yards, and again abundant plant beds for the remainder of the study area.

The West Branch of the Sturgeon River was inhabited by the following fishes: brown trout, Salmo trutta; rainbow trout, Salmo gairdnerii; brook trout, Salvelinus fontinalis; eastern slimy sculpin, Cottus cognatus; and northern mottled sculpin, Cottus bairdii.

Aquatic insects present in the stream were those species associated with swift currents and cold and clean waters. The orders found in the stream were Odonata, Ephemeroptera, Plecoptera, Trichoptera, Diptera, Megaloptera, Coleoptera, and Hemiptera.

Sampling Stations

In a stream system such as the West Branch, a variety of habitats exist. In former years, 16 stations were established which included such factors as shade, stream flow, bottom type, and vegetation composition. Of these established sampling points, 6 were used as stations in the 1961 experiment and were numbered 3, 5, 8, 12, 14, and 16 (Figure 1).

Collections of aquatic plants, periphyton, and aquatic invertebrates were made at stations 3, 8, 12, and 14; fish and lampreys were collected only at stations 8 and 12. The following is a description of the permanent collecting stations and also of those stations used to collect water samples.

Station 3 This site was located 300 yards below the addition point. The immediate area is well shaded, but above the station, the stream is fairly open to sunlight. Vegetation in the area consists of *Chara sp.*; sparse water cress, *Nasturtium officinale*; *Potomogeton pectinatus*; and some *Ranunculus sp.* The water has an average depth of 12.8 inches (Zettelmaier, 1962). The stream flow at this point was 38.73 cfs.

Station 5 This station was used only for the collection of water samples. It is located 550 yards from the addition point. The majority of the area between 3 and 5 is heavily shaded. The flow at this point was 38.73 cfs.

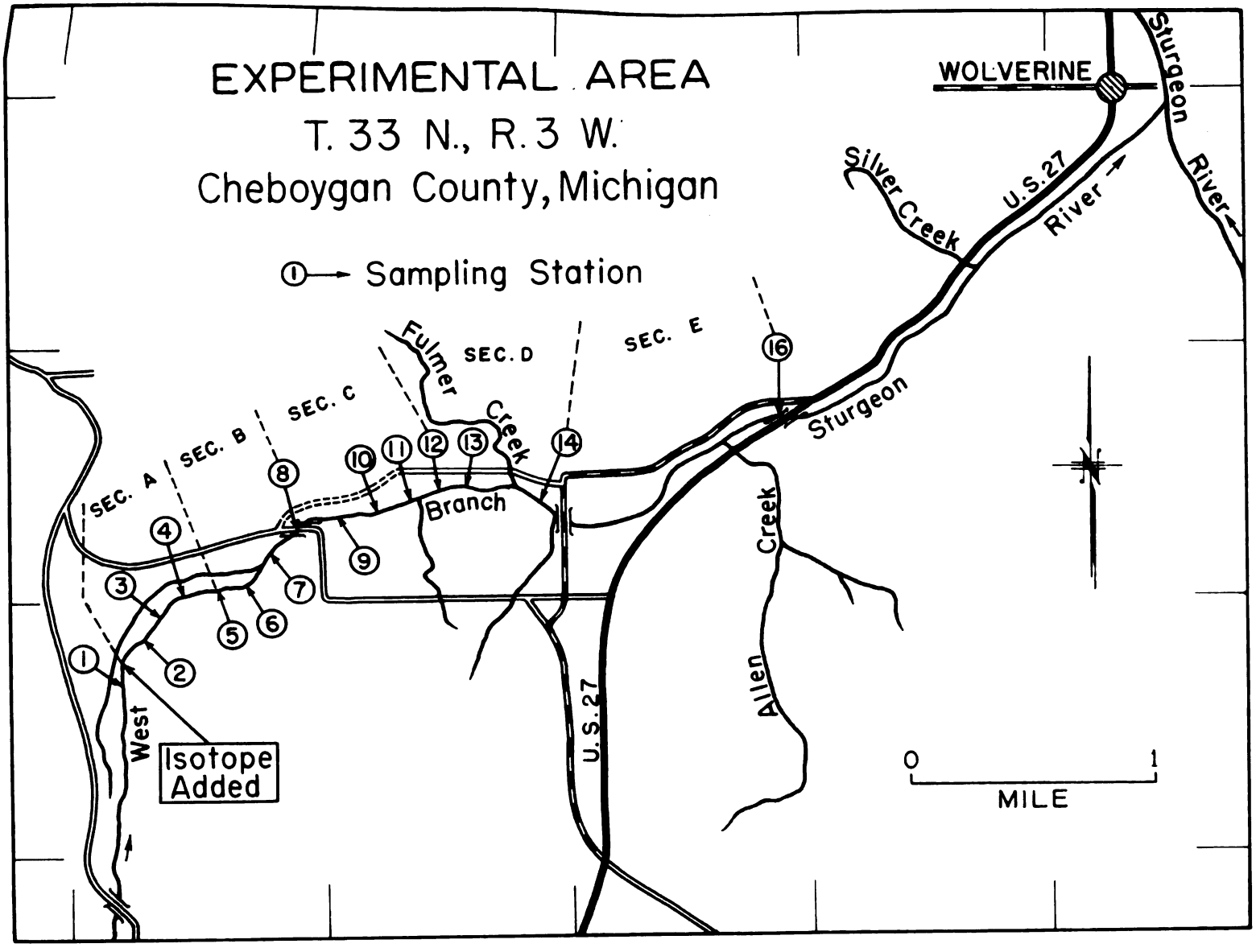
Station 8 This site was located in an open portion of the stream 1,030 yards below the addition point. The vegetation in this segment consists mainly of Chara sp.; water moss, Fontinalis antipyretica; Potomogeton pectinatus; and Ranunculus sp. The mean water depth at this site was 17.2 inches (Zettelmaier, ibid.). The stream flow was 43.48 cfs.

Station 12 This station was located in an open section of the stream 2,580 yards below the isotope addition point. The vegetation consisted mainly of Chara sp.; Ranunculus sp.; Potomogeton pectinatus; and water moss, Fontinalis antipyretica. The mean water depth was 13.3 inches (Zettelmaier, ibid.). The flow at this site was 47.53 cfs.

Station 14 This station was located in a fairly open section of the stream 3,280 yards below the point of isotope entry. Vegetation consisted of Chara sp.; Potomogeton pectinatus; Ranunculus sp.; water moss, Fontinalis antipyretica; and water cress, Nasturtium officinale. The mean depth in this area was 12.2 inches (Zettelmaier, ibid.). The stream flow was 49.72 cfs.

Station 16 The partially shaded site chosen for this station was located 5,280 yards below the addition point and was used only for the collection of water samples. The water flow at this site was 52.5 cfs.

Figure I. Map of the West Branch of the Sturgeon River area, showing 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

METHODS AND PROCEDURES

General Methods

The general methods of following the phosphorus cycle through the stream system are as follows:

1. A study area approximately 5,280 yards in length was established on the West Branch of the Sturgeon River. Taking into consideration various ecological factors, sixteen sampling stations were established in the study area.
2. A fluorescein dye marker, to alert the sampling crews, was added to the stream each year preceding addition of the isotope, and in 1961, preceding all bacteria additions.
3. The isotope was added to the stream as inorganic P^{32} in the first three years of the experiment. Each year approximately 23mc. were added to the stream at a constant rate over a period of 30 minutes.
4. Six sampling stations (3, 5, 8, 12, 14, and 16) were used to follow the initial isotope passage over a period of 1 to 2 hours.
5. Four of the sampling stations (3, 8, 12, and 14) were used to sample the activity of the stream biota throughout the remainder of the study period.

The Isotope

The selection of an isotope for the study of an aquatic environment is dependent upon the element which is to be studied and upon the physical characteristics of the isotope

which may make it acceptable as a study tool. The radioisotope of phosphorus (P^{32}) has been used in various investigations of the phosphorus cycle in lakes and on the West Branch of the Sturgeon River.

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

The procedures used to incorporate the isotope into the bacterial cells will be described fully in a later section.

The method by which the isotope was added to the stream has been described by Borgeson (1959), Clifford (1959), and Knight (1961).

Measurement of Activity

Activity was measured with a Nuclear Measurements Corporation windowless,gas-flow,proportional counter, model PCC-10A; coupled to a decade scaler, model DS-1A; and a single unit counter and scaler, model number PC-3A.

In order to represent activity values obtained in a meaningful fashion, the following correction factors have been given by Robeck, et.al. (1954).

Self Adsorption:

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

Back Scatter:

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

Decay Factor:

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

Background:

Background radiation is caused by natural cosmic radiation, and/or radioactive substances in or near the counter. The determination of background was carried out daily by operating the counters for 30 minutes with muffled planchets. The background observed on that day was then subtracted from the readings made that day.

Volume Factor:

Due to the differences in sample size of the various materials collected, all counts were corrected to counts per gram or to counts per milliliter.

During this study period, counts were corrected as indicated below:

corrected value = (cpm - Bg) x (Vf) x (Df)

where:

Bg = background

Vf = volume

Df = decay

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

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

1 microcurie (uc) = 3.7×10^{4} dps

= 2.22×10^{6} dpm

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

If results are desired in terms of microcuries, then

microcuries = (cpm - Bg) x (Vf) x (Df) x (1.001×10^{-6})

with a counter efficiency of 45%

The Bacterial Carrier

Escherichia coli was selected as the carrier organism for the radioactive phosphorus in the 1961 experiment on the West Branch of the Sturgeon River. E. coli belongs to the coliform group of bacteria which are restricted in habitat to the intestinal tract of man and other animals and can be distinguished from the normal stream flora which is lacking in coliform organisms.

The organism can be characterized by the properties found in the Appendix I.

Many strains of the bacterium have been separated on the basis of biochemical differences and phage specificity. Strain B was originally selected as the carrier, but due to difficulty resulting from low pH of the unbuffered medium or possible mutant phage, strain 0111 was substituted later in the experiment.

Labaw, et.al. (1950) stated that 2.72% of the dry weight of an 8 hour culture of E. coli consisted of phosphorus. The partitioning of phosphorus within the organism was divided as follows: 66% in ribose compounds, 19% desoxyribose nucleic acid, 12% phospholipids, and 3% unknown. Their experiments on uptake of P^{32} by E. coli closely paralleled the findings of this investigation. The authors found that bound phosphorus was not released into the medium as cell death proceeded. Uptake of phosphorus corresponded to an increase in optical density and to cell multiplication as it did in this study.

The usual growth medium for coliform organisms contains beef extract and other phosphorus carrying substances. In order to eliminate the normal phosphorus content of the media, the growth medium given in Appendix II was utilized.

Test For Presence of Members of Coliform Group by Membrane Filter Technique

The filter technique for determination of coliforms is considered as a standard test for coliform enumeration in the 11th edition of Standard Methods 1960. The method consists of filtration of a water sample through a membrane which retains the bacteria, transfer of the membrane to suitable culture media, and enumeration of the type colonies after incubation.

In this study the sample was obtained by placing the sample bottle completely below the surface of the water with the neck pointed in the direction of the current. Samples

were filtered through an appropriate membrane (eg. HA Millipore with a pore diameter of .45 μ) and the filter funnel rinsed three times with 20 to 30 ml. of buffered water solution. Membranes were removed and placed on a surface of an agar plate.

The cultures were then incubated 20 ± 2 hours at 35°C. in an inverted position in an incubator with 100% humidity.

The only deviation in the methods outlined above from those listed in Standard Methods was the use of agar surface instead of an adsorbent pad upon which the membranes were placed.

The colonies developing on eosine methylene blue agar may be described as (1) typical- nucleated, with or without metallic sheen; (2) atypical- opaque, unnucleated mucoid after 24 hours incubation, pink; (3) negative, all others.

Preliminary Bacterial Studies on the West Branch of the Sturgeon

Before utilization of the coliform organism, E. coli, as a carrier of radioactive phosphorus in the stream system, the following points were investigated.

Normal Bacterial Flora

The normal bacterial flora of the stream was analysed to detect the presence of normal coliform organisms.

On June 27,1961, a 500 ml. water sample was collected at station 8. The sample was diluted and aliquots of it filtered and plated on E.M.B. agar. The stream water seemed

fairly high in normal flora (1.27×10^3) organisms per ml., but no coliforms were found.

Samples for normal flora were taken at stations 8, 12, 14, and 16 on July 1 and July 10; no coliforms were found.

It seems safe to believe that coliforms are not normally present in the stream system, therefore, any detected in the system originated from experimental addition.

Rate of Coliform Movement in the Stream System

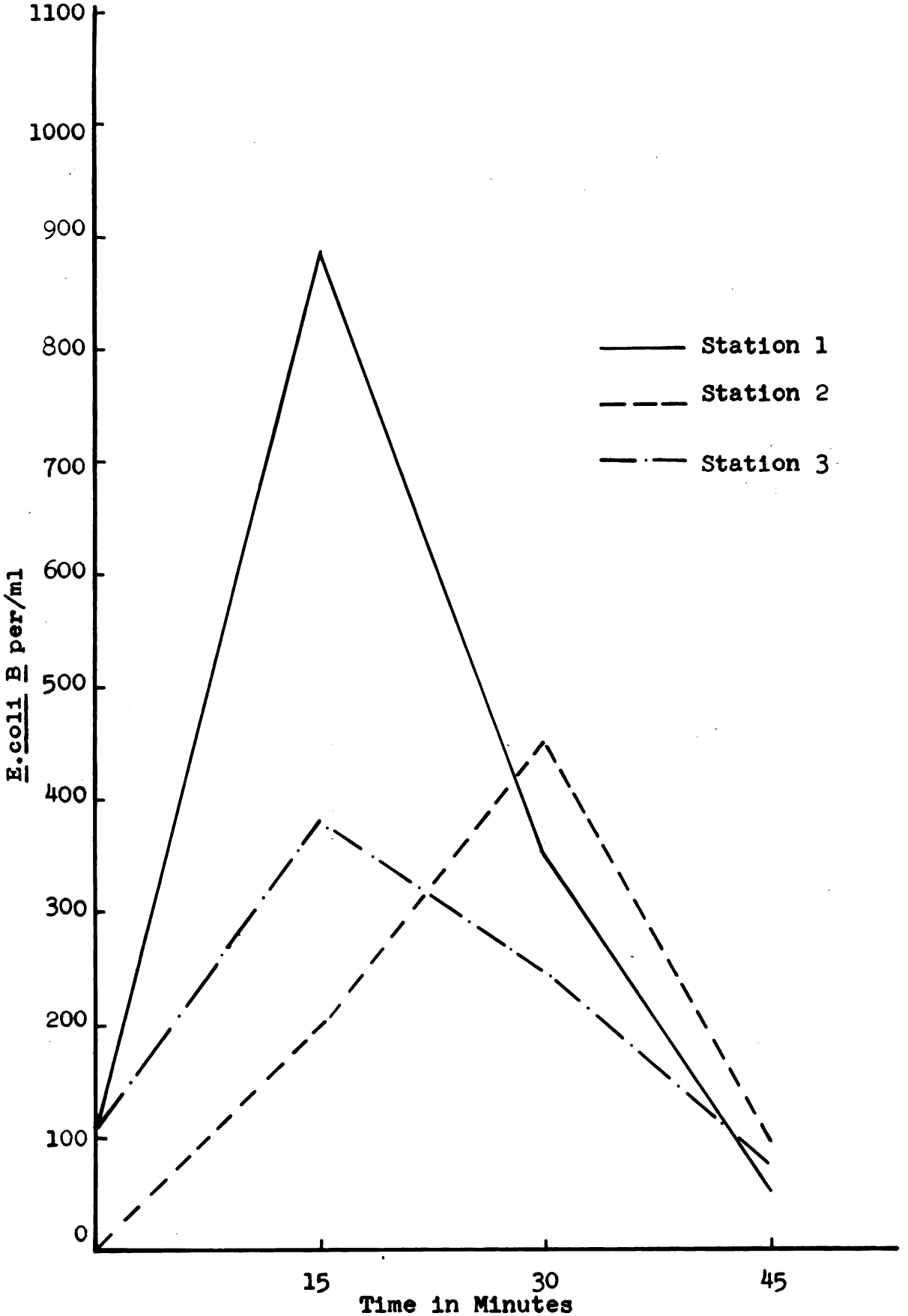
In order to have an idea of sampling intervals needed to detect the movement of radioactive E. coli, the following experiments were conducted.

Three temporary sampling stations were located at 200, 400, and 500 yards below permanent station 14. On June 30, July 1, and July 10, E. coli B was added to the West Branch of the Sturgeon River below station 14. The cultures of bacteria were diluted in 55 gallons of stream water and added to the stream at a constant rate over a period of 30 minutes. Before addition of the bacteria, a fluorescein dye marker was released and samples were taken at 15 minute intervals after passage of the dye.

The first trial showed inconclusive results due to sampling errors or faulty addition methods.

The results of the second and third trials on rate of movement of bacteria showed that sampling intervals of 15 min. could detect movement with accuracy. Figure 2 shows the passage of E. coli past the sampling stations during the

Figure II. Flow of E. coli B through the stream system.


1100
1000
900
800
700
600
500
400
300
200
100
0
E.coli B per/ml
Station 1
Station 2
Station 3
15
30
45
Time in Minutes

third trial.

The total number of bacteria added to the stream on the third trial was 1.3×10^{10}; the results show that a number of this magnitude could easily be detected downstream.

Growth of E. coli and Uptake of P^{32}

E. coli is a facultative aerobe but will attain a higher growth peak and optical density with adequate aeration. The organism can survive between 15^{0}C. to 48^{0}C., indicating wide temperature tolerance, with optimum growth conditions from 35^{0}C to 37^{0}C. E. coli O111 was found to reach the peak of its growth curve between 12-15 hours at 35^{0}C.

During the final uptake experiment with E. coli B, an initial uptake of 85% of the P^{32} occurred within four hours. The culture continued at this uptake level until 12 hours of growth had elapsed; upon filtration of this culture, all of the activity was found in the filtrate. These results could be attributed to several factors: the media being used contained no buffer and the pH of the culture at this time was 4.8, thus lysis of the cells due to low pH is a possibility. E. coli organisms are, however, active fermentors of lactose and can usually withstand fairly acid conditions. Another possibility exists, that of a lysogenic bacteria phage (i.e. a stage in which virulent phage coexists with resistant bacteria) which by spontaneous mutation could have caused near complete lysis of the culture. The real nature of the difficulty could not be determined with the

available data, therefore a phage resistant strain of E. coli O111 was substituted for strain B for the remainder of the experiment.

Using E. coli O111, analytical studies of its growth and uptake of P^{32} were conducted. The object of these studies was to determine how the greatest percent uptake of P^{32} could be achieved and maintained.

Krumholz and Foster (1957) review some factors which have been observed to have an effect upon the uptake of radioisotopes; one of these factors is the concentration of the material in the environment. Boroughs, et.al. (1957) have shown by using the algae Nitzchia sp., that as the concentration of zinc in the environment decreased there was a greater percent of uptake by the cells.

In preliminary studies, E. coli B showed P^{32} uptakes of 74 and 84 percent when 6 milligrams of P^{31} per 100 ml. of media were used as a growth supplement. It was believed that by decreasing the normal phosphorus to a level just sufficient for good growth that a maximum uptake of P^{32} could be obtained.

The growth curve for a bacterial population consists of three areas defined as follows:

(1) The lag phase is a period when only a slight increase in the bacterial population is noted. The length of this phase is directly related to the effective inoculum.

(2) The exponential growth phase is the phase during which the cells are replicating at a constant and geometric rate.

The rate of bacterial growth in this phase is determined by limiting factors. These may be intrinsic in the physiological potentiality of the cell, or they may be extrinsic in that they may be environmental factors, such as concentration of an essential element.

(3) The plateau phase is the phase in which cells are dying and multiplying at equal rates. The decreasing rate of cell division can be accounted for in many ways with the factors differing under different environmental conditions. One of the most important factors is the accumulation of end products of metabolism such as alcohols and organic acids from carbohydrates, to toxic concentrations.

Under constant cultural conditions, one factor in the environment, such as concentration of a nutrient substance, can be varied so that its growth determining factors can be evaluated. Thus, when an element is limiting in the medium, a slower rate of growth might be expected; also the plateau in the growth curve should be reached before the normally produced toxic products have accumulated (Burrows,1959).

The relative amount of phosphorus used by a bacterial culture can therefore be determined by a slower growth rate and by leveling of the normal growth curve at an earlier time than would occur in unlimited phosphorus media.

Four cultures of E. coli 0111 were inoculated, and all conditions held constant except for varying phosphorus concentrations. At one hour intervals, samples were removed from each culture and optical densities were determined.

At the plateau in each growth curve, a sample of the culture was analysed for phosphorus utilization by calculating the ratio of total phosphorus present to organic phosphorus. Figure 3 shows the growth curves of the cultures in varying concentrations of phosphorus.

The cultures containing the following concentration of phosphorus showed corresponding percent uptakes of total phosphorus present.

.3 mg	of phosphorus per/100 ml	97.6%
1.5 mg	of phosphorus per/100 ml	43.4%
3.0 mg	of phosphorus per/100 ml	53.1%
6.0 mg	of phosphorus per/100 ml	7.0%

The results of the above experiment show a high per cent utilization of phosphorus when .3 mg of phosphorus per 100 ml of media were used as a growth supplement.

A trial uptake experiment was run in which .3 mg of phosphorus per 100 ml were used as a growth supplement to .1 of a millicurie of P^{32}. The optical density of the culture was brought to 40 Klett units with an inoculum culture. The culture was incubated at 35^{0}C. and aeriated with a magnetic stirring bar. Within two hours, the bacteria had accomplished a 99.9% incorportation of P^{32}. Figure 4 shows uptake percentage plotted along with Klett units to indicate increasing growth.

On July 12,1961, 11.1 millicuries of P^{32} were added to each of four 1,000 ml flasks containing 500 ml of broth. Each flask was inoculated with enough E. coli O111 to bring

Figure III. Phosphorus utilization by *E. coli* O111.

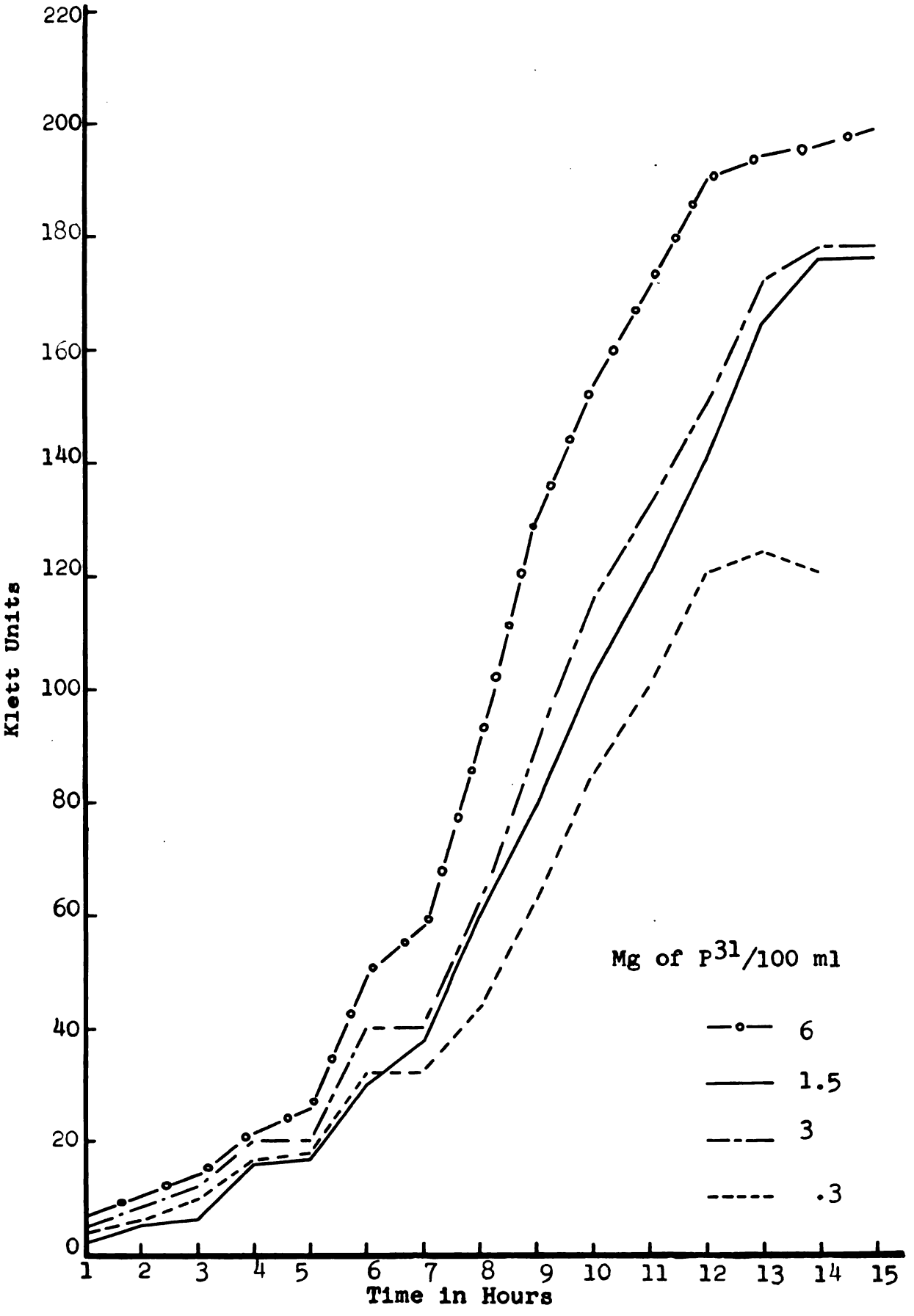
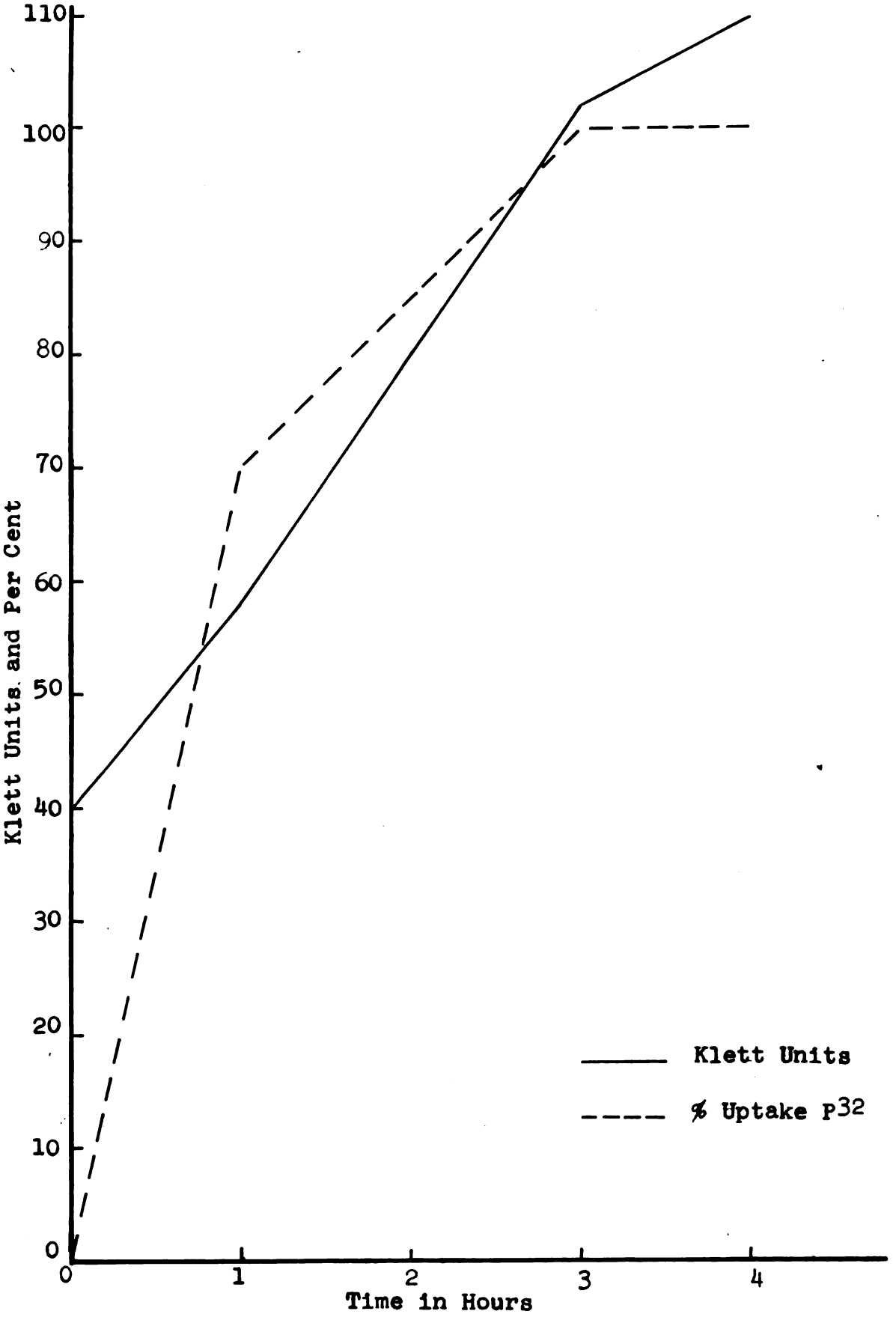

220
200
180
160
140
120
100
80
60
40
20
0
Klett Units
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Time in Hours
Mg of P31/100 ml
6
1.5
3
.3

Figure IV. Uptake of P^{32} by *E. coli* O111.


110
100
90
80
70
60
50
40
30
20
10
0
Klett Units and Per Cent
0
1
2
3
4
Time in Hours
Klett Units
% Uptake P32

the optical density to 20 Klett units*. Carrier phosphorus was added to bring the total phosphorus concentration to .3 mg per 100 ml. The cultures were incubated at 35°C and aeriated with magnetic stirring bars. Samples were removed every hour, checked for percent uptake, and a portion of the sample was used to give growth curve information. Figure 5 shows the percent uptake by the culture of E. coli 0111, and Figure 6 shows the growth curve of the organism.

Fifty ml.-aliquots of the cultures of bacteria were filtered through a 10-gram covering of Celite and a Millipore membrane. The Celite (SiO_2) and membrane were then removed and placed in an ice bath to slow bacterial metabolism. The procedure was repeated until 1,020 ml of the culture had been filtered. Final assay of this material showed that 25.0 millicuries were present in bacterial cells. These cells were then stored in the ice bath until addition to the stream system.

Bacterial Methods

Field

Stations 3, 5, 8, 12, and 14 were designated as collecting sites for bacterial samples. The samples were collected in 1,000-ml polyethylene bottles. After a sample was collected, it was placed in a burlap bag suspended in the stream to prevent warming and hence subsequent multiplication of the bacteria. One sample was collected about

* The formula relating Klett units to opical density is as follows: $\frac{1000 \times D}{2} = R$ where: D is density R is Klett reading

Figure V. Final uptake of P^{32} by E. coli O111.

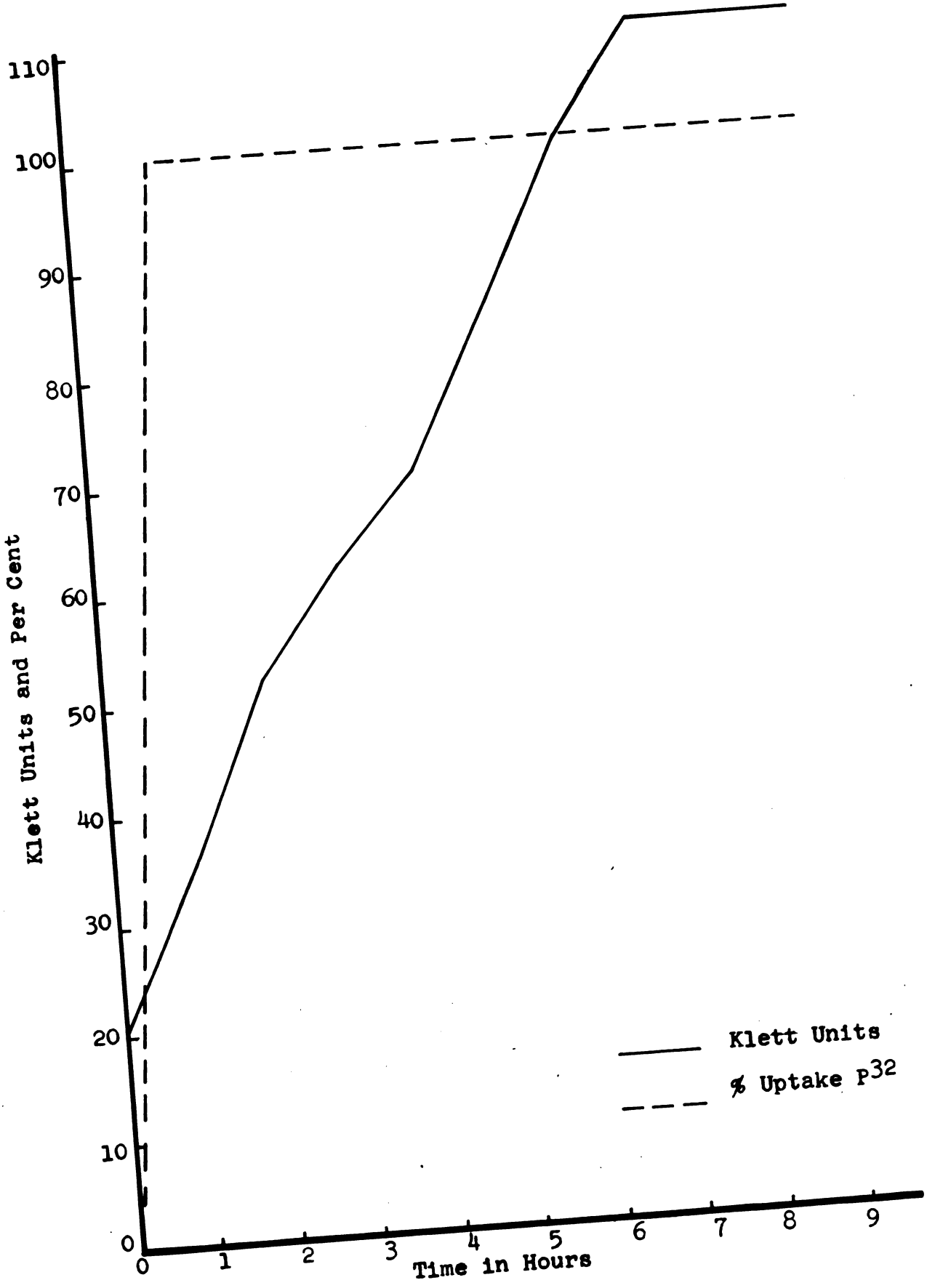
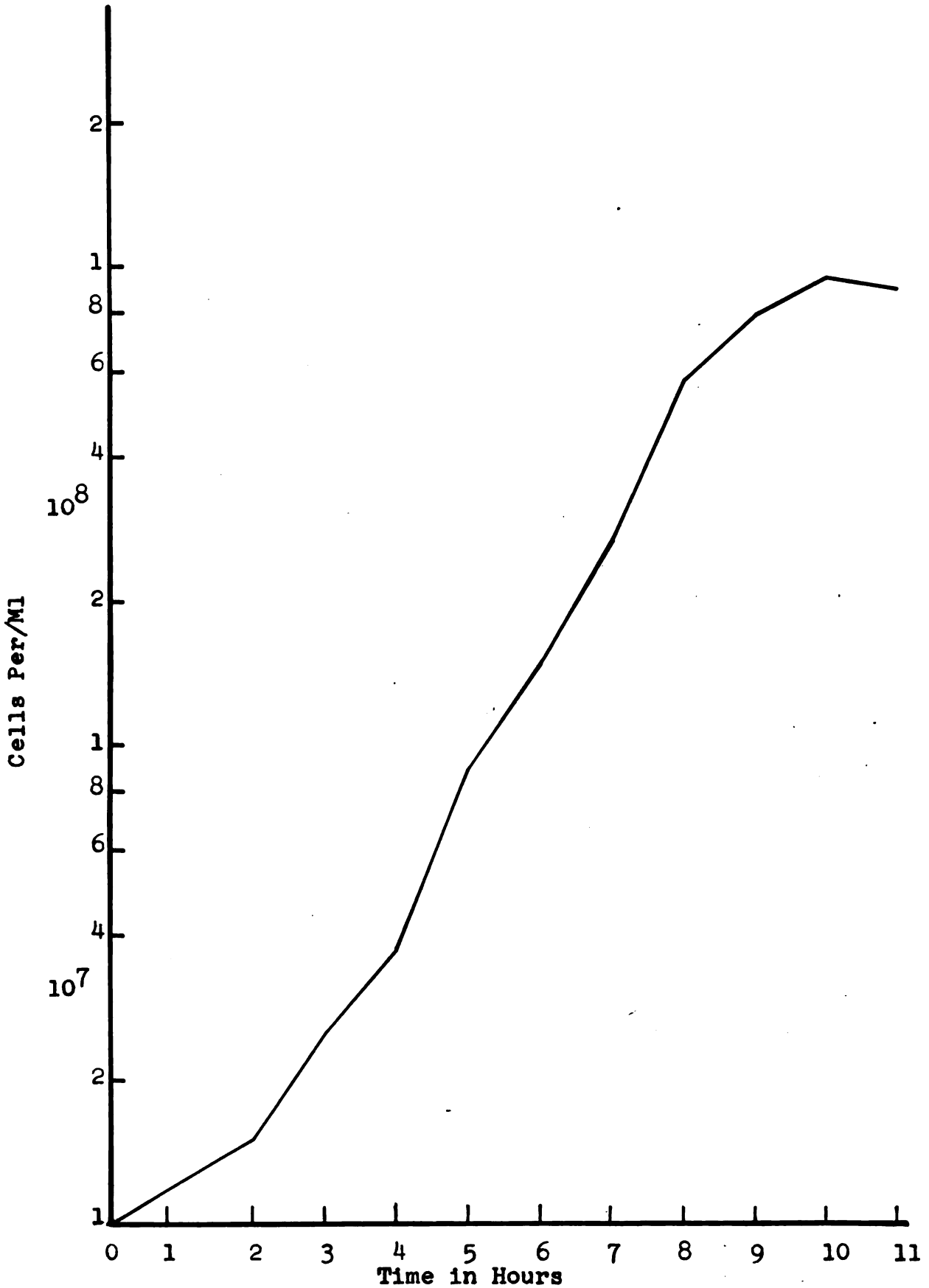

110
100
90
80
70
60
50
40
30
20
10
0
Klett Units and Per Cent
0
1
2
3
4
5
6
7
8
9
Time in Hours
Klett Units
% Uptake P32

Figure VI. Growth of E. coli O111 in the P^{32} medium.


Cells Per/Ml
2
1
8
6
4
10^8
2
1
8
6
4
10^7
2
1
0 1 2 3 4 5 6 7 8 9 10 11
Time in Hours

15 - 20 minutes prior to the anticipated arrival of the dye; and then six samples were collected, beginning 10 minutes after arrival of the dye, at 10 minute intervals.

Laboratory

The samples were brought to the laboratory and stored on ice until processing was begun.

Analysis to determine the number of E. coli organisms per/ml was carried out first because undue holding of the samples might have resulted in erratic counts. Dilutions of the samples, expressed in terms of 1 ml of stream water, were carried out as follows:

(1) Each liter bottle was rotated at least ten times to insure complete suspension of the material.

(2) Two ml of the 1 liter sample were removed with a sterile 2 ml pipette and added to 20 ml of distilled water in order to yield a 10^{-1} dilution.

(3) One ml of 1 liter sample was removed and added to 100 ml of distilled water to give a 10^{-2} dilution.

(4) One ml of the 10^{-2} dilution was removed and added to 10 ml of distilled water to yield a 10^{-3} dilution.

Beginning with station number 14, dilutions of the samples for each sampling period were filtered through Millipore membranes. Filtration of the samples taken prior to the addition of the isotope were carried out before any other samples were filtered in order to determine if any coliforms were present. The filtrations of all other samples were begun with the highest dilution of that sample

(i.e. 10^{-3}), and filtration progressed to the lowest (10^{-1}) dilution. The filters were rinsed with 10 ml of distilled water after each filtration was completed. Between each sample filtration, the filter apparatus was rinsed with 100 ml of distilled water. Filter membranes were removed with forceps and placed in prelabeled petri dishes of E.M.B. agar. The petri dishes were incubated at 35^{0}C in an inverted position and were removed for counting at 20 and 40 hours.

Following the processing of the water samples for determination of bacterial numbers, samples to determine activity levels were processed.

Beginning with station number 14, a 200-ml aliquot of each sample taken at that station was filtered and washed with 100 ml of distilled water. The filter membranes were removed and glued to planchets which were placed in a drying oven at 60^{0}C. The filtrates were placed in prelabeled beakers and processed as water samples.

Filters and flasks were washed with .01 N HCl and then with distilled H_2O before the next sample was processed. After completing the processing of this group of samples, an identical procedure was followed using 100 ml of .01 N HCl acid as a wash in place of the water wash.

The following flow sheet shows the procedure in sequence.

1000 Ml SAMPLE

200 ml	200 ml	3 ml
1. Filtered	1. Filtered	1. Serial dilutions made up to 10^{-3}
2. Rinsed with 100 ml HOH	2. Rinsed with 100 ml of .01 N HCl	2. Filtered
Filter:	Filter:	3. Filters removed and transfered to E.M.B. agar for bacterial enumeration
(a) Removed	(a) Removed	
(b) Glued to a planchet	(b) Glued to a planchet	4. Incubated at 37^{0}C for 20 and 40 hours
(c) Dried at 60^{0}C.	(c) Dried at 60^{0}C.	
(d) Counted:	(d) Counted:	
counts equal total solids	counts equal incorporated activity	
Filtrate:	Filtrate:	
(a) Boiled	(a) Boiled	
(b) Acidified	(b) Acidified	
(c) Transfered to a planchet	(c) Transfered to a planchet	
(d) Counted:	(d) Counted:	
counts equal water soluble	counts, in excess of water soluble, equal acid soluble or adsorbed activity	

Water Methods

Field

Water collection sites were designated at stations 3, 5, 8, 12, 14, and 16. Samples were collected in 140 ml polyethylene bottles. Sampling was begun at the initial sighting of the dye, and samples were collected at 5 minute intervals for a period of 70 minutes and at 10 minute intervals at stations 14 and 16 for a period of 140 minutes.

Laboratory

The water samples were prepared for activity determinations as outlined by Robeck, et. al. (ibid.) with the exception of modification of volumes due to differences in sizes.

Each sample bottle (140 ml) was acidified by an addition of three milliliters of concentrated nitric acid. The sample was mixed, and a 50 ml aliquot was removed for evaporation. The 50 ml aliquot was placed in a beaker and and evaporated until the contents had been reduced to a volume which could be placed in a planchet. The beaker was then rinsed with 2 N nitric acid, and the washings were then added to the planchet. The material in the planchet was evaporated to dryness and placed in a muffle furnace at 600^{0}C until red hot. The planchet was then placed in an aluminium tray and transfered to the counting laboratory where the activity was determined.

Fish and Lampreys

Field Methods

Fish and lampreys were collected at two week intervals at stations 8 and 12 for radiological analysis. Brown trout, brook trout, rainbow trout, muddlers, and lamprey were collected at station 8. The above species were collected at station 12, except for the brook trout which only rarely occurs at station 12.

All species were collected by electro-fishing with a 230 volt, direct current generator as the power source. The generator was placed in a small boat which had a 10 foot copper strip on the bottom to serve as the negative pole. The boat was pulled behind the shocking crew in which each of two men carried small poles with a copper sheet wrapped at one end to serve as positive poles.

Lampreys were collected from Chara or silt beds, and muddlers were collected from under logs and along rocks.

In order to obtain a representative sample of each species, at least four individuals of a species were collected.

Laboratory Methods

Usually, the fish and lampreys from each station were placed in plastic bags and frozen until the following morning when processing was begun. On a few occasions, however, the samples were processed on the same day in which they were collected.

All fish were measured and weighed before processing. Individuals weighing 30 or more grams were homogenized with

an equal volume of distilled water. If an individual weighed less than 30 grams, twice its weight in water was usually added. All samples were homogenized in a Waring blender for 30 seconds or until complete homogenization had been completed.

A sample of the homogenate was then removed with a pipetting device, placed on a preweighed planchet, weighed, and covered with concentrated nitric acid. After the sample was completely digested, the planchet was placed in a muffle furnace at 600^{0}C and removed for counting when it became red hot.

Samples of muddlers and lampreys usually consisted of 3 or 4 individuals.

Fish Biomass

Estimations of the trout and muddler populations of the West Branch of the Sturgeon River were conducted at stations 3, 8, 12, and 14.

A segment of stream 100 yards in length was selected at each station for estimation of the trout population and 50 feet in length for the muddler population. The area was enclosed on the upstream end by a chicken wire fence, and the shocking was conducted in an upstream direction.

The muddler population was estimated before the trout shocking was begun. Muddlers were captured in the section, placed in M.S. 222, measured, clipped on their left pectoral fin, and then weighed as a group. The fish, after undergoing the above procedures, were placed in fresh

water. After they had revived, they were redistributed in the 50 foot section. A 15 - 20 minute waiting period was allowed to elapse, and the procedure was carried out again until enough returns were obtained to yield a fair estimate.

All species and size groups of trout in a 100 yard section were captured; lengths and weights were recorded; and the caudal fins were clipped. The trout were then redistributed in the section, and 20 - 30 minutes were allowed to elapse in order to facilitate redistribution. The section was then reshocked.

Addition of the Isotope

On July 13, 1961, the bacteria and Celite filtering powder were transported to the stream in an ice chest. The mixture was added to a 55 gallon barrel and the barrel was then filled with stream water. A fluoescein dye marker was released 10 minutes before the addition of the isotope was begun. The bacteria were then siphoned into the stream at a constant rate for a period of 33 minutes.

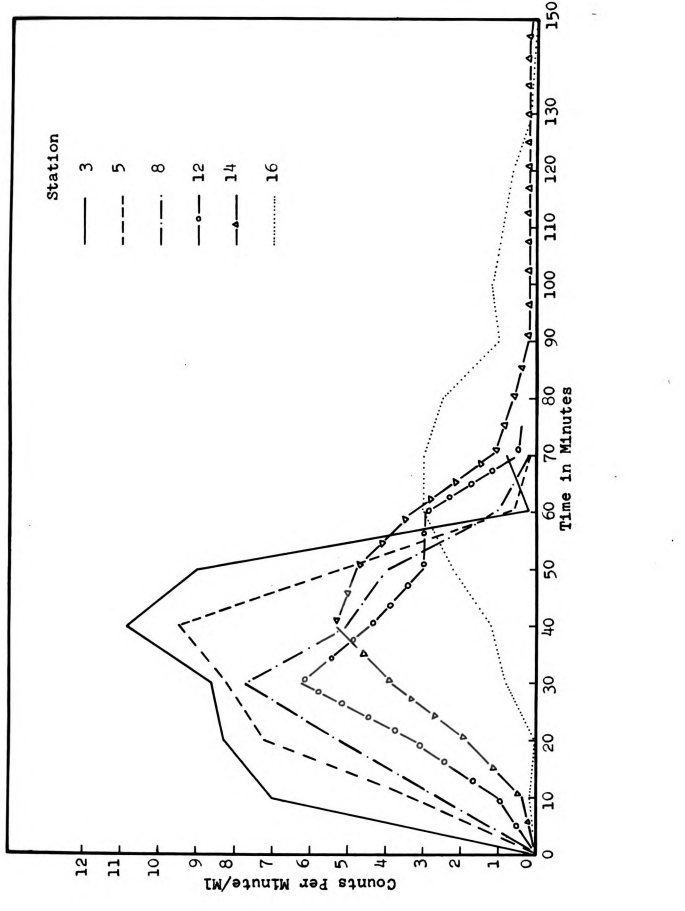
RESULTS

When radioactive phosphorus is released into an aquatic environment, it rapidly disappears from the inorganic form. In lakes, aquatic bacteria and phytoplankton seem to be the organisms responsible for its removal. Bacteria have shown turnover times of 5 minutes in their relationship to radioactive phosphorus in the water (Hayes and Phillips, 1958), hence the removal of phosphorus by bacteria is extremely rapid. Reid (1961) states that if bacteria receive a large share of the available inorganic phosphorus, they may seriously limit the production of the animal mass in the community. The extent to which bacteria are utilized by consumer organisms in the food web is not known.

Earlier experiments on the phosphorus cycle in the West Branch of the Sturgeon River have indicated rapid incorporation of phosphorus into particulate matter (Knight, 1961). The cycle of phosphorus in the stream has been followed extensively through the biota in each of 3 separate experiments, and food chain relationships have been well established (Ball and Hooper, 1962). It was the object of this investigation to add the isotope in the form of bacterial cells and to follow the phosphorus cycle beginning at a different trophic level. If a similar phosphorus cycle was observed, the bacteria might be considered the phosphorus fixers in the stream system.

The activity flow curves are shown in Figure 7.

Figure VII. Total water activity at collecting stations during passage of the isotope. Time zero is arrival at station of water mass carrying P^{32}.


Station
3
5
8
12
14
16
Counts Per Minute/Ml
Time in Minutes
0
1
2
3
4
5
6
7
8
9
10
11
12
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150

These curves were computed from the data obtained by filtration of 200 ml of stream water and addition of activity of the filters and filtrates. The values plotted for station 16 were obtained from the 50-ml water samples.

The general shape of the curves at stations 3, 5, 8, and 12 compare to the curves found in other years (Knight, ibid., Zettelmaier, ibid.). The curves at stations 14 and 16, however, are drawn out much longer than was heretofore encountered. This condition is due to the small amount of uptake at the upper stations with a progressive lenthening of the spike* due to mixing and dilution.

The time at which the peak was reached varied between 30 and 40 minutes from arrival of the water mass bearing P^{32} at stations 3 through 14, but was not reached until 60 minutes at station 16. Graphic representation of the peak values is shown in Figure 8.

Bacteria as Phosphorus Carriers

In order to determine if all the activity observed in the solid state was incorporated into the bacterial cells, an acid wash of 100 ml of .01 N HCl was applied to the filter membranes. After this treatment, the filter should show lower activity values than a comparable sample without the acid treatment if the activity was adsorbed to the surface of the cell. Figure 9 shows the activity values of acid and water washed membranes.

---

* The term spike is used to designate the P^{32} added to the stream water.

Figure VIII. Peak activity levels reached at the collecting stations during isotope flow.

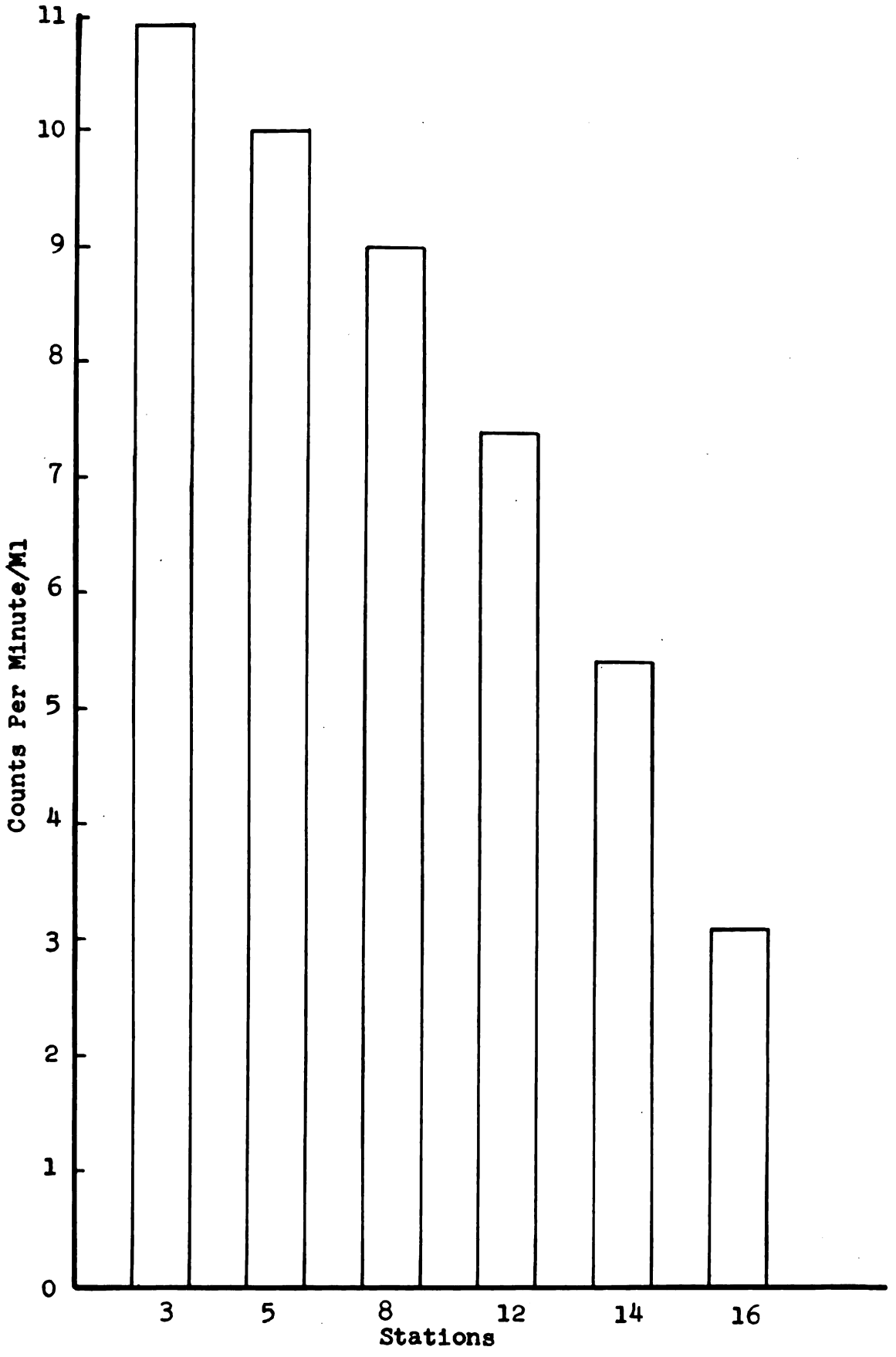
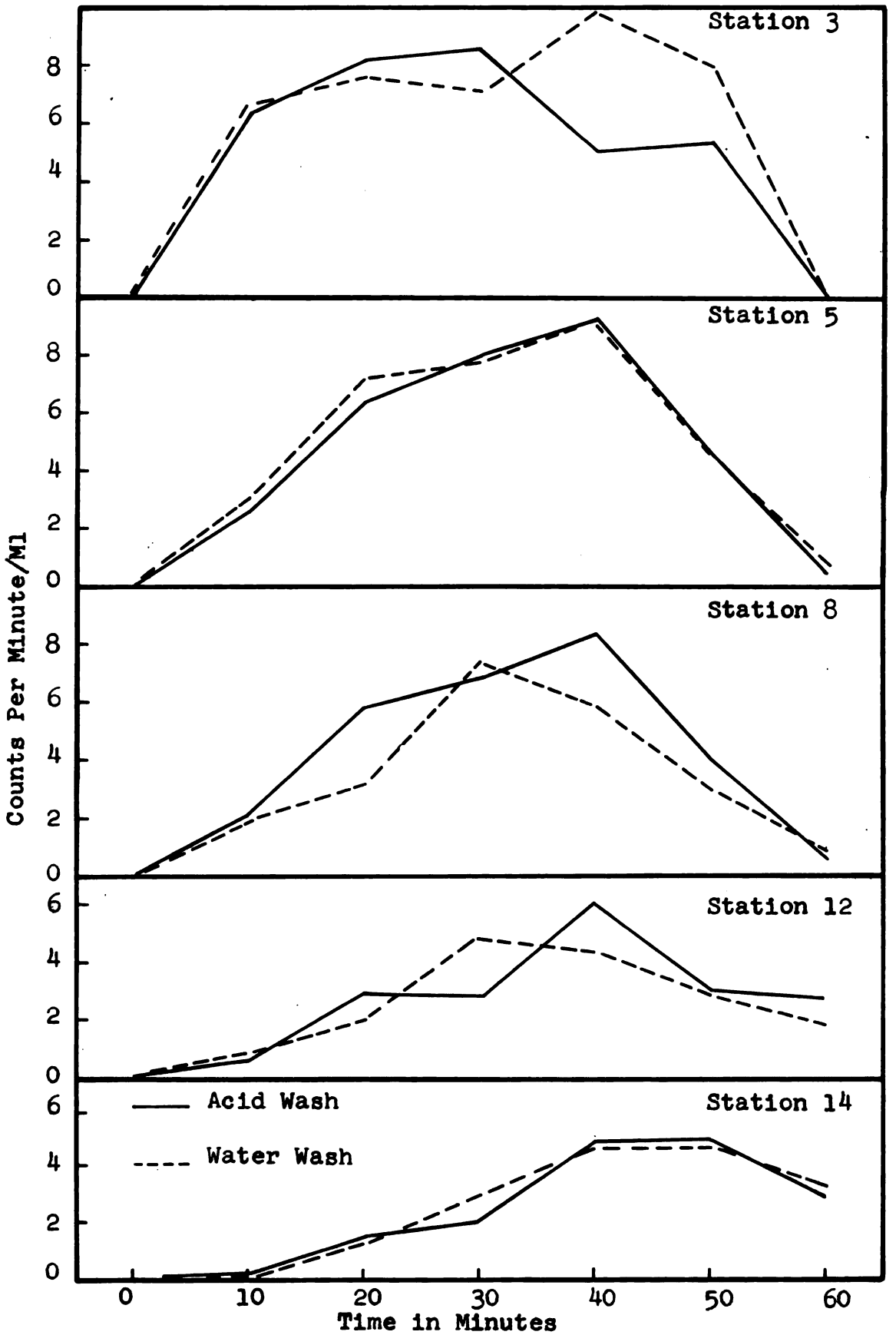

11
10
9
8
7
6
5
4
3
2
1
0
Counts Per Minute/Ml
3
5
8
12
14
16
Stations

Figure IX. Activity of acid and water wash filter membranes at the collecting sites.


Station 3
Station 5
Station 8
Station 12
Station 14
Acid Wash
Water Wash
Counts Per Minute/Ml
Time in Minutes
0
10
20
30
40
50
60
0
2
4
6
8

The small and inconsistant variations between the activity levels for the two types of rinse indicate that the activity was retained within the cells and not adsorbed to the cell surface. The variations were probably due to differences in the aliquots taken from the main sample bottle.

The activity levels for the filtrates of these samples, Figure 10, correspond in magnitude to the values obtained from the filters.

Mean Activity Levels for E. coli Cells

The cell count in the final culture of E. coli which was added to the stream was 9×10^8 per ml ; 1,020 ml of this culture were filtered and the total activity calculated to be 25 millicuries. Assuming each cell in the culture had incorporated an equal amount of P^{32}, a cell should contain .0278 counts.

Figure 11 shows the cell count obtained at each station plotted with corresponding activity values. Utilizing the points for which a cell count was recorded and the corresponding activity values for that time period, the mean activity for each cell was calculated to be .0218 cpm with confidence limits at the 95% level of $.0148 < u < .0288$ cpm (shown in Table 1). These data indicate that each cell had incorporated an equal amount of P^{32}.

The occurrence of activity values, in Figure 11, with no detectable cell count is thought to be due to the greater sensitivity of the radioactive counting procedure.

Figure X. Activity of the acid and water wash filtrates at the various collecting sites.

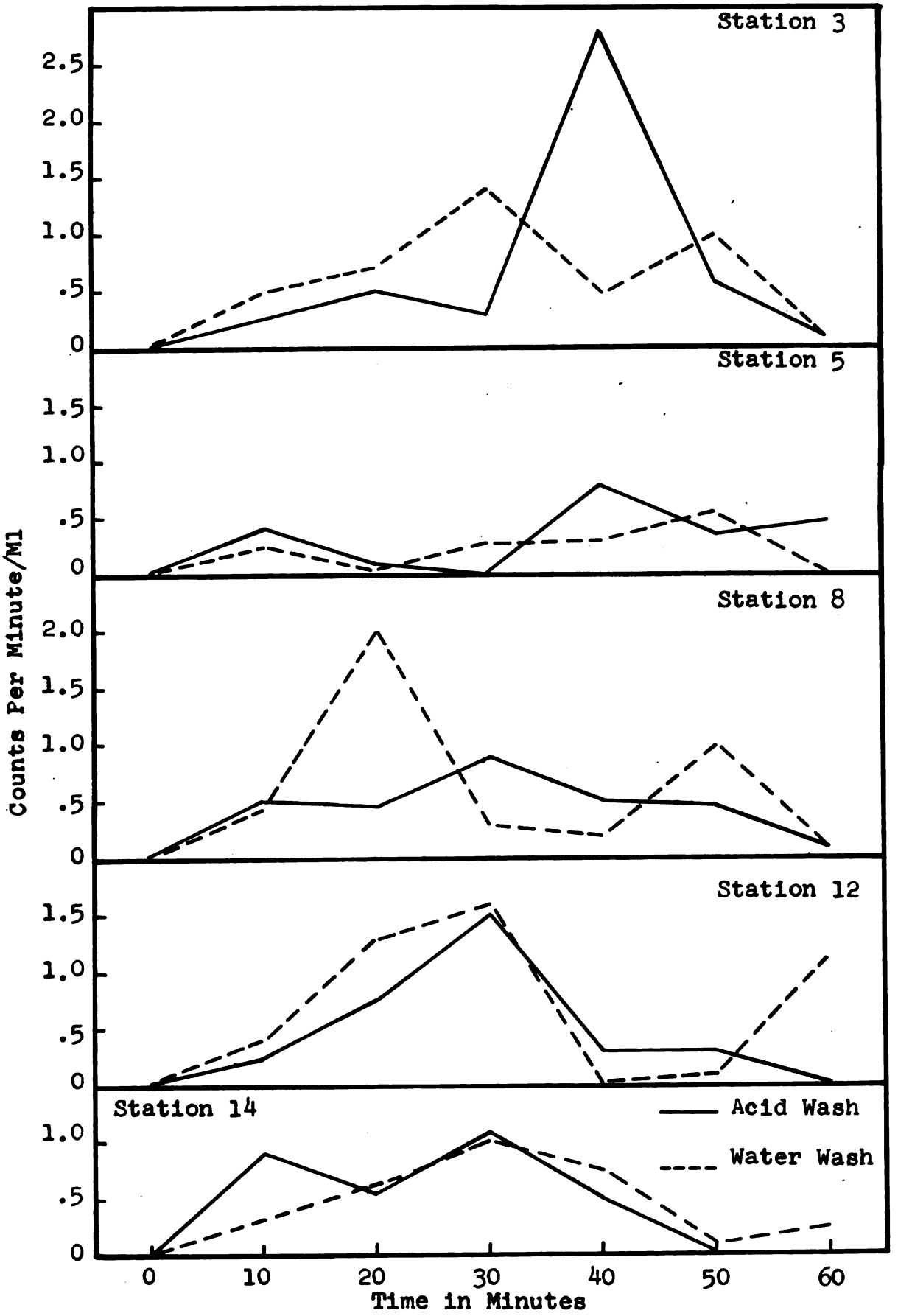

Station 3
Station 5
Station 8
Station 12
Station 14
Acid Wash
Water Wash
Counts Per Minute/Ml
Time in Minutes
0
10
20
30
40
50
60
2.5
2.0
1.5
1.0
.5
0

Table 1

Mean Cell Counts

Time	Station	Counts	Per Cell	Cells
10	3	6.40	.0304	210
20	3	8.10	.0337	240
30	3	8.50	.0146	580
40	3	5.10	.0182	280
50	3	5.50	0	0
60	3	.02	0	0
10	5	2.50	.0104	240
20	5	6.40	.0200	320
30	5	8.00	.0235	340
40	5	9.30	.0232	400
50	5	4.60	.0270	170
60	5	.40	0	0
10	8	2.30	.0135	170
20	8	5.80	.0145	140
30	8	6.80	.0106	640
40	8	8.50	.0121	700
50	8	4.10	.0410	100
60	8	.60	0	0
10	12	.60	.0085	70
20	12	2.90	.0111	260
30	12	2.70	.0128	210
40	12	5.90	.0256	230
50	12	3.00	.0115	260
60	12	2.70	.0900	30
10	14	.20	0	0
20	14	1.50	.0150	100
30	14	2.10	.0150	140
40	14	4.80	.0184	260
50	14	4.90	.0233	210
60	14	2.90	0	0

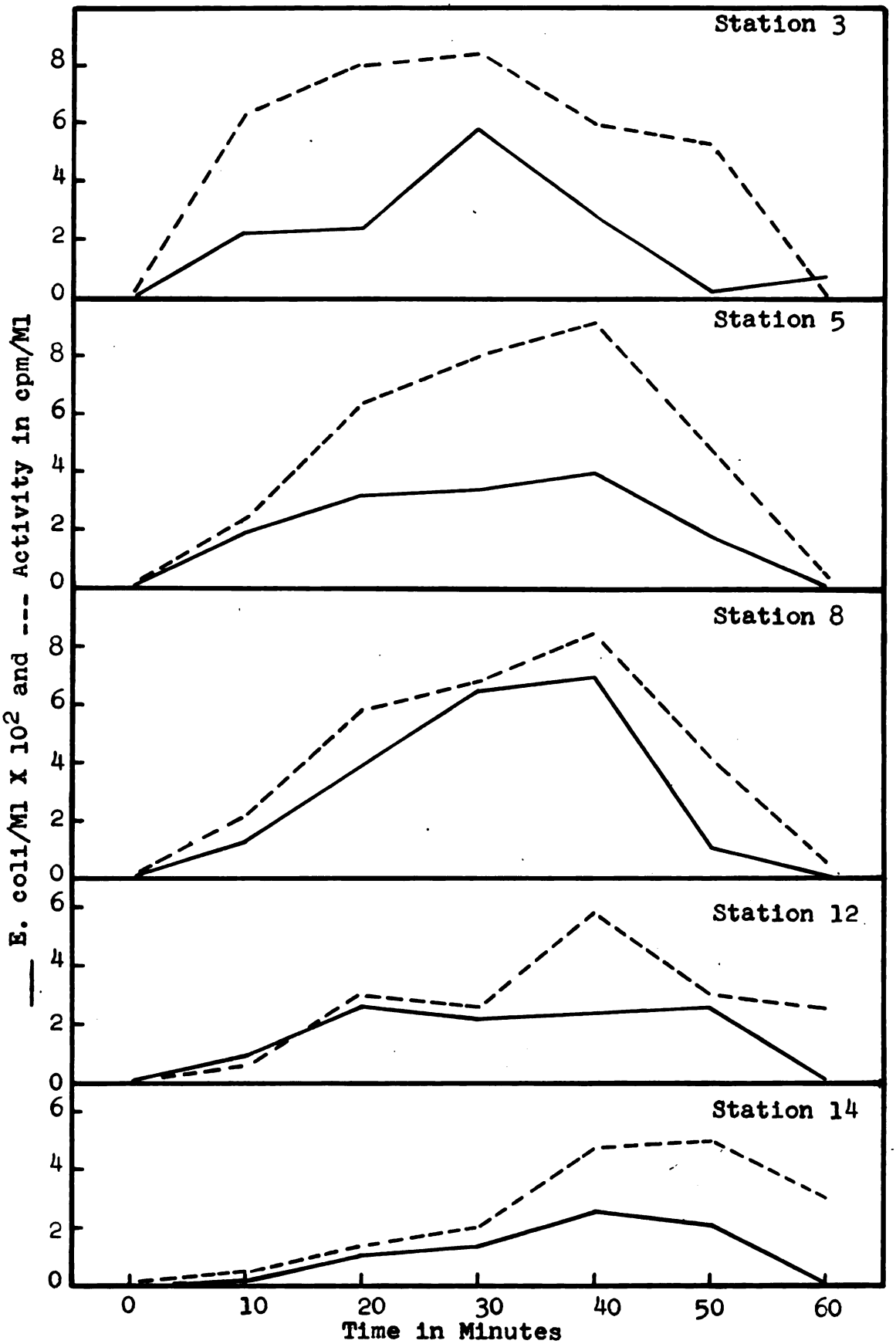
$S^2 = .00027853$

$S = .016689$

$\bar{X} = .0218$

Confidence Limits = $.0148 < u < .0288$

Figure XI. Activity of the filter membranes and number of *E. coli* O111 organisms per milliliter at the collecting sites.


Station 3
Station 5
Station 8
Station 12
Station 14
0
2
4
6
8
0
10
20
30
40
50
60
Time in Minutes
— E. coli/Ml X 10² and --- Activity in cpm/Ml

Effect of the Stream Environment on the Bacterial Cells

In order to detect the effect of the stream environment upon the retention of activity by the cells, an analysis of variance was computed on the data. If the cells as they passed through the stream system lost appreciable activity by transfer of phosphorus ^{32} with the normal phosphorus present, the analysis should detect heterogeneity in cell activity between the stations. The results of this analysis, Table 2, showed at the 95% level that there was no significant difference in activity per cell between the different stations. These data indicate that there was no appreciable loss of P^{32} from the bacterial cells after the initial loss upon addition to the stream.

Hayes and Phillips (ibid.) and others have shown that upon addition of P^{32} to a lake the bacteria equilibrate with the inorganic state in 5 to 20 minutes. In this experiment, the filtrable activity passing each station, Figure 12, remained constant except for one period when a near complete loss of filtrable activity occurred. Immediately after this loss, the level of filtrable activity returned to the concentration at which it was present before the loss. These data indicate a constant exchange level between the bacteria and the water, but it is not possible to say that the filtrable activity was due to inorganic P^{32}. Whittaker (1961) states that loss from algae back to the water may be in the form of some organic compound.

Table 2

Variation in Mean Cell Count Between Stations

Station	Time 20	30	40		R^2
3	337	146	182	665 R_1	442,225
5	200	235	232	667 R_2	444,889
8	145	106	121	372 R_3	138,384
12	111	128	256	495 R_4	245,025
14	150	150	184	484 R_5	234,256
Total	943 T_2	765 T_3	975 T_4	2,683 T	
T^2	889,249	585,225	950,625		

$$S\ S\ \text{rows} = \frac{\epsilon R^2}{3} - \frac{T^2}{15}$$

$$S\ S\ \text{col.} = \frac{\epsilon T^2}{5} - \frac{T^2}{15}$$

$$S\ S\ \text{total} = \epsilon X^2 - \frac{T^2}{15}$$

$$S\ S\ \text{error} = S\ S\ \text{total} - S\ S\ \text{rows} - S\ S\ \text{col.}$$

$$S^2\ \text{rows} = \frac{S\ S\ \text{rows}}{4}$$

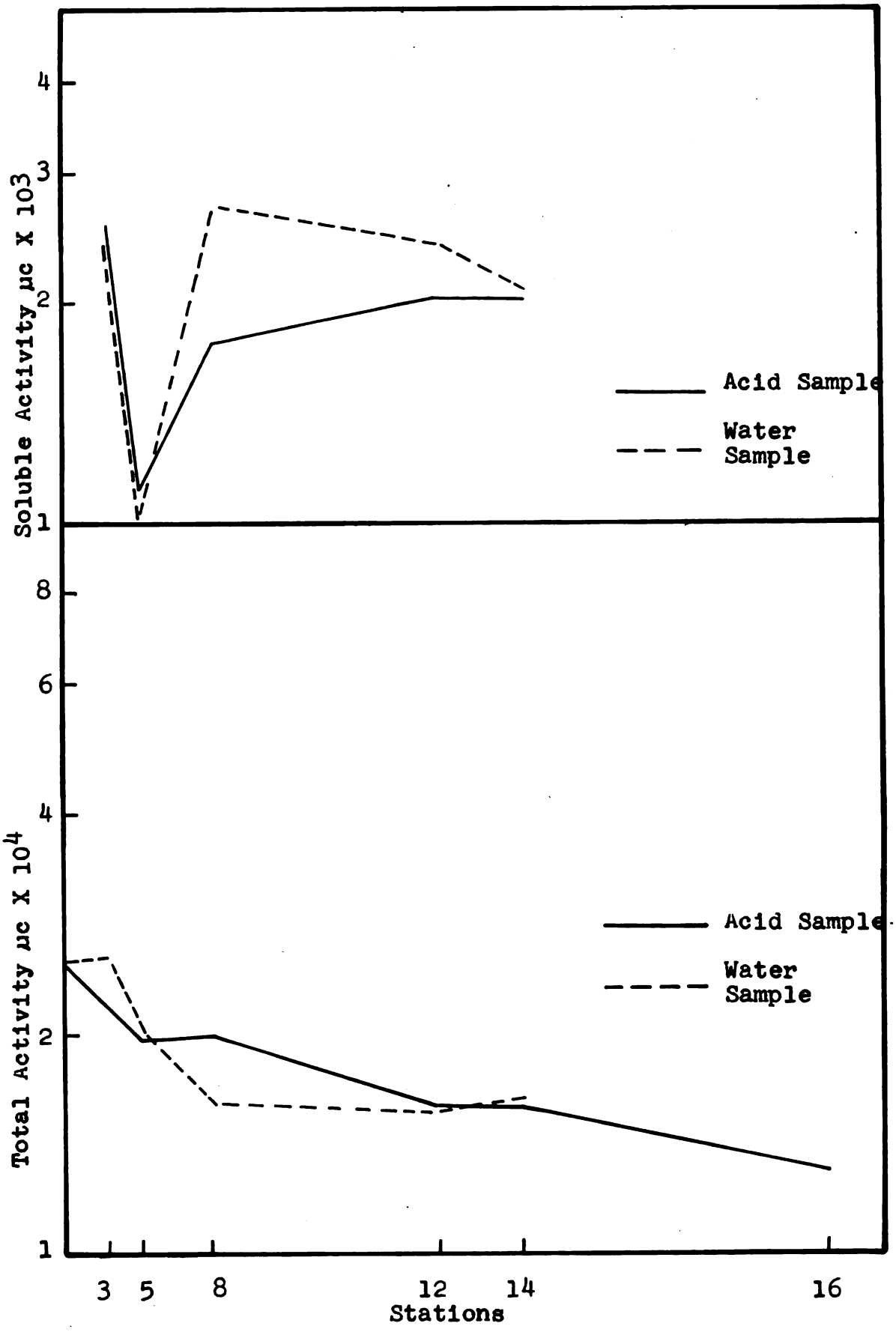
$$S^2\ \text{col.} = \frac{S\ S\ \text{col.}}{2}$$

$$S^2\ \text{error} = \frac{S\ S\ \text{error}}{8}$$

$$F = \frac{S^2\ \text{rows}}{S^2\ \text{error}}$$

$$F = \frac{5423}{3793} = 1.429$$

Figure XII. Total and soluble activity passage by the collecting stations in microcuries.


Soluble Activity µc X 10³
4
3
2
1
Acid Sample
Water Sample
Total Activity µc X 10⁴
8
6
4
2
1
Acid Sample
Water Sample
3
5
8
12
14
16
Stations

Activity Flow and Uptake in Various Stream Sections

Differences or similarities in the uptake of P^{32} by various stream sections may have significance in the basic productivity and in the distribution of phosphorus within the stream system. In order to determine the uptake of the isotope in various stream sections, the total passage of the isotope by each station was computed. The values found in the computation are shown in Table 3. Figure 12 shows the activity flow values plotted logarithmically with distance from the entry point. A comparison of the activity flow values for the years 1958, 1959, 1960, and 1961 is shown if Figure 13. A large difference can be noted between the passage of the isotope in the bacteria and inorganic form. In the 1958 experiment, less than 1 mc passed out of the area; in 1959, all of the P^{32} was removed within the area; in 1960, 1.5 mc passed through the system, but in the 1961 experiment, 13 mc passed through the study area. The large amount of activity passing through is an indication that the bacteria were not the organisms responsible for the distribution of phosphorus in the stream system. Since the experimental conditions were similar on all additions of the isotope, the same general pattern of uptake might be expected if the bacteria were the organisms responsible for the removal of P^{32} from the inorganic state.

The area of each stream section was computed and the uptake of P^{32} in the sections was determined (Table 3). Figure 14 shows the uptake in microcuries per square yard

Figure XIII. Total microcuries passing the sampling points in the indicated years.

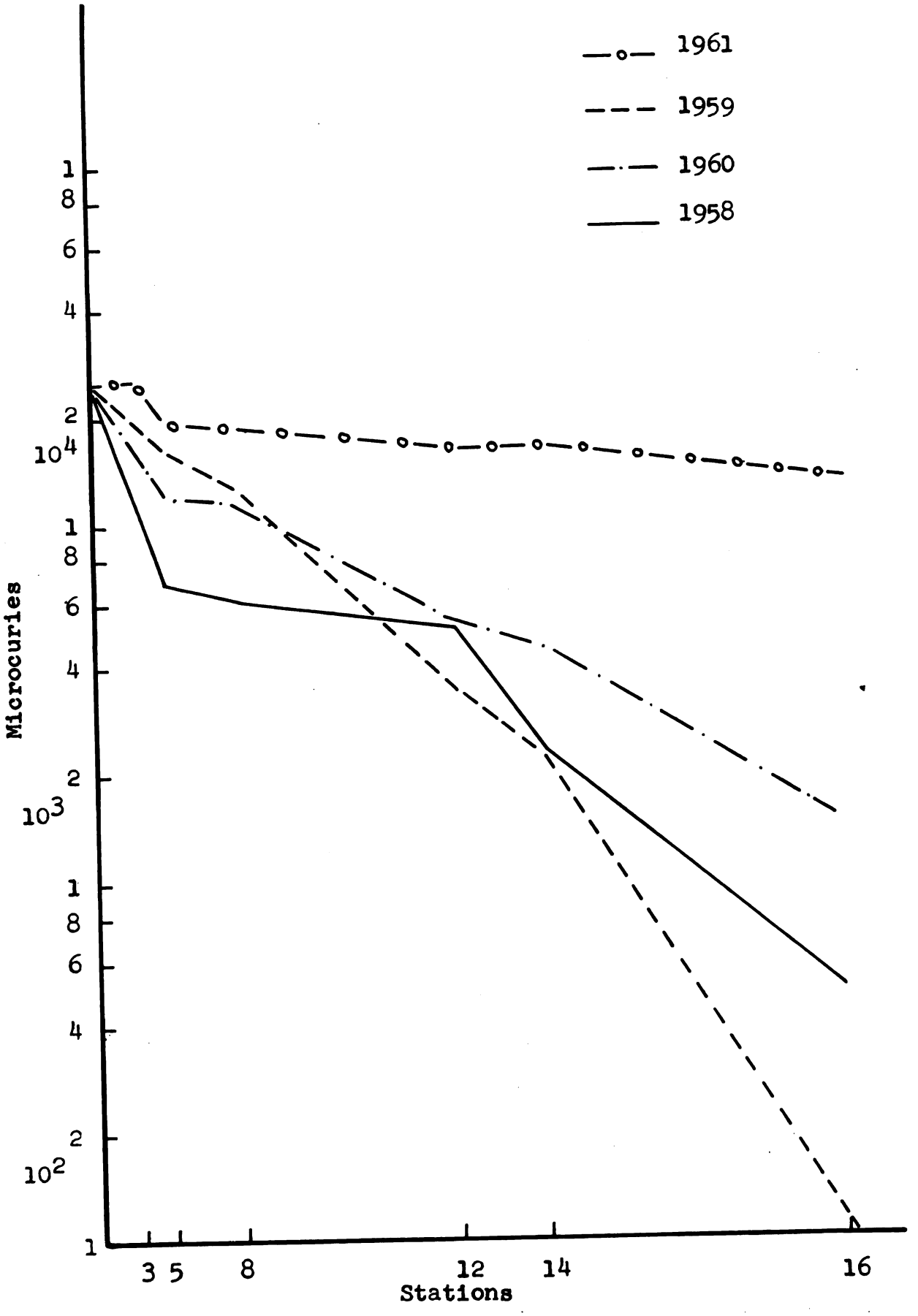
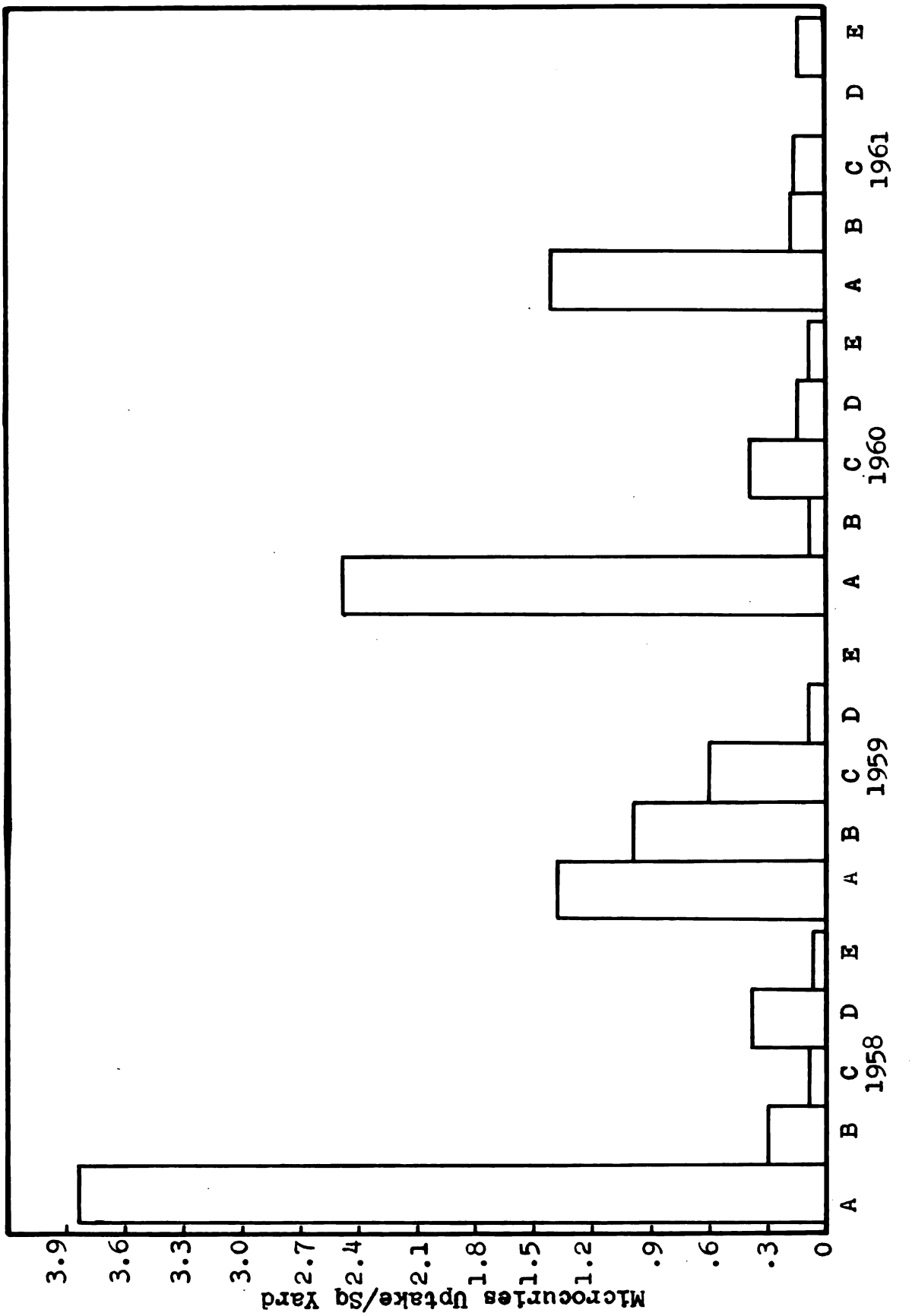

1961
1959
1960
1958
Microcuries
1
8
6
4
2
10^4
1
8
6
4
2
10^3
1
8
6
4
2
10^2
1
3 5 8 12 14 16
Stations

Table 3

Section	Sq. Yards	Uptake	Uptake Per Sq. Yard	Proportional Uptake	Year
2-5	4,378	16,189	3.723	.708	1958
5-8	4,036	625	.154	.091	
8-12	12,037	1,225	.101	.196	
12-14	6,209	2,634	.424	.488	
14-16	20,700	1,803	.087	.707	
2-5	4,378	6,382	1.457	.311	1959
5-8	4,036	4,084	1.011	.289	
8-12	14,926	9,080	.608	.904	
12-14	5,322	962	.180	.999	
14-16	18,900				
2-5	4,378	10,996	2.511	.478	1960
5-8	4,036	479	.118	.036	
8-12	14,926	6,021	.403	.480	
12-14	5,322	982	.184	.150	
14-16	18,900	3,085	.163	.554	
2-5	4,378	6,219	1.420	.242	1961
5-8	4,036	857	.212	.044	
8-12	14,926	2,478	.205	.164	
12-14	5,322				
14-16	18,900	3,183	.177	.202	

Figure XIV. Microcuries uptake per square yard of the stream sections in the indicated years.


Microcuries Uptake/Sq Yard
3.9
3.6
3.3
3.0
2.7
2.4
2.1
1.8
1.5
1.2
.9
.6
.3
0
A B C D E A B C D E A B C D E A B C D E
1958
1959
1960
1961

of stream section in all four years of the investigation. Upon examination of Figure 14, it is evident that section A shows the greatest amount of uptake in all years; unfortunately, these figures can not be related to productivity since the P^{32} available for uptake does not remain constant in all sections. The uptake values were transformed into proportions of the amount of available P^{32} per square yard to the amount of uptake per square yard forming a more logical relationship. The proportions found in the analysis are shown in Table 3 and are graphically represented in Figure 15. A Friedman two-way analysis of variance was computed on the data, Table 4; the analysis showed heterogeneity at the 99% level. Thus the analysis indicates that the proportional uptake rates do not remain constant throughout the study area. Two possible explanations for these results are: (1) the phosphorus is more available for uptake in some sections than in others; (2) some sections of the stream are lower in productivity, populations of energy fixers, and therefore can not remove similar proportions of P^{32}. The stream section between stations 5 and 8 (sectionB) shows low proportional uptake in all four years. The habitat in section B is predominantly a riffle and run area which should allow growth of energy fixing periphyton and higher aquatic plants. In 1960, the only year for which separate plant biomass estimates are available, this section had the lowest biomass estimate. Hence,

Figure XV. Proportion of the activity available to the activity uptake per square yard in the various stream sections in the indicated years.

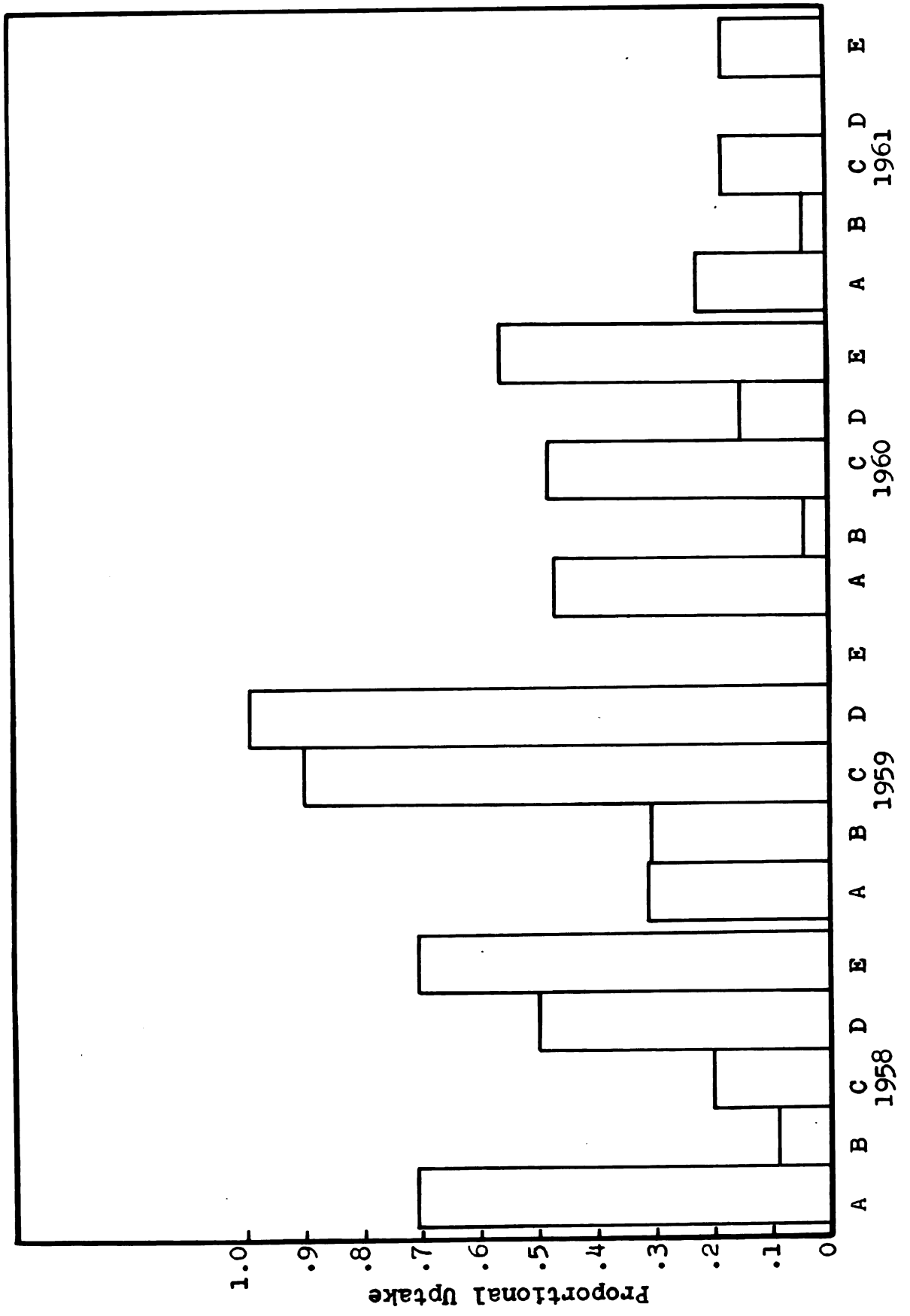

Proportional Uptake
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
A B C D E A B C D E A B C D E A B C D E
1958
1959
1960
1961

Table 4

Friedman Analysis of Variance

	A	B	C	D	E
1958	5	1	2	3	4
1959	3	2	4	5	1
1960	3	1	4	2	5
1961	5	2	3	1	4
	16	6	13	11	14

$$X^2 = \frac{12}{N\,k\,(k+1)} \sum_{j=1}^{k} (Rj)^2 - 3N\,(k+1)$$

$$X^2 = \frac{1}{10} \cdot 716 = 71.6$$

where N = number of rows

k = number of columns

Rj = sum of ranks in jth column and the section with the lowest uptake was ranked as 1

a possible explanation for the lower uptake of section B could be that of a lower population of primary producers to fix inorganic P^{32}. Uptake values in microcuries per gram of aquatic plants, the day following isotope entry, in the various sections during 1960 are shown in Table 5. The amount of uptake per gram of plants was analysed by an analysis of variance which at the 95% level showed no heterogeneity between stream sections. Therefore, the low proportional uptake in section B can not be attributed to a lower plant biomass because the uptake per gram of plant remained fairly constant throughout the stream.

Table 5. Uptake of P^{32} by aquatic plants during 1960 in designated areas.

Plant Grams	Biomass Per Yard ^{2}	Uptake Per Yard ^{2}	Proportional Uptake Per Gram	Uptake Per Gram
A	501	.7713	.03	.0003419
B	359	.1261	.04	.0003412
C	864	.1830	.21	.0002118
D	1,602	.3201	.30	.0001998

Several possible hypothesis for a stable uptake of P^{32} by plants in the various sections are as follows:

(1) The recycling of P^{32} by periphyton from the upper stations allowed uptake of P^{32} by plants in the lower sections, while plants in the upper sections could only lose activity.

(2) The uptake of P^{32} per gram of plant represents only a portion of the P^{32} present, but it is a constant proportion to the amount of P^{32} in a usable state. This hypothesis does not seem reasonable when one considers the proportional uptake of P^{32} (i.e. the amount of P^{32} available to the amount incorporated) by the plants in different sections, as is shown in Table 5. The plants in the lower stream sections removed a greater proportion of the activity present than did the plants in the upper sections.

(3) Physiological differences in plant metabolism due to sunlight, rate of stream flow, and other factors would influence P^{32} uptake, but the importance of these factors is not known.

The low proportional uptake in section B can not be attributed to low plant biomass, and since no periphyton production figures are available, the reasons for the section's low proportional uptake can only be hypothesized. Section B is predominantly a riffle and run area supplied with abundant sunshine; the possibility of photoinhibition of the existing periphyton populations is a consideration. The area may not have had comparable populations of periphyton due to a type of substrate less productive in periphyton or other limiting features not presently recognized.

Fish Activity Levels

The uptake of radioisotopes of biologically essential elements by aquatic organisms may occur by absorption through membranes exposed to the surrounding water, through

engulfment of food or inert particles which contain the radioactive material, or by adsorption onto exposed surface areas (Foster, 1959). Phillips, et.al. (1957) have shown that brook trout in aquaria were able to take up P^{32} from the water, but the utilization from the diet was over 200 times that of dissolved P^{32}. Krumholz and Foster (ibid.) also stated that the major mode of accumulation of radioactive materials by fish occurs through ingestion.

The activity levels of the fish food organisms during this study were generally 10-fold lower than those of previous years. The relationship and importance of a high level of activity in these food organisms can be seen in a comparison of the activity values for fish from 1959 to 1961, Figures 16 and 17.

Due to the large differences in accumulation of P^{32} by fish in the 1959 and 1961 experiments, the patterns of P^{32} uptake were obscured. To make possible a comparison of uptake patterns in these experiments, regression equations for uptake rates were computed; these are found in Appendices III, IV and V. The slopes of the regressions (the rate of P^{32} uptake) are dependent on several factors such as water temperature, basal metabolism, species differences, and food substances. The uptake rates for different years and species were compared by an analysis of variance and no significant differences were found. If the P^{32} were reaching the fish through radically different pathways due to incorporation of the P^{32} into the bacterial cells, different rates of

Figure XVI. Comparison of fish activity levels in 1959 and 1961.

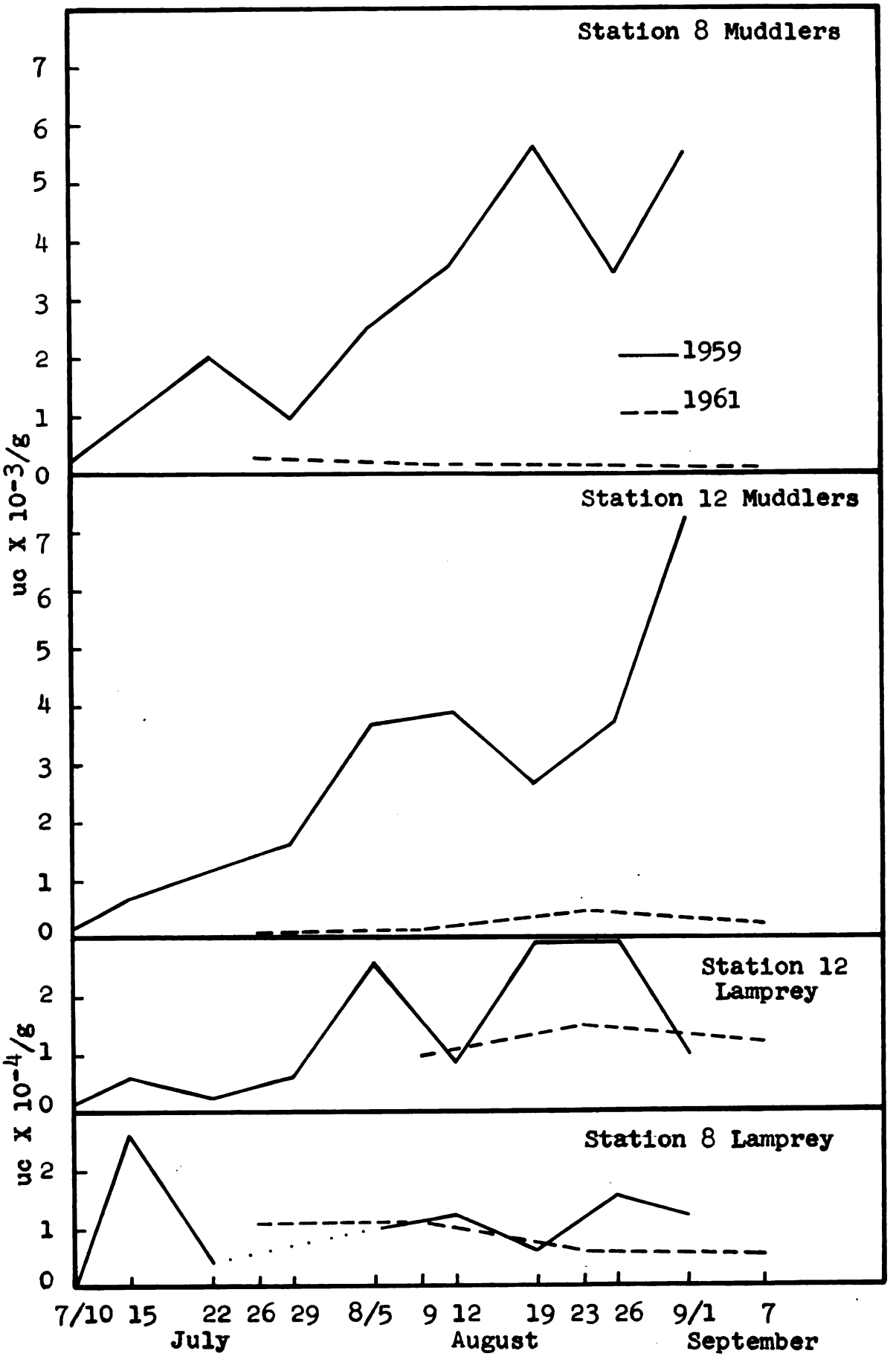
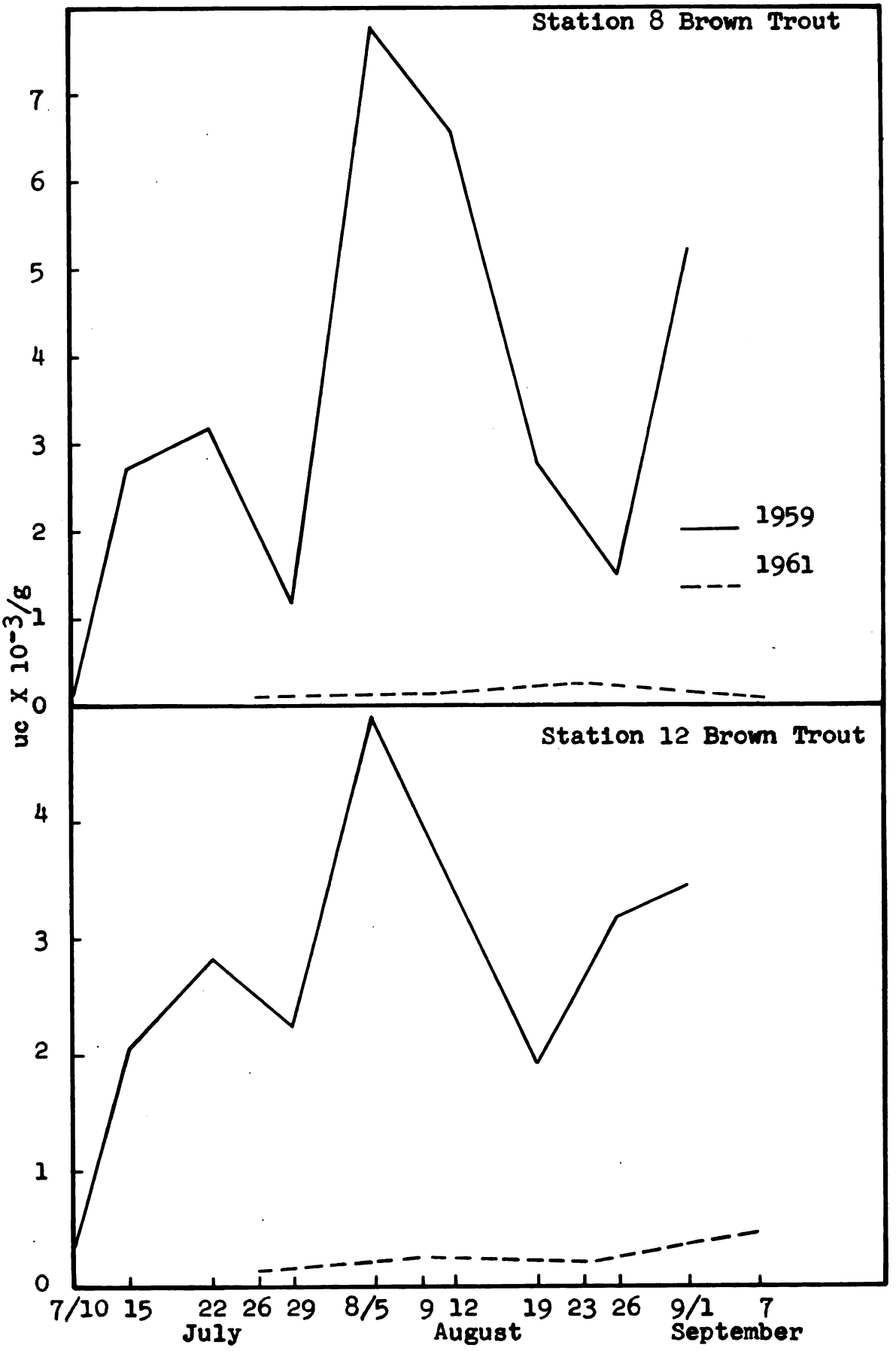

Station 8 Muddlers
1959
1961
uc X 10-3/g
Station 12 Muddlers
Station 12 Lamprey
uc X 10-4/g
Station 8 Lamprey
7/10 15 22 26 29 8/5 9 12 19 23 26 9/1 7
July
August
September

Figure XVII. Comparison of fish activity levels in 1959 and 1961.


Station 8 Brown Trout
Station 12 Brown Trout
1959
1961
uc X 10⁻³/g
0
1
2
3
4
5
6
7
7/10
15
22
26
29
8/5
9
12
19
23
26
9/1
7
July
August
September

uptake might be expected. The finding of similar uptake rates indicates that the fish received the P^{32} from a source similar to that of other years. The magnitudes of activity density, however, are so reduced that the densities could have originated from trophic pathways followed by the small amount of soluble P^{32} present at the time of addition of the isotope.

Figure 18 shows the activity curves for all fish collected during the 1961 study; the uptake rates for these fish are shown in Figures 19 and 20.

The mean counts per gram of each species considering the entire study period were as follows:

	Station 8	Station 12
Lamprey	93	114
Brown Trout	130	224
Brook Trout	138	292 (2 fish)
Rainbow Trout	172	258
Muddler	167	221

The mean activity levels for brown and rainbow trout at stations 8 and 12 were compared by a two way analysis of variance. The analysis showed a significant difference in activity between the stations at the 95% level. Comparison between the species by a one-tailed test showed that the rainbows had significantly greater mean activity levels than the browns at the 90% level. Differences in activity levels for fish at stations 8 and 12 have also been recognized by

Figure XVIII. Activity density of fish at collecting sites 8 and 12.

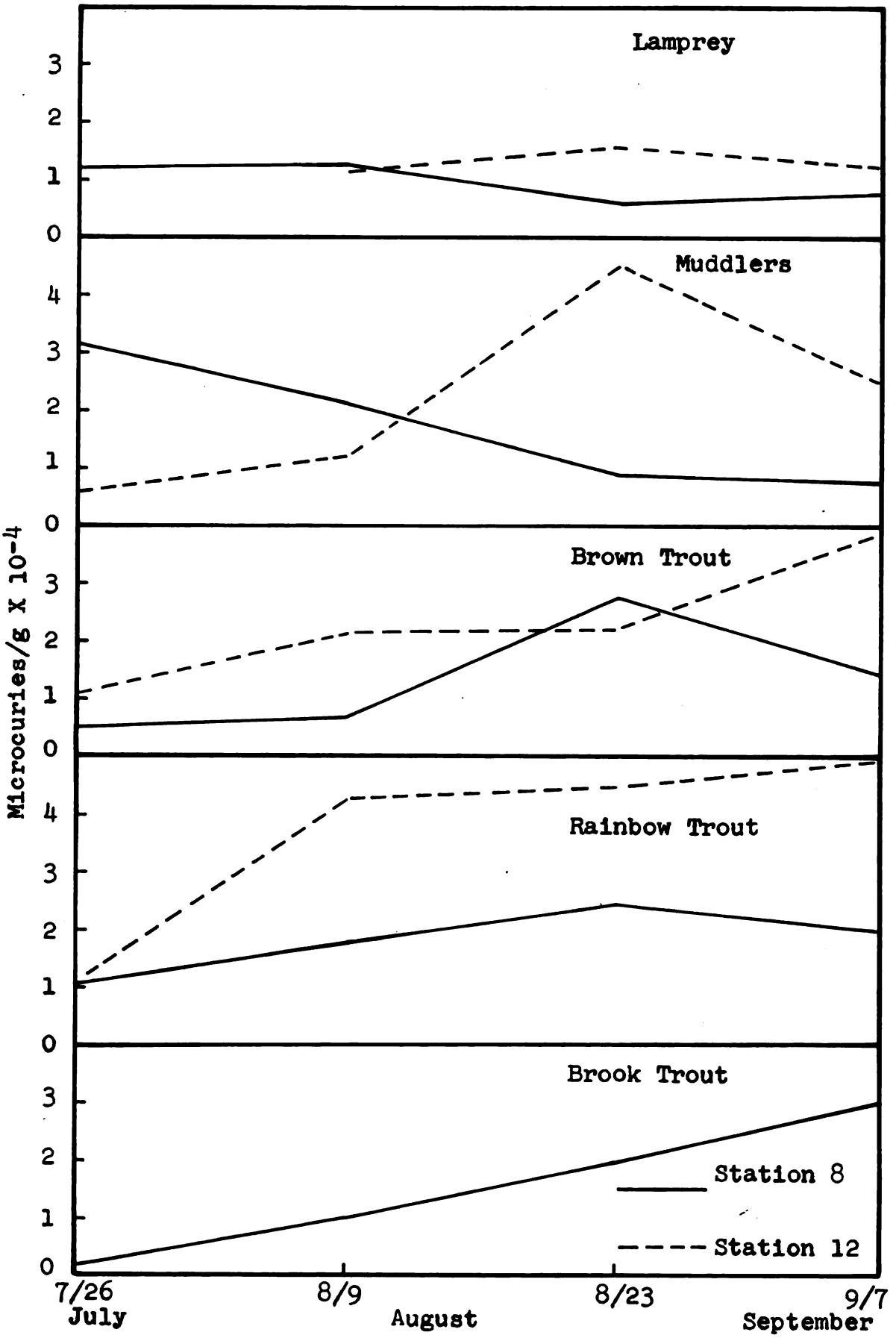

Lamprey
Muddlers
Brown Trout
Rainbow Trout
Brook Trout
Station 8
Station 12
Microcuries/g X 10⁻⁴
7/26
July
8/9
August
8/23
9/7
September

Figure XIX. Activity uptake rates for the fish at station 8.

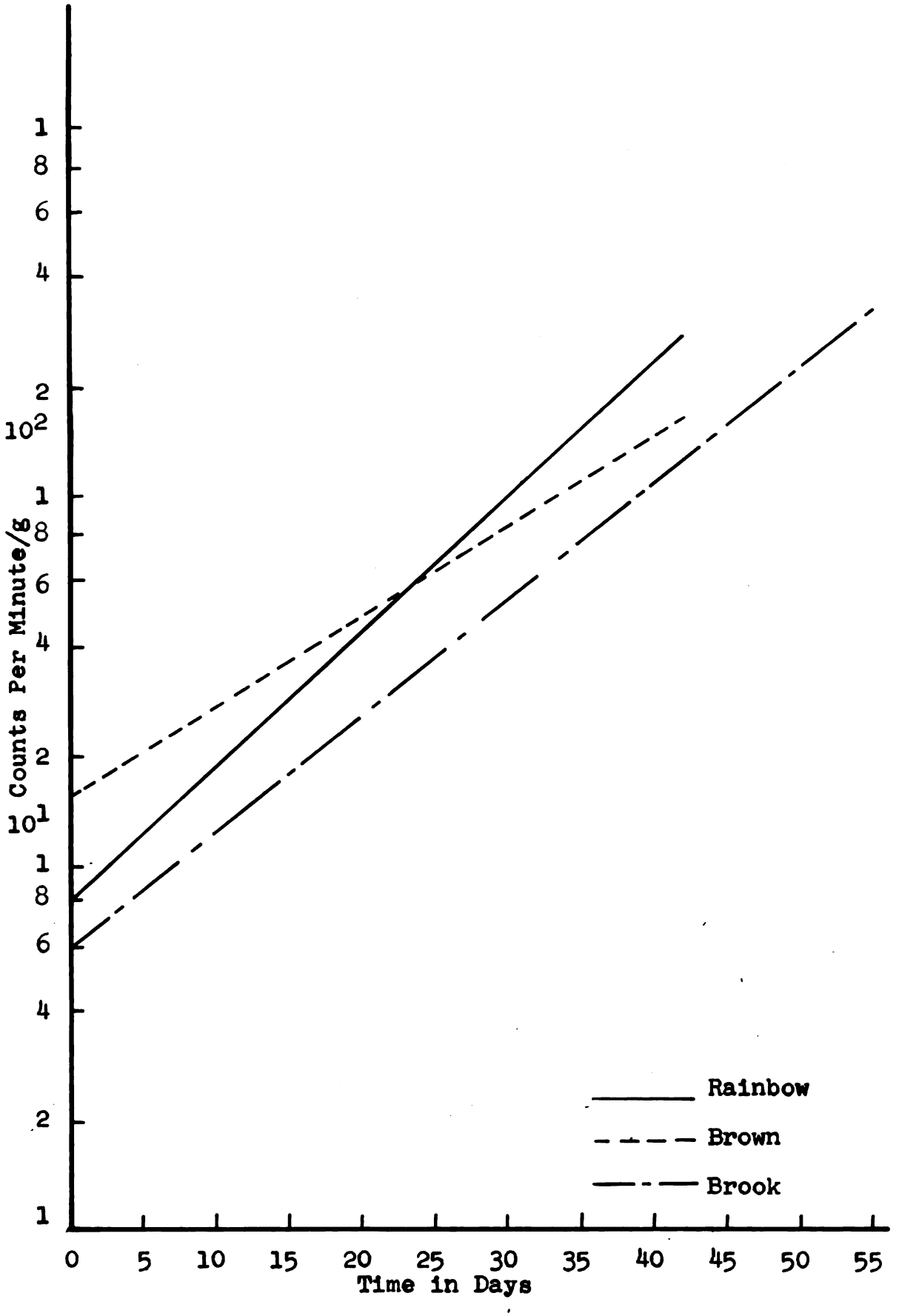
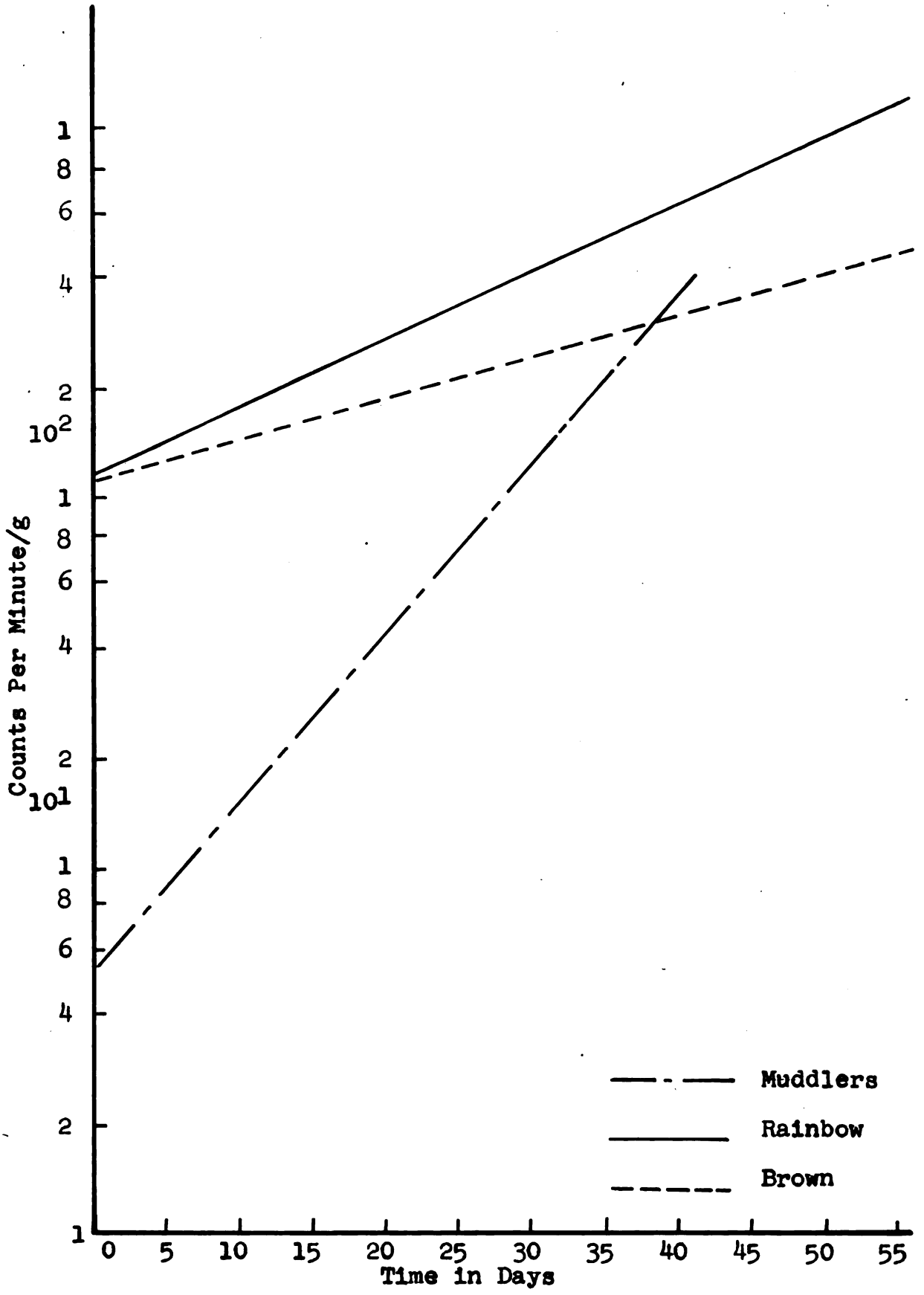

1
8
6
4
2
10²
1
8
6
4
2
10¹
1
8
6
4
2
1
Counts Per Minute/g
0
5
10
15
20
25
30
35
40
45
50
55
Time in Days
Rainbow
Brown
Brook

Figure XX. Activity uptake rates of the fish at station 12.


Counts Per Minute/g
1
8
6
4
2
10²
1
8
6
4
2
10¹
1
8
6
4
2
1
0
5
10
15
20
25
30
35
40
45
50
55
Time in Days
Muddlers
Rainbow
Brown

Knight (ibid.) who attributes the findings to downstream drift. Nelson (1958) noted that rainbow trout accumulated more activity than brook trout in a lake system.

Fish Biomass

The fish population of the West Branch of the Sturgeon River during the 1961 investigation had the following composition.

Station	Species	No. in 100 Yd.	No. per Acre	Lb. per Acre
3	Brook	22	125	3.4
	Brown	21	119	29.0
	Rainbow	19	102	4.7
	Muddler	115	3,928	27.6
8	Brook	115	654	19.5
	Brown	19	108	8.9
	Rainbow	27	153	6.2
	Muddler	114	3,894	27.4
12	Brook	3	16	1.9
	Brown	22	121	18.9
	Rainbow	17	93	2.8
	Muddler	333	11,028	77.9
14	Brook	6	33	4.5
	Brown	26	143	20.5
	Rainbow	27	149	3.7
	Muddler	199	3,941	27.7

SUMMARY

Bacterial cells have been shown to play an important role in the distribution of P^{32} in lakes. The results of this study, however, seem to negate a similarity between lake and stream environments in the transfer of phosphorus, at least at the primary level. Fixation of P^{32} by the stream biota in earlier years of the investigation of the West Branch proved more efficient than in the present study. It seems possible, in fact, that the bacteria lost to the stream system held most of their P^{32} or did not transfer it in a form available to the stream biota.

The exchange of P^{32} with normal P^{31} in the stream system was demonstrated to be of low magnitude, 10 - 15% of the incorporated P^{32}. Rice (1953) using algal cells showed that cells grown in low phosphorus media incorporated more P^{32} into the organic and unexchangeable state than did cells grown in media of high phosphorus content. The bacteria utilized in this experiment were grown under minimal phosphorus concentrations which may account for the small portion of exchangeable activity.

The activity levels reached at all trophic levels investigated were 5-to-20 fold lower than in previous years. Since an amount of P^{32} approximately one half of other years was retained in the area, the activity levels reached should theoretically have been higher if the same paths were followed. The uptake rates of P^{32} for fish have been

shown to be similar to those observed when the P^{32} was added in the inorganic form; this seems to indicate pathways of phosphorus transfer not radically different from earlier years. Uptake of P^{32} by periphyton and plants showed levels of activity easily attributable to the soluble activity present (Bacon,unpublished). The above considerations indicate that the organisms responsible for fixation of P^{32} within the stream are most probably not bacteria.

Uptake of P^{32} per square yard by the uppermost stream section was high in all years, while the uptake in the lower sections was greatly reduced. This is probably due to uptake of the isotope decreasing its availabilty per unit area in the lower sections. Proportional uptake levels for the stream sections revealed that all sections of the stream were fairly uniform in proportional uptake, except for section B. It was thought that the low uptake observed in section B was due to low plant biomass, but upon analysis it was discovered that the uptake of P^{32} per gram of plant in all sections was fairly constant.

LITERATURE CITED

Alexander, Gaylord R. 1956. The fertilization of a marl lake. Master's Thesis, Michigan State University.

American Public Health Association. 1960. Standard methods for the examination of water, sewage and industrial wastes. 11th ed. Amer. Public Health Assoc., New York. 522 pp.

Ball, Robert C. and Frank F. Hooper. 1962. Translocation of P^{32} in a trout stream ecosystem. (in press) Atomic Energy Proceedings, Radioecology Conference.

Borgeson, David P. 1959. The movement of radioactive phosphorus through a stream ecosystem. Master's Thesis, Michigan State University.

Boroughs, Howard, Walter A. Chipman, and Theodore R. Rice. 1957. Laboratory experiments on the uptake, accumulation, and loss of radionuclides by marine organism. Nat. Acad. Science -- Nat. Res. Council. Publ. No. 551: 80 - 87.

Bryant, William C. 1960. Movement of radiophosphorus through the invertebrate community of a trout stream. Master's Thesis, Michigan State University.

Burrows, William. 1959. Textbook of microbiology. W.B. Saunders Company, Philadelphia. 954 pp.

Chase, Grafton D. 1960. Principles of Radioisotope Methodology. Burgess Publishing Company, Minneapolis, Minn. 286 pp.

Clifford, Hugh F. 1959. Response of periphyton to phosphorus introduced into a Michigan trout stream. Master's Thesis, Michigan State University.

Foster, R.F. 1959. Radioactive tracing of the movement of an essential element through an aquatic community with specific reference to radiophosphorus. Pub. della stazione Zool. di napolic (Press), 31: 34 - 62.

Grzenda, Alfred R. 1955. The biological response of a trout stream to headwater fertilization. Master's Thesis, Michigan State University.

Hayes, F.R., J.A. McCarter, M.L. Cameron, and D.A. Livingstone. 1952. On the kinetics of phosphorus exchange in lakes. Jour. Ecology, 40: 202 - 216.

Hayes, F.R., and J.E. Phillips. 1958. Lake water and sediment. IV. Radiophosphorus equilibrium with mud,plants,and bacteria under oxidized and reduced conditions. Limnol. Oceanogr., 3: 459 - 475.

Hutchinson, G.E., and V. T. Bowen. 1950. Limnological studies in Connecticut. IX. A quantitative radiochemical study of the phosphorus cycle in Linsley Pond. Ecology, 31: 194 - 203.

Kinsman, S. 1957. Radiological health handbook. U.S. Dept. of Health, Education, and Welfare, Public Health Service. Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. 355 pp.

Knight, Allen W. 1961. The translocation of radiophosphorus through an aquatic ecosystem. Master's Thesis, Michigan State University.

Krumholz, Louis A., and Richard F. Foster. 1957. Accumulation and retention of radioactivity from fission products and other radiomaterials by fresh-water organisms. Nat. Acad. Science -- Nat. Res. Coúncil. Publ. No. 551: 88 - 95.

Labaw, L.W., V.M. Mosley, and R.W.G. Wyckoff. 1950. Radioactive studies of the phosphorus metabolism of Escherichia coli. Jour. Bacteriol. 59: 251 - 262.

Nelson, Wesley C. 1958. The uptake of radioactive phosphorus by the fish species of upper Camp Lake, Colorado. State of Colorado, Dept. of Game and Fish, Progress Report.

Odum, Eugene P. 1959. Fundamentals of ecology. W.B. Saunders Company, Philadelphia. 546 pp.

Phillips, A.M., Jr., H.A. Podoliak, D.R. Brockway, and R.R. Vaughn. 1957. The nutrition of trout. Cortland Hatchery Rept. 26, New York Cons. Dept., Fish. Res. Bull. 21. 93 pp.

Reid, George K. 1961. Ecology of inland waters and estuaries. Reinhold Publishing Corporation, New York. 375 pp.

Rice, T.R. 1953. Phosphorus exchange in marine phytoplankton. Fishery Bull., U.S. Fish and Wildl. Serv., 80: 77 - 89.

Rigler, F.H. 1956. A tracer study of phosphorus cycle in lake water. Ecol., 37: 550 - 556.

Robeck, G.G., Croswell Henderson, and Ralph C. Palange. 1954. Water quality studies on the Columbia River. U.S. Dept. of Health, Education and Welfare, Public Health Service Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

Whittaker, R.H. 1961. Experiments with radiophosphorus tracer in aquarium microcosms. Ecol. Monogr., 31: 157 - 188.

Zettelmaier, John L. 1961. The translocation of radiophosphorus through a lotic ecosystem. Master's Thesis, Michigan State University.

APPENDIX I

Biochemical Characteristics of *E. coli*

Litmus Milk	A.C.G.R.	Xylose	A.G.
Nitrates reduced	X	Inositol	-
Bouillon	X	Arabinose	A.G.
Potato	X	Mannitol	A.G.
Dulcitol	A.G.	Indol	X
Maltose	A.G.	Voges-Proskauer	-
Dextrose	A.G.	Methyl Red	-
Lactose	A.G.	H_2S	-
Sucrose	-		

A = Acid

C = Coagulation

G = Gas

R = Reduction

X = Activity

- = No Activity

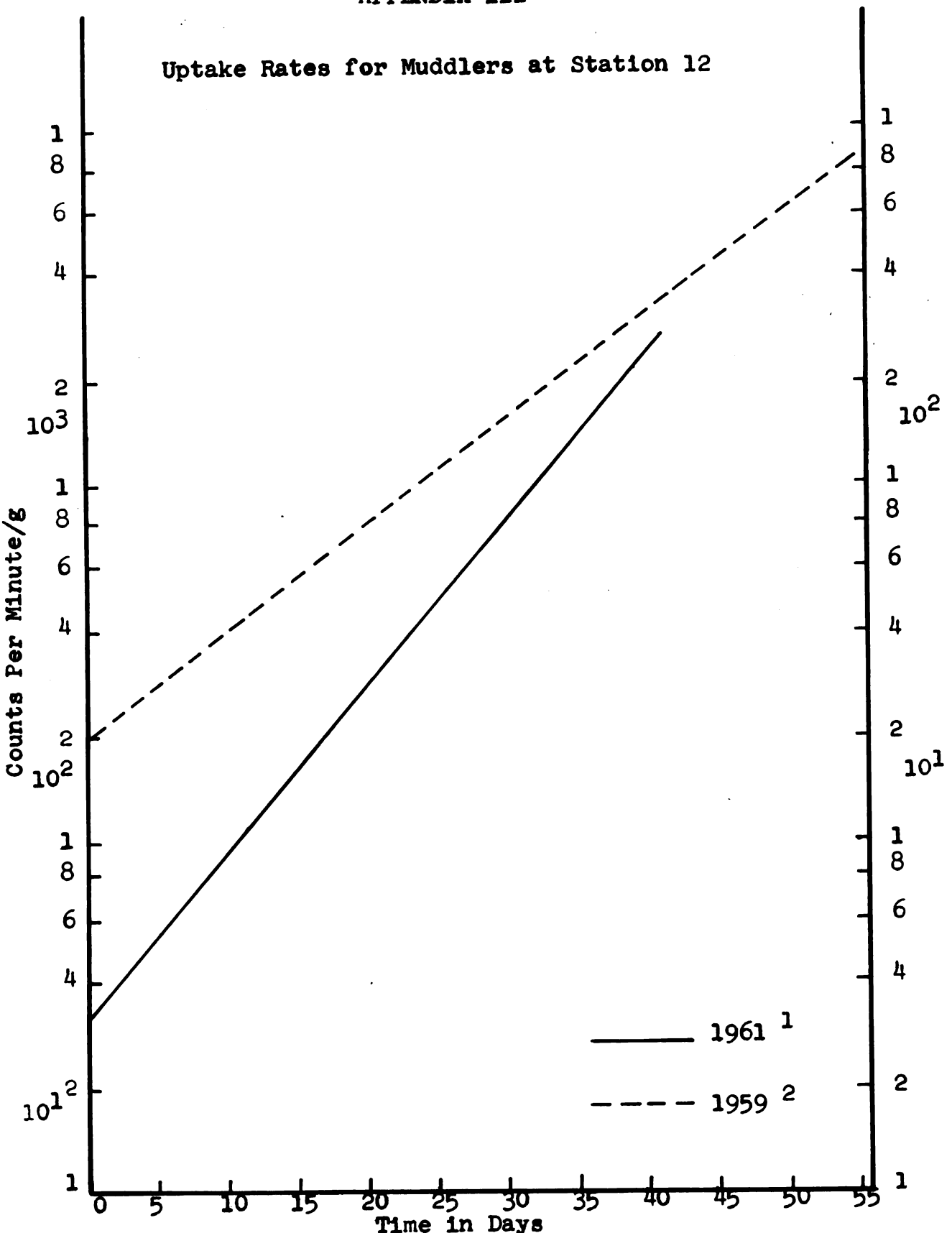
APPENDIX II

Growth Medium for *E. coli* 0111

L-Glutamic Acid	2.0 g	Distilled Water	1,000 ml
Glycine	7.5 g	Boron	.01 g
Glucose	2.0 g	Copper	.10 g
Glycerol	1.0 g	Iron	.20 g
NaCl	5.9 g	Manganese	.02 g
$MgSO_4$	.1 g	Molybdenum	.02 g
$CaCl_2$	.1 g	Zinc	2.00 g

APPENDIX III

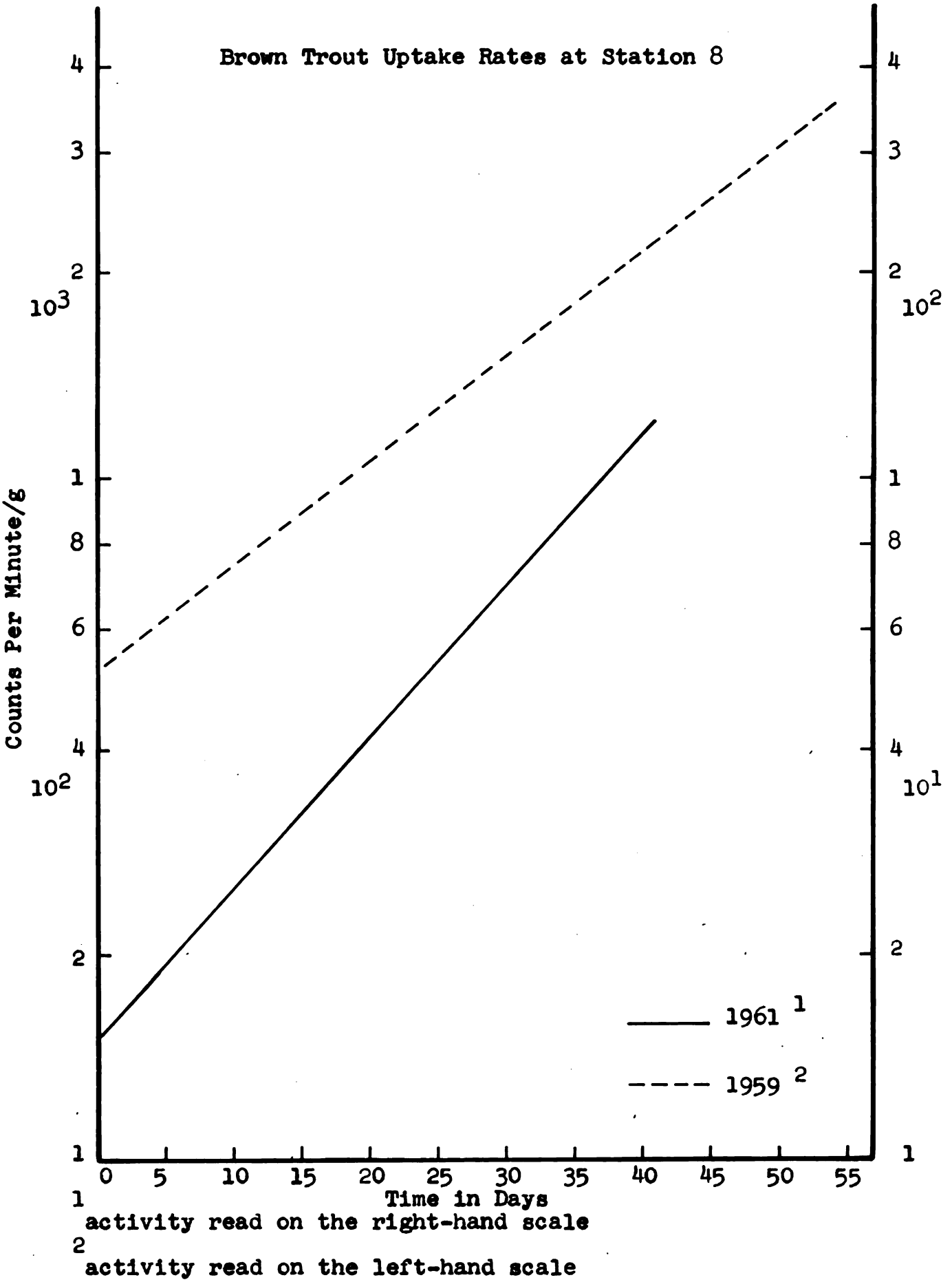
Uptake Rates for Muddlers at Station 12



[1] activity read on the right-hand scale

[2] activity read on the left-hand scale

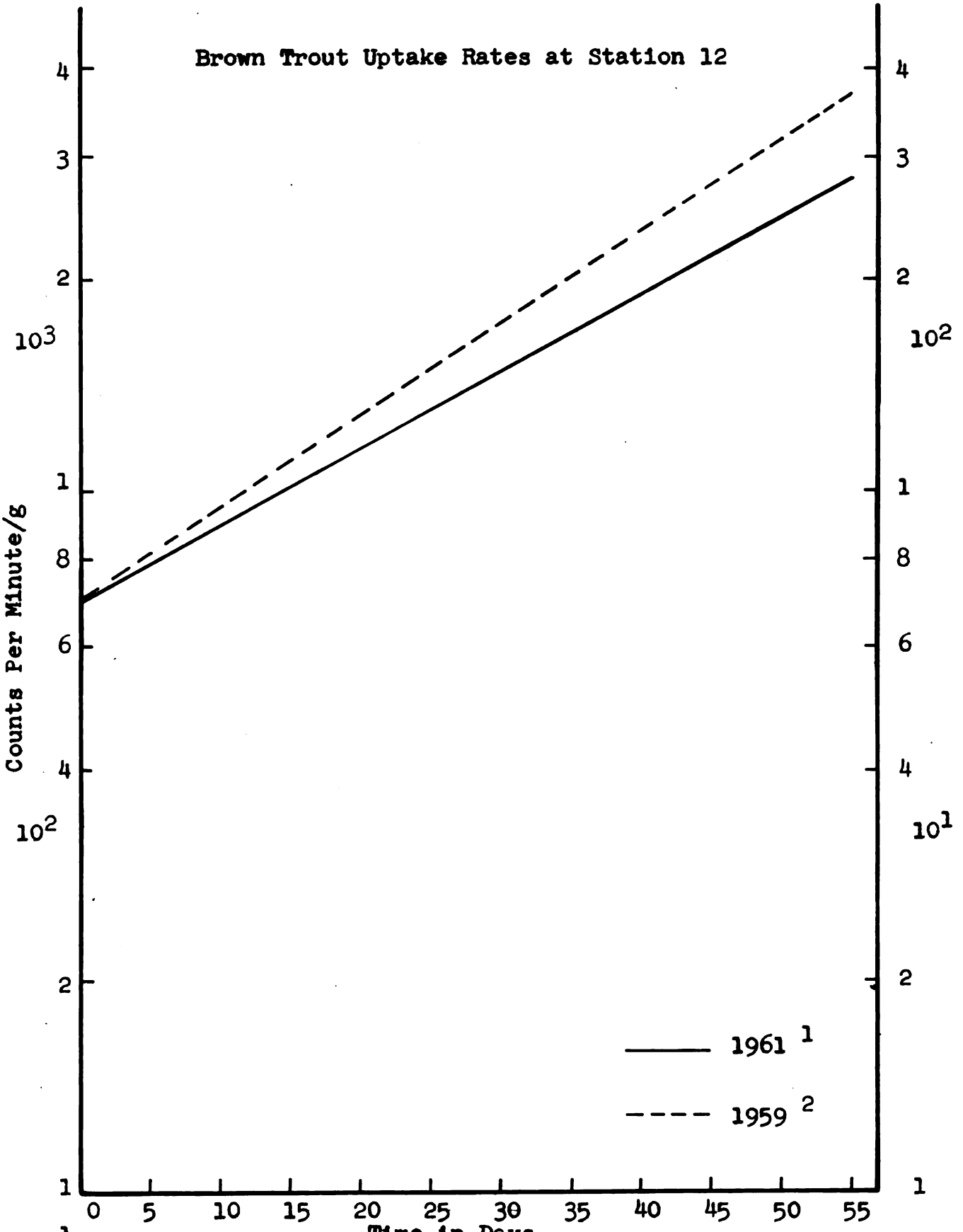
APPENDIX IV



1 activity read on the right-hand scale

2 activity read on the left-hand scale

APPENDIX V



1 activity read on the right-hand scale

2 activity read on the left-hand scale

APPENDIX VI

Activity Values from Acid and Water Wash Samples During the Isotope Passage

Station	Total Activity Passing [1]	Total Activity Passing [2]	Mean Total Passing	Acid Soluble Passing	Water Soluble Passing	Mean Soluble Passing
3	25,790	22,599	25,644	2,551	2,422	2,527
5	19,872	18,999	19,425	1,106	943	984
8	16,972	20,178	18,568	1,734	2,628	2,334
12	15,935	15,890	16,090	2,051	2,386	2,342
14	16,517	15,999	16,251	2,024	2,085	2,161
16	_____	13,068	13,068	_____	_____	_____

[1] from water wash samples

[2] from acid wash samples

APPENDIX VII

Fish Biomass Estimations of the West Branch for the Years 1958, 1959, 1960, and 1961

Year	Pound of Trout per Acre	Muddlers Lb. per Acre	Total
1958 (Bryant, 1960)	86.0	16.0	101.8
1959 (Knight, 1961)	20.0	106.6	126.6
1960 (Zettelmaier, 1962)	55.0	18.5	61.5
1961	30.2	40.0	70.2