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MOVEMENT OF RADIOPHOSPHORUS THROUGH THE INVERTEBRATE COMMUNITY OF A TROUT STREAM

WILLIAM CLARK BRYANT

1960

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By

WILLIAM CLARK BRYANT

A THESIS

Submitted to the College of Agriculture of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

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ABSTRACT

On August 5, 1958, 23.1 millicures of P^{32} was released in a 37-minute period and at a constant rate into the West Branch of the Sturgeon River, Michigan. As the P^{32} flowed downstream it became more dilute but was detectable in the water mass for longer periods of time.

Organisms representing all trophic levels in the stream were assayed for radioactivity. The isotope was rapidly taken up by the plants and appeared to be taken up next by organisms feeding on the plants. This thesis reports the movement of P^{32} through the invertebrate community.

The concentration of P^{32} in bottom fauna collected 100 yards below the isotope release point was of the same magnitude as in similar organisms collected at greater distances downstream.

Invertebrates living in riffles took up a greater amount of P^{32} than was taken up by those of the silt deposits.

Invertebrates that feed on plants had the highest radioactivity. Predators and omnivores had the next highest radioactivity and scavengers had the least radioactivity.

P^{32} was deposited in greater amounts in the flesh parts of snails than in the shells. The amount of P^{32} lost from snails by way of their waste materials decreased with passing time.

Estimates were made of the ratio of the concentration of P^{32} in bottom fauna over that of the water.

The biomass of invertebrates and fish in a 1000 yard section of the stream exposed to the isotope was estimated.

W. C. B.

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
Description of P^{32}	2
Description of the Study Area	3
Flora	3
Fauna	5
Sampling Stations	8
METHODS AND PROCEDURES	12
Radiological Techniques	12
Adding P^{32} to the Stream	12
Sampling Procedure	13
Preparation for Assay	13
Assaying Invertebrate Radioactivity	14
Biomass Estimate Technique	15
RESULTS AND DISCUSSION	19
Factors Affecting Biological Uptake of P^{32}	19
Method of Uptake of P^{32} by the Invertebrates	24
Uptake of P^{32} by Invertebrates of Different Habitats	24
Activity of Invertebrates Representing Different Trophic Levels	31
Differences in P^{32} Uptake by Invertebrates of Different Families	37
Variation in P^{32} Uptake by Invertebrates of the Same Genus	37
Atherix variegata	46
Brachycentrus	46
Cordulegaster	49
Hexagenia limbata	49
Physa	54
Pteronarcys	54
Simulium	54
P^{32} Deposition in Various Parts of the Snail, Physa	54
Snail Eggs	61
Snail Shells	61

Snail Feces 64
Snail Flesh 65
Entire Snail 65

P^{32} Concentration Factors in Invertebrates 66
Radioactivity in Control Organisms 70
An Estimate of the Animal Biomass 71

SUMMARY 76

LITERATURE CITED 81

LIST OF TABLES

Table		Page
I.	Water temperature and stream stage at Station 8 of the West Branch of the Sturgeon River, 1958	4
II.	A listing of the insects found in the West Branch of the Sturgeon River, 1958	7
III.	P^{32} concentrations in bottom fauna expressed as counts per minute per gram, corrected for background and decay	38
IV.	The amount of P^{32} eliminated by snails	64
V.	The estimated concentration factors for invertebrates	67
VI.	Population and biomass estimates of fish in a 1000 yard section of the West Branch of the Sturgeon River ..	73
VII.	A comparison of the trout and invertebrates of 4 streams .	75

LIST OF FIGURES

Figure		Page
1.	Experimental area and sampling stations	9
2.	Activity of 7 invertebrates at Station 2	20
3.	Comparison of activity of the riffle invertebrates, Brachycentridae, Pteronarcidae, and Simuliidae with activity of the silt-bed invertebrates, Cordulegasteridae and Ephemeridae	25
4.	Activity of periphyton and Pteronarcys at Station 2	28
5.	Activity of an omnivore, Physa, and of the herbivores, Simulium, Pteronarcys, and Hexagenia at Station 8	33
6.	Activity of a predator, Atherix, and of the herbivores, Simulium and Pteronarcys	35
7.	Activity of 7 invertebrates at Station 8	40
8.	Activity of 7 invertebrates at Station 13	42
9.	Activity of the larvae of the snipe fly, Atherix variegata	44
10.	Activity of the larvae of the caddis fly, Brachycentrus	47
11.	Activity of the naiads of the dragonfly, Cordulegaster	50
12.	Activity of the nymphs of the mayfly, Hexagenia limbata	52
13.	Activity of the snails (Physa)	55
14.	Activity of the naiads of the stonefly, Pteronarcys	57
15.	Activity of the black fly larvae (Simulium)	59
16.	Activity of entire snails (with shells) and of parts of snails at Station 8	62
17.	Comparison of the concentration factors in Physa with tha that of the average activity of the water on August 5, 1958	68

INTRODUCTION

The feasibility of increasing the production of fish and other organisms in streams by the application of inorganic fertilizer has been little investigated. Huntsman (1948) was the first worker to experiment with the chemical fertilization of a stream. He placed bags of fertilizer in a Nova Scotian stream and observed some local increase in the quantity of algae and fish attributable to the fertilizer.

In the summer of 1954 a research project was undertaken on Hoffman Lake (Charlevoix County, Michigan). Commercial fertilizer was added to the lake that summer (Grzenda, 1955 and Alexander, 1956) as well as during the summers of 1955 (Anton, 1957 and Colby, 1957) and 1956 (Carr, 1959 and Plosila, 1958). The movement of the fertilizer nutrients and the biological responses induced by them were studied not only in the lake but in the West Branch of the Sturgeon River which drains the lake.

During the summer of 1957 Keup (1958) and Correll (1958) added di-ammonium phosphate fertilizer directly to the stream and determined its biological and chemical effects.

The use of radiophosphorus as a research tool in freshwater biology has opened new fields which were almost impossible to explore adequately by other means (Krumholz and Foster, 1957). In the summer of 1958 the cycling of phosphorus through the ecosystem of the West Branch was traced by the employment of radiophosphorus (P^{32}).

Two milliliters of P^{32} solution assayed at 23.1 millicuries arrived at the Pellston, Michigan airport at 8:00 A.M. on August 5, 1958. The entire shipment of P^{32} was released at a constant rate into the stream between 2:01 P.M. and 2:38 P.M. on August 5. No radioecological study has been previously performed on the type of stream represented by the West Branch. The chief factor which distinguished this project from earlier studies of the West Branch was the vast increase in detection sensitivity of phosphorus.

Specialization of effort by the investigators was necessary since the movement of P^{32} through the entire stream ecosystem was studied. The writer's investigations were centered on the invertebrates while Clifford's (1959) research was focused on the periphyton and Borgeson (1959) investigated the water, the higher aquatic plants, and the fish.

The limnological information gained from the experiment contributes to our knowledge of a potential human health hazard: the fate of a metabolically important radioactive pollutant.

Description of P^{32}

P^{32} is an artificially created isotope of phosphorus, most of which is produced in chain-reacting piles. It is a high energy radioisotope emitting negative beta particles with an upper energy limit of 1.69 million electron volts (Kamen, 1947). It has a radioactive half-life period of 14.3 days. Several words are used in the thesis as

synonyms for P^{32} including: radiophosphorus, radioisotope, labeled phosphorus, isotope, and tracer.

Description of the Study Area

The West Branch of the Sturgeon River is a cold, clear trout stream having its origin at the outlet of Hoffman Lake, Otsego County, Michigan. The stream flows northeast to its confluence with the Sturgeon River near Wolverine, Michigan (Cheboygan County).

Springs supply most of the volume of water of the West Branch. The drainage area of the watershed is approximately 14 acres (Grzenda, op. cit.) and most of it is heavily forested. During the summer of 1958 fluctuations in stream stage from highest to lowest value amounted to only 0.46 foot. The extremes in recorded water temperatures for the entire summer differed by only 13° F. Table I lists the stream stages and water temperatures recorded during the summer of 1958.

Total alkalinity and total hardness of the stream water were both 181 PPM on July 12, 1958. Marl concretions of more than 5 inches diameter were present throughout the stream section studied. There were patches of soft marl as much as 4 feet thick underlying some sand and gravel areas of the stream bottom.

Flora

With few exceptions, aquatic Spermatophytes grew only in backwater areas and included: Hippuris vulgaris, two species of

TABLE I. --Water temperature and stream stage at Station 8 of the West Branch of the Sturgeon River, 1958

Date		Time*	Water Temperature (°F.)	Stream Guage Reading (Feet)	Date		Time*	Water Temperature (°F.)	Stream Guage Reading (Feet)
July	16	8:30 P. M.	57	5.82	August	6	6:57 P. M.	57	5.69
	17	9:25	50	8.81		8	Afternoon	61	5.69
	18	11:00	54	5.80		11	. . .	...	5.76
	19	12:25 P. M.	54	5.79		12	9:18	54	5.77
	20	12:05 P. M.	53	5.82		15	. . .	...	5.74
	21	5:10 P. M.	60	5.76		16	12:10 P. M.	52	5.72
	22	12:35 P. M.	56	5.76		20	2:26 P. M.	58	5.90
	23	2:25 P. M.	60	5.76		21	9:45	53	5.82
	25	. . .	...	5.74		25	8:35	50	5.82
	27	. . .	...	5.73		27	9:25	48	5.78
	29	11:30	56	5.73		28	8:45	53	5.84
	30	11:25	55	5.74		30	11:30 P. M.	57	5.87
	30	1:13	58	. . .	September	1	5:15 P. M.	54	5.85
	31	10:08	52	5.72		2	2:00 P. M.	53	5.81
August	1	10:30	53	5.72		8	1:25 P. M.	54	6.15
	2	1:33 P. M.	60	5.71		10	11:45	50	5.96
	3	10:00	56	5.70		14	3:00 P. M.	57	5.86
	4	11:40	56	5.70		15	1:45 P. M.	55	5.86
	5	11:05	57	5.70		17	3:40 P. M.	51	5.85

*All times are A. M. except for those primed.

. . . indicates no data.

Potamogeton, Vallisneria sp., and Nasturtium officinale. Two species of aquatic moss (Bryophyta) were collected, one of which was Fissidens grandifrons (identification was by Dr. H. T. Darlington, retired, Michigan State University). The mosses grew as mats on logs and rocks a foot or more below the water's surface in swifter moving parts of the stream.

Species of algae lived in the riffles as well as in slow water areas. Chara and Spirogyra were present. Filamentous growths of Spirogyra were occasionally found in quiet backwater pools. Chara grew upon silt deposits throughout the length of the stream. Diatoms grew as brownish-colored encrustments on the solid substrates of the stream.

Fauna

The fish fauna was composed of 4 species: rainbow trout (Salmo gairdnerii), brown trout (S. trutta fario), brook trout (Salvelinus fontinalis) and a muddler (Cottus sp.). One specimen each of the brook stickleback (Eucalia inconstans) and golden shiner (Notemigonus crysoleucas) was collected. These 2 species were not established in the stream and are believed to have originated in Hoffman Lake. Larval lampreys were found in most silted areas. The specimens collected belonged to the genus Ichthyomyzon. The only amphibian observed in the stream was the green frog (Rana clamitans).

Aquatic invertebrates of 5 taxonomic classes were collected. Class Pelecypoda was represented by the sphaeriids (finger-nail clams);

Class Gastropoda by physid snails; Class Oligochaeta by lumbricid and tubificid worms; Class Hirudinea by leeches; and Class Insecta by members of 23 families. Table II presents the complete list of insects found in the stream.

The following 7 invertebrates were collected on each sampling date for measurement of their radioactivity:

Family	Genus-Species	Common Name
Pteronarcidae	Pteronarcys sp.	Stonefly nymph
Simuliidae	Simulium sp.	Black fly larvae
Ephemeridae	Hexagenia limbata	Mayfly nymph
Cordulegasteridae	Cordulegaster sp.	Dragonfly naiad
Rhagionidae	Atherix variegata	Snipefly larvae
Brachycentridae	Brachycentrus sp.	Caddis fly larvae
Physidae	Physa sp.	Pond snail

Other organisms collected for radioactivity assay on a less regular basis included:

Family	Genus-Species	Common Name
Corydalidae	Chauliodes sp.	Fish fly larvae
Petromyzontidae	Ichthyomyzon sp.	Lamprey amnocoete
Glossiphoniidae	Not known	Leech
Lumbricidae	Not known	Aquatic earthworm
Ranidae	Rana clamitans	Green frog

TABLE II. --A listing of the insects found in the West Branch of the Sturgeon River, 1958*

- Coleoptera
  - Elmidae
- Diptera
  - Ceratopogonidae
  - Empididae
  - Ptychopteridae
  - Rhagionidae
    - Atherix variegata
  - Simuliidae
    - Simulium
  - Tendipedidae
  - Tipulidae
- Ephemeroptera
  - Baetidae
  - Ephemeridae
    - Hexagenia limbata
  - Heptageniidae
- Hemiptera
  - Gerridae
- Odonata
  - Cordulegasteridae
    - Cordulegaster
- Megaloptera
  - Corydalidae
    - Chauliodes
- Plecoptera
  - Chloroperlidae
  - Perlidae
  - Pteronarcidae
    - Pteronarcys
- Trichoptera
  - Brachycentridae
    - Brachycentrus
  - Hydropsychidae
  - Hydroptilidae
  - Philopotamidae
  - Psychomyiidae
  - Rhyacophilidae

Sampling Stations

The experimental section of the stream, including the control station for invertebrates, is approximately 3.5 miles long. Human dwellings (summer cottages) occur only at the downstream end of the study area more than 2000 yards below the radioisotope source. Figure 1 is a map of the study area and indicates the position of the sampling stations.

Station 16, the farthest downstream station, was located at the State Highway Park next to US Route 27 (Sec. 33, T. 33 N., R. 3 W.). Station 15 was approximately 1 mile upstream from Station 16. The farthest upstream that sampling was conducted was at Station 1-A at the bridge (Sec. 28, T. 33 N., R. 3 W.) one mile upstream from the point of P^{32} release. This was the control area site for collecting invertebrates.

Station 1 was the control site for sampling plants and fish. It was located 100 yards upstream from the tracer point of application. Station 2 was 200 yards downstream from Station 1 and was used as a site for collecting invertebrates for the P^{32} study. Stations 2 through 15 were spaced apart at regular intervals of 150 yards.

Station 8, near the center of the experimental area, was 1000 yards below the isotope source. Invertebrates were collected here for assay of their radioactivity. Normal stream discharge rate

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*Identifications were based on the taxonomic keys of Pennak (1953), Usinger (1956) and Burks (1953).

Figure 1.--Experimental area and sampling stations. Station 1 was the control station for fish and periphyton. Station 1-A was the control station for invertebrates.

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at this station is 37 to 38 cubic feet per second (USGS measurements). Station 13 was 1850 yards below the P^{32} release point. This was the farthest downstream that invertebrates were collected for radioactivity assay.

METHODS AND PROCEDURES

Radiological Techniques

Adding P^{32} to the Stream

The isotope was added to the stream in a simple but efficient manner. A metal drum of 55 gallons capacity was mounted in a midstream position upon the trunk of a Tamarack tree which was inclined almost horizontally across the stream.

It was decided that the isotope would be mixed with 50 gallons of water in the drum and that this solution would be siphoned into the stream at a rate of 1.3 gallon per minute. This is a rate which would bring about a calculated dilution of P^{32} in the stream less than the maximum concentration (2×10^{-4} microcuries per ml) recommended for drinking water by the International Committee on Radiation Protection. It was found by experimentation that with a water "head" of 13.6 inches, the drum would empty via the siphon at the prescribed rate of 1.3 gallon per minute. The constant "head" was maintained by lowering the output nozzle of the siphon 0.8 inch per minute. This operation was facilitated by driving nails into a board at 0.8 inch intervals. One end of the board was anchored in the stream bottom and the discharge end of the siphon initially rested on the nail which was 13.6 inches below the surface level of the water in the drum. The end of the siphon was lowered 1 nail (0.8 inch) per minute. The siphon was attached to a long bamboo pole which enabled

the operator to move the siphon at a hazard-free distance from the radioactivity. At 2:01 P. M., August 5, the isotope began to enter the stream and the drum was empty 37. 5 minutes later.

Sampling Procedure

Organisms for P^{32} assay were collected by the methods which were most convenient and efficient. Insect larvae of the riffles were collected with a Surber stream-bottom square-foot sampler. The larvae were separated from gravel and other debris by using the sugar-flotation method described by Anderson (1959).

Organisms inhabiting silt were collected by pulling masses of silt, bound together by Chara, onto the stream bank. The mass was then inspected and the desired organisms collected with forceps. Snails were collected from logs and branches in slow-water areas.

With one exception, invertebrates were preserved in the field at the time of collection with 50 percent alcohol. This procedure stopped elimination and excretion processes and thus prevented them from losing isotope from these sources. It is believed that this solution of alcohol did not dissolve out fat and contained P^{32} from the tissues of the organisms.

Preparation for Assay

Invertebrates were weighed and their wet-weight values recorded. Most samples weighed about 1 gram but others weighed as much as 2 grams and still others weighed as little as 0. 1 gram.

The organisms were then digested in nitric acid and subsequently placed in a muffle furnace where combustion of their organic portions took place. Details of the process are outlined by Robeck et al. (1954).

Radioactivity was measured by means of a proportional counter, Model PC-3A (Nuclear Measurements Corporation). A gas mixture consisting of methane and argon (P-10) was used in the counting chamber as an ionization medium for emitted beta particles. Actual measurement of radiophosphorus was in terms of beta particles emitted in a unit time. Beta radiation detected by the instrument was registered on a scaling device as counts per minute.

Two operations were performed each day before the counting of samples began: (1) the counter was checked for operating accuracy by performing a test count of a radium standard and, (2) background was counted for a minimum period of 15 minutes.

Assaying Invertebrate Radioactivity

Each sample was counted for 2 minutes. A longer period of count would have resulted in greater accuracy but the radioactivity of most organisms was of such a magnitude that counting errors for 2 minute counts were not significant. Limiting the counts to 2 minutes made it possible to process more samples than could have been achieved with longer periods of counts.

Several correction factors were applied to each sample count to give it meaning. First, the sample radioactivity due to contained

isotope was established by subtracting the background count. Background for this instrument ranged from 47 to 54 counts per minute (C. P. M.) with an average of 50 C. P. M. The adjusted count minus background was divided by the number of minutes that the sample was counted. This established the count on a per minute basis. The count was next put on a per gram basis by dividing it by the wet-weight of the sample organisms. Finally a correction factor for radioactive decay of the P^{32} was applied to the count.

There are other correction factors to apply to counts to give them full meaning. These factors include corrections for the geometry of the counting chamber, for backscattering, and for self absorption. These corrections were not applied to the counts since it would add nothing to the interpretations and comparisons made in the thesis. Borgeson (op. cit.) and Clifford (op. cit.) also did not correct counts by these factors and the radioactivity data in the 3 theses can be directly compared with each other.

In the text, all counts are per minute, per gram of organism and are corrected for background and decay. The concentration of P^{32} in organisms, expressed as counts per minute per gram, is referred to as "activity" in the text.

Biomass Estimate Technique

Benthic organisms were quantitatively sampled with a Surber square-foot sampler. The organisms were not removed from

the debris in the field. Instead, the debris, with contained invertebrates was placed in wide-mouth pint jars. Enough formalin was added to cover the detritus. The samples were shipped to the laboratory of the Institute for Fisheries Research at Ann Arbor, Michigan. Here trained personnel removed the organisms. Each sample of bottom fauna was placed in an individual vial of ethyl alcohol. The vials were then sent to Michigan State University where the organisms were weighed.

The organisms were initially weighed after having been prepared and centrifuged by a procedure used by Welch (1959). The weights of centrifuged, preserved organisms were converted to their live weight values in order to be comparable to the live weight measurements of the fish.

The weight of preserved invertebrates that had been centrifuged was found to be approximately 35 percent of their weights as determined by volumetric displacement of alcohol.

$$\left(\begin{matrix}\text{Weight of the centrifuged} \\ \text{preserved organisms}\end{matrix}\right)\left(\frac{1}{.35}\right) = \begin{matrix}\text{Weight of the organisms} \\ \text{by volumetric displacement}\end{matrix}$$

Live weight of the organisms.

Samples of the fish population were collected by electrofishing. A portable, 220 volt, DC generator mounted in a small flat-bottomed skiff furnished the electric power.

On August 21 and 25, 1958, fish were captured, their lengths and weights recorded, marked by distinctive fin clip and then

released. On August 28, fish were again collected and the total number of fish in the collection recorded as well as the number of marked recaptures. The trout population estimate was calculated by the Petersen method:

$$\frac{\text{Number of marked fish recaptured (R)}}{\text{Total number of fish captured (C)}} = \frac{\text{Total number of marked fish (N)}}{\text{Total fish population (P)}}$$

or

$$P = \frac{CN}{R}.$$

The population of the muddlers was not estimated by the Petersen method because it was difficult to clip their small, folded fins. It was even more difficult to recognize the fin clips of muddlers when they were recaptured in later sampling. It was decided that the population estimate of the muddlers would be based on the population of brown trout:

$$\text{Estimated population of muddlers} = \frac{\text{(Total number of muddlers caught)}}{\text{(Total number of brown trout caught)}} = \text{Population estimate of brown trout}$$

The section of stream in which the biomass was estimated was from Station 8 upstream to the point of P^{32} release. The average width of the stream in this section was determined on the basis of 51 measurements of its width at random intervals. The length of the stream was measured with a 100-foot steel tape. The area was then computed.

The biomass of invertebrates was calculated by multiplying the average weight of organisms in square-foot bottom samples by the

area of the stream in square feet. The product was converted to pounds of invertebrates per acre. The biomass of the fish was computed by multiplying the average weight by the estimated population.

RESULTS AND DISCUSSION

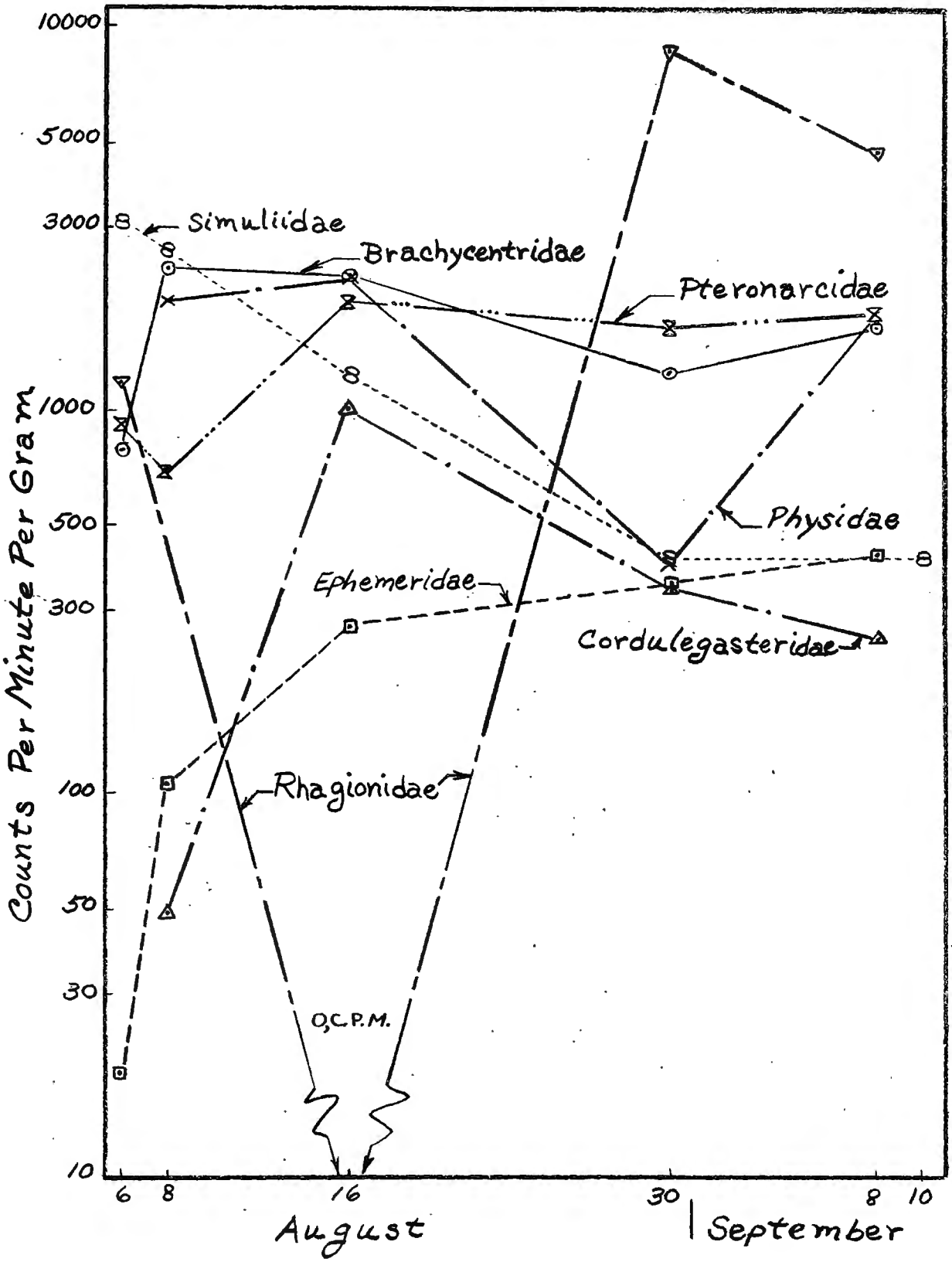
Factors Affecting Biological Uptake of P^{32}

The uptake of P^{32} was in part governed by physical factors. There were changes in the activity of organisms that were attributable to increasing distances downstream from the point of isotope release and there were changes with the passage of time.

Activity of invertebrates at Station 8 was of the same order of magnitude as those at Station 2. Station 8 was 900 yards downstream from Station 2. This finding may be explained by the manner in which the isotope entered the stream. The entire supply of radiophosphorus was introduced into the stream in a 37-minute period. Releasing the P^{32} in this period of time resulted in its dilution as it proceeded downstream. The stream biota was exposed to P^{32} for longer periods but at decreasing concentrations as it flowed downstream. The radioactivity of the water was sustained for approximately twice as long a period at Station 11 as at Station 2 but average activity of water was 4 times greater at Station 2 than at Station 11. Krumholz et al. (1957) have noted that chronic exposure of aquatic organisms to low concentrations of radiomaterials usually resulted in accumulation of greater amounts than from an acute exposure for a short period.

There is evidence that the tracer was not completely mixed in the water mass by the time it arrived at Station 2. Certain areas may have been exposed to concentrations of P^{32} lower than calculated.

Figure 2. --Activity of 7 invertebrates at Station 2. Activity of Cordulegasteridae and Physidae was zero on August 6. Counts are corrected for background and decay.


10000
5000
3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
Simuliidae
Brachycentridae
Pteronarcidae
Physidae
Ephemeridae
Cordulegasteridae
Rhagionidae
O.C.P.M.
6
8
16
30
8
10
August
September

If this is correct, other nearby areas received amounts higher than the calculated concentration. Fluctuations in activity of invertebrates decreased at Station 2 after August 16 as Figure 2 indicates. Mobile organisms were presumed to have moved into adjoining areas of greater or lesser isotopic exposure.

By August 30, most invertebrates were losing activity at a faster rate at Station 8 than at Station 2. There was more streamside vegetation shading the stream at Station 2 than at Station 8. Chara and other aquatic plants were more plentiful at Station 8. The greater available light is presumed to bring about a higher rate of production and growth rate. The organisms of a community dilute a given amount of P^{32} in proportion to their rate of cell division. Rice and Willis (1959) refer to this as "biological dilution."

Throughout the period of the experiment, invertebrates at Station 13 had less activity than those at upstream stations. Nevertheless, in more than half the samples at Station 13, activities of invertebrates were 30 percent as high as at Station 8. Station 13 was approximately twice as far from the point of P^{32} release as Station 8.

With the passage of time, the difference in radioactivity of black fly larvae at the several stations became less and less. The rate of decline in activity of Simuliidae at Station 13 was less than it was for the larvae at upstream stations. This suggests that there was more radioactive drift material upon which to feed, at Station 13 than at upstream stations. Filter-feeders such as the simuliids, retained P^{32}

in a given area by feeding on radioactive drift material. In proceeding upstream from any given station towards the point of isotope entry. the total area of stream exposed to P^{32} decreased. The biota had a correspondingly reduced source from which to receive tracer replenishment.

Digestive tracts of the Ephemerid nymph, Hexagenia limbata, and the aquatic earthworms (Lumbricidae) collected in this area were full of mud. These 2 organisms (and leeches) had the lowest activities of all organisms sampled which indicated that the activity of bottom mud was low. Clifford (op. cit.) concluded that activity of marl concretions of the West Branch was less than that of periphyton. The high activity of the biota compared with the low activity of stream bottom deposits contrasts with the results reported for a fresh water lake by Hayes and Coffin (1951) who found that when P^{32} was released 3 feet from the bottom of a lake the activity of the bottom mud reached high values and most of the tracer was removed by it.

Contributing to the amount of accumulation of P^{32} by the biota was the low level of natural phosphorus concentration in the West Branch to be 7 parts per billion. Uptake of P^{32} is likely to be much greater in a nutrient-poor environment than in a nutrient-rich one (Odum, 1959).

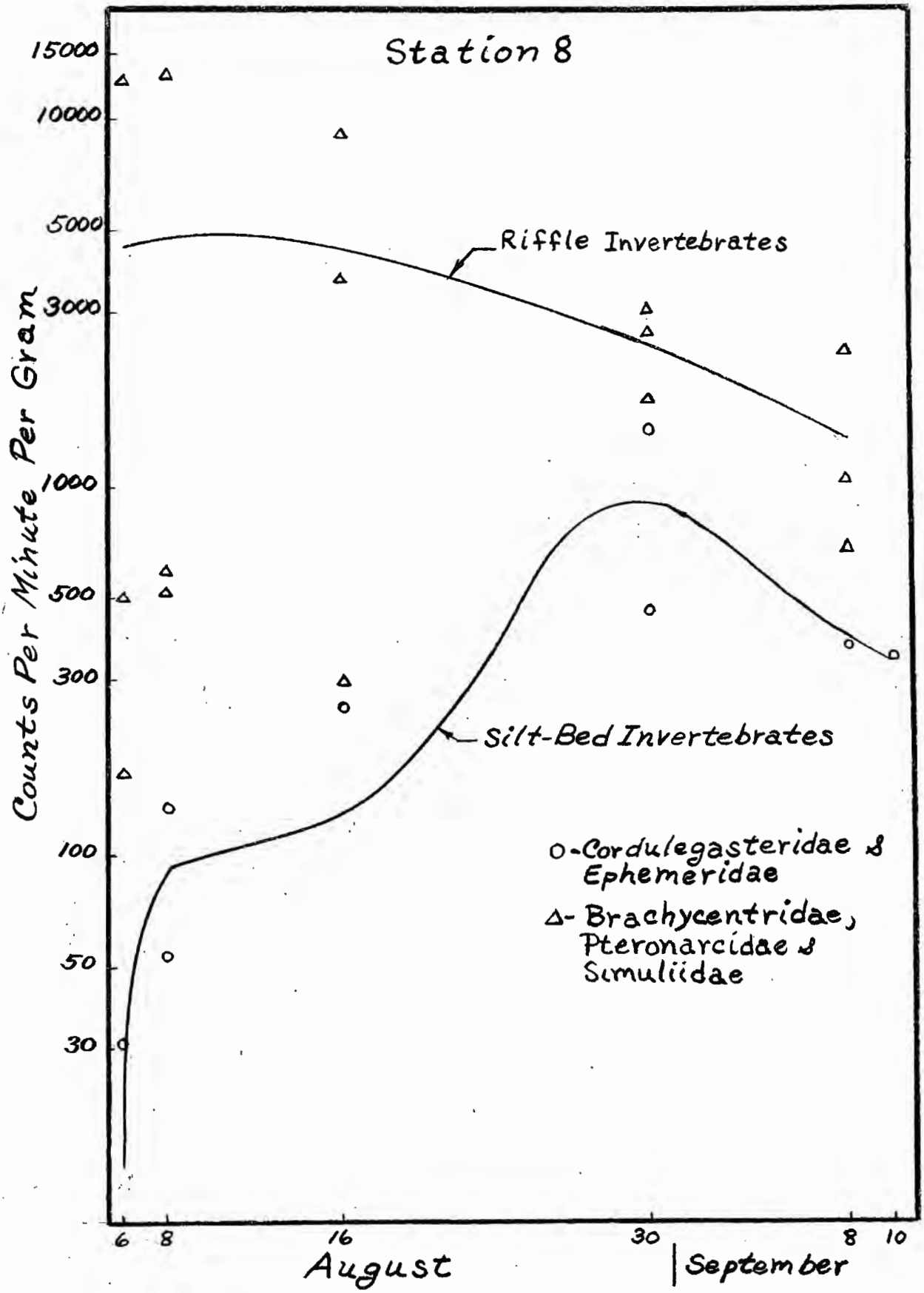
Method of Uptake of P^{32} by the Invertebrates

There are only two known methods for P^{32} to gain entrance to animal tissue: (1) absorption through the gut wall, (2) absorption across skin and gill surfaces. There was no evidence of cutaneous absorption of tracer by invertebrates of the West Branch. Robeck et al. (op. cit.) found no evidence of invertebrates absorbing isotope through their integuments in Columbia River studies. One day after the isotope was released, there was no activity in cordulegasterid naiads from any of the sampling stations. Radioactivity attributable to cutaneous absorption would have been detected in invertebrates in this initial collection day. Nymphs of Cordulegasteridae typically lie covered with sand or silt, with only the tips of their eyes and respiratory aperatures exposed to the water (Walker, 1958). In this habitat, it is probable that they were not in contact with P^{32} in the water mass. But Ephemeridae, Physidae, and Rhagionidae also had no activity on the same day at one or more stations. Physid snails live in an exposed habitat where cutaneous exposure to the isotope was probable.

Uptake of P^{32} by Invertebrates of Different Habitats

The type of food available to an invertebrate is dependent on its habitat. Figure 3 indicates that invertebrates in different habitats fed on material of different activity. Invertebrates living in silt beds had lower activity throughout the study than those inhabiting riffles.

Figure 3. -- Comparison of activity of the riffle invertebrates, Brachycentridae, Pteronarcidae, and Simuliidae with activity of the silt-bed invertebrates, Cordulegasteridae and Ephemeridae. Activity was zero in Cordulegasteridae on August 6 and 16. Counts are corrected for background and decay.


Station 8
15000
10000
5000
3000
1000
500
300
100
50
30
Counts Per Minute Per Gram
Riffle Invertebrates
Silt-Bed Invertebrates
o-Cordulegasteridae & Ephemeridae
Δ- Brachycentridae, Pteronarcidae & Simuliidae
6
8
16
30
8
10
August
September

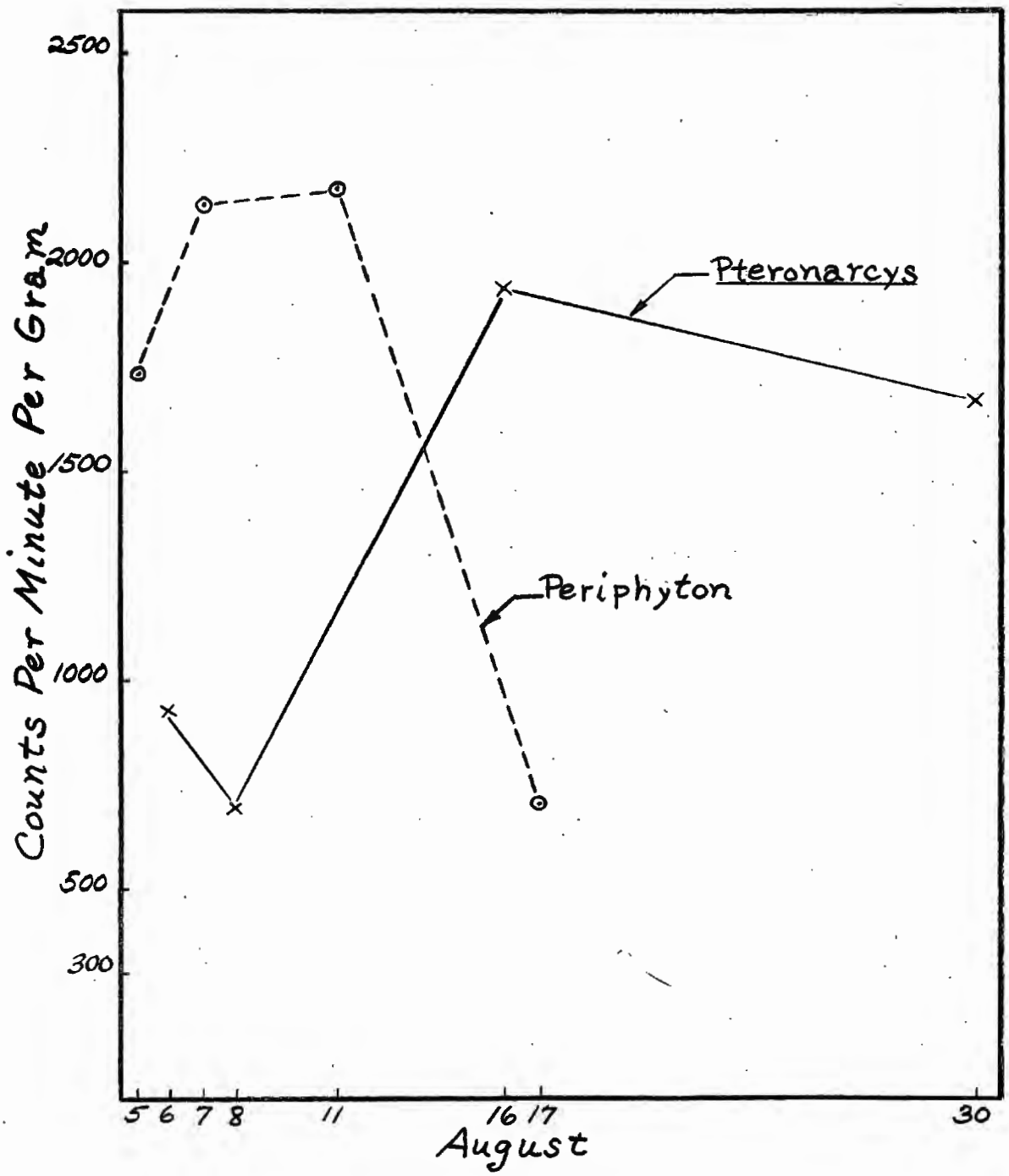
Invertebrates were collected from silt deposits supporting growths of Chara and aquatic spermatophytes. No difference in activity level was noted between these invertebrates and those living in silt beds without vegetation. Hexagenia limbata, which was collected in silt deposits, is presumed to not feed on either Chara or the epiphytic algae growing on their stems. The activity of Chara was greater than that of organisms living in silt which supported Chara beds.

Mayfly nymphs feed largely on dead vegetable substance (Ward and Whipple, 1918). The mayfly nymph, H. limbata appears to be a mud-eater, deriving nourishment from contained organic material (Hunt, 1953). Morgan (1913) found diatoms to be a principal food for the genus Hexagenia. In the West Branch, diatoms are the major source of primary production in riffle areas. This algal material, when washed off substrates and deposited downstream in silt beds, becomes available as food for Hexagenia. Diatom detritus devoured by these nymphs is presumed to have lost most of its activity during organic decomposition. The breakdown of protoplasmic components releases some phosphorus in the soluble state. Clarke (1954) states, " . . . fats break down into these materials [carbon dioxide and water] and also release phosphate."

Pteronarcid nymphs belong to the group of stoneflies which are herbivorous, feeding on algae and vegetable debris (Pennak, op. cit.). Microscopical examination of one of these larvae revealed that the gut contents were composed entirely of diatoms. Figure 4 compares the radioactivity of periphyton with that of Pteronarcys from the same riffle.

28

Figure 4. --Activity of periphyton and *Pteronarcys* at Station 2. Counts are corrected for background and decay.


2500
2000
1500
1000
500
300
Counts Per Minute Per Gram
Pteronarcys
Periphyton
5 6 7 8 11 16 17 30
August

By August 18, activity of Pteronarcys had surpassed that of periphyton at Station 2. At Station 2, this riffle inhabitant attained activity as much as 49 times that of the silt-inhabitant, Hexagenia.

Black fly larvae, Simulium, had the highest activity found in either plants or animals. Three days after release of the isotope, activity of these riffle organisms at Station 13 was more than 7 times that of periphyton at the same station. Diatoms were the main component of periphyton in the West Branch and were believed to be the chief food of the simuliids. In the Maple River of northern Michigan, Simulium feed almost entirely on diatoms (Wu, 1930). It is presumed that a high rate of food intake accounted for the high activity of these larvae. Their need for food for metabolic processes is believed to be high since their growth rate is such that the larval stage lasts but 2 to 6 weeks (Pennak, op. cit.). Wu, (op. cit.) found that most specimens of S. vittatum complete their entire larval stage in 13 to 17 days.

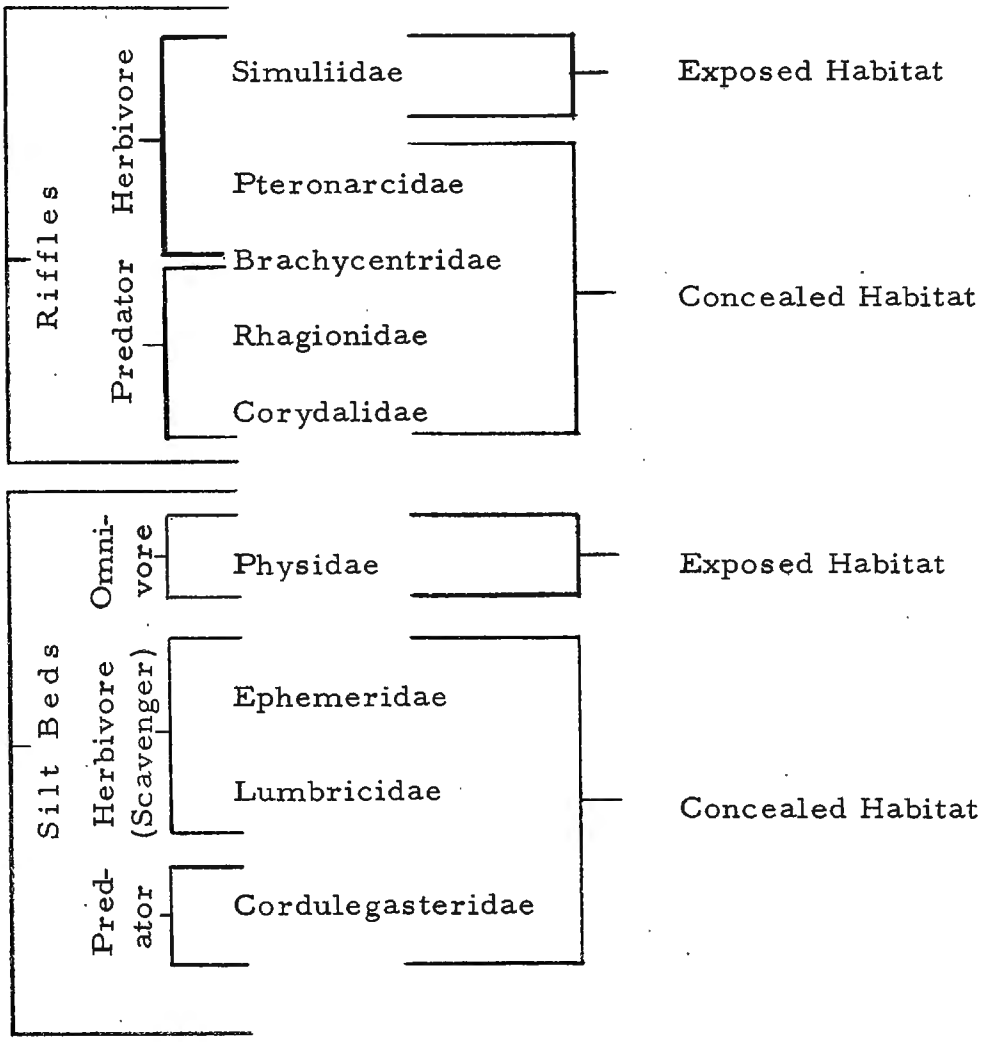
It is possible that Simulium also fed on bacteria which are known to have the greatest powers to concentrate P^{32} of all fresh-water organisms. They may concentrate an isotope over one million times that found in the surrounding environment (Krumholz and Foster, op. cit.).

Some adsorption of P^{32} is also possible. The viscous silken threads secreted by simuliid larvae as a hold-fast mechanism may entangle particulate radiophosphorus on its surface. Rice and Willis (op. cit.) reported that part of the radioactivity of certain marine algae was caused by the adsorption of a particular radionuclide onto their viscous cell walls.

At Stations 8 and 13, simuliids attained peak activity 3 days instead of 1 day after P^{32} was released. Water samples collected following the day on which the P^{32} was released showed no activity above background. This suggests that this filter-feeder was extracting radioactive drift material from the water in amounts undetectable in 500 cc samples.

Activity of Invertebrates Representing Different Trophic Levels

Both primary and secondary consumer invertebrates were sampled. The diagram indicates where they were collected and their position in the food chain.



At Station 2, Pteronarcys, a primary consumer had about one-half the activity of periphyton one day following release of the isotope. The activity of Pteronarcys collected 10 days later was greater than one of its foods, periphyton. However, it did not at any time equal the maximum activity of periphyton. Figure 5 compares activities of invertebrates which feed entirely or partly on plant material. Simulium and Pteronarcys are primarily herbivorous. Physa is essentially omnivorous and like Pteronarcys feeds on periphyton. Physa is also a scavenger, feeding on decaying plant and animal matter (Pennak, op. cit.). It obtains additional nutrition by eating its own mucus secretions which entrap microscopic plants and animals (Dawson, 1911). Hexagenia limbata is herbivorous but is primarily a scavenger deriving nutrition from decaying plant material.

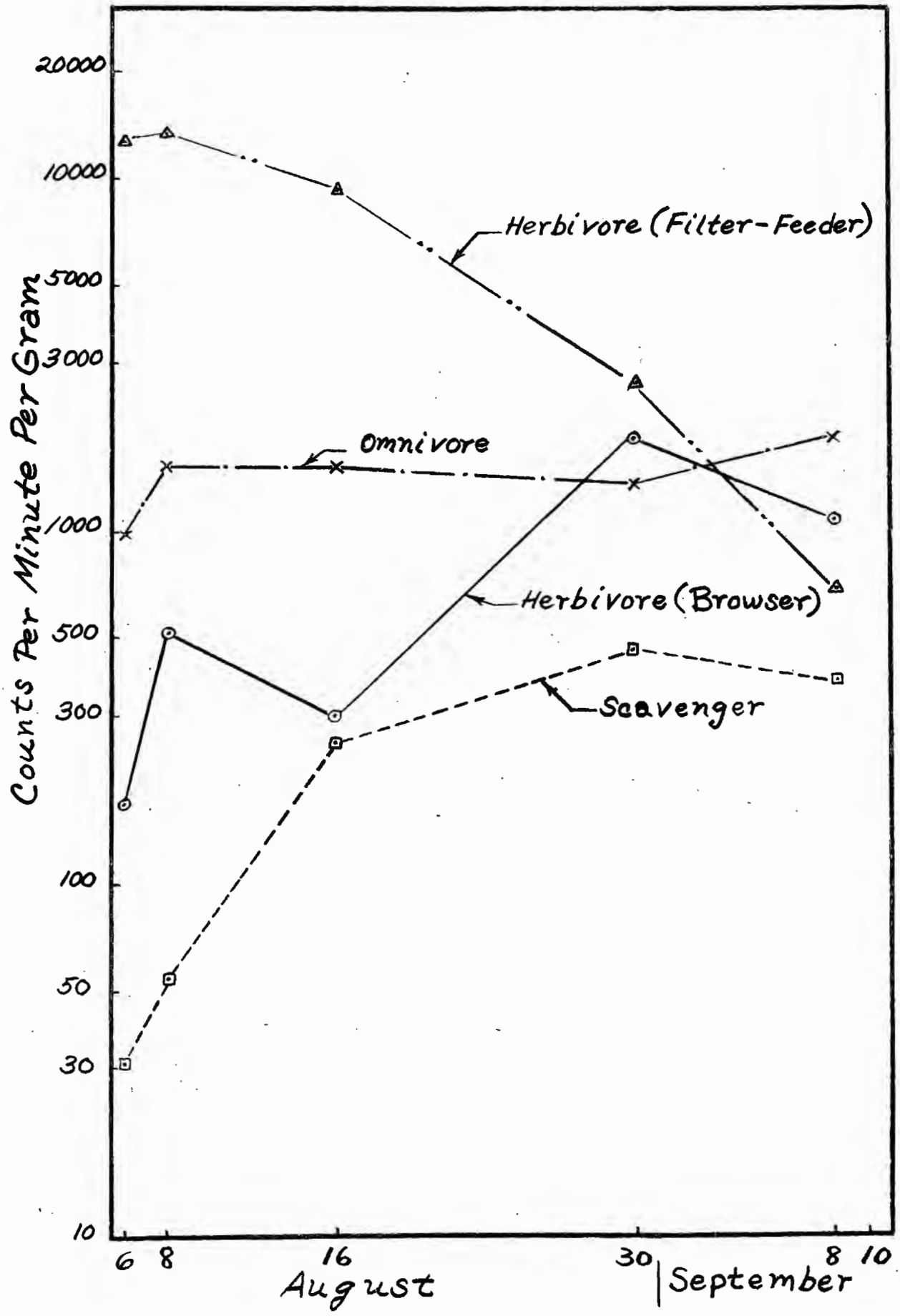
Figure 6 shows that prior to September, activity of a filter-feeding herbivore was higher than that of an omnivore and a scavenger. The omnivore had the next highest activity and fed on material eaten by the herbivore and material eaten by the scavenger.

Predaceous insect larvae included Cordulegaster, Atherix variegata and Chauliodes. After 6 weeks of larval development, Brachycentrus changes from an entirely phytophagous habit to a mainly carnivorous one (Murphy, 1919).

The snipe fly larvae, A. variegata, feeds on soft-bodied insects and other organisms (Clausen, 1940). Activity of this predator and of the herbivorous Simulium and Pteronarcys is shown in Figure 6.

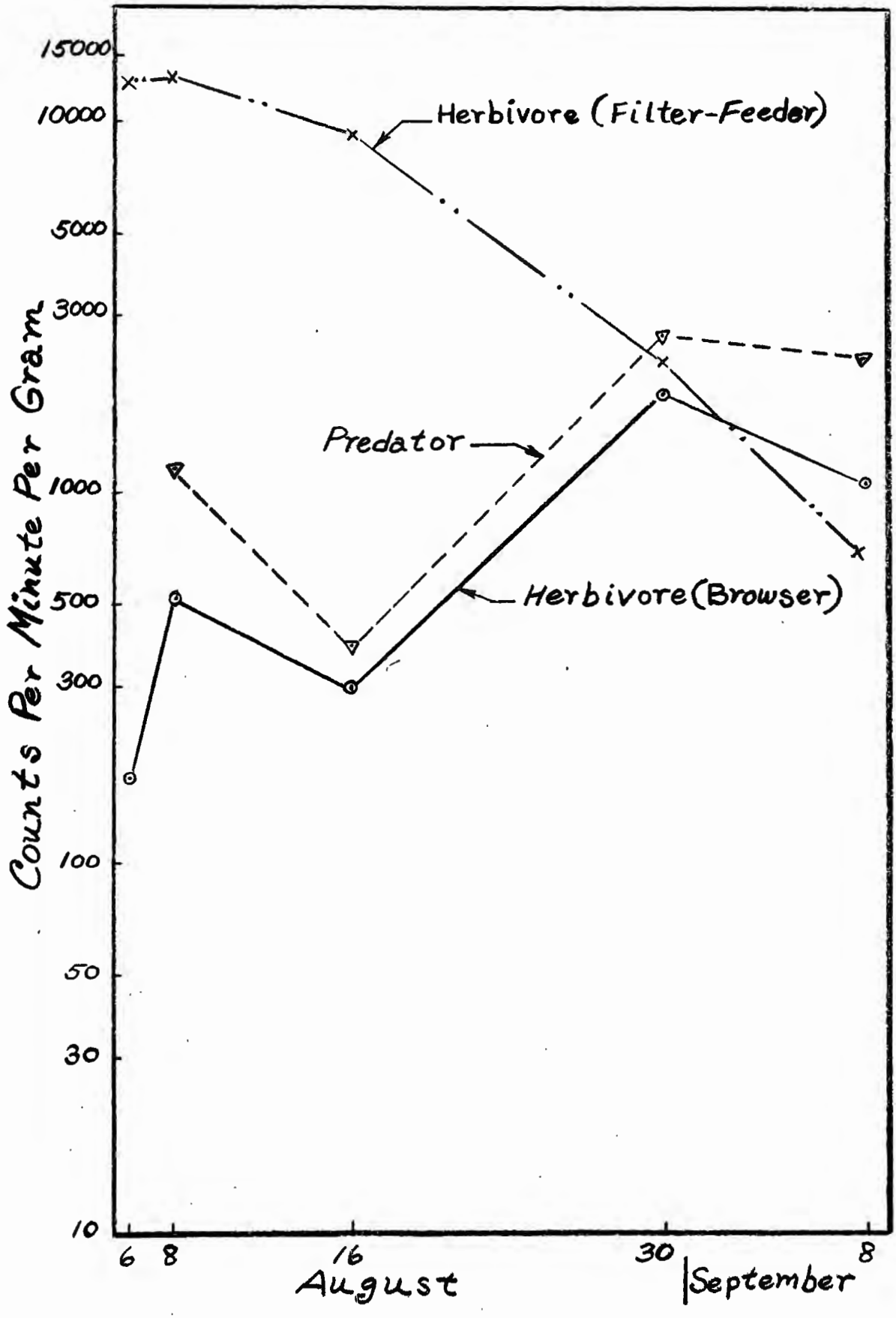
33

Figure 5. --Activity of an omnivore, Physa, and of the herbivores, Simulium (a filter-feeder), Pteronarcys (a browser) and Hexagenia (a scavenger) at Station 8. Counts are corrected for background and decay.


20000
10000
5000
3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
Herbivore (Filter-Feeder)
Omnivore
Herbivore (Browser)
Scavenger
6
8
16
30
8
10
August
September

35

Figure 6. --Activity of a predator, Atherix, and of the herbivores, Simulium (a filter-feeder), and Pteronarcys (a browser). Activity was zero in Atherix on August 6. Counts are corrected for background and decay.


15000
10000
5000
3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
Herbivore (Filter-Feeder)
Predator
Herbivore (Browser)
6
8
16
30
August
September
8

Whether Atherix did or did not prey on these particular species is not known, but they all lived in the same riffle habitat. Pteronarcys had one-half to three-fourths as much activity as Atherix on all sampling dates. Increases in activity of both organisms occurred at the same time and they both lost activity at the same time. The filter-feeding herbivore, the black fly larvae, had greater maximum activity than the predaceous snipe fly larvae, but had the least activity of the 3 species on September 8.

Differences in P^{32} Uptake by Invertebrates of Different Families

The concentration of radiophosphorus was different in invertebrates of different taxonomic families. Table III summarizes the activities of all invertebrates and of the frogs and lampreys. Figures 7 and 8 show these differences in activity of invertebrates of different families. The previous discussion of food, feeding habits, and growth rates help explain the basis for these interfamily differences in activity of organisms.

Variation in P^{32} Uptake by Invertebrates of the Same Genus

Individuals within a genus differed from each other in activity. Factors known to have contributed to this variation were changes in environment and in time. Some variation in activity was expected due to chance.

TABLE III. --P^{32} concentrations in bottom fauna expressed as counts per minute per gram, corrected for background and decay

Date (1958)	August 6				August 8				August 16			
Stations	2	8	13	Control	2	8	13	Control	2	8	13	Control
Invertebrates												
Brachycentridae	800	514	3900	0	2375	1600	203	0	2288	3758	767	...
Cordulegasteridae	0	0	0	30	49	138	0	...	1017	0	430	...
Ephemeridae	19	31	0	0	108	54	0	0	277	254	74	0
Physidae	0	978	344	15	1931	1506	690	...	2261	1512	767	0
Pteronarcidae	928	169	38	5	700	523	185	11	1939	300	565	0
Rhagionidae	1200	0	162	...	...	1181	...	0	0	386	2386	...
Simuliidae	3167	12933	1667	0	2660	13200	8273	...	1255	9225	4200	...
Other Organisms												
Glossiphoniidae	...	...	...	...	...	...	0	0	...	...	0	...
Lumbricidae	...	...	...	...	...	...	...	...	...	0	67	...
Petromyzontidae	...	...	...	...	0	0	...	0	...	...	...	...
Physidae												
Eggs	...	...	...	...	...	...	...	...	42	1438	512	0
Feces	...	...	...	...	...	...	...	...	2950	5333	...	...
Flesh	...	...	...	...	...	...	...	...	5690	2973	1164	...
Shells	...	...	...	...	...	...	...	...	1443	995	275	...
Ranidae												
Small adult	...	...	...	...	...	...	...	...	59	22	...	0
Adult	...	...	...	...	...	...	...	...	...	...	16	...
Tadpole	...	...	...	...	...	...	...	...	46	...	...	...

TABLE III. --Continued

Date (1958)	August 30			September 30			September 10			
Stations	2	8	13	2	8	13	2	8	13	Control
Invertebrates										
Brachycentridae	1259	3086	1964	1675	2379	1639	...	...	...	...
Cordulegasteridae	345	1450	...	255	...	...	55	348	73	15
Ephemeridae	356	468	397	425	378	...	...	...	448	...
Physidae	394	1372	655	1776	1825	841	...	...	...	...
Pteronarcidae	1669	1858	844	1795	1077	279	...	...	...	6
Rhagionidae	8743	2685	877	4779	2305	1345	...	...	...	...
Simuliidae	410	2661	1920	...	685	808	415	...	...	...
Other Organisms										
Corydalidae	651	850	178	399	462	771	...	...	...	57
Physidae										
Feces	587	1167	1150	...	...	...	...	...	...	...
Feces[1]	...	...	...	380	150	0	...	...	...	...
Flesh	2252	3290	...	3170	2444	...	...	...	1294	...
Flesh[2]	...	...	...	3115	2766	1557	...	...	...	...
Shells	0	84	...	0	111	0	...	...	...	...
Shells[2]	...	...	...	0	152	438	...	...	...	...
Preserved	...	...	906	...	...	...	...	...	...	...
Ranidae										
Small adult	64	22	...	...	24	80	...	...	...	...
Adult	...	...	34	...	...	...	...	...	...	...

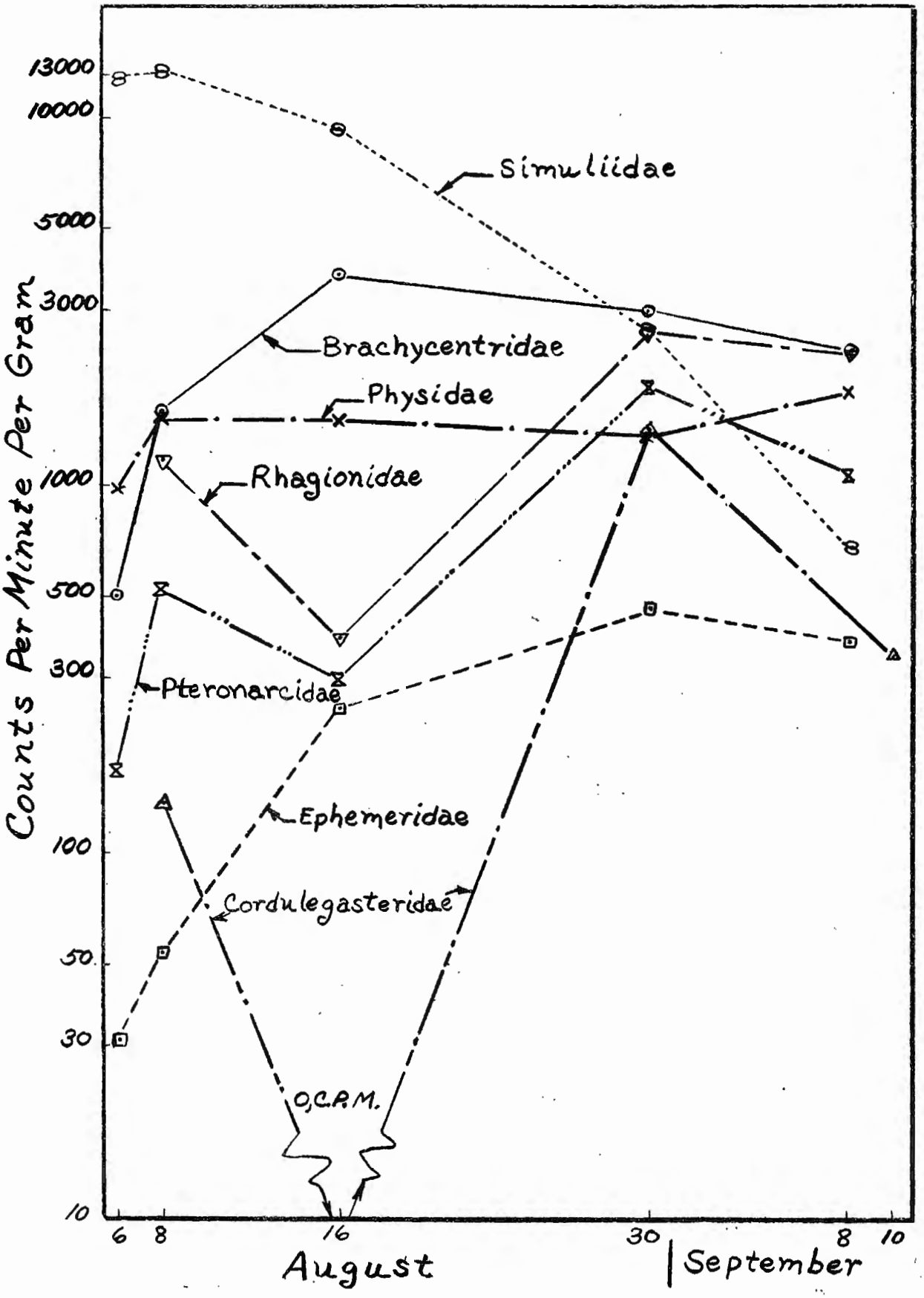
[1] Accumulated from September 8 to September 9, 8:00 P. M.

[2] Alive until September 9, 8:00 P. M.

. . . indicates no data.

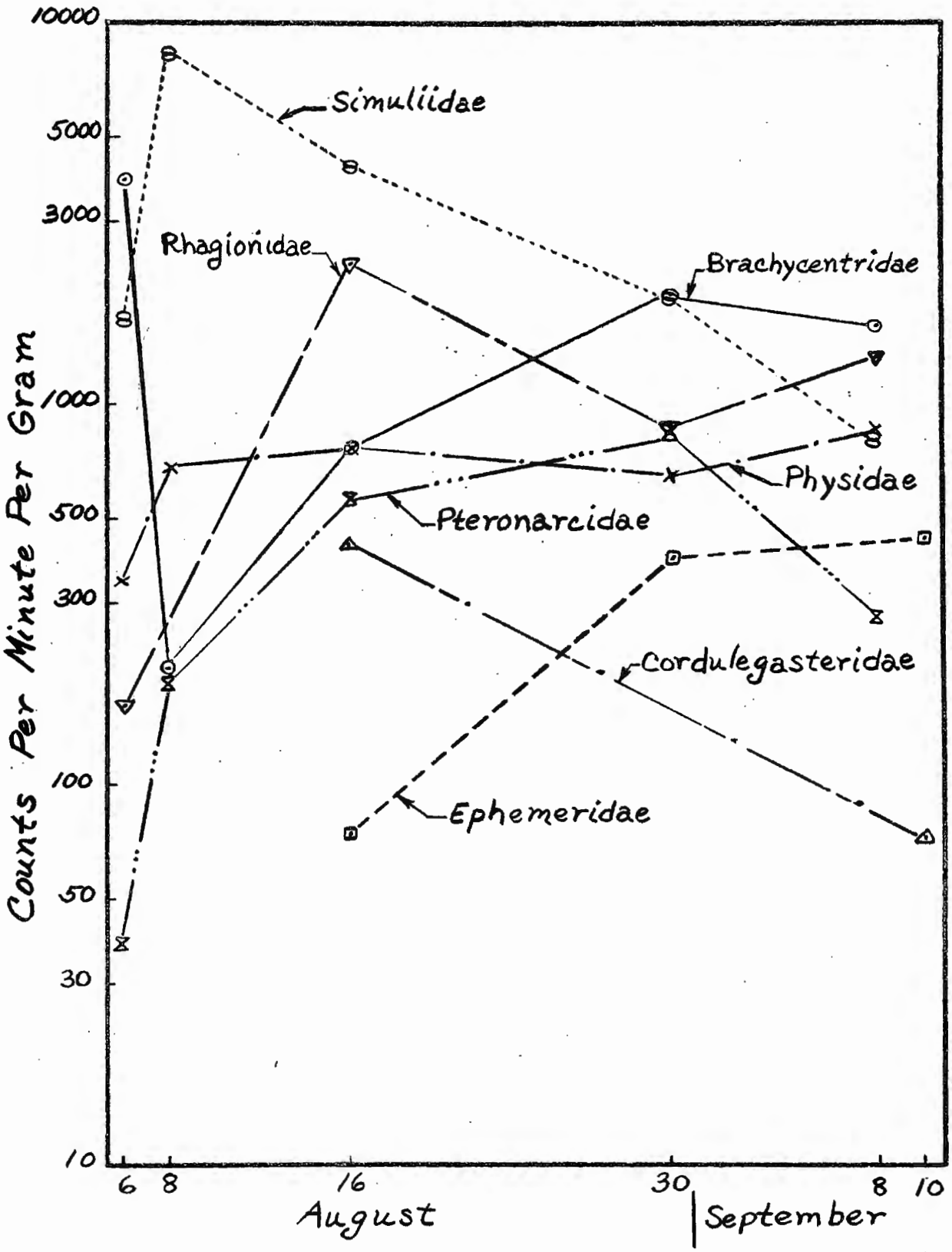
40

Figure 7. --Activity of 7 invertebrates at Station 8. Activity was zero in Cordulegasteridae and Rhagionidae on August 6. Counts are corrected for background and decay.


13000
10000
5000
3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
Simuliidae
Brachycentridae
Physidae
Rhagionidae
Pteronarcidae
Ephemeridae
Cordulegasteridae
O.C.A.M.
6
8
16
30
8
10
August
September

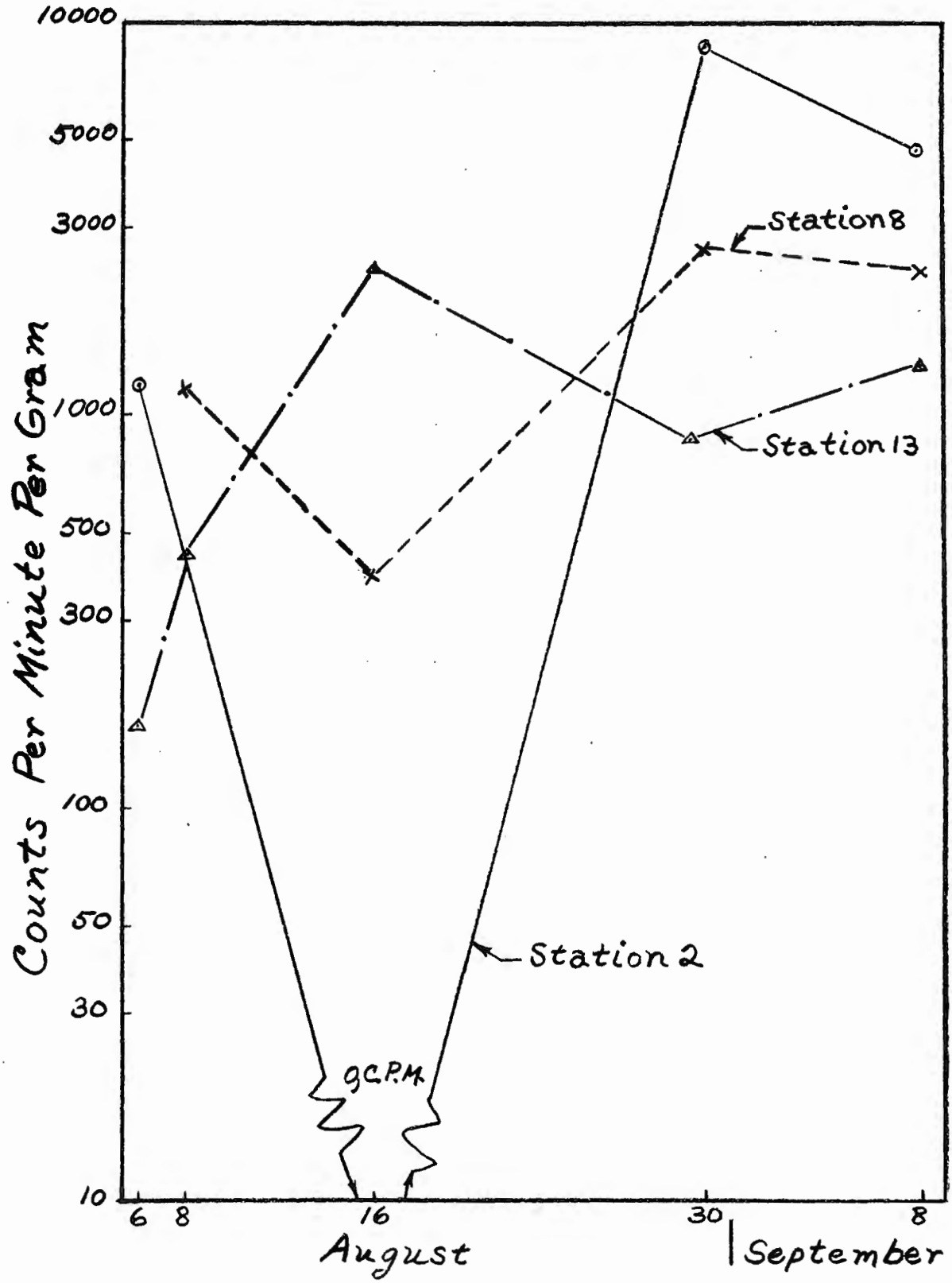
42

Figure 8. --Activity of 7 invertebrates at Station 13. Activity was zero in Cordulegasteridae and Ephemeridae on August 6 and 8. Counts are corrected for background and decay.


10000
5000
3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
Simuliidae
Rhagionidae
Brachycentridae
Physidae
Pteronarcidae
Cordulegasteridae
Ephemeridae
6
8
16
30
8
10
August
September

44

Figure 9. --Activity of the larvae of the snipe fly, Atherix variegata. This organism is predaceous on soft-bodied invertebrates. Activity was zero at Station 13 on August 6. Counts are corrected for background and decay.


10000
5000
3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
Station 8
Station 13
Station 2
G.C.P.M.
6
8
16
30
8
August
September

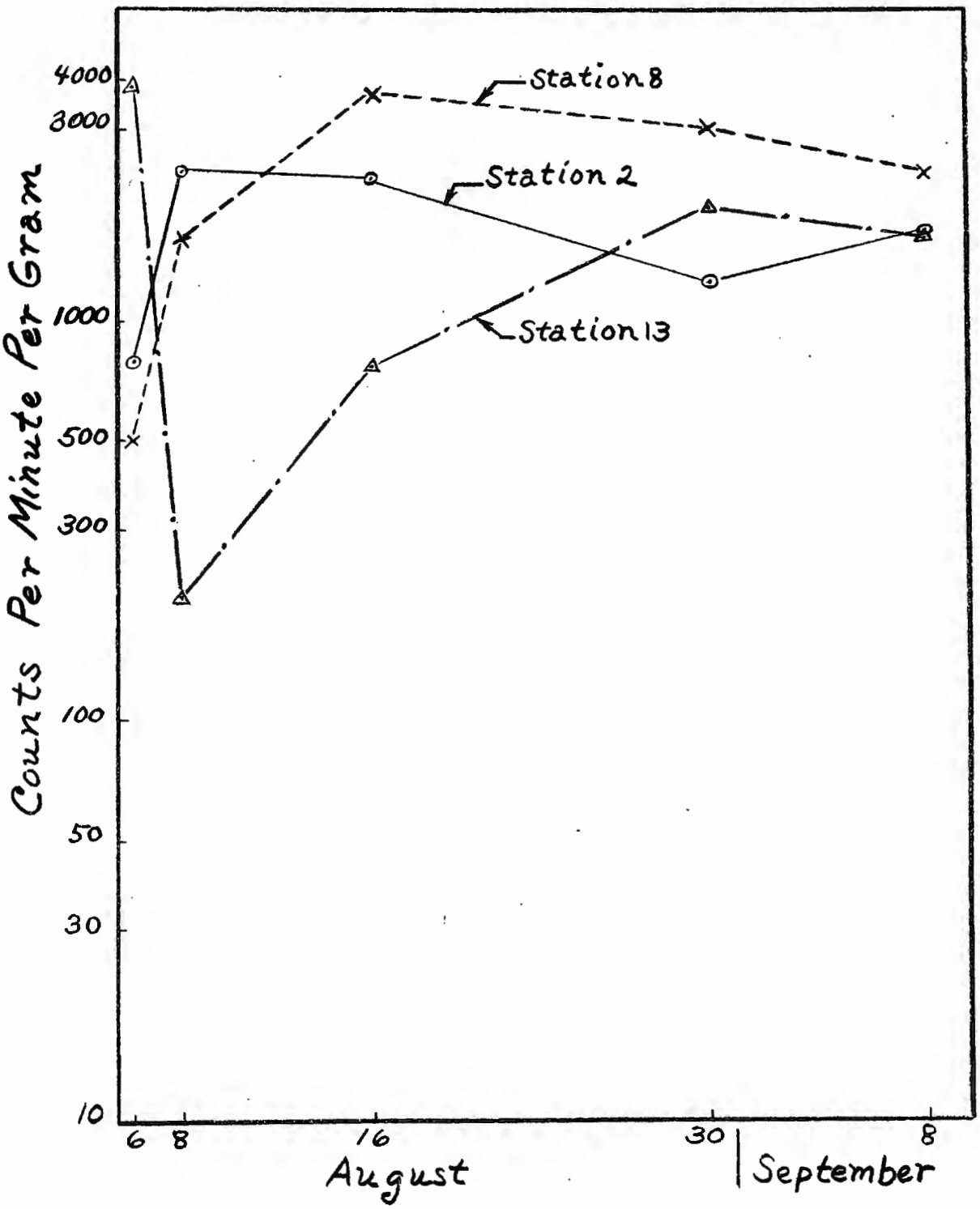
Atherix variegata. --Figure 9 shows the activity of the snipe fly larvae (A. variegata). These larvae, lacking the anchoring support of true legs, are subject to much downstream transport when exposed to the force of the current. The activity of these larvae was more variable than that of any other invertebrate genus. This could have been due to variations in the P^{32} content of their food. Their diet includes different species of invertebrates which are presumed to have differed from each other in activity.

The lack of activity of the Station 2 larvae on August 16 could have been due to: (1) the larvae being washed down from above the point in the stream where the isotope was released (100 yards upstream) or (2) their prey being washed down from above the area of isotopic exposure.

Brachycentrus. --The activity of the larvae of the caddis fly, Brachycentrus, is shown in Figure 10. It is believed to not be representative of the population of Brachycentrus at Station 13 that they had maximum activity one day after release of the P^{32}. On that day only one larva was collected from that station. On all other occasions samples consisted of at least 6 larvae. This single specimen is presumed to have captured and eaten drift material containing more radioactivity than was available to most of the larvae. These organisms were observed to depend on the current to bring them food which they grasped with their legs. They may filter-feed as well. Murphy (op. cit.) reports, "the spines on the femur of the meso--and metathoracic legs . . . may serve as plankton sieves."

47

Figure 10. --Activity of the larvae of the caddis fly, Brachycentrus. This organism feeds primarily on drift organisms. Counts are corrected for background and decay.

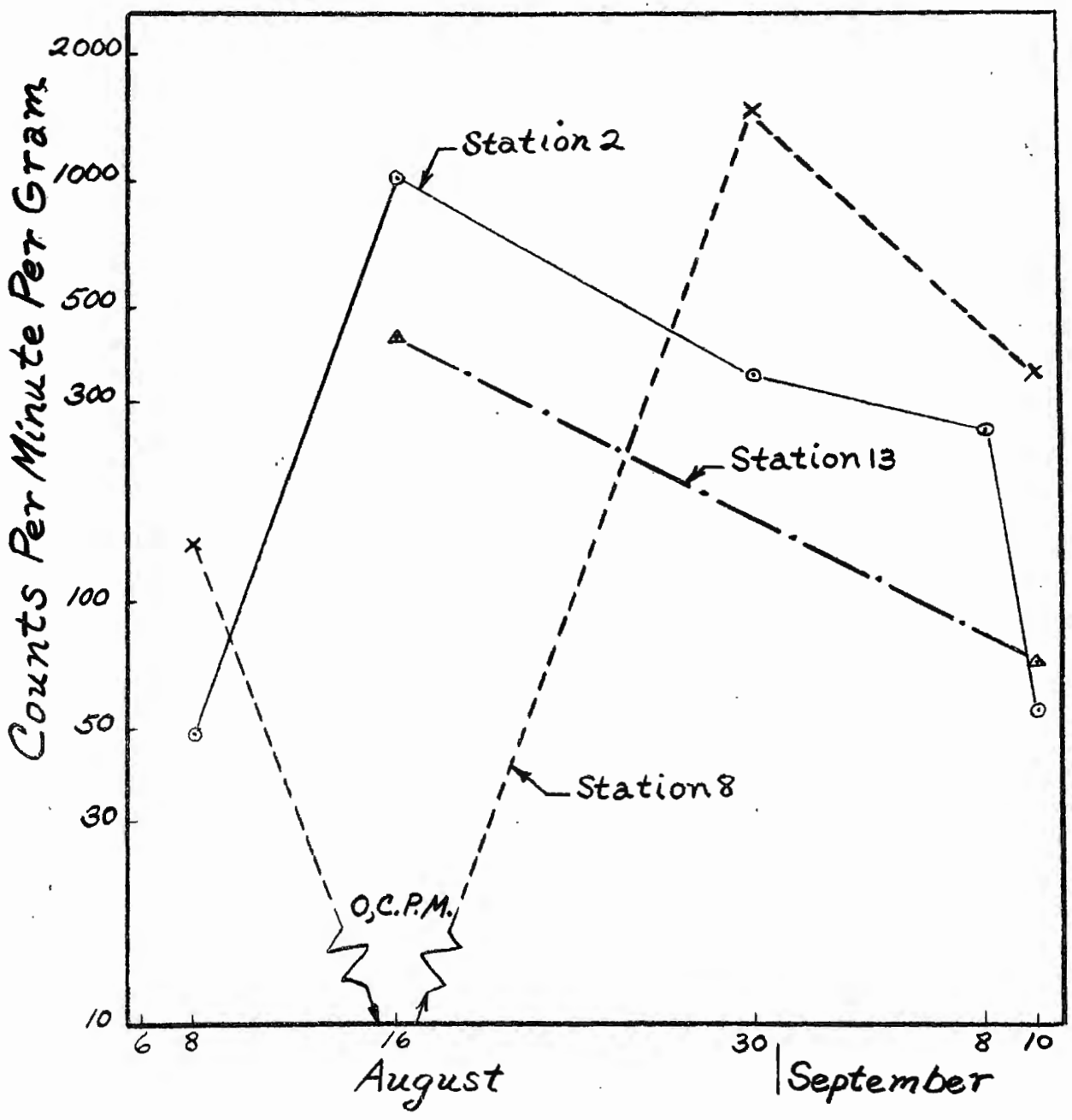

Station 8
Station 2
Station 13
4000
3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
6
8
16
30
8
August
September

Cordulegaster. --Figure 11 indicates that naiads of the dragonfly, Cordulegaster, differed from each other in activity similarly to that of A. variegata. On most occasions only one Cordulegaster naiad was collected at each station. Consequently, the variation shown is that between individuals. No radiophosphorus was found in any of the specimens collected on August 6 nor in the Station 13 specimen collected on August 8. There are two possible reasons for this lack of activity: (1) the naiad may have fed on organisms such as lumbricid oligochaetes which contained little or no P^{32}, or (2) the naiad may have not fed since the tracer was released. These organisms have been known to remain motionless for weeks. They depend on their prey to venture close enough to be seized by the prehensile labium (Needham and Heywood, 1929).

Hexagenia limbata. --The activity of the mayfly nymph, H. limbata, is shown in Figure 12. At the end of the study differences in the activity of nymphs from Stations 2, 8, and 13 were less than 70 C. P. M. per gram. This suggests that the decaying material upon which the nymphs fed had approximately the same activity at all stations. Hexagenia from Stations 2 and 13 had peak recorded activity at the termination of the project. This indicates that with the passage of time radioactive vegetable detritus became an increasingly abundant material for Hexagenia to feed on.

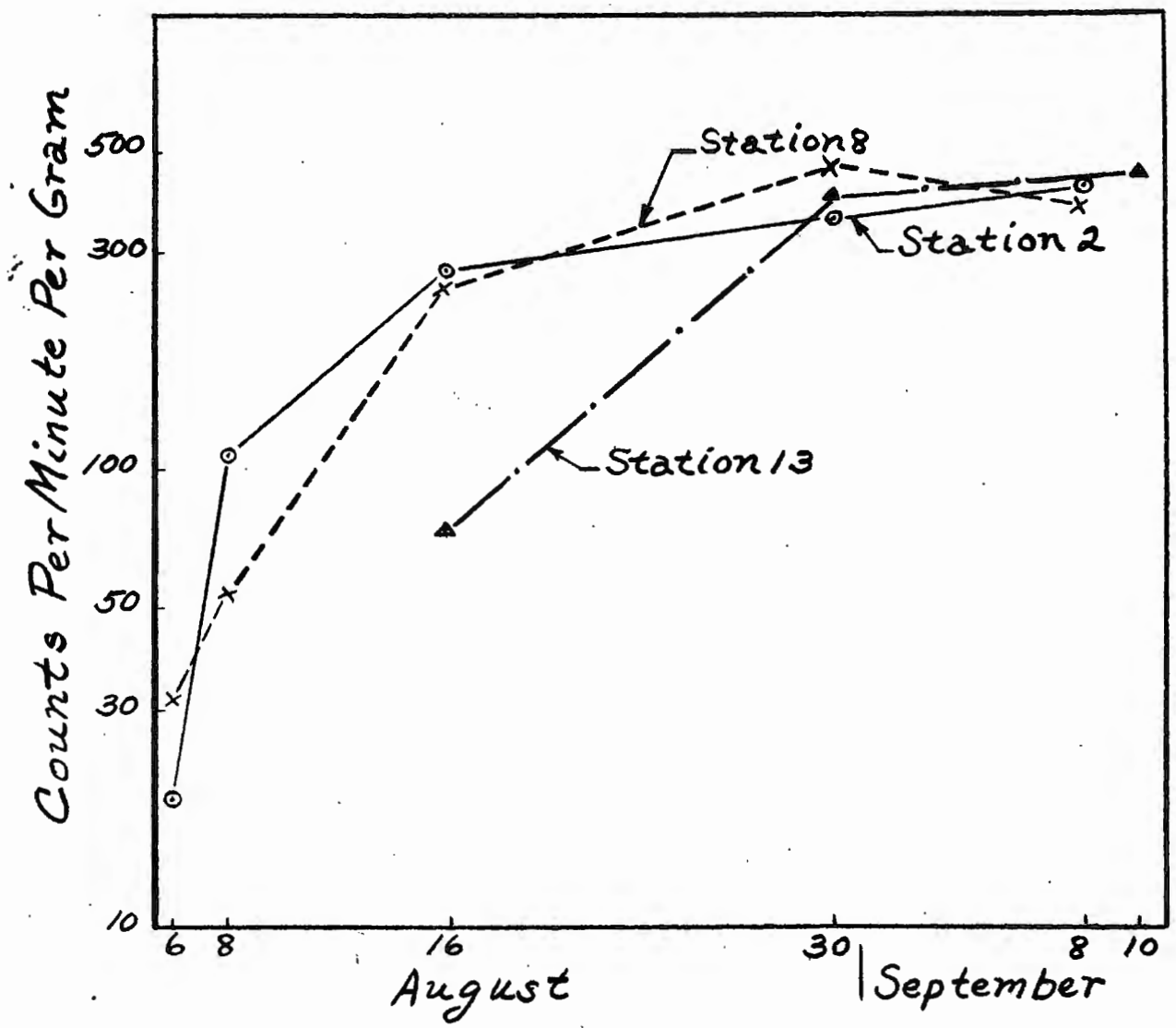
50

Figure 11. --Activity of the naiads of the dragonfly, Cordulegaster. This organism preys on invertebrates and small fish. Activity at all stations of August 6 was zero and zero at Station 13 on August 8. Counts are corrected for background and decay.


Counts Per Minute Per Gram
2000
1000
500
300
100
50
30
10
Station 2
Station 13
Station 8
0, C.P.M.
6
8
16
30
8
10
August
September

52

Figure 12. --Activity of the nymphs of the mayfly, Hexagenia limbata. This organism feeds on vegetable detritus. Activity at Station 13 was zero on August 6 and 8. Counts are corrected for background and decay.


Counts Per Minute Per Gram
500
300
100
50
30
10
Station 8
Station 2
Station 13
6
8
16
30
8
10
August
September

Physa. --Figure 13 shows the amounts of radioactivity in snails (Physa). Activity of Physa at Stations 8 and 13 remained at a more constant level throughout the experiment than that of any insect. It is believed that one reason for the uniformity of the activity of snails was a result of the fact that they do not molt such as occurs in insects. Krumholz, et al. (op. cit.) report that insects lose radio-material from instar to instar through molting.

After August 6, the minimum activity of snails was more than 75 percent of their maximum activity at a given station (at Stations 8 and 13).

Pteronarcys. --Figure 14 shows the amount of radioactivity in larvae of the stonefly, Pteronarcys. At Station 13, activity of the naiads increased with each sampling period until the maximum value was attained on August 30. Activity of Pteronarcys decreased at Stations 8 and 13 after the maximum value recorded on August 30.

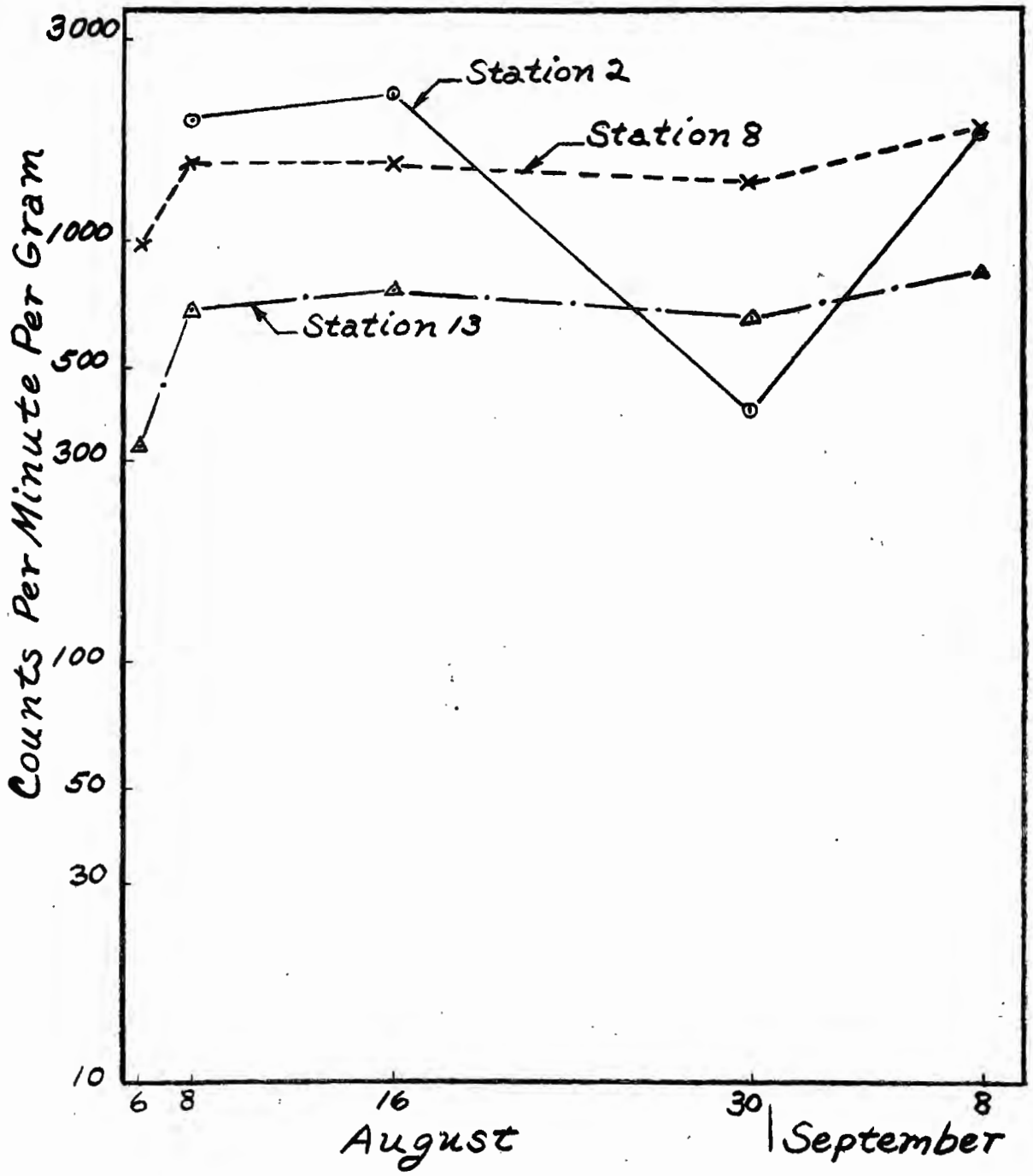
Simulium. --The difference in activity of Simulium collected from the three stations decreased with time as Figure 15 indicates. Explanations for variation in activity of these larvae have been previously discussed.

P^{32} Deposition in Various Parts if the Snail, Physa

P^{32} is known to be accumulated at different concentrations in different tissues and organs of an invertebrate. Hevesy (1948) reports that a radioautograph of a moth larvae showed P^{32} selectively

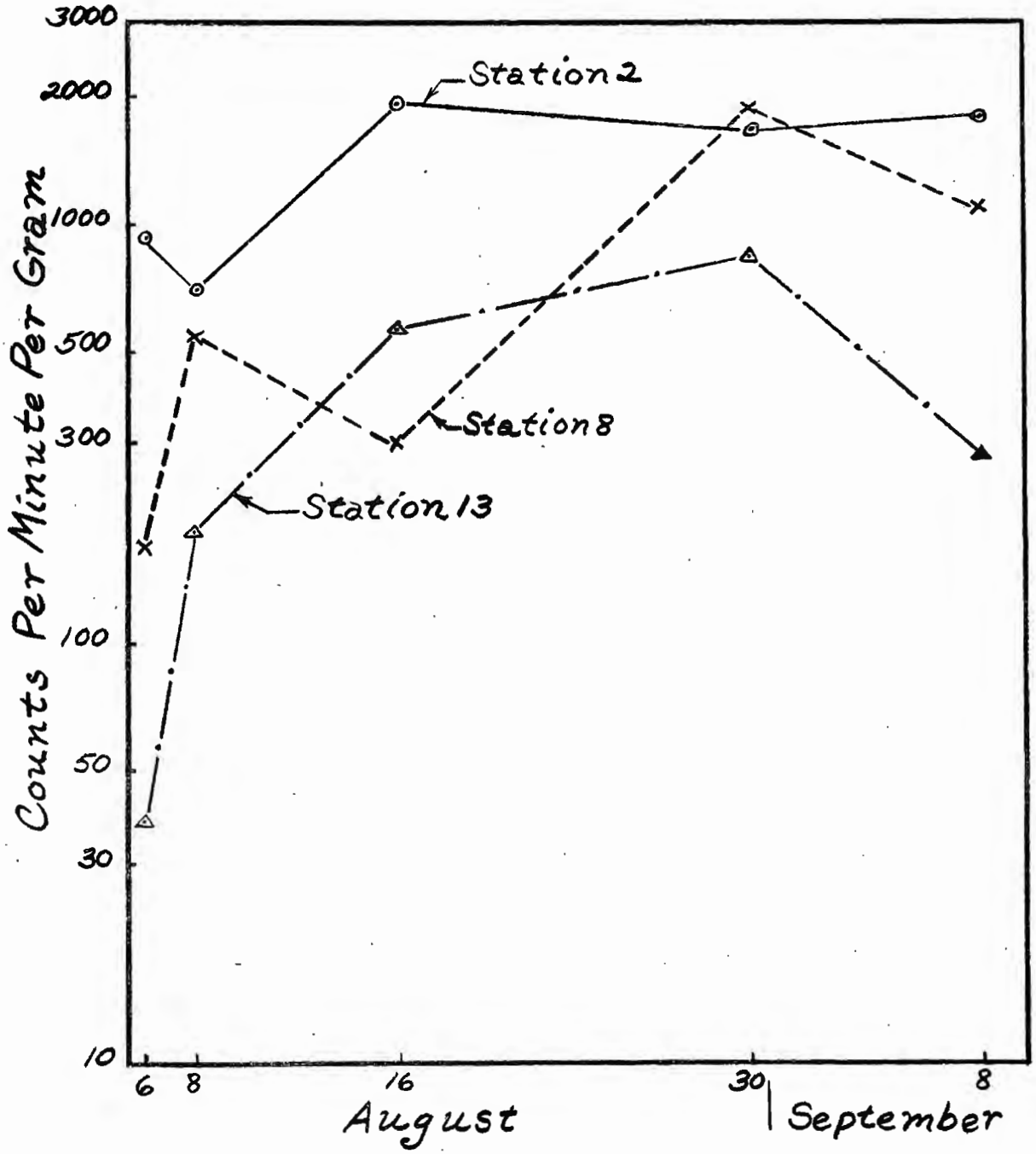
55

Figure 13. --Activity of the snails (Physa). This animal feeds on living and dead plant and animal material. Activity was zero at Station 2 on August 6. Counts are corrected for background and decay.


3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
Station 2
Station 8
Station 13
6
8
16
30
8
August
September

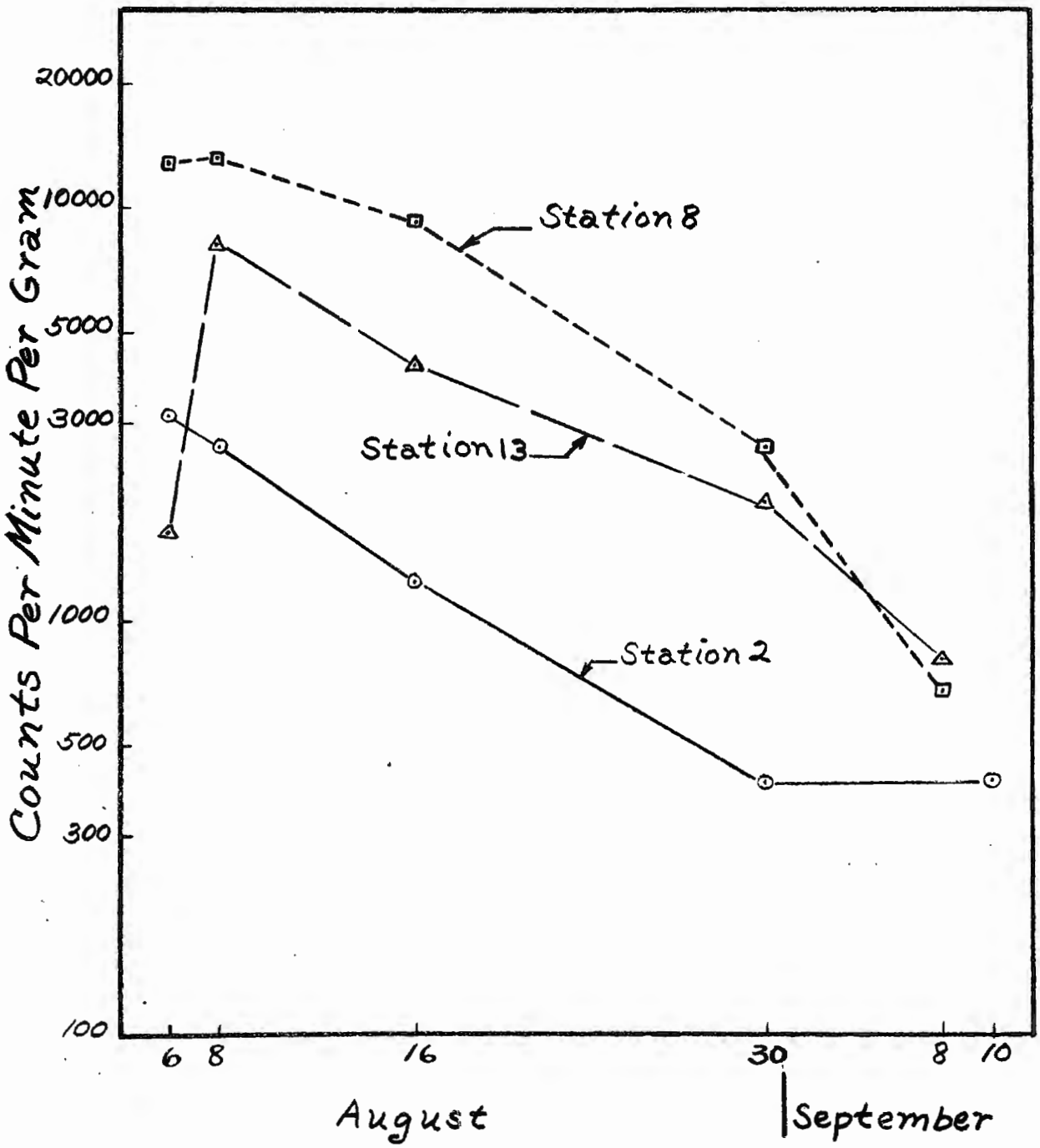
57

Figure 14. --Activity of the naiads of the stonefly, Pteronarcys. This organism feeds on periphytic detritus. Counts are corrected for background and decay.


3000
2000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
Station 2
Station 8
Station 13
6
8
16
30
8
August
September

59

Figure 15. --Activity of the black fly larvae (Simulium). This organism is a filter-feeder. Counts are corrected for background and decay.

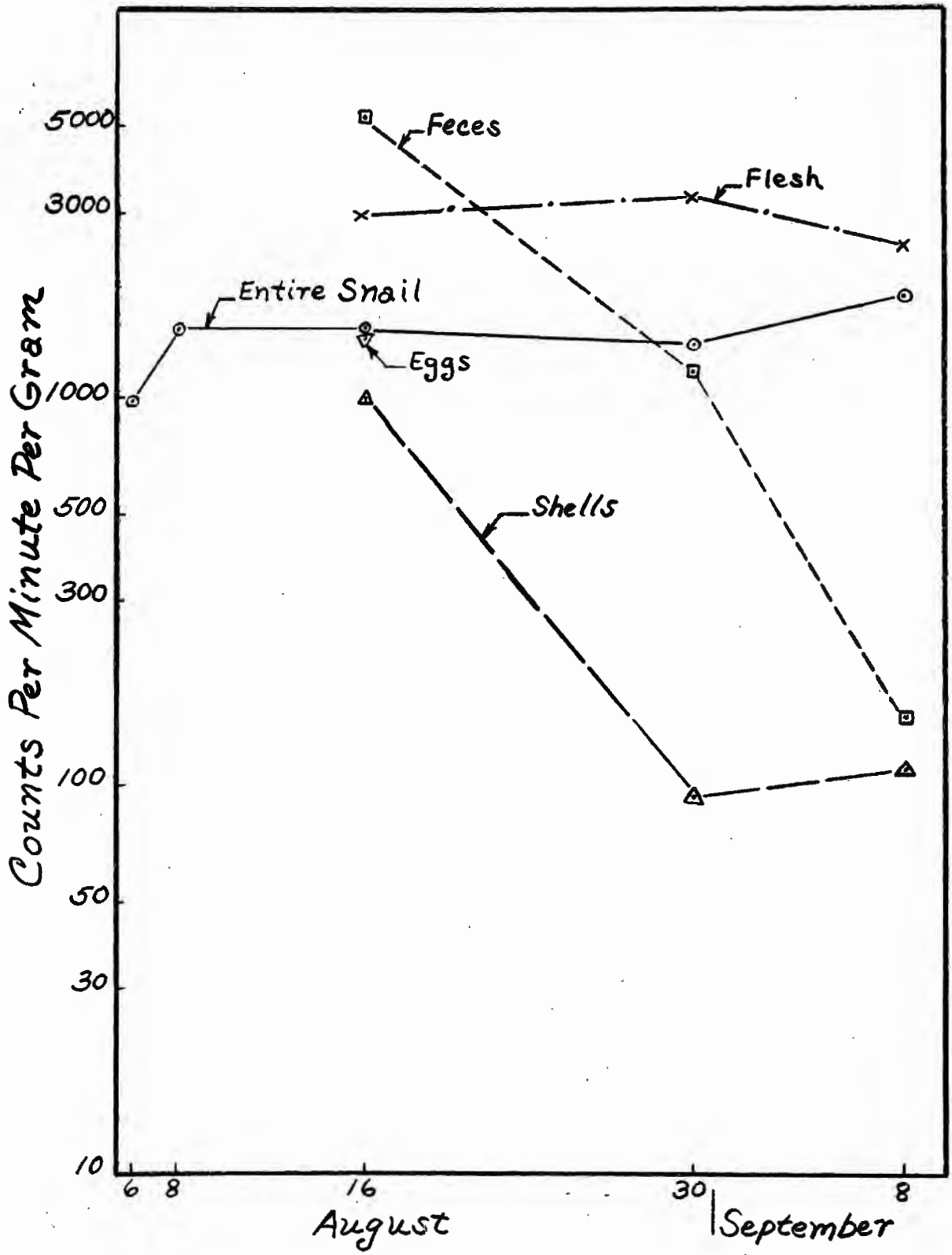

Counts Per Minute Per Gram
20000
10000
5000
3000
1000
500
300
100
Station 8
Station 13
Station 2
6
8
16
30
8
10
August
September

accumulated in the walls of the digestive tract, in the salivary glands, and in the ducts of the silk glands. In the West Branch, activity of the flesh, shells, and feces of snails (Physa) was measured beginning with the August 16 sampling. Physa eggs were collected on August 16 only. Figure 16 presents the results for Station 8 and the activity of snail parts in Stations 2 and 13 samples was summarized in Table III. Two samples of at least 6 snails each were collected at a station. One of the paired samples was used to assay the activity of entire snails (shells intact). In the other sample, shells were separated from the snail bodies and fecal accumulations were collected. These 3 snail parts were measured separately for activity.

Snail eggs. --The snail eggs at Station 8 were greater in activity than the shells but less than the flesh portions.

Snail shells. --Activity of shells was one-third or less of that of snail flesh on all sampling dates. Snail shells in Columbia River studies had activity that was almost negligible compared to that of the flesh (Robeck, et al., op. cit.). At Station 8, August 30, the activity of shells was 8 percent of that of shells collected 2 weeks earlier. This suggests that labeled phosphate was not a tightly bound component of the shell material. Most of the radioactivity of the shells could have been contained in algae which is known to grow on Physa shells. Algae lost activity at a rate similar to the rate of loss in the shells.

Figure 16. --Activity of entire snails (with shells) and of parts of snails at Station 8. Counts are corrected for background and decay.


Feces
Flesh
Entire Snail
Eggs
Shells
5000
3000
1000
500
300
100
50
30
10
Counts Per Minute Per Gram
6
8
16
30
8
August
September

Snail feces. --Snails from Stations 2, 8, and 13 were kept in separate jars without food for approximately a day while their waste products accumulated. The snails and their fecal accumulations were then weighed and their activity assayed.

The amount of P^{32} in feces decreased after August 16 but was detectable in feces eliminated 23 days later. Activity of excrements from snails collected on September 8, at Station 8 was 3 percent of that of the August 16 sample. Activity of feces was greater than that of shells but less than that of the flesh on all but one occasion. The amount of radioactivity in fecal material produced by Physa was calculated for 2 September 8 samples:

TABLE IV. --The amount of P^{32} eliminated by snails on September 8

Station	Feces produced by a live weight gram of Physa in a day	P^{32} content of the feces (in C. P. M.) produced by 1 gm of Physa in a day	Percent of the activity of Physa lost in a day by elimination
2	0.21 gram (wet weight)	80	4.5
8	0.19 gram (wet weight)	28	1.5

Activity of the feces was indicative of the amount of P^{32} not absorbed by the snails from their ingested food material. It does not include excretory losses of isotope since snails do not secrete metabolic wastes into the gut via the Malpighian tubules as insects do.

Sheer (1948) states: "The typical molluscan nephridium is a tube, opening at one end into the pericardium through a ciliated nephrostome, and at the other to the exterior."

Snail flesh. --Activity was greater in flesh portions of snails than in shells or feces on all but one occasion. Activity declined more slowly in the flesh than it did in the feces. At Station 2 there was an increase in activity of the flesh during the period August 30 to September 8. This suggests that: (1) snail tissue retained P^{32} for a longer period than did the snails' food and (2) snails were still obtaining isotope from their ingested food 34 days after the tracer was released.

Entire snail. --Entire snails had higher activities than the shells but less than that of the flesh on all sampling dates. It was assumed that as the activity of shells decreased the difference in activity between the flesh and entire snail would increase. The results from snails collected on September 8 did not verify this assumption. This was believed to have been due to: (1) chance variation between pairs of samples from the same station, and (2) the decrease in activity of fecal matter after August 16. It was estimated that at Station 8, August 16, one-third of the activity of snail flesh was actually that of ingested food and would be lost by elimination. The estimate is based on data of September 8 on the daily production of feces by Physa.

P^{32} Concentration Factors in Invertebrates

The ratio of concentration of radionuclide in an organism to that in the surrounding environment has been termed concentration factor (C. F.) (Odum, op. cit.). At present there is very little information on the chemical composition of fresh-water organisms and, consequently, virtually nothing is known of concentration factors to be expected for different elements in the organisms (Krumholz and Foster, op. cit.).

An estimate of concentration factors in invertebrates of the West Branch was made in which:

$$\text{C. F.} = \frac{\text{(C. P. M. per gram of organism)}}{\text{(C. P. M. per ml of water)}}$$

The amount of isotope per ml of water was an average value for the period during which P^{32} was detectable in water samples, on August 5, only. Table V summarizes the estimated concentration factors of 7 invertebrates. Water samples were not collected for radioactivity assay at Station 13. Concentration factors for invertebrates of Station 13 were calculated by using the average water activity value at Station 11, 300 yards upstream. It is presumed that average activity of the water at Station 13 was less than it was at Station 11 and the concentration factors of invertebrates should be higher than their calculated values.

The tracer was not entirely mixed in the water mass when it reached Station 2. The calculated average activity of the water and

TABLE V. --The estimated concentration factors for invertebrates

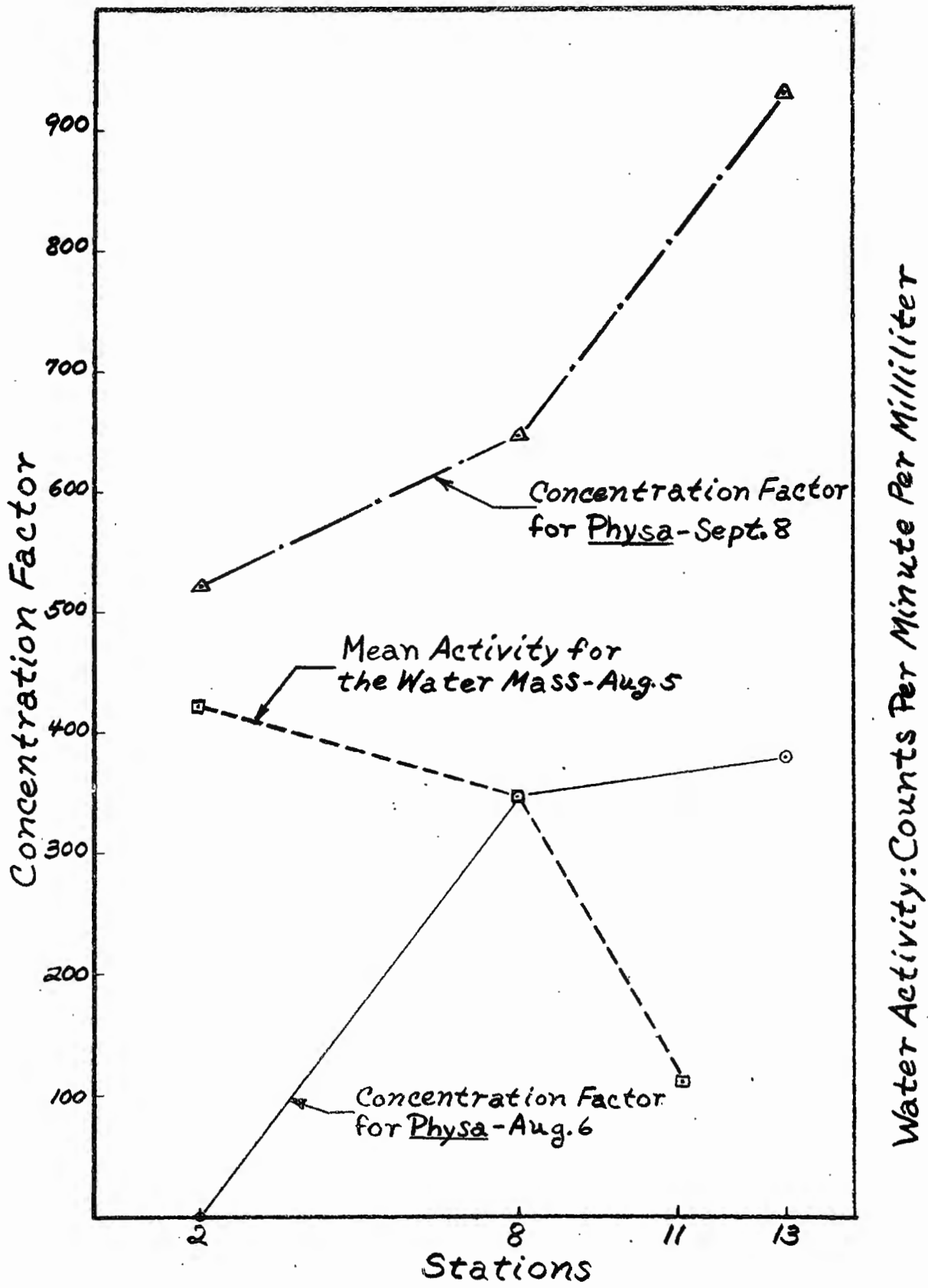
Date (1958)	August 6			August 8			August 16		
Stations	2	8	13	2	8	13	2	8	13
Organisms									
Brachycentridae	235	184	4333	698	571	226	673	1342	852
Cordulegasteridae	0	0	0	14	49	0	299	0	478
Ephemeridae	6	11	0	32	19	0	82	91	82
Physidae	0	349	382	569	538	767	665	540	852
Pteronarcidae	273	60	42	206	187	206	570	107	628
Rhagionidae	353	0	180	...	422	492	0	138	2651
Simuliidae	932	4619	1852	782	4714	9192	369	3295	4667

Date (1958)	August 30			September 8			September 10		
Stations	2	8	13	2	8	13	2	8	13
Brachycentridae	370	1102	2182	493	850	1821	...	...	...
Cordulegasteridae	102	518	...	75	...	...	16	124	81
Ephemeridae	105	167	441	125	135	...	...	...	498
Physidae	116	490	728	522	652	934	...	...	...
Pteronarcidae	491	664	938	528	385	310	...	...	...
Rhagionidae	2572	959	974	1406	823	1494	...	...	...
Simuliidae	121	950	2133	...	245	898	122	...	...

... indicates no data.

68

Figure 17. --Comparison of the concentration factors in Physa with that of the average activity of the water on August 5, 1958.


900
800
700
600
500
400
300
200
100
Concentration Factor
Concentration Factor for Physa - Sept. 8
Mean Activity for the Water Mass - Aug. 5
Concentration Factor for Physa - Aug. 6
2
8
11
13
Stations
Water Activity: Counts Per Minute Per Milliliter

the concentration factors of invertebrates compiled from its value may be more in error, here, than at Station 8. Figure 17 indicates that Physa had its highest C. F. where the activity of the water was the lowest but activity above background was the longest sustained. The largest concentration factors of the snails were at Station 13 throughout the 33 days of sampling. There was a greater maximum activity of the water at Station 8 than at Station 11 but P^{32} could be detected in the water for a longer period at Station 11. The lowest concentration factors for the snails were at Station 2. The maximum recorded activity of the water as it flowed past Station 2 was 3 times that at Station 11. Nevertheless, activity of water was significantly above background for a longer period at Station 11.

It was believed that 2 factors were responsible for most invertebrates having their largest concentration factors at Station 13: (1) P^{32} was taken up by the biota for a longer period of time at Station 13 than upstream nearer the isotope source, and (2) radioactive drift material not available to organisms upstream was fed on by invertebrates of Station 13 and this contributed to their activity.

Radioactivity in Control Organisms

Radioactivity above background was occasionally detected in aquatic plants and animals and terrestrial vegetation, all collected from control areas. Robeck et al. (op. cit.) reported cases of unaccountable radioactivity in plankton from Columbia River control

areas. There was radioactivity in bottom organisms in 7 out of 20 samples from the control area (Station 1-A) of the West Branch. The highest activity above background of control station invertebrates was 57 C. P. M. per gram in a sample of Chauliodes larvae. The activity of the organisms in the other 6 samples was from 5 to 30 C. P. M. per gram. There were samples of vegetation in control areas with higher activity than that of any control station invertebrates.

It is believed that radioactivity in control-area organisms was caused by their uptake of residual nuclear radioactive fallout from atomic bomb explosions. In September, 1958, 40 samples of terrestrial vegetation were collected from control areas of the West Branch. The samples were sent to Michigan State University for identification of radiomaterials contained in them. No activity above background was found in any of the vegetation when assayed in December, 1958. It was impossible to determine whether the vegetation had previously contained short-lived radiomaterial.

An Estimate of the Animal Biomass

An estimate was made of the standing crop of animals in the first 1000 yards of stream below the point of isotope introduction. This was the area from Station 8 to the point of isotope release and contained approximately 1. 77 acres. Riffles occupied about 86 percent of this area and the remainder was silt beds supporting Chara. The methods employed in obtaining the biomass and area estimates were

described previously. Table VI summarizes the population and biomass estimates of the standing crop of fish for the period August 21-28, 1958. Rainbow trout were numerically the most abundant species and muddlers were the next most abundant. More than three-fourths of the total fish biomass was contributed by brown trout.

The average live weight of invertebrates was 0.55 gram per square foot of stream bottom. Oligochaetes constituted 46 percent of the weight of organisms in bottom samples. Grzenda (op. cit.) reported similar results in 1954 bottom sampling of the West Branch. He calculated that 48 percent of the biomass of bottom fauna was contributed by oligochoetes. The average live weight of invertebrates of the riffles was 0.411 gram per square foot and 1.39 grams of organisms per square foot of silt bottom supporting Chara. The invertebrate populations of silt beds weighed 3.4 times more per unit area than populations in riffles. Keup (op. cit.) estimated for a larger section of the West Branch that the bottom organisms of Chara-silt beds weighed 5 times more per unit area than bottom organisms of the riffles.

The standing crop of animals in the 1000 yard section of stream on August 21 was as follows on page 74.

Assuming that the standing crop was of equal biomass on August 5, 273 pounds of animal were in this section of stream when the isotope was released.

TABLE VI. --Population and biomass estimates of fish in a 1000 yard section of the West Branch of the Sturgeon River

Length Class (Inches)	Population (Number of Fish)	Mean Weight per Trout (Grams)	Biomass (Kilograms)
		Brown Trout	
1.0-2.9	385	3.0	1.155
3.0-3.9	602	6.0	3.612
4.0-4.9	7	15.0	0.105
5.0-5.9	77	29.5	2.272
6.0-6.9	52	44.0	2.288
7.0-7.9	34	78.0	2.652
8.0-8.9	30	110.0	3.300
9.0-9.9	35	149.0	5.215
10.0-10.9	12	189.0	2.268
11.0-14.9	36	365.0	13.140
15.0-19.9	10	813.0	8.130
Total	1280	. . .	44.137
		Brook Trout	
1.0-3.9	442	4.0	1.768
4.0-5.9	70	28.1	1.967
6.0-6.9	23	46.8	1.076
7.0-7.9	6	65.8	.395
Total	541	. . .	5.206
		Rainbow Trout	
1.0-1.9	405	2.00	0.810
2.0-2.9	2123	2.75	5.838
3.0-3.9	88	6.00	0.528
4.0-4.9	147	16.30	2.396
5.0-5.9	197	27.20	5.358
6.0-6.9	56	43.10	2.414
7.0-7.9	12	73.50	0.882
8.0-9.9	16	97.10	1.554
Total	3044	. . .	19.780
		Muddlers	
1.0-2.9	2426	3.40	8.248
3.0-3.9	397	10.75	4.268
Total	2823	. . .	12.516

	Pounds in 1000 Yards of Stream (1.77 Acres)	Pounds per Acre
Brown Trout	97.4	55.0
Rainbow Trout	43.7	24.7
Brook Trout	11.5	6.5
Trout Total	152.6	86.2
Muddlers	27.6	15.6
Fish Total	180.2	101.8
Invertebrates	92.6	52.6
Total Fish and Invertebrates	272.8	154.4

Estimates of the standing crop of trout have been made for other Michigan streams. Shetter (1942) reported that the standing crop of trout was 94.4 pounds per acre for a section of Hunt Creek, Montmorency County, Michigan. He reported that the standing crop of fish of all species was 104 pounds per acre. This is only 2 pounds per acre more than was estimated for all fish species in the West Branch. In both streams, muddlers were the only established species of fish besides trout. Ellis and Gowing (1957) estimated that there was a standing crop of 120 to 127 pounds of trout per acre in sections of Houghton Creek, Ogemaw County, Michigan.

The standing crop of trout weighed almost twice as much as the standing crop of invertebrates. Allen (1951) estimated that 6 pounds of fish-food organisms were needed to produce and maintain 1 pound of trout in a New Zealand stream. At this rate of food conversion,

12 times the standing crop of bottom fauna would have been needed to produce and maintain the standing crop of trout in the West Branch. Hayne and Ball (1956) calculated that in a 150 day growing season, sunfish (Lepomis spp.) removed 27 times the standing crop of benthic organisms in a southern Michigan pond. Allen (op. cit.) estimated that trout in a New Zealand stream consumed in one year, 40 to 150 times the standing crop of bottom fauna. Trout in the West Branch would annually consume 2104 pounds of aquatic invertebrates per acre at Allen's minimum estimated rate.

Comparisons of the standing crops of trout and bottom fauna in three Michigan streams and one New Zealand stream are shown in the following:

TABLE VII. --A comparison of the trout and invertebrates of 4 streams

Stream	Pounds of Invertebrates per Acre	Pounds of Trout per Acre	Standing Crop Ratio of Invertebrates to Trout
West Branch of the Sturgeon River	53	86	.62
Hunt Creek	72	94	.77
Houghton Creek	138-411	120-127	...
Horokiwi Stream (New Zealand)	19-86	100-400	...

SUMMARY

On August 5, 1958, radiophosphorus (P^{32}) was released into the West Branch of the Sturgeon River. As the P^{32} flowed downstream, it was progressively diluted but the period during which there was radioactivity in the water increased. The isotope was detectable in the water at the downstream boundary of the study area, more than 2 miles below the entry point.

There was activity in periphyton collected 4 hours after the tracer was released. Bottom organisms were collected on the following day and most of them contained P^{32}. There was zero activity in 4 species of invertebrates at one or more stations. This was evidence that there was little absorption of isotope through the external surfaces of these organisms. Activity attributable to cutaneous absorption would have been detected in the organisms in this initial sampling.

Mayfly nymphs and aquatic earthworms are mud-eaters and had less activity than that of any other organism. From this it was concluded that the organic detritus (mud) contained only small amounts of P^{32} during the period in which these organisms were collected. The activity of Hexagenia (mayflies) which live in the detritus increased as the summer progressed. This was interpreted to mean that the activity of the "muds" which constituted their food was slowly receiving material containing isotope.

The activity was of the same order of magnitude in invertebrates collected at Station 2 and at Station 8 a distance ten times as far downstream. Initially, there was less activity in most invertebrates collected at Station 13 a distance of 1850 yards downstream from the point of P^{32} release. As time passed, activity of drift-feeding organisms of this station became higher than similar organisms upstream. The indication was that there was a relatively larger amount of radioactive drift material for these organisms to feed on with increasing time and distance downstream.

The primary source of food for invertebrates was periphyton in riffle areas and periphyton detritus in silt deposits. Activity of the bottom fauna of the riffles was higher than of the organisms living in silt beds. This suggested that periphyton growing on the riffle substrate had higher activity than periphyton detritus deposited in the silt.

In riffle areas, the highest activity was in filter-feeding black fly larvae and the lowest was in stonefly naiads. The black fly larvae were extracting radioactive drift material from the current 2 days after activity could no longer be detected in the water samples.

Scavenging mayfly nymphs from all stations had approximately the same activity at the end of the period of study. This suggested that the activity of their plant detritus-food was of a uniform value throughout the 1850 yards in which it was studied.

Omnivorous snails had lower maximum activity than

herbivores but more than the scavengers. The snails' activity remained at a more constant level throughout the experiment than that of any larval insects. One explanation is that they do not lose P^{32} by molting of their exoskeletons such as is known to occur in insects.

There were interfamily differences in activity of invertebrates. The black fly larvae had the highest activity of any organism and also lost their activity at a rate greater than other organisms.

There were variations in activity within the population of a given genus or species. The greatest variability of activity was in predaceous insect larvae. An explanation for this is that their prey may be selected from many species which may be quite variable in activity.

In snails, P^{32} was deposited in the flesh in greater concentration than in the shells. Radiophosphorus was not a tightly bound constituent of snail shells; most of the activity of shells was gone by August 30. Algae growing on the shells was believed to have contained most of the radioactivity attributed to the shells.

Snail feces decreased in activity after August 16 but isotope could still be detected in excrements eliminated 34 days after release of P^{32}. Snails do not secrete metabolic waste materials into the gut as insects do. One-third of the activity attributed to snail flesh on August 16 was estimated to be that of ingested food material and would be lost to the organisms by elimination. Thereafter, lesser amounts of isotope were lost by snails through elimination of waste material.

Concentration factors (C. F.) were estimated for the invertebrates:

$$C.F. = \frac{\text{C. P. M. per gram of organism}}{\text{C. P. M. per ml of water}}.$$

The activity of the water was an average value for the period during which P^{32} was detectable in the water mass after its application, August 5. On this basis, the maximum invertebrate C. F. was 9192 found in the black fly larvae. Most invertebrates had their highest concentration factors at Station 13 for which there are 2 explanations: (1) P^{32} was believed to have been taken up by the biota of Station 13 for a longer period of time than was true for the biota nearer the isotope source and, (2) invertebrates of Station 13 took up P^{32} from radioactive drift material not available to organisms of upstream stations.

Radioactivity significantly above background was occasionally detected in aquatic plants and animals as well as terrestrial vegetation of control areas. The identity of the radiomaterial could not be determined but was presumed to have been from radioactive fallout from atomic bomb tests.

The biomass of animals was estimated for the first 1000 yards of stream below the source of the radioisotope. This was an area of 1.77 acres of which 86 percent was riffles. There was 86 pounds of trout per acre and 53 pounds of bottom fauna per acre in this section. The biomass of the population of bottom organisms was more than 3 times as much per unit area in silt deposits as in riffles.

It is estimated that trout in the West Branch would annually consume more than 2100 pounds of bottom fauna per acre if they fed at the minimum rate estimated for trout in the Horokiwi Stream of New Zealand.

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