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TRANSLOCATION OF PHOSPHORUS IN A TROUT STREAM ECOSYSTEM

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The objectives of this research were to explore the movement of phosphorus in a cold-water stream and to better understand relationships between phosphorus and stream productivity. Our method has been to apply a phosphorus-32 spike[1] to the stream so as to fix the radioactivity in the producer level of the food chain within a short span of time. We have then followed the activity through all parts of the stream ecosystem. Previous stream investigations have dealt with chronic exposure of the stream biota to phosphorus-32 activity as contrasted to the very brief exposure in the present study.

Classical data regarding the distribution of phosphorus in an aquatic environment were presented by Juday and Birge (1931) and by Ohle (1934). Hutchinson (1941) recognized that the amount of phosphorus present at certain times in lakes was insufficient to account for many of the biological processes taking place. His studies indicated a more rapid movement of phosphorus through the lake ecosystem than had been previously suggested. This finding was verified when radiophosphorus was first used experimentally in a lake (Hutchinson and Bowen, 1947). Hayes et al. (1952) and Rigler (1956) and others further explored the dynamics of phosphorus movement and exchange in lakes and other

[1] The term spike is used to designate the phosphorus-32 added to the stream water.

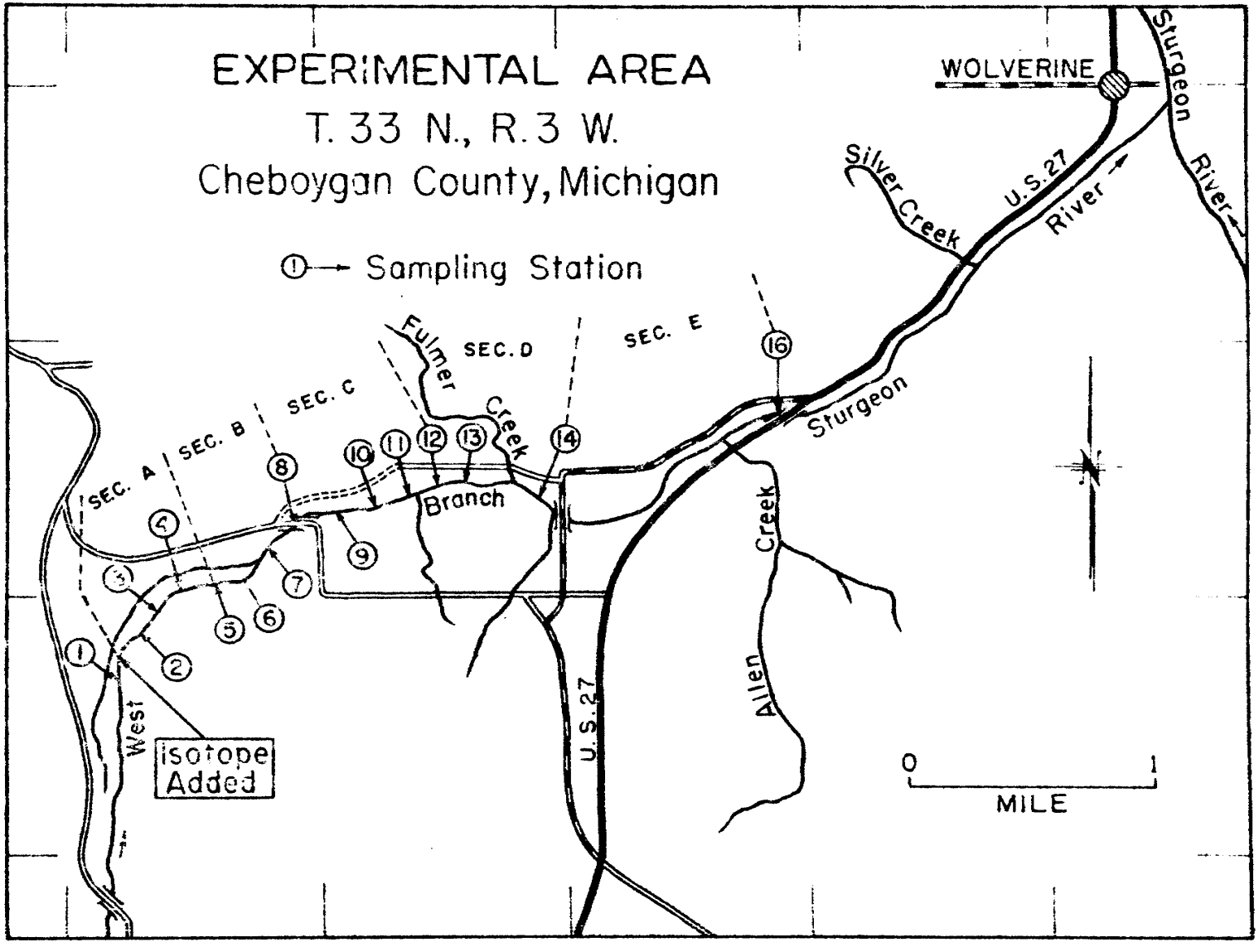
standing-water systems. These studies in which phosphorus-32 has been used have resolved many of the questions concerning the phosphorus cycle in lakes which confronted limnologists two decades ago.

Foster (1959) and Davis and Foster (1958) studied the biota of the Columbia River which receives a continuous supply of radionuclides. Of these, phosphorus-32 was one of the most important because it was strongly concentrated by living organisms. These authors showed that phosphorus intake by higher levels of the food chain was chiefly through ingestion of radioactive food. Foster determined the specific activity of the water and of the animals making up various trophic levels of the Columbia River. He found that the decrease in specific activity among organisms was related to their turnover time and their position along the food chain. Other stream studies with radiophosphorus have been concerned largely with waste disposal problems (Simpson et al., 1958).

Study Area

The section of the West Branch of the Sturgeon River which was studied is located in the northern part of the Lower Peninsula of Michigan, southwest of the town of Wolverine. The addition of the isotope was made in Section 21, Township 33 North, Range 3 West, Cheboygan County, Michigan (Figure 1). The site selected was a remote section of the stream approximately one-half mile from the nearest road. The flow at this point was 37.1 cubic feet per second. The area was comparatively free from disturbance; fishermen were frequently encountered at roads and bridges, but rarely reached the point at which the spike was introduced. We collected samples downstream a distance of approximately two and one-half miles from the point at which the tracer was released. Fifteen sampling stations were established within this area for the purpose of comparing biological uptake and other features (Figure 1). The

Figure 1. Map of experimental area showing location of sampling stations.


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

study area was divided into five sections (A through E). Within each of these sections there was one station at which all types of data were gathered. The data gathered at Station 3 were considered representative of the section farthest upstream (Section A); Station 8 was representative of Section B; Station 12 was considered representative of Section C; and Station 14 was used for Section D. Only water activity was studied in Section E.

Compared with similar trout streams in this part of Michigan, the West Branch of the Sturgeon River is infertile in character. The invertebrate and fish faunas are sparse. One cause of low productivity seems to be temperature. The downstream area which has the highest temperature had an average temperature which fluctuated between 14° and 16° centigrade during July and August. The temperature is lowest in the absence of rainfall when the flow is made up entirely of ground water. Since the ground water resources are remarkably constant, the water level fluctuations ordinarily are small during July and August. Downstream as far as Station 14, the vegetation surrounding the stream is coniferous forest and mixed hardwoods. From this point to Station 16, the surrounding area is more open and there is considerable cleared land. Since this area is readily accessible, there are a few summer cottages near the stream.

Methods

The equipment used to introduce the radionuclide into the stream consisted of a 55-gallon oil drum and a siphon of polyethylene tubing. The drum was supported over the center of the stream by a platform built on a fallen tree. Discharge from the siphon entered the stream near the point of maximum flow. In operation the drum was partially filled with stream water, 23 millicuries of phosphorus-32 (H_3PO_4) were poured in, the bottle containing the tracer was thoroughly rinsed, and the drum was filled completely. When full, the water

and nuclide were thoroughly mixed. Before it was used on the stream, the rate of discharge of the siphon had been carefully measured using various heads. It was found that by maintaining a head of 13 inches, the barrel would discharge its contents at a rate which would give a water activity of 1.22×10^{-5} microcuries per milliliter. This head was maintained by lowering the discharge nozzle along a series of nails placed at the proper intervals in a board which was attached to the drum. The starting position of the siphon was 13 inches below the water level in the barrel, and it was lowered one notch (nail) each minute for 33 minutes. This gave a flow that was very nearly uniform. At the end of the period the barrel was thoroughly cleaned by rinsing several times with water and dilute acid.

To measure water radioactivity as the spike moved downstream, personnel were stationed at certain stations to collect samples of water. Location of collecting stations is shown in Figure 1; distance between selected stations is given in Table III. Five minutes prior to the start of release of the spike from the barrel, a marker of fluorescein was placed in the current. Personnel collecting water at downstream stations took their first sample as the marker passed by. The second was taken exactly five minutes after the marker, and subsequent samples were taken at five-minute intervals. After the drum was emptied and washed out, a second dye marker was put in the stream to mark the upstream end of the spike. Since there was considerable dilution of the dye markers as they moved downstream, they were renewed at three points. After the main nuclide-bearing water mass had passed, water samples were collected at ten-minute intervals.

To collect a sample, a 140 milliliter polyethylene bottle was filled with water from the center of the stream. In 1959 and 1960 in addition to the above samples, a one-liter sample was taken at each station at the time the peak concentration of nuclide was expected. Following collection of the above water

samples as the spike moved by each station, automatic water samplers were put into operation in 1959 and 1960 at Stations 8, 11, and 14. These samplers collected samples of the stream water for the next 48 hours.'

Two milliliters of concentrated nitric acid were added to each of the 140-milliliter water samples; a 50 milliliter aliquot was removed and processed for counting radioactivity using the procedure suggested by Robeck, Henderson, and Palange (1954). The one-liter samples taken at the peak of water radioactivity were used to determine the state in which the nuclide existed as it moved downstream. A 500 milliliter aliquot was filtered through a type HA, Millipore filter. The filter was washed with distilled water and the filtrate processed and counted. This gave an indication of the amount of water-soluble phosphorus-32. The filter was then washed with 0.1 normal hydrochloric acid. Filter and filtrate were processed separately and counted. The radioactivity of the second filtrate is that from adsorbed phosphorus-32 released by the acid wash. The radioactivity of the filter pad is that from phosphorus-32 incorporated into solids.

Uptake of phosphorus-32 by periphyton was determined by suspending plexiglass plates (two by five inches) in the stream, allowing a coating of periphyton to accumulate, removing the plates from the stream and then processing the periphyton for counting. Metal stands containing 35 plexiglass plates were installed at each sampling station two weeks prior to isotope treatment. In addition to the plates (two by five inches), one large plexiglass plate (four by ten inches) was placed at each station. These large plates were attached to logs, roots, and branches below the water surface. Sampling stations maintained in the periphyton study were Stations 3, 8, 12, and 14 (Figure 1). The first set of plexiglass plates was removed ten minutes after the spike passed each station. Additional samples were removed four, 24, and 96 hours after passage of the spike. Thereafter, samples were obtained once each week for the remainder

of the study period. Each sample consisted of four of the small plates (two by five inches) and one large plate (four by ten inches). In addition to the above sampling, a series of plates were added to the stream which were not allowed to come in contact with the spike as it moved downstream. Some of these were installed before the spike passage but were removed temporarily as the spike passed each station; others were installed after passage of the spike. Periphyton analyses were continued for 35 days after the isotope was introduced into the stream. Plexiglass plates were transported to the laboratory for phosphorus-32 analysis. All animal forms, such as blackfly larvae, were picked from the plexiglass upon removal from the stream.

The periphyton on each substrate was scraped into a large beaker, using a polished glass slide. Substrates were then rinsed to remove any periphyton that might remain. The mixture of periphyton and water was filtered through a type HA Millipore filter pad of known weight. The filter pad was then washed with three milliliters of 0.01 normal hydrochloric acid. This was followed with a rinse of five cubic centimeters of distilled water. When the filter pad was observed to be free of all visible moisture, it was removed from the filter apparatus and placed in a planchet of known weight. The wet weight of the periphyton was obtained by subtracting the combined weight of the planchet and filter pad from the total weight. The planchet was treated with concentrated nitric acid and placed under a heat lamp until the periphyton was completely digested. The digestate was heated to 600° centigrade in a muffle furnace. Samples were then cooled and counted. The digestion procedure given above was adapted from the method outlined by Robeck, Henderson, and Palange (1954) for the preparation of samples of filamentous algae.

Aquatic plants were collected routinely at Stations 3, 8, 12, and 14. Collections were made four, 24, and 96 hours after the nuclide arrived at each station, and thereafter at weekly intervals for seven weeks after the introduction of the radiophosphorus in 1959 and 1960 and for five weeks in 1958.

Plant samples consisted of the entire plant, except for roots when roots were present. Samples were rinsed in the stream water to remove adhering materials and then transported to the laboratory. In the laboratory a subsample (one to two grams) of each plant type was washed with distilled water and dried with blotting paper. The blotted sample was weighed, concentrated nitric acid was then added, and the sample was digested on a hot plate. The digestate was heated to red heat in a muffle furnace, cooled, and counted.

In 1959 and 1960 aquatic invertebrates were collected for radiophosphorus analysis at Stations 3, 8, 12, and 14 at periods of four, 24, and 96 hours after the spike was added to the stream, and then at weekly intervals for a period of approximately 55 days. At this time the radioactivity in most organisms approached background levels. In 1958 sampling was continued for only 35 days. Invertebrates were collected by one or a combination of several methods which included use of a Surber sampler, hand picking from logs, stones and bottom deposits, and by washing of aquatic vegetation. A direct-current electric shocker was used to collect fish, and mayflies and larval lampreys in silt beds. Invertebrates were transported alive to the laboratory. They were then rinsed with 0.01 normal hydrochloric acid to remove adsorbed phosphorus. Duplicates of all organisms were collected for identification by specialists.

The procedure for preparation of aquatic invertebrates was modified from the method presented by Robeck, Henderson, and Palange (1954). Organisms were rinsed with 0.01 normal hydrochloric acid and placed in a wire basket that could be introduced into a centrifuge. The basket was centrifuged at 1,840 revolutions per minute for 15 seconds. At the termination of the 15-second period, the power was shut off and the centrifuge was allowed to run to a complete stop. This gave uniformity in the removal of moisture for all types of organisms. Specimens were weighed on an analytical balance and then transferred

into a stainless steel planchet for digestion. Concentrated nitric acid was added and the samples were placed under a heat lamp until they were completely digested. The digestate was transferred to a muffle furnace and heated at 600° centigrade for five to ten minutes. Planchets were then removed from the furnace, allowed to cool, and counted. Processing of fish followed the same general pattern as invertebrates except that they were first treated in a Waring blender and a one to two gram aliquot was used as a sample.

The counting time was adjusted to the radioactivity of the samples. Higher radioactivity levels required less counting time than low levels. As the radioactivity diminished, the counting time was increased to allow our computations to remain within the 0.10 level of significance (Jarrett, 1945). Appropriate corrections for self-absorption and back-scatter were made on all samples. Since the goal of the project was to determine movement and relative concentrations of phosphorus, all values have been corrected for radioactive decay to zero time. Concentration of phosphorus-32 in periphyton, macrophytes, invertebrates, and fish has been recorded as counts per minute per gram. From calibration of counting procedures, it was found that concentration in terms of microcuries per gram can be obtained from counts per minute per gram by multiplying by the factor 1.02×10^{-6}. To obtain accuracy in weighing, processing, and counting the radioactivity of small organisms, it was necessary to pool a number of specimens into a single sample. Samples containing many specimens usually gave consistent determinations of concentration. Larger invertebrates which were plated as individuals showed considerable variation. As a general practice we did not secure replicates of invertebrate samples. To do so would have so seriously depleted the insect population at some stations that few if any organisms would have remained by late summer.

Before adding phosphorus-32 to the stream, collections of periphyton, macrophytes, invertebrates, and fish were made each year at several points in an

attempt to detect background radiation not due to the tracer. We also made frequent collections during the study from a control station (Station 1) located above the point at which the phosphorus-32 was introduced. None of the samples collected in the stream showed radioactivity that was significantly above background. In 1958 samples of tamarack (Larex) and alder (Alnus) collected ten to 30 feet from the stream showed a low level of radioactivity. This was not true in 1959.

Geochemistry of Phosphorus in the Sturgeon River

Previous to the present investigations we devoted four summers to studies of the stream which included analysis of phosphorus using conventional chemical techniques. During this period, we accumulated information on the phosphorus content of the water and periphyton when the stream was in an undisturbed condition and after treatments with a variety of chemical fertilizers containing phosphorus. In the course of these studies, it was found that: (1) the phosphorus content of the water remained remarkably uniform throughout the summer as long as base-flow conditions prevailed, (2) the phosphorus content increased somewhat with rains but fell back to the steady-state level as the stream returned to base-flow, and (3) the phosphorus concentration of the stream during base-flow conditions did not differ significantly from that of a tributary spring (Table I).

If the phosphorus in the stream system is in a steady-state, then there is an equilibrium between input of phosphorus atoms from the ground water and removal by the biota. Since the phosphorus level appears to vary little with space and time (Table I), it would seem that some storage system or regulating mechanism is necessary to preserve this uniformity. The nature of such a regulating mechanism is not well understood but it seems likely that it could be accomplished by the plants themselves by means of their phosphorus-storing capacity (cf. Rhode, 1948). Solids which are capable of adsorbing phosphorus may also act as regulators. In any case, regeneration by decay, release of adsorbed phosphorus from

Table I. Total and soluble phosphorus of water from experimental area of West Branch of Sturgeon River and from a tributary spring (1954-1957). Base-flow condition prevailed at time of these measurements. Single determinations.

Dates	Phosphorus (parts per billion)							
	Shingle-Mill Bridge		Station 8		Station 14		Tributary spring	
	Soluble	Total	Soluble	Total	Soluble	Total	Soluble	Total
1954								
July 26	1	4	1	4	1	6		
1955								
July 7	1	3	1	6	1	6	1	3
11	1	3	1	3	1	3	*	3
18	*	5	*	8	*	7	*	6
28	1	6	1	6	1	6	1	5
1956								
July 2				2				
9				5				
16				8				5
30								8
1957								
July 11		6		5		5		
18		4		4		4		
25		7		5		5		
Average	4.75 ± 0.53[1]		5.09 ± 0.56		5.25 ± 0.46		5.00 ± 0.77	

*Less than 1 part per billion

[1]Average ± one standard error of the mean.

solids, and input from ground water must balance uptake by bacteria and plants. Disturbance of the phosphorus steady-state by the addition of fertilizer brings only a temporary increase in phosphorus content. Within 48 hours after the supply of fertilizer is cut off, the stream returns to the equilibrium level. If the hypothesis of a steady-state of phosphorus in the stream is correct, then it would be expected that a spike of phosphorus-32 added to the flow would act exactly like soluble phosphorus atoms released by decay and like atoms made available from ground water entering the stream at some point.

The quantity of phosphorus-32 added appears to be small when compared to the amount of natural phosphorus-31 in the water. If a value of one part per billion is used for soluble phosphorus and an activity of 1.22×10^{-5} microcuries of phosphorus-32 per milliliter is used for radioactivity of the water, the specific activity of phosphorus in the water becomes 1.22×10^{4} microcuries per gram. This means that about one atom in every 3,300 phosphorus atoms was an atom of phosphorus-32. This figure is open to some question since the value of one part per billion for soluble phosphorus is at the lower limit of the sensitivity of the molybdate method used in the phosphorus analysis but it suggests that the spike did not appreciably increase the phosphorus content of the water.

Total phosphorus concentration averaged about five parts per billion at three collecting localities and at a spring tributary (Table I). Differences in the values of total phosphorus between localities were not statistically significant as based on a one-way analysis of variance (computed $F_{3,29}=0.128$). The nature of the organic components of the phosphorus burden carried by the stream is not known but it is certain that it must include phosphorus incorporated into the bacteria and diatoms suspended in the water. The equilibration of soluble phosphorus atoms with the organic fraction appears to be rapid. In 1959 and 1960 a sizeable fraction of the tracer was incorporated into particulate material by the time it reached Station 3 which was 200 yards downstream from the site of the

radionuclide release. It is clear that a much greater fraction of phosphorus was incorporated in the solids in 1959 than in 1960 (Table II). The 1959 treatment followed a period of heavy rainfall and there may have been a greater quantity of organic detritus and bacteria than in 1960. In recent work, we have found that bacteria increased five to tenfold during such a rainy period. Thus, a greater quantity of water-borne bacteria in 1959 might have brought about greater particulate uptake than in either 1958 or 1960. This rapid partitioning into soluble and organic components provides two fractions which very likely have separate and distinct routes in the food web.

Loss of Radioactivity from a Phosphorus-32 Spike

The spike had distributed itself rather thoroughly in the stream channel by the time it had moved 200 yards downstream from the point of introduction. However, during its passage downstream there were few, if any points at which the distribution of radioactivity was uniform from bank to bank. Measurements of the distribution of radioactivity in the stream channel indicated that as the spike passed a given point, radioactivity was initially highest in the center channel and later increased along the stream margin. During the latter stages of passage, water in the center channel was diluted by nuclide-free water, while high radioactivity remained along the bank as a result of the slow movement and storage of nuclide as it moved in and out of the backwaters. The time necessary for the spike to pass a given point increased as it moved downstream. It was 33 minutes at the release site, 60 at Station 8, 70 at Station 12, and 80 at Station 16. These times represent the elapsed time between the passage of a dye marker released before the spike, and the time at which water radioactivity fell to a level at which it could not be distinguished from background when a 50 milliliter sample was evaporated to dryness and counted for three minutes.

An effort was made to trace any residual radioactivity that might be passing downstream after the spike. For these analyses, 500-milliliter samples were

Table II. Percentage of water phosphorus-32 in soluble, particulate, and adsorbed forms following addition of spikes of phosphorus-32 in 1959 and 1960. Soluble fraction is that passing an HA millipore filter. Adsorbed fraction is that recovered by washing filter with 0.1 normal hydrochloric acid. Single determination at peak of concentration.

	Station 3		Station 5	Station 8		Station 14
	1959	1960	1960	1959	1960	1960
Soluble	12	65	48	47	81	67
Particulate	70	13	32	47	0	18
Adsorbed	18	22	20	6	19	15

evaporated to ten cubic centimeters. The residue was transferred to a planchet which was processed in the usual manner. This proved to be of limited usefulness because the minerals concentrated during evaporation seriously interfered with counting procedures. There was a large loss due to self-absorption and the residue was hygroscopic and difficult to count in a gas-flow counter.

Counts of water radioactivity were plotted against time and a smooth curve was drawn for the plotted points. In most instances the curves were bell-shaped with the peak radioactivity at the mid-point of the time interval. In some instances curves were slightly skewed. This may have been due to the observer changing the site of water collection from the bank toward the center or vice versa as the spike passed by. It became apparent during the three years of sampling that an observer must collect each sample from exactly the same point in the stream. The total radioactivity passing a given station was calculated by summing the area under these curves and multiplying by a factor appropriate for the rate of flow at each station. By subtracting the radioactivity passing successive stations, the uptake by the stream between the two stations was calculated (Table III). These uptake figures are an estimate of phosphorus-32 transferred from the water to the sessile organisms and to the stream bottom.

Loss of radioactivity from a spike as it moves downstream is mathematically analogous to loss of radioactivity by decay. It can be calculated in the same way as turnover loss (cf. Whittaker, 1961) using the formula $A_t = A_{ie}^{-Bt}$ in which A_i is the activity of the water at time i and A_t is the radioactivity present at time t and B is the rate constant for biological turnover (analogous to T for radioactive decay). If the time selected is that required for loss of one-half of the water radioactivity ($T_{1/2}$), this equation is reduced to $B = \frac{0.693}{T_{1/2}}$. In a stream system, it is convenient to substitute the distance a spike moves for a given fraction of uptake for the time required for this fraction of uptake. Thus one might use half-distance ($D_{1/2}$), which is analogous to half-time ($T_{1/2}$) as

Table III. Uptake of radiophosphorus by sections of the West Branch of the Sturgeon River, 1958-1960. Twenty-three millicuries of phosphorus-32 added each year.

Section (Stations) (flow in cubic feet per second)	Length (yards)	1958 Uptake Total (microcuries)	1958 Uptake Percentage[1]	1958 Loss from water Half distance, $D_{1/2}$ (yards)	1958 Loss from water Average distance, D_{-}[5] (yards)	1959 Uptake Total (microcuries)	1959 Uptake Percentage[1]	1959 Loss from water Half distance, $D_{1/2}$ (yards)	1959 Loss from water Average distance, D_{-}[5] (yards)	1960 Uptake Total (microcuries)	1960 Uptake Percentage[1]	1960 Loss from water Half distance, $D_{1/2}$ (yards)	1960 Loss from water Average distance, D_{-}[5] (yards)
A (2-5) (38.73)	550	16,189	70.4	312	450	6,382	27.8	1,155	1,667	10,996	47.8	586	847
B (5-8) (43.48)	450	625	9.2	3,122	4,504	4,084	24.5	1.114	1,608	479	4.0	7,804	11,236
C (8-12) (47.53)	1,550[2]	1,225	24.7	3,093	5,525	9,080	72.4	832	1,201	6,021	52.2	1,453	2,096
D (12-14) (49.72)	600[3]	2,634	53.1	636	917	962	27.9	1,260	1,818	982	17.8	2,081	3,003
E (14-16) (52.5)	2,100[4]	1,803	77.5	1,084	1,565					3,085	68.2	1,828	1,267

[1] Percentage of nuclide entering a section that was taken up by the section.
[2] 1,250 yards in 1958.
[3] 700 yards in 1958.
[4] 2,300 yards in 1958.
[5] Average distance a phosphorus atom travels before loss.

an expression of the distance which the spike must go before losing one-half of its radioactivity.

Half-distance ($D_{1/2}$) can then be calculated using the expression $\underline{\emptyset} = \frac{0.693}{D1/2}$ where $\underline{\emptyset}$ is a rate constant analogous to $\underline{T}$ and $\underline{B}$. In this case $\underline{\emptyset}$ represents the rate of loss of radioactivity per unit distance of stream. Phi is calculated from the fraction of radioactivity lost ($\frac{A_t}{A_1} = a$) as the spike passes through each section by means of the expression $\underline{\emptyset}$ total $= \log e^{(1-a)}$. Phi (total) is then divided by the number of yards in the stream section to give the rate of loss per yard ($\underline{\emptyset}$).

The distance in which one half of the radioactivity is lost (half distance) is not equal to the average distance a phosphorus atom moves downstream before being removed from the water. The average distance an atom remains in the water is analogous to the quantity referred to as average lifetime in the decay of radioactivity. Average distance ($D_{\overline{x}}$) is the reciprocal of the rate constant coefficient $D_{\overline{x}} = \frac{1}{\emptyset}$.

Factors Influencing Uptake Rate

Average distance, half-distance, total uptake and percentage uptake are given for stream sections in 1958, 1959, and 1960 (Table III). It is clear that in some instances there was rapid cycling of phosphorus atoms as they moved downstream. The average distance an atom traveled before being removed from the water ranged from as little as 450 yards to a maximum of 11,236 yards. If steady-state conditions prevail, then, as an average, one atom of phosphorus would reappear for each atom lost in these distances. Most values ranged between 1,000 to 2,000 yards but it is clear there were wide variations in uptake. A host of environmental factors exist which might influence uptake and produce differences between sections in different years. In this study we have not been able to obtain indisputable evidence concerning factors which influence uptake but, since this subject is of considerable ecological interest, some discussion of possible

causes is in order.

Stream gradient would influence uptake rates, but the gradient throughout the study area was remarkably constant. Average velocity was somewhat greater in Section B than in other sections and in this Section the stream channel was almost completely straight and was without pools. Stream temperatures were quite constant in the study area but were slightly higher in Sections D and E. Light conditions might have a strong influence upon uptake rates. Bright sunshine prevailed during all these applications. In 1958 the spike moved through the experimental area between two and six P.M. In 1959 and 1960 it passed through the area between nine A.M. and one P.M. Low light intensities prevailed only in Sections D and E during 1958. Section A is most heavily shaded by stream-side vegetation. Sections D and E have the least shade. Average depth is quite constant but is perhaps the lowest in Section B and greatest in Section A.

The observed uptake patterns did not appear to be well correlated with any of the above environmental differences. Year-to-year differences stand out more than differences between stream sections. In 1959 average distances phosphorus-32 atoms traveled were remarkably constant throughout the experimental area (Table III). In 1958 and 1960 there was a low average distance in Section A; an extremely high average distance in Section B, and downstream sections were intermediate. The only conditions which appear to be related to these year-to-year differences are: (1) the fraction of water radioactivity that appeared in the solid form below the site of introduction and (2) the previous history of fertilization of the stream sections.

There was a far greater uptake of phosphorus-32 into water solids in 1959 than in 1960 (Table II). With a smaller fraction of soluble phosphorus-32 available, differences between sections in uptake by plants may have been minimized and the solids which contained radioactivity may have distributed themselves in a more uniform manner.

High uptake rates (lowest average distances) were noted in sections which had received an application of liquid commercial fertilizer prior to addition of the spike. High uptake rates coincided with fertilization in Sections A and D in 1958 and Section A in 1960. On the other hand, minimum uptake rates occurred in sections downstream from the section that received fertilization (Section B in 1960 and Sections B and C in 1958). In 1959 there were no additions of liquid fertilizer made to the stream. Granular fertilizer was added to sections A and C in order to accelerate growth of periphyton on artificial substrates before the spike was released. The amount of phosphorus added in 1959 was about ten per cent of that added in 1958.

It is tempting to assign the higher uptake rate in 1958 and 1960 as compared to 1959 to differences in size of the phosphorus pool of various stream sections since the three highest uptake figures were associated with enrichments with liquid fertilizer. Not all the data at hand support the hypothesis that the prior history of fertilization accounts for the uptake pattern. The point of disagreement is Section C in 1958 which had an exceedingly low uptake rate despite a sizeable application of soluble fertilizer a week before treatment with the radionuclide.

Uptake by Periphyton

The film of microscopic plants covering rocks and other substrates of the stream (periphyton) probably is the most important single source of biological uptake. Clifford (1959) studied the composition of periphyton communities of the experimental area. The diatom Synedra ulna was the predominant species but diatoms of the genera Cymbella, Navicula, and Cocconeis were also important.

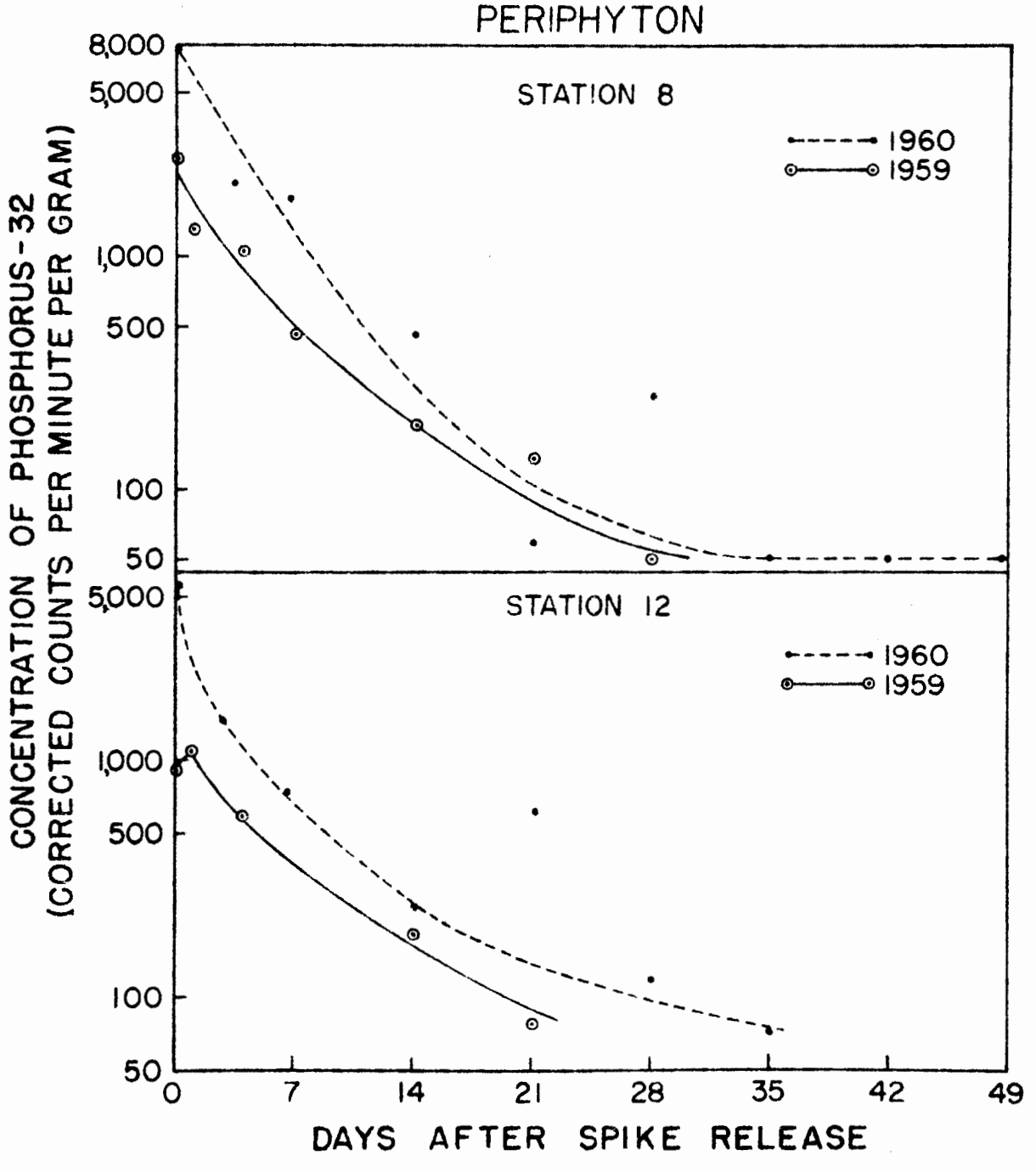
To analyze the uptake of phosphorus-32 by periphyton, we collected samples from plexiglass substrates and from boulders and rocks in the stream bed. Samples of the latter type were difficult to process since they often contained marl and sand grains. These particles caused inaccuracies in weighing. Thus,

all our quantitative data are from artificial substrates mounted on racks and exposed to the current about four inches below the water surface. Periphyton uptake data were highly variable, even when plexiglass plates were used and all procedures were carefully standardized. Some sources of variability are: (1) position effects in the stream, that is, the relation of the plate to current, shading, etc. and (2) variation in the growth rate, in species composition of periphyton, in the thickness of periphyton film, in the relative amount of living versus dead periphyton, and in the rate at which cells are sloughed off in the current. The position effects are probably the most important and in some instances were so large that upstream-downstream differences in uptake were obscured. Year-to-year differences in uptake, however, were large and in most cases were beyond the range of station-to-station variation.

The phosphorus-32 concentration curves for periphyton reflect changes in radioactivity due not only to the exchange of phosphorus-32 with the water but also to the influence of biological dilution (growth of periphyton) as well as to losses due to the sloughing off of old cells and ingestion of cells by consumer organisms. Little is known concerning the position of phosphorus-32 in the algal cell. Much of the activity may be adsorbed on the cell surface and not incorporated into the cell structure (cf. Odum et al., 1958). This may have been the location of some of the radioactivity in the upstream area soon after treatment which later was translocated downstream. On the other hand, it appears likely that most of the phosphorus in periphyton, that has been washed in dilute acid as has been done in the present study, is intercellular.

A plot of phosphorus-32 concentration values on a logarithmic scale shows a somewhat linear decrease as long as the radioactivity is above 1,000 counts per minute (Figure 2). Below this level, however, curvature is apparent, indicating that the rate of loss of phosphorus-32 is decreasing. The decrease in loss rate is probably due to the uptake of re-cycled phosphorus-32 as has been

Figure 2. Concentration--time curves for periphyton at Stations 8 and 12 in 1959 and 1960. Curves fitted by eye. Each plotted point is based on a single observation.


PERIPHYTON
STATION 8
1960
1959
STATION 12
1960
1959
CONCENTRATION OF PHOSPHORUS-32
(CORRECTED COUNTS PER MINUTE PER GRAM)
8,000
5,000
1,000
500
100
50
5,000
1,000
500
100
50
0
7
14
21
28
35
42
49
DAYS AFTER SPIKE RELEASE

noted in other aquatic ecosystems (Hayes et al., 1952; Rigler, 1956; Whittaker, 1960). In this instance, however, the level of re-cycled phosphorus-32 is much lower because it is being continuously removed by the current. Twenty to thirty days after treatment, phosphorus-32 approached an equilibrium level which persisted with little change for the remainder of the summer. Slopes of the curves appeared to be quite similar during the initial logarithmic decrease (Figure 2). The equilibrium level was slightly higher in 1958 but was of similar magnitude all three years.

The greatest uptake at all stations occurred in 1960; the least in 1959; and in 1958 uptake was intermediate (Table IV). Average uptake values were calculated by summing the area under the concentration curves and dividing by the number of days. Average values for the first 20 days after treatment were up to fourfold greater in 1960 than in 1958. Average concentrations were as much as three times greater in 1958 than in 1959. The trend in periphyton uptake from year to year is perhaps related to the year-to-year differences in loss of phosphorus-32 from the waters noted above. Uniform rates of loss in 1959 were associated with low periphyton uptake. In 1959 and 1960 when there were extreme variations from section to section in loss of radioactivity from the water, there was high periphyton uptake.

The pattern of uptake by periphyton from station to station was quite different in various years. This pattern was not always related to the loss of radioactivity in various sections as the spike moved downstream because at some stations the periphyton continued to accumulate phosphorus-32 activity after the spike had passed. In 1959 the concentration increased at Station 12 for the first 24 hours after passage of the spike (Figure 1). In 1958 concentration in periphyton increased at four successive Stations (8 to 12) for the first 48 hours. In 1958 radioactivity at Station 14 showed little loss for a 12-day period after passage of the spike.

Table IV. Concentration of phosphorus-32 in periphyton from artificial substrates exposed to phosphorus-32 spike. Substrates exposed in stream two weeks before treatment with phosphorus-32. The periphyton from 4 substrates was pooled for each activity measurement.

Station	Activity density during 20-day period after the release of spike (corrected counts per minute per gram)								
	1958			1959			1960		
	Maximum	End of Period	Average[2]	Maximum	End of Period	Average[2]	Maximum	End of Period	Average[2]
3	7,900	280	1,600	926	24	440	36,700	584	6,840
8	2,220	350	1,350	2,750	138	1,020	7,837	120	2,760
12[1]	1,390	350	1,256	1,100	78	840	5,642	150	1,570
14[1]	1,934	400	1,100	750	100	454	28,594	100	4,340

[1]In 1958 this station located 150 yards upstream from site in 1959 and 1960.

[2]Average calculated by summing the area under concentration curve and dividing by 20.

Delayed uptake by periphyton was observed only at the downstream stations (below Station 8). The source of radioactivity for the delayed uptake must have been phosphorus-32 lost by periphyton and some other parts of the ecosystem upstream. Hence delayed uptake was highest at downstream stations which received the drift from a long section of stream. In 1960 when the uptake from the spike was high downstream, drift released by the upstream area did not give a detectable increase in radioactivity at any station. On the other hand, large increases were noted whenever uptake from the spike was very low, e.g., Stations 8 to 12 in 1958.

Uptake by Aquatic Macrophytes

The principal aquatic macrophytes of the stream were Potamogeton pectinatus, Fontinalis antipyretica, Chara sp., Nasturtium officinale, Elodea canadensis, and Ranunculus sp. Of these species, only the first three are of quantitative interest. Chara and Potamogeton occur along the stream margin and in backwaters where deposits of sand and silt are found. Chara is often found in midstream at the bottom of the deeper pools. The moss Fontinalis encrusts logs and other substrates.

Curves showing the change in concentration of phosphorus-32 for macrophytes were similar to those for periphyton (Figure 2). There was a similar logarithmic decrease at high radioactivity levels followed by a curve at levels below 1,000 counts per minute per gram. The curve becomes parallel to the X-axis in 15 to 20 days. As in the case of periphyton, the decrease in rate of loss of radioactivity was probably due to uptake of re-cycled phosphorus-32 from the water.

Concentration curves were similar for Chara and Potamogeton. The moss Fontinalis, however, consistently had a higher equilibrium level than Chara and Potamogeton (in most instances, two or three times greater). Another interesting feature of the radioactivity curves is that concentration curves of Nasturtium and Fontinalis were in some instances bimodal, that is, both showed a rise in phosphorus-32 concentration in late summer. This occurred only in 1960 and probably

resulted from uptake from decay of detritus in the bottom sediments rather than from the removal of phosphorus-32 from the stream water.

The concentration of phosphorus-32 by aquatic macrophytes never reached the levels found in periphyton (Table V). In many instances the maximum concentrations were very similar at a given station for all three species of macrophytes. Large and somewhat erratic differences in radioactivity noted between stations in the case of periphyton were not found in the case of macrophytes. In 1958 and 1959 maximum concentrations occurred at Station 3 and decreased downstream. In 1960 levels were somewhat more uniform throughout the experimental area.

Uptake by macrophytes was highest in 1959 and lowest in 1960 (Table V). In 1958 maximum concentrations were well below 1959 levels but averages for the 20-day period were only slightly less and in some instances were greater than those in 1959. As in the case of periphyton, an increase in radioactivity which occurred at downstream stations for several days after passage of the spike indicated that there had been a downstream drift of re-cycled phosphorus-32 which was taken up by plants. The large averages in 1958 may have arisen from a greater supply of re-cycled phosphorus.

The year-to-year changes in uptake of phosphorus-32 by periphyton were the opposite of aquatic macrophytes. The year of maximum uptake by macrophytes, 1959, was lowest in uptake by periphyton. In 1960, a year of high periphyton uptake, aquatic plants accumulated little phosphorus-32.

Re-cycled Phosphorus in the Stream Water

The concentration curves of periphyton and macrophytes clearly indicated that plants were removing phosphorus-32 continuously from the water during the period of study. Sources of this nuclide must have been: (1) the plants themselves which continue to lose phosphorus-32 that was adsorbed or incorporated by plant cells as the spike moved downstream, (2) the decay and mineralization of

Table V. Concentration of phosphorus-32 of three species of macrophytes during a 20-day period following treatment with phosphorus-32 spike.

Station	Species	Activity density (corrected counts per minute per gram) 1958 Maximum	1958 End of period	1958 Average[2]	1959 Maximum	1959 End of period	1959 Average[2]	1960 Maximum	1960 End of period	1960 Average[2]
3	Potamogeton	840	40	296	1,760	100	256	300	*	*
	Fontinalis	1,623	200	704	1,600	280	536	450	130	224
	Chara	682	80	172	560	40	200	430	20	90
8	Potamogeton	422	60	160	1,120	80	160	300	*	*
	Fontinalis	420	120	268	800	320	326	530	120	102
	Chara	519	80	168	540	80	166	570	50	93
12[1]	Potamogeton	183	75	122	300	20	165	100	*	*
	Fontinalis				300	160	156	450	100	102
	Chara	330	40	128	300	30	76	335	55	121
14	Pontamogeton				140	30	140	270	*	*
	Fontinalis				300	60	162	265	80	70
	Chara				110	30	110	180	30	58

[1]In 1958 this station 150 yards upstream from 1959 and 1960 location.

[2]Average calculated by integrating area under concentration curve using a graphic method and dividing by 20.

*Radioactivity not detectable for 20-day period.

plant and animal matter which had accumulated phosphorus-32, and (3) the release of phosphorus-32 adsorbed on sediments.

The level of re-cycled phosphorus-32 was generally below that which could be measured by the usual procedure of concentrating samples by evaporation and wet ashing. The only measures of this radioactivity that could be obtained were indirect measures secured by exposing artificial substrates to the stream water and allowing periphyton to concentrate the phosphorus-32. This procedure could be standardized to give a precise relative measure of re-cycled phosphorus-32. In this present study, however, exposure times for substrates introduced into the stream after the spike release have not been fully standardized, hence our data are of limited usefulness. There appears to have been a decrease in re-cycled phosphorus-32 with increasing time elapsed after the spike was introduced (Table VI). Although year-to-year comparisons are difficult, re-cycled phosphorus-32 apparently was lower in 1959 than either 1958 or 1960. The highest level was recorded in 1960 in the case of substrates exposed to phosphorus-32 passing downstream at Station 12 during the first 24 hours after the spike. Concentrations were much lower in 1960 for exposures of from 288 to 576 hours after passage of the spike than for substrates exposed from 288 to 456 hours after the spike in 1958. In most instances, as might be predicted, the level of re-cycled phosphorus-32 was higher at downstream stations than at upstream stations.

In 1960 a direct measurement was made of the level of re-cycled phosphorus-32 during the first 24 hours after the spike passed downstream. Samples were collected by the automatic water sampler, a device which samples water continuously for five minutes out of each 15-minute period. The average concentration for this period was 0.088 ± 0.016 counts per minute[1] per milliliter at Station 8, and 0.065 ± 0.0022 counts at Station 12, based on five samples at each station. The corresponding concentrations in periphyton substrates exposed during this 24-hour

[1]All averages in this paper $\pm$ one standard error of the mean.

Table VI. Level of re-cycled phosphorus-32 as measured by the concentration in periphyton exposed to this source of radioactivity. The periphyton from 4 substrates was pooled for each activity measurement.

Exposure in stream (hours after spike release)	Activity density (corrected counts per minute per gram) 1958 Station 3	8	12	1959 Station 3	8	1960 Station 8	12
1 to 4				60	80		
1 to 24				95	200	510	840
1 to 96				180	200		
1 to 168				100	250		
1 to 288						610	100
120 to 288		310	616				
288 to 456	284	447	321				
288 to 576						50	60
456 to 624	268	318	357				

period were 510 counts per minute per gram at Station 8 and 840 counts at Station 12. This gives a concentration factor at Station 8 of 5.8×10^3 and a factor of 1.29×10^4 at Station 12.

Uptake by Consumer Organisms

The uptake by a variety of invertebrates and fish was followed each year. An effort was made to collect sufficient data to plot a phosphorus-32 concentration curve for each species. Although a variety of animals were collected and analyzed for phosphorus-32, collection was soon discontinued for forms of rare occurrence and ones which were transient in the stream fauna. The species studied extensively then were: (1) forms found at all stations in sufficient numbers to provide a sample each week of at least one gram (wet weight) and (2) species that had one generation or a single brood that did not emerge from the stream during the period of our observation.

The concentrations in certain forms fluctuated so extensively that the results are of limited value. The small Physa sp. was abundant in the stream and several concentration measurements were made of this species. Even when a large number of specimens were pooled into single samples, concentrations varied erratically from place to place and from one collection date to the next. The concentration in fish also varied in an unpredictable manner. These fluctuations seemingly were superimposed upon a trend of increasing phosphorus-32 and probably arose from differences in the diet or feeding intensity of individual fish. Factors such as weather conditions and emergence flights of insects might also contribute to such fluctuations.

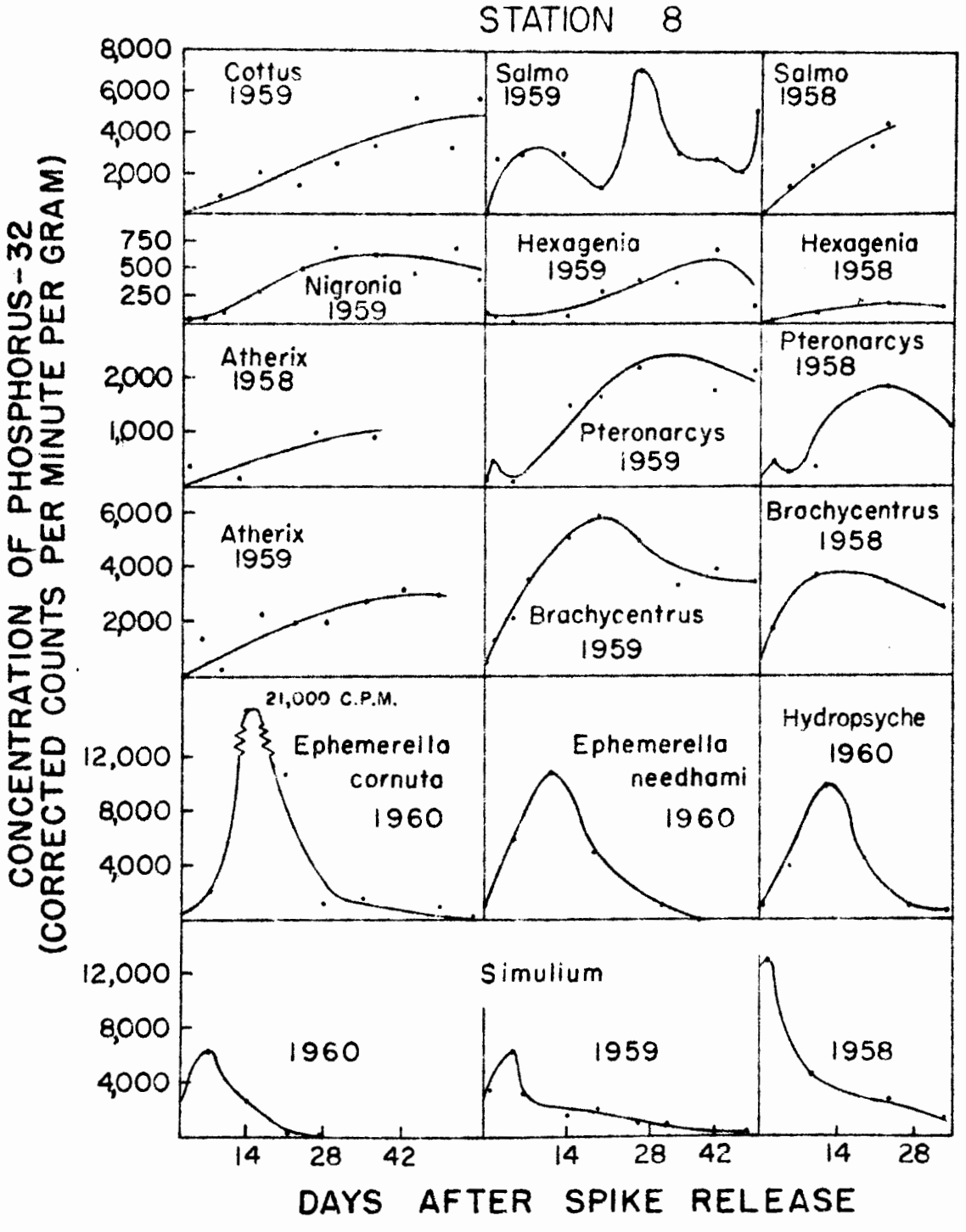
The downstream drift of immature insects in a trout stream is wide-spread in occurrence (cf. Waters, 1961) and undoubtedly accounts for the variability in uptake data for certain species. It was particularly noticeable at the most upstream station (Station 3) in 1958. In this year Station 3 was located scarcely 200 yards below the point at which the spike was introduced and it seems clear

that insects of low phosphorus-32 concentration from the untreated area above drifted downstream into our samples and gave erratic data. Forms showing variability which appeared to be due to drift were the snipe fly, Atherix, the caddis flies, Brachycentrus and Hydropsyche, and the stonefly, Pteronarcys. Less variability was noted in the concentration curves for burrowing forms such as the mayfly, Hexagenia limbata.

From concentration curves (Figure 3) it is clear that there were large differences between species in the magnitude and time of phosphorus-32 uptake. It is also clear that most of the curves differ considerably in shape from comparable curves for closed ecosystems treated with phosphorus-32 in which continuous re-cycling of a large fraction of the tracer took place (cf. Whittaker, 1961, p. 180). In the stream situation where re-cycling was minimized, a large number of the concentrations fell to zero or to a concentration which was small compared to the maximum within a 45-day period. In closed systems nearly all concentrations decrease only to a plateau or equilibrium level.

The passage of phosphorus-32 through the higher levels of the food chain appears to be chiefly by means of the ingestion of food (Davis and Foster, 1958). Although algae may hold considerable phosphorus-32 without assimilation, direct uptake is not a major source of phosphorus-32 in invertebrates and fish. Concentration curves are believed to reflect both metabolic turnover rates and the phosphorus-32 concentration of food assimilated by the organisms (Whittaker, 1961). It is difficult to distinguish between these two effects and it can be done only in certain instances. If the rate of elimination of phosphorus by organisms remains relatively constant then it would seem that the changes in slope of the concentration curve would reflect changes in the concentration of phosphorus-32 activity in assimilated food, namely, a sharp decrease in slope would indicate an assimilation of food of lower phosphorus-32 content and an increase in slope would indicate assimilation of food of higher content. In cases in which a slope

Figure 3. Concentration--time curves for a selected series of consumer organisms collected at Station 8 in 1958, 1959 and 1960. Each plotted point is based on one observation.


STATION 8
CONCENTRATION OF PHOSPHORUS-32
(CORRECTED COUNTS PER MINUTE PER GRAM)
8,000
6,000
4,000
2,000
Cottus
1959
Salmo
1959
Salmo
1958
750
500
250
Nigronia
1959
Hexagenia
1959
Hexagenia
1958
Atherix
1958
2,000
1,000
Pteronarcys
1959
Pteronarcys
1958
6,000
4,000
2,000
Atherix
1959
Brachycentrus
1959
Brachycentrus
1958
21,000 C.P.M.
12,000
8,000
4,000
Ephemerella
cornuta
1960
Ephemerella
needhami
1960
Hydropsyche
1960
12,000
8,000
4,000
Simulium
1960
1959
1958
14
28
42
14
28
42
14
28
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of one species is increasing while the slope of a second is decreasing it would appear that the food of the two species differs in phosphorus-32 concentration even though the two forms have different metabolic turnover rates.

The following phenomena are suggested by the characteristics of the concentration curves (Figure 3). (1) The shape of the curve for a given species is similar from year to year and from station to station. This suggests that the gross shape of the curve is a characteristic of the animal rather than of the environment. (2) The maximum concentration differed widely from year to year and from station to station for a given species. The maximum thus appears to be more closely related to stream conditions at the time of the spike and during the following period at which samples were collected. (3) Curves were similar for closely related species which are known to live in different microhabitats. For example, curves for nymphs of the mayfly Ephemerella cornuta which lives on sticks and submerged logs were similar to those of nymphs of Ephemerella needhami which were nearly always found in the moss Fontinalis. (4) The smaller forms, for example, Simulium sp. and Ephemerella consistently showed higher concentrations than larger forms, for example, Hexagenia limbata, Nigronia sp., and Pteronarcys. Phosphorus-32 concentration increased rapidly to a peak and decreased sharply thereafter for small forms while larger invertebrates showed a much slower rise and decline. This relationship between size and concentration has been noted by Whittaker (1961) and is undoubtedly due to differences in relative growth and metabolic turnover rates between small and large animals. (5) Many differences in the slope of the concentration curves cannot be explained by size differences, for example, Atherix variegata, a small predacious soft-bodied larva, reached a relatively low concentration which increased slowly during the entire period of study. (6) In many instances phosphorus-32 was being lost in one species while being taken up at an increasing rate by another, for example, Simulium sp. and Ephemerella cornuta in 1960 (Figure 3). Such differences appear

to be more closely associated with the differences in concentrations in assimilated food than with other factors.

To assist in comparison of the concentration curves, the areas under the curves were summed using graph paper. From the area the median time and the time quartiles for the concentration curves were determined. In instances in which the concentrations had fallen to a low level they were extrapolated to the base line for the purpose of calculating the median. In instances in which concentration curves were increasing, remaining constant or at a high level at the end of the period of observation, no attempt was made to calculate the median or quartiles. Maximum concentrations, time of maximum concentration, and median time for consumer organisms are given in Table VII.

Concentration data were collected during all three years for only one species, the blackfly, Simulium. This species had its highest concentration in 1958 at Stations 3 and 8 but in 1960 was highest at Station 12. Concentrations were lowest in 1959 for this species at all stations except Station 12. Comparisons between 1958 and 1959 can be made for seven additional species (Table VII). Concentrations were highest in 1959 in the caddis fly, Brachycentrus sp., the stonefly, Pteronarcys sp., and the fishfly, Nigronia sp., the sculpin, Cottus cognatus, and for the brown trout, Salmo trutta. The concentration curves were very similar for the two years for the snipe fly, Atherix, although higher at Station 12 in 1959. Values for the burrowing mayfly, Hexagenia, which inhabits mud banks were highest in 1958 at all stations except Station 8.

The factors responsible for the year-to-year differences are not apparent in some instances but some possible relations are suggested by the data. Low uptake by the filter feeder, Simulium in the upstream area in 1959 was associated with low uptake by periphyton and it is known that a larger fraction of water phosphorus-32 was in particulate form in this area in 1959 than in 1960. Thus there was a smaller fraction of soluble phosphorus-32 available for suspended

Table VII. Maximum concentration of phosphorus-32 and median time of concentration--time curve for consumer organism of stream ecosystem. Data collected for 35 days after phosphorus-32 spike in 1958, 55 days in 1959 and 56 days in 1960.

Species	Year	Maximum Corrected counts per minute per gram				Activity density Time (days after spike)				Median time (days after spike)			
		Station[1]				Station[1]				Station[1]			
		3	8	12	14	3	8	12	14	3	8	12	14
Simulium sp.	1958	8,300	13,200	3,100		2	2	1		10	10	10	
	1959	3,200	5,900	4,500	5,100	4	4	7	7	8	8	8	11
	1960	2,600	7,000	12,000	9,500	6	6	6	6	10	10	9	10
Hydropsyche sp.	1960		10,000	4,600	10,200		13	13	6		14	16	14
Ephemerella needhami	1960	23,000	12,000	6,500	31,000	20	13	6	20	17	15	16	20
Ephemerella cornuta	1960	17,000	21,000	4,750	9,500	20	13	20	20	19	16	18	19
Gammarus sp.	1960				1,300				27				20
Brachycentrus sp.[2]	1958	2,350	3,700	1,950		2	10	24		19	22	30	
	1959	3,200	6,000	3,850	4,000	28	21	21	21	27	*	30	27
Cordulegaster sp.	1958		1,500				24				24		
Pteronarcys[2]	1958	1,900	1,800	825		10	24	24		*	25	19	
	1959	4,000	2,300	1,600	1,000	28	35	28	35	32	*	*	31
Entosphenus lamottenii[2]	1959	160	150	300	200	28	49	42	28	22	36	39	27
Nigronia sp.	1958	600	800	700		24	24	33		*	*	*	
	1959	2,500	600	1,700	700	35	28	35	21	*	*	*	*
Hexagenia limbata	1958	425	470	450		33	24	35		*	*	*	
	1959	250	750	200	400	35	49	49		37	34	*	*
Atherix variegata	1958	8,700	2,700	2,400		24	24	10		*	*	*	
	1959	8,000	3,000	6,400	2,900	49	42	42	28	*	40	37	23
Salmo trutta	1958	5,700	4,600	3,800		20	23	23		*	*	*	
	1959	7,100	7,200	4,800	3,800	28	28	28	49	*	*	*	*
Cottus cognatus	1958	7,200	4,500	3,100		20	20	23		*	*	*	
	1959	7,600	5,700	7,500	4,500	49	55	55	28	*	*	*	*

[1]In 1958 Station 3 located 150 yards upstream and Station 12 150 yards downstream from locations in 1959 and 1960.

[2]Concentration curves extrapolated to baseline to calculate median.

*Median could not be determined. (Activity density increasing or at high level at time of final measurement.)

particles such as diatoms which are removed from the water by blackflies. The high concentration in Hexagenia in 1958 might be associated with a greater concentration in periphyton detritus which settles into mud banks. Trout and sculpin were highest in phosphorus-32 in 1959 when the concentration was high in Brachycentrus, Pteronarcys, and Nigronia rather than in 1958 when it was high in blackflies and burrowing mayflies.

For most species concentrations tended to be high upstream and to decrease downstream. An exception to this rule was Simulium in which concentrations increased from Station 3 to Station 12 in 1960 and from Station 3 to Station 8 in 1958 and 1959. A greater supply of phosphorus-32 in the food for this filter feeder might be expected for individuals living in the downstream area. In 1960 the pattern of concentration at various stations for the periphyton feeder Ephemerella needhami, was similar to the pattern of distribution of phosphorus-32 in periphyton, that is, it was high at Stations 3 and 14 and considerably lower at Stations 8 and 12.

The organisms listed in Table VII have been arranged in order of increasing median time of their concentration. This order follows closely the order of time of maximum concentration for all species in which the concentration curves were complete. Curves were not complete for the nine species of the lower part of the list. For these forms it was not possible to calculate a median and the arrangement is based upon the trend of concentration curves. Species with curves indicating persistence of phosphorus-32 are last on the list. For the last five species the maximum concentration values given were the highest radioactivity recorded during the period of observation. For these forms higher values might have occurred after the period of study. The order in which forms are listed gives an index as to the rate at which phosphorus moves through this community. It passes rapidly through the smaller filter feeders (Similium and Hydropsyche) and periphyton scrapers (Ephemerella). Phosphorus-32 persists somewhat longer

in the omnivorous caddis fly (Brachycentrus), while the large detritus feeders such as Pteronarcys and ammocoetes of the brook lamprey Entosphenus accumulate and hold phosphorus-32 a still longer period. Phosphorus-32 is retained longest by small (Atherix) and large (Nigronia) invertebrate predators as well as by vertebrate predators (Salmo and Cottus). The ooze browser Hexagenia had a concentration curve similar to those of predators insomuch as its concentration of phosphorus-32 continues to increase for an extended period (over 49 days). Despite a steady increase its radioactivity remained at a comparatively low level. This suggests a continuous supply of food with low phosphorus-32 concentration from which Hexagenia continued to concentrate phosphorus-32. Radioactive detritus moving into the mud banks is probably diluted with ooze of low phosphorus-32 concentration.

Discussion

Year-to-year differences in phosphorus uptake are perhaps the most interesting data of the present investigation. In 1959 release of the spike of Phosphorus-32 gave a comparatively uniform distribution of radioactivity within the experimental area, high uptake by aquatic macrophytes, low uptake by periphyton, a low level of re-cycled phosphorus-32, and high uptake by certain omnivores and detritus feeders and by fish. In 1958 and 1960 introduction of a spike in exactly the same manner led to much higher uptake by periphyton and lower uptake by aquatic macrophytes, a higher level of re-cycled phosphorus-32 and a comparatively high uptake by ooze browsers and filter feeders. These major differences in the cycling of radioactivity may have been related to a rather subtle factor, namely, the extent to which the phosphorus-32 distributed itself between soluble and particulate phases in the stream water. This emphasizes that the superstructure of the ecosystem may be drastically influenced by seemingly minor changes at the nutrient level.

It has been suggested that phosphorus-32 can be used to analyze community

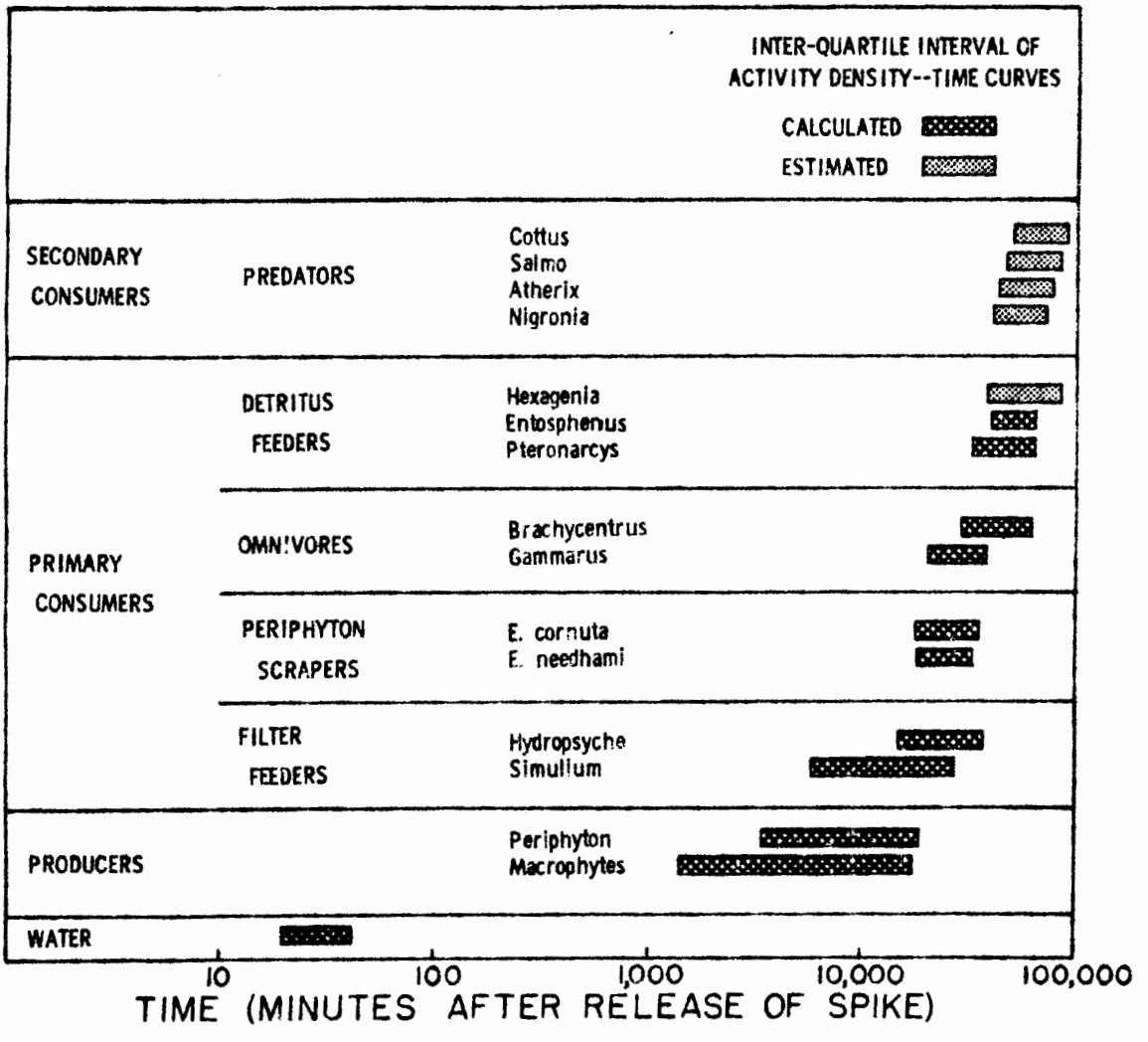
energetics. Whittaker (1961) has constructed a food pyramid based upon the uptake rates of phosphorus-32 entering various community fractions. Beyond question the broad trophic levels can be quantitatively distinguished by careful study of concentration curves (Figure 4). These levels are apparent in the data of the present study (Figure 4). But to proceed with analysis of community energetics, one must assume that phosphorus uptake is rather precisely related to a unit of energy fixation in plants and that this energy relationship is retained quantitatively through various transfers in the food pyramid. Since many uncertainties exist regarding these assumptions, it would seem that at present phosphorus-32 is most useful in defining pathways of energy flow rather than in giving quantitative expressions to energy transfers. It would appear to be especially useful in defining more closely the trophic position of many omnivores and detritus feeders whose energy input is not wholly from either the producer or consumer levels but perhaps from a combination of producers, consumers, and decomposers. The phosphorus-32 uptake of such forms must reflect the relative importance of these sources. In the present investigation there are certain detritus feeders whose food was on the average farther removed in time from energy fixation than certain predators. To proceed farther along such lines of investigation requires a background of information on metabolic turnover rates of the fauna and flora.

Summary

The movement of radiophosphorus through the ecosystem of a cold water stream was studied by adding a spike of approximately 23 millicuries of phosphorus-32 to the water during the summers of 1958, 1959, and 1960. The period of time required for the spike to pass various points in the study area was from 33 minutes at the most upstream point to 180 minutes at a point farthest downstream. The theoretical maximum water concentration from the spike was 1.22×10^{-5} microcuries per milliliter.

Figure 4. Time of phosphorus-32 activity of components of the streams ecosystem. The inter-quartile interval for time has been determined by summing the area under the activity density curves. The time interval plotted therefore represents the central 50 per cent of the time various components had a detectable activity. All activities were corrected for decay. Estimated quartiles have been calculated by extrapolation of activity density curves and are therefore rough approximations. Data used are from Stations 8 and 12 in 1959 and 1960.

TIME OF PHOSPHORUS-32 ACTIVITY



Although the same amount of phosphorus-32 was added to the stream for each of the three years, the pattern of loss of phosphorus-32 from the water differed considerably from year to year. In 1958 and 1960 the rate of loss was exceedingly variable from section to section within the study area. The average distance a phosphorus-32 atom traveled before being removed from the water varied from 450 to 11,236 yards. In 1959 loss from the water was at a much more uniform rate; average distance varied between 1,201 and 1,818 yards. There was also a large year-to-year difference in the form in which the phosphorus-32 was present while in the stream water. In 1959 by the time the water had traveled 200 yards from the release point, 70 per cent of the tracer was incorporated into solids and only 12 per cent was in soluble form. By contrast in 1960 at the same point in the stream 65 per cent was in soluble form and 13 per cent had been incorporated into solids.

Much of the uptake from the water appeared to be by periphyton and by three species of aquatic macrophytes (Potamogeton, Chara, and Fontinalis). All of these plants had a maximum concentration of phosphorus-32 soon after the spike passed through the area. The rate of loss of phosphorus-32 from the plant to the water was initially high but decreased with time. The concentration of phosphorus-32 approached an equilibrium level in 15 to 20 days after the spike was released, and the change in the rate of decrease suggested that plants were removing recycled phosphorus from the stream water. Uptake by plants tended to be highest near the site of release and decreased downstream. The highest uptake by periphyton was in 1958 and 1960. Uptake by periphyton was exceedingly low in 1959. The year-to-year pattern in uptake by aquatic macrophytes was the reverse of that for periphyton. Maximum uptake values were highest in 1959 and were lowest in 1960--in 1958 they were in most instances intermediate. Uptake was somewhat higher by the aquatic moss Fontinalis and by Potamogeton than by Chara. Fontinalis had a higher equilibrium level than other species.

A measure of the level of regenerated or re-cycled phosphorus in the stream water was obtained by exposing the periphyton growing on artificial substrates to this source of phosphorus. Using this method it appeared that the lowest level of re-cycled phosphorus was in 1959 and that the level was considerably higher in 1958 and 1960.

Concentration curves for consumer organisms within the ecosystem reflected both differences in metabolic turnover rates and food relationships. Concentrations rose to a high level in a short period of time in the case of the small filter feeder Simulium. This species appeared to accumulate much of its phosphorus during passage of the spike. Other small filter feeders and periphyton scrapers also accumulated phosphorus-32 rapidly and reached a high level. These invertebrates also lost phosphorus-32 rapidly. Accumulation of phosphorus-32 was much slower for large omnivorous stream insects and for predacious forms even though they were of a comparatively small size.

Uptake by the filter feeder Simulium was high in 1958 and 1960, and low in 1959. Uptake was also higher in 1958 than in 1959 for the burrowing mayflies. On the other hand, somewhat higher concentrations of phosphorus-32 were noted for fish and certain of the large omnivorous insects in 1959 as compared with 1958.

Differences in the manner in which the spike circulated through the ecosystem in 1959 as compared to 1958 and 1960 are believed to be largely the result of a difference in distribution of phosphorus-32 between the soluble and particulate phases following its addition to the stream water.

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