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# PROGRESS REPORT TO THE ATOMIC ENERGY COMMISSION

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## A third report on translocation of radioactive phosphorus ($P^{32}$) in a Michigan trout stream

by

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Introduction.--Features of the project emphasized during 1960 were as follows: (1) We secured further data on the uptake of phosphorus by various stream sections and attempted to relate uptake to the amount of phosphorus present in each section. We again added fertilizer containing phosphorus to certain stream sections. (2) We explored further the relationship between the form of activity and uptake. Earlier work had suggested that much of the activity passed into particulate form. (3) We explored the possibility that activity in the stream could be displaced significantly by the addition of a chelating agent. (4) We secured more data on the level of regenerated phosphorus activity for an extended period after $P^{32}$ treatment.

(5) The level of activity in adult insects emerging from the stream was determined. (6) We gathered further data on a possible trophic classification of trout stream invertebrates based upon their activity curves.

Study area and techniques.--The study area was identical to that used in 1958 and 1959 (Fig. 1). The same amount of isotope was applied to the stream as in previous years (23 millicuries). Application was made on July 5. The technique used in applying the isotope to the stream was identical to that used in 1958 and 1959; hence there was the same theoretical maximum concentration of isotopes as in 1958 and 1959 ($1.22 \times 10^{-5}$ microcuries per milliliter).

Comparison of $P^{32}$ uptake in various sections of the Sturgeon River, 1958-1960.--Since identical amounts of radiophosphorus were used each of the 3 years, comparisons can be made of relative uptake by different parts of the stream. For this analysis the stream was divided into sections which had been sampled each year (Table 1). From activity profiles secured from samples taken at the station furthest downstream in each section (stations 5, 8, 12, 14 and 16; see Table 1), the total activity passing through each section was calculated. The activity passing through a section was subtracted from that entering to obtain total uptake. From the total uptake the uptake per yard, percentage uptake and percentage uptake per 100 yards, were calculated (Table 1). Of these data, percentage uptake per 100 yards is perhaps most significant since it indicates how well a section removed the radiophosphorus that was available.

There were vast differences in uptake during the 3 years. In 1958, 70 percent of the activity was removed in section A (see Table 1 for location of sections) giving 12.8 percent uptake per 100 yards; in

Figure 1 - West Branch of Sturgeon River.
Stream sections designated by
letters and stations by numbers.

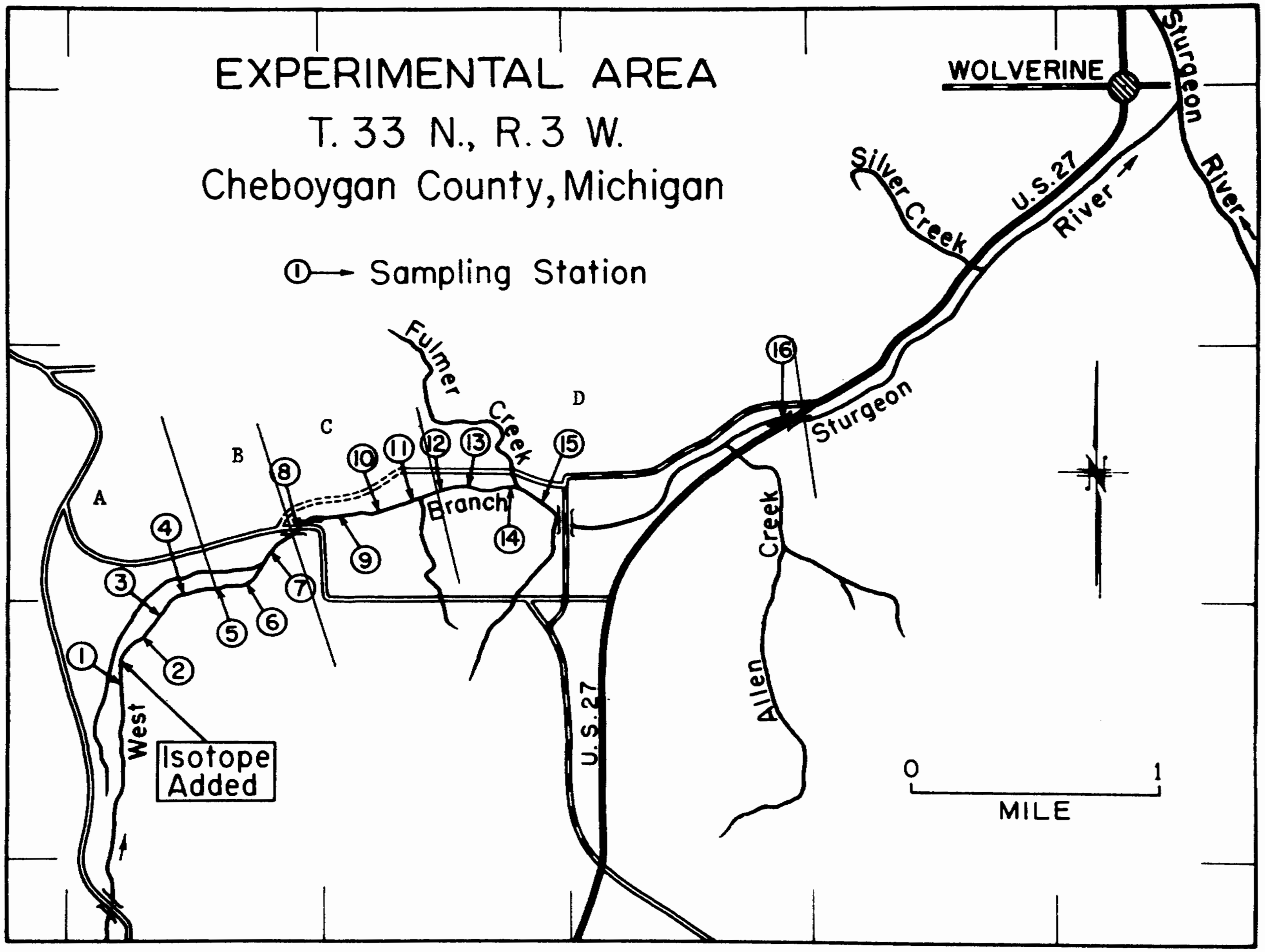
EXPERIMENTAL AREA
T. 33 N., R. 3 W.
Cheboygan County, Michigan
① Sampling Station
WOLVERINE
Sturgeon River
Silver Creek
U.S. 27
River
Fulmer Creek
Sturgeon
Branch
West
Isotope Added
Allen Creek
U.S. 27
A
B
C
D
0
1
MILE

Figure 1

Table 1.--Uptake of radiophosphorous by various sections of the West Branch of the Sturgeon River, 1958-1960. Twenty-three millicuries of $P^{32}$ added each year.

| | | Activity in Microcuries | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1958 | | | | | 1959 | | | | | 1960 | | | | |
| | | Passing through | Uptake | | | | Passing through | Uptake | | | | Passing through | Uptake | | | |
| Section (Stations) (flow cfs) | Length (yards) | | Total | Per yard | Percentage[1] | Percentage per 100 yards | | Total | Per yard | Percentage[1] | Percentage per 100 yards | | Total | Per yard | Percentage[1] | Percentage per 100 yards |
| A (1-5) (38.73) | 550 | 6,811 | 16,189 | 29.4 | 70.4 | 12.8 | 16,618 | 6,382 | 11.6 | 27.8 | 5.05 | 12,004 | 10,996 | 19.99 | 47.81 | 8.69 |
| B (5-8) (43.48) | 450 | 6,186 | 625 | 1.39 | 9.18 | 2.04 | 12,534 | 4,084 | 9.08 | 24.5 | 5.44 | 11,525 | 479 | 1.06 | 3.99 | 0.87 |
| C (8-12) (47.53) | 1,550[2] | 4,961 | 1,225 | 0.98 | 24.7 | 1.59 | 3,454 | 9,080 | 5.86 | 72.4 | 4.67 | 5,504 | 6,021 | 3.88 | 52.2 | 3.67 |
| D (12-14) (49.72) | 600[3] | 2,327 | 2,634 | 3.76 | 53.1 | 7.59 | 2,492 | 962 | 1.60 | 27.9 | 4.65 | 4,522 | 982 | 1.64 | 17.8 | 2.97 |
| E (14-16) (52.5) | 2,100[4] | 524 | 1,803 | 0.78 | 77.5 | 3.37 | | | | | | 1,437 | 3,085 | 1.47 | 68.2 | 3.24 |

[1] Percentage of isotope entering section that was taken up by section .

[2] 1,250 yards in 1958.

[3] 700 yards in 1958.

[4] 2,300 yards in 1958.

sections B and C little activity was removed (2 percent or less per 100 yards); in section D the uptake increased markedly and it remained relatively high in section E. In 1959, percentage uptake per 100 yards was almost identical in sections A through D (4.6 to 5.0 percent). Because of an artificial disturbance to section E in 1959, no uptake data were secured for this section. In 1960, the pattern was similar to that in 1958, i.e., relatively high uptake in section A, very little uptake in section B, and a higher rate in sections D through E.

The size of the pool of participating phosphorus in the environment has been suggested as a factor which influences uptake rate. It is assumed that radioactive atoms are freely exchanged with non-radioactive atoms throughout the system. In all 3 years the size of the phosphorus pool was artificially increased by additions of fertilizer. Treatment of the stream with diammonium phosphate has been shown to increase the phosphorus content of the periphyton for a distance of 1,000 yards or more downstream.[1/]

As might have been predicted, there appears to be a relationship between uptake of $P^{32}$ and the history of fertilization (Fig. 2). The high uptake in section A in 1958 and 1960 was associated with substantial additions of diammonium phosphate. A smaller amount of phosphate appears to have increased uptake in section D in 1958. In 1959, diammonium phosphate was not added to the stream, only small quantities of a granulated commercial fertilizer (10-10-10). These treatments served only to accelerate periphyton growth on certain sets of shingles prior to the addition of the isotope and probably had little effect upon the size of

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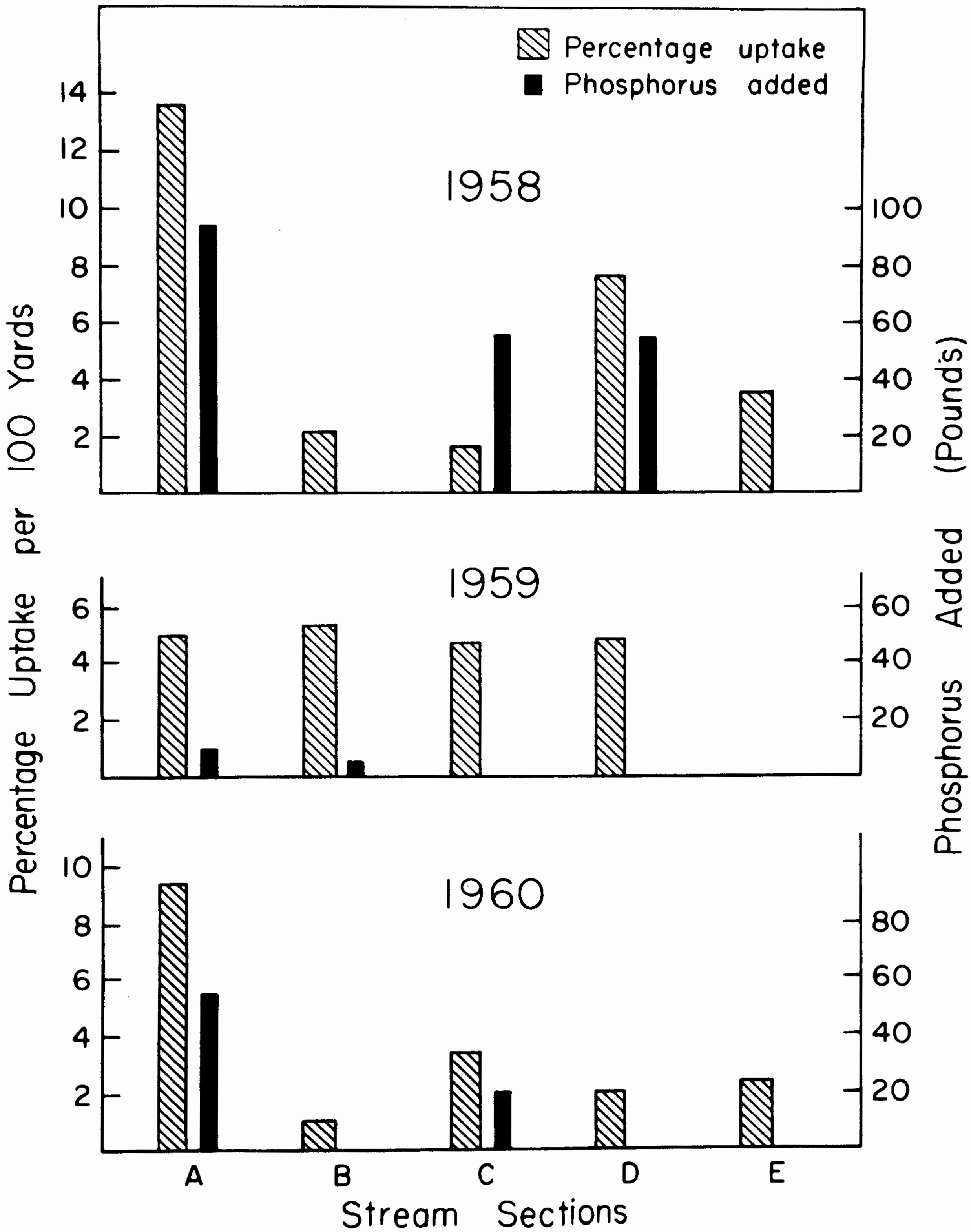
1/ Clifford, Hugh F., 1959. Response of periphyton to phosphorus introduced into a Michigan trout stream. M.S. thesis, Michigan State University.

Figure 2.--Relationships of uptake of $P^{32}$ by stream sections to amount of phosphorus added to sections as fertilizer prior to treatment with isotope.



Percentage uptake
Phosphorus added
1958
1959
1960
14
12
10
8
6
4
2
100
80
60
40
20
Percentage Uptake per 100 Yards
Phosphorus Added (Pounds)
A
B
C
D
E
Stream Sections


**Figure 2**

the phosphorus pool. The relatively constant uptake rates for various sections in 1959 as compared with 1958 and 1960 is believed to be due in large part to the fact that, in 1959, additions of phosphorus to the stream were small and insignificant.

Some facts do not fit the hypothesis that differences in uptake rates were associated with the amount of fertilizer received by various stream sections. For example, in 1958 section C received 56 pounds of phosphorus but showed an uptake of only 1.59 percent per 100 yards. Similarly some increase in the size of the phosphorus pool in section B in 1958 and 1960 might be expected from additions of phosphorus to section A. However, there was a very low uptake in section B in both of these years. Rather than an enhancement of uptake it appears that uptake in B was suppressed by the addition of phosphorus to section A.

Evidence that low uptake in sections B and C in 1958 was associated with low periphyton uptake, comes from the activity of periphyton removed from the stream on July 5, 4 hours after dosage with $P^{32}$ (Table 2). There was high periphyton uptake at stations 3 to 7 but much lower uptake at stations 8 through 10 (lower portions of section B and most of section C). Uptake was again high at station 11, immediately downstream from a point at which fertilizer was applied two times during the previous month. Periphyton at station 8 continued to build up activity during the period August 5-7. During the same period periphyton activity remained almost constant at stations 9-12, but there were large losses of activity at stations 3-7, as well as at stations 13 and 14. Thus, there is a clear suggestion that so little activity was taken up by periphyton at station 8 while the major dose passed downstream, that regenerated $P^{32}$ (at a much lower activity level) was able to increase its activity. This was not true at stations which had a high initial uptake.

Table 2.--Uptake of $P^{32}$ by periphyton after treatment with isotope, August 5, 1958

(Counts per minute per gram)

| Station | Time elapsed after treatment | | |
| --- | --- | --- | --- |
| | 4 Hours | 3 Days | 7 Days |
| 1 | 0 | 0 | 0 |
| 2 | 1,733 | 2,139 | 2,178 |
| 3 | 7,972 | 2,910 | 1,167 |
| 4 | 5,611 | 5,298 | 991 |
| 5 | 4,266 | 1,427 | 440 |
| 6 | 4,222 | 1,368 | ... |
| 7 | 4,236 | 1,202 | 350 |
| 8 | 2,222 | 3,559 | 2,191 |
| 9 | 1,482 | ... | ... |
| 10 | 1,481 | 1,504 | 1,943 |
| 11 | 3,707 | 3,834 | 1,491 |
| 12 | 1,390 | 1,393 | ... |
| 13 | 1,728 | 1,107 | 910 |
| 14 | 1,934 | 182 | 1,174 |

A somewhat tentative hypothesis can be advanced to explain the low uptake zones downstream from a section receiving heavy fertilization (e.g., sections B and C, 1958, section B, 1960). A massive addition of soluble fertilizer may create such a large pool of participating phosphorus that an adjacent section downstream may become phosphorus-starved. Natural phosphorus may be rather completely stripped from the water in moving downstream through such a large pool. Thus after fertilization of section A the supply of natural phosphorus to section B may have been cut off or drastically reduced. Very little phosphorus may have been accumulated during the period between fertilization and the addition of radioactive phosphorus. By the time the radiophosphorus was added this section may have had a smaller than normal phosphorus pool. Some support for this hypothesis comes from an earlier finding that the phosphorus content of periphyton was lower than normal on substrates placed downstream from an area in which the periphyton was unusually high in phosphorus (cf. Table 3, stations 9 through 12).

Form of phosphorus in stream water.--By filtering samples through a millipore filter we demonstrated, in 1959, that a large fraction of the radiophosphorus was in solid form. Autoradiographs showed that this activity was dispersed rather uniformly over the filter pad and was in the form of small particles. It was believed to be $P^{32}$ incorporated into bacteria or small diatoms. In 1960, we measured the fraction of activity present in the soluble, acid soluble, and particulate form. Acid soluble phosphorus is the phosphorus brought into solution by dilute HCl.

Comparison of the 1959 and 1960 data (Fig. 3) indicates that a larger fraction of activity in 1959 was in particulate form. This difference may in part account for the more uniform distribution of activity

Table 3.--Mean value of total phosphorus content of periphyton on substrates exposed for one week in the stream at substations between stations 11 and 13.

(Phosphorus in parts per billion)

| Substation | Date | |
|---|---|---|
| | July 1-8 1958 | July 11-18 1958 |
| 1 | 107 | 56 |
| 2 | 50 | 79 |
| 3 | 117 | 60 |
| 4 | 48 | 44 |
| 5 | 76 | 60 |
| 6 | 89 | 35 |
| 7 | 125* | 64 |
| 8 | 144* | 111 |
| 9 | 94* | 42 |
| 10 | 153* | 47 |
| 11 | 119* | 42 |
| 12 | 166* | 47 |

* Stations exposed to 80 pounds of fertilizer, July 3-5.

Figure 3.--Percentage of activity of stream water attributed to soluble, acid soluble, and particulate matter.

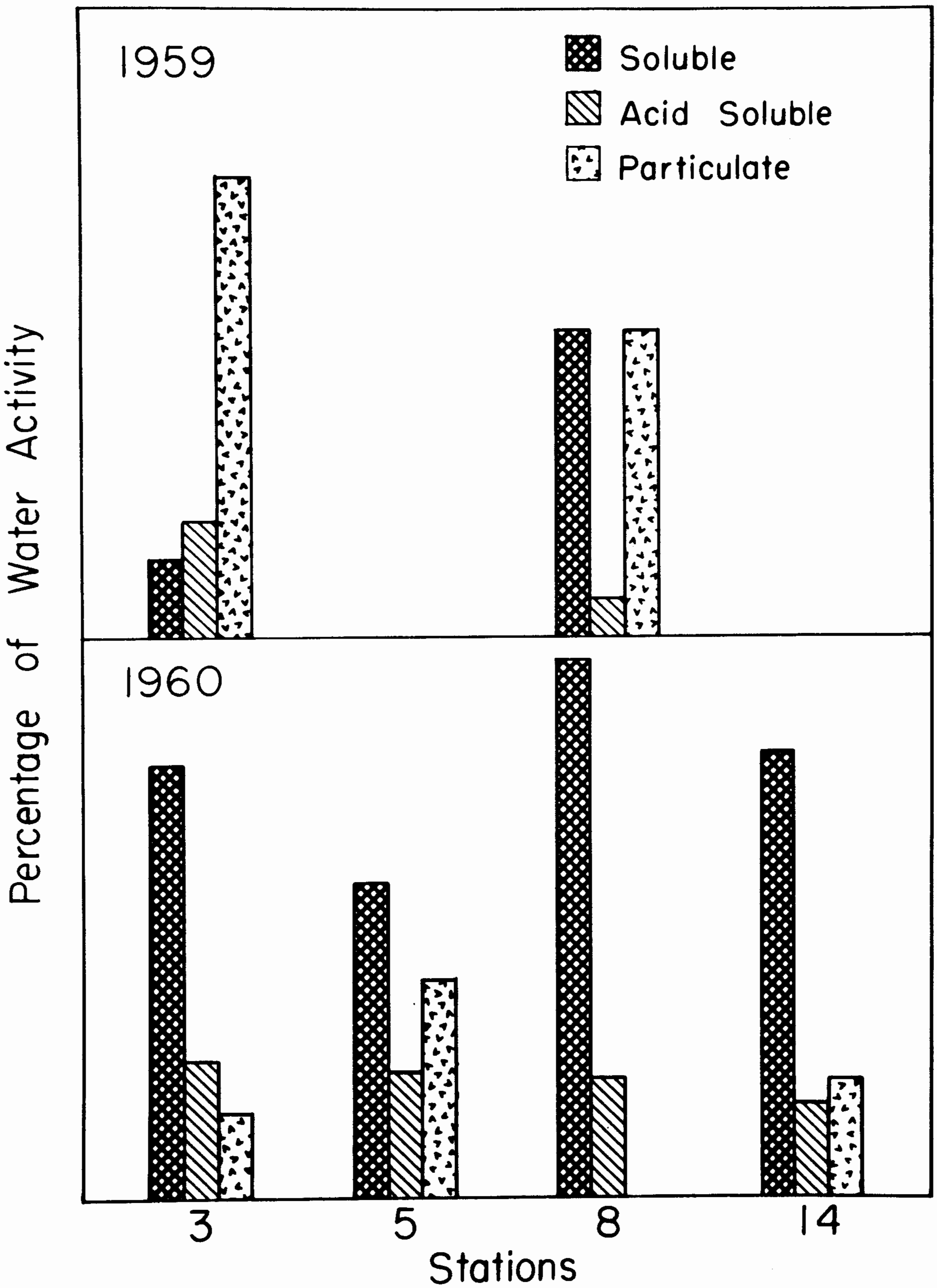

1959
Soluble
Acid Soluble
Particulate
1960
Percentage of Water Activity
3
5
8
14
Stations

Figure 3

through various sections in 1959, as compared to 1960. The percentage of solid activity in 1959 was much greater at station 3 than at station 8. This suggests that activity was first incorporated into solids upon being added to the stream water but passed back into soluble form as it moved downstream.

The difference in ratio of particulate to soluble phosphorus in 1959 as compared to 1960 may have been related to the condition of the stream prior to treatment. In 1959, the stream had just returned to normal stage after a week of heavy rains. Rains have the effect of flushing bacteria and organic materials from bogs and back-waters. Thus more particulate matter and its attached bacterial flora may have been present in the stream in 1959 than in 1960. By contrast, a period of warm, dry weather preceded treatment in 1960 and the stream was exceptionally clear.

The hypothesis that differences in the ratio of particulate to soluble activity influenced the distribution of phosphorus in the stream system is a subject worthy of further study. As pointed out below, there were major differences in the flow of phosphorus through the food chain in 1959, as compared to 1960. Demonstration that a change of form of phosphorus led directly to these differences would be a finding of considerable ecological interest. Thus for 1961 we are proposing treatment of the stream with radioactive bacteria.

To further analyze the activity of floating particulate matter we filtered 20-quart samples of water through a plankton net of No. 20 bolting silk. These samples of macro-particulate material were collected at stations 8 and 12, approximately 30 minutes after the isotope reached these stations. These solids were digested and processed for counting in the usual manner. Our results indicate that only a small fraction of the stream activity at this time was concentrated in particles large enough to be held by a No. 20 mesh net (173 meshes per inch). At station 8, 0.023 percent and at station 12, 0.233 percent of total water activity was in this form.

Level of regenerated activity.--When marked phosphorus atoms are introduced into the phosphorus pool of a stream ecosystem, the length of time the marked atoms stay in the pool and the rate at which they are fed back into the water is a function of the number of phosphorus atoms contained in the pool. The activity level of this regenerated phosphorus in the stream is low and cannot be measured directly. It would appear that the best available measure of this activity is that obtained by suspending substrates in the stream and determining the activity picked up by periphyton. Although such a bio-assay type of measurement lacks precision, it appears to be a useful tool in studying the dynamics of the phosphorus movement.

The only data from the 1958 experiment which provided a measurement of the regenerated phosphorus level were from substrates introduced into the stream 19 days after treatment and removed 26 days after treatment. Periphyton on these substrates accumulated between 268 and 621 counts per minute of activity per gram. The data suggest a rather uniform uptake through most of the experimental area. It was, however, somewhat lower at the station furthest upstream and higher at the station furthest downstream. In 1959, the only data obtained were from substrates exposed to regenerated activity for the first 168 hours following treatment; during this period activity from regenerated phosphorus remained between 200 and 250 counts per minute per gram at station 8 and between 100 and 175 counts per minute per gram at station 3. The 1960 information (Fig. 4) indicates a high level for the first 24 hours at both stations 8 and 12. Activity remained high for the first two weeks

Figure 4.--Activity levels of periphyton. The only source of this activity was from regenerated isotope.

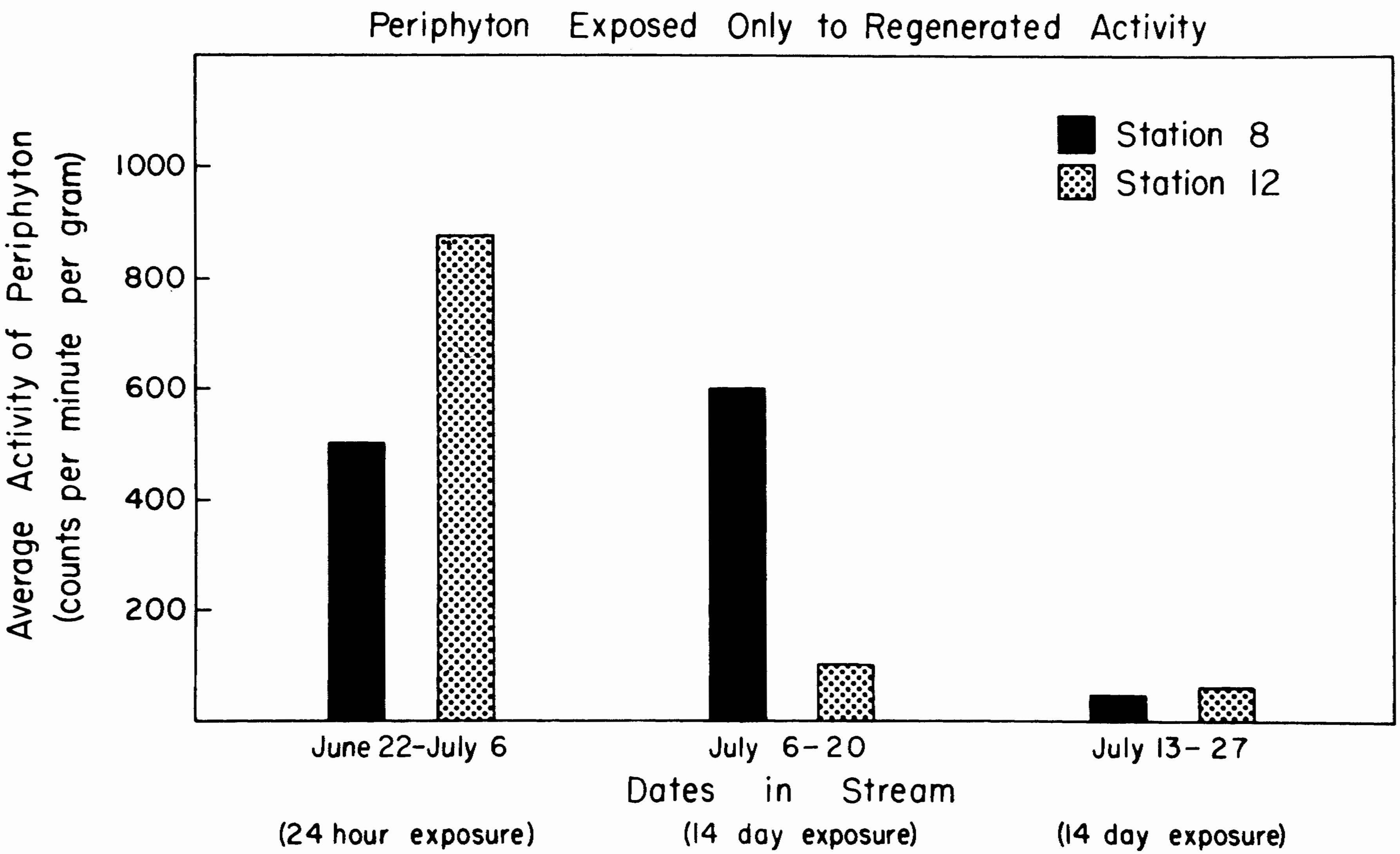

Periphyton Exposed Only to Regenerated Activity
Station 8
Station 12
Average Activity of Periphyton
(counts per minute per gram)
1000
800
600
400
200
June 22–July 6
July 6–20
July 13–27
Dates in Stream
(24 hour exposure)
(14 day exposure)
(14 day exposure)

Figure 4

at station 8 but fell sharply at station 12. Substrates exposed to the regenerated activity present in the stream from 7 to 21 days after treatment had an activity of 50 counts per minute per gram or less at stations 8 and 12. This is far below the levels encountered for exposure between 19 and 26 days in 1958. Thus the somewhat fragmentary data that can be compared in various years suggests that the stream sustained the highest level of regenerated activity (hence presumably had the largest pool of participating phosphorus) in 1958. The level was lowest in 1959 and seemed to be somewhat intermediate in 1960.

Influence of chelating agents upon the distribution of activity.--In an effort to find out whether or not chelating agents would displace the activity in the stream measureably, we added chelated iron to the stream 48 hours after the isotope treatment. The iron chelate used was iron, sodium N-hydroxyetheylethylenediamine-triacetate (NaFeEEDTA). This compound was bled into the stream at station 8 for a 24-hour period. The concentration of chelated iron in the stream was approximately 1 part per million. During the application period water samples were collected at several points downstream. The activity level, phosphorus content, and iron content of these samples were later accurately determined. At the present time these data have not been thoroughly analyzed but superficial inspection has not revealed significant changes in either activity or in the phosphorus content of the water during the release of chelated iron. Further analyses of these data are in progress.

Trophic classification of trout stream invertebrates.--Activity curves of a number of immature insects studied in the 3 years are shown in Figure 5. Several features of these activity curves deserve comment.

Figure 5.--Activity curves of a number of immature aquatic insects.

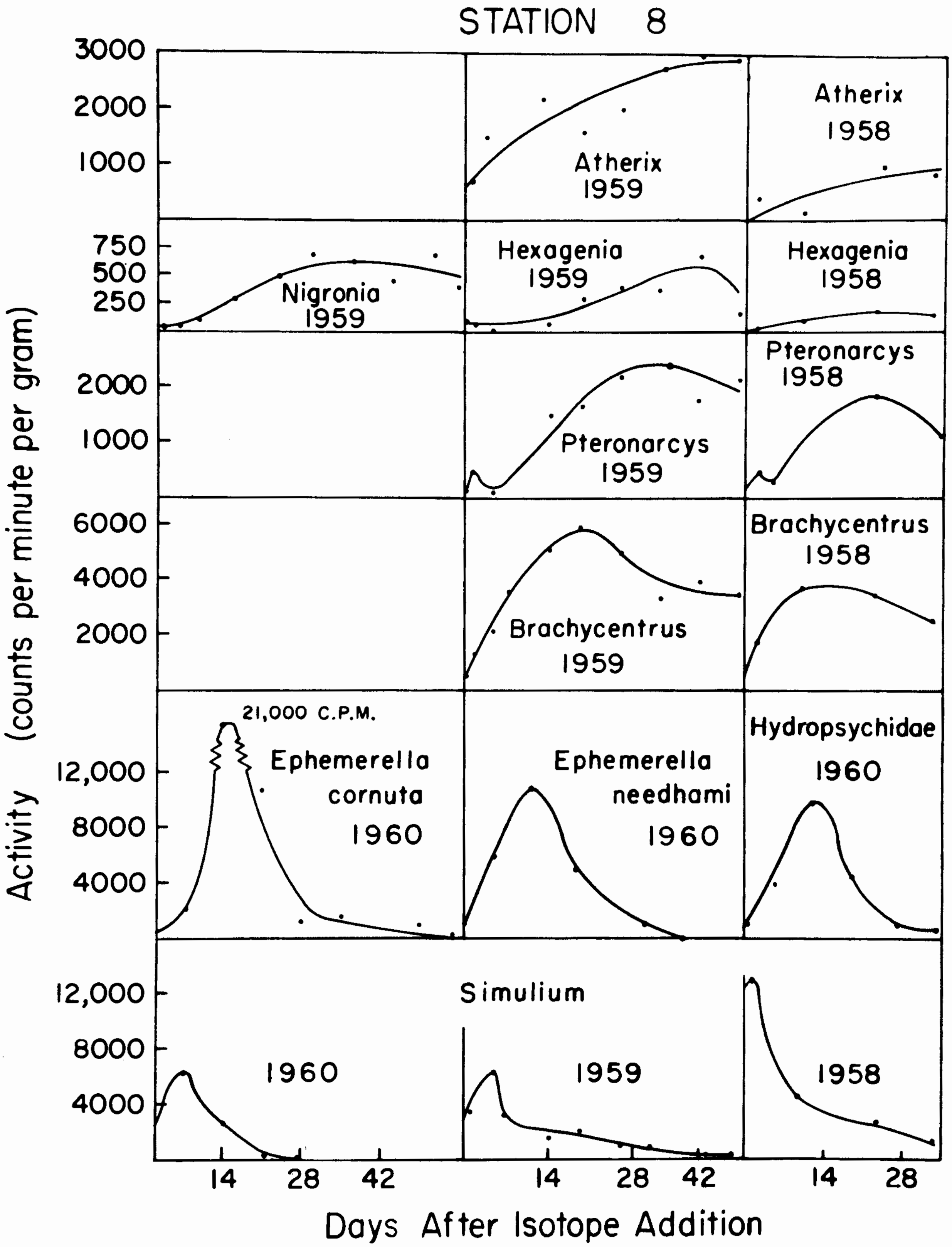

STATION 8
Activity (counts per minute per gram)
3000
2000
1000
Atherix
1959
Atherix
1958
750
500
250
Nigronia
1959
Hexagenia
1959
Hexagenia
1958
2000
1000
Pteronarcys
1959
Pteronarcys
1958
6000
4000
2000
Brachycentrus
1959
Brachycentrus
1958
21,000 C.P.M.
12,000
8000
4000
Ephemerella
cornuta
1960
Ephemerella
needhami
1960
Hydropsychidae
1960
12,000
8000
4000
Simulium
1960
1959
1958
14
28
42
14
28
42
14
28
Days After Isotope Addition

Figure 5

1. The shape of curves appears to be consistent from year to year for a given species.
2. The magnitude of the peak activity (height of curve) for a given species varies from year to year.
3. Time of the activity peak appears to have a distinct relationship to the position of the animal in the food chain. Insects feeding upon living algae and/or bacteria which have removed activity from the water directly, show maxima soon after the isotope treatment. Insects feeding largely on detrital and drifting plant materials appear to have a somewhat later peak, while those insects feeding entirely upon dead and decaying detritus have a much later activity maximum. The activity peaks of predaceous insects come last.
4. Differences between species in maximum activity level is probably in part due to such factors as size, relative proportions of living and dead structures, and feeding rates (growth rates). Thus a small, rapidly growing, soft-bodied animal would be expected to have greater levels of activity than a large, heavily chitinized insect. Large insects such as Nigronia and Hexagenia never had a high activity.
5. It appears that the activity levels of certain invertebrates tend to fluctuate together as a group from year to year. For example, at station 8 activities of Atherix, Hexagenia, Pteronarcys and Brachycentrus were higher in 1959 than in 1958. On the other hand, the activity of Simulium was higher in 1958 than in 1959. Since the total amount of activity taken up in section B (the section including station 8) was low in 1958 but high in 1959, it is believed

that this difference in uptake by the ecosystem is reflected in all of these insects except _Simulium_. The source of $P^{32}$ for invertebrates other than _Simulium_ must be a food chain beginning with periphyton. It is believed that _Simulium_ differs from the other species in its original source of $P^{32}$. _Simulium_ shows an activity peak earlier than any of the periphyton feeders; therefore, its activity source very likely is the water itself (perhaps drifting bacteria).

6. These data suggest that activity curves can be used to classify invertebrates according to feeding habits. Thus an insect feeding on detritus made up of both living and dead material has a peak intermediate in time between that of a plant feeder and that of a detrital feeder. Such a classification perhaps will give us a more quantitative approach to the study of complex food webs.

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