Copies to: Fish Division
Education-Game
J. W. Leonard
I. F. R. (2)
Pigeon R. Station
Hunt Creek Station
C. T. Yoder
R. C. Ball
F. F. Hooper

March 27, 1959

Report No. 1567
(Institute for Fisheries Research)

PROGRESS REPORT TO THE ATOMIC ENERGY COMMISSION

Division of Biology and Medicine

June 14, 1958 --- March 11, 1959

Contract No. AT(11-1)-655

Contract Title. A Study of Productivity in a stream Ecosystem.

Preliminary report on translocation of radioactive phosphorus (P_{32}) in a Michigan trout stream

by

R. C. Ball, Department of Fisheries and Wildlife,
Michigan State University

and

F. F. Hooper, Institute for Fisheries Research,
Michigan Department of Conservation

Introduction. On August 5, 1958, an addition of radioactive phosphorus (P_{32}) was made to the West Branch of the Sturgeon River in the vicinity of Wolverine, Michigan. The objectives of this experiment were: (1) to determine how the naturally occurring phosphorus of a trout stream is used by animals and plants and (2) to determine by what mechanisms phosphorus is transported from place to place in a trout stream. This information will enable us to better understand how phosphorus in the form of fertilizers can be used in streams to increase the production of trout.

From earlier studies (Grzenda 1955, Colby 1956, Carr 1958), it was found that an increased growth of periphyton followed each addition of fertilizer to this stream. In several instances the apparent fertilization effect extended downstream a distance of 6 or 7 miles. However, an increase in the phosphorus content of the water after fertilization could be detected only 1 or 2 miles. Since we could not demonstrate an increase in the phosphorus content of water at downstream stations, the mechanism of the stimulation given to periphyton growth was obscure. In fact, it was difficult to say with certainty that the growth of periphyton was related to the addition of fertilizer. The present experiment with radiophosphorus was designed to again study downstream transport of phosphorus but with a much more sensitive technique than was used in earlier work.

At the time of preparing this report only a portion of the data collected have been assembled. Most of the data presented here are from Station No. 8 located about midway in the study area. This station was selected because information at this point was typical of a large section of the stream during the experiment. At Station No. 8, the public had free access to the experimental section of the stream and the potential health hazard was judged to be greatest. Since only a portion of the data have been assembled, many of the interpretations given in this report must be considered tentative. Further analysis of the data should reveal new and interesting aspects of the experiment not presented here.

The grant from the Atomic Energy Commission was not made until late in the spring of 1958. Since this gave little time for assembling equipment, scheduling the work, and devising appropriate techniques, it was felt that the 1958 test would be little more than a trial-run which would provide opportunity to learn techniques and to check-out our calculations on stream dosage. Soon after the addition of isotope on August 5, it became evident that our dosage estimates were in the proper range and that our techniques were adequate. Hence it is felt that the information obtained from the 1958 experiment is all usable and that a second trial of exactly the same type will not be necessary.

Study Area. The section of the West Branch of the Sturgeon River studied lies southwest of the town of Wolverine. The addition of isotope was made in Section 21, T. 31 N., R. 3 W. We collected samples downstream from this point for a distance of approximately 2 1/2 miles. The point farthest downstream at which there was a monitoring of isotope activity was at the State Highway park adjacent to US-27 (Station 16, Section 14 of T. 33 N., R. 3 W.). Fourteen sampling stations were established between the point of addition and the park (Figure 1).

Figure 1.--Experimental area and sampling stations. Station 1 was the control station and was upstream from point at which isotope was added.

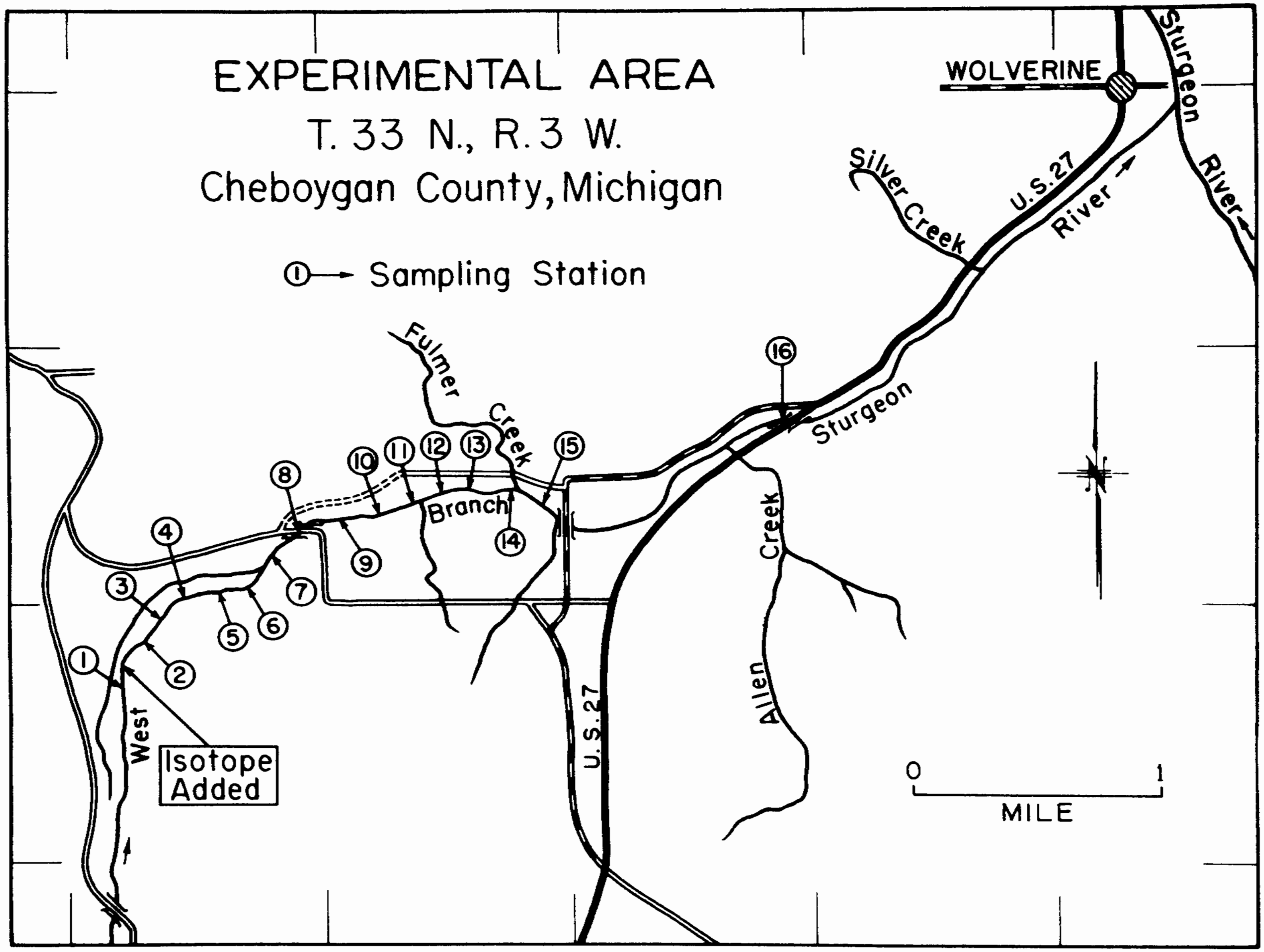

EXPERIMENTAL AREA
T. 33 N., R. 3 W.
Cheboygan County, Michigan
Sampling Station
WOLVERINE
Sturgeon River
Silver Creek
U.S.27
River
Fulmer Creek
Sturgeon
Branch
West
Isotope Added
Allen Creek
U.S.27
0
1
MILE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

Figure 1

Addition of isotope. A shipment of 23.1 millicuries of P_{32} from Oak Ridge was diluted with 55 gallons of stream water. This mixture was siphoned into the stream at a constant rate. The entire dose entered the stream in a 30-minute period. The flow of the stream at the point of addition was approximately 37 cubic feet per second. Using this stream flow and the flow-rate of the diluted isotope given above, the theoretical concentration of isotope in the water was calculated to be 1.22×10^{-5} microcuries per milliliter.

Radioactivity in the water. Water samples were taken before, during, and after the isotope addition. While the dose of radiophosphorus was moving downstream, intensive sampling was undertaken at eight stations. By releasing a marker of fluorescein dye ahead of and behind the isotope dose, we were able to time sample collections at all stations so that they were spaced approximately the same in relation to the mass of moving isotope. Water samples were collected in 140 milliliter polyethylene bottles. Samples were evaporated and prepared for counting on a planchet using the procedure given by Robeck, Henderson and Palange (1954).

The graph showing the passage of isotope by Station No. 8 (Figure 2) is more or less typical of all of the upstream stations. There was a steady increase in activity to a peak at something over one-half of the time-span followed by a sudden drop to almost background level. Such an asymmetrical curve of activity results from purely physical processes; i.e., the water mass containing the first chemicals added is greatly diluted by water lacking isotope as it moves downstream. Water receiving a dosage of isotope at progressively latter times is diluted by water from eddies and backwaters which contains more and more isotope. Thus a higher and higher concentration is built up in the flow passing a given point until the addition of isotope is stopped. Thereafter there is a sharp decrease, the tail of the activity curve represents isotope re-entering the main current from backwaters and embayments.

Figure 2.--Activity of stream water during passage of isotope dosage. Counts have been corrected for background and decay.

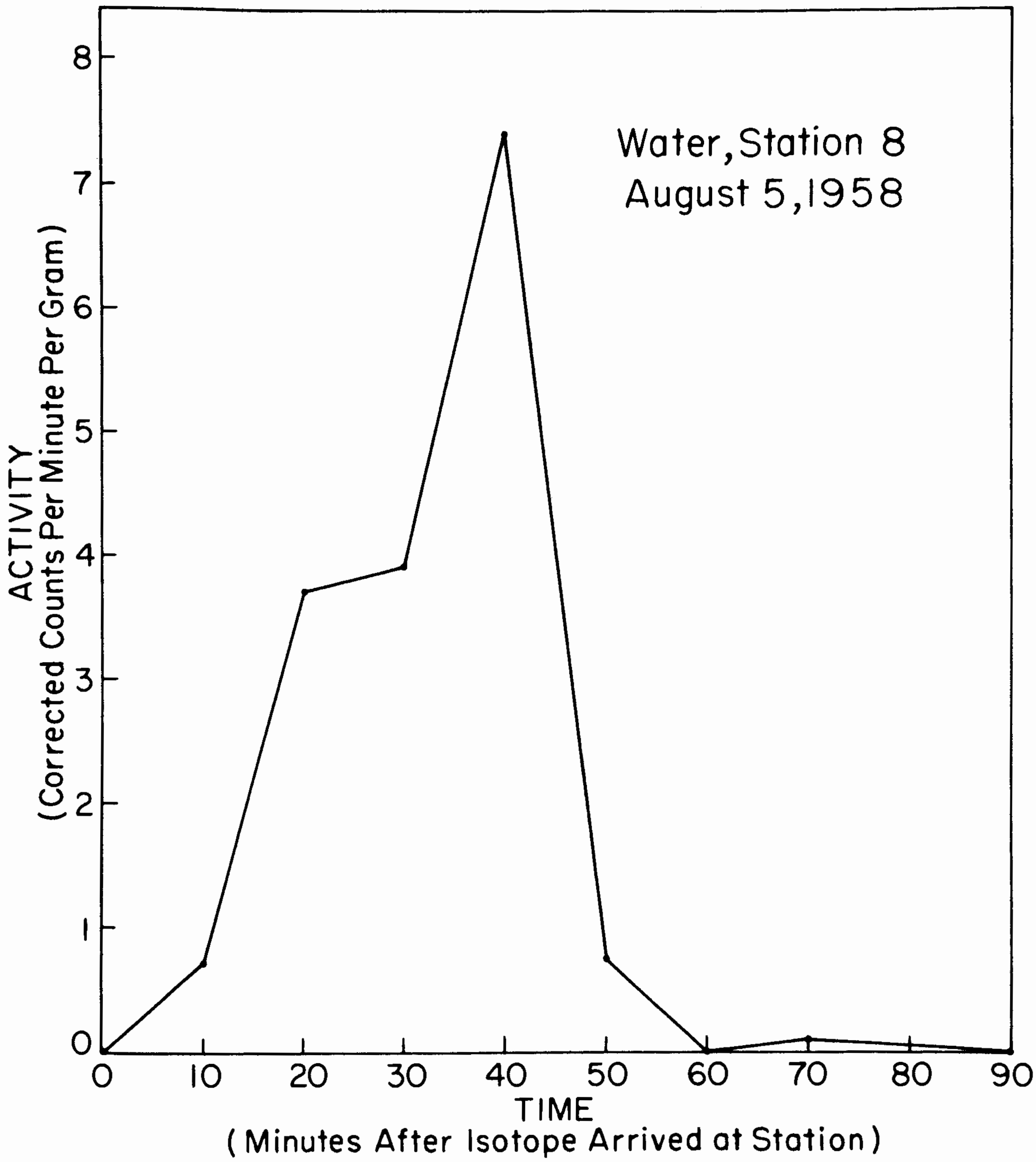

Water, Station 8
August 5, 1958
ACTIVITY
(Corrected Counts Per Minute Per Gram)
0
1
2
3
4
5
6
7
8
0 10 20 30 40 50 60 70 80 90
TIME
(Minutes After Isotope Arrived at Station)

Figure 2

Peak activity (11 counts per minute per gram) occurred at Station No. 3, the first station at which the isotope was completely mixed with the stream water. Downstream there was progressive decrease in activity. At the station farthest downstream (Station No. 16), the maximum activity for a 50 ml. sample was only 20 counts per minute above background. The length of time at which a detectable amount of activity occurred in the water increased downstream. At Station No. 3 counts dropped to a background rate 50 minutes after the isotope first appeared. While at Station No. 14 water giving an activity above background continued to move past this station for over two hours. This "stretching out" of the dose of isotope as it moved downstream is believed to be related to physical process of water transport.

Uptake of radiophosphorus by periphyton.--In the West Branch of the Sturgeon River the periphyton growth on rocks and other substrates appears to be the chief source of energy fixation in the community. Passage of radiophosphorus through this segment of the food chain was studied by (1) measuring the activity of the periphyton growth on plastic shingles suspended in the stream, and by (2) removing the periphyton growth from rocks on the stream bottom. Shingles were supported in the water by a steel stake and were about 8 inches below the water surface. They were set in the stream 6 days before the addition of the isotope so that a measurable amount of periphyton would be present on these substrates by the time the isotope was added. On August 1 the stream was fertilized by an addition of di-ammonium phosphate. The addition of fertilizer stimulated growth of the periphyton on substrates and also acted to increase the total phosphorus "pool" in the stream section under investigation (c.f. Foster, 1958). It was thought that by enlarging the "pool" of phosphorus in this section there would be less chance of water containing a high activity passing beyond the study area where it would have become a much greater health hazard.

On the first three collecting dates, four small (2 x 5 inch) shingles and one large (4 x 10 inch) shingle were removed from each of the fourteen sampling stations. Thereafter shingles were removed only from Stations 1, 2, 3, 5, 8, 12 and 14. Samples of periphyton from rocks were collected only at the latter series of stations. In the laboratory periphyton was scraped from the shingle and placed on a planchet of known weight. The excess water was then removed, and the sample was weighed. Further processing of the sample for counting followed rather closely procedures outlined by Robeck, Henderson, and Palange (1954).

There was a rapid uptake of phosphorous by periphyton. Substrates collected four hours after the isotope was added were high in activity (Figure 3). The second and third collections made August 7 and 11 indicated a further increase in activity at Station No. 8. The increase between August 5 and 7 was perhaps due to isotope re-entering the main stream current from eddies after the August 5 collection. It seems clear that the uptake of radiophosphorus must have exceeded loss to the water between August 5 and 11. Since the activity of the water was scarcely above background level, this net increase must have come from a slow "feed-back" of radiophosphorus into the main current from either (1) eddies or backwaters where there was physical storage, or (2) algae and bacteria where there was biological storage. Further evidence of "feed-back" into the stream comes from the accumulation of activity on shingles placed in stream after the addition of isotope on August 5. On August 13 (8 days after the addition of isotope), shingles were sampled that were put into the stream before and after release of the isotope. It was found that the activity of those put in after release was 33 percent of the activity of those put in before release. These data suggest that biological "feed-back" was quantitatively significant and that phosphorus moves downstream through

Figure 3.--Activity of periphyton taken from artificial substrates (shingles) at stations 2 and 8. Counts were corrected for background and decay.

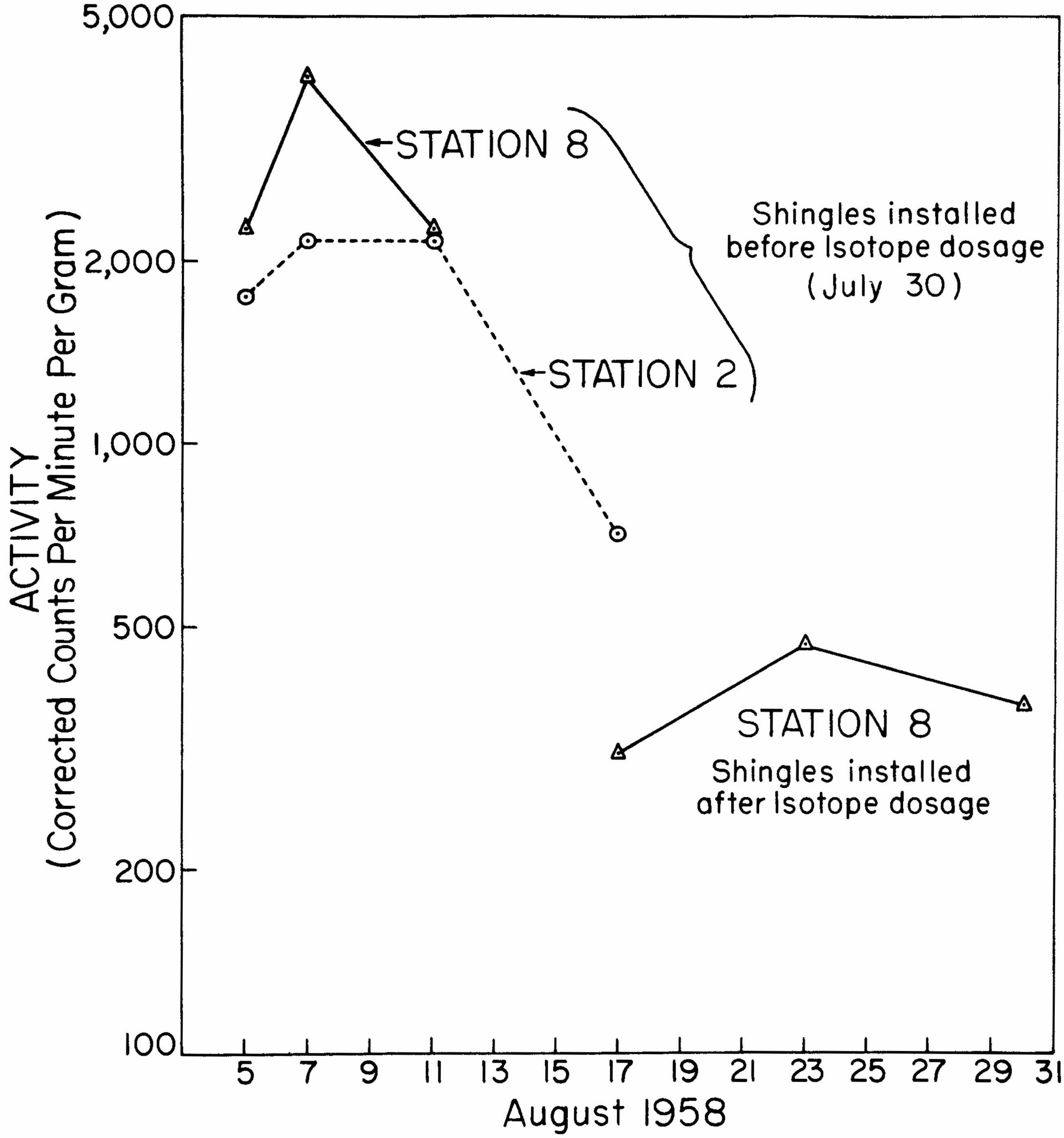

5,000
2,000
1,000
500
200
100
ACTIVITY
(Corrected Counts Per Minute Per Gram)
STATION 8
STATION 2
Shingles installed
before Isotope dosage
(July 30)
STATION 8
Shingles installed
after Isotope dosage
5 7 9 11 13 15 17 19 21 23 25 27 29 31
August 1958

Figure 3

successive periods of absorption by the biota, re-exchange with the stream water, water transport, and re-absorption by the biota.

Uptake of radio-phosphorus by Chara and Potamogeton.--Potamogeton and Chara were collected at 6 stations. Samples weighing approximately 2 grams were washed thoroughly in stream water and then weighed and processed for counting as suggested by Robeck, and Henderson, and Palange, 1954. A plot of activity against time (Figure 4) shows that most of the uptake occurred on August 5. Thereafter activity decreased in both species at a logarithmic rate. The decrease appears to be almost linear on a log scale until August 19. Thereafter there was decrease in activity loss. This plateauing effect in the activity curve suggests a continued uptake of radio-phosphorus. This is probably the "feed-back" phosphorus noted in the case of periphyton. Since the "pool" of stored phosphorus (both radioactive and non-radioactive) in these plants might be expected to be large, uptake of "feed-back" phosphorus would not be detected until there was a considerable decrease in activity level.

Uptake of radio-phosphorus by invertebrates.--The translocation of radiophosphorus was followed through seven stream invertebrates (Figures 5-7). These seven forms were from three habitats. Simulium and the tabanids inhabited gravel riffles while the gomphine dragonfly, the stone fly, Pteronarcys, and the caddis fly Brachycentrus inhabited Chara beds, logs and other detritus of fast water. Hexagenia and the Physid snail, on the other hand, inhabited pools and backwaters. Hexagenia occurred in silt beds. Snails occurred on the logs, stones and detritus.

Collections of invertebrates were made at 4 stations (3 experimental, 1 control). A single collection preceded, and five collections followed, the isotope addition.

Differences in the rate of uptake and the rate of loss of radiophosphorus are apparent among these invertebrates (Figures 5-7). Uptake by Simulium was

Figure 4.--Activity of stream vegetation at Station 8. Counts were corrected for background and decay.

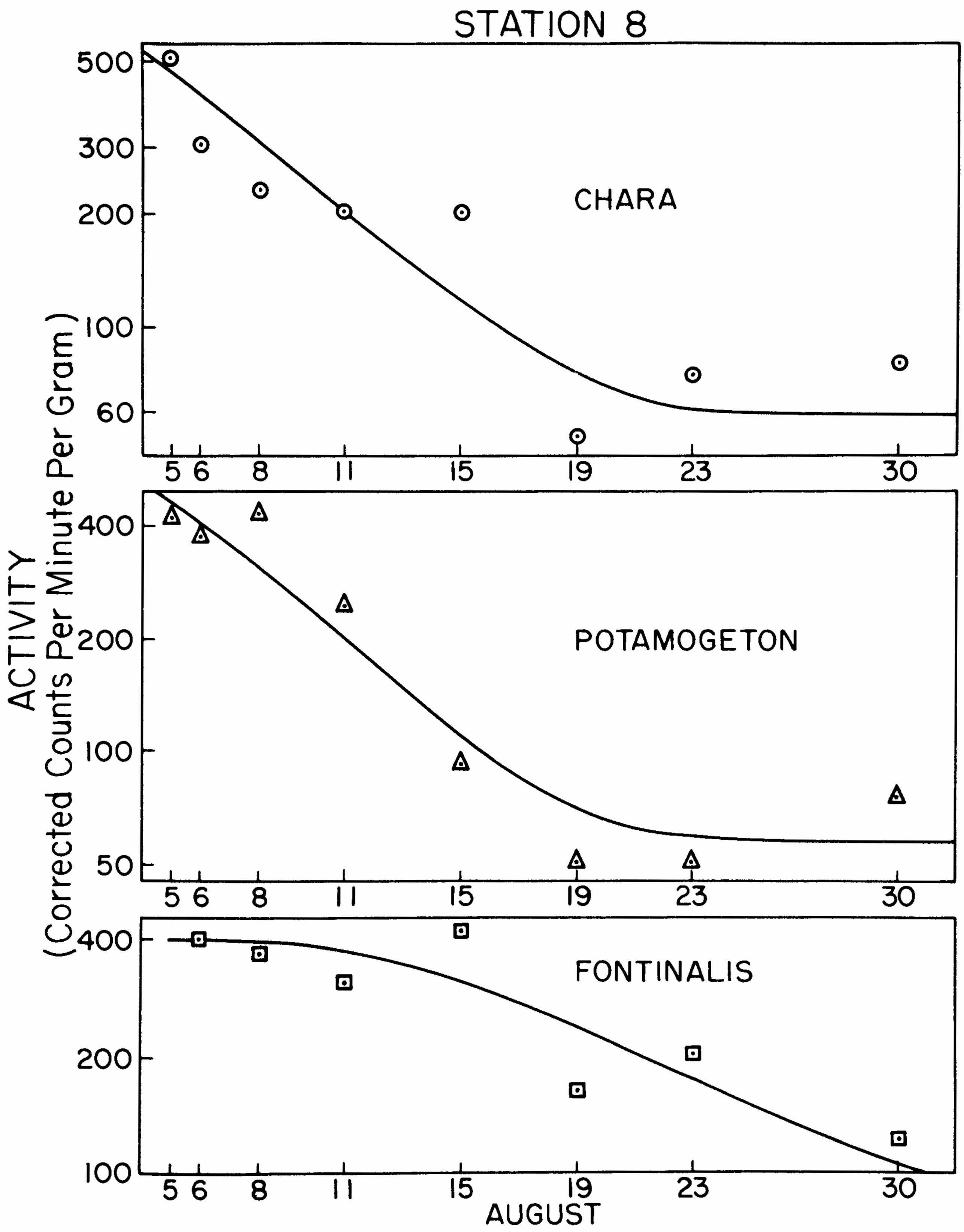
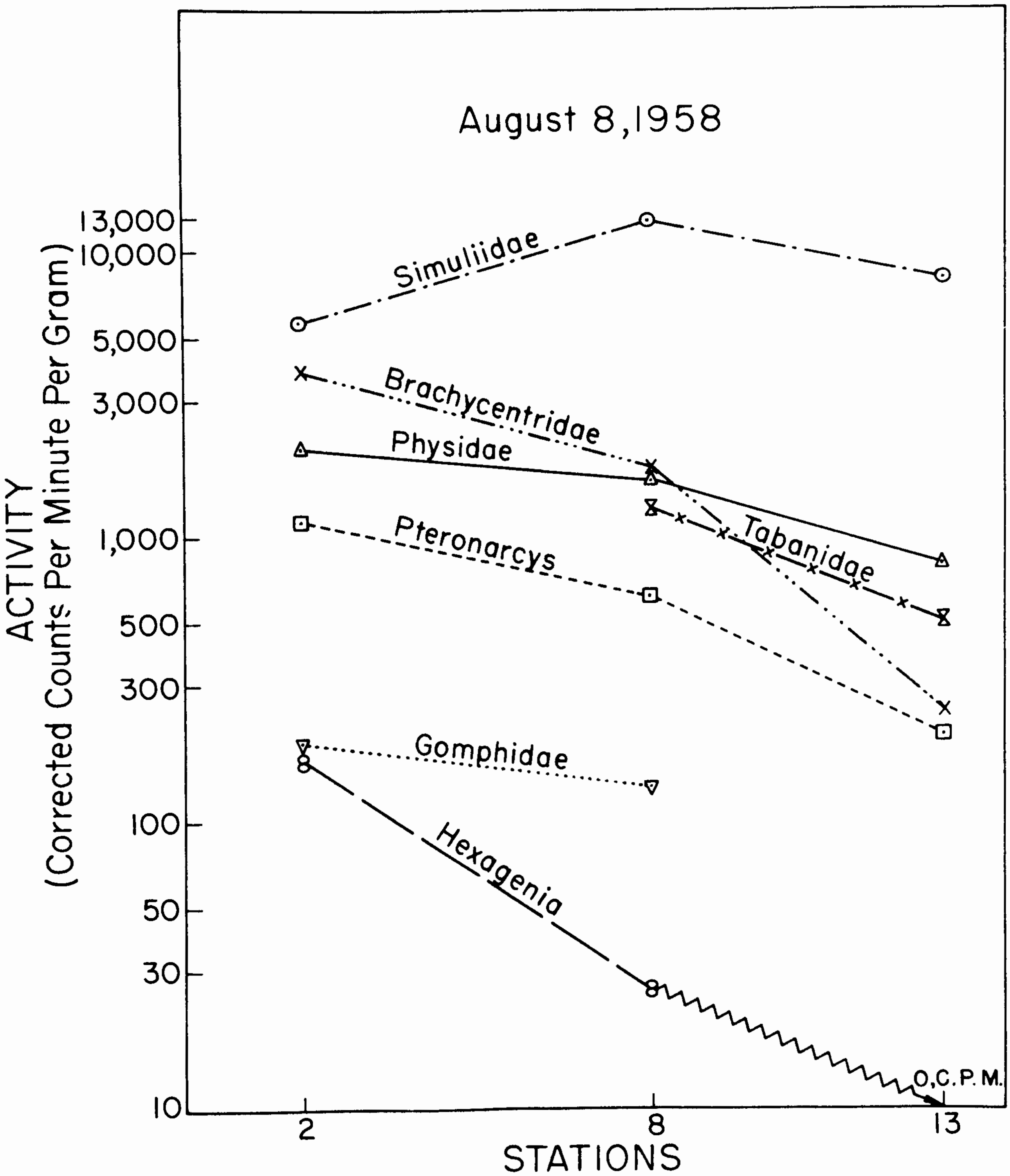

STATION 8
CHARA
POTAMOGETON
FONTINALIS
ACTIVITY
(Corrected Counts Per Minute Per Gram)
500
300
200
100
60
400
200
100
50
400
200
100
5 6 8 11 15 19 23 30
AUGUST

Figure 4

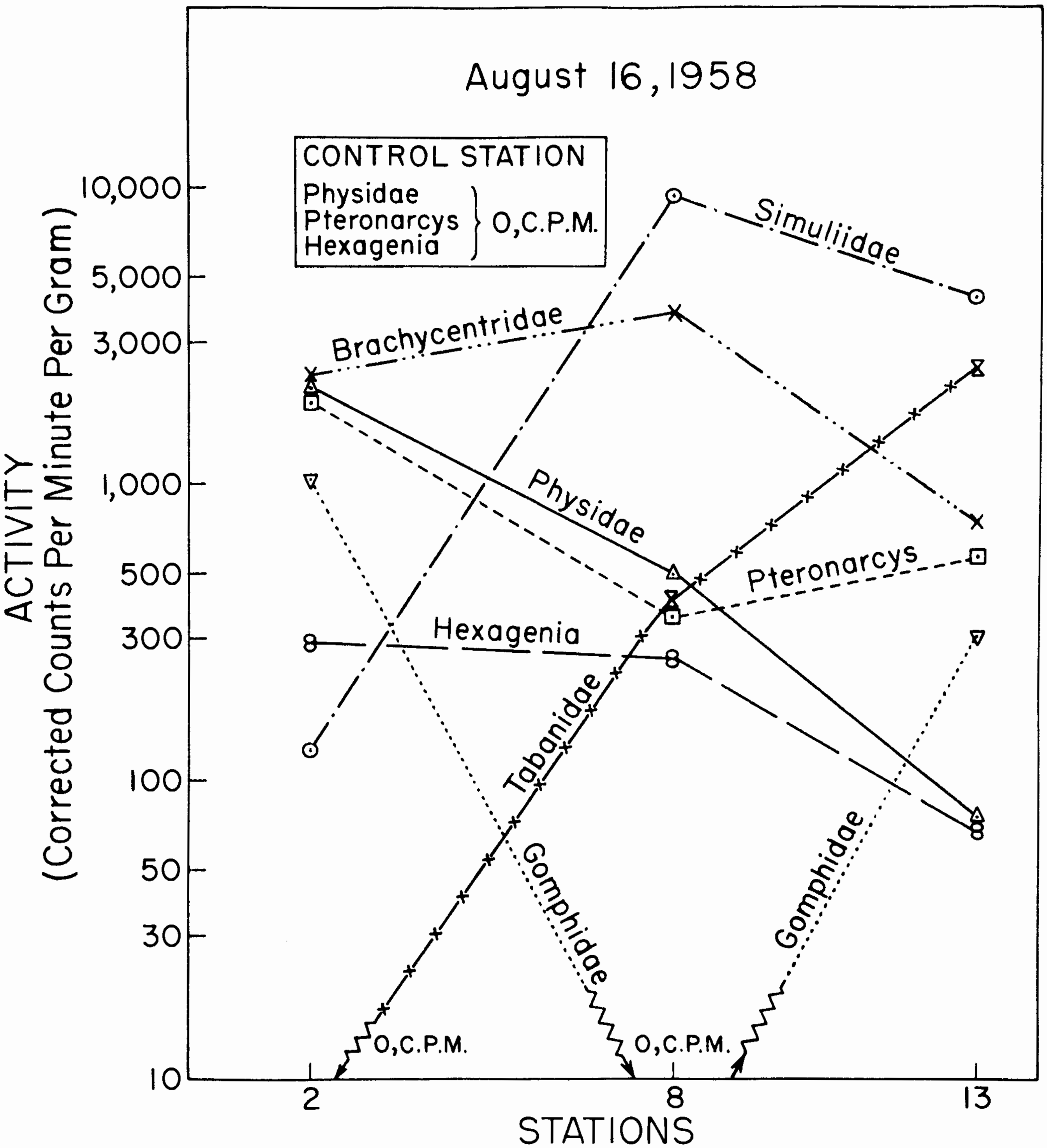
Figure 5.--Activity of bottom invertebrates on August 8, 1958. Counts were corrected for background and decay.



August 8,1958
ACTIVITY
(Corrected Counts Per Minute Per Gram)
13,000
10,000
5,000
3,000
1,000
500
300
100
50
30
10
Simuliidae
Brachycentridae
Physidae
Pteronarcys
Tabanidae
Gomphidae
Hexagenia
O.C.P.M.
2
8
13
STATIONS

Figure 5

Figure 6.--Activity of bottom invertebrates on August 16, 1958. Counts were corrected for background and decay.



August 16, 1958
CONTROL STATION
Physidae
Pteronarcys
Hexagenia
O,C.P.M.
ACTIVITY
(Corrected Counts Per Minute Per Gram)
10,000
5,000
3,000
1,000
500
300
100
50
30
10
Simuliidae
Brachycentridae
Physidae
Pteronarcys
Hexagenia
Tabanidae
Gomphidae
Gomphidae
O,C.P.M.
O,C.P.M.
O,C.P.M.
2
8
13
STATIONS

Figure 6

Figure 7.--Activity of bottom invertebrates on September 8-10, 1958. Counts were corrected for background and decay.

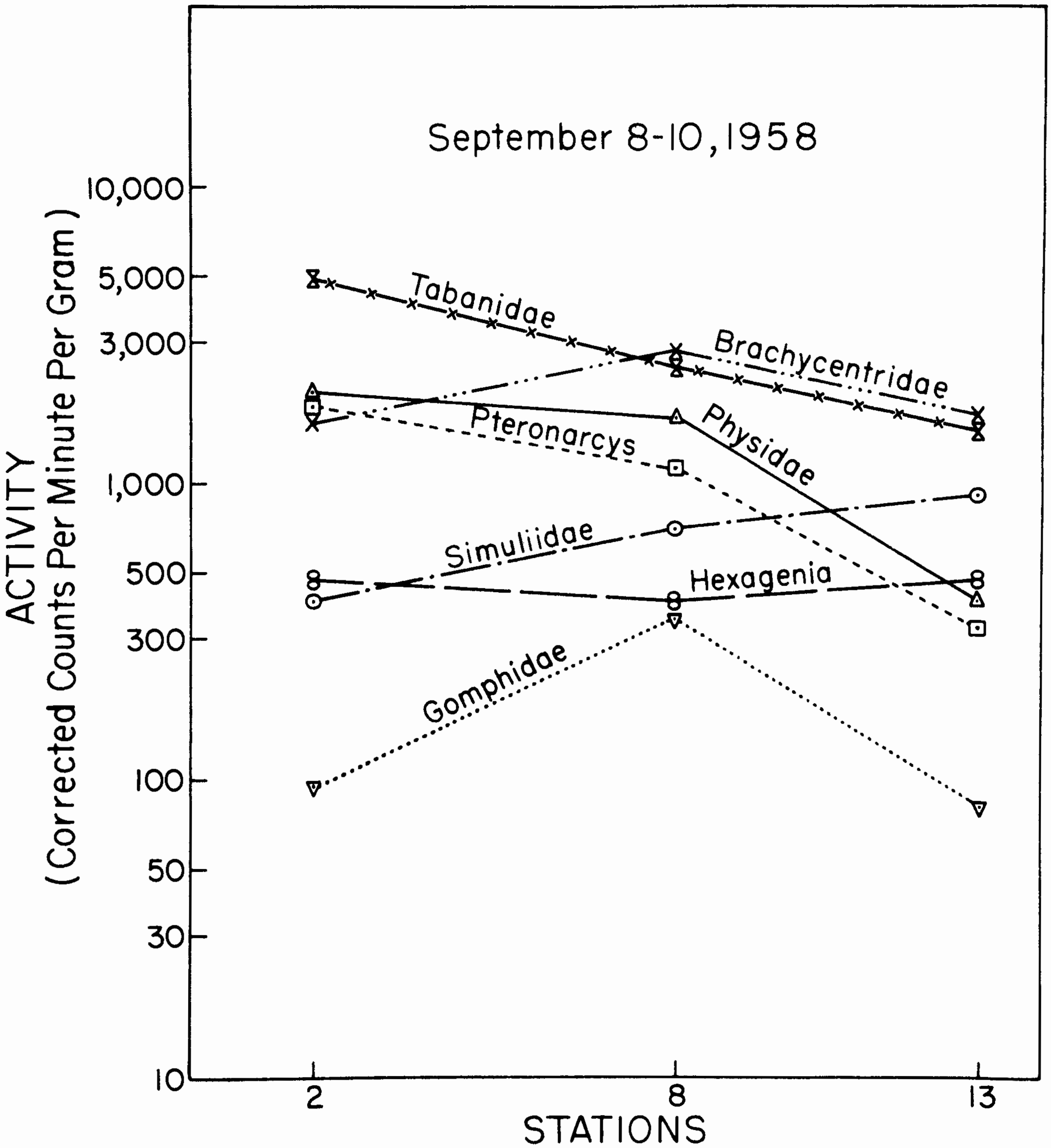

September 8-10, 1958
ACTIVITY
(Corrected Counts Per Minute Per Gram)
10,000
5,000
3,000
1,000
500
300
100
50
30
10
Tabanidae
Brachycentridae
Pteronarcys
Physidae
Simuliidae
Hexagenia
Gomphidae
2
8
13
STATIONS

Figure 7

most rapid. Twenty-four hours after the addition of isotope (August 6) black-flies at Station 8 had a corrected count of approximately 13,000 counts per minute per gram. After August 8 there was a decrease in activity at an exponential rate. The specific activity of the black flies on August 6-8 was higher than the specific activity of any periphyton collection. This suggests that these insects were feeding upon food of higher activity than any food collected in our samples. A food of such activity could have been either (1) bacteria and algae that drifted downstream, or (2) periphyton growing on the rocks with a higher specific activity than the periphyton on our artificial substrates. The first possibility does not appear to be likely since black flies collected immediately below (200 yards) the source of the isotope had a high activity. To account for the activity of these flies from drift it is necessary to assume a rapid uptake of radiophosphorus followed by release of a sizable quantity of radioactive drift within a comparatively short section of stream. If the flies received their high activity food from stream drift, it would seem that animals farthest downstream (Station 13) would in time accumulate the highest activity. This was not the case; maximum activity was at Station 8.

The uptake of radiophosphorus by other invertebrates at Station No. 8 was slower. Snails and Brachycentridae had accumulated a maximum activity by August 16. Thereafter there was a steady decrease in activity of the caddis fly at the upstream stations.

The activity of the snails (corrected for decay) remained almost constant from August 16 until September 8 when the final set of collections was made. The shells were considered separately and showed very little activity at any time. Pteronarcys stored radiophosphorus until August 30 before a decrease was noted. Similarly, peak activity in the tabanid larvae was not encountered until August 30 at Station 2. At downstream stations activity peaks were earlier for this form. This delay in peak activity suggests that these two insects are one step removed in the food chain from snails and <u>Brachycentridae</u>. The burrowing mayfly, <u>Hexagenia</u>, showed the slowest rate of uptake. The activity of this invertebrate did not rise significantly above background level until August 8 and like the <u>Pteronarcys</u> and the <u>Tabanidae</u> did not reach its maximum until August 30. Since <u>Hexagenia</u> feeds upon organic detritus associated with the silt of the backwaters, such a slow rise and fall of activity in <u>Hexagenia</u> might be expected. Organic material eaten by <u>Hexagenia</u> must arise from dead and decaying periphyton which has sloughed off various substrates and settled into the silt beds. Such material is a step removed in time from its fixation by autotrophic plants.

<u>Uptake of radiophosphorus by fish</u>.--The accumulation of activity was followed in two species of fish. Two brown trout (<u>Salmo trutta</u>) and two muddlers (<u>Cottus bairdi</u>) were collected from 8 stations. In the laboratory the fish were homogenized in a Waring blender and samples weighing approximately 2 grams (wet weight) were treated with nitric acid and prepared for counting as suggested by Robeck, Henderson, and Palange (1954). The pattern of uptake by muddlers seems to have differed from the uptake by brown trout. In the case of muddlers, activity (corrected for decay) increased at almost a steady rate up until the last samples were taken on August 28. Uptake by brown trout appears to have been more erratic (Figure 8). There appears to have been a very rapid uptake between August 21 and August 25. This was followed by a decrease between August 25 and August 28.

<u>Radiological safety</u>.--Maximum level of activity encountered in the water and various parts of the stream biota at Station No. 8 are given in Table 1. Activities are not corrected for decay and are given in terms of microcuries per gram. Accumulation by fish is perhaps the most hazardous, but in this case, a high percentage of the activity is concentrated in scales and bones rather

Figure 8.--Activity of fish at Station 8 following the addition of isotope. Counts have been corrected for background and decay.

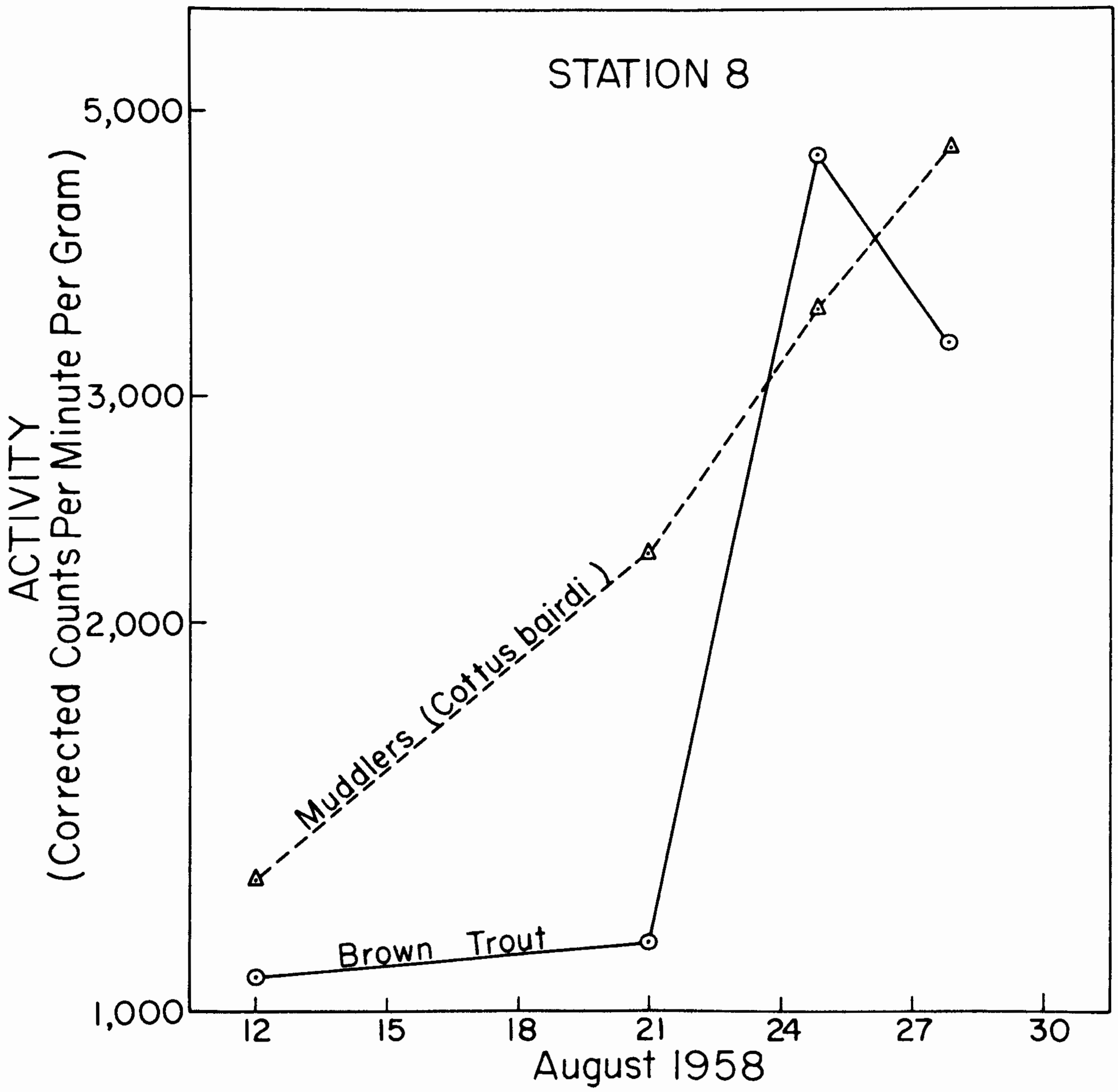

STATION 8
ACTIVITY
(Corrected Counts Per Minute Per Gram)
5,000
3,000
2,000
1,000
Muddlers (Cottus bairdi)
Brown Trout
12
15
18
21
24
27
30
August 1958

Figure 8

Table 1.--Highest activity recorded in aquatic organisms from West Branch of Sturgeon River after addition of radiophosphorus

	Activity (microcuries per gram at time of collection)	Location and date
FISH		
Trout (*Salmo trutta*)		
Whole	1.87×10^{-3}	Station 8 8/28/58
Whole	2.24×10^{-3}	Station 5 8/25/58
Viscera	1.42×10^{-3}	Station 3 8/21/58
Viscera	5.07×10^{-4}	Station 5 8/25/58
Head and gills	1.35×10^{-3}	Station 3 8/21/58
Head and gills	6.47×10^{-4}	Station 5 8/25/58
Skeleton	9.3×10^{-4}	Station 3 8/21/58
Skeleton	1.28×10^{-3}	Station 5 8/25/58
Muscle	1.85×10^{-4}	Station 3 8/21/58
Muscle	2.42×10^{-4}	Station 5 8/25/58
Muddlers (*Cottus bairdi*)	9.3×10^{-4}	Station 8 8/23/58
INVERTEBRATES		
Simulidae	8.41×10^{-2}	Station 8 8/30/58
Brachycentridae	1.46×10^{-3}	Station 8 8/30/58
Snails	1.2×10^{-3}	Station 8 8/30/58
Tabanidae	5.47×10^{-4}	Station 8 8/30/58
Pteronarcys	3.3×10^{-4}	Station 8 8/30/58
Gomphidae	2.76×10^{-4}	Station 8 8/30/58
Hexagenia	9.18×10^{-5}	Station 8 8/30/58
PLANTS		
Periphyton	2.41×10^{-3}	Station 8 8/5/58
Moss	7.5×10^{-4}	Station 8 8/6/58
Chara	6.3×10^{-4}	Station 5 8/5/58
Potamageton	5.7×10^{-4}	Station 4 8/5/58
WATER OF STREAM	5.8×10^{-6}	Station 8 8/5/58

than flesh (c.f. Robeck, Henderson and Palange, 1954). It has been shown that the rate of accumulation in juvenile fish is somewhat greater than accumulation in adult fish (Ibid, 1954). Thus the activity of legal fish reaching the angler's creel might be expected to be somewhat below the activities of the fish that were sampled.

Permissible levels of radiophosphorus of fish used for human consumption have been discussed by Donaldson and Foster (1957). They calculated the amount of radiophosphorus that would be expected in edible parts of fish assuming an intake of $3 \mu c P_{32}$ per week. This figure was based upon uptake from water containing the maximum permissible concentration for drinking water (International Committee on Radiation Protection). Donaldson's and Foster's suggested maximum level is $7 \times 10^{-4} \mu c P_{32}$ per gram for fish flesh, a figure which apparently includes a safety factor of 10. This activity is substantially greater than the maximum we recorded in trout muscle (2.42×10^{-4}). Bone, viscera and head and gills of some of our fish exceeded this activity level slightly. The high activity of the viscera was probably from undigested food.

Summary and conclusions.--The experiment has demonstrated that the natural phosphorus of the stream water is continuously being exchanged with the phosphorus in living plants and animals. Thus a single phosphorus atom follows a devious course through a succession of plant and animal forms with interspersed periods of downstream transport by the water. The evidence of the great amount of biological feed-back of phosphorus suggests that in the case of a fertilization experiment, even though the added phosphorus disappeared rapidly downstream, it would be re-cycled and fed back slowly into the water so that the fertilization effect would extend many miles beyond the point at which an increase could no longer be detected chemically. The evidence of downstream re-cycling may also mean that there is a greater biological use made of phosphorus in stream fertilization than in the case of lakes where there are usually large and prohibitive losses to the lake bottom.