AN ABSTRACT OF THE THESIS OF

Stanley Vincent Gregory for the degree Master of Science
(Name of student) (Degree)

in Fisheries and Wildlife presented on July 1, 1974
(Major department) (Date)

Title: ANALYSIS OF A ^{32}P MATERIAL BALANCE METHOD FOR MEASURING PERIPHYTON PRODUCTION IN FLOWING WATER

Abstract approved: Redacted for Privacy
(Dr. James Hall)

The ^{32}P material balance method for measuring periphyton production and grazing rates on periphyton in streams developed by Elwood and Nelson (1972) was evaluated by laboratory and field experiments. Three basic assumptions of the method were examined: 1) sloughing of periphyton from substrates is negligible, 2) sorption of ^{32}P onto substrates is a biological process, and 3) recycling of ^{32}P into the periphyton after ingestion by grazers does not occur. Sloughing of periphyton from stream substrates was assumed negligible except in the case of freshets and dense algal mats. Sorption of ^{32}P onto stream substrates was mainly a biological process except on very fine sediments. Sorption of ^{32}P onto leaf material was a function of microbial activity, which was a function of residence time of the leaves in the stream. Recycling of ^{32}P into

the periphyton after ingestion by grazers was a potential problem for estimating grazing rates in slow current.

The ^{32}P material balance method was verified against the change-in-standing crop method in the laboratory. The estimates of 576 mg/m^2/day and 522 mg/m^2/day respectively were not significantly different at the 95% confidence level.

The application of the ^{32}P material balance method in the field was not successful. This difficulty was most likely due to the low retention time in the high gradient Cascade stream in which it was applied. The high cost of the method, extensive sampling time, and difficulties in obtaining authorization in streams with public access warranted the development of a suitable modification. The proposed modification involves the incubation of individual substrates within a net enclosure in the stream.

Analysis of a ^{32}P Material Balance Method for Measuring Periphyton Production in Flowing Water

by

Stanley Vincent Gregory

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1975

APPROVED:

Redacted for Privacy

Associate Professor of Fisheries
in charge of major

Redacted for Privacy

Head of Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

Date thesis is presented July 1, 1974

Typed by Lyndalu Sikes for Stanley Vincent Gregory

Sometimes the magic works . . .

Sometimes it doesn't.

From Thomas Burger's
Little Big Man

FOREWARD

In any thesis an author should find a place to "wing it" and openly express himself. I shall take that opportunity here in thanking the many friends who aided and abetted this effort.

I am particularly indebted to Dr. D. J. Nelson and Dr. J. W. Elwood of the Oak Ridge National Laboratory for their unselfish donation of data, advice, and support. I would also like to thank Dr. C. E. Cushing for his suggestions on many of the problems.

Dr. Jack Donaldson constantly gave me encouragement and the freedom to pursue the problem as I saw fit. This independence was one of the most important experiences of my graduate career. The guidance of Dr. Jim Hall was the source of whatever degree of discipline there is in this thesis. His editorial comments decreased my verbosity and increased the clarity of the presentation. I wish to thank Dr. Bob Holton for his encouragement and direction.

To say Dr. Jim Sedell had a great influence on my graduate experience is an understatement. He was a teacher, collaborator, critic, and friend. He is an experience in himself.

The International Biological Program offered a unique opportunity to work with many scientists in many fields. Their advice was invaluable. In particular, the members of the Stream Project of the

Coniferous Biome were always ready to help, ridicule, or contribute in whatever way possible. Dr. Frank Triska, Dr. Norm Anderson, Dr. Jack Lyford, Dr. Dave McIntire, Dick Aho, Ed Grafius, Cary Kerst, and the whole gang receive my special thanks. In particular, I will always be indebted to Ms. Nancy McLean and Ms. Barb Buckley for their assistance in processing my samples and making life altogether enjoyable.

The hardest part of any research program is maintaining one's sanity. For that I owe a great deal to Mark and Sally Hutton. A necessary component of a research project is a good fishin' buddy.

I especially want to thank my wife Vicki mainly for putting up with me and secondly for her support.

This research was supported by a National Science Foundation grant GB-20963 to the Coniferous Biome.

This thesis is dedicated to several men who have greatly influenced my education in aquatic biology: Dr. D. A. Etnier, Bob Hollingsworth, Dr. Jack Donaldson, Dr. Jim Hall, and Dr. Jim Sedell.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
Methods Using Reactants of Photosynthesis	3
Colonization Method	5
^{32}P Material Balance Method	5
EXAMINATION OF ASSUMPTIONS	10
Sloughing of Periphyton	10
^{32}P Sorption Processes	10
Effect of Residence Time in the Stream on Sorption of ^{32}P onto Leaves	15
Recycling of ^{32}P in the Periphyton	20
LABORATORY VERIFICATION OF THE ^{32}P METHOD	23
APPLICATION OF THE ^{32}P MATERIAL BALANCE METHOD IN MACK CREEK	30
DISCUSSION	41
BIBLIOGRAPHY	47

LIST OF FIGURES

Figure		Page
1	Translocation of ^{32}P through a stream ecosystem.	6
2	Uptake of ^{32}P onto bigleaf maple and conifer incubated in Berry Creek from 3 to 60 days during February - March 1973.	17
3	Consumption of oxygen by bigleaf maple and conifer incubated in Berry Creek from 3 to 60 days during February - March 1973.	18
4	Percentage of nitrogen in bigleaf maple leaves and conifer needles for residence times of 3 to 60 days of incubation in Berry Creek during February - March 1973.	19
5	Uptake of ^{32}P versus consumption of O_2 by bigleaf maple and conifer incubated in Berry Creek during February - March 1973.	21
6	Mean percentage of initial ^{32}P per unit area and weight for 30 days in laboratory stream chamber in April 1973.	26
7	Standing crop of periphyton in laboratory stream chamber for 30 days in April 1973.	27
8	Continuous water sampler modified from Klock and Fowler (1972).	34
9	Concentration of ^{32}P in the water leaving the study area during the ^{32}P release in Mack Creek, July 1973.	36
10	Decrease in ^{32}P/mg of periphyton in Mack Creek, July 1973.	39
11	Colonization of bare substrates by algae in Mack Creek in July 1973.	40

LIST OF TABLES

Table		Page
1	^{32}P concentrations on sterilized and unsterilized stream substrates following a 1 hour exposure to ^{32}P at 9°C. in December 1972.	14
2	Calculation of net periphyton production.	28
3	Flow rate and loss of ^{32}P from the study section of Mack Creek, July 1973.	37

ANALYSIS OF A ^{32}P MATERIAL BALANCE METHOD FOR MEASURING PERIPHYTON PRODUCTION IN FLOWING WATER

INTRODUCTION

The complex assemblage of algae, bacteria, and fungi attached to stream substrates forms a community known as periphyton or Aufwuchs. For many years the study of this community was concentrated on the structural and distributional aspects of the algal component of the community. As the importance of the dynamics of energy flow through ecosystems became apparent, methods were needed to measure periphyton production in streams.

A material balance method of measuring net periphyton production and grazing rate on periphyton was recently developed by Elwood and Nelson (1972) at Oak Ridge National Laboratory. The method uses radioactive phosphorus, ^{32}P, to tag the periphyton in the stream. Biological changes in the periphyton are discernable once the community has been labelled. The ^{32}P material balance method has the distinct advantage of being an in situ method which can be used in streams in which the fluctuations of dissolved gases are not detectable. The method has not been verified against another independent method of measuring periphyton production, therefore, its validity is based solely on theoretical grounds. The method has

been used only in one stream, Walker Branch, Tennessee. The degree to which it can be applied to other stream systems has not been examined.

The objectives of my research were to: 1) examine the assumptions of the ^{32}P material balance method, 2) attempt to verify the method against an independent method of measuring periphyton production, 3) attempt to apply the method to streams in the Cascade Mountains of Oregon, and 4) suggest possible improvements or alternatives.

The methods used in measuring periphyton production in streams can be separated into two basic strategies. In one approach primary production is measured indirectly through changes in concentrations of the reactants of photosynthesis. This group of methods utilizes the basic formulation of photosynthesis:

$$6\ CO_2 + 6\ H_2O \xrightarrow{\text{Light}} C_6H_{12}O_6 + 6\ O_2$$

These techniques consist of 1) the dissolved oxygen method, 2) the pH method, which relates changes in pH to changes in CO_2, and 3) the ^{14}C method, which uses radioactive carbon in the form of $Na_2{}^{14}CO_3$ as a tracer. In the other approach total periphyton production is measured by changes in biomass. These methods are 1) the colonization method, which estimates net periphyton production from colonization of periphyton onto bare substrates and 2) the ^{32}P material

balance method, recently developed by Elwood and Nelson (1972). It must be emphasized that the former group estimates primary production and the later group estimates periphyton production.

Methods Using Reactants of Photosynthesis

The changes in dissolved oxygen or carbon dioxide used to measure primary production are often masked in swift, shallow, turbulent streams by diffusion, which maintains dissolved gas concentrations at equilibrium with atmospheric gases. Many streams are sufficiently buffered to prevent discernable changes in pH. In these streams the dissolved gas methods cannot be used in situ and can only be applied to periphyton communities enclosed within a chamber (Odum, 1956; McConnell and Sigler, 1959; Kobayasi, 1961; McIntire et al., 1964; Lane, 1965; Hansmann, 1969). The difficulties involved in the long half-life of ^{14}C require any estimation of carbon fixation by the ^{14}C technique to be conducted within a chamber.

Any enclosure of the periphyton community within a chamber potentially may alter the temperature, light, nutrient, or current of the natural periphyton community. The metabolism of periphyton has been shown to be sensitive to each of these parameters (Whitford, 1960; Whitford and Schumacher, 1961; Kevern and Ball, 1965; McIntire and Phinney, 1965; McIntire, 1966; Thomas and O'Connell, 1966). Any manipulations of the periphyton community within a

chamber, therefore, must control these parameters and eliminate changes between what the periphyton community experiences under natural conditions and what it experiences in the chamber.

Expansion of results from chambers to the total stream is subject to several sources of error. The heterogeneous distribution of periphyton in streams prevents the simple expansion of net production per unit area of substrate within the chamber to the net production per unit area of stream bottom. Mapping of benthic vegetation minimizes but does not eliminate the distributional error. Shallow, turbulent streams possess a wide range of currents, but all chamber studies to date have used a single velocity. Adjustment for this problem would require mapping of the stream into velocity ranges and controlling the current within the chamber to match these ranges. Any chamber study must be of sufficiently short duration to avoid depletion of the nutrient content of the water. This time limitation requires several estimations of primary production throughout the day to expand short term results to daily rates of net primary production. Recent studies by Jackson and Volk (1970) suggest that respiration rate in the dark is not equal to the respiration rate during photosynthesis, therefore, addition of dark chamber results to light chamber results may not yield an accurate assessment of gross primary production. This error would be difficult to eliminate. The use of chambers eliminates many problems but

introduces a wide range of others, therefore any researcher using a chamber system must accept the inaccuracies involved and do extensive studies to minimize them.

Colonization Method

The colonization of various substrates by periphyton in streams has been used as a method of estimating net periphyton production (Sladeckova, 1962; Kevern, Wilhm, and Van Dyne, 1966; King and Ball, 1966; Stockner, 1968; Vladimirova, 1969). This method measures a combination of net periphyton production, grazing, sloughing, and colonization by drift; therefore, it cannot be considered a true estimate of net primary production.

^{32}P Material Balance Method

Elwood and Nelson (1972) developed a material balance method, which uses radioactive phosphorus to determine the net periphyton production and grazing rates on periphyton in streams. The ^{32}P tags the biomass of periphyton with radioactive phosphorus. Net production is then determined by measuring the dilution of the ^{32}P as a result of the increase in biomass. The processes involved in the translocation of ^{32}P in streams are represented in the schematic diagram in Figure 1. A fraction of the ^{32}P introduced into the stream is taken up by the periphyton community. The remaining ^{32}P passes

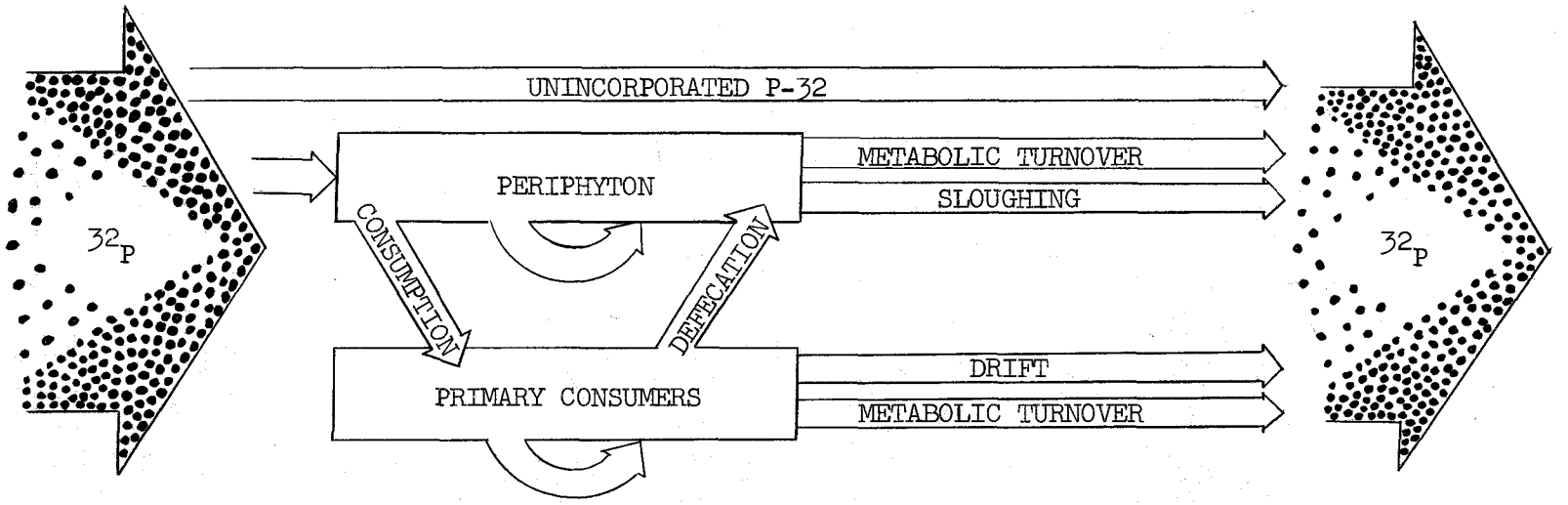

Figure 1. Translocation of ^{32}P through a stream ecosystem (modified from Elwood and Nelson (1972)).

through the stream study section unincorporated. The ^{32}P in the periphyton community can be lost by metabolic turnover or sloughing from the stream substrates. In addition, ^{32}P in the periphyton can be transferred to primary consumers by consumption. ^{32}P in the consumers can be lost from the study section as drift or metabolic turnover. ^{32}P in primary consumers can also be recycled into the periphyton as waste products are excreted onto the periphyton.

A known amount of ^{32}P is introduced into the stream. The radioactivity per unit weight, radioactivity per unit area, and total radioactivity in the study section are measured for 30 to 40 days. The radioactivity per unit weight of periphyton changes as either radioactivity is lost as metabolic turnover of phosphorus or the periphyton increases in biomass. This change in radioactivity per unit weight of periphyton can be expressed by the following differential equation:

$$\frac{dW}{dt} = -(P + T)W$$

where W is the ^{32}P per unit weight of periphyton, P is the turnover rate of periphyton biomass (per unit time), and T is the metabolic turnover rate of ^{32}P per unit ^{32}P (per unit time). The radioactivity per unit area of substrate will change as cells containing ^{32}P are sloughed off, as ^{32}P is turned over metabolically, or cells containing ^{32}P are grazed by benthic invertebrates. The change in radioactivity

per unit area can be represented by the following differential equation:

$$\frac{dA}{dt} = -(G + S + T)A$$

where A is the ^{32}P per unit area of substrate, G is the percentage of the periphyton standing crop grazed (per unit time), S is the sloughing of periphyton per unit area of substrate (per unit time), and T is as previously defined. The total amount of ^{32}P within the study reach will change as ^{32}P is exported downstream in sloughed cells or in solution as a result of metabolic turnover of ^{32}P by cells in the study section. This change in total radioactivity in the study section can be described by the following differential equation:

$$\frac{dC}{dt} = -(T + S)C$$

where C is the ^{32}P retained in the study section of stream and T and S are as previously defined. S can be deleted from the above equations by assuming that sloughing is minimal.

The biomass turnover rate of periphyton is equal to the difference between the rate of change of radioactivity per unit weight of periphyton and the rate of change of total radioactivity in the study stretch. The net rate of periphyton production can be obtained by multiplying the biomass turnover rate by the mean standing crop of periphyton during the study.

The percentage of the periphyton standing crop grazed per unit time can be derived by subtracting the rate of change in radioactivity in the study section from the rate of change in radioactivity per unit area. The grazing rate is equal to the product of the percent of the periphyton standing crop grazed per unit time and the mean standing crop of periphyton during the study.

Three major assumptions must be made in using the ^{32}P material balance as outlined by Elwood and Nelson (1972): 1) sloughing of periphyton from stream substrates is negligible, 2) uptake of ^{32}P is a biological process of the periphyton and not a physical adsorption phenomenon, and 3) ^{32}P is not recycled into the periphyton after ingestion by grazers. The last assumption was not explicitly stated by Elwood and Nelson (1972), but an underestimate of grazing rate would result if the assumption is not valid.

EXAMINATION OF ASSUMPTIONS

Sloughing of Periphyton

The first assumption of the ^{32}P material balance method, that sloughing of periphyton out of the study section is negligible, has been supported by studies by Nelson et al. (1969) and Maciolek and Tunzi (1968). The rate of sloughing of periphyton from substrates is dependent on the type of benthic vegetation, its stage of development, and current velocities in the stream. The assumption is probably valid as long as the periphyton community is composed principally of diatoms and there are no freshets, but it should be evaluated for each site intended for the use of the ^{32}P material balance method.

^{32}P Sorption Processes

The second assumption, that uptake of ^{32}P is a biological function of the periphyton and not a physical sorption process, depends on the substrate material in the particular stream. Sorption of ^{32}P onto inorganic materials has been shown to be a small fraction of the ^{32}P retained in the system by Nelson et al. (1969) on a streambed composed of chert residuals and weathered limestone and Garder and Skulberg (1966) on marine clay. Organic stream sediments are

another potential sorption site in streams, but little work has been done with this material (Nelson et al., 1969). The amount of ^{32}P sorbed onto leaves in Walker Branch was variable (Nelson et al., 1969). They attributed this variation to the amount of periphyton on the leaves and that, in turn, to the amount of time the leaves had been in the stream. Inorganic stream substrates, organic sediments, and leaves provide potential sorption sites; therefore, it must be determined whether ^{32}P sorbed onto these materials is taken up by the associated periphyton or adsorbed physically onto the surface of the substrate. An estimate of physical sorption of ^{32}P can be obtained by irradiating samples of each substrate type with gamma radiation from a ^{60}Co source. In this manner sorption processes onto each substrate type were examined.

Rock samples of volcanic origin were collected from streams in the H. J. Andrews Experimental Forest in Oregon, scrubbed free of periphyton, and rinsed in 90% acetone. Benthic core samples were collected from Oak Creek, Benton County, Oregon and separated into size classes of 10 mm - 0.991 mm, 0.990 - 0.500 mm, 0.499 - 0.175 mm, and 0.174 - 0.116 mm. Alder leaves were obtained from natural leaf accumulations in Oak Creek. Portions of each substrate type were sterilized by ^{60}Co gamma irradiation.

A plexiglass chamber developed by McIntire et al. (1964) was used to simulate stream conditions. Rock samples were placed in

two rows at the head of the chamber. Two discs, 2.1 cm in diameter, were cut from each leaf and placed on pins secured to a plexiglass plate in the chamber. Sediment samples were contained in PVC tubes 5.0 cm in diameter and sealed at both ends with 0.116 mm Nitex netting. A plexiglass plate in each tube separated irradiated samples from non-irradiated samples.

The method for introducing the radioactive phosphorus described by Nelson et al. (1969) was used to release 300 uCi of ^{32}P into the chamber during a 1 hour interval. The flow rate in the chamber was 1×10^4 ml/min; therefore, the maximum ^{32}P concentration was 1.1×10^4 dpm/ml of water. The samples were removed at the end of the hour and were prepared for radiation measurement.

Rock samples were soaked in a 2.0 N HCl 1% HF solution for 24 hours, rinsed, and removed for measurement of surface area and weight. The 2.0 N HCl 1% HF solution was neutralized and evaporated to a crystalline state. The crystal was dried at 50°C, weighed, and subsampled. The crystal subsamples were placed in tared planchets, weighed, and monitored for radioactivity. The total radioactivity in the original rock sample was then determined by extrapolating from the subsamples by weight. Surface area of the rock samples was determined by coating the rocks with collodion, removing the film, outlining the film on graph paper, and measuring the area with a polar planimeter. Leaf discs and sediment samples

were each placed in tared planchets, dried at 50°C for 24 hours, weighed, and measured for radioactivity. After radiation measurements, the leaf discs and sediment samples were placed in a muffle furnace at 500°C for 4 hours to determine ash-free dry weights. A Nuclear Measurements Corporation gas-flow Geiger-Muller detector was used for radiation measurements; all ^{32}P data were corrected for radioactive decay and background noise.

Sorption of phosphorus onto sterilized stream substrates was less than the uptake of phosphorus onto unsterilized substrates (Table 1). The sterilized rock samples sorbed 226.4 dpm/cm^2 during the 1 hour study period. Later studies in the streams in the Cascade Mountains indicated that similar natural rock substrates sorbed 10 times as much ^{32}P for a 75 minute release as did the sterilized substrates. Nelson et al. (1969) in White Oak Creek, Tennessee obtained ^{32}P concentrations 5 times higher than those on sterile substrates. Unsterilized leaf discs sorbed 7 times as much ^{32}P per unit ash-free dry weight and 8 times as much ^{32}P per unit area as the sterilized leaf discs. In the three lower size classes of sediments, the unsterilized samples sorbed twice as much ^{32}P as the sterilized samples; the unsterilized sediment sample in the 10 mm - 0.991 mm size class sorbed 10 times as much ^{32}P as the sterile sample. Sorption onto sediment samples may have been slightly hindered by reduction of flow by the 0.116 mm Nitex net

Table 1. ^{32}P concentrations on sterilized and unsterilized stream substrates following a 1 hour exposure to ^{32}P at 9°C in December 1972 (mean ± one standard error).

Sample	Sterile			Nonsterile		
	dpm/mg Dry Weight	dpm/mg Ash-Free Dry Weight	dpm/cm^2	dpm/mg Dry Weight	dpm/mg Ash Free Dry Weight	dpm/cm^2
Basaltic Rocks	218.1 ± 42.2		226.4 ± 47.2			
Leaf Discs	17.9 ± 2.1	22.5 ± 4.7	83.6 ± 10.4	135.2 ± 12.2	159.5 ± 12.6	651.1 ± 57.4
Sediment Cores[1]						
10 mm - 0.991 mm	0.05	0.77		0.49	8.86	
0.991 - 0.500 mm	0.26	2.33		0.74	6.00	
0.500 - 0.175 mm	1.16	10.82		2.65	17.80	
0.175 - 0.116 mm	2.74	18.74		6.77	36.32	

[1]Sediment cores were analyzed as single samples, therefore, there is no standard error.

covering the tubes. Sorption of ^{32}P was consistently greater on unsterilized stream substrate samples than on sterilized samples. Except for fine stream sediments, the amount of ^{32}P on sterilized samples was always less than 20% of the unsterilized, natural substrates. Fine stream sediments are likely more refractory organic material. The lessened biological activity and greater surface to volume ratio on these materials would account for the greater importance of physical adsorption on fine sediments.

Effect of Residence Time in the Stream on Sorption of ^{32}P onto Leaves

Nelson et al. (1969) reported that the uptake of ^{32}P onto leaves was very variable. They suggested that this variability was related to the residence time of the leaves in the stream and therefore the degree to which the leaves had been colonized by periphyton. To examine this hypothesis, leaf bags of bigleaf maple and conifer needles were placed in Berry Creek, Benton County, Oregon at 3 day intervals for 60 days. At the end of 60 days the leaf bags were removed and taken into the laboratory at Oregon State University. Discs were cut from each leaf and needles were separated into two groups for respiration measurement and ^{32}P uptake. The remaining material was used for nitrogen analysis.

The leaf discs and conifer needles to be used for ^{32}P uptake measurement were separated into two groups, one of which was sterilized by gamma radiation from a ^{60}Co source. The leaves and needles were then treated identically to the leaves in the previous sorption study. The leaf discs and conifer needles to be used for the measurement of respiration were placed in Warburg flasks in a Gilson Differential Respirometer at 13°C. Temperatures in Berry Creek during the 60 day incubation period ranged from 9°C. to 12.5°C. The respirometer was allowed to equilibrate for 1 hour prior to measurement of respiration. Oxygen consumption was measured for three 1-hour periods. The leaf discs and needles were then dried at 50°C. for 24 hours and weighed. Results for each leaf type were expressed as dpm/g of leaf (1 hour exposure) and ul O_2/g of leaf/hr.

Conifer needles showed a definite relationship between both uptake of ^{32}P and consumption of O_2 and residence time in the stream (Figure 2). The same type of response was found with the bigleaf maple leaves, but the rates of ^{32}P uptake and O_2 consumption with increasing residence time were greater than those found on conifer needles (Figure 3). The concentration of nitrogen in the two types of leaf material increased with increasing residence time in the stream (Figure 4). The rate of nitrogen increase was greater in bigleaf maple than in conifer needles. The results of nitrogen increases are consistent with ^{32}P uptake and respiration on the two leaf types.

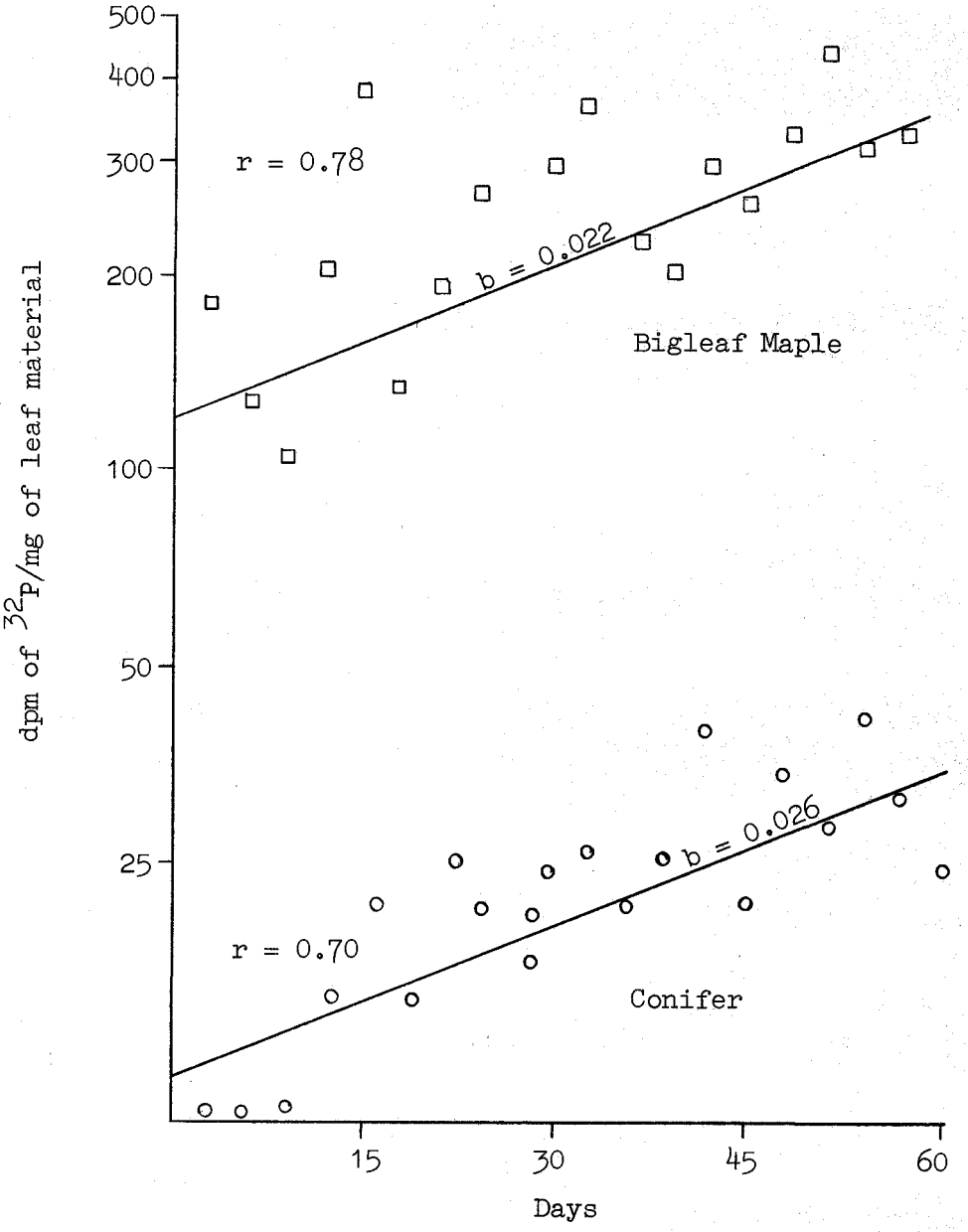

Figure 2. Uptake of ^{32}P onto bigleaf maple and conifer incubated in Berry Creek from 3 to 60 days during February - March 1973.

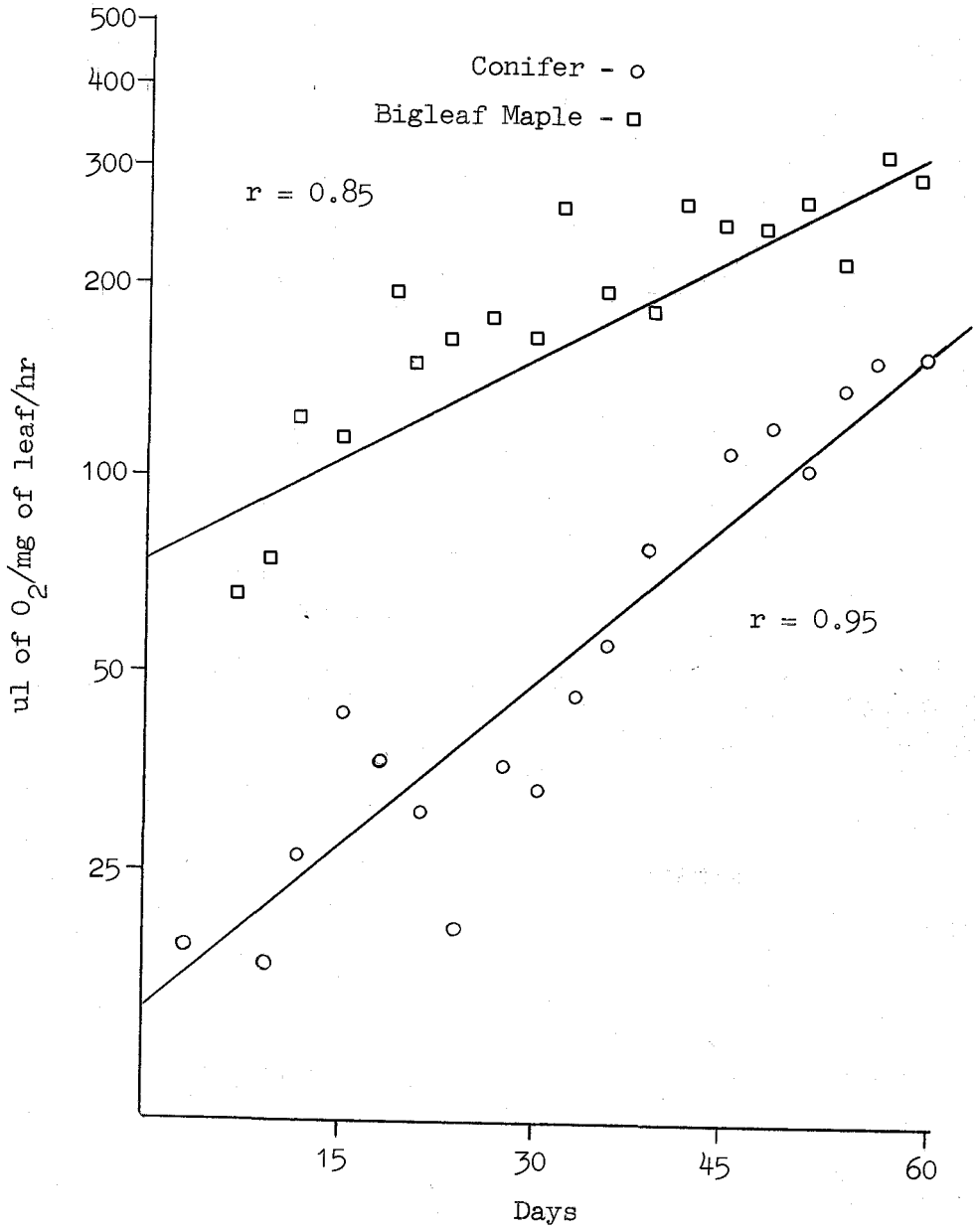

Figure 3. Consumption of oxygen by bigleaf maple and conifer incubated in Berry Creek from 3 to 60 days during February - March 1973.

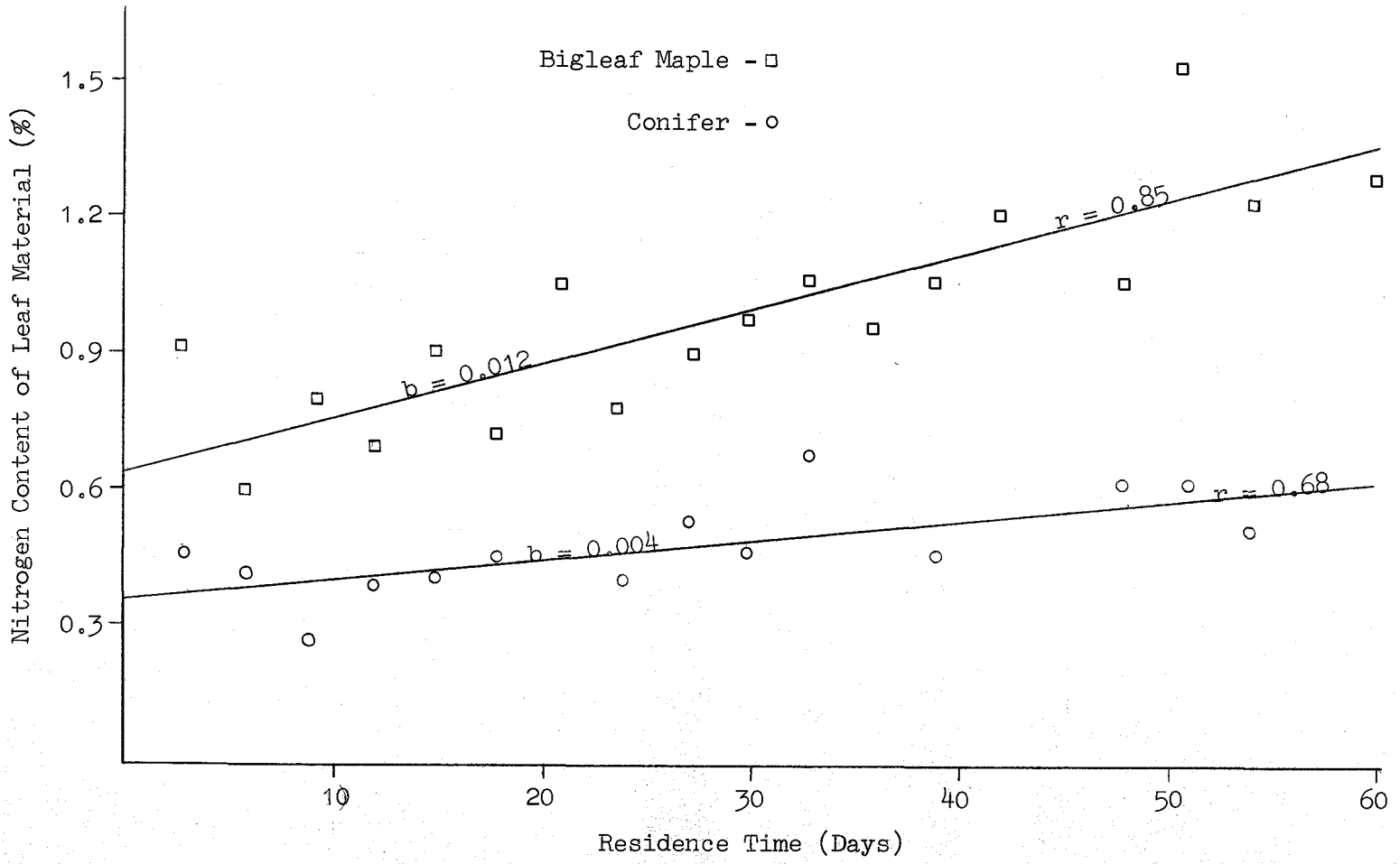

Figure 4. Percentage of nitrogen in bigleaf maple leaves and conifer needles for residence times of 3 to 60 days of incubation in Berry Creek during February - March 1973.

The sorption of ^{32}P/mg of leaf material was positively related to the microbial respiration on the leaves (Figure 5). The rate of ^{32}P uptake with increasing oxygen consumption was greater on bigleaf maple than on conifer needles.

Recycling of ^{32}P in the Periphyton

The third assumption, that ^{32}P is not recycled into the periphyton after ingestion by grazers, is valid only if all feces are washed off substrate surfaces immediately after defecation. This would depend on the type of grazer, substrate morphology, and current velocity. The manner in which a grazer defecates determines where the feces are deposited in relation to the current. Saddle case caddisflies, such as Glossosoma, deposit their feces on the substrate surface under their cases, thus their feces would be less likely to be washed off the substrate than the feces of a grazer such as the grazing mayflies, which deposit their feces slightly off the surface in the current. The structure of the substrate surface would also influence retention of fecal material; smooth surfaces would retain less than a surface with many cracks, crevices, and pockets. Substrates in swift current would retain much less fecal material than those in backwaters and pools. Retention of fecal material of the caddisfly Glossosoma and the snail Oxytrema silicula on several substrate types in a range of currents was

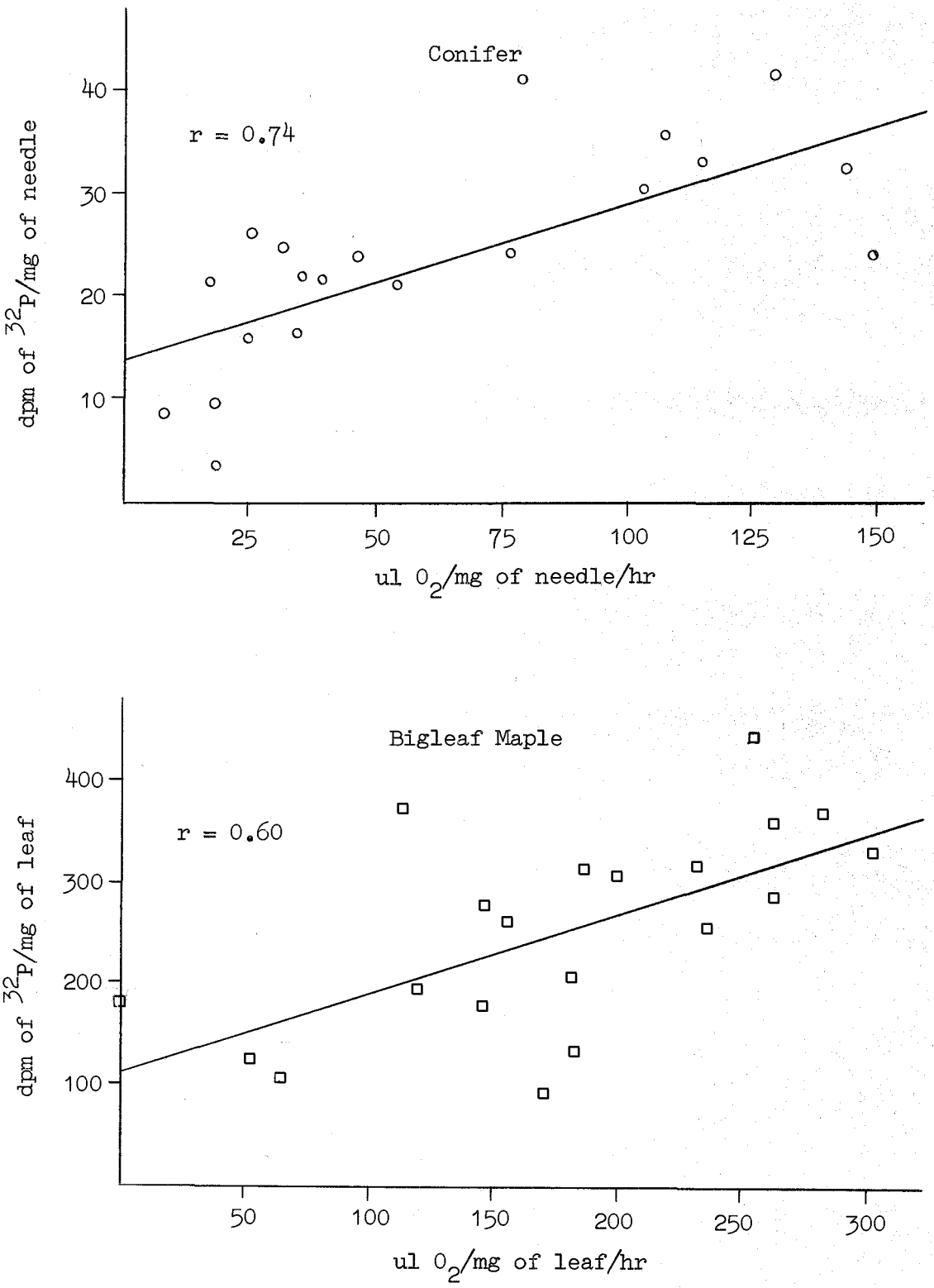

Figure 5. Uptake of ^{32}P versus consumption of O_2 by bigleaf maple and conifer incubated in Berry Creek during February - March 1973.

examined in the clear plexiglass chamber previously described. The study was made by observation only and no efforts to quantify results were made.

All substrate surface types retained some fecal material except for very round, smooth substrates. On these, gravity as well as current removed almost all the feces. In fast current (0.6 m/sec) only substrates with crevices and pockets tended to retain fecal material. In slow current (0.07 m/sec) almost all substrates retained a significant amount of feces. Retention of fecal material containing ^{32}P and subsequent recycling into the periphyton would lead to an underestimate of the grazing rate. As Elwood and Nelson (1972) pointed out, recycling of ^{32}P within the periphyton does not affect the estimates of periphyton production rates or grazing rates. The underestimates of the rates of decrease of ^{32}P/unit weight, ^{32}P/unit area, and ^{32}P in the total stream cancel each other out. However, the recycling of ^{32}P from feces causes an underestimate of the rate of decrease of ^{32}P/unit area but does not affect the rate of change of ^{32}P in the total study area. Therefore, the grazing rate on periphyton is underestimated if ^{32}P is recycled into the periphyton from feces.

LABORATORY VERIFICATION OF THE ^{32}P METHOD

The ^{32}P material balance method for measuring net periphyton production was derived theoretically by Elwood and Nelson (1972) and applied in the field, but the method had never been verified against an independent measurement. The simplest method for measuring net periphyton production is measuring changes in standing crop of periphyton, but this is only valid in the absence of grazers, drift, and sloughing. A test of both methods in the plexiglass chamber provided a direct comparison of the two methods. Grazers were excluded, drift was lacking, and sloughing was minimal since the community was in the early stages of growth. A periphyton community was established on a plexiglass plate in the laboratory with samples from the streams in the H. J. Andrews Experimental Forest as a source of periphyton. The community was allowed to develop for 3 weeks in an aquarium equipped with a stirrer to provide a current. It was then placed in the plexiglass chamber for a week prior to initiating the experiment.

The ^{32}P material balance method was modified because measuring the ^{32}P leaving the chamber would be impossible due to the small amount of periphyton. In the absence of grazers the rate of change of radioactivity per unit area can be expressed as:

$$\frac{dA}{dt} = -(T + S)A$$

therefore, subtracting the rate of change of radioactivity per unit area from the rate of change per unit weight gives the instantaneous growth rate necessary for calculating the net periphyton production rate. This modification was used to measure the rate of periphyton production.

To introduce the ^{32}P, flow through the chamber was stopped and the plate was removed temporarily to allow all water to be removed. The plate was replaced and 3 liters of water were put in the chamber, to which was added 7.28 uCi of ^{32}P as orthophosphate. The ^{32}P solution was held for 2 hours at the end of which two 100-ml water samples were taken. Five 1-ml aliquots were taken from each 100-ml water sample and placed in a planchet, evaporated, and monitored for radioactivity. The chamber was then flushed for an hour to remove unbound ^{32}P. At the end of the flushing period five periphyton samples were taken from the plate using a funnel, a rubber scraper, and a plastic suction tube. The tip of the funnel had been cut to allow sufficient sampling area and leveled to permit it to seal properly against the plate. The rubber scraper was used to remove the bulk of the periphyton from the surface and the nylon brush was used to remove any residual periphyton after the scraper had been used. The periphyton in suspension in the funnel was

removed with the plastic suction tube. The funnel was rinsed and emptied twice after each sampler was used and the scraper and brush were then rinsed in the sample collection flask. Periphyton samples were killed with formalin, filtered through pre-weighed 0.45 u Millipore filters, dried at 50°C. for 24 hours, cooled to room temperature in a dessicator, weighed, and monitored for radioactivity. Weights were corrected for filter leaching and radioactivity was corrected for background and radioactive decay. Groups of five periphyton samples were taken on the 2nd, 4th, 6th, 8th, 10th, 13th, 16th, 19th, 22nd, 26th, and 30th days. Each sample taken to determine the radioactivity per unit area and weight was also used to measure standing crop.

The changes in radioactivity per unit area and weight as well as standing crop were fitted to exponential models of growth. An instantaneous growth rate of 3.9% per day was obtained by subtracting the rate of change of ^{32}P per unit area from the rate of change in ^{32}P per unit weight (Figure 6). The net periphyton production rate as determined by the change in standing crop method was 0.576 $g/m^2/day \pm 0.100\ g/m^2/day$ (95% C. I.) (Figure 7). The product of the instantaneous growth rate and the mean standing crop during the 30 days gave an estimate of a net periphyton production rate of 0.522 $g/m^2/day \pm 0.128\ g/m^2/day$ (95% C. I.) as determined by the ^{32}P material balance method (Table 2). T tests revealed no

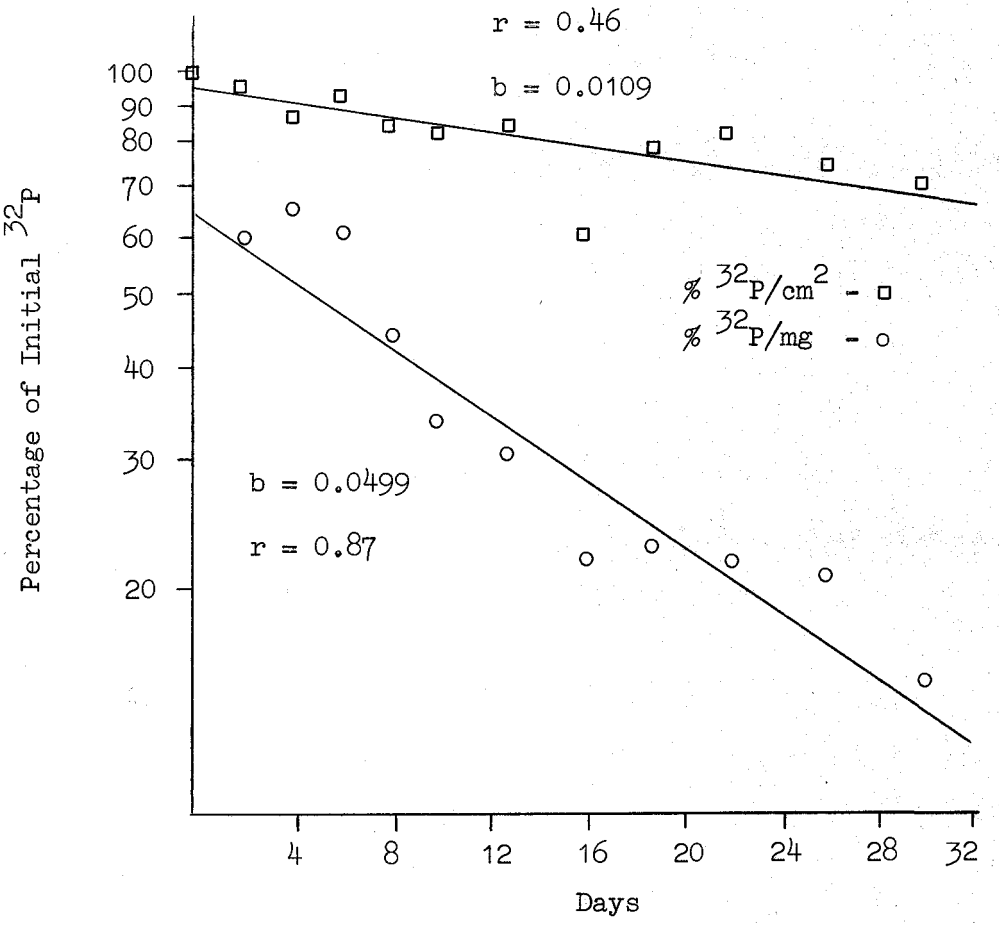

Figure 6. Mean percentage of initial ^{32}P per unit area and weight for 30 days in laboratory stream chamber in April 1973.

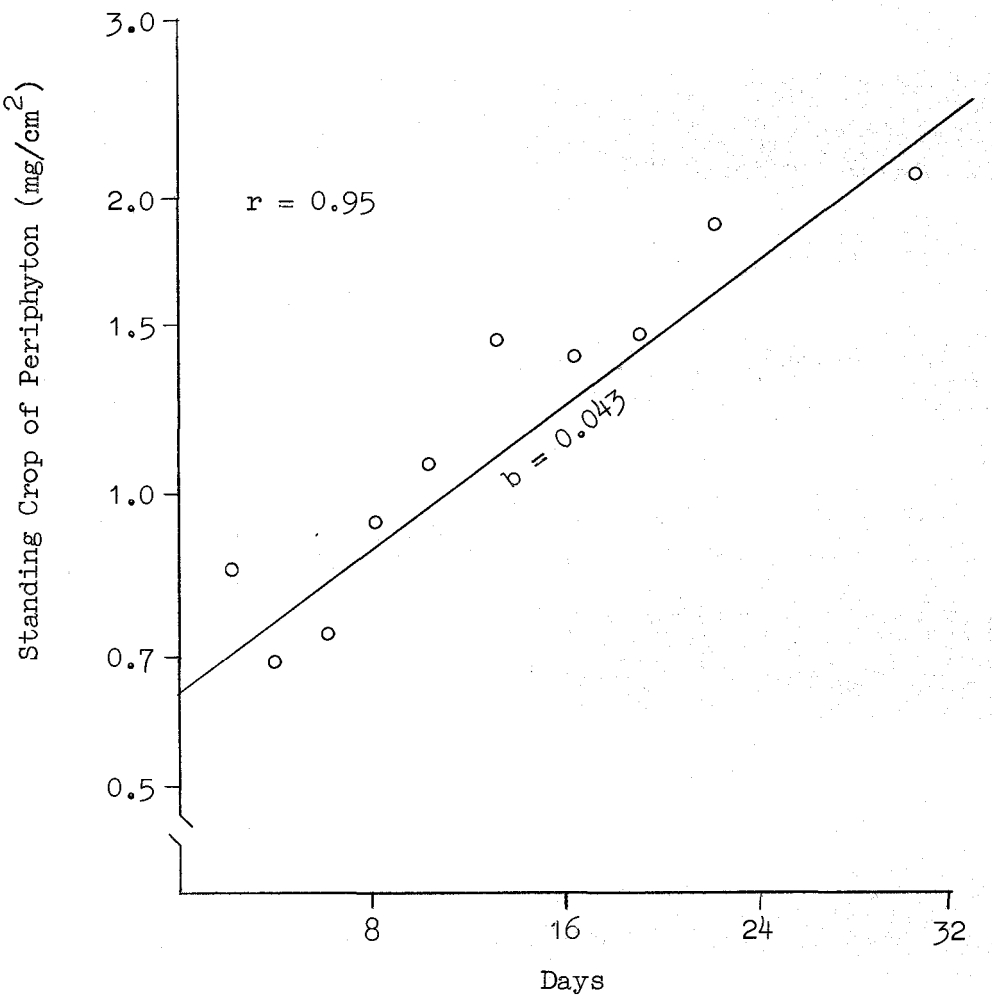

Figure 7. Standing crop of periphyton in laboratory stream chamber for 30 days in April 1973.

Table 2. Calculation of net periphyton production.

^{32}P Material Balance Method		Standing Crop Method
$b_{^{32}P/\text{unit weight}} - b_{^{32}P/\text{unit area}}$	Instantaneous Growth Rate	Slope of Logarithmic Increase
0.050 - 0.011 = 0.039		0.043
IGR X Mean Standing Crop	Net Periphyton Production	IGR X Mean Standing Crop
0.039 X 13.39 g/m^2		0.043 X 13.39
0.522 g/m^2/day $\pm$ 0.128 g/m^2/day		0.576 g/m^2/day $\pm$ 0.100 g/m^2/day

significant difference between the two estimates at the 95% confidence level. The close agreement of the two independent methods supports the validity of the ^{32}P material balance method.

APPLICATION OF THE ^{32}P MATERIAL BALANCE METHOD IN MACK CREEK

The ^{32}P material balance method was used to measure net periphyton production in Mack Creek in the H. J. Andrews Experimental Forest in the Cascade Mountain Range in Oregon during July 1973. Mack Creek drains a 650 heactare watershed with side slopes with a 77% gradient and a stream gradient of 10% (Froehlich, 1973). The section of Mack Creek studied was 65 m long and 3 m wide. The stream section flows north with the west side of the stream bordered with a Douglas-fir-Hemlock forest and the east side by a 10-year-old clearcut. The streambed is mainly composed of rocks of volcanic origin. The size distribution of the substrate is very heterogeneous, ranging from fine particles to boulders up to 2 m in diameter. Stream flow during the study ranged from 110 to 200 liters/sec. Rhodamine G dye was used to measure the flushing rate through the study section. The leading edge of the dye passed through the 65 m long study area in 3 minutes; no visible dye remained after 8 minutes. Stream temperatures ranged from 10°C. to 18°C. Streams in the area are very low in dissolved nutrients with an average total dissolved solids of 40 mg/liter (Fredriksen, 1971). Diatoms and scattered clumps of Zygnema and Prassiola are the predominant forms of algae in the periphyton in the summer.

Because the stream was on Federal land, authorization to release ^{32}P to the stream was obtained through the U. S. Forest Service. The release was designed to maintain ^{32}P concentrations in the stream below the maximum permissable concentration in water of 5×10^{-4} uCi/ml (ICRP, 1959). Mack Creek flowed into Lookout Creek approximately 500 m below the study section. Lookout Creek is about 5 times larger than Mack Creek and flows approximately 15 kilometers before leaving the H. J. Andrews Experimental Forest. Therefore, it was unlikely that significant amounts of ^{32}P would reach areas of public access.

Introduction of the ^{32}P into the stream was similar to that described by Nelson et al. (1969). A drippery bottle was assembled by using a rubber stopper with a glass tube to allow the ^{32}P solution to drip out and another tube to allow air into the top of the bottle. A 9-liter glass carboy that had been soaked for 24 hours in a solution of 2 N HCl 1% HF was used as a spike bottle. Less adsorption has been found on glass treated in this manner than on untreated glass or polyethylene (Hassenteufel, Jagitsch, and Koczy, 1963). The ^{32}P spike solution was prepared by adding 350 mCi of ^{32}P as orthophosphate to 7.0 liters of stream water. A standard of the ^{32}P solution was prepared by adding 0.2 ml of the spike solution to 1.5 ml of 0.66 N HCl 0.3% HF in a serum bottle.

When the spike bottle was inverted over the stream, Rhodamine G dye was released into the stream. As the dye passed the downstream station at 65 m the first water sample was taken in a 300 ml BOD bottle, the second sample was taken 2 minutes after the dye had passed, the third at 5 minutes, and subsequent samples were taken at 5 minute intervals. The ^{32}P solution was released in 75 minutes, after which the spike bottle was rinsed into the stream three times with stream water and another solution of Rhodamine G was introduced. As the second pulse of dye passed the downstream station the time was noted and water sampling was continued for 30 minutes after the spike had passed out of the study area. Water samples were taken back to the laboratory at Oregon State University. Ten ml of 2 N HCl 1% HF were added to each sample and a 2 ml aliquot of the sample was evaporated on a planchet and monitored for radioactivity on a Nuclear-Chicago gas-flow Geiger-Muller detector. Radioactivity measurements were corrected for radioactive decay, background, and instrument efficiency.

One hour after the ^{32}P release, samples of periphyton were taken at 10 m intervals through the study section. Two rocks were removed at each station, placed in zip-lock bags, and covered with formalin. Samples of filamentous algae were also taken if present. The rocks were taken back to the laboratory where they were thoroughly scraped with a nylon brush on a Dremel Moto-Tool and

rinsed with distilled water. The solution containing the periphyton was then filtered through a 0.8 u Millipore filter, dried at 50°C. for 24 hours, weighed, ashed at 500°C. for 4 hours, weighed, and monitored for radioactivity. Radioactivity measurements were corrected for radioactive decay, background, and detector efficiency. Weights were corrected for filter ash and leaching.

The stream water was sampled with a continuous water sampler (Figure 8) which was modified from a sampler developed by Klock and Fowler (1972). The stream water ran through an 8 m plastic hose into a plastic reservoir with an overflow pipe and a glass tube leading into a glass carboy below the reservoir. The carboy was sealed with a rubber stopper with two additional tubes. One glass tube was sealed to a 30-gauge needle and the other was closed off by a two-way valve rubber bulb. By adjusting the pressure in the carboy with the two-way bulb, the drip rate could be controlled, because the air leaving the carboy controlled the amount of water entering the carboy. Two liter composite samples were then taken to the laboratory, 1 liter of which was evaporated into a planchet and monitored for radioactivity.

Colonization of algae onto bare stream substrates was estimated by using chlorophyll a as an index of biomass. Rocks from the stream were cleaned in 90% acetone, rinsed with distilled water, and placed in baskets with a surface area of 100 cm^2. At the

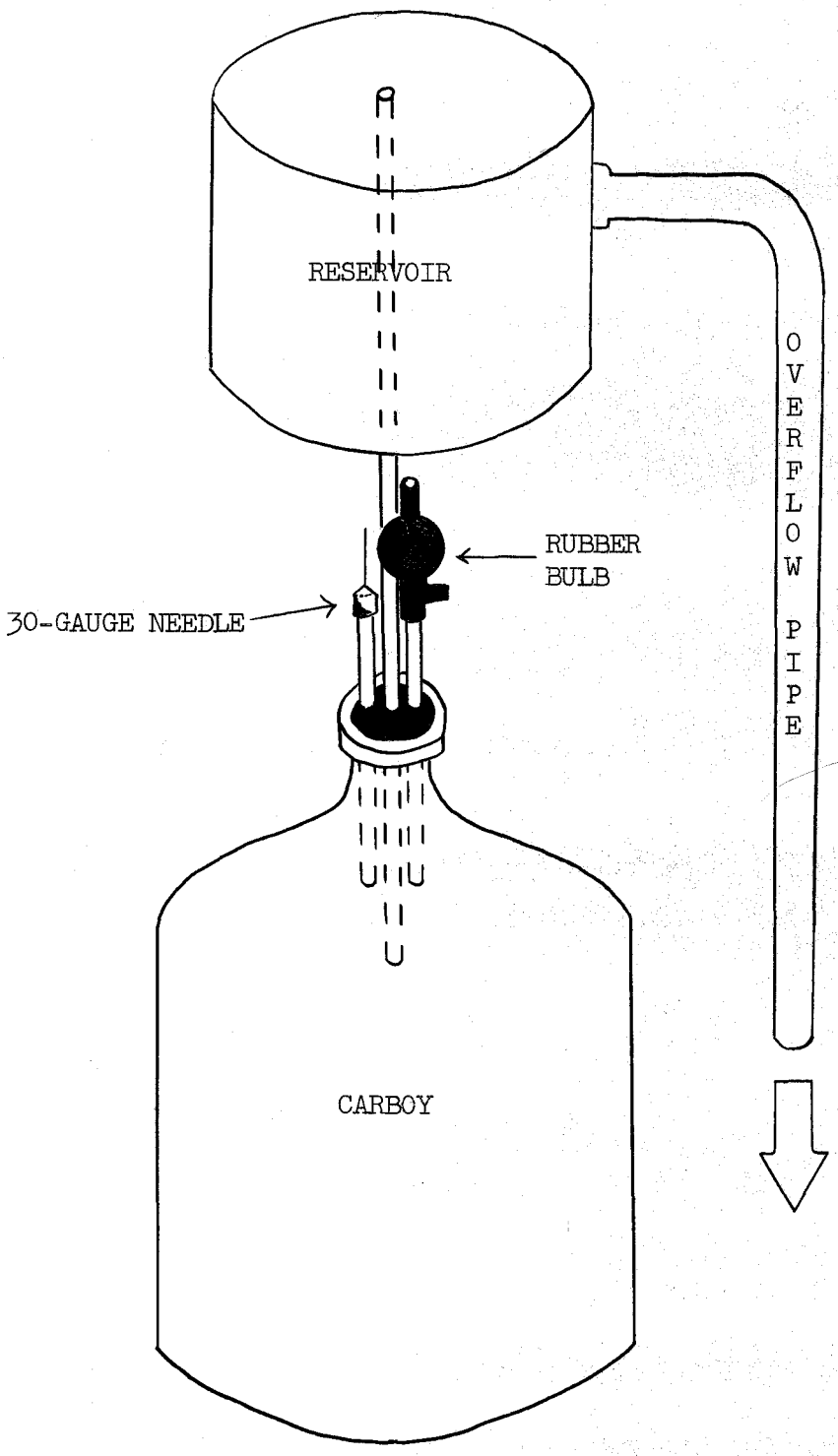

Figure 8. Continuous water sampler modified from Klock and Fowler (1972).

beginning of the experiment they were placed at three of the stations within the study section. On each sampling date three baskets were removed and two baskets of natural substrates were collected and taken to the laboratory for chlorophyll analysis. Samples were held in 90% acetone on ice for 24 hours. Fifteen ml of the acetone solution was centrifuged for 5 minutes and decanted; absorbance at 665 nm, 750 nm, and 665 nm after addition of 0.1 N HCl was read on a Beckman Model DB Spectrophotometer. The amount of chlorophyll a present was calculated by equations described by Wetzel and Westlake (1969).

The concentration of ^{32}P in the water samples taken during the release of ^{32}P was plotted against time (Figure 9). The amount of ^{32}P passing the downstream station was calculated by measuring the area under the curve and multiplying the area by the time of release. A loss of 603 mCi was calculated, which was not possible because only 350 mCi was released.

The amount of ^{32}P actually retained in the study section was calculated indirectly by expanding the concentration of ^{32}P/unit area to include the whole study area. A simple expansion of the ^{32}P/unit area (1.28×10^{-9} mCi/cm^2) to the total stream area (195 m^2) yielded an estimate of 2.5 mCi. An approximate estimate of the ^{32}P retained in the area was obtained by multiplying the simple areal estimate by the ratio of the actual surface area available for colonization to

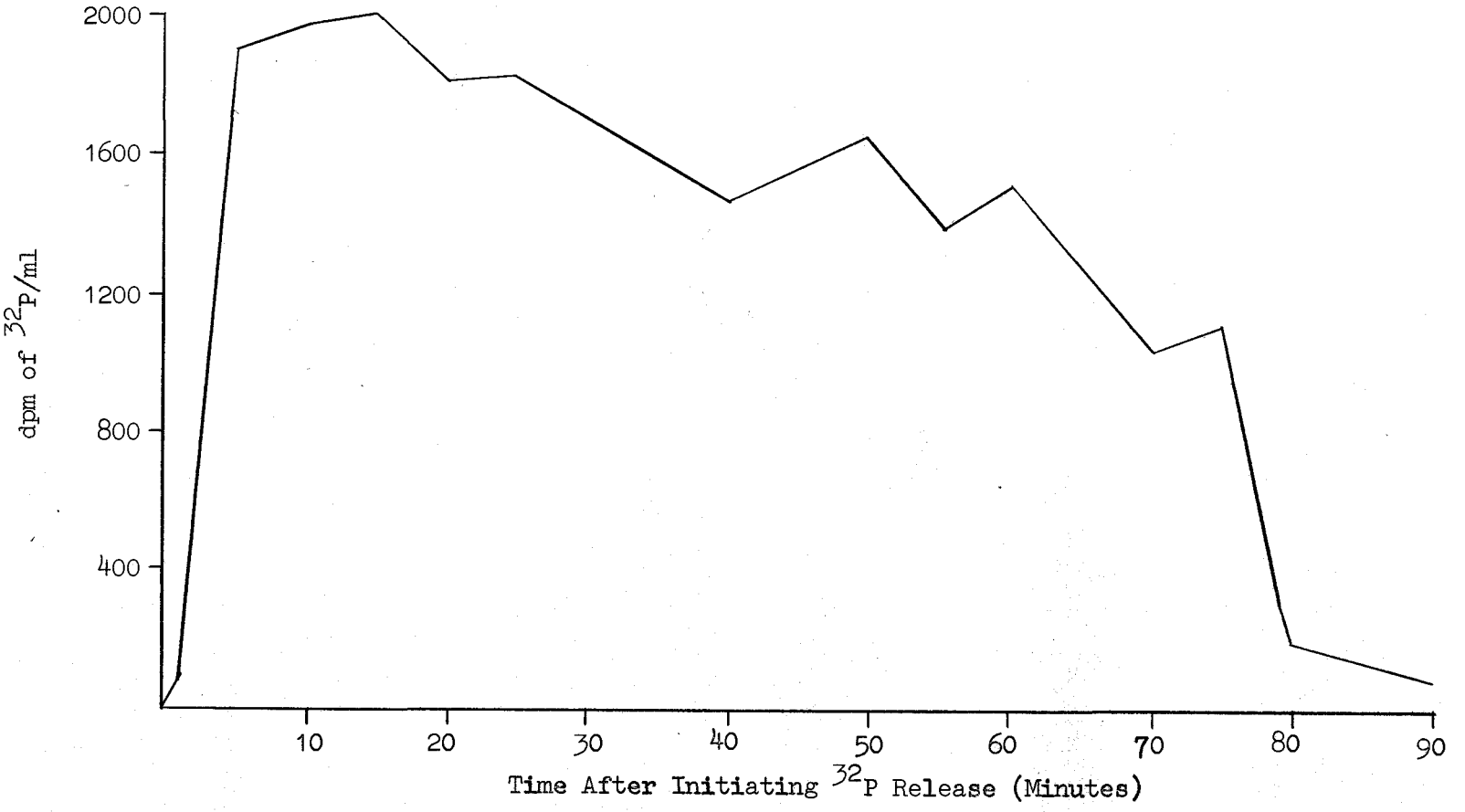

Figure 9. Concentration of ^{32}P in the water leaving the study area during the ^{32}P release in Mack Creek, July 1973.

the surface area of the stream obtained by Nelson et al. (1969) in White Oak Creek, Tennessee. The ^{32}P retained in the study section (2.5 mCi) multiplied by the factor of 4 yielded an estimate of 10.0 mCi retained. If this estimate is correct only 3% of the ^{32}P released was retained in the study area. It would have required 2.5 kilometers of stream to take up 50% of the ^{32}P released.

Analysis of composite water samples throughout the study indicated that 61.0 mCi of ^{32}P left the study area during the experiment (Table 3). This represented 6 times the amount of ^{32}P that was estimated to have been retained in the study section.

Table 3. Flow rate and loss of ^{32}P from the study section of Mack Creek, July 1973.

Interval of Study Period (days)	Flow Rate (liters/sec.)	Loss of ^{32}P (mCi)
0 to 2	197.0	27.8
2 to 4	173.0	6.1
4 to 6	155.7	4.5
6 to 8	150.5	2.6
8 to 10	139.2	6.3
10 to 13	137.3	4.7
13 to 16	137.3	4.3
16 to 20	127.4	4.7
Total		61.0 mCi

The concentration of ^{32}P/unit weight of periphyton over the 20 day study period decreased in a typical exponential manner (Figure 10). After 20 days only 9% of the initial ^{32}P/unit weight remained. The rate of decrease of ^{32}P/unit weight was 12.3% per day.

The colonization rate of periphyton onto bare substrates as measured by the extraction of chlorophyll a was analyzed using an exponential model of growth (Figure 11). The colonization rate of algae has been used to approximate the net periphyton production rate (Sladeckova, 1962; Kevern, Wilhm, and Van Dyne, 1966; King and Ball, 1966; Stockner, 1968; Vladimirova, 1969). A daily instantaneous growth rate of 0.186 was calculated from the exponential rate of increase. An estimate of net periphyton production of 360 mg/m^2/day was obtained by multiplying the instantaneous growth rate by the mean standing crop during the study.

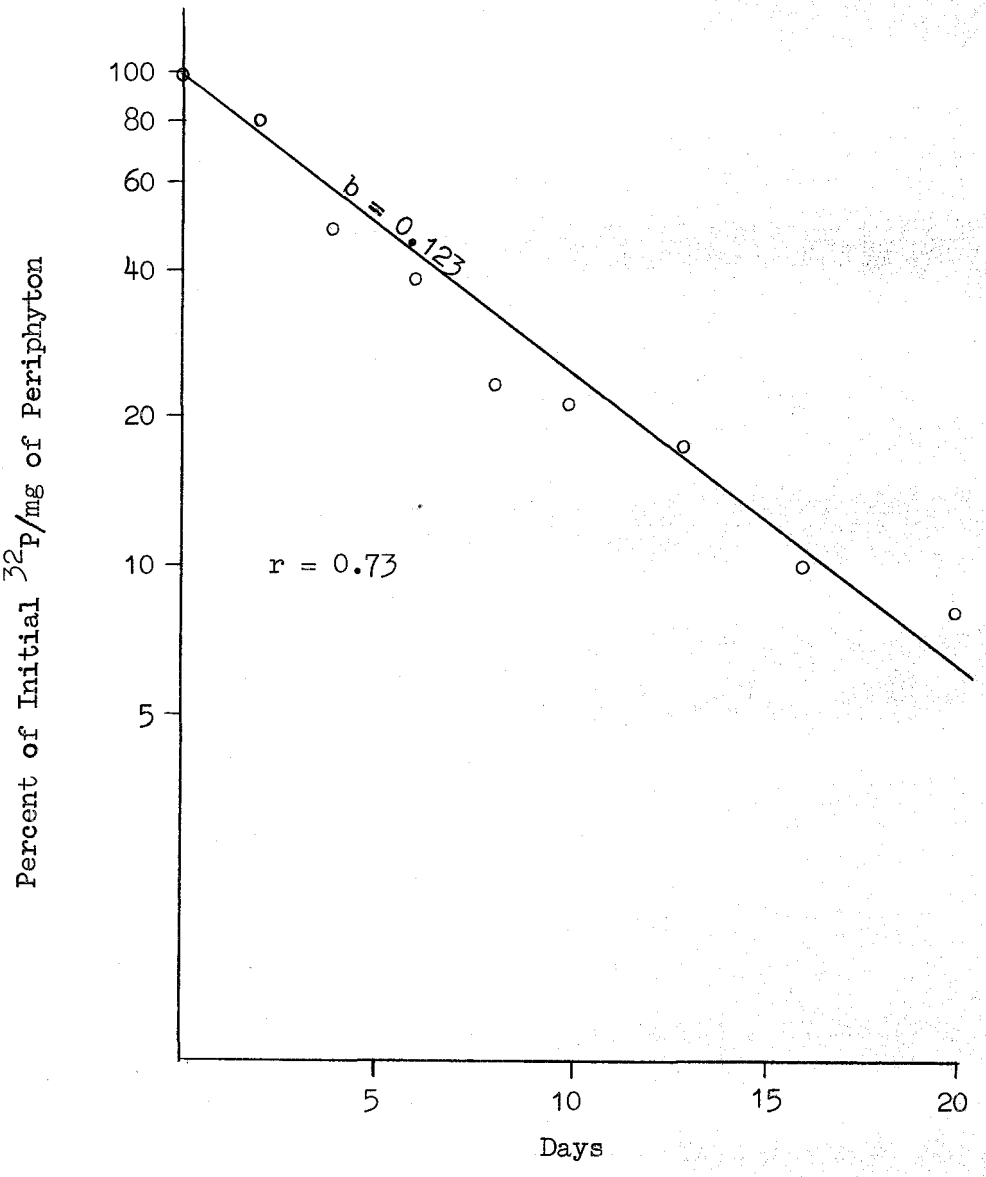

Figure 10. Decrease in ^{32}P/mg of periphyton in Mack Creek, July 1973.

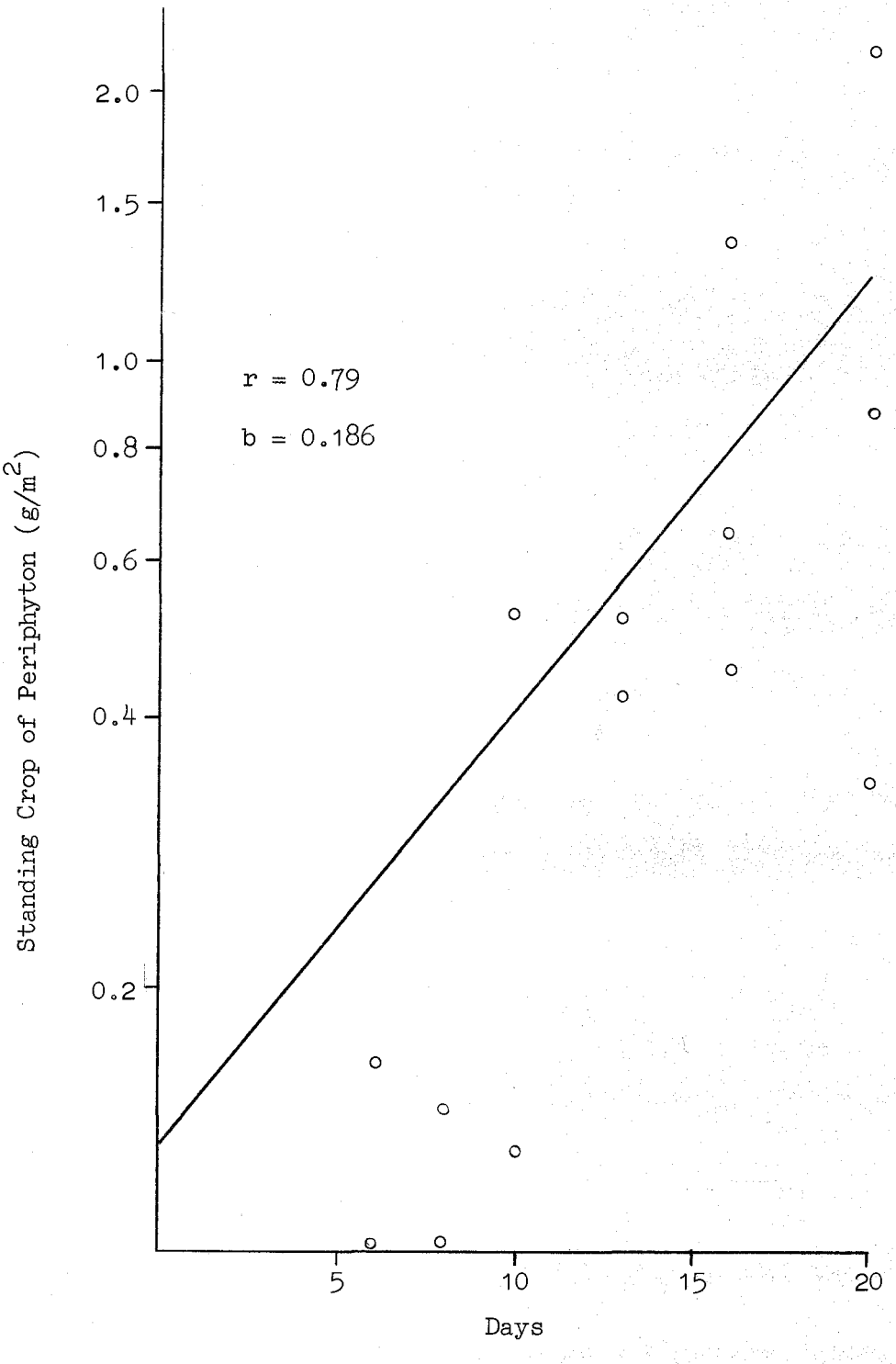

Figure 11. Colonization of bare substrates by algae in Mack Creek in July 1973.

DISCUSSION

The measurement of periphyton production rates in shallow, turbulent streams presents many problems. Methods normally used in flowing water must be used in chambers and are no longer in situ measurements. The ^{32}P material balance method of Elwood and Nelson (1972) offers a possible solution to this problem for the measurement of periphyton production rates. The close agreement between the ^{32}P method and the standing crop method in the laboratory supports the validity of the method as developed by Elwood and Nelson (1972). Natural systems have many more variables than a laboratory stream chamber; therefore, the method must eventually be evaluated for the system in which it will be used.

The validity of the assumptions made by the ^{32}P material balance method varies under different conditions, and any investigator using the method must evaluate the assumptions for his particular situation. The assumption concerning sorption onto stream substrates is likely to be valid under most conditions, having been tested for a wide range of materials. This is not to say that uptake of ^{32}P in streams is a simple biological process, but rather a complex phenomenon predominantly involving the organisms attached to stream substrates. Uptake of ^{32}P onto leaves was clearly a function of the residence time in the stream, and microbial respiration also correlated

very closely with the residence time. The uptake of ^{32}P was not identically related to oxygen consumption on bigleaf maple and conifer needles. Even the rate of ^{32}P uptake onto sterilized leaf material, which should be a simple adsorption process, did not resolve the difference between bigleaf maple and conifer. This suggests that the populations on the two types of leaf material are different or are processing nutrients differently. Nonetheless, uptake of ^{32}P onto stream substrates is mainly a function of the periphyton and, therefore, compatible with the use of the ^{32}P material balance method.

The third assumption involved only the measurement of grazing rates on periphyton. Retention of grazer feces in the periphyton introduces error into the measurement and leads to an underestimate of grazing rates. The velocity in the stream being studied and the substrate type will determine the magnitude of this error. Once the periphyton has been tagged with ^{32}P, a wide range of feeding experiments are possible. Feeding experiments with different food type, different grazers, gut unloading rates, and even dual tagging with another isotope to determine assimilation efficiency offer valuable information on the interaction between the periphyton and primary consumers. This information is preferable in many ways to the total community grazing rate even if it can be assumed that no ingested ^{32}P is recycled into the periphyton.

The ^{32}P material balance method was not successfully applied to the small stream in the Cascade Mountain Range, Mack Creek. A possible explanation of this failure is that the retention time in the study section (8 minutes) was too short to allow a significant fraction of the release to be retained. The retention time of Walker Branch in Elwood and Nelson's study was 1 hour for 100 m of stream (J. W. Elwood, personal communication). The magnitude of the error suggests, however, that additional factors were involved. Since the ^{32}P standards were prepared from the spike solution, an error in the absolute amount of ^{32}P obtained from the radioisotope supply company cannot account for the discrepancy. The possibility of incomplete mixing of the initial ^{32}P solution was examined by trying to recreate the mixing as closely as possible and adding dye. This test indicated complete mixing could be achieved with a minimum of effort. Standards were duplicated so there was little chance of a mistake in preparation of the standards. Several methods of calculation of the ^{32}P leaving the study area were used and all gave similar results, therefore, the error is not mathematical. Water samples were measured for radioactivity on two separate occasions, therefore, there should be no error in the detection of radioactivity. After extensive examination of the procedures and consultation with Dr. J. W. Elwood and Dr. D. J. Nelson at the Oak Ridge National Laboratory and Dr. C. E. Cushing at Batelle Northwest Laboratory,

Hanford, Washington, I can give no logical explanation for the magnitude of the discrepancy involved in constructing the ^{32}P material balance.

Even though the material balance was not resolved, it appears that an extremely small fraction of the ^{32}P released was retained in the area. It was calculated that 2.5 kilometers of stream would have been required to retain 50% of the ^{32}P released to the stream. The use of the ^{32}P method with such a distance of stream would not be feasible in many streams in the Cascades for logistic reasons as well as the fact that the same stream often changes its characteristics within that distance. The only streams in which the ^{32}P material balance method has been used successfully are small streams with long retention times. For these reasons I feel the ^{32}P material balance method is not very applicable to streams with low retention times.

There are several distinct disadvantages in using the ^{32}P material balance method in certain streams in addition to the difficulties encountered with high gradient streams. The use of the maximum permissable concentrations of ^{32}P in water, as suggested by Elwood and Nelson (1972), involves a considerable amount of ^{32}P even for relatively small streams. A release for 1 hour in a stream with a flow of 0.57 m^3/sec (20 ft^3/sec) would require slightly over 1 Curie of ^{32}P, which would cost approximately $1,000. This

problem can be circumvented to some extent by using less than the maximum permissable concentration of ^{32}P or shorter release times. One must be certain to use sufficient amounts of ^{32}P to permit detection of radioactivity in the periphyton for at least 20 days. The method involves a great deal of time in sampling and sample preparation, such that for a single estimate of periphyton production inordinately high amounts of time and effort are required. Streams in which the method can be used are limited because the agencies responsible for authorization are reluctant to authorize the release of radioactive materials in areas open to the public.

A modification of the ^{32}P material balance method would permit a wider and less costly utilization. The modification used in the laboratory verification of the method could be applied to field studies by placing tagged rocks inside a 330 u mesh nylon net enclosure. The enclosure would keep large, actively moving grazers off the rocks. Thirty to 50 rocks could be selected and placed in a ^{32}P solution. The rocks would then be removed, rinsed, and placed inside the net enclosure. The enclosure would be V-shaped, pointing upstream to minimize clogging of the net and slanting toward the banks to minimize shading. As long as the net did not reduce the current appreciably, the estimate of periphyton production would not be affected. The estimate would be assumed to be representative of <u>in situ</u> periphyton production. Several rocks would be sampled at

each sampling date for approximately 20 days and processed to determine the radioactivity per unit area and weight. The instantaneous growth rate and subsequent net production rate would be calculated as in the laboratory verification study. If one is willing to accept the errors involved in recycling of ^{32}P from the feces of the grazers, a conservative estimate of grazing rates on periphyton can be obtained by subtracting the rate of change of ^{32}P per unit area of samples from inside the enclosure from the rate of change of samples outside the enclosure. The product of the difference between the two rates and the mean standing crop would be the grazing rate on periphyton.

Less than 50 uCi of ^{32}P would be required by the modified ^{32}P method, which would be much more economical than the original method. The cost of ^{32}P for a single station would be approximately $10 for any size stream. This quantity of ^{32}P is sufficiently small to permit the use of the method in streams with public access if adequate safety precautions are taken. This modification would permit use of the method in any size stream with a wide range of materials in several types of habitats as often as desired. The enclosure would force it to no longer be considered an in situ method but less manipulated than most chamber studies. This modification of the ^{32}P material balance method developed by Elwood and Nelson (1972) would be a suitable periphyton production method for any size or type of stream.

BIBLIOGRAPHY

Elwood, J. W., and D. J. Nelson. 1972. Periphyton production and grazing rates in a stream measured with a ^{32}P material balance method. Oikos 23: 295-303.

Fredriksen, R. L. 1972. Nutrient budget of a Douglas-fir forest on an experimental watershed. In: Proceedings-Research on Coniferous Forest Ecosystems-A symposium. Bellingham, Washington - March 23-24, 1972. p. 115-131.

Froehlich, H. L. 1973. Natural and manmade slash in streams. Pacific Logging Congress. Loggers Handbook Vol. 33.

Garder, K., and O. Skulberg. 1966. An experimental investigation on the accumulation of radioisotopes by fresh water biota. Arch. Hydrobiol. 62: 50-69.

Hansmann, E. W. 1969. The effects of logging on periphyton communities of coastal streams. Ph. D. Thesis, Oregon State University. 120 pp.

Hassenteufel, W., R. Jagitsch, and F. F. Koczy. 1967. Impregnation of glass surface against sorption of phosphate traces. Limnol. Oceanogr. 8: 152-156.

International Commission of Radiological Protection. 1959. Report of Committee II on Permissable Dose for Internal Radiation. ICRP Publication 2. Pergamon Press, New York. 233 pp.

Jackson, W. A., and R. J. Volk. 1970. Photorespiration. Annual Review of Plant Physiology 22: 385-432.

Kevern, N. R., and R. C. Ball. 1965. Primary productivity and energy relationships in artificial streams. Limnol. Oceanogr. 10: 74-87.

Kevern, N. R., J. L. Wilhm, and G. M. Van Dyne. 1966. Use of artificial substrata to estimate the productivity of periphyton. Limnol. Oceanogr. 11: 499-502.

King, D. L., and R. C. Ball. 1966. A qualitative and quantitative measure of aufwuchs production. Trans. Amer. Microsc. Soc. 85: 232-240.

Klock, G. O., and W. B. Fowler. 1972. An inexpensive water sampler. USDA Forest Service Research Note PNW-188. Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

Kobaysi, H. 1961. Productivity in sessile algal community of Japanese mountain river. Bot. Mag., Tokyo, 74: 228-235.

Lane, C. B. 1965. Metabolism of periphyton communities in two small streams. Masters Thesis. Corvallis, Oregon State University. 57 pp.

Maciolek, J. A., and M. G. Tunzi. 1968. Microseston dynamics in a simple Sierra Nevada lake stream system. Ecology 49: 60-75.

McConnell, W. J., and W. F. Sigler. 1959. Chlorophyll and productivity in a mountain river. Limnol. Oceanogr. 4: 335-351.

McIntire, C. D., R. L. Garrison, H. K. Phinney, and C. E. Warren. 1964. Primary production in laboratory streams. Limnol. Oceanogr. 9: 92-102.

McIntire, C. D. 1966. Some effects of current velocity on periphyton communities in laboratory streams. Hydrobiologia 27: 559-570.

McIntire, C. D. and H. K. Phinney. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monogr. 35(3): 237-258.

Nelson, D. J., N. R. Kevern, J. L. Wilhm, and N. A. Griffith. 1969. Estimates of periphyton mass and stream bottom area using phosphorus-32. Water Res. 3: 367-373.

Odum, H. T. 1956. Primary production in flowing waters. Limnol. Oceanogr. 1: 103-117.

Sladeckova, A. 1962. Limnological investigation methods for periphyton (Aufwuchs) community. Bot. Rev. 28: 286-350.

Stockner, J. G. 1968. Algal growth and primary productivity in a thermal stream. J. Fish. Res. Bd. Canada 25: 2037-2058.

Thomas, N. A., and R. L. O'Connell. 1966. A method for measuring primary production by stream benthos. Limnol. Oceanogr. 11: 386-392.

Vladimirova, K. S. 1969. Procedure for studying the primary production of benthic algae. Hydrobiological Journal 5: 78-80.

Wetzel, R. G. and D. F. Westlake. 1969. Periphyton. In: A Manual on Methods for Measuring Primary Production in Aquatic Environments. R. A. Vollenweider (ed). Blackwell Scientific Publications, Oxford, England.

Whitford, L. A. 1960. The current effect and growth of freshwater algae. Trans. Amer. Microsc. Soc. 79: 302-309.

Whitford, L. A., and C. J. Schumacher. 1961. Effect of current on mineral uptake and respiration by a freshwater alga. Limnol. Oceanogr. 6: 423-425.