A precipitation technique, involving three precipitations, was used to chemically separate and concentrate phosphorus from Columbia River fish for radioanalysis. The technique was adapted to allow direct calculation of $^{32}$P specific activity based on dried precipitate weight and cpm obtained.

Values of $^{32}$P specific activity found in marked fingerling chinook salmon, released into the Columbia River at Ringold, Washington and collected downriver, indicated that there was a lag period in the initial uptake of $^{32}$P. Within three to four weeks, marked fish collected at The Dalles Dam appeared to approach an equilibrium value of 17 nCi $^{32}$P/g P. Calculations based on the rate of $^{32}$P accumulation yielded a biological half-life for phosphorus equal to 7.6 days, which corresponds to a phosphorus turnover rate of 9.1%
per day.

A model was developed which allows certain conclusions to be drawn about the feeding and migration behavior of Columbia River fish on the basis of their $^{32}\text{P}$ specific activity and $^{65}\text{Zn}$ concentration. The basic assumptions of the model were successfully tested by using values of activity found in marked young chinook salmon collected from the Columbia River. Levels of radioactivity found in unmarked fish collected from several locations in the Columbia River indicated that many were new to the river while others had probably remained there since the previous summer.
Determination and Application of $^{32}\text{P}$ Specific Activity in Columbia River Fish

by

Gerald Patrick Romberg

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Master of Science

June 1970
APPROVED:

Redacted for Privacy
Professor of Oceanography
in charge of major

Redacted for Privacy
Professor of Oceanography
in charge of major

Redacted for Privacy
Chairman of Department of Oceanography

Redacted for Privacy
Dean of Graduate School

Date thesis is presented ______________ July 31, 1969 ______________

Typed by Opal Grossnicklaus for ______________ Gerald Patrick Romberg ______________
ACKNOWLEDGMENTS

To my two major professors, Dr. William C. Renfro and Dr. William O. Forster, I express my appreciation and thanks for their help, encouragement, and patience. I also thank Dr. Charles L. Osterberg for my traineeship appointment and introducing me to the field of radioecology.

I am grateful for the assistance of my fellow graduate students, Messrs. Walter H. Delaplane, Robert S. Dyer, Stephen V. W. Pope, and Richard D. Tomlinson. Thanks are also due Mr. I. Lauren Larsen for help with data reduction.

I am indebted to the biologists of the Bureau of Commercial Fisheries who collected and mailed samples of young chinook salmon.

Finally, I thank my wife, Gail, for cheerfully typing the original manuscript; and my parents, who have been a continual encouragement throughout my schooling.
TABLE OF CONTENTS

INTRODUCTION 1

BACKGROUND INFORMATION 4

COUNTING CONSIDERATIONS 12

METHOD FOR DETERMINATION OF $^{32}$P SPECIFIC ACTIVITY 18

Dissolving Fish Sample 18
Phosphorus Precipitation Technique 18
Precipitation Efficiency 18
Upper Limit of Constant Extraction 25
Properties of Final Precipitate 26

APPLICATION OF $^{32}$P SPECIFIC ACTIVITY 31

Preparation of Fish Samples 31
Uptake Study 34
Behavior Model 41

SUMMARY 51

BIBLIOGRAPHY 54

APPENDIX 61
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Counting efficiency of low background beta counter for $^{32}$P.</td>
<td>15.</td>
</tr>
<tr>
<td>2.</td>
<td>Phosphorus-32 self-absorption curve.</td>
<td>16.</td>
</tr>
<tr>
<td>4.</td>
<td>Radiochemical precipitation efficiency.</td>
<td>22.</td>
</tr>
<tr>
<td>5.</td>
<td>Gravimetric precipitation efficiency.</td>
<td>23.</td>
</tr>
<tr>
<td>6.</td>
<td>Upper limit of constant precipitation.</td>
<td>27.</td>
</tr>
<tr>
<td>8.</td>
<td>The Columbia River below Hanford showing dam locations and sampling sites.</td>
<td>32.</td>
</tr>
<tr>
<td>9.</td>
<td>Increase in $^{32}$P specific activity of marked fingerling chinook salmon released into the Columbia River.</td>
<td>36.</td>
</tr>
<tr>
<td>10.</td>
<td>A general model describing the behavior of a fish based on its $^{32}$P specific activity and $^{65}$Zn concentration.</td>
<td>42.</td>
</tr>
<tr>
<td>11.</td>
<td>Testing the model using values of radioactivity found in marked fingerling chinook salmon. A representative standard deviation is shown.</td>
<td>46.</td>
</tr>
<tr>
<td>12.</td>
<td>Use of model to determine behavior of fingerling salmon collected at Boardman, Oregon.</td>
<td>48.</td>
</tr>
</tbody>
</table>
DETERMINATION AND APPLICATION OF $^{32}\text{P}$ SPECIFIC ACTIVITY IN COLUMBIA RIVER FISH

INTRODUCTION

Chemical elements tend to circulate within the biosphere via biogeochemical cycles. The behavior of nutrient elements is of particular interest because they affect primary productivity and hence all productivity. The nutrient elements, however, must be considered individually because chemical and biological processes affect each differently (Barnes, 1957). The nutrient phosphorus is an essential element of protoplasm and often an important factor in limiting and controlling the abundance of organisms (Odum, 1959). A thorough understanding of the cycling of this element in estuaries could contribute greatly to man's endeavor to increase the sea food yield from an area that is already one of the most productive in the world (Duke and Rice, 1966).

Radioisotope techniques have provided an easy and direct method for following the movement of elements in organisms and the biosphere. Phosphorus-32, a beta emitting radionuclide, can be used as a tracer to study the turnover rates, transfer routes, and reservoirs of phosphorus in various components of the phosphorus cycle (Rigler, 1956; Foster, 1959). The most meaningful and useful information obtained from such tracer studies seldom comes from carefully controlled
laboratory experiments, but rather comes from field studies in the natural environment (Foster and McConnon, 1962). Areas near atomic energy installations or nuclear testing grounds are ideal for these studies because detectable amounts of radioactive elements are introduced into the environment.

Small but measurable quantities of radioactive elements have been introduced into the Columbia River from the reactors at Hanford since the start of plutonium production in 1944. The majority of the radionuclides are produced by neutron activation of impurities in the coolant water and consist of both gamma and beta emitting radionuclides, including $^{32}$P (Foster and McConnon, 1962). The radioecology section, of the Department of Oceanography at Oregon State University, for several years had conducted radioecological studies in the Columbia River and adjacent Pacific Ocean using gamma emitting radionuclides from Hanford (Osterberg, Pearcy and Curl, 1964; Osterberg, Pattullo and Pearcy, 1964; Osterberg, Cutshall and Cronin, 1965; Carey, Pearcy and Osterberg, 1966; Johnson, Cutshall and Osterberg, 1967). However, no study has been done at Oregon State University using the beta emitting radionuclides originating from the same source, particularly the biologically important beta emitter $^{32}$P. The Columbia River and its estuary have all the conditions necessary for gaining information on the behavior of phosphorus in the biosphere, they need only to be utilized.
The objectives of this thesis were (1) to adapt and test a technique which could be routinely used to determine $^{32}$P specific activities of Columbia River organisms, and (2) to utilize such measurements for studying phosphorus cycling in particular fish species and gaining information on their feeding and migration behavior. Fingerling chinook salmon (Oncorhynchus tshawytscha) and fingerling coho salmon (Oncorhynchus kisutch) were the species primarily used. It is hoped that this work may lead the way for an extensive study of phosphorus behavior in the Columbia River and its estuary while the Hanford reactors still provide the necessary activity levels. Should these reactors be discontinued or converted such that radioactivity would no longer be introduced into the river, this ideal opportunity would be lost.
BACKGROUND INFORMATION

Phosphorus is a vital element in the biochemical processes of living organisms because phosphorus compounds form the chemical basis for cellular energy and metabolism. Two classes of organic phosphates play a role in metabolism. One group is comprised of simple esters and are quite numerous, occurring in intermediary metabolism. The other group is composed of the high-energy phosphate compounds which are easily hydrolized, with large quantities of energy being released. Adenosine triphosphate (ATP) is the most readily available high-energy phosphate compound and is the major immediate storage reservoir of cellular energy. Even resting tissue requires a continuous supply of ATP to maintain a constant composition and continue metabolism (White, Handler and Smith, 1964).

Other important organic phosphorus compounds are phosphatides which are abundant in the nuclear, mitochondrial, and ribosomal fractions of cells. Phospholipids are found associated with major structural units of cell membranes and nerve fibers (Conn and Stumpf, 1963). In addition to these compounds, large quantities of calcium phosphate are found in the mineral fraction of bone. The skeleton is also capable of storing phosphate which is withdrawn when the serum phosphate concentration decreases or is deposited when the concentration of serum phosphate increases (White, Handler and
In nature, phosphorus is never found as the free element. The great phosphorus reservoir is the rock and mineral deposits (\(\text{Ca}_3(\text{PO}_4)_2\)) and apatite, \(\text{CaF}_2 \cdot 3\text{Ca}_3(\text{PO}_4)_2\). As these erode, inorganic phosphate is released to the biosphere and becomes available to the primary producers. Once it has been taken up and converted to organic compounds, phosphorus can be transferred through the food chains and gradually returned to the environment. Fecal matter, metabolites excreted in the urine, and dead organisms are eventually decomposed by bacteria and the associated phosphorus regenerated as inorganic phosphate. This movement from the environment into the biota and back to the environment is called the phosphorus cycle. Although a long-term equilibrium tends to be established, there are discrete periods of net increase and net decrease. Transfer of phosphorus between components of the cycle is dependent on seasonal water properties and the activities of organisms. At any one time, the majority of phosphorus in an ecosystem is tied up either in organisms or in sediments (Hutchinson and Bowen, 1947, 1950).

For a large river, such as the Columbia, there are many influences which affect phosphorus concentration in the water. A large number of tributaries empty into the Columbia River resulting in a total drainage basin of 672,000 km\(^2\), the largest in Western America.
(U. S. Bureau of Reclamation, 1947). Various geological and climatological conditions within this vast area affect properties of the Columbia. Silker (1964) found Columbia River water at Richland, Washington to have a yearly phosphorus range of 0.03-1.61 micromoles per liter (μM/l). The lowest concentration occurs during the spring freshet. The freshet flow originates primarily from snow melt in the Canadian snow fields and contains smaller amounts of phosphorus, thus decreasing the concentration in river water by dilution (Silker, 1964).

Phosphorus concentrations in Columbia River water at the estuary had yearly ranges for 1966 and 1967 of 0.27-1.05 μM/l and 0.10-0.62 μM/l respectively (Park, Osterberg and Forster, 1969). Seasonal trends at the estuary are much the same as those at Richland. Total phosphorus passing through the estuary in a year was calculated to be $1.2 \times 10^8$ moles (844 tons) for 1966 and $0.8 \times 10^8$ moles (562 tons) for 1967 (Park et al., 1969).

There are seven isotopes of phosphorus with varying numbers of neutrons, ranging from 13 to 18. The only stable form is $^{31}$P with 16 neutrons, and it is 100% abundant in nature (Goldman and Stehn, 1965). Phosphorus-32 is the only radioisotope of phosphorus with a sufficiently long half-life and strong enough beta to be useful for tracer studies. The half-life of $^{32}$P is 14.3 days and it emits a beta particle with a mean energy ($E_{\text{mean}}$) of 1.70 million electron
volts (MeV). A beta particle (negatron) is similar to an orbital electron except for origin. The beta particle is ejected from the atomic nucleus, resulting in the conversion of a neutron to a proton (Wang and Willis, 1965). Upon losing a beta particle and gaining a proton, the $^{32}\text{P}$ isotope becomes the stable sulfur isotope $^{32}\text{S}$. Beta decay differs from both gamma ray and alpha particle decay because beta particles are emitted with a spectrum of energies up to a maximum energy ($E_{\text{max}}$). Each beta emission has an associated total energy equivalent to that of $E_{\text{max}}$; however, this is shared in varying proportions between a neutrino and the beta particle. Only a very small fraction of the betas are emitted at $E_{\text{max}}$. The largest fraction is generally emitted at some mean energy ($E_{\text{mean}}$), which is 0.70 MeV for $^{32}\text{P}$ (Wang and Willis, 1965). Gamma and alpha emitting nuclides can easily be identified by the discrete energy of their emissions, but this is difficult for beta emitters because of overlap in the large range of energies involved.

Phosphorus-32 is introduced into the Columbia River by coolant water from the Hanford reactors. In this single-pass system, purified river water is cycled through the reactor cooling tubes and then expelled into a holding pond from which it soon returns to the river. As the water passes through the reactor, it is subjected to a high flux of neutrons that react with impurities in the water. Phosphorus-32 is primarily produced by the neutron activation of either $^{31}\text{P}$ by thermal
neutrons in the reaction $^{31}\text{P} (n, \gamma)^{32}\text{P}$ or $^{32}\text{S}$ by fast neutrons in the reaction $^{32}\text{S}(\text{n, p})^{32}\text{P}$ (Perkins, 1961b). The quantity produced by each reaction is dependent on the element's probability for neutron activation, the ratio of fast neutrons to thermal neutrons, and the concentration of parent element present. Perkins (1961b) calculated that in process water $^{32}\text{S}$ interactions account for 30 to 60 times more $^{32}\text{P}$ than do $^{31}\text{P}$ interactions. He found, however, that "the majority of $^{32}\text{P}$ is produced from parent materials which are continually absorbed on and eroded from the corrosion film of reactor process tubing and fuel element jackets" (Perkins, 1961b, p. 44). Measurements of $^{32}\text{S}$ and $^{31}\text{P}$ content in corrosion film indicate that about 85% of $^{32}\text{P}$ from this source is produced by $^{32}\text{S}$ activation.

Contribution of $^{32}\text{P}$ to the Columbia River by reactor effluent remains relatively constant. Therefore, the majority of variations in $^{32}\text{P}$ concentration are a result of physical and biological influences in the environment (Cushing and Watson, 1966). Once effluent mixes with river water, the $^{32}\text{P}$ concentration in the water continually declines through dilution, biological uptake, and physical decay. The yearly range of $^{32}\text{P}$ concentration in Columbia River water near Richland, Washington was 4.5 to 4200 picocuries per liter (pCi/l) in 1965 (Nelson, 1968). In the Columbia River estuary the $^{32}\text{P}$ levels for 1965 were less than 45 pCi/l (Toombs and Culter, 1968). Seasonal trends in $^{32}\text{P}$ concentration are similar to those of stable
phosphorus, with the lowest values occurring during spring freshet (Foster and McConnon, 1962). These levels pose no potential health hazard, but they provide a considerable opportunity for studying the phosphorus cycle in a natural environment by radiotracer techniques.

Healy (1947), as cited in Kornberg and Davis (1966), reported that in 1945, shortly after the Hanford reactors started operation, $^{32}\text{P}$ was found to be a major contributor to the total beta activity in Columbia River fish living near reactor coolant outfalls. Further studies have shown that in most aquatic organisms and waterfowl of the upper Columbia River, $^{32}\text{P}$ is the dominant and most highly concentrated radionuclide (Davis et al., 1958; Foster and McConnon, 1962). Because it is so greatly concentrated by organisms, $^{32}\text{P}$ is the nuclide that could most likely constitute a health hazard to man if the activity level in river water is not held to only a small percentage of that permissible for drinking water. Columbia River plankton concentrate $^{32}\text{P}$ to levels 5,000 to 118,000 times greater than that in the water (Cushing and Watson, 1966). Animals in higher trophic levels of the food chain take up $^{32}\text{P}$ by ingesting radioactive food and, therefore, are affected more by the concentration in their food than by the concentration in the river water (Davis and Foster, 1958).

A useful method of expressing $^{32}\text{P}$ levels is by its specific activity. This is the activity of isotope per unit mass of the total element ($\text{pCi}^{32}\text{P/gm total phosphorus}$). There are many advantages
of this measurement over those of activity per gram of organism or concentration factors. Specific activity will show whether the radioisotope is being distributed in proportion to the abundance of the stable element (Duke and Rice, 1966). It is often less variable than activity per unit weight (pCi/g tissue) or concentration of total element (mg P/g tissue) (Renfro, 1967). It eliminates the effects of biological dilution observed by Baptist and Price (1962). Measurements of $^{32}$P specific activity are essential for determining exchange rates and transfer routes in the phosphorus cycle. Foster (1959b) thoroughly discusses how specific activity measurements can be used to determine nutrient source, exchange rates, feeding rates, and food chains. Even though uptake and exchange are experimentally observed, it is impossible to calculate the actual quantities of element involved unless specific activities are measured (Rigler, 1956).

In previous studies, several different methods have been used to measure $^{32}$P in organisms. In many cases, the organisms were dissolved in nitric acid, either directly in a stainless steel counting planchet or in a beaker and then transferred to a planchet, within which they were evaporated to dryness (Ball and Hooper, 1963; Foster, 1959a). The major difficulty in this method is that any other beta emitting nuclide present in the sample will be counted in addition to $^{32}$P. Even under controlled experimental conditions where $^{32}$P is the only radionuclide added, there may be naturally occurring beta emitters
present. Studies have shown that natural $^{40}$K accounts for a substantial amount of beta activity in organisms (Hatfield, Skaven and Rankin, 1963). Perkins (1957) has used differential absorber techniques to eliminate some of the counting interferences due to other beta emitting nuclides; however, it is still difficult to eliminate the contribution from beta emitters which have an $E_{\text{max}}$ not greatly different from that of $^{32}$P. The best method of eliminating interferences from other radioisotopes is to chemically separate the phosphorus from other elements. This can be accomplished by several methods, such as solvent extraction, ion exchange, and precipitation (Mullins and Leddicotte, 1962). The common analytical method for quantitatively determining phosphorus content is by precipitation (Hillebrand et al., 1953; Lundell, Hoffman, Bright, 1931). Setser and Rozzell (1965) adapted this method to extract phosphorus from dissolved oyster soft parts.
COUNTING CONSIDERATIONS

Beta particles can be detected indirectly with a scintillation crystal by their bremsstrahlung radiations and directly with several instruments such as an end window G-M tube, a liquid scintillation detector, a proportional counter and a low background beta detector. Liquid scintillation and low background beta counting are the most widely used methods for accurate analysis.

Liquid scintillation is a popular method of beta detection because it provides a high counting efficiency and many liquid scintillation instruments are equipped to do spectrum scanning which aids in distinguishing between two beta emitting radionuclides in one sample. Limitations with this type counting are that the sample size must be small and a clear solution to avoid problems with quenching. A sample of dissolved fish would cause many problems in liquid scintillation counting because when concentrated it is seldom clear and when greatly diluted the activity in a small aliquot would be very low.

Low background beta counting generally has a lower counting efficiency than liquid scintillation counting and does not lend to spectrum scanning. Samples are plated on a counting planchet and counted in 2π geometry which increases the chance for self-absorption. Labbe, however, states that in many instances "... the difficulties encountered in uniform plating and in self-absorption are somewhat easier to
handle than the quenching problem in liquid scintillation counting" (Labbe, 1965, p. 16). He also believes that the loss in counting efficiency is more than compensated for by the much lower background available with low background beta counting. The minimum detectable level of activity is dependent on both counting efficiency and background count rate. The difference between a background of 1.6 counts per minute (cpm) for low background beta counting and 25 cpm for liquid scintillation is more significant than the difference in counting efficiencies for the two systems. Low background beta counting was, therefore, considered to be the better method of detection for this type work, despite the fact that samples must be free from all beta emitting radionuclides other than $^{32}$P.

The beta counting instrument used for this study was a Nuclear Chicago Model C115 low background counter in conjunction with a Model C11B time interval printer. The detector was a gas flow type having a thin 'Micromil' window with a density of less than 150 micrograms per square centimeter. A mixture of 99.05% helium and 0.95% isobutane at a pressure of five pounds per square inch (approximately 45 milliliters/minute) was the operating gas used. Low background is achieved by graded shielding and a halogen-quenched guard detector equipped with anticoincidence circuits to act as a cosmic ray shield. For short counting times the background was unstable, but for longer periods it became nearly constant at 1.5-1.6 cpm. For this reason,
most samples were counted with preset counts large enough to allow at least 100 minutes of counting.

Counting efficiency of the instrument for $^{32}$P was determined by plating a known amount of $^{32}$P activity on a stainless steel counting planchet and counting to obtain the detected activity. Duplicate samples at three concentrations were prepared by pipeting various volumes of $^{32}$P standard into planchets, diluting with water, adding one drop of surface tension reducer (Polysorbate 80), and evaporating to dryness under an infrared heat lamp. Samples were counted four times over a period of three and one-half months. The results are shown in Figure 1. A least squares analysis yields a slope of $0.220 \pm 0.001$ cpm/nanocurie (nCi) at the 95% confidence level (ASTM, Committee E-11). When the $^{32}$P activity (nCi) is expressed in disintegrations per minute (dpm), the slope equals the counting efficiency which was calculated to be $9.91 \pm 0.5\%$. The error term is two standard deviations and reflects pipetting and counting errors of the samples. The $^{32}$P standard was purchased from Cambridge Nuclear and had an overall accuracy of $\pm 5\%$.

Self-absorption was determined by comparing observed count rates for different amounts of precipitate which had equal specific activities. Self-absorption was found to be significant only in precipitate weights greater than 200 mg as shown in Figure 2. The correction curve supplies a factor to correct the observed count rate for self-absorption.
Figure 1. Counting efficiency of low background beta counter for $^{32}$P.

$CPM = 0.220 \pm 0.001 \text{ ACTIVITY ADDED}$

At 95% Confidence Level
Figure 2. Phosphorus-32 self-absorption curve.
Other factors which must be considered when using a low background beta counter are coincidence loss and backscatter. Coincidence loss can result when count rates are appreciably high in relation to the resolving times, thus resulting in no significant coincidence loss at counting rates less than several hundred thousand counts per minute (Wang and Willis, 1965). The straight line in Figure 1 confirms the belief that there was no significant coincidence loss in the range of activities used.

Backscatter is the radiation that is emitted in the direction of the backing material (sample holder) and subsequently scattered back towards the detector. The count rate increases in direct proportion to the thickness of the backing material up to a saturation value. It is, therefore, necessary to use the same type of backing for all samples in order to compare counting data, and the thickness must exceed that required for saturation. In practice, the saturation value occurs at two-tenths the beta particle range in the backing material (Wang and Willis, 1965). Stainless steel planchets used in this study had a thickness of 0.305 mm which exceeds the calculated thickness for $^{32}$P (0.142 mm in iron) by 0.163 mm. The straight line relationships in Figure 2 also indicates that there is no significant variation due to backscatter.
METHOD FOR DETERMINATION OF 32P SPECIFIC ACTIVITY

Dissolving Fish Sample

Fish samples were dissolved with concentrated nitric acid (25 ml) in 400 ml beakers by heating and occasional stirring. Once dissolved, the sample was evaporated to dryness and baked for 20 minutes. The charred residue was moistened with concentrated nitric acid (1 ml) and again baked to break down fats which otherwise remain as a film on the solution when cooled. If fats remained after multiple baking treatments, they were removed by vacuum filtering the cooled solution through cheese cloth. Concentrated nitric acid (10 ml) was again added and heated until the residue was in solution and black. After cooling, 30% hydrogen peroxide (2 ml) was added and allowed to stand 20 minutes until the black solution became a clear, pale yellow. This was then evaporated to about 5 ml and cooled for the precipitation process.

Phosphorus Precipitation Technique

Precipitation was the method used to remove phosphorus from the fish solution. This was found to be a more satisfactory method than solvent extraction. It was much easier to remove larger quantities of phosphorus by precipitation, and the precipitate presented
fewer problems when mounting on a planchet than did the extracted solvent.

The precipitation procedure used was that adapted by Setser and Rozzel (1965) to analyze $^{32}\text{P}$ in shellfish, but modified to more closely follow the common analytical method for phosphorus analysis (Hillebrand et al., 1953). A detailed outline of reagent preparation, extraction procedure and the reasons for each step are given in the Appendix, along with the chemical equations involved. The procedure involves three precipitations; the first being a preliminary separation, followed by two purification precipitations. The first precipitate was yellow ammonium phosphomolybdate precipitated from an acid solution. The other two precipitates were white magnesium ammonium phosphate. The first of these was precipitated from a cold alkaline solution. The second was modified to induce precipitation by slowly increasing the pH to 9 in an acid solution of the first precipitate (Hillebrand et al., 1953). Figure 3 schematically shows the steps involved in analyzing a sample.

**Precipitation Efficiency**

Efficiency and precision of the precipitation procedure were tested both radiochemically and gravimetrically. To do this, stock acid solutions containing potassium phosphate ($\text{KH}_2\text{PO}_4$) and a $^{32}\text{P}$ spike were aliquoted into five replicates, weighed and processed
Figure 3. Method for determination of $^{32}$P specific activity in fish.
through the precipitation procedure. Sample precipitates were dried, weighed, and counted for $^{32}$P. All counts were corrected for decay to a standard reference time and for self-absorption. The activity per gram of stock solution was determined by evaporating in duplicate a known weight of stock solution and counting for $^{32}$P. For these two samples it was necessary to subtract the $^{40}$K beta contribution before activity was decay corrected. A plot of cpm extracted vs cpm added (Figure 4) has a least squares slope of 0.791, indicating a radiochemical precipitation efficiency of 79.1%. Standard error of the estimate about the line is ±3.13 cpm which yields a range in precision of the samples from 1.4 to 7.8% at one standard deviation.

Total phosphorus in the precipitates was calculated on the basis of a precipitate phosphorus concentration equal to 21.37%. This value was determined experimentally by analyzing the precipitate for phosphorus content as will be discussed later. Total phosphorus per gram of stock solution was determined by the common molybdenum blue colorimetric method using a Beckman DU spectrophotometer Model 2400. A plot of phosphorus extracted vs phosphorus added (Figure 5) indicates a gravimetric extraction efficiency of 89.9%, as determined by the least squares slope. Standard error of the estimate about the line is ±0.45 mgs, which yields a range in precisions of the samples from 0.5 to 3.8% at one standard deviation. Theoretically, there should be no significant difference between the two extraction
Figure 4. Radiochemical precipitation efficiency.

CPM EXT. = 0.791 CPM ADDED

Standard Error of Estimate = ±3.13 CPM
Figure 5. Gravimetric precipitation efficiency.
efficiencies because the radioactive isotope of an element should react chemically the same as the stable isotope (Mullins and Leddicotte, 1962).

The radiochemical value of 79% recovery was reproduced in a similar experiment using an acid stock solution containing trisodium phosphate (Na$_3$PO$_4$·12H$_2$O), and a larger $^{32}$P spike. A check was made to determine if a geometry effect could be causing an added difference in counting between an evaporated sample and a precipitated sample. Duplicate weighed aliquots of the above stock solution were placed in plastic vials and radioanalyzed in a Packard TRI-CARB liquid scintillation counter by detecting Cerenkov radiation. Two other weighed samples of a similar size were processed through the extraction procedure with the final precipitate dissolved in 6N hydrochloric acid, placed in plastic vials, and counted for Cerenkov radiation. A radiochemical precipitation efficiency of 76% was calculated which correlates with the previous value and shows no significant geometry effect. It also indicates that the $^{40}$K correction used in the first experiment was valid. Precipitation efficiency determined gravimetrically was found to be the same as before by another experiment in which initial phosphorus was determined colorimetrically. A value of 90% was also obtained by a method for which initial phosphorus was calculated from the accurately weighed amount of potassium phosphate used for the sample. A definite difference between the
radiochemical and gravimetric extraction efficiencies was therefore believed to exist. One possible explanation is that some of the $^{32}$P (about 11%) in the spike was in a chemical form which was not converted to orthophosphate by the chemical treatment, and consequently did not precipitate as ammonium phosphomolybdate. This could be tested by evaporating the supernatant liquid from the first precipitation and counting for $^{32}$P.

To calculate the $^{32}$P specific activity of a sample directly from $^{32}$P activity and total phosphorus in the precipitate, it is not necessary to know the exact precipitation efficiency. However, both radiochemical and gravimetric extraction efficiencies must be the same. For a dissolved fish sample, these are considered to be the same. The acid and hydrogen peroxide treatment is believed to convert all phosphorus to the orthophosphate, which will precipitate. Specific activity of the precipitate is then assumed to be a valid measure of specific activity in the fish sample.

**Upper Limit of Constant Extraction**

Due to stoichiometry of the chemical reactions involved in the extraction procedure, the total amount of phosphorus which can be removed is dependent on the quantities of reagents used. By using constant volumes of reagents, it was possible to determine an upper limit of constant extraction. Stoichiometry calculations indicated
that 100 ml of ammonium molybdate reagent should precipitate 107.3 mg of phosphorus (Hillebrand et al., 1953). Experimental results, however, showed the actual upper limit for the precipitation procedure to be lower than that calculated for ammonium phosphomolybdate precipitation. This was determined by preparing a stock solution of dissolved starry flounder tissue at a concentration of 0.40 gm wet weight per ml solution. Aliquots were analyzed and the recovered weight of dried precipitate measured. As shown in Figure 6, the upper limit of constant extraction was taken as the point where precipitate yield ceases to have a linear relationship with the weight of fish analyzed. Samples that yield a precipitate weight greater than 425 mg are, therefore, considered not to have undergone a constant precipitation. The specific activity of such a sample would be a valid measurement, but this could not be used to determine the activity per gram dry weight or the percent concentration of the phosphorus in the sample. The actual amount of phosphorus in 425 mg of precipitate is equal to 90.8 mg based on a value of 21.37% phosphorus per gram precipitate.

Properties of Final Precipitate

The drying time required for a precipitate to reach a nearly constant weight was found to be 36 hours at 100°C. This was determined by drying a number of fresh precipitates with eventual dry
Figure 6. Upper limit of constant precipitation.
weights ranging from 58 mg to 399 mg for a total of 60 hours. At 12 hour intervals they were removed from the drying oven, cooled in a desiccator, weighed, and returned to the oven. The change in weight between 36 hours and 60 hours of drying was less than 1% of the weight at 60 hours drying. The precipitate was found to be extremely hygroscopic and in a few cases, absorbed enough moisture during cooling and weighing to yield a weight after 12 hours of drying slightly greater than at the previous weighing. The cooling time must, therefore, be standardized and weighing performed rapidly. Weight increase of a dry precipitate when exposed to air was quite rapid, as shown in Figure 7.

To calculate $^{32}$P specific activity of a sample, it is necessary to know the weight of total phosphorus present, as well as the activity of $^{32}$P. The percent phosphorus composition of the final precipitate was determined so that a measurement of the precipitate weight obtained could be converted directly to a measure of the total phosphorus present. Although this precipitate should be pure magnesium ammonium phosphate, a percent composition based on the chemical formula could not be used because it was not known exactly how many waters of hydration were lost during drying. The percent phosphorus composition of the precipitate was, therefore, determined experimentally by analyzing the precipitate for phosphorus content using the common molybdenum blue colorimetric method. Samples of 18 dried
Figure 7. Hydration of magnesium ammonium phosphate precipitate.
precipitates were dissolved, diluted $10^6$ times, and analyzed for total phosphorus. Average concentration of phosphorus in the precipitate was found to be $21.37 \pm 0.9\%$ at the 95% confidence level. Should this value be in error, it would most likely be slightly low, as a result of precipitate hydration during weighing. The value indicates that there is less than one water of hydration associated with the dried precipitate.
APPLICATION OF $^{32}$P SPECIFIC ACTIVITY

Measurements of $^{32}$P specific activities were made on fingerling salmon taken from the Columbia River during the winter and spring of 1969. From these values, the apparent time required for $^{32}$P specific activity of a fish to approach equilibrium was determined. This, in turn, made possible the calculation of a phosphorus turnover rate for young chinook salmon. It was also found that $^{32}$P specific activity in conjunction with the $^{65}$Zn activities can be used to study feeding and migratory behavior of fish. The area of study and sampling locations are as illustrated in Figure 8.

Preparation of Fish Samples

The majority of fish used in this study were less than 15 centimeters long and under 20 grams live weight. Two different methods of fish preparation were used depending on the information desired. If only the $^{32}$P present in the muscle and skeleton was of interest, a flesh and bone sample was used. For these samples, the head, fins, and entrails were removed and the fish skinned. It was found that fish preserved for a few days in 10% formalin became stiff and were much easier to skin than fresh or freeze preserved fish. A procedure which proved satisfactory for skinning fresh and freeze preserved fish was to enclose them individually in water-tight plastic
Figure 8. The Columbia River below Hanford showing dam locations and sampling sites.
bags and submerge in boiling water for three to five minutes. This cooking allowed the skin to be removed easily and also made it possible to separate the flesh from the bone if desired. Renfro (1969) has shown that both formalin and freeze preservation may affect the zinc elemental composition over long storage periods. In a short storage period, he detected no significant change; however, formalin preserved fish appeared to have less variation than freeze preserved fish. On this basis, formalin is believed to be the best method of sample preservation for fish which are to be analyzed for $^{32}$P. When concerned with differences in total $^{32}$P concentration among fish, the entire organisms were used as a sample with only the gut and intestinal contents removed. After the samples were prepared, they were hung from a drying rack and dried at 100°C for 24 hours. Suspending the fish in this manner eliminated the large tare weight of a container, prevented the sample from adhering to a drying surface, and allowed complete air circulation for faster drying. A dry weight was taken and the fish placed anterior end down in a 12 ml plastic counting tube. They were then analyzed for gamma emitting radio-nuclides by counting for 100 minutes in a 5" x 5" sodium iodide scintillation well detector coupled with a Nuclear Data 512 channel analyzer. After gamma ray analysis, samples were removed from the counting tubes and placed in 400 ml beakers. They were then dissolved, extracted, and counted for $^{32}$P as described previously.
An uptake study was conducted on marked young chinook salmon released from a hatchery at Ringold, Washington. The hatchery is located on a creek which is utilized as its source of water. Two hundred thousand young chinook salmon, cold branded with an upright T on their left anterior side, were released into the creek at 2100 hours on May 12, 1969. Approximately 300 yards downstream from the hatchery this creek empties into the Columbia River at which point the fish received their first exposure to the radionuclides produced at Hanford. Marked fish were collected at McNary Dam and The Dalles Dam by biologists of the Bureau of Commercial Fisheries as they dipped the gate wells. Five fish were taken on various days, placed in plastic bottles containing 10% formalin, and mailed to Corvallis, Oregon. For a background level, fish taken directly from the hatchery pond were also mailed to Corvallis. Whole fish with stomach and intestine contents removed were used as samples.

Twenty-eight chinook salmon, ranging in standard length from 6.5 to 10.3 cm, were analyzed for $^{32}$P specific activity: one group of three from the hatchery; two groups of five from McNary Dam; and three groups of five from The Dalles Dam. All fish taken at the dams were assumed to have entered the Columbia River at 2400 hours on May 12, and to have been removed at 1200 hours the date of
collection. Hatchery fish were found to contain little or no $^{32}$P. As shown in Figure 9, there is a rapid increase of $^{32}$P specific activity which tends to approach a constant value of 17 nanocuries per gram phosphorus (nCi/g P). Interpretation as to the degree of initial increase is dependent on whether McNary and The Dalles Dam samples are considered separately or together. The median value is used as an estimate of the central value for each sample group (Dean and Dixon, 1951). The error associated with each value is small in comparison to the range of values found within a sample group.

Studies by Olson (1952) have shown that consumption of radioactive food is the primary method by which $^{32}$P is accumulated in trout. Whittaker (unpublished data in Watson, 1957) found young salmon to assimilate approximately 70% of the $^{32}$P consumed. Because fish at the hatchery were not exposed to radioactive water or food, their $^{32}$P specific activity was essentially zero. However, once these young chinook salmon enter the Columbia River, their $^{32}$P specific activity increases as they eat food containing $^{32}$P. To measure this increase, only fish collected from the same location should be compared. In this way, they will all have traveled the same distance and can be assumed to have been exposed to similar concentrations of $^{32}$P. The second group of McNary Dam fish may have anomalously low $^{32}$P specific activities and will be discussed later. Therefore, only The Dalles Dam samples are used for comparison.
Figure 9. Increase in $^{32}$P specific activity of marked fingerling chinook salmon released into the Columbia River.
Increase in $^{32}\text{P}$ specific activity is approximated by a smooth curve which approaches a constant value of 17 nCi $^{32}\text{P}/g\text{ P}$ within three to four weeks. Watson et al. (1959) found that $^{32}\text{P}$ concentration in soft tissues of brook trout also reached a constant level in three to four weeks. Studies of $^{32}\text{P}$ uptake by juvenile shiners in the Columbia River near Hanford show a constant level of $^{32}\text{P}$ being reached in four to five weeks (Watson et al., 1969). The solid curve in Figure 9 reflects the integrated increase in $^{32}\text{P}$ specific activity between the time fish entered the Columbia River and the time they reached The Dalles Dam. If the fish accumulate $^{32}\text{P}$ at some regular rate, then the $^{32}\text{P}$ specific activity of fish at some point before The Dalles should be approximated by a value on the solid curve.

The first group of samples from both McNary and The Dalles were collected on the first day that a large number of marked fish appeared at the dams. It is probable then that the first marked fish collected at The Dalles must have passed through McNary Dam near the time that the first McNary samples were taken. Values of $^{32}\text{P}$ specific activity in the McNary samples are lower in relation to The Dalles samples than is predicted by the solid curve in Figure 9. The relationship between the two groups of samples is shown by a dashed curve. This curve indicates that the rate of $^{32}\text{P}$ accumulation does change as a fish travels down river. Because $^{32}\text{P}$ concentration in the water decreases with distance from Hanford, the most rapid
uptake of $^{32}\text{P}$ would be expected to occur when a fish first enters the river. The data, however, show there is a lag in time before $^{32}\text{P}$ specific activity in young chinook salmon increases greatly. This lag may reflect the time required for a hatchery fish to acclimatize to the Columbia River environment and start feeding. Efficiency of a hatchery fish in obtaining natural food would undoubtedly increase at first, as would the amount of food needed, due to added activity. The quantity and type of food available in different portions of the river could also affect the rate of $^{32}\text{P}$ uptake. The dashed curve in Figure 9 is, therefore, believed to more accurately show the actual change in $^{32}\text{P}$ specific activity as fish move downriver to The Dalles Dam.

When an organism maintains a constant $^{32}\text{P}$ specific activity, it is considered to be in equilibrium with the environment relative to phosphorus. The amount of $^{32}\text{P}$ lost through physical decay and biological turnover must be continually replaced by $^{32}\text{P}$ assimilated from ingested food. If the total amount of phosphorus (the phosphorus pool) in an organism remains nearly constant, then an amount of phosphorus equal to that assimilated from food must be eliminated.

The rate at which this continuous exchange occurs is called the turnover rate ($k$), and is defined as the portion of body burden turned over per unit time. Turnover rate is related to biological half-life ($T_{b/2}$) of an element by the equation $k = \frac{0.693}{T_{b/2}}$. Biological half-life is
defined as the time required for 50% of the body burden to be eliminated and can be determined experimentally. The $T_{b/2}$, however, is not a constant, even within one species of fish. It is a measure of the phosphorus exchange at a particular time and under the particular experimental conditions. Size, age, physiological condition and activity of a fish all affect the $T_{b/2}$ in addition to environmental factors such as temperature.

The classical method for determining $T_{b/2}$ is by a retention study. This involves monitoring the decline of radioactivity in organisms with time to establish the length of time required for the activity to be reduced by one-half. Another method which can be used to determine $T_{b/2}$ is an accumulation study which involves monitoring the increase of radioactivity in organisms with time. The rate of accumulation is expressed by the equation $Q_t / Q_e = 1 - e^{-\delta t}$, where $Q_t =$ activity present at some time $t$, $Q_e =$ activity present at equilibrium, and $\delta =$ turnover rate (Davis and Foster, 1958). Because these calculations are based on changing radionuclide concentrations, the turnover rate ($\delta$) also takes into account physical half-life ($T_{p/2}$) of the radioisotope by the equation $\delta = \frac{0.693}{T_{b/2}} + \frac{0.693}{T_{p/2}}$. For practical purposes, equilibrium can be considered to occur when $Q_t = 0.9Q_e$. Using this value in the accumulation equation and solving for the time required to reach nine-tenths of the equilibrium value ($t_{0.9}$), yields the expression $t_{0.9} = 2.303 \left( \frac{0.693}{T_{b/2}} + \frac{0.693}{T_{p/2}} \right)$. Rearranging
to solve for $T_{p/2}$ the expression becomes $T_{b/2} = (0.693)(T_{p/2})(t_{0.9})/\left(2.303 - (0.693)(t_{0.9})\right)$.

Assuming that fingerling chinook salmon reaching The Dalles Dam approach an equilibrium $^{32}\text{P}$ specific activity of 17 nCi $^{32}\text{P}$/g P, the time required to reach nine-tenths of this value is 16.5 days, as determined from Figure 9. Using this value, the calculated $T_{b/2}$ of phosphorus is found to be 7.6 days, which corresponds to a phosphorus turnover rate of 9.1% per day. The turnover of phosphorus in chinook salmon, as determined by these calculations, is much faster than that determined for the brook trout by Watson et al. (1959). By retention studies he found the shortest $T_{b/2}$ to be 20 days for liver with other internal organs having slightly higher values. The $T_{b/2}$ of muscles and bone was much longer resulting in a $T_{b/2}$ for the entire fish equal to 79 days.

There are several factors which could contribute to experimentally determined differences in the $T_{b/2}$ of phosphorus for brook trout and young chinook salmon. Chinook salmon analyzed were about one-fifth the size of brook trout used by Watson et al. (1959). Smaller organisms are often more active and have high metabolic rates which could result in a greater phosphorus turnover rate. Probably most important is that the chinook salmon were free in the environmental while the brook trout were maintained under controlled conditions in a pond. Reichle (1967), by measuring radiócesium turnover in terrestrial
isopods, found that energy requirements under natural environmental conditions were 24 to 31% greater than under laboratory conditions.

**Behavior Model**

To gain information on the feeding and migratory behavior of fish in the natural environment, a model has been developed which allows certain conclusions to be drawn based on the $^{32}$P specific activity and $^{65}$Zn concentration present in a fish. Basic assumptions for the model have been tested using levels of activity determined for chinook salmon in the uptake study. Meat and bone samples of 36 fingerling salmon collected at several locations in the Columbia River were also radioanalyzed, and the apparent behavior of these fish determined by using the model. Fish ranged in size from 8.3 to 13.4 cm (standard length) and were captured with a 50 foot hand seine. Six fish were taken near Portland, Oregon on February 27, 1969, and also near Warrenton, Oregon on April 19, 1969. The other 24 fish were taken near Boardman, Oregon on March 23, 1969.

The model, shown in Figure 10, is based on the assumption that a fish which remains in the Columbia River and actively feeds, accumulates $^{32}$P and $^{65}$Zn in a definite pattern. Should levels of activity found in a fish fall outside the limits of normal variability, then abnormal feeding or migration are assumed to be the cause.
Figure 10. A general model describing the behavior of a fish based on its $^{32}$P specific activity and $^{65}$Zn concentration.
Uptake of both $^{32}$P and $^{65}$Zn in fish is considered to be mainly through assimilation from food (Watson et al., 1959). Accumulation of these radionuclides in fish is, therefore, dependent on concentrations of activity in food, quantity of food consumed, assimilation efficiency, and element turnover rate in the fish. For fish of the same species and near uniform size, the assimilation efficiency and turnover rate for each element can be considered relatively constant under similar environmental conditions. Phosphorus turnover is assumed to be more rapid than zinc turnover. This assumption is supported by results of the chinook uptake study which showed $^{32}$P specific activity approaching equilibrium in three weeks, whereas, $^{65}$Zn concentration was still increasing steadily. Nakatani (1966) also found the loss of zinc in trout to be much slower than reported values for phosphorus loss. Fish of the same species living in an area of the Columbia River would all be exposed to the same food source and similar radionuclide concentrations. These fish would take up $^{32}$P and $^{65}$Zn in definite proportions and approach the respective equilibrium concentrations. Different rates of food consumption remain as the factor which should cause the major differences in radioactivity concentrations between individual fish. A certain amount of variability could be expected to occur normally, due to actual differences in assimilation efficiencies and turnover rates; however, a large variation must be attributed to differences in food consumption.
These differences could be due to either abnormal feeding behavior of a fish living in the area or insufficient time for a fish migrating into the area to reach equilibrium.

If a fish stopped feeding or was restricted in its food consumption, its $^{32}\text{P}$ specific activity would decrease much more rapidly than its $^{65}\text{Zn}$ concentration. The same effect would occur if a fish moved into a nonradioactive environment for a period of time and then returned to the river. A point on the model corresponding to concentrations of radioactivity resulting from such behavior would fall outside the limits of normal variability with a $^{32}\text{P}$ specific activity low in relation to the $^{65}\text{Zn}$ concentration. Uptake of radioactivity by a fish, previously containing no radioactivity and migrating into the Columbia River, would proceed as described by the model with $^{32}\text{P}$ equilibrium being reached first. The slope of this increase is a function of phosphorus and zinc turnover rates. A normal variability between individual fish can be expected to occur due to differences in assimilation efficiencies, biological turnover rates, and feeding rates.

Fish migrating down the Columbia River below Hanford will be exposed to food that continually decreases in radioactivity. The $^{32}\text{P}$ level in this food decreases much faster than the $^{65}\text{Zn}$ level because of physical decay. Actual levels of radioactivity which a fish could attain at equilibrium would also decrease correspondingly with
distance from Hanford. The $^{32}\text{P}$ specific activity of fish, which approach equilibrium before migrating downriver, would decrease rapidly during migration because of physical decay and fast biological turnover. In a short time, fish migrating downriver would have a $^{65}\text{Zn}$ concentration too large in relation to the $^{32}\text{P}$ specific activity and fall outside the limits of normal variability for the model. A logical decision as to whether values result from abnormal feeding or migration can usually be made by considering river conditions, season of the year, location of collection, and expected behavior of the fish in relation to where the point is located in the model.

In Figure 11, the model is tested by using values of $^{32}\text{P}$ specific activity and $^{65}\text{Zn}$ concentration determined for young chinook salmon in the uptake study. Stomach content indicated these fish were actively feeding; therefore, they are assumed to show normal behavior for fingerling salmon in the river. Increase in radioactivity follows the predicted relationship with the values for each set of samples also being grouped together and arranged chronologically. The second group of McNary samples, numbered one through five, are an exception to this trend. Values in fish numbers one through three fall within the limits of normal variability for the model, but their location in the model relative to the location of the first Dalles samples (designated by X) indicate that time spent in the river was only six to ten days. Values for fish number four and five suggest
Figure 11. Testing the model using values of radioactivity found in marked fingerling chinook salmon. A representative standard deviation is shown.
abnormal feeding behavior or return to the river from a nonradioactive environment. If these young chinook salmon had actually remained in the Columbia River above McNary Dam for 15.5 days, their concentrations of radioactivity should have been greater than that found in similar fish collected earlier at The Dalles Dam. It appears that fish numbers one through three either remained in the hatchery creek for several days before entering the Columbia River or entered another tributary and remained there several days before returning to the river. Fish number four and five also must have entered a tributary, but only after having spent some time feeding in the Columbia River. The largest tributary entering the Columbia above McNary Dam is the Snake River. The Snake River contributes about one-third as much water to the upper end of McNary reservoir as does the Columbia (U. S. Geological Survey, 1968). Furthermore, the Snake River plume, extending along the eastern bank of upper McNary reservoir, constitutes an environment in which fish would not be exposed to the higher levels of radionuclides present in Columbia River water.

Radioactivity levels in fingerling salmon collected near Boardman, Oregon are shown in Figure 12. Only ten samples fall within the limits of normal variability for the model, and these are all fish which must be relatively new to the Columbia River. Of the other 14 samples, only five have a $^{32}$P specific activity larger than 1.05 nCi
Figure 12. Use of model to determine behavior of fingerling salmon collected at Boardman, Oregon.
$^{32}$P/g P with the largest being 5.5 nCi $^{32}$P/g P. These fish were collected in March when the levels of radioactivity are normally low, due to less food consumption and less radioactivity in the food (Davis and Foster, 1958; Foster and McComion, 1962). The values are also representative of meat and bone and not the entire fish. Both factors could reduce the equilibrium value for $^{32}$P specific activity under these conditions. Samples number 5 and 17 indicate that some feeding must be taking place. Fish with a high $^{32}$P specific activity could possibly be at equilibrium for this time of year or have recently migrated from upriver. The remaining samples with a high $^{65}$Zn concentration and a low $^{32}$P specific activity are believed to be fish that had previously reached high equilibrium values in the Columbia River and were remaining there through the winter. This indicates that they must be at least second year fish having obtained most of their $^{65}$Zn the previous year.

Fingerling salmon collected near both Portland, Oregon and Warrenton, Oregon all contained low levels of radioactivity ($^{32}$P specific activity < 1.5 nCi $^{32}$P/g P and $^{65}$Zn concentration < 9 pCi $^{65}$Zn/gm dry wt.) indicating they were new to the Columbia River. The actual length of time these fish had spent in the river could vary greatly. During winter when there is limited food intake, accumulation of radioactivity would be much slower than observed for young chinook salmon in May. Fish collected at Portland are suspected to
have come out of the Willamette River, which enters the Columbia about one mile below the collection site.
SUMMARY

Phosphorus, a biologically important element, tends to circulate within the biosphere via a biogeochemical cycle called the phosphorus cycle. An easy and direct method of studying this movement is by the use of radiotracer techniques. Environmental studies usually provide the most useful information on the behavior of phosphorus in the biosphere. Phosphorus-32 is the only radioisotope of phosphorus with a sufficiently long half-life and strong enough beta emission to be useful in such tracer studies. An ideal opportunity for studying the phosphorus cycle in a natural environment using $^{32}$P as a tracer is present in the Columbia River. A level of $^{32}$P sufficient for tracer studies is introduced into the river by expelled coolant water from the Hanford reactors. The objectives of this study were to utilize the opportunity by (1) adapting and testing a technique which could be routinely used to measure $^{32}$P specific activity of Columbia River organisms, and (2) to utilize such measurements for studying phosphorus cycling in particular fish species and gaining information on their feeding and migration behavior.

A precipitation technique which involves three precipitations was used to chemically separate and concentrate phosphorus from Columbia River fish. The procedure was tested and further adapted to make possible the calculation of $^{32}$P specific activity based on
precipitation weight and cpm obtained. Precipitates were placed in 2.5 x 0.8 cm stainless steel counting planchets and counted in a low background beta counter with a counting efficiency of 9.91 ±0.5% at the 95% confidence level. No significant change in precipitate weight occurred after 36 hours of drying at 100°C; however, it was very hygroscopic when exposed to the air. Phosphorus content of the dried precipitate was determined to be 21.37 ± 0.9% at the 95% confidence level.

The rate of $^{32}$P accumulation by young chinook salmon was studied by measuring the change in $^{32}$P specific activity of marked fish collected from the Columbia River. Initial uptake indicated a lag period due to time required for fish to acclimatize to the river. Within three to four weeks, $^{32}$P specific activity appears to approach an equilibrium value of 17 nCi $^{32}$P/g P. Calculations based on the rate of $^{32}$P accumulation yield a biological half-life for phosphorus equal to 7.6 days, which corresponds to a phosphorus turnover rate of 9.1% per day. These values indicate a much greater phosphorus turnover in young chinook salmon than has been reported for brook trout, but this is believed to be partially the result of differences between an environmental study and a controlled laboratory study. A model has also been developed which allows certain conclusions to be made about the behavior of a fish based on its $^{32}$P specific activity and $^{65}$Zn concentration. Validity of the model was
confirmed by the concentrations of radioactivity found in young chinook salmon of the uptake study. Levels of radioactivity found in unmarked fish collected from several locations in the Columbia River indicate that many were new to the river while others had probably remained there since the previous summer.

It is concluded that $^{32}$P specific activity measurements can be made routinely and that such values will contribute greatly to a better understanding of the phosphorus cycle, as well as behavioral patterns of organisms in the environment.
BIBLIOGRAPHY

American Society for testing and materials. Committee E-11 on quality control of materials. 1962. ASTM manual on fitting straight lines. Philadelphia. 28 p. (Special Technical Publication no. 313)


Davis, J. J. et al. 1958. Radioactive materials in aquatic and terrestrial organisms exposed to reactor effluent water. In:


APPENDIX
REAGENTS

1. Deionized distilled water.
2. Concentrated nitric acid (70 to 71% HNO₃).
3. 6N nitric acid solution. Dilute 400 ml of concentrated nitric acid to one liter in a volumetric flask.
4. Hydrogen peroxide (30% H₂O₂).
5. Citric acid solution. Dissolve 12.5 grams of citric acid in distilled water and dilute to 250 ml in a volumetric flask.
6. Ammonium molybdate solution. Dissolve 100 grams of ammonium molybdate in distilled water and dilute to one liter in a volumetric flask (10% w/v).
7. Magnesia mix. Dissolve 50 grams of magnesium chloride and 100 grams of ammonium chloride in water and dilute to one liter in a volumetric flask.
8. Concentrated ammonium hydroxide (28% NH₄OH).
9. One to one ammonium hydroxide solution. Dilute 100 ml of concentrated ammonium hydroxide with 100 ml of distilled water.
10. Ammonium nitrate solution. Dissolve 50 grams of ammonium nitrate in water and dilute to one liter in a volumetric flask (5% w/v).
EXTRACTION PROCEDURE

1. To a cooled sample approximately 5 ml in volume, add 50 ml 6N nitric acid solution with stirring followed by 10 ml citric acid solution.

2. Heat solution to 45°C on hotplate.

3. Add 100 ml ammonium molybdate solution with stirring to precipitate the phosphorus as ammonium phosphomolybdate. Allow the precipitated solution to stand at 45°C for 30 minutes.

4. Transfer precipitate and solution to a 250 ml centrifuge cone and centrifuge at about 1500 rpm for ten minutes. Decant the supernatant liquid.

5. Wash the precipitate with 50 ml of 5% ammonium nitrate solution. Centrifuge and decant the liquid.

6. Dissolve precipitate in 30 ml of 1:1 ammonium hydroxide solution. Cool in an ice bath for 20 minutes or until a fine crystalline precipitate starts to form. Precipitate the phosphorus as magnesium ammonium phosphate by adding 50 ml magnesia mix with vigorous stirring. Centrifuge and decant.

7. Redissolve the precipitate in 25 ml 6N nitric acid solution. Add six to ten milliliters of 50 ml magnesia mix. Reprecipitate the phosphorus as magnesium ammonium phosphate by adjusting the pH to 9 with the slow addition of 20 ml concentrated ammonium
hydroxide while stirring. Add remaining 40 to 44 ml of mag-
nesia mix.

8. Filter precipitate using a preweighed 2.5 cm diameter What-
man #42 filter paper in a filter chimney apparatus.

9. Place paper and precipitate in a preweighed 2.5 x 0.8 cm stain-
less steel counting planchet. Add a few drops of distilled water
and spread precipitate to give a uniform thickness.

10. Dry in an oven at 100°C for 36 hours. Remove and weigh on
an analytical balance. The sample is now ready for counting.

EQUATIONS FOR CHEMICAL REACTIONS INVOLVED

Ammonium Phosphomolybdate Precipitation

\[ \text{H}_3\text{PO}_4 + 12\text{H}_2\text{MoO}_4 + 3\text{NH}_4\text{NO}_3 \Leftrightarrow (\text{NH}_4)_3\text{PO}_4 \cdot 12\text{MoO}_3 \cdot 2\text{HNO}_3 \cdot \text{H}_2\text{O} + \text{HNO}_3 + 11\text{H}_2\text{O} \]

Dissolving Ammonium Phosphomolybdate

\[ (\text{NH}_4)_3\text{PO}_4 \cdot 12\text{MoO}_3 + 24\text{NH}_4\text{OH} \Leftrightarrow (\text{NH}_4)_3\text{PO}_4 + 12(\text{NH}_4)_2\text{MoO}_4 \]

\[ + 12\text{H}_2\text{O} \]

Magnesium Ammonium Phosphate Precipitation

\[ (\text{NH}_4)_3\text{PO}_4 + \text{MgCl}_2 + 6\text{H}_2\text{O} \Leftrightarrow \text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O} + 2\text{NH}_4\text{Cl} \]
EXPLANATION OF EXTRACTION PROCEDURE

Step 1: Acid converts most phosphorus to orthophosphate which will precipitate. Nitric acid provides the acid solution necessary and will not interfere with precipitation as do most other acids. Citric acid adds citrate which speeds the precipitation and helps to prevent contaminants from precipitating.

Step 2-3: Precipitation is faster at higher temperature. However, precipitates formed at temperatures over 50°C contain excess amounts of MoO₃ and are more apt to contain other contaminants. For routine analysis, 30 minutes is considered sufficient for precipitation. Care must be taken not to scratch the sides or bottom of the beaker while stirring because this creates a site for precipitation, thus leaving streaks of precipitate firmly adhering to the glass.

Step 4: A rubber policeman can be used to loosen the precipitate adhering to the beaker walls. A 5 ml volumetric pipet connected by vacuum provides an easy and quite controllable method of decanting.

Step 5: The 5% NH₄NO₃ solution will not dissolve the precipitate and is recommended as the best wash when there is to be further precipitation as magnesium ammonium phosphate.

Step 6: Rotating the centrifuge cone as ammonium hydroxide is slowly
poured down the side will remove precipitate adhering to the upper portion of the cone. In solutions containing large amounts of contaminants (resulting in a deep yellow color) a fine crystalline precipitate will often begin to form before 20 minutes of cooling. A cold alkaline solution will prevent some of the contaminants from being precipitated.

Step 7: Precipitation by slowly adjusting the pH also prevents certain contaminants from precipitating. A volume of 20 ml was experimentally found to be the amount of ammonium hydroxide necessary to yield a final pH of 9 after all the magnesia mix was added.