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인의 우주기원 방사능 동위원소를 이용한 동물플랑크톤 군집의 대사율 측정기법 개발

Development of Technique for the Measurement of Zooplankton Community Metabolic Rate Using Cosmogenic Phosphorus Radionuclides

1995. 7

한국해양연구소

제 출 문

한국해양연구소장 귀하:

본 보고서를 "인의 우주기원 방사성 동위원소를 이용한 동물플랑크톤 군집의 대사율 측정기법 개발" 연구사업의 최종 보고서로 제출합니다.

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한국해양연구소
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요 약 문

I. 제목

인의 우주기원 방사성 동위원소를 이용한 동물플랑크톤 군집의 대사율 측정기법 개발

II. 연구개발의 목적 및 중요성

해양의 동물플랑크톤 체내에 우주기원의 인 (phosphorus)의 방사능 동위원소가 측정가능한 정도로 함유되어 있을 것으로 예상되는 바, 이 방사능 원소를 예전과는 달리 최근에 개발된 저준위 scintillation 측정 기법을 적용하여 측정이 가능한지 여부를 판정하고 가능성이 확인된 경우 이들을 추적자로 이용하여 동물플랑크톤 군집의 대사 속도를 측정하는 새로운 연구기법을 개발함.

III. 연구개발의 내용 및 범위

1. 새로운 측정기기를 이용한 인의 방사성 동위원소 측정을 위하여 종래의 방사능 화학 분석법을 일부 개량하여 확립함
2. 저준위 방사능 측정용 scintillation 측정기기를 이용한 인의 쌍동이 동위원소의 동시 측정 가능성을 타진함
3. 인의 쌍동이 동위원소의 활동도 비를 이용하여 동물플랑크톤 군집의 인의 대사율을 결정하는 모델을 개발함

IV. 연구개발결과의 내용 및 활용에 관한 건의

1. 대기중에서 생성된 인의 쌍둥이 동위원소 ^{32}P (반감기 14.3 일)와 ^{33}P (반감기 25.3 일)가 해수로 공급이 원활하게 일어나는지를 검증하기 위하여 빗물을 채집하여 기초과학지원연구소가 보유하고 있는 Quantalus 1220 Scintillation counter로 측정해 본 결과 ^{32}P와 ^{33}P의 확연한 2개의 스펙트럼을 확인하였다. 이는 해양시료로부터 ^{32}P와 ^{33}P가 측정가능함을 시사하였다.

2. Lee (1991)의 인의 방사화학적 순수분리 방법으로 처리하여 Scintillation counter로 빗물과 동물플랑크톤 시료를 계수하여 본 바 동물플랑크톤에서 약간의 잡신호가 나타났다. 새로운 측정 방법이 발생되는 빛의 양을 재는 만큼 매우 투명한 시료가 요구되므로 화학처리 과정에서 1차적으로 침전시킨 ammonium phosphomolybdate 결정을 암모니아로 다시 용해시켜 2차 침전을 만들어 불순물과 나머지 간섭을 일으키는 방사능 물질을 제거하는 것이 권장된다.

3. $^{33}P/^{32}P$의 활동도 비를 이용하여 동물플랑크톤의 인의 대사 속도를 측정하는 매우 간단한 모델이 개발되었다. 모델은 해수로의 countinuous injection을 가정하여 개발하였다. 모델에 의하면 동물플랑크톤의 활동도 비는 식물플랑크톤에 비해 1.0에서 1.5배 범위의 값을 가지게되며 이에 해당하는 인의 순환 시간은 최대 70일 규모로 현장 적용이 가능한 것으로 판단된다.

4. 금년 4월 동해에서 채집한 동물 플랑크톤에 함유된 ^{32}P와 ^{33}P를 측정해본 결과 반감기가 상대적으로 긴 ^{33}P만 존재하였다. 이는 지난해 가을부터 봄철까지 이상 가뭄에 의해 방사성 인이 원활하게 공급되지 못한 것에 기인한 것으로 예상된다. 이를 해석하기 위해 pulse input model을 개발하여 해석해 본 결과 봄철에 동해의 동물플랑크톤은 3-4주에 한번씩 인의 순환이 일어나고 있는 것으로 판명되었다.

5. 이 연구는 새로운 연구기법의 적용 가능성을 타진하는데 목적을 둔 것으로 개발된 continuous injection model이 약 2달 이상 가뭄이 지속되는 경우 적용이 곤란한 것으로 드러났다. 그러나 비가 잦은 봄부터 늦가을 까지는 적용이 가능할 것으로 확신하며 바다는 물론 호소나 강에로 적용이 가능할 것으로 판단된다.

6. 우주기원 방사성 원소는 다른 분야에서도 활용이 가능할 것으로 예상된다. 한가지는 모델에 의하면 인의 쌍동이 동위원소를 이용하는 방법은 해수로부터 어류에 이르는 거의 전단계 먹이사슬에 대해 적용이 가능할 것으로 예견된다는 점이며, 또 다른 가능성은 빗물에 포함된 ^{7}Be, ^{32}P와 ^{33}P를 동시에 측정하여 대기권의 물질이 얼마나 빠른 시간 규모로 씻겨 나가는지 (tropospheric washout time)를 추적하는 것이다.

Summary

I. Title

Development of Technique for the Measurement of Zooplankton Community Metabolic Rate Using Cosmogenic Phosphorus Radionuclides

II. Research Objectives

Since cosmogenic phosphorus twin radionuclides are expected to be contained in zooplankton by a trophic transfer, their existence is probed using low-level scintillation counting technique and upon confirmation models are constructed for the calculation of phosphorus turnover rate of zooplankton community using these radionuclides as tracers.

III. Research Activities

1. Modify the conventional radiochemical purification procedure of phosphorus for the scintillation counting of beta particles.
2. Test the possibility of direct measurement of twin cosmogenic phosphorus using low-level scintillation counting technique.
3. Construct a model for phosphorus turnover of zooplankton community utilizing the activity ratio of $^{33}P/^{32}P$.

IV. Results and Discussions

1. Rain water was counted for ^{32}P (half-life, 14.3 d) and ^{33}P (half-life, 25.3 d) using Quantalus 1220 Scintillation counter in Korea Basic Science Institute to investigate the injection of these radionuclides into surface ocean. Two peaks of ^{32}P and ^{33}P spectra are clearly seen and this explicitly confirms that existence of these radionuclides in marine samples.

2. Samples treated with radiochemical procedure of Lee (1991) yields few noises in scintillation spectra. It is recommended to prepare cleaner samples adding one more purification step: reprecipitation of primary precipitation of ammonium phosphomolybdate by dissolving with ammonia solution. Added step will eliminate the remaining interfering metals and beta emitters.

3. Simple model for phosphorus turnover utilizing the activity ratio of $^{33}P/^{32}P$ is constructed. Model assumes the continuous injection of the activity ratio of ^{33}P and ^{32}P from atmosphere. Model predicts that zooplankton can have up to 1.5 times the activity ratio of $^{33}P/^{32}P$ over phytoplankton. The ratio is translated to turnover time of up to 70 days which seems to be a satisfactory time range for field application.

4. Contrary to expectation zooplankton taken in the East Sea in April '95 contained only long-lived ^{33}P. The only possible explanation for this is decay out of short-live ^{32}P during unusual prolonged drought in previous season resulted in abnormal source input . Pulse input model is used for interpretation data. Model yields best likely turnover time range of zooplankton is 3 to 4 weeks.

5. The feasibility study points out that continuous input model can only be applicable when wet precipitation occurs at least bimonthly. New

model seems applicable in wet season. Model is also expected to be applicable to other aquatic ecosystem such as lacustrine and river environment.

6. Cosmogenic phosphorus radionuclides have potentials as tracers in other fields. Firstly, model predicts measurable activities in planktivorous fishes, which implied ^{32}P and ^{33}P method can deal almost entire food chain. Secondly, simultaneous measurement of ^{32}P and ^{33}P with ^{7}Be (half-life, 53.3 d) has strong potential for the estimation of tropospheric washout time.

CONTENTS

요 약 문 iii

SUMMARY vi

CONTENTS ix

LIST OF FIGURES x

LIST OF TABLES x

CHAPTER 1. INTRODUCTION 1

CHAPTER 2. MATERIALS AND METHODS FOR THE MEASUREMENT OF COSMOGENIC ^{32}P AND ^{33}P 7

2.1 COLLECTION AND EXTRACTION OF COSMOGENIC PHOSPHORUS 8

2.1.1 Zooplankton sampling and pretreatment *8*

2.1.2 Rain water sampling *8*

2.2 RADIOCHEMICAL PURIFICATION OF PHOSPHORUS 10

2.3 LOW LEVEL SCINTILLATION COUNTING OF ^{32}P AND ^{33}P 11

CHAPTER 3. MODEL CONSTRUCTION 13

3.1 SOURCE FUNCTIONS OF COSMOGENIC PHOSPHORUS 13

3.2 ZOOPLANKTON P TURNOVER MODEL WITH ^{32}P AND ^{33}P 15

CHAPTER 4. RESULTS AND DISCUSSIONS 21

4.1 TROPOSPHERIC FALLOUT RATIO OF $^{33}P/^{32}P$ 21

4.2 $^{33}P/^{32}P$ RATIO IN ZOOPLANKTON SAMPLES 24

4.3 DISCUSSIONS 32

REFERENCES 36

List of Figures

Figure 1. Turnover time (days) of phosphorus in a target pool calculated from continuous input model utilizing activity ratio of $^{33}P/^{32}P$ between source and target pools. ____ 19

Figure 2. Scintillation beta counting spectra of rain water collected in May 15, 1995. a) raw spectrum counted for 10 hours and b) background corrected and deconvoluted spectrum of ^{32}P and ^{33}P using exponential fit to raw spectrum. ________ 23

Figure 3. Scintillation beta counting spectrum of ^{32}P and ^{33}P in zooplankton collected on April 18, 1995 in the East Sea. Spectrum of zooplankton caught by 333 µm mesh net (a) and 555 µm (b). ________ 26

Figure 4. Change of relative specific activities of ^{32}P and ^{33}P with time after a pulse input to seawater. Shown curves are calculated for 50 days of seawater P turnover time, 5 and 21 days of turnover times for phytoplankton and zooplankton, respectively. ___ 31

List of Tables

Table 1. Oceanic source functions of cosmogenic ^{32}P and ^{33}P (Lal and Lee, 1988). ______ 15

Table 2. Possible time range of phosphorus turnover time of zooplankton samples taken in the East Sea in April 18, 1995, with the best estimates on seawater turnover time ranged from 1 to 2 months, and fixed turnover time of phosphorus in phytoplankton community as 5 days. ________ 32

Chapter 1. Introduction

In the ocean, microscopic algae convert the solar energy into the chemical energy that fuels the entire ecosystem. The process called photosynthesys is possible in principle only at sunlit area where enough solar energy penetrates. Regardless of such limitation oceans teem with organisms in the deep as well as in the surface indicating fluent energy flow from surface to the deep.

The processes governing energy flow through the entire ecosystem have been central research theme from the beginning of the modern oceanography traced back to the H. M. S. Challenger Expedition of one century ago. The fundamental question of how much fish can we catch without reducing fish stock still need to work out. Marine food web interactions are so complex there exists no simple way of linking primary production to fish production.

Various approaches have been conducted for above question and in the mean time numerous techniques have been developed as a consequence or in some special cases targeted as itself. The highlight of developed techniques is incubation of photosysnthetic algae using radiocarbon tracer.

Major efforts were diverted to the fundamentals - various aspects of photosysnthetic algae. Others were on fishes and the minimal efforts were on zooplankton. Zooplankton is a collective term describing animals in the water column with limited swimming capability against water movement such as current and wave. One of major functions of zooplankton in the ecosystem is to pack small food particles large enough for fish. Studies on how efficiently they utilized their food, microscopic algae, require huge efforts as long as current approaches based on population interactions are adopted because of the heterogeneous nature of zooplankton community.

The role of zooplankton in trophic relationship was solely a matters of concern to the biological oceanographers until the global change emerged as a central issue to the public recently. Now their role in the marine food web is regarded as important not only because they control the phytoplankton community that is primary responsible for carbon fixation, but also they mediate deep burial of surface carbon via their fast sinking fecal pellet production. The latter can not be resolved easily by conventional method. With the advent of sediment trap specifically designed for collection of sinking particles, it is now possible to grasp the better pictures on the material cycling, which is currently a main theme of a burgeoning discipline, biogeochemistry.

The information from sediment trap studies stimulates wide field of research activities regarding the material flux to the deep. Although invaluable knowledge is augmented by deploying sediment traps, this costs a lot and consequently data are limited to critical area of concern such as Antarctic, Black Sea, North Pacific and North Atlantic, that is, area of concern for developed nations.

The purpose of this study is to develop an algorithm for new biogeochemical tracer technique utilizing naturally occurring twin radionuclides of phosphorus. The twin radionuclides of phosphorus, ^{32}P (half-life, 14.3 days) and ^{33}P (half-life, 25.3 days) are produced continuously by interactions between high energy cosmic rays and earth's materials. Since phosphorus is a limiting nutrient to photosysnthetic algae cosmogenic P nuclides once taken up by algae are supposed to follow the trophic transfer until they decay out by their relatively short half-lives.

Material cycling in the ocean can be viewed as exchanges among pools of distinct chemical characteristics. In view of biogeochemistry, phosphorus pools can be classified largely as dissolved inorganic phosphorus, dissolved organic phosphorus, and particulate organic phosphorus. The last pool can further be divided into phytoplankton, zooplankton and fishes, *etc*.. Each pool is characterized by its own size (total number of P atoms) and exchange rates (number of P atoms/unit time) with other pools.

Assuming stead-state, that is, pool size does not vary with time of concern, pool size divided by incoming flux has a dimension of time and defined as residence time of a given chemical. In such case amount of chemical goes out from the pool should be equal to amount comming in by definition of steady state. When single radiotracer of relatively short half-life is involved in exchange between pools, its activity decreased as time. This characteristic can be exploited as a time keeper for trophic interactions.

The art of using twin radionuclides of phosphorus lies in that what concerns to scientist is not absolute activities of radionuclides but the changes of activity ratio of two radionuclides because two radionuclides of different half-life follow exactly same pathways. As long as each pool has residence time of less than 3 months (= 8 times the half-life of short lived ^{32}P), the ratio of two P radionuclides can be used for estimation of residence time of target pools.

The rationale for this twin tracer method is straightforward. Whenever twin P radionuclides enter a pool and stay for a while, their activity ratio will be changed according to the length of stay in that pool. This is the underlying concept for developing P residence time model of zooplankton community in a natural setting.

There are well defined tracer techniques for phytoplankton productivity such as ^{14}C or ^{15}N labeling methods. These are widely accepted oceanographic research tools. However, there are no such method available for zooplankton because they are heterotrphic that do not uptake any labeled inorganic molecules at all. Also spiking of artificial tracer can only be applied for short incubation time in artificial environment such as in a bottle, zooplankton are not suitable for such lebeling.

The existence of cosmogenic radio-phosphorus was known in 50s (Marquez and Costa, 1955 cited in Lee, 1991). Then analytical capability put away from application of these radionuclides in the field of oceanography. Reevaluation of feasibility of cosmogenic phosphorus in oceanography was performed only in the late 80s in two major American oceanographic institutions; Lal's group in Scripps Institution of Oceanography, Bacon's group in Woods Hole Oceanographic Institution.

Earlier works by Lal 's group in the Southern California Bight suffered from the scarcity of target nuclides due to little rain fall, which is the main route of input to seawater. Bacon's group conducted similar experiments of Lal in the Bermuda where wet precipitation is enough. Results from feasibility studies are quite promising except for counting techniques. Both laboratories used low-level gas-flow beta counters that require multi-step radiochemical purification followed by long term (> 1 month) beta counting which certainly sets a limitation on a number of samples per measurement.

Though expensive, low-level scintillation counter specifically developed for natural tritium and radiocarbon (^{14}C) measurement has a potential for ^{32}P and ^{33}P measurement. Since the energy of ^{33}P is relatively week, E_{max} = 0.249 MeV compared to E_{max} of ^{32}P, 1.71 MeV, direct measurement of ^{33}P by previous gas-flow counting was not possible due to absorption of weak betas. Therefore activity of ^{33}P was inferred by place an absorber in front of sample that cuts off weak betas of ^{33}P to enter the detector.

In case of scintillation counting, beta particles of low energy are directly measurable with same efficiency of high energy betas with energy resolution.

In addition to this feature, large number of samples can be counted due to high counting efficiency. Details will be mentioned in the following chapters.

Subsequent chapters describe radiochemistry and beta counting technique of ^{32}P and ^{33}P using a low-level scintillation counter (chapter 2), simplified phosphorus flow model through food chain utilizing $^{33}P/^{32}P$ activity ratio (chapter 3). Final chapter 4 deals with the results obtain from rain water and zooplankton samples taken in the East Sea in April 1995 and discussions.

Chapter 2. Materials and Methods for the Measurement of Cosmogenic ^{32}P and ^{33}P

Existence of cosmogenic ^{32}P (half-life, 14.3 d) and ^{33}P (half-life, 25.3 d) in marine environment is confirmed only recently (Lal and Lee, 1988; Lal et al., 1988; Lee et al.; 1991; Lee et al., 1992). Despite the recognized application potentials of these phosphorus radionuclides in the biogeochemical cycling, it took 3 decades to find their own applications. Major impediment for using cosmogenic radionuclides in oceanography is solely due to their relative low activities in seawater.

Prior to this study, cosmogenic P radionuclides can only be measured by low-level beta counters consisting of detector and anticoincidence unit to lower the background. This kind of system requires preconcentration of target nuclides or filtration of particles from a large volume of seawater. Since Geiger-Müller type counters do not have ability to discriminate the betas of different energy radiochemical purification steps are indispensable.

In case of cosmogenic P, existence of two sister nuclides having quite different maximum beta energy makes direct measurement simultaneously with new radiation measurement technology. E_{max} of ^{32}P is 1.71 MeV, whereas that of ^{33}P is 0.249 MeV. Due to very low energy, ^{33}P is absorbed very easily by thin materials. Conventional way of measuring ^{33}P is using absorber of known composition and density. By comparing the data with and without absorber activities of ^{32}P and ^{33}P are deconvoluted. Another drawback of Geiger-Müller type counter is that it requires long measurement time typically a month since this needs initial counting, decay curves, and background for good statistics. Accordingly number of samples are limited per each counters.

New way of measuring cosmogenic P radionuclides is now available with advanced low-level scintillation counters in recent years. Several versions are available in the market. Most appropriate one is supplied from Quantas, Australia, specifically designed for low level natural radiocarbon (^{14}C) and tritium in natural samples.

Strongest feature of scintillation counting technique is that data are acquired using a MCA (multi-channel analyzer), that is, energy spectrum makes direct measurement of ^{32}P and ^{33}P possible. Since scintillation counter gives peak spectra of each nuclide using anticoincidence technique, total counting time does not exceed one day foe several samples and fool proof from contamination. Besides counting efficiency is as high as near 100% comparing to about 30% of low-level gas-flow counters. This implies scintillation technique requires much less amount of sample or reduced counting time over gas-flow counting.

Subsequent sections deal sample collection, radiochemical purification procedures, and finally scintillation beta counting of ^{32}P and ^{33}P.

2.1 Collection and extraction of cosmogenic phosphorus

For the study of phosphorus turnover rate of bulk zooplankton using cosmogenic P radionuclides, zooplankton and rain water samples were analyzed. The activities cosmogenic P in rain water was measured to inspect source input to surface ocean and to obtain an initial injection ratio of $^{33}P/^{32}P$.

2.1.1 Zooplankton sampling and pretreatment

Zooplankton was sampled in the East Sea during the East Sea Eddy Cruise of April 1995. Samples were taken from the surface mixed layer using Bongo net equipped with 333 and 505 μm mesh size nets before dawn of 18th April at the south western station of the East Sea. Net was towed obliquely for

20 minutes to depths of 50 m. Collected samples was quickly frozen on board ship and transferred to the laboratory for further analysis.

Thawed zooplankton samples in laboratory were dried in oven at 60°C then combusted in muffle furnace at 350°C. Ashes along with salts were grounded in a mortar and digested with concentrated nitric acid with heating. At near dryness solution was fumed with concentrated nitric acid to expel excess chloride ions. Dried precipitates were washed with concentrated nitric acid and remaining salts were removed by centrifugation. Prepared suppurate are ready for further purification as described in section 2.2. Since zooplankton has plenty amount of phosphorus in their tissue it is not necessary to add phosphorus carrier.

2.1.2 Rain water sampling

Rain water was collected in the backyard of Korea Ocean Research and Development on 9th May 1995. A volume of about 20 liters rain water was acidified with hydrochloric acid to pH around 1. About 10 mg of P (as KH_2PO_4) is added to solution then 5 mg ferric ion (as $FeCl_3 \cdot 6H_2O$) per liter samples was added. After a vigorous stirring, NH_4OH is added to sample solution with mixing until ferric hydroxide precipitates appeared. pH of the sample solution was adjusted to 9 by slowly adding NH_4OH with mixing.

Phosphorus coprecipitated with ferric hydroxide was filtered on fast filtering Whatman filter paper No 541. Filtered ppt were rinsed with deionized water then dissolved with hydrochloric acid. Solution was dried completely by gentle heating and cooled to a room temperature. Precipitates were dissolved with 9 N HCl and ferric ions were remove by repeated solvent extraction with isopropyl ether.

Water portion containing P was dried completely and dissolved with 6N HNO_3. Prepared solution is ready for the radiochemical purification steps describe in the next section.

2.2 *Radiochemical purification of phosphorus*

Chemical purification of P is a series of specific P compound precipitates. The method is a well established procedure for a gravimetric analysis of P (Whaley and Ferrara, 1973). Phosphorus in prepared sample solution were converted to phosphate ions by nitric acid oxidation during previous preparation.

When phosphate ions in solution with excessive nitrate ion is added ammonium molybdate and heated bright yellow precipitates of ammonium phosphomolybdate ($(NH_4)_3PO_4(MoO_3)_{12}$) crystals are formed immediately and settle rapidly. This step is an effective preliminary separation of heavy metals such as Fe, Co, Ni, Cr, Ti and Zr (Mullins and Leddicote, 1962 cited in Waser, 1993). Prolonged heating results in molybdic acid precipitation which is subject to contamination of Si, As, and V (Hillebrand et al., cited in Waser, 1993) so overheating should be avoided. Biological samples can be purified further by repeated precipitation of phosphorus as ammonium phosphomolybdate. If color of ppts is not bright yellow or suspicious of contamination repeat the step by dissolving ppt with NH_4OH followed by HNO_3 addition.

Ammonium phosphomolybdate ppts are rinsed with 0.3 N HNO_3 then dissolved with NH_4OH. With excess of ammonia in solution magnesia reagent is added to precipitate phosphate as white magnesium ammonium ortho-phosphate ($NH_4MgPO_4 . 6H_2O$) ppts. Reaction is facilitated if the solution is kept cool. This step is not for removal of interfering ions but simply changing a chemical form of phosphorus ppt to facilitates the next ion exchange purification. Since $NH_4MgPO_4 . 6H_2O$ ppts is dissolved extremely well in HCl contact with HCl fume should be avoided.

$NH_4MgPO_4 . 6H_2O$ ppts are filtered or centrifuged then redissolved in 0.3 N HCl. A cation exchange column is prepared with washed AG-50W-X8, Cl-form 100-200 mesh resins. Volume of resin is prepared about 1/3 volume of solution as a rule of thumb. Column was preconditioned using 0.3 N HCl at least the 3 times the volume of resin. Cation exchange removes remaining cations of Ca, K, Fe, Al, V, W, Zn, Zr, and Ti which interfere with $NH_4MgPO_4 . 6H_2O$. Ammonia is added to effluent from column and reprecipitated as $NH_4MgPO_4 . 6H_2O$ by adding magnesia reagents. Final ppts were filtered, redissolved with 0.1 N HCl and stored in a scintillation vial for counting.

Ammonium molybdate reagent was prepared by dissolving 100 g of analytical grade ammonium molybdate ($(NH_4)6Mo_7O_{24} . 4H_2O$; F.W.=1235.95) in 1 liter of distilled water. 80 ml of concentrate ammonia solution was added to above solution. This solution was added to a 1 liter of 6 N HNO_3 with rapid mixing.

Magnesia reagent was prepared by dissolving 50 g of $MgCl_2 . 6H_2O$ (F.W.=203.3) and 100 g of NH_4Cl (F.W.=53.49) in 500 ml of distilled water. 10 ml of concentrated ammonia solution was added and diluted to a final volume of 1 liter. Solution was kept overnight then filtered before use.

2.3 Low level scintillation counting of ^{32}P and ^{33}P

Measurement of radiochemically purified P samples with beta emitters in low-level scintillation counter is straightforward. Here, low-level designates very weak radioactivity frequently met in natural environmental samples. Samples of about 10 ml volumes in weak HCl solution were transferred to scintillation vial and added scintillation cocktail to full volume of vial (20 ml).

Samples were counted in Quantalus Scintillation counter (Model 1220) installed in Korea Basic Science Institute. The core of system consists of 4 photomultiplier tubes. System also monitors signals from outer environmental origin and by adopting anticoincindence method, by canceling the signals from other than sample system is secured for lower background.

Data acquisition part of the system equipped with rather small amount of 1 K (1,024) channels compared to competing models of other company with 4 K channel MCAs. The disadvantage of small memory buffer is wider energy allocation per channel that restricts separation of overlapped peaks when two or more beta emitting radionuclides are measured together. The system configured is best for single beta measurement such as tritium, radiocarbon or ^{32}P.

Counting efficiency of system measured by certified reference material of known activities of radionuclides showed very high counting efficiency over 85%. Since the objective of this study is the activity ratio between $^{33}P/^{32}P$ counting efficiency is not considered seriously assuming more or less constant counting efficiency over entire energy (0 - 2 MeV).

Samples were counted using all 1,024 channels to allow simultaneous measurement of ^{32}P and ^{33}P. Background was measured only with scintillation cocktails. Samples were counted for 10 hours and background were counted for 100 minutes.

Chapter 3. Model Construction

For a proper application of cosmogenic P radionuclides in the ocean, source function as well as sink terms in various pools should be precisely described. Since these radionuclides are produced in surface seawater as well as in atmosphere, cosmogenic P present in seawater is a mixture of different origins. Uncertainty in source function will limit the applicability of cosmogenic P techniques. First section will review current status known source functions of cosmogenic P.

This study strongly relies on the assumption that twin P radionuclides behave exactly the same way as its stable partner, ^{31}P, a prerequisite property to be used as tracers for the trophic transfer in marine food web. Upon entering marine food web as a nutrient to microscopic algae along with stable P, a model describing the transfer of cosmogenic P through simplified conventional food chain is modeled utilizing $^{33}P/^{32}P$ activity ratio in the second section.

3.1 Source functions of cosmogenic phosphorus

Earth's atmosphere is continually bombarded by cosmic rays originating from stellar objects such as stars. They are composed primarily of protons and alpha particles with wide range of kinetic energy from 10^{-3} to more than 10^{10} GeV. When energetic cosmic ray particles of more than 10 MeV penetrate a target nucleus one or more nucleons (collective term describing a particle consist of only neutron and proton) are emitted, resulting in the formation of nucleus of lower atomic number. This mode of element production is called spallation.

Spallation of Ar which comprise 1% of earth's atmosphere in volume basis is the primary source of ^{32}P and ^{33}P production. Because earth's magnetic field acts like a shield for charged particles, cosmic rays available for spallation production of cosmogenic P nuclides in seawater is much less than in the atmosphere. Target elements in seawater are Cl, S, and K. Theoretical calculation expects that less than 5% of cosmogenic P in seawater is produced in surface ocean. Estimated production and fluxes of P radionuclides based on Lal and Peters (1967) are summarized in Table 1.

Cosmogenic P nuclides produced in the atmosphere are believed to be rapidly oxidized to phosphate and scavenged by aerosols. Aerosols are ultimately removed from the troposphere by precipitation. Wet precipitation is pointed out as a main route of injection to surface earth for the short lived radionuclides (Lal *et al.*, 1957). Mean tropospheric flux shown in Table 1 is based on the model calculation using 40 days of washout time of troposphere (Lal *et al.*, 1988). Productions in stratosphere is about two times the tropospheric ones, however, stratosphere is strongly stratified as name stands for that doesn't mixes well with troposphere. Therefore injection of cosmogenic P from stratosphere is considered as negligible.

Cosmogenic P atoms in seawater are of mixed origin so called garden variety; mostly of atmospheric origin injected directly to sea surface via wet or dry fallout plus minor ingredients produced in situ. In coastal waters some of cosmogenic P atoms are transported from elsewhere by riverine input and subsequent mixing.

Activities (disintegrations per minute per unit volume) of cosmogenic P are calculated for hypothetical surface ocean of 50 m thickness of upper mixed layer and turnover time of P as 30 days. These are calculated to have ballpark figures for a development of analytical procedures. As shown in Table 1 activities are extremely low. We are expected to see less than one disintegration per minute from 1 ton of seawater or in the other sense less than 400 atoms per 1 cm^2 area water column

Table 1. Oceanic source functions of cosmogenic ^{32}P and ^{33}P (Lal and Lee, 1988).

Parameter	^{32}P	^{33}P
Mean global staratospheric production (atoms $\cdot cm^{-2} \cdot min^{-1}$)	3.2×10^{-2}	2.8×10^{-2}
Mean global tropospheric production (atoms $\cdot cm^{-2} \cdot min^{-1}$)	1.6×10^{-2}	1.3×10^{-2}
Mean tropospheric flux (atoms $\cdot cm^{-2} \cdot min^{-1}$)	5.8×10^{-3}	6.9×10^{-3}
Mean oceanic production (atoms $\cdot cm^{-2} \cdot min^{-1}$)	7.6×10^{-4}	2.9×10^{-4}
Mean inventory in the ocean (atoms $\cdot cm^{-2}$)	1.9×10^{2}	3.8×10^{2}
Activity in the hypothetical mixed layer* (dpm $\cdot m^{-3}$)	0.78	0.65

*: Thickness=50 m, P turnover time=30 days

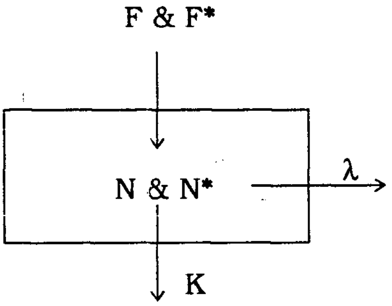
According to earlier studies, production of cosmogenic P in troposphere is nearly independent on latitude (Lal and Peters, 1967). This give an idea of relatively constant ratio of $^{33}P/^{32}P$ in world wide wet precipitations if the presumed washout time of 40 days is plausible. Grant that, $^{33}P/^{32}P$ can be used for direct comparison of world wide biological samples without initial ratio of injection, for example $^{33}P/^{32}P$ of bacteria, phytoplankton, and zooplankton, possibly fish.. However, this idea is yet to be proved experimentally.

3.2 Zooplankton P turnover model with ^{32}P and ^{33}P

In this study, activity ratio of $^{33}P/^{32}P$ will be used as tracer of assessing residence time of phosphorus in zooplankton community. The rationale behind its application is that the ratio increased with time due to longer half-life of ^{33}P over ^{32}P. An increase of ratio relative to that of time of injection (that of rain water) in any particulate organic phosphorus pools such as bacteria, phytoplankton, zooplankton is interpreted as aging of phosphorus in that pool according to the residence time of cosmogenic P.

Simplified model requires at least two pools, donor and recipient. To consider a zooplankton community, phytoplankton and zooplankton pools are necessary in principle. Their relationship is modelled in this study using two-box model for simplicity.

Imaginary pools of phytoplankton and zooplankton are characterized by inventory, N (number of P atoms) and flux, F (number of atoms per unit time) and turnover rate, K (time^{-1}) of given pool. Since P atoms consist of stable and radioisotopes * is used to designate radioactive isotopes. In a box containing both stable and radioactive isotopes, change in number of atoms with time are described as follows:



$$\frac{dN}{dt} = F - KN \tag{1}$$

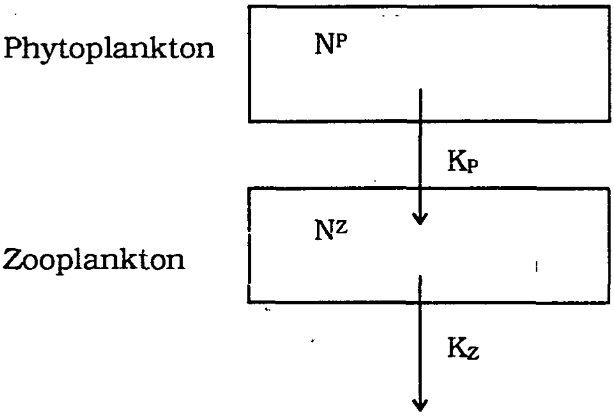
$$\frac{dN^*}{dt} = F^* - (K + \lambda)N^* \tag{2}$$

Where λ is a decay constant of a given radionuclide. When steady-state condition meets, dN/dt = 0, therefore equations (1) and (2) are modified as:

$$F = KN \tag{3}$$

$$F^* = (K + \lambda)N^* \tag{4}$$

With above basic properties, two box model can be developed. For simplicity, trophic transfer of P is assume to be one directional. Schematic of the bulk energy flow looks like following diagram:



Fluxes of cosmogenic P from phytoplankton pool to zooplankton pool are given as:

$$F_{32} = N_{32}^{P} K_P \tag{5}$$

$$F_{33} = N_{33}^{P} K_P \tag{6}$$

Assuming steady-state, mass balance of ^{32}P and ^{33}P dictates:

$$N_{32}^{P} K_P = N_{32}^{Z}(\lambda_{32} + K_Z) \tag{7}$$

$$N_{33}^{P} K_P = N_{33}^{Z}(\lambda_{33} + K_Z) \tag{8}$$

Dividing equation (8) by (7) yields,

$$\frac{N_{33}^{P}}{N_{32}^{P}}=\frac{N_{33}^{Z}}{N_{32}^{Z}}\left(\frac{\lambda_{33}+K_{Z}}{\lambda_{32}+K_{Z}}\right) \tag{9}$$

Multiplying λ_{33} and λ_{32} to denominator and nominator of equation (9) gives,

$$\frac{\lambda_{33}N_{33}^{P}}{\lambda_{32}N_{32}^{P}}=\frac{\lambda_{33}N_{33}^{Z}}{\lambda_{32}N_{32}^{Z}}\left(\frac{\lambda_{33}+K_{Z}}{\lambda_{32}+K_{Z}}\right) \tag{10}$$

According to definition of radioactive decay, λN is a activity of given radionuclide. So, equation (10) can be expressed as:

$$R_{33/32}^{P}=R_{33/32}^{Z}\left(\frac{\lambda_{33}+K_{Z}}{\lambda_{32}+K_{Z}}\right) \tag{11}$$

where, R is the activity ratio of $^{33}P/^{32}P$ in phytoplankton and zooplankton, respectively. Since R's are measurable quantities, and λ's are known constants, turnover rate of P in bulk zooplankton community, K_Z, can be calculated from following equation. Rearranging equation (11),

$$K_{Z}=\frac{\lambda_{32}-\lambda_{33}R_{Z/P}}{R_{Z/P}-1} \tag{12}$$

where, $R_{Z/P}$ is a activity ratio of $^{33}P/^{32}P$ in zooplankton over phytoplankton samples.

Since turnover rate should be a positive number, valid $R_{Z/P}$ has a very narrow range between 1.0 to 1.75. Strictly speaking, more realistic values of $R_{Z/P}$ are in the range of 1.0 to 1.5. In these case, corresponding turnover times ($1/K_Z$) are 0 and 68 days, respectively (Fig. 1).

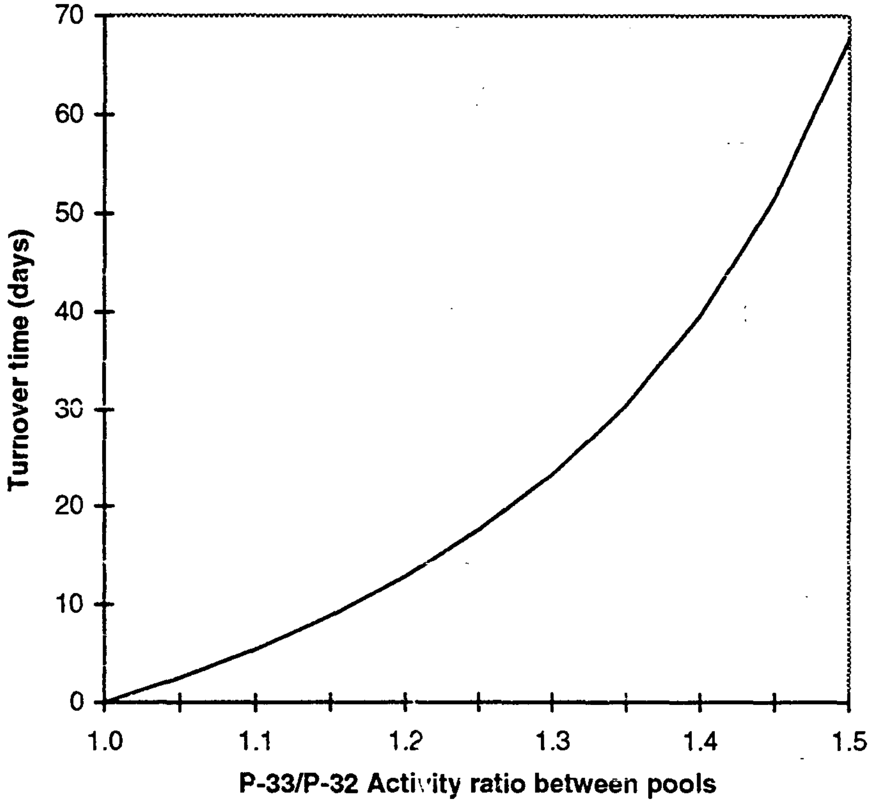


Figure 1. Turnover time (days) of phosphorus in a target pool calculated from continuous input model utilizing activity ratio of $^{33}P/^{32}P$ between source and target pools.

Chapter 4. Results and Discussions

A total of 3 samples were analyzed for the measurements of ^{32}P and ^{33}P activity ratio: one rain water sample and two zooplankton samples. The algorithm developed in previous section, requires the activity ratio in the phytoplankton. However, it is not possible to have large quantity of pure natural phytoplankton using conventional net-towing method due to a contamination of zooplankton. Instead, this study compares activity ratio of zooplankton with that of rain water. The bottom line of comparison is based on current knowledge of fast phytoplankton growth rate of about 1 day in exponential growth stage and 2-3 days in normal growth stage.

When trophic transfer and residence time of P in that pool are fast enough, the activity ratio between two pools should be close to unity. Model (eqn. 12) predicts activity ratio of 1.05 when phytoplankton residence time is 2.5 days (Fig. 1).

This chapter describes the results of the measurement and future refinement of the techniques developed in this study. As will be mentioned in following sections, there exist many problems yet to cope with. Despite some drawbacks, this method finds other possible applications. Future application is also mentioned briefly in the last section.

4.1 Tropospheric fallout ratio of $^{33}P/^{32}P$

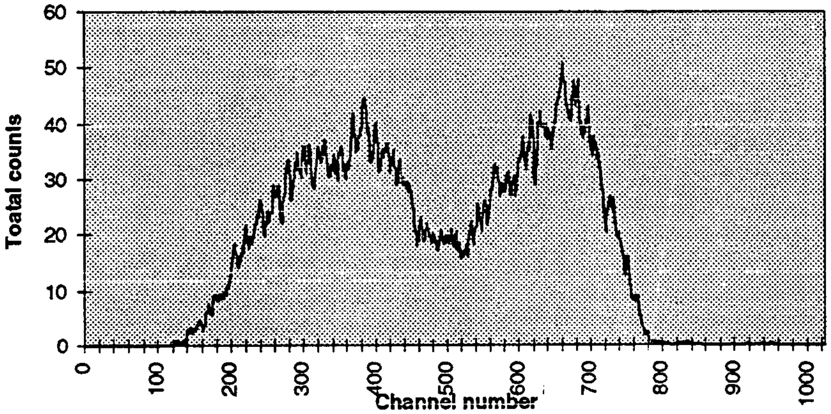
For short-lived cosmogenic radionulides, spallation production in stratosphere does not contribute to flux to ground. Due to about 2 year residence time in stratosphere, most of short-lived cosmogenic radionuclides decay out where they are produced. Cosmic ray particles that strongly influenced by the existence of earth's magnetic shield are primarily used up in the stratosphere. Therefore cosmogenic P radionuclides injected into earth's surface consist mainly of what produced in the troposphere. Troposphere mixes fast enough that injection rate are supposed to be fairly independent of latitude.

Theoretical prediction on spallation production and fallout of cosmogenic ^{32}P and ^{33}P (Table 1) based on Lal and Peters (1967) expects $^{33}P/^{32}P$ activity ratio of 1.9 at the time of input. There are limited number of experimental data available on spalltaion production using target nuclides of Cl, S, K as in seawater but not of Ar as in atmosphere. Published data $^{33}P/^{32}P$ production ratio (in atom number/time) varies widely from 0.2 to 0.5 (Waser, 1993).

Only one rain water sample is analyzed in this study. The result of scintillation measurement of rain water are shown in Fig. 2. Upper panel in Fig. 2 is the raw data spectrum without background subtraction and decay correction. Bottom panel shows spectra of ^{32}P and ^{33}P background corrected and overlapped peaks are separated using exponential extrapolation.

The activity ratio of $^{33}P/^{32}P$ in rain water was 0.8 ± 0.2 at the time of precipitation. Year-long time series data of $^{33}P/^{32}P$ activity ratio in rain water monitored at the Bermuda Biological Station in '91-'92 period showed annual mean of 0.96 with the range of 0.66 to 1.20 (Waser, 1993) quite consistent with our result. Waser (1993) reported that individual activities of each rain event showed large variation, however, due to their same experience in the atmosphere, the activity ratio showed remarkable stability.

(a) Raw Counting Spectrum



(b) Deconvoluted Spectrum of (a)

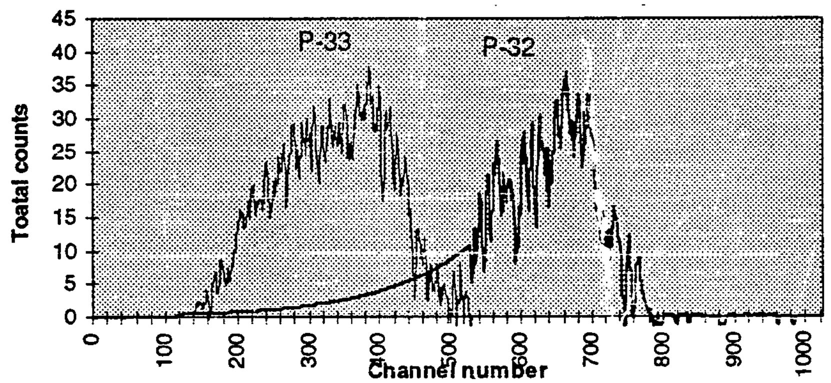


Figure 2. Scintillation beta counting spectra of rain water collected in May 15, 1995. a) raw spectrum counted for 10 hours and b) background corrected and deconvoluted spectrum of ^{32}P and ^{33}P using exponential fit to raw spectrum.

Since atmospheric injection is influenced by the stratosphere-troposphere exchange, activity ratio showed seasonal pattern of higher ratio in winter-spring season and lower ones in summer-fall season in Bermuda. The reason for higher activity ratio in winter-spring season is due to the stratopheric injection when flux through tropopause is strengthened (Lal et al., 1988).

The results of Waser (1993) are lower than those of Goel et al. (1959, cited in Waser, 1993) of 0.8-1.6 but higher than 0.3-0.4 to 1.0 of Luyanas et al., (1970) and 0.46 of Lal et al., (1960). All the previous studies have experienced technical difficulties measuring weak betas of ^{33}P. Year-long monitoring of ^{32}P and ^{33}P in rain water using low-level scintillation technique is strongly recommended afterwards.

4.2 $^{33}P/^{32}P$ ratio in zooplankton samples

Measurements of cosmogenic phosphorus yield unexpected results as shown in Fig. 3. Only one peak in the region of ^{33}P is identified and the signals in the ^{32}P energy are completely dead. In this case model developed using the $^{33}P/^{32}P$ ratio can not be applied at all.

Channels recording beta signal is widened in zooplankton counting spectrum (Fig. 3). Unlike to rain water, zooplankton are expected to contain more beta emitters originate from land. This requires more careful radiochemical purification for zooplankton. Firstly, combustion of dried zooplankton specimen needs higher temperature, possibly 550 or 600°C for complete removal of carbon that makes solution turbid. Secondly, repurification by second precipitation of ammonium phosphomolybdate by dissolving it with ammonia helps removal of remaining interfering metal ions and beta emitters.

The reason why zooplankton has only ^{33}P with relatively longer half-life can be traced from different points of view. Assuming activity ratio of 0.8 measured in rain water as the initial injection ratio into sea surface it will increase as a function of time. Considering the master radioactive decay equation activities after time t are given as:

$$\lambda_{32} N_t^{32} = \lambda_{32} N_0^{32} e^{-\lambda_{32} t} \qquad (13)$$

$$\lambda_{33} N_t^{33} = \lambda_{33} N_0^{33} e^{-\lambda_{33} t} \qquad (14)$$

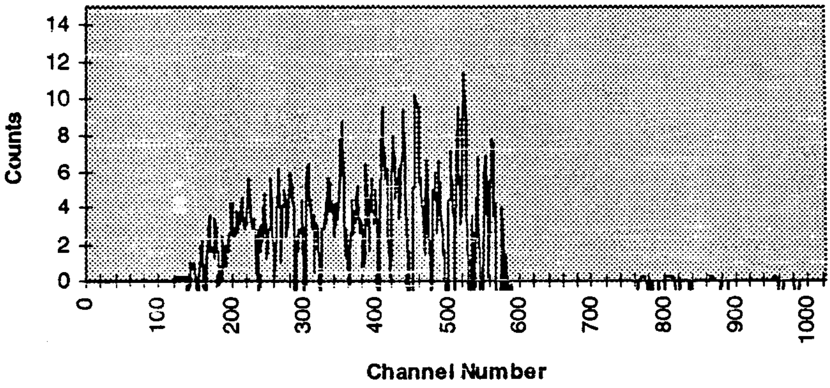
where, λs are decay constants, N is the number of atoms, and attributes 32, 33, 0, t designate properties belonging to ^{32}P, ^{33}P and time zero and after time t, respectively. The activity ratio of ^{33}P over ^{33}P at time t ($A_{33/32,t}$) is then ,

$$A_{33/32,t} = A_{33/32,0} e^{(\lambda_{32} - \lambda_{33})t} \qquad (15)$$

From above equation , after 100 days $A_{33/32,t}$ becomes 8.2 times $A_{33/32,0}$ and 50 times after 6 months. However, it is unlikely that there were no appreciable wet precipitation's during last 6 months.

More plausible explanation is due to the short half-life of ^{32}P. Measurable activity of low level radioactivity lasts less than 8 times the half-life. After 8 times the half-life only 1/128 of original activity remains that is hardly measurable by radioactivity counting. Since the half-life of ^{32}P is 14.3 days, time required for dead signal is 114 days without turnover. Considering the time elapsed between specimen collection and radioactive measurement of 43 days, it is expected that there were no significant input of cosmogenic P for previous 70 days, i.e., since early February, 1995 in the study area. In case of ^{33}P, after 114 days 4.4% of original activity remains thus signals can be seen as in Fig. 3. Since turnover of P by zooplankton facilitates reducing radioactivity, it is not unreasonable to guess such situation.

(a) Raw Counting Spectrum of Small Zooplankton



(b) Raw Counting Spectrum of Larger Zooplankton

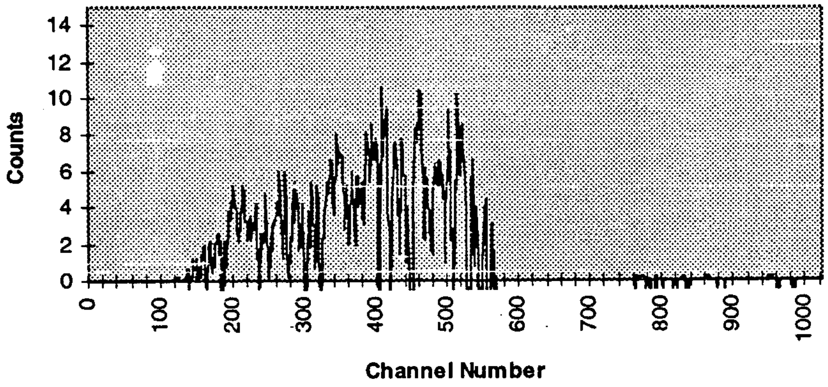


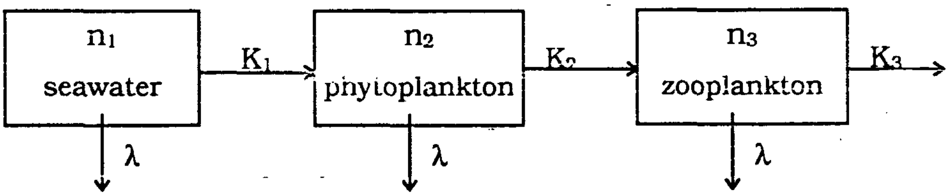
Figure 3. Scintillation beta counting spectrum of ^{32}P and ^{33}P in zooplankton collected on April 18, 1995 in the East Sea. Spectrum of zooplankton caught by 333 μm mesh net (a) and 505 μm (b).

Model developed in section 3.2 assumes at least continual input of cosmogenic P hence maintaining measurable amount of ^{32}P and subsequent use of $^{33}P/^{32}P$ ratio. When dry season lasts for considerable amount of time as evidenced in this study (2 or more months) pulse input model of point injection describes the situation better.

Pervious model called continuous input model hereafter, is simple to use but can only be used when wet precipitation interval is short enough to maintain $^{33}P/^{32}P$ ratio relatively constant. Last year is remembered as abnormal dry season. In normal years with winter snow or rain, it is expected we can safely apply continuous input model.

Pulse input model requires dead signal before injection. Because any e signal at time zero that makes interpretation obscure continuous input model is preferred when applicable.

Let's begin with a box model consists of 3 boxes representing seawater pool, phytoplankton pool, and zooplankton pool, respectively. A very simplified schematic diagram of trophic transfer of cosmogenic P looks as follows:



Each pool is characterized by the number of cosmogenic P atoms (n_i) or specific activity (disintegration per unit time per unit mass) and turnover rates

(K_i). Other sink term λ represents disintegration constant specific to the radioisotope's half-life (= *ln*(2)/half-life).

Changes in the number of atoms or specific activity (say, dpm/g-P) in each pool are described as:

$$\frac{dn_1}{dt} = n_1(-\lambda - K_1) \tag{16}$$

$$\frac{dn_2}{dt} = n_1K_1 - n_2(\lambda + K_2) \tag{17}$$

$$\frac{dn_3}{dt} = n_2K_2 - n_3(\lambda + K_3) \tag{18}$$

Solutions for above equations are:

$$n_1 = n_{1,0}e^{-(\lambda+K_1)t} \tag{19}$$

$$n_2 = n_{2,0}e^{-(\lambda+K_2)t} - \frac{n_{1,0}K_1e^{-(\lambda+K_1)t}}{K_1 - K_2} \tag{20}$$

$$\begin{aligned} n_3 &= n_{3,0}e^{-(\lambda+K_3)t} + \frac{e^{-(2\lambda+K_1+K_2)t}}{(K_1-K_2)(K_1-K_3)(K_3-K_2)} \cdot \\ &(K_1K_2n_{2,0}e^{(\lambda+K_1)t} - K_1K_2^2n_{2,0}e^{(\lambda+K_1)t} - K_1K_2^2n_{1,0}e^{(\lambda+K_2)t} - \\ &K_1K_2K_3n_{2,0}e^{(\lambda+K_1)t} + K_1K_2K_3n_{1,0}e^{(\lambda+K_2)t} + K_2^2K_3n_{2,0}e^{(\lambda+K_1)t}) \end{aligned} \tag{21}$$

where, $n_{1,0}$, $n_{2,0}$, and $n_{3,0}$, designate initial values when t=0.

At the time of pulse input, $n_{2,0}$ and $n_{3,0}$ assume to be 0, and this simplifies n_2 and n_3 as

$$n_2 \approx -\frac{n_{1,0}K_1e^{-(\lambda+K_1)t}}{K_1 - K_2} = \frac{K_1}{K_2 - K_1}n_1 \tag{22}$$

$$
\begin{aligned}
n_3 &\approx \frac{e^{-(2\lambda+K_1+K_2)t}}{(K_1-K_2)(K_1-K_3)(K_3-K_2)} \cdot (-K_1K_2^2 n_{1,0} e^{(\lambda+K_2)t} + K_1K_2K_3 n_{1,0} e^{(\lambda+K_2)t}) \\
&= \frac{K_1K_2}{(K_1-K_2)(K_1-K_3)} n_{1,0} e^{-(\lambda+K_1)t} \\
&= \frac{K_1K_2}{(K_1-K_2)(K_1-K_3)} n_1, \ or \ \frac{K_2}{K_3-K_1} n_2 \text{..........................} (23)
\end{aligned}
$$

Model requires several constants to solve the equations for K_3. The initial concentration, $n_{1,0}$, and turnover rates of K_1, and K_2 should be known. The value of K_1 is one of properties difficult to measure to date. No precise values were known but it is estimated to be on the order of month.

Model depends on the values of K_2-K_1, and K_3-K_1 that should be positive numbers, meaning K_2 and $K_3 > K_1$. The implication of this is that turnover of seawater pool is slowest. Otherwise system fails to sustain phosphorus prerequisite to organisms. Since phytoplankton can double the population within 5 days that K_2 is much higher than K_1. Remaining problem is the magnitude of K_3.

Theoretical calculation constrains the turnover of P in zooplankton community should be faster than that of seawater pool, unless zooplankton has fair amount of cosmogenic P at time zero. Figure 4 illustrates the imaginary situation of system with turnover times of P in pools of seawater, phytoplankton and zooplankton assigned as 60 days, 5 days and 20 days. Initial specific activity of seawater is given as arbitrary number as dpm/mg-P. Due to slowest turnover rate seawater pool loses its activity slowly for over 70 days. In contrast to seawater, specific activity of phytoplankton is weaker than that of zooplankton due to its fast turnover over zooplankton.

Specific activity of ^{32}P in zooplankton in Fig. 4 is very sensitive to the relative magnitude of K_1 and K_3. When turnover time is longer than 25 days in presumed situation, specific activity of zooplankton exceeds that of seawater indicating net accumulation in zooplankton. Offshore data of ^{32}P specific activities in zooplankton showed less than 100 dpm/g-P in Southern California Bight (Lee, 1991) to 3,000 dpm/g-P in Bermuda (Waser, 1993). In former case specific activity of seawater and zooplankton are on same order of magnitude, but the latter case exhibits possibly one order of magnitude higher values in zooplankton. When the specific activity of zooplankton is higher than that of seawater, there is high possibility we can find measurable specific activities in higher trophic levels such as fishes eat on plankton.

According to pulse input model a situation of ^{33}P monopoly is possible when the appreciable wet fallout was not occurred for about 2 months prior to experiments. Since we have experienced unusual drought in late '94, this situation was likely to be happened. Although samples and data taken *in situ* were not enough for the exact solution of equations, unique situation helps to confine possible range of K_3 values by assigning reasonable values of K_1 and K_2. However, it should be mentioned that this kind of solution is not possible in general.

Range of K_3 is examined with turnover time of P in seawater from 30 days to 60 days and that of phytoplankton as 5 days. As the growth rate of phytoplankton in winter is slow, 5 days turnover time seems reasonable. Furthermore pulse model showed, the specific activities of seawater and zooplankton are not very sensitive to K_2 value. Possible turnover time of P in zooplankton is tested from each pairs of K_1 and K_2 as shown in Fig. 4.

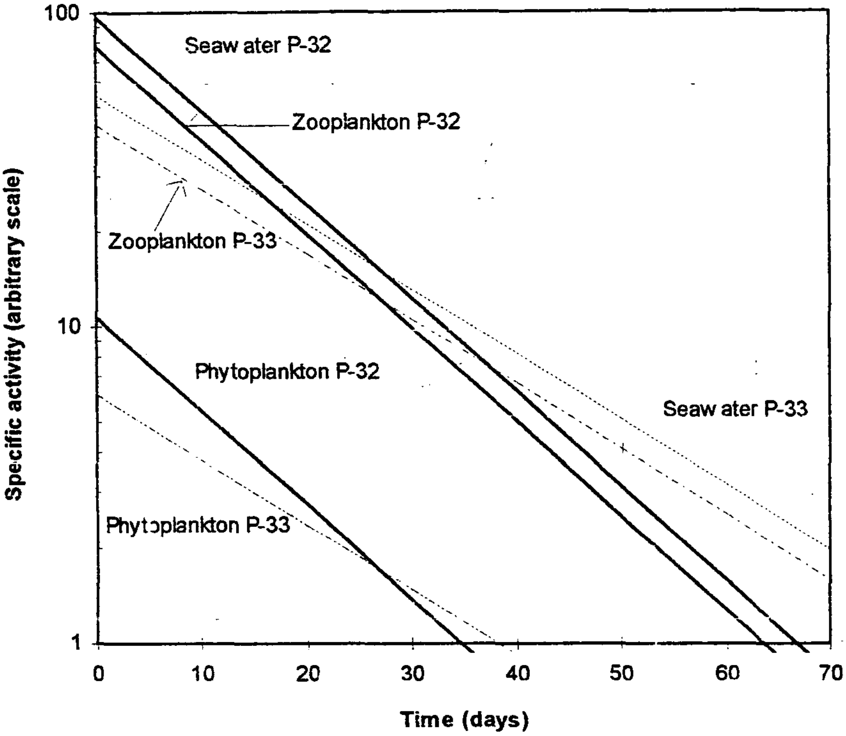


Figure 1. Change of relative specific activities of ^{32}P and ^{33}P with time after a pulse input to seawater. Shown curves are calculated for 50 days of seawater P turnover time, 5 and 21 days of turnover times for phytoplankton and zooplankton, respectively.

Table 2. Possible time range of phosphorus turnover time of zooplankton samples taken in the East Sea in April 18, 1995, with the best estimates on seawater turnover time ranged from 1 to 2 months, and fixed turnover time of phosphorus in phytoplankton community as 5 days.

Turnover time of seawater P (days)	Estimated turnover time of zooplankton P (days)
30	15 - 22
40	15 - 24
50	16 - 25
60	17 - 28

For each pair of K_1 and fixed value of K_2=0.2, range of K_3 is chosen that can reproduce the situation of ^{33}P monopoly indicating all ^{32}P are decayed out but still measurable amount of ^{33}P remains. Despite the lack of information of required parameters, estimated turnover times of P in zooplankton community showed remarkable convergence to 2 - 4 weeks with given wide seawater P turnover time ranged from 30 to 60 days (Table 2). Pronounced ^{33}P peak is more evident in the model calculation (see Fig. 4) when turnover time is more than 3 weeks so that best likely P turnover time is estimated sometime in 3 to 4 weeks. To exhibit only long-lived ^{33}P, time required from pulse input is calculated as 40 to 50 days in all situation, which is not inconsistent with previous assumption of abnormal winter drought.

In summary, pulse input model with the assumption on the scale of turnover time of seawater and phytoplankton pools as 1 to 2 months, and 5 days, respectively, interpreted 3 to 4 weeks as best likely turnover time of phosphorus in zooplankton taken on April 18, 1995 in the East Sea.

4.3 Discussions

This study is the first measurement of cosmogenic P in zooplankton using low-level scintillation counting techniques. A simple algorithm utilizing the activity ratio of $^{33}P/^{32}P$ is developed for the calculation of P turnover rate in zooplankton. This noble idea depends only on the ratio of $^{33}P/^{32}P$, however, it depends on the assumption of continuos injection of cosmogenic P into surface ocean.

Unfortunately zooplankton samples taken in the April in the East Sea contain no measurable ^{32}P. This makes application of new technique impossible. Although P turnover time of zooplankton can be successfully estimated with limited confidence using pulse input model, it requires other parameters to solve the equation. Prerequisites are P turnover time of seawater pool and phytoplankton pool. This in turn requires biomass of phytoplankton and zooplankton, concentration of dissolved phosphorus in seawater. Hence the pulse input model is not as simple as continuous input model thereby difficult to use.

If winter-spring drought is a usual phenomenon then use of pulse model is indispensable option. It is expected that in wet season continuous input model can be successfully adopted. However, this study points out that sample should be measured as soon as recovered to see the statistically good ^{32}P peak from zooplankton.

This study concentrates only on the ratio of $^{33}P/^{32}P$ as a single variable. In the future, it is recommended to measure data on specific activity (disintegration per unit mass of sample) basis. By doing so, one can use other model when poor ^{32}P peak was found as accidentally faced in this study.

It seems very promising to apply $^{33}P/^{32}P$ ratio tracer method for other aquatic ecosystem such as river and lake ecological study. Since inland waters have stronger activities total food chain including fishes are expected to be scrutinized with this methods. Field application is strongly recommended.

Considering the model results ^{33}P and ^{32}P are expected to be measurable in fishes eating phytoplankton and zooplankton at least in wet season. One handicap to this methods is that it requires large amount of specimen (handful of dried plankton) because of very low activities of naturally occurring cosmogenic P. Conventional way of collecting plankton using meshed net is not able to provide pure phytoplankton and zooplankton.

Like other tracer methods utilizing essential element for organism such as ^{14}C or ^{15}N, this method also relies on two assumptions. First one is that there are no isotopic discrimination by organism. Because of mass difference between isotopes, it seems some discrimination is possible during the chemical reactions inside the cell. Generally artificial tracer techniques use high activity spiking techniques with short incubation time. On the other hand this technique uses short-lived natural radioisotopes. Time scale involved for this study is longer than the half-lives of the radionuclides so that isotopic discrimination is more serious problem than the conventional artificial labeling when it happens. However, there are no firm evidence of isotopic discrimination during uptake by microscopic organisms yet.

The other assumption is single phosphorus pool inside cell, or organism. If there are two or more phosphorus pools in the cell with quite different turnover rates, then use of cosmogenic P as trophic tracer subjects to serious misunderstanding. Currently it is believed that there are at least two pools of fast and slow turnover phosphorus pools in the cell, however, time scale is assumed much smaller than that of population or community level turnover that this is not considered seriously in the model.

Despite the recent advances in the field of radioactivity counting techniques, measuring absolute concentration of low-level radioactivity is not an easy task. In many cases, counting peaks of statistically satisfactory are not obtained. Models depend on absolute activity requires huge efforts to have data and even then often faced with relatively large counting errors. Therefore using ratios as parameters has tremendous benefit over using absolute values as evidence in the continuous input model. In that kind of models various parameters are eliminated and consequently fewer kinds of data are necessary to run a model.

Cosmogenic ^{32}P and ^{33}P are applied to the turnover of material (P, more specifically) in the zooplankton community in this study. Since phosphorus is an essential nutrient to aquatic organisms, cosmogenic P have other potential applications. Firstly, there is high possibility to have signals from fishes feed on plankton which means food chain can be trace to higher trophic level. The specific activities and activity ratio can be used to find out how fast the food chain works. Feasibility can be tested in lake or river ecosystem as mentioned before because of easy access and higher activities in fresh water.

Secondly, simultaneous measurement with other cosmogenic radionuclides can be used to calculated tropospheric washout time that is not precisely known yet. Most promising cosmogenic radionuclide is ^{7}Be (half-life, 53.3 days). Due to its small mass ^{7}Be is produced much more in the troposphere and injected to ground exactly same route with ^{32}P and ^{33}P. Since ^{7}Be is a gamma emitter its measurement is very simple with a high purity germanium gamma counter.

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